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Bioremoval of arsenic and antimony from wastewater by a mixed culture of sulfate-reducing bacteria using lactate and ethanol as carbon sources

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ABSTRACT

We investigated the remediation of wastewater containing As and Sb through the application of a mixed culture of sulfate-reducing bacteria (SRB). The effect of Fe(II) and different carbon sources on the removal of As and Sb was evaluated. The wastewater initially contained 5 mg L^{-1} of both As(V) and Sb(V), and the treatment was conducted over a 12-d period. The produced precipitates were characterized by TEM and XRD to elucidate the metalloid removal mechanism. In the absence of Fe(II), Sb was efficiently removed (97.6% and 97.8% with lactate and ethanol as carbon sources, respectively, hereinafter the same), whereas only a relatively small fraction (27.8% and 26.4%) of As was removed. The addition of 200 mg L^{-1} Fe(II) greatly improved the removal of As (78% and 98.2%) and further increased the removal of Sb (98.8% and 99.4%). We hypothesized that As was removed through sorption/co-precipitation by FeS instead of the formation of As_2S_3. The use of ethanol as a carbon source generated a relatively lower yield of sulfide compared to the use of lactate, but it resulted in a higher removal of As and Sb. This may be attributed to the low production of sulfide, which possibly resulted in the slow precipitation of FeS that enhanced the sorption/co-precipitation of ions. This work demonstrates the high application potential of ethanol as a carbon source and the addition of Fe(II) in the bioremoval of As and Sb from wastewater by SRB.

1. Introduction

The exploitation of sulfide minerals in the mining industry usually results in the exposure of large amounts of sulfide ores at the surface. In the presence of oxygen, water, and bacteria, strong oxidation of sulfide minerals (e.g., pyrite) can occur, resulting in the generation of acid mine drainage (AMD) (Johnson and Hallberg, 2005). AMD usually contains high concentrations of metals and has the potential to degrade surface and ground waters and severely affect human health, so the treatment of AMD is a crucial issue (Tsukamoto and Miller, 1999).

Many attempts have been made to remove metals from AMD, with the most widely used treatment process for AMD being lime precipitation (Tsukamoto and Miller, 1999), which is based on the chemical neutralization of acidity, the hydroxide precipitation of metals, and the sorption/co-precipitation of metals on Fe and Al (hydr)oxides (Kaksonen et al., 2006; Martins et al., 2011). However, lime precipitation produces large amounts of sludge contaminated with metals, and it is also expensive and labor intensive (Wakao et al., 1979). The sulfide precipitation of metals has been demonstrated to have several benefits over lime precipitation, such as lower effluent metal concentrations, reduced sludge volumes, and the possibility of recovering valuable metals (Tsukamoto and Miller, 1999; Kaksonen et al., 2006). However, metal precipitation by the direct addition of sulfide is not used as widely as it could be because the dosing of sulfide is seen as difficult to control, and there are concerns about the toxicity and corrosiveness of excess sulfide (Veeken et al., 2003; Huisman et al., 2006). A promising alternative is the biologically induced precipitation of metal sulfides, which is based on hydrogen sulfide production by sulfate-reducing bacteria (SRB) (Kaksonen et al., 2003). SRB use sulfate as the terminal electron acceptor during the metabolism of organic matter, resulting in the production of sulfide, and the generated sulfide is able to remove metal(loid)s by forming insoluble metal sulfide precipitates (Dvorak et al., 1992; Jong and Parry, 2003; Kieu et al., 2011) or inducing the reduction and subsequent hydrolysis and precipitation of metals (Yi et al., 2007; Pagnanelli et al., 2012).

Arsenic and Sb are toxic and carcinogenic metalloids of global concern (Amarasiriwardena and Wu, 2011; Kulp et al., 2014) and are considered as pollutants of priority interest by the European Union and the United States Environmental Protection Agency (Ungureanu et al., 2015). Mining residues from Sb mines and Carlin-type Au mines usually

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constitute an important source of Sb and As pollution because Sb-bearing minerals (e.g., stibnite and pyrite) and As-bearing minerals (e.g., arsenopyrite, pyrite, orpiment, and realgar) are frequently concomitant in the sulfide ores of these mines (Ashley et al., 2003; Wilson et al., 2004; Casiot et al., 2007; Zhang et al., 2009). In an Sb (stibnite) deposit at Hillgrove, Australia, Sb and As concentrations approach 55 and 7.2 mg L^{-1} in tailings dam seepage water and reach 0.47–1.8 and 0.01–0.28 mg L^{-1} in strongly contaminated creek water (Ashley et al., 2003). Although some previous studies have reported the bioremoval of As or Sb as a sole contaminant (Altun et al., 2014; Sahinkaya et al., 2015; Zhang et al., 2016), little research has been conducted on the treatment of combined As and Sb pollution by SRB. Furthermore, As and Sb in waters are present in the form of negatively charged oxyanions (Filella et al., 2002; Smedley and Kinniburgh, 2002), so the sorption of As and Sb tends to decrease at higher pH values (Jones et al., 1997; Tighe et al., 2005). Consequently, the traditional lime precipitation method might be less effective for As and Sb removal.

In this work, batch experiments were performed to examine the biotreatment of wastewater containing As and Sb by a mixed culture of SRB. In particular, the effectiveness of Fe(II) on As removal was investigated because high levels of dissolved iron usually exist in AMD (Wang et al., 2003), and As can be sequestrated by FeS through sorption/co-precipitation (Jong and Parry, 2003; Kocar et al., 2010). Additionally, lactate and ethanol were used as carbon sources for a comparison of their applicability for metalloid removal. Lactate, a good substrate for most SRB, has been widely used in lab-scale experiments, but its application in wastewater treatment processes would imply high operational costs (Kaksonen et al., 2003; Kousi et al., 2011). Ethanol is a competitive alternative because of its ease of availability and relative low cost (Kousi et al., 2011; Zhang and Wang, 2013).

2. Materials and methods

2.1. Reagents, glassware, and plastic ware

High-purity deionized water (HPW) (resistivity: 18.2 MΩ cm) was prepared with a Milli-Q system (Millipore, Bedford, MA, USA) and was used throughout the batch experiments. Sodium arsenate heptahydrate ($Na_2HAsO_4{\cdot}7H_2O$, 98.5% purity) was purchased from Sigma Inc. (Mississauga, ON, Canada). Potassium hexahydroxoantimonate ($KSb(OH)_6$, 99% purity) was purchased from Fluka Inc. (Steinheim, Germany). Ferrous sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$) and the other chemicals were analytical grade.

2.2. Culture medium and the SRB source

Modified Postgate's medium B was used for the selection and enrichment of SRB and in the treatment experiments. It had the following composition (in g L^{-1}): KH_2PO_4 (0.5); NH_4Cl (1); Na_2SO_4 (1); $MgSO_4{\cdot}7H_2O$ (2); sodium lactate (3.65); ascorbic acid (0.1); $CaCl_2$ (0.1); and yeast extract (1). In the first step of incubation, 0.05 g/L of $FeSO_4$ was added to the medium for the indication of successful incubation of SRB. The culture medium was purged with nitrogen gas (99.9% purity) for degassing of oxygen and sterilized by autoclaving at 121 °C for 20 min.

A mixed culture of SRB was enriched from the mine tailing slurry of an Sb mine in Guangxi, China. Approximately 2 g of mine tailing was collected and mixed with 100 mL of the modified Postgate's medium B. The medium was then placed into an incubator (Model 855-ACB, Plas-Labs Inc., China) at 30 °C. After 7 d, blackening of the medium (precipitate of FeS) indicated the growth of SRB. Then, 10 mL of the resultant culture was transferred to 100 mL of the modified Postgate's medium B. This process was repeated five times. The final culture containing SRB was then employed in batch experiments.

To identify the strains in the culture, 1 mL of the liquid culture was anaerobically transferred into 9 mL modified Postgate's medium B and then serially diluted through a 10^{-1} dilution. Dilutions of 10^{-5} were used for isolating these strains by streaking inoculation onto agar plates (agar plates contained the same components with the modified Postgate's medium B in addition to 16 g L^{-1} agar). The plates were incubated anaerobically at 30 °C for approximately 7 d.

Plates with distinct isolates were sent to the Beijing Genomics Institute (BGI) to identify the strains. DNA was extracted with a PowerSoil DNA kit (MoBio). Extracted DNA samples were stored at −20 °C and were used as templates for polymerase chain reaction (PCR) without further treatment. Whole 16S rRNA gene sequences were amplified with the universal bacterial primers 27F (5′ AGAGGTTTGATCMTGGCTCAG3′) and 1492R (5′TACGGYTAC CTTGTTACGACTT3' (Invitrogen, Carlsbad, CA, USA) and a DYAD DNA Engine thermocycler (MJ Research, Watertown, MA, USA). A 30-μL PCR mixture contained 3 μL of PCR buffer, 0.2 μL of Taq polymerase, 2 μL deoxynucleoside triphosphate, 1 μL of each primer, and 2 μL of template DNA. PCR was performed with the following thermocycler program: denaturation at 95 °C for 5 min, then 35 cycles of denaturation at 95 °C for 30 s, annealing at 62 °C for 30 s, and extension at 72 °C for 1 min; and final extension at 72 °C for 10 min. The amplified product was screened by electrophoresis in a 1% agarose gel, then excised and purified using a MagBead DNA Purification Kit. Purified PCR products were sequenced by an ABI-3730XL DNA Sequencer.

The 16S rRNA gene sequence was aligned with the closely related sequences in GenBank using BlastN (for 16S rRNA). Bacteria with 99% similarity were removed from the final analysis in order to simplify the dataset and reduce redundancy (Ziemer, 2014). The 16S rRNA gene nucleotide sequences have been deposited in GenBank under the accession numbers MF175254 to MF175256.

2.3. Biotreatment experiments

In water, As is mostly found as the oxyanions $HAsO_4^{2-}$ (As(V)) and H_3AsO_3 (As(III)) (Smedley and Kinniburgh, 2002), and Sb mostly as oxyanions $Sb(OH)_6^-$ (Sb(V)) and $Sb(OH)_3$ (Sb(III)) (Filella et al., 2002). In surface (oxic) waters at circumneutral pH, As(V) and Sb(V) are the most thermodynamically stable and dominant species (Mitsunobu et al., 2006; Kang et al., 2014; Bowell and Craw, 2014). Therefore, As(V) and Sb(V) were used as the initial As and Sb species in the batch treatments.

Stock solutions of As(V) (500 mg L^{-1}), Sb(V) (500 mg L^{-1}), and Fe (II) (20,000 mg L^{-1}) were prepared by dissolving sodium arsenate heptahydrate, potassium hexahydroxoantimonate, and ferrous sulfate heptahydrate separately in HPW. These stock solutions were filtered through a pre-sterilized syringe-filter (0.22-μm cellulose membrane, Millipore).

Batch kinetic experiments were performed simultaneously in 200-mL serum vials. Six types of batch kinetic treatments are listed in Table 1. The mixed SRB culture was first grown to a late exponential phase. Then, 12 mL of inoculum of the SRB mixed culture was incubated with 192 mL pre-sterilized modified Postgate's medium B. After 2 d of pre-incubation, HPW (6 mL) was added into the culture for treatments 1 and 4, As(V) and Sb(V) stock solutions (2 mL each) and HPW (2 mL) were added into the culture for treatments 2 and 5, and As (V), Sb(V), and Fe(II) stock solutions (2 mL each) were added into the

Table 1
Six types of batch kinetic treatments.

Treatment number	Lactate	Ethanol	As(V) and Sb(V)	Fe(II)
1	+	–	–	–
2	+	–	+	–
3	+	–	+	+
4	–	+	–	–
5	–	+	+	–
6	–	+	+	+