Influence of Reactive Media on Microbial Sulfate Reduction at Two Distinct Temperatures

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Extended Abstract

Mining activities are a major source of sulfate (SO_4^{2-}) to surrounding freshwater systems, as it commonly results from the oxidation of sulfide minerals found in mine waste. High concentrations can be detrimental for aquatic life and surface water quality criteria on sulfate are starting to be imposed in Sweden (Soucek and Kennedy 2005; Swedish Agency for Marine and Water Management 2018). To meet the quality criteria, mining companies must implement removal strategies. Biotechnologies using sulfate-reducing bacteria (SRB) offer an effective method for removing sulfate by reducing it to hydrogen sulfide (H_2S), which can be precipitated as a solid phase metal sulfide (Runtti *et al.* 2018; Pudi *et al.* 2022). Microbial sulfate reduction is influenced by various key factors including pH, temperature, organic matter availability, microbial competition and other physical factors (Middleton and Lawrence 1977). This study aims to evaluate the effects of different solid reactive media as carbon sources and temperature on microbial sulfate reduction to optimize conditions for enhanced sulfate removal.

In this study, woodchips (WC), woodchips with biochar (BC) and woodchips with potato peels (PP) were selected for column experiments at 5 °C and 15 °C. Woodchips and woodchips-biochar were chosen based on previous experiments at 22 °C showing over 90% sulfate removal with lactate as a carbon source (Parvage and Herbert 2023). Biochar was included due to its high porosity, which supports microbial activity, including sulfate-reducing bacteria (Lehmann *et al.* 2011; Easton *et al.* 2015). To sustain sulfate reduction without external carbon sources, a material that releases substantial dissolved organic carbon (DOC) is needed. Potato peel were selected due to its high DOC release (Kiani *et al.* 2020). The columns (40 cm in length and 10 cm in inner diameter) were run in triplicate for each medium with an upward flow direction of 0.2 mL/min yielding a theoretical hydraulic residence time of 5 days. Prior to the start of the experiment, each medium was inoculated overnight with a mixture of activated sewage sludge (10 mL/100 g material) and inflow water to establish an initial microbial community. The inflow was composed of 10 mM sulfate and calcium, major components of waste rock leachate. After 39 days, lactate ($CH_3CH(OH)COO^-$, 16.5 mM), commonly used as an external carbon source to promote sulfate reduction (Widdel 1988), was added to the inflow solution. This addition would theoretically contribute to a maximum of 82.5% sulfate removal based on stoichiometry (reaction 1). The removal produces acetate (CH_3COO^-) and carbonate alkalinity as bicarbonate (HCO_3^-). The 5 °C experiment concluded after 117 days, while the 15 °C experiment extended to 213 days. In the latter, the effects of a higher lactate concentration (33 mM) added on day 150 and the supplementation of macronutrients (phosphate 5 mM and ammonium 18.7 mM) on day 176 on sulfate removal rates were evaluated.

$$SO_4^{2-} + 2CH_3CH(OH)COO^- = H_2S + 2CH_3COO^- + 2HCO_3^- \quad (1)$$

At the start of the experiment, sulfate concentrations remained relatively unchanged, and no sulfide production was observed (Fig. 1). Following lactate addition, sulfate concentrations decreased in treatments, with BC columns showing the fastest and highest reduction in the first 30 days (approximately 40% sulfate removal at 5 °C and >90% at 15 °C) before gradually declining in efficiency (Fig. 1.B). This suggests that SRB are inhibited by hydrogen sulfide that is likely adsorbed by the biochar material (Janyasuthiwong *et al*. 2016; Kanjanarong *et al*. 2017). WC (Fig. 1.A) and PP (Fig. 1.C) columns displayed a slower increase in sulfate removal, stabilizing at 24% and 55% at 5 °C and 58% and 24% at 15 °C, respectively. Although higher temperature benefited the process in WC and BC columns, PP columns performed better at 5 °C. Other microbial processes can influence sulfate reduction, either by releasing additional carbon sources for SRB or competing for them (Zhang *et al*. 2022), which is currently under investigation. Additionally, excess lactate improved sulfate reduction and it could be further improved by nutrient addition, allowing a maximum of 58%, >90% and 47% sulfate removal for WC, BC and PP respectively.

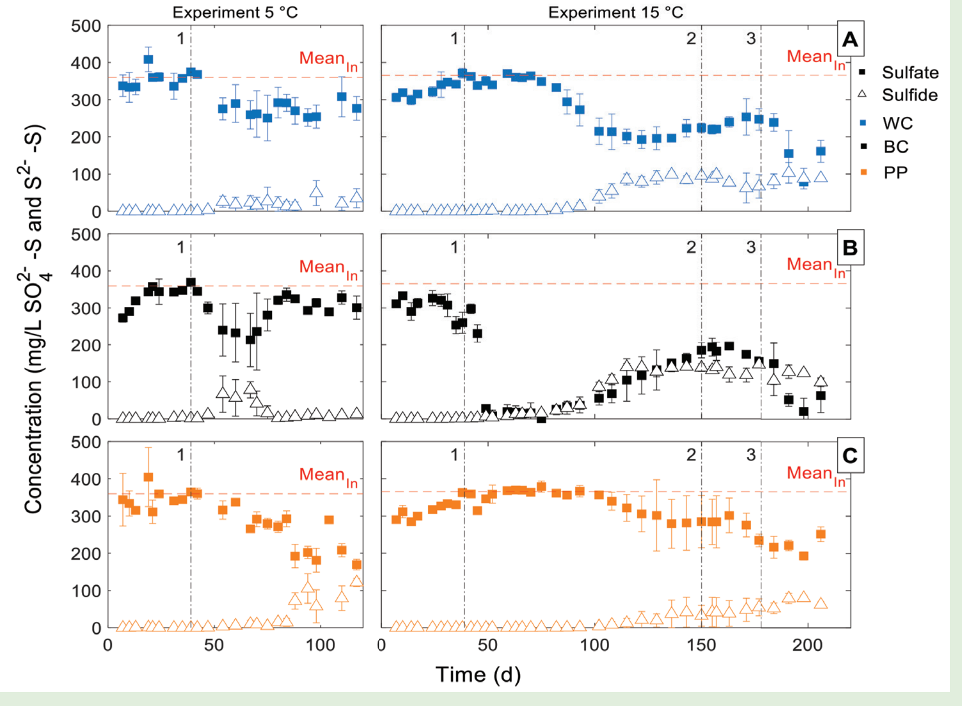

Figure 1 Mean outflow concentrations of sulfate-sulfur (square) and sulfide-sulfur (triangle) from columns containing (A) woodchips, (B) woodchips with biochar and (C) woodchips with potato peels during the experiment at 5 °C (left) and 15 °C (right). The red dashed line denotes the average SO_4^{2-}-S concentration at the inlet. Error bars represent standard deviations on the mean values. The vertical dashed line indicates changes in the inlet composition with (1) lactate addition, (2) double lactate addition and (3) nutrients addition.

This study highlights the significance of both temperature and carbon source selection in optimizing sulfate reduction for mine water treatment. The enhanced sulfate removal observed in BC columns followed by a decline in treatment performance, particularly at higher temperatures, underscores the role of biochar in influencing microbial activity and hydrogen sulfide dynamics. While WC and PP columns exhibited variable performance

across temperatures, the results suggest that microbial competition and carbon source availability play crucial roles in sustaining sulfate reduction. Additionally, the positive effect of lactate and nutrient supplementation reinforces the need for tailored dosing strategies to maximize efficiency. Future research should further explore microbial interactions and long-term stability to refine biotechnological approaches for sulfate removal in mining-affected waters.

Keywords: Biological sulfate reduction, bioreactor efficiency, carbon source, column experiments, temperature effect

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