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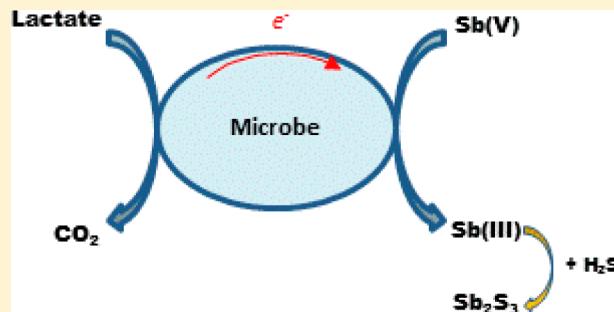
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ABSTRACT: Microbiological reduction of millimolar concentrations of Sb(V) to Sb(III) was observed in anoxic sediments from two freshwater settings: (1) a Sb- and As-contaminated mine site (Stibnite Mine) in central Idaho and 2) an uncontaminated suburban lake (Searsville Lake) in the San Francisco Bay Area. Rates of Sb(V) reduction in anoxic sediment microcosms and enrichment cultures were enhanced by amendment with lactate or acetate as electron donors but not by H₂, and no reduction occurred in sterilized controls. Addition of 2-¹⁴C-acetate to Stibnite Mine microcosms resulted in the production of ¹⁴CO₂ coupled to Sb(V) reduction, suggesting that this process proceeds by a dissimilatory respiratory pathway in those sediments. Antimony(V) reduction in Searsville Lake sediments was not coupled to acetate mineralization and may be associated with Sb-resistance. The microcosms and enrichment cultures also reduced sulfate, and the precipitation of insoluble Sb(III)-sulfide complexes was a major sink for reduced Sb. The reduction of Sb(V) by Stibnite Mine sediments was inhibited by As(V), suggesting that As(V) is a preferred electron acceptor for the indigenous community. These findings indicate a novel pathway for anaerobic microbiological respiration and suggest that communities capable of reducing high concentrations of Sb(V) commonly occur naturally in the environment.



INTRODUCTION

Microbiological oxidation and reduction (redox) reactions directly control the chemical speciation of redox-sensitive toxic metalloids, such as As, Se, or Te, thereby affecting the mobility, toxicity, and bioavailability of these elements in the environment (e.g., ref 1). Redox reactions involving metalloids are conducted by phylogenetically diverse prokaryotes as a strategy of biochemical detoxification or, alternately, through heterotrophic or chemoautotrophic metabolic pathways that provide energy for growth. Particular progress has been made in documenting the widespread natural occurrence of bacteria that reduce the arsenate anion [As(V)] to arsenite [As(III)] as a terminal electron accepting process for anaerobic respiration. Numerous studies have reported dissimilatory arsenic reduction in a diversity of freshwater, terrestrial, marine, and extremophilic settings,^{2–4} while other workers have characterized novel cellular enzymatic systems⁵ and genetic operons^{6,7} that encode for As-based anaerobic respiration.

By contrast, the potential for prokaryotic communities to achieve similar growth via dissimilatory pathways that utilize arsenic's group 15 neighbor, Sb, have not been previously reported. Antimony is a toxic element of emerging global environmental concern, especially, in Australia, China, and parts of Europe^{8–12} where the element often co-occurs with As and other chalcophile elements around contaminated mine

tailing sites. Antimony and its compounds are considered to be pollutants of priority interest by the U.S. Environmental Protection Agency, which sets the maximum contaminant level (MCL) for the element in drinking water at 6 µg/L.¹³ Although there is a scarcity of peer-reviewed studies that document the extent of Sb ecotoxicity and its environmental behavior, the toxicology and geochemistry of Sb are generally thought to be analogous to that of As.¹⁴ Like As, Sb occurs in four different oxidation states [Sb(V), Sb(III), Sb(0), and Sb(–III)]. Antimonate [Sb(V)] and antimonite [Sb(III)] are the predominant aqueous forms under environmentally relevant conditions, and Sb(III) is reported to be the more toxic of these two species.¹⁵

Antimony(V) is thermodynamically stable in oxidized waters while Sb(III) persists in reducing settings, although these species have both been observed to occur outside of their predicted thermodynamic stability ranges in natural waters. Filella and others¹⁶ have commented that the presence of Sb(III) in oxygenated waters and Sb(V) under suboxic conditions may be largely attributable to biological processes.

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Several studies have reported biological Sb(III) oxidation, either as a cellular detoxification mechanism in bacteria and algae^{17–19} or as a possible chemo-autotrophic process for the bacterium *Stibiobacter senarmontii*.²⁰ Enzymatic detoxification pathways that reduce Sb(V) in bacteria and protozoa^{21–23} or biomethylate Sb compounds in bacteria and fungi^{24,25} also appear to be widespread. In this study, we report the first observation of dissimilatory Sb(V) reduction to Sb(III) by sedimentary prokaryotic communities. Respiratory As(V) reduction and autotrophic As(III) oxidation by microorganisms are integral fluxes within the biogeochemical cycle of As in nature. These microbial processes control the environmental speciation and mobility of As.³ Our demonstration of anaerobic respiration that utilizes Sb(V) as a terminal electron acceptor implies that Sb's environmental behavior may also be closely tied to geomicrobiological cycling.

EXPERIMENTAL SECTION

Study Areas and Sample Collection. Microbiological Sb reduction activity was contrasted in sediments from a Sb- and As-contaminated mine site with that of an uncontaminated small suburban lake. The contaminated sediments were collected from a flooded mine pit located at Stibnite Mine, a gold and antimony mine near Yellow Pine, Idaho which has been in intermittent operation since the 1920s. The mine area is currently proposed for inclusion on the U.S. EPA National Priorities List because of high concentrations of As, Sb, cyanide, and other contaminants that leach from mine tailings piles.²⁶ Submerged sediments were collected by hand from the shoreline of the flooded pit using a garden trowel. Uncontaminated sediments were collected from Searsville Lake, a freshwater reservoir located in the Jasper Ridge Biological Station, Stanford, CA. Searsville Lake sediments were collected from 3 m water depth using an Ekman grab sampler. Samples from both sites were transported to the laboratory on ice in completely filled, sealed glass jars and stored at 4 °C for up to 4 months prior to use in microcosm experiments. Water samples were also collected from each location, filtered in the field (0.45 µm) and stored in evacuated tubes on ice (Vacutainer, BD Company) to be transported to the lab for Sb and As determination.

Sediment Microcosms and Enrichment Cultures. Anaerobic sediment slurry microcosms (30 mL of total volume) were prepared under a N₂ atmosphere (or under H₂ for H₂-amended slurries) in 50 mL serum bottles as described in ref 4. The microcosms were prepared using SeFr1 artificial freshwater media (28 mL) without yeast extract²⁷ and Stibnite Mine or Searsville Lake sediment (2 cm³, or approximately 3.0 g, wet weight). Triplicate microcosms were prepared for each experimental condition. Electron acceptors and electron donors were added by syringe from sterile, anoxic stock solutions. A set of triplicate abiotic (killed) control microcosms were prepared for each condition by twice autoclaving (121 °C, 250 kPa, 50 min) the sediment/media mixture prior to further sterile amendments. Antimony(V) and/or As(V) were added as K₂SbO₃·3H₂O or Na₂HAsO₄, respectively, in millimolar concentrations as discussed for each experiment below. Electron donors were added as sodium lactate or sodium acetate. The microcosms were incubated in the dark at 25 °C on a rotary shaking table (150 rpm) and periodic subsamples (0.35 mL) were collected using a N₂-flushed syringe, filtered by centrifuging (14,000 rpm, 3 min) in 0.45 µm filter microcentrifuge tubes (Costar, Inc.), and analyzed to monitor

changes in dissolved Sb(V), Sb(III), As(V), As(III), lactate, and acetate. For sulfide measurements an additional 0.5 mL subsample was collected by syringe and mixed with 0.25 mL of 10% zinc acetate in a Vacutainer prior to analysis. Following the incubation experiments, solid precipitated residues were collected from the microcosms by filtration, dried at 60 °C in a drying oven for 48 h, weighed, and digested in a 3:1 mixture of concentrated trace metal grade nitric acid and hydrochloric acid as described in EPA Method 3051A²⁸ for Sb determination.

Following the microcosm experiments one Stibnite Mine sediment slurry that showed Sb(V) reduction activity with lactate amendment was selected to serve as an inoculum for enrichment cultures. Culture tubes were prepared using either SeFr1 media or an alternate mineral salts media that lacked sulfate (SeFr2²⁹). Media were bubbled (200 mL for 20 min) with O₂-free N₂ and 13 mL was transferred into each tube under a flow of N₂. The tubes were crimp sealed with butyl rubber stoppers and autoclaved prior to amendment with vitamins, 1 mM Sb(V), and 1 mM lactate using sterile stock solutions. The tubes were inoculated by syringe with 0.1 mL of the selected sediment microcosm. Triplicate tubes were prepared for both respective media types, and autoclaved control tubes were prepared in triplicate for each condition by first inoculating the tube, then autoclaving, followed by sterile vitamin, Sb(V), and lactate amendments.

Radioisotope Experiments. Slurries were prepared using Stibnite Mine and Searsville Lake sediments and sterile freshwater mineral salts media lacking sulfate (SeFr2). Sediment and media were mixed 1:5 (v/v) in a beaker using flowing O₂-free N₂ and a stirring rod to mix. After 30 min of vigorous sparging, 2 mL of diluted sediment was added to 5 mL of anaerobic media contained in serum bottles (13 mL) also under flowing N₂ whence the serum bottles were sealed and crimped. Acetate (1.0–1.4 mM) and varying amounts of antimonate (Sb (V); 0, 0.5, 1.0, 2.0, and 5.0 mM) were added aseptically by syringe to sealed serum bottles several hours before initiating the experiment. Autoclaved control slurries were prepared with added acetate and 5.0 mM Sb (V). Serum bottles were flushed for 5 min with O₂-free N₂ followed by addition of 4–5 µCi of 2-¹⁴C-acetate (45–60 µCi/µmol; sodium salt in sterile water; American Radiolabeled Chemicals, Inc., St. Louis, MO). Slurries were incubated at 27 °C without shaking.

Headspace gases (0.1 mL) were collected by syringe for analysis of ¹⁴CH₄ and ¹⁴CO₂. Initial samples were collected on the first day of the experiment followed by repeated measurements within days to weeks of the start of incubations. Final headspace samples were collected following 68 days incubation for Stibnite Mine, after which the slurry was acidified with 0.2 mL of 1.2 N HCl to cause dissolved inorganic carbon (DIC = HCO₃⁻ + CO₃²⁻) to react to form gaseous CO₂. Searsville Lake incubations were handled somewhat differently. Following headspace sampling at 4 and 11 days, a 1.5 mL aliquot of slurry was removed by syringe and transferred to a 2.5 mL evacuated tube containing 0.5 mL 1.2 N HCl. This headspace was sampled by syringe (0.1 mL) after one hour to measure DIC as ¹⁴CO₂. The collected headspace ¹⁴CH₄ and the total ¹⁴CO₂ (headspace + aqueous phase) were then compared with the amount of ¹⁴C-acetate added to obtain the fraction of the label converted (×100 for percent) to methane and carbon dioxide, respectively.

X-ray Spectroscopy. Samples of precipitated mineral residue from sediment microcosms and enrichment cultures

were collected by vacuum filtration on a nylon filter ($0.45\text{ }\mu\text{m}$, Whatman Co.) immediately following the incubation experiments. X-ray spectroscopy of the precipitates was conducted at the Stanford Synchrotron Radiation Lightsource (SSRL) using beamline 4–3. The incident X-ray energy was calibrated to L3 absorption edge of metallic Sb (4132 eV). The energy of the X-rays was selected using a Si (111) double crystal monochromator with the Stanford Positron Electron Accelerating Ring (SPEAR) storage ring containing 300 mA at 3.0 GeV in top-off mode. The fluorescence of the sample was monitored using a PIPS (Passivated Implanted Planar silicon) diode detector. Samples were mounted at 45° to the incident X-ray beam and were maintained in a He purged sample environment. Standard reference compounds for oxidation state and coordination environment were measured, including Sb(III) oxide and Sb(V) oxide which were ground to a fine powder and placed on layers of thin mylar tape, and a chemically precipitated stibnite (Sb_2S_3) which was supported on a nylon filter membrane. Data were collected through the L3, L2, and L1 edges of Sb, from 3900 to 4900 eV. Spectra were processed, background subtracted, and analyzed following standard procedures using the SIXPACK analysis package.³⁰ Fitting to determine approximate oxidation state composition was performed using a linear combination least-squares fitting procedure of the unknown sample to the standards described above over the range from 4130 to 4180 eV. The energy positions were not allowed to float in the fitting process as all samples were carefully energy calibrated during data collection.

Analytical. Total concentrations of Sb and As in water from the Stibnite Mine pit and from Searsville Lake were measured by inductively coupled plasma mass spectrometry (ICP-MS; Perkin-Elmer, ELAN II). Species specific determination of As(V) and As(III) in water samples was accomplished by high performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICP-MS; ref 31) using a Perkin-Elmer Series 200 HPLC interfaced with a Perkin-Elmer ELAN II ICP-MS (Perkin-Elmer Inc., Shelton, CT). Species specific determination of Sb(V) and Sb(III) in water samples and microcosm subsamples was accomplished by HPLC-ICP-MS as described by Liu and others.³² Arsenic(V), As(III), lactate, and acetate in microcosms were measured by HPLC with UV detection.³³ Sulfide was measured spectrophotometrically using the method of Cline.³⁴ Total Sb concentrations in digested sediment microcosm residues were determined by ICP-MS or inductively coupled plasma optical emission spectroscopy (ICP-OES; Varian, Vista-MPX).

RESULTS AND DISCUSSION

Water Concentrations of As and Sb. The water from the flooded Stibnite Mine pit contained $0.9\text{ }\mu\text{M}$ ($64.9\text{ }\mu\text{g/L}$) total As and $0.2\text{ }\mu\text{M}$ ($21.2\text{ }\mu\text{g/L}$) total Sb. The As was present as 67% As(V) and 33% As(III), while the Sb was present as Sb(V) only. The total concentrations of As and Sb in the Searsville Lake water were below the detectable limit ($<13\text{ nM}$ or $<1\text{ }\mu\text{g/L}$).

Sediment Microcosm Experiments. Live, anoxic sediment microcosms from both study locations demonstrated an ability to reduce millimolar concentrations of Sb(V) (Figures 1 and 2). Sediment slurries from the Stibnite Mine completely removed 1 mM Sb(V) from solution at a potential rate of $0.39\text{ }\mu\text{mol mL}^{-1}\text{ d}^{-1}$ under the experimental conditions, with $\sim 40\%$ recovery as aqueous Sb(III) after 4 d (Figure 1A). The rate of Sb(V) reduction in the Stibnite Mine microcosms was not

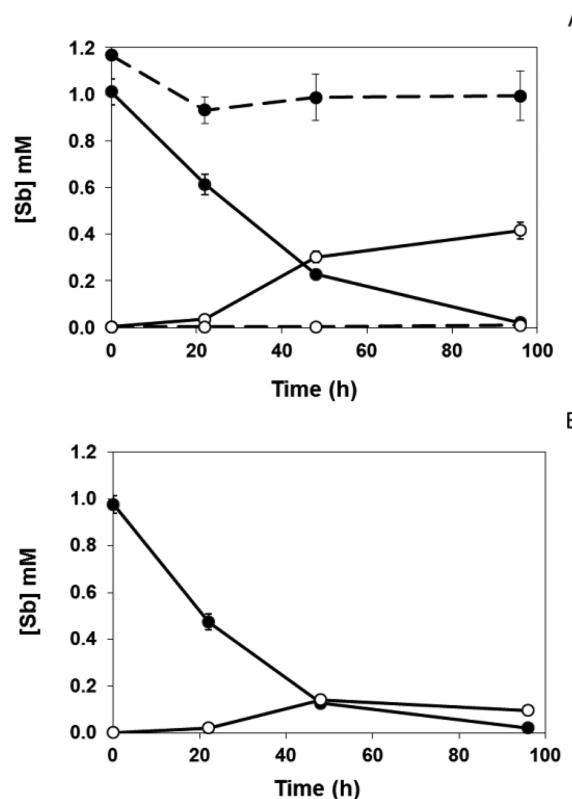


Figure 1. Sb(V) (closed circles) and Sb(III) (open circles) in live anoxic microcosms (solid lines) and autoclaved control sediments (dashed lines) from Stibnite Mine (A). Sb(V) reduction was stimulated by amendment with lactate (B). Recovery of dissolved Sb(III) was poor due to the formation of insoluble Sb(III)-sulfide precipitates during incubation. Points represent the mean of three replicate samples and error bars represent ± 1 standard deviation. The absence of bars indicates that the error was smaller than the symbol.

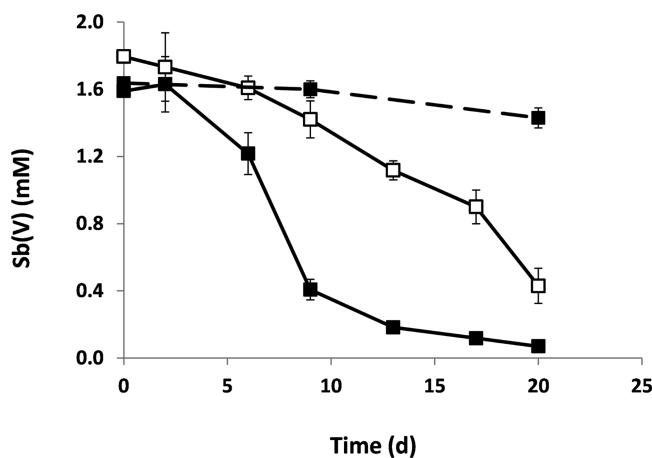


Figure 2. Removal of amended Sb(V) from anaerobic Searsville Lake sediment slurries incubated without electron donor amendment (open symbols, solid line) or with equimolar acetate (closed symbols, solid line), or in an autoclaved control (closed symbols, dashed line). Points represent the mean of three replicate samples and error bars represent ± 1 standard deviation. The absence of bars indicates that the error was smaller than the symbol.

stimulated by the presence of H_2 (data not shown), but did increase slightly to $0.43\text{ }\mu\text{mol mL}^{-1}\text{ d}^{-1}$ in microcosms amended with 1 mM lactate, which also had the effect of diminishing the

final recovery of aqueous Sb(III) to ~10% (Figure 1B). In the autoclaved control microcosms a slight decrease in Sb(V) concentration from 1.17 to 0.93 mM was observed between 0–20 h, with no further loss of Sb(V) during the incubation (Figure 1A). This initial loss of Sb(V) in the killed microcosms may be due to adsorption on the sediment surface. The pH of live Sb(V) reducing microcosms increased from 7.8 to 8.0 during the course of Sb(V) reduction, with no pH change observed in killed controls (data not shown). Searsville Lake microcosms also demonstrated a biologically mediated loss of dissolved Sb(V), but at a significantly slower rate than was observed for Stibnite Mine, and with no corresponding recovery as Sb(III) in solution. Figure 2 shows that without electron donor amendment the Searsville sediments removed 76% of 1.8 mM Sb(V) from solution by 20 d at a maximum potential rate of $0.08 \mu\text{mol mL}^{-1} \text{d}^{-1}$, compared to >95% of this amount in the same time period (at $0.17 \mu\text{mol mL}^{-1} \text{d}^{-1}$) when amended with equimolar acetate. No loss of Sb(V) occurred in autoclaved controls (Figure 2). As we observed with the Stibnite Mine slurries above, no stimulation of Sb(V) removal occurred when Searsville Lake microcosms were amended with H₂ (data not shown). The selective enhancement of Sb(V) removal with lactate (Stibnite) or acetate (Searsville) additions but not with H₂ is indicative of substrate-specificity typically characteristic of a microbiological process.

Anaerobic enrichment cultures inoculated from the Stibnite Mine microcosms also demonstrated Sb(V) reduction with lactate through several dilution transfers in both sulfate-amended and sulfate-free freshwater media (Figure 3). Enrichment transfers in both media preparations reduced two sequential 1.2 mM Sb(V) amendments within two days, respectively. No dissolved Sb(III) was detected in sulfate amended cultures during Sb(V) reduction (Figure 3A), compared to an average final recovery of 0.43 mM Sb(III) in sulfate free media (Figure 3B). No Sb(V) reduction was observed in autoclaved control tubes (data not shown).

The formation of Sb(III) as a product in the Stibnite Mine sediment microcosms as well as in the enrichment cultures was indicative of an Sb(V) reductive process rather than one of Sb(V) adsorptive removal onto a solid-phase mineral matrix (e.g., ferrihydrite). The poor recovery of reduced Sb(V) as aqueous Sb(III) in the Stibnite Mine slurries (Figure 1) and the complete lack of any detectable aqueous Sb(III) in the Searsville Lake slurries (Figure 2) indicated removal of antimony species via precipitation. The formation of sulfide along with the absence of aqueous Sb(III) in the sulfate-amended enrichment culture from Stibnite Mine (Figure 3A) strongly contrasted with the presence of aqueous Sb(III) and the absence of sulfide in the sulfate-free condition (Figure 3B). Moreover, only the sulfate-amended enrichment culture produced a copious amount of a bright orange precipitate (Figure 4) which we suspected was because of the formation of amorphous stibnite (Sb_2S_3). This orange precipitate was also visually present in the sediment slurry residues in microcosms from both study sites that were incubated with sulfate-containing media. Presumably, this insoluble mineral phase resulted from abiotic reaction of Sb(III) with sulfide that was synchronously produced by bacterial sulfate reduction. The identification of the precipitate as amorphous stibnite was confirmed by XANES analysis and comparison to known compounds (Figure 5 and Table 1). Approximate mass balance was achieved between Sb(V) removed from solution by live microcosms and the sum of aqueous-Sb(III) and stibnite-

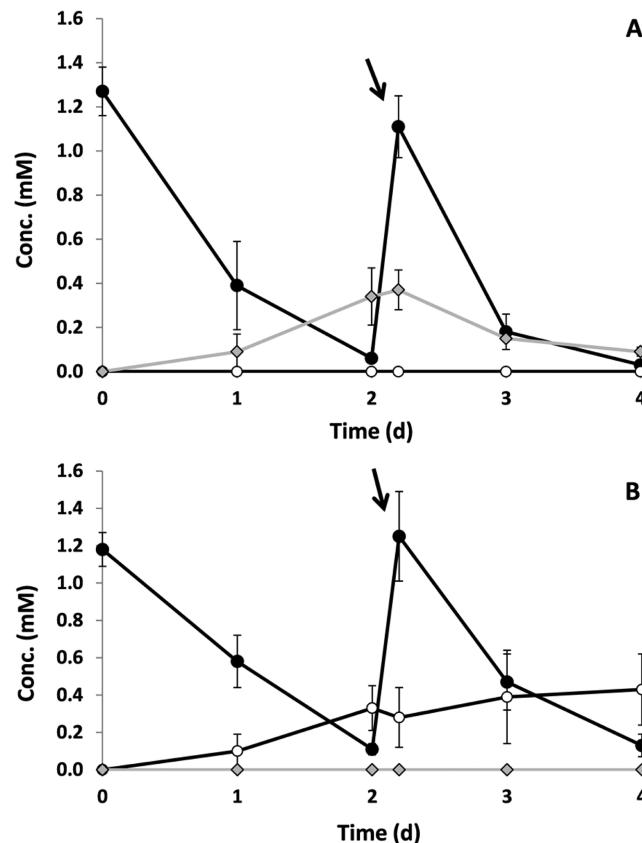


Figure 3. Time course showing concentrations of Sb(V) (closed circles), Sb(III) (open circles), and sulfide (gray diamonds) during biological Sb(V) reduction in Stibnite Mine enrichment cultures grown in mineral salts media containing 2.2 mM sulfate (A), or 0 mM sulfate (B). Arrows denote the timing of supplemental Sb(V) amendment. Points represent the mean of three replicate samples and error bars represent ± 1 standard deviation. The absence of bars indicates that the error was smaller than the symbol.

Sb(III) that was formed. This was confirmed by digestion and analysis of the microcosm residues after incubation, which yielded an average Sb(III) recovery of $91.1 \pm 3.5\%$ from Stibnite Mine sediment slurries and $77.7 \pm 4.3\%$ from Searsville Lake slurries. Similarly, sulfate-amended enrichment cultures from Stibnite Mine sediments yielded an average recovery of $93.5 \pm 1.8\%$ of added Sb(V) as Sb(III) in the form of precipitated Sb_2S_3 .

The precipitation of amorphous Sb-sulfide phases is analogous to reports of amorphous orpiment (As_2S_3), realgar (AsS), and other thioarsenite species which precipitate during bacterial As(V) reduction in sulfidic settings^{35–37} or through abiotic reduction of As(V) by dissolved sulfide.^{38,39} O'Day et al.⁴⁰ suggested that AsS is the primary arsenic-bearing phase in sulfate reducing conditions and that the sequestration of As in sulfide minerals is strongly dependent on the precipitation rate of iron versus arsenic sulfides. In highly reducing environments dissolved sulfide has been shown to chemically reduce Sb(V) to amorphous Sb_2S_3 and other stable Sb(III)-S complexes.⁴¹ The elimination of Sb(V) reduction in our microcosms and enrichment cultures by autoclaving, along with the observed substrate-specific effects on reduction rate, indicate that Sb(V) reduction in sediments from both Stibnite Mine and Searsville Lake is a biological process mediated by components of the microbial flora present in these sediments. We explore whether



Figure 4. Amorphous orange stibnite (Sb_2S_3) precipitate formed after 4 days incubation in live enrichment cultures grown from Stibnite Mine sediments in media containing 1 mM Sb(V), 2.2 mM sulfate, and 1 mM lactate (right tube). A heat sterilized control tube is shown on the left.

this reduction is biologically controlled or biologically induced in the following sections.

Radiosotope Experiments. To test whether microbial reduction of Sb(V) to Sb(III) in these sediments was a dissimilatory process linked to anaerobic respiration, we conducted experiments with microcosms incubated with radiolabeled ^{14}C -acetate in the presence of varying concentrations (0–5 mM) of Sb(V). The results presented in Figure 6A show that for Stibnite Mine sediments the oxidation of ^{14}C -acetate to $^{14}\text{CO}_2$ was enhanced, and $^{14}\text{CH}_4$ production was diminished, in proportion to increasing Sb(V) concentration as the microbial community transitioned from methanogenesis to that of dissimilatory Sb(V) reduction. Similar results were obtained with $2\text{-}^{14}\text{C}$ -acetate when anoxic sediments were assayed for the presence of selenate-respiration⁴² and arsenate-respiration.⁴³ By contrast, Sb(V) concentration had no discernible effect on $^{14}\text{CO}_2$ production in Searsville Lake microcosms, where methanogenesis predominated at all Sb(V) concentrations (Figure 6B). These results demonstrate that heterotrophic anaerobic respiration is the predominant mechanism of Sb(V) reduction in the Stibnite Mine sedimentary community, allowing microorganisms that utilize Sb(V) as an electron acceptor to outcompete methanogens when sufficient Sb(V) and substrate concentrations are present. Antimonate reduction in Searsville Lake sediments, on the other hand, may result from either reductive enzymatic

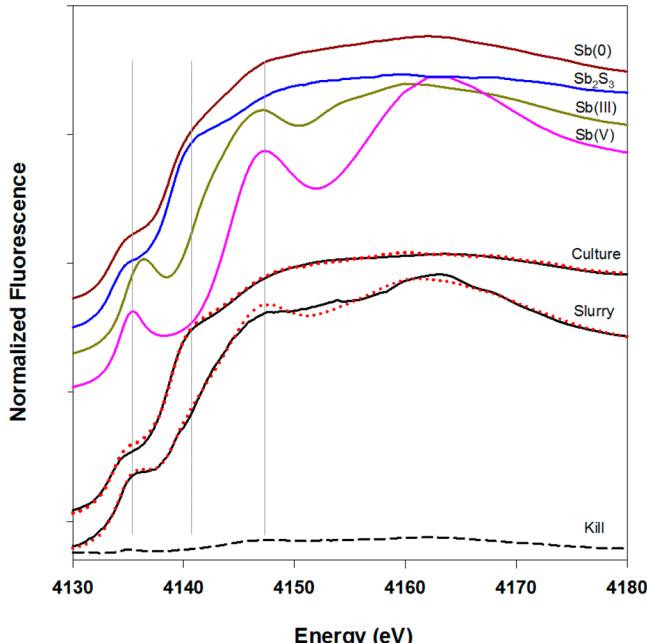


Figure 5. Sb L_{III} -edge XANES spectra of the insoluble residue precipitated during incubation of Sb(V)-reducing microcosms (slurry) and enrichment cultures (culture) and the linear combination fit to the spectra (red dotted lines). The autoclaved control slurry (Kill) is shown in dashed lines. Spectra of chemically precipitated amorphous stibnite (Sb_2S_3), as well as Sb(V) or Sb(III) oxides and Sb(0) foil are shown for comparison. Vertical lines denote regions of distinction among the standard spectra.

d detoxification pathways or from indirect reduction by bacterially produced sulfide.

Effect of Arsenate upon Antimonate Reduction by Sediment Microcosms. A set of microcosm experiments was conducted to investigate the capacity for the Stibnite Mine sediments to reduce As(V) and the potential for co-occurrence of As and Sb reduction. In media amended with 2 mM lactate, Stibnite Mine sediment microcosms reduced 2 mM As(V) to As(III) within 3.8 d, with good recovery of dissolved As(III) and no activity in autoclaved controls (Figure 7A). These sediments also completely removed 3 mM Sb(V) within 5 d when incubated with lactate (Figure 7B), but unlike the slurries presented in Figure 1 no aqueous Sb(III) was detected. Notably, the presence of 2 mM As(V) completely inhibited the reduction of 2.5 mM Sb(V) when both oxyanions were provided simultaneously, and Sb(V) reduction did not commence until all As(V) in the microcosms had been depleted (Figure 7C). This can be interpreted as evidence that As(V) acts as a preferred electron acceptor to Sb(V) for dissimilatory reduction at circumneutral pH. One mechanism by which this may occur is for the electrochemical potential of the As(V)/As(III) couple to exceed that of the Sb(V)/Sb(III) couple and thereby confer a selective energy gain advantage for As(V) respiration.

At first glance this explanation seems reasonable. Calculations using ΔG_f^0 values reported in refs 44–46 for the relevant ionic species predict a slightly higher standard electrochemical potential for the $\text{HAsO}_4^{2-}/\text{H}_3\text{AsO}_3^0$ couple ($E^0 = 842$ mV) than the $\text{Sb}(\text{OH})_6^-/\text{Sb}(\text{OH})_3^-$ couple ($E^0 = 764$ mV) at circumneutral pH. The inverse relationship may be predicted for pH conditions below 7 or above 9, where the $\text{Sb}(\text{OH})_6^-/\text{Sb}(\text{OH})_3^-$ forms persist as the predominant aqueous ionic Sb

Table 1. Results of Linear Combination Modeling Showing the Least Squares Fit of Sb L_{III}-Edge XANES Spectra from Reference Materials [Sb(V) and Sb(III) Oxides, Chemically Precipitated Sb₂S₃, and Sb(0) Foil] Compared to Precipitates from Stibnite Mine Sediment Microcosms and Enrichment Cultures^a

sample	sample information	normalized to a sum of 100%				sum (%)	NSSR ^b
		Sb(III)	Sb(V)	Sb ₂ S ₃ (abiotic)	Sb(0)		
slurry	precipitate from sediment microcosm	27.3	25.7	47.0	0.0	100.0	3.3
culture	precipitate from enrichment culture	0.0	4.2	95.8	0.0	100.0	1.5

^aResults are normalized to 100%. ^bSum of the squared residuals (NSSR (%)) = $100\sum(\text{data}_i - \text{fit}_i)^2 / \sum(\text{data}_i)^2$.

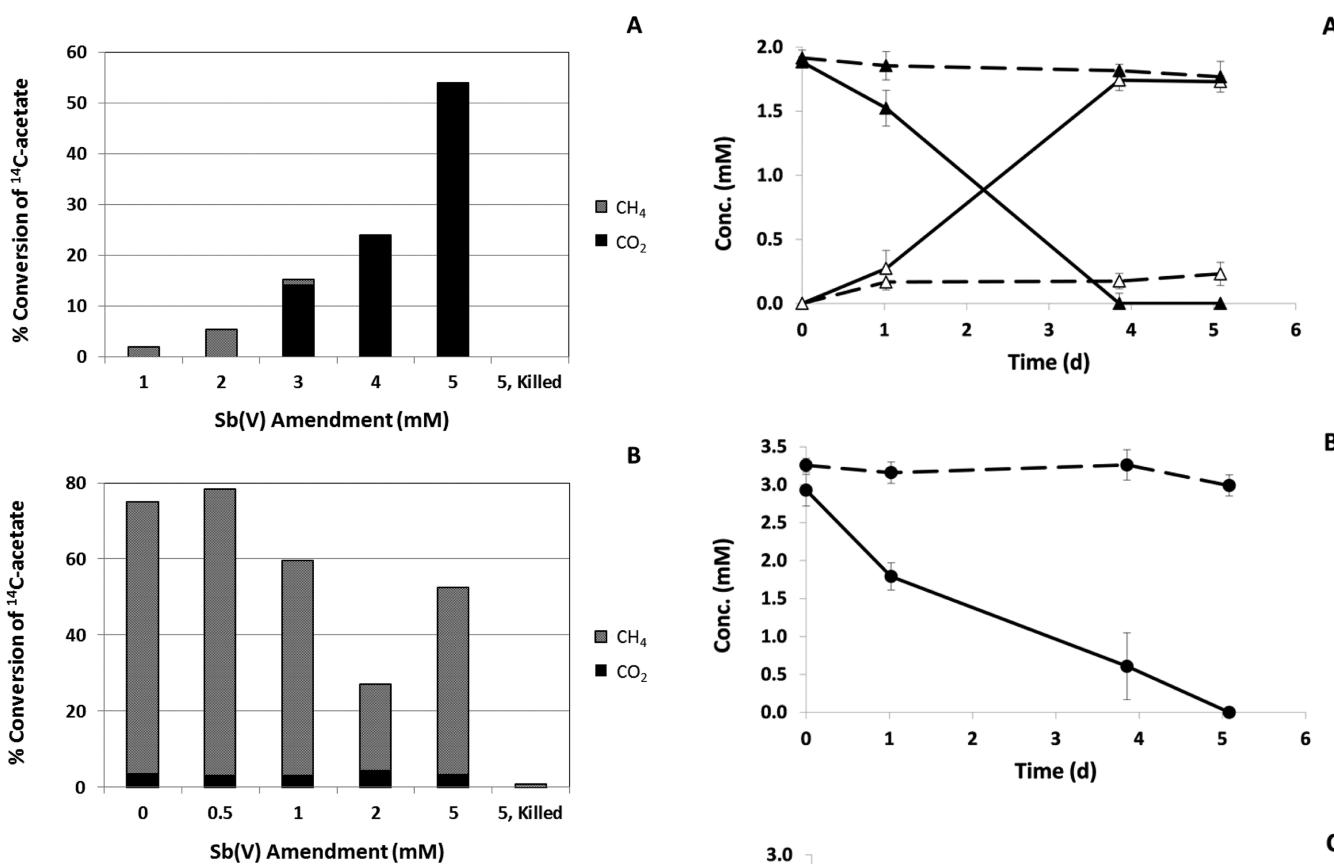


Figure 6. Anoxic sediment microcosms from Stibnite Mine that were incubated with radiolabeled ¹⁴C-acetate (5 μ Ci) demonstrated Sb(V)-dependent oxidation of acetate to CO₂ (A). Searsville Lake sediment slurries did not exhibit Sb(V)-dependent production of ¹⁴CO₂, and methanogenesis predominated at all Sb(V) concentrations (B). No significant ¹⁴CO₂ or ¹⁴CH₄ was produced by autoclaved sediments from either location.

species⁴⁷ but the relevant As couples, H₂AsO₄⁻/H₃AsO₃⁰ ($E^0 = 642$ mV) and HAsO₄²⁻/H₂AsO₃⁻ ($E^0 = 569$ mV) are less energetic. As an alternative to thermodynamic explanations, the predominance of biological As(V) reduction over Sb(V) reduction may simply reflect a sedimentary microbial community that is adapted for, and enzymatically predisposed to, the 4.5-fold greater abundance of As(V) compared to Sb(V) (0.9 vs 0.2 μ M, respectively) in the contaminated mine waters and sediments. In either case, the sequential reduction of As(V) followed by Sb(V) argues that these processes at Stibnite Mine are tied to metabolic pathways that operate in a competitive manner, possibly via shared enzymatic systems. If so, this may explain the persistence of dissolved As(III) and the absence of Sb(III) in the mine pit water.

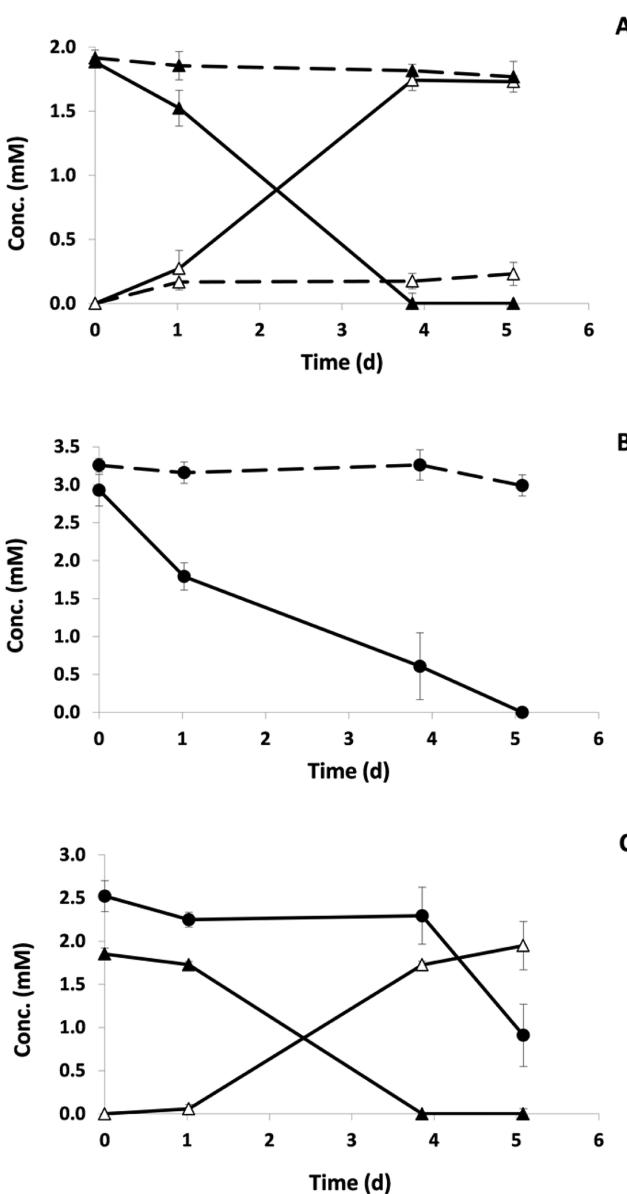


Figure 7. Live Stibnite Mine sediments (solid lines) amended with equimolar As(V) and lactate reduced As(V) (closed triangles) to As(III) (open triangles) with no activity in autoclaved controls (dashed lines) (A). These sediments also removed Sb(V) (closed circles) from solution in live microcosms with lactate (solid line), but not in autoclaved controls (dashed line) (B). When both oxyanions were provided simultaneously, As(V) was reduced preferentially before Sb(V) (C).

In sediments and groundwater aquifers at circumneutral pH, As(V) is highly sorptive to the surface of common minerals,

particularly oxides and oxyhydroxides of Fe(III), Mn, or Al.^{48,49} Antimony oxyanions have also shown a high affinity to adsorb to Fe- and Al-oxides,⁵⁰ as well as clay minerals.⁵¹ In the case of As, these sorptive properties serve to limit the concentration of dissolved As(V) in the liquid phase and, consequently, also limit the rates of uptake and reductive detoxification of As by microorganisms. Reductive detoxification of As(V) is an endocellular process whereby As(V) is passively taken up by phosphate transporters, such as the *Pit* and *Pst* systems in *Escherichia coli*.⁵² It has not been established whether Sb(V) is taken up by these same biochemical pathways, however differences in structure between the octahedral Sb(V) anion [e.g., $\text{Sb}(\text{OH})_6^-$] and tetrahedral As(V) [e.g., H_2AsO_4^-] may make this unlikely. Following uptake into the cytoplasm, As(V) reduction to As(III) is catalyzed by proteins that are encoded by genes of the *ars* operon, including an As(V) reductase (ArsC), and an efflux system (ArsA and ArsB) which transports As(III) out of the cell (reviewed in⁵²). The *ars* system is expressed by oxyanions of As, Sb, and Bi and confers Sb resistance by extruding inorganic Sb(III) in addition to As(III).^{53–55} Unlike these cytoplasmic reactions that confer As- and Sb-resistance, however, the respiratory reductase of As(V) in prokaryotes is associated with an electron transport chain and is located in the periplasm and outer cell membrane. Because of its location near the cellular surface, respiratory As(V) reduction is able to utilize sediment-bound As(V), and is therefore the only mechanism for direct microbiological As(V) reduction in Fe-oxide containing sediments.⁷ Arsenic(V) respiration is associated with the *arr* gene cluster which encodes a periplasmic heterodimer reductase enzyme with two subunits, ArrA and ArrB. This reductase appears to be specific for As(V) in the bacterium *Chrysiogenes arsenatis* and does not function using nitrate, sulfate, selenate, or fumarate as alternative electron acceptors.⁵⁶ In *Bacillus selenitireducens*, Arr is reported to reduce arsenate, arsenite, selenate, and selenite,⁵⁷ but its capacity to reduce Sb(V) has not been examined in any organism. Our observation of the inhibition of Sb(V) reduction by As(V) may suggest that, despite their geometric differences, Sb(V) respiration is accomplished via an established As(V) reductase that exhibits a greater affinity for As than Sb. However, detailed characterization of the putative Sb(V) reductase and its relationship to As(V) enzymatic pathways will require further study of Sb-respiring isolates.

The isolation of a dissimilatory Sb(V)-respiring organism in pure culture would provide a novel and potentially revealing opportunity to further elucidate the processes and genomic operons involved in the biogeochemical cycling of Sb. We note that an anaerobic, spore-forming bacillus was recently isolated that demonstrated Sb(V)-dependent growth linked to lactate oxidation and formation of Sb(III).⁵⁸ Whether or not microbial Sb(V) reduction proceeds by enzymatic pathways established for As(V), or if it has its own Sb-specific metabolism and genes remains to be determined in future experiments with Sb-metabolizing strains.

Significance. These data provide the first evidence of a natural microbiological population that utilizes Sb(V) as a terminal electron acceptor for anaerobic heterotrophic respiration. Our results with sediments from both Sb-contaminated and Sb-free environments suggest that the capacity for microorganisms to reduce Sb(V) to Sb(III) may be environmentally widespread, but that in populations which are not adapted to high Sb concentrations this reduction may be accomplished by detoxification mechanisms or other

enzymatic pathways not linked to respiration. In sulfidic settings, Sb(V) reduction can proceed biologically or abiologically, and results in the sedimentary sequestration of Sb as Sb_2S_3 and other metastable Sb-S minerals (e.g., Sb_2S_5). In cases where Sb contamination co-occurs with high As concentrations, dissimilatory As(V) reduction may be expected to be favored over Sb(V) reduction. Our results demonstrate the complexity of predicting Sb speciation and biogeochemical behavior in the presence of sulfide and As(V), two geochemical species that commonly co-occur with the element. These findings also evoke the prospect of a microbial biogeochemical Sb cycle in nature, the magnitude and significance of which should be evaluated through studies of Sb-reducing communities from other Sb-impacted areas.

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Notes

The authors declare no competing financial interest.

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