

Proceedings of

The 26th International Biohydrometallurgy Symposium

The invisible miners: from laboratory to operation

28th to 30th September 2026
Centre de Conférences, Orléans, France

Edited by: Christopher G Bryan and Benjamin Monneron-Enaud
Hosted by the Bureau de Recherches Géologiques et Minières (BRGM), France



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"*It is a long way from theoretical considerations and laboratory experiments to technical applications, especially with the economic uncertainties lurking in the background*"

Wolfgang Schwartz opened the proceedings of the first International Biohydrometallurgy Symposium in 1977 with these words, at a time when microbial leaching was applied on a broad scale only to sulfidic copper and uranium ores. Almost fifty years later, the 26th Symposium meets in Orléans under the theme "*The invisible miners: from laboratory to operation*".

Reading the forewords of earlier proceedings is humbling. In 1977, Schwartz listed the "new" directions of leaching research: leaching of ore concentrates in suspension, combined electrochemical and microbial leaching, microbial separation of metal mixtures, leaching at high temperatures and high hydrostatic pressures, the use of brackish and sea water, mixed microbial populations, heterotrophic leaching, and the treatment of industrial wastes and mine waters. In 1987, Paul Norris and Don Kelly observed that the state of the art in commercial biohydrometallurgy was not fully reflected in their proceedings, because process details were protected, and hoped that commercial success would bring greater openness.

Almost every one of Schwartz's topics reappears in this volume. A notable newcomer is the recovery of metals from electronic waste and spent batteries, waste streams that barely existed in 1977. Even so, the idea has deep roots in Orléans: Claude Guillemin, then scientific director of BRGM, the French Geological Survey, launched an experimental facility for sorting and recycling urban waste in 1974, and by 1975 was already speaking about recycling discarded electrical and electronic equipment.

Much has changed since 1977. Genomics, microbial ecology and interfacial science now let us ask what the invisible miners are doing, how, and why, in ways the founders could not.

Biohydrometallurgy at BRGM began in the 1980s under Dominique Morin and the late Pierre Ollivier, who with their colleagues led the early work at Kasese, Uganda. There, the bioleaching of cobalt-bearing pyrite tailings took about a decade to progress from the laboratory to an industrial plant, which went on to reprocess the entire stockpile. It remains one of very few commercial examples of tank bioleaching of mine waste, and its microbial consortium is still extensively used in Orléans. That work laid the foundation for much of what followed, and the legacy of these pioneers continues in BRGM's work today.

IBS 2026 places operation alongside discovery. Almost 200 delegates from more than 30 countries bring 71 oral presentations and 91 posters, which are complemented by keynote and invited lectures from industry and research, start-up pitches, and a round table that takes up the questions this foreword raises. After nearly five decades, how far have we advanced on the challenges our predecessors set out? Have we brought the disciplines together without diluting the depth each requires? Does knowledge now flow in both directions between industry and research? And with so much expertise gathered in one room, are we challenging ourselves enough? We hope this volume, and the discussion it accompanies, will help to answer them.

I look forward to an exciting week in Orléans, and to continuing the conversation in Cape Town in 2028.

Christopher G. Bryan

Chair, Local Organising Committee, IBS 2026

Orléans, September 2026

Acknowledgements

The IBS 2026 Local Organising Committee thanks, above all, the authors who submitted abstracts and whose work fills this volume; the members of the IBS International Scientific Committee for reviewing them; our sponsors Ausenco, PEPR Recyclage and PROMETIA; our start-up sponsors 1849 Bio, Transition Metal Solutions and Novonesis; our media sponsor MEI; the exhibitors and partners whose support made the symposium possible; and our colleagues at BRGM for their support throughout.

Sponsors

Ausenco is a global engineering, consulting and project delivery firm built for the mining industry. Founded in Brisbane, Australia, in 1991, it now works from 21 offices across five continents. Ausenco works alongside its clients through every phase of a project, from conceptual design and feasibility studies, through engineering, procurement and construction management and commissioning, to asset performance, mine waste management, and closure, remediation and reclamation. Its stated aim is to help the industry produce the minerals and metals needed for the future more sustainably, with less impact on the planet and its resources.

PEPR Recyclage, Recyclabilité et Ré-utilisation des matières (Recycling, Recyclability and Re-use of Materials; PEPR: Programmes et Équipements Prioritaires de Recherche) is a national research programme under the France 2030 acceleration strategy, led by CNRS. It brings together more than 100 partners across France, with the aim of establishing a French model for secondary raw materials from recycling and their reincorporation into new products. IBS 2026 is supported through the Orléans-Centre-Val de Loire lieu-totem. Established together with the programme's academic and socio-economic partners, the lieux-totem of PEPR Recyclage are some twenty sites that host local activities on the circularity of materials. Built around cross-cutting themes, they will foster interdisciplinary links and interaction between the different actors in the field.

PROMETIA, the Mineral Processing and Extractive Metallurgy for Mining and Recycling Innovation Association, is an international non-profit association (AISBL) established in Brussels in December 2014. Created at the initiative of CEA with 13 founding partners from industry, SMEs, piloting facilities, research organisations and academia, including BRGM, it promotes innovation in mineral processing and extractive metallurgy and aims to strengthen European technical skills and industrial know-how in raw materials processing. It gives industrial partners easier access to leading European R&D teams and up-scaling facilities, promotes innovative research results towards industry, and helps members access funding opportunities. By training a new generation of engineers and metallurgists, it also seeks to boost the long-term innovation capacity of Europe's raw materials sectors.

Media sponsor

Start-ups

Alongside its focus on industry and applications, IBS 2026 includes a section dedicated to biomining start-ups, reflecting the excitement and energy around young companies in the field. A dedicated start-up package gives each company space on the IBS 2026 website and a guaranteed slot to pitch to delegates in the opening session on Monday 28 September. Pitching on the first day puts the companies on a platform and gives them the rest of the symposium to engage with the community. The three companies below are pitching at IBS 2026 and have also supported the symposium through the start-up package.

1849 Bio engineers microbes to improve metal recovery from low-grade ore and e-waste, with a focus on refractory minerals like chalcopyrite. By leveraging state-of-the-art synthetic biology techniques, we aim to transform uneconomic materials into valuable sources of metals.

Transition Metal Solutions develops custom chemical additives that enhance wild microbial communities in sulfide ores to improve metal extraction. The company's platform analyses the indigenous microbiome already at work in a mining operation to formulate targeted products that increase recovery, reduce acid consumption, and accelerate extraction, without new infrastructure or changes to existing flow sheets. Starting with copper, a metal key to the energy transition, the technology is designed to extend to other critical minerals. The company is backed by leading climate and deeptech investors across the US, Europe, and Australia.

Novonesis, a global leader in industrial biosolutions, has launched an initiative to support the mining industry. Its core competence is the efficient, high-quality production of biology. Rather than developing new processes or identifying new organisms itself, Novonesis aims to take responsibility for producing the biology, allowing start-ups and operators to focus on process and application technology.

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Conference venue: Centre de Conférences, 9 place du 6 juin 1944, 45000 Orléans, France. Telephone +33 2 38 56 97 10.



In memoriam and honours

The biohydrometallurgy community remembers colleagues who have passed, and honours those who have recently retired from the field.

Obituary for Professor Stoyan Groudev



Written by: Dr Plamen Georgiev

Photo provided by: Assoc. Prof. PhD. Marina Nicolova

Professor Stoyan Groudev passed away on 14th February 2024.

Prof. Groudev was born in 1941, in a period of political turbulence. After finishing secondary school, the political regime banned him from enrolling in Sofia University "St. Kliment Ohridski". A few years later, he got permission to study at Sofia University, where he graduated from the Department of Microbiology.

The whole professional career of Prof. Groudev was closely related to the University of Mining and Geology "St. Ivan Rilski," where he ascended from microbiologist to professor. He was the director of the Center for Research and Training in Mineral Biotechnology, founding the Department of Engineering Geoecology and introducing specialty Biotechnology to the university's portfolio. In 1999 - 2003, he was a Rector of the University of Mining and Geology "St. Ivan Rilski".

Prof. Groudev's influence extended far beyond his home country. He was a sought-after lecturer, sharing his knowledge and expertise at over 70 foreign universities, scientific institutes, and companies worldwide.

Prof. Groudev's research was not only extensive but also highly impactful. He authored over 660 scientific papers, published in prestigious journals, and had over 2000 citations. His research, spanning biohydrometallurgy, microbiology, ecology, and environmental protection, played a pivotal role in the construction of bioleaching installations in Bulgaria for various metals (copper, uranium, gold) and the removal of heavy metals, radionuclides, arsenic, and toxic organic compounds from soils and waters using environmental biotechnology methods.

Prof. Groudev's career was marked by his active participation in scientific councils for Mining Sciences, Microbiology, and more. His leadership extended to serving as the President of the Scientific Council at the Institute of Microbiology at the Bulgarian Academy of Sciences. His influence was further recognized when he became a member of the New York Academy of Sciences in 1996 and an academician in the International Academy of Ecology and Environmental Protection (IAELPS) in St. Petersburg in 1998.

Professor Groudev's impact on the global scientific community is undeniable. His ranking as the 137th position out of 27 888 scientists globally, according to the 2019 ranking by Stanford University, is a testament to his significant contribution to Mining, Ecology, and Environmental Biotechnology.

Obituary for Marja-Liisa Riekkola-Vanhanen (1941-2025)



Text and photo by Anna Kaksonen

Marja completed a MSc in Biochemist and Licentiate in Biochemistry at the University of Helsinki. She worked in the field of chemistry and biochemistry over five decades and an impactful career in the mining and metals industry. Among others her roles included Senior Research Metallurgist (Biohydrometallurgy) in Outokumpu 1990-2004, Chief Technology Officer in Talvivaara mining Company 2005-2009, Senior Advisor in Talvivaara Mining Company 2009-2013, and Senior Advisor consultant 2009-2025. Marja is most known for her pioneering role in the developer of the heap bioleaching process for Talvivaara Mining Company. The mine has been estimated to contribute up to one sixth of the gross domestic product of the Kainuu region in Finland. Currently the heap bioleaching is operated by Terrafame Mine. Her skills spanned from mineral exploration, biohydrometallurgy, process control, base metals such as copper and nickel, and water treatment. Marja is remembered for being kind and generous, sharing her time to graduate students and contributing to community and social services, e.g. by volunteering to teach the younger generation as a scout leader in Finnish Schouts Union 1953-2005.

Here is a selection of some of Marja's publications:

Riekkola-Vanhanen, M. 2013. Talvivaara mining company – From a project to a mine. Minerals Engineering 48: 2-9. https://doi.org/10.1016/j.mineng.2013.04.018

Halinen, A.-K., Beecroft, N.J., Määttä, K., Nurmi, P., Laukkanen, K., Kaksonen, A.H., **Riekkola-Vanhanen, M.**, Puhakka, J.A. 2012. Microbial community dynamics during a demonstration-scale bioheap leaching operation. Hydrometallurgy 125–126: 34-41. https://doi.org/10.1016/j.hydromet.2012.05.001

Saari, P., **Riekkola-Vanhanen, M**. 2011. Talvivaara bioheapleaching process. Journal of the Southern African Institute of Mining and Metallurgy 112(12):1013-1020

Nurmi, P., Özkaya, B., Sasaki, K., Kaksonen, A.H., **Riekkola-Vanhanen, M.**, Tuovinen, O.H., Puhakka, J.A. 2010. Biooxidation and precipitation for iron and sulfate removal from heap bioleaching effluent streams. Hydrometallurgy 101(1–2): 7-14. https://doi.org/10.1016/j.hydromet.2009.11.004

Wakeman, K.D., Erving, L., **Riekkola-Vanhanen, M.L.**, Puhakka, J.A. 2010. Silage supports sulfate reduction in the treatment of metals- and sulfate-containing waste waters. Water Research 44(17): 4932-4939. https://doi.org/10.1016/j.watres.2010.07.025

Bhatti, T.M., Bigham, J.M., **Riekkola-Vanhanen, M.**, Tuovinen, O.H. 2010. Altered mineralogy associated with stirred tank bioreactor leaching of a black schist ore. Hydrometallurgy 100(3–4): 181-184. https://doi.org/10.1016/j.hydromet.2009.11.010

Dopson, M., Halinen, A.-K., Rahunen, N., Boström, D., Sundkvist, J.-E., **Riekkola-Vanhanen, M**., Kaksonen, A.H. and Puhakka, J.A. 2008. Silicate mineral dissolution during heap bioleaching. Biotechnology and Bioengineering 99: 811-820. https://doi.org/10.1002/bit.21628

Puhakka, J.A., Kaksonen, A.H., Riekkola-Vanhanen, M. (2007). Heap Leaching of Black Schist. In: Rawlings, D.E., Johnson, D.B. (eds) Biomining. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-34911-2_7

Riekkola-Vanhanen, M. (2007). Talvivaara Black Schist Bioheapleaching Demonstration Plant. Advanced Materials Research, 20–21:30–33. https://doi.org/10.4028/www.scientific.net/amr.20-21.30

Kinnunen, P.H.-M., Heimala, S., **Riekkola-Vanhanen, M.-L.**, Puhakka, J.A. 2006. Chalcopyrite concentrate leaching with biologically produced ferric sulphate. Bioresource Technology 97(14): 1727-1734. https://doi.org/10.1016/j.biortech.2005.07.016

Kaksonen, A.H., Plumb, J.J., Robertson, W.J., **Riekkola-Vanhanen, M**., Franzmann, P.D., Puhakka, J.A. 2006. The performance, kinetics and microbiology of sulfidogenic fluidized-bed treatment of acidic metal- and sulfate-containing wastewater. Hydrometallurgy 83(1–4): 204-213. https://doi.org/10.1016/j.hydromet.2006.03.025

Honouring Professor Jaakko Antero Puhakka



Text and photo by Anna Kaksonen

With the transitioning of Prof Jaakko Puhakka into retirement it is timely to honour his excellent contributions to the field of biohydrometallurgy over several decades. Prof. Jaakko Antero Puhakka obtained his PhD (1990) in General Microbiology from the University of Helsinki. He worked as a Researcher at the University of Helsinki (1981-1983) and Tampere University of Technology (TUT) (1983, 1985-1989) and Associate Professor (1995-1997) and full Professor of Environmental Biotechnology(1998-2022) at TUT. He has worked as visiting scientist/assistant professor in the USA (1984-1985, 1991-1994) and Australia (2001-2002), Headed the Chemistry and Bioengineering Department (1997-2013) and became Vice-President for Research (1014) of TUT, the Academic Rector for the University of Eastern Finland (2015-2017) and the Rector of TUT (2019). Jaakko has published extensively with >240 publications in Scopus and has served in the International Scientific Committee of the International Biohydrometallurgy Symposium (IBS) since 2005 and the Finnish Academy of Technical Sciences since 2014. He has made significant contributions to bioleaching of ores and metal-containing wastes, and (bio)hydrometallurgical process and mine water treatment, including the development of the process for heapbioleaching black schist ore at the Talvivaara Mine in Finland, now operated as the Terrafame Mine. Jaakko is known as a very supportive supervisor of many post-graduate students, sharing his time generously to teach the next generation.

Here is a selection of some of Jaakko's publications:

Rasoulnia, P., Hajdu-Rahkama, R., **Puhakka, J.A.** 2023. High-rate and -yield continuous fluidized-bed bioconversion of glucose-to-gluconic acid for enhanced metal leaching. Chemical Engineering Journal. 462: 142088. https://doi.org/10.1016/j.cej.2023.142088

Hajdu-Rahkama, R., Özkaya, B., Lakaniemi, A. M., **Puhakka, J. A.** 2020. Kinetics and modelling of thiosulphate biotransformations by haloalkaliphilic *Thioalkalivibrio versutus*. Chemical Engineering Journal 401, 126047. https://doi.org/10.1016/j.cej.2020.126047

Papirio, S., Ylinen, A., Zou, G. Peltola, M., Esposito, G., **Puhakka, J.A.** 2014. Fluidized-bed denitrification for mine waters. Part I: low pH and temperature operation, Biodegradation 25 (3): 425-435. https://doi.org/10.1007/s10532-013-9671-0

Halinen, A.-K., Beecroft, N.J., Määttä, K., Nurmi, P., Laukkanen, K., Kaksonen, A.H., Riekkola-Vanhanen, M., **Puhakka, J.A.** 2012. Microbial community dynamics during a demonstration-scale bioheap leaching operation. Hydrometallurgy 125–126: 34-41. https://doi.org/10.1016/j.hydromet.2012.05.001

Nurmi, P., Özkaya, B., Sasaki, K., Kaksonen, A.H., Riekkola-Vanhanen, M., Tuovinen, O.H., **Puhakka, J.A.** 2010. Biooxidation and precipitation for iron and sulfate removal from heap bioleaching effluent streams. Hydrometallurgy 101(1–2): 7-14. https://doi.org/10.1016/j.hydromet.2009.11.004

Halinen, A.-K., Rahunen, N., Kaksonen, A.H., **Puhakka, J.A.** 2009. Heap bioleaching of a complex sulfide ore: Part I: Effect of pH on metal extraction and microbial composition in pH controlled columns. Hydrometallurgy 98(1–2): 92-100. https://doi.org/10.1016/j.hydromet.2009.04.005

Puhakka, J.A., Kaksonen, A.H., Riekkola-Vanhanen, M. 2007. Heap Leaching of Black Schist. In: Rawlings, D.E., Johnson, D.B. (eds) Biomining. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-34911-2_7

Kinnunen, P.H.-M., Heimala, S., Riekkola-Vanhanen, M.-L., **Puhakka, J.A.** 2006. Chalcopyrite concentrate leaching with biologically produced ferric sulphate. Bioresource Technology 97(14): 1727-1734. https://doi.org/10.1016/j.biortech.2005.07.016

Kaksonen, A.H., Plumb, J.J., Robertson, W.J., Riekkola-Vanhanen, M., Franzmann, P.D., **Puhakka, J.A.** 2006. The performance, kinetics and microbiology of sulfidogenic fluidized-bed treatment of acidic metal- and sulfate-containing wastewater. Hydrometallurgy 83(1–4): 204-213. https://doi.org/10.1016/j.hydromet.2006.03.025

Puhakka, J., Tuovinen, O.H. 1986. Microbiological solubilization of metals from complex sulfide ore material in aerated column reactors. Acta Biotechnology 6:233–238. https://doi.org/10.1002/abio.370060307

Puhakka, J., Hiltunen P, Tuovinen, O.H. 1985. Patterns of substrate utilization and metal solubilization from sulfide ore material by mixed cultures of microorganisms enriched from mine waters. Systematic and Applied Microbiology 6:302–307. https://doi.org/10.1016/S0723-2020(85)80036-9

Honouring Mariekie Gericke



Text and photo by Anna Kaksonen

With the transitioning of Mariekie Gericke into retirement it is timely to honour her impactful work in the field of biohydrometallurgy. Mariekie holds a BSc in Chemistry and Microbiology and a BSc (Hons) in Microbiology from the University of the Free State, and an MSc in Microbiology from the University of Pretoria. Over the years, Mariekie has worked as a Project Leader at the Council for Scientific and Industrial Research (CSIR) (1987-1995), and in several roles in Mintek, including Head of Biometallurgy (1995-2016), Executive Manager of the Biometallurgy Division (2016-2022) and Group Executive for Extractive Metallurgy (2022-2025). Mariekie's research has spanned from bioleaching metal sulfides and laterites to bioflotation and biological metal nanoparticle production and she has made great advancements in tank bioleaching of base metals. Currently she continues to contribute to the field as a Freelance Consultant. Mariekie became a member of the International Scientific Committee of the International Biohydrometallurgy Symposium in 2011 and served as the Secretary of the Committee 2013-2017.

Here is a selection of some of Mariekie's publications:

Neale, J., Seppala, J., Laukka, A., van Aswegen, P., Barnett, S., **Gericke, M.** 2017. The MONDO minerals nickel sulfide bioleach project: from test work to early plant operation. Solid State Phenomena 262: 28-32. https://doi.org/10.4028/www.scientific.net/SSP.262.28

Erasmus, M., Cason, E.D., van Marwijk, J., Botes, E., **Gericke, M**., van Heerden, E. 2014. Gold nanoparticle synthesis using the thermophilic bacterium *Thermus scotoductus* SA-01 and the purification and characterization of its unusual gold reducing protein. Gold Bul*l* 47: 245–253. https://doi.org/10.1007/s13404-014-0147-8

Gericke, M., Govender, Y. 2011. Bioleaching strategies for the treatment of nickel–copper sulphide concentrates. Minerals Engineering 24(11): 1106-1112. https://doi.org/10.1016/j.mineng.2011.02.006

Govender, Y., **Gericke, M.** 2011. Extracellular polymeric substances (EPS) from bioleaching systems and its application in bioflotation. Minerals Engineering 24(11): 1122-1127. https://doi.org/10.1016/j.mineng.2011.02.016

Gericke, M., Govender, Y., Pinches, A. 2010. Tank bioleaching of low-grade chalcopyrite concentrates using redox control. Hydrometallurgy 104(3–4): 414-419. https://doi.org/10.1016/j.hydromet.2010.02.024

Gericke, M., Neale, J.W., van Staden, P.J. 2009. A Mintek perspective of the past 25 years in minerals bioleaching. Journal of the Southern African Institute of Mining and Metallurgy 109(10):567-585.

Simate, G.S., Ndlovu, S., **Gericke, M.** 2009. Bacterial leaching of nickel laterites using chemolithotrophic microorganisms: Process optimisation using response surface methodology and central composite rotatable design. Hydrometallurgy 98(3–4): 241-246. https://doi.org/10.1016/j.hydromet.2009.05.007

Gericke, M., Muller, H.H., van Staden, P.J., Pinches, A., 2008. Development of a tank bioleaching process for the treatment of complex Cu-polymetallic concentrates. Hydrometallurgy 94(1–4): 23-28. https://doi.org/10.1016/j.hydromet.2008.05.018

Gericke, M., Pinches, A. 2006. Biological synthesis of metal nanoparticles. Hydrometallurgy 83(1–4): 132-140. https://doi.org/10.1016/j.hydromet.2006.03.019

Okibe, N., **Gericke, M.,** Hallberg, K.B., Johnson, D.B. 2003. Enumeration and Characterization of Acidophilic Microorganisms Isolated from a Pilot Plant Stirred-Tank Bioleaching Operation. Applied and Environmental Microbiology 69(4): 1936-1943. https://doi.org/10.1128/AEM.69.4.1936-1943.2003

Gericke, M., Pinches, A., van Rooyen, J.V. 2001. Bioleaching of a chalcopyrite concentrate using an extremely thermophilic culture. International Journal of Mineral Processing 62(1–4): 243-255. https://doi.org/10.1016/S0301-7516(00)00056-9

Gericke, M., Pinches, A. 1999. Bioleaching of copper sulphide concentrate using extreme thermophilic bacteria. Minerals Engineering 12(8): 893-904. https://doi.org/10.1016/S0892-6875(99)00076-X

Using these proceedings

The technical and poster programmes that follow serve as the index to this volume. Each entry gives the abstract identifier and the page on which the abstract begins, and in the electronic version each entry links directly to the abstract. Every abstract page carries a link back to the programme.

Technical programme

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Biohydrometallurgy Through Time: People, Processes and Future Frontiers

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Over the past 50 years, biohydrometallurgy has evolved from a deceptively simple idea into one of the most complex and intriguing interfaces between biology and minerals, a transformation driven in large part by the extraordinary IBS community. Early models reduced the process to a handful of microorganisms catalyzing iron and sulfur oxidation. These frameworks were powerful, but incomplete. What has since emerged is a far richer picture: dynamic microbial networks tightly coupled to mineral surfaces and governed by the rules of geochemistry.

We now recognise bioleaching as a living system, where the key question is no longer just *what happens*, but *who is doing what, to whom, why and how*. Yet even with advances in metagenomics, these systems remain difficult to control, predict, and scale.

At the industrial level, heap leaching has shown that microbial processes can operate across enormous scales, effectively turning ore bodies into bioreactors. But this success highlights the central challenge: harnessing fragile biological networks within highly heterogeneous geochemical systems, something the IBS community has repeatedly tackled with creativity and rigor. The result is a process that works, but not yet with the precision required for the next generation of mining.

New opportunities are now emerging. Advances in synthetic and systems biology offer the potential to design or steer microbial consortia with defined functions, while AI-driven modelling and real-time data integration promise to transform bioleaching into a predictive, actively managed process rather than an empirical one.

At the same time, biohydrometallurgy could potentially expand beyond Earth, for example mining the moon or M type asteroids. Acidophilic iron- and sulfur-based metabolisms provide analogs for early Earth and other planetary environments such as Mars, linking biomining to the limits of life and the potential for chemolithotrophy beyond our planet. As the field turns toward lower-grade ores, saline systems, and stricter sustainability demands, incremental advances will not suffice. The future lies in a shift from observation to control, integrating biology, geochemistry, and data science.

This talk will argue that biohydrometallurgy is poised for this transition, building on the legacy of the IBS community and the contributions of its researchers and students, where new biology and AI converge to create a predictive, systems-level discipline that bridges industrial practice with fundamental questions about life on Earth and beyond.

The talk will also share two anecdotes from past IBS meetings, with the hope that future gatherings continue to embody the same spirit of camaraderie and scientific excellence.

Dynamic heat balance modelling of industrial bioleaching plant: influence of both environment and operating conditions and heat management improvement

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A heat balance model was developed for bioleaching processes in stirred tank reactors, based on mechanistic and semi-empirical models to describe the contribution of each heat transfer phenomena (either due to operating or environmental conditions). It was validated based on the cooling power required for two industrial case studies (KCC plant, Kasese, Uganda and BIOX primary tank, Sao Bento, Brazil) at typical bioleaching temperature of 40 °C and sulfide concentrations of 42.7 and 33.5 %.

Various scenarios were then investigated on the KCC-cascade configuration. At high sulfide concentration, heat transfer from pulp addition and aeration was more significant than environmental transfers. This was validated using environmental ranges of values (temperature, wind speed, relative humidity, cloud cover) from either subarctic or equatorial climates. In the case of a simulation in Finland, 12 % more power was predicted in summer than in winter. Regarding the number of primary tanks, one tank may be removed on the KCC-cascade system to save investments, by accepting a 4 % reduction in conversion rate. Finally, heat loss due to environmental conditions increased as the pyrite concentration decreased, passing from only 12 % with a concentration of 80 to 53 % with a concentration of 10 %. Control of the feeding pulp temperature could reduce the use of heat exchangers, and even eliminate them at low sulfide concentrations.

The model was ultimately applied to simulate a cascade configuration incorporating novel pond-based bioleaching systems, which utilise floating agitators in place of conventional stirred-tank bioreactors. This approach enabled the determination of optimal operating conditions and pond dimensions for a bioleaching process that eliminates the need for heat exchangers.

Keywords: Bioleaching, heat balance, heat transfer modelling.

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Numerical design of a pond bioleaching reactor

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Abstract:

Bioleaching has traditionally been operated either in heaps or in stirred tank reactors (STRs). “Pond bioleaching” is a new concept of mechanically stirred reactor that uses floating agitators to supply gas and to maintain solids in suspension. This technology enables to perform bioleaching in ponds rather than in expensive tanks. It represents a compromise between the low cost of heaps and the high kinetics and recoveries of STRs. However, its industrial application raises three key challenges: the suspension of the particles, the transfer of the oxygen and the control of the temperature.

Different numerical approaches were combined to scale up the concept from laboratory scale to industrial scale. First, two computational fluid dynamics (CFD) simulations were used to characterise the multiphase flows: one Navier-Stokes (NS) with ANSYS Fluent software and one lattice-Boltzmann (LB) with MSTAR software. Both were validated against pilot-scale experiments with a ¼-scale floating agitator. In parallel, a heat-balance model was developed to predict temperature profiles in a full-scale bioleaching pond.

The NS model accurately predicted the degree of particle suspension in the pilot bioreactor but could not simulate gas/liquid transfer, in contrary to LB. Both CFD models were then used to determine the volume of influence of one floating agitator, defined as the pulp volume where particles are properly suspended and gas/liquid mass transfer is sufficient to fulfil the oxygen demand. These results were used to design a bioleaching pond operation at full scale. The temperature was assessed for various operating conditions and ore characteristics using the heat balance model. Simulations showed that, despite the large free surface of pulp in contact with the ambient atmosphere, pond temperature is mainly driven by the exothermic reactions and operating equipment (agitation and aeration), rather than by the weather conditions.

Keywords: bioleaching, pond, floating agitator, CFD, heat balance.

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This project has received funding from two European Union Programmes for Research and Innovation: (i) “Horizon 2020” programme, via NEMO project [Grant agreement N° 7768466], and (ii) “Horizon Europe” programme, via project CICERO [Grant agreement N° 101137560]. Authors are also grateful to David Dew for his support that helped to improve the study.

Heat Management and Thermophile Transition in Chalcopyrite Heap Bioleaching

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Abstract: Heap bioleaching of copper ores has become well established over the past 30 years and has been shown to achieve good extractions of 80-90% from low grade secondary sulfide ores. However, bioleaching of the key primary copper mineral, chalcopyrite, has remained elusive and achieves reasonable recoveries only through the action of thermophile organisms at elevated temperatures (>55 °C). Achieving and maintaining such temperatures consistently within a heap operation is challenging, especially under adverse conditions, such as high altitude, low ambient temperatures and high precipitation. Careful manipulation of the irrigation and aeration rates as well as the availability of a suitable fuel in the form of pyrite or pyrrhotite (present in the ore or added to the heap) are the key parameters that determine the successful management of heat in the heap and its transition to the thermophile temperature region.

The HeapSim model, developed further by the authors, offers a detailed modelling tool for heap bioleaching, which includes simulation of temperature profiles. The present paper offers an introduction to the modelling tool and a demonstration of its capabilities in the context of modelling the temporal and spatial evolution of temperature profiles within a heap of a typical chalcopyrite rich copper ore under a variety of climatic conditions, as well as the effect of available pyrite concentration, on achieving and sustaining the high temperatures required. Findings show that the ratio of irrigation to aeration rate is critical for achieving high temperatures, which may need to be manipulated in the context of ingress of water due to rain or melting snow. Heap permeability is a critical parameter to maintain targeted aeration. The availability of pyrite as a 'fuel' for maintaining high temperatures, especially during the later part of the process, is critical and not necessarily sustained by what the ore can provide, necessitating supplementation.

Keywords: chalcopyrite, thermophiles, heap leaching, modelling, heat management

ACCELERATING OXIDATIVE ACTIVITY DURING LOW-TEMPERATURE START-UP OF COPPER SULFIDE BIOLEACH COLUMNS

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Keywords: heap bioleaching, inoculum development, microbial diversity, psychrotolerant

Abstract

Heap bioleaching is an established technology that employs microorganisms to facilitate metal solubilisation from sulfide ores and has been successfully applied to the extraction of copper (Cu) from secondary copper sulfides. However, efficient bioleaching of chalcopyrite, the most abundant primary copper sulfide mineral, requires elevated temperatures, making temperature transition and microbial succession critical for effective Cu recovery. The initiation of microbial activity at low temperatures is particularly important, as sulfide oxidation contributes to the heat accumulation required for self-heating within bioheaps. This study investigated the performance of a low-temperature-adapted microbial consortium (MC20) relative to its mesophilic parent culture (MC25) in bioleaching columns operated at 15 °C. The effect of weekly re-inoculation on oxidative activity was also evaluated. Columns inoculated with MC20 exhibited an earlier onset of oxygen uptake and faster attainment of redox potentials near 700 mV compared to MC25-inoculated columns. Weekly re-inoculation with MC20 was associated with a further increase in oxidative activity, whereas minimal benefit was observed following re-inoculation with MC25. The improved performance of the MC20-inoculated columns was likely associated with a more diverse microbial community and the greater representation of psychrotolerant species, including *Acidithiobacillus ferrivorans* and *Acidithiobacillus ferriphilus* in the inoculum. These findings suggest that low-temperature-adapted inocula may reduce the lag in oxidative activity during bioheap start-up, although further work is required to confirm the abundance and activity of the psychrotolerant species within the ore bed.

Introduction

Heap bioleaching is an established, cost-effective, and environmentally favourable alternative to conventional metal extraction [1]. It relies on microorganisms to oxidise ferrous iron (Fe^{2+}) and reduced sulfur species, thereby generating ferric iron (Fe^{3+}) and protons (H^+), which promote mineral dissolution and metal liberation. These oxidative reactions are also exothermic and contribute to heat generation within bioheaps [2].

Bioleaching of chalcopyrite-containing ores benefits from elevated temperatures, with moderately thermophilic and thermophilic conditions promoting more effective copper dissolution [2], [3]. The transition to these conditions depends partly on heat generated through the oxidation of sulfide minerals and reduced sulfur species. During heap start-up at low ambient temperatures, however, limited microbial activity may delay the onset of oxidation and consequently restrict early heat accumulation.

One potential strategy for addressing this challenge is to use microbial communities containing psychrotolerant microorganisms, organisms that remain active below 25 °C [4]. Earlier initiation of oxidative activity by such communities could improve the potential for heat accumulation during bioheap

start-up. This study therefore compared a low-temperature-adapted microbial culture with its mesophilic parent culture in copper sulfide bioleach columns operated at 15 °C. The effects of inoculum source and weekly re-inoculation on the onset of oxidative activity were evaluated using oxygen utilisation rate (OUR) and solution-based indicators.

Methods

Bioleaching culture inocula. A mixed mesophilic culture maintained at 25 °C (MC25) and a low-temperature-adapted culture maintained at 20 °C (MC20) were cultivated in 6 L stirred reactors on a blend of pyrite and chalcopyrite concentrates at 1.6% w/v solids in a defined basal salt medium comprising 0.813 g/L $(NH_4)_2SO_4$; 0.128 g/L K_2HPO_4; 0.149 g/L K_2SO_4; and 1.01 g/L $MgSO_4.7H_2O$. The medium was adjusted to pH 2.0 using concentrated H_2SO_4 (98%). Reactors were sparged with CO_2-enriched air (0.35 % CO_2) and operated in fed-batch mode with an average residence time of 14 days. Performance was monitored using pH, redox potential, cell concentration, Cu concentration, and iron speciation. The relative microbial compositions of MC20 and MC25 are presented in Figure 1.

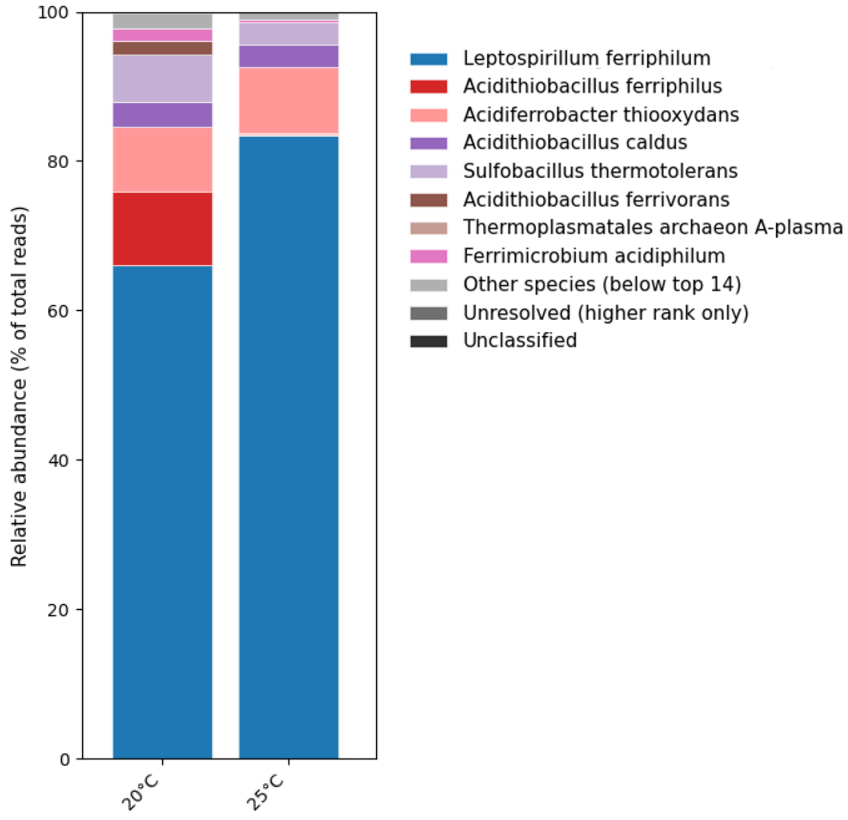


Figure 1. Relative abundance of MC20 and MC25 microbial communities.

Bioleaching column experiments. Four polypropylene columns (1 m height, 160 mm ID) were loaded with ~ 31 kg of non-sterile, low-grade copper-bearing ore (0.32% Cu, ~ 4% pyrite), with chalcopyrite as the dominant Cu-bearing mineral phase (95%) with minor proportions of enargite (3.1%), bornite (0.84%), and chalcocite (0.43%).

The ore was agglomerated with 5 kg/t H_2SO_4 and raffinate, inoculated with 2.5 x 10^{10} cells/t, and then cured at 15 °C for 14 days prior to irrigation. Columns C3_20 and C3_20R received the low-temperature-adapted consortium (MC20), while C4_25 and C4_25R received the 25 °C mesophilic culture (MC25). C3_20R and C4_25R were additionally re-inoculated weekly with 2.5 x 10^9 cells/t of their respective cultures through the irrigation feed.

All columns were maintained at 15 °C and irrigated at 2 L/m²/h with solution containing 8 g/L H_2SO_4, 3 g/L total Fe (1 g/L Fe^{2+}, 2 g/L Fe^{3+}), and 54 g/L total SO_4^{2-}. Air was supplied at 0.67 kg/m²/h.

Table 1. Test matrix for the column bioleaching experiments

Column ID	Culture	Initial inoculation (cells/t)	Re-inoculation	Re-inoculation dosage (cells/t)
C3_20	MC20	2.5×10^{10}	None	-
C4_25	MC25	2.5×10^{10}	None	-
C3_20R	MC20	2.5×10^{10}	Weekly	2.5×10^{9}
C4_25R	MC25	2.5×10^{10}	Weekly	2.5×10^{9}

Analysis. Column performance was monitored using oxygen utilisation rate (OUR) determined from off-gas analysis, together with pregnant leach solution (PLS) redox potential (vs Ag/AgCl), pH, Fe speciation, acid and Cu concentration.

Results and Discussion

Initial column experiments. The progressive transition from mesophilic to thermophilic conditions in chalcopyrite bioheaps requires microbial communities that remain active across a broad temperature **[5]**. In a preceding set of column tests, a mixed inoculum comprising cultures maintained at 25, 35, 49 and 65 °C was evaluated in columns initiated at either 25 °C or 15 °C.

A pronounced temperature-related difference in the onset of oxidative activity was observed (Figure 2). Columns C1_25a and C1_25b (duplicate columns), which were initiated at 25 °C, exhibited a rapid increase in OUR. In contrast, columns C2_25a and C2_25b (duplicate columns), which were initiated at 15 °C showed an extended lag before appreciable OUR was observed, despite weekly re-inoculation with mesophilic cultures. These results demonstrated the operational challenge associated with initiating oxidative activity at 15 °C and motivated the development of a low-temperature-adapted culture.

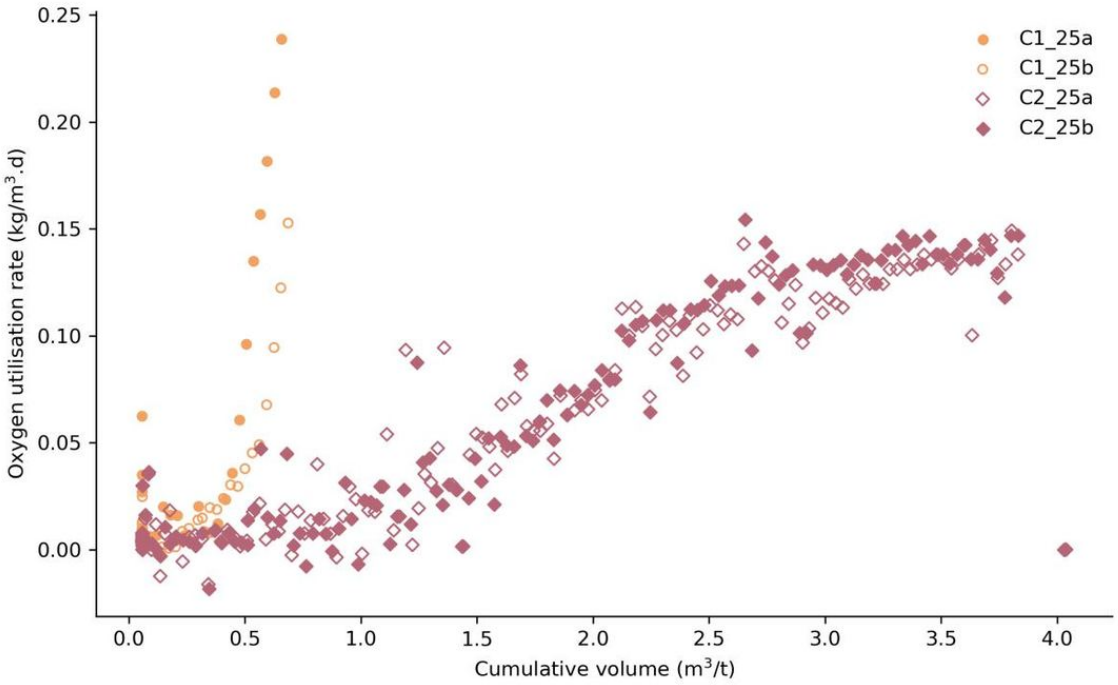


Figure 2. Oxygen utilisation profiles from preceding bioleach column tests initiated at 25 °C and 15 °C, showing the extended lag in oxidative activity at the lower start-up temperature. C1_25a and C1_25b were duplicate columns initiated at 25 °C, C2_25a and C2_25b were duplicate columns initiated at 15 °C.

Low-temperature-adapted microbial consortium in column experiments. MC25 was subsequently adapted to 20 °C to enrich for psychrotolerant species and potentially reduce the lag in oxidative activity. Following adaptation, the relative abundance of *Leptospirillum ferriphilum* decreased from approximately 84% in MC25 to 66% in MC20. The adapted culture contained greater relative abundances of *Acidithiobacillus ferriphilus* and *Acidithiobacillus ferrivorans*, at approximately 10% and 2%, respectively.

Overall, columns inoculated with MC20 exhibited an earlier onset of oxygen uptake than those inoculated with MC25 (Figure 3a). C3_20R showed the earliest and most rapid rise, exceeding an OUR of 0.1 kg/m³.d at 3.0 m³/t cumulative volume. C3_20 trailed slightly, reaching 0.1 kg/m³.d at 3.5 m³/t, whereas C4_25 and C4_25R reached the threshold OUR closer to 4.0 m³/t. A similar trend was observed in the PLS redox potential profiles (Figure 3b).

Figure 3.shows that C3_20R, which was repeatedly re-inoculated with MC20, exhibited a marked improvement in activity compared to its control (C3_20). Conversely, the column re-inoculated with MC25 (C4_25R) showed limited improvement relative to the corresponding single-inoculation MC25 column (C4_25).

The pH profiles (Figure 3c) showed little variation across all the columns. However, PLS pH reflects the combined effects of acid addition, mineral-related acid consumption and biologically mediated acid generation. Similar pH profiles therefore do not necessarily demonstrate equivalent rates of sulfur oxidation or acid generation.

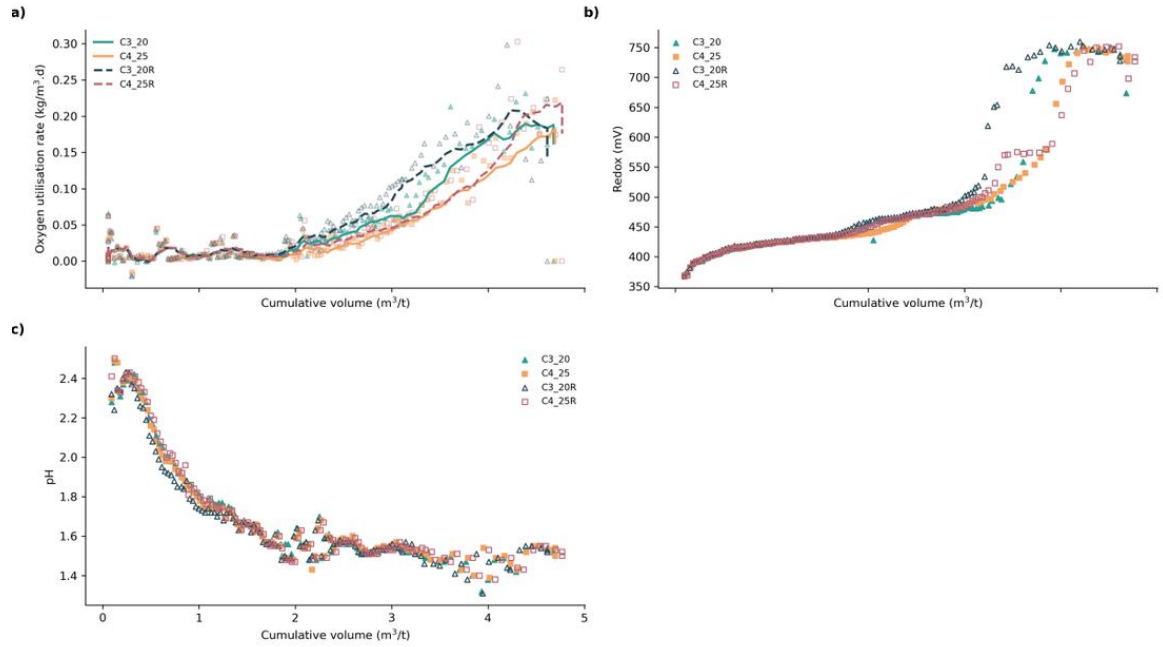


Figure 3. Comparative plots of column performance over the course of the experiment as a function of the cumulative irrigation ratio (m³/t) where (a) OUR (scattered data points) and rolling average OUR (smoothed lines, solid and broken), (b) PLS redox potential, (c) PLS pH profiles, for C3_20, C4_25, C3_20R, and C4_25R

Direct quantitative comparison between the columns in Figures 2 and 3 is limited by differences in inoculum composition and dosage. The columns in Figure 2 were inoculated with 1 x 10^{11} cells/t, comprising cultures from the full temperature suite, whereas those in Figure 3 received 2.5 x 10^{10} cells/t of either MC20 or MC25, representing only the low-temperature mesophilic component of the broader inoculum. Despite this limitation, the trends observed in Figure 3 suggest that incorporating MC20 into the standard inoculum complement would further reduce the lag in the onset of oxidative activity at 15 °C.

The results shown in Figure 3 suggest that the improvement in bioleaching performance, particularly Fe^{2+} oxidation at low temperatures (Figure 3b), was associated with greater diversity in the Fe^{2+}-oxidising community and greater representation of psychrotolerant species in the MC20 inoculum. Across all measured variables, the columns inoculated with the 20 °C consortium (MC20) showed a more favourable response than those inoculated with the 25 °C culture (MC25), suggesting that community composition plays an important role in enabling oxidation activity at low temperatures.

Although approximately 95% of microorganisms in both consortia were capable of Fe^{2+} oxidation, the composition of this population differed significantly. MC25 was strongly dominated by *L. ferriphilum* (84%), with lesser proportions of *Acidiferrobacter thiooxidans* (9%), and *Sulfobacillus thermotolerans* (2.9%). Psychrotolerant species are only present in low abundance (< 0.2%). In contrast, MC20 remained dominated by *L. ferriphilum*, but at a lower relative abundance (66%), had a higher proportion of *Sb. thermotolerans* (5.3%), equivalent *Acidiferrobacter thiooxidans* (9%), and measurable proportions of psychrotolerant *At. ferriphilus* (10%) and *At. ferrivorans* (2%).

It is postulated that the difference in OUR between MC20- and MC25-inoculated columns may be related to this compositional shift in the microbial community and the temperature-dependent physiology of the dominant taxa. Although *L. ferriphilum* remained an important component in both consortia, its competitive advantage is temperature dependent. At lower temperatures, *L. ferriphilum* loses its innate tolerance to Fe^{3+}, with affinity coefficients becoming comparable to those of *Acidithiobacillus species* [6]. Additionally, lower temperatures constrain its growth, with a greater proportion of available energy directed toward cellular maintenance. In contrast, *At. ferrivorans*, and potentially *At. ferriphilus*, can continue growing at lowered temperatures without the maintenance-energy penalty [6]. These physiological differences provide a plausible explanation for the earlier onset of OUR and more rapid Fe^{2+} turnover in MC20-inoculated columns.

The differential response to re-inoculation was also consistent with this hypothesis. C3_20R (MC20) showed improved performance relative to the single-inoculation control (C3_20), suggesting that repeated addition of MC20 may have increased the supply of metabolically active Fe^{2+}-oxidising species and accelerated oxidative activity. Conversely, C4_25R (MC25) showed limited improvement relative to C4_25 despite receiving an equivalent increase in inoculum dosage. This limited response may be consistent with the restricted growth capacity of *L. ferriphilum* at 15 °C. However, as microbial community composition and activity were not determined within the columns, the microorganisms responsible for the observed differences cannot be confirmed.

Although heat generation was neither measured directly nor estimated, the earlier oxygen uptake indicates increased potential for exothermic oxidation during low-temperature heap start-up. Accelerating the onset of these reactions could support earlier heat accumulation and facilitate the subsequent transition towards temperatures more favourable for chalcopyrite bioleaching.

Conclusions

The low temperature-adapted consortium, which contained a more diverse Fe^{2+}-oxidising community and greater representation of psychrotolerant species, was associated with an earlier onset of oxidative activity during copper sulfide column bioleaching at 15 °C. Although heat generation was neither measured nor estimated, earlier oxidative activity could increase the potential for heat accumulation, support microbial succession and enable a smoother transition towards thermophilic conditions, which are essential for efficient Cu extraction from chalcopyrite ores. Further studies will evaluate the contribution of psychrotolerant species to Fe^{2+} oxidation and confirm their abundance and activity within the ore bed.

Acknowledgements

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CARBON DIOXIDE AVAILABILITY AND ITS CORRELATION WITH OXYGEN UPTAKE DURING COLUMN BIOLEACHING OF A CARBONATE-POOR COPPER SULFIDE ORE

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Keywords: Bioleaching; Heap leaching; Carbon dioxide; Oxygen uptake rate; Carbon uptake rate

Abstract

Bioleaching relies on adequate oxygen supply to sustain microbial oxidation of ferrous iron and reduced sulfur species, while carbon dioxide serves as the carbon source for the autotrophic microorganisms that drive these processes. In carbonate-poor ores, the CO_2 supplied by ambient air and the dissolution of carbonate minerals may be insufficient to sustain microbial activity. The relationship between CO_2 availability, oxygen uptake and leaching performance, however, remains poorly quantified. Two 2 m bioleach columns (C1 and C2), each comprising two 1 m sections in series, were used to investigate this relationship across the full mesophile-to-thermophile range (25-65 °C over 220 days). C1 received ambient air, while C2 received air enriched to approximately 1000 ppm CO_2. CO_2 enrichment increased and sustained oxygen and carbon uptake rates at 35 °C, particularly where sections were supplied with CO_2-depleted off-gas. Inlet CO_2 below approximately 100 ppm restricted mesophilic growth, while concentrations below 50 ppm were severely limiting. CUR/OUR yield coefficients decreased from 0.022-0.030 to 0.012-0.016 mol C/mol O_2 on transition to thermophilic conditions, consistent with the lower carbon requirement of thermophilic cultures. Despite declining gas solubility with temperature, oxygen uptake recovered following thermophilic adaptation. CO_2 supplementation is therefore most beneficial during the mesophilic heat-up phase of carbonate-poor copper sulfide ores and may be reduced once thermophilic conditions are established.

Introduction

Heap bioleaching enables the economic recovery of copper from low-grade ores, but bioleaching of primary sulfides such as chalcopyrite has so far seen limited industrial adoption, partly owing to sluggish kinetics relative to conventional processing, solution management challenges and uncertainty around the robustness of microbial cultures at heap scale. Successful heap bioleaching also depends on effective microbial succession from mesophilic to thermophilic population to generate sufficient heat and sustain ferric iron regeneration as the heap temperature increases. Sustaining the activity and growth of these autotrophic iron-and sulfur-oxidising microorganisms requires adequate supplies of oxygen (O_2) and carbon dioxide (CO_2). The biomass of bioleaching microbes consists of approximately 49% carbon by dry mass [1]. An adequate carbon supply is therefore essential to sustaining microbial growth. In industrial heaps, CO_2 is supplied mainly by ambient air (≈400 ppm) and from the dissolution of carbonate minerals in the ore. For carbonate-poor ores these sources may be insufficient. However, the interacting supply and demand of CO_2 and O_2 within a heap-like bed remains poorly quantified.

Some agitated reactor studies have examined the effects of O_2 and CO_2 on bioleaching [2-5], whereas *in situ* gas demand measurements in heaps remain limited. Petersen et al. [6] used a segmented column to show that CO_2 is consumed rapidly from the base of a bed upward, causing substantial depletion in the upper sections even under CO_2-enriched air, with oxidation declining but persisting in the depleted

zones, consistent with maintenance-level metabolism. In a follow-up study using a controlled mesophile-to-thermophile temperature ramp, Petersen et al. [7] found that CO_2 availability under ambient air restricts simultaneous oxidation of an entire bed during the mesophile stage, and that CO_2 demand falls markedly once thermophilic conditions are established. However, it remains unclear how CO_2 availability, oxygen uptake, microbial carbon yield and gas solubility evolve and interact as a bed progresses through staged mesophilic to thermophilic conditions.

This study tests and extends this line of enquiry using two 2 m columns, each split into two 1 m sections connected in series. One column was supplied with ambient air and the other with CO_2-enriched air, while both were subjected to an identical staged temperature ramp from 25 to 65 °C. The objectives were to: (i) quantify the effect of CO_2 supply on the oxygen uptake rate (OUR) and carbon uptake rate (CUR) in each section during the mesophilic and thermophilic stages; (ii) establish threshold CO_2 concentrations associated with restricted microbial growth; (iii) characterise CUR/OUR yield behaviour during the transition to, and establishment of, thermophilic conditions, and compare it with literature values; and (iv) determine CO_2 and O_2 gas solubility at different stages of the leach. Together, these objectives provide an integrated, mechanistic understanding of carbon and oxygen supply, demand and utilisation during staged column bioleaching under simulated heap conditions.

Methods

Materials. Two columns (C1 and C2), each comprising two 1 m segments connected in series (upper, U; lower, L; 160 mm internal diameter; 60 kg ore charge total), were irrigated from the top at 2 L/m²/h with a synthetic raffinate solution containing 9.2 g/L sulfuric acid (H_2SO_4) and a background sulfate concentration of 48.5 g/L. The columns were aerated from the base of the lower section at 0.3 kg/m²/h, thereafter increased to 0.6 kg/m²/h from day 101. C1 received ambient air (≈400 ppm CO_2) and C2 received air enriched to ≈1000 ppm CO_2. The ore was agglomerated with a mixed mesophilic and thermophilic microbial consortium (active at 25 -65 °C) and sulfuric acid (2 kg/t). The columns were subsequently supplemented with additional inoculum at specified intervals in response to observed declines in microbial activity, particularly during the transitions from mesophilic to moderate thermophilic conditions and from moderate to extreme thermophilic conditions. Both columns followed an identical staged temperature ramp: 25 °C (day 0-65), 35 °C (day 71-125), 49 °C (day 130-143) and 65 °C (from day 151) (Fig. 1).

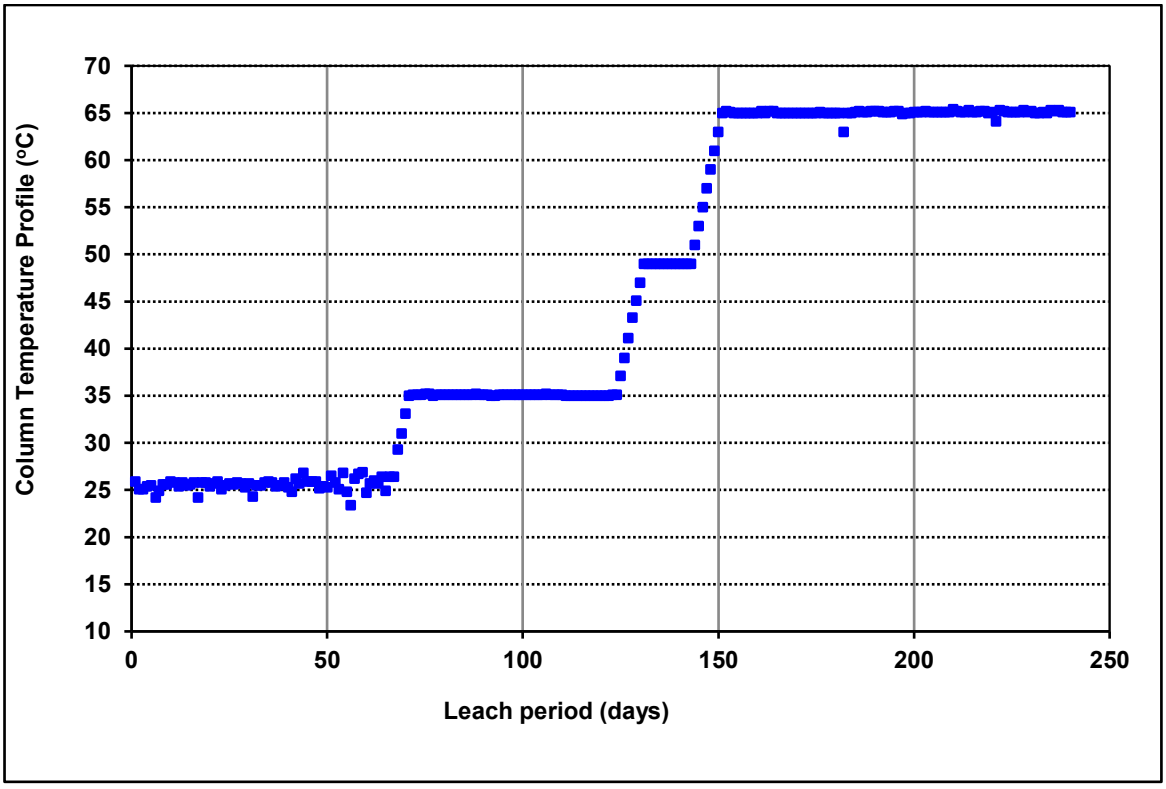


Figure 1.1 C1 and C2 temperature ramp-up profile

Analysis. O_2 and CO_2 concentrations were measured daily in the feed gas, at the adjoining segment and at the final outlet of each column, from which daily oxygen and carbon uptake rates for each 1 m section were calculated. Drainage pH and redox potential (Ag/AgCl reference) were also monitored daily. CUR/OUR yield coefficients were obtained by linear regression of carbon uptake rate against oxygen uptake rate during periods of unrestricted and restricted carbon dioxide supply, and dissolved O_2 and CO_2 concentrations were calculated from gas-phase mass balances corrected for temperature-dependent gas solubility.

Results and Discussion

At 35 °C, CO_2 availability governed both O_2 and CO_2 uptake in both columns. In C1 (ambient air), CO_2 in the off-gas leaving the lower section fell from 400 ppm in the feed to 40-70 ppm by day 65, leaving the upper section severely carbon-limited (average OUR 2.2 g/T/h, versus 6-8 g/T/h in the lower section). In C2 (CO_2-enriched), the lower section supported an OUR of ≈9 g/T/h and a CUR roughly three times that of C1-L, but the off-gas leaving C2-L was itself depleted to 50-100 ppm CO_2, again limiting the upper section until the aeration rate was doubled on day 101, after which OUR in C2-U rose to ≈12 g/T/h. Across both columns, periods of CO_2 depletion were mirrored by a proportional decline in O_2 uptake, indicating that CO_2 supply was the proximate control on both O_2 and CO_2 consumption during the mesophilic stage (Fig. 2, Table 1). Under the conditions of this study, combined analysis of the CUR/OUR and gas concentration trends (not shown) indicated an apparent CO_2 threshold of ≈100 ppm, below which mesophilic growth was inferred to become restricted, and severely restricted below 50 ppm. This interpretation is supported by the established proportionality between the rate of microbial growth and the CO_2 utilisation rate [8].

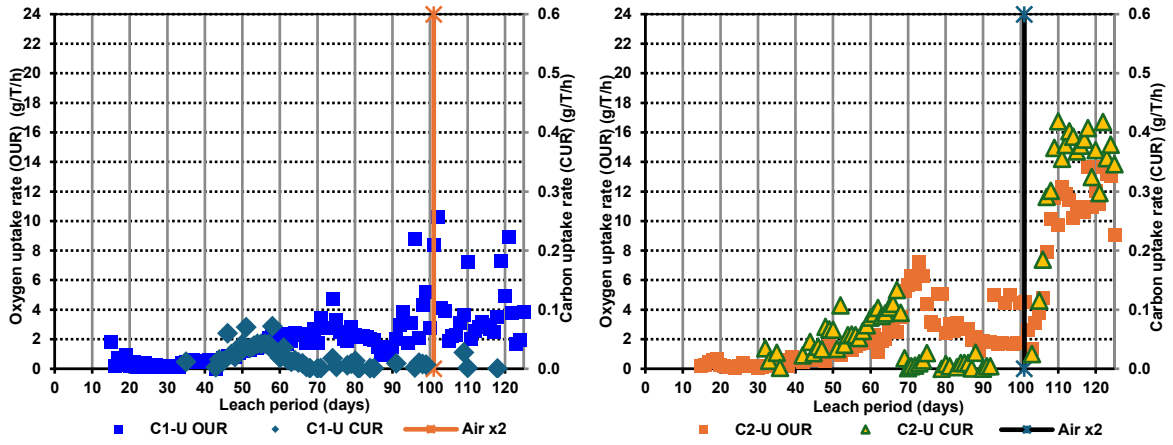


Figure 2.2 Representative O_2 and CO_2 uptake rate profile for column section C1-U and C2-U, showing carbon dioxide limitation at 35 °C, before doubling of air flow (day 101)

Following the staged transition to 65 °C, oxygen uptake rates recovered strongly in all sections (maximum OUR 16-23 g/T/h). This occurred despite a temporary dip in redox potential and gas uptake during the temperature ramp, consistent with a temperature-driven lag in microbial adaptation rather than a loss of activity. The CUR/OUR relationship provides more than a statistical correlation: because essentially all carbon taken up by autotrophic bioleaching cultures is predominantly fixed into new cell material, its slope represents the microbial carbon yield coefficient. Under unrestricted carbon supply, the microbial carbon yield decreased from 0.022-0.030 mol C/mol O_2 at 35 °C to 0.012-0.016 mol C/mol O_2 at 65 °C (Table 1). These values closely match those reported independently for mesophilic and thermophilic cultures (0.015-0.038 mol C/mol O_2 for a large isothermal column operated at 40 °C [6], and 0.0128 mol C/mol O_2 for a column operated at 70 °C and 2-300 ppm CO_2 [7]).

Table 1.1 Summary of key CO_2 thresholds, CUR/OUR yield coefficients and oxygen solubility values

Parameter	Units	Value	Notes
Apparent CO_2 threshold for mesophilic growth restriction	ppm	≈100	Inlet concentration
Apparent critical CO_2 limitation threshold	ppm	≈50	Inlet concentration
CUR/OUR yield coefficient, 35 °C (unrestricted CO_2)	mol C/mol O_2	0.022 - 0.030	Derived from C2-U, C2-L
CUR/OUR yield coefficient, 65 °C	mol C/mol O_2	0.012 - 0.016	Derived from C1-U, C2-U, C2-L
Median dissolved O_2 (equilibrium dissolved oxygen), 25 → 65 °C	mg/L	4.0 → 2.6	Gas-phase mass balance

Calculated cell growth rates (Fig. 3) for the overall columns (C1 and C2) show that the mean growth rate in C2 (1.31×10^7 cells·t^{-1}·h^{-1}) was roughly twice that of C1 (6.17×10^6 cells·t^{-1}·h^{-1}) over the entire test duration. During the temperature ramp, mean growth rates increased from 5.04×10^6 and 1.31×10^7 cells·t^{-1}·h^{-1} at 35 °C (pre-aeration increase) to 9.08×10^6 and 2.80×10^7 cells·t^{-1}·h^{-1} following the aeration increase at 35 °C for C1 and C2, respectively, before decreasing to 8.25×10^6 and 1.50×10^7 cells·t^{-1}·h^{-1} at 49 °C and converging at 7.32×10^6 and 8.69×10^6 cells·t^{-1}·h^{-1} under thermophilic conditions (65 °C). Correspondingly, the C2:C1 growth-rate ratio, which ran at 1.7-3.1 throughout the mesophilic and moderate thermophilic stages (25-49 °C), declined to 1.2 once thermophilic conditions were established (days 151-220). This indicates that once the microbial culture became thermophilic, the two columns were proliferating at comparable rates despite C2 still receiving roughly 2.5 times more CO_2 than C1. This suggests a reduced response to increased CO_2 supply and is consistent with the lower CUR/OUR yield observed at 65 °C.

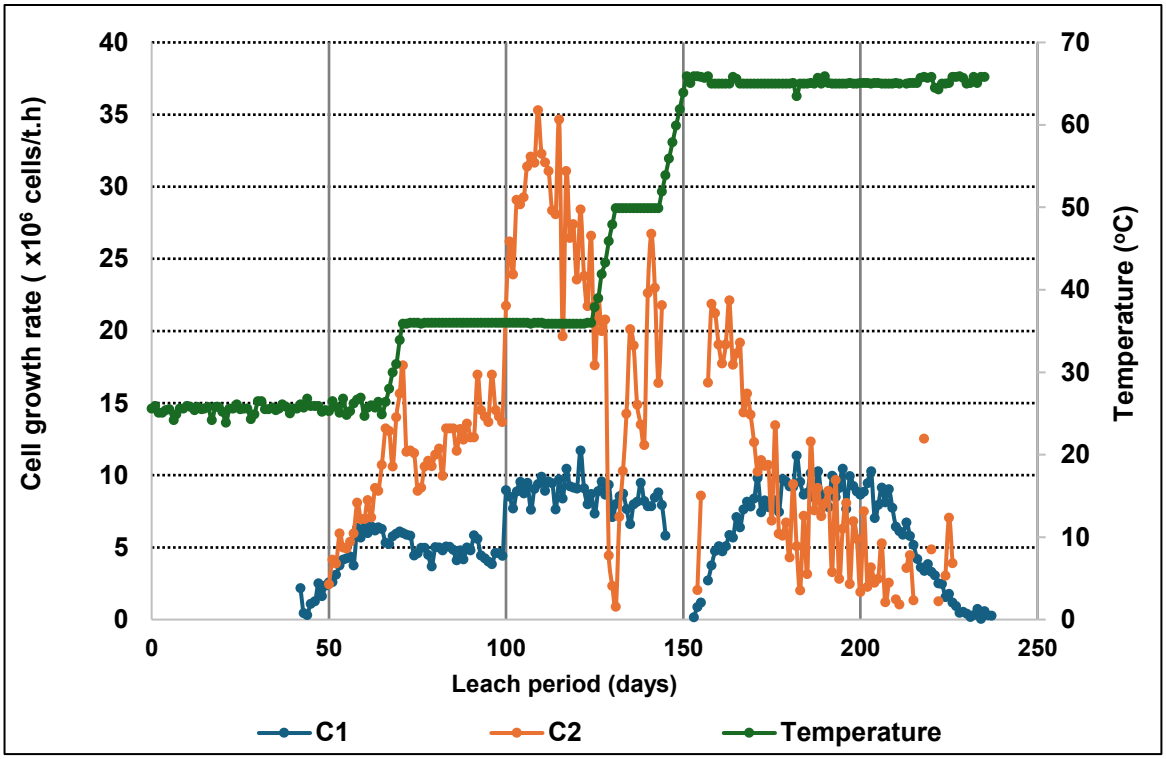


Figure 3.3 C1 and C2 cell growth rates over entire test duration

Calculated dissolved O_2 and CO_2 concentrations (not shown) declined with increasing temperature as expected from reduced gas solubility (median equilibrium dissolved O_2 falling from ≈4.0 to ≈2.6 mg/L between 25 and 65 °C), yet a sustained O_2 uptake was maintained throughout, indicating that oxygen was still sufficient under the applied aeration. Guezennec et al. [9] reported efficient bacterial oxidation and sulfide dissolution at dissolved O_2 concentrations between 4 and 13 mg/L in a stirred-tank bioleaching system, whereas de Kock et al. [3] found an optimum range of 1.5 to 4.1 mg/L, above which oxidation was inhibited. In the present study, sustained O_2 uptake was maintained at calculated dissolved O_2 concentrations varying between 2.6 and 4.0 mg/L. Although lower than the range reported in [9], these values fall within the optimum range reported in [3]. The lower dissolved O_2 concentrations observed here are likely sufficient because of the intrinsically slower sulfide oxidation rates in column/heap bioleaching systems compared to stirred-tank systems.

Conclusions

Carbon dioxide availability strongly influenced microbial activity and sulfide oxidation during the mesophilic stage of bioleaching of a carbonate-poor copper sulfide ore. Progressive CO_2 depletion through the lower sections of the ambient air-fed columns restricted activity in the upper sections, whereas CO_2 enrichment to ≈1000 ppm produced substantially higher and more sustained O_2 and CO_2 uptake. Gas concentration trends in conjunction with CUR/OUR data showed that, under the conditions of this study, microbial activity was restricted below 100 ppm CO_2 and severely restricted below 50 ppm.

CUR/OUR yield coefficients declined from 0.022-0.030 to 0.012-0.016 mol C/mol O_2, and cell growth ratios (C2:C1) decreased from 1.7-3.1 to 1.2 on transition to thermophilic conditions, consistent with the lower carbon requirement of thermophilic cultures reported previously [6,7]. Despite declining gas solubility with temperature, OUR recovered fully following thermophilic adaptation. Overall, by measuring gas availability, gas uptake, microbial carbon yield and gas solubility together within a single staged experiment, this study provides an integrated mechanistic understanding of how CO_2 availability influences bioleaching performance in carbonate-poor copper sulfide ores. These findings suggest that CO_2 enrichment is most beneficial during the mesophilic heat-up phase and may be reduced once thermophilic conditions are established.

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A *Ferroplasma*-dominated heap bioleaching for copper sulfide ore: industrial practice and mechanism analysis

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Abstract: In the bioleaching process of copper sulfide ores, microbial community and activity was influenced with the ore character and industrial operation, and significantly affect copper leaching efficiency and acid-iron balance during heap bioleaching. The formation process of a heap bioleaching system dominated by the archaeon *Ferroplasma for* copper sulfide ores, including industrial heap bioleaching operations, the succession process and mechanism of microbial community and activity, current production challenges, and future solutions for industrial production were introduced.

The Letpadaung Copper Mine in Myanmar was dominated by chalcocite, with a proportion of chalcopyrite and a high pyrite content exceeding 12%. During the start-up of heap bioleaching, high-efficiency iron-sulfur oxidising microorganisms dominated by *Acidithiobacillus and Leptospirillum were* inoculated. Sulfuric acid and the oxidant ferric iron were supplied through the oxidation of pyrite in the ore, realising heap leaching without additional sulfuric acid addition. However, with the passage of time, pyrite oxidation in the heap and the closed-circuit circulation of leachate (without external discharge) led to the massive accumulation of acidity, iron concentration, and impurity ions in the solution, resulting in microbial stress. Meanwhile, insufficient oxygen supply in the multi-layer permanent heap facilitated the dominance of *Ferroplasma*, which is more tolerant to extreme acidity and capable of growing under both aerobic and anaerobic conditions. Further laboratory simulation experiments were conducted to study the microbial community succession under different environmental conditions, confirming that extreme environments drive the formation of *Ferroplasma*-dominated microbial communities.

The *Ferroplasma*-dominated microbial community resulted in low microbial ferrous oxidation activity in the heap leaching system, thus maintaining a relatively low redox potential (460 mV, vs. Ag/AgCl), which is close to the rest potential of pyrite oxidation. This is conducive to inhibiting the massive oxidation of pyrite but may simultaneously exert a certain negative impact on copper leaching, especially the leaching of chalcopyrite. Future research should focus on continuously inhibiting pyrite oxidation and reducing the accumulation rate of acid and iron under low microbial activity conditions. Meanwhile, it is necessary to explore methods to improve chalcopyrite leaching efficiency under such microbial and industrial conditions.

Keywords: heap bioleaching, copper sulfide ore, *Ferroplasma*, pyrite oxidation, chalcopyrite

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Influence of Flotation Reagent Chemistry on Mesophilic Bioleaching of Arsenic-Bearing Cobaltite Concentrates

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Abstract:

The progressive depletion of high-grade mineral deposits has intensified interest in integrated processing strategies for low-grade and chemically complex ores. Cobalt is frequently recovered as a by-product, particularly from arsenic-bearing minerals such as cobaltite, where achieving both effective flotation selectivity and efficient downstream hydrometallurgical recovery remains challenging. Although flotation and bioleaching have individually been applied to cobalt-bearing systems, the combined influence of flotation operating conditions and residual flotation reagents on the subsequent bioleaching performance of arsenic-rich cobaltite concentrates has not been systematically examined. In this study, the flotation response of cobaltite and its subsequent mesophilic bioleaching were systematically investigated, with particular emphasis on the effects of flotation reagent type, activation strategy, and operating parameters on downstream cobalt dissolution. Flotation experiments were carried out using potassium amyl xanthate (PAX) and potassium ethyl xanthate (PEX), with and without sodium sulfide activation, over a range of pH values, pulp densities, and reagent dosages. The resulting flotation concentrates were then subjected to mesophilic bioleaching to evaluate the effects of solid content (2–10%) and selected nutrient additives, including pyrite, silver, iron, sulfur, and L-cysteine, on cobalt recovery. The flotation results demonstrated that acidic conditions significantly enhanced cobaltite recovery, with a maximum recovery of 71.42% achieved at pH 5 using PAX. Sodium sulfide activation markedly improved xanthate performance, yielding cobalt recoveries of 67.79% and 62.02% for PAX and PEX, respectively. Bioleaching experiments showed that increasing solid content adversely affected cobalt dissolution from arsenic-rich concentrates, whereas the highest cobalt recovery (approximately 68.8%) was obtained at a low solid content of 2% in the presence of iron and sulfur additives under mesophilic conditions. Overall, this study provides the first systematic evaluation of how flotation reagent chemistry influences subsequent bioleaching performance for arsenic-bearing cobaltite concentrates and offers practical guidance for the design of integrated flotation–bioleaching flowsheets for complex cobalt ores.

Keywords: Cobalt recovery; Arsenic-bearing cobaltite; Flotation reagent chemistry; Flotation–bioleaching integration; Mesophilic microorganisms.

Indirect bioleaching triggered by MBR showed much higher pre-oxidation performance than direct one on gold extraction from gold ores

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Abstract: With the continuous rise in gold prices, green and efficient gold leaching technologies have become a research hot spot in the industry. Microbial gold leaching has attracted significant attention due to its environmental friendliness, low energy consumption, and controllable costs. However, its industrial-scale application remains limited by low efficiency and prolonged processing times. In this study, a membrane bioreactor (MBR) was employed to efficiently enrich functional microorganisms and produce bioleachate. Integrated with membrane separation technology, three distinct microbial pre-oxidation systems were constructed: (1) bioleachate-only system, (2) microorganism + bioleachate system, and (3) microorganism+bioleachate+nutrient substrate system. The effects of these pre-oxidation strategies on subsequent thiourea-based gold recovery were systematically investigated. Results revealed significant differences in gold leaching performance: the bioleachate-only system achieved the highest gold extraction rate of 92%; the nutrient substrate-containing system performed worst, with only 70% extraction; and the control group — thiourea leaching without microbial pretreatment—yielded merely 53%. Notably, this study found that indirect microbial leaching (dominated by bioleachate) outperformed both direct leaching (using live microbial suspension) and direct–indirect synergistic leaching—contradicting the prevailing consensus in most existing literature that direct or synergistic leaching is superior. This finding provides a novel perspective for optimising microbial leaching of refractory gold ores. Further analysis indicated that nutrient substrates (e.g., elemental sulfur, pyrite) generate passivating layers such as poly-sulfides and jarosite—on the ore surface during pre-oxidation, thereby impeding contact between thiourea and gold and significantly reducing leaching efficiency.

Keywords: gold leaching, MBR, microbial pre-oxidation, indirect leaching, passivating layers

Zn-selective bioleaching of electric arc furnace (EAF) dust using an iron-oxidizing acidophile

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Keywords: EAF dust, $ZnFe_2O_4$, iron-oxidizing acidophile, Zn-selective bioleaching, activated carbon

Abstract

Zinc is widely used as a protective coating for steel because of its excellent corrosion resistance. Consequently, electric arc furnace (EAF) dust generated during steel recycling commonly contains substantial amounts of Zn-bearing phases, primarily ZnO and $ZnFe_2O_4$. The supplied EAF dust was characterised as a mixture of ZnO, $ZnFe_2O_4$ and Fe_3O_4, using XRD, XRF and XANES Fe K-edge. While ZnO readily dissolves under acidic conditions, $ZnFe_2O_4$ is quite refractory. In this study, the bio-assisted leaching of Zn from EAF dust at ambient temperature was investigated using the iron-oxidizing bacterium *Acidimicrobium ferrooxidans* ICP. The initial pH, the pulp density, the additives and the mixed culture with sulfur-oxidizing bacterium *Acidithiobacillus caldus* KU were optimised, aiming to maximise the leaching recovery and the selectivity of Zn over Fe. In the presence of activated carbon with the pulp density of 0.1%, Zn recovery increased from 60% to 88% while Fe dissolution remained suppressed.

Introduction

Toward a carbon-neutral society, the steelmaking process using an electric arc furnace is becoming increasingly important. Steel scrap serves as the primary feedstock in an electric arc furnace, resulting in substantially lower CO_2 emissions than conventional blast furnace steelmaking, and is therefore regarded as a green steelmaking process. The spent ZnO-coated steel products are one of Fe scraps, since ZnO is effectively used as an anti-corrosion coating [1]. So, it is known that ZnO and $ZnFe_2O_4$ are one of major components in EAF dusts. Several recovery technologies of Zn from EAF dusts have been investigated: selective reduction roasting [2], reduced wet grinding [3], hydrothermal reduction under alkaline conditions [4], extraction in biolixivilant inducing ligand promoted dissolution [5]. Due to its smaller scale, hydrometallurgical process has been much paid attention to dissolve refractory $ZnFe_2O_4$, while ZnO is easily dissolved under acidic condition. The present work aims to maximise the recovery and the selectivity of Zn over Fe applying acidiphile bacteria.

Methods

Materials. Electric arc furnace (EAF) dust was provided by UJICY, Uzbekistan. Elemental compositions are as follows: 3.24% Na, 0.403% Mg, 0.316% Al, 2.66% Si, 0.15% P, 1.57% Si, 5.63% Cl, 4.71% K, 2.80% Ca, 0.228% Cr, 2.09% Mn, 39.8% Fe, 0.227% Cu, 33.5% Zn, 2.37% Pb, 0.306% others. Crystal phases of Fe_3O_4, $ZnFe_2O_4$ and ZnO were detected by XRD. Because Fe_3O_4 and $ZnFe_2O_4$ have very similar d-spacings, they cannot be readily distinguished by XRD alone. The molar ratio of Fe(0): Fe(II): Fe(III) was determined to be 0.05: 0.183: 0.767 by XANES K-edge analysis at SAGALS BL06. Based on the combined XRD, XRF, and XANES results, the molar ratio of Fe_3O_4: $ZnFe_2O_4$: ZnO can be estimated to 1.00 :1.10: 2.64. Measurements of the particle size distribution revealed the supplied sample showed a normal probability distribution with P_{80} = 2218. 58 nm.

Microorganisms. *Acidimicrobium ferrooxidans* ICP and *Acidithiobacillus caldus* KU were used. They were pre-cultured separately. *Am. ferrooxidans* was in ABS medium (22.5 g $(NH_4)_2SO_4$, 2.5 g KCl, 2.5 g KH_2PO_4, 2.5 g $MgSO_4 \cdot 7H_2O$, 4.5 g $Na_2SO_4 \cdot 10H_2O$, 0.7 g $Ca(NO_3)_2 \cdot 4H_2O$ per 1 L) including 10 mM $FeSO_4$ and 0.02% yeast extract at pH 2.0, 45 °C and agitated at 150 rpm. *Ac. caldus*

was also pre-cultured in ABS medium including 1 g/L sterilised elemental sulfur after dry-heat sterilisation at pH 2.0 and 45 °C. A preculture of each microorganism was carried out in same medium composition suspending the EAF dust mentioned previously with 0.5(w/v)% pulp density. After confirming a sufficient increase in microbial numbers, centrifugation was performed at 8300 rpm, for 15 min, at 4 °C to prepare a concentrated solution. After obtaining a 10 times concentrated cell suspension, each microbial strain was added to the medium (ABS medium, pulp density of 0.5%, 0.02% yeast extract, 10 mM $FeSO_4$, 10 mM glucose at pH 2.0 and 45℃) to achieve a cell density of 1.0×10^7 cells /mL.

Bio-assisted leaching of Zn from EAF dust. The experiment was then conducted using a bio-shaker (TAITEC G-BR-300, Tokyo, Japan). 0.75 g of EAF dust was added to 150 mL of ABS medium at pH 2.0 supplemented by 10 mM $FeSO_4$, 10 mM glucose and 0.002% yeast extract. *Am. ferrooxidans* ICP and/or *Ac. caldus* KU were inoculated at 45 °C in a duplicate and agitated at 150 rpm. In some cases, activated carbon was also added with 0.1-0.2(w/v)%. At intervals, the supernatants were provided for measurements of pH (TOA-DKK, GST-5841C, Tokyo, Japan), redox potential (vs SHE; TOA-DKK, PST 5821C, Tokyo, Japan), and dissolved Fe and Zn concentrations (ICP-OES, Agilent Technologies 5100, Tokyo, Japan), and solids were for X-ray diffraction (XRD, Rigaku Mini Flex II, Akishima, Japan). After three weeks of incubation, the solid residues were recovered to collect X-ray diffraction patterns.

Results and Discussion

In abiotic leaching, pH rapidly increased to around 6.0, mainly due to $ZnO + 2H^+ = Zn^{2+} + H_2O$, and Fe dissolution was well supressed. In bio-assisted leaching, pH increase was well suppressed, especially by addition of *Ac. caldus* KU (sulfur-oxidizing bacteria). Regarding Zn leaching, the addition of activated carbon significantly enhanced the Zn recovery from 60% to 88% after 21 days in case of *Am. ferrooxidans* ICP (iron-oxidizing microorganism) alone inoculation. The coexistence of *Ac. caldus* KU did not cause the enhancement of Zn recovery, probably due to the lower Zn recovery by co-precipitation with secondarily formed jarosite, supported by XRD.

When the concentration of activated carbon increased from 0.1 to 0.2%, the extraction of Zn gradually decreased. This trend was already observed after 1 day leaching. Activated carbon may have imposed stress on the cell membrane of *Am. ferrooxidans* ICP, inhibiting the metabolic activities.

In the present sample, Zn sources consist of 70% ZnO and 30% $ZnFe_2O_4$, where the former is easily dissolved in acidic condition, and the later has minimal dissolution. Microbial oxidation of Fe^{2+} to Fe^{3+} is accelerated through electron shuttles on the surfaces of activated carbon by back reduction by electrons derived from partially oxidative dissolution of coexisting Fe_3O_4 in EAF dust. The dissolved Fe concentration was under the detection limit in ICP-OES. Coexisting *Ac. caldus* KU induced the recovery loss of Zn^{2+} because of the formation of insoluble jarosite. To increase the extraction efficiency of Zn further, both of the iron-oxidizing activity of *Am. ferrooxidans* ICP and the electron donating system based on powder technology of activated carbon should be improved.

Conclusions

The bio-assisted leaching of $ZnFe_2O_4$ in EAF dust was investigated applying an iron-oxidizing bacteria, *Am. ferrooxidans* ICP. While the bio-assisted leaching rate of $ZnFe_2O_4$ under acidic condition was still low, the leaching rate was enhanced in the presence of a suitable pulp density of activated carbon, enhancing the electron transfer in the redox reaction of Fe^{3+}/Fe^{2+}. An excessively high activated carbon loading inhibited the microbial activities, leading to the declined leaching rate.

Acknowledgements

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Statistical Optimisation of Key Parameters Affecting Bioleaching of Critical Metals from Superalloy Wastes Using Response Surface Methodology

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Keywords: Bioleaching; Critical metals; Superalloy grinding wastes; Response Surface Methodology; Design of Experiments

Abstract

Significant quantities of critical metals are present in wastes generated during superalloy processing. Due to superalloy design and requirements for oxidation resistance and toughness, such wastes are commonly downgraded or recycled through stainless steel production, an approach that limits effective recovery of valuable critical metals. To support a circular economy, this study investigates the effect of key parameters: temperature (T), solid content (S%) and hydrogen peroxide (H_2O_2), on bioleaching of superalloy grinding wastes using the Design of Experiment (DOE) method. Biologically produced acid (pH 0.5–0.6) generated by a mixed *Acidithiobacillus thiooxidans* culture grown in modified 9K medium was used in 19 Central Composite Design (CCD-DOE) experiments. The effectiveness and mutual interactions of key parameters were evaluated through an ANOVA test, and consequently, validated response functions modelled solid dissolution yield, Re, Ni, and Co recoveries to the solution. For all response functions, S% had a negative effect while H_2O_2 and T had positive effect. In most of the modelled response surfaces, the mutual interaction between T and S% was found to be 95% statistically significant. Optimisation of models for maximising responses suggested: T:60-65 °C, H_2O_2:5-8%, and S%:2-2.2%. The obtained results demonstrated the capability of bioleaching as a promising and sustainable approach for critical metal recycling.

1. Introduction

The transition towards low-carbon technologies, rapid expansion of renewable energy systems, and advanced material manufacturing (to achieve exceptional mechanical strength, corrosion resistance and high-temperature stability) have substantially increased the demand for critical metals such as nickel (Ni), cobalt (Co) and rhenium (Re). At the same time, the limited availability of primary resources, increasing extraction costs and growing environmental concerns have intensified interest in recovering critical metals from secondary resources. On the other hand, Metal-containing wastes such as dusts, tailings, and slags are largely landfilled or stockpiled due to the lack of effective recovery approaches, or poorly recycled through conventional methods, which pose significant environmental hazards [1]. Superalloy grinding wastes generated during machining and finishing operations represent a valuable secondary resource as they contain concentrations of critical metals considerably higher than those found in many natural ores. Despite their high intrinsic value, these wastes are commonly recycled through conventional methods which downgrades them into lower-value material, resulting in irreversible dilution and loss of strategic elements such as Re and Co.

Bioleaching has emerged as a promising alternative for critical metal recovery. In bioleaching metal dissolution is facilitated by microbial metabolites, such as organic and inorganic acids, or biogenic oxidising agents, produced by acidophilic bacteria and fungi. Compared to conventional metallurgical methods, bioleaching offers an energy- and cost-effective approach for recovering metals from a range of sources, including low-grade ores, mining waste, and solid wastes [2].

Recent studies confirm the effectiveness of acidophilic bioleaching for Ni and Co recovery across diverse waste streams. Yun et al., isolated an indigenous strain, *Acidithiobacillus ferriphilus* 9P1, which achieved 47% Ni and 53% Co recovery from spent lithium-ion battery black mass within 24 h at a high pulp density of 100 g/L [3]. Constantin er al., achieved over 60% leaching rates for Ni and Co once they employed bio-acid for indirect bioleaching of superalloy wastes [4]. Tian et al., used response surface methodology to optimise bioleaching of electroplating sludge, achieving 100% Ni, 96.5% Cu, 100% Zn, and 76.1% Cr recovery [5]. Deng et al., in their study on cobalt-bearing sulfide tailings found that a moderately thermophilic strain (*n (Sulfobacillus thermosulfidooxidans*) achieved substantially higher cobalt extraction (77.14%) than a mesophilic strai*A. ferrooxidans*, 54.69%) after 29 days [6]. Together, these findings show that bioleaching performance for metal recovery is strongly influenced by microbial strain, waste matrix, and operating conditions, motivating a systematic, statistically driven approach to parameter optimisation.

The present study investigates the bioleaching of critical metals such as Ni, Co, and Re (in total >65% of mass in this waste) from superalloy grinding wastes using biogenic acid produced by *Acidithiobacillus thiooxidans*. A Central Composite Design (CCD) was employed to evaluate the effects of temperature, H_2O_2 concentration and solid content on the dissolution of Ni, Co and Re. Statistical models were developed to identify the dominant process variables, quantify interaction effects and determine optimum operating conditions for maximising critical metal recovery. The outcomes provide both mechanistic insight into the dissolution behaviour of superalloy wastes and a statistically validated basis for process optimisation and future industrial implementation.

2. Methods

Biogenic acid was produced using *Acidithiobacillus thiooxidans* obtained from the Coventry University microbial culture collection. The bacteria were cultivated in a 5 L aerated bioreactor containing modified 9K medium (elemental sulfur, $FeSO_4$, KCl, $(NH_4)_2SO_4$, K_2HPO_4 and $MgSO_4$) at 30 ± 1 °C with an aeration rate of 2.5 L min^{-1}. The reactor was inoculated with 10% (v/v) actively growing culture. Microbial growth was monitored through pH, oxidation-reduction potential (ORP, Ag/AgCl reference electrode) and cell concentration. Acid production was considered complete when the pH stabilised at approximately 0.5. The biogenic acid was subsequently separated from the biomass by vacuum filtration (Whatman No. 1) followed by centrifugation at 5000 rpm and stored at 4 °C prior to leaching experiments.

Superalloy grinding waste was collected from the production line of Advanced Alloy Services Ltd. The material was screened through a 2 mm sieve to remove coarse debris, thoroughly homogenised and divided into representative subsamples for chemical characterisation and leaching experiments

2.1. Experimental design

Response Surface Methodology (RSM) based on a Central Composite Design (CCD) was employed to evaluate the effects of three operating variables on metal dissolution: temperature, H_2O_2 concentration and solid content. Compared with conventional one-factor-at-a-time experimentation, RSM enables simultaneous evaluation of multiple variables while identifying statistically significant interaction effects and reducing the total number of experiments required. The investigated operating ranges were selected based on preliminary leaching experiments and are presented in Table 1. The CCD consisted of 19 experiments, including five centre-point replicates to estimate experimental error and assess model reproducibility.

Table 1. Independent experimental variables and investigated ranges.

Variable (unit)	Coded name	Variable level		
		-1	0	1
Temperature (°C)	A	25	45	65
H_2O_2 (%)	B	0	5	10
Solid content (%)	C	2	5	8

2.2. Bioleaching experiments and analytical methods

Leaching experiments were performed in 250 mL Erlenmeyer flasks containing 100 mL of biogenic acid under continuous stirring (hotplate stirrer, 400 rpm) at the prescribed temperature. The required amount of superalloy powder was added to achieve the desired solid content, H_2O_2 was introduced at the beginning of each experiment when specified by the experimental design. Each experiment was conducted for 7 h, after which the pregnant leach solution (PLS) was separated by filtration (Whatman No. 2). Solid residues were washed, dried at 90 °C for 24 h, and weighed to determine total mass dissolution.

The chemical composition of the superalloy waste was determined using a Rigaku NEX DE X-ray fluorescence (XRF) analyser. Dissolved metal concentrations in the PLS were measured using PerkinElmer AVIO Max 220 ICP-OES.

Experimental results were analysed using analysis of variance (ANOVA), and statistically significant terms ($p < 0.05$) were incorporated into quadratic response surface models describing the dissolution behaviour of Ni, Co and Re. The validated models were subsequently used to evaluate factor interactions and determine the optimum operating conditions for maximising metal recovery.

3. Results and Discussion

3.1. Characterisation of superalloy grinding waste

The chemical composition of the superalloy grinding waste is presented in Table 2. The material contains high concentrations of Ni (55.5 wt.%), Co (7.93 wt.%) and Re (1.59 wt.%), together with Cr, Al, W and Ta. These concentrations are considerably higher than those found in many primary ores and are comparable to, or exceed, those reported for several secondary resources such as electronic wastes. Consequently, superalloy grinding wastes represent an attractive secondary source of critical metals and provide strong justification for the development of selective recycling technologies.

Table 2. Chemical composition of superalloy processing waste

Ni	Co	Re	W	Ta	Al	Cr	Fe	Ti	Mo	Si	Zr
55.5	7.93	1.59	5.19	3.6	9.08	10	1.48	2.06	0.95	2	0.7

3.2. Biogenic acid production

The evolution of bacterial growth, pH and oxidation-reduction potential (ORP) during cultivation of *A. thiooxidans* is shown in Figure 1. The microbial culture exhibited the typical lag, exponential and stationary growth phases. During exponential growth, microbial oxidation of sulfur generated acidic lixiviant, resulting in a continuous decrease in pH accompanied by an increase in ORP.

After approximately 20 days, the culture reached a stable stationary phase with a final pH of 0.46 and an ORP of approximately 630 mV (Ag/AgCl), indicating the production of a highly acidic and oxidising biogenic lixiviant suitable for subsequent leaching experiments.

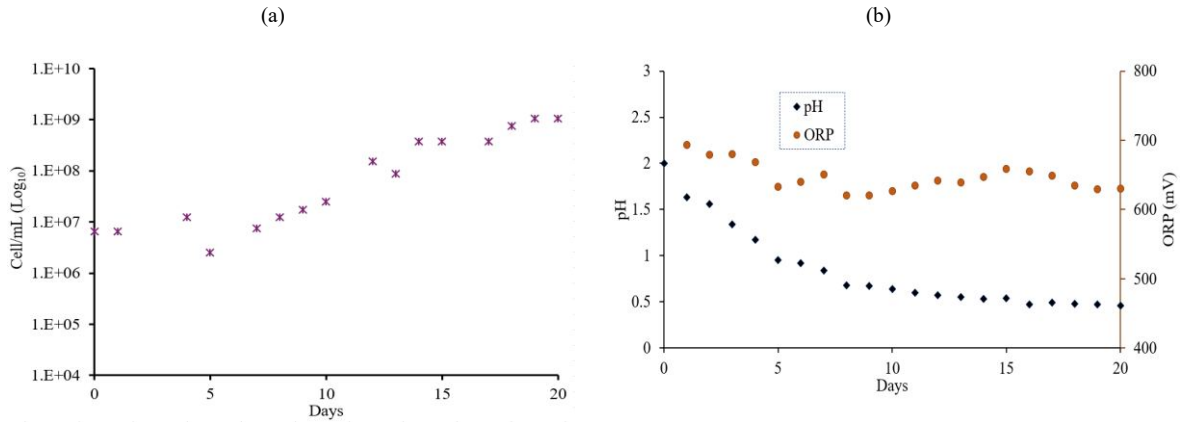


Figure 1. Biogenic acid production monitoring, (a): cell count, (b) pH and ORP (vs. Ag/AgCl) changes

3.3. Critical metal dissolution modelling

The effects of temperature, H_2O_2 concentration and solid content on Ni, Co and Re dissolution were evaluated using RSM. Analysis of variance (ANOVA) demonstrated that the developed quadratic models adequately represented the experimental data (Table 3). The models for all three critical metals exhibited good predictive capability, with acceptable coefficients of determinations (R^2).

Table 3. ANOVA test results for Ni, Co and Re responses.

Source	Ni Dissolution (%)		Co Dissolution (%)		Re Dissolution (%)	
	F-Value	P-Value Prob > F	F-Value	P-Value Prob > F	F-Value	P-Value Prob > F
Model	15.02	< 0.0001	15.66	< 0.0001	9.69	0.0017
A-Temperature	19.07	0.0008	30.89	< 0.0001	--	--
B-H_2O_2	8.56	0.0118	4.35	0.0573	12.76	0.0025
C-Solid content	19.89	0.0006	19.58	0.0007	6.62	0.0204
AC	8.62	0.0116	7.02	0.0201	--	--
B^2	18.94	0.0008	16.47	0.0014	--	--
R^2	0.8524		0.8576		0.5478	
Adj. R^2	0.7956		0.8029		0.4913	
Std. Dev	2.19		2.43		5.55	

For Ni dissolution, temperature, H_2O_2 concentration and solid content all exerted statistically significant effects ($p < 0.05$). In addition, the interaction between temperature and solid content (AC) was found significant, indicating non-linear behaviour and demonstrating that the influence of one parameter depended on the level of the other. A similar trend was observed for Co dissolution, where temperature and solid content were the dominant variables. Although the linear effect of H_2O_2 was only marginally significant, suggesting the existence of an optimum oxidising condition rather than a continuously increasing benefit.

In contrast, Re dissolution was primarily governed by H_2O_2 concentration and solid content, while temperature did not significantly influence the dissolution. This indicates that oxidation potential, rather than reaction kinetics, controls Re dissolution under the present experimental conditions.

Residual plots (Figure 2) showed a random distribution of studentised residuals around zero without any systematic trend, confirming that the regression assumptions were satisfied and that the developed models were suitable for optimisation within the investigated design space.

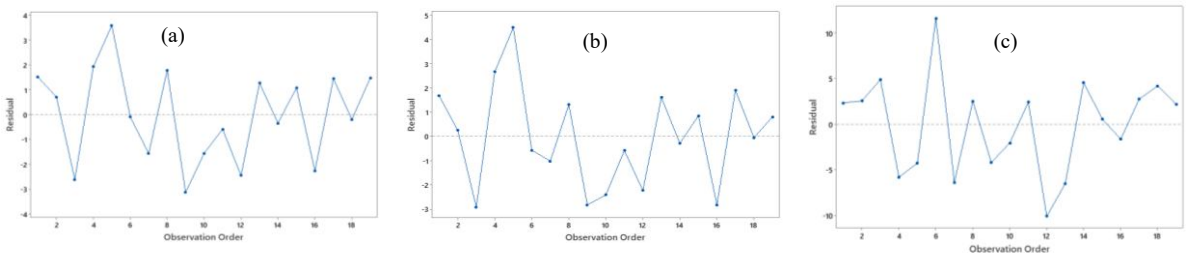


Figure 2. Studentised residuals versus experiment numbers, (a): Ni model, (b): Co model, (c): Re model.

3.4. Effects of operating parameters

The coded response equations (Equations 1–3) quantitatively describe the influence of each variable on metal dissolution. Because the models were developed using coded variables, the magnitude of each regression coefficient directly reflects the relative importance of the corresponding factor.

$$Ni_{Dissolution\ (\%)} = 13.43+3.02A+2.03B-3.09C-2.27AC-4.38B^2 \quad (1)$$

$$Co_{Dissolution\ (\%)} = 15.28+4.26A+1.60B-3.39C-2.27AC-4.52B^2 \quad (2)$$

$$Re_{Dissolution\ (\%)} = 8.37+6.27B-4.52C \quad (3)$$

Temperature exerted a positive effect on the dissolution of Ni and Co (Figure 3), reflecting accelerated dissolution kinetics and improved mass transfer at elevated temperatures. Increasing temperature enhances both chemical reaction rates and diffusion processes, thereby promoting the release of superalloy constituents into solution. However, temperature had no statistically significant influence on Re dissolution, suggesting that Re oxidation is not kinetically limited under the investigated conditions.

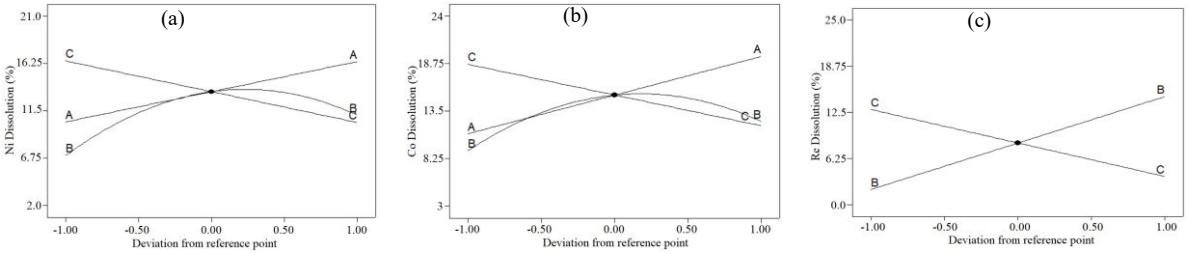


Figure 3. Effect of individual parameters on responses (A=Temperature, B=H_2O_2 (%), C=solid content (%))

The H_2O_2 acted as an auxiliary oxidising agent, increasing the solution ORP and promoting oxidation of metallic phases. Moderate H_2O_2 additions significantly improved Ni and Co dissolution, whereas excessive additions resulted in diminishing returns, as reflected by the significant quadratic terms in the response models. This behaviour may be attributed to surface passivation or the formation of less soluble oxidation products under highly oxidising conditions.

For Re, H_2O_2 was the dominant controlling factor. This behaviour is consistent with the electrochemical characteristics of Re, which is stabilised as soluble perrhenate (ReO_4^-) under strongly oxidising acidic conditions according to its available Pourbaix diagrams. Consequently, increasing ORP through H_2O_2 addition substantially enhanced Re dissolution.

Solid content exhibited a consistently negative effect on all responses. Increasing pulp density reduced the effective acid-to-solid ratio and accelerated acid consumption during dissolution, thereby limiting proton availability and reducing metal extraction. This effect became particularly significant at higher temperatures, where dissolution kinetics were accelerated and acid consumption increased.

3.5. Interaction effects and process optimisation

One of the principal advantages of RSM is its ability to identify statistically significant interactions between operating variables. ANOVA revealed that the interaction between temperature and solid content (AC) significantly affected both Ni and Co dissolution ($p < 0.05$), whereas this interaction was not significant for Re.

The contour and three-dimensional response surface plots (Figure 4 and 5) demonstrate that increasing temperature enhances metal dissolution only when sufficient biogenic acid is available. At higher solid contents, the beneficial effect of temperature is progressively reduced because acid consumption becomes the dominant limiting factor. Such interaction effects cannot be identified using conventional one-factor-at-a-time experimentation and highlight the value of multivariate statistical optimisation.

The validated response models were subsequently employed to determine operating conditions that maximise metal recovery (Table 4). Both Ni and Co exhibited similar optimum conditions, requiring temperatures of approximately 61–65 °C, H_2O_2 concentrations of 6%, and solid contents of 2–2.2%. The similarity of these optima reflects the comparable dissolution behaviour of the two critical metals within the superalloy matrix.

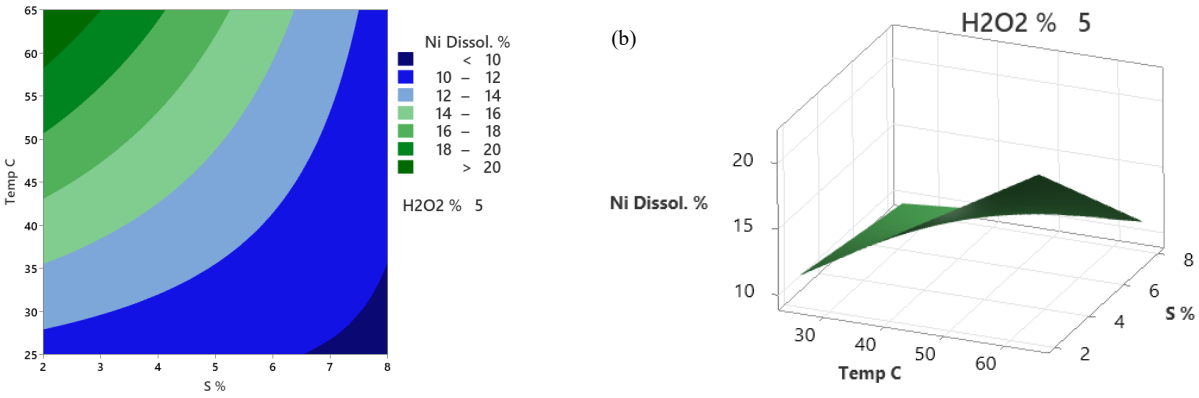


Figure 4. Interaction of temperature and solid content on Ni dissolution at H_2O_2=5%, (a) contour plot, (b) 3D plot

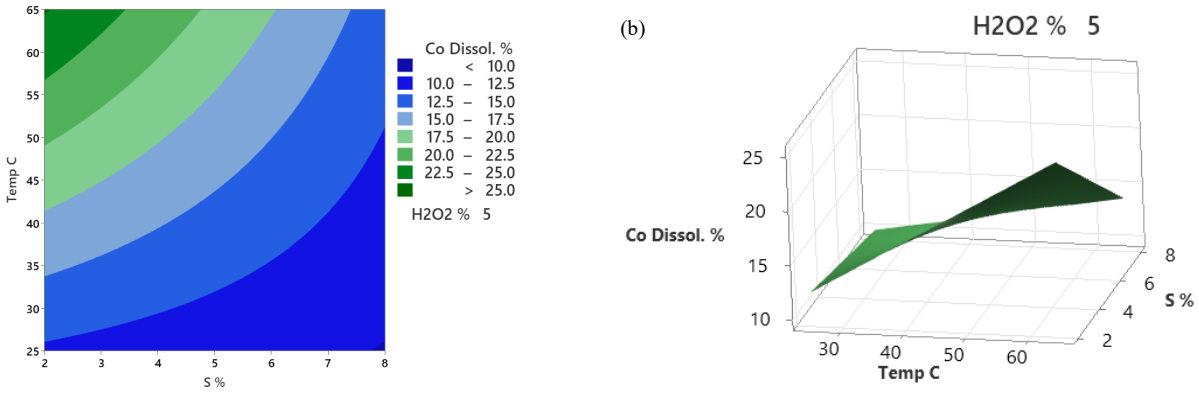


Figure 5. Interaction of temperature and solid content on Co dissolution at H_2O_2=5%, (a) contour plot, (b) 3D plot

For Re, the optimisation suggested the lowest investigated solid content and the highest H_2O_2 concentration, whereas temperature remained insignificant. These findings further support the hypothesis that Re dissolution is controlled predominantly by oxidation potential rather than ionic capacity of the solution or thermal activation.

Table 4. parameters' suggested for maximisation of each response

	Temperature (°C)	H_2O_2 (%)	Solid content (%)
Ni Dissolution (%)	61-63	5.9-6.4	2-2.2
Co Dissolution (%)	61-65	6-6.3	2-2.2
Re Dissolution (%)	25-65	10	2

3.6. Validation of the optimised conditions

A confirmation experiment was performed under the optimised operating conditions (65 °C, 6% H_2O_2 and 2% solids) to validate the developed models and investigate dissolution kinetics over 144 h (Figure 6) Ni and Co exhibited rapid dissolution during the initial stage of leaching, reaching steady state after approximately 30 h. This behaviour indicates that dissolution of the major superalloy constituents is primarily controlled by acid availability.

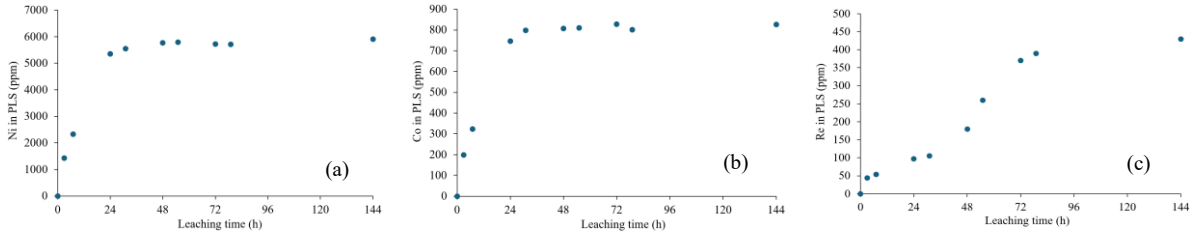


Figure 6. Kinetics of dissolution of Ni, Co, and Re (biogenic acid, temperature=65 °C, H_2O_2=6%, solid content=2%)

In contrast, Re dissolution proceeded considerably more slowly and required approximately 80 h to approach steady-state conditions. The slower dissolution kinetics may reflect the lower abundance of Re within the superalloy microstructure together with its dependence on sustained oxidising conditions for the formation of soluble perrhenate species.

Overall, the validation experiment confirmed the reliability of the response surface models and demonstrated that the statistically optimised conditions are suitable for maximising recovery of critical metals from superalloy grinding waste using biogenic acid.

4. Conclusions

This study demonstrated the feasibility of recovering critical metals from superalloy grinding wastes using biogenic acid produced by *Acidithiobacillus thiooxidans*. RSM coupled with a CCD proved to be an effective approach for evaluating and optimising the combined effects of temperature, H_2O_2 concentration and solid content on the dissolution of Ni, Co and Re.

Statistical analysis showed that solid content had the most pronounced negative influence on metal dissolution, whereas increasing temperature and H_2O_2 concentration generally enhanced metal dissolution. Significant interaction effects between temperature and solid loading were identified for both Ni and Co, highlighting the importance of multivariable optimisation over conventional one-factor-at-a-time experimentation. In contrast, Re dissolution was governed primarily by oxidation potential, with H_2O_2 acting as the dominant controlling factor.

Model optimisation identified operating conditions of approximately 60–65 °C, 5–8% H_2O_2, and 2–2.2% solid loading as favourable for maximising overall metal dissolution. Validation experiments performed under the optimised conditions showed that Ni and Co concentration in solution reached steady state within approximately 30 h, whereas Re required a substantially longer leaching period owing to its slower oxidation-controlled dissolution behaviour. Overall, the results demonstrate that biogenic acid leaching represents a promising capacity and environmentally sustainable strategy for recovering critical metals from superalloy processing wastes.

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Microbial Recycling Process for Sustainable Metal Recovery from End-of-Life Lithium-Ion Batteries for the Circular Economy

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Keywords: **[lithium-ion batteries, bioleaching, metal recovery, cathode regeneration, graphite recycling]**

Abstract

The rapid expansion of the lithium-ion battery (LIB) market and the associated increase in depleted battery waste necessitate the development of environmentally sustainable recycling solutions. This study presents a closed-loop biohydrometallurgical process for valorising commercial black masses derived from spent LIBs. Two acidophilic species, *Acidithiobacillus ferroxidans* and *Acidithiobacillus thiooxidans*, were utilised to generate inorganic metabolites that enabled the solubilisation of key metals, including Li^{+}, Co^{2+}, Ni^{2+}, and Mn^{2+}, through bioleaching. The resulting metal-rich bioleachate underwent selective oxalate co-precipitation to produce metal oxalate precursors, which were subsequently converted into NMC oxide cathode active material via lithium incorporation and high-temperature calcination. Concurrently, the solid leaching residue, composed of graphite and other non-leached metals, was subjected to mild acid washing and thermal treatment to yield pure graphite suitable for use as an anode material. The regenerated cathode and recovered anode materials were assembled into new Li-ion cells, thereby establishing a circular material loop. The electrochemical performance of the recovered graphite surpassed that of commercial graphite, while the regenerated NMC622 cathode material demonstrated performance comparable to its commercial counterpart. The integrated bioleaching-to- new battery process offers a cleaner, greener solution for recovering electrode components, paving the way for a truly circular economy in battery materials.

Introduction

Lithium-ion batteries (LIBs) are essential energy sources for contemporary electronic devices, electric vehicles, and various energy storage systems. The increasing volume of waste generated by LIBs presents a substantial challenge for global electronic waste management. Recycling spent LIBs to recover their metallic constituents can reduce environmental impact and provide a reliable supply of metals for manufacturing new batteries. Currently, pyrometallurgy and hydrometallurgy are the conventional methods for extracting metals from waste LIBs. Pyrometallurgical techniques are generally discouraged due to lithium loss as gas, elevated costs, hazardous gas emissions, and high resource consumption. In contrast, hydrometallurgical methods demonstrate superior sustainability, extraction efficiency, energy requirements, and capital costs. Nevertheless, hydrometallurgical processes are associated with complex operational procedures, significant acid waste generation, and emissions of hazardous gases such as Cl_2, SO_3, and NOx. These emissions pose risks to human health and the environment, and their management increases operational expenses. Therefore, the development of environmentally friendly approaches for metal extraction from depleted LIBs is essential. Our group has been working on the bioleaching process for many years, which uses microorganisms and their metabolic by-products to extract metals from spent LIBs,

Materials

The indigenous strains *A. ferrooxidans* and *A. thiooxidans* were obtained from the DSMZ culture collection centre in Germany. A "Modified 9K medium" was prepared containing all essential inorganic salts required for the growth of both bacterial species. The composition included $(NH_4)_2SO_4$ (3.00 g/L), KCl (0.06 g/L), $K_2HPO_4 \cdot 3H_2O$ (0.20 g/L), $MgSO_4 \cdot 7H_2O$ (0.50 g/L), $Ca(NO_3)_2$ (0.02 g/L), and $FeSO_4 \cdot 7H_2O$ (45 to 150.0 g/L) to support the growth of *A. ferrooxidans*. The final pH of the medium was adjusted to 2.0 using 2N H_2SO_4. *A. thiobacillus were* cultured in Medium 670, which contained $(NH_4)_2SO_4$ (2 g/L), KCl (0.1 g/L), $K_2HPO_4 \cdot 3H_2O$ (0.1 g/L), $MgSO_4 \cdot 7H_2O$ (0.25 g/L), and elemental S^0 (5.0 g/L) as the primary energy substrate. The pH of the medium was adjusted to 3.5 using 2N H_2SO_4.

Methods

Both bacterial strains were cultured using a 10% inoculum for 7 days for A. ferrooxidans and 14 days for A. thiooxidans. During cultivation, pH, redox potential (ORP), Fe^{3+} concentration, and H_2SO_4 concentration were monitored. Bioleaching was performed by adding commercial black masses with pulp densities of 20, 50, 70, 100, and 150 g/L into flasks containing spent culture and incubating in a shaking incubator at 200 rpm and 30 °C.

Analysis

A Metler Toledo pH meter (Seven Compact S220) measured the pH and redox potential. All measurements were conducted at 25 °C. Metal concentrations were determined using ICP-OES (PerkinElmer Optima 8000). Biogenic sulfuric acid (H2SO4) production during bacterial growth and in the bioleaching solution was estimated by acid–base titration. Fe^{3+} ions were quantified using 5-sulfosalicylic acid (SSA). Surface morphology and metal mapping in the black mass and bioleaching residues were examined using scanning electron microscopy (SEM–EDX) (JSM-7600F Jeol, Japan). X-ray diffraction (XRD) analysis was performed using a Bruker powder diffractometer (AXS D8 Advance) with Cu Kα radiation (WL = 1.54060), a 40 kV acceleration voltage, and a 40 mA current to analyse the composition of black mass and leaching residues.

Results and Discussion

Through bioleaching, valuable metals such as lithium, cobalt, nickel, and manganese were extracted from lithium-ion batteries (LIBs) with various chemistries, including LCO, NMC, and LFP. Metal extraction from LCO- and NMC-based spent LIB black mass at high pulp densities presents challenges due to microbial inhibition caused by elevated metal toxicity and substrate (iron) limitation. This study investigated the bioleaching of NMC, LCO, and LFP black material at a high pulp density (150 g/L) using the cost-effective autotrophic bacterium *Acidithiobacillus ferrooxidans*. Enhanced biogenic H_2SO_4 generation in the culture media and replenishment of the bacterial culture for three cycles resulted in a recovery of 85% to 95% of valuable metals, including nickel, manganese, cobalt, and lithium, at a pulp density of 150 g/L. XRD, SEM, and ICP-OES analyses of LIB black mass before and after bioleaching confirmed that most metals were extracted. The extracted metals were subsequently used to synthesize new cathode materials from NMC black mass. These materials demonstrated high purity, robust crystal structure, and homogeneous morphology suitable for battery applications. Regenerated NMC111 and NMC622 exhibited electrochemical performance comparable to commercial NMC, retaining 85% of their capacity after 50 cycles. Additionally, graphite was recycled from the bioleaching residue for use in new lithium-ion battery fabrication.

The regenerated graphite achieved a purity of 99.78%, and its morphology and structure were verified to be indistinguishable from commercial graphite. Regenerated graphite samples demonstrated exceptional electrochemical performance, exceeding 400 mAh/g in charging/discharging capacity and maintaining a retention rate of 100% after 200 cycles. This performance surpasses that of commercial graphite, which has a capacity of 366 mAh/g.

Conclusions

At a pulp density of 100 g/L (S/L), bioleaching mediated by A. ferrooxidans resulted in recoveries of 85.50% Ni, 91.83% Mn, 90.37% Co, and 89.89% Li over three cycles in 6 h. XRD, SEM, and ICP-OES analyses of NMC and LFP black masses before and after bioleaching confirmed that more than 85% of metals were leached from the black mass. The regenerated NMC111 and NMC622 cathode materials exhibited a well-ordered structure, low levels of cation mixing, and electrochemical properties comparable to those of commercial NMC cathodes. NMC111 and NMC622 demonstrated initial discharge capacities of 112 and 109 mA h g−1, respectively, while commercial NMC exhibited an initial discharge capacity of 118 mA h g−1. After 50 cycles at 100 mA h, the capacity retention of regenerated NMC111, NMC622, and commercial NMC reached 89%, 84%, and 85%, respectively. Notably, recycled graphite exhibited a higher specific capacity (approximately 401–413 mAh/g at 100 mA/g) after calcination compared to commercial graphite (366 mAh/g at 100 mA/g).

Acknowledgements

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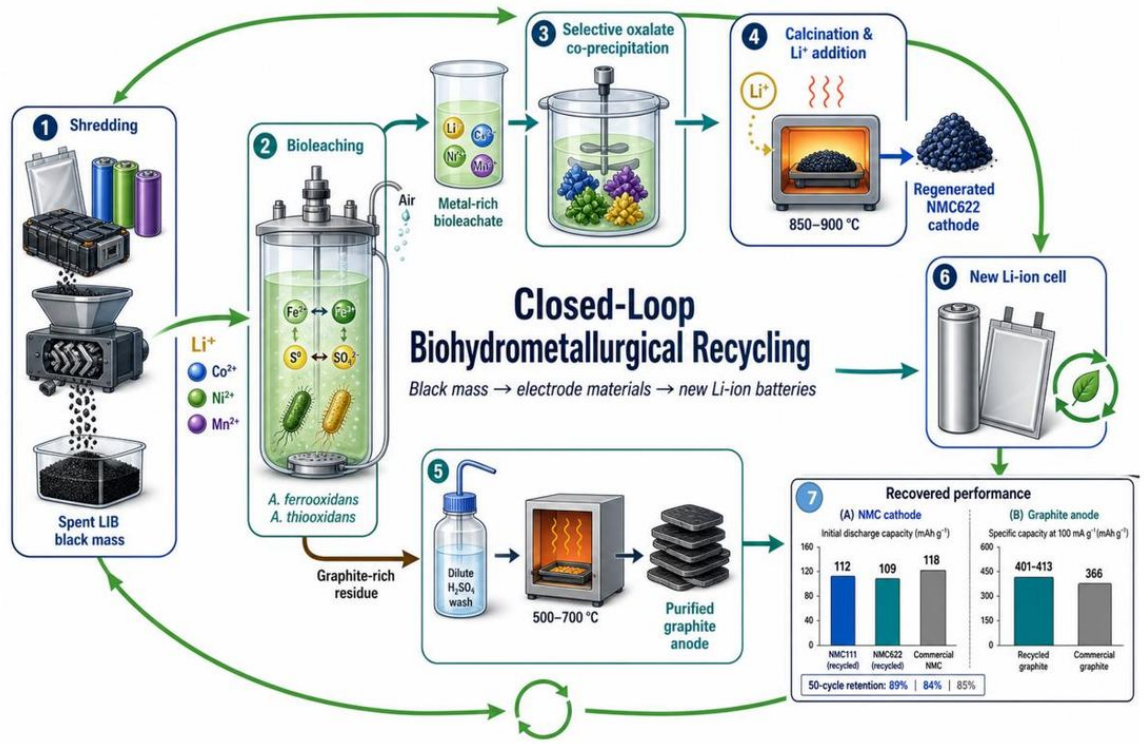
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Graphical Abstract



Microbial effects on Lithium ore flotation processes

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Abstract: Microorganisms can be found in virtually any environment if conditions allow their growth. Mineral processing environments have been shown to host abundant microbial populations across different stages of the process water cycle, including raw feed, flotation, waste streams, and concentrates. Although microbial interactions can play an essential role in the performance of existing processes, microbial activity is not considered in the design of mineral processes. The objective of the LITHOS-project is to account for microbial effects of naturally occurring microorganisms on the flotation of Li-bearing minerals when closing the water loops already during the process research phase.

During the project, microorganisms potentially thriving in Li-processing environments were enriched from lithium ores—spodumene, lepidolite, and petalite—under pH conditions typical for flotation processes, either acidic (pH 2–3) or alkaline (pH 8–10). The enriched communities were subsequently adapted to flotation reagents at approximately one-fifth of the concentrations planned for flotation. Microflotation tests were conducted by comparing standard flotation without microbial addition to flotation water supplemented with enriched microorganisms at concentrations around 10^7 cells/mL, which corresponds to levels observed in mineral processing waters. Preliminary findings revealed that microbial addition had a measurable impact on lepidolite flotation, slightly increasing the amount of floatable material (21.6% to 26.7%) while simultaneously reducing lithium recovery (53.5% to 40.6%). These results suggest that microbial presence may alter flotation behaviour and recovery efficiency. Further experiments are ongoing with other lithium ores to better understand these interactions and to support the development of operational strategies for managing microbial populations in flotation circuits under circular, low water intensity processing conditions.

Keywords: Lepidolite, spodumene, microbe-mineral interaction, Hallimond-tube, bacteria

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Progress in the understanding of dissolution and leaching reactions

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Key to understanding bioleaching is an understanding of dissolution reactions. About a quarter of a century ago our team demonstrated through detailed experimental work that the mechanism of bacterial leaching is through the regeneration of ferric ions. This result, and the realisation that the slow step in this process is the dissolution of the mineral by ferric ions meant that focus was required on fully understanding the chemical dissolution of sulfide minerals. Furthermore, because both the chemical dissolution of sulfides and the growth of the microorganisms require an acidic environment and the gangue minerals consume acid, a detailed understanding of bacterial leaching and heap leaching requires an in-depth understanding of dissolution reactions. In this presentation, the three classes of dissolution reactions, that is, redox reactions, acid-base reactions, and salt reactions, are discussed. Critical experimental results are summarised, the different approaches to interpreting the kinetic parameters, and the advantages and disadvantages of the principal models are evaluated. Finally, it is argued that a clear model of dissolution emerges from this survey of all classes of dissolution reactions.

Geomicrobiology of the deep subsurface of the Iberian Pyrite Belt: Biohydrometallurgical implications

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Keywords: dark biosphere, continental deep subsurface, biogeochemical cycles, Iberian Pyrite Belt, insitu bioleaching

Abstract

Samples from two boreholes drilled in the Iberian Pyrite Belt (IPBSL project) were analysed using complementary methodologies (massive sequencing, immunological identification, fluorescence in situ hybridization, enrichment cultures as well as phenotypic and genotypic characterization of isolated microorganisms). The results allowed to describe the coupled operation of the most important biogeochemical cycles (C, H, N, S and Fe) in the deep subsurface of the IPB. Given that the extreme conditions detected in the Río Tinto basin: acidity and high concentration of heavy metals (Fe, Cu, Ni, Co and Zn) are the result of the activity of this underground bioreactor, a better understanding of its operation should facilitate the development of efficient *in situ* bioleaching methodologies, considered the future of biomining.

Introduction

The Iberian Pyrite Belt (IPB) located in the southwestern part of the Iberian Peninsula holds the largest concentration of metal sulfides in the Earth [1]. Río Tinto with its almost 100 km of length hosts a high concentration of heavy metals, mainly Fe, which are in solution due to the acidic conditions of the river. Until recently it has been considered that the origin of the extreme conditions of the Tinto basin was the very active mining activity in the area during 5.000 years. But dating the oldest terraces of the river gave us an age of millions of years [3], several orders of magnitude older that the first known mining activity, requesting another explanation for the origin of Río Tinto. Recently, the results of a drilling project in the area (IPBSL), close to the head of the river, strongly suggested the existence of a huge underground bioreactor in which metal sulfides are an important energy source, and the metabolic products, mostly ferric iron and sulfate, drain into the river [2]. The results obtained with the analysis of the deepest circumneutral borehole, BH10, allowed to describe the coupled operation of the most important biogeochemical cycles in the subsurface [2]. In this communication we discuss the preliminary results obtained with the analysis of the second borehole, BH11, in which the first 120 m are acidic (close to 4), and as a consequence the need to pay attention to the biogeochemical cycles operating in the deep subsurface for the design and operation of future *in situ* bioleaching operations.

Methods

A detail description of the IPBSL drilling project, the drilled boreholes (BH10 and BH11), and their geochemical and geomicrobiological analysis are described in detail in [2].

Results and Discussion

Most studies on the deep subsurface microbial diversity are based on the analysis of groundwater obtained in artesian wells, which although providing useful data, cannot be directly linked to the geological features of the extremely complex mineral matrix in which the microorganisms dwell.

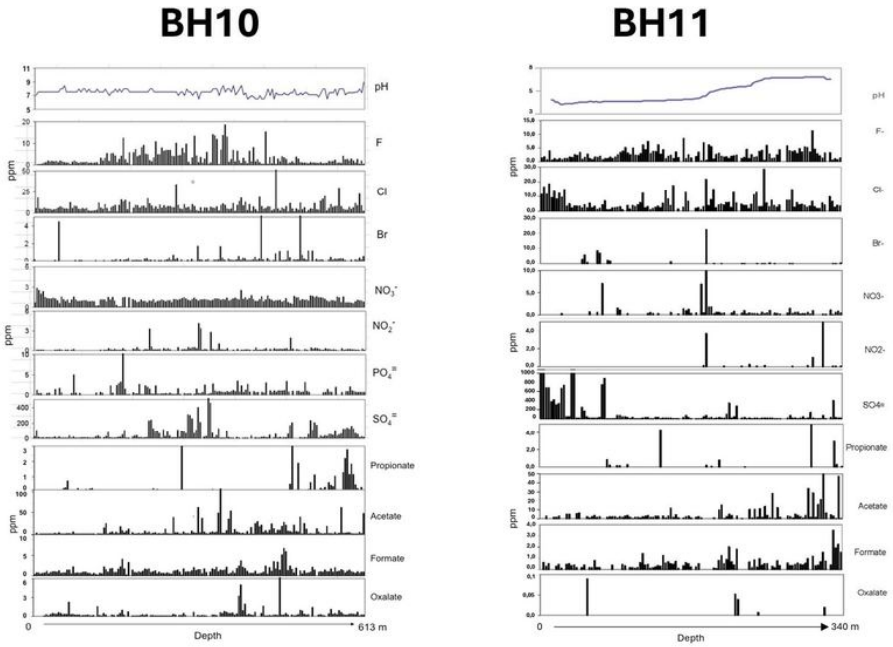


Figure 1. Geochemical data for boreholes BH10 and BH11.

Despite recent progress in continental drilling operations, information on abundance, diversity and the operation of the biogeochemical cycles are scarce [2 and references therein]. In addition, and due to economic reasons, in general most of the drilling projects only use one methodology of analysis to describe the diversity, massive sequencing, which as any methodology has its own bias. In the IPBSL project two boreholes, BH10, 620 meters below surface (mbs), and BH11, 340 mbs were drilled.

Table 1. Genera detected in borehole BH11 at different depths and pH compared with those detected in the circumneutral borehole BH10. Methodologies: H: hybridization, S: enrichment cultures sequencing, √: isolation. For borehole BH10, ++: genera detected by 2 methodologies and at 5 five different depths intervals, +: detected by one methodology.

borehole	BH11	BH11	BH11	BH10
pH	4.2-4.3	5.9	7.5-7.8	
depth	79-93 mbs	145 mbs	320-340 mbs	
Genus				
Acidiphilium	H		H	++
Acidovorax	H		S	++
Acidithiobacillus	H	H	S	++
Bacillus	S	S		++
Cellulomonas	√	S		+
Clostridium	S	S	S	
Desulforispora	S			+
Desulfosporosinus	S H	S H	H	++
Desulfovibrio			S	++
Kleibsella	S	S	S	
Paenibacillus	S	S	S	+
Propionibacterium	S	S	S	++
Pseudomonas	S	S	S	++
Rhodoplanes	S H	S		++
Sulfobacillus	H		H	++
Tessaracoccus	S H	S	S	++

The analysis of samples obtained at different depths of the circumneutral borehole BH10 using complementary methodologies (massive sequencing, immunological identification, fluorescence in

situ hybridization, enrichment cultures as well as phenotypic and genotypic characterization of isolated microorganisms) allowed to describe for the first time the coupled operation of the most important biogeochemical cycles (C, H, N, S and Fe) in the deep subsurface of the IPB in the absence of light and O_2 [2].

The correspondent analysis of borehole BH11, which has the additional interest that its first 120 m are acidic, while the last 120 m are circumneutral (Fig. 1), offers the possibility to compare microbial diversity in the deep subsurface at different pH in the same column and also with the diversity identified in the circumneutral borehole BH10. As shown in Figure 1, in borehole BH11 the highest concentration of sulfates appears in the acidic zone, which is quite different than the distribution observed in the circumneutral borehole BH10. Another important difference is that nitrate concentrations are much lower and almost not existent in the acidic part of borehole BH11. The concentration of organic acids, mainly acetate and formate, are stochastically distributed and similar in both boreholes

A preliminary unexpected result was the high diversity at genus level observed at acidic pH which is comparable with the diversity observed at a neutral pH of the same borehole and the diversity reported for the circumneutral borehole BH10 (Table 1). In this case 63% identity with the most representative genera of borehole BH10 identified by at least two independent methodologies and at five different depth intervals. Interestingly enough, according to the sulfate concentration in borehole BH11 it seems that sulfide oxidation is very active in its acidic part. We should keep in mind that this oxidation must be done in the anoxic conditions prevailing in the deep subsurface.

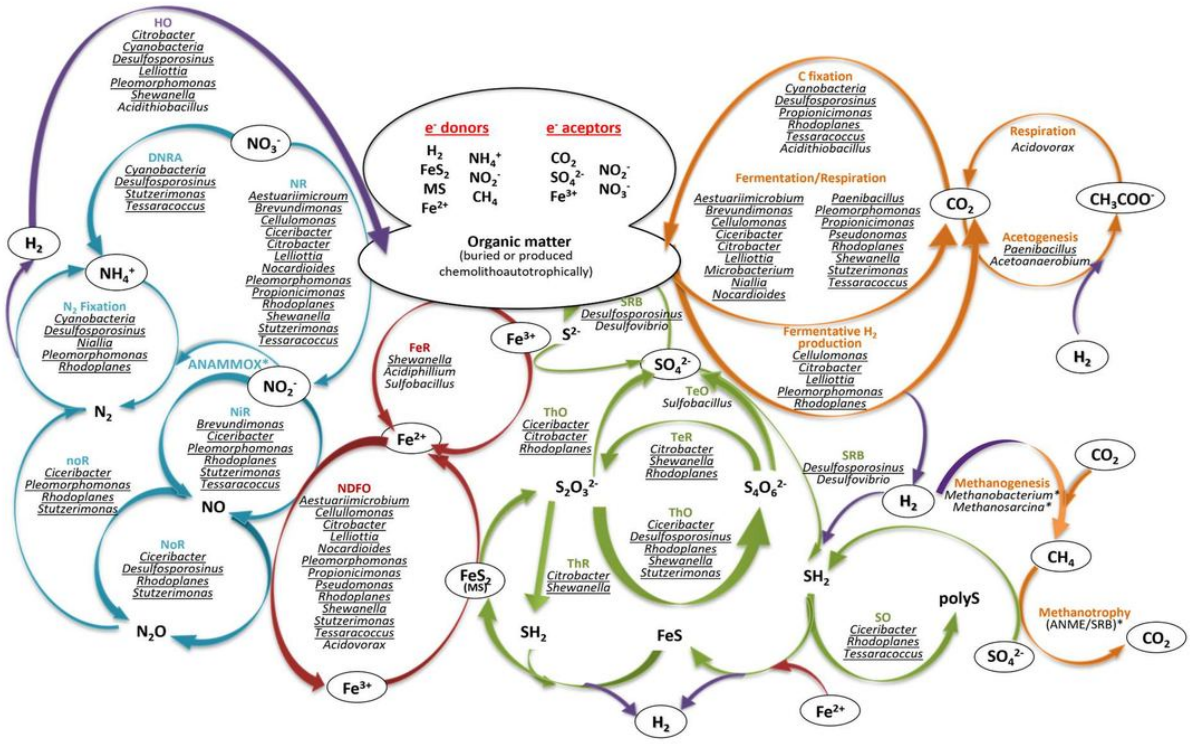


Figure 2. Geomicrobiological model of the biogeochemical cycles operating in the deep subsurface of the IPB in anoxic conditions according to the geochemical and genomic analysis of isolated microorganisms from enrichment cultures using samples from cores obtained at different depths in boreholes BH10 and BH11. For enzymatic details see [2], not underlined genus correspond to microorganisms detected but not isolated, * detected only by CARD-FISH.

At circumneutral pH and anoxic conditions it has been suggested that the oxidation of pyrite and other metal sulfides is promoted by the high concentration of ferric iron generated by the great oxidizing capacity of nitrite, produced as the consequence of using nitrate as electron acceptor for respiration [4, 5, 6]. This situation is quite different than the well documented acidic aerobic

oxidation of pyrite, and other metal sulfides, produced by aerobic iron oxidizing microorganisms [7]. This means that an efficient anoxic oxidation of metal sulfides, related with the generation of acidity, could be functioning in the deep subsurface, requiring a clarification of the involved mechanisms, its relation with pH and the biogeochemical cycles operating in the deep subsurface (Fig. 2).

Conclusions

Considering that *in situ* bioleaching has to be done in the subsurface, in anoxic conditions, it will be convenient to pay attention to the biogeochemical cycles operating in these conditions, to facilitate its further development and efficiency.

Acknowledgements

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FACING THE CHALLENGE OF VARIABILITY OF MINERALS IN BIOLEACHING OPERATIONS

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Keywords: copper bioleaching; microbial activity; coper recovery; LOM life of mine; complex events processing; digital mine; Stream processing; Clustering classification

Abstract

Over the life of a mine, there is significant variability in terms of mineralogy and lithology, with consequences for metallurgical performance in bioleaching processes. In addition, the number and frequency of batches available for loading into bioleaching heaps vary, affecting microbial activity and copper recovery. One approach to managing this variability and optimising the overall process is to properly classify the batches loaded into bioleaching heaps and to consider the impact of the frequency of new loads on microbial activity and copper recovery.

Rigorous, ongoing, long-term monitoring, including microbial activity, together with the use of a carefully designed clustering algorithm enabled the assignment of each load to its corresponding cluster, column, and lift within the heap. Then, a complex event processing (CEP or stream processing) approach was used to identify the heaps that showed a clear correlation between microbial activity, new mineral loading, and copper recovery performance. After that, we estimated the additional Cu recovered from new loads for each column. Furthermore, we analysed the impact of new loads on recovery by comparing loading frequencies.

The results of this analysis supports decision-making regarding mine planning and determining where and when to spread extracted material in the heap columns, following the most suitable loading frequency to optimise Cu recovery.

Introduction

So far, it is well known that the heap bioleaching process is used for copper recovery and has had a huge impact on mining by addressing sustainability challenges in terms of energy and water consumption and pollution, particularly in the context of declining copper ore grades in older mines [1]. In addition, heap bioleaching has enabled the exploitation of non-traditional resources such as mining residues, and processing ways such as dump or *in situ* processing, e.g. [2, 3]. In this context, combined efforts across scientific and technological fields are required to provide the necessary answers. A particular challenge is to identify the microbial activity associated with a particular mineralogical composition, its quantitative impact on copper recovery, and how this impact is distributed over time. As previously mentioned, the large amount of industrial data recorded at the heap bioleaching process at Minera Escondida since the start of industrial operations in 2006 represents an opportunity to increase knowledge about complex bioleaching processes [4]. From a methodological point of view, the availability of non-continuous data on microbial activity and mineral load poses an additional challenge. For this reason, an event-correlation approach is required to thoroughly review all historical data and identify interesting patterns. The definition of these patterns as a mix of microbial activity variables and the timing of mineral loading in a specific heap is a key piece of knowledge. This definition was itself an objective, after identifying the heaps where interesting patterns occur and those where they do not. Based on this information, the goal is to evaluate the impact of identified patterns on copper recovery in specific columns.This requires a rigorous characterisation of microbial activity during the life cycle of bio-heap, and the mineralogical composition of the material loaded into the heap.

Figure 1.shows a schematic view of this particular scope previously described in depth [5], with process variables and environmental conditions as inputs.

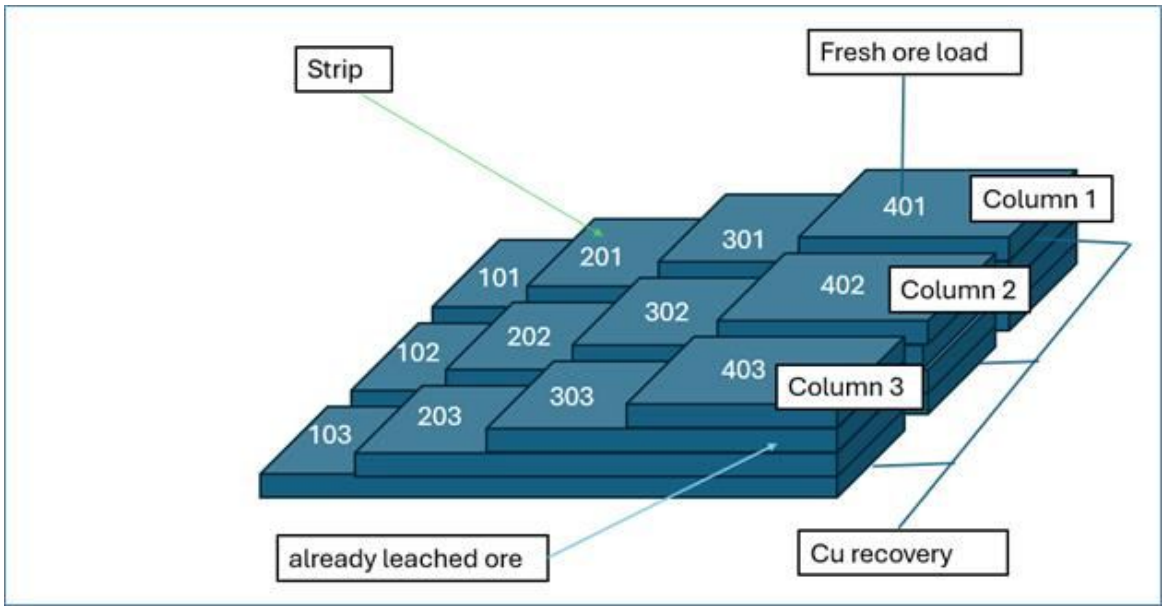


Figure 1. Schematic view of bioleaching Heap in Escondida Mine.

Methods

Capability and Materials. A major baseline for progress in this analysis was the knowledge and raw data from the SHL Escondida Heap Leaching Process. We used a solution developed by KIM21, which incorporates a Complex event processing tool (Esper CEP), a relational database and its coding capabilities (Postgresql), as well as traditional office tools.

Selection of relevant variables. All available historical data were used in the analysis, including the grouping of strips by heap column for the final assessment of their impact on daily copper recovery. The following variables were considered: irrigation start date and duration, copper grade; as a measure of microbial activity, the slope of the Fe(II) oxidation curve [4].

Event-based model for the quantitative analysis of the impact of microbial activity on recovery. As a useful approach to comprehensively identify the behavioural patterns of interest, such as the correlation between fresh loads and microbial activity, a simple events model was developed. A schematic view of this model is shown in Figure 2. The available historical data are then adequate to search for the interesting patterns.

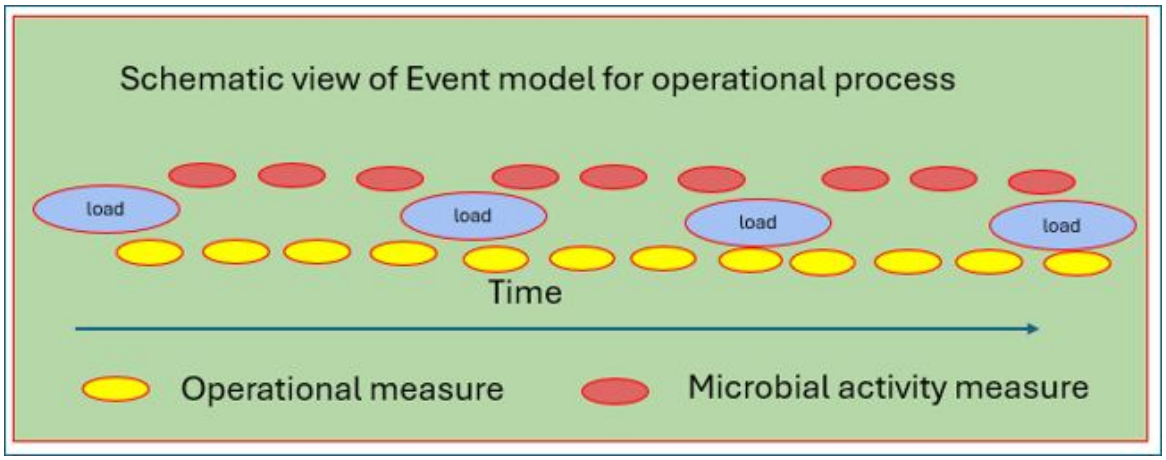


Figure 2. Schematic view of the Event model for the operational process.

Comparison of the impact on extracted copper across cases with different levels of microbial activity. With this information—having identified the columns that showed improvements in microbial

activity and the impact on the recovery of fresh loads—we can compare the impact of fresh loads on a specific column.

Results and Discussion

Two particular patterns were analysed: **Pattern 1** – increase in microbial activity: Loading events followed by at least two measurement events showing increased microbial activity (with an oxidation time lower than that recorded prior to the load), particularly for instances where the variable had deteriorated before a loading event (oxidation time > 20 days). **Pattern 2** – Deterioration of microbial activity: Loading events followed by at least two measurement events showing a longer oxidation time than that recorded prior to the load—specifically for cases where the variable had already deteriorated (> 20 days). Additional patterns were identified during the process.

Table 1.summarises the analysis of the event patterns identified for each column within the available historical data, along with the subsequent estimation of the impact on copper recovery for cases where mineralogical clustering data were available. These results are presented in qualitative terms due to information confidentiality.

Table 1. Summary of the results obtained from the events identified for different patterns

Patterns considered	New load reference	Findings from model runs	Impact on additional extracted Cu
Pattern 1 – increase in microbial activity	After load	70% of cases	Positive and considerable
Pattern 2 – deterioration of microbial activity	After load	30% of cases	Positive but less pronounced
Pattern 1 – increase in microbial activity	Pre load	10 % of cases	Not considered in the model
Pattern 2 – deterioration of microbial activity	Pre Load	90 % of cases	Not considered in the model

To perform the quantitative analysis of copper recovery, it is necessary to estimate the copper recovery for a specific ore group –more precisely, to characterise the average recovery as a function of time. This is shown in Figure 3, where the best representation is a composition of three straight lines rather than a higher-order polynomial.

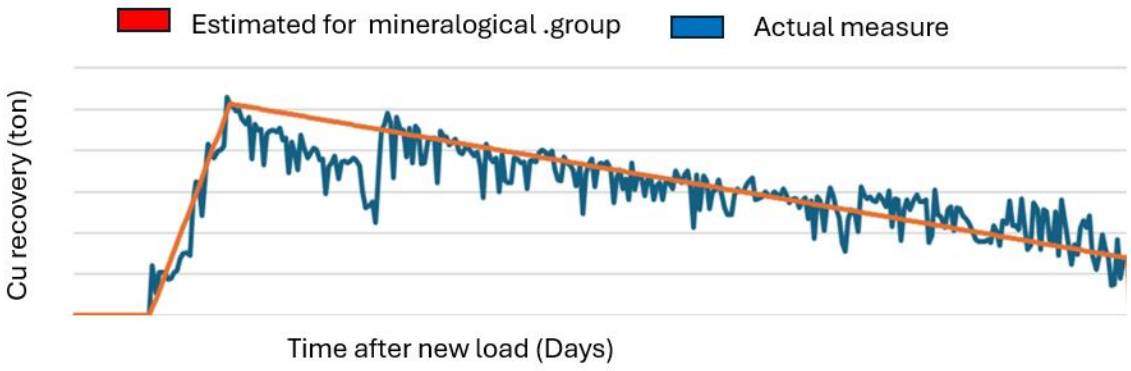


Figure 3. Example of estimate recovery function for specific mineralogical group

An approximation of the copper recovery performance of a specific column following a new load, of cases identified by the model, was obtained using the curve estimated in the previous step (Fig. 3). For this purpose, the following were estimated: a) the amount of copper that would be extracted from the new charge if it were processed in isolation (estimated extracted Cu for specific mineralogical group); b) the amount of copper that would have been extracted if no new charge had been added; this was determined by projecting the extracted Cu levels prior to the new charge; c) the actual extracted Cu from the column following the new charge. Figure 4 illustrates this approach. An estimate of the impact of the fresh charge is given by:

Impact = Actual extracted Cu – (estimated extracted Cu+ projected extracted Cu before last load)

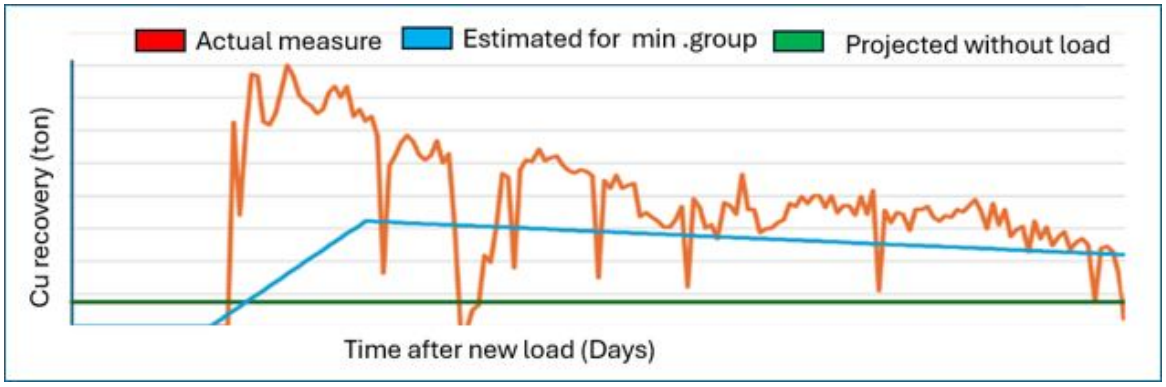


Figure 4. a) Estimated Cu extracted vs time for a heap column from specific mineralogical group (blue curve). b) The projection of the extracted Cu levels prior to the charge (green curve). c) The actual extracted Cu from the column following the new charge (orange curve).

Figure 5.summarises the comparison of oxidation activity, mineral loading frequency, the relative abundance of *At. ferrooxidans* and *Leptospirillum*, mineralogy, and copper recovery in heap columns where Pattern 1 events were identified.

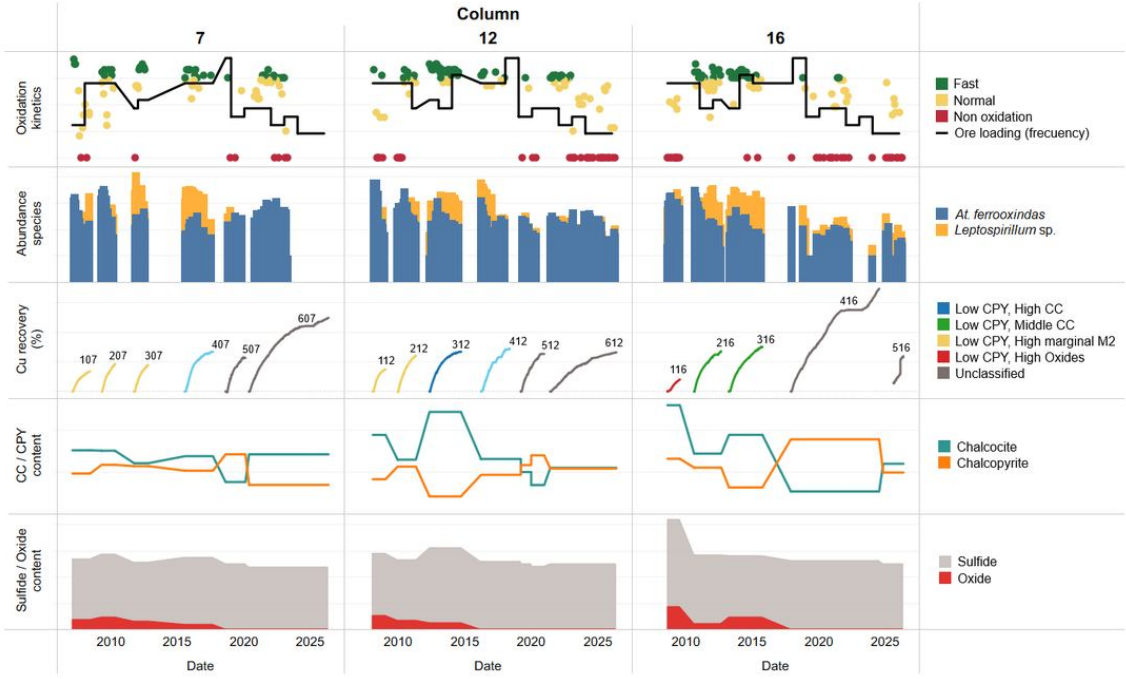


Figure 5. Summary of metabolic oxidation activity, loading fresh ore frequency (number of new strips in operation), microbial abundance, Cu recovery, sulfide species content, and total sulfide and oxide contents in the three selected cases with Pattern 1. Columns 7 and 12 represent cases in which the loaded mineral belongs to the same group, whereas Column 16 exemplifies a case in which the loaded mineral belongs to different groups. Fast, Normal, and Non-oxidation represent the FeII consumption kinetics [5]. CC= Chalcocite, CPY=Chalcopyrite. Colours in the Cu recovery graph represent the group to which each strip belongs, e.g. Low CPY, High CC. Sulfide=% of Cu in sulfide minerals, Oxide=% of Cu in oxide minerals.

The impact of the frequency of fresh mineral loading on microbial activity in the system is easily explained by the substrate requirements needed to support the community, in this case, sulfide minerals. Furthermore, the impact of the composition of the loaded mineral—the oxidation ratio of the charge (Cu Sulfides/Cu Oxides), and the sulfide species—on the structure of the microbial assemblage and microbial activity is consistent with the expected results, considering the capacity of *At. ferrooxidans* vs *Leptospirillum* to oxidise FeII and the differential leaching kinetics of sulfide species [6, 7]. Finally, Cu recovery follows the microbial activity profile, as evidenced, e.g. in the low recovery kinetics coinciding with the density of non-oxidant records of metabolic activity in strip 612 and the low number of new strips, i.e. fresh mineral loaded (Fig. 5).

Conclusions

There is a clear relationship between the frequency of loading and microbial activity within the bioleaching heap, and consequently, its impact on copper extraction. In instances where heap microbial activity improved (Pattern 1), the additional Cu recovery yield was higher; conversely, where microbial activity did not improve (Pattern 2), the impact on copper extraction was less pronounced.

The most significant finding regarding the performance of the bioleaching heap is that the impact of increased microbial activity on copper extraction comes from Cu released from deeper levels within the heap. This means that, with an appropriate loading frequency, it is possible to maintain a level of microbial activity capable of recovering this copper from greater depths; otherwise, this recovery might not occur within the strip's lifecycle**.**

The challenge at this stage is to devise a loading plan that maintains adequate microbial activity levels, or to implement other measures to mitigate the decline in microbial activity, in order to prevent potential losses in copper recovery that might otherwise be irretrievable.

Another significant challenge is to maintain measurements and records covering mineralogical groups in the loads, microbial activity, and other variables of interest, allowing the determination of optimal loading frequencies or appropriate measures to mitigate the decline in microbial activity.

The greatest opportunity lies in fostering dialogue between the mine planners and the experts on microbial behaviour within the heaps. This entails, on the one hand, communicating how microbial activity is affected by loading frequency, the loading sequence within a column or the specific column selected for loading, and the specific mineralogical groups involved; and on the other hand, considering the operational possibilities offered by the mining sequence—such as the various phases and benches. By integrating these considerations into medium- and short-term planning, the impact on microbial activity—and consequently on copper recovery—can be factored into the mine plan, resulting in a more integrated and highly optimised strategy. This approach could even give rise to a new transdisciplinary field that integrates the different dimensions necessary to facilitate such conversations.

Acknowledgements

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High-rate iron oxidation by *Leptospirillum ferriphilum* and psychrotolerant *Acidithiobacillus ferrivorans* for bioleaching and AMD remediation

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Keywords: Iron oxidation, fluidised bed reactor, activated carbon, *Leptospirillum ferriphilum*, *Acidithiobacillus ferrivorans*

Abstract

Leptospirillum ferriphilum efficiently oxidised ferrous iron at redox potentials (Eh) above +950 mV within the optimal temperature range of 35–45 °C. Its high affinity for Fe^{2+} and low susceptibility to Fe^{3+} inhibition relative to *Acidithiobacillus species* exhibits superior kinetic performance.

These physiological traits, combined with growth at extremely low pH (≈1), enable the sustained generation of strongly oxidising, iron-rich acidic liquors. In contrast, *Acidithiobacillus ferrivorans* was the only strain capable of exponential growth on ferrous iron at 5 °C, but it exhibited higher sensitivity to low pH. At low temperatures, the increased solubility of ferric iron allows *At. ferrivorans* to achieve higher redox potentials, expanding the potential for Fe^{2+} oxidation under cold conditions.

To maintain high iron oxidation rates and prevent cell washout, Fe^{2+} (bio)oxidation was further investigated in a bottom-up fluidised bed column reactor using activated carbon as a support matrix. Activated carbon acts as an efficient electron shuttle, facilitating electron transfer from Fe^{2+} to dissolved O_2 driving the generation of hydroxyl radicals (•OH).. High biomass retention supported elevated iron oxidation under sufficient oxygen supply, while oxygen limitation was observed at higher Fe-loading rates.

Introduction

Leptospirillum ferriphilum efficiently oxidises ferrous iron in batch and continuous modes at redox potentials (Eh) above +950 mV within the optimal temperature range of 35–45 °C. Its high affinity for Fe^{2+} and low susceptibility to Fe^{3+} inhibition relative to *Acidithiobacillus species* exhibits superior kinetic performance (1-5). These physiological and kinetic characteristics are highly advantageous for bioleaching operations, where high redox potentials and rapid iron turnover are critical, and for environmental applications such as acid mine drainage remediation. Separating bacterial Fe^{2+} oxidation from chemical sulfide leaching allows each process to operate at optimal conditions, reducing bacterial stress and enhancing mineral dissolution and overall metal recovery (6, 7). To maintain high iron oxidation rates while preventing biomass washout during continuous operation, fluidised bed reactors (FBRs) represent an attractive reactor configuration. Fluidised bed bioreactor (FBR) is a specialised type of bioreactor that utilises fluidisation to enhance biological processes for wastewater treatment and other bioprocesses (8-10).

AC is widely used in contaminant removal, gas separation, energy storage, and various industrial processes (11, 12). AC is a particularly attractive support material for FBRs owing to its high specific surface area, well-developed porous structure, chemical inertness, and excellent mechanical stability (8). The skeletal density of activated carbon (typically 1.8–2.2 g cm^{-3}) provides favourable fluidisation characteristics while ensuring sufficient particle stability under continuous mixing. Furthermore, the extensive surface area of AC facilitates microbial attachment and biofilm development, allowing high biomass retention and enhancing reactor productivity.

In addition to its role as an adsorbent, AC exhibits strong redox activity and can serve as an intermediary, accepting or donating electrons, thereby participating in elemental cycling and pollutant transformation. AC serves as an efficient electron shuttle between Fe^{2+} and dissolved O_2, driving significant •OH production (11).

In the present study, kinetic analysis demonstrated that the presence of AC significantly accelerated ferrous iron oxidation. The resulting higher iron oxidation rates enable the use of more compact reactors or, alternatively, operation at shorter hydraulic retention times while maintaining the same treatment efficiency.

Methods

Materials.

Chemolithotrophic, iron-oxidizing bacteria *Leptospirillum ferriphilum* (DSM 14647) were used in the experiments. The bacterial cultures were maintained in a liquid mineral medium composed of 0.1 g $(NH_4)_2SO_4$, 0.04 g K_2HPO_4, 0.4 g $MgSO_4.7H_2O$, 0.013 g $CaCl_2.2H_2O$ and 44.2 g $FeSO_4.7H_2O$, in 1 litre of 80 mM H_2SO_4. The final pH of the medium was 1.2. Cultivation was carried out at 40 °C under aerobic conditions in batch mode.

Norit® ROW 0.8 mm steam-activated carbon pellets (CAS No. 7440-44-0) were used as the support material in the fluidised bed reactor (FBR). The material was acid washed by 1 M H_2SO_4 followed by rinsing with tap and deionised water and dried at 40 °C. 250 mL of dry carrier material was added to each FBR.

Batch incubation was carried out in 500 mL magnetically stirred reactors equipped with the pH and ORP probes. Air supplied to the reactors was sterilised by filtration through 0.2 µm membrane filters.

The fluidised bed reactor system comprised a fluidisation column hydraulically integrated with an aeration column. Fluidisation of the carrier particles was achieved by the upward flow of recirculating liquid, ensuring controlled bed expansion and uniform hydrodynamic conditions throughout the reactor. Liquid from the upper section of the fluidisation column, above the expanded bed region, overflowed across a tube weir and was transferred by gravity into the aeration column positioned below the FBR. The oxygen saturated liquid was pumped back from the aeration column to the bottom of FBR, providing sustained reactor operation under closed-loop recirculation conditions.

Analysis.

Fe^{3+} was determined by a UV-spectrophotometric method at 300 nm (13). Fe^{2+} concentrations were determined using a modified o-phenanthroline spectrophotometric method insensitive to Fe^{3+} interference (14).

Consort D230 multiparameter datalogger/data acquisition system was used for pH and ORP determination during the experiments using Mettler Toledo InLab® Micro Pro-ISM probes for pH and InLab Redox-L type probes for ORP.

The rates of chemical (abiotic) and bacterial ferrous iron oxidation, as well as bacterial growth, in the batch and FBR reactors were monitored through on-line measurements of oxygen consumption and carbon dioxide production/consumption in the gas phase. The dissolved oxygen (DO) was measured from solution above the fluidised carrier material bed.

The carrier material shape and surface were examined by scanning electron microscope MIRA 3 FE-SEM (TESCAN) coupled with an EDX detector (Oxford Instruments).

Results and Discussion

Iron oxidation experiments were conducted in batch reactors and fluidised bed reactors.

Batch incubation:

The specific O_2 and CO_2 fixation rates of *Aciditiobacillus species* started to decline as the ferrous iron concentration decreased below 40 mmol L^{-1}, while cultures with *Leptospirillum incubated* at

temperatures > 35°C kept constant specific rates until Fe^{2+} depletion and were capable of iron oxidation at redox potentials close to +980 mV (2, 5).

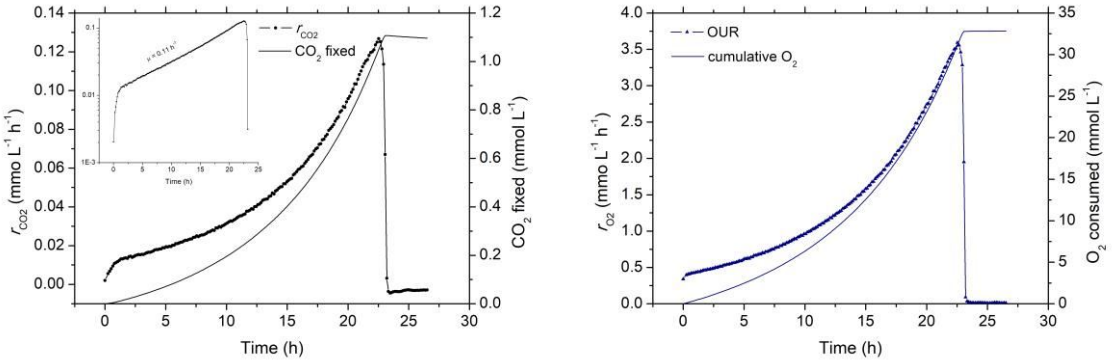


Figure 1. (A) Biomass production rate (r_X) measured as the rate of CO_2 utilisation ($-r_{CO2}$) and cumulative amount of net CO_2 fixed, and (B) oxygen consumption rate and cumulative oxygen uptake by autotrophically grown culture of *L ferriphilum* on ferrous iron at t = 40 °C.

Fluidised bed reactor:

Each FBR contained 250 mL of dry activated carbon carrier material. During operation, the liquid recirculation flow rate was maintained at 300 mL min^{-1}, resulting in a bed expansion of ≈ 20%. Under these conditions, the effective expanded bed volume was 300 mL, while the total liquid volume within the reactor system, including the recirculation loop, was 620 mL. The presence of AC markedly enhanced the kinetics of abiotic ferrous iron oxidation by molecular oxygen in acidic solutions (Fig. 2B) compared with homogeneous abiotic iron oxidation in batch reactors (data not shown). AC acts as an efficient electron shuttle, facilitating electron transfer from Fe^{2+} to dissolved O_2 driving the generation of hydroxyl radicals (•OH) (11).

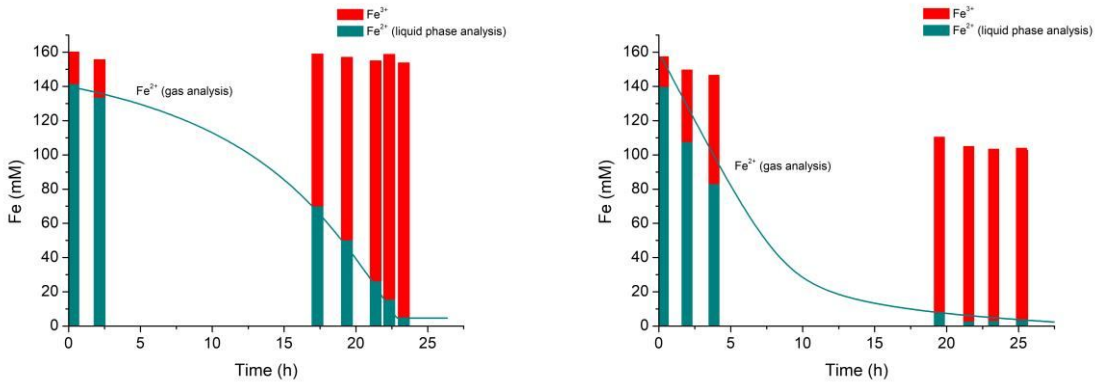


Figure 2. Fe^{2+} and Fe^{3+} concentrations determined by chemical analyses of liquid media and residual Fe^{2+} concentrations calculated from gas analysis (A) Biological oxidation of Fe^{2+} by *L. ferriphilum* in batch reactor. (B) Abiotic oxidation of Fe^{2+} catalysed by AC in FBR.

Figure 2.compares biological Fe^{2+} oxidation mediated by *L. ferriphilum* in batch culture with abiotic Fe^{2+} oxidation in an activated carbon-based FBR under aerobic conditions at 40 °C. This comparison demonstrates the distinct contributions of microbial activity and activated carbon as catalyst for enhanced iron oxidation.

A concurrent reaction accompanying Fe^{2+} oxidation was the production of CO_2 resulting from the oxidation of the activated carbon matrix itself. CO_2 evolution was also observed in parallel control experiments containing acidified deionised water (80 mM H_2SO_4) and AC, indicating that the carbonaceous material undergoes oxidative degradation even in the absence of dissolved iron. Because activated carbon consists predominantly of elemental carbon, it can be oxidised by various oxidising agents under

acidic conditions to produce CO_2. The exact reaction depends on the oxidant and reaction conditions (15).

Conclusions

Fluidised bed bioreactors provide an efficient platform for iron oxidation by maintaining suspended biomass and support particles under continuous fluidisation, thereby enhancing mass transfer and microbial activity. The intensive mixing improves the transport of oxygen, nutrients, and dissolved iron species, while high biomass retention enables compact reactor designs with reduced hydraulic retention times.

Activated carbon is particularly well suited as the support material owing to its high specific surface area, well-developed pore structure, excellent mechanical stability, and resistance to attrition during prolonged fluidisation. In addition to providing a durable support for biofilm growth, activated carbon actively enhances iron oxidation by serving as a heterogeneous redox mediator. Its electrically conductive surface facilitates electron transfer between dissolved Fe^{2+} and molecular oxygen, thereby accelerating the oxidation of ferrous iron and enhancing the overall reaction kinetics.

Acknowledgements

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Adaptation and performance of the BRGM-KCC bioleaching consortium under extremely low pH conditions

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Abstract: pH control is a key operational parameter in bioleaching processes, as it directly influences microbial activity, mineral dissolution kinetics, and operating costs. Industrial bioleaching systems are commonly operated at pH values above 1.0 to preserve microbial performance; however, maintaining such conditions often requires continuous addition of alkaline reagents, leading to economic and environmental impacts. This study investigates the feasibility of operating bioleaching systems at very low pH using the specialized mesophilic to moderate thermophilic BRGM-KCC microbial consortium, composed mainly of *Leptospirillum ferriphilum, Acidithiobacillus caldus* and *Sulfobacillus* spp., during the treatment of a pyrite-rich material.

Batch experiments were conducted in 2 L reactors under controlled and uncontrolled pH conditions to assess microbial adaptation to low pH through monitoring of redox potential, mineral dissolution, and microbial community dynamics across successive generations. An adapted culture obtained after successive batch experiments and condition screening was then selected for continuous operation in a two-reactor cascade (total working volume 6 L). Two operating conditions were evaluated, with pH control of the primary reactor at either pH 1.2 or pH 1.0, while the secondary reactor remained unregulated.

Results demonstrate that the consortium maintained high redox potentials (> 820 mV vs. SHE) and sustained iron oxidation activity even when pH dropped below 0.8, in both batch and continuous modes. Although lower pH conditions slightly affected biomass levels, community structure, and induced longer lag phases, microbial oxidative activity remained high, indicating strong functional resilience. Continuous operation at very low pH (< 1.0) did not compromise system performance.

These findings highlight the robustness of the BRGM-KCC consortium under highly acidic conditions and demonstrate the potential to operate bioleaching processes at lower pH than conventionally applied. Such strategies could reduce alkaline reagent consumption and precipitation phenomena during the bioleaching step, contributing to more cost-effective and environmentally sustainable biohydrometallurgical operations.

Keywords: Bioleaching, low pH, mesophiles, pyrite oxidation, CSTR

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Cooperative Effects of Stress Tolerance Among Species of Acidophilic Bioleaching Bacteria

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Extremely acidophilic microorganisms coexist in bioleaching and other acidic environments where they are exposed to multiple stress factors. In this study, we investigated whether acidophilic bacteria grown under stress conditions produce extracellular compounds that enhance the stress tolerance of other acidophilic species. We obtained supernatants from cultures of *Sulfobacillus thermosulfidooxidans*, *Leptospirillum ferriphilum*, *Acidithiobacillus thiooxidans*, and *Acidithiobacillus ferrivorans* grown under control or stress conditions (heat, osmotic, oxidative, or cold stress). The supernatants from *S. thermosulfidooxidans* were used to supplement cultures of *L. ferriphilum,* and the supernatants of *L. ferriphillum, At. thiooxidans,* and *At. ferrivorans* were used separately to supplement (5-20% v/v) cultures of *At. ferrooxidans* exposed to stress conditions. Growth performance was evaluated by monitoring cell density, pH, and redox potential. Supernatants from *S. thermosulfidooxidans* significantly stimulated the growth of *L. ferriphilum* under both optimal and elevated temperatures, with supernatants derived from heat-stressed cultures providing the strongest protective effect under heat stress. Similarly, supernatants from *L. ferriphilum* improved the tolerance of *At. ferrooxidans* to osmotic and oxidative stress, with supernatants from non-stressed cultures showing a more pronounced effect. Supernatants from *At. thiooxidans* also enhanced the growth of *At. ferrooxidans* in a concentration-dependent manner. In addition, growth curves of *At. ferrooxidans* exposed to cold stress at 9 °C revealed that supplementation with supernatants from *At. ferrivorans* previously subjected to cold stress significantly improved growth compared to control cells. Analytical characterisation of selected supernatants by ESI-FT-ICR-MS revealed a limited number of dominant molecular signals, particularly in *L. ferriphilum* supernatants. Preliminary molecular formula assignments suggest a release of lipid-derived metabolites, including oleamide, erucamide, and sphinganine-like molecules. Overall, these results demonstrate that acidophilic bacteria release stress-induced extracellular metabolites that promote interspecies stress tolerance, highlighting an important mechanism of microbial interaction in extreme environments with potential relevance for bioleaching processes and the stability of industrial consortia.

Key words: acidophilic bacteria, bioleaching bacteria, stress tolerance, culture supernatants, extracellular metabolites

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Fluoride Toxicity and its Mitigation in Acidophilic Bioleaching Microorganisms

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Abstract:

Bioleaching using acidophilic bacteria is a promising approach for recovering metals from industrial residues. However, compared to bioleaching of ores industrial residues introduce several challenges for overall process. A significant problem is the presence of toxic compounds such as fluoride, which can inhibit bacterial activity and reduce bioleaching efficiency. Especially at low pH, fluoride predominantly occurs as HF, which is highly toxic for bacteria. This study systematically investigated the influence of fluoride on the metabolic activity of various acidophilic iron- and sulfur-oxidizing bioleaching bacteria, including *Acidithiobacillus* (*A.*) spp., *Leptospirillum spp.*, and *Sulfobacillus (S.) thermosulfidooxidans* in pure and mixed cultures. Fluoride inhibition was mitigated by lowering HF levels through complexation with aluminium or ferric iron.

Fluoride toxicity was found to be substrate-dependent. Among the tested strains, sulfur-grown *A. thiooxidans* exhibited the highest fluoride tolerance (0.5 mM F^-), while sulfur-grown *S. thermosulfidooxidans* was completely inhibited. In contrast, ferrous iron-grown cultures demonstrated higher fluoride tolerance, with *S. thermosulfidooxidans* even remaining active at fluoride concentrations of up to 1.5 mM. Mixed cultures exhibited enhanced fluoride tolerance during sulfur oxidation but reduced tolerance during iron oxidation. Studies on fluoride complexation revealed that iron or sulfur oxidation resumed at Fe^{3+}: F^- ratios of 7.5:1 or higher, whereas Al: F^- ratios between 1:1 and 2:1 were required.

Overall, the data show that fluoride inhibition during bioleaching is influenced by multiple factors, including mainly pH and ferric iron concentration. Early addition of aluminium is recommended to maintain bioleaching performance by preventing microbial inhibition. This study offers insights into the toxic effects of fluoride during bioleaching of fluoride-containing materials and highlights practical strategies to reduce microbial inhibition.

Keywords: fluoride inhibition, acidophilic bacteria, bioleaching

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Rhodanese-like sulfurtransferase in the heterodisulfide reductase gene cluster of *Acidithiobacillus ferrooxidans*

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Keywords: *Acidithiobacillus ferrooxidans*; rhodanese; sulfurtransferase; sulfane sulfur; heterodisulfide reductase

Abstract

Rhodaneses are widespread sulfurtransferases implicated not only in cyanide detoxification, but also in intracellular sulfur trafficking, iron–sulfur cluster maintenance, and redox processes. Acidophilic bacteria of the genus *Acidithiobacillus drive* metal sulfide dissolution by coupling iron and reduced sulfur compound oxidation, yet cytoplasmic sulfur-transfer steps in these pathways remain incompletely understood. In *At. ferrooxidans*, a rhodanese-like protein (Rhd11) is encoded in the conserved *hdrABC–dsrE–tusA–rhd11* gene cluster associated with sulfur-oxidizing heterodisulfide reductase-like systems proposed to function in dissimilatory sulfur oxidation. Here, we established heterologous production and purification of Rhd11 and performed initial activity profiling. Three recombinant variants were expressed in *Escherichia coli* using either a C-terminal or N-terminal polyhistidine tag or an N-terminal self-cleaving affinity tag. Rhd11 displayed clear sulfurtransferase activity with thiosulfate, whereas no activity was detected with a common low-molecular-weight thiol (2-mercaptoethanol). Notably, Rhd11 also exhibited weak sulfurtransferase activity with glutathione persulfide/polysulfide (GSS_nH), suggesting a possible role in a sulfur relay involving TusA and DsrE that may deliver sulfane sulfur to the sulfur-oxidizing heterodisulfide reductase-like system. Together, these results provide a biochemical basis for investigating the role of Rhd11 in sulfur trafficking during sulfur oxidation in *At. ferrooxidans*.

Introduction

Rhodaneses (thiosulfate:cyanide sulfurtransferases) form a ubiquitous enzyme superfamily whose members participate in cyanide detoxification, intracellular sulfur trafficking, iron–sulfur cluster maintenance, and redox homeostasis [1]. Rhodanese activity was among the first enzymatic activities of sulfur metabolism reported in an acidophilic iron and sulfur oxidiser, detected in extracts of *Ferrobacillus ferrooxidans* (now *Acidithiobacillus ferrooxidans*) over five decades ago [2]. Subsequent genome analyses showed that *At. ferrooxidans* encodes multiple rhodanese-domain proteins, several of which were tentatively linked to sulfur oxidation based on their genomic context and expression [3].

Acidithiobacillus spp. dissolve metal sulfides by coupling the oxidation of ferrous iron and reduced inorganic sulfur compounds, a process central to biomining [4]. Sulfur oxidation in these bacteria proceeds in part through a cytoplasmic heterodisulfide reductase-like (Hdr) system [5], which across diverse sulfur oxidisers receives sulfane sulfur from a DsrE–TusA–rhodanese relay [6]. The individual sulfur-transfer steps feeding this system, however, remain incompletely resolved. In *At. ferrooxidans*, the rhodanese-like protein Rhd11 is encoded within the conserved *hdrABC–dsrE–tusA–rhd11* cluster, suggesting a role in this relay. To test this hypothesis, we established heterologous production and purification of Rhd11 and performed an initial characterisation of its sulfurtransferase activity.

Methods

Bacterial strain and plasmid construction. The *rhd11* gene (DN052_09145), encoding the rhodanese-like protein Rhd11, was amplified from the genomic DNA of *At. ferrooxidans* CCM 4253 using Q5 High-Fidelity DNA Polymerase (New England Biolabs, NEB). Three expression constructs were

generated using NEBuilder HiFi DNA Assembly (NEB): Rhd11 fused to a C-terminal octahistidine tag in pET-21a (Rhd11-His_8), Rhd11 fused to an N-terminal heptahistidine tag followed by a TEV protease cleavage site in pET-28a (Rhd11-His_7), and Rhd11 fused to an N-terminal self-cleaving affinity tag in pET-28a (Rhd11-PS; Cytiva Protein Select). The assembled plasmids were propagated in *Escherichia coli* DH5α, verified by Sanger sequencing (Eurofins Genomics), and subsequently transformed into *E. coli* BL21(DE3) for recombinant protein production.

Recombinant protein production. Cultures were grown overnight at 30 °C in 200 mL ZYM-5052 autoinduction medium [7] supplemented with either 100 µg mL^{-1} ampicillin or 50 µg mL^{-1} kanamycin. Cells were harvested by centrifugation and stored at −70 °C until further use.

Protein purification. Cell pellets were resuspended in the appropriate binding buffer, disrupted by sonication, and clarified by centrifugation at 14,000 × g for 15 min at 4 °C. Rhd11-His_8 and Rhd11-His_7 were purified by Ni^{2+}-immobilised metal affinity chromatography using an ÄKTA Pure 25M system (Cytiva). The N-terminal His tag of Rhd11-His_7 was removed by digestion with TEV protease (NEB), and the cleaved tag and protease were removed by a second Ni^{2+} affinity step, yielding tag-free Rhd11. Rhd11-PS was captured on Cytiva Protein Select resin, and the tag-free protein was released by on-column self-cleavage overnight at 4 °C. Protein concentrations were determined fluorometrically using a Qubit 4 fluorometer (Thermo Fisher Scientific).

Sulfurtransferase activity assay. Sulfurtransferase activity was determined colorimetrically in a microplate format by the cyanolysis assay [8], with minor modifications. Reaction mixtures (250 µL) contained 100 mM HEPES (pH 8.0), 20 mM sodium thiosulfate, and purified enzyme. After a short preincubation at 30 °C, reactions were initiated by adding 20 mM sodium cyanide, allowed to proceed for 10 min, and stopped with formaldehyde. The thiocyanate formed was then detected by reaction with ferric nitrate in nitric acid, and the resulting ferric thiocyanate complex was quantified at 450 nm. To assess sulfur-donor specificity, sodium thiosulfate was replaced with 10 mM 2-mercaptoethanol or 10 mM glutathione persulfide/polysulfides (GSS_nH). One unit corresponds to 1 µmol thiocyanate formed per minute.

Results and Discussion

All four purified Rhd11 preparations transferred sulfur from thiosulfate to cyanide (Table 1), confirming that Rhd11 is an active rhodanese-type sulfurtransferase. None of them showed activity with the low-molecular-weight thiol 2-mercaptoethanol. Rhd11 thus does not appear to use a simple thiol as a sulfur donor, consistent with a restricted substrate specificity rather than broad substrate promiscuity.

Table 1. Specific sulfurtransferase activity (U mg^{-1}) of freshly purified Rhd11 preparations measured with three sulfur donors.

Preparation	Tag / cleavage	Thiosulfate	2-ME	GSS_nH
Rhd11-His_8	C-terminal His_8	0.26 ± 0.09	n.d.	n.d.
Rhd11-His_7	N-terminal His_7	0.77 ± 0.18	n.d.	0.18 ± 0.01
Rhd11 (His_7 cleaved)	Tag-free (TEV)	0.79 ± 0.05	n.d.	0.20 ± 0.02
Rhd11 (PS-cleaved)	Tag-free (Protein Select)	5.87 ± 0.17	n.d.	0.32 ± 0.02

Values are mean ± SD. 1 U = 1 µmol thiocyanate formed per minute. n.d., not detected (not distinguishable from the assay background). 2-ME, 2-mercaptoethanol; GSS_nH, glutathione persulfide/polysulfide.

Rhd11 also transferred sulfane sulfur from GSS_nH, at roughly 5–25% of the thiosulfate rate depending on the preparation (Table 1). Thiosulfate is a standard donor in this assay and is itself an intermediate of sulfur oxidation in *Acidithiobacillus*, whereas GSS_nH represents the persulfidated low-molecular-weight thiol pool that carries sulfane sulfur in the cytoplasm. Activity with a persulfidic donor, together with the absence of activity with a simple thiol, is consistent with a role in cytoplasmic sulfane-sulfur trafficking rather than in cyanide detoxification alone [1]. The genomic context points in the same direction. Within the conserved *hdrABC–dsrE–tusA–rhd11* cluster, Rhd11 is likely part of the DsrE–TusA–rhodanese relay that delivers sulfane sulfur to the heterodisulfide reductase-like system during dissimilatory sulfur oxidation in *Acidithiobacillus* [5,6].

Specific activity depended strongly on the affinity tag and its removal. The C-terminally His-tagged form was the least active (0.26 U mg^{-1}), whereas TEV removal of the N-terminal His_7 tag left activity essentially unchanged relative to its tagged precursor (0.79 vs 0.77 U mg^{-1}), indicating that the N-terminal tag does not measurably impair enzyme activity. The tag-free protein released by the self-cleaving Protein Select system was about sevenfold more active (5.87 U mg^{-1}) than any His-tagged preparation. The two tag-free proteins differ only at the N-terminus, where the TEV-cleaved form carries an additional serine and the Protein Select-cleaved form lacks the initiator methionine. Such minor N-terminal differences are unlikely to account for a sevenfold change in catalytic activity, which more probably reflects preparation-dependent factors, such as purification conditions or protein quantitation. This remains to be confirmed with independent batches. For downstream work, the tag-free enzyme is clearly preferable.

Activity was highest in freshly purified enzyme. Storage overnight at 4 °C and freezing at –20 °C for one week reduced thiosulfate activity, with the largest decrease observed for the Protein Select-derived preparation (2.3 and 1.2 U mg^{-1}, respectively). In contrast, activity toward GSS_nH was better retained. The enzyme was therefore assayed fresh whenever possible.

Although rhodanese activity has long been known in this organism [2] and several sulfurtransferase genes have been predicted from its genome [3], Rhd11 is, to our knowledge, the first rhodanese-family protein encoded within the conserved *hdrABC–dsrE–tusA* sulfur-oxidation gene cluster to be characterised biochemically.

Conclusions

Rhd11 from the *hdrABC–dsrE–tusA–rhd11* cluster of *At. ferrooxidans* was produced heterologously, purified in His-tagged and tag-free forms, and shown to be an active rhodanese-type sulfurtransferase. It uses thiosulfate and the persulfidic donor GSS_nH, but not the thiol 2-mercaptoethanol, a substrate profile consistent with participation in a cytoplasmic sulfane-sulfur relay that feeds the sulfur-oxidizing heterodisulfide reductase-like system rather than functioning solely in cyanide detoxification. The tag-free enzyme provided the most active preparation and is therefore the preferred form for further study. These results provide a biochemical foundation for testing direct sulfur transfer between Rhd11 and its proposed partners TusA and DsrE, and for defining its role in dissimilatory sulfur oxidation in *Acidithiobacillus spp.*

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Reconstruction of an extremophile electron transfer pathway in *Escherichia coli*: Functional validation of the *Acidithiobacillus ferrooxidans* iron reduction pathway

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Abstract: This study provides the first experimental validation of the extracellular iron reduction pathway of *Acidithiobacillus ferrooxidans*, an extreme acidophile capable of anaerobic Fe(III) reduction. The proposed electron transfer cascade – comprising the inner membrane cytochrome CycA2, periplasmic cytochromes Cyc1A and Cyc2A, and the copper-containing redox protein rusticyanin (Rus) – was reconstructed in four heterologous hosts: *Escherichia coli* C43(DE3), BL21(DE3), T7 Express, and *Vibrio natriegens* Vmax X2. Expression analysis revealed that only *E. coli* C43(DE3) and Vmax X2 produced all four redox proteins in a redox-active state and with correct subcellular localisation. Notably, C43(DE3) exhibited robust expression of functional CycA2, Cyc1A, Cyc2A, and Rus, whereas BL21(DE3) yielded minimal holo-Cyc2A, and T7 Express failed to produce holo-CycA2. Functional assessment demonstrated a 2-fold increase in Fe(III)-citrate reduction in C43(DE3) harboring the full pathway compared to the wild-type control. The recombinant pathway in C43(DE3) also accepted a graphite felt electrode as terminal electron acceptor and enabled a 5-fold increase in current generation in a bioelectrochemical H-cell, confirming active extracellular electron transfer (EET) under turnover conditions. Further experiments confirmed that the inner membrane cytochrome CycA2 is essential for efficient Fe(III) reduction in engineered *E. coli* strains. Despite successful protein expression, Vmax X2 failed to support Fe(III) reduction, suggesting host-specific limitations in redox coupling or electron channeling. These results constitute the first direct experimental evidence for the composition of the *At. ferrooxidans* iron reduction pathway and demonstrate that *E. coli* C43(DE3) is capable of producing functional, redox-active electron transfer proteins from an extreme acidophile. This highlights C43(DE3) as a highly competent chassis for the synthetic biology of extremophile-derived EET systems, with significant implications for advancing characterisation studies of proteins originating from acidophiles by taking advantage of *E. coli*'s fast growth rate and high biomass production.

Keywords: extracellular electron transfer, *Acidithiobacillus ferrooxidans*, iron reduction, redox proteins, *Escherichia coli*

Exploring Acidophilic Microbial Communities in a Polymetallic Sulfide-Rich Magmatic Ore Deposit in Russia: A Novel *Acidithiobacillus Species*

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Keywords: acidophilic microbial communities; sulfur- and iron-oxidizing acidophiles; sulfidic ore deposit; *Acidithiobacillus*; biomining

Abstract

In this study, we investigated an acidophilic microbial community associated with a magmatic Cu–Ni–platinum-group-element-bearing sulfide ore deposit in Siberia, Russia. The indigenous community was dominated by several *Acidithiobacillus species*, followed by *Ferroplasma*, *Oxyplasma*, a potentially novel genus of the family *Thermoplasmataceae*, and two potentially new *Alicyclobacillus species*. Analysis of the *Acidithiobacillus fraction* revealed a novel, extremely acidophilic species, *At. sibiricus*, with pronounced sulfur-oxidizing capacity. It dominated the sulfide ore community and was detected in diverse environments across six continents, indicating broad ecological and geographic distribution. Whole-genome analysis of *At. sibiricus* revealed complete sulfur oxidation pathways and a conjugative plasmid potentially involved in biofilm formation and metal/redox responses. The chromosome encoded multiple metal(loid)-resistance systems and a complete glutathione redox cycle, indicating adaptation to oxidative and metal stress. These findings identify the Siberian ore deposit as a source of novel acidophiles with biotechnological potential, including *At. sibiricus*, a promising sulfur oxidiser for sustainable biomining.

Introduction

With the growing importance of biomining for the recovery of critical and precious metals, efficient acid- and metal-resistant sulfur- and iron-oxidizing microorganisms remain a global research priority. These bacteria and archaea inhabit natural and human-impacted environments, including sulfide ore deposits, hot springs, solfataric fields, mines, and acid mine drainage, and are central to the extraction of non-ferrous metals from sulfide ores and related materials. Common bioleaching taxa are *Acidithiobacillus (At.)*, *Leptospirillum (L.)*, *Sulfobacillus (S.)*, *Acidimicrobium (Am.)*, *Thermoplasmatales*, *Acidihalobacter (Ah.)*, *Alicyclobacillus (Al.)*, and some others [1].

Microbial communities in sulfide ore deposits and mines have been characterised at numerous sites. At Richmond Mine (USA), communities were relatively simple and dominated by *Leptospirillum and Ferroplasma*, with *Sulfobacillus enriched* in warmer zones [2]. At the cold Cae Coch pyrite mine (Wales), *At. ferrivorans*, *L. ferrooxidans*, "*Ferrovum myxofaciens*," and *Gallionella*-related iron oxidisers occupied distinct microhabitats [3]. In biofilms from the Drei Kronen und Ehrt pyrite mine (Germany), *Leptospirillum and Acidithiobacillus dominated* oxygenated regions, whereas *Thermoplasmatales* occurred under oxygen limitation [4]. Broader diversity has been reported in Pyhäsalmi mine waters (Finland) [5], Reiche Zeche biofilms and pools (Germany) [6], and Mynydd Parys mineral samples (Wales) [7], including poorly characterised taxa. The deep Iberian Pyrite Belt hosts diverse subsurface microorganisms linked to carbon, hydrogen, nitrogen, sulfur, and iron cycling [8]. Expanding biodiversity surveys of such environments is important for understanding ecological interactions and discovering novel taxa and metabolic traits relevant to metal recovery. This study aimed to characterise the microbial community associated with a magmatic polymetallic sulfide ore from northern Siberia, Russia, and to characterise its dominant sulfur oxidiser.

Methods

Materials. A microbial community was recovered from a northern Siberian sulfide ore containing (%): Fe, 35.8; S, 21.6; Ni, 2.46; Cu, 2.68; Co, 0.11; SiO_2, 10.8; Al_2O_3, 3.42; CaO, 3.11; and MgO, 1.68. The mineral composition was as follows (%): pyrrhotite, 29; chalcopyrite, 8; pentlandite, 7; goethite, 13; aluminosilicate mineral group, 23; quartz, 5; bassanite, 5; anhydrite, 5; elemental sulfur, 5. Cells were washed from the ore with the salt base of 9K medium (pH 2.5) [9]. Strain A1 was isolated by serial dilution in iron-free 9K medium containing elemental sulfur.

Analysis. Community composition was assessed by 16S rRNA V3–V4 metabarcoding, which in some cases enabled reliable species-level assignment of acidophiles [9]. Strain A1 growth was tested at different temperatures and initial pH values, as well as on sulfur compounds, ferrous iron, and organic substrates. Further characterisation included microscopy, phylogenetic and chemotaxonomic analyses, genome sequencing, and comparative genomics [10]. To assess the environmental occurrence, the 16S rRNA gene sequence of strain A1 was queried against public nucleotide databases using BLAST, and public shotgun metagenomes were screened with Branchwater (https://branchwater.jgi.doe.gov/).

Results and Discussion

A sulfur-oxidizing strain A1 was isolated from polymetallic ore collected from a magmatic sulfide deposit in Siberia. Based on phylogenetic, chemotaxonomic, and genomic analyses, it represents a novel species of the genus *Acidithiobacillus*, for which the name *Acidithiobacillus sibiricus* sp. nov. was proposed, with A1^T as the type strain. At the time of the study, the genus comprised 11 validly described species: *At. thiooxidans*, *At. concretivorus*, *At. ferrooxidans*, *At. albertensis*, *At. caldus*, *At. ferrivorans*, *At. ferridurans*, *At. ferriphilus*, *At. sulfuriphilus*, *At. ferrianus*, and *At. acidisediminis* [1]. *At. sibiricus* clustered with its closest relative, *At. acidisediminis*, forming a distinct, well-supported lineage separated from all other *Acidithiobacillus species*.

Analysis of the ore-associated community showed that *Acidithiobacillus dominated* (~80%), with *At. sibiricus*-related sequences comprising ~33%. Other abundant taxa included *At. ferrooxidans*, *At. ferrianus*, *At. ferriphilus*, *Ferroplasma sp.*, *Oxyplasma meridianum*, and a potentially novel genus within the *Thermoplasmataceae*, while two potentially novel *Alicyclobacillus species* occurred at low abundance (Fig. 1). The community combined sulfur and iron oxidisers with the organotroph *O. meridianum*, an aerobic hyperacidophile from Vulcano Island, Italy, that utilises complex organic substrates [11]. On the same island, the hydrothermal soil community was dominated by sulfur-cycling bacteria, particularly *Sulfurimonas and Acidihalobacter* [12].

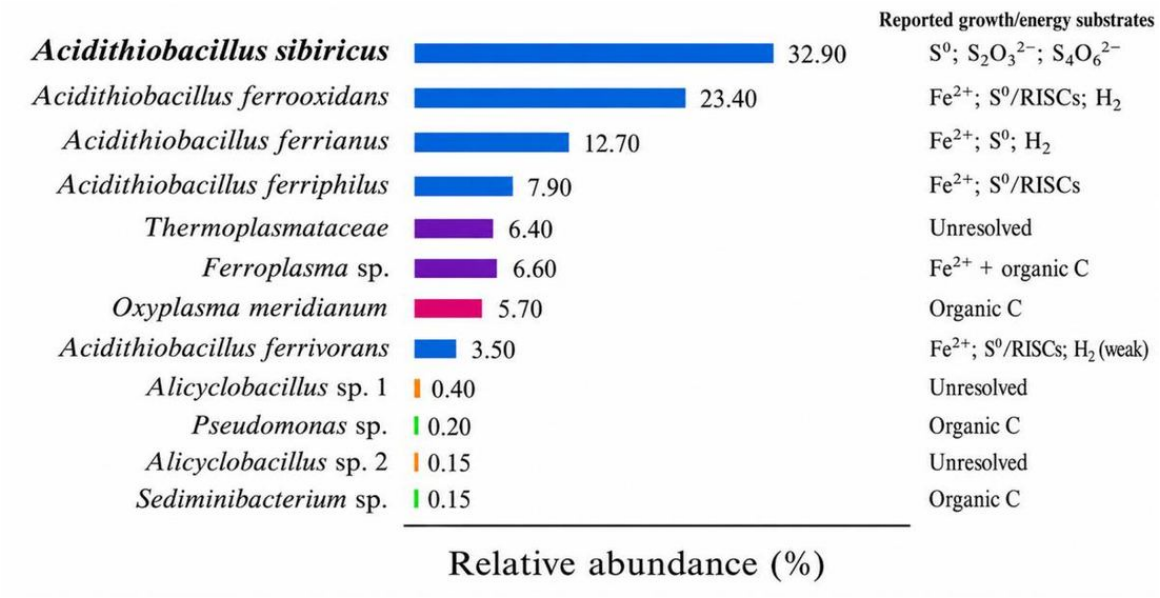


Figure 1. Relative abundance of microbial taxa in the original sulfide-ore community and their reported growth and energy substrates. RISCs, reduced inorganic sulfur compounds; C, carbon. Unresolved, energy substrates could not be assigned reliably at the available taxonomic resolution.

Strain A1 was an extremely acidophilic, halotolerant sulfur oxidiser that grew at 20–43 °C, showed its highest μ_{max} of 0.040 h^{-1} at 35 °C, and lowered the culture pH to 0.38–0.72 depending on temperature (Fig. 2). It oxidised S^0, thiosulfate, and tetrathionate, but not Fe^{2+}, and tolerated up to 7%(w/v) NaCl. Strain A1 cells were rod-shaped and formed large cell aggregates on elemental sulfur particles during growth. Among other features, genome analysis identified complete SOX and S4I sulfur-oxidation pathways, carbon-fixation and carboxysome genes, metal-resistance systems, and a conjugative plasmid potentially involved in biofilm formation and stress adaptation. The chromosome of *At. sibiricus* encoded comprehensive oxidative-stress defenses, including the complete glutathione redox cycle absent from its closest relatives, *At. acidisediminis* and *At. caldus*.

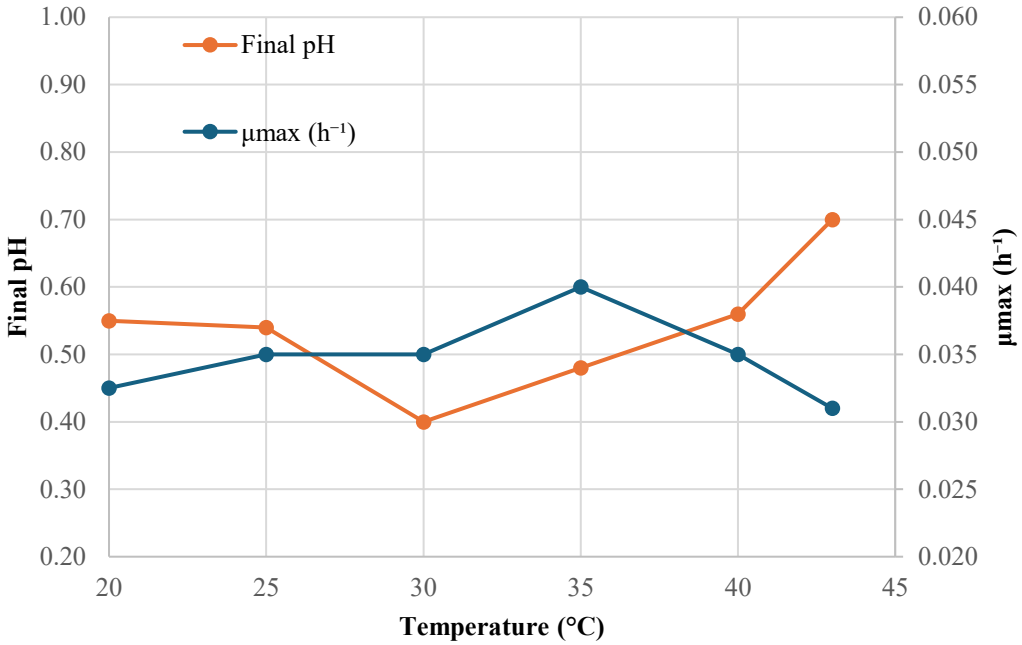


Figure 2. Effect of temperature on the maximum specific growth rate (μ_{max}) and final culture pH of *Acidithiobacillus sibiricus* A1^T during growth on elemental sulfur.

Genome comparisons revealed a close relationship between *At. sibiricus* A1 and the Copahue isolates, which shared the same 16S rRNA gene oligotype as the salt-tolerant Vulcano isolate *Acidithiobacillus sp.* V1 [13,14]. Closely related *At. sibiricus* sequences were also detected in heap-leaching and bioreactor systems, mine waters and effluents, contaminated soils, thermal vents, hot springs, sulfur-rich waters, and other environments. Positive metagenomes were distributed across North America, Europe, Asia, Africa, Oceania, and Antarctica, indicating a broad intercontinental occurrence of the species.

Conclusions

Taken together, these results expand current knowledge of acidophile ecology in magmatic sulfide ores and identify the Siberian deposit as a source of previously uncharacterised acidophilic microorganisms with biotechnological potential. Among them, the sulfur-oxidizing and halotolerant *At. sibiricus* is a promising candidate for biomining under both acidic freshwater and saline conditions, with potential value for developing more sustainable metal-recovery biotechnologies.

Acknowledgements

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Enhanced chalcopyrite heap bioleaching using a UCT–MiCCuR mixed microbial consortium

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Keywords: chalcopyrite; heap bioleaching; microbial consortium; redox control; salinity

Abstract

Chalcopyrite heap bioleaching is constrained by slow kinetics and by the difficulty of maintaining an electrochemical environment that promotes dissolution without accelerating passivation. A designed mesophilic–thermophilic UCT–MiCCuR consortium, comprising strong and weak iron oxidisers together with sulfur-oxidising microorganisms, was evaluated in 4 kg heap-simulating columns operated at 40 °C or subjected to temperature ramping to 65 °C, with and without 150–300 mM NaCl. The consortium combined *Leptospirillum ferriphilum* and *Metallosphaera hakonensis* from the UCT culture (University of Cape Town) with *Sulfobacillus thermosulfidooxidans*, *Sulfobacillus acidophilus* and *Acidithiobacillus caldus* from MiCCuR project (Microbial Consortia for enhanced Copper Recovery), thereby providing microbial functions across mesophilic, moderately thermophilic and thermophilic temperature ranges.

NaCl additions temporarily decreased redox potential and disturbed iron cycling, while temperature ramping produced the strongest improvement in copper extraction. At the end of the experiment, the inoculated sterilised-ore columns achieved total copper recoveries of 48.41 % at fixed 40 °C, 57.81 % after temperature ramping without NaCl, and 50.34 % after temperature ramping with NaCl. The corresponding uninoculated columns achieved 12.86 %, 38.51 % and 37.53 %. The results demonstrate that the mixed-temperature consortium enhanced copper recovery under fixed-temperature and combined salt–thermal conditions.

Introduction

Heap bioleaching is an attractive route for recovering copper from low-grade ores because it has lower energy and capital requirements than conventional concentration and smelting. Its application to chalcopyrite, however, remains limited by slow dissolution, surface passivation and the difficulty of controlling ferric/ferrous iron cycling in an unsaturated and spatially heterogeneous ore bed (Ghorbani et al., 2016). Chalcopyrite does not simply dissolve faster as oxidation potential increases; maximum rates are often associated with an intermediate ferric/ferrous ratio, while strongly oxidising conditions favour pyrite oxidation, with passivation and reduced copper release (Petersen & Dixon, 2006, Kotsiopoulos et al., 2010).

Microbial community design offers a route to biological redox management. Strong iron oxidisers rapidly regenerate Fe^{3+}, scavenging Fe^{2+} to very low concentration and driving the bulk potential above the preferred operating window for $CuFeS_2$ leaching. Weak iron oxidisers sustain a lower ferric flux as Fe^{2+} concentration reduces, and sulfur oxidisers limit the accumulation of reduced sulfur intermediates. The UCT–MiCCuR consortium was assembled to combine these functions across the mesophilic to thermophilic range. Such complementarity is relevant to industrial heaps, where communities must respond to changing temperature, salinity and substrate availability (Christel et al., 2018, Ríos et al., 2024).

This study evaluated the UCT–MiCCuR consortium in heap-simulating columns under fixed temperature without NaCl, temperature ramping without NaCl, and temperature ramping with NaCl, each against a matched uninoculated column. The objective was to determine whether the consortium

could establish effective iron cycling, whether chloride perturbation reduced impact of strong Fe-oxidisers and, together, sustained copper recovery across heap-relevant thermal gradients.

Materials and Methods

Materials and column operation. Low-grade chalcopyrite ore from Spence Mine, Chile, contained 0.68 wt.% Cu, 1.66 wt.% chalcopyrite, 4.69 wt.% pyrite and 92.92 wt.% gangue. The coarse ore was acid-agglomerated using 2 kg H_2SO_4 t^{-1} at approximately 5.5% moisture. Selected portions were sterilised by 50 kGy γ-irradiation; as-received ore was retained in parallel treatments. Cylindrical PVC columns (100 mm internal diameter, 360 mm height) were packed with approximately 4 kg ore and irrigated at 5 L m^{-2} h^{-1} with acidic solution at pH 1.7.

The UCT–MiCCuR inoculum was prepared by combining the UCT and MiCCuR cultures in equal proportions on a cell-number basis. The UCT fraction constituted 50% of the final inoculum and contained equal proportions of the mesophilic culture dominated by *Leptospirillum ferriphilum* and the thermophilic culture dominated by *Metallosphaera hakonensis*. The MiCCuR fraction constituted the remaining 50% and contained equal proportions of *Sulfobacillus thermosulfidooxidans*, *Sulfobacillus acidophilus* and *Acidithiobacillus caldus*. The inoculum was applied at a total loading of 10^{10} cells per tonne of ore.

Five UCT–MiCCuR columns were operated at 40 °C or ramped from 40 to 48 °C between Days 105 and 113, held for one week, and then increased to 65 °C from Day 120. NaCl-treated columns received 150 mM pulses on Days 22, 27 and 42, 300 mM additions on Days 46, 52 and 53, and continuous 300 mM NaCl from Day 61, as summarised in Figure 1. Matched uninoculated columns were operated as controls.

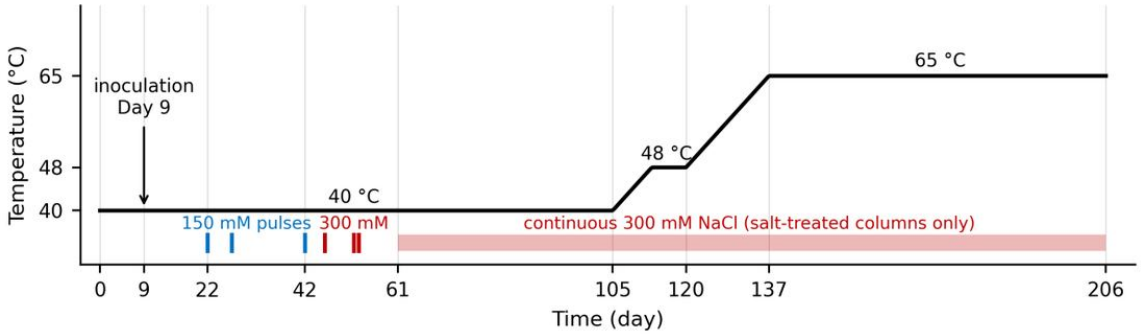


Figure 1. Operating programme, showing the temperature profile and the chloride dosing schedule. Chloride was applied only to the salt-treated columns; matched salt-free columns ran in parallel.

Table 1. Operating conditions of columns

Column	1	2	3	4	5	7	11	12	17	18
Inoculum	None					10^{10} Cells/ton ore UCT – MiCCuR mixed consortium				
Temperature (°C)	fixed 40°C	fixed 40**°C**	ramp to 65	fixed 40**°C**	ramp to 65	fixed 40**°C**	fixed 40**°C**	ramp to 65	ramp to 65	ramp to 65
NaCl (mM)	no	150, 300	150, 300	no	no	no	150, 300	150, 300	no	no
Ore	sterilized	as received	sterilized	as received	sterilized	sterilized	as received	sterilized	as received	sterilized

Analysis

Effluent pH and redox potential were measured using an Ag/AgCl reference electrode. Ferric and total dissolved iron were determined spectrophotometrically, with ferrous iron obtained by difference. Copper concentration in the effluent was measured using bicinchoninic acid (BCA)-based colorimetric assay and cumulative recovery calculated from the copper head grade and dry ore mass. Effluent cell concentrations were used as an indicator of planktonic biomass, while the coupled trends in redox potential, iron speciation and copper extraction were used to assess iron-cycling activity.

Copper recovery was expressed as a percentage of the copper contained in the ore charge and is counted from Day 9, when irrigation with ferrous-containing raffinate and inoculation commenced. Copper released during the preceding acid-wash period was acid-soluble copper, not attributable to bioleaching, and was excluded.

Results and Discussion

Start-up and early copper recovery

After inoculation the UCT–MiCCuR columns showed increasing planktonic cell concentrations, ferric iron accumulation and progressive Fe^{2+} depletion, confirming establishment of active microbial iron oxidation. Copper extraction was greatest within a bulk redox range of approximately 420–550 mV versus Ag/AgCl, and this range differed between columns. By Day 22 the three sterilised-ore UCT–MiCCuR columns had recovered on average 6.79 % of the contained copper, against 2.89 % for their matched uninoculated columns. The steeper cumulative-recovery slopes after inoculation show that the consortium accelerated the transition from acid washing of readily soluble copper phases to biologically supported chalcopyrite dissolution, and support the concept that performance depends on ferric flux and mineral–biofilm interactions rather than on the bulk redox value alone (Kotsiopoulos et al., 2010, Christel et al., 2018).

Effect of NaCl on the process

Individual NaCl additions caused immediate redox decreases of up to ~80–100 mV, accompanied by short-lived increases in dissolved ferric iron, total iron and copper one to two days later. The inverse relationship between lower redox and higher measured ferric iron is consistent with simultaneous biological and chemical effects. Chloride temporarily inhibits Fe^{2+} oxidation, while increased ionic strength and Fe-Cl complexation mobilise iron retained on mineral surfaces, secondary precipitates or extracellular polymeric material (Bevilaqua et al., 2013). The smaller response to the third 150 mM pulse suggested partial adaptation. Continuous 300 mM NaCl nevertheless produced a flatter cumulative-copper slope. Between Days 22 and 105 the salt-treated sterilised column (C12) recovered 13.38 % of the contained copper, whereas the two salt-free sterilised columns (C7, C18) recovered 17.81 and 20.49 %. Chloride therefore shifted the bulk redox environment but did not, by itself, improve copper extraction.

Temperature response and final recovery

Temperature ramping produced the largest improvement in cumulative extraction. For matched sterilised-ore treatments, the UCT–MiCCuR consortium achieved 48.4 % total recovery at fixed 40 °C without NaCl (C7), 57.8 % when ramped to 65 °C without NaCl (C18), and 50.3 % when ramped with NaCl (C12). The corresponding uninoculated columns recovered 12.9 %, 38.5 % and 37.5 % respectively (Figure 2). The highest recovery was therefore obtained in the ramped, salt-free column. Relative to the fixed-temperature inoculated treatment, ramping increased recovery by 9.4 percentage points. Under combined chloride and thermal stress, the consortium still exceeded its matched control by 12.8 percentage points and the fixed temperature inoculated column by 27.2 percentage points, indicating that the community retained substantial functionality despite chloride inhibition, particularly of *Leptospirillum*.

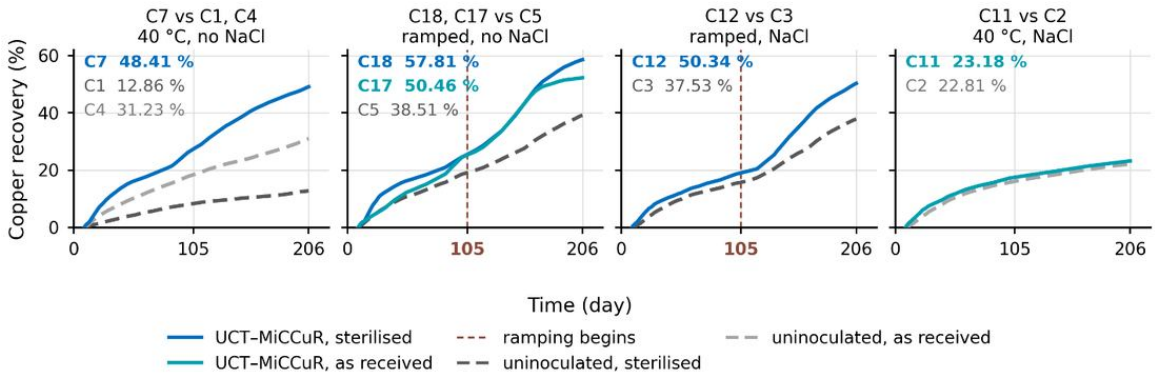


Figure 2. Cumulative copper recovery, expressed as a percentage of the copper contained in the ore charge and counted from Day 9. Each panel groups one operating condition, with the inoculated columns drawn solid and their uninoculated columns dashed, sterilised ore in dark shades and as-received ore in light shades. Final recoveries are listed in each panel and the dashed vertical line marks the onset of temperature ramping. C11 and C17 were reinoculated on Day 29 after weak establishment.

In the salt-free ramped columns, redox potential rose to 703 and 706 mV by Day 147, accompanied by copper extraction of 135 to 144 mg per day. During prolonged operation at 65 °C both potential and daily extraction declined, reaching 421 and 425 mV with extraction of 19 to 51 mg per day over the final twenty-five days, indicating loss of stable iron-oxidation activity as the system exceeded the sustained tolerance of key community members. The salted ramped column behaved differently and never exceeded 548 mV, demonstrating the role of NaCl in inhibiting strong iron oxidisers. However, its Cu extraction did not exceed the unsalted column. This is consistent with a consortium containing organisms of different kinetic capability and temperature optima. Ramping can activate thermophilic functions and accelerate mineral dissolution, but prolonged exposure at the upper limit may remove mesophilic iron oxidisers before a stable thermophilic iron-cycling community is established.

Overall, the UCT–MiCCuR consortium broadened the operating envelope of the column system. At a fixed 40 °C it provided a large inoculation advantage, while temperature ramping raised the maximum recovery and partially offset the inhibitory effect of NaCl.

The benefit of inoculation depended strongly on ore treatment. On sterilised ore the inoculated consortium exceeded its matched uninoculated column by 12.8 to 35.6 percentage points across all three temperature and chloride combinations. On as-received ore at 40 °C with chloride the inoculated column recovered 23.18 % against 22.81 % for its control. Under ramping without NaCl, however, the as-received inoculated column C17 reached 50.46 %, within 7.4 percentage points of the inoculated column of sterilised ore C18 at 57.81 % and well above their shared uninoculated column C5 at 38.51 %. The two uninoculated columns at 40 °C without NaCl differed by 18.4 percentage points on ore treatment alone, 12.86 % for the sterilised C1 against 31.23 % for the as-received C4. Inoculated performance was therefore reproducible across ore treatments, while uninoculated performance depended on the degree of endemic species on as-received ore compared with incidental colonisation occurring in controlled columns as these were not operated under abiotic conditions.

Bulk redox potential did not order the columns by performance. The column sustaining the lowest mean potential before ramping, 468 mV, also gave the lowest recovery at 23.18 %, while the highest recovery of 57.81 % occurred in a column averaging 578 mV. Sustained dissolution required active ferric and ferrous turnover, sulfur oxidation and microbial adaptation across the ore bed rather than a particular bulk potential. The uninoculated columns also developed measurable biological activity during the long experiment, through incidental contamination and, in the as-received ore, indigenous microorganisms, so they represent low-inoculum systems rather than abiotic references.

Operational implications

These comparisons indicated that redox control should be a dynamic operating strategy rather than a single target potential. Short NaCl pulses perturbed excessive oxidation and the diminishing response suggested acclimation, but continuous 300 mM exposure imposed a persistent kinetic penalty before heating. Temperature ramping increased copper release, yet extended exposure at 65 °C destabilised the

community, so a practical heap strategy would use staged heating with chloride limited to the minimum needed to moderate strong iron oxidation. Further, the nature of the microorganisms thriving at 65 °C and in the presence of salt needs to be assessed. Redox potential should be interpreted together with iron speciation and copper flux, to enable both a stable bulk potential and oxidant flux at the mineral surface to be sustained.

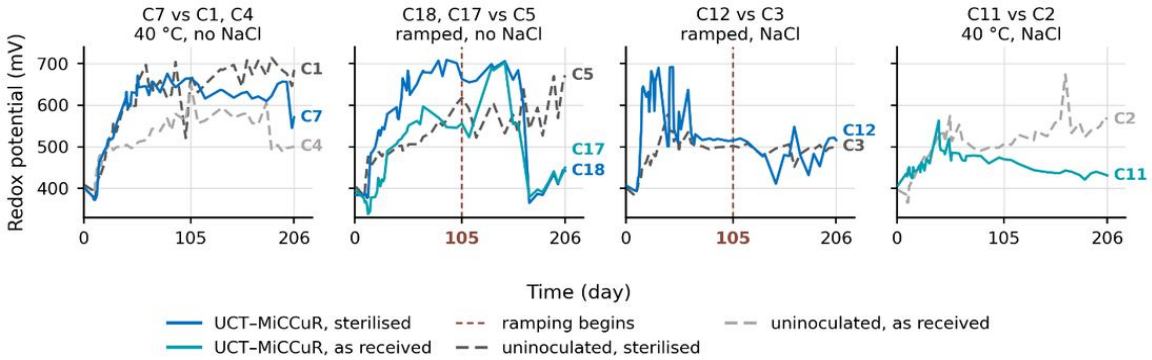


Figure 3. Redox potential of the column effluent versus Ag/AgCl, for the same column groups; conventions as in Figure 2.

Experimental limitations

The long-duration column matrix prioritised breadth of operating conditions over replication, and the microbial composition of the attached and planktonic populations was not quantified during the salt and thermal transitions. The recovery differences therefore demonstrate treatment performance under the tested conditions but do not resolve species-specific mechanisms. Replicated columns and molecular community tracking are needed to fully explain the loss of activity at 65 °C.

Conclusions

The UCT–MiCCuR consortium enhanced chalcopyrite dissolution under fixed-temperature, temperature-ramped and combined salt–thermal conditions. On sterilised ore the inoculated columns showed an early onset of Cu recovery and the highest final recovery, 57.81 %, was achieved by temperature ramping without NaCl. NaCl pulses temporarily moderated redox potential, but continuous 300 mM NaCl reduced pre-ramp extraction. Temperature ramping restored substantial Cu recovery to give 50.34 % under combined stress. Cu recovery in inoculated columns was reproducible across ore treatments, whereas uninoculated recovery depended on the degree of incidental colonisation. Prolonged operation at 65 °C eventually destabilised iron cycling, suggesting temperature transition is controlled rather than maximised or re-inoculation to support Fe oxidation at 65 °C is practised. The consortium is therefore a promising basis for redox-managed heap bioleaching, provided that salinity, microbial succession and the upper thermal exposure are actively managed.

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Enhancement of Copper recovery from mine tailings through Electro-bioleaching with Native Acidophilic Consortia

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***Keywords*:** Chalcopyrite tailings; Copper extraction; acidophilic consortia; Electrobioleaching;

Abstract

With the increasing global emphasis on renewable energy, innovative approaches to recycling mining waste have become critical. This study focuses on the valorisation of chalcopyrite tailings from the Malanjkhand Copper mines in India, which contain 0.34% copper, 3.38% iron, and a high amount of silica. While conventional bioleaching using acidophilic microorganisms has been well documented for many sulfide ores, including chalcopyrite, chalcocite (Cu_2S), and covellite (CuS), the dissolution of copper from chalcopyrite is often slow due to mineral complexity and hindered by surface passivation during bioleaching. Electrochemical leaching (ECL), with and without active acidophilic microbes, has been reported for some sulfide ores, concentrates, and secondary sulfides, with accelerated dissolution rates; however, it has yet to be explored for chalcopyrite tailings. In this study, an accelerated method for copper extraction via electro-bioleaching (EBL) was employed on chalcopyrite tailings through the synergistic application of electric potential and native acidophilic cultures, namely AT1 and TD1, collected from two different acidic mine drainage sites of the Malanjkhand copper mines in Madhya Pradesh, India. The EBL experiments were conducted using pulverised tailings at 5% pulp density, 150 mL volume of media, in a leaching reactor integrated with a DC supply, under continuous agitation at 200 rpm, at pH 1.8(±0.05). The EBL experiments were compared with control bioleaching and ECL conducted under similar parameters. Further, using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), the electrochemical behaviour of the EBL system were studied in detail. Transmission electron microscopy (TEM) confirmed the biofilm formation and attachment to electrodes. The results show a significant breakthrough in the rate of copper solubilization under EBL with native cultures, with copper recovery of 10.76% day^{-1}, as compared to 6.66 % day^{-1} by ECL and 2.1% day^{-1} by control bioleaching. In contrast, copper recovery from Indian chalcopyrite through heap bioleaching is reported at a rate of 0.14% day^{-1}. The dissolved copper was further selectively separated from EBL leach liquor by solvent extraction without any pre-treatment. The findings demonstrate that electro-bioleaching is a potentially economical and sustainable strategy, yielding enhanced recovery rates in the reclamation of copper from mine tailings.

Introduction

Copper is designated as a critical metal globally by several countries, including India, and it plays a crucial role as we transition towards green technology in enabling clean energy systems. The excellent electrical and thermal conductivity makes copper indispensable for electric vehicles, solar panels, wind turbines, power grids, and energy-efficient infrastructure. With ever increasing demand and depletion of primary mineral reserves, it is essential to efficiently recover copper from various secondary sources, including mine waste and tailings. Currently the most economically exploitable copper reserves in the world are reportedly of low grade (≤0.5% Cu) chalcopyrite ores, making 70% of the world reserves. Recovering copper from tailings, predominantly present as

chalcopyrite, is therefore important not only for meeting industrial demand but also for improving the overall sustainability of mining operations [1]. Bioleaching has been a widely applied approach for copper recovery from sulphide ores, particularly for secondary sulphides below commercial grades that cannot be economically extracted by conventional pyro-hydrometallurgical processes. It employs acidophilic microorganisms, which derive energy by oxidising sulphide compounds, and Fe^{2+} to Fe^{3+}, which in turn acts as a strong oxidant with a reduction potential of 0.77 V, and promotes the galvanic dissolution of Cu from the chalcopyrite matrix [1-4]. Some important microbes used in Cu bioleaching include *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans,* and thermoacidophilic organisms such as *A. Caldus* and *Metallosphaera hakonensis, etc. A. ferrooxidans,* in particular, plays a crucial role as it can oxidise a number of mineral sulphides and contribute to metal solubilization. Bioleaching of Cu is slow, and typically it is improved by the addition of external Fe^{2+}, Fe^{3+} and pyrite particles [5], however, this further contributes to surface passivation, where the mineral surface becomes coated with reaction products such as sulphur, polysulfide, jarosite, or other intermediate products that hinder metal solubility [4-8]. This calls for modification in chalcopyrite bioleaching approach that can reduce surface passivation and maintain favourable conditions for metal dissolution, such as electrobioleaching. In Electrobioleaching, external electric potential is applied to the leaching system to enhance microbial activity and improve metal solubility, under controlled electrochemical conditions. It accelerates Cu dissolution by creating conditions that are favourable for both microbial activity and mineral oxidation, simultaneously delay or slow down jarosite formation [2,3]. It has been reported that low potentials (400-430 mV) yielded 77% Cu in 10 days from chalcopyrite concentrates using thermo-acidophilic bacteria, 1.5times as compared to bioleaching, and also showed reduced passivation by limiting jarosite formation [2]. In the present study, native acidophilic cultures AT1 & TD1, predominantly containing iron-oxidising bacteria, were collected from two different sites of the Malanjkhand copper mines, Madhya Pradesh, India, and investigated for copper extraction from chalcopyrite tailings. This study also compares bioleaching (BL) with electrobioleaching (EBL) and electrochemical leaching (ECL) to assess the effectiveness of electric potential on microbial activity and copper solubilisation from tailings. Additionally, Transmission electron microscopy (TEM), Cyclic voltammetry (CV), Electrochemical impedance spectroscopy (EIS), X-ray diffraction (XRD) and Microscopy analyses were carried out to understand the mineralogical and electrochemical changes during the process. This study aims to further the understanding of microbe-mineral interactions, metal recovery, surface passivation and electrochemistry of the system, towards the development of an effective bio-hydrometallurgical strategy for copper extraction from chalcopyrite tailings.

Methods

Raw Material

The raw material used in this study was chalcopyrite tailings rejected after mining, collected from the Malanjkhand mines and graciously provided by Hindustan Copper Limited, Malanjkhand Copper Project (HCL MCP), Malanjkhand, Madhya Pradesh, India. The sample was crushed, pulverised and applied to all bioleaching and electrobioleaching experiments. The average metal composition of the tailings sample from aqua regia digestion and AAS was found to be: 0.34% Cu, 3.38% Fe, and the rest being acid-insoluble aluminosilicates.

Microorganisms collection and growth

Two native acidophilic microbial consortia (labelled AT1 and TD1) developed from sludge samples collected from different acid mine drainage (AMD) sites in the Malanjkhand copper mines, India were used in the study. During collection, the sample pH ranged from 2.9 (TD1) to 3.5 (AT1). The collected samples were adapted in 9K medium [4]. After sterilisation, either 40 g L^{-1} $FeSO_4 \cdot 7H_2O$ or 2g/L elemental S^0 was added as an energy source, and media pH was adjusted to 1.8(±0.05) using H_2SO_4. About 20%(v/v) sludge was added as inoculum from each sample to individual 250 mL flasks containing sterile medium and incubated at 150 rpm at 29(±2ºC) in incubator. Both the

consortia showed quick growth and adaptation with $FeSO_4$, while slow and limited growth with S^0 alone as an energy source. The consortia were transferred to fresh 9K medium every 7 days and after six repeated subcultures, the iron enriched cultures were used for BL and EBL experiments.

Experimental Procedure

Electrobioleaching and bioleaching experiments were performed with both AT1 &TD1 cultures. BL experiments served as controls and were conducted in duplicates in 250 mL Erlenmeyer flasks incubated in an orbital shaker at 29(±2ºC) for duration of 15 days. Electro-bioleaching experiments were carried out in a custom glass bioreactor fitted with teflon lid, agitator, and platinum-coated titanium electrodes as both the anode and cathode. All experiments were conducted at working volume of 150 mL with 20% active culture as inoculum, and 5% (w/v) chalcopyrite tailings added as energy source with continuous agitation up to 200 rpm. The initial pH was adjusted to 1.8±0.05 using H_2SO_4 and the pH was monitored throughout the experiments. A constant potential of 400(±10) mV was applied during EBL from a potentiostat (Aplab) to the electrodes. Two different particle sizes, that is (-)150 µm and (-)45µm were tested. All the EBL and control ECL experiments (without inoculum) were done in triplicates. After leaching Cu was extracted from the EBL liquor using LIX 84I as the extractant, and the preliminary solvent extraction experiments were carried out in a single stage at an organic to aqueous [O: A] phase ratio of [1:1] to establish feasibility.

Analysis

The leaching filtrates were analysed using Atomic Absorption Spectroscopy (AAS) to determine the percentage metal recovery. Transmission electron microscopy (TEM, FEI) was employed to investigate the cellular features and components of the biofilm and residue, while mineralogical analysis was carried out by X-ray diffraction (XRD, PANalytical) and petrographic microscope (Leica). Cyclic voltammetry (CV) was initiated at the open-circuit potential and performed in the positive potential direction. The working and counter electrodes were Pt, and Ag/AgCl electrode was used as reference. Electrochemical impedance spectroscopy (EIS) was conducted as a non-destructive analysis during the EBL experiments (Electrochemical work station, CH instruments), measured between 10,000Hz and 0.01Hz, using a sine-wave perturbation with an alternating current amplitude of 10 mV.

Results and Discussion

The X-ray diffraction and petrographic microscopy studies showed that the tailings consisted of copper predominantly as chalcopyrite ($CuFeS_2$), along with pyrite (FeS_2), and feldspar-like K-aluminosilicate (orthoclase) major matrix, as shown in Fig.1. The microscopic images reveal that the chalcopyrite and pyrite grains are present within aluminosilicate. The yellowish golden grains point to the chalcopyrite, and bright golden grains indicate the pyrite, both within the darker orthoclase mineral matrix of the ore material (Fig. 1a and b). There were no secondary sulphide phases in the tailing sample.

The extraction trends of Cu leaching from tailings are summarised in Fig. 2 for the EBL, BL, and ECL systems under various experimental conditions. In simple bioleaching experiments carried out for 15 days, the native cultures showed different rate of Cu recovery, with TD1 showing higher recovery at 2.10% day^{-1}, and AT1 at 0.86% day^{-1} (Fig.2a). However, when the same cultures were subjected to EBL at a potential of 400(±10)mV, both AT1 and TD1 extracted copper more effectively at over 31% and 32.3% in 72 hours, equivalent to 10.3% day^{-1} and 10.76% day^{-1}, respectively (Fig.2b). Further increase in duration did not increase the extraction rate. The difference in the rate of Cu extraction between the cultures in the control BL experiments seems to have equalised during EBL, indicating adaptability of the organisms to the applied EBL conditions. The rate is also higher than earlier reported rates of chalcopyrite EBL at 7.7% day^{-1} copper [2]. The control ECL exhibited 6.66% day^{-1}, which is mainly attributed to galvanic dissolution in presence of Fe. The difference in extraction rates between EBL and ECL indicates the contribution of the microbial consortium towards oxidative dissolution of chalcopyrite. The pH changed from 1.8±0.05 to 1.65±0.05 during EBL, and the ORP remained in the range 410-500mV.

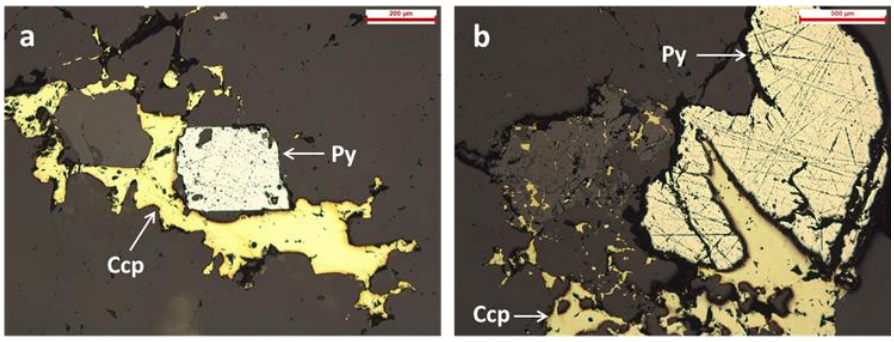


Fig. 1 Microscopic imaging of crushed and polished chalcopyrite tailing samples showing grains of Ccp-chalcopyrite and Py-pyrite within orthoclase (a-scale 200µm; b-scale 500µm)

The particle size variation study with AT1 culture showed a minor difference in the extraction, with 10.4% day^{-1} Cu recovery at (-) 45µm, and 12.01% day^{-1} at (-)150µm (fig.2c), possibly indicating incomplete liberation of the target mineral chalcopyrite. Further, the saturation of maximum Cu extraction at 30-35% in both BL and EBL irrespective of duration, again likely indicates insufficient mineral liberation in the present study.

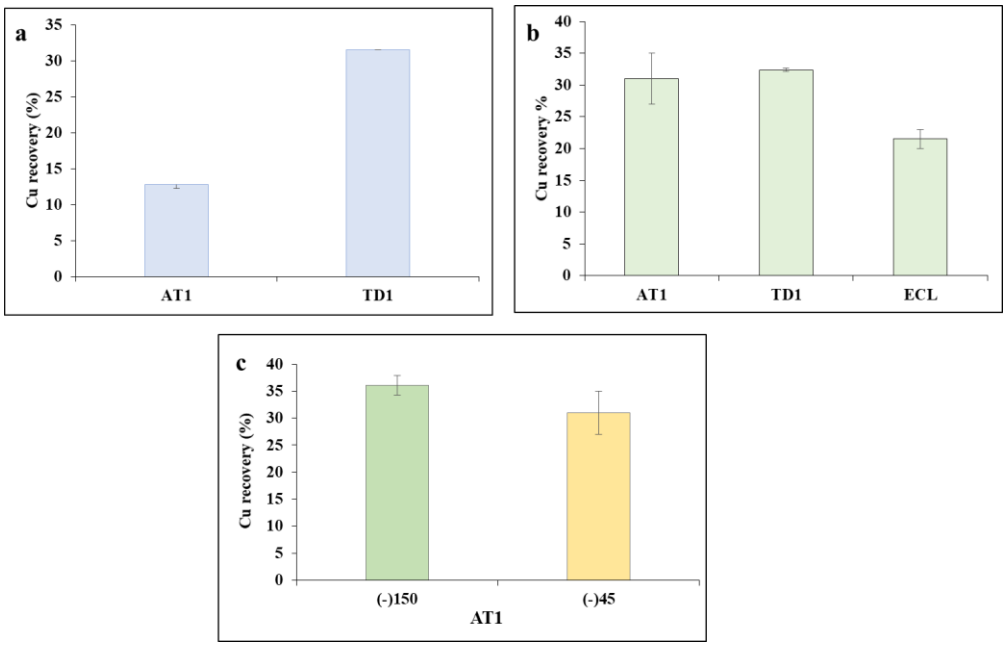


Fig.2 Effect of copper recovery in (a) BL study of AT1 & TD1 native cultures in 15 days, (b) EBL study of AT1, TD1 cultures& ECL (control) in 3 days (particle size -45micron), (c) Particle size variation study in AT1 culture.

Electrochemical characterisation and Electron microscopy study

Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) measurements were performed on Day 0 and Day 3 during the EBL of chalcopyrite tailings at a constant oxidative potential of +400 mV to elucidate the electrochemical dynamics within the system. The impedance spectra were analysed using the equivalent circuit Rs-(Rct||CPE)-W, where Rs represents the solution resistance, Rct the charge transfer resistance, CPE the constant phase element, and W the Warburg diffusion element. The fitted spectra showed good agreement with the experimental data, confirming that the selected equivalent circuit adequately describes the electrochemical behaviour of the bioleaching system. The Nyquist impedance spectra obtained for AT1 and TD1 tailings at Day 0 and Day 3 are presented in Fig. 3, together with the fitted spectra using the equivalent circuit Rs-(Rct||CPE)-W. The extracted fitting parameters are summarised in Table 1.

Table 1. Electrochemical impedance parameters obtained from equivalent circuit fitting of the EIS spectra during electro-bioleaching of chalcopyrite tailings.

Sample	Day	R_s (Ω)	R_{ct} (Ω)	CPE (n)	Warburg Impedance	Metal Extraction (%)
AT1	0	15.30	8.67	0.798	27.87	–
AT1	3	21.83	1149.90	0.529	5843	28
TD1	0	17.56	4.20	0.691	41.56	–
TD1	3	34.07	1182.00	0.800	18642	32

The impedance spectra show a pronounced increase in interfacial impedance after three days of EBL for both native consortia. The solution resistance (R_s) increased only slightly from 15.30 to 21.83 Ω for AT1 and from 17.56 to 34.07 Ω for TD1, indicating that the conductivity of the bulk electrolyte remained largely unchanged throughout the experiment. In contrast, the charge transfer resistance (R_{ct}) increased substantially from 8.67 to 1149.9 Ω for AT1 and from 4.20 to 1182 Ω for TD1. The CPE exponent decreased from 0.798 to 0.529 for AT1, indicating increased interfacial heterogeneity, whereas it exhibited an increase from 0.691 to 0.800 for TD1, suggesting the development of a comparatively uniform electrochemical interface. Similarly, the Warburg impedance element increased from 27.9 to 5843 for AT1 and from 41.6 to 18642 for TD1, demonstrating a significant increase in diffusion-related impedance during electro-bioleaching.

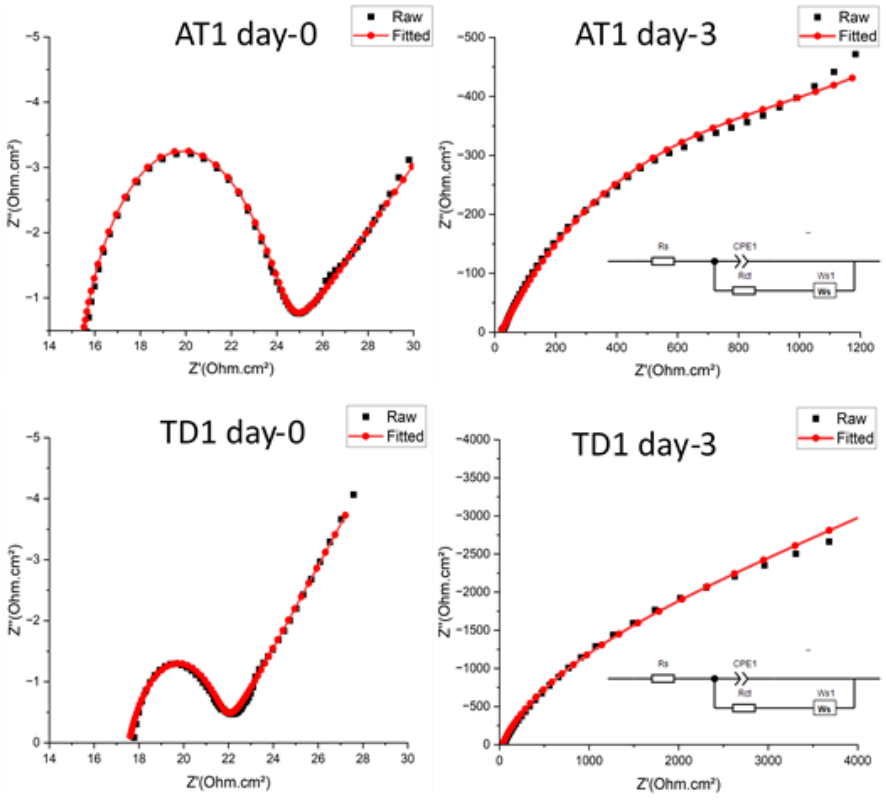


Figure 3. Experimental and fitted Nyquist plots for electro-bioleaching of chalcopyrite tailings (AT1 and TD1) at Day 0 and Day 3.

The cyclic voltammograms recorded during EBL are consolidated in Figure 4. At the beginning (day 0), both AT1 and TD1 exhibited well-defined anodic and cathodic peaks, indicating active, electrochemically reversible interfacial reactions. On day 3, these peaks were markedly suppressed, and the overall current response decreased substantially, resulting in an almost featureless voltammetric profile. The loss of distinct Faradaic features indicates that electrochemical reactions at the interface became increasingly kinetically restricted during bioleaching. This behaviour is fully consistent with the simultaneous increase in charge-transfer resistance and diffusion impedance observed by EIS, indicating a progressive reduction in electrochemical activity at the electrode interface.

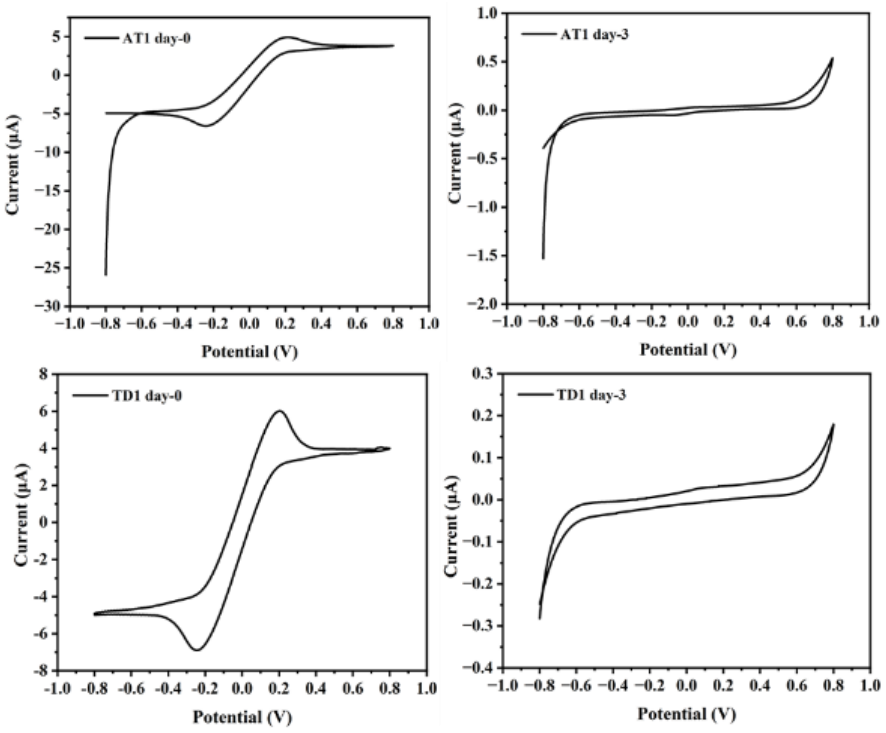


Figure 4. Cyclic voltammograms of the AT1 and TD1 electro-bioleaching systems recorded on Day 0 and Day 3.

The observed changes primarily reflect the progressive evolution of charge-transfer and mass-transport characteristics during EBL. The substantial increase in R_{ct} demonstrates that the impedance evolution is primarily attributed to the electrode-biofilm-mineral interface rather than to changes in the bulk electrolyte, indicating an overall diffusion-influenced electrochemical response. Despite the considerable increase in interfacial impedance and the suppression of reversible electrochemical reactions, copper extraction efficiencies of 31% and 32% were achieved for the AT1 and TD1 systems, respectively, after only 72 hours of EBL. These observations demonstrate that mineral dissolution continued under more resistive electrochemical conditions, indicating a transition from an initially charge-transfer-controlled regime towards a mixed charge-transfer and diffusion-controlled process, rather than complete electrochemical passivation. TEM observations from the experiment further support this and confirm bacterial attachment as shown below.

The TEM micrograph (Fig. 5a) of an anode biofilm sample shows that bacterial cells were attached to the electrode, along with mineral particles, providing direct evidence of microbial attachment and mineral solubilization. It also shows cells surrounded by a visible extracellular polymeric substance (EPS), which appears as a light, diffuse region or layer around the cells that helps the bacteria adhere strongly to the mineral or electrode surface. Additionally, dense surface precipitation was observed on cells (Fig. 5a, red circle), characteristic of iron precipitates in chalcopyrite biofilms [9-11]. This suggests that the bacteria were actively forming a biofilm, facilitating cell attachment and electron transfer in the system and resulting in product layer formation. In Fig. 5(b) (arrow A), the observed rhombic-structured crystals attached to EPS are characteristic jarosite precipitates generated during the process, with the diffraction pattern showing their crystalline feature. It is understood that the formation of the jarosite layer is controlled by pH, temperature, and bacterial cell attachment to the mineral surface [9-11].

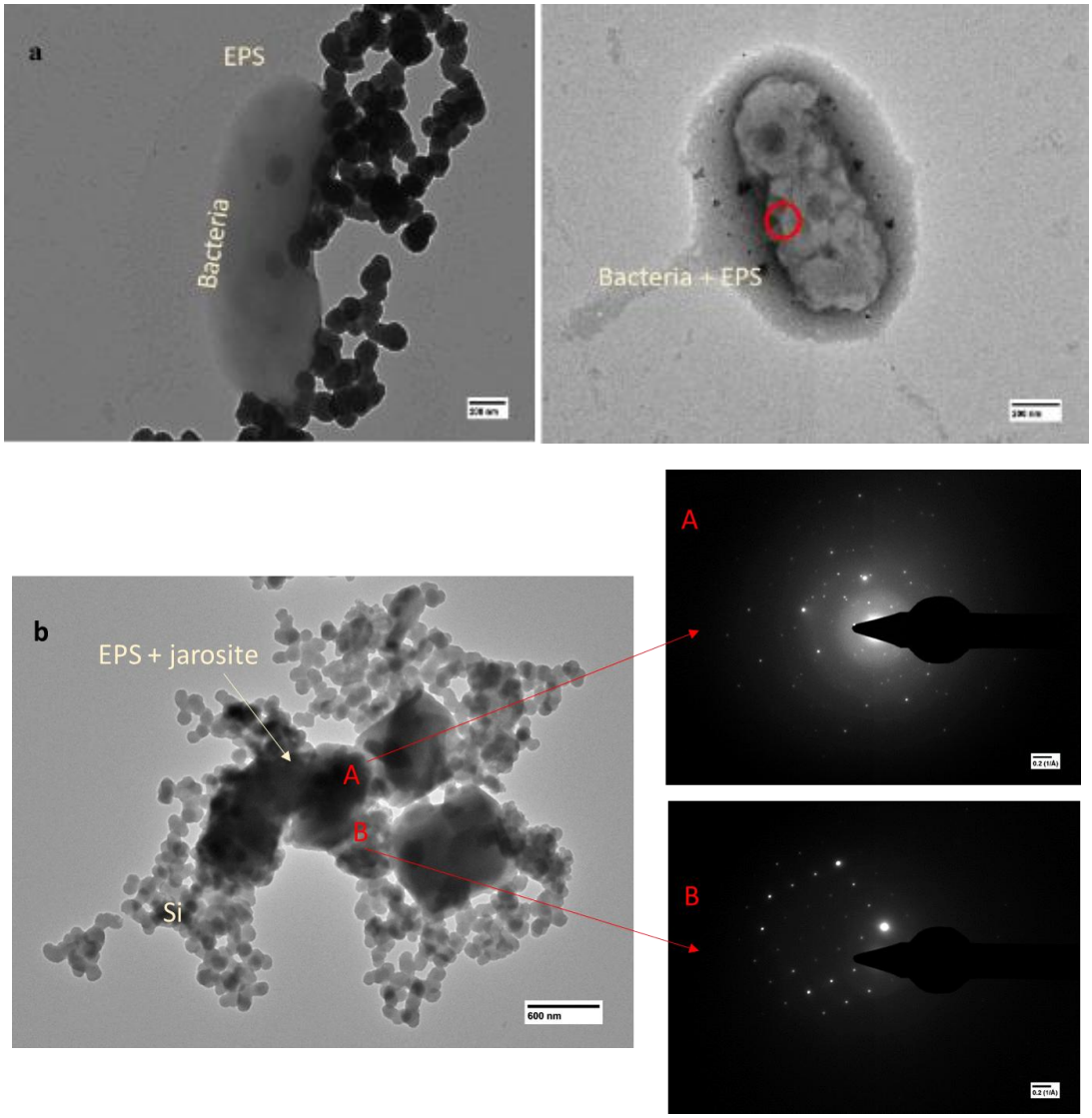


Fig. 5 TEM images showing (a) bacterial attachment on the electrode along with the mineral, (b) sample of EBL leach slurry of chalcopyrite.

The smaller mineral particles attached to both the cell surface (Fig.5a) and in EPS (Fig.5b- arrow B) are consistent with crystalline Si mineral particles observed on chalcopyrite-grown cells, as reported earlier [11]. It is clear from EIS, CV, and TEM observations that the mineral precipitation attached to the biofilm on the electrode is a key contributor to the rise in impedance and the shift from charge-transfer control to diffusion control during the EBL process, thereby saturating the rate of metal recovery.

Solvent Extraction of Cu from EBL Leach Liquor

The bioleach liquor containing 47(±3) ppm Cu and 6(±0.5) g/L Fe was treated by solvent extraction with LIX 84I diluted in kerosene. Extraction was performed at the natural pH of the bioleach liquor without any pH adjustment to evaluate the performance of the extractant under the original leaching conditions. Accordingly, the extraction was carried out within an initial aqueous pH range of 2.3(±0.2), at [1:1] ratio of [O:A] in a single stage. The results showed that 0.5%LIX 84I extracted about 82% Cu, while 1% LIX 84I achieved a higher Cu extraction of 92% under the same operating conditions. These results indicate that 1% LIX 84I can effectively extract Cu directly from bioleach liquor without any pH adjustment. However, further experiments will be needed to optimise the number of stages to maximise the extraction.

Conclusions

The study demonstrated enhanced Cu dissolution from chalcopyrite tailings under eletrobioleaching using native acidophilic cultures, and elucidates the electrochemical process dynamics. The native consortium TD1 showed the highest Cu extraction in the EBL mode, achieving 32.3% in 72 hours at 5% pulp density. This was 1.6x higher than ECL and over 5x higher than conventional bioleaching. Particle-size variation experiments indicated recovery rates of 12.01% day^{-1} at (-)150µm, and 10.4% day-1 at (-)45µm, requiring further optimisation. The EIS study revealed a shift from a charge-transfer mediated process to a diffusion-limited EBL process, which is further corroborated by TEM analysis of the anode biofilm, revealing bacterial attachment with EPS layer, and precipitation of jarosite and silica-like particles at the cellular interface and in the EPS. Finally, solvent extraction of the EBL leach liquor using 1% LIX 84I selectively recovered about 92% of dissolved copper without any pre-treatment, demonstrating that electrobioleaching is a promising strategy with high Cu recovery rates for converting chalcopyrite-bearing mine tailings into a usable secondary resource, contributing to sustainable extraction.

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Biohydrometallurgical Extraction of Critical Metals from Spent Lithium-Ion Batteries via Direct Bioleaching by Mixotrophic Bacteria

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Abstract: The rapid expansion of electric mobility and portable electronic technologies has led to an unprecedented increase in the consumption of lithium-ion (Li-ion) batteries, accompanied by the growing accumulation of end-of-life battery waste. While spent Li-ion batteries represent an environmental concern, they also constitute an increasingly important secondary source of critical metals, including lithium, cobalt, nickel, and manganese. However, the complex and heterogeneous composition of battery black mass poses significant challenges for selective metal recovery. Conventional recycling routes, such as pyrometallurgy and hydrometallurgy, although industrially established, are often associated with high energy demand, elevated operational costs, and the generation of secondary pollutants.

As a sustainable alternative, biohydrometallurgical processing exploits microbial metabolic activity to promote metal solubilisation under mild operating conditions. In this study, direct bioleaching of spent Li-ion battery black mass was investigated using five mixotrophic bacteria, including *Priestia aryabhattai* SKC-5, *Proteus mirabilis* SKC-7, *Bacillus velezensis* SKC/S-2 and SKC/S-4, and *Pseudomonas plecoglossicida* SKC/SH-9, over a seven-day leaching period. The bioleaching system exhibited high selectivity toward lithium extraction, while only limited dissolution of transition metals such as nickel, cobalt, copper, manganese, and aluminium was observed. The evolution of pH and redox potential (Eh) during leaching reflected the metabolic activity of the bacteria and their role in controlling metal solubilisation behaviour. Bioleaching selectivity was quantitatively confirmed, demonstrating the ability of mixotrophic bacteria to preferentially mobilise lithium from complex battery matrices, thereby facilitating downstream separation and recovery processes. Post-leaching solid residues were characterised by SEM-EDS, FTIR, and XRD analyses, providing further evidence of selective metal extraction and structural modification of the black mass.

This study demonstrates that mixotrophic bacteria can achieve highly selective, direct bioleaching of lithium from spent Li-ion batteries without chemical pre-treatment, providing a low-energy and environmentally benign route for valorising battery waste as a secondary metal resource. These findings highlight the potential of biohydrometallurgical approaches to complement conventional recycling technologies and support circular economy strategies for sustainable battery material management.

Keywords: Lithium-ion battery recycling, Selective bioleaching, Mixotrophic bacteria, Secondary critical metals, Sustainable metallurgy

From bio-hydrometallurgical mineral refinement, metal recovery, and waste recycling to the biosynthesis of nanoparticles

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Keywords: Biohydrometallurgy; Biosynthesis; Nanoparticles; Bio-flotation; Recycling

Abstract

This paper synthesises twenty years of research contributions spanning four interconnected themes in biotechnology applied to mineral processing. The work began with fungal bioleaching to beneficiate kaolin and evolved into bio-separation of industrial minerals using *Bacillus licheniformis* as a bioflocculant and bio-collector. In parallel, the microbial synthesis of semiconductor nanoparticles from mine wastewaters was established for the first time, opening a route to convert environmental liabilities into high-value materials. These themes converge in a sustainable philosophy: biological reagents replace or reduce toxic chemicals, secondary resources replace primary ores, and waste streams yield commercial products.

Introduction

Conventional mineral processing relies heavily on toxic chemicals, strong acids, synthetic flocculants, cyanide, and organic collectors, whose manufacture and disposal impose significant environmental costs. The growing scarcity of high-grade ores further intensifies these pressures, forcing the industry to treat lower-grade, more complex, and secondary resources. Biological approaches offer an alternative that is inherently gentler: microorganisms produce acids, extracellular polymers, and reducing agents under mild conditions, consume renewable carbon sources, and leave behind biodegradable residues [1,2]. The body of work reviewed here addresses three practical problems that bio-hydrometallurgy must solve: (i) how to remove iron impurity from industrial clay minerals such as kaolin without damaging the host lattice; (ii) how to separate one mineral from another without synthetic flotation reagents; and (iii) how to recover metals from acid mine drainage (AMD) and other process liquors as value-added nanomaterials rather than as polluting sludge. A fourth strand, environmental remediation, addresses the treatment of chromium-contaminated waters and the valorisation of the voluminous bauxite residue (red mud) produced by the alumina industry.

Results and Discussion

Fungal bioleaching for kaolin beneficiation. The programme began with an investigation of iron removal from a highly contaminated kaolin (11% Fe_2O_3) using *Aspergillus niger* [1]. The pistachio-shell isolate removed 42.8% of total iron, far outperforming the reference NCIM 548 strain. The mechanism was elaborated in a follow-up study using response surface methodology (RSM) based on a central composite design [2]. Initial pH, sucrose concentration, and spore density were the controlling variables for oxalic and citric acid production by the same pistachio-shell *A. niger* isolate. The optimised conditions (pH 2, 70 g/L sucrose, 35×10^7 spores/L) yielded 311.3 mg/L dissolved iron, corresponding to 67.4% removal, a striking improvement over the earlier baseline, and provided a quantitative model that can guide scale-up. A subsequent kinetic study [3] confirmed that bioleaching of iron from kaolin followed shrinking-core kinetics, with product-layer diffusion as the rate-limiting step, providing the fundamental engineering data required for reactor design.

Bioflocculation and bio-separation of industrial minerals. A second major strand exploited the extracellular polymeric substances (EPS) and intact cells of *Bacillus licheniformis* to separate minerals through differential flocculation and flotation. An initial study [4] examined the effects of *B. licheniformis* cells, metabolite, polysaccharide, and protein on the settling behaviour of kaolin and quartz as a

function of pH and solid concentration. Polysaccharide was the more effective flocculant for kaolin, whereas protein drove quartz agglomeration. This mechanistic understanding was then applied to iron oxide removal from kaolin and quartz [5], demonstrating differential bioflocculation in which hematite and goethite settled preferentially relative to the host minerals at pH 7. The selectivity was attributed not to differential EPS adsorption but to differences in zeta potential: the negatively charged polysaccharide flocculated the positively charged iron oxides more strongly, leaving kaolin and quartz dispersed. Complementary work [6] extended the system to the direct biological separation of quartz from kaolinite in alkaline conditions (pH 10–12): using bacterial cells, 98.3% of kaolinite was recovered with a significant reduction in quartz content. The bio-collector concept was extended to barite flotation [7]. *B. licheniformis* cells adsorbed onto barite at densities six times higher than onto quartz at pH 3, following Langmuir isotherms and pseudo-second-order kinetics. Single-mineral flotation recovered 87% of barite; in a 1:1 mixture, 76% barite recovery was achieved at 96.3% product grade with only 4% quartz co-flotation. An earlier companion paper specifically characterised bioflocculation of hematite and goethite using *B. licheniformis* [8], showing that bacterial polysaccharides acted as bridging flocculants via charge-neutralisation on the positively charged iron oxide surfaces.

Microbial synthesis of semiconductor nanoparticles. A third strand converted the metal-laden effluents produced by bioleaching into functional nanomaterials. Copper sulfide (CuS) nanoparticles were produced extracellularly from $CuSO_4$ solution for the first time using *Fusarium oxysporum* [9]. Spherical particles of 2–5 nm were encapsulated in peptide shells approximately 20 nm in diameter. A subsequent study produced covellite-phase CuS nanoparticles (10–40 nm) directly from the AMD of the Sarcheshmeh Copper Complex [10]. The rate limitation of biological synthesis was tackled by applying electric current to *F. oxysporum* cultures before CuS biosynthesis [11]. Moderate currents tripled the biosynthesis rate relative to unstimulated cultures, though the total final yield was unaffected. Zinc sulfide (ZnS) nanoparticles (~42 nm, spherical) were biosynthesised by *F. oxysporum* from $ZnSO_4$ solution [12]. Cadmium sulfide (CdS) quantum dots (2–10 nm) were produced using extracellular polymeric substances of *B. licheniformis* as both reductant and stabilising agent, from both pure $CdSO_4$ and from an impure industrial cadmium sulfate solution [13, 14]. The stabilising role of bacterial EPS, preventing nanoparticle agglomeration, connects this work directly to the bioflocculation strand [4,8], since the same polysaccharides that flocculate mineral particles here arrest nanoparticle growth. The nanoparticle programme was extended to silver phosphate (Ag_3PO_4) using *Sporosarcina pasteurii* [15]. The ~20 nm particles showed strong antimicrobial activity against *E. coli, S. typhimurium, S. aureus*, and *B. cereus*, illustrating how the biosynthesis platform bridges mining biotechnology and biomedical nanotechnology. Bio-synthesised CdS nanoparticles immobilised on diatomite were evaluated as photocatalysts for methylene blue decomposition under UV light [16].

Bio-cyanidation and gold recovery. A fourth theme applied cyanogenic bacteria to precious metal recovery. *Bacillus megaterium* and *Pseudomonas aeruginosa* were compared for biological cyanide production from glycine and subsequent gold dissolution from an oxidised copper-gold ore [17]. Box-Behnken optimisation of medium composition (glycine, $FeSO_4$, NaH_2PO_4, $MgSO_4$) yielded up to 67 mg/L biogenic CN. A pH increase to 10 after bio-cyanidation raised Au recovery to above 80%, and pre-leaching to remove copper further improved gold concentrations in the leachate. The study established a quantitative framework for optimising the inherently complex, multi-variable biological cyanidation process. A follow-up study [18] developed a fully biological analogue of industrial ammoniacal cyanidation for copper-gold ores using *B. megaterium* alone. The bacterium produced both ammonia (via urease) and cyanide (via glycine decarboxylation), separately or simultaneously. Using vinasse, an industrial ethanol-production waste, as the culture medium instead of nutrient broth increased cyanide biogenesis by 60% and gold recovery to 97% via a sequential bio-ammonia pretreatment/bio-cyanidation strategy.

Environmental remediation and secondary resource valorisation. A magnetic nano-biocomposite was synthesised by attaching magnetite nanoparticles (prepared from iron machining scraps) to alkaline-activated *A. niger* biomass [19]. The composite adsorbed ~92% of Cr(VI) from a synthetic solution at pH 5.8 within 11 minutes, following Temkin isotherms indicative of physical adsorption, and could be recovered magnetically for reuse. This paper connects AMD treatment, fungal biomass utilisation, and nanoparticle synthesis in a single material. Bauxite residue (red mud), generated at ~160 million tonnes

per year globally, was bioleached using *A. niger* strains isolated from pistachio husk and grape skin [20]. Grape-skin-strain *A. niger* extracted 92% Al, 88% V, 67% Ti, 66% Sr, and 29% Sc from mechanically activated residue, with citric acid (~15 g/L) as the dominant lixiviant. Kinetic analysis showed that the Luedeking-Piret model described acid production and that shrinking-core, product-layer diffusion-controlled metal dissolution, directly paralleling the kaolin bioleaching kinetics established in [3] and confirming the generality of the kinetic framework across heterogeneous mineral matrices. Copper recovery from flotation tailings of the Sungun copper processing plant was achieved using indigenous mesophilic and thermophilic iron- and sulfur-oxidising microorganisms [21]. Thermophilic microorganisms in Norris medium with nanobubble-enriched water achieved the highest Cu dissolution; the average Cu recovery reached 92.8% after one month.

Conclusions

Locally isolated *A. niger* strains are more effective than reference strains for kaolin bioleaching, and RSM-optimised acid production combined with shrinking-core kinetics provides a complete engineering framework for scale-up. *B. licheniformis* and its EPS are remarkably versatile bio-reagents that can act as selective bio-collectors for barite flotation. The fungus *F. oxysporum* and the bacterium *B. licheniformis* enable the biosynthesis of CuS, ZnS, and CdS semiconductor nanoparticles from metal sulfate solutions. Cyanogenic bacteria produce biogenic cyanide and ammonia for gold and copper recovery, and waste substrates such as vinasse can replace synthetic growth media. Finally, magnetic nano-biocomposites from waste iron and fungal biomass remediate Cr(VI)-contaminated water, *A. niger* valorises bauxite residue by extracting Al, V, Ti, and rare earths, and nanobubble-assisted bioleaching recovers Cu from flotation tailings with >92% efficiency.

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Sustainable recovery of critical raw materials from spent lithium-ion batteries through bioleaching with halotolerant bacteria

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Abstract: The recovery of Critical Raw Materials (CRM) has been targeted by the European Union as a priority, to meet sustainable development goals. CRMs (such as cobalt, lithium, nickel, manganese, and graphite), present in the manufacture of lithium-ion batteries (LIBs), are essential for the development of electric vehicles and energy storage. In 2022, only 22.4% of the 62 Mt of global e-waste generation was properly collected and recycled. Considering the low recycling rates of CRM in 2023: less than 1% for Li, 9% for Mn, 16% for Ni or 22% for Co, its recycling is mandatory to ensure future demand for these key elements for the EU's technological sector and to achieve sustainable environmental goals.

This work aims for the recovery of Co, Li, Ni and Mn from the cathode and graphite from the anode of LIBs starting from black mass powder and using the biohydrometallurgy route. Bioleaching experiments were conducted using a bacterial strain of *Acidihalobacter ferrooxidans* sp and two different black mass samples, from computer and electric vehicle batteries, which were previously characterised by XRF, XRD, elemental microanalysis and acid digestion. The effect of different variables on the yields of metal bioleaching was assessed: (a) direct versus indirect bioleaching; (b) pulp density and (c) type of spent lithium-ion battery. In addition, graphite recovery was evaluated by XRD analysis of the bioleaching residue obtained.

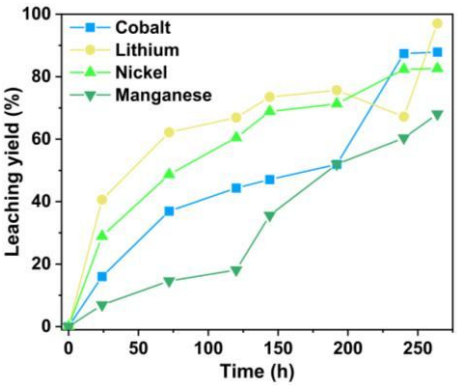


Figure 1. Indirect bioleaching of black mass from a computer battery.

The experimental results confirmed that the indirect bioleaching and a pulp density of 1 g/L were the optimal parameters to achieve bioleaching efficiencies of 88% for Co^{2+}, 97% for Li^{+}, 83% for Ni^{2+}, and 68% for Mn^{2+}, after 270 hours. Under such conditions, the halotolerant bacteria were able to produce a combination of acid and oxidising agents which enhanced the biochemical attack and the extraction of metals from the solid e-waste.

Keywords: Bioleaching, lithium-ion batteries, acidophile, halophile, critical materials

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SynBiomining for Critical Metal Recovery, Carbon Storage and Mine Closure

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Keywords: Critical metals; biomining; synthetic biology; proteins; mineral carbonation

Abstract

Mining and processing residues contain unrecovered critical metals and create substantial environmental and mine-closure liabilities. This work presents SynBiomining, a modular synthetic-biology platform for circular, low-carbon mining. The platform integrates biological mobilisation of critical metals from complex residues, selective capture of dissolved metals using metal-binding biomolecules, and microbial mineral carbonation for stabilisation of suitable Mg-rich tailings. High-throughput cultivation, adaptive evolution, quantitative proteomics and environmental-process validation support development of these modules. Progress establishes waste-adapted microbial systems, candidate biomolecular recovery tools and cyanobacterial carbonation capability, providing a foundation for future feedstock-specific hybrid biological–hydrometallurgical flowsheets for metal recovery, carbon storage and mine rehabilitation.

Introduction

The mining sector must transition from linear extraction to circular, low-carbon practices that meet growing demand for critical metals while reducing environmental impacts. Meanwhile, waste rock and tailings often contain recoverable critical metals, but conventional hydrometallurgical and pyrometallurgical processes remain constrained by poor selectivity and high costs in complex, low-grade matrices [1, 2].

Biomining provides a lower-energy route for metal recovery by using microorganisms to promote dissolution of metal-bearing solids. Current industrial biomining applications rely mainly on acidophilic iron- and sulfur-oxidising bacteria and archaea for sulfidic copper, gold, nickel and cobalt ores [3]. However, critical metals remain unrecovered in many mining and processing residues that lie outside established biomining practice. Bauxite residue, for example, is an alkaline, saline and metal-rich waste stream with potential for critical-metal recovery. Extending biomining to such residues requires microorganisms that tolerate challenging chemical conditions and selectively mobilise target metals. Extremotolerant fungi offer one promising approach, as demonstrated for organic-acid-mediated recovery of rare earth elements (REEs), gallium and vanadium from bauxite residue [4].

Biomining can be integrated with hydrometallurgical processing to support both metal extraction and selective recovery from complex waste streams. Following biological or conventional leaching, dilute polymetallic solutions require downstream separation and concentration of target metals. Biosorption

and protein- or peptide-based biorecovery offer lower-impact approaches for selectively capturing metal ions from multi-metal leachates using metal-binding proteins and engineered peptides [5]. These approaches can complement biological or conventional leaching [6, 7].

A third component of this integrated approach is the management of residual tailings after metal recovery. Rather than being treated solely as a mine-closure liability, suitable residues may support biologically mediated mineral carbonation, linking carbon storage with tailings rehabilitation. In magnesium-rich mafic and ultramafic tailings, microbial processes can enhance CO_2 dissolution and promote the precipitation of stable magnesium carbonate minerals under ambient conditions, reducing the energy and reagent requirements associated with engineered carbonation. Carbonate formation may also improve tailings surface stability, reduce dust generation and create conditions more conducive to ecological succession during mine closure. This potential has been demonstrated in laboratory and pilot-scale systems using ultramafic mine wastes [8, 9].

Collectively, these studies establish three complementary but independently demonstrated capabilities for circular mining: biological mobilisation of critical metals, selective biomolecular metal capture and microbially mediated mineral carbonation. Using red mud for fungal bioleaching, metal-exposed microbial cultures for protein discovery, and bischofite as an Mg-rich substrate for cyanobacterial carbonation, we present SynBiomining: a synthetic-biology platform being developed at the UQ Biosustainability Hub. SynBiomining is designed to configure biological or hybrid biological–hydrometallurgical extraction, biomolecular recovery from leachates, and stabilisation and carbonation of suitable post-processing residues into feedstock-specific flowsheets that support critical-metal recovery, carbon storage and mine-closure outcomes (Figure 1). These functions are supported by a synthetic-biology toolkit encompassing microbiome discovery, omics, high-throughput screening, strain and process optimisation, and engineered metal-binding biomolecules.

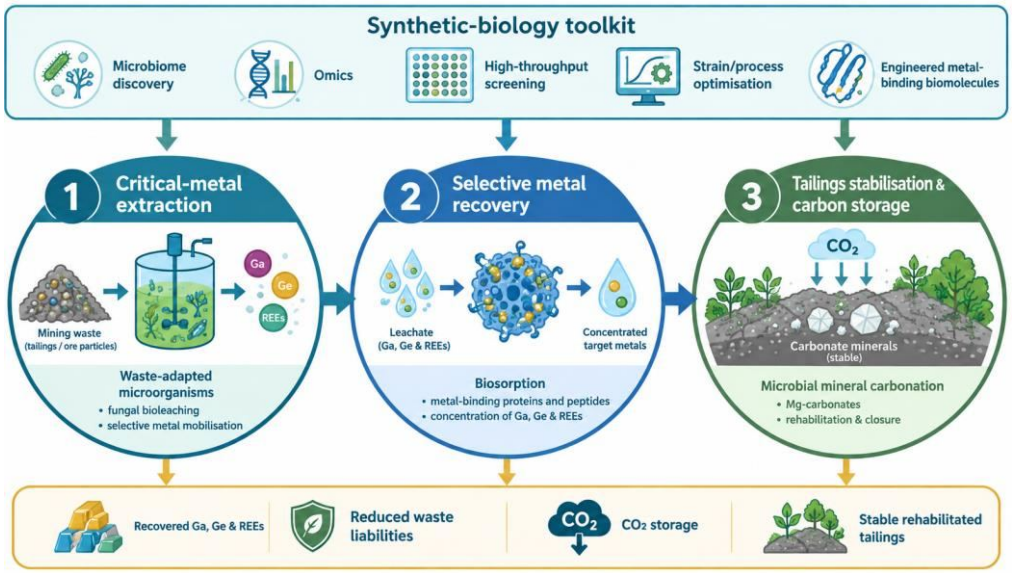


Figure 1. SynBiomining framework for future feedstock-specific critical-metal recovery and mine-waste management. The conceptual artwork was generated with assistance from Perplexity AI and reviewed, edited and verified by the authors.

Methods

SynBiomining was evaluated as a modular synthetic-biology platform for the future, feedstock-specific integration of critical-metal extraction, selective biomolecular recovery and mine-waste stabilisation. The approach was enabled by two co-located NCRIS-supported facilities at the UQ Biosustainability Hub, IDEA Bio, a synthetic-biology biofoundry for automated cultivation, bioreactor-based characterisation and adaptive evolution, and Q-MAP, a metabolomics, proteomics

and systems-biology facility with Orbitrap mass-spectrometry capability, together with the Environmental Engineering Laboratory in the School of Civil Engineering for environmental-process validation.

The platform followed an iterative design-test-learn-optimise workflow. Candidate microorganisms and metal-binding biomolecules were first selected for their potential to tolerate mining-relevant conditions, mobilise target metals, selectively bind dissolved metal ions or promote mineral carbonation. High-throughput cultivation and bioreactor experiments then tested performance across chemical and environmental gradients relevant to mineral residues, including pH, salinity, solids loading, metal exposure and light regime. Process performance was assessed using biomass development, metal mobilisation, stress-response metabolites, extracellular polymer production and mineral-precipitation indicators. Where required, adaptive evolution and mutagenesis were applied to extend microbial tolerance and performance under increasingly challenging conditions.

Quantitative proteomics, metal-affinity enrichment and bioinformatic screening were used to identify metal-responsive and metal-binding proteins, which were prioritised for recombinant expression and functional validation as selective recovery materials for Ga, Ge and REEs. In parallel, Mg-rich model systems were used to assess cyanobacterial growth, stress adaptation, biofilm-associated polymer production and mineral-carbonation potential. Together, these complementary workflows generate the organisms, biomolecular tools, operating windows and molecular datasets needed to configure future residue-specific, hybrid biological–hydrometallurgical flowsheets.

Results and Discussion

The results establish three complementary, independently demonstrated modules within the SynBiomining platform: biological mobilisation of critical metals from challenging residues, selective metal capture using biomolecular tools, and microbially mediated stabilisation of Mg-rich materials through mineral carbonation.These studies do not yet constitute a single coupled flowsheet; rather, they provide the organisms, biomolecules, analytical workflows and operating windows required to develop future feedstock-specific processes compatible with hydrometallurgical operations.

Critical-metal extraction

The extraction module identified effective organic acids for red-mud bioleaching and improved the tolerance of acid-producing fungi to alkaline tailings. Organic acids substantially outperformed conventional mineral acids for critical-metal mobilisation, achieving 2.0–7.1-fold greater light-REE solubilisation, 3.3–4.7-fold greater heavy-REE solubilisation, and 2.5–10.8-fold greater scandium mobilisation than sulfuric acid. Vanadium extraction reached 27.9%, whereas mineral acids showed negligible vanadium solubilisation. Gluconic acid provided the strongest overall performance, recovering 13.17% of light REEs, 13.21% of Sc and 27.9% of V.

These findings indicate that fungal organic acids promote metal mobilisation through mechanisms beyond simple acidification, including ligand complexation and mineral-surface interactions. Adaptive laboratory evolution increased fungal tolerance to red mud from approximately 5% (w/v) in the wild-type strains to 25% (w/v), with evolved strains also producing more organic acids than their parental isolates. This improvement increases the potential for operation at higher solids loadings, reducing dilution requirements for alkaline residue treatment. Ongoing metabolic analysis will identify pathways associated with organic-acid production, including a potential tartaric-acid pathway in *Aspergillus niger*, to inform future strain engineering for improved metal selectivity.

Selective metal recovery

The selective-recovery module established a lanthanum-immobilised metal-affinity chromatography (La-IMAC) workflow for identifying lanthanide-binding proteins from complex microbial proteomes. The pipeline combines metal exposure, protein extraction, affinity enrichment and

quantitative proteomics to prioritise biomolecular candidates for downstream metal capture.

Application of the workflow to *Pseudomonas putida* identified two previously uncharacterised lanthanide-binding proteins, Psy1 and Psy2. Recombinant production and biochemical characterisation confirmed lanthanide-specific binding, including metal-induced structural changes and micromolar binding interactions. These candidates therefore provide experimentally supported starting points for developing selective biomaterials for lanthanide recovery. Current work is assessing their selectivity, binding stoichiometry and performance in complex metal-bearing solutions before application to Ga-, Ge- and REE-containing leachates.

Carbonation and stabilisation

The tailings-management module assessed whether a wild cyanobacterial consortium could tolerate Mg-rich saline conditions and promote magnesium-carbonate biomineralisation. Bischofite was used as a controlled $MgCl_2$-rich model substrate, rather than as a post-leaching red-mud residue.

The consortium sustained growth under low-light conditions and maintained biomass at bischofite concentrations up to 62 g/L. At 31 and 62 g/L bischofite, cultures produced increased trehalose and capsular extracellular polymeric substances (EPS), indicating osmoprotection and biofilm formation under magnesium and salinity stress. These EPS matrices can retain Mg^{2+} ions and provide nucleation sites for carbonate precipitation, while cyanobacterial photosynthesis can promote conditions favourable for mineral formation. SEM analysis further revealed filament-associated mineral structures consistent with magnesium-carbonate precipitation, demonstrating potential for biological stabilisation and carbonation of suitable Mg-rich residues.

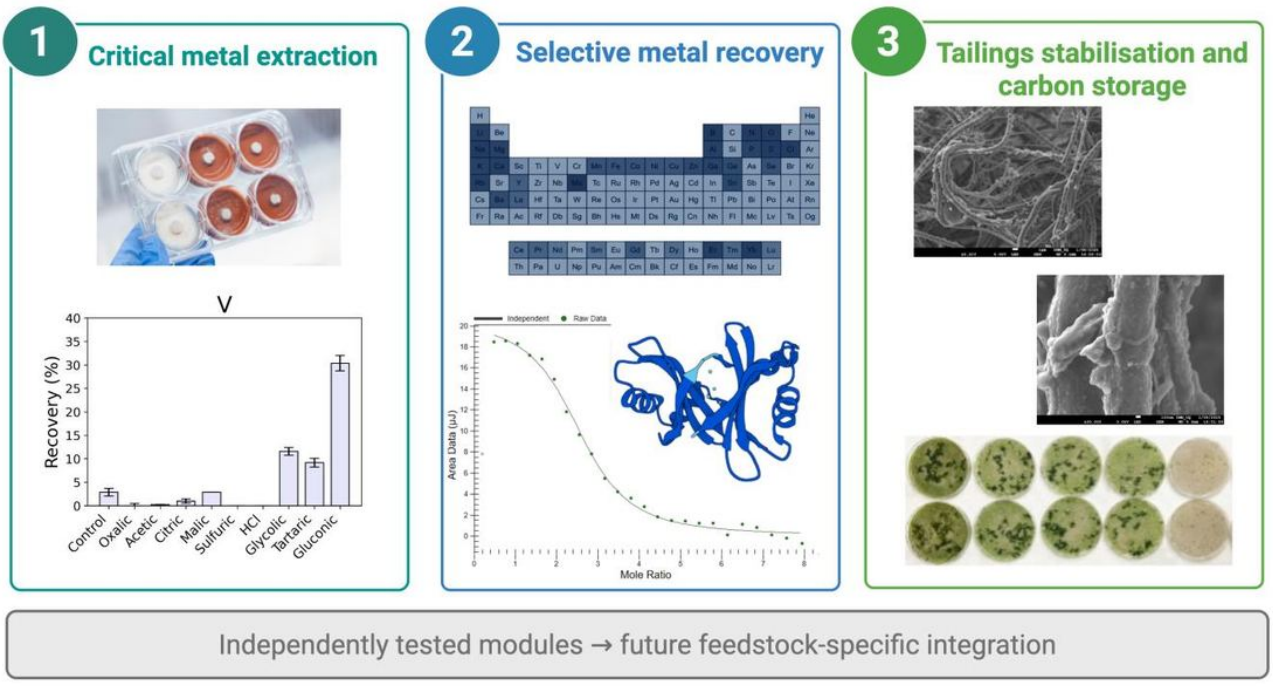


Figure 2. SynBiomining approach (1) Critical-metal extraction: extremotolerant fungi were cultivated under red-mud-relevant conditions, and fungal-derived organic acids enhanced mobilisation of REEs, Sc and V from bauxite residue. (2) Selective metal recovery: lanthanum-affinity enrichment and proteomics identified the candidate proteins Psy1 and Psy2 from *Pseudomonas putida*; recombinant and biophysical analyses confirmed lanthanide binding, while selectivity testing is ongoing. (3) Tailings stabilisation and carbon storage: a wild cyanobacterial consortium tolerated Mg-rich bischofite conditions and formed filament-associated structures consistent with Mg-carbonate biomineralisation. The three modules were tested independently using distinct model systems and provide building blocks for future feedstock-specific SynBiomining flowsheets.

Platform readiness

Together, the results move SynBiomining beyond a conceptual framework by establishing experimentally supported extraction, selective-recovery and carbonation capabilities. The fungal

module provides waste-adapted strains, effective organic-acid leaching conditions and targets for metabolic engineering. The protein-discovery module provides lanthanide-binding candidates and a transferable route towards selective biomolecular recovery materials. The cyanobacterial module demonstrates tolerance, protective biofilm formation and potential Mg-carbonate precipitation under Mg-rich conditions.

SynBiomining is not proposed as a universal or already fully coupled treatment train. Instead, it provides a design–test–learn–optimise framework for assembling biological functions into residue-specific hybrid biological–hydrometallurgical flowsheets. For example, a red-mud process may combine fungal or organic-acid extraction with selective recovery of dissolved REEs, Sc and V, whereas Mg-rich ultramafic tailings may be more suited to cyanobacterial mineral carbonation and surface stabilisation. Future work will couple extraction and recovery modules using representative leachates and evaluate carbonation on actual Mg-bearing mine wastes, guided by residue mineralogy, target-metal inventory, leachate chemistry and mine-closure requirements.

Acknowledgements

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Abstract Title: Integrated biomining approaches for critical mineral recovery and CO_2 mineralization from mine wastes

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Abstract: Mining and metallurgical operations generate billions of tonnes of mineral wastes each year including metallurgical slag, ultramafic/mafic tailings, and bauxite residue. These mineral resources are commonly landfilled despite their residual metal content and reactivity. Integrating these industries within a circular economy framework offers a pathway toward near-zero waste through the recovery, recycling and reuse of by-products. These mineral resources can be re-envisioned as secondary sources of critical minerals (CMs) and value-added products. Concurrently, the carbonation of mineral wastes provides a permanent route for atmospheric CO_2 sequestration. This study investigates whether valuable base and critical metals can be recovered from alkaline mineral wastes through heterotrophic bioleaching while preserving their potential for subsequent CO_2 mineralization. Industrial mineral wastes are typically alkaline, silica-rich, and heterogeneous in nature; therefore, samples were first subjected to physico-chemical pre-treatment. Then, a two-step process was applied, consisting of heterotrophic bioleaching followed by CO_2 mineralization at 30 °C. Heterotrophic bacterial and fungal isolates were employed for bioleaching based on their capacity for organic acid production. The isolates were screened and identified by 16S/18S rRNA gene sequencing, and demonstrated substantial production of gluconic and citric acids during glucose metabolism through fermentative and oxidative pathways. Metal solubilization results and mineralogical analyses revealed strain-dependent and metal-specific mobilization from the mineral wastes, alongside maintained carbonation potential. X-ray diffraction (XRD) and scanning electron microscopy (SEM) confirmed partial dissolution and reduced crystallinity of key mineral phases following microbial treatment. Overall, these findings demonstrate that heterotrophic bioleaching can selectively mobilize labile metals from alkaline mineral wastes while retaining their capacity for CO_2 mineralization. This integrated approach supports the development of climate-positive, circular mineral processing strategies that simultaneously address critical mineral recovery and carbon sequestration.

Keywords: Heterotrophic bioleaching; Industrial waste; Critical and base metal recovery; CO_2 mineralization

When Microbes Team Up: Synergistic Drivers of Rare Earth Element Bioleaching from Monazite

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Abstract:

Rare earth elements (REEs) are critical minerals that are essential to modern technologies and are vital to the global energy transition. However, geopolitical tensions and environmentally harmful traditional mining practices have exposed major vulnerabilities in the global supply chain of REEs. Consequently, the development of sustainable and eco-friendly REEs extraction methods is of increasing importance, with microbial biotechnology representing a promising alternative.

Mount Weld in Western Australia hosts one of the world's highest-grade REEs ore deposits, primarily as the phosphate mineral monazite. This project aims to develop sustainable bioprocessing strategies for REEs recovery from monazite by investigating the microbial ecology of monazite ores and identifying how interactions between native monazite-associated microbial communities and exogenously introduced phosphate-solubilizing microorganisms (PSMs) influence mineral dissolution and REEs mobilisation.

Experiments were designed using varying levels of phosphate concentrations in modified growth media to understand the effects of the microorganisms on mineral dissolution kinetics. X-ray fluorescence (XRF) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analysis were applied to characterise temporal shifts in elemental composition of the liquid and solid remanent before, during and after bioleaching. This integrative approach has demonstrated synergistic interactions between indigenous monazite-associated communities and introduced PSMs, resulting in a more than twofold increase in REEs concentrations in the leachate compared to systems containing either the same PSMs with sterile monazite or non-sterile monazite with only native microbes. Results also revealed interesting temporal patterns in the elemental composition of the leachate, suggesting that higher phosphorus consumption by the complex microbial community may be an important mechanism underlying this synergistic effect.

Diversity profiling through amplicon sequencing is now being applied to characterise temporal shifts in community structure during the bioleaching process to assist in the identification of microbial taxa potentially associated with enhanced phosphate and REE mobilisation from monazite. These results will provide insights for the development of sustainable bioleaching strategies using a combination of native microorganisms and PSMs.

Keywords: Rare Earth Elements, Bioleaching, Monazite, Microbial ecology

Biorecovery of Critical Metals through Protein-mediated Affinity Separations

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Abstract: An increasing demand for critical metals to support the low carbon economy, combined with decreasing grades of minerals and ores from which they have typically been extracted and geo-political tensions, destabilise supply chains and drive the need to recover metals from a broader range of mineral ores and secondary resources. These include secondary resources such as mine wastes, mine waters and brines and waste electrical or electronic equipment (WEEE). While these sources present an attractive avenue to increase metal supply in a shorter time scale than new mine developments (also required), they pose the challenge of efficiently and economically recovering multiple metals from a single resource; it is widely recognised that high complexity in source material increases costs and reduces the overall metal recovery efficiency.

These complex materials will require alternative metal recovery strategies compared to conventional minerals. Affinity separations using metal-binding proteins (biochelators) offer an attractive solution because they often have inherent high selectivity and affinity for specific metals and can enable more efficient separations than common multi-step industrial processes that rely on differences in charge, solubility or hydrophobicity (Webster et al. 2026). Furthermore, biochelators can be fine-tuned using protein engineering tools to alter selectivity and enhance stability. Importantly, while many proteins with preference for lighter metals (including various transition metals) are known, it was the recent discovery of proteins with high affinity and selectivity for Rare Earth Elements (REEs) that triggered growing interest in designing optimal biochelators for the recovery of critical elements from mixed metal aqueous streams (Zurier et al. 2025).

This paper reviews the potential of such biochelators for highly selective metal recovery from aqueous, mixed metal-bearing streams. Particular focus is on the application of this emerging technology for the extraction of REEs and lithium, but includes considerations of this approach to a broader spectrum of critical metals sourced from mine waste and WEEE. The paper also explores process design toward scaled applications, including an early-stage techno-economic analysis to identify areas and pathways with greatest potential for impact.

Keywords: Metal-protein binding, metal recovery, process development, critical metals, REE

1. INTRODUCTION

The current demand for critical metals is driven by the digital transformation, energy transition and low carbon economy; supply of primary sources is limiting, owing to their location in increasingly 'difficult to access' resources as well as the time to bring new mineral resources online through new mines. While acceleration of the time to initiate new mine projects is receiving significant attention, targeting of critical metal recovery from non-traditional sources, including low grade materials (often from mine wastes), complex resources, dilute aqueous streams and through recycling of resources is gaining

traction (Harrison, 2016; Soto-Montandon et al., 2025; Lubben et al. 2026). Conventional hydro- and pyrometallurgical approaches are often not suitable for these waste materials and streams, necessitating new technological approaches. Further, some of these new approaches may be suited to intensive, smaller footprint processes rather than capital-intensive, large-scale mines. Bio-based modules have the potential to integrate into conventional mining approaches, especially using approaches that enable high affinity separations from complex metal-bearing solutions.

The specificity of protein-metal interactions to enable metal binding with high selectivity has long been recognised through the role of metals such as copper, zinc, iron, magnesium, manganese, cobalt, nickel and others as critical cofactors in enzyme activity (Waldron et al., 2009). The affinity of the proteins for these metals is governed by the coordination environment, spatial geometry, functional groups, charge density, among others. While the drivers for understanding these interactions have been driven by maximising or controlling enzyme activity (Pedroso et al., 2017), such affinity binding between metal and protein that is reversible can also provide the basis of selective recovery of metals from complex aqueous environments. This affinity for metals has been expanded with the recent recognition of REEs as cofactors in biological molecules (Cotruvo et al., 2018), which have been suggested to form capture and release of metals for the recovery and/or separation of these critical metals.To apply such an approach to metal separation for its recovery, it is necessary to identify appropriate protein–metal couples with high affinity, rapid, high loading and reversible binding. Cost effective production of proteins with high stability should be coupled with effective immobilisation strategies that allow a high number of separation cycles.

Here, we explore the growing knowledge of proteins suited to affinity separations of varied critical metals, from rare earth elements (REEs) such as lanmodulin and its variants (Diep et al., 2026), through lithium binding peptides to critical metals such as copper and cobalt. While we recognise the potential role for biohydrometallurgy in solubilisation of these varied metals (Harrison, 2016; Soto-Montandon et al., 2025), here we focus on utilising selective proteins for affinity separations within intensive, compact, and highly selective unit operations. The need for protein discovery, biophysical characterisation, binding rates and conditions, protein expression, immobilisation and design and operation of contacting separators for affinity binding of proteins and subsequent elution are highlighted in this review (Figure 1). We discuss three metal families: REEs, copper and lithium.

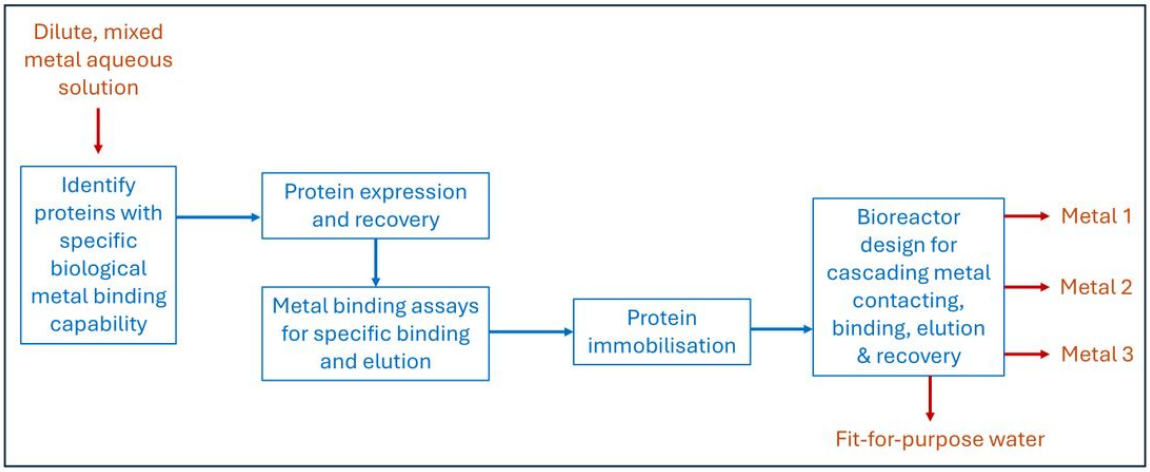


Figure 1. Schematic outlining the stages of development of the affinity binding metal recovery process

PROTEINS FOR AFFINITY SEPARATION OF REEs

REE separations are challenging because the lanthanides possess very similar chemical properties and subtle differences in ionic radius. Conventional organic solvents and acids require separation processes with multistage solvent extraction systems. Protein-based affinity separations offer a promising option by exploiting highly selective protein-metal interactions based on the small differences in ionic radii (Gutenthaler et al., 2022). The best-characterised is lanmodulin (LanM), a bacterial lanthanide-binding protein that exhibits exceptional affinity and selectivity for REEs and has been used to recover and separate REEs, including challenging metal pairs such as Nd and Dy, under entirely aqueous conditions

(Cotruvo Jr. et al., 2018, Mattocks et al., 2023). Discovery of hundreds of LanM homologues with distinct selectivity profiles illustrates substantial unexplored diversity of REE-binding proteins remains in nature, creating opportunities to discover novel biochelators for sustainable and efficient REE recovery and separation technologies (Diep et al., 2026). Such multi-metal recovery platforms show potential to reduce environmental footprint of REE production while strengthening critical mineral supply chains needed for permanent magnets, renewable energy technologies, and electrification.

AFFINITY SEPARATION OF COPPER, COBALT AND OTHER CRITICAL METALS

While affinity binding of metals to proteins for their recovery has largely been considered for the critical metals of lesser availability, especially the REEs, the concept is also promising for the separation of more common critical metals such as Cu, Co, Ni, Zn, Sn. Recovery of multiple metals from mixed metal solutions currently requires many-step flowsheets of sequential extraction, where losses across each step lead to poor metal recoveries and reduce cost-effectiveness. Selective recovery of transition metals such as cobalt and nickel from acidic mine waters, leachates and electronic waste remains challenging because of the Irving-Williams series, which predicts increasing stability of divalent metal complexes across the first-row transition metals, culminating in copper. As a result, conventional chelators preferentially bind copper, leaving lower-affinity metals unrecovered.

Biological systems provide opportunities to circumvent these thermodynamic constraints. One strategy for this bio-based affinity approach targets proteins from the electron transport chain of acidiphilic chemolithoautotrophics involved with redox cycling under metal rich conditions, thereby exploiting redox-dependent metal speciation. Copper-binding proteins such as rusticyanin (Wilson et al., 2024) selectively stabilise copper in its reduced Cu^{+} state through sulfur-rich coordination environments. By separating copper into a distinct redox pool, competing divalent metals such as cobalt and nickel become more accessible for downstream recovery. A second strategy relies on protein-based coordination site engineering. Unlike small-molecule chelators, proteins can create highly defined three-dimensional binding environments that favour specific metal geometries. By designing rigid coordination pockets that disfavour the distorted geometries preferred by Cu^{2+} while stabilising those favoured by Co^{2+} or Ni^{2+}, metalloproteins can achieve selective metal capture even in complex, multi-metal process streams. Together, these approaches illustrate how protein engineering can overcome fundamental thermodynamic limitations and enable highly selective recovery of critical transition metals from challenging resources.

AFFINITY SEPARATION OF LITHIUM

Affinity-based direct lithium extraction (Bio-DLE) utilises engineered metal-binding peptides and proteins (biochelators) to achieve Angstrom-scale molecular selectivity, representing a highly precise biological pathway to address the "Mg/Li challenge". In natural continental brines and clay-hosted mineral leachates, the strong physicochemical similarities between lithium (Li^{+}) and competing magnesium (Mg^{2+}) ions limit the efficiency of conventional precipitation and standard inorganic adsorption methods. Molecular recognition in biology overcomes these limitations by utilising specific structural coordination environments rather than relying strictly on differences in ionic charge or hydrated size. For instance, molecular modelling of the designer peptide sequence GPGAP reveals a stable cyclic conformation wherein the carbonyl oxygens of proline (2), glycine (3), and proline (5) act as the primary coordination sites for lithium binding, while the inclusion of glycine residues, which possess an inherently high affinity for lithium, enhances target recognition (Bhargawa et al., 2024).

To optimise loading capacities, researchers have investigated multimeric repeats of these target sequences, demonstrating that metal adsorption increases with the number of peptide repeats up to a trimeric limit, before encountering steric or structural hindrance. A trimeric repeat of GPGAP displayed on the outer membrane of *Escherichia coli* using the OmpC anchoring motif achieved an exceptional adsorption capacity of 3240 µmol/g dry cell weight (DCW) at 20 mM LiCl, maintaining a high selectivity for lithium over competing cobalt, chromium, and copper ions (Bhargawa et al. 2024). When translated to solid-phase supports, magnetic beads functionalised with a trimeric GPGDP sequence (Pep-D3-Bead) captured 85.5 mg of lithium per gram of bead, representing a more than 10-fold increase over bare beads, and fit a Langmuir adsorption model with a projected maximum capacity (q_{max}) of 126.3 mg Li/g bead. Furthermore, replacing the third residue with aspartic acid (D) in GPGDP ensures the

retention of a net negative surface charge even after polymerisation, which promotes strong electrostatic interactions with lithium cations under neutral pH conditions.

ENGINEERING APPLICATION APPROACHES FOR PROTEIN-BASED AFFINITY SEPARATIONS

Translating soluble biochelators from laboratory-scale molecular binding to large volume continuous-flow operations requires robust engineering design strategies that preserve protein folding, structural stability, and binding kinetics under diverse operating conditions. Operating conditions are expected to vary greatly with large differences in total particulate concentration, total pH and volume. This drives the design rationale towards immobilisation of the biochelators preserving their ability to chelate target metals while affording reusability and stability. Immobilisation approaches include binding onto a support matrix, membrane encapsulation or entrapment of the protein. Design of the immobilisation matrix requires a proactive approach with soluble biochelators being treated as a sub-unit of the platform, allowing substitution of biochelators for application in different feedstocks.

An example of the first immobilisation approach is covalent binding of proteins to polymer particles or ionic binding onto a suitable matrix. Covalent binding of proteins to immobilisation matrices offers high stability and low leaching loss due to the strength and permanency of the immobilising bond (Robescu and Bavaro, 2025). The immobilisation matrix is designed with the operating conditions in mind, allowing long operating lifetimes, even in harsh conditions. Covalent immobilisation offers great control over the orientation of the biochelator in the matrix with target side chains in the amino acid sequence binding to the matrix. This has been seen with thiol-maleimide immobilisation offering stable immobilisation at acidic pH ranges and reusability over many cycles and covalent binding to nanocellulose fibres via carbodiimide-mediated coupling (Dong et al., 2021; Bua et al. 2026).

An example of the second immobilisation approach is the entrapment of engineered selective proteins within polymeric hydrogel beads, which have been optimised for high structural stability and low fouling in water purification processes, including acid mine drainage (Yin et al., 2025; 2026). Entrapment offers a simpler approach than the covalent immobilisation allowing fast iteration and testing of novel biochelators. Each immobilisation approach has their use cases, with larger proteins better suited for entrapment; hence, both are pursued due to the diversity of biochelators available.

The wide breadth of possible process streams considered, from tailings to e-waste to dilute aqueous streams, impacts reactor design. Process stream dilution imposes a large-volume reactor as a design constraint and, as mentioned earlier, total particulate concentration is expected to vary and must be designed for to minimise upstream operation requirements. These hybrid biomaterials are designed to be packed into modular, "plug-and-play" cartridges housed within continuous-flow columns, reducing process downtime and maximising biochelator exposure to the process stream. Another reactor implementation considered is the use of the hybrid biomaterials in packed or expanded bed reactors. By operating with mild desorbing agents (such as citric acid gradients, 1 mM EDTA or hydrochloric acid gradients) rather than corrosive mineral acids, these platforms achieve high elution yields while mitigating sorbent degradation and secondary waste generation.

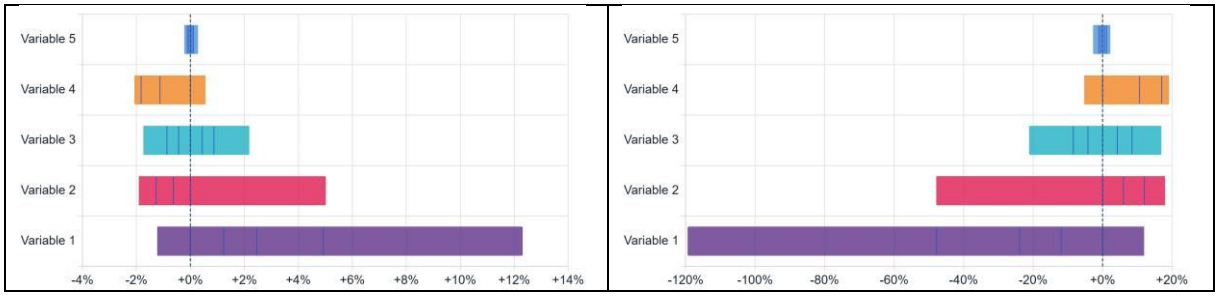


Figure 2. Exploring the impact of protein and bead cost, protein binding and loading, and bead lifespan on the operating costs (left) and margin (right) of metal recovery through protein binding

Initial techno-economic analyses (TEAs) have been performed to determine which operational parameters offer the greatest effect on process economics. A tornado diagram highlighting these results can be

seen in Figure 2. Clearly, cost-effective separation via immobilised biochelators is maximised on achieving an extended operational lifetime, where the protein and accompanying matrix is stable across many process cycles. The system is designed to maximise robustness against harsh operating conditions, afford high protein stability and be reusable for many cycles.

FUTURE OUTLOOK AND POTENTIAL

The selectivity and design flexibility of protein-based separations open up extraordinary opportunities for the multi-stage valorisation of complex secondary resources, such as industrial leachates, geothermal fluids, mine tailings, and electronic waste (WEEE). By leveraging high-throughput functional metagenomic screening to mine the "mineral microbiome" and using generative AI for protein design refinement, researchers can identify novel, non-physiological metal-binding motifs adapted to extreme pH and hypersaline conditions. This molecular recognition platform can be expanded to design sequential capture architectures that selectively isolate adjacent Rare Earth Elements (REEs) as well as valuable co-products like cobalt, nickel, lithium, magnesium, and strontium. Specifically, recovering strontium (Sr) from lithium-depleted tailings streams not only improves the overall economics of DLE operations but also provides high-purity photocatalysts essential for green hydrogen production, aligning future mineral processing directly with the global transition to a circular, low-carbon economy.

These proteins, where produced through efficient expression and in a stable form, can be inserted into affinity metal separation reactor systems through immobilisation. Appropriate contacting can then drive removal and recovery of metals from aqueous streams and secondary resources. Focussed techno-economic analysis (TEA) and life cycle analysis (LCA) serve as essential tools to benchmark these emerging process parameters, such as energy demand, lixiviant recycling, and biosorbent lifespan, against the standard process options. These tools also help to identify the cost offsets provided by reduced chemical consumption.

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A Preliminary Investigation into the Bioleaching of Deep-Sea Fe/Mn Nodules by Deep-Sea Sediment Enrichment Cultures under Simulated Deep-Sea Conditions

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Abstract: Deep-sea minerals represent a substantial and strategically significant resource for the procurement of critical raw materials that are indispensable to the global energy transition. Of particular significance among these deposits are manganese nodules, which are of economic importance due to their abundance and high nickel, cobalt, and copper content. Although biohydrometallurgy offers an eco-friendly processing route, there is still a paucity of knowledge regarding the adaptability of microorganisms and the dominant reaction pathways under the extreme high-pressure conditions of the deep ocean. This study presents a preliminary investigation into the bioleaching characteristics of manganese nodules using sulfur-oxidizing enrichment cultures derived from deep-sea sediments within a custom-designed simulated deep-sea environment.

Experiments were conducted within a high-pressure bioreactor system that was designed to simulate the key parameters of deep-sea environments [insert pressure/temp, e.g., 30 MPa, 4 °C]. Preliminary results demonstrated that the indigenous microbial community was capable of successfully initiating mineral dissolution. A critical examination of the data unearthed a discernible structural shift, marked by a concurrent surge in the prevalence of both iron-reducing and sulfur-oxidising microorganisms. This specific enrichment pattern suggests that, in contrast to surface environments, bioleaching in deep-sea conditions may be predominantly driven by a coupled sulfur-oxidation and iron-reduction process. In this pathway, the cycling of iron is hypothesised to act as a mediator for electron transfer, facilitating the reductive dissolution of manganese oxides.

Furthermore, surface characterisation of the leached nodules detected a significant accumulation of sulfur oxidation intermediates (e.g., polysulfides) and extracellular metabolic products. These findings confirm active interfacial bio-corrosion and suggest that the microbial consortium creates a localised microenvironment favourable for mineral breakdown. Collectively, these results indicate that deep-sea sediments are a reservoir of diverse, robust microbial candidates capable of metal extraction. This work underscores the considerable potential inherent in the exploration of indigenous deep-sea microflora for the purpose of developing future in-situ or shipboard marine biohydrometallurgy technologies.

Keywords: Deep-sea polymetallic nodules; Deep-sea microbial consortia; Bioleaching; Deep-sea minerals; Deeps-sea biogeochemical cycle.

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Bioleaching of mine tailings fractions for metal extraction and purification in the project ReTail

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Keywords: tailings, Rammelsberg mine, cobalt, flotation, bioleaching

Abstract

In reprocessing of mine tailings the extraction of metals from solids via (bio)leaching plays an important role, on the one hand to recover valuable metals and on the other hand to purify tailings fractions for their use as construction material for example. Material from the Bollrich tailings pond of the former Rammelsberg mine in Goslar, Harz mountains, Germany was reprocessed and three tailings fractions were obtained via flotation. Bioleaching experiments with mesophilic or moderate thermophilic lithotrophic, acidophilic ferrous iron- and sulfur-oxidizing mixed cultures showed a high extraction efficiency for cobalt and other metals besides lead, which could be extracted using organoheterotrophic bacteria or fungi. The results allow to further develop a concept for large-scale recycling of the complete tailings within the current joint research project ReTail.

Introduction

Processing residues from mining, such as tailings, often contain considerable amounts of recyclable materials. Worldwide work is being done on the recording of the raw material potential and on extraction processes for the reprocessing of tailings including bioleaching [1, 2]. Scientists of the technical university TU Clausthal (Institute of Geotechnology and Mineral Resources), Harz mountains, Germany have been working together with several partner institutions and companies for several years on a concept for the reprocessing of the tailings of the former Rammelsberg ore mine in Goslar, Germany, which operated for >1000 years until its closure in 1988 (today it is a UNESCO World Heritage Site). In the years 1935/36 the construction of a flotation plant allowed for an efficient extraction of the target metals copper, zinc and lead from the complex polymetallic sulfide ore, and the flotation tailings have been stored in the Bollrich pond, which is estimated to contain approximately 3.6×10^6 m^3 and 7 Mt. of material with an average water content of 30%. [3]. Based on results of two previous research projects REWITA and REMINTA funded by the German federal ministry of research, the current project ReTail aims, with regard to a later large-scale recycling of the complete tailings, to further develop technical and logistical aspects up to a technology readiness level of TRL 6, as well as decommissioning planning, process control of the complex processing procedures, and the overall structuring of the value chains involving a variety of industrial actors and research institutions. Chemical leaching and bioleaching steps [4, 5, 6] are foreseen in the process scheme, and the goal of the partner BGR is the development of bioleaching processes for the extraction of contaminating lead and arsenic from various tailings fractions, as well as for the extraction of cobalt and other valuable metals from the sulfide fraction obtained from the partner TU Clausthal via flotation of the tailings. Here we report about published joint laboratory leaching experiments done by Tamara Azevedo Schueler [6] at TU Clausthal and BGR in this context (https://www.bgr.bund.de/EN/Themen/Rohstoffe/Projekte/Lagerstaettenforschung_laufend_en/ReTail_BMFTR_en/ReTail_BMFTR_en.html).

Methods

Bioleaching experiments with lithotrophic, acidophilic ferrous iron- and sulfur-oxidizing mixed cultures were done in 2 L or 20 L stirred tank aerated bioreactors using either a consortium of mesophiles at 30 °C or a consortium of moderate thermophiles at 45 °C [6]. Bioleaching experiments with organoheterotrophic microorganisms were done in 250 mL Erlenmeyer flask shaken at 30 °C. Control tests were also performed without the addition of microorganisms (Figure 1).

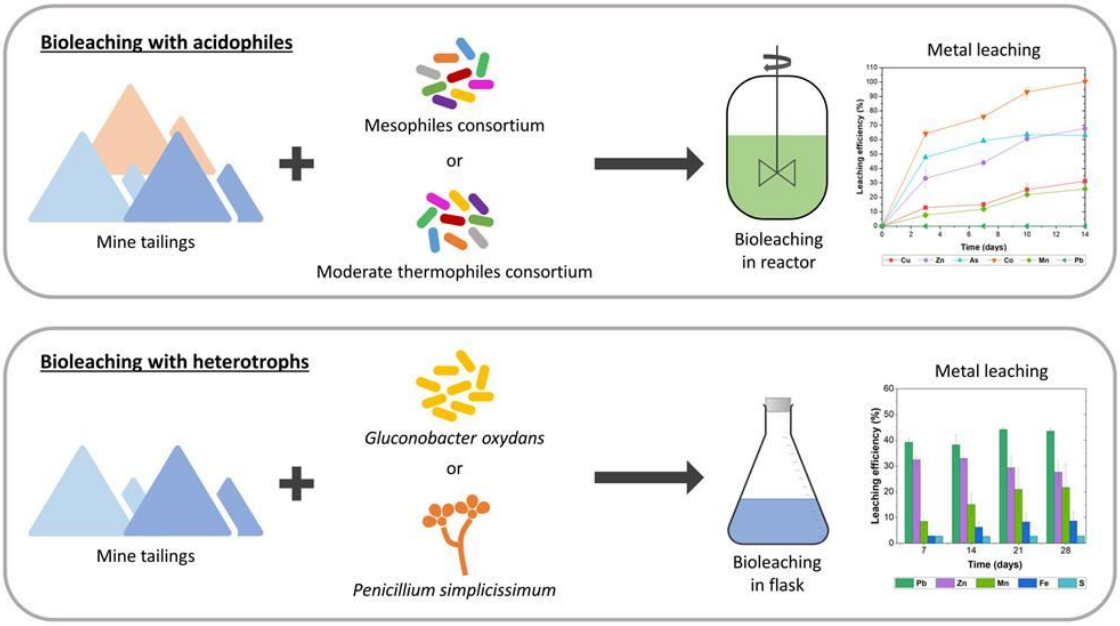


Figure 1. Experiment scheme for tailings bioleaching with consortia of acidophilic, lithotrophic mesophilic or moderate thermophilic bacteria and archaea for metal sulfide dissolution and extraction of Co and Cu (and other metals; top), or with chemoorganoheterotrophic bacteria or fungi for mainly Pb extraction (bottom; taken from [6] Graphical Abstract).

Materials

The bulk Bollrich tailings were processed via flotation, and three fractions were generated and analysed. Data are given in Table 1.

Table 1. Physical properties, chemical and mineralogical compositions of the samples from the bulk Bollrich tailings (BB) and its flotation fractions barite tailings (BT), barite concentrate (BC), and sulfide concentrate (SC), obtained via flotation of the bulk tailings (taken from [4-6]).

		Mine tailings samples			
		BB	**BT**	**BC**	**SC**
Physical properties	Density (g/cm³)	3.53	3.13	4.13	4.21
	Surface area (m²/g)	8.08	5.7	2.3	3.6
Minerals (% w/w)	Ankerite		19	7	3
	Barite		11	72	7
	Chlorite		8	5	4
	Muscovite		26	5	6
	Pyrite		6	4	58
	Quartz		12	3	3
Major elements (% w/w)	Al	3.9	5.6	1.5	1.5
	Ba	11.3	8.2	42	3.2
	Fe	11.6	7.2	3.7	30.2
	S	11.6	4.5	10.1	34.6
	Si	8.8	14.3	2.8	2.9
Minor elements (% w/w)	As	0.09	0.04	0.03	0.3
	Co	0.019	0.007	0.006	0.06
	Cu	0.14	0.06	0.03	0.5
	Pb	1.36	0.8	0.4	3.7
	Zn	1.48	0.7	0.4	4.4

Results and Discussion

Bioleaching laboratory experiment results clearly showed efficient metal extraction from bulk Bollrich tailings (BB) as well as the flotation fractions barite tailings (BT), barite concentrate (BC), and sulfide concentrate (SC). Lithotrophic, acidophilic ferrous iron- and sulfur-oxidizing mixed cultures showed a high extraction efficiency for cobalt, zinc and arsenic in 20 L stirred bioreactors. About 67% or 72% of Co, 28% or 50% of Zn, 56% or 28% of As, 28% or 36% of Cu, and 6% or 5% of Mn were extracted from SC using mesophiles or moderate thermophiles, respectively. Lead was not detected in solution due to $PbSO_4$ precipitation. However, lead could be extracted using organoheterotrophic bacteria or fungi excreting organic acids [5, 6]. Selected results are shown for bioleaching of BT in Figs. 1 and 2. Citric acid has also shown to be most efficient in lead extraction in chemical leaching experiments with the same material [4].

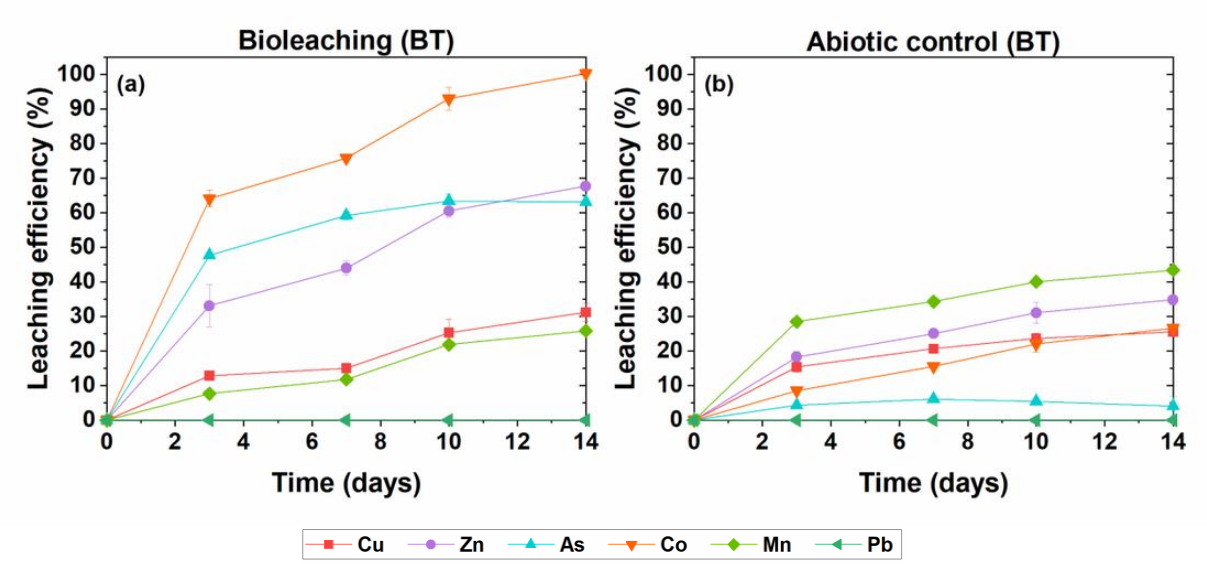


Figure 2. Metal extraction from barite tailings (BT, 10% (w/v)) in bioleaching tests with acidophilic mesophiles (a) and abiotic control tests (b) in 2 L-stirred bioreactors at 30 °C over 14 days (taken from [6]).

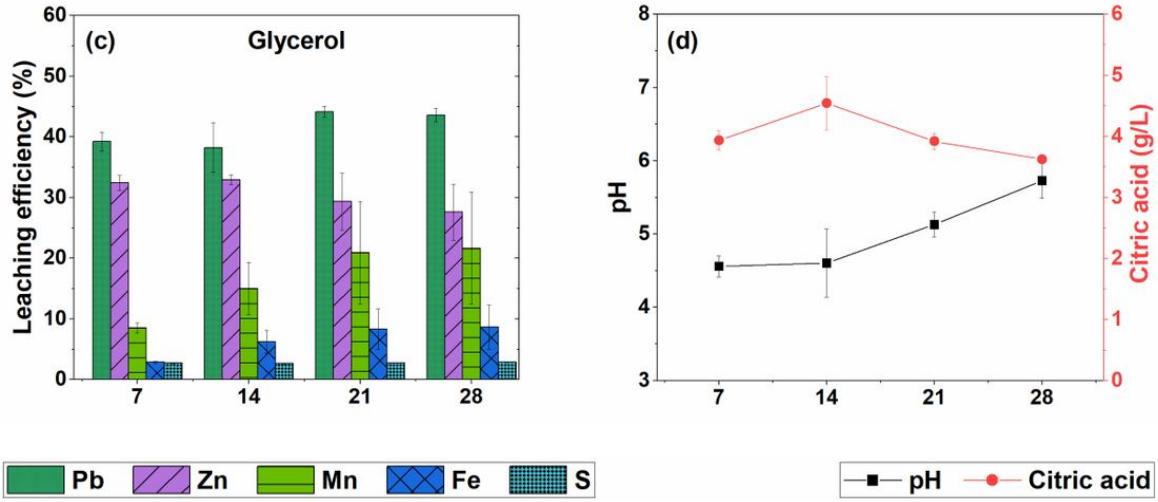


Figure 3. Metal extraction from barite tailings (BT, 10% (w/v)) c), and pH and citric acid concentration (d) in bioleaching tests with the fungus *Penicillium simplicissimum* in medium containing glycerol at 30 °C over 28 days. The concentrations of Cu, Co, and As were below the limit of detection (< 0.5 mg/L, not shown). Abiotic control assay showed leaching efficiencies of <5% for all metals (not shown; taken from [6]).

Conclusions

Based on published laboratory leaching experiments at TU Clausthal and BGR summarised here, various bioleaching variants, including combinations of inorganic-chemical and bioleaching methods, will be tested and optimised within the ReTail project as part of a process development for recycling of the complete material from the tailings pond of the Rammelsberg ore mine.

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Towards sustainable copper supply from WEEE: Configuration of a two-stage bioleaching system for PCB treatment

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Keywords: Printed Circuit Boards; Bioleaching; Microbial Oxidation; E-waste; WEEE

Abstract

Recycling waste metal streams, including printed circuit boards (PCBs), can contribute to the demand for critical metals for low-carbon technologies while simultaneously alleviating the environmental burden associated with increasing waste electrical and electronic equipment (WEEE). Extraction of base metals from PCBs both allows their recovery to contribute to the current critical metal supply deficit and enhances recovery of gold, silver, and platinum group metals. To minimise the reagent supply needed, we have reported the potential of microbial ferrous iron oxidation for the supply of ferric iron using Cu-adapted immobilised microbial consortia consisting of *At caldus*, *L ferriphilum*, and *Acidiplasma* (*Ap*) *cupricumulans*. Here, we expand on our two-stage bioleaching approach through an open, semi-continuous two-stage bioleaching reactor system consisting of a stirred tank reactor (STR) for ferric leaching of PCBs coupled to a packed-bed bioreactor for microbial ferric iron regeneration. The reactor system is fitted with an outlet stream to enable inter-stage copper recovery and control the maximum metal concentrations in the system. Using a 1 litre STR for PCB leaching and a set of packed bed columns for ferrous iron oxidation, we varied the outlet or purge rate (0.01 – 0.03 L/h) to recycle rate at increasing PCB solid loading (0 – 36 % w/v). The volumetric microbial rates of Fe^{3+} regeneration were compared to the Fe^{3+} reduction rates associated with metal leaching from PCBs at these varying reactor configurations. The Fe^{3+} reduction rate was maintained around 0.194 g/L/h, while Fe^{2+} oxidation rate decreased with increasing PCB loading. On introduction of a purge stream, Fe^{2+} oxidation improved by 19%. Increasing the purge rate from 0.01 to 0.03 L/h further improved the rate by 57%. These findings are anticipated to improve the design of the PCB bioleaching process and enable its upscaling towards subsequent commercialisation.

Introduction

Owing to their high metal content, printed circuit boards (PCBs), a core component of waste electrical and electronic equipment (WEEE), receive attention as a secondary resource for valuable metals. In PCBs, concentrations of metals of value such as Cu, Zn, Ni, Co, Au, Al, are often high and in purer form than in their typical ores [1–3]. For example, Cu content is as high as approximately 20% w/w in PCBs, compared to approximately 0.2 – 0.7% w/w in low grade Cu ores currently being processed [4,5]. The traditional landfilling as a disposal strategy for waste PCBs are associated with the well-known socio-environmental and economic liabilities and no longer considered acceptable. Further the demand for PCB-associated metals is predicted to soon outstrip supply, driven by the transition to a low carbon economy and rapid digitisation. Thus, it is imperative to develop technologies that enable the sustainable recovery of critical metals from anthropogenic activities, including WEEE, to contribute to resource efficiency and the circular economy, while alleviating environmental burden.

Bioleaching offers a potentially environmentally friendly and sustainable route for the extraction of base metals from PCBs prior to the extraction of precious metals. The *in-situ* microbial regeneration of the

primary Fe^{3+} oxidant in bioleaching is a key advantage over the traditional pyro- and hydrometallurgical routes. The addition of fresh lixiviant and effluent generation is thus minimised. Together with a low required operating temperature, bioleaching offers a potentially economically viable option, particularly for low grade PCBs. Further, the extraction of mixed metals into an aqueous stream is expected to enable selective recovery of multiple key metals of value. Extraction of more than 90% of base metals has been reported during the bioleaching of PCBs with pure or mixed consortia, including *Acidithiobacillus* (*At*) *ferrooxidans*, *At thiooxidans*, *Sulfobacillus* (*S*) *thermosulfidooxidans*, *Leptospirillum* (*L*) *ferriphilum*, *L ferrooxidans*, etc. in shake flask experiments at low PCB concentrations[6–9]. However, with increasing PCB loading (solid-to-liquid ratio (%w/v) and dissolved metal concentrations, microbial activity becomes limited. An optimal PCB loading of 1% or below is typically reported in the literature, with a threshold of 5% [10–12].

We have previously reported high bioleaching efficiency of 96% Cu, and >79% Ni, Zn, Pb, and Cr at high PCB loading of 18% w/v in a two-stage reactor system [13]. A purge stream was fitted in the two-stage reactor system to minimise metal accumulation[10]. Here, we explore varying purge rates for optimum conditions for the maximum delivery rate of the Fe^{3+} oxidant at increasing PCB loading.

Methods

Custom-made template PCBs used were acquired from Trax Interconnect (Pty) Ltd, Cape Town, South Africa. The mechanical pretreatment of the PCBs and their metal content is described in Maluleke et al [13]. A two-stage reactor system used comprises a stirred tank reactor (STR) for ferric leaching of PCBs coupled to a packed-bed bioreactor for microbial ferric iron regeneration [13]. The column bioreactors in series were packed with polyurethane foam particles colonised with Cu-adapted mixed acidophilic culture dominated by *At caldus*, *L ferriphilum*, and *Acidiplasma* (*Ap*) *cupricumulans*. Post Fe^{3+} oxidation of the PCBs, the flowrate of the Fe^{2+}-rich stream through the packed-bed column bioreactors was 0.05 L/h, and the microbially regenerated Fe^{3+}-rich stream was circulated back into the STR. An outlet stream, purge stream, was fitted between the outlet of the chemical PCB leach reactor and the inlet of the column bioreactors to allow the reactor system to operate as an open reactor system. The purge rate was varied between 0.01 and 0.03 L/h. The reactor was operated with no purge stream up to 15% w/v PCBs. The purge stream was introduced from 18% w/v, and the purge rate was increased from 0.010 L/h, through 0.015 L/h, to 0.030 L/h. PCBs were loaded intermittently, at a 3% w/v dosage, into the chemical leach reactor to a cumulative 36%. Bioleaching efficiency was monitored and evaluated from the changes in pH, redox potential, Fe (Fe^{3+}/Fe^{2+} and total Fe) concentration, and leached metal concentrations.

Results and Discussion

Owing to the microbial regeneration rate of Fe^{3+} being the rate-determining step in bioleaching, the bioleaching efficiency was evaluated by investigating the effect of PCB loading on Fe^{2+} oxidation rates. The volumetric Fe^{3+} reduction and Fe^{2+} oxidation rates with increasing cumulative PCB loading are presented in Figure 1. The volumetric rates were calculated from the change in the concentration of Fe^{3+} and Fe^{2+} over time. The volumetric Fe^{3+} reduction rate, as a result of metal oxidation in PCBs, was maintained between 0.177 and 0.218 g/L/h, with an overall average of 0.194 g/L/h, with increasing PCB loading up to 36% w/v. This is as expected, given that the Fe^{3+} reduction is a chemical reaction and there were no changes in the reactor conditions, i.e. temperature and pH. Conversely, as a result of the inhibitory effects of accumulated metal ions released from the PCBs, the volumetric Fe^{2+} oxidation rates, as a function of metabolic microbial activity, decreased from 0.047 g/L/h to 0.038 g/L/h with an increase in PCB loading from 3 to 12% w/v, respectively. A purge stream was introduced at a cumulative 18% w/v PCBs to mitigate metal accumulation within the reactor, and its associated inhibition of microbial Fe^{2+} oxidation. At a purge rate of 0.01 L/h, the volumetric Fe^{2+} oxidation rate improved from 0.038 to 0.048 g/L/h. Despite the increasing cumulative PCB loading (to 24% w/v), it further increased slightly to 0.050 g/L/h at the same purge rate. Increasing the purge rate to 0.015 g/L/h further increased the Fe^{2+} oxidation rate to 0.074 g/L/h. Due to tube blockage and leakage, the rate decreased to 0.022 g/L/h over the period the reactor was operated at 30 % loading. However, on restoring operation and ramping the purge to 0.03 L/h, Fe^{2+} oxidation rate recovered to stabilise around 0.058 g/L/h.

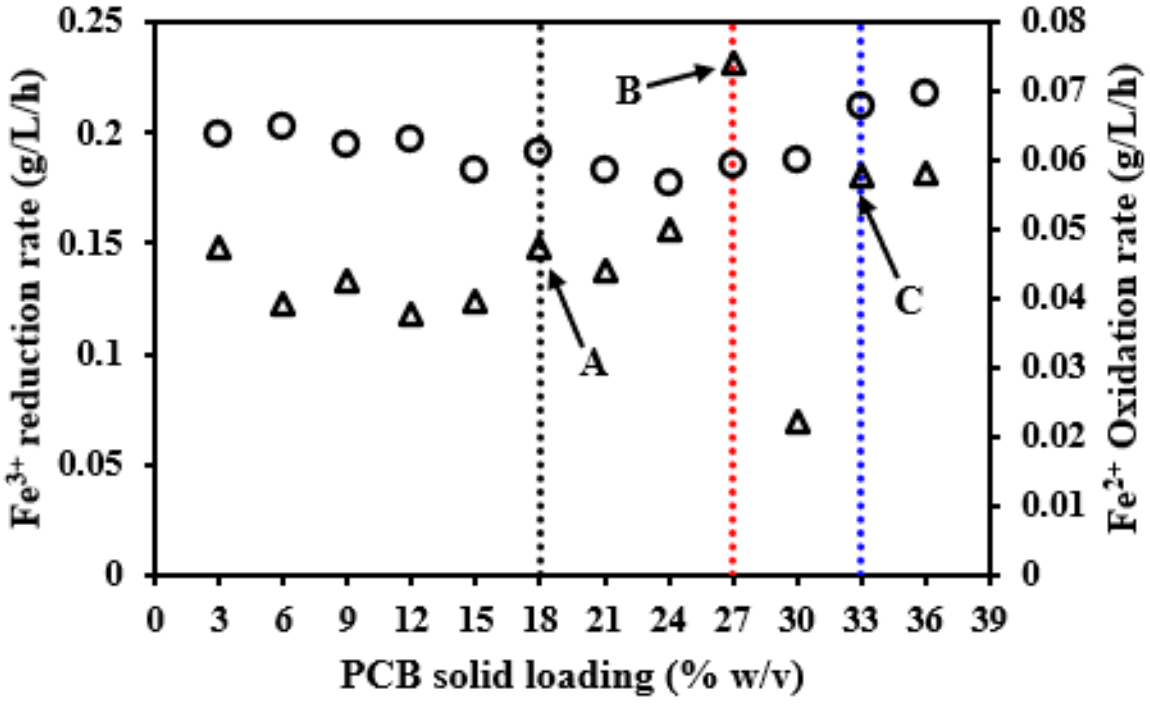


Figure 1. Volumetric ferrous (Fe^{2+}) oxidation (Δ) and ferric (Fe^{3+}) reduction (O) rates at increasing cumulative PCB solid loading during bioleaching of PCB in a two-stage reactor system. The vertical dotted black line represents the point at which a purge stream was introduced: A: 0.01 L/h, with increase at B: 0.015 L/h, and C: 0.03 L/h purge rate.

The high volumetric Fe^{2+} oxidation rates with increasing solid PCB loading in the open system reported in this study present a promising approach to mitigating microbial inhibition with accumulating metal ions at high PCB loadings, one of the major drawbacks in PCB bioleaching systems. The 19% increase in Fe^{2+} oxidation on the introduction of the purge stream (18% w/v, purge rate:0.01 L/h) and 57% increase at the 0.03 L/h purge rate relative to the lowest Fe^{2+} oxidation rate of 0.038 g/L/h (at no purge stream) was attributed to reduced metal ion accumulation through introducing the outlet stream. The increasing purge rate may also improve microbial conversion by increasing the residence time in the bioreactor, allowing increased contact time for Fe^{2+} oxidation. To the best of our knowledge, this study is the first to report on sustained microbial activity at high PCB loading of 36% w/v. Further optimisation of the reactor study may include the use of Fe source such as pyrite to provide in-situ Fe^{3+} to compensate for the fraction lost from the reactor in the outlet stream generated to recover the critical metals of value, with the concomitant generation of acid to maintain the required acidic condition.

Conclusions

The open reactor configuration explored in this study allowed improved volumetric Fe^{2+} oxidation rates at increasing PCB loading. The introduction of an outlet or purge stream improved the Fe^{2+} oxidation rate by about 19% relative to a reactor configuration with no outlet stream. Increasing the outlet rate to 0.03 L/h further increased the Fe^{2+} oxidation by 57% relative to the closed system. The high PCB solid loading achieved here, with sustained microbial activity, offers an opportunity to optimise both the rate and extent of metal recovery. Such an approach has great potential to improve the economic feasibility of the PCB bioleaching process, a critical step in technology development and implementation, allowing selective recovery of critical metals from PCBs.

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E-waste bioleaching in a bioreactor: Experimental and numerical studies of particles suspensions

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Abstract:

Bioleaching with acidophiles has been proven to be an efficient process to recover base metals, such as copper, contained in Printed Circuit Boards (PCBs).

Prior to bioleaching, waste PCBs are grinded into particles of dozens µm to a few mm size in order to enhance the reactive surface, the metals accessibility and reduce the process duration. A sufficient agitation rate is required to ensure particles suspension; this generally means a higher power consumption, which must also be limited to prevent microbial damage. PCBs particles are, however, heterogeneous in size, shape, and composition (mean density of 2.446). A sufficient particles suspension is thus hard to manage.

The study of this dynamic and heterogeneous system was performed by coupling computational fluid dynamics (CFD) using Lattice-Boltzmann approach (LB) for fluid flow modelling and Discrete Element Method (DEM) for particles modelling. Three representative classes of particles, namely metals (7549 $kg \cdot m^{-3}$), plastic (2715 $kg \cdot m^{-3}$) and glass (1029 $kg \cdot m^{-3}$) were simulated. The case study consists in a 2 L bioreactor filled with a culture medium and aerated with gas bubbles. The minimal agitation rate for suspension (*Njs*) was experimentally determined using the Zwietering criterion to assess the effects of solid loading (40, 100 and 200 $g \cdot L^{-1}$) particle granulometry (63 µm to 2 mm) and gas flow rate (15, 30, 60 and 100 $L \cdot h^{-1}$). A least 122 measurements were performed at different conditions.

Simulations showed a heterogeneous particle distribution within the bioreactor. Most particles found at the tank bottom were of metals type (60,5%) with a low concentration of plastic (1.2%). Disperse N_{js} values were found, ranging from 300 to 1500 RPM. N_{js} is most sensitive to solid concentration followed by gas flow rate and mean particle diameter with respective power-law exponents of 0.226, 0.155, and 0.114. Studies with a similar system yielded lower values of N_{js} (around 700 RPM).

Keywords: Bioleaching, modelling, Computational Fluid Dynamics (CFD), multiphase fluid flow, Printed Circuit Board.

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Sustainable recovery of copper and gold from waste Printed Circuit Boards by biogenic ferric iron and thiosulphate

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Abstract: (200-300 words)

Recycling metals from electronic waste is recognised as an effective approach to address the environmental challenges, ensuring human safety and conserving natural resources. Among recycling methods, bioleaching is a sustainable and green technology for metal solubilisation using microorganisms. This research investigates a two-step bioleaching process for recovering copper and gold from waste printed circuit boards. In the first step, pure copper was recovered using an indirect bioleaching and electrowinning. The cultivation of the bacterium *Acidithiobacillus ferrooxidans* in the 2 L continuous stirred tank reactor (CSTR) produced Fe^{3+} at high concentration (11.5 g/l), which ensured a consistent supply for the following leaching step. Then, leaching of copper in 2 litre CSTR resulted in 100% of copper solubilisation at 40 ˚C, pulp density of 6%, and a hydraulic residence time of 96 hours with pH control, and later around 70.85% of pure copper was recovered from the metal rich solution by the electrowinning process with a current density of 0.0168 A/cm^2 over 8 hours. To validate scalability, the process was further performed in a 6-litre CSTR. A closed-loop system was proposed, where the post-electrowinning solution was recycled as a nutrient source (Fe^{2+}) for bacterial growth, significantly reducing material costs and improving sustainability. In the second step, biogenic thiosulphate produced by the bacterium *Acidithiobacillus thiooxidans* was investigated as an environmentally friendly leaching agent for gold leaching from residue waste. Biogenic thiosulphate is an unstable intermediate produced during the sulphur oxidation pathway of bacteria, stabilises by pH adjustment and inhibitors. The highest gold leaching rate, 9.6% (0.0614 mg/L), was achieved after 3 days under conditions of 30 °C, 120 mg/L biogenic thiosulphate, 0.02 M Cu^{2+}, 1 M ammonia, and 1% pulp density. The use of biogenic thiosulphate proved to be a practical alternative to chemical thiosulphate, opening the door for green gold recovery process.

Keywords: Waste printed circuit board, Bioleaching, Continuous stirred tank reactor, Gold, Acidophilic microorganism

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MAXIMISING RESOURCE EFFICIENCY THROUGH BIOLEACHING OF COPPER AND ZINC FROM BAAL GAMMON MINE WASTES: TOWARDS A CIRCULAR ECONOMY

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Keywords: Mine wastes; Copper; Zinc; Baal Gammon; Bioleaching

Abstract

Mine wastes from the Baal Gammon site in north-eastern Queensland, Australia, represent potential secondary resources for copper (Cu) and zinc (Zn). This study evaluated the mineralogy of the Baal Gammon mine waste and potential to recover metals of interest through bioleaching. Bioleaching was compared on inoculation with an indigenous microbial consortium enriched from Baal Gammon and with an enriched consortium from Mt Morgan, both dominated by acidophilic iron- and sulfur-oxidizing microorganisms implicated in metal extraction from sulfidic minerals.

Mineralogical characterisation revealed a complex silicate-sulfide matrix, with 45% quartz, 5% plagioclase and 15% Fe-oxide, 1.17% chalcopyrite, 0.6% pyrite, 8% pyrrhotite and 0.08% sphalerite. Batch bioleaching experiments using 0.5-1.0 mm particle size demonstrated a clear link between microbial community and metal solubilisation. The Baal Gammon culture, enriched as a consortium containing *Acidithiobacillus and Leptospirillum species* amongst others, achieved the highest Zn recovery (~50%) within 50 days, significantly outperforming the Mt Morgan culture and uninoculated controls. Copper recovery reached ~30% across experiments inoculated with enrichment cultures from Mt Morgan, Baal Gammon and the un-inoculated experiment, indicating a shared mechanism for Cu solubilisation. These bioleaching systems were set up with a starting pH of 2.3 maintaining stable acidic pH values (2.0–2.5) throughout and microbial populations stabilising between 1.0×108 and 3.2×108 cells mL^{-1} leachate.

Post-leaching mineralogical analysis indicated an increase in amorphous content in the bioleached residues, attributable to secondary precipitate formation and/or silicate weathering. Secondary precipitate formation or incomplete liberation both contribute to restricting extent of metal recovery.

These findings demonstrate that endogenous microbial consortia, tailored to specific sulfidic mineralogy, can facilitate metal recovery from polymetallic waste rock offering potential for sustainable valorisation of mine wastes and reduction of environmental liabilities through bio-hydrometallurgical processes.

Introduction

The mining industry increasingly faces the challenge of processing low-grade ores, which demands energy-intensive processing methods and generates vast quantities of solid waste. Queensland's extensive mining history and abundant repositories of mine waste offer a unique opportunity to recover valuable

metals from these mine wastes. Historically, poor mineral liberation and a focus on single-metal extraction have left these resources largely untapped[1-3]. Therefore, this study investigated bioleaching of Cu and Zn from Baal Gammon mine waste using indigenous iron- and sulfur-oxidising consortium enriched from Baal Gammon site and non-indigenous consortium from the abandoned Mt Morgan mine (also in northern Queensland[4-5]) By tailoring bioleaching to site-specific attributes (e.g., AMD risk, mineralogy, and 16S rRNA microbial analysis), this approach maximises recovery while minimising environmental harm, contributing to sustainable management of legacy mining liabilities.

Methods

Baal Gammon Mine Waste Sample Collection and Characterisation

Baal Gammon mine waste samples were collected as part of the Mine Waste Transformation through Characterisation (MIWATCH) project at the Sustainable Minerals Institute (SMI), University of Queensland, which evaluated the endowment of critical and strategic metals in sulfidic waste as a basis for potential reprocessing[6-7]. Bulk chemical analysis of a representative subsample of Cu-bearing waste rock was performed at ALS Geochemistry Laboratory (Brisbane). Quantitative X-ray diffraction (XRD) analysis was conducted at Sietronics and automated mineral liberation analysis (MLA) was conducted at UQ's JKMRC.

Microbial Inoculum Characterisation and Bioleaching Experiments

Mesophilic acidophilic microbial consortia were sourced as biofilms from two abandoned mine sites in Queensland, Australia: Baal Gammon (indigenous culture) and Mt Morgan (non-indigenous inoculum). Enrichment and maintenance of the cultures were performed in 250 mL shake flasks incubated on rotary shakers at 130 rpm and 30 °C. The basal medium contained 0.4 g L^{-1} $(NH_4)_2SO_4$, 0.4 g L^{-1} $MgSO_4 \cdot 7H_2O$, and 0.1 g L^{-1} K_2HPO_4, supplemented with 3.33 g L^{-1} $FeSO_4 \cdot 7H_2O$ as the sole energy source to support growth of iron- and sulfur-oxidising acidophiles. The microbial community composition of the Baal Gammon biofilm was characterised by high throughput 16S rRNA gene amplicon sequencing, performed on the samples collected before and after bioleaching experiments.

Batch bioleaching experiments using 0.5-1.0 mm particle size Baal Gammon mine tailings were conducted to evaluate the performance of indigenous and inoculated microbial consortia relative to un-inoculated controls. The un-inoculated control experiments were performed in 250 mL Erlenmeyer flasks containing 100 mL of sterile basal salts liquid medium without microbial inoculum. To suppress growth of indigenous bacteria in the BG control, glutaraldehyde was added at a final concentration of 0.5% (v/v). A summary of the experimental conditions is provided in Table 1. The experiments were conducted using Erlenmeyer flasks containing 90 mL of sterile mineral salts medium and 10 mL of the microbial culture in exponential growth phase (initial cell density 1.4×10^8 cells mL^{-1}), resulting in a final volume of 100 mL.

Table 1. Conditions of the Baal Gammon sample bioleaching experiments using Mt Morgan and Baal Gammon culture

Test*	m9K medium (mL)	Microbial strain (mL)	Time (days)	Glutaraldehyde (mL)	BG (g)	Culture type
Control-MM	100	-	50	-	10	-
Bioleach-MM	90	10	50	-	10	Mt. Morgan
Control-BG	100	-	50	1	10	-
Bioleach-BG	90	10	50	-	10	Baal Gammon

*All experiments were performed in triplicate for 50 days at initial pH 2.3 (adjusted with 5 M H_2SO_4), constant temperature of 27 °C, and orbital shaking at 130 rpm. Weekly sampling (5 mL) was conducted for solution analysis by inductively coupled plasma–optical emission spectrometry (ICP-OES) at the Australian Centre for Water and Environmental Biotechnology (ACWEB) and bacterial enumeration. Solution pH and redox potential (Eh, mV vs. SHE) were monitored using a EUTECH pH/Eh meter.

Results and Discussion

Mineralogical and Chemical Characterisation of Baal Gammon Mine Waste Sample

Figure 1.presents the mineralogical and elemental composition of the Baal Gammon mine waste used for this study. Mineralogically, the sulfide minerals included 1.17% chalcopyrite, 0.6% pyrite, 8% pyrrhotite, 0.08% sphalerite. Further, there was 45% quartz, 15% Fe-oxide and 5% plagioclase present as key gangue (Fig. 1a-1b). Bulk elemental analysis (Fig 1c) shows elevated concentrations of critical and valuable elements, including 24.1 ppm silver (Ag), 5,570 ppm arsenic (As), 5,753 ppm copper (Cu), 337 ppm lead (Pb), 554 ppm zinc (Zn), and 44.4 ppm indium (In) (Fig. 1c).

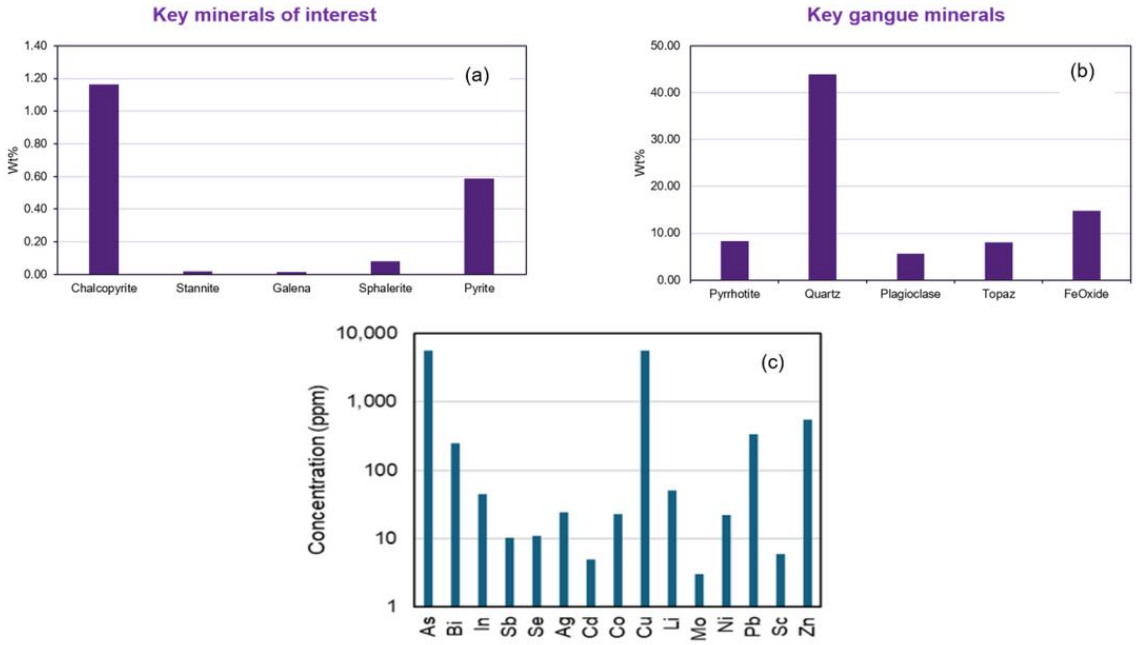


Figure 1. 1 Elemental composition and MLA quantitative mineralogy of Baal Gammon mine waste: (a) key sulfide minerals of interest; (b) key gangue minerals; (c) concentration of key elements present.

Bioleaching Results

In Figure 2, the copper (Cu) and zinc (Zn) leaching from the Baal Gammon mine waste is presented. Cu leaching reached approximately 35% within 50 days in the Bioleach-MM with glutaraldehyde, Control-MM without glutaraldehyde, and Bioleach-BG treatments, coinciding with a stable pH range of 2.0 - 2.5 (following a minor initial increase) (Fig. 2a). Further, the microbial populations in Bioleach-BG and Bioleach-MM groups showed steady growth, stabilising between 1×10^{8} and 3.2×10^{8} cells mL^{-1} after 20 days. Zinc (Zn) recovery (Fig. 2b) followed a similar trend to Cu. The highest Zn extraction was achieved in Bioleach-BG (~50%), likely facilitated by enhanced Fe^{3+}-mediated oxidative dissolution of sphalerite (ZnS). Bioleach-MM and Control-MM yielded comparable Zn recoveries (~25%), while Control-BG showed the lowest recovery (<5%).

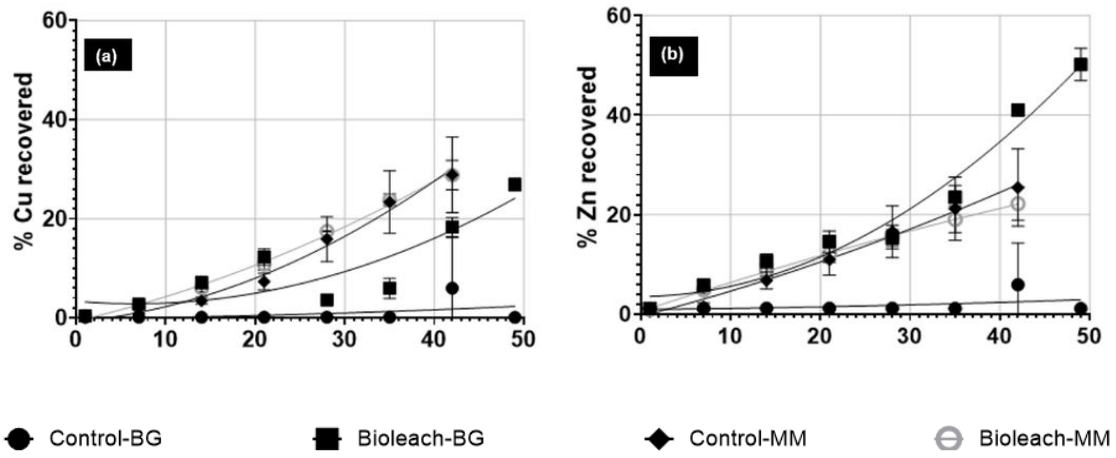


Figure 2. 2(a) Copper (Cu) and (b) zinc (Zn) mobilisation from Baal Gammon mine waste

Figure 3.provides an estimation of the taxonomic breakdown at the genus level. The BG inoculum was dominated by chemolithotroph acidophiles: *Acidithiobacillus* (55%), *Leptospirillum* (14.9%), *Acidiphilium* (14%), and *Acetobacteraceae* (12.6%). Following bioleaching of the Baal Gammon tailings, these key taxa remained dominant across both the Baal Gammon and Mt Morgan cultures, with shifts in their relative abundances. These compositional changes indicate that bioleaching conditions, particularly in the presence of the indigenous Baal Gammon and Mt Morgan consortium, selectively enriched for acidophilic iron-and sulfur-oxidizers. In contrast, the Control-BG experiment showed a lack of iron- and sulfur oxidisers, consistent with the poor leaching of metals from mineral sulfides. This was supported by undetectable cell numbers in the Control-BG whereas similar growth and cell numbers were observed in terms of cell numbers in the other treatments.

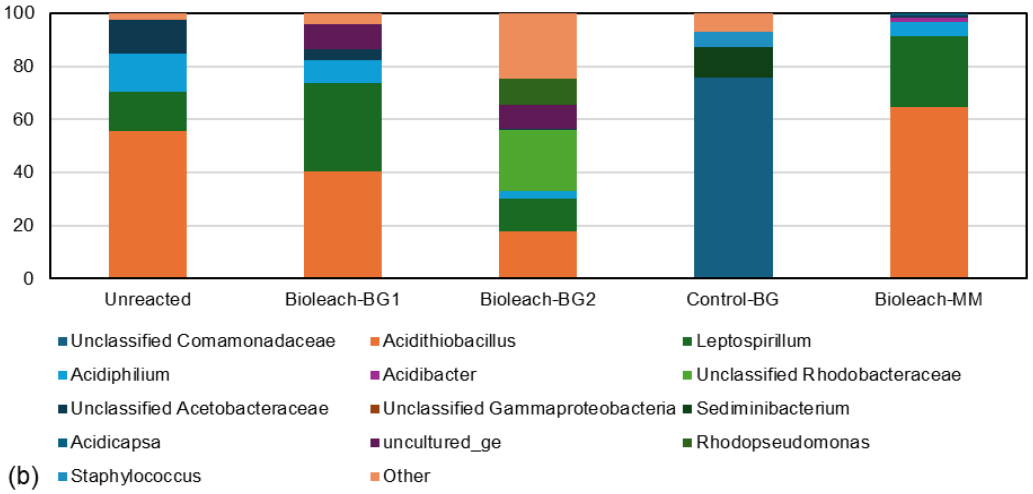


Figure 3. 3 Microbial taxa changes before and after leaching

Figure 4.highlights the mineralogical composition (wt%) of leached residues determined by X-ray diffraction (XRD) after 50 days of leaching. All residues exhibited a significant amorphous fraction. This suggests enhanced formation of secondary amorphous phases (e.g., precipitates or silicate alteration products) during biologically mediated oxidative processes. These mineralogical comparisons highlight the selective reactivity of sulfide minerals (pyrrhotite, and to a lesser extent chalcopyrite) during bioleaching, particularly with the indigenous Baal Gammon consortium, alongside accumulation of amorphous material likely linked to secondary precipitation and silicate weathering.

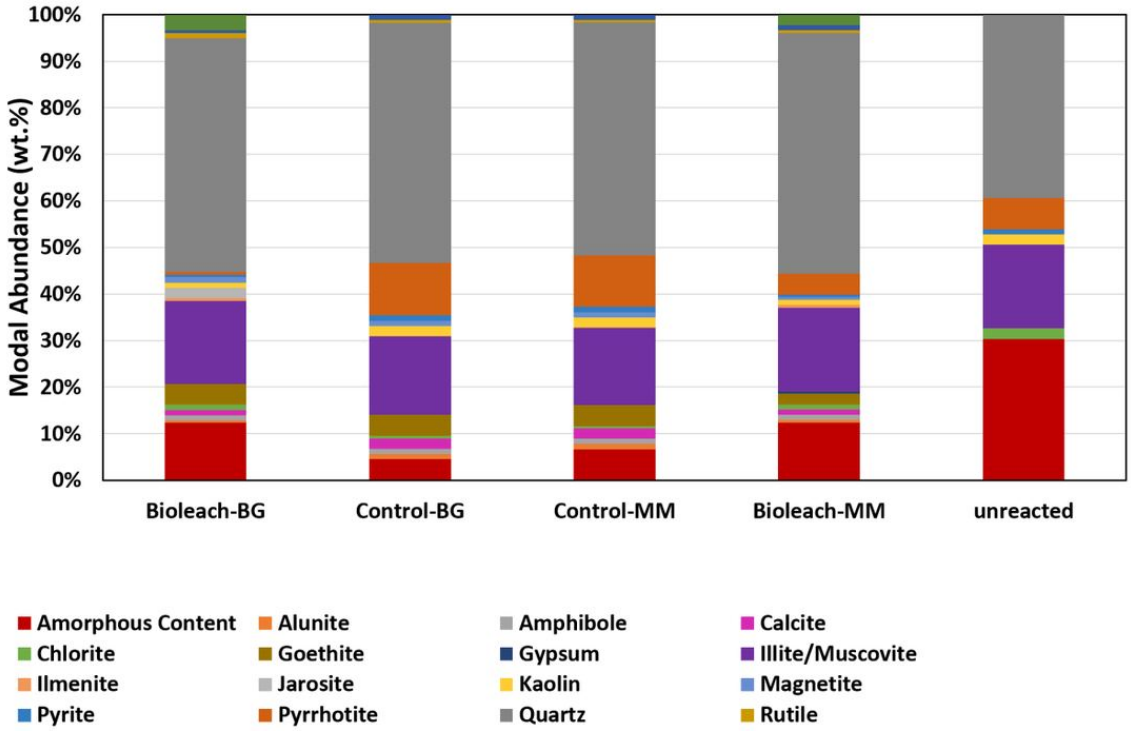


Figure 4. 4 Mineralogical composition of leached residue against unreacted sample per XRD

Conclusion and Ongoing Work

This study shows that abandoned sulfidic mine waste at Baal Gammon mine represents a potential secondary resource for recovery of valuable metals e.g. copper (Cu) and zinc (Zn), through bioleaching with acidophilic microbial Fe- and S oxidisers. Using an enriched culture indigenous to Baal Gammon, bioleaching of coarse Baal Gammon mine tailings (0.5 – 1.0 mm) achieved ~35% Cu and ~50% Zn recovery within 50 days, giving an average leach rate of 40.3 mg Cu kg^{-1} tailings day^{-1} and 5.54 mg Zn kg^{-1} tailings day^{-1}. These rates were significantly higher than those obtained with the inoculated Mt Morgan culture or un-inoculated controls, highlighting advantage of site-adapted chemolithotrophic communities dominated by *Acidithiobacillus and Leptospirillum which* sustain acidic conditions (pH 2.0 – 2.5) and regeneration of Fe^{3+} for leaching. Several challenges remain for industrial application. The complex mineralogy, including high clay content and sulfide encapsulation within gangue, limits rate and extent of metal recovery and may contribute to reagent consumption, pH instability, and reduced oxidative capacity. The continuing study addresses particle size and mineral liberation to enhance recovery. This work demonstrates potential to contribute to the circular economy and resource efficiency through responsible valorisation of legacy polymetallic mine waste to yield valuable metals.

Acknowledgements

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Mitigating iron inhibition in the accelerated bioleaching of South African coal waste through targeted high-iron harvesting cycles for ferrous sulfate heptahydrate production

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Keywords: mineral bioleaching; ferric iron inhibition; ferrous sulfate heptahydrate; pyritic coal waste; circular economy

Abstract

Waste materials from coal mining and beneficiation often contain significant quantities of pyrite (FeS_2), representing both an environmental liability and a potential secondary source of iron and sulfur. This study investigated the time-dependent bioleaching behaviour of pyritic coal waste, identified iron-induced inhibition thresholds, and evaluated strategies to sustain bioleaching while enabling recovery of ferrous sulfate heptahydrate ($FeSO_4.7H_2O$). Laboratory-scale experiments using pyritic coal discards from Mpumalanga, South Africa, showed that continuous solution recycling resulted in ferric iron accumulation, with bioleaching performance declining in iron concentrations of roughly 60 $g \cdot L^{-1}$. Periodic harvesting of iron-rich leachates and solution replacement maintained iron concentrations below inhibitory levels, sustaining microbial activity and accelerating pyrite dissolution. Ferric iron-rich leachates were subsequently reduced under anaerobic conditions, and ferrous sulfate heptahydrate was recovered by ethanol precipitation. X-ray diffraction confirmed ferrous sulfate heptahydrate content exceeding 90%. These findings demonstrate that controlled iron accumulation enables bioleaching efficiency and recovery of valuable iron products, supporting circular biohydrometallurgical valorisation of pyritic coal waste.

Introduction

While renewable energy is increasing in South Africa, coal is still expected to play the major role in electricity generation at least until 2040, hence minimising its environmental burden is key for the achievement of long term sustainable development. Discard waste materials from the extraction and beneficiation of coal deposits contain considerable amounts of sulfidic minerals, particularly pyrite (FeS_2). This contributes to major environmental challenge, as the exposure of pyritic sulfur-containing residues to water and oxygen results in the pyrite being oxidised to produce a highly acidic and saline effluent with high sulfate concentrations, commonly referred to as acid rock drainage (ARD) and with potential to enable mobilisation of dissolved toxic elements. As mining industries are encouraged to move towards more sustainable practices, the development of a circular economy chain, with resource cycles moving towards closure via maximising resource extraction, re-using and re-cycling product, and re-purposing of wastes, is increasingly important (Harrison et al., 2013; Mbamba et al., 2012). Several studies have investigated the feasibility of producing sulfur and iron-based materials that can be used in several industrial sectors including, but not limited to, ferric sulfate coagulant, ferrous sulfate heptahydrate, sulfuric acid and iron oxides (goethite, hematite and magnetite) from pyrite present in mine waste (Colling et al., 2011; Menezes et al., 2017; Vigânico, 2014). In bioleaching, microorganisms produce lixiviants necessary to extract metals from low-grade ores, offering a simple, environmentally friendly alternative to traditional methods requiring high energy processing and chemical addition. In bioleaching of the pyrite rich coal discards, the acidic ferric iron rich lixiviant is recycled in the system to promote further oxidative dissolution of pyrite. The South African coal discards often contain high

levels of gangue materials such as carbonates and alumino-silicates, and understanding the effect of gangue materials on leaching behaviour and kinetics of pyrite is important for value recovery from coal waste. High levels of acid-neutralising gangue materials can negatively impact acidophilic bioleaching. To enhance crystallisation of ferrous sulfate heptahydrate formed, a high concentration iron sulfate leachate stream is desirable which requires recycle of the lixiviant; however, this may affect the efficiency of pyrite leaching. To address these requirements, inhibition was mitigated through periodic harvesting of high-iron leachates with solution replacement to maintain iron concentrations within a non-inhibitory range and sustain microbial activity for accelerated leaching. This study evaluates the integrated flow-through pre-leaching followed by bioleaching of pyrite-enriched South African coal discards with recycle to produce high-grade ferrous sulfate heptahydrate.

Materials and Methods

Two pyrite-bearing coal discard samples (2–50 mm) were collected from the eMalahleni area, South Africa. Following crushing, the samples were characterised for mineralogy, elemental composition, total sulfur, and acid rock drainage (ARD) potential using standard analytical techniques. Bioleaching experiments were performed in lab-scale packed bed glass columns (7 cm × 30 cm) under conditions summarised in **Table 1** and using the experimental rig is shown in **Fig. 1**. The first experiment evaluated natural acidification and the onset of microbial pyrite oxidation under recycle conditions. Two reactor columns (A_1, A_2) packed with pyrite-enriched coal discards (sample 1) were irrigated with deionised water, after which one column was inoculated with 10^9 cells/g coal discards using a mixed iron- and sulfur-oxidising consortium comprising *Leptospirillum ferriphilum*, *Acidithiobacillus caldus*, *Sulfobacillus thermosulfidooxidans*. The second experiment investigated the effect of removing acid-neutralising capacity (ANC) prior to recycle bioleaching. Sample 2 was first subjected to flow-through leaching to remove ANC before changing to recycle operation. Five columns were operated comprising two inoculated columns (C_1 and C_3), two acidified abiotic controls at pH 1.6 (C_2 and C_4), and one deionised water control (C_5). Iron rich leachates were periodically harvested and replaced with fresh feed to maintain dissolved iron concentrations below inhibitory levels. Leachates were analysed daily for pH, redox potential, ferric and total iron. Following anaerobic ferric iron reduction, ferrous sulfate heptahydrate was recovered by ethanol precipitation (80%, 1:1 v/v) and characterised by XRD and ICP-MS.

Table 1. Experimental setup of the two leaching runs. In Run 1, Columns A_1 and A_2 were initially irrigated with deionised water, with Column A_1 subsequently inoculated with a mixed iron- and sulfur-oxidising microbial consortium. In Run 2, Columns C_1 and C_3 were inoculated, Columns C_2 and C_4 served as abiotic controls, and Column C_5 (deionised water) served as the control.

Sample	Column	Ore sterilization	Irrigation	Inoculation
1	A_1	Non-sterilised	Deionised- H_2O	Inoculated
	A_2	Non-sterilised	Deionised -H_2O	Non-inoculated
2	C_1	Sterilised	Nutrient solution	Inoculated
	C_2	Sterilised	Acidified- H_2O	Non-inoculated
	C_3	Non-sterilised	Nutrient solution	Inoculated
	C_4	Non-sterilised	Acidified- H_2O	Non-inoculated
	C_5	Non-sterilised	Deionised -H_2O	Non-inoculated

Results and Discussion

XRD analysis confirmed that pyrite was the major sulfide mineral in both coal discard samples, comprising 51.3 wt.% in Sample 1 and 77.4 wt.% in Sample 2. Dolomite and calcite were identified as the most abundant acid neutralising minerals (**Fig 2**). Total sulfur contents were 22.3 wt% for Sample 1 and 32.3 wt% for Sample 2, indicating greater pyrite enrichment in the latter. Static acid-base accounting (ABA) classified both materials as acid-forming. Sample 1 showed an acid-neutralising capacity (ANC) of 127.7 kg H_2SO_4/t and a net acid-producing potential (NAPP) of 554.7 kg H_2SO_4/t, while Sample 2 had a higher ANC of 253.5 kg H_2SO_4/t with significantly higher NAPP of 734.9kg H_2SO_4/t owing to its high sulfur content.

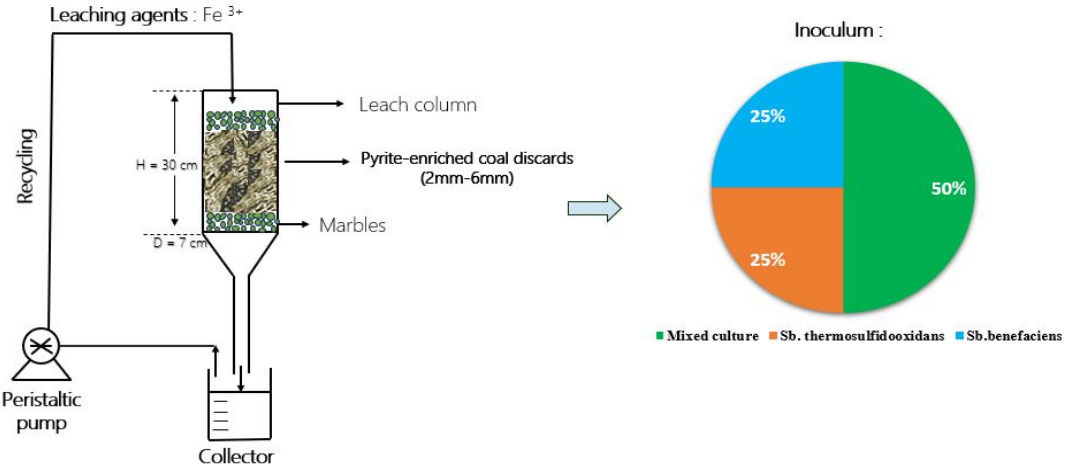


Figure 1. (a) A reactor column packed with pyrite enriched coal discards sandwiched between two layers of glass marbles, and (b*)* mixed microbial consortium used in this study.

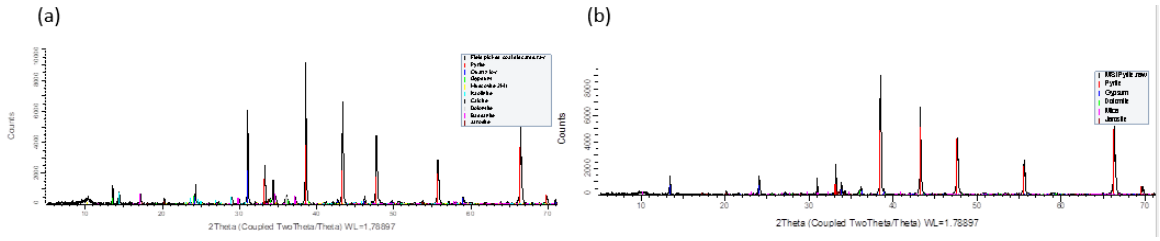


Figure 2. XRD pattern for samples 1 (a) and 2 (b) used in this study.

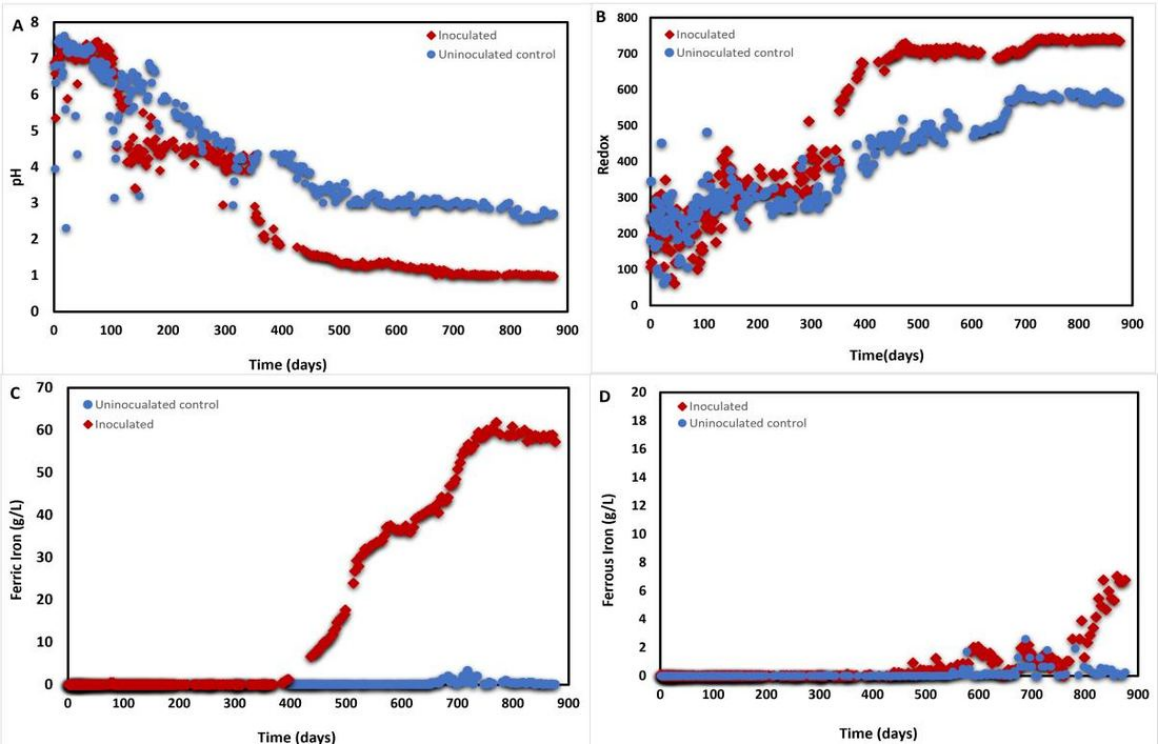


Figure 3. Solution pH (a), Redox potential (b), Ferric iron (c) and Ferrous iron (d) profiles generated through bioleaching of inoculated (A_1) and uninoculated control (A_2) of pyrite-enriched coal discards, Sample 1.

During the first bioleaching experiment, dissolution of acid-neutralising minerals, particularly dolomite and calcite resulted in increased solution pH and precipitation of iron (**Fig 3**). Periodic replacement of

the leachate with fresh deionised water progressively removed dissolved alkalinity, allowing the solution pH to decrease below 5 after approximately 260 days. Following inoculation of Column A_1 on day 352, the pH decreased further while the redox potential increased to approximately 700 mV, indicating active microbial oxidation of ferrous to ferric iron. This was accompanied by sustained ferric iron regeneration and increasing ferric iron concentrations. Once dissolved ferric iron reached approximately 60 g/L, microbial iron oxidation declined, evidenced by a sharp increase in ferrous iron concentration to ~7 g/L. These results indicate iron accumulation-driven inhibition of the microbial consortium, with ferric iron concentrations of approximately 60 g/L representing the onset of inhibition under the conditions investigated.

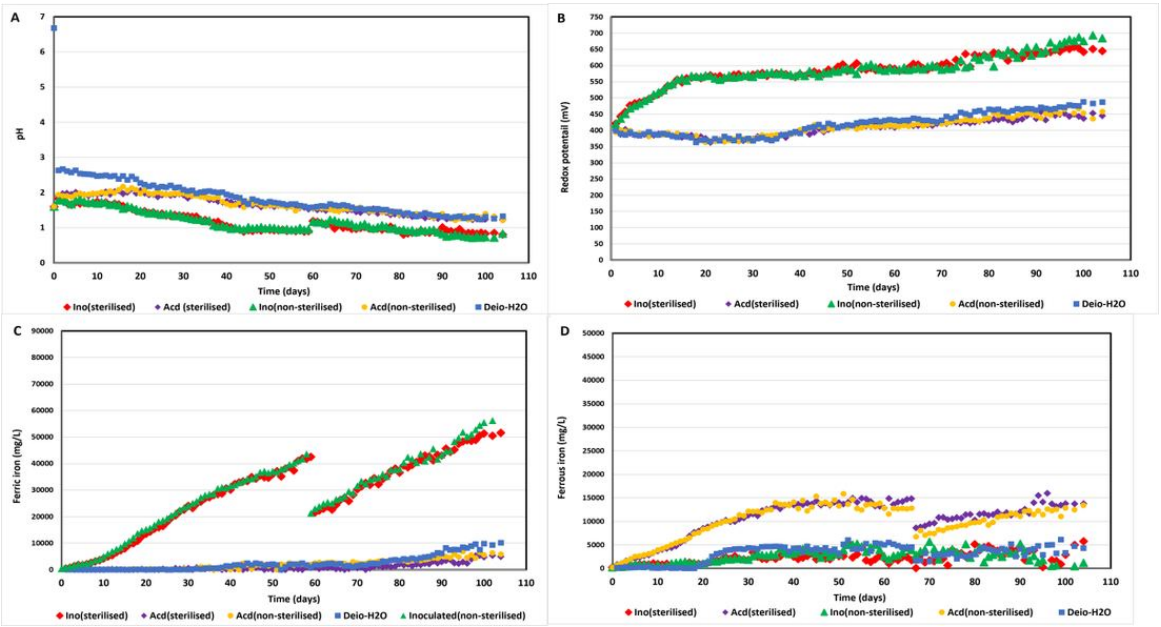


Figure 4. Solution pH (a), redox potential (b), ferric iron (c) and ferrous iron (d) profiles generated through bioleaching (C_1, C_3), acid leaching (C_2, C_4) and water leaching (C_5) of pyrite-enriched coal discards in Experiment 2.

During the flow-through pretreatment in Experiment 2 **(Fig. 4)**, rapid increase in leachate pH was observed during the first 12 days accompanied by increase in Ca and Mg concentrations (amongst others), indicating dissolution of acid-neutralising minerals. After switching to recycle operation, the inoculated columns maintained redox potentials of approximately 650 mV, demonstrating sustained microbial regeneration of ferric iron. Based on the inhibition threshold identified in the first experiment, iron-rich leachates were harvested at approximately 42 g/L dissolved iron in column C_1 and C_3 and replaced with fresh solution to avoid decline in microbial activity. Bioleaching continued with no decrease in rate with dissolved iron concentrations increasing to approximately 60 g/L where leaching rates slowed, confirming the previously identified inhibition threshold. This cyclic harvesting strategy maintained microbial activity while enabling continued pyrite dissolution. Based on this understanding, a continuous process can be designed for generation of the iron-based products.

Following anaerobic ferric iron reduction, the ferrous iron-rich solution was treated with 80% ethanol to recover ferrous sulfate heptahydrate. XRD **(Fig. 5)** confirmed melanterite ($FeSO_4 \cdot 7H_2O$) as the dominant crystalline phase (~92% of the major mineral phases), while ICP-MS showed impurity levels below those reported for analytical-grade material. These results demonstrate that periodic harvesting of iron-rich leachates provides an effective strategy for mitigating iron accumulation-driven inhibition while enabling recovery of a high-purity ferrous sulfate product.

On acid leaching (C_2, C_4), iron accumulated in solution as ferrous iron at a lower rate to ~15 g/L where it stabilised until solutions were refreshed. In dH2O (C_5), iron dissolution was negligible. These results demonstrate the benefit of bioleaching.

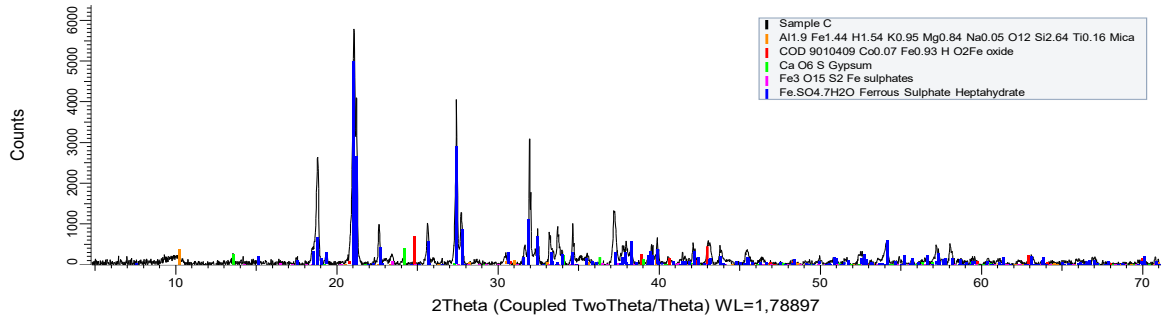


Figure 5. XRD pattern of ferrous sulfate heptahydrate crystals precipitated in Experiment 2 of this study.

Conclusions

This study demonstrated the beneficial bioleaching of high-pyrite coal discards to yield concentrated aqueous iron streams for product recovery. Dissolved iron accumulation is a key factor limiting the bioleaching of pyritic coal discards, with microbial iron oxidation, declining when iron concentrations approached approximately 60 g/L. A flow-through pretreatment effectively removed acid-neutralising minerals to enable the rapid onset of bioleaching, while periodic harvesting of iron-rich leachates before the onset of inhibition sustained microbial activity and enhanced pyrite dissolution. The harvested leachate was successfully converted into high-purity ferrous sulfate heptahydrate by anaerobic ferric iron reduction followed by ethanol precipitation. These findings establish a practical operating strategy for integrating bioleaching with selective iron recovery, supporting the circular valorisation of pyritic coal waste into a valuable iron-based product.

Acknowledgements

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Efficiency of assisted phytostabilisation with white lupin (*Lupinus albus* L.)

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Keywords: phytoremediation; heavy metals; metalloids; arsenic; barium

Abstract

Human activities have contributed to soil contamination with metal(loid)s, which can be toxic to organisms in terrestrial and aquatic ecosystems. Several plant species can reduce metal(loid) uptake and leaching. White lupin seeds were sown and grown for 3 weeks in a greenhouse on agricultural soil of Limousin (France) taken near waste from a former tin mine, with or without amendments: 5% composted sewage sludge, 2% and 5% biochar, and 2% red mud. The results showed a low initial metal(loid) bioavailability and a positive effect of all amendments in further reducing this bioavailability. Metal(loid) concentrations in white lupin were low and with bioconcentration and translocation factors below 1. As and Ba were present in non-negligible quantities in the roots (around 45-85 and 20-45 mg. kg^{-1} dry weight, according to the amendments) but the concentrations were not considered toxic for most plant species. The condition with 2% biochar was the most favourable, with a higher aerial biomass, higher chlorophyll, total soluble carbohydrate, and total soluble protein contents, as well as a reduction in leaf lipid peroxidation. This suggests that white lupin could be an effective excluding species and that amendments could improve metal(loid) phytostabilisation.

Introduction

Metal(loid)s used in various sectors accumulated in soils and aquatic ecosystems, posing risks of toxicity for all organisms along the food chain. Phytostabilisation is a nature-based solution that involves establishing vegetation cover to reduce soil erosion and metal(loid)s leaching. It requires the use of exclusionary plants, meaning plants limiting root metal(loid) uptake and subsequent translocation to the aboveground organs (Tang et al. 2026, Shabrina et al. 2024). Soil amendments can improve phytostabilisation by reducing metal(loid) bioavailability [1]. Organic amendments are routinely used in phytoremediation and not only modify metal(loid) bioavailability but also enrich the soil, improve plant growth, help to recycle waste and thus to reduce process costs [2]. Inorganic amendments can also be used and have shown a certain degree of effectiveness in terms of binding or solubilising metal(loid)s [3]. Fabaceae can enrich the soil with nitrogen, which helps improve soil fertility while reducing inputs [4]. In a previous study, we also showed the effectiveness of white lupin for phytostabilisation at sites contaminated by multiple metal(loid)s and exhibiting different physicochemical properties [5]. White lupin exhibits characteristics of metal(loid) excluder [6]. However, the addition of amendments can have different effects on metal(loid) uptake by white lupin, depending of the culture conditions [6,7]. The objective of our study was therefore to test various organic amendments (composted sewage sludge, biochar, and red mud) on acidic, low-fertility soil contaminated with several metal(loid)s in order to 1) determine whether the amendments reduce the bioavailability of the metal(loid)s and their uptake by the plants, and 2) investigate their effects on the growth and defense mechanisms of white lupin.

Methods

Materials.

Soil was collected in Haute-Vienne (France) near the tailings of a former tin and tungsten mine. After drying and sieving, it was moistened and incubated with 4 amendments: 5% (w/w) composted sewage

sludge (CSS), 2 and 5% (w/w) wood biochar (BC2 and BC5), and 2% (w/w) red mud (RM). A control soil was prepared without amendment. White lupin seeds were sown in a greenhouse in pots (5 seeds per pot) containing amended or unamended soil. Plants were harvested 3 weeks later: the stems and roots were measured, and the organs (leaves, stems, roots) were weighed and stored at -20 °C until analysis. A portion of the samples was dried to obtain dry weight (DW) and for metal(loid) quantification.

Analysis.

The soil pH was measured according NF ISO 31-130. Organic C and total N contents were performed according to NF ISO 10694. The concentration of dissolved organic carbon (DOC) was obtained after mixing dry soil with $CaCl_2$ and using a TOC-VWP analyser. P concentration was obtained with a colorimetric assay [5]. The content of photosynthetic pigments was determined using Lichtenthaler's equations [8]. Total soluble protein (TSP), total soluble carbohydrate (TSC), starch, total free amino acid (TFAA), total free proline, total non-protein thiols (NPT), total phenolic compounds (TPC), H_2O_2, and thiobarbituric acid reactive substances (TBARS) contents were obtained with colorimetric assays [5,9]. Total soil metal(loid) concentrations were determined from pressed pellets by X-ray fluorescence analyses [10]. The metal(loid) potentially bioavailable fraction was obtained with extraction with $CaCl_2$ [11]. Dry samples of plant organs were mineralised [9]. All samples (plant organs and $CaCl_2$-extractable fractions) were filtered and the metal(loid) concentrations were determined by ICP-MS. For soil characterisation, duplicate analyses were performed for all parameters. For plant analysis, results were the mean of 5 independent experiments with 5 seeds per pot and per condition. The statistical analyses were conducted using the PAST 5.3 software (Natural History Museum, University of Oslo, Norway). One-factor analysis of variance (ANOVA) was used to assess significant differences among treatment conditions and Tuckey's test was performed to separate means that were significantly different ($p \leq 0.05$).

Results and Discussion

The CSS amendment led to the greatest increase in soil parameters such as CEC and OM, while RM treatment increased pH (table 1). Despite high total concentrations of metal(loid)s in the control soil, notably As and Ba which exceed moderate to high anomaly thresholds in French soils, these elements exhibited low bioavailability. The addition of amendments generally reduced metal(loid) bioavailability (all data not shown), except for As in the CSS treatment, likely due to increased dissolved organic carbon and P competing for soil retention sites [12]. White lupin displayed an exclusion strategy with metal(loid) concentrations being highest in roots, followed by stems and leaves. Metal(loid) levels measured in lupin tissues in the control soil remained below toxicity thresholds, with As concentrations significantly lower than levels reported in other studies to cause damage [13]. All amendments reduced metal(loid) uptake, particularly for As and Ba, confirming previous findings that white lupin is an excluder plant [5,14]. Calculated BCF and TF values remained below 1, further supporting the plant's suitability for phytostabilisation.

Table 1. Soil characteristics and As and Ba concentrations in soil and roots of lupin.

	pH	CEC	OM	As			Ba		
				Total	Extractable	Roots	Total	Extractable	Roots
Control	4.48	3.1	3.03	839	0.26	0.101 a	784	8.04	0.062 a
CSS	5.22	8.1	7.78	813	1.29	0.085 a	635	2.63	0.018 d
BC2	4.57	3.5	3.64	720	0.34	0.062 b	536	7.36	0.048 b
BC5	5.00	4.0	5.31	772	0.35	0.06 3b	585	5.39	0.039 c
RM	5.51	5.2	5.60	738	0.33	0.046 c	623	1.69 c	0.021 d

CEC and OM are expressed in cmol(+) kg^{-1} and % (w/w), respectively. As and Ba concentrations are expressed in mg kg^{-1} soil and kg^{-1} DW for roots.

Results showed that the BC2 amendment was most favourable for lupin development, significantly increasing leaf DW and stem height compared to the control (table 2). This amendment also significantly stimulated chlorophyll a synthesis and improved N and C metabolism. Similar positive effects of biochar

on photosynthetic activity and antioxidant defense modulation have been observed in other plant species [15]. Moreover, BC2 tended to reduce the level of antioxidants defense such as free proline and TPC (data not shown), as well as the level of H_2O_2 (data not shown) and TBARS. The observed increase in root NPT across all amended soils suggests however a general activation of defense responses to scavenge metal(loid)s and limit translocation.

Table 2. Effect of amendments on white lupin.

	Leaf DW	Stem height	Chlorophyll a	Leaf TSC	Leaf starch	Leaf TSP	Leaf TBARS	Root NPT
Control	353 b	9.4 b	10.9 b	121 a	4.3 c	121 a	0.075 bc	3.3 d
CSS	395 ab	11.6 ab	10.9 b	154 a	5.4 b	154 a	0.087 abc	9.4 a
BC2	431 a	13.5 a	13.1 a	138 a	14.2 a	138 a	0.069 c	5.6 c
BC5	372 ab	13.1 a	13.9 a	123 a	12.4 a	123 a	0.089 b	6.4 bc
RM	296 c	9.5 b	10.5 b	141 a	12.9 a	141 a	0.100 a	8.8 ab

DW and height are expressed in mg and cm, respectively. Chlorophyll a and starch contents are expressed in mg g^{-1} DW. TSC, TSP, NPT and TBARS contents are expressed in µmol g^{-1} DW. For each column and each organ, different letters between two means correspond to significant differences ($p < 0.05$).

In contrast, RM amendment reduced biomass and increased oxidative stress markers such as TBARS, possibly due to substrate salinity or physical properties limiting root development [16]. While CSS improved soil characteristics, its impact on plant growth was limited, potentially due to the short 3-weeks duration of the experiment, as higher proportion of CSS might be required for optimal nutrient release [17].

Conclusions

Although metal(loid)s were not very bioavailable in the tested soil tested, the amendments used further reduced the metal(loid) bioavailability in most cases. BC2 emerged as the best compromise between metal(loid) phytostabilisation and lupin growth. To enhance the process, it could be interesting to test a mixture of amendments, more specifically CSS and BC. Indeed, BC2 improved plant growth but CSS was better for metal(loid) reduction of bioavailability (except As but BC2 can limit the leaching of this metalloid). Moreover, it could be interesting to extend the exposure time in order to know if metal(loid)s remain mostly in roots or if they tend to be translocated over time and accumulate in the seeds.

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QS molecules modulate subpopulation distribution and inhibit bioleaching in mock thermophilic consortia

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Keywords: Bioleaching, metal sulfides, moderately thermophilic bacteria, EFM microscopy, proteomics

Abstract

Biomining is a sustainable alternative to conventional mineral processing that uses acidophilic microorganisms to catalyse the extraction of valuable metals from sulfide minerals. Mixed microbial consortia composed of moderate thermophiles (MT) such as *Acidithiobacillus caldus*^{T}, *Sulfobacillus thermosulfidooxidans*^{T} and *Leptospirillum ferriphilum*^{T}, improve metal extraction efficiency at higher temperatures compared to pure cultures of mesophiles. Although biofilm formation and cell communication are critical for mineral colonisation and sulfide oxidation, the impact of diffusible signal factor (DSF) signalling in MT species remains unclear. Using bioleaching assays, high-throughput microscopy, 16S rDNA sequencing and proteomics, we evaluated the role of DSF in mixed MT cultures. Exogenous DSF impaired pyrite and chalcopyrite colonisation and dissolution, reducing copper recovery. DSF also altered community structure by driving *At. caldus*^{T} dispersal with a concomitant increase of *L. ferriphilum*^{T} on pyrite surface. Furthermore, DSF induced differential changes in *At. caldus*^{T} and *L. ferriphilum*^{T} proteomes depending on the mineral in which they were grown. In pyrite DSF triggers robust, growth-promoting metabolic adaptations specially benefiting *At. caldus*^{T}. In chalcopyrite, these molecules exert a broad inhibitory and stress-inducing effects, likely reinforced by high copper concentrations. Overall, DSF regulated key target microbial interactions, that opens the possibility to improve chalcopyrite bioleaching in the studied consortia.

Introduction

Biomining is a low-energy and environmental-friendly alternative for recovering valuable metals from low-grade sulfide ores using extremely acidophilic microorganisms [1]. Moderately thermophilic (MT) acidophiles such as *Sulfobacillus and* some *Leptospirillum species*, offer a practical balance of high metabolic rates to accelerate mineral dissolution in high-temperature bioleaching setups [2,3]. Although quorum sensing (QS) signalling via acyl-homoserine lactones and diffusible signal factors (DSF) regulates adaptive bacterial behaviours, its inhibitory effects have been studied primarily in axenic cultures [4,5,6]. Consequently, the broader relationships between DSF signalling, microbial community dynamics, and overall bioleaching performance in MT consortia remain largely unexplored. To address this gap, this study investigated the effects of DSF signalling on iron oxidation, biofilm development, and community composition using a defined MT consortium to evaluate its proteome response and its impact on mineral colonisation, and copper recovery.

Methods

Bacterial strains and growth conditions. L. ferriphilum DSM 14647^T, *At. caldus* DSM 8584^T and *S. thermosulfidooxidans* DSM 9293^T were grown in K medium pH 2 or 2.5 at 37 °C with constant shaking at 140 rpm. Specific electron donors and nutritional supplements (ferrous ion, elemental sulfur and yeast extract) were supplied depending on the species as detailed in Salas et al. [7].

Bioleaching assays. Assays were performed at 37 °C in Erlenmeyer flasks with K medium pH 2 and 2 % (wt/vol) pyrite or chalcopyrite grains, plus 34 mM Fe^{2+}. Mineral cultures were prepared in triplicate (*n = 3*). Medium was inoculated with a mixture of the three species each at $2 \cdot 10^7$ cells/mL. DSF-signal compounds were added directly along with the inoculation. Cultures were maintained for 1 to 7 or 9 days, when cells were recovered for total DNA and protein extraction.

Monitoring of bioleaching experiments. Assays were monitored testing pH and redox potential, in addition to photometric determination of total copper concentration [8]. Cell counts were performed by optical microscopy using a Neubauer-improved chamber.

Quorum sensing signal compounds. The DSF-family signal compounds DSF or *cis*-11-methyl-2-dodecenoic acid (CAS 677354-23-3; Cayman) and BDSF or *cis*-2-dodecenoic acid (CAS 55928-65-9; Cayman) were used in combination at 2 µM per compound. Stock solutions were prepared in dimethyl sulfoxide (DMSO) at 5 mM. Control assays were amended with equal volume of DMSO.

Extraction of DNA and proteins. DNA was extracted from planktonic and pyrite-attached cells while proteins were only extracted from planktonic cells. After 7 or 14 days, the recovered cells were subjected to DNA/proteins extraction using hot-phenol (pH 8) extraction as previously described [9]. Extracted proteins were separated by SDS-PAGE, directly digested with trypsin, and recovered from the gel using acetonitrile prior to mass spectrometry analysis.

16S rRNA gene-based microbial community development. Partial 16S rRNA gene regions were amplified with 175F and 512R primers [10] and sequenced on an Illumina MiSeq platform with MiSeq Reagent Kit v3. Taxonomical identification was performed with the QIIME 2 platform [11] using either the SILVA [12] or Greengenes [13] databases.

Mass spectrometry. Peptides were analysed by LC-ESI-MS/MS on a nanoAcquity UHPLC coupled to an LTQ Orbitrap Elite mass spectrometer. Protein identification and label-free quantification (LFQ) were performed with Andromeda and MaxQuant version 2.6.2.0 against UniProt reference proteomes. Analyses of Differentially abundant proteins (DAPs) were done using normalised LFQ values with Perseus software version 2.1.0.0.

High throughput epifluorescence microscopy (EFM). Mineral grains were withdrawn from cultures at 1 and 9 days, and cells were differentially stained with DAPI. Automatic massive image acquisition was done using an inverted epifluorescence microscope (Zeiss Axio Observer Z1/7) with a motorised microscopy stage. EFM image analysis was conducted following an in-house procedure [14]. Statistical analyses to determine differences between the control and DSF/BDSF treated groups were carried out using the *car* package in R and the non-parametric Kruskal-Wallis test.

Results and Discussion

Effect of DSF/BDSF signal compounds on MT consortium planktonic growth, iron oxidation and mineral colonisation. This study evaluated whether DSF/BDSF treatment can affect oxidising activity and/or colonisation capacity of iron oxidiser species, in order to reduce and maintain ORP values in the range required for optimal chalcopyrite lixiviation (420–550 mV) [15]. First tests in pyrite mineral revealed that external addition of DSF/BDSF to MT consortium, significantly reduced cell density (between 1 – 3 days) and ORP (at 3 days; Figure 1). Furthermore, colonisation on pyrite surfaces was inhibited by DSF/BDSF treatment at 1 day but not at 9 days [shown in 7].

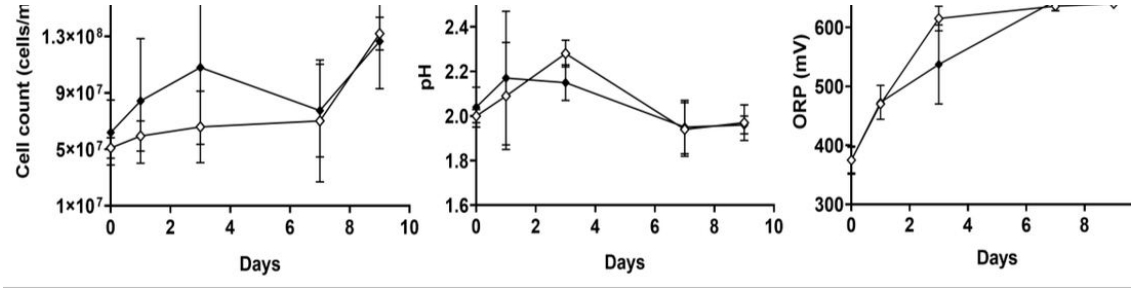


Figure 1. Effect of DSF/BDSF on growth and oxidising activity of MT consortium grown in pyrite. Planktonic cell numbers (left), pH (middle) and ORP (right) of MT consortium are shown. DSF/BDSF treated cultures are depicted as filled symbols and control cultures in white symbols. Assays were performed in triplicates ($n = 3$) and significant differences are shown according to results of *t-test* (* $p < 0.05$).

Effect of DSF/BDSF signal compounds on MT consortium mediated chalcopyrite dissolution and mineral colonisation. Evaluation of DSF/BDSF in MT consortium in chalcopyrite leaching assays confirmed the inhibitory effects of these molecules.Treatment with DSF/BDSF markedly decreased cell counts, ORP levels, and copper dissolution in chalcopyrite (Figure 2), which correlated with impaired bacterial attachment on chalcopyrite mineral [shown in 7]. Comparing colonisation in pyrite, the MT consortium grown on chalcopyrite was more sensitive to DSF/BDSF, while inhibitory effects were observed only in short timeframes in pyrite, DSF/BDSF addition prevented chalcopyrite colonisation for 9 days and resulted in a lowered mineral dissolution.

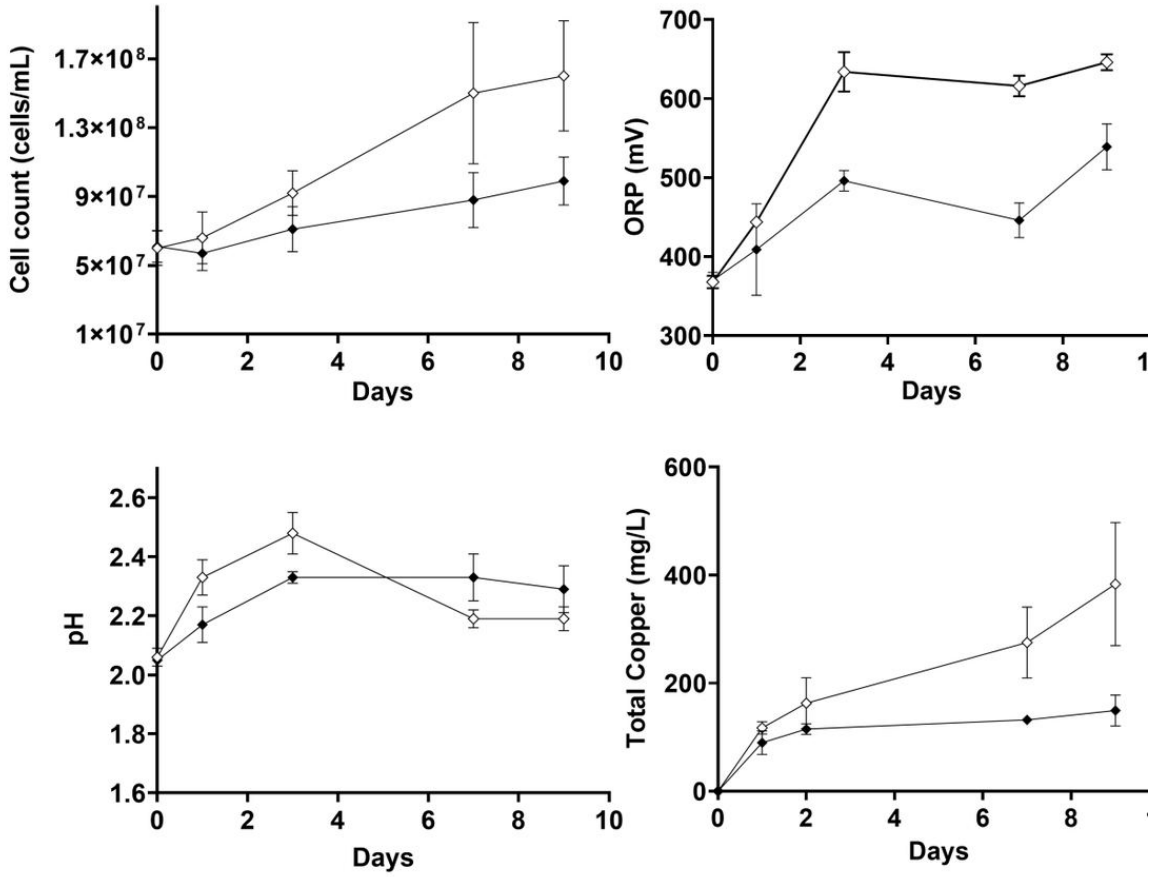


Figure 2. Effect of DSF/BDSF on MT consortium chalcopyrite bioleaching. Planktonic cell numbers (cell counts), redox potential vs Ag/AgCl (ORP), pH, and released copper (Total copper). DSF/BDSF treated cultures are shown in black

diamonds with control cultures in white diamonds. Assays were triplicates ($n = 3$) and significant differences are shown according to results of *t-test* (* $p < 0.05$).

Effect of QS molecules on MT consortium development in the planktonic and mineral attached cell subpopulations grown in pyrite. 16S rRNA gene profiling revealed that the addition of DSF/BDSF significantly disrupted the MT community distribution compared to control cultures. In planktonic subpopulation, DSF/BDSF increased the relative proportion of *At. caldus*^{T} to ~45% while decreasing *L. ferriphilum*^{T} to ~50% within 7 days. Conversely, in the biofilm subpopulation, DSF/BDSF promoted the attachment and expansion of *L. ferriphilum*^{T} while driving the dispersal of *At. caldus*^{T} from the mineral surface by day 14. Meanwhile, *S. thermosulfidooxidans*^{T} biofilm levels remained largely unaffected by DSF/BDSF, though its planktonic fraction declined to near zero by day 14 [shown in 7]. Overall, DSF/BDSF signal compounds induced contrasting subpopulation shifts in pyrite cultures, promoting a planktonic lifestyle in *At. caldus*^{T} and a sessile biofilm lifestyle in *L. ferriphilum*^{T}.

Importantly changes in MT community composition in chalcopyrite could not been analysed since DSF/BDSF seems to induce a harmful effect on cell viability under these conditions, which resulted in extremely low biomass and low-quality DNA obtained, specially from the mineral fractions. Although the planktonic population could be analysed, no significant changes were observed (data not shown). Therefore, the DSF/BDSF treatment likely resulted in some cell death within the biofilm subpopulation due to the low or absent iron oxidation and resulting in biomass recovery from the mineral samples being challenging.

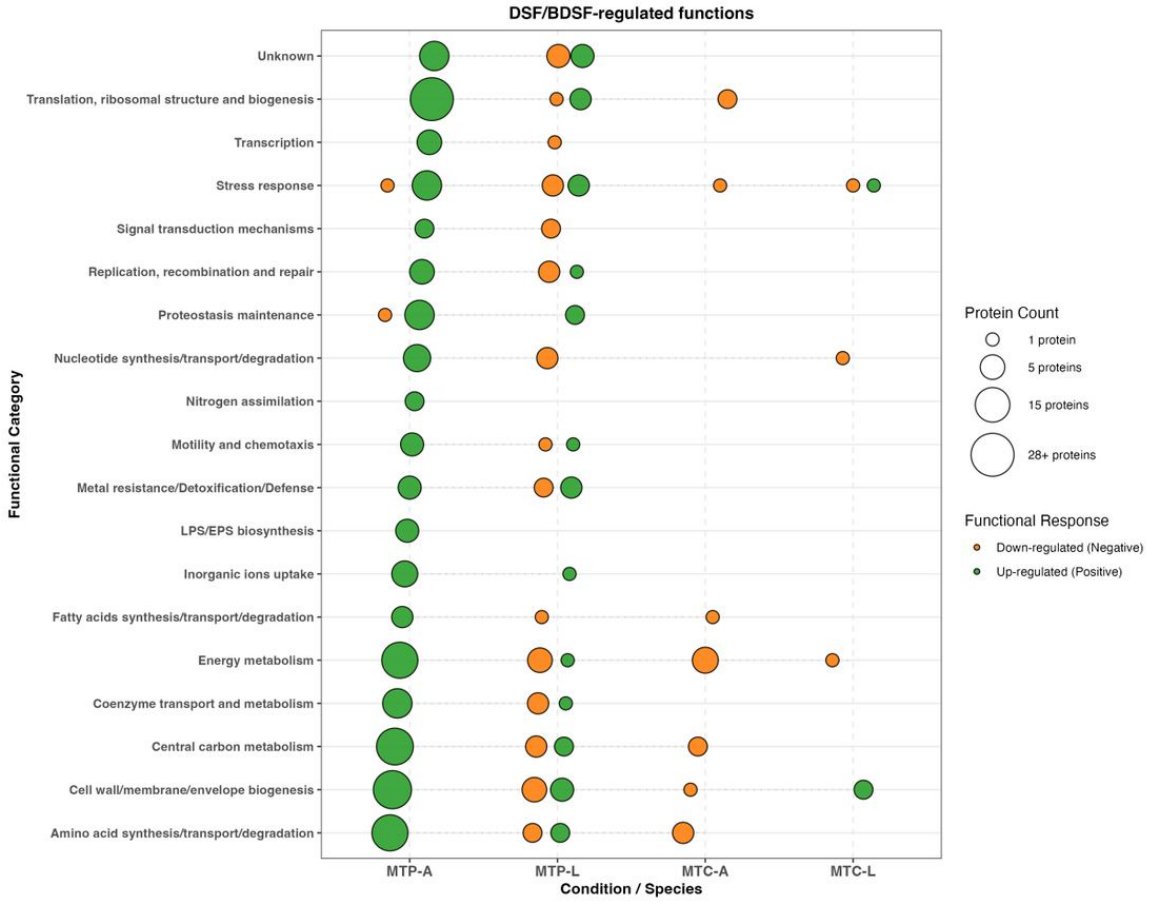


Figure 3. Effect of DSF/BDSF on MT proteome grown in pyrite and chalcopyrite. Key functions are plotted along the y-axis, and experimental conditions/species are displayed along the x-axis. Bubble size corresponds to the cumulative count of differentially affected proteins (DAPs). Bubble fill colour indicates the functional response direction: green for positive response and orange for negative response. MTP and MTC are proteome data collected for MT consortium grown in pyrite and

chalcopyrite, respectively, all treated with DSF/BDSF for 7 days. Data are shown according to each MT species. L: *L. ferriphilum*^{T} and A: *At. caldus*^{T}, while although *S. thermosulfidooxidans*^{T} was part of the MT consortium, it was excluded since no DAPs were identified in these experiments.

Effect of DSF/BDSF treatment on MT consortium proteome response in pyrite. Determination of DAPs in planktonic fraction of MT consortium grown in pyrite, identified 58 total DAPs for *L. ferriphilum*^{T}, divided into 25 increased and 33 decreased proteins, while in *At. caldus*^{T} total DAPs were 167 proteins, all increased, except for one protein detected as biologically relevant with decreased abundance. In the case of *S. thermosulfidooxidans*^{T} no DAPs were detected (Figure 7).

Per species analyses showed that in *At. caldus*^{T}, DSF/BDSF induced a broad upregulation across translation, central carbon, energy, and cell envelope biogenesis, driving energy harvesting, *de novo* amino acid biosynthesis, and translational machinery for biofilm formation and stress adaptation. Conversely, *L. ferriphilum*^{T} exhibited an induced response in translation, metal resistance, and inorganic ion uptake alongside decreased energy metabolism, nucleotide synthesis, and replication, reflecting cell cycle arrest, which likely promote biofilm formation and cell wall remodeling.

Effect of DSF/BDSF treatment on MT consortium proteome response in chalcopyrite. On chalcopyrite, the proteomic response was far more constrained for both species. Results showed that *L. ferriphilum*^{T} DAPs were divided into 3 and 2 proteins with increased and decreased levels, respectively, while *At. caldus*^{T} showed 15 proteins with decreased abundance. Importantly, again no DAPs were identified in *S. thermosulfidooxidans*^{T}. Functional classification of *At. caldus*^{T} DAPs indicated a suppressed energy, amino acid, and central carbon metabolism, including impaired respiratory chain components (NADH dehydrogenase, NQO) and sulfur mobilisation systems. Meanwhile, *L. ferriphilum*^{T} proteome pointed toward a reduced electron transfer via downregulated cytochrome c oxidase (CcoN), paired with cell wall reinforcement and Hsp20-mediated protein folding stress (Figure 7).

It is important to note that obtained proteomes only reflects the effects on the planktonic subpopulation after 7 days of treatment. To gain a comprehensive understanding, complementary assays were indeed performed at a shorter timeframe (1 day) and evaluated not only planktonic, but biofilm subpopulation as well. As mentioned, several samples could not be analysed due to low biomass recovered, which rendered proteins samples unsuitable for HPLC-MS/MS. Although some early-timeframe planktonic samples were successfully processed by MS, the high biological variability among replicates precluded any reliable or in-depth statistical analysis. Considering that previous transcriptomic analyses have also mainly focused on planktonic subpopulations after 7 or 14 days of DSF addition [7, 16], the early molecular responses of mineral-attached cells remain mainly uncharacterised.

Conclusions

External DSF/BDSF addition to MT consortium grown in pyrite DSF/BDSF induces a displacement of *At. caldus*^{T} from mineral surface by *L. ferriphilum*^{T}, concomitantly with a drop in ORP values below the optimal range for copper dissolution. Therefore, DSF/BDSF treatment did not improve pyrite and chalcopyrite bioleaching. Furthermore, the MT consortium grown on pyrite was less sensitive to DSF/BDSF compared to chalcopyrite cultures. This effect on mineral colonisation added to reduced iron oxidation and a certain degree of cell death induced by DSF/BDSF especially in mineral-attached fractions, explains the low copper recovery in chalcopyrite assays.

Proteome analyses showed a complex cellular response to DSF/BDSF molecules in *L. ferriphilum*^{T} and *At. caldus*^{T} depending on the mineral they were grown. When *L. ferriphilum*^{T} as part of the MT consortium grown on pyrite, DSF/BDSF promotes its capacity for pyrite colonisation, displacing *At. caldus*^{T} from the mineral-attached fraction. These changes in the community composition increased RISC liberation, and consequently, *At. caldus*^{T} metabolism was activated, but at the same time both species were under oxidative stress.

Within the MT consortium in chalcopyrite cultures, external DSF/BDSF addition most severely affected *At. caldus*^{T}, diminishing an extensive set of its central functions. Conversely, the central metabolism of planktonic *L. ferriphilum*^{T} remained mostly unchanged. This behaviour is likely related

to the copper tolerance of each species. Chalcopyrite dissolution releases large amounts of Cu^{2+}, which is better tolerated by *L. ferriphilum*^{T}. In the case of *At. caldus*^{T} the stress exerted by DSF/BDSF molecules combined with the copper stress, collapsed its metabolic state producing a detrimental effect on its growth and RISC-oxidizing activity. This low oxidation activity of *At. caldus*^{T} suggested this species suggested that this species could not dissolve the passivation layer produced during chalcopyrite leaching explaining in part the low copper recovery.

Acknowledgements

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Microbial communities in column bioleaching: leachates vs mineral and implications for heap bioleaching

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Abstract: Laboratory-scale columns are used to simulate heap bioleaching and provide invaluable data for determining the best operational parameters at small scale. Parameters such as pH, redox potential, and metal dissolution rates are used to assess bioleaching efficiency. However, bioleaching depends on the action of microorganisms which are normally not closely monitored. A series of column experiments were performed to assess bioleaching efficiency of a Ni-Cu low-grade ore at different temperatures (30 °C, 48 °C, 60 °C). Microbial community composition was determined on the inocula (mixture of leachate and solid), and on the leachates (or drainage solutions, no solids). The inocula used at 30 °C and at 48 °C were dominated by *Acidithiobacillus sp.* and *Sulfobacillus sp.* The microbial communities in drainage solution were dominated mostly by *Leptospirillum sp.* with the presence of *Acidiferrobacter sp.* and *Acidimicrobium sp.* in some columns depending on experimental settings and sampling date. At 60 °C, the results showed that the microbial community composition of the inocula differed substantially from the drainage solutions. The inocula were dominated by *Sulfolobus sp.* and the drainage solutions were dominated by *Sulfobacillus sp.* These discrepancies between inocula and drainage solutions may suggest inadequate sampling approach. Therefore, two further column experiments were carried out at 60 °C where a different sampling strategy was used which included microbial community determination of drainage solutions and the ore. The results showed that the microbial community composition of drainage solutions was similar to previous experiments while the mineral's community was similar to the inoculum's. These results evidence the bias of only sampling drainage solutions in heap bioleaching which inform heap performance and inoculation strategy. At a pilot scale and commercial operations, monitoring should extend beyond leach performance and include the monitoring of microbial populations. This is essential for properly informing and optimising the operations.

Keywords: microbial community, bacteria, archaea, heap bioleaching, mineral

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Microbial Community Succession in Chalcopyrite Leach Columns Underlines the Benefits of Biological Process Control

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Abstract: Iron and sulfur oxidising microbes drive solution chemistry and heat generation in copper sulfide leaching, yet their viability and performance remain a black box for operators seeking to optimise leach parameters at commercial scale. To highlight the limitations of unmanaged, ore-derived microbes versus Endolith's continuous bioaugmentation approach, leach tests were conducted in 1 kg columns containing low-grade chalcopyrite ore. Columns were irrigated with synthetic raffinate and ramped from 30–65 °C to simulate exothermic, microbial pyrite oxidation, and sampled destructively between 21–105 days to correlate microbial community composition with leachate chemistry and residue mineralogy. Bioaugmented columns consistently exhibited higher oxidation/reduction potential, increasing availability of ferric iron serving as the oxidant to solubilise further sulfide ores across the temperature gradient. In consequence, they also exhibited a higher rate of pyrite oxidation, indicating enhanced microbial activity and autogenous heat generation critical for dissolution of recalcitrant primary sulfides. 16S microbial community profiling confirmed faster establishment, greater diversity, and superior adaptability of Endolith inoculants compared to indigenous populations. Having demonstrated the tangible benefits of optimising, controlling, and managing microbial activity in sulfide leaching, Endolith also recognises the need for transparent microbial performance indicators commercial scale. To bridge this gap, Endolith is developing SentinelHeap, a comprehensive bioheap monitoring platform. SentinelHeap integrates near real-time bio-performance indicators such as metabolic activity markers and population density with operational chemistry and geology to give mine operators actionable insights beyond reactive management. This enables proactive optimisation of microbial communities in sulfide leach operations, maintaining conditions ideal for heat generation and copper recovery.

Keywords: Copper sulfide leaching, bioaugmentation, chalcopyrite, 16S amplicon sequencing, process control

Decoupling the effects of ore, novel catalyst, and temperature in bioleaching. Implications for microbial tolerance and oxidant capacity.

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Keywords: [Bioleaching; oxidant capacity; microbial resilience; temperature adaptation; catalyst biocompatibility]

Abstract

Bioleaching of copper sulfides depends on microbial consortia that regenerate Fe(III) by oxidising Fe(II) to Fe(III). This function is particularly important for chalcopyrite-bearing primary sulfides, whose leaching is limited by slow dissolution and surface passivation. Jetti's thiocarbonyl-based catalyst is designed to enhance chalcopyrite leaching by interacting with the mineral surface and facilitating electron transfer through the passivation layer. This study evaluated whether mineral–microbe interaction, catalyst addition and temperature affected microbial abundance and the ability of adapted consortia to oxidise Fe(II) to Fe(III).

Mineral–microbe interaction assays used a primary-sulfide-dominant ore and a mixed secondary/primary sulfide ore with enriched consortia at 30 and 45 °C. Catalyst compatibility was assessed using mine-raffinate-adapted consortia in batch reactors at 25, 35 and 45 °C, followed by continuous catalyst addition/stop-addition at 25 °C. During mineral–microbe interaction assays, planktonic cells decreased immediately, with the strongest response observed for the primary-sulfide-dominant ore; however, post-contact tests showed that both consortia retained the ability to oxidise Fe(II) to Fe(III). In batch catalyst tests, control and catalysed systems reached ORP >800 mV(SHE), maintained comparable bacterial counts and oxidised Fe(II) after catalyst addition, with temperature mainly affecting the time required to develop oxidising conditions. In continuous operation, ORP, bacterial abundance and Fe(II)-to-Fe(III) oxidation remained stable during catalyst addition and after catalyst removal, despite changes in relative biomass distribution. Overall, the presence of the catalyst did not compromise total bacterial abundance or the Fe(II)-oxidising function required for bioleaching.

1. Introduction

Chalcopyrite is the dominant copper-bearing mineral in hypogene porphyry ores, but its recovery by heap leaching is limited by slow dissolution and surface passivation. Under acidic ferric leaching conditions, microbial consortia play a key role in sustaining oxidising conditions by regenerating Fe(III) through Fe(II) oxidation. Therefore, any strategy aimed at enhancing chalcopyrite leaching must also be compatible with the microbial function required to maintain oxidant capacity. This study evaluates whether mineral–microbe interaction, catalyst addition, and temperature affect microbial abundance, Fe(II)-oxidising capacity and ability to sustain bioleaching conditions.

2. Methods

This work integrates two experimental studies designed to separate mineral-driven microbial responses from catalyst-compatibility effects. The first study evaluated how enriched bioleaching consortia responded to contact with ores of contrasting mineralogy, focusing on solution-phase microbial abundance and Fe(II)-oxidising capacity. The second study evaluated catalyst compatibility using mine-raffinate-adapted bioleaching consortia through batch and continuous reactor configurations.

2.1. Mineral – microbe interactions studies

Mineral contact tests were conducted using two enriched bioleaching consortia cultivated at 30 and 45 °C and stabilised through successive subcultures. Tests were performed in acidic K medium at pH 1.7 with 1 g/L Fe(II), an initial cell concentration of approximately 1E+08 cells/mL, duplicate mineral additions of 2.5–10% w/v, and untreated controls. Solution-phase cell concentration, pH and ORP were monitored during mineral exposure, and post-exposure Fe(II) oxidation assays were used to evaluate recovery of oxidative capacity after contact with the ores.

2.2. Batch catalyst-addition tests

Batch catalyst-addition tests were conducted at 25, 35 and 45 °C using mine-raffinate-adapted bioleaching consortia in 2 L reactors. Paired control and catalysed systems were operated with continuous aeration at 1 L/min, agitation at 300 rpm, and free acid of 7–8 g/L, Fe(II) supplementation as the energy source, and 50% v/v inoculation. These tests evaluated whether catalyst presence inhibited the ability of the consortia to achieve Fe(II) oxidising conditions. pH, ORP, total iron and Fe(II) were monitored during operation, while total cell counts and post-test Fe(II) oxidation capacity were used to assess microbial abundance and Fe(II) oxidative function.

2.3. Continuous operation during catalyst addition and dosing cessation

Continuous catalyst addition and post-addition tests were conducted at 25 °C using mine-raffinate-adapted bioleaching consortia in 2 L reactors. Reactors were operated with continuous aeration at 1 L/min, agitation at 300 rpm, and free acid of 7–8 g/L, Fe(II) supplementation as the energy source, and 50% v/v inoculation. Each test was operated sequentially under three conditions: baseline operation without catalyst addition, operation with catalyst addition, and post-addition operation after catalyst dosing was ceased. These tests evaluated whether ORP, total bacterial abundance and Fe(II)-oxidising capacity were maintained during catalyst addition and after dosing was stopped. pH, ORP, total iron, Fe(II), total cell counts and post-stage Fe(II) oxidation capacity were used to assess operational stability, microbial abundance and Fe(II) oxidative function. For the continuous 25 °C test, relative biomass distribution was also evaluated by grouping the detected biomass as ferrooxidants, sulfooxidants and heterotrophs.

3. Results and Discussion

3.1. Ore toxicity

The ore-toxicity study was conducted to evaluate whether mineral interaction affected the microbial consortium in solution and whether this response depended on copper sulfide content and deportment. Two ores with contrasting copper and iron deportment were selected for this comparison (Table 1). Ore 1 contained higher Cu and total Fe and was dominated by primary sulfide mineralogy, with most of the copper hosted in chalcopyrite and a higher proportion of locked copper sulfides. This indicates that Fe in Ore 1 was contributed by multiple Fe-bearing phases, not only by pyrite. In contrast, Ore 2 represented a mixed secondary/primary sulfide ore, with a larger contribution from chalcocite and lower locked sulfide content. Although Ore 2 had higher modal pyrite content than Ore 1, 3.50% versus 1.95%, its total Fe content was lower than Ore 1.

Table 1. Copper deportment, iron grade, and sulfide liberation characteristics of the tested ores

#	Cu (%)	Fe (%)	Main Cu deportment	Cu sulfide exposure / liberation
1	0.58	2.60	Chalcopyrite-dominant; ~85.9% of Cu in chalcopyrite	Higher locked Cu sulfides; ~54.8% locked
2	0.30	1.98	Secondary-sulfide-rich; ~57.7% of Cu in chalcocite and ~32.9% in chalcopyrite	Lower locked Cu sulfides; ~31.7% locked

The biological response was assessed using solution-phase cell concentration and post-exposure Fe(II) oxidation recovery tests (Figure 1). Although additional solid loadings of 2.5 and 5% w/v were tested, the 10% w/v condition is shown because it represents the highest mineral–microbe interaction condition

and the clearest response. Attempts were also made to evaluate mineral-associated cells; however, reliable quantification of this fraction was limited by the difficulty of separating cells from fine mineral particles and by variable DNA quality. Therefore, the interpretation focuses on the measured planktonic cells and the subsequent Fe(II) oxidation recovery assays.

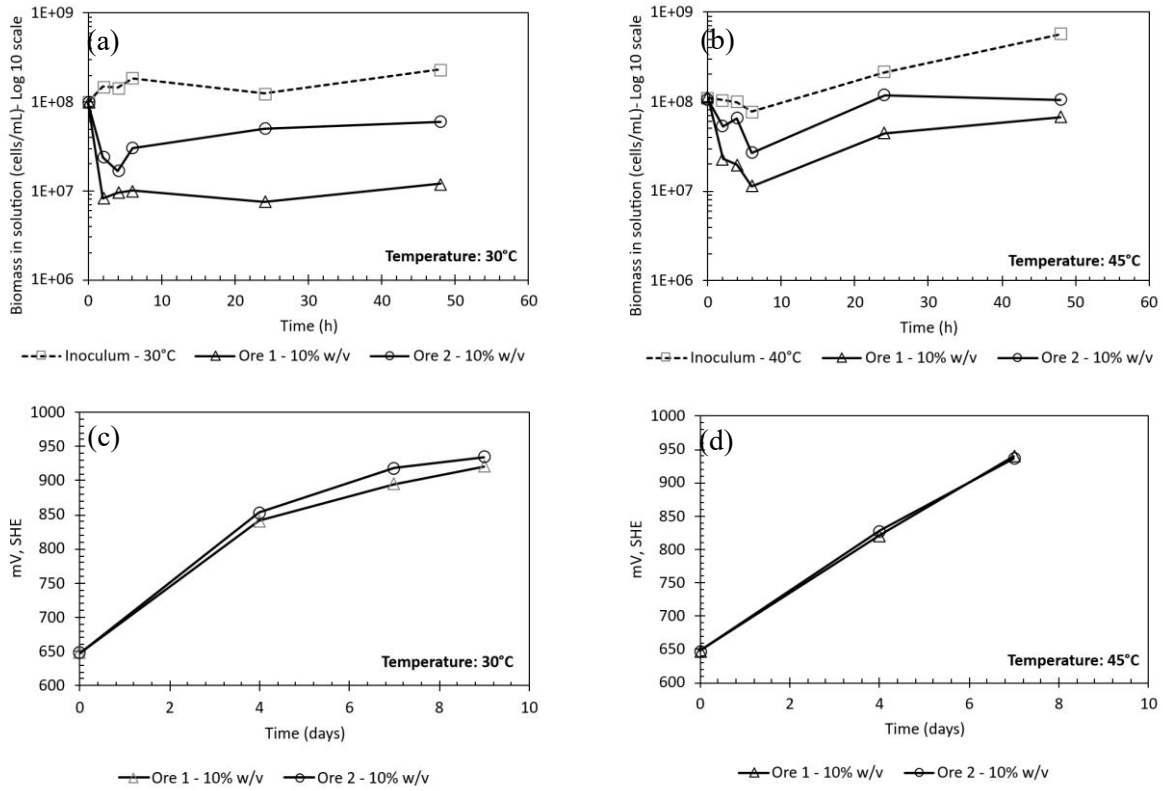


Figure 1. Effect of ore interaction on planktonic cells and recovery of Fe(II)-oxidising capacity at 30 and 45 °C. (a) Planktonic cell concentration during the 30 °C ore-exposure test. (b) ORP development during the post-exposure Fe(II) oxidation recovery assay at 30 °C. (c) Planktonic cell concentration during the 45 °C ore-exposure test. (d) ORP development during the post-exposure Fe(II) oxidation recovery assay at 45 °C.

Mineral–microbe interaction caused an immediate decrease in planktonic cells, with a stronger response for the primary-sulfide-dominant Ore 1 than for the mixed secondary/primary Ore 2 (Figure 1a,c). Based on the available measurements, this decrease indicates a reduction of the planktonic fraction and may reflect cell attachment or adsorption onto mineral surfaces, transient physiological stress, or both; however, these mechanisms cannot be separated from cell counts alone. At 30 °C, Ore 1 showed a more persistent decrease over the 48-h period, whereas Ore 2 partially recovered after the initial decrease (Figure 1a). At 45 °C, both ores also caused an initial decrease, but recovery was more evident, particularly for Ore 2 (Figure 1c). This response should be interpreted considering that microbial and chemical Fe(II)-to-Fe(III) oxidation kinetics are expected to increase at higher temperature.

Despite these temperature-dependent effects and the different short-term cell responses, post-contact Fe(II) oxidation assays showed that both consortia retained the ability to oxidise Fe(II) to Fe(III), with ORP increasing above 800 mV (Figure 1b,d). Therefore, the initial decrease in planktonic cells indicates a mineral-dependent response rather than a persistent loss of Fe(II)-oxidising function in either ore.

3.2. Establishment of oxidising bioleaching conditions in batch systems under catalyst addition

The catalyst-tolerance test was designed to evaluate whether the presence of Jetti's catalyst affected the ability of the bioleaching system to maintain suitable oxidising conditions (defined as, ORP > 800 mV(SHE)). In contrast to the ore-exposure study, this test did not evaluate mineral response but focused on the interaction between the microbial consortia and mine raffinate chemistry (or catalyst). The comparison was made between control and catalysed systems at 25, 35, and 45 °C.

Both control and catalysed systems reached an ORP above 800 mV(SHE) within 30 days at all temperatures tested (Figure 2a). The shorter time observed at higher temperature is consistent with faster microbial and chemical Fe(II) oxidation kinetics under adapted conditions. Post-test Fe(II) oxidation assays showed that all systems recovered oxidative capacity within the 20-day criterion (Figure 2b). Although the 25 °C catalysed test showed faster post-test oxidation than the control, this trend was not consistent across temperature and was therefore not attributed directly to the catalyst enhancing bacterial capability. Initial and final bacterial counts were comparable between control and catalysed systems (Figure 2c), indicating that catalyst presence did not negatively affect microbial growth or viability during the recovery of oxidising bioleaching conditions.

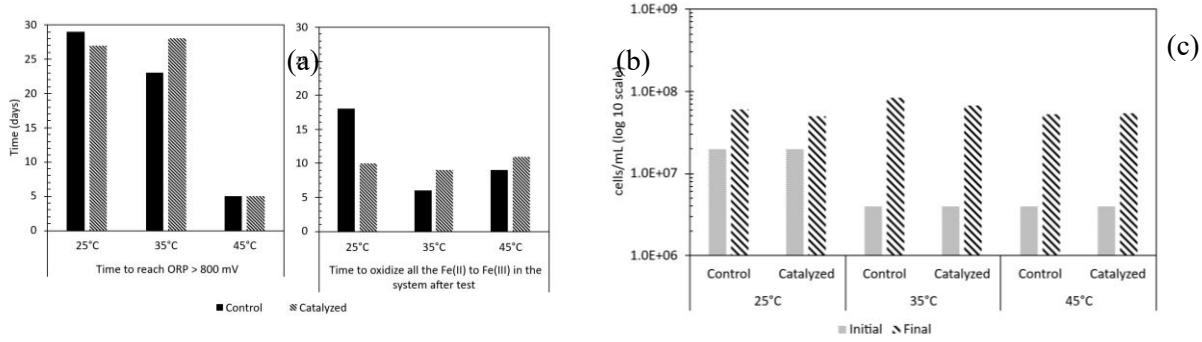


Figure 2. Batch catalyst compatibility in mine raffinate at 25, 35 and 45 °C. (a) Time required to reach ORP >800 mV (SHE) and (b) time to oxidise all the Fe(II) to Fe(III) after test for control and catalysed systems. (c) Initial and final bacterial counts for control and catalysed systems.

Overall, the catalyst did not prevent the system from reaching suitable oxidising bioleaching conditions or retaining post-test Fe(II)-oxidising capacity. The comparable bacterial counts and oxidative responses between control and catalysed systems indicate that catalyst exposure did not compromise the microbial function required to sustain bioleaching activity.

3.3. Maintenance of oxidising bioleaching conditions in continuous systems during catalyst addition and cessation

The continuous test was designed to evaluate whether a bioleaching system that had already developed Fe(II) oxidising conditions could maintain its oxidative performance after the introduction of Jetti's catalyst and if any potential changes observed were reversible after catalyst was stopped. This differs from the previous test, which evaluated whether the system could reach Fe (II) oxidising conditions. Here, the focus was on operational stability and reversibility under continuous operation.

Across the three temperatures evaluated, ORP remained within the oxidising range during baseline operation, catalyst addition and post-dosing operation, and bacterial counts were consistently within the same order of magnitude throughout the test sequence (Figure 3a). No systematic decrease in ORP or bacterial abundance was observed after catalyst addition or after its cessation, indicating that the catalyst did not destabilise the operating system. The post-stage Fe(II) oxidation tests confirmed that the cultures remained functionally active after each operating condition (Figure 3b). Oxidation times were comparable between baseline, catalysed and post-dosing stages, showing that Fe(II)-oxidising capacity in less than 10 days.

Figure 3c.shows the relative microbial distribution at 25 °C for the inoculum, baseline, catalysed and post-dosing conditions, grouped as ferrooxidants, sulfooxidants and heterotrophs. The distribution changed between the inoculum and the baseline condition, with an increase in the sulfooxidant fraction. Under catalyst addition, ferrooxidants became the dominant group, whereas after catalyst removal the heterotrophic fraction increased to approximately 60%. Therefore, catalyst addition and removal modified the relative microbial distribution, but this shift was not associated with a decrease in total bacterial abundance.

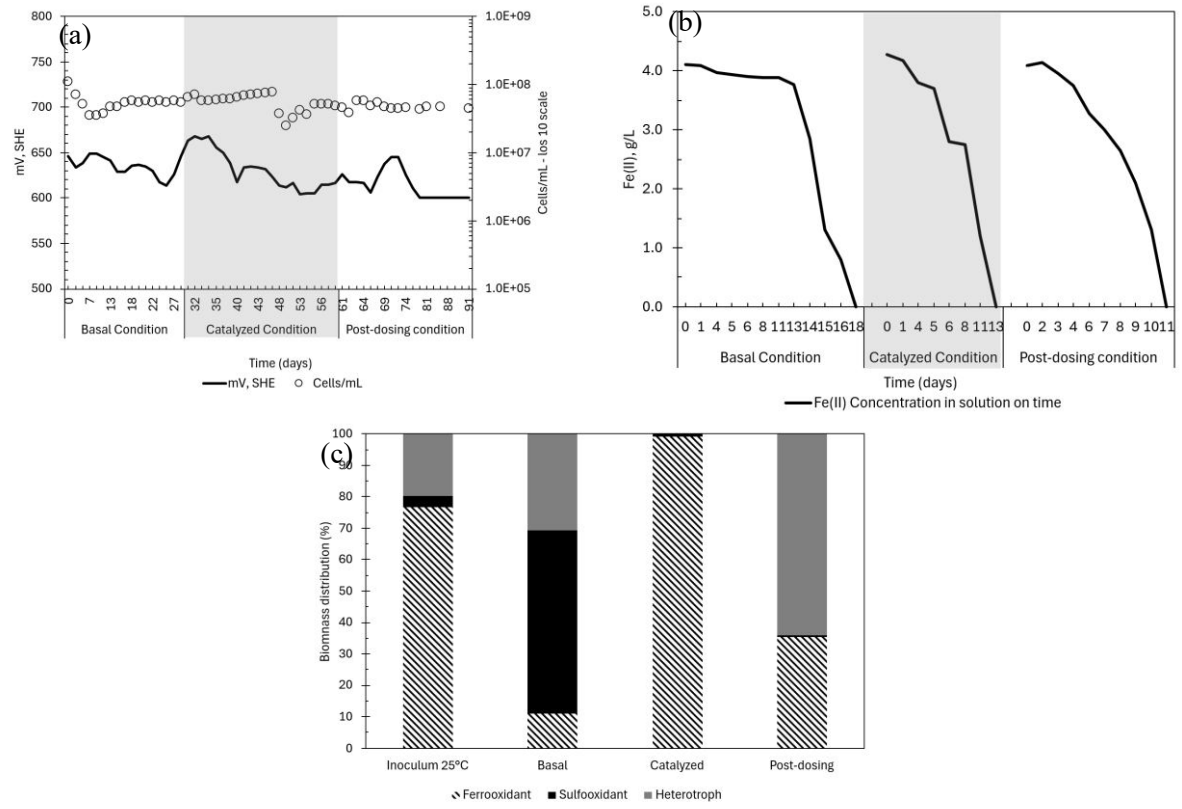


Figure 3. Continuous stability and reversibility of the bioleaching system under catalyst addition. (a) ORP and bacterial counts during baseline, catalysed and post-dosing operation at 25 °C. (b) Post-stage Fe(II) oxidation capacity after each operating condition at 25 °C. (c) Biomass distribution during baseline, catalysed and post-dosing condition.

4. Conclusions

Mineral–microbe interaction produced a transient decrease in planktonic cells, particularly for the primary-sulfide-dominant Ore 1. This response should be interpreted as a change in the liquid-phase microbial fraction rather than direct evidence of total biomass loss, since attached cells can represent a substantial fraction of the microbial population during chalcopyrite bioleaching (Wang et al, 2014). Post-contact Fe(II) oxidation tests confirmed that both ores retained the ability to oxidise Fe(II) to Fe(III), indicating no persistent inhibition of iron-oxidizing activity. Batch and continuous catalyst-addition tests showed that adapted consortia maintained bacterial abundance, ORP stability and Fe(II)-oxidising function. Although catalyst addition and dosing cessation modified the relative biomass distribution, these changes were not associated with loss of total bacterial abundance or Fe(II)-oxidising function.

Acknowledgements

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Bacteria-ore interaction in bioleaching: a CryoSEM/EDS approach to assess the influence of ore chemical composition and surface porosity.

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Abstract: Bioleaching bioprocess relies on the activity of specific acidophilic bacteria able to attack metallic ores. Their interactions with the surface of ore particles are known to significantly influence the process. However, the mechanisms of bacteria attachment are not yet fully understood.

Cryogenic Scanning Electron Microscopy (SEM) coupled to EDS (Energy Dispersive Spectrometry) was used to characterise the attachment of bioleaching bacteria and biofilm formation according to substratum properties and composition. This tool enables to observe ore surfaces and to analyse ore chemical composition at a micrometre scale. Experiments were performed by using bioleaching bacteria as pure strains of *Acidithiobacillus* or multispecies consortium (BRGM-KCC), and various sulfidic ores as well as sulfur particles. Particles were sampled at different times of bacterial growth.

Results strongly suggested that whatever the bacterium and whatever the support/substratum, bacteria during bioleaching mainly form monolayer biofilms. Biofilms developed mainly in cavities. Sulfur particles and pyrite were the most colonised surfaces, but biofilms were also observed on other mineral phases, such as quartz or amphibole. This is probably due to their surface porosity. In contrary, no biofilm was observed on pyrrhotite, chalcopyrite or pentlandite. With the consortium, various bacterial shapes (vibrio, rod or spirilla) were observed on all types of minerals revealing a multispecies biofilm. At last, biofilm formation and bacterial growth occurred concomitantly, suggesting a direct link between bacteria attachment and bioleaching activity.

This study confirms that the new generation of SEM under cryogenic conditions is a powerful tool to investigate biofilm formation and substrate/microorganisms interaction. The simple and rapid preparation of samples (cryogenic freezing in nitrogen slush (-210 °C) followed by steps of sublimation and then Pt coating) preserves the bacteria at the surface of the particle, while EDS enables to combine observation of the biofilm and chemical characterisation of the particles at the micrometre scale.

Keywords: bioleaching, biofilm, bacteria/surface interaction, Cryo-SEM, EDS.

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Sugar Supplementation Enhances Biofilm Formation and Extracellular Polysaccharide Production in *Sulfobacillus acidophilus*

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Keywords: *Sulfobacillus*; Lectin binding; Extracellular polysaccharide; Epifluorescence microscopy; Sugar transporters

Abstract

Sulfobacillus is an acidophilic bacterial genus that plays a key role in bioleaching of metal sulfides at moderately thermophilic temperatures (45–55 °C), owing to its mixotrophic metabolism and capacity to form biofilms by synthesising an extracellular polysaccharide (PS)-rich matrix. The composition these PS remain poorly characterised. A genome-wide survey of *Sulfobacillus acidophilus* DSM 10332^T identified a diverse repertoire of putative sugar transporters. Supplementation with glucose, fructose, xylose or sucrose increased biofilm formation on elemental sulfur, quantified using large-scale image analysis, and increased extracellular PS predominantly composed of mannose and glucose residues, as shown by fluorescent lectin binding and confirmed by HPLC-UV-ESI-MS. Sugar supplementation stimulates biofilm development by enhancing PS biosynthesis and establish *Sulfobacillus* as a model for studying sugar uptake, extracellular PS biosynthesis and carbon cycling in thermophile bioleaching systems.

Introduction

Biomining relies on extremely acidophilic microorganisms that oxidise ferrous iron and/or reduced inorganic sulfur compounds to recover metals from sulfide ores [1]. *Sulfobacillus comprises* moderately thermophilic, mixotrophic, non-motile bacteria capable of both chemolithoautotrophic and heterotrophic growth [2]. Like other bioleaching bacteria, *Sulfobacillus attaches* to mineral surfaces and forms biofilms stabilised by a matrix of extracellular polymeric substances (EPS), of which polysaccharides (PS) are a major component [3]. Genome analyses of environmental *Sulfobacillus spp.* have revealed genes for glycolysis, the pentose phosphate pathway and putative sugar ABC transporters [4], and other species grow heterotrophically on sugars and α-glucans [5]. However, these transport systems have not been functionally validated, and whether imported sugars are channelled into PS biosynthesis remains unknown. This study combined bioinformatic and experimental approaches to (i) identify putative sugar transporters encoded in the genome of *S. acidophilus* DSM 10332^T, (ii) evaluate the effect of sugar supplementation on biofilm formation, and (iii) determine whether supplementation enhances extracellular PS production and characterise its monomeric composition.

Methods

Bioinformatic analysis. Complete genomes of *S. acidophilus*^{T} and *S. thermosulfidooxidans*^{T} were searched against the Transporter Classification Database using GBlast [6]. Membrane topology, signal peptides and conserved functional domains were predicted using standard bioinformatic tools, and genomic context was annotated using subsystem-based genome annotation.

Biofilm formation assays. *S. acidophilus*^{T} (10^7 cells/mL) was cultured in K medium (pH 1.7) with 2 g/L Fe(II), 0.02% yeast extract and 1% elemental sulfur (S^0) pearls in static 6-well plates at 37 °C for 96 h, with individual sugars (glucose, fructose, xylose, sucrose) or extracellular DNA supplemented at 1 mM or 1 ng/µL, respectively.

Epifluorescence microscopy and image analysis. Sulfur pearls were stained with DAPI and, for Fluorescent Lectin Binding Analysis (FLBA), with FITC-/TRITC-conjugated lectins, then imaged on a Zeiss Axio Observer Z1/7 (40× objective, tiled z-stacks). Colony density and percentage colonised area were quantified using a custom Python image-analysis pipeline [7], with 768–1536 tiles analysed per condition; differences were assessed by Kruskal–Wallis and Dunn's tests.

Extracellular PS production and analysis. For PS production, *S. acidophilus*^{T} was grown in shaken flasks (150 rpm, 50 °C, 168 h) in K medium with sugars (5 mM monosaccharides, 2.5 mM sucrose). Residual sugars were monitored using a colorimetric method. PS were precipitated from cell-free supernatants with isopropanol and their monosaccharide composition determined by HPLC-UV-ESI-MS/MS following acid hydrolysis and PMP-derivatisation [8].

Results and Discussion

***Sulfobacillus encodes* a diverse repertoire of sugar transporters.** Comparative genomic analysis identified 17 and 16 putative sugar transporters in *S. acidophilus*^{T} and *S. thermosulfidooxidans*^{T}, respectively, distributed among three families: the Major Facilitator Superfamily (8 transporters in each genome), ABC transporters of the CUT-1 (5 systems) and CUT-2 (3 systems) subfamilies, and the phosphotransferase system (PTS). A complete fructose-specific PTS system, and CUT-1 transporters predicted to mediate trehalose and xylose uptake, were identified exclusively in *S. acidophilus*^{T}, pointing to species-specific sugar-uptake capabilities. Both genomes additionally encoded multiple Nucleobase:Cation Symporter (NCS-1) transporters (10 in *S. acidophilus*^{T}, 7 in *S. thermosulfidooxidans*^{T}), consistent with a capacity to scavenge nucleosides and nucleobases released by acid hydrolysis of DNA under the strongly acidic growth conditions.

Sugar supplementation enhances biofilm formation. Using a high-throughput image-analysis workflow [8], static cultures supplemented with fructose, xylose or sucrose (1 mM) showed significantly increased colony density and colonised surface area on S^0 pearls relative to unsupplemented controls, whereas glucose increased colony density without increasing colonised area. Supplementation with extracellular DNA (1 ng/µL) also significantly enhanced biofilm formation, consistent with the genomic capacity for nucleobase/nucleoside uptake (Figure 1).

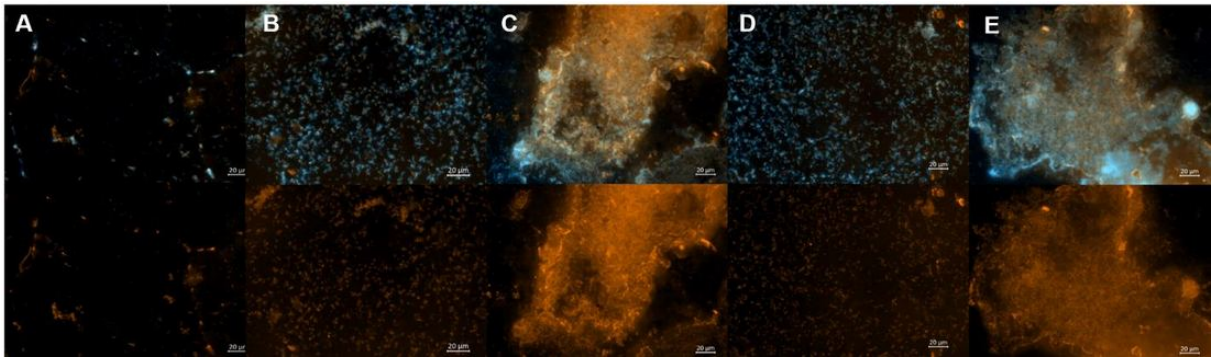


Figure 1. Sugar supplementation enhances extracellular PS production in *S. acidophilus*^{T}. Biofilms of *S. acidophilus*^{T} on S^0 pearls, stained with DAPI and HHA-TRITC. The upper panel shows the merged TRITC-DAPI channels, and the lower panel shows images of the TRITC channel. A) Control, B) DNA, C) Fructose, D) Xilose E) Trehalose. Binding to the capsular EPS is observed. Size bars represent 20 µm.

Increased extracellular PS accompanies enhanced biofilm formation. Lectin screening identified HHA and ConA — both mannose/glucose-specific — as the strongest and most consistent binders to the *S. acidophilus*^{T} biofilm matrix, indicating a mannose- and glucose-rich PS matrix, consistent with previous FLBA studies of acidophilic biofilms [10]. Binding intensity of both lectins increased markedly upon sugar supplementation without a change in staining pattern, indicating a rise in PS abundance rather than a shift in composition.

Sugar supplementation increases planktonic growth and total PS yield under agitation. In shaken-flask cultures, fructose, glucose, xylose and sucrose supplementation significantly increased planktonic cell densities relative to sugar-free controls, accompanied by progressive depletion of the supplemented sugar (fructose and sucrose were nearly fully consumed within 96 h). HPLC-UV-ESI-MS/MS analysis of culture supernatants confirmed a pronounced increase in total PS production under all sugar-supplemented conditions. The monomeric composition of the PS was dominated by mannose, with glucose as the second most abundant sugar and minor contributions of ribose, galactose, glucosamine and galactosamine across all conditions; rhamnose was detected only upon sugar supplementation (Table 1). This composition is consistent with capsular PS reported for *A. ferrooxidans* [4] and supports mannose/glucose as conserved building blocks of *Sulfobacillus EPS*.

Table 1. **Sugar monomer composition of the PS produced by *S. acidophilus*^{T}.** The proportion of each monosaccharide was calculated by averaging the values of each replicate. All components were analysed and quantified via HPLC-ESI-MS/MS.

Monosaccharide (molar ratio)	Xylose	Glucose	Fructose	Sucrose	Control
Xylose	n.d.	n.d.	n.d.	n.d.	0.08
Ribose	0.07	0.11	0.11	0.11	0.06
Rhamnose	n.d.	0.04	0.07	0.04	n.d.
Mannose	0.73	0.68	0.60	0.61	0.71
Glucose	0.17	0.15	0.15	0.18	0.14
Glucosamine	n.d.	n.d.	0.02	0.02	n.d.
Galactose	n.d.	n.d.	0.03	0.03	n.d.
Galactosamine	0.02	n.d.	n.d.	n.d.	n.d.
Total	1.00	1.00	1.00	1.01	1.01

Taken together, these results indicate that *S. acidophilus*^{T} can import exogenous sugars, channeling them into extracellular PS biosynthesis, thereby enhancing biofilm formation on elemental sulfur.

Conclusions

This study provides the first genome-wide survey of sugar transporters in *Sulfobacillus and* demonstrates that supplementation with sugars enhances both biofilm formation and extracellular PS production in *S. acidophilus*^{T}, without altering its mannose/glucose-dominated composition. These findings establish a functional link between sugar uptake and EPS biosynthesis during biofilm development and position *Sulfobacillus* as a promising model for studying sugar metabolism, EPS biosynthesis and carbon cycling in bioleaching systems.

Acknowledgements

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Integrated bioleaching and resource recovery from sulfidic tailings for critical metals and iron mineral production

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Abstract:

Increasing demand for critical raw materials and the environmental impacts of mining waste require sustainable strategies. Within the ReMining Plus and RAWMINA projects, two different mining sites were investigated to develop a scalable and environmentally friendly approach integrating microbial metal recovery and the valorisation of tailings within a circular economy framework.

ReMining Plus focused on historical sulfide tailings from lead–zinc–silver mining in Germany. The residues used in the experiments contained 730 mg/kg zinc and 20 mg/kg indium. Bioleaching was performed in a pilot-scale airlift reactor, resulting in leachate concentrations up to 1.6 g/L zinc and 2 mg/L indium, demonstrating efficient recovery of sphalerite-hosted metals.

RAWMINA project tested at pilot scale in a stirred tank reactor two different materials, tailings and run of mine of Iberian pyrite belt area contained <0.4% copper and around 200 mg/kg cobalt. Valuable minerals like pyrite, chalcopyrite and enargite were microbiologically leached, yielding up to 0.5 g/L copper and 25 mg/L cobalt in the pregnant leach solution.

The bioleaching of both tailings was carried out using sulfur- and iron-oxidising species of the genera *Acidithiobacillus und Leptospirillum* in reactors (bio-airlift and stirred tank reactors)

The oxidation of sulfide minerals generated high iron and sulfate concentrations in solution. These were further processed to produce iron-based materials. Precipitation of iron was optimised through the controlled formation of schwertmannite (Fe(III)-oxyhydroxysulfate) at pH 2.0–3.5 using magnesium oxide as a neutralising agent, while minimising co-precipitation of valuable metals. Process parameters were carefully adjusted to prevent gel formation and to ensure efficient solid–liquid separation. The resulting schwertmannite was subsequently converted into nano-scale magnetite (Fe_3O_4) via reaction with ferrous sulfate under controlled heating and pH adjustment to 9–10.

Iron-bearing secondary products generated during hydrometallurgical processing can be utilised for environmental and material applications. Schwertmannite is characterised by high adsorption capacity and may be utilised as a sorbent for oxo-anions such as arsenate, phosphate, and chromate. It can also be processed into pigment materials. Nano-scale magnetite has properties comparable to low-grade magnetite ores and may therefore be considered a potential substitute in relevant industrial applications.

The results demonstrate the technical feasibility of bioleaching as an integrated approach for the holistic utilisation of sulfidic tailings, including the recovery of dissolved metals and the production of by-products, thereby supporting more sustainable management of mining residues.

Keywords: Bioleaching, tailings, pilot plant, metal recovery, environmental benefits

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Cobalt extraction from pyrite concentrates through bioleaching: developing by-product valorisation strategies at the mine design stage

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Abstract: The biohydrometallurgical recovery of strategic metals from mining by-products represents a sustainable industrial pathway for resource valorisation. This study investigates cobalt extraction from a cobaltiferous pyrite concentrate recovered from copper-iron ore flotation tailings at the Dominga mining project in Chile, employing the BRGM-KCC acidophilic microbial consortium, primarily composed of *Leptospirillum ferriphilum*, *Acidithiobacillus caldus* and *Sulfobacillus spp.*

Mineralogical characterisation confirmed cobalt association with pyrite phases in a fine-grained material (P80 = 39 µm) with elevated sulfide content (~60 %). Bioleaching screening tests in agitated flask systems validated the consortium's metabolic capabilities to bioleach the concentrate, demonstrating effective iron and sulfur biooxidation with temperature optimisation at 40 °C.

Scale-up trials conducted in 2 L stirred-tank bioreactors revealed robust microbial kinetics, achieving solution redox potentials exceeding 900 mV (vs. SHE) and pronounced pH reduction, consistent with intensive biooxidation processes. Cobalt solubilisation yield reached close to 100% while iron dissolution yield achieved 76%. Extrapolation to continuous stirred-tank reactor (CSTR) operation using residence time distribution analysis and first-order kinetics (as a first simple approach) indicates that 95% cobalt dissolution currently requires a hydraulic residence time of 6 days. However, a modest 30% increase in the biooxidation kinetic constant would reduce this to 4 days.

This demonstrates that biohydrometallurgy represents a viable industrial approach for cobalt recovery from Dominga copper flotation tailings. Further process optimisation is expected to enhance biooxidation kinetics and achieve industrial competitiveness for sustainable metal valorisation within the circular economy framework.

Keywords: cobalt recovery, pyrite bioleaching, tailings valorisation, acidophilic consortium, mine design optimisation.

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Bioleaching of Gold from Cyanide Heap Leach Tailings with Iodide-Oxidising Microorganisms

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Abstract:

With declining ore grades and the global shift towards a circular economy, interest in mine waste valorisation is increasing. This project explored the bioleaching of gold from cyanide heap leach tailings using iodide-oxidising microorganisms. The tailings contained 67% quartz, 22% muscovite, 2% kaolinite, 1% goethite, 1% hematite and 7% amorphous material, with a gold content of 96 ppb. Iodide-oxidising microorganisms enriched from the tailings and mine water were used as inoculum for the bioleaching experiments. The bioleaching of the tailings was evaluated in four bench-scale columns simulating heap bioleaching. Each column was hydraulically connected to an external leach liquor tank and operated for 186 days. Two columns were aerated directly from the base, whereas the leach liquor of the two non-aerated columns was aerated in the recirculation tanks. The columns were irrigated with mine water amended with KI, CH_3COONa, NH_4NO_3 and Na_2HPO_4 as substrates and nutrients. From day 102, the leach liquor pH was regularly adjusted to 7.5-8.5 using HCl. Results indicated that soluble gold concentrations increased in all columns over time, reaching 44 µg L^{-1} in one of the aerated columns. The final gold leaching yields were 24.2-24.8% for non-aerated columns and 29.1-30.0% for aerated columns, indicating that direct aeration of the columns significantly improved gold leaching by 5% ($p = 0.011$) compared to aerating the leach liquor in the tanks. Some of the most abundant prokaryotic genera in the column residues at the end of the experiment were *Pseudomonas* (0-69.3%), *Thalassospira* (0-62.8%), *Phaeobacter* (0-46.6%), *Marinobacter* (0-38.3%), *Rheinheimera* (0-16.4%), *Pseudoalteromonas* (0-16.3%) and *Marinomonas* (0-12.4%). In the liquor tanks, predominant genera included *Phaeobacter* (0-92.7%), *Pseudomonas* (0-71.9%), *Thalassospira* (0-54.0%), *Parvibaculum* (0-33.6%), *Ruegeria* (0-28.0%), *Anaerobacillus* (0-24.7%) and *Agaricicola* (0-15.2%). This study demonstrated the technical feasibility of using iodide-oxidising microorganisms for bioleaching gold in aerated and non-aerated columns, highlighting their potential for valorising cyanide heap leach tailings.

Keywords: bioleaching, gold, iodide oxidation, mine waste valorisation, tailings

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Circular Biohydrometallurgy: Moderate-Thermophilic Bioleaching of Co–Ni from Chilean Copper Tailings

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Keywords: heap bioleaching; microbial consortia; cobalt; nickel; mining tailings

Abstract

Chile's copper-mining tailings retain significant cobalt and nickel not captured by conventional flotation, a potential unexploited secondary resource for critical-metal recovery. Unlike the low-grade primary sulfides treated by industrially proven heap bioleaching (ROM or ¾-inch ore) [1], tailings are a fine-grained secondary material that requires prior agglomeration onto a coarse, chemically inert support to sustain percolation [2]. This work evaluates a thermotolerant native consortium ("Kobold B (S)", DSM 35389) for bioleaching cobalt from the CS-CL scavenger-tailings fraction of the San José mineral processing plant (Pucobre, Copiapó, Chile), an IOCG-type deposit whose pyrite-concentrated CL scavenger tail averages 1400 ppm Co, 741 ppm Ni and 2117 ppm Cu. Flask assays inoculated with Kobold B reached >90% cobalt recovery by day 35, versus ~46% when non inoculated. Agglomerated-column bioleaching (45 °C, pH 1.5, 80% acid curing, 1×10^7 cells g^{-1}) achieved, at a common 91-day point, 95% Co, 84% Ni, 54% Cu and 55% Fe recovery. Given the scale of Chilean tailings and the feed's high pyrite content, expected to self-heat a full-scale heap, heap configurations offer a viable route to implementation of cobalt recovery. Beyond metal recovery, controlled dissolution of the cobalt-hosting pyrite removes the tailings' principal acid-generating phase, reducing acid-drainage risk.

Introduction

Chile hosts 836 tailings facilities as of December 2025, of which only 129 are active, 223 inactive and 455 abandoned or under review [3,4]. Although not intrinsically hazardous, these deposits often retain arsenic, copper, lead and other elements that mobilise through sulfide oxidation and acid mine drainage (AMD) once exposed to oxygen and water [5,6], with attendant disaster-risk and governance challenges under a changing climate [7]. In January 2026, Chile's Ministry of Mining launched the National Strategy for Critical Minerals (Estrategia Nacional de Minerales Críticos, EMC), which explicitly identifies cobalt among 14 priority elements and promotes the recovery of critical metals from secondary sources, including mining residues, as part of a circular-economy agenda [8].

Cobalt has gained strategic relevance owing to its central role in battery supply chains for electro mobility and energy storage, while conventional hydrometallurgical routes for its recovery from low-grade residues remain constrained by high capital and operating costs and secondary-effluent generation [9–11]. Microbial bioleaching, using acidophilic chemolithotrophic consortia to oxidise iron and sulfur and thereby release associated metals, offers a lower-energy, lower-carbon alternative [9,12–14], although tailings bioleaching is often limited by slow initial kinetics and low indigenous microbial titres [1,15–17]. Pre-adapted inoculation strategies have therefore become central to reducing lag phases and improving reproducibility [1,15,16,18,19].

The present study pursues four objectives: (i) to characterise the feed resource, including its cobalt and nickel content and mineralogy; (ii) to describe how the native microbial consortia were isolated, taxonomically characterised and applied; (iii) to quantify the recovery of cobalt and associated metals from both flask and column (percolation) bioleaching; and (iv) to set out the technical and economic basis for heap (percolation) leaching, rather than stirred-tank leaching, as the appropriate configuration for treating tailings of this tonnage and grade.

Methods

Materials. The feedstock was the CS-CL scavenger-tailings fraction from the cleaning circuit of the San José flotation plant of the company Pucobre (Copiapó, Atacama Region, Chile, Table 1), processing an IOCG-type deposit copper ore. Two independent campaigns (60 kg and 150 kg) were collected, quartered, crushed and homogenised. Cobalt content was 1100–1400 ppm across the two campaigns, determined by atomic absorption spectroscopy (AAS). The specific head feed agglomerated onto the inert quartz support for the minicolumn bioleaching trials averaged, by AAS, 1400 ppm Co, 741 ppm Ni, 2117 ppm Cu and 24.4 wt% Fe. This grade represents the outcome of a deliberate pyrite-concentration strategy: baseline scouting characterisation of the same scavenger circuit (September 2024) had identified an average feed grade of ~4 wt% pyrite and 257 ppm Co, with an explicit target of exceeding 1000 ppm Co by increasing the pyrite content of the scavenger fraction in coordination with the plant's operations team [20]; the 27–40 wt% pyrite content and 1100–1400 ppm Co achieved in the CS-CL feed used for the present bioleaching campaigns exceed that target. Mineralogy was assessed by TESCAN Integrated Mineral Analyser (TIMA) and X-ray diffraction (XRD): pyrite was the dominant sulfide (27–40 wt%), accompanied by a gangue of quartz, chlorite, K-feldspar, albite and minor magnetite/hematite and calcite. Granulometric analysis (TIMA) gave a median particle size of 27–28 µm (range 5–360 µm), with pyrite surface liberation exceeding 80% in more than 95% of grains, favoring microbial attachment and oxidative dissolution.

Table 1. Characterisation of the CS-CL scavenger-tailings feedstock.

Parameter	Value	Method / Notes
Source	CS-CL scavenger fraction, cleaning circuit, San José de Pucobre flotation plant (Copiapó, Chile)	IOCG-type Cu ore; two campaigns (60 kg, 150 kg)
Cobalt (Co)	1100–1400 ppm (column-feed head average 1400 ppm, AAS)	Atomic absorption spectroscopy (AAS)
Nickel (Ni)	741 ppm (column-feed head average, AAS)	AAS
Copper (Cu)	2117 ppm (0.21 wt%; column-feed head average, AAS)	AAS
Iron (Fe)	24.4 wt% (243,941 ppm; column-feed head average, AAS)	AAS
Dominant sulfide	Pyrite: 27% (XRD) – 40% (TIMA)	TIMA + XRD
Gangue	Quartz, chlorite, K-feldspar, albite, magnetite/hematite, minor calcite	TIMA + XRD
Particle size (median)	27–28 µm (range 5–360 µm)	TIMA granulometry
Pyrite liberation	>80% surface exposure in >95% of grains	TIMA

Microbial consortium acquisition and selection. Eleven samples were collected across the flotation circuit (raw material, concentrate, sulfated-pool solids, Cpy-pool solids, scavenger tailings, column tailings, rougher feed and column feed) and cultured in KMD medium at 30, 45 and 55 °C, supplemented with Fe(II) and/or elemental sulfur. Growth and iron/sulfur-oxidizing activity were confirmed at 30 and 45 °C in six of eleven samples; no activity was observed at 55 °C. Two consortia were retained for further work: Kobold A (mesophilic, 30 °C) and Kobold B (thermotolerant, 45 °C). Cell-viability assays showed 100% viability for Kobold B under optimal growth conditions and >90% viability when directly exposed to the Cola Scavenger tailings.

Taxonomic characterisation. Both consortia were characterised by 16S rRNA gene sequencing (16S Barcoding Kit SQK-16S024, Oxford Nanopore Technologies). At 30 °C Kobold A was dominated by *Acidithiobacillus caldus*, *Sulfobacillus sp.*, *Acidithiobacillus sp.*, *Acidithiobacillus ferrooxidans* and *Leptospirillum sp.* Kobold B comprised a thermotolerant community of *Sulfobacillus thermosulfidooxidans*, *Acidithiobacillus caldus*, *Sulfobacillus sp.*, *Acidithiobacillus sp.*, *Leptospirillum sp.* and *Lepto-*

spirillum ferriphilum; at 45 °C it was dominated by *Sulfobacillus thermosulfidooxidans*, with *Acidithiobacillus caldus* and *Leptospirillum sp.* in lower abundance. Kobold B was selected for scale-up; its deposit and patent status are given below.

Bioleaching assays. Preliminary flask assays used 0.5X KMD medium supplemented with Fe(II) at 20% (w/w) pulp density, in six flasks: M1 (non inoculated, 30 °C), M2–M3 (Kobold A, 30 °C), M4 (non inoculated, 45 °C) and M5–M6 (Kobold B, 45 °C), with pH, redox potential (Eh, Ag/AgCl) and cobalt recovery monitored throughout. Column bioleaching used 30-cm minicolumns covered by heating jackets (30/45 °C) and pH (1.0/1.5); acid-curing intensity (60/80%); inoculum density (1×10^6–1×10^8 cells g^{-1} total solids); and inoculation method (irrigation vs. post-curing application). Dissolved cobalt, nickel, copper and iron, together with pH and Eh, were monitored periodically by AAS to determine leaching kinetics and overall recovery.

Results and Discussion

Cobalt grades of 1100–1400 ppm in the CS-CL feed consistently exceed the 1000 ppm threshold established for viable secondary resources, and the high degree of pyrite liberation (>80% in >95% of grains; Table 1) provides a favourable substrate for oxidative, microbial mediated dissolution, since cobalt substitutes for Fe within the pyrite lattice.

Inoculation was the decisive factor for recovery performance (Table 2). In flask assays, Kobold B (45 °C) reached >90% cobalt recovery by day 35, compared with ~82% for Kobold A (30 °C) and only ~46% for the non inoculated control, confirming that a robust population of iron- and sulfur-oxidizing microorganisms shortens the lag phase associated with the low indigenous microbial titres typical of tailings. Column bioleaching, which more closely emulates industrial percolation conditions, reproduced this trend at larger scale: under optimised conditions (Kobold B, 45 °C, pH 1.5, 80% acid curing, 1×10^7 cells g^{-1} applied post-curing), cobalt recovery reached ~95% by day 91, the common time point adopted here for cross-metal comparison, against ~71% for the non inoculated control over the same period. The full cobalt- and nickel-recovery kinetics of the minicolumn assays up to day 91 are shown in Figure 1.

Table 2. Cobalt recovery in flask and minicolumn bioleaching assays.

System	Conditions	Time	Co recovery
Flask assay, non inoculated control	20% pulp (w/w), 30–45 °C	Day 35	~46%
Flask assay, Kobold A	30 °C, 20% pulp	Day 35	~82%
Flask assay, Kobold B	45 °C, 20% pulp	Day 35	>90%
Minicolumn, non inoculated control	45 °C, pH 1.5, 80% acid curing	Day 84	~70%
Minicolumn, Kobold B (optimized)	45 °C, pH 1.5, 80% acid curing, 1×10^7 cells g^{-1}, post-curing inoculation	Day 84	90%

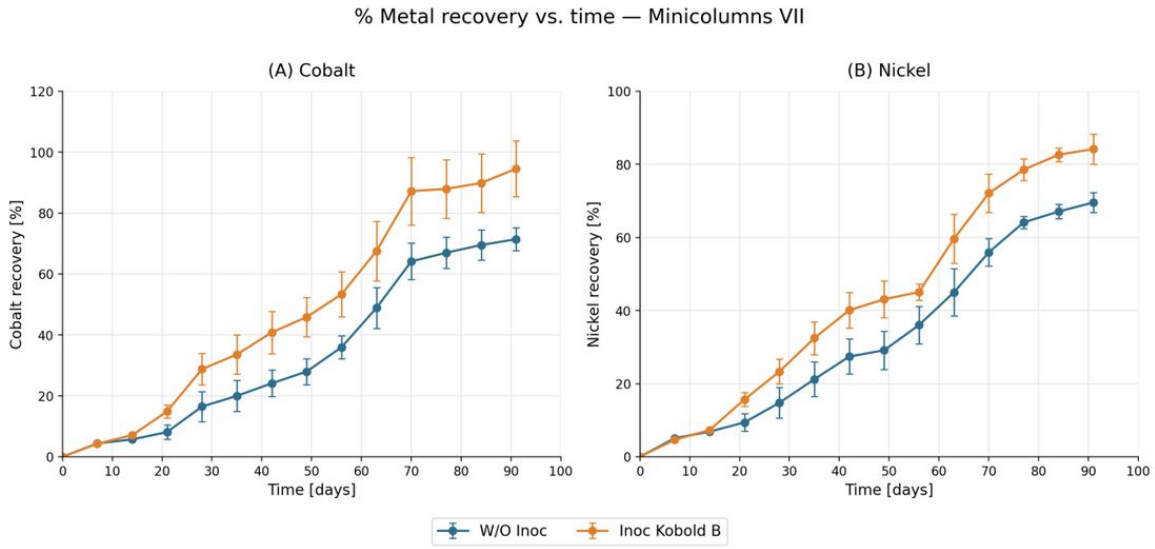


Figure 1. Metal recovery versus time in the Minicolumns VII assays (up to day 91). (A) Cobalt recovery and (B) nickel recovery, for the non inoculated control (W/O Inoc) versus Kobold B inoculation (Inoc Kobold B).

Beyond cobalt, the optimised column system co-recovered, at the same 91-day point, other economically relevant metals (Table 3): 84% nickel, 54% copper and 55% iron, demonstrating polymetallic valorisation potential beyond a single-metal process.

Table 3. Polymetallic recovery from optimised minicolumn bioleaching, day 91.

Co	Ni	Cu	Fe
95%	84%	54%	55%

Basis for heap (percolation) rather than stirred-tank leaching

Two process-engineering considerations govern scale-up: the achievable thermal regime and the reactor configuration. Sulfide oxidation is strongly exothermic, and heap bioleaching operations self-heat from ambient into the moderate-thermophilic range (40–55 °C) purely from pyrite oxidation when adequately aerated and insulated [21,22]; as little as ~5 wt% pyrite suffices to drive a heap into the thermophilic range [21], so the 27–40 wt% pyrite of the CS-CL feed (Table 1) comfortably supports autogenous heating to 45 °C, further aided by thermal covers over the irrigated surface [23]. Stirred-tank bioleaching, by contrast, is reserved for higher-value concentrates given its fine-grinding, aeration and cooling demands and long residence times (5–12 days) [24]; at the hundreds-of-millions-of-tons scale of Chilean tailings [3], heap leaching is the only economically viable route, treating run-of-tailings material *in situ* or in constructed piles at a fraction of the cost per ton [25,26]. Heap bioleaching is therefore the appropriate configuration, currently being validated in 1–4 m column and semi-industrial pilot trials (see Conclusions).

The microbial consortium and its patent deposit

The consortium is a defined, multi-species culture deposited with the Leibniz Institute DSMZ (International Depositary Authority) under the Budapest Treaty, accession DSM 35389 (original deposit 2025-02-13; viability confirmed 2025-02-21; certified 2025-04-15), and associated with international patent application PCT/CL2025/050085. Under Rule 9 of the Budapest Treaty, patent-purpose deposits are held in confidentiality and are not listed in public catalogues until a legitimate right of access is established [27,28]. 16S rRNA characterisation of Kobold B at 45 °C confirms a multi-species consortium dominated by *Sulfobacillus thermosulfidooxidans*, with *Acidithiobacillus caldus* and *Leptospirillum sp.* as minor but consistently detected components.

Conclusions

The CS-CL scavenger-tailings fraction from the San José de Pucobre plant is a technically viable secondary cobalt resource (1100–1400 ppm Co) hosted in highly liberated pyrite. Inoculation with the thermotolerant consortium Kobold B (DSM 35389) is the decisive factor enabling >90% cobalt recovery

within 35 days in batch flasks and ~95% cobalt recovery by day 91 in percolation-type minicolumns, together with substantial co-recovery of nickel (84%), copper (54%) and iron (55%) at the same 91-day comparison point. Critically, the approach delivers value on two fronts: it not only recovers critical metals from a waste material, but also controllably and progressively dissolves the cobalt-bearing pyrite, the phase responsible for acid mine drainage, thereby removing the principal source of long-term acid generation from the tailings and reducing the risk of future acidic effluents, spills and potential heavy metals contamination. The high pyrite content of the feed (27–40 wt%) is expected to sustain autogenous heating of a full-scale heap into the operating temperature range demonstrated here, and the tonnage and grade of Chilean tailings favour heap over stirred-tank configurations on both technical and economic grounds. These findings, together with the documented, currently valid DSMZ deposit of the Kobold B consortium, support continued development toward semi-industrial piloting under the "Cobalto Sostenible" next coming project (CORFO 25CODT-286726, 2027–2029), building on the current Cobalto Verde project (ANID/Pucobre, TA23I10022, 2024-2027).

Acknowledgements

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Bioleaching of Critical Metals from Low-Grade Tailings: Process Development for the COMAN Pilot Scale

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Abstract: Tailings represent the residual waste generated in mineral extraction and often retain economically significant metal concentrations that remain unrecovered due to limited cost-effective processing options. In sulfide-rich tailings, oxidative reactions further pose a risk of acid rock drainage, creating additional environmental challenges.

In COMAN process critical metals, Co, Cu and Mn, are recovered from low-grade secondary sources and mining tailings through pre-treatment (comminution, gravity separation and flotation) followed by a hydrometallurgical process based on a biological leaching and the precipitation of valuable metals as sulfides.

This work describes the bioleaching phase of the COMAN process where iron- and sulfur-oxidizing microorganisms were utilised to liberate cobalt, copper, manganese, nickel and zinc from pyrite-rich tailings. Microbiological culture was first adapted to the material, and then several batch bioleaching tests in shake flasks and stirred tank reactors were conducted to optimise the parameters for continuous-batch mode pilot run in 100 L 904L stainless steel reactor. The results indicated that the pH substantially affects the release behaviour of Cu and Mn. Lower pH values (pH 1.25) preferentially promote Cu leaching, whereas higher pH levels (pH 1.75) enhance the mobilisation of Mn into solution. In contrast, Co and Zn exhibit consistent release across the entire pH range investigated.

Keywords: Bioleaching, tailings, mining wastes, secondary resources

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Pilot scale operation for nickel bioleaching from pyrrhotite tailings

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Keywords: nickel, pyrrhotite, pilot-scale, bioreactor, operations

Abstract

A 30 L multistage pilot plant modelled after the BIOX® process was constructed to bioleach nickel from pyrrhotite-rich mine tailings while recovering elemental sulfur. The plant was operated with 10% (w/w) fresh tailings, 30 °C, pH 2, and with a target residence time of 10 days. Although steady-state was not achieved, the operation identified key indicators for several scale-up challenges including consistent feed and solids accumulation. These early indicators included variations in oxygen uptake rate, dissolved oxygen and oxidation-reduction. Quantification of the acid and base consumption in each reactor pointed to excess acid production in the later stage reactors likely produced by complete sulfur oxidation through sulfate, as it corresponded with low elemental sulfur yields. However, this could be taken advantage of by introducing a recycle to reduce operating costs. This work highlights valuable lessons learned and has identified possible process improvements for common scale-up challenges.

Introduction

Nickel mining operations in Sudbury, Canada produce an estimated 1000 kt of pyrrhotite-rich tailings annually [1]. Not only do these tailings present an environmental challenge due to their acid rock drainage potential, but they contain 0.6-0.8 wt-% nickel which is currently lost value [1,2]. Previous studies demonstrated the use of bioleaching to extract nickel from legacy tailings with various success in operation and leaching efficiency [3,4]. This work scales a previously benchtop reactor modelled after Metso's BIOX® process [4], to a 30 L continuous pilot plant. Key metrics identified for process optimisation are the acid and base addition, the nickel extraction efficiency, and the elemental sulfur yield.

This presentation will focus on the operation of the 30 L system at 10% solids using fresh pyrrhotite-rich tailings, at pH 2 and with a target residence time of 10 days. In particular, lessons learned from the operation including challenges faced, process indicators, and opportunities for improvement.

Methods

Pilot Plant Materials The 30 L pilot plant modelled the BIOX® process configuration was constructed with glass reactors; a 15 L primary reactor (P1), three 5 L secondary reactors (S1, S2, S3), and a 15 L stock tank to feed to P1. The reactor agitation and aeration were independently controlled (Eurostar 60, IKA; SLA5850S, Brooks Instruments). The temperature was maintained by a circulating waterbath (AP28R-30-A11B, VWR). The material flow through the reactor was controlled by a peristaltic pump and a feed splitter (Masterflex L/S, VWR; Afromix). The pump drew slurry from the bottom of the stock tank and pumped it through a feed splitter which in the default position directed the feed to recirculate back to the stock tank, and when open passed the feed to P1. The feed splitter allowed for high pump rates of 1.2 L/min to keep solids suspended, and slow overall residence times in the cascade. Effluent mass flow was continuously monitored using a scale under the collection bucket.

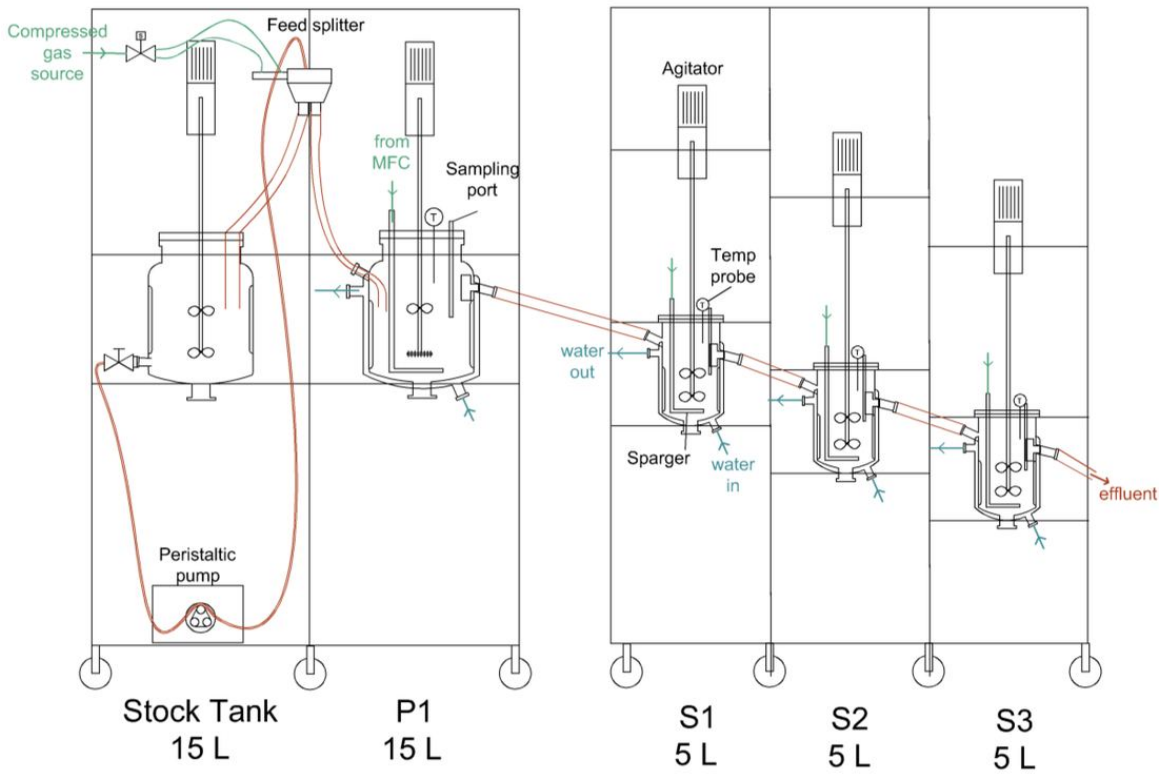


Figure 1. Schematic of multistage 30 L pilot plant showing the stock tank, 15 L primary tank (P1) and three 5 L secondary tanks (S1, S2, S3) in series. Coloured lines indicate flows of slurry (orange), water (blue) and air (green). MFC = mass flow controller.

Pilot Operation and Monitoring All pilot operations were performed using fresh pyrrhotite rich tailings produced from milling ore from the Sudbury basin, Ontario, Canada. The average elemental composition of the tailings was 45% iron (w/w), 21% sulfur, and 0.6% nickel. The P1 reactor was inoculated with a mixed community of acidophilic iron and sulfur-oxidizing microbes, dominated by *Acidithiobacillus and Sulfobacillus*, enriched on tailings waste originating from Sudbury sites. The inoculum was adapted to fresh tailings at 10% solids loading for at least 4 months prior to their use as inoculum.

All pilot operations were run at a temperature of 30 °C, 0.5 g/L of $(NH_4)_2SO_4$ was supplied as the nitrogen source, the pH was manually maintained at 2.0 ± 0.1 using H_2SO_4 or $MgCO_3$, and the dissolved oxygen (DO) was maintained at a concentration above 2 mg/L. The pilot campaign was initiated by a batch stage in P1 to build up the inoculum and achieve high microbial activity.

The reactor was monitored daily, and the following metrics were collected: pH, ORP, DO, oxygen uptake rate ([OUR], using an offline method adapted from [5]), ferrous and total iron, and sulfate. Liquid and solid fractions were analysed by inductively coupled plasma-optical emission spectrometry (ICP-OES; Agilent Dual View 720), and elemental sulfur was quantified by CS_2 extraction. The microbial community was monitored with microscopy and nanopore full length 16S gene sequencing, processed with an EMU pipeline (in-house and Flow Genomics Toronto, ON).

Results and Discussion

The 30 L pilot plant was successfully commissioned and operated for a period of 35 days at a target residence time of 10 days, but steady-state was not achieved. Initially the target flow was not achieved due to frequent clogging of the peristaltic pump line and bleed from the feed splitter. Modifications to the pump and tubing configuration and an additional pre-screen of the tailings to remove inert non-mineral particles (e.g. wood and plastic pieces) introduced through the collection and processing allowed the target flow to be maintained for 14 days.

The tailings were loaded as a 10% (w/w) slurry as it was found in batch experiments to retain high biomass density. The solids loading and density in each tank was monitored daily. Progressive solids accumulation in P1 resulted in three process upsets. Sharp decreases in OUR (and increase in DO) and ORP in P1 indicated process deterioration and allowed quick intervention. In Figure 2A, the points of upset can be seen by drops in the P1 OUR when the density is high. In these incidents, solids were removed from P1 and either disposed of (in the case of the first incident) or redistributed to tanks S1 and S2, which allowed microbial activity to rebound quickly.

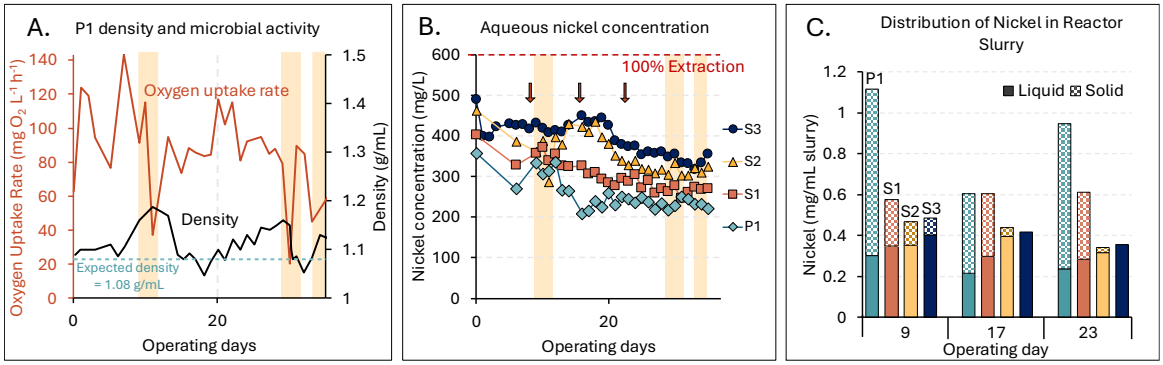


Figure 2. Process data during operation at a 10-day residence time. (A) Oxygen uptake rate (red) and slurry density (black) in P1, the expected density is denoted in light blue. (B) The liquid nickel concentration in mg/L in each pilot tank, the expected concentration for 100% extraction is shown as a red dashed line, arrows indicate where solid samples were analysed. (C) The distribution of nickel (mg/mL slurry) in liquid and solid fractions in each reactor (colours match reactor specified above first bars). Yellow highlights indicate the process upset identification.

While the daily process monitoring data displayed clear signs of upset, the nickel dissolution data was not as apparent (Figure 2B). During the process upsets the liquid nickel concentration in P1 was consistent at 230-250 mg/L after day 14. However, the nickel concentration in the later part of the reactor dropped steadily during this time even though 100% extraction efficiency was achieved in S3 as determined through solid sample analysis (Figure 2C). This trend indicates that only a portion of the material flowed through the system, while much remained in P1. Future improvements will be made to the P1 agitation and weir designs. The key learning from this is that close solids monitoring is essential both for microbial health and accurate process interpretations.

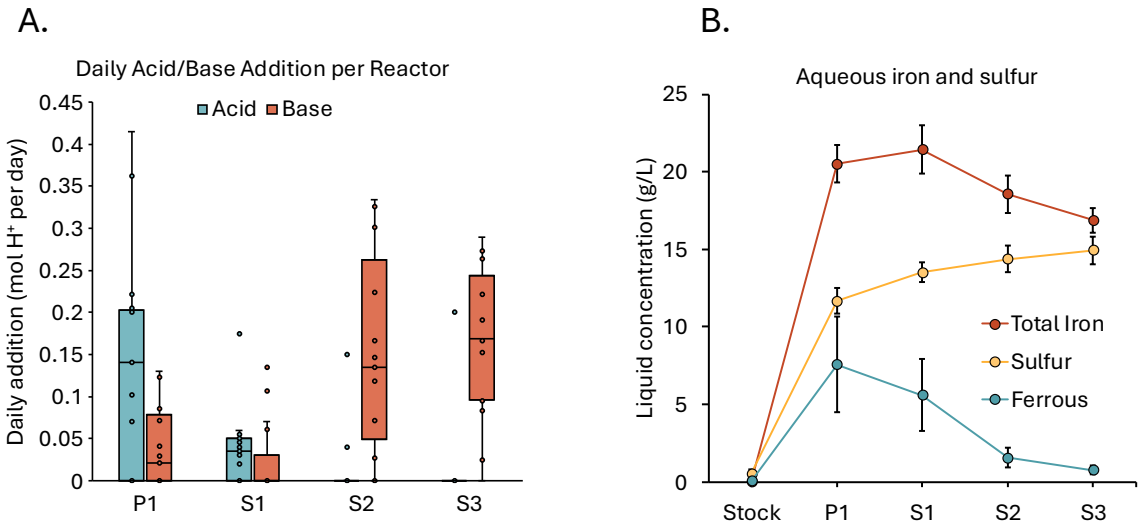


Figure 3. Average process data in each tank over 15 days of operation at a 10-day residence time. (A) The acid (blue) and base (red) addition in each tank in equivalents of mol H^+ per day, assuming one mole of base consumes two moles of H^+. (B) The average liquid concentrations of total (red) and fer-

rous (blue) iron and sulfur (yellow). The error bars are standard deviation of daily measurements from the 15-day period.

In addition to the nickel extraction efficiency, two key metrics influence the economic viability of this process: 1) the acid/base consumption (cost driver), and 2) the yield of elemental sulfur (saleable product). In addition to acid consumption by ferrous oxidation, the tailings entering P1 contain substantial alkalinity; 101.4 g H_2SO_4/kg tailings are required to reach pH 2. However, *in situ* sulfur oxidation and ferric precipitation occur throughout the process and generate acidity (Figure 3B). This causes considerable acid addition at the beginning of the process where the acid is consumed by tailings entering and early ferrous oxidation, and then a pivot into base consumption at and the end of the process where acid generating processes are more dominant (Figure 3A). A logical future improvement would be to introduce a recycle from S3 to P1. This has the potential to reduce external acid addition, and to improve leaching efficiency by introducing additional ferric iron. It is also worth noting that during periods of flow disruption, increased base additions were required to maintain a stable pH in all reactors, and when microbial activity was low from process upset, greater acid additions were required.

While sulfur oxidation is beneficial in reducing the acid requirements, elemental sulfur is a saleable product. During this operation ~17-23% of the sulfur remained as elemental sulfur. Future work will evaluate the economic trade-offs between improving sulfur-oxidation vs elemental sulfur yields and identify conditions that favour sulfur-oxidizing microbes for better sulfur management.

Conclusions

Operation of a continuous 30 L pilot plant identified key scale-up considerations including feed pre-processing, solids transport, and process monitoring. Process performance was primarily limited by inefficient solids transfer rather than microbial kinetics. Combining ORP with OUR and DO provided reliable early indicators for process deterioration caused by solids accumulation and microbial rebound. The acid/base consumption trends indicated that using a recycle might be a viable process improvement to reduce operational costs. Overall, these findings support the importance and challenges of engineering design in continuous bioleaching operations and can be used to help direct future scale-up efforts.

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Field trial of a sulfate-reducing bioreactor treating an arsenic-rich acid mine drainage

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Abstract:

Mining activities are a major source of arsenic (As) contamination in water and soil worldwide. Due to its strong affinity for sulfide mineral ores, As is frequently released into acid mine drainage (AMD). Sulfate-reducing (SR) bioreactors have emerged as an effective and sustainable solution for treating various types of AMD. This study evaluated the performance of a field-scale SR bioreactor treating AMD from the former Carnoulès closed mine (France), showing high dissolved concentrations of As, Fe, and Zn of approximately 100, 1000, and 20 mg/L, respectively. The objectives were to (i) assess the robustness of a pozzolana fixed-bed SR bioreactor under field conditions on site, (ii) evaluate the impact of decreasing hydraulic retention time (HRT) on treatment efficiency, and (iii) investigate the relationship between microbial communities and environmental changes. The bioreactor operated for 375 days on site and was fed with a mixture of glycerol and natural AMD. High and stable removal efficiencies for As and Zn (up to 99%) were achieved despite a gradual reduction in HRT from 29 to 4 days, seasonal variations in AMD chemistry, and changes in feeding mode (batch or continuous). The main phase suspected of immobilising As within the bioreactor was amorphous orpiment (As_2S_3), consistent with the observation of yellow-gold crystal precipitates at the bottom of the reactor. Enrichment of anaerobic sulfate-reducing bacteria occurred, primarily *Desulfatirhabdium* and *Desulfovibrio*, with *Desulfosporosinus present* in lower proportions. The abundance of *Desulfatirhabdium*, capable of degrading acetate, was positively correlated with the sulfate reduction rate and negatively associated with acetate concentrations in the effluent. Diversification of the sulfate-reducing community appeared to positively influence bioreactor performance. This field trial demonstrates the adaptability of the bioreactor microbial community to in-situ temperature fluctuations, seasonal changes in water chemistry, and operational variations, while maintaining high performances for treating the AMD.

Keywords: AMD, arsenic, sulfate-reducing bacteria, bioreactor, field trial

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Remediation of mine-impacted water with high chloride concentration using a sulfate-reducing consortium in a continuous bioreactor and a bioelectrochemical system

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Abstract

Water scarcity has introduced new challenges for copper mining in Chile. Currently, several mining companies use raw seawater in their processes, though its impact on water quality remains unclear, particularly when sulfide minerals in tailings oxidise, generating high sulfate concentrations that contaminate groundwater through infiltration. A known scenario occurs in abandoned coal mines operating near the coast, where seawater intrusion occurs and saline ocean water flows through the shafts, affecting groundwater. This process is observed in Chile at the mine "Chiflón del Diablo," an abandoned coal mine on the tentative list for UNESCO World Heritage Status. The mine has been a tourist attraction due to the exploitation of coal beneath the seafloor. Since flooding of the mine was observed, saline groundwater has been discharged directly into the nearshore zone to maintain tourist activities. This water, pumped from the mine to the beach, contains high concentrations of chloride (~600 mM), ferrous iron (between 2-5 mM), and sulfate (~33 mM) due to the oxidation of pyrite, the main sulfide mineral associated with the mine. This study describes the use of microbial enrichments obtained from sediment samples from the "Salar de Huasco" in the Atacama Desert, which possess dissimilatory sulfate reduction to remediate mine-impacted water with high chloride concentrations. The consortium was evaluated in a continuous sulfidogenic biofilm reactor and a bioelectrochemical system for its efficiency in removing sulfate. The results confirm that in both systems, genera in the order Desulfovibrionales were the most abundant sulfate-reducing bacteria (SRB), and more than 90% of sulfate can be removed. In the bioelectrochemical system, we have tested different electrochemical potentials, with or without organic electron donors, demonstrating the electrochemical capabilities of SRB from the consortium. This study highlights the use of a halophilic sulfate-reducing consortium to promote sulfidogenesis in mine water with a high chloride concentration.

Keywords: Halophilic sulfate-reducing consortium, Sulfate removal, Sulfidogenic systems

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U(VI) Immobilisation by *Sulfobacillus thermosulfidooxidans* under Acidic and Aerobic Conditions

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Keywords: Uranium immobilisation, *Sulfobacillus thermosulfidooxidans*, Fe/S oxidising bacteria, Mining sites

Abstract

Uranium released from mining activities poses a threat to human health. Bioleaching bacteria are abundant in mining environments and play a significant role in uranium mobility. However, the relationship between the Fe/S-oxidizing bacterium *Sulfobacillus thermosulfidooxidans* and uranium is not well understood. In this study, we investigated the interaction between uranium (VI) and *S. thermosulfidooxidans* isolated from cultures grown on FeS_2, under sulfate-rich and aerobic conditions. The strain exhibited uranium immobilisation capacity under acidic and aerobic conditions. To know the mechanism of uranium adsorption by *S. thermosulfidooxidans*, the adsorption kinetics and isotherm analysis were studied. The results show that the adsorption process fits both the pseudo-first-order and pseudo-second-order models, suggesting that this interaction process may involve multiple rate-controlling steps, including physical and chemical reactions. Uranium uptake by *S. thermosulfidooxidans* aligns more closely with the Freundlich isotherm model than with the Langmuir model. The deviation may be attributed to concurrent processes such as precipitation or reduction during the interaction. The finding that the bioleaching bacterium *S. thermosulfidooxidans* can adsorb U(VI) in cells under acidic and aerobic conditions provides new insights into the biotransformation of uranium at mining sites.

Introduction

Uranium (U) is a significant resource for the nuclear industry and plays a critical role in power generation. However, U contamination near mining sites worldwide poses a serious threat to human health and the environment because of its radiological and chemical toxicity [1]. Therefore, understanding the fate and mobility of U in mining environments, as well as controlling its release from mine sites, is essential for protecting both human health and the environment.

Bacterial bioremediation is a cost-effective and environmentally friendly approach for the treatment of heavy metal contamination, including U [2]. Bacteria can immobilise uranium through four main pathways: biosorption, bioaccumulation, biomineralisation, and bioreduction. The abundance of functional groups on bacterial cell surfaces provides favourable binding sites for U adsorption. In addition, some bacteria are capable of transporting and accumulating U within their cells. Certain U-resistant bacteria can biomineralise U through releasing phosphate, and formatting uranium-phosphate (U–P) minerals either on the cell surface or intracellularly. Bioreduction is can convert soluble and mobile hexavalent uranium (U(VI)) into insoluble tetravalent uranium (U(IV)), thereby reducing uranium mobility and environmental risk.

Sulfobacillus thermosulfidooxidans (*S. thermosulfidooxidans*), an iron- and sulfur-oxidizing bacterium, is one of the key microorganisms commonly found in mining environments. *S. thermosulfidooxidans* has also been identified in U mine sites, where it plays a critical role in U mobility and transformation [3]. However, despite the important role of *S. thermosulfidooxidans* in U-contaminated mining environments, studies investigating its capacity and mechanisms for U immobilisation remain extremely limited. In this study, U immobilisation by *S. thermosulfidooxidans* was investigated to understand the

mechanisms of U–bacteria interactions and to evaluate the potential role of this microorganism in controlling U mobility in mining environments.

Methods

Cultivation and Harvest of Bacteria. The bacteria *S. thermosulfidooxidans* (GenBank accession number: DQ650351) were provided by the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Australia. The cultivation of bacteria was conducted by adding cells into 100 mL of 9K medium in a 250 mL Erlenmeyer flask, and the culture was kept at 45 °C for 20 days with a shaking speed of 140 rpm. Pyrite was used as the energy substrate in this process. Bacteria were harvested by separating them from the solution and solid residues for subsequent adsorption experiments.

U uptake by *S. thermosulfidooxidans*. A stock solution of 1 g/L U(VI) was prepared by dissolving $UO_2(NO_3)_2 \cdot 6H_2O$ in Milli-Q water. Subsequent working solutions of varying concentrations were obtained by serial dilution using 0.3% $(NH_4)_2SO_4$ solution. The initial pH of each solution was adjusted to the desired value using HNO_3 and NaOH.

The bacterial concentration was determined by using optical microscope and a cell counting chamber. A specific volume of the concentrated bacterial suspension was added to the prepared U(VI) solution for uranium uptake experiments, and the mixture was incubated in a shaker at 90 rpm under pH4.2 and ambient temperature. The pH of the solution was monitored throughout the adsorption process using a Mettler-Toledo pH meter. At regular time intervals, the upper-layer solutions were collected and separated by centrifugation at 10,000 rpm. The supernatant was then diluted to below 100 ppb with 2% HNO_3 for further analysis by Agilent 7700x Inductively Coupled Plasma Mass Spectrometer (ICP-MS).

Adsorption kinetics analysis. The pseudo-first-order adsorption equation is typically expressed as:

$$\frac{dq}{dt} = -k_1(q_e - q_t) \quad \text{(Eq 1)}$$

Where q represents the amount of uranyl ions adsorbed on the surface of *S. thermosulfidooxidans*, q_e denotes the amount adsorbed at equilibrium, q_t is the amount adsorbed on the surface at time t, and k_1 (h^{-1}) is the rate constant for pseudo-first-order adsorption.

The pseudo-second-order adsorption equation is commonly expressed as:

$$\frac{dq}{dt} = -k_2(q_e - q_t)^2 \quad \text{(Eq 2)}$$

where k_2 (cell counts mg^{-1} h^{-1}) is the rate constant for pseudo-second-order adsorption, and the remaining parameters are consistent with those described earlier.

Adsorption isotherm analysis. The adsorption isotherms for *S. thermosulfidooxidans* were analysed using the Langmuir and Freundlich equations to characterise the adsorption behaviour. The Langmuir isotherm can be expressed as:

$$q_e = \frac{q_{max} K_L C_e}{1 + K_L C_e} \quad \text{(Eq 4)}$$

where K_L is the adsorption equilibrium constant, C_e and q_e have the same meanings as described earlier. q_m represents the maximum amount of U(VI) adsorbed in the cells.

The empirical Freundlich isotherm can be expressed as:

$$q_e = K_F C_e^{1/n} \quad \text{(Eq 5)}$$

where K_F represents the constant related to the adsorption capacity, and n is the constant associated with the intensity of adsorption.

Results and Discussion

Kinetics study. Kinetic studies provide important information on the rate and mechanism of adsorption, which is essential for optimising operating conditions in large-scale treatment processes [4]. The kinetics study of U(VI) uptake by *S. thermosulfidooxidans* was investigated (Fig. 1). The experimental data

demonstrated that the U(VI) uptake process was well-described by both the pseudo-first-order and pseudo-second-order models. However, lower RMSE values for the pseudo-second-order model suggests a better fit. This indicates that U(VI) uptake by *S. thermosulfidooxidans* is governed by both physical and chemical adsorption processes, with the chemical adsorption mechanism being the predominant pathway. Steeper adsorptions were observed with increased bacterial concentration suggest that a greater number of bacteria in the solution accelerates the adsorption process. This is likely due to the availability of more active sites for U(VI) capture, facilitating a faster uptake of U(VI) by the cells. Cell concentration is an important factor in U(VI) biosorption, also occurred from other bacteria, such as *Shewanella putrefaciens* [5].

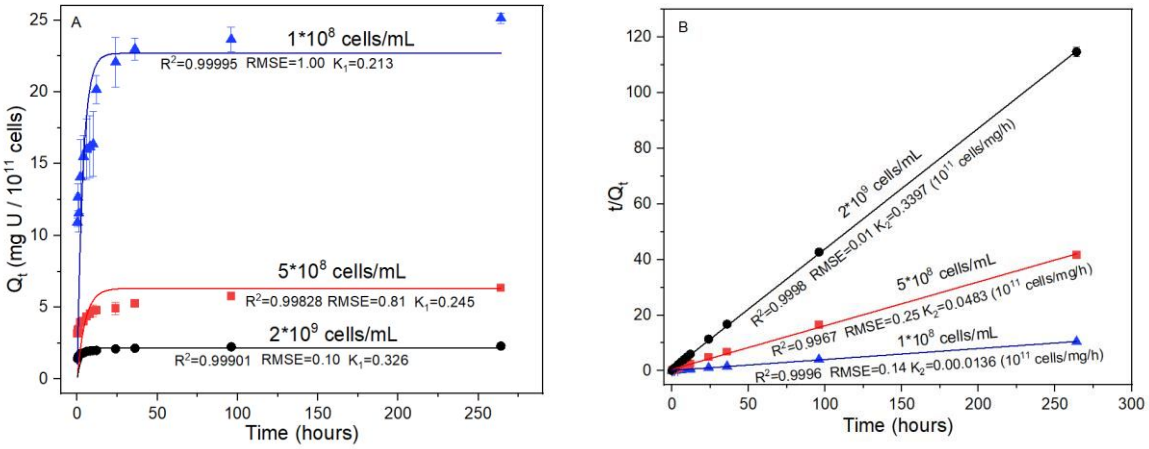


Figure 1. Pseudo-first-order (A) and pseudo-second-order (B) kinetic analyses of U(VI) uptake by *S. thermosulfidooxidans* cultured at varying U(VI) concentrations. The data represent the mean ± SD from three independent experiments.

Adsorption isotherm. Adsorption isotherm analysis (**Fig. 2**) was performed using the Langmuir and Freundlich models. The result shows that the adsorption process aligns more closely with the Freundlich isotherm. This implies that the U(VI) adsorption process by *S. thermosulfidooxidans* is likely governed by a heterogeneous distribution of adsorption sites with different affinities, allowing for multi-layer adsorption, rather than a uniform surface with a single type of adsorption site as assumed by the Langmuir model [6]. The 1/n value ranged between 0 and 1, suggesting that U(VI) biosorption onto the biomass was favourable under the studied conditions. The maximum U(VI) loading capacity was calculated to be 64.9 mg/g, indicating that *S. thermosulfidooxidans* has a strong capacity for U(VI) immobilisation.

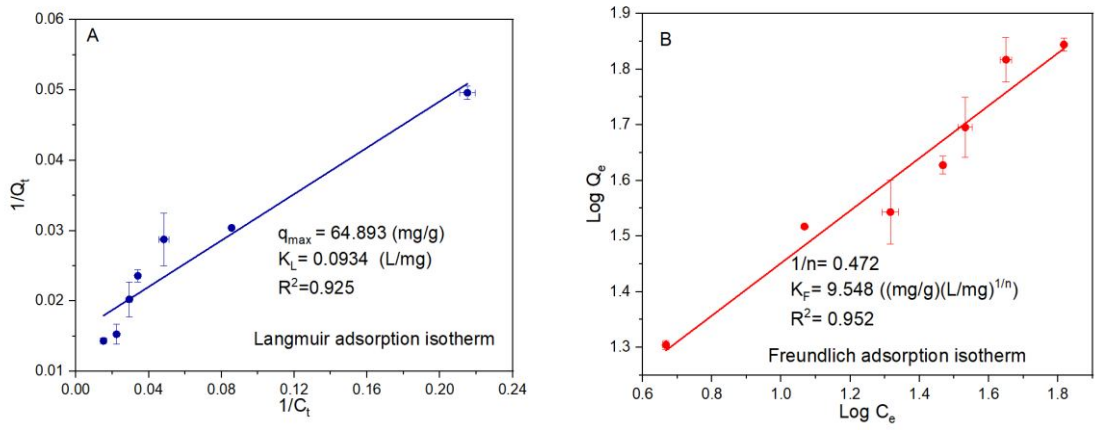


Figure 2. Langmuir (a) and Freundlich adsorption isotherm (b) analysis of U(VI) uptake by *S. thermosulfidooxidans* cultured at pH 4.2 and room temperature. The data represent the mean ± SD from three independent experiments.

Conclusions

This study investigated U(VI) immobilisation by the Fe/S-oxidizing bacterium *S. thermosulfidooxidans* under acidic and aerobic conditions. The results demonstrated that *S. thermosulfidooxidans* possesses a significant capacity for U(VI) immobilisation in environments relevant to U(VI) mining systems. Kinetic analyses showed that the immobilisation process was well described by both pseudo-first-order and pseudo-second-order models, suggesting that both physical adsorption and chemical interactions contributed to U(VI) uptake. Furthermore, adsorption isotherm analysis revealed that the Freundlich model provided a better fit than the Langmuir model, indicating heterogeneous and multilayer adsorption behaviour on the bacterial surface. These findings improve the understanding of U(VI)–microbe interactions in acidic environments and highlight the potential role of *S. thermosulfidooxidans* in U(VI) bioremediation and mobility control in mining-impacted systems.

Acknowledgements

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Development of biobased adsorbents to remove arsenic and selenium from sulfate-rich wastewaters

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Keywords: arsenic; selenium; wastewater; biosorbent; biosorption

Abstract

A porous multiphase iron-based pea starch hydrogel was developed for selective removal of arsenic (As) and selenium (Se) from sulfate-rich wastewaters typical of mining, metallurgical, and flue gas desulfurization processes. The material was synthesized by incorporating ferric iron, nanoscale zero-valent iron (nZVI), and sodium carbonate during pea starch gel formation. Iron enabled speciation changes and adsorption of As and Se, while sodium carbonate generated internal porosity that increased surface area and adsorption capacity. The hydrogel effectively removed selenite (Se(IV)) and selenate (Se(VI)) from simulated wastewater, and it remained stable in the range pH 2–10 for Se(IV) and pH 2–6 for Se(VI), with higher temperatures enhancing Se(VI) removal. In sulfate-rich real wastewater, it maintained >98.5% Se removal despite SO_4^{2-} concentrations exceeding Se by 1000-fold. Mechanistic analysis indicates contributions from surface complexation, hydrogen bonding, electrostatic interactions, and Fe^{2+}/nZVI-mediated reduction. A related hydrogel formulation also removed As(III) and As(V) with >99.1% efficiency across broad pH, temperature, and competitive-ion conditions. These results demonstrate the hydrogel's strong potential as an effective, environmentally friendly material for As and Se remediation.

Introduction

Arsenic (As) is a toxic metalloid of major environmental and public health concern. Water contamination arises from natural geological processes—such as weathering of As-bearing minerals—and from mining, pesticide use, and industrial discharge. Chronic exposure to As-contaminated water can cause skin lesions, cardiovascular disease, and increased risks of liver, lung, and bladder cancers. Selenium (Se) contamination, driven by coal mining, agricultural runoff, and electronics manufacturing, is also problematic; excessive Se in aquatic systems leads to bioaccumulation, reproductive toxicity, and deformities in aquatic organisms. In water, As and Se occur mainly as arsenite (AsO_3^{3-}), arsenate (AsO_4^{3-}), selenite (SeO_3^{2-}), and selenate (SeO_4^{2-}). Arsenite, the more toxic As species, is neutral at environmentally relevant pH and therefore difficult to remove via conventional adsorption. Arsenate, in contrast, forms negatively charged oxyanions that bind strongly to metal-based sorbents. Selenate is particularly challenging to remove due to its high solubility, mobility, and strong competition with sulfate (SO_4^{2-}) for adsorption sites. Composite biobased hydrogels have recently gained attention for heavy-metal remediation because they are renewable and combine porous 3D network properties of the hydrogel with the sorption and chemical reduction capacities of inorganic compounds embedded in the hydrogel. Although these hydrogels typically perform quite well for selenite, they struggle to remove selenate in sulfate-rich waters due to its chemical similarity to sulfate [1,2,3]. This limitations highlight the need for advanced hydrogel formulations capable of selectively removing selenate in complex wastewater matrices. Biopolymer-based hydrogels offer a promising route due to their biodegradability, renewability, and tunable porous structures. Pea starch, composed of amylose and amylopectin, gelatinizes upon heating and forms stable 3D networks during cooling through hydrogen bonding, chain entanglement, and retrogradation. This structure enhances mechanical strength, water retention, and provides an effective scaffold for incorporating active components such as iron-based adsorbents, enabling synergistic adsorption and coprecipitation processes. In this study, a multiphase iron-based porous pea starch

hydrogel was synthesized using pea starch, Fe^{3+}, and Na_2CO_3. Hydrogel synthesis conditions were first optimized for As removal, resulting in PMIPS-hydrogel, and subsequently for Se removal, resulting in IPMIPS-hydrogel. The objectives of this study are to: 1. Assess As(III), As(V), Se(IV), and Se(VI) removal by (I)PMIPS-hydrogel across varying pH conditions. 2. Evaluate hydrogel stability by quantifying Fe leaching during As and Se removal. 3. Determine performance in presence of anions, including high sulfate concentrations, to assess suitability for complex wastewater. 4. Investigate the underlying removal mechanisms. This work was originally presented in the PhD thesis of Jingya Ren [4]. A full description of methodology and discussion of the data can be found in this work.

Methods

Materials. Pea starch (≥90%) was obtained from Xinxiang Liangrun Whole Grain Food Co., Ltd. (China). Nano zero-valent iron (nZVI, ≥80%) was supplied by NANO IRON, s.r.o (Czech Republic). Iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, ≥99%) and sodium carbonate (Na_2CO_3, ≥99.5%) were purchased from Sigma-Aldrich (Germany). Sodium arsenite ($NaAsO_2$, 98%), disodium hydrogen arsenate heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$, 98.5%), sodium selenate (Na_2SeO_4, 99%), and sodium selenite (Na_2SeO_3, 98%) were obtained from Merck Life Science (Belgium).

Synthesis of PMIPS-hydrogel (As removal) and IPMIPS-hydrogel (Se removal). Pea starch (5 g) and $FeCl_3 \cdot 6H_2O$ (3.4 g) were dissolved in 50 mL deionised water and stirred at 500 rpm for 1 h. nZVI was added to obtain Fe^{3+}:Fe molar ratios of 1:1.5 (PMIPS) or 1:2.5 (IPMIPS), followed by 10 min stirring. Sodium carbonate was then added (0.38 mol/L) and mixed for another 10 min. The mixture was heated to 100 °C (≈5 °C/min). Gelation occurred at ~70 °C, accompanied by CO_2 release. After reaching 100 °C, the paste was transferred to Petri dishes and cooled at 4 °C for 30 min to stabilise the hydrogel network. The hydrogel was washed three times with deionised water, blotted, cut into ~3 mm cubes, and stored at room temperature.

Batch Sorption Experiments. Hydrogel (0.2 g) was added to 10 mL solutions containing 10 mg/L As(III), As(V), Se(IV), or Se(VI). Samples were shaken at 400 rpm for up to 16 h and filtered through 0.45 µm membranes. Residual As and Se concentrations were measured by ICP-OES.

Influence of pH, Temperature, and Competing Anions. For pH studies, 10 mL of 100 mg/L As or Se solutions were adjusted to pH 2–11 using 0.01–1 M HCl or NaOH before adding 0.2 g hydrogel. pH was recorded before and after adsorption. Temperature effects were evaluated at pH 6 and 0, 5, 20, and 35 °C. Competitive anion effects were assessed by adding NaCl, Na_2CO_3, $NaNO_3$, Na_2SO_4, or Na_2HPO_4 at 10, 100, or 1000 mg/L to 10 mg/L As or Se solutions.

Isotherms. Hydrogel was added to solutions containing 0.25–20 mmol/L As or Se at pH 6 and shaken for 16 h. Equilibrium concentrations were measured by ICP-OES and fitted to Langmuir, Freundlich, and Redlich–Peterson models.

Column Experiments. Real metallurgical wastewater was pre-filtered (4–12 µm). A glass column (20 cm × 2.5 cm) was packed with 50 g hydrogel and capped with 5 g glass wool. One liter of wastewater was passed through the column at 2.00 mL/min (empty bed contact time: 30 min). Effluent samples (10 mL) were collected between 0 and 540 min and analyzed for As, Se, Cl^-, NO_3^-, SO_4^{2-}, and $H_2PO_4^-$.

Results and Discussion

During the optimisation of the hydrogel synthesis conditions for Se removal, wherein Fe^{3+}:Fe molar ratios were varied, Se(IV) removal by the hydrogel was found to remain consistently high across all Fe^{3+}/Fe ratios, exceeding 98% even in the presence of 2000 mg/L SO_4^{2-}. In contrast, Se(VI) removal improved markedly with increasing Fe dosage: efficiency declined at a 1:1.5 Fe^{3+}/Fe ratio but reached ~99% at 1:2.5. Iron release remained below 0.8 mg/L for all ratios. Under optimal conditions (Fe^{3+}/Fe = 1:2.5), residual Se(IV) and Se(VI) concentrations were reduced to 0.08 mg/L and 0.07 mg/L, respectively—far below regulatory discharge limits. The superior Se(IV) and Se(VI) removal at higher Fe^{3+}/Fe ratios (1:2.5) is attributed to combined adsorption and reduction processes. Both Se species probably initially adsorb onto multiphase iron phases such as $Fe(OH)_3$ and FeOOH. Se(VI) may then be reduced to Se(IV) by residual nZVI and Fe^{2+} (Fan et al., 2019; Liang et al., 2014), followed by further reduction to insoluble Se^0, which precipitates within the hydrogel. Se(IV) undergoes direct

reduction to Se^0 via Fe^{2+} and nZVI (Tang et al., 2016). The multiphase iron species formed during synthesis (e.g., $Fe(OH)_2$, $FeCO_3$, $Fe(OH)_3$, FeOOH) provide abundant reactive sites for surface complexation and adsorption (Das et al., 2013; Li et al., 2024), while the porous starch matrix enhances retention of reduced Se. Under sulfate-rich conditions, rapid Se(VI) reduction to Se(IV) minimizes competition from SO_4^{2-}, as Se(IV) has a stronger affinity for iron phases. The low Fe release (<0.5 mg/L) at the optimal 1:2.5 Fe^{3+}/Fe ratio further indicates good structural stability and environmental safety, supporting the hydrogel's suitability for efficient and sustainable selenium removal.

The adsorption isotherms of Se(IV) and Se(VI) were best described by the Redlich–Peterson model ($R^2 > 0.99$), indicating that Se uptake occurs through mixed adsorption mechanisms on heterogeneous active sites. This behavior aligns with the synergistic adsorption–reduction processes characteristic of IPMIPS-hydrogel. A higher adsorption capacity observed for Se(IV) reflects its greater reactivity, as nZVI and Fe^{2+} within the hydrogel readily reduce Se(IV) to insoluble Se^0, enhancing removal efficiency. In contrast, Se(VI) must first be reduced to Se(IV), a kinetically slower step that limits its overall adsorption performance. Adsorption data for As on the PMIPS-hydrogel were best described by the Freundlich model (R^2 = 0.991 for As(III) and 0.997 for As(V)), reflecting heterogeneous adsorption with sites of varying affinity.

The Se(IV) and Se(VI) removal capacities of IPMIPS-hydrogel were evaluated in the pH range 2–11 (Figure 1). Se(IV) removal remained high (>4.8 mg/g) between pH 2–10, decreasing only at pH 11 (3.7 mg/g). Se(VI) removal peaked under strongly acidic conditions (4.4 mg/g at pH 2–3) and remained above 2.4 mg/g from pH 5–11, with a maximum of 3.1 mg/g at pH 6. Across initial pH 4–11, the equilibrium pH stabilized near 8, and iron release stayed below 1 mg/L, indicating good hydrogel stability. High Se(IV) removal over pH 2–10 is attributed to combined reduction and adsorption. Under acidic conditions, nZVI and Fe^{2+} probably promote Se(IV) reduction to Se^0, which is immobilized on the hydrogel. At pH 11, competition from OH^- and reduced reductive activity may suppress removal. Se(VI) removal is lower overall due to the additional reduction step to Se(IV), slower kinetics, and weaker affinity for $Fe(OH)_3$/FeOOH surfaces. However, at pH 2–3, rapid nZVI- and Fe^{2+}-mediated reduction enhances Se(VI) removal, while partial reduction and adsorption sustain moderate performance from pH 4 to 10. The hydrogel's buffering effect, maintaining pH ~8, supports structural stability and limits iron release, though strongly acidic conditions remain most favorable for Se(VI) reduction.

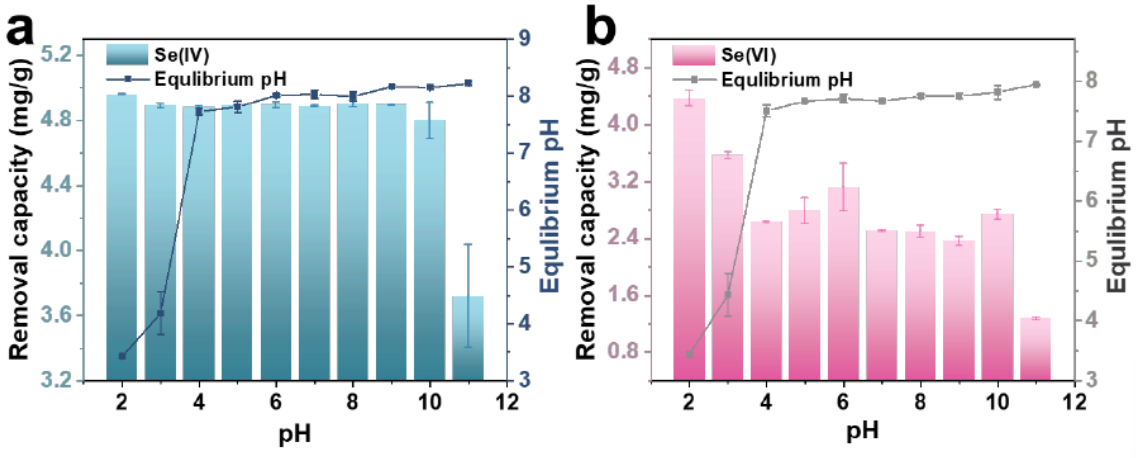


Figure 1. Influence of pH on removal capacity of (a) Se(IV) and (b) Se(VI) by IPMIPS-hydrogel at 20 °C (initial concentrations 100 mg/L), with the corresponding equilibrium pH after adsorption. Error bars represent the standard deviation of replicate measurements. Source: [4]

The removal capacities of As(III) and As(V) by the PMIPS-hydrogel showed distinct pH-dependent trends (Figure 2). As(III) removal remained stable (>3.2 mg/g) from pH 3 to 9, decreased sharply at pH 2 (1.4 mg/g), and declined gradually above pH 9. As(V) removal peaked at pH 3 (4.4 mg/g), remained above 3.3 mg/g between pH 3–6, and dropped markedly at higher pH, reaching 0.9 mg/g at

pH 11. In As(III) solutions, equilibrium pH increased with initial pH, whereas in As(V) solutions it was buffered at ~7.5 from pH 4–11. Iron release remained low (<1 mg/L) between pH 5–11, indicating hydrogel stability. These behaviors reflect differences in As speciation and interactions with iron phases. Between pH 3 and 9, As(III), present mainly as neutral H_3AsO_3, is removed via surface complexation with $Fe(OH)_3$/FeOOH through hydrogen bonding and ligand exchange. Above pH 9, deprotonation to $H_2AsO_3^-$ and increased OH^- competition reduce adsorption. The rising equilibrium pH indicates limited buffering in As(III) systems. As(V), present as $H_2AsO_4^-$/$HAsO_4^{2-}$, is removed through electrostatic attraction, surface complexation, and coprecipitation. At pH 3, coprecipitation with Fe^{3+} as $FeAsO_4$ enhances removal and lowers equilibrium pH. At higher pH, reduced surface protonation, OH^- competition, and partial $Fe(OH)_3$ dissolution diminish adsorption efficiency. The stable equilibrium pH (~7.5) from pH 4–10 reflects buffering by $Fe(OH)_3$ and residual carbonate in the hydrogel.

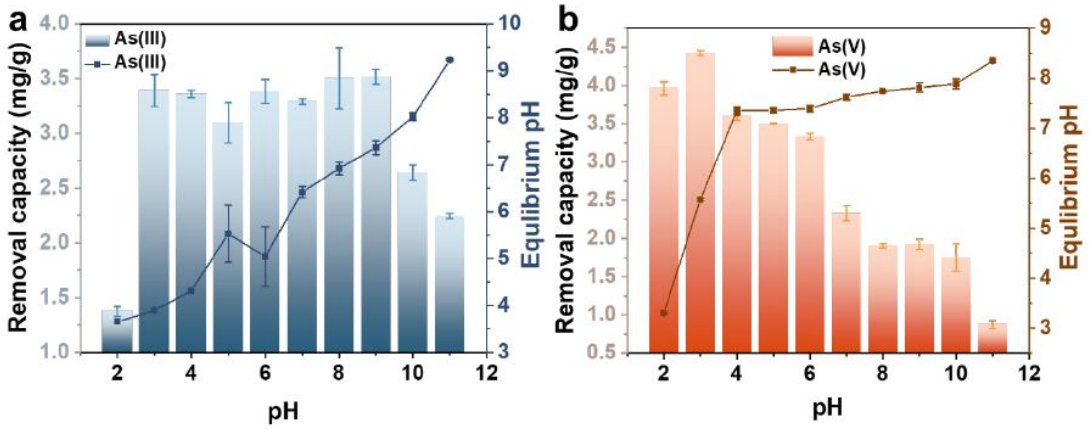


Figure 2. Influence of pH on removal capacity of (a) As(III) and (b) As(V) by PMIPS-hydrogel at 20 °C (initial concentrations 100 mg/L), with the corresponding equilibrium pH after adsorption. Error bars represent the standard deviation of replicate measurements. Source: [4]

The effects of coexisting anions on Se(IV) and Se(VI) removal by IPMIPS-hydrogel are shown in Figure 3. Chloride and sulfate had no measurable impact, with removal efficiencies remaining above 99.1% even at concentrations 1000-fold higher than selenium (1 mg/L). In contrast, NO_3^-, CO_3^{2-}, and HPO_4^{2-} significantly inhibited removal, particularly for Se(VI). At 1000-fold excess, Se(IV) removal decreased to 95.2%, 84.2%, and 68.6%, while Se(VI) removal dropped more sharply to 34.0%, 66.9%, and 29.8%, respectively. Temperature had little influence on Se(IV) removal in simulated wastewater. However, Se(VI) removal improved markedly with increasing temperature in both simulated and real wastewater, reaching 99.4% and 99.3% at 20 °C, indicating enhanced reduction and adsorption kinetics. The presence of Cl^-, SO_4^{2-}, and NO_3^- - even at 100-fold excess relative to As (10 mg/L) - had minimal impact on removal, with efficiencies consistently above 99.2% (data presented in [4]). In contrast, CO_3^{2-} and HPO_4^{2-} strongly inhibited As(V) removal, reducing efficiencies to 57.2% and 3.7% at 100-fold concentrations. As(III) removal was less affected under the same conditions. Temperature had little influence on As removal in simulated wastewater; however, in real wastewater, higher temperatures improved performance, reaching 99.5% at 20 °C.

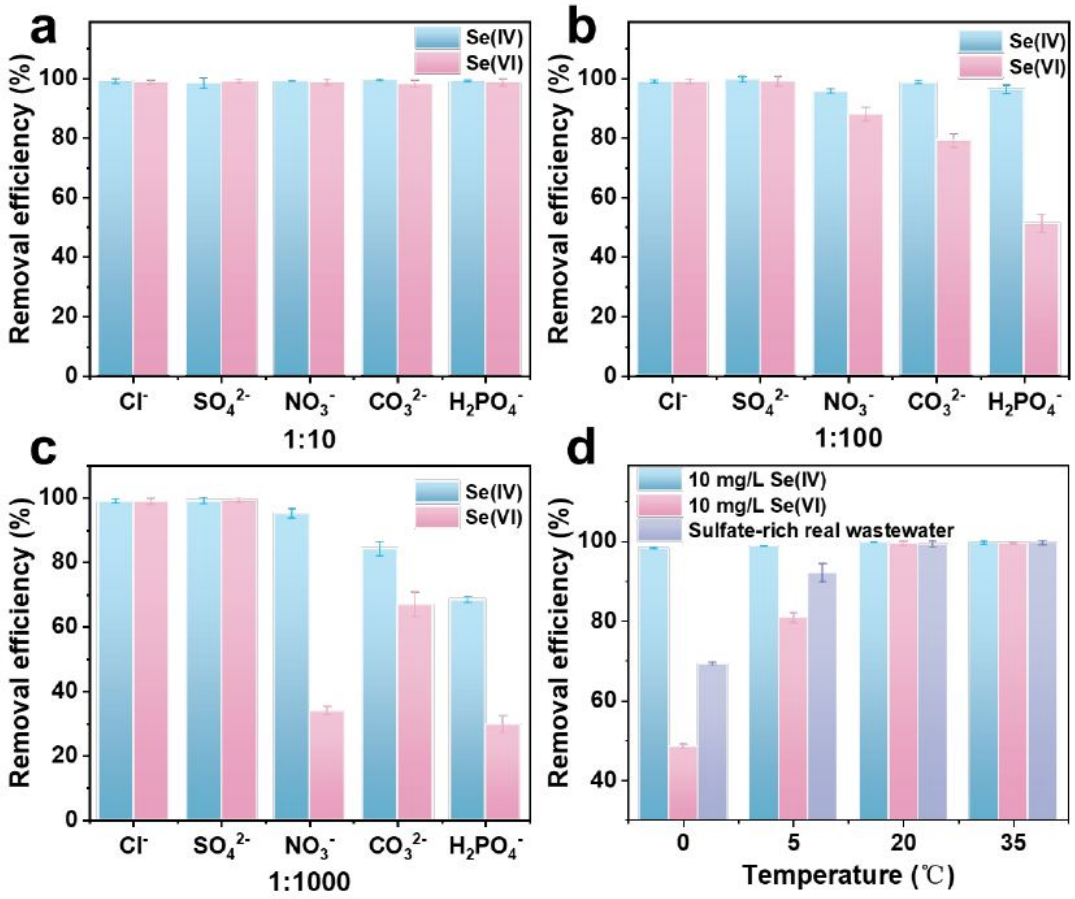


Figure 3. Effects of temperature (d) and co-existing ions on 1 mg/L of Se(IV) and Se(VI) at concentration ratios of (a) 1:10, (b) 1:100, and (c) 1:1000. Error bars represent the standard deviation of replicate measurements. Source: [4]

The hydrogels were further evaluated in a column setup using real sulfate-rich industrial wastewaters containing As(III), As(V), Se(IV) and Se(VI) and high concentrations of competing anions (full data presented in [4]). The initial wastewaters contained 1.91 mg/L total Se (34% Se(IV), 66% Se(VI)) and 24.5 mg/L total As (71% As(III), 29% As(V)), along with elevated Cl^- (5703-5728 mg/L) and SO_4^{2-} (2540-2587 mg/L), and lower levels of NO_3^-, CO_3^{2-}, and PO_4^{3-}. Effluent Se and As concentrations remained below 0.06 mg/L for 10 hours, demonstrating stable and efficient removal in the real wastewater. The hydrogel effectively immobilized both As and Se species despite the high sulfate background, which typically suppresses adsorption. The effluent pH gradually decreased from ~8 to ~7.5 and then stabilized, likely due to interactions with the hydrogel matrix.

From SEM, FTIR and XRD analyses (data presented in [4]) it could be concluded that the Se removal mechanism involves surface complexation, hydrogen bonding, electrostatic interactions, and reduction facilitated by Fe^{2+} and nZVI, which were critical to its high efficiency. Arsenic removal was attributed to a combination of $FeAsO_4$ precipitation and adsorption, including surface complexation, hydrogen bonding, and electrostatic interactions.

Conclusions

The results of our study highlight the potential of (I)PMIPS-hydrogel as an effective and environmentally friendly solution for As and Se remediation in real wastewater environments. The hydrogel showed strong effectiveness, selectivity and stability in treating Se- and As-contaminated, sulfate-rich industrial wastewater, indicating its suitability for further optimization and practical application. However, future work should still further explore its long-term stability in real wastewater environments and investigate its potential applications for other contaminants. Further optimization of the synthesis process could enhance its scalability and efficiency for industrial applications.

Acknowledgements

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Reducing Alkali Consumption in the Dewatering of Mine Pit Lakes via Ferrous Iron Biooxidation

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Keywords: Valorisation; Bioremediation; Closured Mine; Continuous Biooxidation; Acid Mine Drainage

Abstract

The reactivation of decommissioned and flooded mines requires comprehensive dewatering programs. In metal-sulfide deposits, the accumulated water is highly acidic and enriched with heavy metals, with Fe^{2+} dominating deeper zones. Conventional remediation relies on neutralisation by lime addition. However, managing millions of m^3 via this treatment presents profound logistical and economic challenges.

This study proposes a strategy to reduce alkali consumption by promoting *in situ* Fe and sulfate precipitation within Fe^{2+} biooxidation reactors. To enhance performance, cell recirculation from the effluent is proposed by using ferric precipitates as carriers, following their maturation under saturation conditions in a crystalliser and subsequent concentration in a thickener. This hypothesis was evaluated by continuous biooxidation of water collected from a mine pit lake within the Iberian Pyrite Belt ($[Fe^{2+}]$ = 18.5 $g \cdot L^{-1}$, pH =2.65) in three bioreactor configurations: packed-bed, bubble column, and stirred tank.

While the packed-bed suffered severe clogging due to the accumulation of iron precipitates, biooxidation rates >1.2 $g \cdot L^{-1} \cdot h^{-1}$ were obtained in the bubble column and stirred tank at Fe conversion higher 95%. More than 20% of the Fe precipitated inside these bioreactors, reaching 35% including the crystalliser.

Introduction

Following the cessation of open-pit mining operations, pit lakes typically form as a result of the deactivation of dewatering systems and the subsequent recovery of the piezometric level. Over time, the weathering of minerals on the pit walls transfers acidity and heavy metals to the water body [1]. In metal sulfide deposits, natural mineral oxidation processes catalysed by sulfur- and iron-oxidizing acidophilic microorganisms [2] can decrease the water pH to below 3 and increase heavy metal concentrations to several $g \cdot L^{-1}$, well above those normally observed in typical acid mine drainage (AMD).

The chemical composition and large volume of this water (millions of cubic meters) pose a significant environmental threat, potentially compromising the biodiversity and water resources of surrounding ecosystems [3]. Beyond this environmental risk, these impounded waters hinder the resumption of mining activities in deposits that could be economically exploitable. The economic viability of these deposits is driven by rising demand and metal prices associated with the expansion of digital technologies, sustainable mobility, and the clean energy transition.

The conventional treatment for purifying acidic, metal-rich waters consists of chemical neutralisation via alkali addition, which precipitates metals as hydroxides [4]. Lime and limestone are the most widely used neutralising agents due to their availability and low cost. However, supplying the large quantities of neutralising agents required represents a major economic and logistical challenge owing to the large water volumes, their high acidity, and metal, and sulfate concentrations.

Fe is the most abundant heavy metal in mine pit lakes. As a result of meromictic stratification, stable gradients are formed based on oxygen availability; while Fe^{3+} concentrates in the oxygenated surface layers, Fe^{2+} dominates in the deep anoxic zones, and its concentration can even reach tens of $g \cdot L^{-1}$ [1].

Iron removal via alkalisation is facilitated when Fe is exclusively present as Fe^{3+}. To reduce the Fe concentration below 1 mg·L^{-1}, Fe^{3+} requires a pH close to 4, whereas Fe^{2+} requires a pH above 9. An additional advantage of precipitating iron at a pH around 4 is the potential recovery of metals such as Zn, which remain in solution under these acidic conditions [5].

Biooxidation is highly advantageous for complete Fe^{2+} oxidation to Fe^{3+} (Eq. 1). On one hand, this process requires only air supply; on the other hand, iron-oxidizing microorganisms—typically belonging to the genera *Acidithiobacillus and Leptospirillum*—generate local chemical gradients that promote the co-precipitation of iron and sulfates as jarosites (Eq. 2), thereby producing the acidity required for the biooxidation (1) [6].

(1)

(2)

where X^+ is a monovalent cation (such as , , or).

Under mesophilic range, the formation of these biogenic precipitates depends primarily on the medium composition and the time available for crystal nucleation and growth [7].

This work proposes the continuous biooxidation of Fe^{2+} in mine pit lake water to promote jarosite precipitation, thereby reducing the alkali dosage required for water purification. To this end, three bioreactor configurations were evaluated: packed-bed, bubble column, and stirred-tank. To improve the performance of the latter two, cell recirculation from the effluent is proposed. This cell recycling is achieved using the ferric precipitates as a carrier, once they have matured under saturation conditions in a crystalliser and have been concentrated in a thickener (Figure 1).

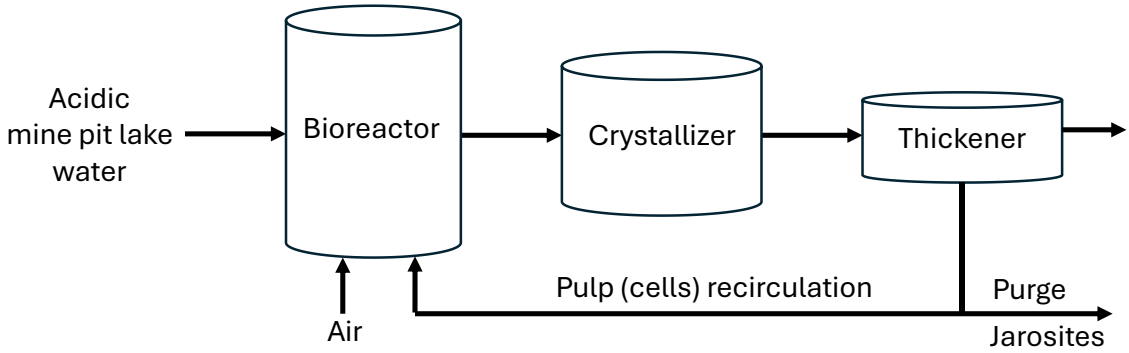


Figure 1. Conceptual flowsheet for *in situ* iron precipitation via ferrous iron biooxidation.

Materials and methods

Culture. The culture used as inoculum was isolated from acid drainage at the Río Tinto mine (Spain) and maintained by subculturing in 9K medium. The dominant microbial species are *Acidithiobacillus ferrooxidans* and *Leptospirillum ferriphilum*.

Liquid media. Standard 9K medium, modified 9K+ medium with double the ferrous sulfate concentration ([$FeSO_4 \cdot 7\ H_2O$ = 88.44 g·L^{-1}), and a raw sample collected from an acid mine pit lake in the Iberian Pyrite Belt (RW) were used as culture media. The main physicochemical characteristics of the RW were as follows: ORP = 345 mV, pH = 2.65, and mayor metals concentrations [Fe] = 19.5 g·L^{-1}, [Fe^{2+}] = 18.5 g·L^{-1}, and [Zn] =2.8 g·L^{-1}. Additionally, a modified RW+ medium was tested, which was prepared by supplementing the raw RW sample with 1.5 g·L^{-1} of and 0.25 g·L^{-1} of .

Bioreactors. For continuous ferrous iron biooxidation, flooded packed-bed (FPBB), bubble column (BC), and continuous stirred-tank (CSTR) bioreactors were tested at . A summary of their main geometric characteristics and operating conditions is presented in Table 1.

Table 1. Reactors. FPBB: flooded packed bed. BC: bubble columna. CSTR: continuous stirred tank reactor. Dilution rate: liquid flow rate divided by working volume. $k_L \cdot a$: volumetric mass transfer coefficient for aeration.

Parameter	FPBB	BC	CSTR
Total volume (mL)	1108	1862	1090
Working volume (mL)	659	1849	1020
Maximum dilution rate (h^{-1})	0.16	0.16	0.15
Air flow rate ($mL \cdot h^{-1}$)	750	2000	750
Air flow rate/working volume (h^{-1})	1.13	1.08	0.74
$k_L \cdot a$ (h^{-1})	110	146	215

Batch biooxidation tests were conducted in Erlenmeyer flasks shaken at and , mixing of inoculum with of either 9K, 9K+, RW, or RW+ medium.

Continuous biooxidation tests were performed in FPBB, BC, and CSTR using 9K+ and RW+ media. The effluent from the BC and CSTR was directed to a continuous stirred-tank operating as a crystalliser (figure 1). The crystalliser slurry was settled for , and the thickened underflow was recirculated to the BC and CSTR at a flow rate equal to 3% of the feed flow rate. The ferric precipitates were characterised by XRD.

Metal precipitation tests were carried out on RW and fully biooxidised RW+. Lime was dosed while monitoring the pH of the agitated liquor.

Results and Discussion

Batch biooxidation tests demonstrated that the maximum specific biooxidation rate was similar (0.062±0.003 h^{-1}) across the synthetic 9K, 9K+, and RW+ media. Without nutrient supplementation, biooxidation in the raw RW ceased after three successive subcultures. Phosphate and ammonium concentrations in flooded massive sulfide pit lakes can be extremely low because these ions are scarce in the mineralised rock, and their solubility is heavily limited in the presence of Fe ions within the pH range of jarosite precipitation.

Continuous biooxidation studies were initially conducted in the flooded packed-bed reactor, as it offers the highest performance and operational flexibility regarding feed composition (Mazuelos, 2023). Biooxidation rates exceeded 1.5 $g \cdot L^{-1} \cdot h\text{-}^{1}$ with complete Fe^{2+} oxidation at a liquid flow rate of 99 ± 15 mL/h. However, after 360 hours of operation, the flow of air and liquid was impeded by the massive accumulation of iron precipitates within the bed.

Due to the unstable operation of the packed-bed reactor, continuous biooxidation was evaluated in the bubble column and stirred-tank reactors. These configurations lack immobile internal components that could retain ferric precipitates and obstruct the flow. For these reactors, feed flow rate limitations are expected to prevent maintaining chemostat conditions. Therefore, cell recirculation using ferric precipitates as carriers was evaluated. After maturing in the crystalliser and settling, the ferric precipitate concentration in the underflow was 23 ± 0.7 $g \cdot L^{-1}$. This stream was partially recirculated to the bioreactor at a recycle ratio of 3% relative to the feed flow. Figure 2 shows the results obtained.

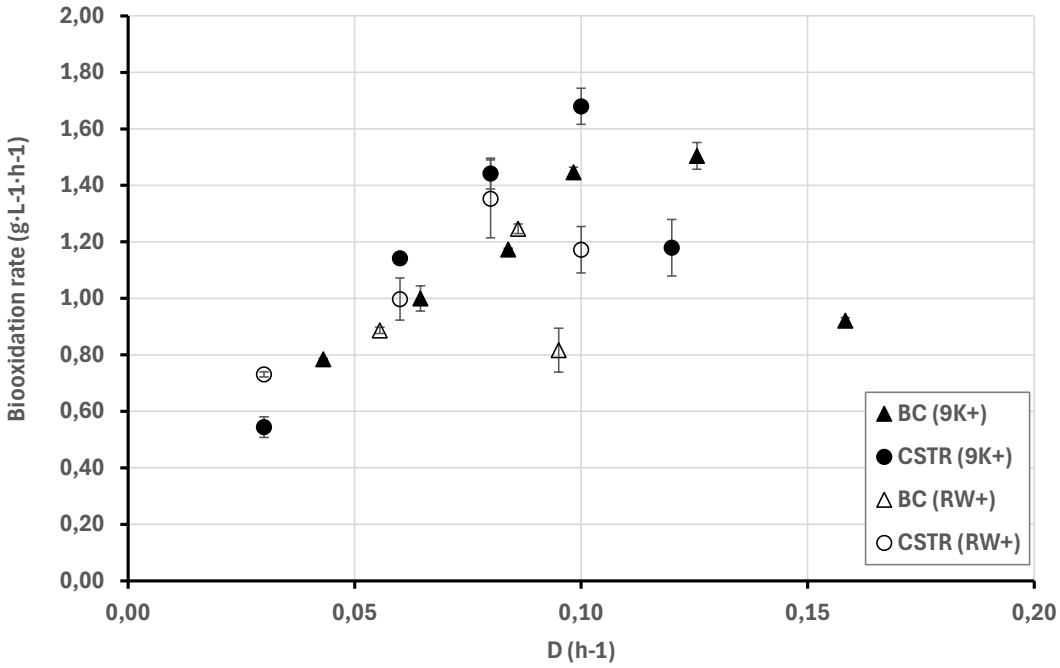


Figure 2. Continuous biooxidation tests in stirred tank (CSTR) and bubble column (BC) with synthetic medium 9K+ and real sample (RW+). Dilution rate (D): liquid flow rate divided by working volume.

Both BC and CSTR achieved biooxidation rates comparable to the peak values obtained with the FPBB; In contrast to the FPBB, stable operation was maintained for nearly 1000 h without flow disturbances other than those associated with scheduled changes in the feed flow rate. In these reactors, over 20% of the Fe precipitated *in situ*, predominantly as ammonium, hydronium, and potassium jarosites. When precipitation in the crystalliser was also considered, approximately 35% of the total iron fed to the system was removed by precipitation.

A comparison of the alkali consumption required to decrease the iron concentration below7 $mg \cdot L^{-1}$ in raw RW (at pH 9.1) and below 1 $mg \cdot L^{-1}$ bio-oxidised RW+ (at pH 4.1) shows that the biooxidation strategy decreased lime consumption by 38.5%. This reduction represents an estimated savings of 18000 t of lime per cubic hectometre of water treated for Fe removal. Furthermore, the supernatant contained 2.41 $g \cdot L^{-1}$ of Zn, enabling a maximum recovery yield of 86%.

Conclusions

Efficient biooxidation of acid mine pit lake waters may require ammonium and phosphate supplementation. For treating these acidic waters without prior pH adjustment, packed-bed reactors are unsuitable due to the accumulation of ferric precipitates that clog the bed. These operational limitations can be overcome by using bubble column and stirred-tank reactors. Biomass recirculation using matured and thickened ferric precipitates as carriers enabled biooxidation rates exceeding 1.2 $g \cdot L^{-1} \cdot h^{-1}$ when treating mine pit lake water. More than 20% of the influent iron precipitated *in situ* within the bioreactors, reaching approximately 35% when precipitation in the crystalliser was included, thereby significantly reducing alkali consumption for Fe removal during water remediation. Complete Fe precipitation was achieved by adjusting the biooxidised effluent to pH 4.1 using lime, leaving 86% of the Zn in the supernatant for potential recovery.

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Turning Acid Mine Drainage into Mineral Pigments through Biological Iron Oxidation

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Keywords: Acid mine drainage, Biological iron oxidation, Microbial consortium, Smolník, Mineral pigments

Abstract

Biological oxidation of ferrous iron was investigated as the central step in a resource-oriented treatment of mining influenced water from the abandoned Smolník mine in Slovakia. The water contained approximately 230 mg/L total iron and had an initial pH close to 4, at which abiotic Fe^{2+} oxidation is slow. A native microbial consortium was therefore cultivated directly in Smolník mine water and used to accelerate iron oxidation. Following an initial 30 h aeration step, the water was inoculated with 50 mL of an exponentially growing. Fe^{2+} decreased from approximately 230 mg/L to below 2 mg/L within 29 h, corresponding to more than 99% oxidation. In the non-enriched comparison, oxidation reached only approximately 11%. Biological oxidation and subsequent filtration reduced dissolved total iron to 15.7 mg/L without deliberate pH adjustment, while Cu, Co, Mn and Mg remained predominantly in solution. The resulting Fe-rich precipitate was amorphous to poorly crystalline and consistent with a schwertmannite-like phase and displayed an orange-brown ochre colour. The results demonstrate that enrichment of indigenous iron-oxidising microorganisms can combine rapid iron removal with production of a potentially valuable mineral pigment precursor.

Introduction

Acid mine drainage and other mining-influenced waters commonly contain elevated concentrations of dissolved iron, sulfate and associated metals as a consequence of sulfide-mineral oxidation. Conventional treatment generally relies on neutralisation followed by precipitation of dissolved metals and acidity-generating constituents (1, 2). Although effective, this approach may generate large quantities of chemically heterogeneous Fe- and Al-rich sludge, which can complicate handling, disposal and subsequent material utilisation. Controlled biological oxidation provides an alternative in which dissolved Fe^{2+} is converted to Fe^{3+} before precipitation, allowing improved control over iron removal and the formation of Fe-rich secondary minerals (1, 3).

The oxidation step is particularly important under acidic conditions. At pH values below approximately 4, abiotic Fe^{2+} oxidation by dissolved oxygen proceeds slowly, whereas acidophilic microorganisms can accelerate the process by several orders of magnitude (4). The generated Fe^{3+} subsequently hydrolyses and precipitates as poorly soluble minerals such as schwertmannite, ferrihydrite, jarosite or goethite, depending primarily on pH, sulfate concentration and solution composition (5).

The main mine water discharge from the Pech shaft at Smolník typically has a pH in a range of approximately 3.95–4.12 and a flow of about 6.5–10 L/s. Recent total Fe concentrations generally range from approximately 190 to 260 mg/L, while sulfate concentrations exceed 2 g/L in many samples. The water also contains Al, Mn, Mg, Zn, Cu, Co and As (6).

Previous 16S rRNA analysis showed that the Smolník microbial community was diverse. *Acidithiobacillus was* not abundant in the Pech shaft water, whereas the iron-oxidising genus *Gallionella was* prominent, and more than 40% of sequences remained unclassified at genus level. After transferring sediment from the Pech shaft to fresh 9K medium, the microbial community shifted, with *Acidithiobacillus becoming* the dominant genus. (7). This indicates that selective Fe^{2+} rich cultivation can enrich functionally important acidophilic iron oxidisers that occur only at low abundance in the original mine water.

The objective of the present study was to evaluate Fe^{2+} oxidation in real Smolník mine water using a pre-cultivated native microbial consortium and to determine whether the resulting Fe precipitate could be considered as a precursor for mineral pigments.

Methods

For the purposes of the experiment, native microbial consortia were cultivated in mine water from the main discharge shaft (Pech), supplemented with 9K salts (1.6 mmol/L $MgSO_4 \cdot 7H_2O$, 0.76 mmol/L $(NH_4)_2SO_4$, 0.23 mmol/L K_2HPO_4, and 120 mmol/L $FeSO_4 \cdot 7H_2O$). Cultivation was carried out in magnetically stirred, aerated baffled reactors (0.5 L working volume) at 25 °C and 240 rpm.

A combined glass electrode (InLab Micro, Mettler Toledo) was used for pH measurement, and oxidation–reduction potential (ORP) was monitored using a Pt–Ag/AgCl redox electrode (InLab Redox Micro, Mettler Toledo). Measurements were performed with a Seven2Go S2 meter (Mettler Toledo).

Ferric iron was determined with a UV-spectrophotometric method at 300 nm (8). Ferrous iron concentrations were determined by a modified o-phenantroline spectrophotometric method, insensitive to Fe^{3+} interference (9).

Elemental analysis of the water was done using AAS (Varian AA240Z, AA240FS) and ICP-MS 7700 (Agilent). Sulfate concentration was analysed by ion chromatography (Dionex ICS 5000).

Results and Discussion

The initial Fe^{2+} concentration was approximately 230 mg/L. In the inoculated sample, Fe^{2+} decreased rapidly during the first hours of cultivation and reached approximately 123 mg/L after 6.5 h. The most pronounced decline occurred between 6.5 and 21.5 h, after which only approximately 3.5 mg/L Fe^{2+} remained. At 29.5 h, the concentration had decreased to approximately 1.4 mg/L, corresponding to an oxidation efficiency of 99.4%.

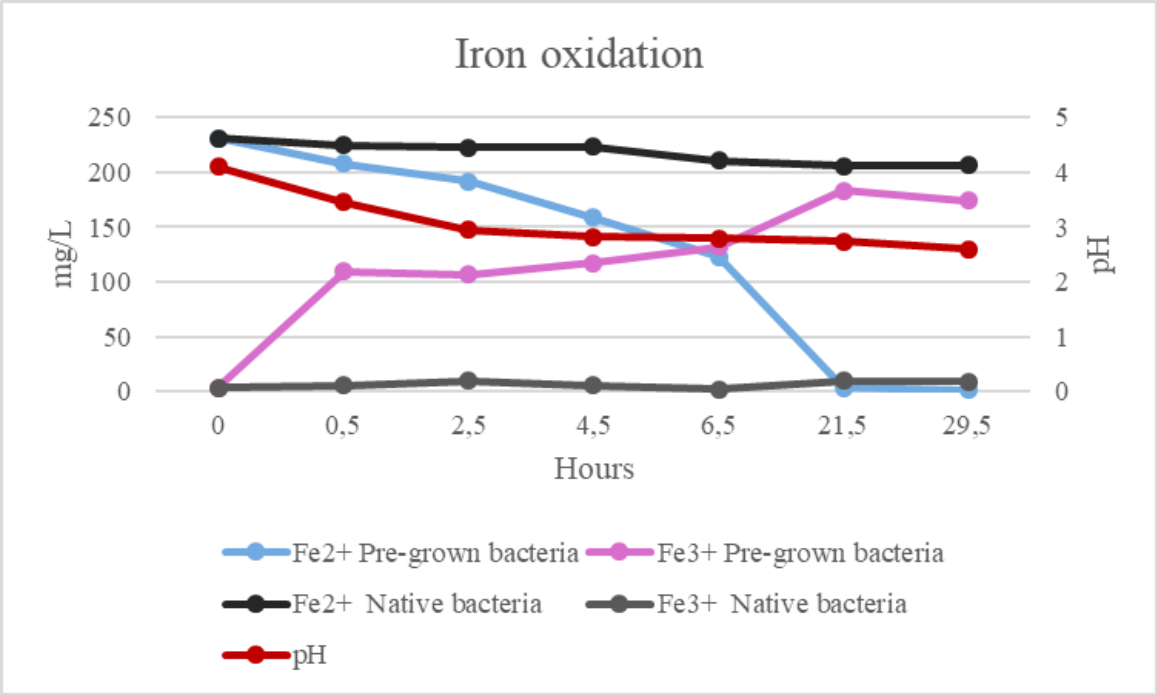


Figure 1. Iron oxidation monitored via Fe^{2+} and Fe^{3+} concentration change over 30 h

In contrast, approximately 206 mg/L Fe^{2+} remained in the uninoculated sample after 29.5 h. The corresponding oxidation efficiency was only about 10.7%.

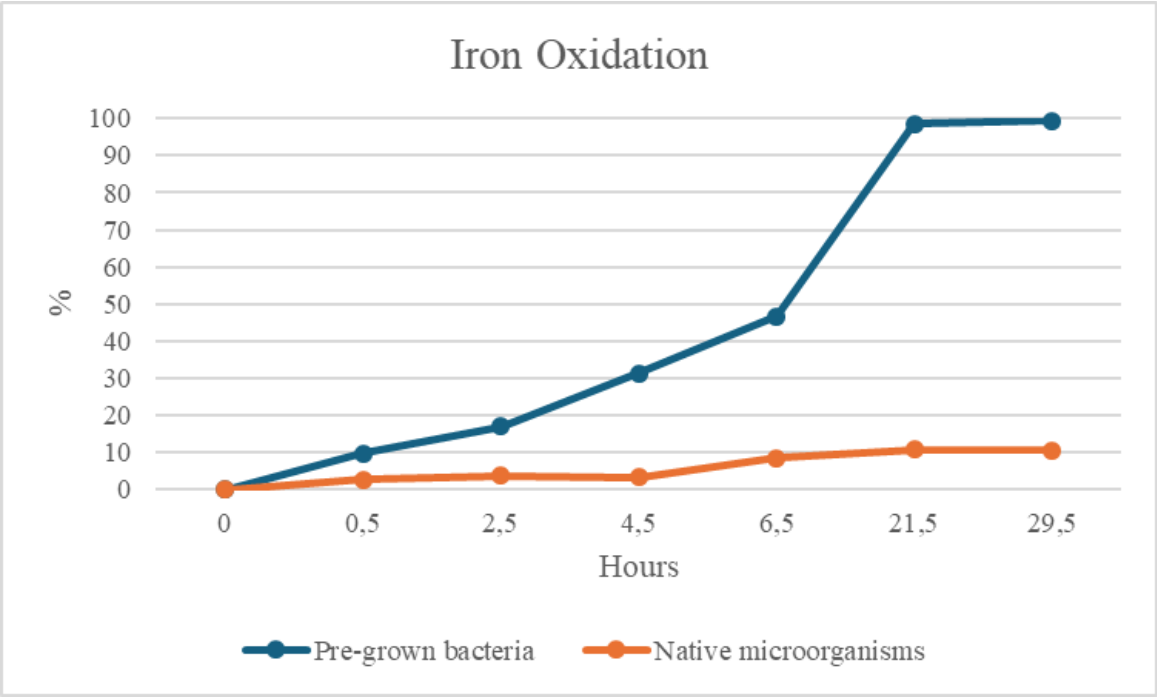


Figure 2. Efficiency of Fe^{2+} removal/oxidation exceeding 99%

The pH decreased from approximately 4.1 to 2.6 during oxidation.

Initial aeration reduced dissolved total Fe from 238.8 to 196.5 mg/L. After bacterial oxidation and filtration, the concentration decreased to 15.7 mg/L without deliberate pH adjustment. The majority of Fe removal therefore occurred during the microbial stage through coupled Fe^{2+} oxidation, Fe^{3+} hydrolysis and precipitation.

The first Fe fraction acted as an effective arsenic-scavenging phase before production of the main pigment material.

Conclusions

Enrichment of a native Smolník microbial consortium enabled more than 99% Fe^{2+} oxidation within approximately 29 h, compared with only about 11% in the non-enriched cultivation. Biological oxidation remained effective as pH decreased to 2.6 and reduced dissolved total Fe from 238.8 to 15.68 mg/L before deliberate pH adjustment.

Cu, Co, Mn and Mg remained predominantly dissolved during the low-pH oxidation stage. This selectivity is advantageous because the Fe-free solution can subsequently be considered for recovery of these elements by separate precipitation, adsorption or other separation processes.

The results demonstrate that microbial enrichment is essential for converting the diverse indigenous community of Smolník mine water into an efficient Fe^{2+}-oxidising consortium. Biological iron oxidation can consequently provide both an effective AMD treatment step and a route for transforming dissolved iron into a potentially useful mineral pigment.

Acknowledgements

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Operational transition from simplified matrices to real PCBs in a continuous pilot-scale copper bioleaching system under aluminium accumulation

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ABSTRACT:

Scaling up bioleaching processes from laboratory to pilot scale using real electronic waste remains a major challenge for urban mining applications. While simplified waste matrices are commonly employed during start-up, real printed circuit boards (PCBs) introduce additional metals, particularly aluminium (Al), which promote copper (Cu) cementation. This study describes the operational transition from a simplified Cu-only matrix to real shredded computer PCBs in an integrated continuous pilot-scale system (104 L total volume).

The plant comprises a 50 L bioreactor for biological Fe^{3+} regeneration (maintained at ~10 g L^{-1} Fe^{3+}), dominated by *Acidithiobacillus ferrooxidans*, coupled to a 27 L leaching reactor and a 27 L electrochemical recovery unit operating in semi-continuous recirculation mode. During a four-month start-up period, 14 complete multi-stage cycles were performed using 1 kg loads of simplified matrix. Under these conditions, Cu recovery exceeded 85% in most cycles, while stable Fe^{2+} bio-oxidation rates between 0.04 and 0.09 g L^{-1} h^{-1} were established.

Following the transition to real PCBs (particle size 0.5–4 mm), a distinct four-cycle operational profile emerged due to competitive metal dissolution phenomena. The first cycle enabled selective Al dissolution, reaching 2.89 g L^{-1} Al^{3+} after 24 h with minimal Cu solubilisation (0.88 mg L^{-1}). During the second cycle, dissolved Cu increased to 580 mg L^{-1}, requiring a short electrochemical depletion step (3.5 h) before solution recirculation to the bioreactor. Subsequent cycles operated under Cu-rich leaching conditions, reaching up to 3.4 g L^{-1} Cu^{2+} after 46 h and 3.7 g L^{-1} Cu^{2+} after 68 h (> 90% total Cu recovery).

Despite progressive Al accumulation (1.4 g L^{-1}), Fe^{2+} oxidation rates increased to 0.13 g L^{-1} h^{-1}, indicating adaptive microbial acclimation and demonstrating operational robustness during the transition to real PCB processing, supporting the feasibility of long-term continuous operation with multi-metal electronic waste.

KEYWORDS: Bioleaching; printed circuit boards; *Acidithiobacillus ferrooxidans*; pilot-scale operation; copper recovery.

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Advancing Continuous Bioleaching of Pyrrhotite-Rich Mine Tailings: Pilot-Scale Evaluation of Two Sudbury Legacy Tailings Using the CanmetMINING Process

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Keywords: tailings reprocessing, pilot scale, pyrrhotite, acidophiles, nickel.

Abstract

Continuous bioleaching tests were conducted on two pyrrhotite-rich legacy tailings from Sudbury to evaluate the performance and scalability of the CanmetMINING process for nickel (Ni), cobalt (Co), iron (Fe), and associated metals recovery. Pilot-scale experiments were performed using a cascade of continuously operated stirred tank bioreactors (50 L each), building on prior optimisation studies.

Results demonstrated effective Ni and Co solubilisation under moderately elevated pH conditions (2.0–3.0), which promoted Fe precipitation and maintained low dissolved Fe concentrations (0.03–0.3 g L^{-1}), thereby reducing downstream processing requirements. Comparative testing of tailings with varying pyrrhotite content confirmed the robustness of the process, while highlighting variability linked to feed characteristics.

Operational challenges were identified, including limitations in slurry pumping and uneven solids transfer, which affected steady-state conditions. Process control required careful management of acid and base consumption due to pH value fluctuations during continuous operation. Additionally, microbial community shifts were observed, with reduced planktonic populations and increasing influence of indigenous microorganisms.

Overall, the study demonstrated the feasibility of continuous bioleaching of Sudbury tailings, achieving Ni and Co recoveries of 64–93% and 52–95%, respectively, while emphasising the importance of process optimisation and microbial monitoring for scale-up.

Introduction

Bioleaching, as a sustainable alternative to conventional extractive metallurgy, is particularly relevant for the reprocessing of pyrrhotite-rich legacy tailings in the Sudbury region, where significant nickel (Ni) and cobalt (Co) values remain unrecovered. While pyrrhotite biooxidation is typically most effective at pH 1.5–2.5 [1, 2], performance is often constrained by mineralogical variability, passivation of mineral surfaces, and relatively slow oxidation kinetics compared to other sulfides, posing challenges for process efficiency and scale-up [3, 4].

To address these limitations, the CanmetMINING process was developed to enhance selective Ni and Co recovery from pyrrhotite-rich materials while minimising iron (Fe) dissolution and downstream processing requirements. Unlike conventional systems operating at low pH, this approach employs moderately elevated pH conditions to suppress the solubilisation of unwanted elements such as Fe, Al, Mg, and Si [3, 5]. Previous studies demonstrated that operating at elevated pH can improve Ni recovery

while reducing acid consumption, highlighting pH as a critical control parameter in bioleaching system optimisation [6].

Subsequent investigations refined the process through optimisation of operating conditions, microbial community characterisation, and development of a simplified nutrient medium to support cost-effective microbial activity [5, 7]. These studies confirmed robust Ni and Co recoveries across a range of sulfide ores and tailings, while demonstrating the importance of microbial diversity and adaptation in sustaining leaching performance after testing the developed microbial culture with six nickel sulfide ores with differing mineralogy. Additional process enhancements, including magnetic separation, flotation integration, and ion exchange recovery, further improved metal extraction efficiency and reinforced the scalability potential of the CanmetMINING approach [8, 9].

The downstream recovery strategy also considered the production of mixed sulfide precipitate (MSP), leveraging the selective precipitation of Ni and Co at low pH to reduce reagent consumption, simplify Fe management, and support subsequent production of electric vehicle (EV)-grade battery metals [10, 11].

Despite these advances, continuous bioleaching of pyrrhotite remains challenging due to operational constraints such as slurry handling, mass transfer limitations, process control under dynamic conditions, and microbial adaptation. Within this context, the present study evaluates the continuous bioleaching performance of two Sudbury tailings with contrasting pyrrhotite contents using a pilot-scale cascade of stirred tank bioreactors. By assessing metal recovery, operational stability, and microbial dynamics, this work aims to support process optimisation and advance the CanmetMINING technology toward industrial implementation.

Methods

Two pyrrhotite-rich tailings from legacy Sudbury impoundments were selected to represent contrasting mineralogical compositions. Tailing A, used in bioleaching Test 1, contained low sulfide content (8.46 wt.% pyrrhotite), whereas tailing B, used in Test 2, was significantly enriched in pyrrhotite (±69 wt.% including interstitial phases), with corresponding variations in sulfur (S), Fe, and base metal content (**Table 1**). Both materials were homogenised and ground to <106 µm (>99% passing) to ensure consistent particle size distribution and minimise variability in bioleaching kinetics associated with particle size effects.

A mixed acidophilic consortium of Fe- and S-oxidizing microorganisms, originally enriched from Sudbury mining environments and maintained following Cameron et al. [5], was used as inoculum. The culture was adapted in 5 L stirred tank reactors containing 10 wt.% tailings in modified Tuovinen and Kelly (TK) medium [12] at pH 3.0 under aerobic conditions. Triplicate cultures were grown to late exponential phase (cell densities (8.25–9.25) × 10^8 cells mL^{-1}; ORP 618–635 mV – Ag/AgCl), then pooled and used to inoculate the pilot-scale system. Microbial characterisation of the inoculum was performed using 16S rRNA gene sequencing to establish baseline community composition for subsequent comparison.

Table 1. Key mineralogy & stock base concentration differences for the pilot-scale pyrrhotite bioleaching tests.

Description		Test 1	Test 2
Tailing's particle size		< 106 µm	< 106 µm
Mineralogy (%)	**Pyrrhotite**	8.46	69.36
	Pyrite	0.01	0.59
	Pentlandite	0.1	0.41
	Chalcopyrite	0.11	0.07
	Iron	14.29	12.47
Concentration of Na_2CO_3 (% w/v)		20	Initial 20, later 30

Pilot-scale continuous bioleaching was conducted in a cascade of three 50 L stirred tank reactors fed by a 70 L slurry tank, operated at 40 ± 1 °C, 350 rpm, and 4.5 L min^{-1} air supply. Following an initial batch phase, the system was transitioned to continuous mode at a feed rate corresponding to a 10-day residence time. The CanmetMINING process strategy was applied, with no pH control in the primary reactor and

controlled pH conditions (2.5 and 3.0) in two downstream reactors respectively, using Na_2CO_3. Two tests were performed (for 50 and 71 days) to evaluate the influence of tailings mineralogy. Process performance was monitored through daily sampling for pH, ORP, Fe speciation, sulfate, and dissolved metals (ICP-MS), alongside microbial cell counts and community analysis. Mass balance calculations were performed to determine metal solubilisation efficiencies.

Metal sulfide precipitation tests were conducted on pregnant leach solution (PLS) under controlled reducing conditions, using NaSH, to evaluate the feasibility of directly producing mixed Ni/Co sulfide precipitates (MSP) at pH 3.2–3.3 without prior Fe removal or pH adjustment.

Results and Discussion

The CanmetMINING process was implemented under continuous pilot-scale conditions using a cascade of stirred tank reactors designed to integrate sulfide biooxidation with *in situ* iron management. Through staged pH control, the reactor configuration promoted initial sulfide oxidation in the upstream stage while enabling the progressive precipitation and removal of ferric iron in downstream stages, thereby maintaining conditions favourable for sustained bioleaching performance. This configuration effectively limited dissolved Fe concentrations, although continuous feeding of pyrrhotite introduced variability in pH control and required frequency of base additions. Notably, Test 2 exhibited significantly higher reagent consumption, reflecting increased acid generation associated with higher sulfide content in tailing B and highlighting the influence of feed mineralogy on process stability (**Table 2**).

Table 2. Consumption rates of acid and base throughout the pilot-scale bioleaching of pyrrhotite-rich tailings. Legend: "Cont" refers to sequential 10-day continuous runs; Po = pyrrhotite.

Utilization (kg/kg Po)		Test 1					Test 2				
		Batch	Cont1	Cont2	Cont3	Total	Batch	Cont1	Cont2	Cont3	Total
Acid (H_2SO_4)	**STR 1**	0.0004	-	-	-	**0.0004**	0.0109	-	-	-	**0.0109**
	STR 2	0.0005	-	-	-	**0.0005**	0.0094	-	-	-	**0.0094**
	STR 3	0.0005	-	-	-	**0.0005**	0.0088	-	-	-	**0.0088**
	Total	**0.0014**	**-**	**-**	**-**	**0.0014**	**0.0291**	**-**	**-**	**-**	**0.0291**
Base (Na_2CO_3)	**STR 1**	-	-	-	-	-	0.0111	-	-	-	**0.0111**
	STR 2	0.0123	0.0184	0.0299	0.0280	**0.0836**	0.0256	0.0379	0.0283	0.0264	**0.1182**
	STR 3	0.0071	0.0041	0.0085	0.0070	**0.0267**	0.0269	0.0208	0.0265	0.0240	**0.0982**
	Total	**0.0194**	**0.0225**	**0.0383**	**0.0300**	**0.1102**	**0.0636**	**0.0588**	**0.0548**	**0.0504**	**0.2276**

The redox conditions and Fe speciation highlighted significant constraints that vary with scale. Throughout the continuous operation during Test 2, the oxidation-reduction potential (ORP) values in the primary reactor gradually decreased due to an inadequate microbial oxidation capability compared to the incoming load of Fe^{2+}. This led to an accumulation of Fe and its transfer to the downstream reactors. In contrast, the controlled reactors were able to sustain higher ORP values; however, they still faced challenges with incomplete oxidation of Fe^{2+} and fluctuating ratios of Fe^{3+} to Fe^{2+}, which pointed to limitations in the sulfide oxidation capacity, especially at high loading of pyrrhotite. Despite these challenges, effective precipitation of Fe^{3+} was successfully achieved in Test 1, while Test 2 exhibited increased concentrations of dissolved Fe. This underscores the process sensitivity to variations in mineral composition and the amount of Fe present. (**Figure 1**).

Microbial population dynamics were pivotal to overall process efficiency. In both trials, a short lag phase was followed by rapid proliferation, and Test 2 achieved higher cell densities because more substrate was available, compared to Test 1. When the system shifted to continuous operation, planktonic populations declined slightly, likely due to increased dissolved Fe and altered environmental conditions. Community analysis also showed changes in the dominant taxa during the transition from batch to continuous mode, reflecting adaptation to reactor conditions and a possible influence of indigenous microorganisms introduced with the fresh tailings feed.

Metal solubilisation data demonstrated the effectiveness of the process while highlighting operational limitations. Test 1 achieved high extraction efficiencies (93.4% Ni, 95.4% Co), whereas Test 2 showed reduced recoveries (64.5% Ni, 52.2% Co), consistent with less favourable Fe speciation and higher

dissolved Fe levels (**Figure 2**). Although slightly higher leaching rates were observed in Test 2 due to increased Fe^{3+} availability, overall performance was constrained by suboptimal redox conditions and process instability. These findings underscore the importance of controlling Fe chemistry, pH, and microbial activity to optimise continuous bioleaching performance and ensure scalability of the CanmetMINING process.

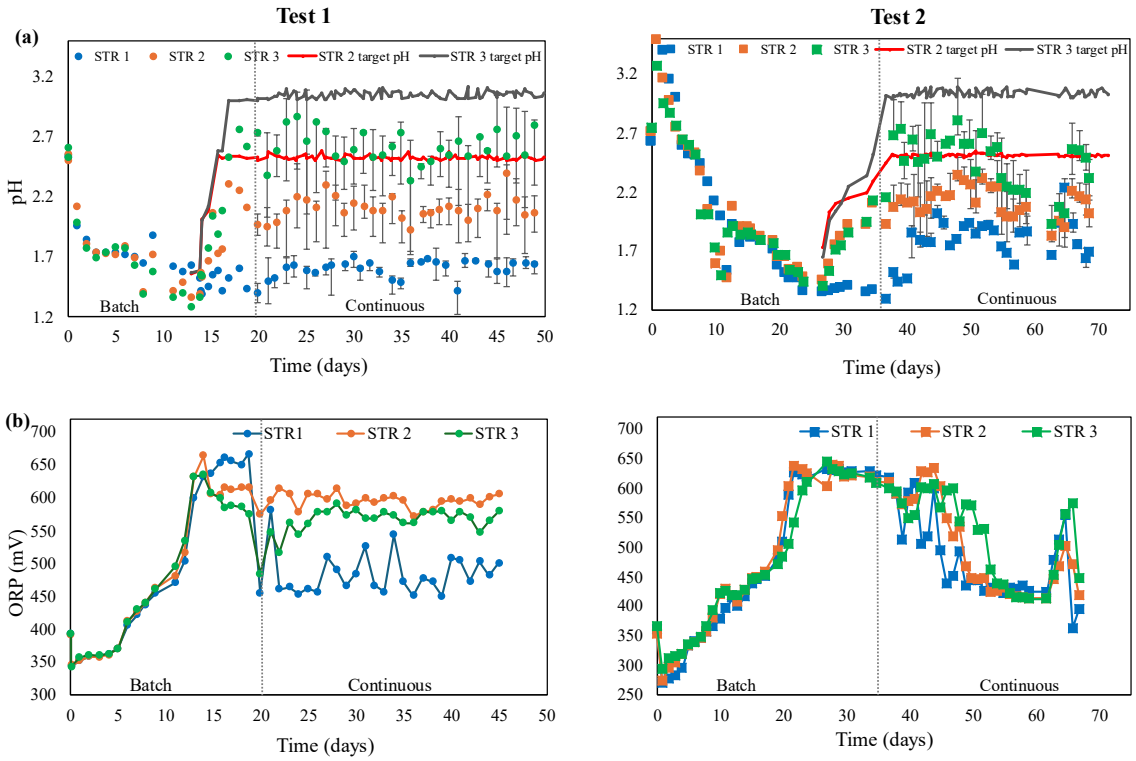


Figure 1. Changes in (a) pH, and (b) ORP during bioleaching of pyrrhotite-rich tailings in Test 1 and Test 2. In plot (a), the points indicate pH values measured mid-day (prior to pH adjustment), with the error bars showing pH values measured in the early morning (low values) and late afternoon (high values), respectively; the solid lines indicate the mean pH values obtained after pH adjustments (n = 3).

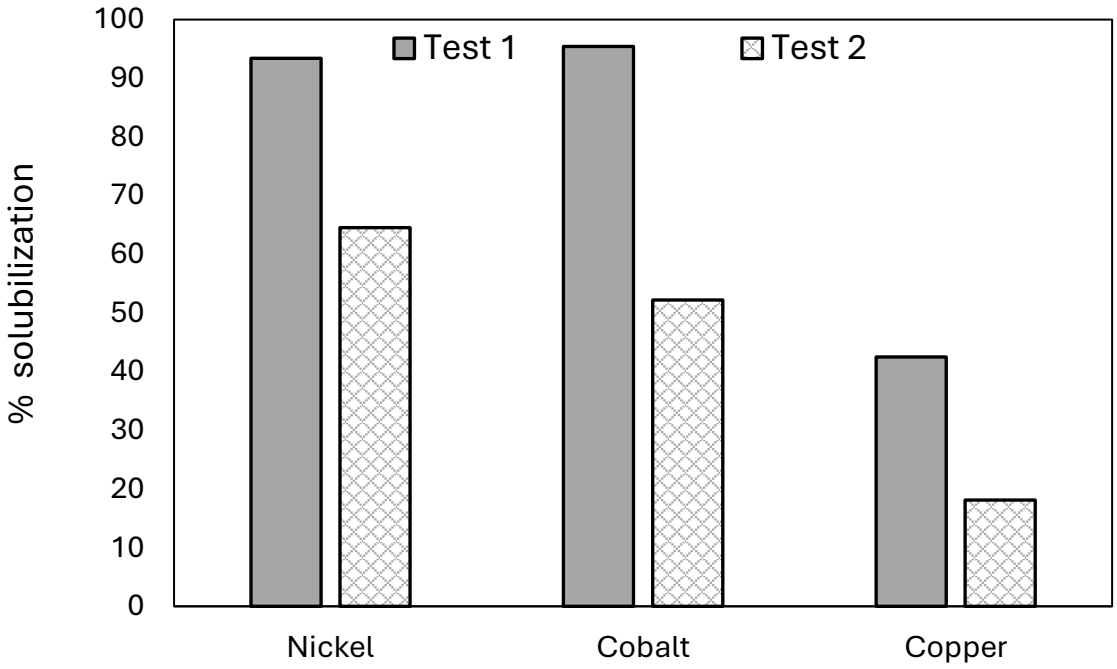


Figure 2. Ni, Co, and Cu solubilisation during the bioleaching of two pyrrhotite-rich tailings achieved during Test 1 and Test 2.

Metal sulfide precipitation tests conducted on two pregnant leach solution (PLS) samples obtained in Test 1 demonstrated that slight modifications to solution chemistry significantly improved Ni and Co precipitation efficiency, with most metal removal occurring within the first 15 minutes of reaction time (**Table 3**).

Table 3. Composition of original and modified PLS samples and corresponding Ni and Co precipitation efficiencies during MSP production obtained for Test 1 (* Based on comparing solution chemistry).

	pH	ORP vs. Ag/AgCl (mV)	Al (ppm)	Ca (ppm)	Co (ppm)	Cu (ppm)	Fe (ppm)	Mg (ppm)	Na (ppm)	Ni (ppm)	Efficacy*
Original PLS	2.94	588	120	275	5.27	14.1	28.3	196	4,070	227	**52% Ni 58% Co**
Modified PLS	3.60	582	107	272	4.9	13.8	2.5	193	4,290	216	**94% Ni >99% Co**

Conclusions

This study demonstrates the technical feasibility of continuous bioleaching of pyrrhotite-rich Sudbury tailings using the CanmetMINING process under pilot-scale conditions. The staged reactor configuration, combining uncontrolled primary leaching with downstream pH-controlled Fe precipitation, proved effective in reducing dissolved Fe concentrations while enabling efficient Ni and Co recovery through the MSP of the PLS.

Process performance was strongly influenced by feed mineralogy, with higher pyrrhotite content leading to increased Fe loading, greater reagent consumption, and limitations in microbial oxidation capacity. While high Ni and Co recoveries were achieved for the lower-sulfide material, reduced performance under elevated Fe conditions highlighted the sensitivity of the system to Fe speciation and redox balance.

Microbial activity and community dynamics were closely linked to process stability, with shifts observed during the transition from batch to continuous operation and under varying chemical conditions. These findings emphasize the importance of integrating microbial monitoring with process control strategies.

Overall, this work confirms the potential of the CanmetMINING process for sustainable reprocessing of legacy tailings, while identifying key scale-up challenges related to slurry handling, mass transfer,

and Fe management. Addressing these constraints will be critical for advancing toward robust, cost-effective, and industrial implementation of continuous bioleaching systems.

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Integration of Bioleaching with sulfur Capture

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Abstract: Bioleaching is commercially used for metal leaching from sulfidic minerals, such as low-grade copper sulfides. However, bioleaching of oxidic material streams is challenging due to the absence of reduced sulfur compounds necessary for biological sulfur oxidation. Metal-containing oxidic waste streams present a promising, low-cost alternative to primary adsorbents in industrial gas cleaning for H_2S removal through adsorption, thereby converting oxidic materials into sulfides. Acidophilic sulfur- and iron-oxidising microorganisms can then oxidise the sulfided mineral and leach metals with reduced chemicals consumption. This study explored how integrating H_2S removal with the bioleaching of metals from sulfided materials can help recover both metal and sulfur values. Chosen oxidic wastes first adsorbed H_2S, and bioleaching was then compared before and after sulfidation. This new concept enables efficient gas cleaning and metal leaching with minimal use of external chemicals.

Solution and residue analyses of experiments demonstrated that sulfur- and iron-oxidising microorganisms significantly enhanced leaching from the sulfided samples. Acid utilised for leaching was generated directly from the sulfided material, resulting in a notably reduced reliance on external chemical inputs. This study demonstrated the feasibility of integrating high-temperature H_2S adsorption with oxidic metal containing waste materials, with metal recovery from sulfided material through bioleaching processes.

Keywords: Hydrogen sulfide adsorption, bioleaching, circular economy, metal recovery

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Design and Performance of Iron Biooxidation Systems for In-Situ Bioleaching Applications

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Abstract: *In-situ* bioleaching is an emerging biomining strategy that enables the recovery of metals from low-grade ores and inaccessible ore bodies while minimising excavation, surface disturbance, and energy consumption. Within the XTRACT project (funded by the EU Commission in the frame of Horizon Europe program), an integrated in-situ bioleaching concept is being developed in which ferric iron is biologically regenerated in a dedicated bio-oxidation reactor and used as the primary lixiviant for the oxidative dissolution of sulfidic minerals in an underground ore vein.

Ferric iron is produced *ex-situ* by acidophilic iron-oxidising microorganisms and injected into a fractured ore body, where it oxidises metal sulfides and leaches valuable metals. The ferrous iron generated during leaching is recirculated to the bio-oxidation reactor for regeneration, enabling a closed-loop lixiviant system. Laboratory-scale studies were conducted in column bioreactors operated in continuous mode to investigate the influence of key operational parameters relevant to underground deployment, particularly temperature and carbon dioxide availability, on iron bio-oxidation kinetics.

Results demonstrate that iron oxidation by *Leptospirillum ferriphilum* remains effective under reduced temperature and CO_2 concentrations representative of underground mine environments, although reaction rates decrease compared to optimal laboratory conditions. These findings indicate that stable iron bio-oxidation can be sustained without active temperature control or CO_2 supplementation, which are often impractical or restricted in underground settings.

The outcomes provide essential design and operational data for the implementation of pilot-scale *in-situ* bioleaching systems and confirm the feasibility of microbial iron bio-oxidation as a robust and adaptable driver for sustainable underground biomining applications.

Keywords: *in-situ* bioleaching; iron biooxidation; biomining; *Leptospirillum ferriphilum*; underground mining

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Development of protein resins for REE separation using high-throughput screening

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Abstract: Rare earth elements (REEs) are a class of metals containing the 15 lanthanides, yttrium, and scandium. They share similar chemical and physical properties and are used in renewable energy applications including magnets and batteries. As these metal ions generally have the same charge (3+) and similar atomic radii, industrial separation relies on many solvent-intensive, liquid-liquid extraction steps to exploit small differences, resulting in costly, energy-intensive processes. Recently, a small number of proteins with high selectively (10^8) for REEs were discovered and used at laboratory scale to separate specific REEs from each other. Identifying more proteins with high selectivity for REEs generally and specifically would enhance separations of these metals, however finding these proteins poses a challenge. Here we demonstrate progress towards a high-throughput pipeline to identify natural proteins with REE-specific binding sites. Protein candidates are first selected *in silico* by mining bacterial genomes associated with lanthanide utilisation using structural prediction and molecular dynamics. These proteins are qualitatively screened for selectivity using inductively coupled plasma-mass spectrometry and hits advanced to quantitative binding characterisation using differential scanning fluorimetry. Top hits are then immobilised to chromatography resins using a procedure that combines protein purification and immobilisation in a single step. These protein resins are then evaluated for use through automated metal binding-elution cycling system that we have developed. Implementation of this pipeline will allow us to quickly and effectively identify proteins with different selectivity to REEs, will shed light on biophysical questions about poorly understood protein coordination of lanthanide ions, and could be used beyond REEs to identify proteins that bind to any metal of interest.

Keywords: Rare earth elements, lanthanides, protein biochemistry, metal separation, high-throughput

Bioleaching of Rare Earth Elements from a Hyper Enriched Black Shale: Effects of Microbes, Temperature and Energy Source

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Abstract: The transition to low-carbon technologies is driving a strong increase in demand for rare earth elements (REE), motivating the development of more sustainable extraction routes from complex ores and secondary resources. Biohydrometallurgy offers a promising alternative to conventional high-temperature or acid-intensive processes, particularly for fine-grained, sulfidic resources that are difficult to treat using traditional methods. In this study, the bioleaching of REE from a hyper-enriched black shale (HEBS) was investigated using controlled batch experiments focusing on the effects of microbial inoculation, temperature and energy source.

Leaching tests were conducted at 30 °C and 45 °C for 28 days, with and without an acidophilic inoculum, and, with or without Fe^{2+} and S^0 as energy sources. Dissolved elements were quantified by inductively coupled plasma-optical emission spectrometry/mass spectrometry. Across all tested conditions, total dissolved REE concentrations increased by approximately one order of magnitude over the leaching period, indicating substantial solubilisation from the shale. Flasks containing an active inoculum consistently exhibited higher soluble REE concentrations than abiotic controls, with enhancements ranging from ~20% in ore-only systems to more than threefold in Fe–S-amended systems at 30 °C. Elevated temperature generally accelerated REE leaching, particularly in inoculated flasks.

Energy source supplementation exerted a dual role, stimulating biolixiviant generation and indirect bioleaching in inoculated systems while suppressing REE dissolution in abiotic controls. This highlighted the importance of microbially mediated iron oxidation for effective bioleaching performance. Light and heavy REEs exhibited similar dissolution behaviour. Overall, the results demonstrated that rare earth bioleaching from HEBS can be notably enhanced by appropriate microbial inoculation, increased temperature and energy source supplementation, and provided insights for the development of industrial REE biomining flowsheets.

Keywords: acidophiles, bioleaching, hyper-enriched black shale, iron- and sulfur oxidation mesophiles, rare earth elements.

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Circular Extraction of Rare Earth Elements via Bioleaching and Phage Display-Based Purification

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Keywords: [Bioleaching; Yttrium; Bacteriophage; Circularity; Acidophile]

Abstract

The Rare Earth Elements (REE) are a series of 17 D and F block elements notable for their widespread use in modern technology such as electronics, motors, wind turbines, glasses, ceramics, catalysts, and in defence applications. This research describes a circular low-cost method of REE extraction from Yttrium rich industrial feedstocks using Acidithiobacillus ferrooxidans a gram-negative chemolithoautotrophic bacteria capable of producing sulfuric acid via the metabolism of sulfur compounds and oxidation of divalent iron. Using bacterially derived leaching solutions, 99.8% of the yttrium and europium content was extracted from rare earth-rich scrap following a 24-hour leaching. To further purify this leachate, a library of novel phage display-derived proteins were created using a bio-panning method to select for pH-sensitive dodecapeptide sequences capable of selectively binding to target divalent and trivalent cations. For validation, these proteins were expressed in tandem on the N terminus of the OMPa protein in E. coli to produce modified biosorbent bacteria for sequestration. This was followed by the precipitation of yttrium carbonate product with significantly decreased levels of calcium and europium compared to the untreated product and a 100% increase in europium concentration in the europium raffinate portion.

Introduction

The recovery of rare earth elements from industrial waste is a challenge due to a number of factors. Despite the high value and myriad industrial applications of REE, the low concentrations relative to other metals, concomitance of multiple REE in a single feedstock and highly similar precipitation profiles result in a highly challenging set of circumstances to overcome when creating new REE refinement workflows [1]. As a result, this research aimed to develop a bioleaching-based system to recover REE from phosphor powder waste. Phosphor powders were selected as a model REE bearing feedstock due to their widespread use, high REE concentration, the presence of multiple REE in the feedstock and the limited previous research into bioleaching of Yttrium Europium phosphors. Previous research into the bioleaching of yttrium europium lamp phosphors through biological means has focused primarily on either single-stage direct bioleaching experiments utilising iron and sulfur oxidising bacteria as presented by [2], direct acid-based leaching as demonstrated by [3] or complexiolytic leaching as shown in [4]. From this research, several key findings can be drawn to direct future research. Firstly, while acid-based bioleaching was effective at solubilising both Y and Eu from phosphor powder waste, the presence of trivalent iron in solution rapidly accelerated the rate of solubilisation and the total quantity of material solubilised. Secondly, while the extraction efficiency of the bioleaching process is often cited as between 95-100%, there has been little consideration of the quantity of off-target dissolution during bioleaching. Due to the requirements of high purity in most REE applications, the optimisation of contaminant removal from rare earth leachates is of prime importance in addition to the solubilisation of the REE. Further to this point, this research aimed to identify selective metalloproteins for the efficient room temperature separation of metal ions in the liquid phase. To achieve this goal, phage display was employed as performed by [5] with the aim of developing specific short binding peptides capable of adsorbing and desorbing target metal ions at specific pH gradients. Previous work by [6] was able to analyse larger proteins with similar binding profiles; however, via the application of phage display, this work aimed to identify *de novo* minimally sized binding epitopes for separation.

This work therefore, had three objectives:

Objective 1: Optimise the bioleaching process using iron and sulfur oxidising bacteria for the solubilisation of REE from phosphor powders.

Objective 2: Develop metal-binding proteins for key contaminant metals (Eu, Ca) in phosphor powder leachates

Objective 3: Express binding proteins on a bacterial membrane to separate ions present in phosphor powder leachates.

Methods *At. ferrooxidans* ATCC23270 was cultured at 30 °C in Acidophile Basal Salt and Trace Element liquid media, shaking at 160 rpm. Y_2O_3:Eu^{3+} phosphor powders from fluorescent lamps provided by Dr Daniel Ray were used in all leaching reactions. Analysis of phosphor powder leachates was performed using a Perkin Elmer NexION 2000 mass spectrometer operating under helium KED. Samples were diluted 1/50,000 in 2% HNO_3 prior to analysis.

Molecular Biology New England Biolabs Ph.D.-12 Phage Display Peptide Library Kit v2 was used to perform repeated rounds of biopanning against Ca^{2+} and Eu^{3+} metal ions immobilised on IDA ion exchange resin inoculated with bacteriophages and washed using TBST (0.1%). Bound epitopes were desorbed using a pH 4 citrate buffer and repropagated. Following three rounds of biopanning, bound epitopes were sequenced by Azenta Life Sciences. Following identification of the binding epitopes, six tandem repeats of the binding epitope were cloned into a pET21-b plasmid expression vector bound to the N terminus of the OMPa protein. These fusion proteins were expressed in *E.coli* BL21(DE3). Validation of protein expression was performed using SDS-PAGE. Following validation, the solutions were incubated with the bacterial cultures for 1 hour and centrifuged. The bacterial pellets were resuspended in a pH 4 citrate buffer to desorb Eu and Ca ions.

Leaching experiments were performed using cultures of *At. ferrooxidans* ATCC23270. Protocol optimisation experiments were performed by comparing 1 step, 2 step and abiotic direct leaching compared to abiotic and acid digest controls. Acid digestion samples were digested in 97% sulfuric acid for 6 hours at 200 °C. Leachates were precipitated using by adding an equal volume of $0.35mL^{-1}$ of sodium bicarbonate.

Results and Discussion

In order to optimise the solubilisation of yttrium from phosphor powders, a series of four leaching experiments were performed, each at 1% pulp density (PD), with the aim of identifying the most effective solubilisation method compared to heat-assisted acid digestion. Figure 1 displays a comparison of direct single-step bioleaching, spent media two-step bioleaching, acid digestion and abiotic digestion, in which aseptic bacterial media was used in place of bacterial culture.

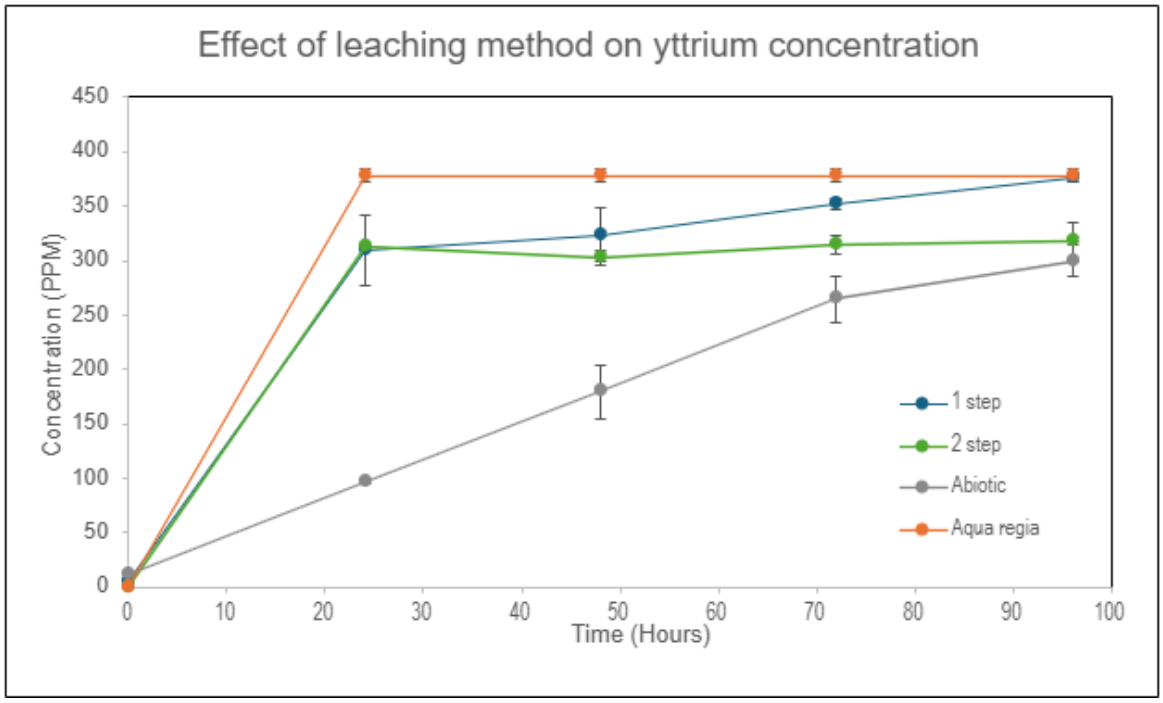


Figure 1. Effect of leaching method on yttrium concentration. Concentrations measured using ICP-MS N=3

As displayed in Figure 1, while all four leaching methodologies solubilised in excess of 300 PPM of yttrium, abiotic leaching lagged significantly behind in terms of solubilisation rate. Furthermore, following 24 hours, single and dual step leaching began to diverge, resulting in single step leaching reaching parity with acid digestion. In total, single-step leaching solubilised 99.8% of the yttrium present in the phosphor powder solution. Following validation of the ability of *At. ferrooxidans* to solubilise yttrium. In order to remove solubilised calcium and europium impurities in the leachate, a series of phage display experiments were performed to identify Eu^{3+} and Ca^{2+} binding epitopes. These binding epitopes were then expressed in tandem on the N terminus of the OMPa protein. Figure 2 depicts pairwise alignments of these fusion proteins with the canonical OMPa protein.

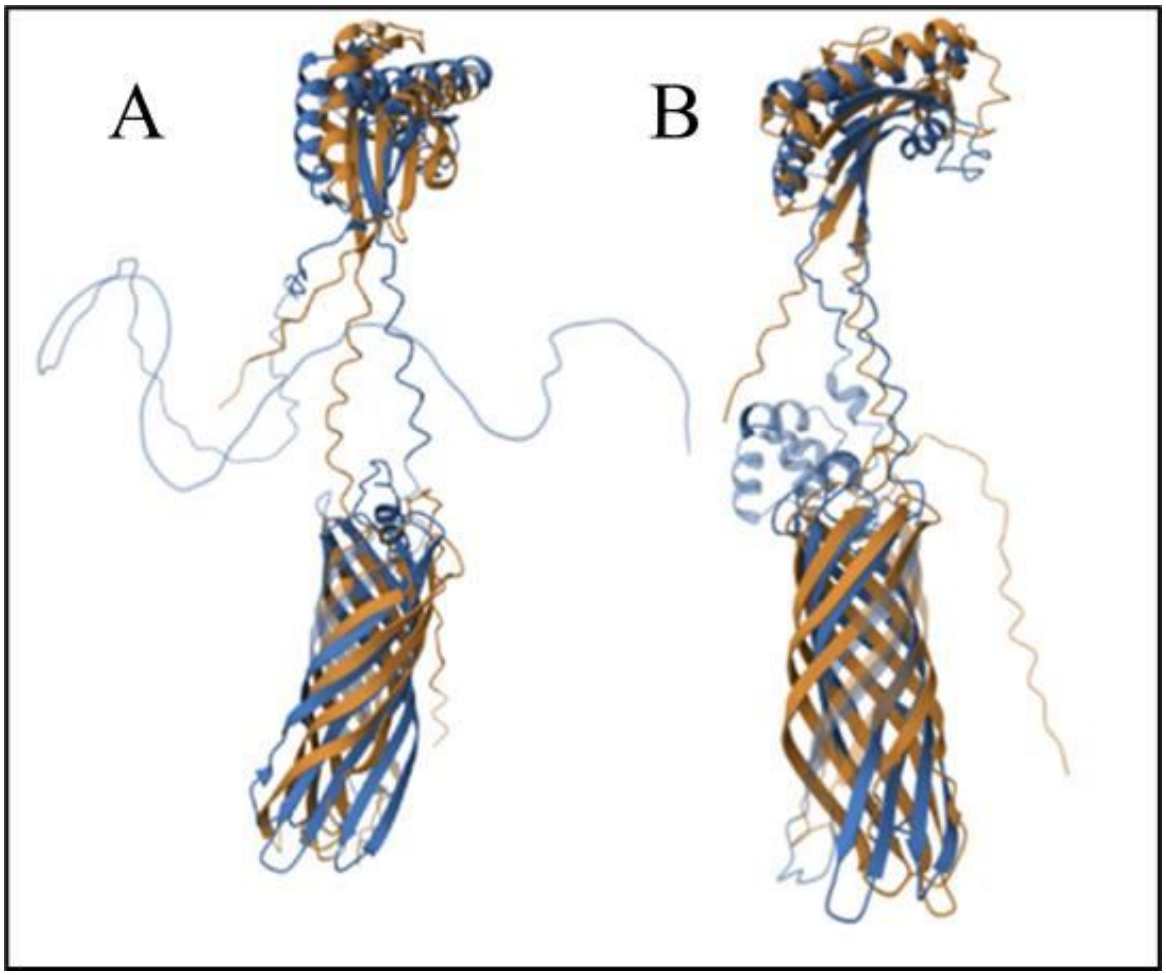


Figure 2. Ca (A) and Eu (B) ion binding proteins (Blue). Modelled using Alphafold3 aligned with canonical OMPa protein (Orange) using RCSB Pairwise Alignment Tool

As shown in Figure 2, the affixation of six tandem short binding proteins to the N terminus of the OMPa protein yielded little change to the tertiary structure of the membrane-bound domain. As such, following proteomic expression under a T7 promoter, the membrane-bound proteins were washed with a pH 4 citrate buffer to remove any residual metal ions and pelleted. Subsequently, the supernatant was removed, and samples of the leachate derived from Figure 1 were used to suspend the biosorbent bacteria. Following 60 minutes of agitation, the solution was once again pelleted and the supernatant collected to create the yttrium raffinate. The pellet was washed with a pH 4 citrate buffer to suspend the sequestered Eu^{3+}. A comparison of the Eu concentration across the leachate, yttrium raffinate and Eu raffinate is presented in Figure 3.

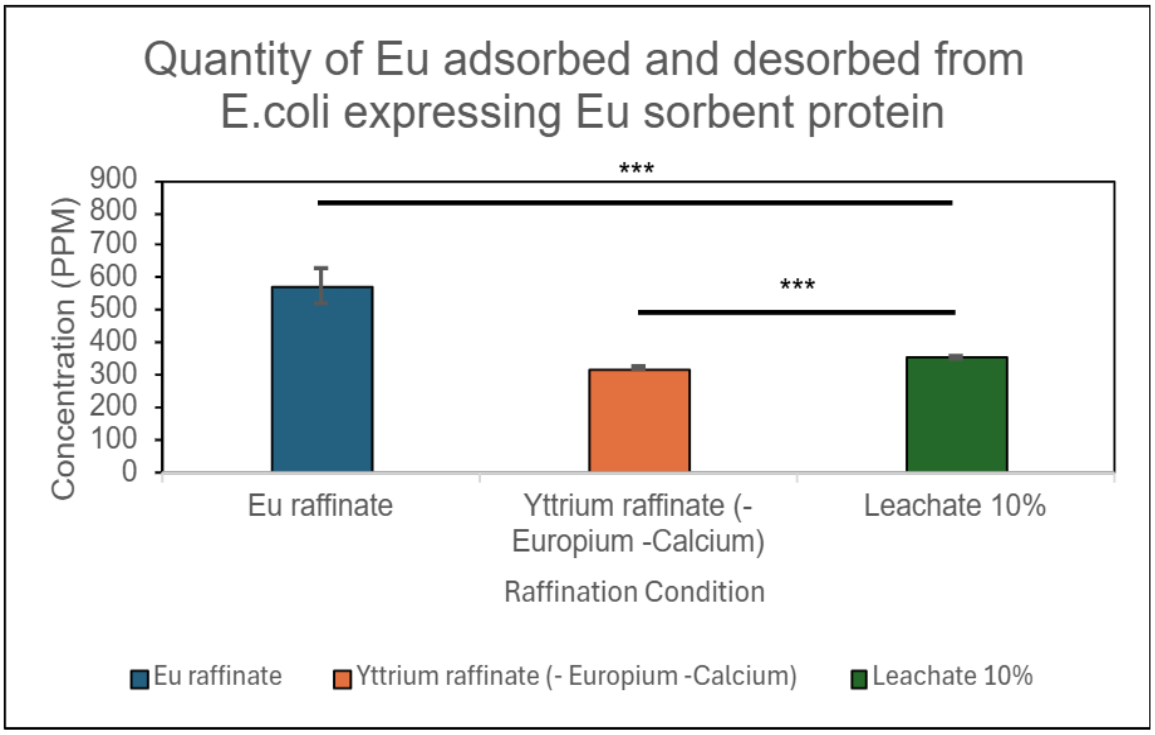


Figure 3. Quantity of Eu adsorbed and desorbed from E.coli expressing Eu sorbent protein. ***=p>0.001 significance analysed using students T test. Input volume of 50mL. Eluent volume of 5mL. N=3

As displayed in Figure 3, following washing the biosorbent *E.coli* culture with bioleachates resulted in a sequestration of 50 PPM of Eu^{3+} ions from the solution, which were subsequently eluted in 1/10 the initial loading volume, causing a significant increase in the europium concentration of the solution.

Conclusions

In summary, this research has evaluated the bioleaching of REE from phosphor powder waste using *At. ferrooxidans*. In the course of this optimisation, it was found that single-step leaching resulted in the most effective solubilisation of material. Recovering 99.8% of the yttrium present in solution compared to heat-assisted acid digestion. Furthermore, this research has expanded upon previously developed workflows by describing a method of liquid phase separation of europium from yttrium-rich bioleachates. In which phage display-derived binding proteins were successfully used to recover 50PPM of Eu^{3+} from solution, which was later successfully eluted. This finding, therefore, expands the domain of biological processing from leaching to purification, providing a framework for future larger-scale biological REE separation methodologies. The use of phage display in the development of metal-binding proteins is an emergent and rapidly changing field of study. To date, many applications have focused on biologically relevant metals such as copper or calcium. However, the downstream applications of this research present a starting point for future work on de novo metal ion protein design. Future research will aim to increase the binding of the biosorbent protein membrane via optimisation of the binding protein active site via computational mutagenesis. Additionally, while the use of phosphor powders as a model feedstock provides a useful supply of both europium and yttrium, future research may benefit from additional consideration of a wider range of feedstocks for leaching and a wider range of biosorbent proteins for separation.

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Siderophore assisted platform technology for the recovery of critical metals from low concentrated feeds

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Recovery of critical metals like gallium, germanium, and indium from their low concentrated feed such as industrial wastewater or e-waste leachates is challenging due to high concentration of contaminants, and low concentrations of the target metals. Conventional techniques such as solvent extraction and ion-exchange are either technically challenging or economically unfeasible. Thus, a highly selective and sensitive process is needed for the technology development. Desferrioxamine B (DFOB) has been demonstrated to complex gallium, indium and germanium under different conditions. However, to develop the technology, separation of the metals from the wastewater or leachate needs to be achieved.

In this study, DFOB was immobilised on the carboxylated polystyrene beads. After the successful immobilisation of the DFOB, there was no effect on selectivity of DFOB towards Ga. Thus, 100 g of DFOB immobilised carboxylated beads was packed in a column. The breakthrough was achieved at 11.4 L of the wafer industry wastewater containing 33 mg/L of Ga and 40 mg/L of As. To scale up, 300 g of DFOB immobilised carboxylated beads were packed in the column, the system was able to treat 80 L/day of the above-mentioned wafer industry wastewater after optimising the flowrate. The recovery rate of Ga in the columns was more than 90% while As retention in the column was less than 5%. The elution of Ga from the column was carried out with 50 mM EDTA leading to almost 100% elution of Ga. The Ga concentration in the elution was 5 times more than the initial concentration. The life cycle analysis demonstrated that siderophores based process has more than 90% less than environmental impact compared to the gallium recovery from the primary production. This technology can be implemented to other critical metals due to the complexing ability of DFOB towards other critical metals like germanium, indium.

Keywords: Siderophores, gallium, low concentrated wastewater, carboxylated beads, columns

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Harnessing Lanmodulin Proteins for REE Separation

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Abstract: Rare earth element (REEs: Sc, Y, La- Lu) are irreplaceable components in many energy and consumer technologies. However, the extraction and subsequent separation of individual REEs from feedstocks remains a significant economic and environmental challenge. We are working to develop a biology-based, all-aqueous REE extraction and separation approach as a potential alternative or augmentation to conventional hydrometallurgical processes. This process leverages lanmodulin proteins, bacterial proteins that have evolved as part of lanthanide uptake pathways and exhibit exceptionally high REE binding affinity and selectivity. Through immobilisation of lanmodulin onto porous resin, we have demonstrated selective capture of REE ions from complex metal ion mixtures, ranging from low-grade ores to coal by-products. More recently, we have focused on intra-REE separations, including high-purity (>99%) Dy vs Nd/Pr separation from mixed rare earth oxides generated from a range of end-of-life magnet feedstocks. Guided by first principles modelling, we have further refined our separation methodology to enable high-purity, high-yield separations of feedstock-relevant, near-adjacent middle and heavy REEs (Sm, Gd, Dy, and Y) at gram-scale. Building on this foundation, we are expanding the lanmodulin toolkit through protein engineering and bioprospecting. Using a newly developed high-throughput screening platform for quantitative determination of intra-REE selectivity, we have characterised thousands of natural and engineered REE-binding proteins, leading to the discovery of variants with improved selectivity among light and middle/heavy REEs. These new variants have enabled separation of energy critical Pr from abundant, low-value La and separation of the adjacent pair, Tb/Dy, in single column processes. Current efforts are focused on scaling this separation technology in partnership with industry.

Keywords: lanmodulin, Protein-based extractant, Rare Earth Elements, REE separation, biomolecular separations

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Enhancement of chalcopyrite bioleaching with *Acidithiobacillus ferrooxidans* using a non-ionic organic surfactant

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Abstract:

As the primary source of the world's copper, chalcopyrite is critical to the green energy transition. However, its refractory nature creates significant processing bottlenecks amid surging global demand. Consequently, unlocking the full potential of chalcopyrite reserves requires urgent attention to process optimisation that overcomes the mineral's inherent kinetic limitations. Recent literature demonstrates that organic biodegradable surfactants can help to overcome the refractory nature of chalcopyrite, as they significantly reduce interfacial tension and inhibit the formation of passivating layers. By modifying the mineral's surface wettability, these additives facilitate deeper lixiviant penetration and sustained dissolution kinetics, thereby unlocking superior copper recovery rates compared to conventional methods.

In this research, a non-ionic biodegradable surfactant was evaluated in chalcopyrite bioleaching using non-adapted mixed mesophilic bacteria, mainly comprised of *Acidithiobacillus ferrooxidans*. The study investigated the effect of surfactant concentration on both copper extraction efficiency and bacterial compatibility. Results indicated that the addition of the surfactant within a specific low-concentration range significantly enhanced final copper extraction compared to control tests. However, the study identified a critical threshold beyond which the surfactant exhibited diminishing returns. Furthermore, distinct inhibitory effects on bacterial growth and overall copper yield were observed at elevated concentrations. Specifically, higher dosages were found to extend the lag phase of bacterial metabolism and, in extreme cases, hinder the bioleaching process entirely. Throughout the experiments, operational parameters including pH, oxidation-reduction potential (ORP), bacterial count, surface tension, and ferric concentration were monitored to correlate biological activity with leaching performance. Bioleaching residues were also subjected to characterisation analyses, such as XRD and SEM/EDS, to identify phase and morphological changes induced by the surfactant.

Keywords: Chalcopyrite, Copper, Surfactant, Bioleaching, Mesophile

Fine-tuning strain design and growth conditions for improved efficiency for biomining in *Acidithiobacillus ferridurans* JAGS

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Keywords: Strain engineering; Carbon fixation; Nitrogen fixation; Iron oxidation; Bioleaching

Abstract

Biomining uses microbial iron oxidation to extract remnant metals from mine tailings. To develop an efficient biomining process, a proficient microbial population is required. Though the acidophilic chemolithoautotrophs such as *Acidithiobacillus ferridurans* JAGS have potential, they are constrained by native metabolism. Identifying efforts to improve the iron oxidation rate might help improve the leaching process. *A. ferridurans* JAGS is an auto-diazotroph that derives its energy from iron oxidation to support the energetically demanding processes of carbon and nitrogen fixation. Therefore, we hypothesized that enhancing the high energy demand for carbon and nitrogen fixation can improve iron oxidation efficiency. CRISPR-mediated genome editing technique was employed for pathway engineering in the strain. Cells grown under nitrogen fixing conditions showed 1.2-fold higher iron oxidation and 1.3-fold higher per cell respiration rate as compared to supplemented ammonium. However, the biomass accumulation was observed to be lower which was also predicted by the Thermodynamic Electron Equivalents Model. Further overexpression of the Carboxysome-Rubisco complex along with the genes in the electron transport chain showed 1.9-fold higher iron oxidation rates and 2.3-fold improvement in biomass as compared to wildtype. This study demonstrates the importance of strain fine-tuning to understand the bottlenecks and achieve maximum process productivity.

Introduction

The increase in the global demand for electric automobiles, batteries, solar panels had led to increased mining activities for valuable metals. Biomining provides an environment-friendly alternative to traditional mining for low grade ores and mine tailings where significant amount of valuable metals are still contained. The Sudbury basin, Ontario, Canada is one of the active mine sites and has been reported to have approximately 50-100 million tonnes of pyrrhotite tailings with 0.6-0.8 wt% of residual nickel [1], [2]. Biomining uses microbial community to develop a self-sustainable mineral extraction process from the tailings [3]. These tailings are host to several microbial communities that thrive under these extreme harsh conditions of acidity, heavy metals toxicity. Enriching and characterising these microbes provide a repertoire of strains with potential for biomining [4]. The most dominant bacteria found in these samples is the genus of *Acidithiobacillus*, a Gram-negative chemolithoautotroph that can thrive in pH as low as 1.5 and high metal concentrations. Recently, a tolerant acidophile, *Acidithiobacillus ferridurans* JAGS (JAGS) was enriched and isolated from one of these tailing samples in our lab, which exhibited potential as host for biomining [5], [6]. Despite the genomic characteristics and features, the native microbes have been found to be constrained by their metabolic capabilities. To establish an efficient biomining process, high performing microbes with efficient iron oxidation rate are necessary because oxidised iron acts as the lixiviant in the leaching process. Metabolic engineering efforts have proved to be successful for other microbes [7], [8], [9], [10], [11]. However, limited studies are available for the acidophilic microbial community [7], [12], [13]. Recently, some studies on isolated gene overexpression such as *rusticyanin* have demonstrated the importance of engineering strategies to improve microbial performance [14].

Acidithiobacillus ferridurans JAGS is capable of sequestering atmospheric carbon dioxide and nitrogen as their carbon, nitrogen source respectively and harness the power of iron/sulfur oxidation to drive their metabolism [5], [7]. While carbon and nitrogen fixation pathways are energy extensive processes, iron oxidation produces ATP. We hypothesized that the increase in demand of energy equivalents will re-wire the microbial metabolism to perform more iron oxidation. Therefore, we have explored different strategies to fine tune the microbial metabolism either by modifying growth conditions or genome engineering. The state-of-the-art CRISPR-mediated genome editing technique [14] was deployed to over-express genes expression by changing the native promoters with in-house characterised synthetic promoters. This presentation will focus on the different strategies and the outcomes with regards to strain performance.

Methods

Materials: Enzymes and kits for cloning work were purchased from New England Biolabs (NEB, Ipswich, MA), and Qiagen (Hilden, Germany). All other chemicals used were of analytical/HPLC grade procured from Sigma-Aldrich (St. Louis, MO). All primers used in this study were ordered from Integrated DNA Technologies (IDT, Coralville, IA). The DNA fragments for constructing the CRISPR-Cas9 plasmid were designed and ordered from Twist Bioscience (San Francisco, CA). The strain of interest *Acidithiobacillus ferridurans* JAGS [5] was isolated and enriched in our lab from an acid mine drainage (AMD) sample obtained from Sudbury, Ontario, Canada. The cargo strain SM10 [15] was used for conjugating plasmids in JAGS. DH5a strain was used as the plasmid maintenance strain.

Strain Growth and maintenance: A. ferridurans JAGS is cultivated either in a shake flask or 48 well microtiter plate (for high-throughput growth) in a 9K medium supplemented with $FeSO_4$ at a temperature of 30 °C with rotation speed of 200 rpm. Single colonies were obtained on 2:2 agar plates with 0.4% agar and appropriate antibiotics. The *E. coli* strains are grown at 37 °C in LB medium with or without appropriate antibiotics.

Strain Construction: Genome editing in JAGS was performed using CRISPR-Cas9 system (Figure 1a), where the pAF plasmid [14] was digested with *ApaI* and *XhoI* and the gRNA and repair template are cloned as a single fragment using either traditional cloning or seamless cloning technique. The constructed CRISPR-editing plasmids were then sequenced for conformation. The confirmed plasmids were first transformed into the conjugal strain (SM10) to be used conjugation in JAGS (Figure 1b). The conjugation protocol is based on a previously published protocol [14]. The single colonies on selective plates were inoculated, grown and confirmed. The strains were then cured of the CRISPR plasmid by multiple streaking on non-antibiotic plates, to either store in a freezer or to make further combinatorial mutants. The strains were stored in 6% Betaine in the -80 °C freezer for long-term storage.

Growth characterisation: The engineered strains and the wildtype JAGS were inoculated from the freezer stock and maintained on a 2:2 agar plate. The cells were grown in a 9K-Fe media in a 48 well microtiter plate at 30 °C incubated in a plate reader for continuous reading at an rpm of 500 (linear shaking). For high-throughput continuous characterisation, OD 400nm was used as a proxy for iron oxidation and the slope calculated was used to determine the rate of oxidation [14]. When cells are grown in flask, we perform the following measurements: OD400nm, pH, ORP, respiration rates (DO levels), cell count under microscope.

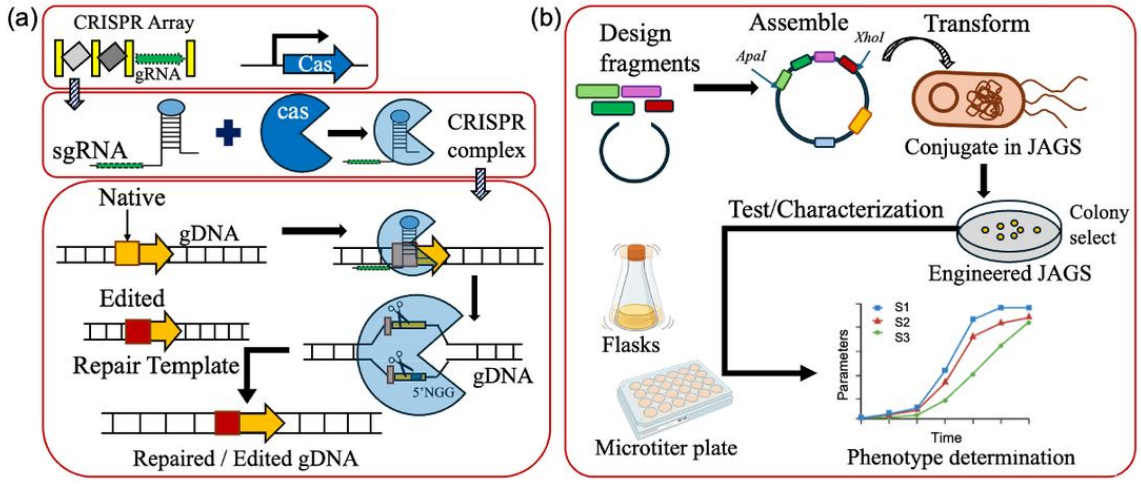


Figure 1. (a) The workflow and mechanism of CRISPR-mediated genome editing. (b) The workflow schematic showing the steps for generating engineered strains of JAGS and the high throughput characterisation to determine the phenotype of the engineered strains

Results and Discussion

A. ferridurans JAGS is a chemolithoautotroph deriving energy from iron oxidation and capable of fixing carbon dioxide and nitrogen. Figure 2a shows the reactions for the pathways of interest and how they interplay. To investigate the effect of nitrogen fixation on iron oxidation, cells were grown with and without the available ammonium salt. The analysis indicated that under nitrogen fixing condition the strain exhibited higher iron oxidation rate (Figure 2b) and respiration rates (Figure 2c) as compared to the ammonium supplemented medium. The higher rates of respiration suggested the probable mechanism for aerobic nitrogen fixation like Rhizobium species [16]. However, the biomass accumulation was observed to be lower under the nitrogen fixing state (Figure 2c), due to more energy being diverted towards fixing nitrogen rather than biomass. To address this biomass issue and further increase the iron oxidation rate the enzymes of the carbon fixation pathway and electron transport chain (ETC) were overexpressed to create several single, double and triple combination strains. While some of the combination strains exhibited improvement, there were a few combinations that showed additional burden of overexpression as compared to wild type strain. The triple combination strain that so far performed the best has an overexpression of the carboxysome and rubisco enzymes of the carbon fixation pathway along with the Cyc2 enzyme of the ETC. This strain AS213 showed 1.9-fold higher iron oxidation rate and 2.3-fold improvement in biomass accumulation as compared to the wild type strain (Figure 2d, e). Further combinations and characterisation are in progress.

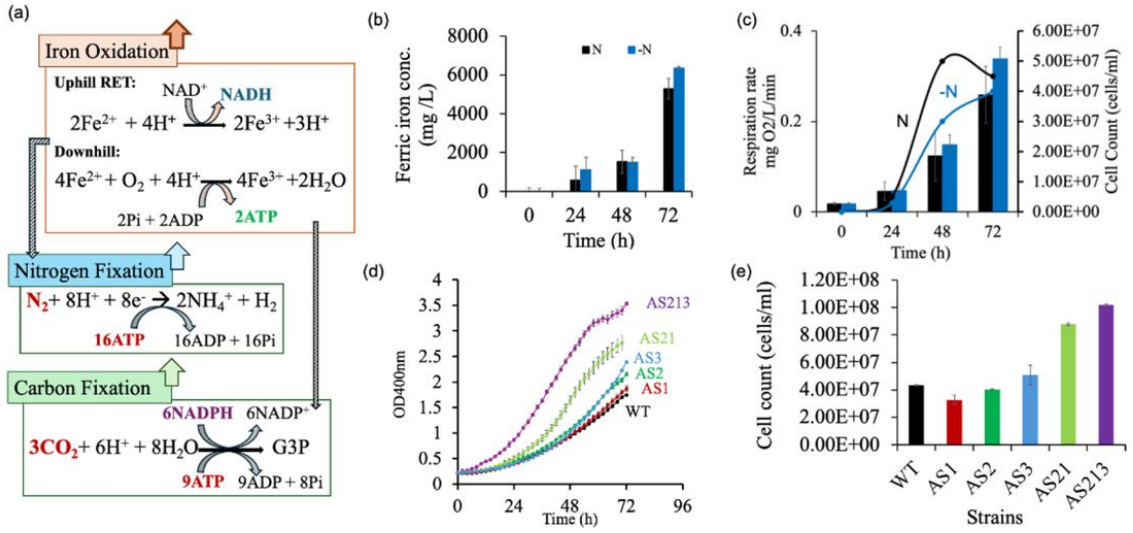


Figure 2. (a) The energy interplay between the three pathways. The effect of iron oxidation when JAGS cells were grown with (N) or without (-N) added ammonium salt. (b) The ferric iron concentration, (c) The respiration rate and cell count under this condition. (d) The Iron oxidation dynamics (OD400nm) of subset of engineered JAGS. (d) The cell biomass accumulation of these strains after a growth of 72h.

Conclusions

This study demonstrates the native metabolic constrains of the wildtype strain can be re-wired either by fine tuning the media composition or targeted genetic engineering for application of interest. Systematic targeted engineering strategies to balance the demand and supply of energy for cellular metabolism have shown to improve both iron oxidation rates and biomass accumulation in *A. ferridurans* JAGS. While not all genetic interventions are expected to produce synergistic benefits, systematic and iterative optimisation can identify combinations that maximise strain performance. Thus, continued advances in strain engineering will pave the way for the development of acidophilic microbial hosts with enhanced performance, enabling next-generation biomining technologies and bridging the gap between fundamental microbial biology and industrial bio-hydrometallurgical applications.

Acknowledgements

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EFFECT OF MICROBIAL ACTIVITY ON LEACHING PERFORMANCE AND PRECIPITATE SORPTION PROPERTY

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Keywords: Bioleaching; Microbial activity; Jarosite; Metal adsorption; Heap leaching

Abstract

With growing interest in bioleaching as a sustainable hydrometallurgy practice and rising global demand for copper, it has become necessary to efficiently optimize the system performance. Metal sorption by secondary precipitates scavenges dissolved metals and adversely reduces bioleaching performance. This study investigated the effect of mesophilic consortia activity on the performance of a typical heap leaching system through a comparative study of copper sorption using jarosite formed from both column bio- and chemical leaching under similar initial solution pH (1.9 and 2.4), temperature (40 ^{o}C) and redox conditions. The obtained jarosite precipitates were characterized using XRD, FTIR, SEM and pH_{pzc} analyses. Microbial activity modified jarosite morphology and pHpzc resulting in higher copper sorption removal of 60.4 – 79.4% within adsorption equilibrium of 80 min at 150 mg/L and 40 ^{o}C. Adsorption data fitted both Langmuir and Freundlich models with higher adsorption capacity obtained for bio-jarosite (21.25 and 14.89 mg/g) than chemical jarosite (19.44 and 13.45 mg/g). Adsorption kinetics aligned well with the pseudo-second order rate expression having high regression coefficients. This indicates a chemical interaction between Cu^{2+} and jarosite with lower activation energy estimations of 15.17 and 33.02 kJ/mol in bio-jarosite than 18.33 and 37.89 kJ/mol in chemical jarosite across 40 – 55 ^{o}C and pH 1.9 and 2.4 respectively. Thermodynamics data obtained show that both adsorption system are endothermic, favourable and non-spontaneous. These findings demonstrate that microbial activity facilitates favorable adsorption pathways, thereby decreasing bioleaching performance.

1 Introduction

Bioleaching of low-grade copper sulfide minerals currently accounts for more than 30% of the worldwide production of cathode copper. The bioleaching dynamics of copper sulfide heap involve multiple industrial procedures, multi-phase media and multi-physics providing intervention opportunities for researchers towards optimizing the process [1]. Bioleaching heap heterogeneity and dynamic conditions, affects microbial community composition, microbial activity and leaching performance. These variations can affect ferrous to ferric cycle which is important for metal dissolution. The activity of microorganisms in bioleaching system can either enhance or inhibit dissolution rate. For instance, increased microbial activity may lead to high ferric concentrations and to maintain ferric-ferrous ion balance, jarosite precipitate forms on the surface of ore particles. While jarosite precipitate is important for microbial attachment and biofilm formation, it limits the penetration of leaching agent and bacteria cells to the mineral surfaces [2]. The accumulation of jarosite over long periods causes blockage of pipes and adsorption of dissolved metals, further compromising system performance [3]. It has been shown through simulation that surface structure of particle influences its adsorption property. The surface structure of jarosite precipitates has been reported to depend on pH, however in bioleaching processes, the operating conditions are a function of the microorganism used. It is not clear if the surface characteristics is a result of the microbial activity or solely dependent on operating conditions. Studies have demonstrated that extracellular polymeric substances (EPS) from microbial activity in bioleaching system, could be adsorbed onto iron precipitates leading to the modification of their surface reactivities [4]. Microbial charged cell walls and EPS consisting of organic acids, enzymes and other biologically reactive compounds,

provides active sites for adsorption and complexation of dissolved aqueous metals [5]. It also induces active jarosite nucleation sites by reducing the activation energy barriers [5]. Besides, the predominant presence of negatively charged functional groups, such as hydroxyl (−OH), sulphate ($-SO_4^{2-}$) and amino ($-NH_2$) groups, on the surface of the jarosite precipitate [6], may increase its ability to interact with protons or metal ions.

The performance of bioleaching of copper sulfide have focused on the effect of microbial consortia, operating conditions and its regulation towards minimizing precipitate formation and efficiently dissolving metals from their parent ores. Biooxidation and jarosite precipitation are concurrent process during bioleaching, and it has been established that jarosite precipitates trap dissolved metals. Hence, this study seeks to access the contribution of microbial activity on the performance of leaching system with regards to precipitate-metal interaction in the system as metals trapped in jarosite are not easily re-dissolved under typical leaching conditions, representing a loss in the overall process.

2 Methodology

Heap bio- and chemical leaching of pyrite were simulated using jacketed borosilicate glass column reactors (260 mm × 40 mm) operated at 40 ºC for 118 days. Columns were aerated at 200 mL/min and fed with leach solution at 1 mL/min at initial solution pH of 1.9 and 2.4. Bioleaching media comprised of 90% Norris growth medium and 10% mesophilic microbial consortium obtained from the Centre for Bioprocess Engineering Research (CeBER) at the University of Cape Town, Cape Town (UCT) in South Africa. The development of bioleaching redox potential was maintained in chemical leaching through gradual addition of 30% H_2O_2 to mimic bioleaching system while isolating microbial effect. The process was repeated in triplicates for reproducibility.

The precipitates obtained after leaching experiments were characterised using XRD, SEM, FTIR and point of zero charge (pH_{pzc}). To access the sorption property of the precipitate, adsorption experiments were performed with 0.5 g precipitate in 50 mL of 150 mg/L Cu^{2+} solution at 40 ºC for 80 min and initial pH of 4 in Erlenmeyer flasks. The adsorbed copper at the end of the experiment was determined by ICP-OES.

3 Results and discussion

The ferric and ferrous concentration in the bio- and chemical leach medium (Fig. 1) were similar to a reasonable extent showing a successful replication of bioleaching in chemical leaching. The XRD analyses (Fig. 2) show that the precipitates formed across all pH levels have identical peaks of well-defined crystalline K-Na jarosite solid solution and minor pyrite and quartz phase existing as impurities from mineral ore. Bioleaching jarosite (BioJar) exhibited lower relative crystallinity and crystallite sizes compared to jarosite obtained from chemical leaching (ChmJar). This is attributable to microbial cells and EPS providing active nucleation sites that favour rapid formation of defect-rich crystallites over well-ordered crystal growth [7]. The FT-IR spectra (Fig. 3) identified vibrational modes corresponding to SO_4^{2-}, H_2O, OH, and FeO_6 groups in jarosite samples. SEM micrographs (Fig. 4) of BioJar showed stacked rose-flower like aggregates interspersed with loosely packed cauliflower-like particles whereas ChmJar comprised of low crystalline assemblages characterized by cubic-shaped structure with sharp edges. Increasing the pH transitioned poorly crystalline aggregates to well-defined crystal structure.

The pH_{pzc} (Fig. 5) values decreased with microbial activity influence for all initial pH considered increasing the negatively charged surface. The smaller pH_{pzc} of BioJar in this study is attributable to microbial modification resulting from partial or total coverage of microorganism during attachment on jarosite surface. This modification brings the precipitate closer to the pH_{pzc} of the present microbial community [8]. It was further confirmed that pH and microbial activity are interrelated as increasing the pH increased the pH_{pzc} which is indicative of the extent of EPS coverage on jarosite precipitate.

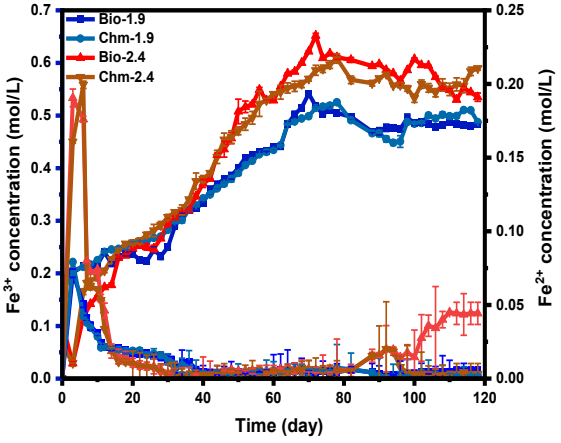


Figure 1. Ferric and ferrous ion concentration at pH 1.9 and 2.4

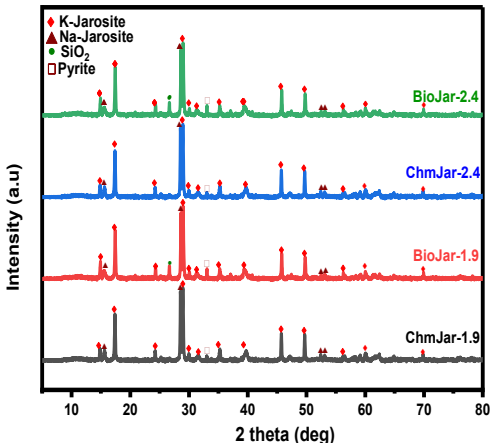


Figure 2. XRD graph of BioJar and ChmJar at pH 1.9 and 2.4

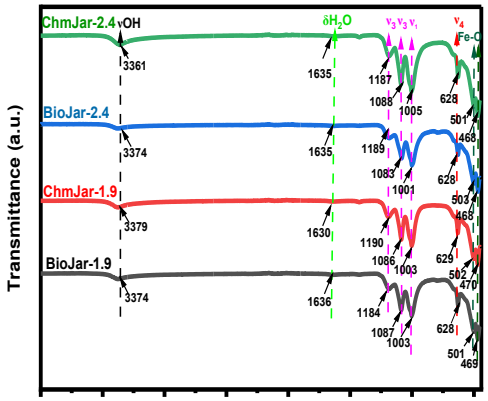


Figure 3. FT-IR of BioJar and ChmJar at pH 1.9 and 2.4

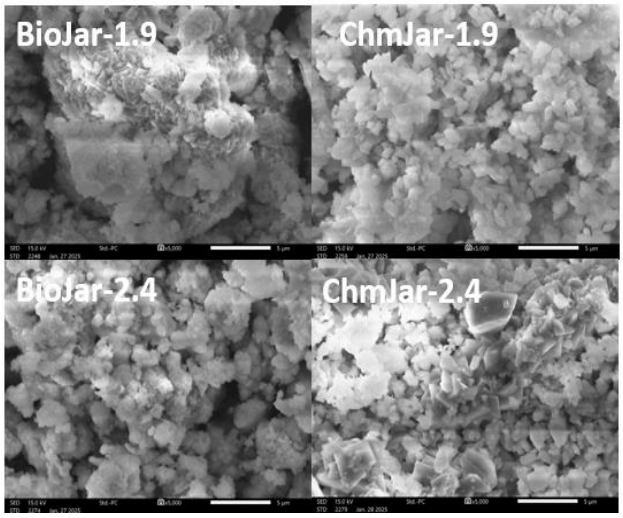


Figure 4. SEM micrograph of BioJar and ChmJar at pH 1.9 and 2.4

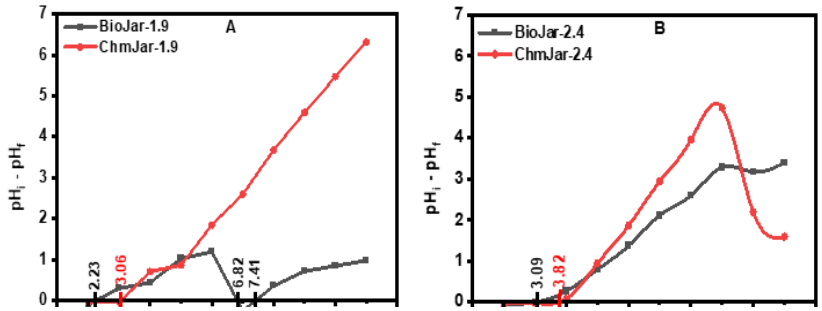


Figure 5. pH at point of zero charge of BioJar and ChmJar at pH 1.9 and 2.4

Table 1: Relative crystallinity and crystallite size of jarosite precipitates at different pH

pH	Relative Crystallinity (%)		Relative crystallite size (nm)	
	BioJar	ChmJar	BioJar	ChmJar
1.9	41.16	54.37	27.47	27.89
2.4	57.35	70.35	22.38	24.39

Adsorption of copper ions proceeded in the order of BioJar and ChmJar formation pH 1.9 > 2.4. At all pH levels, BioJar showed higher monomolecular adsorption capacity over ChmJar. Copper sorption onto BioJar and ChmJar followed pseudo-second model indicating a chemisorption-controlled process. The smaller activation energy of BioJar compared to ChmJar for all initial solution pH, revealed easier and faster adsorption rate requiring less energy. The magnitude and positive ΔH^o for both BioJar and ChmJar depicts a physico-chemical adsorption and an endothermic process resulting in displacement of water molecules by the copper ions [9]. The entropy change observed for both jarosite copper sorption suggests greater randomness at the adsorbate-adsorbent interface. The non-spontaneity and feasibility of the adsorption process is shown in the positive values of ΔG^o while the decrease in ΔG^o with increase in temperature indicates ascend in the rate of adsorption. Lower values of ΔG^o observed for BioJar revealed a significant reduction in the energy barrier required for the sorption to occur compared to ChmJar which is attributable to the structural defect residual in BioJar. Microbial activity introduced more heterogeneous hydroxyl-rich surfaces providing higher energy chemisorption sites that release protons per copper bound unlike ChmJar with lower density of reactive-OH groups.

Table 2: Maximum adsorption capacity and energetic parameter of copper sorption onto BioJar and ChmJar

Jarosite	q_m (mg/g)	E_a (kJ/mol)	ΔH^o (kJ/mol)	ΔS^o (kJ/mol K)	ΔG^o (kJ/mol)			
					313 K	318 K	323 K	328 K
BioJar-1.9	21.25	15.13	21.45	0.060	2.58	2.28	1.98	1.67
ChmJar-1.9	19.44	18.33	23.31	0.057	5.46	5.17	4.89	4.60
BioJar-2.4	14.89	33.02	11.24	0.020	4.86	4.76	4.65	4.55
ChmJar-2.4	13.45	37.89	9.76	0.007	7.53	7.49	7.46	7.42

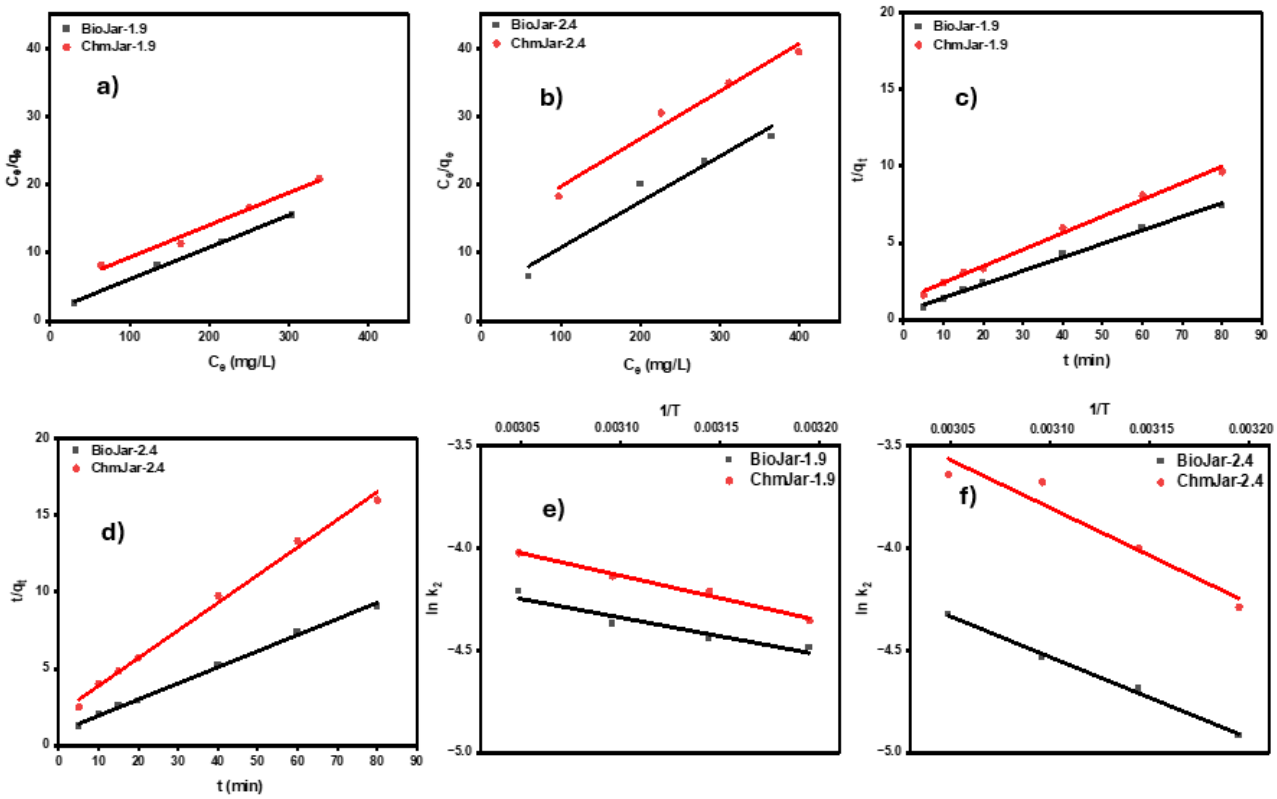


Figure 6. Graph of models of copper sorption onto BioJar and ChmJar at pH 1.9 and 2.4: Langmuir isotherm (a and b), Pseudo-second order (c and d) and activation energy plot (e and f).

4 Conclusion

The precipitates obtained from bio- and chemical leaching were a mixture of K-Na jarosite solid solutions. Microbial activity modified the surface morphology and relative crystallinity of the formed jarosite. The pH_{pzc} results showed that the highly negatively charged BioJar surface enhanced Cu^{2+} adsorption ability due to electrostatic attraction while the less negatively charged ChmJar surface suppressed sorption because of high electrostatic repulsion forces. Copper sorption onto BioJar showed higher monomolecular adsorption capacity than onto ChmJar samples. The thermodynamic analysis showed copper adsorption for both system is a physicochemical, non-spontaneous and endothermic adsorption process. The results presented suggest that microbial modification of jarosite surfaces properties causes a more rapid adsorption resulting from more accessible and reactive sites which reduces overall bioleaching performance.

Acknowledgements

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Managing Iron Precipitation Behaviour in Ferrous Iron Bio-Oxidation Processes and Its Implications for Bioleaching Performance

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Abstract: Ferrous iron bio-oxidation is a critical sub-process governing the efficiency of bioleaching systems, as it drives the oxidative dissolution of many economically important sulfide minerals. Ferric iron precipitation during bio-oxidation is strongly influenced by operational parameters such as pH, temperature, and solution chemistry, and the accumulation of iron precipitates can adversely affect metal recovery due to their surface-driven scavenging behaviour. This study investigates the influence of initial pH and temperature on the physicochemical properties of iron precipitates formed during ferrous iron bio-oxidation. The effect of initial pH was examined in a continuous stirred bioreactor operated at 35 °C using three initial pH values (1.7, 1.9, and 2.2), while the effect of temperature was evaluated in Erlenmeyer flask experiments conducted at a constant initial pH of 1.7 and mesophilic temperatures of 30, 35, and 40 °C. The results showed that jarosite precipitates formed at higher initial pH exhibited increased specific surface areas, ranging from 8.32 to 11.00 $m^2\ g^{-1}$ at 35 °C. In contrast, at constant pH 1.7, increasing the bio-oxidation temperature resulted in comparatively lower surface areas (0.30–0.68 $m^2\ g^{-1}$). These findings indicate that temperature plays a significant role in controlling jarosite surface area and, consequently, its metal-scavenging capacity. It may offer a potential route for improved management of iron precipitation in biohydrometallurgical processes.

Keywords: Bio-oxidation, Iron precipitation, Jarosite, Solution pH, Mesophiles

Incorporation of (K^+, NH_4^+) cations into jarosite phases in conditions similar to those of bioleaching processes

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Abstract: Jarosite-group compounds ($AFe_3(SO_4)_2(OH)_6$ where A is a monovalent or divalent cation) precipitate from iron- and sulfate-rich acidic aqueous solutions(pH 1-3), including those encountered in (bio)leaching systems [1], [2]. Bioleaching media contain cations such as H_3O^+, Na^+, K^+ or NH_4^+, which can incorporate into the A site of the jarosite structure, alone or as solid solutions. The precipitation reactions of jarosite are yet not completely described, especially for (H_3O^+, K^+, NH_4^+) solid solutions at moderate temperature, commonly found in bioleaching processes. Jones et al. demonstrated in biotic conditions that in presence of (K^+, Na^+) or (K^+, NH_4^+) cations, ternary (K, NH_4/Na, H_3O)-jarosite solid solutions precipitate [3], [4]. This work provides an in-depth structural analysis of the products after two weeks but lacks of intermediate data accounting for kinetics of reaction (especially the order of incorporation of the cations). The present work aims at determining whether the cations precipitate simultaneously or if they incorporate one after the other into the jarosite phase.

This work focuses on the experimental characterisation of jarosite precipitation phenomena, in synthetic solutions containing K^+ and NH_4^+. Experiments were performed in a sulfate medium at 42 °C with 0.05 and 0.008 mol/kg_s (kg_s = kg of solution) of NH_4^+ and K^+, respectively, a fixed pH of 1.3 or 2.0 and an iron(III) concentration of 0.1 or 0.7 mol/kg_s. The experiments were monitored using turbidity and targeted element concentrations during the 14-days runs. The intermediate and final solids were characterised by XRD, TGA, SEM, FTIR, K-Fe-S elemental analysis. Results suggest that K-jarosite firstly precipitates and then, NH_4^+ precipitates with similar kinetics. A comparison of the experimental results with thermodynamic computations (Phreeqc software with Thermoddem database) shows that equilibrium is rarely reached in bioleaching environment at moderate temperature and precipitation kinetics are ruling the mechanisms of jarosite solid solutions formation.

Keywords: jarosite, precipitation, kinetics, thermodynamics

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KINETIC CONTROLS ON BIOLOGICALLY MEDIATED IRON OXIDATION AND SECONDARY MINERAL FORMATION IN ACID MINE DRAINAGE

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Keywords: Fe-oxidizing bacteria, secondary iron minerals, metal recovery, mining-influenced water

Abstract

Mining-influenced water (MIW) generated at abandoned mine sites represents both an environmental challenge and a potential secondary source of critical raw materials. This study investigated the biologically mediated oxidation of ferrous iron in MIW collected from the flooded Manó–Gabriela siderite mine in the Slovak Ore Mountains. Batch reactor experiments were conducted under controlled aerobic conditions using indigenous iron-oxidizing microorganisms. The oxidation of Fe^{2+}, changes in physicochemical parameters, dissolved metal concentrations, oxygen consumption, and secondary mineral formation were monitored using spectrophotometric, chemical, and X-ray diffraction analyses. Initial Fe^{2+} oxidation was dominated by abiotic processes; however, following a decrease in pH from 5.6 to 3.5, chemical oxidation slowed considerably, allowing biological oxidation to become the dominant mechanism after an approximately 140 h lag phase. Nearly complete oxidation of 1400 $mg.L^{-1}$ Fe^{2+} was achieved within 190 h. XRD analysis confirmed the formation of jarosite with minor schwertmannite. Importantly, dissolved concentrations of Mn, Ni, and Co remained essentially unchanged, indicating selective iron removal while retaining valuable metals in solution. These findings demonstrate that biologically mediated iron oxidation represents a promising pre-treatment strategy for MIW, enabling the recovery of critical raw materials through subsequent downstream processing while reducing iron-related environmental impacts.

Introduction

Slovakia has a long and distinguished mining history that has substantially contributed to the economic development of several regions. However, the decline of the mining industry following the political and economic transition in the 1990s resulted in the closure of numerous ore mines and left behind a notable environmental legacy [1]. Slovakia contains nearly 17,000 old mining works, including tunnels, shafts, heaps, sludge ponds and other works. Many of them pose environmental challenges due to the generation of mining-influenced water (MIW) [2,3]. MIW is increasingly being recognised not only as an environmental burden but also as a potential secondary resource containing valuable and critical raw materials, thereby supporting the transition towards a circular economy [4].

The mine water investigated in this study originates from a flooded siderite mine located in the Spiš–Gemer Ore Mountains. It is one of the most important historical mining districts in Slovakia, with notably MIW sites such as Smolník and Nižná Slaná [1]. The MIW is highly supersaturated with respect to ferric iron minerals and represents an appropriate model water sample for investigating biologically mediated iron oxidation [5]. This study focuses on the initial stage of bacterial Fe^{2+} oxidation under acidic conditions and the subsequent formation of secondary iron minerals. The biological process promotes the selective precipitation of ferric iron, while maintaining divalent metals

such as Mn^{2+}, Ni^{2+}, Co^{2+}, and Mg^{2+} in solution. This selective partitioning reduces sludge production and enables subsequent recovery of critical raw materials.

Methods

Materials

Mine water samples are originated from Slovak Ore Mountains. The mine water was poured into three sterile 500 mL magnetically stirred reactors equipped with the pH and oxidation–reduction potential (ORP) probes. Air supplied to the reactors was sterilised by filtration through 0.2 µm membrane filters. All experiments were conducted at 30 °C, corresponding to the in-situ temperature of the mine water at the time of sampling.

Analysis

Concentration of metals in mine water was determined using atomic absorption spectrometer VARIAN AA240FS. Sulfate, magnesium, calcium and potassium concentrations were analysed by ion chromatography Dionex ICS 5000. Ferric iron was determined by a UV-spectrophotometric method at 300 nm [6]. Ferrous iron concentrations were determined using a modified o-phenanthroline spectrophotometric method insensitive to Fe^{3+} interference [7]. Physical-chemical parameters, such as pH and ORP were measured using probes Radiometer Analytical/HACH Lange for pH and Mettler Toledo InLab Redox-L type for ORP. Gas analysis, in term of inlet and outlet of O_2 and CO_2 measurements, were carried out using a paramagnetic oxygen analyser and an infrared carbon dioxide analyser (Sable Systems, USA), respectively. The analysers were calibrated using high-purity nitrogen (99.99% N2) for zero point and ambient outdoor air for span calibration of O_2 (20.95%) and CO_2 (0.042%) concentrations. Air of the same quality was used to aerate the reactors. The data acquisition was performed by a software application developed in LabVIEW ® (National Instruments).

The sludge was characterised by X-ray diffraction (XRD) using a D8 Advance diffractometer (Bruker AXS, Germany). The diffraction data were interpreted using X'Pert HighScore Plus 2.0a (version 2.0.1) software. The measured diffractograms were further processed using Diffracplus Basic and Microsoft Excel. Mineral abbreviation was supported by Warr [8].

Results and Discussion

The mining-influenced water used in this study originated from a flooded siderite mine located in the Slovak Ore Mountains. The model mine water was characterised by pH of 5.62 and a high dissolved iron concentration (1,400 $mg.L^{-1}$). In addition to iron, the water contained elevated concentrations of potentially toxic metals and metalloids, including Mn, Co, Ni, As, Zn, and Cu. The concentrations of the monitored elements are summarised in Table 1.

Table 1. Characterisation of mining-influenced water

Parameter	Units	Value	Parameter	Units	Value
SO_4^{2-}	$mg.L^{-1}$	29700	As	$mg.L^{-1}$	12.7
Na^+	$mg.L^{-1}$	60.2	Co	$mg.L^{-1}$	0.34
K^+	$mg.L^{-1}$	89.9	Ni	$mg.L^{-1}$	2.7
Mg^{2+}	$mg.L^{-1}$	5500	Zn	$mg.L^{-1}$	0.88
Ca^{2+}	$mg.L^{-1}$	490	Cu	$mg.L^{-1}$	0.77
Mn	$mg.L^{-1}$	92.8	pH	$mg.L^{-1}$	5.62
Fe	$mg.L^{-1}$	1140			

The model mine water was treated under aerated conditions. During the initial stage of the experiment, ferrous iron was predominantly oxidised through abiotic processes. However, the rate of chemical ferrous iron oxidation is strongly pH-dependent [9,10], following decrease in pH from 5.62 to 3.5 substantially reduced the oxidation rate after 140 h. The onset of exponential growth of indigenous iron-oxidising bacteria was observed up 140 h h after the mine water equilibrated with atmospheric conditions (Fig. 1a). Prolonged bacterial lag phase was observed in reactor R5. Consequently, the dissolved ferrous iron was completely oxidised from initial concentration of 1140 $mg.L^{-1}$. The bacteria

completely oxidised ferrous iron to ferric iron within the next 48 h. The exponential increase in O_2 consumption rate (Fig. 1a) accompanied by CO_2 consumption, indicates the activity of indigenous autotrophic iron-oxidizing bacteria naturally present in mine water [5].

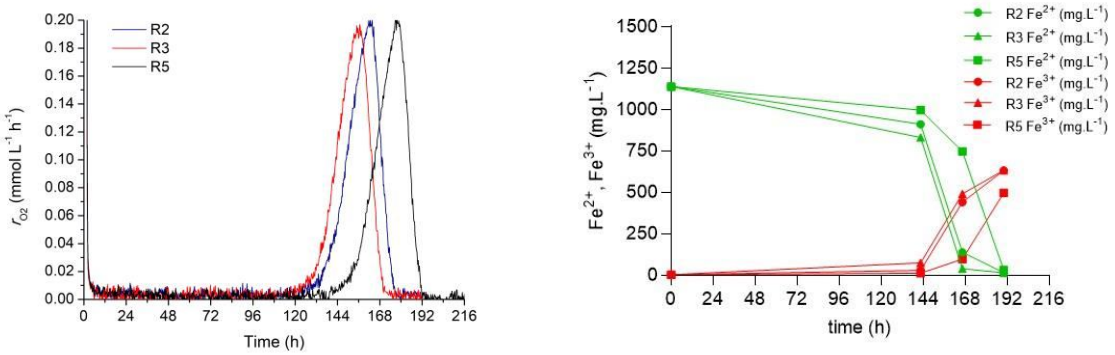


Figure 1. *Oxidation of ferrous iron in the mine water under aerobic conditions. A) O_2 consumption rate and cumulative O_2 consumption related to Fe^{2+} oxidation. Chemical iron oxidation was followed by increased O_2 consumption due to exponential growth of indigenous Fe-oxidizing bacteria. B) Concentrations of Fe^{2+} and soluble Fe^{3+} in the water.*

Hydrolysis of ferric iron resulted in drop of pH to value 2.56. Under acidic conditions, ferric iron precipitation was less pronounced due to hydrolysis reactions were limited [5]. Fraction of the oxidised iron precipitated as secondary iron minerals. X-ray diffraction (XRD) analysis confirmed the formation of jarosite ($KFe^{3+}_3(SO_4)_2(OH)_6$) as the dominant mineral phase (Fig. 2). The formation of jarosite (Fig. 2) was promoted by the addition of K_2SO_4. However, detail characterisation of the sludge morphology and mineralogical composition is still ongoing.

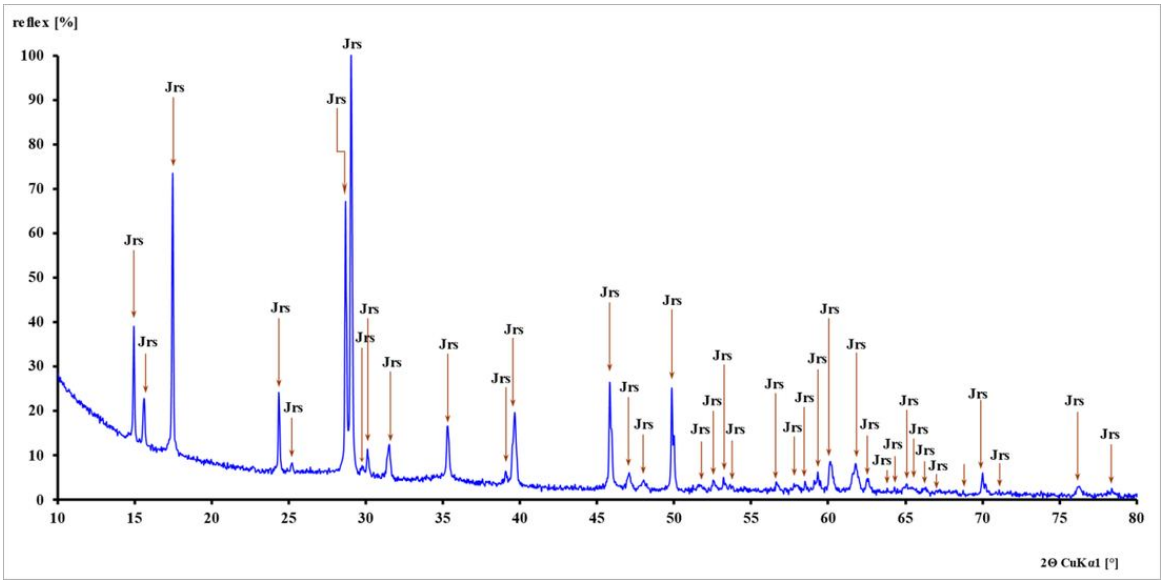


Figure 2. *XRD detection of jarosite Jrs - jarosite $KFe^{3+}_3(SO_4)_2(OH)_6$ formation after artificial increase of K^+ and SO_4^{2-} ions in the solution and equilibration.*

Selective biological oxidation of ferrous iron resulted in the precipitation of secondary iron minerals without notably affecting the dissolved concentrations of Mn^{2+}, Ni^{2+}, and Co^{2+}. Consequently, these valuable metals remained available for further recovery processes, demonstrating the potential of biological iron oxidation as an effective pre-treatment technology.

Conclusions

Biologically mediated oxidation of ferrous iron proved to be an effective pre-treatment strategy for mining-influenced water from the flooded siderite mine. Following the initial abiotic oxidation stage,

indigenous iron-oxidizing bacteria promoted efficient ferrous iron oxidation and the precipitation of secondary iron minerals, predominantly jarosite with minor schwertmannite. Importantly, valuable divalent metals, including Mn, Ni, and Co, remained dissolved, demonstrating the potential of this approach for selective iron removal while preserving critical raw materials for subsequent recovery. Further research will focus on optimising continuous reactor operation and developing other technologies for the recovery of dissolved critical metals.

Acknowledgements

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Electro-assisted biostimulation of a native microbial community from cobalt-bearing pyrite tailing: Effect of an iron-restricted culture medium on copper and cobalt recovery

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Abstract:

Chile is the world's leading copper producer, and mining is the country's main economic activity. However, mining generates around 900 million tonnes of mine waste per year, better known as tailings, and its disposal causes environmental problems and health risks. The energy transition to renewable sources has increased demand for cobalt, a key resource for lithium batteries. It is estimated that tailings from Chile's second region alone represent a potential extraction of 17,000 tonnes of cobalt. Therefore, bioleaching is a sustainable and efficient technology for the revaluation of tailings and the recovery of critical metals.

This study investigated biological leaching through biostimulation using an electroactive-system process (BES) to eliminate the need for aeration. Control was provided by a known microbial community. The efficiency of copper and cobalt recovery was evaluated using a restrictive 0K culture medium to validate the use of iron compounds from pyrite tailing as an energy source.

Metal recoveries were determined using X-ray fluorescence (XRF) analysis. The native microbial community of the tailings at BES-0K achieved the best performance, recovering 34% of cobalt and 79% of copper, while at BES-9K it recovered 16% of cobalt and 76% of copper. The control community was similar, with BES-0K recovering 25% of cobalt and 79% of copper, while BES-9K recovered 10% of cobalt and the same amount of copper.

The microbial characterisation showed that the electroactive-system stimulated the dominance of *Brevundimonas*, whose relative abundance reached 48% in the native communities and a slightly lower in the controls. The second (8%) and third (4%) taxonomic dominance was alternately occupied by the genera *Enterobacter* and *Fibrobacter*, whose variability in subdominance depended directly on the study condition. It has been established that the biostimulation of native communities from tailings allowed valuable metals, such as copper and cobalt, to be recovered from mining waste.

Keywords: bioleaching, native-bacteria, metal-recovery, bio-electrochemical system, iron oxidising metabolism.

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Towards 2050 Climate Goals: Selective recovery of copper from low-grade tailings through bioleaching

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Abstract: The increasing demand for strategic and critical metals, particularly copper (Cu), driven by the global energy transition toward climate neutrality targeted by the European Union, underscores the need to explore alternative sources beyond primary ores. Recovering metals from mine tailings generated by primary ore processing has attracted attention as a potential solution, as the latest data indicate that 46 million tonnes of Cu are sitting idle in waste dumps at mines worldwide [1]. However, the low-grade nature and heterogeneity of tailings make sustainable and economically viable metal recovery strategies challenging. Bioleaching, which involves the metabolic activity of microorganisms to solubilize metals, offers a cost-effective and environmentally sustainable alternative to conventional processing routes [2,3]. This work aims to take the first steps toward the selective recovery of Cu from low-grade mine tailings through bioleaching using an acidophilic bacterium. Attention is given to the role of biofilm formation and extracellular polymeric substances (EPS) production on the mineral surface, and to their influence on Cu extraction efficiency. Preliminary experiments were performed at a a pulp density of 1.25% (w/v) for 30 days under controlled temperature and agitation conditions, using *Acidithiobacillus ferridurans*, well known for its Fe^{2+}-oxidizing and sulfur-oxidizing capabilities in bioleaching. The results reveal positive bacterial activity and progressive Cu solubilization, with high extraction efficiencies in biofilm-based bioleaching assays, suggesting that the colonization of mineral surfaces by biofilm-forming cells contributes to improved leaching kinetics and, consequently, high bioleaching extraction efficiency.

Keywords: Bioleaching; biofilm formation; circular economy; copper; *Acidithiobacillus ferridurans*

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Exploring flame spherodisation as a pre-treatment for bioleaching of Li-rich mine waste

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Abstract: The rapid growth in global lithium demand, driven by electric vehicles and renewable energy storage, has exposed environmental and geographical constraints associated with conventional lithium extraction from brines and hard rock deposits. These routes are energy- and water-intensive, motivating the development of more sustainable alternatives. Lithium-bearing micas such as lepidolite and zinnwaldite are abundant but challenging resources, as lithium is strongly retained within their layered aluminosilicate structures, requiring extensive processing for release. This study investigates an integrated material-processing and biohydrometallurgical strategy to enhance lithium recovery from mica minerals using flame spheroidisation (FS) and microbial leaching.

Flame spheroidisation converts platy mica particles into microspherical forms while partially amorphising the crystal structure, thereby increasing mineral reactivity and leachability. FS-treated mica (MicaMS) and unprocessed mica were evaluated in two bioleaching systems: (i) a native heterotrophic microbial consortium enriched from mica material, and (ii) the acidophilic sulfur-oxidising bacterium *Acidithiobacillus caldus*. Heterotrophic bioleaching was conducted in shaking flasks at 30 °C and 2% pulp density, where pH decreased from neutral to ~3.0 within three days. Under these conditions, MicaMS yielded lithium concentrations up to 35 mg L^{-1}, nearly two orders of magnitude higher than untreated mica, although overall recovery remained modest (~8%).

In contrast, a two-step bioacid leaching approach using biogenic sulfuric acid generated by *At. caldus* achieved substantially higher performance, with ~50% lithium recovery at pH 1.0 and up to 90% at pH 0.7 within 24 h. SEM–EDX analysis revealed limited surface alteration following heterotrophic leaching, whereas acidophilic leaching induced extensive surface cracking correlated with enhanced dissolution. Overall, this study demonstrates that flame spheroidisation combined with targeted microbial processes can significantly improve lithium mobilisation from mica under environmentally compatible conditions.

Keywords: lithium recovery, bioleaching, flame spheroidisation, mica microspheres, biogenic acids

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Cobalt Recovery from Mine Tailings via Electro-assisted Bioleaching using Bacterial Community

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Abstract:

The RDC dominates global cobalt production; however, persistent socio-environmental challenges bring significant supply risks. In contrast, Chile generates approximately 537 Mton/yr of mining tailings containing metals of interest, such as copper and cobalt. In parallel, global cobalt demand is projected to increase 68% by 2030, driven by the electromobility industry. Although bioleaching is a promising recovery technology, conventional approaches are limited by long processing times (~9 months) and aeration requirements. In this context, electro-assisted anoxic bioleaching emerges as an innovative alternative for mineral recovery without oxygen supply.

This study evaluates cobalt recovery from tailings using electro-assisted anoxic bioleaching using a acidophilic bacterial community.

Experiments were conducted in 500 mL Schott bottles under aerobic, anoxic, and bioelectrochemical (BES) conditions at 30 °C, 350 rpm, 5% pulp density, and a 0.4 ΔV. Ferrous iron (Fe^{2+}) concentration was monitored as an indicator of microbial oxidative activity. In BES reactors, Fe^{2+} decreased by 27.5% after 28 days, and the final Fe^{2+}/Fe^{3+} mass ratio approached unity, indicating sustained availability of both redox species. This balanced redox environment favors microbial metabolic flexibility, allowing iron to function effectively as either an electron donor or acceptor. In contrast, aerobic and anoxic reactors exhibited pronounced Fe^{2+} losses (73% and 98%, respectively), likely enhanced by jarosite precipitation, which limits iron availability in solution.

Cobalt solubilisation increased in all systems, with aerobic reactors achieving the highest concentrations (1467.1 ± 151.2 mg/L), followed by BES (685.7 ± 174.6 mg/L) and anoxic reactors (247.6 ± 79.3 mg/L). Notably, BES achieved nearly threefold higher cobalt recovery than anoxic conditions. Microbial community analysis revealed that BES imposed a strong selective pressure, leading to marked shifts in community composition, likely favoring electroactive microorganisms such as *Brevundimonas* and *Enterobacter*. These results position electro-assisted anoxic bioleaching as a robust and sustainable alternative for cobalt recovery from secondary resources.

Keywords: Bioleaching; Mine Tailings; Cobalt Recovery; Bioelectrochemical Systems; Electroactive community.

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Scale-up and demonstration of bioleaching to recover critical raw materials from sulfidic tailings

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Abstract

Several billions of mining waste (MW) are disseminated as stocks and flows in mining countries causing high environmental and stability risks. Rehabilitation operations are now considering the integration of MW into their scenarios in order to recover remaining valuable raw materials. Among techniques that could be applied, biohydrometallurgy is an interesting option to leach critical raw materials (CRM) and/or desulfurize fine grained tailings.

EU-funded RAWMINA project aims to evaluate the whole value chain to recover CRM and other products from three sulfidic MW containing Cu, Co, Sb and W. Technical steps included (i) MW sampling and pre-treatment (crushing/grinding, gravimetry concentration), (ii) bioleaching in stirred-tank reactors, (iii) valorization of Fe and (iv) recovery of CRM through innovative development of nanofibers.

BRGM was involved in the first two steps. Bioleaching scale-up experiments were performed from lab scale (flask, 2 L reactor) to continuous pilot scale (114 L) with the acidophilic BRGM-KCC consortium, composed mainly of *Leptospirillum ferriphilum, Acidithiobacillus caldus* and *Sulfobacillus* spp. Influence of mesophilic to moderate thermophilic temperatures (38°C-45°C) was tested, as well as pH control using two types of alkaline reagents (calcite and basic Mg carbonate). The influence of air supplementation with CO_2 and O_2 on the kinetics was studied.

The best bioleaching performances were obtained at 42°C and thermodynamic considerations confirmed the interest of this moderate working temperature to treat materials with very high content of Fe-sulfides (several tens %) and reduce temperature fluctuations harmful to microbial consortium and bioleaching yield.

pH control with basic Mg carbonate did not impact microbial activity and facilitated the downstream recovery of Fe as a valuable product. CO_2 concentration significantly influenced the leaching kinetics and the overall design of bioleach unit operation. Without CO_2 supplementation, the residence time in the primary bioleaching reactor must be increased from two to four days to maintain the final metal recovery close to 90%.

Keywords: bioleaching, sulfides, cobalt, copper, tungsten, microorganisms

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Modelling of a Continuous Pilot-Scale Pyrrhotite Bioleaching System for Nickel and Sulfur Recovery

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Keywords: bioleaching; bioremediation; pyrrhotite; process modelling; process optimisation

Abstract

Decades of nickel mining in Sudbury, Ontario, Canada have resulted in over 100 Mt of pyrrhotite tailings containing ~0.6-0.8 wt% nickel. Bioleaching has the potential to recover this nickel at a lower cost and greater efficiency than traditional hydrometallurgical techniques. However, optimised process conditions are required for economic feasibility. A process model is a necessary tool for process optimisation and economic assessment. In this work, we present a bioleaching process model that accounts for mineral dissolution, microbial growth and oxidation of ferrous iron and elemental sulfur, and gas transfer. Mineral dissolution is modelled by shrinking core kinetics. Microbial activity is modelled using Monod kinetics; it is found that a product inhibition parameter for ferric iron is necessary. The fitted parameters suggest that microbial populations experience a ~10-fold reduction in activity under real bioleaching solution conditions compared to experiments with synthetic bioleaching solutions containing no solids.

Introduction

Bioleaching is a promising technology for the processing of tailings from the mining industry due to its low cost and the potential for value recovery from the tailings. To this end, a group at the University of Toronto is investigating the scale-up of a bioleaching process for the treatment of Ni-bearing pyrrhotite tailings in Sudbury, Ontario. The process chemistry is summarised in Table 1.

Table 1. Biotic and abiotic conceptual reactions modelled and their stoichiometry

Reaction	Stoichiometry
R1: Ferric attack [1]	$FeS_{(s)} + 2Fe^{3+}_{(aq)} \rightarrow 3Fe^{2+}_{(aq)} + S^0_{(s)}$
R2: Ferrous bio-oxidation [1]	$2Fe^{2+}_{(aq)} + 0.5O_{2(aq)} + 2H^+_{(aq)} \xrightarrow{\text{microbes}} 2Fe^{3+}_{(aq)} + H_2O_{(l)}$
R3: Sulfur bio-oxidation [1]	$S^0_{(s)} + 1.5O_{2(aq)} + H_2O_{(l)} \xrightarrow{\text{microbes}} SO^{2-}_{4\ (aq)} + 2H^+_{(aq)}$
R4: Ferric hydroxide precipitation [1]	$Fe^{3+}_{(aq)} + 3H_2O_{(l)} \rightleftharpoons Fe(OH)_{3(s)} + 3H^+_{(aq)}$
R5: Oxygen dissolution	$O_{2(g)} \rightarrow O_{2(aq)}$

The above chemistry requires modifications from the commercially successful BIOX™ process. The latter pre-treats refractory gold ores, whereas our modified BIOX™ process aims to recover low-value Ni and elemental sulfur while remediating the tailings from their AMD potential [2].

Many works in the bioleaching modelling literature address the subprocesses of bioleaching separately. For example, some works give careful consideration to the evolution of particle size distribution (PSD) but are decoupled from a global mass balance [3]. Others include a global mass balance to describe the kinetics but simplify the dissolution of the mineral as a homogeneous reaction rather than a heterogeneous one that depends on the PSD [4]. By contrast, Crundwell reported a model that includes

global mass balances and mineral dissolution kinetics accounting for PSDs [5]. We follow the Crundwell approach.

In this work, a process model variant for pyrrhotite bioleaching is validated on its aqueous iron concentration prediction ability in a case with no ferric hydroxide precipitation in a hypothetical case with pH less than 2.

Methods

The process model consists of a component *i* mass balance for reactions *j* around each reactor in the cascade as shown in Eq. (1). The concentration of each component in each reactor is determined by a least squares optimiser until its mass balance closes.

$$\frac{dn_i}{dt} = \sum_{\text{in}} \dot{n}_\iota - \sum_{\text{out}} \dot{n}_\iota + \sum_j \nu_{ij} R_j = 0 \tag{1}$$

The composition in the feed to the first reactor in the cascade is fixed, and the resulting slurry of the first reactor defines the composition of the inlet to the second reactor. In this way, the mass balance is solved sequentially for every reactor in the cascade.

The need to control elemental sulfur production requires two microbial populations be included in the model: sole sulfur oxidisers (X_{S^0}) and sole iron oxidisers (X_{Fe}). Gangue minerals are considered inert and therefore do not participate in the mass balance. The reactive, Ni-bearing phases consist of pyrrhotite (Po) and pentlandite (Pn). Ni reports roughly equally between the two phases even though Pn makes up <2 wt% of the overall mineral mass [6]. However, because of the similar leaching kinetics and elemental makeup of Po and Pn, we treat them both as a single phase for mass balance calculations [7]. The addition of sulfuric acid for pH control is considered in the mass balances.

The rate equations used are shown in Table 2. The bio-oxidation of ferrous iron and elemental sulfur are modelled according to Monod kinetics. For the iron oxidisers, an inhibition factor for ferric iron was necessary. The kinetics of precipitation of ferric hydroxide are modelled using a driving force based on the degree of supersaturation of ferric iron. Lastly, the dissolution rate of oxygen from the gas into the aqueous phase is based on the mass transfer coefficient $k_L a$.

The yield parameters are $Y_{X/S,Fe}$= 0.288 g biomass/mol Fe^{2+} oxidised (experimentally determined [8]) and $Y_{X/S,S^0}$ = 3 g biomass/mol S^0 oxidised (estimated from the thermodynamic electron equivalents model [9]). The terms $k_{d,Fe}$ and k_{d,S^0} represent cell decay and were selected to be 5% of μ_{max} for each microbial population.

Table 2. Biotic and abiotic reactions modelled and their rate equations

Reaction	Rate equation ($mol \cdot m^{-3} \cdot s^{-1}$)
R1: Ferric attack	Shrinking core model – see below.
R2: Ferrous bio-oxidation [10]	$\mu_{Fe} = \mu_{max,Fe}\left(\frac{[Fe^{2+}]}{[Fe^{2+}]+K_{s,Fe}}\right)\left(\frac{K_{i,Fe}}{[Fe^{3+}]+K_{i,Fe}}\right) - k_{d,Fe}$ (2) $r_2 = \mu_{\mathrm{Fe}} \cdot [X_{\mathrm{Fe}}] \cdot Y^{-1}_{X/S,\mathrm{Fe}}$
R3: Sulfur bio-oxidation [10]	$\mu_{S^0} = \mu_{\max,S^0}\left(\frac{[S^0]}{[S^0]+K_{s,S^0}}\right) - k_{d,S^0}$ (3) $r_3 = \mu_{S^0} \cdot [X_{S^0}] \cdot Y^{-1}_{X/S,S^0}$
R4: Ferric hydroxide precipitation [11]	$r_4 = k_{ppt}\left(1 - \frac{Q}{K_{sp}}\right)$ (4)
R5: Oxygen dissolution [12]	$r_5 = k_L a\left([O_2^*] - [O_2]\right)$ (5)

Based on previous experimental results [13], the surface reaction-controlled version of the shrinking core model was selected (Eq. (6)).

$$k_s = \frac{a}{b}\frac{MW_A}{\rho_A D_0} k' C_B^n$$
$$x(D_0, t) = 1 - (1 - k_s t)^3 \quad (6)$$

Eq. (6) describes the leaching of a monosized particle (A) population with initial diameter D_0 by a lixiviant (B) over time. The residence time distribution (RTD) and particle size distribution (PSD) were accounted for to calculate the conversion and the PSD of the leached particles entering the next reactor[14]. The reaction order is $n=0.47$, and the PSD of the feed particles was modelled by the Rosin-Rammler distribution (Eq. (7)) with $\bar{D}=19.2\cdot10^{-6}$ m and $s=0.899$, based on experimental measurements [13].

$$f(D) = \frac{s}{\bar{D}^s} D^{s-1} exp\left[-\left(\frac{D}{\bar{D}}\right)^s\right] \quad (7)$$

Experimental data from a continuous reactor cascade was used to fit the model parameters. The surface reaction constant in Eq. (6), k', was fitted using a fixed, known steady-state concentration and experimental conversion value. Then, the Monod parameters $\mu_{\max,Fe}$, $\mu_{\max,S^0}$, $K_{s,Fe}$, K_{s,S^0}, and $K_{i,Fe}$ were fitted to minimise the residuals between experimental and predicted steady-state concentrations.

Results and Discussion

Validation data were sourced from a benchtop cascade consisting of four reactors (primary reactor P1, 7.5L; secondary reactors S1-S3, 2.5L each). The model produced good agreement with experimental measurements (Figure 1).

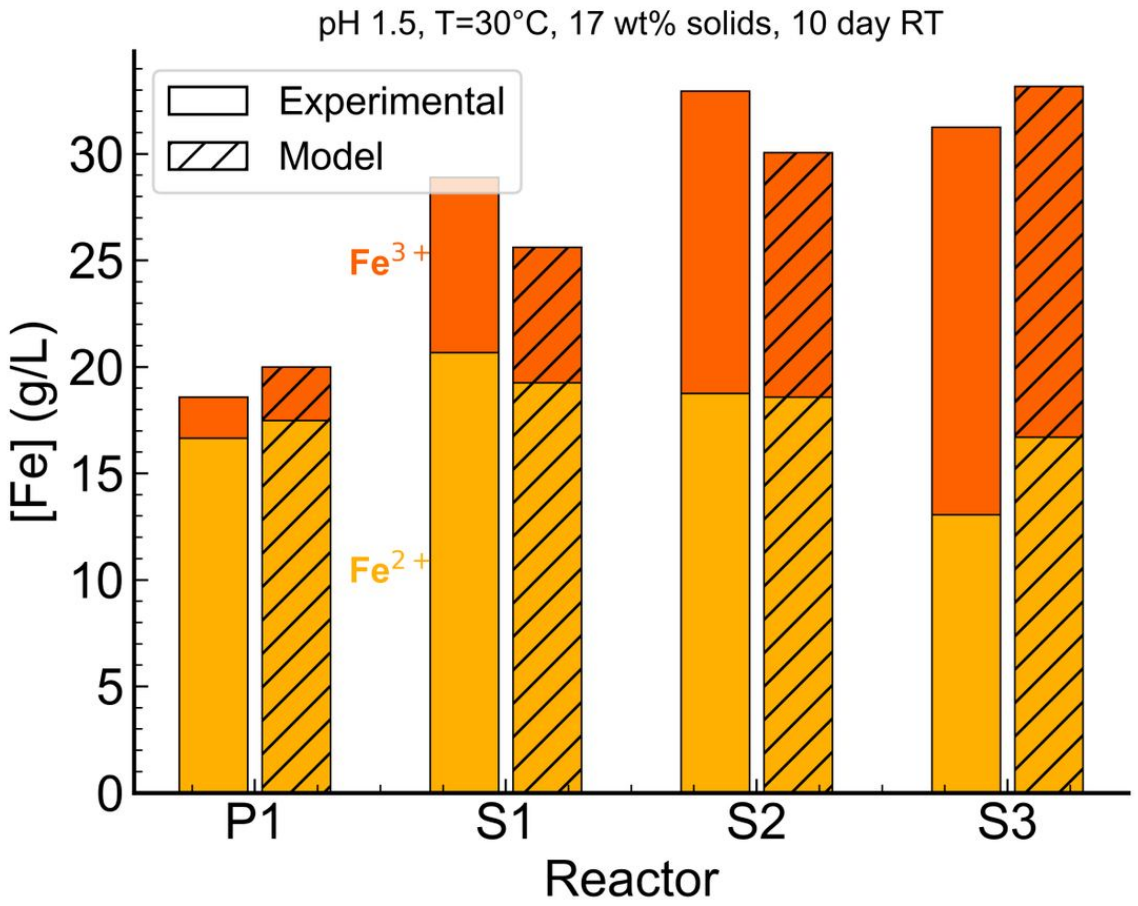


Figure 1. Comparison of model predictions and experimental data. Steady-state concentrations of Fe^{2+}, Fe^{3+}, H^{+} (measured), and SO_4^{2-} (calculated) were used to fit model parameters.

The parameters fitted from this model suggest slower growth of iron oxidising microbes in a 17% w/w solids feed slurry than in a synthetic $FeSO_4$ solution with no solids [8]. The value of μ_{max} decreases approximately 4-5-fold to 0.019 h^{-1}. Similarly, K_s increases by ~1.5-6x; increases in K_s correspond to slower growth at the same substrate concentration.

Conclusions

A first-principles model for continuous bioleaching of pyrrhotite tailings using a BIOX™ reactor configuration was developed and compared against experimental data. The kinetics of dissolution were represented by the shrinking core model, while microbial activity was modelled using Monod rates. The model includes separate iron and sulfur oxidising microbial populations. A comparison of the fitted Monod parameters in experiments with and without slurry suggest that the introduction of slurry slows microbial activity. Future work will use the present model to analyse a wider range of operating conditions. In addition, the inclusion of biological data from an ongoing pilot (cell counts and relative abundance of different species) will provide more insights for model fitting and validation.

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Column bioleaching of a low-grade Cu-Ni ore: a mineralogical perspective

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Abstract:

The heap bioleaching of mine wastes supposes a challenge due to their complex mineralogy. During the NEMO project, bioleaching efficiency of a low-grade Ni-Cu ore (also referred to as secondary ore), produced as residue from a primary leach stage, was assessed. This secondary ore comprised 3.19% sulfides as pyrite, pyrrhotite, sphalerite, chalcopyrite, pentlandite and violarite. Rapid dissolution of metals was observed during the first days of ore irrigation (before inoculation) in laboratory-scale column experiments. The initial irrigation generated high metal containing solutions (e.g., up to 18 g/L Al, 60 g/L Fe, 8 g/L Mn, 6 g/L Ni, 8 g/L Zn) which was expected to be severely inhibitory to microbial activity and cell growth if experiments had been inoculated on day zero, prior to initial irrigation of the ore. This high metal release suggested the presence of a highly-soluble metal-containing mineral fraction not identified by quantitative mineralogical analysis of the secondary ore. In order to characterise the secondary ore, in terms of acid/water soluble and more refractory fractions, a seven-step sequential extraction of the secondary ore was performed. This showed high metal concentration in the water/acid leach fraction (easily-soluble salts) which accounted for ~34% Co, ~22 % Cu, ~36% Mn, ~65 % Ni, ~34 Zn. The mineralogical analysis determined by QEMSCAN® did not show easily soluble salts, explained by dissolution during sample preparation. The combination of QEMSCAN® and sequential extraction analyses provided valuable data to understand the behaviour of the secondary ore during (bio)leaching. This methodology is of particular relevance when considering (bio)leaching of mine wastes with complex mineralogy, where the results of analysis may be used to inform (bio)leaching strategies to treat such material. A two-stage process may be more suitable when dealing with mineral containing easily-leached fraction, where the first step consists on acid leaching of the easily-soluble fraction and followed by bioleaching targeting more refractory sulfide minerals.

Keywords: mineralogy, pyrite, metal sulfides, bioleaching, secondary ore

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Bioreductive Pre-treatment of Low-Grade Nickel Laterites Using *Shewanella oneidensis* for Enhanced Nickel and Cobalt Recovery

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Abstract: The ever-increasing demand for electric vehicles and a global push for a green energy transition has placed considerable pressure on critical battery metals such as nickel and cobalt. With current resources unable to match projected demand due to depletion of high-grade sulfide ores, attention has diverted to low-grade nickel laterite ores which present considerable mineralogical, economic and environmental challenges for conventional techniques. Therefore, sustainable alternatives must be developed for eco-efficient nickel and cobalt extraction from these low-grade deposits.

This study investigates the application of Shewanella oneidensis MR-1 as a Dissimilatory Iron-Reducing Bacteria (DIRB) in a bioreductive pre-treatment to combat mineralogical challenges and enhance nickel and cobalt recovery from Australian limonitic and saprolitic laterite ores. Early characterisation of the raw laterite ores using advanced techniques such as X-ray diffraction, automated mineralogy, electron microscopy and mass spectroscopy show heterogeneous distribution of nickel and cobalt within larger, dominant metal-oxide matrices such as goethite, consistent with the atmospheric acid leaching behaviour of the untreated samples.

Destabilisation of the laterite ore structure via reduction of the acid resistant, Ni/Co-bearing mineral phases enhances the accessibility of target metals during subsequent atmospheric acid leaching. Dissimilatory Iron-Reducing Bacteria such as Shewanella oneidensis MR-1 uses available Fe(III) and Mn(IV) oxides as terminal electron acceptors under anaerobic conditions, mediating the reduction of the resistant phases in the ore. The performance of the bioreductive pre-treatment process is compared with conventional pyrometallurgy pathways in benchmarking cost-effectiveness and sustainable benefits of the bioreductive process. The work highlights relevant literature and demonstrates the potential of DIRB-mediated bioreduction as a low-intensity, environmentally sustainable alternative for laterite processing, contributing to scalable biohydrometallurgical strategies for critical metal recovery.

Keywords: Biohydrometallurgy, Dissimilatory iron-reducing bacteria, Nickel laterites, Bioreductive pretreatment

Critical raw materials bioleaching from Hungarian bauxite residue by *Aspergillus sp.*

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Abstract:

Bauxite residue (BR), a waste of alumina production, contains significant concentrations of critical raw materials (CRMs), including Al, Ti, V, Zr, and rare earth elements (REEs), making it a promising target for bioleaching-based recovery processes. In this study, an *Aspergillus sp.* was applied to bioleach Hungarian BR under low-temperature (22 °C) and short contact time (5 days) conditions. Bioleaching experiments were conducted in shaken flasks (30 and 240 mL) using varying BR loadings (1–20%). Sucrose was used as a reference carbon source, while alternative waste-derived substrates, corn-processing waste and brewery waste, were evaluated as potential sustainable substitutes.

The highest CRM concentrations in the leachate were obtained at 5% BR, reaching 2281 mg/L Al, 177 mg/L Ti, 107 mg/L Fe, 15.1 mg/L V, 6.88 mg/L Zr, 4.92 mg/L Ce, 3.50 mg/L Y, and 2.35 mg/L La. In contrast, the tested waste streams yielded markedly lower extraction efficiencies than sucrose, potentially due to adsorption of mobilised metals onto the organic components of the alternative carbon sources. Ecotoxicological assessment of the solid leaching residue revealed increased toxicity compared with untreated BR, underscoring the importance of addressing residue management in bioleaching processes.

Overall, low-temperature fungal bioleaching with short contact times is a promising, environmentally friendly approach for the recovery of CRM from bauxite residue.

Keywords: bauxite residue, *Aspergillus sp.*, critical raw materials

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Valorisation of Red Mud as a Secondary Mining Resource for Improving Nutrient Availability and Maize Growth in Tropical Peatland Soils

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Keywords: Bauxite residue; KCl fertiliser; Maize cultivation; Peatland amelioration; Red mud valorisation; Tropical peatland

Abstract

Tropical peatlands in West Kalimantan are characterised by extreme soil acidity (pH < 4.5), which severely limits crop productivity and promotes unsustainable land management practices such as peat burning. This study evaluated the valorisation of red mud, a secondary resource from bauxite mining, combined with potassium chloride (KCl) fertiliser as a soil ameliorant to improve nutrient availability and maize (*Zea mays* L.) growth on acidic peatland soils. A randomised complete block design examined five treatments with five replications (n = 25). Four treatment levels (R1–R4) consisted of increasing red mud doses (0.75–3.0 kg plot^{-1}) combined with KCl fertiliser (42.6 g), with a control. Red mud exhibited alkaline properties (pH 10–12) with heavy metal concentrations below regulatory thresholds. Treatments significantly elevated soil pH from 4.41 (control) to 5.45–5.67. Exchangeable K increased by 118%, from 2.02 to 4.40 cmol(+) kg^{-1}, while available P improved by 13.4%. The optimal treatment (R4: 3.0 kg red mud + 42.6 g KCl) demonstrated superior maize performance in plant height, stem diameter, and ear weight—exceeding control yields by 84–87%. These findings confirm the potential of red mud valorisation as a sustainable strategy for improving peatland soil fertility through circular reuse of bauxite mining waste.

The global imperative to expand food production on marginal lands while maintaining environmental integrity has intensified scientific interest in tropical peatlands as agricultural frontiers [1]. West Kalimantan's peatlands cover approximately 1.73 million hectares—11.6% of the provincial territory—yet their agricultural potential remains largely unrealised owing to severe pedological constraints [2]. Chief among these is extreme soil acidity, with pH values routinely below 4.5, which restricts maize (*Zea mays* L.) yields to 15–35% of genetic potential [3]. Low pH engenders nutrient unavailability, elevated phytotoxic compound concentrations, and suppression of beneficial microbial communities [4], collectively rendering these soils inhospitable for high-productivity cropping systems.

Conventional peat management by burning temporarily alleviates acidity through ash alkalinisation but generates severe environmental and public health consequences [5]. Combustion releases substantial particulate matter ($PM_{2.5}$ and PM_{10}), elevating respiratory infection incidence by 40–60% during burning seasons [6], while CO_2 emissions reach 500–1,500 t CO_2-equivalent per event [7]. These impacts erode peatlands' irreplaceable role as global carbon sinks [8] and underscore the urgency for sustainable amelioration alternatives.

Red mud, the principal alkaline residue of alumina refining via the Bayer process, is generated at approximately 2.5 million tonnes annually in West Kalimantan alone [9]. This strongly alkaline material (pH 10–12) contains agriculturally relevant concentrations of Ca, Al, Fe_2O_3, P, and K [10], and previous studies demonstrate its capacity to neutralise peat acidity and enhance nutrient availability [11, 12]. However, red mud's high Na content predominantly as NaOH and sodium aluminosilicates—can induce osmotic stress and reduce maize yields by 30–50% through ionic

antagonism [13]. Combining red mud with KCl fertiliser addresses this limitation: high K^+ levels competitively suppress Na^+ absorption at root membranes while supplying K for crop nutrition [14, 15].

Despite individual component data, the interactive biogeochemical effects of red mud–KCl combinations on peat soil chemistry and maize performance remain insufficiently characterised. This study therefore comprehensively examines the valorisation of red mud as a secondary bauxite mining resource when co-applied with KCl fertiliser in tropical peatland maize cultivation, focusing on soil pH dynamics, macronutrient availability patterns, and crop growth responses. The findings advance sustainable peatland management, circular economy principles for mining waste, and climate change mitigation through preserved peat carbon stocks.

2 Materials

2.1 Experimental Site and Initial Soil Properties

A field experiment was conducted on hemic peatland at Punggur Kecil Village, Kubu Raya Regency, West Kalimantan, Indonesia (GPS: Lat −0.169966°, Long 109.292299°; Fig. 1) from March to September 2024. Initial soil characterisation revealed extreme acidity (pH 3.08), very high organic C (55.74%), very high total N (1.68%), very high available P (71.47 ppm), low exchangeable Ca (4.21 me 100 g^{-1}), critically low base saturation (8.51%), and very high CEC (115.55 me 100 g^{-1}) (Table 1). These properties are representative of the pedological limitations characteristic of tropical histosols in this region.

Table 1. Initial peat soil properties at the experimental site.

Parameter	Value	Criteria
pH (H_2O)	3.08	Extremely acidic
Organic C (%)	55.74	Very high
Total N (%)	1.68	Very high
Available P (ppm)	71.47	Very high
Exchangeable Ca (me 100 g^{-1})	4.21	Low
Exchangeable Mg (me 100 g^{-1})	4.20	High
Exchangeable K (me 100 g^{-1})	0.98	High
Exchangeable Na (me 100 g^{-1})	0.57	Moderate
CEC (me 100 g^{-1})	115.55	Very high
Base saturation (%)	8.51	Very low

Classification based on Eviati et al. [16].

2.2 Experimental Design and Treatments

A randomised complete block design (RCBD) with five treatments and five replications (n = 25 plots, each 1.5 m × 3.0 m) was implemented. Treatments comprised: R0 (control, unamended); R1 (red mud 3 kg $plot^{-1}$ only); R2 (red mud 3 kg $plot^{-1}$ + KCl 10.6 g); R3 (red mud 3 kg $plot^{-1}$ + KCl 21.1 g); and R4 (red mud 3 kg $plot^{-1}$ + KCl 42.6 g). Red mud dosage was established through preliminary incubation targeting pH 5.5, yielding a 1:70 red mud-to-peat mass ratio. KCl rates were computed based on stoichiometric equivalence with exchangeable Na in red mud, exploiting competitive monovalent cation displacement. Red mud sourced from PT Indonesia Chemical Alumina (Tayan Hilir, West Kalimantan) exhibited pH 10.87, organic C 0.95%, and base cation composition of K 0.11, Ca 11.37, Mg 0.06, and Na 68.76 cmol(+) kg^{-1}. The KCl fertiliser (60% K_2O) was thoroughly incorporated with red mud before surface application. Basal fertilisation at 230–144–180 kg ha^{-1} N–P_2O_5–K_2O was applied uniformly across all plots [17].

2.3 Analytical Methods and Growth Measurements

Soil samples were collected after 28-day incubation and analysed at the Soil Chemistry Laboratory, Universitas Tanjungpura. Soil pH was determined in a 1:5 soil-to-water suspension; electrical conductivity (EC) and salinity in saturated paste extracts; total N by Kjeldahl digestion; available P by the Bray-1 method (0.03 M NH_4F + 0.025 M HCl); and exchangeable K and Na by extraction with 1 M NH_4OAc at pH 7.0, followed by atomic absorption spectrophotometry (AAS) [16]. Maize plant

height (from soil surface to highest growing point) and stem diameter (5 cm above soil surface using digital calipers) were monitored weekly over seven weeks [18]. Yield was assessed by ear weight with husk (EWH) and ear weight without husk (EWOH) at harvest. Data were subjected to Shapiro–Wilk normality testing, Levene's homogeneity test, ANOVA ($\alpha = 0.05$), and Duncan's Multiple Range Test (DMRT) for mean separation [19].

3 Results and Discussion

3.1 Effects on Peat Soil Chemical Properties

Red mud–KCl amendments significantly elevated peat soil pH from 4.41 in the untreated control to 5.45–5.67 across all treated plots (Table 2), representing a fundamental transition from strongly acidic to moderately acidic conditions. This pH elevation reflects the neutralisation capacity of red mud's alkaline constituents—CaO (52.48%), Na_2O (41.9%), MgO (0.07%), and K_2O (0.03%)—which displace H^+ ions from the peat sorption complex while releasing OH^- into the soil solution [20]. A dose-dependent pH increase from R1 (5.45) to R4 (5.67) was observed, though statistical differences among red mud treatments were non-significant, indicating that even the lowest application rate achieved near-maximum pH buffering within the experimental timeframe. Enhanced pH conditions facilitate improved nutrient solubility, reduced Al toxicity, and stimulated microbial activity [21].

Exchangeable K rose from 2.02 cmol(+) kg^{-1} in the control to 4.40 cmol(+) kg^{-1} in R4—a 118% augmentation effectively doubling available K reserves (Table 2). This enhancement stems from direct K contribution from K-bearing red mud compounds and improved K retention at elevated pH [22]. Available P increased consistently from 85.50 ppm (R0) to 96.97 ppm (R4), a 13.4% improvement consistent with pH–P solubility relationships [23]. Electrical conductivity (2.99–3.74 mS cm^{-1}) and salinity (0.08–0.09 ppt) remained statistically invariant, confirming that red mud did not induce problematic salt accumulation at the doses applied [24].

Exchangeable Na increased dramatically from 1.20 cmol(+) kg^{-1} (control) to 9.24–10.15 cmol(+) kg^{-1} across all red mud treatments, reflecting the predominantly Na-based alkalinity (NaOH and sodium aluminosilicates) of the red mud matrix [25]. Sodium concentrations exceeding 2.5 cmol(+) kg^{-1} in peat soils can disrupt K and Ca uptake through ionic antagonism [26]; however, the concurrent substantial elevation of exchangeable K in R2–R4, driven by KCl addition, mitigated Na-associated risks through competitive displacement of Na^+ at root membrane transporters [15]. Heavy metal concentrations in amended soils remained below regulatory thresholds, consistent with findings of low leaching potential under typical field conditions [27].

Table 2. Chemical properties of peat soil under various red mud and KCl treatments (mean ± SE, n = 5).

Treat-ment	pH	Salinity (ppt)	EC (mS cm^{-1})	Total N (%)	Avail. P (ppm)	Exch. K (cmol(+) kg^{-1})	Exch. Na (cmol(+) kg^{-1})
R0	4.41±0.05b	0.08±0.01a	2.99±0.54a	3.01±0.09a	85.50±1.58a	2.02±0.71b	1.20±0.13b
R1	5.45±0.15a	0.09±0.01a	3.04±0.31a	2.83±0.07a	86.48±3.52a	2.19±0.78a	9.24±0.82a
R2	5.52±0.15a	0.09±0.01a	3.07±0.28a	3.07±0.08a	87.92±1.35a	2.79±0.05a	9.83±1.11a
R3	5.64±0.20a	0.09±0.01a	3.30±0.52a	3.00±0.16a	90.82±2.04a	3.23±0.87a	9.86±2.52a
R4	5.67±0.09a	0.09±0.01a	3.74±0.55a	3.33±0.50a	96.97±10.80a	4.40±0.82a	10.15±0.82a

Values followed by different letters within the same column differ significantly (DMRT, $\alpha = 0.05$). EC = electrical conductivity.

3.2 Maize Growth: Plant Height and Stem Diameter

Maize plant height monitoring over seven weeks revealed initially uniform growth during weeks I–II, followed by progressive differentiation from week III onward (Fig. 2a). Treatment R4 consistently achieved the greatest height throughout the accelerated vegetative phase (weeks III–VII), while the untreated control exhibited markedly restricted growth. Superior height development in R4 directly correlates with the 118% enhancement in K availability—critical for cell elongation and protein synthesis—and the pH shift from strongly to moderately acidic conditions [28]. This pattern corroborates findings that peat soil amelioration with polyvalent cation-rich materials substantially enhances nutrient uptake efficiency and vegetative growth in maize [17].

Stem diameter development paralleled plant height patterns, with statistically significant differentiation between the untreated control and red mud–KCl combinations from week III onward (Fig. 2b). Treatment R4 attained the maximum stem diameter by week V, reflecting enhanced vascular tissue development supported by elevated K supply for cell division and turgor maintenance [29]. Stem diameter serves as a critical indicator of the plant's capacity for water and assimilate translocation from roots to reproductive organs [30]. The synergistic effects of red mud–KCl on soil rhizosphere conditions are clearly expressed in the visual contrast between control and amended plots (Figs. 3 and 4).



Figure 1. (a) Experimental field site at Kecamatan Sungai Kakap, Kabupaten Kubu Raya, West Kalimantan (GPS: Lat −0.169966°, Long 109.292299°), showing plot layout at establishment with red mud application visible in plot A3U2 (foreground). Penelitian DIPA 2024, Fakultas Pertanian UNTAN. (b) Field research team conducting maize seedling measurements and data recording in the treated peatland plots.



Figure 2. Maize crop at the advanced vegetative stage under red mud–KCl treatment (plot R2U2), showing vigorous canopy development and healthy stands on amended peatland. Lush vegetation in adjacent plots reflects consistent ameliorative effects across all treatment blocks.



Figure 3. Overview of all treatment blocks at the advanced vegetative stage (plot A1U1 visible in the foreground), demonstrating the uniform stand establishment and vigorous growth achieved under red mud–KCl amendments on the tropical peatland site.

3.3 Maize Yield: Ear Weight Parameters

Red mud–KCl application exerted a significant, dose-dependent positive effect on maize ear weight (Fig. 5). Treatment R4 achieved the highest EWH (1,552 g) and EWOH (926 g), substantially exceeding control values of 843 g and 496 g, respectively—yield increases of 84% (EWH) and 87% (EWOH) over the unamended control. The EWOH/EWH ratio of 59.7% in R4 indicates superior grain-filling efficiency, reflecting optimised photosynthate partitioning to reproductive sinks. Enhanced ear weight in amended plots correlates mechanistically with elevated K availability driving photosynthate translocation to developing ears [31], improved P bioavailability supporting energy transfer during grain filling, and reduced Al toxicity at elevated pH allowing unrestricted root proliferation and nutrient acquisition.

The visual contrast in ear morphology between treatment groups is striking (Figs. 5 and 6): ears from R1U4 and R4U1 display uniform, tightly packed grain rows of consistent length, while ears from control plots (R0U5) are noticeably smaller, sparsely filled, and exhibit incomplete kernel development—hallmarks of severe K and P deficiency on unamended acidic peat. Progressive improvement from R1 to R4 for both EWH and EWOH demonstrates a positive dose–response relationship. The marginal yield gain between R3 and R4, however, warrants cost–benefit analysis prior to wide-scale adoption. While red mud demonstrates considerable agronomic potential, systematic monitoring of heavy metals in soil and harvested grain remains imperative for long-term food safety [32].



Figure 4. Harvested maize ears from treatment R1U4 (red mud 3 kg plot⁻¹, no KCl, Block 4), showing 15 uniformly well-filled ears with consistent grain development, indicative of effective pH amelioration by red mud alone.



Figure 5. Harvested ears from treatment R2U1 (red mud 3 kg plot⁻¹ + KCl 10.6 g, Block 1), displaying 14 ears with generally good fill; some size variability in the lower row reflects within-plot heterogeneity typical of peatland field experiments.



Figure 6. Harvested ears from treatment R4U1 (red mud 3 kg plot^{-1} + KCl 42.6 g, Block 1), showing notably large, well-filled ears in the upper row alongside smaller ears in the lower row—reflecting the high but variable productivity achievable under optimal amendment.



Figure 7. Harvested ears from treatment R4U4 (red mud 3 kg plot^{-1} + KCl 42.6 g, Block 4), confirming consistently large ear size across replications under the R4 treatment regime.



Figure 8. Harvested ears from the unamended control (R0U5, Block 5), illustrating severe growth suppression on untreated acidic peatland: only six ears recovered, two of which are pale, malformed, and incompletely filled—symptomatic of acute K and P deficiency under pH < 4.5 conditions.

4 Conclusions

The valorisation of red mud as a secondary bauxite mining resource through combined application with KCl fertiliser successfully ameliorated strongly acidic tropical peatland soils, elevating pH from 4.41 to 5.67, doubling exchangeable K reserves (118% increase), and improving P bioavailability by 13.4%. The optimal treatment—R4 (3.0 kg red mud + 42.6 g KCl per plot)—maximised maize performance, yielding ear weights 84–87% above the untreated control and demonstrating superior grain-filling efficiency. These outcomes substantiate the circular economy value of bauxite mining waste valorisation for sustainable tropical peatland agriculture, simultaneously addressing industrial waste management, food security, and carbon conservation objectives. However, the substantial Na loading associated with red mud application requires continued monitoring of long-term soil sodicity, heavy metal accumulation, and food safety parameters. Future research should prioritise techno-economic feasibility analyses, multi-season agronomic assessments, and stakeholder engagement to operationalise this amelioration approach at landscape scale in tropical peatland systems.

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Siderophore-assisted recovery of cobalt from mining tailings and spent lithium-ion battery cathodes using an extremophilic bacterium from the Andean Altiplano of northern Chile

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Abstract: This study evaluates the potential of siderophores produced by an extremophilic bacterium isolated from the Salar de Huasco (Tarapacá Region, northern Chile), located in the Andean Altiplano at approximately 4,000 m above sea level, for the recovery of cobalt from mining and urban residues. Siderophores are low-molecular-weight chelating compounds synthesized by microorganisms to acquire ferric iron (Fe^{3+}) essential for their metabolism; however, several studies have demonstrated that certain siderophores also exhibit high affinity constants toward other metal ions such as gallium (Ga^{3+}) and cobalt (Co^{2+}/Co^{3+}), enabling their application in selective metal recovery processes. Cobalt is considered a critical raw material due to its key role in lithium-ion battery cathodes and energy technologies, coupled with increasing global demand and supply-chain vulnerability driven by electromobility.

The bacterial strain, designated ATCHN, was taxonomically characterised by 16S rRNA gene analysis, revealing an Average Nucleotide Identity (ANI) of 91% with its closest related species, *Halomonas alkaliantarctica*. Whole-genome sequencing and analysis of NRPS/PKS biosynthetic gene clusters predicted the production of amphiphilic siderophores related to amphibactins (Ser–Orn–Ser–Orn, with variants containing D-β-OH-Asp) or marinobactins (β-OH-Asp–Orn–Ser–Orn), forming potashchelin-type structures.

A minimal culture medium was optimised to maximise siderophore production, quantified using the Chrome Azurol S (CAS) assay. Crude siderophore extracts were applied to cobalt-bearing mining tailings and lithium-ion battery (LIB) cathodes of the LCO type. Experiments were conducted at a solid-to-liquid ratio of 5 $g \cdot L^{-1}$ and pH 5.0, and cobalt extraction was monitored over 14 days.

Cobalt recovery reached 33.3% from mining tailings and 19.4% from spent LIB cathodes. These results demonstrate that siderophores produced by extremophilic microorganisms from high-altitude saline environments can effectively mobilise cobalt from primary and secondary resources under mild conditions, highlighting siderophore-assisted extraction as an environmentally friendly strategy for the recovery of critical elements.

Keywords: Siderophores, Cobalt recovery, Biohydrometallurgy, Mining tailings, Lithium-ion battery recycling.

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Integrated bio-hydrometallurgical recycling of spent Li-ion batteries for producing critical mineral powders

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Keywords: Battery; Biohydrometallurgy; Critical minerals; Recycling; Solvent extraction

Abstract

The rapid expansion of Li-ion battery (LIB) production and consumption has intensified the need for efficient, low-impact recycling technologies capable of recovering critical minerals. This study presents an integrated bio-hydrometallurgical process for the recycling of spent NMC532 batteries, combining mechanical shredding, black mass stripping, biological leaching, and solvent-extraction purification to produce high-purity critical-mineral powders. Initially, end-of-life LIBs were shredded to generate cathode and anode electrode particles covered by black mass enriched in Li, Ni, Co, Mn, and graphite. An environmentally benign chemical separation step was applied to delaminate and detach cathode active materials (CAM) and graphite particles from foils, enabling the production of clean aluminium and copper foils and a metal and graphite-rich black mass. The recovered black mass was subjected to bioleaching using sulfur-oxidizing microorganisms under optimised conditions. The biogenic lixiviant efficiently solubilised transition metals, achieving high extraction yields while operating at ambient temperature and low acid consumption compared to conventional leaching methods. The resulting pregnant leach solution was then purified using solvent extraction (SX) to selectively separate Li, Ni, Co, and Mn into individual streams suitable for downstream synthesis. Final precipitation and crystallisation yielded critical-mineral powders including nickel sulfate, cobalt sulfate, manganese oxide, and lithium carbonate.

Introduction

The global transition toward electrification and renewable energy technologies has led to a dramatic increase in the production and use of lithium-ion batteries (LIBs), particularly in electric vehicles and portable electronic devices [1]. Among various LIB chemistries, nickel–manganese–cobalt (NMC) batteries are widely utilised due to their high energy density and long cycle life [2]. However, the rapid growth in LIB consumption has generated significant volumes of end-of-life batteries, creating both environmental concerns and a growing demand for sustainable recycling technologies [3]. Spent LIBs contain substantial quantities of critical minerals such as lithium, nickel, cobalt, and manganese, which are essential for battery manufacturing but are associated with limited natural reserves, high market volatility, and environmentally intensive mining operations [4].

Bioleaching has emerged as a promising alternative for LIB recycling due to its ability to solubilise metals through biologically generated lixiviants under mild operating conditions. sulfur-oxidizing microorganisms can produce acidic environments and oxidising agents capable of dissolving valuable metals from battery materials with lower energy requirements and reduced chemical usage compared to conventional processes [5]. Nevertheless, the integration of bioleaching with upstream mechanical separation and downstream hydrometallurgical processes remains insufficiently explored, particularly to produce battery-grade recovered materials suitable for closed-loop recycling.

In this study, an integrated bio-hydrometallurgical process was developed for the recycling of spent NMC532 batteries. The process combines shredding, wet black mass stripping, bioleaching, and solvent extraction, and crystallisation/precipitation to recover critical-minerals. The proposed approach aims to

improve resource recovery efficiency while reducing environmental impact, supporting the development of sustainable and circular battery supply chains.

Methods

1 kg of spent Li-ion batteries (NMC532) were discharged and then shredded. The shredded materials were mixed with dimethyl sulfoxide (DMSO) to delaminate black mass from the electrodes. The chemical composition of the black mass was obtained after digestion in aqua regia by ICP-MS. Two bacterial strains, *A. thiooxidans* and *Alicyclobacillus sp.* were cultured in a bioreactor using 9K medium and sulfur powder. The produced biogenic sulfuric acid was collected after 10 days. Indirect bioleaching was carried out using the biogenic sulfuric acid at 60 °C, and optimised using a full factorial design with the following variables: slurry densitiy, hydrogen peroxide dosage, and time. Extraction percentage (Eq. 1) of Mn, Co, Ni, and Li were considered as the response variables.

$$E\,(\%) = 100 \times (C \times V) / (G \times M) \quad (1)$$

Where E is the metal extraction, C is the metal concentration in the leachate, V is the volume of the leachate, G is the metal assay in the black mass, and M is the mass of the black mass.

The obtained bio-leachate with the inherent pH of ~4 was subjected to three solvent extraction (SX) stages using (1) Cyanex® 272 to extract Mn and Co, (2) Mextral® 204P to separate Mn from Co, and (3) Versatic™ Acid 10 to extract Ni and leave Li in the final solution. Three separate full factorial designs were implemented to optimise the process variables such as temperature, organic/aqueous ratio, extractant percentage, and pH. The aqueous phase was sampled for metal analysis, and separation factors (Eq. 2) were calculated and used as the response variables.

$$\alpha = D_A/D_B \quad (2)$$

Where D is the distribution ratio; A and B were Co and Ni, Mn and Co, and Ni and Li, for SX stages 1 to 3, respectively. Finally, the organic phases were collected, stripped and used for metal recovery.

In the last step, Ni and Co solutions were concentrated by evaporation and left at room temperature to crystallise metal sulfates. Mn oxide was precipitated by adding potassium permanganate to the metal solution at 50 °C. Li carbonate was precipitated by adding sodium carbonate at

The metal concentrations of the obtained solutions were measured by inductively coupled plasma (ICP-MS), Xseries2, Thermo-Scientific. Powder X-ray diffraction (PXRD) analyses were performed using a STOE STADI P (STOE & Cie GmbH, Darmstadt, Germany) equipped with a Cu X-ray source and a double-bounce monochromator, yielding monochromatic Cu $K\alpha_1$ radiation ($\lambda = 1.5406$ Å). Furthermore, all the experiments were designed and analysed using Minitab 22 software.

Results and Discussion

As the applied bioleaching method is an indirect process, the first stage involves the bioproduction of sulfuric acid to be used in the leaching phase. According to Fig. 1a, pH decreased continuously to <0.5 in two weeks because of the bacterial growth (Fig. 1b) and sulfur oxidation activity (Fig. 1c).

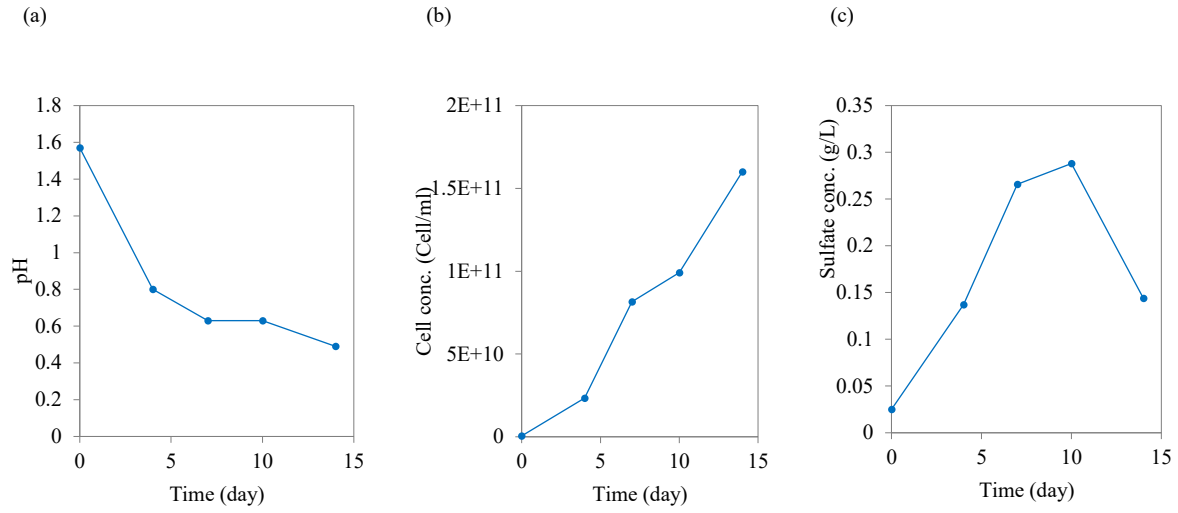


Figure 1. Variation of (a) pH, (b) cell and (c) sulfate concentrations during the bio-production of sulfuric acid

The next stage is to bioleach the black mass delaminated from electrode foils. The leaching process was optimised in two stages. At the first stage, slurry density was the only statistically significant parameter with the best value at 3% (Fig. 2a). As the time and hydrogen peroxide dosage did not significantly affect the metal extraction at the tested ranges, in the second design, the values were reduced to less than 30 min and 3%, respectively. Fig. 2b obviously shows that 30 min rection time and 2% hydrogen peroxide are the optimum values of the investigated parameters.

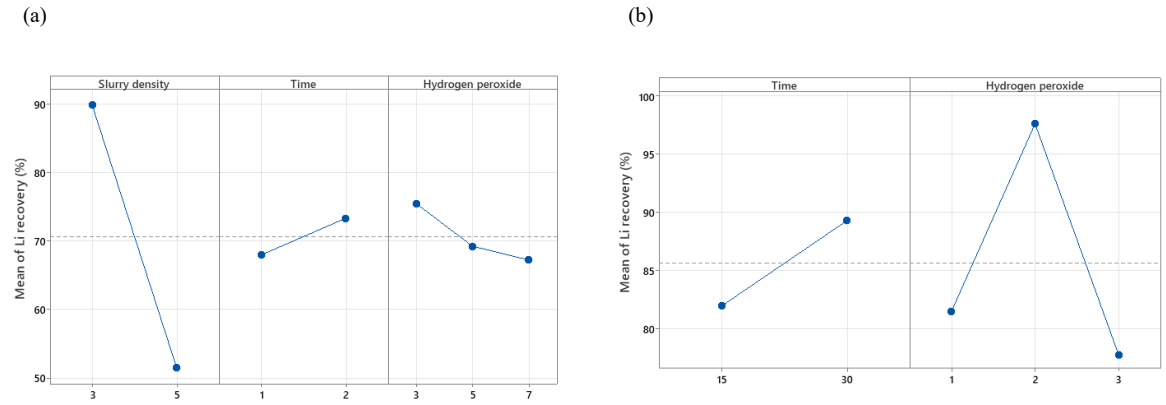


Figure 2. Effects of independent variables including slurry density, time and hydrogen peroxide on the metal extraction efficiency in the (a) first and (b) second experimental designs

Figure 3a.presents the results of the solvent extraction (SX) experiments conducted using Cyanex 272. Statistical analyses (not shown) indicated that neither temperature nor the extractant concentration in kerosene had a significant effect on the Co–Ni separation factor. The optimal operating conditions were determined to be pH 5.75 and an organic-to-aqueous (O/A) ratio of 2. As shown in Fig. 3b, all investigated variables except temperature significantly influenced the Ni–Li separation factor. The highest separation factor was achieved at pH 7.0, an O/A ratio of 1, and a Versatic 10 concentration of 40%. Regarding Fig. 3c, Mn was effectively separated from Co using Mextral 204P at pH 3.5. The remaining variables were found to be statistically insignificant within the investigated range.

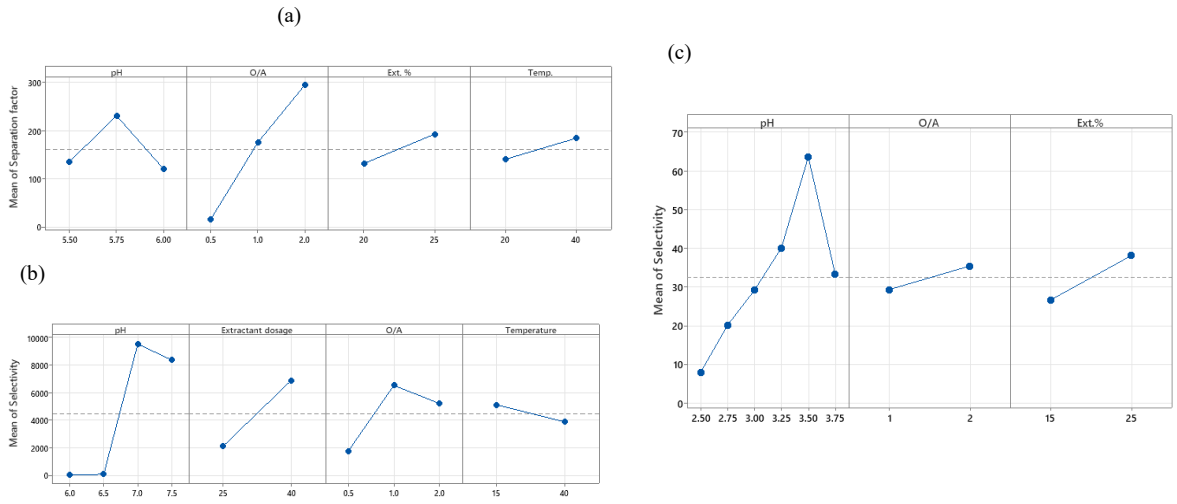


Figure 3. Effects of independent variables on the solvent extraction using (a) Cyanex272, (b) Versatic10, and (c) Mextral 204P

The purified Mn, Ni, Co, and Li solutions were used for metal recovery by crystallisation and precipitation. Nickel and cobalt were crystallised as nickel sulfate and cobalt sulfate as evident from the XRD diagrams presented in Fig. 4a and b. Manganese was precipitated as manganese oxide. However, as seen in Fig. 4c, probably due to the low crystallinity of the particles, the phase was not detectable by XRD. Finally, precipitation of lithium carbonate is clear in the graph shown fin Fig. 4d.

Conclusions

This work demonstrates the feasibility of an integrated bio-hydrometallurgical route for the recovery of critical minerals from spent NMC532 lithium-ion batteries, combining mechanical pre-treatment, green solvent-assisted black mass liberation, bioleaching, solvent extraction, and crystallisation/precipitation into a coherent closed-loop flowsheet. The process enables efficient solubilisation of transition metals using biogenically produced sulfuric acid under mild operating conditions, significantly reducing reliance on conventional strong acids and associated environmental burdens. Subsequent solvent extraction stages achieved effective and selective separation of Li, Ni, Co, and Mn into distinct process streams, demonstrating strong controllability of phase chemistry through optimised pH and organic-to-aqueous ratios. Final recovery steps produced crystalline metal sulfates, manganese oxide, and lithium carbonate with confirmed phase purity by PXRD, indicating suitability for downstream battery precursor applications. Overall, the integrated approach highlights a scalable and environmentally improved alternative to conventional recycling routes, supporting circular recovery of critical battery materials and providing a foundation for further process intensification and industrial scale-up.

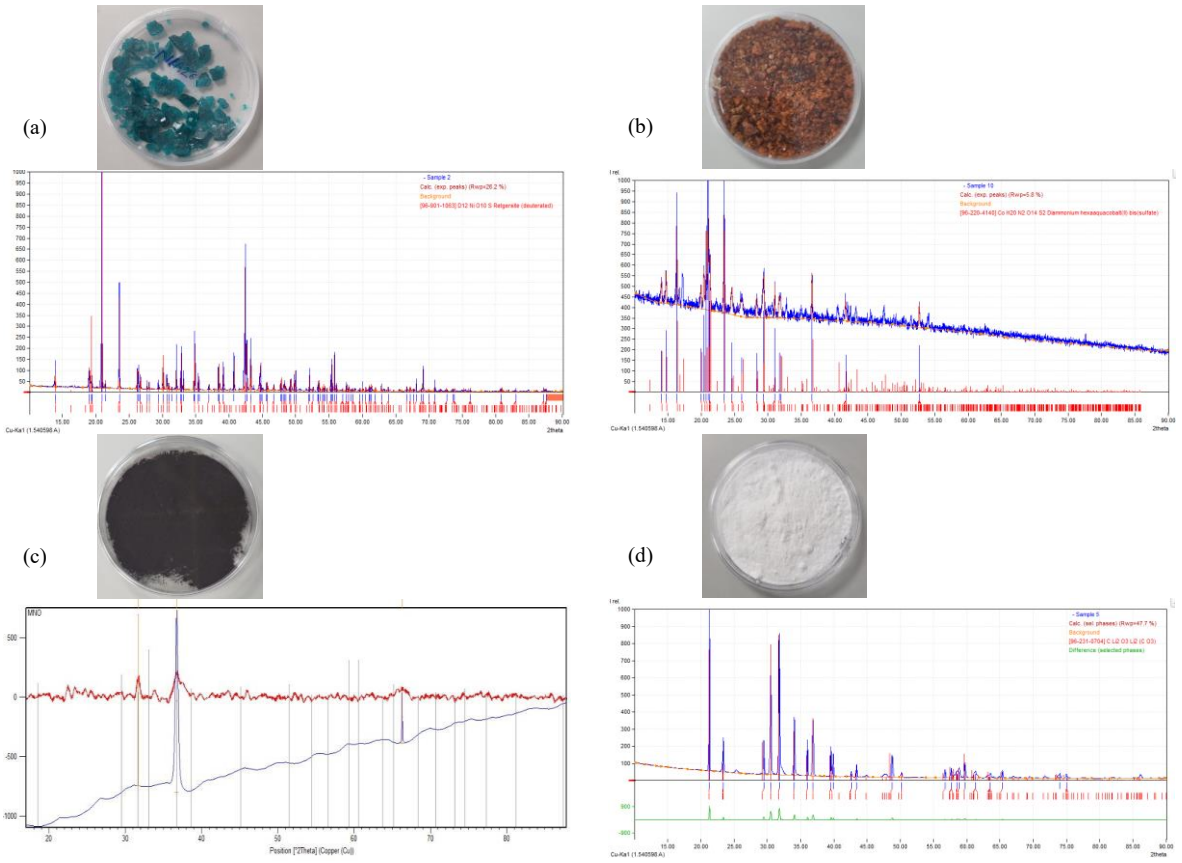


Figure 4. XRD patterns of (a) nickel sulfate crystals, (b) cobalt sulfate crystals, (c) manganese oxide powder, and (d) lithium carbonate

Acknowledgements

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Fungal bioleaching of spent Li-ion batteries for low-emission lithium extraction

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Abstract: The rapidly growing demands for rechargeable lithium-ion batteries in portable devices, electric vehicles, and mobile phones has led to an unprecedented increase in spent battery volume. In parallel, supply constraints for critical elements used in battery electrode materials have made the recycling of spent lithium-ion batteries increasingly essential. Conventional recycling technologies, which rely on energy-intensive smelting and aggressive chemical leaching, generate significant secondary waste and environmental burdens. Consequently, environmentally benign biotechnological alternatives are being actively explored.

In this study, a fungi-based bioleaching approach was investigated for the recovery of lithium from spent $LiCoO_2$ cathode material using *Aspergillus niger* as a biological source of metabolically excreted organic acids. Both one-step and two-step bioleaching processes were evaluated at a liquid-to-solid ratio (L/S) of 50. The one-step bioleaching achieved a lithium extraction efficiency of about 91 wt.%, while the two-step process yielded approximately 95 wt.% bioleaching. In contrast, cobalt solubilisation remained below 1 wt.% under both bioleaching conditions.

These findings demonstrate the strong potential of *Aspergillus niger* for selective lithium extraction from spent $LiCoO_2$ cathode powder. The results provide a foundation for further optimisation of bioleaching strategies aimed at developing environmentally sustainable processes for the recovery of lithium and, ultimately, cobalt from lithium-ion battery waste.

Keywords: Spent Li-ion battery, Fungal bioleaching, Circular economy, One- and two-step microbial activity.

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Use of *Acidithiobacillus ferrivorans* ACH for recycling used lithium-ion batteries

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Urban biomining enables the sustainable recovery of secondary materials from anthropogenic sources, addressing resource scarcity. Lithium-ion batteries (LIBs) play a key role in electromobility and renewable energies. This study contributes to the development of sustainable recycling strategies by establishing a method aimed at solubilising critical elements, specifically lithium and cobalt, from the cathode materials of spent LIBs.

Although conventional recycling is effective, it has high energy and environmental costs. Biological alternatives, such as bioleaching and bioremediation, provide a greener solution for recovering lithium and cobalt from discarded lithium-ion batteries (LIBs). The isolation and characterisation of new strains from an underexplored extreme environment expands the microbial toolkit available for sustainable urban mining and reduce the current pressure that exists on the Chilean salt flats by the conventional extraction of lithium.

A bioleaching process using *Acidithiobacillus ferrivorans* ACH was carried out to recover lithium and cobalt from the cathodes of discarded lithium-ion batteries. The batteries were collected, disassembled, and subjected to a two-step bioleaching process with microorganisms. Bioleaching tests were performed in orbital shaking flasks for 16 days, and four quantities of cathode powder (1, 2, 4, and 6% w/v) were tested in the presence of the bacterium. ICP-OES was used to determine the concentrations of lithium and cobalt present in the leached solutions. Finally, it is concluded that the two-step methodology proved to be more efficient for lithium recovery (98%), compared to the one-step method.

Keywords: Bioleaching, Lithium-ion batteries, Secondary resources, *Acidithiobacillus ferrivorans*.

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Recovery of critical raw materials from NMC and LFP batteries using biohydrometallurgical processes

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Abstract:

The transition to electric mobility in Europe by 2030 will increase the demand for lithium-ion batteries (LIBs) and critical raw materials, emphasizing the need for sustainable management of batteries at the end of their useful life (EOL). In this context, developing innovative strategies for the recovery of strategic metals is essential.

This study investigates biohydrometallurgical approaches to recover metals from end-of-life lithium-ion batteries, including nickel-manganese-cobalt (NMC) and lithium iron phosphate (LFP) chemistries. Specific black mass pretreatments are being explored to improve metal availability and optimize subsequent bioleaching processes. In the case of NMC, flotation and alkaline corrosion pretreatments improved graphite recovery and aluminum leaching (>70% in 24 hours), with low copper mobilization (12%). Chemical leaching tests showed that 10% w/v black mass with a particle size >100 µm in contact with 0.5 M citric acid followed by 0.5 M sulfuric acid solubilized >50% of Li, Mn, Co, and Ni, and >80% of Al and Cu after 4 days. Biogenic organic acids produced by *Aspergillus niger* enhanced metal mobilization compared to synthetic acids, highlighting the potential of microbial metabolites in biological leaching. Preliminary studies on LFP black mass showed that treatments with sulfuric acid, with or without hydrogen peroxide, showed good metal solubilization, including lithium. The black mass initially floats, limiting contact between the solid and the liquid, so surfactants were tested to improve dispersion and the initial leaching rate. Promising trends were also observed with the use of organic acids. These observations will guide the selection of microorganisms for upcoming bioleaching experiments, with the aim of maximizing the recovery of lithium and other metals.

These findings highlight the potential of pretreatments and bioleaching strategies to improve metal recovery from end-of-life lithium-ion batteries, providing useful insights for sustainable recycling processes and supporting circular economy initiatives.

Keywords: lithium-ion bateries, biohydrometallurgy, black mass, metal recovery, bioleaching

Acknowledgements: This work has been supported by the Ministry of Science and Innovation (MCIN) and the AEI (MCIN /AEI /10.13039/501100011033) and ERDF

Coupling biogenic H_2SO_4 production with reductants for enhanced bioleaching of metals from spent lithium-ion batteries

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Abstract:

Electric vehicles powered by lithium-ion batteries (LIBs) have rapidly gained prominence in the global market due to their high energy storage density and long lifespan. However, the treatment and recycling of spent LIBs are expected to become pressing global issues in the coming decades. Compared with pyrometallurgy and hydrometallurgy, biohydrometallurgy offers a more sustainable and cost-effective way of recovering valuable metals from spent LIBs.

In this study, we propose an efficient and sustainable strategy for metal bioleaching. This approach involves enriching chemo-lithotrophic sulfur-oxidizing bacteria (SOB) from sewage sludge in both a continuous stirred-tank reactor (CSTR) and a biofilm reactor to produce biogenic sulfuric acid (H_2SO_4) continuously. The process is then coupled with low concentrations of reductants (e.g., H_2O_2 or ascorbic acid) to achieve high metal leaching efficiency.

Enrichment experiments demonstrated that the concentration of biogenic H_2SO_4 gradually increased to above 0.5 mol/L through the long-term operation of the CSTR, accompanied by an increase in sulfate concentration and oxygen uptake rate. Leaching experiments showed that a combination of 0.5 mol/L biogenic H_2SO_4 and 0.5 vol% H_2O_2 could completely dissolve 30 g/L of spent LIBs (e.g., $LiCo_xNi_yMn_zO_2$) within 24 h. The leaching efficiency of lithium, cobalt, manganese and nickel was approximately 100%. Additionally, biofilm formation was also observed on the surfaces of the carrier materials in the biofilm reactor. Microbial community analysis of the CSTR system revealed the presence of SOB (e.g., *Acidithiobacillus spp.*) and sulfur/iron-oxidizing bacteria (e.g., *Alicyclobacillus spp.*) in the enrichment broth, which are capable of efficiently utilising elemental sulfur.

These findings highlight the significant potential for optimising enrichment conditions to reduce the time required for biogenic H_2SO_4 production at room temperature, facilitating the solubilisation of critical cathodic metals.

Keywords: Lithium-ion batteries (LIBs); Biohydrometallurgy; Sulfur-oxidizing bacteria (SOB); Biogenic sulfuric acid.

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Bioleaching for recovery of phosphates from sewage sludge ash

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Abstract:

Phosphorus (P) and phosphate rock are essential yet finite nutrients central to global fertiliser production. While the immediacy of mineral deposit depletion remains debated, the emerging global issue of an insecure, geopolitically sensitive supply chain persists. Phosphate rock is a limited, regionally concentrated primary resource of P often extracted under environmentally and socially harmful conditions, which is why the European Commission classified phosphate rock as a critical raw material.

Sewage sludge ash (SSA), typically containing 5–11% P, is considered an important secondary phosphorus resource. However, current recovery technologies are often energy-intensive, costly, and environmentally burdensome, relying on strong acids, high temperatures, or complex downstream purification. In this approach, we assess solubilisation via acidic contact leaching using enriched acid-producing cultures derived from activated sludge in a municipal wastewater treatment plant, alongside pure strains of established bioleaching organisms such as *Acidithiobacillus thiooxidans*. Our aim is to develop an effective, scalable, and environmentally friendly alternative to recover phosphates from mono-incinerated sewage sludge.

Bioleaching, well studied for metal recovery, is here adapted to mobilise phosphate from SSA. To optimise the leaching process, all experiments were conducted on pilot-scale SSA obtained from a partner university, observing parameters such as pH evolution, temperature, solid-to-liquid ratios, phosphate and sulfate concentration. The approach supports reduced chemical inputs, lower environmental impact, and meets regulatory goals.

Keywords: Phosphorus recovery, bioleaching, sewage sludge ash, mono-incineration, fertiliser production

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Biological sulfuric acid production by *Acidithiobacillus thiooxidans* for phosphate recovery from sewage sludge ash

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The recovery of secondary resources is dominated by energy-demanding pyrometallurgy and chemical-intensive hydrometallurgy, relying on inorganic acids such as sulfuric acid. Although sulfuric acid is a ubiquitous industrial reagent, its production remains closely tied to oil and gas. Sulfur-oxidizing species, such as *Acidithiobacillus thiooxidans*, are able to oxidise reduced inorganic sulfur compounds to sulfate, resulting in the release of protons and subsequent acidification of the surrounding medium. For biological sulfuric acid production, *A. thiooxidans* was cultivated in a basal salts medium in a 3 L stirred-tank reactor and supplemented with high doses of elemental sulfur as an energy source. At the end of the cultivation period, the biolixiviant was sterile-filtrated, yielding a sulfate concentration close to 1 M.

Phosphorus (P), indispensable for producing phosphate fertilisers, is a critical resource for the food industry. The geographic concentration of rock phosphate and the challenges associated with its mining highlight the need for alternatives to conventional sourcing. Since May 2014, P has been included on the EU list of critical raw materials, with rock phosphate added in 2017. Sewage sludge ash is a promising P source, with legislative changes mandating the recovery of P from sewage sludge by 2030 in Austria. Effective solubilisation of P from sewage sludge ash can be achieved using diluted sulfuric acid, including the biogenic acid produced by *A. thiooxidans*. In contrast to conventional bioleaching, the separation of biolixiviant generation and chemical leaching, allows greater process control and higher pulp densities. This, in turn, improves the ecological footprint and economic competitiveness.

Keywords: Biogenic acid, phosphate recovery, sewage sludge ash, spent medium leaching

Acknowledgements:

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Biohydrometallurgical approaches for the recovery of critical metals from municipal waste

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Abstract: In 2023, municipal waste (MW) generation in the European Union averaged 511 kg per capita, with significant differences between countries: Austria, Denmark, and Luxembourg exceeded 700 kg per capita, while Romania, Poland, and Estonia generated less than 380 kg. Of the total, 48% was recycled, while 25.2% was incinerated and 22.5% was landfilled, reflecting progress in circularity but also remaining challenges. Energy recovery of RM generates significant volumes of slag and fly ash, underutilized resources for construction materials and the recovery of metals of industrial and critical value. Biohydrometallurgical processes offer a sustainable and selective alternative to traditional pyrometallurgical and hydrometallurgical methods, mobilizing metal ions from complex matrices using acids and other metabolites produced by microorganisms such as acidophilic bacteria and fungi. At flask-scale, tests with consortium of *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans* showed that adding 1% of fly ash after bacterial growth achieved solubilization rates above 50% for Li, Co, Mg, Al, Mn, Cu, and Ni. At the same time, the process was evaluated with organic acid-producing fungi (*Aspergillus niger*), achieving solubilization of more than 55% of Li, 88% of Mg, 38% of Ni, 90% of Al, 100% of Mn, 72 % of Co, and 46% of Cu with 3% ash. These preliminary results show high recovery potential, although it is still necessary to optimize conditions to maximize process efficiency.
As future steps, it is planned to treat the leachates using electrochemical and physicochemical processes, as well as to evaluate the development of construction materials using the solids exhausted after bioleaching, assessing their mechanical and environmental properties to ensure their viability and safety. These preliminary results confirm the potential of biohydrometallurgy as a sustainable and selective strategy for the recovery of critical metals, promoting circularity and sustainability in RM management.

Keywords: biohydrometallurgy, municipal waste (RM), microorganisms, critical metals, bioleaching.

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Bio-Based Recovery of Critical and Valuable Metals from Industrial and Municipal Incineration Waste

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Global demand for critical and strategic raw materials is accelerating due to industrialisation, digitalisation, and the transition toward climate neutrality. This trend underscores the need for circular economic solutions that minimise environmental impact while securing resource availability. In Sweden, significant quantities of ash are generated from waste-to-energy facilities and wood-pulping mills. These residues contain economically valuable elements, including Zn, Cu, Ni, Co, and rare-earth elements (REEs). Despite this potential, large amounts of ash are currently landfilled, resulting in low recycling rates and therefore loss of valuable resources.

This research investigates the potential of bioaccumulation and biosorption by plants, algae and fungi as sustainable approaches for recovering metals from ash-derived waste streams. Anticipated challenges include low organic matter, extreme pH values and limited microbial activity, which may limit biomass growth and metal uptake. To address these constraints, amendments, including chelating agents, will be evaluated to enhance bioavailability and improve recovery efficiency. In addition to element uptake, the research will explore methods for recovering accumulated elements from the biomass itself, ensuring that the process achieves full resource recovery and economic viability.

The first step focuses on detailed characterisation of the selected waste streams and target metal speciation, which are critical for designing tailored recovery strategies. Analytical work will include ICP-MS for precise quantification after sequential fractionation of base metals and REEs. These methods, together with the results, will be presented and discussed.

By integrating biological processes with targeted chemical strategies, this work aims to develop a low-footprint method for recycling critical raw materials from industrial residues. The outcomes will contribute to advancing circular economy principles and reducing dependency on primary mining, supporting sustainable resource management for future industrial systems.

Keywords: Incineration ash, Critical raw materials, Biomining, Circular economy

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Bioleaching of hazardous waste incineration bottom ashes: metal recovery potential and limitations

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Incineration enables the reduction of volume and toxicity of non-recyclable waste. Metal-rich solid residues, such as incineration bottom ashes (IBA), produced in high volumes, may be considered as environmentally hazardous materials or resources for secondary metal recovery.

In this study, bottom ash from a hazardous waste incinerator with high metal contents (in mg/kg, Cu: 3700, Ni: 1480, Zn: 3770, Cr 1500, V: 980, Mo: 522, Co: 290) was submitted to a bioleaching procedure to assess the material's potential for a biohydrometallurgical recovery of metals. The IBA was leached in solutions with two bacterial strains, *Acidithiobacillus thiooxidans* and *Pseudomonas fluorescens*, supported by specific growth media that involved low pH and elemental sulfur for *A. thiooxidans* and circumneutral pH and succinic acid for *P. fluorescens*. The experiment was conducted in triplicate, with three pulp densities (1%, 2%, and 5%) over 18 days.

Leaching with *A. thiooxidans* provided much higher ranges of metal recoveries (up to 80% Cu, 86% Ni, 69% Zn, 77% Cr, 52% V, 43% Mo, and 41% Co). In contrast, *P. fluorescens* treatments were less efficient (up to 11% Cu, 9% Ni, 0.5% Zn, 0% Cr, 1.3% V, 12% Mo, 6% Co), which might be directly attributed to the pH level (with no significant contribution of a biomass uptake in the overall metal recovery). The results indicate that low pH plays more significant role in metal leaching from IBA than the presence of *A. thiooxidans*, and satisfactory metal recoveries can be achieved only at pulp density of 1%. This poses a question regarding the IBA potential for a biohydrometallurgical metal recovery.

However, slightly increased releases of V, Mo and Co were noticed for the biotic treatment with *A. thiooxidans* (up to 52% V, 43% Mo, 41% Co) compared to the abiotic one (up to 45% V, 25% Mo, 37% Co). Additionally, higher pulp densities provided similar outcomes as the 1% pulp density in the case of *P. fluorescens* treatment. This leaves a space for potential optimisation of biotic conditions or testing other experimental settings (e.g. physical separation of some phases from IBA, indirect bioleaching reactor) in future investigations to improve metal recovery.

Keywords:

- Higher metal mobility in treatments associated with *A. thiooxidans* and low pH comparing to treatments associated with *P. fluorescens* and circumneutral pH
- **Higher influence of low pH comparing to presence of bacteria**
- **Improved leaching of V, Mo and Co by *A. thiooxidans* comparing to abiotic control**

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Assessing the Bioleaching Potential of Copper-Containing Slags using Acidophilic Bacteria

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Keywords: bioleaching, copper slag, acidophilic bacteria

Abstract

Copper slags are produced globally in vast amounts and represent an increasingly important secondary resource for metal recovery. Bioleaching has been shown as potential approach for extracting metals from slags, however, its application remains challenging. In this study, the bioleaching of various copper slags was investigated using typical autotrophic iron- and sulfur-oxidizing bacteria. To enable a direct comparison, all bioleaching experiments were performed under identical experimental conditions in shake flasks. Finally, two copper slags were particularly promising for bioleaching application. At a solid load of 5% (w/v), up to 65-89% Cu, were extracted within 14 days. Following this, further tests in 2-L stirred tank bioreactors were performed to assess the scale-up potential. Overall, the results confirm the potential of bioleaching for processing copper slags, and highlight the remaining challenges that need to be addressed for practical implementation.

Introduction

Metallurgical slags represent potential secondary metal resources due to their content of valuable metals [1]. Bioleaching has become important as an environmentally-friendly alternative to conventional hydrometallurgical processes. The bioleaching of copper slags has already been investigated using fungi [3,4] as well as heterotrophic and autotrophic bacteria [5,6]. Although most studies have so far been limited to laboratory scale, the feasibility in batch stirred-tank reactors, has already been demonstrated [7]. In addition, several technical challenges still limit the commercial application of slag bioleaching, including maintaining a stable pH, operating at high solid loads, and preventing silica gel formation [1]. Since the composition of slag varies considerably depending on the industrial process, the development of bioleaching processes is generally adapted to the specific types of slag [1]. Potysz et al. (2018) proposed a first adaptable systematic workflow for developing slag bioleaching processes.

The present study aims to evaluate the suitability of different copper slags for bioleaching in a standardised experimental approach. To enable a direct comparison between different slags, the operational parameters are kept constant and were not adapted for each slag. The specific objectives were (i) to improve the understanding of slag bioleaching by identifying the mineral phases suitable to bioleaching by iron- and sulfur-oxidizing acidophilic bacteria, as well as those that are resistant; (ii) to evaluate if the standardised methodology is suitable for rapidly assessing the feasibility of bioleaching for different slag materials; and (iii) to identify the most promising slag materials for subsequent bioreactor-scale optimisation.

Methods

Slag Characteristics

Three copper slags originating from different mine sites were investigated: Eliseyna (Bulgaria), historical slag from São Domingos (Spain), and two samples from Mansfeld (Germany). A preliminary overview of the major elemental composition of the slags is provided in Table 1. Initial mineralogical analyses indicate that the Bulgarian slag is predominantly crystalline and is characterized by silicate

phases (fayalite and augite) and oxide phases (magnetite and sulfides (chalcopyrite, pyrite, and galena). The São Domingos slag contain silicates (fayalite and quartz), oxides (magnetite and hematite) and sulfides (chalcopyrite, bornite, galena, and sphalerite). The Mansfeld slag contains a substantial amorphous fraction as well as silicates (wollastonite, quartz, and leucite), magnetite and sulfides comprise chalcopyrite, bornite, chalcocite–covellite, galena and sphalerite, and metallic copper.

Because of the alkaline nature of the slags, the consumption of mineral sulfuric acid to maintain acidic pH was determined prior for respective solid loads.

Table 1. Chemical composition of the selected copper-slags [wt%]

Slag	Fe	Al	Ca	K	Mg	Si	Zn	S	Cu
Mansfeld (spl. 1)	3.5	7.2	15.4	3.7	1.6	23.6	0.1	0.2	0.6
Mansfeld (spl. 2)	2.1	7.4	15.0	3.5	3.4	22.3	0.7	0.3	0.2
Eliseyna	27.2	2.8	6.9	1.0	1.9	15.7	1.9	0.9	0.4
São Domingos	33.0	1.00	4.0	0.2	0.2	14.3	2.0	2.9	0.2

Bioleaching experiments

Adaptation and pre-bioleaching tests with up to 5% (w/v) of slag showed that the moderately thermophilic mixed culture comprising *Sulfobacillus (S.) thermosulfidooxidans*^{T}, *Leptospirillum (L.) ferriphilum*^{T} and *Acidithiobacillus (At.) caldus*^{T} exhibited the highest microbial activity and bioleaching performance. Cultivation was carried out using minimal basal salt medium (HBS) adjusted to pH 1.8 with sulfuric acid [10]. Furthermore, 1% (w/v) S° was used as substrate as well as 40 mM of ferrous iron (supplied as $FeSO_4*7H_2O$) for the Mansfeld slag samples. An inoculum of 10% (v/v) was applied, and cultivation was performed in shake flasks at 42 °C at 120 rpm for 14 days.

Bioleaching shake flask experiments were conducted in 50 mL working volume with at 5 % (w/v) of slag. Biotic approaches were performed in triplicates. Additionally, a negative control (growth medium without inoculum) and an abiotic control (without inoculum, but with pH adjusted to match the biotic conditions) were included. Samples were collected at regular intervals to monitor pH (Mettler Toledo InLab® Semi-Micro probe), redox potential (ORP, Mettler Toledo InLab® Redox Micro electrode referenced to Ag/AgCl), and concentrations of Fe^{2+}, Fe^{3+}, and total iron by the Ferrozine® assay [11,12]. The leaching solutions were analysed for dissolved metal concentrations by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES, SPECTRO ARCOS FHX3X). For the Eliseyna and São Domingos slags, bioleaching experiments were performed in triplicate in a pH- and temperature-controlled, aerated 2-L stirred-tank bioreactor (Labfors 5, Infors AG) with a working volume of 1 L. The same experimental conditions as in the 5% (w/v) shake flask experiments were applied, using a stirring speed of 500 rpm. Sulfuric acid was added automatically when the pH exceeded 2.0. Samples were collected at regular intervals according to the same procedure described for the previous experiment. The bioreactor was stopped once a pH of 1.0 was reached. The solid residue was washed with deionised water of pH 1.0 and analysed by handheld X-ray fluorescence analysis (h-XRF) to assess a first view on the residue composition.

Results and Discussion

A standardised experimental approach was established for the development and comparison of the bioleaching process of the slags. The experimental workflow started with a comprehensive mineralogical and chemical characterisation of each slag. In parallel, the chemical behaviour of the slags was evaluated to assess their effects in growth medium. The microbial consortium was subsequently adapted to increasing solid loads of slag. The starting pH was maintained ~1.8 throughout all experiments, as this acidity is required for the activity of acidophilic iron- and sulfur-oxidizing microorganisms. For comparison an abiotic chemical controls are required to distinguish biologically mediated metal dissolution from purely chemical leaching. This short article focuses on the results of the experiments conducted at a 5% (w/v) solid load. During shake flaks bioleaching of both Mansfeld slag samples, the pH in the abiotic control experiments increased markedly within the first 2–4 days. In the biotic systems, the pH increase was less pronounced, due to the microbial

production of sulfuric acid. For sample 1, the pH decreased to 0.98 and the ORP increased to 654 mV after 11 days indicating active microbial oxidation of sulfur and ferrous iron. In contrast, for sample 2, the pH remained at 1.35 even after 16 days, while the ORP reached 608 mV by day 7. The pronounced buffering effect observed during leaching is a well-known characteristic of metallurgical slags [1]. Nevertheless, the higher Ca and Mg content, which indicates a greater abundance of Ca- as well as Mg-bearing phases, may have contributed to the buffering effect. Overall, the final metal recovery was generally lower for both samples, as shown in Figure 1. Cu extraction ranged from 30 to 70%, primarily due to the limited pH decrease, particularly in sample 2. Although, recoveries were approximately similar to the abiotic control, suggesting that leaching was predominantly driven by acid dissolution. Due to the low metal extraction and the pronounced buffering effect, the Mansfeld slag was found to be less suitable for bioleaching. For the copper slags from Eliseyna and São Domingos, the abiotic control experiments showed an initial minor increase in pH, whereas the biotic systems maintained stable or decreasing pH values. During bioleaching of the Eliseyna slag, the pH decreased to 0.93 after 14 days, accompanied by an increase in ORP to 650 mV by day 7. Similarly, the São Domingos slag exhibited a decrease in pH to 1.02 after 14 days, while the ORP started to increase after day 7 and reached 645 mV. Overall, higher metal recoveries were achieved for both slags, with Cu recoveries reaching 89% for the Eliseyna slag and 65% for the São Domingos slag (Figure 1). For both slags, recoveries were approximately similar to the abiotic control, showing leaching was predominantly driven by acid dissolution. Since both slags showed high microbial activity and promising recovery rates, initial bioreactor tests were conducted at a solid load of 5% (w/v) slag. For the Eliseyna slag, duration time could be reduced to 7 days while achieving reproducible results in triplicate experiments. In addition, metal recoveries were further improved, reaching a recovery of 97% Cu. Residue analysis confirmed the high Cu dissolution. The bioreactor experiments with the São Domingos slag revealed variations in duration time of 9 to 11 days. However, no improvement in Cu recovery was achieved, with a final 54% Cu. Residue analyses confirmed that a significant fraction of the copper remained in the solid residue and was not dissolved. Furthermore, high concentrations of iron and silica were detected in the leachate. The formation of silica gel may pose a challenge for downstream processing, as dissolved silica can hinder solid–liquid separation due to its poor filterability and tendency to form colloidal phases. Considerable amounts of elemental sulfur remained in the residues of both slags, indicating that future optimisation should focus on reducing sulfur addition during bioleaching. Another important optimisation step will be reducing sulfuric acid demand for pH stabilisation through two-step bioleaching or stepwise slag addition. Furthermore, comprehensive mineralogical characterisation is required to determine which mineral phases are dissolved, remain resistant, or become enriched during bioleaching, and to assess its potential for reducing environmental impacts associated by toxic components. Subsequently, downstream processing and a life cycle assessment will be conducted to evaluate the economic feasibility and sustainability of the technology following bioleaching, with particular emphasis on silica removal, which remains a key challenge. Finally, microbial community analyses will provide further insights, including shifts in community composition.

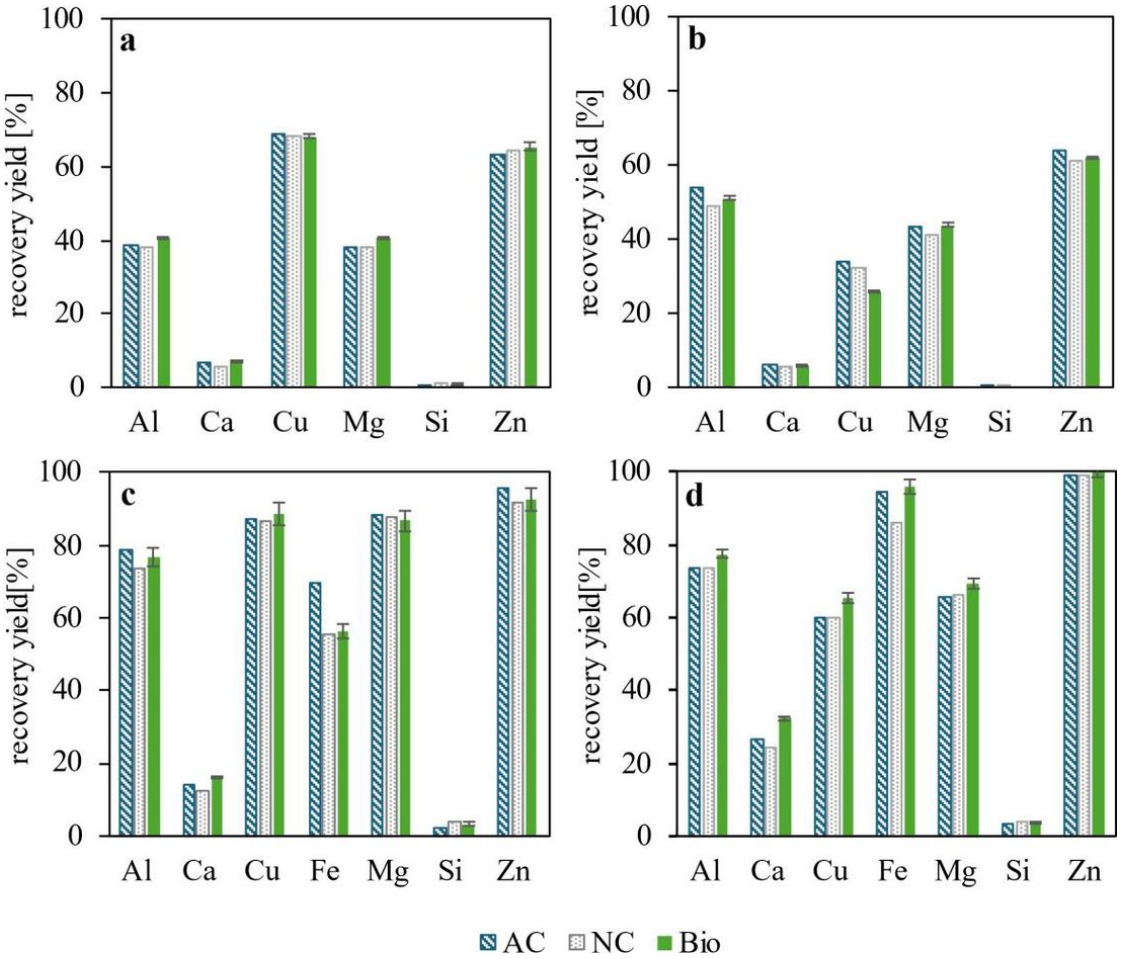


Figure 1. Final metal recoveries after bioleaching of 5% (w/v) copper slag by a moderately thermophilic mixed culture (42 °C) in shake flasks; (a) Mansfeld slag sample 1, (b) Mansfeld slag sample 2, (c) Eliseyna slag (d) São Domingos historical slag; BIO= mean and standard deviation (error bars) of biotic approaches, AC = abiotic controls (pH acidified analogously to the biotic approaches); NC = negative control (without inoculum).

Conclusions

In this study, the suitability of three copper slags, Eliseyna (Bulgaria), historical slag from São Domingos (Spain), and Mansfeld (Germany), for bioleaching was investigated using a standardised experimental approach in shake flasks. A moderately thermophilic mixed culture of sulfur- and iron-oxidizing microorganisms was applied. The Mansfeld slag showed lower metal recovery rates and a strong buffering effect, which negatively affected bioleaching performance. Therefore, this slag was considered unsuitable for further bioleaching application. In contrast, the Eliseyna and São Domingos slags exhibited promising results and were further investigated in bioreactor experiments. Copper recovery rates of 97% and 54% were achieved, respectively. Further optimisation measures are needed to improve metal recovery, process efficiency, and the sustainability of the overall process, with a focus on downstream processing.

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EFFECT OF MICROBIAL SPECIES WITH HETEROTROPHIC METABOLISM ON THE NON-FERROUS METAL BIOLEACHING FROM COPPER SLAG

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Keywords: copper slag; copper; zinc; cobalt; bioleaching

Abstract

The main objective of this study was to compare the efficiency of bioleaching of copper, zinc, and cobalt from pyrometallurgical copper slag in the presence of different heterotrophic microbial species. The copper slag contained 0.36% copper, 1.93% zinc, and 0.09% cobalt, with a pH of 7.36. Approximately 90% of the total zinc and cobalt content was in secondary forms that were easily leached at acidic pH. The copper content in this phase was 75% of the total amount; the remaining 25% was in an oxidisable phase. The main mineral constituents in the sample were protomangano-ferro-anthophyllite, fayalite, and clinopyroxene. Bioleaching was performed using a direct two-stage shake-flask technique, in which biological recovery began after preliminary accumulation of microbial biomass for one week, at 5% pulp density and at 25 °C with fungi (*Aspergillus niger, Penicillium ochrochloron*) and 35 °C with bacteria (*Sporosarcina ureae* or *Bacillus megaterium*), using finely ground (minus 100 µm) copper slag. Microbial tolerance to heavy-metal ion concentrations and the effect of microbial metabolites on pulp pH strongly influenced the biological recovery of zinc and copper, as well as the co-leaching of iron and silicon.

Introduction

Complexity in the chemical composition and structure of organic matter determines the participation of different groups of heterotrophic microorganisms that utilise various metabolic pathways and thrive under different environmental conditions. At the same time, the decomposition of organic compounds, in addition to its direct effect on the redox potential of the environment, has both direct and indirect effects on the dissolution of non-ferrous metals from the minerals that make up the biotope through acid-generating or alkali-generating processes, as well as through the formation of suitable ligands, such as organic acids, ammonium ions, and others. The main aim of this study was to investigate the effects of selected groups of heterotrophic microorganisms on the extraction of non-ferrous metals, iron, and silicon from pyrometallurgical copper slag.

Methods

Materials. The copper slag sample was collected from the surface of a dump near Eliseyna, Northwestern Bulgaria. The sample was ground to a dominant particle size of 75 µm to 25 µm. In that form, the copper slag was used in bioleaching experiments. The analysis revealed that the contents of copper, zinc, and cobalt in the sample were 0.36%, 1.93%, and 0.09%, respectively, and that the pH was 7.36. The copper slag contained iron (27.2%) and silicon (15.9%), and was composed of protomangano-ferro-anthophyllite (56.8%), fayalite (33.2%), and clinopyroxene (10%) as main mineral constituents.

pH-dependent leaching with sulfuric acid revealed that 90% of zinc and cobalt contained in the copper slag leached easily at acidic pH. In comparison, 75% of copper leached under the same conditions, and the residual amount was an oxidisable fraction.

The following microbial strains were used in the bioleaching of copper slag: *Aspergillus niger BC383,* obtained from the Microbial Culture Collection at the Department of General and Industrial Microbiology, University of Sofia; *Penicillium ochrochloron CCM F-158; Sporosarcina ureae CCM 981;* and *Bacillus megaterium CCM 3360.* The last three microbial strains were obtained from the Czech Collection of Microorganisms at the Department of Experimental Biology at Masaryk University. The fungal strains were cultivated in a medium containing sucrose and under conditions optimal for citric acid synthesis [1]. *Sporosarcina ureae* and *Bacillus megaterium* were cultivated in a nutrient broth containing 2% peptone and 2% urea as the main constituents [2] and in Ashby's medium [3], respectively. All growth media and glassware were sterilised for 15 min at 12 °C prior to use, and all manipulations were performed under sterile conditions.

Bioleaching of copper slag. The direct two-stage shake-flask technique was used to bioleach the non-ferrous metals from the copper slag sample. The first stage involved inoculating 150 ml of the relevant nutrient medium with 10 ml of active late-log-phase microbial culture of the relevant microbial strain. The cultivation was carried out on an orbital shaker (150 rpm) for a week at optimal temperature (25 °C for fungal strains and 35 °C for bacterial strains). The second stage of that bioleaching technique began with the addition of 12.5 g of copper slag, which underwent preliminary dry-heat sterilisation at 180 °C for 2 h in a hot-air oven, to each Erlenmeyer flask. Bioleaching continued for a week for the bacterial strains and for three weeks for the fungal strains, respectively, under the conditions mentioned above. The water losses due to evaporation were compensated for weekly with distilled water. Control experiments were conducted utilising fresh nutrient medium and sterile copper slag. All experiments were run in three replicates, and the results were reported as the mean value.

Analysis. The pulp pH across all variants during bioleaching was monitored weekly using a combined pH electrode. Weekly, a transparent pregnant solution from all variants was obtained by centrifugation aseptically at 10,000 rpm for 10 minutes. The concentrations of citric and oxalic acids were determined by titration and spectrophotometry, respectively [4,5]. Concentrations of NH_4^+ and TOC were determined by the salicylate colourimetric method [6], and the standard method 5310D (at a temperature of 200 °C, highly acidic pH and in the presence of P_2O_5 and CrO_3 as chemical oxidants), respectively.

Results and Discussion

The optimal growth conditions maintained during the first stage of the direct two-stage bioleaching technique of mineral raw materials result in the accumulation of microbial biomass and high concentrations of suitable ligands in the production solution, which facilitates the adaptation of microbial culture to the mineral and chemical composition of the copper slag as the process transitions to the second stage. As a result, the recovery of non-ferrous metals from raw minerals is generally more efficient than that achieved through a direct one-stage bioleaching technique [7].

Aspergillus niger and *Penicillium ochrochloron* were cultured on a nutrient medium containing 185 g/L sucrose under conditions that favoured the synthesis and accumulation of primarily citric acid and, to a lesser extent, oxalic acid. At the end of the first stage, the spent medium of *A. niger* had a pH of 2.58 and citric and oxalic acid concentrations of 24.8 and 8.1 g/L, respectively. The weight of fungal biomass was 19.5 g. The spent medium of *P. ochrochloron* had a pH of 3.24, and the concentrations of oxalic and citric acids were 10.9 and 18.9 g/L, respectively. As the process progressed from the first to the second stage of bioleaching upon the addition of the solid sample, fungal mycelium from both microbial strains covered the mineral particle surfaces, resulting in pellet formation in the variant with *A. niger*, acidolysis of minerals, and leaching of non-ferrous metals, all of which occurred at a higher rate. It resulted in the accumulation of a significant concentration of base metals, which may, in some cases, be toxic to microbial cells. *A. niger* is a more sensitive species and, at aluminium, manganese, and copper concentrations of 27 mM, 2.2 mM, and 0.44 mM in the pregnant solution, responds by overproducing oxalic acid. For this reason, the pH of the pulp was nearly half a unit lower than that of *P. ochrochloron* (Table 1), thereby further facilitating acidolysis, improving the recovery of zinc and cobalt, and leading to significant co-leaching of silicon and iron. Under these conditions, however, copper recovery decreased significantly due to its secondary precipitation as copper oxalate.

Table 1. pH at the end of the direct two-stage bioleaching technique of copper slag from Eliseyna, Bulgaria, and recovery of non-ferrous metals, iron, and silicon with the relevant microbial strains

Parameter	Units	*Aspergillus niger*	*Penicillium ochrochloron*	*Sporosarcina ureae*	*Bacillus megaterium*
pH	-	4.47	4.95	9.63	7.83
Cu	%	16.7	25.0	30.5	41.7
Zn	%	66.3	41.4	12.4	41.4
Co	%	78.9	58.9	16.7	43.3
Fe	%	36.4	4.0	4.8	35.3
Si	%	20.1	27.0	1.2	22.4

Sporosarcina ureae thrives in an alkaline pH environment because it possesses enzyme urease, which allows it to participate in the breakdown of amino acids and urea through ammonification and the accumulation of ammonium ions in the solution. For example, the spent medium of the bacterial strain had a pH of 9.43 and contained 11.7 g/L ammonium ions at the end of the first stage of bioleaching. Copper leached efficiently from the copper slag sample under these conditions during the second stage, and its final recovery was higher than the results obtained in the presence of both fungal strains. In that case, the weight of bacterial biomass determined at the end of copper slag bioleaching was 1.23 g. The bioleaching of copper was highly selective, with negligible co-leaching of silicon and iron. Under these conditions, however, the extraction of the other non-ferrous metals contained in the copper slag—zinc and cobalt—was negligible (Table 1).

Bacillus megaterium belongs to the group of silicate-solubilising microorganisms that grow in environments with extremely low phosphorus content. Biofilm formation enriched with weakly acidic polysaccharides, proteins, and poly-β-hydroxybutyrate on the surface of mineral particles is the most used mechanism for silica solubilisation [8]. The weight of microbial biomass determined at the end of copper slag bioleaching was 1.18 g. Another mechanism is the secretion of metabolites, such as amino acids, phenolic compounds, organic acids, and siderophores [9,10], which are strong ligands to non-ferrous metals and iron. In that case, *B. megaterium* tolerates higher concentrations of heavy metals and metalloids, such as copper, zinc, cobalt, and mercury, in the environment due to the presence of specific operons that confer bacterial resistance to their toxicity [11]. The spent medium formed after a week of cultivation had a pH of 5.63 and contained dissolved organic compounds with a total TOC content of 1180 mg/L. Copper recovery under these conditions was the highest (41.7%; Table 1), compared with other heterotrophic strains tested in this study, despite a slightly alkaline pH and a second-stage duration of only a week. In that case, the recovery of non-ferrous metals was also non-selective due to the significant co-leaching of iron and silicon.

Conclusions

The copper slag sample contained zinc and cobalt, and a significant amount of copper as oxide forms, which leached easily at an acidic pH. The efficiency of their biorecovery, due to the growth of heterotrophic microorganisms, depends on the amount and type of biofilm formed on mineral particle surfaces, the types and concentrations of secreted ligands, and the tolerance of each microbial strain to the mix of non-ferrous metals accumulated in the pregnant solution. The practical potential of the results is limited due to the process operation at lower pulp density, the need for a permanent supply of a suitable organic compound and the need to isolate heterotrophic contaminants by maintaining a closed bioleaching system.

Acknowledgements

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Controlled Recovery of Indium and Gallium from LCD and LED Waste via Siderophore-Assisted Leaching

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Abstract: The accelerating use of indium (In) and gallium (Ga) in advanced electronic and low-carbon technologies has intensified the need for sustainable and selective recycling strategies. End-of-life liquid crystal displays (LCDs) and surface-mounted device (SMD) light-emitting diodes (LEDs) constitute important secondary sources of these critical metals. Although conventional acid leaching processes are effective, they typically lack selectivity and promote extensive co-dissolution of matrix elements such as Al, Cu, Fe, and Pb, thereby increasing the complexity of downstream separation.

Here, we propose a bio-inspired leaching approach based on the microbial siderophore desferrioxamine E (DFOE) to selectively mobilise In and Ga from complex electronic waste. For LCD waste, a dedicated pre-treatment step enabled the recovery of an indium-rich glass fraction. Subsequent DFOE-assisted leaching revealed competitive binding between In and Al, with maximum In recovery of 32% obtained under mild conditions (pH 5.5, 25 °C, 1 g/L solid-to-liquid ratio, 5 mM DFOE) after 90 days, corresponding to an indium dissolution rate approximately ten times higher than that of aluminium.

The applicability of DFOE was further evaluated for spent SMD LEDs as an alternative to aggressive acid leaching. While conventional 3 M HCl combined with H_2O_2 achieved near-quantitative extraction of Ga and In (97–98%) without selectivity, siderophore-mediated leaching enabled more controlled and selective metal recovery. Under optimised conditions, up to 12% of Ga (pH 4) and 24% of In (pH 7.3) were mobilised, with minimal dissolution of competing elements.

This study provides the first evidence of siderophore-driven selective leaching of indium and gallium from heterogeneous electronic waste. The results demonstrate the promise of microbial chelators as environmentally benign agents for low-energy, selective metal recovery and for producing metal-enriched leachates compatible with downstream separation processes such as GaLIophore.

Keywords: Indium and gallium recycling, Siderophore-assisted leaching, Electronic waste (e-waste), Selective metal recovery, Biohydrometallurgy

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Bioleaching of Steel Milling Dust for Polymetallic Recovery

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Keywords: bioleaching; steel milling dust; *Acidithiobacillus ferrooxidans*; polymetallic recovery; circular economy

Abstract

Secondary wastes from the steel industry are valuable resources for critical metal recovery within a circular economy. This study evaluated the bioleaching of milling dust from a steel industry using *Acidithiobacillus ferrooxidans*. Shake-flask experiments (30 °C, 150 rpm) compared two low pulp densities (0.25% and 0.5% w/v) against abiotic controls, with triplicate biotic reactors and daily monitoring of pH, redox potential and temperature. Endpoint Mn mobilisation reached >30% but was largely acid-driven, showing minimal difference from controls. Zn mobilisation (8.9%) was enhanced by bacterial activity at 0.5% pulp density. Dissolved Pb declined over time in biotic reactors, consistent with sulfate-mediated re-precipitation. Rising redox potential (580-600 mV) confirmed active iron oxidation. The results demonstrate the feasibility of biohydrometallurgical processing of steel dust while revealing strongly metal-specific behaviour that must guide process design.

Introduction

Steel manufacturing produces large quantities of fine particulate residues such as milling dust, which are typically landfilled despite containing economically and strategically significant metals [1]. Recovering valuable metals from secondary sources reduces primary extraction pressure, decreases the environmental burden of leachable heavy metals, and supports circular economy targets for critical raw metals [2]. Bioleaching, the mobilisation of metals by acidophilic chemolithotrophic microorganisms, is an established low-energy, low-reagent alternative to conventional methods like pyro and hydrometallurgy for low-grade and complex feedstocks [3]. *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) oxidises ferrous iron and reduced inorganic sulfur compounds, regenerating the ferric iron and protons that act as the principal leaching agents and promote metal solubilisation [3]. The approach has been demonstrated for the recovery of Zn and other metals from iron-rich steelmaking and metallurgical dusts [4].

This study investigates the bioleaching of steel milling dust by *A. ferrooxidans*, compares biotic and abiotic metal mobilisation, and evaluates the effect of low pulp densities (0.25% and 0.5% w/v) on the recovery of individual metals.

Methods

Feedstock characterisation. The steel milling dust obtained from a steel manufacturing industry in the Brandenburg State of Germany. Elemental composition was determined by energy-dispersive X-ray fluorescence (XRF); results are summarised in Table 1.

Microorganism and medium. *A. ferrooxidans* was maintained in DSMZ Medium 882 (autoclaved Solution A of mineral salts, pH 1.8; filter-sterilised Solution B of 20g/L $FeSO_4 \cdot 7H_2O$; combined 20:1 with 1 mL/L trace-element solution). Active inocula were subcultured in 100 mL baffled flasks (30 °C, 105 rpm, 3 to 4 days) and used for the experiment.

Bioleaching experiments. Shake-flask bioleaching was performed at two pulp densities - 0.25 g and 0.5 g dust per flask (0.25% and 0.5% w/v) - in triplicate biotic reactors (A, B, C) along with abiotic

control without inoculum. Dust was suspended in 75 mL medium and the pH stabilised at 2.0 with 10 N H_2SO_4; reactors were then inoculated the following day with 25 mL bacterial culture (100 mL final working volume), delaying inoculation until after the initial acid demand had subsided to avoid pH shock to the cells. The experiment ran for 16 days at 30 °C and 150 rpm. pH was checked daily and re-adjusted to 2.0 with 10 N H_2SO_4; temperature and oxidation - reduction potential (ORP) was logged daily.

Analysis. Leachate samples were drawn on days 2, 9 and 16 for dissolved-metal quantification by inductively coupled plasma optical emission spectrometry (ICP-OES). Parallel samples were collected at each timepoint for DNA-based microbial community analysis to confirm an active bacterial population (analysis pending). Extraction efficiency was calculated as dissolved metal mass relative to total metal content of the dust, assuming 100 mL working volume.

Results and Discussion

Table 1. Elemental composition of steel milling dust by XRF

Metal	Content [%]	Metal	Content [%]
Fe	56.60	Bi	1.41
Zn	19.50	Cu	0.83
Si	9.77	Co	0.72
P	2.98	Ti	0.41
Mn	2.52	Cr	0.24
Pb	1.47	Others (Zr, V, Mo, Nb, Mg)	<0.35 each

Endpoint (day 16) dissolved concentrations and gross extraction efficiencies for the three target metals are given in Table 2, in which dissolved concentrations (biotic triplicate mean ± SD; single abiotic control) and gross extraction efficiency relative to XRF-estimated total content (100 mL working volume) is given. Mn showed the highest extraction (30%), Zn intermediate (10%), and Pb the lowest and most complex behaviour.

Table 2. Endpoint dissolved concentrations for the three target metals

Metal	Pulp density [%]	Biotic concentration [mg/L]	Abiotic concentration [mg/L]	Biotic extraction [%]	Abiotic extraction [%]
Mn	0.25	19.8 ± 0.2	19.7	31.4	31.3
Mn	0.5	37.5 ± 4.9	34.5	29.7	27.4
Zn	0.25	50.0 ± 1.1	57.4	10.2	11.7
Zn	0.5	87.0 ± 9.5	69.7	8.9	7.1
Pb	0.25	1.32 ± 0.05	2.37	3.6	6.5
Pb	0.5	2.29 ± 0.37	3.98	3.1	5.4

Manganese. Mn mobilisation rose steadily over the run (31% at 0.25% pulp density) but was essentially indistinguishable between biotic and abiotic reactors, indicating that Mn release is governed by proton attack at pH 2 rather than by bacterial oxidation. Only at 0.5% pulp density did the biotic reactors show a small day-16 margin over controls (29.7% vs 27.4%).

Zinc. At 0.25% pulp density, biotic Zn extraction was equal to or below the abiotic control, indicating no bacterial enhancement and possible re-adsorption. At 0.5%, biotic reactors clearly exceeded controls (8.9% vs 7.1%), showing that the bacterial contribution to Zn mobilisation becomes measurable at higher solid loading.

Figure 1 shows the dissolved metal concentrations over the entire experimental period under the different experimental conditions.

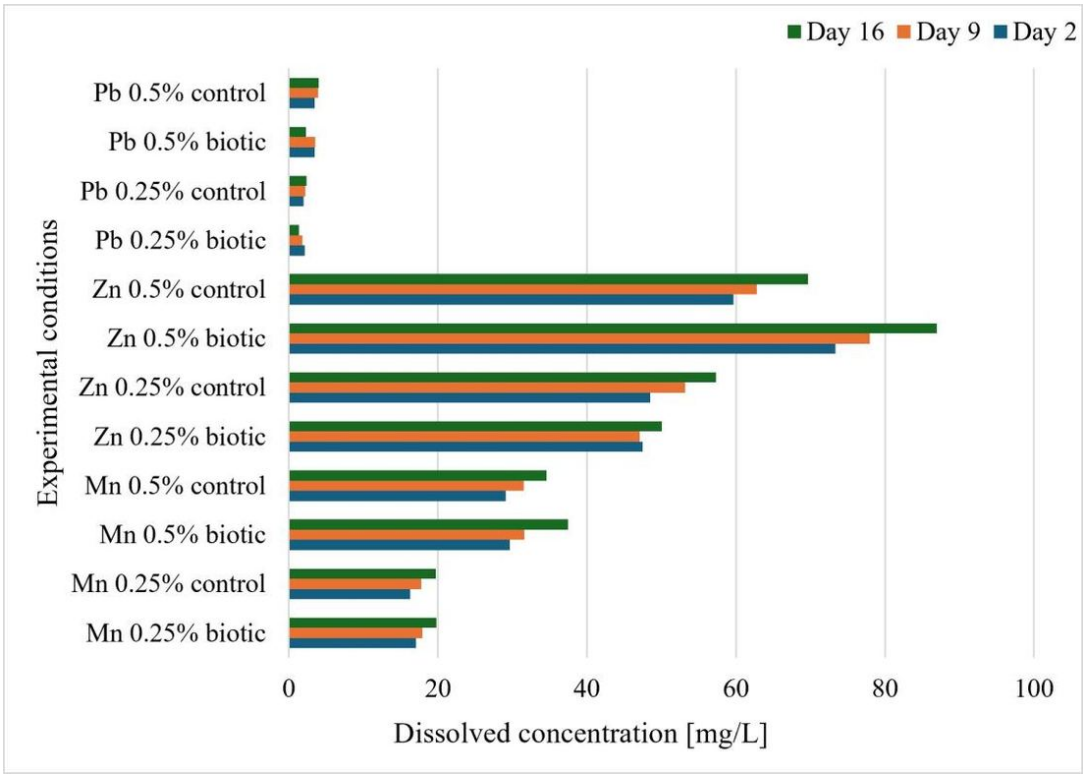


Figure 1. Time-course of dissolved Mn, Zn and Pb at days 2, 9 and 16 for biotic and abiotic control reactors at 0.25% and 0.5% pulp density.

Lead. Pb behaved opposite to the other metals: dissolved Pb declined over time in biotic reactors while rising in abiotic controls, giving lower biotic than control extraction at both pulp densities. This is consistent with secondary precipitation of Pb as anglesite ($PbSO_4$) and/or co-precipitation with jarosite, as bacterial iron and sulfur oxidation raised sulfate and ferric-iron concentrations; anglesite formation has been shown to hold dissolved Pb at low levels in *A. ferrooxidans* cultures in sulfate-rich systems [5]. Pb is therefore mobilised by acid but re-immobilised under active biotic conditions, an important constraint for any Pb-recovery route.

Redox and pH dynamics. ORP rose over the run to 580-600 mV in the active biotic reactors, confirming microbial oxidation of Fe^{2+} to Fe^{3+}. Reactors required repeated H_2SO_4 additions in the first days to hold pH 2.0, after which pH stabilised, consistent with establishment of an acid-generating population.

Implications. The dust is a genuinely polymetallic feedstock, but the target metals respond very differently: Mn and (partly) Zn report to the leachate, whereas Pb is re-immobilised under bioleaching conditions. Hence the process design must therefore be metal-specific.

Conclusions

Bioleaching of steel milling dust with *A. ferrooxidans* achieved endpoint mobilisation of nearly 30% Mn and 10% Zn at lower pulp densities over 16 days, with active iron oxidation confirmed by rising redox potential. The bacterial contribution was modest and metal-specific: significant for Zn only at higher pulp density, negligible for the strongly acid-driven Mn, and negative for Pb, which re-precipitated under biotic conditions. These findings confirm the technical feasibility of biohydrometallurgical valorisation of steel dust while underlining that metal-specific chemistry must govern process optimisation and downstream recovery within a circular-economy framework.

Acknowledgements

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Use of autotrophic cyanobacteria for gold leaching from printed circuit boards

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The present study investigates the potential of bio-cyanidation for gold recovery from waste printed circuit boards (PCBs), a significant source of electronic waste containing valuable metals. Two strains of cyanobacteria, Jaaginema sp. TAU-MAC 0110 and Cyanocohniella hyphalmyra TAU-MAC 3117, were employed as biological cyanide producers. Through process optimisation, cyanide production was significantly enhanced, with increases of 28% and 54% for the respective strains, demonstrating the effectiveness of strain-specific improvements.

Initial leaching experiments using Jaaginema sp. TAU-MAC 0110 achieved 30% gold recovery from PCB samples. While these results are promising, further optimisations—such as adjusting pH and nutrient availability—are required to improve extraction efficiency. Future research should focus on scaling up bio-cyanidation processes, investigating microbial consortia for enhanced cyanide synthesis, and assessing the economic viability of this method compared to traditional cyanidation.

This study contributes to the development of eco-friendly, biobased metallurgical techniques, offering a sustainable alternative for precious metal recovery from complex waste streams. Further advancements in bio-cyanidation could revolutionise gold extraction, particularly in regions with stringent environmental regulations.

Development of a monitored bio-leaching process for precious metal recovery from Waste Electrical and Electronic Equipment

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Abstract:

The increased usage of electronic devices has led to a rise in the production of Waste Electrical and Electronic Equipment (WEEE), which is still a poorly recycled waste product. In addition to the dispersion of toxic metals, valuable resources such as critical and precious metals are lost due to the industry's inefficient methods. This situation calls for new methods that are more environmentally friendly and efficient. We have developed a global approach that represents a paradigm shift in Printed Circuit Boards (PCB) recycling. First, PCBs are disassembled, and then the recovered loose electronic components (ECs) are sorted based on their chemical composition. [1-2] This enables the ECs to be sorted into bins that are enriched in the targeted (and usually spent/lost) metals therefore reducing the complexity of the overall bins' composition. Therefore, dedicated and economically viable hydrometallurgical post-processing can now be used to extract these metals, with a reduced environmental impact. This could be achieved by increasing the use of bio-hydrometallurgical methods that rely on acidophilic microorganisms to regenerate an oxidant in an aqueous medium. [3-4]. These bacteria could be used in the overall process for the recovery of critical or precious metals.

In this presentation, we will report on our yet unpublished results using iron-oxidising bacteria for the oxidation of PCBs and recovery of these metals. We will also discuss how key elements such as metal and sulfate concentrations, as well as biomass evolution, could be successfully tracked on-line and in real time using faster & integrated techniques. This approach enabled us to maintain sufficiently low concentrations of metals that are toxic to bacteria, allowing for continuous extraction with minimal need for new oxidants in the regeneration step, and to recover metals not only in an economically viable fashion but with a lower carbon footprint.

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Keywords:

WEEE, iron-oxidising bacteria, metal recovery, circular economy

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Biorecovery of copper from printed circuit boards using seawater

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Abstract: Printed circuit boards (PCBs) are among the most generated waste streams worldwide, representing up to 6% of the total weight of electronic waste. PCBs contain up to 40 wt% of metals, with copper being the main metallic source (up to 25 wt%), which, along with PMGs, is the primary target for e-waste recyclers.

The recycling of the metal fraction of PCBs can be addressed through pyrometallurgical and hydrometallurgical methods. In the latter case, the use of seawater appears to be an attractive approach since the presence of chloride ions could have a positive impact on the leaching process. Attempts to bioleach metals from PCBs in chloride media require the use of halotolerant bacteria that would accelerate the chemical process through a joint action of inorganic acids (acidolysis) and oxidizing agents (Fe^{3+}) (redoxolysis) excreted by the bacteria and complexing agents (Cl^-) (complexolysis) capable of dissolving the metals by chelation.

The experiments were conducted on solder-free custom-made unpopulated PCBs of known composition. The PCBs were leached in stirred tank reactors under mechanical or magnetic stirring at room temperature or 40º C, respectively. Periodically, pH and redox potential were monitored and copper in liquid samples was determined by XRF. Two complementary approaches were evaluated: chloride chemical leaching versus chloride-based bioleaching using a bacterial strain of *Acidihalobacter ferrooxidans* sp, at different Fe^{3+}concentrations and using a pulp density of 10 g/L. Experiments were run for a period of ≥ 55 hours, resulting in higher copper extractions with milled than with shredded PCBs. In addition, toxicity tests showed that bacterial cells were sensitive to copper concentrations above 500 mg/L.

Keywords: Bioleaching, chemical leaching, printed circuit boards, halotolerant bacteria, seawater

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Optimisation of a biological process for recovering metals from eco-designed, bio-based Printed Circuit Board (PCB) substrates.

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The recycling rate for electronic waste is less than 43% on all continents, while 62 million tons of electronic waste were produced in 2022[1]. The DESIRE4EU project aims to create a sustainable printed circuit board assembly (PCBA) with an eco-friendly life cycle. It requires simultaneous consideration of the design, production and end-of-life. This study aims to optimise metal recovery with a specific bioleaching process from a PCBA designed with a bio-sourced and biodegradable substrate (composite made from flax-fiber reinforced, flame-retarded polylactic acid: PLA/flax).

The effectiveness of bioleaching depends on biological, physicochemical variables and design of the boards. Our micro-reactor tests first demonstrated that designing a card with flame-retarded PLA/flax, instead of traditional FR4 (composite made of fiberglass and brominated flame retarded epoxy) did not affect the bioleaching process, which permitted to recover 77% of Cu in less than 6 days. The bioleaching process was found to be under the control of leached metals toxicity to bacterial strains (in particular *Acidithiobacillus*). A bacterial consortium was therefore isolated from a Cu-rich mining waste and grown under controlled reproducible conditions. The consortium retained sufficient metabolic capacity even when increasing exposure to metal-rich leachates of PCBA. Tests are underway to identify the impact of different variables on the bioleaching process (composite materials, inoculum, pH, temperature, stirring, ferric iron concentration). A response surface plan was applied to minimise experimental execution while considering the effects of interactions between variables. The process will ultimately be modelled using the CORICO software to determine the optimal conditions of metal recovery rates.

These results will contribute to the development of innovative biotechnologies which will permit to anticipate the end of life of these new PCBA and confer them a virtuous life cycle.

Keywords: Metals bioleaching; Printed circuit boards (PCBs); Response surface methodology (RSM).

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Integrated Biotechnological Systems for Sustainable Metal Recovery and Plastic Upcycling from E-Waste

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Abstract: The rapidly increasing volume of electronic waste (e-waste) calls for sustainable recycling strategies to recover valuable metals and reduce the environmental burdens associated with traditional extraction processes. As part of a circular bioeconomy, this study investigates the integration of biotechnological approaches—microbial bioleaching, enzymatic bio-recovery, and biomineralisation—with mechanical and chemical recycling methods to enhance metal recovery from complex e-waste matrices. The combined application of biological, hydrometallurgical, and pyrometallurgical processes offers an environmentally friendly and efficient strategy to maximise yields while reducing secondary pollution and economic impacts. Genetically engineered microorganisms, including specialised yeasts, fungi, and bacteria, are developed to enhance tolerance and specificity toward targeted metals.

Additionally, the biotransformation of plastics from e-waste into value-added compounds is explored, emphasising microbial attachment, enzyme efficacy, transformation of polymer monomers induced mechanical, thermal and chemical changes in the polymers and additives, and mass variation in polymer blends. Predictive modelling of microbial dynamics and enzyme kinetics supports process optimisation, thereby enabling efficient and scalable bioprocessing methodologies that require reduced pre-processing of plastic and metals and lower energy-intensive operations for circular recovery.

Keywords: *Biotechnology, Circular Economy, Electronic Waste, Metal Recovery, Plastic*.

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Mobilisation of metals from e-waste by redox-active microorganisms

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The ever-increasing demand for electronics is challenging due to scarcity of critical raw materials while current practices for mining and disposal of end-of-life products create hazards for both humans and environment. Current approaches such as hydro- and pyrometallurgy straggle with low specificity, dissipation of metal along the process, high-cost and negative environmental impact. Therefore, innovative and sustainable recycling processes are needed. In this study, we set out to determine whether redox-active microorganisms can be employed for metal recovery from printed circuit boards (PCB's).

The capacity of Fe(II)-oxidising and Fe(III)-reducing bacterial strains to leach metals from PCB's was assessed via batch incubations. Metal concentrations were monitored via Inductively Coupled Plasma – Mass Spectrometry.

To test the effect of microbially mediated oxidative metal dissolution from PCB pieces, an acidophilic (pH 2) 50% v/v culture of *Acidithiobacillus ferrooxidans* (Af) was compared to filter-sterilized spent medium from the Af culture and an abiotic control (PCB in medium). Spent medium proved most effective in bioleaching metals (amongst which: Fe, Cu, Mg, Ni, Ba, Zn Cr, Au) followed closely by *Acidithiobacillus*. The abiotic control released metals at only a slightly lower level (e.g. Cu release 89% of the spent medium release) indicating low pH and/or absence of microbial biomass known to sorb metal ions, is the main driver.

Potential for metal mobilisation via reductive dissolution was evaluated by incubations of PCB piece with *Geobacter metallireducens* (GM), *Geobacter sulfurreducens* (GS), *Shewanella oneidensis* (SO) or an abiotic control (PCBM). Only GM exhibited increases in iron and manganese, while copper increased in all incubations. Palladium increased for all except SO and GS, which suggests microbially driven redox changes of palladium lead to its precipitation with these strains.

Overall, bacterial strains proved to affect metal release from PCB's differently and *Aciditihiobacilus ferrooxidans* and *Geobacter metallireducens* were identified as promising candidates for further studies.

Topic: Bioprocessing, Industrial Ecology and the Circular Economy in Mining and Minerals Processing

Microalgae-Enabled Biorefining Metallurgy: Converting CO_2 and Metallurgical Wastes into Sustainable Battery Material Precursors

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Abstract: The increasing demand for nickel-based materials driven by battery and energy-transition technologies has intensified metallurgical activities, resulting in the generation of large volumes of ferronickel slag and substantial greenhouse gas emissions. While ferronickel slag is commonly regarded as waste, it represents a chemically reactive secondary resource that is rich in alkaline earth and transition metals, offering opportunities for integrated waste valorisation and carbon management. This study introduces a microalgae-driven biorefining metallurgy concept that integrates CO_2 utilisation with the valorisation of metallurgical wastes into sustainable battery material precursors**,** advancing circular economy-based mineral processing.

Ferronickel slag from PT ANTAM Tbk was subjected to direct bioleaching using *Bacillus nitratereducens* strain SKC-2a, producing a metal-rich pregnant leach solution (PLS) containing Ca, Mg, and Fe. Bioleaching experiments were conducted under facultative anaerobic conditions for 10 days using −200 +270 mesh (53–74 μm) slag. The addition of acetic acid enhanced metal extraction, achieving maximum recoveries on day 10 of approximately 42% Ca, 3% Mg, and 10% Fe. Mineralogical and compositional changes in the slag before and after bioleaching were confirmed by XRF, XRD, FTIR, and SEM-EDS analyses, indicating effective mobilisation of target elements.

The resulting PLS was subsequently utilised for biologically assisted CO_2 mineralisation by combining carbonic anhydrase derived from spent microalgae medium with controlled CO_2 injection. The effects of enzyme concentration, pH regulation, and gas flow rate on carbonate precipitation were systematically investigated. Optimal bio-CCUS (carbon capture, utilisation, and storage) performance was achieved at 10% enzyme addition, a controlled pH of 11, and a CO_2 flow rate of 0.15 L min^{-1}, yielding high conversion efficiencies of approximately 82% Ca, 17% Mg, and 90% Fe into stable carbonate phases.

These results demonstrate the feasibility of integrating bacterial bioleaching and microalgae-assisted CO_2 utilisation into a unified biorefining metallurgy approach. By converting metallurgical waste and captured CO_2 into carbonate-based precursors for battery-related and energy storage materials, this work highlights the role of bioprocessing in enabling low-carbon, circular pathways within mining and minerals processing.

Keywords: Biorefining metallurgy, Microalgae-assisted CO_2 utilisation, Bio-CCUS, Ferronickel slag, Carbonate mineralisation

Acknowledgements: The authors acknowledge PT ANTAM Tbk for the provision of ferronickel slag samples.

Green Approaches to Heavy Metal Removal: Copper Biosorption Using Alga Biomass and Agro-Wastes

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Keywords: [biosorption; Fucus vesiculosus; agricultural wastes; copper removal; sustainable water remediation

Abstract

Heavy metal contamination of aquatic environments, particularly by copper (Cu), poses serious risks to ecosystems and human health, creating a need for sustainable and cost-effective remediation technologies. Biosorption has emerged as an environmentally friendly alternative to conventional treatment methods due to its efficiency and use of abundant natural materials. This study evaluated the Cu biosorption potential of *Fucus vesiculosus* biomass and agricultural waste-derived biosorbents (pomegranate peel, almond shell, walnut shell, eggshell, and corn husks) under different pH conditions. Comprehensive characterisation was conducted to examine the influence of biomass composition and surface functional groups on adsorption performance. Among the tested materials, *F.vesiculosus* demonstrated the highest adsorption capacity, achieving up to 81% Cu^{2+} removal from synthetic solutions at pH 6. Kinetic analyses showed that copper uptake followed both pseudo-first-order and pseudo-second-order models, suggesting the combined contribution of physical adsorption and chemisorption, while solution pH strongly influenced adsorption efficiency. These findings demonstrate that low-cost biosorbents derived from marine biomass and agricultural wastes offer an effective and sustainable alternative for copper removal from aqueous systems. The study highlights the potential of biosorption to support green remediation technologies, resource recovery, and the protection of aquatic environments from heavy metal pollution.

Introduction

Heavy metal contamination caused by mining, metallurgy, electroplating, and other industrial activities has become a major environmental concern due to the persistence and toxicity of metal ions [1]. Among these contaminants, copper (Cu^{2+}) is extensively used in industrial processes and is frequently detected in contaminated wastewater. Conventional treatment technologies, including chemical precipitation, ion exchange, and membrane filtration, are effective but often involve high operational costs, significant energy consumption, and the generation of secondary waste [2, 3]. Therefore, the development of sustainable and cost-effective alternatives for copper removal has become an important research priority. Biosorption has emerged as an environmentally friendly and economical technology for heavy metal remediation, utilising biological materials rich in functional groups capable of binding metal ions [4, 5]. Agricultural wastes and marine macroalgae are particularly attractive biosorbents because they are abundant, renewable, inexpensive, and contain carboxyl, hydroxyl, and sulfate groups with high affinity for metal ions [6, 7]. Among macroalgae, *F. vesiculosus* has demonstrated remarkable adsorption capacity owing to its alginate- and fucoidan-rich cell walls, which provide numerous active binding sites for divalent metal ions [8, 9].

Although the biosorption potential of algae and agricultural residues has been widely investigated, comparative studies evaluating *F. vesiculosus* alongside locally available agricultural waste biosorbents under identical experimental conditions remain limited. In addition, the influence of solution pH on adsorption performance and biosorption kinetics requires further investigation to optimise the process for wastewater treatment practical applications. Therefore, the aim of this study was to compare the Cu^{2+} biosorption performance of *F. vesiculosus* and selected agricultural waste

biosorbents, evaluate the effect of solution pH on adsorption efficiency, and investigate the biosorption kinetics of the most efficient biosorbent to identify the optimal parameters and conditions for copper removal.

Methods

All procedures are explained in Figure 1.

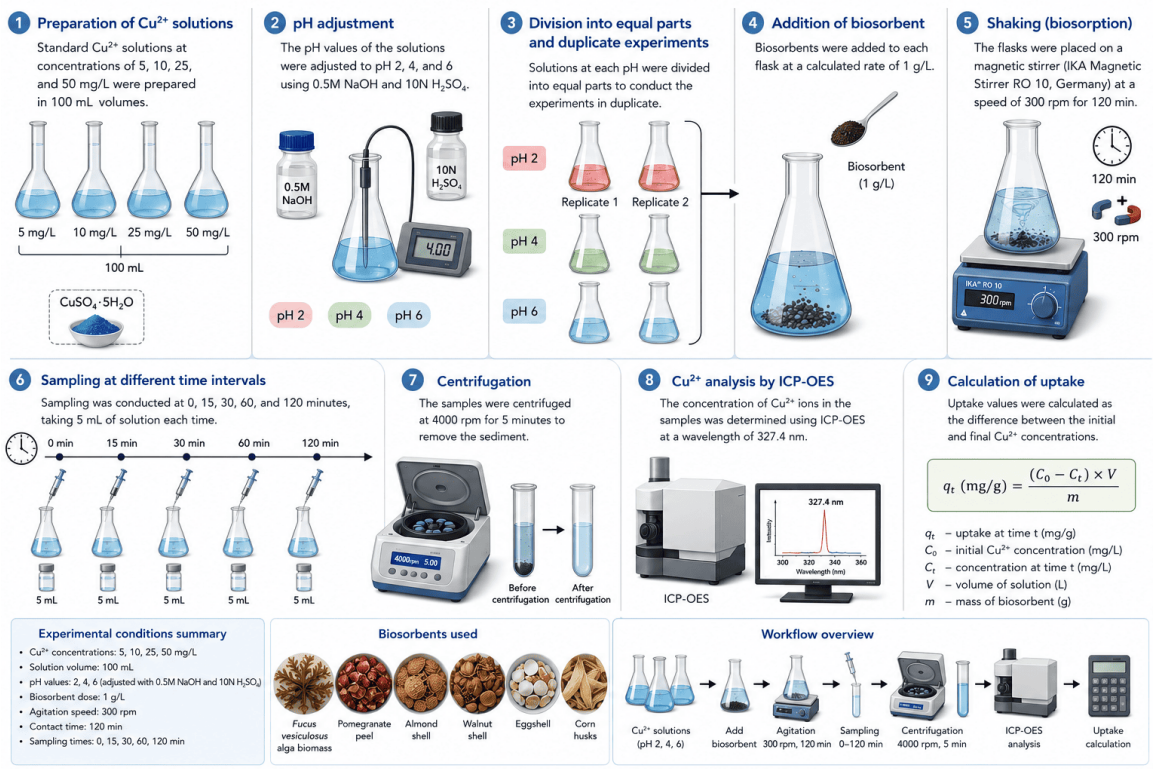


Figure 1. Overview of the experimental procedure employed to evaluate Cu^{2+} biosorption by *F. vesiculosus* biomass and agricultural waste biosorbents under different pH conditions, including sample preparation, adsorption experiments, ICP-OES analysis, and adsorption capacity calculations.

Results and Discussion

The preliminary screening of the investigated biosorbents revealed that *F. vesiculosus* exhibited the highest affinity for Cu^{2+} ions, reaching a removal efficiency of 81% (Figure 2). In contrast, the remaining biosorbents achieved considerably lower adsorption efficiencies, with maximum values below 52.1%. Based on its superior performance, *F.vesiculosus* was selected for further investigation. The influence of solution pH on Cu^{2+} biosorption was evaluated at pH 2, 4, and 6. For nearly all biosorbents, adsorption increased with increasing pH, with the highest metal removal observed at pH 6. The only exception was grape residue, which exhibited its maximum adsorption capacity at pH 4. These findings indicate that solution pH plays a crucial role in regulating the availability of active binding sites and the interaction between Cu^{2+} and the biosorbent surface. The outstanding adsorption performance of *F. vesiculosus* is attributed to the abundance of functional groups, including carboxyl and sulfate moieties, associated with alginate and fucoidan present in the brown algal cell wall. These groups provide numerous binding sites capable of forming stable complexes with metal ions. Similar observations have been reported in previous studies investigating the biosorption properties of brown algae [10]. The enhanced copper uptake at pH 6 is also consistent with the established mechanism of metal biosorption, where reduced proton competition at near-neutral pH promotes interaction between Cu^{2+} ions and negatively charged functional groups [11]. Overall, these results identify *F. vesiculosus* as the most promising biosorbent among the tested materials and confirm that pH optimisation is essential for maximising Cu^{2+} removal efficiency.

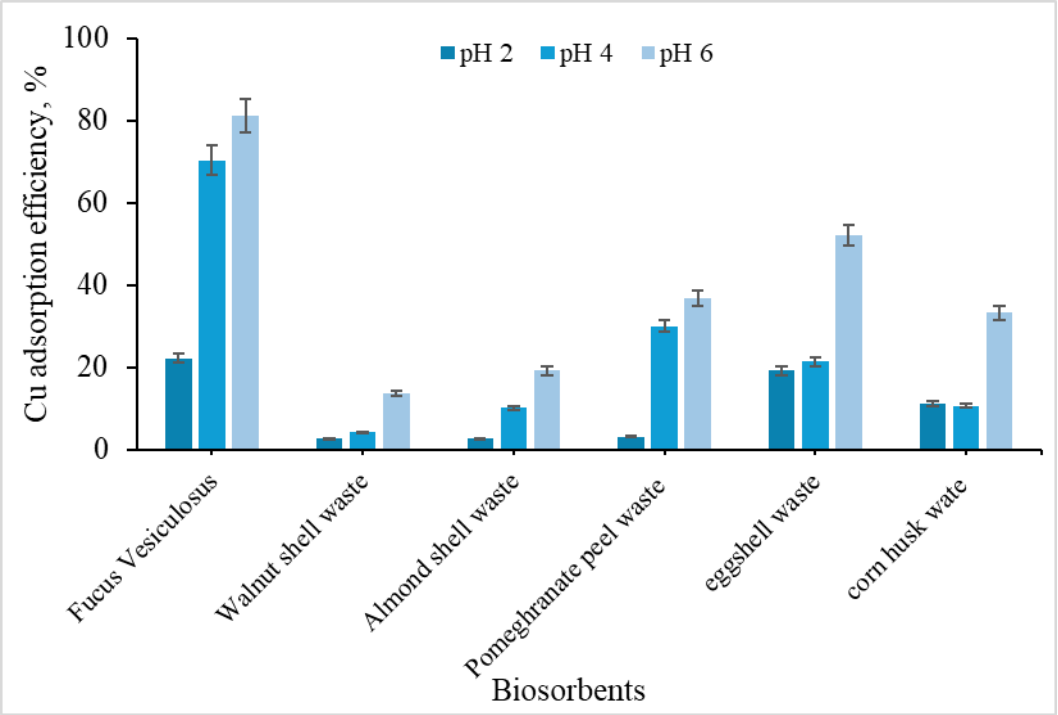


Figure 2. Removal of copper ions (%) from solution using different biosorbents (each biosorbent dose 1 g/L, copper concentration 25 mg/L).

The kinetic analysis of Cu^{2+} biosorption by *F. vesiculosus* demonstrated a clear influence of solution pH on the adsorption mechanism (not provided). At pH 4, the experimental data were best described by the pseudo-first-order and pseudo-second-order kinetic models, indicating favourable adsorption conditions with chemisorption as the predominant mechanism. In contrast, the Elovich model provided the best fit at pH 6, suggesting increased surface heterogeneity and more complex adsorption interactions resulting from changes in surface charge and Cu^{2+} speciation. These findings confirm that pH is a key factor governing biosorption kinetics by affecting the protonation state of functional groups and the availability of active binding sites, highlighting the importance of pH optimisation for efficient copper removal.

Conclusions

The results demonstrate that *F. vesiculosus* is an efficient and sustainable biosorbent for Cu^{2+} removal, exhibiting the highest adsorption performance among the investigated biosorbents. Solution pH significantly influenced biosorption efficiency, with maximum copper uptake achieved at pH 6. Kinetic modelling indicated that adsorption behaviour varied with pH, with the pseudo-second-order model describing the process under optimal conditions, suggesting the predominance of chemisorption. Overall, these findings highlight the importance of biosorbent selection and pH optimisation for enhancing copper removal and support the potential application of *F. vesiculosus* in environmentally friendly wastewater treatment technologies.

Acknowledgements

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Bio-Electrochemical Lithium Recovery from Coal Mine Wastewater: A Circular Economy Approach

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Abstract: The increasing global demand for lithium, driven by the rapid expansion of energy storage and electric vehicle technologies, has intensified the need for sustainable and alternative lithium recovery methods. The global surge in electric vehicle production and energy storage deployment has turned lithium into a critical strategic resource, sparking a search for new sustainable recovery methods and resources. Coal mine wastewater often overlooked and environmentally problematic presents a promising source, though its complex nature and trace lithium concentrations make extraction difficult.

In this study, we propose the feasibility of using a Microbial Electrolysis Cell (MEC) as an innovative, low-energy approach for lithium recovery from coal mine wastewater. Coal mine wastewater collected from the Lingaraj site in Orissa (India), was characterized prior to treatment and contained 200 ppm lithium (Li), along with 60 ppm NH_4^+, and 155 ppm SO_4^{2-}. The MEC system employed electroactive microorganisms at the anode to drive bioelectrochemical oxidation under an externally applied voltage, facilitating selective ion migration and lithium enrichment at the cathode through selective ion-exchange membranes in the presence of competing ions. Operational parameters including applied voltage, pH, and ionic strength were optimized to enhance lithium recovery efficiency and selectivity. In addition, the potential integration of evapotranspiration-based concentration as a pre-treatment or hybrid step was evaluated conceptually. Evapotranspiration can reduce wastewater volume and further increase lithium concentration prior to bioelectrochemical processing, thereby improving overall recovery efficiency.

Experimental results demonstrate that the MEC achieved significant Li enrichment in the catholyte, with recovery efficiencies exceeding conventional physicochemical methods under comparable conditions. Additionally, the system can reduce energy consumption and lower chemical input requirements, highlighting its potential as a cost-effective and environmentally sustainable alternative. This work presents a proof-of-concept for coupling wastewater treatment with critical mineral recovery using microbial electrochemical technologies. The findings suggest that MEC-based lithium recovery could play a valuable role in circular economy strategies for mining-impacted waters.

Keywords: Coal-mine wastewater, Lithium recovery, Microbial electrolysis cell, Bioelectrochemical Technology.

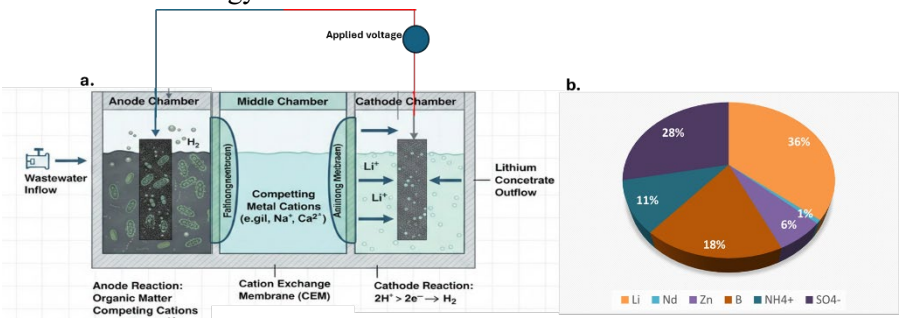


Figure 1a shows the MEC setup for Li recovery, and 1b shows the concentration of ions in coal-mine wastewater.

Toward Pilot-Scale Bioleaching of Printed Circuit Boards: Effects of Solder Mask Removal and Lixiviant Reuse

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Keywords: Bioleaching, PCB-recycling, Copper recovery, Surface-pretreatment, Lixiviant reuse

Abstract

The increasing generation of electronic waste and demand for secondary metals motivate the development of lower energy processes for recovering copper from waste printed circuit boards (PCBs). This evaluates two process intensification strategies for the bioleaching of whole, unsorted PCBs: chemical removal of the solder mask and sequential use of the bioleaching lixiviant. The solder mask polymer layer on PCBs hinders leaching efficiency. PCB samples were leached in shake flasks using either a mixed acidophilic consortium comprising *Aciditiobacillus ferooxidans*, *Leptospirillum ferrooxidans* and *Acidithiobacillus thiooxidans*, or a pure *A. ferrooxidans* culture. Dissolved copper was subsequently recovered by cementation with iron. Solder mask removal increased copper recovery from 30.38% to 50.83%, corresponding to an increase of 20.45 percentage points, and shortened the mean leaching time by 1.5 days. For decoated PCBs, the mixed consortium achieved a higher copper recovery than the pure *A. ferooxidans*, with values of 54.6% and 36.9%, respectively. Sequential lixiviant reuse initially improved copper recovery, but later reuse cycles resulted in lower purity and reduced copper recovery. This deterioration was associated with the accumulation and precipitation of iron introduced during cementation and became apparent at estimated iron concentrations near or above 20 g/L. The results demonstrate that solder mask removal can improve the bioleaching of whole PCBs. However, controlled lixiviant replacement, iron management, and further biological characterisation are required before repeated reuse can be implemented in a pilot-scale process.

Introduction

As the production of electronic devices increases, so will the amount of e-waste, in addition to the growing demand for metals. Conventional recycling methods often require high energy consumption and generate highly acidic or basic waste, resulting in high operational costs [1]. Bioleaching is therefore gaining traction as an alternative to recycle electronic waste, such as waste printed circuit boards (PCBs). A large proportion of these studies cut, shredded or pulverised the PCBs before further treatment and leaching to speed up the slow kinetics associated with bioleaching by increasing the reaction surface area through comminution. However, this method has several disadvantages such as metal loss through dust, difficulty to separate the non-metallic PCBs remains from the formed precipitate complicating recycling of the non-metallic remains [2], a risk of a sudden increase in heavy metal toxicity to the bacteria, and the extra implementation cost. On the other hand, using whole PCBs could create complications in calculations and experiments by the heterogeneity of electronics and PCBs, as identifying the metal content in PCBs before leaching would be complicated.

Previous laboratory trials within the framework of a preceding project indicated slow kinetics during leaching with sub-optimal recovery yield. This is largely attributed to the effect of a polymer soldering mask layer sprayed on the plates during production (so called solder resist) that prevents bacteria and iron(III) from reacting directly with the copper in the plates [3]. It was therefore decided to investigate the possibility of employing pretreatments for the PCBs as a mitigation measure. In preparation for

scaling up the entire PCB recycling method to pilot plant scale, the feasibility of reusing lixiviant was also investigated.

Methods

Material

Bioleaching experiments were performed using two established bacterial cultures, which were maintained in self-designed fermenters. The cultures had been cultivated previously, one consisting of a mixed consortium of *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans* and *Acidithiobacillus thiooxidans* and the other of a pure culture of *Acidithiobacillus ferrooxidans*. The cultures were cultivated under acidic conditions and used as inocula for the bioleaching experiments.

The bacterial cultures were cultivated in a 9K nutrient medium containing iron and mineral salts (3.0 g/L $(NH_4)_2SO_4$, 0.5 g/L K_2HPO_4, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.1 g/L KCl, 0.01g/L $Ca(NO_3)_2$ and 44.2 g/L $FeSO_4 \cdot 7H_2O$, corresponding to approximately 9 g/L of iron). The nutrient medium was prepared by separately dissolving the mineral salts and the ferrous sulfate source, before combining both solutions. The final medium pH was adjusted to 2.3. 1 mL of a trace element solution containing cobalt, manganese, zinc, molybdenum, nickel, copper, selenium and boron was added to ensure the availability of micronutrients for bacterial growth and metabolic activity.

PCBs from a variety of manually recycled electronics, were used as the primary recycling material in this study. To better simulate the real-life operation of the pilot plant, the PCBs were not sorted further by type. The study used an array of cell phone boards, computer random access memory (RAM) sticks and other unidentifiable PCBs. Due to the heterogeneity, the initial copper mass in the PCBs were estimated to be 24.7% [4] of the PCB weight after pretreatment.

Bioleaching Procedure

The bioleaching procedure is divided into three main parts: decoating as a pre-treatment, copper leaching and cementation to recover copper. When applicable, the decoating process involves submerging PCBs in a solution based on 10% (w/w) KOH at 120 °C for at least 90 minutes, until the soldering mask layer is completely detached. The PCBs are then removed and rinsed with deionised water (pH 4.4) before being dried and weighed.

Bioleaching experiments were conducted in 500 mL Erlenmeyer flasks using lixiviants cultivated from laboratory fermenters. The aforementioned consortia and pure *Ac. ferrooxidans* cultures were introduced to evaluate their performance. Pre-weighed PCB samples (6–8 g) were submerged in the lixiviants and 10 N sulfuric acid was added dropwise to adjust the pH value to <2.0. The flasks were then incubated in an orbital shaker at 40 °C and 105 rpm. The pH value, temperature and oxidation-reduction potential (ORP) were monitored twice daily, with 10 N H_2SO_4 added dropwise as required to maintain a pH <2.0. In both pH control procedure, the number of H_2SO_4 drops were recorded. The PCBs were removed when no visible brown copper layer remained on the surface, showing no further dissolution of copper as Cu^{2+}. This simulates a plant-scale operation, where the overall batch would consist of multiple PCBs and individual readiness could not be guaranteed. After leaching, the remaining PCBs were removed, rinsed with deionised water and dried. The leachate was first passed through a 1 mm sieve to retrieve the larger components that fell from the PCBs (mostly ICs), then the fluid through filter paper to filter a sludge-like substance containing the metals from the detached solder joints. These fractions were separately washed before being dried, weighed and passed through X-ray fluorescence (XRF) to determine the metal fractions.

For cementation, dissolved copper was recovered using pre-weighed, non-stainless steel plates measuring 35 x 35 x 2 mm with a mass of around 10 g as sacrificial iron plate. During this process, the iron in the sacrificial iron plate reduced the dissolved copper ions, resulting in the precipitation of elemental copper. The flasks were placed in an orbital shaker at a temperature of 40 °C and a speed of 105 rpm. The pH and temperature were monitored twice daily, and 10 N H_2SO_4 was added dropwise as required to maintain a pH of < 2.0. The process was considered complete when the characteristic blue colour of the copper ions had disappeared and no further elemental copper formation was observed. The sacrificial iron plate and the precipitated copper were then washed and dried at 40 °C before being weighed. The

purity of the recovered copper fractions was determined by XRF. To test whether decoating improves the leaching yield, four batches consisting of ten trials in total were conducted: four without decoating pretreatment and six with it. All batches were conducted in duplicate, with one set using the consortia culture and the other set using the pure *A. ferrooxidans* culture as lixiviants.

To evaluate the effect of reusing lixiviants, two batches comprising four consecutive trials were conducted using a mixture of 200 mL consortia culture and 100 mL pure culture as lixiviants. After the aforementioned procedures, including decoating, leaching and cementation, the batches were reused in a new round with new PCBs to be recycled. Ten percent (v/v) of fresh, unused lixiviant (20 mL consortia culture and 10 mL pure *A. ferrooxidans* culture) was replenished between rounds to compensate for evaporation and potential nutrient loss. The reuse cycle continued several times.

Results and Discussion

Several bioleaching trials demonstrated that the chemical pretreatment effectively removed the solder mask layer from the PCB surface, thereby exposing more of the underlying copper. In trials comparing decoating effectiveness, the Cu yield with and without decoating pretreatment was 30.38% and 50.83%, respectively ($p < 0.01$), while the leaching procedure took an average of 1.5 days less with decoating pretreatment. This suggests that decoating is an effective pretreatment method for increasing the effectiveness of leaching.

Conversely, for decoated PCBs, the lixiviant solutions produced by the consortia culture outperformed those produced by the *A. ferrooxidans* culture alone, with yields of 54.6% and 36.9%, respectively ($p < 0.01$). This suggests that consortia should be favoured in future experiments.

In the reuse experiments, one of the two reuse batches (batch 5) was reused twice (three cycles), while the other (batch 6 and 8) continued to a fourth reuse (five cycles in total). During cementation, a brown or orange precipitation mixed with the copper recovery was noticed, which always occurred with an overnight spike in the pH value to around 3.5. Figure 1(a) shows a steady increase in copper yield up to the second reuse (R2) cycle, after which the purity of the recovered copper decreased, resulting in a fall in the actual copper yield from the third reuse (R3) onwards. A further trial involving the mixing of half of the fourth reuse (R4) solution and half of a fresh solution (1:1 R4:fresh) was conducted, but the actual copper yield remained at the same level as R4, despite the impurities doubling. This corresponds to the excessive iron introduced from the sacrificial iron plate (Figure 1(b)), which accumulates in the system. This is calculated by the mass difference of the iron plate during cementation and removal by precipitation. It seems that there is an empirical threshold to the iron concentration in the solution at around 20 g/L, where an iron concentration exceeding that introduces excessive iron precipitation, which lowers the purity of the yielded copper.

Decoating was shown to effectively improve yield and speed of bioleaching by removing the soldering mask, which limits the reaction surface area. However, further investigation is needed before it can be implemented in the pilot plant, as the basic solution at an elevated temperature is corrosive to most conventional materials. Additionally, decoating took up to two hours before no greenish colour could be observed on the PCBs, suggesting the possibility of optimisation.

As shown in Figure 1(b), iron concentration was also demonstrated to affect purity and therefore copper yield. The Cu yield increased until the second round of reuse, suggesting an increase in the iron concentration helps increasing the total copper yield. The improvement during the first reuse cycles may reflect changes in microbial activity, iron speciation, residual acidity, or a combination of these effects. Additional biological and chemical measurements are required to distinguish between these mechanisms. However, an iron concentration above 20 g/L is, as shown in the experiments, counterproductive to the process by reducing the purity of recovered copper. Therefore, it may be sensible to plan a larger portion of fresh lixiviant each round to maintain a stable iron concentration.

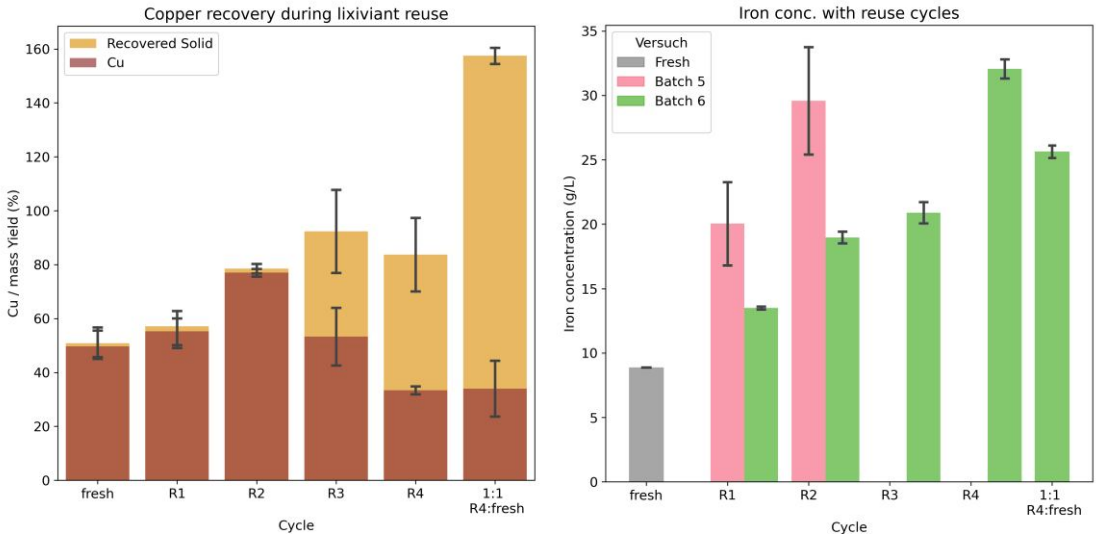


Figure 1. Comparison of (a) the mass of copper recovery with impurities ("Recovered Solid") and copper yield ("Cu") confirmed by XRF, and (b) total iron concentration at the start of each batch. R1 to R4 indicate the reusing cycles rounds, where 1:1 R4:Fresh denotes a 1:1 4th reused and fresh solution. Error bars represent 95% confidant level.

Conclusions

Surface pretreatment with a basic mixture showed promising improvements in leaching efficiency, as evidenced by shorter leaching times and higher copper yields. Although an increased yield was observed in the first and second reuse rounds, further investigation into the mixing ratio of the reuse and fresh solutions to control the iron content is needed.

Acknowledgements

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Particle Size-Dependent Metal Recovery from Waste Printed Circuit Boards by Two-Stage Bioleaching with *Acidithiobacillus ferrooxidans*

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Keywords: printed circuit boards; bioleaching; metal recovery; particle size effect; sustainable recycling

Abstract

The rapid growth of electronic waste (e-waste), driven by increasing demand for electronic devices and their short lifespan, poses major environmental and resource management challenges. Printed circuit boards (PCBs) contain high concentrations of valuable base and precious metals, making them an important secondary resource. This study evaluated the efficiency of a two-stage bioleaching process using biogenic ferric ions produced by *Acidithiobacillus ferrooxidans* 61 as a bio-lixiviant for sustainable metal recovery from waste PCBs. PCB particles were classified into four size fractions, pretreated with 10% NaOH, washed, dried, and subjected to bioleaching. The results showed that copper recovery was highest 100% for particles sized 125–630 μm, providing an optimal balance between surface area and reduced particle agglomeration. Zinc dissolution was more efficient in larger particles (>800 μm), about 87.1%, whereas aluminium recovery was greatest in the finest fraction (<125 μm), 15.58%, highlighting the need for metal-specific particle size optimisation. X-ray diffraction (XRD) analysis revealed significant mineralogical changes, including decreased metallic copper peaks, formation of minor oxide phases, and increased amorphous content, confirming effective copper biooxidation and solubilisation by *A. ferrooxidans*. Overall, the findings demonstrate that bioleaching is an efficient and environmentally friendly approach for recovering valuable metals from e-waste.

Introduction

Advancements in technology and the growing demand for electronic devices have led to shorter lifespans and increased e-waste generation [1]. Global e-waste reached 53.6 Mt in 2019 and is projected to rise to 74.7 Mt by 2030 [2]. Waste printed circuit boards (WPCBs) are a highly valuable fraction due to their high metal content compared to primary ores [3], but they also contain hazardous substances such as flame retardants and heavy metals [4]. PCBs account for 3–5% of e-waste and consist of ~28% metals, 23% plastics, and ceramic/fibrous materials [5]. Copper is the dominant metal (25–30% of total metals), occurring at levels 20–40 times higher than in natural ores [6]. Although precious metals are present only at 0.01–1 wt%, they contribute nearly 90% of PCB economic value [7]. PCBs also contain base metals (Cu, Fe, Sn, Pb, Zn), ceramic phases, and polymeric materials such as epoxy resins [8].

Conventional recycling methods include pyrometallurgy and hydrometallurgy. Pyrometallurgy is energy-intensive and emits toxic compounds such as dioxins [9], while hydrometallurgy consumes large amounts of chemicals and generates contaminated effluents [10]. Despite high recovery efficiency, both methods have significant environmental drawbacks. Biohydrometallurgy provides a more sustainable alternative by using microorganisms for metal extraction under mild conditions with lower energy demand and environmental impact [11]. Acidophilic bacteria such as *A.ferrooxidans*

exhibit strong leaching potential [12], but bioleaching remains limited by slow kinetics and process sensitivity [13]. Two-step bioleaching improves metal recovery by regenerating Fe^{3+}, enhancing stability, and reducing microbial stress [14]. Particle size strongly influences metal liberation, with finer fractions generally improving recovery efficiency [15]. Therefore, this study investigates the effect of particle size on Cu, Al, and Zn recovery from PCBs using a two-step bioleaching process with *A.ferrooxidans* 61. Unlike previous studies that evaluated limited particle-size ranges, this work systematically investigates the influence of PCB particle size on the two-stage bioleaching behaviour of multiple metals using *A. ferrooxidans*, providing guidance for optimising mechanical pre-treatment prior to bioleaching.

Methods

All procedures are presented in Figure 1.

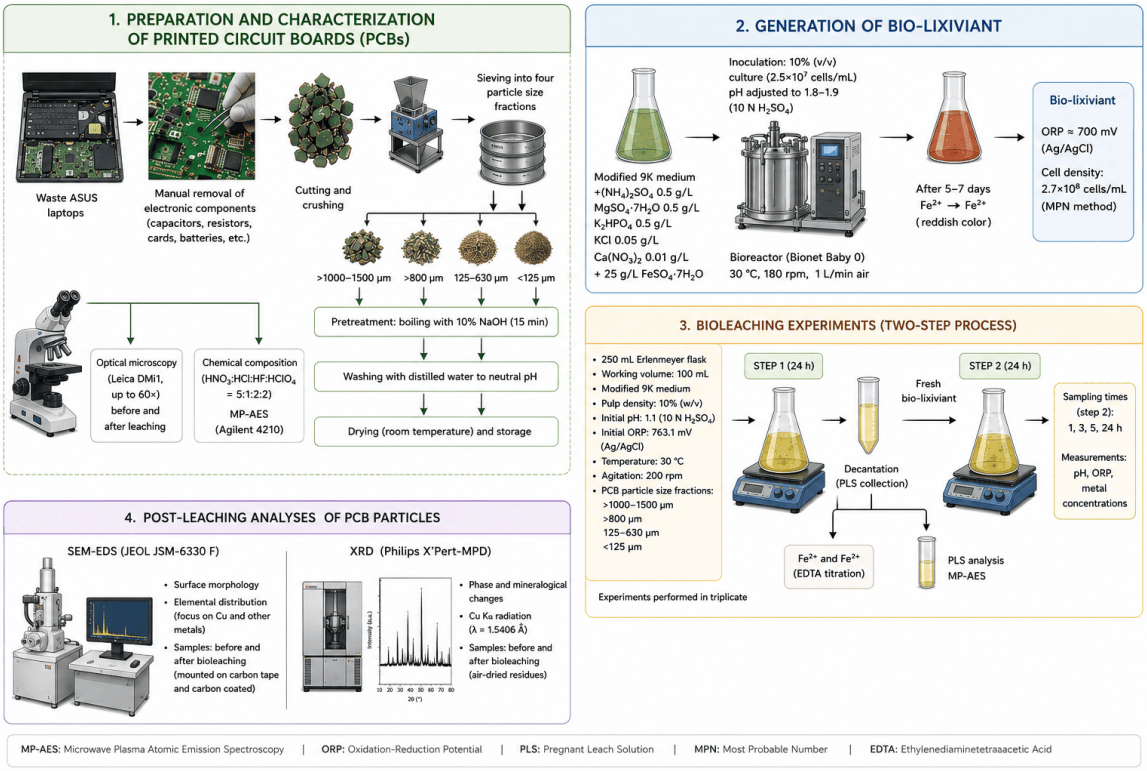


Figure 1. Preparation and characterisation of PCBs, Generation of Bio-lixiviant and Bioleaching experiments.

Results and Discussion

Chemical composition of waste PCBs

The elemental composition of the waste PCBs was determined following acid digestion, and copper was the dominant metal, accounting for 22.56% of the total metal composition, followed by aluminium (6.1%), zinc (3.24%). Other metals are not detected.

Influence of particle size on metal bioleaching

The efficiency of metal recovery varied considerably with PCB particle size (Figure 2). Among the tested fractions, particles ranging from 125 to 630 µm yielded nearly complete copper recovery (99.3%) during the two-stage bioleaching process. In contrast, zinc showed the highest extraction efficiency in the coarsest fraction (>800 µm; 94.3%), whereas aluminium recovery was greatest in the finest fraction (<125 µm, 17.8%). These results demonstrate that the optimal particle size depends on the target metal. Although smaller particles generally provide a larger reactive surface area, reducing

particle size below a certain threshold did not further improve copper extraction. Excessively fine particles may promote agglomeration and increase interactions between particles and bacterial cells, thereby reducing bioleaching efficiency. The observed differences among metals recovery also suggest that bioleaching efficiency is influenced by factors such as metal distribution within the PCB matrix, mineral accessibility, and microbial activity. Overall, the first bioleaching stage accounted for most of the metal dissolution (within the first 24 h). The second stage contributed only marginally to the recovery of Zn and Al, whereas it contributed approximately equally to the recovery of Cu.

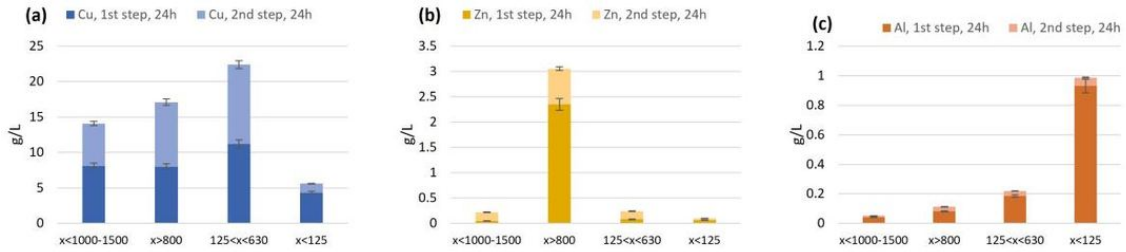


Figure 2. Recovery of metals from PCBs via two-step bioleaching (bio-lixiviant- 20g/L Fe^{3+}; pH 1.0, temp. 30 °C, PD-10%, duration 24 h + 24 h)

Conclusions

Particle size plays a key role in the bioleaching efficiency of metals from waste PCBs. Nearly complete copper recovery (99.3%) was achieved with particle sizes of 125–630 µm, while zinc and aluminium showed the highest recovery at >800 µm and <125 µm, respectively, demonstrating that the optimal particle size is metal-specific. Most metal dissolution occurred during the first 24 h of the two-stage bioleaching process, although the second stage contributed substantially to copper recovery while providing only limited additional recovery of zinc and aluminium. XRD analysis confirmed the biooxidation of copper-bearing phases by *A. ferrooxidans*, as evidenced by mineralogical changes following bioleaching. Overall, these findings demonstrate that optimising particle size and understanding PCB material characteristics are essential for maximising metal recovery from e-waste, thereby supporting the development of more efficient and sustainable biohydrometallurgical recycling processes.

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Investigating Cell Mechanical Adaptation of Chemolithoautotrophic Bacteria in Acidic, Metal-Rich Environments

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Abstract: Chemolithoautotrophic bacteria inhabiting mine ores, tailings, and waste sediments are exposed to extreme environmental conditions, including high metal concentrations, acidity, and variable tonicity, imposing strong selective pressures. To thrive under such stress, these microorganisms undergo dynamic changes in their cell mechanical properties. This adaptation influences survival, nutrient acquisition, and metal uptake. The nature of bacterial cell mechanical changes are multifaceted: shifts in biophysical properties, like turgor pressure, and molecular mechanisms, responsible for setting the architecture of the cell wall, both are critical contributors.

Here, we report on our studies to understand why chemolithoautotrophic bacteria undergo cell mechanical adaptation. Using isopycnic buoyancy centrifugation as a bulk measurement, we assay the limits of mechanical change exogenous stressors impose on bacteria. To define the biophysical dynamics of cell mechanical adaptation, we consequently use an advanced imaging approach, quantitative phase spectroscopy. We unravel how stressors driving mechanical modulation of cells impact both the biochemical properties of bacteria and density.

We offer a foundational framework for defining how and why chemolithoautotrophic bacteria undergo cell mechanical adaptation. These insights reveal how cell mechanical plasticity contributes to survival in shifting environmental stress conditions. These fundamental studies will inform novel strategies for biomining, bioremediation, and metal recovery from mine-impacted sites.

Keywords: chemolithoautotrophs, cell mechanical adaptation, acidic metal-rich environments, buoyancy centrifugation

Metabolic and evolutionary mechanisms of microbial taxa in acid mine drainage

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Acid mine drainage (AMD) is an extreme environment characterised by extreme acidity, high heavy metal pollution and lack of organic materials, which is typically used as models to study the crucial roles of acidophilic microbes in aquatic environments. In our work, metagenomics and culturomics-based approaches were employed to explore the diversity, composition, and ecological functions of microbiomes in global AMD environments with different mineral types. The results revealed a high level of specialisation in the diversity structures and ecological functions of AMD microbial communities based on mineral-type conditions (Fig. 1 & Fig. 2). Additionally, twelve bacterial and two archaeal lineages were discovered for the first time in AMD environments.

Low abundance and richness acidophiles, particularly iron and sulfur metabolism microbes, are functional species in AMD and are integral to the formation of AMD and the geochemical cycling of iron, sulfur, carbon and nitrogen. These strains can also contribute to the improvement of bioleaching efficiency and treatment of pollution. In this study, the microbial biobank of AMD (mbAMD) containing 42 species (including 21 novel species) was constructed by culturomics. mbAMD represented over 80% of the core bacterial taxa identified from global AMD metagenomics data (n = 226), in which 36 taxa were detected as iron or sulfur metaboliser which occupies 70% reported functional species (Fig. 3). A comparative genomic analysis revealed multiple horizontal gene transfer events wide spreading in AMD genomes (Fig. 4). The study expanded the functional microbial groups, and provided new insight into the evolutionary mechanisms of extreme microbes.

Keywords: Acid Mine Drainage, Culturomics, Functional microbes, Metabolic Mechanisms, Horizontal Gene Transfer

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The Project „Green Tech“: Innovative Biorecovery

of Valuable Metals from Mining and Metallurgy Wastes (BioRecoM)

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Abstract: A joint project of interdisciplinary research groups with the title „Innovative Biorecovery of Valuable Metals from Mining and Metallurgy Wastes (BioRecoM)“ started now in Armenia and Germany. Aim of the project is the development of innovative processes of valorisation of mining wastes (dumps, tailings, slags) with biotechnological processes. On the field of microbiology, new microorganism strains and consortia shall be isolated and cultivated, respectively, and identified. Armenian porphyry copper ores shall be further investigated as well as the potential of biohydrometallurgical processes for the utilisation of these ores. The challenge is consisting in the complex mineralogy in the ores and complex gangue mineralisations. The application of modern geoscientific methods like Remote Sensing methods is planned, and microscopical analysis of ore mineralisation and of bioleaching products shall help to unravel the fate of the valuable trace metals during the processing. A valorisation of dumps and tailings shall help to reduce the environmental impact and to improve the saving of resources. The project is an interdisciplinary approach of microbiologists, geologists and biotechnologists. Aim of the project is a sustainable, innovative and efficient utilisation of the given mineral resources in the context of global challenges of raw material support.

Keywords: Bioleaching, microorganisms, complex porphyry copper ores, dump material

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Sulfuric Acid Production by different *Acidithiobacillus thiooxidans* strains

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Abstract: One of the most widely used industrial chemicals for hydrometallurgy is sulfuric acid, yet its conventional production relies on chemical catalysts, and energy-intensive processes. In contrast, sulfur-oxidizing bacteria can convert reduced inorganic sulfur compounds into sulfuric acid under mild conditions. Here, biological sulfuric acid production by different *Acidithiobacillus thiooxidans* on chemical sulfur was investigated. Among them, a representative originally isolated from clay, exhibited the highest sulfur oxidation activity. The highest-, intermediate-, and lowest-performing strains were subsequently investigated on biogas-derived sulfur obtained from various sampling locations of biogas plants. Remarkably, sulfate production from biogas-derived sulfur was 2.5-fold higher than with chemical sulfur for all strains. At 1% sulfur loading, nearly complete sulfur oxidation was seen. Increasing the biogas-derived sulfur load to 6% further enhanced sulfate levels by 7-fold compared to chemical sulfur. The biogas-derived sulfur was analyzed by scanning electron microscopy, water contact angle measurement, ATR-FTIR, and elemental composition. The superior performance of biogas-derived sulfur most likely results from its smaller particle size, higher hydrophilicity as indicated by lower contact angles and due to the presence of organic additives. These findings demonstrate that low-cost biogas-derived sulfur is an excellent source for environmentally friendly microbial sulfuric acid production, offering an alternative to conventional methods.

Keywords: waste sulfur, biogenic sulfuric acid, *A. thiooxidans*

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From biomining to biomanufacturing: scaling up omics-ready sampling in acidic mine waters

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Abstract: Acidophilic microbial communities are central to biomining through bioleaching and metal recovery, yet mine-impacted waters also represent an underexploited reservoir of enzymes and metabolites for wider bioprocessing. Centimetre-scale heterogeneity at redoxclines in mine lakes are biodiversity hotspots with pH shifts of up to two units over one meter. However, However, constrained sample volumes and depth resolution can prevent capturing key metabolic activities. On top of this, waters with low pH harvest low volumetric biomass.

As an exemplary sampling campaign, in May 2025 we sampled the stratified acidic pit lake Cueva de la Mora (Huelva, Spain) with our standard protocol. We sampled seven depths along the water column using a 5-L Van Dorn bottle. Manual syringe-vacuum filtration was labor-intensive and required ~1.5 h per depth. High organic and metal loads limited processed volumes to as little as 0.5 L at some depths. These constraints impacted biomass collection and nucleic acid extraction. Consequently, metagenomes were obtained for only four depths (DNA yield 1.5 ± 1.1 ng mL^{-1} filtered, n=4), and metatranscriptomics for a single depth (RNA yield 0.8 ng mL^{-1} filtered). This campaign underscored the operational limits of sampling corrosive, metal-rich waters.

To overcome this, we are designing 1) high-throughput manual filtration procedures and 2) robotics-assisted sampling that can operate reliably in difficult mining waters. The methodological developments target (i) increasing the number of litres filtered per layer while preserving RNA integrity, (ii) maintaining *in situ* redox/anoxic conditions during collection and transfer, and (iii) improving depth targeting to resolve redoxclines with high resolution. As a result, we generate richer omics datasets, strengthen geochemistry-activity linkages, and accelerate discovery of acidophile functions that extend biomining beyond metal recovery towards production of high-value molecules.

Keywords: acidophiles, acid mine drainage, high throughout sampling, bioprospecting, multiomics.

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Comparative genomics of iron and sulfur-oxidizing bacteria isolated from pyrrhotite tailings from Sudbury, Ontario, Canada and applications to a nickel bioleaching pilot

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Keywords: genomics; pyrrhotite; nickel; microbial metabolism; synergism

Abstract

Nickeliferous pyrrhotite tailings in Sudbury, Ontario, Canada contain diverse communities of iron- and sulfur-oxidizing microorganisms, which were used as the inoculum for a pilot-scale nickel bioleaching study at University of Toronto. To understand their individual metabolic contributions to the process and adaptations to the Sudbury tailings, several of these microorganisms were enriched on selective media, isolated, and underwent whole genome sequencing. Four strains were isolated from Sudbury pyrrhotite tailings and sequenced: *Leptospirillum ferriphilum*, *Sulfobacillus thermosulfidooxidans*, *Acidithiobacillus caldus*, and *Acidithiobacillus ferriphilus*. The organisms contained genes and mobile genetic elements unique from all other sequenced members of their genera, including various plasmids bearing metal resistance genes, highlighting specific selection pressures associated with the Sudbury tailings environment. Genomic data will be used to inform synthetic community design for future nickel bioleaching studies and OMICS-based investigations of metabolism and metal resistance in the reactor community. Plasmids and CRISPR systems discovered in the strains may also be leveraged as tools for genetic engineering of acidophiles.

Introduction

Decades of nickel mining in Sudbury, Ontario, Canada has led to the accumulation of ~100 million dry metric tonnes of tailings containing ~0.8 wt% nickel, mostly within the mineral pyrrhotite ($Fe_{(1-x)}S$, x = 0 to 0.125) rejected from the traditional flotation process [1, 2, 3]. If exposed to oxidising conditions in the environment, tailings can generate acid mine drainage (AMD) which may require costly remediation. The goal of the University of Toronto nickel bioleaching pilot project is to use native iron and sulfur-oxidizing microorganisms from Sudbury pyrrhotite tailings to solubilise this nickel into solution for electrochemical separation, creating value from low-grade material. When coupled with the extraction of elemental sulfur as a marketable product and the formation of environmentally stable iron precipitates, this process can concurrently prevent AMD generation.

While natural communities from fresh tailings have been used as the inoculum for our current operation, the question remains why certain organisms dominate the process and if a synthetic community could perform better. However, the roles of individual community members are difficult to discern in mixed cultures with several variables fluctuating. The Sudbury tailings are known to be hotbeds of microbial diversity, with a novel isolate (*At. ferridurans* JAGS) having been previously isolated from the tailings [4, 5, 6, 7]. The strain had unique physiology and genomic adaptations, including an abundance of genomic islands indicating frequent horizontal gene transfer (HGT), and CRISPR-based gene editing of

the strain allowed for increased iron oxidation and reduced sulfur oxidation with no impact on nickel leaching rates [4, 5, 6]. Two other strains, *Leptospirillum spp.* and *Sulfobacillus thermosulfidooxidans*, were also enriched and isolated from Sudbury pyrrhotite but never sequenced [8]. In the current phase of our pilot project, a wider array of organisms were detected in our reactor using 16S sequencing: *At. ferriphilus, At. caldus, At. ferrianus, S. thermotolerans,* and *Alicyclobacillus aeris*. Our objective is to isolate, sequence, and monitor gene and protein expression of these species under controlled conditions to understand their individual roles in nickel bioleaching and facilitate strain engineering for desired characteristics.

Methods

Enrichment and isolation. Moist Sudbury tailings were incubated in pH 1.7 liquid media at 30 °C or 40 °C until microbial growth was observed by microscopy [8]. Cultures were transferred to media containing 20 mM ferrous sulfate or elemental sulfur, with or without the addition of 0.2% w/v yeast extract, at both temperatures [8]. When microbial growth was observed in 20 mM ferrous sulfate media without yeast extract and elemental sulfur media with 0.2% w/v yeast extract at 40 °C, cultures were streak inoculated onto overlaid solid medium, resulting in purified colonies [8, 9]. Morphological analysis identified the ferrous sulfate culture as a strain of *Leptospirillum*, while the sulfur culture was confirmed to be *S. thermosulfidooxidans* by 16S sequencing [8]. Pure strains were preserved, shipped to the University of Toronto, and transferred to TK medium at 40 °C and pH 1.64 containing the same electron donors and carbon sources with which they were enriched.

Samples of a 5% w/v slurry of Sudbury pyrrhotite were taken from the continuous nickel bioleaching pilot reactor at University of Toronto operating at 30 °C and pH 2 and transferred to flasks of liquid TK media incubated at 30 °C. Liquid cultures were then plated onto double-layered solid medium and incubated at 30 °C. Two colonies from the solid media were inoculated on four different types of TK media at pH 1.64 and 30 °C: elemental sulfur or ferrous sulfate with or without 0.2% w/v yeast extract. Microscopy was used to confirm microbial growth.

Genomic DNA extraction, quality control (QC), and sequencing. Cultures were pelleted and washed twice with oxalate buffer (pH 3). Genomic DNA was extracted using the SOX Microbiome Kit (Metagenom Bio) and quantified using the NanoDrop Spectrophotometer (ThermoFisher) and Qubit fluorometer (Invitrogen). DNA was sent for 16S sequencing to ensure purity of the cultures. If pure and selected for whole genome sequencing, the DNA from a culture was purified using the Monarch DNA and PCR clean-up kit (New England BioLabs) and visualised on 0.8% agarose gel to see if < 3kb fragments were present, making the DNA unfit for long-read sequencing. Once DNA passed QC, it was sent for long-read whole genome sequencing using the Oxford Nanopore MinION sequencer.

Bioinformatic analysis. Genomes were assembled into contigs using Flye [10]. Average nucleotide identities (%) of the genomes of all Sudbury strains were compared to all other members of their respective genera with sequenced genomes in GenBank using FastANI, with 99% ANI being the cut-off for novel strain designation [11]. All genomes of Sudbury strains and genomes of the genera downloaded from NCBI were annotated using Bakta, while only the Sudbury strain genomes and plasmids were annotated using SeqHub, an online platform using the AI-enabled gLM2 model [12, 13, 14]. Pan-genome analysis was conducted on the Bakta annotations using Panaroo to find genes unique to the Sudbury strains [15].

Results and Discussion

Leptospirillum ferriphilum. Whole genome sequencing identified the *Leptospirillum strain* to the species level as *L. ferriphilum* (Table 1). FastANI analysis revealed that the Sudbury *L. ferriphilum* strain shared >99% ANI with 5 other *L. ferriphilum* strains from NCBI, indicating that it could not be classified as a novel strain. However, pan-genome analysis found 67 genes unique to the Sudbury strain, including mercury and arsenic resistance clusters (*arsR, merA, merD, merE, merP, merR, merT*) as well as genes for nitrogen metabolism (*nasA, nirB*, *glnB,* oxidoreductase molybdopterin-binding domain-containing protein) and its required molybdenum co-factors (*moaA*, *moaB*, *moaC, moeA, moaE*).

Sulfobacillus thermosulfidooxidans. The *S. thermosulfidooxidans* strain yielded a 3.8 Mbp genome, consisting of a 3.6 Mbp chromosome and a 206 kbp megaplasmid (Table 1). The strain had 208 unique genes, including many related to antibiotic resistance and bacterial immune defense, carbohydrate metabolism, membrane transporters, metal resistance, oxidative stress responses, and an adenylyl-sulfate kinase involved in sulfur metabolism. FastANI analysis revealed that the strain did not share >99% ANI with any other strains from NCBI, with or without the megaplasmid included, indicating that it could be classified as a novel strain.

The *S. thermosulfidooxidans* plasmid, to our knowledge, is one of the largest ever sequenced in any acidophilic chemolithotroph and the largest in any *Sulfobacillus species* [16, 17]. About half of the coding sequences on the plasmid were found solely on the plasmid, with no homolog on the chromosome. Some of the plasmid-unique functions include elements of inorganic and organic carbon metabolism, mercuric transport (*merC*), various toxin-antitoxin systems (likely involved in maintaining the large plasmid within the cell), and machinery for conjugation and horizontal gene transfer.

Acidithiobacillus ferriphilus. A strain of *At. ferriphilus* was isolated from the bioreactor sample inoculated on ferrous sulfate medium without yeast extract. The strain had a 2.77 Mbp chromosome and three plasmids (41.6 kbp, 36.1 kbp, 11.8 kbp) (Table 1). The strain had the fewest unique genes out of all Sudbury strains (25), mostly related to immune defense and mobile genetic element transfer. The plasmids contained multiple genes related to metal tolerance, oxidative stress responses, DNA repair, and biofilm formation. FastANI analysis showed only one other *At. ferriphilus* strain, C52, as having over 99% ANI with the Sudbury strain. However, the C52 strain contains only two plasmids (pYL1 and pYL2), showing a much greater divergence that qualifies the Sudbury strain for novel strain designation.

Acidithiobacillus caldus. A strain of *At. caldus* was isolated from the bioreactor sample inoculated on elemental sulfur medium with 0.2% w/v yeast extract. The *At. caldus* strain had a 2.83 Mbp chromosome, a 276.3 kbp genomic island, and three plasmids (43.5 kbp, 19.8 kbp, 12.0 kbp) (Table 1). The strain had 62 unique genes, most notably a CRISPR Cas2 protein endoribonuclease. The genomic island and plasmids contained multiple genes related to metal tolerance. While FastANI analysis showed >99% ANI to 7 other strains in GenBank when plasmids were included, the composition of the genomic islands and plasmids is likely unique to the strain, much like the Sudbury *At. ferriphilus*.

Table 1. Isolation conditions and genome assembly statistics of the Sudbury strains as generated by Bakta and SeqHub. YE = yeast extract, C = chromosome, C* = chromosomal fragment, P = plasmid.

Strain	Isolation Conditions (Electron donor, temperature in °C)	Genome Completeness (%)	Contig	Size (kbp)	Type	CDS (Bakta)	CDS (SeqHub)	CRISPRs
L. ferriphilum	Fe^{2+}, 40	93.09	1	2436	C	2346	2389	0
S. thermosulfidooxidans	S^0 + YE, 40	99.00	1	3560	C	3464	3494	3
			2	206.6	P	233	236	0
At. ferriphilus	Fe^{2+}, 30	99.34	1	2774	C	2803	2771	0
			2	41.6	P	45	46	0
			3	36.1	P	34	35	0
			4	11.8	P	12	14	0
At. caldus	S^0 + YE, 30	99.38	1	2829	C	2788	2783	3
			2	276.3	C*	306	305	0
			3	43.5	P	47	47	0
			4	19.8	P	28	28	0
			5	12.0	P	10	20	0

Cross-species comparisons. Three of the strains (*At. caldus, At. ferriphilus, and S. thermosulfidooxidans*) harbored plasmids with metal tolerance genes. Genes coding for TonB receptors involved in iron siderophore uptake were found in both *Acidithiobacillus species* (*At. caldus* genomic island and plasmid 1, *At. ferriphilus* plasmid 1), suggesting that iron regulation provides a selective advantage [18]. Transport proteins from the ExbD/TolR family, which coordinate with TonB, were also found on the

At. caldus genomic island and plasmid 1, as well as *At. ferriphilus* plasmid 1, while related MotA/TolQ/ExbB family proteins were found on the former two genetic elements. The *copZ* gene, a copper-binding chaperone protein, was found to be shared between the *At. caldus* genomic island and *At. ferriphilus* plasmid 2, reflecting the high copper content of the tailings. Various *czc* genes coding for cobalt, zinc, and cadmium resistance efflux pumps were also found on the *At. caldus* genomic island (*czcC*) and *At. ferriphilus* plasmid 2 (*czcA*). Across species, the *dnaJ* gene coding for a repair protein was identified in the *S. thermosulfidooxidans* megaplasmid and *At. ferriphilus* plasmid 2.

In addition to tolerance mechanisms, the Sudbury strains show diversity in iron and sulfur oxidation pathways. Gene annotations indicated that *At. caldus* is the only known member of the reactor community with a truncated Sox pathway ($S_2O_3^{2-} \rightarrow S_8 + SO_4^{2-}$), which could contribute to its success in the nickel bioleaching process (Fig. 1). In the bioreactor, *At. caldus* comprised roughly 35% of the community, while *S. thermotolerans* comprised about 50%. Smaller proportions consisted of *At. ferriphilus* and *At. ferridurans* (~5% each) and *S. thermosulfidooxidans* (~2.5%). The *At. ferriphilus* and *At. ferridurans* JAGS species are the strongest iron oxidation candidates in the community based on their suite of rusticyanin genes, while *At. ferridurans* JAGS and *S. thermosulfidooxidans* are the only sequenced members with pathways for oxidation of thiosulfate to tetrathionate (Fig. 1).

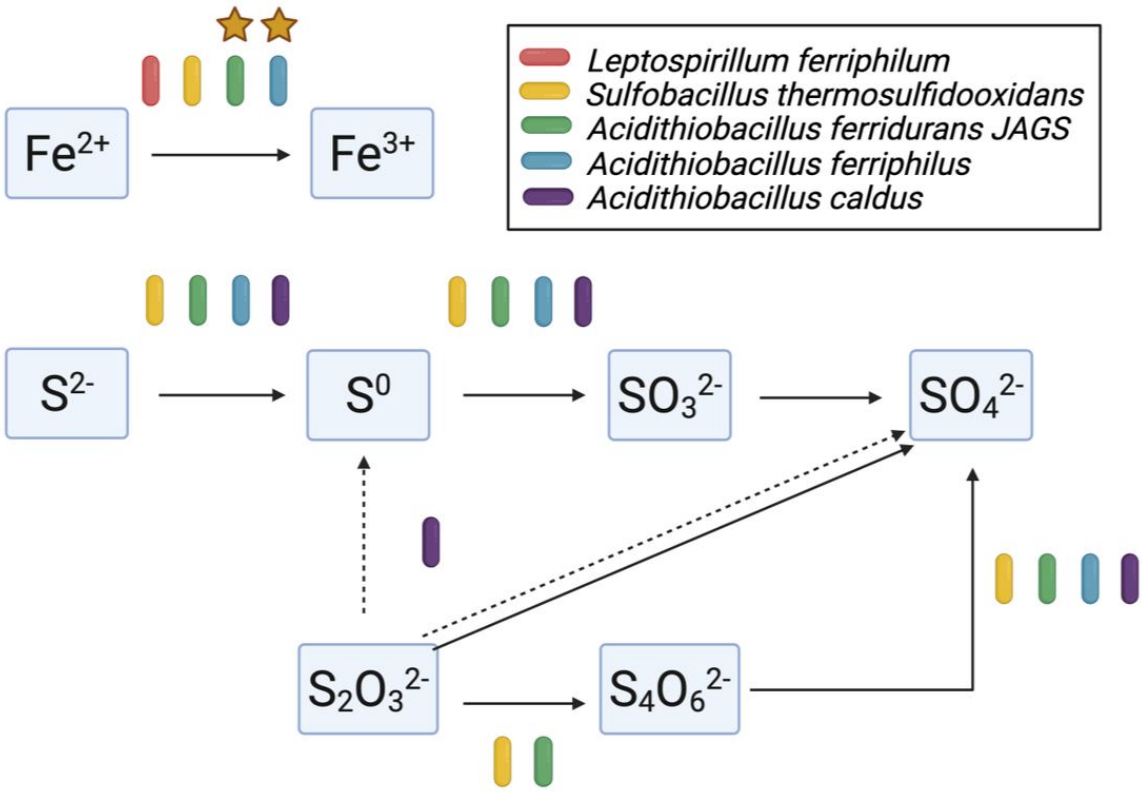


Figure 1. Flow diagram of possible iron and sulfur oxidation functions of sequenced members of the bioreactor community based on annotated genes from whole genome sequences as generated by Bakta. Stars indicate that the species are the strongest candidates for this reaction based on their iron oxidation pathways. Dotted lines indicate both reactions are involved in the pathway catalysed by the adjacent organism (*At. caldus*). Created with Biorender.com.

Conclusions

Microorganisms in the Sudbury tailings environment have unique adaptations unlike any other members of their genera, several being shared across the four sequenced species amongst mobile genetic elements. Many of these genes relate to the stressors imposed by the Sudbury tailings; iron homeostasis, metal tolerance, oxidative stress, ionic transport, biofilm formation, phage defense, lipid biosynthesis, nitrogen and carbon metabolism, and DNA repair. Many of these functions point to the metal-rich nature of the Sudbury tailings, particularly iron, nickel, copper, and cobalt-related defenses. Furthermore, the strains

seem to benefit from genes related to quorum sensing and biofilm formation, perhaps cooperating with other community members to form protective biofilms on mineral surfaces. Selection for elevated nitrogen and carbon metabolism and immune defense may be driven by the organic matter used to remediate legacy tailings, which contain plant biomass and higher bacterial load, possibly causing competition for resources and exposure to phages [7]. Various genes involved in nucleotide uptake, biosynthesis, and DNA repair indicate this as a protective mechanism against stressors across species. Whether the presence of similar genetic elements across species indicates HGT or convergent evolution remains to be investigated.

Isolation is currently being attempted on other strains from the nickel bioleaching reactor. Transcriptomic and proteomics studies of these isolates will help us understand which features are responsible for the success of these strains and which pathways are active at different points of the bioleaching process. OMICs based studies will also allow us to understand the advantages imparted to the strains by the plasmids, and narrow down targets for strain engineering to create more efficient biomining organisms, a goal which may be assisted by characterisation of the CRISPR elements.

Acknowledgements

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Transcriptomic Responses of *Leptospirillum ferriphilum* and *Acidithiobacillus thiooxidans* to Iron, Sulfur, and Copper Stress

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Keywords: chemolithotrophic bacteria, transcriptomics, metal stress response, iron and sulfur oxidation pathways

Abstract

Acidophilic chemolithotrophic bacteria play a crucial role in bioleaching processes by driving iron and sulfur oxidation under extreme environmental conditions. Understanding their molecular responses to metal and substrate stress is essential for improving bioleaching efficiency and stability. In this study, a comparative transcriptomic analysis of *Leptospirillum ferriphilum* and *Acidithiobacillus thiooxidans* to elucidate their adaptive responses to iron (Fe^{2+}), sulfur (S), and copper (Cu^{2+}) stress was performed. The results revealed that *L. ferriphilum* exhibited pronounced transcriptional changes under copper stress, with significant upregulation of genes involved in metal detoxification, including metal efflux systems and copper resistance determinants, as well as oxidative stress defense and energy metabolism. In contrast, *A. thiooxidans* displayed a strong transcriptional response to elemental sulfur, characterised by enhanced expression of genes associated with sulfur oxidation pathways, supporting efficient energy generation. Exposure to copper induced the activation of detoxification mechanisms, including metal-binding proteins, efflux transporters, and oxidative stress response regulators. Overall, this study provides novel insights into the molecular basis of microbial adaptation in bioleaching systems and identifies key genetic pathways associated with metal resistance and energy metabolism. These findings offer valuable targets for enhancing microbial robustness and optimising bioleaching performance for sustainable metal recovery and environmental remediation.

Introduction

Acidophilic bacteria are a group of extremophilic microorganisms that thrive in harsh conditions and are extensively used in the industrial bioleaching of copper and other sulfide ores [1]. The unique physiological and metabolic traits of these bacteria have attracted considerable scientific attention [2]. *Leptospirillum ferrooxidans* and *Acidithiobacillus ferrooxidans* are two of the most prevalent iron-oxidizing bacteria, making up over 70% of the prokaryotic population is extreme environment with high concentration of metals [3]. This capability is essential for the biogeochemical cycles of iron and sulfur and has significant potential for biotechnological applications, including bioleaching [4]. Copper ions are essential for many organisms, primarily as enzyme cofactors and for protein synthesis. However, during the advanced stages of bioleaching, microorganisms are exposed to elevated copper ion concentrations due to the accelerated dissolution of copper-bearing minerals [5,6]. Excessive copper induces oxidative stress in biomining microorganisms and disrupts the activity of intracellular enzymes related to iron-sulfur metabolism [7,8]. This study aims to comprehensively investigate the transcriptional responses of key bioleaching microorganisms, including *L. ferriphilum* and *A. thiooxidans*, to various stress conditions relevant to bioleaching processes. Particularly the study examines the transcriptional responses of *A. thiooxidans* to Fe^{2+} stress, elevated S^{o} concentrations, and high copper levels, with a specific emphasis on identifying genetic and regulatory pathways that enable survival and activity under these challenging conditions.

Methods

L. ferriphilum CC (OM272948), «KJ» indigenous consortium and *A. thiooxidans* SO-1 (KP455985) were cultivated in Mackintosh medium, containing Fe^{2+} or sulfur (S^o) as the energy source. In the logarithmic phase of the growth, the bacterial cells were collected by centrifugation at 5000 g x10 min. Total RNA is extracted from samples using the Trizol method. The library is constructed using the NEBNext® Ultra II Directional RNA Library Prep Kit for Illumina.

Results and Discussion

The effect of Fe^{2+} (20 mM) ions (T1) on the growth of *L. ferriphilum* CC, the effect of Fe^{2+} (20 mM) and S^o (1 g/L) (T2) on the growth of *At. thiooxidans* SO-1 and "KJ" consortium and the effect of Fe^{2+} (20 mM), S° (1 g/L), and Cu^{2+} (10 mM) (T3) on *At. thiooxidans* SO-1 and "KJ" consortium were investigated for transcriptomic analysis. It should be noted the Principal Component Analysis (PCA) plot (Figure 1) illustrates the variance in gene expression profiles across the three samples. The spatial separation of T1 (Fe^{2+} only), T2 (Fe^{2+} + S^o), and T3 (Fe^{2+} + S^o + Cu^{2+}) along PCA suggests distinct transcriptional responses under each condition.

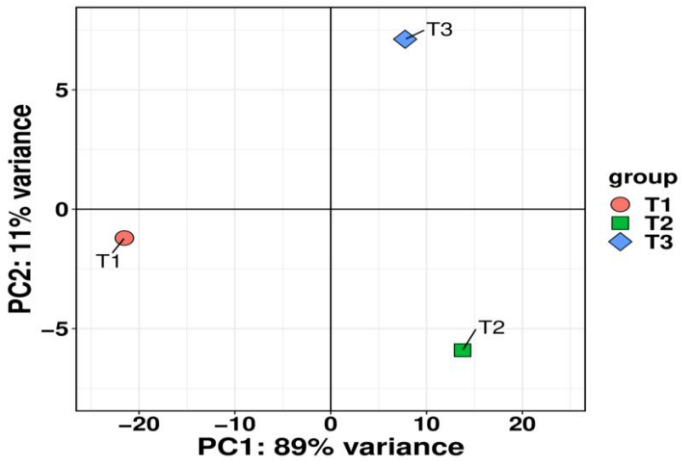


Figure 1. Principal Component Analysis (PCA) of transcriptomic data.

KEGG (Kyoto Encyclopedia of Genes and Genomes) Level 1 functional classification was performed to characterise the biological functions of differentially expressed genes (DEGs) identified under the T1, T2, and T3 treatment conditions. A total of 569 DEGs were identified, comprising 331 upregulated and 238 downregulated genes. Remarkably, among the annotated DEGs, Metabolism was the most abundant KEGG Level 1 functional category, with 372 gene annotations (65.38%), including 191 upregulated and 181 downregulated genes. Overall, the second largest category was Environmental Information Processing, followed by Genetic Information Processing and Cellular Processes. The DEGs are categorised into Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) at Gene Ontology (GO) Level 2. In the T2 vs. T1 comparison, the Biological Process (BP) category contained a higher number of upregulated than downregulated genes. CC class entity shows a high number of DEGs, suggesting alterations in the expression of genes associated with cellular structures and subcellular compartments in response to elemental sulfur (S^0). Genes associated with Protein-containing complexes were predominantly upregulated, potentially reflecting increased synthesis of proteins involved in sulfur metabolism. Within the MF category, genes associated with binding functions have the highest numbers of DEGs, which consistent with metabolic adaptations required for growth in the presence of S^0.

The comparative transcriptomic analysis between T1 and T2 revealed substantial transcriptional changes in *A. thiooxidans* SO-1 and the indigenous consortium KJ in response to sulfur supplementation. The volcano plot shows a large number of DEGs, including significantly upregulated (red) and downregulated (green) transcripts, indicating that the presence of elemental sulfur (S^0) elicited extensive changes in gene expression. These transcriptional changes suggest metabolic and stress-response adaptations to sulfur supplementation.

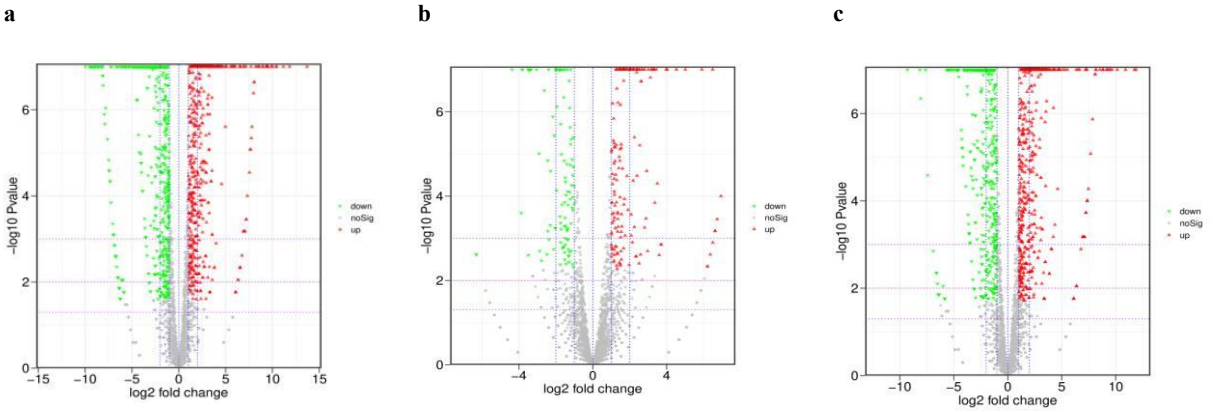


Figure 2. Volcano plot between T1-T2 (a), T3-T1(b) and T3-T2(c).

The transcriptomic analysis comparing T3 and T1 provides insights into the differential gene expression patterns induced by the presence of S° and Cu^{2+} in *At. thiooxidans* SO-1 and the "KJ" consortium. A considerable number of upregulated genes in T3 suggest an active cellular response to Cu^{2+} and sulfur metabolism. Conversely, the downregulated genes indicate metabolic and regulatory pathways that are suppressed under T3 conditions. The presence of Cu^{2+} may disrupt iron homeostasis in acidophilic microorganisms by interfering with iron uptake and transport systems, leading to the downregulation of genes involved in iron acquisition. Moreover, genes associated with metabolic processes were strongly upregulated in T3, likely reflecting enhanced sulfur metabolism and cellular adaptation to Cu^{2+} stress. The Categories Catalytic activity and binding have the highest number of DEGs, suggesting an increase in enzymatic functions related to sulfur metabolism and metal homeostasis. Transporter activity is markedly upregulated, suggesting the activation of metal transport systems and efflux mechanisms involved in Cu^{2+} detoxification. The heatmap highlights the major regulatory shifts in metabolic pathways related to sulfur oxidation, stress response mechanisms, and heavy metal homeostasis.

The heavy metal response regulator transcription factor (ACRY_RS00010) was markedly upregulated in T2, accounting for 78.04% of its normalised expression, compared with 68.74% in T1 and 26.28% in T3. In contrast, the heavy metal sensor histidine kinase (*ACRY_RS00015*) displayed its highest expression in T1 (14.85 %), with reduced expression in T2 (7.95 %) and T3 (4.07%), suggesting treatment-specific regulation of the sensor component of the two-component system.

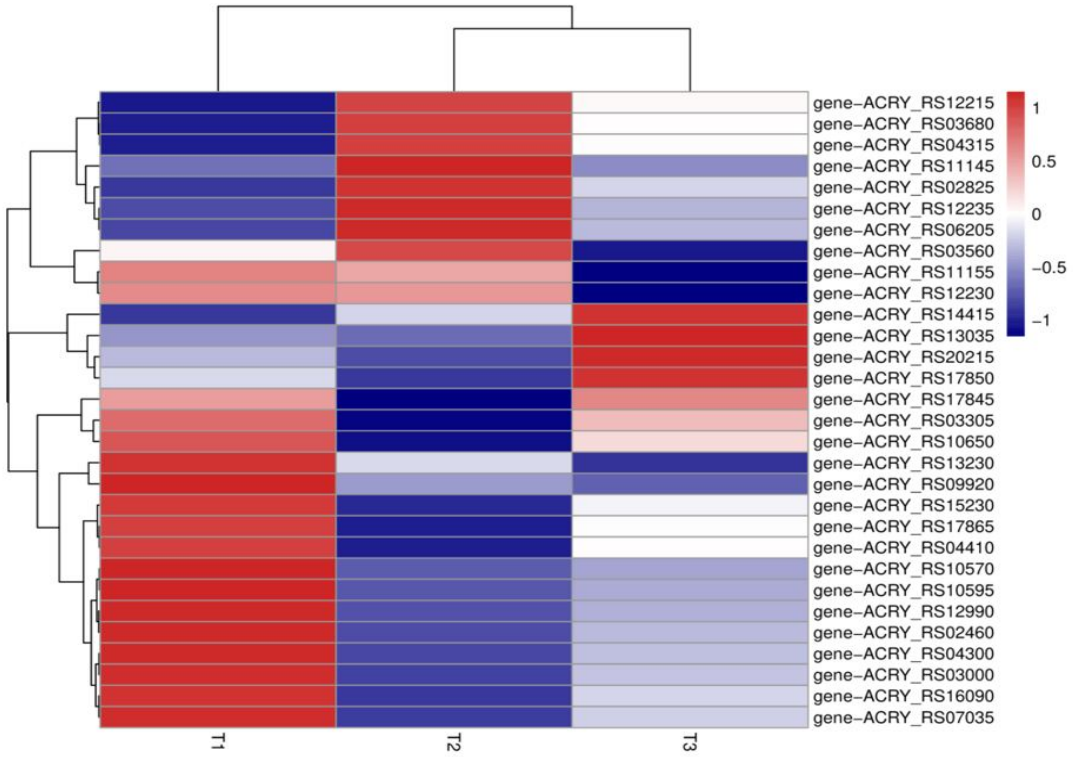


Figure 3. A heatmap of the microbial genera in samples T1, T2, and T3 was created based on unique gene assignments to the NCBI-NR database

Comparative transcriptomic analysis identifies several metabolism-related genes, including ACRY_RS08320, ACRY_RS11385, ACRY_RS14975, ACRY_RS03270, ACRY_RS17240, ACRY_RS12690, ACRY_RS10310, ACRY_RS08540, ACRY_RS10865, and ACRY_RS11350. Overall, these findings provide new insights into the molecular mechanisms underlying microbial adaptation, metal resistance, and energy metabolism in bioleaching systems.

Conclusions

This study provides a comprehensive transcriptomic analysis of *L. ferriphilum* and *A. thiooxidans* under different environmental stress conditions relevant to bioleaching processes.

Comparative analysis of transcriptional responses of these microorganisms to Fe^{2+}, S° and Cu^{2+}, identified key metabolic and stress adaptation pathways that enable their survival and functional activity in extreme environments. *L. ferriphilum* exhibited pronounced transcriptional responses to copper stress, including the upregulation of genes associated with metal detoxification, oxidative stress defense, energy metabolism, and metal efflux, highlighting its capacity to maintain metal homeostasis. These findings identify potential genetic targets for improving microbial resilience and bioleaching performance.

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Impact of Quorum sensing modulation on e-waste bioleaching by *Leptospirillum ferriphilum*

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Abstract:

Iron-oxidizing bacteria such as *Leptospirillum ferriphilum* are widely involved in bioleaching processes for strategic metal recovery (Cu, Co …) from ores. The possibility to extract metals from electronic waste thanks to the bioleaching activity of this strain is under way. However, e-waste leaching is an unfriendly environment for microorganisms, with high concentrations of mixed metals known to inhibit their activity (1). Bioleaching has been shown to be affected by Quorum Sensing (QS), an inter-microbial communication pattern based on production of molecules enabling bacterial behavior coordination. QS activation can enhance both bioleaching yield and bacterial-surface interaction (2,3). However, some studies suggested some QS molecules could lead to surface attachment without enhancement of bioleaching activity. Thus, identifying QS molecules capable of enhancing oxidation activity and promoting *L. ferriphilum* adhesion in e-waste bioleaching conditions is crucial to potentially enhance the e-waste bioleaching process. Within this framework, this work (PEPR Cyclamet, ANR-22-PERE-0003, France 2030) aims to assess QS-based strategies to enhance bioleaching of e-waste PCBs through targeted supplementation of signaling molecules.

Here, the effect of exogenous N-acyl-L-homoserine lactones (C8-HSL, 3-oxo-C8-HSL and 3-hydroxy-C8-AHL at 5 µM) was first assessed on growth, oxidation potential and adhesion (on glass) of *L. ferriphilum* in well microplates in iron synthetic medium by monitoring pH, Eh (mV vs SHE) as a proxy of FeII oxidation, and biomass (DAPI staining and fluorescence microscopy).

Results showed no negative impact of these molecules on iron oxidation by *L. ferriphilum*, and suggested a positive impact of C8-HSL and 3-hydroxy-C8-AHL on bacterial surface colonization with micro-aggregate formation. The next step focuses on the potential impact of these molecules on *L. ferriphilum* growth and activity in the presence of e-waste leachate, known to be stressful conditions. Additional signaling molecules (C14-HSL) are also being evaluated, and potential synergistic effects of molecular combinations will likewise be assessed.

Keywords: Bioleaching, Strategic metals, E-wastes, *L. ferriphilum*, Quorum Sensing. Biofilms

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Exploring pyrite-driven biofilm formation as a bioengineering strategy for enhanced bioleaching

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Abstract: Extreme ecosystems, such as mine waste, host a wide array of microorganisms with specialised functions, including metal solubilisation, acid and metal tolerance, and biofilm formation; traits highly relevant to bioengineering technologies. In this study, we monitored biofilm formation of a naturally occurring bioleaching consortium, as well as individual species via SEM imaging. Although a critical process for effective bioleaching, the physiochemical and biological traits that trigger biofilm formation onto mineral surfaces remain poorly understood.

We compared biofilm formation by axenic cultures of *Acidithiobacillus ferrooxidans*, the model organism for bioleaching microbes, and a naturally occurring bioleaching consortium on pyrite (FeS_2). Robust biofilm was observed on the mineral surface in both systems after 40 days of incubation; however, consortium-derived biofilms embedded morphologically diverse microorganisms within the matrix, suggesting potential cooperation and ecological interactions that may benefit the consortium. Structurally, there was no significant difference between the biofilm produced by axenic cultures of *Acidithiobacillus spp.* and the bioleaching consortium, which may suggest that (i) key species are responsible for biofilm formation, and (ii), there is a recurrent pattern for biofilm formation, which can be due to both biological (e.g., activation of specific metabolic pathways) and chemical (e.g., presence of Fe, S) properties.

To investigate underlying mechanisms, we are analysing the proteome of axenic *At. ferrooxidans* CF3 cultures grown in the presence and absence of pyrite. Our hypothesis is that the mineral chemical composition, rather than the nutrient composition, may trigger biofilm formation. By characterising these strategies, we aim to gain insights into microbial interactions with pyrite, by looking at different stages of growth of biofilm formation and the importance of this structure for cell adhesion and mineral dissolution. Understanding these mechanisms can inform the design of synthetic consortia, as well as gene-directed strategies to enhance biofilm formation for the bioleaching of minerals.

Keywords: Biofilm formation, Bioleaching, SEM, Proteomics

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Adaptive laboratory evolution to develop a specialized acid, temperature and nitrate tolerant iron oxidizing culture

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Abstract: In acid mine drainage, bioleaching heaps and geothermal systems, microbial communities often restructure along thermal and pH gradients, with functional selection for Fe/S oxidation and tolerance to high ionic strength. Nitrate inputs in mine waters can similarly restructure communities and favour denitrification in engineered settings.

To investigate how microbial communities adapt to simultaneous environmental stressors and determine how these conditions drive changes in community composition and functional potential, we performed adaptive laboratory evolution on a community from bioleaching reactors and a mixed community collected at circumneutral pH and ambient temperature from an abandoned saltpeter processing site in northern Chile, where nitrate concentrations reached 61.7 g/L.

The experiment sequentially modified pH, temperature, and nitrate concentrations. We tracked Fe(II) oxidation, nitrate reduction, and 16S rRNA profiles and predicted functionality. The adapted communities grew and oxidized Fe(II) and reduced nitrate, but at levels insufficient to support it as a primary electron acceptor.

Overall, the analyses show that nitrate exposure led to the restructuring of exposed communities, manifested by changes in composition and functional potential. These changes did not involve the emergence of dominant species or a small number of specialists, but rather a broad community reorganization.

In terms of matabolic processes, exposure to nitrate produced a distributed reconfiguration of the community (activation/recruitment of rare taxa and changes in relative abundances). This was coupled with a functional selection dominated by two axes: (1) nitrate respiration/denitrification as an energy acquisition strategy and (2) osmoadaptation required to tolerate the increased ionic strength associated with the addition of nitrate.

Therefore, the system not only changes who is present, but also what ecological capacities emerge: greater effective diversity, greater nitrate respiration potential (with indications of more complete denitrification), and an expanded repertoire for organic carbon processing under the new redox-chemical regime.

Keywords: nitrate, iron oxidation, community ecology, denitrification potential, osmoadaptation

Acknowledgements: Any acknowledgements (optional)

The Effect of Ferrous Iron on Indigenous Microbial Isolation and Bioleaching Potential in Diverse Lithium-Bearing Ores

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Abstract:

Lithium is considered an economically critical raw material with a rapidly increasing global demand, driven by uses in batteries and energy storage devices. Conventional lithium extraction methods, while effective, come with high energy consumption and environmental burdens, including production of toxic by-products. Bioleaching is a type of biological metallurgy used for the dissolution of metals from their mineral sources using microorganisms. Bioleaching offers a complementary approach to conventional chemical and physical methods for easier, more environmentally conscious processing.

Various bioleaching-capable microbes have been identified, most of which are autotrophic acidophiles. In this work, the aim was to isolate and characterise indigenous microbial communities present in 5 different lithium-bearing mineral ores across Europe, including lithium clays, lepidolite, petalite, zinnwaldite and zinnwaldite tailings. Indigenous microorganisms are predicted to improve bioleaching efficiency compared to exogenous microorganisms due to evolutionary adaptation to the native mineralogical conditions. While the overall aim is to identify mineral type specific microbes, this work tested the concentration of added ferrous iron in the bioleaching reaction to assess whether this also determines a mineral dependent selective pressure on microbial populations, along with determining the ideal bioleaching conditions for each mineral type.

The indigenous microorganisms were isolated in liquid cultures at 25 °C in 0K media supplemented with four ferrous iron concentrations (0-1000 mg/L). Cultures were maintained at a pH of ~2.0 using sulfuric acid. Throughout the experiments, pH, oxidation-reduction potential, ferrous iron, and lithium concentrations were monitored every ten days from the leach solution. On the completion of the experiment, all samples were filtered, and the final lithium recovery was measured. Samples of the leach solution were taken for DNA extraction and sent for next-generation sequencing. This study is still ongoing; however, preliminary results have successfully demonstrated variation in the indigenous microorganisms present in the different lithium-bearing mineral ore types.

Keywords: Bioleaching, Lithium, Microorganisms, Ferrous iron,

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THIOCYANATE TOLERANCE, INHIBITION, AND ADAPTATION IN KEY BIOOXIDATION MICROORGANISMS

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Keywords: *L. ferriphilum; Ap. cupricumulans; At. caldus;* thiocyanate inhibition; adaptive evolution

Abstract

Biooxidation pre-treatment of refractory gold ores relies on the combined activity of iron- and sulfur-oxidising acidophiles. Water recycling from cyanidation tailings is constrained by microbial sensitivity to thiocyanate (SCN^-), which persists at inhibitory concentrations even after bioremediation. This work characterises SCN^- tolerance, inhibition kinetics, and adaptive capacity in three biooxidation isolates from an industrial consortium, *Leptospirillum ferriphilum*, *Acidiplasma cupricumulans*, and *Acidithiobacillus caldus*; and integrates these findings into an unstructured kinetic modelling framework. Onset and critical inhibition concentrations were established for each species with respect to substrate oxidation and growth, revealing a tolerance ranking of *At. caldus* ≫ *Ap. cupricumulans* > *L. ferriphilum*. Model fitting showed SCN^- acts as a competitive inhibitor in *L. ferriphilum* and *At. caldus* but a non-competitive inhibitor in *Ap. cupricumulans*, despite a conserved haem-copper oxidase target. Adaptive laboratory evolution improved tolerance in the bacterial isolates; markedly in *L. ferriphilum*, moderately in *At. caldus* but increased SCN^- sensitivity in *Ap. cupricumulans*. These species-specific differences are attributed to metabolic flexibility, terminal oxidase bioenergetics, membrane configuration, and bacterial versus archaeal genomic plasticity. The resulting kinetic framework supports prediction of biooxidation performance under closed-loop water management and informs monitoring requirements for circuits recycling remediated cyanidation tailings water.

Introduction

Biooxidation is an established pre-treatment for sulfidic gold-bearing refractory ores, in which iron- and sulfur-oxidising microbial consortia generate ferric iron (Fe^{3+}) and acid (H^+) that catalyse sulfide mineral dissolution and liberate occluded gold ahead of cyanidation. Biooxidation performance, and downstream gold recovery, is therefore directly dependent on the health and functional stability of the resident microbial community.

Increasing pressure on water resources has driven the mining and minerals processing sector toward water reuse and recycling. Biooxidation circuits have historically avoided recycling process water from cyanidation tailings because of microbial sensitivity to thiocyanate (SCN^-); while tailings SCN^- concentrations may reach up to 5000 mg/L, inhibition of biooxidation microorganisms has been reported from as low as 1 mg/L [1], [2], [3]. Advances in cyanide and SCN^- bioremediation now allow treated effluents to reach residual SCN^- concentrations below 1 mg/L, and several commercial biooxidation operations already recycle detoxified tailings water [4]. However, fluctuations in residual SCN^- within remediated effluent remain a risk to microbial stability and associated biooxidation process performance. Further, the mechanistic basis for species-specific SCN^- sensitivity has not been systematically resolved.

This study addresses that gap by quantifying SCN^- tolerance, inhibition kinetics, and adaptive response in three key biooxidation microorganisms isolated from an industrial consortium: *L. ferriphilum* (Fe^{2+}-oxidising bacterium), *Ap. cupricumulans* (Fe^{2+}-oxidising archaeon), and *At. caldus* (S^0-oxidising bacterium). Additionally, these findings are integrated into a kinetic modelling framework to support prediction of biooxidation performance under closed-loop water management.

Methods

Materials. *L. ferriphilum*, *Ap. cupricumulans*, and *At. caldus* were isolated from an industrial biooxidation consortium and cultivated at 45 °C: *L. ferriphilum* on Fe^{2+} in DSMZ 882 or ABS medium [5], [6], *Ap. cupricumulans* on Fe^{2+} with yeast extract (0.02% w/v) in MM medium [7], and *At. caldus* on elemental sulfur (0.5% w/v) in 0K medium [8]. SCN^- tolerance was assessed across a concentration gradient for each species. Thiocyanate-adapted strains were generated by adaptive laboratory evolution (ALE) at fixed SCN^- concentrations (0.5 mg/L and 1 mg/L for *L. ferriphilum* and *Ap. cupricumulans* respectively; 1 and 20 mg/L for *At. caldus*) [6], [8], [9].

Analysis. Fe^{2+} oxidation and sulfate generation were tracked over time and fitted to unstructured kinetic models. A modified Michaelis-Menten-form expression (accounting for Fe^{3+} competitive inhibition) for the Fe^{2+} oxidisers [10], and the Contois equation for the solid-substrate S^0 oxidiser were applied. SCN^- inhibition was resolved into competitive and non-competitive terms via non-linear regression. Additionally, the onset and critical inhibition concentrations were defined with respect to substrate oxidation and cell growth.

Results and Discussion

Thiocyanate tolerance and mechanistic basis

Onset and critical SCN^- inhibition concentrations (Table 1) establish a clear tolerance ranking: *At. caldus* ≫ *Ap. cupricumulans* > *L. ferriphilum*. This tolerance pattern illustrates the metabolic and bioenergetic differences between the species. *At. caldus* possesses a branched respiratory chain (bo_3- and bd-type terminal oxidases) and derives up to six electrons per sulfur oxidation event, generating a large potential difference across the electron transport chain and affording redundancy under SCN^- stress [11], [12]. Fe^{2+} oxidisers, by contrast, gain only one electron per Fe^{2+} oxidised via a single electron transport chain, restricting metabolic versatility [13], [14]. However, the archaeon *Ap. cupricumulans* is mixotrophic and is able to draw on organic carbon as an auxiliary electron donor [15] and possesses a more bioenergetically efficient aa_3 terminal oxidase [16] than *L. ferriphilum*'s cbb_3 oxidase [17], [18]. Additionally, its tetraether membrane lipids more tightly restrict SCN^--driven H^+ influx relative to *L. ferriphilum*'s bacterial membrane [19], [20]. *L. ferriphilum*, wholly dependent on Fe^{2+} oxidation via a single, less efficient oxidase, is the most metabolically constrained of the three and correspondingly the most SCN^--sensitive.

Table 1. Comparison of onset and critical SCN^- inhibition concentrations for *L. ferriphilum* and *Ap. cupricumulans* (grown on Fe^{2+}) and *At. caldus* (grown on S^0) at 45 °C

Microorganism	Onset concentration- substrate oxidation (mg/L)	Onset concentration- cell growth (mg/L)	Critical inhibition concentration (mg/L)
L. ferriphilum	0.5	1.0	1.75
Ap. cupricumulans	1.0-1.5	1.0	6.0
At. caldus	< 1.0	< 5.0	20.0

Inhibition kinetics and mechanism

SCN^- is understood to act at the haem-copper oxidase (HCO), impairing O_2 reduction and dissipating the proton motive force (PMF) [21]. Although all three species possess at least one HCO, model fitting showed SCN^- acts as a competitive inhibitor in *L. ferriphilum* and *At. caldus*, but as a non-competitive inhibitor in *Ap. cupricumulans* [5], [7], [8]. This divergence is attributed to *Ap. cupricumulans'* ability to potentially derive electrons from organic carbon as well as Fe^{2+}. While the mode of inhibition differs between these bacteria and archaeon, both result in reduced electron flow across the respiratory chain, limiting the rate of H^+ translocation across the cell membrane and lowering the PMF.

Additionally, all inhibition rate expressions were structured as discontinuous functions with species-specific transition points to reflect the shift in the rate-controlling mechanism with increasing SCN^- concentration. It is postulated that at low SCN^- concentrations inhibition is attributed to compromised O_2 reduction at the HCO with pH homeostasis modulations effectively counteracting the decline of the PMF. At higher SCN^- concentrations the decline in PMF outpaces compensatory pH-homeostatic mechanisms and the PMF becomes limiting [5], [8], [9].

Adaptive response to SCN^-

Thiocyanate-adapted strains showed markedly divergent adaptive outcomes across species (Table 2). Adaptation eliminated detectable SCN^- inhibition in *L. ferriphilum* across the concentration range tested, and substantially improved SCN^- inhibition in *At. caldus*. In contrast, the adapted *Ap. cupricumulans* strain showed reduced tolerance relative to its unadapted control, with complete inhibition at 3 mg/L SCN^- (down from 6 mg/L). This bacterial-versus-archaeal divergence is consistent with genome size and coding capacity: bacterial genomes (~3–5 Mb) confer greater physiological plasticity under selective pressure than the comparatively constrained archaeal genome (~2 Mb), which is thought to have evolved under chronic energy stress [20], [22]. This aligns with the broader view of adaptation as a function of genetic potential shaped by persistent selective pressure [22]. Kinetic re-characterisation supports this picture as adapted *At. caldus* strains showed increased K_i (improved tolerance) and a shift from competitive to non-competitive inhibition, while the adapted *Ap. cupricumulans* strain showed decreased K_i (increased sensitivity).

Table 2. Comparison of onset and critical SCN^- for both control and SCN^--adapted species of *L. ferriphilum* and *Ap. cupricumulans* (grown on Fe^{2+}) and *At. caldus* (grown on S^0) at 45 °C

Microorganism	Onset concentration- substrate oxidation (mg/L)	Onset concentration- cell growth (mg/L)	Critical inhibition concentration (mg/L)
L. ferriphilum (control)	0.5	1.0	1.75
L. ferriphilum (adapted to 0.5 mg/L)	Not detected [a]	Not detected [a]	Not detected [a]
Ap. cupricumulans (control)	1.0-1.5	1.0	6.0
Ap. cupricumulans (adapted to 1 mg/L)	1.5	1.0	3.0
At. caldus (control)	< 1.0	< 5.0	20.0
At. caldus (adapted to 1 mg/L)	5.0	20	Not detected [b]
At. caldus (adapted to 20 mg/L)	5.0	Not detected [b]	Not detected [b]

[a] *where SCN^- concentration range explored was 0-2.0 mg/L*

[b] *where SCN^- concentration range explored was 0-20.0 mg/L*

Conclusions

Thiocyanate tolerance among key biooxidation microorganisms follows the order *At. caldus* ≫ *Ap. cupricumulans* > *L. ferriphilum*, driven by differences in metabolic flexibility, terminal oxidase bioenergetics, and membrane configuration. Thiocyanate inhibits competitively in the bacterial isolates and non-competitively in the archaeon, despite a shared HCO target. Adaptive laboratory evolution improved tolerance in both bacterial species but exacerbated sensitivity in the archaeon. Together, these species-resolved tolerance and kinetic parameters provide a foundation for predicting biooxidation performance under closed-loop water management and for defining SCN^- monitoring thresholds in circuits recycling remediated cyanidation tailings water.

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Salt-enhanced growth and aggregation in novel acidophile *Igneacidithiobacillus copahuensis* relevant to saline bioleaching.

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Salinity is an increasing constraint in biomining, particularly in chalcopyrite processing, as chloride severely inhibits the growth and activity of most acidophilic microorganisms, compromising process stability under fluctuating conditions. *Igneacidithiobacillus copahuensis*, a recently described sulfur-oxidizing acidophile from volcanic environments, represents a novel lineage within the *Acidithiobacillia.* Unlike most of acidophiles, certain strains not only tolerate but exhibit optimal growth under saline conditions (10 ± 2.5 g/LNaCl). This uncommon phenotype makes *I. copahuensis* a valuable model to investigate microbial robustness and strain-level variability relevant to saline bioleaching systems. We examined growth performance, acclimation capacity, and salt responses of multiple *I. copahuensis* strains with contrasting tolerance phenotypes under constant and fluctuating NaCl regimes (0–30 g/L). After approximately 30 generations, constant versus fluctuating selection did not significantly alter overall growth performance. However, fluctuating acclimation enhanced salt tolerance in previously non-tolerant strains, enabling cell yields at 15 g/L NaCl comparable to those observed without salt. In contrast, salt-tolerant strains maintained stable performance across treatments, displaying maximal growth at 12.5 g/L NaCl and sustained growth up to 30 g/L. Comparative genomic analyses revealed a streamlined genome architecture and a reduced representation of canonical salt stress determinants, including compatible solute biosynthesis pathways and *kdp* potassium uptake system. Salt-enhanced growth was associated with strain-specific aggregation and capsular polysaccharide production, supported by the presence of an additional *pel* polysaccharide biosynthesis cluster in salt-tolerant strains. These aggregation phenotypes may contribute to cellular cohesion and surface-associated lifestyles under saline conditions, traits considered relevant in bioleaching processes. Together, these results identify *I. copahuensis* as an unconventional acidophile combining intrinsic salt-enhanced growth with strain-dependent acclimation potential, highlighting the importance of strain-level selection for saline biomining systems.

Keywords: salinity, *Acidithiobacillia*, acclimation, Pel polysaccharide, aggregation

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Transcriptomic Insights into Chloride Stress Tolerance Mechanisms in *Sulfobacillus thermosulfidooxidans*

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Abstract: Elevated chloride concentrations in process waters pose a major challenge for biomining, as chloride ions inhibit growth and iron oxidation in most acidophilic microorganisms. *Sulfobacillus thermosulfidooxidans*, a moderately thermophilic, Gram-positive iron- and sulfur-oxidizing bacterium, is among the few acidophiles capable of maintaining activity under saline conditions. However, the molecular mechanisms underlying chloride tolerance in this organism remain poorly understood.

In this study, the chloride stress response of *Sb. thermosulfidooxidans* DSM 9293 was investigated using RNA sequencing. Cultures were grown under control (0 mM NaCl) and chloride-stressed (100 mM NaCl) conditions at pH 1.8 and 45 °C. Principal component analysis revealed a clear separation between treatments, with chloride accounting for approximately 61% of the total variance in gene expression (PC1). Differential expression analysis identified 93 significantly regulated genes ($|\log_2 FC| \geq 1$, padj < 0.05), indicating a targeted yet coordinated transcriptomic response.

Chloride stress led to strong upregulation of membrane transport systems, including ABC and MFS transporters and EmrB/QacA-family efflux pumps, as well as genes involved in redox protection such as peroxiredoxins and flavin-associated enzymes. In contrast, genes associated with cell division, sporulation, respiration, and polysaccharide biosynthesis were broadly downregulated. Notably, no transcriptional activation of classical compatible-solute synthesis pathways was observed, suggesting an alternative chloride tolerance strategy.

Overall, *Sb. thermosulfidooxidans* responds to chloride by reprogramming ion transport, reinforcing redox homeostasis, and suppressing growth-related processes, resulting in a low-activity, maintenance-oriented physiological state. These findings provide new insight into halotolerance strategies of acidophilic Firmicutes and are relevant for the application of bioleaching processes using saline water resources. This adaptive strategy may contribute to the robustness of *Sb. thermosulfidooxidans* in industrial biomining systems exposed to saline process waters.

Keywords: *Sulfobacillus thermosulfidooxidans*; chloride stress; RNA-seq; halotolerance; bioleaching

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A comparison of metal tolerance in acidophiles comprising 15 type species and 10 different an- and cations

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Abstract: The tolerance of acidophilic bacteria and archaea to high concentrations of various metals and sulfate in acidic solution is a prerequisite for metal bioleaching. Metal tolerance or resistance of acidophiles has been investigated mechanistically and for particular species of bacteria and archaea in separate studies. Acidophiles have an internal positive cytoplasmic transmembrane potential allowing them to tolerate high concentrations of metal cations such as Fe^{3+}, Fe^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, among others. In addition, in low pH environments where acidophiles prevail, usually high concentrations of sulfate allow for complexing of metal cations, lowering the concentrations of free metal cations in solution largely. Vice versa, besides sulfate, acidophiles are usually very sensitive to elevated concentrations of anions such as Cl^- and F^-, including metal oxyanions such as CrO_4^{2-} and MoO_4^{2-}, and low molecular weight organic acids being dissociated at low pH. Here, 15 type species of acidophilic bacteria and archaea were comparatively tested for their growth in pure culture at various growth conditions at different concentrations of 10 different ions comprising Cl^-, SO_4^{2-}, CrO_4^{2-}, MoO_4^{2-}, VO_4^{2-}, WO_4^{2-}, Ni^{2+}, Cu^{2+}, Co^{2+}, and Cr^{3+}. Growth was observed via microscopic evidence of increasing cell numbers in the overall about 1500 cultivation assays. Tolerance variation between species was less pronounced than for the different ions tested. For example, more than 1 µM WO_4^{2-} was not tolerated by most acidophiles while at least 10 mM Cr^{3+} was tolerated, meaning a 10,000-fold higher concentration. A comprehensive dataset on metal tolerance of acidophiles will be presented.

Keywords: acidophiles, metal tolerance, metal resistance, cations, anions

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Membrane H_2-fed bioreactor applied to high-sulfate mine water for Cu recovery

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Abstract:

Mining-influenced waters (MIW) pose environmental risks but also represent potential secondary sources of Cu, Zn and other elements. Using the process simulator software USIM PAC, a flow sheet was designed for selective Cu recovery from high-sulfate MIW (HSMIW). It consisted in sequential precipitation for recovery of valuable elements and removal of potential contaminants (recovery of Cu as sulfide, removal of Fe as schwertmannite, recovery of Zn as sulfide) with step increase of pH followed first by lime treatment for main SO_4 removal, and then by a sulfate-reducing bioreactor operating at neutral pH, supplying H_2S for upstream sulfide precipitation and enabling SO_4 polishing. Based on this design, a continuous H_2-fed bioreactor was implemented. The HSMIW (pH 2.7), simulating a real high flowrate and metal rich MIW, was prepared by spiking real MIW and contained 86.0 g/L SO_4, 742 mg/L Cu, and 198 mg/L Zn; after liming to pH 7, these decreased to 29.4 g/L SO_4, 0.02 mg/L Cu, and 0.03 mg/L Zn. The inoculum, sourced from a long-running BRGM SRB bioreactor, was adapted to lime-treated HSMIW and H_2 as electron donor. The system comprised two connected columns: a pozzolana-filled biofilter and an H_2- diffusion and dissolution column with porous fibers. Operated for 160 days, it achieved a maximum SO_4 reduction rate of ~70 mg/L·h. Sulfate-reducing bacteria dominated the microbial community, notably *Solidesulfovibrio carbinoliphilus* and *Desulfosporosinus acidiphilus* representing 8% and 4% of the 16S gene sequences respectively. H_2S was stripped via CO_2 bubbling and injected into HSMIW (prior to lime treatment) to precipitate Cu sulfide, achieving 98% Cu recovery in a solid phase containing 50% Cu and only 0.05% Zn. These results provide a foundation for process development, including enhanced sulfate reduction through optimised H_2 delivery.

Keywords: Mining-influenced water; process simulation; Sulfate-reducing bacteria; Hydrogen; membrane bioreactor

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Enrichment and Characterisation of Sulfate-Reducing Microbial Communities for Mine Water Treatment

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Abstract: The Erzgebirge region and surrounding areas comprises numerous abandoned mine sites that continue to pose environmental challenges due to the uncontrolled release of metals, metalloids, and sulfate. Biological treatment systems utilising sulfate-reducing microorganisms represent a promising approach for sustainable remediation and simultaneous resource recovery from metal-contaminated waters.

To establish robust biological water treatment systems, anaerobic enrichment cultures microorganisms were established using samples collected at the Reiche Zeche mine and from the associated mine drainage "Roter Graben" in Freiberg (Germany). Enrichments were set up under varying conditions (pH, substrate, T) using media designed for sulfate-reducing microorganisms. Initial results confirmed active sulfate reduction, particularly in Postgate B medium.

Microbial community composition of the enrichment cultures was analysed via next-generation sequencing, revealing diverse sulfate-reducing bacterial communities adapted to mining-impacted environments. Dominant genera included *Desulfosporosinus, Solidesulfovibrio, Humidesulfovibrio,* and *Desulfofarcimen*, alongside other microbial groups, indicating complex and resilient community structures. To compare metabolic activity among the enrichment cultures sulfate reduction rates were determined.

Ongoing work focuses on the development of quantitative *real-time* PCR (qPCR) assays targeting functional genes involved in dissimilatory sulfate reduction to enable quantitative comparison of sulfate-reducing populations across different enrichment conditions. These molecular tools will provide insights into community dynamics and functional activity during process optimisation. The obtained results will be transferred to bioreactor studies to systematically evaluate key parameters influencing the efficiency of mine water treatment. Overall, this study contributes to the advancement of sustainable biotechnological solutions for remediation of mining-impacted water.

Keywords: microbial sulfate-reduction, mine-impacted water treatment, enrichment, community analysis

Progress in biotechnical mine and process water treatment and resource recovery

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Abstract:

Mine sites and metallurgical operations generate diverse mine and process waters that require treatment before disposal to meet environmental and regulatory requirements. These waters can range from acidic to alkaline, and may contain a range of contaminants, including oxyanions (e.g., sulfate, selenate, and nitrate), metals and metalloids (M&M), rare earth elements (REEs), radionuclides (RNs), cyanide and organic impurities originating from either the ore itself or processing reagents. Biotechnology offers various pathways for purifying these streams while enabling the recovery of valuable resources such as elemental sulfur (S^0) and selenium (Se^0), M&M, REEs, RNs and caustic soda (NaOH), thereby supporting circularity. Depending on water characteristics, treatment and resource recovery can be achieved through bioreduction, biooxidation, bioprecipitation, biosorption, uptake and bioaccumulation. These processes can be facilitated by microorganisms, seaweed, plants, and biological or bioinspired materials such as biomimetic membranes. Engineering solutions range from passive to active treatment systems that integrate biological, physico-chemical and/or bioelectrochemical unit processes. This presentation provides an overview of the mechanisms underpinning biotechnical mine and process water treatment and resource recovery, together with engineering approaches for their implementation. Selected case studies are presented for various water qualities and recoverable commodities. Recent advances, emerging opportunities and future research priorities in this field are also highlighted.

Keywords: bioprocess, mine water, process water, resource recovery, treatment

Acknowledgements: Funding from CSIRO Environment is gratefully acknowledged.

High-rate bioprocess for complex acid mine drainage treatment and metals recovery

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Abstract:

Acid mine drainage (AMD) has often been treated by chemical neutralisation with lime, which facilitates metal hydroxide precipitation and partial removal of sulfate as gypsum. Biological sulfate reduction offers an alternative nature-based solution for the removal of acidity, sulfate and metals with potential for the selective recovery of chalcophile metals that precipitate as metal sulfides. This study evaluated the feasibility of an integrated biotechnical process for treating high-strength complex AMD and recovering metals. The lab-scale reactor system included unit processes for biofilm-driven sulfate reduction, metal precipitation, excess dissolved sulfide oxidation to elemental sulfur and solids settling. Lactate and ethanol were evaluated as electron donors and carbon sources for sulfate reducers. The integrated system was fed with synthetic mine water (pH ~2) with up to 11.4 g L^{-1} sulfate and various metals (mg L^{-1}): 680 Al, 165 Mn, 89.9 Cu, 32.9 Fe, 32.3 Zn, 10.1 Co, 10.0 Ni and 1.32 Cd. The process effectively neutralised the acidic water increasing pH from 2 to 8-9 and achieved high volumetric sulfate reduction rates (>8 g L^{-1} d^{-1} calculated based on biomass carrier volume) and efficiencies (up to 80%). Cu precipitated selectively at low pH of ~2 with biogenic H_2S, whereas other metals formed mixed precipitates as pH increased. Average metal recoveries (%) were: >99.9 for Al, Cu and Co; >99.8 for Ni and Cd; >99.7 for Zn and Fe; and >99.5 for Mn. Final average effluent metal concentrations (mg L^{-1}) were: <0.002 Cd; <0.003 Co; <0.02 Cu and Ni; <0.06 Fe and Zn; <0.2 for Al; and <0.5 Mn. The integrated system facilitated the effective removal of acidity, sulfate and metals and showed promise for treating high-strength complex mine water and enabling metal recovery.

Keywords: acid mine drainage, biological sulfate reduction, bioprocess, resource recovery, treatment

Acknowledgements: Funding from Rio Tinto Services Limited and CSIRO Environment is gratefully acknowledged.

Acid Mine Drainage-Driven Biooxidation of a Refractory Sulfide Gold Ore

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Keywords: Acid mine drainage; Biooxidation; Refractory sulfidic ore; Gold; Jarosite

Abstract

Acid mine drainage (AMD) is usually considered an environmental liability, yet it also contains acidophilic iron- and sulfur-oxidizing microorganisms that may be repurposed for biohydrometallurgy. In this study, AMD collected from a sulfide-bearing gold mine was evaluated as a biocatalytic medium for the oxidation of a high-grade refractory pyritic gold ore and compared with previously cultured microbial consortia (PCM). The tests were carried out at 35 and 45 °C, at initial pH values of 1.5 and 2 under local-water conditions. The AMD consortium showed faster adaptation, stronger sulfide oxidation, and higher gold recovery than the PCM. Most importantly, Biooxidation using AMD increased gold extraction after cyanidation from 73.0% to 99.3%. These results indicate that AMD can serve as an effective and environmentally meaningful feedstock for the pre-oxidation of refractory sulfide gold ores.

Introduction

Refractory gold ores remain difficult to process because gold is finely encapsulated within sulfide minerals, most often pyrite and related iron sulfides. Conventional pre-oxidation routes such as roasting, pressure oxidation, and chemical oxidation can be effective, but they are capital-intensive and may generate additional environmental burdens. Biooxidation offers a lower-temperature alternative in which acidophilic microorganisms oxidise sulfide minerals and expose the locked gold for downstream cyanidation. AMD is a particularly interesting inoculum because it naturally contains indigenous acidophiles already adapted to low pH and metal-rich conditions, which may reduce lag time and improve process resilience [1-4].

The main objective of this work was to determine whether AMD from a sulfide-rich gold mine could outperform PCM in the biooxidation of a refractory gold ore, and to evaluate how pH, temperature, and ferrous/ferric iron additions affect oxidation behaviour, precipitate formation, and final gold recovery [5-7].

Methods

Materials. The ore sample was obtained from a high-grade sulfide deposit in the Mouteh gold mine area (Chah Khatun, Meimeh, Isfahan). After staged crushing and milling, a particle size of D80 = 75 µm was achieved. XRF analysis in Fig. 1 showed a silicate-rich ore with 66.63% SiO_2, 11.42% Al_2O_3, 6.57% Fe_2O_3, 2.63% SO_3, and 5.96% loss on ignition, indicating that a substantial portion of the ore matrix was gangue associated with iron-sulfide-bearing phases. The AMD inoculum was collected from the mine drainage pond (Fig. 2) and contained elevated concentrations of Fe, sulfate, Cu, Al, Mg, and Ca, making it both acidic and metal rich.

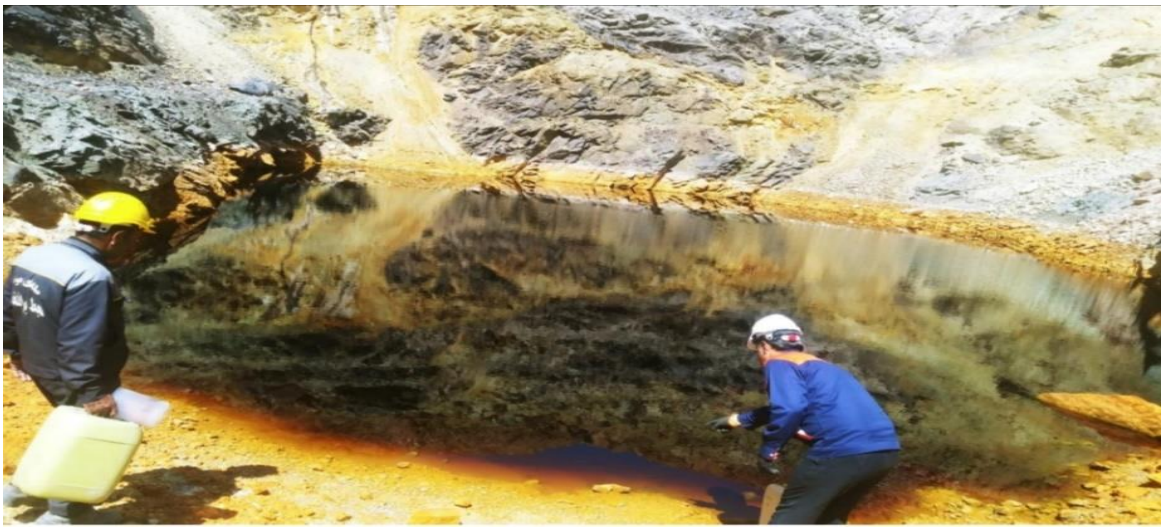


Figure 1. Sampling from the AMD of the Mouteh gold mine

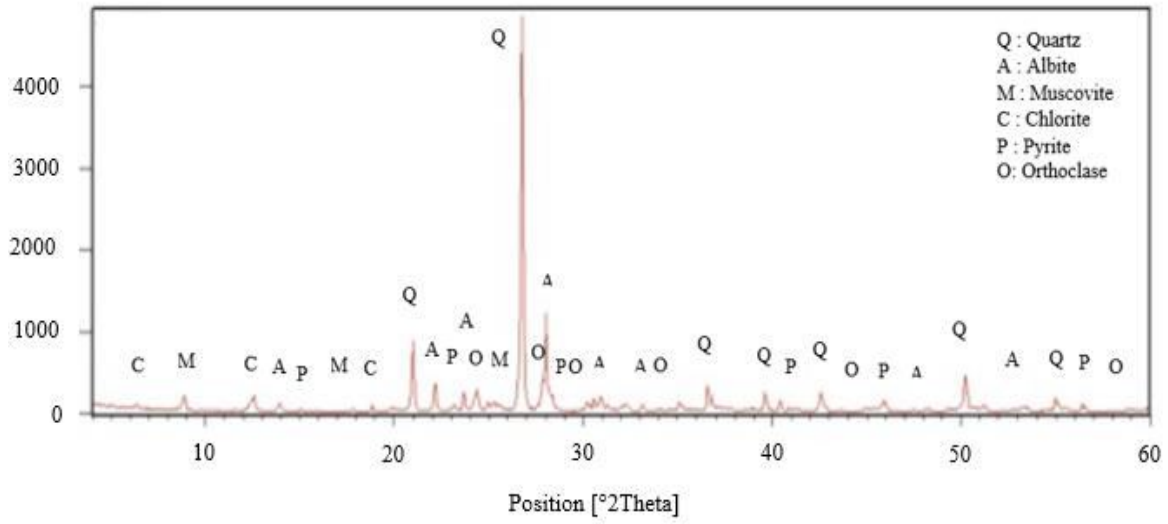


Figure 2. XRD analysis of the run of mine ore sample

Two microbial sources were studied. The native AMD consortium was sampled directly from the mine drainage system containing (mg/L) Fe, 1247; SO_4, 19643; Cu, 98; Al, 1709; Mg, 996, whereas the reference cultures (PCM) were previously cultured mesophilic and moderate thermophilic microorganisms from a microbial bank. The mesophilic reference group included *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans,* and *Leptospirillum ferrooxidans*, and the moderate thermophilic group included *Acidithiobacillus caldus, Leptospirillum ferriphilum,* and *Sulfobacillus thermosulfidooxidans*. These organisms are well known for their ability to oxidise iron and sulfur species under strongly acidic conditions.

Biooxidation experiments were performed in 500 mL Erlenmeyer flasks with Norris medium under controlled pH and temperature. These tests compared AMD and culture-bank (PCM) inoculated at 35 and 45 °C using local water (Table 1).

Analysis. Process performance was tracked by pH and ORP, because lower pH together with a rise in ORP indicates active oxidation of ferrous iron and sulfide minerals. The oxidised residues were characterised by XRD to identify mineralogical changes and inhibitory precipitates. Gold contents in solids and solution were determined by fire assay, ICP, and AAS, and cyanide concentration was measured during bottle-roll cyanidation tests [1].

Table 1. Summary of experimental stages and headline outcomes.

Experiment set	Inoculum	Main condition	Main outcome
Biooxidation tests	AMD vs. PCM	35/45 °C; pH 1.5; local water	AMD showed a shorter lag phase and stronger oxidation
Cyanidation tests	Oxidized residues	Bottle-roll leach, 72 h	Gold extraction recovery increased from 73.0% to 99.3%

Results and Discussion

Microbial adaptation and oxidation kinetics. The native AMD consortium consistently adapted faster than the culture-bank inoculated. This was reflected in a shorter lag phase and a more rapid increase in oxidation indicators, showing that microorganisms already exposed to the mine environment were better prepared to attack the sulfide matrix. In the preliminary tests, the AMD mesophiles were particularly active, suggesting that the local microbial community was well matched to the physicochemical conditions of the drainage water. The thermophilic cultures also oxidised the ore, but they generally required a longer acclimation period.

This behaviour is important because biooxidation performance is not determined only by the final oxidation state; the early activation period strongly affects reactor productivity. A shorter lag phase means that the microbial community can establish itself more quickly on the mineral surface, generate ferric iron more efficiently, and begin exposing gold-bearing particles sooner.

pH/ORP evolution and iron cycling. The pH and ORP trends supported the same interpretation (Fig. 3). As oxidation progressed, pH decreased or remained strongly acidic, while ORP increased, indicating continuing ferrous-to-ferric cycling and progressive sulfide oxidation. The AMD-derived organisms showed a more pronounced shift in both signals than the reference cultures, which implies a more vigorous bioleaching metabolism. The behaviour observed here is consistent with previous reports showing that indigenous AMD microorganisms can be highly effective in sulfide oxidation because they are already pre-adapted to harsh mine drainage conditions [3,4,7].

In Fig. 4, the amount of dissolved iron in the AMD culture at 35 °C (Fe → min=1, max=1.8 g/l) is slightly higher than that in the solution containing mesophilic PCM (Fe → min= 0.4, max= 1.7 g/l). But in general, iron dissolved in the AMD culture is more than that in the PCM culture, which leads to the higher dissolution of the sulfide minerals in the AMD culture because of more adaptability and higher ferric concentration, as illustrated in Fig. 3.

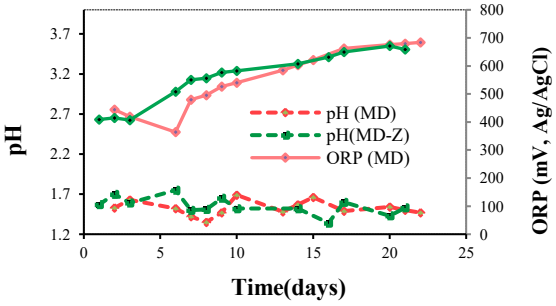


Figure 3. pH-ORP variation of (Norris culture medium prepared from deionized water (D), MD-Z= mesophilic AMD microorganisms, MD= mesophilic PCM

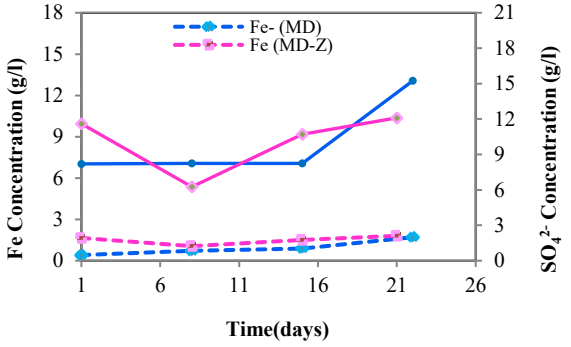


Figure 4. Comparison of AMD and PCM cultures by examining changes in the concentration of dissolved iron and sulfate ions (Norris culture medium prepared from deionized water (D), diagram (a) TD-Z= AMD thermophiles at 45°C, TD=PCM thermophiles, (b) MD-Z=AMD mesophiles at 35°C, MD=PCM mesophiles

Gold recovery after cyanidation. The most important outcome of the study was the improvement in downstream gold extraction. The untreated or weakly oxidised ore yielded substantially lower gold recovery, whereas pre-oxidation with AMD in saline local water lifted extraction from 73.0% to 99.3% after cyanidation. In addition, the gold content in the solid sample was 25.3 g/t before cyanidation and

0.18 g/t after cyanidation, which further confirms the increase in gold recovery to the desirable level reported above. Also, the results of atomic absorption analysis are presented in Table 3, indicating that the amount of dissolved gold in the first sample after 2 h was 8.6 mg/L (equivalent to 25.8 g/t). The results indicated that almost 99% of the gold dissolved within the first 2 h of cyanidation, likely due to the disintegration of sulfide networks and the release of locked gold particles by AMD microorganisms during biooxidation. Figure 5 shows that sodium cyanide consumption increased sharply during the first 2 h because of rapid gold dissolution and gold–cyanide complex formation, and then continued more gradually, probably due to the dissolution of other ore components. Finally, the total sodium-cyanide consumption reached 720 g/t, which appears to be favourable for the industrial cyanidation process. These findings indicate that native AMD effectively enhanced sulfide oxidation and reagent access during leaching, demonstrating its potential as a robust and cost-effective inoculum for refractory sulfide gold ores.

Table 3. Gold concentration in solution samples

Sampling time (hours)	The concentration of gold in the solution (mg/l)
2	8.6
6	7.4
24	8.6
48	8.0
72	8.6

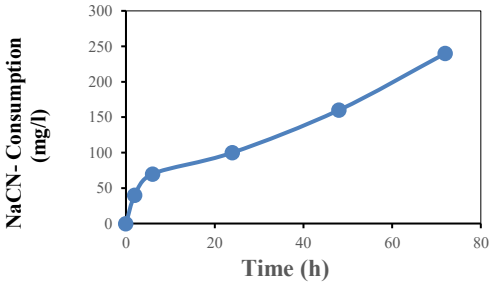


Figure 5. The consumption of sodium cyanide as a function of leaching time

Saline local water did not inhibit indigenous AMD activity, enabling high gold extraction and highlighting the potential for water reuse. Consistent with previous studies [3,4,6], AMD-derived consortia promoted pre-oxidation of refractory gold ore prior to cyanidation, demonstrating their value as practical, site-adapted inocula. Process performance depended on both inoculum source and iron control to prevent passivation.

Conclusions

AMD should not be viewed only as a waste stream. In this work, it acted as a productive microbial source for the biooxidation of a refractory sulfide gold ore. Compared with the reference culture-bank consortium, AMD microorganisms showed faster activation, stronger sulfide oxidation, and better gold extraction.

The practical significance of the study is that a hazardous mine drainage can be converted into a useful bioprocessing resource, simultaneously improving gold recovery and offering a pathway toward more sustainable mine-site management.

Acknowledgements

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BioGeoMetals – A Research School for Sustainable Metal Recycling

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Abstract: The BioGeoMetals Research School addresses the urgent need for sustainable methods to secure Europe's supply of Strategic and Critical Raw Materials, which are vital for renewable energy, mobility, and digital technologies. Traditional mining is energy-intensive, highly polluting, and generates vast waste streams in solid, liquid, and slurry forms that harm ecosystems and public health. This doctoral school will pioneer biotechnology-based solutions, i.e., biomining and bioremediation, involving naturally occurring microorganisms to reclaim valuable metals from mine waste with lower costs, reduced energy and chemical use, and minimal environmental impact. The program is jointly organised by Linnaeus University, Luleå University of Technology, and KTH Royal Institute of Technology, complemented by international partners and industry stakeholders. Seven interlinked PhD projects will span geochemical exploration of mine water and waste, microbial bioprospecting to identify candidate populations for metal solubilisation and selective metal recovery, biomining processes, selective recovery of metals via e.g., sulfate reduction and biosorption, controlled crystallisation for recovery of metal salts and solid phase characterisation, biotransformation and bioremediation, and sustainability assessments to address the environmental, economic, and social dimensions of biomining-based metal recovery systems. The students will receive multidisciplinary training in geochemistry, microbiology, bio-process engineering, and environmental science, alongside courses on ethics and circular economy principles. By combining fundamental research with applied innovation, the school will educate a new generation of experts in sustainable mining and mineral research. Its outcomes will secure a resilient supply of Strategic and Critical Raw Materials for Europe's green and digital transitions.

Keywords: Geochemical exploration, Bioprospecting, Biomining, Metal recovery and remediation, and Sustainability

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Sustainable copper removal using different biosorbents

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Keywords: yeast; green alga; agricultural wastes; biosorption; copper removal

Abstract

Water pollution is escalating global concerns, with acid mine drainage (AMD) representing a major source of heavy metal contamination, highlighting the need for sustainable metal recovery strategies. This study evaluates the potential of various biosorbents, including *Chlorella*, an AMD-derived yeast isolate, for Cu^{2+} removal from AMD-affected waters. Biosorption experiments were conducted at pH 2, 4, and 6, with initial Cu^{2+} concentrations of 25 mg/L. Results revealed that pH 4 and 6 were optimal for biosorption, with removal efficiencies varying among materials. Yeast isolate *Rhodotorula* sp. achieved 48.1% Cu^{2+} removal at a biomass concentration of 1 g/L, which increased to 71.5% at 3 g/L. For comparison, reported Cu^{2+} removal efficiencies at 1 g/L biomass were 51.2% for *Chlorella*, which increased to 65.9% at 3g/L. Kinetic modelling for yeast indicated a pseudo-second-order fit (R^2 > 0.99), consistent with chemisorption-dominated mechanisms. Langmuir isotherms (R^2 = 0.93) confirmed monolayer adsorption on homogeneous sites. These findings highlight the effectiveness of yeast as an eco-friendly, low-cost biosorbents for copper recovery from AMD-contaminated waters.

Introduction

Heavy metal pollution is a major environmental problem resulting from industrial activities such as mining, metallurgy, electroplating, and energy production. Toxic metals, including copper, zinc, cadmium, chromium, and lead, persist in the environment, accumulating in ecosystems [1,2]. Acid mine drainage (AMD) is one of the most significant sources of heavy metal contamination. It is generated through the oxidation of sulfide minerals exposed to oxygen and water, which is often accelerated by iron-oxidizing microorganisms. AMD is characterised by low pH and high concentrations of dissolved metals and sulfates, making it difficult to remediate [3]. Among the available treatment methods, biosorption is an eco-friendly and cost-effective alternative to conventional physicochemical technologies utilising biological materials to bind metal ions through functional groups such as carboxyl, amino, hydroxyl, and phosphate groups of cell surfaces [4,5]. Metals can be regenerated through desorption, which gives it additional advantage for wastewater remediation [6]. Various biosorbents, including microorganisms, algae, and agricultural by-products, have demonstrated promising metal removal capacities [7,8,9]. In this study, the Cu^{2+} biosorption potential of *Chlorella*, an AMD-derived *Rhodotorula* yeast isolate, was evaluated under different experimental conditions. The adsorption mechanism of the most effective biosorbent was further investigated using kinetic and isotherm models.

Methods

The overall methodology used in this study is summarised schematically in Figure 1.

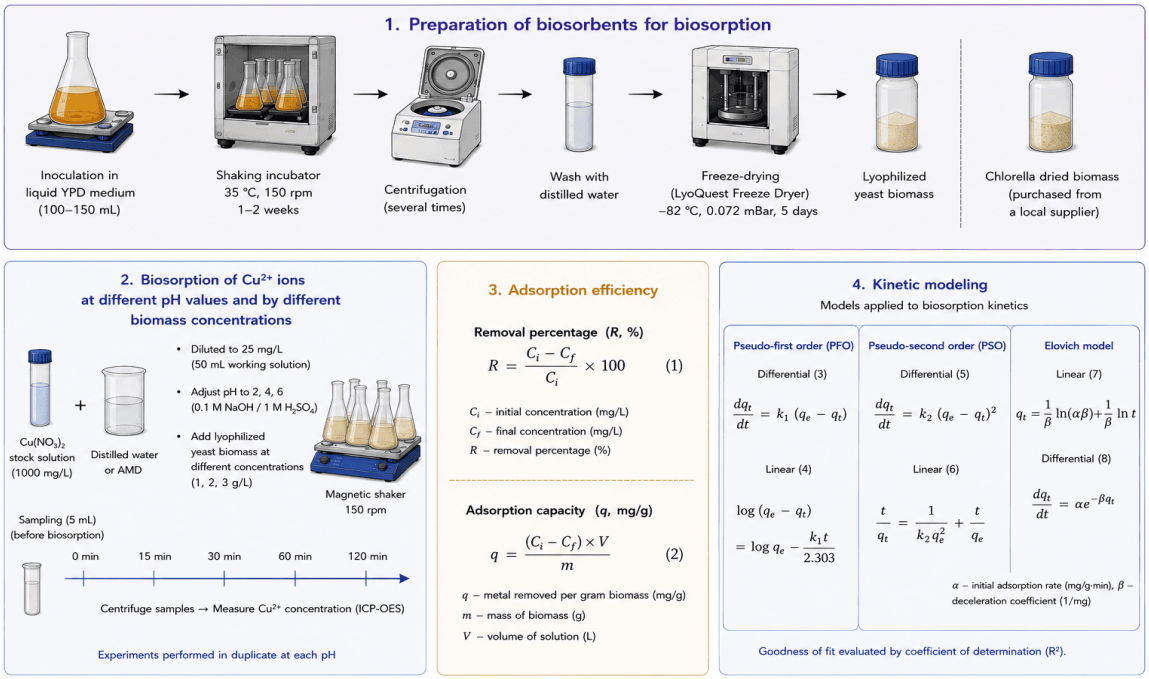


Figure 1. Schematic overview of the experimental design, including preparation of yeast and *Chlorella biomass*, Cu^{2+} biosorption experiments, and kinetic modelling of the biosorbent.

Results and Discussion

Biosorption experiments

The AMD-derived yeast isolate PX677341, identified as *Rhodotorula* sp. KT strain, was cultivated, harvested, dried, and used as a biosorbent in the experiments. Dried *Chlorella biomass* purchased commercially was used for comparison. The biosorption efficiency of both biosorbents was strongly influenced by the solution pH, with the highest Cu^{2+} removal observed at pH 6. The solution pH decreased from 6.0 to approximately 4.0–4.5 during the biosorption process. This pH reduction likely decreased the availability of negatively charged binding sites on the biomass surface and increased competition between H^+ and Cu^{2+} ions for adsorption sites, resulting in a lower biosorption capacity. The removal efficiencies obtained under different biosorbent concentrations are presented in Figure 2. As shown in the figure, increasing the biomass concentration from 1 g/L to 3 g/L enhanced Cu^{2+} removal for both biosorbents. For *Rhodotorula* sp., the removal efficiency increased approximately 1.5-fold, while for *Chlorella biomass* it increased by about 1.3-fold. These results indicate that a higher biomass dosage provides a greater number of available binding sites, thereby improving copper biosorption.

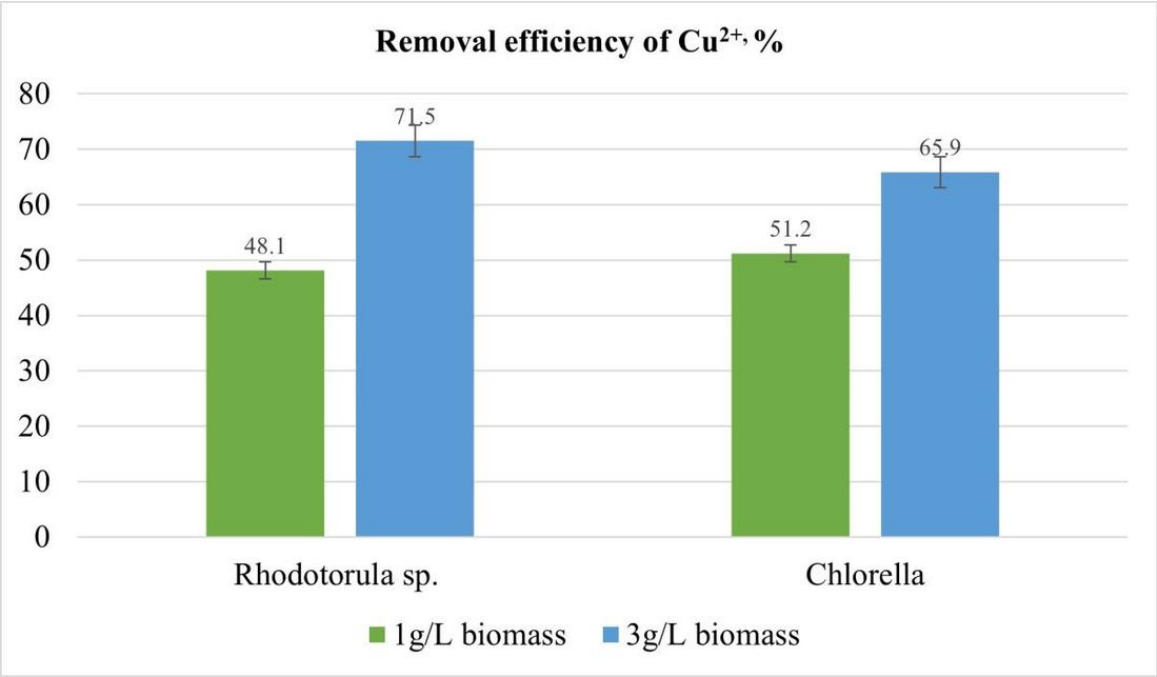


Figure 2. Cu^{2+} removal efficiency at different biomass concentrations of *Rhodotorula* sp. and *Chlorella*.

Kinetic analysis and Isotherm modelling

The biosorption kinetics of Cu^{2+} onto the AMD-derived *Rhodotorula* sp. biomass were evaluated using the pseudo-first-order (PFO), pseudo-second-order (PSO), and Elovich models. The PSO model provided the best fit to the experimental data ($R^2 > 0.99$), indicating that chemisorption is the predominant mechanism governing copper uptake. In contrast, the lower goodness-of-fit obtained with the PFO model suggests that physical adsorption is not the primary rate-controlling process. The comparatively poorer fit of the Elovich model indicates that adsorption occurred mainly on relatively homogeneous active sites of the yeast biomass. The kinetic analysis therefore confirms the high affinity of the biosorbent for Cu^{2+} and supports its potential application for the treatment of AMD-contaminated waters.

The adsorption isotherm data were analysed using the Langmuir and the Freundlich models. The Langmuir model provided the best fit to the experimental data ($R^2 = 0.93$), indicating that Cu^{2+} adsorption occurred predominantly as monolayer adsorption on a finite number of relatively homogeneous active sites. The rapid initial adsorption followed by a slower approach to equilibrium suggests progressive saturation of the available binding sites. At higher initial Cu^{2+} concentrations, a reduction in adsorption efficiency was observed, which was likely associated with the decrease in solution pH during the biosorption process.

Overall, the AMD-derived *Rhodotorula* sp. demonstrated high potential as a biosorbent for Cu^{2+} removal. Biosorption efficiency was strongly influenced by solution pH and biomass concentration, while kinetic and isotherm analyses indicated that Cu^{2+} uptake was predominantly governed by chemisorption through monolayer adsorption.

Conclusions

A wild *Rhodotorula* sp. isolated from acid mine drainage exhibited promising biosorption capacity for Cu^{2+} removal. The highest copper removal efficiency (71.5%) was achieved at pH 6 using a biomass concentration of 3 g/L, compared with 65.9% obtained under the same conditions using dried *Chlorella biomass*. Kinetic analysis demonstrated that the pseudo-second-order model best described the adsorption process, indicating that chemisorption is the predominant mechanism governing Cu^{2+}

uptake. SEM–EDS analysis confirmed the presence of copper on the yeast biomass, while the Langmuir isotherm indicated predominantly monolayer adsorption on relatively homogeneous active sites. These findings demonstrate that the AMD-derived *Rhodotorula* sp. is an efficient, low-cost, and environmentally friendly biosorbent with considerable potential for the remediation of acid mine drainage and other metal-contaminated wastewaters.

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Evaluating the Potential of Microbially Induced Calcite Precipitation to Overcome Shortcomings of Conventional Co-Disposal

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Abstract: Acid rock drainage (ARD) arises when sulfide-bearing mine wastes undergo oxidative weathering, producing acidic leachate upon exposure to oxygen and water. Preventing or minimising contact between oxidants and sulfide minerals is therefore essential to suppress the reactions responsible for acid generation. One widely applied mitigation strategy is co-disposal, in which mine wastes with contrasting ARD potential and particle-size distributions are blended to restrict oxidant transport to acid-producing components.

This study investigates the integration of co-disposal with microbially induced calcite precipitation (MICP) as an enhanced ARD control approach. MICP relies on ureolytic microorganisms to precipitate calcium carbonate, which can progressively obstruct pore spaces in granular media. The resulting calcite accumulation acts as a reactive barrier that limits oxidant ingress into sulfide-rich coal waste rock, thereby delaying ARD initiation. Simultaneously, calcite precipitation improves the mechanical integrity of the waste matrix, reducing permeability and seepage associated with oxidative degradation.

A total of twelve laboratory-scale bioreactors were prepared using distinct packing arrangements to identify the physical conditions most conducive to effective MICP development. Two bacterial inoculation methods, irrigation and agglomeration, were evaluated to determine their influence on calcite distribution and penetration depth. This work represents the first reported demonstration of successful MICP formation within a co-disposed mine waste system. Results indicate that packing configuration exerts a dominant control on MICP kinetics, spatial extent, and overall yield, while the effectiveness of inoculation strategies is strongly contingent upon the chosen packing protocol.

Keywords: acid rock drainage, co-disposal, microbially-induced calcite precipitation, mine waste, prevention

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Microalgal polycultures for carbon cycling and metal removal from metal-contaminated mine waters

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Abstract:

Where sulfidic mine waste is generated, including pyritic coal discards, acid mine drainage (AMD) remains a persistent challenge. On exposure to oxygen and water, the endemic microbial communities catalyse oxidation of mineral sulfides resulting in acidic, sulfate-rich solutions with elevated concentrations of dissolved metals. In this study, we investigate potential of a micro-algal polyculture developed from endemic micro-algal species from the Mpumalanga coal mining area of South Africa, identified for both their rapid carbon assimilation and metal concentration, as a potential biological treatment for metal-contaminated mine waters. We seek to produce fit-for-purpose water and enable a resource-recovery route with an emphasis on metal removal, pH improvement, carbon assimilation and biomass generation for potential energy recovery.

Microalgal isolates from the Mpumalanga coal-mining region were screened for growth performance, metal uptake capacity and carbon assimilation. Four isolates were selected to formulate the polyculture. The polyculture performance in the presence of a metal cocktail typical of acid mine drainage from the Mpumalanga coal fields was assessed in 3.2 L airlift photobioreactors under lab conditions and 100 L raceway ponds under outdoor conditions. Biomass growth, and its carbohydrate, protein, and pigment content, as well as pH, nitrate concentration and dissolved metal concentration in the aqueous stream were monitored.

The polyculture remained viable under 1x and 5x metal treatments relative to typical AMD, confirming tolerance to metal-rich conditions. Cultivation increased pH towards circumneutral to alkaline conditions. Dissolved metal concentrations decreased during cultivation to levels acceptable for irrigation according to the IRMA regulations. Bioconcentration factors for target metals showed values in the range of 2000 to 4000. The findings demonstrate that microalgal polycultures can function as a nature-based treatment for low-pH and metal-rich mine waters, combining treatment with resource recovery, yielding both metal concentrates and bio-energy.

Keywords: micro-algae, metal concentration, carbon capture, bio-energy

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The effects of microbial inoculation, substrates and initial pH on the leaching of critical minerals from black shale

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Abstract:

The bioleaching of black shales has been shown to be commercially viable for extracting various base metals such as nickel, zinc, cobalt and copper in Finland. This study explored the bioleaching and chemical leaching of critical minerals from ore from the Byro rare earth and lithium deposit in Western Australia. Leaching experiments were conducted in 250 mL shake flasks containing 100 mL liquid at 1% (w/v) ore pulp density for 21 days. The effects of various substrates (none, glucose, Fe^{2+} only, S^0 only, and glucose + Fe^{2+} + S^0) on bioleaching were evaluated using basal salts medium (BSM, pH 2) inoculated with a mixed biomining culture. Chemical leaching was evaluated at initial pH values ranging from 0.53 to 3.0, adjusted with H_2SO_4 in BSM or deionised water. Several rare earth elements, lithium, vanadium, rubidium and nickel were solubilised from the Byro ore during bioleaching and chemical acid leaching. Initial pH, microbial inoculation and supplementation with glucose, Fe^{2+} and S^0 notably influenced leaching performance. Bioleaching enabled higher yields than chemical leaching for most rare earth elements. The highest bioleaching yields for Y (65.3%), Pr (67.2%), Nd (68.2%), Sm (51.7%), Eu (73.0%), Gd (81.3%), Tb (80.3%), Dy (65.0%), Ho (62.4%), Er (65.5%), Tm (51.9%), Yb (46.2%), Lu (47.6%), Li (62.0%) and V (42.7%) were achieved when the ore was bioleached with S^0 as the sole amendment. This indicated that the acid generated via microbial S^0 oxidation enhanced the leaching. In contrast, the highest yields for Sc (41.7%), La (65.6%), Ce (70.2%) and Rb (32.1%) were reached through chemical leaching in deionised water at an initial pH of 0.53, while the highest Ni leaching (80.0%) occurred in deionised water at an initial pH of 0.75-1.0. Overall, the results demonstrated the technical feasibility of extracting critical minerals from the Byro black shale using bioleaching.

Keywords: bioleaching, black shale, chemical leaching, critical minerals, rare earth elements

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Seawater Bioleaching of Chalcopyrite: Adaptation of Acidophilic Consortia to Seawater for Improved Chalcopyrite Bioleaching

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Abstract: The copper mining industry operates under constant limitations imposed by decreasing ore grades, which are further exacerbated by increasing freshwater scarcity, particularly in arid mining regions. Addressing these constraints requires alternative extraction strategies that minimise environmental impact, reduce dependence on freshwater, and ensure economically viable copper production. Bioleaching represents a potential alternative, as it generally requires less water and fewer chemical reagents than conventional leaching. However, large-scale application remains limited by slow reaction kinetics, chalcopyrite surface passivation, and operational limitations.

This study addresses these challenges from a microbial perspective by exploring how acidophilic communities physiologically and metabolically respond to saline environments, to enhance chalcopyrite bioleaching under seawater-based conditions. Two microbial consortia were considered: a natural consortium obtained from a copper mine in Cyprus and a synthetic consortium containing sulfur and iron-oxidising microorganisms. Both consortia were exposed to gradual increases in seawater concentration to induce microbial adaptation, while metabolic activity, growth, and iron oxidation were monitored.

Following microbial adaptation, chalcopyrite bioleaching under 40% seawater and control conditions was evaluated through bioreactor and column experiments to assess process scalability. Different redox trajectories were observed, identifying the redox potential as a key parameter to differentiate the consortia. The seawater-adapted consortia maintained lower ORP values, between 320 and 450 mV, whereas the non-adapted consortia evolved toward higher ORP values in the range of 540 to 670 mV, conditions commonly associated with iron precipitation and mineral surface passivation in chalcopyrite bioleaching.

Consistent with these trends, the seawater-adapted consortia exhibited the highest bioleaching performance, with the natural consortium showing superior tolerance to elevated salinities while sustaining favourable conditions for chalcopyrite dissolution. Overall, this study underscores the critical role of microbial adaptation and redox management in enabling seawater-based bioleaching as a sustainable and water-efficient alternative for future copper extraction in water-scarce mining regions.

Keywords: Seawater bioleaching, Chalcopyrite dissolution.

The effects of microbial inoculation, temperature and organic carbon on the leaching of low-grade chalcopyrite ore in saline water

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Abstract:

Chalcopyrite is one of the most abundant, yet refractory copper minerals. Although the use of saline water has been shown to enhance chalcopyrite leaching, the commonly used biomining microbes are intolerant to high concentrations of both chloride and copper. This study evaluated the leaching of copper from low-grade chalcopyrite ore (0.54% Cu) in saline water with and without a salt-tolerant mixed microbial consortium. The experiments were conducted in duplicate 250 mL shake flasks containing 100 mL of medium, agitated at 150 rpm for 21 days. Tested conditions included: artificial seawater (SW) concentration (0.5 × or 1 ×), organic carbon addition (with or without 0.01 % yeast extract), temperature (30 °C or 45 °C), and microbial inoculation (with or without 10% inoculum). At 30 °C, the highest Cu yield (68.9 %) was achieved in inoculated flasks with 0.5 × SW without yeast extract, whereas at 45 °C the highest Cu yield (81.5 %) occurred in inoculated flasks with 1 × SW with yeast extract. Overall, the study demonstrated that elevated temperature effectively accelerated bioleaching and chemical leaching of Cu from low-grade chalcopyrite-bearing ore under saline conditions in both 0.5 × SW and 1 × SW (analysis of variance (ANOVA) p-values 0.0029-0.046). Microbial inoculum increased Cu leaching in 0.5 × SW at 30 °C (p-value 0.038) and in 1.0 × SW at 45 °C (p-value 0.0085). Organic carbon addition slightly further enhanced Cu bioleaching at 45 °C, but the increase was not statistically significant (p-value 0.068).

Keywords: bioleaching, chalcopyrite, chemical leaching, saline water, salt tolerant microorganisms

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Biooxidation of a sulfidic refractory gold ore at different particle sizes

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Abstract: sulfide oxidation using microbial enhancement is one of the alternative processes to roasting or pressure oxidation for refractory sulfide gold ores. Pressure oxidation and roasting have been used in a large scale globally. Both technologies require particle size of less than 75 micron or smaller for faster kinetics with higher sulfide oxidation rate. Comminution cost is the highest among various unit metallurgical processes and often impacts negatively on overall project economics. Heap biooxidation has been demonstrated with much larger particle sizes. The goal of the research is to investigate the potential processing option using coarser particles at 0.6 mm (30 mesh) and a conventional percolation system. Two ore samples were received from a project site in Northern Nevada. Sample A contains 3.42 g/t gold, 1.22% total sulfur, and 0.38% total carbon. Sample B contains 3.18 g/t gold, 0.68% sulfur, and 0.38% carbon. As received ores showed gold extraction by cyanidation of 61.2 and 61.6%, respectively.

Coarse particle biooxidation was carried out using agglomerated samples in a stainless-steel reactor. A nutrient solution was fed prior to irrigate bio-solution containing a consortium of mesophilic bacteria. The system is similar to column operation with low irrigation rate and collecting percolates at the bottom of the reactor. To compare with the conventional process, biooxidation tests were also performed at 75 μm in a stirred reactor. After biooxidation, bottle roll cyanidation was improved up to 83.1% from fine particle biooxidation and 71.4% from coarse particle percolation.

Keywords: biooxidation, refractory gold ores, mesophiles, coarse particles biooxidation

Validation of a Custom Pilot Stirred Tank Bioreactor via Comparative Bench- and Pilot-Scale Biooxidation of Refractory Gold-bearing Arsenopyrite

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Keywords: arsenopyrite biooxidation, stirred tank reactor, pilot scale, acidophiles, refractory gold

Abstract

Biooxidation is an established pre-treatment for refractory gold ores containing arsenopyrite and pyrite, where post-process cyanidation outcomes are improved by the sulfide oxidation. However, biooxidation performance is mineralogy-specific and requires pilot-scale validation. This study compares the biooxidation performance of an arsenopyrite/pyrite-rich material (8.0% As, 42 ppm Au, 37 ppm Ag) at bench-scale and at pilot-scale with a custom-built bioreactor system.

A mixed acidophilic consortium was adapted via serial passaging and trialled in 5 L and 50 L stirred tank reactors (STRs) (10% w/v, 35 °C, 400 rpm, 4.5 L min^{-1} air). Bench-scale results showed rapid Fe^{2+} oxidation (>98%), ORP 670–700 mV, liquor sulfate concentration up to 7 g L^{-1}, and cell density of 1.8×10^{8} cells mL^{-1}.

The 50 L reactor exhibited a longer lag phase in microbial growth, but improved metal oxidation outcomes, with 22 g L^{-1} Fe^{3+}, 50 g L^{-1} SO_4, 8.2×10^{8} cells mL^{-1}, and Fe leaching rate of 0.088 kg m^{-3} h^{-1}. This achieved 80–92.5% As removal and enabled >99.9% subsequent Au recovery, with lower Ag recovery (26.2%).

These results confirm a successful scale-up and support the process optimisation of the custom-built pilot STRs.

Introduction

In Canada, gold remains the most valuable mined metal, with exports reaching $52.1 billion in 2025 [1]. However, global mineable reserves are declining, and a significant portion of the remaining resources occur as refractory gold associated with sulfide minerals, which complicates extraction [2].

Conventional gold extraction methods, including gravity concentration, cyanidation, and adsorption processes, are effective for free-milling ores but are inefficient for refractory ores where gold is encapsulated in sulfide matrices [3, 4]. These ores require pre-treatment to oxidise sulfides and expose microscopic gold, prior to its dissolution and extraction via cyanidation [5, 6]. Biooxidation, using iron- and sulfur-oxidizing microorganisms in aerated and agitated systems, is an established and environmentally favourable approach. Technologies such as the BACOX™ process have demonstrated high gold recovery efficiencies (up to 98%) from a gold-bearing quartz-carbonate-sulfide deposit by enhancing sulfide oxidation and improving downstream leaching performance [7, 8].

Pilot-scale testing is a critical step in advancing biooxidation from laboratory to industrial application. While laboratory studies provide insight into microbial and kinetic behaviour, they do not fully capture the hydrodynamic and operational complexities of large-scale systems. Pilot systems enable evaluation of key parameters such as feed rates, residence time, gas mass transfer, temperature control, and continuous operation, while providing realistic data on kinetics, microbial stability, and process robustness. This information is essential for scale-up, techno-economic assessment, and risk reduction, and is often mineral-specific [9, 10, 11]. Consequently, existing pilot studies cannot directly validate the performance of a newly commissioned, custom-built reactor configuration for a distinct arsenopyrite-rich feed.

This study addresses this gap by evaluates the biooxidation performance of a refractory gold-bearing arsenopyrite material at both bench scale (5 L) and pilot scale (50 L), with the objective of assessing scalability and supporting process validation following the commissioning of the MIRARCO custom-built pilot system under comparable operating conditions, and to provide reactor-specific scale-up data [12, 13].

Methods

An arsenopyrite-rich feed was prepared from blended refractory ore, flotation concentrate, and tailings from a gold mine in Ecuador, ground to <75 µm. The material contained ~62% sulfides (mainly arsenopyrite and pyrite), assayed 42 g t^{-1} Au (±75% refractory) and 8% As, with a soluble Fe:As ratio of 10.17.

A mixed acidophilic culture (35 °C, basal salts medium [14], 5 g L^{-1} Fe^{2+}, pH 1.5) was adapted to arsenopyrite and maintained with chalcopyrite. The inoculum consortium was dominated by *Leptospirillum (L.) ferrooxidans* (90.8%) and *Sulfobacillus (S.) spp.* (8.8%), confirmed using 16S rRNA sequencing.

Batch biooxidation was conducted in 5 L and 50 L STRs (**Table 1**) at 10% (m/v), pH 2.5, 35 °C, 400 rpm, and 4.5 L min^{-1} air supply. Leaching was completed in 12 and 9 weeks, respectively. Monitoring included pH, ORP, Fe speciation, sulfate, microbial counts, and dissolved metal determinations. Washed residues were analysed (fire assay, ICP-MS) and cyanide treated for gold recovery. Microbial communities for the pilot-scale were assessed by 16S rRNA sequencing [15].

Table 1. System specifications of the bench-scale and the pilot-scale bioreactors used for the arsenopyrite biooxidation tests.

Parameters	Bioreactor specifications	
	5 L STR (Bench-scale)	50 L STR (Custom pilot-scale)
Max. STR volume	6 L	75.4 L
Operating volume	5 L	50.3 L
Total STR height	322 mm	517 mm
STR inside diameter	157 mm	431 mm
Height to diameter ratio	0.48	0.83
Tank to impeller ratio	0.41	0.33
Impeller diameter	64.3 mm	140.2 mm
Impeller design	Axial 4 pitched blades up-pumping propellers	
Number of baffles	4 baffles fixed to the inside wall at 90 º from each other	
Aeration supply	Side L-shape sparger	Through impeller shaft
STR material	PVC	Stainless steel

Results and Discussion

Bench-scale testing provided controlled conditions for evaluating biooxidation, whereas the pilot-scale reactor introduced operational complexity, enabling assessment of scale-up performance. Distinct differences in pH and redox behaviour were observed. In the bench reactor, pH decreased from 3.1 to 1.5 within 15 days and remained stable thereafter. In contrast, the pilot reactor showed an initial pH rise (2.5 to 2.76), followed by a decline to <1.0 and subsequent pH values of 0.94–0.97 (**Figure 1b**). This was likely associated with more extensive sulfide oxidation and the resulting acid generation, consistent with the higher sulfate concentration measured in the pilot system.

ORP trends reflected iron oxidation dynamics. The bench reactor reached 666–675 mV during active biooxidation before declining slightly, whereas the pilot system achieved higher values (up to 714 mV) and subsequently remained at 720–734 mV. Active biooxidation, which is indicated by the constant increase in Fe^{3+} in the system, occurred earlier in the bench system (days 10–26) compared to the pilot-

scale (days 25–41) (**Figure 1a, c**). Maximum Fe^{3+} concentrations reached 17.9 g L^{-1} (bench) and 22.0 g L^{-1} (pilot), with low residual Fe^{2+}, indicating efficient oxidation.

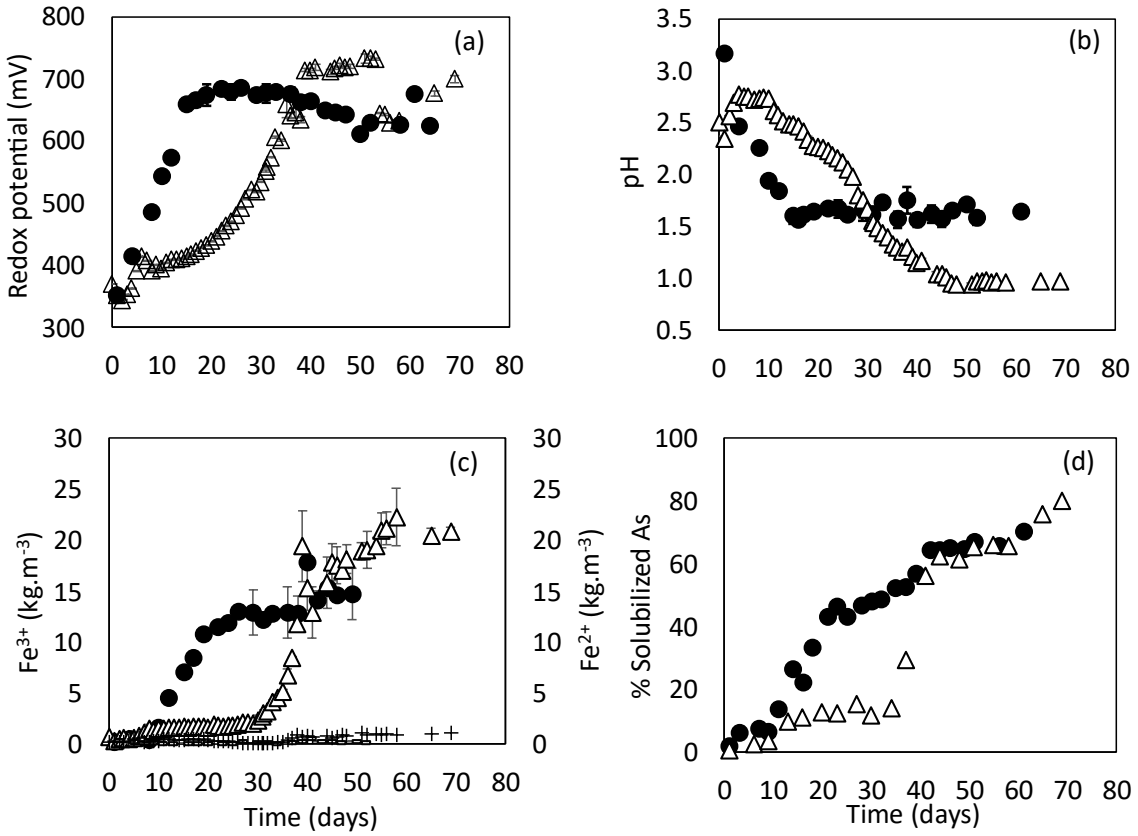


Figure 1. Changes in (a) ORP, (b) pH, (c) Fe^{3+} (● and Δ) and Fe^{2+} (- and +) concentrations, and (d) concentration of solubilised As. Bench-top STR (●) vs. custom pilot-scale STR (Δ).

Iron solubilisation reached 94.6% (bench) and 97.5% (pilot), with a higher Fe oxidation rate in the pilot reactor (0.088 vs 0.039 kg m^{-3} h^{-1}) (**Table 2**).

Table 2. Maximum Fe leach rates and extent of Fe solubilisation at the end of arsenopyrite biooxidation in bench-top and custom pilot-scale bioreactors.

STR	Max Fe leaching rate [kg $m^{-3}h^{-1}$]	R^2	Extent of Fe solubilization [%]	Lag phase prior to Fe oxidation [days]	Time for total Fe oxidation [days]
Bench-scale (5 L)	0.039	0.98	94.59	8.21 ± 0.38	45.1
Custom pilot-scale (50 L)	0.088	0.98	97.45	33.48 ± 1.74	43.6

The pilot system exhibited a longer lag phase (33.5 vs 8.2 days) but slightly shorter total oxidation time (44 vs 45 days), indicating improved overall kinetics despite delayed onset (**Table 2**). Arsenic solubilisation based on solution analysis achieved 70.4% (bench) and 80.2% (pilot) (**Figure 1d**), with residue analysis indicating higher values of 83.2% and 92.5%, respectively. Performance differences could be attributed to reactor design, influencing solids suspension and gas–liquid mass transfer, notably the height-to-diameter (0.48 in bench vs 0.83 in pilot STR) and tank-to-impeller ratios (0.41 vs 0.33) with the delay in oxidation onset in the pilot-scale STR attributed to the substrate adapted inoculum (chalcopyrite).

Following biooxidation, cyanidation achieved near-complete gold recovery in the pilot system and <90% in the bench reactor (**Figure 2**). In contrast, silver recovery remained low in both cases, likely due to partial encapsulation associated with jarosite formation [16].

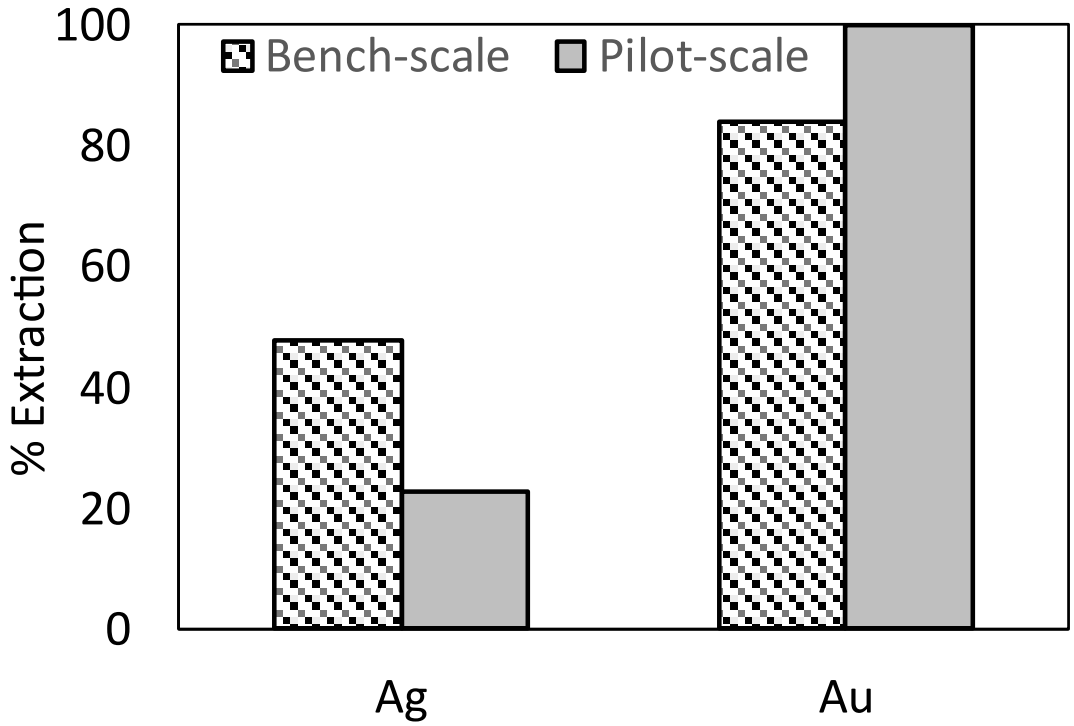


Figure 2. Gold and silver recovery following the cyanidation of the biooxidised residues.

Microbial dynamics in the pilot-scale reactor were monitored to assess population shifts during biooxidation. The inoculum was initially dominated by *L. ferrooxidans* and *S. thermosulfidooxidans*, with minor taxa including *Pseudomonas antarctica*, *Acidibacillus ferrooxidans*, and *Alicyclobacillus aeris*.

During operation, *S. thermosulfidooxidans* progressively succeeded *L. ferrooxidans*, reflecting a transition from iron-dominated to mixed iron- and sulfur-oxidizing activity, consistent with previous studies [17, 18, 19]. Additional acidophilic chemolithotrophs remained at low abundance. These shifts highlight the advantage of mixed microbial consortia, particularly those adapted to the mineral environment, in establishing a resilient system capable of coordinated iron and sulfur oxidation, thereby improving overall leaching performance.

Conclusions

This study demonstrates successful scale-up of arsenopyrite biooxidation from 5 L to a custom 50 L reactor for pre-treatment of a refractory gold feed. Although the pilot system exhibited a longer adaptation phase, it achieved superior performance, including higher iron oxidation rates and greater arsenic solubilisation, enabling near-complete gold recovery. Results highlight the critical role of reactor design, mass transfer, and microbial dynamics in scale-up. Continuous monitoring of redox conditions and microbial succession proved essential for process control and optimisation. Overall, the findings confirm the technical feasibility of the custom-built pilot-scale biooxidation and provide key insights for further optimisation and industrial application.

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Facile and efficient sequence of Au and Ag recovery from various DRGOs: biooxidation of sulfides followed by thiourea leaching under the strongly acidic conditions

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Keywords: Double refractory gold ores (DRGOs), thiourea, biooxidation, *Acidimicrobium ferrooxidans*, *Acidithiobacillus caldus*

Abstract

Double refractory gold ores (DRGOs) are defined as gold ores in which gold grains are encapsulated in sulfides in accompany with organic carbonaceous matter and abandoned to develop in the world. DRGOs are distributed in various regions, including west and south Africa, central and southeast Asia, Australia, and USA. Various DRGO samples were supplied from Mali (SYM), Uzbekistan (UZB) and South Africa (PFC), including different gold and carbon contents. After biooxidation with the mixed culture of *Acidimicrobium ferrooxidans*, and *Acidithiobacillus caldus* at pH 2 and 45 °C, oxalic acid washing was performed to dissolve jarosite and surface-adsorbed Fe^{3+} species for DRGOs. During thiourea leaching at pH 1 and 60 °C, thiourea and formamide disulfide concentrations were separately determined by HPLC. Oxalic acid washing is inevitable, because Fe^{3+} ions act as an oxidant to thiourea as well as an oxidant to Au(0) in DRGO. In thiourea leaching of Au from SYM and UZB, thiourea was gradually converted into formamide disulfide, which is the oxidation product of thiourea and does not act as a ligand to Au. Meanwhile, thiourea concentrations are maintained in leaching of DRGO from PFC. Au recovery reached ~100% in all DRGOs within 30 h.

Introduction

Double refractory gold ores (DRGOs) are regarded as abandoned gold ores and have not been developed because of economically unacceptable types of gold ore causing gold robbing on the surfaces of carbonaceous matter in cyanidation of DRGOs in spite of the relatively higher gold contents than conventional gold ores [1]. There have been several trials to pretreat DRGOs in environmentally friendly methods: one of them is the sequential pretreatment of DRGOs with bio-oxidation and enzymatic treatment, leading to ~90% recovery of gold [2, 3]. Such procedures are environmentally friendly because of no high temperature and no combustion to emit pollutant gas but complicate through several steps at different pHs.

Thiourea is known as one of ligands to Au under the acidic conditions [4]. It is advantageous to set the process after biooxidation of sulfides in DRGOs. Additionally, thiourea is significantly less toxic than cyanide, and higher extraction rate than cyanide. Meanwhile, it is known there are disadvantages of low stability, especially in the coexisting Fe^{3+} ions, and difficulties to find efficient and reasonable adsorbents for recovery of gold thiourea complex [5].

In the present work, the biooxidation (BIOX) pretreatment of different types of DRGOs prior to thiourea leaching and the adsorbent of gold thiourea complex was optimised.

Experimental

Different DRGO samples were supplied from Mali (SYM), Uzbekistan (UZB) and South Africa (PFC). Major elemental compositions are 24.0 g/t Au, 14.5% Fe, 4.16% organic carbon in SYM; 30.1 g/t Au, 4.3 g/t Ag, 18.3 wt% Fe and 4.02% organic carbon in UZB; 5.5 g/t Au, 5.51 wt% Fe, 2.15% organic carbon in PFC. The mineralogical compositions of the original DRGO from SYM and UZB

consist of quartz, pyrite, magnesite, chlorite, muscovite, albite, and dolomite, and those from PFC consists of quartz, much smaller content of pyrite, chlorite, muscovite, albite, confirmed by XRD.

To decompose sulfides for the liberation of gold grains, BIOX was performed by inoculating iron-oxidizing bacteria, *Acidimicrobium ferrooxidans* ICP and sulfur-oxidizing bacteria *Acidithiobacillus caldus* KU, to make the final cell density 1.0 x 10^7 cells /mL in all batches. The initial cell density was inoculated to 1.0 x 10^7 cells/mL for each. Both species grow actively at acidic pH and 45 °C. After 14 days, XRD sulfide peaks disappeared. The solid residues were washed with distilled water and then provided for thiourea leaching for gold and silver extraction.

The gold and silver extraction was performed by thiourea at acidic pH and cyanide at alkaline pH for each solid residue, separately. In thiourea leaching, 1.250 g of each powdery ore sample was suspended in 100 mL of a solution containing 5.00 mM thiourea ($(NH_2)_2CS$) and 4.7 uM $Fe_2(SO_4)_3$ as an oxidant at pH 1 adjusted with H_2SO_4. at 60 °C and 125 rpm for 48 h in duplicate. The dissolved Au and Ag concentrations were determined by ICP-MS.

Cyanidation of Au from the same pretreated ores was also conducted to compare with thiourea leaching. A 0.200 g of each solid sample was suspended in 4 mL of 2.5 mM KCN adjusted to pH 12 with 1 M KOH in 50 mL flasks. The mixtures were shaken at room temperature and 134 rpm for 24 h. These extracted solutions were supplied to determine the dissolved Au concentration by ICP-MS.

Remaining thiourea concentrations were determined by HPLC (LC-Net II/ADC, JASCO, Hachioji, Japan), which was equipped with octadecyl silyl (ODS) column using 95% 2.5 mM tetrabutylammonium hydrogen sulfate (TBA-HS)/ 5% methanol as a mobile phase at pH 1.0 and 30 °C, and by UV-VIS detector at 214 nm.

Results and Discussion

After biooxidation, sulfides exposed on DRGOs were mostly dissolved to liberate gold grains in SYM, UZB and PFC, but the carbonaceous matter were not altered, confirmed by Raman spectroscopy. In comparison of ICP alone with the mixed cultures of ICP and KU, the mixed culture improved Au recovery, probably due to oxidative dissolution of elemental sulfur secondarily formed in biooxidation of pyrite. Carbonaceous matter was not degraded by the mixed cultures of ICP and KU, confirmed by Raman spectroscopy.

When solid residues were treated by cyanide at pH 11, re-adsorption of $Au(CN)_2^-$ was clearly observed with SYM and UZB in Fig. 1 (d), (e), but not clearly with PFC in Fig. 1(f). It is probably due to the organic carbon contents and the graphiticities, because the decrease in Au extraction is mainly caused by re-adsorption of $Au(CN)_2^-$ on carbonaceous matter. Cyanide extraction of Au was improved after biooxidation,

For the same solid residues, thiourea leaching of Au was conducted under the acidic condition. After biooxidation of all DRGOs, thiourea leaching recovered Au completely within half one day. Re-adsorption did not happen in any sample, implying that the complex of $Au(CS(NH_2)_2)_2^+$ does not have affinity with carbonaceous matter. This means that activated carbon cannot be used for recovery of the extracted Au complex like after cyanidation. Therefore, ion-exchange resin was investigated instead of activated carbon. A cation exchange resin (DIAION PK216L) was applied for the extracted solution from SYM, in which 4.86 uM Au and 4.20 mM Fe are included. 96.09% of Au was adsorbed on the resin in addition to 100% Fe. This suggests that $Au(CS(NH_2)_2)_2^+$ ions were quantitatively recovered on the conventional type of cation exchange resin even in the coexistence of higher concentration of Fe ions the three orders of magnitudes than Au concentrations.

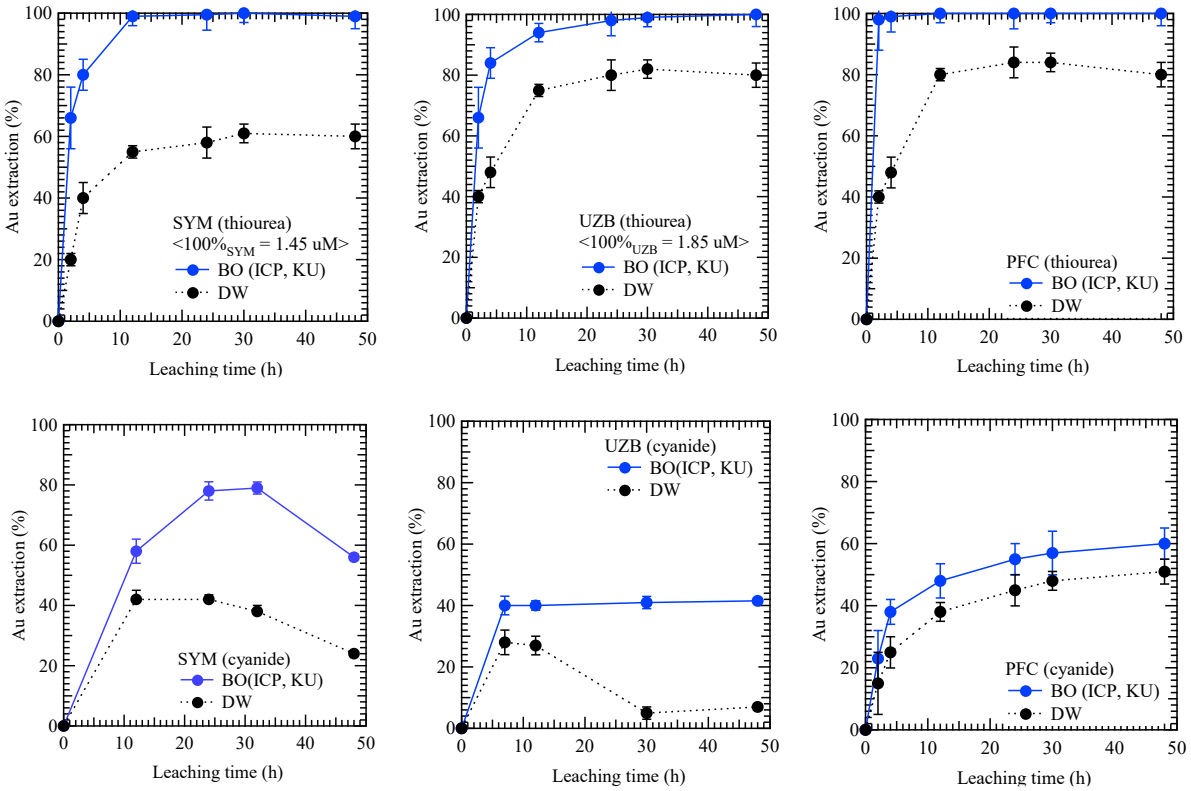


Figure 1. Thiourea leaching behaviours of Au from (a) SYM, (b) UZB, and (b) PFC, in comparison with cyanide leaching behaviours of Au from (d) SYM, (e) UZB and (f) PFC before and after biooxidation. 100% Au extractions correspond 1.45 uM in SYM, 1.85 uM in UZB and 0.332 uM in PFC. Black and blue symbols indicate Au extraction percentages in duplicate before and after

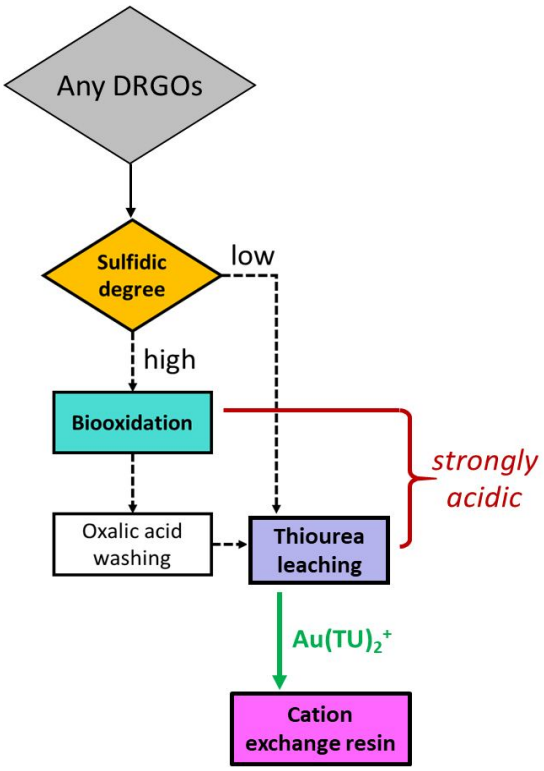


Figure 2. Proposed flowsheet of pretreatment and extraction for Au recovery from DRGOs.

Conclusions

In thiourea extraction, the samples after bio-oxidation showed the complete Au extraction, suggesting that the degradation of organic matter is needless. In cyanidation, the highest extraction rate was observed after bio-oxidation with the mixed culture including organic decomposing bacteria, but re-adsorption still happened. 96% of $Au(TU)_2^+$ was recovered using cation exchange resin from solution even coexisting mM orders of Fe concentrations. Less affinity of $Au(TU)_2^+$ with activated carbon was overcome. For extraction and recovery of Au from double refractory gold ores (DRGOs) which is undeveloped natural gold resources, thiourea extraction has advantages over cyanidation from a view point of efficiency, simplification of process, environmental friendliness.

Acknowledgements

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The BioGold Paradigm: Redefining Urban Waste as a Living Gold Resource

'The BioGold project demonstrates how tailored made biological systems can transform urban waste into a sustainable source of gold, redefining cities as living mines within the circular bioeconomy'.

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Abstract

Urban environments are rapidly accumulating gold-bearing materials from electronic waste and industrial residues, creating novel anthropogenic substrates for biological metal transformation. The PX Urban Mining Initiative is a collaborative industrial and research effort led by PX Precinox to recover valuable metals — especially precious metals like gold — from urban waste streams such as electronic waste and other side streams. PX PRECINOX, together with BRAIN Biotech, AG, has advanced the Biogold™ technology from laboratory to pilot-scale operation (2 × 500 L). The project delivers a disruptive, bio-based solution for sustainable recovery of precious metals from secondary raw materials, targeting the multi-billion-euro urban mining and metal recycling market.

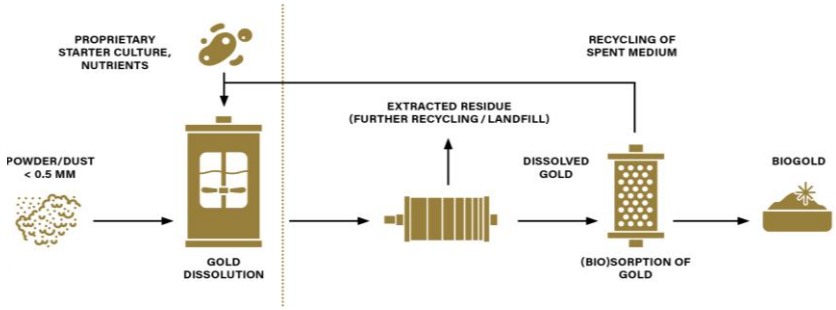


Figure 1. Process flow diagram illustrating the bioleaching of industrial waste for biogold recovery.

The **BioGold project** advances a paradigm shift by reframing urban waste as an anthropogenic biosystem in which microbial and enzymatic processes actively mediate gold mobilization by efficiently using BSL-1 microorganisms. A mobile, container-based pilot plant called the *BioXtractor V2* was developed in partnership with BRAIN Biotech at PX Group's site, Switzerland to scale the process and produce the first BioGold™ nuggets from urban waste streams.



Figure 2. First Module 'The BioXtractor': From Lab to Pilot Scale: Proof-of-Concept Accomplished; First tangible, refined biogold nuggets.

This approach extends biomining beyond geological ores to human-made substrates, investigating how microbial communities adapt to heterogeneous, metal-rich environments and how enzymatic systems selectively solubilize gold under mild physicochemical conditions. Experimental studies explore the influence of pH, nutrient availability, redox potential, and microbial community structure on gold mobilization dynamics, revealing adaptive strategies that maintain functional activity despite inhibitory compounds commonly present in urban materials.

Compared to existing bioleaching approaches, Biogold™ speculated to achieves gold recovery efficiencies of ≥70%, reduces chemical reagent use by ≥30%, and operates in modular, mobile units for on-site deployment, lowering capital and operational costs. Exploitation pathways include technology licensing, commercialization of mobile units, and partnerships with European recycling and metallurgical companies, positioning PX PRECINOX to capture a significant share of the circular metals market and strengthen Europe's strategic autonomy in critical raw materials.

Keywords: Urban Mining, E-waste, Bioleaching, Circular bioeconomy, BioGold

ABSTRACT IBS 2026

Investigating the biomineralisation potential of *Lysinibacillus sphaericus* PG22 in the circular recovery of critical raw materials

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Abstract:

The recovery of critical raw materials from industrial waste is emerging as a critical necessity within the green transition framework. Different categories of waste streams, including Mine Tailings, spent Lithium-ion Batteries and Printed Circuit Boards from End-of-Life Vehicles, exhibit a hidden metal value and represent critical secondary sources of valuable and strategic raw materials [1].

Geochemically enriched marine sediments represent underexplored reservoirs of microbial life with potential applications for transforming waste into resources. Our work focuses on isolating bacterial strains from these environments, particularly those that display resilience to potentially toxic elements (PTEs), with the aim of leveraging them in biomining processes to develop environmentally sustainable approaches.

Our biorecovery strategy involves exploiting ureolytic marine strains for Microbially Induced Carbonate Precipitation (MICP) to selectively precipitate and remove competing ions, such as PTEs, from waste leachates, allowing cleaner recovery of the precious elements. MICP is one of the most known biomineralising processes producing high amounts of $CaCO_3$ in a very short time [2]. We demonstrated that the MICP potential of *Lysinibacillus sphaericus* PG22, a marine gram-positive sporulating bacterium, relied on its strong ureolytic activity [3]. In presence of Pb^{2+}, Zn^{2+}, and Cd^{2+}, PG22 promoted the formation of $PbCO_3$, $ZnCO_3$, and $CdCO_3$. Currently, we are evaluating, through ICP, XRD, and ESEM-EDX analyses, PG22 fitness in the treatment of the aforementioned anthropogenic waste matrices enriched with PTEs and precious elements. Preliminary results showed the ability of PG22 to efficiently mineralise already at 0.5X waste leachates. The presence of $CaCO_3$ was confirmed by XRD, and ICP-OES data will further assess the removal rates of other divalent cations. These findings highlight PG22's potential for MICP-based strategies, offering a novel solution for an integrated marine biotechnological platform for the sustainable, high-purity separation and recovery of strategic elements.

Keywords: biomineralisation, MICP, biorecovery, marine bacteria, CRMs

Acknowledgements: This research is funded by the Project “Biotechnologies for the recovery of critical RAw materials from VEhicles” (BRAVE, Project ID: I53C25000570001), funded under the Italian Strategic Investment Fund of the Ministry of University and Research (FIS-2023-03296) and the project BIOMINA PRIN-PNRR 2022.

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Hybrid approach for rare earth elements extraction from oil sand tailings

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Abstract: (200-300 words) Various waste streams including as oil sand tailings are reported to contain potential strategic elements such as Rare Earth Elements (REEs). Initially, the occurrence and distribution of REEs in oil sands tailings was examined. Chemical and chemical-biological leaching approaches were applied to tailings to degrade complex mineral structures and as an initial step to easily access to the mineral matrix. A mixed lixiviant containing hydrochloric acid and hydrogen peroxide appeared to be the most suitable leaching agent. The recovery rates of Tb and Zn attained 82.8% and 35.9% with 2M HCl + H2O2, which further improved to 100% and 66.7% with 4M HCl + H2O2 assisted leaching, respectively. However, optimisation of temperature, HCl and H2O2 concentrations, shaking speed, and leaching time needs to be optimised. During chemical-biological leaching, oil sand tailings were treated with 0.5M sulfuric acid initially which resulted in 76% recovery of Tb and 25.3% of Zn. The development of a chemically-biologically based processes using *Acidithiobacillus ferrooxidans* DSM 14882 and *Leptospirillum ferrooxidans* DSM 2391 resulted in modest solubilisation rates for Dy, Eu, Gd, Sm and V (aproximately 12-19%), while Zn recovery reached around 50%. The overall performance of these acidophilic bacteria was limited, likely due to the presence of residual hydrocarbons, which appeared to hinder bioleaching efficiency. An understanding of the biochemical leaching process, especially in relation to limiting factors is then necessary to the development of biochemical leaching of oil sands tailings as a secondary resource of REE and other critical metals

Keywords: REEs, oil sand tailings, chemical leaching, biological leaching,

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Determination of operation parameters of an *in-situ* bioleaching pilot

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Abstract:
In-situ bioleaching is a promising process that enables the extraction of metals from an ore body without excavating the mineral. It directly leaches the metals out of the ore by injecting the biolixivant into the fractured ore vein. In the context of the Horizon Europe XTRACT project, a pilot operation is planned to demonstrate the industrial feasibility of an *in-situ* operation. The pilot site is located in Germany in the Erzgebirge at the Tellerhäuser mine in a polymetallic deposit (mainly Zn, Sn, Cu, In). A sphalerite-rich vein from which a block measuring 1.5 x 1.6 x 15 m, estimated at 132 tons, awaits being fractured and connected to a 500 L ferrous iron-oxidizing bioreactor producing an acidic ferric iron- rich leachate.
This study shows preliminary calculations to determine the operating parameters of the pilot demonstration. The bubble column reactor was designed to accommodate the required oxygen transfer rate, which drives the iron oxidation capacity. To provide realistic data, calculations were based on lab studies performed in continuous mode, that evaluated the kinetics of bacterial oxidation and mineral acid consumption using an iron-oxidizing culture dominated by *Leptospirillum ferriphilum*. Bioreaction was modelled in the condition of the mine, where the temperature is not controlled. The oxidation rate at 20°C is 20% of the rate at 37°C (optimum), resulting in an iron oxidation rate of 0.18 g/L/h at a hydraulic retention time of 48 h. Zinc production of 1.2 kg/day is expected out of the ore vein, considering all ferric iron being consumed by sphalerite. For this case study, calculations showed the ferric iron demand of the vein is larger than the bioreactor production rate, thus the rate of the bioreactor controls the overall kinetics of the operation. Main leverage to improve metal production was identified to be the addition of a bioreactor temperature control.

Keywords (5 max): *In-situ* bioleaching; Bioleaching; Iron bio-oxidation; Sphalerite; Pilot operation

Acknowledgements: The authors acknowledge the European Union's Horizon Europe Research and Innovation program for funding the project XTRACT under Grant Agreement no. 101138432, and all collaborators of the project.

Evolution of multiscale pore-fracture networks and seepage pathways in porphyry copper ore under simulated *in situ* bioleaching conditions

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Keywords: *In situ* bioleaching; porphyry copper ore; Staged loading; reconstruction of pore networks; seepage pathway

Abstract

Deep porphyry copper deposits have a tight matrix and extremely low permeability, restricting lixiviant penetration and microbial migration in bio-ISL systems. Single-fractured specimens were prepared by stepwise loading and leached for 48 days at 50 °C and pH 2. Pore structure evolution was characterised by three-dimensional CT reconstruction and low-field NMR T_2 spectra. After leaching, outer-ring porosity (0–8 mm) increased by 55.1%, while the inner ring (16–25 mm) increased by only 0.082 percentage points, indicating that penetration depth is primarily controlled by fracture location. T_2 spectral analysis resolved four leaching stages: dissolution-dominated, precipitation-enhanced, mesopore recovery, and quasi-equilibrium, with mesopores (10 ms < T_2 < 100 ms) driving late-stage seepage recovery. SEM-EDS confirmed secondary $CaSO_4$ precipitation at macropores and fracture tips (Ca:S = 1.52). Kozeny-Carman estimates yielded a post-/pre-leaching seepage capacity ratio of 3.90, the highest among three fracture configurations. The expanded intermediate throats (26.5–50.1 μm) exceed typical bioleaching bacteria cell dimensions by over an order of magnitude, indicating that the post-leaching pore network can support microbial transport and biofilm formation, providing a quantitative reference for fracture network design in bio-ISL.

Introduction

In situ leaching (ISL) has attracted growing attention as a technique for recovering copper from deep, low-grade porphyry deposits owing to its reduced surface disturbance and lower production costs [1,2]. Bio-ISL further improves copper recovery by introducing acidophilic microorganisms to catalyse the oxidative dissolution of sulfide minerals [3,4]. Implementation of bio-ISL requires not only effective lixiviant penetration into the ore but also sufficient pore-throat space for microbial transport and colonisation at mineral surfaces. Deep porphyry copper deposits, however, are characterised by a tight matrix and extremely low permeability [5], which severely constrains both lixiviant seepage and microbial transport.

Hydraulic fracturing can create artificial fracture networks in tight ore bodies to improve seepage conditions [6], yet the fracture architecture does not remain static. During prolonged acidic leaching, dissolution of primary minerals and precipitation of secondary phases such as $CaSO_4$ continuously alter pore-throat dimensions and connectivity [7,8]. When pore throats contract to dimensions approaching bacterial cell sizes (e.g., *A. ferrooxidans*, approximately 0.5–1.0 μm), mechanical straining prevents microbial passage. The evolution of throat-size distributions during leaching therefore directly determines whether the post-leaching pore network can provide effective pathways for microbial penetration.

Existing studies on pore structure evolution during ISL have focused primarily on medium- to high-permeability reservoirs such as sandstone-hosted uranium deposits [9], with limited attention to pore-throat reorganisation in tight porphyry copper ores after artificial fracturing. This study prepares single-fractured porphyry copper ore specimens by stepwise loading and subjects them to 48-day column leaching at 50 °C and pH 2. Three-dimensional CT reconstruction is combined with LF-NMR T_2 spectra to characterise the spatiotemporal evolution of pore structures. CT reconstruction provides

spatial topology of the pore-throat network, while LF-NMR T_2 spectra capture volumetric changes across different pore-size classes; the combination enables cross-scale characterisation from microstructure to macroscopic seepage. On this basis, the implications of these structural changes for lixiviant seepage and microbial migration are discussed.

Methods

Materials. Porphyry copper ore specimens were sourced from a deposit in Northeast China and prepared as standard cylinders (50 mm in diameter and 100 mm in height). XRD analysis indicated a primary mineralogical composition of quartz (73.8%), muscovite 3T (13.5%), and calcite (12.7%), with an average Cu grade of 0.67%. No $CaSO_4$ was detected in the source ore. Using a VIS HPTAS triaxial testing system, a constant axial load of 0.65σc (σ_c = 69.70 MPa) was applied and sustained for 8 h to generate a single through-going macrofracture on the specimen surface. Non-fractured and double-fractured specimens were prepared at 0.55σc and 0.60σc, respectively, as controls.

Column leaching. Specimens were vertically installed in custom-built double-ring acrylic column leaching apparatuses. A sulfuric acid solution (pH = 2) was continuously injected from the column inlet at 4 mL/min, with circulating water at 50 °C flowing through the outer jacket to maintain a stable temperature field. This temperature is consistent with subsurface geothermal conditions at depth and falls within or near the lower bound of the activity range of moderately thermophilic bioleaching organisms commonly used in bio-ISL, including *Sulfobacillus spp.* (optimum 45–55 °C) and the thermophilic archaeon *Sulfolobus spp.* (optimum 65–80 °C) [3,4]; the chemical leaching data reported here can therefore serve as a direct baseline for subsequent inoculation experiments. Prior to leaching, specimens were pre-washed at a flow rate of 7 mL/min to remove surface-adherent fine particles and eliminate air bubbles, after which the flow rate was reduced to 4 mL/min for the leaching phase. The experiment lasted 48 days. Specimens were scanned at 4-day intervals during the initial (Days 0–4) and final (Days 44–48) stages, and at 8-day intervals during the intermediate phase (Days 4–44), yielding a total of eight scanning cycles. During each measurement interval, fluid supply was suspended, and specimens were removed and sealed with plastic film to prevent moisture evaporation. After CT and LF-NMR measurements, specimens were returned to the apparatus to resume leaching.

Analysis. High-resolution 3D CT was performed using a Phoenix v|tome|x s system (160 kV, 160 μA, voxel size 53 μm). Scan data were imported into Avizo for three-dimensional reconstruction and extraction of pore-throat network parameters including porosity, coordination number, equivalent throat radius, and tortuosity. Pores (aspect ratio AR < 3) and fractures (AR > 3) were distinguished based on the aspect ratio of equivalent ellipsoids. LF-NMR measurements were conducted using a MesoMR12-60H-I analyser with a CPMG pulse sequence to acquire T_2 relaxation spectra. Post-leach fracture surface samples were analysed by SEM-EDS to identify secondary precipitate phases.

Results and Discussion

Annular porosity evolution. The cylindrical specimen was concentrically partitioned into three radial zones: an outer ring (0–8 mm from the curved surface), a middle ring (8–16 mm), and an inner ring (16–25 mm). Prior to leaching, the LF-NMR-derived total porosity of the single-fractured specimen was only 0.61%. After 48 days of leaching, outer-ring porosity increased from 0.476% to 0.738%, an increment of 55.1%; the middle ring increased by 20.1%; and the inner ring increased by only 0.082 percentage points. These results indicate that the lixiviant penetrated primarily along the fracture and its adjacent zones, producing pronounced modification in the near-fracture region. The low matrix porosity, however, prevented further penetration into the inner ring. This radial attenuation pattern demonstrates that fractures significantly enhance mass transfer in the near-fracture zone, while deep modification remains a slow diffusion-controlled process governed by the matrix permeability. From the perspective of microbial transport, the expansion of outer-ring throats provides increased physical space for microbial entry and attachment, whereas the negligible porosity increment in the inner ring implies that bioleaching microorganisms would face severe spatial constraints when migrating toward the specimen core. The biologically active zone in bio-ISL is therefore likely to remain confined to the fracture and its immediate vicinity over extended leaching periods.

Pore-throat structure evolution. Pore coordination number, equivalent throat radius, and tortuosity were extracted from the Avizo-reconstructed pore-throat network models. After leaching, low-coordination pores (CN = 1–3) increased from 6,785 to 24,007, indicating that acidic dissolution fragmented well-connected pores into numerous small discrete units. High-coordination nodes (CN > 7), by contrast, remained nearly unchanged (44 to 43), indicating that the multiple parallel flow paths provided by the fracture promoted seepage continuity and that localised blockage did not compromise the overall network connectivity. In terms of throat-size distribution, intermediate throats (26.5–50.1 μm) increased markedly, while the proportion of large throats decreased. SEM-EDS analysis (Figure 1) revealed dense tabular gypsum crystals on the fracture surface (Ca: 21.84 at.%, S: 14.35 at.%, Ca:S = 1.52), identified as secondary $CaSO_4$ precipitated from locally supersaturated Ca^{2+} and SO_4^{2-} following H_2SO_4-driven calcite dissolution. These precipitates formed preferentially in large throats and at fracture tips where flow velocity was low and ion residence time was prolonged, causing intermediate throats to become the primary seepage channels. The intermediate throats (26.5–50.1 μm) exceed the cell dimensions of *A. ferrooxidans* (~0.4 × 0.8 μm) by over an order of magnitude, well above the critical threshold for mechanical straining of bacteria, and are therefore sufficient to permit the passage of bioleaching bacteria and the formation of biofilms on throat walls, providing the physical space prerequisite for microbially catalysed oxidation reactions in bio-ISL systems.

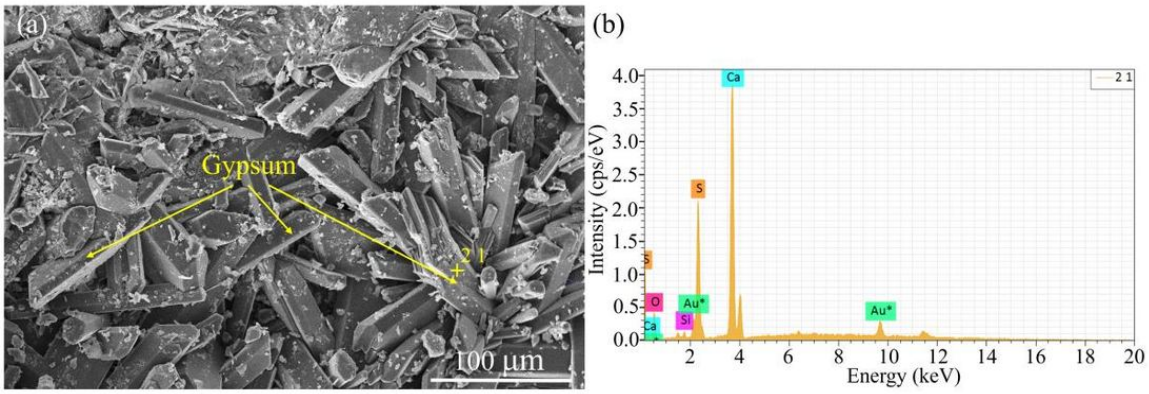


Figure 1. Post-leach SEM-EDS of the single-fractured specimen: (a) SEM image (500×) showing tabular gypsum crystals at the fracture surface; (b) EDS spectrum confirming Ca and S as dominant elements (Ca:S = 1.52).

T_2 spectral evolution. Based on the temporal trend of T_2 spectral area (Figure 2), the 48-day leaching process was resolved into four stages. Stage I (Days 0–24) was dissolution-dominated: the lixiviant infiltrated the matrix along fractures, and the macropore spectral area increased rapidly. In Stage II (Days 24–32), precipitation intensified as Ca^{2+} and SO_4^{2-} accumulated at macropores and fracture tips until the ion activity product exceeded the solubility product of gypsum, triggering $CaSO_4$ precipitation that suppressed macropore spectral area growth. Stage III (Days 32–48) was characterised by mesopore recovery: locally elevated acidity in blocked regions drove partial redissolution of precipitates, and the mesopore (10 ms < T_2 < 100 ms) spectral area recovered steadily, dominating the late-stage increase in total spectral area. In Stage IV (approaching Day 48), the system approached dynamic equilibrium. The leaching process ultimately produced a seepage structure dominated by mesopores with passivated macropores (Figure 3). This structural characteristic has direct relevance for bio-ISL: as leaching progresses, macropores and fracture tips are gradually occupied by $CaSO_4$ precipitates, and the active space available for microorganisms may shift from the initial fracture–macropore system to the mesopore network. Maintaining the connectivity of the mesopore network in bio-ISL operations is therefore critical not only for seepage recovery but also for sustaining the metabolic activity of acidophilic microbial communities and Fe^{3+} regeneration efficiency, both of which are essential for continued bioleaching.

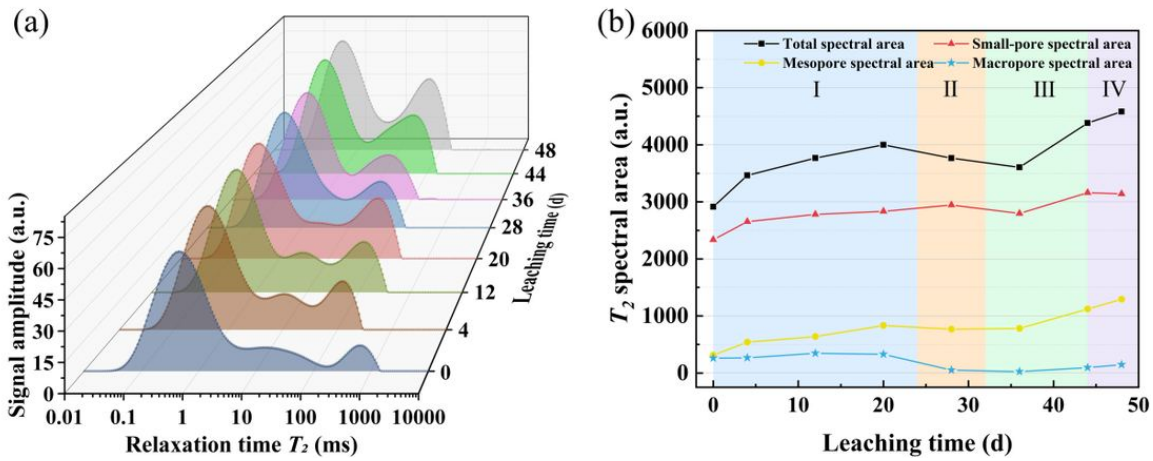


Figure 2. LF-NMR T_2 spectra evolution for the single-fractured specimen: (a) three-dimensional T_2 spectra over 48 days of leaching; (b) T_2 spectral area versus leaching time, with background shading marking four stages (I–IV).

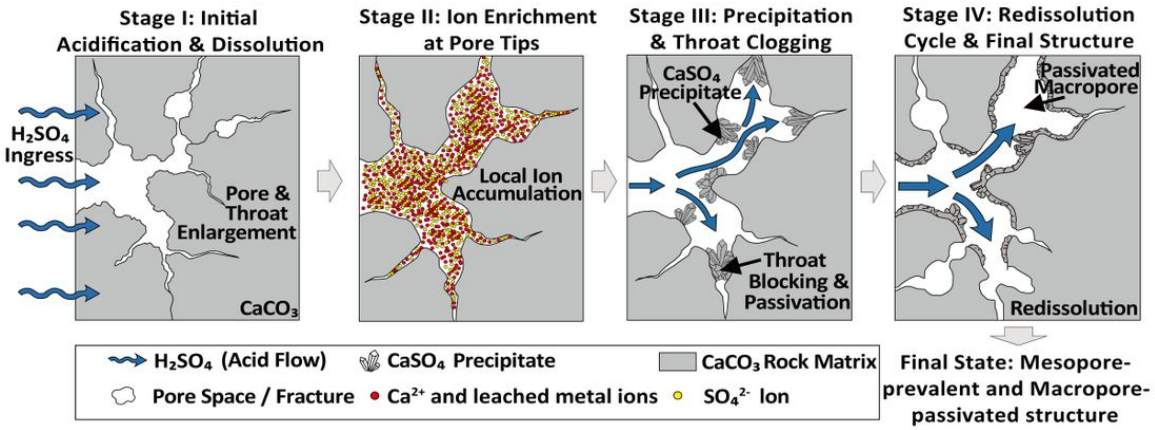


Figure 3. Schematic of pore evolution during acidic leaching of porphyry copper ore, illustrating the four-stage process from initial dissolution to the final mesopore-prevalent structure.

Seepage capacity estimation. Using the measured porosity, throat diameter, and tortuosity data (Table 1), the Kozeny-Carman equation in ratio form was applied to estimate the relative change in seepage capacity before and after leaching. The single-fractured specimen yielded K_{post}/K_{pre} = 3.90, indicating that the seepage capacity increased to nearly four times the pre-leaching value. For reference, non-fractured and double-fractured specimens from the same experimental batch yielded ratios of 2.66 and 1.31, respectively (Table 1), with the single-fractured configuration showing the greatest improvement. Although the double-fractured specimen exhibited a larger porosity increment, throat narrowing and increased tortuosity offset this gain. These results indicate that increasing fracture density does not monotonically improve seepage; an intermediate fracture density provides the optimal balance between connectivity enhancement and precipitation-induced blockage. Furthermore, to mitigate the progressive passivation of macropores and fracture tips by $CaSO_4$, bio-ISL operations could incorporate periodic adjustment of lixiviant acidity or planned rest cycles to promote redissolution of precipitates, thereby delaying the deterioration of seepage channels and maintaining accessible migration pathways for bioleaching microorganisms.

Table 1. Kozeny-Carman semi-quantitative estimation of relative permeability evolution

Specimen	φ_{pre} (%)	φ_{post} (%)	d_{pre} (μm)	d_{post} (μm)	τ_{pre}	τ_{post}	K_{post}/K_{pre}
Non-fractured	1.1357	1.6453	22.72	19.71	1.180	1.095	2.66
Single-fractured	0.6096	1.0641	49.23	42.71	1.162	1.178	3.90
Double-fractured	0.5559	0.6957	47.43	39.40	1.147	1.165	1.31

Conclusions

(1) Porosity growth in the single-fractured specimen after leaching exhibited pronounced radial differentiation: the outer-ring increment reached 55.1%, while the inner ring increased by only 0.082 percentage points, indicating that lixiviant penetration extent is primarily controlled by the spatial location of fractures.

(2) LF-NMR T_2 spectra resolved the 48-day leaching process into four successive stages: dissolution-dominated, precipitation-enhanced, mesopore recovery, and quasi-equilibrium. Secondary $CaSO_4$ precipitation blocked macropores and fracture tips in the later stages, while mesopores dominated the late-stage seepage recovery.

(3) The Kozeny-Carman-estimated K_{post}/K_{pre} of 3.90 for the single-fractured specimen was the highest among three configurations, whereas the double-fractured specimen yielded only 1.31 due to throat narrowing and increased tortuosity, demonstrating that fracture density and seepage improvement do not follow a monotonically increasing relationship.

(4) The substantially expanded intermediate throats (26.5–50.1 μm) exceed typical bioleaching bacteria cell dimensions by over an order of magnitude, providing sufficient space for microbial transport and colonisation. These data can serve as a quantitative reference at the pore scale for fracture network design and microbial inoculation strategies in bio-ISL.

Acknowledgements

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Agglomeration of Basalt Powder for heap bioleaching

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Abstract: Residual materials from mining and metallurgical processes such as heap material, tailings, slags, filter residues, ashes and dusts are mostly fine-grained and contain valuable metals as targets for “urban mining”. The metals need to be extracted from such residues, on the one hand for their recovery, and on the other hand to allow the usage of the residual material after metal extraction (e.g. as construction material) to avoid waste deposition. Since large amounts of metal-containing residual materials have to be processed, stirred tank reactor bioleaching, being applied for metal-rich ore concentrates, is likely too costly for processing of residual material. Heap bioleaching is less costly, however, the fine-grained material has to be agglomerated before to allow for percolation of solutions in a heap. Agglomeration is an established technology for heap leaching of milled ores but not for residual materials. For example, large amounts of basalt powder are generated by crushing of basalt rocks mined in quarries worldwide. The project "INNOCycle" aims to develop a cost-effective and environmentally friendly method for extracting metals from basalt powder and slags based on heap bioleaching. The goals of the partner BGR in the project are, on the one hand, the technological development of agglomeration of basalt powders and, on the other hand, the use of the agglomerates in percolators for the extraction of metals from the basalt powders via bioleaching and comparative chemical leaching with sulfuric acid. To achieve these goals, various agglomeration variants are tested, and bioleaching experiments are carried out in the laboratory for optimisation. The evaluation is based on physico-chemical and mineralogical analyses of the original samples, agglomerates, and leaching residues.

Keywords: Agglomeration, basalt powder, slags, heap bioleaching, urban mining

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Non-ferrous metal leaching from metallurgical slags and regeneration of leaching solutions by microorganisms immobilised on natural carriers

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Keywords: Chemolithotrophic bacteria, natural carriers, metallurgical slags, bioleaching, copper extraction

Abstract

The ongoing progress of the mining industry worldwide has resulted in the accumulation of technogenic slag, which still contains a significant amount of non-ferrous metals. A two-stage efficient biohydrometallurgical process for the extraction of valuable metals from slag has been developed and proposed. Bioleaching a microbial process in which iron-oxidizing microorganisms generate ferric iron (Fe^{3+}), a strong oxidiser, through the oxidation of ferrous iron (Fe^{2+}). Increasing the bacterial biomass density via immobilisation can significantly enhance the rate of oxidant regeneration, thereby improving metal dissolution. Comparative studies showed that when zeolite and shungite were used as carriers, the SG consortium exhibited an increased Fe^{2+} oxidation rate, reaching a maximum at adhesion degrees of 100% and 98%, respectively, on the third day. While in case of *Sulfobacillus thermosulfidooxidans* strain 41 immobilised on shungite, an iron oxidation peak was observed on the 5th day of immobilisation. Comparative studies of bioleaching have shown that in the presence of biogenic iron, copper extraction from slag increases by about 2 times, reaching 97-98%. It can be concluded that the efficiency of bioleaching from slags directly depends on the ability of bacteria to adhere to the slag surface and promote jarosite formation.

Introduction

The ongoing growth of the mining industry worldwide has led to serious environmental problems associated with the accumulation of large volumes of technogenic waste, including slags. However, slags still contain significant amounts of valuable metals that can be efficiently extracted. To address this challenge, a two-stage biohydrometallurgical process for the bioleaching of complex concentrates has been developed and proposed based on previous studies [1,2]. The key component of leaching liquor (lixiviant) used in the second stage is ferric ion (Fe^{3+}), which represents a strong oxidiser. However, during the bioleaching of metallurgical slags, the activity of the lixiviant gradually decreases. Therefore, efficient bioregeneration of the lixiviant is a crucial step in the development of sustainable two-stage biohydrometallurgical technologies [3–7]. The use of iron-oxidizing bacteria (IOB) allows for simplifying the process of regeneration of leaching solutions and significantly decreases costs, as microorganisms are considered to be unexhausted catalysts. Besides, these microorganisms can be used for the regeneration of Fe^{3+}, consequently for leaching liquors [8-10].

The SG natural consortium and the pure culture of *S. thermosulfidooxidans* strain 41 were used for immobilisation and generation of oxidant (Fe^{3+}). Immobilisation of bacteria on natural carriers such as zeolite and shungite were performed in Macintosh medium (MAC) [11]. The experiments were carried out in 250 mL flasks under batch mode and shaking conditions (40 °C, 160 rpm) (ZQZY-CF8ES). The adhesion intensity was assessed by the difference between the initial titer of bacteria and the final cell number observed in the liquid phase. Cell adhesion to zeolite and shungite surfaces was studied using a HITACHI SU8100 Emission Scanning Electron Microscope (SEM) equipped with Microsystem (x

3, 5, 10, 30, 50k SEM) software. Chemical analysis of the leaching solution was performed via Agilent 5800 ICP-OES. The pH and ORP of the leaching slags were monitored over time.

Results and Discussion

Bioleaching using biogenic ferric iron (Fe^{3+}) is a microbial process in which iron-oxidizing microorganisms generate ferric iron and protons through the oxidation of ferrous iron (Fe^{2+}) and sulfur compounds, thereby promoting the dissolution of metals from slag.

In the first stage of slag bioleaching, the adhesion of the SG natural consortium and *S. thermosulfidooxidans* strain 41 of iron-oxidizing bacteria to the surface of natural carriers, including zeolite and shungite, was investigated. Afterward, immobilised bacteria and bacterial consortium were used for the generation of leaching liquid. The ferric iron-containing lixiviant obtained from immobilised bacterial activity was used for slag leaching in the second stage.

The comparative results of the studies showed that among the tested bacterial cultures, the new SG natural consortium exhibits high adhesion ability. In the presence of both zeolite and shungite, an increase in the rate of Fe^{2+} oxidation was observed by the SG consortium, which reached a maximum on the 3rd day of cultivation. The number of bacteria in the liquid decreased sharply, and the degree of adhesion was 100% and 98%, respectively. Figure 1 shows the adhesion of the tested bacteria *S.thermosulfidooxidans* 41 and SG consortium to the surface of zeolite and shungite via SEM.

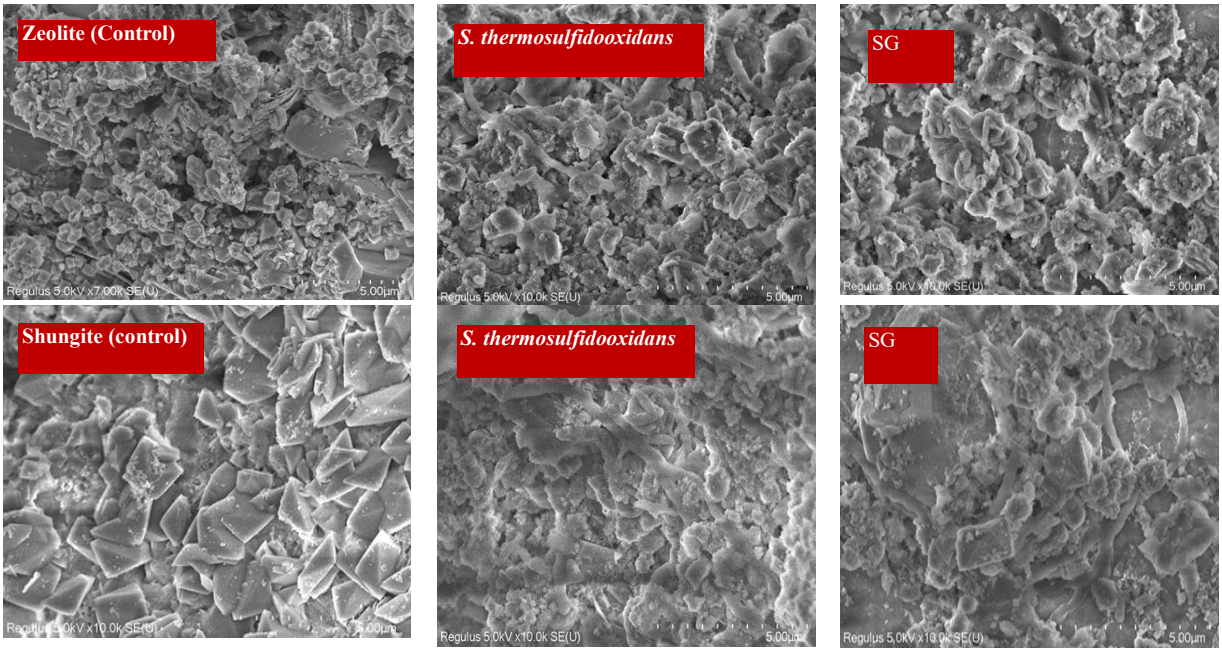


Figure 2. SEM images showing adhesion of SG natural microbial consortium and *S. thermosulfidooxidans* strain 41 to the surfaces of zeolite and shungite.

Zeolite and shungite are negatively charged minerals. On the other hand, it is known that acidophilic leaching bacteria acquire a positive charge due to the accumulation of Fe^{3+} ions in the surface structures of the cells, which allows them to attach to negatively charged carriers. Therefore, it is assumed that the adhesion of positively charged bacteria to the surface of negatively charged zeolite and shungite can occur by the mechanism of electrostatic interaction.

The adhesion of bacteria on neutral carriers contributes to the intensification of the regeneration process of ferric iron (Fe^{3+}). By increasing the density of bacterial biomass, it will be possible to significantly increase the rate of oxidiser (Fe^{3+}) regeneration.

Comparative studies indicated that the iron- and/or sulfur-oxidizing bacterium *S. thermosulfidooxidans* strain 41 exhibited the ability to adhere to zeolite, with the amount of oxidised iron being 3.6 and 3.9 g/L on days 3 and 4, respectively (Fig.2a). At the same time, data have shown that in the presence of shungite, the oxidation of Fe^{2+} by *S. thermosulfidooxidans* 41 reached its maximum on day 5 (Fig 2b).

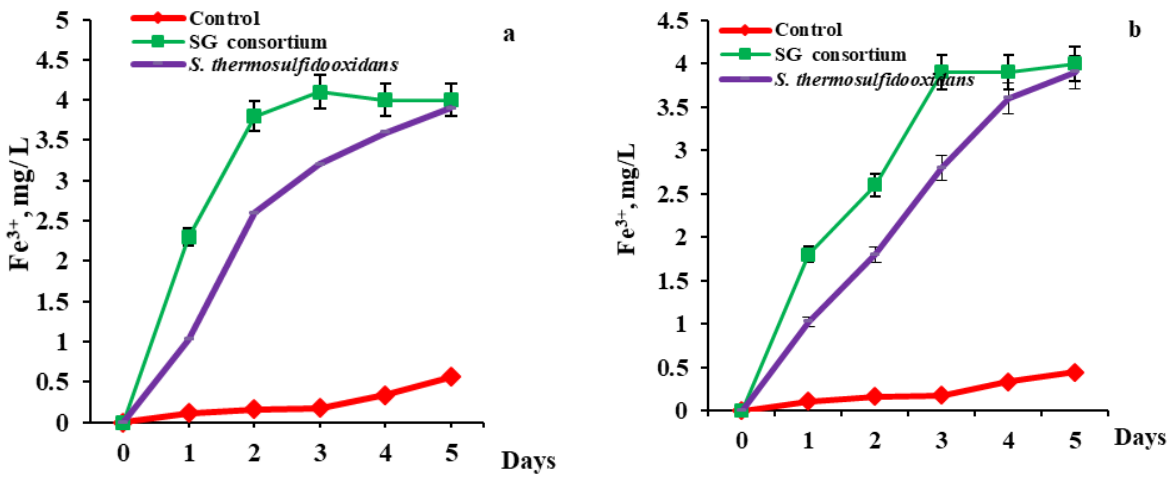


Figure 2. The dynamics of oxidation of Fe^{2+} by *S. thermosulfidooxidans* 41 and SG consortium immobilised on Zeolite (a) and Shungite (b).

To determine the optimal conditions for metal extraction by biogenic ferric iron, leaching experiments were performed at different pulp densities (1%, 2%, and 5%) using a 4 g/L Fe^{3+} lixiviant. Data obtained are presented in Figure 3.

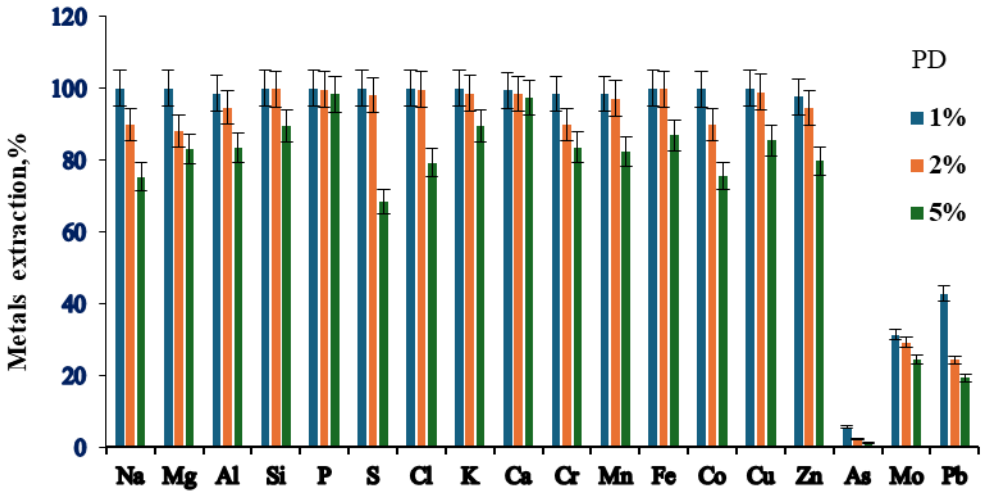


Figure 3. Metal extraction from slag (%) by biogenic ferric iron (Fe^{3+}) (Duration 20 days).

It was shown that, pulp density significantly influenced the extraction efficiency of elements from the slag. The highest extraction was achieved at 1% pulp density, with most major elements (Na, Mg, Al, Si, P, S, Cl, K, Ca, Cr, Mn, Fe, Co, Cu, and Zn) showing recoveries close to or above 95% (Fig. 3). Increasing the pulp density to 2% and 5% resulted in a gradual decrease in element extraction efficiency, particularly for S, Na, Co, Cl, Mn, and Zn. In contrast, As, Mo, and Pb exhibited considerably lower extraction under all tested conditions, with As showing the lowest recovery (<5%). Overall, the results demonstrate that 1% pulp density provided the most favourable conditions for bioleaching of slag, while higher solid loadings reduced metal extraction efficiency.

Conclusions

Increasing the bacterial biomass density can significantly enhance the rate of oxidant regeneration, thereby improving metal dissolution. The results also demonstrate that, when biogenic ferric iron (Fe^{3+}) is used as the lixiviant, the slag bioleaching process becomes cyclic and continuous because the Fe^{3+} consumed during metal extraction is continuously regenerated by iron-oxidizing bacteria. This regeneration enhances the overall efficiency and sustainability of the bioleaching process.

Acknowledgements

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Continuous Bioleaching of Pyrrhotite Tailings for Ni Recovery and S^0 Production

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Keywords: Bioleaching, Pyrrhotite Tailings, Biomining, Process Simulation, BIOX®

Abstract

Over 50 million metric tons of nickel-bearing pyrrhotite tailings (~0.8 wt% Ni) stored in northern Ontario pose a severe acid mine drainage risk. To assess eco-friendly remediation and resource recovery, a continuous BIOX®-style bioleaching process was adapted. Benchtop continuous experiments evaluated the effects of temperature (30–40 °C), pH (1.5–2.0), and residence time (10–15 days) on nickel extraction, elemental sulfur (S^0) production, reagent consumption, and effluent processability, alongside 16S rRNA community profiling. Maximum Ni recovery (84%) occurred at 40 °C, pH 1.5, and a 15-day residence time; however, operating at pH 1.5 increased acid consumption by 6-fold compared to pH 2.0. Despite a dominance of S-oxidizing species (e.g., *Acidithiobacillus ferrooxidans* and *Sulfobacillus thermotolerans*), substantial S^0 was generated (>10% yield), with higher yields achieved at pH 2.2 compared to pH 1.8 (22% vs. 10%).

Introduction

The nickel mining industry in Sudbury, Canada, has generated 50-100 million tons of rejected pyrrhotite ($Fe_{1-x}S$) tailings containing ~0.8 wt% residual nickel [1]. Underwater storage mitigates immediate atmospheric oxidation, but these untreated solids present a long-term acid mine drainage risk. Past pyrometallurgical treatment attempts had limited successes due to SO_2 emission and economic infeasibility [2]. Biomining, specifically the BIOX® process, offers an environmentally friendly alternative that eliminates SO_2 emission. However, applying BIOX® to pyrrhotite tailings deviates fundamentally from its conventional application in gold-bearing pyrite biooxidation [3]. Unlike pyrite, pyrrhotite is acid-soluble, follows a non-uniform dissolution mechanism that releases iron before sulfur, and generates different intermediate sulfur species (polysulfides vs. thiosulfate) [4][5][6]. Furthermore, the operational goals shift from solid-phase gold liberation to recovery of aqueous nickel and solid elemental sulfur (S^0). Due to these mechanistic and operational differences, the kinetics and behaviour of pyrrhotite bioleaching under a continuous BIOX® reactor configuration remain poorly understood. The end objectives of this study are to evaluate nickel extraction kinetics, elemental sulfur production, and overall process performance.

Methods

Materials. Two tailings from Vales Base Metals ("legacy tailings", 34 wt% of pyrrhotite and 0.8 wt% of Ni) and Glencore Canada ("fresh tailings", 56 wt% of pyrrhotite and 0.6 wt% of Ni) were used. A mixed indigenous culture collected from the Sudbury area [7], containing primarily *Acidithiobacillus ferrooxidans* and *Acidithiobacillus ferridurans* was used for reactor experiments. Bioleaching experiments were conducted in a benchtop reactor system (**Figure 1**) mimicking the BIOX® process. A nutrient solution (0.4 $g \cdot L^{-1}$ of each of $MgSO_4 \cdot 5H_2O$, $(NH_4)_2SO_4$, and K_2HPO_4) [8] mixed with pyrrhotite tailings was prepared as the daily semi-batch feed to the reactor system.

Analysis. The pH, redox potential, effective hydraulic retention time, and acid/base consumption were monitored daily. Aqueous and solid phase elemental compositions were measured via ICP-OES (Agilent 5900). The Fe^{2+} concentration was determined via potentiometric titration against $K_2Cr_2O_7$. The S^0 content was determined through a solvent-based (CS_2) extraction method. The microbial concentration was measured through direct cell counting, while the genomic analysis was done via S16 rRNA sequencing (GenBac and GenArch).

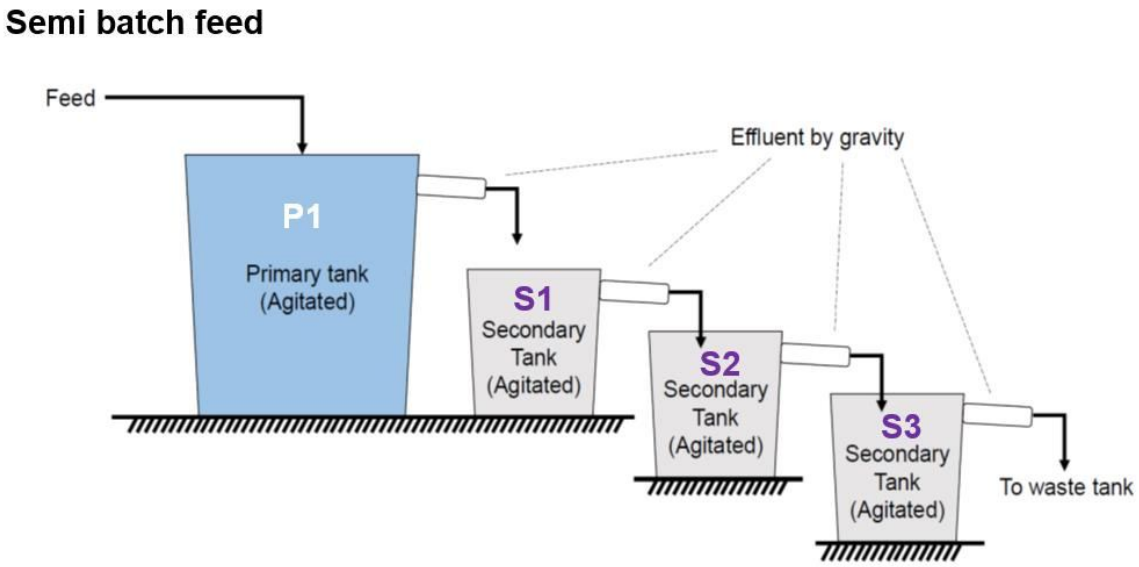


Figure 1. Benchtop reactor system setup containing a primary tank (P1), and three secondary tanks (S1-S3) connected in series

Results and Discussion

The extraction profiles of Fe and Ni are shown in **Figure 2**, the extractions were calculated based on the aqueous and solid elemental mass balances in each slurry sample taken.

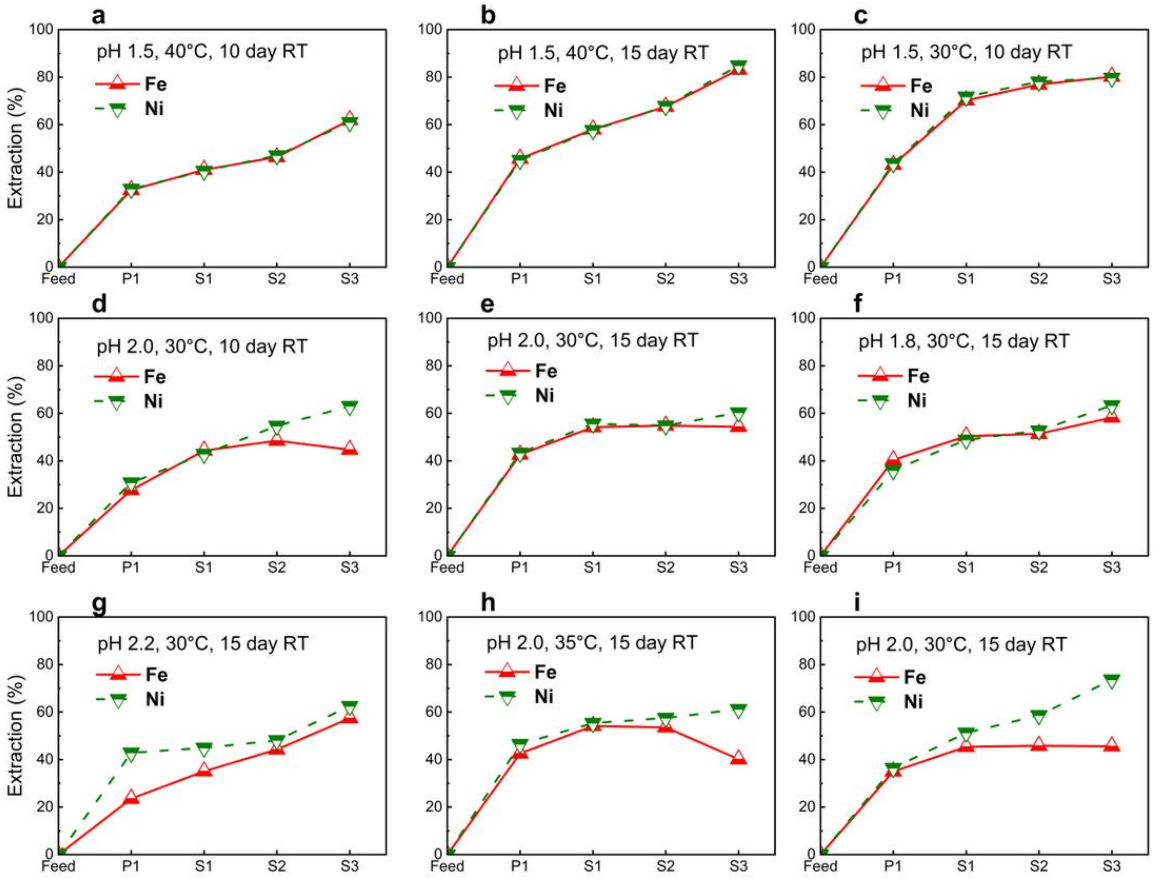


Figure 2. Extraction profiles of Fe & Ni in BIOX reactor train as per Figure 1. Panels a to d represent experiments conducted with legacy tailings, while e to i with fresh tailings. All tests were maintained at 20% (w/v) solids loading, with the exception of i, which ran at 10% (w/v). Other conditions as indicated. RT: Residence Time

In a previous mineralogical study, we found that nickel partitions evenly between pyrrhotite and a nickel-rich pentlandite phase [9]. Subsequent batch abiotic leaching experiments demonstrated that nickel in these two mineral phases exhibits similar dissolution kinetics [10]. This aligns with observations from the present continuous reactor study at pH=1.5 in the absence of *in situ* Fe precipitation, as illustrated in **Figure 2a** to **c**. Higher operating temperature did not enhance leaching kinetics (**Figure 2a** vs. **2c**), suggesting that the leaching is mainly influenced by the solution redox potential. This hypothesis is supported by the faster nickel kinetics observed at low pH (84% (Figure 1c) vs. 61% (Figure 1d)), where higher Fe^{3+} solubility produces a higher redox potential (517 mV vs. 459 mV). However, from an operating cost perspective, increasing the pH from 1.5 to 2.0 can achieve an 80% reduction in acid consumption (**Figure 3**).

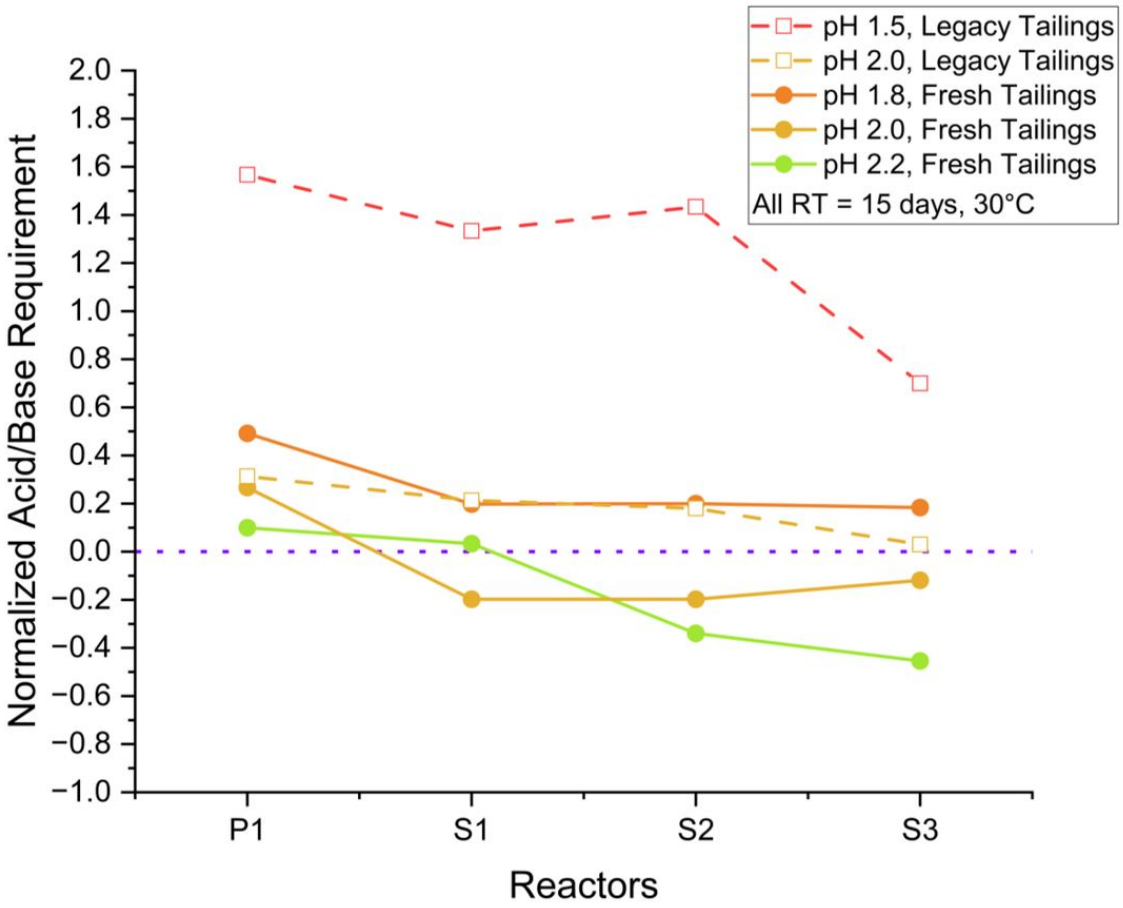


Figure 3. Recorded acid (1M H_2SO_4) and base (powder $MgCO_3$) consumptions, normalised to moles of H^+/OH^- required per kilogram of dry tailings feed to keep constant pH.

Across the continuous bioleaching runs, although the microbial consortia consistently contained S-oxidizing species (*Acidithiobacillus ferrooxidans*, *Sulfobacillus thermotolerans*, and *Sulfobacillus thermosulfidooxidans*), S^0 oxidation was incomplete due to kinetic limitations. High S^0 yield was observed at pH=2 to 2.2 (**Figure 4**), where rapid Fe^{3+} precipitation occurs due to increased supersaturation [11]. These conditions decrease the redox potential, creating conditions more favourable for microbial Fe^{2+} utilisation as an electron donor [12][13].

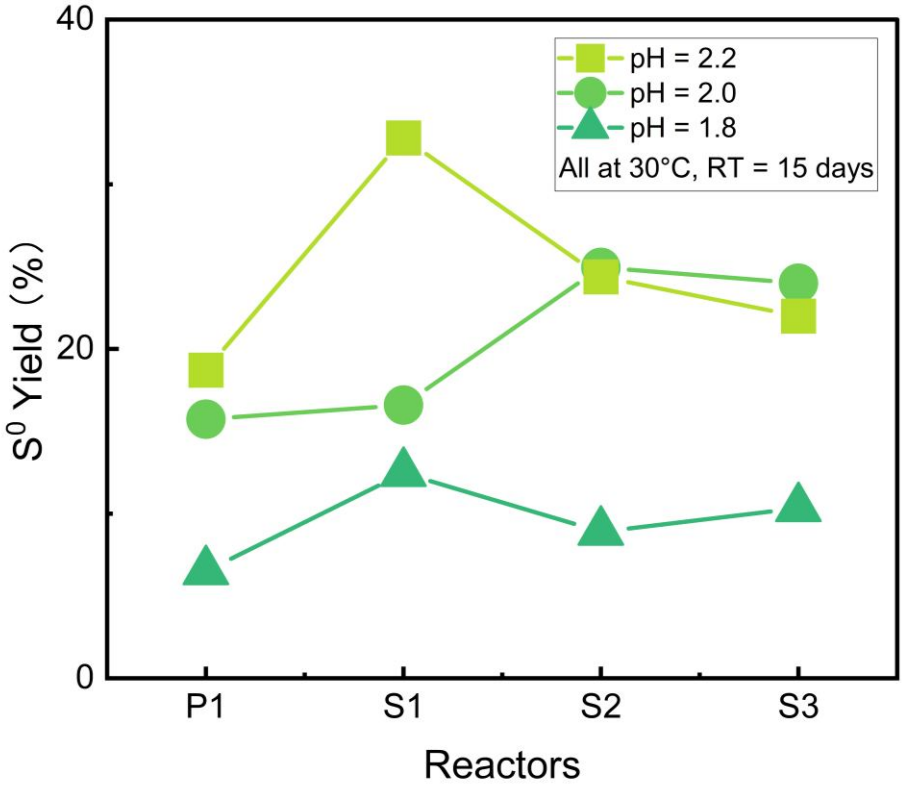


Figure 4. Elemental Sulfur Yield

Conclusions

This study demonstrates the technical feasibility of adapting the BIOX® reactor configuration for treating nickel-bearing pyrrhotite tailings. The maximum nickel extraction of 84% (pH 1.5, RT = 15 days, T = 40 °C) is influenced by the solution redox potential rather than the operating temperature, requiring a lower pH (1.5) to maintain high Fe^{3+} solubility. However, increasing the operating pH to 2 reduces acid consumption by 80%, offering a significant trade-off to optimise process economics. Furthermore, an elemental sulfur yield of 24% is achieved in the presence of S-oxidizing microbes. More rapid Fe^{3+} precipitation at higher pH decreases the redox potential and kinetically favours microbial Fe^{2+} utilisation, resulting in a higher S^0 yield. Overall, bioleaching of pyrrhotite with BIOX® reactor configuration provides a viable strategy for simultaneous nickel recovery, S^0 co-production, and tailings remediation.

Acknowledgements

The author would like to thank NSERC, Genome Ontario, Genome Canada, Ontario Ministry of Research & Innovation, Ontario Ministry of Northern Development and Mines, as well as Glencore, Metso and Vale for funding and technical support.

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Reductive dissolution and biomineralisation of magnetite: assessing a novel biomining approach for iron extraction on Mars

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Sustainable human exploration of Mars will require in-situ resource utilisation, including metals from the Martian regolith as construction materials. Biomining is promising due to low energy/chemicals requirements, but faces challenges such as a low sulfur content in the regolith. A novel approach proposes that dissimilatory iron-reducing microorganisms (DIRM) could convert Fe(III) iron oxides in the regolith into magnetite, a magnetically extractable mineral. However, only one species has been tested so far, using a Mars simulant of low mineralogical accuracy (1).

In this study, three bacterial species of DIRM using different reduction mechanisms were compared for their ability to anaerobically reduce iron oxides and the Mars global simulant MGS-1. *Geobacter sulfurreducens* and *Shewanella oneidensis* are gram-negative mesophiles, while *Carboxydothermus ferrireducens* is a gram-positive thermophile. An adapted spectrophotometric assay was used to quantify solid Fe(II), and minerals were characterised through XRPD Rietveld refinements.

Pure ferrihydrite was completely converted to magnetite and siderite (following ≈35 % reduction) by all three species. Pure hematite was partially reduced (≈12 %), and vivianite precipitated. By contrast, important differences were found with MGS-1. *Geobacter significantly* reduced the solid powder (≈21 % reduction of ferrihydrite in MGS-1), with chukanovite detected as probable bioproduct. *Shewanella did* not significantly reduce the powder (≈-2.5 %). Finally, reduction by *Carboxydothermus* was correlated with sulfate consumption in MGS-1 (≈45 mM), enabling up to 81 % reduction and the formation of pyrite.

These results suggest that Martian regolith is a less favourable substrate than pure iron oxides for DIRM, with the exception of sulfide-mediated bioreduction. This may be explained by the low iron oxide content of MGS-1, or other unknown microbial inhibitions. The higher direct reduction by *Geobacter may* be enabled by its unique nanowires. Overall, magnetite biomineralisation appears more challenging than previously thought, owing to the mineral complexity of the Martian soil.

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Keywords: space biomining – Mars – iron reduction – biomineralisation – iron oxides.

Bioregenerative Biomining of Lunar Regolith: Integrated Extraction and Precipitation Processes

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Abstract: EcoMine™ is a bioregenerative mining platform developed to enable sustainable extraction and recovery of mineral resources from low-grade ores, with a primary focus on lunar regolith and future applicability to asteroids, Mars, and terrestrial environments. The system integrates biological mineral processing with a closed-loop regenerative architecture that minimizes external reagent inputs while maintaining continuous operation.

The EcoMine approach employs heterotrophic microorganisms to solubilise target elements through biologically mediated acidolysis, supported by photoautotrophic production of oxygen and organic carbon sources. Following controlled contact between the microbial consortia and the regolith, minerals of interest are leached into solution, producing a complex, multicomponent aqueous phase enriched in major elements such as aluminium, iron, silicon, titanium, and magnesium.

Subsequent processing focuses on recovery of the dissolved elements from the leachate. EcoMine incorporates downstream extraction steps to isolate individual dissolved minerals for purification, enabling separation of target species from competing ions within the solution. Once purified, controlled precipitation strategies are applied to recover the elements as discrete mineral phases suitable for storage, utilization, or further processing. These extraction and precipitation steps are designed to be compatible with closed-loop operation and in situ resource utilization constraints.

Preliminary results are presented for both the bioleaching stage and the downstream mineral extraction and precipitation processes, demonstrating the technical feasibility of biologically driven, regenerative mineral recovery. Together, these results highlight EcoMine's potential to reduce energy demand, reagent mass, and environmental impact while supporting autonomous and scalable resource utilisation in space and on Earth.

The EcoMine™ project is supported by the National Aeronautics and Space Administration under Grants No. 80NSSC24CA217

Keywords: Bioregenerative mining, biomining, lunar regolith, *in situ* resource utilisation (ISRU), mineral extraction and precipitation.

Indirect bioleaching by MBR show wide application in selectively deep extraction of valuable/toxic metals from industrial wastes for both metal recovery and waste full-use

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Abstract

Huge quantity of industrial wastes has long been generated for energy & metals /non -metals materials production, including coal ash, blast furnace slag, steel slag, red mud, phosphogypsum, waste rock, tailing, metallurgical slag, etc. The industrial wastes often cover SiO_2, Fe_2O_3, $Al2O_3$, CaO and MgO as macroelements as well as As, Cr, Cd, Pb and Hg and In, Ga, Ge, Au, PGMs and REEs as microelements, having both ecological risk and recovery value. The indirect bioleaching (spent medium process) exhibits unique advantages over the traditional direct one towards selective and deep extraction of low dosage of valuable or toxic metals from industrial wastes, with very short time, exempt from waste toxicity and harsh leaching conditions. The indirect bioleaching technology launched by advanced membrane biological reactor (MBR) realises a much high-density biomass to efficiently produce active leachate in a continuous and easy way to greatly rise bioleaching speed, shorten the bioleaching duration and improve the bioleaching efficiency, representing the new generation of bioleaching processes. The MBR-based indirect bioleaching not only extract the high price metals such as rare metals, PGMs and REEs from the industrial solid wastes to achieve great economical interests but also remove the toxic metals and harmful alkaline matters to create a beneficial condition for the full-use and scale-application of the industrial wastes in a manner as ecological return.

Keywords: indirect bioleaching, MBR, industrial solid wastes, metals recovery, full-use.

Application Example

1. Indirect Bioleaching of Molybdenum from Molybdenum-Bearing Waste Rock

Using waste sulfur paste from coking plant and waste rock containing pyrite as the energy substrate, a sulfur/iron oxidising bacterial consortium was cultured in a 200 L MBR to continuously produce an active leachate (pH 0.6) containing H^+, Fe^{3+}, Fe^{2+}, amino acids and EPS as active molecules. At a pulp density of 50% (w/v), an indirect bioleaching efficiency of Mo from molybdenum-containing waste rock was 69%, and the leaching concentration of Mo was 1099 mg/L; whereas a lower leaching efficiency of 59% for Mo was attained with H_2SO_4 solution at pH 0.6 as control, indicating a better performance of indirect bioleaching over H_2SO_4 as chemical leaching. However, the indirect bioleaching efficiency of Mo decreased to 48% when the pulp density lifted to 100% due to the shortage of H^+. When indirect bioleaching was enhanced by addition of sulfuric acid, the Mo extraction efficiency sharply reached 86% and the dissolution concentration of Mo evidently increased to 2800 mg/L, exhibiting a good practical application of enhanced bioleaching on the extraction of Mo from the waste rock.

2. Aspartic Acid Enhances Bioleaching of Cobalt and Molybdenum from Spent Co-Mo Catalyst

Only an addition of Asp at 0.05 g/L effectively modulates both the two-component system and quorum sensing signalling pathways, motivating an overproduction of extracellular polymeric substances (EPS) by a mixed culture of sulfur/ferrous oxidising cells. And the induced EPS reduces evidently the surface tension of the spent Co-Mo catalyst to improve its hydrophilicity and enhances a contact &

interaction between the spent catalyst and the active small molecules in the bioleachate, thereby promoting the efficient extraction of both Mo and Co. As a result, the maximum release concentrations of Co and Mo respectively reach 1973.7 and 6125.4 mg/L at a pulp density of 10%. Accordingly, the leaching efficiencies of Co and Mo is 73.1% and 74.7%, an increase of 22.1% and 26.6% compared with that no Asp addition, respectively, suggesting that Asp-induced bioleaching has a better application potential on the release of both Co and Mo from spent Co-Mo catalyst through precise metabolism regulation.

3. Selective Zn Extraction from Blast Furnace Bag Ash with MBR Bioleachate

The active leachate at pH 0.8 generated by using MBR is successfully employed for the selective extraction of Zn from blast furnace bag filter ash, achieving a zinc leaching efficiency of 99.5% and a zinc leaching concentration of 8.5 g/L. Meanwhile, the Fe leaching efficiency is below 3% and an iron released concentration is only 0.5 g/L. In contrast, H_2SO_4 leaching gives a zinc extraction of only 85.5%, accompanied by a much high iron dissolution of 10%. After a simple and cheap purification operation, the bioleachate containing a high concentration of Zn is reacted with sodium carbonate to yield high-purity zinc carbonate at a production cost of merely 1000 US dollar per tonne. However, H_2SO_4 leaching process requires expensive purification, causing a high cost of production (1800 US dollar per tonne) and a poor purity of $ZnCO_3$ as a chemical product. The result showed that a selective bioleaching is advantageous over traditional chemical H_2SO_4 leaching on the extraction of Zn from Zn-containing steel metallurgic ash and subsequent cheap synthesis of high purity of $ZnCO_3$.

The Bioleaching Potential of diverse *Acidithiobacillus ferrooxidans* Strains for Cobalt Recovery from Mine Tailings

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Abstract: Mine tailings are the remaining waste after the extraction of valuable metals from ores. They are characterised by small particle size and are usually disposed in tailing ponds. Their chemical composition can vary depending on the mine location but sometimes they contain metals which largely remain unexploited. Cobalt is one such metal which plays an important role in industrial applications, e.g. serving as a crucial component in high strength alloys and in many semi-conductor materials. An environmentally friendly process for extracting cobalt from mine tailings is bioleaching, where acidophilic microorganisms like *Acidithiobacillus ferrooxidans* help solubilise the metals through their metabolic activity. Here we studied the variation in leaching efficiency among seven different strains of *A. ferrooxidans* related to leaching of cobalt from low grade ore tailings.

The screening was done in a stepwise approach, starting with iron sulfate ($FeSO_4$) as an energy source, to evaluate the iron oxidation kinetics under controlled conditions. The strains were then tested on pyrite and a combination of $FeSO_4$ and pyrite to assess their ability to oxidise complex sulfide minerals. Based on pH, redox potential (ORP) and iron oxidation efficiency the best, middle and worst performer were chosen for further leaching trials with cobalt bearing mine tailings. Preliminary results displayed clear variation between the seven strains. During the screening with $FeSO_4$ and pyrite, one strain showed the lowest average pH (1.66 on day 14) and the highest total iron leached (15.35 mM). This value was almost double compared to the worst performer (8.05 mM) indicating an enhanced oxidative activity. The findings will help with evaluating the variation in strain performance among *A. ferrooxidans* and demonstrate the importance of systematic investigation of bioleaching processes.

Keywords: *Acidithiobacillus ferrooxidans*, pyrite, mine tailings, iron sulfate, cobalt.

Acknowledgements: This work is supported by the Horizon Europe OPTIMINER project (Grant Agreement No. 101178354).

Biosurfactant-enhanced ion flotation for sustainable treatment of metal-bearing effluents

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Abstract: Ion flotation offers a promising approach for recovering metals from low-concentration solutions by employing chemical surfactants. To overcome challenges like poor selectivity and secondary pollution from conventional surfactants, bio-based alternatives are increasingly sought to widen ion flotation's applicability.

Biosurfactants with their amphiphilic properties and biodegradability emerge as superior ecofriendly collectors for ion flotation. This research evaluates their practical utility, delving into metal complexation dynamics and the selectivity mechanism. Forming part of broader efforts to elucidate biosurfactant-metal interactions, this work lays foundational insights for sustainable metal extraction processes. In this work, rhamnolipid biosurfactant was used as a flotation reagent and investigated for the recovery of metal ions from battery process water generated during the recycling of spent lithium-ion batteries. The effect of various metal ions (Al, Mn, Ni, Co, and Li) on the surface and interfacial activity of rhamnolipid was examined using dynamic tensiometry and metal binding affinity by isothermal titration calorimetry. Additionally, the influence of key operating parameters such as pH and biosurfactant concentration on the process was investigated to enhance the metal recovery.

This approach offers a green solution for treating such process water as well as industrial effluents, mining waters, leachates, etc., aligning with circular economy principles by enabling metal recycling while minimising environmental impact.

Keywords: Rhamnolipid, metal recovery, battery, process water, recycling

Environmental Mobility of Metallic and Metalloid Elements in Mining Technosols

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Keywords: ***Metallic and metalloid elements, Characterisation test, Conformity test, Leachability.***

Abstract

Metal(loid) elements are naturally present in the environment, but they can also be introduced by anthropogenic activities. In high amounts, these elements are toxic to the environment. Mining wastes are rich in metal(loid) elements and can have a negative impact on the environment; therefore, it is important to assess the hazardousness of tailings to predict this impact. Conformity and characterisation tests are the first steps in understanding the behaviour of metal(loid) elements. The results highlight significant differences both within and between sites. These differences are likely due to variability in the bearing phases and in the extraction methods. These findings improve the understanding of element behaviour in technosols and contribute to a better assessment of their environmental risk.

Introduction

Metallic and metalloid elements in the environment can affect human health. Their presence has diverse origins, notably mining activities[1], which generate large quantities of tailings. Over time, these residues can evolve into technosols through spontaneous revegetation.

The environmental impact of technosols is assessed using both conformity and characterisation tests. Conformity tests, such as EN 12457-2, are used to evaluate the leaching potential of metallic and metalloid elements and to classify mining wastes according to their environmental hazardousness [2]. The behaviour of metallic and metalloid elements (here: V, Cr, Mn, Co, Ni, Cu, Zn, As, Cd, Sn, Sb, Ba, W and Pb) is characterised using complementary experimental approaches. The pH leaching test allows the assessment of the influence of pH (0.5, 1, 2, 3, 6, 9, 11 and 13) [3,4] on element mobilisation, while sequential extractions of the BCR type make it possible to operationally associate the behaviour of trace elements with the main bearing phases of the soil. Sequential extraction allows obtaining four different fractions: F1 — easily exchangeable and acid-soluble; F2 — adsorbed on Fe and Mn oxides; F3 — associated with organic matter and sulfur; F4 — residual fraction included in mineral structures [4].

The objective of this work is to determine the hazardousness of different tailings and to compare their leachability using various methods.

Methods

First, the samples needed to be evaluated and classified according to their potential environmental impact. Then, the behaviour of metallic and metalloid elements was investigated to improve the understanding of their mobility and their associated fractions.

Samples used in this study came from three abandoned mining sites located in France. All of them were tailings with limited natural revegetation. These residues could contain Zn, As, Sb, and Pb.
Three samples from Vaulry in Haute Vienne (VLY) and four samples from Abbaretz in Loire Atlantique (ABZ) corresponded to residues from tin mining, whereas two samples from La Petite Faye in Creuse (LPF) originated from gold mining and one sample from Pontgibaud in Puy-de-Dôme (PGB) which is a residue from silver mining.

Measurement of total element: total element was obtained by X-ray fluorescence.

EN 12457-2 test: 1 g of soil was precisely weighed and placed in contact with 10 mL of ultra-pure water in a centrifuge tube for 24 hours under agitation (150 rpm). The suspension was then centrifuged at 3000 g for 20 min and filtered using a syringe equipped with a 0.45 µm filter. The leachate was analysed by ICP-MS.

pH leaching test: 1 g of soil was precisely weighed and placed in contact with 10 mL of solution with a pH between 0.5 and 13 (0.5, 1, 2, 3, 6, 9, 11, and 13) in a centrifuge tube for 24 hours under agitation (150 rpm). These solutions were prepared using NaOH or HNO_3 and ultra-pure water. Following the EN 12457-2 procedure, the suspension was then centrifuged at 3000 g for 20 min and filtered using a syringe equipped with a 0.45 µm filter. The leachate was analysed by ICP-MS.

Sequential extraction of the BCR type:
-Solution A: 0.11 M acetic acid.
-Solution B: 0.5 M hydroxylammonium chloride.
-Solution C: H_2O_2 8.8 M.
-Solution D: 1 M ammonium acetate at pH 2.

Step 1: 1 g of soil was precisely weighed and placed in contact with 40 mL of solution A in a 100 mL centrifuge tube for 16 hours under agitation (150 rpm). The suspension was then centrifuged for 20 min at 3000 g and filtered using a syringe equipped with a 0.45 µm filter. This fraction corresponded to F1.
The soil residue was rinsed twice with 20 mL of ultra-pure water, agitated for 15 minutes, then centrifuged for 20 min at 3000 g; the supernatant was discarded.

Step 2: 40 mL of solution B were added to the residue from Step 1 and agitated for 16 hours (150 rpm). The suspension was then centrifuged for 20 min at 3000 g and filtered using a syringe equipped with a 0.45 µm filter. This fraction corresponded to F2.
The residue was rinsed following the same procedure as in Step 1.

Step 3: The tube was weighed, then 10 mL of solution C were added, and the tube was weighed again. The suspension was digested for 1 hour at room temperature and subsequently for 1 hour at 85 °C. The volume was reduced to 3 mL or less, and the tube was weighed again. Another aliquot of solution C was added and digested for 1 hour at 85 °C. The volume was then reduced to 1 mL, and 50 mL of solution D were added and agitated (150 rpm) for 16 hours. The suspension was then centrifuged for 20 min at 3000 g and filtered using a syringe equipped with a 0.45 µm filter. This fraction corresponded to F3.
The residue was rinsed following the same procedure as in Steps 1 and 2.

All fractions were analysed by ICP-MS.

Results and Discussion

The EN 12457-2 leaching test is a standardised compliance procedure used to assess the release of elements from waste materials, with results compared against threshold values defined by the European

Landfill Directive (EUFLD, 2002) to determine their classification into inert, non-hazardous, hazardous landfill categories, or eligibility for underground storage.

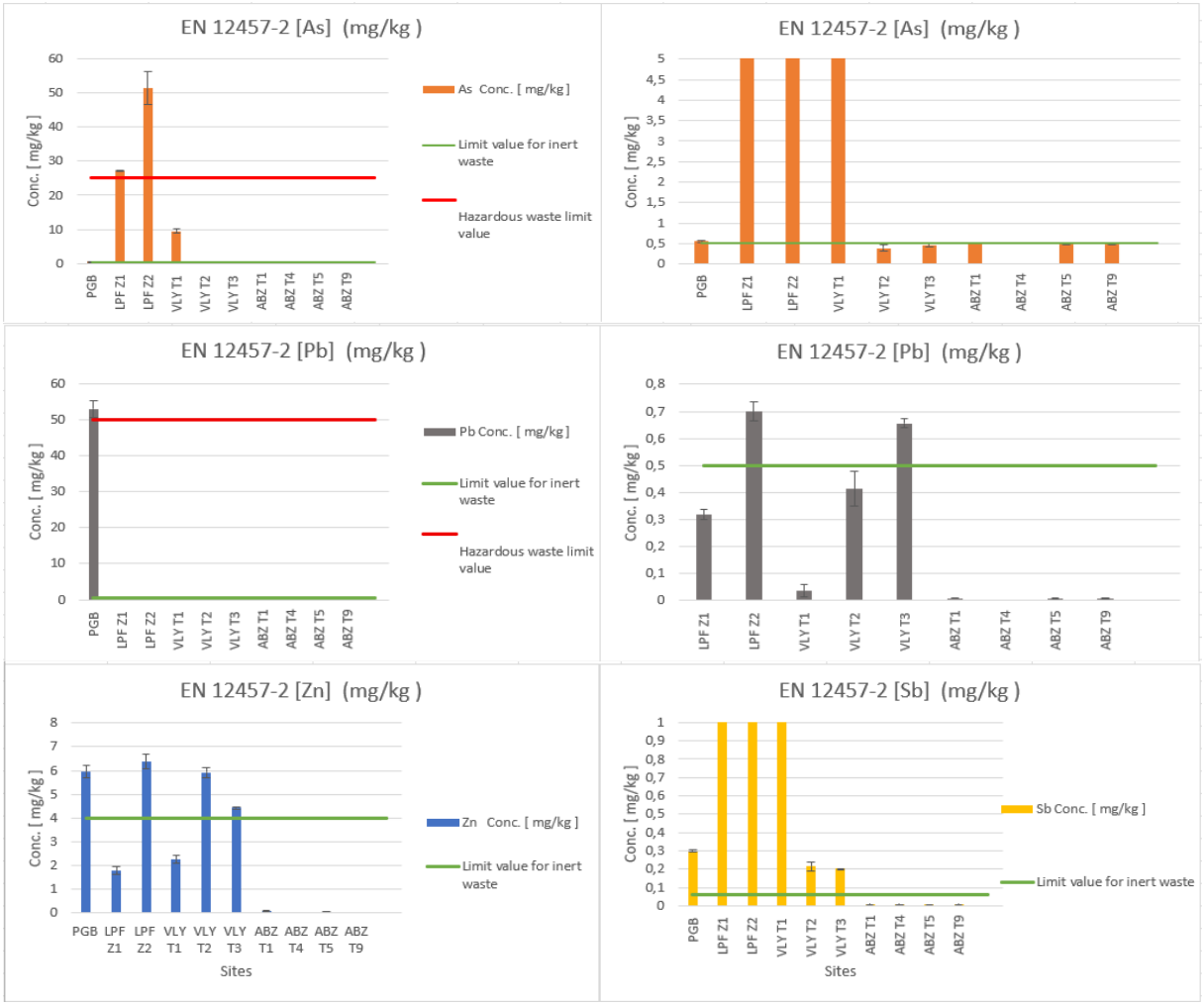


Figure 1.1 Results of the EN 12457-2 test compared with hazardous waste limit values

Each site met at least one limit value for inert waste for at least one element (

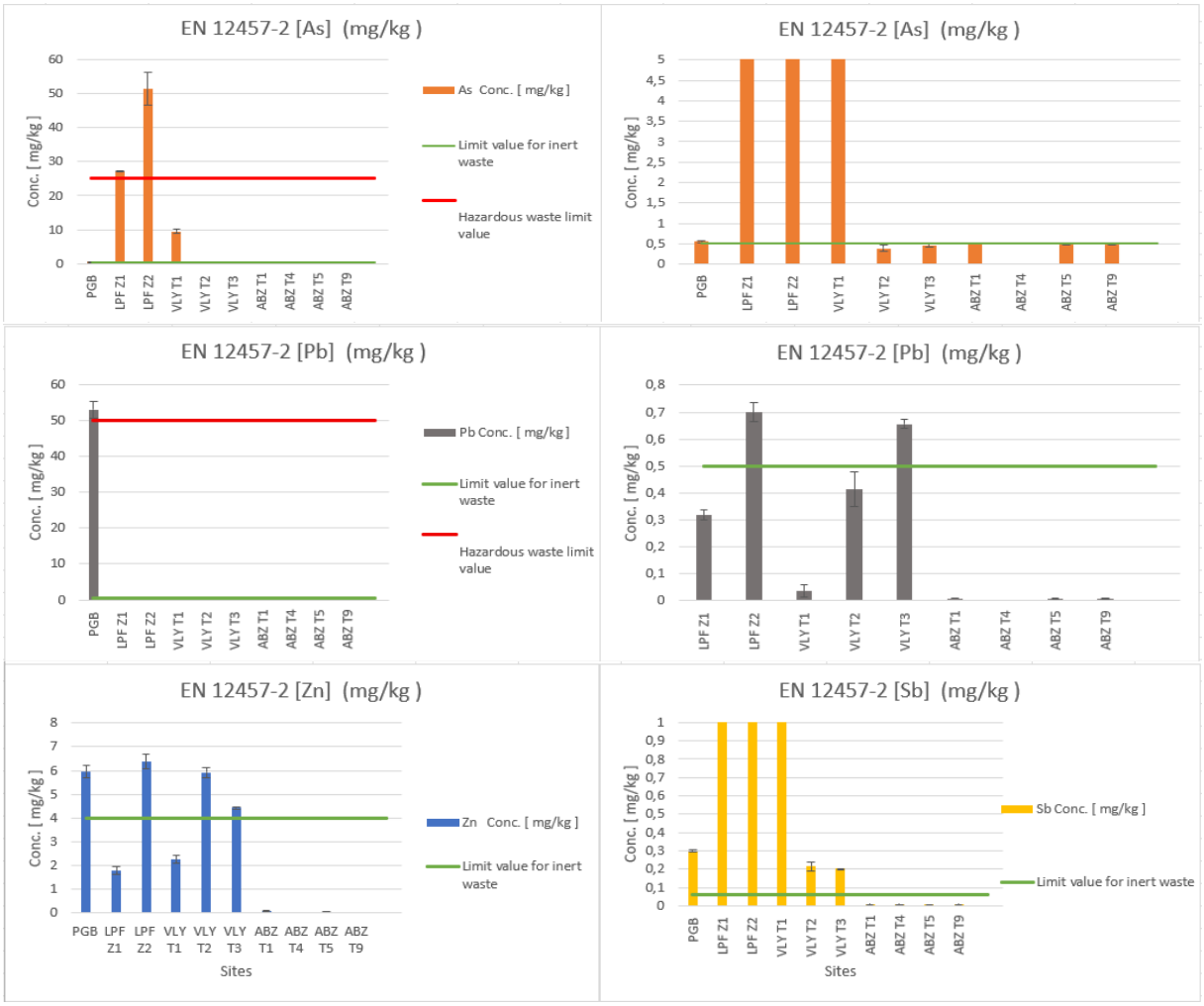


Figure 1.1), and sites LPF Z1 and Z2 met the limit value for hazardous waste for arsenic, while site PGB met the limit value for hazardous waste for lead. Site ABZ appeared to be the least problematic, with the lowest leaching values. All samples were classified as non-inert waste, while LPF and PGB were classified as hazardous waste.

The F1 fraction could be compared to the values obtained from pH 2 and pH 3 solutions in the pH leaching test. Indeed, F1 was obtained using 0.11 M acetic acid (pH ≈ 2.8), so its values should theoretically have lain between those obtained at pH 2 and pH 3.

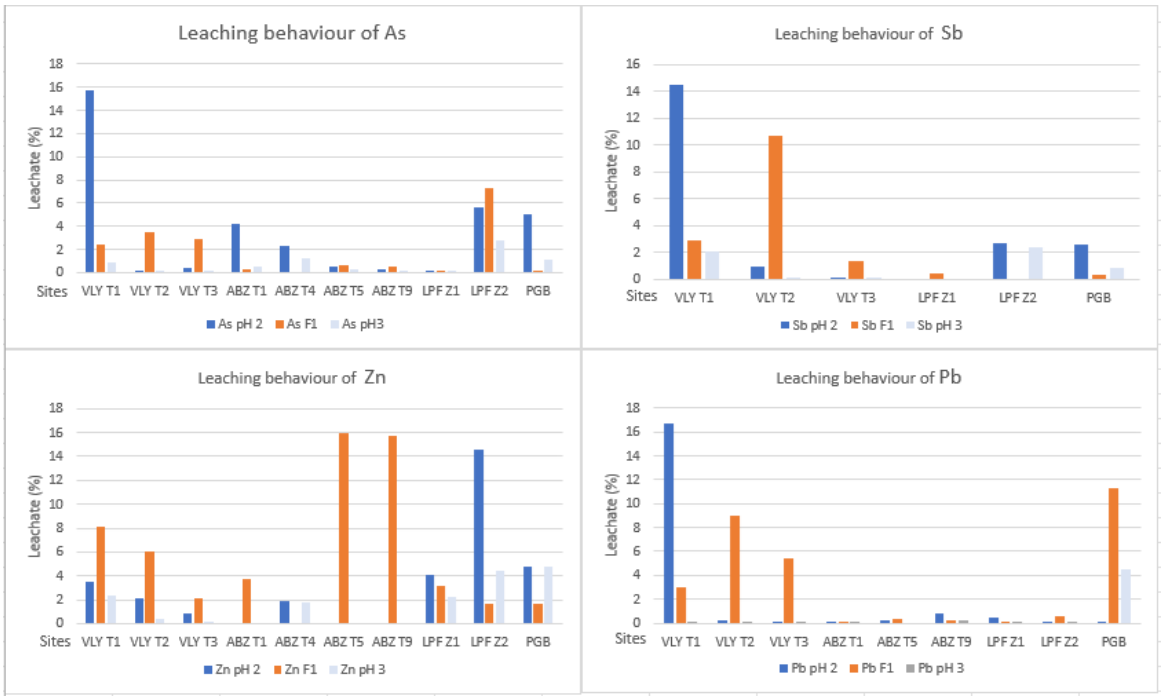


Figure 2.2 Comparison of leaching behaviour between the pH leaching test and the F1 fraction of the BCR sequential extraction

The results showed a variability of behaviour between elements within the same site, and between samples as well as between sites (**Figure 2**.2). The F1 fraction values should theoretically have lain between those obtained at pH 2 and pH 3. However, higher F1 values were observed for: As: VLY T2, T3; ABZ T5, T9; LPF Z2 – Sb: VLY T2, T3; LPF Z1 – Zn: VLY T1, T2, T3; ABZ T1, T5, T9 – Pb: VLY T2, T3; PGB. Conversely, lower F1 values were observed for: As: ABZ T1, T4 and PGB – Sb: LPF Z2 and PGB – Zn: ABZ T4, LPF Z2 and PGB. These differences were likely due to both the nature of the acids and variations in metal(loid)-bearing phase stability. Acetic acid could form organic complexes that enhanced solubility, explaining higher F1 values compared to pH 2–3 HNO_3 leaching. At these pH levels, the oxidative power of HNO_3 could have partially dissolved F2 phases (Fe/Mn oxides), and differences in phase stability also contributed significantly to the observed solubility.

Conclusions

The results reveal differences both within sites (between samples from a same site) and between elements. These differences are likely due to the distinct extraction mechanisms of the two methods, which demonstrate that pH alone does not control metal(loid) solubilisation in tailings. Solution composition and mineralogy also play crucial roles. Mineralogical analysis would confirm the influence of leaching solutions and provide deeper insight into metal(loid) behaviour.

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Column bioleaching of critical elements from mining waste: influence of surface-active reagents

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Abstract: Recovery of critical elements from secondary mineral resources, including mining waste, is environmentally and economically relevant and in accordance with the circular economy principles. The development of new technologies is driving growing demand for metals, hence the need to explore alternative resources.

Surfactants, both naturally and chemically produced, can modulate bioleaching efficiency by altering interfacial properties, bacterial adhesion, and mass transfer characteristics. This offers new opportunities for optimising bioleaching, including column bioreactors that simulate heap leaching operations.

The research involved bioextraction of mining waste in a closed-loop column. Arsenic was identified as the main element. The mineral material was modified by the adsorption of surface-active compounds in order to check whether their presence at the mineral surface affects the process. Various types of surfactants were tested: ionic, non-ionic, and biosurfactants. It was shown that surface-active reagents influence the process efficiency. In the case of non-modified particles, arsenic recovery reached over 360 mg/L, while the best extraction was for the cationic surfactant (620 mg/L). The final results confirmed the presence of copper (0.82 - 2.6 mg/L), barium (0.18 - 0.28 mg/L), magnesium (349 - 369 mg/L), manganese (8.7 - 9.3 mg/L), phosphorus (7.4 - 31 mg/L), and vanadium (0.020-0.055 mg/L). The surface morphology of leaching residues and secondary products was analyzed by X-ray diffraction and scanning electron microscopy. The surface charge of the solid residues was also examined.

Keywords: acidophilic microorganisms, arsenic, mining waste, zeta potential

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The rate of pyrite oxidation with ferric iron in the presence and absence of microorganisms

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Abstract:

Refractory sulfidic gold ores often harbour gold in a pyrite matrix that requires oxidation before gold leaching. The aim of this study was to determine the rate of pyrite oxidation with ferric iron in the presence and absence of microorganisms. Two computer-controlled continuously stirred tank reactors (CSTRs) were operated with 20% pulp density of Simberi ore. Reactor A (RA) was inoculated with microorganisms to facilitate biological oxidation of ferrous iron, whereas uninoculated reactor B (RB) was dosed with hydrogen peroxide (H_2O_2) as a chemical oxidant. Gas purging was switched between air and nitrogen to reach target redox potentials of +670 mV and +550 mV vs. Ag/AgCl, respectively. During the 10 days of the experiment, a total of 18 and 56 redox cycles between +550 mV and +670 mV vs. Ag/AgCl were detected in RA and RB, respectively. In general, RA was operated predominately at a higher redox potential range than RB. After reaching the lowest redox potential set point of +550 mV vs. Ag/AgCl, a longer time was required for RA to reach the maximum redox potential set point of +670 mV vs. Ag/AgCl than in the RB. The longer operational time under the higher redox potential condition in RA improved pyrite oxidation when compared to RB, as revealed by the higher soluble Fe and S, and a lower residue sulfide content in RA. Moreover, the redox cycle lengths were remarkably increased with time in both reactors and were generally longer in RA than in RB. The redox potential drop rate in RA was generally lower than in RB, likely due to the simultaneous biooxidation of ferrous iron and the higher soluble Fe. Overall, the study gave valuable information on the rate of pyrite oxidation in the presence and absence of microorganisms as a function of redox potential.

Keywords: biooxidation, chemical oxidation, ferric iron, pyrite, reactor

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EFFECT OF MICROBIAL-MINERAL INTERACTION ON THE SURFACE PROPERTIES OF JAROSITE PRECIPITATE DURING BIOLEACHING OF SULFIDE MINERALS

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Bioleaching heaps are complex multiphase systems, and the dynamics of microbial-mineral interactions are poorly understood. Microbial-mineral interactions may be directly influenced by microbial modes of attachment. This study investigated the influence of microbial activity on the surface properties of jarosite precipitates that facilitate metal sorption, a critical factor for bioleaching optimization. Bioleaching and Chemical leaching of pyrite were conducted at 40 ºC and pH 1.9–2.4. The Chemical leaching was conducted to mimic the bioleaching. The fit of the kinetic data to the Hansford model revealed that similar maximum Fe^{2+} oxidation rates for bioleaching at pH 1.9, 2.2, and 2.4. One-way analysis of variance confirmed that both systems exhibited statistically equivalent Fe^{2+} oxidation rate kinetics at a given pH. The characterization of the jarosite precipitates revealed that bio- and chemical jarosites consisted of K-Na jarosite solid solutions but varied in weight percent. Bio-jarosite exhibited lower crystallinity (41-57%), and smaller crystallites (22–27 nm) compared to Na-dominated chemical jarosite with 54-71% crystallinity. All formed jarosites exhibited mesoporous particles however, the surface area of bio-jarosites was slightly higher than chemical jarosites. Morphology of bio-jarosite showed rose-flower aggregates with cauliflower-like particles, whereas chemical jarosite exhibited cubic crystal shape. Isoelectric point of bio-jarosites was lower than chemical jarosite, indicating that microbial modification increased the negative surface charge, which may be responsible for metal occlusion during leaching. These findings provide a fundamental understanding of the role of microbial activity in shaping precipitate properties and can inform strategies for optimizing and managing bioleaching processes.

Keywords: bioleaching; microbial-mineral interaction; extracellular polymeric substances; surface properties; jarosite;

1 Introduction

Microbial-mineral interactions are critical for understanding the influence of microbial activity on the surface properties of jarosite precipitates in bioleaching processes. Bioleaching proceeds directly through redox-dependent microbial attachment to mineral surfaces or indirectly through extracellular polymeric (EPS) secreted upon cell attachment [1]. The EPS microenvironment binds ferric ions, and promotes jarosite nucleation through electrostatic interaction, charge accumulation, geometric matching of crystal, and complementary structure in space [2]. Jarosite precipitation is often associated with diminishing ferric ions and occlusion of valuable metals from the leach solution thereby reducing metal recovery. The surface reactivity of jarosite precipitates plays a key role in its capacity to adsorb and retain dissolved metals. Studies have linked the surface properties to process conditions under which the precipitate forms [3]. However, process conditions employed during bioleaching are optimized for microbial growth and metabolic activity, and the extent to which microbial activity modifies jarosite surface properties and in turn metal occlusion remains insufficiently understood. Thus, this study aims to understand the effect of microbial activity on the surface properties of jarosite precipitates during bioleach operation.

2 Materials and Methods

Jarosite precipitates were obtained from bio- and chemical leaching (BioL and ChmL) of pyrite under the same conditions; initial pH of leaching solution medium (1.9, 2.2 and 2.4), temperature (40 °C), and redox potential using column reactor. The development of bioleaching redox potential was maintained in chemical leaching through gradual addition of 30% H_2O_2 to mimic bioleaching system and isolate microbial effect on jarosite precipitate. The obtained jarosite precipitates were analyzed with XRD, SEM/EDS, FT-IR, PSD and BET/BJH. The surface charge was measured via pH at point of zero charge (pH_{pzc}).

3 Results and Discussion

The ferrous-ion oxidation kinetics fitted to the Hansford model accurately with precision ($R^2 > 0.92$). One-way ANOVA ($p > 0.05$) confirmed that bio- and chemical leaching exhibit statistically equivalent oxidation rates under similar redox potentials (Table 1). XRD analysis revealed characteristic peaks of K-Na jarosite solid solution in both BioJar and ChmJar at different pH with minor peaks of pyrite and quartz (Fig. 1). However, peak analysis (d_{113}) indicated varying cation composition: BioJar favored potassium incorporation (~61% K and ~39% Na), while ChmJar incorporated higher sodium (56-57% K and 43-44% Na). Microbial EPS and cell surfaces likely serve as selective K^+-binding promoting K-jarosite and limiting Na^+ incorporation. From Table 1, the relative crystallinity and crystallite sizes of BioJar were lower than ChmJar across all pH levels. The reduction may be due to microbial cells and EPS creating localized zones of extreme supersaturation, favoring rapid nucleation of numerous defect-rich crystallites rather than fewer well-ordered one [4]. FT-IR spectroscopy (Fig. 2) identified vibrational modes corresponding to SO_4^{2-}, H_2O, OH, and FeO_6 groups which are consistent with standard jarosite.

Distinct morphologies were observed for BioJar and ChmJar across all pH levels (Fig. 3). At pH 1.9, BioJar formed stacked rose-like aggregates mixed with loose, cauliflower-like particles while ChmJar comprised of low crystalline assemblages of sharp-edged cubic structure. The morphological difference may be a result of EPS which promotes cell adhesion of jarosite particles. Also, microbial activity may alter process conditions to enhance heterocoagulation between bacteria and jarosite particles thereby promoting particle aggregation and crystal growth. As pH increased, a progressive transition from poor crystalline aggregates to a well-defined crystalline structure was observed. The higher surface area of BioJar at different pH may be a result of poor relative crystallinity and amorphous nature from EPS-mediated attachment providing many nucleation sites and trapped within the jarosite crystals[5].

Table 1: Maximum rate of oxidation ($r_{Fe^{2+}}^{max}$) of bio- and chemical leaching, relative crystallinity, relative crystallite sizes, and surface area of BioJar and ChmJar at pH 1.9, 2.2 and 2.4

pH	$r_{Fe^{2+}}^{max}$ (mmol/L.hr)		Relative crystallinity (%)		Relative crystallite size (nm)		Surface area (m^2/g)	
	BioL	ChmL	BioJar	ChmJar	BioJar	ChmJar	BioJar	ChmJar
1.9	4.98	4.41	41.16	54.37	27.47	27.89	4.99 ± 0.026	4.47 ± 0.019
2.2	4.15	3.44	47.53	56.56	27.40	27.71	2.28 ± 0.016	1.47 ± 0.009
2.4	2.45	2.11	57.35	70.35	22.38	24.39	1.91 ± 0.014	1.39 ± 0.010

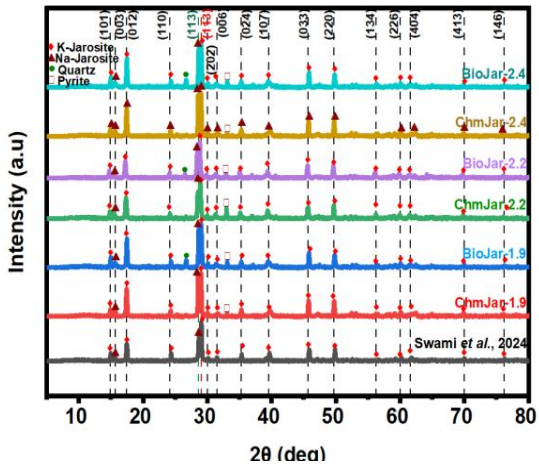


Figure 1. XRD peaks and identification of BioJar and ChmJar at pH 1.9 and 2.4

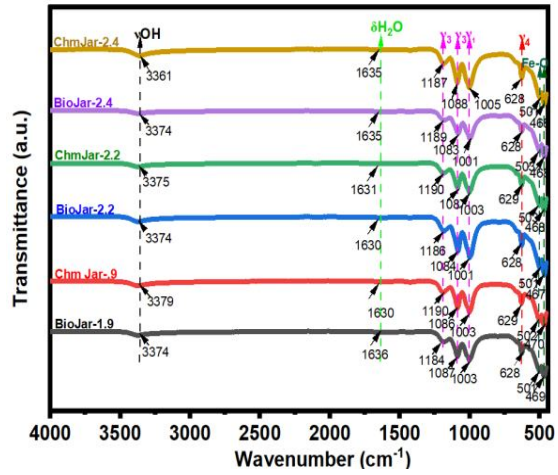


Figure 2. FTIR bands of BioJar and ChmJar at pH 1.9 and 2.4

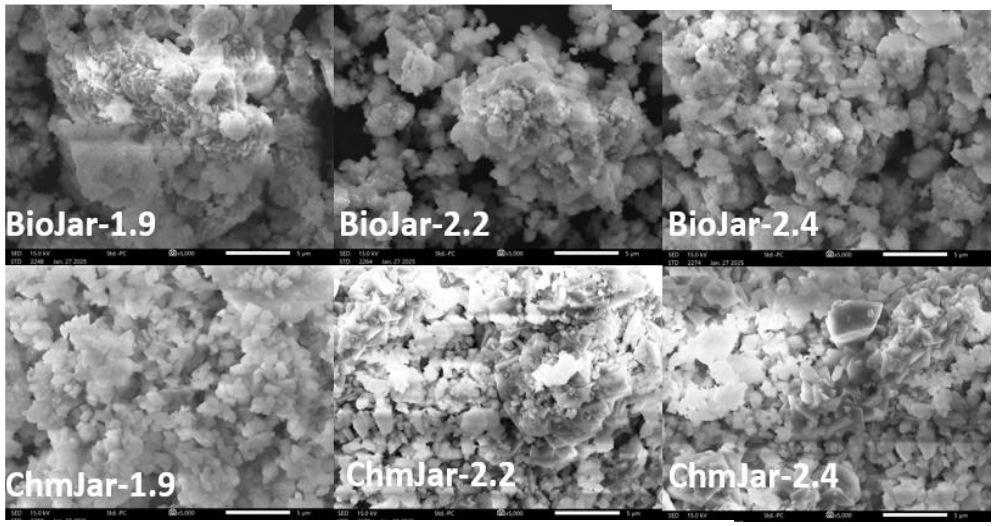


Figure 3. SEM morphology of BioJar and ChmJar at pH 1.9 and 2.4

The elemental analysis confirmed a non-stoichiometric molecular formula characterized by variable occupancy of Fe^{3+} at the B-site and K-Na-H_3O^+ at the A-site (Table 2) [6]. The A-site occupancy in BioJar crystal lattice was predominantly occupied by 73–76% K^+ while Na^+ contributed 2–23%. ChmJar showed lower K^+ occupancy (17–38%) and higher Na^+ incorporation (19–43%). This further validates the XRD findings of preferentially incorporating K^+ over Na^+ in BioJar at across all pH levels. From Table 2, the average particle size at pH 1.9 and 2.2 for BioJar was smaller compared to ChmJar, indicates a finer particle population comprised of nanoscale and uniform particles due to controlled nucleation by EPS which alters growth kinetics [7]. The higher average particle size observed for BioJar compared to ChmJar at pH 2.4 may be attributed to variations in the ionic radii of the jarosite phases identified via XRD.

From Fig. 5, microbial activity decreased the pH_{pzc} values for all initial pH considered resulting in a higher density of negatively charged surface on the jarosite precipitates. This modification of BioJar may stem from partial or total coverage of microorganism during attachment on jarosite surface, which brings the precipitate closer to the pH_{pzc} of the colonizing microbial community [8]. Also, the increase in pH_{pzc} at higher initial pH levels highlights the interplay between microbial activity and pH.

Table 2: Nonstoichiometric molecular formula and average particle size of BioJar and ChmJar at pH 1.9, 2.2 and 2.4

pH	Molecular Formula		Particle size, D50 (µm)	
	BioJar	ChmJar	BioJar	ChmJar
1.9	$K_{0.73}Na_{0.15}[H_3O^+]_{0.12}Fe_{2.82}[SO_4]_2[OH]_{5.47}$	$K_{0.38}Na_{0.26}[H_3O^+]_{0.36}Fe_{2.86}[SO_4]_2[OH]_{5.57}$	19.40	36.37
2.2	$K_{0.76}Na_{0.23}[H_3O^+]_{0.01}Fe_{2.89}[SO_4]_2[OH]_{5.67}$	$K_{0.27}Na_{0.19}[H_3O^+]_{0.56}Fe_{2.92}[SO_4]_2[OH]_{5.76}$	40.10	58.00
2.4	$K_{0.76}Na_{0.02}[H_3O^+]_{0.22}Fe_{2.64}[SO_4]_2[OH]_{4.93}$	$K_{0.17}Na_{0.43}[H_3O^+]_{0.42}Fe_{2.77}[SO_4]_2[OH]_{5.30}$	33.33	9.17

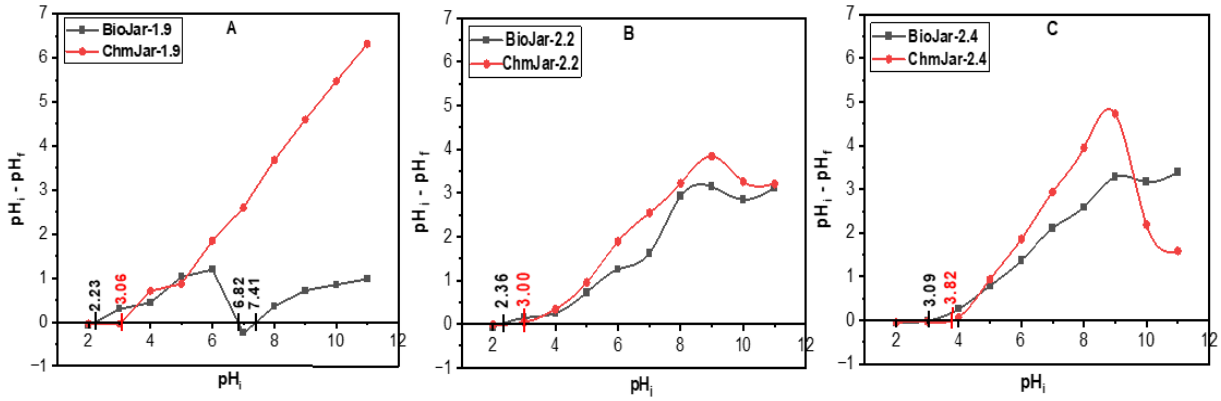


Figure 4. pH_{pzc} of BioJar and ChmJar at pH 1.9 and 2.4

4 Conclusion

Bioleaching conditions fundamentally modified jarosite surface properties compared to purely chemical synthesis. Microbial activity via EPS favored the nucleation of jarosite with higher K^+ over Na^+, finer crystallite growth, and higher surface areas. Microbial activity modified the morphology and pH_{pzc} of jarosite precipitate resulting in higher density of negatively surface charge. These surface modifications highlight the influence of microbial activity in shaping precipitate properties beyond standard process conditions and may offer fundamental insights for bioleaching optimization.

Acknowledgments. The financial support of the Centre for Postgraduate Studies (CPGS) of the Cape Peninsula University of Technology (CPUT) is acknowledged.

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STUDY OF SYNTHESIS OF Fe(III)-HYDROXYSULFATE MINERALS IN ACIDIC SULFATE SYSTEMS

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Keywords: Fe(III)-hydroxysulfates, schwertmannite, jarosite, iron-oxidizing bacteria

Abstract

This study investigates the controlled synthesis of Fe(III)-hydroxysulfate phases mediated by an inoculated iron-oxidizing bacterial culture of *Acidithiobacillus ferrivorans* SS3 (DSM 17398) under acidic aerobic conditions at 25 °C. Mineral formation was directed by medium composition. Schwertmannite was synthesized in a potassium-free medium. Jarosite synthesis was induced by adding 50 mM K^+ as K_2SO_4 to a medium containing (per litre) $FeSO_4 \cdot 7\ H_2O$ (33.4 g), $MgSO_4 \cdot 7\ H_2O$ (0.39 g), $NH_4H_2PO_4$ (0.70 g), K_2SO_4 (4.36 g). The initial pH of both systems was adjusted to 2.5. As a result of bacterial oxidation, the concentration of Fe^{2+} gradually decreased from an initial concentration of 124 mM to zero after about 96 h, while the oxidation-reduction potential (Eh) increased from 620 mV to ~880 mV. Hydrolysis of Fe^{3+} resulted in the precipitation of iron and the formation of schwertmannite ($Fe_8O_8(OH)_6SO_4$) and jarosite ($KFe_3(SO_4)_2(OH)_6$). Chemical analyses of residual Fe, SO_4^{2-}, and monovalent cations revealed a progressive decrease in potassium, iron, and sulfate concentrations in near-stoichiometric proportions consistent with the ideal jarosite composition, whereas schwertmannite formation was characterised by an Fe/S molar ratio of ~8. Mineral phases were identified by powder X-ray diffraction (XRD) (D8 Advance, Bruker), and scanning electron microscopy (SEM; MIRA 3 FE-SEM, TESCAN) revealed globular needle-like schwertmannite aggregates and smooth globular jarosite aggregates.

Introduction

The bacterial species *Acidithiobacillus* (*At.*) *ferrivorans* SS3 is a psychrotolerant acidophile capable of growing in the temperature range of 5–35 °C (optimum ~25 °C), which derives energy from the oxidation of ferrous cations and inorganic sulfur compounds (ISCs). As a carbon source, this bacterium utilises CO_2. At low temperatures, *At. ferrivorans* catalyses the dissolution of metals in a biotechnological process known as bioleaching and acts as the dominant species in the dissolution of metal sulfides at 7 °C [1]. Mixed cultures dominated by *At. ferrivorans* are being studied at low temperatures for the removal of ISCs from water in mining operations. Precipitates composed mainly of Fe compounds, known as "yellow boy," serve as a good indicator of surface water contamination by acid mine drainage (AMD). The formation of precipitates poses a significant environmental burden because the precipitates easily become suspended, negatively affecting river and wetland systems, the service life of limestone reservoirs and engineered reservoirs, and also play an important role in the binding and transport of toxic elements [2].

The primary reaction leading to the formation of AMD is the oxidation of pyrite. The process of pyrite oxidation and the formation of secondary minerals is a complex biochemical process involving hydration, hydrolysis, oxidation, and microbial catalysis [3]. Under appropriately selected conditions, the acidophilic bacterial species *At. ferrivorans* promotes the oxidation of Fe^{2+} and the subsequent precipitation of Fe^{3+} hydroxysulfate minerals such as schwermannite ($Fe_8O_8(OH)_6SO_4$) or jarosite ($KFe_3(SO_4)_2(OH)_6$) [4]. Schwermannite (1) is a metastable, reddish-brown mineral. It has a relatively large surface area and is capable of adsorbing metals and oxyanions such as arsenates, phosphates, and sulfates. When observed under an SEM, its crystals have a "needle-like" shape that forms a typical

"hedgehog" structure. Depending on the pH of the environment, the transformation of schwertmannite results in jarosite (2) or goethite [5]. Jarosite is a mineral with a well-defined crystalline structure; it has a pseudocubic crystal shape and a straw-yellow colour. Hydroxysulfate-type minerals are present almost everywhere in streams and lakes affected by mine waters in the form of ochre-colored precipitates [6].

$$3Fe^{3+} + SO_4^{2-} + 14H_2O \rightarrow Fe_8O_8\left(OH\right)_6\left(SO_4\right) + 22H^+ \quad (1)$$

$$3Fe^{3+} + K^+ + 2SO_4^{2-} + 6H_2O \rightarrow KFe_3\left(SO_4\right)_2\left(OH\right)_6 + 6H^+ \quad (2)$$

Methods

The growth and replication of the *At. ferrivorans* SS3 bacterial culture took place in batch cultivation. A total of 495 mL of nutrient medium and 5 mL of the grown bacterial culture were prepared in sterilised culture bottles. The synthesis of schwertmannite took place in a medium free of monovalent cations K^+ (per 1 L of medium: $MgSO_4 \cdot 7H_2O$ (0.33 g), $NH_4H_2PO_4$ (0.70 g), $FeSO_4 \cdot 7H_2O$ (33.4 g)). The synthesis of jarosite took place in a solution with the addition of 50 mM K^+ in the form of potassium sulfate (per 1 L of medium— $FeSO_4 \cdot 7H_2O$ (33.4 g), $MgSO_4 \cdot 7H_2O$ (0.39 g), $NH_4H_2PO_4$ (0.70 g), K_2SO_4 (4.36 g). The initial pH of these solutions was 2.5.

Cultivation took place at 25 °C, the optimal temperature for the growth of *At. ferrivorans* SS3 under aerobic conditions. The culture bottles were magnetically stirred at 180 rpm, and the air flow rate was set to 250 mL/min. The inoculum was added 24 hours after the start of the analysis. Samples were collected regularly every 24 hours in 1.2-mL volumes into test tubes, which were then centrifuged (10,000 rpm, 10 min). The separated supernatant was then analysed for changes in the concentration of selected ions. The concentrations of K^+ and SO_4^{2-} ions were determined using ion chromatography (Dionex ICS 5000, Sunnyvale, CA, USA). The mineralogy of the precipitates was determined by powder X-ray diffraction (XRD) (D8 Advance, Bruker). Morphology and chemical composition were investigated using scanning electron microscopy (SEM; MIRA 3 FE-SEM, TESCAN, Czech Republic) and elemental analysis using an EDX detector (Oxford Instrument, Oxford, UK).

Results and Discussion

As a result of the catalytic activity of the A. ferrivorans bacterial isolate, oxidation of divalent iron occurred in the presence of atmospheric oxygen. Trivalent iron, as a product of this oxidation, subsequently hydrolysed and precipitated with sulfates and other associated elements to form the minerals schwertmannite ($Fe_8O_8(OH)_6SO_4$) and jarosite. The formation of schwertmannite was observed in a solution without monovalent cations. On the other hand, jarosite ($KFe_3(SO_4)_2(OH)_6$) precipitated in a solution supplemented with 25 mM K_2SO_4.

As a result of bacterial oxidation, the Fe^{2+} concentration gradually decreased from the initial concentration of 124 mM after approximately 96 hours to zero. The oxidation-reduction potential (ORP) increased during the oxidation of Fe^{2+} to Fe^{3+} from 460 mV, reaching a value of approximately 870 mV (absolute values) (Fig. 1). Hydrolysis of Fe^{3+} leads to the precipitation of iron and the formation of Fe(III) hydroxysulfate minerals. The maximum concentration of Fe^{3+} in the medium for schwertmannite reached 90 mM after approximately 120 hours, and in the medium for jarosite, it reached 60 mM after approximately 96 hours (Fig. 1).

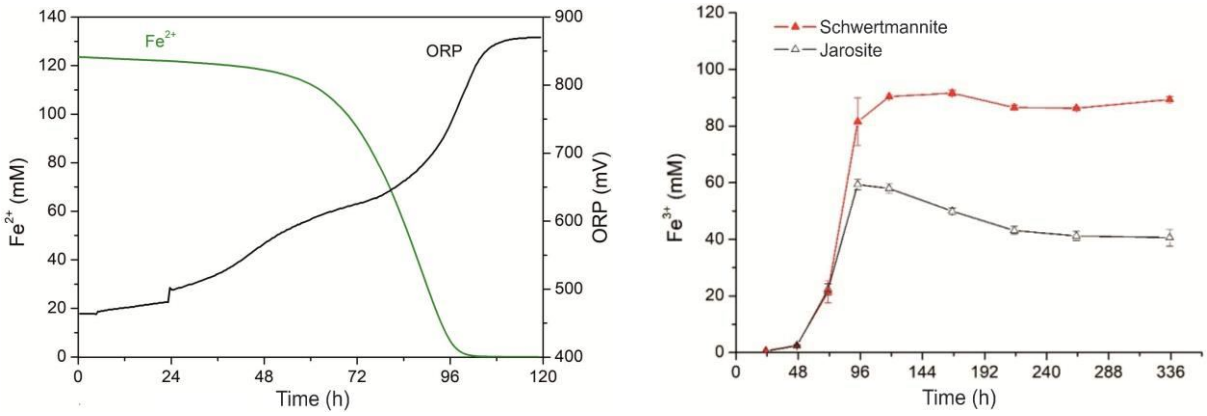


Figure 1. Changes in Fe^{2+} concentration and ORP in solutions (left), changes in Fe^{3+} concentration due to bacterial oxidation and subsequent Fe^{3+} hydrolysis (right).

During the oxidation of Fe^{2+} and the hydrolysis of Fe^{3+}, changes occurred in the concentration of SO_4^{2-} anions (Fig. 2). As a result of the hydrolysis of Fe^{3+} and the formation of jarosite, changes were also observed in the concentration of monovalent K^+ cations (Fig.2).

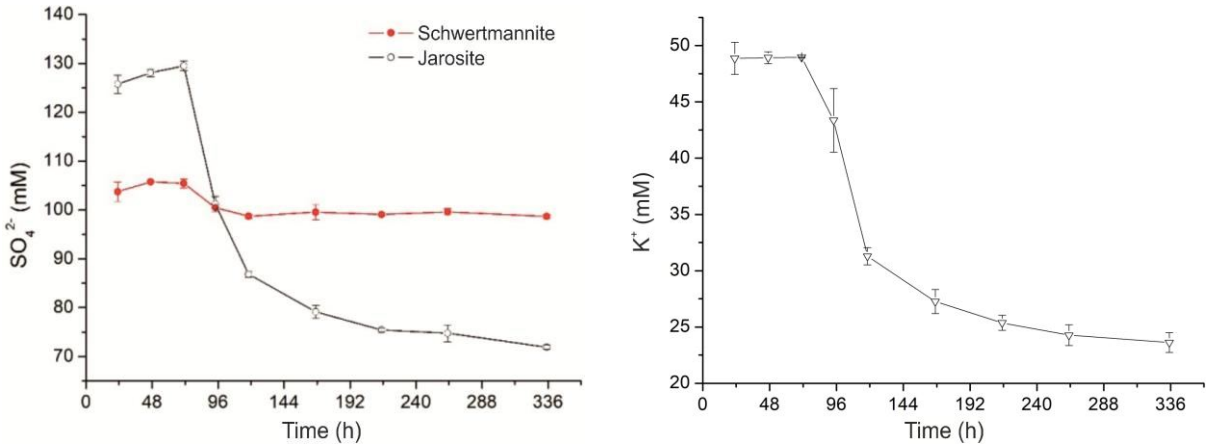


Figure 2. Changes in the concentration of SO_4^{2-} anions during the synthesis of schwertmannite and jarosite (left), changes in the concentration of K^+ cations during the synthesis of jarosite (right).

The mineral composition of the synthesized precipitates was evaluated using elemental analysis (SEM-EDX) of solid-phase samples for schwertmannite (At% - O, 71.9; Fe, 21.8; S, 5.1; P, 1.2) and jarosite (At% - O, 72.6; Fe, 13.9; S, 9.1; K, 3.8; P, 0.6). The atomic percentage composition of jarosite revealed the incorporation of Fe, S, and monovalent K^+ cations into the mineral. In both cases, the phosphate anion was incorporated into the structure. Using a scanning electron microscope (SEM), the morphology typical of schwermannite was observed, consisting of globular aggregates with protruding needles. The synthesized jarosite appeared as smooth globular aggregates.

Solid phases were also determined by the powder X-ray diffraction (XRD). Schwertmannite has a unique X-ray diffraction pattern that can be distinguished from other minerals provided the sample is sufficiently pure. However, identification is difficult not only in the presence of mixed minerals, but also when there is high background noise and the sample has a low degree of crystallinity. X-ray diffraction patterns show seven broad peaks with an interplanar spacing greater than 1.4 Å and a relatively high background, indicating nanoscale particles and a low degree of crystallinity. For the solid phase of schwertmannite, the sharpest reflection occurs at 2.54 Å, corresponding to the hkl (212) plane. Other reflections were observed at 3.38 (310), 2.27 (302), 1.94 (113), 1.64 (522), 1.51 (004), and 1.45 Å (204), which correspond to the reference sample $Fe_8O_8(OH)_6SO_4$.

Unlike other mineral phases, minerals of the jarosite group are typically well-crystallized and, as a result, are easily identifiable by X-ray diffraction analysis. Their reflections are sharp and narrow. The X-ray diffraction pattern of jarosite synthesized at 25 °C shows the sharpest reflections at 5.09 (012), 3.11

(021), and 3.07 Å (113), and three moderately intense reflections at 2.27 (107), 1.97 (303), and 1.82 Å (220). These interplanar distances correspond to the reference sample $KFe_3(OH)_6(SO_4)_2$.

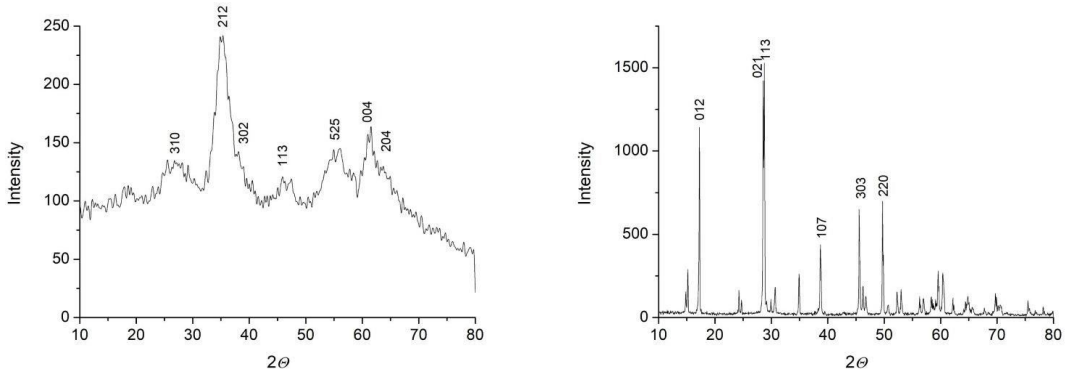


Figure 3. X-ray patterns of schwertmannite (left) and jarosite (right).

Conclusions

The biotechnological utilisation of microorganisms capable of oxidising Fe^{2+} to Fe^{3+} can be applied in the processing of mineral resources and waste, as well as in the monitoring and remediation of contaminated water and soil, including acidic mine drainage (AMD). The results demonstrate that medium composition is the key factor controlling the bacterial synthesis of Fe(III)-hydroxysulfate minerals. Potassium-free conditions favoured schwertmannite formation, whereas K+ addition induced jarosite precipitation.

Acknowledgements

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BIOLEACHING OF Ag-BEARING TETRAHEDRITE USING IRON-OXIDIZING BACTERIA UNDER MESOPHILIC CONDITIONS

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Keywords: tetrahedrite; bioleaching; iron-oxidizing bacteria; ferric ions

Abstract

This study investigates the bioleaching of natural Ag-bearing tetrahedrite originating from the Strieborná vein of the Mária mine (Rožňava, Slovakia). The high contents of Cu (40-46 wt%), Sb (26 wt%), and a relatively significant content of Ag (~1 wt%) make this mineral an economically attractive primary source of critical and strategic raw materials. Tetrahedrite concentrate was bioleached by iron-oxidizing bacteria (*Acidithiobacillus* (*A.*) *ferrivorans* SS3, *Leptospirillum* (*L.*) *ferriphilum*) in acidic sulfate media (pH 1.6-2) under aerobic conditions at 30 °C. Bioleaching was performed in stirred slurry flasks at a pulp density of 2%. Elevated temperature supported biological regeneration of ferric ions, resulting in a high redox potential and enhanced oxidative dissolution of the tetrahedrite concentrate. After 30 days of bioleaching, the maximum Cu concentration reached 2517 mg L^{-1} (*A. ferrivorans* SS3) and 3753 mg L^{-1} (*L. ferriphilum*). The bacterial strain *L. ferriphilum* also exhibited higher efficiency in mobilising additional elements, achieving maximum concentrations of 856 mg L^{-1} Sb, 62 mg L^{-1} Zn, and 109 mg L^{-1} As. In contrast, silver concentrations in the leachates remained very low, not exceeding $0.5 \cdot 10^{-3}$ mg L^{-1} throughout the bioleaching experiment. The results demonstrate that bioleaching at 30 °C provides favourable conditions for efficient tetrahedrite bioleaching using mesophilic iron-oxidizing bacteria.

Introduction

The Strieborná vein deposit (Mária Mine, Rožňava) is characterised by the presence of siderite-sulfide veins. The Strieborná vein represents a significant mineralogical assemblage dominated by tetrahedrite, chalcopyrite, pyrite, and arsenopyrite [1]. Currently, the Mária Mine in Rožňava is of economic interest, primarily due to the presence of natural tetrahedrite ($Cu_{12}Sb_4S_{13}$) containing silver. This sulfide mineral of copper and antimony is characterised by a high content of copper (40–46 wt%), antimony (29 wt%), and a relatively high content of silver (1 wt %). In natural tetrahedrites, elements such as Cu, Sb, and S are often partially substituted by other elements [2]. The presence of Sb, Hg, As, and S poses not only technological challenges in pyrometallurgical processing but also a significant environmental risk, particularly due to emissions of mercury and volatile oxides of antimony, arsenic, and sulfur.

Acid mine drainage (AMD) is typically characterised by elevated concentrations of sulfates, metals, and metalloids such as iron, zinc, cadmium, aluminium, arsenic, and others. The main factors influencing the microbiome in such an environment include pH, temperature, high concentrations of metals and metalloids, total organic carbon (TOC), and dissolved oxygen (DO) [3]. Mine drainage water with a neutral pH (6.9) and concentrations of 402 mg L^{-1} of sulfates and 4.65 mg L^{-1} of iron flows out of the Mária Mine. Metagenomic analysis of the microbiome in the mine drainage water from the Mária Mine revealed, in particular, the presence of iron-oxidizing neutrophils. This environment was dominated by neutrophilic microorganisms, including *Sulfuritalea hydrogenivorans* (6.93%), *Ferrigenium kumadai* (5.45%), and *Gallionella capsiferriformans* (3.79%). Acidophilic groups (e.g., *Thiobacillus sp.* at 0.43%, *Ferrovum myxofaciens*, *Acidithiobacillus ferrivorans*, *Leptospirillum ferrooxidans*) together accounted for less than 1% of the community. Metal resistance systems for Ni, Ag, As, Cu, and Zn

(including CzcD, CzcA, CznA, ArsD, and AioX/AoxX) were widely represented. This integrated taxonomic-functional picture points to microbial communities precisely adapted to specific geochemical conditions, combining lithotrophic metabolic pathways with multi-element resistance [4].

Bioleaching and biooxidation are used to extract metals from low-grade ores with a low content of the target component, as well as from complex ores and ore concentrates. Ferrous iron is a key oxidising agent in the dissolution of sulfide minerals under acidic conditions. The role of iron-oxidizing acidophiles in this process is to oxidise divalent iron (Fe^{2+}) to trivalent iron (Fe^{3+}) and to maintain a high oxidation-reduction potential, defined by the Fe^{3+}/Fe^{2+} ratio. At the same time, most hydrometallurgical processes for processing tetrahedrite–tennantite ores utilise alkaline leaching with Na_2S [5, 6]. Following these findings, experiments were conducted on the bioleaching of tetrahedrite concentrate to evaluate the recovery rates of metals and metalloids at 25 °C in an acidic medium (pH 1.5). Bioleaching of tetrahedrite concentrate in the presence of iron- and sulfur-oxidizing acidophiles resulted in the recovery of >80% Cu (>4 g/L) [7]. In subsequent bioleaching tests conducted at a slightly elevated pH (≈ 2), an increase in the Sb concentration to 665 mg/L (recovery rate of 12.7%) was also observed [8].

This study focuses on the biological leaching of tetrahedrite concentrate under mesophilic conditions (30 °C) in the presence of iron-oxidizing acidophiles, with the aim of extracting metals such as Cu, Zn, and Ag into solution.

Methods

Tetrahedrite concentrate, obtained by flotation processing of a complex siderite-tetrahedrite ore rich in Cu and Ag from the Strieborná vein in Rožňava, was used in the bioleaching process. Bioleaching of the concentrate took place under aerobic conditions at a temperature of 30 °C in an acidic solution (mineral medium) with the following composition per litre: 0,4 g $MgSO_4$ $_7H2O$; 0,1 g $(NH_4)_2SO_4$; 0,04 g K_2HPO_4; 33,6 g $FeSO_4$ $7H_2O$, and 5 mL 5M H_2SO_4. The pH of the solution was 1.6. The autotrophic, acidophilic iron-oxidizing bacterial species *Acidithiobacillus* (*A.*) *ferrivorans* SS3 (DSM 17398) and *Leptospirillum (L.) ferriphilum* (DSM 14647) were used for bioleaching. The bacterial culture was maintained in a liquid nutrient medium containing ferrous sulfate (9K medium). The entire actively growing culture was used as a bioleaching medium, and the tetrahedrite concentrate was added directly at a pulp density of 20 g/L. The flasks were placed on an orbital shaker and stirred at 190 rpm.

Bioleaching was conducted in three parallel experiments. Sterile control experiments consisted of an acidic mineral medium of the same composition without bacterial inoculation. Leachate samples were collected at regular intervals and analysed using AAS, ICP-MS, and ion chromatography. The solid residues after leaching were characterised for elemental composition using a scanning electron microscope MIRA 3 FE-SEM (TESCAN) with an EDX detector (Oxford Instruments).

Results and Discussion

The course of bioleaching of tetrahedrite concentrate in the presence of *L. ferriphilum* and *At. ferrivorans* SS3 at 30 °C differed significantly from the abiotic control. The differences were evident not only in the concentrations of the monitored elements in the leachate but also in the trends of the oxidation-reduction potential (ORP), pH, and the proportions of individual iron oxidation states.

In the biological experiment, rapid oxidation of Fe^{2+} was observed, as evidenced by a sharp drop in its concentration during the initial leaching phase. During the first few days of the experiment, the Fe^{2+} concentration fell from approximately 1,000 mg/L to near-zero levels, while Fe^{3+} became the dominant form of iron in the leachate. During bioleaching with both *At. ferrivorans* SS3 and *L. ferriphilum*, the Fe^{3+} concentration remained close to 6,000 mg/L. The opposite trend was observed in the abiotic control. The concentration of Fe^{2+} remained high throughout the experiment, whereas that of Fe^{3+} was significantly lower and gradually increased to approximately 1,200 mg/L (Figure 1).

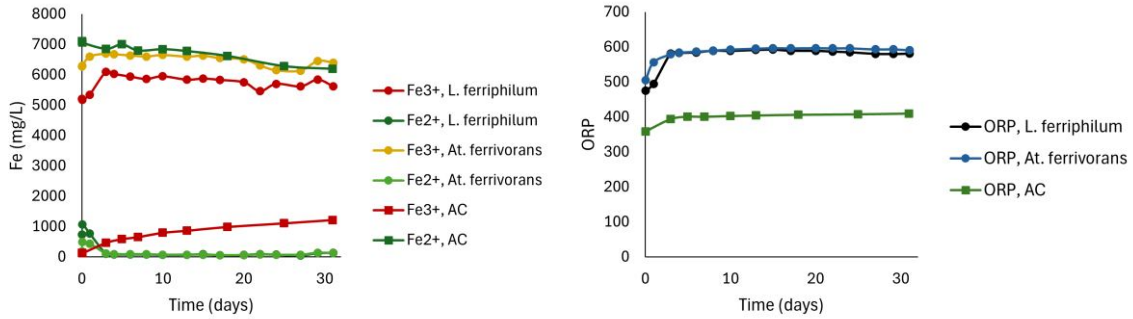


Figure 1. Iron concentration (left) and trends in measured ORP (right) during bioleaching of tetrahedrite concentrate in the presence of *L. ferriphilum*, *At. ferrivorans* SS3 and in the abiotic control, AC – abiotic control.

This difference was also reflected in the ORP values. In the biological experiments, the ORP reached values of approximately 600 mV, while in the abiotic control, it ranged from 360 to 410 mV (Figure 1). In the abiotic control, the pH also gradually increased from 1.65 to 2.2. During bioleaching, a slight decrease in pH was observed, reaching a minimum value of 2.1 in the presence of *L. ferriphilum* and 1.93 in the presence of *At. ferrivorans* SS3.

Significant differences were also observed in the concentrations of Cu and Sb. In the presence of bacteria, the Cu concentration increased continuously throughout the experiment, reaching approximately 2,500 mg/L in the presence of *At. ferrivorans* SS3 and 3,800 mg/L in the presence of *L. ferriphilum* after 30 days. In the abiotic control, the increase in Cu was significantly lower, and the final concentration reached approximately 600 mg/L. In the case of Sb, the concentration gradually increased to approximately 560 mg/L for *At. ferrivorans* SS3 and 850 mg/L for *L. ferriphilum*, while in the abiotic control, the maximum Sb concentration after 30 days was approximately 20 mg/L (Figure 2). The concentrations of As and Zn exhibited different trends. In the case of As, there was a significant increase in concentration during the first few days of bioleaching, with a maximum concentration of approximately 100 mg/L for *At. ferrivorans* and 110 mg/L for *L. ferriphilum*. The concentration of Zn during the bioleaching of tetrahedrite gradually increased throughout the experiment, reaching approximately 34 mg/L and 60 mg/L by the end. In the abiotic control, Zn concentrations remained minimal throughout the leaching test.

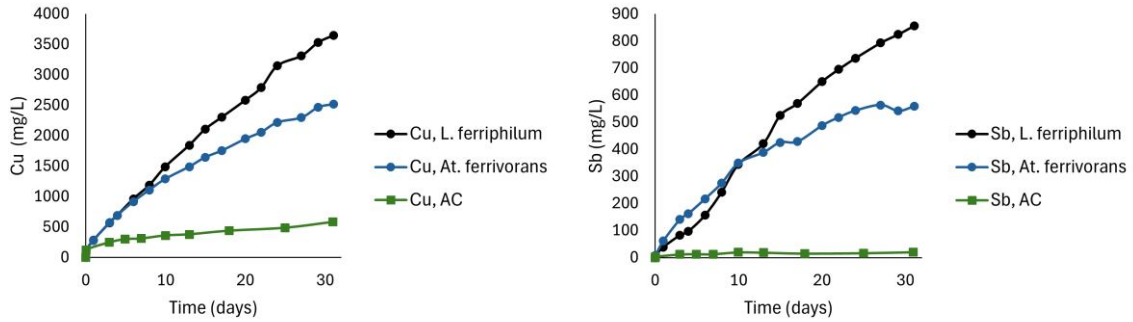


Figure 2. Concentrations of Cu (left) and Sb (right) during the bioleaching of tetrahedrite concentrate in the presence of *L. ferriphilum*, *At. ferrivorans* SS3 and in the abiotic control, AC – abiotic control.

The overall results indicate significant differences between biological and abiotic leaching, particularly for Cu and Sb, where substantially higher concentrations were achieved in the leachate in the presence of bacteria.

Conclusions

The bioleaching tests performed at 30 °C confirmed that the presence of iron-oxidizing acidophiles significantly affected the dissolution of tetrahedrite concentration and the mobilisation of metals and metalloids into solution. Compared to the abiotic control, the biological system showed rapid oxidation of iron, resulting in higher ORP values and enhanced sulfide mineral dissolution. The dominant presence

of ferric iron during the test indicated that the bacterial iron oxidation rate exceeded the rate of chemical reduction of ferric iron during tetrahedrite bioleaching. Under these conditions, the rate-limiting step was likely the heterogeneous reaction between ferric iron and the tetrahedrite surface rather than microbial iron oxidation itself.

The obtained extraction efficiencies demonstrate the mobilisation of individual elements from tetrahedrite. The highest recoveries were achieved with *L. feriphilum* reaching 57 % Cu , followed by 38 % Zn, 32 % As, and 16 % Sb, whereas *At. ferrivorans* reached 38% Cu, 21 % Zn, 29 % As, and 11 % Sb. Ag extraction remained negligible (<1%). Cu showed continuous release throughout the experiment, indicating effective oxidative dissolution of the tetrahedrite matrix. The relatively lower Sb extraction compared to Cu may be related to the precipitation of secondary Sb-bearing phases. Such as tripuhyite (FeSbO4), which is known to limit antimony solubility in acidic oxidising systems.

Overall, the results demonstrate that bioleaching under mesophilic conditions is a promising alternative for processing complex tetrahedrite concentrates compared with conventional pyrometallurgical methods, which are associated with high energy consumption and the release of toxic emissions containing Sb, As, Hg, and sulfur oxides.

Acknowledgements

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Influence of Ferric ion concentration and Redox potential on the Chemical Leaching of Skutterudite ($CoAs_3$) and Nickeline (NiAs) under acidic conditions.

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Abstract:

The global transition toward electromobility has intensified demand for battery-related metals such as cobalt and nickel, prompting interest in unconventional arsenic (As)- bearing ores containing skutterudite ($CoAs_3$) and nickeline (NiAs). Although bioleaching approaches have proven effective for $CoAs_3$, a clear assessment of the chemical leaching and the kinetics remains limited, particularly for NiAs. In this study, we evaluated the influence of ferric iron (Fe^{3+}) concentration (50-400 mM), oxidation–reduction potential (ORP), and temperature (30, 45, 60 °C) on the leaching of both minerals at 1% w/v at pH 1.8. Cobalt, nickel, and total arsenic leaching were quantified by ICP-OES, while arsenic release and speciation were also analysed by ion chromatography (IC). For both minerals, leaching efficiency was strongly dependent on Fe^{3+} concentration and a high ORP. Near-complete metal extraction (~100%) was achieved in less than 24 h with 400 mM Fe^{3+}. Increasing the temperature to 60 °C further improved cobalt recovery from $CoAs_3$, reducing the time to reach the maximum yield to 6 h, whereas only a moderate temperature effect was observed for NiAs. Arsenic speciation analysis revealed distinct release patterns, with $CoAs_3$ leaching producing both As(III) and As(V), whereas nickeline leaching resulted exclusively in As(III) release. These results suggest that biogenic Fe^{3+} may well be applied for the leaching of arsenides. Additional experiments are being carried out to oxidise As(III) to As(V) for safe disposal.

Keywords: Arsenic, arsenide leaching, cobalt, nickel, bioleaching

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Microbial strategies for the recovery of critical metals from tungsten-rich mining residues: a comparative study of biostimulation and bioaugmentation

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Abstract: (200-300 words)

The treatment of tungsten-rich silicate mine tailings poses a major challenge from environmental, health and economic perspectives. This study aimed to treat this type of waste using two biological approaches, either individually or in combination: the stimulation of indigenous microorganisms through the addition of nutrients (biostimulation) or the inoculation of a pre-adapted microbial consortium (bioaugmentation). Metal bioleaching trials were conducted over 60 days in batch reactors. Sulphur-based amendments were also tested to optimise metal recovery. Analyses identified several bacterial genera capable of oxidising the Fe, S and Mn present but inactive in the waste. The addition of nutrients both stimulated the growth and diversity of bacteria of biotechnological interest and induced metal leaching. The use of a microbial inoculum rich in Acidithiobacillus also compensated for the inactivity of the indigenous bacteria. The addition of sulphur and its oxidation induced acidification of the reactors and primarily contributed to the leaching of Mn and Mg, but reduced extraction yields for other metals. Finally, bioaugmentation with sulphur addition and biostimulation without sulphur addition were the treatments yielding the highest metal recovery (100% Zn, 100% Mn, 23% Cu, 73% Mg, 9% W). Biostimulation, however, appears to be more robust than bioaugmentation and easier to implement on a large scale. In both cases, bioleaching is a promising strategy for the recovery of critical metals and the decontamination of tungsten-rich mining waste.

Keywords: Mining waste, Critical metals, bioleaching, Acid Mine Drainage, Metabarcoding, iron and sulfooxidizing bacteria

Effect of particle size on bioleaching of low-grade copper sulfide ore containing silicate, chloride and fluoride gangue materials in column reactors

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Keywords: Heap bioleaching, chalcopyrite, hexahydrate, jarosite, mixed cultures of mesophilic microorganisms

Abstract

The rapid depletion of the world's reserves of high-grade copper ores has resulted into escalation of development of bioleaching process as an alternative technology for metal recovery from copper low-grade ores. Consequently, this study employed ore sizes of -50+25, -25+10, -10+5, -5+1 mm which were investigated for the dry mass varying between 450 kg and 470 kg. The bioleaching solution was inoculated with 10^6 cells/cm^3 of mixed culture of mesophilic microorganisms, at operating conditions of solution and air flowrate of 20 L/hr.m^2 and 2 L/min respectively. The experimental trials were conducted in the column reactors of 500 mm diameter and 2800 mm long. The solution pH was daily adjusted to pH 1.5 and, redox potential was measured regularly.

Copper dissolution corresponded to an efficiency of (75.54%) at particle size of -5+1 mm owing to high compaction in column, (79.12%) at -10+5 mm, (65.42%) at -25+10 mm and (52.92%) at -50+25 mm. However, the redox potential was low (350-450 mV vs. Ag/AgCl) throughout 60 days of experiment. This scenario could be attributed to dissolution of fluoride in ore that affects micro-organisms activities. Also, jarosite precipitation affected copper recovery. The results obtained are crucial in contributing to optimisation application of the bioleaching process at industrial scale.

1. Introduction

The depletion of high-grade copper ore reserves has increased the need for sustainable and cost-effective methods to recover copper from low-grade sulfide ores [1, 2, 10, 13]. Bioleaching is an environmentally friendly technology that uses micro-organisms to oxidise ferrous-iron (Fe^{2+}) to ferric-iron (Fe^{3+}) [4, 12, 16-17] and sulfur oxidising bacteria oxidise the sulfur moieties to sulfur and sulfuric acids [6, 7] which sustains the bioleaching process, facilitating the dissolution and extraction of copper. Micro-organisms play a crucial role by regenerating ferric iron, the main oxidising agent in sulfide mineral dissolution, and by producing sulfuric acid that sustains the leaching process. Despite its advantages, heap bioleaching is often limited by slow leaching kinetics compared to stirred-tank systems.

In spite of considerable previous studies conducted in various aspects such as microbial kinetics [1-2, 4, 6], growth conditions [2, 4, 7, 13], and reactor design [24], limited attention has been given to the influence of particle size on heap bioleaching performance using mixed culture of mesophile. Therefore, this study investigated the effect of particle size on the bioleaching of low-grade sulfide copper ore and examines the impact of dissolved anions on mixed cultures of *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans.*

2. Materials and Methods

2.1 Materials

2.1.1 Ore

The low-grade sulfide copper ore used for this test work was acid consuming with magnesium high grade, contained fluoride and chloride, as shown in the Table 1 below:

2.1.2 Microorganisms

A mixed culture of mesophiles containing *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* were used for all the test work. *Acidithiobacillus thiooxidans* was cultured in Starkey medium at temperature varying between 28-35 °C and *Leptospirillum ferrooxidans* was cultured in 9K medium (Silverman &Lundgren) temperature varying between 30-40 °C**.**

2.2 Methods

Four samples with different weights were heap leached in column reactors for 60 days at pH 1.5. The solution irrigation flowrate was 20 L/m^2/h and air flowrate from the bottom of column to the top was 2 L/min. The peristaltic pumps *Lead Fluid*, Model wG600S was used to irrigate columns reactors by feeding blue buckets on top of each column to keep the irrigation flowrate constant. Silicon tubes were used to transport the leach solution in the blue buckets on top of each column. The irrigation solution inoculated twice with 10^6 cells/cm^3 of *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* percolated through the stacked ore and was collected to the bottom of the column in the container and recycled to the top of column in closed circuit. The contact between acid solution and the ore facilitated copper solubilisation.

Daily routine tasks

Heap leaching process was monitored daily. The pH was adjusted by adding sulfuric acid at 98% A.R. Dissolved ions were determined by ICP in the laboratory.

These below were daily tasks:

- The columns were frequently checked, to observe if there were any leakages, overflowing, etc.
- Also, the irrigation and air flowrate monitoring were done to ensure that were constant throughout the experimental trials.
- Measuring of the slump as well taking note of the compaction, by measuring the distance from the top of the column to the top of the solids.
- Assays of PLS for: Cu, Co, total Fe, Mn, Ca, Mg, Zn, F, Cl, Free Acid, ect were frequently analysed.
- pH adjustments with sulfuric acid 98% A.R were done, checking if irrigation was still running, sufficient irrigation solution, if not water was added to adjust the solution level in container at the bottom of each column

For all experiments in calculating the bioleaching efficiency of copper, a correction factor was used to consider the volume losses due to sampling [8].

$$\eta_{M,i} = \frac{(V - \sum_{i=1}^{i-1} v_i)C_{M,i} + \sum_{i=1}^{i-1} v_i C_{M,i}}{m(C_M/100)} \quad (1)$$

Where:

$\eta_{M,i}$: is the percentage of copper extracted corresponding to the sample i,

V : is the initial volume of the bioleaching solution in the bottle in L,

v_i : is the volume of the sample i taken from the bottle containing bioleaching solution, v_i was equivalent to 0.02 L (20 mL),

$C_{M,i}$: is the copper concentration in sample i (20 mL) in g/L,

m : is the initial mass of ore in grams fed in the column reactor,

C_M : is the initial percentage of copper in the ore.

3. Heap leaching test results and discussion

The effect of particle size on copper dissolution was evaluated over 60 days using mixed cultures of mesophilic microorganisms. For the first 10 days, copper dissolution occurred rapidly in the column packed with -25+10 mm particles, indicating a surface reaction-controlled process [14, 16, 24]. As leaching progressed, dissolution became diffusion-controlled [14, 16, 19, 24] due to the formation of precipitates such as jarosite and gypsum, which increased diffusion pathways and reduced overall copper recovery [14, 16, 19]. Although the redox potential shows a sudden increase after the second inoculation to reach a maximum 450 mV but started to decrease after two days to reach around 390 mV except column packed with -50+25mm where the ORP remained above 400 mV Ag/AgCl. This situation was as a result of inhibition of micro-organisms activities which could be attributed to presence of fluoride and chloride in leached solution [12, 18-19]. The closed-circuit system promoted the accumulation of impurities, particularly fluoride and chloride, which adversely affected microbial activity [11, 17, 23] and caused oxidation-reduction potential (ORP) to decline after inoculation.

Particle size had a significant influence on copper recovery. The highest recovery (79.12%) was achieved in the column packed with -10+5 mm particles, followed by -5+1 mm (75.54%), -25+10 mm (65.42%), and -50+25 mm (52.92%). The high performance of the -10+5 mm fraction was attributed to high acid–ore contact, higher permeability. In contrast, the -5+1 mm column experienced excessive compaction and fines migration, which reduced permeability and promoted jarosite precipitation.

The study also showed that acid consumption was relatively high as shown in Table 1B due to the dissolution of silicate gangue minerals [11, 15,16,19]. Fluoride and chloride concentrations increased during leaching [9-11, 18, 20, 21] but decreased alongside iron and copper concentrations, indicating their incorporation into jarosite precipitates [11, 18, 22]. This finding is consistence with [23] who reported that jarosite formation depended on concentration of monovalent cations, ferric iron, sulfate ions in the solution not only the pH of solution. These findings are in line with the study of [13] and [11] who reported jarosite formation in the pH range of 1.35-1.5. However, the ferric iron precipitation did not stop completely copper dissolution but affected overall copper recovery. Ferric iron precipitation did not completely stop copper dissolution; it significantly reduced leaching efficiency [8, 11, 12, 14, 24]. Overall, the results demonstrate that an intermediate particle size (-10+5 mm) provides the best balance between permeability, acid distribution, and copper recovery, while fines impact negatively heap bioleaching performance through compaction, poor solution flow, and precipitation phenomena. Although permeability and solution flow were satisfactory in the coarse-particle columns, copper recovery was lower than expected in both cases.

Table 1. (A) elementary composition of low-grade sulfide used for this experience, (B) Copper heap bioleaching recovery, column slump and acid consumption from the test

A

	Size	%Cu	%Co	%Fe	%Mn	%Mg	%Ca	%F	%S	%Zn	%Cl
COLUMN 1	-5+1mm	0,401	0,017	2,49	0,079	7,25	0,645	0,74	0,89	0,01	0,96
COLUMN 2	-10+5mm	0,525	0,015	2,42	0,059	6,96	0,58	0,85	0,95	0,0085	1,012
COLUMN 3	-25+10mm	0,361	0,012	1,96	0,046	5,63	0,22	0,59	0,67	0,0065	0,895
COLUMN 4	-50+25mm	0,299	0,011	2,223	0,05	5,02	0,28	0,41	0,43	0,0055	0,65

B

Samples size	Copper recovery in %	Column Slump in %	Gangue Acid Consumption in T acid/T of Cu
-5+1 mm	75.54	56	58.87
-10+5 mm	79.12	48	56.95
-25+10 mm	65.42	32	53.56
-25+50 mm	52.92	24	53.64

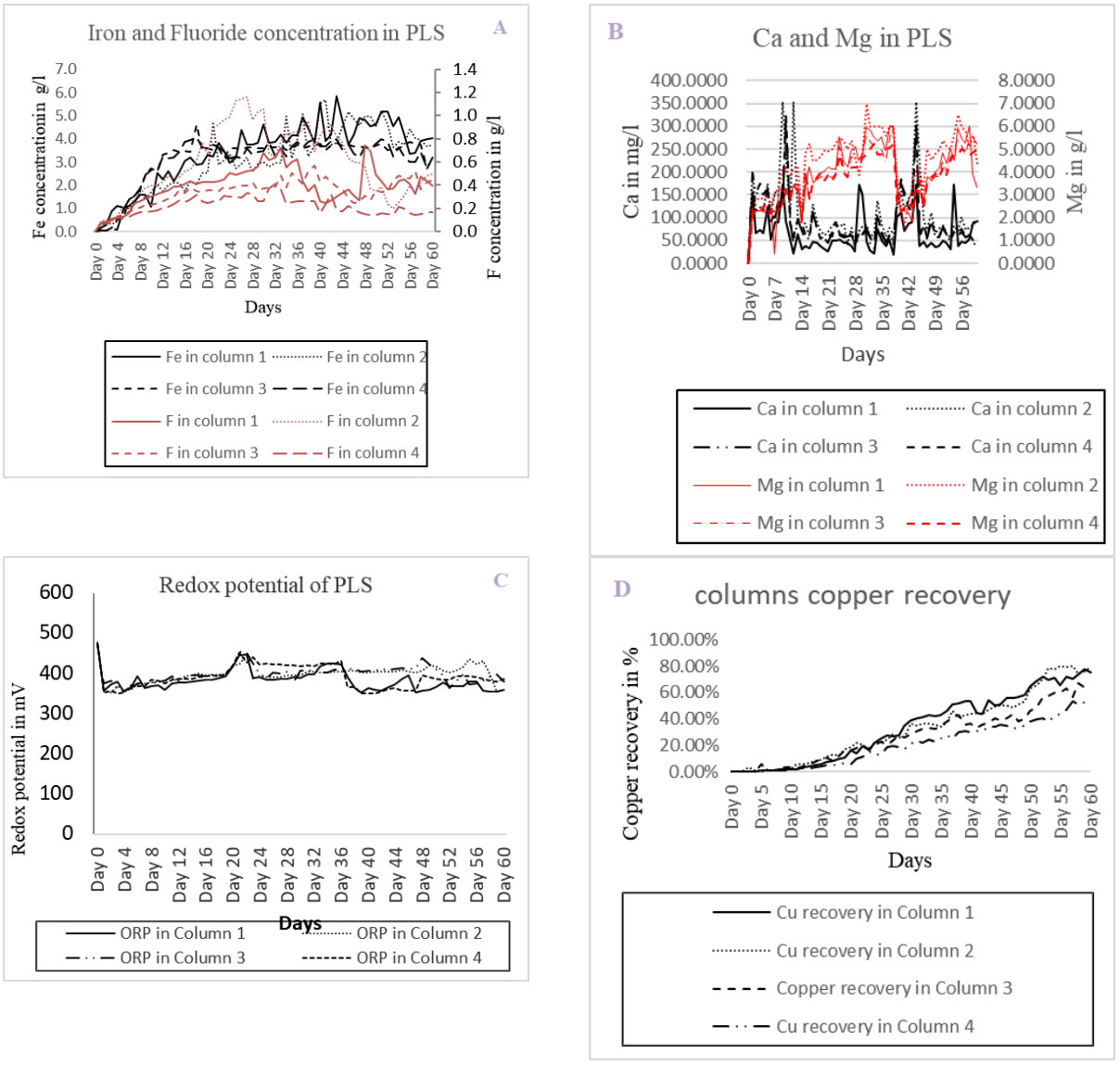


Figure 1. (A) Redox potential and PLS pH versus period of bioleaching, (B) Concentration of metals and fluoride extracted versus bioleaching period, (C) Effect of pre-leaching on metals and fluoride concentration during bioleaching and (D) Effect of PLS pH adjustment frequency on metals and fluoride concentration during bioleaching. All experiments were terminated after 80 days of bioleaching.

Conclusions

Results obtained in this study show that copper sulfide ore heap bioleaching performance is strongly influenced by mineralogy, copper distribution, porosity, particle size, and compaction. Precipitate formation on ore surfaces increased diffusion pathway, reduced copper recovery, and increased acid consumption. Closed-circuit operation caused impurity accumulation, while fluoride and chloride ions inhibited microbial activity that impacted negatively on the PLS redox potential (< 450mV) but did not stop completely copper solubilisation. Although smaller particle sizes improved dissolution rates, excessive compaction reduced permeability and overall copper recovery.

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Bioleaching of Li-minerals

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Abstract: Bioleaching offers benefits for Lithium (Li) recovery over traditional leaching such as sustainability, energy efficiency and lower chemical load. In case of Li minerals, bioleaching of hard-rock zinnwaldite, spodumene, petalite and lepidolite have so far demonstrated limited efficiency whereas carbonate-clay such as jadarite has shown comparatively higher amenability to lithium bioleaching. Here we aimed to compare the bioleaching of three Li ores: carbonate-clay, lepidolite and zinnwaldite with an iron and sulfur-oxidizing mixed culture. The inoculum originated from the Pyhäsalmi mine, Finland, previously confirmed having active sulfur and iron oxidising mixed communities at 40 °C consisting of sulfur oxidising *Sulfobacillus* sp., *Acidithiobacillus caldus* and iron oxidising *Leptospirillum ferriphilum*, and to a lesser extent other species.

The inoculum was at first cultured in 0K-medium amended with elemental sulfur and ferrous iron before adaptation to ores together with 0K-medium and sulfur. Prior to the start of bioleaching, the duplicate ore-culture media bottles were adjusted to pH below 2 using sulfuric acid. During the bioleaching the experiments were monitored weekly for changes in pH and redox potential, and at selected times, the dissolution of lithium was measured. In addition, the live/dead ratio of microorganisms was microscopically assessed. Preliminary results indicate that Li dissolved most efficiently from the carbonate-clay mineral, as expected. In one week over half of the lithium was dissolved and after four weeks around 90%. From zinnwaldite and lepidolite about 16% and 8% of Li, respectively, was dissolved in four weeks by sulfur-oxidizing microorganisms. Thereafter Li bioleaching did not progress further, possible due to precipitation or sorption limiting additional lithium dissolution or the inherently slow breakdown of the silicate structure of the minerals. This study indicated that bioleaching shows promise as an alternative in carbonate-clay minerals processing.

Keywords: Lepidolite, Zinnwaldite, carbonate-clay, bacteria, bioleaching

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INFLUENCE OF TEMPERATURE ON BIOLEACHING OF NICKEL SULFIDE ORE BY AN ACIDOPHILIC MICROBIAL COMMUNITY

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Keywords: nickel ore; temperature effect; bioleaching; community composition

Abstract

The principal challenge in biohydrometallurgy is the rate of metal leaching from minerals, making process optimisation a critical technological endeavor. It is known that temperature exerts the most substantial influence on reaction rates, whereas the rate of biologically mediated reactions is shaped by optimal conditions for the oxidative activity of the microbial community. The present study investigates the dependency of bioleaching efficiency of nickel sulfide ore on temperature within the range of 40 to 52 °C. The ore, which contains 5.41% nickel, 0.77% copper, 21.0% sulfur, and 23.1% iron, serves as the focus of the investigation. Copper was found in the form of chalcopyrite ($CuFeS_2$), while nickel was present as pentlandite ($(Ni,Fe)_9S_8$) and violarite ($FeNi_2S_4$). Bioleaching was performed over a period of 15 days in batch mode within bioreactors. Inoculation involved a community represented by a mixture of communities cultivated on iron and elemental sulfur at the aforementioned temperatures. The iron and/or sulfur-oxidizing microorganisms present in the mixed community included *Acidithiobacillus caldus*, *Sulfobacillus spp.*, *Leptospirillum ferriphilum*, and *Acidiplasma sp.* This study establishes a correlation between the degree of element leaching and mineral conversion and temperature within the specified range. Additionally, changes in community composition were examined throughout the process at various temperatures.

Introduction

Biohydrometallurgy has been successfully commercialised for the recovery of non-ferrous metals, primarily copper, from sulfide ores, as well as for the treatment of refractory gold-bearing sulfide ores and concentrates. Although biohydrometallurgical technologies are considered more environmentally sustainable than pyrometallurgical processes and chemical oxidation methods, they are limited by relatively slow process kinetics and the sensitivity of microbial communities to variations in operating conditions. In addition, studies focusing on the bioprocessing of copper–nickel ores remain relatively scarce in the scientific literature. Available data on the (bio)leaching of nickel from sulfide minerals are limited, and many of the underlying mechanisms and governing factors remain poorly understood [1]. The largest industrial heap bioleaching operation for nickel is located at the Talvivaara Sotkamo mine, while the Vuonos plant in Finland represents a notable example of pilot-scale reactor bioleaching of nickel-bearing sulfide materials [2]. The efficiency of bioleaching and the composition of microbial communities are strongly influenced by temperature. To date, all industrial biohydrometallurgical operations employ moderately thermophilic microbial consortia, which typically exhibit optimal growth at temperatures between 40 and 50 °C. Nevertheless, previous studies have demonstrated that even relatively small temperature changes within the moderately thermophilic range can substantially affect bioleaching performance [3]. Therefore, investigating the influence of temperature within the moderately thermophilic range on both bioleaching efficiency and the structure of microbial communities is of considerable scientific interest.

Methods

Materials. The object of study was copper–nickel sulfide ore from the Shanouch deposit (Kamchatka, Russia). Copper was present in the form of chalcopyrite ($CuFeS_2$), while nickel occurred as pentlandite ($(Ni,Fe)_9S_8$), violarite ($FeNi_2S_4$), and Ni-bearing pyrrhotite ($(Fe,Ni)_{1-x}S$). The particle size distribution

had a P_{80} of 44 μm. The quantitative composition of major elements and minerals in the ore is presented in Table 1 and Table 2, respectively.

Table 1. Content of main elements (wt.%).

Ni	Cu	Fe	S	Ca	Si
5.41	0.77	23.1	21.0	1.34	13.8

Table 2. Content of sulfide minerals (wt.%).

Pentlandite	Violarite	Chalcopyrite	Pyrrhotite	Pyrite
4	4	2	7	5

To carry out the bioleaching of the ore, enrichment cultures were obtained from copper–nickel ore samples at 40, 44, 48, 50, and 52 °C, with or without yeast extract (0.01% (w/v)). These cultures were then combined in equal proportions. The resulting consortium contained chemolithoautotrophic and mixotrophic iron and sulfur oxidisers: *Acidithiobacillus* (*At.*) *caldus*, *Leptospirillum* (*L.*) *ferriphilum*, *Ferroplasma acidiphilum*, *Acidiplasma sp.*, *Sulfobacillus (S.) thermotolerans*, *S. thermosulfidooxidans*, *S. acidophilus*, *S. benefaciens*, *Acidibacillus sp.*, *Ferrimicrobium acidiphilum*. It also included chemoorganoheterotrophic acidophiles such as *Alicyclobacillus* (*Al.*) *tolerans* and *Cuniculiplasma sp.* This consortium was added (10%(v/v)) to the medium and cultured in 2.5-L flasks containing 1 L of the solution for two days at 40–52 °C. A modified 9K medium (initial pH 1.5) with the following composition (g/L) was used: (NH4)2SO4, 0.75; KCl, 0.025; K2HPO4·3H2O, 0.125; MgSO4·7H2O, 0.125; Ca(NO3)2·4H2O, 0.0025. Ferrous iron (Fe^{2+}, 5.0 g/L) in the form of $FeSO_4 \cdot 7H_2O$ and elemental sulfur (1 %(w/v)) served as energy sources. Additionally, 0.01%(w/v) yeast extract was added to the medium to support the growth of microorganisms requiring organic compounds for their metabolism. The obtained communities were subsequently combined in equal proportions, and their dominant members were identified. The combined community was used as the original inoculum for the bioleaching experiments. Thus, the inoculum prepared in this manner contained 5.0 g/L Fe^{3+} and a cell density of $1 \cdot 10^{10}$ cells/mL. The pH was adjusted to 1.2 by adding concentrated sulfuric acid. This solution was then separated from the sulfur precipitate by filtration through a paper filter and used for subsequent bioleaching tests. Bioleaching experiments were carried out at 40–52 °C in 2-L bioreactors containing 1 L of suspension, with agitation provided by an overhead stirrer operating at 500 rpm, and air sparging at a flow rate of 4 L/min. Each bioreactor was loaded with 100 g of solid phase and 1 L of inoculum (thus, the inoculum quantity was 100%). Thus, the pulp density in the experiments was 10%(w/v). The duration of bioleaching was 15 days.

Analysis. The concentrations of copper and nickel ions were determined by flame atomisation atomic absorption spectroscopy (Perkin Elmer 3100, USA). The concentration of the ferric iron in the aqueous phase was determined from the reaction with ammonium thiocyanate [4] using a PE-5400UF photometer (Russia) at λ=480 nm. The total iron concentration was determined by the same method after the reaction of the sample with ammonium persulfate. The copper and nickel contents in the solids were analysed using the referenced wet chemical method [5], followed by the flame atomisation atomic absorption (Perkin Elmer AAnalyst-800, USA). The microbial community structure was determined by metabarcoding analysis with specific molecular markers (V3-V4 hypervariable region of the 16S rRNA gene) as previously reported. This analysis enabled identification of the major acidophiles at both the genus and species levels due to the high sequence variability of the V3–V4 region of the 16S rRNA gene among these microorganisms [6].

Results and Discussion

The results of the analysis of iron concentrations during the bioleaching of nickel ore are presented in Figure 1. At the initial stage of bioleaching, the concentration of ferrous iron increased sharply due to the oxidation of sulfide minerals by the ferric iron present in the inoculum, as well as the dissolution of pyrrhotite. At 40–48 °C, ferrous iron was almost completely oxidised by day 8 of the process. At 50 °C, its oxidation was nearly complete by day 14, whereas at 52 °C the concentration of ferrous iron gradually decreased, reaching 5.5 g/L by the end of the experiment.

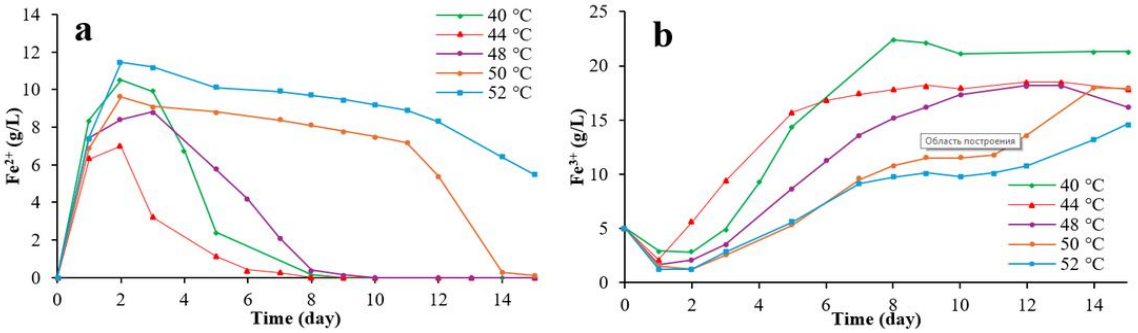


Figure 1. Concentration of ferrous (**a**) and ferric (**b**) iron during bioleaching.

The results of the analysis of the bioleaching residues after 15 days are presented in Table 3. Although the highest nickel bioleaching efficiency was observed at 44–48 °C, no marked decline in nickel recovery was detected at 50–52 °C. In contrast, copper bioleaching reached its highest values at 50–52 °C, likely due to the presence of ferrous iron under these temperature conditions, which lowered the redox potential of the leaching medium.

Table 3. Characteristics of the residues after bioleaching for 15 days.

Temperature (°C)	Residue yield (wt.%)	Recovery (wt.%)		Content in residue (wt.%)	
		Ni	Cu	Ni	Cu
40	64.0	90.5	37.7	0.800	0.750
44	65.1	94.1	33.7	0.493	0.784
48	65.7	95.2	48.0	0.329	0.609
50	63.8	87.7	63.2	1.04	0.444
52	52.0	81.1	56.5	1.65	0.540

16S rRNA gene metabarcoding showed that the original community, prepared by combining inocula grown at 40–52 °C, consisted mainly of the sulfur-oxidizing chemolithoautotroph *At. caldus* and the moderately thermophilic mixotroph *S. thermosulfidooxidans*, with minor amounts of *S. benefaciens*, *S. thermotolerans*, and other taxa (Table 4).

Table 4. Relative abundance (%) of microbial taxa in the original community and in the communities formed by the end of copper–nickel ore bioleaching at different temperatures.

Microbial taxon	Original community	Final community				
		40 °C	44 °C	48 °C	50 °C	52 °C
At. caldus	87	62	71	57	1	1
S. thermosulfidooxidans	12	ND	19	37	41	71
S. thermotolerans	0.2	23	6	5	ND	ND
S. benefaciens	0.2	4	3	0.7	ND	ND
S. acidophilus	ND	ND	ND	0.2	ND	1
L. ferriphilum	ND	11	0.1	ND	ND	ND
Al. tolerans	ND	ND	ND	0.1	ND	ND
Acidiplasma sp.	ND	ND	ND	ND	58	21
Other taxa	<1	ND	<1	<1	<1	6

Relative abundance was calculated as the proportion of 16S rRNA gene amplicon sequences obtained by V3–V4 region metabarcoding. ND, not detected. “Other taxa” comprise microorganisms each representing <0.02% of the total 16S rRNA gene sequences in the community.

By the end of bioleaching, the composition of the microbial communities differed considerably depending on the process temperature. At 40 °C, the community was dominated by *At. caldus*, *S. thermotolerans*, and *L. ferriphilum*, with *S. benefaciens* present as a minor component. At 44 °C, *At. caldus* remained dominant, followed by *S. thermosulfidooxidans* and *S. thermotolerans*, whereas at 48 °C, the predominant community members were *At. caldus* and *S. thermosulfidooxidans*, with several minor *Sulfobacillus species* and *Al. tolerans* also detected.

A marked shift in community structure occurred at higher temperatures. At 50 °C, *Acidiplasma sp.* and *S. thermosulfidooxidans* dominated, while the relative abundance of *At. caldus* decreased to a very low

level. At 52 °C, *S. thermosulfidooxidans* became the predominant species, accompanied by *Acidiplasma sp.*, whereas *At. caldus* remained a minor component.

Overall, the communities at 40–48 °C were dominated by members of the genera *Acidithiobacillus and Sulfobacillus*, while increasing the temperature to 50–52 °C favoured the moderately thermophilic *S. thermosulfidooxidans* and *Acidiplasma sp.* The community at 48 °C exhibited the greatest phylogenetic diversity, whereas the presence of the iron-oxidizing bacterium *L. ferriphilum* among the dominant community members at 40 °C was likely associated with the proximity of the process temperature to the optimum growth temperature of this species.

Conclusions

The highest nickel extraction efficiency was achieved during bioleaching at 44–48 °C, a temperature range characterised by the greatest microbial diversity, with *At. caldus* and *Sulfobacillus spp.* as the dominant microorganisms.

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Empirical Evidence of REE–Organic Acid Complex Formation in Bioleaching

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Abstract

Rare earth elements (REEs) are essential for advanced technologies, yet their recovery from primary resources such as monazite remains challenging due to the environmental and economic costs of conventional extraction. Biohydrometallurgy offers a sustainable alternative, with phosphate-solubilising microorganisms (PSMs) enabling REE release from phosphate minerals through organic acid production. While acidolysis, the dissolution of phosphate by microbial acids, is well established, the hypothesised secondary mechanism of complexolysis for REE-phosphate minerals involves REE–organic acid (REE–OA) complex formation, which has lacked empirical evidence. This study provides the first confirmation of REE–OA complex formation under both abiotic and biotic leaching conditions using advanced microanalytical techniques, including STEM-XEDS and ToF-SIMS. Results show that microbial biofilms significantly enhance complex formation compared to abiotic systems, with up to 3.6 fold higher REE–OA signals in bioleached samples. Complexes between REEs and formate, acetate, malate, citrate, and gluconate were detected, with acetate complexes showing the strongest signal. Biofilm extracellular polymeric substances (EPS) could play a key role in concentrating REE cations and entrapping organic acids, thereby promoting complex formation. These findings highlight complexolysis as a key contributor to REE mobilisation and suggest that engineering biofilms could improve bioleaching efficiency. Although this work advances the mechanistic understanding of REE bioleaching and opens new avenues for biofilm-based strategies in sustainable REE recovery from phosphate minerals and secondary resources, further research is necessary to fully understand how biofilm, particularly EPS, affects bioleaching.

Keywords: Rare earth elements, bioleaching, phosphate-solubilising microorganisms, complexolysis, organic acids, ToF-SIMS, STEM-XEDS, biofilm engineering.