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Abiotic Reduction of Nitrite and Nitrate by Nanoscale Chemogenic Magnetite: Pathways for Significant Greenhouse Gas Production

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ABBIOTIC REDUCTION OF NITRITE AND NITRATE BY NANOSCALE CHEMOGENIC MAGNETITE: PATHWAYS FOR SIGNIFICANT GREENHOUSE GAS PRODUCTION

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

By

ADAM C BURDSALL
B.S., Wittenberg University, 2011

2013
Wright State University
I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Adam C Burdsall ENTITLED Abiotic Reduction of Nitrite and Nitrate by Nanoscale Chemogenic Magnetite: Pathways for Significant Greenhouse Gas Production BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science

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ABSTRACT


The biogeochemical cycling of nitrogen in terrestrial systems is a major source of nitrous oxide (N₂O), which is among key greenhouse gases (GHG). While biotic processes are commonly accepted as the major drivers of N₂O production, the role of abiotic processes is less understood, and their importance may be underestimated. This study investigates abiotic reactions in the formation and breakdown of N trace gases, including nitric oxide (NO) and nitrous oxide (N₂O) by naturally occurring nanoscale magnetite in soil as denitrification (reduction of nitrite) intermediates. Nitrogen biogeochemical cycling has been studied extensively with respect to microbial processes, atmospheric pollution and greenhouse gases. In addition to being potent greenhouse gases, NO catalyzes ozone production in the troposphere and N₂O contributes to the destruction of ozone in the stratosphere.

Iron oxyhydroxides such as lepidocrocite were reported to reduce nitrite and nitrate to NO and N₂O. Likewise, Magnetite's power to reduce pollutants like carbon tetrachloride (CT) and nitrobenzenes was also demonstrated in past works, suggesting that magnetite may have the ability to reduce nitrite and nitrate. In this study, the potential of chemogenic magnetite nanoparticles to abiotically reduce nitrite and nitrate to other nitrogen species (i.e. NO, N₂O, N₂ and NH₃) was investigated in bench-scale
batch reactors by characterizing reaction kinetics and quantifying various product mole fractions. The study focused on mass-fractions of NO and N$_2$O that may be produced from nitrite and nitrate with magnetite.

The results confirm that chemogenic magnetite was capable of rapidly degrading nitrite into N$_2$O with some N$_2$ exhibiting pseudo first order reaction kinetics. Results show that 1.16 g L$^{-1}$ (5 mM) magnetite in batch experiments under anaerobic conditions at pH 7 with no Fe$^{2+}$ degraded almost all of 0.025 millimoles of nitrite resulting in about 50% N$_2$O-N in about 2 days. N$_2$O-N production was reduced by increases in pH and the amount of magnetite used. Ammonia was produced under basic conditions and N$_2$ gas yields increased under basic conditions and in the presence of aqueous Fe(II). The presence of aqueous Fe(II) also increased the rate of the reaction such that nearly all of 0.025 millimoles of nitrite were removed within two to three hours. NO became a major product when the initial rate constant of the reaction ($k_{obs}$) was low or when the magnetite was insufficient to degrade the nitrite that was present. Fe(II) additions degraded the NO and the reaction continued until the NO concentration stabilized again, suggesting a relationship between NO concentration and denitrification reaction. Increasing magnetite concentration increased $k_{obs}$. Although nitrate has been said to be reactive in some cases, the reaction of nitrate to magnetite was nearly negligible in this investigation. Any reaction that did take place appeared to have only N$_2$ as a product.

Magnetite reactions toward nitrite and nitrate are newly reported and the implications of this redox system are not yet clearly indicated. However, it is suggested that in interface zones of Fe$^{3+}$ reduction that may form magnetite, denitrification of nitrite may take place, especially in areas where farm practices include excessive fertilization.
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Chapter 1

1.0 BACKGROUND

The biogeochemical cycling of nitrogen in terrestrial systems is a major source of nitrous oxide (N\textsubscript{2}O), which is among key greenhouse gases (GHG). Biological processes are commonly accepted as the major drivers of N\textsubscript{2}O production (Kampschreur et al., 2011) through the reduction of nitrate (NO\textsubscript{3}\textsuperscript{-}) or the reduction of nitrite (NO\textsubscript{2}\textsuperscript{-}). Nitrite may become concentrated in soil when fertilizers like urea, ammonium carbonate, diammonium phosphate, and monoammonium phosphate are applied in bands on soils (van Cleemput, 1998). The role of abiotic processes of denitrification in the subsurface is less understood than biological processes, and their importance may be underestimated. This study investigated abiotic reactions in the formation and breakdown of N trace gases, including denitrification intermediates like nitric oxide (NO) and N\textsubscript{2}O by chemogenic nanoscale magnetite (Fe\textsubscript{3}O\textsubscript{4}). The results of this investigation could be applied to natural settings in which nitrite and nitrate accumulate in magnetite rich soil.

Magnetite is a common mineral that may be formed by DIRB or by chemogenic processes when dissolved Fe(II) interacts with sources of Fe(III) in the proper proportions. Denitrification of nitrite and nitrate in the subsurface was expected to follow a pattern similar to the reactions that have been observed for biological systems, where
nitrate or nitrite is reduced to NO, N₂O, and finally to N₂. This progression of nitrogen reduction followed a pattern illustrated by Eq. 1.

\[
\text{NO}_3 \rightarrow \text{NO}_2 \rightarrow \text{NO} \rightarrow \text{N}_2\text{O} \rightarrow \text{N}_2
\] (1)

### 1.1 Abiotic Denitrification

Venterea's experiments in 2007 used soil batch reactors to monitor N₂O production in soils. Killed control reactors were γ irradiated to examine the biological source of N₂O. However, it was found that cultivated γ irradiated soils exhibited 60 to 75% N₂O production (Venterea, 2007). Although not stated in the paper by Venterea, other studies on abiotic denitrification point to the presence of minerals and dissolved Fe(II) that adsorbs to the mineral's surface, producing reactive sites for nitrite and perhaps nitrate.

Kampschreur et al. (2011) investigated the reaction of KNO₂ in solution in a batch reactor containing FeSO₄ solution. NO and N₂O formation were monitored until dissolved Fe(II) was depleted (Kampschreur et al., 2011). The reactor started out as a homogeneous liquid, but as nitrite reacted with and oxidized Fe(II) in solution, it formed Fe(III) precipitates (Kampschreur et al., 2011). The precipitate that formed as Fe(II) oxidized was presumed to be green rust based on the greenish color of the precipitate (Summers and Chang, 1993; Kampschreur et al., 2011). Kampschreur et al. (2011) proposed the following reactions that lead to the production of NO and N₂O in natural systems (Eqs. 2a and 2b below), which add to make an overall reaction mechanism (Eq. 2). The Gibbs free energy calculations (ΔGₜₚₚ), however, suggest that the overall reaction may not be highly thermodynamically favorable (Eq. 3) (Kampschreur et al., 2011). The
Gibbs free energies of Eq. 2 suggest that the initial reaction between nitrite and Fe(II) to produce Fe(III) and NO (Eq. 2a) is the rate limiting step (Kampschreur et al., 2011).

\[
\begin{align*}
\text{NO}_2^- + \text{Fe}^{2+} + 2\text{H}^+ &\rightarrow \text{Fe}^{3+} + \text{NO} + \text{H}_2\text{O} \quad [\Delta G = -43.1 \text{ kJ/mol}] \\
+ \quad \text{NO} + \text{Fe}^{2+} + \text{H}^+ &\rightarrow \text{Fe}^{3+} + 0.5\text{N}_2\text{O} + 0.5\text{H}_2\text{O} \quad [\Delta G = -80.1 \text{ kJ/mol}]
\end{align*}
\] (2)

\[\Delta G = -43.1 \text{ kJ/mol} + -80.1 \text{ kJ/mol} = -123.2 \text{ kJ/mol} \quad (3)\]

For nitrite reduction with the green rust precipitate, the pH affected the product distribution. NO and N$_2$O were produced under slightly acidic conditions and ammonia was observed at around pH 7.6 (Eq. 4) (Summers and Chang, 1993).

\[6\text{Fe}^{2+} + 7\text{H}^+ + \text{NO}_2^- \rightarrow 6\text{Fe}^{3+} + 2\text{H}_2\text{O} + \text{NH}_3 \] (4)

The mechanisms for denitrification with magnetite are proposed to be very similar to those proposed by Kampschreur et al. (2011) and Summers and Chang (1993). These reduction reactions can instead include the anodic magnetite half reaction shown by White, et al. (1994) whereby magnetite was oxidized to γ-maghemite (Eq. 5).

\[3[\text{Fe}^{2+}\text{Fe}_2^{3+}]\text{O}_4\text{(magnetite)} \rightarrow 4\gamma[\text{Fe}_2^{3+}]\text{O}_3\text{(maghemite)} + \text{Fe}^{2+} + 2e^- \] (5)

When this half reaction replaces the Fe(II) oxidation half reaction in equations 2 and 4, the reactions should function as shown in Eqs. 6a, 6b, 6, and 7 below (modified from White et al., 1994; Kampschreur et al., 2011; and Summers and Chang, 1993).

\[3\text{Fe}_3\text{O}_4 + 2\text{NO}_2^- + 4\text{H}^+ \rightarrow 4\text{Fe}_2\text{O}_3 + \text{Fe}^{2+} + 2\text{NO} + 2\text{H}_2\text{O} \quad [\Delta G = -236.1 \text{ kJ/mol}] \quad (6a)\]

\[+ \quad 3\text{Fe}_3\text{O}_4 + 2\text{NO} + 2\text{H}^+ \rightarrow 4\text{Fe}_2\text{O}_3 + \text{Fe}^{2+} + \text{N}_2\text{O} + \text{H}_2\text{O} \quad [\Delta G = -547.2 \text{ kJ/mol}] \quad (6b)\]
6Fe₃O₄ + 2NO₂⁻ + 6H⁺ → 8Fe₂O₃ + 2Fe²⁺ + N₂O + 3H₂O [ΔG = -783.3 kJ/mol] (6)

And

9Fe₃O₄ + NO₂⁻ + 8H⁺ → 12Fe₂O₃ + 3Fe²⁺ + NH₄⁺ + 2H₂O [ΔG = -525.8 kJ/mol] (7)

In reactions between nitrite and aqueous Fe²⁺, the $k_{obs}$ of the denitrification was proportional to the concentration of nitrite (Summers and Chang, 1993; Kampschreur, 2011) and temperature (Summers and Chang, 1993). The starting concentration of Fe(II) in the reactor had little effect on the rates of NO and nitrite reaction (Kampschreur et al., 2011). NO reduction was a function of the concentration of Fe(II) that was adsorbed to the green rust precipitate (Kampschreur et al., 2011). This result agreed with the results of Tai and Dempsey (2009), which showed that hydrous ferric oxide (HFO) was also highly reactive toward nitrite at pH 6.8 in the presence of dissolved Fe(II) and Fe(II) bound to Fe(III) solid. The second order kinetics of nitrite reduction by HFO was directly proportional to the concentration of solid bound Fe(II) (Tai and Dempsey, 2009). It should be noted, however, that sometimes Fe²⁺ solutions did not react to reduce nitrogen compounds, as was the case when Klausen (1995) tried to degrade nitrobenzene with only Fe²⁺ solutions. Klausen (1995) was able to degrade nitrobenzene with lepidocrocite, magnetite, and goethite with Fe²⁺ in solution.

The equations modified from Kampschreur and others (2011) proposed mechanisms for the formation of NO and N₂O in natural systems by abiotic reduction by magnetite (Eqs. 6 and 7). A third step could be added to this process in which NO can also react to form N₂ (Eq. 8, modified from White et al., 1994 and Clayton et al., 2008).
The iron oxyhydroxide, Lepidocrocite ($\gamma$-FeOOH), can degrade nitrite to N$_2$O so long as it is in the presence of a solution with dissolved Fe(II) (Sørensen and Thorling, 1991). Fe(II) controls initiated denitrification without lepidocrocite, but the reaction was very slow (Sørensen and Thorling, 1991). Chemodenitrification could be written in either of two ways (Eqs. 9 and 10), but Eq. 9, which resulted in magnetite, was preferred in a N$_2$ purged, room temperature environment (Sørensen and Thorling, 1991). Regardless, Fe(II) adsorbed to a mineral's surface provided the reactive surface needed for the nucleophilic addition that produced N$_2$O (Sørensen and Thorling, 1991).

\[
6\text{Fe}^{2+} + 2\text{NO}_2^- + 5\text{H}_2\text{O} \rightarrow 2\text{Fe}_3\text{O}_4 + \text{N}_2\text{O} + 10\text{H}^+ \quad (9)
\]

\[
4\text{Fe}^{2+} + 2\text{NO}_2^- + 5\text{H}_2\text{O} \rightarrow 4\text{FeOOH} + \text{N}_2\text{O} + 6\text{H}^+ \quad (10)
\]

Abiotic denitrification of nitrate may also take place. Four pertinent methods were described here. First, the paper by Sørensen and Thorling (1991) cites Postma (1990), indicating that Fe(II) can degrade nitrate, but only if a solid phase iron-oxyhydroxide is present. The degradation of nitrate with a ferromagnesian mineral like pyroxene was possible after Fe(II) in solution oxidized to Fe(III) during dissolution of the iron-rich silicate (Postma, 1990). A third mechanism shows that the bacteria Gallionella ferruginea reduces nitrate by Fe(II) oxidation (Postma, 1990). A fourth notable method of abiotic denitrification of nitrate to produce nitrite and ultimately N$_2$O is by exposure and reaction to light at a wavelength of 305 nm (Rubasinghege et al., 2011).
1.2 Magnetite Reactivity Toward Nitrogen Species

Magnetite (Fe₃O₄) is a polar, highly reactive iron oxide. It consists of 1 ferrous (Fe(II)) and two ferric (Fe(III)) iron atoms. Different methods of magnetite synthesis have been shown to produce magnetite particles of differing shapes and reactivities. It has been found by Vikesland and others (2007) that nanoparticles of magnetite are far more reactive than granular magnetite due to their greater surface area. Varying pH or ionic strength can strongly affect particle size, where, increasing pH and ionic strength of solutions creates smaller nanoparticles (Vayssières et al., 1998). Likewise, increases in temperature increases mean particle diameter (Vayssières et al., 1998).

The paper by Regazzoni et al. (1981) describes a wide variety of methods for producing magnetite nanoparticles including a method to produce uniform spherical particles. The method of magnetite synthesis used for this investigation was adapted from Vikesland et al. (2007). Using different methods for magnetite synthesis could produce different results (Table 1). Magnetite may also be formed by biological processes. *Geobacter metallireducens* (GS-15) has been found to utilize Fe(III) phases as electron acceptors in natural environments to form a black poorly crystallized form of magnetite (McCormick et al., 2004; Heijman et al., 1993). GS-15 continues to produce magnetite until one third of the Fe(III) present in a system has been reduced (Heijman et al., 1993).

One of the most important factors in the reactivity of synthesized nanoparticle magnetite is the stoichiometry of the particles. Perfectly stoichiometric magnetite nanoparticles, in which the ratio of Fe(II) to Fe(III) is 0.5 is the most reactive magnetite
The Fe(II)/Fe(III) value can vary from 0 to 0.5 in magnetite (Gorski et al., 2010). If the ratio is 0, the magnetite is completely oxidized and is referred to as maghemite ($\gamma$-Fe$_2$O$_3$) instead of magnetite (Gorski et al., 2010). In experiments described in Gorski et al. (2010) nitrobenzene (ArNO$_2$) was reduced by magnetite of varying levels of oxidation. The Fe(II)/Fe(III) ratio was increased from 0.31 to 0.50, which caused the rate of ArNO$_2$ reduction to increase by 5 orders of magnitude (Gorski et al., 2010). Stoichiometric magnetite was created by Gorski and Scherer (2009) by preparing a solution of 1:2 Fe(II)/Fe(III) solution under anoxic conditions and titrating with NaOH to a pH greater than 10. The mixture was allowed to mix overnight. To produce magnetite batches of different levels of oxidation, the stoichiometric magnetite powders were exposed to hydrogen peroxide (H$_2$O$_2$) (Gorski et al., 2010). Oxidation due to extended exposure to air may also change the magnetite into maghemite (Fe$_2$O$_3$), a hematite isomorph (Peterson et al., 1996). Gorski and Scherer (2009) suggest that this oxidation over several months would reduce the effectiveness of the magnetite. The paper by Gorski and Scherer (2009) cautioned against excessive washing of the fresh magnetite because excessive washing could remove structural Fe$^{2+}$.

Exposing oxidized magnetite to a source of Fe(II) restored the 0.5 ratio and the magnetite's reactivity (Gorski and Scherer, 2009). Gorski and Scherer (2009) analyzed a batch of non-stoichiometric magnetite before and after a reaction with Fe$^{2+}$ solution. They used Mössbauer spectroscopy to analyze samples, in which they observed a 12% increase in the reduced and more stoichiometric magnetite and an 11% decrease in the oxidized magnetite signature (Gorski and Scherer, 2009).
Magnetite can be an important reductant in natural soils, which has been shown to reduce 4-chloronitrobenzene (Heijman et al., 1993), nitrobenzene (Klausen et al., 1995; Gorski and Scherer, 2009), and carbon tetrachloride (CT) (McCormick et al., 2002; McCormick et al., 2004). Magnetite was found to degrade 4-chloronitrobenzene to 4-chloroaniline with pseudo first order reaction kinetics (Heijman et al., 1993). Likewise, Gorski and Scherer (2009) found magnetite to be strongly reactive toward and readily reduce nitrobenzenes to anilines. It was found that nitrobenzene was reduced to aniline within one hour when the magnetite had a ratio of Fe(II)/Fe(III) around 0.5 (Gorski and Scherer, 2009). Gorski and Scherer (2009) also underlined the observation that stoichiometric magnetite reduced the nitrobenzene without the presence of aqueous Fe(II). Magnetite tends to react with CT to produce maghemite (Fe$_2$O$_3$) (Vikesland et al., 2007; McCormick et al., 2002; McCormick et al., 2004). There are three pathways of CT degradation that lead to the formation of mostly carbon monoxide (CO) and chloroform (CF) (Danielsen et al., 2004).

1.3 Research Objectives

This study examined the degradation potential of nitrite and nitrate with chemogenic magnetite and its gaseous N reaction products, such as N$_2$O and NO. The goal of the experiments was to characterize the rate of loss of the reactants and the production of the products. This research expanded on previous works that describe abiotic sources of N$_2$O and NO. Magnetite was believed to be reactive toward nitrite because past research has shown that magnetite is reactive toward compounds like nitrobenzene and CT and that other sources of iron are highly reactive toward nitrite and nitrate.
Objective 1: Investigate nitrite and nitrate degradation with magnetite particles and the production of their byproducts (N₂, N₂O, NO, and NH₃).

Objective 2: Examine the effects of pH and concentration of dissolved Fe(II) on nitrite/nitrate degradation kinetics and product distribution as possible controls on the system.

Objective 3: Examine the effects of changing the concentration of nitrite or nitrate and the concentration of magnetite on reaction kinetics and byproduct distribution.
Chapter 2

2.0 MATERIALS AND METHODS

2.1 Magnetite Synthesis

Magnetite was freshly synthesized on the day before starting each degradation experiment using a procedure adapted from Vikesland et al. (2007). Magnetite was synthesized in batches in an anaerobic chamber with a N₂ atmosphere by adding a mixture of 5 mL of 100 mM ferrous sulfate heptahydrate (FeSO₄•7H₂O) and 5 mL of 200 mM ferric chloride hexahydrate (FeCl₃•6H₂O) drop wise using a burette to a 6.67 mL solution of 1000 mM sodium hydroxide (NaOH) and 1000 mM sodium chloride (NaCl). The NaOH + NaCl solution was swirled gently as the FeSO₄ + FeCl₃ mixture was added, immediately producing a black precipitate of magnetite. The slurry was set aside on a non-active magnetic stir plate to let the magnetite settle. If the supernatant had an orange tint, the solution was swirled more to encourage more reaction. This usually precipitated the remaining iron solution as magnetite. The clear supernatant was decanted into a waste beaker. The remaining slurry containing magnetite for each batch was transferred into separate 15 mL serum bottles and sealed with butyl rubber stoppers before removing them from the anaerobic chamber for washing with anaerobic Milli-Q water.

The magnetite slurry was centrifuged for 5 minutes at 3861 RPM (Eppendorf Centrifuge model 5810). Supernatant was carefully extracted with a syringe and replaced
with high purity gaseous N₂ using another syringe. Then 7 or 8 mL of deoxygenated Milli-Q water was added allowing the headspace gas to vent into a second syringe. Both syringes were removed and the bottle was thoroughly mixed on a Fisher Vortex Genie 2 until all of the magnetite was mobile in the slurry. The washing procedure was repeated as described until the pH of the extracted supernatant was between 10.5 and 11.5. If the pH of the supernatant dropped near and below 10.5, the supernatant carried a slight grayish tint, which may have been from removal of structural Fe(II) as described by Gorski and Scherer (2009), resulting in maghemite particles, which did not agglomerate and were not magnetic. This was not significant and did not appear to affect results. The batches were placed back in the anaerobic chamber to be assembled into reactors.

### 2.2 Batch Reactor Set-up

After washing, magnetite batches were rinsed into separate 160 mL borosilicate serum bottles inside the anaerobic chamber. The reactors were filled with 20 mL of a solution of deoxygenated 50 mM TAPSO buffer at pH 6, 7, or 8, a known volume of ferrous sulfate solution if the investigation required a concentration of Fe(II), and deoxygenated Milli-Q water. The desired volume of 0.1 M sodium nitrite (NaNO₂) or sodium nitrate (NaNO₃) was left out of the reactor prior to sealing it. Initial pH readings were measured and the reactors were sealed and removed from the chamber. All reactors were purged for about 50 minutes with grade 5 helium gas. Helium was passed into the reactor through a 23 gauge needle. The reactors were all tilted so that the needle would be angled down into the reactors' liquid. A second venting needle was also inserted into the reactors angled so that the point of the needle pointed up out of the liquid.
Each experiment consisted of four reactors with magnetite. Two of these reactors were combined with a control reactor with the same conditions without magnetite (See Supplemental Information (SI): Fig. S.1). This first group was used for headspace analysis for N₂, N₂O, and NO. The remaining two reactors with magnetite were combined with a second control and used for liquid analysis of nitrite/nitrate and either total dissolved iron or NH₃. On the day of the experiment, an initial gas analysis reading was taken from the gas analysis reactors to find the initial concentration of N₂ prior to the start of the experiment. Following this, the needed volume of helium purged 100 mM NaNO₂ or NaNO₃ solution was added at t0 using a disposable 1 mL syringe to bring the total liquid volume to 100 mL. The reactors were vortexed for about 15 to 30 seconds each to ensure thorough mixing. All of the reactors were sampled 3 or 4 times on day 1, typically at one to two hour intervals so that initial rates of reaction could be determined. On day 2, reactors were sampled 2 or 3 times or perhaps only once if only minute changes were observed between t4 on day one and t5 on day two. This was done only in cases when the reaction rate was greatest or when reactivity was low. Reactors were sampled on day 3 if there was cause to believe that the reactions were not finished at the end of day 2. Gas sampling was reinforced by two replicate injections for each time step. The values of each were averaged. As soon as the gas reactors were sampled, a liquid sample was taken from the corresponding reactor for liquid analysis (e.g. the two control reactors were sampled together, gas followed by liquid).
Liquid sampling consisted of 1 mL liquid extractions from the reactors filtered through a 13 mm plastic filter with a 0.22 micron mesh size into a small vial under a helium stream which already contained 1 mL of filtered Milli-Q water purged with helium. The samples were allowed to mix with the DI water and were separated. 1 mL of the sample was for either total Fe analysis or for ammonia. If the sample was used for total Fe, a small amount of 12 N HCl (~40 µL) was added to stabilize the sample. The remaining sample was used for the ion chromatograph and had ~50 µL of 1 N NaOH added to precipitate any dissolved Fe(II).

2.3 Effect of pH

The concentration of magnetite for the pH analysis was 1.16 g/L (5 mM) for all nitrite experiments. No Fe(II) solution was added for these experiments. Total volume of liquid in the reactors prior to the NaNO₂ amendment was 99.75 mL. 0.25 mL NaNO₂ solution was added at the time the experiment began to give an initial concentration of around 0.25 mM nitrite. For the first experiment, 20 mL of 50 mM TAPSO buffer at pH 7 was used. These conditions became the foundational experimental condition for each of the investigations following (including the pH investigation). To test the effect of pH on the reaction rate and the products, 50 mM TAPSO buffers were made at pH 6 and 8. A separate set of 6 reactors was assembled to analyze the reactions at pH 6 and pH 8. The $k_{obs}$ values were calculated for the first two or three hours of the experiment and normalized to magnetite mass concentration.

To analyze the difference between pH 6 and pH 7 for the nitrate analysis, the reactors were assembled with 2.32 g/L (10 mM) magnetite and 0.1 mM Fe(II) so that any
change in reactivity due to pH might be accentuated. The concentration of nitrate was kept the same as the concentration of nitrite (0.25 mM). Just like in the nitrite analysis, 20 mL of 50 mM TAPSO buffer was used (pH 7 and pH 6). It was determined based on results from the pH 6 and pH 7 experiments that a nitrate experiment at pH 8 was unnecessary.

2.4 Effect of [Fe(II)]

Overall conditions of experiments monitoring nitrite degradation consisted of 1.16 g/L magnetite with 20 mL of 50 mM TAPSO buffer at pH 7 and 0.025 mmol of NaNO₂ injected at the beginning of the experiment. The foundational experiment in this series consisted of 0 mL of FeSO₄ solution added. Other sets of six reactors were assembled with all of these same characteristics containing an added ferrous sulfate concentration of 0.25, 0.50, and 1.0 mM.

The effect of Fe(II) analysis with reactions to nitrate was determined in much the same way as the nitrite series. The reactors were assembled in the same way as in the nitrite series, using 1.16 g/L magnetite and 0.25 mM NaNO₃. However, in the nitrate series, only 0, 0.50, and 1.0 mM ferrous sulfate was examined.

A threshold experiment was also designed to determine the point at which nitrate might degrade in the presence of magnetite and aqueous Fe(II). In the reactor was 4.64 g/L magnetite with 0.25 mM nitrate with 0.1 mmol aqueous Fe(II) at t₀. As the experiment continued, two more amendments of 0.1 mmol Fe(II) were added, increasing the final concentration of Fe(II) added to 0.3 mM.
2.5 Effect of [Nitrite]

Experiments for the analysis of nitrite concentration were assembled using 1.16 g/L (5 mM) magnetite and 20 mL of 50 mM TAPSO buffer at pH 7. No Fe(II) was added for this series initially. The reactors were filled to a volume of 100 – x mL where x was the number of mL of NaNO₂ solution that was added.

Preliminary experiments included a high concentration of nitrite in the reactors: around 5 mM NaNO₂. The concentration of nitrite was decreased to 0.25 mM for most of the experiments that have been used for this investigation. Later, the concentration of NaNO₂ was increased again to 1.0 mM to examine the difference in reaction products. In the experiment with 1.0 mM nitrite, an amendment of 0.05 mmol Fe(II) was added to the reactor when product levels appeared to stabilize. No Fe(II) was added to reactors in the 0.25 mM nitrite experiment because the magnetite was sufficient to degrade the 0.025 mmol nitrite.

2.6 Restoration of Magnetite Reactivity Toward Nitrite by Fe(II) Amendment

There were two instances where restoration of magnetite reactivity was tested in which the amount of magnetite was insufficient to degrade all of the nitrite injected into a reactor. Reactors were monitored with headspace analysis until the amounts of N₂, NO, and N₂O were nearly stable and constant. Then, the reactors were injected with a known volume of 100 mM ferrous sulfate solution. The reactors were then monitored for any changes.
This was first attempted near the conclusion of the experiment to monitor the effect of nitrite concentrations on degradation products with 1.0 mM NaNO₂. An amendment of 0.05 mmol Fe(II) was added by injection 2 days after starting the experiment and then again at t = 15 days. Results from this series were compared to results in the foundation experiment in which 0.25 mM NaNO₂ was used.

The second experiment in which this was attempted was during the "effect of [magnetite]" series described below. The experiment that included 0.58 g/L (2.5 mM) magnetite had shown that at t = 6 days, some nitrite had not reacted, so 0.05 mmol Fe(II) was added to the reactors in that series.

2.7 Effect of [Magnetite]

In this part of the investigation, five experiments were conducted for the nitrite investigation. Five concentrations of magnetite were tested at pH 7 with no initial Fe(II) and 0.25 mM NaNO₂. The concentrations of magnetite tested were 0.58, 1.16, 2.32, 2.90, and 3.48 g/L (2.5, 5, 10, 12.5, and 15 mM) in batch reactors. An experiment with 20 mM magnetite was conducted but the results were not shown in figures because no liquid analysis was completed.

The overall reactor set-up for the nitrate reactors included 20 mL of 50 mM TAPSO buffer, 1.0 mM Fe(II) added, and 0.25 mM NaNO₃. The concentrations of magnetite tested were 1.16, 2.32, and 4.64 g/L.
2.8 NO Degradation with Chemogenic Magnetite

Another experimental design was used for the analysis of the fate of NO and N₂O, reaction intermediates or products. The set consisted of 4 reactors (1 control and 3 experimental reactors) prepared in the same way as mentioned in sections 2.1 and 2.2. This experiment required a different kind of set-up because instead of a liquid reactant amendment, large quantities of a gaseous mix of helium and NO were added by 20 mL injections. To minimize air contamination and headspace loss during the amendments, the process of adding the NO amendments was carried out underwater in a chest cooler (SI: Fig. S.2).

For the first NO amendment at t0, all reactors were placed into the cooler cap-down to avoid gas exchange with the atmosphere. A helium purged 20 ml syringe with a locking stopcock was used to extract 20 mL of headspace from the reactor. Holding the plunger at 20 mL, the stopcock was locked and left in the water until the NO amendment was ready. 20 mL of 1% NO were withdrawn from a small NO stock bottle with a helium purged 20 mL syringe with a locking stopcock. The stock bottle from which the NO was extracted was connected to a gas line containing 1% NO with helium balance. Without removing it from the water, the syringe was locked and the syringe was inserted into the reactor with 20 mL of headspace removed. The stopcock was opened and the NO was injected into the reactor. The syringe was locked and both were carefully removed from the bottle to avoid gas or liquid exchange. The reactor was kept under water until the time came for sampling. Sampling time t1 was within 15 minutes of the NO amendment. Sampling t2 was about two hours later, and t3 for that iteration was the following day.
Then, the reactors were submerged again for another NO amendment, which would start the next iteration of three sampling times named t4, t5, and t6. For each of these three NO amendments, the process was similar to the first amendment except that there was no volume of headspace removed, eliminating one of the syringes that was used. This way the results would be cumulative and the changes in products could be monitored as more NO was added. However, gas pressure in the reactor increased with each addition.

2.9 Analysis

Gas analyses were performed by gas chromatograph (Hewlett Packard 5890) by extracting 50 µL samples from the headspace with a helium flushed 500 µL Hamilton lockable syringe. The primary gases examined were N₂, N₂O, and NO. Oxygen (O₂) and carbon dioxide (CO₂) were the secondary gases analyzed. Gas analysis values were calculated from an average of two injections at each time step.

For each liquid sample, 1 mL was extracted for use for either total iron or for an NH₃-N analysis. NH₃ was primarily shown to be a major product at basic pH, so NH₃ analyses were infrequent after this observation was made. NH₃ analyses were performed using the Phenol hypochlorite method as outlined in Solorzano (1969) on a spectrophotometer using the LAMBDA 45 program (Appendix B). When Fe(II) was added to reactors, instead of an ammonia analysis, ~40 µL of 12 N HCl was added to stabilize the iron for a total iron analysis. Total iron was analyzed using the phenanthroline spectrophotometric method using hydroxylamine to reduce all iron to Fe(II) (Appendix B).
The remaining sample volume was combined with \(~50 \, \mu\text{L}\) of 1 N NaOH to precipitate out any remaining Fe(II) for ion chromatography analysis of nitrate and nitrite (Dionex ICS 2500). Samples were filtered again for the analysis to remove all precipitates possible. The ion chromatograph was used to monitor nitrite and nitrate. Calibration curves for each analysis were created. Some analyses also included a chloride internal standard.

Data from the experiments was expressed either as mole fractions (decimals) or molar amounts in millimoles (mmol). Full details of determination of gas products are in Appendix A. Generally, NO and N\textsubscript{2}O amounts were determined by finding the partial pressure of each gas in the syringe based on a calibration curve using stock gases composed of 1\% NO with helium balance and 1\% N\textsubscript{2}O with helium balance. The aqueous partitioning coefficient using the dimensionless Henry's Constant ($k'\text{H}$) was used to determine the amount of each gas in the liquid phase and the amounts in the headspace phase.

To determine N\textsubscript{2}, it was presumed that oxygen present in the reactor was a result of air contamination either in the reactor or in the needle just prior to injection. An ideal peak area of N\textsubscript{2} from air contamination was determined by multiplying the O\textsubscript{2} peak area by the N\textsubscript{2}/O\textsubscript{2} peak area ratio as determined from low volume air standards (for full details, see Appendix A, sec. A.1.1). The resulting contamination N\textsubscript{2} value was subtracted from the raw N\textsubscript{2} peak area to obtain a N\textsubscript{2} value for the reactor without contamination. Any negative values were considered to have no N\textsubscript{2} present. If the initial N\textsubscript{2}-N value taken on day one was at zero or slightly negative, then the assumption that no air was present in the reactor was presumed to be accurate.
To analyze liquid analysis data, the sample values were calculated from a 1 mL sample and corrected for the volume that was lost during filtering, which was around 0.1 mL. Initial nitrite or nitrate values were calculated from an average of reliable control measurements. This accounted for nitrite or nitrate loss due to adsorption and tolerance of the syringe used. The initial rate of reaction ($k_{obs}$) was found using the first hypothetical data point and the data point nearest to $t=0.1$ days. For the NO and total iron analyses, the first two points were used for determining reaction rate constants. The $k_{obs}$ value was then normalized to the mass concentration ($\rho_m$ in g L$^{-1}$) of magnetite to find $k_m$ by dividing $k_{obs}$ by the mass concentration of magnetite.

During the analysis of NO degradation with magnetite, only gas analyses were completed. Additionally, anomalous peak areas were eliminated (e.g. negative values and outliers). At any point during the NO analysis, though, results from at least two reactors contributed to the averages shown. A correction factor was implemented in the data analysis by estimating a 20 mL increase in headspace volume to compensate for the increase in pressure inside the reactors.
Chapter 3

3.0 RESULTS AND DISCUSSION

3.0.1 Nitrite degradation with chemogenic magnetite

Degradation of nitrite with magnetite was observed to follow pseudo-first order reaction kinetics. Most experiments were complete after about 48 hours from the time of nitrite amendment, though some ran longer. Usually, almost all of the nitrite was consumed within 6 hours of the nitrite addition.

Observations of all experiments in which magnetite reduced nitrite indicated that nitrite is quickly degraded in the presence of magnetite, generally producing a mole fraction of about 0.1 N₂-N and about 0.5 N₂O-N with some other remaining products (Fig. 1). This result varies with the parameters examined, but the common theme was a significant output of N₂O. This investigation has shown that nitrite was readily reduced by the magnetite without the need for aqueous Fe(II) to trigger the reaction. In order for denitrification to take place with other minerals as described in the "Background," the solution medium needed aqueous Fe²⁺. It is presumed that because Fe(II) is part of the structure that it is reactive toward nitrite in the reactors, the Fe²⁺ is not necessary for the reaction to proceed.

Initial rate constants ($k_{obs}$ and therefore $k_m$) varied due to heterogeneities in the reactors created during the magnetite rinsing process because the magnetite was never a
single mixture from the time of synthesis. Error bars for N\textsubscript{2}-N values were also variable because of variation in the raw N\textsubscript{2} values, which was caused by air contamination during sampling.

3.0.2 Nitrate degradation with chemogenic magnetite

In magnetite experiments in this investigation, nitrate appears to be nearly unreactive with magnetite. For any variation in the parameters examined, only a very small amount of N\textsubscript{2} was produced during the course of the experiments. However, it is as yet unclear whether the source of this N\textsubscript{2} product was from a reaction with nitrate. The low reactivity of nitrate and the low product yields made the ability to quantify the reactions in nitrate somewhat limited. Sørensen and Thorling (1991) have cited Postma (1990) suggesting that nitrate reduction was possible if an Fe(II)-containing silicate such as augite formed an oxyhydroxide precipitate on the crushed mineral's surface in a fluidized bed reactor. During dissolution of the mineral particles, an oxyhydroxide precipitate formed (Postma, 1990). The identity of that precipitate was unclear, but Postma (1990) suggested that nitrate reacts with the secondary products from silicate dissolution with Fe\textsuperscript{2+}.

Possible alternate sources of this N\textsubscript{2} include nitrite impurities in the nitrate stock or even in the sodium nitrate reagents that react with magnetite. Trace amounts of N\textsubscript{2} may have remained in the reactors after the helium purging process was complete. N\textsubscript{2} trapped in the water would have partitioned into the headspace as the experiment progressed. Small amounts of N\textsubscript{2} may have also seeped into the reactor through the holes
in the bottle caps at the time of sample extraction. Magnetite in the reactors may have reacted with the oxygen that entered the reactors to produce a false N₂ signature.

In order for nitrate to be reduced through magnetite alone, it must first be changed to nitrite, which is much less stable (van Cleemput and Samater, 1998). In addition to methods of nitrate reduction mentioned near the end of section 1.1, biological methods may reduce nitrate to nitrite. Nitrate may also be made available for degradation with magnetite by adding electron shuttles to the reactors to enhance the rate of reaction with nitrate. Further study in this area would reveal more.

3.1 Effect of pH

3.1.1 Nitrite degradation with chemogenic magnetite

Altering the pH changed the speed at which the reaction proceeded and the mole fractions of products that appeared, especially at higher pH. Mass normalized degradation rate constants (kₘₐ) seemed to decrease by nearly half as pH dropped from 7 to 6 (Fig. 2). This meant that the reaction was slower under more acidic conditions. Vikesland and others (2007) and White and others (1994) suggested that magnetite would react with protons in the water to produce maghemite (Fe₂O₃) by way of Eq. 5. This result is different from results by Dhakal et al. (2013). However, since the magnetite samples in this investigation were allowed to be in contact with acidic conditions overnight before injection of nitrite, the magnetite may have been partially oxidized at t₀ by the protons in solution, suggesting that while denitrification may proceed as fast or faster in acidic conditions, it may be unlikely that the magnetite would last long in those conditions. The reaction was believed to be rapid at low pH but nearly undetectable at
high pH. In White and others (1994), magnetite underwent anodic oxidation through the half reaction shown in Eq. 5. The reactivity of Maghemite was not analyzed in this investigation, but its structure's lack of Fe(II) atoms, suggests that it is less reactive toward nitrite because of the lower Fe(II)/Fe(III) ratio. If maghemite was less reactive than magnetite, then the added protons in the solution from having a lower pH could reduce the magnetite's effectiveness at reducing nitrite.

As pH increased from 7 to 8, the initial $k_m$ seemed to decrease more than the drop to acidic conditions (Fig. 2). Results agreed with the observations of Dhakal et al. (2013) when they observed a drop in reaction rate as pH increased from 6.5 to 7.5. A possible explanation for these observations could be found in comparing the reaction of nitrite to become NO and N$_2$O (Eq. 6) to the reaction to reduce nitrite to ammonia, which is among the major products at pH 8 (Eq. 7). Reducing nitrite to ammonia as described in Eq. 7 required 50% more magnetite, which means that the magnetite was oxidized more quickly and its potential to reduce nitrite at higher pH was lower, resulting in lower product yields and a slower nitrite reaction.

Changes to reactor pH were also observed to cause changes in product distribution. Under neutral to acidic conditions, the primary reaction products included N$_2$O and N$_2$. As pH was lowered from neutral to pH 6, the amount of N$_2$O and N$_2$ produced was not significantly affected, however NO became visible in small amounts. The mole fraction of N$_2$O-N produced was about 0.50, whereas the N$_2$-N mole fraction remained near 0.10. Nevertheless, the trend was that neutral conditions seemed to produce more N$_2$O than acidic or basic conditions. These results agreed with the results
with green rust found by Kampschreur et al. (2011). Through the course of nitrite experiments, most of the nitrite degraded by the end of the second day.

This was not true at pH 8, where nearly half of the nitrite that was added to the system was still present after the experiment was done. If more magnetite was needed to degrade the nitrite by the mechanism shown in Eq. 7, then 1.16 g/L magnetite may have been inadequate to degrade the nitrite. Of the nitrite that did degrade, the NH$_3$-N, N$_2$-N, and N$_2$O-N mole fractions were nearly equal (Fig. 3). NH$_3$ production was negligible under neutral and acidic conditions, but became a more important product at pH 8, composing about 0.154 of the products (Fig. 3).

In the environment, neutral pH levels would exhibit the highest yield of N$_2$O and show the greatest rate of nitrite reduction in the subsurface. Raising the pH would increase the NH$_3$-N yield and work to inhibit the overall reaction with magnetite. This result supports the observations of Summers and Chang (1993), in which their denitrification process that produced ammonia increased as pH rose higher than 7.6. However, Summers and Chang (1993) did not describe a reaction taking place at a measurable rate below pH 7.3. Acidic environments would mainly serve to slow the reaction and may stimulate NO formation, but such changes had little effect on yields of N$_2$O and N$_2$. This investigation illustrates abiotic pathways from nitrite to NO. Neutral and basic conditions do not show this relation, but rather appears to demonstrate abiotic pathways from nitrite to N$_2$O and from nitrite to N$_2$ directly.

3.1.2 Nitrate degradation with chemogenic magnetite
Nitrate experiments showed very little reaction at pH 7 and pH 6. Considering the effect of basic conditions on nitrite degradation, no experiment was attempted for nitrate at pH 8. At pH 7 about 0.0023 mmol of N$_2$-N was produced. The amount of N$_2$-N found in reactors at pH 6 nearly doubled to 0.0039 mmol N$_2$-N. However, the amount of nitrogen produced compared to the amount of nitrate added was not particularly significant, leaving room to speculate that the N$_2$ might also be explained by the errors outlined at the end of section 3.0.2.

3.2 Effect of [Fe(II)]

3.2.1 Nitrite degradation with chemogenic magnetite

When nitrite reacted with magnetite, there was already sufficient electron donor present in the magnetite structure to cause nitrite reduction. When aqueous Fe(II) was added to a reactor with magnetite and nitrite, the magnitude of the initial nitrite degradation rate constants increased, agreeing with results seen by Dhakal et al. (2013). Initial degradation rate constants ($k_{obs}$) were normalized to the concentration of magnetite in g/L ($k_m$). Values of $k_m$ were proportional to the amount of Fe(II) added to the reactors (Fig. 4). According to the experiments conducted, the introduction of aqueous Fe(II) to a site of pH 7 groundwater where magnetite was already degrading nitrite would cause the rate of the reaction to increase in roughly linear proportion to the concentration of Fe(II). This correlated well to similar experiments conducted with HFO (Tai et al., 2009). In their experiments, Tai and others (2009) found that $k_{obs}$ for the amount of nitrite lost increased as the concentration of adsorbed Fe(II) increased.
The amount of Fe(II) added also affected product distribution. The mole fraction of the total Nitrogen consisting of N$_2$O-N varied around a mean of 0.51 with a standard deviation of 0.034 as Fe(II) amendments were increased to 0.1 mmol. The yield of N$_2$O-N decreased slightly when 0.10 mmol Fe(II) was added. Further study will verify whether there is a link between Fe(II) concentration and N$_2$O-N yield as the yield was largely unaffected in these experiments. As Fe(II) amendments increased, the fraction of N$_2$-N that was produced also seemed to increase from about 0.09 to 0.22 as Fe(II) concentration increased from 0 to 0.10 mmol respectively (Fig. 5). Increasing the amount of Fe(II) present in the reactor provided more electron donor to the system allowing the reduction of N$_2$ to proceed at a faster rate, as seen in Fig. 4. As the rate of reaction increased, it was likely that more of the products would be weighted toward the N$_2$ reaction product. However, the increase in the N$_2$-N fraction was only by 0.02 when the amount of Fe(II) added was increased from .050 to 0.10 mmol (Fig. 5). This may mean that a point was reached where the rate of reaction was constant and independent of the concentration of Fe(II) in the system.

It was observed later in the investigation that Fe(II) had an additional significant effect on product yields when NO was a product of the reaction between nitrite and magnetite. Amendments of Fe(II) caused the degradation of existing NO. If enough Fe(II) was added to the reactors, the NO yield was reduced or eliminated.

Fe(II) and total iron were analyzed during this section of the investigation. Fe(II) was analyzed by itself in the experiment in which 0.025 mmol Fe(II) was added. This data showed an instantaneous drop in Fe(II) to undetectable levels, suggesting that any Fe(II) that was present in the reactor had either oxidized or adsorbed to the magnetite. In
other experiments during this investigation, total iron analyses were conducted on filtered samples. Total iron in solution analyses showed exponential decay following pseudo-
first order kinetics (Fig. 6). Some of the Fe(II) was missing immediately from both experimental reactors as well as control reactors. This was likely due to adsorption to the glass in the reactor. Some of the Fe(II) may have been adsorbed to the magnetite particles' surfaces. The rate of iron loss was similar overall, but seemed slightly faster with the 0.10 mmol Fe(II) experiment. Other analyses may determine which reaction was taking place: direct Fe(II) reaction to the nitrite utilizing the oxidized magnetite's surface or restoration of the stoichiometry of magnetite.

To facilitate the reduction of nitrite or nitrate, a source of electrons must be provided. As a mixed species, magnetite has within its structure a ferrous iron (Fe(II)), which could donate an electron to the nitrite or nitrate. This would explain the lack of need for Fe(II) in solution. Adding more of this electron donor in the form of aqueous Fe(II) increased the reaction rate and perhaps alter the distribution of reaction products. Gorski and Scherer (2009) also cite evidence that the Fe(II) would react with oxidized magnetite to restore some of its reactivity.

In the environment, magnetite might be mixed with aqueous Fe(II) in groundwater at times when reducing conditions exist in the presence of iron oxide minerals. The iron oxides would be reduced and dissolve into groundwater to be transported to another area rich in magnetite. If nitrite is present and reacting with magnetite, the rate of the reaction may be enhanced. The amount of nitrite that the magnetite can reduce may also be increased by the presence of the aqueous Fe(II). The
addition of this electron donor may either react with the magnetite to restore the 0.5 Fe(II)/Fe(III) ratio or react directly with the nitrite using the magnetite's surface.

3.2.2 Nitrate degradation with chemogenic magnetite

In nitrate experiments when Fe(II) amendments were increased, the $k_m$ values for nitrate were extremely low or negligible. Results for studying the effect of Fe(II) amendments on nitrate degradation were inconclusive. Nevertheless, $N_2$-N data suggested that increasing the amount of Fe(II) added increased the $N_2$-N fraction from 0.064 to 0.188 when 0.05 mmol of Fe(II) was added. In the experiment where 0.10 mmol Fe(II) was added, the $N_2$-N fraction dropped back to near 0.06, suggesting that changes in $N_2$ yields may be from one of the errors described in section 3.0.2 rather than an effect of the added Fe(II).

In the threshold experiment, $N_2$O and NO were never produced and only about 0.01 mmol of $N_2$-N was observed. $N_2$ values may have been overestimated, especially since the amendment was made between sampling times in which the sample after the amendment showed a sudden increase in $N_2$, suggesting $N_2$ contamination from the glovebox during the iron amendment.

Total iron consumption was much less for the nitrate experiments than for the nitrite experiments (Supplemental Information: Fig. S.9). Small iron loss in all reactors suggests adsorption to the glass surface of the reactor or to magnetite particles in experimental reactors. There may also be a small amount of nitrate reduction associated with the addition of Fe(II). The smaller Fe(II) consumption in the nitrate experiments
suggests that the Fe(II) in nitrite experiments took an active role in either the
denitrification reaction or reduction of magnetite.

3.3 Effect of [Nitrite]:

Increasing the concentration of nitrite in the reactors was found to decrease the
rate of the reaction, which was consistent with pseudo first order reaction kinetics (Fig.
7). Fig. 7 shows the results of an initial experiment that used about 5 mM nitrite. There
was little loss of the nitrite in the experiment and the loss that took place was slow. At
low concentration, the denitrification reaction was limited only by the time taken for
nitrite to attach to magnetite and for either N₂O or N₂ to be given off. At high
concentrations, the competition for reactive sites became the rate limiting process.

In Fig. 8, comparisons of experiments A and B along with D and E show the
effect of increasing the nitrite concentration on product fractions. In terms of mole
fractions, less N₂O-N and N₂-N were formed (in terms of molar amounts, these product
yields were tripled; see SI Fig. S.10). In experiment B, it was found that after 2 days of
reaction, only around 55% of the nitrite had reacted (Fig. 8).

Whenever the initial rate constant (and therefore the overall apparent rate of the
reaction) was low, NO was a major reaction product. NO was no longer a significant
product when the amendments of nitrite were reduced from 0.5 mmol to 0.025 mmol
NaNO₂, an amount that the 1.16 g/L magnetite had demonstrated the capacity to degrade.
Therefore, the higher nitrite concentration indirectly resulted in a greater yield of NO by
slowing the denitrification reaction (Fig. 8).
3.4 Restoration of Magnetite Reactivity Toward Nitrite by Fe(II) Amendment

Aqueous Fe(II) had already been observed to enhance the reaction between magnetite and nitrite. However, other research suggested that contact with aqueous Fe$^{2+}$ would restore the 0.5 ratio of Fe(II) to Fe(III) (Gorski and Scherer, 2009). When the magnetite concentration was insufficient to reduce the nitrite present, FeSO$_4$$ \cdot $7H$_2$O was added. In both of the experiments that used a Fe(II) amendment, the evidence suggested that the restoration of magnetite's stoichiometry was taking place. However, it was not completely clear with these simple tests, nor was it the overall goal of the amendments to prove or disprove the observations of Gorski and Scherer (2009). Rather, the goal was to observe the effect of an influx of Fe(II) on a system that was mostly finished reacting. However, some clues in those observations suggested the stoichiometric restoration was taking place.

The first experiment in which this was done (described in Section 2.6) showed the most dramatic changes. Observations included a jump in N$_2$O and N$_2$ formation along with a sudden drop in NO levels in the reactors, which had stabilized around 0.026 mM at the time of the Fe(II) injection. NO then gradually increased toward 0.020 mM, where it peaked once again. This agreed with observations of Kampschreur et al. (2011) who suggested that denitrification would lead to a constant aqueous NO concentration. By the next day, NO-N levels were lower at 0.010 mM (Fig. 9). NO likely continued to react with the magnetite as reactive sites became available (Kampschreur et al., 2011). The addition of Fe(II) accelerated this reaction. Similar observations were made in the second experiment where 0.58 g/L magnetite was exposed to 0.025 mmol nitrite (SI Fig. S.12).
In the experiment with 1.0 mM nitrite, some visible signs of possible magnetite restoration were observed. Visual inspection of filtered liquid samples showed a gray tint in the samples collected before an Fe(II) amendment. This gray coloration was lessened or not observed in samples collected immediately after an Fe(II) amendment. Although not tested in this experiment, it was suggested that oxidized magnetite which had lost its magnetism passed through the 0.22 micron filter and gave the liquid sample a gray tint. This was removed with the addition of Fe(II). This effect was observed in the second experiment with 0.58 g/L magnetite as well (SI: Fig. S.12).

The system for these conditions seemed to use more Fe(II) than the systems set up for the Fe(II) investigation (SI Fig. S.11). At first, the system had no added Fe(II) for the first 2 days. At about t = 2 days, an amendment of 0.050 mmol Fe(II) was added. In the control, the iron behaved much the same way as in previous analyses. In the experimental reactors, however, there was a dramatic loss of nearly all of the iron almost immediately. In the experiment with 1 mM nitrite the rate constant of loss from the hypothetical initial point to the first collected data point was higher for these Fe(II) amendments than for the "effect of [Fe(II)]" in section 3.2. Most likely the loss of Fe(II) depended on the level of magnetite oxidation that had taken place. In experiments where Fe(II) was added prior to the start of the reaction, the Fe(II) would not react until some of the magnetite had oxidized and would then be controlled by the rate of magnetite oxidation. In the experiments where Fe(II) was added after the reaction, the magnetite reduction was almost instantaneous because the oxidation had already taken place.
3.5 Effect of [Magnetite]

3.5.1 Nitrite degradation with chemogenic magnetite

The last parameter examined in this investigation of the controls on anaerobic nitrite and nitrate degradation was the concentration of magnetite. An increase in magnetite added to the reactors was expected to increase the rate of nitrite degradation and perhaps change the distribution of products. With each experiment, as magnetite increased, $k_m$ increased slowly until it peaked at the experiment with 2.32 g/L magnetite (Fig. 10).

Increasing the amount of magnetite caused a general decrease in N$_2$O-N mole fractions with the mole fractions varying around a mean of 0.45 (Fig. 11). N$_2$ levels remained low for most of the experiments, but were greater in the experiment with 2.90 g/L and the 3.48 g/L experiment. At the lowest concentration of magnetite (0.58 g/L), NO was visible as a product. The differences in product yields at variable magnetite concentrations may be explained by a combination of the increase in the rate of reaction and the heterogeneities that form during synthesis. Other observations of relationships between product yields and $k_{obs}$ suggested that N$_2$ yields increased with increasing $k_{obs}$. However, for the magnetite concentration experiments, there was not a clear increase in N$_2$-N yields except as the magnetite concentration increased to 2.9 g/L (12.5 mM).

3.5.2 Nitrate degradation with chemogenic magnetite

The results of nitrite reactions toward increasing concentrations of magnetite were dramatic. However the same could not be said for similar reactors with nitrate.
Increasing the amount of magnetite in the reactor had no significant effect on the amount of N\textsubscript{2} produced in reactors where nitrate degradation was examined.

### 3.6 Fate of reaction intermediates

In an experiment in which gaseous NO was introduced to a batch reactor with magnetite, it was observed that the magnetite reacts immediately with the NO to produce N\textsubscript{2}O and N\textsubscript{2} (Fig. 12 B). Heterogeneity between various reactors and their conditions under higher than 1 atmosphere of pressure made some of the determination of products somewhat ambiguous, but it was clear that two major reduction products of NO were N\textsubscript{2} and N\textsubscript{2}O. Kampschreur et al. (2011) also noted that N\textsubscript{2}O was a product of NO reduction. In the first two days of the experiment, the amount of N\textsubscript{2}-N produced was half or less than half the amount of N\textsubscript{2}O-N produced (Fig. 12 B). However, as the reaction progressed and the gas pressure inside the reactor approached 2 atm, N\textsubscript{2} became the more dominant product. Over the span of the experiment, there was no loss of N\textsubscript{2}O apparent in the results suggesting that it is a stable product of the reactions of NO with magnetite and of nitrite with magnetite.

This experiment demonstrated two different abiotic pathways. NO reacts with magnetite to produce both N\textsubscript{2} and N\textsubscript{2}O. The path producing N\textsubscript{2}O was the dominant path under low atmospheric pressure. Whether the NO reacts directly to produce N\textsubscript{2} or whether it first became N\textsubscript{2}O as an intermediate was not clear from these results.
3.7 Environmental Implications

The mass normalized rate of reaction \( (k_m) \) helped to determine which of the three main denitrification products were observed and in what proportions (Eq. 1). While \( N_2 \) is relatively innocuous, NO and \( N_2O \) are powerful greenhouse gases. In addition, NO can catalyze ozone production in the troposphere (Tai and Dempsey, 2009). Likewise, \( N_2O \) may contribute to the destruction of ozone in the stratosphere (van Cleemput and Samater, 1996; Tai and Dempsey, 2009; Kampschreur et al., 2011; Rubasinghege et al., 2011). However, NO may be reduced either by magnetite, the addition of Fe(II) to the system, or by contacting another Fe-oxide with Fe(II) in solution.

On the other hand, in systems in which nitrite reduction occurs quickly, it may be that NO does not appear and in some cases the \( N_2 \) yields may be more favored. Although evidence in this investigation suggested such a relationship between reaction rate and final yields of products, more investigation is needed to determine definitively whether \( N_2 \) and \( N_2O \) were more or less favored as a function of degradation rate. The reaction rate of such degradation could significantly affect the emission of \( N_2O \) from environments where abiotic denitrification takes place. However, such a relationship was suggested by comparison of results from the Fe(II) investigation and the magnetite concentration investigation (SI Fig. S.16).

All of the parameters tested demonstrated some measurable level of control on the rate of the reaction, consistent with other work (Dhakal, et al., 2013). Of those tested, the denitrification of nitrite was most sensitive to the concentration of Fe(II), where a higher concentration of Fe(II) caused a greater increase in mass normalized degradation rate.
constant than increases in magnetite concentration. Fig. 4 shows that $k_m$ increased roughly linearly as Fe(II) concentrations rose, but this pattern may not hold at higher concentrations of Fe(II). In Fig. 10, a plot of $k_m$ against magnetite concentration (in g/L) shows a curve where $k_m$ peaked at 2.32 g/L and started to decrease again, suggesting that a concentration could be reached at which the reaction proceeded at a maximum rate.

In Fig. 13, both biological and abiotic pathways described in Venterea (2007), or elsewhere were combined with pathways observed in this investigation. Biological pathways shown in the diagram were not used or observed in this investigation. The pathway from nitrite to ammonia was only observed at pH 8. Venterea (2007) reported a direct pathway from nitrite to N$_2$O. This path may have contributed to the N$_2$O yields when N$_2$O-N yields far exceeded the N$_2$-N yields. However, this pathway was not directly observed in this investigation and was likely not as important as the pathways from nitrite to NO and from NO to N$_2$O. In the intermediate investigation, the degradation rate constant of NO was always above 35 (Fig. 12A), which was nearly 3 times greater than the degradation rate constant for the nitrite experiment with equivalent pH and magnetite concentration. Therefore in the environment, the reaction of nitrite to NO is more likely to be a rate limiting step (Eqs. 2a and 6a), making NO unlikely to accumulate unless reactive sites are scarce. Pathway 5 from N$_2$O to N$_2$ was suggested as a possible fate of a small portion of N$_2$O and may explain momentary drops in N$_2$O-N yields, but N$_2$O was shown to be highly stable and resistant to reduction by magnetite. Unless another method for N$_2$O degradation were present in the environment, the N$_2$O would likely remain stable and perhaps be emitted. A possible pathway was suggested in the nitrate experiments that would link nitrate with N$_2$ directly. However, a more likely
scenario was that the reaction followed through pathways 1, 2, and 4 with pathway 1 as the rate limiting step assuming the N\textsubscript{2} observed was evidence of such reduction.

In environmental settings such as the edges of Fe(III) reducing zones where magnetite may be formed or where DIRB produce magnetite, the input of natural or anthropogenic nitrite could produce a significant amount of N\textsubscript{2}O and perhaps NO. While there would likely not be a significant reaction between nitrate and magnetite, nitrate could be transformed into nitrite (van Cleemput and Samater, 1998; Dhakal et al., 2013), which could then be reduced to NO, N\textsubscript{2}O, and N\textsubscript{2}. At this early time in the research it is unclear to what degree abiotic denitrification with magnetite contributes to the NO and N\textsubscript{2}O gas emissions in the environment. Further study is needed to examine the competition between abiotic and biological processes and examine other controls on the degradation of nitrite and nitrate with chemogenic magnetite.
Chapter 4

4.0 CONCLUSIONS

The purpose of this investigation was to determine reaction kinetics and examine some of the controls on abiotic denitrification of nitrite and nitrate to intermediate GHGs like N\(_2\)O and NO using chemogenic magnetite nanoparticles. It has been found through the investigation that magnetite is a strong reducer of nitrite in the subsurface without aqueous Fe(II) in solution. At neutral or slightly acidic conditions, about 50% of the nitrogen that was reduced formed N\(_2\)O. In environments where nitrate may be reduced to nitrite by biological activity or through other means in the subsurface (Dhakal et al., 2013), a nearly 50% N\(_2\)O product yield could become a significant contributor to the total N\(_2\)O emission budget. If nitrite is present at the margin of iron reducing zones or where DIRB are producing magnetite, denitrification may take place, particularly at localities where biological denitrification is minimal or nonexistent.

Other conclusions about the controls for this system are as follows. Neutral conditions produced the strongest nitrite reactions. Acidic and basic conditions resulted in smaller \(k_{\text{obs}}\). Under basic conditions, ammonia was produced with less N\(_2\)O. Higher competition for reactive sites caused NO accumulation when \(k_{\text{obs}}\) was small. Aqueous Fe(II) enhanced denitrification in magnetite systems without significantly decreasing the N\(_2\)O yield. Fe(II) was also quickly removed and appeared to restore the stoichiometric
ratio of Fe(II)/Fe(III) in magnetite. Abiotic denitrification follows pseudo first order reaction kinetics. The mass normalized nitrite degradation rate constant \( (k_m) \) was more sensitive to the concentration of Fe(II) than to the concentration of magnetite, though increases in both increased \( k_m \). Nitrate was more stable and very little, if any, reacted with magnetite.
5.0 REFERENCES


Clayton, R., Harold, M., and Balakotaiah, V. Selective Catalytic Reduction of NO by H$_2$ in O$_2$ on Pt/BaO/Al$_2$O$_3$ Monolith NO$_x$ Storage Catalysts. *Applied Catalysis B: Environmental* **2008**, *81*, 161-181.


Figure 1. This diagram shows the product distribution over time as nitrite was consumed in a reaction with magnetite at pH 7 with 5 mM magnetite, 0 mM Fe(II), and 0.25 mM NaNO₂. The results from these conditions were the initial results for all of the subsequent analyses. Parameters were changed from these conditions. The main products were N₂O and N₂. Nitric oxide and ammonia levels were below detection. Error bars represented 1 standard deviation in values. Graphs for products at pH 6 and 8 are included in the supplemental information (Fig. S.4 and Fig. S.5).
Figure 2. This figure analyzes the effect of pH on pseudo-first order nitrite degradation kinetics of the first and often third data point (whatever was nearest to \( t = 0.1 \) days) within one standard deviation. Initial conditions were 5 mM magnetite, 0 mM Fe(II), and 0.25 mM NaNO\(_2\). The main products were N\(_2\)O and N\(_2\). The highest reaction rate was observed at pH 7, whereas the lowest was observed at pH 8. Rates varied widely because of heterogeneities in the magnetite preparation between reactors as the magnetite was synthesized separately for each reactor instead of as a single large batch to be divided among reactors.
Figure 3. This diagram shows the effect of pH on the distribution of final products of nitrite reduction by magnetite. Initial conditions were 5 mM magnetite, 0 mM Fe(II), and 0.25 mM NaNO₂. The main products were N₂O and N₂. Nitric oxide was very small at pH 6 and undetected at other pH values. The ammonia fractions were only above detection limits for pH 8. Error bars represented 1 standard deviation in values. At pH 8, less nitrite had reacted and the fraction of N₂O-N and NH₃-N were nearly equal, and N₂O-N fractions at pH 6 and 7 were similar at 0.475 and 0.498 respectively.
**Figure 4.** This figure analyzes the effect of Fe(II) concentration on pseudo-first order degradation kinetics of the first 3 data points for nitrite degradation kinetics within one standard deviation. Initial conditions were pH 7, 5 mM magnetite, and 0.25 mM NaNO₂. The main products were N₂O and N₂. The rate of reaction increased exponentially as Fe(II) was increased from 0 to 0.1 mM. The rate constant for the experiment in which no Fe(II) was added ("0") used the same data as the pH 7 experiment in the pH investigation.
Figure 5. This diagram shows the effect of Fe(II) on the distribution of final products of nitrite reduction by magnetite. Initial conditions were pH value of ~7, 5 mM magnetite, and 0.25 mM NaNO₂. The main products were N₂O and N₂. Nitric oxide was below the detection limit. Ammonia was not analyzed. Error bars represented 1 standard deviation in values. The fraction of N₂O-N was not strongly affected by concentration of Fe(II); remaining at approximately 0.5 for the 0, 0.25, and 0.5 mM Fe(II), but the N₂-N fraction steadily increased from 0.092 to 0.224.
Figure 6. This diagram shows the loss of total iron over time as nitrite reacted with magnetite at pH 7 with 5 mM magnetite, 0.050 mM (squares) and 1.0 mM (diamonds) Fe(II), and 0.25 mM NaNO₂. White symbols are data from control reactors, while black symbols are experimental reactors. Error bars represented 1 standard deviation in values. The data for the 0.50 mM Fe(II) experiment showed a greater proportion of loss than the 1.0 mM Fe(II) experiment, but only because there was less Fe(II) added initially. All reactors show a small amount of iron loss, including the controls. This was likely due to adsorption to the glass bottle.
Figure 7. This figure analyzes the effect of the concentration of nitrite on pseudo-first order nitrite degradation kinetics of the first and often third data point (whatever was nearest to $t = 0.1$ days) within one standard deviation (the experiment with nitrite concentration = 5 mM consisted of one reactor because it was from an initial experiment performed during procedural set-up, making the data point suspect. Initial conditions were 5 mM magnetite, 0 mM Fe(II), and 0.25 mM NaNO$_2$. The main products were N$_2$O and N$_2$. The highest reaction rate was observed at the lowest concentration of nitrite and the initial rate constant decreased in a roughly exponential manner. Rates varied because of heterogeneities in the magnetite preparation between reactors as the magnetite was synthesized separately for each reactor instead of as a single large batch to be divided among reactors.
Figure 8. This diagram shows the effect of nitrite concentration on the distribution of final products of nitrite reduction by magnetite. Initial conditions were pH value of ~7, 5 mM magnetite, and 0 mM Fe(II). The final results of Experiment E were results from experiment B, but after a 0.05 mmol FeSO₄ amendment. The main products were N₂O and N₂. Nitric oxide was also visible when nitrite concentration was 1.0 mM. Ammonia was not analyzed. Error bars represented 1 standard deviation in values. The fraction of N₂O-N decreased with the increase in the amount of nitrite added from 0.498 to 0.334 without Fe(II) added. It should also be noted that the yield of any product was not directly proportional to the concentration of nitrite. The mole fraction of N₂O-N approached 0.5 when 0.05 mmol Fe(II) was added.
Figure 9. This diagram analyzes the effect of differences in nitrite concentration and shows the product distribution over time as nitrite was consumed in a reaction with magnetite at pH 7 with 5 mM magnetite, 0 mM Fe(II) at first, followed by an addition of 0.05 mmol Fe(II) in solution after ~48 hours (See the graph break), and 1.0 mM NaNO₂. The main products were N₂O and N₂. Nitric oxide (NO) became a detectable product in this experiment and is plotted on the secondary y-axis. Ammonia was not analyzed. Error bars represented 1 standard deviation in values. The reaction continued after the Fe(II) amendment at day 2. Results on day 15 suggested that a small amount of reaction was still taking place in that time. NO decreased upon adding Fe(II), stabilized again at around the same mole fraction by the end of the second part of the experiment after the Fe(II) amendment.
Figure 10. This figure analyzes the effect of magnetite concentration on pseudo-first order nitrite degradation kinetics of the first and usually 3rd data point (or whatever point is at 0.1 days) within one standard deviation. Initial conditions were pH 7, 0 mM Fe(II), and 0.25 mM NaNO₂. The main products were N₂O and N₂. The reaction rate increased as magnetite concentration rose from 0.58 to 2.9 g/L (2.5 to 12.5 mM). The rate constant for the experiment in which the magnetite concentration was 5 mM used the same data as the pH 7 experiment in the pH investigation.
Figure 11. This diagram shows the effect of magnetite concentration on the distribution of final products of nitrite reduction by magnetite. Initial conditions were pH value of ~7, 0 mM Fe(II), and 0.25 mM NaNO₂ following an amendment of 0.05 mmol Fe(II). The main products were N₂O and N₂. Nitric oxide was visible when magnetite concentration was 0.58 g/L, but the value was very small and is not represented on this graph. Ammonia was below the detection limit. Error bars represented 1 standard deviation in values. The fraction of N₂O-N largely decreased with the increase in the magnetite concentration from 0.785 to 0.407, while the N₂-N fraction seemed to triple at higher magnetite concentrations. In the 0.58 g/L magnetite experiment, NO decreased to 0 after adding Fe(II).
Figure 12. This diagram shows the product distribution over time as nitric oxide was consumed in a reaction with magnetite at pH 7 with 5 mM magnetite, 0 mM Fe(II), and 4 amendments of 20 mL of gas containing 1% NO with helium balance totaling around 0.048 mmol. The only observed products were N₂ and N₂O. No liquid analysis was conducted for this investigation. Graph A shows the NO degradation with $k_{obs}$ for the first 2 data points (3 in the first amendment) (black symbols). The average of $k_{obs}$ is 48.98 ± 10.67. The control (white symbols) shows no significant loss of NO. Changes in symbol shape indicates a separate NO amendment. Graph B shows the product distribution with total nitrogen with each additional amendment.
Figure 13. This figure is modified from Venterea (2007). In this figure, all abiotic pathways are represented by dashed lines and biological processes are represented by solid lines. Abiotic pathways consistently observed or suspected in the magnetite investigations included pathways 2, 3, 4, and 7. Abiotic pathways 2 and 6 were given by Venterea (2007). An abiotic pathway 3 was also reported in Kampschreur, (2011) (see Eq. 2b). Evidence collected in this investigation suggested that the abiotic pathway from nitrite to N\textsubscript{2}O may be less important for N\textsubscript{2}O production than paths 2 and 3. The finer line abiotic pathway 1 from nitrate to nitrite was described in Rubasinghege et al., (2011), Sørensen and Thorling (1991), and Postma, (1990). The biotic part of pathway 1 was described in Postma, (1990).
Appendix A: Analysis and Calculations

A.1 CALCULATIONS FOR DETERMINING TOTAL AMOUNTS AND MOLE FRACTIONS OF PERMANENT GASES IN REACTORS (ADAPTED FROM POWELL AND AGRAWAL 2011)

A.1.1 Nitrogen (N$_2$-N)

1. Determine the amount of average peak area that is not from air contamination

   a. $A_{\text{contamination}} = \frac{A_{\text{oxygen}}}{X}$

   Where $A_{\text{contamination}}$ is nitrogen peak area of the air contamination, $A_{\text{oxygen}}$ is the peak area of oxygen, and $X$ is the ratio of oxygen peak area to nitrogen peak area as determined by air injections (This was determined to be 0.242842 ± 0.003701 for this investigation).

2. Determine nitrogen peak area in reactor.

   a. $A_{\text{reactor}} = A_{\text{Total}} - A_{\text{contamination}}$

   where $A_{\text{reactor}}$ is the peak area of nitrogen in the reactor and $A_{\text{Total}}$ is the raw nitrogen peak area from the injection.

3. Using the ideal gas law, determine the number of moles ($n$) in the calibration curve

   a. $n = \frac{pV}{RT}$
where \( R = \text{gas constant, } 0.0821 \text{ atm liter mole}^{-1} \text{ K}^{-1}; \)
\( T = \text{temperature, } 298 \text{ K}; \ P = \text{Pressure, } 1 \text{ atm}; \ V = \)
volume of gas injected.

4. These determinations were done for the calibration, which was created by
ingjecting a known volume of pure \( N_2 \) into a 160 mL serum bottle with 100
mL water so that partitioning was accounted for. \( A_{\text{reactor}} \) values in
experiments were set to the calibration curve to find the number of mmol
of \( N_2 \).

5. Find the amount of \( N_2\)-N:
   a. \( n_{N_2-N} = n_{N_2} \times 2 \)

6. Find the mole fraction
   a. \( mf = n_t/n_{\text{nitrite}}^o \)

Where \( n_{\text{nitrite}}^o \) is the initial number of moles of nitrite in
the reactor as determined by finding the average of the
amount of nitrite determined to be in the control
reactor.

A.1.2 **Nitrous Oxide (N\(_2\)O) and Nitric Oxide (NO)**

1. Create a calibration curve from 1% \( N_2\)O with helium balance
   a. Analyze 10, 30, and 50 µL ambient air samples (\( Samp_{\nu} \)) with a
      50 µL gas-tight syringe (\( Syr_{\nu} \)) on a HP 5890 series gas
      chromatograph system with a thermal conductivity detector, and a
      packed column with helium carrier gas
   b. Determine partial pressure, in atm, of air in samples (\( P_i \))
i. \[ Pi = \left( \frac{S_{amp,v}}{S_{yr,v}} \right) 0.01 \]

Where 0.01 = percent \( N_2 \)O or NO in tank.

2. Plot a standard curve with peak area on the y-axis and \( Pi \) on the x-axis

3. Find the aqueous partitioning coefficient \( (f_w) \)
   i. \[ f_w = \frac{1}{(1 + \frac{1}{k'_{H}}) \frac{V_a}{V_w}} \]
   where \( k'_{H} \) = Dimensionless Henry’s constant
   
   \((C_{water} / C_{gas}) \) at 20 °C, (0.604654771 for \( N_2 \)O and 0.04403844 for NO) [D] (Sander 1999); \( V_a \) = volume of headspace; \( V_w \) = volume of liquid

   ii. \[ f_a = 1 - f_w \]

4. Find total amount (in mmol) of \( N_2 \)O-N or NO-N in reactor
   i. \[ n_t = (n_g \div f_a) \times N \]

   Where \( n_g \) is the number of mmol of \( N_2 \) in gas phase and 

   \( N \) is the number of N atoms in the desired gas molecule.

5. Find the mole fraction of \( N_2 \)O-N in the reactor

   \[ mf = n_t \div n^o_{nitrite} \]

   Where \( n^o_{nitrite} \) is the initial number of moles of nitrite in the reactor as determined by finding the average of the amount of nitrite determined to be in the control reactor.
A.1.3 References


Supplemental Information

Figure S.1. A normal batch reactor set to be analyzed within one week consists of a total of six reactors. From left to right, Control 1 had no magnetite and was used for headspace sampling. Experimental Gas 1 and 2 had a known amount of magnetite and were made identically. These were also used for headspace sampling. Control 2 was made to be like Control 1, but for liquid analysis only, followed by Experimental liquid 1 and 2 reactors, which were the same as Experimental Gas 1 and 2, but they were sampled for liquid using the 1 mL syringes attached to the reactor bottles.
Figure S.2. This cooler rig was used to make NO amendments that were not contaminated with air. The clamp around the NO stock bottle (black arrow) kept the NO stock bottle and syringe tip underwater while preparing the amendment. When 20 mL was obtained, a stopcock on the syringe was closed and the gas was transferred to a reactor (white arrow). The syringe and the bottle cap were constantly submerged during the amendment process. The reactor was only removed from the water after the amendment was done and the needle had been carefully removed from the cap.
Figure S.3. TEM micrographs of magnetite nanoparticles. In A, the nanoparticles were fresh with 10 mM TAPSO buffer at pH 7. The average diameter of 60 of the clearest grains was 10.06 ± 2.27 nm. In B, the particles have aged on contact with 0.2 mmol FeSO$_4$, 10 mM TAPSO buffer at pH 7, and have reacted with 0.25 mM NaNO$_2$. The pictures also have XRD spectra underneath. The most obvious difference is the significant increase in size of magnetite particles as they age and are exposed to Fe(II) and nitrite. A diffraction pattern taken from the particles in A were identified as either magnetite or maghemite. Further distinction was not possible because of the similarity of diffraction patterns between magnetite and maghemite.
Figure S.4. This diagram analyzes the effect of pH and shows the product distribution over time as nitrite was consumed in a reaction with magnetite at pH 6 with 5 mM magnetite, 0 mM Fe(II), and 0.25 mM NaNO₂. The main products were N₂O and N₂. Nitric oxide (NO) was visible and was plotted on the secondary y-axis. Ammonia levels were below detection. Error bars represented 1 standard deviation in values. This graph is supplemental to Fig. 1. Nitrite loss was slower than for the pH 7 analysis (black diamonds).
Figure S.5. This diagram analyzes the effect of pH and shows the product distribution over time as nitrite was consumed in a reaction with magnetite at pH 8 with 5 mM magnetite, 0 mM Fe(II), and 0.25 mM NaNO₂. The main products were N₂O and N₂. NO was below detection, but ammonia became a significant product. Error bars represented 1 standard deviation in values. This graph is supplemental to Fig. 1. Nitrite loss was much slower than for the pH 7 analysis (black diamonds).
**Figure S.6.** This diagram analyzes the effect of Fe(II) concentration and shows the product distribution over time as nitrite was consumed in a reaction with magnetite at pH 7 with 5 mM magnetite, 0.25 mM Fe(II), and 0.25 mM NaNO₂. The main products were N₂O and N₂. NO levels were below detection. Ammonia was not analyzed. Error bars represented 1 standard deviation in values. Compared to Fig. 1, N₂O-N levels were similar at 0.54 and N₂-N was produced. Nitrite degradation rate constants were expressed in Fig. 4. Totals in mole fractions were expressed in Fig. 5.
Figure S.7. This diagram analyzes the effect of Fe(II) concentration and shows the product distribution over time as nitrite was consumed in a reaction with magnetite at pH 7 with 5 mM magnetite, 0.50 mM Fe(II), and 0.25 mM NaNO₂. The main products were N₂O and N₂. NO levels were below detection. Ammonia was not analyzed. Error bars represented 1 standard deviation in values. Compared to Fig. 1 and S.6, N₂O-N levels look similar at 0.54, but nitrite concentrations dropped faster. Nitrite degradation rate constants were expressed in Fig. 4. Totals in mole fractions were expressed in Fig. 5.
Figure S.8. This diagram analyzes the effect of Fe(II) concentration and shows the product distribution over time as nitrite was consumed in a reaction with magnetite at pH 7 with 5 mM magnetite, 1.0 mM Fe(II), and 0.25 mM NaNO₂. The main products were N₂O and N₂. NO levels were below detection. Ammonia was not analyzed. Error bars represented 1 standard deviation in values. Compared to Fig. 1, S.6, and S.7, N₂O-N levels dropped slightly to 0.47 and nitrite concentrations dropped faster than in the previous analysis. Nitrite degradation rate constants were expressed in Fig. 4. Totals in mole fractions were expressed in Fig. 5.
Figure S.9. This diagram shows the loss of total iron over time as nitrate reacted with magnetite at pH 7 with 5 mM magnetite, 0.050 mM (squares) and 1.0 mM (diamonds) Fe(II), and 0.25 mM NaNO₂. Error bars represented 1 standard deviation in values. Degradation rate constants for the iron in these experiments were much lower than for the nitrite experiments (Fig. 6). All reactors show a small amount of iron loss, including the controls (white symbols). This was likely due to adsorption to the glass bottle.
Figure S.10. This diagram analyzes the effect of nitrite concentration and shows the effect of nitrite concentration on the distribution of final products of nitrite reduction by magnetite. Initial conditions were pH value of ~7, 5 mM magnetite, and 0 mM Fe(II). Experiment E represents the same experiment as B, but with 0.50 mM Fe(II). The main products were N2O and N2. Nitric oxide was visible when nitrite concentration was 1.0 mM. Ammonia was not analyzed. Error bars represented 1 standard deviation in values. The yield of N2O-N increased as the amount of nitrite added increased from 0.122 to 0.301. The mole fraction approached 0.5 when 0.05 mmol Fe(II) was added. The yield of any product was not directly proportional to the concentration of nitrite. NO decreased upon adding Fe(II), stabilized again at around the same mole fraction by the end of the second part of the experiment after the Fe(II) amendment. The mass balance of experiment C was labeled and extends above the top of the graph.
Figure S.11. This diagram shows the loss of total iron over time as nitrite reacted with magnetite at pH 7 with 5 mM magnetite and 1.00 mM NaNO$_2$ with Fe(II) amendments. Error bars represented 1 standard deviation in values. The amount of detectable desorbed iron stabilized slightly less than the calculated amounts of added Fe(II). Very little of the Fe(II) added to the experimental reactors remained in a detectable form and the rate of consumption was much greater than in the Fe(II) experiments (Fig. 6). It was believed that the Fe(II) reacted with the magnetite, which had oxidized, to restore the 0.5 Fe(II)/Fe(III) ratio in the reactors. This interpretation operates under the premise that the magnetite is mostly oxidized at the times of Fe(II) amendments (black arrows).
Figure S.12. This diagram analyzes the effect of magnetite concentration and shows the product distribution over time as nitrite was consumed in a reaction with magnetite at pH 7 with 0.58 g/L (2.5 mM) magnetite, 0 mM Fe(II), and 0.25 mM NaNO₂. The main products were N₂O and N₂. NO was also a major product of this analysis. Ammonia was not analyzed. Error bars represented 1 standard deviation in values. Nitric oxide was observed to drop when 0.050 mmol of Fe(II) was added (gray line) Nitrite levels also continued to drop after the amendment. Nitrite degradation rate constants are expressed in Fig. 7. The final amounts are expressed as mole fractions in Fig. 8.
Figure S.13. This diagram analyzes the effect of magnetite concentration and shows the product distribution over time as nitrite was consumed in a reaction with magnetite at pH 7 with 2.32 g/L (10 mM) magnetite, 0 mM Fe(II), and 0.25 mM NaNO₂. The main products were N₂O and N₂. NO was below detection limit. Ammonia was not analyzed. Error bars represented 1 standard deviation in values. Nitrite degradation rate constants are expressed in Fig. 10 and show that the degradation rate was much greater than the experiments represented by Fig. 1 and S.12. The final amounts are expressed as mole fractions in Fig. 11.
Figure S.14. This diagram analyzes the effect of magnetite concentration and shows the product distribution over time as nitrite was consumed in a reaction with magnetite at pH 7 with 2.90 g/L (12.5 mM) magnetite, 0 mM Fe(II), and 0.25 mM NaNO₂. The main products were N₂O and N₂. NO was below detection limit. Ammonia was not analyzed. Error bars represented 1 standard deviation in values. Nitrite degradation rate constants are expressed in Fig. 10 and show that the degradation rate was much greater than the experiments represented by Fig. 1, S.12, and S.13. The final amounts are expressed as mole fractions in Fig. 11.
Figure S.15. This diagram analyzes the effect of magnetite concentration and shows the product distribution over time as nitrite was consumed in a reaction with magnetite at pH 7 with 3.48 g/L (15 mM) magnetite, 0 mM Fe(II), and 0.25 mM NaNO₂. The main products were N₂O and N₂. NO was below detection limit. Ammonia was not analyzed. Error bars represented 1 standard deviation in values. Nitrite degradation rate constants are expressed in Fig. 10 and show that the degradation rate was much greater than the experiments represented by Fig. 1, S.12, S.13, and S.14 and was complete within the first day. The final amounts are expressed as mole fractions in Fig. 11.
Figure S.16. This diagram shows the relationship between initial reaction rate constant ($k_{obs}$) and final molar yield of major products. The experiments represented were the increasing Fe(II) concentration experiments and the increasing magnetite concentration experiments. These two investigations generally showed increases in $N_2$-N yield and decreases in $N_2O$ and NO with increasing $k_{obs}$. The nitrite concentration experiment could not be used in this graph because of the greater amount of material to be degraded, skewing molar amount results. Likewise, a different reaction mechanism created a further complicating factor when trying to use the pH investigation.
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<td>coprecipitation from solution with 2 M Fe(NO)3, 1 M FeCl2, and a salt by adding the salt's corresponding base</td>
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*This method was adapted for use in this investigation.
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*One of the serem bottles containing magnetite broke during the washing process. A replacement was not made due to time constraints

**In this examination, no liquid analysis was conducted.

***This experiment was very early in the investigation and a method had not yet been developed to analyze for N₂ gas.
Table S.2 Continued:

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79
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Ending results from All Nitrite Investigations

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Appendix B: Spectrophotometric Procedures

STANDARD OPERATING PROCEDURE FOR REACTOR TOTAL DISSOLVED IRON DETERMINATION BY PHENANTHROLINE METHOD (adapted from Fortune and Mellon 1938).

Required Materials:
Glass vials, 5.1 mM, 1000 mg L\(^{-1}\) iron stock solution (Lab Chem Inc.), 1,10-Phenanthroline, 10% hydroxylamine solution, 1.2M ammonium acetate buffer, Iso-Disc Filter, N-25-4 Nylon 25 mm x 0.45 μm filters, 1-1000 μL Eppendorf pipette (1-5 or 1-10 mL pipette optional), Fisher Vortex Genie 2

Preparation of Fe(II) Standards:
1. A 1000 mg L\(^{-1}\) iron stock solution was used (LabChem Inc.).
2. The iron stock solution from 1000 mg L\(^{-1}\) was diluted to 50 mg L\(^{-1}\).
   a. 2.5 mL iron stock solution was placed into a 50 mL volumetric flask using a 0-1000 μL Eppendorf pipette, and filled to the mark with Milli-Q water.
   b. Flask was shaken well to make sure the contents were thoroughly mixed.
   c. The final concentration was 50 mg L\(^{-1}\).
3. Seven 15 mL centrifuge tubes were labeled with STD1, STD2, STD3, STD4, STD5, and STD6 and each tube was filled, using a 10 mL Eppendorf pipette with 10 mL of Milli-Q water
4. The following volume of DIW was removed from each tube:
a) 0 µL DIW from blank tube (STD1-1)
b) 300 µL DIW out of 1.5 mg/L (STD2-1)
c) 600 µL DIW out of 3mg/L (STD3-1)
d) 900 µL DIW out of 4.5mg/L (STD4-1)
e) 1800 µL DIW out of 9mg/L (STD5-1)
f) 3600 µL DIW out of 18mg/L (STD6-1)
g) 5400 µL DIW out of 27mg/L (STD7-1)

5. The same volumes of 50 ppm Fe stock solution was added to each tube respectively.
   a) These volumes may be adjusted to account for needs of the experiment.

6. The contents of the tubes were mixed thoroughly using the vortex mixer (Fisher Vortex Genie 2).

7. Reagent Addition: 7 new 15 mL centrifuge tubes were labeled as before (STD1, STD2, STD3, STD4, STD5, and STD6), and 1 mL of each of the above standard solutions was transferred to the newly labeled tubes. These tubes were prepared for mixing with the reagents, as described below. In addition to the 1 mL Fe(II) standards, the following reagents were added to each tube:
   a) Add 0.5 mL of 10% hydroxylamine solution
   b) Add 0.5 mL of 5.1 mM 1, 10-phenanthroline to each tube
   c) Add 1 mL of 1.2 M sodium acetate buffer solution (RICCA)

8. Addition of the above reagents brought the final volume to 3 mL. Ferrous iron present in the sample turned orange due to the addition of the coloring agent.

9. The Fe(II) standard solution and the reagents in each tube were mixed using the vortex mixer.
10. Solutions were allowed to react for at least 30 minutes.

11. Standards were analyzed using the Perkin Elmer Lambda 45 UV/VIS spectrophotometer.

12. Acidified unknown samples (1 mL each were added to 15 mL vials of their own

13. Reagents described in step 7 a, b, and c were added to the unknown samples. Steps 8, 9, and 10 were carried out for the unknown samples along with the standards, which were prepared fresh for each analysis.

**Fe(II) Standard Concentration Calculations:**

Concentrations for the first dilution were calculated as follows (using STD2 as an example):

\[ C_1 \times V_1 = C_2 \times V_2 \]

Where,

- \( C_1 = 50 \text{ mg L}^{-1} \)
- \( V_1 = 300 \text{ μL} \)
- \( C_2 = \text{unknown concentration in test tube to be calculated} \)
- \( V_2 = 10 \text{ mL} \) (in the tube)

\[ C_2 = \frac{(C_1 \times V_1)}{V_2} = \frac{\left(\frac{50 \text{ mg L}^{-1} \times 0.0003 \text{ L}}{0.010 \text{ L}}\right)}{1.5 \text{ mg L}^{-1}} = 1.5 \text{ mg L}^{-1} \]

Concentration for the addition of the color reagent, reductant, and buffer was calculated as follows (using STD2 as an example):

Where,

- \( C_1 = 1.5 \text{ mg L}^{-1} \)
- \( V_1 = 1 \text{ mL} \) (of 1.5 mg L\(^{-1}\) solution)
- \( C_2 = \text{unknown} \)
\[ V_2 = 3 \text{ mL (in the centrifuge tube for analysis)} \]

\[ C_2 = \frac{(C_1 \times V_1)}{V_2} = \frac{(1.5 \text{ mg L}^{-1} \times 1.0 \text{ mL})}{3 \text{ mL}} = 0.25 \text{ mg L}^{-1} \]

**Procedure for Spectrophotometric Analysis of Fe(II) in Aqueous Samples:**

1. The spectrophotometer was turned on and allowed to warm-up.

2. When the machine was ready (about 10 minutes after start-up) the LAMBDA 45 icon was opened.

3. The appropriate analysis method was double-clicked (An Fe analysis).

4. The information for all of the icons to the left of the sample spreadsheet was checked (data collection, instrument, corrections, Beer's Law Quant, parameters, calibration) to make sure all of the information was correct.

5. In the Accessory tab only the cell changer was checked (not peltier or sipper).

6. After all of the information was checked for setting accuracy in the Sample Info page, the number of samples to be analyzed was entered.

7. The Run button was clicked.

8. The cuvette was filled with 3 mL of sample to be analyzed.

9. The outside of the cuvette was gently cleaned with Kim Wipes to make sure no liquid or fingerprints were present.

10. The cuvette was placed in the cell changer to run the sample.

11. The sample was poured into a waste jar. The cuvette was rinsed with a small amount of the next sample and poured out.

12. Steps 8-11 were repeated for each sample.

13. Data was recorded in a notebook for analysis
14. When all data was collected and recorded, the Lambda 45 program was closed and the spectrophotometer was shut off.

15. Waste was disposed of in a non-chlorinated hazardous waste container
STANDARD OPERATING PROCEDURE FOR REACTOR TOTAL DISSOLVED NITROGEN FROM AMMONIA (NH₃-N) DETERMINATION BY PHENOL HYPOCHLORITE METHOD (adapted from Solorzano, 1969).

**Required Materials:**

Glass vials, 1000 mg/L ammonia standard Solution as NH₃-N (HACH company), 100 g/L phenol solution in 95% v/v ethanol solution, 0.5% sodium nitroprusside solution, alkaline solution (200g/L trisodium citrate and 10 g/L sodium hydroxide in Milli-Q water), Sodium hypochlorite solution (commercial Clorox or some other bleach at least 1.5 N.), Iso-Disc Filter, N-25-4 Nylon 25 mm x 0.45 μm filters, 1-1000 µL Eppendorf pipette (1-5 or 1-10 mL pipette optional), Fisher Vortex Genie 2

**Preparation of Ammonia Standards:**

1. A 1000 mg L⁻¹ ammonia stock solution was used (Hach).
2. The ammonia stock solution was diluted from 1000 mg L⁻¹ to 1.5 mg L⁻¹.
   a. 0.15 mL stock was placed in a 100 mL volumetric flask using a 0-200 μL Eppendorf pipette and the flask was filled with Milli-Q DI water.
   b. Flask was shaken well to make sure the contents are thoroughly mixed.
   c. The final concentration was 1.5 mg L⁻¹.
3. 4 15 mL centrifuge tubes were labeled with STD1, STD2, STD3, STD4 and each tube was filled, using a 0-1000 μL pipette, with specified volume of Milli-Q water
   a) 3 mL added to blank tube (STD1)
b) 2 mL added to 0.5 mg L\(^{-1}\) (STD2)
c) 1 mL added to 1.0 mg L\(^{-1}\) (STD3)
d) 0 mL added to 1.5 mg L\(^{-1}\) (STD4)

2. The following volumes of the 1.5 mg L\(^{-1}\) solution was added to the standards
   a) 0 mL added to blank tube (STD1)
b) 1 mL added to 0.5 mg L\(^{-1}\) (STD2)
c) 2 mL added to 1.0 mg L\(^{-1}\) (STD3)
d) 3 mL added to 1.5 mg L\(^{-1}\) (STD4)

3. These volumes may be adjusted to account for needs of the experiment

4. Unknown samples consisted of 1 mL of sample diluted to 3 mL with Milli-Q water.

5. Appropriate volumes of the hypochlorite solution and the alkaline solution were mixed for the number of samples to be analyzed the day of analysis.
   a) 1 part hypochlorite solution and 4 parts alkaline solution by volume.

6. Phenol solution and nitroprusside solution were added to each sample: 120 µL of each solution.

7. 300 µL of the bleach and alkaline mix was added from step 16.

8. Addition of the above reagents brought the final volume to 3.54 mL.

9. The mixtures turned yellowish, followed by green initially. The contents of the tubes were mixed thoroughly using the vortex mixer (Fisher Vortex Genie 2).

10. The solutions were allowed to react for at least 1 hour, 30 minutes.

11. Standards were analyzed using the Perkin Elmer Lambda 45 UV/VIS spectrophotometer.
Ammonia Standard Concentration Calculations:

Concentrations for the standard solution dilution were calculated as follows (using
STD2 as an example):

\[ C_1 \times V_1 = C_2 \times V_2 \]

where,

\[ C_1 = 1.5 \text{ mg L}^{-1} \]
\[ V_1 = 0.001 \text{ L} \]
\[ C_2 = \text{unknown concentration in test tube to be calculated} \]
\[ V_2 = 0.003 \text{ mL} \]

(Because the standards and the unknown samples have the same 3.54
mL volume, the calculations could be assumed to have 3 mL in the tube without loss
of fidelity)

\[ C_2 = \frac{(C_1 \times V_1)}{V_2} = \frac{(1.5 \text{ mg L}^{-1} \times 0.001 \text{ L})}{0.003 \text{ mL}} = 0.5 \text{ mg L}^{-1} \]

Concentration for the addition of the color reagents and oxidant were figured to be
negligible because of the low volume added and the fact that the same amount of each
solution was added to all of the vials, standard and unknown sample alike.

Procedure for Spectrophotometric Analysis of NH₃-N in Aqueous Samples:

1. The spectrophotometer was turned on and allowed it to warm-up.
2. When the machine was ready (about 10 minutes after start-up) the LAMBDA 45 icon
   was opened.
3. The appropriate analysis method was selected (an ammonia analysis).
4. The information for all of the icons to the left of the sample spreadsheet (data collection, instrument, corrections, Beer's Law Quant, parameters, calibration) was checked to make sure all of the information was correct.

5. In the Accessory tab only the cell changer was checked (not peltier or sipper).

6. After all of the information was checked for setting accuracy in the Sample Info page, the number of samples to be analyzed was inserted.

7. The Run button was clicked.

8. The cuvette was filled with 3 mL of sample to be analyzed.

9. The outside of the cuvette was gently cleaned with Kim Wipes to make sure no liquid or fingerprints were present.

10. The cuvette was placed in the cell changer to run the sample.

11. The sample was poured into a waste jar. The cuvette was rinsed with a small amount of the next sample and poured out.

12. Steps 8-11 were repeated for each sample.

13. Data was recorded in a notebook for analysis.

14. When all data was collected and recorded, the Lambda 45 program was closed and the spectrophotometer was shut off.

15. Waste was disposed of in a chlorinated hazardous waste container because of the bleach hypochlorite concentration.
References

