



	Experiment title: Role of birnessite in the dissimilatory arsenic and iron reduction by <i>Shewanella</i> sp. ANA-3: Speciation of manganese	Experiment number: ES-13
Beamline: BM26	Date of experiment: From 28.03. to 02.04.2013	Date of report: 28 August 2013
Shifts: 15	Local contact(s): Sergey Nikitenko, Dipanjan Banerjee	<i>Received at ESRF:</i>
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1. Introduction

Elevated concentrations of As in natural waters, soils, and sediments represent a major 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.^{1,2} Elevated As solution concentrations observed in reducing natural systems are usually accompanied by microbially mediated As(V) and Fe(III) reduction.³ A strong correlation has been recognized between reductively dissolved Fe and As released,³ 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 reduction to As(III).⁴

Manganese oxides are ubiquitous in soils and sediments.⁵ They are strong oxidants and have been shown to naturally attenuate As contamination in aquifers.^{6,7} 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.⁸ 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 surface sites by different processes like sorption of oxidized species, presence of other reduced species (e.g. Fe(II), Mn(II)) or bacterial tissue may lower As(III) oxidation rates.^{9,10} Here, we investigated the microbial Fe(III) and As(V) reduction by *Shewanella* sp. ANA-3 in the presence of birnessite. In these experiments we varied the concentration and crystallinity of birnessite and followed the Mn(III/IV) reduction during the course of the experiments by Mn K-edge X-ray absorption spectroscopy (XAS) at beamline BM26. These measurements complement our As K-edge XAS data to be collected at beamline BM01 of ESRF (Exp. 01-01-899) 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(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. Total element concentrations in the solutions and digests were measured with ICP-OES. Samples for the solid-phase speciation of Mn, Fe, and As by XAS were collected by 0.2 μ m filtration of the suspensions and subsequent drying of the filter residues in the glove-box atmosphere (N₂). The dried material was then homogenized, mixed with LicoWax® and BN, and pressed into pellets that were sealed with Kapton® tape. The 23 resulting samples were kept in N₂ atmosphere until the final XAS measurement. Manganese reference compounds were similarly prepared, and in N₂ atmosphere if necessary.

Manganese K-edge (6,539 eV) X-ray absorption near edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) spectra were recorded at ~20 K (He(I) cryostat) in fluorescent-yield mode for the samples using a 13-element Ge detector, and in transmission mode for most reference compounds. The monochromator was calibrated to the first maximum in the first derivative of the K-edge absorption spectrum of a metallic Mn foil (6,539 eV), which was continuously monitored to account for slight energy shifts during the sample measurements. For each sample 3-8 scans were averaged. Spectral processing was done according to standard procedures using the IFFFIT program suite.¹¹ Fourier transforms of the $\chi(k)$ data were calculated over a k -range of 2-12 Å⁻¹ using a Kaiser-Bessel apodization window with a sill width of 3 Å⁻¹.

3. Results

Figure 1 shows Mn K-edge XANES (A) and the corresponding k^3 -weighted EXAFS spectra (B) of Mn reference compounds. Due to the proximity of the Mn K-edge (6,539 eV) to the Fe K-edge position (7,112 eV), Fe containing samples could only be recorded up to $k = 11.2$ Å⁻¹. The quality of the spectra collected in transmission mode was reasonably good and 3 scans per sample resulted in an acceptable signal-to-noise ratio. Figure 2 illustrates the samples' XANES (A) and k^3 weighted EXAFS spectra (B). The birnessite spectra were found to be consistent with Na-birnessite.¹² The XANES region, which is especially sensitive to changes in the Mn oxidation state, was fitted using the combo method.¹³ Table 1 lists the amount of Mn(IV) present in the samples during the course of microbial Mn reduction as inferred from linear combination fitting. For both birnessites, we obtained 88% Mn(IV) at the start of the experiment, a result which will be further verified by wet chemical methods.¹⁴ However, the Fourier-transformed EXAFS spectra of both birnessites revealed a clear difference in the peak height of the Mn-O and Mn-Mn pair correlations (Figure 3). The loss of amplitude with increasing crystallinity can be explained by the effects of the Jahn-Teller distortion as discussed in more detail by Webb et al.¹⁵ We will further explore the structural differences between both birnessites using shell fitting and Rietveld refinements of synchrotron X-ray diffraction data.

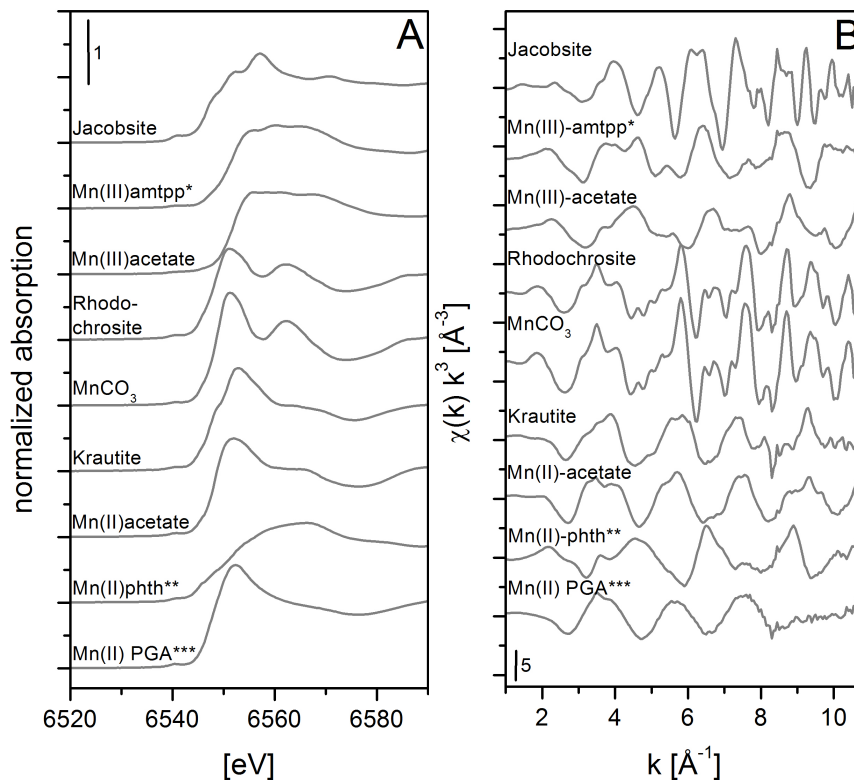


Figure 1: (A) Normalized Mn K-edge XANES spectra of Mn reference compounds, and (B) their k^3 -weighted Mn K-edge EXAFS spectra. *Mn(III)-acetate meso-tetraphenylporphine, **Mn(II)-phthalocyanine, ***Mn(II) sorbed to polygalacturonic acid. Spectra of jacobsonite and natural rhodochrosite were truncated in order to exclude the Fe K-edge.

The percentages of reduced Mn(IV) in the incubated samples indicate that the LC birnessite is reduced faster than the HC birnessite (Table 1, data at 69 h). This result is in line with other studies showing lower reduction rates for more crystalline birnessites.^{8,16} In order to infer Mn species formed after birnessite reduction, we are currently performing linear combination fits. So far, these analyses indicate a decreasing goodness of fit with ongoing sample reduction, which implies the complexation of Mn(II) by Fe minerals and/or bacterial tissue.

In addition to the incubation samples, we also measured a bulk soil sample taken from a river bank of the Ogosta River floodplain (Bulgaria) which is highly polluted with As (Figure 2, sample 'OGS_sB'). The soil contains elevated concentrations of Mn with a small molar Mn/Fe ratio of 0.25. Linear combination fitting of the XANES spectrum revealed that about 60% of Mn is present as Mn oxide, while about 40% could be ascribed to rhodochrosite. This information is valuable as the high Mn contents of the floodplain soils are expected to strongly impact the fate of As.

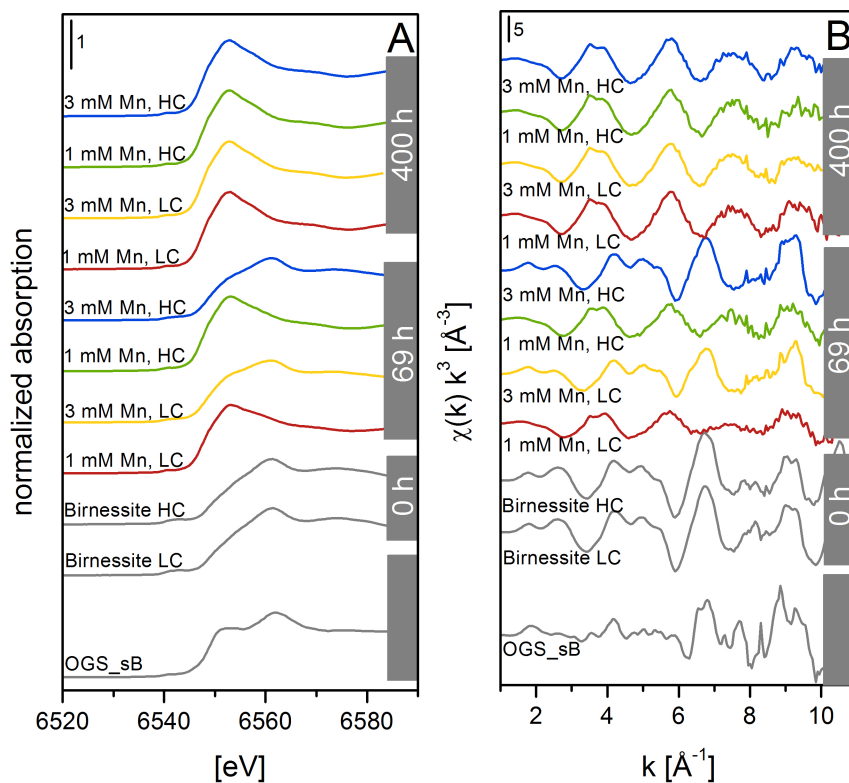


Figure 2: (A) Normalized Mn K-edge XANES spectra of the measured samples as a function of reduction time (LC = low crystalline, HC= high crystalline), and (B) corresponding k^3 -weighted Mn K-edge EXAFS spectra.

Table 1: Percentages of Mn(IV) present in the samples at different incubation times as estimated by linear combination fitting of the Mn K-edge XANES spectra.

Sample	Total Mn(IV) [%]		
	0 h	69 h	397 h
1 mM Mn, LC	88	0	0
3 mM Mn, LC	88	39	0
1 mM Mn, HC	88	0	0
3 mM Mn, HC	88	59	0

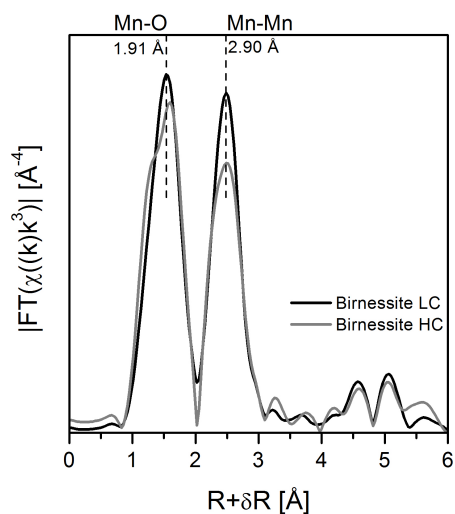


Figure 3: Fourier transformed Mn K-edge EXAFS spectra of the birnessite samples used in the reduction experiments (uncorrected for phase shift).

4. References

1. Smedley, P. L.; Kinniburgh, D. G., A review of the source, behaviour and distribution of arsenic in natural waters. *Appl. Geochem* **2002**, *17*, (5), PII S0883-2927(02)00018-5.
2. Islam, F. S.; Gault, A. G.; Boothman, C.; Polya, D. A.; Charnock, J. M.; Chatterjee, D.; Lloyd, J. R., Role of metal-reducing bacteria in arsenic release from Bengal delta sediments. *Nature* **2004**, *430*, (6995), 68-71.
3. Weber, F.-A.; Hofacker, A. F.; Voegelin, A.; Kretzschmar, R., Temperature Dependence and Coupling of Iron and Arsenic Reduction and Release during Flooding of a Contaminated Soil. *Environ. Sci. Technol.* **2010**, *44*, (1), 116-122.
4. Campbell, K. M.; Malasarn, D.; Saltikov, C. W.; Newman, D. K.; Hering, J. G., Simultaneous microbial reduction of iron(III) and arsenic(V) in suspensions of hydrous ferric oxide. *Environ. Sci. Technol.* **2006**, *40*, (19), 5950-5955.
5. Post, J. E., Manganese oxide minerals: Crystal structures and economic and environmental significance. *Proc. Nat. Acad. Sci. U.S.A.* **1999**, *96*, (7), Natl Acad Sci.
6. Oscarson, D. W.; Huang, P. M.; Defosse, C.; Herbillon, A., Oxidative Power of Mn(IV) and Fe(III) Oxides With Respect To As(III) In Terrestrial and Aquatic Environments. *Nature* **1981**, *291*, (5810), 50-51.
7. Hasan, M. A.; Ahmed, K. M.; Sracek, O.; Bhattacharya, P.; Von Broemssen, M.; Broms, S.; Fogelstroem, J.; Mazumder, M. L.; Jacks, G., Arsenic in shallow groundwater of Bangladesh: investigations from three different physiographic settings. *Hydrogeol. J.* **2007**, *15*, (8), 1507-1522.
8. 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.
9. Parikh, S. J.; Lafeerty, 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.
10. Ying, S. C.; Masue-Slowey, Y.; Kocar, B. D.; Griffis, S. D.; Webb, S.; Marcus, M. A.; Francis, C. A.; Fendorf, S., Distributed microbially- and chemically-mediated redox processes controlling arsenic dynamics within Mn-/Fe-oxide constructed aggregates. *Geochim. Cosmochim. Acta* **2013**, *104*, 29-41.
11. Ravel, B.; Newville, M., ATHENA, ARTEMIS, HEPHAESTUS: data analysis for X-ray absorption spectroscopy using IFEFFIT. *J. Synchrot. Radiat.* **2005**, *12*, 537-541.
12. Friedl, G.; Wehrli, B.; Manceau, A., Solid phases in the cycling of manganese in eutrophic lakes: New insights from EXAFS spectroscopy. *Geochim. Cosmochim. Acta* **1997**, *61*, (2), 275-290.
13. Manceau, A.; Marcus, M. A.; Grangeon, S., Determination of Mn valence states in mixed-valent manganates by XANES spectroscopy. *Am. Mineral.* **2012**, *97*, (5-6), 816-827.
14. Carpenter, J. A. M. E. S. H., The accuracy of the Winkler method for dissolved oxygen analysis. *Limnol. Oceanogr.* **1965**, *10*, ((1)), 135-140.
15. Webb, S. M.; Tebo, B. M.; Bargat, J. R., Structural characterization of biogenic Mn oxides produced in seawater by the marine bacillus sp strain SG-1. *Am. Mineral.* **2005**, *90*, (8-9), 1342-1357.
16. 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.