Chromium(III) Oxidation Coupled with Microbially Mediated Mn(II) Oxidation

Youxian Wu and Baolin Deng
Department of Civil & Environmental Engineering, University of Missouri, Columbia, Missouri 65211, USA

Huifang Xu
Department of Geology and Geophysics, University of Wisconsin, Madison, Wisconsin 53706, USA and Department of Earth and Planetary Sciences, University of New Mexico, Albuquerque, New Mexico 87131, USA

Hiromi Kornishi
Department of Earth and Planetary Sciences, University of New Mexico, Albuquerque, New Mexico 87131, USA

Reductive immobilization of Cr(VI) has been widely explored as a cost-effective approach for Cr-contaminated site remediation. In soils containing manganese oxides, however, the immobilized form of chromium, i.e., Cr(III), could potentially be reoxidized. In this study, batch experiments were conducted to assess whether there were any microbial processes that could accelerate Cr(III) oxidation in aerobic, manganese-containing systems. The results showed that in the presence of at least one species of manganese oxidizers, Pseudomonas putida, Cr(III) oxidation took place at low concentrations of Cr(III). About 30–50% of added Cr(III) (10–200 μM) was oxidized to Cr(VI) within five days in the systems with P. putida and biogenic Mn oxides. The rate of Cr(III) oxidation was approximately proportional to the initial concentration of Cr(III) up to 100 μM, but the growth of P. putida was partially inhibited by Cr(III) at 200 μM and totally stopped when it reached 500 μM. Cr(III) oxidation was dependent upon the biogenic formation of Mn oxides, though the oxidation rate was not directly proportional to the amount of Mn oxides formed. Chromium(III) oxidation took place through a catalytic pathway, in which the microbes mediated Mn(II) oxidation to form Mn-oxides, and Cr(III) was subsequently oxidized by the biogenic Mn-oxides.

Keywords: bioremediation, chromium oxidation, manganese oxidation, Pseudomonas

INTRODUCTION
Chromium (Cr) contamination has been found in many industrial and federal sites in the United States (Nriagu 1988; Thornton and Amonette 1999). Since Cr chemicals are known to be toxic and carcinogenic (Costa 1997), site remediation is often needed to reduce its risk to humans and ecosystems. The mobility of Cr in the environment largely depends on its oxidation states. Cr(VI) species are quite mobile in soils and aquifers, whereas Cr(III) species are generally considered to be less toxic and mostly precipitated as hydroxides and adsorbed onto mineral surfaces. As a result, Cr(VI) could be immobilized and become less bioavailable and toxic when reduced to Cr(III).

Such reductive immobilization for Cr has been widely explored for site remediation of Cr(VI)-contaminated water and soils. Reductants used include zero valent iron (Blowes et al. 1997; Pratt et al. 1997), divalent iron (Eary and Rai 1988, 1989; Seaman et al. 1999), Fe(II)-bearing minerals (Eary and Rai 1989; Anderson et al. 1994), organic compounds (James and Bartlett 1983; Wittbrodt and Palmer 1995; Deng 1996a, 1996b), and H2S (Thornton and Amonette 1999; Kim et al. 2001; Hua and Deng 2003). For example, In-Situ Gas Reduction (ISGR) treatment system developed by the Pacific Northwest National Laboratory (PNNL) uses H2S for effective reduction and immobilization of Cr(VI) in soils (Thornton et al. 1999). Whether
the immobilized Cr(III) species could be remobilized through its reoxidation, however, has not been fully explored. Based on the thermodynamics, molecular oxygen, hydrogen peroxide, and manganese(III, IV) oxides are capable of oxidizing Cr(III) to Cr(VI) at concentrations typically found in the aquatic environment (Deng 1995). When considering the subsurface environment, direct Cr(III) oxidation by O_2 is limited due to the slow kinetics (Nakayama et al. 1981; Rai et al. 1986). While Cr(III) could be oxidized by hydrogen peroxide (Pettine and Millero 1990; Pettine et al. 1991), in the subsurface where hydrogen peroxide production is limited, Cr(III) oxidation by this oxidant is probably not important. Mn(III/IV) oxides could be important oxidants in soil systems for Cr(III) oxidation. It has been reported that in the aqueous suspensions, Cr(III) is oxidized to Cr(VI) by γ-MnOOH (Johnson and Xyla 1991), β-MnO_2 (Eary and Rai 1987), δ-MnO_2 (Fendorf and Zasoski 1992), and Na_2Mn_{13}O_{27}·9H_2O (Silvester et al. 1995). Cr(III) oxidation by Mn oxides in natural soils was also observed (Masscheleyn et al. 1992; Milacic and Stupar 1995; Koza et al. 2000). Mn oxides needed to be “fresh and amorphous” for facile Cr(III) oxidation; aged and well-crystallized MnO_2, usually pyrolusite, was quite weak and slow to oxidize Cr(III) (Bartlett 1996). Nevertheless, Cr(III) oxidation by Mn oxides following the reductive treatment may not be significant because the highly reactive Mn oxides originally present in the soils should also be reduced.

Uncertainties on the long-term stability of immobilized chromium arise from the effects of microorganisms that could potentially accelerate Cr(III) oxidation. In the presence of Mn(II) species, Mn-oxidizing microorganisms could utilize Mn(II) to obtain energy, forming various Mn oxides such as hausmannite (Mn_3O_4), manganite (γ-MnOOH), vernadite (δ-MnO_2), and disordered γ-MnO_2, depending on specific chemical and physical conditions (Ehrlich 1980; Tebo et al. 1997). These Mn-oxidizing microorganisms were found to be widely present in the environments such as rock surface, soil, water and sediments (Gregory and Staley 1982; Ehrlich 1990; Emerson 2000). It was found that genus *Pseudomonas* was the major microorganisms involved in the formation of manganese precipitates (Greene and Madgwick 1991). Some *Pseudomonas* species showed strong resistance to Cr. For example, *P. fluorescens* LB300 and LB304 could grow with as much as 1000 mg/L of Cr(VI) (Bopp and Ehrlich 1988).

This study investigated whether microbial processes could influence Cr(III) oxidation in the presence of Mn through a large number of aerobic batch experiments. The work focused on a Mn oxidizers—*Pseudomonas putida*, because of its known catalytic ability towards Mn(II) oxidation, resulting in the formation of Mn-oxides that could potentially oxidize and remobilize Cr(III).

**MATERIALS AND METHOD**

**Organisms and Growth Conditions**

*Pseudomonas putida* was obtained from American Type Culture Collection (ATCC #23483). The bacteria were grown on the modified LEP medium at 26°C under constant mixing conditions (Boogerd and de Vrind 1987; Caspi et al. 1998). The modified LEP medium contained (per liter of deionized water): 0.50 g of yeast extract (Difco Laboratories), 0.50 g of casamino acids (Difco), 5.0 mM (→)-glucose, 10.0–20.0 mM HEPES (N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic sodium) (to buffer pH at 7.0), 0.50 mM CaCl_2, and 1.00 ml of trace element solution. The trace element solution contained (per liter of deionized water): 10 mg of CuSO_4·5H_2O, 44 mg of ZnSO_4·7H_2O, 20 mg of CoCl_2·6H_2O, and 13 mg of Na_2MoO_4·2H_2O. The trace metals had concentrations several orders of magnitude lower than the added Cr and Mn concentrations in the experimental systems described below, so they were unlikely to have a major impact on the reaction between Cr and Mn-oxides. The medium was autoclaved for 20 min at 121°C followed by adding HEPES, glucose (both 0.2-μm filter sterilized), and Casamino acids (autoclaved for 40 min at 110°C). Preliminary experiments showed that a 20 mM of HEPES buffer solution could maintain a constant pH at 7.0 during the microbial growth and oxidation of Mn(II) and Cr(III) in a wide range of concentrations (10–1000 mM), therefore, this buffer was subsequently used in all experiments.

**Experimental Systems**

**Microbial Growth and Cr(III) Oxidation under Various Mn(II) and Cr(III) Concentrations.** The tests were conducted using 300 ml conical flasks containing 100 ml of the modified LEP medium to determine the effects of Mn(II) and Cr(III) concentrations on the microbial growth, formation of Mn oxides, and Cr(III) oxidation. Cr(III) was added as CrCl_3 at a fixed initial concentration ([Cr(III)]_0) of 50 μM in a set of experiments examining the effects of Mn(II) concentrations (50, 100, 200, 500, 1000 μM of MnCl_2). Another set of experiments were conducted at a fixed Mn(II)/Cl_2 concentration ([Mn(II)]_0) of 100 μM to examine the effects of varying Cr(III) concentrations (10, 20, 50, 100, 200, 500 μM). Mn(II) and Cr(III) solutions were sterilized with 0.2-μm sterile Niflon membrane filters (Fisher brand) and added into the sterile medium followed by the inoculation of *P. putida*. The cultures were incubated in a temperature-controlled orbital shaker (at 150 rpm at 26°C). Three ml of samples was taken from flasks at different time intervals for sample analyses. The experiments were duplicated, with the average value reported in the paper.

**Determination of Adsorbed Chromium.** In the presence of bacterial cells and Mn oxides, Cr(III) and Cr(VI) could be adsorbed by cells and Mn oxides. A set of experiments was therefore designed to determine the adsorbed amount of Cr on Mn oxides as follows. First, the *P. putida* culture in the LEP medium containing 50–1000 μM of Mn(II) was incubated, leading to the formation of Mn-oxides. Cr(III) was then spiked into the culture at a total final concentration of 300 μM. After 120 h of reaction, three ml of the incubated culture sample was taken and filtered through 0.2 μm Niflon membrane filters (Fisher brand), and the filtrate was used for the analyses of soluble Cr(VI) ([Cr(VI)]_S) by colorimetric method and total soluble Cr ([Cr_{T}]_s) by inductively coupled plasma spectroscopy (ICP). The solid phase was
collected on the filter and washed three times, each with 1.0 ml of phosphate buffer solution at pH 7. This filtrate solution was collected and used for the detection of phosphate-exchangeable Cr(VI) ([Cr(VI)]$_p$). To determine the total adsorbed Cr on Mn oxides, another 3.0 ml of sample was taken and adequately mixed with 1.0 ml of 20 mM ascorbic acid and then filtered through the 0.2 μm membrane filters. This process resulted in the reductive dissolution of Mn-oxides and release of sorbed Cr. The filtrate was acidified and used for analysis of total chromium ([Cr$_T$]) by ICP. The total sorbed Cr ([Cr$_T$]$_s$) on Mn oxides was calculated as the difference between total Cr and total soluble Cr in the system ([Cr$_T$]$_s$ = [Cr$_T$] - [Cr$_T$]$_s$). Preliminary experiments showed that Cr(VI) was reduced in coupling with the reductive Mn-oxide dissolution by ascorbic acid, but reduced Cr was still present in the soluble phase in the experimental systems.

**Analyses**

**Cell Density.** Cell optical density (OD600) was measured with spectrophotometric method at 600 nm. Biogenic Mn oxides interfered slightly with the determination of optical density. To correct this interference, OD600 values for different concentrations (0 – 1.0 mM) of Mn oxides were measured and used as blank controls. The cell density in the culture was calculated by the difference in OD600 values between growth cultures and the corresponding concentrations of Mn oxides.

**Mn-(hydr)oxides.** A spectrophotometric method, the Leuco Berbelin blue assay, was adapted for the analysis of Mn(III, IV) (hydr)oxides produced from the microbially mediated Mn(II) oxidation (Boogerd and de Vrind 1987; Francis and Tebo 2002). Samples (0.10 – 0.20 ml) were added to 0.50 ml of 0.04% Leuco Berbelin blue in 45 mM acetic acid. The oxidation of Leuco Berbelin blue proceeded within seconds with the development of a blue color. The absorbance was measured at 620 nm. The presence of microbial cells could interfere with the measurement of Mn(III, IV) (hydr)oxides when cell optical density was higher than 1.0 whereas Mn(III, IV) (hydr)oxides was lower than 10 μM. If samples were diluted by 5–10 times and the absorbance was measured within 15–24 hrs after the color development, the interference was negligible. Standard curves with 0–5 μM of suspending MnO$_2$ showed that the absorbance was linear up to the A$_{620}$ value of 1.50.

**Chromium and Manganese.** Total amounts of soluble Cr and Mn were measured with inductively-coupled plasma spectroscopy after samples were filtrated through 0.2 μm membrane filters, followed by acidification with 10% of concentrated high purity HNO$_3$ suitable for trace metal analysis. Chromate (Cr(VI)) was determined using diphenylcarbazide colorimetric method (Deng and Stone 1996a; APHA 1998). The standard Cr(VI) solution used for calibration was prepared using the consumed LEP medium. The consumed LEP medium was collected by filtering the culture of *P. putida* during the stationary phase of microbial growth in the LEP medium with no Mn(II) addition. Soluble Cr(III) was calculated as the difference between total soluble chromium and soluble chromate.

**TEM Analysis.** All high resolution transmission electron microscopy (TEM) images and X-ray energy-dispersive spectra (EDS) were obtained by using a JEOL 2010F FASTEM equipped with Oxford Instruments EDS system and Gatan GIF system. The precipitates collected through filtration were thoroughly washed using distilled water in order to eliminate interference from soluble salts and nutrients in TEM samples. TEM samples were prepared by dropping precipitate-bearing suspensions onto holey-carbon coated TEM copper grids.

**RESULTS**

**Mn(II) and Cr(III) Oxidation in the Presence of Pseudomonas putida**

Figure 1 shows microbial growth and oxidation of Mn(II) and Cr(III) in the presence (sets A and B) and absence (set C) of *P. putida*. The growth of *P. putida* was comparable for the systems with 100 μM of Mn(II) (A) and without Mn(II) (B), with the bacterial growth peaked at around 12 hrs, followed by the stationary phase (Figure 1a). Apparently, Mn(II) did not significantly affect the microbial growth kinetics. In the sterile control system (C), no microbial growth was observed, as expected, during the experimental time period. In terms of Mn(II) consumption and Mn-oxide production (Figure 1b), [Mn(II)] dropped sharply following the bacterial growth phase in the experiment A, with concurrent rapid Mn-oxide production. In both the system without initially added Mn(II) (B) and in the sterile control with Mn(II) but not the active bacterial cells (C), no change in Mn(II) concentration and no Mn-oxide production were observed. It was noted that the rapid formation of Mn oxides only occurred in the presence of mature *P. putida*, consistent with the observations reported in the literature (DePalma 1993).

Chromium oxidation clearly occurred in the experiment A (Figure 1c), in which *P. putida* was active and Mn oxides were formed. The formation of Mn oxides took place after the growth phase of *P. putida* ended, followed by immediate Cr(VI) production with concurrent decrease in Cr(III) concentration. Soluble Cr(VI) concentration reached 25 μM during the 5 days of incubation, indicating that at least 50% of the 50 μM Cr(III) originally present in the system was oxidized. Neither Cr(VI) increase nor Cr(III) decrease, however, was as quick as the formation of Mn-oxides. Low concentration of Cr(VI) was observed in the experiment B when Mn(II) was not added; but its accumulation rate was much slower than that in Experiment A. No Cr(VI) was found in the experiment C in the absence of *P. putida*, indicating that chemical oxidation of Cr(III) by oxygen alone was insignificant in the experiments.

**Cr(III) Oxidation Under Various Mn(II) Concentrations**

Concentration changes of Mn oxides and Cr(III, VI) in the presence of *P. putida* under various initial concentrations of Mn(II) were presented in Figure 2. Five treatments were compared with initial concentrations of Mn(II) at 50 (Mn50), 100 (Mn100), 200 (Mn200), 500 (Mn500), and 1000 (Mn1000) μM.
systems: it reached zero at lower concentrations (50, 100, and 200 μM) by 24th hour and at 500 μM, by 48th hours. With 1000 μM of initial Mn(II), the concentration decreased to below 200 μM within 24 hrs, but was not completely oxidized during the 144 hrs of experimental time period. Mn-oxides (Figure 2b) were generated concurrently to Mn(II) consumption and reached steady but various levels corresponding to various {[Mn(II)]}. The maximum amounts of Mn oxides detected

![Graph](image)

**FIG. 1.** Oxidation of Mn(II) and Cr(III) in the presence of *P. putida.* (a) microbial growth; (b) aqueous Mn(II) and solid Mn oxides; (c) aqueous Cr(III) and Cr(VI).

Microbial growth curves were all very similar in the systems with different initial Mn(II) concentrations, with the stationary phase starting at around 24 hrs of incubation (data not shown). As illustrated by Figure 2a, [Mn(II)] decreased quickly for all

![Graph](image)

**FIG. 2.** Cr(III) oxidation under different initial concentrations of Mn(II). (a) aqueous Mn(II); (b) solid Mn oxides; (c) aqueous Cr(VI); (d) aqueous total Cr(III, VI); (e) aqueous Cr(III). *(Continued)*
The results suggested that Mn(II) was oxidized to Mn-oxides in the examined concentration range, but a fraction of the manganese was sequestered, present neither as Mn-oxides nor as soluble forms, when higher than 200 mM of Mn was added into the system.

Chromium(VI) production occurred in all systems treated with various [Mn(II)]₀ (Figure 2c). The increase in Cr(VI) concentration with time, however, was not as sharp as the increase of Mn oxides. Soluble amounts of Cr(VI) were essentially the same in Mn50, Mn100, and Mn200, reaching close to 20 μM within 144 hrs. The amount was however only around 12 μM for Mn500 and 5 μM for Mn1000. The production of Cr(VI) was coupled with soluble Cr(III) consumption, as suggested by Figure 2d, and occurred only after Mn-oxides were formed, suggesting the importance of Mn-oxides for its oxidation. The time courses for the tests Mn50, Mn100, and Mn200 were quite similar in terms of both Cr(VI) production and soluble Cr(III) disappearance. Soluble Cr(III) was stable within the first 24 hrs, but gradually decreased afterward. Decreases in total soluble Cr (Figure 2e), which included both soluble Cr(III) and Cr(VI), were less significant compared to soluble Cr(III) in the tests Mn50, Mn100, and Mn200, but comparable to the tests Mn500 and Mn1000.

Comparing the tests with high (Mn500 and Mn1000) and low (Mn50, Mn100, Mn200) initial Mn(II) concentrations, the detected soluble amounts of Cr(VI) in the high [Mn(II)]₀ systems were much less than those with low [Mn(II)]₀, despite that Mn-oxides generated were significantly higher. The concentration profiles for soluble Cr(III) and total soluble Cr were also different. Total soluble Cr was higher at low [Mn(II)]₀ and there was no rapid drop during the time period from 19–32 hours. At high [Mn(II)]₀, it decreased much more significantly in this time period (Figure 2e). The amounts of soluble Cr(III) were higher at low [Mn(II)]₀ than at high [Mn(II)]₀, but all showed a rapid drop during 19–32 hrs (Figure 2d). These results suggested that more Cr was sorbed onto the solid phases in the system when [Mn(II)]₀ was high, because more Mn-oxides were formed.

The sorption of Cr on biogenic Mn oxides/bacterial cells was assessed following the reaction between 300 μM Cr(III) and various amounts of biogenic Mn oxides for 120 hrs (Table 1). Soluble Cr(VI) ([Cr(VI)]ₗₑ) determined by the diphenylcarbazide

| TABLE 1 |
| Adsorption of Cr on biogenic Mn oxides at the 5th day of incubation |

<table>
<thead>
<tr>
<th>Cr concentrations, μM</th>
<th>Total soluble acid-soluble Cr ([Cr(III)]ₗₑ)</th>
<th>Total soluble Cr ([Cr(III)]ₗₑ)</th>
<th>Soluble Cr(VI) ([Cr(VI)]ₗₑ)</th>
<th>Total Cr bound to Mn oxides ([Cr(VI)]ₗₑ)</th>
<th>Phosphate-exchangeable Cr(VI) ([Cr(VI)]ₗₑ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn(II), μM</td>
<td>[Cr(III)]₀</td>
<td>223</td>
<td>215</td>
<td>212</td>
<td>8</td>
</tr>
<tr>
<td>50</td>
<td>300</td>
<td>300</td>
<td>259</td>
<td>251</td>
<td>500</td>
</tr>
<tr>
<td>100</td>
<td>300</td>
<td>259</td>
<td>251</td>
<td>249</td>
<td>11</td>
</tr>
<tr>
<td>200</td>
<td>300</td>
<td>270</td>
<td>259</td>
<td>231</td>
<td>36</td>
</tr>
<tr>
<td>500</td>
<td>300</td>
<td>273</td>
<td>37</td>
<td>115</td>
<td>94</td>
</tr>
<tr>
<td>1000</td>
<td>300</td>
<td>206</td>
<td>112</td>
<td>250</td>
<td>8</td>
</tr>
</tbody>
</table>
colorimetric method ranged from 215 to 250 μM in the systems with 50–500 μM Mn and was 115 μM in the system with 1000 μM Mn, indicating that majority, if not all, of Cr(III) had been oxidized. Total soluble Cr([Cr(VI)]) determined by ICP spectroscopy was essentially the same as [Cr(VI)], determined by the colorimetric method, so soluble Cr(III) species were negligible in this neutrally-buffered culture medium. In another set of tests, ascorbic acid, a known reductant for Mn-oxides (Stone and Morgan 1984), was added to reduce Mn oxide for the analysis of total Cr. The adsorbed amounts of Cr ([Cr(Ia)]) estimated from the difference between total Cr after ascorbic acid reduction and total soluble Cr increased with increasing Mn concentration, ranging from 8 to 94 μM. Phosphate-exchangeable Cr(VI) followed the same trend of increases with increasing Mn concentration, ranging from 6 to 59 μM. It was clear that a fraction of Cr(VI) produced was adsorbed onto the Mn-oxides and/or biomass. The adsorbed amounts of Cr, however, did not appear to be directly proportional to the amount of Mn oxides produced. In the three treatments with low concentrations (50, 100, 200 μM) of Mn(II), sorbed Cr accounted for about 3% of total added Cr, but it reached 10 and 30% respectively in the two treatments with higher concentrations (500 and 1000 μM) of Mn(II).

Transmission Electron Microscopy (TEM) images clearly showed that the bacterial cells were closely associated with the Mn-oxides (Figure 3a). TEM and selected-area electron diffraction (SAED) pattern indicated that the precipitated biogenic Mn-oxides were in poorly crystalline birnessite-like phases, with nano-sheets and nanoporous textures shown in Figures 3a & 3b. The energy-dispersive spectrometry (EDS) showed that the biogenic Mn oxides contained a small amount of K and Ca, in addition to manganese and oxygen (Figure 3c).

**Cr(III) Oxidation at Different Initial Concentrations**

Figure 4 shows the microbial growth, Mn(II) consumption/ Mn-oxide production, and chromium oxidation in the presence of various [Cr(III)]₀. Initial Mn(II) was 0.10 mM in all tests. The results clearly indicated that the microbial growth was affected by Cr(III) concentrations (Figure 4a). The growth of *P. putida* was unhindered in the mediums containing up to 50 μM of Cr(III), but no growth was observed at all in the medium containing 500 μM of Cr (III). When the concentrations of Cr(III) were in between (100 and 200 μM), the growth took place, but the rate was apparently suppressed.

Changes of soluble Mn(II) with time in the cultures was shown in Figure 4b. Dissolved Mn(II) was completely removed in 24 hrs at low Cr(III) concentrations (0–50 μM), and it took 36–48 hrs to completely consume the soluble Mn(II) at higher concentrations (100–200 μM) of Cr(III). The consumption of Mn(II) was concurrent with the production of Mn oxides in the systems (Figure 4b & 4c). In the cultures containing low concentrations of Cr(III) (0–50 μM), Mn oxides were rapidly

FIG. 3. Transmission electron micrographs of (a) *P. putida* associated with oxidized Mn-oxide nano-sheets, and (b) biogenic Mn oxides with nanoporous texture. (c) EDS spectra from biogenic Mn oxides.
produced, and reached a plateau of approximately 80–100 μM. The formation of Mn oxides began at a later time in the presence of higher amounts of Cr(III) (100 and 200 μM), and then reached constant but at slightly lower concentration levels. With 500 μM of Cr(III) in the medium, neither Mn(II) concentration change nor Mn oxides formation was observed. The results on the consumption of Mn(II) and production of Mn-oxides agreed well with the data for the microbial growth presented above.

Cr(VI) production induced by the microbial process of Mn(II) oxidation was shown in Figure 4d. Cr(III) oxidation, as indicated by Cr(VI) production, took place in all systems where Mn-oxides
were produced. There was a lag time between the rapid microbial growth and Cr(III) oxidation, and in fact, Cr(VI) production did not start until microbial growth has reached the stationary phases but was well coupled with the formation of Mn oxides (see Figures 4a–4d). With low initial Cr(III) concentrations (10, 20, and 50 μM), Cr(III) oxidation began at around 24–36 h, consistent with the time needed for Mn-oxide formation. In the system with 100 or 200 μM of Cr(III), the microbial growth ended in 24 h in the systems but Cr(III) oxidation started at around 36–48 h, right after the formation of Mn-oxides. In general, about 50% of soluble Cr(III) or more was oxidized during the 120 h of experiments. No Cr(III) oxidation was found in the system with [Cr(III)]₀ = 500 mM.

DISCUSSION

Our experiments have shown that Cr(III) oxidation is catalyzed by the Mn(II)-oxidizing microorganism, P. putida, in the systems containing Mn(II). The Cr(VI) production is concurrent with the formation of biogenic Mn oxides, which act as primary oxidants for Cr(III) oxidation. When Mn(II) is absent, very small amounts of Cr(VI) (<3 μM) are detected in the presence of Mn(II)-oxidizing microorganisms (Figure 1c). This does not appear to be an analytical artifact considering that the Cr(VI) concentration increases consistently with time in the system, in comparison to no detectable Cr(VI) at all in the sterile system. Possible mechanisms include (i) Cr(III) oxidation through metabolic pathways and (ii) the presence of a trace amount of Mn associated with the culture medium, which catalyzes Cr(III) oxidation. A recent study by Murray and coworkers (Murray et al. 2005) showed that Cr(III) was indirectly oxidized by P. putida in the presence of Mn(II); non-Mn oxidizing mutants of the bacteria did not result in any Cr(III) oxidation. We, therefore, contribute the low Cr(VI) observed in the system without externally added Mn to the latter mechanism, i.e., trace amounts of Mn in the culture medium.

It is proposed that the overall process of Cr(III) oxidation under the experimental conditions be described by the following two steps: (i) formation of Mn oxides through microbial mediated Mn(II) oxidation and (ii) Cr(III) adsorption and reaction on the biogenic Mn oxide surfaces that result in Cr(VI) production. The exact nature of Mn oxides produced in our study is unclear. In another study on Mn(II) oxidation by a Pseudomonas sp., however, Greene and Madgwick (1991) have found by X-ray diffraction and Fourier transform infrared spectroscopy that the product is a semipure disordered γ-MnO₂ with O/Mn ratio of 1.92. Villalobos et al. (2003) obtained similar poorly crystalline Mn(IV) oxides from Mn(II) oxidation by P. putida strain MnB1, with the oxide properties comparable to δ-MnO₂. MnO₂ is therefore used here for Mn oxides to illustrate the two-step process:

\[
2\text{Mn}^{2+} + \text{O}_2 + 2\text{H}_2\text{O} = 2\text{MnO}_2 + 4\text{H}^+ \quad [1]
\]

\[
2\text{Cr}^{3+} + 3\text{MnO}_2 + 2\text{H}_2\text{O} = 2\text{HCrO}_4^- + 3\text{Mn}^{2+} + 2\text{H}^+ \quad [2]
\]

FIG. 5. Relationship between added Cr(III) concentrations and biogenic Mn oxides and Cr(VI) productions.

It is known that microbial oxidation of Mn(II) (Equation 1) occurs widely in natural systems such as soils, sediments, and water. A variety of microorganisms, including heterotrophic bacteria, sheathed bacteria, fungi, and algae can mediate the conversion of soluble Mn(II) to Mn oxides (Greene and Madgwick 1991). The types of oxides formed vary with the types of microorganisms and chemical, physical, and growth conditions of cultures. Our observations show the formation of disordered nano-sized Mn oxides by P. putida at pH 7, which is directly responsible for Cr(III) oxidation (Equation 2). The biogenic formation of Mn oxides is suppressed by Cr(III) when [Cr(III)]₀ ≥ 100 μM (Figure 5). For the system with 500 μM of [Cr(III)]₀, no microbial growth and Mn-oxide production take place at all, accordingly there is no Cr(VI) production. It is concluded that the growth of Mn-oxidizing bacteria and consequently Mn(II) oxidation could be inhibited by high concentrations of Cr(III), which could be an important mechanism to maintain Cr in the more stable Cr(III) species. Murray and coworkers (Murray et al. 2005) also showed that Cr(III) could be toxic to microorganisms, with its toxicity even higher than that of Cr(VI).

Cr(III) oxidation by various types of Mn oxides has been well documented in the literature (Masscheley et al. 1992; Milacic and Stupar 1995; Kozuh et al. 2000). In terms of electron balance, three electrons are involved in oxidizing one mole of Cr(III) to Cr(VI), which would require 1.5 moles of MnO₂ (if all reduced to Mn(II)) or three moles (if only reduced to MnOOH). A 60 μM of Cr(VI) produced in the experimental system with 100 μM Mn(II)/200 μM Cr(III) should, therefore, consume all of the 100 μM Mn-oxides available in the system (Figure 4). The fact that a near constant amount of Mn-oxides (~80 μM) and near zero amount of soluble Mn(II) are detected in the system indicates that rapid re-oxidation of Mn(II) takes place, i.e., Mn-oxides/Mn(II) serves as catalysts by removing electrons from Cr(III) to produce Cr(VI) in the presence of Mn-oxidizing
microorganisms, with O₂ as terminal electron acceptor, thus completing the proposed catalytic cycle.

The Cr(III) oxidation process is complex with its extent and rate influenced by many factors. These include Cr(III) adsorption on Mn oxides, mechanism of electron transfer, pH, desorption and re-adsorption of produced Mn(II) and Cr(VI), and types of Mn oxides (Early and Raï 1987; Fendorf and Zasowski 1992; Fendorf et al. 1993, 1994, 2000; Silvester et al. 1995). Biogenic nanosized Mn-oxides are likely to be more reactive in redox reactions than the well crystallized minerals (Nelson et al. 1999). As demonstrated with soil samples, Cr(III) oxidation is enhanced by “fresh and amorphous” Mn oxides, aged and well-crystallized MnO₂ is weak and slow to oxidize Cr(III) (Bartlett 1996). Biogenic Mn oxides in our systems are certainly fresh as disordered and nanosized particles. Direct comparison between Cr(III) oxidation by biogenic Mn oxides as examined in this study and oxidation by chemically synthesized Mn oxides reported in the literature, however, is difficult because the utilized solution compositions, solid loading, and pH are often different (Early and Raï 1987; Fendorf and Zasowski 1992). Nevertheless, the amount of Cr(VI) produced in Cr(III) oxidation by β-MnO₂ (Early and Raï 1987) is nearly 10 times less than what is observed in our system (Figure 1c) under comparable pH and time conditions.

Because of the presence of mixed organic compounds in the culture, changes observed in the composition of the aqueous solution (i.e., [Cr₃⁺₄⁺], [Mn]₀, [Cr(VI)]₄⁻) are generally not interpretable on the basis of the solubility behaviors of Cr(OH)₃(s) as a function of pH and redox states as reported by Raï et al. (1986). Our observations show that over 70% of added Cr exists in the soluble phase at low initial concentrations (<200 μM) and approximately 40% of added Cr is soluble at the initial concentration of 500 μM at pH 7.

The adsorption of Cr(III, VI) on Mn oxides changes with the initial concentrations of Mn(II) added into the experimental systems. This is caused by the different amounts and types of Mn oxides formed. It has been shown that Mn oxides formed by Bacillus sp. spores are different at different Mn(II) concentrations (Mandernack et al. 1995; Bargar et al. 2005). Biogenic Mn oxides have a higher adsorption capacity of Pb than chemically-synthesized Mn oxides (Nelson et al. 2002), where the influence of specific surface area seems to be crucial (Villalobos et al. 2005). Whether this is the same for metals such as Cr and whether such adsorption is associated with biogenic Mn oxides formed under different conditions need further study.

Manganese is ubiquitous in the aquatic environment. Soluble Mn is mostly around 50 μM (2.75 mg/l) or a little higher in groundwater (Nealson 1983), which is in the concentration range for Cr(III) oxidation in the presence of Mn-oxidizing bacteria, as elucidated in this study. The rate of Mn(II) oxidation can be biologically accelerated by up to several orders of magnitude compared to abiotic Mn(II) oxidation (Nealson et al. 1988; Wehrli et al. 1995; Harvey and Fuller 1998; Morgan 2000). Biological Mn(II) oxidation can also take place anaerobically in the presence of nitrate (Vandenabeele et al. 1995). The production of biogenic Mn oxides might lead to Cr(III) oxidation in some environments. Mn oxide production is, however, dependent upon Cr(III) concentrations, availability of oxygen and/or other electron acceptors, as well as other system constituents such as iron sulfides. Further study is needed to assess the potential of Cr(III) oxidation under field conditions in the presence of Mn(II)-oxidizing organisms.

REFERENCES


Bartlett RJ. 1996. Chromium redox mechanisms in soils: should we worry about Cr(VI)? In: Sequi P Editor, San Miniato, Italy: Chromium Environmental Issues.


Emerson D. 2000. Microbial oxidation of Fe(II) and Mn(II) at circumneutral pH. In: D.R. Lovley (Editor), Environmental Microbe-Metal Interactions. DC: Washington ASM Press, American Society for Microbiology.


