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Simultaneous Microbial Reduction of Iron(III) and Arsenic(V) in Suspensions of Hydrous Ferric Oxide

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Bacterial reduction of arsenic(V) and iron(III) oxides influences the redox cycling and partitioning of arsenic (As) between solid and aqueous phases in sediment-porewater systems. Two types of anaerobic bacterial incubations were designed to probe the relative order of As-(V) and Fe(III) oxide reduction and to measure the effect of adsorbed As species on the rate of iron reduction, using hydrous ferric oxide (HFO) as the iron substrate. In one set of experiments, HFO was pre-equilibrated with As(V) and inoculated with fresh sediment from Haiwee Reservoir (Olancha, CA), an As-impacted field site. The second set of incubations consisted of HFO (without As) and As(III)- and As(V)- equilibrated HFO incubated with *Shewanella* sp. ANA-3 wild-type (WT) and ANA-3Δ*arrA*, a mutant unable to produce the respiratory As(V) reductase. Of the two pathways for microbial As(V) reduction (respiration and detoxification), the respiratory pathway was dominant under these experimental conditions. In addition, As(III) adsorbed onto the surface of HFO enhanced the rate of microbial Fe-(III) reduction. In the sediment and ANA-3 incubations, As-(V) was reduced simultaneously or prior to Fe(III), consistent with thermodynamic calculations based on the chemical conditions of the ANA-3 WT incubations.

Introduction

Arsenic (As) causes severe health effects when ingested, and evidence for this type of poisoning is apparent in countries such as Bangladesh, where millions of people are affected by drinking As-contaminated groundwater (*1*−*4*). In Bangladesh and many other As-impacted areas, As commonly co-occurs with iron (Fe) minerals in sediments as an adsorbed species, and the fate of Fe and As are often closely linked (*5*−*7*). Arsenic mobility can be affected by redox chemistry (i.e., the cycling between the +III and +V oxidation states) and also by sediment transformations, particularly the reductive dissolution of Fe(III) (hydr)oxides.

Microorganisms can mediate redox cycling of both As and Fe (*8*−*10*). Microbially driven As redox transformations have been observed in laboratory and field studies (*8, 11*−*13*). There are two known microbial pathways for reduction of As(V) to As(III). The respiratory pathway (*arrA* pathway) couples the oxidation of an organic substrate to As(V) reduction resulting in cell growth (*9, 14*−*17*). The detoxification pathway (*arsC* pathway) is used by the cell to convert As(V) to As(III), which is actively transported out of the cell; this process requires ATP (*17*).

Dissimilatory Fe reducing bacteria (DIRB) are widespread and considered to be the primary agent in the reductive dissolution of Fe minerals in sedimentary environments (*2*, *6*, *18*). Less crystalline Fe phases with higher surface areas are more susceptible to biological reduction (*19*−*23*). Furthermore, rates of reductive dissolution are affected by Fe mineralogy and accumulation of Fe(II) reaction products. As the parent mineral is reduced, reaction products, including sorbed or precipitated Fe(II), accumulate on the mineral surface, slowing the reduction rate (*21*, *22*, *24*−*26*). The observed rates and products of these microbial reactions depend on experimental conditions such as bacterial strain, initial Fe mineral, and flow-through or batch incubation.

The amount of As released into solution due to the bacterial reductive dissolution of Fe (hydr)oxides has been shown to depend on As oxidation state and Fe mineralogy (*11*−*13*, *27*−*29*). The oxidation state of As at the onset of Fe reduction is crucial to As mobility, since As(V) and As(III) sorption and complexation can be significantly different at environmentally relevant pH values when competing sorbates, such as phosphate, are present (*7*, *30*). As a result, whether As(V) is reduced prior to Fe(III) may partially determine As mobilization. In addition, while the rates of Fe(III) and As(V) reduction are affected by Fe mineralogy, the effect of adsorbed As species on rates of Fe reduction has not been investigated.

This study addresses the relative order of microbial As(V) and Fe(III) reduction as well as the effect of sorbed As species on rates of Fe reduction. Two microcosm studies were performed with a synthetic iron oxyhydroxide slurry and incubated with bacteria from two types of microbial inocula. One inoculum was fresh sediment containing its ambient microbial community from Haiwee Reservoir (Olancha, CA), where Fe- and As-rich sediment have been deposited as a result of in situ, full-scale water treatment (*31*, *32*). Arsenic in Haiwee sediment is primarily adsorbed to a poorly crystalline Fe(III) oxyhydroxide phase. Iron and As in the sediment porewaters are strongly correlated, consistent with reductive dissolution. In the solid phase, As(V) was detectible only in the surficial sediment with As present as As(III) throughout the sediment column (*14*, *32*). The other type of inoculum was a well-studied laboratory strain, *Shewanella* sp. strain ANA-3 wild-type (WT), and a mutant strain with a deletion of the *arrA* gene. ANA-3 WT is capable of both Fe and arsenate reduction. ANA-3 is a reasonable model organism since an isolate of native arsenate-reducing bacteria from Haiwee Reservoir sediments was found to be a strain of *Shewanella* bacteria, also capable of Fe and As reduction (unpublished data).

Inocula of Haiwee sediment or ANA-3 were incubated with hydrous ferric oxide (HFO), an amorphous Fe(III) oxyhydroxide similar to the Fe phase in Haiwee reservoir sediments (*32*). For experiments with Haiwee sediment inoculum, HFO was equilibrated with As(V) prior to inocula-

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TABLE 1. Summary of Experimental Conditions

inoculum	solid	mol_{As}/g_{HFO}	solid-to-soln ratio (g/L)	pH	carbon source	initial carbon concn (mM)
Haiwee sediment	HFO/As(V)	0.002	2.0	7.2	lactate	19
	HFO/As(V)	0.002	2.0	7.2	acetate	17
	HFO/As(V)	0.002	2.0	7.2	no carbon amendment	0
Shewanella sp. strain ANA-3 WT	HFO/As(V)	0.001	2.8	8.0	lactate	14
	HFO/As(III)	0.003	2.7	8.0	lactate	14
	HFO/no As		1.9	8.0	lactate	14
Shewanella sp. strain ANA-3 Δ*arrA*	HFO/As(V)	0.001	2.8	8.0	lactate	14

tion, and experiments were conducted with varying organic substrates (lactate, acetate, or no added organic carbon). For experiments with ANA-3, HFO was equilibrated with either As(V) or As(III) before inoculation or used without exposure to As. The order of microbial As(V) and Fe(III) reduction, the effects of organic substrates on As(V) and Fe-(III) reduction, and the effect of adsorbed As(V) and As(III) on microbial Fe(III) reduction were examined.

Experimental Section

Reagents. All chemicals used were reagent grade and used without further purification. Solutions were prepared with 18 MΩ-cm deionized water (Barnstead, Nanopure infinity) and stored in plastic containers that had been washed in 10% oxalic acid. For bacterial incubations, all solutions were autoclaved before use with the exception of the bicarbonate buffer, which was filter-sterilized (0.2 μm pore size) and added to the autoclaved medium. The bacterial minimal growth medium (Tables S1 and S2, Supporting Information) was buffered with 50 mM bicarbonate and had a total phosphate concentration of 50 μM and an ionic strength of 0.06 M.

Preparation of HFO and As-Equilibrated HFO. HFO was prepared by the dropwise addition of 0.5 M NaOH to 0.05 M $Fe(NO_3)_3$ until the solution stabilized at pH 8 (*34*). The suspension was equilibrated for >3 h under constant stirring, adjusting any pH drift as necessary with 0.5 M NaOH. The HFO was then washed three times with sterile water and centrifuged at 7000×g for 10 min. The HFO was not autoclaved after synthesis to avoid changes in mineralogy.

After the final wash, the HFO was resuspended in an As solution and equilibrated overnight with constant stirring. For the sediment incubations, 15 g of HFO was resuspended in 1 L of 0.05 M Na_2HAsO_4 (Sigma) at pH 7.2. For the ANA-3 incubations, 5 g of HFO was suspended in 0.5 L of 0.02 M Na_2HAsO_4 and 3 g of HFO was suspended in 0.5 L of 0.02 M $NaAsO_2$ (Sigma) at pH 8.0. These conditions ensured that all available surface sites for As sorption were saturated with As at the given pH. In all cases, the HFO was washed twice with sterile water to remove excess As that was not sorbed and resuspended in bacterial minimal medium to a final slurry composition summarized in Table 1. The pH was adjusted to 7.2 for the sediment incubations and pH 8 for the ANA-3 incubations with 0.5 M NaOH prior to inoculation. In the case of HFO without any adsorbed As, the solid was resuspended directly in bacterial medium after the initial washing and adjusted to pH 8. Uninoculated controls were maintained over the course of the experiment to ensure that no contamination was introduced in the synthesis, washing, distribution, and sampling of the HFO slurries.

Incubation Experiments. The HFO suspension was transferred under sterile conditions to acid-washed and autoclaved plastic bottles with a screw-cap lid; 200 mL of HFO slurry was added to each bottle. The bottles were transferred to an anaerobic chamber (80% N_2, 15% CO_2, 5% H_2) and equilibrated for 24 h with the lid loosely covering the bottle mouth to allow for passive gas exchange before inoculation. The bottles were inoculated in the anaerobic chamber and then incubated in the dark at 30 °C in the anaerobic chamber for the remainder of the experiment. The bottles were not stirred during incubation but were shaken vigorously prior to each sampling. All experiments were repeated in triplicate. In each experiment, an additional bottle was left uninoculated as a control and sampled in an identical manner.

Incubations with Haiwee Sediment. Bacterial minimal medium was amended with lactate, acetate, or left unamended (no added carbon source). The slurry consisted of HFO pre-equilibrated with As(V). The bottles were inoculated with fresh sediment from Haiwee Reservoir (2 g wet sediment/200 mL slurry), extracted from a core (5−10 cm depth), and homogenized in the anaerobic chamber. Control bottles consisted of inoculation with pasteurized sediment or autoclaved sediment, but both controls showed both As and Fe reduction. Formaldehyde prevented further reduction in autoclaved sediment control bottles (data not shown), suggesting that the reduction was microbially catalyzed. Uninoculated bottles did not show evidence of growth. Aliquots were taken from the bottles approximately every 12 h for 112 h and analyzed as described below.

Incubations with ANA-3. Incubations were also performed using a laboratory isolate, *Shewanella* sp. strain ANA-3 WT, and mutant ANA-3Δ*arrA*, which has a deletion of the respiratory *arrA* gene (*35*). Slurries were prepared with HFO with adsorbed As(V), HFO with adsorbed As(III), or HFO only (no As). ANA-3 WT was inoculated into bottles with HFO/As(V), HFO/As(III), and HFO. The ANA-3 Δ*arrA* mutant was inoculated into bottles containing HFO/As(V), as a control for respiratory arsenate reduction. Lactate was included in all bottles as the electron donor. Bottles were inoculated to 10^5 total cells initially, corresponding to 500 cells/mL. Samples were taken approximately every 12 h for 131 h. The pH was monitored with pH indicator strips (EM, ColorpHast) at the time of each sampling.

Analytics. At each time point, aliquots were removed and filtered through a 0.2 μm filter (Costar, nylon Spin-X) for determination of dissolved concentrations of Fe(II), total Fe, lactate, acetate, As(III), and As(V). Total concentrations were determined after dissolution of the solid phase by acid addition and measurement of total Fe, Fe(II), As(V), As(III), lactate, and acetate. Total organic carbon was measured in a core sample from Haiwee reservoir. For further details, see the Supporting Information.

Thermodynamic Calculations. The thermodynamic driving force (ΔG) for both arsenate reduction and Fe reduction coupled to lactate oxidation was calculated based on experimental conditions observed in the ANA-3 WT/HFO/As(V) time course (for additional details, see Supporting Information). The reactions and equilibrium constants for the coupled reactions are given below, with the reactions written with the dominant dissolved species at pH 8 and equilibrium constants from ref *37*.

$$CH_3CHOHCOO^- + 2HAsO_4^{2-} + 3H^+ = CH_3COO^- + HCO_3^- + 2H_3AsO_3 + 2H_2O \quad (1)$$

$$4am\text{-}Fe(OH)_{3(s)} + CH_3CHOHOO^- + 7H^+ = 4Fe^{2+} + CH_3COO^- + HCO_3^- + 10H_2O \quad (2)$$

Values of ΔG at 25 °C were calculated from the expression

$$\Delta G = \Delta G^0 + RT(\ln 10) \log Q = \Delta G^0 + 5.7081 \log Q \quad (3)$$

where

$$Q_1 = \frac{[CH_3COO^-][HCO_3^-][H_3AsO_3]^2}{[CH_3CHOCHOO^-][HAsO_4^{2-}]^2\{H^+\}^3} \quad (4)$$

for the arsenate reduction reaction and

$$Q_2 = \frac{[CH_3COO^-][HCO_3^-][Fe^{2+}]^4}{[CH_3CHOCHOO^-]\{H^+\}^7} \quad (5)$$

for the HFO reduction reaction, where the activity of HFO is assumed to be 1, and $\{H^+\}$ corresponds to the measured pH. Total concentrations (dissolved plus adsorbed) of As(V), As(III), lactate, acetate, and Fe(II) were measured at each time point. Total lactate and acetate concentrations were directly substituted into the equations, since their total and dissolved concentrations were approximately equal. The pH was maintained between 7.9 and 8.1 for the entire incubation, and therefore $\{H^+\}$ was assumed to be 10^{-8} for all time points. The bicarbonate concentration was assumed to be constant and in equilibrium with the $CO_{2(g)}$ present in the glove box gas mixture at pH 8. The ionic strength was 0.06 M.

Because dissolved concentrations of H_3AsO_3, $HAsO_4^{2-}$, and Fe^{2+} were below the detection limits of the analytical methods, MINEQL+ was used to calculate these concentrations based on experimental measurements. The contribution of lactate and acetate complexation are neglected in these calculations since complexation with As(III), As(V), and Fe(II) species is weak (*25*).

The surface site density was determined by sorption isotherms with HFO synthesized similarly to this study (As(III) on HFO at pH 8.0, unpublished data) and is defined as

$$[\equiv Fe^{wk}OH] + [\equiv Fe^{st}OH] = 0.12\ Fe_T \quad (6)$$

where Fe_T is measured total Fe. The ratio of strong-to-weak sorption sites is assumed to be 0.025 (*30*). Only ferrihydrite was allowed to precipitate in the model. The double-layer FeOH sorption model was used to calculate the amount of sorbed and dissolved As(V), As(III), and Fe(II) species at each time point. The intrinsic surface complexation constants used in the model are given in Table S4.

Results

The two experimental time courses in this study were designed to investigate the effects of organic substrates on As(V) and Fe(III) reduction and adsorbed As species on the rate of Fe(III) reduction. Dissolved concentrations of Fe and As were undetectable throughout the course of both microcosm experiments. Thus, Fe(II), As(V), and As(III) were predominantly associated with the solid phase.

Incubations with Haiwee Sediment. Both As(V) and Fe(III) were reduced in all of the incubations inoculated with Haiwee sediment (Figure 1). Reduction of As(V) began before Fe(III) reduction and ceased after 40−60 h in the lactate- and acetate-amended bottles. Even though the rate and extent of reduction varied with carbon amendment, the onset of

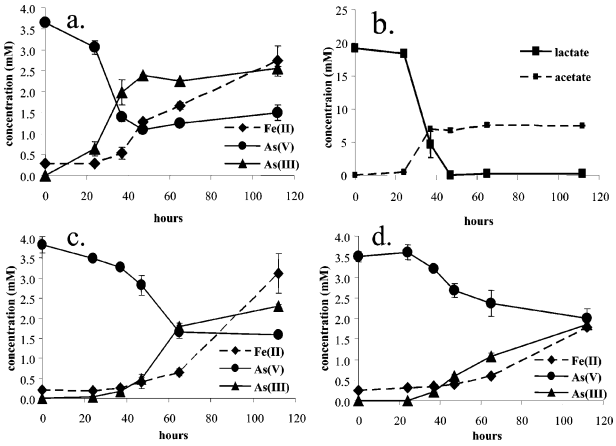

FIGURE 1. Measured total concentrations of As(III), As(V), Fe(II), and organic carbon for sediment incubations (a) and (b) amended with lactate, (c) amended with acetate, and (d) without a carbon amendment. The HFO was pre-equilibrated with As(V) and inoculated with fresh sediment from Haiwee reservoir, introducing a natural bacterial community to the slurry. The error bars represent one standard deviation of measured concentrations of triplicate samples and, if not visible, are smaller than the symbol. For experimental conditions, see Table 1.

As(V) reduction preceded Fe(III) reduction in all cases. For part of the time course, reduction of As(V) and Fe(III) proceeded simultaneously, after which As(V) reduction ceased and Fe(III) reduction continued until the termination of the experiment.

The rate and extent of As(V) and Fe(III) reduction depended on the type of organic substrate, with the greatest amount of reduction occurring in the lactate-amended bottles. As(V) reduction occurred within 50 h with lactate and 70 h with acetate and proceeded from 20 h until the termination of the experiment in the unamended bottles. Although As(V) was not completely reduced, the termination of As(V) reduction in the lactate-amended bottles occurred concurrently with the complete consumption of lactate (~45 h). Fe(III) reduction continued even after the lactate was exhausted, but as the experiments with the unamended bottles show, some As(V) and Fe(III) reduction can occur without organic carbon amendment. About 13−14% of the Fe(III) was reduced in the lactate- and acetate-amended bottles, while 8% of the Fe(III) was reduced in the unamended bottles.

In the lactate-amended bottles, the changes in lactate and acetate concentration exceeded the values expected based on the stoichiometry of Fe(II) and As(III) production. Since the sediment inoculum introduced a mixed microbial community, it is possible that some consumption of lactate proceeded through alternative pathways not involving Fe(III) or As(V) reduction, such as nitrate or manganese reduction.

Reduction of As(V) and Fe(III) was observed in the unamended microcosm inoculated with Haiwee reservoir sediment, indicating that the bacteria were able to utilize the ambient carbon from the small amount of sediment introduced with the inoculum. The carbon content of the sediment is 2.8% on a dry weight basis.

Incubations with ANA-3. As(V) and Fe(III) were simultaneously reduced in the incubations with ANA-3 WT with HFO/As(V) (Figure 2a). As(V) reduction occurred within 40 h of incubation, although it was not completely converted to As(III). HFO reduction continued throughout the course of the experiment. No As(V) reduction was observed in the ANA-3 Δ*arrA* mutant incubation on HFO/As(V), and acetate production (Figure 2c) was coupled to Fe(III) reduction (Figure 3). As(III) concentrations remained constant over

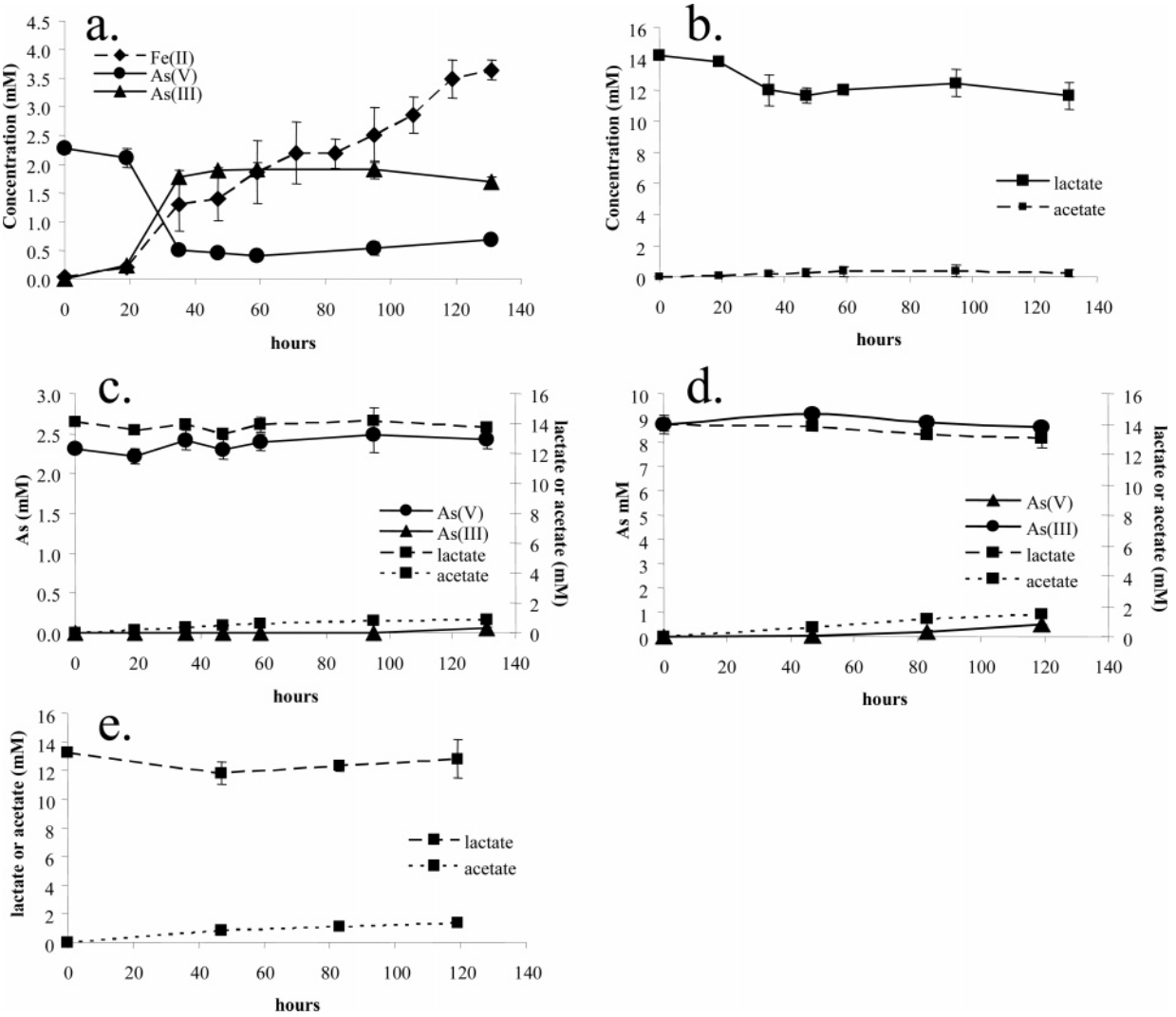

FIGURE 2. Measured total concentrations of As(V), As(III), Fe(II), lactate, and acetate in incubations with (a,b) ANA-3 WT incubated with As(V)-equilibrated HFO, (c) ANA-3 Δ*arrA* mutant incubated with As(V)-equilibrated HFO, (d) ANA-3 WT incubated with As(III)-equilibrated HFO, and (e) ANA-3 WT incubated with HFO (no As). ANA-3 WT or Δ*arrA* mutant was inoculated into the appropriate HFO slurry at a final cell density of 500 cells/mL slurry. For experimental conditions, see Table 1.

time in experiments where ANA-3 WT was incubated with HFO/As(III). In all incubations with ANA-3, conversion of lactate to acetate was observed consistent with previous observations (*38*). Although acetate concentrations were less than expected, lactate was consumed stoichiometrically based on As(III) and Fe(II) production (Table S6). Fe(III) reduction was observed in all incubations with ANA-3, but the rates of Fe(III) reduction varied in the different incubations (Figure 3). The mutant ANA-3 Δ*arrA* showed the lowest rate of Fe(III) reduction. With ANA-3 WT, more Fe(III) reduction was observed with HFO pre-equilibrated with either As(V) or As(III) than with HFO alone.

Discussion

Utilization of Terminal Electron Acceptors. In both sediment and ANA-3 microcosm experiments, As(V) reduction occurs concurrently or even prior to Fe(III) reduction. In the ANA-3 WT microcosms, the utilization of As(V) as a terminal electron acceptor prior to or concurrently with Fe(III) is consistent with calculations of the thermodynamic driving force for As(V) and Fe(III) reduction coupled with lactate oxidation. For conditions where the reactant As(V) and the products As(III) and Fe(II) are predominantly sorbed to HFO, the thermodynamics of As(V) and Fe(III) reduction are nearly the same throughout the course of the experiment (Table 2); ΔG becomes less favorable for both reactions as they progress. Decreased driving force (less negative ΔG) has been calculated in other Fe systems, where Fe(II) accumulation decreases the driving force for bacterial reduction of hematite over time (*26*). In the ANA-3 microcosms, As(V) was not reduced completely to As(III), indicating that the residual As(V) may have been limited by kinetic factors such as inaccessibility of residual As(V) rather than thermodynamic considerations.

Thermodynamics alone do not always predict the utilization of TEAs in these types of reactions, since other factors such as enzyme kinetics may influence the rates and extent of TEA consumption. However, the thermodynamic calculations presented here show that the effects of chemical reactions such as adsorption can greatly affect the energetics of the system.

Our study illustrates the importance of considering the speciation of As and Fe associated with the solid phase both in assessing the full extent of As(V) and Fe(III) reduction and in comparing the energetics of As(V) and Fe(III) reduction. In other studies where As and Fe were measured only in the dissolved phase, Fe(II) either accumulated in solution prior to the increase in dissolved As(III), or As and Fe(II) were released simultaneously into solution (*5, 11*−*13*). However, the amount of Fe and As in solution is dependent not only on the extent of reduction of each element but also the extent

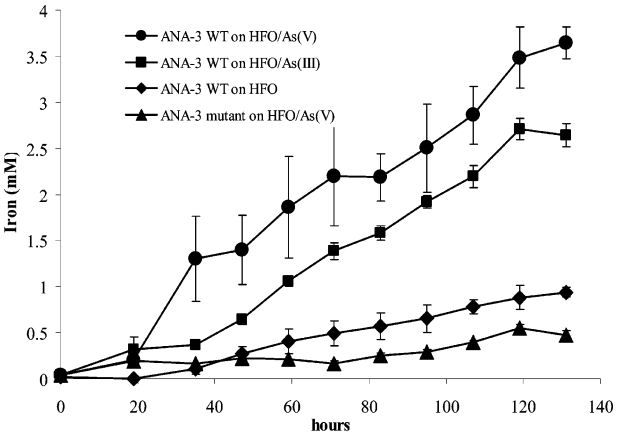

FIGURE 3. Measured total concentrations of Fe(II) for ANA-3 WT grown on HFO pre-equilibrated with As(V), As(III), or HFO only (no As), and ANA-3 Δ*arrA* mutant on HFO pre-equilibrated with As(V). The rates of Fe(II) production after 40 h of incubation are 0.024 mM Fe/h for ANA-3 WT on HFO/As(V), 0.028 mM Fe/h for ANA-3 WT on HFO/As(III), 0.009 mM Fe/h for ANA-3 WT on HFO only, and 0.003 mM Fe/h for ANA-3 Δ*arrA* mutant. The presence of As(III) adsorbed onto HFO enhances the rate of Fe(III) reduction.

TABLE 2. Thermodynamic Driving Force Calculation Results for ANA-3 WT Microcosm

time (h)	$\Delta G_{As(V)}$ (kJ/mol)	ΔG_{HFO} (kJ/mol)
19	−200	−189
35	−144	−143
59	−133	−130

to which they are sorbed onto the solid phase. Therefore, measurement of both dissolved and solid-phase redox speciation is necessary to follow the energetics of the reactions.

Although the bicarbonate, As, and Fe concentrations used in the microcosms are higher than would be generally found in natural systems, the HFO used in this study is a reasonable laboratory model for the Fe floc deposited in Haiwee sediments. Thus, the observed order of terminal electron acceptor utilization may be expected for Haiwee sediment. Our laboratory results are consistent with field observations at Haiwee reservoir, where As(V) was converted to As(III) in the surficial sediments and Fe(III) reduction occurred deeper in the sediment column (*32*). In addition, the *arrA* gene was identified in Haiwee sediments (*14*) indicating that the respiratory pathway of As(V) reduction may be important. This suggests that the redox transitions observed in Haiwee sediments are due to microbial As(V) and Fe(III) reduction.

Respiratory and Detoxification Pathways. The ANA-3 ΔarrA mutant in an HFO/As(V) slurry did not reduce any As(V) (Figure 2c), although both ANA-3 WT and ANA-3ΔarrA mutant are capable of As(V) reduction for the purpose of detoxification using the ArsC reductase. Therefore, the detoxification pathway did not play a significant role in this incubation. The threshold for expressing the *ars* detoxification genes is >100 μM As(V), while the *arr* respiratory genes are expressed at As(V) concentrations of 100 nM (*39*). Dissolved concentrations of As(V) were below the detection limit of 100 μM, below the threshold for *ars* expression. Thus, detoxification should not be a significant pathway for As(V) reduction, consistent with the experimental observations.

In the sediment microcosms, the reduction of arsenate ceased around 40 h of incubation in lactate-amended bottles, which corresponds to the complete consumption of lactate. The correlation between As(V) reduction and lactate consumption is indicative of the respiratory pathway and suggests it may be dominant in the sediment microcosms as well. This is consistent with previous studies that suggest that rates of As(V) reduction via the detoxification pathway may be slower than the respiratory pathway in other bacterial strains (*19*).

It is evident that bacteria can reduce As(V) for respiration or detoxification even under conditions where As(V) is predominantly adsorbed to or associated with an Fe mineral matrix (*11*, *12*, *19*, *28*, *29*). The arsenate reductase ArrA is thought to be located in the periplasm of the bacterial cell on the basis of genetic similarities to other known proteins (*35*, *40*) and to require a phosphate transporter to translocate dissolved As (i.e., after desorption from the solid) into the periplasm. This would imply that the dissolved As(V) in our experiments, although below the detection limit, was sufficient to support the observed microbial reduction. However, the question remains as to whether a bacterial cell can directly utilize adsorbed As(V) or requires desorption of As(V) from the solid phase.

Effect of Sorbed As on Fe(III) Reduction. The presence of adsorbed As(III) increased the rate of Fe reduction in the ANA-3 WT microcosm experiments. In the incubations with HFO/As(V), sorbed As(V) was reduced to As(III) within approximately 40 h. Once most of the As(V) was reduced (>40 h) and As(III) was the dominant species adsorbed onto the HFO, the rate of Fe reduction was comparable to the HFO/As(III) case (0.024 mM Fe/h for HFO/As(V) microcosm and 0.028 mM Fe/h for the HFO/As(III) microcosm) from 40 to 130 h. The HFO only and HFO/As(V)/mutant incubations show substantially less Fe reduction (0.009 and 0.003 mM Fe/h, respectively) than observed in the HFO/As(V) and HFO/As(III) microcosms, which indicates that sorbed As(III) enhances the bacterial reduction of HFO.

The rate of microbial reduction of Fe(III) oxyhydroxides depends on the surface area and crystallinity of the oxide mineral (*12*, *19*, *21*). Changes in these properties could be related to the observed differences in rates of Fe reduction. The time scale of this experiment (~4 days) is considerably less than the time scale of ~15 days over which substantial recrystallization of HFO, with or without adsorbed As, was observed in abiotic batch experiments (*41*). In flow-through experiments, however, a transition from HFO to lepidocrocite has been observed within 2 days of incubation with bacteria (*42*−*43*). The absence of dissolved As in our study suggests that surface area did not decrease substantially over the course of the experiment.

The enhancement of microbial Fe reduction observed when As(III) was adsorbed on the HFO substrate was unexpected and may have significant environmental implications. Bacterial reduction of As(V) to As(III) may increase the rate of reductive dissolution of poorly crystalline Fe hydroxides, possibly leading to an increase in As mobility into groundwater in sediment systems.

Acknowledgments

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Supporting Information Available

Chemical constituents of bacterial minimal medium, vitamins and minerals, intrinsic surface complexation constants, analytical methods, concentrations used to calculate thermodynamic driving forces, and complete description of thermodynamic calculations. This material is available free of charge via the Internet at http://pubs.acs.org.

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