International Biodeterioration & Biodegradation 115 (2016) 148–155

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Bioremoval of antimony from contaminated waters by a mixed batch culture of sulfate-reducing bacteria

Guoping Zhang [a, *], Xiaoxue Ouyang [b], Haixia Li [a], Zhiping Fu [a], Jingjing Chen [a]

[a] State Key Laboratory of Environmental Geochemistry, Institute of Geochemistry, Chinese Academy of Sciences, China
[b] Surveying and Mapping Institute of Geology and Mineral Resources of Guizhou Development Authority, China

ARTICLE INFO

Article history:
Received 12 January 2016
Received in revised form
22 June 2016
Accepted 22 August 2016

Keywords:
Antimony
Wastewater
Sulfate-reducing bacteria
Bioremediation

ABSTRACT

Bioremediation of metal(loid)-contaminated water could be a cost-effective process. In this work, Sb-polluted water was treated by application of a mixed batch culture of sulfate-reducing bacteria (SRB). Aqueous Sb could be efficiently removed by the SRB over an initial pH range 5–8. The SRB was tolerant of at least 50 mg L^{-1} Sb in solution. With an initial pentavalent Sb (Sb(V)) concentration of 5 mg L^{-1}, batch kinetic variations of the treatment were studied over a 11 d period at an initial pH 7 at 30 °C. A high removal (93%) of the aqueous Sb was achieved. The final products were identified microscopically. Before removal of Sb from solution in this treatment, Sb(V) was first reduced to trivalent Sb (Sb(III)). Hydrogen sulfide was proven to be the reducing agent in this reaction. The SRB were not able to reduce Sb(V) enzymatically. Following the chemical reduction of Sb(V) to Sb(III), the latter reacted with excess sulfide, resulting in the formation of insoluble antimony sulfide (Sb_2S_3). Studies on the sorption of Sb species by dead SRB indicated that, in the batch treatment, sorption by bacteria made a relatively small contribution to the removal of Sb.

1. Introduction

Antimony is highly toxic for biota and humans. A possible synergistic effect of Sb in drinking water has been suggested in cancer development (Gebel, 1997). Antimony is of growing interest to industry. A significant input of Sb into the environment can result from some industrial processes, such as the manufacture of alloys, semiconductors, fire retardants, glass, and polyethylene terephthalate (Filella et al., 2002). Mine tailings are important Sb sources in many areas (Filella et al., 2009; He et al., 2012). For example, China has the most abundant Sb resources in the world (He et al., 2012). Mining in China has generated vast amounts of tailings that contain a high level of Sb. Oxidation of Sb-containing sulfide minerals (e.g., stibnite and pyrite) in the mine tailings remobilizes the Sb that originally existed in minerals. Leaching of tailings by rain and surface water usually results in drainage that contains high concentrations of Sb (Ashley et al., 2003; Casiot et al., 2007). The drainage has negative effects on the quality of the environment and the ecosystem and is therefore a crucial environmental concern.

Antimony has an s^2p^3 outer orbital electron configuration and can display in four oxidation states (−3, 0, +3, and +5) but are mostly found in two oxidation states (+3 and +5) in the environmental systems. It can occurs as $Sb(OH)_6^-$ or $Sb(OH)_3$ in an aqueous environment (Filella et al., 2009; Wilson et al., 2010). In contrast to metals (e.g., Cu, Pb, and Zn), Sb shows high mobility in the surface environment (Bowell and Bruce, 1995; Pokrovsky and Schott, 2002; Cidu and Frau, 2009; Zhang et al., 2009). An elevation of pH facilitates the mobilization of Sb (Ashley et al., 2003). These properties of Sb make its removal by traditional methods (such as the addition of lime) difficult. Therefore, it is important to develop new treatment methods for Sb removal from wastewater.

Various methods have been used for the treatment of wastewaters contaminated with metals and metalloids. Among them, biological treatment has become an important approach. It may involve biosorption, bioprecipitation, and biouptake (Pardo et al., 2003). Among biological methods, the use of sulfate-reducing bacteria (SRB) is a very important one. SRB can reduce sulfate to sulfide while oxidizing a suitable carbon source. The sulfide so generated is able to remove metals by forming metal sulfide precipitates. The use of SRB for generating hydrogen sulfide from sulfate with which to treat pollution by metals such as Cu, Zn, Ni, Cr, U, Fe, Mn and Cd has been studied for decades (Dvorak et al., 1992;

* Corresponding author.
E-mail address: zhangguoping@vip.gyig.ac.cn (G. Zhang).

http://dx.doi.org/10.1016/j.ibiod.2016.08.007
0964-8305/

Jong and Parry, 2003; Azabou et al., 2007; Yi et al., 2007; Pagnanelli et al., 2010). Yet, little research has been done on the use of SRB in remediating Sb pollution. To the best of authors' knowledge, only Wang et al. (2013) has demonstrated the feasibility of using SRB to remove Sb from synthetic Sb-containing wastewater. They proposed that pentavalent Sb (Sb(V)) was reduced by sulfide. They did not examine whether the Sb(V) was reduced by the SRB. In addition, they did not examine the contribution of sorption by the bacteria although sorption by SRB can be important in the treatment of metal pollution (Pagnanelli et al., 2010; Ngwenya and Chirwa, 2010). To optimize the performance of the treatment of Sb pollution by SRB, the specific Sb(V) reducing mechanism, and biosorption of Sb by SRB should be addressed.

In this study, we examined bioprecipitation of Sb in water by a mixed batch culture of SRB. The aim was to demonstrate the effectiveness of this process in removing Sb from aqueous waters. Particularly, the mechanism of transformation of specific Sb species in wastewater and the sorption of Sb species by bacteria were considered.

2. Materials and methods

High-purity deionized water (HPW) (resistivity: 18.2 MΩ cm) was prepared with a Milli-Q system (Millipore, Bedford, USA) and was used throughout the batch experiments. All chemicals were analytical grade.

2.1. Isolation and enrichment of SRB

A mixed culture of SRB was obtained from a stream that receives the drainage from a coal mine in Guiyang, southwest China. Stream sediment was used as inoculum. Approximately 2 g of sediment was collected and immediately mixed with 100 mL of modified Postgate's Medium B (0.5 g L^{-1} K_2HPO_4; 1.0 g L^{-1} NH_4Cl; 0.1 g L^{-1} $CaCl_2 \cdot 6H_2O$; 2.0 g L^{-1} $MgSO_4 \cdot 7H_2O$; 1.0 g L^{-1} Na_2SO_4; 1.0 g L^{-1} sodium lactate; 1.0 g L^{-1} yeast extract; pH = 7.0). The culture medium had been previously purged with a stream of pure nitrogen (99.99% purity) for degassing of oxygen and sterilized by autoclaving at 121 °C for 20 min. After inoculation with stream sediment, the medium was incubated in an incubator (Model 855-ACB, Plas-Labs Inc., China) at 30 °C under static conditions. After 7 d, 10 mL of the resultant culture was transferred to 100 mL of modified Postgate's Medium B. This process was repeated five times. The final culture containing SRB was then employed in batch experiments treating Sb-polluted water.

The molecular characterization of the mixed culture of SRB was conducted in Novogene Inc., Beijing, China. The cells were harvested from 10 mL of the culture by centrifugation at 10,000 rpm for 10 min and twice washed with sterilized deionized water. DNA was extracted with FastDNA® spin kit (MP bio, Santa Ana, USA) following the manufacturer's instructions. DNA was amplified following the protocol described previously (Caporaso et al., 2011). 16S rRNA tag-encoded high-throughput sequencing was carried out in Illumina MiSeq platform according to the methods described by Sun et al. (2014).

2.2. Bioprecipitation experiments

Sb(V) and trivalent Sb (Sb(III)) stock solutions of 500 mg L^{-1} were prepared by dissolving potassium hexahydroxoantimonate ($KSb(OH)_6$, 99.0% purity, Fluka Inc., Steinheim, Germany) and potassium antimonyl tartrate sesquihydrate ($C_4H_2KO_6Sb \cdot 1.5H_2O$, >99%, Acros Inc., New Jersey, USA) separately in HPW. The stock solutions of Sb(V) and Sb(III) were filtered through a pre-sterilized syringe-filter (0.22 μm cellulose membrane, Millipore, USA). In batch experiments of bioprecipitation of aqueous Sb by the SRB, the Sb(V) stock solution was used. In experiments of biosorption of Sb, both the Sb(V) and Sb(III) stock solutions were used.

Culture setups to test the effect of initial pH of the medium on the fate of dissolved Sb were run in 100-mL glass bottles with butyl rubber stoppers. The pH of the modified Postgate's Medium B was adjusted using 1 M HCl or NaOH. First, 1 mL of inoculum of the SRB mixed culture was pre-incubated with 98 mL pre-sterilized modified Postgate's Medium B for 2 d. Then, Sb(V) stock solution was added to the inoculated medium for an initial Sb(V) concentration of 5 mg L^{-1}. Seven days after the addition of the Sb(V) stock solution, residual Sb in solution was determined on an aliquot of syringe-filtered culture. Batch experiments to examine the tolerance of the SRB for Sb in solution were carried out in the same procedure at pH 7. The initial concentrations of Sb(V) were 2.5, 5, 10, 25, 50 and 75 mg L^{-1}, respectively. Batch experiments to compare the performance of the treatment in the presence and in the absence of sulfate were also carried out in the same procedure at pH 7. At the end of the experiment, two aliquots of the culture were collected. One aliquot was used for OD measurement and the other one was syringe-filtered for determination of Sb(V) and Sb(III).

Static batch kinetic experiments were run in 200-mL glass bottles with butyl rubber stoppers. First, 2 mL of inoculum of the SRB mixed culture was pre-incubated with 197 mL pre-sterilized modified Postgate's Medium B for 2 d. Then, 1 mL of Sb(V) stock solution was added to the culture for an initial Sb(V) concentration of 5 mg L^{-1}. After the addition of the Sb(V) stock solution, 10-mL aliquots of culture were collected at intervals of 0, 3, 6 and 12 h, and on days 1, 2, 3, 4, 5, 6, 7, 9, and 11. Each aliquot was divided into two portions, one of which was syringe-filtered. The unfiltered portion was used for pH, Eh and OD measurements. The filtered portion was divided into sub-portions for determination of dissolved sulfate, sulfide, total Sb (Sb(T)) and Sb(III). At the end of a batch experiment, an aliquot of culture was centrifuged at 10,000 rpm for 15 min. The precipitate was washed three times with HPW, resuspended in HPW, recentrifuged and freeze-dried in a vacuum freeze-dryer (Model FD-18, Detianyou Technologies, Beijing, China) for further study.

All the batch experiments above were carried out at 30 °C in triplicate in a parallel mode. We showed in preliminary experiments that a temperature of 30 °C was favorable for the growth of SRB. In control experiments, SRB were omitted.

2.3. Biosorption experiments

To avoid generation of hydrogen sulfide in experiment, sulfate was omitted from modified Postgate's Medium B. The incubation of the culture was the same as that in the static batch kinetic experiments. After 7 d, the culture was autoclaved to kill the bacteria. One milliliter of Sb(V) or Sb(III) stock solution was added to 99 mL of autoclaved culture for an initial Sb(V) or Sb(III) concentration of 5 mg L^{-1}. The mixture was kept at 30 °C for 4 d with intermittent magnetic stirring. Then, the supernatant of the mixture was sampled, filtered, and the concentration of Sb(V) or Sb(III) was determined. The pH of the mixture was monitored at the beginning and end of the biosorption experiments. For a control experiment, the autoclaved solution was filtered (0.22-μm, Millipore, USA) to remove biomass prior to the addition of the stock solution of the Sb species.

2.4. Analyses

The pH and Eh of the supernatant samples were immediately measured with a Denver UB-7 pH-meter and a redox potential