Performance of a Sulfate-Reducing Bioreactor for Sulfate Removal under Cold Climate Conditions

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Abstract

Mining activities can elevate sulfate levels in freshwater recipients, prompting stricter discharge limits in Sweden and increasing the need for effective treatment of mine leachate. This study evaluates a pilot-scale sulfate-reducing bioreactor operated under subarctic conditions at the Kiruna mine, examining the effects of temperature, flow rate, and lactate dosing. The woodchip-filled bioreactor achieved up to 88% sulfate removal, with performance strongly driven by lactate availability and enhanced at higher temperatures. Initial DOC release, pipe fouling, and high nitrate levels influenced system behavior. Overall, sulfate-reducing bioreactors show promising potential for sulfate removal in cold climates when properly optimized.

Keywords: Biological sulfate reduction, bioreactor efficiency, field operation, temperature, woodchips

Introduction

In mining areas, waste materials that contain sulfide or sulfate-rich minerals may, under certain environmental conditions, release sulfate (SO_4^{2-}) into nearby freshwater systems. Long-term exposure to elevated sulfate levels can be harmful to freshwater organisms (Soucek and Kennedy 2005; Karjalainen *et al.* 2023). Additionally, rising sulfate concentrations can indirectly impact aquatic ecosystems by enhancing phosphorus availability and increasing the risk of eutrophication (Lamers *et al.* 1998; Smolders *et al.* 2006; Zak *et al.* 2006; Geurts *et al.* 2009), as well as by stimulating mercury methylation (Han *et al.* 2007; Wang *et al.* 2022). In Sweden, recent environmental court decisions have introduced limits on sulfate discharges to surface waters, creating a need for reliable and efficient removal technologies (Swedish Agency for Marine and Water Management 2018).

One example of a removal technology is the woodchip bioreactor, which has previously been applied in mining settings to remove nitrate through denitrification (Nordström and Herbert 2018; Hellman *et al.* 2024). With appropriate modifications, this type of system could also be utilized for sulfate removal (e.g. Parvage and Herbert 2023). In such a configuration, the initial step for sulfate removal would involve dissimilatory sulfate reduction (reaction 1), carried out by sulfate-reducing bacteria (SRB) under anaerobic conditions. When sufficient organic carbon (CH_2O) is available, these microorganisms reduce sulfate to sulfide (H_2S). The produced sulfide can subsequently be immobilized through different pathways, such as by precipitation with metal ions or chemical or biological oxidation, depending on the desired product (Pudi *et al.* 2022).

$$SO_4^{2-} + 2\,CH_2O \rightarrow H_2S + 2\,HCO^{3-} \qquad (1)$$

This study evaluates the influence of temperature, flow rate and an external carbon source on sulfate removal in an on-site SRB-woodchip treatment system at the Kiruna mine in northern Sweden under subarctic conditions.

Methods

Experimental setup

The treatment system was constructed at LKAB's Kiruna iron ore mine located in northern Sweden (67°85′ N, 20°14′ E) and operated from June 2024 to December 2025. During this period, the hourly atmospheric temperature from a nearby weather station ranged from −28.5 to +28.1 °C with an average of +2.7 °C (Swedish Meteorological and Hydrological Institute (SMHI), n.d.). The treatment consists of four main units: (1) a water reservoir, (2) a sulfate-reducing bioreactor, (3) a sulfide precipitation tank, and (4) a sedimentation tank (Fig. 1).

The water reservoir continuously received outflow from a denitrifying woodchip bioreactor treating mine leachate from a nearby waste rock pile (described in Hellman *et al.*, 2024). The collected water was subsequently pumped at rates of 3.45–9.50 m³/d (Fig. 2) to the SRB bioreactor. Prior to reaching the bioreactor, the mine water was amended with lactate at a dosing rate of 4.5–40 kg/d (Fig. 2); lactate was added as a labile carbon source to enhance microbial sulfate reduction (reaction 2).

$$SO_4^{2-} + 2\,CH_3CH(OH)COO^- \rightarrow H_2S + 2\,CH_3COO^- + 2HCO^{3-} \qquad (2)$$

In addition to lactate dosing, the inlet water was also heated before entering the bioreactor (Fig. 1). A 2000 W inline electrical heater installed in the inlet pipeline was used for this purpose.

The sulfate-reducing bioreactor consisted of a subsurface concrete cylindrical tank (2.4 m diameter × 5.4 m height) filled with pine woodchips (size approximately 30 × 30 × 10 mm). A downward flow direction and a 50 cm water layer on top were implemented to limit sulfide emissions. Sampling wells were installed into the bioreactor for chemical profiling at six depths (A and B, Fig. 1). Three weeks after the start of operations, the woodchips were inoculated by adding 500 L of active sewage sludge (100:1 woodchip: sludge volume ratio) at a high lactate dose (27.2 kg/d) to promote the establishment of a microbial community. Flow rate (3.5–9.5 m³/d), lactate dosing (0–40 kg/d), and inflow temperature (10–25 °C), were systematically varied to assess process responses (Fig. 2).

The effluent from the bioreactor was directed to the precipitation tank (Fig. 1), where sodium hydroxide (NaOH) and iron chloride ($FeCl_3$) were added to

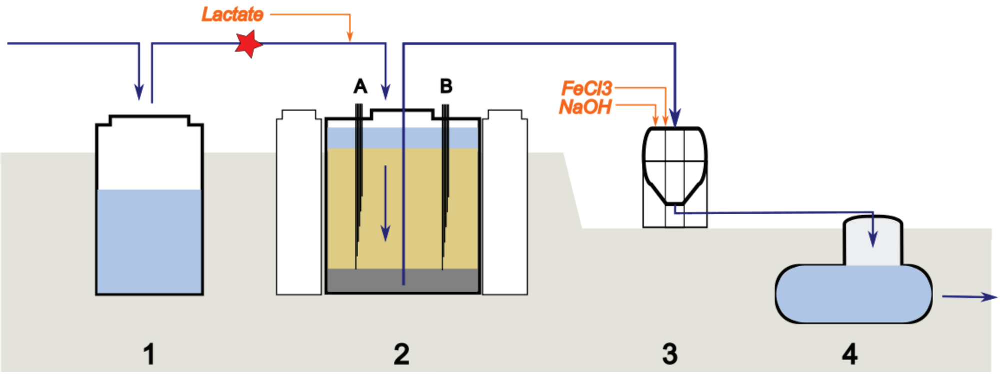

Figure 1 *Cross-section representation of the SRB–woodchip treatment system, consisting of: (1) water reservoir, (2) sulfate-reducing bioreactor including water-sampling wells (A and B), (3) sulfide precipitation tank, and (4) sedimentation tank. The red star indicates the heating apparatus, blue arrows show the flow direction, and orange arrows denote points of chemical addition. The figure is not drawn to scale.*

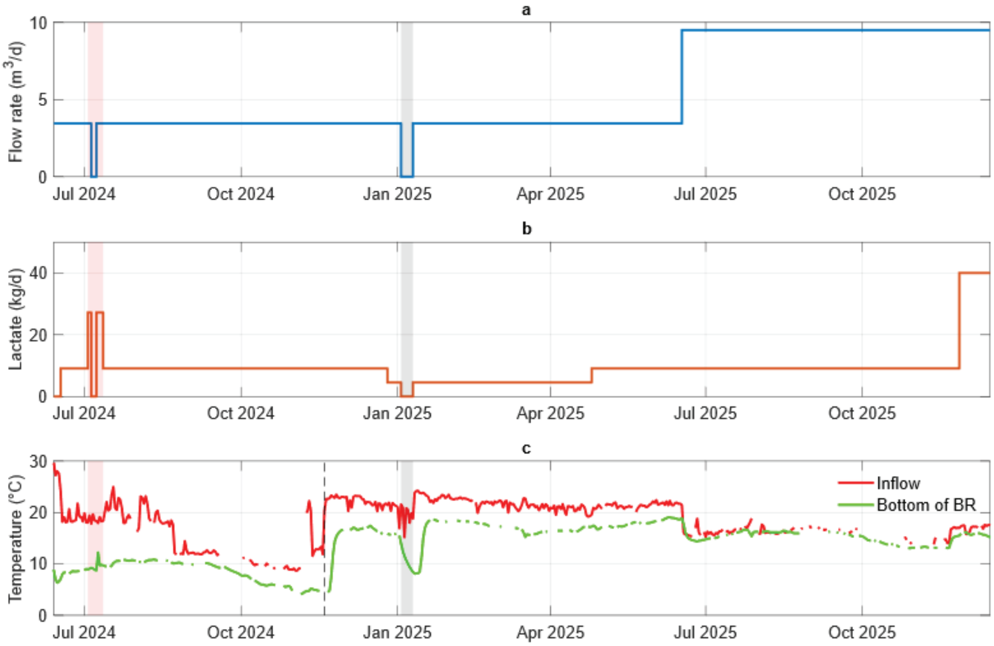

Figure 2 *Flow rate (a), lactate dosing (b), and water temperature (c) of the inflow and the bottom of the SRB bioreactor (BR). The red vertical highlight represents the inoculation period while the grey vertical highlight displays a stop of operation.*

precipitate dissolved sulfide as iron sulfide (FeS) and elemental sulfur (S^0) prior to the sedimentation tank (reactions 3 and 4). The $FeCl^{3-}$ driven precipitation generates acidity, but in the presence of alkalinity (HCO^{3-}), this acidity can be neutralized through the formation of carbonic acid (reaction 4).

$$H_2S + NaOH \rightarrow HS^- + H_2O + Na^+ \qquad (3)$$

$$2\,FeCl_3 + 3\,HS^- + 3\,HCO^{3-} \rightarrow S^0_{(s)} + 2\,FeS_{(s)} + 3\,H_2CO_3 + 6\,Cl^- \qquad (4)$$

Sampling procedure and chemical analysis

Water samples (2 L) were collected weekly by LKAB personnel from the inflow and outflow of the sulfate-reducing bioreactor. For the outflow samples, a $ZnCl_2$ solution (50 mL, 117 mM) was added to precipitate sulfide to ensure safe handling during analysis. All water samples were analyzed by LKAB, which has an accredited laboratory for chemical analyses. Depth profiles of chemical parameters in the bioreactor were obtained during regular sampling campaigns and included analyses of sulfate, sulfide, nitrate, nitrite, and ammonium. Water samples were collected from each sampling well (see Fig. 1) using a peristaltic pump, and approximately 2 L were purged to ensure representative sampling prior to filling 50 mL collection bottles. All analyses were performed within 4 hours of collection to prevent oxidation, using a spectrophotometer (HACH DR1900) in combination with HACH pre-dosed reagent kits.

Sulfate removal was evaluated through the calculation of sulfate removal efficiency based on outlet sulfate sulfur concentrations (C_{out}) relative to their corresponding inlet concentrations (C_{in}) (equation 5).

$$Sulfate\ removal\ efficiency\ (\%) = \frac{C_{in} - C_{out}}{C_{in}} \times 100$$

Results and discussion

During the operational period, the average inflow concentrations were 385 mg/L for SO_4^{2-}–S and 34 mg/L for NO_3^- -N (Tab. 1). The large standard deviations (Tab. 1) were closely linked to seasonal variations.

Table 1 *Mean concentrations at the inflow and outflow of the sulfate-reducing bioreactor. All values are expressed in mg/L, except Al, Cu, Fe, Mn, P, and Zn, which are expressed in µg/L. An asterisk (*) indicates a statistically significant difference ($P < 0.05$) between the median inflow and outflow concentrations, determined using an unpaired Wilcoxon signed-rank test.*

Compound	Inflow	Outflow	Compound	Inflow	Outflow
pH*	7.2 ± 0.2	6.9 ± 0.3	NO_2^- –N*	3.5 ± 2.7	0.5 ± 1.3
Alkalinity*	208.2 ± 42.6	403.3 ± 124.3	NH_4^+ –N*	0.1 ± 0.0	3.7 ± 4.4
DOC*	7.0 ± 19.0	192.3 ± 105.7	Al*	11.1 ± 3.1	6.1 ± 3.9
SO_4^{2-} –S*	384.7 ± 79.6	343.1 ± 123.8	Cu*	1.2 ± 0.7	0.4 ± 0.9
NO_3^- – N*	34.2 ± 19.0	1.2 ± 2.1	Fe*	0.01 ± 0.01	0.08 ± 0.23
Ca	457.8 ± 89.6	453.2 ± 77.9	Mn*	6.2 ± 3.3	13.4 ± 19.6
K	51.4 ± 13.0	51.7 ± 12.0	P	7.8 ± 4.8	8.0 ± 4.3
Mg	51.4 ± 12.8	51.2 ± 12.0	Zn*	22.2 ± 13.5	3.2 ± 10.5

Both SO_4^{2-}–S and NO_3^-–N concentrations decreased following the snowmelt period, which diluted the inflow water (Fig. 3a and c). Similar dilution effects, while not displayed in this article, were observed for major ions (e.g. K, Mg, Ca), whose concentrations did not differ before and after treatment (Tab. 1).

The highest sulfate removal efficiency (88%) was achieved during summer 2025 under low flow (3.45 m³/d), medium lactate dosing (9.1 kg/d), and heating conditions (Fig. 3a). Sulfate concentrations were inversely related to sulfide concentrations, which is consistent with sulfide being produced through sulfate reduction (Fig. 3a and b). At low water inflow, lactate addition played a critical role, with higher dosages corresponding to higher sulfate removal.

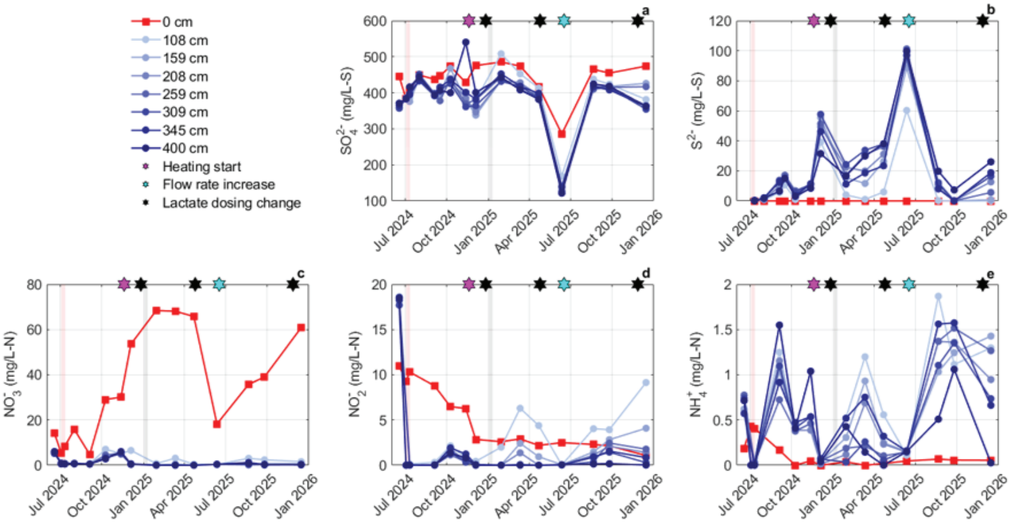

Figure 3 *Change in average A-B wells concentrations with depth in bioreactor for sulfate (a), sulfide (b), nitrate (c), nitrite (d) and ammonium (e). Red squares represent the inflow concentration while blue circles are concentrations at different bioreactor depths (from top light blue to bottom dark blue). The purple star marks the beginning of heating, the blue star indicates a flow increase, and the black stars display main lactate dosage changes (more details in Fig. 2). The red vertical highlight represents the inoculation period while the grey vertical highlight indicates a stop of operation.*

These results indicate that increasing lactate availability directly enhanced sulfate reduction under longer hydraulic retention times. However, when the inflow rate increased, high lactate dosage did not substantially improve sulfate reduction. Whereas low-flow operations provided approximately three days of residence time, increasing the flow reduced this to roughly one day, which likely did not allow sufficient time for sulfate-reducing bacteria to carry out complete reduction.

Despite considerable nitrate loads entering the system, nitrate removal efficiency consistently exceeded 75% throughout the monitoring period. These values indicate strong and stable denitrification performance, highlighting the bioreactor's potential as an effective treatment option for enhanced nitrate removal. Nitrite removal was similarly efficient (Fig. 3d), with low nitrite accumulation (< 10 mg/L–N) observed in the system. In contrast, ammonium concentrations increased within the bioreactor, suggesting net ammonium production. While these values remain low (< 2 mg/L–N), this trend is likely associated with dissimilatory nitrate reduction to ammonium (DNRA). The addition of lactate elevated the C:N ratio, creating conditions favorable for DNRA. Previous studies show that under anaerobic conditions, high availability of electron donors relative to nitrate or nitrite concentration promotes DNRA (Burgin and Hamilton 2007; Parvage and Herbert, 2023).

Additionally, vertical profiling showed that most sulfate and nitrate removal occurred in the upper layers of the bioreactor (Fig. 3), with minimal variation in compound concentrations across the sampling wells. However, at higher flow rates, this variation increased, suggesting a possible shift in removal activity into the deeper layers of the bioreactor. The sulfide precipitation tank achieved complete sulfide removal and maintained a pH of 6.9 ± 0.3.

A significant removal in several trace metals, including Cu, Zn, and Mo, was observed, while Mn, and Fe exhibited net release (Tab. 1). However, the relatively high standard deviation observed for Mn and Fe suggests concentration spikes within the system rather than a continuous release. The removal of selected trace metals is likely attributable to sulfide precipitation, whereas the release of others appears to originate primarily from the woodchips used as the reactive substrate (Lepine *et al.* 2021).

Although the SRB bioreactor was intended to function in a semi-passive manner, it still relied on pumps, chemical inputs, and periodic maintenance, which further complicated operation in an area with limited infrastructure. Continuous carbon dosing was essential to sustain sulfate reduction, reinforcing that the system cannot operate reliably without supplemental electron donors (Bettoni and Herbert 2024). For this reason, adding the carbon source directly within the bioreactor is recommended, as upstream dosing can stimulate biomass growth in the pipes and lead to fouling. In the presence of nitrate, denitrifying bacteria outcompete SRB for the available carbon source. This competition limits the carbon available for SRB and contributes to lower sulfate removal efficiency. Consequently, more effective or complete nitrate removal is required before the bioreactor, which introduces additional operational costs and highlights the need for more sustainable and affordable pre-treatment strategies.

Conclusions

This study demonstrates that SRB-based bioreactors can effectively remove sulfate from carbon-limited mine water under subarctic conditions when key operational parameters (flow rate, lactate dosing, and temperature) are properly optimized. The results provide valuable operational insights for the design and implementation of biological sulfate removal systems in cold mining regions, supporting more sustainable compliance with sulfate discharge standards.

Acknowledgements

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