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Spectromicroscopy of Mn distributions in micronodules produced by biomineralization

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The interaction of single cell microorganisms with inorganic substrates is a central issue in soil and aquatic environmental chemistry. The biochemical pathways of the bacterial metabolism based on redox reactions of transition metal compounds involve the processes of microbial attachment to and subsequent modification of inorganic surfaces (biocorrosion), as well as the precipitation of inorganic compounds (biomineralization). Soft x-ray spectromicroscopy has been used to investigate Mn micronodules collected from freshwater sediments in order to attempt the direct mapping of the distribution of Mn valence states in biologically produced minerals.

Keywords: soft x-ray spectromicroscopy, bacterial metabolism, Mn micronodules.

1. Introduction

Strains of sediment bacteria have been discovered which oxidize Mn or Fe into higher valence states, whereas others will reduce the oxides as part of their energy transport cycle (Chapnick 1982, Nealson 1988). Certain basic facts regarding the reaction products formed by bacteria that metabolize Mn and Fe are still not fully understood. These issues are important for the investigation of biocorrosion and biomineralization phenomena as well as of possible benefits of microbial mineralization products used in bioremediation efforts.

An inherent characteristic of the metabolic activity of bacteria is the fact that the chemical processes involved are spatially inhomogeneous, thus close proximity of different charge states of the transition metals has to be expected. There are different chemical methods to quantify the average oxidation state of solid precipitates formed by biotic or abiotic transformation of solved Mn^{2+} present in lake- or seawater, e.g. iodometry. However, bulk chemical methods are unable to distinguish e.g. between Mn^{3+} and a mixture of Mn^{2+} and Mn^{4+} (Murray 1985). The O/Mn ratio in Mn containing micronodules has been found to vary between 1.4 and 2 depending on sampling sites, generally falling in the range between 1.9 an 2 (Murray 1984). Thus in natural samples low valence states of Mn seem to be present only in trace amounts.

Soft x-ray spectromicroscopy is an ideal tool for the investigation of bacterial reaction products. It offers the spatial resolution required to show micronodules with particle sizes ranging from submicron to several microns. Using scanning or full field imaging transmission techniques, samples can be investigated in their naturally hydrated state. Additionally, by means of the variation of the wavelength (energy) of the incident radiation, the absorption contrast may be adjusted to reveal the spatial distribution of a certain element and/or its valence state in the sample. Finally, within the water window direct imaging of the microorganisms is possible, giving structures of the attachment of the bacteria (e.g. direct contact of microorganisms to mineral sufaces, specific attachment sites etc.).

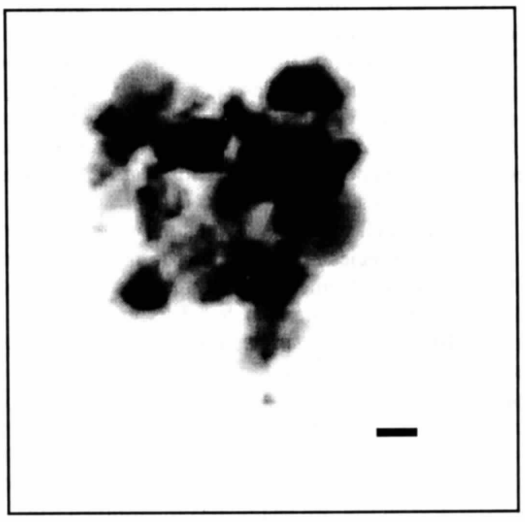

Figure 1: STXM image (10 μm × 10 μm) of Mn micronodules - the bar marks 1 μm.

This report presents the first results obtained for natural Mn nodules collected from Green Bay (Lake Michigan) sediment samples using high resolution soft x-ray spectromicroscopy at the Advanced Light Source (ALS). The first row transition metal L absorption edges, due to 2p→3d core transitions, expose a rich fine structure which allows to differentiate between several valence states of the metal ion (de Groot 1990, van der Laan 1992). Without spacial resolution, these studies have been seriously limited. This is clearly demonstrated by the comparison of the microspectroscopic transmission measurements (STXM) to spatially averaged total electron yield measurements (TEY, see Fig. 2).

2. Results and Discussion

A: Scanning Transmission Microscopy. Fig. 1 shows a 10 μm × 10 μm image from a sample prepared as a thin liquid film of a suspension containing micronodules which was sandwiched between two Si_3N_4 membranes. The image has been taken at the STXM (Scanning Transmission X-ray Microscope, Warwick 1998) endstation at beamline 7.0.1 with a spatial resolution of about 0.2 μm. As generally observed for these samples, the microparticles tend to form irregularly shaped agglomerates of varying density which occur in a wide range of sizes. Mn $L_{2,3}$ x-ray absorption spectra recorded at two different locations inside the agglomerate together with spectra of reference compounds are shown in Fig. 2. All absorption curves are normalized to maximum intensity. These spectra are of special importance since they prove that the chemical composition of the particles varies on a microscopic scale. The comparison of the nodule spectra to those derived from reference samples taken in TEY mode ($Mn(IV)O_2$, $Mn(III)_2O_3$ and

Mn(II)SO$_4$), which contain Mn in tetra-, tri- and divalent state, respectively, clearly exhibits the coexistence of Mn^{2+} (curve a) and Mn^{4+} (curve e). Additionally, in Fig. 2 the corresponding Mn $L_{2,3}$ x-ray absorption of the nodules taken in total electron yield technique is shown (curve f). The spatially averaged measurement of the Mn nodules turns out to expose a fine structure, which can be immediately explained as the superposition of the 2+ and the 4+ Mn spectral features.

The biochemical transformation of the divalent Mn into insoluble Mn^{4+} species is expected to occur via Mn^{3+} as an intermediate. However, from our measurements we did not get any evidence for the Mn^{3+} state so far. This may indicate that Mn^{3+} species exist only on the surface, thus being invisible to the bulk sensitive transmission measurements, or immediately disproportionate into Mn^{2+}/Mn^{4+} under the environmental conditions of the formation of the nodules (Murray 1984). All of the nodules we have looked at contained detectable amounts of divalent Mn species. As our measurements are bulk sensitive, i.e. the radiation penetrates several μm, we can disregard adsorbed Mn^{2+} species as an explanation for the detected pattern of divalent Mn. Bulk Mn^{2+} is expected to be present, e.g. as carbonate, due to chemical precipitation caused by bacterial activity (Nealson 1997). However, we have found no evidence for a regular distribution of the reduced Mn (e.g. some kind of core-shell structure), which rather seems to prevail in certain patches randomly spread over the precipitates.

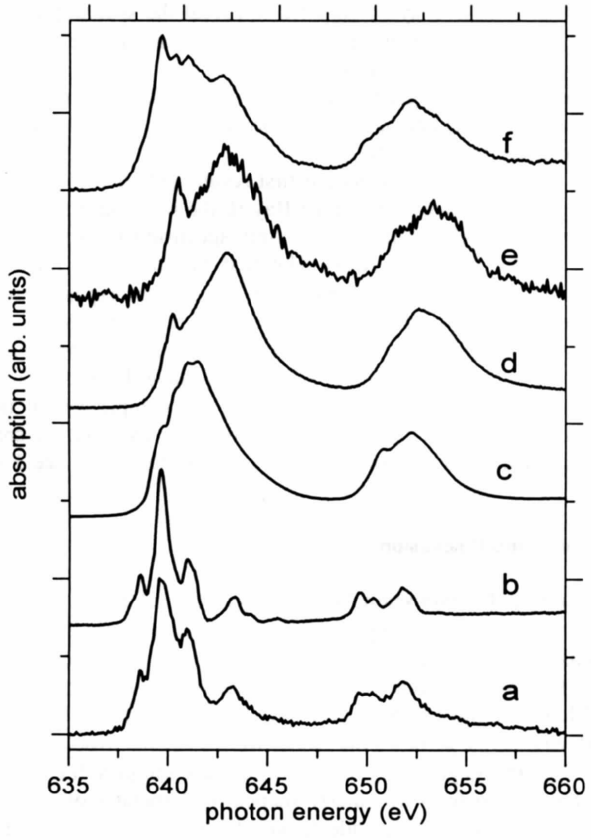

Figure 2: Mn $L_{2,3}$ XANES of micronodules (a, e, diameter of analysis area ≈200 nm) and reference compounds - b: *Mn(II)SO$_4$*, c: *Mn(III)$_2$O$_3$*, d: *Mn(IV)O$_2$*, - f: TEY measurement of nodules (analysis area ≈1×1 mm^2).

B: Full Field Imaging Transmission Microscopy. Additionally, high resolution soft x-ray full field imaging has been performed at the XM-1 endstation at beamline 6.1.2 (Meyer-Ilse 1995). The differential energy contrast at the Mn L_3 absorption edge has been found sufficient to clearly reveal the Mn distribution in a variety of agglomerates of micronodules. For the investigation of precipitates in their naturally aqueous environments, 2 μl of the suspensions containing the micronodules have been sandwiched between two Si_3N_4 membranes. All samples have been checked by optical DIC (Differential Interference Contrast) microscopy prior to x-ray imaging.

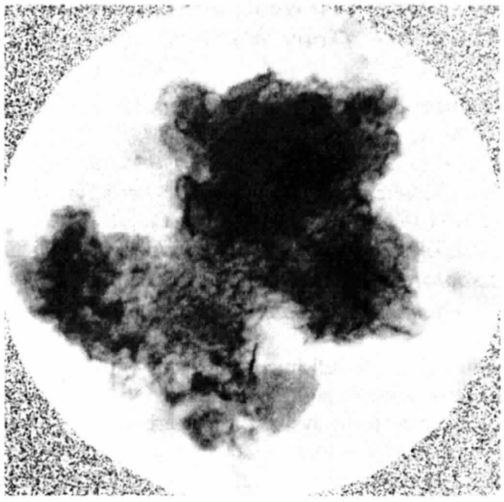

Figure 3: XM-1 image of micronodules (normalized), M=2400×.

A typical image of a precipitate as seen by XM-1 is shown in Fig. 3. Here the field of view covers again an area of about 10 μm in diameter. The image was taken from a dry sediment sample below the Mn L_3 absorption edge (1.97 nm corresponding to 630 eV) at a magnification of 2400×.

It has been normalized by division through a background image taken at the same wavelength at an area of the Si_3N_4 membrane free of precipitates. Although the particles generally tend to

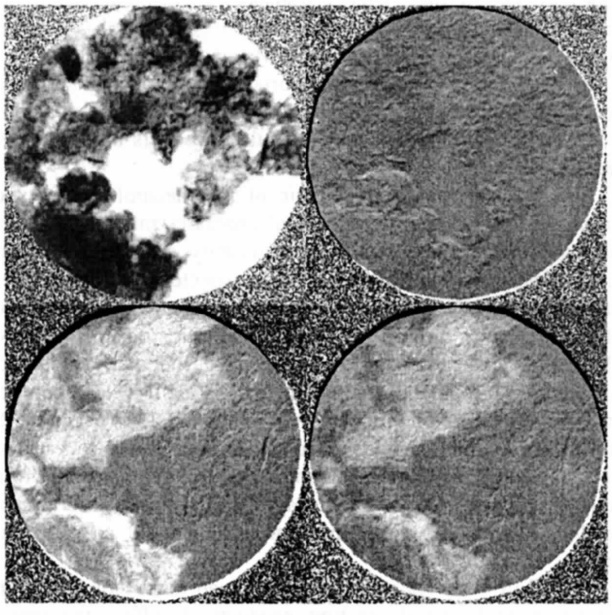

Figure 4: Differential energy contrast imaging of Mn nodules (M= 2400×) – top left: 629 eV, right: 629/636, bottom left: 629/639, right: 629/642.

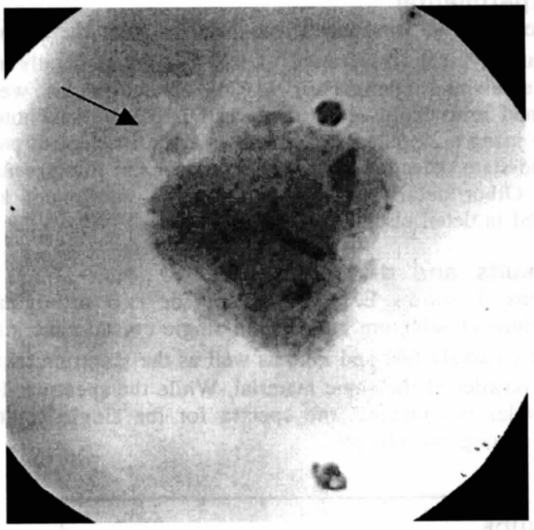

Figure 5: XM-1 wet cell image of micronodule (M=2400×) with attached bacterium (arrow).

form agglomerates of various sizes and shapes, the increase in spacial resolution given by XM-1 (compare to STXM image in Fig. 1) reveals a network of needle-shaped structures, which turned out to be a common characteristic of these particles.

In Fig. 4 a sequence of images taken at four different wavelengths in the vicinity of the Mn L_3 absorption edge is presented. By normalizing with respect to the low energy image (calculation of I_0/I), the Mn distribution appears as bright area in these images. As expected from a Mn^{2+}-sulfate reference measurement, there is no contrast at 636 eV (below the L_3 edge), maximum contrast at 639 eV (on top of the white line) and diminishing contrast at 642 eV. These images can be seen as a clear visualization of the inhomogeneous action of the Mn reducing bacteria. In all agglomerates, which have been investigated by this technique, the Mn distribution appears to be concentrated in diffuse zones surrounding larger, more regularly shaped particles. Here the function of sand particles as anchors for the bacterial attachment and subsequent precipitation of Mn oxides may be hypothesized.

Finally, Fig. 5 shows a wet-cell image of a micronodule taken at 516 eV inside the water window. The exposure time has been 6.4 sec. The arrow points to a bacterium of the size of about 1 μm - even revealing some internal structure (Krause 1996) - which is still attached to the particle.

3. Conclusions and Outlook

Scanning transmission spectromicroscopy and full field differential energy contrast imaging in the vicinity of the Mn L absorption edges have been used to reveal the distribution of Mn and Mn valence states in inorganic precipitates produced by *S. putrefaciens*. It has been demonstrated that the spatial resolution of the x-ray transmission microscope STXM at the Advanced Light Source allows to differentiate between two species of the transition metal ions, which are not homogeneously distributed throughout the agglomerates of microparticles. This can be seen as an important step towards the desired understanding of localized chemical processes induced by single cell microorganisms. Rather than recording absorption spectra at a fixed sample position ('pixel-mode'), a new technique is presently implemented which extracts these spectra from an array of images taken as a function of the incident photon energy ('stack-mode'). This technique will allow us to create more precise oxidation state maps of the entire particles.

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