The Products of Manganese (II) Oxidation

Alana Perkins

Philander Smith College

Jenny Dominguez

Merced Community College

Office of Science, Student Undergraduate Laboratory Internship (SULI)

Stanford Linear Accelerator Center

Menlo Park, CA

August 12, 2004

Prepared in partial fulfillment of the requirements of the Office of Science, Department of Energy's Science Undergraduate Laboratory Internship under the direction of J.Bargar in the Stanford Synchrotron Radiation Laboratory at Stanford Linear Acceleration Center.

Participants: Alana Perkins and Jenny Dominguez
Signature

Research Advisor: John Bargar
Signature

Table of Contents

Abstract	i.i.
Introduction	1
Materials and Methods	2
Results	8
Discussion and Conclusions	11
Acknowledgements	11
References	12

ABSTRACT

The Products of Manganese (II) Oxidation. ALANA PERKINS (Philander Smith College, Little Rock, AR, 72202) JENNY DOMINGUEZ (Merced Community College, Merced, CA, 95342) JOHN BARGAR (Stanford Linear Accelerator Center, Menlo Park, CA, 94025).

Manganese, the second most abundant transition metal in the earth's crust, exists in a number of oxidation states, among which the II, III, and IV oxidation states are of greatest environmental importance. Produced through microbial activity, manganese oxides help to mediate redox reactions with organic and inorganic compounds and help to sequester a variety of metals. The mechanism by which Manganese (II) is oxidized to Manganese (IV) is a biologically catalyzed process. There are at least three different pathways by which Mn(II) can be bacterially oxidized to Mn(IV); the first in which states that Mn(II) can be oxidized to mixed Mn(III, IV), and Mn(IV) oxides and oxyhydroxides. The second of these pathways is that Mn(II) can be directly oxidized to Mn(IV) and the last of these pathways is that Mn(II) follows an enzymatic bond with a Mn(III) intermediate in which Mn(II) oxidizes to Mn(III) and then to Mn(IV). The pathways of focus for this research are the latter two pathways.

INTRODUCTION

Manganese is the second most abundant transition metal that is found within the earth's crust. It exists in a variety of differing oxidation states. Among the most common of those oxidation states are Mn (II), (III), and (IV), in the environment (Tebo et. al, 1997). Biologic oxidation of Mn(II) to Mn (IV) is five orders of magnitude faster than the abiotic oxidation by O₂. Therefore, biologic processes have an enormous impact on the oxidation cycle in the environment. Mn is important because it is an essential micronutrient for life. Mn (IV) oxides are an example of these nutrients and their functions. Biological functions of Mn (IV) oxides are protecting organisms from toxic heavy metals, from reactive oxygen species, UV light, predation, or viruses (Ghirose 1984, Emerson 1989); storage of an electron acceptor for later use in anaerobic respiration (Tebo 1983); breakdown of refractory organic matter into utilizable substrates (Sunda and Kieber 1994); and scavenging of micronutrient trace metals. (Bargar, Webb 298). Mn (III) and Mn (IV) oxides are important mineral phases that play important roles in elemental biogeochemical cycles in sediments, waters, and soils. They are some of the strongest oxidants found naturally in the environment (Tebo, 2004). Uses of Mn oxides applications in water and wastewater treatment include, soil and sediment remediation (of metals and organics) and metal removal and recovery. They also act as catalysts and sorbents (Tebo, 2004). 1

Based on previous laboratory experiments there have been proposed three differing potential pathways concerning the biological oxidation of Mn (II) to Mn (IV). The first of these hypotheses states that Mn (II) can be oxidized first to mixed Mn (III, IV), and Mn (IV) oxides and oxyhydroxides. The process in which this occurs is

analogous to abiotic reactions involving two steps. The first of which begins with the precipitation of Mn (II) to Mn (III). Subsequently these oxyhydroxides or oxides can transform into Mn (IV) oxides (Hem, 1981, Lind, 1983)(Tebo et.al, 1997) because it is a thermodynamically favored reaction (Mandernack, et.al 1995). A second potential pathway is that Mn (II) can be directly oxidized to Mn (IV). The last of the three pathways is that Mn (II) follows an enzymatic bond Mn (III) intermediate in which Mn (II) oxidizes to Mn (III) and then to Mn (IV).

The purposes of these experiments are to study which of these potential pathways are utilized in the biologically catalyzed process in which Mn (II) is oxidized into Mn (IV). The pathways in which bacteria oxidize Mn(II) and precipitates Mn (IV) as a solid is a small portion of the overall project of focus. One of the pathways that will be studied is to determine the intermediate step, which is the formation of Mn (III) as the Mn (II) is being oxidized to Mn (IV). In an experiment conducted by Mandernack (1995), pertaining to the oxidation of Mn (II) to Mn (IV), he hypothesized that Mn (II) is oxidized directly to Mn (IV) without the presence of an intermediate.(Bargar, Tebo, et. al 4). However, there are differing views to whether this statement is conclusive, thus other experiments, such as the one being carried out at the moment, are being conducted to determine if the exact opposite is true.

MATERIALS AND METHODS

This experiment was conducted using the marine Mn (II) *Bacillus* sp. strain SG-1. The SG-1 strain was used because of its ability to use Mn oxides as a terminal electron acceptor for the oxidation of organic matter or H₂ in the absence of oxygen. Using direct or indirect processes bacteria are capable of catalyzing the oxidation of Mn (II). The

method used in this experiment was the indirect catalysis of Mn (II) oxidation by the modification of the pH of the local aqueous environment. In order to carry out this experiment, the following solutions were prepared: .01 M CaCl_2 , 1M Hepes, and 100 μM MnCl_2 . All solutions were placed in a 50ml falcon tube and filled with milli-Q water until the solution reached the 50ml mark and were mixed on an end-over-end rotator for a time period of 24 hours. The following day the solution was removed and the pH was checked. Using .1M NaOH the Mn (II) concentration's pH was adjusted. .1M NaOH was added to the MnCl_2 drop-wise until a pH of 7.75 was reached. Next an HCl solution with a pH 3 was prepared with the addition of milli-Q water. The pH was made so low because if it were to be made any stronger it would cause damage to the cell. The acrylic, equilibrium dialysis membrane was then obtained and the screws within the membrane were removed. The membrane as well as the screws was soaked in the HCl solution for an hour so that there would be no contamination when the experiment was conducted. The membrane and the screws were then removed from the HCl and rinsed with milli-Q water. While the screws and the membrane were soaking, the dialysis membrane was measured and cut so that it would cover the entire cavity of the acrylic, membrane.

See Figure 1 Below:

Dialysis Membrane

Subsequently, the Mn (II) concentration was injected into the bottom cavity with the use of a syringe and needle. The cavity was then covered with the dialysis membrane. Succeeding this, the top portion of the membrane was placed over the bottom portion and the screws were positioned so that the acrylic, equilibrium membrane would be secure. Following that, the spores were injected into the top cavity of the membrane using the same apparatus that was used to inject the Mn (II) concentration. A nylon screw was then placed into the side of the top portion of the membrane so that none of the liquid would leak out of the cavity. The acrylic, equilibrium dialysis membrane was then placed onto a shaker for a period of one week. The membrane was observed every day for that week and pictures were taken of the membrane. The cell was then disassembled. The Mn (II) concentration was removed from the cavity with the use of a syringe and needle and placed into a 15 ml falcon tube so that it could be observed to determine if there were any traces of the oxides within the concentration. The spores were then removed from the top cavity using the same apparatus and placed into a differing 15 ml falcon tube. With the use of a vacuum filtration apparatus, the Mn (II) concentration was then tested for traces of oxides. Pictures of the results were taken and results were recorded. (Experiment was conducted twice)

The experiment that followed is known as pre-trapping. In this experiment Pyrophosphate and Bis-tris were tested to determine which of the two would be a better ligand when trapping Mn (III). A total of thirty four 14 ml falcon tubes were obtained and mixtures were made using milli-Q water, seawater, pyrophosphate, bis-tris, and hepes. The first of the mixtures was water + hepes+ pp₁ and water +hepes + pp₂. The next was water+hepes+Bt₁ and water+hepes+Bt₂. The other sixteen falcon tubes were

composed of seawater in the same mixtures as the water with the exception of the milli-Q water which was replaced by the seawater. The difference between pp_1 and pp_2 as well as Bt_1 and Bt_2 is their molarities. These differences can be seen in the tables below.

The amount of each chemical as well as the water and seawater that was measured is shown in the tables below as well.

Water PP and BT

	A	B	C	D	E	F	G
1							
2	Stock of PP	9.8 mM					
3	Stock 2	0.5488 mM	0.56 mL			10 mL	
4	Total Vol	5 mL					
5							
6	Final Tube	Amt HEPES	Amt PP	Amt PP2	Amt Water		Conc.
7	0	0.060	0	0	5.02		0
8	0.005	0.05		0.04	4.96		0.00435
9	0.01	0.07		0.18	4.97		0.01892
10	0.02	0.07		0.2	4.75		0.02186
11	0.05	0.07		0.5	4.44		0.05477
12	0.1	0.07	0.05		4.99		0.09589
13	0.2	0.05	0.1		4.87		0.19522
14	0.5	0.060	0.24		4.7		0.4704
15	1	0.05	0.60		4.47		1.14844
16							
17							
18	Stock of BT	20.5 mM					
19	Stock 2	0.5 mM	0.25 mL			in 10 ML	
20	Total Vol	5 mL					
21							
22	Final Tube	Amt HEPES	Amt BT	Amt BT2	Amt Water		Conc.
23	0	0.060		0	4.97		0
24	0.005	0.060		0.04	4.95		0.00396
25	0.01	0.05		0.1	4.88		0.00994
26	0.02	0.07		0.21	4.75		0.02087

27	0.05	0.04		0.51	4.48		0.05070
28	0.2	0.05	0.08		4.9		0.32604
29	0.5	0.08	0.12		4.81		0.49102
30	1	0.08	0.24390		4.7		0.99524

Seawater PP and BT

	A	B	C	D	E	F	G
1							
2	Stock of PP	9.8 mM					
3	Stock 2	0.5 mM		0.56 mL		in 10 ML	
4	Total Vol	5 mL					
5							
6	Final Tube	Amt HEPES	Amt PP	Amt PP2	Amt Seawater		CONC.
7	0	0.05		0	4.98		0
8	0.005	0.04		0.05	5		0.00491
9	0.01	0.05		0.1	4.83		0.01004
10	0.02	0.060		0.2	4.75		0.01996
11	0.05	0.05		0.5	4.58		0.04873
12	0.1	0.05	0.07		4.95		0.13531
13	0.2	0.05	0.12		4.86		0.23380
14	0.5	0.05	0.25		4.73		0.48708
15	1	0.05	0.5		4.45		0.98
16							
17							
18	Stock of BT	20.5 mM					
19	Stock 2	0.5 mM		0.25 mL		in 10 ML	
20	Total Vol	5 mL					
21							
22	Final Tube	Amt HEPES	Amt BT	Amt BT2	Amt Water		Conc.
23	0	0.04		0	4.96		0
24	0.005	0.060		0.05	4.91		0.00498
25	0.01	0.060		0.1	4.84		0.01
26	0.02	0.04		0.2	4.75		0.02004

27	0.05	0.05		0.490	4.47		0.04890
28	0.2	0.08	0.04		4.92		0.16270
29	0.5	0.060	0.12		4.83		0.49102
30	1	0.05	0.24		4.7		0.98597

After completing the addition of each mixture to the falcon tubes, the tubes were placed into a test tube holder for a period of two days so that the Pyrophosphate and Bis-Tris would have time to precipitate out of the mixtures. Next, with the use of a spectrometer the absorbance of each of the tubes was measured and graphed.

Following this the actual Trapping experiment was conducted. The *Bacillus* species SG-1 strain spores were used in this experiment as well. However the bacteria oxidized Mn (II) directly rather than indirectly via the production of polysaccharides or proteins (Bargar, Webb, 298). This strain of bacteria oxidizes Mn (II) enzymatically on the exosporium which contains the protein coat and is the outermost layer of the spore. To begin this experiment 5ml of milli-Q water was pipetted into a 13 ml falcon tube. Next 100 μ l of 1M hepes was placed into the same tube as the water. Subsequently a stirrer was added to the tube and it was placed onto a stir plate so that it could mix. The mixture was then referenced with the use of a spectrometer. The *Bacillus* species SG-1 spores were then obtained. The spores were defrosted, sonicated, and vectored to insure the quality of the spores that would be used. While the tube was still on the stirrer, 200 μ l of *Bacillus* species SG-1 exosporium was added to the falcon tube. The graph that appeared was then saved and the solution was referenced. The final solution that was added was 50 μ l of 10mM Mn (II). The experiment remained on the stir plate for 48 hours and the possible formation of Mn (III) was monitored continuously in five minute

intervals using UV/V spectroscopy. The experiment was then repeated with the use of seawater in place of milli-Q water. The results from this experiment were monitored and recorded.

RESULTS

The expected results of the dialysis cell were that Mn (IV) would be soluble and diffuse through the membrane and that the oxide (MnO_2) would be formed on the clean side of the cell. (The clean side refers to the cavity that contained the Mn (II) concentration.) However, it was observed from the results that Mn(IV) did not diffuse through the membrane, therefore, we concluded that Mn(IV) must form an insoluble precipitate rapidly and is unable to be a mobile compound. Instead of the MnO_2 forming on the clean side, it was observed to have formed on the side of the cell in which the spores were in. See Figure 1.1

Figure1.1 Cell Membrane Results: As seen in the picture above, MnO_2 when formed

has a characteristic brown color. The spore side also has this distinctive brown color while the clean side lacks this color. This informs one that the MnO_2 was formed on the spore side rather than on the clean side.

The results of the pre-trapping experiment were to show rather Bis-Tris or Pyrophosphate was a better trapping agent when trapping Mn(III). It was observed that Bis-Tris is a very weak trapping agent in both sea water and milli-Q water. This was known because when graphed, the absorbance spectrum of the Bis-Tris was negative. This infers to one that sea water entirely removes the Bis-Tris. Pyrophosphate appeared to be a good trapping agent in both water and sea water. When graphed the spectrum of the pyrophosphate in both water and sea water produced a positive absorbance pattern. However, the results of the pyrophosphate in sea water were not conclusive because of the fact that a pyrophosphate absorbance resembles a pyrophosphate: Ca^{2+} complex absorbance. See Figure: 1.2; 1.3

Figure 1.2

Figure 1.3

The results of the trapping experiment were to show whether pyrophosphate would form a Mn (III): pyrophosphate complex in sea water. The results showed that this complex was easily formed in milli-Q water, however in sea water the results were negative. In sea water this complex was not formed and the result is believed to be based on the fact that sea water contains a variety of ions. Ca^{2+} is believed to be causing the pyrophosphate not to form a bond with the Mn(III). This is because there is such an abundance of Ca^{2+} in sea water that it is overpowering the Mn(III) and bonding to the pyrophosphate to form a Ca^{2+} :pyrophosphate complex. See Figure 1.4

Figure 1.4 Results of Trapping experiment.

Observance of pyrophosphate in water is shown in blue. Observance of pyrophosphate in sea water is shown in pink. Absorbance spectrum based on first 20 hours of experiment.

DISCUSSION AND CONCLUSION

To conclude, it was found that the pre-trapping experiment gave results that were not accurate. This was shown on the graph that compares the pyrophosphate in milli-Q water to the pyrophosphate in sea water. The graphical representation made it appear as if the pyrophosphate was a good trapping agent in both milli-Q water and seawater.

However, when the actual trapping experiment was performed and the data collected it was seen that the pyrophosphate was a weak trapping agent in seawater. In the dialysis

cell experiment it was concluded that Mn(IV) is insoluble and it must form a insoluble precipitate rapidly and thus is unable to be a mobile compound.

ACKNOWLEDGEMENTS

We would like to thank the U.S. Department of Energy for the opportunity to participate in the SULI program. We would also like to thank DOE for this wonderful experience. We would like to give special thanks to John Bargar and Sam Webb our mentors. Thanks for your patience and understanding and for guiding us through this very exciting and knowledgeable experience. Also, we would like to thank Apurva Mehta for being available to us when we needed a little extra guidance.

REFERENCES

- [1] Bargar, J.R., Bergman, Uwe, Chiu, V.Q., Glatzel, P., Tebo, B.M., Vallalobos, M., Webb, S.M. (2004). Biotic and Abiotic Products of Mn(II) oxidation by spores of the Marine *Bacillus* sp. strain SG-1. Revised manuscript submitted to *American Mineralogist*. Pg.4.
- [2] Tebo, B.M., Bargar, J.R., Clement, B., Dick, G., Murray, K.J., Parker, D., Varsity, R., and Webb, S. (2004) Manganese Biooxides: Properties and Mechanisms of Formation. *Annual Review of Earth and Planetary Science*, pgs. 287,295,296,302,303).
- [3] Tebo, B.M., Ghiorse, W.C., Van Waasbergen, L.G., Siering, P.L., and Caspi, R. (1997) Bacterially Mediated Mineral Formation: Insights into Manganese (II) oxidation from molecular genetic and biochemical studies. *Reviews in mineralogy* vol.35. In J.F. Banfield, and K. H. Nealson, Eds. *Geomicrobiology: Interactions between microbes and Minerals*, p.226,228,229,230. Mineralogical Society of America, Washington, D.C.