INTERACTIONS BETWEEN THE IRON, MANGANESE, AND OXYGEN CYCLES IN THE MARINE ENVIRONMENT

by

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ABSTRACT

The speciation of dissolved Mn (dMn\textsubscript{T}) in oxygenated systems has typically been assumed to be dominated by soluble Mn(II) in addition to Mn(IV) existing as solid MnO\textsubscript{2}. Work in the last few decades has shown that the intermediate state, Mn(III), is stabilized by organic ligands (L) in oxygen depleted waters, making up to 78% of dMn\textsubscript{T} in the oceanic-type surface waters of the Delaware Bay and up to 99% of dMn\textsubscript{T} in surface waters of salt marsh creeks feeding the Broadkill River in Delaware. These values were obtained using a known method involving spectrophotometric determination of the kinetics of Mn(II) complexation with porphyrin (POR). The higher percentage of Mn(III) in creek waters as opposed to the bay was attributed to greater influence of the salt marsh in the form of an influx of humic material as unknown ligands. The complexation of Mn(III) and humic materials to form Mn(III)-L allows for increased transport of Mn and organic material to the oceans or, in this case, the Delaware Bay. Unexpected oxidation of environmental samples was observed, prompting laboratory experiments that uncovered a strong interaction between iron, manganese, and oxygen in a somewhat saline matrix (S = 14 ppt). The interaction observed was due to a series of reactions, beginning with the oxidation of Fe(II) producing superoxide. This superoxide was then able to react with Mn(II) to generate MnO\textsubscript{x}. This previously unconsidered reaction series implies an interaction between iron, manganese, and oxygen cycling in the environment with relevance in not only surface water systems but also in hydrothermal vent systems and banded iron formations. More work is needed to determine the extent to which this interaction is occurring in natural systems.
1.1 Manganese in the Environment

The multiple oxidation states of manganese contribute to its critical role in reduction/oxidation reactions. In the environment, this places manganese in a position of importance in both biogeochemical cycling and in a biological context, as its ability to readily change oxidation state makes it a critical component of the oxygen evolving complex of photosystem II (Siegbahn 2008). Traditionally, environmental chemists have focused primarily on soluble Mn(II) and insoluble Mn(IV) species, as the free Mn(III) ion tends to disproportionate in water; though its potential importance as a one-electron transfer redox species was acknowledged, few believed that this species could persist under normal conditions. However, more recently it has been shown thermodynamically that the formation of Mn(III) by a one-electron transfer during the oxidation of Mn(II) and the reduction of Mn(IV) is favorable, particularly compared to a two-electron pathway that would jump between Mn(II) and Mn(IV) directly (Luther 2005). Environmental data has supported these calculations, and has shown Mn(III) to be a significant component of environmental manganese when bound to ligands particularly in suboxic waters (Trouwborst et al. 2006). In the St. Lawrence Estuary, Mn(III)-L was found to compose up to 85% of the total dissolved manganese (dMn$_T$) found in suboxic samples (Madison et al. 2011). The initial phase of this work sought to expand on this knowledge of manganese in the environment and gain a better understanding of how Mn(III) may be present in oxygenated waters.
1.2 Iron in the Environment

Iron is critical in the marine environment. Phytoplankton, the basis of many marine food webs and the producers of up to 70% of the Earth’s oxygen, are heavily dependent on iron for survival (Sekerci & Petrovskii 2015). It has been well established that, in areas limited by iron, a sudden influx can spark a massive bloom that may last more than a month (Boyd et al. 2000). Iron also serves a vital role to some chemolithotrophic bacteria, which use iron oxidation as a source of energy (Emerson et al. 2010). Some higher order organisms also depend on iron for survival, particularly in oxygen-binding pigments such as hemoglobin and myoglobin (Wilson & Reeder 2007).

In addition to its critical role in sustaining life, iron has a rich biogeochemical cycling that has been well-studied. Sources and sinks of iron in the marine environment are of particular interest, and it has been estimated that up to 50% of the total soluble iron pool in the ocean is recycled weekly by both biological and abiotic mechanisms (Boyd & Ellwood 2010). Hydrothermal vents are considered to be a source of iron to the ocean, notably in the form of kinetically stable nanoparticles which allow for long-distance transport of reduced Fe(II) (Yücel et al. 2011). This source of Fe(II) is particularly notable, as free Fe(II) may readily oxidize in an oxygenated water column (Luther 2010). Oxidized iron species make up a significant portion of marine sediments and are the principle component of banded iron formations (BIFs) which formed during the Precambrian period (Kostka & Luther 1994; Trouwborst et al. 2007). It is believed that, at least for some of these BIFs, the oxidant for the iron was biologically produced oxygen, which is an important example
of the relationship between biology and iron cycling in the environment (Chan et al. 2016).

1.3 Objectives of Thesis

The primary objective of this thesis was to understand how manganese is present in the environment, and how manganese interacts with other chemical species in natural systems. Manganese speciation was examined in environmental samples from local waterways in order to ascertain how manganese behaved in specific systems. After Mn(II) added to samples unexpectedly oxidized to Mn(III) and was consistently observed, laboratory experiments were conducted as a means of exploring potential interactions between iron and manganese reactions in the presence of oxygen. Based on thermodynamic calculations (Luther 2010), it was predicted that Fe(II) would react with O$_2$ to form the reactive oxygen species (ROS) superoxide, O$_2^-$, which would then react with Mn(II) to form Mn(III,IV) phases.

1.4 Sample Site: Lewes, Delaware

Lewes, Delaware is home to the Great Marsh, which lines part of the southeastern shore of the Delaware Bay and is dominated by the cordgrass *Spartina alterniflora*. The Broadkill River and several tributary creeks cut through the Great Marsh on their way to the Delaware Bay, creating a system that is influenced by both marine and freshwater influences and heavily dependent on tidal cycles. The sample site for the environmental phase of this work was chosen due to its location at this freshwater/saltwater interface, as well as its proximity to the lab which ensured rapid processing and analysis of samples. An image of the sample site and surrounding area is provided below.
The sample site itself was located off of a small bridge crossing a Broadkill tributary creek. The inlet seen in the northeastern portion of the satellite image shown is Roosevelt Inlet. The Great Marsh is directly south of the sample site and extends to the west. The University of Delaware Lewes Campus, where the work was carried out for this thesis, is just outside of the image to the southeast.
Chapter 2
ENVIRONMENTAL WORK

2.1 Environmental Methods

2.1.1 Sampling

Samples were taken from Delaware Bay on June 14, 2016 and from a tributary creek on June 14 and 16, 2016. These samples were analyzed for manganese (Mn) speciation as part of an investigation into tidal fluxes and light vs dark impacts. On June 14, samples were collected into clean, plastic 250 mL bottles at falling, low, rising, and high tides from the dock, representative of an oceanic sample, at the Sharp campus of the University of Delaware and at a nearby creek on Pilottown Road. Contamination was reduced by wearing nitrile gloves, using acid cleaned bottles, and ensuring that the bottles were opened only below the surface microlayer. Bottles were triple rinsed with sample before filling and were immediately returned to the lab. All samples were filtered through a 0.2 μm syringe filter (Nylon, Whatman) to remove sediment, organic solids, and MnOx. Samples taken on June 16, 2016 were collected before and after sunrise, into clear and dark bottles. An aliquot of these samples was filtered and analyzed immediately, and a second timepoint was analyzed 24 hours after collection.

2.1.2 Mn(II) and Mn(III) Determination

Samples were analyzed in triplicate within a few hours (in one case, after 24 hours) of collection. Analysis was performed based on a preexisting method developed by Ishii et al. (1982) and modified by Madison et al. (2011) to determine soluble Mn speciation. All reagents were prepared as described in Madison et al.
(2011). A Hewlett Packard 8452 diode array spectrophotometer, coupled with Olis, Inc. Globalworks software, was used to obtain all spectrophotometric data. Prior to all analyses, a calibration curve was created using standards ranging from 0.5 to 11.0 μM made from MnCl$_2$ dissolved in deionized water.

The method of Madison et al. (2011) was used to determine manganese speciation spectrophotometric ligand ([α, β, γ, δ-tetrakis(4-carboxyphenyl)porphine] or T(4-CP)P (ε=95,400M$^{-1}$cm$^{-1}$). The Mn(III)-porphyrin complex exhibits a peak at 468 nm. This peak was measured over the course of 15 minutes to determine kinetic data, which allowed for the determination of soluble Mn(II) and Mn(III) bound to weak ligands. To determine Mn(III) bound to strong ligands, hydroxylamine (HA) was added in excess to a sample and allowed to equilibrate for at least 30 min. This gave the total concentration on soluble Mn in the sample and allowed for the determination of Mn(III) bound to strong ligands by difference.

This method is based on the concept of metal substitution and ligand exchange reactions. Mn(II) will replace the Cd(II) sitting in the porphyrin ring relatively quickly, resulting in a curve that rises steeply and then levels off. Mn(III) bound to a weak ligand will show a slower rise over time, as the ligand exchange is slower than the metal substitution reaction. Mn(III) bound to a strong ligand will not show a curve using this method, as the porphyrin ring in the T(4-CP)P is not a strong enough competitor to exchange with the strong ligand. This is why the hydroxylamine is necessary; to reduce the entire sample to Mn(II) so that Mn(III)-L$_{(strong)}$ can be determined by difference. A sample kinetic curve generated using the Madison et al. 2011 method is shown below.
Figure 2.1.2.1: Sample kinetic curve using the Madison et al. (2011) manganese speciation determination method. Data from the morning falling tide on June 14, 2016 with a 1μM MnCl₂ addition.

2.1.3 Tidal Analysis

Samples were taken on June 14, 2016 at falling (09:11), low (11:12), rising (14:54), and high (17:44) tides from the creek site. Samples were filtered using a 0.2 μm syringe filter (Nylon, Whatman) upon arrival at the lab and analyzed immediately. Triplicate analyses were performed using the porphyrin method described above. These analyses were compared to determine the tidal fluxes of manganese speciation in a system with both terrestrial and marine inputs.

2.1.4 Light vs. Dark Analysis

Samples were collected from the creek on June 16, 2016 before sunrise (04:03) and a few hours after sunrise (09:33), each in a light and dark bottle. An aliquot of
each sample was filtered and analyzed upon arrival at the lab. The rest of the sample was left unfiltered in its collection bottle and was stored at room temperature. The remaining sample was then filtered and analyzed 24 hours after collection.

2.1.5 Mn(II) Addition

In addition to the analyses described above, all collected samples were subjected to a Mn(II) addition experiment. Sample filtered through a 0.2 μm syringe filter (Nylon, Whatman) were spiked with a standard solution of MnCl$_2$ to a total volume of 10 mL and a Mn(II) concentration 1 μM above that of the sample. These treated samples were then analyzed for Mn speciation using the porphyrin method as above.

2.2 Results and Discussion

2.2.1 Tidal Speciation

Samples for the tidal speciation experiment were collected on June 14, 2016 from the marshy tributary creek (Broadkill) site. Environmental conditions at the time of sample collection are summarized below. It should be noted that this was a bright, mostly sunny, breezy day.

<table>
<thead>
<tr>
<th>Time</th>
<th>Tide State</th>
<th>Water Temp. (°C)</th>
<th>Salinity (ppt)</th>
<th>Conductivity (mS)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>09:11</td>
<td>Falling</td>
<td>21.3</td>
<td>26.1</td>
<td>40.80</td>
<td>7.89</td>
</tr>
<tr>
<td>11:12</td>
<td>Low Tide</td>
<td>22.3</td>
<td>21.8</td>
<td>34.66</td>
<td>7.49</td>
</tr>
<tr>
<td>14:54</td>
<td>Rising</td>
<td>22.8</td>
<td>26.1</td>
<td>40.85</td>
<td>8.14</td>
</tr>
<tr>
<td>17:44</td>
<td>High Tide</td>
<td>23.0</td>
<td>26.8</td>
<td>41.69</td>
<td>8.19</td>
</tr>
</tbody>
</table>
The conditions summarized above give insight into the degree of influence that the oceanic system and marsh system had on the samples. Salinity, as a conservative tracer, is a particularly good indicator of this. In the tidal creek, it is expected that the highest influence of the oceanic system would be observed at high tide, and the higher salinity value observed helps to confirm this. The high pH value could also be reflective of oceanic influence, but there are more complex factors that influence pH that make it more desirable to use salinity as an indicator of marine influence.

Figure 2.2.1.1: Tidal manganese speciation in the Broadkill from samples collected June 14, 2016.

The tidal data series collected on June 14, 2016 shows a peak in dMn$_T$ at the low tide data point. This is not altogether unexpected, as this is when the system is more heavily influenced by the marsh surroundings and freshwater inputs than marine
tidal floods. The 1.58 μM increase at low tide is most likely due to the heightened influence of the marsh porewaters and the subsequent influx of organic material to the system.

It is also worth noting that the trends for Mn(II) and Mn(III)-L\(_{\text{weak}}\) follow the trends of dMn\(_T\) – they show a sharp increase at low tide but remain relatively constant at other points in the tidal cycle. This is consistent with the idea that the marine input to the system is effectively diluting the marsh influence, as the trends in dMn\(_T\) mirror the trends in salinity of the samples closely. Madison et al. (2013) found that soluble manganese in estuarine porewaters plays an important role in sedimentary redox reactions, so it is unsurprising that the porewater influence was readily visible in this system. However, this is not the case for Mn(III)-L\(_{\text{strong}}\). Mn(III)-L\(_{\text{strong}}\) remained relatively consistent over time, though with a slight increase at high tide. This trend is not easily explained by tidal cycles.

### 2.2.2 Light vs. Dark Bottle Experiments

Samples for this experiment were collected on June 16, 2016. Samples were obtained at two timepoints; conditions at the time of sampling are presented in Table 2.2.2.1 below. It should be noted that it was raining at the time of sample collection. High tide occurred at 06:28.

<table>
<thead>
<tr>
<th>Time</th>
<th>Tide State</th>
<th>Water Temp. (°C)</th>
<th>Salinity (ppt)</th>
<th>Conductivity (mS)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>04:03</td>
<td>Rising</td>
<td>20.4</td>
<td>26.6</td>
<td>41.40</td>
<td>8.04</td>
</tr>
<tr>
<td>09:33</td>
<td>Falling</td>
<td>20.2</td>
<td>26.9</td>
<td>41.73</td>
<td>8.03</td>
</tr>
</tbody>
</table>
There were two goals of this experiment. The first goal was to observe differences in manganese speciation before sunrise in the absence of photosynthetic activity and after sunrise with the onset of photosynthetic activity. This experiment also aimed to determine if samples should be kept in the dark to prevent photo-oxidation, or if samples would be unaffected by light. The results of this experiment are presented below in Figure 2.2.2.1.

**Figure 2.2.2.1:** A) Percent manganese speciation for the June 16, 2016 04:03 creek sample in a dark bottle. B) Percent manganese speciation for the June 16, 2016 09:33 creek sample in a dark bottle. C) Percent manganese speciation for the June 16, 2016 04:03 creek sample in a light bottle. D) Percent manganese speciation for the June 16, 2016 09:33 creek sample in a light bottle.

From Figures 2.2.2.1C and 2.2.2.1D, it is clear that Mn(II) and Mn(III)-L(weak) make up a greater portion of dMnT after sunrise as opposed to before photosynthetic
activity begins for the day. Before sunrise, Mn(III)-L\textsubscript{(strong)} seems to dominate the total dissolved manganese pool. It should be noted that the total concentration of dissolved manganese at 04:03 was 3.92 ± 0.41 μM, while at 09:33 the total dissolved manganese concentration was 1.58 ± 0.08 μM. This difference in concentration could be due to tidal flushing, as the morning high tide occurred between these two timepoints at 06:28. However, these values were calculated from the light bottle data; the dark bottle data gives dMn\textsubscript{T} as 1.55 ± 0.17 μM and 1.43 ± 0.34 μM for the 04:03 and 09:33 samples, respectively. While there is reasonable agreement between dMn\textsubscript{T} values for the 09:33 samples, the dark and light bottle data for the 04:03 samples are not in agreement. The difference between these samples is not entirely explained by the presence of Mn(II) in the light bottle samples (C = 0.91 ± 0.06 μM). It is uncertain whether this discrepancy was due to contamination, an analytical error, or the formation of MnO\textsubscript{2}.

The samples stored in the light bottles did show a small amount of oxidation of Mn(II) to Mn(III)-L. A conversion of Mn(III)-L\textsubscript{(weak)} to Mn(III)-L\textsubscript{(strong)} was also observed. The samples stored in the dark bottles both showed a conversion of Mn(III)-L\textsubscript{(weak)} to Mn(III)-L\textsubscript{(strong)} over time as well. This result is not entirely unexpected; if the samples had an abundance of strong ligands, it is reasonable that the strong ligands would gradually outcompete the weaker ligands to bind Mn(III). Oddly, though samples were collected at the same time and location into the light and dark bottles, samples in the dark bottles did not appear to contain a significant amount of Mn(II), while the samples collected into light bottles did. To answer the oxidation question initially posed, samples were spiked with 1μM MnCl\textsubscript{2} and analyzed in the same
fashion as the other samples. The results from this experiment are presented below in Figure 2.2.2.2.

Figure 2.2.2.2: Percent manganese speciation for the following samples plus a 1μM MnCl₂ addition. A) June 16, 2016 04:03 creek sample stored in a dark bottle. B) June 16, 2016 09:33 creek sample stored in a dark bottle. C) June 16, 2016 04:03 sample stored in a light bottle. D) June 16, 2016 09:33 sample stored in a light bottle.

Again, the difference in Mn(II) concentration is observed between the dark and light bottle storage due to the initial difference between the samples. However, now oxidation trends can be observed due to the addition of Mn(II). The samples stored in dark bottles from both timepoints, as seen in Figures 2.2.2.2A and 2.2.2.2B, show an oxidation of Mn(II) over time. Interestingly, the samples stored in the light bottles show a slight increase in the percent speciation composed of Mn(II), though the percentage is more consistent than the dramatic decrease shown in the dark bottle.
samples. The conclusion of this experiment is that photooxidation may not be an issue over short timescales for sample storage, but something else may be going on in the sample that alters manganese speciation. Since samples were not filtered before storage as this was an attempt to see how flexible analysis timescales were, the change in speciation could have been due to oxidants produced due to biological activity. However, this should not have been an issue in the dark bottles, as photosynthetic organisms were deprived of light and should have consumed rather than produced oxygen. The reactions observed in this experiment, therefore, are likely not due to biological activity alone.

2.2.3 Addition Experiments

Addition experiments were performed using the samples collected for the tidal speciation experiment. It should be noted that samples were analyzed in triplicate; unaltered samples were processed, and the 1μM addition samples were processed immediately after the completion of the 15 minute kinetic analyses on the unaltered samples (~45 minutes later). Conditions at the time of sampling can be found above in Table 2.2.1.1. A comparison between the unaltered samples and the samples spiked with 1μM MnCl₂ is presented below in Figure 2.2.3.1.
Figure 2.2.3.1: Tidal manganese speciation data for A) unaltered samples collected on June 14, 2016 and B) samples collected on June 14, 2016 subjected to a 1μM MnCl$_2$ addition.

This experiment was initially designed as a method check. If all went as expected, a recovery of 1+dMn$_T$ and 1+Mn(II) should have been observed, and both categories of Mn(III)-L should have remained unchanged in concentration. However, this was not the case. The expected increase in the total dissolved manganese concentration was observed, but this is the end of anticipated outcomes. The Mn(II) concentration did not rise as expected; instead, the added Mn(II) was recovered as Mn(III)-L. In the case of the low tide sample, some of the initial Mn(II) in the sample also oxidized. Unlike the light/dark bottle experiments, these samples were all filtered immediately upon returning to the lab, thus biological activity can be ruled out as a cause for this observed manganese oxidation. These reactions are most likely entirely chemical in nature. It was believed that a reactive oxygen species (ROS) was responsible for these observations, but these samples were not analyzed for the presence of ROS. Instead, laboratory experiments were designed and carried out in the winter of 2018 to explore these observations.
3.1 Experimental Setup and Methods

3.1.1 Manganese and Iron Molar Excess Trials

Stock solutions of Mn(II) and Fe(II) were prepared to carry out these trials. Mn(II) solutions were prepared by dissolving MnCl$_2$ in deionized water. To prepare the Fe(II) stock solution, deionized water was purged with argon. This deoxygenated water was then used to dissolve Fe(NH$_4$)$_2$(SO$_4$)$_2$. This solution was used immediately after preparation to ensure that the Fe(II) did not oxidize.

Early in the process, four separate trials were run per day. Four magnetic stir plates were set up and paired with a 400 mL beaker and a magnetic stir bar. Aquarium bubblers were attached to two of the four beakers. Later excess trials were run without bubblers, as the stirrers alone saturated the solutions with oxygen. 100 mL of filtered seawater (Salinity, S, 35 ppt) were added to each beaker. One beaker with and without a bubbler were designated as “5:1 Mn Excess Trial,” and had 125 mL ~10 mM MnCl$_2$ added. The other two beakers were designated as “5:1 Fe Excess Trials,” and had 25 mL ~10mM MnCl$_2$ added. The stirrers for all plates (and the bubblers, where applicable) were turned on to allow the solutions to equilibrate. The “5:1 Mn Excess Trial” beakers received a 25 mL aliquot of ~10 mM Fe(NH$_4$)$_2$(SO$_4$)$_2$, whereas 125 mL were added to the “5:1 Fe Excess Trial” beakers. The times of the iron additions were recorded, and the reactions were allowed to run for several hours to ensure that they ran to completion.

After the reactions ran to completion, four 50 mL aliquots of the reaction mixture were removed while stirring and filtered using a 0.2 μm syringe filter (Nylon,
Whatman), and the time was recorded. Both the filter and the filtrate were saved. The filters were dried under argon before being stored in labelled petri dishes. The filtrates were stored in labelled 50 mL falcon tubes. The times of removal for each aliquot were recorded so that the total reaction time would be known. The saved filtrate was later analyzed for iron concentration and speciation, while the filters were later analyzed to determine if manganese oxides were present.

### 3.1.2 Acidified Seawater Trials

The acidified seawater trials were run in a manner similar to the above. However, no bubblers were used; instead, the manganese excess and iron excess trials were run in duplicate. Filtered seawater was acidified using HCl to a pH of 5.23, similar to what was measured at diffuse flow hydrothermal vent waters in April 2017. The reactions were the run the same as the above.

### 3.1.3 Ligand Addition Trials

Trials run without the presence of additional ligand showed Mn(II) oxidation to a solid phase. However, in the environmental samples taken in 2016, Mn(II) was oxidized to an aqueous Mn(III)-L phase. In an attempt to replicate these results, reaction vessels were set up in a similar manner to the iron and manganese excess trials, but with the addition of ligands to the system.

#### 3.1.3.1 Tiron

A 103 μM solution of the ligand tiron, (OH)$_2$C$_6$H$_2$(SO$_4$)$_2$, was prepared in filtered seawater. 100 mL of this solution were added to the reaction vessels, which were set up with stir plates as above. For manganese excess trials, 125 mL of ~ 10 μM MnCl$_2$ were added, whereas iron excess trials received an aliquot of 25 mL of the ~ 10
μM MnCl₂ solution. Stirring was turned on to allow the solutions to equilibrate. Manganese excess trial reaction vessels were then spiked with 25 mL of ~10 μM Fe(NH₄)₂(SO₄)₂, whereas iron excess trial beakers had 125 mL added. Another series was later run with triple the iron and manganese concentrations.

After the reactions had gone to completion, 50 mL aliquots were removed from the vessels while stirring and filtered using a 0.2 μm syringe filter (Nylon, Whatman). No solid was collected on the filters. The filtrate was then analyzed using a Hewlett Packard 8452 diode array spectrophotometer coupled with Olis, Inc. Globalworks software.

3.1.3.2 Deferoxamine Mesylate (DFOB)

A 117 μM stock solution of DFOB was prepared in filtered seawater. A 100 mL aliquot was added to a reaction vessel outfitted with a magnetic stir plate. 125 mL of a 40 μM solution of MnCl₂ were added, and the solution was stirred to allow for equilibration. 25 mL of a 40 μM Fe(NH₄)₂(SO₄)₂ solution were then added, and the reaction was allowed to run to completion. 50 mL aliquots were then removed from the vessels while stirring and filtered using a 0.2 μm syringe filter (Nylon, Whatman). No solid was collected on the filters. The filtrate was then analyzed using a Hewlett Packard 8452 diode array spectrophotometer coupled with Olis, Inc. Globalworks software.

An order of addition experiment was also performed. In this experiment, reaction vessels were prepared with a magnetic stir plate, 20 mL of filtered seawater, and 26.7 mL of deionized water. 0.25 mL of 10 mM Fe(NH₄)₂(SO₄)₂ were added while the solution was stirring. Once precipitation was visible (evidenced by a faint yellow color), 2.8 mL of a 3.6 mM solution of DFOB in deionized water and 0.25 mL of 10
mM MnCl\textsubscript{2} were added simultaneously. The reaction was allowed to go to completion, and then aliquots of the reaction mixture were analyzed using a Hewlett Packard 8452 diode array spectrophotometer coupled with Olis, Inc. Globalworks software.

3.1.3.3 1, 4, 8, 11-Tetraazacyclotetradecane (Cyclam)

A 0.52 mM solution of cyclam was prepared in deionized water. To facilitate dissolution, 0.3531 g of NaOH were added. The solution was stirred for eight hours, allowed to sit for 48 hours, and then stirred for 3 hours before use.

The first experiment, designated “Cyclam A,” was prepared by adding 20 mL filtered seawater, 19.1 mL 0.52 mM cyclam, 0.85 mL deionized water, and 0.25 mL 10 mM MnCl\textsubscript{2} to a reaction vessel paired with a magnetic stir plate. 0.6 mL of 5% HCl was added to adjust the pH of the solution. After allowing the solution to stir, 0.25 mL of a 10 mM solution of Fe(NH\textsubscript{4})\textsubscript{2}(SO\textsubscript{4})\textsubscript{2} was added, and the reaction was allowed to go to completion.

The experiment designated “Cyclam B” was prepared in a reaction vessel with a magnetic stir plate by adding 20 mL filtered seawater, 0.85 mL deionized water, and 0.6 mL 5% HCl. 0.25 mL of a 10 mM solution of Fe(NH\textsubscript{4})\textsubscript{2}(SO\textsubscript{4})\textsubscript{2} was added while stirring, followed by a simultaneous addition of 0.25 mL 10 mM MnCl\textsubscript{2} and 19.1 mL 0.52 mM cyclam.

“Cyclam C” was prepared in a reaction vessel equipped with a magnetic stir plate by combining 20 mL filtered seawater, 0.85 mL deionized water, 0.6 mL 5% HCl, and 19.1 mL 0.52 mM cyclam. After allowing this mixture to equilibrate, 0.25 mL 10 mM MnCl\textsubscript{2} and 0.25 mL 10 mM Fe(NH\textsubscript{4})\textsubscript{2}(SO\textsubscript{4})\textsubscript{2} were added simultaneously. These reactions were all allowed to go to completion. Aliquots were then analyzed...
using a Hewlett Packard 8452 diode array spectrophotometer coupled with Olis, Inc. Globalworks software.

### 3.1.4 Peroxide Experimentation

To prove that the superoxide produced during Fe(II) oxidation was responsible for the observed manganese oxidation and not peroxide, a small experiment was designed. In a small beaker, 20 mL filtered seawater, 29 mL deionized water, and 1 mL 10 mM MnCl$_2$ were combined, and allowed to stir on a magnetic stir plate. 0.1 mL 30% H2O2 was added, and the mixture was monitored for the formation of precipitates. When no reaction had occurred after 3 minutes, another 1.0 mL 30% H$_2$O$_2$ was added. A total of 4 1.0 mL aliquots of 30% H$_2$O$_2$, spaced 5 minutes apart, were added, and the mixture was allowed to stir for another 30 minutes. After this period, no reaction was observed, and the trial was terminated.

### 3.2 Sample Analysis Methods

Analysis of all samples generated in this phase of experimentation was primarily spectrophotometric in nature. A Hewlett Packard 8452 diode array spectrophotometer was used alongside Olis, Inc. Globalworks software to make the spectrophotometric measurements. X-Ray diffraction was also used to determine solid species present on the filters (Nylon, Whatman) from the 5:1 excess trials as well as the acidification experiments. Methods are detailed below.

#### 3.2.1 Leukoberbelin Blue I (LBB)

LBB can be used to quantify manganese oxides present in a sample, but does not react with iron oxides. For this reason, this reagent was chosen to analyze the
solids collected on the filters in the above experiments. This method is derived from the work of Altmann (1972) and Stein et al. (2001).

A LBB stock solution was prepared from the solid reagent (65% LBB Dye, Aldrich) and deionized water, brought up to a pH of 10.5-11 with 10M NaOH. This stock solution was 4% LBB. The stock was stored in a refrigerator to preserve freshness. The solution recrystallized upon sitting in the refrigerator and had to be brought to room temperature and re-dissolved before it could be used. Primary (1° LBB) and secondary (2’ LBB) reagents were prepared for sample analysis. 1° LBB, 0.04% LBB, was prepared by diluting the stock solution by a factor of 100 in 1% acetic acid. This primary reagent was the used to prepare the secondary reagent (0.004% LBB) by diluting 1° LBB by a factor of 10 with deionized water. After some experimentation, it was determined that 1° LBB was better for these analyses than 2° LBB, so only the primary reagent was used in subsequent analyses.

Filters (Nylon, Whatman) from the 5:1 excess trials and the acidification trials were weighed before use. After the first set of experiments, an average weight of 0.0125g was used in all subsequent calculations. After filtration, filters were dried thoroughly under argon, and weighed again to get the mass of the solid by difference. Filters were the transferred to the bottom of 15 mL falcon tubes and covered with 3 mL 1° LBB. These samples were allowed to sit overnight and were then analyzed using a Hewlett Packard 8452 diode array spectrophotometer coupled with Olis, Inc. Globalworks software. The reacted reagent has a spectrophotometric peak at λ = 620 nm.

Prior to sample analysis, a calibration curve was generated using a stock solution of MnO₂. This calibration curve is presented below.
3.2.2 Iron Determination

Ferrozine is a reagent used to spectrophotometrically quantify aqueous Fe(II) ($\lambda_{\text{max}} = 562$ nm) as per the work of Stookey (1970) and Gibbs (1976). The ferrozine reagent was prepared by dissolving the solid reagent in deionized water to a concentration of 0.01M. A 2.5M solution of ammonium acetate buffer was also prepared by dissolving solid ammonium acetate into deionized water. Hydroxylamine (HA) was prepared to a concentration of 100 mM by dissolving solid hydroxylamine in deionized water.

Clean 15 mL Falcon tubes were prepared and labelled for the analysis. Tubes were spiked with 0.75 mL 0.01M ferrozine and 0.75 mL 2.5M ammonium acetate. An aliquot of sample was then added, though the volume of this aliquot varied depending on the initial Fe(II) concentration of the reaction mixture; the 5:1 iron trials were spiked with 0.010 mL filtrate, while the 5:1 manganese trials were spiked with 0.250 or 0.100 mL filtrate. The ferrozine mixtures were then diluted to a total volume of 10
mL with deionized water, capped and inverted, and allowed to react for at least 30 minutes. An aliquot of the reacted mixtures was poured into a 1 cm quartz cuvette and analyzed at $\lambda = 562$ nm using a Hewlett Packard 8452 diode array spectrophotometer coupled with Olis, Inc. Globalworks software. The aliquot analyzed was then poured back into the Falcon tube, and the tube was spiked with 0.75 mL HA to reduce any Fe(III) in the sample to Fe(II). The tubes were then capped and inverted, and were allowed to react for at least 30 minutes. Samples were then analyzed again at $\lambda = 562$ nm to get a total aqueous iron concentration for the sample. Fe(III) was then determined by difference.

Prior to sample analysis, a calibration curve was prepared using solutions of known Fe(NH$_4$)$_2$(SO$_4$)$_2$ concentration. This calibration curve is provided below.

![Figure 3.2.2.1: Ferrozine calibration curve. Trendline: A = 0.02725C – 0.00966, R$^2$ = 0.9988.](image)
3.2.3 Manganese Determination

Analysis was performed based on a preexisting method developed by Ishii et al. (1982) and modified by Madison et al. (2011) to determine soluble Mn speciation. All reagents were prepared as described in Madison et al. (2011). The spectrophotometric ligand used was a porphyrin compound ([α, β, γ, δ-tetrakis(4-carboxyphenyl)porphine] or T(4-CP)P (ε=95,400M⁻¹cm⁻¹). The Mn(III)-porphyrin complex exhibits a peak at 468 nm, and was analyzed using a Hewlett Packard 8452 diode array spectrophotometer coupled with Olis, Inc. Globalworks software.

Kinetic analyses using T(4-CP)P allow for the differentiation of Mn(II) and Mn(III)-L species. To perform this, the peak at 468 nm was monitored over the course of 15 minutes, with absorbance measurements taken every three seconds. Kinetic analyses revealed that there was only Mn(II) present in the samples, so subsequent samples were not analyzed kinetically but instead through a modified method in which the T(4-CP)P, CdCl₂, imidazole-tetraborate buffer, deionized water, and sample were combined in that order and allowed to react for 15 minutes before being analyzed at 468 nm.

Prior to sample analysis, a calibration was prepared using a stock solution of MnCl₂. This calibration curve is presented below.
3.2.4 X-Ray Diffraction (XRD)

Sample analysis was performed using a Bruker D8 XRD coupled with a LYNXEYE_XE (0D mode) detector and a copper source tube (1.5418 Å). Filters (Nylon, Whatman) dried under argon from the 5:1 excess and acidification trials were packaged and transported to be analyzed in the Advanced Materials Characterization Lab. Filters were placed on low-background holders and taped at the edges to prevent the sample from moving unexpectedly. Samples were then subjected to a coupled 2θ/θ continuous scan, there 2θ varied from 5° to 69.9890° with an increment of 0.0498° (1306 steps, time = 0.3s). One filter considered representative of each experiment from the 5:1 excess trials and the acidification trials were analyzed. The 5:1 excess trial filters had more material and were allowed to run for an hour. The acidification experiment filters were analyzed for an hour and 47 minutes. Scans were then compared to standards in the database to determine what species may have precipitated out during the experiments.
3.3 Results and Discussion

3.3.1 Manganese and Iron Molar Excess Trials

These experiments were run four at a time. Two reaction vessels contained a 5:1 molar excess of manganese to iron, while the others had a 5:1 molar excess of iron to manganese. These duplicates were further split; one vessel was stirred using a magnetic stir plate, while the other was stirred and outfitted with an aquarium bubbler. An experimental setup is pictured below.

![Experimental Setup](image)

Figure 3.3.1.1: Manganese and iron molar excess trials experimental setup. A) Four reaction vessels at completion, January 8, 2018, from left to right: 5:1 Mn excess no bubbler, 5:1 Fe excess no bubbler, 5:1 Mn excess with bubbler, 5:1 Fe excess with bubbler. B) Comparison of 5:1 Mn excess and 5:1 Fe excess reaction vessels immediately after iron addition, no bubbler, January 9, 2018.

In Figure 3.3.1.1B above, a distinct difference can be seen between the manganese excess trial (left) and the iron excess trial (right). This image was taken immediately after iron was added into the reaction vessels. The reaction in the iron excess trial proceeded faster due to the high concentration of Fe(II), which readily oxidized to give the bright orange color. It should also be noted that the strength of color in the iron excess reaction vessel in Figure 3.3.1.1B closely matches that of the
completed reactions shown in Figure 3.3.1.1A. These reactions arrive at completion rapidly, though they were given several hours to equilibrate to ensure that the reactions were at completion prior to analysis.

The manganese excess trials began with a Mn(II):Fe(II) ratio of 5:1. Iron recovery was determined using ferrozine analysis on known volumes of filtrate. Manganese oxides were quantified using LBB. Soluble manganese was recovered solely as Mn(II), and the percent recovery as Mn(II) was over 100% in all cases; this may be due to issues with the calibration curve, or contamination of the Falcon tubes used to prepare the samples for analysis. The data summarizing the recovered Fe(II)\(_{aq}\) is presented below.

Table 3.3.1.1: Iron recovery for 5:1 manganese excess trials conducted January 8, 2018 through January 16, 2018.

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample Designation</th>
<th>Initial Fe(II) (µM)</th>
<th>Initial Mn(II) (µM)</th>
<th>% Recovered Fe(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01/08/18</td>
<td>A</td>
<td>1004</td>
<td>4762</td>
<td>53.9 ± 0.1</td>
</tr>
<tr>
<td>01/08/18</td>
<td>C</td>
<td>1004</td>
<td>4762</td>
<td>48.3 ± 0.1</td>
</tr>
<tr>
<td>01/09/18</td>
<td>E</td>
<td>992.8</td>
<td>4762</td>
<td>52.4 ± 0.1</td>
</tr>
<tr>
<td>01/10/18</td>
<td>G</td>
<td>1019</td>
<td>4762</td>
<td>53.5 ± 0.7</td>
</tr>
<tr>
<td>01/10/18</td>
<td>I</td>
<td>1019</td>
<td>4762</td>
<td>53.8 ± 0.4</td>
</tr>
<tr>
<td>01/11/18</td>
<td>K</td>
<td>1002</td>
<td>5034</td>
<td>55.3 ± 0.7</td>
</tr>
<tr>
<td>01/11/18</td>
<td>M</td>
<td>1002</td>
<td>5034</td>
<td>56.2 ± 0.5</td>
</tr>
<tr>
<td>01/12/18</td>
<td>O</td>
<td>993.8</td>
<td>5034</td>
<td>53.5 ± 1.3</td>
</tr>
<tr>
<td>01/12/18</td>
<td>Q</td>
<td>993.8</td>
<td>5034</td>
<td>55.2 ± 0.5</td>
</tr>
<tr>
<td>01/16/18</td>
<td>S</td>
<td>998.8</td>
<td>5034</td>
<td>54.4 ± 0.8</td>
</tr>
<tr>
<td>01/16/18</td>
<td>U</td>
<td>998.8</td>
<td>5034</td>
<td>55.7 ± 0.5</td>
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</table>

These trials showed a relatively consistent recovery of Fe(II) from the filtrate, with an average recovery of 54%. No iron was recovered as Fe(III) using this method. A brown-orange precipitate was collected on filters (Nylon, Whatman) during these
trials. It is likely that the remaining iron was present in this precipitate, though no quantifiable iron measurements were made on the solid. However, manganese oxides on the filter were quantifiable through the use of LBB. The results of these analyses are presented below.

Table 3.3.1.2: Solid manganese recovery for 5:1 manganese excess trials conducted January 8, 2018 through January 16, 2018.

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample Designation</th>
<th>Initial Mn(II) (μM)</th>
<th>μmol Mn on Filter</th>
<th>% Mn recovered as solid</th>
</tr>
</thead>
<tbody>
<tr>
<td>01/10/18</td>
<td>G</td>
<td>4762</td>
<td>0.2593</td>
<td>0.11</td>
</tr>
<tr>
<td>01/10/18</td>
<td>I</td>
<td>4762</td>
<td>0.2568</td>
<td>0.11</td>
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<td>01/11/18</td>
<td>K</td>
<td>5034</td>
<td>0.2229</td>
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</tr>
<tr>
<td>01/11/18</td>
<td>M</td>
<td>5034</td>
<td>0.2152</td>
<td>0.09</td>
</tr>
<tr>
<td>01/12/18</td>
<td>O</td>
<td>5034</td>
<td>0.2037</td>
<td>0.08</td>
</tr>
<tr>
<td>01/12/18</td>
<td>Q</td>
<td>5034</td>
<td>0.2013</td>
<td>0.08</td>
</tr>
<tr>
<td>01/16/18</td>
<td>S</td>
<td>5034</td>
<td>0.4006</td>
<td>0.16</td>
</tr>
<tr>
<td>01/16/18</td>
<td>U</td>
<td>5034</td>
<td>0.4091</td>
<td>0.16</td>
</tr>
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Recoveries for the manganese solids were less consistent than recoveries for the soluble Fe(II). This is likely because, after the first 16 filters were weighed prior to use, an average weight of $0.0125 \pm 0.0002$ g was used in all following calculations; had an exact weight been obtained for all clean, dry filters, these results may have been more precise.

Similar analyses were performed for the iron excess trials. Results from these experiments are summarized below.
Table 3.3.1.3: Iron recovery for 5:1 iron excess trials conducted January 8, 2018 through January 16, 2018.

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample Designation</th>
<th>Initial Fe(II) (μM)</th>
<th>Initial Mn(II) (μM)</th>
<th>% Recovered Fe(II)</th>
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<tbody>
<tr>
<td>01/08/18</td>
<td>B</td>
<td>5020</td>
<td>952.3</td>
<td>99.0 ± 0.4</td>
</tr>
<tr>
<td>01/08/18</td>
<td>D</td>
<td>5020</td>
<td>952.3</td>
<td>98.3 ± 0.1</td>
</tr>
<tr>
<td>01/09/18</td>
<td>F</td>
<td>4496</td>
<td>952.3</td>
<td>110 ± 0.5</td>
</tr>
<tr>
<td>01/10/18</td>
<td>H</td>
<td>5096</td>
<td>952.3</td>
<td>85.6 ± 9.5</td>
</tr>
<tr>
<td>01/10/18</td>
<td>J</td>
<td>5096</td>
<td>952.3</td>
<td>90.8 ± 1.1</td>
</tr>
<tr>
<td>01/11/18</td>
<td>L</td>
<td>5012</td>
<td>952.3</td>
<td>87.1 ± 1.8</td>
</tr>
<tr>
<td>01/11/18</td>
<td>N</td>
<td>5012</td>
<td>952.3</td>
<td>88.2 ± 1.1</td>
</tr>
<tr>
<td>01/12/18</td>
<td>P</td>
<td>4969</td>
<td>1007</td>
<td>100 ± 0.3</td>
</tr>
<tr>
<td>01/12/18</td>
<td>R</td>
<td>4969</td>
<td>1007</td>
<td>100 ± 0.6</td>
</tr>
<tr>
<td>01/16/18</td>
<td>T</td>
<td>4994</td>
<td>1007</td>
<td>100 ± 1.1</td>
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<tr>
<td>01/16/18</td>
<td>V</td>
<td>4994</td>
<td>1007</td>
<td>99.4 ± 1.3</td>
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</table>

Table 3.3.1.4: Solid manganese recovery for 5:1 iron excess trials conducted January 8, 2018 through January 16, 2018.

<table>
<thead>
<tr>
<th>Date</th>
<th>Sample Designation</th>
<th>Initial Mn(II) (μM)</th>
<th>μmol Mn on Filter</th>
<th>% Mn recovered as solid</th>
</tr>
</thead>
<tbody>
<tr>
<td>01/10/18</td>
<td>H</td>
<td>952.3</td>
<td>0.3069</td>
<td>0.64</td>
</tr>
<tr>
<td>01/10/18</td>
<td>J</td>
<td>952.3</td>
<td>0.2892</td>
<td>0.61</td>
</tr>
<tr>
<td>01/11/18</td>
<td>L</td>
<td>952.3</td>
<td>0.2551</td>
<td>0.54</td>
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<tr>
<td>01/11/18</td>
<td>N</td>
<td>952.3</td>
<td>0.2889</td>
<td>0.61</td>
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<tr>
<td>01/12/18</td>
<td>P</td>
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<td>0.2971</td>
<td>0.59</td>
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<td>01/12/18</td>
<td>R</td>
<td>1007</td>
<td>0.2407</td>
<td>0.48</td>
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<td>01/16/18</td>
<td>T</td>
<td>1007</td>
<td>0.4617</td>
<td>0.92</td>
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<tr>
<td>01/16/18</td>
<td>V</td>
<td>1007</td>
<td>0.4017</td>
<td>0.80</td>
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</tbody>
</table>

It is interesting to note that, as shown in Tables 3.3.1.4 and 3.3.1.2, the percentage of initial manganese recovered as a solid oxide increased roughly five times in the iron excess trials as compared to the manganese excess trials. This result indicates that whatever is happening in the reaction mixture to oxidize the manganese is dependent on iron, particularly since the percent recovery of manganese as a solid
oxide increased by the same factor that the iron concentration was increased by, despite the fact that manganese was lower in concentration.

The iron excess trials had an overwhelming recovery of iron as Fe(II), though the actual percentage was more variable than that of the manganese excess trials. It is surprising that almost all of the iron was recovered as Fe(II) at first glance, as iron would be expected to oxidize in solution. However, this occurrence may be explained by pH effects.

At the start and end of all of the trials, pH was recorded. The 5:1 manganese excess reactions started off at an average pH of 7.7 and ended at a pH of 5.0. The 5:1 iron excess reactions started at the same pH but tended to end a bit lower at 4.4. As monitored visually, the reactions appeared to go to completion within 15 minutes. However, reactions were allowed to run for 4-8 hours depending on the day in order to ensure the reactions went to completion. This variation in time did not seem to impact results, indicating that these interactions proceed rapidly. It was hypothesized that the drop in pH was the factor that shut down the reaction progress. This belief has theoretical basis, as shown by thermodynamic calculations by Luther (2010). Based on these calculations, it is expected that the oxidation of Fe(II) to insoluble Fe(III) species by molecular oxygen to form superoxide by a one-electron transfer reaction (Equation 1) is only favorable at pH above 5.5; below this threshold, reactions following this mechanism would not be expected to run. It is important to note that, by similar calculations, Mn(II) oxidation to Mn(III) by molecular oxygen by a one-electron transfer process (Equation 2) is not favorable below a pH of 10; however, Mn(II) oxidation to form Mn(III) by superoxide via a one-electron transfer is favorable across all pH (Luther 2010). These theoretical calculations mesh well with the experimental
data to support the idea that, in the presence of oxygen, iron will oxidize to form superoxide which can then go on to oxidize manganese. To further test the pH effect on these reactions, a new experiment was designed.

\[
Fe(II) + O_2 \rightarrow Fe(III) + O_2^- \quad (1)
\]
\[
Mn(II) + O_2^- \rightarrow Mn(III) + H_2O_2 \quad (2)
\]

### 3.3.2 Acidified Seawater Trials

In order to test the pH effects described above on these reactions, a sample of filtered seawater was acidified to a pH of 5.53 and used to prepare the reaction vessels in place of regular filtered seawater. After addition of all reactants except iron, the manganese excess trials started at a pH of 5.48, while the iron excess trials started at a pH of 5.40. After roughly five hours of reaction time, the reactions had visibly not progressed to the extent that the initial experiments had, though some color was still visible. The manganese excess trials ended at an average pH of 5.2, while the iron excess trials ended at a pH of 4.66. An image of these reactions after 2.5 hours of reaction time is presented below.

Figure 3.3.2.1: Acidified seawater experiment from January 18, 2018 after 2.5 hours of reaction time. From left to right: 5:1 manganese excess replicate A, 5:1 manganese excess replicate B, 5:1 iron excess replicate A, 5:1 iron excess replicate B.
The same iron speciation analysis and manganese analyses done for the regular excess trials were also performed on these samples. The results of these experiments are presented below.

Table 3.3.2.1: Iron recovery for 5:1 excess trials conducted in acidified seawater on January 18, 2018.

<table>
<thead>
<tr>
<th>Excess Metal</th>
<th>Replicate</th>
<th>Initial Fe(II) (μM)</th>
<th>Initial Mn(II) (μM)</th>
<th>% Recovered Fe(II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manganese A</td>
<td>1007</td>
<td>4990</td>
<td>92.0 ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Manganese B</td>
<td>1007</td>
<td>4990</td>
<td>94.6 ± 2.0</td>
<td></td>
</tr>
<tr>
<td>Iron A</td>
<td>5034</td>
<td>1007</td>
<td>84.8 ± 8.2</td>
<td></td>
</tr>
<tr>
<td>Iron B</td>
<td>5034</td>
<td>1007</td>
<td>64.0 ± 12</td>
<td></td>
</tr>
</tbody>
</table>

The exceptionally low value for percent recovery Fe(II) for the 5:1 iron excess B replicate is not credible, particularly in light of the large standard deviation. Though the ferrozine method makes use of a buffer to control pH, it is possible that the buffer was not enough to control the pH during analysis of these acidified samples enough for the method to work correctly, resulting in a lower apparent percent recovery and high standard deviations on the values obtained. Recovery of added iron as Fe(III) was highly variable even within triplicates, ranging between 0 and 1.7%.

The results from the solid MnO₅ analyses are presented below.

Table 3.3.2.2: Solid manganese recovery

<table>
<thead>
<tr>
<th>Excess Metal</th>
<th>Replicate</th>
<th>Initial Mn(II) (μM)</th>
<th>μmol Mn on Filter</th>
<th>% Mn recovered as solid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manganese A</td>
<td>4990</td>
<td>0.4001</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Manganese B</td>
<td>4990</td>
<td>0.3749</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Iron A</td>
<td>1007</td>
<td>0.5293</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>Iron B</td>
<td>1007</td>
<td>0.5036</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>
Results for the manganese solids were much more consistent than the results for the iron speciation, indicating that the issue with the iron analysis was likely specific to the methods and not the sample handling and preparation. These results mimic the trends seen in the normal trials, with the percent recovery of manganese in the solid phase being around five times higher in the iron excess trials than in the manganese excess trials. Interestingly, though there was visibly less material on the filters, roughly the same amount of manganese oxide was present on the filters from the acidified trial as in the normal trials. This is likely because the iron oxidation was inhibited by low pH as predicted by Luther (2010), but what iron did go forward was able to produce enough superoxide to oxidize the manganese. As manganese oxides were generated, it likely became slightly easier for manganese oxides to form and precipitate as the crystals could flock together, which allowed more manganese oxides to form with less iron oxidizing and precipitating out of solution.

This result has interesting implications for natural systems, particularly hydrothermal vent systems. As part of Cruise AT37-11 (R/V Atlantis with DSV Alvin) in spring 2017 at the East Pacific Rise (EPR), measurements were made for iron speciation, manganese speciation, and H₂O₂ at and around vents and diffuse-flow sites (Luther et al. 2017). In some areas where peroxide was found, oxidized Mn(III)-L and MnOₓ species were also detected while iron was entirely present as Fe(II), in accordance with Equations 1 and 2 (Luther et al. 2017). The results obtained from these laboratory experiments seem to mimic the results obtained from environmental samples brought up by DSV Alvin. This provides new insights into trace metal chemistry and cycling in deep waters near vents and other natural systems.
These findings also have some bearing on discussions of banded iron formations (BIFs). While BIFs, as the name would suggest, are predominantly composed of iron species, low levels of manganese-containing minerals are also present. Some attempts have been made to explain the presence of manganese oxides by photooxidation; a study by Anbar and Holland (1992) was able to precipitate Mn(II) as birnessite by exposure to light ($\lambda<240$ nm). However, the rates estimated by Anbar and Holland for this pathway under Precambrian conditions were approximately 1000 times slower than the rates that would have been needed to deposit the BIFs seen today. The presence of even a small amount of oxygen, based on the acidified seawater experiments performed as a part of this thesis, could have facilitated some additional iron oxidation and generated superoxide, which would allow for manganese to oxidize and supplement the rates described by photooxidative pathways. This iron-manganese-oxygen interaction provides a new lens to analyze natural systems.

3.3.3 Ligand Addition Trials

Though the production of manganese oxides is an important result and should not be neglected, the original observations that prompted this work noted manganese oxidation forming Mn(III)-L. The hydrothermal vent work of AT37-11 also saw these oxidized species, though conditions at the hydrothermal vents were different from those in the marsh. As such, these ligand addition experiments were designed in an attempt to replicate the original results of the $+1\mu$M MnCl$_2$ additions from 2016.

Ligands in natural systems are highly diverse, poorly characterized, and difficult to isolate. For the purposes of these experiments, a quantifiable ligand was required so that Fe(III)-L and Mn(III)-L could readily be identified and distinguished.
Therefore, a series of spectrophotometrically known compounds were used in an attempt to stop the oxidation of Mn(II) at Mn(III), and to hold any oxidized iron as Fe(III)-L before it was precipitated by a drop in pH.

The first ligand used was tiron ((OH)$_2$C$_6$H$_2$(SO$_3$Na)$_2$), which consists of an aromatic ring substituted with two ortho hydroxy groups and two meta -SO$_3$- Na$^+$ pairs. A structure of this molecule is provided in Figure 3.3.3.1 below.

![Figure 3.3.3.1: Molecular structure of tiron (produced using ChemDraw).](image)

This ligand has been spectrophotometrically characterized for both iron and manganese. A summary of wavelengths of maximum absorption for different iron and manganese complexes are presented in Table 3.3.3.1 below.

<table>
<thead>
<tr>
<th>Metal Ion</th>
<th>Mono</th>
<th>Bis</th>
<th>Tris</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mn(III)</td>
<td>440</td>
<td>420</td>
<td>575</td>
</tr>
<tr>
<td>Fe(III)</td>
<td>356</td>
<td>355</td>
<td>476</td>
</tr>
<tr>
<td></td>
<td>417</td>
<td>561</td>
<td></td>
</tr>
<tr>
<td></td>
<td>670</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The molar absorptivity coefficients for the Mn(III)-tiron complexes are an order of magnitude smaller than the coefficients for the iron-containing complexes (Sever & Wilker 2004). Since some of the peaks are expected to be close together, drowning out the Mn(III)-tiron signal was a concern. An experimental spectrum of the reaction mixture with tiron is given in Figure 3.3.3.2 below.

Figure 3.3.3.2: Sample spectrum of Mn(III)-tiron and Fe(III) tiron as experimentally obtained January 22, 2018. Inset an expanded view of 400-600 nm.

As seen in Figure 3.3.3.2 above, the reaction mixture exhibited a broad peak between 400 and 600 nm. Upon expansion, it was revealed that this peak was actually composed of several smaller peaks occurring at $\lambda = 446$, 486, 580, 584, and 592 nm. The peak at 446 nm may correspond to the anticipated mono Mn(III)-tiron complex, while the peak at 580 nm may reflect the tris Mn(III) species observed by Sever & Wilker 2004. If this is the case, then this experiment was able to replicate the natural system observed in the initial phase of experimentation – iron was able to react with
oxygen to produce superoxide which was able to go on and oxidize Mn(II) to Mn(III) via a one electron transfer, which was then trapped to form a Mn(III)-L species. However, iron has some signals nearby with a higher molar absorptivity that may have overlapped with the Mn(III) signal; therefore, other ligands were used to clarify results.

Desferoxamine mesylate (DFOB, C$_{25}$H$_{48}$N$_{6}$O$_{8}$·CH$_{4}$O$_{3}$S) was the next ligand used. A structure of the molecule, both alone and complexed with Mn(III), is shown in Figure 3.3.3.3 below.
Figure 3.3.3.3: A) Linear structure of DFOB, un-complexed. B) Complexed structure of DFOB with Mn(III), η⁶. Structures produced using ChemDraw.

The DFOB complexes iron in a similar manner to manganese. A sample spectrum of the reaction mixture with DFOB is presented in Figure 3.3.3.4 below.
As seen in Figure 3.3.3.4 above, DFOB was not an ideal ligand to use for this experiment. One large peak is visible between 380 and 480 nm, but even in an expanded view iron cannot be differentiated from manganese. The order of addition experiments described in the methods section yielded similar results.

1,4,8,11-Tetraazacyclotetradecane (cyclam) was the final ligand used in this experiment. A structure of the molecule is provided in Figure 3.3.3.5 below.
There were three experiments carried out using this ligand. Of the three, “Cyclam B” yielded the most promising results. In this experiment, a reaction vessel was prepared with filtered seawater, DI, and Fe(II) and pH adjusted using 5% HCl based on the volume required to pH adjust “Cyclam A.” Mn(II) and cyclam were then added simultaneously. A spectrum of the products of this reaction is reproduced below in Figure 3.3.3.6.

Figure 3.3.3.6: Spectrum of the products of “Cyclam B,” run on January 31, 2018. Inset an expanded view of 230-460 nm.

The broad slope from around 220 to 450 nm, when expanded, reveals shoulders in the curve. Spectra collected of Fe(III)-Cyclam complexes revealed a broad signal around 244 and 360 nm, which indicates that the broad sloping peak seen in the reaction mixture is likely due to an iron-cyclam complex. A prepared sample of a Mn(III)-cyclam complex displayed a peak at 296 nm. It is believed that the first shoulder observed in the inset of Figure 3.3.3.6 is due to the presence of a Mn(III)-cyclam complex as a result.
Though experiments with DFOB were not successful, spectrophotometric peaks for manganese (III) complexes with tiron and cyclam were potentially identified. This serves as a proof of concept that the presence of ligands in a system can halt the oxidation of manganese at an aqueous state, especially since no precipitates could be collected during any of the ligand experiments.

### 3.3.4 Peroxide Experimentation

The goal of the peroxide experiment was to determine whether peroxide ($H_2O_2$) or superoxide ($O_2^-$) was responsible for the oxidation of manganese during the experiments described above, as both may form during the oxidation of Fe(II) to Fe(III). By adding peroxide to a solution of Mn(II) in the absence of Fe(II), dependence on peroxide could be confirmed or rejected.

The reaction was monitored visually, as all other reactions run over the course of laboratory experimentation exhibited some kind of visible color change and/or precipitation over the course of the reaction. The peroxide did not visibly react with the Mn(II), nor were any precipitates formed. It was therefore concluded that peroxide generated by iron oxidation was not responsible for the oxidation of manganese, but rather superoxide was most likely the oxidant.

### 3.3.5 X-Ray Diffraction

The XRD patterns for the 5:1 manganese excess trials and the 5:1 iron excess trials were nearly identical. A sample XRD pattern is presented in Figure 3.3.5 below.
Figure 3.3.5.1: Background-subtracted XRD pattern of a 5:1 iron excess trial with a bubbler run on January 10, 2018. XRD analysis performed January 26, 2018.

There are some notable features in the XRD pattern in Figure 3.3.5.1 above. The broad peaks around $2\theta = 35$ and 62 are indicative of the presence of ferrihydrite, which is an oxidized form of iron (Um 2014). These peaks are nearly absent in the XRD patterns of the acidified seawater trial filters, which further indicates that the lower pH prevented iron oxidation to some extent.

Some evidence is found in the above XRD pattern for oxidized manganese species as well. Amorphous, poorly crystallized MnO$_2$ can display a dip in the spectrum at $2\theta = 45$ (Lafferty et al. 2010). This may be present in the XRD spectrum above, but the signal is too noisy to be able to tell for sure. Other manganese minerals, including buserite, biressite, and todorokite, have strong signals in the region $2\theta = 10$-20 (Lee & Xu 2016). Unfortunately, this region in the XRD is incredibly indistinct, so
it is difficult to identify if any of these are present in the samples analyzed. Although it would be interesting to determine exactly which manganese oxide species formed over the course of the experiments performed in the laboratory, the XRD data are ultimately not necessary to achieve the aims of this thesis. The LBB method proved that the manganese oxides formed, and the ligand addition experiments were able to confirm ligand trapping of Mn(III) as Mn(II) was oxidized by superoxide generated by Fe(II) oxidation by O$_2$. 
Chapter 4

CONCLUSIONS

4.1 Environmental Manganese Speciation

Mn(III)-L is a significant component of the total dissolved manganese pool. In a tidal creek setting, up to 99% of the total manganese recovered was composed of Mn(II)-L species. This number was slightly lower in the Delaware bay surface water samples (78%), likely due to the higher influx of organic material from the marsh at the creek site. These high percentages of Mn(III) are particularly significant since they come from oxygenated systems; previous Mn(III) work has predominantly been focused on suboxic zones. Some more recent work has also documented the presence of Mn(III)-L in oxygenated waters (Oldham et al. 2017; Oldham et al. 2017).

Tidal fluctuations of manganese concentrations were observed, primarily due to the interactions between the higher-concentration marsh porewater influences and the dilution that occurs upon interaction with lower-concentration oceanic-type waters of the Delaware Bay. A sharp increase of 1.58μM dMnT was observed at low tide in comparison to other points in the tidal cycle. Mn(II) and Mn(III)-L_{(weak)} concentrations followed the tidal fluctuations of dMnT. However, Mn(III)-L_{(strong)} did not follow this scheme. More work, namely another tidal series collection with more time-points, is needed in order to gain a clearer understanding of tidal influences on Mn(III)-L_{(strong)} in tidal marshes.

The light/dark bottle experiments, though imperfect, helped to clarify that photo-oxidation of samples was not a major concern on short timescales. However, as Mn(II) was oxidizing over time in the dark bottles, it is clear that some unexpected
reactivity is present and occurring in environmental samples that was unaccounted for previously.

   The addition experiments to the tidal series confirmed these findings. Though these experiments were carried out prior to the light/dark bottle experiment as an expectedly simple method check, the results make more sense in light of the findings from the light/dark bottle experiment. All added Mn(II) was oxidized in the 45-minute time window before analysis, and in the case of the low tide sample some of the additional Mn(II) present in the environmental sample was oxidized as well. As these samples were all filtered, biological activity can be ruled out as a cause of this oxidation. These findings laid the groundwork for the laboratory experimentation that followed in 2018.

4.2 Interactions Between Iron, Manganese, and Oxygen

   In a mixture of filtered seawater, deionized water, and manganese (II) chloride, an addition of ferrous ammonium sulfate resulted in an immediate, visibly apparent reaction. This reaction was believed to be a single-electron transfer pathway which began with the oxidation of Fe(II) by molecular oxygen to form Fe(III) and superoxide. The superoxide could then go on to react with Mn(II) through single electron pathway, oxidizing the Mn(II) to Mn(III) (Luther 2010). This Mn(III) could precipitate out as a manganese oxide, oxidize further to an insoluble Mn(IV) oxide, or be complexed by a ligand to remain trapped in solution.

   Over the course of the laboratory experiments carried out during the winter of 2018, it was shown that this reaction series was possible, and that superoxide (not peroxide) was the oxidant responsible for the formation of Mn(III). It was also shown that these reactions depend heavily on pH, as predicted by Luther (2010). Ligands
were shown to be able to halt the reaction pathway by trapping the Fe(III) and Mn(III) in solution, preventing the formation of precipitates.

These results offer a possible explanation for the unexpected sample oxidation observed during the environmental phase of this thesis. They also have implications for a variety of natural systems beyond the marsh from which the environmental samples were taken. For example, the hydrothermal vent system of the East Pacific Rise showed, in some locations, an abundance of Mn(III)-L and reactive oxygen species, and yet all iron was recovered as Fe(II) (Luther et al. 2017). The result of oxidized manganese being present while the more reduced form of iron is recovered was replicated by these laboratory experiments, including at lower pH values that are frequently found surrounding these vent systems. Findings from these laboratory experiments may also be applicable to banded iron formations. Previous theories of manganese incorporation, such as photooxidation, could only account for some of the manganese found associated with the BIFs (Anbar & Holland 1992; Trouwborst et al. 2007). This work has shown that iron, in the presence of oxygen, can produce reactive oxygen species capable of oxidizing manganese. While this may not be the only mechanism in play for BIF deposition, it helps to account for some of the manganese present in these formations.

4.3 Implications for Future Work

This work has uncovered potential interactions between the iron, manganese, and oxygen cycles in the marine environment. Both salt marsh and vent samples showed that Mn(II) oxidized at pH values where the reaction of Mn(II) and O₂ is thermodynamically unfavorable. Laboratory experiments using natural seawater with natural organics were able to mimic the natural systems when Fe(II) and Mn(II) were
added to the seawater. Using a spectrophotometrically discernable ligand to halt Mn(II) oxidation at Mn(III)-L and Fe(II) oxidation at Fe(III)-L is a point of interest and could potentially be of use in determining the kinetics of these interactions.
REFERENCES


Madison AS, Tebo BM, Luther GW III (2011) Simultaneous Determination of Soluble Manganese (III), Manganese (II), and Total Manganese in Natural (Pore)Waters. *Talanta* **84**:374-381.


