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Additive and Competitive Effects of Bacteria and Mn Oxides on Arsenite Oxidation Kinetics

L. Camille Jones,[†,*] Brandon J. Lafferty,[†,‡] and Donald L. Sparks[†]

[†]Department of Plant & Soil Sciences, University of Delaware, Newark, Delaware, United States

[‡]U.S. Army Corps of Engineers, Engineer Research and Development Center, Vicksburg, Mississippi, United States

ABSTRACT: Arsenic (As) is a redox-active metalloid whose toxicity and mobility in soil depend on oxidation state. Arsenite [As(III)] can be oxidized to arsenate [As(V)] by both minerals and microbes in soil however, the interaction between these abiotic and biotic processes is not well understood. In this study, the time dependency of As(III) oxidation by two heterotrophic soil bacteria (*Agrobacterium tumefaciens* and *Pseudomonas fluorescens*) and a poorly crystalline manganese (Mn) oxide mineral (δ-MnO_2) was determined using batch experiments. The apparent rate of As(V) appearance in solution was greater for the combined batch experiments in which bacteria and δ-MnO_2 were oxidizing As(III) at the same time than for either component alone. The additive effect of the mixed cell- δ-MnO_2 system was consistent for short (<1 h) and long (24 h) term coincubation indicating that mineral surface inhibition by cells has little effect the As(III) oxidation rate. Surface interactions between cells and the mineral surface were indicated by sorption and pH-induced desorption results. Total sorption of As on the mineral was lower with bacteria present (16.1 ± 0.8% As sorbed) and higher with δ-MnO_2 alone (23.4 ± 1%) and As was more easily desorbed from the cell-δ-MnO_2 system than from δ-MnO_2 alone. Therefore, the presence of bacteria inhibited As sorption and decreased the stability of sorbed As on δ-MnO_2 even though As(III) was oxidized fastest in a mixed cell-δ-MnO_2 system. The additive effect of biotic (As-oxidizing bacteria) and abiotic (δ-MnO_2 mineral) oxidation processes in a system containing both oxidants suggests that mineral-only results may underestimate the oxidative capacity of natural systems with biotic and abiotic As(III) oxidation pathways.

INTRODUCTION

With millions of people being poisoned by arsenic (As) laden drinking and irrigation water in Southeast Asia, the question of how As is mobilized in the environment is urgently relevant to human health.[1,2] Arsenic mobility and toxicity in soil depend on As oxidation state and the biogeochemical processes that drive electron transfer (redox) in soil.[3,4] Arsenic oxidation is kinetically slow with atmospheric O_2 so it is thought that other soil components, including minerals and microorganisms, drive As(III) oxidation in oxic soil and sediments.[5] The oxidized form, As(V), whose behavior is similar to phosphate in soils, is less mobile and less toxic than the reduced form, As(III).[6] Therefore, the rates and mechanisms of biogeochemical As(III) oxidation impact As remediation and risk assessment.

One pathway for As(III) oxidation in soil is via mineral oxidants. Manganese (Mn) oxides are common soil minerals with a high capacity to oxidize and sorb heavy metals and metalloids.[7] Many Mn oxide minerals in the environment are poorly crystalline with high surface areas, making them very reactive even at low concentrations.[7,8] Arsenite oxidation by Mn oxides results in As(V) sorption as inner-sphere ligand complexes and production of Mn(II) and Mn(III) by direct reduction and conproportionation.[9−11] Laboratory studies have demonstrated rapid kinetics of As(III) oxidation by Mn oxide minerals accompanied by rapid product [As(V), Mn(II), Mn(III)] release into solution and mineral sorption.[9−15] Passivation is the process by which products block reactive sites on a mineral surface and thereby inhibit the oxidation reaction. Arsenite oxidation by Mn oxides is slowed by mineral surface passivation resulting from adsorption of As(V), Mn(II), and Mn(III).[9−13,15,16] It has also been noted that oxidation enhances As removal from solution because more total As is sorbed by reacting Mn oxides with As(III) than with As(V).[17−19]

Despite its toxicity, As oxidation and reduction can be carried out by many bacteria and archaea. Arsenite oxidation may occur through chemolithoautotropic metabolism, in which bacteria gain energy from As(III), and through detoxification mechanisms in heterotrophic bacteria, which do not grow with As as their sole electron donor.[20−23] An As oxidase enzyme, with corresponding genes (*aro*, *aso*, and *aox*), has been identified and it is suspected that other enzymes and mechanisms could also be involved in As(III) oxidation.[24−27] In addition to isolating As oxidizing bacteria from the environment, primers for As oxidase genes have been used to identify their activity in

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contaminated soils, providing evidence that microbial As(III) oxidation may be widespread.[28−30]

The coexistence of biotic and abiotic As(III) oxidation pathways in soil raises the question of how As(III) oxidation proceeds when these two pathways are concurrent. Both processes have temporal variability: As sorption and oxidation mechanisms can change over time and As(III) oxidizing bacteria exhibit a range of oxidation rates.[9,26,31] Therefore, studying the time dependency of As(III) oxidation in mixed microbe-mineral systems is the logical step forward from prior research on biotic oxidation, abiotic oxidation, and sorption processes. This reaction pair is relevant for oxic soil environments and soil colloids where microbial and mineral As(III) oxidants are found. The objective of this study was to characterize the kinetics of As(III) oxidation in mixed cell-mineral batch experiments using a model Mn oxide mineral, δ-MnO_2, and heterotrophic As(III) oxidizing soil bacteria *Pseudomonas fluorescens* and *Agrobacterium tumefaciens*. This study is one of the first to link the effects of these bacteria, isolated from a contaminated soil, and a highly reactive Mn oxide mineral on the kinetics of As(III) oxidation and As sorption.[16,31]

MATERIALS AND METHODS

δ-MnO_2. The poorly crystalline Mn oxide, δ-MnO_2, was selected because of its similarity to natural biogenic Mn oxides and its higher surface area and reactivity than crystalline Mn oxides.[8,32] δ-MnO_2 was synthesized following established procedures.[9,10,15,16,33] Synthesis began by adding a solution containing $Mn(II)(NO_3)_2$*$4H_2O$ and 18.2 MΩ deionized (DI) water to a solution containing $KMn(VII)O_4$ and NaOH corresponding to a final $Mn(II):Mn(VII):OH^-$ ratio of 3:2:4. The solution was then stirred for at least 12 h or until the $KMn(VII)O_4$ was completely reacted. The mixture was centrifuged at 10 000*g* for 15 min, supernatant decanted, replaced with DI water, and resuspended by sonication. This washing step was repeated three times. Following washing, the mineral was dialyzed for at least 48 h or until the conductivity of dialysis water remained unchanged for at least 12 h. δ-MnO_2 was stored at 4 °C in the dark, and used within three weeks of synthesis.

The point of zero charge (PZC) of δ-MnO_2 used in these experiments was 1.85, determined using the prolonged salt titration method (PST).[34] X-ray diffraction (XRD) was conducted on δ-MnO_2 synthesized using the synthesis method used in these studies. XRD analysis confirmed that only poorly crystalline δ-MnO_2 is produced from this synthesis method.[10] The surface area of δ-MnO_2 was determined, via BET analysis, to be 273.5 $m^2\ g^{-1}$. The average particle size of δ-MnO_2 was 450 nm as determined by dynamic light scattering (Zetasizer Nano Series, Malvern Instruments Ltd.).

As(III) and As(V) Solutions. All As solutions were made using $NaAsO_2$ and 18.2 MΩ DI water, and concentrations were verified with high performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICP/MS) using the method described below. Batch experiments were conducted at a concentration of 65−77 μM As(III) and no added As(V) (concentration below detection). Solutions and mineral were kept in oxic atmospheric conditions at 4 °C in the dark.

Bacterial Growth. Isolates of the aerobic heterotrophic bacteria *Agrobacterium tumefaciens* and *Pseudomonas fluorescens* were obtained from Dr. Richard Macur (Montana State University) and cryogenically preserved (−80 °C). These bacteria were isolated from an As-affected soil in Montana.[31] They require an organic carbon source (e.g., glucose) for growth and their rapid As(III) oxidation was hypothesized to be a detoxification mechanism as opposed to energy generation.[31]

Bacteria from the cryogenic reserve were used to inoculate starter plates, consisting of Petri dishes with nutrient rich agar (R2A) that did not contain As. Defined growth media, modified from the media used to isolate these bacteria, consisted of sterilized As(III) (75 μM), 3-(N-morpholino)propanesulfonic acid (MOPS) buffer (5 mM, pH 7.2), glucose (20 mM), NH_4NO_3 (1.25 mM), $CaSO_4$ (2 mM), $MgCl_2$ (2 mM), KH_2PO_4 (0.064 mM), KOH (1.25 mM), $FeCl_2$ (5 μM), micronutrients and vitamins.[31] The same media without As(III) or glucose was used in batch kinetics experiments.

Liquid cultures, agitated on a rotary shaker at 100 rpm, consisted of defined growth media with 75 μM As(III) in sterile plastic Erlenmeyer flasks inoculated with single colonies from plates or starter liquid cultures. Growth was monitored by optical density (OD) at 650 nm using a spectrophotometer (Spectronic 21, Bausch & Lomb). When liquid cultures reached late exponential phase (16−24 h depending on the strain) cells were rinsed three times in defined media lacking glucose and As(III) at 8000*g* for 10 min, stored at 4 °C, and used in batch experiments within 24 h.

Cell count and protein content were correlated to OD using direct cell counts on a Zeiss AxioImager microscope (phase contrast) and a protein assay. Protein was measured using the Bradford Assay method in which frozen resting cell cultures were centrifuged, digested with HCl, neutralized with NaOH, and diluted with $NaPO_4$.[35] This solution was then assayed with a Coomassie blue dye reagent and OD was measured at 595 nm. The amount of protein was quantified using a standard curve created with bovine serum albumin (BSA).

Batch Oxidation and Sorption Kinetics Experiments. Batch experiments were conducted to determine the apparent rate of As(III) oxidation and As sorption. All experiments with cells included washed resting cell suspensions of bacteria in late exponential growth phase and no carbon source in the media.[29] This method ensured that the cells were in the same growth phase and at a constant cell number for kinetics experiments. Experiments were carried out in triplicate using sterile plastic Erlenmeyer flasks with 100 mL of defined media (see above) buffered with MOPS (5 mM at pH 7.2), pristine δ-MnO_2 (0.05 g L^{-1}), and resting cell cultures of *A. tumefaciens* (9.8 μg protein mL^{-1}) or *P. fluorescens* (6.9 μg protein mL^{-1}). Cell protein concentrations in mixed experiments were different for the two isolates to normalize for variability in cellular protein and As(III) oxidation rate. All experiments were performed with the same concentrations of cells and minerals to achieve oxidation rates that could be measured within the temporal resolution of the batch technique.

The reaction was initiated (time = 0) by adding As(III) ($NaAsO_2$) to achieve a final concentration of 75 ± 5 μM for mixed microbe-mineral experiments. Experiments were initiated at two microbe-mineral coincubation times: several minutes after mixing the cells and mineral together and 24 h after mixing. The short and 24 h coincubation times tested whether the contact time between cells and mineral affected the oxidation kinetics, sorption, and desorption. During the experiments, 1 mL samples were removed periodically from the flasks, filtered through 0.22 μm nylon filters to stop the

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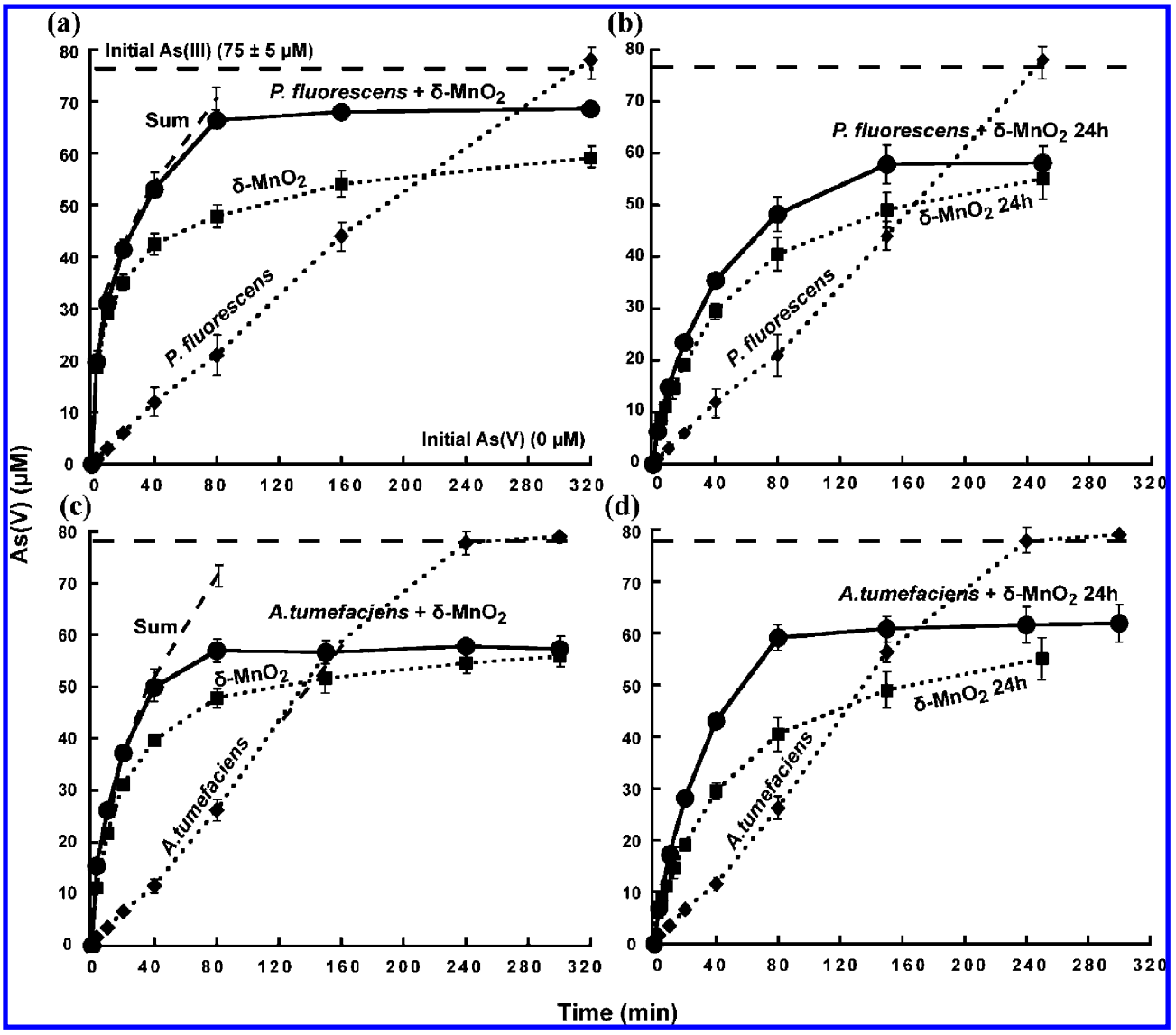

Figure 1. Appearance of As(V) in solution over time in batch reactions measuring As(III) oxidation by δ-MnO_2 with *P. fluorescens* (a, b) and *A. tumefaciens* (c, d) alone (dashed lines) and as mixtures (solid lines) with a short (a, c) and 24 h coincubation (b, d). The initial As(III) concentration in solution is marked by a horizontal dashed line and the initial As(V) concentration was below detection.

reaction, and stored in the dark at 4 °C until As speciation analysis.

Sorbed As was calculated using the following mass balance eq 1:

$$[(C_{0\mathrm{AsTotal}}V_{0\mathrm{AsTotal}}) - (C_{\mathrm{fAs(III)}}V_{\mathrm{fAs(III)}} + C_{\mathrm{fAs}(V)}V_{\mathrm{fAs}(V)})] /(C_{0\mathrm{AsTotal}}V_{0\mathrm{AsTotal}}) = q \times 100\% \quad (1)$$

where $C_{0\mathrm{AsTotal}}$ and $V_{0\mathrm{AsTotal}}$ are the initial total As in μM and volume in L, $C_{\mathrm{fAs(III)}}V_{\mathrm{fAs(III)}} + C_{\mathrm{fAs(V)}}V_{\mathrm{fAs(V)}}$ are final (300 or 320 min) values, and q is the amount of sorption. The variable q is unitless. It was inferred that all sorbed As was associated with the mineral phase because sorption by bacteria was not detected.

The apparent first order rate constant (k_{obs}) for the δ-MnO_2-only and mixed microbe-mineral batch experiments was described by the rate eq 2:[14]

$$-\mathrm{d}[\mathrm{As(III)}]/\mathrm{d}t = k_{obs}[\mathrm{As(III)}] \quad (2)$$

and k_{obs} was calculated using the integrated form of eq 2:

$$\ln([\mathrm{As(III)}]_t/[\mathrm{As(III)}]_0) = -k_{obs}t \quad (3)$$

for the first 80 min of reaction. Half-life ($t_{1/2}$) for the apparent first order reactions was calculated using eq 4:

$$t_{1/2} = \ln 2/k_{obs} \quad (4)$$

These kinetic calculations reflect apparent rates and do not imply any reaction mechanism. k_{obs} and $t_{1/2}$ were calculated for each experiment in order to quantitatively compare batch experiment results.

Control experiments were conducted with killed cells to check whether As(III) oxidation required living bacteria. Cultures were sterilized by autoclaving twice (45 min, 121 °C). Oxidation batch experiments were carried out using the killed cells with the same method described above.

Batch Desorption Experiments. Replenishment desorption experiments were conducted to investigate the stability of

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sorbed As on δ-MnO_2 and microbial reactants as a function of pH. Desorption does not occur over the time scale of these experiments at pH 7.2. Previous studies reported that decreasing the pH of As sorbed on Mn oxide minerals releases As into solution.[36] Batch experiments (as described above) were reacted until steady-state, centrifuged at 10 000*g* for 15 min and decanted. Desorption experiments were initiated by resuspending the microbe-mineral paste in 100 mL of defined media buffered with 2-(*N*-morpholino)ethanesulfonic acid (MES) at pH 5.8. Solutions were briefly vortexed to mix and reacted for 250 min. Samples were filtered through 0.22 μm filters (Cole-Parmer, NY membrane) and stored in the dark at 4 °C until they were analyzed. The amount of sorbed As was calculated based on percent of sorption (eq 1) and μmoles As_{sorbed} per mg δ-MnO_2.

HPLC-ICP/MS Analysis. Aqueous As(III) and As(V) were monitored throughout all experiments. As_{Total} was calculated from the sum of As(III) and As(V) and sorbed As was calculated as described above. Dissolved concentrations of As were determined by HPLC-ICP/MS (HPLC: Agilent 1200; ICP/MS: Agilent 7500cx) following an established protocol.[16,19]

FESEM Analysis. Batch reaction products including δ-MnO_2-only, cell-only, and mixed cell- δ-MnO_2 samples were imaged by field-emission scanning electron microscopy (FESEM) within 3 h of reaction completion. Samples were filtered onto 0.22 μm filter papers (Nucleopore, PC membrane) and attached to a Gatan sample holder with a thin layer of Tissue Freezing Medium (Electron Microscopy Sciences) mixed with graphite powder (Polysciences, Inc.). The samples were plunged into liquid nitrogen slush and transferred into the Gatan Alto 2500 cryo-stage. Surface ice was removed through sublimation at −90 °C for 10 min. Samples were recooled to −125 °C and sputter coated with a 2−3 nm layer of gold−palladium. Samples were imaged in a Hitachi S-4700 FESEM at −125 °C using a 3.0 kV accelerating voltage and working distances of 6.8 and 7.8 mm.

RESULTS AND DISCUSSION

As(III) Oxidation in Mixed Microbe-Mn Oxide Experiments. A series of four mixed cell-mineral batch experiments were conducted using each model bacterium and δ-MnO_2, with short (0−10 min) and 24 h coincubation prior to starting the reaction. The two coincubation times were selected to address whether reaction time before As(III) addition changed oxidant reactivity. The bacteria were in the same growth phase and resting state (no glucose available) during the 24 h and short coincubations to minimize differences in cell metabolic and physiological state. Figure 1 summarizes time series results from all batch experiments including the mixed cell-mineral experiments and separate cell suspension and mineral experiments.

Arsenite oxidation in mineral-only (dashed lines in Figure 1) and mixed microbe-mineral (solid lines in Figure 1) batch experiments exhibited biphasic apparent first order reaction kinetics. The rapid initial As(III) oxidation phase followed by a slow phase approaching steady state was consistent with rates from previous studies on δ-MnO_2.[9,10,33] The bacteria, however, oxidized As(III) in apparent zero order reaction kinetics with the rate of As(V) appearance constant until all As(III) in the batch was oxidized. These cell-only As(III) oxidation rates were 3.2×10^{-2} and 3.9×10^{-2} μmol As(III) min^{-1} mg $protein^{-1}$ for *A. tumefaciens* and *P. fluorescens* respectively. Only As(V) results were shown in Figure 1, but As mass balance confirmed that the cells quantitatively oxidized As(III) and did not retain any As(III) or As(V).

In the initial phase of the reaction (0−80 min), there was an additive effect of the two oxidation pathways when both the bacteria and the mineral were present. In this period, the appearance of As(V) in solution was more rapid in all mixed cell- δ-MnO_2 batches than in either the cell or the mineral batches alone. In mixed cell- δ-MnO_2 experiments, As(III) was oxidized fastest in the initial (0−80 min) reaction phase followed by a slower second phase (>80 min). This suggests that in the initial part of the reaction, the cells and mineral were oxidizing As(III) in parallel and not inhibiting the other component's oxidation rate.

In the second phase, we hypothesize that As and Mn sorption on the mineral surface affected As(V) appearance in solution. The rate of As(V) appearance for all experiments with Mn oxide (mixed cell- δ-MnO_2 and δ-MnO_2 alone) was characteristic of previously reported surface passivation.[9−13,15,16] As the reaction proceeded, the products, As(V), Mn(II), and Mn(III), adsorbed to the mineral surface and blocked reactive sites.[9−13,15,16] This physical inhibition of As(III) oxidation sites on δ-MnO_2 accounts for the slower rate of As(V) appearance in solution after the initial reaction phase. In other words, As(III) oxidation slows down once As(V), Mn(II), and Mn(III) begin to saturate δ-MnO_2 edge sites.[9,10]

The sums of mineral-only and cell suspension-only experiments, shown for comparison to the mixed cell-δ-MnO_2 data, are evidence of additive As(III) oxidation kinetics in the mixed cell- δ-MnO_2 experiments ("Sum" dashed lines in Figure 1a and c). The sum was determined by adding data points for *P. fluorescens* or *A. tumefaciens* alone to δ-MnO_2 alone. The sums were within the error of the mixed cell-δ-MnO_2 data for the first 80 (Figure 1a) and 40 min of reaction (Figure 1c). The sum deviated from the mixed cell-δ-MnO_2 data because of the decrease in As(III) oxidation rate for δ-MnO_2-alone as a result of surface passivation by sorbed species as was observed for δ-MnO_2 in prior work.[9−13,15,16] The sum trend was the same for the short and 24 h coincubated experiments. The similarity of summed single oxidizer and mixed cell-δ-MnO_2 As(V) appearance rates indicated that As(III) oxidation by cells and mineral components were additive in this initial reaction phase.

A first order kinetic model provided a more quantitative assessment of As(III) oxidation rates in mixed cell-mineral and singular δ-MnO_2 experiments (Figure 2).

Apparent As(III) oxidation rate parameters for δ-MnO_2-only experiments were similar to published results on Mn oxides despite differences between studies regarding mineral crystallinity, surface area, pH, and electrolyte concentration differences.[14,17,18] We constrained kinetic calculations to the initial reaction phase because the rates deviated from apparent first order after 40−80 min due to the surface passivation discussed above. In the cell- δ-MnO_2 experiments for both incubation times, rate constants (k_{obs}) were higher and half-lives were shorter than in the δ-MnO_2-only experiments (Table 1).

However, the rates of 24 h coincubated experiments were lower than those with a shorter coincubation time. This could have been a result of mineral surface passivation by cellular products or components of the media solution such as PO_4^{2-}.[9,10,37,38] Overall, As(III) oxidation was most rapid when cell and mineral oxidants were mixed.

Influence of Cell-Mineral Interactions on As(III) Oxidation. The rapid As(III) oxidation in mixed cell-mineral experiments compared with the mineral alone was the first line

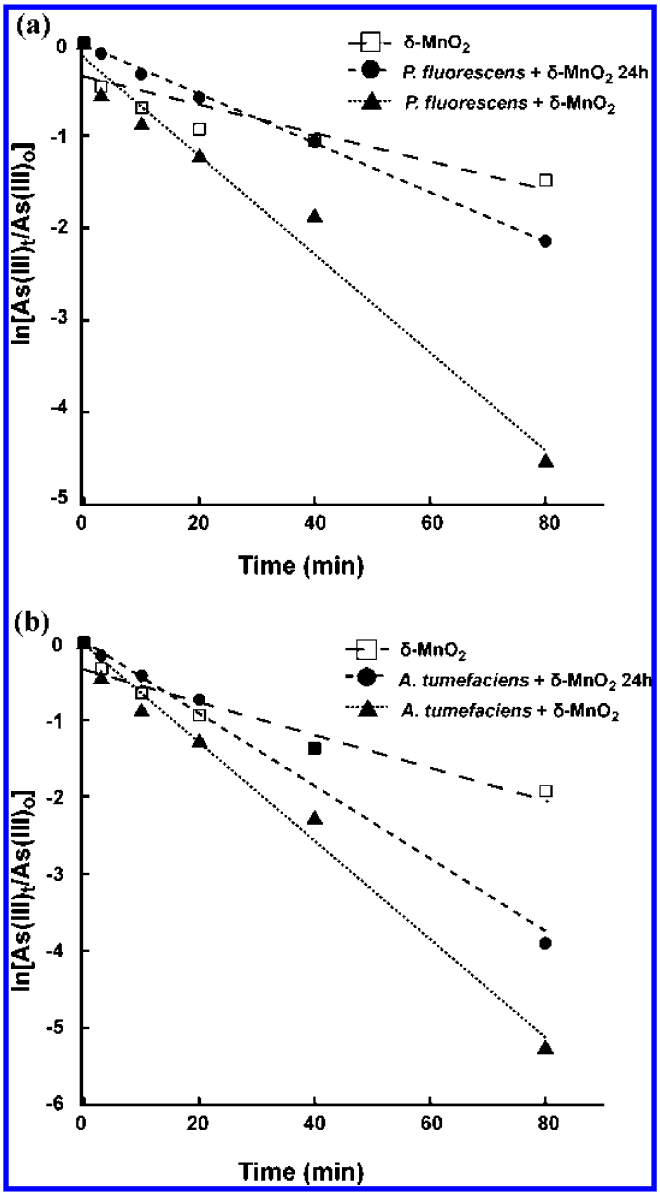

Figure 2. Apparent first order kinetics for δ-MnO_2-only and dual mixtures of δ-MnO_2 with *P. fluorescens* (a) and *A. tumefaciens* (b) cells. k_{obs} and R^2 values are included in Table 1.

Table 1. Apparent First Order Kinetic Parameters for δ-MnO_2-Only and Mixed Cell δ-MnO_2 Batch Experiments[a]

	k_{obs} (h^{-1})	half-life (h)	R^2
A. tumefaciens + δ-MnO_2	3.76	0.185	0.990
P. fluorescens + δ-MnO_2	3.13	0.221	0.976
A. tumefaciens + δ-MnO_2 24 h	2.84	0.244	0.975
P. fluorescens + δ-MnO_2 24 h	1.58	0.438	0.999
δ-MnO_2	1.33	0.523	0.918

[a]The error was less than 2 standard deviations based on triplicate replicates.

of evidence that the cell suspension and δ-MnO_2 did not interact in a way that inhibited As(III) oxidation (Figure 1). Complementary evidence was apparent from SEM imaging (Figure 3), which showed minimal macroscopic association between cells and mineral particles.

The cells and δ-MnO_2 imaged after batch experiments appeared to be discrete with neither component coating or blocking the other (Figure 3). The poorly crystalline appearance of the mineral surface was also unaltered in the cell-δ-MnO_2 system compared with the δ-MnO_2 only system. This macroscopic result does not eliminate the possibility of an extracellular polymeric substance (EPS) coating on δ-MnO_2 because EPS could have been invisible at this image resolution. We therefore conclude that any cell-mineral interaction in this study did not affect the appearance of the reactants by steady state but it may have slowed the initial rate of As(III) oxidation (Figure 2). This aspect could be investigated in future work by Fourier transform infrared (FTIR) spectroscopy and other in situ techniques.[16,19,38]

As Sorption and Desorption. Sorption (removal of As from solution) was observed in all batch experiments with δ-MnO_2 (Table 2).

Once the reactions reached steady state (250−320 min), 15.2−24.5% of initial As in solution was sorbed to the mineral phase. Overall this result agrees with sorption previously reported on Mn oxides.[9,10,33] The speciation of sorbed As was expected to be all As(V) based on prior work with the same mineral.[10,15]

More As was sorbed by δ-MnO_2 alone (21.3−24.5%) than by the cell-δ-MnO_2 batch experiments. Sorption in mixed cell-mineral experiments was similar for both *A. tumefaciens* and *P. fluorescens* mixed with δ-MnO_2 (15.2−17.1%) (Table 2). Less sorption may have been a result of mineral surface passivation by EPS, which have phosphate functional groups that could compete with As for sorption sites.[16,37] The short and 24 h coincubations exhibited similar amounts of As sorption. The cells were in a resting state during the coincubation, therefore cell-mineral associations were expected to have been unchanged after the extended interaction time.

A recent paper with the same δ-MnO_2 and bacteria reported slower As(III) oxidation reaction rates on δ-MnO_2 in the presence of these bacteria using attenuated total reflectance (ATR) FTIR spectroscopy.[16] To conform to the detection limit of ATR-FTIR, Parikh et al.,[16] used a concentration of up to 25 mM As(III) for their oxidation experiments, whereas our experiments were conducted at an initial concentration of 75 μM As(III). Cell-only As(III) oxidation data from both studies show that *A. tumefaciens* and *P. fluorescens* can grow at the higher As concentration, but their rate of As(III) oxidation is diminished perhaps as a result of As toxicity. Arsenic sorption results in the present study support the hypothesis of Parikh et al.[16] However, our results demonstrated that rapid microbial As(III) oxidation was additive to δ-MnO_2 at the lower As(III) concentration, whereas it was shown to be inhibitory at higher As(III) concentrations.[16]

Desorption experiments, conducted with a replenishment technique, demonstrated that sorbed As could be released from the surface of δ-MnO_2 by decreasing the pH from 7.2 to 5.8 (Table 2). Previous research has shown that desorption can be induced by a change in electrolyte, competitive cations, competition between cell-associated phosphate, and decreased pH.[33,36−40] Soils are typically buffered to changes in pH but a drop in pH could occur in the case of extreme conditions such as acid mine drainage or human gastric systems. In our experiments, the presence of bacterial cells decreased the amount of As retained on δ-MnO_2 in the case of a pH change. More As was desorbed from mixed *A. tumefaciens*- and *P. fluorescens*-δ-MnO_2 experiments (61.0−64.5%) than from δ-

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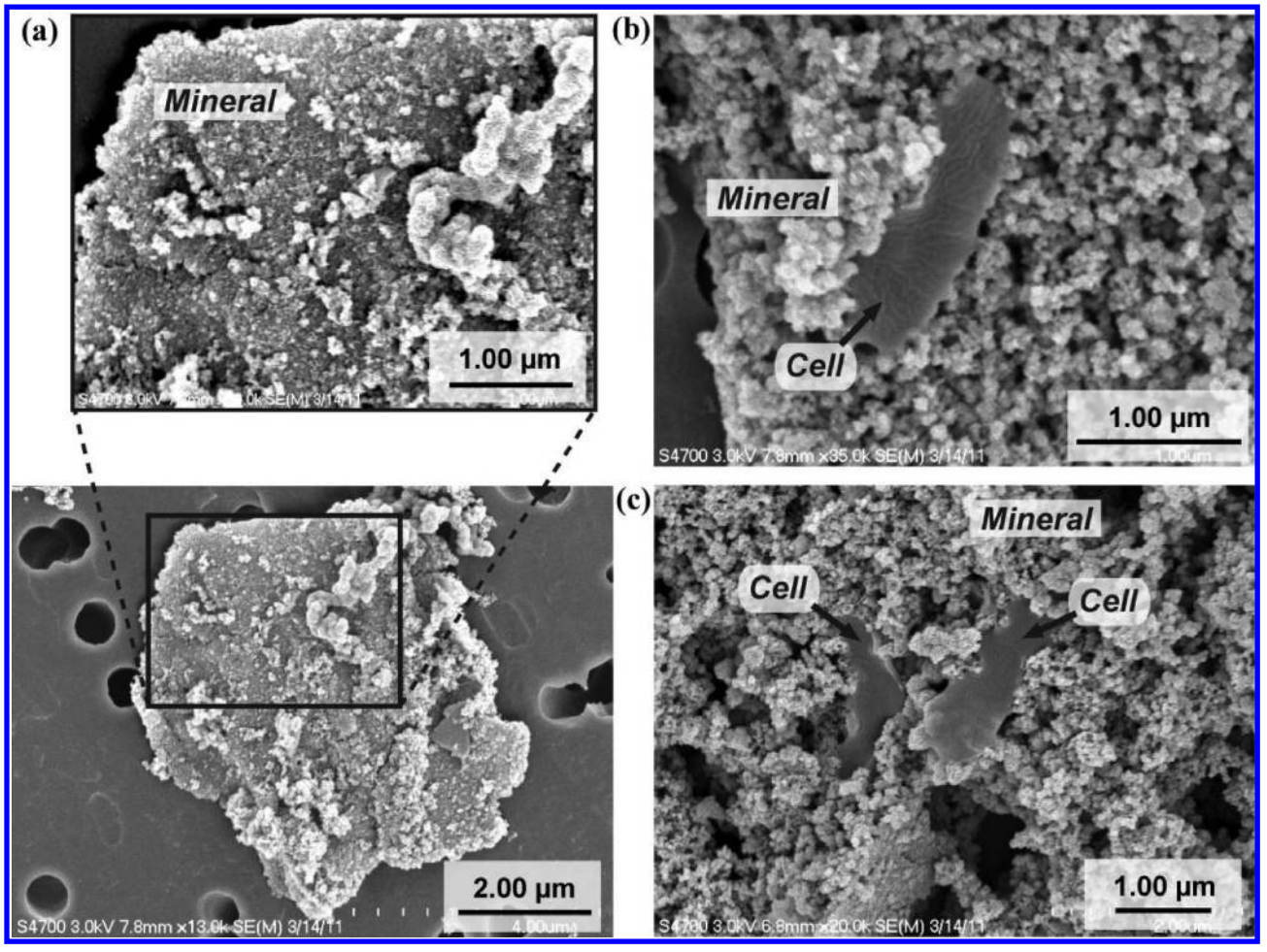

Figure 3. Field emission scanning electron microscopy (FESEM) images of δ-MnO_2 alone (a), mixtures of δ-MnO_2 with *P. fluorescens* (b), and δ-MnO_2 with *A. tumefaciens* (c).

Table 2. Amount of As Sorbed and Desorbed from Mineral-Only and Mixed cell-δ-MnO_2 Batch Experiments[a]

	Sorbed As		Desorbed As	
	mg kg^{-1} solid	±	mg kg^{-1} solid	±
δ-MnO_2	27.44	3.91	12.86	1.41
P. fluorescens + δ-MnO_2 24 h	16.80	3.67	10.86	0.79
P. fluorescens + δ-MnO_2	18.00	5.09	10.94	1.62
A. tumefaciens + δ-MnO_2 24 h	18.00	3.28	11.36	1.23
A. tumefaciens + δ-MnO_2	17.10	5.75	10.94	1.08

[a]Desorption was conducted via replenishment by decreasing the pH from 7.2 to 5.8.

MnO_2 alone (46.7−58.3%). Identifying the mechanism of desorption in the mixed cell- δ-MnO_2 system requires further study, but we hypothesize that it was a result of (1) differences in the amount of mineral surface passivation by As(V), Mn(III), and Mn(II), (2) mineral surface passivation by bacteria EPS, or (3) a combination of those effects.

Implications for As Mobility in the Environment. The additive effects of *P. fluorescens* and *A. tumefaciens* cells on the rate of As(III) oxidation by δ-MnO_2 reported here bridges prior work on separate mineral and microbial As oxidation.[17,24,31] In addition to the more rapid As(III) oxidation rate, *P. fluorescens* and *A. tumefaciens* cells decreased As sorption and enhanced desorption at the δ-MnO_2 surface most likely via passivation and surface inhibition.[9−13,15,16] Similar effects on the rate of As(III) oxidation and As sorption by As-oxidizing bacteria in the presence of Mn oxide minerals are expected in oxic soil environments. However, the initial rapid rates of As(III) oxidation observed here may overestimate rates in soil, which could be slowed as a result of organic coatings on minerals, competing sorptives, and lower concentrations of As(III) oxidizing cells, warranting further study on coupled biotic and abiotic systems and environmental As mobility.

AUTHOR INFORMATION

Corresponding Author

*Phone: (650) 723 − 4152; e-mail: l.camille.jones@gmail.com.

Present Address

Department of Environmental Earth System Science, Stanford University, Stanford, California, United States.

Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Smith, A. H.; Lingas, E. O.; Rahman, M. Contamination of drinking-water by arsenic in Bangladesh: a public health emergency. *Bull. W. H. O.* **2000**, *78* (9), 1093−1103.

(2) Bhattacharya, P.; Welch, A. H.; Stollenwerk, K. G.; McLaughlin, M. J.; Bundschuh, J.; Panaullah, G. Arsenic in the environment: Biology and Chemistry. *Sci. Total Environ.* **2007**, *379* (2−3), 109−120, DOI: 10.1016/j.scitotenv.2007.02.037.

(3) Nordstrom, D. K. Worldwide occurrences of arsenic in ground water. *Science* **2002**, *296* (5576), 2143−2145, DOI: 10.1126/science.1072375.

(4) Borch, T.; Kretzschmar, R.; Kappler, A.; Van Cappellen, P.; Ginder-Vogel, M.; Voegelin, A.; Campbell, K. Biogeochemical redox processes and their impact on contaminant dynamics. *Environ. Sci. Technol.* **2010**, *44* (1), 15−23, DOI: 10.1021/es9026248.

(5) Eary, L. E.; Schramke, J. A. Rates of inorganic oxidation reactions involving dissolved oxygen. In *Chemical Modeling of Aqueous Systems II*; Melchior, D., et al., Eds.; ACS Symposium Series; American Chemical Society: Washington DC, 1990.

(6) Duker, A. A.; Carranza, E. J. M.; Hale, M. Arsenic geochemistry and health. *Environ. Int.* **2005**, *31* (5), 631−641, DOI: 10.1016/j.envint.2004.10.020.

(7) Post, J. E. Manganese oxide minerals: Crystal structures and economic and environmental significance. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96* (7), 3447−3454, DOI: 10.1073/pnas.96.7.3447.

(8) Tebo, B. M.; Bargar, J. R.; Clement, B. G.; Dick, G. J.; Murray, K. J.; Parker, D.; Verity, R.; Webb, S. M. Biogenic manganese oxides: Properties and mechanisms of formation. *Annu. Rev. Earth Planet. Sci.* **2004**, *32*, 287−328, DOI: 10.1146/annurev.earth.32.101802.120213.

(9) Lafferty, B. J.; Ginder-Vogel, M.; Sparks, D. L. Arsenite oxidation by a poorly crystalline manganese-oxide: 1. Stirred-flow experiments. *Environ. Sci. Technol.* **2010**, *44* (22), 8460−8466, DOI: 10.1021/es102013p.

(10) Lafferty, B. J.; Ginder-Vogel, M.; Zhu, M.; Livi, K. J. T.; Sparks, D. L. Arsenite oxidation by a poorly crystalline manganese-oxide: 2. Results from x-ray absorption spectroscopy and x-ray diffraction. *Environ. Sci. Technol.* **2010**, *44* (22), 8467−8472, DOI: 10.1021/es102016c.

(11) Zhu, M.; Paul, K. W.; Kubicki, J. D.; Sparks, D. L. Quantum chemical study of arsenic (III, V) adsorption on Mn-oxides: Implications for arsenic(III) oxidation. *Environ. Sci. Technol.* **2009**, *43* (17), 6655−6661, DOI: 10.1021/es900537e.

(12) Scott, M. J.; Morgan, J. J. Reactions at oxide surfaces. 1. Oxidation of As(III) by synthetic birnessite. *Environ. Sci. Technol.* **1995**, *29* (8), 1898−1905, DOI: 10.1021/es00008a006.

(13) Nesbitt, H. W.; Canning, G. W.; Bancroft, G. M. XPS study of reductive dissolution of 7Å-birnessite by H_3AsO_3, with constraints on reaction mechanism. *Geochim. Cosmochim. Acta* **1998**, *62* (12), 2097−2110, DOI: 10.1016/S0016-7037(98)00146-X.

(14) Tournassat, C.; Charlet, L.; Bosbach, D.; Manceau, A. Arsenic(III) oxidation by birnessite and precipitation of manganese(II) arsenate. *Environ. Sci. Technol.* **2002**, *36* (3), 493−500, DOI: 10.1021/es0109500.

(15) Ginder-Vogel, M.; Landrot, G.; Fischel, J. S.; Sparks, D. L. Quantification of rapid environmental redox processes with quick-scanning x-ray absorption spectroscopy (Q-XAS). *Proc. Natl. Acad. Sci.* **2009**, *106* (38), 16124−16128, DOI: 10.1073/pnas.0908186106.

(16) Parikh, S. J.; Lafferty, B. J.; Meade, T. G.; Sparks, D. L. Evaluating environmental influences on As(III) oxidation kinetics by a poorly crystalline Mn-oxide. *Environ. Sci. Technol.* **2010**, *44* (10), 3772−3778, DOI: 10.1021/es903408g.

(17) Oscarson, D. W.; Huang, P. M.; Liaw, W. K.; Hammer, U. T. Kinetics of oxidation of arsenite by various manganese dioxides. *Soil Sci. Soc. Am. J.* **1983**, *47*, 644−648.

(18) Manning, B. A.; Fendorf, S. E.; Bostick, B.; Suarez, D. L. Arsenic(III) oxidation and arsenic(V) adsorption reactions on synthetic birnessite. *Environ. Sci. Technol.* **2002**, *36* (5), 976−981, DOI: 10.1021/es0110170.

(19) Parikh, S. J.; Lafferty, B. J.; Sparks, D. L. An ATR-FTIR spectroscopic approach for measuring rapid kinetics at the mineral/water interface. *J. Colloid Interface Sci.* **2008**, *320* (1), 177−185, DOI: 10.1016/j.jcis.2007.12.017.

(20) Mukhopadhyay, R.; Rosen, B. P.; Phung, L. T.; Silver, S. Microbial arsenic: from geocycles to genes and enzymes. *FEMS Microbiol. Rev.* **2002**, *26* (3), 311−325, DOI: 10.1111/j.1574-6976.2002.tb00617.x.

(21) Oremland, R. S.; Stolz, J. F. The ecology of arsenic. *Science* **2003**, *300* (5621), 939−944, DOI: 10.1126/science.1081903.

(22) Rhine, E. D.; Garcia-Dominguez, E.; Phelps, C. D.; Young, L. Y. Environmental microbes can speciate and cycle arsenic. *Environ. Sci. Technol.* **2005**, *39* (24), 9569−9573, DOI: 10.1021/es051047t.

(23) Garcia-Dominguez, E.; Mumford, A.; Rhine, E. D.; Paschal, A.; Young, L. Novel autotrophic arsenite-oxidizing bacteria isolated from soil and sediments. *FEMS Microbiol. Ecol.* **2008**, *66* (2), 401−410, DOI: 10.1111/j.1574-6941.2008.00569.x.

(24) Anderson, G. L.; Williams, J.; Hille, R. The purification and characterization of arsenite oxidase from Alcaligenes faecalis, a molybdenum-containing hydroxylase. *J. Biol. Chem.* **1992**, *267* (33), 23674−23682.

(25) Ellis, P. J.; Conrads, T.; Hille, R.; Kuhn, P. Crystal structure of the 100 kDa arsenite oxidase from *Alcaligenes faecalis* in two crystal forms at 1.64 Å and 2.03 Å. *Structure* **2001**, *9* (2), 125−132, DOI: 10.1016/S0969-2126(01)00566-4.

(26) Silver, S.; Phung, L. T. Genes and enzymes involved in bacterial oxidation and reduction of inorganic arsenic. *Appl. Environ. Microbiol.* **2005**, *71* (2), 599−608, DOI: 10.1128/ AEM.71.2.599-608.2005.

(27) Kashyap, D. R.; Botero, L. M.; Franck, W. L.; Hassett, D. J.; McDermott, T. R. Complex regulation of arsenite oxidation in *Agrobacterium tumefaciens*. *J. Bacteriol.* **2006**, *188* (3), 1081−1088, DOI: 10.1128/ JB.188.3.1081-1088.2006.

(28) Inskeep, W. P.; Macur, R. E.; Hamamura, N.; Warelow, T. P.; Ward, S. A.; Santini, J. M. Detection, diversity and expression of aerobic bacterial arsenite oxidase genes. *Environ. Microbiol.* **2007**, *9* (4), 934−943, DOI: 10.1111/j.1462-2920.2006.01215.x.

(29) Achour, A. R.; Bauda, P.; Billard, P. Diversity of arsenite transporter genes from arsenic-resistant soil bacteria. *Res. Microbiol.* **2007**, *158* (2), 128−137, DOI: 10.1016/j.resmic.2006.11.006.

(30) Quemeneur, M.; Heinrich-Salmeron, A.; Muller, D.; Lievremont, D.; Jauzein, M.; Bertin, P. N.; Garrido, F.; Joulain, C. Diversity surveys and evolutionary relationships of *aoxB* genes in aerobic arsenite-oxidizing bacteria. *Appl. Environ. Microbiol.* **2008**, *74* (14), 4567−4573, DOI: 10.1128/ AEM.02851-07.

(31) Macur, R. E.; Jackson, C. R.; Botero, L. M.; McDermott, T. R.; Inskeep, W. P. Bacterial populations associated with the oxidation and reduction of arsenic in an unsaturated soil. *Environ. Sci. Technol.* **2004**, *38* (1), 104−111, DOI: 10.1021/es034455a.

(32) Villalobos, M.; Toner, B.; Bargar, J.; Sposito, G. Characterization of the manganese oxide produced by *Pseudomonas putida* strain MnB1. *Geochim. Cosmochim. Acta* **2003**, *67* (14), 2649−2662, DOI: 10.1016/S0016-7037(03)00217-5.

(33) Lafferty, B. J.; Ginder-Vogel, M.; Sparks, D. L. Arsenite oxidation by a poorly-crystalline manganese-oxide: 3. Arsenic and manganese desorption. *Environ. Sci. Technol.* **2011**, *45* (21), 9218−9223, DOI: 10.1021/es201281u.

(34) Tan, W.-F.; Lu, S.-J.; Liu, F.; Feng, X.-H.; He, J.-Z.; Koopal, L. K. Determination of the point-of-zero charge of manganese oxides with different methods including an improved salt titration method. *Soil Sci.* **2008**, *173* (4), 277−286.

(35) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle

of protein-dye binding. *Anal. Biochem.* **1976**, *72* (1−2), 248−254, DOI: 10.1016/0003-2697(76)90527-3.

(36) Amirbahman, A.; Kent, D. B.; Curtis, G. P.; Davis, J. A. Kinetics of sorption and abiotic oxidation of arsenic(III) by aquifer materials. *Geochim. Cosmochim. Acta* **2006**, *70* (3), 533−547, DOI: 10.1016/j.gca.2005.10.036.

(37) Huang, J.-H.; Elzinga, E. J.; Brechbuehl, Y.; Voegelin, A; Kretzschmar, R. Impacts of *Shewanella putrefaciens* strain CN-32 cells and extracellular polymeric substances on the sorption of As(V) and As(III) on Fe(III)-(hydr)oxides. *Environ. Sci. Technol.* **2011**, *45* (7), 2804−2810, DOI: 10.1021/es103978r.

(38) Huang, J.-H.; Voegelin, A.; Pombo, S. A.; Lazzaro, A.; Zeyer, J.; Kretzschmar, R. Influence of arsenate adsorption to ferrihydrite, goethite, and boehmite on the kinetics of arsenate reduction by *Shewanella putrefaciens* strain CN-32. *Environ. Sci. Technol.* **2011**, *45* (18), 7701−7709, DOI: 10.1021/es201503g.

(39) Chiu, V. Q.; Hering, J. G. Arsenic adsorption and oxidation at Manganite surfaces: 1. Method for simultaneous determination of adsorbed and dissolved arsenic species. *Environ. Sci. Technol.* **2000**, *34* (10), 2029−2034, DOI: 10.1021/es990788p.

(40) Scheidegger, A. M.; Sparks, D. L. A critical assessment of sorption-desorption mechanisms at the soil mineral/water interface. *Soil Science* **1996**, *161* (12), 813−831.