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ABIOTIC NITRATE AND NITRITE REACTIVITY WITH IRON OXIDE MINERALS

Prakash Dhakal
University of Kentucky, dhakalajneya@gmail.com
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Prakash Dhakal, Student
Dr. Christopher J. Matocha, Major Professor
Dr. Mark S. Coyne, Director of Graduate Studies
ABIOTIC NITRATE AND NITRITE REACTIVITY WITH IRON OXIDE MINERALS

DISSERTATION

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the College of Agriculture at the University of Kentucky

By
Prakash Dhakal
Lexington, KY

Director: Dr. Christopher J. Matocha
Lexington, KY

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ABSTRACT OF DISSERTATION

ABIOTIC NITRATE AND NITRITE REACTIVITY WITH IRON OXIDE MINERALS

Under iron (Fe$^{3+}$)-reducing conditions where aqueous Fe$^{2+}$ and unreduced solid Fe$^{3+}$-oxides commonly coexist, soil Fe$^{2+}$ oxidation has been shown to be coupled with nitrate (NO$_3^-$) reduction. One possible secondary reaction is the involvement of NO$_3^-$ and nitrite (NO$_2^-$) with Fe-oxide minerals found in many natural environments. Yet, spectroscopic measurements and kinetic data on reactivity of NO$_3^-$ and NO$_2^-$ with Fe-containing oxide minerals such as goethite (α-FeOOH), and magnetite (Fe$_3$O$_4$) are not found in the literature. The reactivity of goethite and magnetite with NO$_3^-$ and NO$_2^-$ was studied over a range of environmentally relevant pH conditions (5.5-7.5) with and without added Fe$^{2+}$(aq) under anoxic conditions. Laboratory experiments were conducted using stirred batch experiments and reaction products were analyzed using ion chromatography (IC), gas chromatography (GC), ultraviolet visible near infrared spectroscopy (UV-VIS-NIR), x-ray diffraction (XRD), scanning electron microscopy (SEM), Mössbauer, and Attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy. Nitrate removal by goethite and magnetite was much slower when compared with NO$_2^-$. There was a pH-dependence in the reduction of NO$_2^-$, and the initial rate of NO$_2^-$ removal was nearly 2 and 8 times faster at pH 5.5 than at pH 7.5 by magnetite and goethite, respectively. Nitric oxide (NO) and nitrous oxide (N$_2$O) were identified as products when NO$_2^-$ has reacted with magnetite, whereas N$_2$O is the major reaction product in the experiment with goethite. In comparison to experiments containing magnetite or goethite alone, addition of Fe$^{3+}$ greatly accelerated the NO$_2^-$ removal rate. Wet chemical experiments combined with the Mössbauer study reveals that NO$_2^-$ reduction to NO and subsequently to N$_2$O by magnetite occurs via a heterogeneous electron transfer process. ATR-FTIR and diffuse reflectance spectroscopy (DRS) results from the studies with goethite indicate that NO$_2^-$ was removed from solution by adsorption in a surface complex involving the oxygen atoms, and a portion of the nitrite is reduced to NO and N$_2$O.
This study suggests that under anaerobic conditions soil and sediments that contain goethite, magnetite, and other Fe$^{3+}$-oxides can catalyze abiotic NO$_2^-$ reduction and the kinetics data from this study can be used to predict the NO$_2^-$ removal under such conditions.

KEYWORDS: Nitrate, nitrite, reduction, magnetite, goethite

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Prakash Dhakal
Student’s Signature

______________
9/16/2013
Date
ABIOTIC NITRATE AND NITRITE REACTIVITY WITH IRON OXIDE MINERALS

By
Prakash Dhakal

Dr. Christopher J. Matocha
Director of Dissertation

Dr. Mark S. Coyne
Director of Graduate Studies

9/16/2013
Date
Dedicated to my family
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Chapter One: Abiotic Nitrate and Nitrite Reactivity with Iron Oxide Minerals

1. Introduction

Nitrogen (N) is an essential nutrient for all life forms. In biological cells, nitrogen is held as a base element of nucleic acids and protein structure. Total percent of organic-N pools in the Earth’s biosphere is significantly small (<0.1%) compared to inorganic-N pool that includes N-in the atmosphere (~2%) and rocks (~97%) (Ehrlich, 1995). If global N content fixed in rocks were excluded, most of the inorganic-N occurs as gases in the atmosphere such as ammonia (NH₃), dinitrogen (N₂), nitrous oxide (N₂O), and nitric oxide (NO), and in the form of dissolved species in water as ammonium (NH₄⁺), nitrate (NO₃⁻), and nitrite (NO₂⁻) (Lindsay, 1979). Of these, N₂O and NO are important greenhouse gases, and dissolved N-species are readily available to be transformed by microbes enzymatically (Sylvia et al., 2005).

Among different N-species, N₂(g) is abundant in the earth’s atmosphere (78%). Despite its abundance, it is not readily available to plants except to those that can fix N₂ from the atmosphere in symbiotic association with N-fixing prokaryotes (Sylvia et al., 2005; Canfield et al., 2010). Because N is a major nutrient element that limits the productivity of plants (Vitousek et al., 2009), anthropogenic inputs of N have become an essential agricultural practice to enhance crop production and they provide nearly 45% of the total fixed N produced annually on Earth (Canfield et al., 2010). The principal forms of N taken up by plants are NO₃⁻ and NH₄⁺ (Fig. 1.1). However, unused N applied in excess of plant requirements can be lost to groundwater and nearby water bodies and deteriorate water quality (Dowdell et al., 1979; Xing and Zhu, 2000). It is therefore important to maximize crop production to meet food and energy demands of the world in a sustainable fashion and yet, minimize N losses to the environment (Tilman, 1999; Gallagher et al., 2011). Human health problems linked to an increase in NO₃⁻ and NO₂⁻ loading in water resources (Fan and Steinberg, 1996; Fewtrell, 2004) from anthropogenic fertilizer use worldwide (Spalding and Exner, 1993; Goolsby et al., 2001; Fields, 2004; Ahrens et al., 2008) has therefore stimulated research in understanding processes that lead to NO₃⁻ /NO₂⁻ removal in soil environments.
Leaching and denitrification are two major pathways of N loss from the soil and sediments. First, the ability of the NO$_3^-$ anion to bind with soil particles, often with negative surface charge, is very weak unless the soil contains significant pH-dependent charged minerals (Toner et al., 1989). As a result, NO$_3^-$ is highly susceptible to leaching through the soil-pore water. The denitrification process is the sequential reduction of NO$_3^-$ to NO$_2^-$, which further reduces to NO, N$_2$O and finally to N$_2$ (Tiedje, 1994). When oxygen (O$_2$) is depleted in the subsurface, microorganisms utilize NO$_3^-$ as the next most favorable electron acceptor (Table 1.1) and reduce it to N$_2$ coupled to oxidation of organic carbon (Lovley, 2001). Biological denitrification involves nitrate reductase, nitrite reductase, nitric oxide reductase, and nitrous oxide reductase enzymes to fully reduce NO$_3^-$ to N$_2$ as shown in scheme 1 (Tiedje, 1988; Tiedje, 1994; Canfield et al., 2010).

\[
\text{NO}_3^- \xrightarrow{\text{Nitrate reductase}} \text{NO}_2^- \xrightarrow{\text{Nitrite reductase}} \text{NO} \xrightarrow{\text{Nitric oxide reductase}} \text{N}_2\text{O} \xrightarrow{\text{Nitrous oxide reductase}} \text{N}_2
\]

Scheme 1

A global estimate of N lost via denitrification from terrestrial land is ~22% indicating the importance of denitrification in the N cycle (Seitzinger et al., 2006). This renders the N unavailable for plants. Where denitrification is incomplete, N$_2$O accumulates and its release to the atmosphere is undesirable because it plays a role as a greenhouse gas (Mosier et al., 1998; Baggs, 2008), with a greater warming potential than carbon dioxide (CO$_2$) gas. Nitrous oxide emitted from agricultural soils accounts for approximately 25% of the total N$_2$O released on a global basis (Mosier et al., 1998). Nitrous oxide can also be produced by nitrification (Wrage et al., 2001). Another important intermediate N-species produced during incomplete denitrification is NO. Another important source of NO is from industrial related fossil fuel combustion (Liu et al., 1987; Cheng et al., 2004) and agricultural soil where N-fertilizer has been applied (Warneck, 2000). Combined NO and N$_2$O is commonly referred to as a single quantity NO$_x$ that is a precursor for the photochemical formation of gaseous nitric acid (HNO$_3$), and thus contributes to the acidity of clouds and precipitation (Tost et al., 2007).
Historically, it has been assumed that biogeochemistry of N was entirely dependent on organic carbon (OC) availability, and reduction–oxidation (redox) reactions mediated by microbes (Seitzinger et al., 2006; Wallenstein et al., 2006). Consequently, microbial denitrification has been linked to the carbon cycle. An environmental conditions that stimulate anaerobic microbial denitrification include the presence of readily usable OC, NO₃⁻ availability, and circumneutral pH (Tiedje, 1994; Seitzinger et al., 2006). Because energy obtained from O₂ is higher, microbes consume NO₃⁻, manganese (Mn³+/Mn⁴⁺), iron (Fe³⁺), sulfate (SO₄²⁻), and carbon dioxide (CO₂) in sequential order for next highest energy source only after O₂ is depleted (Lovley and Chapelle, 1995) (Table 1.1). In these terminal electron accepting processes (TEAP), heterotrophic denitrifying microbes transfer electrons from oxidation of OC to electron accepting oxidized species such as NO₃⁻ for their metabolic activity. These microbes can couple denitrification to growth and energy production (Strohm et al., 2007).

1.0.1 Soil Fe chemistry

Iron (Fe) is the fourth most abundant element in the earth’s crust, and it exists in two important oxidation states, Fe²⁺ and Fe³⁺ (Ehrlich, 1995). The valence state of iron is primarily controlled by the ambient redox conditions. This makes Fe the most prevalent redox-sensitive metal commonly available on the boundary between the oxidized and reduced soil-water zones (Lovley, 1991; Lovley and Chapelle, 1995; Hiemstra and van Riemsdijk, 2007). Iron minerals exert a significant influence on soil and sediment geochemistry (Ponnamperuma, 1972). Under aerobic conditions, iron exists as Fe³⁺ in different mineral phases ranging from poorly crystalline materials, such as ferrihydrite (Fe(OH)₃), to well crystalline minerals such as goethite (α-FeOOH), lepidocrocite (γ-FeOOH), hematite (Fe₂O₃), and magnetite (Fe₃O₄) (Sposito, 1989; Cornell and Schwertmann, 2003). Dissolved Fe³⁺-organic carbon complexes have also emerged as important in the Fe cycle.

The microbial reduction of solid iron Fe³⁺-hydr(oxide) minerals is an important biogeochemical process in soil, groundwater and sedimentary environments that influences the cycling of nutrients and metals. Under Fe³⁺-reducing conditions, soil Fe³⁺ is reduced to Fe²⁺. Fe²⁺ can remain dissolved, adsorbed, or reprecipitate. Siderite
green rust (Fe$^{2+}$CO$_3$(s)), magnetite (Fe$^{2+}$Fe$^{3+}$$_2$O$_4$(s)), and lepidocrocite (Fe$^{2+}$Fe$^{3+}$$_2$O$_4$(s)) are potentially precipitating Fe$^{2+}$ minerals (Lovley et al., 1987; Fredrickson et al., 1998; Peretyazhko and Sposito, 2005). Also, in anoxic environments, dissolved Fe$^{2+}$(aq) can resorb to unreduced ferrihydrite to produce magnetite, goethite, and lepidocrocite depending on initial dissolved Fe$^{2+}$(aq), ligand type, and pH (Hansel et al., 2003; Hansel et al., 2005). The effect of Fe$^{2+}$(aq) is illustrated in Figure 1.2. Low initial concentrations of Fe$^{2+}$(aq) (0.2 mM), result in lepidocrocite and goethite precipitation whereas concentrations of 2.0 mM catalyze magnetite precipitation. The ability of reduced Fe to adsorb and re-precipitate depends on the nature of the minerals present in the soil and the soil solution conditions such as pH and ionic strength. Adsorbed and re-precipitated forms of Fe$^{2+}$ are more abundant than dissolved Fe$^{2+}$ forms based on chemical extractions (Peretyazhko and Sposito, 2005; Matocha and Coyne, 2007).

Microbial Fe$^{3+}$-reduction coupled to oxidation of organic carbon occurs after O$_2$, NO$_3^-$, and Mn$^{4+}$ reduction and before sulfate reduction based on free energy available (Chapelle and Lovley, 1992; Lovley and Chapelle, 1995; Appelo and Postma, 2007) (Table 1.1). Overlap of these reduction zones is not uncommon (Postma and Jakobsen, 1996), and could give rise to a biogeochemical scenario where coupling of NO$_3^-$ reduction and Fe$^{2+}$ oxidation is possible.

1.0.2 Coupling of N and Fe cycles

Recent studies have indicated that the Fe$^{2+}$ produced from microbial Fe$^{3+}$ reduction can participate in electron transfer reactions involving nitrate reduction, highlighting the need to look beyond OC alone (Burgin et al., 2011; Schlesinger et al., 2011). Field and laboratory studies have shown that Fe$^{2+}$ can be oxidized during NO$_3^-$ reduction (Yasuhiko et al., 1978; Obuekwe et al., 1981; DiChristana, 1992; Cooper et al., 2003; Weber et al., 2006a; Matocha and Coyne, 2007). This process is called NO$_3^-$-dependent Fe$^{2+}$-oxidation (NDIO) and it can involve both biological, abiotic, and coupled biological/abiotic components (Coby and Picardal, 2005; Weber et al., 2006b; Matocha et al., 2012). Abiotic reduction of nitrate can occur on time scales comparable to biological denitrification when mineral Fe$^{2+}$-forms are present such as green rust and wüstite (Fe$^{2+}$O) (Hansen et al., 1996; Rakshit et al., 2005). A coupled biological/abiotic pathway
might be operative in which nitrate reductase enzyme performs the first two-electron reduction to form nitrite (Scheme 1). The resulting NO$_2^-$ would then be available for reaction with mineral Fe$^{2+}$ forms such as green rust and siderite in an abiotic process (Hansen et al., 1996; Rakshit et al., 2008). Picardal (2012) pointed out that this secondary abiotic reaction between biologically produced NO$_2^-$ and Fe$^{2+}$ would be obscured in abiotic controls, presumably due to the halting of the first step of NO$_3^-$ reduction due to sterilization. Abiotic NO and N$_2$O emission from a lake environment was reported to occur by an abiotic reaction coupling mineral Fe$^{2+}$ oxidation to NO$_2^-$ reduction (Samarkin et al., 2010) and also in the laboratory batch studies (Kampschreur et al., 2011). Table 1.2 summarizes the literature to date on abiotic reduction of nitrate and nitrite on Fe minerals compared with other contaminants. The role of goethite and magnetite in the reduction of NO$_3^-/NO_2^-$ is not known and is the focus of this dissertation.

1.1 Magnetite mediated NO$_3^-/NO_2^-$ removal

In a secondary reaction, Fe$^{2+}$ produced during dissimilatory reduction of poorly crystalline ferrihydrite by iron reducing bacteria (DIRB) has shown to precipitate magnetite (Lovley and Phillips, 1987; Fredrickson et al., 1998). The illustration in Fig. 1.2 provides a simplified diagram for the process. In addition, a magnetotactic bacterium has also shown to precipitate as magnetite in its cell surface (Bazylinski et al., 2007). Because Fe$^{2+}$ is included in the crystalline structure, magnetite (Fe$_3$O$_4$) is regarded as a mixed valence Fe$^{2+}$/Fe$^{3+}$ mineral which has been identified in natural environments such as soils and sediments (Lovley et al., 1987; Maher and Taylor, 1988; Cornell and Schwertmann, 2003). Previous studies have shown that structural Fe$^{2+}$ positioned in magnetite crystals allows it to serve as a reductant in the transformation of organic (Gregory et al., 2004; Gorski and Scherer, 2009) and inorganic contaminants such as chromate (White and Peterson, 1996; Peterson et al., 1997; Jung et al., 2007) and uranium (U$^{6+}$) (Das et al., 2010; Latta et al., 2012a; Singer et al., 2012). Contaminant reducing capacity of magnetite is attributed to its ability to transfer electrons both within the solid state and also across the solid-liquid interface (White et al., 1994; Scherer et al., 1999).
Ideally, the ratio of \( \text{Fe}^{2+}:\text{Fe}^{3+} \) in stoichiometric magnetite is 1:2 (Gorski and Scherer, 2009). In general, \( \text{Fe}^{3+} \) occupies tetrahedral sites and both \( \text{Fe}^{2+} \) and \( \text{Fe}^{3+} \) ions occupy octahedral sites of a magnetite spinel structure (Daniels and Rosencwaig, 1969; Tronc et al., 1984; Gorski et al., 2010). In a condition where the magnetite stoichiometry \( x = \frac{\text{OctFe}^{2+}}{(\text{TetFe}^{3+} + \text{OctFe}^{3+})} \) differs from 1:2, a wide range of possibilities can exist (0 < x < 0.5).

In previous studies, magnetite is reported to form when \( \text{NO}_3^- \) is chemically reduced by wüüstite (Rakshit et al., 2005), green rust (Hansen et al., 1996), and also when surface \( \text{Fe}^{2+} \)-lepidocrocite is used to reduce \( \text{NO}_2^- \) (Sørensen and Thorling, 1991). In the presence of continuous hydrogen (\( \text{H}_2 \)) flow, reduction of \( \text{NO}_3^-/\text{NO}_2^- \) catalyzed by monometallic Pd/\( \text{Fe}_3\text{O}_4 \) has recently been reported (Sun et al., 2012). However, there is no clear evidence if \( \text{NO}_3^- \) or \( \text{NO}_2^- \) could be reduced by magnetite. Because the role of abiotic \( \text{NO}_2^- \) removal mediated by magnetite is less understood, its importance in anaerobic cycling of \( \text{NO}_3^-/\text{NO}_2^- \) may be underestimated. Importantly, there is a thermodynamic driving force for \( \text{NO}_2^- \) reduction by magnetite because the \( \text{Fe}_3\text{O}_4/\text{Fe}^{2+} \) redox couple lies well below that of \( \text{NO}_2^-/\text{NO} \) and \( \text{NO}_2^-/\text{N}_2\text{O} \) over a wide range of pH values (Fig. 1.3). Therefore, it is a timely pursuit to investigate abiotic reduction of \( \text{NO}_2^- \) by magnetite.

### 1.2 Goethite mediated \( \text{NO}_3^-/\text{NO}_2^- \) removal

Goethite has been identified as a common iron oxide mineral in many natural environments (Cornell and Schwertmann, 2003). It can be produced under oxic conditions, for example, when structural \( \text{Fe}^{2+} \) in biotite is oxidized by \( \text{O}_2 \) (Essington, 2004). Goethite can also be produced under anoxic conditions. For example, microbial reduction of poorly crystalline ferrihydrite by DIRB results in production of \( \text{Fe}^{2+}_{(aq)} \) which can resorb to unreduced ferrihydrite forming goethite at both low and high concentrations of initial \( \text{Fe}^{2+} \) as shown in Figure 1.2 (Glasauer et al., 2003). In addition, nitrate-dependent iron (\( \text{Fe}^{2+} \)) oxidizing bacteria have been discovered which produce goethite anoxically (Senko et al., 2005; Miot et al., 2009; Larese-Casanova et al., 2010). Photoautotrophic \( \text{Fe}^{2+} \) oxidizers (Kappler and Newman, 2004) have also been shown to produce crystalline goethite as their oxidation product in the absence of oxygen. Thus,
goethite has been used extensively as a model iron oxide mineral in many studies carried out to investigate sorption and reduction of contaminants (Grossl et al., 1997; Amonette et al., 2000; Williams and Scherer, 2004; Catalano et al., 2011; Um et al., 2011).

Goethite is often written as the product of dissolved Fe$^{2+}$ oxidation by NO$_2^-$ and NO$_3^-$ (Picardal, 2012). Previous studies in our lab showed abiotic goethite and lepidocrocite precipitation occurred during NO$_2^-$ reduction by siderite, an Fe$^{2+}$ mineral, under anoxic conditions (Rakshit et al., 2008). Kinetic modeling of the time series data assumed that NO$_2^-$ removal from solution was entirely due to surface sites on siderite. However, it is possible that the appearance of goethite provides reactive surface sites that can bind anions such as NO$_2^-$ and remove them from solution. A survey of the literature reveals only one study which documented the reactivity of NO$_2^-$ with goethite (Coby and Picardal, 2005), however it was restricted to one pH value (pH 7.0) and only N$_2$O production was measured as the metric of reactivity.

1.3 Influence of surface sorbed Fe$^{2+}$ in NO$_3^-$/NO$_2^-$ removal

The reductive dissolution of Fe$^{3+}$-oxides by DIRB releases Fe$^{2+}$ (aq) to solution (Fig. 1.2). The resulting Fe$^{2+}$ (aq), like other first row transition metals, is capable of adsorbing to unreduced Fe$^{3+}$-oxides or other mineral surfaces (Essington, 2004). In various studies, surface complexed Fe$^{2+}$ has shown to be a more effective reductant than dissolved, hexaquo Fe$^{2+}$-(Fe(H$_2$O)$_6$)$_2^+$ (Wehrli et al., 1989; Stumm and Sulzberger, 1992; Strathmann and Stone, 2003; Tai and Dempsey, 2009). Increased reactivity of surface sorbed Fe$^{2+}$-species can be explained using molecular orbital theory (Luther et al., 1992). The coordination of Fe$^{2+}$ to surface hydroxyls allows electron density to be pushed through the pi system of OH$^-$ to Fe$^{2+}$ (t$_{2g}$ (π) orbital) to an oxidant with unoccupied orbitals of appropriate symmetry, making electron transfer favorable. This is analogous to the acceleration of Fe$^{2+}$ oxidation by O$_2$ whose rate increases with increasing OH$^-$ added to solution (Luther et al., 1992). In this study, an attempt will be made to explore how reduction of NO$_3^-$/NO$_2^-$ by surface sorbed Fe$^{2+}$ on magnetite and goethite will vary compared to the system with mineral alone.
1.3.1 NO$_3^-$/NO$_2^-$ removal by surface sorbed Fe$^{2+}$ on magnetite

Studies have shown that adding Fe$^{2+}$(aq) to a magnetite slurry enhanced the rate of polyhalogenated methane, RDX, nitrobenzene (Pecher et al., 2002; Gregory et al., 2004; Gorski and Scherer, 2009), and U$^{6+}$ (Latta et al., 2011) reduction. In these studies, it was observed that structural Fe$^{2+}$ in magnetite participated in the electron transfer process and reduction of contaminants. Evidence from Mössbauer spectroscopy revealed that the ratio of Fe$^{2+}$:Fe$^{3+}$ in the reacted magnetite decreased (Gorski and Scherer, 2009; Latta et al., 2011). This is a positive indication that oxidation of structural Fe$^{2+}$ in magnetite stimulated the reduction process. Further, reductions of contaminants were more favored by stoichiometric magnetite, and the rate progressively decreased with more non-stoichiometric magnetite (Gorski et al., 2012). Results from Fe$^{2+}$ sorption experiments on magnetite showed that less stoichiometric magnetite retained more Fe$^{2+}$. With increasing Fe$^{2+}$ uptake, the ratio of Fe$^{2+}$ in magnetite crystal increased. It was observed that with added Fe$^{2+}$, non-stoichiometric magnetite not only replenished oxidized Fe$^{3+}$ in the crystal arrangement, but also continued to maintain reducing capacity of the mineral surface (Latta et al., 2011; Gorski et al., 2012). Here, we raise a question of whether removal of NO$_3^-$ and NO$_2^-$ from solution by magnetite is changed in the presence of added Fe$^{2+}$(aq). To our knowledge, this abiotic reduction of NO$_3^-$/NO$_2^-$ has not been studied.

1.3.2 NO$_3^-$/NO$_2^-$ removal by surface sorbed Fe$^{2+}$ on goethite

Compared to the system without added Fe$^{2+}$, goethite-bound Fe$^{2+}$ substantially promoted the reductive transformation of CCl$_4$ (Amonette et al., 2000), polyhalogenated methane (Pecher et al., 2002), and 2-nitrophenol (Tao et al., 2010). Results from laboratory studies suggest that Fe$^{2+}$-bound to lepidocrocite and hydrous ferric oxide reduced NO$_2^-$ more rapidly than the system with only aqueous Fe$^{2+}$ or oxide-mineral slurry (Sørensen and Thorling, 1991; Tai and Dempsey, 2009). In anoxic subsurface environments, where NO$_3^-$ reduction and Fe$^{2+}$ oxidation are potentially coupled, biologically produced NO$_2^-$ could further undergo abiotic reduction. In the presence of Fe$^{2+}$(aq) produced by DIRB, soil and sediments dominated by goethite could potentially yield surface bound Fe$^{2+}$-goethite complex that could further reduce NO$_2^-$ more rapidly than the systems with goethite alone. In addition, thermodynamic calculations suggest
that NO$_2^-$ reduction by goethite/Fe$^{2+}$ couple is feasible (Picardal, 2012) (Fig. 1.3). However, kinetic data on NO$_2^-$ reactivity with goethite and Fe$^{2+}$-treated goethite are not yet published.

1.4 Spectroscopic study of NO$_3^-$/NO$_2^-$ reactivity with magnetite and goethite

The influence of aqueous Fe$^{2+}$ (Moraghan and Buresh, 1977; Kampschreur et al., 2011) and mineral Fe$^{2+}$ on NO$_2^-$ reduction (Sørensen and Thorling, 1991; Rakshit et al., 2005; Rakshit et al., 2008; Tai and Dempsey, 2009) has been studied previously. These studies employed wet chemical methods, using changes in total nitrate and nitrite concentration over time to understand the electron transfer process. In order to understand mechanisms of electron transfer, spectroscopic measurements of reacted solid phases in the presence of water (*in situ*) are necessary where possible. Notably, spectroscopic data of NO$_3^-$/NO$_2^-$ reactivity with magnetite and goethite minerals are not found in the literature. Commonly, spectroscopic techniques such as Mössbauer, Diffuse Reflectance Spectroscopy (DRS), and Infrared (IR) Spectroscopy are used to identify changes in surface chemical properties of reacted metal oxides.

Magnetite consists of TetFe$^{3+}$ and Oct(Fe$^{2+}$, Fe$^{3+}$) in the crystal structure, and stoichiometry (x= Fe$^{2+}$/Fe$^{3+}$) of magnetite can often vary (0<x<0.5). As stoichiometric magnetite (x=0.5) becomes oxidized, the Fe$^{2+}$/Fe$^{3+}$ ratio decreases. Completely oxidized magnetite (x=0) is known as maghemite ($\gamma$-Fe$_2$O$_3$). Common methods used to measure magnetite stoichiometry include: (a) $^{57}$Fe Mössbauer spectroscopy (Daniels and Rosencwaig, 1969; da Costa et al., 1995); and (b) complete acidic dissolution (Tamura et al., 1974; Tronc et al., 1984; Gorski and Scherer, 2009). The iron-specific Mössbauer spectroscopy technique utilizes detection of the $^{57}$Fe isotope. A mineral sample is subjected to gamma rays, some of which are absorbed by the sample, resulting in nuclear transitions. This technique is widely used in detecting changes in Fe$^{2+}$/Fe$^{3+}$ ratio in mineral solids as well as hydrated mineral oxide surfaces. With careful comparison of the Fe$^{2+}$/Fe$^{3+}$ ratio obtained by fitting relative peaks in a Mössbauer spectrum of reacted and unreacted iron oxides, one can quantify changes in the Fe$^{2+}$/Fe$^{3+}$ ratio (Gorski and Scherer, 2011). In this study, the role of structural OctFe$^{2+}$ in magnetite for NO$_3^-$/NO$_2^-$
reduction will be studied using Mössbauer spectroscopy and compared with wet chemical (acid) dissolution.

Infrared spectroscopy (IR) is employed to probe vibrations of atoms or groups of atoms within a molecule subject to selection rules developed in quantum mechanics (Huheey et al., 1993). Conventional IR spectroscopy was limited by the strong absorption of water, which complicated interpretations. Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) has emerged as a technique which enables one to study reactions at the mineral-water interface, thus it offers the possibility for in situ spectroscopic investigations. Previously, adsorption mechanisms of several oxyanions on metal oxides such as goethite have been investigated using ATR-FTIR spectroscopy (Peak et al., 1999; Arai and Sparks, 2001; Villalobos and Leckie, 2001; Lefèvre, 2004). Virtually no work has been carried out to understand the NO$_2^-$ sorption mechanism and reaction intermediates using iron oxide minerals such as goethite. Nitrite is a versatile ligand that can participate in acid/base coordination or oxygen transfer chemistry (Hitchman and Rowbottom, 1982). Five possible modes of NO$_2^-$ bonding to metal cations (denoted by M) have been previously suggested (Scheme 2, shown below) (Finney et al., 1981; da Cunha et al., 1996; Hadjiivanov, 2000).
ATR-FTIR analytical techniques will be utilized to establish the nature of coordination of NO$_2^-$ with surface sites on goethite and possibly magnetite by observing N and O bond stretching/bending vibration frequencies. These experiments will be performed first with NO$_2^-$ alone and then in the presence of Fe$^{2+}$-bound onto minerals. A close monitoring of the energy regions will be performed as a function of surface Fe$^{2+}$ onto these minerals with NO$_2^-$ loading as a function of pH, and ionic strength. It is hypothesized that results from this study will help to identify possible modes of NO$_2^-$ bonding to metal cations. Such investigations will allow us to better understand the role of chemical reoxidation of Fe$^{2+}$ by NO$_2^-$ in the inhibition of Fe$^{3+}$ reduction by NO$_3^-$.

In addition to ATR-FTIR, DRS within UV to NIR electromagnetic spectrum will be used to measure electronic spectra of adsorbed Fe$^{2+}$ onto goethite and obtain direct information about ground state and the excited state of such complexes. Electronic spectroscopy thus collected could provide evidence of electron transfer between adsorbed Fe$^{2+}$ and Fe$^{3+}$OOH (goethite), and also during redox reactions of goethite, and the Fe$^{2+}$-goethite complex with NO$_2^-$.
1.5 Objectives of the present study

The overall aim of this research project is to investigate abiotic transformations of NO$_3^-$ and NO$_2^-$ under anoxic conditions in stirred-batch experiments containing iron oxide slurries, in the presence and absence of added Fe$^{2+}_{(aq)}$. Specifically, the objectives are to determine:

(i) the reactivity of NO$_3^-$ and NO$_2^-$ with magnetite and goethite,
(ii) the impact of surface adsorbed Fe$^{2+}$ on the ability of magnetite and goethite to remove NO$_2^-$ from solution,
(iii) the changes in reacted magnetite and goethite samples by means of Mössbauer, ATR-FTIR, and DRS techniques to elucidate mechanisms of reactivity at the mineral-water-interface.

It is believed that this research has important implications in biogeochemical cycling of nutrients and contaminant transport.
Table 1.1 Sequence of naturally occurring microbial terminal electron accepting process (TEAP) in the anoxic subsurface, and corresponding standard reduction potential (Eh°). Calculation was performed at pH 7.0 (modified after Hall et al. (1996); Davidson and Kingerlee (1997)).

<table>
<thead>
<tr>
<th>Less energy (ΔG°) for microbes</th>
<th>Microbes involved</th>
<th>Redox couple</th>
<th>E_h°(V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerobes/oxidizers</td>
<td>O_2/H_2O</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>Nitrate reducers</td>
<td>NO_3^-/N_2</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>Manganese reducers</td>
<td>MnO_2(s)/Mn^{2+}</td>
<td>0.61</td>
<td></td>
</tr>
<tr>
<td>Iron reducers</td>
<td>FeOOH(s)/Fe^{2+}</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Sulfate reducers</td>
<td>SO_4^{2-}/H_2S</td>
<td>-0.22</td>
<td></td>
</tr>
<tr>
<td>Methanogens</td>
<td>CO_2/CH_4</td>
<td>-0.24</td>
<td></td>
</tr>
</tbody>
</table>
Table 1.2 Common iron oxides used in laboratory batch experiments to study abiotic reduction/transformation of contaminants.

<table>
<thead>
<tr>
<th>Fe-Oxides</th>
<th>Added Fe$^{2+}_{(aq)}$</th>
<th>Contaminants</th>
<th>Reduction Products</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Green Rust</td>
<td>No NO$_3^-$</td>
<td>NH$_4^+$, magnetite</td>
<td>Hansen et al., 1996</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No NO$_3^-$</td>
<td>NH$_4^+$, magnetite</td>
<td>Choi and Batchelor, 2008</td>
<td></td>
</tr>
<tr>
<td>Ferrihydrite</td>
<td>Yes NO$_2^-$</td>
<td>N$_2$O, goethite, magnetite</td>
<td>Tai and Dempsey, 2009</td>
<td></td>
</tr>
<tr>
<td>Wüstite</td>
<td>No NO$_3^-$</td>
<td>NH$_4^+$, magnetite</td>
<td>Rakshit et al., 2005</td>
<td></td>
</tr>
<tr>
<td>Lepidocrocite</td>
<td>Yes NO$_2^-$</td>
<td>N$_2$O, magnetite</td>
<td>Sørensen and Thorling, 1991</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes ArNO$_2$</td>
<td>ArNH$_2$</td>
<td>Klausen et al., 1995</td>
<td></td>
</tr>
<tr>
<td>Siderite</td>
<td>No NO$_2^-$</td>
<td>N$_2$O, lepidocrocite</td>
<td>Rakshit et al., 2008</td>
<td></td>
</tr>
<tr>
<td>Goethite</td>
<td>No NO$_3^-$</td>
<td>N$_2$O</td>
<td>Cooper et al., 2003</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes 2-nitrophenol</td>
<td>NA, reductive transformation chloroform, goethite</td>
<td>Tao et al., 2010</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes CCl$_4$</td>
<td>NA, reductive transformation chloroform, goethite</td>
<td>Amonette et al., 2000</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes As</td>
<td>As$^{3+}$ to As$^{5+}$, goethite</td>
<td>Amstaetter et al., 2009</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes ArNO$_2$</td>
<td>ArNH$_2$</td>
<td>Klausen et al., 1995</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No NO$_2^-$</td>
<td>N$_2$O$_2$, goethite</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>Magnetite</td>
<td>Yes ArNO$_2$</td>
<td>ArNH$_2$</td>
<td>Gorski and Scherer, 2009</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes Cr$^{6+}$</td>
<td>Cr$^{3+}$, oxidized magnetite</td>
<td>Jung et al., 2007</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No Cr$^{3+}$</td>
<td>Cr$^{3+}$, maghemite</td>
<td>Peterson et al., 1997</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No Hg$_2^{2+}$</td>
<td>Hg$^0$, oxidized magnetite</td>
<td>Wiatrowski et al., 2009</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yes CCl$_4$</td>
<td>MNX, DNX, TNX</td>
<td>Gregory et al., 2004</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No NO$_3^-$</td>
<td>chloroform (CHCl$_3$),</td>
<td>Vikesland et al., 2007</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No NO$_3^-$/NO$_2^-$</td>
<td>NO, N$_2$O, magnetite</td>
<td>This study</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1.1 A portion of the nitrogen cycle, emphasizing the transformation of ammonium and nitrate applied to agricultural land. The chemical reaction illustrated in the box indicates that there is no clear understanding of how nitrate and nitrite react with various iron (II,III) oxides in the presence of dissolved or surface adsorbed Fe$^{2+}$. 
Figure 1.2 Conceptual illustration showing formation of a reactive Fe$^{2+}$/Fe$^{3+}$ phase formed in the presence of Fe$^{2+}$(aq) and poorly crystalline iron oxide, and consequential abiotic phase transformation (modified from Hansel et al. (2003); Hansel et al. (2005)).

a Initial Fe$^{2+}$(aq) concentration of 2.0mM.

b Initial Fe$^{2+}$(aq) concentration of 0.2mM.

c dissolution and (re)precipitation

--- Defined pathways ---

--- Unknown pathways ---
Figure 1.3 Redox potential ($E_h$) versus pH of relevant nitrogen and iron couples. The standard redox potential ($E_h^0$) of each half reaction shown in the diagram is taken from Bard et al. (1985). Equimolar NO$_3^-$ and NO$_2^-$ concentrations were used in the plot ($5 \times 10^{-4}$ M). Nitric oxide (NO) concentration was $4 \times 10^{-7}$, and nitrous oxide (N$_2$O) concentration was $1.5 \times 10^{-8}$ molL$^{-1}$, respectively. The $E_h^0$ for the Fe$_3$O$_4$/Fe$^{2+}$ and $\alpha$-FeOOH/Fe$^{2+}$ couple was estimated from Gibbs free energy of formation values to be $1.5894 - 0.236$ pH for Fe$^{2+}$*(aq)* concentration of $1 \times 10^{-8}$ molL$^{-1}$ and $1.182 - 0.1182$ pH for Fe$^{2+}$*(aq)* concentration of $1 \times 10^{-7}$ molL$^{-1}$ based on the equation proposed for reductive dissolution of magnetite and goethite by (White and Peterson, 1996) and (Essington, 2004), respectively. Except for $\alpha$-FeOOH/Fe$^{2+}$, and Fe$_3$O$_4$/Fe$^{2+}$ redox couples, information on $E_h^0$ for the N-Fe system is adopted from (Matocha et al., 2012) and references therein. Shaded area brackets range of experimental pH conditions considered in this study (5.5-7.5)

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Chapter Two: Nitrite Reactivity with Magnetite


2. Introduction

Magnetite (Fe$_3$O$_4$) is a mixed valence Fe$^{2+}$/Fe$^{3+}$ mineral which has been identified in natural environments such as soils and sediments (Lovley et al., 1987; Maher and Taylor, 1988; Cornell and Schwertmann, 2003). It is formed in various ways: (i) as a product of respiration during dissimilatory reduction of poorly crystalline ferrihydrite by Fe$^{3+}$-reducing bacteria, (Lovley et al., 1987; Fredrickson et al., 1998); (ii) by magnetotactic bacteria, (Bazylinski et al., 2007); (iii) during corrosion of metallic iron (Lee and Wilkin, 2010; Saheb et al., 2010). Magnetite contributes to the magnetic properties of soils and sediments and its presence is being used to reconstruct past climates (Maher, 2009).

The structural Fe$^{2+}$ in magnetite allows it to serve as a reductant in the transformation of organic, (Gregory et al., 2004; Gorski and Scherer, 2009) and inorganic contaminants (Peterson et al., 1997; Jung et al., 2007; Das et al., 2010; Latta et al., 2011; Singer et al., 2012). Tetrahedral sites in the magnetite spinel structure are occupied by Fe$^{3+}$, and both Fe$^{2+}$ and Fe$^{3+}$ ions occupy octahedral sites (Daniels and Rosencwaig, 1969; Gorski et al., 2010) in an overall Fe$^{2+}$:Fe$^{3+}$ ratio of 1:2 for stoichiometric magnetite. The presence of both Fe$^{2+}$ and Fe$^{3+}$ ions on the same site in magnetite offers unique electronic, structure and redox properties (Kündig and Hargrove, 1969; White et al., 1994). It is suggested that magnetite is capable of transferring electrons both within the solid state and also across the solid-liquid interface (White and Peterson, 1996; Scherer et al., 1999). Magnetite stoichiometry (x=OctFe$^{2+}$/(TetFe$^{3+}$ + OctFe$^{3+}$) can have a wide range (0<x<0.5) due to the possible existence of solid solution between magnetite (x=0.5) and maghemite (γ-Fe$_2$O$_3$) (x=0) (Trone et al., 1984; White and Peterson, 1996; Gorski and Scherer, 2009). Experimental results have shown that stoichiometric magnetite is more reactive than oxidized (non stoichiometric) magnetite (Gorski and Scherer, 2009). During reduction of hexavalent uranium (U$^{VI}$), solid magnetite is transformed to maghemite, (Das et al., 2010) or more oxidized magnetite (Latta et al., 2011). Addition of dissolved
Fe$^{2+}$ to magnetite can recharge partially oxidized magnetite and enhance reactivity (Gorski and Scherer, 2009; Latta et al., 2011).

Microbial denitrification involves the reduction of nitrate (NO$_3^-$) to nitrite (NO$_2^-$), which sequentially reduces further to nitric oxide (NO), nitrous oxide (N$_2$O), and eventually to dinitrogen (N$_2$), (Tiedje, 1994). This process is usually assumed to be mediated by organic carbon oxidation (Burford and Bremner, 1975). In anoxic environments, however, nitrate reduction and Fe$^{2+}$ oxidation are potentially coupled. Coupling of NO$_3^-$ reduction to Fe$^{2+}$-oxidation is probable when NO$_3^-$ from still oxidized soil layers penetrates into Fe$^{3+}$-reducing zones. Nitrate can be abiotically reduced by mineral Fe$^{2+}$ forms such as green rust and wüstite (Hansen et al., 1996; Rakshit et al., 2005). A second possible scenario is the biological reduction of NO$_3^-$ to NO$_2^-$ and the subsequent further reduction in abiotic reactions involving mineral Fe$^{2+}$ (Chaudhuri et al., 2001; Rakshit et al., 2008; Samarkin et al., 2010) and dissolved Fe$^{2+}$ (Kampschreur et al., 2011). Addition of Fe$^{2+}$ to Fe$^{3+}$-oxides results in surface-bound Fe$^{2+}$ which functions as a good reductant of NO$_2^-$ (Sørensen and Thorling, 1991; Tai and Dempsey, 2009; Matocha et al., 2012). In fact, observed N$_2$O fluxes were attributed to abiotic mineral Fe$^{2+}$- NO$_2^-$ reactions in a hypersaline lake (Samarkin et al., 2010).

Magnetite was the reaction product when NO$_3^-$ was abiotically reduced by wüstite (Rakshit et al., 2005) and green rust, (Hansen et al., 1996) and also when surface Fe$^{2+}$-lepidocrocite (Sørensen and Thorling, 1991) was used to reduce NO$_2^-$. There is a thermodynamic driving force for NO$_2^-$ reduction by Fe$_3$O$_4$ because the Fe$_3$O$_4$/Fe$^{2+}$ redox couple lies well below that of NO$_2^-$/NO and NO$_2^-$/N$_2$O over a wide range of pH values (Fig. 1.3). There has been one study that evaluated the ability of structural Fe$^{2+}$ in Fe$_3$O$_4$ to reduce NO$_3^-$ and NO$_2^-$, (Sun et al., 2012) however, the Fe$_3$O$_4$ was coated with a palladium catalyst. To our knowledge, chemical reduction of NO$_2^-$ by magnetite alone has not been studied nor has the impact of added Fe$^{2+}$. The aim of this study is to investigate NO$_2^-$ reactivity with magnetite at various pH values under anaerobic conditions and in the presence of added Fe$^{2+}$. 
2.1 Experimental section

Unless otherwise indicated, all the reactions in this study were performed in an anaerobic glove box (COY Laboratory Products, Grass Lake, MI) purged with 95:5 argon (Ar)-H₂ and equipped with palladium catalyst for O₂ removal. Before transport into the glove box, all aqueous solutions were prepared in double deionized water (18.3 MOhm cm⁻¹) and purged for 3hr with ultra pure Ar. Dissolved oxygen (O₂) in the solution was measured using an 782 oxygen meter (Strathkelvin Instruments, Scotland, UK) to ensure anoxic conditions. Before use, solid magnetite, glass vials, reaction vessel, and other experimental accessories were equilibrated for a few days inside the glove box to reduce sorbed oxygen. To verify the solid phase only contributed to abiotic reaction paths, magnetite solid samples were analyzed for microbial DNA. The 16S rDNA fragments profile pattern obtained from gel electrophoresis (GE) indicate that the mineral is free of any microbes or below the detection limit (Fig. 2.1).

2.1.1 Characterization of magnetite

Magnetite used in this study was purchased from Alfa Aesar (PURATRONIC® powder, Ward Hill, MA) and used as received. The specific surface area was determined by Brunauer-Emmet-Teller (BET) method with N₂ adsorption to be 1.54±0.18 m² g⁻¹. The solid magnetite powder, both control and reacted, was characterized by x-ray diffraction (XRD), and scanning electron microscopy (SEM). After extraction, control and treated magnetite samples were evenly layered over a glass slide. A drop or two of Ar-degassed glycerin was mixed with samples on the glass slide to minimize mineral Fe²⁺ oxidation pending X-ray analysis. XRD scans were taken from 10 to 60° 2θ with CuKα radiation at 40 kV and 30 mA using Siemens D500 Diffractometer (Bruker AXS, Madison, WI). Data were analyzed using the software Match!® (ver 1.11, Crystal Impact, Bonn, Germany). X-ray diffraction (XRD) scans revealed peaks 4.84, 2.97, 2.53, 2.10, 1.71, and 1.61Å representing diagnostic d-spacings for synthetic magnetite (Fig. 2.2).

Control and treated magnetite samples were dried inside the glove box to maintain anaerobic conditions, and mounted on a carbon tape attached to an aluminum holder and coated with Au/Pd for SEM analysis. A Hitachi (Tokyo, Japan) S-3200 SEM was used to image these samples for various magnifications and a working distance of 30mm. Energy
dispersive x-ray spectroscopy (EDX) analysis of the magnetite was also performed with EDX module attached to S-3200. Bulk magnetite seen under SEM micrograph showed porous aggregate structure with an average particle size 0.85-1.1µm, and EDX analysis showed that the chemical composition of those crystals was Fe and O (Fig. 2.3).

The estimated pH at the isoelectric point (pH_{IEP}) of magnetite suspensions was 6.05±0.05 as determined by measuring zeta potentials as a function of ionic strength and pH (Fig. 2.4). The stoichiometry of untreated magnetite determined by complete dissolution in 5 M HCl was lower (x_{diss}=0.45) than that of Mössbauer spectroscopy (x_{MS}=0.47) (see detail below).

2.1.2 Nitrate and nitrite reduction studies

Analytical stock solutions of NO$_3^-$ and NO$_2^-$ were prepared from certified ACS-grade NaNO$_3$ and NaNO$_2$ using deoxygenated deionized water (DDIW) inside an anaerobic glove box.

Initial tests were performed to evaluate the potential of the biological buffer MES [2-(N-morpholino) ethane sulfonic acid] to interact with magnetite. MES buffered to pH 5.5 was added at two different concentrations (5 mM and 25 mM) and compared with a MES-free control in which pH was adjusted manually using HCl or NaOH over the span of 14 days. Significant amounts of aqueous Fe$^{2+}$ were detected in solution, and Fe$^{2+}$ concentration steadily increased during this period (Fig. 2.5). An increase in MES concentration in the samples had a positive effect on Fe$^{2+}$ release. The amount of aqueous Fe$^{2+}$ released nearly doubled when MES concentration was raised from 5mM to 25mM in identical solid-solution conditions. Anomalous buffer interferences have been reported in previous studies that used MES, HEPES and TAPS to buffer experimental solution pH (Zhang and Huang, 2005 & 2006). MOPS and HEPES have also been shown to cause substantial release of previously sorbed Fe$^{2+}$ from iron oxide (Buchholz et al., 2011). It is suggested that the organic pH buffer can potentially increase reaction rates, vary product yield, and alter the number of available reaction sites (Danielsen et al., 2004; Buchholz et al., 2011).

To avoid buffer interference to the reaction, all subsequent experiments were performed by manually adjusting the pH. Stirred-batch kinetic reactions were conducted
in duplicate glass vials with 10 gL\(^{-1}\) of prehydrated magnetite over a range in pH values (pH 5.5-7.5) at room temperature. Reactions were initiated by spiking with 0.5 mM NO\(_2\)\(^-\) and the batch reactors were kept shaking with an end-to-end shaker at 120 rpm to allow complete mixing. Separate experiments were performed using 0.5 mM NO\(_3\)\(^-\). Aliquots were removed at increasing time intervals and filtered using 0.22µm filter paper (Fisher Scientific, Hampton, NH). Part of the filtrate was immediately complexed with ferrozine [3-(2-pyridyl)-5,6 bis(4-phenylsulfonic acid)-1,2,4-triazine, monosodium salt] for Fe\(^{2+}\) quantification and the remaining filtrate was used for NO\(_3\)^-, NO\(_2\)^-, and NH\(_4\)^+ quantification. Slope of the line for NO\(_3\)^-, and NO\(_2\)^- decay recorded between 0 and 168 hr was used to calculate “initial rate” of removal and rate constants (k\(_{obs}\)). Parallel experiments were performed to characterize possible gaseous products of NO\(_2\)^- reduction under comparable experimental conditions as described above by sampling the headspace periodically from 30mL crimp sealed glass vials using a 1 mL syringe. This headspace gas was injected into a gas chromatograph. Parallel experiments were performed using NO\(_3\)^-.

2.1.3 Nitrite reduction in the presence of surface Fe\(^{2+}\)

Fe\(^{2+}\) stock solutions were prepared from zerovalent iron (Fe\(^0\)) mixed with 0.1M HCl stock solution and shaken in an end-over-end rotator for 24 hr inside an anaerobic glove box. Residual Fe\(^0\) was removed from the solution with a magnet. Then, the solutions were filtered through a 0.2µm membrane filter, and stored inside a glove box to prevent Fe\(^{2+}\) oxidation. Nitrite reduction by magnetite preconditioned with added Fe\(^{2+}\)\(_{(aq)}\) was investigated under similar conditions as described above (10 gL\(^{-1}\) magnetite, pH 5.5-7.5). Approximately 0.1mM anoxic Fe\(^{2+}\)\(_{(aq)}\) solution was added to the magnetite slurry and equilibrated for 12 hr before reacting with NO\(_2\)^- to study surface Fe\(^{2+}\) dependence on the reaction. The sorption of Fe\(^{2+}\) on magnetite-alone slurries was studied under comparable conditions at various pH values (pH 4.0-10.5).

2.1.4 Analyses and characterization of products.

The ferrozine method was used to quantify dissolved Fe\(^{2+}\) (Stookey, 1970) in reacted filtrates at 562 nm wavelength using an ultraviolet-visible-near-infrared (UV-VIS-NIR) scanning spectrophotometer (Shimadzu, UV-3101 PC, Columbia, MD). Nitrite
and nitrate were measured using a Model 819 ion chromatograph (IC) (Metrohm, Switzerland) equipped with an anion exchange column with retention times 12.7 and 20 min, respectively. Following the manufacturer’s recommendation, interference due to Fe\(^{2+}\) oxidation in the column was minimized by rinsing the column with 1-2% methanol each time a sample was injected. Additionally, a 20 mM oxalic acid solution was used to clean oxidized Fe\(^{2+}\) in the column every 4-5 samples. Ammonium was measured using the indophenol-blue method and interferences expected from the presence of dissolved Fe\(^{2+}\) were corrected by adding EDTA (as Fe chelator) (Ngo et al., 1982).

Nitric oxide (NO) was measured using a real time ISP-NO micro-sensor (World Precision Instruments Inc, Sarasota, FL) in a batch reactor at 25\(^{\circ}\)C following the procedure in Zhang et al. (2000). An isothermal water bath was used to maintain constant temperature throughout the experimental period before and after spiking the solution with NO\(^{2-}\). Electrical impulses were recorded as volts for real time NO emission and converted to concentration (nM scale) using LabScribe2® software (iWorx Systems, Inc. NH).

Evolution of N\(_2\)O was measured with increasing time intervals by analyzing headspace gas using a \(^{63}\)Ni source Electron Capture Detector Gas Chromatograph (ECD-GC-8A, Shimadzu, Columbia, MD). Samples were prepared at pH 5.5, 6.5, and 7.5 to investigate the pH dependence of the reaction. Henry’s constant for N\(_2\)O was used to convert gas volume into concentration.

Solid phase reaction products from the reaction of magnetite and NO\(^{2-}\) were characterized using XRD, SEM-EDX, and \(^{57}\)Fe Mössbauer Spectroscopy (see below).

\(^{57}\)Fe Mössbauer Spectroscopy. Magnetite in the presence and absence of 0.5mM NO\(^{2-}\) at pH 5.5 was studied using \(^{57}\)Fe Mössbauer spectroscopy. To minimize oxidation by O\(_2\), reacted samples were dried inside an anoxic chamber and prepared for analysis using a standard protocol (Kamali-M et al., 2006). Transmission (\(^{57}\)Fe) Mössbauer spectroscopy was performed at room temperature and spectra were collected to estimate stoichiometry (\(x_{MS}\)). This was compared with the wet chemical method for stoichiometry (\(x_{diss}\)) whereby 5 M HCl was added to the reacted and unreacted magnetite slurries in the glovebox to effect complete dissolution as described by Tamura et al. (1974).
2.2 Results and discussion

2.2.1 Reduction of NO$_3^-$ / NO$_2^-$ by magnetite

Nitrite was more rapidly removed from solution than NO$_3^-$ by magnetite at pH 5.5 (Fig. 2.6A, B). Initial rates of removal were $1.75 \pm 0.04 \times 10^{-11}$ and $8.14 \pm 0.4 \times 10^{-10}$ M s$^{-1}$ for NO$_3^-$ and NO$_2^-$, respectively (Table 2.1). The lack of microbial DNA in GE bands in magnetite slurries prior to NO$_3^-$ or NO$_2^-$ addition indicates that our reactions were strictly abiotic (Fig. 2.1). The kinetics of nitrite removal from solution at pH 5.5 reasonably agreed with a first order model at early reaction times (0-72 hr), with a best fit pseudo-first order rate coefficient of $1.2 \pm 0.1 \times 10^{-3}$ hr$^{-1}$, and there were slight deviations thereafter (Fig. 2.6B and Table 2.1). In the control samples (Fe$_3$O$_4$ free), no significant NO$_2^-$ loss occurred at pH 5.5 during the same time frame. This agrees with previous studies in which self decomposition of NO$_2^-$ is negligible at pH >5.0 (Van Cleemput and Baert, 1984).

The greater removal rate of NO$_2^-$ by magnetite when compared with NO$_3^-$ at pH 5.5 can be rationalized on the basis of the shared charge concept and steric considerations assuming that sorption is the mechanism of removal from solution. The shared charge of nitrate (calculated by dividing the valence of the N atom by the number of O atoms) is 1.67 whereas that of nitrite is 1.50 (McBride, 1994). The lower the shared charge, the greater the effective negative charge residing on each O atom, allowing NO$_2^-$ to bind more strongly to mineral surface sites on Fe$_3$O$_4$ than NO$_3^-$. The shared charge concept qualitatively describes sorption behavior of other oxyanions on Fe oxide mineral surfaces such as phosphate (McBride, 1994). Steric considerations might also play a role because the angular molecular shape of NO$_2^-$ (Shriver et al., 1994) could allow it to approach reactive surface sites on Fe$_3$O$_4$ more effectively than the trigonal-planar geometry of NO$_3^-$. 

2.2.2 NO and N$_2$O emission

It is possible that the NO$_2^-$ removed from solution, presumably by sorption, further engaged in electron transfer reactions because the NO$_2^-$/N$_2$O and NO$_2^-$/NO couples lie above the Fe$_3$O$_4$/Fe$^{2+}$ redox couple (Fig. 1.3). It was observed that immediately after NO$_2^-$ addition to the magnetite slurry at pH 5.5, NO appeared (Fig.
2.7A). The concentration of NO reached a plateau of ~42 nM after 1 hour followed by a decrease to below detection. Experiments carried out with NO$_3^-$ did not show detectable NO concentrations. A control sample performed at pH 5.5 in the absence of solid magnetite that only contained NO$_2^-$ did not show detectable concentration of dissolved NO ruling out self decomposition of NO$_2^-$ to form NO. At longer time scales, N$_2$O is generated as a product of NO$_2^-$ reduction (Fig. 2.7B). The N$_2$O emission was detected after 6hr and reached a plateau after 14 days. Green rust and siderite, both of which contain structural Fe$^{2+}$, were reported to reduce NO$_2^-$ to N$_2$O (Hansen et al., 1994; Rakshit et al., 2008). Nitrite treated with dissolved Fe$^{2+}$ has been shown to induce similar NO and N$_2$O emission with concomitant Fe$^{2+}$ oxidation (Kampschreur et al., 2011). In another study, when NO$_2^-$ was reacted with Fe$^{2+}$ bound to lepidocrocite, N$_2$O was evolved after 5hr (Sørensen and Thorling, 1991).

The sum of NO and N$_2$O did not account for the NO$_2^-$ removed from solution by magnetite at pH 5.5. One possible explanation for this nonstoichiometry is that NO$_2^-$ was reduced all the way to N$_2$, which was not measured in our study. We did measure NH$_4^+$ and levels were below detection (data not shown). Another possible explanation is that some of the NO produced was immediately bound on the magnetite surface because it is known to adsorb to mineral surfaces and to Fe$^{2+}$ in enzyme active sites (Mortland, 1965; Collman et al., 2008). Lastly, there might have been some NO$_2^-$ removed from solution to form a surface-bound complex to magnetite surface sites but it remained unreduced. Magnetite is known to sorb anions prior to electron transfer (Cui and Eriksen, 1996).

2.2.3 Changes in magnetite

During the removal of NO$_2^-$ from solution, aqueous Fe$^{2+}$ concentrations were consistently below the detection limit of ~0.5µM at pH 5.5 in NO$_2^-$-magnetite and magnetite-only slurries (inset Fig, 2.6B). Results from this study indicate that the reaction likely occurred as a heterogeneous process, whereby aqueous NO$_2^-$ reacted with structural Fe$^{2+}$ in magnetite. Magnetite is known to be quite insoluble (Walker, 1983; Kennedy et al., 1998). Heterogeneous redox reactions have also been observed during Cr$^{6+}$ reduction by magnetite (White and Peterson, 1996). Other research studies have shown that reduction of U$^{6+}$ led to enhanced release of Fe$^{2+}$ from magnetite to solution.
(Singer et al., 2012). This latter study was performed under continuous flow conditions rather than batch, which might explain the differences. To explore the possibility of heterogeneous electron transfer further, we followed changes in magnetite using XRD, Mössbauer spectroscopy, and SEM-EDX.

X-ray diffraction peaks at 4.84, 2.96, 2.53, 2.42, 2.10, 1.71, 1.61 Å represent the diagnostic d-spacings for magnetite. Reacted solids after exposure to NO$_2^-$ showed residual magnetite peaks and subtle increases in the intensity of maghemite based on peaks located 4.81, 2.63, 2.40, 1.702 Å (Fig. 2.2).

The Mössbauer spectra of magnetite-alone and NO$_2^-$-reacted magnetite are shown in Fig. 2.8. In magnetite, both Fe$^{2+}$ and Fe$^{3+}$ occupy the octahedral sites (B sites). Electron hopping between Fe$^{2+}$ and Fe$^{3+}$ at room temperature in these sites is fast with respect to the Mössbauer measurements and the ions appear to the technique to be equivalent and have an average iron oxidation state of 2.5 (Greenwood and Gibbs, 1971). Because these ions are also magnetically coupled, they give rise to the inner six-line pattern (sextet). Area measured for inner six-line pattern is denoted as $\text{octFe}^{2.5+}$. A second outer sextet is present arising from Fe$^{3+}$ in type A (tetrahedral) sites and is written as $\text{tetFe}^{3+}$. The relative peak areas after fitting the Mössbauer spectrum of the magnetite-alone sample indicate that the Fe$^{2+}$/Fe$^{3+}$ ratio, $x_{\text{MS}}$, was 0.47 (Fig. 2.8, Table 2.2). After reaction with NO$_2^-$, $x_{\text{MS}}$ decreased to 0.46. The decrease in magnetite stoichiometry from 0.47 to 0.46 for the unreacted and NO$_2^-$ treated sample based on Mössbauer results is within the error of the Mössbauer spectroscopic fitting technique. However, the wet chemical data ($x_{\text{diss}}$ in Table 2.2) also show a decrease in magnetite stoichiometry which provides strong evidence of structural Fe$^{2+}$ oxidation in the NO$_2^-$ treated sample (Appendix A). Theoretical calculations shown in Appendix A demonstrate NO$_2^-$ reduction via structural Fe$^{2+}$ would progressively yield a less stoichiometric magnetite. These findings agree with a recent study, which noted that structural Fe$^{2+}$ in Fe$_3$O$_4$ can serve as a reductant of NO$_2^-$ (Sun et al., 2012), however, the results from this latter study are not directly comparable because palladium was attached to the magnetite mineral as a catalyst.
Structural Fe\(^{2+}\) in magnetite has been reported to reduce U\(^{6+}\) to U\(^{4+}\) and Hg\(^{2+}\) to Hg\(^0\) based on decreases in Fe\(^{2+}\)/Fe\(^{3+}\) ratios determined using Mössbauer spectroscopy (Wiatrowski et al., 2009; Das et al., 2010; Latta et al., 2011). Some of the oxidized Fe\(^{2+}\) might have been utilized to form a trace amount of maghemite, as this reaction has been reported to occur, (Sidhu et al., 1977; Jolivet and Tronc, 1988) and we did observe a slight increase in diagnostic XRD peaks as noted above. Production of maghemite might serve to passivate the magnetite surface and prevent further reduction of NO\(_2^-\), which reaches steady-state levels after 168 h (Fig. 2.6B). Chromate (Cr\(^{6+}\)) reduction by magnetite was initially rapid followed by a halting of the process due to passivation (Peterson et al., 1997). This limited the capacity of the magnetite to reduce Cr\(^{6+}\) and a similar phenomenon might be operative in our studies.

### 2.2.4 Effect of pH on NO\(_2^-\) reduction

There was pH-dependence in the removal of NO\(_2^-\) from solution by magnetite; the initial rate was two times faster at pH 5.5 than at pH 7.5 (Fig. 2.6B). This enhancement of the reaction rate at lower pH can be attributed to the chemical speciation of magnetite surface sites and NO\(_2^-\). The pH dependence of magnetite surface speciation is related to the following equilibria:

\[
\equiv S-OH_2^+ \rightarrow \equiv S-OH^- + H^+ \quad pK_{a1} = 4.4 \quad (1)
\]

\[
\equiv S-OH^- \rightarrow \equiv S-O^- + H^+ \quad pK_{a2} = 9.0 \quad (2)
\]

where \(\equiv S-OH_2^+\), \(\equiv S-OH^-\), and \(\equiv S-O^-\) represent the fully protonated, neutral, and deprotonated surface sites, and \(pK_{a1}\) and \(pK_{a2}\) are the intrinsic acidity constants determined by Regazzoni et al. (1983) for magnetite. The surface speciation of magnetite shows that positively charged sites become more important as pH is lowered (Fig. 2.9A).

The NO\(_2^-\) anion can protonate at lower pH values to form HNO\(_2\) (nitrous acid) with a dissociation constant \((K_a)\) value of 6.9x10\(^{-4}\), corresponding to a \(pK_a\) value of 3.16 (da Silva et al., 2006). Both HNO\(_2\) and NO\(_2^-\) merit consideration in explaining the pH dependence in total NO\(_2^-\) removal from solution. Using the approach by Yao and Millero (1993), we calculated possible surface complexes that may form assuming precursor surface complex formation is the rate-limiting step. The product of \([\equiv S-OH_2^+]\) and [NO\(_2^-\)]
([≡S-OH\textsuperscript{2-}][\text{NO}_2\textsuperscript{-}]) becomes more important as pH decreases (Fig. 2.9B). This trend mimics the greater removal rate of total \text{NO}_2\textsuperscript{-} at lower pH (Fig. 2.6B and Table 2.1) and indicates that the anionic \text{NO}_2\textsuperscript{-} ion is attracted to positively charged sites on magnetite. Another important complex is the binding of \text{NO}_2\textsuperscript{-} to the ≡S-OH\textsuperscript{o} sites ([≡S-OH\textsuperscript{o}][\text{NO}_2\textsuperscript{-}]) (Fig. 2.9C). This might occur if ligand exchange is the operative mechanism of \text{NO}_2\textsuperscript{-} removal from solution whereby the surface hydroxyl group is replaced by \text{NO}_2\textsuperscript{-}. Ligand exchange of anions is favored by a decrease in pH because \text{OH}\textsuperscript{-} is released and appears on the product side (McBride, 1994). The neutral surface site on magnetite was implicated in a ligand exchange complexation reaction with the anionic pertechnetate (\text{TcO}_4\textsuperscript{-}) ion (Cui and Eriksen, 1996). The [≡S-OH\textsuperscript{2+}][\text{HNO}_2\textsuperscript{-}] complex plays a role at more acidic pH values than those employed in our study (pH<5) and the negatively charged surface sites ([≡S-O\textsuperscript{-}]) interacting with \text{NO}_2\textsuperscript{-} become important only at higher pH values (Fig. 2.9D), thus these can be ruled out as possible precursor surface complexes. The exact nature of the reactive surface species between nitrite and magnetite awaits spectroscopic confirmation.

**2.2.5 Significance of surface bound \text{Fe}^{2+} in \text{NO}_2\textsuperscript{-} reduction**

Past studies have noted that addition of dissolved \text{Fe}^{2+} to magnetite, which has been partially oxidized, can recharge the structural \text{Fe}^{2+} within the mineral and enhance reactivity (Gregory et al., 2004; Gorski and Scherer, 2009). Added \text{Fe}^{2+} removed \text{NO}_2\textsuperscript{-} from solution more rapidly than magnetite-alone at a given pH value. For example, surface \text{Fe}^{2+} on magnetite accelerated the rates of \text{NO}_2\textsuperscript{-} removal by three-fold over magnetite-alone at pH 5.5 (Fig. 2.6B,C). To account for the possible homogeneous reaction between dissolved \text{Fe}^{2+} and dissolved \text{NO}_2\textsuperscript{-}, separate controls were run (0.1 mM \text{Fe}^{2+} and 0.5 mM \text{NO}_2\textsuperscript{-}) without magnetite under comparable conditions. The data indicate that there were no changes in \text{NO}_2\textsuperscript{-} concentration (open circles in Fig. 2.6C) nor dissolved \text{Fe}^{2+} (open symbols, Fig. 2.10), thus, indicating that the homogeneous reduction of \text{NO}_2\textsuperscript{-} was not operative under our experimental conditions. Our observation runs parallel to a previous study in which \text{NO}_2\textsuperscript{-} reduction and \text{Fe}^{2+} oxidation were insignificant in the absence of solid hydrous ferric oxide (Tai and Dempsey, 2009).
The rate of NO$_2^-$ loss from solution where Fe$^{2+}$ was added to magnetite increased with a decrease in pH (Fig. 2.6C). The uptake of Fe$^{2+}$(aq) by magnetite is strongly pH dependent (Fig. 2.11A) and the literature indicates that reduction rates of other oxidants increase positively with an increase in surface-bound Fe$^{2+}$ (Klausen et al., 1995; Gregory et al., 2004). In our study, however, we found that NO$_2^-$ reduction rates decreased with an increase in surface-bound Fe$^{2+}$ as pH was raised (Fig. 2.11B). The slope value of the log $k_{obs}$ versus log [Fe$^{2+}]_{ads}$ plot was -2.2, suggesting an inverse dependence of NO$_2^-$ removal on surface Fe$^{2+}$. It is difficult to assign a definite cause for this behavior, particularly in light of the contrasting removal rates of dissolved Fe$^{2+}$ with pH in the presence of nitrite (closed symbols, Fig. 2.10). To tease out the role of pH and surface Fe$^{2+}$ loading further, additional experiments are needed by varying the surface loading at a constant pH as described elsewhere (Gregory et al., 2004).

2.3 Implications

This study suggests that magnetite-bearing soils and sediments can catalyze abiotic NO$_2^-$ reduction with negligible reaction towards NO$_3^-$ . One might envision a scenario in natural environments under Fe$^{3+}$-reducing conditions where the first step of NO$_3^-$ reduction to NO$_2^-$ could be biologically mediated. Secondary reactions involving NO$_2^-$ and magnetite are possible and would lead to some NO and N$_2$O production (Fig. 2.12). Back-of–the envelope calculations based on initial NO$_2^-$ removal rates observed in this study (Table 2.1) indicate abiotic NO$_2^-$ reduction mediated by magnetite is comparable or even more important to that of microbial nitrite reduction catalyzed by nitrite reductase in anoxic soil and sediments (assuming 2% by weight magnetite) (Appendix B). Abiotic magnetite-induced NO$_2^-$ removal becomes increasingly important when dissolved Fe$^{2+}$ is present, as the rate increases to nearly 12 fold the capacity of NO$_2^-$ reducers.

Another possible environment in which this process may be observed is where permeable reactive barriers containing metallic iron (Fe$^0$) are utilized to treat groundwater containing co-contaminants such as NO$_3^-$ (Blowes et al., 2000). Magnetite is a commonly identified secondary corrosion product, (Lee and Wilkin, 2010) and could potentially engage in electron transfer reactions if NO$_2^-$ appears as an intermediate.
Interestingly, NO$_3^-$ reduction exhibits complex kinetic behavior with Fe$^0$, green rusts, and FeO$_{(s)}$ in lab studies, (Hansen and Koch, 1998; Alowitz and Scherer, 2002; Rakshit et al., 2005) perhaps due in part to secondary reactions involving Fe$_3$O$_4$ and NO$_2^-$. 
Table 2.1. Initial rate and $k_{obs}$ measured for initial 0.5 mM of NO$_2^-$ (and NO$_3^-$) removal from solution by magnetite with or without added Fe$^{2+}$ at various pH.

<table>
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<th>pH</th>
<th>Treatment</th>
<th>Ini. Rate (Ms$^{-1}$)</th>
<th>$k_{obs}$ (hr$^{-1}$)</th>
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<td>5.5</td>
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<td>1.2±0.14×10$^{-3}$</td>
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<td></td>
<td>Fe(II)-Magnetite</td>
<td>2.60±0.13×10$^{-9}$</td>
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<td>Fe(II)-Magnetite</td>
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<td>2.6±0.17×10$^{-3}$</td>
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<tr>
<td>7.5</td>
<td>Magnetite</td>
<td>4.05±0.16×10$^{-10}$</td>
<td>6.0±0.06×10$^{-4}$</td>
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<tr>
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<td>1.2±0.21×10$^{-5}$</td>
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</table>

<table>
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<tr>
<th>pH</th>
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<th>Ini. Rate (Ms$^{-1}$)</th>
<th>$k_{obs}$ (hr$^{-1}$)</th>
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<tbody>
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</tbody>
</table>
Table 2.2. Fitted hyperfine parameters from Mössbauer spectra at 293K for magnetite samples, before and after reaction with NO$_2^-$ as shown in Fig. 2.8. Stoichiometry of untreated magnetite and NO$_2^-$-reacted magnetite were also analyzed by the dissolution ($x_{\text{diss}}$) method (Tamura et al., 1974).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tet Fe$^{3+}$ (A-Parameter)</th>
<th>Oct Fe$^{2.5+}$ (B-Parameter)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IS</td>
<td>QS</td>
</tr>
<tr>
<td></td>
<td>mm s$^{-1}$</td>
<td>mm s$^{-1}$</td>
</tr>
<tr>
<td>Untreated Magnetite</td>
<td>0.279</td>
<td>0.002</td>
</tr>
<tr>
<td>NO$_2^-$ Treated Magnetite</td>
<td>0.282</td>
<td>0.002</td>
</tr>
</tbody>
</table>

IS = Isomer shift  
QS = Quadrupole splitting  
$H_o$ = Hyperfine field in kGauss

Fe(%)= Relative spectral area

*Magnetite Stoichiometry by Mössbauer, $x_{MS} = \frac{1}{2} \frac{(OctFe^{2.5+})}{(TetFe^{3+})} = 0.47$

**Magnetite Stoichiometry by dissolution, $x_{diss} = 0.45$
Figure 2.1 Gel electrophoresis (GE) results showing no microbial 16S rDNA bands from synthetic magnetite and goethite minerals used in this study. Samples were analyzed directly from the container as it was used for batch experiments. Results suggest that the mineral(s) is free of any microbes or they are present below detection.
Figure 2.2 Plot of X-ray diffraction pattern observed from untreated magnetite and those reacted with NO$_2^-$ at pH 5.5 and 7.5. Major peak lines for a reference magnetite (Fe$_3$O$_4$), hematite (Fe$_2$O$_3$), maghemite ($\gamma$-Fe$_2$O$_3$) are shown as solid lines at bottom. All peaks correspond to magnetite. Peaks marked by green arrows (4.81, 2.63, 2.40, 1.702 Å) observed in the residual magnetite samples treated with NO$_2^-$ matched with reference maghemite pattern.
Figure 2.3 Characterization of untreated and treated magnetite by SEM. (A) Untreated magnetite showing porous aggregate structure. (B) Average size of the untreated magnetite particles. (C) Samples treated with NO$_2^-$ at pH 5.5 for 14 days. (D) Energy dispersive spectroscopy data of untreated magnetite.
Figure 2.4 The effect of ionic strength on the $\zeta$-potential, and electrophoretic mobility of untreated magnetite. Measured pH$_{\text{IEP}}$ of magnetite is 6.05±0.05, when solid-solution was 10 gL$^{-1}$.

Figure 2.5 The effect of MES buffer concentration on dissolved Fe$^{2+}$ release from magnetite compared with buffer-free samples (open circles) with a magnetite concentration of 10 gL$^{-1}$ at pH 5.5. Error bars represent the standard deviation from the mean for duplicate runs.
Figure 2.6 Removal of (A) NO$_3^-$ (at pH 5.5), and (B) NO$_2^-$ from solution by magnetite at pH 5.5 (●), 6.5 (▲), and 7.5 (◇) (B) without and (C) with added Fe$^{2+}$. Initial NO$_3^-$/NO$_2^-$ and magnetite concentration were ~0.5 mM and 10 gL$^{-1}$ respectively. Open circle in (B) represents blank (magnetite free) samples at pH 5.5 whereas open circles in (C) represent 0.1 mM Fe$^{2+}$ and 0.5 mM NO$_2^-$ controls. Symbols indicate experimentally determined data, and solid lines represent the kinetic model fit. Error bars represent the standard derivation from the mean of duplicate analysis. Inset figure in (B) shows dissolved Fe$^{2+}$ measured in the samples.
Figure 2.7 Nitric oxide (A) and nitrous oxide (B) emission recorded in the experiment when 0.5 mM NO$_2^-$ was reacted with magnetite at pH 5.5. Open circle indicate the control samples without magnetite and open triangle represents NO emission from sample treated with NO$_3^-$. Error bars represent the standard deviation from the mean for duplicate runs.
Figure 2.8 Room temperature Mössbauer spectrum of (A) untreated magnetite, and (B) magnetite treated with 0.5 mM NO$_2^-$ alone for 14 days at pH 5.5. Open circle and solid lines represents observed values and total fit respectively. Fitting parameters are tabulated in Table 2.2.
Figure 2.9 (A) The predicted surface speciation of magnetite as a function of pH:

\[
\begin{align*}
\text{(---) } & \equiv \text{S-OH}_2^+, \quad \text{(— ▲ —) } \equiv \text{S-OH}^0, \quad \text{(— ▼ —) } \equiv \text{S-O}^- \\
\text{using a total iron concentration (10gL}^{-1}\text{) of 43mM and experimentally derived pK}_{a1}(=4.4) \text{ and pK}_{a2}(=9.0) \text{ values (Regazzoni et al., 1983). Possible precursor surface complexes calculated in terms of reactant concentrations as a function of pH for (B) } & \equiv \text{S-OH}_2^+][\text{HNO}_2] \\
& \text{and } \equiv \text{S-OH}_2^+][\text{NO}_2^-, (C) } \equiv \text{S-OH}^0][\text{HNO}_2] \text{ and } \equiv \text{S-OH}^0][\text{NO}_2^-, \text{ and (D) } \equiv \text{S-O}^-][\text{HNO}_2] \text{ and } \equiv \text{S-O}^-][\text{NO}_2^-.} \\
\text{The } \text{HNO}_2 \text{ and } \text{NO}_2^- \text{ species distribution for various pH is derived by using } & K_a=6.9 \times 10^{-4} \text{ (pKa=3.16 at 25°C) (da Silva et al., 2006) and a total } \text{NO}_2^- \text{ concentration of 0.5 mM.}
\end{align*}
\]
Figure 2.10 Dissolved Fe$^{2+}_{(aq)}$ concentrations in the presence of 0.5 mM NO$_2^-$ as a function of pH with and without magnetite present. Control samples without magnetite were shown with open symbols. Error bars represent standard deviation calculated from replicate samples.
Figure 2.11 (A) Adsorption of 0.1 mM Fe$^{2+}$ on 10 g L$^{-1}$ magnetite measured after 1 hr equilibrium time and (B) apparent reaction order plot ($k_{obs}$) for reduction of NO$_2^-$ as a function of pH. Linear regression of the data sets is represented by a line for varying pH. In the top figure, symbols indicate experimental points.
Figure 2.12 Scheme illustrating possible redox reactions mediated by electron transfer from structural Fe$^{2+}$ in magnetite-NO$_2^-$ system. Electron shuttling from structural Fe$^{2+}$ in magnetite possibly stimulate NO$_2^-$ reduction to N$_2$O that can concomitantly decrease stoichiometry of the reacted magnetite by successively lowering of Fe$^{2+}$/Fe$^{3+}$ ratio. The nitrogen species are drawn as Lewis structures taken from Luther (2010). Solid arrow indicates possible reaction product/pathways that produce N-intermediates.
Chapter Three: Reaction of Nitrite with Goethite and Surface Fe$^{2+}$-Goethite Complex

3. Introduction

The microbial reduction of solid iron oxide minerals such as goethite is an important biogeochemical process in soil, groundwater, and sedimentary environments that influences cycling of nutrients and metal contamination. Goethite (α-FeOOH) has been identified in many natural environments such as soils and sediments (Cornell and Schwertmann, 2003) due to its high thermodynamic stability (Majzlan et al., 2003), and accounts for yellowish colors of many soils (Schwertmann and Cornell, 2000; Huang et al., 2012). Goethite is formed via abiotic secondary phase transformation when poorly crystalline ferrihydrite, Fe(OH)$_3$ is aged in the presence of Fe$^{2+}$(aq) and certain ligand such as chloride, sulfate and carbonate (Hansel et al., 2005), however, in many natural environments it is formed via coupled biotic-abiotic pathways when Fe$^{2+}$ produced by dissimilatory iron reducing bacteria (DIRB) oxidizes (Fredrickson et al., 1998; Zachara et al., 2002; Hansel et al., 2003). Goethite has been studied extensively as a model iron oxide mineral in understanding sorption of inorganic anions such as phosphate (Kim et al., 2011), sulfate (Peak et al., 1999), arsenate (Dixit and Hering, 2006; Catalano et al., 2011), chromate (Grossl et al., 1997; Kim et al., 2007), and pertechnetate (Um et al., 2011). It has also been used as the mineral for organic contaminant remediation (via reduction) studies in which target reactant is carbon tetrachloride (CCl$_4$) (Amonette et al., 2000), polyhalogenated methane (Pecher et al., 2002), nitrobenzene (Williams and Scherer, 2004), and 2-nitrophenol (Tao et al., 2010).

Due to extensive use of N fertilizer in modern agro-ecosystems, excess nitrate (NO$_3^-$) is often leached to deeper soil layers and nearby water bodies causing a decline in water quality (Spalding and Exner, 1993; Goolsby et al., 2001; Fields, 2004; Ahrens et al., 2008). Where all of the O$_2$ has been exhausted, NO$_3^-$ is the next most favorable electron acceptor (Table 1.1). Nitrate undergoes sequential biological denitrification process where it is first reduced to nitrite (NO$_2^-$), which further reduces to nitric oxide (NO), nitrous oxide (N$_2$O) and finally to dinitrogen (N$_2$) (Tiedje, 1994; Canfield et al., 2010). Notably, nitrite is often observed in water and soil-water systems as a toxic anion.
that occurs as an intermediate in microbial nitrification (Wrage et al., 2001) and
denitrification (Tiedje, 1994) processes. Human health problems linked to an increase in
nitrate and nitrite loading in water resources (Fan and Steinberg, 1996; Fewtrell, 2004)
from anthropogenic fertilizer use worldwide (Spalding and Exner, 1993; Goolsby et al.,
2001; Fields, 2004; Ahrens et al., 2008) has therefore stimulated research in
understanding processes that lead to NO$_3^-$/NO$_2^-$ removal.

Historically, it is assumed that biogeochemistry of N-transformation is essentially
mediated by heterotrophic and autotrophic denitrifiers (Korom, 1992; Tiedje, 1994), and
little attention was given to N-cycle mediated by other elements. In recent studies,
simultaneous reduction of NO$_3^-$ and Fe$^{3+}$ measured in laboratory incubations of soil
slurries (Yasuhiko et al., 1978) and pure cultures (Weber et al., 2001; Cooper et al., 2003)
have indicated strong coupling between Fe and N cycle. Further, strains of lithotrophic
microorganisms isolated from natural sediments can acquire energy by oxidizing reduced
Fe$^{2+}$ concurrent with NO$_3^-$ reduction (Straub et al., 1996; Weber et al., 2006c; Konhauser
et al., 2011). This process is now widely known as NO$_3^-$-dependent Fe$^{2+}$-oxidation. It
contains a biological and an abiological component. An example of the abiotic process is
demonstrated by the rapid reduction of NO$_3^-$ to NO$_2^-$ by mineral Fe$^{2+}$ forms usually found
in Fe$^{3+}$-reducing environments such as green rust (Hansen et al., 1996) and wüstitite
(Rakshit et al., 2005). In such systems, a second possible chemical reaction is the
production of NO$_2^-$ via biological reduction of NO$_3^-$ which can undergo further (abiotic)
reduction catalyzed by mineral Fe$^{2+}$ (Rakshit et al., 2008; Tai and Dempsey, 2009;
Dhakal et al., 2013). Several studies have hypothesized this phenomenon in agricultural
soil slurries (Yasuhiko et al., 1978), activated sludge amendment (Nielsen and Nielsen,
1998) and pure cultures (Obuekwe et al., 1981; Cooper et al., 2003).

Biogenic Fe$^{2+}_{(aq)}$ produced by DIRB commonly exists in sorbed or precipitated
form (Fredrickson et al., 1998; Zachara et al., 2002). There is evidence in the literature
that structural and surface Fe$^{2+}$ is more reactive towards NO$_2^-$. In an experiment
conducted to study NO$_2^-$ reactivity with lepidocrocite ($\gamma$-FeOOH), Sørensen and Thorling
(1991) found that surface bound Fe$^{2+}$ on lepidocrocite reduced NO$_2^-$ at a faster rate than
aqueous Fe$^{2+}$ (Sørensen and Thorling, 1991). In another study, Tai and Dempsey (2009)
reported that NO$_2^-$ reduction by Fe$^{2+}$ sorbed to hydrous ferric oxide (HFO) is roughly 4-
fold greater than that of surface bound Fe$^{2+}$ on lepidocrocite (Tai and Dempsey, 2009). Similarly, Coby and Picardal (2005) observed reduction of NO$_2^-$ and N$_2$O by Fe$^{2+}$ sorbed on goethite in microbial cell, however, it is inconclusive if the reaction is entirely of abiotic origin (Coby and Picardal, 2005). In a recent study, we reported that NO$_2^-$ removal from solution by magnetite was accelerated in the presence of surface Fe$^{2+}$-treated magnetite (Dhakal et al., 2013). Goethite formation occurs during NO$_3^-$-dependent Fe$^{2+}$-oxidation (Senko et al., 2005; Weber et al., 2006a; Larese-Casanova et al., 2010), and NO$_2^-$ appears as an intermediate product. It is possible that NO$_2^-$ could react with goethite and surface-bound Fe$^{2+}$ on goethite, however, there is very little information in this regard. Notably, thermodynamic calculations predict that under anaerobic conditions NO$_2^-$ reduction to NO and N$_2$O would be favorable over a wide range of environmentally relevant pH levels (Fig. 1.3). Additionally, studies have shown that reduction potential ($E_h^0$) of surface Fe$^{2+}$ on goethite is lowered well below $\alpha$-FeOOH/Fe$^{2+}$ redox couple making Fe$^{2+}$-goethite complex far more effective reductant than dissolved, hexaaqua Fe(H$_2$O)$_6^{2+}$ (Wehrli, 1990; Strathmann and Stone, 2003).

In this study, we conducted laboratory stirred-batch experiments to investigate NO$_2^-$ reactivity with goethite at various pH values (5.5-7.5) under anaerobic conditions and evaluated the impact of added Fe$^{2+}$ (at pH 5.5). Here, we present kinetic data on reactivity of NO$_2^-$ with goethite and Fe$^{2+}$-goethite complex. These results of abiotic NO$_2^-$ removal by goethite and Fe$^{2+}$-goethite have implications for the possible role of chemodenitrification in water saturated soil and groundwater with elevated NO$_2^-$.

3.1 Materials and methods

3.1.1 Chemicals and minerals: Chemicals used in this study were analytical grade. All the reactions were performed at room temperature inside an anoxic glove box (Coy Laboratory Products, Grass Lake, MI) purged with 95% argon (Ar) and 5% hydrogen (H$_2$) mixture, and equipped with palladium (Pd) catalyst to remove trace oxygen (O$_2$). To eliminate O$_2$ interference, all aqueous solutions were prepared using doubly deionized water (18.3MOhm cm$^{-1}$) that had been previously purged with ultra pure Ar for 3 hr and was stored in the glove box. To ensure anoxic conditions, dissolved oxygen (O$_2$) in the solution was measured using a 782 oxygen meter (Strathkelvin Instruments, Scotland,
Before use, solid goethite minerals, all glass vials, reaction vessel, and other experimental accessories were equilibrated for a few days inside the glove box to reduce sorbed oxygen. Reaction vessels used in this study were wrapped with aluminum foil to prevent light exposure and minimize photoredox dissolution of goethite (Waite and Morel, 1984; Waite, 1987). To isolate only abiotic reaction paths, a fraction of goethite solid samples prepared for this study were analyzed for microbial DNA. Results obtained from gel electrophoresis (GE) profile pattern obtained from 16S rDNA fragments indicate that the minerals were devoid of any microbes or below the detection limit (Fig. 2.1).

Nitrite stock solution was prepared from certified ACS-grade sodium nitrite salt (NaNO₂) inside the glove box using deoxygenated doubly deionized water (DDIW). Separate batches of 25 mM buffered solutions were prepared with [2-(N-morpholino) ethane sulfonic acid] (MES), and (1,4-piperazine diethane sulfonic acid) (PIPES) to buffer the pH of the experimental solution at 5.5, 6.5, and 7.5, respectively. Ionic strength of the buffer solution was maintained by using NaCl at 0.001 M for a total ionic strength of 0.0251 M. The Fe²⁺ stock solution was prepared from zerovalent iron (Fe⁰) mixed with 0.1 M HCl stock solutions and shaken over end-to-end rotator for 24 hr inside the anaerobic glove box. After isolating residual Fe⁰ using magnet, the suspension was filtered through 0.22µm membrane filter and stored inside the glove box to prevent Fe²⁺ oxidation.

3.1.2 Goethite synthesis and characterization: Goethite (α-FeOOH) used in this study was synthesized in the laboratory using a method proposed by (Schwertmann and Cornell, 2000). Freshly prepared ferric chloride, FeCl₃·6H₂O (1 M) suspension was rapidly titrated with KOH (5 M) until the pH reached ~12. The suspension was then heated at 70°C for 60 hr for aging, centrifuged and transported into 7 Spectra/Por® dialysis membranes (Spectrum Laboratories, Inc. USA). Sample contained inside the dialysis membrane was washed several times for several days with doubly deionized water (18.3MOhm cm⁻¹) until the pH and conductivity of the solution containing goethite crystals matched that of deionized water. Later, goethite crystals were freeze dried and stored in a vacuum desiccator. The specific surface area of goethite synthesized for this study as determined by Brunauer-Emmett-Teller (BET) method on a Tristar 3000
Micrometrics surface area analyzer (Micrometrics Instruments Corp., GA, USA) with N$_2$
adsorption was 51.43±0.03 m$^2$/g, which is occasionally abbreviated in the text as GT$_{51}$.
Both control and reacted goethite samples have been characterized by x-ray diffraction
(XRD), and scanning electron microscopy energy dispersive spectroscopy (SEM-EDX).

Solids were characterized before and after reacting with nitrite (NO$_2^-$) without and
with added Fe$^{2+}$. Solid samples were dried inside the glove box to maintain anaerobic
conditions after mounting on a glass slide for XRD analysis. XRD samples were prepared
by adding a drop or two of Ar-degassed glycerin over the filtered solid mounted over a
glass slide to maintain anoxic environment and prevent Fe$^{2+}$ oxidation. XRD scans were
taken from 10 to 70° 2θ with CuKα radiation at 40 kV and 30 mA using Siemens D500
Diffractometer (Bruker AXS, Madison, WI). Data were analyzed using the software
Match!® (ver 1.11, Crystal Impact, Bonn, Germany).

For SEM analysis, samples were mounted on a carbon tape attached to an
aluminum holder and coated with Au/Pd. A Hitachi (Tokyo, Japan) S-3200 SEM was
used to image these samples for various magnifications at a working distance of 30mm.
Energy dispersive x-ray spectroscopy (EDX) analysis of the goethite was also carried out
with an EDX module attached to S-3200.

X-ray diffraction scans revealed peaks at 4.98, 4.19, 3.38, 2.69, 2.46, 2.25, 2.19,
1.72 and 1.45Å representing diagnostic d-spacing for unreacted synthetic goethite (Fig.
3.1). Scanning electron microscopy (SEM) with energy dispersive x-ray spectroscopy
(EDX) showed that the sample consisted of well-formed acicular goethite rods with an
average length, width, and particle size of 1.46±0.36, 0.14±0.04, and 0.79±0.23µm
respectively, and a chemical composition of Fe and O (Table 3.1, and Fig. 3.1). The
estimated pH at the point of salt effect (PZSE) of goethite suspension was 8.45±0.1 as
determined by acid-base titration method (Fig. 3.2).

3.1.3 Nitrite reaction with goethite experiment: Stirred batch kinetic reactions were
conducted in duplicate glass serum vials (30 mL) that contained ~2 g L$^{-1}$ (100 m$^2$ surface
area/L) of prehydrated goethite over a range of pH values (pH 5.5, 6.5, and 7.5) at room
temperature. A Teflon coated magnetic stirrer was inserted into the reactor. To initiate the
reaction, stock NO$_2^-$ was added to the reactor to reach an initial NO$_2^-$ concentration of
~0.1 mM then the reactor was temporarily sealed with a silicon stopper and aluminum crimp seals (Wheaton, Millville, NJ) and placed over a magnetic stirrer to allow complete mixing at 200 rpm. With increasing time intervals, ranging from 15 min to 7 days, aliquots were removed and filtered using 0.22 µm filter paper (Fisher Scientific, Hampton, NH). A part of the filtrate was immediately complexed with ferrozine [3-(2-pyridyl)-5,6 bis(4-phenylsulfonic acid)-1,2,4-triazine, monosodium salt] for dissolved Fe$^{2+}$ measurement (Stookey, 1970). Remaining filtrate was used for NO$_3^-$, NO$_2^-$, and NH$_4^+$ quantification. Control reactors were included with NO$_2^-$ alone. The solution pH did not deviate more than ±0.15 for the duration of the experiment.

To characterize possible gaseous product of NO$_2^-$, parallel reactors (20 mL sample volume and 10 mL headspace) were set up under comparable experimental conditions as described above. For nitrous oxide (N$_2$O) measurement, headspace was sampled periodically for 14-21 days from a permanently crimp sealed reactor vials using a 1mL glass syringe and injected into a gas chromatograph. Nitric oxide (NO) was measured using a real time ISP-NO micro-sensor (World Precision Instruments Inc, Sarasota, FL) in a reactor vial at room temperature following previously suggested method (Zhang et al., 2000).

3.1.4 Fe$^{2+}$-sorption measurements: The sorption of Fe$^{2+}$ onto goethite was measured using 20 mL glass vial under anaerobic condition as a function of pH (5.5 to 7.5) and ionic strength (1 and 10 mM NaCl). Solution pH was buffered using 25mM MES (5.5 and 6.5), and 25mM PIPES (7.5). Aliquots of stock Fe$^{2+}$ were added to achieve nominal concentration of ~1.5 mM in a glass vial containing prehydrated goethite suspension for a solid loading of ~2 g L$^{-1}$. Vials were placed over the end-over-end rotator and sampling began at predetermined time intervals starting from 5 min. Aliquots removed from the vials at each time intervals were filtered and measured for solution Fe$^{2+}$ using the ferrozine method as described previously. Fe$^{2+}$ sorption reached equilibrium in 24 hr for all pH conditions (Fig. 3.3). Consequently, experiments conducted to investigate NO$_2^-$ reaction with Fe$^{2+}$-treated goethite were equilibrated for 24 hr before spiking with NO$_2^-$.
3.1.5 Nitrite reactivity with Fe$^{2+}$- goethite complex: To study surface Fe$^{2+}$ dependence on NO$_2^-$ reaction with goethite, approximately 1.5 mM Fe$^{2+}$(aq) solution was added to the prehydrated goethite suspension and equilibrated for 24 hr before spiking with NO$_2^-$. Aliquots were removed from the reactors with increasing time interval to quantify Fe$^{2+}$, NO, and N$_2$O, and NO$_2^-$. Sorbed Fe$^{2+}$ was calculated from the difference between the initial and final Fe$^{2+}$(aq) concentrations.

3.1.6 Analysis and characterization of the reaction product: Nitrite and NO$_3^-$ with retention time 12.7 and 20 min, respectively were measured in reacted filtrate by a Model 819 ion chromatograph (IC) (Metrohm, Switzerland) equipped with an anion exchange column. The indophenol-blue method (Ngo et al., 1982) was modified by adding EDTA (as Fe chelator) to correct for interference arising from dissolved Fe$^{2+}$ to quantify ammonium (NH$_4^+$). Dissolved Fe$^{2+}$ in the reacted filtrates was quantified by the ferrozine assay method at 562 nm wavelength with an ultraviolet-visible-near-infrared (UV-VIS-NIR) scanning spectrophotometer (Shimadzu, UV-3101 PC, Columbia, MD).

For NO measurement, reactors were removed from the glove box and briefly opened to insert Ar purging to sustain solution anoxia. Before NO$_2^-$ injection, solution temperature was equilibrated at 25°C by an isothermal water bath until steady temperature was achieved and continued for the entire experiment period. Electrical impulses acquired by volt recorder for real time NO emission were converted to concentration using LabScribe2® software (iWrox Systems, Inc. NH). Nitrous oxide evolution was measured at increasing intervals by analyzing headspace gas with a $^{63}$Ni source electron capture detector gas chromatograph (ECD-GC-8A, Shimadzu, Columbia, MD). Henry’s constant for N$_2$O was used to convert gas volume into concentration.

Solid goethite samples treated with NO$_2^-$ with or without added Fe$^{2+}$, and retained after filtering the liquid solution were partly mounted on a glass slide inside an anoxic chamber pending XRD analysis. Remaining fraction of reacted goethite was dried and mounted on a carbon tape attached to an aluminum holder for SEM analysis.
3.2 Results and discussion

3.2.1 Removal of NO$_2^-$ by goethite: Nitrite removal by goethite as a function of pH (5.5-7.5) is shown in Fig. 3.4A. Initial rates of NO$_2^-$ removal were 1.06±0.2×10$^{-9}$, 2.20±0.15×10$^{-10}$, 1.25±0.05×10$^{-10}$ Ms$^{-1}$ for pH 5.5, 6.5 and 7.5, respectively (Table 3.2). Removal of NO$_2^-$ from solution at a constant goethite concentration agrees with a pseudo first order model that can be represented by single exponential decay equation.

$$A(t) = A(o)e^{-k\cdot t}$$ \[1\]

in which $A_o$ is initial (t = 0) NO$_2^-$ concentration (M), and k is rate coefficient (hr$^{-1}$, determined from initial rates). The integrated expression [1] for rate law is derived from the differential rate equation by the initial rate method (Lasaga, 1981)

$$-\frac{d[NO_2^-]}{dt} = k' [NO_2^-]^x$$ \[2\]

in which the initial rate of NO$_2^-$ disappearance (Mhr$^{-1}$) is represented by $-\frac{d[NO_2^-]}{dt}$ in expression [2], k’ is the overall rate coefficient. Because the initial concentration of goethite (22 mM) was much higher than nitrite (0.1 mM) k’ represent k[FeOOH].

Nitrite removal by goethite was pH dependent. Rate coefficient of NO$_2^-$ removal calculated using equation [1] decreased from 1.40±0.05×10$^{-1}$ to 4.80±0.15×10$^{-2}$ hr$^{-1}$ as pH increased from 5.5 to 7.5 (Table 3.2). Although there were sufficient reactive surface sites available in goethite (Appendix C) to completely remove all of the initial NO$_2^-$ (0.1 mM), only about 30% of the initial NO$_2^-$ was lost from the solution at pH 5.5. No significant NO$_2^-$ loss occurred in our control samples (goethite free) (Fig. 3.4A). This rules out self decomposition of NO$_2^-$ in our samples, which has been reported as a key NO$_2^-$ loss mechanism at pH <5.0 (Van Cleemput and Baert, 1984). Furthermore, because the reaction vessels were shielded from light exposure, and the pH of the solution was above 5.5, photo-catalytic NO$_2^-$ disappearance observed in Fe$^{3+}$-NO$_2^-$ system at low pH (Zhang and Bartlett, 2000) was minimized in our samples. Dissolved Fe$^{2+}$ in the solution extracted from the reaction vessel, for the entire experimental time span, was below detection level (data not shown). This indicates that biological iron reduction did not occur. In addition, lack of detectable microbial DNA in goethite slurry prior to NO$_2^-$
addition indicates that removal reaction was strictly abiotic (Fig. 2.1). During the first 24 hr, \( \text{NO}_2^- \) removal was rapid then it reached an apparent equilibrium. Nearly, 31, 20, and 9\% of initial 0.1 mM \( \text{NO}_2^- \) was removed from the solution at pH 5.5, 6.5, and 7.5, respectively (Fig. 3.4A). Although the calculated reactive surface sites concentration in goethite was 0.23 mM (Appendix C) there was not an equal amount of \( \text{NO}_2^- \) removal.

The greater rate of \( \text{NO}_2^- \) removal by goethite at lower pH can be attributed to the surface reactive sites of goethite and \( \text{NO}_2^- \). Potentiometric titration data shows that electrical charge at the goethite surface falls to zero at \( \text{pH}_{\text{PZSE}} 8.45\pm0.1 \) (Fig. 3.2). This indicates that goethite bears positive surface charges at \( \text{pH} < \text{pH}_{\text{PZSE}} \). Reactive surface sites in goethite are commonly represented by singly (≡\( \text{Fe}-\text{OH}-0.5 \)) and triply (≡\( \text{Fe}_3\text{-OH}-0.5 \)) coordinated surface functional groups, in which ≡\( \text{Fe} \) refers to a surface site (Sigg and Stumm, 1981; Essington, 2004). The doubly coordinated ≡\( \text{Fe}_2\text{-OH}^0 \) group is predicted to be relatively inert because the coordination environment is complete. Fraction of protonated hydroxyls, ≡\( \text{Fe}-\text{OH}^+0.5 \) (and ≡\( \text{Fe}_3\text{-OH}^+0.5 \)) increases as pH decreases from \( \text{pH}_{\text{PZC}} \), and positive reactive sites progressively become more dominant, which result in surfaces with net anion exchange capacity (Sigg and Stumm, 1981; Essington, 2004). Thus, two possible mechanisms could be discussed to explain \( \text{NO}_2^- \) loss observed in this study. First, \( \text{NO}_2^- \) nitrite might be binding via an anion exchange (outer-sphere) complex with positively charged sites on goethite driven by favorable electrostatic interactions (Essington, 2004). This might produce a complex such as ≡\( \text{Fe}-\text{OH}^-0.5 \)--\( \text{NO}_2^- \) surface species at \( \text{pH} < \text{pH}_{\text{PZSE}} \) (Sigg and Stumm, 1981; Boily et al., 2001). Secondly, \( \text{NO}_2^- \) might also adsorb directly as an inner-sphere surface complex onto the surface Fe\(^{3+} \) center on goethite (Yates and Healy, 1975). A spectroscopic technique such as ATR-FTIR will be employed to investigate the exact nature of the reactive surface species between Fe and N (see chapter 4).

3.2.2 Impact of surface sorbed \( \text{Fe}^{2+} \) in \( \text{NO}_2^- \) removal: The impact of surface bound \( \text{Fe}^{2+} \) on \( \text{NO}_2^- \) reactivity was examined at pH 5.5. Specifically, pH 5.5 was selected to isolate maximum \( \text{NO}_2^- \) removal condition as observed in earlier experiments with only goethite. We anticipated rapid as well as complete removal of \( \text{NO}_2^- \) by \( \text{Fe}^{2+} \)-treated goethite because the \( \text{NO}_2^- \) removal by goethite alone was greatest at this pH (Fig. 3.4A).
Fig. 3.4B shows the effect of added Fe$^{2+}$ on the kinetics of NO$_2^-$ removal from solution by Fe$^{2+}$-goethite complex. We observed pseudo-first order rate kinetics and the rate constants were calculated from the first 48 hr experimental data. The rate of NO$_2^-$ removal was nearly five times faster for the samples conditioned with Fe$^{2+}$ than with samples containing only goethite (Table 3.2). The effect of Fe$^{2+}$ on NO$_2^-$ transformation is considerable. With added Fe$^{2+}$, NO$_2^-$ was reduced nearly 100% when compared to the system without Fe$^{2+}$.

It is suggested that Fe$^{2+}$-minerals and sorbed Fe$^{2+}$ species are more effective reductants than dissolved Fe$^{2+}$ (Stumm and Sulzberger, 1992). Sorption of Fe$^{2+}_{(aq)}$ onto iron oxide develops a reactive Fe$^{2+}$-oxide surface at the mineral-water interface (Tronc et al., 1984; Amstaetter et al., 2009). In this process, redox potential of the surface Fe$^{2+}$-goethite complex is lowered which results in increased reducing power than dissolved Fe$^{2+}$ alone (Wehrli, 1990; Strathmann and Stone, 2003; Gorski and Scherer, 2011). Previous studies have established clear evidence that surface sorbed Fe$^{2+}$ on goethite enhances reactivity compared to the system without Fe$^{2+}$. Compared to the system with goethite alone, sorption of arsenate increased nearly 10% (Catalano et al., 2011), and reductive transformation of nitrite (Tai and Dempsey, 2009), 2-nitrophenol (Tao et al., 2010), and carbon tetrachloride (Amonette et al., 2000) increased to nearly 100% when goethite was treated with Fe$^{2+}_{(aq)}$. Hence, results from this study where nearly 100% NO$_2^-$ removal is achieved when Fe$^{2+}$-was pre-sorbed onto goethite are consistent with previous studies.

3.2.3 Characterization of reaction products: Nitrite added to goethite alone did not produce nitrous oxide (N$_2$O) at pH 5.5 (Fig. 3.5), the point where the most rapid NO$_2^-$ removal was observed. This suggests that NO$_2^-$ removal in the presence of goethite was likely due to adsorption alone rather than electron transfer. In the goethite samples treated with Fe$^{2+}$ there was immediate production of N$_2$O. In later experiments, N$_2$O production began within the first 15 min after adding NO$_2^-$ and then continued thereafter. Production of N$_2$O reached a maximum at 48-72 hr and declined. Cumulative N$_2$O emission during the experiment reached a plateaued after 72 hr. These results agree with previous work which shows that NO$_2^-$ is reduced to N$_2$O by mineral Fe$^{2+}$ and surface Fe$^{2+}$ forms
Nitrous oxide produced in this study can be accounted for by Fe$^{2+}$ sorbed onto goethite (Fig. 3.4B and 3.6) and the decrease in Fe$^{2+}$ concentration from solution as NO$_2^-$ was reduced. Results from these experiments clearly demonstrate that NO$_2^-$ is more rapidly reduced to N$_2$O by surface bound Fe$^{2+}$ in goethite than by Fe$^{2+}$($aq$) alone.

Nitric oxide production during abiotic NO$_2^-$ reduction is thermodynamically feasible as shown in Fig. 1.3, and also documented previously when NO$_2^-$ is reacted with Fe$^{2+}$ (Kampschreur et al., 2011) and Fe$^{2+}$-treated magnetite (Dhakal et al., 2013). Our attempt to quantify NO in this study was severely impaired when goethite mineral blocked the NO detector membrane. Production of NO during NO$_2^-$ reduction in a Fe$^{2+}$-NO$_2^-$-goethite system could not be verified in this study, but would be a fertile area for further study by employing a different analytical technique.

Morphological analysis carried out using XRD and SEM images of untreated goethite compared to reacted Fe$^{2+}$ conditioned goethite indicate overlapping values, however, there is a tendency for the particle size to increase (Table 3.1). For the same sample weight, diagnostic d-spacing (4.98, 4.19, 3.38, 2.46, 1.72, 1.45 Å) intensity, particularly 4.19 Å (110), of Fe$^{2+}$ treated samples have slightly increased compared to untreated and goethite treated with NO$_2^-$ alone (Fig. 3.7). Studies have shown that the 110 reflection plane in the goethite crystal is sensitive to aggregation and crystal growth (Anderson et al., 1985; Weidler et al., 1998). Thus, one possibility is that the Fe$^{2+}$ is sorbing to goethite surface sites and promoting coagulation, reflected by the increase in intensity of the 110 reflection. Another possibility is that addition of Fe$^{2+}$($aq$) to the system stimulated electron transfer between surface sorbed Fe$^{2+}$ and bulk structural Fe$^{3+}$, thus producing more goethite (Williams and Scherer, 2004; Lares-Casanova and Scherer, 2007; Latta et al., 2012b). Specifically, if the sorbent is goethite, added Fe$^{2+}$ in the system has been shown to promote precipitation of a “goethite-like” reactive surface (Silvester et al., 2005; Amstaetter et al., 2009; Handler et al., 2009). Spectroscopic evidence from previously published literature indicates that reacted goethite produced Fe$^{3+}$-mineral precipitate that totally resembled untreated mineral when sorbed Fe$^{2+}$ on goethite was oxidized by with concomitant NO$_2^-$ reduction could precipitate goethite-like
phase on existing goethite nitrobenzene (Chun et al., 2006; Larese-Casanova et al., 2012). In this study, we observed an increase in crystal length and size of the goethite treated with Fe$^{2+}$ during NO$_2^-$ reduction (Table 3.1, Fig. 3.7 and 3.8). It is proposed that the oxidation of redox-active Fe$^{2+}$ surface complex surface. As a result, length and crystal size of reacted goethite most probably have increased as observed in XRD and SEM image compared to its unreacted counterpart.

We performed theoretical calculations to evaluate the possibility that added Fe$^{2+}$ might have precipitated as Fe(OH)$_2$ (see Appendix C) in the goethite-mineral-water interface. These calculations indicate that our conditions were undersaturated with respect to Fe(OH)$_2$ precipitation.

Several studies have demonstrated that Fe$^{2+}$ uptake by iron oxide is influenced by particle size (Cwiertny et al., 2008) and aggregation (Amonette et al., 2000). Changes in surface area of such oxide directly resulting from the differences in particle size have shown to dramatically influence reductive dissolution (Anschutz and Penn, 2005; Cwiertny et al., 2009), and thus reduction kinetics of nitrobenzene (Cwiertny et al., 2009) and CCl$_4$ (Vikesland et al., 2007). In the future, particle size effects on NO$_2^-$ reduction kinetics could shed light into predicting the influence of NO$_2^-$ reactivity with goethite on a surface area basis.

### 3.3 Environmental implications

Evidence is presented for the importance of goethite and the Fe$^{2+}$-goethite system in removing NO$_2^-$ under anoxic conditions. Our study shows that under anaerobic conditions, the simultaneous presence of Fe$^{2+}_{(aq)}$ and goethite-bearing soil and sediments can catalyze abiotic NO$_2^-$ reduction over a range of environmentally relevant pH levels. Goethite and other various iron oxides are common soil components, and have shown potential in reductive transformation of pollutants. Reactivity of iron oxides is often very low once the reactive surface sites are exhausted. However, reactivity of iron oxide such as goethite towards NO$_2^-$ can continue over long periods as Fe$^{2+}$ becomes available under iron reducing conditions. Surface sorbed Fe$^{2+}$ not only enhances reducing capacity of the system, but also continuously regenerates reactive surface sites by uptake of Fe$^{2+}_{(aq)}$. 

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In nature, microbial reduction of $\text{NO}_3^-$ to $\text{NO}_2^-$ by nitrate reductase is the first step in microbial denitrification process. This could lead to secondary chemical reactions in which $\text{NO}_2^-$ reacts with goethite, and or $\text{Fe}^{2+}$-goethite complex that would lead to $\text{N}_2\text{O}$ production. Recent studies have shown that abiotic $\text{N}_2\text{O}$ production by mineral $\text{Fe}^{2+}$, and or surface sorbed $\text{Fe}^{2+}$ is an important process in the Fe-N cycle (Sørensen and Thorling, 1991; Rakshit et al., 2008; Kampschreur et al., 2011; Dhakal et al., 2013). In addition, coupling of mineral $\text{Fe}^{2+}$ oxidation by $\text{NO}_2^-$ reduction has shown to produce $\text{N}_2\text{O}$ via chemical (Samarkin et al., 2010), and mixed chemical-biological pathways (Cooper et al., 2003).

A recent study provides evidence that suggests that electron transfer between $\text{Fe}^{2+}_{\text{(aq)}}$ and goethite is severely impaired by the presence of long chain phospholipid, a common organic molecule present in the microbial cell wall (Latta et al., 2012b). Because electron transfer between $\text{Fe}^{2+}$ and the solid oxide phase is the first step to stir reductive transformation of contaminants, abiotic $\text{NO}_2^-$ reduction by mineral $\text{Fe}^{2+}$ and surface sorbed $\text{Fe}^{2+}$ could be more important where biomass loading is minimal or absent.
### Table 3.1. Crystal morphology of untreated and treated goethite at pH 5.5 after 72 hr incubation.

<table>
<thead>
<tr>
<th></th>
<th>GT\textsubscript{51} Untreated</th>
<th>GT\textsubscript{51}+NO\textsubscript{2}\textsuperscript{-}</th>
<th>GT\textsubscript{51}+Fe\textsuperscript{2+}</th>
<th>GT\textsubscript{51}+Fe\textsuperscript{2+}+NO\textsubscript{2}\textsuperscript{-}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avg. length\textsuperscript{†}</td>
<td>1.46±0.35</td>
<td>0.82±0.25</td>
<td>1.51±0.28</td>
<td>1.69±0.46</td>
</tr>
<tr>
<td>Avg. width\textsuperscript{†}</td>
<td>0.14±0.05</td>
<td>0.08±0.02</td>
<td>0.13±0.02</td>
<td>0.13±0.03</td>
</tr>
<tr>
<td>L/W ratio</td>
<td>11.0±1.3</td>
<td>10.81±0.74</td>
<td>11.61±1.45</td>
<td>12.33±1.5</td>
</tr>
<tr>
<td>Avg. crystal size (measured)\textsuperscript{†}</td>
<td>0.79±0.23</td>
<td>0.50±0.12</td>
<td>0.80±0.09</td>
<td>0.84±0.19</td>
</tr>
<tr>
<td>Avg. particle size (calculated using Scherrer equation)\textsuperscript{††}</td>
<td>0.62±0.12</td>
<td>0.36±0.14</td>
<td>0.66±0.21</td>
<td>0.99±0.41</td>
</tr>
</tbody>
</table>

\[ n > 25 \]

\textsuperscript{†} Length, width, crystal size (in µm) measured using SEM image.

\textsuperscript{††} Particle size calculated from XRD using Scherrer equation (Scherrer, 1918):

\[
\text{Particle size (in µm)} = \frac{k\lambda}{B \cos\theta},
\]

where B is the width or full width at half-maximum (FWHM) of a diffraction spot (110), λ is x-ray wavelength, and θ is the Bragg angle.

Scherrer constant k is commonly cited in the literature as having a value of ~0.9.

### Table 3.2. Initial rate and \( k_{\text{obs}} \) values measured for NO\textsubscript{2}\textsuperscript{-} removal (initial concentration of 0.1 mM) from solution by goethite (2 gL\textsuperscript{-1}) with or without added Fe\textsuperscript{2+} at various pH. The \( k_{\text{obs}} \) values were derived from a first order rate expression.

<table>
<thead>
<tr>
<th>pH</th>
<th>Treatment</th>
<th>Removal Rate (Ms\textsuperscript{-1})</th>
<th>( k_{\text{obs}} ) (hr\textsuperscript{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.5</td>
<td>Goethite</td>
<td>1.06±0.20×10\textsuperscript{-9}</td>
<td>1.40±0.05×10\textsuperscript{-1}</td>
</tr>
<tr>
<td></td>
<td>Fe\textsuperscript{2+}-Goethite</td>
<td>5.15±0.10×10\textsuperscript{-9}</td>
<td>4.97±0.25×10\textsuperscript{-1}</td>
</tr>
<tr>
<td>6.5</td>
<td>Goethite</td>
<td>2.20±0.15×10\textsuperscript{-10}</td>
<td>7.95±0.10×10\textsuperscript{-2}</td>
</tr>
<tr>
<td>7.5</td>
<td>Goethite</td>
<td>1.25±0.05×10\textsuperscript{-10}</td>
<td>4.80±0.15×10\textsuperscript{-2}</td>
</tr>
</tbody>
</table>
Figure 3.1 X-ray diffraction patterns of control (untreated) goethite (GT$_{51}$) in MES [2-(N-morpholino) ethane sulfonic acid] at pH 5.5. Scanning electron micrograph (SEM) of the control goethite is shown on top. Graph below XRD spectra shows composition microanalysis of untreated goethite by EDX.
Figure 3.2 Acid-base titration data for goethite (GT51) at three different ionic strengths on a surface area basis (100 and 330 m²L⁻¹). Solid lines are experimental data point connectors.

Figure 3.3 Plot showing sorption of Fe²⁺(aq) onto goethite (GT51). Initially, 1.5 mM of Fe²⁺(aq) was injected into reaction vials containing ~2 gL⁻¹ (~100 m²L⁻¹) of goethite slurry. Open and solid symbols indicate samples that have 1 and 10 mM NaCl respectively. Equilibrium Fe²⁺ sorption was observed after 24 hr at pH 5.5 (open and closed circles). pH buffer used for pH 5.5 and 6.5 samples is 25 mM MES, and for 7.5 is 25 mM PIPES.
Figure 3.4 Removal (%) of NO\textsubscript{2} from solution by goethite (GT\textsubscript{51}) at pH 5.5 (●), 6.5 (◇), and 7.5 (▲) (A), without (●) and with (◇) added Fe\textsuperscript{2+} at pH 5.5 (B). Initial nitrite concentration was ~0.1 mM. Experiments conducted with preconditioned Fe\textsuperscript{2+} contained initial ~1.5 mM Fe\textsuperscript{2+}. Equilibrium Fe\textsuperscript{2+} concentration after 24 hr was ~1.07 mM before NO\textsubscript{2} injection. Loss (in %) of 1.07 mM Fe\textsuperscript{2+} from the solution after NO\textsubscript{2} injection is represented by symbol (□). Symbols indicate experimentally determined data, and solid lines represent the pseudo first-order kinetic model fit. Open symbols represent control experiments at pH 5.5 without goethite in the absence (A) and presence (B) of added Fe\textsuperscript{2+}. Error bars represent the standard deviation from the mean for duplicate runs; bars not visible are smaller than the symbols.
Figure 3.5 Nitrous oxide (N₂O) emission recorded in the experiment (at pH 5.5) after adding 0.1 mM NO₂⁻ in the reaction vessel that contained goethite slurry in the absence (●), and presence (○) of Fe²⁺(aq). Inset figure shows cumulative N₂O evolution. Open symbols indicate the control samples without goethite for respective experiment. Error bars represent the standard deviation from the mean for duplicate runs; bars not visible are smaller than the symbols. In all samples, changes in the pH, from its initial 5.5, are within ±0.1.
Figure 3.6 Surface Fe$^{2+}$ loading (○) with respect to NO$_2^-$ reduced (△) per gram of goethite at pH 5.5 over the span of 96 hr in a Fe$^{2+}$-NO$_2^-$-goethite system. Error bars represent the standard deviation from the mean for duplicate runs; bars not visible are smaller than the symbols.
Figure 3.7 Plot of a powder x-ray diffraction pattern observed from untreated goethite (GT51) and that reacted with NO$_2^-$ at pH 5.5. XRD spectra of the goethite samples conditioned with added Fe$^{2+}$ before treating with NO$_2^-$ are stacked on the top. Standard reference spectra for goethite, magnetite, and hematite are shown as solid lines at the bottom of the figure. Samples treated with NO$_2^-$ in the presence of added Fe$^{2+}$ match with goethite spectra, and the peak (110) intensity is larger than untreated samples (inset).
Figure 3.8 Scanning electron micrographs (SEMs) of untreated goethite (A), goethite treated with 0.1 mM NO$_2^-$ at pH 5.5 for 72 hr (B), and Fe$^{2+}$-conditioned (1.5 mM) goethite treated with 0.1 mM NO$_2^-$ at pH 5.5 for 72 hr (C & D) showing the changes in size and morphology of the goethite particles for various treatment. Composition microanalysis of untreated goethite by EDX (E).
Figure 3.9 Possible scheme showing NO$_2^-$ redox transformation mediated by surface sorbed Fe$^{2+}$ on goethite-water geomedia. Electron shuttling from Fe$^{2+}$-goethite complex possibly stimulates NO$_2^-$ reduction to N$_2$O. The nitrogen species are drawn as Lewis structures taken from Luther (2010). Solid arrow indicates possible reaction product/pathways that produce N-intermediates.
Chapter Four: An *In Situ* Spectroscopic Study of Nitrite Interaction with Goethite and Fe$^{2+}$-Goethite Mineral Water Interface

4. Introduction

Due to its ability to probe chemical bonds, Fourier transform infrared (FTIR) spectroscopy has been applied for years to collect IR spectra of organic and inorganic compounds. Because elemental composition and chemical bonds are unique to each compound, IR spectra can serve as a fingerprint (Stuart, 2004). Conventional FTIR methods involve drying the mineral of interest to remove water, which absorbs strongly in the IR region with intense OH stretching and bending bands (Farmer, 1974). This precludes the study of *in situ* surface investigations. Attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy is a modified FTIR tool which offers the possibility for the *in situ* spectroscopic investigation of adsorbed oxyanions at metal (hydr)oxide-water interfaces (Tejedor-Tejedor and Anderson, 1986). In this novel technique, the IR beam is allowed to pass through an ATR crystal and is reflected at the crystal-sample interface, forming an transient wave and minimizing the contribution from the liquid (Stuart, 2004). By careful spectral subtractions, one can obtain information at the surface-water interface (Peak et al., 1999; Arai and Sparks, 2001; Zhang and Peak, 2007).

ATR-FTIR spectroscopy is extensively being used to examine interactions of inorganic anions with various minerals. Previously, the sorption mechanism of phosphate (Arai and Sparks, 2001), carbonate (Villalobos and Leckie, 2001), and sulfate (Peak et al., 1999) on goethite has been investigated using this technique. Although the relationship between the symmetry of sulfur (S), carbon(C), and phosphorus (P) complexes and their IR spectra is well established, virtually no work has been carried out to understand the nitrite (NO$_2^-$) sorption mechanism and reaction intermediates using iron oxide minerals such as goethite.

Nitrite is a versatile ambidentate ligand that can participate in acid/base, coordination or oxygen transfer chemistry by donating a pair of electrons from either N or O atoms (Hitchman and Rowbottom, 1982). A summary of possible modes of NO$_2^-$ bonding to metal cations (denoted by M) is shown in Scheme 1 (see below). Nitrite
coordination to cations and metals can occur through one or two of its oxygen atoms (nitrito) (Scheme 1, a & b). Oxygen is the ligating atom with Mg\(^{2+}\) (Cerruti et al., 1974) and Cu\(^{2+}\) (Hitchman and Rowbottom, 1982). Nitrite coordination with aqueous Fe\(^{2+}\) and membrane bound reactive Fe\(^{2+}\) sites have shown to favor N-ligation (Scheme 1 e, (Hitchman and Rowbottom, 1982; Einsle et al., 2002). These different binding modes can be distinguished using IR spectroscopy (Hitchman and Rowbottom, 1982).

**Scheme 1**

Diffuse reflectance spectroscopy (DRS) in the ultraviolet-visible-near infrared (UV-VIS-NIR) region allows one to probe metal d-d transitions, ligand-to-metal transitions, and metal-to-ligand transitions (Burns, 1993; Figgis and Hitchman, 1999). Previously, this technique has been used to quantify Fe-oxide minerals in soil (Scheinost et al., 1998), aluminum substitution in goethite (Scheinost et al., 1999a), oxidation states of iron in sodium silicate glass (Jeoung et al., 2001), and also in the study of electron transfer between metal and nitrite in solution (Barnes et al., 1972). The use of UV-VIS-NIR spectroscopy on wet pastes of clay minerals reacted with nickel (Ni\(^{2+}\)) allowed
Scheinost et al. (1999b) to evaluate the mechanism of Ni$^{2+}$ removal from solution. They found under certain conditions, Ni$^{2+}$ formed surface precipitates on the clay minerals.

In Chapter 3, the removal of NO$_2^-$ by goethite was quantified using wet chemical (macroscopic) techniques. The rate of NO$_2^-$ removal by goethite was accelerated at lower pH and in the presence of surface Fe$^{2+}$. To better understand the mechanism of NO$_2^-$ removal by goethite, spectroscopic techniques are needed to provide molecular level information. The objective of this study is to examine the interaction of NO$_2^-$ with goethite and Fe$^{2+}$-amended goethite as a function of pH (5.5-7.5), initial NO$_2^-$ concentration (0.1-100 mM), and goethite concentration (2 and 10 g/L) using in situ ATR-FTIR and UV-VIS-NIR spectroscopies. This will provide insight in the nature of coordination between Fe and N at mineral-water-interface.

4.1 Experimental sections

4.1.1 Reagents, minerals synthesis, and characterization: Reagent grade or better chemicals were used as received. Nitrate and nitrite stock solutions were prepared by certified ACS-grade sodium nitrate (NaNO$_3$) and sodium nitrite (NaNO$_2$) salts inside an anaerobic glove box (Coy Laboratory Products, Grass Lake, MI) purged with 95% argon (Ar) and 5% hydrogen (H$_2$) mixture. All solutions were prepared using deoxygenated doubly deionized water (DDIW) (18.3 MOhm cm$^{-1}$) purged for 3 hr with ultra pure argon (Ar) before transporting into the glove box. Solution pH was buffered using 25 mM [2-(N-morpholino) ethane sulfonic acid] (MES) solution combined with a 0.1 mM NaCl solution, for a total ionic strength of 25.1 mM.

Preparation, purity, size distribution, and detailed characterization of goethite ($\alpha$-FeOOH) minerals used in this study are described previously (Chapter 3). The specific surface area of the goethite used in this study was 51.43±0.03 m$^2$/g, and occasionally abbreviated in the text as GT$_{51}$.

4.1.2 Nitrite interaction with goethite studies: A goethite suspension (2 and 10 g L$^{-1}$) was prepared at pH 5.5 in water, and pH of the solution was stabilized using 25 mM MES solution. The suspension was well stirred in an end-to-end rotator overnight for prehydration. Ionic strength of the solution was maintained using 0.1 mM NaCl, for a
total ionic strength of 26 mM. To begin sample precondition, goethite slurry (~5 µL) was spread over the ATR crystals under Ar purging. Then, the sample was allowed to partially evaporate excess water until a thin goethite layer was developed on the ATR crystal. On average, goethite layer was developed from the slurry within 15-20 min. Development of goethite layer in the form of “paste” on the ATR crystal is confirmed by continuously monitoring IR peak at 3140, 897 and 792 cm\(^{-1}\) as suggested previously (Tejedor-Tejedor and Anderson, 1986). Proper in situ pre-treatment is necessary to produce an evenly distributed goethite layer over the ATR crystal. Even distribution of goethite layer was tested via series of experiments using exact same volume of slurry, and monitoring fingerprint IR peak intensity as mentioned earlier. Reaction began by injecting ~5 µL of 0.1, 0.5, and 100 mM NO\(_2^-\) solution via an injection port (Fig. 4.1A,B). Infrared spectra were collected at 5, 15, 30, 45 min, 1 hr and 2 hr after introduction of NO\(_2^-\) solution.

Effect of pH (5.5-7.5) on NO\(_2^-\) interaction at the goethite-water interface was studied using IR measurement on nitrite treated goethite slurry. Goethite slurry (10 gL\(^{-1}\)) was prepared in 3 different reactor vials (10 mL) at designated pH values and prehydrated overnight (24 hr) inside a glove box. Goethite slurry at each pH was spiked with 0.1 mM NO\(_2^-\) solution, mixed thoroughly on end-over-end rotator for 1 hr, and transported out of anaerobic glove for IR measurement (see detail below).

To study NO\(_2^-\) interaction with Fe\(^{2+}\)-goethite complex using ATR-FTIR, goethite slurry (10 gL\(^{-1}\)) prepared in a similar method as mentioned previously was first spiked with 1.5 mM Fe\(^{2+}\) at pH 5.5 and agitated inside the glove box for 24 hr. Fe\(^{2+}\) stock solutions were prepared from zerovalent iron (Fe\(^0\)) by complete dissolution in 100 mM HCl stock solution. After 24 hr of agitation, residual Fe\(^0\) fraction was retained in a 0.22µm membrane filter (Fisher Scientific, Hampton, NH) and stored inside anoxic chamber to prevent oxidation of Fe\(^{2+}\). To begin experiment, sealed sample vial was transported out of glove box and under Ar purging ~5µL of Fe\(^{2+}\)-goethite slurry was spread over on ATR crystal using a 10 µL syringe. Sample precondition, and IR measurement of NO\(_2^-\) (0.1 mM) treated Fe\(^{2+}\)-goethite slurry was performed in the same manner as described earlier.
In addition to ATR-FTIR study, a separate experiment was conducted to study the interaction of \( \text{NO}_2^- \) with \( \text{Fe}^{2+} \)-goethite complex. In this method, goethite slurry (10 g L\(^{-1}\)) preconditioned with 1.5 mM \( \text{Fe}^{2+} \) at pH 5.5 was spiked with 0.1 mM \( \text{NO}_2^- \). Initially, \( \text{Fe}^{2+} \) was added to goethite slurry prepared in 20 mL glass vials inside the glove box and equilibrated for 24 hr before reacting with \( \text{NO}_2^- \). Reactor vials were kept inside the glove box shaking with an end-to-end rotator at 30 rpm to allow complete mixing. With increasing time interval, ranging from 15 min to 48 hr, aliquots were removed and filtered through 0.22\( \mu \)m filter paper (Fisher Scientific, Hampton, NH). Wet sample retained on the filter paper was immediately mounted on a glass slide pending analysis using UV-VIS-NIR spectrophotometer (detail below).

4.1.3 \textit{In situ} ATR-FTIR measurement: All IR spectra were collected by using a Thermo Nicolet 6700 spectrometer (Thermo Electron Corp., Madison, WI) equipped with SmartDuraScope\textsuperscript{TM} (Fig. 4.1). Spectra were collected at 8 cm\(^{-1}\) resolution, 200 scans, data spacing of 3.857 cm\(^{-1}\), and mirror velocity of 0.66 cm\(^{-1}\)s\(^{-1}\). SmartDuraScope\textsuperscript{TM} is equipped with a 1.5mm diamond sample holder with a capacity of probing a 2 \( \mu \)m sample depth at a rate of 3 reflections, and has an integrated digital magnifier (100X).

Initially, IR spectra for various concentrations of \( \text{NO}_2^- \) and \( \text{NO}_3^- \) standard, 25 mM MES solution, dry goethite, and goethite paste prepared in 25 mM MES were collected by spreading the solution over a diamond crystal in the SmartDuraScope. Later, spectral data were collected for various treated and untreated goethite samples as discussed previously. ATR-FTIR spectra obtained from experimental samples were then subtracted for water, \( \text{NO}_2^- \), and MES solution for analysis. Also, IR spectra for solid \( \text{NaNO}_3 \), and \( \text{NaNO}_2 \) were recorded as standards. Spectral manipulation such as baseline correction, spectral subtraction, peak fitting, and normalization was performed using the OMNIC 7.3 software package (Thermo Electron Corp., Madison, WI).

4.1.4 \textit{In situ} Diffuse Reflectance Spectroscopy (DRS): A wet paste of \( \text{Fe}^{2+} \)-conditioned goethite reacted with \( \text{NO}_2^- \) was retrieved after filtering, mounted on a glass slide, then immediately transferred from the glove box to an IRS 3100 integrating sphere attachment assembled to a UV-VIS-NIR spectrophotometer (Shimadzu, UV-3101PC, Columbia, MD) for spectral measurement. The sample port in the spectrophotometer was constantly...
purged with ultra pure Ar to minimize oxidation. Spectra were acquired in the range of 9500-35000 cm\(^{-1}\) (1053-285 nm) at 2 nm increments. In addition to experimental samples, optical spectra of Fe\(^{2+}\) and Fe\(^{3+}\)-oxide(s) were collected as standard for comparison. Raw wavelength-dependent reflectance data were converted into Kubelka-Monk remission function (Kubelka and Munk, 1931). Within the range of wavelength investigated, assuming scattering coefficient is relatively constant, the shapes of the Kubelka-Monk spectra are equivalent to the actual absorption spectra. Spectral analysis was carried out using GRAMS32AI® software (Thermo Electron Corp., Madison, WI).

4.2 Results and discussion

4.2.1 Theoretical IR vibration of NO\(_2^–\)

Theoretically, the number of IR active bands of a nonlinear molecule is given by 3N-6, where N is the number of atoms (Huheey et al., 1993). Polyatomic nitrite exhibits C\(_{2v}\) symmetry and its three IR vibrational modes are IR active. Table 4.1 summarizes the vast majority of well characterized ionic nitrites in the solid state. The three IR vibrational modes occur in the following regions: (i) the symmetric stretching (\(\nu_s = 1380-1305\text{ cm}^{-1}\)) (ii) the symmetric bending (\(\delta_{\text{NO}_2} = 845-805\text{ cm}^{-1}\)) and (iii) the asymmetric stretching (\(\nu_{as} = 1235-1208\text{ cm}^{-1}\)) (Miller and Wilkins, 1952; Weston and Brodasky, 1957; Hitchman and Rowbottom, 1982; Nakamoto, 1986). Nitrite is unusual in that the asymmetric stretching bond energy is lower than the symmetric one, due to the high interaction force constant between the terminal atoms, similar to that observed in other molecules such as ozone (O\(_3\)) and OF\(_2\) molecules (Hitchman and Rowbottom, 1982) and reference therein). Complexed nitrite species, however, exhibit asymmetrical (\(\nu_{as}\)) stretching vibration at a higher energy than symmetric (\(\nu_s\)) stretching (Hitchman and Rowbottom, 1982; Laane and Ohlsen, 1986; Nakamoto, 1986). Notably, \(\nu_s\) and \(\nu_{as}\) band assignment for “nitro complexes” is observed in the 1470-1250 cm\(^{-1}\), and 1650-1340 cm\(^{-1}\) region, respectively (Table 4.1 and Scheme 1e). Commonly, \(\nu_s\) and \(\nu_{as}\) band assignment in “nitrito complex” is in the lower frequency region compared to “nitro complex” (Nakamoto, 1986). This is because O\(^2–\) impose weaker ligand-field and positioned to lower energy regions in the spectrochemical series than N; therefore N binding with metals is expected to occur at higher energy (Figgis and Hitchman, 1999).
Due to reduced NO$_2^-$ symmetry in monodentate surface species, IR active symmetric N-O and N=O stretching modes are fairly distinguishable. Commonly, weaker N-O stretching bond is observed at lower frequency (1206-1050 cm$^{-1}$) then stronger N=O bond (1485-1375 cm$^{-1}$) (Nakamoto, 1986; Hadjiivanov, 2000). Although it is difficult to discern bridging from non-bridging structure from IR data, N=O bond frequency arising from bridging compounds are at higher energy values than those from non-bridging compounds (Hadjiivanov, 2000). Because the chelating and the bridging bidentate surface species (Scheme 1, c&d) preserve the structural symmetry ($C_{2v}$), IR active bands arising from such nitrito species resembles those of NO$_2^-$ (aq) ion (Nakamoto, 1986). Finally the NO$_2^-$ anion can also coordinate via N atom (nitro) to a metal surface site (Scheme 1, e) with higher N-O stretching frequencies than the corresponding vibrations for bidentate nitrito species (Hadjiivanov, 2000).

An ATR-FTIR spectrum of solid NaNO$_2$ shown in Fig. 4.2(A) demonstrates three major IR bands (1324, 1229, and 825 cm$^{-1}$). The broad and intense peak centered at 1229 cm$^{-1}$ is assigned to a $\nu_{as}$ N-O stretch and the absorption peaks at 1324, and 825 cm$^{-1}$ are assigned to a $\nu_{s}$ N-O stretch and $\delta$NO$_2$ (N-O bending), respectively. The free aqueous nitrite anion in ATR-FTIR mode displays an absorption band at 1232 cm$^{-1}$, assigned to $\nu_{as}$ (Fig. 4.2B). The peak at 830 cm$^{-1}$ in aqueous nitrite is attributed to the $\delta$NO$_2$ vibration (Fig. 4.2B). The $\nu_{s}$ band was below detection in aqueous nitrite, which is not uncommon (Cerruti et al., 1974; Hitchman and Rowbottom, 1982). These assignments for the standards are consistent with the literature, where $\nu_{as}$ ranges from 1260-1208 cm$^{-1}$ and the range for $\delta$NO$_2$ is 833-785 cm$^{-1}$ (Weston and Brodasky, 1957; Cerruti et al., 1974; Hitchman and Rowbottom, 1982) as summarized in Table 4.1. Where $\nu_{s}$ appears, it is found in the range of 1335-1303 cm$^{-1}$.

4.2.2 Characterization of goethite-water related ATR-FTIR spectral feature

Before studying the nitrite-goethite interface, one has to account for the effect of the pH buffer (MES), the presence of water, and the properties of goethite in contributing to the IR spectra. The IR absorption spectrum of liquid water alone at pH 5.5 is shown in Figure 4.3a. There is a broad absorbance peak centered at 3289 cm$^{-1}$ which is a combination of overlapping peaks; peak deconvolution (inset Fig. 4.3) reveals peak at
3343 cm\(^{-1}\) and 3205 cm\(^{-1}\), assigned to asymmetric O-H and symmetric O-H stretching vibrations (Lappi et al., 2004). The peak at 1636 cm\(^{-1}\) is assigned to an H-O-H bending mode. These features are attributed to H-bonding (Lawrence and Skinner, 2003). Similar to the spectrum (a) shown in Fig 4.3, absorption spectrum of 25mM MES (pH 5.5) solution observed in Fig. 4.3b is also identical to spectra of liquid water reported previously (Al-Abadleh and Grassian, 2003; Lappi et al., 2004). The O-H stretching vibrations in goethite paste buffered at pH 5.5 with 25mM MES\(_{\text{aq}}\) resulted in a slight shift to lower energy, with a broad and strong absorption peak centered at 3243 cm\(^{-1}\) (Fig. 4.3c). No significant absorption peak for MES is observed, presumably due to the low concentration.

ATR-FTIR spectra of the MES buffered goethite paste (Fig. 4.3c) display three absorption peaks dominated by water O-H vibrations (3243, 2107, and 1636 cm\(^{-1}\)) (Villalobos and Leckie, 2001; Walrafen and Pugh, 2004). In addition, two sharp absorption peaks at 885 and 784 are noted (Fig. 4.3c). These two bands can be assigned to the bending O-H vibrations of the bulk hydroxyl group in (δ\(_{\text{OH}}\)) and out (γ\(_{\text{OH}}\)) of the 001 goethite crystallographic plane (parallel to c axis) (Cambier, 1986; Cwiertny et al., 2009). ATR-FTIR spectra of the water-goethite paste and MES buffered goethite paste were identical (data not shown).

Although there is a significant increase in the absorption intensity from a wet goethite paste, spectra obtained from untreated dry (room temperature) and MES subtracted goethite samples are similar (Fig 4.3 d&e). A strong and broad absorption peak observed at 3243 cm\(^{-1}\) (H\(_2\)O + O-H: overtone of O-H stretching vibration) in goethite paste is shifted to 3101 cm\(^{-1}\) after subtracting with MES solution. Narrowing and shifting of peak 3243 cm\(^{-1}\) could be due to decreased residual water in the surface of water saturated goethite paste. A water-subtracted IR spectrum of goethite does not show the broadening feature but rather emerges into strong peak at 3101 cm\(^{-1}\). This peak is attributed to the stretching vibration of bulk O-H in the goethite structure (ν\(_{\text{OH}}\)), similar to the peak observed at 3124 cm\(^{-1}\) in dry goethite sample. Similar IR shift in water saturated goethite paste has been reported previously (Tejedor-Tejedor and Anderson, 1986). In fact, an absorption band at 3124 cm\(^{-1}\) is reportedly sensitive to dehydroxylation, and or hydration (Ruan et al., 2002a). Similarly, decrease in peak intensity at 1636 cm\(^{-1}\) is due to
removal of excess surface water at the goethite-water interface. Because the peaks at 3124 cm\(^{-1}\), and 893 and 791 cm\(^{-1}\) were retained in the spectra subtracted from the water, these bands are related to the bulk O-H vibration in the goethite structure and not from surface O-H energy. The band near 634 cm\(^{-1}\) is due to a symmetric Fe-O stretch (Cambier, 1986; Ruan et al., 2002b).

**4.2.3 ATR-FTIR of NO\(_2^-\)\(_{\text{aq}}\) treated goethite**

Figure 4.4 shows time series ATR-FTIR data associated with goethite (10 gL\(^{-1}\)) that had been reacted with 0.1 mM NO\(_2^-\)\(_{\text{aq}}\) (pH =5.5). Absorption peaks at 3099, 2091, 1633, 855, 787, and 635 cm\(^{-1}\), and their assignment have been discussed in the previous sections. Within 1000- 1300 cm\(^{-1}\) region, reacted samples show four distinct IR absorption peaks (1265, 1231, 1186, and 1121 cm\(^{-1}\)). Another two peaks at 1791 and 2306 cm\(^{-1}\) emerge, well above the water bending frequency (1633 cm\(^{-1}\)), from samples treated with NO\(_2^-\). Absorption peak for \(\delta\)NO\(_2^-\) observed earlier (833-785 cm\(^{-1}\)) in NO\(_2^-\)\(_{\text{aq}}\) standard no longer existed, and could be hidden under OH-peak at 892-797 cm\(^{-1}\).

After 15 minutes of reaction time between NO\(_2^-\) and goethite at pH 5.5 and 10 g goethite L\(^{-1}\), there was appearance of an absorption peak at 1791 cm\(^{-1}\) (Fig. 4.4). This band can be assigned to a surface bound NO species. Gaseous NO has a peak near 1876 cm\(^{-1}\) and is red-shifted upon complexation. The presence of IR absorption at 1791 cm\(^{-1}\) in our study is likely due to the \(\nu_{\text{as}}\) (N=O) stretching vibration of adsorbed NO. IR studies have confirmed that mineral surfaces provide suitable sites for NO adsorption (Mortland, 1965). This assignment is consistent with a recent study conducted at ambient room temperatures that have shown a similar vibrational mode of adsorbed NO onto pyrite (FeS\(_2\)) (Singireddy et al., 2012). Comparable vibrational mode of gaseous NO adsorbed on a variety of metal surfaces is found in the literature (Hadjiivanov, 2000; Davydov, 2003; Sá and Anderson, 2008). At low temperature, it is found that NO molecules tend to dimerize, and the N-O stretching modes can fall below 1876 cm\(^{-1}\) due to \(\pi\)-back donation with cations rich in d-electrons (Hadjiivanov, 2000). Efforts to measure NO\(_g\) as a reaction product from the reactor containing NO\(_2^-\) treated goethite slurry, as done in Chapter 2 with magnetite, were severely impaired because the NO detector membrane was blocked by the goethite slurry (Chapter 3).
Another distinct IR vibration is observed at 2306 cm$^{-1}$ (Fig. 4.4). This peak is assigned to an N-N stretching vibration from a linear N-N-O structure of dissolved or weakly adsorbed N$_2$O molecule at the goethite-water interface. It is noted that this assignment is also consistent with the vibrational frequencies (cm$^{-1}$) for gaseous N$_2$O adsorbed to various transitional metals as reported by Laane and Ohlesen (1980) and Hadjiivanov (2000). Results from the wet chemical experiment also supports this data where N$_2$O$_{(g)}$ was measured in the headspace of reaction vessel containing NO$_2^-$ and goethite (Chapter 3). Notably, the absorption peak for NO$_{(ads)}$ and N$_2$O$_{(ads)}$ observed in this study is relatively stronger in the beginning (15-45 min) and tends to diminish at longer reaction times (>2 hr). This indicates that these species are relatively unstable and short lived. IR absorbance data presented here shows evidence of goethite mediated NO$_2^-$ reduction to N$_2$O preceded by the production of NO (see Chapter 3).

The ATR-FTIR spectra of NO$_2^-$ reacted goethite samples also show additional IR absorption peaks in the 1000-1300 cm$^{-1}$ region (Fig. 4.4). This region was isolated by varying NO$_2^-$$_{(aq)}$ (0.1, 0.5, and 100 mM) and goethite (2 and 10 gL$^{-1}$) loading at pH 5.5 over time (Fig. 4.5). Reaction of 2 g goethite L$^{-1}$ with 0.1 mM NO$_2^-$ resulted in appearance of four IR peaks at 1256, 1221, 1192, and 1121 cm$^{-1}$ (Fig. 4.5A). All four absorption peaks intensify with longer reaction time. On the basis of behavior in relation to the coverage these four components are related as couples at 1256, 1190 cm$^{-1}$ and 1221, 1121 cm$^{-1}$. NO$_2^-$ adsorption onto magnesium oxide at 25°C resulted in 4 IR absorption peaks positioned in the same wavenumber range (Cerruti et al., 1974). On theoretical and experimental grounds, Cerruti et al. (1974) grouped these 4 peaks into pairs and assigned them to two separately adsorbed NO$_2^-$ species, specifically one for excited and the other for ground state nitrite species.

Data indicate that NO$_2^-$ ion can exhibit singlet $^1$B$_1$ ($v_s$ at 1018 cm$^{-1}$) and triplet $^3$B$_1$ ($v_a$ at 1170 cm$^{-1}$, and $v_{as}$ at 1124 cm$^{-1}$) excited states (Sidman, 1957; Harris, 1973). Based on electron occupancy in 6a$_1$, and 2b$_1$ orbitals, NO$_2^-$ can be either in the ground ($^1$A$_1$) or excited ($^1$B$_1$ & $^3$B$_1$) state (Cerruti et al., 1974). In ground state, 6a$_1$ orbital in NO$_2^-$ is doubly occupied, and in excited states electron from 6a$_1$ orbital is split into 6a$_1$ and 2b$_1$. Thus, while excited 6a$_1$ & 2b$_1$ orbital in NO$_2^-$ is singly occupied as in the ground state of NO$_2^2$-. From steric consideration, NO$_2^-$ molecule approaching reactive surface sites such
as goethite or magnesium oxides could stir electronic crowding in such a way that molecules spread to excited states forcing electron occupancy in the orbital to changed.

On the basis of spectroscopic data alone, it is often challenging to distinguish between the species NO$_2^-$ ($^1$A$_1$), and NO$_2^-$ ($^1$B$_1$ & $^3$B$_1$), particularly when sensitivity of NO$_2^-$ stretching frequencies is weak. However O-N-O bond length and angles in the excited species are greater and increase (by 9° and 15°) in the $^1$B$_1$ and $^3$B$_1$ states (Harris, 1973; Cerruti et al., 1974). In fact, increase of bond length results in lowering of N-O stretching frequency as the bond becomes unstable. This explanation justifies our assignment to absorption peak couples at lower wavenumber to excited surface NO$_2^-$. Commonly observed $\nu_s$, and $\nu_{as}$ N-O vibrations, summarized in Table 4.1, arises from ground state, $^1$A$_1$ electronic configuration of NO$_2^-$. The stated consideration suggests that absorption peak couple at 1256, 1190 cm$^{-1}$ and 1221, 1121 cm$^{-1}$ can be attributable to ground and excited states of NO$_2^-$ adsorbed onto goethite surface, respectively. Assigned to surface sorbed ground state and excited NO$_2^-$ species, absorption peak couples 1256, 1190 cm$^{-1}$ and 1221, 1121 cm$^{-1}$ become more resolved with increasing reaction time (Fig. 4.5A). As of now it is not clear if the IR pair 1221, 1121 cm$^{-1}$ is essentially due to adsorbed excited NO$_2^-$ ion, an issue that awaits further study. IR vibration data on excited NO$_2^-$ ion are the results of extrapolation of spectroscopic data refering to dimer species Na$_4$N$_2$O$_4$ and electronic and vibrational state of nitrite ions (Gray and Yoffe, 1955; Cerruti et al., 1974). No other study has even been reported on excited NO$_2^-$ or ground NO$_2^{2-}$ ion species; it presents a unique opportunity in NO$_2^-$ transformation studies, and deserves further investigation.

The geometry of NO$_2^-$ coordination to goethite can be analyzed on the basis of theoretical and experimental vibrational frequencies assigned to various surface nitrite structures. If the goethite-NO$_2^-$ complex had been of the nitro type, both $\nu_s$ and $\nu_{as}$ should have shifted to higher frequencies compared to the free nitrite ion (Table 4.1). This rules out the structure e in Scheme 1 and leaves open the possibility of formation of several different surface nitrito complexes. In nitrito complexes, the shifting of $\nu_{as}$ is expected to lie higher and $\nu_s$ to lower frequencies than aqueous nitrite ions (Hitchman and Rowbottom, 1982; Nakamoto, 1986; Hadjiivanov, 2000). Thus, we assign the peak at 1256 cm$^{-1}$ as $\nu_{as}$ and the peak at 1190 cm$^{-1}$ as $\nu_s$. Based on this argument, it is suggested that a nitrito-type complex is formed when NO$_2^-$ reacts with goethite. Furthermore, it
seems very reasonable to exclude the structures a&b illustrated in Scheme 1 because these structures have allowed IR bands for $\nu_s$ well above those observed in our study. In addition, the values for $\nu_{as}$ in our study (1256-1265 cm$^{-1}$) are greater than those reported for the monodentate (structure a, Scheme 1) and bridging monodentate nitrito (structure b, Scheme 1). On the contrary, our values for $\nu_{as}$ and $\nu_s$ of adsorbed NO$_2^-$ fit within the ranges of vibrational frequencies specified in Table 4.1 for structure c&d (Scheme 1).

On the basis of IR data alone, it is often difficult to distinguish between chelating and bridging geometries; however, a further refinement of the possible nitrite surface structure can be made by recognizing that $\nu_{as}$ for the bridging structure is IR inactive (Table 4.1). The fact that we observed a $\nu_{as}$ band suggests that NO$_2^-$ is not binding to goethite surface sites in the geometry illustrated by structure d (Scheme 1). Therefore, this structure can also be excluded from a potential nitrite surface species on goethite. Thus, our IR data are most consistent with NO$_2^-$ ions bound to the goethite surface through the two oxygen atoms (structure c, chelating nitrito complex, Scheme 1). Nitrite sorbed to magnesium oxides has also been shown to produce chelating nitrito structure within comparable wavenumbers as reported here (Cerruti et al., 1974).

For NO$_2^-$ to chelate Fe$^{3+}$ atoms (denoted as M in structure c, Scheme 1) on the goethite surface, a displacement of hydroxide or water surface functional groups would be necessary. As the surface protonation state is pH-dependent on goethite, it might be expected that there would be a change in the IR spectra with pH as well.

### 4.2.4 Effect of pH on NO$_2^-$ interaction with goethite

The effect of pH on NO$_2^-$ interactions at the goethite-water interface was studied at pH 5.5, 6.5, and 7.5 after 60 minutes of reaction time (Fig. 4.6). Two important observations can be made from these data. First, the IR peak intensities at $\approx$1260, 1220, 1185, and 1120 cm$^{-1}$ are all greater at pH 5.5 when compared with pH 6.5 and pH 7.5. This indicates more NO$_2^-$ ions are present as adsorbed species and agrees with our macroscopic data showing greater NO$_2^-$ removal from solution as the pH is lowered (see Chapter 3). As mentioned in Chapter 3, a reactive surface site in goethite is often represented by singly ($\equiv$Fe-OH$^{0.5}$), doubly ($\equiv$Fe$_2$-OH$^0$), and triply ($\equiv$Fe$_3$-OH$^{1.5}$) coordinated surface functional groups where singly and triply coordinated surface
functional group can only protonate or deprotonate. The pH_{PZSE} of GT_{51} is 8.45±0.1,
thus, the electrical charge at the surface of goethite becomes progressively positive when
the pH falls below pH_{PZC}, allowing more protonated reactive surface sites, such as
≡FeOH_{2}^{0.5+} to form. Nitrite can displace the OH_{2} group (or OH groups) and directly
bond (chelate) the Fe^{3+} metal in a process referred to as ligand exchange (McBride,
1994). This process would be favored at lower pH, corroborating the IR data. We cannot
rule out the possibility that some NO_{2}^{-} might undergo anion exchange as well, where an
electrostatic bond is formed between anionic NO_{2}^{-} and positively charged goethite
surface sites (Sigg and Stumm, 1981; Essington, 2004).

A second main observation is the change in spectral appearance as the pH is
increased. There is a gradual shift of the peak at 1227 cm\(^{-1}\) to ~1220 cm\(^{-1}\) and the peaks
at 1255 cm\(^{-1}\) and 1185 cm\(^{-1}\) become weak shoulders at pH 7.5 when compared with pH
5.5 (Fig. 4.6). The peak at 1121 cm\(^{-1}\) is red-shifted and becomes attenuated as well. This
could indicate that the speciation of adsorbed NO_{2}^{-} is varying with pH. Additional
experiments employing deuterium oxide (D_{2}O) would be useful to elucidate the exact
role of H^{+} and OH^{-} in the configuration of the adsorbed nitrite-goethite surface complex.

4.2.5 Effect of goethite and NO_{2}^{-} loading levels

The IR spectra of the goethite samples reacted with increasing NO_{2}^{-} concentration
at pH 5.5 are shown in Fig. 4.5. With 2 gL\(^{-1}\) goethite, an increase in initial NO_{2}^{-}
concentration (0.1, 0.5, and 100 mM) resulted in no significant changes in IR spectra
(Fig. 4.5A,C, E). Regardless of NO_{2}^{-} loading levels, all spectra show four well-resolved
absorption peaks which were assigned previously. This finding can be reconciled when
one calculates the reactive surface site concentration for goethite. Assuming 1.7 sites per
nm\(^{2}\) (Pivovarov, 1997), the reactive surface sites in 2 gL\(^{-1}\) were calculated to be 0.23
mM. Thus, addition of 0.5 and 100mM initial NO_{2}^{-} concentrations would be in excess of
the surface site concentration and likely resulted in saturation and a negligible change in
the IR spectra.

At higher goethite concentration (10 gL\(^{-1}\), corresponding to a surface site
centration of 1.15 mM, the absorption peaks intensify for the 0.1 mM NO_{2}^{-} level
(Fig. 4.5B). The IR peaks at 1256 cm\(^{-1}\), 1221 cm\(^{-1}\), 1182 cm\(^{-1}\) and 1121 cm\(^{-1}\) are well-
resolved and increase in intensity over time, up to 120 min of reaction time. A similar trend was observed where 0.5 mM NO$_2^-$ was added (Fig. 4.5D). The IR spectra appeared different upon the addition of 100 mM NO$_2^-$ to 10 g L$^{-1}$ goethite (Fig. 4.5E) and the reason for this is not clear at this point. It is suggested that excited nitrite ion vibration (1121 and 1221 cm$^{-1}$) dominates at higher goethite loading. It is seen that nitrite absorption peaks at 1121 and 1256 cm$^{-1}$ increase (Fig. 4.5B, D).

These studies demonstrate that the number of active surface sites in goethite is an important factor in nitrite-goethite complexation. When excess surface sites on goethite are available, IR peaks corresponding to chelating nitrito surface complexes (Scheme 1c) increase in intensity. Where initial NO$_2^-$ concentrations are in excess over surface sites, there was a negligible change in IR band intensities for the lower goethite concentration, suggesting that saturation of surface sites was reached. These data interpretations can be supported by results presented earlier where NO$_2^-$ removal from the solution plateaus at longer reaction times at all pH conditions (5.5-7.5) (Chapter 3).

4.2.6 Effect of Fe$^{2+}$ addition on NO$_2^-$ interaction with goethite

The ATR-FTIR spectra of the Fe$^{2+}$-conditioned goethite (equilibrated for 24 hr) (Fig. 4.7A) shows intensification of absorption peaks associated with $\nu_s$ and $\nu_{as}$ vibration of adsorbed NO$_2^-$ ions at 1191 and 1257 cm$^{-1}$ overtime. However, peak vibrations assigned to the excited state of NO$_2^-$ (1121 and 1221 cm$^{-1}$) observed in non-Fe$^{2+}$ conditioned experiments (Fig. 4.4 and 4.5) were not seen. It is assumed that metastable excited surface NO$_2^-$ species are consumed rapidly concurrent to rapid NO$_2^-$ reduction by Fe$^{2+}$-conditioned goethite (Chapter 3). There was appearance of an IR peak at 2305 cm$^{-1}$, assigned to adsorbed N$_2$O, which increases with increasing time. This N$_2$O peak was more intense than that observed in the NO$_2^-$-goethite alone experiments (see Fig. 4.4) which suggests that more NO$_2^-$ underwent electron transfer reactions when Fe$^{2+}$ was added. Formation of N$_2$O in a Fe$^{2+}$-goethite-nitrite system was higher than in a system without added Fe$^{2+}$ (Fig. 3.5, Chapter 3). In addition, the vibrational frequency assigned to NO$_{\text{ads}}$ at 1792 cm$^{-1}$ was observed after 15 min and 1 hr and was negligible after 2 hr (Fig. 4.7A). This suggests that NO is a short lived intermediate during NO$_2^-$ reduction to N$_2$O.
Optical spectra, equivalent to $F(R)$, of hydrated goethite without added Fe$^{2+}$ and NO$_2^-$ revealed five distinct absorption peaks (10448, 15212, 20162, 23995, and 29079 cm$^{-1}$) in the range of 8000 to 34000 cm$^{-1}$ (solid dark spectrum in Fig 4.7B). The SCF-α calculation of Tossel (1978) suggests that the broad band system centered around 29079 cm$^{-1}$ may be assigned to transitions between the oxygen non-bonding and metal d-orbitals (O$\rightarrow$Fe$^{3+}$ charge transfer). All other four bands observed in the spectrum of control goethite are analogous, and also within the molecular orbital calculations reported previously (Strens and Wood, 1979; Sherman and Waite, 1985; Scheinost et al., 1999a).

Based on these studies, weak absorption maxima observed at 10448 cm$^{-1}$ and 15212 cm$^{-1}$ from untreated goethite paste in Fig 4.7B are assigned to the $^6A_1\rightarrow^4T_1$ and $^6A_1\rightarrow^4T_2$ transitions of octahedrally coordinated Fe$^{3+}$ in goethite structure. Another absorption feature at 23995 cm$^{-1}$ corresponds to that of $^6A_1\rightarrow^4E; ^4A_1$ ligand field transition. This band is commonly absent in the spectra of Fe$^{3+}$-minerals without magnetic coupling to other Fe$^{3+}$ ions (Sherman and Waite, 1985). The band that occurs as inflection at 20162 cm$^{-1}$ can be assigned to d-d electron pair transition (EPT), ($^6A_1+^6A_1$) $\rightarrow$ ($^4T_1+^4T_1$) of a goethite structure resulting from magnetic coupling of adjacent edge or face sharing octahedral Fe$^{3+}$ cations similar to that in Fe(O,OH)$_6$ and ferrihydrite (Sherman and Waite, 1985; Scheinost et al., 1998). Although discrepancy can occur from magnetic coupling, EPT band energy is approximately the sum of two single ion Fe$^{3+}$ ligand field transitions, $2(^6A_1) \rightarrow 2(^4T_1)$ (Sherman and Waite, 1985).

Diffuse reflectance spectrum obtained from relatively unoxidized Tutton’s salt show splitting (10800, and 8400 cm$^{-1}$) of the peak centered at ~10500 cm$^{-1}$ (~950 nm) indicate the presence of Fe$^{2+}$ (Hawthorne, 1988) (Fig 4.7B). This band corresponds to the d-d spin ($d^6$) allowed electronic transition of $^5T_{2g} \leftarrow ^5E_g$ in the octahedral sites of hexaaquo Fe$^{2+}$-Fe(H$_2$O)$_6^{2+}$ (Cotton and Meyers, 1960). Doublet structure separated by nearly 2000 cm$^{-1}$ is due to Jahn-Teller distortion associated with the orbital degeneracy of the $T_{2g}$ ground term (Figgis and Hitchman, 1999). Goethite paste equilibrated with Fe$^{2+}$ for 24 hr shows significant shifts at 10448, 15212, and 20162 cm$^{-1}$ electron transition bands. The band assigned to $^6A_1\rightarrow^4T_1$ (10448 cm$^{-1}$) and $^6A_1\rightarrow^4T_2$ (15212 cm$^{-1}$) shift to lower wavenumber by ~150 cm$^{-1}$ (green dotted spectrum in Fig 4.7B).
This shift of absorption band ($^6A_1 \rightarrow ^4T_1$) towards lower energy might indicate that Fe$^{2+}$ binds on surface hydroxyls of goethite in an inner-sphere surface complex. Because OH$^-$ and O$^{2-}$ ligands both lie below H$_2$O in the spectrochemical series (Holleman and Wiberg, 2001), absorption band shift to lower energy level when Fe$^{2+}$ binds onto these goethite surface hydroxyls. Within the experimental pH condition considered for this study, and the first pK$_a$ (7.7) value reported for $\equiv$Fe-OH$^{0.5}$ surface complex for goethite (Hiemstra et al., 1989; Boily et al., 2001), it is more reasonable to propose Fe$^{2+}$ binding with OH$^-$ ligand than with O$^{2-}$. If Fe$^{2+}$ was bound as an outer-sphere complex, where the waters of hydration are retained upon adsorption (Essington, 2004), absorption peak would look similar to Tutton’s salt (hexaaquo standard). Notably, band $^6A_1 \rightarrow ^4T_1$ shift to lower energy is less than expected based on ligand field theory. In case of complete inner sphere complexation, the band shift should lie, at least in theory, below 8960 cm$^{-1}$. Close monitoring of shifts in band position (cm$^{-1}$) during Fe$^{2+}$-goethite complexation over a range of Fe$^{2+}$ loading could provide more accurate estimate of metal ligand bond distance.

Goethite samples equilibrated with Fe$^{2+}$($aq$) also show increase in absorption intensity and the band (15212 cm$^{-1}$) shift towards low wavenumber (15026 cm$^{-1}$). This change observed after Fe$^{2+}$ addition could be attributed to the Fe$^{2+} \rightarrow$Fe$^{3+}$ charge transfer, also known as intervalence charge transfer (IVCT), between adjacent Fe$^{2+}$ and Fe$^{3+}$ ions in edge- or face sharing goethite polyhedra as suggested previously (Burns et al., 1980). The increased intensity indicates that Fe$^{3+}$ within the octahedral sheets is being reduced via Fe$^{2+}$($ads$)→Fe$^{3+}$ electron transfer. Reduction of Fe$^{3+}$ in octahedral structure of nontronite mineral have shown similar band shift (Merola and McGuire, 2009). Evidence of Fe$^{2+}$($ads$)→Fe$^{3+}$ electron transfer have been reported in the past (Williams and Scherer, 2004; Larese-Casanova and Scherer, 2007). IVCT from Fe$^{2+} \rightarrow$ Fe$^{3+}$ is reported to occur at 14285 cm$^{-1}$ (700 nm) in amphibole, 15384 cm$^{-1}$ (650 nm) in green rust, and 13330 cm$^{-1}$ (750 nm) in Fe$^{2+}$-added nontronite (Hawthorne, 1988; Hansen, 1989; Merola et al., 2006).
Importantly, EPT band, \(2(6A_1) \rightarrow 2(4T_1)\) (20162 cm\(^{-1}\)) shifts towards higher energy values after Fe\(^{2+}\) addition. Shifts in EPT to higher energy are caused when magnetic coupling between neighboring Fe\(^{3+}\) centers is increased in face sharing (octahedral) iron oxide structures (Sherman and Waite, 1985). The fitting of EPT band at 20162 cm\(^{-1}\) was satisfactory, and the shifts to higher wave number with added Fe\(^{2+}\) are in line with spectroscopic results of (Kosmas et al., 1986). In their study, Kosmas et al (1986) observed EPT band shift towards lower wavelength in Al-substituted goethite compared to samples with no Al-substitution. As suggested previously by Scheinost et al (1999a), decrease in goethite crystal symmetry as a result of Al-substitution could shift the EPT and \(6A_1 \rightarrow 4E; 4A_1\) band to higher energy levels, and a similar process might be operational in our study after Fe\(^{2+}\) addition. Although the electronic transition band \(6A_1 \rightarrow 4E, 4A_1\) did not increase, the EPT transition to higher energy values is strong evidence to support electron transfer between adsorbed Fe\(^{2+}\) and octahedral Fe\(^{3+}\) in goethite. Alternatively, increase in absorbance observed in the EPT band also suggests formation of Fe–oxyhydroxide cluster. Optical spectroscopic investigation of a neutralizing acid mine drainage (AMD) showed progressive increase in absorbance intensity in the EPT region while the solution was precipitating Fe-oxyhydroxide (Zhu et al., 2012). A strong absorption feature observed in the EPT band in this study was attributed to the Fe cluster or particles formed during AMD neutralization. Most probably, adsorbed Fe\(^{2+}\) species at the goethite interface acted as a cluster to facilitate Fe\(^{2+}\)→Fe\(^{3+}\) electronic transition.

Immediately after NO\(_2^−\) addition, there was a quick increase in EPT intensity which then began to drop gradually at longer time intervals (Fig. 4.7B). Absorption bands of \(6A_1 \rightarrow 4T_1\), and \(6A_1 \rightarrow 4T_2\) of Fe\(^{2+}\)-goethite samples treated with NO\(_2^−\) are relatively more intense than goethite alone. However, with longer reaction time, absorption intensity decreases, and the band \(6A_1 \rightarrow 4T_1, 6A_1 \rightarrow 4T_2\) shifts towards longer wavelengths (Table 4.2). Mean crystal size measurements tabulated in Table 4.2 indicate overlapping values showing there is a tendency for the particle size to increase in the order: goethite control > Fe\(^{2+}\)-goethite control > NO\(_2^−\) treated Fe\(^{2+}\)-goethite samples. This evidence supports that goethite like Fe-(oxyhydr)oxide could precipitate during oxidation of Fe\(^{2+}\)\(_\text{(ads)}\) by NO\(_2^−\). In the beginning, immediately after NO\(_2^−\) addition, oxidation of Fe\(^{2+}\)\(_\text{(ads)}\) could yield low crystalline Fe-(oxyhydr)oxide on goethite-water interface. As time
progresses, low crystalline precipitate could aggregate or grow larger. Based on previous studies, Scheinost et al (1999a) suggested deviation in particle size from ~0.8 µm decrease Mio scattering, and oscillation of the absorption and the diffusion efficiency. This ultimately results in a shift in visible intensity to darkening and reddening causing absorption intensity to decline. With increasing goethite mineral crystallinity, Williams et al. (2002) observed shift of absorption peak towards higher energy region and decrease in absorption intensity (Williams et al., 2002). Probably, goethite-like early precipitate produced from Fe$^{2+}$ oxidation by NO$_2^-$ in our study is gradually aging and converting to well crystallized particles, and thus causing observed shifts in band peak and depth of $^6A_1 \rightarrow ^4T_1$, $^6A_1 \rightarrow ^4T_2$ electronic transition (Table 4.2).

As the reaction progressed after NO$_2^-$ addition, there was a steady drop in absorption intensity of band $^6A_1 \rightarrow ^4E; ~ ^4A_1$ (23995 cm$^{-1}$). Yet, the shift in peak position was subtle, and towards low energy. Based on a study from Barnes et al (1972), electronic transition from Fe$^{2+}$-NO$_2^-$ complexes is likely to occur in this region. This is because the lowest energy allowed region for optically reducible metal (Fe$^{2+}$) to NO$_2^-$ electron transfer transition is expected in the 24000-25000 wavenumber region. Although small, energy transition observed in 23995 cm$^{-1}$ absorption band suggests Fe$^{2+}$ (ads) binding with NO$_2^-$ via oxygen atom. If the Fe$^{2+}$-NO$_2^-$ binding had been via N ligation, the transition would have occurred at much higher energy (Barnes et al., 1972).

The absorption peak at 27079 cm$^{-1}$, assigned to O→Fe$^{3+}$ charge transfer, is almost unchanged. Assuming the partially adsorbed Fe$^{2+}$ in the goethite interface could have changed to surface precipitated form, an unchanged peak position is assumed to be due to similar Fe-O bond length of goethite and precipitated iron oxyhydroxide. Bond length of Fe-O in poorly crystalline ferrihydrite or similar iron oxyhydroxides and goethite are similar (Jambor and Dutrizac, 1998). Data from x-ray and scanning electron microscopy (SEM) corroborates our spectroscopic results. Goethite and Fe$^{2+}$-goethite samples retrieved after reaction with NO$_2^-$ were identical in mineralogy (Fig. 3.7 and 3.8 from Chapter 3).
4.3 Conclusion

For the first time, \textit{in situ} ATR-FTIR and UV-VIS-NIR spectra of NO$_2^-$ treated goethite and Fe$^{2+}$-goethite complex have been presented. Spectral evidence shows that NO$_2^-$ is adsorbed and transformed in ambient temperature and pH (5.5) conditions at the goethite, and Fe$^{2+}$-goethite water interface for reaction times less than 2 hr. The coordination of nitrite with goethite was found to be chelating in nature (nitrito structure) whereby two oxygens of NO$_2^-$ are bound to metal (surface Fe$^{3+}$) cations. Thus, nitrite adsorbs to goethite as an inner-sphere surface complex. In addition, ATR-FTIR data indicate the presence of surface-bound NO and surface N$_2$O species on goethite. This novel finding might explain the nonstoichiometry between nitrite removal and N$_2$O production observed in Chapter 2. At higher pH conditions (6.5 and 7.5), nitrite adsorption onto goethite is limited or low.

Although the metal-ligand bond distance could not be quantified, spectroscopic shift in the $^6\text{A}_1 \rightarrow ^4\text{T}_1$, electronic transition towards lower energy suggests Fe$^{2+}$ is binding directly to surface hydroxyl sites of goethite. An electronic transition observed at 15026 cm$^{-1}$ provides spectroscopic evidence for Fe$^{2+}$→Fe$^{3+}$ electron transfers (IVCT). Findings from spectroscopic study and wet chemical study suggest oxidation of Fe$^{2+}_{(\text{ads})}$ could be reason in particle size increasing tendency of reacted goethite. The formation of surface NO$_2^-$ species and reaction product of Fe$^{2+}$-goethite mediated NO$_2^-$ reduction revealed in this study shows strong N-Fe coupling in soil and sediments under Fe$^{2+}$ reducing conditions.
Table 4.1 Position of IR peak maxima of dissolved nitrate, nitrite, solid NaNO₂, and metal-nitrite possible surface complexes.

<table>
<thead>
<tr>
<th>Species</th>
<th>Phase</th>
<th>IR active band(cm⁻¹)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ν₁ (νₛ)</strong></td>
<td><strong>ν₃ (νₘₐ)</strong></td>
<td><strong>ν₂ (δNO₂⁻)</strong></td>
<td></td>
</tr>
<tr>
<td><em>Free NO₃⁻ ion</em></td>
<td>NO₃⁻(aq)</td>
<td>1348(s), 1035-970 (m,w)</td>
<td>† Ref 1</td>
</tr>
<tr>
<td><em>Free NO₂⁻ ion</em></td>
<td>NO₂⁻(aq)</td>
<td>1335-1303(m), 1235-1208(s), 833-785(w)</td>
<td>† Ref 1,&amp;2</td>
</tr>
<tr>
<td><em>Solid NaNO₂</em></td>
<td>Ionic</td>
<td>1380-1305(w), 1270-1208(s), 845-805(m)</td>
<td>† Ref 2,3,5,6 &amp;</td>
</tr>
<tr>
<td>M—O—N=O (monodentate nitrito)</td>
<td>Structure</td>
<td>1485-1375 (νₑ¹⁻), 1206-1050 (νₑ¹⁻)</td>
<td>Ref 1,4,&amp;7</td>
</tr>
<tr>
<td>(M₂—O)—N=O (bridging monodentate nitrito)</td>
<td>Structure</td>
<td>1495-1380 (νₑ¹⁻), 1235-1055 (νₑ¹⁻)</td>
<td>Ref 1,4, &amp;7</td>
</tr>
<tr>
<td>(M—O₂)2—N (chelating bidentate nitrito)</td>
<td>=Surface</td>
<td>1192-1182 (νₛ NO₂⁻), 1261-1256 (νₘ NO₂⁻)</td>
<td>Ref 1,4,7, &amp;8</td>
</tr>
<tr>
<td>=Surface</td>
<td>1121-1112 (νₛ NO₂⁻), 1227-1218 (νₘ NO₂⁻)</td>
<td>† Ref 6</td>
<td></td>
</tr>
<tr>
<td>(M—O₂)=N (bridging bidentate nitrito)</td>
<td>=Surface</td>
<td>1225-1192 (νₛ NO₂⁻), IR inactive</td>
<td>Ref 1,4, &amp;9</td>
</tr>
<tr>
<td>M—NO₂ (nitro-compound)</td>
<td>Structural</td>
<td>1365-1250 (νₛ NO₂⁻), 1650-1340 (νₘ NO₂⁻)</td>
<td>Ref 1,4,5, &amp;7</td>
</tr>
<tr>
<td>NO and N₂O</td>
<td>≡Surface</td>
<td>~1791 (N=O of NO), ~2306 (N=N of N₂O)</td>
<td>† Ref 10</td>
</tr>
</tbody>
</table>

s=strong peak; m=moderate peak; w=weak peak; † See Fig. 2A&B; Surface species proposed by Ref 1 (Table 4)

† = This Study  
1= Hadjiivanov (2000)  
2= Weston and Brodasky (1957)  
3= Miller and Wilkins (1952)  
4= Hitchman and Rowbottom (1982)  
5= Nakamoto (1986)  
6= Cerruti et al. (1974)  
7= da Cunha et al. (1996)  
8= Rima et al. (2010)  
9= Davydov (2003)  
10= Singireddy et al. (2012)  
11= Laane and Ohlsen (1986)
Table 4.2 Differences in band position and absorption intensity measured from the DRS spectra shown in Fig. 4.7B, and average crystal size measured from corresponding treatment obtained after 48 hr using scanning electron microscopy (SEM) image.

<table>
<thead>
<tr>
<th></th>
<th>$^6A_1 \rightarrow ^4T_1$</th>
<th>$^6A_1 \rightarrow ^4T_2$</th>
<th>$2(^6A_1) \rightarrow 2(^4T_1)$</th>
<th>Avg. crystal size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GT$_{51}$ paste- Ctrl</td>
<td>10448 0.58</td>
<td>15212 0.51</td>
<td>20162 3.0</td>
<td>0.79±0.23</td>
</tr>
<tr>
<td>GT$_{51}$+ Fe$^{2+}$ paste-Ctrl</td>
<td>10314 2.3</td>
<td>15026 1.6</td>
<td>21122 5.1</td>
<td>0.80±0.09</td>
</tr>
<tr>
<td>GT$_{51}$+ Fe$^{2+}$-NO$_2$-15 min</td>
<td>10468 1.7</td>
<td>15218 1.2</td>
<td>21109 5.3</td>
<td>-</td>
</tr>
<tr>
<td>GT$_{51}$+ Fe$^{2+}$-NO$_2$-2hr</td>
<td>10612 0.9</td>
<td>15349 0.7</td>
<td>21103 3.8</td>
<td>0.84±0.19</td>
</tr>
</tbody>
</table>

† Band (Peak) position in cm$^{-1}$
†† Absorption intensity (Kubelka-Munk)
††† Crystal size measured from 48 hr treated samples, n>25
Figure 4.1 ATR-FTIR (Thermo Nicolet 6300) set up used for collecting IR spectra in this study. Inset (A) shows custom set up built to maintain anoxic environment during data collection by continuously purging the sample with Ar. Inset (B) shows the diamond crystal sample holder seen on digital recording magnifier before (without sample) and during experiment.
Figure 4.2 (A) ATR-FTIR spectrum of solid NaNO₂. A solid dark line is experimental data collected in this study, and a light grey line represents data from Hitchman and Rowbottom (1982), reprinted by permission of Elsevier Ltd. (B) IR spectrum of aqueous NO₃⁻ and NO₂⁻ (0.1 and 100 mM) at pH 5.5. Scheme in the top figure illustrates the possible structure of the assigned surface NO₂⁻ species.
Figure 4.3 ATR-FTIR spectrum of (a) water at pH 5.5, (b) 25 mM MES buffer solution at pH 5.5, (c) goethite slurry at pH 5.5 (in MES solution), (d) MES subtracted goethite paste, and (d) a dry goethite. Inset shows magnified portion of a deconvoluted ATR-FTIR spectrum of water at pH 5.5.
Figure 4.4 Time series ATR-FTIR spectra obtained during the reaction of NO$_2^-$ (0.1 mM) with goethite (10 g L$^{-1}$). Spectrum (e) is control goethite paste without NO$_2^-$, and (f) is dry goethite. Detail spectral changes observed in the region inside inset for various NO$_2^-$ goethite loading are presented in Fig. 4.5.
Figure 4.5 Time series ATR-FTIR spectra: Effect of NO$_2^-$ loading (0.1, 0.5, 100 mM) and surface sites (2 and 10 gL$^{-1}$) on NO$_2^-$ adsorption onto goethite. GT$_{51}$ represents ATR-FTIR of an unreacted goethite sample.
Figure 4.6 ATR-FTIR spectra: pH effect (5.5, 6.5, and 7.5) on NO$_2^-$ (0.1 mM) adsorption (1 hr) on goethite (10 gL$^{-1}$) at the goethite-water interface. GT$_{51}$ represents ATR-FTIR of an unreacted goethite sample.
Figure 4.7 ATR-FTIR spectra: (A) Effect of added Fe$^{2+}$ (0.1 mM) on 0.1 mM NO$_2^-$ adsorption at goethite (10 g L$^{-1}$) - water interface at pH 5.5. (B) Kubelka-Munk remission function for a goethite (GT$_{51}$) paste as control and Fe$^{2+}$-conditioned goethite paste extracted before and after reaction with NO$_2^-$. Spectrum of Tutton’s salt (dry) represents hexaqua [Fe$^{2+}$-Fe(H$_2$O$_6^{2+}$)]-standard.

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Chapter Five: Summary and Future Research

Summary

Nitrite was more rapidly removed from solution than NO$_3^-$ by both magnetite and goethite at ambient temperature and pH (5.5) conditions. In a system containing only magnetite or goethite, NO$_2^-$ removal was highest at pH 5.5. With increasing pH (6.5 and 7.5), nitrite removal gradually decreased indicating pH dependence of the reaction. At pH 5.5, initial rate of NO$_2^-$ removal by only magnetite or goethite were comparable, and the rate increased nearly three (in magnetite) and five (in goethite) fold in a system conditioned with Fe$^{2+}$. Addition of Fe$^{2+}$ to magnetite or goethite slurries resulted in adsorption and an accelerated the rate of NO$_2^-$ reduction at pH values considered in this study. Regardless of Fe$^{2+}$ addition, the kinetics of NO$_2^-$ removal from solution by magnetite and goethite at pH 5.5 reasonably agreed with a first order model at early reaction times (0-72 hr), respectively. The main products of NO$_2^-$ reaction with magnetite were N$_2$O and NO, and a partially oxidized magnetite. The XRD pattern of oxidized magnetite closely resembles maghemite. N$_2$O remained the main reaction product when NO$_2^-$ was reacted with goethite amended with Fe$^{2+}$.

Results from Mössbauer spectroscopy suggested that structural Fe$^{2+}$ on magnetite served as a reductant of NO$_2^-$. The ATR-FTIR study of NO$_2^-$ reactivity with goethite and Fe$^{2+}$-goethite complex revealed that NO$_2^-$ is present in the surface-adsorbed species where two oxygens of nitrite bind with metal cation via chelation. Nitric oxide and N$_2$O were observed as reaction products and were short-lived. Also, by observing characteristic shifts in energy bands related to electronic transition, evidence of direct Fe$^{2+}$ binding on surface hydroxyl of goethite, and NO$_2^-$ adsorption to metal was probed using diffuse reflectance spectroscopy.

Taken together as a whole, results of the experiment presented in this study have shown that abiotic NO$_2^-$ reduction, and or removal from solution is mediated by iron oxide(s) such as goethite and magnetite. Because magnetite and goethite are ubiquitous iron oxide minerals found in a wide range of environmental conditions, soil and sediments that contain these minerals can catalyze abiotic NO$_2^-$ reduction with negligible reactions towards NO$_3^-$. An example of a natural environment where this process could
be important would be an anoxic zone where NO$_3^-$ percolated from still oxidizing layer intersects Fe$^{3+}$-reducing zone. In such a natural setting, NO$_3^-$ reduction to NO$_2^-$ could be the first step that is biologically mediated. Parallel to NO$_3^-$ reduction, Fe$^{2+}$ accumulated in the solution oxidizes, and or precipitates as goethite and magnetite. Observed results from this study provide strong evidence of secondary reactions involving NO$_2^-$ and magnetite or goethite that would further lead to some NO and N$_2$O production. This potentially explains why NO$_2^-$ is short lived in the anoxic soil and groundwater under Fe$^{3+}$-reducing condition.

**Future research thrust**

A next logical step would be to follow these abiotic processes in column experiments to investigate and compare nitrite reduction rates in natural settings. By following relevant redox species in a goethite-coated sand-packed column with sampling ports located at various depths would allow one to vary the redox conditions and nitrite concentrations in water flowing through the column.

Another gap in the literature would be to study siderite (Fe$^{2+}$CO$_3$) and pyrite (Fe$^{2+}$S$_2$) which have been shown to reduce NO$_2^-$ at ambient temperature (22°C) (Singireddy et al., 2012; Rakshit et al., 2008). In both studies, the rate of NO$_2^-$ reduction accelerated at higher temperature. Nitric oxide (NO) formed during NO$_2^-$ reduction by pyrite (Fe$^{2+}$S$_2$) slurry was measured as adsorbed species to pyrite. Based on these results, it would not be speculative to say NO$_2^-$ reduction by goethite or magnetite would be higher at higher temperature. This could be investigated in the future in order to understand temperature dependence of NO$_2^-$ reduction by goethite and magnetite. Also, there is a large research gap in understanding particle size control on nitrite removal rate. This could be accomplished using macro and nano size iron oxide minerals.
Appendices

Appendix A: Calculation of Magnetite Stoichiometry

Because each magnetite molecule consists of 3 iron (Fe) atoms, total Fe for 10gL⁻¹ stoichiometric magnetite (Fe₃O₄) would be:

\[
10 \frac{g}{L} Fe_3O_4 \times \frac{1 \text{ mol} Fe_3O_4}{231.54 g} \times \frac{3 \text{ Fe}}{1 \text{ mol} Fe_3O_4} = 129.5 \text{ mM Fe}
\]

Ratio of Fe²⁺/Fe³⁺ for stoichiometric magnetite: \( x = \frac{Fe^{2+}}{Fe^{3+}} = 0.50 \)

Let Fe³⁺ = y. Then:

129.5 mM Fe = (0.5y + y)

Fe³⁺ = y = 86.33 mM Fe³⁺

Fe²⁺ = 129.5 - 86.33 = 43.16 mM Fe²⁺

For 10gL⁻¹ stoichiometric maghemite (γ-Fe₂O₃)

\[
10 \frac{g}{L} Fe_2O_3 \times \frac{1 \text{ mol} Fe_2O_3}{159.69 g} \times \frac{2 \text{ Fe}}{1 \text{ mol} Fe_2O_3} = 125.25 \text{ mM Fe}
\]

Based on Mössbauer results, Fe²⁺/Fe³⁺ stoichiometry of the unreacted magnetite used in this study was measured as 0.47, and using the scheme proposed previously, (Gorski and Scherer, 2009; Latta et al., 2011) relative proportions of maghemite (Magh) and magnetite (Mag) are used to calculate the concentration:

Mag = fraction of Fe₃O₄, and Magh = Fraction of γ-Fe₂O₃

\[
[Fe^{2+}] = Mag \times 43.16
\]

\[
[Fe^{3+}] = 125.25 \times Magh + 86.33 \times Mag
\]

Mass balance: Magh + Mag = 1

\[
[Fe^{3+}] = 125.25 \times (1 - Mag) + 86.33 \times Mag
\]

\[
[Fe^{3+}] = 125.25 - 38.92 \times Mag
\]

\[
\frac{Fe^{2+}}{Fe^{3+}} = \frac{43.16 \times Mag}{125.25 - 38.92 \times Mag}
\]

Rearranging for Mag:

\[
x = \frac{Fe^{2+}}{Fe^{3+}} = \frac{43.16 \times Mag}{125.25 - 38.92 \times Mag}
\]


Mag = \frac{125.25 \times x (=0.47)}{43.16 + 38.92 \times x (=0.47)} = 0.958

[Fe^{2+}] = Mag \times 43.16 = 0.958 \times 43.16 = 41.35 \text{mM Fe}^{2+}

[Fe^{3+}] = 125.25 - 38.92 \times Mag = 125.25 - 38.92 \times 0.958 = 87.96 \text{mM Fe}^{3+}

On the basis of our wet chemical data, nearly 150µM NO_2^− reduced in 14 days at pH 5.5. Reduction of NO_2^− to N_2O requires 2 electrons. If structural Fe^{2+} in magnetite is taken as source of electron supply for the reduction of NO_2^− to proceed, reduction of 150µM NO_2^− would require 0.3mM e- equivalents from magnetite Fe^{2+}.

Final x is calculated as follows:

x = \frac{41.35 - 0.3}{87.96 + 0.3} = 0.46

Therefore, if x of the magnetite in the beginning is 0.47, reacted magnetite stoichiometry would be nearly 0.46. Stoichiometric values calculated here for untreated and treated magnetite samples is nearly identical to those obtained from Mössbauer studies.
Appendix B: A Back-of-the envelope calculation showing the extent of abiotic and microbial NO$_2^-$ reduction in soil (Kg NO$_2^-$N ha$^{-1}$ day$^{-1}$) and sediments that composes ~2% magnetite, and 0.1% nitrite reducers per kg of soil, respectively.

Assumption:

a) Abiotic NO$_2^-$ reduction rate by magnetite at pH 5.5 in the absence or presence of Fe$^{2+}$ is taken from Table 1.

b) Microbial NO$_2^-$ reduction rate by mixed culture nitrite reducers in soils is assumed 0.42×10$^{-9}$ (M g$^{-1}$ sec$^{-1}$) (Wilderer et al., 1987). A population of anaerobic NO$_2^-$ reducing bacteria was assumed to be present at a level of 10$^9$ per gram of soil. As a rule of thumb, 1 bacterium is equal to 10$^{-12}$g. Therefore, ~1.0g nitrite reducers are estimated in 1Kg of soil.

c) Anoxic soil layer thickness is assumed 15 cm with density of 1.2g soil/cm$^3$.

Case I: Kg of NO$_2^-$N removed per acre of soil per day by magnetite (abiotic) without Fe$^{2+}$

\[
\frac{\text{Kg NO}_2^-=\text{N}}{\text{ha.day}} = \frac{8.14 \times 10^{-10} \text{M NO}_2^-=\text{N}}{10 \text{ g Fe}_3\text{O}_4 \cdot \text{sec}} \times \frac{86400 \text{ sec}}{1 \text{ day}} \times \frac{1.2 \text{g soil}}{1 \text{ cm}$^3$} \times \frac{15 \text{cm}}{1} \times \frac{100^2 \text{ cm}^2}{1 \text{ m}$^2$} \times \frac{10,000 \text{ m}^2}{1 \text{ ha}} \times \frac{20 \text{g Fe}_3\text{O}_4}{1000 \text{g soil}} \times \frac{14 \text{g NO}_2^-=\text{N}}{1 \text{M NO}_2^-=\text{N}} \times \frac{1 \text{Kg NO}_2^-=\text{N}}{1000 \text{g NO}_2^-=\text{N}}
\]

\[
\frac{\text{Kg NO}_2^-=\text{N}}{\text{ha.day}} = 3.55
\]

Similarly,

Case II: Kg of NO$_2^-$N removed per acre of soil per day by magnetite (abiotic) with Fe$^{2+}$

\[
\frac{\text{Kg NO}_2^-=\text{N}}{\text{ha.day}} = \frac{2.60 \times 10^{-9} \text{M NO}_2^-=\text{N}}{10 \text{ g Fe}_3\text{O}_4 \cdot \text{sec}} \times \frac{86400 \text{ sec}}{1 \text{ day}} \times \frac{1.2 \text{g soil}}{1 \text{ cm}$^3$} \times \frac{15 \text{cm}}{1} \times \frac{100^2 \text{ cm}^2}{1 \text{ m}$^2$} \times \frac{10,000 \text{ m}^2}{1 \text{ ha}} \times \frac{20 \text{g Fe}_3\text{O}_4}{1000 \text{g soil}} \times \frac{14 \text{g NO}_2^-=\text{N}}{1 \text{M NO}_2^-=\text{N}} \times \frac{1 \text{Kg NO}_2^-=\text{N}}{1000 \text{g NO}_2^-=\text{N}}
\]

\[
\frac{\text{Kg NO}_2^-=\text{N}}{\text{ha.day}} = 11.32
\]

Case III: Kg of NO$_2^-$N removed per acre of soil per day by mixed culture NO$_2^-$ reducers

\[
\frac{\text{Kg NO}_2^-=\text{N}}{\text{ha.day}} = \frac{0.42 \times 10^{-9} \text{M NO}_2^-=\text{N}}{1 \text{ g soil.sec}} \times \frac{86400 \text{ sec}}{1 \text{ day}} \times \frac{1.2 \text{g soil}}{1 \text{ cm}$^3$} \times \frac{15 \text{cm}}{1} \times \frac{100^2 \text{ cm}^2}{1 \text{ m}$^2$} \times \frac{10,000 \text{ m}^2}{1 \text{ ha}} \times \frac{1 \text{g microbes}}{1000 \text{g soil}} \times \frac{14 \text{g NO}_2^-=\text{N}}{1 \text{M NO}_2^-=\text{N}} \times \frac{1 \text{Kg NO}_2^-=\text{N}}{1000 \text{g NO}_2^-=\text{N}}
\]

\[
\frac{\text{Kg NO}_2^-=\text{N}}{\text{ha.day}} = 0.92
\]
Appendix C: Reactive surface sites, Fe$^{2+}$ addition and potential for Fe(OH)$_2$ precipitation.

Before NO$_2^-$ addition, nearly 1.5mM of Fe$^{2+}$ was added to goethite suspension and equilibrated for 24 hr. Observation shows that nearly 0.6mM of Fe$^{2+}$ was removed from the reaction vessel prior to NO$_2^-$ addition (Figure Fe$^{2+}$ sorption). Assuming 1.7 site per nm$^2$ (Pivovarov, 1997) for goethite, approximately 0.23mM reactive surface area were present in our experimental solution. This is much lower than the initial Fe$^{2+}$ solution removed (0.6mM). This indicates that Fe$^{2+}$ removed from the solution was in excess of the amount of Fe$^{2+}$ needed to form complete monolayer coverage from reacted Fe$^{2+}$. Assuming one Fe$^{2+}$ binding sites for each reactive sites in goethite, ratio of Fe$^{2+}$ removed by goethite results in 1:2.6 (Fe$^{2+}$: Fe$^{3+}$).

Importantly, removal of Fe$^{2+}$ continued beyond the calculated monolayer reference point which indicates that Fe$^{2+}$ loading onto goethite is oversaturated in terms of surface coverage. Isotopic signature from a study carried out to investigate arsenic transformation by $^{57}$Fe$^{2+}$-conditioned $^{56}$goethite indicate that nearly 95% of the added Fe$^{2+}$ removed from the solution was oxidized to goethite and 5% formed an unidentified Fe$^{2+}$ species (Amstaetter et al., 2009).

Potential for Fe(OH)$_2$ precipitation: Assuming equilibrium reaction as follows

$$\text{Fe(OH)}_2 (s) \leftrightarrow \text{Fe}^{2+} (aq) + 2\text{OH}^-$$

Fe$^{2+}$ remaining in the goethite solution after 24 hr at pH 5.5 is 0.9mM

Now, \( Q = [\text{Fe}^{2+}][\text{OH}^-] = 0.9 \text{mM} \times (10^{-8})^2 = 9.0 \times 10^{-19} \), and solubility constant for Fe(OH)$_2$ is \( k_{sp} \{\text{Fe(OH)}_2\} \) which is \( =1.0 \times 10^{-15} \). Because calculated \( Q < k_{sp} \), the system is undersaturated with respect to Fe(OH)$_2$ at 0.9mM Fe$^{2+}$ concentration at pH 5.5. Therefore, it is unlikely that Fe(OH)$_2$ precipitation and resulting solid phase to be a possible reactive phase.
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VITA

Prakash Dhakal

Education:

Tribhuvan University (TU), B.Sc. in Geology, 1999
Tribhuvan University (TU), M.Sc. in Engineering Geology and Geoinformatics, 2002
Auburn University (AU), M.S. in Geology, 2008
University of Kentucky (UK), seeking PhD in Soil Sciences, 2009 to present

Research and Professional Experience:

Assistant Lecturer (10/2002-01/2004), Central Department of Geology, TU
Geologist (02/2004-07/2006), Department of Mines and Geology, Kathmandu, Nepal
Research Assistant (08/2006-08/2008), Department of Geology and Geography, AU
(01/2009-Present), Department of Plant and Soil Sciences, UK
Teaching Assistant (05/2007-08/2007), Department of Geology and Geography, AU
(08/2010-12/2010)
(01/2012-05/2012) Department of Plants and Soil Sciences, UK
(01/2013-05/2013)

Referred Publications:


Other publication:

Presentations at International/National Meetings:


