Microbial Systems for Sustainable Resource Recovery

Denys Villa Gomez[1,2,3], Rosie Gillane[1,2,3], Luke Webster[1,2,3], Fernanda Soto[1,2,3] and Esteban Marcellin[1,3]

[1] *ARC Centre of Excellence in Synthetic Biology (CoESB), The University of Queensland, Brisbane, Queensland, Australia*

[2] *School of Civil Engineering, The University of Queensland, 4072 QLD, Australia*

[3] *Australian Institute for Bioengineering and Nanotechnology, The University of Queensland, 4072, QLD, Australia*

Abstract

The mining industry supplies critical metals essential for the net-zero transition but generates challenging alkaline wastes like red-mud tailings. SynBiomining develops modular biotechnologies that integrate microbiome discovery, extremophile bioleaching, and protein-based biorecovery to selectively extract metals while neutralising residues. High-throughput omics and validation workflows establish reusable pipelines that accelerate bioprocess design across diverse mine wastes. This framework enhances circularity in mining, transforming liabilities into critical-metal resources and minimising environmental impacts.

Keywords: Critical metal recovery, mine waste, extremophile isolation, fungal bioleaching, metal-binding proteins

Introduction

The mining industry occupies a paradoxical position in the global drive toward net-zero emissions: it is simultaneously a major source of greenhouse gases and a critical supplier of the metals required for electrification, renewable energy and advanced manufacturing (Lord, 2019). Meeting rapidly growing demand for critical and strategic elements such as rare earth elements (REEs), nickel and cobalt while reducing environmental impacts is a formidable challenge. Conventional hydrometallurgical and pyrometallurgical routes for metal production are energy-intensive, chemically aggressive and often poorly suited to low-grade ores, complex residues and legacy mine wastes. At the same time, mine waters and solid wastes frequently contain significant inventories of dissolved or mineral-bound metals that remain under-utilised, representing both a contamination risk and a lost resource.

Biotechnological approaches offer an attractive alternative by harnessing the metabolic capabilities of microorganisms to mobilise, transform and recover metals under comparatively mild conditions (Nancharaiah *et al.*, 2016). Classical biomining has already demonstrated the viability of microbially assisted leaching at industrial scale, particularly for sulfide ores and base metals (Kaksonen *et al.*, 2020). However, these applications have remained largely focused on a narrow set of autotrophic acidophiles and have not been broadly extended to the recovery of many critical metals or to more diverse, heterotrophic and extremotolerant microbial communities. This constrains the applicability of biomining to other waste streams with critical-metal recovery potential such as red-mud tailings, alkaline tailings and complex mine waters.

In response to these challenges, we are advancing biomining through a framework that combines microbiome characterisation, omics-driven discovery and high-throughput screening with targeted bioprocess development. Within the UQ Biosustainability Hub, our work focuses on two key application areas, bioleaching and biorecovery, and uses genome- and proteome-resolved microbiome analyses to identify candidate organisms, pathways and metal-binding functions from mining environments.

Extremotolerant fungi are developed for bioleaching of critical metals from alkaline and saline wastes, while proteomic mining of metal-resistant strains yields metal-binding proteins and biomaterials for selective recovery from leachates. Synthetic biology provides a forward-looking layer to enhance these systems by improving strain performance and tailoring binding specificity (Capeness and Horsfall, 2020), while the present study demonstrates how omics and high-throughput tools can accelerate the development and optimisation of biomining processes across these two domains.

The aim of this paper is to demonstrate how microbiome-informed, omics-driven and high-throughput techniques can be used to build and refine biomining pipelines for critical-metal recovery (Figure 1). By showing how these tools guide the development of extremotolerant fungal bioleaching systems and protein-based biorecovery platforms, we highlight a methodology for accelerating bioprocess design rather than a single, fixed process configuration. In doing so, this work outlines how SynBiomining (Synthetic biology and mining) approaches can expand biomining beyond classical organisms and commodities, contribute to circular-economy strategies that transform mine wastes into sources of critical metals while reducing their environmental footprint.

Materials and Methods

Microbiome characterisation. Metagenomic sequencing was used to characterise microbial communities associated with red-mud tailings and related mine wastes and to identify candidate taxa, functions and strains. Approximately 250–500 mg

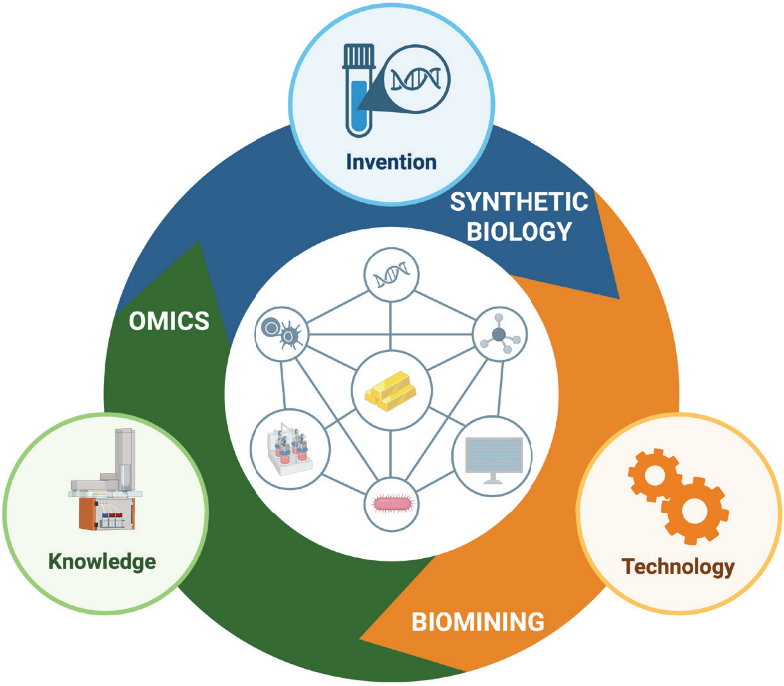

Figure 1 *Conceptual overview of the SynBiomining framework, highlighting how omics driven knowledge generation, synthetic biology enabled invention, and biomining technology are coupled in a cyclical design build test learn loop to recover critical metals from mine wastes. The central panel depicts engineered microbial systems and process units connected in a network around a critical metal product, while the surrounding colored segments emphasize the integration of (i) omics platforms that generate mechanistic insight and design rules, (ii) synthetic biology tools that create and optimise new biomining functions, and (iii) biomining technologies that implement and validate these functions at process scale.*

of material per sample was submitted for DNA extraction and shotgun sequencing, followed by high-stringency quality control, assembly, binning and taxonomic assignment to obtain medium- to high-quality metagenome-assembled genomes and a non-redundant protein catalogue annotated with DRAM. Community profiles derived from MAGs and single-copy marker genes resolved bacterial and archaeal lineages associated with metal resistance, organic-acid production and extracellular polymer formation, providing a reusable template for microbiome-informed strain and target selection in other mining residues. In parallel, microorganisms were isolated from red-mud tailings on a suite of alkaline and general media, purified, and identified by 16S rRNA gene amplicon sequencing; a subset of unique isolates was further subjected to PacBio whole-genome sequencing and functional annotation.

Fungal bioleaching of critical metals. *Aspergillus niger* (DSM 2466) and *Penicillium oxalicum* (DSM 898) were used to develop an extremotolerant bioleaching pipeline for red-mud tailings that can be adapted to other alkaline wastes. Initial screening assessed pH (7–12), salinity (5–20% w/v NaCl) and red-mud tolerance (2–40% w/v) on modified Vogel's minimal medium (1.5% sucrose, 1.5% agar). Both strains showed high neutralisation capacity, decreasing pH by 0.6–2.7 units (*A. niger*) and 0.2–0.9 units (*P. oxalicum*), and prior work showed that their organic acids outperform mineral acids for V and REE recovery from red mud (Soto-Montandon, 2025). To extend tolerance, fungal strains were subjected to adaptive laboratory evolution by chemical and physical mutagenesis (EMS at 75, 150, 300 µg/mL; UV exposure for 10, 15, 20 min) and then adaptively passaged to increasing red-mud loadings.

Discovery and testing of metal-binding proteins. To establish a discovery pipeline for metal-binding proteins that can be used to extract critical metals from mine influenced waters and leachates, we applied a common metal-induced proteomics, REE-IMAC enrichment and bioinformatics workflow across biomining organisms (*Pseudomonas putida, Geobacter metallireducens, Shewanella oneidensis, A. niger, P. oxalicum*). Metal-tolerance ranges were first determined in microplate cultures in defined minimal media, and sub-inhibitory exposure concentrations, for example 100 µM La and Dy, 2 µM Ga, 2000 µM Ge for *P. putida* were selected for proteomic analysis (Webster *et al.*, 2025). Triplicate shake-flask cultures were grown to mid-exponential phase, harvested, and cell lysates passed through a custom rare-earth–immobilised metal affinity chromatography (REE-IMAC) column to enrich putative metal-binding proteins before S-Trap™ digestion and Orbitrap Exploris 480 LC–MS analysis against species-specific UniProt reference proteomes. Differentially abundant and/or REE-enriched proteins were identified at false discovery rate <5% and $|\log_2$ fold-change$| > 0.58$ (~1.5-fold). A composite metal-binding protein database was built from UniProt, the Metal-binding Protein Atlas and MetalPDB, and each proteome was aligned against it, integrating metal-binding motifs, subcellular localisation (PSORTb, CELLO, UniProt) and domain information (InterProScan). Cross-referencing these annotations with the REE-IMAC-enriched proteomics in R yielded a short list of candidate metal-binding scaffolds that can be carried forward to functional testing for a range of leachates and residues, not only the systems studied here.

Results and Discussion

Native extremophile pipelines from red mud microbiomes

Across red mud tailings, over 1,000 colonies were screened, with 60 isolates subjected to 16S whole operon identification and 19 to whole genome sequencing, yielding 18 assembled genomes with functional and secondary metabolite annotations. Key isolates included *Priestia megaterium*, *Halomonas spp.*, *Aliidiomarina haloalkalitolerans*, *Nesterenkonia salmonea* and *Bacillus spp.*, several of which may represent novel taxa. Growth and tolerance tests showed that strains affiliated with *Halomonas*, *Bacillus* and *Nesterenkonia* maintained colony formation even at 15% red mud loading and under elevated Na^+, Fe^{2+} and lanthanide concentrations. *Halomonas sp.* (RG49) in particular displayed consistent growth across ionic stresses, whereas *Bacillus subtilis* responses were more variable, especially in the presence of lanthanides. Although mechanisms were not resolved here, these patterns are consistent with haloalkaliphilic strategies and demonstrate that red mud tailings harbour native, extremotolerant communities. From a pipeline perspective, this microbiome work establishes a strain and genome resource that can be reused as chassis or community members for future bioleaching and biorecovery processes in mine waste streams.

Scalable fungal bioleaching for alkaline residues

A. niger and *P. oxalicum* bioleaching agents for red mud tailings were adapted through mutagenesis and stepwise exposure to increasing red mud loadings to further improved growth, tolerance to red mud tailings and capacity to produce organic acids that both decreased pH and complex REE. Both fungal strains were capable to decrease the pH and to improve tolerance to red mud, but more importantly, they allowed the creation of a protocol using high throughput tolerance screens and iteratively improving strains for operation on chemically challenging residues, with output solutions tailored for downstream selective recovery.

High-throughput metal-binding protein discovery

A biorecovery pipeline was established based on metal induced proteomics, REE IMAC enrichment and informatics across P. putida, G. metallireducens, S. oneidensis, A. niger and P. oxalicum. Across La, Dy, Ga and Ge exposures, 434 proteins showed significant abundance changes, with La and Dy sharing 125 differentially expressed proteins and strong up regulation of periplasmic cation ABC transporters and TonB dependent receptors, traits consistent with enhanced metal trafficking at the cell surface. Integration of these data with a curated metal binding database indicated that ~300 responsive proteins carried predicted metal binding functions, many located in membranes or periplasm and therefore well positioned for biosorption or transport. Gene set enrichment analysis showed, for example, that 78% of predicted Ni binding proteins were enriched under Dy exposure, pointing to overlapping machinery for lanthanide and transition metal handling that could be co-opted for critical metal recovery. Cloning of the most promising candidates will allow to test these proteins for biorecovery of REE, Ni, Co and other critical metals. These outcomes illustrate how the pipeline moves from broad proteomic reprogramming to a short, rationally selected list of protein scaffolds ready for engineering and deployment.

Conclusions

This study demonstrates modular, transferable pipelines for recovering critical metals from mine wastes. These results show how microbiome informed strain discovery, extremotolerant fungal bioleaching, and protein based biorecovery are being developed as modular, reusable pipelines. Each module is demonstrated on red mud tailings and selected mine water chemistries but is explicitly structured so it can be transferred to other residues, metals and sites as the SynBiomining program expands.

References

Capeness, M.J., Horsfall, L.E., 2020. Synthetic biology approaches towards the recycling of metals from the environment. Biochemical Society Transactions 48(4), 1367-1378.

Kaksonen, A.H., Deng, X., Bohu, T., Zea, L., Khaleque, H.N., Gumulya, Y., Boxall, N.J., Morris, C., Cheng, K.Y., 2020. Prospective directions for biohydrometallurgy. Hydrometallurgy 195, 105376.

Lord, M., 2019. From mining to making - Australia's future in zero-emissions metal, Energy Transition Hub. The University of Melbourne.

Nancharaiah, Y.V., Mohan, S.V., Lens, P.N., 2016. Biological and bioelectrochemical recovery of critical and scarce metals. Trends in Biotechnology 34(2), 137-155.

Soto-Montandon, F., Webster, Luke, Gillane, Rosemary, Marcellin, Esteban, Harrison, Susan T. L., and Villa-Gomez, Denys, 2025. Valorisation of bauxite residue for critical metal recovery using fungal bioleaching: a pathway toward circular economy., Global Bioeconomy Alliance Annual Conference 2025. Copenhagen, Denmark, p. 45.

Webster, L.J., Palfreyman, R., Gillane, R., Soto, F., Marcellin, E., Villa-Gomez, D., 2025. Protein Identification and Binding Capacity of Pseudomonas putida to Critical Metals, in: Metallurgy, Materials Society of, C.I.M. (Eds.), Proceedings of the 63rd Conference of Metallurgists, COM 2024. Springer Nature Switzerland, Cham, pp. 1185-1188.