

Bioremediation of Acid and Metalliferous Drainage using Sulfate-Reducing Bacteria

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Abstract

This study examined acid-tolerant sulfate-reducing bacteria (SRB)-based AMD treatment using an inoculum cultured from sediment exposed to a pH 3.5 AMD stream at the Mt. Morgan gold and copper legacy mine site in Central Queensland, Australia. The SRB-consortium was enriched in a selective growth medium and inoculated into a continuously running anaerobic-bioreactor operated for 290 days at 33 hours hydraulic retention time with gradually increased synthetic AMD load. Ethanol was used as the carbon source at a 0.67 COD:sulfate ratio by mass and 2–4 mm size porous beads were added for SRB colonization. Following bioreactor stabilisation, the effluent analysis showed Desulfobacterota as the most abundant bacterial phylum (almost 45% relative abundance) in the bioreactor system. Operation at 0.2 mL/min synthetic AMD feed achieved a maximum sulfate removal of 71% accompanied by acetate and sulfide production (600 mg/L sulfide). Increasing COD:sulfate ratio to 1 at 0.5 mL/min synthetic AMD feed resulted in enhanced sulfate removal and a pH increased from 5.7 to 6.5. Moreover, dissolved Cu, Fe, and Zn in the synthetic AMD were fully removed as metal sulfides, highlighting the potential for both AMD remediation and metal recovery valorisation.

Keywords: Acid Metalliferous Drainage (AMD), bioremediation, sulfate-reducing bacteria

Introduction

Acid and Metalliferous Drainage (AMD) remains an enduring challenge for the mining sector. A study by Werner *et al.* (2020) estimated that Australia has approximately 80,000 abandoned mines, and around 2,825 of them are located in Queensland. One of the mine sites with AMD issue in Queensland is the Mount Morgan gold and copper legacy mine site in Central Queensland (Kaur *et al.* 2018). Mining operations at the site ceased in 1990 and remediation efforts applied at the historic mine site including operation of a lime-dosing water treatment plant

(Queensland Government, 2021). Despite the state government efforts, environmental concerns related to AMD generated from the site persist until today.

Limestone or lime addition is among the most widely used AMD treatment methods. While effective in neutralizing acidity and precipitating most metals, it generates chemical sludge requiring further management (Villa Gomez *et al.* 2023) and contributes to greenhouse gas emissions through limestone production and CO₂ release during the acid-neutralization process (Iizuka *et al.* 2022). Nature-based treatments,



such as biologically mediated sulfate reduction, offer advantages over conventional alkaline methods, including lower CO₂ footprint and maintenance cost (Kim *et al.* 2025), as well as the potential for sustained sulfate reduction and metal recovery through metal sulfide formation (Liu *et al.* 2021). Extensive research on sulfate-reducing bacteria (SRB)-mediated AMD treatment have been conducted with focus on non-native or model SRB strains to mediate AMD bioremediation. However, comparatively little attention has been given to the potential of indigenous, in situ microbial communities to drive AMD bioremediation. Investigating these naturally adapted communities could provide significant advantages, as their intrinsic tolerance to acidic and metal-rich environments may reduce the need for pH buffering and enable sustained bioprocess performance under variable AMD loading conditions.

This study aims to evaluate the potential of indigenous, acid-tolerant SRB to sustain sulfate reduction and metal recovery under variable carbon, acidity, and metal loading conditions typical of AMD systems.

Methods

Study Location and Sample Collection.

The Mt. Morgan site is a legacy gold and copper mine located 35 km southwest

of Rockhampton in Central Queensland (Queensland Government, 2021). Water, sediment, and biofilm samples were collected in March 2023 and June 2024 from the Mine Pit, Frog Hollow Tailing Dump, Linda Gully, and Dee River Downstream (Figure 1). AMD collected from Frog Hollow Tailing Dump was used to prepare synthetic AMD for this study.

Enrichment of Sulfate Reducing Bacterial Consortium

One (1) mg of sediment sample collected from underneath a pH 3.5 AMD stream at Frog Hollow Outlet was inoculated into enrichment media consist of 6 mL/L lactate (60% w/w), 10 g/L tryptone, 1 g/L yeast, 2 g/L MgSO₄·7H₂O, 0.5 g/L FeSO₄·7H₂O, and 0.75 g/L ascorbic acid to enrich sulfate reducing bacterial consortium.

Experimental Set-Up

The enriched SRB culture was used as inoculum in a 2L working volume anaerobic bioreactor supplemented with ethanol as a carbon source (indicated as Chemical Oxygen Demand/COD loading) and 2–4 mm Poraver® porous beads for SRB colonization. The experiment was operated for 290 days at 33-hour hydraulic retention time (HRT) and 1 mL/min inlet flow rate. The reactor inlet solution was growth media consisting of 0.5 g/L KH₂PO₄, 0.2 g/L NH₄Cl, 2.5 g/L

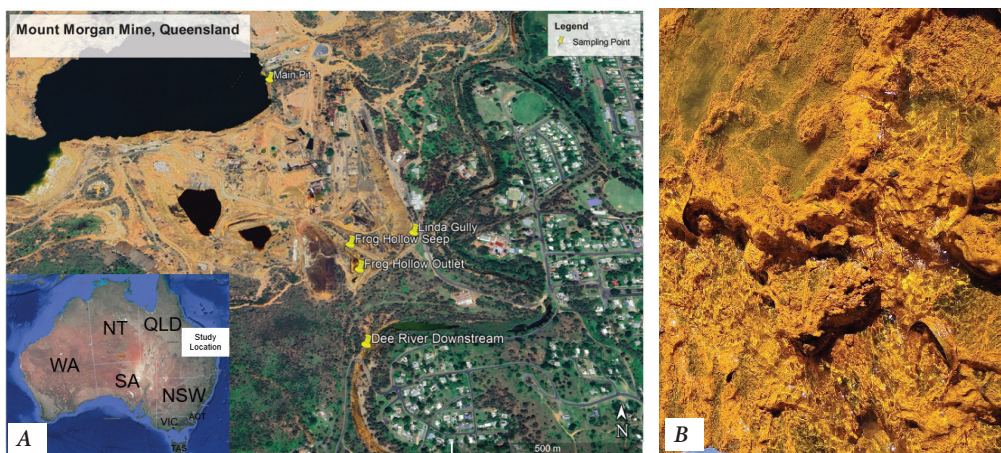


Figure 1 Mt. Morgan gold and copper mine site sampling locations: (A) Frog Hollow Seep, Frog Hollow Outlet, Main Pit, Linda Gully, and Dee River Downstream. Source: Mt. Morgan Ltd. (Queensland Government Department of Resources, 2023), (B) Frog Hollow Outlet AMD stream SRB inoculum collection point.



CaCl₂·2H₂O, 2.5 g/L MgSO₄·7H₂O, 1.51 g/L FeSO₄·7H₂O, and 0.75 g/L ascorbic acid. The inlet solution was gradually transitioned from 100% growth media to increasing load of synthetic AMD, applied sequentially as 0.2, 0.5, and 1 mL/min AMD, with the remainder of each mixture consisting of growth media. The acidity and metal concentration in the bioreactor system were gradually increased along with increasing synthetic AMD in the feed solution over the experiment (Table 1). The synthetic AMD consisted of 1.5 g/L CaSO₄·2H₂O, 0.3 g/L CuSO₄·5H₂O, 5 g/L MgSO₄·7H₂O, 0.4 g/L Na₂SO₄, 6.8 g/L Al₂K(SO₄)₂·12H₂O, 3.7 FeSO₄·7H₂O, 0.3 g/L MnSO₄·H₂O, and 0.1 g/L ZnSO₄·7H₂O adjusted to pH 3.5 with 0.5M H₂SO₄.

Chemistry and Electron Microscopy Analysis

Triplicate influent and effluent samples were collected for pH, metals, ethanol, acetic acid, sulfate, sulfide and total organic carbon (TOC) analyses. pH values were measured using an Eutech Instruments pH 700 pH meter. Metal samples were filtered using 0.2 µm syringe filters, then analysed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) analysis using Thermo Fisher iCAP PRO XP Duo. Ethanol and acetic acid analysis was conducted using gas chromatography system with Agilent

Technologies 7890A. Sulfate concentrations were measured using Dionex ICS 2000 IC. Sulfide concentrations were determined using colorimetric method as described by Cord-Ruwisch (1985). TOC and bicarbonate (measured as total inorganic carbon) was determined using the difference method on a Shimadzu TOC-L analyzer Inoculated porous beads were fixed with 2.5% glutaraldehyde and dehydrated and dried according to Jones *et al.* (2023), and analysed using a Secondary Electron Microscopy Energy (SEM) using a JEOLJSM – 7800F.

Molecular Identification using Amplicon Sequencing

Biomass pellets from the SRB enrichment culture and bioreactor effluent were submitted for DNA extraction and amplicon sequencing at the Australian Centre for Ecogenomics employing Polymerase Chain Reaction (PCR) amplification of 16S rRNA gene using universal primers (Jones *et al.*, 2023). The resulting raw DNA sequences were then processed using MOTHUR software version 1.48.0 (Schloss *et al.*, 2009) following the pipeline reported in Gagen *et al.* (2018).

Results and Discussion

SEM analysis showed extensive microbial colonization and growth within the porous beads, indicating that the beads matrix

Table 1 SRB bioreactor operating conditions.

Phase	Day of Experiment	Inlet pH	COD loading (g/L)	Sulfate loading (g/L)	COD:sulfate	Synthetic AMD flow rate (mL/m)	Note
I	0-7	7.0	1	1.4	0.67	0	Batch
II	8-56	7.0	1	1.4	0.67	0	Continuous
III	57-70	6.0	1	1.4	0.67	0	Continuous
IV	71-114	7.0	1	1.4	0.67	0	Continuous
V	115-134	7.0	1.5	2.0	0.67	0	Continuous
VI	135-156	6.7	1.5	2.0	0.67	0	Continuous
VII	157-184	6.4	2.3	3.4	0.67	0.2	Continuous
VIII	185-194	6.4	1.6	1.6	1	0.2	Continuous
IX	195-215	5.8	4.1	4.1	1	0.5	Continuous
X	216-243	5.8	6.1	4.1	1.5	0.5	Continuous
XI	244-268	5.8	4.1	4.1	1	0.5	Continuous
XII	269-290	3.6	8.1	8.1	1	1	Continuous



provides effective attachment surface and protected microenvironment for biofilm development. This condition likely contributed to the SRB community ability to maintain their dominance under acidic AMD conditions throughout the long-term bioreactor operation (Figure 2). Microbial community development was further confirmed through 16S rRNA sequencing (Figure 3), which showed that microbial diversity grew from predominantly sulfate-reducing bacteria to including fermentative bacteria over time, consistent with an AMD environment. After more than 100 days of bioreactor operation, *Desulfobacterota*, the incomplete carbon-oxidizing SRB (Dyksma & Pester, 2024), thrive and became the most abundant microbial group in the system. Their dominance was consistent with the observed incomplete ethanol oxidation to acetic acid in the system.

The bioreactor stabilized after approximately 70 days of operation (stage I-III), marking the transition from SRB community acclimation to a steady sulfate-reduction system (stage IV-IX). During 200 days of bioreactor operation, SRB metabolic activity generated bicarbonates that buffered the system and maintained neutral pH (Figure 3). A stress-response phase was evident during stages X-XII. After stage IX (0.5 mL/min synthetic AMD), increased acidity and dissolved metal concentrations likely inhibited SRB metabolic activity, reducing bicarbonate production, and driving the effluent pH below 6. Increasing the COD:sulfate ratio in stage X did not restore the system performance, demonstrating that increasing the carbon load alone is insufficient to overcome inhibition caused by acidity and metal stress. Therefore, the COD:sulfate ratio was returned to the prior level in stage XI. At the highest synthetic AMD loading tested (stage XII, 1 mL/min synthetic AMD), effluent pH was higher than the influent pH, suggesting an improved SRB metabolic activity due to reduced H₂S toxicity as a result of increased metal loading that consumed sulfide through precipitation.

Despite the stress response observed during stage X-XII, operation at stage IX

with 0.5 mL/min synthetic AMD flow rate and COD:sulfate ratio 1 enhanced sulfate removal, peaking at 1.3 g sulfate removed/L/day (Figure 4). This performance is comparable to sulfate removal rates reported in recent studies with similar AMD inflow rate, lower sulfate loading, and shorter experimental durations (Hernandez *et al.* 2022; Liu *et al.* 2021). Metal analysis under optimum bioreactor performance (stage 9) showed complete removal of dissolved iron, aluminium, copper, and zinc, suggesting effective precipitation as metal sulfides (Figure 5). In contrast, the SRB bioreactor removed only about half of the dissolved manganese, consistent with the relatively high solubility of Mn compared with metal sulfides such as FeS, ZnS, and CuS, (Clark *et al.* 1998). Overall, these results demonstrated the potential of the indigenous, acid-tolerant SRB adapted to high acidity and metal concentrations for site-specific AMD remediation, while also indicating the opportunity to further system performance optimization through SRB community amendments and carbon loading control. While the experiment was conducted at a maximum synthetic AMD rate of

1 mL/min under laboratory conditions, practical applications would require scaling to much higher flow rates (e.g., on the order of m³/s), potentially through multi-unit operation or reactor redesign.

Conclusions

This study demonstrated that indigenous, acid-tolerant SRB can be successfully enriched and sustain sulfate reduction and metal recovery within a bioreactor system under prolonged and variable carbon, acid, and metal loading conditions to treat AMD. The findings also highlight opportunities for performance optimization through microbial community diversity amendments, reduced AMD loading, and improved control of COD:sulfate ratio and sulfide levels. Effective removal of iron, aluminium, copper, and zinc demonstrated sulfide-driven metal recovery, while incomplete manganese removal indicated the need for staged or integrated treatment process for comprehensive metal recovery.

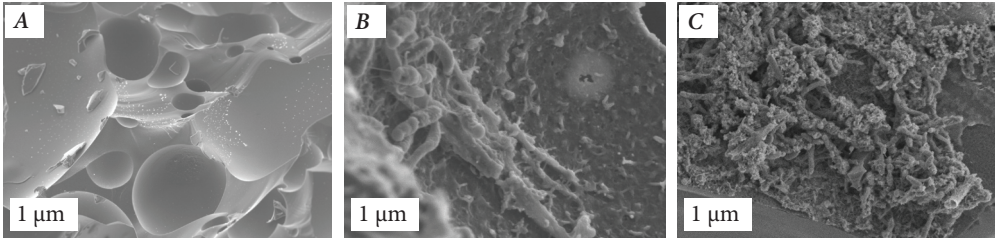


Figure 2 Secondary images of porous beads from (A) day 0, (B) 71, and (C) 290 of SRB bioreactor operation showing development of microbial community in the system.

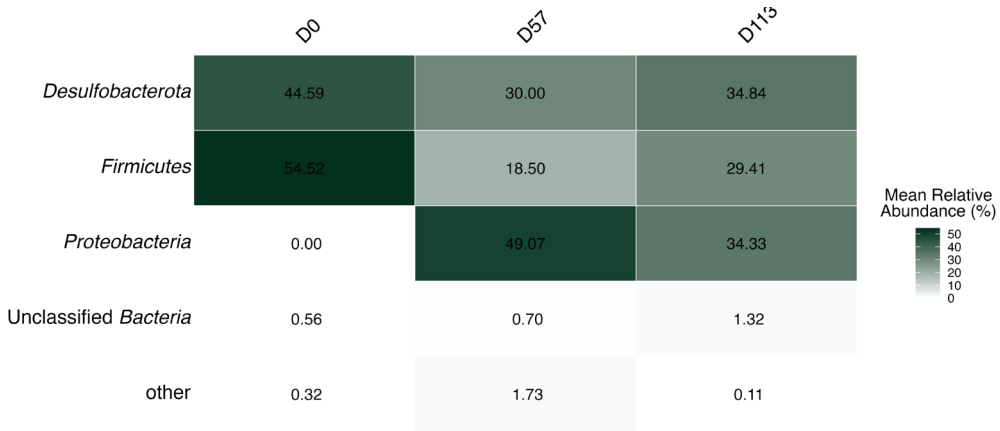


Figure 3 Microbial community composition at phylum level in the bioreactor system at day 0, 57, and 113 shows *Desulfobacterota* as the most abundant phyla in the bioreactor system.

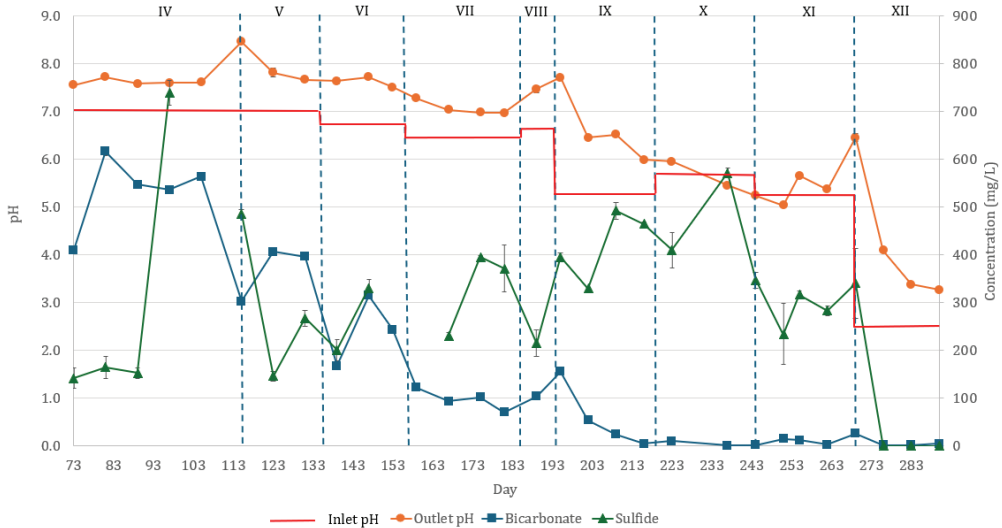


Figure 4 Bicarbonate production influence on bioreactor effluent pH after bioreactor system stabilization.

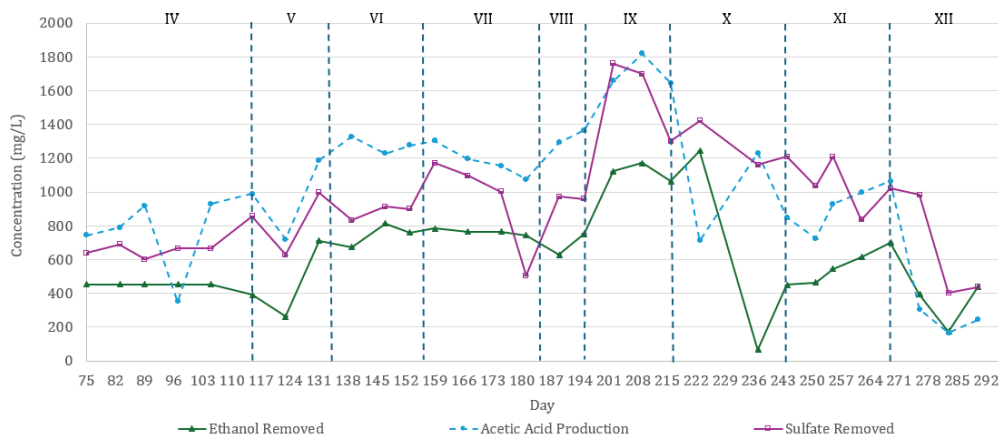


Figure 5 Bioreactor system dynamic over experiment time after bioreactor system stabilization showing acetic acid production as by product of incomplete ethanol oxidation. The chemical parameters dynamic also shows that increased in ethanol loading did not always correspond to sulfate removal improvements.

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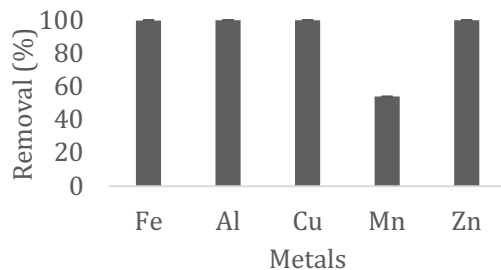


Figure 6 Metals removal efficiency at 0.5 mL/min AMD highlight effectiveness of SRB bioreactor system in recovering Fe, Al, Cu, and Zn as metal sulfide precipitates.



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