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Removal of antimonate from wastewater by dissimilatory bacterial reduction: Role of the coexisting sulfate

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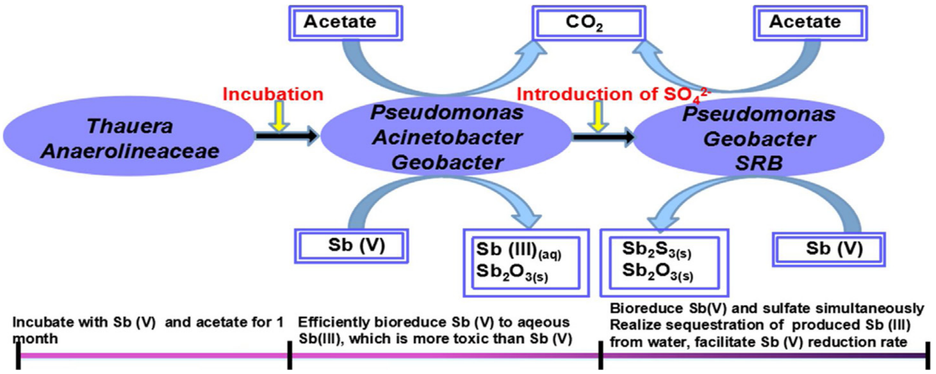
HIGHLIGHTS

- 0.8 mM Sb(V) could be completely reduced with 48.6% Sb(III) recovery within 20 h.
- SO_4^{2-} can be simultaneously reduced to HS^- in Sb-acclimated anaerobic sludge.
- The formed S^{2-} participated in both Sb(V) reduction and Sb(III) precipitation.
- Formation of Sb_2O_3 and Sb_2S_3 precipitates were confirmed.
- SO_4^{2-} stimulated the growth of *Pseudomonas* and sulfate-reducing bacteria.

GRAPHICAL ABSTRACT



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ABSTRACT

The priority pollutant antimony (Sb) exists primarily as Sb(V) and Sb(III) in natural waters, and Sb(III) is generally with greater mobility and toxicity than Sb(V). The bio-reduction of Sb(V) would not become a meaningful Sb-removal process unless the accumulation of produced dissolved Sb(III) could be controlled. Here, we examined the dissimilatory antimonate bio-reduction with or without the coexistence of sulfate using Sb-acclimated biomass. Results demonstrated that 0.8 mM Sb(V) was almost completely bio-reduced within 20 h along with 48.6% Sb(III) recovery. Kinetic parameters q_{max} and K_s calculated were 0.54 mg-Sb mg-DW^{-1} h^{-1} and 41.96 mg L^{-1}, respectively. When the concentrations of coexisting sulfate were 0.8 mM, 1.6 mM, and 4 mM, the reduction of 0.8 mM Sb(V) was accomplished within 17, 9, and 5 h, respectively, along with no final Sb(III) recovery. Also, the bio-reduction of sulfate occurred synchronously. The precipitated Sb_2O_3 and Sb_2S_3 were characterized by scanning electron microscopy coupled with energy dispersive spectrometer, X-ray diffraction, and X-ray photoelectron spectroscopy. Compared with bacterial compositions of the seed sludge obtained from anaerobic digestion tank in a wastewater treatment plant, new genera of *Pseudomonas* and *Geobacter* emerged with large proportions in both Sb-fed and Sb-sulfate-fed sludge, and a small portion of sulfate reduction bacteria emerged only in Sb-sulfate-fed culture.

1. Introduction

Antimony (Sb), is currently a toxic metalloid of emerging global environmental concern [1–3]. As the ninth-most mined metal worldwide [4], Sb is primarily released into waters, soils and sedi-

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ments by mining and smelting activities [5,6], especially in China, which produces the greatest amount of antimony (≥80%) and possesses the largest antimony mine (Xikuangshan in Hunan Province) in the world [7]. Also, the wide use of Sb in manufacture of flame retardants, semiconductors, alloys for ammunition and storage batteries, additives for glassware and ceramics, paint pigment, and catalyst increased its transfer into the environment [8]. Though the ecotoxicity of Sb is insufficiently known [9,10], trivalent Sb compounds are generally considered to be more toxic than pentavalent Sb, similar to that of As [6,11–13]. Considering its toxicity and suspected carcinogenicity to human, Sb and its compounds have been labeled as priority pollutants by the United States Environmental Protection Agency (US-EPA) [14] and the European Union [15] since 1970s, and in 2009 the US-EPA has imposed a maximum contaminant level (MCL) of 6 $\mu g\,L^{-1}$ in drinking water [16].

Sb can exist in four different oxidation states (V, III, 0, −III), with antimonate (Sb(V)) and antimonite (Sb(III)) being the common oxidation states observed in aqueous media [17]. The existing species mainly depend on the redox property of the system. Sb(V) is the predominant species in oxic to slightly reducing systems, while Sb(III) appears to be thermodynamically favored form in anoxic settings. While in natural Sb-contaminated waters, both Sb(III) and Sb(V) have been observed to occur outside of thermodynamically stable setting largely attributed to biological activities [8]. In addition, antimony is a strongly chalcophile element and thus most frequently occurs as mineral Sb_2S_3 (stibnite) and Sb_2O_3 (the transformation product of stibnite) [8,18], both of which have limited solubility in aqueous solutions and can precipitate readily over a wide range of pH [19]. As such, there is a great interest in realizing the immobilization of aqueous Sb via the bio-reduction of Sb(V) to Sb(III) coupling to the subsequent formation of Sb(III) precipitates.

Several studies have documented the feasibility of Sb(V) bioreduction or biomethylation by bacteria, fungi, or even protozoa in the last two decades [20–24]. In anoxic sediments, a novel anaerobic microbiological respiration that utilizing antimonate and organic carbon as the terminal electron acceptor (TEA) and electron donor, respectively, was observed [1], and two different kinds of involved antimonate-respiring bacteria (strain MLFW-2 and JUK-1) were successively identified and isolated [25,26]. And recently, the process of autotrophic antimonate bio-reduction using hydrogen instead of organic carbon as the electron donor was also reported [27]. Therefore, the microbe-mediated process proved feasible to realize the reduction of Sb(V) to Sb(III) in reducing environment, while if the yielding Sb(III) would completely precipitate seemed a significant concern when applying the process for the detoxification of antimony. According to the solubility of Sb_2O_3 in aqueous solutions [19,28] and the E_h–pH diagram for the Sb–S–H_2O system on the basis of thermodynamic calculations [17,29,30], under reducing conditions, antimony is predominantly present as soluble $Sb(OH)_3$ in the absence of sulfur, but replaced by solid Sb_2S_3 in the presence of sulfur at circumneutral pH. Therefore, the presence of sulfide may provide a promising niche for the precipitation and separation of Sb(III) during the bio-reduction of Sb(V). Indeed, the abiotic reduction of Sb(V) by dissolved sulfide has been observed under reducing conditions with the formation of Sb_2S_3 precipitation during the process [31]. In another study, the bio-reduction of sulfate by sulfate-reducing bacteria (SRB) and the redox reaction of Sb(V) and sulfide have been combined to realize the removal of Sb(V) from Sb mine drainage in the form of stibnite [32]. Despite these encouraging studies, the relatively slow reduction rate (about 0.1–4 $mg\,L^{-1}\,d^{-1}$) at circumneutral pH was the major obstacle with abiotic reduction. Overall, although these data provide evidence for a potent bio-remediation process of Sb-contamination, the role of sulfate in the process is not clear yet, also little is known about antimonate bio-reaction kinetics and involved microbial community.

Actually, mining and smelting wastewater containing high levels of Sb also commonly contain high concentrations of sulfate. For example, according to a survey on the water components of three rivers around the Xikuangshan Sb mine in Hunan Province, China [33], the concentrations of sulfate (4.86–322.2 $mg\,L^{-1}$) in contaminated rivers were approximately as high as 100 times those of Sb (2–6384 $\mu g\,L^{-1}$). Therefore, the main goals of the experiments included (i) to identify the processes of microbe-mediated Sb(V) reduction and associated reaction kinetics, (ii) to evaluate the influence of sulfate concentration on the Sb(V) reduction rate, (iii) to identify the solid produced from Sb(V) reduction, and (iv) to examine if different experimental conditions induce changes in the microbial community structure. The results of this study provide further insight into the behavior and fate of Sb in the anoxic aquatic environment and fundamental information for the development of biological treatment of water containing Sb and sulfate.

2. Materials and methods

2.1. Source of biomass

Originally, anaerobically-digested sludge taken from Quyang Wastewater Treatment Plant in Shanghai, China, was used as the inoculum for the enrichment of Sb(V) reducing culture. The total Sb content of the seed sludge was measured (using aqua regia for sludge digestion first and further analysis by inductively coupled plasma mass spectrometry according to the method in Section 2.4.) below detection limit.

2.2. Preincubation

The basal salts medium (BSM) was prepared using deionized water and contained the following ingredients (analytical grade) per liter: $K_2HPO_4{\cdot}3H_2O$ 0.75 g, $NaH_2PO_4{\cdot}H_2O$ 0.4 g, $NH_4H_2PO_4$ 0.25 g, $MgCl_2{\cdot}6H_2O$ 0.083 g, $CaCl_2{\cdot}2H_2O$ 0.01 g, and 1 mL trace mineral solution (2 g of $FeCl_2{\cdot}4H_2O$, 0.5 g of $MnCl_2{\cdot}4H_2O$, 0.05 g of $ZnCl_2{\cdot}7H_2O$, 0.09 g of $AlCl_3{\cdot}6H_2O$, 0.03 g of $CuCl_2{\cdot}2H_2O$, 2 g of $CoCl_2{\cdot}6H_2O$, 0.05 g of H_3BO_3, 0.05 g of $NiCl_2{\cdot}6H_2O$, 0.03 g of $Na_2MoO_4{\cdot}2H_2O$, 0.05 g of $NaSeO_3{\cdot}5H_2O$, 0.05 g of $Na_2WO_4{\cdot}2H_2O$, and 0.3 g of Na_2EDTA per liter) [34]. The pH value was maintained at 7.0 ± 0.2 using phosphate buffer included in the media. Sterile stock solutions of ACS reagent-grade sodium acetate, $KSb(OH)_6$ and Na_2SO_4 were prepared using deionized water and added in millimolar concentrations as discussed for each experiment below.

The enrichments were initiated by adding 0.2 L of the gravity-settled seed sludge and 1.4 L of BSM amended with 1 mM $KSb(OH)_6$ and 2 mM sodium acetate to an autoclaved 2-L glass serum bottle, and then the culture was flushed with pure N_2 for 15 min (reducing the dissolved oxygen level to less than 1 $mg\,L^{-1}$) before sealed with rubber septa and aluminum crimp cap. Subsequently, the culture was incubated in a thermostat orbital shaker at 150 rpm and 35 °C. Periodically, the enrichments were subcultured by successively discarding about two thirds of the supernatant, replacing with the same volume of fresh BSM containing $KSb(OH)_6$ and sodium acetate, and incubating as described above. During incubation, the changes in dissolved total Sb, Sb(V), and Sb(III) were monitored by analyzing the collected subsamples. After one month of acclimation, the consortium can finish the reduction of 1 mM of Sb(V) within 24 h and was used as the inoculum for further experiments. All the manipulations during the experiment were performed in an anaerobic chamber.

2.3. Experimental set-up

2.3.1. Sb(V) reduction experiments in the absence of $SO_4{}^{2-}$

Aliquots of the inocula were dispensed in triplicate into each of three sets of 250 mL glass serum bottles containing sterile