



Sulfate removal from mine drainage at low temperature: Effects of three reactive porous media on microbial sulfate reduction

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Abstract

Sulfate removal by microbial sulfate reduction can be promoted in anaerobic treatment systems where there is a supply of organic carbon for bacterial growth. This study evaluates sulfate removal using three organic carbon media (woodchips, woodchips with biochar and woodchips with potato peels), with and without supplemental organic carbon (lactate), and at 5 °C. The study highlights the different factors that could have affected sulfate reduction and how it can be implemented in a field-based bioreactor. It concludes that organic carbon media composition and lactate dosing need to be considered when designing a bioremediation system.

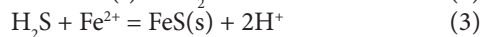
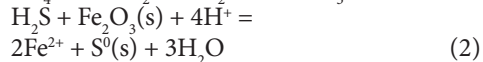
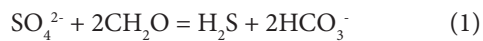
Keywords: Biological sulfate reduction, column experiments, bioreactor efficiency, carbon source, low temperature

Introduction

Sulfate (SO_4^{2-}) is often found at high concentrations in mining waters, as it is commonly derived from the oxidation of sulfide minerals in mine wastes. The chronic effect of SO_4^{2-} can cause serious harm to some freshwater species (Soucek & Kennedy 2005). In addition, elevated SO_4^{2-} concentrations may have an indirect effect on aquatic ecosystems by increasing phosphorus availability and susceptibility to eutrophication (Zak et al. 2006), and by promoting mercury methylation (Han et al. 2007). As a result, the regulation of SO_4^{2-} discharge to water bodies has recently received increased attention in Sweden and new surface water quality criteria have been proposed (Swedish Agency for Marine and Water Management 2018). To be able to meet these criteria in the future, Swedish mining companies will need to implement sulfate treatment technologies.

Several methods have been assessed to remove sulfate from water, spanning from simple (e.g. chemical precipitation as gypsum) to more advanced techniques (e.g. nanofiltration; Runtti et al. 2018). However, active treatment methods can be energy-demanding and therefore costly, such that passive or semi-passive treatment solutions are often preferred for cost reduction (Gadd 2010). Woodchip bioreactors are an example

of passive treatment systems that have been previously used for nitrate removal by denitrification in mining environments (Nordström & Herbert 2018; Nordström et al. 2021), and could potentially be adapted for sulfate removal (e.g. Parvage & Herbert 2023). In such a bioreactor, dissimilatory sulfate reduction (reaction 1) would be used as a first step for sulfate removal. This reaction is driven by sulfate reducing bacteria (SRB) under anaerobic conditions in the presence of an abundance of organic carbon (CH_2O) where sulfate is reduced to sulfide (H_2S) (reaction 1). Sulfide can then be captured with various methods, such as by reacting H_2S with ferric oxide (Fe_2O_3) to precipitate ferrous sulfide (FeS) and elemental sulfur (S^0 , reactions 2 and 3) (Pudi et al. 2022; Parvage & Herbert 2023).



The growth of SRB, and hence the sulfate reduction rate, is controlled by a number of factors, such as temperature and organic carbon availability (Middleton & Lawrence 1977). Sulfate reducing bacteria can tolerate and adapt easily to temperatures spanning

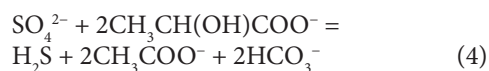
from $-5\text{ }^{\circ}\text{C}$ to $75\text{ }^{\circ}\text{C}$ (Cocos et al. 2002). However, under cold conditions, the microbial metabolic rate tends to decrease (Mara & Horan 2003) resulting in lower reaction rates. Furthermore, the establishment of an SRB community in a bioreactor is dependent on the properties of the supporting media on which the SRB grow (e.g. porosity, surface functional groups, organic carbon content). Porous supporting media are advantageous as they present a high surface area-to-volume ratio, increasing reactivity. The aim of this study was to test different reactive porous media, rich in carbon, for sulfate removal under cold conditions, which later on can be implemented in a full-scale bioreactor for the sustainable and efficient bioremediation of mine water in a Swedish sub-Arctic climate.

Method

Sulfate reduction in the presence of three reactive carbon media (woodchips, woodchips with biochar [volume ratio 1:2] and woodchips with potato peels [volume ratio 1:3]) was investigated using column experiments for 117 days. Woodchips and woodchips with biochar reactive media were selected based on the results of previous column experiments performed under room temperature conditions, which achieved more than 90% sulfate removal after lactate addition as an external carbon source (Parvage & Herbert 2023). Additionally, biochar was selected since it is highly porous (high intragrain porosity) and may host a large community of SRB (Lehmann et al. 2011), and thus has an ability to enhance microbial activity (Easton et al. 2015). However, if sulfate reduction is to be sustained for an extended period of time without the addition of an external carbon source (e.g. lactate), an organic carbon medium that releases substantial amounts of dissolved organic carbon (DOC) is required. One such material is potato peel (Kiani et al. 2020), which mixed with woodchips was selected as the third reactive carbon medium.

Prior to the start of the experiment, each material was inoculated individually overnight with a mixture of activated sewage sludge (10 mL/100 g material) and inlet water, with the purpose of establishing an

initial microbial community. To prepare the inlet water, deionized water was mixed with 10 mM Ca^{2+} (as calcium chloride) and 10 mM SO_4^{2-} (as sodium sulfate), since Ca^{2+} and SO_4^{2-} are major compounds found in the mine drainage. No additional compounds were introduced into the solution to maintain the focus on sulfate reduction. After an initial 39-day period when limited sulfate reduction was observed, 16.5 mM of lactate ($\text{CH}_3\text{CH}(\text{OH})\text{COO}^-$) was added to the system as it is commonly used as an external carbon source promoting sulfate reduction (reaction 4) (Widdel 1988). With lactate as the only electron donor, a maximum of 82.5% sulfate removal can be achieved based on stoichiometry. This reaction produces carbonate alkalinity as bicarbonate (HCO_3^-).



The plexiglas columns used for the experiments were 40 cm in length and 10 cm in inner diameter. They were filled (fig. 1.a), from the bottom to the top, with a layer of glass beads, the reactive media and then sampling bags. A synthetic mesh was placed at the top to prevent clogging. The flow rate was constant ($\approx 0.2\text{ mL/min}$) and yielded a theoretical hydraulic residence time (HRT) of 5 days based on the measured column intergrain porosity (0.58, 0.49 and 0.51 for woodchips, woodchip with biochar, and woodchips with potato peels, respectively). Both the upward flow direction and the glass bead layer aimed to prevent the development of preferential flow paths. Each reactive media was used in triplicate columns and placed into an incubator set to a constant temperature of $5\text{ }^{\circ}\text{C}$ (fig. 1.b).

Throughout the experiment, water samples were collected regularly, on a weekly basis, from all of the column outlets for subsequent analysis of sulfate, sulfide, calcium, nitrate, nitrite, ammonium, pH, alkalinity and total organic carbon (TOC). All chemical compounds were analyzed using spectrophotometry (HACH DR1900) with HACH pre-dosed reagents. All dilutions were made with nitrogen purged deionized water. As sulfide is easily-oxidizable, it should

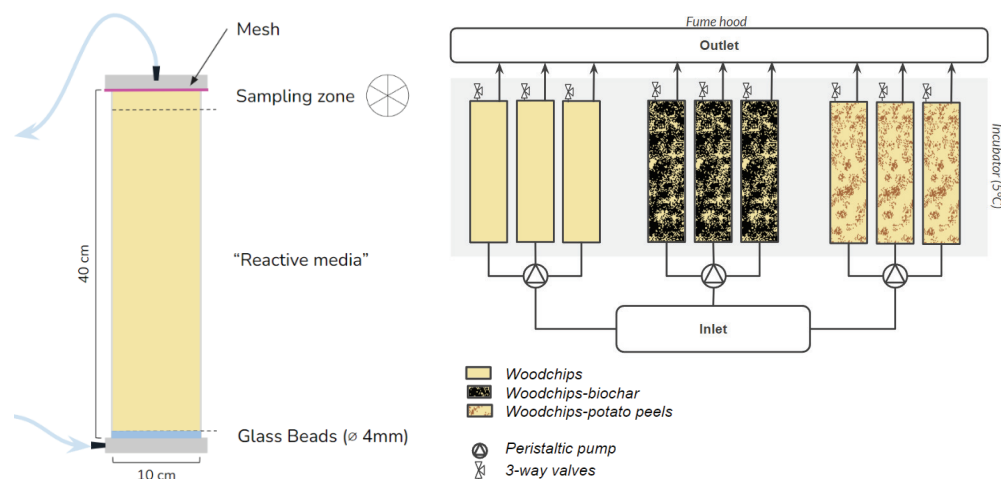


Figure 1 Column design (a) and column experiment set-up (b)

be noted that some oxidation might have occurred during the analysis. The reported sulfide concentrations should therefore be regarded as minimum values. The pH was measured using a pH electrode and the alkalinity was determined by titration with 0.005 M H₂SO₄. TOC was measured on a Shimadzu TOC-L TNM instrument.

Results and discussion

Column experiment performance

During the experiment, the average inflow sulfate concentration was 342±35 mg/L SO₄²⁻-S and the alkalinity did not exceed 10 mg/L CaCO₃. The pH was 5.7±0.5 which is linked to the low buffering capacity of the deionized water used to make the inflow solution (fig. 2). The outflow water quality presented more variability, depending on the reactive media and the experiment phase. In total, three main phases were identified based on several sulfate reduction indicators (sulfate and sulfide concentration, alkalinity, and pH): (1) start-up phase, prior to lactate addition, to investigate if a high degree of sulfate reduction could be achieved without an external carbon source, (2) 30-day period of sulfate reduction following lactate addition and (3) final phase following the 30-day period.

Prior to lactate addition, outflow sulfate concentrations were similar to the inflow mean concentration, regardless of the

reactive media (fig. 2.a, b and c). Additionally, sulfide concentrations were low (< 4.3 mg/L S²⁻-S), there was no measurable production of alkalinity (fig. 2.d), and pH (fig. 2.e) was lower than the pH of the inflow. According to reaction (1), this suggests that sulfate reduction was minimal in the first phase of the experiment.

The introduction of lactate into the system led to measurable sulfate reduction within the first two weeks and a continuous decrease in sulfate concentrations for an approximately 30-day period. Sulfate removal in the lactate-amended woodchip columns (WC) stayed relatively constant (15–24% removal), while sulfate removal in the woodchip-biochar columns (BC) tripled from 12 to 38%. Sulfide production increased up to 26 and 78 mg/L S²⁻-S for WC and BC, respectively. In contrast, sulfate reduction in the woodchip with potato peel columns (PP) showed a slower start with sulfide < 10 mg/L S²⁻-S and only reached a similar reduction rate, relative to the WC and BC columns, toward the end of the period with 22% removal on day 67. Alkalinity and pH reflected this trend with higher values being positively correlated to the columns with a higher degree of sulfate reduction during this specific phase.

From day 70 to 84, there was a period of decreased sulfate reduction with increases

in sulfate concentration and decreases in sulfide concentration, pH and alkalinity for all the columns. Although the precise cause of the decrease in sulfate reduction remains unclear, a plausible hypothesis is that this was associated with a short period of pump malfunction combined with nutrient depletion following the first peak of sulfate reduction. During this period, oxygen was likely introduced into all the columns by a syphoning effect and affected sulfate reduction. After the pump malfunction was corrected, WC rapidly returned to its formal removal values under lactate conditions and BC stabilized around 10% removal. In contrast, PP demonstrated a relatively constant improvement in sulfate removal efficiency and achieved the best results overall with 53% sulfate removal and a sulfide production exceeding 120 mg/L S^{2-} -S. This result suggests that, during the temporary occurrence of oxic conditions in the PP columns, organic matter in the potato peels may have oxidized through aerobic respiration, producing readily available organic carbon for subsequent utilization by SRB once

anaerobic conditions were re-established in the columns. In terms of nutrients, nitrogen concentrations were low in the columns (tab. 1); phosphorus concentrations, while not measured, were likely low as well. The deficiency in nitrogen and phosphorus, in addition to low trace element concentrations, may have constrained bacterial growth due to inadequate nutrient availability after the first peak of sulfate reduction.

Total organic carbon and nitrogen content

During the start-up phase of the experiment, TOC concentrations exceeding 200 mg/L (fig. 2.f) were detected in the column outflows. Although TOC concentrations in the WC and BC column outflows rapidly reached a background concentration of < 45 mg/L, reflecting consumption by sulfate reducing bacteria, TOC concentrations in the PP columns nearly doubled (1134 mg/L) prior to a gradual decline to 72 mg/L on day 42. After lactate addition, which corresponded to a TOC addition of 594 mg/L (i.e. carbon concentration in 16.5 mM lactate), TOC increased for all columns to

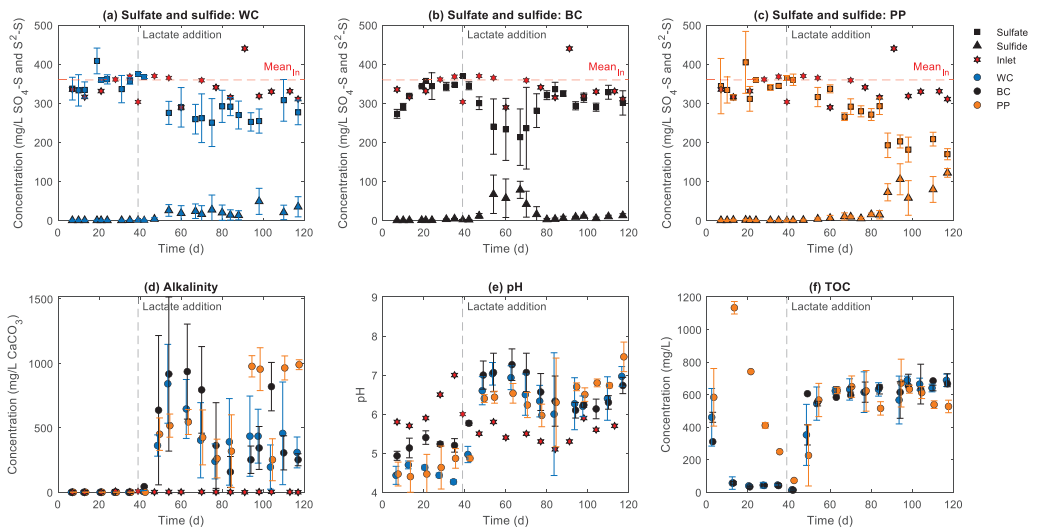


Figure 2 Outflow concentrations of sulfate-sulfur (square) and sulfide-sulfur (triangle) from columns containing WC (woodchips) (a), BC (woodchips with biochar) (b), and PP (woodchips with potato peel) (c). The red dashed line denotes the average SO_4^{2-} -S concentration at the inlet. Outflow alkalinity (d), pH (e), and TOC (f) are depicted for WC (blue), BC (black), and PP (orange). Error bars represent standard errors. Inflow values are marked with red stars, and the vertical dashed line indicates the commencement of lactate addition

an average concentration of 603 mg/L and remained relatively constant. Based on the stoichiometry of reaction 4, a maximum of 82.5% sulfate removal could have been achieved with lactate as the only electron donor. However, none of the reactive media achieved such sulfate reduction (instead, a maximum sulfate removal of 24%, 38% and 53% was observed for WC, BC and PP columns, respectively), compared with the experiments by Parvage & Herbert (2023) that attained >90% removal at 22 °C. This experiment was run at a lower temperature of 5 °C, which suggests that temperature may have limited sulfate reduction kinetics and prevented complete sulfated reduction within the 5-day HRT.

An important factor for the growth of SRB is the presence of nutrients such as nitrogen (N) compounds. During the column experiments, nitrogen compounds were released from the porous reactive media; N concentrations were generally highest in the outflows from the PP columns prior to and after lactate addition (tab. 1), potentially enhancing the activity of sulfate reducing bacteria (SRB) and leading to superior sulfate reduction compared to WC and BC. Aside from nitrogen being an essential nutrient for bacterial growth, some SRB can couple ammonium oxidation with sulfate reduction in a process called sulfammox (Dominika et al. 2021). This

suggests that other microbial processes might have been involved, apart from dissimilatory sulfate reduction, in removing sulfate from solution, but a microbial study is necessary to support this hypothesis.

Field applications

Similarly, to a previous study performed at room temperature (Parvage & Herbert 2023), the column experiments with woodchip-based porous media demonstrated that high concentrations of sulfate removal through microbial sulfate reduction cannot be achieved at low temperature without the addition of a labile carbon source. Assuming a $\text{SO}_4^{2-}\text{-S}$ concentration of 321 mg/L and a water flow of 0.5 L/s in a field-based system, 77 kg of lactate would be consumed each day to achieve a similar degree of sulfate reduction. For variable sulfate concentrations in mine drainage, lactate dosing would need to be regulated using an automated system to avoid under- or overdosing.

In addition to the production of H_2S , which needs to be efficiently captured from treatment system effluents and is not discussed in this study, the discharge of TOC into receiving water bodies from bioreactor treatment could have detrimental effects on aquatic ecosystems. Hence, the selection of porous reactive media is not only relevant for supporting the growth of the SRB community, but also in terms of organic

Table 1 Mean nitrogen concentrations in outflows during the different phase for woodchips (WC), woodchips with biochar (BC) and woodchips with potato peels (PP)

	Nitrate-N mg/L N	Nitrite-N mg/L N	Ammonium-N mg/L N
Phase 1- Prior to lactate addition			
WC	0.34	0.01	0.29
BC	0.23	0.03	0.25
PP	0.68	1.40	*4.28
Phase 2-After lactate addition			
WC	0.59	0.09	0.47
BC	0.63	0.12	0.52
PP	0.43	0.22	*4.12
Phase 3-After lactate addition			
WC	0.86	0.06	0.28
BC	0.38	0.04	0.30
PP	0.81	0.08	0.42

*Ammonium concentration >15 N- mg/L for day 35 and day 42.

carbon leaching and oxygen consumption in water bodies. While the woodchip with potato peels mixture proved to be the most effective porous reactive medium, likely related to the high nutrient and initial carbon content, a post-bioreactor treatment unit would be required for TOC removal. This requirement applies not only after lactate addition but also during the initial start-up phase, especially if this material is chosen for a field-scale treatment system.

Conclusion

To conclude, major reductions of sulfate concentrations did not occur prior to lactate addition, highlighting the importance of labile carbon addition in sulfate removal systems operating at low temperature. However, while the BC columns lost efficiency over time, the WC columns showed relatively constant treatment and the PP columns achieved the highest concentrations of sulfate removal with the highest nitrogen concentrations. This suggests that both lactate addition and the choice of reactive media play a role in the relative amount of sulfate removal. Other parameters such as nutrient concentrations and temperature exert a strong control on long-term treatment and reaction kinetics, respectively. The insights garnered from this study can be applied for the design of a full-scale bioreactor for the sustainable and efficient bioremediation of mine water.

Acknowledgements

This study is part of the SULFREM project, funded by the Swedish Innovation Agency (VINNOVA, project number 2021-04669), Boliden Mineral AB and LKAB.

References

- Cocos, I.A., Zagury, G.J., Clément, B. & Samson, R. (2002). Multiple factor design for reactive mixture selection for use in reactive walls in mine drainage treatment. *Water Research*, 36 (1), 167–177. [https://doi.org/10.1016/S0043-1354\(01\)00238-X](https://doi.org/10.1016/S0043-1354(01)00238-X)
- Dominika, G., Joanna, M. & Jacek, M. (2021). Sulfate reducing ammonium oxidation (SULFAMMOX) process under anaerobic conditions. *Environmental Technology & Innovation*, 22, 101416. <https://doi.org/10.1016/j.eti.2021.101416>
- Easton, Z.M., Rogers, M., Davis, M., Wade, J., Eick, M. & Bock, E. (2015). Mitigation of sulfate reduction and nitrous oxide emission in denitrifying environments with amorphous iron oxide and biochar. *Ecological Engineering*, 82, 605–613. <https://doi.org/10.1016/j.ecoleng.2015.05.008>
- Gadd, G.M. (2010). Metals, minerals and microbes: geomicrobiology and bioremediation. *Microbiology*, 156 (3), 609–643. <https://doi.org/10.1099/mic.0.037143-0>
- Han, S., Obratsova, A., Pretto, P., Choe, K.-Y., Gieskes, J., Deheyn, D.D. & Tebo, B.M. (2007). Biogeochemical factors affecting mercury methylation in sediments of the Venice Lagoon, Italy. *Environmental Toxicology and Chemistry*, 26 (4), 655–663. <https://doi.org/10.1897/06-392R.1>
- Kiani, S., Kujala, K., T. Pulkkinen, J., Aalto, S.L., Suurnäkki, S., Kiuru, T., Tiirola, M., Kløve, B. & Ronkanen, A.-K. (2020). Enhanced nitrogen removal of low carbon wastewater in denitrification bioreactors by utilizing industrial waste toward circular economy. *Journal of Cleaner Production*, 254, 119973. <https://doi.org/10.1016/j.jclepro.2020.119973>
- Lehmann, J., Rillig, M., Thies, J., Masiello, C., Hockaday, W. & Crowley, D. (2011). Biochar effects on soil biota - A review. *Soil biology & biochemistry*, 43, 1812–1836. <https://doi.org/10.1016/j.soilbio.2011.04.022>
- Mara, D. & Horan, N.J. (2003). *Handbook of Water and Wastewater Microbiology*. Elsevier.
- Middleton, A.C. & Lawrence, A.Wm. (1977). Kinetics of Microbial Sulfate Reduction. *Journal (Water Pollution Control Federation)*, 49 (7), 1659–1670
- Nordström, A., Hellman, M., Hallin, S. & Herbert, R.B. (2021). Microbial controls on net production of nitrous oxide in a denitrifying woodchip bioreactor. *Journal of Environmental Quality*, 50 (1), 228–240. <https://doi.org/10.1002/jeq2.20181>
- Nordström, A. & Herbert, R.B. (2018). Determination of major biogeochemical processes in a denitrifying woodchip bioreactor for treating mine drainage. *Ecological Engineering*, 110, 54–66. <https://doi.org/10.1016/j.ecoleng.2017.09.018>
- Parvage, M.M. & Herbert, R. (2023). Sequential removal of nitrate and sulfate in woodchip and hematite – coated biochar bioreactor.

- Environmental Science: Water Research & Technology,. <https://doi.org/10.1039/D2EW00499B>
- Pudi, A., Rezaei, M., Signorini, V., Andersson, M.P., Baschetti, M.G. & Mansouri, S.S. (2022). Hydrogen sulfide capture and removal technologies: A comprehensive review of recent developments and emerging trends. *Separation and Purification Technology*, 298, 121448. <https://doi.org/10.1016/j.seppur.2022.121448>
- Runtti, H., Tolonen, E.-T., Tuomikoski, S., Luukkonen, T. & Lassi, U. (2018). How to tackle the stringent sulfate removal requirements in mine water treatment—A review of potential methods. *Environmental Research*, 167, 207–222. <https://doi.org/10.1016/j.envres.2018.07.018>
- Soucek, D.J. & Kennedy, A.J. (2005). Effects of hardness, chloride, and acclimation on the acute toxicity of sulfate to freshwater invertebrates. *Environmental Toxicology and Chemistry*, 24 (5), 1204–1210. <https://doi.org/10.1897/04-142.1>
- Swedish Agency for Marine and Water Management (2018). Remittance on the revision of regulations (HVMFS 2013:19) on the classification on environmental quality standards with regard to surface water, Havs- och vattenmyndigheten (in Swedish).
- Widdel, F. (1988). Sulfate- and sulfur-reducing bacteria. *Biology of Anaerobic Microorganisms*., <https://cir.nii.ac.jp/crid/1573105974571710720> [2023-12-20]
- Zak, D., Kleeberg, A. & Hupfer, M. (2006). Sulphate-mediated phosphorus mobilization in riverine sediments at increasing sulphate concentration, River Spree, NE Germany. *Biogeochemistry*, 80 (2), 109–119. <https://doi.org/10.1007/s10533-006-0003-x>