Kinetics of Mn(II) oxidation by spores of the marine *Bacillus* sp. SG-1

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Abstract

The kinetics of Mn(II) oxidation by spores of the marine *Bacillus* sp. SG-1 was measured under controlled conditions of the initial Mn(II) concentration, spore concentration, chemical speciation, pH, O₂, and temperature. Mn(II) oxidation experiments were performed with spore concentrations ranging from 0.7 to 11 x 10⁹ spores/L, a pH range from 5.8 to 8.1, temperatures between 4°C and 58°C, a range of dissolved oxygen from 2 to 270 µM, and initial Mn(II) concentrations from 1 µM to 200 µM. The Mn(II) oxidation rates were directly proportional to the spore concentrations over these ranges of concentration. The Mn(II) oxidation rate increased with increasing initial Mn(II) concentration to a critical concentration, as described by the Michaelis-Menten model (K_m = ca. 3 µM). Whereas with starting Mn(II) concentrations above the critical concentration, the rate was almost constant in low ionic solution (I = 0.05, 0.08). At high ionic solution (I = 0.53, 0.68), the rate was inversely correlated with Mn(II) concentration. Increase in the Mn(II) oxidation rate with the dissolved oxygen concentration followed the Michaelis-Menten model (K_m = 12-19 µM DO) in both a HEPES-buffered commercial drinking (soft) water and in artificial and natural seawater. Overall, our results suggest that the mass transport limitations of Mn(II) ions due to secondary Mn oxide products accumulating on the spores cause a significant decrease of the oxidation rate at higher initial Mn(II) concentration on a spore basis, as well as in more concentrated ionic solutions. The optimum pH for Mn(II) oxidation was approximately 7.0 in low ionic solutions (I = 0.08). The high rates at the alkaline side (pH > 7.5) may suggest a contribution by heterogeneous reactions on manganese bio-oxides. The effect of temperature on the Mn(II) oxidation rate was studied in three solutions (500 mM NaCl, ASW, NSW solutions). Thermal denaturation occurred at 58°C and spore germination was evident at 40°C in all three solutions. The activation energies

1 calculated from the Arrhenius plots are consistent with the observation that Ca ions stimulate the
2 Mn(II) oxidation rate.

3 4 1. INTRODUCTION

5
6 Mn(II) oxidation governs not only the global cycling of Mn in aquatic environments, but also
7 the cycling of other toxic and nutrient metals and organic carbon by an array of biogeochemical
8 processes. These include adsorption, oxidation/reduction, ligand binding reactions, mineral
9 precipitation/dissolution, and uptake by biological organisms. Biological catalysis is generally
10 believed to be the dominant mechanism of Mn(II) oxidation in natural waters and sediments
11 (Nealson et al., 1988; Tebo et al., 1997; Tebo et al. 2004). The effect of biological rate controls
12 on Mn(II) oxidation is strongly supported by the isolation of Mn(II)-oxidizing enzymes and the
13 identification of the putative genes responsible for Mn(II) oxidation in several different
14 Mn(II)-oxidizing bacteria: *Pseudomonas putida* MnB1 and GB-1, *Bacillus* SG-1, *Pedomicrobium*
15 sp. ACM 3067 and *Leptothrix discophora* SS-1 (van Waasbergen et al., 1996; Corstjens et al.,
16 1997; Brouwers et al., 1999, 2000; Francis and Tebo, 2001, 2002; Tebo et al., 2005; Ridge et al.,
17 2007; Geszvain and Tebo, 2010). The biologically oxidized Mn oxides typically have higher
18 specific surface areas than the abiotic Mn oxides widely employed in adsorption studies (e.g.,
19 Nelson et al., 1999; Tebo et al., 2004; Spiro et al., 2010). Thus, understanding the kinetics of
20 biogenic Mn oxide formation is required to generate the quantitative mechanistic models that
21 predict Mn cycling and the fate of toxic metals.

22 Though abiotic Mn(II) oxidation occurs on mineral surfaces, it is kinetically slow at pH
23 values < 9.0 (Davies and Morgan, 1989). The rate of production of primary Mn oxide is subject
24 to biological control at most ambient pH values (Diem and Stumm, 1984; Nealson et al., 1988).

1 The environmental rate of Mn(II) oxidation follows Michaelis-Menten kinetics (Tebo and
2 Emerson, 1986; Clement et al., 2009) and is extremely variable from environment to environment
3 (Tebo et al., 1997). For example, the rate of Mn(II) oxidation varies from around 2 pM/h in
4 deep-sea hydrothermal vent plumes (Cowen et al. 1990; Mandernack and Tebo, 1993) to 65 nM/h
5 in nearshore waters of the Black Sea (Tebo, 1991). Reported values of the Mn(II) oxidation rate
6 in freshwater can be higher, up to 350 nM/h (Tipping, 1984). A variety of different parameters
7 including oxygen, temperature, pH and metal concentration affect the rate of microbial Mn(II)
8 oxidation in the environment (e.g., Tebo et al., 1984). Many quantitative in-situ studies of
9 microbial Mn(II) oxidation in the environment have been performed (e.g., Tebo and Emerson,
10 1986; Clement et al., 2009), but knowledge of the rate determining factors is limited.

11 Previously, Zhang et al. (2002) investigated the kinetics of Mn(II) oxidation by *L. discophora*
12 SS1 in a mineral salt medium under bioreactor conditions with control of various parameters (cell
13 concentration, pH, Cu concentration, dissolved oxygen concentration, and temperature) and
14 constant chemical speciation. Toyoda and Tebo (2013) examined the chemical speciation effect
15 on bacterial Mn(II) oxidation by spores of the marine *Bacillus* sp. SG-1. They found that Ca^{2+}
16 ions strongly promote microbial Mn(II) oxidation and suggest that Ca^{2+} may exert a direct effect
17 on the enzyme as a stabilizing bridge between the components. They also observed that high
18 salt content inhibits the oxidation rate in higher ionic strength solutions ($I > 0.2$), probably due to
19 the coagulation and precipitation of Mn oxide products around the spore. Webb et al. (2005)
20 showed that Mn(II) oxidation by the spores is a result of two sequential one-step electron transfer
21 processes and the Mn(II) to Mn(III) step is the rate limiting step. Further factors that influence
22 bacterial Mn(II) oxidation are examined in the present paper, including spore concentration,
23 initial Mn(II) concentration ($[\text{Mn}]_I$), temperature, oxygen concentration, and pH. Rate laws for
24 Mn(II) oxidation by spores of the marine *Bacillus* sp. SG-1 are then proposed in terms of these

factors. To more broadly understand the kinetics of bacterial Mn(II) oxidation, comparison of the results with previous reports (e.g., Zhang et al., 2002) and relations with the Ca-effect and ionic strength effects on the rate are considered.

2. MATERIAL AND METHODS

Preparation of artificial seawater (ASW) and spores of *Bacillus* sp. strain SG-1 for incubation experiments, and measurement of Mn(II) concentration with time, are as described in Toyoda and Tebo (2013). For the kinetic experiments, 400 mL of pH-adjusted 2 mM HEPES solution was augmented with Mn(II) and placed in 500 mL acid-washed 500 mL Erlenmeyer (Pyrex) flasks at room temperature (16-24°C) or another fixed temperature. HEPES is a non-chelating buffer, and should not affect the speciation of Mn(II) (Lambeth et al., 1992). To examine the effect of temperature, incubation experiments were done at 4°C and 12°C in low-temperature rooms, and at 40 and 58°C in incubators. We performed continuous sparging incubations in which CO₂-scrubbed air or gas was supplied and designated these incubations as semi-closed systems. For the study of the effect of oxygen, we used N₂ and O₂ gas mixtures (0.195% O₂; 0.996% O₂) distributed from Airgas Inc. and the oxygen pressures were certified with an uncertainty of 2.0% by the company. The Mn(II) concentration and the pH of the media were checked and readjusted if necessary before the start of the experiment. Thawed spores were suspended in ca. 15 mL of milli-Q water and homogenized. Some of the spore suspension was pipetted into weighed 400-mL solutions so that the spore concentration was equal in different flasks in each experimental run. The number of spores in the flask was determined microscopically using a Petroff-Hauser counting chamber. The rate of Mn(II) removal was monitored by removing 1 mL aliquots at intervals, filtering through 0.2 µm disc-syringe HT Tuffryn filters (polysulfone) to

remove cells and oxidized Mn, and then measuring the dissolved Mn(II) concentration. The Mn (II) concentration was measured using the formaldoxime colorimetric technique (Brewer and Spencer, 1971) or by ICP-AES on a Seiko SPS7700 instrument. Because the minimum display value in the UV-Vis spectrometer with a 1-cm cell is equivalent to 0.11 μM , the analytical error of the colorimetric technique is $\pm 0.11 \mu\text{M}$. Replication of Mn concentrations around 1 μM and over 10 μM by ICP-AES analysis were below 5% and 1–2% relative standard deviation, respectively. Statistical analysis of regression lines and curve-fitting to the Michaelis-Menten plot were carried out using StatPlus and KaleidaGraph softwares.

Abiotic Mn(II) oxidation is inhibited at pH values < 9.0 , and a rate law for the autocatalytic abiotic oxidation of Mn(II) has been developed by Morgan and Stumm (1964). The primary solid biogenic product of Mn(II) oxidation is an X-ray amorphous oxide similar to δ - MnO_2 , which has a Mn oxidation state between 3.7 and 4.0 (Bargar et al., 2000). Reaction of Mn(II) with the primary biogenic oxide results in the production of abiotic secondary products (Bargar et al., 2005; Spiro et al., 2010). The observed decrease in the dissolved Mn(II) concentration is therefore attributed to Mn(II) adsorption on spores and subsequent Mn(II) oxidation, and is then followed by mineralogical transformation of the secondary products. Based on our preceding study (Toyoda and Tebo, 2013), an initially linear decrease in Mn(II) concentration is expected, both after saturation of the active site by Mn(II) binding, and before the production of significant amounts of MnO_x under the above conditions of incubation with bubbling of filtered air or gas. An example of the linear decrease in Mn(II) concentration is shown in Fig. 1(a). In summary, after the initial period of Mn(II) binding, the observed decrease in Mn(II) concentration within a few or several days after adding the spores is approximately equal to the biological Mn(II) oxidation rate. Mn(II) concentrations below one-half of the initial Mn(II) concentrations were

omitted from the calculation of oxidation rates of the experimental runs in this study (the complete experimental data is provided in the appendices, Tables S1–S9).

For one set of experiments in 50 mM NaCl/10 mM CaCl₂ we also determined kinetics from progress curves by measuring the Mn precipitation rate versus Mn(II) concentration in single incubation experiments at 3 different Mn(II) concentrations (10, 20 and 102 μM). Time course experiments of 10 hrs (for 10 μM Mn(II)) to about 7 d (for 102 μM) were conducted and the Mn precipitation rate and average Mn(II) concentration between two sequential time points for 8-16 time points were determined. From the resulting progress curve data (rate vs. [Mn(II)]) we analyzed the Michaelis-Menten kinetics using Eadie-Hofstee plots to derive the kinetic parameters K_M and V_{max} .

3. RESULTS

3.1 Effect of spore concentration

The relation between spore concentration and the rate of Mn(II) removal from 12 solutions with initial Mn(II) concentration ($[Mn]_i$) of 20-21 μM are shown in Figure 1. These experiments were performed at six different spore concentrations ranging from 0.7 to 11.1×10^9 spores/L at 20°C, 100% air saturation, and pH 7.4. The Mn(II) oxidation rate was perfectly proportional to the spore concentration, with a gradient of 0.098 and 0.075×10^{-9} μM/h per spore in lower ionic solution ($I = 0.08$ M; open circles in Fig.1b) and higher ionic solution ($I = 0.53$ M; closed circles in Fig.1b), respectively. The rate of Mn(II) oxidation in 50 mM NaCl and 10 mM CaCl₂ solution ($I = 0.08$ M) was 1.3 times that in 500 mM NaCl and 10 mM CaCl₂ solution ($I = 0.53$ M). In the experiments reported here (Fig. 1) the stock spore suspension contained ca. 181 mg (wet weight) of spores and 2.35×10^{10} spores total giving a concentration of 1.3×10^8

spores/mg. Although there is variability in the spore concentration and activity from preparation to preparation, we use this value to calculate the rates constants from in this paper.

3.2 Effect of initial Mn(II) concentration

The relation between Mn(II) oxidation rates and initial Mn(II) concentrations in high ionic solutions ($I = 0.53, 0.65$) at pH 7.5 is shown in Figs. 2a and 2b. The rates in the two solutions with initial Mn(II) concentration ($[Mn]_i$) in the range of 5-200 μM have a strong negative relation with the $[Mn]_i$ (Fig. 2a), with the exception of below 25 μM of Mn(II) concentration in the lower ionic solution (open circles in Fig.2a).

To investigate the effect of Mn(II) concentration in more detail in the range of 1-25 μM of $[Mn]_i$, two kinetic experiments were performed in the solutions containing 500 mM NaCl and 10 mM $CaCl_2$ ($I = 0.53$), maintaining pH 7.5, 16°C, and with other conditions constant (Fig. 2b). The Mn(II) oxidation rate at $[Mn]_i > 5 \mu M$ and 3 μM for 2 different spore concentrations was almost constant or decreased slightly. The subtle variation of the rate at $[Mn]_i$ from 3-5 to 25 μM in the experiment shown in Fig. 2b is consistent with the modest change of the rate at $[Mn]_i$ below 10-20 μM in the separate experiment shown in Fig. 2a (first three open circles) in solutions with the same chemical composition.

Biological Mn(II) oxidation is generally expected to follow Michaelis-Menten (M-M) enzyme kinetics, according to the rate law:

$$-dMn(II)/dt = V [Mn(II)]/(K_m+[Mn(II)]) = V_{max} [X] [Mn(II)]/(K_m+[Mn(II)]) \quad (1)$$

where K_m is the half velocity constant (the concentration of Mn(II) when the oxidation rate is half of the maximum), V_{max} is the maximum Mn(II) oxidation rate, and $[X]$ is the spore concentration (mg/L). The change of the rate at initial Mn(II) concentrations below 5 μM and 3 μM appears to follow the M-M model in the first and second runs, respectively. The dotted

curve in Fig. 2b is extrapolated by the data fitting to equation (1), giving $K_m = 6.3 \pm 2.9 \mu\text{mol Mn(II)/L}$ and $V^* = 0.034 \mu\text{mol Mn(II)/(hour} \cdot \text{mg spore)}$ for the first experiments (Table 1).

To investigate the effect of Mn(II) concentration in lower ionic solutions ($I = 0.05$ or 0.08 M), kinetic experiments were also performed in three solutions: 50 mM NaCl ; 50 mM NaCl and 10 mM CaCl_2 ; 50 mM NaCl and 10 mM MgCl_2 . Figure 3a shows the relation between Mn(II) oxidation rates and $[\text{Mn}]_i$ in the range of $5\text{-}200 \mu\text{M}$ and $5\text{-}50 \mu\text{M}$ at 16°C or 24°C . The Mn(II) concentration in 50 mM NaCl and 10 mM CaCl_2 solution (closed circles in Fig. 3a) was measured by ICP-AES, and the rates at $[\text{Mn}]_i$ below $10 \mu\text{M}$ are therefore less precise. Nevertheless, the changes at $[\text{Mn}]_i$ below 5 or $10 \mu\text{M}$ in the two solutions (open circles and open squares in Fig. 3a) appear to follow the M-M model; the broken curves in Fig. 3a were extrapolated by fitting equation (1) to the data, giving the parameters shown in Table 1. The rates in the two higher ionic solutions ($I = 0.08$) have a fairly negative relation with the $[\text{Mn}]_i$ (closed circles and open squares in Fig.3a). In contrast, the rate in lower ionic solution ($I = 0.05$) is constant with Mn(II) concentrations above $20 \mu\text{M}$ (closed circles in Fig.3a). The effect of ionic strength on the relation between $[\text{Mn}]_i$ and the Mn precipitation rate is shown in Appendix Fig. S1.

Plots of the progression of Mn precipitation rates versus Mn(II) concentration during time course experiments with $[\text{Mn}]_i = 10, 20$ and $102 \mu\text{M}$ for the $50 \text{ mM NaCl}/10 \text{ mM CaCl}_2$ experiments from Fig. 3a (closed circles) and the lines fitted from the Michaelis-Menten equation (eq.1) are shown in Fig. 3b. The K_M and V_{max} value derived from Eadie-Hofstee plots of the data were between $1.2\text{-}3.7 \mu\text{M/h}$ and $0.8\text{-}1.3 \mu\text{M/h}$, respectively, in close agreement with the values determined for the other kinetic measurements in low ionic solutions (Table 1, Fig. 3a). As with the experiments at high ionic strength (Fig. 2), the Mn precipitation rate at low ionic

1 strength is also retarded at high concentrations (Figs. 3a, 3b). There appears to be little
2 difference in the K_m values between high and low ionic solutions (Table 1).

3 In all experiments, there was consistently observed a decrease in the Mn precipitation rate at
4 high $[Mn]_i$, generally above a threshold value between 10-25 μM , depending on the spore
5 concentration (Table 1, Appendix Fig. S2). For example, the threshold values of $[Mn]_i$ in Fig.
6 3a were in the range of ca.10 μM and ca.20 μM with 2.0×10^9 spores/L and 5.3×10^9 spores/L,
7 respectively. In high ionic solutions ($I = 0.53$), estimated threshold values of initial Mn(II)
8 concentration in Figs. 2a and 2b were in the range of ca. 10 μM , ca. 5 μM and ca. 3 μM with 8.0
9 $\times 10^9$ spores/L, 2.0×10^9 spores/L and 1.7×10^9 spores/L, respectively (Table 1). We also
10 observed that a threshold value of $[Mn]_i$ in high ionic solution ($I = 0.68$) exists at lower than 5
11 μM with 6.0×10^9 spores/L in Fig 2a. These threshold values have positive correlations with
12 spore concentrations (Table 1; Appendix Fig. S2), but an apparent difference in maximum $[Mn]_i$
13 under M-M range per spore concentration is found between lower and higher ionic solutions ($I =$
14 0.05-0.08 and 0.53-0.68).

16 3.3 Effect of pH

17 The effect of pH on Mn(II) oxidation in low ionic solutions ($I = 0.08$) was examined for pH
18 values varying from 5.8 to 8.1, at 20°C, 100% air saturation, $[Mn]_i$ of 28-30 μM , augmented with
19 7.8×10^9 spores/L, with all other conditions constant (Fig. 4). Mn(II) oxidation rates in both 50
20 mM NaCl/10 mM $CaCl_2$ and 50 mM NaCl/10 mM $MgCl_2$ solutions indicate a pH optimum at
21 around 7.0. The rates, including the maximum rate (V_{max}), in the 50 mM NaCl/10 mM $CaCl_2$
22 solution (open circles) were between about 3.5-5 times that in the 50 mM NaCl/10 mM $MgCl_2$
23 solution (closed circles) at pH values varying from 6 to 8 (the ratio is plotted as a thin solid curve
24 in Fig.4).

Previous investigations have found that the rate of biological Mn(II) oxidation is strongly influenced by pH, and is very slow at pH values less than 6 or greater than 8.5 (e.g., Adams and Ghiorse, 1987; Zhang et al., 2002). Bailey and Ollis (1986) indicate that the effect of pH on the maximum rate of an enzymatic reaction can be modeled by the equations of the following form:

$$V = k V_{max} / (1 + [H^+]/K_1 + K_2/[H^+]) \quad (2)$$

$$(\text{pH})_{\text{optimum}} = (\text{p}K_1 + \text{p}K_2) / 2$$

where V is the Mn(II) oxidation rate per cell, V_{max} is the Mn(II) oxidation rate per cell at optimum pH, and k , K_1 and K_2 are constants. Least squares data analysis for the experimental data at pH values in the range from 6.4 to 7.1, shown in the broken and dotted curves in Figure 4, gives $k = 9.3$, $K_1 = 0.22 \times 10^{-7}$ and $K_2 = 3.8 \times 10^{-7}$ with a resulting value of $V_{max} = 1.47 \mu\text{M/h}$ at the optimum pH of 7.04 in 50 mM NaCl/10 mM CaCl_2 solution and $k = 58.4$, $K_1 = 0.04 \times 10^{-7}$ and $K_2 = 33 \times 10^{-7}$ with a resulting value of $V_{max} = 0.38 \mu\text{M/h}$ at the optimum pH of 6.94 in 50 mM NaCl/10 mM MgCl_2 solution.

3.4 Effect of dissolved oxygen (DO) concentration

The effect of O_2 on Mn(II) oxidation was investigated by measuring Mn(II) precipitation rates in three solutions at different dissolved oxygen levels (1%, 5% and 100% Air), all with 6.4×10^9 spore/L at 23°C (Fig. 5). Closed circles indicate the Mn(II) oxidation rates in commercial Volvic water, which was used as an analogue of groundwater and is essentially soft water, with pH adjusted to 7.6. Open circles and closed squares show the rates in artificial seawater (ASW) and natural seawater (NSW) that was aged and filtered to reduce the organic matter content. In all three waters, the Mn(II) precipitation rate increased with increasing dissolved oxygen concentration (DO), as described by Michaelis-Menten kinetics (Fig. 5). The following relation is used to account for the influence of O_2 on the rate of Mn(II) oxidation:

$$d\text{Mn(II)}/dt = V^{*'} [\text{DO}]/(K_m' + [\text{DO}]) [X] \quad (4)$$

where K_m' is the half velocity constant for DO, $V^{*'}$ is the maximum Mn(II) oxidation rate for DO, and $[X]$ is the spore concentration (mg/L). Upon substituting $[X] = 49 \text{ mg/L}$ (6.4×10^9 spore/L) to Eq. (4) in the text, the $V^{*'}$ values are calculated as 6.5, 3.0, 2.8 nmol Mn(II)/(hour · mg spore) for Volvic water, ASW, NSW, respectively. The three dotted curves of the rates fitted to Eq. (4) (Fig.5) gave K_m' values of 18.7 ± 1.1 , 12.5 ± 2.4 , $14.7 \pm 2.7 \text{ } \mu\text{M}$ DO for Volvic water, ASW and NSW, respectively.

3.5 Effect of temperature

Mn(II) oxidation rates were determined at different temperatures (4, 12, 23, 40, 58°C) in three solutions (500 mM NaCl, ASW, filtered NSW solutions) at pH = 7.4-7.7 with bubbling of CO₂-scrubbed-air with approximately 35 μM Mn(II) and augmented with 1.2×10^9 spores/L, with all other conditions constant (Fig. 6). Fig. 6a shows the variation in Mn(II) removal in 500 mM NaCl. At 58°C, no Mn(II) oxidation is observed. Mn(II) oxidation activity was recovered after cooling to room temperature from 58°C, but the rate is clearly slower than that at 23°C from the start of the experiment. At 40°C the Mn(II) oxidation rate is initially rapid but gradually slows down, whereas the rates at 4, 12, 23°C are constant across the range of times measured. These phenomena at 40°C and 58°C are also observed in both ASW and NSW solutions (Figs. 6b and 6c).

Taking the initial rate at 40°C (the broken line in Fig. 6a), the increase in the Mn(II) oxidation rate between 4 and 40°C is clearly observed in 500 mM NaCl, and can be attributed to the activation temperature of the reaction. The increase in the rate between 4 and 40°C is also observed in ASW and filtered NSW solutions (Figs. 6b and 6c), but the variations are smaller than in 500 mM NaCl solution (Fig. 6a). The rate is expected to vary in this region according to

the Arrhenius equation:

$$d[\text{Mn(II)}]/dt = (k)_T [X] = A \exp(-Ea/RT) [X] \quad (5)$$

where $(k)_T$ is the reaction rate at absolute temperature T , Ea is the activation energy, A is a constant, and $[X]$ is the spore concentration (mg/L). A plot of $\ln V$ versus $1/T$ (Fig. 7) results in a straight line of slope $-Ea/R$ in each solution, giving the apparent activation energies (Ea) for the Mn(II) oxidation reaction as 33, 15.5 and 2.2 kJ/mol in 500 mM NaCl, NSW and ASW solutions, respectively. The difference between the calculated activation energies in the three solutions is apparent even though the conditions are almost the same (pH= 7.4 - 7.7; DO = 8.2 mg/L; $[\text{Mn}]_i$ = ca. 35 μM ; 1.2×10^9 spore/L) except for Ca^{2+} , the possible presence of very small amounts of organic matter (in NSW) and ionic strength.

4. DISCUSSION

4.1 Effect of spore concentrations

The rates of Mn(II) oxidation are dependent on the concentration of the spores used in the experiments. There is significant variability of the value from spore preparation to spore preparation because of difficulty in obtaining accurate spore counts due to spore clumping, differences in the percentage of active spores in each preparation, differences in the properties of the exosporium where the Mn oxidase is found (Francis and Tebo, 2002), different site density of the active enzyme and/or some other factor. In our experiments reported here we estimated a concentration of 1.3×10^8 spores per milligram and used this value in determining the number of spores used in our experiments and when specifying rates constants. This value is similar to previously reported values. De Vrind et al. (1986) reported a value of $1.5 \times 10^8 \pm 0.2 \times 10^8$ spores per milligram (wet weight), and Mandernack et al. (1995) reported a value of 0.64×10^8

1 spores per milligram (wet weight).

2 We observed a linear relationship between the concentration of spores in both lower and
3 higher ionic solutions with rates 30% slower at higher ionic strength (Fig. 1, Appendix Fig. S2).
4 This is consistent with previous studies conducted with an initial Mn(II) concentration of 50 μ M
5 and pH 7.7 which showed a 2-fold difference in the rates between these same two solutions
6 (Toyoda and Tebo, 2013). We previously concluded that the slower rate was due to coagulation
7 and precipitation of Mn oxides around spores which interfered with Mn(II) binding and oxidation
8 in solutions of ionic strength higher than 0.2 M (Toyoda and Tebo, 2013). The difference in the
9 ratios (1.3 versus 2.0) suggests the effect on coagulation and precipitation around the spores is
10 greater at higher $[Mn]_i$.

12 4.2 Effect of initial Mn(II) concentration

13 The effect of $[Mn]_i$ is different in high versus low ionic strength solutions. We saw
14 inhibition of Mn precipitation in high ionic strength solutions at $[Mn]_i$ above about 5 μ M and
15 inhibition above about 25 μ M in low ionic strength solutions (Figs. 2 and 3). Like the
16 differences observed in the spore concentration experiments, we believe that the decline with
17 Mn(II) concentration is due to the coagulation and precipitation of Mn oxides around spores
18 (Toyoda and Tebo, 2013). As spores become encrusted with Mn oxide the rates of Mn(II)
19 oxidation decrease, apparently as a result of masking of the active Mn(II) binding sites (de Vrind
20 et al., 1986; Hastings and Emerson, 1986; Pecher et al., 2003). It is possible that increasing
21 Mn(II) concentration in the solution generates thicker encapsulation of secondary Mn oxide
22 around the spore inducing the decline in the Mn precipitation rate. A similar phenomenon is
23 also observed with Mn(II) concentrations over 200 μ M in a solution with lower ionic strength (I
24 < 0.05). Mass transport limitations of Mn(II) may limit the Mn precipitation rate in the solution

of higher $[Mn]_i$ and thereby mask the normal M-M type kinetic response. High concentrations of manganese have also been observed to inhibit the manganese oxidation rate in a marine environment (Tebo and Emerson, 1985).

We determined relatively consistent K_m values for both low and high ionic strength solutions (Table 1). These values are comparable to the K_m values of about 6 μM for both *P. putida* strain GB-1 and *L. discophora* (Okazaki et al., 1997; Adams and Ghiorse, 1987; Zhang et al., 2002) but higher than observed in the environment. For example, in the seasonally stratified fjord, Saanich Inlet, British Columbia Canada, at 85-115 m depth the environmental K_m values for Mn(II) ranged from 0.4 to 0.9 μM (Tebo and Emerson, 1986).

4.3 Effect of pH

We observed an optimum pH of ~ 7.0 in two solutions of low but equal ionic strengths, 50 mM NaCl/10 mM $CaCl_2$ and 50 mM NaCl/10 mM $MgCl_2$ (Fig. 4). The optimum pH matches well the pH optimum of ~ 7 for the partially purified Mn^{2+} -oxidizing factor of *Pseudomonas putida* strain GB-1 (Okazaki et al., 1997). Ca^{2+} strongly stimulated Mn(II) oxidation consistent with results reported previously for high ionic strength solutions (Toyoda and Tebo, 2013). For both solutions, although the ability of Eqn. (2) to fit the experimental data was good in the pH range of 6.0 to 7.1, the rates at $pH > 7.5$ were higher than the fitted curves (Fig. 4). This discrepancy of the rates at $pH > 7.5$ (Fig.4) is likely due to the contribution of heterogeneous Mn(II) oxidation at the Mn oxide surface as shown by Bargar et al. (2005).

The kinetics of abiotic Mn(II) oxidation follows the rate law:

$$-d[Mn(II)]/dt = k_1[O_2][OH^-]^2[Mn(II)] + k_2[O_2][OH^-]^2[Mn(II)][MnO_x]$$

(3)

1 where $k_1 = 4 \times 10^{12} \text{ M}^{-3} \text{ day}^{-1}$ and $k_2 = 1 \times 10^{18} \text{ M}^{-4} \text{ day}^{-1}$ for experiments with $\text{pH} > 8$ at 20°C (Diem
 2 and Stumm, 1984; von Langen et al., 1997). The first term on the right hand side of the
 3 equation represents homogeneous oxidation and the second term describes a heterogeneous
 4 mechanism due to oxidation at the manganese oxide surface. Considering the inorganic Mn
 5 oxidation rate in a solution containing $5 \text{ } \mu\text{M}$ Mn oxide and $25 \text{ } \mu\text{M}$ of Mn(II) at $\text{pH} = 8$ with
 6 air-bubbling and substituting $[\text{O}_2] = 230 \text{ } \mu\text{M}$ and $[\text{OH}^-] = 1 \times 10^{-6} \text{ M}$ to Eq.(3), the removal rate
 7 of Mn(II) was calculated as $-\text{d}[\text{Mn(II)}]/\text{dt} = 0.96 \times 10^{-3} + 1.2 \times 10^{-3} \text{ } \mu\text{M/h}$. Thus, both terms of
 8 Eq.(3) are negligible in this experiment. However, the velocity constant k_2 in Eq.(3) was
 9 measured as the autocatalytic reaction on the surface of inorganically-synthesized Mn oxides, but
 10 not biogenic Mn oxides (Diem and Stumm, 1984; von Langen et al., 1997). The primary
 11 biogenic oxide produced by SG-1 and other Mn(II)-oxidizing bacteria is a highly reactive catalyst
 12 for further Mn(II) oxidation reactions (Bargar et al., 2000, 2005; Learman et al., 2011). Thus,
 13 the surface of the initial Mn(IV) bio-oxide appears to promote the autocatalytic reaction more
 14 than that of synthetic Mn(IV) oxide such that the contribution can be significant at the higher pH
 15 range studied. We previously showed that higher ionic strength of the solution ($I > 0.2$)
 16 accelerates the precipitation of Mn oxides around the spores (Toyoda and Tebo, 2013). If the
 17 autocatalytic reaction on the bio-oxide contributes significantly to the total rate in the higher pH
 18 range as shown in the second term of Eq.(3), the contribution could shift the apparent optimum
 19 pH to a higher value. If so, this may explain the higher maximum pH (7.6) in artificial
 20 seawater (ASW) in the presence of 10 mM Ca^{2+} with $[\text{Mn}]_i = \text{ca. } 50 \text{ } \mu\text{M}$ as previously observed
 21 (Toyoda and Tebo, 2013). In the absence of Ca^{2+} , the contribution of the autocatalytic reaction
 22 should be more prominent. Although we initially assumed that autocatalytic Mn oxidation
 23 would be negligible in the pH range of 6-8, it appears that the discrepancy in optimum pH

1 between the low and high ionic strength experiments can be explained by the highly reactive
2 biogenic Mn oxides (Spiro et al., 2010; Learman et al., 2011).

4 4.4 Effect of dissolved oxygen (DO) concentration

5 The rate of Mn(II) oxidation with respect to O₂ concentration follows Michaelis-Menten
6 kinetics with the K_M' being in the range of 12.5 – 18.7 μM for different media (Fig. 5). This
7 result contrasts with the Mn(II) oxidation rate with *L. discophora* SS1, which is directly
8 proportional to dissolved oxygen concentration (Zhang et al., 2002). These K_M' values are
9 relatively larger than the environmental O₂ half-saturation constants for Mn(II) removal rates
10 (0.30-10.5 μM DO) in the Black Sea suboxic zone (Clement et al., 2009) and in the hydrothermal
11 plume at the Guaymas deep-sea Basin (~10 μM DO; Dick et al., 2009). Nevertheless, our result
12 in Volvic water ($K_M' = \text{ca. } 19 \mu\text{M}$ DO) also indicates rapid microbial Mn(II) oxidation can occur
13 in suboxic (dissolved oxygen < 30 μM ; Langmuir, 1997) groundwater as well as in suboxic
14 seawater. The small difference in the K_M' values (ca. 19 μM and 12-15 μM) between low ionic
15 strength Volvic water and high ionic strength solutions (ASW and NSW; Fig.5) is likely due to
16 the mass transport limitations of dissolved O₂ and/or Mn(II) ions to the spore surface caused by
17 the flocculation of Mn(III, IV)-bearing secondary reaction products around spores in the solution
18 of higher ionic strength. The ionic strength of the NSW and ASW is ca. 0.70 and the $[\text{Mn}]_i$ in
19 these experiments were in the range of 48 to 50 μM (Fig.5), a range where significant inhibition
20 of the Mn precipitation rate occurs in NSW and ASW (Fig. 2a). The similarity in the K_M'
21 values in the three solutions therefore indicates that the significant decrease in the Mn
22 precipitation rate is caused by mass transport limitations of Mn(II) ions, rather than by dissolved
23 oxygen.

4.5 Effect of temperature

The Mn precipitation rate was completely inhibited at 58°C, however, the spores were still capable of oxidizing Mn(II) when the temperature was shifted to 23°C, albeit at a slower rate than without the heat treatment (Fig. 6a). This result suggests that denaturation of the enzyme is severe at 58°C, but is reversible to some extent. Thermal inhibition might begin at around 40°C as suggested by the non-linear rate of Mn(II) removal which decreases with time. In addition to denaturation of the enzymes this may be due to heat shock of the spores. Whereas dormant spores have the ability to oxidize Mn(II), as they begin to germinate they lose this ability; the vegetative SG-1 cells do not oxidize Mn but are capable of reducing Mn oxides during cellular growth (Tebo et al., 1997). High temperature is known to stimulate spores to germinate (Turnbull et al., 2007), and spore germination at 40°C could account for the gradual decrease in the Mn(II) removal rate. This is in agreement with observations by Rosson and Nealson (1982), who reported that temperatures greater than 45°C inhibited manganese binding and oxidation.

We determined a range of apparent activation energies (E_a) for the three different solutions tested (500 mM NaCl, NSW and ASW with 10 mM Ca^{2+}). We know of no other reports of the activation energies for microbial Mn oxidation. However, Zhang et al. (2002) reported that the Mn(II) oxidation rate by *L.discophora* SS1 increases by about ten times per 20°C increase from 10°C to 30°C; from an Arrhenius plot of their data the apparent activation energy can be estimated to be about 96 kJ/mol (not shown in Zhang et al., 2002), significantly higher than our results with SG-1 spores.

In Figs. 6a, 6b and 6c, the Mn(II) precipitation rates at 23°C in 500 mM NaCl, filtered NSW, ASW solutions are 34, 74, 83 $\mu\text{M/h}$, respectively. The larger rate in ASW and filtered NSW solutions ($\text{Ca}^{2+} = 10 \text{ mM}$; $I = \text{ca. } 0.7$) than in 500 mM NaCl solution ($\text{Ca}^{2+} = 0 \text{ mM}$; $I = 0.5$) is due to the effect of Ca and the difference in the ionic strength of the solutions. The rate in ASW

(Ca²⁺ = 10 mM; *I* = ca. 0.7) at 23°C in Fig.6c was 2.5 times of that in the 500 mM NaCl solution (Ca²⁺ = 0 mM; *I* = 0.5) at 23°C in Fig.6a. This is consistent with the magnitude of the relative activation energy values (31 and 2.5 kJ/mol) calculated in Fig. 7. We have shown that the rate in Ca²⁺-containing solutions is about 4 times that in the absence of Ca²⁺ at 20°C with varying pH values from 6 to 8 at ionic strength (*I* = 0.08) in Fig. 4.

The lower rate in filtered NSW solutions than in ASW solution may be due to reductive reaction by dissolved natural organic matter in the NSW solution (Toyoda and Tebo, 2013). This may also explain why the calculated activation energy (11 kJ/mol) in filtered NSW is larger than that (2.5 kJ/mol) in ASW. The three experiments in Fig. 7 were performed in high ionic solutions (*I* = 0.5, 0.7) with [Mn]_i = 34-36 µM and 1.2 x 10⁹ spores/L (Fig.6). Under these conditions, the mass transport limitations of Mn(II) ions due to secondary Mn oxide products gathering round the spores cause a significant decrease of the oxidation rate which affects the activation energy of the enzyme. Thus our results may be higher than the actual activation energy values. The estimation of activation energies of the kinetics on the spore surface will require future work in lower ionic solutions with lower initial Mn(II) concentrations.

5. CONCLUSION

Our kinetics study at different initial Mn(II) concentrations suggests that the formation of secondary Mn oxide products around the spores significantly decreases the Mn(II) oxidation rate at higher initial Mn(II) concentrations, as well as in higher ionic strength solutions as previously reported (Toyoda and Tebo, 2013). Changes of the Mn(II) oxidation rate with the DO concentration also follow the Michaelis-Menten model (*K*_m = ca. 17 µM) in both seawater and freshwater and provides laboratory evidence for rapid microbial Mn(II) oxidation under suboxic

1 conditions as previously observed in the environment (e.g., Clement et al., 2009). The effect of
2 pH on Mn(II) oxidation in low ionic strength solutions suggests the contribution from the
3 auto-oxidation of Mn(II) on the surface of extremely reactive preformed bacteriogenic Mn oxides
4 by SG-1 spores (Spiro et al., 2010) at pH values above the optimum pH, even at pH values as low
5 as 7.6, where abiotic oxidation is believed to be extremely slow. The temperature experiments
6 and calculation of the energies of activation along with the other experiments in the presence and
7 absence of Ca^{2+} also support the role of Ca^{2+} in the catalytic activity of the Mn(II)-oxidizing
8 enzyme. The major findings in this study are summarized in Table 2, which compares
9 parameters between low and high ionic strength solutions.

10 Our research also demonstrates that each Mn oxidizing bacterium might be different and have
11 different kinetics and effects of environmental conditions. The rate equations (Eqn.1, 2, 3)
12 describing the results presented in this research are significantly different than the kinetics of
13 Mn(II) oxidation by *Leptothrix discophora* SS1 (Zhang et al., 2002). The Mn(II)-oxidizing
14 bacteria *Pedomicrobium*, *Leptothrix discophora* and *Pseudomonas putida* are all Proteobacteria,
15 whereas the *Bacillus* sp. Strain SG-1 used in this study belong to the Firmicutes phylum.
16 Additionally, it is the spores that oxidize Mn(II) and not the vegetative cells. Dick et al. (2008)
17 and Butterfield et al. (2013) concluded that the Mn(II)-oxidizing protein found in SG-1 is a new
18 class of MCO. Recently, a non-MCO type of manganese-oxidizing enzyme was identified in
19 different α -proteobacteria (Johnson and Tebo, 2008; Anderson et al., 2009; Andeer et al., 2015).
20 Thus, because Mn(II) oxidizing bacteria are extremely phylogenetically diverse (Tebo et al.,
21 1997; Francis and Tebo, 2002), further integration of the kinetics for biogenic Mn oxide
22 formation are needed to predict Mn cycling and related chemical fates.

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2
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Figure Captions:

Fig. 1 (a) Example of a time-course experiment: Linear decreases in Mn(II) concentration. (b) Effect of spore concentration from 0.7 to 11×10^9 spores/L on the initial rate of Mn(II) oxidation in 50 mM NaCl/ 10 mM CaCl_2 solution ((a) and open circles in (b)) and 500 mM NaCl/ 10 mM CaCl_2 solution (closed circles in (b)), maintaining pH 7.5 with CO_2 -scrubbed-air and with 2 mM HEPES buffer, at 20°C . The rates are directly proportional to the spore concentration. Error bars represent one standard error of the rate measurements.

Fig. 2 Mn oxidation rates as a function of initial Mn(II) concentration in high ionic solutions ($I = 0.53, 0.68$), maintaining pH at 7.5 with CO_2 -scrubbed-air and with 2 mM HEPES buffer. Error bars represent one standard error of the rate measurements. (a) Initial Mn(II) concentration from 5 to 200 μM : solution of 500 mM NaCl and 10 mM CaCl_2 ($I = 0.53$) with 8.0×10^9 spores/L at 20°C (open circles); solution of 650 mM NaCl and 10 mM CaCl_2 ($I = 0.68$) with 6.0×10^9 spores/L at 24°C (closed circles). (b) Initial Mn(II) concentration from 1 to 25 μM in 500 mM NaCl/ 10 mM CaCl_2 ($I = 0.53$) at 16°C . The spore concentrations were 2.0×10^9 spore/L in first run solutions (open circles) and 1.7×10^9 spore/L in second run solutions (open squares). The rate corresponding to Michaelis-Menten kinetics for Mn(II) (dotted curve) is limited in solutions of Mn(II) concentrations below ~ 5 μM , while the rate is fairly constant in Mn(II) concentrations from 5 to 25 μM .

Fig. 3 Mn oxidation rates as a function of Mn(II) concentration in low ionic solutions ($I = 0.05$,

0.08), maintaining pH at 7.5 with CO₂-scrubbed-air and with 2 mM HEPES buffer. The resulting parameters K_m and V^* are listed in Table 1: 50 mM NaCl solution (open circles) with 5.3×10^9 spores/L at 24°C, 50 mM NaCl/10 mM CaCl₂ solutions (closed circles) with 2.0×10^9 spores/L at 16°C, and 50 mM NaCl/10 mM MgCl₂ solutions (open square) with 3.9×10^9 spores/L at 24°C. The rate corresponding to Michaelis-Menten kinetics for the initial Mn(II) concentrations (dashed curves) is limited to solutions with Mn(II) concentrations below 10 or 20 μ M, while the rate is fairly constant in Mn(II) concentrations above 20 μ M. Error bars represent one standard error of the rate measurements. (b) The progress curves (dashed lines) fit the data from the time course of Mn precipitation rates with Mn(II) concentration with $[Mn]_i = 10, 20, \text{ or } 102 \mu\text{M}$ (see closed circles in Fig. 3a).

Fig. 4 The effect of pH on Mn(II) oxidation was examined in low ionic solutions ($I = 0.08$) with 2 mM HEPES buffer and 7.8×10^9 spores/L, at 20°C. The initial Mn(II) concentrations are in the range of 27 to 31 μ M. In both experiments, 50 mM NaCl and 10 mM CaCl₂ solutions (open circles) and 50 mM NaCl and 10 mM MgCl₂ solutions (closed circles), the maxima in the Mn(II) oxidation rate were observed at around pH 7.0. Error bars represent one standard error of the rate measurements. The thin solid curve indicates the change of the ratio in rates between the two low-ionic strength solutions ($I = 0.08$) with or without 10 mM of CaCl₂ as a function of pH. The broken and dotted bell-shaped curves indicate Bailey and Ollis (1986)'s equation fitting to the data of rates by least squares data analysis.

Fig. 5 Effect of dissolved oxygen concentration on Mn oxidation rates in three solutions gas-sparged with different dissolved oxygen levels (0.195%, 0.996% and 20.9% O₂),

maintaining pH at 7.6 to 7.9 with CO₂-scrubbed-gas, 2 mM HEPES buffer, and 6.4×10^9 spores/L, at 23°C. The O₂ concentrations in the air-sparged waters were calculated using a fitting equation for the dependence of O₂ solubility on salinity and temperature (Benson and Krause, 1984). Closed circles indicate Mn oxidation rates in commercial Volvic water (salinity 0.1‰; 0.4 mM Na; 0.2 mM Ca) at pH = 7.6. Open circles show the rates in artificial seawater (ASW; salinity 34‰; at pH = 7.8). Closed squares indicate the rates in aged and filtered natural seawater (NSW; salinity 34‰) at pH = 7.9. The curve-fitting of the rates to the Michaelis-Menten plot gave K_m' values as shown in the figure and V_{max} values of 0.320 ± 0.005 , 0.145 ± 0.007 , 0.136 ± 0.006 mg/L DO ($R^2 = 0.9998$, 0.9941 , 0.9957) for Volvic water, ASW and NSW, respectively.

Fig. 6 Change in Mn(II) oxidation rates with varying temperature (4, 12, 23, 40, 58°C) at 100% Air saturation for each temperature: (a) 500 mM NaCl solutions at pH = 7.5, (b) Filtered natural seawater at pH = 7.65, (c) Artificial seawater at pH = 7.4. Heat stimulates spores to germinate at 40°C, and spore germination by heat shock gradually slows the Mn removal rate. Thermal deactivation of the enzyme at 58°C is observed, but Mn oxidation activity restarted after cooling to room temperature. The three experiments were performed in 2 mM HEPES buffer with bubbling of CO₂-scrubbed-air, augmented with 1.2×10^9 spores/L.

Fig. 7 Effect of temperature on Mn oxidation rates in 500 mM NaCl, ASW, and NSW. The rates measured in the experiments of Figs. 6a-6c are described by Arrhenius plots ($\ln V$ versus $1/T$) for the Mn oxidation rates (V) at a given absolute temperature (T). The apparent activation energies for the Mn(II) oxidation reaction were determined as 32.8 ± 2.9 , $15.5 \pm$

1 3.6, and 2.2 ± 0.3 kJ/mol in the respective solutions of 500 mM NaCl, NSW, and ASW.

2 Error bars represent one standard error of the rate measurements.

Table 1. Experimental parameters for kinetic analysis of Mn oxidation and the resulting Michaelis-Menten parameters (pH ≈ 7.5).

Fig. no. and symbol for experimental data	initial [Mn] (μM)	Ionic strength of solution (M)	[Ca ²⁺] (mM)	Temp. (°C)	Spore concentration (x10 ⁹ spore/L)	Maximum [Mn] _i (μM) [#]	Michaelis-Menten kinetics			
							<i>K_m</i> (μmol Mn(II)/L)	<i>V_{max}</i> (μmol Mn(II)/L·hr)	<i>V_{max}</i> (μmol Mn(II) /(hr·mg spore))*	<i>R</i>
● in Fig.2(a)	5 - 194	0.68	10	24	6.0	< 5	---	---	---	---
○ in Fig.2(a)	5 - 200	0.53	10	24	8.0	10 - 20	---	---	---	---
○ in Fig.2(b)	1 - 25	0.53	10	16	2.0	ca.5	6.3 ± 2.9	0.53 ± 0.16	0.034 ± 0.010	0.992
□ in Fig.2(b)	2 - 14	0.53	10	16	1.7	ca.3	---	---	---	---
○ in Fig.3(a)	5 - 198	0.05	0	24	5.3	ca.20	4.9 ± 0.9	1.29 ± 0.08	0.032 ± 0.002	0.997
□ in Fig.3(a)	2 - 52	0.08	0	16	2.0	ca.10	2.6 ± 1.1	0.61 ± 0.11	0.040 ± 0.007	0.995
● in Fig.3(a)	5 - 200	0.08	10	24	3.9	(< 10)	---	---	---	---
□ in Fig.3(b)	9.99	0.08	10	24	3.9	---	1.25 ± 0.72	1.40 ± 0.22	0.047 ± 0.007	0.917
○ in Fig.3(b)	20.2	0.08	10	24	3.9	17	3.4 ± 0.4	1.39 ± 0.06	0.046 ± 0.002	0.990
● in Fig.3(b)	102	0.08	10	24	3.9	12 - 23	1.53 ± 0.43	0.81 ± 0.08	0.027 ± 0.003	0.986

±: standard deviation calculated for each parameter by kaleidagraph software
*: Calculated by the value of 1.3 x 10⁹ spores per miligram
#: The maximum [Mn]_i below which kinetics follow Michaelis-Menten kinetics

Table 2. Summary comparison of the experimental parameters examined and their effect on the Mn oxidation rate in low and high ionic strength solutions.

Parameter*	Low ionic strength soln. (I = 0.05, 0.08 or less)	High ionic strength soln. (I = 0.53, 0.68 or NSW)	Reference figure
Spore concentration	positively correlated	positively correlated	Fig.1
Effect of $[Mn]_i$ on the Mn oxidation rate#	strongly inversely correlated below 100 μM of $[Mn]$	weakly inversely correlated or constant rate	Figs.2(a), 3(a)
Mn concentration range of Michaelis-Menten kinetics	$[Mn] < \text{ca. } 20 \mu M^{\wedge}$	$[Mn] < \text{less than } 10 \mu M^{\wedge}$	Fig. S3
K_m of $[Mn]$	ca. 3 μM	$6 \pm 3 \mu M$	Figs.2, 3
max pH	7.0	7.5	Fig.4,
Effect of Ca^{2+} on the Mn oxidation rate	$[Ca] = 10 \mu M$ accelerates 3.5-5 times (pH = 6 to 8)	$[Ca] = 10 \mu M$ accelerates 4-5 times (pH = 7.5, 7.8, and 7.95)	Toyoda and Tebo (2013)
K_m of $[O_2]$	ca. 14 μM O_2	ca. 19 μM O_2	Fig.5

*Parameters tested in this study that affect the rate of Mn oxidation

#For the range of $[Mn]_i$ that follows Michaelis-Menten kinetics

⁹
^at 5×10^9 spore/L, pH = 7.5

Figure

Fig.1(a)

Figure

Fig.1(b)

Fig.2 (a)

Fig.2 (b)

Fig.3 (a)

Figure

Fig.3 (b)

Figure

Fig. 4

Fig.5

Fig.6a

Figure

Fig.6b

Figure

Fig.6c

Figure

Fig.7