



	Experiment title: Role of birnessite in the dissimilatory arsenic and iron reduction by <i>Shewanella</i> sp. ANA-3: Speciation of arsenic	Experiment number: 01-01-899
Beamline: BM01B	Date of experiment: From 10.02. to 13.02.2014	Date of report: 1 June 2014
Shifts: 6	Local contact: Hermann Emerich	<i>Received at ESRF:</i>
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1. Introduction

Elevated concentrations of As in natural waters, soils, and sediments represent a severe problem for millions of people worldwide. Reducing conditions have been identified as the major reason for As mobilization in soils, caused by a redox speciation change of As and thus a change in its adsorption behavior.¹ Elevated As solution concentrations observed in reducing natural systems are usually accompanied by microbially mediated As(V) and Fe(III) reduction.² A strong correlation has been recognized between reductively dissolved Fe and As released,² and it is widely accepted that As is mainly mobilized by the desorption of As(V) during reductive dissolution of Fe(III)-(hydr)oxides and subsequent its subsequent reduction to As(III).³

Manganese oxides are ubiquitous in soils and sediments.⁴ They are strong oxidants and have been shown to naturally attenuate As contamination in aquifers.⁵ Recent studies have found a rapid oxidation of As(III) by Mn-oxides and that As(III) oxidation rates are dependent on Mn-oxide crystallinity.⁶ Manganese oxides are known to be thermodynamically more favorable electron acceptors for microorganisms than Fe(III)-(hydr)oxides. Consequently, a preferential reduction of Mn(III/IV)-oxides over Fe(III)-(hydr)oxides would retard the release of As from the latter phases, and thus reduce the amount of dissolved As(V) potentially available for dissimilatory As-reducing bacteria. However, passivation of reactive Mn-oxide surfaces by different processes like adsorption of reduced metal species (e.g. Fe(II), Mn(II)) or bacterial tissue may lower As(III) oxidation rates.^{7,8} Therefore, we investigated the microbial Fe(III) and As(V) reduction by *Shewanella* sp. ANA-3 in the presence of birnessite and followed the As(V) reduction during the course of the experiments by As K-edge X-ray absorption spectroscopy (XAS) at beamline BM01B. These measurements were meant to complement our Mn K-edge XAS data collected at the DUBBLE beamline of ESRF (Exp. ES13) and our Fe K-edge XAS measurements performed at the XAFS beamline at the Elettra Synchrotron facility (Trieste, Italy).

2. Materials and Methods

The kinetics of Fe(III) and As(V) reduction and release were studied in simplified batch systems containing microorganisms and mineral electron acceptors in serum bottles sealed with gas-tight butyl rubber septa. We used *Shewanella* ANA-3 wild type, which is capable of Fe(III), As(V), and Mn(III/IV) reduction. Ferrihydrite (30 mM Fe) coprecipitated with As(V) at a molar Fe/As ratio of 20 was used as potential Fe(III) and As(V) source, while birnessite (0, 1, 3 mM Mn) was used as potential Mn(III/IV) source. After synthesis, the birnessite batch was split and different crystallinities were produced by ageing for 48 h at 105°C (low crystalline, LC) and for 168 h at 140°C in an autoclave (high crystalline, HC), respectively. All incubation experiments were performed at pH 7.2 (20 mM HEPES) in 2 mM NaCl. The initial solutions contained 20 mM lactate and cell densities of 1×10^8 mL⁻¹. A growth medium was avoided to prevent interference of other ions with As sorption to Fe or Mn mineral phases. Over a time course of 400 h, the solutions and solids were continuously sampled in an anoxic glove box. Arsenic solution speciation was performed by HG-AFS and Fe speciation changes were followed by colorimetry using the 1,10-phenanthroline method. Speciation of Fe (Fe(II) vs. Fe(III)) and As (As(III) vs. As(V)) in the solid phase was performed with the mentioned analytical methods after total digestion of unfiltered samples. Samples for the solid-phase speciation of Mn, Fe, and As by XAS were collected by 0.2 µm filtration of the suspensions and subsequent drying of the filter residues in the glove-box atmosphere (N₂).

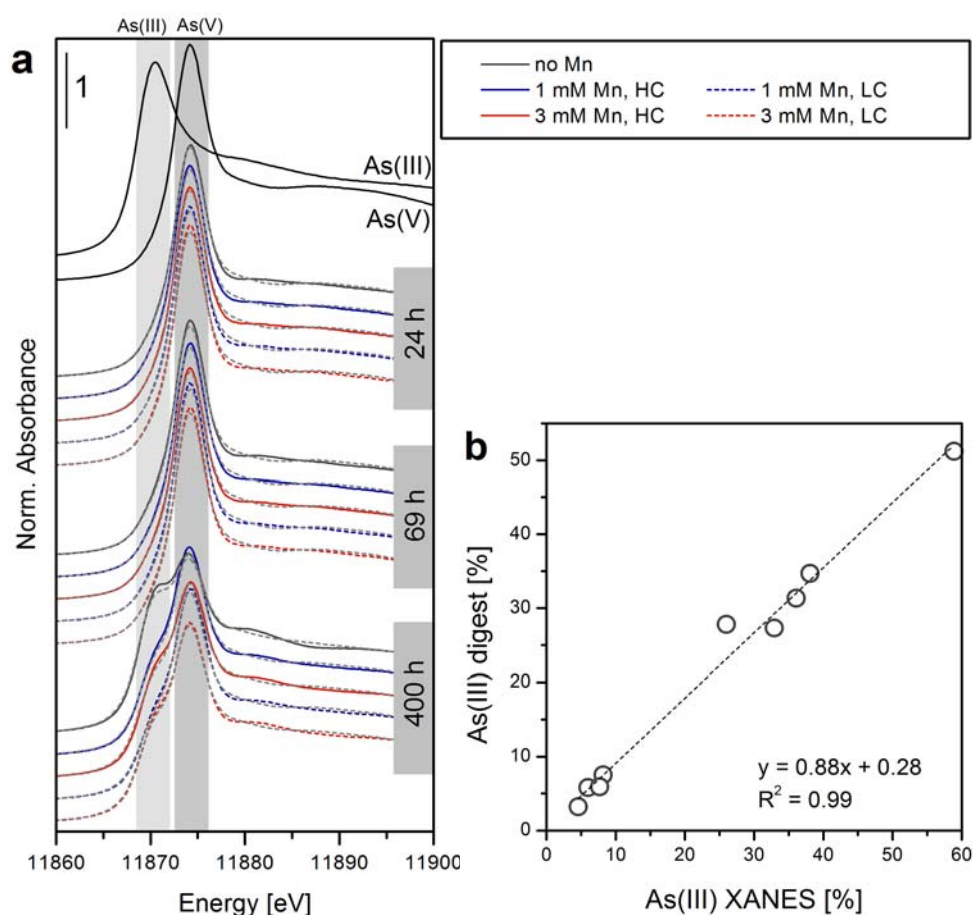


Figure 1: (a) Normalized As K-edge XANES spectra of two As reference compounds (As(III)- and As(V)-sorbed ferrihydrite) as well as sample spectra of the five treatments (HC = high crystallinity, LC= low crystallinity) at times 24 h, 69 h and 400 h of the experiment. Results of LCF of the sample spectra with the reference substances are plotted in grey on top of each spectrum. (b) Correlation between wet chemical As speciation results and LCF results from As XANES spectroscopy.

The dried material was then homogeneized, mixed with LicoWax® and BN, and pressed into pellets that were sealed with Kapton® tape. The 15 resulting samples were kept in N₂ atmosphere until the final XAS measurement. Arsenic reference compounds were similarly prepared (N₂ atmosphere if necessary).

Arsenic K-edge (11.867 eV) X-ray absorption near edge structure (XANES) spectra were recorded at ~86 K (N₂ cryostream) in fluorescent-yield mode for the samples using a 13-element Ge detector (12 active channels). Aluminum foil was placed in front of the detector in order to minimize the Fe fluorescence. The monochromator was detuned to 60 % of maximal intensity and calibrated to the first derivative maximum of the K-edge absorption spectrum of a metallic Au foil (11.919 eV), which was continuously monitored in transmission mode to account for slight energy shifts during the sample measurements. For each sample 1-3 scans were collected and averaged. Spectral processing was done according to standard procedures using the IFEFIT program suite.⁹

3. Results

Arsenic K-edge XANES spectra of the reference compounds As(III)- and As(V)-sorbed ferrihydrite and the samples are displayed in Figure 1. Linear combination fits (LCF) based on these references are plotted in grey on top of each sample spectrum, and Table 1 summarizes the LCF results. In all samples we observed significant reduction of As(V) to As(III) after 400 h, which is consistent with the respiratory reduction of As(V) as an alternative electron acceptor in the course of the incubation experiment. The reduction was most pronounced in the birnessite-free control treatment (no Mn). In this treatment about 1.3-1.5 times more acetate was produced from lactate after 200 h onwards compared to all other treatments with similar acetate trends (data not shown). This effect was accounted for by correcting the As speciation data of the control treatment (Table 1).

Table 1: Arsenic speciation as determined by LCF of the sample spectra with As(III)- and As(V)-sorbed ferrihydrite as fit references (Figure 1a). ^aNormalized sum of the squared residuals (NSSR (%) = $100 \times \Sigma(\text{data}_i - \text{fit}_i)^2 / \Sigma(\text{data}_i)^2$). ^bPercentages of As(III) and As(V) after normalization to the amount of acetate formed.

Treatment	Time [h]	As(III) (%, normalized to a sum of 100)	As(V)	Fitted Sum [%]	NSSR ^a [%]
1 mM Mn, LC	0	0	100	100	0
	24	3	97	101	0.04
	69	6	94	101	0.03
	400	36	64	101	0.03
3 mM Mn, LC	0	0	100	100	0
	24	4	96	101	0.04
	69	5	95	101	0.04
	400	26	74	101	0.04
1 mM Mn, HC	0	0	100	100	0
	24	5	95	100	0.03
	69	8	92	100	0.03
	400	38	62	101	0.04
3 mM Mn, HC	0	0	100	100	0
	24	5	95	100	0.03
	69	6	94	101	0.03
	400	33	68	102	0.05
No Mn	0	0	100	100	0
	24	5	95	100	0.03
	69	8	92	101	0.05
	400	58 (39) ^b	42 (61) ^b	102	0.06

After correction, the average effect of birnessite addition was a decrease in As(III) formation by $2\pm 1\%$ and $10\pm 5\%$ in the 1 mM Mn and 3 mM Mn treatments, respectively. There was no significant difference between the As(III) accumulation in treatments with different birnessite crystallinities. Figure 1b shows the relationship between As speciation results obtained by HG-AFS after acid solid-phase digestion and XAS for samples with comparatively low Mn concentrations. The good linear correlation ($R^2 = 0.99$) even for very low As(III) fractions indicates that both methods yield comparable results, and that As speciation data obtained by wet chemical analyses were unbiased from $t = 69$ h onwards. However, our XAS results also revealed that in the high-Mn treatments residual Mn(III/V) significantly biased the wet-chemical speciation results for As due to As(III) re-oxidation in the acid digests. This finding testifies to the need for synchrotron radiation in order to accurately determine the solid-phase speciation of redox-sensitive elements. In summary, our experiment at BM01B has been successful and completed our data set, which is currently in preparation for publication.

4. References

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