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Mobilization of Arsenite by Dissimilatory Reduction of Adsorbed Arsenate

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Sulfurospirillum barnesii is capable of anaerobic growth using ferric iron or arsenate as electron acceptors. Cell suspensions of *S. barnesii* were able to reduce arsenate to arsenite when the former oxyanion was dissolved in solution, or when it was adsorbed onto the surface of ferrihydrite, a common soil mineral, by a variety of mechanisms (e.g., coprecipitation, presorption). Reduction of Fe(III) in ferrihydrite to soluble Fe(II) also occurred, but dissolution of ferrihydrite was not required in order for adsorbed arsenate reduction to be achieved. This was illustrated by bacterial reduction of arsenate coprecipitated with aluminum hydroxide, a mineral that does not undergo reductive dissolution. The rate of arsenate reduction was influenced by the method in which arsenate became associated with the mineral phases and may have been strongly coupled with arsenate desorption rates. The extent of release of arsenite into solution was governed by adsorption of arsenite onto the ferrihydrite or alumina phases. The results of these experiments have interpretive significance to the mobilization of arsenic in large alluvial aquifers, such as those of the Ganges in India and Bangladesh, and in the hyporheic zones of contaminated streams.

Introduction

Arsenic (As) is well recognized for its broad toxicity (*1*), and it has a complex hydrochemistry that governs the speciation and distribution of its two most prevalent oxidation states found in nature: arsenate [As(V)] and arsenite [As(III)] (*2*−*4*). As(V) is typically the predominant, but not exclusive, oxidation state found in surface waters (*5*, *6*). The concentrations of both As(V) and As(III) may be controlled by adsorption onto iron and manganese oxyhydroxides (*7*−*9*), and the extent of adsorption is highly dependent upon pH. Under anoxic conditions in the presence of sulfide, arsenic(III) sulfide phases form (*3*), and pyrite can fix a substantial quantity of arsenic within its crystal lattice (*10*, *11*). Thus, changes in chemical conditions can greatly influence the concentrations and mobility of arsenic observed in natural waters (*12*).

Iron oxide and hydroxide mineral phases play a key role in the biogeochemical cycle of As. Both As(V) and As(III) species are strongly adsorbed by iron oxides (*8*, *13*, *14*). Spectroscopic studies have shown that these species form bidentate, inner-sphere complexes on ferrihydrite (hereafter abbreviated as Fhy), and goethite surfaces and also reveal that As(III) is not oxidized by the goethite surface (*15*−*17*).

Biological processes can mobilize arsenic by either direct or indirect mechanisms. When ferric oxide or oxyhydroxide phases are buried in sediments and become exposed to anoxic conditions, reductive dissolution of the ferric oxides can release mineral-bound arsenic and other trace metals (*7*, *18*−*20*). Solubilization of As(V) was reported when an Fe(III)-respiring bacterium was incubated with contaminated lake sediments (*21*).

It has been recently discovered that several newly described species of anaerobic bacteria with very diverse phylogenies achieve growth by respiratory reduction of As(V) to As(III) (*22*−*24*). This process is called "dissimilatory reduction", and two closely related strains of the genus *Sulfurospirillum*, namely, *Sulfurospirillum arsenophilum* (MIT-13) and *Sulfurospirillum barnesii* (SES-3) (*25*), have been investigated in detail when As(V) occurs as a free oxyanion (*26*, *27*). In experiments with the Fe(III)-respiring *Shewanella alga*, an organism that does not respire As(V), Cummings et al. (*21*) demonstrated that reduction of Fe(III) in synthetic scorodite ($FeAsO_4 \cdot 2H_2O$) resulted in the release of As(V) and Fe(II) into solution. Although this result illustrated a mechanism for biotic As(V) release, this element's mobility in sediments and aquifers would still be severely constrained by readsorption reactions onto Fhy or other metal oxides present at the aerobic/anaerobic interface (*19*, *20*). Furthermore, the question of reduction of As(V) to As(III) was left unanswered. Ahmann et al. (*28*) conducted As(V) reductive dissolution experiments with sterile sediment microcosms amended with ferric arsenate and *S. arsenophilum*. A release of As(III) into solution occurred, but the ability of *S. arsenophilum* to mobilize As(V) adsorbed to iron oxides was not investigated. Furthermore, although some Fe(II) release was noted, it is not clear if *S. arsenophilum* actually has a dissimilatory ferric reductase (*25*). Langner and Inskeep (*29*) found that CN8, a glucose-fermenting microorganism, was capable of reducing aqueous As(V) but was not capable of reducing Fe(III) in Fhy or As(V) that had been adsorbed to Fhy for 4 days before addition of the cell suspension. These authors argued that solubilization of As in contaminated sediments would likely only occur at a significant rate under conditions favoring Fe(III) dissimilatory reduction.

In this paper, we report experimental results with cell suspensions of *S. barnesii*, a bacterium that respires both As(V) and Fe(III). We report on its ability to reduce As(V) adsorbed onto Fhy (a poorly crystalline, commonly occurring ferric oxyhydroxide mineral) and As(V) adsorbed onto aluminum hydroxide. In theory, we have been able to decouple the process of As(V) reduction from what had been previously assumed to be the required first step, namely, reductive dissolution of the adsorbent mineral phase.

Materials and Methods

Preparation of Ferric and Aluminum (Hydr)oxides. The 2-line ferrihydrite (*30*) was synthesized in a polyethylene vessel under a N_2 atmosphere by dropwise addition of dissolved NaOH (300 mM) to 100 mL of a stirred and acidified solution of $FeCl_3 \cdot 9H_2O$ (50 mM) until a pH value of 8 was stable for 1 h (*8*). Ferric chloride was used instead of ferric nitrate in order to avoid any use of nitrate as an electron acceptor by bacteria.

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Fhy with presorbed As(V) (before incubations with bacteria) was prepared by adding 10 mL of $Na_2HAsO_4 \cdot 7H_2O$ (280 mM) to 200 mL of a stirred suspension of Fhy (14 mM as Fe) aged for 5 days. After 1 day, the suspension was centrifuged, made up to 200 mL with water, and used immediately.

Fhy with coprecipitated As(V) was formed by adding 50 mL of $Na_2HAsO_4 \cdot 7H_2O$ (14 mM) and 50 mL of dissolved $FeCl_3 \cdot 9H_2O$ (70 mM) to 100 mL of water in a closed reaction vessel. The solutions were added with a double syringe pump at a continuous flow rate of 0.5 mL min^{-1}. Dissolved NaOH (300 mM) was added simultaneously with a second syringe pump at a rate that maintained a pH of 7.5−8.0 in the reaction vessel (*8*). The suspension produced was aged for 5 days, centrifuged at 4000*g* for 20 min, and then washed in water, centrifuged, and resuspended in water to achieve an Fe concentration of 14 mM in suspension. Aluminum hydroxide with coprecipitated As(V) was synthesized analogously.

All chemicals used were of reagent grade or better. Water was double-deionized and passed through a Milli-Q system. All solutions, except analytical reagents, were degassed with N_2 gas.

Bacterial Cell Suspensions. *S. barnesii* strain SES-3 was grown in batch culture (1.5 L) with nitrate as the electron acceptor, a condition in which cells are also able to reduce As(V) (*27*). Cell suspensions were generated according to Oremland et al. (*31*). Nitrate was chosen as the growth electron acceptor in lieu of As(V) or Fe(III) because cell yields are low on the former (*27*), and we required the suspensions to be initially free of exogenous Fe-particles, a situation which would have occurred with the latter. Cells were harvested by centrifugation (5000*g*, 20 min at 4 °C) and washed twice with a solution containing HEPES buffer pH 7.3 (21 mM) and neutral salts (NaCl, 14 mM; $CaCl_2$, 1.4 mM; $MgCl_2$,1.4 mM). The washing solution did not contain any phosphate that could interact with the Fhy or $Al(OH)_3$ phases. In addition, the washing solution was kept free of N and P to prevent additional growth of the resuspended cell population. The final volume of concentrated cell suspensions resuspended in the above buffer plus salts solution was 200 mL, with typical cell densities of $1-3 \times 10^9$ cells mL^{-1} as determined by direct counts (*32*). All manipulations of the suspensions were made in an anaerobic glove chamber. Assays were conducted in serum bottles (100 mL) that contained (a) 25 mL of washed cell suspension, (b) 10 mL of sodium lactate (final concentration = 1 mM), (c) 10 mL of water, and (d) 25 mL of $Al(OH)_3$ with coprecipitated As(V) or Fhy suspension with either no As(V), presorbed As(V), or coprecipitated As(V). For the experiments with no As(V) bound to Fhy initially, 10 mL of $Na_2HAsO_4 \cdot 7H_2O$ (7 mM) solution was added instead of the pure water. Bottles were sealed with butyl rubber stoppers. Additional experiments included cell suspensions incubated with only dissolved As(V) or Fhy as electron acceptors to ensure that cells were capable of dissimilatory reduction of either. All biological experiments were performed in duplicate.

In chemical control experiments, the kinetics of As(V) desorption and As(III) adsorption by the mineral phases were measured without added bacteria. To determine if chemical reduction of As(V) by Fe(II) was possible under the experimental conditions, 10 mL of $FeSO_4 \cdot 2H_2O$ (3.5 mM) solution and 10 mL of $Na_2HAsO_4 \cdot 7H_2O$ (7 mM) solution were added to Fhy with coprecipitated As(V) in serum bottles without bacteria. All assays and controls contained the same final concentrations of HEPES pH 7.3 buffer (7.5 mM), lactate (1 mM), and neutral salts: NaCl (5 mM), $CaCl_2$ (0.5 mM), and $MgCl_2$ (0.5 mM). Bottles were incubated with continuous rotary shaking (250 rpm) at 25 °C. Samples were periodically removed from the serum bottles by syringe over the course of the experimental incubation. Samples were frozen immediately to preserve arsenic speciation until analyzed.

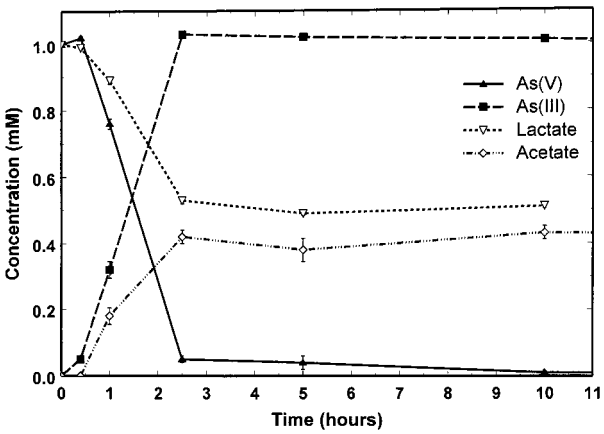

FIGURE 1. Microbial reduction of As(V) to As(III) and simultaneous oxidation of 1 mM lactate to acetate by a washed cell suspension of *S. barnesii*. Initial cell density was 0.7×10^9 cells/mL. Symbols represent the average of two cell suspensions, and bars indicate range in values. In most cases, ranges are smaller than the symbols and are not evident.

Analyses. As(III), As(V), lactate, and acetate concentrations were determined by high-performance liquid chromatography (HPLC) using 1.5 mL of filtered (0.2 μm) injectates and analyzed within a few hours of sampling (*27*). The detection limits were 0.02 mM for As(III), 0.04 mM for As(V), 0.06 mM for lactate, and 0.1 mM for acetate. To measure Fe(II) produced during incubations, the sampled suspensions were first acidified by adding 3 mL of 0.7 M HCl to 1 mL of suspension to desorb Fe(II). The suspensions were then filtered (0.2 μm) after 30 min exposure to the acidified conditions, and dissolved Fe(II) was measured photometrically by the ferrozine method (*33*).

For the determination of total Fe or Al and As(V) or As(III) associated with mineral phases, 1-mL suspensions were centrifuged (1000*g*, 10 min) and decanted. The residue was dissolved completely in 100 μL of 5 M HCl, and after 1 h the solution volume was made up to 1 mL with water. Samples were analyzed within a few hours of sample collection. Fe, Al, and total As were measured by ICP−OES (*34*) after a 20-fold dilution with Milli-Q water. As detection limit by ICP−OES was 0.67 μM. As(V) and As(III) were also determined by HPLC. Total As concentrations determined by ICP−OES differed by less than 10% from that determined by the sum of As(V) and As(III) from HPLC analysis.

Results

Microbiological Experiments with Cell Suspensions. *S. barnesii* reduced ~95% of dissolved As(V) to As(III) within 3 h, with concurrent oxidation of lactate to acetate (Figure 1). The reduction rate was ~700 nmol of As(V) $(10^9 \text{ cells})^{-1}$ h^{-1}. The results were in stoichiometric conformity with the following reaction (*27*):

$$\text{lactate}^{1-} + 2\text{HAsO}_4^{1-} + \text{H}^+ \rightarrow \text{acetate}^{1-} + 2\text{H}_2\text{AsO}_3^{1-} + \text{HCO}_3^{1-}$$

The cells also had the ability to reduce Fhy to Fe(II). The rate of Fe(II) production was studied in two different batches, and although the concentrations of dissolved Fe(II) were always within a factor of 2, the rates in the two experiments varied considerably when normalized to cell density (Table 1). When averaged over the first 10 h there was a difference of ~9-fold, but this disparity decreased to only ~3-fold when averaged over 46 h of incubation. However, the highest rates

TABLE 1. Production of Fe(II) from Fhy in Two Cell Suspensions of Nitrate-Grown *S. barnesii* Incubated without As(V)[a]

10 h incubation		46 h incubation	
Fe(II) (mM)	rate (nmol/10^9 cells/h)	Fe(II) (mM)	rate (nmol/10^9 cells/h)
0.52 (0.51−0.53)[b]	45.8	1.01 (0.97−1.04)	18.5
0.20 (0.19−0.21)[c]	5.0	0.70 (0.69−0.71)	6.1

[a] Values represent the average of two cell suspensions with parentheses indicating range. [b] Cell density = 1.2×10^9 cells/mL. [c] Cell density = 2.5×10^9 cells/mL.

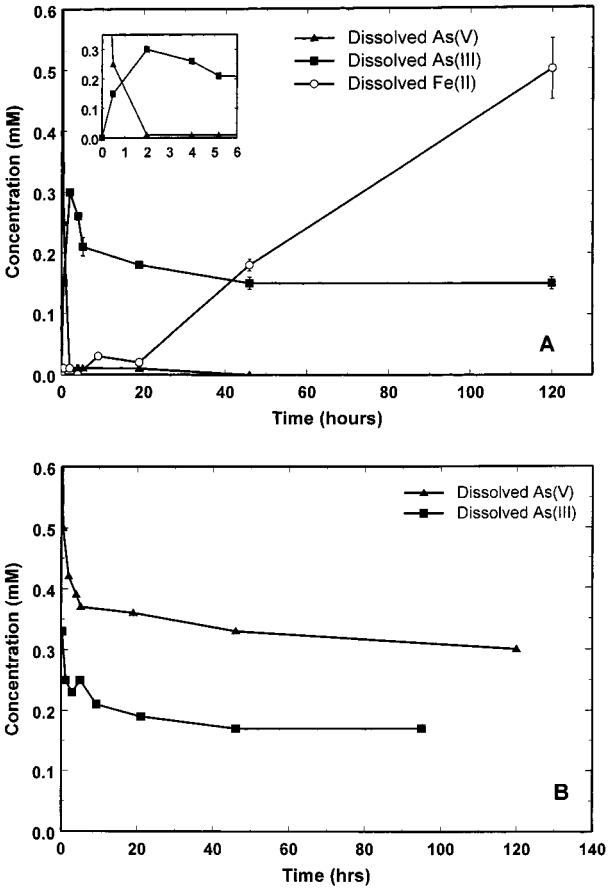

FIGURE 2. Batch experiments with 1 mM dissolved As(V) added to a suspension of Fhy particles (5 mM as Fe) at $t = 0$. (A) Incubation with cell suspensions of *S. barnesii* (2.5×10^9 cells/mL). Symbols represent the average of two separate experiments, and bars indicate the range of values. Most ranges are smaller than the symbols and are not evident. (B) Kinetics of As(V) or As(III) adsorption on Fhy in separate abiotic experiments, 1 mM initial As(V) or As(III).

of Fe(II) production in these experiments were about 2 orders of magnitude lower than that achieved during growth of *S. barnesii* (*27*) with soluble (chelated) Fe(III) as the electron acceptor [~1850 nmol of Fe (10^9 cells)$^{-1}$ h^{-1}].

In assays conducted with cell suspensions mixed with pure Fhy and dissolved As(V) at the start of the experiment, aqueous As(V) concentrations decreased to near or less than the detection limit, and 30% of the total As in the experiments was present as dissolved As(III) 2 h after the incubation began (Figure 2A, inset). Dissolved As(III) declined to 20% of the total As after 5 h and to 15% by 120 h (Figure 2A). In separate abiotic adsorption experiments under identical chemical conditions, about 65% of As(V) or 80% of As(III) was adsorbed by pure Fhy within a few hours (Figure 2B).

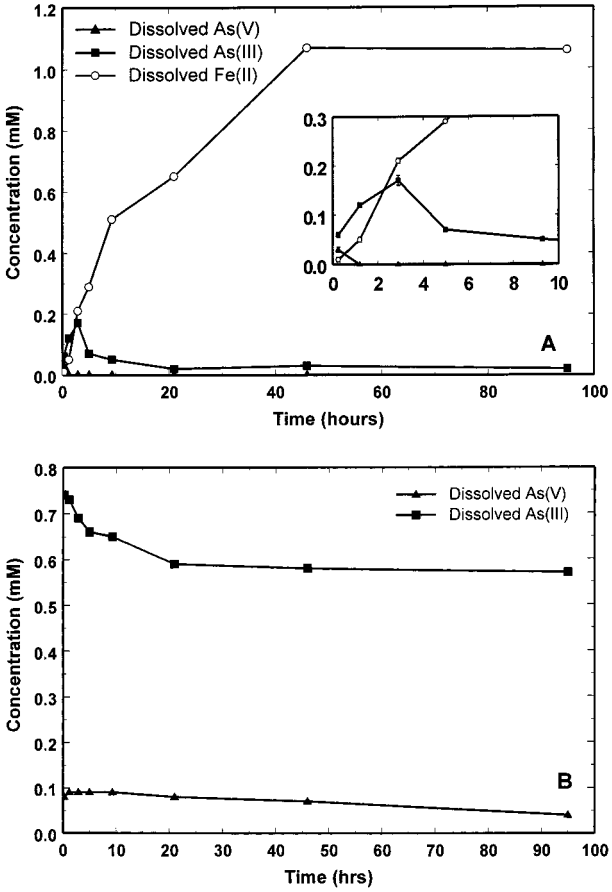

FIGURE 3. Batch experiments with 0.8 mM As(V) presorbed on Fhy particles (5 mM as Fe) for 1 day prior to the beginning of the experiment. (A) Incubation with cell suspensions of *S. barnesii* (2.5×10^9 cells/mL). Symbols represent the average of two separate experiments. The range of values is not displayed because all were smaller than the symbols themselves. (B) Separate abiotic experiments: desorption of As(V) from Fhy (triangles) and adsorption of As(III) (1 mM initial) on Fhy with 0.8 mM As(V) presorbed (squares).

The incubation experiments showed that at least 30% of the As(V) was reduced to As(III) within the first 2 h. The remaining 70% of the total As was adsorbed either as As(V) or As(III). If the adsorptive properties of Fhy were unaffected by the cell suspension, one can conclude that most of the adsorbed As was present as As(III), because adsorbed As(III) can be estimated as 4 times greater than the solution concentration under these conditions (Figure 2B).

Dissimilatory reduction of Fe(III) was not significant until after 20 h of incubation, long after all the As(V) had disappeared from solution. The rate of Fe(III) reduction, as inferred from accumulation of Fe(II), was ~2 nmol of Fe (10^9 cells)$^{-1}$ h^{-1}, which was comparable to the Fhy experiments without As(V) (Table 1). In contrast, the rate of As(V) removal was much more rapid. Although not accurately assessed, it was roughly ~200 nmol of As(V) (10^9 cells)$^{-1}$ h^{-1}, comparable to the reduction occurring in the absence of Fhy (Figure 1). However, this rate may have reflected the combined effects of biological reduction to As(III) plus chemical adsorption of As(V) onto Fhy.

Incubation of cell suspensions with As(V) presorbed on Fhy for 1 day gave different results (Figure 3A). Only a small amount of dissolved As(V) (3.1% of total As) was detectable at the first sampling point at 15 min; after that time As(V) was below detection. Dissolved As(III) increased during the first 3 h to 22% of the total As and then declined to about 2−3% of the total As after 20 h (Figure 3A). In an abiotic desorption experiment with the same initial concentration

of presorbed As(V), dissolved As(V) declined slowly from 11% of total As at the start to 5% of total As at 96 h (Figure 3B, triangles). Thus, in the absence of As(V) reduction by the cells, a low but measurable concentration of dissolved As(V) was maintained due to desorption from Fhy.

In another abiotic experiment, adsorption of As(III) was measured on Fhy with the same concentration of presorbed As(V). Only about 40% of the As(III) was adsorbed (Figure 3B, squares) in comparison to 80% adsorbed on pure Fhy (Figure 2B). Thus, competition for adsorption sites with presorbed As(V) decreases As(III) adsorption on Fhy.

Assuming similar adsorption behavior for As(III) to that shown in Figure 3B, it can be estimated that at least 31% of the As(V) was reduced to As(III) within the first 3 h of the incubation experiment (Figure 3A). After that time, it is difficult to know the amount and rate at which As(III) was reduced since the relative concentrations of As(V) and As(III) adsorbed on the solid phase are unknown. However, less dissolved As(III) was present in solution at 96 h (Figure 3A) than was observed in the previous experiment (Figure 2A), implying that less As(V) was reduced by the cells in the experiment with presorbed As(V). The combined results of these biotic and abiotic experiments indicated the need to examine arsenic speciation with the solid phase as well as in solution.

In contrast to the previously described experiment (Figure 2A), Fe(II) production proceeded vigorously (Figure 3A) for the first 10 h [rate = ~20 nmol of Fe(II) $(10^9$ cells$)^{-1}$ h^{-1}] but then declined and ended. The initial rate was comparable to that observed in the absence of As (Table 1).

Results of incubation of cell suspensions with washed Fhy coprecipitated with As(V) are shown in Figure 4A, including the speciation of As(V) and As(III) associated with the solid phase. Dissolved As(V) concentrations were below detection throughout the experiment. As(V) adsorbed by the solid phase decreased steadily over time, equivalent to a cell-normalized rate of reduction of ~7.7 nmol of As(V) $(10^9$ cells$)^{-1}$ h^{-1}. Both dissolved and adsorbed As(III) increased with time, with the mass balance for total As in the experiment indicating total recovery (98−104%) within experimental error. After nearly 200 h, about 75% of the As(V) had been reduced. Production of Fe(II) occurred at a steady but slow rate [~2.6 nmol of Fe(II) $(10^9$ cells$)^{-1}$ h^{-1}] over the duration of the experiment.

In an abiotic desorption experiment with the same concentration of As(V) in the washed Fhy coprecipitate, dissolved As(V) was below detection throughout the experiment (Figure 4B, triangles). Thus, it is possible that the rate of As reduction in the presence of cells was controlled or tightly coupled with the rate of As(V) desorption from the coprecipitate (*8*, *17*). In a separate abiotic experiment, adsorption of As(III) was measured on Fhy with the coprecipitated As(V). About 50% of the As(III) was adsorbed (Figure 4B, squares) in comparison to 80% adsorbed on pure Fhy (Figure 2B). As in the previous experiment, competition for adsorption sites with presorbed As(V) decreased As(III) adsorption. In the incubation experiment, about 80% of the As(III) was adsorbed by the end of the experiment (Figure 4A), suggesting that the competitive adsorption effect had been removed as As(V) was reduced.

In another abiotic experiment (Figure 5), it was shown that Fhy coprecipitated with As(V) still had the capacity to absorb more As(V) than was added to the solution. These experiments were conducted in the presence of Fe(II), but As(III) was never detected either in solution or sorbed on Fhy. Therefore, Fe(II) could not act as a chemical reductant of As(V) under these conditions. In summary, these experiments demonstrated that *S. barnesii* could achieve a dissimilatory reduction of both Fe(III) and As(V) when they occurred in the solid phase as coprecipitates and that most

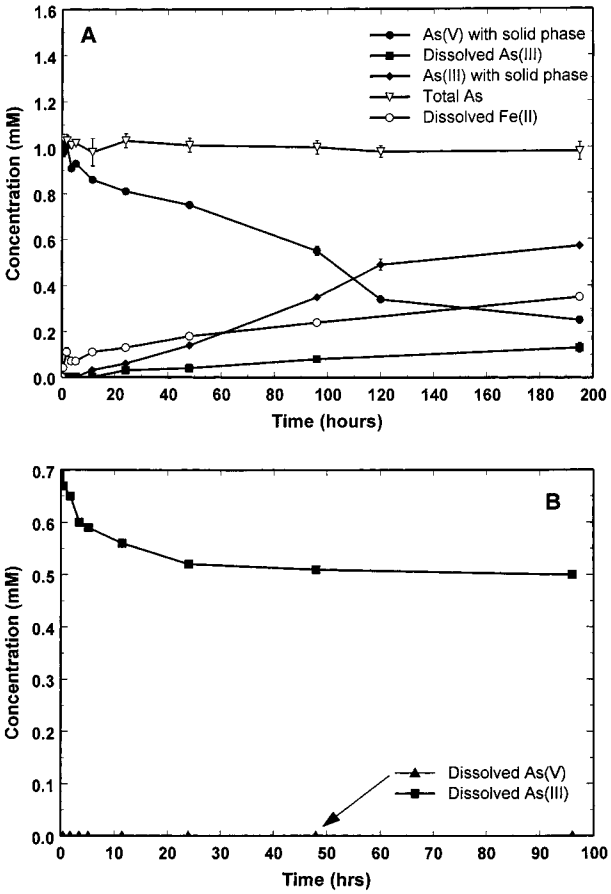

FIGURE 4. Batch experiments with a washed Fhy coprecipitate (5 mM as Fe) containing 1 mM As(V). (A) Incubation with cell suspensions of *S. barnesii* (0.7×10^9 cells/mL). Symbols represent the average of two separate experiments, and bars indicate the range of values. Most ranges are smaller than symbols and are not displayed. (B) Separate abiotic experiments: desorption of As(V) from Fhy (triangles) and adsorption of As(III) (1 mM initial) on a washed Fhy coprecipitate (5 mM as Fe) containing 1 mM As(V).

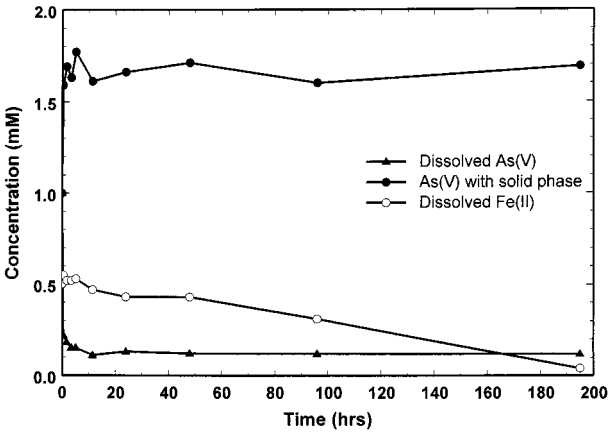

FIGURE 5. Abiotic batch experiments with a washed Fhy coprecipitate (5 mM as Fe) containing 1 mM As(V), 0.5 mM Fe(II), and 1 mM As(V) added at the beginning of the experiment. As(III) was undetectable through the time course either in solution or on the solid phase (data not shown).

of the As(III) formed under these conditions was retained on the remaining Fhy.

To determine if dissimilatory As(V) reduction could occur with a biologically inert solid-phase adsorbent in lieu of Fhy, we conducted experiments using As(V) coprecipitated with $Al(OH)_3$ (Figure 6). In the experiment with cell suspensions

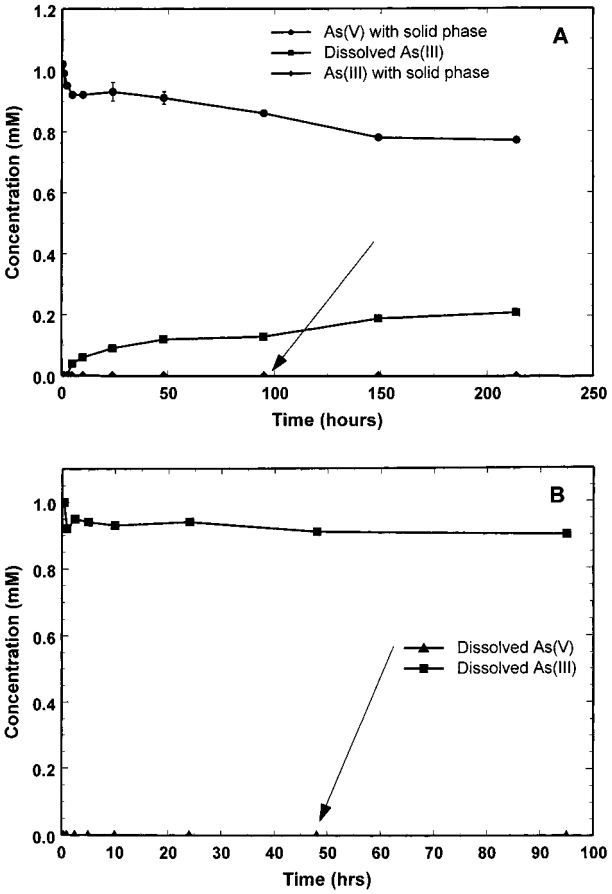

FIGURE 6. Batch experiments with a washed $Al(OH)_3$ coprecipitate (5 mM as Al) containing 1 mM As(V). (A) Incubation with cell suspensions of *S. barnesii* (1.4×10^9 cells/mL). Symbols represent the average of two separate experiments, and bars indicate the range. In most cases the ranges are smaller than the symbols and are not displayed. (B) Separate abiotic experiments: desorption of As(V) from $Al(OH)_3$ (triangles) and adsorption of As(III) (1 mM initial) on a washed $Al(OH)_3$ coprecipitate (5 mM as Al) containing 1 mM As(V).

(Figure 6A), As(V) adsorbed by the solid phase decreased more slowly with time than was observed in the analogous experiments with Fhy (Figure 4A). Dissolved As(V) and adsorbed As(III) concentrations were below detection throughout the experiment. Only about 20% of the As(V) was reduced during the 215 h of incubation, with about 10% reduced in the first 10 h. As As(V) was reduced, an equivalent amount of As(III) appeared in the aqueous phase. In separate abiotic experiments (Figure 6B), it was found that As(V) did not desorb from the coprecipitate with $Al(OH)_3$ and that As(III) adsorption was very weak.

Discussion

S. barnesii is capable of growth with a diversity of electron acceptors in addition to As(V). These include Fe(III), selenate, nitrate, nitrite, trimethylamine *N*-oxide, fumarate, thiosulfate, elemental sulfur, and low concentrations of oxygen (*25*, *27*, *31*). The dissimilatory arsenate reductase of *S. barnesii* is membrane-bound and hydrophobic (*35*), while that of *Chrysiogenes arsenatis*, the only other characterized enzyme, occurs in the periplasm and is soluble (*36*). Both of these reductases are located in the cell envelope and are fundamentally different from the cytoplasmic *ars C* type system of *Escherichia coli*, which has a detoxifying function (*22*, *23*). It is clear that *S. barnesii* expresses arsenate reductase activity when grown on nitrate (Figure 1). Although cells also had the ability to reduce Fhy to Fe(II) (Table 1), the rates of two different batches were variable and were 2−3 orders of magnitude lower than when *S. barnesii* actually grows on solubilized Fe(III) (*27*). Therefore, a kinetic limitation on Fe(III) reduction occurred with solid phase Fhy in the suspensions when compared to readily available chelated Fe(III). The fact that there was variability between batches of nitrate-grown cells with respect to their ability to reduce Fhy is therefore not surprising. The key point is that both batches of nitrate-grown cells possessed the ability to reduce Fe(III) to Fe(II).

Nitrate-grown cells also had the ability to reduce Fe(III) and As(V) simultaneously (Figures 2A, 3A, and 4A), and it was not surprising that the reduction rates for adsorbed As(V) were slower than that observed for dissolved As(V) (Figure 1). Furthermore, the lack of chemical reduction of As(V) by Fe(II) (Figure 5) proves that the observed As(V) reduction was achieved directly by the arsenate reductase of *S. barnesii* rather than by indirect formation of Fe(II) derived from biotic reduction of Fhy. Presumably, Fe(III) reduction was also achieved by a ferric reductase; however, the nature and physiological controls on expression of this enzyme have not been investigated in this organism (*37*).

The rate of As(V) reduction by *S. barnesii* depended upon whether As(V) was coprecipitated, presorbed, or added as a soluble ion at the start of the incubation (Figures 2A, 3A, and 4A). Understanding the rate-limiting processes in these systems is complex and could include coupled physical, chemical, and biological processes.

As(V) added as a soluble ion at the beginning of the incubation is most available to the cells, remaining present either as a dissolved ion or adsorbed to exterior surface sites of the Fhy aggregates (*8*, *38*). Fresh Fhy precipitates have a primary particle size of the order of 30 Å, but coagulation quickly leads to micron-sized aggregates of these particles (*15*). As(V) that is adsorbed to the exterior surface sites of the aggregates is relatively easily desorbed (*8*, *38*), making possible its enzymatic reduction to As(III) in solution. Alternatively, the arsenate reductase may be able to directly attack the As(V)-containing exterior sites of Fhy particles because the arsenate reductase of *S. barnesii* is membrane-bound and Fhy aggregates are electrostatically attracted to the cell membranes.

Presorbed As(V) desorbs more slowly, as its initial adsorption is followed by diffusion into Fhy aggregates and the formation of stronger surface complexes with time that are slower to dissociate (*8*, *38*). For As(V) to reach the solution phase outside of the aggregates, it must diffuse back out of the aggregates in response to low As(V) concentrations in solution around the aggregate, which is a slow process (compare dissolved As(V) in Figures 2B and 3B).

As(V) desorbs from the washed Fhy coprecipitate much more slowly (ref *8* and Figure 4B), as most of the As(V) is located in the interior of Fhy aggregates and the structure is stabilized (*15*). This resulted in a significant decrease in the rates of reduction of both As(V) and of Fhy (Figure 4A). In a similar abiotic desorption experiment with coprecipitated As(V), Fuller et al. (*8*) found that about 15% of coprecipitated As(V) desorbed in the period of 120−320 h after precipitation (our experiments included 120 h of Fhy aging and washing prior to the onset of the experiment). Using these data, an estimate of 0.75 μmol L^{-1} h^{-1} can be derived for the As(V) desorption rate in this study (Figure 4). The volume-normalized rate of As(V) reduction in the incubation experiment (Figure 4A) is estimated to be 5.4 μmol L^{-1} h^{-1}, which suggests that the rate of As(V) reduction proceeded faster than the desorption rate alone.

S. barnesii could destabilize the aggregates as a consequence of its respiration of Fe(III). In the experiment discussed above, the Fe(II) production rate from dissolution

of Fhy was 1.7 μmol L^{-1} h^{-1} (Figure 4A), and assuming that As(V) was uniformly distributed within the coprecipitate (0.2 mol of As/mol of Fe), this process could release 0.34 μmol of As(V) L^{-1} h^{-1}. Indeed, while there was extensive As(V) reduction in the coprecipitated Fhy experiments in 200 h (Figure 4A), it was quite limited in the analogous experiments with $Al(OH)_3$ (Figure 6A). This could be due to the ability of *S. barnesii* to destroy particles of Fhy by dissimilatory reduction but not those of $Al(OH)_3$. Alternatively, it could mean that the As(V) desorption rate from $Al(OH)_3$ was considerably slower than that from Fhy.

Our results with *S. barnesii* stand in contrast to those of Langner and Inskeep (*29*), who detected no reduction of As(V) adsorbed to Fhy with an arsenic-resistant soil clostridium although the organism was able to reduce As(V) in solution. The *ars C* encoded detoxifying reductase systems of As(V) resistant microbes are located internally within the cytoplasm of the organisms (*22*, *23*), while the dissimilatory reductases of As(V) respirers are located on their periphery, within the cell envelope (*35*, *36*) that forms the boundary with the external mileu. We speculate that this peripheral location of the dissimilatory As(V) reductase of *S. barnesii* may have been a critical facet that accounted for its ability to achieve reductive dissolution of As(V) associated with the solid phases of Fhy and $Al(OH)_3$.

The loss of As(III) from solution after As(V) reduction occurred (Figure 2A and 3A) is expected from adsorption on and diffusion into the structure of the Fhy aggregates (Figures 2B and 3B). This explains the lack of stoichiometric release of As(III) into solution in some of the biological experiments. In the experiments with coprecipitated As(V), release of As(III) was relatively minor as compared to the replacement of adsorbed As(V) with adsorbed As(III) (Figure 4A), and this can be explained by adsorption of As(III) by Fhy (Figures 2B and 4B).

Our results with *S. barnesii* illustrate that both dissimilatory As(V) and Fe(III) reduction of Fhy with adsorbed As(V) results in the formation of Fe(II) and As(III). The release of As(III) into solution is controlled by its adsorption onto the remaining Fhy solid phase. The experiments with $Al(OH)_3$ demonstrated that As(V) adsorbed by an unreactive mineral phase can undergo reduction to As(III) without the a priori reductive dissolution of the mineral phase (Figure 6A). While extrapolation of our results to field conditions must be tempered by our use of dense cell suspensions of *S. barnesii* ($\sim 10^9$ cell mL^{-1}), an organism capable of respiring a broad spectrum of potential electron acceptors, we have nonetheless shown that As(V) desorption and reduction is feasible in theory, and this contrasts with the results of Langner and Inskeep (*29*). In addition to this process, As(III) mobilization is facilitated by reductive dissolution of an adsorbing mineral phase provided that phase can also supply anaerobic bacteria with an abundant electron acceptor, such as iron(III) oxides. We recognize that crystalline iron oxides (e.g., goethite) are more resistant to dissimilatory reduction than is Fhy (*39*), which would constrain the release of any arsenic from the interior of these minerals.

It now is clear that mobilization of mineral-bound arsenic in nature can potentially be achieved a number of different ways and by a number of different bacteria in addition to those we demonstrated for *S. barnesii*. These include those described for *Sh. alba* (*21*), and *S. arsenophilum* (*28*), but not for arsenic-resistant clostridia (*29*). We conclude that depending upon the particular bacterial flora present in a sediment or aquifer milieu, any one or all of these mechanisms may be operative.

These collective theoretical investigations with different microbes have implications with regard to the mobility of arsenic in large drinking water aquifers, such as those of the Ganges Delta (*40*). The waters of these alluvial aquifers contain naturally occurring arsenic, and the consumption of water from the constructed wells in this region has caused wide-scale, serious human health problems. However, the mechanism(s) for arsenic mobilization into solution from the alluvial material is(are) still unclear. It was recently shown that sedimentary organic carbon served as an electron donor for bacterial iron oxide reduction in the Ganges aquifer, which was linked with arsenic solubilization. Since arsenic speciation was not determined, it is unclear whether the increase in dissolved arsenic was due to microbially mediated mobilization of As(V) from dissolved iron oxides or dissimilatory reduction of aqueous and adsorbed As(V) or both.

Although As(III) can be found in surface waters near inputs from geothermal springs (*6*), our results support the hypothesis that As(III) may appear in surface waters because of the reduction of dissolved and adsorbed As(V) at oxic–anoxic interfaces found in the hyporheic zone of streams (*18*) and freshwater sediments (*19*). After reduction, As(III) may remain adsorbed, although As(III) is generally more mobile than As(V) (*14*). The ultimate fate of the As(III) mobilized depends on where it is transported and the chemical conditions of that environment. It may be reoxidized to As(V) by manganese oxides or by aerobic, chemoautotrophic bacteria that conserve energy from As(III) oxidation (*41*); thus continuing the arsenic cycling. Alternatively, it may remain as As(III) under more reducing conditions (*3*). However, our results confirm that a biological mechanism exists that can mobilize As(III) in the environment, the oxidation state of arsenic with the greatest toxicity.

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