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Fate of Arsenic during Microbial Reduction of Biogenic versus Abiogenic As–Fe(III)–Mineral Coprecipitates

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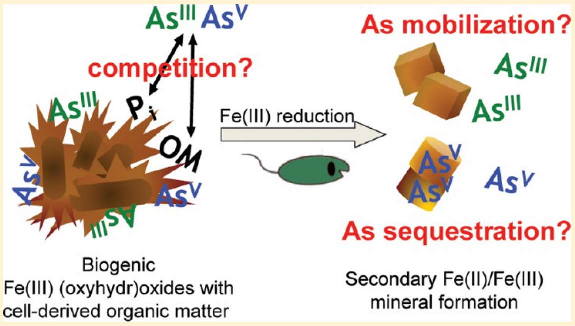
Supporting Information



ABSTRACT: Fe(III) (oxyhydr)oxide minerals exhibit a high sorption affinity for arsenic (As) and the reductive dissolution of As-bearing Fe(III) (oxyhydr)oxides is considered to be the primary mechanism for As release into groundwater. To date, research has focused on the reactivity of abiogenic Fe(III) (oxyhydr)oxides, yet in nature biogenic Fe(III) (oxyhydr)oxides, precipitated by Fe(II)-oxidizing bacteria are also present. These biominerals contain cell-derived organic matter (CDOM), leading to different properties than their abiogenic counterparts. Here, we follow Fe mineralogy and As mobility during the reduction of As-loaded biogenic and abiogenic Fe(III) minerals by *Shewanella oneidensis* MR-1. We found that microbial reduction of As(III)-bearing biogenic Fe(III) (oxyhydr)oxides released more As than reduction of abiogenic Fe(III) (oxyhydr)oxides. In contrast, As was immobilized more effectively during reduction of As(V)-loaded biogenic than abiogenic Fe(III) (oxyhydr)oxides during secondary Fe mineral formation. During sterile incubation of minerals and after microbial Fe(III) reduction stopped, As(V) was mobilized from biogenic Fe(III) (oxyhydr)oxides probably by sorption competition with phosphate and CDOM. Our data show that the presence of CDOM significantly influences As mobility during reduction of Fe(III) minerals and we suggest that it is essential to consider both biogenic and abiogenic Fe(III) (oxyhydr)oxides to further understand the environmental fate of As.

INTRODUCTION

Although the worldwide anthropogenic input of arsenic (As) into the environment has decreased over recent decades, a severe As problem has most prominently arisen in Southeast Asia where millions of people are exposed to As-contaminated drinking water.[1] Erosion from Himalayan rocks followed by fluvial transport causes the deposition of As-bearing sulfide minerals in Southeast Asian river deltas,[1] which are weathered to As-bearing Fe(III) (oxyhydr)oxides further downstream.[2] Potential mechanisms that lead to the mobilization of As from these sediments have been intensely studied but are still a matter of debate. Oxidative destabilization of As-bearing sulfidic minerals,[3,4] competition of As with other oxyanions such as phosphate[5] or bicarbonate,[6] and the release of sorbed As as a consequence of microbial reduction of As(V) to the more mobile As(III)[7−9] have been suggested to cause As mobilization. Recent studies have also proposed that organic material (OM) mobilizes As by sorption competition and complex formation.[10−12] However, the reductive dissolution of As-bearing Fe(III) (oxyhydr)oxides as a consequence of oxidation of allochthonous and autochthonous OM is generally considered to be the most important process causing the release of As.[2,13] The high As-binding capacity of Fe(III) (oxyhydr)oxide minerals[14] and their abundance in nature[15] qualifies these minerals to play a major role for As mobility.

Microbial Fe(III) reduction requires anoxic conditions as well as an electron donor such as organic carbon.[16] These geochemical conditions are met in the heterogeneous Southeast Asian aquifers either by the presence of organic rich peat lenses,[1,17] the input of fresh organic carbon due to water percolation from surface water[18] or the migration of naturally occurring petroleum.[19] The presence of dissimilatory metal-reducing bacteria was demonstrated in Southeast Asian aquifers and their abundance was correlated to the mobilization of As in sediment microcosms.[7,13,20] Based on these results it was suggested that the activity of Fe(III)-reducing bacteria causes As mobilization in As-contaminated environments.[2,13,17] However, laboratory-based studies have shown that microbial Fe(III) reduction does not result in the dissolution of Fe minerals and the mobilization of As under all circumstances.[21,22] Depending on the geochemical conditions and the concentration and identity of the initial Fe(III) mineral substrate, the formation of secondary Fe mineral phases such as siderite, vivianite, green rust, or magnetite can be observed during microbial Fe(III) reduction.[16,23−25] To determine the mobility of As during the microbial formation of secondary Fe mineral phases, aqueous As

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concentrations were monitored in microcosms containing either abiogenic, chemically synthesized As(V)- and As(III)-loaded Fe(III) (oxyhydr)oxides[21,22,26,27] or natural As-bearing Southeast Asian sediments.[26,28] These studies demonstrated that the formation of secondary Fe(II) and mixed Fe(II)/(III) minerals may actually lead to a partial sequestration, or at least a retention of As rather than mobilization.

So far most of the knowledge about As mobilization from Fe(III) minerals was obtained from studies using abiogenic, synthetic Fe(III) minerals. However, it was shown that As-contaminated environments contain not only Fe(III)-reducing but also Fe(II)-oxidizing bacteria, including the genus *Acidovorax* and other Proteobacteria.[29−31] During microbial Fe(II) oxidation and Fe(III) mineral precipitation, these bacteria produce aggregates of Fe(III) minerals and cell-derived organic matter (CDOM).[32−34] These biominerals often exhibit different properties compared to their abiotic counterparts.[33,35−37] Biogenic minerals tend to be smaller in size, are less crystalline, exhibit a larger surface area and are negatively charged at neutral pH in contrast to the positively charged abiotic minerals,[33] suggesting that these biogenic Fe(III) (oxyhydr)oxides have different properties regarding metal-binding[36,38−40] and are probably more easily reduced by microorganisms.[41,42] The natural occurrence of organics and cells associated with As-loaded biogenic Fe oxides was recently shown.[43]

Furthermore, it has been demonstrated that the nitrate-reducing Fe(II)-oxidizer *Acidovorax* sp. strain BoFeN1 can not only metabolize in the presence of high As concentrations but it can efficiently immobilize As by sorption to and coprecipitation with the newly formed biogenic Fe(III) (oxyhydr)oxides.[34,40] It is unknown however, how stable these biogenic As-loaded Fe(III) (oxyhydr)oxides are toward microbial Fe(III) reduction. The main objectives of the present study therefore are to compare reduction rates and extent of reduction of As-loaded and As-free biogenic Fe(III) (oxyhydr)oxides produced by strain BoFeN1 (mainly nanocrystalline goethite with minor amounts of ferrihydrite) by the dissimilative Fe(III)−reducer *Shewanella oneidensis* MR-1 to the reduction of abiogenic, chemically synthesized As-loaded Fe(III) minerals (goethite and ferrihydrite). Furthermore, we quantified the extent of As mobilization or sequestration by the secondary Fe phases formed during the microbial Fe(III) reduction process.

■ MATERIALS AND METHODS

Source of Microorganism and Growth Conditions. *Shewanella oneidensis* strain MR-1 is a Fe(III)-reducing β-Proteobacterium able to utilize a wide range of substrates.[44] Iron-free precultures of strain MR-1 were adapted stepwise to the mineral medium used for the actual experiments. First, strain MR-1 was streaked out from a −80 °C frozen stock and grown aerobically at 28 °C for 24 h on lysogeny broth agar plates. Second, single colonies were transferred to 10 mL of anoxic mineral medium (pH 7; N_2/CO_2 80/20 v/v; 0.14 g/L KH_2PO_4, 0.2 g/L NaCl, 0.3 g/L NH_4Cl, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.1 g/L $CaCl \cdot 2H_2O$, 22 mM bicarbonate buffer, 1 mL/L vitamin solution, 1 mL/L trace element solution, 1 mL/L selenate-tungstate solution[40]) containing 20 mM Na-lactate and 40 mM Na-fumarate. After 3 days of growth at 28 °C, 200 μL were transferred to a fresh tube and incubated for another 2 days. The cell number was determined by absorption measurements at 660 nm, previously calibrated against microscopic cell counts using a Thoma-chamber (Axioscope 2, Zeiss, Germany),[45] and diluted to 2×10^7 cells/mL in mineral medium. For experiments (set up in duplicates), 20 mM Na-lactate (electron donor), 0.1 mM AQDS (electron shuttle) and As-free and As-bearing Fe(III) minerals (normalized to 6−8 mM Fe) were added to anoxic mineral medium in butyl-stoppered glass bottles and inoculated with strain MR-1 to a starting cell number of 2×10^5 cells/mL. The bottles were incubated in the dark at 28 °C. Independent of the initial Fe(III) minerals, the pH increased in all bottles from 7.0 to 7.3 during microbial Fe(III) reduction. In preliminary experiments with the same medium amended with lactate and fumarate, the presence of aqueous As(III) and As(V) concentrations between 0 and 200 μM did not affect MR-1 growth significantly (data not shown). Strain MR-1 was also unable to reduce As(V) under these conditions (data not shown).

Source of Abiogenic and Biogenic Fe(III) Minerals. As-loaded abiogenic ferrihydrite and goethite were synthesized sterilely by adding two different As solutions ($NaAsO_2$ and $Na_2HAsO_4 \times 7H_2O$) from anoxic stocks into solutions of 0.2 and 1 M $Fe^{III}(NO_3)_3$, respectively, in Fe:As ratios of 50:1 and 250:1 (mg:mg) for both As(III) and As(V). As-bearing abiogenic ferrihydrite was then synthesized by neutralization of the As−$Fe^{III}(NO_3)_3$ solution using 1 M KOH.[15] Goethite was obtained by neutralizing the As−$Fe^{III}(NO_3)_3$ solution using 5 M KOH and aging the reddish As−Fe precipitate at 65 °C for 3 days.[15] The purity of the synthesized abiogenic Fe(III) minerals was verified by XRD. As-bearing biogenic minerals were synthesized using the Fe(II)-oxidizer *Acidovorax* sp. BoFeN1. The growth conditions including medium composition are provided in Kappler et al. (2005).[46] The medium was amended with $FeCl_2$ (10 mM) and filtered after 48 h to remove all Fe(II) precipitates present.[34,47] Subsequently, Na-nitrate (5 mM), Na-acetate (10 mM) and either As(III) or As(V) were added at the target Fe:As ratios of 50:1 and 250:1 before inoculation with 5% of a second-generation iron-free preculture of strain BoFeN1 (grown on acetate and nitrate). Within 2 weeks at 28 °C more than 90% of the Fe(II) was oxidized and the As−Fe coprecipitates were harvested anoxically and washed once with sterile, anoxic Millipore water. According to EXAFS analysis in Hohmann et al.,[40] As-free and As(III)-bearing biogenic Fe(III) (oxyhydr)oxides consist mainly of goethite and approximately 13 ± 5% ferrihydrite, while As(V)-bearing biogenic Fe(III) (oxyhydr)oxides contain approximately 49 ± 10% ferrihydrite and 51 ± 10% goethite. Biogenic and abiogenic minerals were collected by centrifugation at 7100*g* for 12 min, resuspended in anoxic, sterile MQ water and used within two weeks of synthesis. The actual amount of As in the coprecipitates was determined by quantifying dissolved As concentrations prior to and after Fe mineral precipitation (Supporting Information (SI) Table S1). The actual Fe:As ratios were close to the targeted values (50:1 and 250:1) for abiogenic ferrihydrite and biogenic Fe(III) (oxyhydr)oxides, but were slightly higher for abiogenic goethite as As coprecipitated to a lower extent during goethite precipitation (SI Table S1). For simplicity, the ratios are still named 250:1 and 50:1 throughout the paper.

Sampling of Batch Experiments. For Fe and As analyses, 2-mL-samples were withdrawn from each bottle in the glovebox at all time points. For quantification of total Fe(II) and Fe(III), 100 μL of sample were added to 0.9 mL of deoxygenated 6 M HCl to dissolve all minerals and to prevent Fe(II) oxidation until measurement after 24 h.[48] The remaining 1.9 mL were centrifuged at 13 200*g* for 2 min. 100 μL of the supernatant was diluted in 0.4 mL anoxic 1 M HCl for determining dissolved Fe(II) and Fe(III). 990 μL of the supernatant was stabilized by

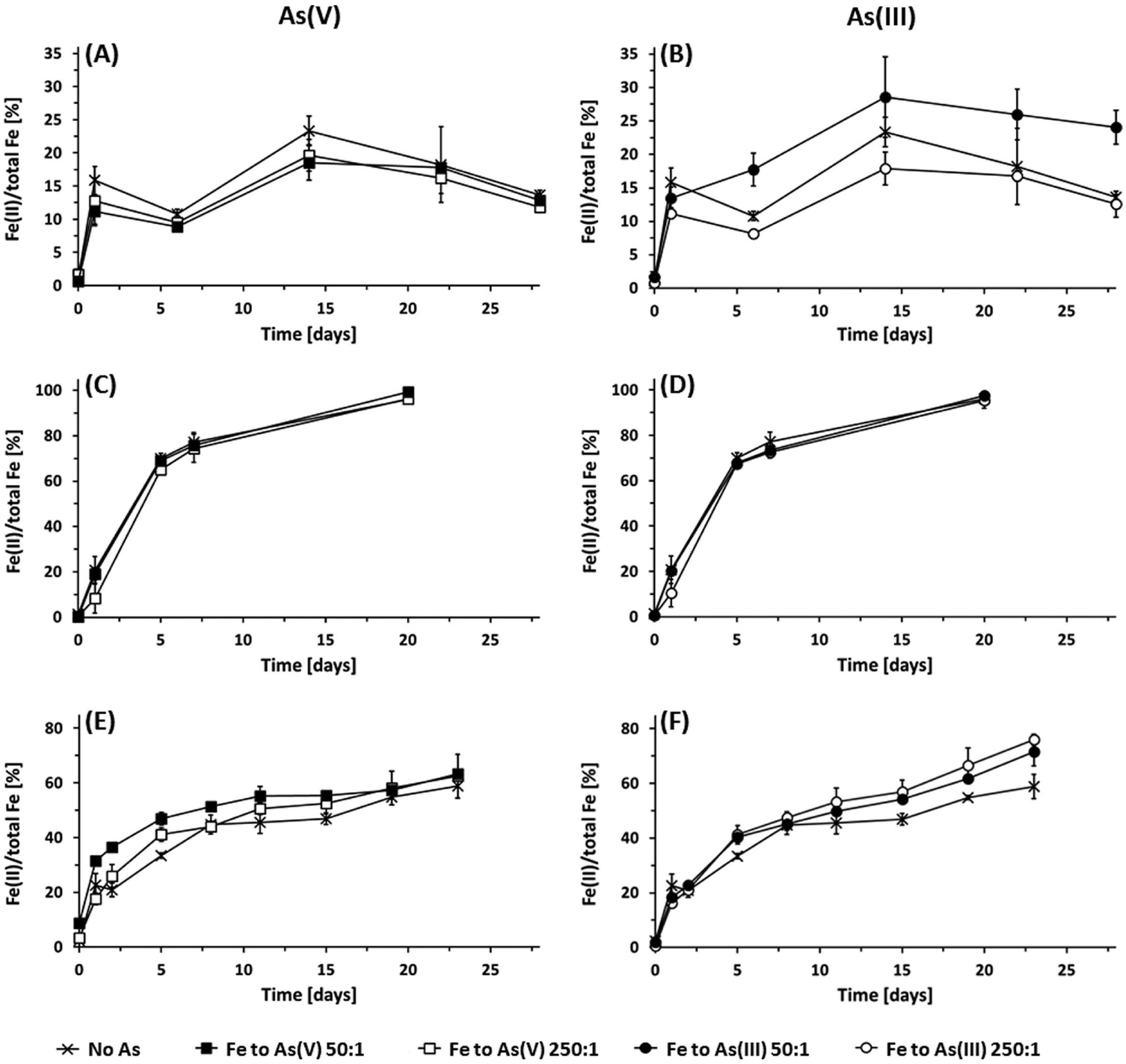

Figure 1. Fe(II) formation in % of total Fe over time during reduction of abiogenic goethite (A, B), abiogenic ferrihydrite (C, D) and biogenic Fe(III) (oxyhydr)oxides (E, F) by *S. oneidensis* MR-1. The Fe(III) minerals contained either no As (×) or As(V) (A,C,E) at Fe:As(V) ratios of 250:1 (□) and 50:1 (■) or in the presence of As(III) (B,D,F) at Fe:As(III) ratios of 250:1 (○) and 50:1 (●). Data represent the mean of duplicate cultures. Fe(II) in abiotic controls was below 10% throughout the experiments and is not shown. Absolute Fe(II) concentrations in mM are given in SI Figure S1.

10 μL of 65% HNO_3 and stored at −20 °C for total dissolved As quantification. For As species analysis in the beginning and at the end of the experiment, 0.5 mL supernatant was diluted in 9.5 mL of 10 mM phosphoric acid and kept at 4 °C until measurement.[49] Additionally, minerals were harvested by centrifugation (13 200*g*, 2 min) and vacuum drying for scanning electron microscopy (SEM) and X-ray diffraction (XRD).

Analytical Methods. Fe(II) and Fe(III) were determined spectrophotometrically using the ferrozine assay.[50] Total dissolved As was quantified after dilution in 2% HNO_3 by ICP-MS (7700 series ICP-MS, Agilent Technologies). Dissolved As species were identified and quantified by HPLC-ICP-MS (Agilent 7700). Details of the speciation method are described elsewhere.[51] Samples for SEM were prepared anoxically in an N_2-filled glovebox using Al stubs covered with sticky graphite tape,[32] sputtered under vacuum for 60 s with a 6−8 nm thick Pt layer (SCD 005/CEA 035 sputter, BAL-TEC, 35 mm working distance, 30 mA, 0.05 mbar argon) and images were taken by an electron microscope (SEM LEO-1450 VP, LEO Electron Microscopy Ltd.) with an acceleration voltage of 15 kV at a working distance of 8−9 mm. For XRD, the vacuum-dried minerals were ground in an agate mortar in an anoxic glovebox, and transferred onto a silicon wafer. Each measurement was done oxically between 5 and 75 degrees within 3−5 min to minimize exposure to O_2 using a μ-XRD (Bruker D8 Discover XRD instrument, Bruker AXS GmbH, Germany) equipped with a Co K_α source.[45]

■ RESULTS AND DISCUSSION

Microbial Reduction of Biogenic vs Abiogenic Fe(III) Minerals. Fe(III) reduction by *Shewanella oneidensis* strain MR-1 was quantitatively followed over time for three different Fe(III) (oxyhydr)oxides: abiogenic goethite, abiogenic ferrihydrite, and biogenic Fe(III) (oxyhydr)oxides. They contained either no As, or As(III) or As(V) with Fe:As ratios of 250:1 and 50:1 (mg/mg) (Figure 1 and SI Figure S1). The extent of microbial Fe(III) reduction differed significantly among the three Fe(III) (oxyhydr)oxides. After 20 days, 96% of Fe(II) was measured in

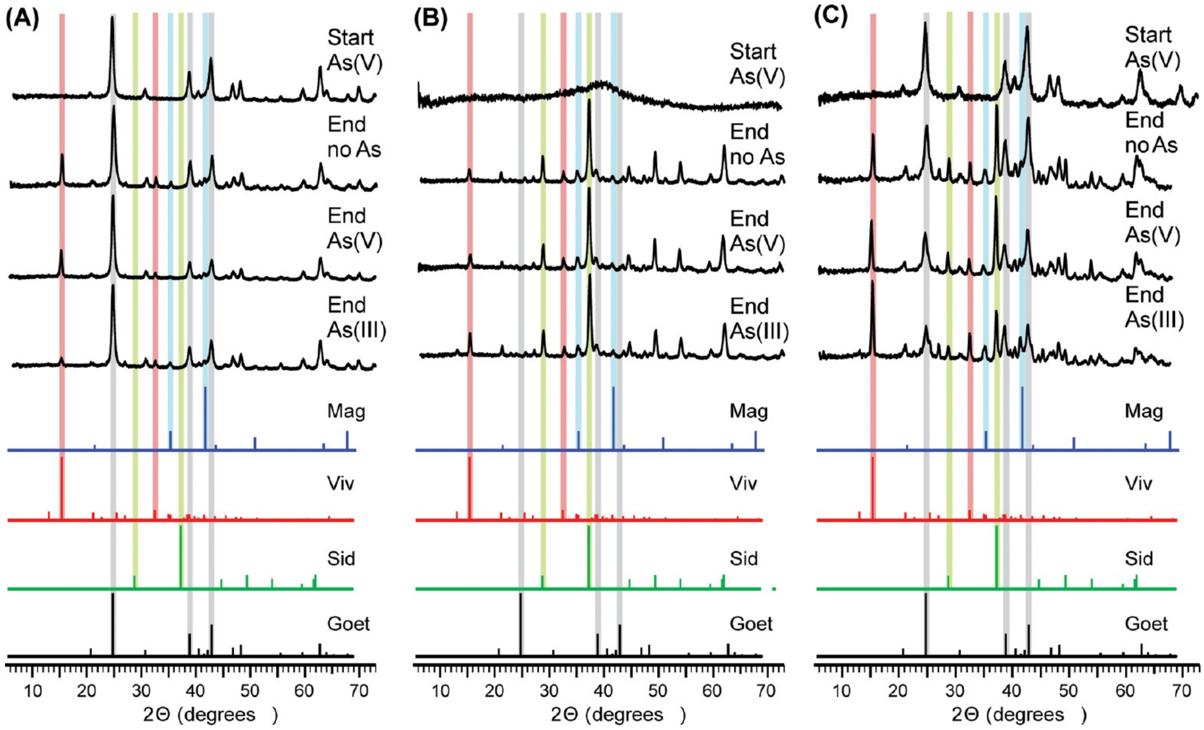

Figure 2. μ-X-ray diffractograms of As-free, As(V)-, and As(III)-bearing abiogenic goethite (A), abiogenic ferrihydrite (B), and biogenic Fe(III) (oxyhydr)oxides (C) before and after reduction by *S. oneidensis* strain MR-1. The diffractograms of the As(V)-loaded Fe(III) minerals with an Fe to As(V) ratio of 50:1 are shown as representatives of all starting samples prior to inoculation (Start As(V)) since they were virtually identical to the diffractograms of the As(III)-loaded and the As-free minerals. Microbially reduced samples for each Fe(III) mineral without As (End no As), with a Fe:As(V) ratio of 50:1 (End As(V)) and a Fe:As(III) ratio of 50:1 (End As(III)) are plotted below the start sample. The main reflections of the reference data for goethite (Goet in gray), vivianite (Viv in red), siderite (Sid in green), and magnetite (Mag in blue) are depicted at the bottom of the graphs and highlighted with their respective color for comparison. The data is given in relative counts.

solution after reduction of 6 mM abiogenic ferrihydrite (Figure 1C,D) compared to about 59–75% Fe(II) formed from 6 to 8 mM biogenic Fe(III) (oxyhydr)oxides after 23 days (Figure 1E,F) and only about 15–25% Fe(II) from 6 mM abiogenic goethite (Figure 1A,B) after 28 days. Microbial reduction of all three types of Fe(III) (oxyhydr)oxides started within 1 day after inoculation. The initial rate of Fe(III) reduction between time point 0 (time of inoculation) and 1 day was similar for biogenic Fe(III) (oxyhydr)oxides and abiogenic ferrihydrite with 0.222 ± 0.053 and 0.195 ± 0.066 Fe(II)/Fe(tot) day^{-1}, respectively, but lower for abiogenic goethite with 0.148 ± 0.011 Fe(II)/Fe(tot) day^{-1} (SI Table S2). In summary, our data suggest that abiogenic ferrihydrite was reduced best, followed by biogenic Fe(III) (oxyhydr)oxides, and then abiogenic goethite.

The observed trend in Fe(III) reduction is reasonable since the biogenic Fe(III) (oxyhydr)oxides mainly consist of nanoparticulate goethite and minor amounts of poorly crystalline Fe(III) minerals as has been demonstrated in a previous study.[34] The observed lower extent of abiogenic goethite reduction in our study confirms previous work by Zachara et al.,[24] who found 17% Fe(III) reduction of goethite within 39 days of incubation with *Shewanella putrefaciens* strain CN32 under similar conditions. An almost complete reduction of ferrihydrite as observed in our experiments has been shown before using different *Shewanella* strains.[23,25,45]

Both the extent and the rates of Fe(III) reduction correlate to the chemical properties of the different Fe(III) minerals tested. Highly crystalline Fe(III) (oxyhydr)oxides such as goethite are more stable against microbial Fe(III) reduction than smaller sized, poorly crystalline ferric (oxyhydr)oxide mineral phases such as ferrihydrite.[24,42,52,53] Poorly crystalline minerals like ferrihydrite exhibit a large surface area (e.g., 200–400 m^{2}g^{-1})[15] and, thus, have more accessible surface sites for Fe(III) reduction than goethite (e.g., 2–50 m^{2}g^{-1})[15] and the biogenic Fe(III) (oxyhydr)oxides produced by strain BoFeN1 (i.e., biogenic goethite with a surface area of 158 m^{2}g^{-1}).[46]

The differences in microbial reduction of biogenic versus abiogenic Fe(III) (oxyhydr)oxides are not only caused by differences in crystallinity and size of the minerals, but also high amounts of cell derived organic matter (CDOM) probably including lysed cell material and exudates that is associated with biogenic Fe(III) minerals.[33] These organics could potentially be used as additional electron donors for microbial Fe(III) reduction,[42] although we expect this to be of minor importance in our experiments as the electron donor lactate was provided in excess. However, CDOM has the ability to complex the formed Fe^{2+}, which on the one hand is then not able to block mineral surface sites for further Fe(III) reduction[42] and on the other hand makes Fe(III) reduction thermodynamically more favorable.[54] The complexation of Fe^{2+} by CDOM is also expected to prevent or at least reduce Fe(II)-catalyzed mineral transformation[55] and to retard the precipitation of secondary Fe(II) and Fe(II)/Fe(III) mineral phases. Both processes will also facilitate further Fe(III) reduction. Evidence for the complexation and thus solubilization of Fe^{2+} by CDOM comes from the high dissolved Fe^{2+} concentrations in the setups with biogenic Fe(III) (oxyhydr)oxides after microbial Fe(III) reduction (SI Figure S2). Additionally, CDOM may change mineral surface processes by sorbing itself to the mineral surface

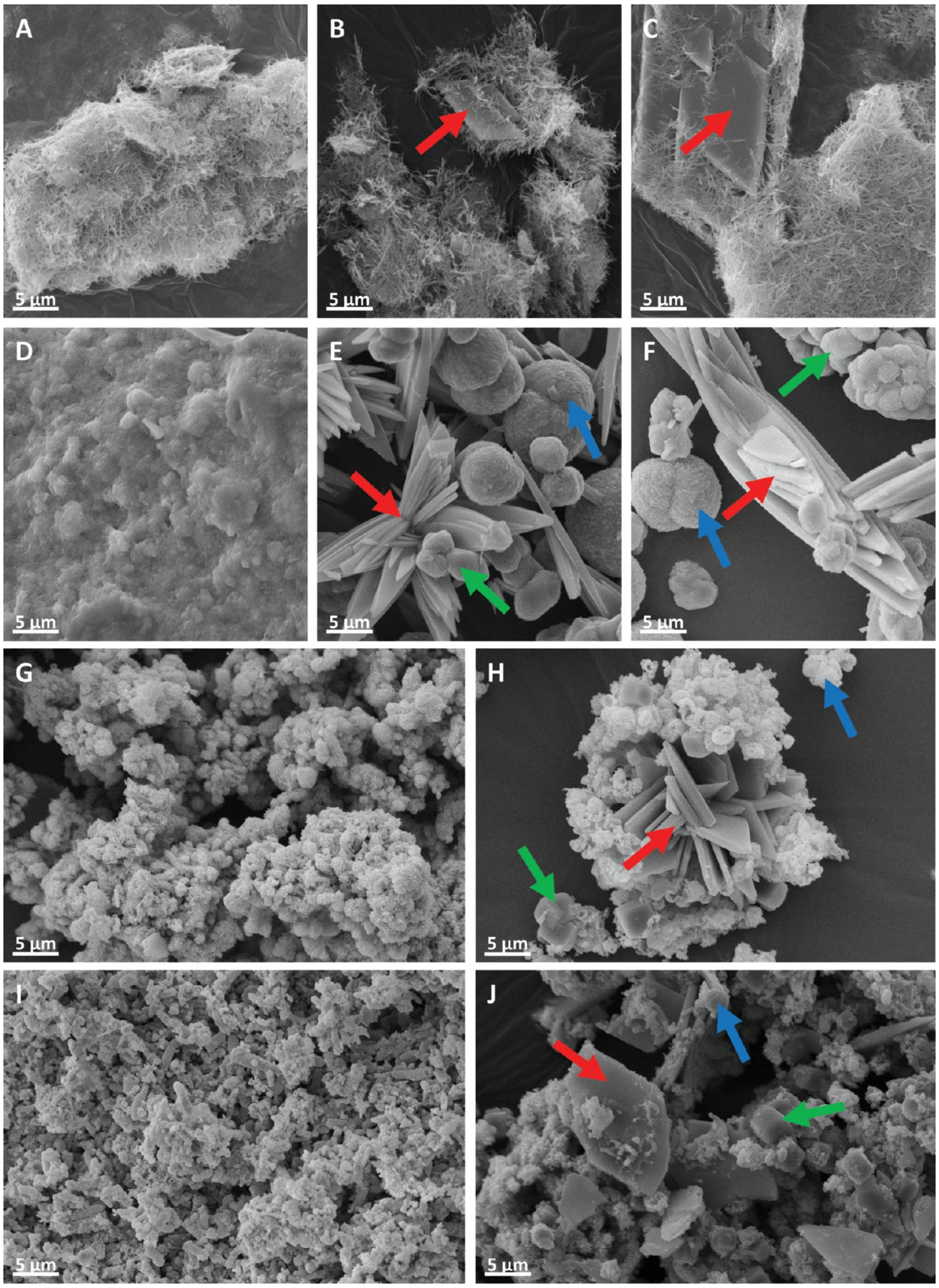

Figure 3. Scanning electron micrographs of As(III)- and As(V)-loaded abiogenic goethite (A,B,C), abiogenic ferrihydrite (D,E,F) and biogenic Fe(III) (oxyhydr)oxides (G,H,I,J) with a Fe:As ratio of 50:1 before (A,D,G,I) and after (B,C,E,F,H,J) Fe(III) reduction by *S. oneidensis* MR-1. Representative micrographs of As(III)-loaded abiogenic goethite (A) and As(III)-loaded abiogenic ferrihydrite (D) starting minerals are shown as no visual difference was observed to As(V)-loaded abiogenic goethite and ferrihydrite. The secondary Fe mineral products after microbial reduction of the abiogenic As(V)-loaded goethite (B), As(V)-loaded ferrihydrite (E), As(III)-loaded goethite (C), and As(III)-loaded ferrihydrite (F) are shown. For the biogenic Fe(III) (oxyhydr)oxides, As(III)- and As(V)-loaded minerals before (G and I, respectively) and after (H and J, respectively) reduction by strain MR-1 are depicted. Colored arrows indicate the different morphologies of the newly formed secondary Fe mineral phases after Fe(III) reduction.

and blocking surface sites for Fe(III) reduction and Fe(II)-catalyzed mineral transformation.[55]

Identity and Crystallinity of Secondary Fe Mineral Products Formed during Microbial Fe(III) Reduction. XRD analysis of the As-free and As-bearing abiogenic goethite before and after microbial reduction showed that it was partially transformed to vivianite ($Fe_3(PO_4)_2{\cdot}8H_2O$) and magnetite (Fe_3O_4), whereas the bulk still remained as goethite (Figure 2A). Reduction of As-free and As-bearing ferrihydrite (Figure 2B) and biogenic Fe(III) (oxyhydr)oxide (Figure 2C) resulted in the formation of vivianite, siderite ($FeCO_3$) and magnetite. Since the biogenic Fe(III) (oxyhydr)oxide was not completely reduced by MR-1, some of the initially present biogenic goethite remained visible at the end of the experiment (ca. 25−40% based on Fe speciation analysis, see Figure 1). The XRD data is supported by SEM images (Figure 3) that revealed three new mineral phases with different morphologies after reduction. These minerals were either of a platelet shape developing radially from one point (Figure 3 red arrows), showed structures of two associated globules (Figure 3 blue arrows) or were present as

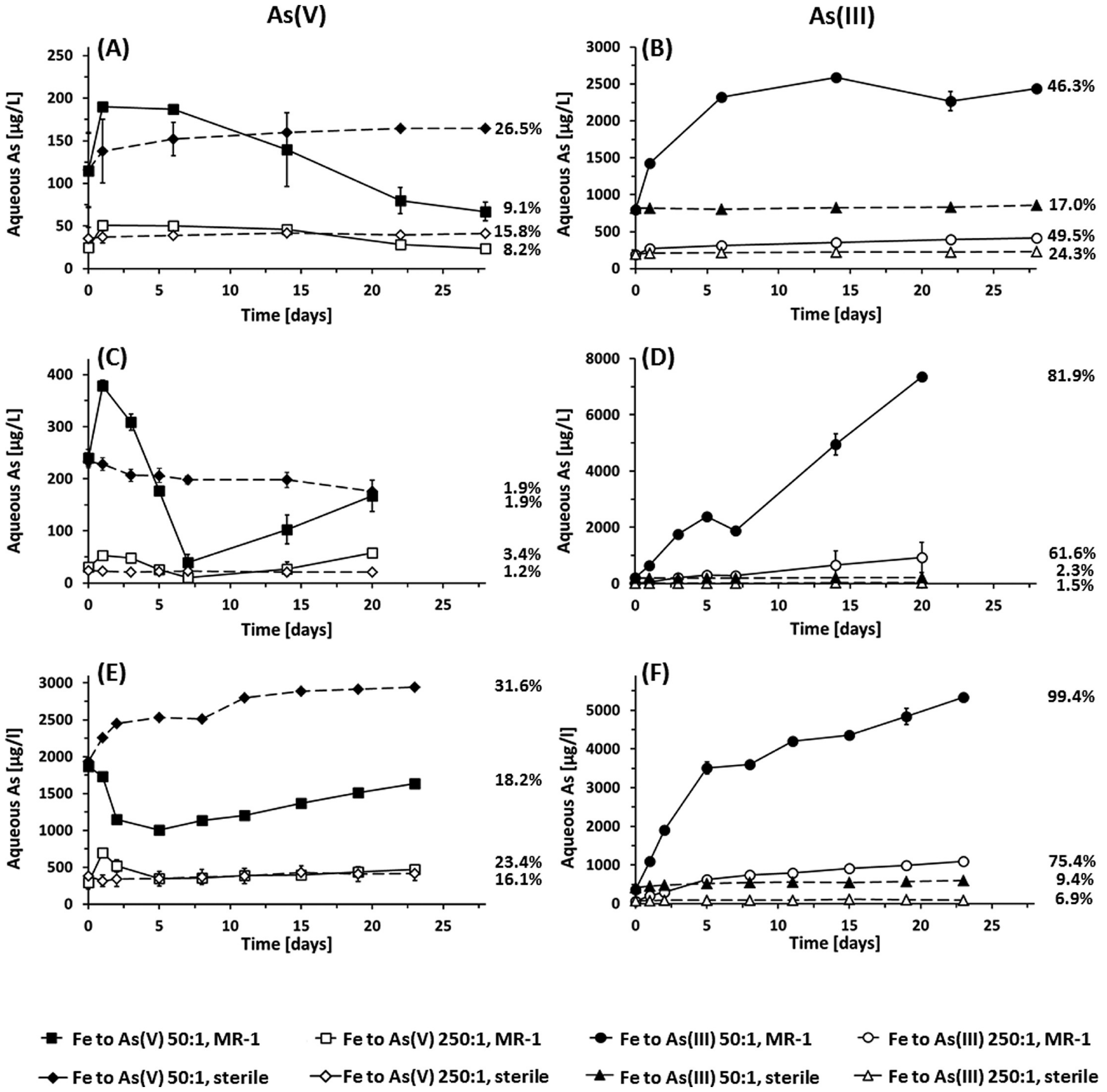

Figure 4. Aqueous As concentrations over time during microbial reduction of abiogenic goethite (A,B), abiogenic ferrihydrite (C,D), and biogenic Fe(III) (oxyhydr)oxides (E,F) by *S. oneidensis* MR-1. The Fe(III) minerals were synthesized either in the presence of As(V) (A,C,E) at Fe:As(V) ratios of 250:1 (□, ◇) and 50:1 (■, ◆) or in the presence of As(III) (B,D,F) at Fe:As(III) ratios of 250:1 (○, △) and 50:1 (●, ▲). The extent of dissolved As in % of total As is given for the last time point of the experiment. Abiotic controls are shown in open and closed triangles and diamonds. Data represent the mean of duplicate cultures.

small cubes (Figure 3 green arrows). All three shapes were found in samples of microbially reduced As-free and As-bearing abiogenic ferrihydrite (Figure 3E,F) and biogenic Fe(III) (oxyhydr)oxides (Figure 3H,L), while only the platelet-type mineral phase and remaining goethite needles were evident in the abiogenic goethite (Figure 3B,C).

It is well-known that Fe(II)-bearing minerals such as vivianite, siderite and magnetite are formed during microbial reduction of Fe(III) (oxyhydr)oxides[16,21,23−26,45,53,56,57] and due to the presence of 1 mM phosphate and 22 mM bicarbonate within the growth medium used, both vivianite and siderite could be expected to form.[24,25,45] However, vivianite exhibits a lower solubility at 25 °C with a log K_{sp} of −36,[58] than siderite which has a log K_{sp} of −10,[58] leading to the precipitation of vivianite before siderite. Due to the low Fe(II) concentrations in the abiogenic goethite setup, siderite is most likely not formed due to its higher solubility product. Additionally, the formation of vivianite and siderite has been linked to fast Fe(III) reduction, while magnetite is formed at lower Fe(II) formation rates,[25] although some previous studies also identified magnetite as the sole end product of rapid ferrihydrite reduction in the absence of bicarbonate buffer.[26,27] Magnetite formation is inhibited by high phosphate concentrations,[25] needs at least 25% Fe(II) of the Fe(tot) and has also been shown to be further reduced to siderite by continued Fe(III) reduction.[45,57] Due to the geochemical composition of the medium used (high phosphate and carbonate concentrations) and the fast Fe(III) reduction rates of strain MR-1, magnetite only accounted for a small fraction of secondary Fe minerals formed. Since phosphate and carbonate have been observed in As-contaminated aquifers in Southeast Asia,[59] the formation of siderite and vivianite is of environmental relevance and these Fe(II) minerals were suggested to play a role for As sequestration.[28]

Impact of As on Extent of Microbial Fe(III) Reduction and Secondary Mineral Formation. The impact of As on the extent of microbial reduction of the three different Fe(III) (oxyhydr)oxides tested and on secondary Fe mineral formation was also investigated. It was found that coprecipitation of As with abiogenic ferrihydrite did not affect the extent of ferrihydrite reduction (Figure 1C,D, SI Table S2). In contrast, 75% of the biogenic Fe(III) (oxyhydr)oxides were reduced to Fe(II) within 23 days when they contained As(III) compared to about 60% Fe(II) formation for the As-free or As(V)-bearing biominerals (Figure 1E,F). Similarly, high loads of As(III) decreased the stability of abiogenic goethite, leading to the formation of 25% Fe(II) as opposed to 15% Fe(II) (on a mM basis) from As-free or As(V)-bearing abiogenic goethite (Figure 1A,B). In summary, the presence of As(III) but not As(V) led to a higher extent of microbial Fe(III) reduction for abiogenic goethite and biogenic Fe(III) (oxyhydr)oxides, whereas abiogenic ferrihydrite was still reduced completely in the presence of As.

It has been described before that the presence of ions such as As(III) and As(V) decreases particle size and crystallinity of the minerals synthesized,[15,60] potentially increasing the bioavailability of the Fe(III) compounds for metal-reducing bacteria. This has been shown before for biogenic Fe(III) minerals formed by strain BoFeN1 by Hohmann et al. (2010 and 2011).[34,40] They found that As−free biogenic Fe(III) (oxyhydr)oxides exclusively consisted of goethite, whereas increasing As concentrations led to the formation of poorly crystalline ferrihydrite, thus explaining the increased extent of Fe(III) reduction observed here for the As-loaded biominerals. However, in our experiments, the presence of As(III) resulted in a higher extent of microbial reduction of the Fe(III) (oxyhdr)oxides, whereas the presence of As(V) did not affect Fe(III) reduction. The production of As-bearing goethite and biogenic minerals resulted in more As(III) than As(V) per Fe (Table S1). Higher As/Fe ratios have been shown to increase reduction rates[61] however, it remains unknown which conditions favor increased Fe(III) mineral dissolution.[62]

Based on XRD and SEM analysis (Figure 2 and 3), As did not influence the identity and crystallinity of the end products of all three Fe minerals after microbial reduction by strain MR-1, relative to As-free controls. This is in contrast to a study by Ona-Nguema et al.,[63] who used room temperature backscattering Mössbauer spectroscopy and HRTEM−XEDS−EELS and observed a shift to more poorly crystalline mineral phases during reduction of As-loaded lepidocrocite. The difference between these results could be due to differences in the abilities of Mössbauer vs XRD to elucidate certain mineral phases.

Impact of Phosphate and Cell-Derived Organic Matter on As Mobility. Already in the absence of the Fe(III)-reducer MR-1, some leaching of As(V), and to a low extent also of As(III), from the Fe(III) minerals was observed when incubated in growth medium (Figure 4). Arsenic was mobilized abiotically over time in particular from As(V)-bearing biogenic Fe(III) (oxyhydr)oxides and abiogenic goethite, but not from ferrihydrite. Abiotic leaching of As(V) during incubation of As-loaded goethite- or ferrihydrite-coated sand has previously been described,[11,27,37] and was reasoned by the presence of phosphate and/or OM in the microbial growth medium.[14,15,64] Phosphate has a high affinity to positively charged Fe(III) (oxyhydr)oxide surfaces, competes with As for sorption sites,[14] and displaces it from the Fe(III) mineral surfaces (Figure 4A,E). Whereas the presence of phosphate reduced As(III) sorption to the surface of poorly crystalline Fe(III) (oxyhydr)oxides more strongly than As(V) sorption at neutral pH, both As species were equally replaced from the surface of goethite.[14] We observed a stronger release of As(V) from biogenic Fe(III) (oxyhydr)oxides compared to abiogenic goethite (Figure 4E,A, respectively), probably due to the presence of CDOM, which, additionally to phosphate, strongly competes with As(V) for sorption sites.[37,65] Moreover, the anionic character of CDOM changes the Fe mineral surface charge from positive to negative,[33,37] further enhancing the mobilization of the negatively charged As(V) compared to the noncharged As(III). As(V) was not leached from abiogenic ferrihydrite, maybe because the ferrihydrite still has a large number of vacant sorption sites due to its large surface and functional group density of 16.8 μmol sites/m^2 compared to goethite with 5.7 μmol sites/m^2.[66]

Fate of As(III) during the Reduction of Fe(III) Minerals. Aqueous As(III) concentrations remained nearly constant in all sterile setups with As(III)-loaded biogenic and abiogenic minerals, suggesting low competition between As(III) and medium components such as phosphate. According to the literature, phosphate competes more effectively with As(V) than As(III) for sorption sites on minerals.[14,67] Microbial reduction of all three As(III)-bearing Fe(III) minerals resulted in significant increases in aqueous As(III) concentrations. Most As(III) was released from biogenic Fe(III) (oxyhydr)oxides (up to 99% of total As), followed by abiogenic ferrihydrite (up to 82%) and abiogenic goethite (50%) (Figure 4). The significant mobilization of As(III) during reduction of abiogenic and biogenic Fe(III) minerals in our study shows that there is either no or only minor As readsorption onto the remaining minerals or sorption/coprecipitation to/with secondarily formed Fe(II)-bearing minerals, for example, vivianite, siderite, and magnetite. Minimal As(III) binding to natural vivianite has been shown previously[68] and can potentially be explained by vivianite's low point of zero charge (PZC) of 5.3,[69] giving vivianite a negative surface charge at neutral pH. Assuming a similar PZC for the vivianite present in our growth medium, negatively charged As(V) as well as uncharged As(III) will possibly not sorb to the vivianite to a large extent, although it should be noted that the PZC of 5.3 for vivianite has been determined in water[69] and might be different in growth medium. As(III) sorption to siderite is also not expected to a large extent since it was shown that at neutral pH As(III) does not bind to siderite as efficiently as As(V).[70] Alternatively, magnetite produced during Fe(III) reduction could potentially immobilize As(III) as previously reported by Dixit and Hering (2003),[14] but the fraction of magnetite that was formed in our experiments was probably too small (only a few %) to significantly contribute to As(III) immobilization. We conclude that microbial reduction of As(III)-bearing Fe(III) minerals, especially biogenic Fe(III) (oxyhydr)oxides, resulted in a strong mobilization of As(III) due to low binding to secondary Fe(II)-bearing minerals. In case of biogenic Fe(III) (oxyhydr)oxide reduction, the released As(III) may even be complexed by the CDOM originating from biomineral synthesis[10] and is thus prevented from (re)adsorption to the remaining mineral surface or to the secondarily formed Fe(II)(/Fe(III)) phases, causing particularly high elevated As(III) concentrations.

Fate of As(V) during the Microbial Reduction of Fe(III) Minerals. All noninoculated setups with As(V)-loaded minerals showed dissolved As(V) concentrations of less than 250 μM for abiogenic goethite and ferrihydrite and about 3000 μM for

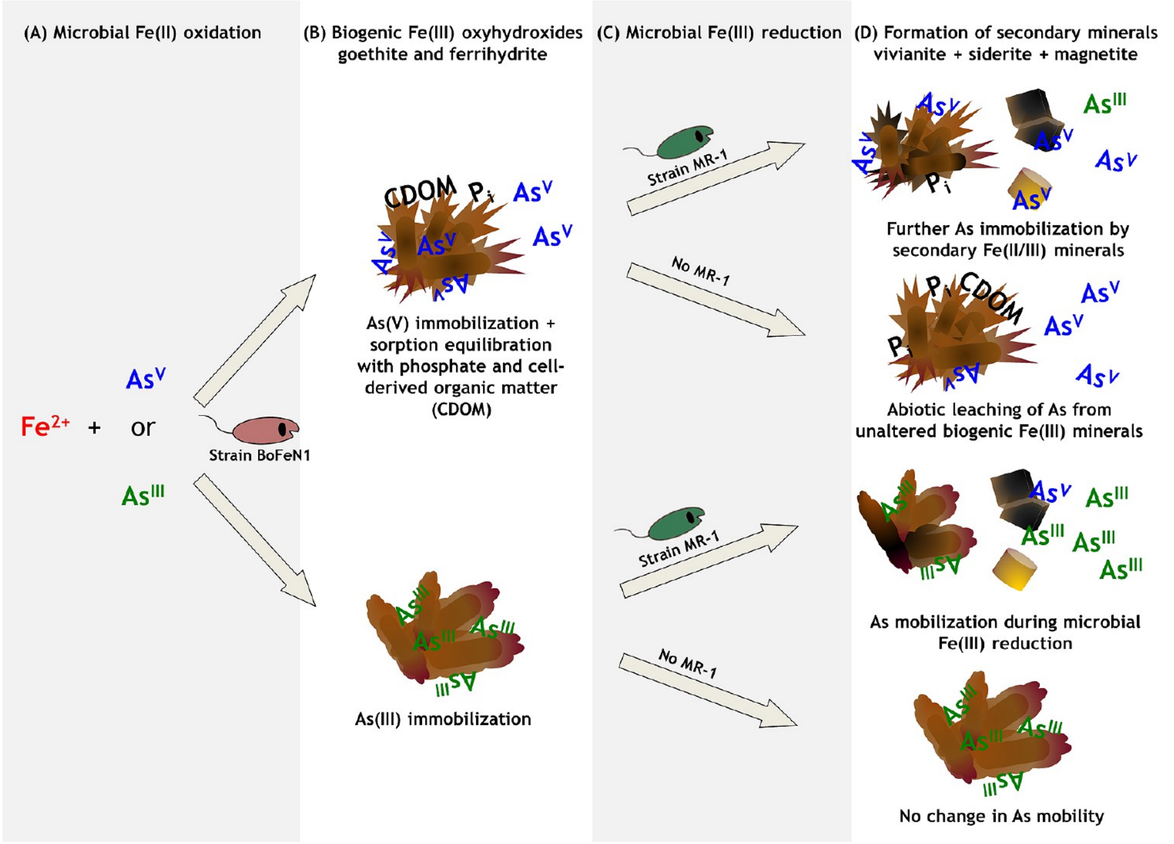

Figure 5. Scheme summarizing the fate of As(V) and As(III) during the formation of As(V)- and As(III)-bearing biogenic Fe(III) (oxyhydr)oxides followed by microbial Fe(III) reduction. As-bearing biogenic Fe(III) (oxyhydr)oxides (identified as goethite) were synthesized by the nitrate-reducing *Acidovorax* sp. strain BoFeN1 (B) in growth medium containing dissolved Fe(II), nitrate, acetate, ca. 40 μM phosphate and the As species As(V) or As(III) (A). The biogenic Fe(III) (oxyhydr)oxides were then incubated in 1 mM phosphate-containing mineral medium amended with lactate and AQDS in the absence or presence of the Fe(III)-reducing bacterium *Shewanella oneidensis* strain MR-1 (C). The mobilization or immobilization of As(III) or As(V) due to microbial Fe(III) reduction is attributed to the competing effect of inorganic phosphate (Pi) and cell-derived organic matter (CDOM) with As(V) only and/or the formation of secondary Fe(II) and Fe(II)/Fe(III) mixed mineral phases (D).

biogenic Fe(III) (oxyhydr)oxides, due to sorption competition of medium components, probably phosphate, with the bound As (Figure 4A,C,E). In contrast to the setups with As(III), microbial reduction of As(V)-bearing Fe(III) minerals did not lead to further As mobilization, but rather to a net sequestration. The behavior of As(V) during microbial reduction of the Fe(III) minerals can be described in three phases. In phase 1 (within the first 24 h of inoculation), aqueous As(V) concentrations increased rapidly (with the exception of the setup with biogenic Fe(III) oxyhydroxides with an initial Fe:As(V) ratio of 50:1). After the first day, in phase 2, aqueous As(V) decreased to a minimum within 27, 7, and 5 days of continuing Fe(III) reduction for abiogenic goethite, abiogenic ferriyhdrite and biogenic Fe(III) (oxyhydr)oxide, respectively. In phase 3, after microbial Fe(III) reduction halted, aqueous As(V) slowly increased again. Overall, the fastest decrease as well as the highest extent of As(V) removal relative to the initial aqueous As concentration was observed for biogenic Fe(III) minerals with a removal of ca. 800 μg As/L, whereas abiogenic ferrihydrite and goethite removed 350 and 120 μg As/L, respectively. However, the total aqueous As concentrations were about 10 times lower for the abiogenic than for biogenic minerals.

The behavior of As(V) during the three described phases is probably controlled by sorption competition processes (phases 1 and 3) and by mineral transformation during and after Fe(III) reduction (phase 2 and maybe even phase 3). Within the first 24 h, As(V) is released from the abiogenic As(V)-loaded goethite and ferrihydrite directly after inoculation with bacteria due to sorption competition of the added cells (CDOM) with As(V).[15,37,65] This effect is not seen for the As(V)-bearing biogenic Fe(III) (oxyhydr)oxides since they already contained a large fraction of CDOM from their synthesis. In the second phase, As(V) is continuously released from the surface of the Fe(III) minerals during microbial Fe(III) reduction and Fe(III) mineral dissolution (SI Figure S2). However, the formation of secondary Fe(II) and Fe(II)/Fe(III) minerals led to a net immobilization of the released As(V) as has already been observed in previous studies.[26,71] As(V) immobilization during Fe(III) mineral reduction could be due to three different mechanisms. First, at neutral pH As(V) could be sequestered via sorption to the secondary Fe(II)-bearing minerals siderite,[26,70,72] magnetite[14,26,73] and vivianite.[26,68] A second reason why As(V) is immobilized could be the removal of the competing anion phosphate by the precipitation of vivianite. Third, As(V) could potentially exchange for phosphate in vivianite due to the structural homology between phosphate and As(V). This might result in the formation of a vivianite-symplesite mixed mineral phase ($Fe_3(PO_4/AsO_4)_2 \cdot 8H_2O$). In the third phase, after microbial Fe(III) reduction halted, As(V) is mobilized again since competition with phosphate and OM and/or mineral aging toward more crystalline particles with lower surface area dominates over continued formation of secondary Fe(II) and

Fe(II)/Fe(III) minerals (see SI Figure S2 for dissolved Fe(II) data). In summary, microbial reduction of As(V)-bearing Fe(III) minerals leads to a net As(V) immobilization mainly due to binding to secondary Fe(II) and Fe(II)/Fe(III) minerals.

Speciation of Dissolved As and Implications for As Removal by Secondary Fe(II) Mineral Phases. Although the redox state of As associated with the minerals has not been analyzed in our study (e.g., using synchrotron based X-ray absorption analysis), speciation analysis of dissolved As present at the start and end of the experiments indicated that in most experiments the As redox state changed at least to some extent. As(III) was oxidized to As(V) during reduction of As(III)-bearing goethite, ferrihydrite and biogenic Fe(III) (oxyhydr)-oxide to 21−25, 20−43, and 32−34%, respectively, based on analysis of the dissolved As fraction (SI Table S3). Twenty and 40% As(V) of the total dissolved As was reduced to As(III) during microbial reduction of As-bearing abiogenic ferrihydrite and biogenic Fe(III) (oxyhydr)oxide, respectively, but not during reduction of abiogenic goethite (SI Table S3).

Control experiments showed that *S. oneidensis* MR-1 was not able to microbially reduce As(V) in the used medium amended with lactate and fumarate both in the presence or absence of the electron shuttle AQDS (data not shown), even though some *Shewanella* strains are known to be able to reduce As(V) under certain conditions .[63,74] The present results also confirm a previous study by our group where we showed that reduced AQDS, that is, the hydroquinone AH_2QDS, does not reduce As(V) to a large extent at neutral pH.[75] We therefore conclude that the As(V) reduction observed here is probably caused by reactive Fe(III)/Fe(II) mineral phases that are known to function as reductants for inorganic and organic contaminants.[15] The oxidation of As(III) observed in our experiments could either be due to the oxidizing power of reactive semiquinone radicals formed during AQDS reduction[75] or by Fe(II)-containing Fe(III) minerals that have been shown to form surface complexes that can oxidize As(III) to As(V).[50]

Considering the redox transformation of As during microbial reduction of the three different Fe(III) (oxyhydr)oxides, it is puzzling that the setups that contained initially either As(III) or As(V) yielded fundamentally different results regarding As mobility, even though at the end of the incubation always a mixture of As(III) and As(V) was present. However, maybe this allows us to draw conclusions regarding the sequence of the different processes, that is, Fe(III) reduction, Fe(III) mineral dissolution, secondary mineral formation, As(III) oxidation, and As(V) reduction (Figure 5). In a scenario with As(V) initially present, As(V) is mobilized during microbial Fe(III) reduction and can be simultaneously removed by the newly forming secondary Fe minerals. In setups with As(III) initially present, As(III) is also mobilized during microbial Fe(III) reduction, but not incorporated into the secondary Fe minerals. However, As(III) oxidation to As(V) could have happened only after most secondary Fe minerals were already formed, resulting in a net mobilization of As.

Implications for As Mobility in Environments with Biogenic Fe(III) (Oxyhydr)Oxides. In the present study we have shown that the fate of As(III) and As(V) during microbial reduction of biogenic Fe(III) (oxyhydr)oxides shows significant differences compared to the reduction of the abiotic counterparts goethite and ferrihydrite. Since more and more evidence is indicating that microbes are involved in Fe(II) oxidation and Fe(III) mineral precipitation in the environment,[43,76−80] clearly the properties of biominerals[33,38] and their association to organics[32] have to be considered when evaluating the fate of contaminants such as As but also other metals including U or Cr in the environment. However, in the present study we only tested Fe(III) minerals produced by one metabolic type of Fe(II) oxidation (coupled to nitrate reduction) and by one strain. In nature, Fe(II) oxidation is also catalyzed by microaerophilic and even phototrophic organisms[78] and is linked to Fe(III) reduction leading to efficient Fe cycling.[81] To fully understand the influence of microbial Fe cycling on the fate of As, the impact of CDOM and the resulting abiotic and biotic processes need to be considered in future studies.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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