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Application of Synchrotron μ-XRF-XAFS to the Speciation of Fe on a Single Stalk in Bacteriogenic Iron Oxides (BIOS)

Sakiko Kikuchi,*[1] Hiroko Makita,[2] Satoshi Mitsunobu,[3] Yasuko Terada,[4] Noriko Yamaguchi,[5] Ken Takai,[2] and Yoshio Takahashi[1]

[1]*Graduate School of Science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8526*
[2]*JAMSTEC, 2-15 Natsushima-cho, Yokosuka, Kanagawa 237-0061*
[3]*University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526*
[4]*SPring-8 JASRI, 1-1-1 Koto, Sayo-gun, Hyogo 679-5189*
[5]*National Institute for Agro-Environmental Science, 3-1-3 Kannondai, Tsukuba, Ibaraki 305-8604*

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Speciation of iron (Fe) precipitated on bacterial stalks was studied by Fe K-edge μ-XRF-XAFS. Micro-XRF analysis of the stalk samples showed that Fe is precipitated around the stalk. All the μ-XANES spectra at several Fe-accumulated parts showed similar spectra. This study indicated that synchrotron μ-XRF-XAFS is a powerful tool for chemical speciation of metals at single-cell level for bacteria bearing metal.

Bacteriogenic iron oxides (BIOS) are widely known as a form of iron (Fe) oxide in the natural environment observed as Fe oxide precipitates around bacterial-induced extracellular polysaccharide (stalk and sheath).[1] Since Fe oxides are important host phases of trace elements through adsorption, the adsorption behavior of natural BIOS has been of great interest in aqueous environmental chemistry.[1–3] It has been suggested that intermixed Fe oxides and organic matter exhibit unique and complex adsorption properties of BIOS. For example, laboratory adsorption experiments suggest that uranium adsorption onto BIOS shows different adsorption behavior than synthesized Fe oxides.[4] Thus, characterization of Fe species in BIOS is important to understand its role as adsorbents in natural environments. It has been reported that poorly ordered Fe oxide (e.g., ferrihydrite) is the dominant Fe species of BIOS by X-ray diffraction (XRD) and high-resolution transmission electron microscopy (HR-TEM).[1,2] Although HR-TEM combined with electron diffraction analysis is one of the most effective tools to study mineralogy particularly at nanoscale, poorly ordered Fe oxide only gives broad diffraction patterns which cannot indicate the mineral phase precisely. Since BIOS is usually attached to stalks (or sheaths) the size of which is micrometer scale, point by point analysis at micrometer scale will provide better insight into Fe-oxide biomineralization at different locations of the stalk.

Here, we examined the Fe species of BIOS by micro Fe K-edge X-ray absorption fine structure (μ-XAFS). The distribution of Fe was analyzed by micro X-ray fluorescence (μ-XRF) spectrometry. After the μ-XRF imaging, the mineral species of the Fe-accumulated area on the stalk were investigated by micro X-ray absorption near edge structure (μ-XANES) at the Fe K-edge.

BIOS analyzed in this study was produced by an Fe-oxidizing bacterium *Mariprofundus ferroxydans*.[5] It is the only isolated chemolithoautotrophic Fe-oxidizing bacteria widely observed in Fe-rich hydrothermal sediment and plays predominant roles in Fe and carbon cycles. *M. ferroxydans* was cultured in artificial seawater containing trace minerals and vitamins.[6] Iron monosulfide (FeS) was applied as an energy source for the bacterial growth. About 24 h after the beginning of culture at 298 K, fluffy BIOS were precipitated in the bottom of the bottle. The suspension containing BIOS was pipetted onto formvar-coated copper grids designed for TEM analysis and air-dried for μ-XRF-XAFS analysis.

Micro-XRF and Fe K-edge μ-XANES spectra were collected at BL37XU in SPring-8, Japan. The beamline consisted of a Si(111) double crystal monochromator with a Kirkpatric-Baez mirror to obtain a $1.5 \times 1.6\,\mu m^2$ X-ray beam at the sample. Spectra of BIOS samples were collected in fluorescence mode using a silicon drift detector. Reference spectra were collected in transmission mode measured either at BL37XU or at BL01B1. The Fe XANES collected at both beamlines suggested that the energy resolutions of the spectra were identical between the two beamlines, showing that we can compare the spectra. Measurements in BL37XU were conducted with an energy step of 0.5 eV and exposure time of 2 or 5 s. Mapping of Fe by μ-XRF was carried out at irradiation energy at 8 keV. The step size was set to be 1 μm both in x and y directions. The measurement time of each pixel was 0.1 s.

Figures 1A and 1B show scanning electron microscopy (SEM) images of the stalk. Helical twisted stalks are thought to be typical products of *M. ferroxydans*.[7] Figures 1C and 1D show the distribution of Fe in the stalk structure. It is revealed that Fe is distributed broadly around the stalk, whereas the density of existing Fe is highly different in the location of the stalk at a microscale (Figure 1D). According to a previous study, Fe-oxidizing bacteria are capable of localizing Fe mineral outside the cells to avoid encrustation of the cell by Fe oxides.[7] The distribution of Fe by μ-XRF was spatially associated with the stalk structure visualized by SEM, which indicates that μ-XRF imaging was successfully conducted at single stalk-level resolution.

Figure 2 shows Fe K-edge XANES spectra at four Fe concentrated spots represented by the number shown in Figures 1A and 1B. Spectra obtained from different points were approximately identical. The adsorption edge was around 7.13 keV (dashed line in Figure 2), similar to that of ferrihydrite. Least-squares fitting of the XANES spectra showed that inclusion of Fe carboxylate species in the fitting in addition to ferrihydrite results in the decrease of R value (a parameter of the goodness of fit, see Supporting Information (SI) for more details[13]) from 0.00110 to 0.00016 in spectrum1 in Figure 2. This indicates that the Fe species is not simple ferrihydrite but consists of

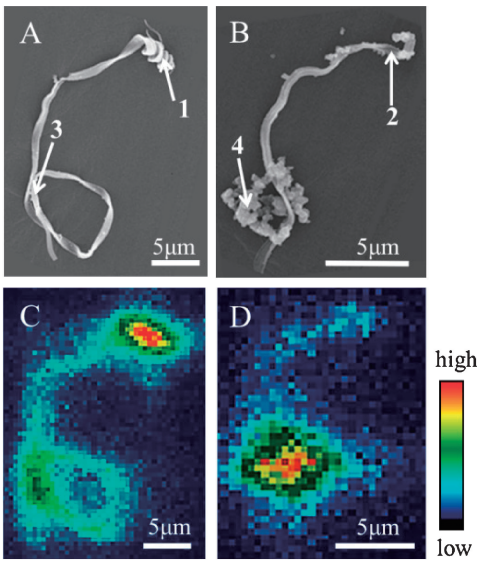

Figure 1. SEM and μ-XRF images of samples. (A), (B) SEM images of stalk structure in BIOS. The numbers show the measured points by μ-XANES (C), (D) μ-XRF images of Fe. X-ray beam size, $1.5 \times 1.6\,\mu m^2$; Step size, 1 μm; Measurement time, 0.1 s/point.

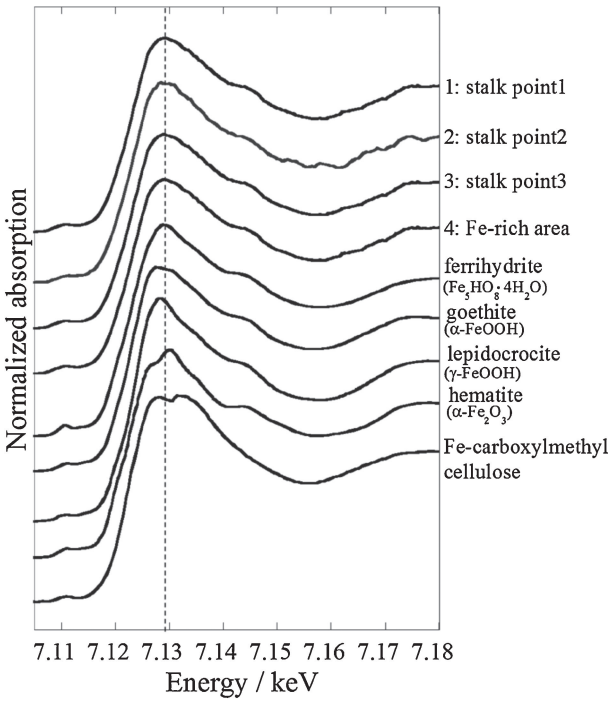

Figure 2. Normalized Fe K-edge μ-XANES spectra of samples and reference minerals. The numbers indicate the measurement points as shown in Figure 1. The vertical dashed lines indicate the adsorption edge of samples.

ferrihydrite and Fe carboxylate species (ferrihydrite: 74%; Fe carboxylate species: 26%; Table S1 and Figure S1 in SI[13]).

In previous studies, various analytical tools were used to study mineralogy of BIOS, which include TEM, scanning transmission X-ray microscopy (STXM), and photoelectron emission microscopy (PEEM).[1,2,7–9] Although TEM has proved to be an effective analytical tool to study mineralogy at a nanometer scale, it has difficulty in the diffraction analysis of Fe species if they are amorphous forms, for which diffraction does not exhibit clear patterns. Furthermore, TEM cannot be employed for electron beam-sensitive materials.[8,10] On the contrary, as a nondestructive measurement technique, Fe L-edge XANES analysis has been conducted by STXM and PEEM.[7–9] However, it is difficult to distinguish various Fe oxides such as ferrihydrite and lepidocrocite by these method, since the spectra are quite similar. In addition, PEEM measurement is performed under ultrahigh vacuum (10^{-10} Torr), which may lead to the alteration of Fe species during the measurement. Thus, all these measurements discussed above have some disadvantages.

In this study, we have successfully applied μ-XRF-XAFS to examine the chemical speciation of Fe at single stalk resolution. Iron K-edge is a valuable tool to identify various Fe species.[11] As shown in Figure 2, XANES spectra can distinguish each Fe species by their peak edges and spectra shapes, regardless of the crystallinity of Fe oxides. There is a report applying μ-XAFS for the analysis of biofilm.[12] However, the measurement was conducted using a $16 \times 7\,\mu m^2$ X-ray beam which only gives bulk information. In this study, XANES measurements were conducted with a $1.5 \times 1.6\,\mu m^2$ beam. The resolution is high enough for the Fe speciation at various points in BIOS.

To our knowledge, this study is the first attempt to apply μ-XRF-XAFS measurement for the chemical speciation in biominerals at single stalk level. It was demonstrated that μ-XANES at Fe K-edge could be a new analytical method to identify Fe species in BIOS, which can contribute to better understanding of the formation process of BIOS. In addition, μ-XRF-XAFS can be further applied to the speciation of trace elements adsorbed and/or incorporated in BIOS, which will provide a great insight into the significant role of BIOS in the migration of trace elements in natural waters.

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13 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.

