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Autotrophic antimonate bio-reduction using hydrogen as the electron donor

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ABSTRACT

Antimony (Sb), a toxic metalloid, is soluble as antimonate (Sb(V)). While bio-reduction of Sb(V) is an effective Sb-removal approach, its bio-reduction has been coupled to oxidation of only organic electron donors. In this study, we demonstrate, for the first time, the feasibility of autotrophic microbial Sb(V) reduction using hydrogen gas (H_2) as the electron donor without extra organic carbon source. SEM and EDS analysis confirmed the production of the mineral precipitate Sb_2O_3. When H_2 was utilized as the electron donor, the consortium was able to fully reduce 650 μM of Sb(V) to Sb(III) in 10 days, a rate comparable to the culture using lactate as the electron donor. The H_2-fed culture directed a much larger fraction of it donor electrons to Sb(V) reduction than did the lactate-fed culture. While 98% of the electrons from H_2 were used to reduce Sb(V) by the H_2-fed culture, only 12% of the electrons from lactate was used to reduce Sb(V) by the lactate-fed culture. The rest of the electrons from lactate went to acetate and propionate through fermentation, to methane through methanogenesis, and to biomass synthesis. High-throughput sequencing confirmed that the microbial community for the lactate-fed culture was much more diverse than that for the H_2-fed culture, which was dominated by a short rod-shaped phylotype of *Rhizobium* (*α-Protobacteria*) that may have been active in Sb(V) reduction.

1. Introduction

Metalloid Antimony (Sb), the ninth most mined metal (Scheinost et al., 2006), is widely used in manufacture of semiconductors, flame retardants, alloys for storage batteries and catalysts (Filella et al., 2002a). Antimony has serious negative effects on human health. For instance, prolonged exposure to antimony will induce pneumoconiosis, abdominal pain, diarrhea, dermatitis, spontaneous abortion and increased blood pressure (Sundar and Chakravarty, 2010). Antimony also has acute toxicity, which can damage the gastrointestinal system: e. g., oral exposure to antimony at relatively high concentrations can lead to burning stomach pains and colic (Sundar and Chakravarty, 2010). The U. S. EPA has established a maximum contaminant level (MCL) for Sb in drinking water at 6 μg/L (USEPA, 2009). China produces the most antimony in the world (88%), and Hunan province possesses the most abundant antimony reserves (Liu et al., 2010). Wastewaters from mines and smelting factories contain large amounts of antimony and frequently are discharged directly to receiving waters in China (Liu et al., 2010; He et al., 2012).

The common oxidation states of antimony are antimonate (Sb(V)), antimonite (Sb(III)), elemental antimony (Sb^0), and antimonide (Sb(-III)). Sb(V) and Sb(III) are the states most frequently observed in natural water, usually in the form of $Sb(OH)_6^-$ and $Sb(OH)_3$, respectively (Filella et al., 2002b). Although trivalent antimony compounds are generally considered to be more toxic that the pentavalent state, biological reduction of Sb(V) to Sb(III) may have significance as a remediation technology for Sb contamination, because Sb(III) can readily precipitate with sulfide or be strongly adsorbed by Fe(III) hydroxides at neutral pH; thus, Sb(III) easily can be removed by centrifugation or filtration (Xiao et al., 2013).

Several physico-chemical treatment technologies are common for Sb(V) removal, such as adsorption on iron oxides or nano

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materials, coagulation, and electrochemical methods (Miao et al., 2014; Shan et al., 2014; Wu et al., 2010; Lan et al., 2014). Though biological Sb(V) reduction has gained interest in the past decade, the process remains poorly understood and was not demonstrated until very recently. Kulp et al. (2014) first reported the anaerobic microbial reduction of Sb(V) when they incubated Sb(V) with sediments collected from a stibnite mine. Abin and Hollibaugh (2014) isolated a Bacillales order strain (MLFW-2) able to reduce Sb(V) using lactate as the electron donor. Wang et al. (2013) employed sulfate-reducing bacteria (SRB) to convert Sb(V) to Sb (III) using lactate as electron donor. So far, all the Sb(V) microbial reduction studies reported were performed by the heterotrophic microorganisms using organic carbon as the carbon source and electron donor.

Chemolithotrophic microorganisms obtain energy from the oxidation of inorganic compounds, and many of them are autotrophs because they obtain their carbon from CO_2 (Madigan et al., 2009). For example, hydrogenotrophic denitrification involves denitrifying bacteria (DB) that reduce nitrate (NO_3^-) or nitrite (NO_2^-) to nitrogen gas (N_2) while oxidizing hydrogen (H_2) as the electron donor (Mansell and Schroeder, 2002). Thus, the chemoautotrophic process does not need addition of organic carbon, which makes wastewater treatment safer, simpler, more reliable, and less costly (Lee and Rittmann, 2002; Nerenberg et al., 2002). Using H_2 as the electron donor for respiration is a promising pathway for metals and metalloid reduction, because H_2 is inexpensive and non-toxic, leaves no residual organic substrate, and has been applied for microbiological reductions of various oxidized contaminants, such as NO_3^-, perchlorate (ClO_4^-), selenate (SeO_4^{2-}), chromate (CrO_4^{2-}) and arsenate (AsO_4^{3-}) (Nerenberg et al., 2002; Chung et al., 2006a, b; Lai et al., 2014; Marsh and Mclnerney, 2001; Santini et al., 2002; Zhao et al., 2011). The H_2-based membrane biofilm reactor (MBfR), in which H_2 is transferred through the hollow fiber membrane to biofilm attached on the fiber to drive the microbial respiration, is a promising technical approach for bioreduction of oxidized contaminants (Lee and Rittmann, 2002; Nerenberg et al., 2002; Chung et al., 2006a, b; Lai et al., 2014; Marsh and Mclnerney, 2001; Zhao et al., 2011). So far, no study has reported microbial Sb(V) reduction coupled to the oxidation of H_2 as the electron donor.

In this study, we enriched a microbial consortium able to reduce Sb(V) using H_2 as the sole added electron donor, and we studied the stoichiometry of Sb(V) reduction using H_2 or lactate as the electron donor. In particular, the reduction patterns and the metabolic products for the two donors were compared. Energy dispersive X-ray analysis (EDS) and scanning electron microscopy (SEM) were used to identify the reduction product. We also characterized the bacterial communities in both cultures using high-throughput sequencing. These insights document the novel microbial process of Sb(V) reduction and removal by H_2-oxidizing autotrophs, providing information that gives a solid foundation for developing biological treatment of water containing Sb(V).

2. Materials and methods

2.1. Sediment sample collection

We collected sediment samples from a flooded mine pit located at the Zitong mine (N 118.7E, 29.6N), an antimony mine in Hangzhou, China. Anoxic sediments were collected using a sediment sampler, and stored on ice in plastic bags prefilled with nitrogen gas (N_2) before arriving at Zhejiang University.

2.2. Preincubation

The medium pH was adjusted to 7.0 ± 0.2 and contained the following ingredients (analytical grade) per L of deionized H_2O: NaCl 0.2 g, $MgCl_2 \cdot 6H_2O$ 0.203 g, $NaHCO_3$ 0.2 g, $NaH_2PO_4 \cdot 2H_2O$ 1.44 g, $Na_2HPO_4 \cdot 12H_2O$ 2.164 g, $CaCl_2$ 1.35 mg, NH_4Cl 3.15 mg, $FeCl_3 \cdot 6H_2O$ 1 mg, 5 mL vitamin solution (Oremland et al., 1994) and 1 mL trace element solution which contained the same trace salts in Zhao et al. (2011). The medium was continuously sparged with N_2–CO_2 (95:5, vol/vol) for 25 min prior to use. Sterile stock solutions of sodium lactate (1 mol/L) and $KSb(OH)_6$ (1000 mg Sb/L) were dissolved in ultrapure water and kept anaerobically as described above.

We added 0.1 g sediment sample into two 120-mL glass serum bottles holding 60 mL of medium, sparged them with $N_2 + CO_2$ for 15 min, tightly closed the bottles with butyl rubber stoppers, and sealed them with crimped aluminum caps. $KSb(OH)_6$ was then added to the medium at a final concentration of 1 mM serving as electron acceptor, and 20 mL of H_2 was injected into one bottle while 0.15 mmol of lactate was added into another serving as the sole electron donor afterwards. The H_2 or lactate was provided in excess of the mass needed to fully reduce all Sb(V) to Sb(III). Then the culture was incubated at 30 °C and in the dark in order to avoid any oxygenic photosynthesis and keep the cultures anaerobic (Brennan and Owende, 2010). When both consortiums were able to reduce all of 1 mM of Sb(V) in 14 days, we chosen the H_2 pre-incubated consortium as the inoculum to do subsequent experiments.

2.3. Sb(V) reduction experiments with H_2 or lactate as the electron donor

We transferred 25 mL medium, 1 mL ATCC vitamin supplement and 80.5 mg/L (0.66 mM) of Sb(V) into 120-mL glass serum bottles. Before transferring 15 mL of inoculum culture into each bottle, we added 1.67 mM lactate (final concentration) or 9.2 mL (5.1 mM) of H_2 to each bottle. Based on 12 mol e^- per mol of lactate and 2 mol e^- per mol of H_2, the concentration of lactate and H_2 were the same in terms of electron donating equivalents (800 μmol electron equivalents) and 15 times the stoichiometric concentration needed to fully reduce Sb(V) to Sb(III). The working volume was 40 mL of liquid with 80 mL of headspace. 0.68 mM Sb(V) was re-introduced after Sb(V) was completely consumed, which occurred at day 10. We also set up a positive control inoculated with the same consortium, but without providing any electron donors. Another two bottles inoculated with same autoclaved consortium and supplied by the same amount of H_2 or lactate, were as negative controls. All experiments were performed in duplicates, and results are presented as the average values from the two runs.

2.4. Chemical analysis

We took liquid samples using 200-μL syringes and immediately filtered them through a 0.2-μm membrane filter (LC + PVDF membrane, Shanghai Xinya, China). The liquid samples were centrifuged at 15,000 g for 10 min to remove precipitated Sb(III) and assayed the liquid portion for Sb(V) + Sb(III) using HPLC-ICP-MS (Nexlon 300X, PekinElmer, USA) (Liu et al., 2010). HPLC equipped with an anion exchange column (PRP-X100, 250 mm, 10 μm, Hamilton) was used to separate Sb(V) and Sb(III). The mobile phase was 20 mM EDTA and 2 mM potassium hydrogen-phthalate at pH 4.5, and it was delivered at a constant flow rate of 1 ml/min. We run the inductively coupled plasma-mass spectrometry in standard mode for Sb determination.

The organic acids such as lactate, acetate, formate, and propionate were determined by high-performance liquid chromatography (HPLC, Agilent 1200 series, Agilent Technologies) as described by Parameswaran et al. (2011). Methane (CH_4) was measured by