Chromium(III) Oxidation by Biogenic Manganese Oxides of Varying Structural Ripening

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Environmental Impact

Chromium (Cr) is a widespread anthropogenic contaminant that compromises ecosystem and human health. Transformation of the micronutrient and less mobile species Cr(III) to highly mobile and toxic Cr(VI) is strongly mediated by manganese (Mn) oxides. In fact, Mn oxides are the only oxidants of Cr(III) under most environmental conditions. Although Mn oxides are generally considered biogenic in origin, few studies have addressed the coupled role of reaction conditions and biogenic Mn oxide structure in Cr(III) oxidation. In this study we examined the effect of light, organic carbon content, pH, and structural ripening of biogenic manganese oxides on Cr(III) oxidation using Mn oxides produced by a marine bacterium within the widespread *Roseobacter* clade.

Abstract

Manganese (Mn) oxides, which are generally considered biogenic in origin within natural systems, are the only oxidants of Cr(III) under typical environmental conditions. Yet the influence of Mn biooxide mineral structural evolution on Cr(III) oxidation under varying geochemical conditions is unknown. In this study we examined the role of light, organic carbon, pH, and structure of biogenic Mn oxides on Cr(III) oxidation. Aging of Mn oxides produced by a marine bacterium within the widespread *Roseobacter* clade resulted in structural ripening from a colloidal hexagonal to particulate triclinic birnessite phase. The structurally diverse Mn oxides were then reacted with aqueous Cr(III) within artificial seawater in the presence or absence of carbon and light. Here we found that Cr(III) oxidation capacity was highest at near neutral pH and in the combined presence of carbon and light. Mn oxide ripening from a hexagonal to triclinic birnessite phase led to decreased Cr(III) oxidation in the presence of carbon and light,

whereas no change in reactivity was observed in the absence of carbon and/or in the dark. As only minimal Cr(III) oxidation was observed in the absence of Mn oxides, these results strongly point to coupled Mn oxide- and photo-induced generation of organic and/or oxygen radicals involved in Cr(III) oxidation. Based on Mn oxide concentration and structural trends, we postulate that Mn(II) produced from the oxidation of Cr(III) by the primary Mn oxide is recycled in the presence of organics and light conditions, (re)generating secondary hexagonal birnessite and thereby allowing for continuous oxidation of Cr(III). In the absence of this Mn oxide regeneration, Cr(III) induced structural ripening of the hexagonal birnessite precludes further Cr(III) oxidation. These results highlight the complexity of reactions involved in Mn oxide mediated Cr(III) oxidation and suggest that photochemical carbon reactions are requisite for sustained Cr(III) oxidation and persistence of reactive Mn oxides.

Key words: Bacteria, Roseobacter, Biogenic manganese oxides, Chromium, Oxidation, Organics, X-ray absorption spectroscopy (XAS, XANES, EXAFS)

1 **1. Introduction**

2   Chromium (Cr) is a significant anthropogenic metal contaminant in soils and aquatic

3 systems due to its widespread industrial applications. The toxicity and transport behavior of Cr

4 depend strongly on its valence state, with hexavalent and trivalent being the most common

5 oxidation states. Cr(VI) compounds are typically soluble, mobile, bioaccessible, and are

6 considered carcinogenic upon inhalation exposure. Cr(III) generally forms insoluble

7 (oxyhydr)oxides and is an essential micronutrient [1,2].

8   In terrestrial and aquatic systems, a variety of compounds are capable of reducing Cr(VI)

9 to Cr(III), such as organic matter, Fe(II), and reduced sulfur species [1,3-5]. Following reduction,

10 Cr(III) precipitates as pure Cr(III) or mixed Cr(III)-Fe(III) (oxy)hydroxide phases that have low

11 solubility and typically lack long range structural order [6,7]. However, within common

12 environmental conditions, Mn(III, IV) (oxyhydr)oxides (hereafter referred to as Mn oxides) are

13 capable of oxidizing Cr(III) to Cr(VI) and are, in fact, considered the only environmentally

14 relevant Cr(III) oxidant [5,8,9]. Given the widespread presence of Mn oxides within the

15 environment, understanding reaction conditions and mechanisms of Mn oxide mediated Cr(III)

16 oxidation is important for predicting Cr mobility and transport. Several previous studies have

17 investigated the kinetics and mechanisms of Cr(III) oxidation by abiotic (synthetic or natural)

18 Mn oxides, such as δ-MnO$_2$ [3,10-12], birnessite [11-17], manganite [18], todorokite [15], pyrolusite [5], and

19 hydrous manganese oxide [19]. It has generally found that the rate and extent of Cr(III) oxidation

20 was affected by the type and structure of Mn oxides [15,20-22]. In particular, Mn oxides with

21 hexagonal symmetry (biogenic Mn oxide and birnessite) have shown the highest oxidation

22 capacity for Cr(III) [22].

23   Natural Mn oxides are believed to form primarily by Mn(II) oxidation via either direct or

24   indirect microbial activity [23]. The predominant type of biogenic Mn oxide formed at

25   circumneutral pH is a highly disordered, nanocrystalline, phyllomanganate phase, similar to

26   hexagonal birnessite [24-26]. This phase is highly reactive and can undergo abiotic transformation

27   and ripening to form more ordered and crystalline phases such as todorokite, feitknechtite, and

28   triclinic birnessite [24, 27-29]. Due to their greater disorder and surface area, biogenic oxides are

29   considered more reactive than their synthetic analogs [24, 25, 30]. Yet, only a few studies have

30   examined Cr(III) oxidation by Mn oxides produced by microorganisms, such as the Mn(II)-

31   oxidizing bacteria *Pseudomonas putida* [22, 31-33] and *Bacillus* sp. [22, 32]. These studies either used

32   the fresh biogenic Mn oxides (with a layered structure similar to δ-MnO_2) or characterized the

33   coupled Mn(II)-Cr(III) oxidation in the presence of active microbial Mn(II)-oxidizing activity.

34   To our knowledge, no previous studies have examined the impact of natural structural

35   transformations of biogenic Mn oxides on their reactivity toward Cr(III) oxidation.

36   Microorganisms (both bacteria and fungi) that are capable of oxidizing Mn(II) to

37   Mn(III,IV) oxides are widespread in both marine and terrestrial settings, as well as contaminated

38   sites [23, 34, 35]. Recently, Mn(II) oxidation by some bacterial and fungal species has been linked to

39   the extracellular production of the reactive oxygen species (ROS) superoxide [34-36]. Oxidation by

40   a bacterium (*Roseobacter* sp. AzwK-3b) within the widespread and abundant *Roseobacter* clade

41   was attributed to an exoprotein (extracellular), such that superoxide production and Mn(II)

42   oxidation activity occurred outside the cell and thus activity was maintained in cell-free filtrate [36,

43   [37]. The initial oxidation of Mn(II) by these exoproteins within the cell-free filtrate results in the

44   formation of a highly reactive colloidal birnessite phase with hexagonal symmetry and similar in

45   structure to δ-MnO_2. This initial hexagonal birnessite phase is highly reactive and induces the

46   secondary abiotic oxidation of Mn(II). Mn(II)-induced aging of this colloidal phase introduces

47   structural reorganization and the gradual transformation to a particulate phase with trigonal

48   symmetry and minimal reactivity toward aqueous Mn(II) [27]. Abiotic oxidation of Mn(II) at the

49   colloidal hexagonal birnessite surface was substantially enhanced in the presence of light and

50   organic carbon and diminished in the presence of superoxide scavengers (e.g., superoxide

51   dismutase – SOD), indicating a role for ROS and organic radicals in Mn(II) oxidation. Further, it

52   illustrates a tight coupling between microbial and mineral surface chemical processes responsible

53   for the oxidation of Mn(II). Given the structural evolution and subsequent changes in reactivity

54   of biogenic Mn oxides, this system provides an ideal opportunity for studying the structure-

55   reactivity relationship of biogenic Mn oxides in the oxidation of Cr(III).

56   Accordingly, in this study, we examined the oxidation of Cr(III) by Mn oxides produced

57   by biogenic extracellular superoxide over the course of mineral structural evolution. We further

58   explored the impact of pH, organic carbon content, and light on Cr(III) oxidation by these

59   structurally diverse biogenic oxides. Given the widespread of presence of Mn(II) oxidizing

60   microorganisms in the environment, results from this study provide important insights regarding

61   the reactivity of biogenic Mn oxides, as well as the fundamental basis for predicting and

62   understanding Cr fate and transport behavior in nature.

63

64   **2. Materials and methods**

65   *2.1. Bacterial growth and biogenic Mn Oxide harvesting process*

66   *Roseobacter* sp. AzwK-3b was grown in an organic carbon rich (K) medium containing 2

67   g/L peptone, 0.5 g/L yeast extract, and 20 mM HEPES buffer prepared with 75 vol.% artificial

68   seawater (ASW), pH 7.2 at 25 °C and 150 rpm [37] without $MnCl_2$. The ASW contains 0.3 mol/L

69   NaCl, 0.05 mol/L MgSO$_4$, 0.01 mol/L CaCl$_2$, and 0.01 mol/L KCl. After growing *R*. AzwK-3b to

70   mid exponential phase (OD$_{600}$ ~0.12), cell free filtrate was collected by centrifuging the growth

71   culture at 8,000 rpm for 10 min and passing the supernatant through a 0.45 μm filter. The

72   resulting solution is hereafter referred to as a cell free filtrate, which maintains the activity for

73   extracellular enzymatic superoxide production [27, 36] and is used for producing biogenic Mn

74   oxides.

75   Biogenic Mn oxides (hereinafter referred to as bioMnOx) were produced by reacting the

76   cell free filtrate with 100 μM MnCl$_2$ at 25° C and 150 rpm in the presence of ambient light.

77   BioMnOx were aged and harvested at various time points (4–211 h) by centrifuging at 9,000 rpm

78   for 25 min and washing with deionized water (18 MΩ-cm).

79

80   *2.2. Cr(III) oxidation experiments*

81   Harvested bioMnOx were resuspended in 50 mL of sterile K medium or ASW at pH 6.2

82   or 7.2 (buffered by 20 mM HEPES) and tested for Cr(III)-oxidizing capacity in the presence or

83   absence of light. The initial bioMnOx concentration was ~40 μM, measured as Mn(III,IV) oxide

84   concentration using the Leucoberlelin blue (LBB) method (see details below) [38]. Cr(III) was

85   added as CrCl$_3$ from a 100 mM sterile stock solution to achieve an initial concentration of 50 μM.

86   Flasks containing the suspension were shaken at 25 °C and 150 rpm for ~200 h in the absence

87   (dark condition) or presence of ambient light (light condition). For experiments conducted in

88   dark conditions, all solutions were prepared in bottles wrapped with aluminum foil, and the

89   reaction flasks further covered with a cardboard box. Sampling was done without direct light

90   exposure. Control experiments were conducted at the same Cr(III) concentration and reaction

91   conditions without the addition of bioMnOx. The effect of bioMnOx concentration on the Cr(III)

92   oxidation was also explored using up to 4 times concentrated bioMnOx (i.e., 1× to 4× of 40 μM

93   Mn(III,IV) oxide) and an initial Cr(III) concentration of 50 μM. All experiments were conducted

94   in duplicate.

95   During the course of reaction, Mn oxide loss and Cr(VI) production were constantly

96   monitored. Concentration of Mn(III, IV) oxides were quantified using the Leucoberlelin blue

97   (LBB) method with a UV-Vis spectrophotometer (Cary 50, Varian) [38]. Standard curves were

98   obtained using LBB and synthetic δ-MnO_2 [25]. The concentration of Cr(VI) in the reacting

99   suspension was analyzed using the s-diphenyl carbazide method [9]. To account for unavoidable

100   minor variations in initial Mn oxide content, a Cr oxidation capacity is used to normalize the

101   amount of Cr(VI) production to the initial Mn(III, IV) oxide concentration, defined as:

102   Cr oxidation capacity = [Cr(VI)] / [Mn(III,IV) oxide]$_{ini,}$

103

104   ***2.3. X-ray absorption spectroscopy analysis***

105   X-ray absorption spectroscopy (XAS) data were collected on the bioMnOx before and

106   after reaction with Cr(III) to identify speciation and structural changes. Suspensions containing

107   Mn oxides were vacuum filtered through 0.2 μm polycarbonate membranes and rinsed with DI

108   water. The moist filter membranes loaded with Mn oxide wet pastes were then mounted in a

109   Teflon sample holder covered with Kapton tape for XAS data analysis. Samples were frozen at -

110   20 °C and thawed prior to analysis. Manganese K-edge XAS spectra were collected at beamline

111   11-2 at Stanford Synchrotron Radiation Lightsource (SSRL) and beamline X18B at National

112   Synchrotron Light Source, Brookhaven National Laboratory (NSLS-BNL) with a Si (220) or

113   (111) double crystal monochromator (40% detuning), respectively. Energy calibration was

114   achieved using a Mn foil (6539 eV). Data were collected in both fluorescence and transmission

115   mode using a 30-element Ge solid-state detector with a Cr filter at beam line 11-2 (SSRL) or a

116   PIPS detector at beam line X18B (NSLS). Analysis of the near edge region of consecutive XAS

117   spectra for each sample showed no photo-induced reduction of Mn oxides under the X-ray beam.

118   Analysis of the bulk XAS data was performed using the programs SIXPACK [39] and Ifeffit

119   [40]. The composition and structure of Mn oxides were determined using both the XANES (X-ray

120   absorption near edge structure) and EXAFS (extended X-ray absorption fine structure) regions.

121   For EXAFS analysis, spectra were background-subtracted, k^3-weighted and analyzed from 3–12

122   Å^{-1}. As previously described [24, 27], principal component analysis (PCA), combined with target

123   transformation and linear combination fitting (LCF), were performed on the EXAFS spectra to

124   establish the number of components representing the entire data set. A spectral reference library

125   of model Mn compounds was used to identify and quantify the structural components. The

126   model compounds used were previously described [24], and include: δ-MnO_2, triclinic Na-

127   birnessite, hexagonal Ca-birnessite, groutite (α-MnOOH), feitknechtite (β-MnOOH), manganite

128   (γ-MnOOH), hausmannite (Mn_3O_4), synthetic todorokite $[(Na,Ca,K)(Mg,Mn)Mn_6O_{14}\cdot5H_2O]$,

129   pyrolusite (β-MnO_2), synthetic Mn_2O_3, aqueous Mn(III) pyrophosphate, aqueous $MnCl_2$, and

130   aqueous $MnSO_4$.

131   To determine the detailed structure of the nanoparticulate and highly disordered

132   manganese oxides, a full multiple scattering model [26] that is sensitive to the bending of the Mn

133   octahedral layer and Mn site vacancies was used to conduct shell-by-shell EXAFS fitting. This

134   model has been previously described in detail [26, 27] and is briefly summarized below. The model

135   consists of all single scattering (SS) paths and three types of collinear multiple scattering (MS)

136   paths. The amplitude of all MS paths is strongly affected by the level of distortion of the

137   phyllomanganate layers, which is accounted for as an out-of-plane bending angle β (along the *a*-

138 axis) [26, 41]. A parameter f_{occ} characterizes the lattice occupancy and accounts for both the

139 vacancies within the phyllomanganate layer as well as edge effects in nanoparticulate Mn oxides.

140 .

141 **3. Results and Discussions**

142 *3.1. Production of structurally evolved Mn oxides*

143 Similar to previous observations [24-26], the predominant form of biogenic Mn oxides

144 formed at circumneutral pH was a highly disordered layered phyllomanganate phase with

145 hexagonal symmetry. Principle component analysis (PCA) using a suite of Mn reference

146 compounds indicated that two components were needed to reconstruct all of the unreacted

147 bioMnOx Mn EXAFS spectra, and these components were identified as a hexagonal birnessite

148 phase (δ-MnO_2) and a triclinic birnessite phase (Na-birnessite) (Figure 1a). The difference

149 between these two structures are most distinctive at around 7–10 Å^{-1} in k space (gray shading in

150 Figure 1a). The hexagonal phase shows two distinct peaks at ~8 and 9 Å^{-1}, whereas for the

151 triclinic phase the 8 Å^{-1} oscillation decreases in amplitude accompanied with a broadening of the

152 feature at ~9 Å^{-1}. Linear combination fitting (LCF) using the two reference compounds yielded

153 good fits for all bioMnOx samples (Table S1 and Figure 1b dotted line). The structure of the

154 fresh (4 h) bioMnOx was composed of 100% hexagonal birnessite, and appeared as brownish

155 suspended colloids. Upon aging, a triclinic birnessite structure was needed to fit the bioMnOx

156 spectra. With increased aging time, the fraction of the triclinic birnessite phase increased, and the

157 bioMnOx also aggregated to larger dark brown to black particles. At 211 h, the bioMnOx

158 structure was best fit with 56% hexagonal and 44% triclinic birnessite. A near linear relationship

159 ($R^2 = 0.9238$) between the fraction of hexagonal birnessite phase and aging time was observed

160 (Figure 1b), suggesting a strong correlation between bioMnOx structure and aging time.

161   These results are consistent with previous experiments using the same organism and

162   similar experimental conditions [27]. In detail, Learman et al [27] reported the transformation of

163   bioMnOx from a pure hexagonal birnessite phase at 4 h to a 45% hexagonal/55% triclinic phase

164   at 96 h. Similarly, using synchrotron X-ray diffraction and absorption spectroscopy, Webb et al

165   [26] found that the initial bioMnOx produced by the spores of a marine *Bacillus sp.* was a layered

166   phyllomanganate with hexagonal sheet symmetry similar to that of δ-MnO_2 and transformed to

167   pseudo-orthogonal sheet symmetry with a structure similar to that of triclinic birnessite. These

168   results are in agreement with our findings and suggest that a gradual structural transformation of

169   bioMnOx can be common in the nature, and might play an important role in the bioMnOx

170   reactivity toward controlling metal speciation such as the oxidation of Cr(III).

171   Detailed structural fitting of the initial Mn oxides were consistent with the LCF results

172   (Figure 2, Table S2). In particular, the initial colloidal Mn oxide phase (4 h) had a structure with

173   a negligible out-of-plane bending of the octahedral layers ($\beta_a = 0$), a high number of corner-

174   sharing Mn octahera (CN = 3.2), and low fraction of occupied Mn octahedral sites ($f_{occ} = 0.64$).

175   These values are in good agreement with the structural model for hexagonal birnessite [26] with a

176   slightly lower f_{occ} value. Upon aging, the bioMnOx gradually transformed with structural

177   parameters intermediate between hexagonal and triclinic birnessite. The out-of-plane bending

178   angle β_a increased from zero to ~1.6, 6.9, and 7.4 for bioMnOx aged for 31, 96 and 211 hours,

179   respectively, suggesting a gradual transformation of the bioMnOx from hexagonal to triclinic

180   symmetry over time and is consistent with the LCF results. Triclinic birnessite has pseudo-

181   orthogonal symmetry and is characterized by a larger proportion (up to 1/3) of Mn(III) octahedra

182   in the sheets compared to hexagonal birnessite [42, 43]. The large angular non-linearity along *a*-axis

183    $(\beta_a\ 12–17)$ [26, 41] of triclinic birnessite is a result of lattice strain produced in the planar sheet

184   buckling upon Mn(III)-rich row formation [42].

185   Further and as expected, the dominant oxidation state of the bioMnOx was Mn(IV) with a

186   minor component of Mn(III). Comparing the peak crest positions of bioMnOx with those of the

187   reference compounds (vertical dotted lines), indicated that the bioMnOx all have a Mn valence

188   state similar to that of δ-MnO$_2$, which has an average oxidation state of 3.9–4.0 (Figure S1).

189   However, all bioMnOx spectra also showed peak broadening, with a small shoulder at ~6550 eV

190   that is similar to that of the feitknechtite spectra, suggesting the presence of minor amounts of

191   Mn(III) in the structure.

192

193   *3.2. Cr(III) oxidation by structurally diverse biogenic Mn oxides under varying light and*

194   *carbon conditions*

195   The extent of Cr(VI) production was 2–10 times higher in the presence relative to

196   absence of bioMnOx, indicating that the biogenic Mn oxides induced the oxidation of aqueous

197   Cr(III) (Figure 3). In detail, Cr(VI) production in the absence of Mn oxides averaged 0.55 μM,

198   (1.1% of added Cr(III)) which was substantially lower than that in the presence of bioMnOx

199   (Figure 3). Interestingly, control experiments conducted in the absence of Mn oxides and within

200   the dark yielded Cr(VI) production close to zero, whereas those in the light within either cell-free

201   filtrate, ASW, or K medium yielded small amounts of Cr(VI) production (0.5–1.5 μM). These

202   values, although far less than those in the presence of bioMnOx, suggest a potential contribution

203   of photo-active factors, such as reactive oxygen species (ROS) or organic radicals produced in

204   the presence of light, in oxidizing Cr(III).

205   In the presence of bioMnOx, the rate and extent of Cr(III) oxidation varied as a function

206   of Mn oxide structure (age), light, and organic carbon. In the absence of organic carbon (i.e., in

207   ASW) and/or under dark conditions, a significant difference between the Cr(III) oxidation

208   capacity of differently aged MnOx was not observed (Figure 4). However, in organic carbon-rich

209   K medium in the presence of light, the Cr(III) oxidation capacity is highly dependent on MnOx

210   structure (age). In particular, more aged bioMnOx phases with a higher proportion of triclinic

211   birnessite demonstrated lower reactivity. In fact, a linear correlation ($R^2 = 0.9897$; dotted line in

212   Figure 4) between Cr(III) oxidation capacity and MnOx age can be established. Note that all

213   experiments were conducted with the same concentration of initial MnOx and Cr(III), therefore

214   the differences in reactivity was likely due to variation in the intrinsic reactivity as the oxides

215   aged from hexagonal to triclinic birnessite.

216   Despite many previous studies, the detailed reaction mechanism(s) of Cr(III) oxidation by

217   Mn oxides remain unclear. It was showed that the Cr(III) oxidation ability of different

218   manganese oxides can be related to, although not directly proportional to, the presence of

219   structural Mn(III) [21]. The availability of Mn(III) during the oxidation of Cr(III) by δ-MnO_2 was

220   also shown to be an important controlling factor in that the presence of Mn(III) complexing

221   ligand pyrophosphate during the reaction between Cr(III) and Mn oxide drastically reduced

222   Cr(III) oxidation rate [13]. The presence of Mn(II) has also been shown to enhance Cr(III)

223   oxidation by synthetic manganese oxides (e.g., hausmannite, Mn_3O_4) [21] or in systems containing

224   Mn(II)-oxidizing microorganisms [22, 31-33]. For these biotic systems, it is generally recognized that

225   the oxidation of Cr(III) is mainly due to the formation of biogenic Mn oxides that are produced

226   from the oxidation of Mn(II) by microorganisms [23, 34-36]. However, in these studies, the presence

227   of active cells complicates the investigations due to cell metabolism and/or the toxicity effect of

228 Cr(VI), thus making it difficult to isolate the structural effect of the biologically formed Mn

229 oxides. Here, we show too that light and organic carbon (Figure 3–5, Table 1) complicates the

230 direct assessment of the role of Mn oxide structure and composition on Cr(III) oxidation.

231 In particular, a substantial structural influence in the presence of carbon and light was

232 observed for the initial hexagonal birnessite dominated Mn oxide phases (aged less than 50

233 hours). For 4 h old Mn oxides, the Cr(III) oxidation capacity was highest under combined light

234 and carbon conditions, reaching ~0.20 at near steady state; whereas all other conditions (K dark,

235 ASW light, or ASW dark) showed similar Cr(III) oxidation capacity of ~0.07 (Figure 5 a). As

236 minimal Cr(VI) was produced in K light condition in the absence of Mn oxides, a mineral

237 surface produced photoactive factor was likely at play. Mn oxides are versatile oxidants of a

238 wide range of organic compounds such as hydroquinone, oxalic acid, and humic substances [44-47],

239 and the Mn oxide catalyzed oxidation of organics are known to produce reactive organic radicals

240 [45, 48]. Similarly, Learman et al [27] implicated both mineral induced organic and oxygen radical

241 formation in accelerated Mn(II) oxidation rates by colloidal hexagonal birnessite formed by this

242 *Roseobacter* species. On the other hand, it is worth noting that Cr(III) can strongly complex with

243 organic ligands such as citrate and EDTA, which can prevent the release of Cr(III) ions and

244 subsequent oxidation by Mn oxides [14, 17]. Although we observed enhanced Cr(VI) formation in

245 the presence of organics, complexation of Cr(III) by media components (K medium is a rich

246 medium containing peptone and yeast extract) could hypothetically limit the extent of Cr(VI)

247 formation. At this stage, the exact photo-active factor or organic compound(s) in our system is

248 not clearly understood; partially due to the fact the K medium is a rich organic matrix composed

249 of a large diversity of organic nitrogen and carbon molecules. The identity of the reactive

250 organic(s) and the reaction mechanisms are currently under investigation.

251   Interestingly, despite differences in Cr(VI) production by the initial hexagonal birnessite

252   phase incubated under different conditions (Figure 5a), a concomitant change in Mn oxide

253   concentration was not observed (Figure 5b). In detail, all experiments showed a similar sharp

254   decrease in the Mn oxide concentration to ~65% of the initial concentration within 20 h,

255   followed by a pseudo steady state level that was maintained at ~60% throughout the remainder

256   of the reaction (Figure 5b). The 4 and 31 h aged bioMnOx showed similar Cr(VI) production and

257   MnOx consumption trends (Figure 5a-d). For the more triclinic bioMnOx (211 h aged), however,

258   differences in Cr(VI) production and MnOx consumption varied based on the experimental

259   conditions, with the carbon replete (K) light incubations still yielding the highest Cr(VI)

260   production and the carbon-deplete (ASW) dark condition showed the largest loss of Mn(III,IV)

261   oxide.

262   The change in Mn oxide concentration likely involved a number of reactions, including

263   decreased MnOx concentration through the redox reaction with Cr(III), photo-induced

264   dissolution, and increased MnOx concentration via the abiotic oxidation of aqueous Mn(II) at the

265   Mn oxide surface (production of secondary Mn oxides) [49]. Mn oxides [50, 51], especially highly

266   reactive layered phyllomanganates [52], can accelerate the oxidation of Mn(II) by O_2 through

267   autocatalysis. Further, Learman et al [27] reported hexagonal birnessite- and light-mediated

268   formation of carbon and oxygen radicals can lead to Mn(II) oxidation rates in excess of the

269   competing photodissolution process [27]. Based on our Mn oxide and Cr(VI) concentration data,

270   we postulate that this Mn (re)cycling is a likely mechanism responsible for the high Cr(III)

271   oxidation capacity in the combined presence of light and organics in our system. In detail, during

272   the initial 20 h, during which Mn(III, IV) oxide concentration experienced a sharp decrease, the

273   system is dominated by the redox reaction between primary bioMnOx and Cr(III), which

274 produces Cr(VI) and presumably aqueous Mn(II). The produced Mn(II) can then be oxidized by

275 carbon and/or oxygen radicals produced by reaction with the remaining bioMnOx to produce

276 more secondary MnOx, which are also capable of oxidizing Cr(III). This process (cycling of

277 Mn(II)) might explain the apparent higher Cr(III) oxidation capacity yet near steady state

278 concentration of Mn oxide in the system. Alternatively, the carbon- and/or oxygen-radicals could

279 directly oxidize Cr(III) – yet, this would not explain the Mn oxide concentration trends (Figure

280 5b) in combination with the persistence of hexagonal birnessite in the presence of light and

281 carbon (discussed below).

282

3.3. Rates of Cr(III) Oxidation

284 For all conditions, the rates of Cr(III) oxidation were fastest during the initial 20 h,

285 slowly decreasing over time and plateauing between 50 and 150 hours (e.g., Figure 5a, c, and e).

286 Initial Cr(III) oxidation rates, based on the amount of Cr(VI) production during the first 20 h,

287 spanned an order of magnitude and were highly dependent upon the structure of the Mn oxide

288 and the aqueous conditions (Table 1). For instance, the initial Cr(III) oxidation rate for the 4 h

289 old bioMnOx was $0.11–0.29 \ \mu M \ h^{-1}$ under K light condition, $0.02–0.03 \ \mu M \ h^{-1}$ at K dark

290 condition, and $0.02–0.11 \ \mu M \ h^{-1}$ at ASW conditions (light and dark).

291 Previous studies on the oxidation of Cr(III) by various manganese oxides showed varied

292 reaction rates and capacity. Using Q-XAFS (quick X-ray absorption fine structure spectroscopy),

293 Landort et al [11] showed that the oxidation of Cr(III) by three difference types of synthetic poorly

294 crystalline layered manganese oxides (δ-MnO_2, random stacked birnessite, and acid birnessite)

295 were fast within the first 30 min and ceased between 30 min and 1 h, which was explained as the

296 sorption of Cr(VI) species on the Mn oxides. Dai et al [17] also observed a fast increase of Cr(VI)

16

297   levels in the presence of δ-MnO$_2$ within the initial ~50 min, followed by very slow increase of

298   Cr(VI) after 300 min, which was explained as the saturation of available surface adsorption sites.

299   Due to the difference in experimental conditions, it is difficult to directly compare the

300   oxidation rates from our system with previous studies. Therefore we compared our results with

301   Mn(II) oxidation rates by these same biooxides [27]. Consistent with Cr(III) oxidation, Learman et

302   al [27] found that more aged (more triclinic) bioMnOx phases yielded lower Mn(II) oxidation.

303   Using 4 h aged colloidal hexagonal birnessite, they reported Mn(II) oxidation rates of 0.56–2.30

304   μM h^{-1} in the presence of organic carbon (K media) and light, 0.21–0.62 μM h^{-1} in K within the

305   dark, and 0.08–0.27 μM h^{-1} in the absence of organic carbon (ASW). These values are

306   substantially higher than the Cr(III) oxidation rates observed here, likely due to the difference in

307   the nature of the reaction mechanism(s), e.g., 3 electrons are needed to oxidize Cr(III) to Cr(VI),

308   whereas only 1–2 electrons are needed to oxidize Mn(II) to Mn(III) or Mn(IV). However, both

309   datasets follow a general trend that the combination of light and organic carbon yielded higher

310   oxidation rates.

311

312   *3.4. Mn oxide structural changes upon reaction with Cr(III)*

313   The reacted bioMnOx samples appeared to be more triclinic as compared to the reference

314   compound δ-MnO$_2$ (hexagonal birnessite structure), with a diminished oscillation at ~8 Å^{-1} and

315   broad feature at ~9 Å^{-1} (Figure 2). Detailed structural analysis revealed that all reacted samples

316   transformed from the highly disordered hexagonal birnessite phase into more ordered phases

317   similar to triclinic birnessite (Figure 6 and Table S2). For instance, the out-of-plane bending

318   angles β$_a$ are generally much higher than those of the unreacted samples. Interestingly, the 4 h

319   aged bioMnOx, which possessed the highest oxidative capacity for Cr(III) in the presence of

320   light and organic carbon (Figure 4) yielded final Mn oxides with a lower β_a angle than the other

321   three conditions (K dark, ASW light, and ASW dark) (Figure 6). The 31 h aged bioMnOx also

322   showed a lower β_a angle in K light condition compared to the ASW dark condition. These

323   observations are consistent with our hypothesis that Mn(II) produced from the redox reaction

324   between Cr(III) and the (primary) bioMnOx was recycled in the system and induced the

325   (re)precipitation of (secondary) hexagonal birnessite. Such cycling was dominant in carbon-

326   replete light conditions and the produced secondary Mn oxides restored the hexagonal Mn oxide

327   reservoir in the system, thus resulting in an apparent steady state of MnOx concentration (Figure

328   5b and d) as well as lower β_a angles that correspond with the presence of more hexagonal phases

329   (Figure 6).

330   In contrast, the 211 h aged bioMnOx had similar β_a angles after reaction under carbon-

331   replete (K) light and -deplete (ASW) dark conditions. This is consistent with a similar lack in

332   Cr(VI) production under these conditions (Figure 4) and is likely due to the low reactivity of

333   these more aged bioMnOx toward Cr(III) oxidation, Mn(II) oxidation, and thus production of

334   secondary MnOx. The fact that both β_a angles were lower than those of the 4 and 31 h aged

335   bioMnOx under dark and carbon-deplete (ASW) conditions is consistent with the overall low

336   reactivity of 211 h aged bioMnOx.

337

338   *3.5. Effect of pH and Mn oxide concentration*

339   Cr(III) oxidation by abiotic Mn oxides has a strong pH dependence [3, 15]. Here the

340   oxidation of Cr(III) by 96 h aged bioMnOx reacted in the organic rich medium (K) under light

341   conditions was substantially greater at pH 7.2 relative to 6.2 (Figure 7b). At pH 7.2, Cr(III)

342   oxidation had a fast initial reaction (within ~40 h) followed by a slow yet continuous process that

18

343   reached steady state only after ~150 h, resulting in a final Cr oxidation capacity of ~0.16 (Figure

344   7b). Correspondingly, the amount of bioMnOx in the reacting suspension quickly dropped to

345   87% within ~40 h, and slowly continued to ~80% after 160 h (Figure 7a). The increase in Cr(VI)

346   and decrease of bioMnOx levels was presumably dominated by the redox reaction between

347   Cr(III) and bioMnOx, which involved the oxidation of Cr(III) to Cr(VI) and the reduction of

348   Mn(III, IV) oxides to soluble Mn(II) species.

349   Interestingly, at pH 6.2 the Cr(III) oxidation capacity quickly increased to ~0.06 within

350   ~10 h at a rate similar to that at pH 7.2, but then remained at this level throughout 160 h of

351   reaction with no further significant production of Cr(VI) (Figure 7b). Mn oxide concentration

352   decreased to ~85% during the first 10 h, and drastically dropped to only ~25% after 160 h

353   reaction time (Figure 7a). These observations are strikingly different from that at pH 7.2. At low

354   pH, less Cr(VI) was produced but there was much greater loss of Mn(III, IV) oxides. In contrast,

355   higher pH resulted in a much higher and continuous production of Cr(VI), accompanied by a

356   near steady-state concentration of Mn oxides.

357   Several studies have examined the effect of pH on Cr(III) oxidation by synthetic Mn

358   oxides [3, 10, 15], with an emphasis at acidic pH ranges. These studies observed that Mn oxides can

359   strongly oxidize Cr(III) at lower pH ranges, and the pH effects were mainly contributed to two

360   processes: (1) the adsorption of Cr(III) species on the surface of Mn oxides, which increases with

361   increasing pH due to increased surface negative charge, and is favorable for more Cr(III)

362   oxidation; and (2) the complexation of Cr(III) with hydroxyl groups and potential precipitation

363   of Cr(III)-(oxyhydr)oxide phases, which is enhanced with increasing Cr(III) concentration and

364   pH, and significantly decreases Cr(III) oxidation by Mn oxides. In our system, with the low

365   Cr(III) concentration employed (50 μM), we do not expect the second mechanism to be

366 significant. In fact, speciation calculations using the program Phreeqc [53] indicated that at both pH

367 values our system was undersaturated with respect to solid Cr(III) (oxyhydr)oxide phases and the

368 dominant Cr(III) species were $Cr(OH)_2^+$ and $CrOH^{2+}$.

369 　　The observations in our system appeared to be contradictory to previous studies in that a

370 higher pH value yielded higher Cr(III) oxidation in our system. However, it is worth noting that

371 all those studies used synthetic Mn oxides. In a few studies examining the oxidation of Cr(III) by

372 biogenic manganese oxides formed *in situ*, pH were controlled at near circumneutral values to

373 maintain cell activity [22, 31-33]. It is possible that (1) the biogenic Mn oxides formed under these

374 conditions (including our system) might undergo proton-promoted dissolution at reduced pH

375 conditions; and/or (2) that the activity of these biogenic Mn oxides toward Mn(II) oxidation and

376 production of secondary Mn oxides is lower at low pH values. The detailed reaction

377 mechanism(s) as well as the changed reactivity of bioMnOx under low pH conditions are under

378 active investigation.

379 　　The Cr(III) oxidation capacity was also dependent on the initial bioMnOx concentration.

380 Under both carbon-rich (K) light and -deplete (ASW) dark conditions, higher initial bioMnOx

381 concentrations resulted in decreased Cr(III) oxidation capacity (Figure S2). Consistent with our

382 previous observations, Cr(VI) oxidation efficiencies were higher under K light than ASW dark

383 condition. All values were obtained after the reaction proceeded for ~150 h, representing near

384 steady state values. Given all the same reaction conditions, one might expect the Cr(III)

385 oxidation capacity to maintain a constant value regardless of Mn oxide concentration. The fact

386 that it decreased with increasing Mn oxide concentration was likely due to aggregation effects.

387 Mineral particle aggregation and/or collision at high concentrations can decrease the effective

388 surface area and result in lower degrees of contact with Cr(III).

389

4. Environmental Implications

390

391   To our knowledge, this is the first study exploring the effect of structural evolution of

392 biogenic Mn oxides on the oxidation of Cr(III) under various geochemical conditions. Here, as

393 schematically illustrated in Figure 8, we show that Cr(III) oxidation occurs to a much greater

394 extent via reaction with hexagonal birnessite in the presence of both light and organic carbon.

395 The production of photo-active factors, presumably organic- and/or oxygen- radicals, likely

396 induce the (re)cycling of Mn in the system yielding substantially higher Cr(III) oxidation extent

397 and rates. These surface mediated reactions in the presence of light and organics is apparently

398 great enough to offset photo-dissolution of Mn oxides and allow for sustained oxidative capacity.

399 The regeneration of reactive hexagonal birnessite under these conditions suggests that the

400 speciation and fate of Cr in the natural environment might be dictated by even just a small

401 amount of Mn oxides. Given the ubiquitous presence of Mn oxides in nature and that

402 nanoparticulate hexagonal birnessite is the dominant Mn oxide formed by both terrestrial and

403 marine microorganisms, this cycling scenario might play a substantial role in controlling the

404 speciation of numerous redox sensitive elements, such as other metals and organic compounds.

Acknowledgements

Electronic Supplementary Information Available

XANES of reference compounds, unreacted, and reacted Mn oxides; Mn K-edge EXAFS fitting results of reference compounds and bioMnOx before and after reaction with Cr(III); Cr oxidation capacity as a function of bioMnOx concentration. This information is available at DOI:xxx.

Figure Captions

Figure 1. (a) Linear combination fitting (LCF) results of unreacted bioMnOx at different ages, with raw and fitted data in solid and dotted black lines, respectively. Also shown are the two end member spectra (gray lines) used for LCF, a hexagonal birnessite (δ-MnO_2) and triclinic Na-birnessite. (b) Corresponding LCF fraction of hexagonal and triclinic birnessite for the bioMnOx. Dotted line is a linear fitting of the hexagonal data ($R^2 = 0.9238$).

Figure 2. Mn K-edge EXAFS data (a) and corresponding Fourier transforms (b) of reference compounds (grey lines) and bioMnOx before (blue lines) or after (black lines) reacting with Cr(III) (~200 h). Raw and fitted data are shown in solid and dashed lines, respectively. Square with dotted line in (a) shows the "indicator" region of Mn EXAFS. Vertical dotted line in (b) labels the peak with major contributions from multiple scattering.

Figure 3. Amount of Cr(VI) produced in the presence of 4 h aged bioMnOx (filled bars) in artificial sea water (ASW) or K medium (K) at pH 7.2 under light (L) or dark (D) conditions. Also shown are the Cr(VI) production in control experiments (open bars), with the same amount of initial Cr(III) and no bioMnOx addition. They are conducted either in the cell-free filtrate, ASW, or K medium under light or dark conditions. Vertical dotted line is the averaged Cr(VI) production from all control experiments (0.55 μM). All values were measured when reactions reached near steady state (~200 h).

Figure 4. Cr oxidation capacity as a function of bioMnOx age when reacted in K medium or ASW under light or dark conditions. Dotted line is a linear fitting of the K light data ($R^2 = 0.9897$). Reactions were conducted at pH 7.2.

Figure 5. Cr(III) oxidation capacity (a, c, e) and percent change of Mn(III, IV) oxide concentration (b, d, f)) as a function of reaction time in the K medium or ASW under light or dark conditions. BioMnOx ages are 4 h (a and b), 31 h (d and e), and 211 h (e and f)

Figure 6. Comparison of the out-of-plane bending along a-axis (β_a angle) derived from EXAFS fitting of bioMnOx with different age and structural ripening before and after reaction with Cr(III) under different conditions.

Figure 7. Percent change of Mn(III,IV) oxide concentration (a) and Cr(III) oxidation capacity (b) as a function of reaction time at pH 6.2 and 7.2. Reaction condition: 96 h aged bioMnOx, K medium, light condition.

Figure 8. Schematic of possible reaction mechanism. Orange arrows represent reactions that occur only in the combined presence of organics and light.

Table 1. Initial Cr(III) oxidation rate and total Cr(VI) production. All reactions conducted at pH

7.2 in either K medium or ASW under light or dark conditions.

Reaction condition	BioMnOx age (h)	n[a]	Initial Cr(III) oxidation rate[b] (μmol L^{-1} h^{-1})	Total Cr(VI) production[c] (μM)
K light	4.5	6	0.11 - 0.29	9.92 ± 3.11
	12	2	0.29 - 0.34	10.42 ± 0.36
	31	1	0.2	11.20
	96	3	0.15	6.15 ± 0.05
	211	2	0.09	1.97 ± 0.32
K dark	4.5	3	0.02 - 0.03	3.53 ± 1.30
	31	1	0.06	4.36
	211	2	0.02	1.45 ± 0.08
ASW light	4.5	5	0.03 - 0.09	3.76 ±0.75
	12	2	0.15	4.46 ± 0.05
	24	3	0.08 - 0.09	3.23 ± 0.12
	31	1	0.09	2.72
	96	3	0.13 - 0.16	4.56 ± 0.18
	211	2	0.06	2.29 ± 0.12
ASW dark	4.5	4	0.02 - 0.11	3.70 ± 1.24
	31	1	0.08	2.66
	211	2	0.05	3.04

[a] Total number of experiments conducted at this condition;

[b] The rate of Cr(VI) production within initial 20 h;

[c] The total amount of Cr(VI) produced in the reaction systems at near steady state (~200 h).

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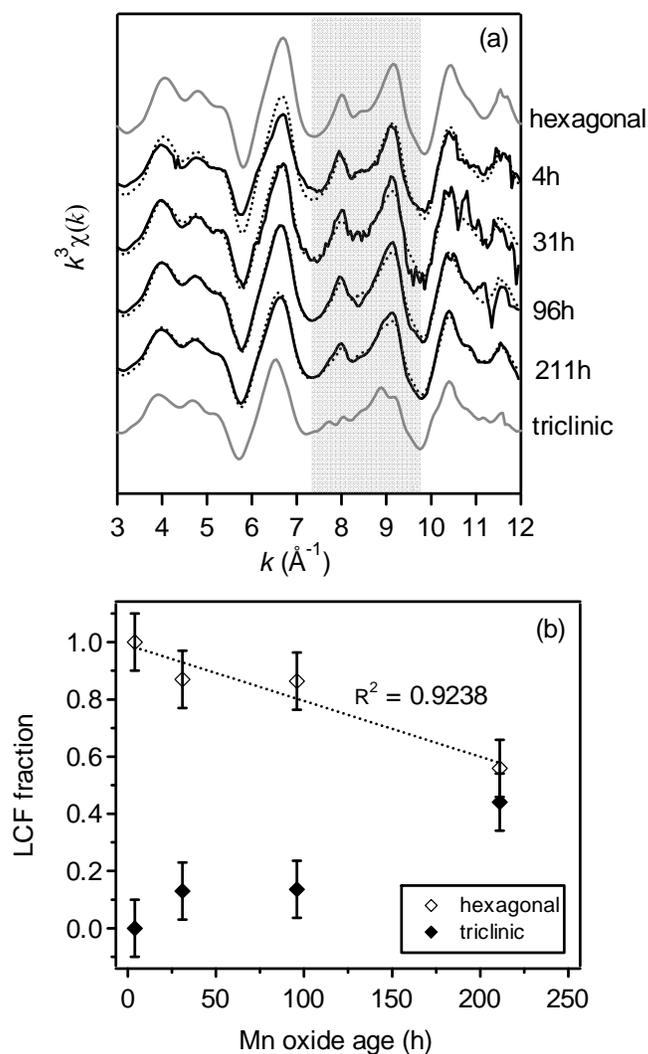
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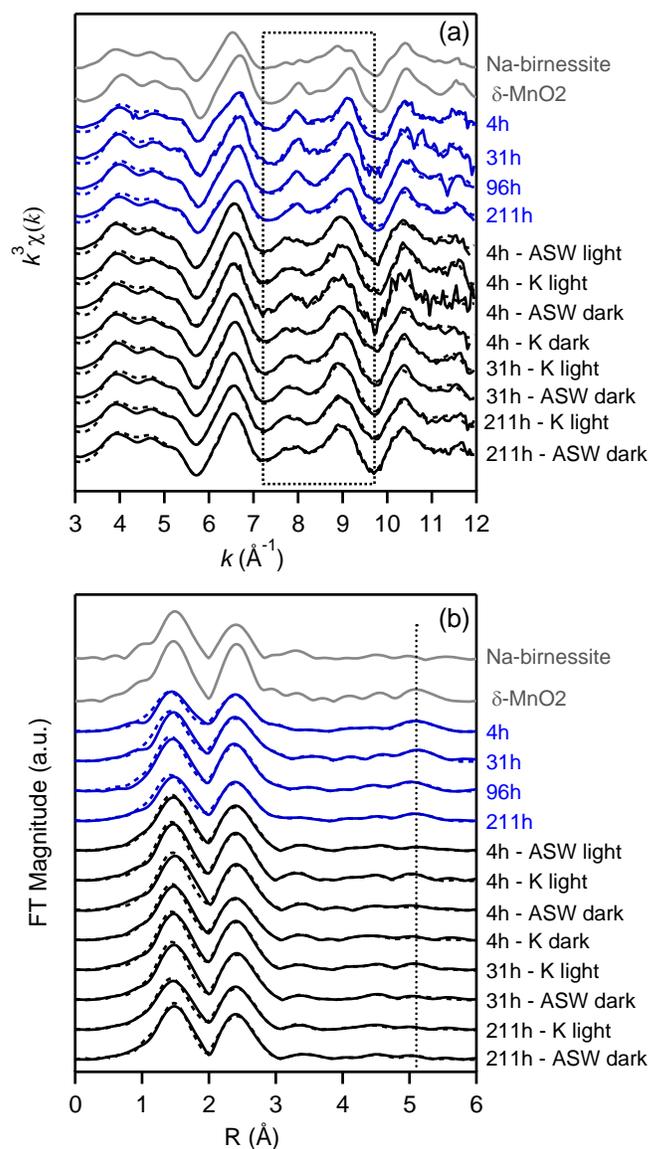
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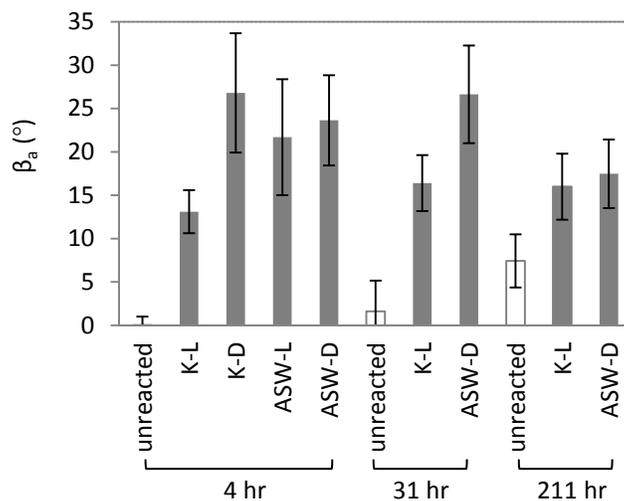
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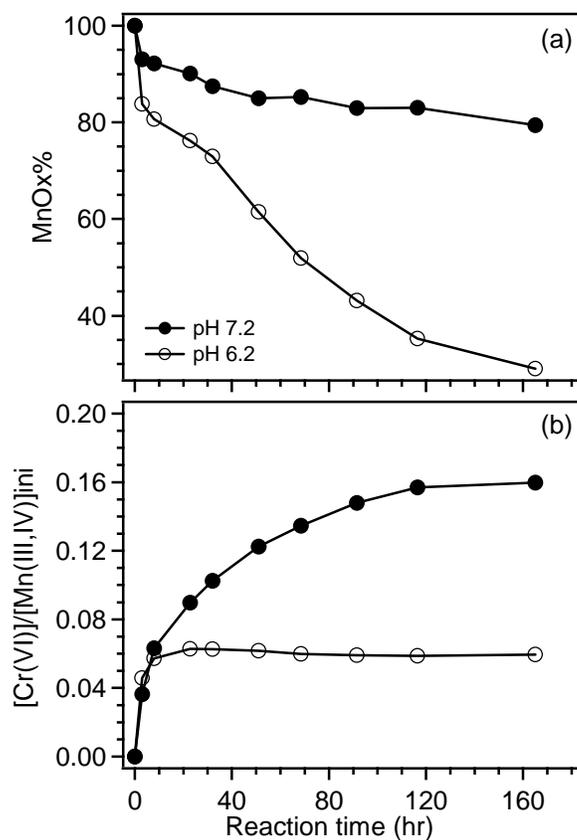
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