Operation of a Continuous Sulfidogenic System for Treating Mine Water with a High Concentration of Chloride

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Abstract

In Chile, coal was extracted from underground mines beneath the Pacific Ocean, in the Arauco Basin in the Southern Biobío Region, with the operation ceasing in 1997. The mine "Chiflón del Diablo" is an abandoned coal mine and part of the Lota mining complex, registered on the tentative list to be awarded UNESCO World Heritage status. The mine has been a tourist attraction due to the exploitation beneath the coastal area. Unfortunately, since the last massive earthquake that hit the Biobio Region (February 2010), flooding of the mine was observed, requiring saline groundwater to be dewatered directly into the nearshore zone to maintain tourist activities. This water pumped from the mine out onto the beach, possessed a high concentration of chloride (~600 mM; 21.272 ppm) as a consequence of the seawater intrusion process into the shafts, a phenomenon reported at many mines near the coast. The mine water discharged to the sea from the Chilean mine additionally contained elevated ferrous iron (between 2–5 mM; 112–279 ppm) and sulfate (~33 mM;) due to the oxidation of pyrite, the main sulfide mineral associated with the Arauco Basin. This study describes the removal of iron and sulfate in mine water with high chloride concentration by using a continuous sulfidogenic biofilm reactor inoculated with sediment samples from the "Salar of Huasco," Chile. In the samples analyzed in the biofilm, *Desulfomicrobium*, a genus belonging to the order Desulfovibrionales, was the most abundant SRB, with a relative abundance of about ~30%. Feeding the mine water, with a hydraulic retention time of 25 h, it was possible to remove more than 90% of sulfate and iron by using lactate as electron and carbon source. This study highlights the use of a halophilic sulfate-reducing consortium to promote sulfidogenesis in mine water with a high chloride concentration.

Keywords: Sulfate reduction, Chloride, Continuous system

Introduction

Chile's mining industry faces important challenges in terms of environmental sustainability and water resource management that must be addressed. In the current scenario, one of the biggest environmental problems is the generation of mine drainage. In addition, climate change has led to increased water scarcity, affecting the use of water in industrial processes with the mining industry being forced to use different water sources for mineral extraction, including seawater. A major problem associated with this strategy is that few reports have evaluated the impact of seawater on mine drainage mitigation strategies (Texeira *et al.*, 2023). In the southern part of Chile, mine water impacted by seawater ("Chiflón del diablo" mine) is currently being discharged into the coastal sea. This is an example of untreated saline mine water that generates a plume containing highly visible ochre particles, where mine water treatment has yet to be implemented. Conventional AMD treatment is not cost-effective, pointing to the urgent need for a sustainable solution, particularly for mine waters impacted with seawater. A sustainable alternative approach for the

removal of sulfate in mine drainage compared with other physicochemical treatment technologies is bioreactors promoting sulfate reduction. By doing this, sulfate is reduced to sulfide that can be used as metal precipitant or oxidized to elemental sulfur as demonstrated in different studies and full-scale applications using sulfate-reducing microorganisms (SRMs) at moderately low pH. While sulfidogenic bioreactors are becoming increasingly targeted for the remediation of (i.e., Johnson and Santos, 2020), few reports targeting sulfate-reducing consortia with the ability to grow under saline conditions to remediate the mine drainage originating from mine operations impacted with seawater have been described. In a recent study by Barton and Fauque (2022), on polyextremophilic microorganisms, it was found that *Desulfovibrio tunisiensis* and *Desulfohalobium retbaense* are sulfate reducers that can survive in moderately acidic pH ranging from 4.5 to 5.5 with optimal salinities of 4% and 10% NaCl, respectively. Chile harbors a huge diversity of salars in the Altiplano and Atacama Desert. The Salar de Huasco is an athalassohaline system, high-altitude (3.800 m above sea level) salt flat with neutral pH, water salinities ranging from freshwater to saturated salt waters. The concentration of sulfate up to ~70 mM was determined within the Salar the Huasco, and neutrophilic SRMs have been detected (belonging to the order of Desulfovibrionales and Desulfobacterales) in ponds with mats (Molina *et al.*, 2018). In this study, microbial enrichments were obtained from sediment samples from the Salar de Huasco, possessing dissimilarity sulfate reducing bacteria to set up and operate a sulfidogenic metal remediation process for the treatment of the water discharged from the mine "Chiflón del Diablo" characterized with elevated concentrations of iron, sulfate and chloride.

Materials and Methods

Sulfidogenic bioreactor

An upflow sulfidogenic biofilm bioreactor (Electrolab, UK) with a working volume of 2.3 L was set up and operated under continuous flow mode, based on a system previously described by Gonzalez and colleagues (2019). A pre-trial bioreactor was set up housing sulfidogenic bacteria obtained from different ponds in Salar de Huasco (Nancucheo *et al.*, 2023) with occurrence of blackened sediments that served as source material to enrich for sulfate reducing microbial communities. Enrichments were grown on 1–2 mm diameter porous beads made from recycled glass (Poraver Dennert GmbH, Germany), which occupied ~50% of the total volume of the vessel. The bioreactor was filled with SRMs medium with 10 mM of sulfate at pH 7 and 5 mM of lactate as electron donor and carbon source. The medium was maintained in batch mode for 20 days, recirculating the liquid through the bioreactor to encourage attachment to the beads. The bioreactor was maintained at 30 °C and stirred at 50 rpm with a continuous stream of nitrogen to remove H_2S produced. A pre-trial continuous bioreactor was operated and fed with synthetic water, with chloride increased from 150 to 500 mM, and pH modified from 6 to 8. During this period, the HTR was decreased by up to 25 hours prior to feeding with the real mine water. For 50 days, the sulfidogenic bioreactor system was fed with real mine water discharged from the "Chiflón del Diablo mine" which contained sulfate (~33 mM) and chloride (~600 mM) with an HRT of 25 h. Due to the variable amount of iron for each discharge process (ranging from 2.3 to 5 mM), mine waters were collected three times over a period of 40 days to fed the reactor with "fresh" iron water. During this phase, the feed liquor was sparged with N2 to minimize ferrous iron oxidation. An additional bottle was used to supply essential nutrients for the SRMs, including salts/trace elements (Nancucheo *et al.*, 2016), lactate (45 to 90 mM) and yeast extract (0.1 to 0.3 %).

Miscellaneous analysis

The pH of the filtered liquid samples was measured using a pH meter (model HI 2221; Hanna Instrument, Inc.). The concentration of sulfate was determined with Sulfaver Kits (HACH, based on SM4500-SO42- E, APHA, 2005). Lactate and acetate in the effluent liquor were measured by HPLC coupled with a UV detector (Agilent Technologies, Santa Clara, CA, USA), although acetate

was not detected during the course of the experiment. Total iron was quantified by the method of o-phenanthroline. A sample from the bioreactor was collected at the end of the experiments to analyze the microbial community using high-throughput 16S rRNA gene sequencing targeting the V4 region, as described elsewhere (Schwarz *et al.*, 2020).

Results and discussion

Fig. 1 shows the measured values of total iron in the influent and effluent liquor, as well as sulfate removal. During the operation to remove high concentrations of sulfate in the influent liquor (33 mM), two steady states were observed. Between days 10 and 35, approximately 60% of the sulfate was removed, and lactate was entirely consumed as a carbon and electron donor (data not shown). Between days 35 and 50, the major objective was to increase the removal of sulfate. by increasing the amount of lactate (45 to 90 mM) and yeast extract (0.1 to 0.3 %) on day 35, it was possible to remove up to approximately 95%. Under these conditions, the rate of sulfate reduction was 3 g sulfate L-1 day-1, which is higher than those reported for other sulfidogenic bioreactors. Importantly, no acetate was found in the effluent, which typically appears as a waste product in sulfate-reducing bioreactors. This presence leads to chemical oxygen demand, which is undesirable. During the operation, most of the iron was removed, presumably as FeS, due to the black coloration observed in the sulfidogenic bioreactor. Although FeS can be unstable, its recovery has attracted widespread interest due to its excellent biocompatibility and multifunctionality in biomedical applications.

Fig. 2 shows the relative abundance of the bacterial community at the end of the operation. The analysis confirmed the presence of two genera of SRMs capable of thriving in high chloride concentrations. *Desulfomicrobium*, a non-spore-forming, gram-negative sulfate-reducing genus, was identified as the dominant microorganism in the bioreactor. One challenge is to increase the abundance of *Desulfomicrobium*, promoting the removal of sulfate and the consumption of carbon sources rather than allowing the growth of other heterotrophs that do not contribute to the sulfidogenesis process.

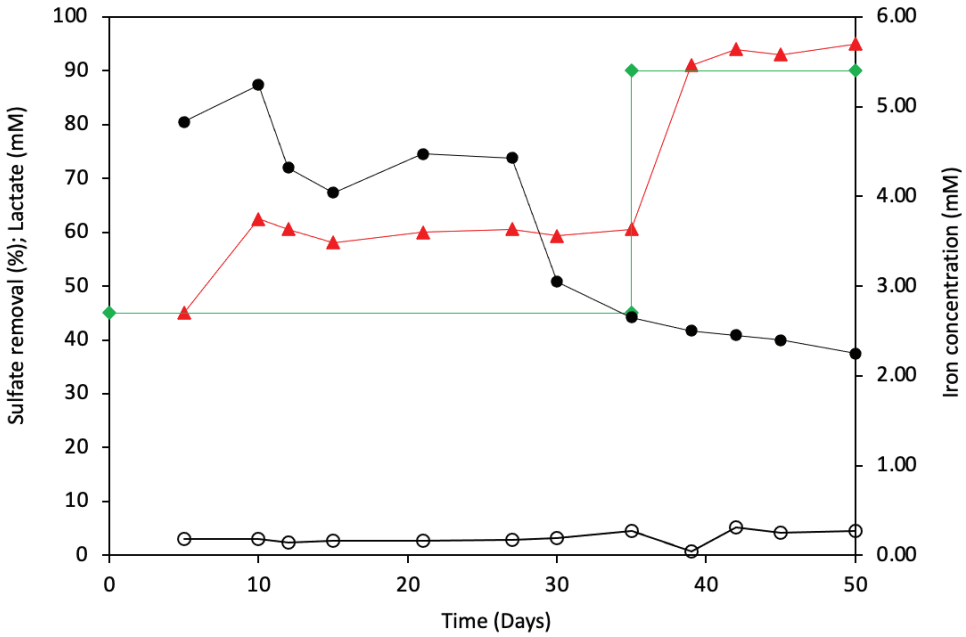

Figure 1 *Removal of sulfate (▲) and concentrations of iron in the feed liquor (●) and in the outflow (❍) of the bioreactor, along with lactate (●) supplied with the mine water with high concentration of chloride.*

Conclusions

The experimental work demonstrated a simple and efficient process for removing sulfate and iron from actual mine water with a high chloride concentration. For this particular and extreme mine, water is crucial for the microbial community that thrives in high concentrations of chloride obtained from a salar in the Atacama Desert in Chile.

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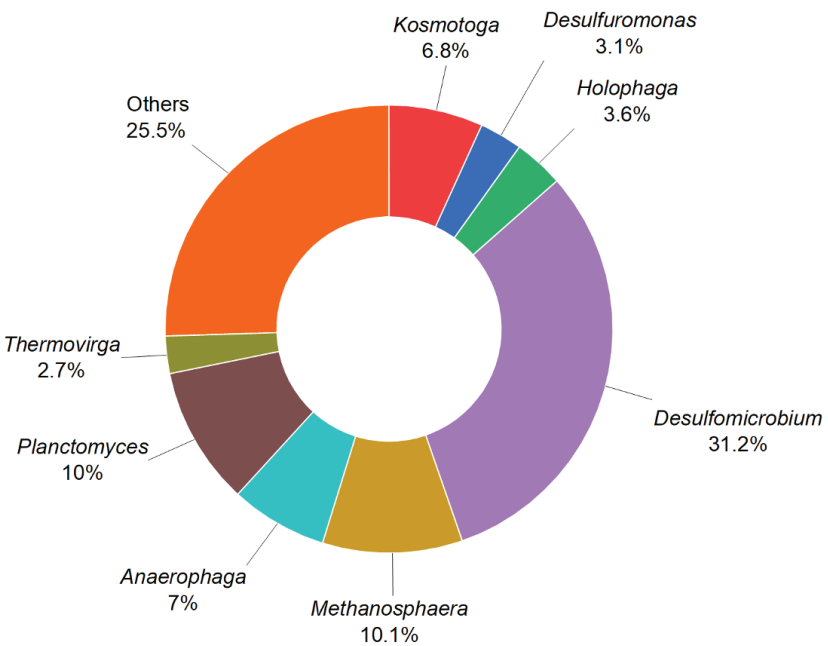

Figure 2 Microbial population determined in the sulfidogenic system.