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Research article

Removal of selenate from brine using anaerobic bacteria and zero valent iron

Jinshu Liu, Jon C. Taylor, Susan A. Baldwin*

Chemical and Biological Engineering, University of British Columbia, 2360 East Mall, Vancouver, B.C., Canada

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ABSTRACT

The mining industry needs to treat large volumes of wastewater highly concentrated in chemical compounds that can adversely affect receiving environments. One promising method of treatment is the use of reverse osmosis to remove most of the dissolved salts. However, the resulting brine reject is a highly saline wastewater that needs further treatment to remove the toxic components, such as selenate, which is a chemical compound of great concern in coal-mining regions. Biological reduction and removal of dissolved selenium from a brine solution was achieved. Microorganisms were enriched from environmental samples collected from two mines, respectively, at different geographic locations through adaptive evolution in the laboratory. Batch treatment of typical brine was tested with two different enrichments with the addition of either of two chemical forms of iron, ferrous chloride or zero valent iron. Successful selenium removal in the presence of high nitrate and sulphate concentrations was achieved with a combination of enriched microorganisms from one particular site and the addition of zero-valent iron. The composition and metabolic potential of the enriched microorganisms revealed *Clostridium*, *Sphaerochaeta*, *Synergistes* and *Desulfosporosinus* species with the metabolic potential for selenate reduction through the YgfK enzymatic process associated with selenium detoxification.

1. Introduction

Metal and coal mining use large quantities of water. For example, in 2009, Canadian mining industries used 2044.9 million cubic meters of water (Statistics Canada, 2012). During mining and mineral processing, this water can become concentrated in chemical compounds such as metals, nitrate, sulphate, and oxyanions of selenium and or arsenic that must be removed to below regulated limits if the water needs to be discharged to the receiving environment. There are several existing technologies for treating mining wastewater, such as chemical precipitation (Fu and Wang, 2011), membrane filtration (Malaeb and Ayoub, 2011), constructed wetlands and other biological treatment methods (Sheoran and Sheoran, 2006; Gadd, 2010; Johnson and Hallberg, 2005; Khoshnoodi et al., 2013; Baldwin et al., 2015a). However, all these technologies are challenged by the high total dissolved solids (TDS) of mine influenced water, which can be in the range of 600 to 7000 mg/L (Zinck and Griffith, 2013). In many instances treatment of high TDS mine influenced water results in production of large volumes of highly concentrated toxic residual. Thus, hybrid processes that combine several different treatment technologies are needed to reduce the risks and liabilities associated with disposal of toxic mine wastewater sludge (Pérez-González et al., 2012). One process concept is to combine reverse osmosis (RO) with biological treatment of the RO brine.

In this study, we focused on the removal of one constituent of concern that leaches from some coal and metal mine waste, namely selenium. Selenium concentration guidelines in receiving aquatic ecosystems are controversial (Luoma and Presser, 2009) but can be as low as 1 µg/L (Canadian Council of Ministers of the Environment water quality guidelines: http://www.ccme.ca/en/resources/canadian_environmental_quality_guidelines/). Such low concentrations are required since selenium bioaccumulates up the food chain (Stewart et al., 2010) and can cause teratogenic effects in fish and birds (Janz et al., 2010). Removal of the dissolved and most mobile form of selenium, selenate (SeO_4^{2-}), can be achieved using biological reductive processes (Tan et al., 2016). But this is proving to be challenging due to the large volumetric flow rates of mine influenced water and high capital and operating costs of large-scale bioreactors. One alternative method considered in this study was to first remove TDS from mine affected water using reverse osmosis and then remove selenium from the concentrated brine product. It was unknown if biological treatment using bacteria capable of reducing selenate to insoluble forms would be possible in a very saline environment. The advantages of combining two processes over treating the raw mine affected water directly with a

* Corresponding author.
E-mail address: sbaldwin@mail.ubc.ca (S.A. Baldwin).

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biological process would be much-reduced volumetric flow rates and smaller footprint bioreactors that might be operated passively, as well as far less residual to manage.

Some bacteria are capable of reducing selenate to selenite and then to elemental Se in order to obtain energy for respiration (Oremland et al., 1989). *Thauera selenatis* is one bacterium that is known to use selenate as its preferred electron acceptor for respiration (Macy et al., 1993). Due to functional flexibility between enzymes that reduce nitrate and selenate, many denitrifying bacteria that respire on nitrate can also reduce selenate (Sabaty et al., 2001). Since nitrate respiration is more thermodynamically favourable and nitrate concentrations in mine influenced water are much higher than those for selenium, nitrate inhibits selenate reduction by denitrifiers. Another co-contaminant with selenium in some mine affected water is sulphate, which is typically present at concentrations much higher than nitrate or selenium. Sulphate reducing bacteria that respire on sulphate have been shown to be capable of reducing selenate as well as sulphate (Hockin and Gadd, 2003, 2006). Thus, the potential for selenate reduction together with denitrification and sulphate reduction exists in the microbiological world and can be leveraged for biological treatment of mine affected water containing all three of these contaminants. However, at the time of writing, it was not known if this could be achieved in saline brine and which types of microorganisms would have the capacity for selenate, nitrate and sulphate reduction under these conditions. Since many microorganisms with the ability to reduce selenate have been isolated from saline environments, such as the San Joaquin Valley (Oremland et al., 1989) and Mono Lake (Stolz et al., 2006) in California, USA, we postulated that similarly halotolerant microorganisms can be enriched from ecosystems exposed over a long period of time to mine affected water containing sulphate, nitrate and selenium. To investigate this, two sites, one on a metal mine and the other located on a coalmine, were used as sources for bacteria that might be capable of reducing selenate in high TDS brine containing also nitrate and sulphate. The site on the metal mine consisted of an organic rich constructed wetland that was removing selenate, nitrate and sulphate from mine tailings water that was previously shown to contain diverse species of sulphate-reducing bacteria (Baldwin et al., 2015b). The coalmine site was a natural wetland receiving waste rock seepage that had already been shown to contain selenate reducing and denitrifying microorganisms (Subedi et al., 2017). Samples from these sites were enriched in growth medium with concentrations typical of that produced after reverse osmosis of coalmine affected water. According to one mine operator, typical brine produced from RO treatment of coalmine affected water contained just over 90 g/L TDS, of which 60 g/L was sulphate, 60 mg/L was nitrate-N and 7 mg/L was total dissolved selenium (personal communication). A chemical precipitation process carried out on the RO brine product removed TDS down to ~3000 mg/L with 1730 mg/L sulphate, 235 mg/L nitrate and 1.9 mg/L total dissolved Se plus other dissolved salts (personal communication). These concentrations were used in a batch trial to test if microbes enriched from the mine environmental samples were capable of reducing dissolved Se as the contaminant of most concern. Simultaneous removal of nitrate and sulphate would be an asset. For biological treatment, the hypothesis that was tested was that sulphate reducing bacteria enriched from the mine environmental samples would adapt to the simulated brine and be capable of removing selenium as well as sulphate and nitrate. This was supported by evidence in the literature for sulphate-reducing bacteria having the ability to metabolize all three of these electron acceptors (Hockin and Gadd, 2006; Marietou, 2016). Iron was also added to the treatments since it is a common ingredient of passive biological mine affected water treatment bioreactors that support sulphate-reducing bacteria in order to sequester sulfide. Additionally, some forms of iron, such as zero valent iron have been shown to enhance selenate reduction in other studies (Lindsay et al., 2008; Jeen et al., 2014; Tang et al., 2014). The addition of two different forms of iron was tested. Metagenomic analyses of the 16S ribosomal ribonucleic acid (rRNA) phylogenetic marker gene and whole genome DNA were used to identify microorganisms in the enrichments that had the metabolic capacity to reduce selenate, nitrate and or sulphate as the species likely responsible for treatment.

2. Methods and materials

2.1. Description of the sites used to source inoculum for the enrichments

Mine site samples as sources for bacteria were removed from anaerobic subaqueous sediments located on two mines. The first site on Mine 1 was a constructed, sub-surface flow anaerobic biological reactor (BCR) treating Se-, nitrate- and sulphate-containing mine tailings pond toe drain water (Baldwin et al., 2015b). The second site on Mine 2 was a natural marsh (Marsh) receiving coalmine affected seepage also containing Se, nitrate and sulphate (Subedi et al., 2017). Samples were stored at room temperature in an anaerobic chamber until inoculating into growth medium.

2.2. Enrichment of bacteria

To select for sulphate-reducing bacteria, 30 g of each environmental sample was inoculated into Postgate B growth medium separately (Postgate, 1983). Culture bottles were filled to the brim, sealed and incubated anaerobically at 30 °C in the dark for one month. A 10 mL syringe was used to collect supernatant at time 0, 1 week and 1 month from the bottles and freshly made growth medium was used to fill the bottles back to the brim. The supernatant sample was filtered through 0.22 µm syringe filters and preserved in 2% w/v zinc acetate until analysis for sulphate concentration using Standard Method 4500-SO_4^{2-} (Eaton et al., 2005a). After one month, the enriched bacteria were adapted to increasing concentrations of a typical RO brine sample obtained from a mine site over three passages of three weeks to one month each. Since the brine did not contain iron, additional $FeCl_2{\cdot}4H_2O$ was added based on the Postgate B medium recipe. This was done to reduce the toxicity of any sulfide produced. Sodium lactate was added as a carbon source based on the stoichiometric requirement of 0.67 g COD (chemical oxygen demand) per 1 g of sulphate. Supernatant samples were collected at the end of the last passage and filtered through 0.22 µm syringe filters. One sample aliquot was preserved with 2% w/v zinc acetate for sulphate concentration measurement and another was preserved with a drop of analytical grade nitric acid for metal analysis (ALS Environmental, 8081 Lougheed Highway, Burnaby, British Columbia V5A 1W9, Canada). The final enrichments were in stored in 0.5 mL glycerol at −80 °C.

2.3. Batch culture tests

In this experiment, the enrichment cultures prepared previously were used to examine to what extent selenium, sulphate and nitrate could be removed from a simulated RO brine wastewater having undergone chemical precipitation. In addition, three treatments with different iron and nitrogen amendments were compared. Ingredients based on the RO brine composition (Table 1) common to all growth media were (in g/L) $MgSO_4$ (0.25), $CaSO_4{\cdot}2H_2O$ (2.74), Na_2SeO_4 (0.0045), NaCl (0.096), $CaCl_2{\cdot}2H_2O$ (0.23), $Ca(NO_3)2{\cdot}4H_2O$ (1.26), KH_2PO_4 (0.50), HNO_3 (0.27). Yeast extract (1.0 g/L), sodium lactate (2.67 mL/L) and trace elements (1.0 mL/L) were also added. No ammonium or iron was present in the brine, but these are ingredients of Postgate B medium. Iron was deemed necessary to precipitate any sulfide formed to reduce the toxicity to microbial growth (Reis et al., 1992), but it was not known if ammonium was required. For condition 1, only iron was added ($FeCl_2{\cdot}2H_2O$ (3.58 g/L)). For condition 2, both ammonium and iron were added (NH_4Cl (1.0 g/L) & $FeCl_2{\cdot}2H_2O$ (3.58 g/L)), whereas for the third condition, iron was added in the form of zero valent iron (ZVI (18.64 g/L)). Growth medium was prepared anaerobically using the Hungate method (Hungate, 1969). Since