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Characterization of diversified Sb(V)-reducing bacterial communities by various organic or inorganic electron donors

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ABSTRACT

This study aims to enrich Sb(V)-reducing bacterial communities from Sb-contaminated soils using various electron donors for bioremediation of Sb-contaminated sites and recovery of Sb from wastewater. When the organic electron donors were used, Sb(V) reduction rates were 2–24 times faster but electron recoveries were 24–59% lower compared to the culture using inorganic electron donor. The morphological crystallizations of the antimony-reduced precipitates were completely different depending on the electron donor. Different microbial populations were enriched with various electron donors but most commonly, only *Proteobacteria* and *Firmicutes* phyla were enriched from a diversified soil microbial community. *Geobacter* sp. seemed to be an important bacterium in organic electron donors-fed cultures whereas an unclassified *Rhodocyclaceae* was dominant in inorganic electron donor-fed cultures. The results indicated that organic electron donors especially sugar groups were preferable options to obtain rapid Sb(V)-reduction whereas inorganic electron donor like H_2 was better option to achieve high electron recovery.

1. Introduction

Antimony (Sb) is a naturally occurring element in Earth's crust. Sb is frequently found as stibnite (Sb_2S_3), which is the most dominant form in Sb mineral mines (USGS, 2016). Sb and its compounds have been considered highly toxic chemicals. The World Health Organization (WHO) set the maximum acceptable concentration of Sb in drinking water at $5\,\mu g\,L^{-1}$ (WHO, 2003). The United States Environmental Protection Agency (US EPA) subsequently regulated the maximum contamination level of Sb in drinking water at $6\,\mu g\,L^{-1}$ as the national drinking water standard (US EPA, 2009).

In spite of its toxicity, Sb has been used in medicine and cosmetics since ancient times. Currently, Sb was considered a key element in electronics and mechanical engineering industry. It is used in flame retardants, plastics, pigments, glassware, ceramics, and alloys. Sb has created a global dilemma because of its multi-faceted uses and toxicity (Herath et al., 2017). The increase in Sb production along with its insufficient recovery has accelerated Sb contamination in surrounding soils and waters. In the aqueous phase, Sb commonly exists as Sb(V) (antimonate) and Sb(III) (antimonite). Aqueous Sb(V) is less toxic and more stable than Sb(III) in oxidative environments (Filella et al., 2002). However, Sb(III) can readily precipitate with sulfide (Zhu et al., 2018) and form solid state $Sb(OH)_3$ (Filella et al., 2002). Conversion of the redox state of Sb might result in different benefits in terms of Sb remediation.

Microbiological control of the redox state of Sb was recently addressed in many studies (Li et al., 2016, 2015, 2013; Nguyen and Lee, 2015; Terry et al., 2015; Wang et al., 2015). Even though the microbial Sb(III) oxidase gene was defined and investigated in some bacteria (Li et al., 2015; Wang et al., 2015), the investigation on microbial Sb(V) reduction has been limited in recent studies on basic metabolisms of Sb (V)-reducing bacteria (Abin and Hollibaugh, 2014; Kulp et al., 2014; Lai et al., 2016; Nguyen and Lee, 2014). Abin and Hollibaugh (2014) successfully isolated a bacterium, later designated as ***Desulfuribacillus stibiiarsenatis*** strain MLFW-2 (Abin and Hollibaugh, 2017) that can produce Sb_2O_3 microcrystals while using lactate as the electron donor. In contrast, Nguyen and Lee (2014) produced amorphous Sb_2O_3 nanoparticles by the heterotrophic growth of *Sinorhizobium* sp. JUK-1 with acetate as the electron donor. Recently, Lai et al. (2016) obtained the autotrophic Sb(V) reduction with H_2 as the electron donor in a microbial mixed culture enriched from the sediments dominated by *Rhizobium*. The Sb_2O_3 obtained in that study was amorphous nanoparticles in the cultures fed with H_2 and lactate.

This study aims to select electron donors useful for Sb(V) reduction by evaluating Sb(V)-reduction rate and electron recovery depending on external electron donor sources. Both heterotrophic growth with

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various organic compounds ranging from low-carbon chains (acetate) to high-carbon chains (glucose) and autotrophic growth with H_2 as the electron donor were investigated using an inoculation source from the Sb-contaminated soil collected near a Sb-producing factory.

2. Materials and methods

2.1. Soil sample collection and pre-incubation

The Sb-contaminated soil used in this study was collected in the summer near a Sb-producing plant in Chungcheongnam-do, South Korea. The soil sample was transported to the laboratory and kept in a refrigerator at 4 °C for subsequent experiments. A portion of the soil sample was air-dried and digested by aqua regia to determine the total Sb concentration. The supernatant after digestion was serially diluted and subjected to a laser ablation microprobe-inductively coupled plasma-mass spectrometer (LAM-ICP-MS Perkin Elmer, Elan DRC-e, UP-213, Massachusetts, USA) to determine the Sb concentration. The total Sb concentration in soil was 3611 mg kg^{-1}.

The pre-incubation was started by adding 1 g soil into a 120-mL serum bottle containing 100 mL mineral salt medium. The mineral salt medium used in this study contained 0.5 g L^{-1} $NaHCO_3$, 0.15 g L^{-1} K_2HPO_4, 0.1 g L^{-1} KH_2PO_4, 0.1 g L^{-1} NH_4Cl, 0.001 g L^{-1} $CaCl_2{\cdot}2H_2O$, 0.001 g L^{-1} $MgCl_2{\cdot}6H_2O$, and 0.001 g L^{-1} $FeSO_4{\cdot}7H_2O$. The pH of the medium was adjusted to 7.0. Sb(V) was added at the concentration of 1 mM from the stock solution of $KSb(OH)_6$. Organic electron donors such as acetate, lactate, propionate, butyrate, glucose, and inositol were added into the pre-incubation medium at the concentration of 2 mM. The unique inorganic electron donor, H_2, was provided by purging the headspace of the serum bottle (20 mL) with a gas mixture containing 80% H_2 and 20% CO_2. The equivalent amount of H_2 supplied to the culture was approximately 700 μmol. The headspace of the cultures supplied with organic electron donors was purged with argon gas to maintain anaerobic conditions. The pre-incubation was continued at ambient temperature (25 °C) for 10 d.

2.2. Sb(V) reduction with various electron donors

Inoculum (10 mL) taken from each pre-incubation chamber was inoculated into a fresh medium containing the same ingredients as the pre-incubation experiment. The initial Sb(V) concentration was maintained at 1 mM by adding a stock solution of $KSb(OH)_6$. Organic electron donors were added at the concentration of 2 mM whereas H_2 was added at 700 μmol. The final volume of each culture was 100 mL. Samples (5 mL) were periodically taken from each culture and filtered through 0.2-μm syringe filters for analysis. When Sb(V) in all cultures was reduced, an amount of Sb(V) similar to the initial Sb(V) concentration was added into each culture without any further addition of electron donor sources (E-Supplementary data). The Sb(V) reduction with the remaining electron donors in each culture was continuously monitored until all of the additionally-supplied Sb(V) was depleted.

2.3. Scanning electron microscopy and energy dispersive X-ray spectroscopy analysis

Micro-crystal precipitates produced by microbial Sb(V) reduction according to electron donors were characterized using scanning electron microscopy (SEM). The medium from each culture was centrifuged at 5000 rpm for 1 min to collect the precipitates of Sb(III). The precipitates were air-dried in a desiccator at ambient temperature (25 °C). The samples were coated with Pt prior to the field emission-scanning electron microscope (FE-SEM SUPRA25; Zeiss, Oberkochen, Germany) analysis. Elemental components of the precipitates were analyzed by energy dispersive X-ray spectroscopy (EDS) using an EDAX instrument (EDAX Inc., Mahwah, NJ, USA) attached to the Zeiss FE-SEM SUPRA25 microscope.

2.4. Microbial community analysis

Microbial community changes depending on electron donors were clarified in detail by 454 GS-FLX pyrosequencing. At the end of the experiment, biomass from each culture was collected by centrifuging 10 mL of the medium at 5000 rpm for 5 min. Genomic DNA was extracted using a PowerSoil™ DNA isolation kit (Mo Bio Lab., Carlsbad, CA, USA) according to the manufacturer's instructions. The bacterial 16S rRNA gene was amplified and introduced to the pyrosequencing by the GS-FLX Titanium Sequencing System (Roche, Branford, CT, USA) as described previously (Park et al., 2017). Short reads were assembled using FLASH (Mag and Salzberg, 2011). Operational Taxonomic Unit (OTU) analysis was performed using CD-HIT-OTU (Li et al., 2012). The rDNA tool (Schloss et al., 2009) was also used during preprocessing and clustering. Taxonomic diversity analysis of the community sequencing data was performed using QIIME (Caporaso et al., 2010). Pyrosequencing and data analysis were carried out by Macrogen Company (Daejeon, South Korea).

2.5. Analytical techniques and calculations

Sb(V) concentrations were determined by an ion chromatography system (ICS-1000, Dionex, Sunnyvale, CA, USA) based on a previous study (Nguyen et al., 2016a). The Sb(V) peak was detected at 2.4 min right after the injection peak and a linear calibration curve ($R^2 > 0.99$) was obtained for Sb(V) concentrations in the range of 10–500 μM. Total soluble Sb concentration was determined by an inductively coupled plasma-mass spectrometer (ICP-MS; Agilent Technologies 7700 series, Wilmington, DE, USA). The Sb(III) concentration was calculated as the difference between the total Sb and Sb(V) concentrations.

Glucose, inositol, and organic acids, such as formate, acetate, lactate, propionate, and butyrate, were analyzed by a high-performance liquid chromatography system (Dionex Ultimate3000, ThermoFisher Scientific Inc., Waltham, MA, USA), set up and operated as described in another study (Nguyen et al., 2016b). Under the same conditions, inositol was eluted at 10.89 min and a linear calibration curve ($R^2 > 0.99$) was obtained in the range of 0.1–2 mM.

H_2 gas in the headspace of the serum bottles was analyzed using a gas chromatography system (GC YL6500, Young Lin Instrument, Anyang, South Korea) coupled with a thermal conductivity detector which is maintained at a temperature of 150 °C.

Electron distribution and recovery during Sb(V) reduction were calculated according to an equation suggested by a previous study (Lai et al., 2016). The number of electron equivalents per mole was calculated as 24 for glucose and inositol, 20 for butyrate, 14 for propionate, 12 for lactate, 8 for acetate, and 2 for formate and H_2.

3. Results and discussion

3.1. Heterotrophic Sb(V)-reduction with various organic electron donors

Microbial Sb(V) reduction was observed using acetate, lactate, glucose, and inositol (Fig. 1) whereas the growth of Sb(V)-reducing bacteria was not observed when propionate and butyrate were used (E-Supplementary data). Sb(V), propionate, and butyrate remain constant during the incubation. The pattern of Sb(V) reduction and electron donor consumption were typically different from other organic electron donors. Simple organic compounds such as acetate were entirely oxidized to produce CO_2 whereas other longer carbon-chain organic compounds such as lactate, glucose, and inositol were simultaneously oxidized and fermented to produce other byproducts such as H_2, formate, acetate, and propionate. The time for Sb(V) depletion and Sb(III) production was also different for various organic electron donors.

With acetate as the electron donor (Fig. 1a), acetate consumption started right after the incubation whereas Sb(V) reduction started after 1 d of incubation. All of Sb(V) was depleted after 5 d of incubation. The

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