Environmental Science & Technology

ARTICLE

pubs.acs.org/est

Biogenic Fe(III) Minerals Lower the Efficiency of Iron-Mineral-Based Commercial Filter Systems for Arsenic Removal

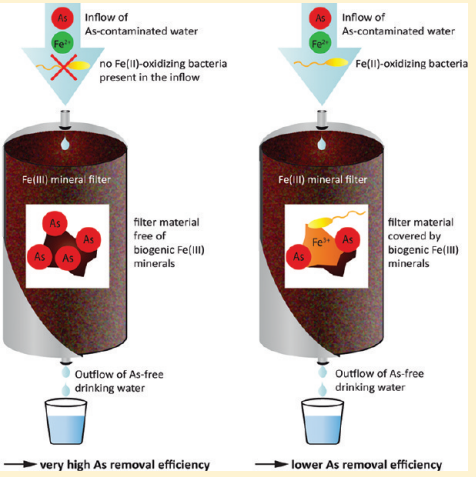
Susanne Kleinert,[†] Eva M. Muehe,[†] Nicole R. Posth,[†] Urs Dippon,[†] Birgit Daus,[‡] and Andreas Kappler*[,†]

[†]Geomicrobiology, Center for Applied Geosciences, University of Tuebingen, Sigwartstrasse 10, D-72076 Tuebingen, Germany

[‡]Department Groundwater Remediation, UFZ – Helmholtz Centre for Environmental Research, Leipzig, Germany

Supporting Information

ABSTRACT: Millions of people worldwide are affected by As (arsenic) contaminated groundwater. Fe(III) (oxy)hydroxides sorb As efficiently and are therefore used in water purification filters. Commercial filters containing abiogenic Fe(III) (oxy)hydroxides (GEH) showed varying As removal, and it was unclear whether Fe(II)-oxidizing bacteria influenced filter efficiency. We found up to 10^7 Fe(II)-oxidizing bacteria/g dry-weight in GEH-filters and determined the performance of filter material in the presence and absence of Fe(II)-oxidizing bacteria. GEH-material sorbed 1.7 mmol As(V)/g Fe and was ~8 times more efficient than biogenic Fe(III) minerals that sorbed only 208.3 μmol As(V)/g Fe. This was also ~5 times more efficient than a 10:1-mixture of GEH-material and biogenic Fe(III) minerals that bound 322.6 μmol As(V)/g Fe. Coprecipitation of As(V) with biogenic Fe(III) minerals removed 343.0 μmol As(V)/g Fe, while As removal by coprecipitation with biogenic minerals in the presence of GEH-material was slightly less efficient as GEH-material only and yielded 1.5 mmol As(V)/g Fe. The present study thus suggests that the formation of biogenic Fe(III) minerals lowers rather than increases As removal efficiency of the filters probably due to the repulsion of the negatively charged arsenate by the negatively charged biogenic minerals. For this reason we recommend excluding microorganisms from filters (e.g., by activated carbon filters) to maintain their high As removal capacity.



INTRODUCTION

Several aquifers around the world possess elevated As concentrations and exceed the 10 μg L^{-1} WHO drinking water limit for As by far.[1] The problem is most prominent in the Bengal Basin (Bangladesh and West Bengal, India).[1] Arsenic enters the groundwater by natural processes: by dissolution of As-containing minerals due to weathering, via microbial activity,[1,2] or by mobilization via competition and complexation by natural organic matter (humic substances).[3,4] Additionally, human activities like mining, burning of fossil fuels, and use of arsenical pesticides and phosphate fertilizers contribute significantly to elevate As concentrations in the groundwater.[1,5,6] Since groundwater is commonly used as drinking water, it is estimated that more than 100 million people are at risk of regular As uptake.[7] Moreover, it is well recognized that long-term As exposure leads to severe health problems including skin diseases, cardiovascular and nervous affections, diabetes and cancer.[7−9]

In order to avoid the health problems caused by As contaminated drinking water, two general approaches are taken: (i) a switch to unpolluted water sources or (ii) removal of the As before water consumption.[10] One very common and effective As removal technology is the use of filters containing either Fe(III) (oxy) hydroxides or iron-mineral-coated sand particles that bind As.[10] Commonly used Fe(III) (oxy)hydroxide sorbents include goethite, akaganeite, and ferrihydrite.[7] Both noncommercial (e.g., sand filters[8,11]) and commercial filter systems (e.g., GEH [12]) are applied worldwide to remove As and other metals from drinking water.

In anoxic groundwater aquifers, As-contaminated water often also contains high amounts of dissolved Fe(II),[11] which does not pose any direct threat to human health.[11] After being brought to the surface, the Fe(II) in the water can be oxidized and precipitated as Fe(III) minerals either chemically by O_2 or by O_2-respiring microorganisms. Microaerophilic Fe(II)-oxidizing bacteria live in habitats with low O_2 where they successfully compete with the chemical oxidation of Fe(II). Under anoxic conditions, nitrate-reducing Fe(II)-oxidizing bacteria can use nitrate as electron acceptor to oxidize Fe(II) and to precipitate biogenic Fe(III) (oxy)hydroxides.[13]

Biogenic Fe(III) minerals differ from abiogenic ones in terms of morphology, mineralogy, composition, particle size, and density.[14,15] Specifically, biogenic Fe(III) minerals are cell-mineral aggregates[14,16] and hence contain significant amounts of organic carbon. A lower crystallinity and an overall smaller

Received: May 4, 2011
Accepted: July 18, 2011
Revised: July 7, 2011
Published: July 18, 2011

crystal size further delineate biogenic from abiogenic Fe(III) minerals.[17,18] Moreover, the bacterial fraction of the cell-mineral aggregate results in an overall negative surface charge of biogenic Fe(III) minerals.[14,19]

Until now it was unknown whether Fe(II)-oxidizing bacteria are present in commercial iron-based filter systems (GEH) and whether their activity influences the As removal efficiency. The presence and activity of Fe(II)-oxidizing bacteria in GEH filters could either increase As removal from solution due to additional coprecipitation of As with freshly formed biogenic Fe(III) minerals or decrease As removal due to competitive effects between As and organic molecules originating from the microbial cells.

Based on these knowledge gaps, the objectives of this study were to (i) determine whether aerobic (microaerophilic) and anaerobic (nitrate-reducing) Fe(II)-oxidizing bacteria are present in iron-mineral-based commercial filter systems (GEH), (ii) quantify the removal of As by sorption to and coprecipitation with biogenic, abiogenic and mixed Fe(III) (oxy)hydroxides, and thus (iii) compare the efficiency of GEH-material in the absence and in the presence of Fe(II)-oxidizing bacteria and their Fe(III) precipitates.

MATERIALS AND METHODS

Source of Microorganism. *Acidovorax* sp. strain BoFeN1 is a chemoheterotrophic, nitrate-reducing β-Proteobacterium and was isolated from Lake Constance sediments.[16] It grows mixotrophically by oxidizing ferrous iron and an organic cosubstrate, such as acetate.[16]

Most Probable Number (MPN) Quantification. Used GEH material was collected from the top and the bottom of three water filters (abbreviated T, W, and D) from three different locations in Germany (Figure 1, Table S1). Samples (1 g) of the filters were diluted serially 1:10 in anoxic freshwater medium[20] up to a dilution of 10^{-8}. Nitrate-reducing Fe(II)-oxidizing bacteria were quantified in freshwater medium[21] amended with 10 mM $FeCl_2$, 5 mM Na-acetate, and 10 mM Na-nitrate. MPNs for Fe(III)-reducing bacteria were supplied with 10 mM ferrihydrite and either 20 mM Na-acetate or Na-lactate. Seven parallels per dilution were set up in 96 deep-well plates containing 900 μL of medium amended with 100 μL of inoculum (from the different dilution steps). Microaerophilic Fe(II)-oxidizing bacteria were quantified by inoculating 100 μL of the dilutions into Fe(II)-O_2-gradient tubes containing 1 mL of a FeS-agarose bottom layer overlaid by 10 mL of growth agar.[22] MPN setups and gradient tubes were incubated for 2 months at 20 °C in the dark. Microbial growth of Fe(III)-reducing and nitrate-reducing Fe(II)-oxidizing microorganisms was confirmed in MPN plates visually by a color change of the Fe(II) or Fe(III) substrate and by visual comparison of Fe(II) concentrations after adding ferrozine, a reagent that forms a magenta colored complex with Fe(II).[23] For details of the ferrozine assay see below. Microbial growth of microaerophilic Fe(II)-oxidizers was confirmed by the presence of orange bands of iron (hydr)oxides and microorganisms forming in the gradients of Fe^{2+} (diffusing upward from the bottom FeS layers) and O_2 (diffusing downward from the oxic headspace). Additionally, the presence of living cells in the bands was confirmed by fluorescence microscopy using a dead/live stain (L7012, Molecular Probes, Eugene, OR, USA).

Microbial Growth Media and Growth Conditions in Batch Experiments. Strain BoFeN1 was cultivated at pH 7 in anoxic mineral medium (0.14 g L^{-1} KH_2PO_4, 0.2 g L^{-1} NaCl, 0.3 g L^{-1} NH_4Cl, 0.5 g L^{-1} $MgSO_4$*$7H_2O$, 0.1 g L^{-1} CaCl*$2H_2O$, 22 mM bicarbonate buffer, 1 mL L^{-1} vitamin solution, 1 mL L^{-1} trace element solution, 1 mL L^{-1} selenate-tungstate solution) modified from Hohmann et al.[24] Fe(II) was added from a sterile 1 M $FeCl_2$ stock solution to the medium (target concentration: 12 mM). The precipitating whitish-green Fe(II) carbonate and phosphate minerals were removed by filtration after 48 h.[25] The remaining dissolved Fe(II) concentration in the filtered medium was approximately 10 mM, and the remaining phosphate concentration was measured to be below 40 μM.[24] Stock and experimental cultures of strain BoFeN1 were cultivated at 28 °C in an N_2/CO_2 (v/v, 80/20) atmosphere and provided with 10 mM Na-nitrate and 5 mM Na-acetate.

Sterile, anoxic As(V) stock solutions (2.5, 100, and 300 mM) were prepared by dissolving sodium arsenate (Na_2HAsO_4) in 25 mL of anoxic Milli-Q water, followed by filtration (0.22 μm, cellulose acetate, Fisher Scientific).

GEH-Material. Unused GEH-filter-material was provided by GEH Wasserchemie GmbH & Co. KG.[26] For experiments, the filter material was ground in a mortar. The GEH surface area of 277.9 m^2 g^{-1} was determined by BET analysis after grinding.

Experimental Setup. Sorption and coprecipitation experiments were done in the same microbial growth medium containing <40 μM phosphate (that was remaining after filtration of the medium, see above). The presence of this low phosphate concentration was necessary for microbial growth. To allow comparability between the different setups, all setups (including the ones not containing microorganisms) were prepared in the same microbial growth medium containing <40 μM phosphate. The anoxic, sterile, and filtered medium was filled into sterile serum bottles (58 mL bottles, 25 mL of medium). To quantify the sorption of As(V) to GEH-material, 0.25 g of ground filter material was added to the medium. To quantify As(V) sorption to biogenic Fe(III) minerals, strain BoFeN1 was inoculated into the medium (2%) and incubated for approximately one month before As(V) was added. Before As(V) addition, the medium was tested for complete Fe(II) oxidation. For the quantification of As(V) sorption to a mixture of GEH-material and biogenic Fe(III) minerals, the latter were formed by strain BoFeN1 in the presence of GEH and prior to the addition of As(V). As(V) sorption was equilibrated for 48 h before determining remaining dissolved and total As(V) concentrations.

The coprecipitation of As(V) with biogenic Fe(III) minerals or with biogenic Fe(III) minerals in the presence of GEH-material was quantified by adding As(V) before inoculation with strain BoFeN1. As(V) was added to the bottles to yield target concentrations of 0.025, 0.05, 0.1, 0.2, 0.5, 1, 2, 5, and 10 mM. Inoculation was carried out with 2% of a second-generation Fe(II)-free-(acetate-only)-grown BoFeN1 preculture. The total amount of Fe present in the different setups was ~0.014 g in setups with biogenic Fe(III) minerals only, 0.152 g in the setup with GEH-material only, and 0.166 g in setups with a mixture of biogenic Fe(III) minerals and GEH-material. Since the filter redox status was oxic (see Table S1) and to exclude influences of As(III) oxidation on As sorption/coprecipitation behavior, only As(V) and not As(III) was used in the sorption and coprecipitation experiments. Arsenic removal was normalized to the amount of Fe present. All experiments were conducted in duplicates.

Analytical Methods. To determine the progress of biogenic Fe(II) oxidation, Fe(II) and Fe(III) were quantified spectrophotometrically using the ferrozine assay.[23] This assay is based

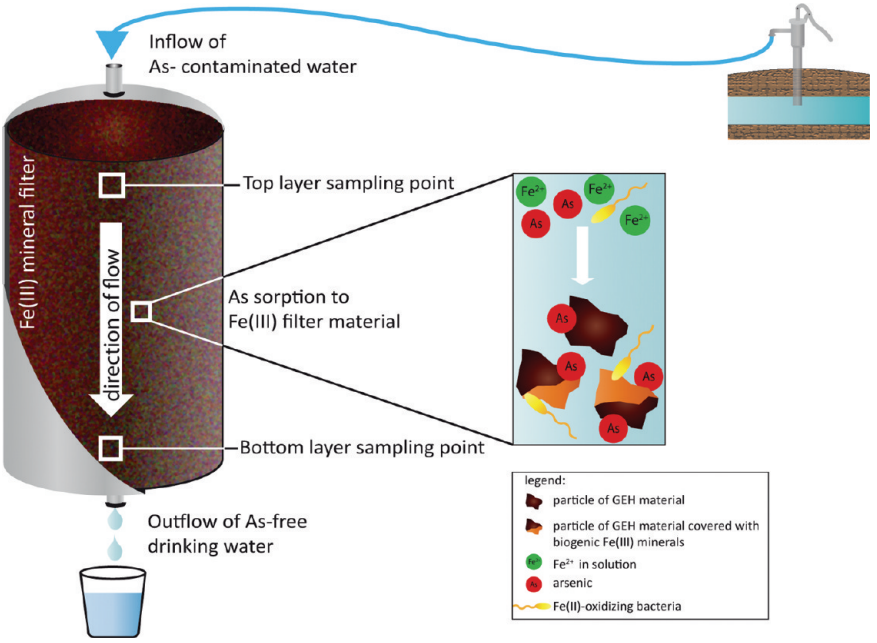


Figure 1. Diagram of a GEH filter system, illustrating microbial processes and As sorption inside the filter as well as the sampling locations for most probable number quantification.

on the principle that Fe(II) forms a magenta colored complex with ferrozine (Na_2 3-(2-pyridyl)-5,6-diphenyl-1,2,4-triazine-p, p-disulfonic acid). 1-mL samples were taken and completely dissolved in 6 M HCl. Fe(II) was quantified directly by the ferrozine assay, while total Fe was determined after mixing 20 μL of sample with 80 μL of the reducing agent hydroxylamine hydrochloride (1 M in HCl).

For analysis of dissolved As(V) concentrations, 800 μL samples were filtered (Costar Spin-X-centrifuge-filters, 0.22 μm), whereas total As(V) concentrations were determined by dissolving 100 μL samples in 900 μL of 6 M HCl each in the anoxic glovebox. Subsequently, 500 μL of filtrate or 500 μL of dissolved sample respectively were acidified with 9.5 mL of phosphoric acid (10.5 mM) to preserve the As redox state.[27] Arsenic concentrations and speciation were quantified by ICP-MS (7700 series ICP-MS, Agilent Technologies) or ICP-OES (CIROS, Spectro A.I.) for concentrations lower than 200 $\mu g\ L^{-1}$ or higher than that, respectively. The limits of quantification were 100 μg/L (ICP-OES) and 0.1 μg/L (ICP-MS). Speciation analysis was done by a simplified method of Daus et al. (ref 28, 8 min of isocratic elution) with He-mode of the ICP-MS to minimize the chlorine effect.

The scanning electron micrographs were taken as described in the Supporting Information (S1). For mineral analysis with ^{57}Fe—Mössbauer spectroscopy, 100 mg of unused, ground GEH-material was placed between two layers of oxygen-impermeable Kapton tape. Measurement and evaluation were performed following Larese-Casanova et al.[29]

RESULTS AND DISCUSSION

Fe(II)-Oxidizing and Fe(III)-Reducing Microorganisms in GEH-Filter-Systems. Aerobic (microaerophilic) and anaerobic (nitrate-reducing) Fe(II)-oxidizing microorganisms were quantified in three different used GEH-filter-systems and compared to the number of Fe(III)-reducing microorganisms in the same samples (Table 1). Samples were taken at two positions in each filter (top and bottom (Figure 1)). In all filters, Fe(II)-oxidizing bacteria were found. Microaerophilic Fe(II)-oxidizers with cell numbers of 10^5—10^7 cells/g dry weight GEH were 10^2—10^5 times more abundant than nitrate-reducing Fe(II)-oxidizers with cell numbers of 10^2—10^3 cells/g GEH (Table 1). In all three filters, 2 to 20 times higher numbers of nitrate-reducing Fe(II)-oxidizers were determined in the bottom sampling points in comparison to the top sampling locations. In one filter (D), 3 times more microaerophilic Fe(II)-oxidizers were present in the bottom sampling point, while the two other filters (W, T) contained higher numbers of microaerophilic Fe(II)-oxidizers at the top. In contrast to Fe(II)-oxidizers, only very low numbers or even no Fe(III)-reducing bacteria (oxidizing lactate or acetate) were found in the three filters (Table 1). If Fe(III)-reducers were present, slightly more lactate- than acetate-oxidizing Fe(III)-reducers were found.

The MPN counts of Fe-utilizing microorganisms in three different GEH filters suggest that Fe(II)-oxidizing bacteria play a more important role than Fe(III)-reducing bacteria. Indeed, evidence for the presence of Fe(II)-oxidizers in As-contaminated aquifers was recently found.[30] Fe(II)-oxidizing bacteria can therefore be expected to enter the filter system together with water inflow. In addition to microaerophilic Fe(II)-oxidizers, we also found low, but still significant, numbers of nitrate-reducing Fe(II)-oxidizers. This could be due to the fact that some of the microaerophilic Fe(II)-oxidizers could replace O_2 by nitrate for Fe(II) oxidation. Indeed, we recently found that *Acidovorax* sp. strain BoFeN1 that was isolated as a nitrate-reducing Fe(II)-oxidizer can also oxidize Fe(II) microaerophilically (unpublished data). Nevertheless, the low concentrations of nitrate found in the raw water entering the filters (Table S1) probably explain the low numbers of nitrate-reducing Fe(II)-oxidizers quantified by MPNs. In contrast to microaerophilic Fe(II)-oxidizers, Fe(III)-reducers typically need anoxic conditions for Fe(III) reduction. Therefore, as analysis of the filters showed oxidizing conditions,

Table 1. Most Probable Number Quantification of Microorganisms in Three Different GEH Groundwater Filters (Abbreviated with "D, W, T") That Were in Use for One to Four Years[a]

			Fe(II)-oxidizing bacteria [cells/g DW GEH]		Fe(III)-reducing bacteria [cells/g DW GEH]	
filter location	sample position in filter	operation time of filter [years]	O_2	nitrate	lactate	acetate
D	top	4	54.0×10^5	10.8×10^2 [57.3×10^1, 23.5×10^2]	22.8×10^1 [11.6×10^1, 42.2×10^1]	45.5 [32.6, 58.9]
	bottom		16.4×10^6	27.3×10^2 [14.8×10^2, 44.0×10^2]	10.4×10^1 [55.1, 22.6×10^1]	59.7 [38.2, 78.3]
W	top	2.5	58.0×10^6	12.0×10^2 [63.7×10^1, 24.3×10^2]	Bdl	Bdl
	bottom		58.0×10^5	25.7×10^3 [13.2×10^3, 45.6×10^3]	Bdl	Bdl
T	top	1	61.0×10^7	44.7×10^2 [26.5×10^2, 61.1×10^2]	Bdl	Bdl
	bottom		60.0×10^6	68.8×10^2 [43.7×10^2, 90.4×10^2]	80.2×10^1 [48.8, 11.3×10^1]	Bdl

[a] Filter from sampling location "T" is located in Solling, Lower Saxony/Germany, location "D" in Sauerland, North Rhine-Westphalia/Germany, and location "W" in Northeastern Hesse/Germany. The top layer and the bottom layer of the filters were sampled. The population size of bacteria oxidizing iron(II) with O_2 or with 10 mM nitrate as electron acceptor and reducing iron(III) with 20 mM lactate or acetate as electron donor were determined. All values are given in cells/g dry filter material. The 95% confidence intervals [lower, upper] for anaerobic bacteria of seven parallels are given in brackets. Bdl: below detection limit. No parallels were carried out for the most probable number quantification of Fe(II)-oxidizing bacteria using O_2 as the electron acceptor.

low numbers of Fe(III)-reducers were expected and confirmed by the MPN results.

Nitrate-Reducing, Fe(II)-Oxidizing Bacteria As Model Strain for As Sorption and Coprecipitation Experiments. In order to determine the effect of Fe(II)-oxidizing bacteria and their biogenic Fe(III) mineral products on the As(V) removal efficiency of the GEH filters, we designed batch experiments with the nitrate-reducing Fe(II)-oxidizing bacterium *Acidovorax* sp. strain BoFeN1. We have recently shown that this strain is able to oxidize Fe(II) in the presence of high concentrations of As(V)/As(III) and efficiently removes As from solution by sorption and coprecipitation.[24] Additionally, closely related strains have been identified in As-contaminated aquifers in Bangladesh and Cambodia.[30,31] Even though the numbers of microaerophilic Fe(II)-oxidizers in our GEH filters were higher than the numbers of nitrate-reducing Fe(II)-oxidizers, we chose a nitrate-reducing Fe(II)-oxidizer as model strain to determine the effect of biogenic Fe(III) minerals. Importantly, chemical Fe(II) oxidation (as it occurs in cultures of microaerophilic Fe(II)-oxidizers in parallel to the microbial Fe(II) oxidation) was hereby prevented, and the formation of new abiogenic Fe(III) minerals in parallel to the biogenic ones avoided. The influence of microbially precipitated Fe(III) (oxy)hydroxides on the As(V) removal efficiency of the GEH filters could thereby be exclusively determined.

Mineralogy in GEH-Filters before and after Microbial Fe(II) Oxidation. Mössbauer spectroscopy analysis showed that GEH consists of two iron oxide mineral phases. 41% of the GEH material were identified as akaganeite (ferric oxyhydroxide, β-FeOOH, a polymorph of goethite and lepidocrocite that has a tunnel-structure stabilized by Cl^- ions[32]) (Figure 2). The second phase accounting to 59% of the GEH material could not be clearly identified. Modeling of the Mössbauer signals yielded a poorly crystalline iron oxide phase with very small particle sizes in the paramagnetic range (<30 nm), e.g. ferrihydrite (a poorly crystalline ferric hydroxide). For the following discussion, it is assumed that this second mineral phase is ferrihydrite although it must be noted that it could not be distinguished from poorly crystalline/nanocrystalline Fe oxyhydroxides such as goethite, lepidocrocite, or even akaganeite. Scanning electron micrographs of untreated GEH-material showed that the particles were granular, not aggregated and irregular in shape and size (Figure 2A). The particles were characterized by sharp edges and rather smooth surfaces. Pulverized GEH-material used for the present experiments was generally smaller in size, varied more in shape, and exhibited smoother edges (Figure 2B). In the absence of GEH-material, strain BoFeN1 cells were mostly covered by needle-like iron mineral structures after Fe(II) oxidation (Figure 2C). In experiments with GEH-particles present during the oxidation of Fe(II) by strain BoFeN1, the GEH particles were completely covered by sharp needle-like structures at the end of Fe(II) oxidation (Figure 2D).

The biogenic Fe(III) minerals covering the GEH-material were very similar in shape to those formed in our study by strain BoFeN1 in the absence of GEH-material and also resembled the iron mineral precipitates formed by strain BoFeN1 cells oxidizing dissolved Fe(II) in recent studies.[16,24] Therefore, it is very likely that the structures covering the GEH-material in the present study were biogenic Fe(III) minerals formed by strain BoFeN1. It was shown that biogenic Fe(III) minerals formed by strain BoFeN1 consist mostly of goethite.[24] However, the mineralogy is altered when As(V) is coprecipitated. A larger content of less crystalline (ferrihydrite-like) iron mineral phases was found with increasing As concentration.[24,33] Additionally, mineral identity was shown to depend on the geochemical conditions (pH, ion composition, presence of organic compounds) during Fe(II) oxidation.[29] Therefore, it is very likely that a variety of Fe(III) (oxy)hydroxides such as ferrihydrite, green rusts, goethite, lepidocrocite, and akaganeite can be found in filter systems in which Fe(II)-oxidizing bacteria and As are present at the same time.

In addition to the differences in the identity of biogenic vs abiogenic Fe(III) minerals, it has to be considered that biogenic Fe(III) minerals are cell mineral aggregates containing organic compounds (exopolysaccharides, microbial exudates, organic metabolites, or cell detritus) which change the surface properties

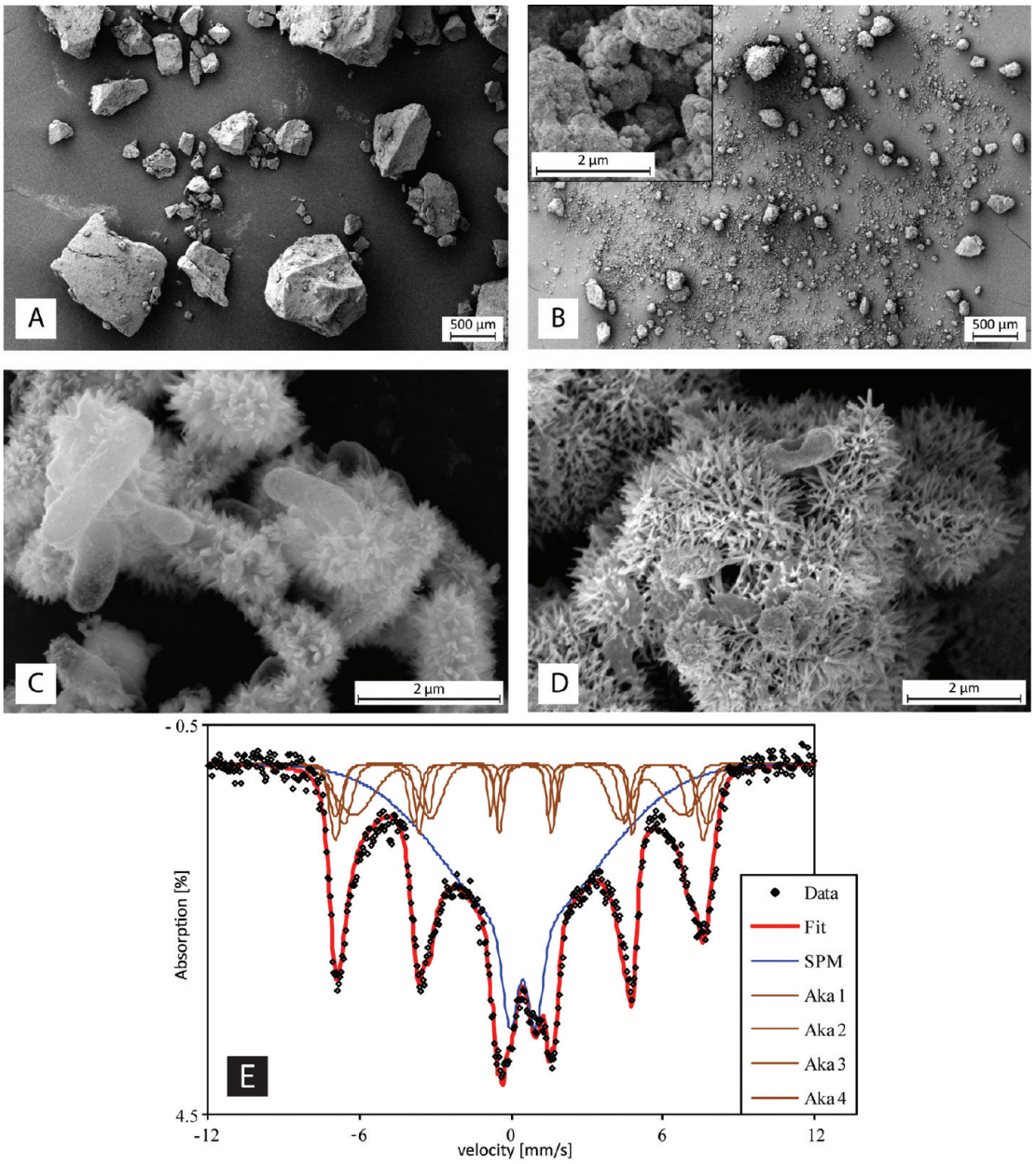


Figure 2. Scanning electron micrographs of GEH filter material and biogenic Fe(III) minerals. A) unused, original GEH-material, B) unused, ground GEH-material with insert showing a close-up of the material to facilitate comparison to the biocoated material (Figure 2D), C) biogenic Fe(III) minerals and encrusted cells of strain BoFeN1 D) ground GEH-material coated with biogenic Fe(III) minerals of strain BoFeN1, E) Mössbauer spectrum of unused GEH-material at 77 K. Aka1−4 represent the four components of the 77K Mössbauer spectra for akaganeite[48] used as references to model the experimentally determined spectrum in our study. Error bars indicate range of values obtained from duplicate setups.

of the biogenic mineral precipitates (see Discussion below). It was also shown that biogenic Fe(III) minerals are significantly more susceptible to reductive dissolution by dissimilatory iron-reducing bacteria than mineralogically similar synthetic iron oxides.[34] Microbial reduction of such biogenic minerals loaded with As could also be stimulated by the presence of microbially derived redox-active organic matter and by the presence of oxyanions such as arsenate.[35,36]

As Removal by GEH Filters in the Absence and Presence of Fe(II)-Oxidizing Bacteria. After the addition of 0.025−10 mM As(V) to 0.25 g of GEH filter material, we found that most As(V) was sorbed to the GEH-material (Figure 3). In contrast, sorption of As(V) to biogenic Fe(III) minerals, sorption to a mixture of GEH-material and biogenic Fe(III) minerals as well as coprecipitation of As(V) with biogenic Fe(III) minerals both in the presence and absence of GEH-material removed significantly less As(V) from solution (Figure 3). Arsenic speciation analysis at the end of the experiments showed that only As(V) was present (data not shown) suggesting the absence of As(V) reduction during the experiments.

Sorption data were fitted with the Langmuir equation ($a = (\eta Kc)/(1+Kc)$), and sorption isotherms (Figure 3) were plotted as described in the Supporting Information (S2). The maximum sorption capacity of As(V) on original (ground)

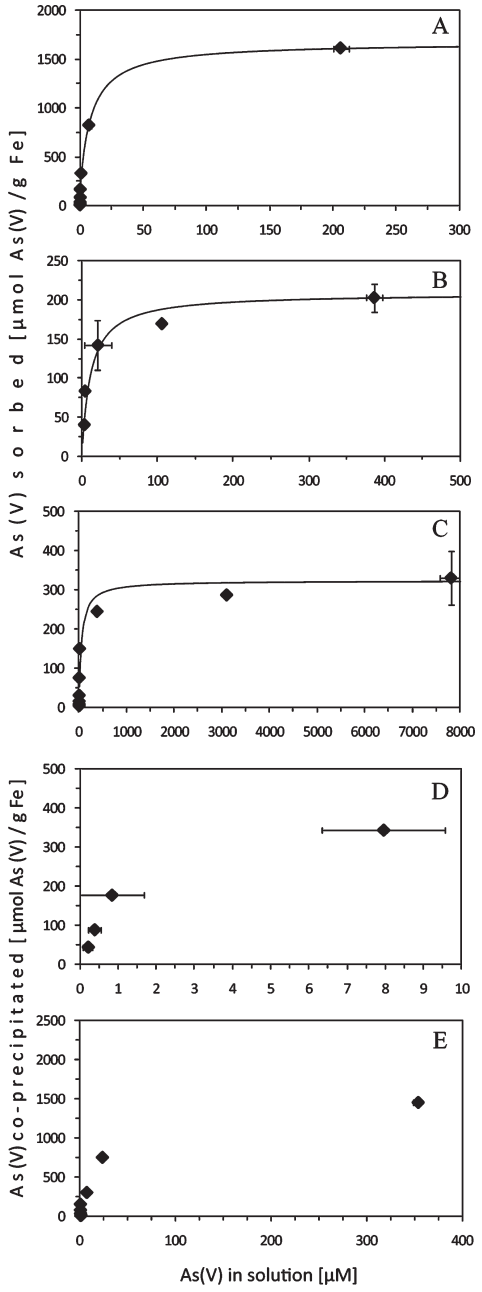


Figure 3. Isotherms for A) As(V) sorption to GEH-material, B) As(V) sorption to biogenic Fe(III) minerals precipitated by strain BoFeN1, C) As(V) sorption to a mixture of GEH-material and biogenic Fe(III) minerals precipitated by strain BoFeN1, D) coprecipitation of As(V) with biogenic Fe(III) minerals precipitated by strain BoFeN1, E) coprecipitation of As(V) with biogenic Fe(III) minerals precipitated by strain BoFeN1 in the presence of GEH-material. The sorbed respectively coprecipitated amount of As(V) in μmol/g Fe is plotted against the remaining As(V) concentration in solution after equilibrium was reached (48 h).

GEH-material was determined to be 1.7 mmol As(V)/g Fe. In comparison, the maximum sorption capacity of As(V) on biogenic Fe(III) minerals was only 208.3 μmol As(V)/g Fe. Therefore, the loading of GEH with As(V) and thus its As removal efficiency was ~8 times higher than that of biogenic Fe(III) minerals. The mixture of GEH-material and biogenic Fe(III) minerals (with the biogenic minerals being precipitated in the presence of GEH material) showed a maximum sorption capacity of 322.6 μmol As(V)/g Fe and thus was ~5 times lower than that of the pure GEH-material but 1.5 times higher than that of the biogenic Fe(III) minerals.

The maximum loading of As(V) in biogenic Fe(III) minerals during coprecipitation was 343.0 μmol As(V)/g Fe (obtained with an initial As(V) concentration of 0.2 mM and leading to an aqueous equilibrium concentration of 7.9 μM (Figure 3)). Thus, the highest loading of biogenic Fe(III) minerals we determined in our coprecipitation experiments was almost 5 times lower than the maximum sorption capacity of pure GEH-material. This was still higher than both the maximum sorption capacities of As(V) on biogenic Fe(III) minerals and on the mixture of GEH-material and biogenic Fe(III) minerals. Coprecipitation of As(V) with biogenic Fe(III) minerals in the presence of GEH-material at an initial As(V) concentration of 10 mM yielded maximum loadings of 1.5 mmol As(V)/g Fe and a remaining aqueous As(V) concentration of 354.3 μM. The highest As(V) loading obtained during Fe(III) minerals precipitation in the presence of GEH minerals was therefore only slightly lower than the maximum loading of GEH-material alone.

Pure GEH-material is more efficient in removing As(V) from solution than systems containing biogenic Fe(III) minerals. GEH consists of ferrihydrite (59%) and akaganeite (41%) with a high surface area of 250−300 $m^2 g^{-1}$ (ground ~278 m^2/g) and a high porosity of 75−80%[12] and therefore a high number of potential sorption sites for As(V). Furthermore, the point of zero charge for ferrihydrite is ~7.9[32] and for akaganeite 7.3[37] implying a positive surface charge of GEH at pH 7 and favored sorption of the relevant As(V) species ($H_2AsO_4^-$, $HAsO_4^{2-}$) to the positively charged GEH surface due to electrostatic forces.[32] Comparison of our data to previous As(V) sorption studies with granulated ferric hydroxide (GFH)[12,38] showed a similarly high As(V) removal efficiency, though with slightly different absolute values of As(V) sorption capacity. Those small differences might be due to experimental variations with regard to (i) the ionic composition of the experimental solutions, (ii) the particle size of the GFH, (iii) the equilibration time of As(V) sorption, or (iv) the solution-adsorbent-ratio used.

Compared to pure GEH material, biogenic minerals showed lower As(V) sorption. This might be due to the roughly two times lower surface area of Fe(III) minerals formed by BoFeN1 (~158 $m^2 g^{-1}$ [16]) compared to ground GEH-material (~278 $m^2 g^{-1}$). Less sorption sites for As(V) may be therefore available on biogenic Fe(III) minerals. Furthermore, ferrihydrite, one of the two main components of GEH-material, has a three times higher functional group density (16.8 μmol sites per m^2) than goethite (5.73 μmol sites per m^2),[39] the Fe(III) mineral formed by strain BoFeN1.[24] Since functional groups, such as hydroxyl groups, are responsible for the sorption of As(V) to Fe(III) (oxy)hydroxide surfaces,[32] higher numbers of functional groups per surface area offer more possibilities for As(V) to bind to GEH-material than to biogenic Fe(III) minerals. However, the differences in mineralogy alone do not fully explain the large differences between As(V) sorption to GEH-material and to biogenic Fe(III) minerals. Biogenic Fe(III) minerals also contain a substantial amount of organic compounds derived from the bacteria.[14] Biomolecules such as proteins, carbohydrates, or even fragments of cells may compete for sorption sites with As(V).[40,41] Additionally, organic molecules can increase As mobility by forming colloids and dissolved complexes consisting of organic matter, iron(III), and

As(V).[3] Furthermore, sorption of negatively charged biomolecules (that have points of zero charge (pzc) of 4.5−5.4[42,43]) to the biogenic Fe(III) minerals can change their surface charge.[14] Indeed, in contrast to abiogenic Fe(III) (oxy)hydroxides such as ferrihydrite and goethite with pzc values between 7 and 8, the pzc of biogenic Fe(III) minerals formed by BoFeN1 was determined to be 4.4.[44] Negatively charged biogenic Fe(III) minerals at neutral pH are expected to repel negatively charged As(V) ions and explain the highly efficient removal of As(V) by positively charged pure GEH-material and the lower As removal efficiency of the biogenic minerals.

When As(V) was sorbed to a mixture of GEH-material and biogenic Fe(III) minerals formed in the presence of the GEH-material, less As(V) (322.6 μmol As(V)/g Fe) was removed from solution compared to sorption to GEH-material alone (1.7 mmol As(V)/g Fe). Scanning electron micrographs of the mixture of the GEH and biogenic minerals showed that the smooth surface of the GEH-material was completely covered by needle-like biogenic Fe(III) minerals. This coating of Fe(III) minerals and cell organic matter lowered the sorption capacity of the GEH-material or even prevented As(V) sorption to GEH-material. Due to the mineral cover with biogenic Fe(III) minerals, As(V) sorption in the mixture was dominated by the properties of the biogenic Fe(III) minerals and not by the properties of the GEH-material. As shown by the sorption studies above, biogenic Fe(III) minerals represent rather poor adsorbents for negatively charged As(V) species explaining the lower As removal efficiency of the mixture of GEH-material and biogenic Fe(III) minerals.

In addition to the effects of the organic matter in the biotic setups on As sorption, it has to be considered that the microbial growth medium used for all experiments contained low concentrations of phosphate (<40 μM). Therefore, in the setups containing the lowest As(V) concentrations tested (25 and 50 μM) probably also competition for sorption sites between the arsenate and phosphate occurred. However, since the same medium containing the same amount of phosphate was used in all experiments, this effect should have been the same in all setups and comparison between the different setups is still possible. Additionally, since the highest concentration of As(V) used (up to 10000 μM) was significantly higher than the phosphate concentrations present (<40 μM), the sorption maxima determined should not have been influenced significantly.

When As(V) was coprecipitated with biogenic Fe(III) minerals it was removed from solution almost twice as efficiently as by sorption to preformed biogenic Fe(III) minerals. The fact that As is removed from solution more efficiently by coprecipitation than by sorption to biogenic Fe(III) minerals has already been shown by several studies.[24,45−47] During coprecipitation, As(V) is permanently coordinated by surface sites during mineral growth and sorbs to the surfaces of small crystallites before larger aggregates are formed.[45] Therefore, coprecipitation leads to sorption, inclusion, and occlusion which cause embedding of As into the mineral. Furthermore, we recently showed that As coprecipitation during microbial Fe(II) oxidation causes a decrease in Fe mineral particle size and crystallinity[24] probably leading to a higher relative surface area and more available sorption sites. In contrast, sorption sites for As on preformed Fe(III) minerals are restricted solely to the mineral surface. Aging of the biominerals reduces these sites and leads to increased crystallinity and lower surface area.

As(V) removal by coprecipitation with biogenic Fe(III) minerals in the presence of GEH material (with simultaneously occurring sorption of As(V) to the GEH-material) was almost as efficient (1.5 mmol As(V)/g Fe) as the removal of As(V) by sorption to GEH-material only (1.7 mmol As(V)/g Fe). This suggests that the removal of As(V) from solution was mainly dominated by sorption to GEH-material but was slightly hindered by As coprecipitation with freshly formed biogenic Fe(III) minerals. Since the biogenic Fe(III) minerals were not present when the As(V) was added, the GEH surface was not coated with biogenic Fe(III) minerals and therefore did not influence initial As(V) sorption to the GEH-material. Consequently, the performance of the GEH-material was not dramatically affected by the bacterial Fe(II) oxidation (as observed in the setup where both biogenic Fe(III) minerals and GEH-material were present before As(V) addition), and the largest amount of As(V) was probably already sorbed to the GEH-material before Fe(II) oxidation and precipitation of the biogenic Fe(III) minerals started. The slight reduction in efficiency observed compared with sorption to pure GEH might be explained by cell organic matter in the biogenic system that blocked sorption sites and displaced As(V) already sorbed or As(V) complexed with iron and organic matter.[3,40,41]

Practical Consequences of Microbial Fe(II) Oxidation in Commercial As Filters. The present study confirms that GEH is a very efficient material for the treatment of As contaminated water and removes As(V) much more efficiently than biogenic Fe(III) minerals. However, we also found that the presence of Fe(II)-oxidizing bacteria and the formation of biogenic Fe(III) minerals that cover the GEH-material are not beneficial for the operation of the filter systems and lowers their efficiency by a factor of ~5. Additionally, no additive effect of As(V) sorption to GEH-material and simultaneous coprecipitation was observed. We conclude that an optimal performance of GEH filter systems is ensured best in the absence of Fe(II)-oxidizing bacteria, i.e. if these organisms are removed e.g. by an activated carbon filter. Another possibility to exclude Fe(II)-oxidizing bacteria from GEH-filters is to remove the Fe(II) from the raw water (e.g., deironing by aeration leading to chemical oxidation and precipitation). A positive side effect would be that part of the arsenic would already be removed by coprecipitation before the raw water enters the GEH-filter.

■ ASSOCIATED CONTENT

Supporting Information. Table of the geochemical parameters of GEH filter systems applied at three different locations in Germany (Table S1) and provides information about the scanning electron microscopes used for taking the scanning electron micrographs presented in this study (S1). Furthermore, S2 shows how sorption isotherms and maximum sorption capacities were determined by fitting the experimental data with the Langmuir equation. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +49-7071-2974992. Fax: +49-7071-295059. E-mail: andreas.kappler@uni-tuebingen.de.

■ ACKNOWLEDGMENT

This work was funded by the DFG and the BMBF within the special program GEOTECHNOLOGIEN (subproject MikroAktiv).

We thank the GEH Wasserchemie GmbH & Co. KG for providing used/unused GEH-material for our experiments and geochemical parameters of the filter systems. Furthermore, we thank the NMI (Reutlingen, Germany) and Fabian Zeitvogel, Nikolas Hagemann, and Claudia Hohmann for providing scanning electron micrographs.

REFERENCES

(1) Smedley, P.; Kinniburgh, D. A review of the source, behaviour and distribution of arsenic in natural waters. *Appl. Geochem.* **2002**, *17* (5), 517–568.

(2) Islam, F.; Gault, A.; Boothman, C.; Polya, D.; Charnock, J.; Chatterjee, D.; Lloyd, J. Role of metal-reducing bacteria in arsenic release from Bengal delta sediments. *Nature* **2004**, *430* (6995), 68–71.

(3) Sharma, P.; Ofner, J.; Kappler, A. Formation of binary and ternary colloids and dissolved complexes of organic matter, Fe and As. *Environ. Sci. Technol.* **2010**, *44* (12), 4479–4485.

(4) Sharma, P.; Rolle, M.; Kocar, B.; Fendorf, S.; Kappler, A. Influence of natural organic matter on As transport and retention. *Environ. Sci. Technol.* **2011**, *45* (2), 546–553.

(5) Bissen, M.; Frimmel, F. Arsenic-a review. part I: occurrence, toxicity, speciation, mobility. *Acta Hydrochim. Hydrobiol.* **2003**, *31* (1), 9–18.

(6) Campos, V. Arsenic in groundwater affected by phosphate fertilizers at Sao Paulo, Brazil. *Environ. Geol.* **2002**, *42* (1), 83–87.

(7) Henke, K. *Arsenic: environmental chemistry, health threats and waste treatment*; John Wiley & Sons: 2009.

(8) Luzi, S.; Berg, M.; Pham, T.; Pham, H.; Schertenleib, R. Household sand filters for arsenic removal-technical report. *Swiss Federal Institute for Environmental Science and Technology (Eawag)* **2004**.

(9) Mazumder, D. Chronic arsenic toxicity: clinical features, epidemiology, and treatment: experience in West Bengal. *J. Environ. Sci. Health, Part A* **2003**, *38* (1), 141–163.

(10) Petrusevski, B.; Sharma, S.; Schippers, J.; Shordt, K.; Wijk, C. *Arsenic in drinking water. Delft*; IRC International Water and Sanitation Centre: 2007; pp 1−11.

(11) Berg, M.; Luzi, S.; Trang, P. T. K.; Viet, P. H.; Giger, W.; Stuben, D. Arsenic removal from groundwater by household sand filters: Comparative field study, model calculations, and health benefits. *Environ. Sci. Technol.* **2006**, *40* (17), 5567–5573.

(12) Driehaus, W.; Jekel, M.; Hildebrandt, U. Granular ferric hydroxide-a new adsorbent for the removal of arsenic from natural water. *Aqua* **1998**, *47*, 30–35.

(13) Kappler, A.; Straub, K. Geomicrobiological cycling of iron. *Rev. Mineral. Geochem.* **2005**, *59* (1), 85.

(14) Posth, N. R.; Huelin, S.; Konhauser, K. O.; Kappler, A. Size, density and composition of cell-mineral aggregates formed during anoxygenic phototrophic Fe (II) oxidation: impact on modern and ancient environments. *Geochim. Cosmochim. Acta* **2010**.

(15) Fortin, D.; Langley, S. Formation and occurrence of biogenic iron-rich minerals. *Earth-Sci. Rev.* **2005**, *72* (1−2), 1–19.

(16) Kappler, A.; Schink, B.; Newman, D. Fe (III) mineral formation and cell encrustation by the nitrate-dependent Fe (II)-oxidizer strain BoFeN1. *Geobiology* **2005**, *3* (4), 235–245.

(17) Châtellier, X.; Fortin, D.; West, M.; Leppard, G.; Ferris, F. Effect of the presence of bacterial surfaces during the synthesis of Fe oxides by oxidation of ferrous ions. *Eur. J. Mineral.* **2001**, *13* (4), 705.

(18) Châtellier, X.; West, M.; Rose, J.; Fortin, D.; Leppard, G.; Ferris, F. Characterization of iron-oxides formed by oxidation of ferrous ions in the presence of various bacterial species and inorganic ligands. *Geomicrobiol. J.* **2004**, *21* (2), 99–112.

(19) Tadanier, C. J.; Schreiber, M. E.; Roller, J. W. Arsenic mobilization through microbially mediated deflocculation of ferrihydrite. *Environ. Sci. Technol.* **2005**, *39* (9), 3061–3068.

(20) Ehrenreich, A.; Widdel, F. Anaerobic oxidation of ferrous iron by purple bacteria, a new type of phototrophic metabolism. *Appl. Environ. Microbiol.* **1994**, *60* (12), 4517.

(21) Straub, K. L.; Kappler, A.; Schink, B. Enrichment and isolation of ferric-iron-and humic-acid-reducing bacteria. In *Methods in Enzymology*; Jared, R. L., Ed.; Academic Press: 2005; pp 58−77.

(22) Emerson, D.; Merrill Floyd, M. Enrichment and isolation of iron-oxidizing bacteria at neutral pH. *Methods Enzymol.* **2005**, *397*, 112–123.

(23) Stookey, L. Ferrozine-a new spectrophotometric reagent for iron. *Anal. Chem.* **1970**, *42* (7), 779–781.

(24) Hohmann, C.; Winkler, E.; Morin, G.; Kappler, A. Anaerobic Fe(II)-oxidizing bacteria show As resistance and immobilize As during Fe(III) mineral precipitation. *Environ. Sci. Technol.* **2010**, *44* (1), 94–101.

(25) Kappler, A.; Newman, D. Formation of Fe (III)-minerals by Fe (II)-oxidizing photoautotrophic bacteria 1. *Geochim. Cosmochim. Acta* **2004**, *68* (6), 1217–1226.

(26) *GEH-Wasserchemie* Website. http://www.geh-wasserchemie.de/1-1-home-en.html (accessed month day, year).

(27) Daus, B.; Mattusch, J.; Wennrich, R.; Weiss, H. Investigation on stability and preservation of arsenic species in iron rich water samples. *Talanta* **2002**, *58* (1), 57–65.

(28) Daus, B.; Mattusch, J.; Wennrich, R.; Weiss, H. Analytical investigations of phenyl arsenicals in groundwater. *Talanta* **2008**, *75* (2), 376–379.

(29) Larese-Casanova, P.; Haderlein, S.; Kappler, A. Biomineralization of lepidocrocite and goethite by nitrate-reducing Fe (II)-oxidizing bacteria: Effect of pH, bicarbonate, phosphate and humic acids. *Geochim. Cosmochim. Acta* **2010**.

(30) Sutton, N. B.; van der Kraan, G. M.; van Loosdrecht, M.; Muyzer, G.; Bruining, J.; Schotting, R. J. Characterization of geochemical constituents and bacterial populations associated with As mobilization in deep and shallow tube wells in Bangladesh. *Water Res.* **2009**, *43* (6), 1720–1730.

(31) Lear, G.; Song, B.; Gault, A.; Polya, D.; Lloyd, J. Molecular analysis of arsenate-reducing bacteria within Cambodian sediments following amendment with acetate. *Appl. Environ. Microbiol.* **2007**, *73* (4), 1041.

(32) Cornell, R.; Schwertmann, U. *The Iron Oxides: Structure, Properties, Reactions, Occurrences, and Uses*; Wiley-VCH Verlag: Weinheim, 2003.

(33) Hohmann, C.; Morin, G.; Ona-Nguema, G.; Guigner, J.-M.; Brown, G. E., Jr.; Kappler, A. Molecular-level modes of As binding to iron (oxy)hydroxides precipitated by the anaerobic nitrate-reducing iron(II)-oxidizing Acidovorax sp. strain BoFeN1. *Geochim. Cosmochim. Acta* (in press).

(34) Langley, S.; Gault, A.; Ibrahim, A.; Renaud, R.; Fortin, D.; Clark, I.; Ferris, F. G. A comparison of the rates of Fe (III) reduction in synthetic and bacteriogenic iron oxides by Shewanella putrefaciens CN32. *Geomicrobiol. J.* **2009**, *26* (2), 57–70.

(35) O'Loughlin, E. J.; Gorski, C. A.; Scherer, M. M.; Boyanov, M. I.; Kemner, K. M. Effects of oxyanions, natural organic matter, and bacterial cell numbers on the bioreduction of lepidocrocite (FeOOH) and the formation of secondary mineralization products. *Environ. Sci. Technol.* **2010**, *44*, 4570–4576.

(36) O'Loughlin, E. J. Effects of electron transfer mediators on the bioreduction of lepidocrocite (γ-FeOOH) by *Shewanella putrefaciens* CN32. *Environ. Sci. Technol.* **2008**, *42*, 6876–6882.

(37) Deliyanni, E.; Bakoyannakis, D.; Zouboulis, A.; Matis, K. Sorption of As (V) ions by akaganeite-type nanocrystals. *Chemosphere* **2003**, *50* (1), 155–163.

(38) Kolbe, F.; Weiss, H.; Morgenstern, P.; Wennrich, R.; Lorenz, W.; Schurk, K.; Stanjek, H.; Daus, B. Sorption of aqueous antimony and arsenic species onto akaganeite. *J. Colloid Interface Sci.* **2011**.

(39) Dzombak, D.; Morel, F. *Surface complexation modeling: Hydrous ferric oxide*; John Wiley & Sons: 1990.

(40) Weng, L.; Van Riemsdijk, W. H.; Hiemstra, T. Effects of fulvic and humic acids on arsenate adsorption to goethite: Experiments and modeling. *Environ. Sci. Technol.* **2009**, *43* (19), 7198–7204.

(41) Mikutta, C.; Frommer, J.; Voegelin, A.; Kaegi, R.; Kretzschmar, R. Effect of citrate on the local Fe coordination in ferrihydrite, arsenate

binding, and ternary arsenate complex formation. *Geochim. Cosmochim. Acta* **2010**.

(42) Plette, A. C. C.; van Riemsdijk, W. H.; Benedetti, M. F.; van der Wal, A. pH dependent charging behavior of isolated cell walls of a gram-positive soil bacterium. *J. Colloid Interface Sci.* **1995**, *173* (2), 354–363.

(43) Sokolov, I.; Smith, D.; Henderson, G.; Gorby, Y.; Ferris, F. Cell surface electrochemical heterogeneity of the Fe (III)-reducing bacteria Shewanella putrefaciens. *Environ. Sci. Technol.* **2001**, *35* (2), 341–347.

(44) Hegler, F. Microbial Fe(II) oxidation: cell-mineral interactions and implications for modern and ancient environments. Ph.D. Dissertation, Eberhard Karls University, Tübingen, Germany, 2011.

(45) Fuller, C.; Davis, J.; Waychunas, G. Surface chemistry of ferrihydrite: Part 2. Kinetics of arsenate adsorption and coprecipitation. *Geochim. Cosmochim. Acta* **1993**.

(46) Roberts, L.; Hug, S.; Ruettimann, T.; Billah, M.; Khan, A.; Rahman, M. Arsenic removal with iron (II) and iron (III) in waters with high silicate and phosphate concentrations. *Environ. Sci. Technol.* **2004**, *38* (1), 307–315.

(47) Zouboulis, A.; Katsoyiannis, I. Recent advances in the bioremediation of arsenic-contaminated groundwaters. *Environment Int.* **2005**, *31* (2), 213–219.

(48) Barrero, C.; García, K.; Morales, A.; Kodjikian, S.; Greneche, J. New analysis of the Mössbauer spectra of akaganeite. *J. Phys.: Condens. Matter* **2006**, *18*, 6827.