Structures of Bacterially Produced Manganese Oxides

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Introduction

Manganese oxides act as catalysts, reactants and products in a suite of sorption and electron transfer reactions that affect the fate, transport and transformation of metals and organic compounds in the natural environment. The oxidation of humic substances to low molecular weight organics [1], the scavenging of trace metals by manganese nodules [2], and the oxidation of As(III), Co(II), Cr(III), and Pu(III) [3] are three examples of these processes. The catalytic redox properties of manganese oxides have also been used in industry (e.g., rechargeable batteries), while new catalytic activities of manganese oxides have been reported recently, including: ozone decomposition [4], low temperature carbon monoxide oxidation [5], alkane functionalization [6], and combustion of C3 hydrocarbons and oxygenates [7].

Over the past few years, there has been a growing interest in expanding and exploring the potential use of microbial processes in engineered systems. The use of biocatalysts has increased the selectivity, reduced the cost, and improved the environmental friendliness of several industrial catalytic processes [8] in the areas of chemicals manufacturing (biocides, polymers, agrochemicals) and processing operations (detergents, food and feed processing). The diversity of both microbes and of the chemical reactions that they perform is remarkable, and it is not surprising that several species of bacteria are able to catalyze the oxidation of manganese. In fact, the formation of manganese oxides in aquatic environments has been recognized for a long time to be controlled by the catalytic activity of various microbes [9]. It is then tempting to speculate that biogenic manganese oxides may have different and untapped reactivities compared to manganese oxides synthesized inorganically.

Although the structural characteristics of several manganese oxides minerals have been studied in details, our understanding of biogenic manganese oxides is far from complete. Characterization of biogenic manganese oxides by traditional techniques (*e.g.*, X-ray or electron diffraction analyses) has been difficult since they often correspond to amorphous structures. Therefore, some important questions remain to be answered: 1. Are biogenic manganese oxides structurally different from their inorganic counterparts? 2. How do they differ amongst themselves? 3. What key variables control their structure and reactivity?

In this work we used X-ray absorption spectroscopy, in addition to electron microscopy, to probe the structures of manganese oxides produced by two manganese-oxidizing bacteria grown under different chemical conditions.

Methods and Materials

Leptothrix discophora SP-6 (ATCC 51168, originally isolated from an artificial iron seep) and one isolate from a Green Bay manganese nodule (Isolate #7) were grown on 0.2 μm polycarbonate membrane filters on top of solid MSVP medium (ATCC Culture Medium 1917). The composition per liter of the growth media was 240 mg $(NH_4)_2SO_4$, 60 mg $MgSO_4 \cdot 7H_2O$, 60 mg $CaCl_2 \cdot 2H_2O$, 20 mg KH_2PO_4, 30 mg Na_2HPO_4, 2.383g HEPES, 15 g Agar Noble, 5 ml 20% sodium pyruvate, and 1 mL 10 mM $FeSO_4$. pH was adjusted to 7.2 with NaOH and $MnSO_4$ was added to give 50 μM Mn(II). The cultures were incubated aerobically at room temperature and the membranes were collected after a noticeable amount of manganese oxides was formed. Two variations in the growth conditions of SP-6 were also tested. The first excluded iron (*i.e.*, $FeSO_4$ was omitted) from the growth media and the second used liquid media instead of agar media. For the liquid culture, the manganese oxides were collected on the membrane by filtration.

The time evolution of the manganese oxidation process was followed for the liquid culture of SP-6 grown in MSVP with no $FeSO_4$. The oxidation process started when 50 μM Mn(II) (as $MnSO_4$) was added to a stationary-phase liquid culture of SP-6 grown without manganese added. Samples were collected from 10 minutes up to three days after the addition of Mn(II).

Each membrane sample taken during these experiments was supported on Kapton© tape and stored in liquid nitrogen until exposure under the photon beam. XAS measurements were carried out at the DuPont-Northwestern-Dow CAT, Advanced Photon Source, Argonne National Laboratory. In order to detect and minimize the possibility of beam damage to the sample, that was observed during our first experiments, we carried all our scans in the Quick-XAS mode (described elsewhere [10]). The incident and transmitted intensities were measured with ion chambers. The fluorescence intensity was measured with a Lytle cell using a Z-1 (Cr) filter. The XAS spectra of various manganese mineral standards were also collected under the same conditions for comparison against the biogenic oxides.

Results

After 3 weeks the cultures grown on agar showed brownish bacterial colonies, which suggested the presence of manganese oxides. *Leptothrix discophora* SP-6 showed the fastest production of manganese oxides (less than one week).

Figure 1 presents the XANES and radial distribution functions of the biological manganese oxides. The data for a Mn(II) species, $MnCl_2$, and a Mn(IV) species, psilomelane, are also plotted as reference.

The spectra in Figure 1a reveal different coordination environments of manganese for the different biogenic manganese oxides. Figure 1a shows that the edges for these oxides are associated to an average redox state that is comprised between II and IV. When iron is added to the growth media, the manganese edge moves closer to a redox state of II, whereas the edge is shifted toward a redox state of IV when iron is absent form the growth media. In the latter, the XANES follows that of psilomelane

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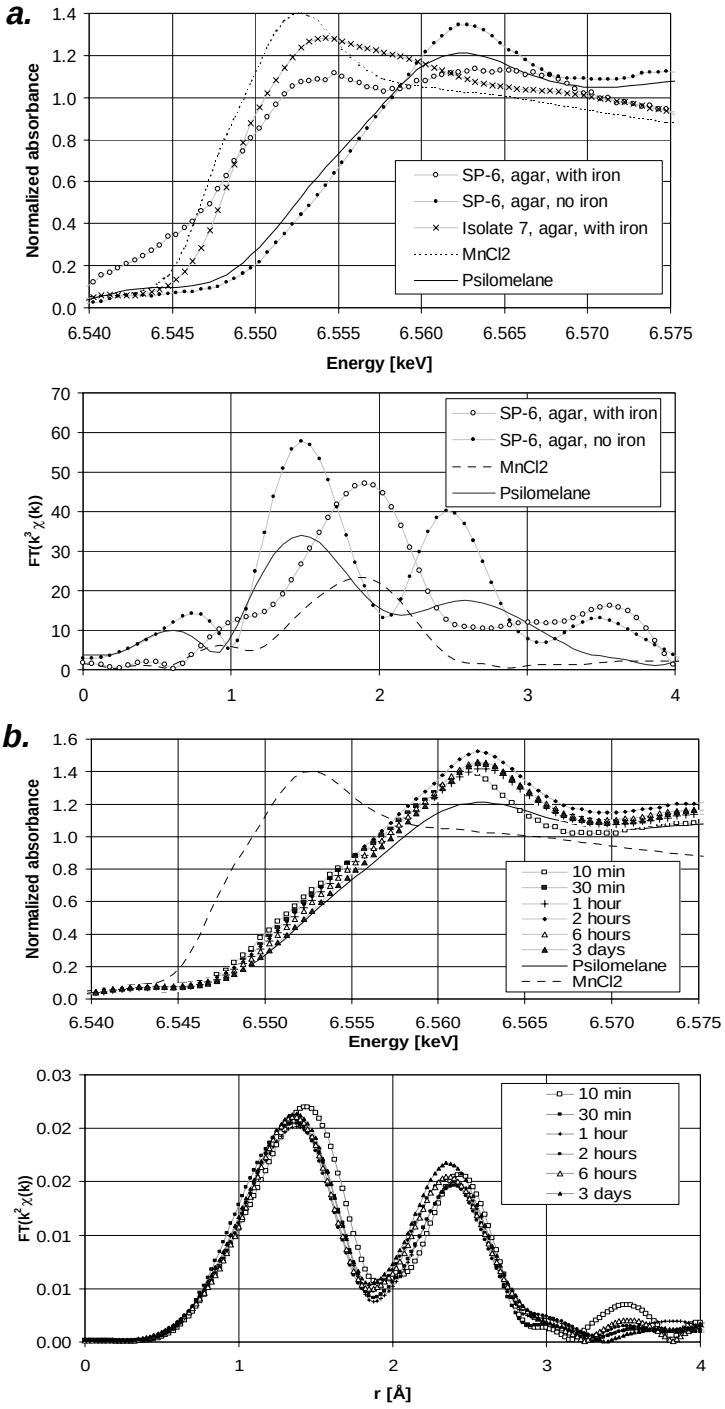

Figure 1. XANES and radial distribution functions of biogenic manganese oxides and reference minerals **a.** Cultures grown in solid media. **b.** Evolution of the oxidation process by SP-6 in liquid culture when no is iron added.

Figure 1b shows that within the observed time window (10 minutes to 3 days), approximately the same coordination environment of manganese predominates on the solids throughout oxidation of Mn(II) by SP-6 (liquid culture, no iron added). This predominant coordination environment corresponds to an oxidation state of IV, with a slight trend towards lower oxidation states for lower elapsed times.

Discussion

Our results show that bacteria can produce diverse forms of manganese oxides. Growth conditions of the bacterial cultures are important factors that determine differences in the structures of the manganese oxides. The presence of iron induces the formation of oxides with lower oxidation states. Presumably, the redox coupling with Fe(II) mediates the reduction of the newly formed manganese oxides. This is consistent with the work of Burdige *et al.* [11] that found reduction of synthetic manganese oxides by Fe(II) in a low organic artificial seawater medium.

The evolution of the manganese oxidation process suggests that SP-6 does not produce long-lived intermediate forms of manganese oxides. However, there is not enough evidence to extrapolate this for different growth conditions or time windows.

This work demonstrates the need for further experiments directed at the characterization of the oxidation process and its products at a molecular level, and a detailed investigation of the biogeochemical conditions under which they occur. This will provide us with a better understanding of the role of biogenic manganese oxides in the natural environment, and it will shed light on how we can take advantage of their catalytic properties.

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