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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	–	+	+	+

culture for treatments 3 and 6. When added, As(V), Sb(V), and Fe(II) in the experiments had initial concentrations of 5, 5, and 200 mg L^{-1}, respectively.

The treatment of As and Sb pollution was over a period from 2 to 14 d. Over the period of experiments, 10-mL aliquots of culture were collected at the beginning and at intervals of 2, 3, 5, 7, 10, and 14 d. At the beginning of the experiments, aliquots were collected after the inoculation of the mixed SRB culture. At 2 d, aliquots of culture were collected after the addition of HPW in treatments 1 and 4, the addition of As(V) and Sb(V) stock solutions in treatments 2 and 3, or the addition of As(V), Sb(V), and Fe(II) stock solutions in treatments 5 and 6. Each aliquot was divided into two portions, one of which was syringe-filtered (0.45-μm pore-size nitrocellulose filter). The unfiltered portion was used for measurement of pH and alkalinity. The filtered portion was further divided into sub-portions for the determination of dissolved sulfate and sulfide, total As (As(T)), As(III), total Sb (Sb(T)), Sb(III), and Fe(II). The concentrations of As(V) and Sb(V) were calculated as the difference between the concentrations of As(T) and As(III) and between the concentrations of Sb(T) and Sb(III), respectively. At the end of batch experiments, the suspension of the precipitate and SRB was collected and centrifuged at 8000 rpm for 10 min. The solid phase was immediately washed with HPW and re-centrifuged, and then was freeze-dried at −40 °C for 48 h in a vacuum freeze dryer (BILON FD-1A-50, Beijing, China).

All the batch experiments were carried out at 30 °C in triplicate, and the mean data were reported.

2.4. Analyses

The pH was immediately measured with a UB-7 pH meter (Denver Instrument, USA). Total alkalinity was analyzed by titrating unfiltered samples with 0.02 M of HCl to pH 4.5 (Kaksonen et al., 2003). The concentrations of sulfate were measured by barium chromate spectrophotometry (MEPC, 2007). Dissolved sulfide was measured immediately using the methylene blue method (Greenberg et al., 1992) on a UV–Vis spectrophotometer (Model 756MC, Jinghua Technologies Ltd., Shanghai, China). Total As, As(III), Sb(T), and Sb(III) concentrations were determined by hydride generation-atomic fluorescence spectrometry (HG-AFS) (AFS-2202E, Haiguang Instruments Corp., Beijing, China). The detection limits for As(T), As(III), Sb(T), and Sb(III) were 0.01, 0.03, 0.004, and 0.05 μg L^{-1} (Fu et al., 2016). Considering that the aliquot of culture was diluted by 100-fold prior to HG-AFS determination, the detection limits were 1, 3, 0.4, and 5 μg L^{-1} for As (T), As(III), Sb(T), and Sb(III), respectively. Fe(II) concentration was measured by a 1,10-phenanthroline spectrophotometric method (APHA, 1998).

The precipitates prepared by the centrifugation of culture aliquots at the end of the culture incubation were examined by a field emission transmission electron microscope (Tecnai G2 F20 S-TWIN, FEI Inc., USA) equipped with an energy dispersive spectrometer. The precipitates from the culture medium were characterized by X-ray diffraction (Empyrean, PANalytical Co., The Netherlands) using a Cu tube and a scanning range from 4° to 60° 2θ with a step of 0.03° and 8 s/step measuring time. Qualitative analysis of the minerals was carried out using the instrument control SW (Empyrean vs. 7.6 20140701).

3. Results and discussion

3.1. Molecular identification of bacteria

Three strains were obtained from the culture containing SRB. These strains were later identified as *Escherichia coli*, *Clostridium* sp., and *Ruminococcaceae* bacterium according to BLAST analysis of their 16S rDNA in GenBank. *E. coli* is a widespread microbe, and the others belong to the Firmicutes phylum, which contains a large group of sulfate-reducing bacteria. Phylogenetic analysis showed that *Clostridium* sp. was closest to two microorganisms referred to as *Clostridium*, which is a genus containing some species with the ability to reduce sulfate (Bufton, 1959; Hernandez-Eugenio et al., 2002). We found no studies confirming that species in the *Ruminococcaceae* family can reduce sulfate.

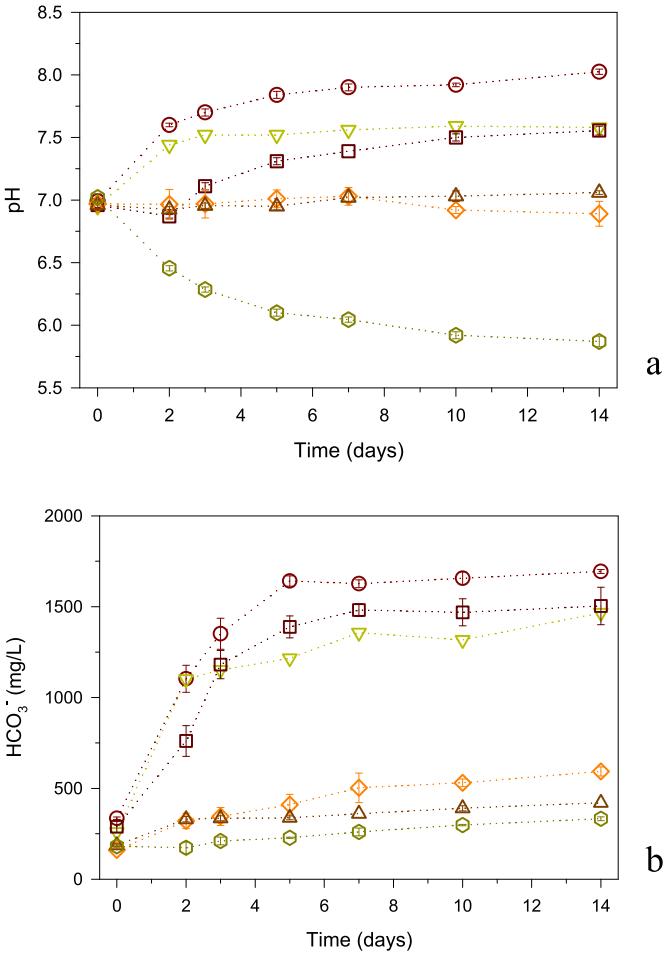

Fig. 1. Evolution of pH and alkalinity over time in the SRB culture (○, treatment 1; ▽, treatment 2; □, treatment 3; ⋄, treatment 4; △, treatment 5; ⬡, treatment 6).

3.2. pH and alkalinity evolution, sulfate reduction, and sulfide production

The temporal evolution of pH and HCO_3^- concentrations in the batch culture are shown in Fig. 1. Treatments 1 and 4 were not affected by the addition of As, Sb, or Fe(II), so these two treatments were suitable for a comparison of the evolution of pH and the HCO_3^- concentration. The pH of treatment 1 increased from 6.99 at the beginning to 8.03 at 14 d, whereas that of treatment 4 was 6.97 at the beginning and then maintained steadily at 7.03 at 14 d. Over the same period, the HCO_3^- concentration in treatment 1 increased greatly from 335 to 1694 mg L^{-1}, whereas that in treatment 4 increased only from 160 to 593 mg L^{-1}. These results indicated that the use of lactate as a carbon source (treatment 1) resulted in higher pH values and HCO_3^- concentrations compared to the use of ethanol (treatment 4). The increase in pH could be related to the generation of HCO_3^- because HCO_3^- in the mixture can increase the pH by consumption of H^+. Lactate and ethanol can be oxidized by SRB to the intermediate acetate, and acetate can be employed as an electron donor and further oxidized to HCO_3^-. These processes are described by the following reactions (Thauer et al., 1977; Nevatalo et al., 2010):

$$2CH_3CHOHCOO^- + SO_4^{2-} \rightarrow 2CH_3COO^- + 2HCO_3^- + HS^- + H^+ \quad (1)$$

$$2CH_3CH_2OH + SO_4^{2-} \rightarrow 2CH_3COO^- + HS^- + H^+ + 2H_2O \quad (2)$$

$$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^- \quad (3)$$

The oxidation of lactate produces HCO_3^- in both reactions (1) and (3), whereas the oxidation of ethanol only produces HCO_3^- in reaction (3). Therefore, the oxidation of lactate can generate more HCO_3^- compared to that of ethanol. The pH of the mixture is considered to be the result of equilibria between the reaction products H_2S, HS^-, S^{2-}, CO_2, HCO_3^-, and CO_3^{2-} (Dvorak et al., 1992; Kousi et al., 2011). The relatively high pH of treatment 1 can be attributed to the high production of HCO_3^- resulting from the use of lactate as a carbon source, whereas the relatively constant pH of approximately 7 in treatment 4 is likely due to the low production of HCO_3^- resulting from the use of ethanol.

Additionally, the culture pH evolution also differed noticeably between treatments using ethanol as a carbon source. In treatments 4 and 5, the pH was relatively constant at approximately 7, but in treatment 6, it declined markedly from 7.02 to 5.87 (Fig. 1a). This difference in pH trend can be explained by the neutralization of acid released in the following reaction between Fe(II) and sulfide in treatment 6:

$$Fe^{2+} + H_2S \rightarrow FeS_{(s)} + 2\,H^+ \quad (4)$$

The low production of HCO_3^- (Fig. 1b) when ethanol was used as a carbon source was likely not enough to neutralize the H^+ released in the precipitation of metal sulfide (Sahinkaya, 2009), resulting in a decline in pH. In a study on the treatment of Cu- and Zn-containing wastewater by SRB grown on ethanol, the pH also decreased from 1.9 to 1.8–1.5 due to acid production during the precipitation of CuS (Sahinkaya et al., 2009). In contrast, treatment 3 using lactate as the carbon source was also in the presence of Fe(II), but the pH of this treatment increased only slightly from 6.96 to 7.55 (Fig. 1a). This can be explained by the fact that the oxidation of lactate resulted in a large amount of HCO_3^-, and consequently, the mixture exhibited a high buffering capacity for the generated acid.

The temporal evolution of sulfate and sulfide concentrations in the batch culture is shown in Fig. 2. The bacterial activity resulting from the use of lactate and ethanol as carbon sources can be compared between treatments 1 and 4. The use of lactate in treatment 1 resulted in generally lower sulfate concentrations and higher sulfide concentrations compared to the use of ethanol in treatment 4 (Fig. 2). The reduction of sulfate and the increase of sulfide directly indicated SRB activity (Pagnanelli et al., 2012), so this finding indicated that lactate was superior to ethanol for the SRB activity in the batch experiments. In treatment 1, the sulfide concentration reached 77 mg L^{-1} at 2 d, whereas in treatment 4 it reached 53.8 mg L^{-1} at 14 d (Fig. 2b). This result demonstrated that the use of ethanol might lengthen the process start-up (Kaksonen et al., 2003).

In addition to the carbon source, the addition of metalloids may also greatly affect the evolution of pH and HCO_3^- and sulfate concentrations in the batch culture. Compared to treatment 1, treatments 2 and 3 showed lower pH and HCO_3^- concentrations (Fig. 1a and b) and higher residual sulfate concentrations (Fig. 2a). This was also true for treatments 5 and 6 when compared to treatment 4. As discussed above, the decrease in pH and HCO_3^- concentration and the increase in residual sulfate concentration implied a decrease in bacterial activity (Pagnanelli et al., 2012), so these results indicated that the SRB were partially inhibited in treatments 2, 3, 5, and 6. The inhibition of the SRB was possibly caused by the presence of As and Sb. Teclu et al. (2009) reported that the growth of SRB was lower as the As concentration increased from 1 to 20 mg L^{-1}, and very little SRB growth occurred at 20 mg L^{-1} As(V) or As(III). In addition, Zhang et al. (2016) reported partial inhibition of the SRB when the Sb(V) concentration was more than 50 mg L^{-1}.

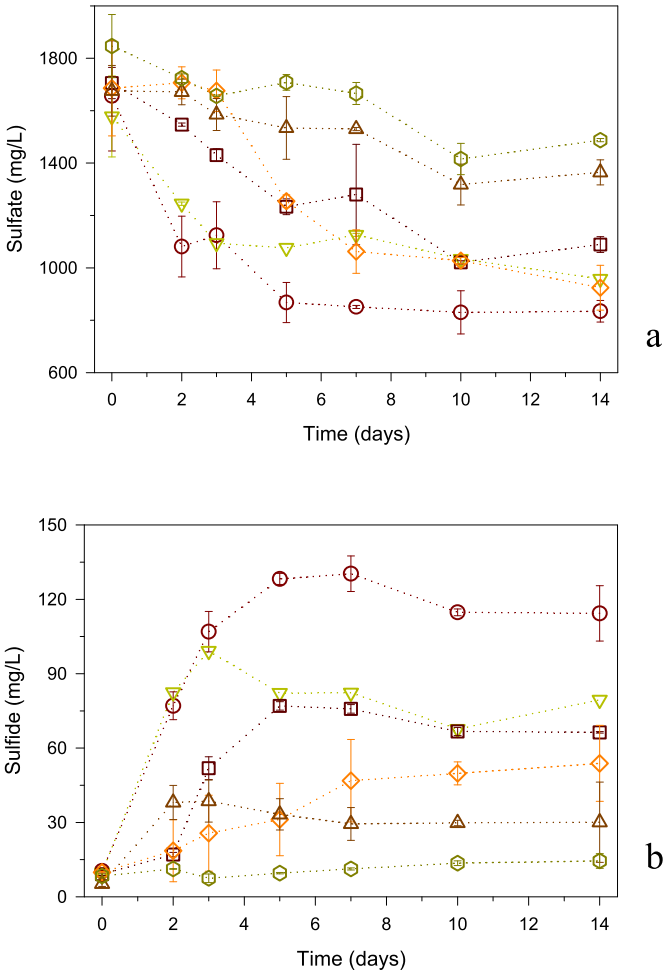

Fig. 2. Evolution of sulfate (a) and sulfide (b) concentrations over time in SRB cultures (legend the same as in Fig. 1).

3.3. Metalloid removal

The temporal evolution of the concentrations of As and Sb species and Fe(II) in the batch treatments with and without the addition of Fe (II) (treatments 2, 3, 5, and 6) is shown in Figs. 3 and 4. The nominal initial As(V), Sb(V), and Fe(II) concentrations are also shown. When lactate was used as the carbon source, the final As(V), As(III), Sb(V), and Sb(III) concentrations were 2.86, 0.75, 0.08, and 0.04 mg L^{-1} (27.8% removal of total As and 97.6% removal of total Sb) in treatment 2 and 0.88, 0.22, 0.06 mg L^{-1}, and bdl (below detection limit) (78% removal of total As and 98.8% removal of total Sb) in treatment 3 (Fig. 3a and b). When ethanol was used as the carbon source, the final As(V), As(III), Sb(V), and Sb(III) concentrations were 3.05, 0.63, 0.08, and 0.03 mg L^{-1} (26.4% removal of total As and 97.8% removal of total Sb) in treatment 5 and 0.09 mg L^{-1}, bdl, 0.03 mg L^{-1}, bdl (98.2% removal of total As and 99.4% removal of total Sb) in treatment 6 (Fig. 4a and b). These results showed that high removal of Sb was achieved with or without the addition of Fe(II) when lactate or ethanol was used as the carbon source. Regardless of which carbon source was used, the addition of Fe(II) not only greatly reduced the residual concentrations of both As(V) and As(III) but also further reduced the residual concentrations of both Sb(V) and Sb(III).

The efficient removal of Sb was consistent with the results previously reported by Wang et al. (2013) and Zhang et al. (2016). It has been proposed that Sb(V) is first reduced to Sb(III) by sulfide, and Sb (III) then reacts with excess sulfide, resulting in a precipitate of Sb_2S_3 (Zhang et al., 2016). The relatively high solubility product of As_2S_3 (log

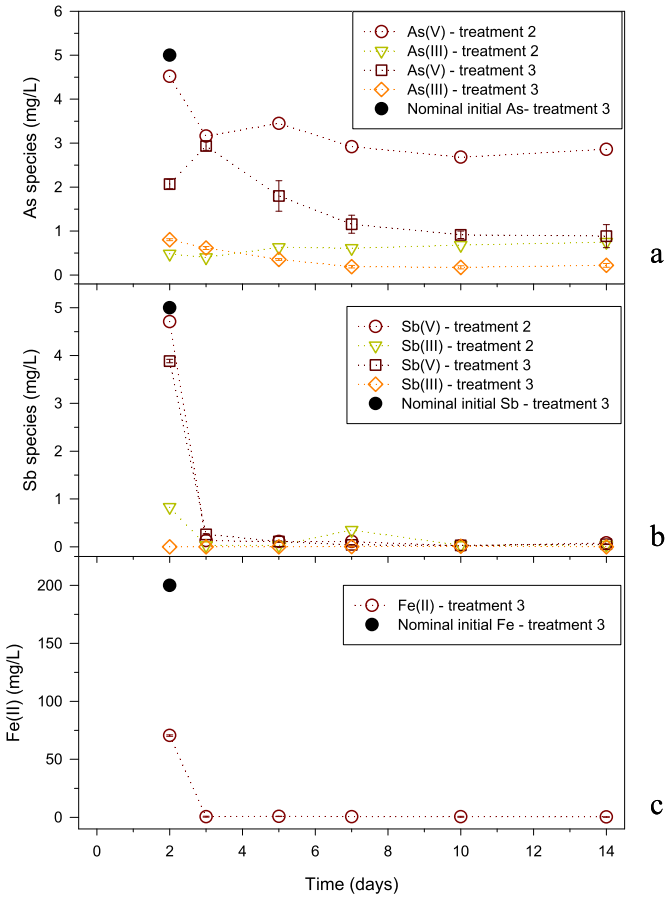

Fig. 3. Removal of As and Sb by SRB grown on lactate.

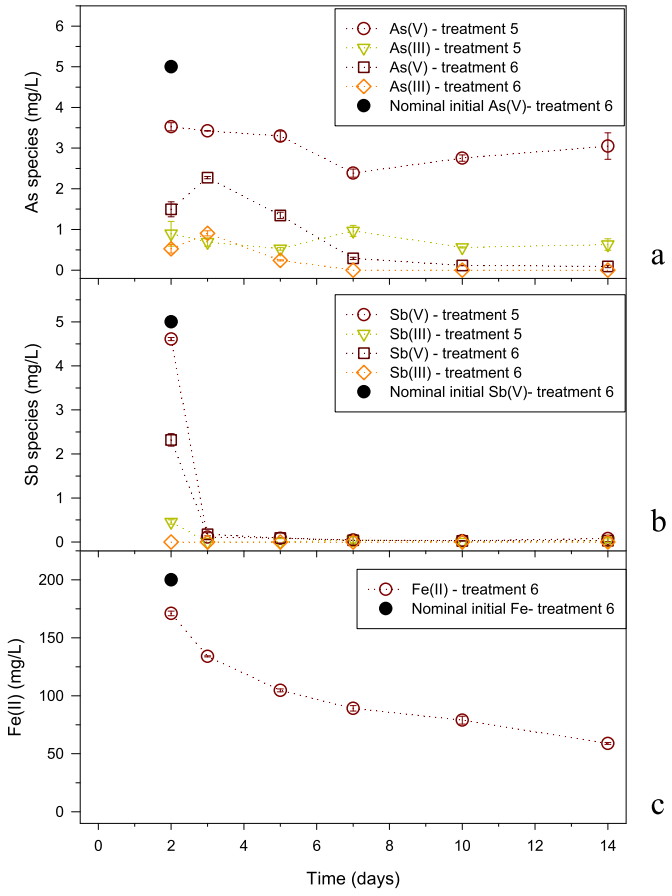

Fig. 4. Removal of As and Sb by SRB grown on ethanol.

$K_s = -11.9$, Eary, 1992) compared to that of Sb_2S_3 (log $K_s = -92.8$, Mane and Lokhande, 2003) indicates that the formation of As_2S_3 is much more difficult than that of Sb_2S_3. Therefore, the low removal of As without the addition of Fe(II) can be related to the relatively high solubility of As_2S_3.

Previous studies have also reported low As removal in the absence of metals. In a sulfidogenic fixed-bed column bioreactor, As removal efficiency was no higher than 8% (Altun et al., 2014). In the biotreatment of As-containing acid mine drainage in an upflow anaerobic sludge blanket reactor, As removal was not detected in the absence of other metals (Sahinkaya et al., 2015). In a bioreactor column for the bioremoval of As and Se using SRB, As removal of 30–60% at pH 6.3 and 40–80% at pH 8.3 was observed (Luo et al., 2008). Previous studies reported the improvement of As removal in the presence of metals (especially Fe(II)). Altun et al. (2014) reported that As removal was increased from 8% to 63% by the addition of 100 mg L^{-1} Fe(II) and was further improved to 85% by the addition of 200 mg L^{-1} Fe(II). Sahinkaya et al. (2015) reported that As removal efficiency increased from 0% when As was the sole contaminant to 98–100% in the presence of Fe, Zn, Ni, and Cu. In a UAPB reactor, As removal by SRB reached 77.5% in the presence of metals such as Cu, Zn, Ni, Fe, Al, and Mg (Jong and Parry, 2003). It has been suggested that, in the presence of other metal(loid)s, their precipitates could also sorb or co-precipitate As (Jong and Parry, 2003; Sahinkaya et al., 2015).

In this study, the 27–27.8% removal of As in the absence of Fe(II) could possibly be related to the co-existence of initial Sb in the wastewater. The sorption/co-precipitation of As by Sb_2S_3 was assumed to have contributed to the removal of As. This view was further confirmed by the co-existence of As in the precipitate of Sb_2S_3 revealed by the TEM analysis later. The high removal of As in the presence of Fe(II) could be associated with Fe(II) precipitates because it has previously been reported that As removal is highly improved by the addition of Fe(II), possibly due to the sorption/co-precipitation of As by FeS precipitate and the formation of FeAsS (Kirk et al., 2004; Luo et al., 2008; Battaglia-Brunet et al., 2012; Altun et al., 2014; Sahinkaya et al., 2015). The FeS produced by SRB has excellent sorptive properties regarding metallic ions (Watson et al., 1995) and As (Wolthers et al., 2005; Teclu et al., 2008).

It is noteworthy that the removal efficiency of As and Sb in the presence of Fe(II) was also related to the carbon source. Because the bacterial activity was relatively low when ethanol was used instead of lactate as a carbon source, the production of biogenic sulfide in treatment 6 was lower than that in treatment 3 (Fig. 2b). However, treatment 6 in the presence of Fe(II) resulted in a higher removal of As (and also Sb) compared to treatment 3 in the absence of Fe(II). For example, the residual As(V), As(III), and Sb(V) concentrations in treatment 3 were 2.1, 0.81, and 3.89 mg L^{-1} at 2 d and 0.88, 0.22, and 0.06 mg L^{-1} at 14 d (Fig. 3a). In contrast, the residual As(V), As(III), and Sb(V) concentrations in treatment 6 were at lower levels of 1.5, 0.53, and 2.32 mg L^{-1} at 2 d and 0.09, bdl, and 0.03 mg L^{-1} at 14 d (Fig. 4a). A comparison of Sb(III) concentrations was not applicable because it was bdl for almost all the sampling intervals in both treatments 3 and 6 (Figs. 3b and 4b). Similar results were also reported in previous studies. Altun et al. (2014) reported that when the influent COD concentration in a bioreactor decreased from 1560 to 780 mg L^{-1}, the sulfide concentration decreased from approximately 475 to 46 mg L^{-1}, but As removal increased from 85% to 96% in the presence of 200 mg L^{-1} Fe (II). Battaglia-Brunet et al. (2012) and Sahinkaya et al. (2015) also reported that As removal efficiency increased at low dissolved sulfide concentrations.

The mechanism for this phenomenon is of significance. In previous studies, it was suggested to be related to the formation and dissolution

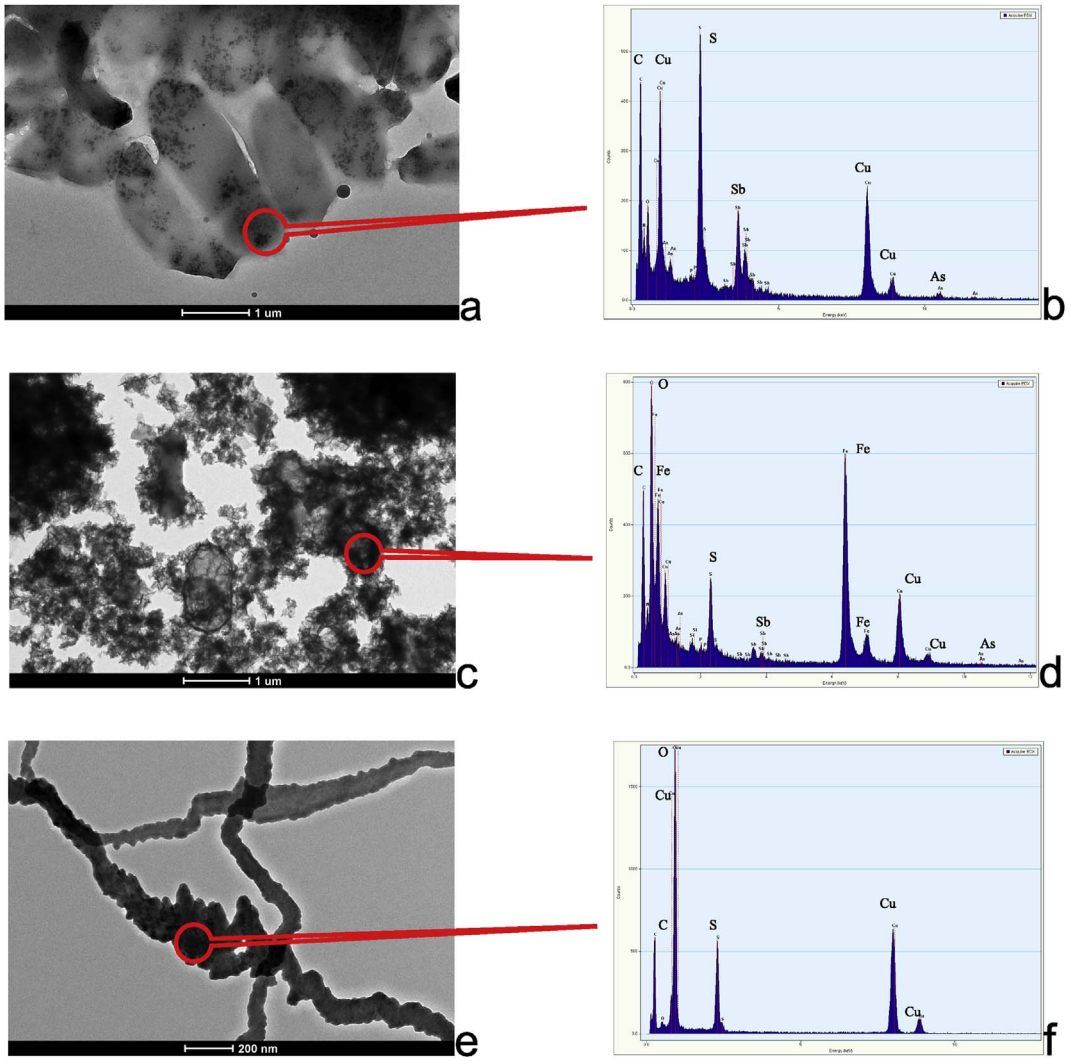

Fig. 5. TEM images and EDS spectra of precipitates (a and b: image and composition of precipitates of treatments without the addition of Fe(II); c and d: with the addition of Fe(II); and e and f: occurrence and EDS evidence of elemental sulfur in precipitates of treatment 6. The signal of Cu originated from the copper support for the sample).

of As_2S_3 because As_2S_3 may dissolve at high sulfide concentrations according to the following reaction (Newman et al., 1997; Battaglia-Brunet et al., 2012; Sahinkaya et al., 2015):

$$3/2\ As_2S_3 + 3/2\ H_2S \rightarrow H_2As_3S_6^- + H^+ \quad (5)$$

In the presence of Fe(II), however, the formation of FeS should be privileged since the log K_s for amorphous FeS (log $K_s = -27.39$, Jong and Parry, 2003) is significantly lower than that for As_2S_3 (log $K_s = -11.9$, Eary, 1992). Additionally, the initial concentration of Fe (II) (200 mg L^{-1}) in both treatments 3 and 6 was much higher than that of As(III), which was lower than 5 mg L^{-1} because As(III) was generated in the reduction of the initial 5 mg L^{-1} As(V). Consequently, the formation of FeS should be much easier than that of As_2S_3 in this experiment. Therefore, the high removal of As achieved by a low sulfide concentration is suggested to be associated with the formation of amorphous FeS instead of the stability of As_2S_3. The low production of sulfide when ethanol was used as the carbon source resulted in a gradual decline of Fe(II) concentrations (Fig. 4c), which was in contrast to the drastic decline of Fe(II) concentrations when lactate was used as the carbon source (Fig. 3c). The gradual decline of Fe(II) concentrations might indicate slow precipitation of FeS, which could have probably enhanced the sorption and co-precipitation of ions and consequently resulted in the high removal of metalloids.

3.4. Characterization of the precipitates

In the TEM images, no significant differences were found between the treatments using lactate and ethanol as carbon sources.

The precipitates from treatments without the addition of Fe(II) were present as amorphous nanoparticles or aggregates of nanoparticles (Fig. 5a). EDS analysis of the aggregates of nanoparticles showed the presence of C, O, N, P, S, and Sb (Fig. 5b). The compositions of C, O, N, and P were considered to have originated from the bacteria. The strong peaks of S and Sb indicated that antimony sulfide (Sb_2S_3) was the product precipitated from the treatment mixtures. Additionally, the EDS analysis also revealed the existence of As in the precipitates of Sb_2S_3 (Fig. 5b), implying that some As was sorbed or co-precipitated by Sb_2S_3.

The precipitates from treatments with the addition of Fe(II) were larger amorphous particles in size compared to those without Fe(II) (Fig. 5c). The EDS analysis showed that the precipitates were mostly composed of C, O, N, P, S, Fe, Sb, and As (Fig. 5d). The strong peaks of Fe and S indicated that the precipitates were primarily composed of FeS. Most of the Sb in the precipitates was possibly present as Sb_2S_3 as indicated above. Additionally, a fraction of Sb in the precipitates was also possibly sorbed or co-precipitated by FeS because the addition of Fe(II) increased the removal of Sb (as mentioned above). EDS analysis showed that the As in the precipitates was highly related to amorphous

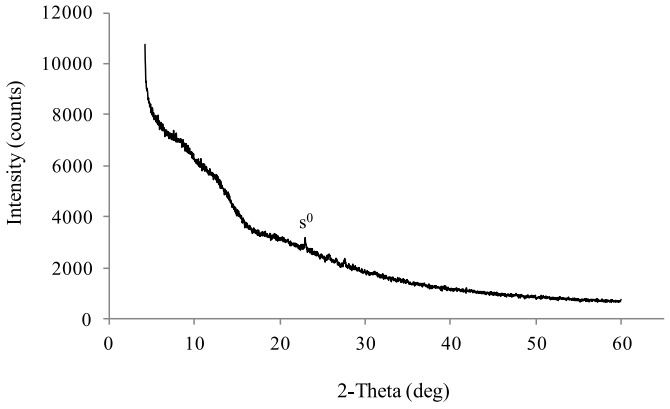

Fig. 6. XRD pattern of precipitates from treatment 2.

FeS, indicating that the removal of As from the wastewater was primarily due to the sorption/co-precipitation by FeS.

The XRD analysis showed no obvious response signals concerning crystal compounds of either As and Sb sulfides or Fe sulfide. However, the XRD analysis indicated the presence of crystal S^0 (Fig. 6) in the precipitates from treatments with the addition of As and Sb. The TEM-EDS analysis also confirmed the presence of elemental sulfur in these precipitates (Fig. 5e and f). The existence of elemental sulfur in the precipitates indicated that, in the reduction of As(V) and Sb(V) by sulfide, the sulfide was oxidized to S^0 (Wang et al., 2013). Similarly, in the reduction of Cr(VI) by sulfide generated by SRB from sulfate, elemental sulfur is also formed from the reaction of sulfide with Cr(VI) (Chang and Kim, 2007; Neculita et al., 2007; Kieu et al., 2011). The possible reactions for the reduction of As(V) and Sb(V) are:

$$HAsO_4^{2-} + H_2S + 2H^+ \rightarrow H_3AsO_3 + S_{(s)} + H_2O \quad (6)$$

$$Sb(OH)_6^- + H_2S + H^+ \rightarrow Sb(OH)_3 + S_{(s)} + 3H_2O \quad (7)$$

4. Conclusions

This study demonstrated the feasibility of the removal of As and Sb from wastewater by using a mixed culture of SRB in batch treatments. When As and Sb were the only metal(loid) ions in wastewater, Sb was efficiently removed, whereas As was only removed to a low extent. The presence of Fe(II) very efficiently improved the removal of As and further increased the removal of Sb from the wastewater. We propose that As was removed through the sorption/co-precipitation by FeS instead of the formation of As_2S_3 precipitates.

The use of ethanol as a carbon source resulted in a higher removal of As and Sb compared to the use of lactate. This was likely attributed to the low production of sulfide, which possibly resulted in the slow precipitation of iron sulfide and consequently enhanced the sorption and co-precipitation of ions.

The results of this study highlight the potential application of SRB treatment to As and Sb pollution generated in mining sites. The use of ethanol instead of lactate as a carbon source showed promise for reducing the operational cost and for noticeably improving As removal in the presence of Fe(II).

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