ABIOTIC REDUCTION TRANSFORMATIONS OF RECALCITRANT
CHLORINATED METHANES, CHLORINATED ETHANES, AND 2,4-
DINITROANISOLE BY REDUCED IRON OXIDES AT BENCH-SCALE

A dissertation submitted in partial fulfillment of the
Requirements for the degree of
Doctor of Philosophy

By

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I HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER MY SUPERVISION BY Adam C. Burdsall ENTITLED Abiotic Reduction Transformations of Recalcitrant Chlorinated Methanes, Chlorinated Ethanes, and 2,4-Dinitroanisole By Reduced Iron Oxides at Bench-Scale BE ACCEPTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Doctor of Philosophy.

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ABSTRACT

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Abiotic Reduction Transformations of Recalcitrant Chlorinated Methanes, Chlorinated Ethanes, and 2,4-Dinitroanisole By Reduced Iron Oxides at Bench-Scale

Sites contaminated with chlorinated hydrocarbons are frequent and widespread, and with the rising use of insensitive high explosive (IHE) compounds, more widespread contamination is inevitable. In the cases of both classes of organic contaminants, natural attenuation is a critical component of our understanding of the environmental fate of these compounds. This dissertation is intended to expand the knowledge of potential abiotic natural attenuation mechanisms and, in the case of the study of chlorinated hydrocarbons, to examine degradation under variable pH conditions in the hopes of helping to develop minimally invasive remediation techniques. The results indicated that precipitated hydrolyzed Fe(II) species are more reactive toward chlorinated hydrocarbons than precipitated magnetite particles alone. The combination of precipitated magnetite with Fe(II) species at high pH were found to have a slightly slower reaction than Fe(II) species but produced more reduced byproducts than either Fe(II) species or magnetite particles alone.

Until this study, reduction of 2,4-dinitroanisole (DNAN) had not been studied with naturally occurring iron oxide minerals. Fe(II) added to hydrous ferric oxide and goethite at neutral to basic pH facilitated nitroreduction of insensitive explosive component, 2,4-dinitroanisole (DNAN) to various nitroaniline byproducts. Magnetite was
found to be a stronger reductant for DNAN, degrading it with and without Fe(II) amendments at pH 6 to 10. The study with magnetite and DNAN demonstrated that structural Fe(II) was more reactive than adsorbed Fe(II).
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Chapter I

Introduction and Purpose

The removal of chlorinated hydrocarbons by mineral phases is important in areas where biologically mediated natural attenuation might not remove recalcitrant pollutants. Most investigations into mineral phases include ferric iron oxides or hydroxides that need a source of ferrous iron to facilitate pollutant reduction. Mixed phase iron oxides were shown to be much stronger at reducing chlorinated hydrocarbons. Chapter II expands on some of the previous work with mixed phase iron oxides by examining them at high pH and without buffer. The work in chapter II also examined the effect of Fe(II) species that form at higher pH both by themselves and in association with magnetite nanoparticles on reaction potency and product distribution of chlorinated pollutants. Results show that Fe(II) alone produces species that may react better than magnetite alone and the combination of magnetite and Fe(II) species had a synergistic effect on pollutant removal at pH 10 or more. This work may be applied to anaerobic natural conditions at high pH with high iron concentrations such as wetlands or at the interface between oxidizing and reducing conditions in the subsurface but may also be applied in engineered conditions where a pH adjustment or minimally invasive techniques are needed.

The chemicals that make up insensitive high explosive (IHE) formulations are of increasing interest to the U.S. armed forces due to the lower heat and shock sensitivity that prevents injury and equipment loss due to unintentional detonation. Their increased use makes
environmental releases more likely, but little is known about their physical properties, their
toxicity to organisms, environmental impact, and their movement and fate in the subsurface.
The goal of review in Chapter III is to summarize the physical properties, partitioning behavior
and movement, transformations, and fate of the emerging insensitive high explosives (IHEs),
focusing on 2,4-dinitroanisole (DNAN), nitrotriazolone (NTO), and nitroguanidine (NQ).

DNAN, NTO, and NQ are organic compounds with nitro functional groups that are
designed to replace TNT and RDX. The solubility of DNAN is low and it is considered slightly
hydrophilic and adsorbs to carbon and organic matter, while NTO and NQ are more soluble.
DNAN shows a moderate toxicity by inhibiting bacterial growth and affecting reproduction of
larger animals. It is highly toxic if ingested. Little is known of NTO's toxicity, but it's believed
to be less toxic than DNAN. NQ is not considered toxic, but its reduction products have
relatively high toxicity.

DNAN, NTO, and NQ tend to undergo either reduction reactions at their nitro
substituents or they can also get oxidized which involves the replacement of a substituent with
a hydroxide group. DNAN may also produce Meisenheimer structures. Both DNAN and NTO
may produce azo dimer products. Degradation to innocuous products involves multiple steps.
Chapter III is a review that examines studies on fate and remediation of IHE compounds and
identifies gaps in the research and understanding of these compounds, offering some
suggestions of research directions. The sections pertaining to DNAN serve as an introduction
for Chapters IV and V in this dissertation.

The batch reactor study in Chapter IV is aimed at understanding the chemical
reactions and fate of DNAN in the reducing conditions of ferric iron minerals such as
hydrous ferric oxide (HFO) and goethite with Fe(II). This study used varying conditions
to predict the response of DNAN in the subsurface. DNAN degradation followed a nitroreduction pathway with iron oxide minerals that was mostly dependent on the concentration of aqueous or adsorbed Fe(II) and on the solution pH. Reaction mechanisms and iron speciation appear to be affected by pH. Mineral concentration had little effect on the potency of the reaction. Results suggest that ferric minerals with low [Fe(II)] would only partially reduce DNAN in the subsurface.

Chapter V is a batch reactor study that expands on the results of the previous study by examining magnetite to understand the effect of structural Fe(II) on nitroreduction reactions with DNAN. This study used various pH, [Fe(II)] and [magnetite] conditions to approximate natural conditions to study natural attenuation of DNAN. DNAN reduction with magnetite was dependent on increases in structural and adsorbed Fe(II) with structural Fe(II) being more important. Solution pH also influenced both reaction potency and mechanisms. Higher concentrations of magnetite nanoparticles produce a potent system that may quickly and completely reduce DNAN in the subsurface. This study also compared reactivity of magnetite to HFO and goethite from Chapter IV in the effort to compare the effects of mineral type on DNAN reduction and compare structural Fe(II) and adsorbed Fe(II).

These studies with DNAN lay the groundwork for future studies on the natural abiotic attenuation and fate of this and related IHE emerging contaminants like NTO and NQ. To the best of this investigator’s knowledge, abiotic reduction with iron oxide and other minerals has been lacking for IHEs and this study begins to delve into those topics. More work will be needed to further strengthen some of the observations made in this dissertation and to expand on the mechanisms observed or suspected in this study.
Additionally, further work is needed to continue the research into the behavior of structural versus adsorbed Fe(II), the speciation of Fe(II), and the behavior of Fe(II) species in the subsurface and in contact with oxidized iron minerals.
Chapter II

Bench-Scale Abiotic Degradation of Select Chlorinated Hydrocarbons (CHCs) with Chemogenic Ferrous Hydroxide and Magnetite Particles: Implications for Treatment and Fate

Abstract

The removal of chlorinated hydrocarbons by mineral phases is important in areas where biologically mediated natural attenuation might not remove recalcitrant pollutants. Most investigations into mineral phases include ferric iron oxides or hydroxides that need a source of ferrous iron to facilitate pollutant reduction. Mixed phase iron oxides were shown to be much stronger at reducing chlorinated hydrocarbons. This work expands on other studies with mixed phase iron oxides by examining them at high pH and without buffer. This work also examined the effect of Fe(II) species that form at higher pH both by themselves and in association with magnetite nanoparticles on reaction potency and product distribution of chlorinated pollutants. Results show that Fe(II) alone produces species that may react better than magnetite alone and the combination of magnetite and Fe(II) species had a synergistic effect on pollutant removal at pH 10 or more.

1.0 Introduction

Various reduced iron oxides have been investigated due to their reactivity towards easily reduced chlorinated hydrocarbons (CHCs) and for chlorinated ethenes in particular because abiotic reduction generates more acetylene or ethene than biological processes.
Various iron oxide minerals containing structural or adsorbed Fe(II) have shown strong reactivity toward common groundwater pollutants like chlorinated hydrocarbons (CHCs); the minerals include magnetite (Agarwal et al., 2011; Danielsen and Hayes, 2004; Liang et al., 2009; McCormick and Adriaens, 2004; Vikesland et al., 2007); sulfate green rust (Liang et al., 2009), chloride green rust (Maithreepala and Doong, 2005; Jeong et al., 2013), Fe(II) treated goethite (Amonette et al., 2000), and Fe(II) treated lepidocrocite (Agarwal et al., 2011). The CHC that has been examined with Fe(II)-bearing iron oxides in abiotic systems most commonly is carbon tetrachloride, and by extension, any of its degradation products, such as chloroform (CF), dichloromethane (DCM), chloromethane, and methane (Agarwal et al., 2011; Amonette et al., 2000; Danielson et al., 2004; Hanoch et al., 2006; Liang et al., 2009; Maithreepala and Doong, 2005; McCormick and Adriaens, 2004; Neumann et al., 2009; Vikesland et al., 2007).

Other CHCs examined in the literature for their potential to degrade by reduction with various Fe(II)-bearing iron oxides like magnetite and chloride green rust in abiotic systems include perchloroethene (PCE), which typically followed reductive dehalogenation, forming trichloroethene (TCE), followed by cis and trans dichloroethene (DCE), vinyl chloride (VC), and finally ethene (Lee et al., 2003; Maithreepala and Doong, 2005). Structural Fe(II) in smectite clays were used to degrade hexachloroethane, which followed dichloroelimination (forming perchloroethene), pentachloroethane, 1,1,2,2-tetrachloroethane (1,1,2,2-TeCA), and 1,1,1,2-TeCA typically followed dehydrochlorination, forming an ethene by removing a chlorine and a hydrogen (Neumann et al., 2009). The speciation of Fe(II) at alkaline pH can enhance transformation kinetics of organic compounds, such as pesticides (Strathmann and Stone,
2002), but the role of Fe(II) speciation under alkaline conditions in CHC transformations has been largely overlooked or, as in Jeong et al. (2013), were not a main focus of the study.

The formation of Fe(II) in natural systems by dissimilatory iron reducing bacteria is ubiquitous in anaerobic environments, which occurs upon bioreduction of Fe(III) oxides, like goethite, hematite, and magnetite (Amonette et al., 2000; Kostka and Nealson, 1995; Roden and Zachara, 1996). Fe(II) is generally understood to be relatively unreactive until it adsorbs to a mineral surface (Maithreepala and Doong, 2005; Amonette et al., 2000). Particularly, goethite can play an important role in holding adsorbed Fe(II) in a position favorable to react with CT (Amonette et al., 2000). Klausen et al. (1995) also reported that aqueous Fe(II) was unreactive toward nitrobenzene unless adsorbed to a mineral such as lepidocrocite, magnetite, or goethite, suggesting that the potential for nitro reduction reactions with Fe(II) may depend on the ambient conditions and the presence of mineral surfaces (e.g., iron oxides) on which to adsorb. For instance, as pH increases, the mineral surface can become more negatively charged and the sorption of Fe$^{2+}$ and FeOH$^+$ cations increases (Amonette et al., 2000), which would increase the number of reactive sites.

Structural Fe(II) is also found to be reactive in mixed phase iron oxides, e.g., magnetite. Perfectly stoichiometric magnetite has an Fe(II)/Fe(III) ratio of 0.5 and structural Fe(II) may reduce pollutants like CT without Fe(II) amendment (Danielsen and Hayes, 2004). However, if Fe$^{2+}$ is added to magnetite, its reactivity increased. Gorski and Scherer (2009) suggested that Fe(II) uptake can occur by partially oxidized magnetite with (Fe(II)/Fe(III) ratio <0.5); they reported that the "adsorbed Fe(II)" increased the
Fe(II)/Fe(III) ratio in magnetite to become 0.5 and the amount of Fe$^{2+}$ uptake was proportional to the amount of Fe(II) needed to restore mineral stoichiometry. Similarly, Amonette et al. (2000) found that goethite's reactivity could be regenerated by additional amendment of aqueous Fe$^{2+}$, which may be due to electron exchange between adsorbed Fe(II) and bulk mineral (Gorski and Scherer 2009). Further, it has been shown that solid ferric oxides mineral may not support stable surface Fe(II) species, but rather the Fe(II) surface species donated an electron to the underlying bulk phase. Schaefer et al. (2011) also observed electron exchange between adsorbed Fe(II) and nontronite. Further, Gorski et al. (2012) suggested that a Fe-atom exchange can occur, whereby minerals such as goethite, hematite, and magnetite, can exchange structural Fe(III) atoms in their lattices with aqueous Fe$^{2+}$.

Fe(II) may also transform into several reactive species (Naka et al., 2006; Strathmann and Stone, 2002) that are explained in greater detail in the supplemental Information (SI). Naka et al. (2006) indicated that aqueous Fe$^{2+}$ may react with hydroxide in water to form FeOH$^+_{(aq)}$ at neutral conditions. Other reactive species that can form at higher pH include Fe(OH)$_2_{(s)}$ (Jeong et al., 2013; Strathmann and Stone, 2002) and Fe(OH)$_3_{(s)}$ (Liu et al., 2013) beginning around pH 8.3. According to Liger et al. (1999), each of these Fe species can also develop an adsorbed form (e.g. =FeOFe$^+$, =FeOFeOH$^0$, and =FeOFe(OH)$_2^-$). Strathmann and Stone (2002) considered the role of FeOH$^+$ as a complicating factor because its contribution to the overall rate constants of carbamate pesticide reduction could not be separated from Fe(II) based on iron speciation, but rather had to be calculated with respect to total Fe(II). Since quantification of the individual
aqueous, solid, and adsorbed Fe(II) species is problematic, the amount of Fe(II) species in this study is expressed in terms of total FeSO₄ added, in mM.

1.1 Research goals and objectives

The interaction between mixed phase iron oxides and aqueous Fe(II) is of great importance to the fate of more persistent pollutants but is not well understood. The objectives of this bench-scale study are to simulate CHC degradation with Fe(II)-bearing minerals in engineered systems or high pH natural systems, which can occur naturally in wetlands or can be engineered in a constructed wetland. This includes characterizing the reaction kinetics and byproducts: (i) assess the role of chemogenic magnetite synthesized in situ at various pH in CHC degradation, (ii) characterize the effect of Fe(II) amendments at near neutral to basic pH conditions toward chlorinated methanes, ethanes, and ethenes, and (iii) examine the effect of magnetite-Fe(II) combinations on CHC degradation at various pH and characterize the reaction kinetics and byproducts.

2.0 Materials and Methods

2.1 Materials

The chemicals used in magnetite synthesis were as follows: FeSO₄•7H₂O (MP Biomedical, reagent grade), FeCl₃•6H₂O (Fisher Scientific, Certified ACS grade), NaOH (Fisher Scientific, Certified ACS grade), NaCl (Fisher Scientific, Certified ACS grade), hydrochloric acid (Fisher Scientific, 37% pure), carbon tetrachloride, chloroform (Fisher Scientific, Certified ACS grade), trichloroethene (ACROS, Reagent ACS grade), 1,1,2,2 tetrachloroethane (ACROS, 98.5% purity), and 1,1,2 trichlorethane (Sigma Aldrich, 98%
purity), and 1000 ppm Vinyl chloride compressed gas cylinder (Weiler Welding). Other laboratory supplies included 160 mL glass serum bottle (Wheaton; Cat # 223748), PTFE-lined butyl rubber stopper (Kimble-Chase; Cat #: 73811T-21), aluminum seal caps (Kimble-Chase), Vortex Genie 2 lab mixer (Fisher), etc.

2.2 Reactor setup with Fe(II) species

The setup of batch reactors to study CHC degradation with Fe(II) at different initial pH (8 through 12) was accomplished entirely inside the anaerobic chamber (Coy Lab MI) filled with ~3% H₂ and balance N₂. It began with preparation of a 0.1 M FeSO₄•7H₂O solution in deoxygenated water, which was then transferred into a burette. The aqueous medium for each reactor was prepared separately in a 125 mL Erlenmeyer flask with ~75 mL solution containing deoxygenated, deionized Milli-Q water and 6.67 mL of a 1:1 mix of 1 M NaOH and 1 M NaCl. (adapted from Leussing and Kolthoff, 1953). The calculated volume of the FeSO₄•7H₂O solution in the burette was added dropwise to the aqueous mixture in the Erlenmeyer flask. The pH of the mixture in the Erlenmeyer flask was adjusted to near the target pH with 1 N HCl, as necessary. The pH was monitored using a pH meter (model AP10, Denver Instrument). During drop-wise addition of FeSO₄ solution, the flask was swirled and pH was adjusted as needed to keep it close to target value until all of FeSO₄ solution had been added (Fig. S1 in SI). The pH often dropped rapidly during synthesis because precipitation of iron oxide phase removed OH⁻ from the solution, and frequent pH adjustment with NaOH was necessary. During drop-wise addition of FeSO₄, a white to very pale green precipitate formed and appeared to make the solution cloudy, as described in Leussing and Kolthoff (1953). Sometimes, 1 N HCl was used for final pH adjustment of the aqueous media to the desired initial pH.
However, buffer was not used during the set-up to avoid its potentially undesirable effect on the outcome of the experiment (cf. Jeong et al., 2013). For example, Danielsen et al. (2005) noted that TRIS buffer increased initial rate constants for CT removal with magnetite, but TEEN buffer changed the reaction pathway such that less CF and more carbon monoxide was produced compared to the unbuffered experiment. The volume of the aqueous medium was then increased to 100 mL with deoxygenated Milli-Q water and the pH was adjusted, if necessary. The liquid with any precipitate was transferred to the 160 mL glass serum bottle, sealed with PTFE-lined butyl rubber stopper and aluminum crimp, and wrapped in aluminum foil to simulate darkness. The color of the precipitate sometimes varied slightly with the differences in pH and other conditions (Fig. S3). The precipitate had a greener shade presumably due to oxygen contamination (Leussing and Kolthoff, 1953), which suggests trace development of a Fe(II)-Fe(III) mix phase solid, referred to as ‘green rust’.

2.3 Reactor setup with magnetite

The batch reactor setup to study CHC degradation with magnetite at different initial pH (8 through 12) was quite similar to the procedure described above (section 2.2) and was loosely adapted from Vikesland et al. (2007). The reagents in the burette was a 1:1 volumetric mixture of 0.1 M FeSO₄•7H₂O and 0.2 M FeCl₃•6H₂O so that stoichiometric Fe(II):Fe(III) ratio in magnetite precipitate formed should initially be 1:2. The FeSO₄-FeCl₃ mixture was added drop-wise to an Erlenmeyer flask containing 75 mL mixture of 1N NaOH and 1 N NaCl (in 1:1 ratio), as described in section 2.2. During the synthesis, a gentle swirling of the Erlenmeyer flasks was maintained, and the pH was adjusted as needed to keep it near the target pH. A black precipitate of magnetite was
produced quickly. For batch experiments containing magnetite with Fe(II) amendment, further pH adjustments with NaOH was necessary to maintain pH while additional FeSO₄ solution was slowly added to the Erlenmeyer flask containing freshly-synthesized magnetite. The magnetite slurry, with/without Fe²⁺ amendment, was diluted to 100 mL with DDI water while continuing to slightly adjust the pH to the desired level. The freshly precipitated magnetite slurry was then transferred to a 160 mL borosilicate serum bottle, sealed with a PTFE-lined rubber stopper and aluminum crimp, and wrapped in aluminum foil.

The method outlined in Vikesland et al. (2007) for magnetite synthesis was modified in two ways: (i) buffer was not used for magnetite synthesis in this setup, which is different from the published method (Vikesland et al. 2007); and (ii) magnetite prepared by the published method was synthesized at pH 12 and then washed to remove extra ions. In comparison, the batch reactor setup in this investigation was by synthesizing the magnetite in situ without washing and by synthesizing nanoparticles at the pH used in the experimental conditions. Preliminary experiments during our method development suggested that the reactivity of magnetite synthesized at pH 12 and washed until the reactor approached pH 10 showed CT removal at a faster rate than magnetite synthesized at pH 10 without washing (Fig. S5).

2.4 CHC Degradation Experiments in Batch Reactors

All experiments were carried out in sealed 160 mL borosilicate glass serum bottle reactors in duplicate. Some experiments with magnetite were initially conducted with single reactors, where the purpose was to obtain a baseline so that this experimental setup
might be compared to conditions that have been studied before by others. A control reactor containing DDI water was also prepared in each experiment to estimate initial CHC amounts and various unrelated losses during the experiment. Known volume of the CHC stock solution was injected into the sealed reactors, which were then vigorously shaken on a vortex mixer for ~40 sec to begin the experiment. The reactors were then placed on an end-over-end rotary shaker for continuous mixing at 45 rpm, except during sampling. The calculated initial amounts of CT, CF, 1,1,2 TCA, 1,1,2,2, TeCA, and TCE in the CHC degradation experiments (described below) were 0.071, 0.096, 0.067, 0.059, and 0.059 µmol, respectively. It was attempted to keep initial CHC molar amounts at similar levels for comparison.

2.5 Sampling and Analysis

Headspace sampling and direct injection gas chromatography (7890 model GC; Agilent Technologies) was used to analyze for CT, CF, 1,1,2,2-TeCA, TCE, 1,1,2 TCA and their degradation products (methane, vinyl chloride and ethane) in the reactors. After reactors and standards were injected with the CHCs, all reactors were vigorously shaken on a vortex mixer for 40 seconds to accelerate equilibrium of volatile partitioning into the headspace. A 50 µL headspace sample, T1, was withdrawn immediately by a 250 µL gastight syringe (cat# 81100; Hamilton, Reno, NV) for analysis by gas chromatography. After T1 sampling for each reactor, the reactors were placed on a rotary shaker (Glas Col, IN) for end-over-end mixing at 45 rpm. Typically, two or three samples were taken on the first day and once per day as needed afterward until the experiment concluded.
Upon injection into the GC injection port, the gaseous samples were split into two capillary columns. Methane, ethane and vinyl chloride were separated on a GS GasPro column (30 m x 0.32 mm x 5 µm; cat# CP7351; Agilent J&W Scientific) connected to the flame ionization detector (FID), while other chlorinated volatiles were separated on an HP 624 column (30m x 0.32mm x 1.8 µm; cat# 13870; Agilent Technologies) connected to an electron capture detector (ECD), with high purity helium carrier gas. GC method parameters for chlorinated methane compounds analysis include inlet at 200 ºC, ECD at 350 ºC, FID at 250 ºC, oven at 100 ºC (isothermal); and carrier gas flows were 1.0 mL/min. For chlorinated ethanes and ethenes, the method was modified to have the oven at 120 ºC (isothermal), and carrier gas flow was 1.5 mL/min to shorten the retention times. The make-up gas for ECD was high purity N₂ with a flow rate of 60 mL min⁻¹. The flow rates for high purity H₂ and air to the FID was 40 and 450 mL min⁻¹, respectively. 50 µL gas samples were withdrawn using a 250 µL gastight glass syringe, and immediately injected manually into the GC inlet for analysis.

2.6 Preparation of stocks, standards and calibration curves:

Stock solutions of CHCs were prepared in an aqueous solution in 160 mL serum bottles. The bottles were prepared to have no headspace. 20 µL of individual CHCs were injected into the sealed 160 mL serum bottles by syringe to prepare their respective stock solutions. Each bottle was vortexed and then allowed to equilibrate on the rotator for 48 hours prior to use. All compounds were quantified using calibration curves. At least three standards were prepared for each CHC compound in 160 mL serum bottles sealed with stopper and containing 100 mL DI water. Calculated volumes of CHC stock solution
(except VC) were injected into standards bottles, wrapped in aluminum foil, and allowed to equilibrate on an end-over-end rotary shaker (45 rpm) for at least 2 hrs. The standards for methane, ethane and VC were prepared by injecting calculated volumes of high purity gaseous stocks (cylinders of methane and ethane at 99+% purity, and 1000 ppmv vinyl chloride in nitrogen) in sealed serum bottles as described above and allowed to equilibrate on the rotary shaker for at least 2 hrs (Powell and Agrawal, 2011; Burris et al., 1996). New standards were made for every experiment to quantify the amounts of parent CHC and the detectable daughter products in the batch reactors. Standards were analyzed every day to correct for unforeseen changes in reactor conditions and variability in the instrument. The amount of the chemicals (in μmoles) in each reactor was quantified by multiplying the respective GC peak areas with the slope of calibration curves. The amounts of each chemical (in μmoles) were then converted to mole fractions for each sampling event.

2.7 Data Treatment

The initial amount of CHC injected in the duplicate reactors containing magnetite, $m_0$, at the beginning of the experiment, $t_0$, could not be measured due to rapid CHC degradation with magnetite. In this situation, the initial CHC in the magnetite reactors, $m_0$, was taken from the measured CHC amount in the DI water control reactors. For most experiments, the pseudo-first order degradation kinetics ($k_{obs}$) were calculated using CHC amount-time data pairs that fit an exponential curve with a minimum $r^2$ value = 0.90 till sample $t_3$ (e.g., Fig. 1A in Section 3.1). If CHC degradation was extremely rapid and the sample from the reactor at $t_1$ yielded a 0 peak area, $k_{obs}$ was loosely estimated by putting a
non-zero value of 0.000001 just to complete a regression as above, but admittedly such values were considered “estimates” as \( k_{\text{obs}} \) were determined by the sampling time and were likely slower than actual degradation kinetics. Some of the CHCs were found to be susceptible to loss at high pH conditions in DI water control reactors (containing no magnetite). This resulted in some experimental reactors showing slower kinetics than their DI water controls. In experiments where CHCs degraded in the control reactors, \( t_1 \) was used as the starting point for regression in order to estimate \( k_{\text{obs}} \) and the data for the DI water control reactor is included in plots to quantify the pH effect. This technique was mostly used for the experiments with 1,1,2,2-TeCA and CF as the parent compound.

Scatter plots were prepared to estimate \( k_{\text{obs}} \), showing contaminant amount (\( \mu \)moles) on the ordinate and time (days) on the abscissa. The \( k_{\text{obs}} \) (day\(^{-1}\)) were determined from the regression through the data at selected sampling points for an exponential fit. A similar approach has been employed in numerous other studies to estimate pollutant degradation kinetics; for example, experiments with iron oxides (magnetite) used a pseudo-first order rate model to calculate kinetics (e.g. Gregory et al., 2004; Gorski and Scherer, 2009; Danielsen and Hayes, 2004; McCormick and Adriaens, 2004; and Vikesland et al., 2007). In Vikesland et al. (2007), the concentration of pollutants was held constant for all experiments, facilitating the use of a pseudo-first order rate model. In the present study, the amounts of CHCs were held constant, making Fe(II) (adsorbed and structural) the only variable reactant for determining the reaction order. The quantities of parent and daughter products were transformed into mole fractions \( (m/m_0) \), which was obtained by dividing their amount \( (m, \mu \text{moles}) \) at different sampling times by the initial amount of the parent compound \( (m_0, \mu \text{moles}) \) at \( t_0 \).
A further analysis using R statistical software to examine the full set of data with respect to varying [magnetite], [Fe(II) species], initial pH, and the combined contributions of these variables that will be referred to as "input variables." The analysis was conducted as a linear model to examine those factors' influence on what is being called "output variables" were $k_{\text{obs}}$, mole fraction of parent pollutant, mole fraction of the primary product, and carbon mass balance. P-values were used to confirm the importance of each of the reactor conditions to the dependent variables (shown in SI). The charts showing the relationships between the input variables and the output variables were also plotted (see SI). The correlation values between the input variables and the output variables provided a confirmation for the model (see SI). However, some of the correlations were not completely linear, which led to higher p-values.

3.0 Results

3.1 Mineral characteristics and pH variations during and after synthesis

During reactor setup, the goal was to synthesize Fe(II) solid species or magnetite at the target pH of the experiment. However, the procedures described in section 2 resulted in pH near the inflection point moving rapidly toward acidic conditions upon adding iron solutions and back to basic conditions as NaOH was added in the absence of buffer. This pH adjustment was accomplished manually for individual reactors, which resulted in minor pH and ionic strength differences between reactors with identical starting condition.

The laboratory procedure for iron oxide synthesis was rigorous, and the parameters, such as pH and concentrations of base and iron oxide mix, were kept
consistent as much as possible in order to prepare particles of similar size and shape. The method was adapted from Vikesland et al. (2007), who reported that the size of their magnetite particles was ~9 nm in diameter, which may not be visible by light microscopy, but clusters of agglomerates were clearly visible. Since particle agglomerates produced by Vikesland’s procedure looked similar to particles produced by the adapted titration method and were quite uniform, it was surmised that the particle sizes were similar in both procedures. However, the particles were small enough that their size range could not be estimated by the tools at hand. From the light microscopy photos (Figs. S9 and S10 in SI) in this study, the magnetite agglomerates produced by the titration method (Fig. S10 in SI) appear to be less uniformly-sized than the particles produced by Vikesland et al. (2007) procedure, which were quite uniform (Fig. S9 in SI).

In reactors containing only Fe(II), the ferrous hydroxide precipitates (Fig. S11 in SI) appeared to exist as amorphous agglomerates with looser packing than the magnetite particles (described above). Particles and agglomerates of ferrous hydroxide species appeared amorphous. It appears that an explicit structure of Fe(OH)$_2$ crystalline solid is not available in the literature. However, ferrous hydroxide particles can be synthesized by method based on Leussing and Kolthoff (1953) (see Method section 2.2) for further characterization by future researchers.

The batch reactors with Fe(II) species at pH 9 and 10 showed visual changes and a change in pH over time during an experiment. Fe(II) species experiments turned a darker green color over time (Fig. S8 in SI). The agglomerates of Fe(II) species also appeared to be somewhat larger/courser after 1 week of equilibration on the rotator (continuous end-over-end mixing). The change in color was most clearly visible in the
reactors with high [Fe(II) species] at both pH 9 and 10 (particularly in 15 mM Fe(II) reactors) because of the highly grainy appearance. In contrast, magnetite particles did not change color over time and the changes in particle character were difficult to discern because the liquid appeared black. However, magnetite particles agglomerated and became attached to the sides of the borosilicate glass bottle over time despite constant mixing on the rotary shaker, so that by day 8, a large fraction of magnetite was attached to the inside surface of the reactor bottles.

Changes in pH were observed in reactors that were initially at pH 10 containing 5 mM Fe(II) species, 15 mM Fe(II) species, 1.16 g/L magnetite, 1.16 g/L magnetite combined with 5 mM Fe(II), 2.32 g/L magnetite combined with 5 mM Fe(II), and a control with no iron species. In all cases, the pH drifted downward to near pH 8.5 to 9.5 by the end of the first day. Initial rate constants of the pH decrease using the first four measurements were 0.43, 0.67, 0.47, 0.72, 0.58, and 0.26 d\(^{-1}\) respectively. Reactors with a pH initially at 9 showed a similar drop in pH to the pH 10 reactors, with all but the control and 1 mM Fe(II) reactors drifting into the range of pH 7 and 8 by the end of the first day. The control and 1 mM Fe(II) reactors stayed around pH 8.5. The majority of the drift in the pH values was finished at the end of the first day. Degradation of compounds such as CT and 1,1,2,2 TeCA was also completed by the end of the first day. Many of the other visible changes took place after the majority of the parent pollutant compounds were transformed into daughter species. For subsequent sections, the mentioned pH levels will refer to the initial pH at the time of CHC amendment.

3.2 CHC Degradation by Magnetite
The degradation of various CHCs (CT, CF, 1,1,2,2-TeCA, and 1,1,2-TCA) with freshly synthesized magnetite was characterized in this investigation as a baseline reference to its reactivity and for its comparison to magnetite prepared by the method described in Vikesland et al. (2007).

Experiments with magnetite alone were also completed to provide a baseline for comparison to experiments with Fe(II) species and mixed magnetite and Fe(II) species experiments. With magnetite produced by the titration method at pH 10, the rate of CT removal and the amount of CF produced increased as [magnetite] increased, while the amount of CT remaining decreased. CF was the primary product observed (Fig. 1A). However, at pH 12, there was little difference in the CT degradation and CF production and removal over time. (Fig. 1C). Generally, CT $k_{\text{obs}}$ increased as both [magnetite] and pH increased. Highest removal rates were observed at pH 12, but pH 12 conditions also produced wide variability in kinetics. Despite the marginal difference between the different [magnetite] series on the concentration vs. time plots, CT $k_{\text{obs}}$ appeared to increase more with increases in [magnetite] at pH 12 than they did at pH 10 and 8 (Fig. 1D). In the experiment at pH 8 with 0.29, 0.58, and 1.16 g/L magnetite (equivalent to 1.25, 2.5, and 5 mM Fe(II)$_{\text{structural}}$, respectively), the CT $k_{\text{obs}}$ values did not vary much (0.52, 0.58, and 0.47 d$^{-1}$ respectively) with increasing [magnetite] (Fig. 1D). For the same magnetite concentrations, CT $k_{\text{obs}}$ at pH 10 was 0.25, 0.48, and 1.57 d$^{-1}$ respectively (Fig. 1D).

CT remaining at pH 8 for the three magnetite concentrations above were 0.82, 0.80, and 0.80 mole fraction respectively. At pH 10, CT remaining was 0.18, 0.03, and 0.003 mole fraction respectively. At pH 12, all CT was removed from all reactors. At pH
8, CF mole fraction yields for the three [magnetite] levels were 0.06, 0.08, and 0.12 respectively. At pH 10, those yields were 0.62, 0.88, and 1 respectively. At pH 12, CF was observed to degrade in controls as well as experimental reactors with 0.28, 0.58, and 1.16 g/L magnetite. However, there was very little difference between the control and the reactors. Products of CF removal were not clear. Methane was present in trace amounts. In experiments with CT as the parent compound, mole fraction yields of CF after 10 days for the [magnetite] described in the previous paragraph were 0.39, 0.38, and 0.38 mole fraction respectively.

The multiple linear regression analysis examining all three variables, pH, [Fe(II)], and [magnetite] among all experiments showed that magnetite was the most influential variable on CF yields. Interestingly, the trend was negative, whereby the higher [magnetite] was, the lower the CF final yield was. The p-value for the relationship between CF and [magnetite] was 0.00096 (SI). Mass balance also showed a decreasing trend with increasing [magnetite] (p-value was 0.011).

1,1,2,2 TeCA was found to be only slightly reactive toward magnetite alone at pH 10. While only about 8% of the TeCA degraded with magnetite by the end of the first day, the pH 10 control removed all of the TeCA by the end of the first day (not shown). The experiment showed more rapid removal of TeCA at the beginning of the experiment, which then slowed to a steady rate of removal, creating two phases of TeCA removal. At the end of the experiment, at t=43 days, about 35% of the TeCA remained in the reactor. TCE was the primary product, but it was not observed to degrade at any point in the experiment.
\[ y = 0.93e^{-1.79x} \quad \text{R}^2 = 1.00 \]

\[ y = 0.92e^{-0.79x} \quad \text{R}^2 = 0.99 \]

\[ y = 0.92e^{-0.58x} \quad \text{R}^2 = 0.98 \]
Fig. 1: Effect of [magnetite] on CT degradation at pH 10. No buffer was used to maintain pH. Initial amount of CT in the reactors was 0.076 µmol. (A) Effect of [magnetite] on Pseudo-first order rate kinetics, and (B) product distribution over time. (C) Effect of [magnetite] on CT degradation at pH 12. Note, this preliminary experiment had no error bars because it consisted of one reactor for each [magnetite] for obtaining a reactivity baseline. (D) CT $k_{\text{obs}}$ with increasing magnetite at pH 8, 10, and 12. No buffer was used in these experiments. Error bars were defined by the upper and lower bounds of the data when applicable.

3.3 Effect of [Fe(II) species] on CHC removal
The addition of FeSO₄ solution to a strong base produced a light gray to very pale green colored precipitate. Light microscopy revealed very little color (Fig. SI 11). The precipitate formed agglomerates of loosely packed particles that did not appear to agglomerate at low [Fe(II)]. Particles also appeared to become darker green over time and if exposed to oxygen, would turn orange or yellow when completely oxidized. In one instance, where Fe(II) species were precipitated side by side at pH 8 and 9, the particles were a darker green in the pH 8 reactors than in the pH 9 reactors (Fig. SI 3).

Most experiments at different [Fe(II) species] and mixtures of magnetite and Fe(II) species were conducted at pH 10. At pH 10, low [Fe(II)] (1 mM) removed CT more quickly and had less CT remaining 1.16 g/L magnetite alone (which contained 5 mM structural Fe(II)) (Fig. 2A). CT $k_{obs}$ increased with increasing [Fe(II) species]. CF was the primary product initially for all [Fe(II)] (Fig. 2B). At 15 mM and 25 mM Fe(II) species, significant amounts of CF were removed. However, DCM and methane were not observed to increase as CF was removed (not shown).

As expected, CT remaining showed a decreasing trend with increases in [Fe(II)] according to the multiple linear regression study examining all experiments. The p-value was 0.060 (See SI). Mass balance also showed a slight decreasing trend with increasing [Fe(II)]. Its p-value was close to that of CT remaining at 0.062 (See SI).

At pH 10, 1,1,2,2-TeCA was found to readily degrade in reactors with 1, 5, and 15 mM Fe(II), but the same was observed in the pH 10 DI water control (Fig 3A and Table 1). As stated in section 3.2, magnetite alone showed little 1,1,2,2-TeCA removal by comparison. The dominant product of 1,1,2,2-TeCA degradation for all Fe(II) species
experiments, including the control, was TCE, which was not observed to degrade in these experiments (Fig. 3B).

1,1,2-TCA degraded slowly with 5, 15, and 25 mM Fe(II) species at pH 10, but there was not much difference between the three [Fe(II)] levels (Fig 3C and Table 1). However, more VC was produced as [Fe(II)] increased. No ethene or ethane was observed in these experiments, nor were there other observed byproducts that would account for the difference in VC yields when 1,1,2-TCA removal did not change with increasing [Fe(II) species].

Different CHC compounds responded differently to changes in [Fe(II) species] at pH 10 (Table 1). Values of \( k_{\text{obs}} \) increased roughly linearly with increasing [Fe(II) species]. Rate constants for removal of CF and 1,1,2-TCA were largely unaffected at pH 10 for the range of [Fe(II) species] tested. Degradation of 1,1,2,2-TeCA was rapid at low [Fe(II) species], but the rate of removal was largely unpredictable over the range of [Fe(II) species] tested. Yields of the dominant daughter products for these experiments also varied with CHC species at pH 10. CF yields, the product of CT degradation decreased with increasing [Fe(II)] (Table 1). Simultaneously, the product of CF removal, methane, showed an increase with [Fe(II) species]. VC was the product of 1,1,2-TCA removal and increased with increasing [Fe(II) species]. TCE, the product of 1,1,2,2-TeCA removal showed no significant pattern in its yield.
Fig. 2: CT experiments with ferrous hydroxide (Fe(OH)$_2$). (A) Degradation of CT and (B) CF production is shown over time with various concentrations of Fe(II) species at pH 10. 1.16 g/L magnetite data was shown as a comparison, which contained the same molar amount of Fe(II) in the synthesis as the 5 mM Fe(II) experiment.
(A) $y = 0.97e^{-5.97x}$ 
$R^2 = 0.99$

$y = 1.03e^{-2.87x}$ 
$R^2 = 0.78$

$y = 0.93e^{-14.69x}$ 
$R^2 = 0.89$

$y = 0.99e^{-12.38x}$ 
$R^2 = 0.92$

$y = 0.99e^{-12.38x}$ 
$R^2 = 0.95$

(B) $y = 1.16 g/L mag$
$y = 1 mM Fe(II)$
$y = 5 mM Fe(II)$
$y = 15 mM Fe(II)$
Fig. 3: Degradation of (A) 1,1,2,2-TeCA) and the production of (B) TCE over time at pH 10 with various [Fe(II) species] with a magnetite only experiment and control reactor result for comparison. (C) Degradation of 1,1,2-TCA and the production of its dominant product, vinyl chloride (VC) over time at pH 10 with various [Fe(II) species].
Table 1: Chlorinated methane and ethane degradation at pH 10 with various [Fe(II) species]. (See charts in Fig. S1 12)

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<th>Parent</th>
<th>product 1</th>
<th>[Fe(II)] (mM)</th>
<th>Avg $k_{obs}$</th>
<th>Parent remaining (mol frac)</th>
<th>Product (mole frac)</th>
<th>C Mass Balance (mol frac)</th>
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<td>0.842 ±0.077</td>
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<td>0.161 ±0.012</td>
<td>0.917 ±0.016</td>
<td></td>
</tr>
<tr>
<td>112 TCA VC</td>
<td>25</td>
<td>1.75 ±0.25</td>
<td>0.714 ±0.082</td>
<td>0.246 ±0.15</td>
<td>0.961 ±0.070</td>
<td></td>
</tr>
</tbody>
</table>
3.4 Effect of pH on CHC removal with Fe(II) species

Fe(II) Experiments with CT at pH 7, 9, and 10 shows that as [Fe(II) species] increased, so did $k_{\text{obs}}$. In those same experiments, however, the $k_{\text{obs}}$ for given [Fe(II) species] was apparently not dependent on changes in pH (Table 2). For pH 12 data (Table 2), had some $k_{\text{obs}}$ values that were too high to be calculated accurately and had to be estimated by the addition of a non-zero number for $t_1$. Generally, $k_{\text{obs}}$ increased with increasing pH.

Experiments at pH 7 with 5 mM Fe(II) had 0.793 $m/m_0$ CT remaining after 13 days, while 15 mM Fe(II) experiments removed all CT. CF accounts for nearly all (0.95) $m/m_0$ for the pH 7 experiment. Traces of DCM and methane were visible but insufficient for quantification. A carbon monoxide (CO) analysis was carried out during the removal of CT in a pH 10 reactor with 5 mM Fe(II), but yields were below detection limit.

Experiments at pH 9 and 10 showed similar overall $k_{\text{obs}}$ at all [Fe(II)]. As [Fe(II) species] increased, CF final yields decreased at pH 9 and 10 (Table 2), and CF $k_{\text{obs}}$ increased (Table 2). However, at pH 9, CF $k_{\text{obs}}$ increased more slowly than at pH 10 with increasing [Fe(II) species].

The $k_{\text{obs}}$ values of 1,1,2,2-TeCA were observed to drastically increase between pH 9 and 10 (Fig. 4A). 5 mM Fe(II) experiments showed greater $k_{\text{obs}}$ than their corresponding DI water controls. Experiments at pH 8 were observed to have very low $k_{\text{obs}}$ at 5 mM Fe(II), but all experiments with 5 mM Fe(II) were observed to remove at least 75% of 1,1,2,2-TeCA (Fig. 4B). Interestingly, although final 1,1,2,2-TeCA and TCE $m/m_0$ were similar, carbon mass balance was greater for the DI controls than for the 5
mM Fe(II) reactors. Experiments at pH 8 and 9 were run for a second cycle (not shown). Controls and experimental reactors at pH 9 showed 1,1,2,2-TeCA removal for both cycles, while the pH 8 reactors showed that the degradation of 1,1,2,2-TeCA in the DI water control took place only in cycle 1. TCE experiments were conducted with 15 mM Fe(II) at pH 8 and 9. TCE was shown to degrade very slowly, much like 1,1,2-TCA (Fig. 4C). Degradation products from TCE reduction were not identified. TCE was not observed to degrade in 1,1,2,2-TeCA reactors once formed, but experiments with 15 mM Fe(II) conducted over four months at pH 8 and 9 showed gradual TCE removal (Fig. 4C). Removal was faster for the first 10 days and was a slow, roughly linear decline over the remaining days.

Other factors that may have had a role in changing reaction kinetics and product distribution included ionic strength and pH drift. Ionic strength would be high in all experiments because the particles were not washed to remove excess sulfate, chloride, sodium, and hydroxide ions after synthesis. In all iron oxide experiments, pH was observed to drift downward during the first day and stabilize, usually ~0.5 to 1 pH unit below the initial pH. The magnitude of pH drift increased as the target pH approached the inflection point around pH 7, but pH drift was minimal in pH 12 studies and only modestly affected pH 10 experiments. In the case of CT degradation, most of the CT reduction was finished by the end of the first day of the experiment and so likely was not significantly affected by the drop in pH. Likewise, CF removal was not visible until after that first day, so the CF removal took place after the majority of the pH drift.
Table 2: CT degradation at various pH with various [Fe(II) species]. (Expressed graphically in Fig. SI 13)

<table>
<thead>
<tr>
<th>pH</th>
<th>[Fe(II) species] (mM)</th>
<th>CT $k_{obs}$</th>
<th>CT remaining (mol frac)</th>
<th>CF yield (mole frac)</th>
<th>CF $k_{obs}$ ($d^{-1}$)</th>
<th>C Mass Balance (mol frac)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>5</td>
<td>6.36 ± 4.1</td>
<td>0.793 ± 0.094</td>
<td>0.155 ± 0.05</td>
<td>0 ± 0</td>
<td>0.948 ± 0.044</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>20.1 ± 13</td>
<td>0 ± 0</td>
<td>0.940 ± 0.03</td>
<td>0 ± 0</td>
<td>0.940 ± 0.032</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>17.9 ± 5.4 - 5.2</td>
<td>0 ± 0</td>
<td>0.861 ± 0.094 - 0.18</td>
<td>0 ± 0</td>
<td>0.861 ± 0.094 - 0.18</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>107 ± 50</td>
<td>0 ± 0</td>
<td>1.00 ± 0</td>
<td>0.00480 ± 0.0042</td>
<td>1.00 ± 0</td>
</tr>
<tr>
<td>8</td>
<td>2.5</td>
<td>124 ± 40</td>
<td>0 ± 0</td>
<td>1.00 ± 0</td>
<td>0 ± 0</td>
<td>1.00 ± 0</td>
</tr>
<tr>
<td>8</td>
<td>15</td>
<td>146 ± 34</td>
<td>0 ± 0</td>
<td>0.860 ± 0.026</td>
<td>0.0315 ± 0.0095</td>
<td>0.861 ± 0.026</td>
</tr>
<tr>
<td>9</td>
<td>1</td>
<td>18.3 ± 1.2</td>
<td>0 ± 0</td>
<td>1.00 ± 0.0035</td>
<td>0.0175 ± 0.0045</td>
<td>0.999 ± 0.0035</td>
</tr>
<tr>
<td>9</td>
<td>2.5</td>
<td>60.6 ± 26</td>
<td>0 ± 0</td>
<td>0.897 ± 0.054</td>
<td>0.0140 ± 0.0040</td>
<td>0.897 ± 0.054</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>32.7 ± 1.8</td>
<td>0 ± 0</td>
<td>0.750 ± 0.015</td>
<td>0.0110 ± 0.0070</td>
<td>0.750 ± 0.015</td>
</tr>
<tr>
<td>9</td>
<td>5</td>
<td>56.0 ± 3.9</td>
<td>0 ± 0</td>
<td>0.716 ± 0.017</td>
<td>0.00750 ± 0.0015</td>
<td>0.716 ± 0.016</td>
</tr>
<tr>
<td>9</td>
<td>15</td>
<td>77.3 ± 4.3</td>
<td>0 ± 0</td>
<td>0.692 ± 0.021</td>
<td>0.0285 ± 0.0095</td>
<td>0.692 ± 0.021</td>
</tr>
<tr>
<td>10</td>
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<td>12.7 ± 0.88</td>
<td>0 ± 0</td>
<td>1.00 ± 0</td>
<td>0.00145 ± 0.00055</td>
<td>1.00 ± 0</td>
</tr>
<tr>
<td>10</td>
<td>2.5</td>
<td>27.3 ± 5.8</td>
<td>0 ± 0</td>
<td>0.957 ± 0.042</td>
<td>0.00350 ± 0.0015</td>
<td>0.977 ± 0.053</td>
</tr>
<tr>
<td>10</td>
<td>5</td>
<td>57.3 ± 13</td>
<td>0 ± 0</td>
<td>1.00 ± 0</td>
<td>0.00600 ± 0.0030</td>
<td>1.00 ± 0</td>
</tr>
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<td>10</td>
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<td>81.0 ± 5.5</td>
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<td>0.753 ± 0.038</td>
<td>0.0250 ± 0.023</td>
<td>0.753 ± 0.038</td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>134 ± 29</td>
<td>0 ± 0</td>
<td>0.330 ± 0.11</td>
<td>0.0730 ± 0.012</td>
<td>0.409 ± 0.028</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>146 ± 30</td>
<td>1.25E-08 ± 0</td>
<td>1.00 ± 0</td>
<td>0 ± 0</td>
<td>1.00 ± 0</td>
</tr>
<tr>
<td>12</td>
<td>2.5</td>
<td>379 ± 91</td>
<td>1.25E-08 ± 0</td>
<td>1.00 ± 0</td>
<td>0 ± 0</td>
<td>1.00 ± 0</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>310 ± 49</td>
<td>4.1E-05 ± 7.74E-06</td>
<td>0.0202 ± 0.0040</td>
<td>3.23 ± 0.44</td>
<td>0.0202 ± 0.0040</td>
</tr>
</tbody>
</table>
(A) $k_{obs}$ (d$^{-1}$) vs pH

- $1,1,2,2$-TeCA no Fe(II)
- $1,1,2,2$-TeCA 5 mM Fe(II)

(B) $1,1,2,2$-TeCA & Mass balance (mol frac) vs pH

- $1,1,2,2$-TeCA no Fe(II)
- $1,1,2,2$-TeCA 5 mM Fe(II)
- Mass balance no Fe(II)
- Mass balance 5 mM Fe(II)
Fig. 4: (A) $k_{\text{obs}}$ and (B) 1,1,2,2-TeCA remaining mole fractions and total carbon mass balance are expressed for various pH levels in controls and experiments with 5 mM Fe(II). Degradation of (C) TCE over time with control at pH 9 with 15 mM Fe(II) species.

3.5 Effect of [Fe(II)] and [Magnetite] together

Table 3 showed parent $k_{\text{obs}}$ and product yield, respectively, at pH 10 with 1.16 g/L magnetite and increasing [Fe(II)], whereas Table 3 showed $k_{\text{obs}}$ and product yield, respectively, at pH 10 with 5 mM Fe(II) and increasing [magnetite]. At pH 10, experiments with a combination of magnetite and Fe(II) degraded all carbon tetrachloride in their reactors easily. When both magnetite and Fe(II) were in the reactors, the $k_{\text{obs}}$ values for both CT and CF degradation generally increased as the concentration of either iron source increased (Fig. 5A). However, the 5 mM Fe(II) alone experiment had faster kinetics than all but the experiments with 5 mM Fe(II) with 1.74 g/L magnetite and 5 mM Fe(II) with 3.48 g/L magnetite. The experiment with 5 mM Fe(II) and 1.16 g/L magnetite showed the slowest kinetics. For the magnetite concentrations of 0, 1.16, 1.74, 2.32, and 3.48 g/L magnetite with 5 mM Fe(II), CT $k_{\text{obs}}$ values were 57.3 16.09, 61.94, 39.74, and 110.8 d$^{-1}$ respectively (Table 3). For 1.16 g/L magnetite with 10 and 15 mM
Fe(II) had $k_{\text{obs}}$ values of 32.78 and 41.04 d$^{-1}$ respectively. All experiments with both magnetite and Fe(II) species showed some CF removal. Experiments with 5 mM Fe(II) and [magnetite] greater than 1.16 g/L removed more CF than experiments with 1.16 g/L magnetite and [Fe(II)] greater than 5 mM. For experiments with 1.16 g/L magnetite with various [Fe(II)] showed little change in CF removal with increasing [Fe(II)]. A series of experiments with 5 mM Fe(II) and increasing [magnetite] with CF as the starting compound removed nearly all CF within 10 days where 5 mM Fe(II) alone and magnetite alone were not able to remove CF (Table 4). Methane was the dominant product (Table 4). The removal of CF and production of methane was not significantly different as magnetite increased (Fig. 5C and D).

An experiment designed to examine the effects of Fe(II) species and magnetite concentrations on CF degradation showed that the mixture of magnetite and Fe(II) was effective at removing CF, producing primarily methane (Fig. 5C and D). The mole fraction final yields of CF after the experiments with 1.16, 2.32, and 3.48 g/L magnetite with 5 mM Fe(II) was near zero in all cases and 75 to 80% of the mass balance composed of methane. The CF $k_{\text{obs}}$ for the experiments with 5 mM Fe(II) and 1.16, 2.32, and 3.48 g/L magnetite averaged to be 0.543, 0.908, and 0.775 d$^{-1}$ respectively. An experiment in this series that was designed to examine the effect of adding more Fe(II) to a constant concentration of magnetite showed a decrease in the reactivity when increasing the [Fe(II)] from 5 to 10 mM. The $k_{\text{obs}}$ value was 0.19 d$^{-1}$.

Degradation kinetics of 1,1,2,2-TeCA followed a positive linear trend with increasing [Fe(II)] as well (Table 3), but the increase was modest compared to the increase for CT. It was difficult to discern a pattern in TCE yield, the dominant product
for 1,1,2,2-TeCA (Table 3), and it had no distinct pattern with increasing [magnetite] (Table 4). 1,1,2-TCA $k_{\text{obs}}$ did not increase much with [Fe(II)] (Table 3) and only modestly increased with [magnetite] (Table 4), but VC yield increased drastically at higher [magnetite].
Fig. 5: (A) Degradation of CT (with line equation and $R^2$ in order from slowest to fastest from top to bottom and colored to approximate the matching data series) and (B) production and degradation of CF at various concentrations of both Fe(II) and magnetite at pH 10. Results from a separate set of experiments with CF as the parent compound showed (C) relatively rapid removal of CF with (D) a strong increase in methane.
<table>
<thead>
<tr>
<th>Parent</th>
<th>Product</th>
<th>[Fe(II)] (mM)</th>
<th>Parent (k_{\text{obs}}(d^{-1}))</th>
<th>Parent remaining (mol frac)</th>
<th>Product yield (mol frac)</th>
<th>Product (k_{\text{obs}}(d^{-1}))</th>
<th>C mass balance (mol frac)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>CF</td>
<td>0</td>
<td>2.09 ± 0.52</td>
<td>0.00154 ± 0.0015</td>
<td>0.865 ± 0.13</td>
<td>0 ± 0</td>
<td>0.867 ± 0.14</td>
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<tr>
<td>CT</td>
<td>CF</td>
<td>5</td>
<td>16.1 ± 2.2</td>
<td>0 ± 0</td>
<td>0.376 ± 0.18</td>
<td>0.0525 ± 0.020</td>
<td>0.615 ± 0.031</td>
</tr>
<tr>
<td>CT</td>
<td>CF</td>
<td>10</td>
<td>32.7 ± 1.3</td>
<td>0 ± 0</td>
<td>0.501 ± 0.074</td>
<td>0.0490 ± 0.009</td>
<td>0.532 ± 0.043</td>
</tr>
<tr>
<td>CT</td>
<td>CF</td>
<td>15</td>
<td>42.7 ± 14</td>
<td>0 ± 0</td>
<td>0.623 ± 0.13</td>
<td>0.0815 ± 0.012</td>
<td>0.664 ± 0.091</td>
</tr>
<tr>
<td>1122 TECA</td>
<td>TCE</td>
<td>0</td>
<td>2.23 ± 0.13</td>
<td>0.445 ± 0.027</td>
<td>0.329 ± 0.30</td>
<td>0.458 ± 0.23</td>
<td>0.787 ± 0.070</td>
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<tr>
<td>1122 TECA</td>
<td>TCE</td>
<td>5</td>
<td>7.59 ± 0.48</td>
<td>0.759 ± 0.048</td>
<td>0.0117 ± 0.0053</td>
<td>0.475 ± 0.029</td>
<td>0.487 ± 0.023</td>
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<td>1122 TECA</td>
<td>TCE</td>
<td>10</td>
<td>5.17 ± 0.86</td>
<td>0.345 ± 0.057</td>
<td>0.0026 ± 0.0026</td>
<td>0.958 ± 0.042</td>
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<tr>
<td>1122 TECA</td>
<td>TCE</td>
<td>15</td>
<td>5.83 ± 0.79</td>
<td>0.291 ± 0.039</td>
<td>0.0012 ± 0.0013</td>
<td>0.715 ± 0.044</td>
<td>0.716 ± 0.045</td>
</tr>
<tr>
<td>112 TCA</td>
<td>VC</td>
<td>5</td>
<td>0.666 ± 0.18</td>
<td>0.0665 ± 0.018</td>
<td>0.848 ± 0.032</td>
<td>0.00713 ± 0.0071</td>
<td>0.855 ± 0.025</td>
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<td>112 TCA</td>
<td>VC</td>
<td>10</td>
<td>1.30 ± 0.04</td>
<td>0.0866 ± 0.0027</td>
<td>0.903 ± 0.030</td>
<td>0 ± 0</td>
<td>0.903 ± 0.030</td>
</tr>
<tr>
<td>112 TCA</td>
<td>VC</td>
<td>15</td>
<td>0.915 ± 0.33</td>
<td>0.0427 ± 0.017</td>
<td>0.923 ± 0.064</td>
<td>0.0429 ± 0.0075</td>
<td>0.955 ± 0.045</td>
</tr>
</tbody>
</table>
Table 4: Effect of [Magnetite] at pH 10 and 5 mM Fe(II) (Expressed graphically in Fig. SI 14C and D)

<table>
<thead>
<tr>
<th>Parent</th>
<th>Product</th>
<th>Magnetite (g/L)</th>
<th>Parent $k_{obs} (d^{-1})$</th>
<th>Parent remaining (mol frac)</th>
<th>Product yield (mol frac)</th>
<th>Product $k_{obs} (d^{-1})$</th>
<th>C mass balance (mol frac)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>CF</td>
<td>0</td>
<td>57.3 ±13</td>
<td>0 ±0</td>
<td>1.00 ±0</td>
<td>0.006 ±0.003</td>
<td>0.000 ±0</td>
</tr>
<tr>
<td>CT</td>
<td>CF</td>
<td>1.16</td>
<td>16.1 ±2.2</td>
<td>0 ±0</td>
<td>0.376 ±0.18</td>
<td>0.052 ±0.020</td>
<td>0.615 ±0.031</td>
</tr>
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<td>CT</td>
<td>CF</td>
<td>1.74</td>
<td>61.9 ±12</td>
<td>0 ±0</td>
<td>0.336 ±0.11</td>
<td>0.190 ±0.070</td>
<td>0.473 ±0.063</td>
</tr>
<tr>
<td>CT</td>
<td>CF</td>
<td>2.32</td>
<td>39.7 ±1.8</td>
<td>0 ±0</td>
<td>0.0166 ±0.009</td>
<td>0.128 ±0.010</td>
<td>0.0166 ±0.009</td>
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<td>CT</td>
<td>CF</td>
<td>3.48</td>
<td>111 ±0.45</td>
<td>0 ±0</td>
<td>0.0296 ±0.018</td>
<td>0.793 ±0.062</td>
<td>0.269 ±0.13</td>
</tr>
<tr>
<td>CF</td>
<td>CH₄</td>
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<td>0.0300 ±0</td>
<td>0.932 ±0.02</td>
<td>0.155 ±0.026</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CF</td>
<td>CH₄</td>
<td>1.16</td>
<td>0.550 ±0.07</td>
<td>0.006 ±0.00</td>
<td>0.769 ±0.004</td>
<td>NA</td>
<td>0.775 ±0.003</td>
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<tr>
<td>CF</td>
<td>CH₄</td>
<td>2.32</td>
<td>0.930 ±0.21</td>
<td>0.009 ±0.00</td>
<td>0.785 ±0.02</td>
<td>NA</td>
<td>0.795 ±0.030</td>
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<tr>
<td>CF</td>
<td>CH₄</td>
<td>3.48</td>
<td>0.880 ±0.45</td>
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<td>0.761 ±0.02</td>
<td>NA</td>
<td>0.781 ±0.008</td>
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<td>TECA</td>
<td>TCE 0</td>
<td>14.1 ±0.57</td>
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<td>0.0038 ±3.4E-5</td>
<td>0.538 ±0.007</td>
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</tr>
<tr>
<td>1122</td>
<td>TECA</td>
<td>TCE 1.16</td>
<td>7.59 ±0.48</td>
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<td>0.0117 ±0.00</td>
<td>0.475 ±0.029</td>
<td>0.487 ±0.023</td>
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<td>TECA</td>
<td>TCE 2.32</td>
<td>12.2 ±3.1</td>
<td>0.810 ±0.21</td>
<td>0.0195 ±0.00</td>
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<td>1122</td>
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<td>TECA</td>
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<td>0.721 ±0.073</td>
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<td>1.74</td>
<td>±0.64</td>
<td>0.348 ±0.13</td>
<td>0.658 ±0.09</td>
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<td>0.721 ±0.073</td>
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<td>6.81</td>
<td>±0.34</td>
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<td>0.680 ±0.016</td>
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<tr>
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<td>2.32</td>
<td>2.24</td>
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<td>0.215 ±0.03</td>
<td>0.903 ±0.060</td>
<td>1.00 ±0</td>
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<tr>
<td></td>
<td>3.48</td>
<td>0.958</td>
<td>±0.92</td>
<td>0.047 ±0.04</td>
<td>0.320 ±0.04</td>
<td>1.00 ±0</td>
<td>1.00 ±0</td>
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</table>
3.6 Effect of pH on the interaction of [Fe(II)] and [Magnetite]

Changes in [magnetite] had little effect on CT $k_{\text{obs}}$ at pH 8 and 9. At pH 9, as magnetite concentrations increased, the CT $k_{\text{obs}}$ decreased (Table 5). The combinations tested with 5 mM Fe(II) species were 0, 1.16, and 2.32 g/L magnetite. CF final yield values at pH 8 and 9 were similar for experiments with only 5 mM Fe(II) and 5 mM Fe(II) with 1.16 g/L magnetite. Yields were between 0.7 and 0.9 $m/m_0$. CF $k_{\text{obs}}$ under these conditions were nearly 0 (Table 5). CF yield at pH 9 with 2.32 g/L magnetite was near 0, but CF $k_{\text{obs}}$ was also 0. CT removal was greatest and fastest at pH 10 for all reactor conditions, and CF $k_{\text{obs}}$ increased in an exponential relationship when [magnetite] approached 3.48 g/L.

The experiment with 5 mM Fe(II) and 1.16 g/L magnetite removes CT much more slowly than 15 mM Fe(II), ($k_{\text{obs}} = 10.72$ and 70.24 d$^{-1}$ for the mix and 15 mM Fe(II) respectively), but they remove CF at approximately the same rate (0.035 and 0.033 d$^{-1}$ respectively) and leave the same CF yield at the end of the experiments (66 and 67% respectively). In the statistical analysis in R of all CT experiments including experiments that contained solid Fe(II) species, the degradation rate constant was better correlated to increases in pH than to increasing magnetite (See SI). The p-values for pH, Fe(II), and magnetite from a multiple linear model with $k_{\text{obs}}$ was 0.00028, 0.152, and 0.108 respectively. In the multiple linear regression, CT $m/m_0$ remaining was inversely correlated to pH and [Fe(II)] (See SI). The rise in CF $m/m_0$ yield had the lowest p-value for the [magnetite] predictor (0.00096) (See SI). Mass balance showed a high dependency on pH, Fe(II), and magnetite, but the most distinctive effect was from pH. The p-values
of the model were $1.46 \times 10^{-5}$, 0.0625, and 0.0112 respectively (See SI). The correlation model check had the highest correlations between the following predictor to dependent variables: pH and $k_{\text{obs}}$ (0.50), pH and $k_m$ (0.45), pH and CT (-0.42), pH and mass balance (-0.58), and magnetite and CF (0.52).
Table 5: CT degradation at various pH with 5 mM [Fe(II)]. (Expressed graphically in Fig. SI 15)

<table>
<thead>
<tr>
<th>pH</th>
<th>[magnetite] (g/L)</th>
<th>CT $k_{obs}$ (d⁻¹)</th>
<th>CT remaining (mol frac)</th>
<th>CF yield (mol frac)</th>
<th>CF $k_{obs}$ (d⁻¹)</th>
<th>C mass balance (mol frac)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>0</td>
<td>6.36 ±4.1</td>
<td>0.793 ±0.094</td>
<td>0.155 ±0.050</td>
<td>0 ±0</td>
<td>0.948 ±0.044</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>17.9 ±5.4 - 5.2</td>
<td>0 ±0</td>
<td>0.861 ±0.094 - 0.18</td>
<td>0 ±0</td>
<td>0.861 ±0.094 - 0.18</td>
</tr>
<tr>
<td>8</td>
<td>1.16</td>
<td>13.8 ±2.1</td>
<td>0 ±0</td>
<td>0.745 ±0.021</td>
<td>0.0480 ±0.0070</td>
<td>0.745 ±0.021</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>32.7 ±1.8</td>
<td>0 ±0</td>
<td>0.750 ±0.015</td>
<td>0.0110 ±0.0070</td>
<td>0.750 ±0.015</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>56.0 ±3.9</td>
<td>0 ±0</td>
<td>0.716 ±0.017</td>
<td>0.00750 ±0.0015</td>
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<td>9</td>
<td>1.16</td>
<td>11.7 ±5.2</td>
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<td>0.725 ±0.14</td>
<td>0.0270 ±0.011</td>
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<td>9</td>
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<td>0 ±0</td>
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<tr>
<td>10</td>
<td>0</td>
<td>57.3 ±13</td>
<td>0 ±0</td>
<td>1.00 ±0</td>
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<td>1.00 ±0</td>
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<tr>
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<td>1.16</td>
<td>16.1 ±2.2</td>
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<td>0.376 ±0.18</td>
<td>0.0525 ±0.020</td>
<td>0.615 ±0.031</td>
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<tr>
<td>10</td>
<td>1.74</td>
<td>61.9 ±12</td>
<td>0 ±0</td>
<td>0.336 ±0.11</td>
<td>0.190 ±0.070</td>
<td>0.473 ±0.063</td>
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<tr>
<td>10</td>
<td>2.32</td>
<td>39.7 ±1.8</td>
<td>0 ±0</td>
<td>0.0166 ±0.0093</td>
<td>0.128 ±0.010</td>
<td>0.01166 ±0.0093</td>
</tr>
<tr>
<td>10</td>
<td>3.48</td>
<td>111 ±0.45</td>
<td>0 ±0</td>
<td>0.0296 ±0.018</td>
<td>0.793 ±0.062</td>
<td>0.269 ±0.13</td>
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<tr>
<td>12</td>
<td>0</td>
<td>310. ±49</td>
<td>4.13E-05 ±7.7E-06</td>
<td>0.0202 ±0.004</td>
<td>3.23 ±0.44</td>
<td>0.0202 ±0.004</td>
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<tr>
<td>12</td>
<td>1.16</td>
<td>256 ±85</td>
<td>2.29E-05 ±3.5E-06</td>
<td>0.00875 ±0.0013</td>
<td>9.19 ±0.10</td>
<td>0.00878 ±0.0013</td>
</tr>
</tbody>
</table>

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3.7 Influence of structural vs. Adsorbed Fe(II)

Experiments at pH 10 that collectively have 15 mM Fe(II) were used to compare whether structural Fe(II) in magnetite, adsorbed Fe(II) on magnetite surface, or Fe(II) species are more potent reducers (Fig. 6). Results for CT $k_{obs}$ values from fastest to slowest were 15 mM Fe(II), 5 mM Fe(II), 5 mM Fe(II) with 2.32 g/L magnetite, and 10 mM Fe(II) with 1.16 g/L magnetite. $K_{obs}$ values were 80.76, 55.64, 39.51, 32.78 d$^{-1}$ respectively (Fig 6A). CF was formed and then removed in order of lowest final yield remaining to highest yield remaining were 5 mM Fe(II) with 2.32 g/L magnetite, 10 mM Fe(II) with 1.16 g/L magnetite, 15 mM Fe(II), and 5 mM Fe(II) (Fig. 6B). CF removal was slow and could be described by a linear model (zero order) or a pseudo-first order model. The CF $k_{obs}$ values in these experiments, if described by a pseudo-first order model, were 0.133, 0.049, 0.034, and ~0 d$^{-1}$ for 5 mM Fe(II) with 2.32 g/L magnetite, 10 mM Fe(II) with 1.16 g/L magnetite, 15 mM Fe(II), and 5 mM Fe(II), respectively (Fig. 6C). Similar experiments with 1,1,2-TCA showed TCA removal, in order from greatest mole fraction removed to least, 5 mM Fe(II) with 2.32 g/L magnetite, 15 mM Fe(II), 10 mM Fe(II) with 1.16 g/L magnetite, 5 mM Fe(II), and control reactor (Fig. 6D). The VC mole fraction yield in order from greatest yield to least was 5 mM Fe(II) with 2.32 g/L magnetite, 15 mM Fe(II), 5 mM Fe(II), and 10 mM Fe(II) with 1.16 g/L magnetite.
Fig. 6: (A) Mole fraction of CT and $k_{\text{obs}}$ in reactors, (B) Mole fraction of CF production and removal over time, and (C) $k_{\text{obs}}$ of CF in pH 10 reactors containing 15 mM total Fe(II) with a 5 mM experimental comparison with a control. 1,1,2-TCA degradation with 15 mM total Fe(II) with varying [Fe(II)] and [magnetite] with a 5 mM experimental comparison with a control.

4.0 Discussion

4.1 Mineral characteristics and pH variations during and after synthesis

Rapid fluctuations between acidic and basic conditions during synthesis resulted in variable conditions in otherwise identical reactors that account for variability observed...
in experiments. Fewer experiments were conducted at pH 8 and 9 because the target pH was closer to the inflection point. At these target pH levels, small additions of iron solutions and NaOH caused large changes in pH. This would make the difference between experiments at pH 8 and pH 9 small, depending on whether the solution spent more time at acidic or basic conditions during the synthesis. Iron oxides could be synthesized before site application in an engineered contaminated site, but distribution of magnetite or ferrous hydroxide in the subsurface as a treatment method may be improved if the solutions were injected separately so that precipitation happens in the subsurface. This study is aimed at estimating how CHCs might behave in such a setting.

According to research by Leussing and Kolthoff (1953), the slightly greenish tint in the Fe(II) precipitates that formed indicates that a small amount of oxygen may be present in the reactor was observed in all reactors that contained only Fe(II) species. This suggests that small amounts of green rust may be forming. Similarly, in reactors with lower pH, hydroxide may be limited, so chloride and sulfate green rusts may form. There may have been oxygen in the glovebox because the measurement of oxygen within the glovebox during synthesis was accurate only to the nearest ppm and may read 0 in when the concentration of oxygen was less than 500 ppb. The increased graininess of the particles seemed to indicate that either particles had agglomerated or particles became larger over time due to crystalline growth. Future studies of crystal growth of solid ferrous hydroxide may require more detailed study of Fe(II) phase diagrams similar to those in Strathmann and Stone (2002). Future electron microscopy analyses of ferrous hydroxide may be possible, but only if the particles are dried anaerobically and gold
coated to prevent oxidation, which was beyond the methods available for this investigation.

For magnetite and presumably for solid Fe(II) species, reductive dechlorination was a surface mediated process by which structural Fe(II) donated electrons through the mineral to the chlorinated pollutant (Vikesland et al., 2007). The kinetics of pollutant destruction was proportional to the concentration of magnetite surface sites. Vikesland’s work also indicated that surface adsorbed Fe(II) would increase reactivity (2007). Vikesland’s procedure resulted in 9 nm particles. Light microscopy suggested that magnetite particles were roughly spherical or amorphous. However, magnetite tends to form octahedral crystal structures (Chesterman, C., 2000). The unit cell had a formula of \((\text{Fe}^3+)_{\text{tet}}(\text{Fe}^3+_8-\text{Fe}^2+_8)_{\text{oct}}\text{O}_{32}\) (Rebodos and Vikesland, 2010). Agglomerates of particles of solid Fe(II) species under light microscopy were colorless and appeared to be completely amorphous.

Changes in pH during the first day of the experiment was likely due to the continued reaction of iron with hydroxide in the aqueous solution. As hydroxide is removed, the pH drops. This was a characteristic of using freshly precipitated Fe(II) species and magnetite. The choice to not use buffer resulted in no protection against the drop in pH, which more closely reflects the conditions of the environment, but adversely affected reproducibility. Given that many others have used buffers to confirm pollutant degradation mechanisms, it was logical to use no buffers to more closely approximate what might happen in natural systems or large scale engineered systems. In experiments in which the parent contaminant takes longer than 24 hours to degrade, a notable drop in the rate of the reaction occurs after the first 24 hours. In experiments such as the CT
experiments with high concentrations of Fe(II) species or a mixture of Fe(II) and magnetite, however, it can be said that much of the CF degradation is taking place abiotically at around pH 9 instead of at the initial pH of 10.

4.2 CHC degradation by magnetite only

Magnetite has been well documented as a strong reducer of carbon tetrachloride (Agarwal et al., 2011; Amonette et al., 2000; Danielsen et al., 2005; McCormick et al., 2008; and Vikesland et al., 2007). Preliminary experiments, which were designed to provide a baseline reference for this work and provide a loose comparison to previous studies, supported the results described in these references. Magnetite preparation methods were relevant in this investigation due to the apparent higher reactivity found in magnetite precipitated at or above pH 12 than the presumed magnetite or mixed phase iron oxides precipitated at lower pH (Fig. S5). Possible explanations for the disparity in behavior include the possibly larger grain size in the titrated magnetite (Fig. S10) than the magnetite produced by the method outlined in Vikesland et al. (2007). Light microscopy images showed a similar color in the aggregates produced by each method, but small amounts of other minerals may have been present as well, which could change the redox behavior of the mineral particles.

CT $k_{obs}$ values increased as the magnetite concentration increased and as the pH increased. In the Amonette et al. (2000) study with CT and magnetite indicated that as pH increased, the mineral’s surface becomes more negatively charged, which increases sorption of cations. Amonette et al. referred to the adsorption of Fe(II) species for this, but such pH increases also improve contaminant reduction when no aqueous Fe(II) was
added to the reactor, suggesting that the negative charge that accumulates on the surface of the particles may assist in CT degradation.

Other research from Danielsen et al. (2005) showed that high concentrations of magnetite, like 25 g/L, produced primarily carbon monoxide (CO) as a reaction product through an elimination pathway with TRIS buffer, TEA buffer, and no buffer experiments. Preliminary experiments with magnetite produced CF as the primary product, but pH 12 data showed that CF yield declined with no other product yield increasing. Sampling did not include CO, which suggests elimination as a possible reaction pathway at high pH. Elimination was not a dominant mechanism for CT in any of the experiments with magnetite in this study (Fig. 1) because of two differences in procedure: (1) the mass concentration of magnetite in Danielsen et al. (2005) was 25 g/L while this study was < 4 g/L, and (2) the particles used in this study were produced near the starting pH of the reactor compared to Danielsen’s, which were synthesized at high pH. When comparing Danielsen's results to this study, it is suggested that the concentration of magnetite affects the pathway of reaction that CT might follow. While high concentrations of magnetite may reduce CT to innocuous products, low magnetite concentrations like those used in this investigation produce CF, a hazardous byproduct that was more persistent in the reactors. Since magnetite concentration was shown in the multiple linear regression to have a strong negative influence on CF yields, some of the CF seen in experiments containing higher [magnetite] likely followed similar reaction pathways to form CO and formate (Danielsen et al., 2004). The removal of CF without producing other detectable byproducts would have decreased the mass balance with increasing [magnetite] as well.
Danielsen and Hayes (2004) also observed that the CF that was produced by reductive dechlorination of CT did not degrade with magnetite, which is consistent with the results from this investigation. However, at pH 12, CF degradation was visible. CF degraded almost as quickly in the control, though, suggesting that the majority of CF degradation when [magnetite] is small is a result of pH effect. Traces of methane were observed (not shown), indicating that CF degrading by pH effect followed a pathway other than reductive dechlorination.

4.3 Effect of [Fe(II) species] on CHC removal

The light gray to pale green precipitate was presumed to be ferrous hydroxide (Fe(OH)$_2$) according to descriptions in Leussing and Kolthoff, 1953. At pH 9 through 12, it is also understood that a portion of the Fe(II) might remain in the Fe$^{2+}$ aqueous form, FeOH$^+$ aqueous form, and the Fe(OH)$_2$ aqueous form (as determined by Fe(OH)$_2$ solubility) and reach an equilibrium distribution of these species dependent on the aqueous conditions (Strathmann and Stone, 2002). The ionic forms may then adsorb to the surfaces of the precipitated solids according the behavior described by Amonette et al. (2000) as described in section 4.1. At higher pH such as 10 and 12, Fe(II) may also form the species Fe(OH)$_3^-$ (Naka et al., 2006). The precipitate becomes more of a greenish color over time and when exposed to low concentrations of oxygen (Leussing and Kolthoff, 1953) or when Fe(III) is present (Strathmann and Stone, 2002).

The measurement of oxygen within the glovebox during a synthesis was accurate only to the nearest ppm, resulting in some variation in the composition of the Fe(II) species if traces of oxygen below detection threshold were present. Lower pH conditions
such as pH 8 may also limit the amount of hydroxide available to produce precipitates and may produce less Fe(OH)$_2$. Experiments with Fe(II) species alone may instead produce sulfate or chloride green rust as both anions are present in the slurry at the time of synthesis (Fig. S3). Other conditions were identical and the two experiments were produced at the same time using the same materials, which makes oxidation less likely. Fe(II) species precipitates tended to form somewhat less readily at pH 7 and 8 as well.

The reaction of Fe(II) species toward CT and CF was drastically greater than magnetite’s reactivity toward CT (Fig. 2A). One possible explanation for this could be a greater [Fe(II)$_{\text{structural}}$] near the surface of the solid because exclusively aqueous Fe(II) has generally been shown to be unreactive toward groundwater pollutants (Klausen et al., 1995). Another reason for the higher reactivity could be due to the lower agglomeration of solid Fe(II) species. At high concentrations, like those particles shown in Fig. S11, particles clearly agglomerate, but at low concentrations it was clear that the packing of agglomerates was looser than those of magnetite (Fig. S9). The greater reactivity of Fe(II) species allowed for the removal of CF at [Fe(II)] of 15 and 25. Although pH 9 and 10 experiments showed similar increases in $k_{\text{obs}}$ for CT with increasing [Fe(II) species], the increase in CF $k_{\text{obs}}$ at pH 9 was modestly less than that for pH 10. This was likely because Fe(II) speciation favored more reactive Fe(II) species and possibly greater concentration of reactive solid Fe(II) species at pH 10.

Studying Fe(II) influence on CT removal over all experiments in the multiple linear regression study was problematic because in some cases, Fe(II) was a group of solid species whereas other times, Fe(II) was likely to be surface bound species in association with magnetite. Despite this interference, it was expected that [Fe(II)] might
exhibit an overall behavior of increasing kinetics and reducing CT removal. The interference factors of including magnetite studies in the multiple linear regression masked the pattern in \( k_{\text{obs}} \) but showed that both CT remaining and mass balance decreased with increasing \([\text{Fe(II)}]\). This is not surprising because solid Fe(II) species were highly reactive toward CT and, in a few cases, were able to remove significant amounts of CF without leaving another detectable byproduct. Other factors (pH and \([\text{magnetite}]\)) interfered with the pattern of lower CF yield with increasing \([\text{Fe(II)}]\).

The behavior of 1,1,2,2-TeCA at pH 10 was problematic because 1,1,2,2-TeCA was highly susceptible to degradation due to the pH effect. Whether degradation took place by the pH effect in the control or due to reduction at the surface of iron oxide particles, the dominant product was TCE. The pH effect complicated any reduction patterns as a function of \([\text{Fe(II)} \text{ species}]\) as seen in Table 1. As seen in Fig. 3A and B, the presence of greater amounts of Fe(II) species and especially magnetite was observed to slow the reaction. Since much of 1,1,2,2-TeCA’s reduction was a function of pH, the iron oxides’ attraction for hydroxide may have caused pH heterogeneities that would locally drive pH down at the surface of the iron oxides, where a reduction reaction would take place. If the concentration of Fe(II) species or magnetite was high, the pH effect was lessened and the reaction was slower (magnetite series and at 15 mM Fe(II)). TCE yields (Table 2) suggest that an intermediate or another reaction pathway may be present, producing products not observed on the GC’s setup. TCE was not observed to degrade in 1,1,2,2-TeCA experiments likely because the experiments were not extended over several months’ time.
Degradation of 1,1,2-TCA was far slower than that of CT and 1,1,2,2-TeCA and it was not susceptible to the pH effect. Values of $k_{\text{obs}}$ for the three [Fe(II) species] that were tested did not change significantly, but the amount of VC produced in each experiment increased with increasing [Fe(II)], suggesting that a stable intermediate might form between 1,1,2-TCA and VC or that a different pathway was favored at lower [Fe(II) species], resulting in byproducts not detected on the GC. In Table 1, parent CHC compounds that were not significantly affected by another degrading mechanism like pH effect (CT, CF, and 1,1,2-TCA) showed linear trends in their degradation kinetics and in their daughter product yields.

4.4 Effect of pH on CHC removal with Fe(II) species

At pH 7, CT removal was very limited, likely because Fe(OH)$_2$ was likely not formed in either the solid or aqueous phase according to Strathmann and Stone (2002), leaving FeOH$^+$ as the main Fe(II) species to react with CHCs. The color of Fe(II) species below pH 8 was also seen to be a slightly darker green than reactors made above pH 9, suggesting that pH 7 and 8 experiments were made up of green rusts. The Fe(II) rich species at neutral pH with 5 mM Fe(II) was insufficient to remove all of the approximately 0.076 micromoles of CT injected, but 15 mM Fe(II) degraded all of the CT in the reactor (this was the reason for the sudden increase in CF yield in Table 2). Under basic conditions, [Fe(II) species] as low as 1 mM were potent reducers of CT. CT $k_{\text{obs}}$ values increase with increasing pH and with increases in concentrations of Fe(II) species at all pH levels, but the pH had only a modest impact on Fe(II)’s effect on CT $k_{\text{obs}}$ and CF yields (Table 2). CF $k_{\text{obs}}$ with increases in [Fe(II)] were more dependent on pH (Table 2).
1,1,2,2 TeCA was far more susceptible to degradation by the pH effect than CT and CF. The data was fitted with linear or exponential trendlines as an approximate model of the effect of increasing pH. More detailed work is necessary to confirm observations in the 1,1,2,2-TeCA part of this study. The check on correlation between pH and $k_{\text{obs}}$ was 0.501 (R output in SI). The pH effect was much lower at pH 8 and 9, which, after the pH drifted downward, was closer to pH 7 and 8.5. This pH drift would explain why a second cycle completed for these experiments showed no degradation in the control. The degradation of 1,1,2,2-TeCA in the second cycle for the experimental reactors at pH 8 (not shown) showed that after the pH drifts downward toward pH 7, pH effect will be less important, but Fe(II) species would continue to be reactive toward 1,1,2,2-TeCA.

In a pH 10 experiment with 5 mM of Fe(II) species that were slightly oxidized (indicated by a greenish color), the yield of TCE was equal to that of the corresponding control. However, in an experiment with an identical amount of Fe(II) species where the precipitates were more reduced (and paler in color), the yield of TCE was smaller and the $k_{\text{obs}}$ of the 1,1,2,2-TeCA was somewhat greater than that of the experiment with the more oxidized Fe(II) species. The lower TCE yield as well as the difference in $k_{\text{obs}}$ that was seen between the DI water controls and the experimental reactors containing Fe(II) species suggests that the mineral may cause the 1,1,2,2-TeCA to follow a different reaction pathway. TCE results from this investigation show that TCE is not susceptible to either the pH effect or to low concentrations of Fe(II) species.

The TCE that was produced in 1,1,2,2-TeCA experiments was not seen to degrade, mainly, because the experiments were not permitted to run for several months.
However, pH 8 and 9 experiments with 15 mM Fe(II) showed over a long period of time that TCE does degrade with Fe(II), but only with a long exposure time. TCE removal at 15 mM Fe(II) over four months (Fig. 4C) suggested a degradation pathway largely unaffected by the pH effect that removed 1,1,2,2-TeCA from controls and produced products not visible on the GC. Since the majority of the post synthesis pH drift took place within one day of the synthesis leveled out after the first day, the TCE losses that were observed were likely not a result of pH effect.

It was also clear that the rate at which these reactions might proceed was affected by other factors such as ionic strength and difficulties with maintaining pH during mineral synthesis as the fluctuation in pH could not be exactly replicated by hand in different reactors and may have affected how the mineral phases were precipitated, creating greater variability in the results. Ionic strength has also been described in other work to have a strong effect on rate kinetics (Schultz and Grundl, 2000). In Strathmann and Stone's investigation (2002), ionic strength from anions like chloride and sulfate had little effect on oxomyl carbamate elimination reactions, but those same ligands greatly increased the rate of oxomyl carbamate reduction. In this investigation, since the primary reaction pathways observed in chlorinated methanes were hydrogenolysis, a reduction reaction, increasing ionic strength may have increased $k_{obs}$ values of chlorinated methane loss. On the other hand, since an elimination reaction was observed in chlorinated ethane degradation, ionic strength may have had less effect on the degradation of 1,1,2,2 TeCA and 1,1,2 TCA, making the results more consistent from one reactor to the next.

4.5 Effect of [Fe(II)] and [Magnetite] together
Many authors have noted that the introduction of Fe(II) can improve reaction of iron oxide solids toward many environmental pollutants (Amonette et al., 2000; Lee et al., 2003; Liang et al., 2009; Klausen et al., 1995). This study also noted a synergistic effect endowed by adding aqueous Fe(II) to magnetite. Ideally, magnetite has a stoichiometric ratio of structural Fe(II) to Fe(III) of 0.5, but that ratio drops as the mineral is oxidized. Past studies using Mössbauer spectroscopy showed that exposing the oxidized magnetite to aqueous Fe(II) increased the structural Fe(II)/Fe(III) ratio toward stoichiometric conditions (Tratnyek et al., 2011). If pollutant reduction was mediated by surface bound Fe(II) species or Fe(II)\textsubscript{structural}, then the introduction of aqueous Fe(II) should increase the reaction rate constants and increase the sorption of various Fe(II) species. If magnetite Fe(II)/Fe(III) in this investigation was at or near 0.5, as expected, the electron uptake by magnetite particles should have been limited because, as described in Gorski and Sherer (2009), electron transfer between adsorbed Fe(II) and stoichiometric magnetite was minimal.

In section 4.2, several reactive species of Fe(II) were described, FeOH\textsuperscript{+}, Fe(OH)\textsubscript{2}\textsuperscript{0} and Fe(OH)\textsubscript{2} solid. However, according to Liger et al. (1999), Fe(II)\textsubscript{adsorbed} may also have speciated, forming =FeOFeOH and =FeOFe(OH)\textsubscript{2} on the magnetite surface, which could also add to the reactivity. At pH 10, there was generally an increase in \(k_{obs}\) with an increase in either magnetite or Fe(II). However, in the case of low concentrations of both Fe(II) and magnetite (5 mM with 1.16 g/L), the CT rate constants were still lower than in the 5 mM Fe(II) species alone (Fig. 5A). The trade-off in these experiments was that while CT degraded more slowly, CF, which was untouched by low concentrations of either Fe(II) or magnetite, was removed when the two phases were used together.
Liger et al.’s (1999) phase diagrams showed that the more reactive Fe(II) species formed in an adsorbed setting at higher pH than they did without an iron-oxide. If speciation of surface-bound Fe(II) did not take place until higher pH levels, the combined experiments might have less reactive surface species, but a stronger overall reductive potential. Magnetite agglomerates were also observed to be more tightly packed than solid Fe(II) species unless the [Fe(II)] was very high. Solid Fe(II) species may therefore have a higher effective surface area than magnetite agglomerates that would increase initial reaction rates, but still have a lower overall reduction potential than the mixture of magnetite and Fe(II) species. This may result in a slower initial reaction in a system that results in more reduced byproducts.

The production of CF in these pH 10 experiments with both Fe(II) and magnetite indicates a reduction reaction. When CF was the parent compound, reductive dechlorination was seen to take place resulting in a significant quantity of methane (75 to 80%) with the remaining carbon mass balance likely belonging to CO and formate (Fig. 5D) (Danielsen et al., 2004). However, methane was not seen in such amounts in experiments where CT was the parent compound, suggesting higher yields of CO and formate. In experiments with CF as the parent pollutant, the magnetite and Fe(II) mix had greater reduction potential whereas when CT was the parent compound, the iron oxides were partly oxidized from reducing CT. Furthermore, when CF was the parent pollutant, CF was exposed to the higher initial pH during the first day of experimentation before the pH drifted downward.

As stated previously, the influence of either [magnetite] alone or [Fe(II)] alone on CT degradation in the multiple linear regression study was problematic due to the fact
that they each interfere with one another. Fe(II) species alone remove CT more quickly than magnetite alone and with small concentrations of Fe(II). Further, when the two are together, Fe(II) has a smaller influence on kinetics and product distribution than magnetite. Since the totality of the data shows a non-linear pattern of behavior, isolating the contribution of either [magnetite] or [Fe(II)] when both are present is problematic.

1,1,2,2-TeCA and 1,1,2-TCA were largely unaffected by changes in [Fe(II)]. Reduction of 1,1,2,2-TeCA had a low $k_{obs}$, but degraded into TCE. 1,1,2,2-TeCA was more susceptible to the pH effect than to reduction by iron oxides, interfering with any potential pattern of degradation. This was true for both variable [Fe(II)] with 1.16 g/L magnetite (Table 3) and for variable [magnetite] with 5 mM Fe(II) (Table 4). Vinyl chloride was too recalcitrant to show a strong trend with increasing [Fe(II)] or [magnetite]. However, higher [magnetite] experiments showed higher VC yields, showing the stronger reduction potential of structural Fe(II) compared to adsorbed Fe(II).

4.6 Effect of pH on the interaction of [Fe(II)] and [Magnetite]

Changes in [magnetite] seemed to have little effect on CT $k_{obs}$ at pH 8 and 9 as though the increased [magnetite] quenched the reaction. This artifact may have been created by unknown interference factors or it may indicate another trend concerning magnetite or other iron oxide particles that precipitate at pH 8 and 9. Quenching the reaction may be a logical analogy of what happened in these experiments if adsorbed Fe(II) does not speciate into potently reactive species until the ambient pH is between 9 and 10. Another possibility was that the mineral particles produced localized lower pH levels at the surface of the particles. If the pH of solution at the surface of the particles
was less than 9 as the mineral was reacting with and removing hydroxide anions from the water, this may have prevented Fe(II) that was freshly adsorbed from speciating into more reactive forms. A third factor that may influence the adsorption is the pH itself. Amonette et al. (2000) has indicated that negative charge builds up on the surface of the mineral as pH increases, which encourages adsorption. At pH 8 and 9, this effect will be less potent than at pH 10 as aqueous Fe(II) species will be less likely to adsorb to magnetite and contribute to the reaction. More research is clearly needed to isolate what mechanisms affected the behavior of Fe(II) with magnetite at those lower pH levels.

The effect of these factors would account for the decrease in reactivity as [magnetite] increased and the apparent lack of reactivity in the 2.32 g/L magnetite with 5 mM Fe(II) system at pH 9, whereas its pH 10 counterpart showed strong reduction characteristics toward both CT and CF (Table 5). The added Fe(II) at lower pH may adsorb as the species ≡FeOFe^+, which was less reactive according to Liger et al. (1999). More work is needed to understand how adsorbed Fe(II) speciation differs from aqueous Fe(II) speciation.

A fourth factor that may affect reactivity at lower pH that should be mentioned is that the magnetite particles themselves may be different from those synthesized at pH 10 or 12. Most chemogenic magnetite synthesis procedures call for precipitating magnetite particles from a basic solution of a mixture of Fe(II) and Fe(III), or by adding an appropriate amount of oxidant to a solution of Fe(II). The method for this study involves precipitation of stoichiometric amounts of Fe(II) and Fe(III) salt solutions near the target pH. At pH 8 and 9, the result could be a less reactive mineral species, or, because of the
fluctuation of pH between more basic and acidic conditions, a mixture of particle species formed at higher and lower pH.

The multiple linear regression study of all CT experiments revealed that pH was a greater influence on $k_{obs}$ than [magnetite] and [Fe(II)] based on individual p-values in a full model (See SI). The $k_{obs}$ values were shown to increase with [magnetite] the most when Fe(II) species were also present in the reactor. The low p-values in the full models indicated an extremely low likelihood that the results of increasing $k_{obs}$ with increasing pH was a result of random error. Inconsistencies in the p-values and the relatively low values of the variable correlation can be explained because Fe(II) species that form when no magnetite was present had a stronger effect on $k_{obs}$ than magnetite. Increases in pH also increased the reactivity with both Fe(II) solid species and magnetite.

4.7 Influence of structural vs. Adsorbed Fe(II)

The potency of experiments with mixtures of magnetite and Fe(II) can be measured by magnitude of $k_{obs}$ or by the product distribution. CT $k_{obs}$ values indicated that the most potent system was 15 mM Fe(II), but the 5 mM Fe(II) with 2.32 g/L magnetite experiment was able to remove more CF at pH 10. In remediation, the goal is to degrade the pollutant to non-toxic byproducts, which was more likely, in this system, with 5 mM Fe(II) and 2.32 g/L magnetite. The trade-off of the rate of reaction for more reduced byproducts would therefore be worthwhile.

1,1,2-TCA was recalcitrant and showed a very similar behavior to CF, but where CF formed methane with a small amount of DCM, 1,1,2-TCA underwent dihaloelimination, forming VC. The initial $k_{obs}$ from 1,1,2-TCA reactors was problematic,
perhaps as a result of pH effect, so final product yields and degradation profiles would be a superior method of determining reactivity in the 1,1,2-TCA experiments. It was determined from this investigation that the combination of magnetite and Fe(II) produced more favorable results than Fe(II) species alone, but only when the Fe(II) concentration was fixed at 5 mM Fe(II) and magnetite concentration was higher (2.32 or 3.48 g/L). This result may be because of differences in Fe(II) speciation across the larger surface area of large concentrations of magnetite.

5.0 Conclusions

- In systems contaminated with highly chlorinated hydrocarbons and more persistent contaminants like CF and 1,1,2 TCA, the order of effectiveness of the reductants examined from least to most effective in all pH 10 experiments attempted was magnetite alone, Fe(II) species alone, and magnetite combined with Fe(II) species. One exception to this condition was seen in the pH 10 1,1,2,2 TeCA investigation, where \( k_{\text{obs}} \) values for TeCA loss in 5 mM Fe(II) alone was greater than those of the iron phase mixtures. However, 1,1,2,2-TeCA was observed to be highly sensitive to pH, degrading to TCE under pH 9 and 10 conditions without any iron oxide phases.
- Of the variations of concentrations tested at pH 10 when examining the combination of magnetite and Fe(II), the combination that was found to be most effective against chloroform, 1,1,2,2 TeCA, and 1,1,2 TCA was a high concentration of magnetite (at least 2 g/L) with a low concentration of Fe(II) (5 mM), but this was done at the expense of parent compound \( k_{\text{obs}} \). The mixture of magnetite and Fe(II) was less effective at pH less than 10.
Abiotic removal of highly chlorinated methanes and ethanes by mixtures of Fe(II) species and magnetite shows promise for natural systems with potentially high iron concentration and pH such as wetlands, the aerobic and anaerobic interface, and in engineered systems.
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Chapter III
A Review of Known Physicochemical Properties, Toxicology, Behavior, and Remediation of 2,4-Dinitroanisole (DNAN), Nitrotriazolone (NTO), and Nitroguanidine (NQ)

ABSTRACT

The chemicals that make up insensitive high explosive (IHE) formulations are of increasing interest to the U.S. armed forces due to their lower heat and shock sensitivity that prevents injury and equipment loss caused by unintentional detonation. These chemicals include 2,4-Dinitroanisole (DNAN), Nitrotriazolone (NTO), and Nitroguanidine (NQ). The increased use of IHEs makes environmental releases more likely, but their physical properties, their toxicity to organisms, environmental impact, and their movement/fate in the subsurface are poorly understood. The goal of this review is to summarize the physical properties, partitioning behavior and movement, transformations, and fate of above IHEs.

DNAN, NTO, and NQ are organic compounds with nitro functional groups that are designed to replace TNT and RDX. The solubility of DNAN is low and it is considered slightly hydrophilic and adsorbs to carbon and organic matter, while NTO and NQ are more soluble. DNAN shows a moderate toxicity by inhibiting bacterial growth and affecting reproduction of larger animals. It is highly toxic if ingested. Little is known of NTO's toxicity, but it's believed to be less toxic than DNAN. NQ is not considered toxic, but its reduction products may have relatively high toxicity.
DNAN, NTO, and NQ tend to undergo either reduction reactions at their nitro substituents or they can also be oxidized, which involves reactions that replace substituents with a hydroxide group. DNAN may also produce Meisenheimer structures. Both DNAN and NTO may produce azo dimer products. Degradation to innocuous products involves multiple steps. This review examines studies on fate and remediation of IHE compounds and identifies gaps in understanding.

Key Words: Insensitive munitions, Organic pollutants, Dinitroanisole, nitrotriazolone, Nitroguanidine, explosives

1.0 Introduction

Unintentional detonation of munitions, often during transport, has cost over 600 lives and caused over $4 billion in equipment damage since 1926 (Walsh et al. 2014). Therefore, there is great interest in the U.S. armed forces to find alternative munitions compounds that are less prone to unwanted detonation or ignition. This class of explosives was called insensitive munitions compounds (IMCs) and was recently renamed insensitive high explosives (IHEs). 2,4-dinitroanisole (DNAN) is a promising replacement for trinitrotoluene (TNT) (Boddu et al. 2008; Hawari et al. 2014; Saad et al. 2012). DNAN also has industrial uses in the synthesis of dyes and insect repellants (Boddu et al. 2009). DNAN was used in the formulation of explosives during World War II as TNT supplies became limited (Boddu et al. 2009). Nitrotriazolone (3-Nitro-1,2,4-triazol-5-one or NTO) is comparable in its performance to RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) (Nandi et al. 2013). Nitroguanidine (NQ) is a relatively new explosive compound that is used as an explosive propellant (Mu et al. 2012). Another use of NQ is as an insecticide (Mu et al. 2012). In recent years, DNAN and NTO have become
widely used in low and high-grade explosive formulations, making environmental releases inevitable. However, their properties and fate in the environment have not been fully characterized. Estimates of their discharge into the environment are also not available from referenced sources, which makes tracking their effects difficult (Kennedy et al. 2017). Another co-contaminant found in insensitive munitions formulations is the oxidizing salt, ammonium perchlorate (AP) (Walsh et al., 2014), but AP is not addressed significantly in this review.

Three common explosive formulations that contain DNAN, NTO, and/or NQ are Insensitive Munitions eXplosive 101 (IMX 101, composed of 43% DNAN, ~14% NTO-360, and 37% NQ), IMX 104 (32% DNAN, 53% NTO, and 15% RDX), and Picatinny Arsenal eXplosive 21 (PAX 21, made of 34% DNAN, about 31% RDX, and 30% AP) (Taylor et al. 2015; Walsh et al. 2014). IMX 104 produces more detonation residues than other formulations (Walsh et al. 2014). The first IHE approved for use was the 60 mm mortar cartridge with PAX 21 (Walsh et al. 2014).

2.0 Physical Properties

2.1 2,4 Dinitroanisole (DNAN)

DNAN is a nitroaromatic compound and solid at room temperature. Its Lewis structure is illustrated in the first diagram in Fig. 7. The formula weight of DNAN is 198.135 g/mol. Three of the characteristics that determine the fate and behavior of a compound in the subsurface are water solubility ($S_w$), octanol-water partition coefficient ($K_{ow}$), and Henry's constant ($K_H$) (Boddu et al. 2008). For DNAN, the reported $S_w$ varies from 213 to 276.2 mg/L in pure water at 298.15 K (Boddu et al. 2008; Hawari et al. 2015; Taylor et al. 2015). DNAN is like most other explosive compounds, showing a low solubility, which means that its
concentrations in the groundwater tend to be small. However, the manufacturing of DNAN, NTO, and the explosive formulations requires a high volume of water, which generates significant amounts of wastewater (Le Campion et al. 1999; Ahn et al. 2011). The log $K_{ow}$ estimated for DNAN is $\sim$1.58 to 1.61, indicating that it is slightly hydrophilic (Boddu et al. 2008; Hawari et al. 2015). The predicted Henry’s constant, $K_H$ for DNAN is 1.37 m$^3$•Pa/mol or 0.013 L•atm/mol (Boddu et al. 2008). The density of solid DNAN is 1.34 g/cm$^3$ (Taylor et al. 2015).

2.2 Nitrotriazolone (NTO)

NTO is a compound (see structure in Fig. 8) that was first created in 1905, but its explosive characteristics were examined later in 1985 (Crouse et al. 2015). The formula weight for NTO is 130.063 g/mol, and the density of solid NTO is 1.93 g/cm$^3$ (Taylor et al. 2015). However, many of the physical properties of NTO remain unknown. Experimental solubility of NTO at 291.2 K ranges from 12.8 to 16.6 g/L, making it much more soluble than DNAN (converted from Spear et al. 1989; Taylor et al. 2015). Considering its higher solubility, the $K_{ow}$ value of NTO may be less than that of DNAN, but it has not yet been determined experimentally. A reliable experimentally determined Henry’s constant for NTO is still largely lacking and should be investigated.

Another important property of NTO is its p$K_a$ of 3.7, making it highly acidic in an aqueous solution (Chipen et al. 1966; Le Campion et al. 1999; Taylor et al. 2015). Taylor et al. (2015) performed outdoor tests to determine the pH of its products that may enter the soil by dissolving the residues of IMX 101 and IMX 104 in rainwater. The pH of the solutions ranged from 2.8 to 3.6 and 2.6 to 3.3 at the beginning of the tests for IMX 101 and IMX 104,
respectively, and approached neutral near the end of the tests (Taylor et al. 2015). Since shallow groundwater systems typically have a low buffering capacity, it can therefore become easily acidified by NTO contamination (Takem et al. 2015). A low pH groundwater may then mobilize divalent cations adsorbed to oxides and clay minerals (Kjøller et al. 2004).

2.3 Nitroguanidine (NQ)

Nitroguanidine is a nitroimino compound that has been used as a military propellant (Kaplan et al. 1982) and found in IHE formulation IMX 101 (Taylor et al. 2015; Walsh et al. 2014). It exists in two forms (Fig. 9, structures (a) and (b)) that are interchangeable and at equilibrium with each other, but form (a) tends to dominate in acidic to slightly basic media (Kaplan et al. 1982). NQ exists as a white powder solid under normal conditions with a molar mass of 104.07 g/mol (Thermo-Fisher 2017) and a density of 1.55 g/cm$^3$ (Taylor et al. 2015). The solubility in water for NQ may range from ~4.2 g/L (Mu et al. 2012) to 4.4 g/L (Jenkins et al. 2012; Rossin et al. 2017), which is more than that for DNAN but less than NTO. It is somewhat less soluble in methanol, at ~0.3 g/L (Mu et al. 2012). The log $K_{ow}$ for NQ is -0.89 and its Henry’s constant is $4.67 \times 10^{-13}$ L·atm/mol at 298 K (Jenkins et al. 2012). It has a low soil-water partition coefficient at 0.15 to 0.43 L/kg and carries no charge between pH -0.5 and 12.2, making it mobile in aqueous and soil systems (Rossin et al. 2017). NQ is stable under normal conditions but it poses a reactive hazard and is incompatible with strong oxidizers (Thermo-Fisher 2017). Most of the other physicochemical properties of NQ are not known at this time. Little work has been done on its environmental impacts and toxicology in residues and wastewater discharge because it was not manufactured on an industrial scale in the United States (Noss and Chyrek 1984).
2.4 *Microbiological Toxicology*

DNAN and NTO may both pose a threat to the environment and organisms in it. While microbes can easily degrade DNAN to 2-methoxy-5-nitroaniline (MENA; aka, 2-amino-4-nitroanisole (2-ANAN) and 2,4-diaminoanisole (DAAN), studies also show that DNAN inhibits some of the activities of methanogens, nitrifying bacteria, aerobic heterotrophic bacteria, and the bioluminescent species *Aliivibrio fischeri* according to Liang et al. (2013), who performed assay experiments in glass flasks exposing several types of bacteria to varying concentrations of DNAN. Methanogenesis was slowed in assays with any DNAN, and oxygen consumption by aerobic heterotrophic bacteria was moderately slowed after 10 hours of exposure to 390 µM DNAN (Liang et al. 2013). Nitrifying bacteria were completely inhibited with more than 260 µM DNAN (Liang et al. 2013). The co-contaminant, AP, is found in large quantities in PAX 21 residues and the presence of DNAN inhibited those bacteria that reduce the AP (Ahn et al. 2011; Liang et al. 2013). DNAN exposure also decreased the growth of the freshwater green algae species, *Pseudokirchneriella subcapitata* and the bioluminescence of the bacteria, *Vibrio fischeri* (Dodard et al. 2013).

However, the effect of NTO concentration on its degradation by microorganisms seems to be largely unstudied for its removal from industrial wastewater. Le Campion et al. (1999) observed NTO degradation by the bacteria, *Bacillus licheniformis*, when the wastewater pH was raised from 3 to neutral. This seems to suggest that the environmental hazard posed by NTO contamination may be caused by decline in groundwater pH that is unsuitable for microbial attenuation of NTO and its co-contaminants.
NQ is considered to be nearly nontoxic, having a toxicity threshold of about 2.2 g/L (Perreault et al. 2012b). However, its degradation products may have varying degrees of toxicity. As described later, NQ may also interfere with degradation of other IHE compounds (Indest et al. 2017). NQ also degrades into chemicals that can be highly toxic (Kennedy et al. 2017). Toxicity of photo-degraded NQ ranged from 0.76 mg/L to 16.1 mg/L depending on the initial concentration for the freshwater species *Ceriodaphnia dubia* while the toxicity of unreacted NQ was considered by Kennedy et al. (2017) to be “practically harmless.”

2.5 Floral and Faunal Toxicology

The toxicity of DNAN may be important to both plants and animals, but the topic has not been investigated in detail. Very little floral toxicity research has been done with DNAN, but the growth of a perennial ryegrass, called *Lolium perenne*, slowed when exposed to DNAN (Dodard et al. 2013). DNAN was found to be less toxic to earthworms than TNT, but the concentration that killed 50% of the population (LC$_{50}$) was 98 and 47 mg/kg soil for the 7-day and 14-day exposures, respectively (Dodard et al. 2013). Earthworms were also observed to avoid sub-lethal concentrations of DNAN (Dodard et al. 2013). The study with rats showed that doses of 80 mg DNAN/kg·day interfered with their male reproductive organs such as decreased mass of testes and atrophy of testicular seminiferous tubules (Sweeney et al. 2015). Spleen enlargement was observed in female rats and neurotoxicity was observed in both males and females at the 80 mg/(kg·d) doses (Sweeney et al. 2015). The maximum rate of DNAN metabolism normalized to body weight was 7 mg/h·kg$^{0.67}$ for rats and estimated to be 35 mg/h·kg$^{0.67}$ for humans assuming similar metabolism rates to the primate species *Rhesus macaques* (Sweeney et al. 2015).
The toxicological properties of NTO and NQ have not been determined in detail thus far, but limited research suggests that NTO is slightly toxic (London and Smith 1985) and that NQ is practically harmless (Kennedy et al. 2017). Tests on Chinese hamsters for genotoxicity showed no significant aberrations or other signs of genotoxicity with NTO (Reddy et al. 2011). The LD$_{50}$ for a population of rats was 5 g NTO/kg of body mass (London and Smith 1985). In male rats, oral toxicity studies revealed that NTO doses of 500 mg NTO/(kg·d) and greater reduced the size of testes (Crouse et al. 2015). Topical tests demonstrated that NTO can cause some skin irritation (Lent et al. 2015; London and Smith 1985).

As stated in section 2.3, toxicity of NQ is far less important than that of its photodegradation byproducts, which can be about as toxic as unaltered DNAN (Kennedy et al. 2017). Since NQ is photosensitive, it is likely that it will degrade after being released, so it is important that careful attention is given to what it will be broken down into by photodegradation. The LC$_{50}$ for fathead minnows with NQ was ~2,700 mg/L, whereas photolyzed NQ LC$_{50}$ was ~34.5 mg/L (van der Schalie 1985). Nitrosoguanidine, a common nitroreduction product of NQ, was also found to be carcinogenic in rats (Rossin et al. 2017). By comparison, the photodegradation products of DNAN and NTO are less toxic than their parent compounds (Kennedy et al. 2017).

3.0 Environmental Mobility

There is a lack of detailed studies to indicate the fate and behavior of DNAN, NTO, and NQ in the subsurface, but there is sufficient information to predict some likely behaviors based on their chemical properties and the behavior of similar compounds. All three chemicals are solids at room temperature and all of the common chemicals in explosive formulations that
would be found in explosive residues are water soluble to various degrees. Their behavior on the ground surface or in a body of water depends mostly on their solubility and their tendency to adsorb to sediment and organic matter. On land, this means that IHE compounds can dissolve in rainwater over time and infiltrate into the subsurface, which may eventually reach an aquifer, lake, or stream. Along the way, the chemicals may adsorb to other solid and organic material.

3.1 Sources of IHEs in the Environment

Industrial wastewaters containing DNAN and NTO may be produced during the manufacture of munitions and their formulations (Wallace et al. 2009; Ahn et al. 2011; Olivares et al. 2013). Specifically, acidic wastewaters containing 10 to 15 g/L NTO can be produced (Campion et al. 1999) during the industrial conditioning of NTO. Typically, the solutions of wastewater are stored until an effective remediation process can be developed (Campion et al. 1999; Wallace et al. 2009), but in the meantime, accidental release of these and other wastewaters can occur.

IHE compounds are likely to be released following their use by dissolution of explosive residues from munition formulations (Taylor et al. 2015). The military uses live rounds for combat training of soldiers (Dodard et al. 2013; Taylor et al. 2015). As a result, testing ranges have large quantities of explosive chemical residues that may dissolve and infiltrate into the subsurface soil and groundwater, and also reach surface water due to run-off. High order detonations involve complete fragmentation of the munitions, where the remaining the organic explosive compounds is 0.006% (e.g., 16 mg of PAX 21 residue remains from a 60 mm mortar round) (Walsh et al. 2013). Low order explosions often leave larger fragments of munitions
behind and residues of explosives (Walsh et al. 2013). Blown in place tests (BIPs) leave some of the largest explosive residues, amounting to 0.2 to 9% (1600-32000 mg in a 60 mm mortar round with PAX 21) of the original organic chemicals (Walsh et al. 2013). These percentages in the residues are small, but they can add up and if they move with the groundwater off the base, regulatory agencies can stop or limit live fire training (Taylor et al. 2015). Additionally, high order detonations that show the greatest efficiency can leave about 14 g of ammonium perchlorate (AP) residues, which is enough to contaminate 7 million liters of groundwater to 2.0 ppb, above the current US drinking water standards (Walsh et al. 2014). BIP tests can create about 35 g perchlorate residue per BIP operation (Walsh et al. 2014). Any contamination of DNAN can lead to significant environmental problems because DNAN can inhibit natural attenuation of AP (Dodard et al. 2013).

3.2 Dissolution Behavior of IHEs

In the outdoor dissolution studies conducted by Taylor et al. (2015) on IMX 101, IMX 104, and PAX 21 residues, about 2.2 L water interacted with the residues between June 2013 and October 2014. While NTO and NQ dissolved more quickly, the chunks of residue did not completely dissolve after 16 months of exposure to the elements and they estimated complete dissolution of particles to take 3 to 21 years (Taylor et al. 2015). This suggests that compounds like DNAN with low aqueous solubility will be long-term sources of DNAN contamination and in some cases may not reach the water table until long after other IHE compounds have been detected.

3.3 Possible Reaction Pathways
Nitroaromatic compounds tend to have multiple reaction pathways because they often have multiple functional groups in their molecular structure. For DNAN, most reaction pathways (Fig. 7) begin with the reduction of the nitro groups in the 2- (ortho) and 4- (para) positions by pathways 2 and 4 respectively, or by substitution of the methoxy substituent in the first position on the aromatic ring (pathway 14). The reaction pathway DNAN follows depends on the method of transformation and the group that is completing the reduction. Reduction pathways that target the nitro groups reduce first one nitro group to produce either 2-amino-4-nitroanisole (2-ANAN) or 4-amino-2-nitroanisole (4-ANAN), and then reduce the other nitro group to produce 2,4-diaminoanisole (DAAN) (Fig. 7: pathways 3 and 4; Ahn et al. 2011; Hawari et al. 2015). Whether the intermediate 2-ANAN or 4-ANAN dominates in the reaction depends upon the competing input of steric effects, which favors the production of 4-ANAN, and electronegativity, which favors 2-ANAN (Hawari et al. 2015). In the investigation by Hawari et al (2015), both biotic and abiotic processes favored 2-ANAN, indicating that electronegativity is dominant in determining the intermediate formed during nitroreduction.

DNAN and its reduction products show increasing solubility in the order of DNAN < 2-ANAN < 4-ANAN < DAAN (Hawari et al. 2015). The solubility of 4-ANAN is several times greater than DNAN whereas 2-ANAN is only slightly more soluble than DNAN (Hawari et al. 2015). The log $K_{ow}$ of DNAN reduction product decreased in the order of DNAN > 2-ANAN > 4-ANAN > DAAN (Hawari et al. 2015). DAAN can also partially ionize in water (Hawari et al. 2015). Declining $K_{ow}$ of DNAN degradation products are therefore more likely to cause their mobility in the subsurface soil and groundwater than the parent compound (Hawari et al. 2015). Ring cleavage products are not observed by reductive transformation of DNAN (Hawari et al. 2015). Sorption behavior of DNAN reduction byproducts indicates that the
intermediate products (2- and 4-ANAN) can adsorb reversibly or irreversibly to soil, and that DAAN sorbs irreversibly to soil indicating that less of the products of DNAN reduction can be recovered from the soil over time (Hawari et al. 2015).

In some conditions, DNAN reduction products can form azo and hydrazine dimer derivatives by joining/coupling of two molecules of DNAN or its reduction products by the nitrogen substituents in the ortho position forming azo structures (Fig. 7: Pathways 5 and 6; Olivares et al. 2013; Platten et al. 2010) or in the para position (Not shown; Perreault et al. 2012a). Olivares et al. (2013) suggested that azo dimers produced by pathways 5 and 6 can undergo several transformations of oxidation (pathways 8 and 9) and substitution (pathway 10), which can be followed by oxidation reactions (pathways 11 and 12) and can ultimately lead back to breaking the dimer apart into DAAN (Pathway 7 and 13) (see Fig. 7).

Substitution reactions can replace DNAN's methoxy group with a hydroxyl group to make 2,4-dinitrophenol (2,4-DNP) and methanol (Fig. 7: Pathway 14) or a methoxy nitrophenol with nitrite (Pathways 17 and 18; Rao et al. 2013). Little is known of 2,4-DNP toxicity, but it was suggested that it may be more toxic to organisms like fish than DNAN (Richard and Weidhaas 2014a). It is possible for 2,4-DNP to be oxidized into nitrate and be transformed into compounds containing -COOH and possibly -C=O structures (Fig. 7: Pathways 15 and 16; Rao et al. 2013). 2,4-DNP has an EPA reference dose of ≤2 µg/(kg·d) for chronic oral intake (Rao et al. 2013). Alternatively, the substitution of a nitro group can produce a methoxy nitrophenol (Rao et al. 2013).

Alkaline hydrolysis of DNAN may make intermediates with hydroxyl group attached to the aromatic ring that may be stable, called Meisenheimer complexes (Salter-Blanc et al.
In Meisenheimer complexes, the hydroxide anion may either attach to one of the unsubstituted positions in the DNAN aromatic ring or at the methoxy group (Fig. 7: Pathway 19). Attachment at the methoxy position may result in substitution of the methoxy group, making 2,4-DNP as described earlier (Fig. 7: Pathway 14; Salter-Blanc et al. 2013). DNAN's structure does not support an initial oxidation reaction because of its electron withdrawing nitro groups and the pollutant's resistance to get mineralized in an oxidative reaction can increase with the number of nitro groups (Shen et al. 2013).

NTO can be reduced or oxidized depending on the conditions to which it is subjected. Reduction reactions may result in 3-amino-1,2,4-triazol-5-one (ATO) with an intermediate of 1,2-dihydro-3H-1,2,4-triazol-3-one (HTO) by transforming the nitro group to an amino group under anaerobic conditions (Fig. 8: Pathway 1; Le Campion et al. 1999; Richard and Weidhaas 2014a; Krzmarzick et al. 2015). Certain reduction reactions may stop at ATO (Krzmarzick et al. 2015). Sometimes, however, ATO can undergo further reduction to 1,2-dihydro-3H-1,2,4-triazol-3-one (Fig. 8: Pathway 4; Richard and Weidhaas 2014a). It has also been demonstrated that the kinetics of NTO reduction may increase under increasingly acidic conditions, suggesting that undissociated NTO is more attracted to reduction sites than its ionic form (Koutsospyros et al. 2012). If the ATO is further degraded, it can follow one of several pathways: (i) it may be mineralized and undergo ring cleavage to produce nitrite and nitrate when exposed to oxygen (Fig. 8: Pathway 2; Krzmarzick et al. 2015); (ii) ATO may alternatively be converted to urea and hydroxyurea, from which there can be some mineralization to CO₂ (Fig. 8: Pathway 3 and 5; Campion et al. 1999); and (iii) ATO may undergo an elimination reaction to form 1,2-dihydro-3H-1,2,4-troazol-3-one (Fig. 8: Pathway 4; Richard and Weidhaas, 2014a). NTO may also form the azo dimer, azoxytriazolone (AZTO)
and a series of other azo dimers (Fig. 8: Pathways 6, 7, 10 and implied pathways 8 and 9; Cronin et al. 2007; Wallace et al. 2011). AZTO is a yellow solid with poor solubility in water and other common solvents (Cronin et al. 2007). The reaction pathway is very similar to the DNAN azo reduction process. AZTO may then be reduced to hydrazotriazolone, followed by a thermal disproportionation reaction that produces azotriazolone (azoTO) and ATO again (Fig. 8: Pathways 7, 8, 9, 10, 11; Wallace et al. 2011). NTO can also be oxidized directly that ultimately results in a combination of nitrate, carbon monoxide, ammonia, carbon dioxide, and nitrous oxide (Fig. 8: Pathways 12 through 20; Wallace et al. 2009).

Similar to the reduction pathways for DNAN and NTO, the initial step for reducing NQ begins at the nitro functional group, producing, first, nitrosoguanidine (Kaplan et al. 1982; Pathway 1 in Fig. 9). Nitrosoguanidine also has explosive properties (Sabetta et al. 1935). It also has the ability to act as an acid or base, causing different hydrolytic and degradation reactions depending on pH. Acidic conditions produce guanidine and nitrous acid (Pathway 6, Fig. 9; Sabetta et al. 1935). Neutral reactions will follow Pathway 2 and produce cyanamide and nitrogen, and basic conditions will, in addition to cyanamide and nitrogen, produce CO₂ and NH₃ (Pathway 11, Fig. 9; Sabetta et al. 1935). If nitroreduction continued, however, nitrosoguanidine would produce aminoguanidine, which was inferred by Kaplan et al. (1982) (Pathways 7 and 8, Fig. 9) and was observed by Leeds and Smith (1951). However, Kaplan’s work did show nitrosoguanidine degrading into cyanamide with nitrosamide (Pathway 2, Fig. 9). This could then be polymerized to guanidine (pathway 3) or cyanoguanidine (pathway 4), which could further polymerize to form cyclic melamine (pathway 4, Fig. 9). In Perreault et al. (2012b), NQ was observed to degrade into nitrourea (Pathway 8) and then continue to break down into CO₂, NH₃, and N₂ (Pathway 10, Fig. 9).
4.0 Transformations of IHE Compounds

4.1 Microbiologically Mediated Transformation

DNAN’s reduction to 2-ANAN and then to DAAN could be carried out using several bacterial strains. Platten et al. (2010; 2013) have conducted studies using anaerobic fluidized bed bioreactors (AFBBs) to treat DNAN and other IMs with anaerobic bacteria using ethanol as an electron donor. The bacteria readily reduced DNAN in both studies (Platten et al. 2010; Platten et al. 2013). DNAN concentrations were increased incrementally to allow the bacteria to acclimate to the energetic compound (Platten et al. 2010). The final influent concentration of DNAN was 83.33 mg/L and the pH was maintained around 7 (±0.2) (Platten et al. 2010). Results mostly showed that DNAN concentrations in the effluent from the anaerobic fluidized bed bioreactor (AFBB) were within detectable limits when there was a lower concentration of ethanol (about 75 mg/L or less) present to support the bacterial activity (Platten et al. 2010). The loss of some of the byproducts was attributed to the formation of the azo dimers shown in Fig. 7 formed by pathway 6, 7, and 10. Platten et al. (2013) demonstrated that a very similar set-up treated DNAN and N-methyl-p-phenylenediamine (MNA) with a range of concentrations of ethanol (electron donor) and with the addition of perchlorate, which provides oxygen in explosive formulations and is a chemical of interest (Platten et al. 2013). Although more ethanol was needed to degrade the pollutants, the AFBB degraded both the perchlorate and the DNAN through the early reduction pathways shown in Fig. 7: pathways 1, 2, 3, and 4. However, they then reacted with air to form azobound dimers like those formed by pathway 5 in Fig. 7 (Platten et al. 2013). Increasing the DNAN concentration gradually as in Platten et al (2013) is similar to the slow dissolution of DNAN in explosive residues, but a sudden release of DNAN like a wastewater spill may be more toxic to the bacteria.
In Arnett et al. (2009), another AFBB experiment was designed to examine how the biodiversity of anaerobic microbes in the reactor are affected by IHE including DNAN. The bioreactor was initially dominated by *Desulfiromonales* sp. (Arnett et al. 2009). After the addition of the DNAN, however, the active microbial community shifted so that *Levilinea* sp. was the dominant species, indicating that some bacteria were inhibited by DNAN (Arnett et al. 2009). Arnett et al. (2009) recommended that future work using AFBB experiments should include real wastewater from the destruction of insensitive munitions that contain DNAN and examine treatment methods for treating their nitroaniline byproducts and azo dimer byproducts.

In Olivares et al. (2013), anaerobic treatment of DNAN in wastewater was conducted using sludge, resulting in the production of 2-ANAN, DAAN (Fig. 7: Pathway 2 and 4), and the azo dimers following the reduction pathways shown in pathways 5, 6, 10, 11 and 12. They examined the rate of transformation of DNAN with unmodified aerobic or anaerobic sludge and sludge with H\(_2\) added to increase microbial activity (Olivares et al. 2013). They found that the highest rates of DNAN reduction that took place under heat killed, endogenous (unmodified), and added H\(_2\) co-substrate conditions were observed under anaerobic conditions (Olivares et al. 2013). DNAN degradation rates with all three co-substrates decreased under increasingly aerobic conditions (Olivares et al. 2013). Further research showing DAAN degradation under aerobic, microaerophilic, and anaerobic conditions might conversely show that aerobic conditions produce the strongest DAAN removal while anaerobic conditions may show the weakest.

Perreault et al. (2012a) conducted DNAN degradation studies with soil slurries under aerobic conditions and examined the effects of adding nitrogen and carbon sources. The bacterial strain of *Bacillus* 13G was found to degrade DNAN to DAAN, but they also detected
an azoxy dimer that may react to form an azo dimer, but instead of being connected at the \textit{para} position on the ring like that shown in pathways 6, 8, and 10 in Fig. 7, they are connected at the \textit{ortho} position (Perreault et al. 2012a). There were also minor amounts of acetylated compounds produced (Perreault et al. 2012a). The reaction was found to be cometabolic because they found no DNAN transformation when the \textit{Bacillus} was isolated with only DNAN as a substrate, i.e., without nitrogen and carbon amendments (Perreault et al. 2012a).

Soil enrichment studies were conducted by Richard and Weidhaas (2014a) by selecting for the fastest growing culture that degraded IMX 101 compounds while other sources of carbon and nitrogen were present. Then they selected from that group cultures that were able to use the compounds in the IMX-101 formulation as their only sources of carbon and nitrogen (Richard and Weidhaas 2014a). This eliminated cometabolic processes. They then conducted batch studies with the enrichment cultures using several concentrations of IMX-101 (Richard and Weidhaas 2014a). In this investigation, DNAN was removed within 48 hours, but generally NTO degraded more slowly than NQ and DNAN in experiments with IMX-101 concentrations between 15 and 100 mg/L (Fig. 10E; Richard and Weidhaas 2014a). For DNAN, they observed that the methoxy group was removed and replaced by a hydroxy group (Pathway 14 in Fig. 7; Richard and Weidhaas 2014a). Enrichment cultures of \textit{Pseudomonas} sp. FK357 and \textit{Rhodococcus imtechensis} RKJ300 were then able to degrade 2,4-DNP producing stoichiometric amounts of nitrite, see pathway 16 in Fig. 7 (Richard and Weidhaas 2014a). They speculated that completely degrading DNAN to innocuous products may require an assortment of microorganisms (Richard and Weidhaas 2014a).

Similarly, Indest et al. (2017) studied degradation of IMX 101 and IMX 104 in surface soils. They examined specific soil samples taken from training ranges in Camp Shelby in
Massachusetts and the Umatilla chemical depot in Oregon (Indest et al. 2017). Their experiments were carried out under aerobic and anaerobic growth conditions. They observed complete degradation of DNAN and NTO under anaerobic conditions in the Camp Shelby soil, but the Umatilla soil did not yield complete degradation (Indest et al. 2017). Microorganisms that thrived under anaerobic conditions were more efficient at removing IHE compounds than aerobic microbes, but evidence suggested that NQ and RDX in the IMX formulations may have affected the growth of organisms that could degrade DNAN and NTO and likewise that the presence of DNAN, NTO, and their degradation byproducts may have inhibited the organisms that would have degraded NQ (Indest et al. 2017). This makes remediation of sites contaminated with IMX formulation residues more complicated because of the combined effects of the IHE compounds on microbial growth. Microbe phylotypes that were associated with explosive degradation under anaerobic conditions were Burkholderiaceae, Bacillaceae, and Paenibacillaceae (Indest et al. 2017). Only the Sphingomonadaceae phylotype was associated with explosive degradation under aerobic conditions (Indest et al. 2017).

Hawari et al. (2015) studied several methods to remediate DNAN including a bacterially mediated anaerobic reaction. They conducted resting cell experiments with Enterobacter strain DM7, Shewanella oneidensis, Pseudomonas fluorescens, and Burkholderia cepacia, harvested late in the exponential growth phase in the presence of 10 mM sodium nitrate and 50 µM DNAN (Hawari et al. 2015). Transformation of DNAN favored reduction of the nitro group in the ortho position to produce 2-ANAN (Fig. 7: Pathway 2) (Hawari et al. 2015). Enterobacter degraded DNAN at the rate of 9.4 µmol/(min·g of protein) (Hawari et al. 2015). B. cepacia and S. oneidensis reduced DNAN in a similar manner but more slowly (Hawari et al. 2015).
Niedźwiecka et al. (2017) used bacterial strain *Geobacter metallireducens* (GS-15) to degrade DNAN to 2-HA-NAN, 2-ANAN, and DAAN with some the degradation following an alternative pathway, producing 4-ANAN instead. The response from GS-15 varied depending on the type and amounts of substrates and electron shuttles (Niedźwiecka et al. 2017). They observed that AQDS as an electron shuttle accelerated DNAN reduction by GS-15.

Different bacteria produce different enzymes that may react with pollutants to form different products. Hawari et al. (2015) observed bacteria that regioselectively reduced the ortho nitro group to an amino group, while Niedźwiecka et al. (2017) showed DNAN reduction at both the ortho and para positions. The research with *Nocardioides* sp. by Fida et al. (2014) showed that its enzymes trigger a rapid hydrolytic release of methanol, producing about 100% 2,4-dinitrophenol, which took place at a much faster rate than reported earlier by Richard and Weidhaas (2014a) (Fig. 10C). Further degradation was possible by forming a hydride-Meisenheimer complex (examples in Fig. 7: pathway 19) but with hydride instead of hydroxide as the nucleophile) followed by the release of nitro groups accounting for most of the nitro group substituents (Fig. 10C; Fida et al. 2014).

Le Campion et al. (1999) studied NTO degradation with *Bacillus licheniformis* in enrichment culture experiments with wastewater at pH 7.4 that were amended with 50 g/L glucose. NTO was reduced to ATO and then converted to urea, hydroxyurea, and ~40% CO$_2$ as shown by pathways 1 and 3 (Fig. 8). Some of the urea and hydroxyurea were converted to inorganic gases (Pathway 5 in Fig. 8). This result may not be significant with soil and water with low natural buffering because NTO tends to acidify groundwater. It may be necessary to adjust the pH in the subsurface with amendments because the acidic conditions may move the pH conditions out of the tolerance range for *Bacillus* and other microbial cultures.
The study by Richard and Weidhaas (2014a) also reported NTO degradation to ATO using enrichment cultures (Pathways 1 and 2 in Fig. 8) rather efficiently in 24 hours; the cultures also degraded ATO further to 1,2-dihydro-3H-1,2,4-triazol-3-one (Pathway 4 in Fig. 8). However, microbial NTO degradation took place at a much slower pace and required a longer acclimation period in comparison DNAN and NQ (Richard and Weidhaas 2014a).

Krzmarzick et al. (2015) investigated NTO degradation in anaerobic batch experiments conducted in 160 mL serum bottle microcosms, flushed with an 80/20% mix of He/CO₂ and H₂ was added as an electron donor. Krzmarzick et al. (2015) also examined NTO degradation under aerobic condition in microcosms made with 200 mL flasks topped with cotton using naturally occurring soil bacteria amended with varying soil textures (percent sand, silt, and clay) and nitrogen and carbon contents (Krzmarzick et al. 2015). Their results showed that NTO did not degrade significantly under aerobic conditions that led the authors (Krzmarzick et al. 2015) to speculate that those NTO degradation in aerobic conditions reported by Richard and Weidhaas (2014a) and Le Campion et al. (1999) were experimental artifacts inadvertently produced under localized oxygen poor conditions due to their high concentrations of organic material and cells. As indicated in previous studies, Krzmarzick et al. (2015) reported microbially-mediated NTO reduction into ATO with HTO as an intermediate (Fig. 8: Pathway 1). Adding additional substrates like 10 mg/L yeast extract (Fig. 11B) increased the rates of NTO reduction were than that under aerobic conditions and also more ATO production (Fig. 11C) (Krzmarzick et al. 2015). Krzmarzick et al. (2015) further showed that adding citrate or pyruvate (electron-donor) amendments to the microcosms decreased lag time before NTO reduction began and adding either of these with yeast extract decreased lag time further to within 5 days. Krzmarzick et al. (2015) also demonstrated that ATO did not degrade under
anaerobic conditions, but it degraded to nitrate and nitrite under aerobic conditions. The study concluded that biodegradation of NTO would form the most favorable byproducts by degrading it to ATO anaerobically followed by aerobic oxidation to reach an endpoint with safe products (Krzmarzick et al. 2015).

Nitroguanidine degradation has been studied under aerobic and anaerobic conditions with activated sludge (Kaplan et al. 1982). NQ was generally unreactive under aerobic conditions, but was reduced cometabolically under anaerobic conditions, producing nitrosoguanidine, but only after the microbes were acclimated. (Kaplan et al. 1982). NQ was completely removed after 12 days in experiments with 4 g/L nutrient broth under batch conditions (Fig. 12). Their continuous culture removed nitroguanidine in a similar manner to their batch reactor experiments, but the yield of nitrosoguanidine was higher and stabilized over an extended period (Kaplan et al. 1982). Nitrosoguanidine was degraded abiotically to produce cyanamide, cyanoguanidine, and melamine (Pathways 2-4, Fig. 9). In a study by Indest et al. (2017), however, NQ was not observed to degrade. However, since NQ was observed to degrade in other studies, it was possible that biotransformation products of DNAN and NTO may have inhibited NQ degradation (Indest et al. 2017).

While Indest et al. (2017) did not see NQ biodegradation under anaerobic or aerobic conditions, Perreault et al. (2012b) demonstrated NQ degradation under aerobic condition. Degradation assays containing 10 g soil and 10 mL of mineral salt medium and 192 µM NQ under aerobic conditions removed NQ in 6 days when only carbon (as glucose and succinate) was added (Perreault et al. 2012b). Further, NQ degradation was much slower in microcosms amended with combined carbon and nitrogen (as NH₄Cl), while the degradation was practically nonexistent in unamended soil. In assays examining the *Variovarax* strain VC1 strain in
particular, most NQ was removed within the first day, producing ammonia and nitrous oxide (Perreault et al. 2012b); they suggested that VC1 would preferentially utilize an alternative nitrogen source in place of NQ, if that was available. They concluded that given the right conditions with an aerobic soil and no more favorable nitrogen source, NQ could be degraded to innocuous byproducts in natural soil (Perreault et al. 2012b).

4.2 Phytoremediation

Compared to microbially-mediated IMC remediation techniques, phytoremediation has been a method that has been largely unexplored for DNAN and NTO. There are several types of phytoremediation defined in Richard and Weidhaas (2014b): (i) phytodegradation, in which the contaminant is taken up and transformed to less toxic products, (ii) phytoextraction, where the contaminant is simply removed from the soil and stored in the plant biomass without much transformation, (iii) rhizodegradation, where the compound is transformed by microbes near plant roots, and (iv) phytovolatilization, where uptake of the pollutant is followed by transpiration. No phytoremediation transformation studies for DNAN and NTO have been identified. The lack of sufficient information to build upon leaves many possible research opportunities in phytoremediation.

In Richard and Weidhaas (2014b), phytoextraction experiments of IMX-101 constituents were conducted with a mixture of big bluestem grass, Nash Indian grass, and switchgrass. Their control, which contained only soil without enrichment showed 83% removal of both DNAN and NTO, which they suspected was due to the activity of native soil microbes or due to irreversible adsorption to soils. With plants, the concentrations of the three IHEs investigated (DNAN, NTO, and NQ) were reduced by more than 96% after 225 days of
exposure (Richard and Weidhaas 2014b). At lower concentrations, the addition of enriched microorganisms increased the rate of DNAN removal. DNAN was detected in 5 of 10 root masses and 2 out of 10 shoot masses while NQ was found in 1 of 10 root masses and 7 of 10 shoot masses (Richard and Weidhaas 2014b). However, they did not detect NTO in plant tissues and they concluded that phytoextraction would account for the fate of a limited amount of the IMX 101 constituent residues (Richard and Weidhaas 2014b). Most of the fate would include microbially mediated transformation, and sorption (Richard and Weidhaas 2014b).

4.3 Abiotically Mediated Adsorption

Sorption behavior for IHEs is important to understand their fate and to develop appropriate remediation techniques. As organic compounds, DNAN, NTO, NQ, and their degradation byproducts might be expected to adsorb predominantly to other organic material, but both organic matter and mineral phases may play an important role in their adsorption behavior in the subsurface (Linker et al. 2015). In Boddu et al. (2009), DNAN adsorption behavior toward activated carbon was tested, as well as its desorption behavior. Boddu et al. (2009) indicated that DNAN removal from aqueous phase by adsorption to granular activated carbon (GAC), GAC with chitosan, acid treated GAC, and alkali treated GAC, was 98.7, 87.3, 98.9, and 98.7%, respectively. DNAN adsorption to activated carbon is strongest under neutral pH conditions (Boddu et al. 2009). Koutsospyros et al. (2012) also cited a technical report (Small 1984) that NQ showed loss from aqueous phase due to adsorption to activated carbon.

Hawari et al. (2015) indicated that DNAN adsorbs to soil reversibly, meaning the DNAN can be remobilized. Since there is still little known about DNAN's behavior, some of its behavior can be predicted based on the behavior of substances that have been more thoroughly
studied. DNAN possesses a similar chemical structure to TNT. DNAN has somewhat greater solubility than TNT, and its log $K_{ow}$ is slightly smaller than that of TNT (Hawari et al. 2015), but DNAN may nonetheless adsorb to organic material in a similar manner to TNT. The study of TNT partitioning behavior has shown that its adsorption was greater in soil samples that contained higher concentrations of organic matter (Rivera et al. 2007). The study also reported that the ability to extract the adsorbed TNT decreases over time (Rivera et al. 2007).

A bench-scale study with a common organic material, lignin, showed that DNAN adsorption expressed pseudo-second order kinetics in which it adsorbed quickly during an initial phase and reaches an equilibrium state in the later phase extending to 24 hours (Saad et al. 2012). Experiments were conducted in stirred 20 mL borosilicate vials with 50 mg/L DNAN and 10 g/L lignin (Saad et al. 2012). In the adsorption phase, about 75% of the DNAN adsorbs to alkali-treated lignin in the first hour and 40% adsorbs to organosolv lignin over the first 3 hours, and then both experiments reach equilibrium over the following 24 hours (Saad et al. 2012). Deionized water recovered only 10% of the DNAN (Saad et al. 2012).

DNAN is predicted to adsorb to clays as well. Computer simulations conducted by Scott and others (2014) indicated that most nitroaromatic compounds adsorb to kaolinite surfaces using a combination of hydrogen bonds and dispersion forces. The simulations with DNAN indicated that it prefers an orientation parallel to the sheet structure, preferably on a tetrahedral side with adsorption energy of -19 kcal/mol (Scott et al. 2014). In the parallel orientation, more hydrogen bonds and dispersion force bonds can be made. The presence of water decreases the adsorption strength of DNAN and other nitroaromatic compounds (Scott et al. 2014). In the subsurface this could mean that DNAN absorbs to soil, organic material, and
layered silicates more readily in the vadose zone than it would in the aquifer. Dissolved DNAN may adsorb to the organic material strongly but may be easily reworked from layered silicates.

Linker et al. (2015) evaluated sorption behavior of DNAN and its degradation products to montmorillonite (a layered silicate clay mineral), birnessite (a manganese oxide), and goethite (an iron oxide). Adsorption was measured by finding the difference between initial pollutant concentration and that at set times during the experiment (Linker et al. 2015). DNAN and its degradation byproduct, 2-ANAN showed a strong affinity for montmorillonite, but 2-ANAN sorption was weaker than that of DNAN, probably because DNAN’s more electronegative nitro groups attract the mineral’s Na\(^+\) and K\(^+\) cations’ exchangeable electrons more than the amino groups that replace them (Linker et al. 2015). DNAN showed little affinity for the hydroxylated surface of goethite and birnessite, but 2-ANAN was attracted to the surface of birnessite more than its parent compound (Linker et al. 2015). 2-ANAN also reacted with birnessite, but its product was unidentified (Linker et al. 2015).

The high solubility of NTO allows it to dissolve quickly (Spear et al. 1989; Taylor et al. 2015) and travel with the groundwater at high concentrations with a lower likelihood to adsorb. Adsorption simulations with NTO by Scott and others (2014) show that, like DNAN, NTO prefers a parallel orientation when adsorbing to kaolinite by way of several hydrogen bonds between the structures. Like TNT, NTO prefers to adsorb to an octahedral layer with very similar adsorption energies (-26.5 and -26 kcal/mol for TNT and NTO respectively). NTO may behave similar to TNT when adsorbing to clay particles and other substrates. Although the adsorption data for NTO is sparse, its adsorption to clay particles may be limited (Richard and Weidhaas 2014b). For the most part, adsorption-based technologies for remediation of NTO in wastewater and groundwater are expected to be largely ineffective (Koutsospyros et al. 2012).
Linker et al. (2015) also showed unique behavior for both NTO and its common daughter product, ATO with montmorillonite, birnessite, and goethite with respect to the minerals’ points of zero net charge (PZNC). The PZNC is the pH at which the net total charge in the particle is zero (Appel et al. 2003). If the pH of the soil is above that value, the particle will have a negative charge and tend to exchange cations, and if the pH is below the PZNC, the particles will have a positive charge and exchange anions (Appel et al. 2003). Both NTO and ATO were found to have poor adsorption potential with montmorillonite, unlike DNAN (Linker et al. 2015). Linker et al. (2015) suggested that NTO and ATO was repelled from montmorillonite because both produced a negative charge at the experimental pH (pH 7). As pH increases, the surfaces of minerals become more negatively charged (Amonette et al. 2000) and because the NTO molecule becomes negatively charged as pH increases, the two repel each other (Linker et al. 2015). Goethite, on the other hand, has a point of zero net charge (PZNC) of 7.5, so its surface charge is slightly positive at pH less than 7.5. Under neutral experimental conditions (Linker et al. 2015), the positive charge of goethite attracted NTO. Both NTO and ATO showed stronger uptake on birnessite as well, but the electrostatic forces responsible for NTO affinity for goethite did not apply to birnessite, whose PZNC is 1.9 (Linker et al. 2015).

In some cases, adsorption can also lead to transformation. As Linker et al. (2015) showed, 2-ANAN adsorbed to birnessite, but then also reacted with it, producing a byproduct that was not identified. However, since birnessite tends to oxidize the pollutants, the possible products are likely similar to those described as "photo-oxidation" products by Rao et al. (2013), as shown in Fig. 7, pathways 14, 15, and 16 (Linker et al. 2015).

4.4 Photodegradation of IHEs
Hawari et al. (2015) conducted photolysis experiments with DNAN using an artificial sunlight generator in 20 mL quartz crucibles that contained 5 mL of 50 mg/L aqueous DNAN. DNAN was removed following first order rate kinetics with a rate constant of 0.22 d\(^{-1}\) (Hawari et al. 2015). Initially, nitrate, ammonium, formaldehyde, and formic acid were found, followed by a rapid production of 2,4-DNP, similar to the pathway shown in Fig. 7, pathway 14 (Hawari et al. 2015). The 2,4-DNP, underwent photolysis under the same conditions at a concentration of 70 mg/L and was found to produce mostly nitrocatechol (Hawari et al. 2015). Rao et al. (2013) had similar findings producing the products seen in Fig. 7, pathway 14 and saw that 2,4-DNP may undergo photolysis. The authors suspected that there was little change in the rate of photolysis because DNAN’s structure has no proton exchange sites (Rao et al. 2013). So far photolysis has not been reported to transform NTO.

NQ and its first reduction step byproduct, nitrosoguanidine, were found to be susceptible to being degraded by UV light (Kaplan et al. 1982; Noss and Chyrek 1984). Fig. 9 is partly derived from work by Kaplan et al. (1982). Pathway 1 was biologically mediated, but pathways 2, 3,4, and 5, which produced cyanamide with nitrosamide, cyanoguanidine, melamine, and guanidine, respectively, proceeded chemically from exposure to UV light. NQ photolysis was observed by Spanggord et al. (1987), which noted that NQ in an aqueous system had a half-life of 0.6 days in summer and 2.3 days in winter. The mechanism in this system was a direct photolytic excitation of NQ, producing nitrite and nitrate, but ultimate products were not identified (Spanggord et al. 1987).

NQ was also seen to degrade over time in this manner, but NQ photodegradation kinetics was slower than that of nitrosoguanidine (Noss and Chyrek 1984). Noss and Chyrek (1984) investigated the degradation of NQ by ozone and hydrogen peroxide as well as by
ozone and hydrogen peroxide in conjunction with photolysis, but their investigation indicated that oxidation methods were not effective or complementary to photolysis, neither did they affect product distribution. NQ photolysis rate was also independent of pH between 3 and 11, but guanidine yields increased as pH increased. (Noss and Chyrek 1984). At 20 mg/L NQ, all NQ was photolyzed after 30 minutes and at 100 mg/L, all NQ was removed within 2 hours (Noss and Chyrek 1984).

4.5 Electrochemical Degradation of NTO and NQ

Electrochemical transformation studies have mainly been done with NTO. Electric current was applied using potentiostats built in-house to either reduce (Cronin et al. 2007; Wallace et al. 2011) or oxidize (Wallace et al. 2009) NTO. The solid yellow precipitate, AZTO, is the primary product in all electrochemical reduction studies (Fig. 8: Pathway 6; Cronin et al. 2007; Wallace et al. 2011). However, Wallace et al. (2011) showed that AZTO can be reduced further at higher pH due to its higher solubility near the electrode. As shown in subsequent reaction pathways after pathway 6, AZTO is reduced to hydrazotriazolone, which reduces thermally to either azoTO or back to ATO (Wallace et al. 2011). This is a method for NTO treatment that is of interest partially because the reduced solid products are possible energetic materials that test well as IHEs (Wallace et al. 2011). The electrochemical oxidation of NTO can mineralize it to produce ammonia, nitrate, and carbon dioxide (Fig. 8: Pathways 12 through 20; Wallace et al. 2009). Future work with this remediation technology could lead to techniques to recover the dimer reduction products for refinement and use as high explosives, especially when treating NTO wastewater.
Leeds and Smith (1951) used electrochemical stimulation to facilitate the reduction of some nitroalkanes to produce $N$-alkylhydroxylamines and in some cases alkylamines. Although NQ was not the primary subject of their research, they described a set-up that they used direct current from a 12-volt, 1.2-kilowatt motor generator to reduce NQ to aminoguanidine (Leeds and Smith 1951).

4.6 Alkaline Hydrolysis of IHEs

In addition to reductive transformations, alkaline hydrolysis has been shown to be the other important abiotic transformation pathway (Salter-Blanc et al. 2013). Alkaline hydrolysis can lead to byproducts that are more water soluble, less explosive, and more easily biodegraded (Hill et al. 2012). In alkaline hydrolysis, a basic nucleophile (a hydroxyl group) complexes with a carbon on DNAN's aromatic ring which then eliminates a leaving group from that same ring position (Fig. 7: Pathways 14, 17, and 18; Salter-Blanc et al. 2013). Hill et al. (2012) compared alkaline hydrolysis for TNT, 2,4-dinitrotoluene (DNT), and DNAN and estimated Gibbs free energy values during the complexation and the formation of products using modeling. DNAN was predicted to be the most stable of the three explosives and required greater activation energy to hydrolyze than TNT and DNT (Hill et al. 2012). Based on Gibbs free energy diagrams in Hill and others (2012), the direct nitro substitution products seen in Fig. 7, pathways 17 and 18 were likely the most favorable products energetically, while a few Meisenheimer complexes similar to those shown after pathway 19 in Fig. 7 had lower activation energy.

Salter-Blanc et al. (2013) examined alkaline hydrolysis of DNAN and TNT experimentally with batch experiments in 20 mL amber vials containing 50 mM phosphate
buffers at pH 11, 11.7, or 12.0. 200 µL of 10 g/L DNAN or TNT in acetonitrile was added, followed by mixing and sampling (Salter-Blanc et al. 2013). DNAN disappearance was modeled using a pseudo-first order model where reaction kinetics was strongly influenced by the increase in pH, demonstrating a sharp increase in the rate of DNAN loss, particularly as pH approached 12 (Fig. 10F; Salter-Blanc et al. 2013). Based on the above behavior of DNAN (Salter-Blanc et al. 2013), it may be argued that DNAN would show slow or no reaction by alkaline hydrolysis at pH ranges that are more environmentally relevant (such as 8 or 9). At pH 12, alkaline hydrolysis removed nearly all DNAN in ~200 hours, which was considerably slower than biodegradation studies (Fig. 10). Future research may examine more environmentally relevant pH ranges or examine feasibility with respect to real wastewaters. DNAN transformation by alkaline hydrolysis may be insufficient to treat dissolution products from IHE formulations, mainly because DNAN degradation was slower than many of the other mechanisms tested. Furthermore, Koutsospyros et al. (2012) showed that alkaline hydrolysis was ineffective to remove NTO over 2 hours of exposure in 0.5 N NaOH, indicating that the kinetics of NTO loss may be similar to or less than that of DNAN, making alkaline hydrolysis impractical for NTO treatment.

4.7 Degradation of IHEs by Metals and Minerals

In the effort to degrade insensitive munitions like DNAN and NTO, biological methods are often environmentally benign and cost effective, but in some cases, such as wastewater containing DNAN, biological processes may not yield sufficient results (Shen et al. 2013). Ahn et al. (2011) found that the DNAN in PAX 21 wastewater was the primary compound that was toxic to the bacteria that would have otherwise degraded ammonium perchlorate in the wastewater. Therefore, some abiotic processes can become important. So far, most of the
abiotic work with metals and minerals have been completed with DNAN and zero valent iron (ZVI) (Ahn et al. 2011; Shen et al. 2013; Hawari et al. 2015).

Ahn et al. (2011) demonstrated a need for a two-step approach to degrading the PAX 21 explosive formulation because DNAN proved toxic to the perchlorate reducing bacteria. AP did not degrade until DNAN was reduced to a less toxic product (i.e. DAAN). The authors used a packed column containing ZVI to abiotically remove DNAN and RDX from water obtained from dissolving PAX 21 at near neutral pH (about pH 7.25) followed by an anaerobic bioreactor to remove the AP (Ahn et al. 2011). They also used some batch studies to analyze ZVI reactions toward DNAN (Ahn et al. 2011). With ZVI granules, DNAN was found to rapidly reduce to DAAN in vials with 5 mL liquid and 1 g of ZVI with small concentrations of intermediates visible for a short time (Fig. 10B where DNAN reduction kinetics with ZVI was comparable to the stronger anaerobic biological processes (Fig. 10; Ahn et al. 2011).

Shen et al. (2013) investigated a two-step DNAN degradation in a bench scale experiment consisting of: (i) a ZVI bed reactor, followed by, (ii) a Fenton reactor to oxidize the effluent of the ZVI bed to create an effective pretreatment of wastewater from the Dongfang Chemical Co. Ltd. Based on previous results (Ahn et al. 2011), Shen et al. (2013) expected DNAN to be reduced to DAAN in the ZVI reactor. The Fenton reactor had a pH between 2.9 and 3.5, a H₂O₂/Fe(II) molar ratio of 15:1, with varying concentrations of Fenton reagent (Shen et al. 2013). Hydrogen peroxide concentrations of 0.216 mol/L gave final products of methanol and formic acid that were degraded microbially in the final step of the treatment process (Shen et al. 2013).
Hawari et al. (2015) conducted batch experiments in 60 mL serum bottles with 0.5 g granular ZVI at room temperature. Like with their biological experiments, ZVI regioselectively reduced the nitro group in the ortho position on the DNAN structure, resulting first in ortho-hydroxylamine (2-HA-NAN), followed by reduction to 2-ANAN, finally producing DAAN. In such treatment scenario, DAAN would persist in the subsurface if subsequent steps to oxidize and mineralize the products were not employed.

Koutsospyros et al. (2012) used bimetallic particles of Fe/Ni and Fe/Cu to study reactions with the high energy explosives, RDX, HMX, and TNT, and the IHE compounds, NTO, NQ, and DNAN. Bimetallic particles have not been thoroughly studied, especially with IHEs like DNAN, NTO, and NQ, but they appear to have an advantage over particles of only ZVI (Koutsospyros et al. 2012). The secondary metals that coat ZVI surface help catalyze the reaction and decrease oxide formation on the surface of the particles (Koutsospyros et al. 2012). All three IHE compounds were degraded by pseudo-first order kinetics. NTO showed the slowest kinetics of degradation (Fig. 11A) while DNAN degradation was the fastest at 3-5 times greater than that of NQ and having a half-life of 2 minutes with the Fe/Cu bimetal experiments (Fig. 10A; Koutsospyros et al. 2012). Among the various degradation processes compared (Figs. 3 and 4), the iron bimetal particles showed the fastest degradation kinetics for both DNAN and NTO. Parameters found to shorten the half-life of NTO were bimetal solid: liquid ratio and the initial pH (Koutsospyros et al. 2012). In particular, changing the pH from 3.0 to 2.8 drastically increased the kinetics of NTO reduction such that NTO was undetectable after 2 minutes at pH 2.8 but was undetectable after 60 minutes (Fig. 11A; Koutsospyros et al. 2012). While increasing the solid:water ratio from 0.5% to 1% increased the degradation kinetics DNAN and NQ only slightly, but such increase in the ratio doubled the degradation
rate constant for NTO. It is as yet unknown how neutral or basic pH conditions will affect reactivity and product distribution.

Fe(II) also has been well documented as a potent reducer of organic contaminants (e.g. Boparai et al. 2010; Gregory et al. 2004). In Niedźwiecka et al. (2017), they examined DNAN degradation with Fe(II) and a palladium catalyst pellets. Alone, the palladium catalyst did not degrade DNAN, but combined with Fe(II), DNAN was reduced to mostly 2-ANAN at pH 7, and to 2-ANAN with the terminal product, DAAN, at pH 8 and 9 (Fig. 10D; Niedźwiecka et al. 2017). The fastest kinetics for DNAN were observed when the mechanism was either completely mediated abiotically, such as with 1.5 mM Fe(II) with palladium catalyst at pH 8 and 9; or when the mechanism was completely biologically mediated, such as when GS-15 was used with 1.5 mM Fe(III) in the form of Fe(III)-citrate with AQDS (section 4.1; Niedźwiecka et al. 2017); there was no observed degradation of DNAN at pH 6 in their abiotic study.

According to Sabetta et al. (1935) nitrosoguanidine was also produced from nitroguanidine (NQ) using zinc in ammonium chloride. In Sabetta et al. (1935), the degradation of NQ produced derivative compounds such as nitrosoguanidine or to produce another explosive compound. Sabetta et al. (1935) also described several pathways nitrosoguanidine may follow in an aqueous solution depending on that solution pH. In acidic solutions, nitrosoguanidine may degrade into guanidine and nitrous acid (Pathway 6, Fig. 9; Sabetta et al. 1935). In neutral solutions, nitrosoguanidine produces cyanamide (Pathway 2, Fig. 9; Sabetta et al. 1935). Under basic conditions, the authors reported CO₂, NH₄⁺, (Pathway 11, Fig. 9), cyanamide and nitrogen (Pathway 2, Fig. 9) as key byproducts (Sabetta et al. 1935).
Titanium(III) with Fe(II) is used to degrade NQ, but not as a groundwater remediation tactic. For example, Brandt et al. (1955) described this as an analytical method to quantify NQ. The reaction is often carried out in a strongly acidic solution (Brandt et al. 1955). Nitroguanidine underwent nitroreduction and followed a pathway similar to pathway 6 (Fig. 9) to form guanidine and ammonia (Brandt et al. 1955).

5.0 Discussion and Future Work

5.1 Gaps in understanding

There are numerous gaps in understanding with respect to the behavior of IHEs. In the safety data sheets for all three chemicals, there are multiple unknown factors that might affect how the chemicals behave and interact with the environment (Sigma-Aldrich 2015; Apollo Scientific Limited; Thermo-Fisher Scientific, revised 2017). Biologically mediated fate studies described in the literature are listed in Table 6 and abiotically mediated fate studies are listed in Table 7. Some of the gaps in the knowledge about these chemicals and their behavior are visible in the blacked-out cells of Tables 6 and 7.

Biologically-mediated sorption consists mostly of phytoextraction. Richard and Weidhaas (2014b) described DNAN and NTO removal from soil by phytoextraction into several grasses. With so few studies on Phytoextraction of DNAN or NTO, and practically none about NQ, there are many opportunities for research such as various trees and wetland plants. DNAN removal by adsorption has been examined extensively, but there are gaps in NTO removal by adsorption. Contaminant removal by sorption to lignin and activated carbon have been studied for DNAN but not NTO, which may be due to high NTO solubility making it unlikely that adsorption technologies will be as effective on it as for DNAN (Koutsospyros et
al. 2012). Nevertheless, Linker et al. (2015) showed that NTO can adsorb to goethite and birnessite and is currently the only study that showed that NTO has adsorptive tendencies. The study of DNAN and NTO adsorption onto kaolinite was a computational study (Scott et al. 2014). The evidence from the kaolinite study suggests that NTO will only adsorb to octahedral sites. Since the octahedral layers in montmorillonite are typically obstructed on both sides by tetrahedral layers, it is a possible explanation for why montmorillonite does not attract NTO. Further research with other clay minerals is necessary to examine the clay structures that might facilitate NTO adsorption in natural and engineered systems, especially since the study of montmorillonite clay shows that NTO does not adsorb (Koutsospyros et al. 2012).

Most studies examining biologically-mediated degradation of DNAN and NTO under anaerobic conditions showed that a reduction reaction of DNAN resulted in DAAN with intermediates is commonly observed (Platten et al. 2010; Platten et al. 2013; Perreault et al. 2012a; Olivares et al. 2013; Hawari et al. 2015) and ATO (Campion et al. 1999; Krzmarzick et al. 2015), respectively. Additional research is needed with NQ by anaerobic biodegradation, as there are only two studies that examine such mechanisms (Kaplan et al. 1982; Indest et al. 2017). The majority of the aerobic bacteria studies show that DNAN follows a substitution pathway shown in Fig. 7, pathway 14, that results in 2,4-DNP (Fida et al. 2014). There is some debate about whether some of the aerobic degradation of DNAN might in fact have been due to unintended reduction reactions under oxygen poor locations that developed due to high substrate concentrations (Krzmarzick et al. 2015). Further, there is some confusion as to whether NQ can be degraded by aerobic bacteria (Kaplan et al. 1982; Perreault et al. 2012b). Therefore, additional research is needed to identify the degradation mechanisms and the controls on pathways and kinetics, especially under aerobic conditions and to better understand
the conditions under which NQ might degrade aerobically. NTO biodegradation under aerobic conditions (Richard and Weidhaas, 2014a) is somewhat unclear, but it seems to suggest that reduction to ATO and sometimes 1,2-dihydro-3H-1,2,4-triazol-3-one is the dominant reaction pathway, but the reaction is generally slower. Other gaps in Table 6 further show that the treatment methods that have been tested for DNAN have not been tested on NTO. In experimental setup, many toxicology and transformation studies adjust the NTO solution pH to neutral. To simulate natural attenuation especially, completing studies without pH adjustments may improve understanding of environmental impact and conditions for natural attenuation.

As described before, very little work has been completed for both DNAN and NTO to evaluate soil/aquifer treatment by phytoremediation, but apparently none has been done for NQ. The phytoremediation method that might be most effective is rhizodegradation. Most of the research examining biological remediation mechanisms suggests that the most effective method for removing insensitive explosives is by first treating them under anaerobic conditions to reduce the pollutant, followed by aerobic conditions to facilitate an oxidation reaction, similar to Krzmarzick et al. (2015), which showed anaerobic degradation of NTO to ATO followed by ATO oxidation under an aerobic condition. A combination of anaerobic and aerobic condition in close proximity can be found in natural systems around the rhizosphere of certain plant roots, where small zones of aerobic conditions might be present in areas where anaerobic methanogens and ammonia oxidizing bacteria might normally thrive (Powell and Agrawal 2011; Powell et al. 2014). These conditions can also be found at the sediment-water interface (Powell and Agrawal, 2011; Powell et al. 2014). Work by Powell and Agrawal (2011) demonstrated that processes that take place near the rhizosphere of wetland plant roots like those of Carex comosa and Scirpus atrovirens have the potential to degrade trichloroethene and
other recalcitrant pollutants. They speculate that similar processes may take place with other species as well (Powell et al. 2011). These studies (Powell and Agrawal 2011; Powell et al. 2011; and Powell et al. 2014) used bench-scale experiments with soil free roots from wetland plants to study the role of methane and ammonia oxidizing bacteria in degrading chlorinated hydrocarbons. A possible limitation for this system may be its effectiveness at degrading DNAN's reduction products and intermediates to innocuous products, since innocuous oxidation products for DAAN and the azo dimers that have been produced are not clearly identified yet in the literature. Products may instead more closely resemble photo-degradation products reported by Rao and others (2013).

In Table 7, it initially appears that in many cases what has been tested on one of the two IHEs of interest has not been tested on the other. However, little research has been done on using adsorption techniques and alkaline hydrolysis to treat NTO because these mechanisms were found to be ineffective to degrade NTO (Koutsospyros et al. 2012). Apparently, studies have not been conducted to examine NTO removal by photolysis or ZVI. Likewise, ZVI and other metal or mineral species (except for Zinc) have not been used to degrade NQ either.

The transformation studies of other explosive compounds, such as RDX, can also provide ideas for other substrates. The investigation by Boparai et al. (2010) used Fe(II) species such as Fe(OH)$_2$ and mixed Fe(II) and Fe(III) iron species such as green rust and magnetite that precipitated at pH conditions ranging from 5.8 to 8.5 to reduce RDX, HMX, and TNT. Fe(II) concentrations ranged from 0 to 2.0 mM (Boparai et al. 2010). A study by Gregory et al. (2004) investigated RDX transformation by Fe(II)-treated magnetite particles in aqueous suspensions. The concentration of magnetite was about 1 g/L in their study with a surface area of 44 m$^2$/L, but neither the magnetite nor the Fe(II) by themselves degraded RDX (Gregory et al. 2004).
Larese-Casanova and Scherer (2008) used 5 to 6 g/L freeze-dried green rust to degrade RDX under neutral conditions, resulting in products of mostly formaldehyde, nitrous oxide gas, and ammonium accounting for 70 and about 50% of the carbon and nitrogen mass balance, respectively. Niedźwiecka et al. (2017) examined DNAN degradation by using a palladium catalyst instead of a natural mineral, but such reduction studies have not been undertaken for either NTO or NQ.

Iron oxide minerals can exist abundantly in the subsurface and have been found to naturally degrade many anthropogenic pollutants ranging from chlorinated hydrocarbons (e.g.: Agarwal et al. 2011; Danielsen 2004; Hanoch et al. 2006) to high explosives (e.g.: Koutsospyros et al. 2012; Williams et al. 2005), but information about their behavior toward DNAN, NTO, NQ are lacking. Further research about IHE reactions with iron oxides would provide insights into their fate in the subsurface and help to implement appropriate treatment strategies to remove them and their byproducts. This research could bring new concerns about the fate of NQ to light if the reaction products are more toxic than their parent, as indicated by Kennedy et al. (2017). Most studies on iron oxides and associated aqueous Fe(II) have demonstrated that they can potentially facilitate reduction reactions. In the case of high explosives that share a similar structure to DNAN or NTO, like TNT, most of the minerals reduced the nitro groups to produce products such as 2-amino-4,6-dinitrotoluene and its further reduced product, 2,4-diamino-6-nitrotoluene, which were more biodegradable products (Boparai et al. 2010). It is likely that similar mineral treatments with DNAN might yield similar results, producing DAAN, by way of the pathways 1 through 4 as illustrated in Fig. 7, and as shown by Niedźwiecka et al. (2017). Product distribution would depend on the mineral and experimental conditions (e.g. pH, mineral concentration, Fe$^{2+}$ concentration, etc.). Reduction
kinetics of DNAN at basic pH such as 8 or 9 would likely increase with rising pH, as suggested by the research by Niedźwiecka et al. (2017), which is contrary to what may be expected. Previous research into denitrification of nitrite by magnetite showed a decrease in the denitrification reaction pseudo-first order rate constants and an increase in the amount of nitrite remaining at the end of experiments (Dhakal et al. 2013).

The degradation behavior of NTO with iron oxide minerals and aqueous Fe(II) may be somewhat difficult to predict, and only an investigation will sufficiently describe its behavior. NTO would probably be reduced to ATO or one of its intermediates based on the reaction pathways shown in Fig. 8, pathway 1 and 3. However, the acidification of the NTO containing solution may corrode iron oxide surfaces and perhaps reduce their effectiveness in systems where pH is not buffered. Conversely, the acidity can be advantageous to degrading NTO, partly because it may degrade more quickly under acidic conditions (Koutsospyros et al. 2012). In a situation where the solution pH is not sufficiently buffered, NTO solutions could mobilize cations like Fe$^{2+}$ and other ions which might assist/facilitate the reduction of NTO and other explosive compounds (Koutsospyros et al. 2012). If the pH is artificially buffered to near neutral conditions, the build-up of negative charge on the iron oxide's surface may repel NTO as montmorillonite did. Further research is needed to clarify understanding of IHE fate.

NQ transformation with reducing minerals would likely follow a nitroreduction pathway, producing a nitrosoguanidine and perhaps aminoguanidine depending on the potency of the mineral. However, kinetics may be strongly affected by changes in pH whether that favors reduction reaction mechanisms; this is because different products may form depending on whether NQ is in an acidic, neutral, or basic solution (Sabetta et al. 1935).
The natural attenuation of DNAN and NTO by iron oxide minerals would likely be much slower than their reduction by ZVI and bimetal compounds. The degradation potentials of IHEs with iron oxide alone would not provide a complete solution at sites polluted by DNAN and NTO because the products these processes leave (e.g. DAAN and ATO) can still be harmful to the environment. However, a combined biotic and abiotic treatment strategy can be effective in treating the daughter compounds (Linker et al. 2015).

The oxidative degradation of reduced daughter products may be facilitated by oxidizing agents that might naturally be present at the root zones of some plants, at the soil-water interface, or as may be provided by artificial means, producing a Fenton reaction or by other advanced oxidation methods that have yet to be analyzed with IHEs. Birnessite was examined primarily from an adsorption perspective in Linker et al. (2015) and its reactive potential was implied, but little research has been completed beyond this first step. Further research into oxidized manganese species, like birnessite and permanganate (MnO$_4^-$) (Chokejaroenrat et al. 2011), could lead to methods to oxidize ATO and possibly DAAN. Chokejaroenrat et al. (2011) demonstrated the oxidation of RDX with MnO$_4^-$, which has been widely accepted for use in in situ chemical oxidation (ISCO). In their study, the authors postulated that RDX is oxidized at its methylene group and is ultimately mineralized to form N$_2$O, CO$_2$, and water (Chokejaroenrat et al. 2011). Similar mineralization reactions are expected to take place if ATO is exposed to MnO$_4^-$ or another oxidizing manganese oxide. DNAN and NTO may undergo direct oxidation under these conditions as well, but their structures do not support that mechanism. Treatment by ozone also represents a potential oxidative method for reduction products of IMCs that has not been studied.

5.2 Methods of Filling Gaps
Most of the gaps in understanding may be addressed by bench-scale experiments in batch system to quantify reactions, products and reaction kinetics. For example, the effects of rhizodegradation of IHEs can be investigated with experiments similar to those found in Powell et al. (2011), in which clipped and washed roots from wetland plants could be placed in serum bottles with growth media. These could escalate to mesocosm experiments in which grown live plants are placed in flow-through reactors containing sediment and contaminated solutions are fed through the column to observe rhizodegradation and phytoextraction.

Likewise, for abiotic experiments with reducing DNAN and NTO, the investigation could begin with batch experiments for reduction or oxidation reactions with minerals. Studies could examine the roles of various experimental parameters such as concentration and type of mineral phase, varying pH, concentrations of aqueous Fe(II), dissolved organic matter concentrations, etc. with different munition formulations instead of solutions containing only one IHE compound. Numerous batch studies have been performed to evaluate the removal of environmental pollutants by metal oxides. Batch studies by Boparai et al. (2010) might be a promising model for similar tests with IHEs due to their approximation of natural conditions like Fe(II) concentrations.

In nearly all fate studies examined in this review (e.g. Cronin et al. 2007; Koutsospyros et al. 2012; Hawari et al. 2015; Krzmarzick et al. 2015) that characterizes DNAN or NTO and their degradation products, the analytical tool of choice is high performance liquid chromatography (HPLC) with a UV-vis detector and an appropriate C-18 or similar column. NQ and its reduction products can also be detected by HPLC (Perreault et al. 2012b). Wallace et al. (2009) indicated that NTO could also be quantified spectrophotometrically. The HPLC is sufficient to detect the organic explosives and their reduction products, but not the products of
oxidation, mineralization, or the Meisenheimer structures in alkaline hydrolysis. Le Campion et al. (1999) used C-14 to help quantify product distribution, which could be measured with a radioactivity monitor on an HPLC. Urea could be measured using a commercial test (Le Campion et al. 1999). Nitrate, nitrite, and ammonium from substitution reactions or mineralization can be detected by ion chromatography (Perreault et al. 2012a; Hawari et al. 2015). Liquid chromatography-mass spectroscopy (LC-MS) may be necessary to quantify other organic products that might form like azo dimers (Olivares et al. 2013) or to analyze pathways and intermediates (Linker et al. 2015). Solid products like AZTO and azoTO can be identified by NMR (Wallace et al. 2011).
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Thermo-Fisher Scientific. SDS for 99% Nitroguanidine Moistened with ca 25% Water.

revised 2017.


Fig. 7: Composite reaction pathways for 2,4-Dinitroanisole. Known pathways are indicated by a solid line, while implied pathways are indicated by dotted lines. Pathways are color coded. Data for this diagram has been collected from several studies that have included figures depicting several pathways (Ahn et al. 2011; Olivares et al. 2013; Rao et al. 2013; Salter-Blanc et al. 2013; Fida et al. 2014).
Fig. 8: Composite reaction pathways for nitrotriazolone. Known pathways are indicated by a solid line, while implied pathways are indicated by dotted lines. Pathways are color coded. Data for this diagram has been collected from several studies that have included figures depicting several pathways (Campion et al. 1999; Cronin et al. 2007; Krzmarzick et al. 2015; Richard and Weidhaas 2014a; Salter-Blanc et al. 2013; Wallace et al. 2009; Wallace et al. 2011).
**Fig. 9:** Composite reaction pathways for Nitroguanidine. Known pathways are indicated by a solid line, while implied pathways are indicated by dotted lines. Pathways are color coded. Data for this diagram was compiled from several studies that have included figures depicting several pathways (Sabetta et al. 1935; Leeds and Smith 1951; Kaplan et al. 1982; and Perreault et al. 2012b).
Fig. 10: This figure compares various concentration versus time plots for DNAN and in some cases products and intermediates under several major conditions examined in this review. They are arranged so that A is the most rapid degradation experimental condition and F is the slowest condition. The axis values have been converted to the same units to facilitate comparisons between studies. A: Shows DNAN degradation at pH 3 (black circles) and 2.8 (white circles) for Fe/Cu bimetal experiments with a solid to liquid ratio of 1% (Modified from Koutsospyros et al. 2012). B: Shows rapid reduction of DNAN (black circles) to DAAN (black squares) with intermediates (open circles) and mass balance (diamonds with dotted line) on contact with ZVI granules under anaerobic conditions at pH 6.7. Error bars representing standard deviations from replicate measurements. C: Biodegradation of DNAN with Nocardioioides sp. Strain JS 1661 produces 2,4-DNP. The 2,4-DNP is removed, producing nitrite. Note: the final concentration of nitrite is nearly double the initial concentration of DNAN (Modified from Fida et al. 2014). D: Degradation of DNAN under various pH with palladium catalyst (Solid symbols) and with added Fe(II) (clear symbols) E: Degradation of DNAN under aerobic conditions beginning with about 80 mg/L DNAN (converted in figure). Black circles represent the experimental reactors’ results, while the killed control (not shown) shows little DNAN degradation (Modified from Richard and Weidhaas 2014a). F: Degradation of DNAN by alkaline hydrolysis at room temperature at pH 11 (squares), 11.7 (diamonds), and 12.0 (triangles).
Fig. 11: This figure compares various concentration versus time plots for NTO and in some cases products and intermediates under several major conditions examined in this review. They are arranged so that A is the most rapid degradation experimental condition and D is the slowest condition. The axis values have been converted to the same units to facilitate comparisons between studies. A: shows NTO degradation at pH 3 (black circles) and 2.8 (white circles) for Fe/Cu bimetal experiments with a solid to liquid ratio of 1% (Modified from Koutsospyros et al. 2012). B: Anaerobic biodegradation of NTO (black squares) to HTO (white circles) as an intermediate, and finally to ATO (white triangles) using H₂ as an electron acceptor, along with 20 mM pyruvate with 10 mg/L yeast extract (Modified from Krzmarzick et al. 2015). The degradation took place quickly, however, the graph shows a lag time for the acclimation of the microbes. C: Degradation of NTO under aerobic conditions beginning with 40 mg/L NTO (converted in figure). The white triangles represent killed control reactors while black triangles represent the experimental reactors’ results (Modified from Richard and Weidhaas 2014a). D: From the same investigation by Krzmarzick and others, (2015) this figure shows the same NTO (black squares) to HTO (white circles) and finally to ATO (white triangles). Like in part B, this graph represents anaerobic biodegradation with H₂ as an electron acceptor without pyruvate, citrate, or yeast extract (Modified from Krzmarzick et al. 2015).
Fig. 12: This figure shows an example of a concentration versus time plots for NQ (black ovals) and nitrosoguanidine (white ovals). The axis values have been converted to the same units as Fig. 4 and 5 to facilitate comparisons between studies and IHE compounds. This graph was from Kaplan et al. (1982) at pH 6 in batch studies with 4.0 g/L nutrient broth.
### Biological Methods of DegradingInsensitive High Explosives Described in Literature

<table>
<thead>
<tr>
<th>Biological Processes</th>
<th>DNAN Products</th>
<th>NTO Products</th>
<th>NQ Products</th>
<th>Bacterial Species</th>
<th>Other Conditions</th>
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<tr>
<td><strong>AFBB</strong> Platten et al. 2010</td>
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<tr>
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<td>Anaerobic</td>
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<td>Activated Sludge Kaplan et al. 1982</td>
<td>Nitrosoguanidine, which degraded abiotically to cyanamide, guanidine and others</td>
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<td>ATO followed by NO$_2$ and NO$_3$- mineralization products</td>
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<td>Anaerobic (ATO) followed by aerobic conditions to mineralize</td>
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<td>Soil Perreault et al. 2012a</td>
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<td>Bacillus sp.</td>
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<td>Several species believed to be important</td>
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<td>Hawari et al. 2015</td>
<td>2-ANAN, DAAN</td>
<td>Enterobacter strain DM7, <em>Shewanella oneidensis</em>, <em>Pseudomonas fluorescens</em>, and <em>Burkholderia cepacia</em></td>
<td>Anaerobic`</td>
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<tr>
<td>Bacteria Strains</td>
<td>Fida et al. 2014</td>
<td>2,4-DNP, Meisenheimer complexes</td>
<td>Nocardioides sp. Strain JS1661</td>
<td>Aerobic</td>
<td></td>
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<tr>
<td>Bacteria strains</td>
<td>Perreault et al. 2012b</td>
<td>Nitrourea, CO$_2$, NH$_3$, N$_2$O</td>
<td><em>Variovorax</em> strain VC1</td>
<td>Aerobic</td>
<td></td>
</tr>
<tr>
<td>Bacteria with electron shuttles</td>
<td>Niedźwiecka et al. 2017</td>
<td>2-ANAN, 4-ANAN, DAAN</td>
<td><em>Geobacter metallireducens</em> (GS-15)</td>
<td>Anaerobic with various e$^-$ shuttles and substrates</td>
<td></td>
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<tr>
<td>Waste Waters</td>
<td>Le Campion et al. 1999</td>
<td>ATO and ring cleavage products</td>
<td><em>Bacillus licheniformis</em></td>
<td>Varying concentration of oxygen</td>
<td></td>
</tr>
<tr>
<td>Adsorption</td>
<td>Richard and Weidhaas 2014b</td>
<td>Compounds are taken up and adsorb to grass plant tissues</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Process</td>
<td>Table 7</td>
<td>Abiotic Methods of Degrading Insensitive High Explosives Described in Literature</td>
<td></td>
<td></td>
<td></td>
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<td>---------------</td>
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<tr>
<td>Adsorption</td>
<td>Saad et al. 2012</td>
<td>DNAN, NTO, NQ products</td>
<td>Adsorption to</td>
<td>Type of reaction</td>
<td></td>
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<tr>
<td>Adsorption</td>
<td>Boddu et al. 2015</td>
<td>DNAN, NTO, NQ products</td>
<td>Activated Carbon</td>
<td>N/A</td>
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</tr>
<tr>
<td>Adsorption</td>
<td>Linker et al. 2015</td>
<td>DNAN, NTO, NQ products</td>
<td>Montmorillonite, Goethite and Birnessite</td>
<td>N/A</td>
<td></td>
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<td>Adsorption</td>
<td>Scott et al. 2014</td>
<td>DNAN, NTO, NQ products</td>
<td>Kaolinite (clay)</td>
<td>N/A</td>
<td></td>
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<tr>
<td>Photolysis</td>
<td>Hawari et al. 2015</td>
<td>DNAN, NTO, NQ products</td>
<td>2,4-DNP, Nitrocatechol</td>
<td>Substitution</td>
<td></td>
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<tr>
<td>Photolysis</td>
<td>Rao et al. 2013</td>
<td>DNAN, NTO, NQ products</td>
<td>2,4-DNP, NO$_3^-$</td>
<td>Substitution, carboxylation</td>
<td></td>
</tr>
<tr>
<td>Photolysis</td>
<td>Kaplan et al. 1982</td>
<td>DNAN, NTO, NQ products</td>
<td>Cyanamide, Nitrosamide, Guanidine, Cyanoguanidine, Melamine</td>
<td>Reduction, polymerization</td>
<td></td>
</tr>
<tr>
<td>Photolysis</td>
<td>Noss and Chyrek 1984</td>
<td>DNAN, NTO, NQ products</td>
<td>Nitrosoamidine, Guanidine, nitrate-nitrogen, 50% not recovered</td>
<td>Reduction</td>
<td></td>
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<td>Electrochemical</td>
<td>Cronin et al. 2007</td>
<td>DNAN, NTO, NQ products</td>
<td>AZTO</td>
<td>Reduction</td>
<td></td>
</tr>
<tr>
<td>Electrochemical</td>
<td>Wallace et al. 2009</td>
<td>DNAN, NTO, NQ products</td>
<td>CO, CO$_2$, NO$_3^-$, N$_2$O, NH$_4^+$</td>
<td>Oxidation</td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td>Authors Year</td>
<td>Products</td>
<td>Process Type</td>
<td></td>
<td></td>
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<tr>
<td>-------------------------------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Electrochemical</td>
<td>Wallace et al. 2011</td>
<td>AZTO, azoTO, ATO</td>
<td>Reduction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Electrochemical</td>
<td>Leeds and Smith 1951</td>
<td>Aminoguanidine</td>
<td>Reduction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thermal</td>
<td>Lee and Jaw 2006</td>
<td>Note: the temperature needed was very high</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline Hydrolysis</td>
<td>Hill et al. 2012</td>
<td>Nitro-substituted structures, Meisenheimer structures</td>
<td>Substitution and complexation</td>
<td></td>
<td></td>
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<tr>
<td>Alkaline Hydrolysis</td>
<td>Salter-Blanc 2013</td>
<td>2,4-DNP, Meisenheimer structures</td>
<td>Substitution and complexation</td>
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<td></td>
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<tr>
<td>Alkaline Hydrolysis</td>
<td>Sviatenko et al. 2014</td>
<td>2,4-DNP</td>
<td>Substitution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline Hydrolysis</td>
<td>Bowden and Presannan 1987</td>
<td>2,4-DNP</td>
<td>Substitution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkaline Hydrolysis</td>
<td>Koutsospyros et al. 2012</td>
<td>No degradation took place</td>
<td>N/A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe(II) with palladium catalyst at pH 7.9</td>
<td>Niedźwiecka et al. 2017</td>
<td>2-ANAN, 4-ANAN, DAAN</td>
<td>Reduction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zero Valent Iron</td>
<td>Ahn et al. 2011</td>
<td>DAAN</td>
<td>Reduction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zero Valent Iron</td>
<td>Hawari et al. 2015</td>
<td>DAAN</td>
<td>Reduction</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Method</td>
<td>Authors</td>
<td>Description</td>
<td></td>
<td></td>
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<td>-------------------------------</td>
<td>------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zero Valent Iron &amp; Fenton Reaction</td>
<td>Shen et al. 2013</td>
<td>(DAAN after ZVI), Methanol, formic acid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bimetal: Fe/Ni &amp; Fe/Cu</td>
<td>Koutsospyros et al. 2012</td>
<td>Not identified</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc and ammonium chloride</td>
<td>Sabetta et al. 1935</td>
<td>Nitrosoguanidine, Guanidine, CO₂, NH₄⁺, Cyanamide, and Nitrogen</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reduction followed by oxidation
Reduction
Reduction followed by amphotolytic degradation effects of nitrosoguanidine
Chapter IV

Bench-Scale Abiotic Degradation of 2,4-Dinitroanisole with Hydrous Ferric Oxide and Goethite: Implications for its Natural Attenuation

Abstract

As the use of insensitive munitions like 2,4-dinitroanisole increases, the chances of accidental release also increase. This batch reactor study is aimed at understanding the chemical reactions and fate of DNAN in the reducing conditions of ferric iron minerals such as hydrous ferric oxide (HFO) and goethite with Fe(II). This study used varying conditions to assess the response of DNAN and predict its behavior in the subsurface. DNAN degradation with iron oxide minerals was mostly dependent on the concentration of aqueous or adsorbed Fe(II) and on the solution pH. Reaction mechanisms and iron speciation appear to be affected by pH. Mineral concentration had little effect on the potency of the reaction. Results suggest that ferric minerals with low [Fe(II)] would only partially reduce DNAN in the subsurface.

1.0 Introduction

2,4-dinitroanisole (DNAN) is an insensitive high explosive (IHE) that is of increasing interest to the U.S. military due to dangers posed by its more heat and shock sensitive counterparts, such as trinitrotoluene (TNT) (Walsh et al., 2014). DNAN is a promising replacement for and has a similar structure (Fig. 13) to TNT (Boddu et al.,
Groundwater contamination by DNAN can occur from wastewater produced during DNAN and IHE manufacturing or from explosive residues at live fire testing and training ranges. DNAN’s toxicity is less than that of TNT, but it can still inhibit the activities of multiple bacterial groups (Liang et al., 2013; Dodard et al., 2013). In rats, DNAN targeted the reproductive organs in males, caused spleen enlargement in females, and neurotoxicity was observed in both males and females at doses of 80 mg DNAN/kg/day (Sweeney et al., 2015). Since DNAN is becoming more widely used by the military and others, it is essential to learn more about its behavior in the environment and its fate in the subsurface.

DNAN is capable of degrading by multiple pathways, usually involving reduction at one of the nitro groups (Fig. 13A) or a substitution at the methoxy group. A reduction reaction typically begins at the nitro in the ortho position, due to higher electronegativity at that site, producing (a) 2-nitroso-4-nitroanisole (2-NO-NAN), (b) 2-hydroxylamino-4-nitroanisole (2-HA-NAN), and (c) 2-amino-4-nitroanisole (2-ANAN) (Hawari et al., 2015). Further reduction of 2-ANAN at the remaining nitro group forms (d) 2-amino-4-nitrosoanisole, followed by (e) 2-amino-4-hydroxylaminoanisole, and finally (f) 2,4-diaminoanisole (DAAN). These were the dominant products observed or inferred in reduction studies like the abiotic experiments conducted by Niedźwiecka et al. (2017). Niedźwiecka’s work examined DNAN reduction primarily using Geobacter metallireducens with electron shuttles and poorly crystalline Fe(III) or Fe(III)-citrate. However, they also completed some abiotic studies with palladium pellets with 1.5 mM Fe(II). Olivares et al. (2013) was able to identify several dimers by mass spectrometer. Azo dimers (g) and (h) may also form by an oxidation reaction that commonly takes place in aerobic conditions.
(Platten et al., 2013), but they may also form under anaerobic conditions if an electron acceptor is present (Olivares et al., 2013). Some intermediates and the azo dimers are inferred by showing them in brackets, but Olivares et al. (2013) identified many of them using a mass spectrometer. Dimers may be reduced back to DAAN. Meisenheimer complexes can form if hydroxyl groups attach to various parts of the DNAN aromatic ring (Fig. 13B) (Salter-Blanc et al., 2013). Hydroxide attaching at the nitro groups can result in substitution reactions (Salter-Blanc et al., 2013).

DNAN degradation was observed by both biological (Platten et al., 2010; Olivares et al., 2013; Hawari et al., 2015) and abiotic (Rao et al., 2013; Salter-Blanc et al., 2013; Ahn et al., 2011; Hawari et al., 2015) processes. While, most aerobic biodegradation studies of DNAN resulted in production of 2,4-dinitrophenol (DNP) and Meisenheimer complexes (Richard and Weidhaas, 2014; Fida et al., 2014), anaerobic biodegradation generally resulted in reduction products like 2-ANAN, DAAN and azo dimers (Platten et al., 2010; Olivares et al., 2013). Abiotic studies have shown DNAN degradation by photolysis (Hawari et al., 2015; Rao et al., 2013) and alkaline hydrolysis (Sviatenko et al., 2014; Bowden and Presannan, 1987; Salter-Blanc et al., 2013; Hill et al., 2012) resulted in DNP and Meisenheimer complexes. Zero valent iron (Ahn et al., 2011; Hawari et al., 2015; Shen et al., 2013) produced DAAN and other reduction products. DNAN degradation has not been examined with reactive iron oxides that are common in soil and sediments. The present study provides a much-needed understanding of abiotic DNAN reactivity and degradation products with Fe(II) containing oxides in natural settings.
Naturally occurring iron oxide minerals mixed with aqueous Fe(II) were shown to reduce various organic contaminants; for example, goethite (Hanoch et al., 2006; Maithreepala and Doong, 2009) and ferrihydrite (Maithreepala and Doong, 2009) mixed with aqueous Fe(II) could degrade carbon tetrachloride. High explosives like TNT, RDX (IUPAC name: 1,3,5-Trinitro-1,3,5-triazine), and HMX (IUPAC name: 1,3,5,7-Tetranitro-1,3,5,7-tetrazocane) were degraded in Fe(II) solutions mixed with various iron minerals (e.g., ferrihydrite) and soils (Boparai et al., 2010).

The goal of this investigation was to study the potential for Fe(II)-treated iron oxides, particularly hydrous ferric oxide (HFO) and goethite to degrade DNAN under conditions simulating an iron reducing environment. The present study evaluates the effects of [Fe(II)], [mineral], and aqueous pH on DNAN degradation with HFO and goethite in bench-scale reactors in order to characterize the reaction byproducts, kinetics and transformation pathways by natural attenuation processes at DNAN contaminated sites. The key objectives of this research are as follows: (i) To describe the behavior of DNAN and its reduction products and degradation kinetics with reactive iron oxides under natural attenuation conditions; (ii) To determine how [Fe(II)] influences DNAN degradation reaction; (iii) To determine how [mineral] influences DNAN degradation reaction and how it interacts with [Fe(II)]; (iv) To determine how pH influences DNAN degradation reaction and the effects of the previous two variables to predict the DNAN transformation pathways in natural attenuation conditions; and (v) To compare the influences of HFO and goethite on DNAN degradation kinetics, product distribution, and transformation pathways.
Fig. 13: (A) Expected pathways for DNAN transformations in the environment, (adapted from Ahn et al., 2011; Olivares et al., 2013). (B) Pathway of the formation of Meisenheimer complexes (adapted from Salter Blanc, 2013 and Hill et al., 2012).

2.0 Materials and Methods

2.1 Materials
Chemicals used included sodium hydroxide pellets (ACS reagent grade, Fisher Scientific), sodium chloride (ACS reagent grade, Fisher Scientific), ferric chloride hexahydrate (ACS reagent grade, RICCA Chemical company), ferrous sulfate heptahydrate (Biomedical grade, MP Biomedical), and ferric nitrate nonahydrate (98% ACS reagent, ACROS Organics). TAPSO buffer (ACS reagent grade, ACROS Organics) was used for pH control. High purity organic chemicals included DNAN (98%, Alpha Aesar), 2-ANAN (95%, Sigma Aldrich), and DAAN (90%, Pfaltz & Bauer).

Other materials included 72 mL borosilicate serum bottles (Cat# 223746, Wheaton), PTFE-lined butyl rubber stoppers (Cat# 73811T-21, Kimble-Chase), aluminum crimps, disposable syringes (BD Syringe; U-100), 0.22 µm PVDF syringe filters (SIMSII), 2 mL Pyrex HPLC autosampler vials with caps and septa. Equipment included an anaerobic chamber (Coy labs, MI), pH meter (Denver Instrument; AP10), Vortex Genie 2 lab mixer (Fisher), rotary shaker (Glas Col, IN), and HPLC (Model 920, Varian) with a photo diode array detector.

2.2 Synthesis of Hydrous Ferric Oxide and Goethite

HFO (Fe(OH)$_3$) was synthesized in bulk based on published methods by mixing of 0.2 mM FeCl$_3$ and 1 M NaOH solution (Lovley and Phillips, 1986; Roden and Zachara, 1996; and Li et al., 2008). The iron oxide particles thus synthesized are described by name as ‘2-line ferrihydrite’ (Roden and Zachara, 1996), and as hydrous ferric oxide (HFO) (Li et al., 2008). Vigorous stirring was used to prevent heterogeneities and to ensure accurate pH measurement during drop-wise addition of NaOH to FeCl$_3$ solution. As the slurry pH stabilized near 7, addition of NaOH was halted and the HFO
slurry was stirred overnight slowly. HFO particles were allowed to settle and then washed with fresh Milli-Q water and the supernatant was discarded. The slurry was agitated and the supernatant was replaced repeatedly until the slurry conductivity was below 5000 µS/cm. After the washing process was complete, NaCl solution was used to bring the ionic strength of the slurry to ~0.1 M. For preparing the reactors, the slurry was put under constant stirring to extract aliquots of homogeneous slurry.

Goethite was synthesized in bulk based on a published method by Atkinson et al. (1967) using 50 mL of 0.3 M Fe(NO$_3$)$_3$•9H$_2$O in Milli-Q water and 12.2 mL of 5 M NaOH solution. Milli-Q water used for goethite synthesis was boiled for 1 hour to drive off traces of dissolved CO$_2$ in order to prevent siderite (FeCO$_3$) precipitation. A minor difference in this procedure from Atkinson et al. (1967) was replacing KOH with NaOH. Further, in the procedure from Atkinson et al. (1967), the base was added to the ferric nitrate solution. In the present procedure, however, ferric nitrate solution was added to 5 M NaOH solution slowly with vigorous mixing on a stir plate to maintain constant slurry pH and uniform precipitation. The mixing of ferric nitrate and NaOH solutions caused a dark red precipitate to develop quickly. The mixture was then aged for 48 hours at 60°C with gentle mixing on a heated stir plate. Within 24 hours, the slurry developed a golden yellow color with goethite as precipitate. The slurry was agitated and the supernatant was replaced repeatedly until the slurry conductivity was below 5000 µS/cm. Multiple batch reactors were then set up after washing the precipitated goethite by the procedure similar to that of the HFO (described above). Given the slightly different methodology for goethite synthesis from what prescribed methods suggest, characterization by XRD and
electron microscopy may be needed to note if there are differences in the size, shape, or uniformity of the particles.

2.3 Batch Reactor Setup

Batch reactors were assembled in duplicate with some triplicate using 72 mL borosilicate glass serum bottles that contained desired aliquots of mineral (HFO or goethite) slurry, TAPSO buffer, and Milli-Q water. Each set of reactors included two control reactors, a blank reactor containing DI water with 10 mM TAPSO buffer, and mineral slurry in DI water with 10 mM TAPSO buffer and no Fe(II). The reactors containing HFO and goethite were then sealed with butyl rubber stoppers and aluminum crimps and purged either with high-purity nitrogen or argon gas for 20 min to deoxygenate the reactors. The reactors containing minerals were then transferred into the anaerobic chamber (Coy Lab, MI) and treated with Fe(II) as needed using 0.1 M FeSO₄ solution. The final pH of the reactors was adjusted as necessary with 1 M NaOH. DNAN stock solution was added to each reactor to make its initial concentration 25 mg/L. The time of DNAN addition was recorded as the beginning of the experiment ($t_0$). The reactors were filled with deoxygenated DI water so that it had no headspace and was resealed with the stopper and crimp.

2.4 Sampling and Analysis

Since DNAN and its degradation byproducts are not volatile, their partitioning into the reactor headspace was expected to be negligible. The first sample ($t_1$) was taken immediately after sealing the reactors. The gap between $t_0$ and $t_1$ was typically ~2 minutes. Either three or four samples were taken on the first day. Liquid samples
withdrawn from the reactors were filtered into HPLC autosampler vials. 1 mL high-purity Ar or N₂ gas was injected into the reactors to maintain pressure. If the reaction was expected to be fast, the reactor was shaken manually for a few seconds and sample \( (t_2) \) was collected prior to removing the reactors from the anaerobic chamber. Sample \( (t_3) \) was taken under Ar stream after reactors were removed from the anaerobic chamber and vortexed for ~40 seconds. The reactors were placed on the rotary shaker for about an hour before collecting sample \( (t_4) \). If the reaction was expected to be slow, sample \( t_2 \) was eliminated. All samples were analyzed for DNAN and its nitroreduction products on the same day by a Varian 920 HPLC with a photo diode array detector and a C-18 Roc column (3 mm x 150 mm, 3 µm particle size; Restek) with a guard column assembly containing C-18 cartridge (Restek; Roc®10 x 4 µm; Cat# 953450210). The flow rate used was 0.4 mL/min of a premixed 60:40 volume ratio of methanol to water. DNAN, 2-NO-NAN, and DAAN were quantified at 220 nm and 2-HA-NAN and 2-ANAN were best quantified at 254 nm. Additional samples were collected and analyzed every day for the next several days.

Standards of known concentrations were prepared for high purity DNAN, 2-ANAN, and for DAAN that were commercially available, and used to generate calibration curves to quantify these compounds in the samples. Two intermediates, 2-NO-NAN and 2-HA-NAN, that were not available from commercial sources, were identified by comparison with the published chromatograms in Hawari et al. (2015), who had a similar HPLC setup. 2-NO-NAN and 2-HA-NAN were quantified in unknown samples by using the calibration curve of the quantified product whose retention time was closest to the byproduct peak. The retention time of 2-HA-NAN was close to that of 2-ANAN,
so the 2-ANAN calibration curve was employed to quantify 2-HA-NAN yields. Likewise, the retention time of 2-NO-NAN peak was near that of DNAN, so the calibration of DNAN was used to quantify 2-NO-NAN. Standards were analyzed and fresh calibration curves were generated on each day of sampling.

2.5 Data Treatment

The concentrations (mM) and amount (in µmoles) of DNAN and its various degradation products in the batch reactors were calculated by using their respective calibration curves. Peak areas were converted to mass concentration using a slope value from a calibration curve. Mass concentration was converted to µmol/L using the molar mass and then to µmol using the volume of the reactor (72 mL). Mole fractions \( \frac{m}{m_0} \) was determined by dividing the amount of DNAN or reduction product at a given time \( t \) (m) by the starting concentration \( m_0 \), which was determined using the first 2 or 3 measurements of DNAN in the control created for those experiments (more detailed calculations in SI).

DNAN degradation seemed to take place in two phases, with the initial degradation phase within the first few minutes and the second phase taking place after that. In order to calculate kinetics in phase 1, \( k_{obs1} \) was calculated using DNAN mole fraction in individual reactors from samples taken at \( t_0 \) and \( t_1 \). The kinetics of the second phase, \( k_{obs2} \), was calculated in individual reactors using DNAN samples at \( t_2 \) and \( t_3 \). The individual reactor \( k_{obs1} \) and \( k_{obs2} \) values were then averaged to obtain average \( k_{obs1} \) and \( k_{obs2} \) for the experimental condition.
Other studies that examined abiotic degradation of DNAN with a solid reductant like zero valent iron (Hawari et al., 2015) and bimetallic iron with copper or nickel (Koutsospyros et al., 2012) exhibited apparent first-order kinetics. Other studies with iron oxides showed reactivity toward other nitroaromatic as well as chlorinated hydrocarbon pollutants exhibiting first-order or pseudo-first order degradation rate kinetics (e.g. Gregory et al., 2004; Gorski and Scherer, 2009; Danielsen and Hayes, 2004; McCormick and Adriaens, 2004; and Vikesland et al., 2007). In Vikesland et al. (2007), the concentrations of pollutants were held constant for all experiments, facilitating the use of a pseudo-first order rate constant. As a result, [DNAN] in this study was held constant, making adsorbed and structural Fe(II) the only variable reactant for determining reaction order. Therefore, $k_{\text{obs}1}$ and $k_{\text{obs}2}$ were treated as pseudo-first order rate constants.

A further analysis of the data using R statistical software was used to examine the full set of data with respect to varying [HFO], [Fe(II)], initial pH, and the contributions of these variables that will be referred to as "input variables." The analysis was conducted as a linear model to examine those factors' influence on what is being called "output variables:" $k_{\text{obs}1}$, $k_{\text{obs}2}$, mole fraction of DNAN remaining, mole fractions of products, and C mass balance. P-values were used to confirm the importance of each of the reactor conditions to the dependent variables (See SI). Graphs showing the relationships between the input variables and the output variables were also produced (See SI). Correlation values between the input variables and the output variables gives a confirmation for the model (See SI). However, some relationships were not completely linear, which would cause p-values to be higher.
DNAN removal and product distribution at any given time are presented in mole fractions \((m/m_0)\), as DNAN remaining, and product yields. The sum of DNAN and its degradation products give the carbon mass balance. Table 8 shows conditions that were examined for this investigation.

Table 8: Experimental Conditions Examined in DNAN Degradation with Respect to pH, [Fe-oxide], and [Fe(II)] Treatment.

<table>
<thead>
<tr>
<th>Fe-oxide</th>
<th>pH</th>
<th>[Fe-oxide] (mM)</th>
<th>[Fe(II)] Amendment (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HFO</td>
<td>6</td>
<td>1.39</td>
<td>0, 0.28, 0.56, 0.83</td>
</tr>
<tr>
<td>HFO</td>
<td>6</td>
<td>2.78, 4.17</td>
<td>0, 0.28</td>
</tr>
<tr>
<td>HFO</td>
<td>7</td>
<td>1.39</td>
<td>0, 0.28, 0.56, 0.83, 1.66, 1.94</td>
</tr>
<tr>
<td>HFO</td>
<td>7</td>
<td>2.78</td>
<td>0, 0.28, 0.56, 0.83, 3.04, 3.32</td>
</tr>
<tr>
<td>HFO</td>
<td>7</td>
<td>4.17</td>
<td>0, 0.28, 0.56, 0.83, 4.86, 5.14</td>
</tr>
<tr>
<td>HFO</td>
<td>8.5</td>
<td>1.39, 2.78, 4.17</td>
<td>0, 0.28, 0.56, 0.83</td>
</tr>
<tr>
<td>HFO</td>
<td>10</td>
<td>1.39</td>
<td>0, 0.28, 0.56, 0.83</td>
</tr>
<tr>
<td>HFO</td>
<td>10</td>
<td>2.78, 4.17</td>
<td>0, 0.28</td>
</tr>
<tr>
<td>Goethite</td>
<td>7</td>
<td>12.5, 25</td>
<td>0, 0.28, 0.56, 0.83</td>
</tr>
<tr>
<td>Goethite</td>
<td>8.5</td>
<td>12.5</td>
<td>0, 0.28, 0.56, 0.83</td>
</tr>
</tbody>
</table>

2.6 Particle Characterization

Samples of mineral slurries were diluted 100x and placed on a micro slide to be examined on a Zeiss Axioskop light microscope with a 100x magnification lens and a 10x magnification eyepiece at the Wright Patterson Air Force Base’s Air Force Institute of Technology (AFIT). 1000x magnification was insufficient to obtain accurate grain size and shape and therefore could not calculate surface area, but some qualitative
comparisons of the particle aggregates could be made. Micrograph photos were taken using Axiovision software (See SI).

3.0 Results

3.1 DNAN degradation by Fe(II)-treated HFO

The treatment of HFO by Fe(II) (as ferrous sulfate) caused the color of its particles to darken and become dark brown in proportion to the amount of Fe(II) amendment. The color of HFO particles with Fe(II) were also darker with increasing pH. The change in HFO color was almost instantaneous upon adding Fe(II). The pH of the slurry containing HFO sometimes declined upon Fe(II) addition if the buffer (TAPSO, 10 mM) was insufficient to maintain the pH. Reactors were adjusted to the desired pH as needed.

DNAN degradation was rapid with 1.39 mM HFO treated with varying amounts of Fe(II) initially at pH 7 (Fig. 14). Freshly prepared HFO treated with 0.83 mM Fe(II) (Fe(II)/Fe(III) molar ratio = 0.6, which was greater than the ratio for stoichiometric magnetite) showed modest DNAN degradation to 2-NO-NAN, 2-HA-NAN and 2-ANAN as reaction intermediates, and DAAN as final product in ~6 days (Fig. 14A). 2-NO-NAN and 2-HA-NAN formed immediately. 2-NO-NAN remained in trace amounts (not shown), and 2-HA-NAN yield ($m/m_0$) was 0.24; both intermediates degraded quickly to form relatively stable 2-ANAN that had the greatest yield ($m/m_0 = 0.47$). The formation of DAAN as end product was in trace amount (yield, $m/m_0 < 0.01$). A modest fraction of DNAN did not degrade (remaining mole fraction, $m/m_0 = 0.31$) in 6 days. DNAN degradation was biphasic (Fig. 14B). There was an initial rapid degradation with separate
kinetics ($k_{obs1}$) followed by a second slower phase ($k_{obs2}$). The two phases could not be modeled with a single best fit curve. With 1.39 mM HFO treated with 0.83 mM Fe(II) at pH 7, the majority of DNAN removal occurred within the first 24 hours (Fig. 14A), and the DNAN remaining ($m/m_0$) reached 0.4 and stabilized by the end of the first day. Fig. 14B shows $k_{obs1}$ and $k_{obs2}$ calculations for each reactor. DI water and HFO control reactors showed no significant DNAN degradation and no reaction byproducts; a slight decrease in DNAN was observed that can be explained by a small amount of sorption to the HFO surface. DNAN mole fractions remaining ($m/m_0$) were typically between 0.95 and 1 for DI water control reactors, and between 0.85 and 0.9 in HFO only control reactors regardless of HFO mass concentration.
3.2 Effect of Fe(II)-treated [HFO] on DNAN degradation

DNAN degradation by 1.39, 2.78, 4.17 mM [HFO] pre-treated with constant 0.56 mM Fe(II) at pH 7 had a modest effect on DNAN degradation and product distribution (2-HA-NAN and 2-ANAN). Most DNAN degradation took place in the first hour after the experiment began and the system stabilized for all three [HFO] within a day (Fig. 15). With increasing [HFO], the amount of DNAN removal decreased modestly; DNAN mole fraction remaining ($m/m_0$) were 0.54, 0.46 and 0.40 at 1.35, 2.78, and 4.17 mM [HFO], respectively (Fig. 15A). The first byproduct, 2-HA-NAN, was produced quickly but degraded slowly over the 5-day period (Fig. 15B). However, increase in [HFO] did not greatly influence on 2-HA-NAN yields at any time during the experiment. The primary byproduct, 2-ANAN, was produced and stabilized over time (Fig. 15C). With 3-fold increase in [HFO], the production of 2-ANAN increased only modestly; its mole fraction...
yields after 5 days were 0.21, 0.27 and 0.26 with 1.35, 2.78, and 4.17 mM [HFO], respectively (Fig. 15C).

Further statistical analysis examining all experiments revealed no distinct linear trend between [HFO] and the output variables of $k_{\text{obs1}}$, $k_{\text{obs2}}$, and final mole fractions of DNAN, 2-ANAN, DAAN and total mass balance. The lowest p-value in the analysis for HFO was 0.02, suggesting that the chances of the trend between [HFO] and DAAN yields being from random fluctuations. However, the trend did not show a strong increase or decrease in DAAN yields with increasing [HFO].
Fig. 15: (A) DNAN degradation (B) 2-HA-NAN production and subsequent degradation, and (C) 2-ANAN production over time with various [HFO] pretreated with 0.56 mM Fe(II) at pH 7. DAAN was either not produced or only produced in trace amounts (not shown).

3.3 Effect of [Fe(II)] with HFO on DNAN degradation

With increasing [Fe(II)] amendments to 1.39 mM HFO in subsequent experiments, there was no significant evidence to suggest that [Fe(II)] had a discernible effect on DNAN $k_{\text{obs1}}$, yet $k_{\text{obs2}}$ increased with increasing [Fe(II)] (Fig. 16A). However,
the byproduct distribution was strongly influenced by the increase in [Fe(II)] (Fig. 16B). The mole fraction of DNAN remaining decreased as [Fe(II)] increased. 2-NO-NAN and 2-HA-NAN were present in trace amounts. 2-ANAN yield showed an increasing trend with increasing [Fe(II)], which made up the majority of the reaction products and was consistent with the dropping DNAN yields. However, at pH 7 with 1.39 mM HFO, DAAN was also present only in trace amounts.

The R statistical analysis comparing the input variable [Fe(II)] to $k_{obs1}$ and $k_{obs2}$ values and final mole fraction values of DNAN, 2-ANAN, DAAN, and mass fraction. Fe(II) was most closely tied to $k_{obs1}$ with a p-value of 0.011. This suggests that the initial rate constant, $k_{obs1}$ was directly correlated by the increase in [Fe(II)], however Fig. 16A did not show a significant difference with increasing [Fe(II)]. Trends in the product distribution from DNAN remaining and 2-ANAN were not detected by running linear regression.

<table>
<thead>
<tr>
<th>[Fe(II)] (mM)</th>
<th>$k_{obs1}$ ($d^{-1}$)</th>
<th>$k_{obs2}$ ($d^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.28</td>
<td>60</td>
<td>2.5</td>
</tr>
<tr>
<td>0.56</td>
<td>70</td>
<td>3.5</td>
</tr>
<tr>
<td>0.83</td>
<td>80</td>
<td>4.5</td>
</tr>
<tr>
<td>1.66</td>
<td>90</td>
<td>5.5</td>
</tr>
<tr>
<td>1.94</td>
<td>100</td>
<td>6.5</td>
</tr>
</tbody>
</table>
Fig. 16: Effect of [Fe(II)] on DNAN degradation and product distribution at 1.39 mM HFO and pH 7. (A) DNAN $k_{obs1}$ and $k_{obs2}$, and (B) DNAN remaining and reaction byproduct yields at varying [Fe(II)] in experiments with 1.39 mM HFO at pH 7.

Additional investigations show that changes in [Fe(II)] in tandem with variations in [HFO] at pH 7 affect DNAN degradation (Fig. 17; Table 9). The rate constants of DNAN degradation ($k_{obs1}$ and $k_{obs2}$) increased 1.1 to 1.4-fold with a 3-fold increase in [HFO] from 1.39 to 4.17 mM at 0.28, 0.56, and 0.83 mM Fe(II) (Figs. 17A and B; Table 9), which supports the observation in section 3.2 that the effect of [HFO] on DNAN degradation kinetics is modest. Further, increasing [Fe(II)] also resulted in smaller DNAN mole fractions remaining and greater 2-ANAN yield. While the increase in DNAN $k_{obs1}$ values was modest with Fe(II) increasing from 0.28 to 0.83 mM at varying [HFO] (Fig. 17A; Table 9), but its effect on increase in $k_{obs2}$ was greater (Fig. 17B; Table 9); for example, a 17-fold increase in Fe(II) affected a ~17-fold increase in $k_{obs2}$ at 4.17 mM HFO. Similarly, increases in [Fe(II)] from 0.28 to 4.86 mM systematically enhanced DNAN removals and 2-ANAN yields, but increases in [HFO] had less discernible effects (Figs. 17C and D; Table 9). The relationship of [HFO] and [Fe(II)] together when
considering all experiments in the R study showed that the pairing of the two *input variables* did not adequately explain the variability of kinetics and product distribution. The coupling of HFO and Fe(II) did, however, show a strong relationship to the final mole fractions of DNAN remaining with a P value of 0.059 (See SI).
### Table 9: The Effect of [Fe(II)] and [HFO] on DNAN Kinetics and Product Distribution at pH 7

<table>
<thead>
<tr>
<th>mM</th>
<th>Fe(II)</th>
<th>$k_{obs1}$</th>
<th>$k_{obs2}$</th>
<th>DNAN</th>
<th>2-ANAN</th>
<th>DAAN</th>
<th>Mass Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.39</td>
<td>0.28</td>
<td>+8.7 ± 6.4</td>
<td>1.32</td>
<td>0.560</td>
<td>0.190</td>
<td>0</td>
<td>0.750 ± 0.020</td>
</tr>
<tr>
<td>1.39</td>
<td>0.56</td>
<td>75.8 ± 14</td>
<td>1.65</td>
<td>0.536</td>
<td>0.210</td>
<td>0</td>
<td>0.759 ± 0.020</td>
</tr>
<tr>
<td>1.39</td>
<td>0.83</td>
<td>82.4 ± 40</td>
<td>1.88</td>
<td>0.319</td>
<td>0.471</td>
<td>0.00743 ± 0.0020</td>
<td>0.797 ± 0.011</td>
</tr>
<tr>
<td>1.39</td>
<td>1.66</td>
<td>60.2 ± 13</td>
<td>4.03</td>
<td>0.195</td>
<td>0.532</td>
<td>0.00705 ± 0.0070</td>
<td>0.734 ± 0.019</td>
</tr>
<tr>
<td>1.39</td>
<td>1.94</td>
<td>59.4 ± 3.4</td>
<td>4.18</td>
<td>0.197</td>
<td>0.531</td>
<td>0.00370 ± 0.00037</td>
<td>0.732 ± 0.0042</td>
</tr>
<tr>
<td>2.78</td>
<td>0.28</td>
<td>70.4 ± 12</td>
<td>1.15</td>
<td>0.584</td>
<td>0.0947 ± 0.0017</td>
<td>0</td>
<td>0.679 ± 0.00088</td>
</tr>
<tr>
<td>2.78</td>
<td>0.56</td>
<td>89.5 ± 0.13</td>
<td>2.15</td>
<td>0.461</td>
<td>0.268</td>
<td>0.0130 ± 0.0023</td>
<td>0.742 ± 0.044</td>
</tr>
<tr>
<td>2.78</td>
<td>0.83</td>
<td>78.7 ± 5.1</td>
<td>2.80</td>
<td>0.393</td>
<td>0.398</td>
<td>0.00557 ± 0.00012</td>
<td>0.796 ± 0.00017</td>
</tr>
<tr>
<td>2.78</td>
<td>3.04</td>
<td>138 ± 11</td>
<td>10.3</td>
<td>0.0271</td>
<td>0.708</td>
<td>0.0281 ± 0.0090</td>
<td>0.763 ± 0.0026</td>
</tr>
<tr>
<td>2.78</td>
<td>3.32</td>
<td>136 ± 14</td>
<td>8.20</td>
<td>0.0291</td>
<td>0.704</td>
<td>0.0206 ± 0.0040</td>
<td>0.754 ± 0.0092</td>
</tr>
<tr>
<td>4.17</td>
<td>0.28</td>
<td>90.1 ±5.0</td>
<td>1.34 ±0.091</td>
<td>0.539 ±0.015</td>
<td>0.130 ±0.0059</td>
<td>0 ±0</td>
<td>0.669 ±0.0093</td>
</tr>
<tr>
<td>4.17</td>
<td>0.56</td>
<td>87.8 ±7.9</td>
<td>2.60 ±0.063</td>
<td>0.395 ±0.0071</td>
<td>0.258 ±0.011</td>
<td>0.0133 ±0.0030</td>
<td>0.667 ±0.00045</td>
</tr>
<tr>
<td>4.17</td>
<td>0.83</td>
<td>106 ±0.30</td>
<td>10.1 ±0.48</td>
<td>0.143 ±0.021</td>
<td>0.621 ±0.017</td>
<td>0.00676 ±0.00068</td>
<td>0.771 ±0.010</td>
</tr>
<tr>
<td>4.17</td>
<td>4.86</td>
<td>142 ±27</td>
<td>22.7 ±4.4</td>
<td>0 ±0</td>
<td>0.732 ±0.0013</td>
<td>0.0294 ±0.00070</td>
<td>0.761 ±0.00064</td>
</tr>
<tr>
<td>4.17</td>
<td>5.14</td>
<td>84.8 ±24</td>
<td>21.7 ±2.2</td>
<td>0 ±0</td>
<td>0.722 ±0.0019</td>
<td>0.0300 ±0.0034</td>
<td>0.752 ±0.0052</td>
</tr>
</tbody>
</table>
**Fig. 17:** Effect of [Fe(II)] with [HFO] on DNAN degradation kinetics and product distribution at pH 7. (A) Initial pseudo-first order rate constant ($k_{obs1}$) values are plotted against [Fe(II)] on the x-axis and series separated by [HFO]. (B) Variations in overall rate constant ($k_{obs2}$) for different [HFO] and increasing [Fe(II)]. (C) DNAN mole fraction remaining and (D) 2-ANAN mole fraction yield, combined for all three HFO series with increasing [Fe(II)]. Minor DAAN yield ($m/m_0 \leq 0.04$) at [Fe(II)] above 2 mM (not shown).

### 3.4 Effect of pH on DNAN degradation with [Fe(II)]-treated HFO
DNAN degradation with 2.78 mM HFO pre-treated with 0.28 mM [Fe(II)] shows that its kinetics and reduced byproducts are affected by variations in initial pH (Fig. 18). The decline in [DNAN] over time followed the same biphasic degradation pattern as described before (Fig. 18A). DNAN removal during the initial 5 to 6-day period increased with the general increase in pH, but the DNAN mole fraction remaining, \((m/m_0)\) in reactors at initial pH 8.5 and 10 were similar (Fig. 18A). 2-HA-NAN was produced quickly in reactors at different initial pH. Its yield increased from pH 6 to 8.5 but declined considerably at pH 10 (Fig. 18B). At initial pH 8.5 and 10, the maximum yields of 2-HA-NAN were ~0.15 each but it was slowly degraded over the next several days (Fig. 18B). Further degradation of 2-HA-NAN formed 2-ANAN as a stable product. At pH 6, 2-ANAN was produced in trace amounts, but its mole fraction yield increased to ~0.1 at pH 7 on day 1 and then stabilized (Fig. 18C). 2-ANAN final yields increased slightly to 0.12 at pH 8.5 but declined to 0.06 at pH 10. In this series, the reactors at initial pH 10 were the ones to produce minor amounts of DAAN \((m/m_0\) was ~0.08).

The statistical analysis showed that \(k_{obs1}\) was influenced by pH with a p-value of 0.058 (See SI). However, \(k_{obs2}\) did not show a strong relationship with increasing pH. When comparing all HFO experiments, pH was observed to be a stronger influence on 2-ANAN yields. The p-value for 2-ANAN yields among all experiments with respect to pH was 0.010, mass balance was most strongly affected showing a negative trend in the graphs and a p-value of 0.0073 (See SI).
Fig. 18: Effect of pH on DNAN degradation and byproduct distribution with 2.78 mM HFO pre-treated with 0.28 mM Fe(II) at pH 6, 7, 8.5 and 10. Time-mole fraction plots of DNAN (A), 2-HA-NAN (B), and 2-ANAN (C) over 6 days. DAAN was not produced in reactors with initial pH at 6, 7, and 8.5, but it formed at pH 10 in trace amounts.

The DNAN mole fraction remaining ($m/m_0$) with 1.39 mM HFO pre-treated with 0.28 mM Fe(II) decreased from 0.84 to 0.56 (Fig. 19A) with an increase in pH from 6 to 7. At pH 6, DNAN remaining was ~0.84, similar to the control reactor at 1.39 mM HFO only. DNAN remaining at pH 8.5 to 10 showed a similar decrease in $m/m_0$ at 1.39 mM HFO at each [Fe(II)] tested (Fig. 19A). However, the change in pH from 8.5 to 10 did not appear to distinctly change DNAN remaining.

At pH 6, there was a nearly 0.001 mole fraction yields of 2-ANAN and 0 DAAN (Removed from Fig. 19B and C). Above pH 6, 2-ANAN yields increased with increases in [Fe(II)]. No significant difference between pH 7, 8.5, and 10 series was observed. At 1.39 mM HFO, pH 10 experiments had the highest DAAN yields, which never exceeded 0.2 mole fraction (Fig. 19C). Final mass balance for this investigation (Fig. 19D) showed that experiments at pH 8.5 and 10 have a lower mass balance than experiments at pH 6.
and 7. This pattern of behavior was also observed when [HFO] was 4.17 mM. Product mass balance was observed to be mostly unchanging at pH 6 regardless of [Fe(II)] or [HFO] (Fig. 19D). Values of $k_{\text{obs1}}$ show a dramatic change at pH 8.5 and 10 (Fig. 19E). The $k_{\text{obs1}}$ increased drastically from pH 7 to 8.5 and less so between 8.5 and 10. Likewise, as [Fe(II)] increased, $k_{\text{obs1}}$ increased drastically for pH 8.5 and 10, but was a slow, steady, linear increase for pH 7. The $k_{\text{obs2}}$ values for this data showed a similar pattern of behavior (data not shown).

The multiple linear regression model examined the interaction of pH with [HFO] and pH with [Fe(II)]. The interaction of pH with [HFO] did not yield strong trends with either kinetics or product distribution. However, the interaction of pH and [Fe(II)] showed strong trends for $k_{\text{obs1}}$, $k_{\text{obs2}}$, DNAN remaining, and yields of 2-ANAN and DAAN. P-values were 4.4E-7, 0.013, 0.00078, 0.00021, and 8.1E-10, respectively (See SI). To confirm some of these correlations, pH showed correlations numbers near 0.5 or above for $k_{\text{obs1}}$, DNAN, DAAN, and mass balance (See SI).
(B) 

![Graph showing 2-ANAN yield (mol frac) vs. [Fe(II)] (mM) for pH 7, pH 8.5, and pH 10.]

(C) 

![Graph showing DAAN yield (mol frac) vs. [Fe(II)] (mM) for pH 7, pH 8.5, and pH 10.]

Fig. 19: Effect of Initial pH on DNAN degradation kinetics and product distribution at 1.39 mM HFO with increasing [Fe(II)]. (A) Final DNAN \(m/m_0\) remaining, (B) 2-ANAN yield, (C) DAAN yield (pH 6 was eliminated), (D) final product mass balance \((m/m_0)\), and (E) DNAN \(k_{obs1}\) values with increasing [Fe(II)]. Series are separated according to initial pH.

3.5 Comparison of DNAN degradation with Fe(II)-treated Goethite and HFO

Similar to HFO, the color of the goethite slurry in the reactor changed immediately with Fe(II) addition, which become a greenish color that was darker than the goethite’s golden color. The color became darker at higher pH and at greater [Fe(II)].
Batch experiments with 12.5 mM goethite and 4.17 mM HFO at pH 7 and at 8.5 showed that the rate constants of DNAN degradation ($k_{obs1}$ and $k_{obs2}$) were consistently greater with HFO than with goethite when [goethite] was far greater than [HFO] and both had equal [Fe(II)] amendment (Fig. 20A). The average $k_{obs}$ values with 12.5 mM goethite at 0.28, 0.56, and 0.83 mM Fe(II) at pH 7 were 29.3, 52.3, and 57.3 d$^{-1}$, respectively. In comparison to goethite, the average $k_{obs}$ values with 4.17 mM HFO at 0.28, 0.56, and 0.83 mM Fe(II) at pH 7 were 90.1, 87.8, and 105.8 d$^{-1}$, respectively. Similarly, the DNAN degradation $k_{obs}$ values at pH 8.5 were much greater with HFO than with goethite, with equal [Fe(II)] amendment (Fig. 20A). Further, DNAN removals during the experiment at pH 7 and 8.5 were greater with HFO than with goethite at the same [Fe(II)] (Fig. 20B). The DNAN removal increased (i.e., DNAN remaining decreased) as pH increased to 8.5.

Comparing the effects of mineral species with product yields was somewhat more complicated. 2-ANAN was the dominant product, but patterns were less specific than for DNAN remaining and $k_{obs1}$ (Fig. 20C). There was less spread in the data between mineral type and pH. 2-ANAN yield increased with increasing [Fe(II)] and HFO had slightly smaller 2-ANAN yield than goethite, but there was no distinct relationship between pH and 2-ANAN yield. DAAN was produced in trace amounts in HFO studies, but not in goethite experiments with 12.5 mM (not shown). Experiments with 25 mM goethite had a maximum DAAN yield of 0.033 ($m/m_0$). In Fig. 20D, it was generally seen that carbon mass balance at pH 8.5 was lower than in pH 7. HFO experiments saw an overall lower mass balance than goethite studies.
Fig. 20: Effect of iron oxide phase (goethite vs. HFO) at various [Fe(II)] and pH. (A) DNAN initial $k_{obs}$ values with increasing [Fe(II)] for data series divided by pH 7 or 8.5 with 4.17 mM and 12.5 mM HFO and goethite, respectively, for each pH level. Final mole fraction of (B) DNAN remaining, (C) 2-ANAN yield and (D) final mass balance divided in the same way as in A.

3.6 Characterization of HFO and goethite nanoparticles

Light microscopy was used to determine the reason for the apparent lower reactivity of goethite. HFO aggregates (See SI) were more amorphous and had a smaller apparent grain size than goethite samples. Grain shape, when visible appeared generally
rounded for HFO particles, but were more rod shape for goethite particles in some aggregates. When looking at aggregate shapes, Edges of goethite aggregates appeared generally smoother than the surfaces of the HFO aggregates (See SI).

4.0 Discussion

4.1 DNAN degradation by Fe(II)-treated HFO

DNAN degradation products were not observed in control reactors containing HFO alone, so the 0.05-0.10 mole fraction DNAN removal may have been due to adsorption to mineral substrates. A Similar portion of the DNAN loss in experimental reactors may be from adsorption. While many experiments showed that mole fraction yields of final products were <1, other experiments show the carbon mass balance to be ~1, suggesting that the loss due to adsorption to HFO was generally small. It was observed both in this experiment and in literature that Fe(II) adsorbed to the surface of HFO mineral particles facilitates pollutant reduction (Gorski and Scherer, 2011). DNAN degradation did not take place until aqueous Fe(II) was added.

A color change in HFO similar to that observed in Schaefer et al. (2011) indicates that a similar change to the mineral species took place. A Mössbauer spectroscopic analysis by Schaefer and others (2011) attributed this to electron exchange by Fe(II)-Fe(III) pairing between the adsorbed Fe(II) and structural Fe(III). Surface spectra of nontronite with adsorbed $^{57}\text{Fe(II)}$ showed high levels of $^{57}\text{Fe(III)}$, indicating electron exchange to have occurred on the mineral surface. The reaction between DNAN and Fe(II)-treated HFO was a two-phase process, beginning with a rapid DNAN removal in the first 3 min (Phase I) followed by a slower degradation that lasted for several hours.
(Phase II) before the concentration of DNAN and products reached equilibrium. In general, 2-NO-NAN was produced in trace amounts and was removed by day 2. The fraction of DNAN removed was limited by the availability of Fe(II) reductant. The primary reactor mechanism at work was reduction of the nitro groups at the ortho and then para positions on the DNAN structure.

2-HA-NAN was produced almost as quickly as DNAN was removed, but it degraded slowly and was removed by day 6. Since 2-NO-NAN and 2-HA-NAN are quite unstable, it’s unclear whether their transformation to 2-ANAN was facilitated by Fe(II). 2-ANAN was a stable byproduct of DNAN reduction when the conditions were not potent enough to reduce the second nitro group and form DAAN. Under the conditions examined in this study, the product distribution was dominated by 2-ANAN, which was produced at the beginning and as 2-HA-NAN degraded. In experiments where DNAN can be reduced completely to DAAN, several intermediate species including dimer species (Fig. 13A) and Meisenheimer complexes (Fig 13B) may form but not be detected by the HPLC method. In cases where carbon mass balance <100%, these undetectable byproducts may account for some or most of the missing carbon. DAAN yields for any HFO experiments and yields were typically small, except at pH 10 with moderate [Fe(II)]. The majority of DNAN degradation and product distribution changes occurred in the first day of the experiment. In Niedźwiecka et al. (2017), palladium catalyst pellets with about 1.5 mM Fe(II) was used to degrade DNAN at pH 6 through 9. Like this investigation, the reaction was mostly finished by the end of 24 hours (Niedźwiecka et al., 2017).

Although the initial rate constant, $k_{obs1}$ values did not strongly increase with the 7-fold increase in [Fe(II)], $k_{obs2}$ and product distribution showed clearly that [Fe(II)]
accelerated DNAN reduction and it was a primary driver for enhancing reaction kinetics and product yields. The multiple linear regression analysis suggested, however, that there was no such trend among all studies containing HFO. This lack of great difference in $k_{obs1}$ suggests that the degradation of DNAN is dependent upon the availability of surface reaction sites. Variability among trace amounts of intermediates and products for all experiments can be explained by random error, however a generally decreasing trend in final yields, particularly 2-HA-NAN, was clearly evident, with 2-ANAN as the dominant product of degradation. Increasing [Fe(II)] at pH 7 did not reduce DNAN to its most reduced form, which was problematic because 2-ANAN may be carcinogenic and may pose additional risks at DNAN contaminated sites (Sigma-Aldrich, 2017).

4.2 Effect of Fe(II)-treated [HFO] on DNAN degradation

At pH 7, increasing the [HFO] caused a very modest increase in DNAN removal and 2-ANAN production and had little effect on the amount of 2-HA-NAN production and removal in between (Fig. 15B). Multiple linear regression analysis calculating correlation between [HFO] and the \textit{output variables} showed no significant dependence (See SI). DNAN’s slight increase in $k_{obs1}$ with increasing [Fe(II)] suggests that the surface area of the mineral species makes little difference with ferric iron oxides. The series of $k_{obs2}$ at 4.17 mM HFO had a large variability, especially at higher [Fe(II)], which may indicate that $k_{obs1}$ may be slightly dependent on [HFO]. However, in experiments with fast kinetics, $k_{obs1}$ and $k_{obs2}$ can be more dependent on the timing of samples, which was limited by the methodology of reactor construction. The amount of electron exchange between adsorbed Fe(II) and HFO mineral should not change with increasing [HFO]. No
significant trend in changing kinetics or product distribution was seen with increasing [HFO] (See SI).

4.3 Effect of $[\text{Fe(II)}]$ with HFO on DNAN degradation

At pH 7 with 1.39 mM HFO, changes in $[\text{Fe(II)}]$ had a greater effect on $k_{\text{obs}2}$ and product distribution than $k_{\text{obs}1}$ (Fig. 16). The effect of increasing $[\text{Fe(II)}]$ was only marginally amplified by increasing [HFO] as well. Fig. 17 (Table 9) showed that product yields and $k_{\text{obs}2}$ were particularly affected by $[\text{Fe(II)}]$, but only marginally affected by changes in [HFO] for those $[\text{Fe(II)}]$. Best fit linear models for 1.35, 2.78, and 4.17 mM HFO $k_{\text{obs}2}$ with increasing $[\text{Fe(II)}]$ indicate that $[\text{Fe(II)}]$, instead of [HFO], was the primary control on the rate of DNAN removal throughout the reaction and the yields of more reduced products like DAAN. The conclusion is that the concentration of [HFO] and its surface area are less important for DNAN removal and the data reinforces that it is $[\text{Fe(II)}]$ that controls the reaction potency.

The multiple linear regression analysis examining all studies showed that $k_{\text{obs}1}$, $k_{\text{obs}2}$, DNAN remaining, 2-ANAN yields, and DAAN yields were directly affected by changes in Fe(II). P-values for these parameters were 7.2E-7, 0.016, 0.0045, 0.0019, and 2.4E-9 respectively, indicating an extremely low probability that the variation in those parameters with changes in $[\text{Fe(II)}]$ could be explained by random variability. When examining the model with respect to both [HFO] and [Fe(II)], kinetics and product distribution was not adequately explained by the model with [HFO] and [Fe(II)] considered together, except for DNAN remaining. One reason for this is that the relationship for kinetics was more complicated than a linear model could explain and the
product distribution was also not linear when DNAN was completely removed or when 2-ANAN composed nearly all of the products. In Fig. 17D, it is clear that DNAN remaining was the most linear in its behavior with increasing [Fe(II)] for all [HFO]. It is also clear that changes in [HFO] did not appear to contribute significantly to the model.

4.4 Effect of pH on DNAN degradation with [Fe(II)]-treated HFO

Starting pH was expected to have some influence on initial $k_{obs}$. DNAN’s behavior was expected to reflect TNT’s lack of significant change in behavior at pH 6 to 8.5 with green rust and magnetite (from Boparai et al., 2010). At pH 6 and 7, this expectation bears out in the data. The amount of DNAN remaining at pH 6 suggests that negligible DNAN removal took place. Fe(II) likely did not speciate to form reactive species under acidic conditions and may not have adsorbed to HFO surfaces. Niedźwiecka et al. (2017) observed a similar behavior when they attempted to degrade DNAN with palladium pellets and 1.5 mM Fe(II). The system for both this study and in Niedźwiecka et al., (2017) was more potent at pH 7. 2-ANAN yield was largely unchanged by increasing pH in Fig. 19B, but at high [Fe(II)], more DAAN was produced at pH 10, showing that pH increased reaction potency. However, the multiple linear regression model showed that 2-ANAN yield was somewhat dependent on pH, showing an upward trend as pH increased (See SI). PH 10 produced stronger reducing conditions than other pH levels, accounting for some of the lower 2-ANAN yields. Abiotic experiments performed by Niedźwiecka et al. (2017) at pH 7 produced traces of 4-ANAN, which was not observed in this study.
The dynamic relationships between aqueous Fe(II), adsorbed Fe(II), and structural Fe(II) may explain the difference in mass balance with increasing pH. In section 4.1, the comparisons to other work in Fe(II) behavior with ferric minerals suggests that electron exchange takes place between mineral and Fe(II) (Schaefer et al., 2011). The adsorbed iron oxidized while reducing the mineral phase. Increasing pH introduced the added complexity of Fe(II) speciation and Meisenheimer complex formation. Work by Strathmann and Stone (2002) described several species that begin to form in an aqueous solution of Fe(II) as pH increases beyond pH 7. Aqueous Fe(II) speciates into aqueous FeOH$^+$ and eventually aqueous Fe(OH)$_2^0$ and solid Fe(OH)$_2$ due to higher hydroxide concentrations (Strathmann and Stone, 2002). The mineral’s surface becomes more negatively charged with rising pH, which increases the adsorption of these aqueous Fe(II) species (Amonette et al., 2000). Adsorbed forms of these Fe(II) species have been further described by Liger and others (1999), including $\equiv$FeOFe$^+$, $\equiv$FeOFeOH$^0$, and $=\equiv$FeOFe(OH)$_2^-$. These species have a greater reduction potential than aqueous Fe(II) and may also account for the increase in $k_{obs1}$, decreases in DNAN remaining, and marginal increases in 2-ANAN and DAAN yields as [Fe(II)] and pH increase.

At pH 8.5 and 10, the mass balance was lower than at other pH levels, suggesting a competing mechanism. This result was supported by a decreasing trend in mass balance as pH increased for all experiments and a strong negative correlation value (-0.83) between pH and mass balance found when confirming the multiple linear regression model (See SI). The competing mechanism expected was alkaline hydrolysis, resulting in Meisenheimer complex formation. Examples of such a reaction pathway with DNAN is shown in Fig. 13B. Alkaline hydrolysis of DNAN was observed by Salter-Blanc et al.
(2013) to take place at pH levels around 11 and 12, whereby a hydroxyl group attaches to various sites on the aromatic ring. These complexes are not detectable by HPLC with methods used in this study. Salter-Blanc et al. suggest that Meisenheimer complexes were favorable over direct substitution of substituents on nitroaromatic explosives sue to high activation energy. Alkaline hydrolysis may also become a favored pathway over nitroreduction. It is important to note that DNAN removal did not take place in either DI or mineral only controls as a result of nitroreduction or alkaline hydrolysis. This suggests that Meisenheimer complexes could be forming with the nitroreduction products and intermediates, which may be subject to this mechanism at pH 8.5 and 10, but this behavior has not been described as far as we know. Until more research to identify other terminal products at basic conditions is conducted, this interpretation remains a theory.

When [Fe(II)] was increased, there were two possible effects that might explain the increase in mass balance seen in Fig. 19D. Aqueous Fe(II) may be reacting with hydroxyl groups to form the Fe(II) species described by other work (Salter-Blanc et al., 2013; Strathmann and Stone, 2002; Liger et al., 1999). This removes –OH from the system, which would decrease the pH over time and decrease the formation of Meisenheimer products and instead favor the formation of either dimer products or DAAN.

4.5 Comparison of DNAN degradation with Fe(II)-treated Goethite and HFO

Goethite (α-FeOOH) preparation was unique in that it included an artificial aging step. The process of aging turned the particles from dark red to light yellow color. Goethite is described as one of the most stable iron oxide minerals that occurs in natural environments (Cornell and Schwertmann, 2003). Goethite’s color changed nearly the instant that aqueous Fe(II) was added to the reactor, similar to the change in HFO and
likely indicates the same electron exchange behavior described near the beginning of Section 4.1.

Lower DNAN $m/m_0$ remaining values in HFO experiments were strong evidence that higher reactivity in HFO experiments was due to a property of the particles. Models of DNAN remaining with increasing [Fe(II)] showed nearly identical rate of change for both HFO and goethite. It has been established that at constant pH, [Fe(II)] is the variable controlling most of the reaction’s potency, which may indicate that the effect of a set increase in [Fe(II)] affects the reaction potency similarly regardless of the amount or type of mineral present, but this must be further studied to be verified. However, mineralogy plays a role independent of [Fe(II)] and its interaction with the mineral. HFO experiments showed a slightly lower yield of 2-ANAN partly because of a slightly higher amount of transformation of 2-ANAN into DAAN (DAAN data not shown). However, the production of more DAAN does not explain why HFO experiments showed a lower C mass balance than Goethite experiments (Fig. 20D).

As mentioned in section 4.4, lower mass balance at pH 8.5 may be partly explained by greater formation of Meisenheimer complexes. However, the formation of Meisenheimer complexes with nitroreduction products should not have been affected by mineralogy, and the degree of Meisenheimer complex formation at pH 8.5 was expected to be modest. HFO studies had lower mass balance than goethite studies with equivalent conditions, suggesting a trend connected to a physicochemical difference between the two mineral species. One reason for this is that control reactors containing only 4.17 mM HFO or 12.5 mM goethite had different final amounts of DNAN remaining, suggesting different amounts of adsorption. The HFO controls had 0.86 mole fraction DNAN
remaining, while goethite controls had 0.91 \( m/m_0 \). The other reason for the mass balance discrepancy, that best explains the discrepancy at pH 7, was that since HFO reactors produced greater yields of DAAN, it is likely that the reaction favored dimer and other intermediates between 2-ANAN and DAAN that were not detectable by HPLC.

4.6 Characterization of HFO and goethite nanoparticles

The results from literature on whether HFO or goethite creates a more potent system was mixed. Hanoch and others (2006) suggest that no significant difference in pseudo-first order rate constants exists between treated ferric minerals by HS\(^-\) for carbon tetrachloride degradation. Another article, by Maithreepala and Doong (2009) contended that when normalized with respect to surface area, rate constants for carbon tetrachloride removal showed that goethite was more effective than ferrihydrite, which is in the same mineral family as HFO.

The shape of the individual mineral particles and aggregates was difficult to determine due to the limits of the magnification of the light microscope. According to Cornell and Schwertmann (2003), HFO exists only as nanoscale crystals, indicating a much higher surface area than the goethite particles. However, goethite formed larger particles and were rod shaped, occasionally reaching lengths of about 1 µm (Fig. S6). These morphology differences suggest that the goethite particles would have a smaller surface area for equivalent mass concentrations of mineral and therefore exhibit less reactivity in general. Therefore, Goethite’s reactivity per unit of surface area may reflect Maithreepala and Doong’s observations (2009), being more potent than HFO. Alternatively, since the nanoparticles had a strong tendency to aggregate, it is possible
that effective surface area of aggregates might have had a stronger effect on reaction potency than the surface area of the individual particles. If aggregate properties have a stronger impact on reaction potency, then HFO would show greater reactivity and potency than goethite because of HFO aggregates’ rough texture and therefore greater effective surface area (See SI).

5.0 Conclusions

In a natural environment, the degradation of DNAN takes place in two phases, including a rapid initial phase followed by a second stabilization phase. The most important parameters experimentally tested in this investigation that determine the potency of a reaction between ferric minerals like HFO and goethite are [Fe(II)] and pH. High [Fe(II)] in ferric oxide-rich soil will degrade DNAN quickly to more reduced byproducts. However, it should be noted that at the somewhat modest mineral and Fe(II) concentrations used here that may be more likely to occur naturally, complete reduction to DAAN could be rarely achieved, leaving the hazardous byproduct, 2-ANAN, as the dominant byproduct. Increases in hydroxyl ions from higher pH changes the species of Fe(II) to more potent species and encourages the production of Meisenheimer products with nitroreduction byproducts in the environment, resulting in competition between the two mechanisms for dominance. Mineral abundance will have little effect on DNAN removal, but particle size and mineral species can have an impact. The inherent stability of goethite (Cornell and Schwertmann, 2003), larger grain size and smoother aggregate shape seen in this study could make goethite less effective in the environment at removing DNAN, assuming these particles are representative of those that occur naturally. Further work in removing IHE compounds with biogenic minerals is needed.
References


Chapter V

Bench-scale Abiotic Degradation of 2,4-Dinitroanisole (DNAN) with Magnetite:
Implications for Natural Attenuation and Fate

Abstract

As the use of insensitive munitions like 2,4-dinitroanisole increases, the chances of accidental release also increase. This batch reactor study is an expansion of the previous study with HFO and goethite that examines magnetite to understand the effect of structural Fe(II). This study used various pH, [Fe(II)] and [magnetite] conditions to approximate natural conditions to study natural attenuation of DNAN. DNAN degradation with magnetite was dependent on increases in structural and adsorbed Fe(II) with structural Fe(II) being more important. Solution pH also influenced both reaction potency and mechanisms. Higher concentrations of magnetite nanoparticles produce a potent system that may quickly and completely reduce DNAN in the subsurface.

1.0 Introduction

Unintentional detonation of munitions (Walsh et al., 2014), have increased the interest in using Insensitive High Explosives (IHEs) instead of conventional munitions. 2,4-dinitroanisole (DNAN) is increasingly used to replace trinitrotoluene (TNT) (Boddu et al., 2008; Saad et al., 2012; Hawari et al., 2015). DNAN contamination sources include wastewater from IHE manufacturing and residues in live munitions testing grounds.
DNAN can inhibit bacterial activity including methanogens and nitrifying bacteria (Liang et al., 2013). It may also pose health hazards to humans and other organisms because DNAN is reported to damage reproductive organs in male rats and shows neurotoxicity in female rats at 80 mg DNAN/kg/day (Sweeney et al., 2015). IHE formulations that use DNAN also contain several co-contaminants, including nitrotriazolone (NTO), nitroguanidine (NQ), ammonium perchlorate (AP), and sometimes hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) (Taylor et al., 2015; Walsh et al., 2014).

DNAN can degrade by multiple pathways, usually involving transformation at its two nitro groups (Fig. 21A) or a substitution at the methoxy group. A review of DNAN degradation products and pathways by reductive transformations (Fig. 21) based on Ahn et al. (2011) and Olivares et al. (2013) shows that DNAN reduction begins at the nitro group at the ortho-position producing the following three intermediates: (a) 2-nitroso-4-nitroanisole (2-NO-NAN), (b) 2-hydroxylamino-4-nitroanisole (2-HA-NAN), (c) 2-amino-4-nitroanisole (2-ANAN). Reductive transformation continues with the second nitro group and forms (d) 2-amino-4-nitrosanisole, followed by (e) 2-amino-4-hydroxylaminoanisole, and finally (f) 2,4-diaminoanisole (DAAN). These were the primary products described in reduction studies. Work by Niedźwiecka et al. (2017) observed this product distribution examining DNAN reduction primarily using Geobacter metallireducens with electron shuttles and poorly crystalline Fe(III) or Fe(III)-citrate. However, they also completed some abiotic studies with palladium pellets with 1.5 mM Fe(II). The intermediates (d) and (e) and azo dimers (g) and (h) shown in brackets are inferred for this study, but Olivares et al. (2013) was able to observe several dimers by mass spectrometer. Azo dimers form by an oxidation reaction that may commonly take place in
aerobic conditions (Platten et al., 2013), but they may also form under anaerobic conditions if an electron acceptor is present (Olivares et al., 2013). Dimers may be reduced back to DAAN. High pH conditions can form Meisenheimer complexes, in which hydroxyl group(s) attach to various C on the aromatic ring (Salter-Blanc et al., 2013) (Fig. 21B). Hydroxide attaching at the nitro groups can result in substitution reactions (Salter-Blanc et al., 2013).

DNAN degradation was observed by both biological (Platten et al., 2010; Olivares et al., 2013; Hawari et al., 2015) and abiotic (Rao et al., 2013; Salter-Blanc, 2013; Ahn et al., 2011; Hawari et al., 2015) processes. While most aerobic biodegradation studies of DNAN resulted in production of 2,4-dinitrophenol (DNP) and Meisenheimer complexes (Richard and Weidhaas, 2014; Fida et al., 2014), anaerobic biodegradation generally resulted in reduction products like 2-ANAN, DAAN and azo dimers (Platten et al., 2010; Olivares et al., 2013). Abiotic studies have shown DNAN degradation by photolysis (Hawari et al., 2015; Rao et al., 2013) and alkaline hydrolysis (Sviatenko et al., 2014; Bowden and Presannan, 1987; Salter-Blanc et al., 2013; Hill et al., 2012) resulting in DNP and Meisenheimer complexes. Experiments with zero valent iron and DNAN (Ahn et al., 2011; Hawari et al., 2015; Shen et al., 2013) resulted in DAAN and other reduction products. DNAN degradation has not been examined with reactive iron oxides that are common in soil and sediments. The present study provides a much-needed understanding of abiotic DNAN reactivity and degradation products with Fe(II) containing oxides in natural settings.

The companion study (Chapter IV) examined DNAN degradation mechanisms with Fe(II) with hydrous ferric oxide (HFO) and goethite that are presumably
nanoparticles. In that study, DNAN was reduced to primarily 2-ANAN with minor DAAN and other unidentified products believed to be a mixture of Meisenheimer complexes (especially at high pH) and azo dimers. Magnetite has reduced various chlorinated hydrocarbons both in the published literature (e.g., Danielsen and Hayes, 2004; Vikesland et al., 2007) and in a companion work (Chapter II). Natural aquifers can contain magnetite and Fe(II) that may potentially degrade contaminants (Liang et al., 2009).

The goal of this investigation was to examine the potential for magnetite to degrade DNAN in batch reactors simulating iron-reducing conditions in the subsurface. As shown in the companion study (Chapter IV), Fe(II) and mineral concentrations, and pH are demonstrated to be important experimental variables. The main objectives of the present research are as follows: (i) Characterize DNAN transformations, reaction byproducts, and its reduction kinetics with magnetite; (ii) Describe the effect of [Fe(II)] amendments and pH on degradation kinetics and byproduct distribution; and (iii) Compare magnetite results to the HFO results in chapter IV to describe the effects of structural versus adsorbed Fe(II). These objectives were achieved by examining DNAN degradation by simulating naturally occurring conditions.
(A) 2-NO-NAN

(B) 2-HA-NAN

(C) 2-ANAN

(D) 2-amino-4-nitrosoanisole

(E) 2-amino-4-hydroxylaminoanisole

(F) DAAN

(G) 3,3’-diamino-4,4’dimethoxy-azobenzene

(H) 3,3’-diamino-4,4’dimethoxy-hydrazobenzene
Fig. 21: (A) Expected pathways for DNAN transformations in the environment, (adapted from Ahn et al., 2011; Olivares et al., 2013). (B) Pathway of the formation of Meisenheimer complexes (adapted from Salter Blanc, 2013 and Hill et al., 2012).

2.0 Materials and Methods

2.1 Materials

Chemicals included sodium hydroxide pellets (ACS reagent grade, Fisher Scientific), sodium chloride (ACS reagent grade, Fisher Scientific), ferric chloride hexahydrate (ACS reagent grade, RICCA Chemical company), ferrous sulfate
heptahydrate (Biomedical grade, MP Biomedical), and ferric nitrate nonahydrate (98% ACS reagent, ACROS Organics), and TAPSO buffer (ACS reagent grade, ACROS Organics). High purity organic chemicals included DNAN (98%, Alpha Aesar), 2-ANAN (95%, Sigma Aldrich), and DAAN (90%, Pfaltz & Bauer). Solutions were prepared with 18.2 MΩ water from a Milli-Q water system (Millipore, MA).

Other materials include 72 mL borosilicate serum bottles (Cat# 223746, Wheaton), PTFE-lined butyl rubber stoppers (Cat# 73811T-21, Kimble-Chase), aluminum crimps, 1 mL disposable syringes (BD Syringe; U-100), 0.22 µm PVDF syringe filters (SIMSII), 2 mL Pyrex HPLC autosampler vials with caps and septa. Analytical equipment included anaerobic chamber (Coy labs, MI), pH meter (Denver Instrument; AP10), rotary shaker (Glas Col, IN), Vortex Genie 2 lab mixer (Fisher), and HPLC (Model 920, Varian) with a photo diode array detector.

2.2 Magnetite Synthesis

Magnetite was synthesized fresh for each experiment almost entirely in an anaerobic chamber to prevent its oxidation in air. The synthesis method was similar to Vikesland et al. (2007), but where they separated precipitated magnetite particles with a magnet, this study used a centrifuge. Initially, equal volumes of 0.2 M FeCl$_3$$\cdot$6H$_2$O and 0.1 M FeSO$_4$$\cdot$7H$_2$O solution mix was placed in a burette inside the anaerobic chamber. Vikesland et al. (2007) indicated that their particles were approximately 9 nm in diameter. Following a strongly similar procedure would also produce nanoparticles of a similar size. The [Fe(II)$_{\text{total}}$] in magnetite experiments were estimated by assuming that its Fe(II)/Fe(III) ratio was 0.5 (stoichiometric) based on the concentrations of FeSO$_4$ and FeCl$_3$ reagent mix used for its synthesis. A 1:1 mixture of 1 M NaOH and 1 M NaCl
solutions was placed in duplicate 72 mL serum bottles. The FeCl$_3$/FeSO$_4$ solution mix was added to the NaOH/NaCl solution dropwise while swirling the serum bottles (reactors). The ratio of total iron added to NaOH solution was 3:2. A black precipitate (magnetite) developed almost immediately. The reactors were sealed with butyl rubber stoppers and aluminum crimps and removed from the anaerobic chamber.

The reactors were centrifuged at 3000 rpm for 10 min to separate magnetite from supernatant. Supernatant was removed by syringe and replaced with deoxygenated DI water. The reactors were pressurized with ultrapure N$_2$ gas during the exchange to prevent air contamination. After each rinse cycle, the reactors were vigorously agitated on a vortex mixer to resuspend the magnetite particles into a slurry form. After ~4 rinse cycles (when the supernatant pH was between 10.5 and 11.5), the bottles were placed back in the anaerobic chamber to finish assembling the reactors, including Fe(II) amendments, TAPSO buffer, and DNAN.

2.3 Batch Reactor Setup

Batch reactors were assembled in duplicate using 72 mL borosilicate glass serum bottles that contained desired aliquots of mineral (HFO or goethite) slurry, TAPSO buffer, and Milli-Q water. The reactor setup in all experiments included a control reactor containing DI water only. After the washing process, the reactors were reopened inside the anaerobic chamber to add deoxygenated TAPSO buffer to the reactors (final concentration of 10 mM). A calculated volume of aqueous Fe(II) was added as 0.1 M FeSO$_4$ solution as indicated in specific experiments. The pH of the reactors was adjusted as needed with 1:1 mix of 1 M NaOH and 1 M NaCl. DNAN stock solution (100 mg/L) was then added to the reactors (initial DNAN conc. in the reactors: 25 mg/L), and its time
was recorded as \( t_0 \). Deoxygenated DI water was added to the reactors until no headspace remained. The reactors were then resealed with PTFE-lined butyl rubber stoppers and aluminum crimps.

2.4 Sampling and Analysis

Since DNAN and its degradation byproducts are not volatile, their partitioning into the reactor headspace was expected to be negligible. Sampling methods, equipment, and product identification were the same as those described in Chapter IV. Immediately after reactors were sealed, a 1 mL sample \( (t_1) \) was withdrawn from the reactors usually \(~2\) minutes after DNAN was injected \( (t_0) \). The samples were filtered through a 0.22 µm filter attached to a 1 mL syringe into an HPLC vial. Either three or four samples were taken on the first day. If the reaction was expected to be rapid, the reactor was shaken manually for a few seconds and a sample “\( t_2 \)” was taken prior to removing reactors from the anaerobic chamber. The reactors were then agitated vigorously on a vortex mixer for 40 seconds and sample \( t_3 \) was taken under argon stream. Reactors were placed on the rotator for about one hour at \(~45\) RPM before taking sample \( t_4 \). All samples were analyzed on the same day by an HPLC equipped with an auto-sampler and a C-18 Roc column (3 mm x 150 mm, 3 µm particle size; Restek) with a guard column assembly containing C-18 cartridge (Restek; Roc\(^\circ\)10 × 4 µm; Cat# 953450210). The flow rate used was 0.4 mL/min of a premixed 60:40 ratio of methanol to water. DNAN and DAAN were quantified at 220 nm and 2-NO-NAN, 2-HA-NAN, and 2-ANAN were best quantified at 254 nm.

Further sampling was done once per day as needed. Standards were generally analyzed every day of sampling.
Various calibration standards of known concentrations were prepared from commercially available high purity DNAN, 2-ANAN, and DAAN. 2-NO-NAN and 2-HA-NAN were identified by comparing chromatograms in this study to those in Hawari et al. (2015), who used a similar HPLC setup. They were quantified by using the calibration curves of compounds with retention times closest to the unknown intermediates (DNAN and 2-ANAN respectively) (Chapter IV). Standards were analyzed and fresh calibration curves were generated on each day of sampling.

2.5 Data Treatment

The concentrations (mM) and amount (in µmoles) of DNAN and its various degradation products in the batch reactors were calculated by using their respective calibration curves. The peak areas of various analytes were converted to mM using the calibration curve slope. The concentration in mM was converted to amount in µmol. The mole fractions ($m/m_0$) of the analytes were estimated by dividing the amount of DNAN remaining or reduction product yield at a given time, $t$ ($m$) by the initial amount of DNAN ($m_0$) at $t_0$, which was determined by averaging the values of 2 or 3 samples taken from the DI water control reactor prepared for each experiment.

DNAN degradation in most experiments typically occurred in two phases, with initial degradation phase within the first 3 minutes and the second phase taking place after that. To calculate kinetics in phase 1, $k_{obs1}$ was calculated using the mole fraction of DNAN at $t_0$ and $t_1$. In some experiments, $k_{obs1}$ was estimated by putting a small non-zero peak area for $t_1$ to estimate kinetics based on sampling time. Initial kinetics of the second phase, $k_{obs2}$, was calculated using $t_2$ and $t_3$. 
Other studies that examined abiotic degradation of DNAN with a solid reductant like zero valent iron (Hawari et al., 2015) and bimetallic iron with copper or nickel (Koutsospyros et al., 2012) exhibited apparent first order kinetics. Other studies with iron oxides showed reactivity toward other nitroaromatic as well as chlorinated hydrocarbon pollutants exhibiting first order or pseudo-first order degradation rate kinetics (e.g. Gregory et al., 2004; Gorski and Scherer, 2009; Danielsen and Hayes, 2004; McCormick and Adriaens, 2004; and Vikesland et al., 2007). In Vikesland et al. (2007), the concentrations of pollutants were held constant for all experiments, facilitating the use of a pseudo-first order rate constant. As a result, [DNAN] in this study was held constant, making adsorbed and structural Fe(II) the only variable reactant for determining reaction order. Therefore, \( k_{\text{obs1}} \) and \( k_{\text{obs2}} \) were treated as pseudo-first order rate constants.

DNAN removal and product distribution at any given time are presented in mole fractions \((m/m_0)\), as DNAN remaining, and product yields. The sum of these products gives the final C mass balance. Table 10 shows all conditions that were examined for this investigation.

A further analysis of the data using R statistical software was used to examine the full set of data with respect to varying \([\text{magnetite}], [\text{Fe(II)}], \text{initial pH}\), and the combined contributions of these variables that will be referred to as "input variables." The analysis was conducted as a linear model to examine those factors' influence on what is being called "output variables:" \( k_{\text{obs1}}, k_{\text{obs2}}, \) mole fraction of DNAN remaining, mole fractions of products, and C mass balance. P-values were used to confirm the importance of each of the reactor conditions to the dependent variables (See SI). Graphs showing the relationships between the input variables and the output variables were also produced.
(See SI). Correlation values between the *input variables* and the *output variables* gives a confirmation for the model (See SI). The companion study (in Chapter IV) examined DNAN degradation with Fe-treated ferric minerals (HFO and goethite) with similar initial conditions to those used with magnetite in this study. Additionally, experiments were done at high concentrations of adsorbed Fe(II) ([Fe(II)\textsubscript{adsorbed}]) that were equal to the amount of Fe(II)\textsubscript{total} present in the magnetite experiments. The purpose was to compare the effect of Fe(II)-treated ferric minerals to the mixed phase, magnetite, and to understand the role of structural Fe(II) versus adsorbed Fe(II) associated with minerals on DNAN degradation. For the comparison of these two data sets, the sum of [Fe(II)\textsubscript{adsorbed}] and [Fe(II)\textsubscript{structural}] was expressed as [Fe(II)\textsubscript{total}]. In order to analyze the effect of [Fe(II)\textsubscript{total}], $k_{\text{obs1}}$ was normalized with respect to [Fe(II)\textsubscript{total}] (by dividing $k_{\text{obs1}}$ by [Fe(II)\textsubscript{total}]) to estimate $k_{\text{Fe(II)}}$ in L/(mmol·d). The values of $k_{\text{obs1}}$ were also normalized with respect to [mineral] by the same approach, expressed as $k_{\text{mineral}}$ in L/(mmol·d).

Table 10: Experimental Conditions of DNAN Degradation by Magnetite:

<table>
<thead>
<tr>
<th>pH</th>
<th>[magnetite] (g/L)</th>
<th>[Fe(II)] (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>1.39, 2.78, 4.86</td>
<td>0, 0.28, 0.56</td>
</tr>
<tr>
<td>7</td>
<td>1.39, 2.78, 4.86</td>
<td>0, 0.28, 0.56</td>
</tr>
<tr>
<td>8</td>
<td>1.39</td>
<td>0, 0.28, 0.56</td>
</tr>
<tr>
<td>9</td>
<td>1.39</td>
<td>0, 0.28, 0.56</td>
</tr>
<tr>
<td>10</td>
<td>1.39, 2.78, 4.86</td>
<td>0, 0.28, 0.56</td>
</tr>
</tbody>
</table>

2.6 Nanoparticle Characterization
Samples of magnetite slurries were diluted 10x and placed on a micro slide to be examined on a Zeiss Axioskop light microscope with a 100x magnification lens and a 10x magnification eyepiece. However, 1000x magnification was insufficient to obtain accurate grain size and shape and therefore could not calculate surface area, but some qualitative comparisons of the particle aggregates could be made. Micrograph were taken using Axiovision software. Micrograph photos were taken using Axiovision software (See SI).

3.0 Results

3.1 Magnetite nanoparticle characterization

Magnetite nanoparticles were produced according to the methodology described by Vikesland et al. (2007). The average diameter of the particles produced by Vikesland was approximately 9 nm. They characterized the surface area of the particles with BET surface area, electron microscopy, and x-ray diffraction. The surface areas determined for the magnetite produced in Vikesland et al. (2007) was 63.5, 115, and 54.6 m²/g, respectively. The methodology used in this study produced magnetite that likely has similar physical properties. Light microscopy images (See SI) indicated that the particles agglomerated into clusters large enough to be visible by a microscope with 1000x zoom.

3.2 DNAN degradation by magnetite

DNAN degradation was observed with 1.39, 2.78, and 4.86 mM (0.32, 0.64, and 1.125 g/L) magnetite that was not pretreated with aqueous Fe(II) (Fig. 22A). 2-HA-NAN was produced quickly and degraded showing greater 2-HA-NAN removal at higher
[magnetite] (Fig. 22B). As 2-HA-NAN degraded, the yields of 2-ANAN and DAAN gradually increased (Figs. 22C and D). While DNAN removal and 2-HA-NAN appearance at various [magnetite] was initially rapid (~15 min), further 2-HA-NAN degradation was also initially rapid but became slower over the course of 24 hrs. The formation of 2-NO-NAN intermediate was not observed at various [magnetite] in this experiment. With 1.39, 2.78, and 4.86 mM magnetite, the $k_{ob1}$ values were 227.25, 809.45, and 1225.5 d$^{-1}$, respectively, while the corresponding $k_{ob2}$ values were 3.0, 14.9, and 399.3 d$^{-1}$, respectively.

The multiple linear regression analysis with respect to [magnetite] showed little correlation between [magnetite] and $k_{ob1}$, but there was a strong relationship to $k_{ob2}$ with a p-value of 0.00015 (See SI). DNAN mole fractions remaining for all experiments showed a strong negative trend as magnetite increased with a p-value of 0.0011. The final yield of DAAN was also strongly correlated to [magnetite] alone with a p-value of 0.0103 (See SI). The correlation function between variables showed correlation coefficients of 0.5 or greater between [magnetite] and $k_{ob1}$, $k_{ob2}$, DNAN, 2-HA-NAN, DAAN, and mass balance. Values were 0.72, 0.50, -0.64, -0.75, 0.88, and 0.72 respectively (See SI).
Fig. 22: DNAN degradation with 1.39, 2.78, and 4.86 mM (0.32, 0.64, and 1.125 g/L) magnetite and product distribution at pH 7. Magnetite was not pretreated with aqueous Fe(II), (A) Rapid DNAN degradation, (B) Formation and subsequent degradation of 2-HA-NAN, (C) Formation and accumulation of 2-ANAN, and (D) Formation and accumulation of DAAN.

3.3 DNAN degradation by Fe(II)-treated magnetite

DNAN degradation with 1.39, 2.78, and 4.86 mM (0.32, 0.64, and 1.125 g/L) magnetite pre-treated with 0.56 mM aqueous Fe(II) at pH 7 are shown in Fig. 23. As [Fe(II)] increased from 0 to 0.56 mM Fe(II) in pH 7 experiments, there was no significant
increase in initial DNAN degradation $k_{obs1}$ at 2.78 mM magnetite, but there was an increase seen at 1.39 mM magnetite (Fig. 23A). Fig. 23A shows that $k_{obs1}$ greatly increases as [magnetite] increases. The $k_{obs1}$ values at 4.86 mM magnetite and 0.28 and 0.56 mM Fe(II) were estimated above 4000 d$^{-1}$ but were too fast to graph or quantify reliably. Values of $k_{obs2}$ had a slight increase for 1.39 mM magnetite and a large increase for 2.78 mM magnetite as [Fe(II)] increased (Fig. 23B). At 4.86 mM magnetite with no Fe(II), $k_{obs2}$ was 399.3 d$^{-1}$ with a large error bar (removed from Fig. 23B), yet it was still much greater than the results from the lower magnetite concentrations. It was not possible to get $k_{obs2}$ for other [Fe(II)] at 4.86 mM magnetite.

As [Fe(II)] increased from 0 to 0.56 mM Fe(II) in 1.39 mM magnetite reactors (Fig. 23C), the final DNAN mole fraction remaining decreased from ~0.35 to 0.05. Further, in reactors with 2.78 and 4.86 mM magnetite, each with 0.56 mM Fe(II), virtually all DNAN was removed. In all reactors, 2-NO-NAN and 2-HA-NAN were present only in trace amounts (data not shown). 2-ANAN was the dominant product in 1.39 and 2.78 mM magnetite reactors, but at 1.39 mM magnetite, its yields were generally increased with increasing Fe(II), while at 2.78 mM magnetite, 2-ANAN showed a decreasing trend with increasing [Fe(II)] (Fig. 23D). At 4.86 mM magnetite, 2-ANAN yield declined sharply with increasing [Fe(II)] (Fig. 23D), and DAAN was the dominant product, with its mole fraction yield ($m/m_0$) ~1. DAAN yield reached ~1 quickly as [Fe(II)] increased as it was the dominant product in 4.86 mM magnetite reactors (Fig. 23E). Magnetite nanoparticles appeared to show significant agglomeration (Fig. S8).
In the multiple linear regression model DNAN remaining was the only parameter that appeared to be affected by [Fe(II)] alone with a p-value of 0.075. Most of the results from multiple linear regression showed dependency on Fe(II) only when interacting with another variable, such as [magnetite]. The interaction between [Fe(II)] and [magnetite] was important to $k_{obs2}$, showing a p-value of 0.024 and 2-ANAN, showing a p-value of 0.016. The interaction of [Fe(II)] and [magnetite] was also the most important variable in the full model for DNAN remaining.
Fig. 23: Combined effect of [Fe(II)] and [magnetite] on DNAN degradation kinetics and product distribution pH 7. (A) DNAN \( k_{obs1} \), (B) DNAN \( k_{obs2} \) (could not be calculated at 4.86 mM magnetite), (C) DNAN remaining, (D) 2-ANAN yield, and (E) DAAN yield in mole fraction with increasing [Fe(II)] and series defined by [magnetite].

3.4 Effect of pH on DNAN degradation with various [Fe(II)] with magnetite

The effect of different initial pH conditions on DNAN degradation with 1.39 mM magnetite and 0.56 mM Fe(II) showed that DNAN removal generally increased with increasing initial pH (Fig. 24A). The mole fractions of 2-HA-NAN and 2-ANAN intermediates produced varied with changes in initial pH (Fig. 24B and 4C). Overall DNAN transformation also increased with increasing pH, shown by the increase in DAAN yields (Fig. 24D). In all reactors, 2-NO-NAN were present only in trace amounts (data not shown).

The multiple linear regression analysis revealed that DNAN remaining and mass balance showed dependence on pH alone with p-values of 0.055 and 0.051 respectively. Although DNAN was dependent on multiple variables simultaneously, mass balance showed a low p-value with variation of pH alone.
y = e^{-200.3x}

y = e^{-554.1x}

y = e^{-703.2x}

y = e^{-936.1x}

y = e^{-934.4x}

y = 0.48e^{-3.92x}

y = 0.25e^{-12.09x}

y = 0.08e^{-14.58x}

y = 0.05e^{-11.97x}

y = 0.04e^{-15.43x}

(A)

DNAN (mol frac)

Time (d)

(B)

2-HA-NAN (mol frac)

Time (d)
Fig. 24: 1.39 mM magnetite and 0.56 mM Fe(II) experiments with various pH show (A) the degradation of DNAN, (B) 2-HA-NAN production and removal, (C) 2-ANAN production, and (D) DAAN production over time.

Fig. 24 and the previous part of this section examined the effect of pH at fixed [Fe(II)]. The effect of different pH levels ranging from 6 to 10 on DNAN degradation kinetics and product distribution was also investigated at increasing [Fe(II)] from 0 to 0.56 mM at 1.39 mM magnetite. As both [Fe(II)] and pH increased, generally, so did $k_{obs1}$, though pH 8 data was bit of an outlier. The pH increase appeared to amplify the effect of Fe(II) so that $k_{obs1}$ increases more at pH 10 with increasing [Fe(II)] than it does
at pH 7 with the same increase in [Fe(II)]. The \( k_{\text{obs}2} \) values showed no distinct trend with increasing [Fe(II)] and increasing pH (not shown).

Final DNAN remaining at 1.39 mM magnetite showed modest dependence on pH (Fig. 25B). However, DNAN mole fraction remaining at pH 6 was greater than at all other pH suggesting little degradation. DNAN remaining did not exhibit a strong pattern of change from pH 7 to 10, and the DNAN remaining was higher at pH 6 than at pH 7 to 10. The dominant products were split between 2-ANAN and DAAN (Figs. 25C and D, respectively). Both products’ yields increased as [Fe(II)] increased, but 2-ANAN yield generally decreased as pH increased. The results show the lowest DAAN yields at pH 6 and 7. The overall C mass balance shows a minor decrease as pH increased (Fig. 25E), suggesting possible shift in transformation pathway at higher pH.
3.5 Effect of pH on DNAN degradation with increasing [magnetite]

The effect of different pH ranging from 6 to 10 on DNAN degradation kinetics and product distribution was examined at [magnetite] from 1.39 to 4.86 mM (Tables 11 and 12). In general, $k_{obs1}$ increased as [magnetite] increased. Without Fe(II) amendment, pH 6 and 7 showed slower kinetics. However, DNAN’s $k_{obs1}$ increase with increasing...
[magnetite] was much smaller at pH 6 and 7 than at pH 10 without Fe(II) (Table 11); DNAN degradation kinetics with 4.86 mM magnetite at pH 10 were too fast to quantify (Table 11). At 0.56 mM Fe(II), \( k_{\text{obs1}} \) also increased with increasing [magnetite], but the rate of increase was somewhat more pronounced, especially at pH 7 (Table 12). The \( k_{\text{obs1}} \) values at pH 6 increased more with increasing [magnetite] than they did with increasing [Fe(II)] (Tables 11 and 12).

The experiments at 2.78 and 4.86 mM magnetite showed almost no DNAN remaining. 2-ANAN was the dominant byproduct in the pH 7 and 10 experiments with 2.78 mM magnetite (Table S1). 2-ANAN yields were lower for pH 10 than for pH 7 and both decreased with increasing [Fe(II)]. 2-ANAN yield did not change at pH 6 with 4.86 mM magnetite with (Table S1). For pH 7 and 10, 2-ANAN yields decreased until it was absent as [Fe(II)] increased (Tables 11 and 12). In 2.78 mM magnetite experiments, final DAAN yield increased with increasing [Fe(II)] and pH (Tables 11 and 12). In experiments with 4.86 mM, final mole fraction yield of DAAN was approximately 1 for experiments that had 0 (Table 11) and 0.56 (Table 12) mM Fe(II) at pH 7 and 10. Total DAAN yield at pH 6 for 4.86 mM Fe(II) remained constant near 0.7 or 0.8 mole fraction at different [Fe(II)].

The final mass balance in experiments without Fe(II) amendments show that mass balance approaches 1 mole fraction as [magnetite] increased (Table 11). Final mass balance at 1.39 mM magnetite without added Fe(II) generally decreased with increasing pH, but with 0.56 mM Fe(II), the data was more variable (Table 12). As [magnetite] increases, the mass balance of detectable products at all pH approached 1 in experiments with both without Fe(II) (Table 11) and with 0.56 mM Fe(II) amendments (Table 12).
With 0.56 mM Fe(II) (Table 12), pH had little difference in C mass balance at each [magnetite] studied. Mass balance at pH 6 was near 1 for all [Fe(II)] at 4.86 mM magnetite (Tables 11 and 12). Average mass balance for all experiments with 1.39 mM magnetite was 0.67 with a standard deviation of 0.17. Average mass balance for all experiments with 2.78 mM magnetite was 0.75 with a standard deviation of 0.11. Average mass balance for all experiments with 4.86 mM magnetite was 0.987 with a standard deviation of 0.03.

In the multiple linear regression study with R software, the interaction between pH and [magnetite] was confirmed in the multiple linear regression study as a strong influence on the behavior or DNAN degradation with magnetite. The full models showed that magnetite and pH together had a strong positive influence on \( k_{\text{obs1}} \) (p-value of 0.0082). Values of \( k_{\text{obs2}} \) also demonstrated a strong negative trend with the interaction between increasing [magnetite] and increasing pH (p-value of 0.0026).
Table 11: DNAN degradation with Magnetite with 0 mM Fe(II)

<table>
<thead>
<tr>
<th>mM magnetite</th>
<th>pH</th>
<th>$k_{obs1}$</th>
<th>$k_{obs2}$</th>
<th>DNAN</th>
<th>2-ANAN</th>
<th>DAAN</th>
<th>Mass Balance (mol frac)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.39</td>
<td>6</td>
<td>185 ±25</td>
<td>0.318 ±0.12</td>
<td>0.321 ±0.030</td>
<td>0.263 ±0.069</td>
<td>0.0113 ±0.0015</td>
<td>0.684 ±0.028</td>
</tr>
<tr>
<td>4.86</td>
<td>6</td>
<td>1640 ±350</td>
<td>352 ±34</td>
<td>0 ±0</td>
<td>0.209 ±0.015</td>
<td>0.834 ±0.0025</td>
<td>1.00 ±0</td>
</tr>
<tr>
<td>1.39</td>
<td>7</td>
<td>227 ±20</td>
<td>3.00 ±0.070</td>
<td>0.357 ±0.013</td>
<td>0.269 ±0.017</td>
<td>0.0135 ±0.0015</td>
<td>0.836 ±0.011</td>
</tr>
<tr>
<td>2.78</td>
<td>7</td>
<td>809 ±130</td>
<td>14.9 ±0.16</td>
<td>0.0392 ±0.014</td>
<td>0.646 ±0.0057</td>
<td>0.0700 ±0.018</td>
<td>0.823 ±0.045</td>
</tr>
<tr>
<td>4.86</td>
<td>7</td>
<td>1230 ±220</td>
<td>399 ±32</td>
<td>0 ±0</td>
<td>0.508 ±0.0017</td>
<td>0.466 ±0.0076</td>
<td>0.974 ±0.0059</td>
</tr>
<tr>
<td>1.39</td>
<td>8</td>
<td>370 ±22</td>
<td>0.0835 ±0.054</td>
<td>0.302 ±0.0028</td>
<td>0.161 ±0.012</td>
<td>0.161 ±0.012</td>
<td>0.810 ±0.033</td>
</tr>
<tr>
<td>1.39</td>
<td>9</td>
<td>322 ±62</td>
<td>2.35 ±0.12</td>
<td>0.294 ±0.018</td>
<td>0.0797 ±0.0054</td>
<td>0.0471 ±0.0099</td>
<td>0.493 ±0.065</td>
</tr>
<tr>
<td>1.39</td>
<td>10</td>
<td>539 ±190</td>
<td>6.42 ±0.45</td>
<td>0.259 ±0.010</td>
<td>0.0457 ±0.0070</td>
<td>0.0844 ±0.00012</td>
<td>0.408 ±0.0043</td>
</tr>
<tr>
<td>2.78</td>
<td>10</td>
<td>915 ±100</td>
<td>4.44 ±0.19</td>
<td>0 ±0</td>
<td>0.403 ±0.00015</td>
<td>0.285 ±0.011</td>
<td>0.693 ±0.010</td>
</tr>
<tr>
<td>4.86</td>
<td>10</td>
<td>TF</td>
<td>0 ±0</td>
<td>0 ±0</td>
<td>0.257 ±0.032</td>
<td>0.713 ±0.074</td>
<td>0.969 ±0.036</td>
</tr>
</tbody>
</table>

$TF$: reaction kinetics were too fast to quantify

$i$: No secondary kinetics were possible because the reaction was too fast

Notes: 0.28 mM Fe(II) data table in SI (Table SI 1). Graphic figures for these data are in SI.
Table 12: DNAN degradation with Magnetite with 0.56 mM Fe(II)

<table>
<thead>
<tr>
<th>mM magnetite</th>
<th>pH</th>
<th>$k_{obs1}$</th>
<th>$k_{obs2}$</th>
<th>DNAN</th>
<th>2-ANAN</th>
<th>DAAN</th>
<th>Mass Balance (mol frac)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.39</td>
<td>6</td>
<td>204 ±23</td>
<td>0.627 ±0.054</td>
<td>0.232 ±0.019</td>
<td>0.342 ±0.000083</td>
<td>0.0120 ±0.000040</td>
<td>0.669 ±0.026</td>
</tr>
<tr>
<td>4.86</td>
<td>6</td>
<td>2940 ±1200</td>
<td>101 ±101</td>
<td>0    ±0</td>
<td>0.252 ±0.016</td>
<td>0.758 ±0.0065</td>
<td>1.00 ±0</td>
</tr>
<tr>
<td>1.39</td>
<td>7</td>
<td>585 ±170</td>
<td>11.8 ±0.82</td>
<td>0.0351 ±0.017</td>
<td>0.380 ±0.17</td>
<td>0.0226 ±0.0037</td>
<td>0.616 ±0.086</td>
</tr>
<tr>
<td>2.78</td>
<td>7</td>
<td>671 ±91</td>
<td>72.7 ±11.6</td>
<td>0    ±0</td>
<td>0.461 ±0.021</td>
<td>0.214 ±0.0096</td>
<td>0.675 ±0.031</td>
</tr>
<tr>
<td>4.86</td>
<td>7</td>
<td>TF</td>
<td>0 ±0</td>
<td>0    ±0</td>
<td>0.0257 ±0.0068</td>
<td>0.944 ±0.056</td>
<td>0.954 ±0.046</td>
</tr>
<tr>
<td>1.39</td>
<td>8</td>
<td>714 ±33</td>
<td>1.14 ±0.063</td>
<td>0.0145 ±0.0021</td>
<td>0.430 ±0.0040</td>
<td>0.430 ±0.0040</td>
<td>1.00 ±0</td>
</tr>
<tr>
<td>1.39</td>
<td>9</td>
<td>937 ±20.</td>
<td>12.5 ±3.36</td>
<td>0.0157 ±0.0028</td>
<td>0.187 ±0.0014</td>
<td>0.136 ±0.016</td>
<td>0.497 ±0.019</td>
</tr>
<tr>
<td>1.39</td>
<td>10</td>
<td>1260 ±330</td>
<td>9.77 ±7.74</td>
<td>0.0206 ±0.0082</td>
<td>0.228 ±0.051</td>
<td>0.312 ±0.11</td>
<td>0.628 ±0.10</td>
</tr>
<tr>
<td>2.78</td>
<td>10</td>
<td>TF</td>
<td>0 ±0</td>
<td>0    ±0</td>
<td>0.249 ±0.019</td>
<td>0.649 ±0.042</td>
<td>0.899 ±0.023</td>
</tr>
<tr>
<td>4.86</td>
<td>10</td>
<td>TF</td>
<td>0 ±0</td>
<td>0    ±0</td>
<td>0    ±0</td>
<td>1.00 ±0</td>
<td>1.00 ±0</td>
</tr>
</tbody>
</table>

$TF$: reaction kinetics were too fast to quantify

$i$: No secondary kinetics were possible because the reaction was too fast

Notes: 0.28 mM Fe(II) data table in SI (Table SI 1). Graphic figures for these data are in SI.
3.6 Comparison of DNAN degradation with magnetite vs. Fe(II)-treated HFO and goethite

The most obvious difference observed when comparing magnetite and ferric minerals was that magnetite was capable of reducing DNAN without aqueous Fe(II) while Hydrous ferric oxide (HFO) and goethite needed aqueous Fe(II) to facilitate DNAN transformation.

Increasing the concentration of \([\text{Fe(II)}_{\text{total}}]\) was observed to increase the potency of reaction in both magnetite and HFO studies. At pH 7, initial \(k_{\text{obs1}}\) increased for both HFO and magnetite experiments as \([\text{Fe(II)}_{\text{total}}]\) increased. In HFO studies, all of the Fe(II)\(_{\text{total}}\) concentration was from aqueous Fe(II) amendment (Fig. 26A). In magnetite studies (Fig. 26A), \([\text{Fe(II)}_{\text{total}}]\) was mostly composed of Fe(II)\(_{\text{structural}}\). \([\text{aqueous Fe(II)}]\) varied between three concentrations, 0, 0.28, and 0.56 mM. Experiments for 4.86 mM magnetite with 0.28 and 0.56 mM Fe(II) were excluded from Fig. 26A and B because the reaction was too fast to obtain accurate \(k_{\text{obs1}}\). Magnetite experiments showed a strong positive trend for \(k_{\text{obs1}}\) with increasing total \([\text{Fe(II)}_{\text{total}}]\). This trend was vastly greater than the upward trend with increasing \([\text{Fe(II)}_{\text{total}}]\) in the HFO system. Values of \(k_{\text{obs2}}\) were distinctly smaller than their corresponding \(k_{\text{obs1}}\) values, but they show similar trends with increasing total \([\text{Fe(II)}]\) (Fig. S5). The \(k_{\text{Fe(II)}}\) values in HFO had a negative trend with increasing \([\text{Fe(II)}_{\text{total}}]\) (Fig. S5A). Values of \(k_{\text{mineral}}\) showed a trend line with a slope near 0 in both magnetite and HFO results (Fig. 26B).

Final DNAN remaining values show that 25 mg/L DNAN was able to be completely removed from an aqueous solution by ~3 mM Fe(II)\(_{\text{total}}\) for both HFO and
magnetite at pH 7, but goethite may remove all DNAN at a lower total concentration (Fig. 26C). The 2-ANAN data shows that at a certain [Fe(II)\text{total}], a maximum point is reached at which 2-ANAN that is produced will be reduced to the next product (Fig. 26D). With magnetite, there was a clear and strong increase toward a DAAN yield of 1 mole fraction as the amount of Fe(II)\text{total}, most especially Fe(II)\text{structural} was increased. HFO and goethite experiments, by contrast, showed a shallow, roughly linear increase in DAAN yield over the same [Fe(II)] range.
$$\text{DNAN remaining (mol frac)}$$

$$\text{[Fe(II)_{total}] mM}$$

(C)

$$\text{DNAN yield (mol frac)}$$

$$\text{[Fe(II)_{total}] mM}$$

(D)
Fig. 26: Combined effect of [Fe(II)\textsubscript{total}] (mM) and mineral species on DNAN degradation kinetics and product distribution. (A) Variations in DNAN $k_{\text{obs}}$ values with increasing [Fe(II)\textsubscript{total}] (mM) at pH 7. (B) Variations in DNAN $k_{\text{mineral}}$ ($k_{\text{obs1}}$ normalized with respect to [mineral]) with increasing [Fe(II)\textsubscript{total}] at pH 7. Goethite data was excluded from 26a and 26b because it showed no significant trend and overlapped HFO data at low Fe(II) amounts. In the magnetite series, 2 observations were excluded above 5 mM Fe(II) because the reaction was too fast to obtain reliable $k_{\text{mn}}$ data. (C) Final DNAN remaining, (D) 2-ANAN yields, and (E) DAAN yields in mole fraction with increasing total [Fe(II)] in mM. The data from goethite results were included in Fig. 26C, 26D, and 26E.

3.7 Structural vs. Adsorbed Fe(II)

In Fig. 26A, the increase in $k_{\text{obs1}}$ as [Fe(II)\textsubscript{total}] increased was much greater when most of the Fe(II) was in the structure of magnetite instead of adsorbed on the surface of HFO. When $k_{\text{obs1}}$ was normalized with respect to [Fe(II)\textsubscript{total}] ($k_{\text{mineral}}$), structural Fe(II) in magnetite continued to show a greater overall $k_{\text{mineral}}$ value than HFO with similar [Fe(II)\textsubscript{adsorbed}]. The comparison of the mixed iron phase mineral, magnetite to completely ferric minerals begins to compare Fe(II)\textsubscript{structural} to Fe(II)\textsubscript{adsorbed}, but the comparison must also be made where the mineralogy used is the same. Earlier in this study, $k_{\text{obs1}}$ was plotted for various [magnetite] with increasing [Fe(II)] (Fig. 23A). The graph showed a slight upward trend with increasing [Fe(II)] for any [magnetite] tested. However, when
[Fe(II)] and [magnetite] were switched, as in Fig. 27A, the lack of apparent trend with increasing adsorbed [Fe(II)] with $k_{\text{obs1}}$ was reinforced by the overlap of error and overall similarity of all [Fe(II)] at constant [magnetite]. However, a distinct increase was observed with increasing [magnetite]. The $k_{\text{obs1}}$ values at 0.28 and 0.56 mM Fe(II) were not measurable at 4.86 mM magnetite. Values of $k_{\text{obs2}}$, by contrast did not show any distinct patterns with either [Fe(II)] or [magnetite] (Fig. 27B).

Another measure of reaction potency to compare structural and adsorbed Fe(II) was DAAN yield In Fig. 27C, series for individual adsorbed [Fe(II)] were plotted against [Fe(II)$_{\text{structural}}$] in magnetite. Linear models did not produce a strong correlation, but their slopes demonstrate that increases in [Fe(II)] and [magnetite] affected the potency of the reaction in much the same way, but their contribution was more complex, having a more synergistic effect. At 1.39 mM magnetite, increasing [Fe(II)] had a weak influence on reaction potency with respect to DAAN production (Fig. 23E), whereas at 4.86 mM magnetite, the same range of [Fe(II)] showed a stronger increase in DAAN yield with increasing [Fe(II)].
Fig. 27: Comparison of \text{Fe(II)}_{\text{adsorbed}} and \text{Fe(II)}_{\text{structural}} on DNAN degradation kinetics and product distribution at pH 7. DNAN (A) $k_{\text{obs1}}$ and (B) $k_{\text{obs2}}$ values with increasing [magnetite] to show whether [magnetite] or [Fe(II)] have a greater influence on DNAN degradation. 4.86 mM magnetite with 0.28 and 0.56 mM data not included because kinetics were too fast to quantify for 27A and 27B. (C) DAAN yield in mole fraction with increasing [magnetite].

4.0 Discussion

4.1 Magnetite nanoparticle characterization
The changes to the magnetite synthesis procedure as described in Vikesland et al. (2007) were minor and not expected to significantly affect the morphology or size distribution of the magnetite. While grain shape was not especially clear from the TEM image from Vikesland et al. (2007), but the general morphology of the particles may resemble the morphology of magnetite crystals. Magnetite tends to form octahedral crystals (Chesterman, C., 2000).

4.2 DNA degradation by magnetite

Magnetite reduced DNAN without aqueous Fe(II) because of the high [Fe(II)\text{structural}] within magnetite Fig. 22A. The pathway of DNAN removal favored by magnetite was nitroreduction, following pathways observed in Fig. 21A. Degradation was rapid during a short-lived phase 1 (usually about \( \leq 15 \) minutes) followed by a slower second phase. The degradation of DNAN may have been limited only by available reaction sites. 2-HA-NAN was produced at near the same rate for all [magnetite], but as [magnetite] increased, more 2-HA-NAN was degraded to 2-ANAN. The \emph{ortho} position (site 2 on the DNAN structure in Fig. 21A) was favored because of an electronegativity advantage over the steric advantage that the nitro group at the \emph{para} position had (Site 4) (Hawari et al., 2015). 2-ANAN was being reduced to DAAN, but the degradation was slower than its production for the first day and leveled off while DAAN rose steadily.

In the multiple linear regression study, [magnetite] was a strong predictor value for \( k_{\text{obs2}} \), DNAN remaining, and DAAN yield. For these experiments, magnetite was the dominant source of Fe(II) for the reaction. Therefore, it was expected that [magnetite] would have a stronger effect on variables that were likely to respond in a more linear fashion, such as \( k_{\text{obs1}}, k_{\text{obs2}}, \) DNAN remaining, and DAAN yields. Intermediates like 2-
ANAN were not expected to show a high correlation in a linear model because of their non-linear behavior, as can be seen in Fig. 26D. As a result, the p-values were higher for the contribution of [magnetite] to a linear model predicting 2-ANAN yields as well as for other intermediates (See SI). For mass balance, however [magnetite] alone had a very high p-value, although a patter appeared in the plot of mass balance against [magnetite] in mM (See SI). It would have been a negative trend if adsorption was important, but since the trend appeared to be more positive, it was expected that any contribution from [magnetite] to the model would have been a result of the increase in reaction potency, producing more reduced products that were visible on the HPLC (i.e.: DAAN). Correlation values between variables validate the contribution of [magnetite] to DNAN and DAAN mole fractions and the effects on kinetics.

In general, the multiple linear regression analysis supported the relationships observed in the investigation. However, some relationships were not completely linear, which would cause p-values to be higher, particularly for the behavior of intermediate final yields. Furthermore, synergistic effects may also cause non-linear or exponential patterns of behavior depending on the behavior of Fe(II) and its various species.

4.3 DNAN degradation by Fe(II)-treated magnetite

In the results for Fig. 23A, $k_{obs1}$ had mixed results, showing limited dependence on increasing [Fe(II)]. Variability in these results was attributed to the rapid degradation in phase 1 and the relationship of kinetics to small changes in Fe(II) could be detected either by looking at $k_{obs1}$ (the 1.39 mM magnetite data) or $k_{obs2}$ (the 2.78 mM magnetite data) depending on sampling timing. In particular, 2.78 mM magnetite had faster kinetics overall, which may have created an artifact in the $k_{obs1}$ data. Experiments at 4.86 mM
magnetite with 0.28 and 0.56 mM Fe(II) were conducted, but all DNAN was removed before sample $t_1$ was taken (therefore, $k_{obs2}$ could not be calculated). Fig. 23B shows that $k_{obs2}$ increased with increasing [Fe(II)] for all [magnetite]. Higher concentrations of magnetite amplified the effect of the [Fe(II)] on $k_{obs2}$. Experiments with 4.86 mM magnetite with 0.28 and 0.56 mM Fe(II) were too fast to quantify either $k_{obs1}$ or $k_{obs2}$. Estimates of $k_{obs1}$ were made based on the sampling time that were >4000 d$^{-1}$, but $k_{obs2}$ could not be estimated in those instances because all DNAN is removed during phase 1.

Most magnetite experiments removed all DNAN within 3 or 4 days. Only experiments with 1.39 mM magnetite left a significant mole fraction of DNAN remaining, 1.39 mM magnetite without Fe(II) removed at least 65% DNAN and was the weakest reaction. At 1.39 mM magnetite, 2-ANAN yield increased because more DNAN was reduced as [Fe(II)] increased, but the 2-ANAN was not removed. At 2.78 mM magnetite, the reaction was potent enough to begin removing 2-ANAN and convert it to DAAN. At 4.86 mM magnetite, nearly all 2-ANAN was reduced to DAAN at pH 7. In the work by Niedźwiecka et al. (2017), palladium catalyst pellets with about 1.5 mM Fe(II) was used to degrade DNAN at pH 6 through 9 and only showed significant DAAN production at pH 8 and 9. Final mass balance showed no distinct pattern between the [magnetite] levels with increasing [Fe(II)], except that 4.86 mM magnetite removed had a higher C mass balance at all [Fe(II)], likely due to the favoring of more reduced products (See SI). If dimer products were formed, reduction pathways may lead back to the formation of DAAN under the stronger reducing conditions (Fig. 21A; Olivares et al., 2013).

In the multiple linear regression, [Fe(II)] was never the only variable that an output variable was dependent on. This was likely because the amount of aqueous Fe(II)
added to the reactors was small. Instead it was the interaction of [Fe(II)] with [magnetite] that was the strongest influence on $k_{obs2}$ and DNAN remaining, suggesting that neither [Fe(II)] nor [magnetite] were singularly the most important variables to the amount of DNAN remaining, but rather their interaction that was responsible for the patterns observed in the data.

4.4 Effect of pH on DNAN degradation with various [Fe(II)] with magnetite

At higher pH, the particles' surfaces build up negative charge and increases Fe(II) adsorption (Amonette et al., 2000), but without added Fe(II) to adsorb to the surface, DNAN reduction would involve the structural Fe(II) donating an electron more easily to explain the results showing more reduced products with increasing pH (Fig. 24). As Fe(II) is added to the reactor, more surface Fe(II) adsorption would take place (Amonette et al., 2000). Adsorbed Fe(II) could speciate to $=\text{FeOFe}$, $=\text{FeOFeOH}$, and $=\text{FeOFe(OH)}_2^-$ (Liger et al., 1999). These behaviors of Fe(II) explain the amplification of the effect of increasing [Fe(II)] on $k_{obs1}$ at higher pH (Fig. 25A). The dependence of DNAN remaining with increasing [Fe(II)] on pH was limited to whether the pH was at least 7 because pH 6 experiments left more DNAN at 1.39 mM magnetite when Fe(II) was present (Fig. 25B). At neutral to basic conditions, added Fe(II) adsorbed to the magnetite surface, making a more potent system. Basic conditions show lower yield for 2-ANAN because more 2-ANAN was reduced to DAAN and because reduction was competing with alkaline hydrolysis, which produces Meisenheimer complexes at pH 9 and 10 (Fig. 21B; Salter-Blanc et al., 2013). Meisenheimer structures are more likely responsible for lower C mass balance than unidentified intermediates because evidence suggests that basic conditions create more potent reactions. At pH 8, Meisenheimer
structures may not form, which may be the reason for pH 8 experiments' higher DAAN yield and mass balance.

In the multiple linear regression analysis, the p-values for DNAN remaining and mass balance with changing pH alone were around 0.05, indicating a roughly 5% chance that the decrease in DNAN remaining and mass balance with increasing pH was a result of random variation. The statistical analysis also confirmed the negative dependence of mass balance on increasing pH. For all experiments, increasing pH caused a general slight decrease in C mass balance.

Azo dimers were another possible reason for a C mass balance of <1. They were visible by mass spectrometer in Olivares and others' work (2013), but dimers were not observed in abiotic studies unless DAAN was exposed to oxygen (Hawari et al., 2013). Dimers could also undergo reduction and split to form DAAN, which would cause DAAN yields to fluctuate (Olivares et al., 2013). However, repressurizing reactors with 99.999% argon helped mitigate oxygen contamination and DAAN yield was not observed to waver. Therefore, it is expected that reduction and alkaline hydrolysis mechanisms compete at pH ≥8, with alkaline hydrolysis becoming more dominant as pH increases, accounting for nearly half of the byproducts at pH 9 and 10.

At 1.39 mM magnetite, pH 8 was an outlier. This may have been an artifact, but it was also possible that at pH 6 and 7, product distribution may have included more intermediates and azo dimers, whereas pH 9 and 10 may have produced Meisenheimer complexes with nitroreduction byproducts. At pH 8, the reactions may have been strong enough to remove less reduced intermediates and dimers that were undetectable on
HPLC, but the conditions may not have had enough hydroxide to produce dimers. More work is needed in this area to clarify these mechanisms.

4.5 Effect of pH on DNAN degradation with increasing [magnetite]

Values of $k_{\text{obs1}}$ increased with increasing [magnetite]. The rate of increase was amplified by increasing both [Fe(II)] and pH. Adding magnetite increases Fe(II)$_\text{structural}$. Additions of extra Fe(II) were small and had a modest effect on $k_{\text{obs1}}$ increase. Table 11 with no Fe(II) and Table 12 with 0.56 mM Fe(II) showed the sharp increase in reactivity for the pH 10 experiments as [magnetite] increased. The estimated $k_{\text{obs1}}$ values made according to the timing of sample $t_1$ should only be considered an estimate (shown as smaller data points in graphs in SI).

The conditions tested were capable of removing all DNAN in most cases. 2-ANAN yield was dependent on reaction potency but a more potent reaction will reduce 2-ANAN to DAAN. 2-ANAN yield increased from 1.39 mM magnetite to 2.78 mM magnetite, indicating that reactivity increased enough to produce more 2-ANAN, but was not potent enough to remove it. 2-ANAN yield increased at 1.39 mM magnetite as [Fe(II)] increased, but 2-ANAN yield decreased as [Fe(II)] increased for 2.78 mM magnetite because 2-ANAN was reduced to DAAN. Higher magnetite concentrations favored DAAN production (Table 11), the modest increase in DAAN yields with rising [Fe(II)] indicated that [magnetite] was the main driver for the increase in reaction potency.

At pH 6, added Fe(II) may remain aqueous and not hydrolyze to reactive forms, accounting for the minimal change in $k_{\text{obs1}}$ and DAAN yield with increased [Fe(II)]
(Liger et al., 1999; Salter-Blanc et al., 2013; Strathmann and Stone, 2002). Therefore, only \([\text{Fe(II)}_{\text{structural}}]\) at pH 6 will govern DNAN reduction.

Final DAAN yields increased with increasing \([\text{Fe(II)}], [\text{magnetite}], \text{or pH}\), reflecting an increase in potency. Final DAAN mole fraction yields were at or near 1, indicating that the reaction reached completion, at high \([\text{Fe(II)}]\) for pH 7 and 10 experiments with 4.86 mM magnetite. With 4.86 mM magnetite, DAAN yields were around 0.8 mole fraction at pH 6, indicating that \(\text{Fe(II)}_{\text{structural}}\) alone could reduce most DNAN. The final DAAN yield showed a very small increasing trend with increasing \([\text{Fe(II)}]\) at pH 7 and 10 (Fig. S3B). At pH 6, the strength of the reaction was exclusively driven by \(\text{Fe(II)}_{\text{structural}}\) in \([\text{magnetite}]\).

Mass balance values increased toward 1 mole fraction as \([\text{magnetite}]\) increased for all pH levels tested and all \([\text{Fe(II)}]\) (Tables 11 and 12). When mass balance was 1, reduction at DNAN's nitro substituents account for around 100% of the observed products. The end result is accounted for by reduction at the nitro groups, though other pathways may be at work. At mass balance <1, it means either (i) that undetected intermediates may be forming, or (ii) that DNAN follows another pathway. At pH 6 with 1.39 mM magnetite, mass balance was ~0.7. The remaining balance at pH 6 would form undetected intermediates and possibly azo dimers in a system not potent enough to produce DAAN since [hydroxide ions] is low. Experiments at 4.86 mM magnetite and pH 6, show ~100% mass balance, indicating complete reduction of DNAN.

At pH 9 and 10, however, lower mass balance at 1.39 mM magnetite indicates that, as in section 4.3, alkaline hydrolysis competes with nitroreduction, forming Meisenheimer complexes (Fig. 25E). PH levels closer to neutral produced less hydroxide.
and therefore less competition between these mechanisms, resulting in a higher mass balance. As \([\text{magnetite}]\) increases, however, it became apparent at both 0 and 0.56 mM \(\text{Fe(II)}\) (Table 11 and 12, respectively), mass balance reaches 1 mole fraction, mostly consisting of DAAN, indicating that high pH and \([\text{magnetite}]\) conditions favor nitroreduction over alkaline hydrolysis.

The influence of the interaction of \([\text{magnetite}]\) and pH was likely higher for kinetics values because \([\text{magnetite}]\) was the primary source of reactant \(\text{Fe(II)}\). The influence of adsorbed \(\text{Fe(II)}\) was more a result of its speciation behavior than its concentration, which explains why pH was also a dominant influence on the model of DNAN degradation.

4.6 Comparison of DNAN degradation with magnetite vs. \(\text{Fe(II)}\)-treated HFO and goethite

Structural \(\text{Fe(II)}\) in magnetite was an effective reductant for DNAN without added \(\text{Fe(II)}\). HFO Experiments were conducted with \([\text{Fe(II)}]\) equal to the molar \([\text{Fe(II)}]_{\text{structural}}\) in stoichiometric magnetite. Adding aqueous \(\text{Fe(II)}\) to non-stoichiometric magnetite was shown by Gorski and others (2012) to cause atom exchange, resulting in an increase in the \(\text{Fe(II)}/\text{Fe(III)}\) ratio approaching 0.5 without significantly changing the structure, size, or morphology of the particles. Magnetite and HFO results in Fig. 26B show that the trend with increasing \([\text{Fe(II)}]_{\text{total}}\) was largely dependent on that \(\text{Fe(II)}]_{\text{total}}\). Results from HFO studies in Chapter IV (Fig. 26B) suggested that both were dependent on mineral concentration. This may be more indicative of a dependency on surface area. This may be less important for HFO than for magnetite, because HFO did not contain structural \(\text{Fe(II)}\).
From Fig. 26C, it appears that for the initial degradation step of DNAN at pH 7, magnetite and HFO had similar potency. A minor trend within the magnetite data showed that in the first three observations, adsorbed [Fe(II)] was 0, 0.28, and 0.56 mM respectively with the same [magnetite]. The second group of three observations (near 3 mM Fe(II)\text{total}) follows the same pattern, suggesting that both Fe(II)\text{adsorbed} and Fe(II)\text{structural} strongly influence the potency of the reaction with respect to product distribution. Goethite data shows that final DNAN remaining was dependent on [Fe(II)\text{total}].

The comparison of the three minerals with 2-ANAN and DAAN yields shows stronger differences. The [Fe(II)\text{total}] at which 2-ANAN yield peaks and declines takes place at a lower [Fe(II)\text{total}] for magnetite than HFO (~3 vs ~4 mM respectively). 2-ANAN yields were consistently lower for magnetite than HFO, suggesting that structural Fe(II) in magnetite was able to reduce more 2-ANAN than HFO systems with similar [Fe(II)\text{adsorbed}]. Fe(II)\text{structural} in magnetite produced almost entirely DAAN above 5 mM Fe(II), especially when that Fe(II)\text{structural} was accompanied by small concentrations of Fe(II)\text{adsorbed}. Fe(II) would speciate on the surface or undergo atom or electron exchanges with the mineral.

4.7 Structural vs. Adsorbed Fe(II)

What takes place when aqueous Fe(II) contacts the ferric minerals is poorly understood. Schaefer and others (2011) indicated that electron exchange between the Fe(II)\text{adsorbed} and ferric minerals reducing structural Fe(III) while the adsorbed iron oxidizes. This suggests that Fe(II)\text{adsorbed} in this investigation and in the previous chapter of this dissertation, after the darker greenish color change when aqueous Fe(II) was added
to ferric minerals, may be Fe(II)$_{\text{structural}}$ after electron exchange. If so, the effect of truly adsorbed Fe(II) may be visible when Fe(II) was added to stoichiometric magnetite.

Increasing adsorbed [Fe(II)] at each [magnetite] showed modest increases at 1.39 mM Fe(II) (Fig. 23A), and a strong positive trend with increasing [Fe(II)$_{\text{structural}}$] (Fig. 27A). This may be partly explained because [Fe(II)$_{\text{structural}}$] was studied at a broader range than [Fe(II)$_{\text{adsorbed}}$]. Whether [Fe(II)$_{\text{adsorbed}}$] or [Fe(II)$_{\text{structural}}$] had the greater influence on $k_{\text{obs2}}$ was not determined. The experiment with 4.86 mM Fe(II) in magnetite with no Fe(II)$_{\text{adsorbed}}$ was dismissed from the analysis of $k_{\text{obs2}}$ as an outlier (Fig. 27B).

Increasing [Fe(II)$_{\text{structural}}$] showed a drastic increase in DAAN mole fraction yields regardless of [Fe(II)$_{\text{adsorbed}}$]. The potency of increasing [Fe(II)$_{\text{adsorbed}}$] could have an equal potency as [Fe(II)$_{\text{structural}}$], but its potency with respect to DAAN yields (Fig. 27C and 23E) showed greater influence by [Fe(II)$_{\text{structural}}$]. In this synergistic effect, the effect of increasing [Fe(II)$_{\text{adsorbed}}$] was enhanced by the presence of higher [Fe(II)$_{\text{structural}}$].

Gorski and Scherer (2009) studied Fe(II) uptake by magnetite for nitrobenzene reduction. They cited Klausen et al., (1995) and Gregory et al., (2004), indicating that magnetite was less reactive toward nitroaromatic pollutants. Gorski and Scherer suggested this was because the magnetite in those studies was oxidized. They suggested that, as observed in their 2009 study, the aqueous Fe(II) donated electrons to the magnetite, partially restoring its stoichiometry to its pure Fe(II)/Fe(III) of 0.5. In this study, however, particles were precipitated in situ, under anaerobic conditions. Modest oxidation may occur during washing, but steps were taken to minimize oxygen exposure and the mole ratio of Fe(II)/Fe(III) in the beginning mix was expected to be 0.5.
If magnetite Fe(II)/Fe(III) in this investigation was \( \sim 0.5 \), electron uptake by magnetite particles should have been limited (Gorski and Scherer, 2009). Gorski's experiments were conducted at pH 7.2 and they found that adsorbed Fe(II) species were not present in the study and they did not discuss Fe(II) species behavior in experiments with pure magnetite. In Strathmann and Stone (2002), Fe(II) speciates to FeOH\(^+\) and solid and dissolved Fe(OH)\(_2\). In Liger and others (1999), these species were shown to form in an adsorbed form beginning with increases in amounts of an adsorbed form of Fe\(^{2+}\) as pH increased from 6 to 7. Phase diagrams from Liger and others (1999) and Strathmann and Stone (2002) suggest that \( =\text{FeOFeOH}^0 \) forms at a higher pH than aqueous FeOH\(^+\). Adsorbed \( =\text{FeOFeOH}^0 \) becomes a more dominant phase of Fe(II) as pH increases past 7 and declines as \( =\text{FeOFe(OH)}_2^- \) forms. These behaviors have been described for ferric minerals like hematite (Liger et al., 1999), but not for magnetite.

Gorski and Scherer (2009) looked for, but did not observe, adsorbed Fe(II) species under the conditions of their experiment (pH 7.2), but it is likely Fe(II) speciation was responsible for some of the increases in reactivity seen in this study, especially at high pH. The ferrous species would adsorb and react directly with the pollutants, donating electrons to the pollutant directly instead of to the mineral particle as would happen for oxidized magnetite and ferric minerals.

Magnetite held a distinct and consistent advantage over the HFO experiments, for which the Fe(II) was either adsorbed or structural by electron exchange. The synergistic effect of magnetite’s Fe(II)\(_{\text{structural}}\) and the added Fe(II)\(_{\text{adsorbed}}\) made determining which form of Fe(II) was more potent difficult. However, comparing the effects of \([\text{Fe(II)}_{\text{structural}}]\) in magnetite to equivalent \([\text{Fe(II)}_{\text{adsorbed}}]\) on pollutant degradation
demonstrated that Fe(II)$_{\text{structural}}$ is more potent than Fe(II)$_{\text{adsorbed}}$. Overall pH trends with Kinetics showed that experiments at pH 6 showed less change in reaction potency as [Fe(II)$_{\text{total}}$] increased than those at pH 7 through 10 (Fig. S6).

5.0 Conclusions

[Fe(II)$_{\text{adsorbed}}$] was important for DNAN degradation with magnetite, but stoichiometric magnetite could reduce DNAN in the environment without the assistance of added Fe(II), unlike the ferric minerals from Chapter IV. Fe(II)$_{\text{structural}}$ in magnetite appeared to be a stronger reductant than Fe(II)$_{\text{adsorbed}}$ and was critical to remove DNAN at pH 6. Aqueous Fe(II) probably did not hydrolyze to form reactive species and did not adsorb at pH <7. Nearly stoichiometric magnetite may take up modest amounts of electrons from aqueous Fe(II) species. The rest will likely adsorb to particles' surfaces. Where both Fe(II)$_{\text{structural}}$ and Fe(II)$_{\text{adsorbed}}$ are present, they have a synergistic effect on DNAN reduction. Higher pH conditions may also favor the formation of Meisenheimer complexes, but higher [Fe(II)] and [magnetite] can make nitroreduction outcompete the formation of the complexes. Based on this study as well as the previous HFO and goethite study, it is apparent that naturally occurring Fe-oxides and Fe(II) species can reduce DNAN to nitroaniline byproducts without human interference, and more reduced products will be found where aqueous Fe(II) and Fe(II)-containing iron oxides are mixed. It is reasonable to expect that green rusts will have a similarly strong reducing effect as magnetite, but this has not been explored.
References


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Chapter II Supplemental Information:

Expanded Fe(II) speciation behavior and information

Naka et al. (2006) indicated that while Fe\(^{2+}\)\(_{(aq)}\) and FeOH\(^+\)\(_{(aq)}\) may be dominant iron species at near neutral conditions, but Fe(OH)\(_2\)\(_{(s)}\) and Fe(OH)\(_3\)\(^-\) can form at more alkaline conditions, suggesting four different Fe(II) species that might be reactive at environmentally significant pH conditions. Strathmann and Stone (2002) considered the role of FeOH\(^+\) a complicating factor because its contribution to the overall rate constants of carbamate reduction could not be separated from Fe(II) based on iron speciation, but rather had to be calculated with respect to total Fe(II). Schultz et al. (2000) suggested that FeOH\(^+\) could be a strong reductant of nitrobenzenes in their system, especially at pH 7.4-7.8; the study showed that the kinetics of 4-chloronitrobenzene degradation increased with pH due to increasing FeOH\(^+\) and greater Fe(II) adsorption to the clay mineral (montmorillonite) (Schultz et al., 2000). FeOH\(^+\) can form by hydrolysis of Fe\(^{2+}\) as in equation 1 below (Liu et al., 2013).

\[
\text{Fe}^{2+} + \text{H}_2\text{O} \rightarrow \text{FeOH}^+ + \text{H}^+ \tag{1}
\]

Of greater consequence to this investigation is the formation and redox potential of the solid Fe(II) species that form. As pH increases from pH 7, an aqueous phase of Fe(OH)\(_2\)\(^0\) begins to form according to equation 2 (Strathmann and Stone, 2002). When the concentration of the Fe(OH)\(_2\)\(^0\) species reaches a critical point and when the pH
reaches a certain range, it begins to precipitate out according to equation 3 (Strathmann and Stone, 2002; Liu et al., 2013). The solid species, ferrous hydroxide (Fe(OH)$_2$) typically precipitates above pH 8.3 (Strathmann and Stone, 2002). Strathmann and Stone (2002) reported that reduction kinetics and elimination kinetics of carbamate increased the most at pH levels where FeOH$^+$ concentrations were already high and when Fe(OH)$_2^0$ began to form. Elimination continued to increase after Fe(OH)$_2$(s) began to precipitate out. Strathmann and Stone (2002) speculated that the aqueous species Fe(OH)$_2^0$ may control carbamate reduction kinetics at Fe(II) total <2.5 mM, but above 2.5 mM, the reduction kinetics may be more strongly influenced by Fe(II) adsorbed to Fe(OH)$_2$(s).

$$\text{Fe}^{2+} + 2\text{H}_2\text{O} \rightarrow \text{Fe(OH)}_2^0 + 2\text{H}^+$$ (2)

$$\text{Fe}^{2+} + 2\text{H}_2\text{O} \rightarrow \text{Fe(OH)}_{2(\text{s})} + 2\text{H}^+$$ (3)

Additionally, the solid species Fe(OH)$_3^-$ may form by a similar hydrolysis reaction, equation 4 (Liu et al., 2013).

$$\text{Fe}^{2+} + 3\text{H}_2\text{O} \rightarrow \text{Fe(OH)}_3^- + 3\text{H}^+$$

Materials and Methods Expanded

Standards and reactors were inoculated from stock solutions created in 160 mL serum bottles with no headspace in which small amounts of pure CHC compounds were injected with a small gastight syringe (usually a 50 µL syringe). Concentration of standards was calculated by equations 1 and 2.

$$\text{Volume injected (µL)} \times \text{density of contaminant} \left(\frac{\text{mg}}{\mu\text{L}}\right) = \text{Mass of CHC (mg)} \quad (1)$$

$$\text{Mass of CHC (mg)} \div \text{Volume of bottle (L)} = \text{CHC concentration} \left(\frac{\text{mg}}{\text{L}}\right) \quad (2)$$
The stock bottle was vortexed and allowed to equilibrate for 48 hours on a rotary shaker. 3 standards were made to help quantify contaminants and their degradation byproducts in unknown reactors. 160 mL serum bottles were filled to 100 mL with Milli-Q water and different volumes of stock solutions were injected into the standard bottles and their corresponding reactors. Contaminants in standards were quantified by calculating the amount of contaminant added to each reactor. The amounts of contaminants added to the reactor was calculated by the following calculations:

\[
\frac{\text{Concentration of stock (mg/L)} \times \text{stock volume stock injected (\mu L)}}{\text{volume of liquid in reactor or standard (mL)}} = \text{Concentration before partitioning (\mu g/L)} \tag{3}
\]

\[
\frac{\text{Concentration before partitioning (\mu g/L)}}{\text{formula weight (\mu g/\mu mol)}} \times \text{liquid volume (L)} = \text{Amount before partitioning (\mu mol)} \tag{4}
\]

The partitioning coefficient was calculated based on dimensionless Henry’s constants:

\[
\frac{1}{1 + \text{dimensionless Henry’s constant (d)} \times \frac{\text{Volume of headspace (mL)}}{\text{Volume of liquid (mL)}}} = \text{fraction in water (d)} \tag{5}
\]

\[
\frac{\text{Mass of CHC in reactor (\mu g)}}{\text{volume of liquid (mL)}} \times \text{fraction in water (d)} = \text{concentration in liquid (mg/L)} \tag{6}
\]

Sampling the three standards produced peak areas, which could be plotted against those known concentrations to produce a calibration curve. Peak area was plotted on the x-axis and the amount (\mu moles) in the bottle on the y-axis. A linear regression was used to find the slope of the line, which was utilized to quantify measured GC peak areas in each
experiment in terms of µg and ultimately converted back to total µmoles and finally mole fractions by the following equations:

\[
\text{Slope of calibration curve line} \times \text{peak area of samples} = \text{mass of CHC in liquid (µg)}
\]

\[
\frac{\text{Concentration of CHC in liquid (mg/L) \times Vol of liquid (L)}}{\text{fraction in water (d)}} = \text{Total mass of CHC (mg)}
\]

\[
\frac{\text{Total mass of CHC (mg)}}{\text{Molar mass (mg/mmol) \times 1000 µmol/mol}} = \text{Total amount of CHC at time t (µmol)}
\]

\[
\frac{\text{Total amount of CHC at time t (µmol)}}{\text{Total amount of CHC at time t0 (µmol)}} = \text{total mole fraction (d)}
\]

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

Required Materials:

Glass vials, 5.1 mM, 1000 mg L\(^{-1}\) iron stock solution (Lab Chem Inc.), 1,10-
Phenanthroline, 10% hydroxylamine solution, 1.2M
ammonium acetate buffer, Iso-Disc Filter, N-25-4 Nylon 25 mm x 0.45 µm filters, 1-1000 µL Eppendorf pipette (1-5 or 1-10 mL pipette optional), Fisher Vortex Genie 2
The first preliminary experiment with magnetite was set up so that the mixture of 5 mL of 100 mM aqueous FeSO₄•7H₂O and 5 mL of 200 mM FeCl₃•6H₂O was added dropwise to 6.67 mL of 1 M NaOH and NaCl solution using a burette according to the procedure outlined in Vikesland and others (2007) (Fig. S1). A black precipitate was produced instantly. The supernatant was removed, and the magnetite was washed with
deoxygenated DI water, placed in 15 mL serum bottles and centrifuged several times until the pH of the extracted supernatant was about 10.5. Then the magnetite was added to the reactor along with 10 mM TAPSO buffer up to 100 mL total volume and sealed using a PTFE-lined butyl rubber stopper and aluminum crimp. CT was added weekly for 5 identical cycles with the accumulation of reaction products. After injection, the reactor was vortexed on a Vortex-T Genie II (Scientific Industries) and placed on an end-over-end rotator between taking samples. Headspace analysis of CT and its degradation products was completed using a Hewlett Packard 6890 GC with an electron capture detector (ECD).
**Fig. S2:** Diagram of reactor setup for magnetite experiments. Before transferring to the serum bottle, any FeSO$_4$ solution that was needed was added and then pH was adjusted as needed and the final volume was brought to 100 mL.

Other preliminary results with magnetite were collected using a technique similar to that of a titration (Fig. S2). A mix of 1M NaOH with 1 M NaCl mixture is diluted by deoxygenated DI water to around 75 to 80 mL (in a 125 mL Erlenmeyer flask) inside the anaerobic chamber. The pH of the solution is adjusted to be somewhat above the target pH value (usually within 0.5 pH. Deoxygenated Fe mix (0.1 M FeSO$_4$•7H$_2$O + 0.2 M FeCl$_3$•6H$_2$O) solution added dropwise to the flask using a burette. As the iron solution was being added, pH was monitored. A black precipitate forms in the solution quickly, however, the particles seem to take longer to settle out using this method than the previous washing method. If, during the preparation, the pH dropped below the target pH, more NaOH mix was added to increase the pH back up to the target. The solution was finely adjusted to the desired pH and nearly 100 mL with NaOH and HCl solutions, transferred to a 100 mL graduated cylinder for final pH reading and final adjustments to the volume and pH. From there it was transferred to a 160 mL serum bottle. The control always contained only water with NaOH mix solution adjusted to the target pH.
The attempts to synthesize iron oxides by hand in situ at a particular pH and the lack of buffer placed limits on the types of experiments that were possible and increased the size of error bars. The goals of this research were to simulate conditions that might be present either in a natural system with higher pH, or an engineered field site. Reactors were made in duplicate because of limitations in sampling. Error bars represent the maximum and minimum range of the data at those conditions. Some early experiments
with magnetite alone used only one reactor. These magnetite experiments were designed to give a baseline comparison for the Fe(II) species and the mixed magnetite and Fe(II) experiments.
Other Results:

*Preliminary Experiments: Magnetite and CT at pH 7.*

In the initial experiments conducted with 1.16 g/L magnetite alone in 10 mM TAPSO buffer at an initial pH of 7 (procedure from Vikesland et al., 2007), CT degraded quickly and produced CF as the sole reaction product for three repeat injections in successive cycles, each lasting ~4 days (Fig. S4). The pseudo-first order CT degradation rate constants (CT \( k_{obs} \)) were 2.18, 1.84, and 1.78 d\(^{-1}\) for cycles 1, 2, and 3, respectively. The maximum CF formed in three cycles, expressed as mole fraction \( (m/m_0) \), were 0.79, 0.71 and 0.77, respectively. In all subsequent degradation experiments, the magnetite was synthesized as close to the target pH as possible without the use of buffer (see *Materials and methods*).

The quantification of this accumulation of products was difficult to maintain reliably when CF concentrations were high. Rate kinetics for each cycle was similar for each cycle. The relatively high degradation kinetics suggest here that reaction sites were relatively abundant. Although the CF yield from one cycle to the next was cumulative, subtracting the previous final yield from the raw value gave a supposed yield for each individual cycle. Cycles 1 to 3 yield fairly consistent results with yields around 80% for CF. After cycle 3, yields are somewhat ambiguous. Minor products observed in these experiments include DCM and theoretically chloromethane and methane (Not shown). Other products may include CO, HCHO, and similar compounds (these were not analyzed). These minor products are expected to make up about 20% of the product yield.
**Fig. S4:** This diagram shows the mass balance of CT and its degradation product, CF for cycles 1 through 4 in the preliminary CT experiment with 1.16 g/L magnetite synthesized according to the procedure outlined in Vikesland and others (2007). In this diagram, CF yields being corrected for the accumulation of CF from previous cycles. Magnetite appears to consistently produce about 80% CF from CT degradation. In cycles 4 and 5...
(Cycle 5 not shown), the calibration curve did not extend to that high of concentration of CF and so had difficulty quantifying the accumulation.

Results from the preliminary magnetite experiment buffered with 10 mM TAPSO at pH 7 with 1.16 g/L magnetite, no aqueous Fe(II), and 69 μg/L aqueous CT at t0 showed strong kinetics over an extended period of time of nearly 40 days. The dominant product was CF and accumulated as approximately 80% of the total products of each cycle in the reactors as more CT was added and degraded. In Figure S4, the CF data is corrected to eliminate the previous cycle's CF so that the figure shows only the CF produced from the degradation of the new pulse of CT associated with that cycle. CT degradation showed similar kinetics over time with little loss of reactivity as new additions of CT were added weekly (Table 11) with $k_{obs}$ values ranging from 1.4 to 2.2 d$^{-1}$.

Preliminary experiments with chemogenic magnetite nanoparticles in buffered batch reactors were highly reactive with the model compound, carbon tetrachloride, and those results are a good comparison for the results seen in unbuffered reactor systems with magnetite and ferrous hydroxide.

Unbuffered experiments with magnetite alone with CT were conducted at several pH levels. Fig. S5 shows the effects of increasing magnetite concentration (0.29 g/L, 0.58 g/L, and 1.16 g/L) on mole fractions of CT and CF as well as the carbon mass balance at pH 8. Fig. S6 shows a similar set of reactors with the same magnetite concentrations over two cycles at pH 10. The same concentrations of magnetite were used in experiments where the pH was adjusted to pH 12 (Fig. S7). Transformation of CT to CF occurred very rapidly in cycle 1 but was considerably slower when another addition of CT was injected for cycle 2 two weeks later. CF yields reached about 80%
and decreased slowly over the course of the experiment. Initial CT degradation rate constants ($k_{obs}$) ranged from 5.56 days$^{-1}$ in R3 ($k_m = 19.21$ L/g•d) to 47.12 days$^{-1}$ in R1 ($k_m = 40.7$ L/g•d) (Table 12).

Fig. S5: Comparison of 2 procedures of magnetite synthesis: the Vikesland et al. (2007) magnetite was washed while the titration method magnetite was not.
washed and was precipitated at around pH 10.

**Fig. S6:** This diagram shows the mole fraction of CT, CF, and the total mass balance with various concentrations of magnetite up to 1.16 g/L. These experiments were conducted at pH 10. These experiments were conducted at pH 12 and contained several cycles, although two of them are shown.
**Fig. S7:** This diagram shows the mole fraction of CT, CF, and the total mass balance with various concentrations of magnetite up to 1.16 g/L. These experiments were conducted at pH 12 and contained several cycles, although two of them are shown.

Other magnetite precipitate pictures are included below. The first group was synthesized by the method used in Vikesland and others (2007) and were closest to the magnetite synthesized for the experiments with TAPSO and whose results are shown in Fig. S4, which created mostly uniformly sized and shaped particles (Fig. S8). Other micrographs from the titration method used to make magnetite in this investigation are pictured in Fig. S9. Other pictures of ferrous hydroxide (Fe(OH)$_2$) is included in Fig.S10.
Fig. S8: Visual changes in Fe(II) species over time at a starting pH of 9 with 1, 5, and 15 mM Fe(II) from left to right, beginning with (A) day 1 and (B) day 2. In C, the far left is a DI control for comparison.
Fig. S9: Extra pictures of magnetite synthesized by the procedure outlined in Vikesland and others (2007). Pictures were obtained by using light microscopy.
**Fig. S10:** Other pictures of magnetite agglomerates synthesized by the titration method at pH 10. Pictures obtained by using light microscopy.
Fig. S11: Other pictures of ferrous hydroxide synthesized by the titration method at pH 10. The first of these pictures was partially oxidized and was loosely packed. Particles and liquid was observed moving between the agglomerates in the gaps. This sample was diluted 10 times, while all of the subsequent figures were undiluted and were prepared to better prevent oxidation.
(D) At pH 12, magnetite alone was capable of removing CF and so was the control by pH effect.
Fig. S12: (A) A comparison of parent compound $k_{obs}$ values for all [Fe(II) species] at pH 10 and (B) dominant product mole fraction final yields at pH 10.
**Fig. S13:** (A) Pseudo-first order reduction kinetics for CT expressed as $k_{\text{obs}}$, (B) Final CF yield in mole fraction, and (C) $k_{\text{obs}}$ values for CF are expressed at various [Fe(II) species] with different pH series. For 1,1,2,2 TeCA.
Fig. S14: For other starting compounds at pH 10, (A) Parent compound $k_{obs}$ and (B) primary product mole fraction yield with 1.16 g/L magnetite with various [Fe(II)]. (C) Parent compound $k_{obs}$ and (D) dominant daughter product yield at 5 mM Fe(II) and various [magnetite].
\[ y = -19.935x + 42.431 \]

\[ R^2 = 0.8065 \]

\[ R^2 = 0.7867 \]
**Fig. S15:** The effect of various [magnetite] at different pH levels on (A) CT $k_{obs}$, (B) CF mole fraction yield, and (C) CF degradation $k_{obs}$ with 5 mM Fe(II).

**Fig. S16:** Fe(II) species $k_{obs}$ with CT with increasing Fe(II) at different pH. This figure includes data from pH 8 and 12, but the pH 8 and 12 data were outliers. The pH 12 $k_{obs}$ experiments had kinetics that were too fast to get reliable kinetics. The procedure to synthesize minerals in this investigation was difficult to reliably accomplish at pH 8 because the pH would fluctuate wildly during synthesis and the pH 8 reactors may have therefore been more reactive if the slurry spent more time at high pH.
Experiments with magnetite and additional doses of Fe(II) consisted of 3 reactor sets containing variable amounts of Fe(II) as FeSO$_4$ (R1, R2, and R3, that had no magnetite): R1 with 1 mM Fe(II), R2 with 2.5 mM Fe(II), and R3 with 5 mM Fe(II). A gray precipitate, presumably Fe(OH)$_2$, formed quickly in all reactors, which was very reactive towards CT. R4 contained 1.16 g/L magnetite amended with 5 mM Fe(II). The control was DIW adjusted to pH 12.

Method: Fe(OH)$_2$ synthesis/Experimental set-up (pH 10 reactors)

The mineral was synthesized in a small Erlenmeyer flask containing 75 mL aqueous solution that was adjusted to around pH 10.5 with NaOH. An iron reagent consisting of 100 mM FeSO$_4$ was added dropwise to the beaker and stirred continuously. The pH was continuously monitored and as the pH dropped below pH 10, NaOH was added dropwise to keep the pH near the target zone of 10. A white or slightly grayish green precipitate formed throughout the synthesis. The final Fe(II) concentration achieved in separate preparations was 1, 2.5, and 5 mM of Fe(II) in R1, R2, and R3 reactors respectively. Starting CT concentration 69 µg/L.

The experimental conditions of experiment 3 were as follows: Just like in the pH 12 system, any minerals synthesized in most of the 100 mL solution of NaOH and adjusted to keep the pH 10. Starting CT concentration 69 µg/L. The contents of R1, R2, R3, and R4 were the same as their respective cousins from the pH 12 experiments' 5 and 6. The mineral phases believed to be in the reactors were Fe(OH)$_2$ and magnetite. Concentrations of the mineral phases in the reactors are as follows (if not stated, it's presumed the concentration is 0):

R1: 1 mM Fe(OH)$_2$
R2: 2.5 mM Fe(OH)₂
R3: 5 mM Fe(OH)₂

R4: 1.16 g/L magnetite and 5 mM Fe(OH)₂

Fig. S17: This diagram shows that 1,1,2,2 tetrachloroethane degrades by hydrodechlorination to produce 100% product yield of trichloroethene over time at pH 9, regardless of the presence or absence of Fe(II).
Fig. S18: This diagram shows that 1,1,2,2 tetrachloroethane degrades by hydodechlorination to produce 100% product yield of trichloroethene over time at pH 8, in the experimental reactors. There is slight degradation of 1,1,2,2 TeCA in the control's first cycle, but not the second.
Fig. S19: (A) This diagram shows that trichloroethene degrades by an unclear pathway over time at pH 9, with 15 mM Fe(II) in the experimental reactors. (B) This diagram shows that trichloroethene degrades by an unclear pathway over time at pH 8, with 15 mM Fe(II) in the experimental reactors.

A visual inspection of the TCE results from the reaction with 15 mM Fe(II) species seems to reveal that there is little difference between pH 8 and 9 reactors. This could be because of the way pH tends to drift toward pH 6 during the course of the experiment, causing the profile of degradation to look similar between the two different experiments. However, these two figures correspond with the image in Fig. S3, where a visual inspection of the reactors at the time of Fe(II) synthesis reveals that the difference in pH causes a distinct difference in the distribution of Fe(II) species present in the reactor.
Experiments with 1,1,2,2 tetrachloroethane were conducted at pH 9 and 8 and were conducted for two cycles to examine the effect of the Fe(II) species over time. Concentrations of Fe(II) in experimental reactors was 5 mM. Generally, the two experiments showed a small reduction in pseudo-first order rate constants between cycles 1 and 2 (Table 11), which were separated by 1 week. Nearly all of the 1,1,2,2 TeCA that was removed was reduced to TCE in both reactors. In terms of $k_m$, which is normalized to molar concentration of Fe(II), the rate constant was decreased by about 0.5 mM$^{-1}$• day$^{-1}$ between cycles 1 and 2 in both experiments. As was expected from previous experiments, the pseudo-first order rate constants were higher at pH 9 than at pH 8.

Experiments were conducted at pH 8, 9, and 12 with 5 mM Fe(II) to examine the reduction potential of TCE with Fe(II) species. At the low concentration of 5 mM, however, no such degradation of TCE was observed at any of the three pH values tested (data not shown), which is also supported by the lack of TCE degradation in 1,1,2,2 TeCA. Two more experiments were designed with controls in which the Fe(II) concentration was increased to 15 mM. In both experiments, degradation of TCE was observed (Table 11). However, the product of this reaction has been unclear experimentally and the degradation was very slow, but at pH 9, 0.048 µmoles of the total 0.067 µmoles of TCE injected had degraded after about 120 days. Pseudo-first order rate constants show that the rate of TCE removal depends on pH, but it also depends on the amount of Fe(II) present. With the increased Fe(II) concentration, the rate of removal is still very slow compared to other CHCs. The controls also showed no significant TCE loss at any pH.
One experiment was completed to examine the reduction potential of 1,1,2 trichloroethane with Fe(II). Reactors contained 5 mM of Fe(II) species, which were synthesized in the reactor at pH 9. The expected product of this reaction was vinyl chloride (VC), produced by dihaloelimination. While a trace of VC was observed in experimental reactors, there was no significant drop in 1,1,2 TCA values observed (Table 11).

![Graph](image)

**Fig. S20:** 1,1,2,2 TeCA degraded completely at pH 9 (above) and at pH 8 (not shown) to produce TCE. However, TCE with 5 mM Fe(II) was persistent and accumulated in the reactors over two cycles.
Fig: S21: Duplicate reactors with 15 mM Fe(II) at pH 9. TCE decreases very slowly over four months. Although slight loss was also observed in the control at pH 9, but more loss was observed in experimental reactors.
The mixed iron phase experiment (circles) contains more overall iron, but both experiments contained the same concentration of total Fe(II), with most of the Fe(II) being structural.

**Fig. S22:** Equal Concentrations of Total Fe(II)
**Fig. S23:** This diagram shows the amount in micromoles of 1,1,2-TCA and VC with 5 mM Fe(II), at pH 9.
**Fig. S24:** This diagram shows the mole fraction of 1,1,2-TCA, VC, and the total mass balance with various concentrations of only Fe(II) up to 25 mM. These experiments were conducted at pH 10.
**Fig. S25:** This diagram shows the mole fraction of 1,1,2-TCA, VC, and the total mass balance with various concentrations of magnetite up to 3.48 g/L with Fe(II) concentration fixed at 5 mM. These experiments were conducted at pH 10.
Fig. S26: This diagram shows the mole fraction of 1,1,2-TCA, VC, and the total mass balance with various concentrations of magnetite up to 3.48 g/L with Fe(II) concentration fixed at 5 mM. These experiments were conducted at pH 10.

### Ending pH of Carbon Tetrachloride reactors

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* For some preliminary experiments, ending pH was not measured.

* For some preliminary experiments, ending pH was not measured.
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**Ending pH of Trichloroethene reactors**

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### Ending pH of 1,1,2-Trichloroethane reactors

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* For some preliminary experiments, ending pH was not measured.

** This was a preliminary experiment during the initial magnetite synthesis method development.
R output for Chapter II multiple linear Regression

Analysis of $k_{obs}$ for all CT analyses

```r
> CTkobs.reg <- lm(kobs ~ pH + Fe2 + magnetite + TotalFe2, data = CTkobs)
> summary(CTkobs.reg)
```

Call:
```
lm(formula = kobs ~ pH + Fe2 + magnetite + TotalFe2, data = CTkobs)
```

Residuals:
```
  Min  1Q Median  3Q  Max
-143.52 -51.91 -10.34  17.71  226.71
```

Coefficients: (1 not defined because of singularities)
```
                     Estimate Std. Error   t value     Pr(>|t|)
(Intercept) -286.514     93.232   -3.073       0.00402 **
pH             36.815      9.145    4.026       0.00028 ***
Fe2             3.549      2.426    1.463       0.15217
magnetite -27.312     16.549   -1.650       0.10756
TotalFe2       NA       NA       NA        NA
```

---

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 84.34 on 36 degrees of freedom
Multiple R-squared:  0.3443,  Adjusted R-squared:  0.2897
F-statistic: 6.301 on 3 and 36 DF,  p-value: 0.001508

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Carbon Tetrachloride

> CTCT.reg <- lm(CT ~ pH + Fe2 + magnetite + TotalFe2, data = CTCT)
> summary(CTCT.reg)

Call:
  lm(formula = CT ~ pH + Fe2 + magnetite + TotalFe2, data = CTCT)

Residuals:
   Min      1Q  Median      3Q     Max
-0.30567 -0.17119 -0.05043  0.08236  0.75781

Coefficients: (1 not defined because of singularities)
               Estimate Std. Error  t value  Pr(>|t|)
(Intercept)     1.031896    0.277185   3.723 0.000671 ***
pH              -0.091383    0.027190  -3.361 0.001851 **
Fe2             -0.014011    0.007212  -1.943 0.059912 .
magnetite       0.064558    0.049201   1.312 0.197782
TotalFe2         NA         NA      NA       NA

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.2507 on 36 degrees of freedom
Multiple R-squared: 0.2875, Adjusted R-squared: 0.2281
F-statistic: 4.842 on 3 and 36 DF, p-value: 0.006231
Chloroform models

```r
> CTCF.reg <- lm(CF ~ pH + Fe2 + magnetite + TotalFe2, data = CTCF)
> summary(CTCF.reg)

Call:
  lm(formula = CF ~ pH + Fe2 + magnetite + TotalFe2, data = CTCF)

Residuals:
   Min      1Q  Median      3Q     Max
-0.63898 -0.14955  0.07794  0.24283  0.44370

Coefficients: (1 not defined because of singularities)
                        Estimate Std. Error t value Pr(>|t|)
(Intercept)         1.069562   0.353207   3.028 0.004530 **
pH                 -0.038520   0.034647  -1.112 0.273598
Fe2                 0.001131   0.009190   0.123 0.902777
magnetite          -0.225464   0.062695  -3.596 0.000961 ***
TotalFe2           NA       NA       NA       NA

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.3195 on 36 degrees of freedom
```
Multiple R-squared: 0.2905, Adjusted R-squared: 0.2314
F-statistic: 4.913 on 3 and 36 DF, p-value: 0.0058
Chloroform $k_{obs}$ Model

> CTCFKobs.reg <- lm(CFKobs ~ pH + Fe2 + magnetite + TotalFe2, data = CTCFKobs)
> summary(CTCFKobs.reg)

Call:
  lm(formula = CFkobs ~ pH + Fe2 + magnetite + TotalFe2, data = CTCFKobs)

Residuals:
  Min      1Q  Median      3Q     Max
-1.5412 -0.6136 -0.2027  0.3776  7.5460

Coefficients: (1 not defined because of singularities)
                         Estimate Std. Error  t value  Pr(>|t|)
(Intercept)            -4.34514  1.70378   -2.550   0.01516 *
pH                     0.48758  0.16713    2.917   0.00605 **
Fe2                    0.01412  0.04433    0.318   0.75195
magnetite              0.05650  0.30242    0.187   0.85284
TotalFe2               NA        NA        NA       NA

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 1.541 on 36 degrees of freedom
Multiple R-squared: 0.1947, Adjusted R-squared: 0.1276
F-statistic: 2.901 on 3 and 36 DF, p-value: 0.04813
> summary(CTMassBalance.reg)
Call:
  lm(formula = MassBalance ~ pH + Fe2 + magnetite + TotalFe2, data = CTMassBalance)
Residuals:
  Min  1Q Median  3Q    Max
-0.50164 -0.10073 -0.01679  0.11009  0.49641
Coefficients: (1 not defined because of singularities)
                  Estimate Std. Error  t value  Pr(>|t|)
(Intercept)        2.060402   0.258485    7.971     1.83e-09 ***
pH                  -0.127041   0.025355   -5.010    1.46e-05 ***
Fe2                 -0.012928   0.006726   -1.922    0.0625 .
magnetite          -0.122693   0.045881  -2.674   0.0112 *
TotalFe2            NA         NA       NA       NA
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Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Residual standard error: 0.2338 on 36 degrees of freedom
Multiple R-squared:  0.4885,    Adjusted R-squared:  0.4458
F-statistic: 11.46 on 3 and 36 DF, p-value: 2.018e-05
Chapter IV Supplemental Information

(A) DNAN (mol frac) vs Time (d)

(B) DNAN (mol frac) vs Time (d)

1.39 mM HFO
2.78 mM HFO
4.17 mM HFO
**Fig. S1:** DNAN mole fraction over time for (A) 0.28 mM Fe(II), (B) 0.56 mM Fe(II), and (C) 0.83 mM Fe(II). Charts are cut off at t=2 days because no significant changes in DNAN was visible beyond that time.
Fig. S2: DNAN degradation over time under conditions of 0.35 g/L HFO with (A) pH 7, (B) pH 8.5, and (C) pH 10. (D) DNAN $k_{obs}$ values with increasing [Fe(II)] with series separated by pH.

Fig. S3: Initial pseudo-first order rate constant ($k_{obs1}$) values are plotted against [HFO] on the x-axis and series separated by [Fe(II)].
Mass Balance (mol frac)

1.04 g/L Ferr: Effect of Fe(II) concentration at various pH on Mass Balance with various [ferrihydrite]

y = -5.90x + 73.47
R² = 0.15

y = 21.59x + 67.78
R² = 0.95

y = 4.07x + 92.63
R² = 0.17
\[ y = 1.90x + 0.61 \]  
\[ R^2 = 0.97 \]

\[ y = 2.70x + 0.57 \]  
\[ R^2 = 0.94 \]

\[ y = 4.01x + 2.32 \]  
\[ R^2 = 0.93 \]
Fig. S4: (A) C mass balance decreased as pH increased. The yield also slightly increased as [Fe(II)] increased. Effect of [Fe(II)] with [HFO] on DNAN degradation kinetics and product distribution at pH 7. (B) Initial pseudo-first order rate constant ($k_{obs1}$) values are plotted against [Fe(II)] on the x-axis and series separated by [HFO]. (C) Variations in overall rate constant ($k_{obs2}$) for different [HFO] and increasing [Fe(II)]. (D) DNAN mole fraction remaining and (E) 2-ANAN mole fraction yield, combined for all three HFO series with increasing [Fe(II)]. Minor DAAN yield ($m/m_0 \leq 0.04$)
**Fig. S5:** Combined effect of [HFO] with increasing pH on DNAN degradation kinetics and product distribution at 0.28 mM Fe(II). (A) DNAN $k_{obs}$ values with increasing [HFO] with series separated by pH. (B) Final DNAN remaining, (C) 2-ANAN yield in mole fraction with increasing [Fe(II)] with the series separated by pH.
Fig. S6: Light microscopy micrograph photos taken at 1000x magnification showing aggregates of (HFO (A and B), and goethite particles.

Additional Mineral characterization photos with light microscopy:

Goethite
### Ending pH of HFO reactors

<table>
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<th>[HFO] mM</th>
<th>[Fe(II)] mM</th>
<th>[Total Fe(II)] mM</th>
<th>Starting pH</th>
<th>Ending pH</th>
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317
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25  0.28  0.28  pH 7  7.18  
25  0.56  0.56  pH 7  7  
25  0.56  0.56  pH 7  7  
25  0.83  0.83  pH 7  6.78  
25  0.83  0.83  pH 7  6.78  
12.5  0.28  0.28  pH 8.5  8.49  
12.5  0.28  0.28  pH 8.5  8.49  
12.5  0.56  0.56  pH 8.5  8.53  
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12.5  0.83  0.83  pH 8.5  8.4  
12.5  0.83  0.83  pH 8.5  8.36  

Chapter IV R Code: Multiple Linear Regression

$K_{obs1}$

Call:
`lm(formula = (kobs1 ~ (HFO + Fe2 + pH)^2), data = HFO)`

Residuals:

<table>
<thead>
<tr>
<th>Min</th>
<th>1Q</th>
<th>Median</th>
<th>3Q</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>-354.73</td>
<td>-87.86</td>
<td>-39.31</td>
<td>90.55</td>
<td>392.71</td>
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</tbody>
</table>

Coefficients:

| Estimate | Std. Error | t value | Pr(>|t|) |
|----------|------------|---------|---------|
| (Intercept) | 1171.056 | 624.546 | 1.875 | 0.0716 . |
| HFO | -138.371 | 164.615 | -0.841 | 0.4080 |
| Fe2 | -4345.403 | 677.784 | -6.411 | 7.21e-07 *** |
| pH | -157.998 | 79.749 | -1.981 | 0.0578 . |
| HFO:Fe2 | 1.047 | 29.292 | 0.036 | 0.9717 |
| HFO:pH | 24.504 | 20.362 | 1.203 | 0.2393 |
| Fe2:pH | 618.726 | 93.744 | 6.600 | 4.42e-07 *** |

---

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 170 on 27 degrees of freedom
Multiple R-squared:  0.804,  Adjusted R-squared:  0.7605
F-statistic: 18.46 on 6 and 27 DF,  p-value: 2.089e-08
\[ K_{\text{obs2}} \]

\[
> \text{kobs2.reg} \leftarrow \text{lm}(\text{kobs2} \sim (\text{HFO} + \text{Fe2} + \text{pH})^2, \text{data=HFO})
\]

\[
> \text{summary(kobs2.reg)}
\]

**Call:**

\[
\text{lm(formula = (kobs2 \sim (\text{HFO} + \text{Fe2} + \text{pH})^2), data = HFO)}
\]

**Residuals:**

\[
\begin{array}{c|c|c|c|c|c|c|c}
\text{Min} & \text{1Q} & \text{Median} & \text{3Q} & \text{Max} \\
-41.348 & -5.525 & -3.117 & -0.494 & 116.287
\end{array}
\]

**Coefficients:**

| Estimate | Std. Error | t value | Pr(>|t|) |
|----------|------------|---------|----------|
| (Intercept) | 147.1770 | 107.3822 | 1.371 | 0.1818 |
| HFO | -14.1286 | 28.3034 | -0.499 | 0.6217 |
| Fe2 | -299.7636 | 116.5358 | -2.572 | 0.0159 * |
| pH | -20.4998 | 13.7117 | -1.495 | 0.1465 |
| HFO:Fe2 | 0.9469 | 5.0363 | 0.188 | 0.8523 |
| HFO:pH | 2.0599 | 3.5009 | 0.588 | 0.5612 |
| Fe2:pH | 42.7990 | 16.1180 | 2.655 | 0.0131 * |

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 . ‘.’ 0.1  ‘ ’ 1
Residual standard error: 29.23 on 27 degrees of freedom
Multiple R-squared: 0.2495,  Adjusted R-squared: 0.08274
F-statistic: 1.496 on 6 and 27 DF,  p-value: 0.2171

DNAN

> DNAN.reg <- lm(DNAN ~ (HFO + Fe2 + pH)^2, data=HFO)
> summary(DNAN.reg)

Call:
  lm(formula = (DNAN ~ (HFO + Fe2 + pH)^2), data = HFO)

Residuals:
   Min     1Q Median     3Q    Max
-0.34035 -0.06894 0.00121 0.04355 0.25970

Coefficients:
 Estimate Std. Error t value Pr(>|t|)
(Intercept) 0.36785 0.52270 0.704 0.48761

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HFO          0.12078    0.13777   0.877 0.388413
Fe2          1.75662    0.56726   3.097 0.004527 **
pH           0.04437    0.06674   0.665 0.511849
HFO:Fe2      0.04826    0.02452   1.969 0.059339 .
HFO:pH       -0.02048   -0.01704   -1.202 0.239854
Fe2:pH       -0.29712   -0.07846   -3.787 0.000775 ***

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 . ‘.’ 0.1 ‘ ’ 1
Residual standard error: 0.1423 on 27 degrees of freedom
Multiple R-squared:  0.8004,    Adjusted R-squared:  0.756
F-statistic: 18.04 on 6 and 27 DF,  p-value: 2.658e-08

2-HA-NAN

> HANAN.reg <- lm((HANAN ~ (HFO + Fe2 + pH)^2), data=HFO)
> summary(HANAN.reg)
Call:
\( \text{lm(formula = (HANAN \sim (HFO + Fe2 + pH)^2), data = HFO)} \)

Residuals:

<table>
<thead>
<tr>
<th></th>
<th>Min</th>
<th>IQR</th>
<th>Median</th>
<th>3Q</th>
<th>Max</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>-0.027271</td>
<td>-0.007660</td>
<td>-0.003539</td>
<td>-0.000216</td>
<td>0.080192</td>
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</tbody>
</table>

Coefficients:

|                | Estimate | Std. Error | t value | Pr(>|t|) |
|----------------|----------|------------|---------|----------|
| (Intercept)    | 0.0821786 | 0.0806681  | 1.019   | 0.317    |
| HFO            | -0.0113864 | 0.0212622  | -0.536  | 0.597    |
| Fe2            | 0.0005158  | 0.0875445  | 0.006   | 0.995    |
| pH             | -0.0076723 | 0.0103006  | -0.745  | 0.463    |
| HFO:Fe2       | 0.0017499  | 0.0037834  | 0.463   | 0.647    |
| HFO:pH        | 0.0011275  | 0.0026300  | 0.429   | 0.672    |
| Fe2:pH        | -0.0015202 | 0.0121082  | -0.126  | 0.901    |

Residual standard error: 0.02196 on 27 degrees of freedom
Multiple R-squared: 0.1291, Adjusted R-squared: -0.06441
F-statistic: 0.6672 on 6 and 27 DF, p-value: 0.6767

2-ANAN

> ANAN.reg <- lm((ANAN ~ (HFO + Fe2 + pH)^2), data=HFO)
> summary(ANAN.reg)

Call:
\( \text{lm(formula = (ANAN \sim (HFO + Fe2 + pH)^2), data = HFO)} \)

Residuals:

<table>
<thead>
<tr>
<th></th>
<th>Min</th>
<th>IQR</th>
<th>Median</th>
<th>3Q</th>
<th>Max</th>
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<tbody>
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Coefficients:

|                | Estimate | Std. Error | t value | Pr(>|t|)     |
|----------------|----------|------------|---------|--------------|
| (Intercept)    | 1.13788  | 0.45030    | 2.527   | 0.017673 *   |
| HFO            | -0.13056 | 0.11869    | -1.100  | 0.281040     |
| Fe2            | -1.67804 | 0.48869    | -3.434  | 0.001936 **  |
| pH             | -0.15898 | 0.05750    | -2.765  | 0.010138 *   |
| HFO:Fe2       | -0.05387 | 0.02112    | -2.551  | 0.016735 *   |
| HFO:pH        | 0.02216  | 0.01468    | 1.509   | 0.142809     |
| Fe2:pH        | 0.28967  | 0.06759    | 4.286   | 0.000207 *** |

---

Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.1226 on 27 degrees of freedom
Multiple R-squared: 0.7981, Adjusted R-squared: 0.7532
F-statistic: 17.78 on 6 and 27 DF, p-value: 3.093e-08
DAAN

> DAAN.reg <- lm(DAAN ~ (HFO + Fe2 + pH)^2), data=HFO)
> summary(DAAN.reg)

Call:
lm(formula = (DAAN ~ (HFO + Fe2 + pH)^2), data = HFO)

Residuals:

                     Min          1Q      Median          3Q         Max
-0.039141 -0.006208  -0.000528   0.008076   0.026607

Coefficients:

                         Estimate Std. Error t value Pr(>|t|)
(Intercept)              0.051708   0.056998   0.907   0.3723
HFO                      0.037067   0.015023   2.467   0.0202 *
Fe2            -0.539819   0.061856  -8.727 2.42e-09 ***
pH                0.008968   0.007278   1.232   0.2285
HFO:Fe2          -0.001631   0.002673  -0.610   0.5469
HFO:pH            -0.004705   0.001858  -2.532   0.0175 *
Fe2:pH            0.078780   0.008555   9.208 8.08e-10 ***

---

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 . ‘.’ 0.1 ‘ ’ 1
Residual standard error: 0.01552 on 27 degrees of freedom
Multiple R-squared: 0.8596,  Adjusted R-squared: 0.8284
F-statistic: 27.54 on 6 and 27 DF,  p-value: 2.629e-10
```
> MassBalance.reg <- lm((MassBalance ~ (HFO + Fe2 + pH)^2), data=HFO)
> summary(MassBalance.reg)

Call:
  lm(formula = (MassBalance ~ (HFO + Fe2 + pH)^2), data = HFO)

Residuals:
     Min       1Q   Median       3Q      Max
-0.22103 -0.03703  0.01865  0.04333  0.14929

Coefficients:
             Estimate Std. Error t value  Pr(>|t|)
(Intercept)   1.52937   0.32333   4.730  6.29e-05 ***
HFO           0.03797   0.08522   0.445     0.65951
Fe2          -0.49240   0.35089  -1.403     0.17193
pH           -0.11982   0.04129  -2.902     0.00729 **

```

Mass Balance

```
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<td>-0.007407</td>
<td>0.015165</td>
<td>-0.488</td>
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Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.08803 on 27 degrees of freedom
Multiple R-squared: 0.7296, Adjusted R-squared: 0.6695

F-statistic: 12.14 on 6 and 27 DF, p-value: 1.353e-06

![Graph 1](image1.png)

![Graph 2](image2.png)
```r
> cor(HFO)

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<th></th>
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<th>Fe2</th>
<th>pH</th>
<th>kobs1</th>
<th>kobs2</th>
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MassBalance 1.00000000
Fig. S1: Mass balance at pH 7 with various [magnetite] and [Fe(II)].
Fig. S2: (A) 2-ANAN and (B) DAAN yields at 2.78 mM magnetite at pH 7 and 10.
Fig. S3: (A) 2-ANAN and (B) DAAN yields at 4.86 mM magnetite at pH 6, 7, and 10.
(C) $y = 1942.2x - 591.3$
$R^2 = 0.8825$

$y = 179.54e^{0.645x}$
$R^2 = 0.8811$

$y = 69.907e^{0.7692x}$
$R^2 = 1$

(D) $y = 0.237x - 0.318$
$R^2 = 1$

$y = 0.183x - 0.1901$
$R^2 = 0.9917$

$y = 0.0036e^{1.0111x}$
$R^2 = 0.9947$
(E) Final DAAN yield (mol frac) vs. [magnetite] (mM)

- pH 6: $y = 0.1127e^{0.455x}$, $R^2 = 0.9939$
- pH 7: $y = 0.0071e^{1.0507x}$, $R^2 = 0.9982$
- pH 8: $y = 0.0021e^{1.2297x}$, $R^2 = 1$
- pH 9: $y = 0.1127e^{0.455x}$, $R^2 = 0.9939$
- pH 10: $y = 0.1127e^{0.455x}$, $R^2 = 0.9939$

(F) Final DAAN yield (mol frac) vs. [magnetite] (mM)

- pH 6: $y = 0.1959x + 0.064$, $R^2 = 0.9895$
- pH 7: $y = 0.0071e^{1.0471x}$, $R^2 = 0.9473$
- pH 8: $y = 0.1127e^{0.455x}$, $R^2 = 1$
- pH 9: $y = 0.1127e^{0.455x}$, $R^2 = 0.9939$
- pH 10: $y = 0.1127e^{0.455x}$, $R^2 = 0.9939$
Fig. S4: Combined effect of [magnetite] with increasing pH on DNAN degradation kinetics and product distribution. (A) DNAN $k_{\text{obs}1}$ with 0 added Fe(II), (B) $k_{\text{obs}1}$ with 0.28 mM Fe(II), (C) DNAN $k_{\text{obs}}$ with 0.56 mM Fe(II), (D) DAAN yield with 0 added Fe(II), (E) DAAN yield with 0.28 mM Fe(II), (F) DAAN yield with 0.56 mM Fe(II), (G) C mass balance with 0 added Fe(II), (H) C mass balance mole fractions with 0.28 mM Fe(II), and (I) mass balance with 0.56 mM Fe(II) in mole fraction with increasing [magnetite]. Series were defined by their starting pH levels. Data that were analyzed by estimating $k_{\text{obs}1}$ based on sampling time are indicated by smaller symbols.
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TF: reaction kinetics were too fast to quantify

i: No secondary kinetics were possible because the reaction was too fast

![Graph A](image1.png)

(A) $k_m$ (wrt Total Fe(II)) w total Fe(II)

- magnetite
- HFO
- Power (HFO)

$y = 203.01e^{0.0436x}$

$y = 86.437x^{-0.854}$

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Fig. S5: Normalized $k_{obs}$ values with respect to mass concentration of (A) magnetite with HFO and goethite and (B) with only HFO with total [Fe(II)] at pH 7.
Fig. S6: (A) Distribution of $k_{obs1}$ with respect to [total Fe(II)] at various pH. Figures B and C show the same data split according to (B) magnetite experiments and (C) HFO experiments.
The following are light microscopy micrographs of magnetite synthesized in the method similar to Vikesland et al., (2007).

**Fig. S7:** $k_{obs2}$ values for magnetite, HFO, and goethite with [total Fe(II)] in mM.
**Fig. S8:** Light microscopy pictures of magnetite nanoparticle agglomerates. Pictures were 1000x magnification.

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R Statistical Analysis

$K_{\text{obs1}}$

```r
> kobs1.reg <- lm((kobs1 ~ (mag + Fe2 + pH)^2), data=magnetite)
> summary(kobs1.reg)
Call:
  lm(formula = (kobs1 ~ (mag + Fe2 + pH)^2), data = magnetite)
Residuals:
     Min      1Q  Median      3Q     Max
-2085.1  -623.4    81.5   222.3  4506.4
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)   2191.3     3261.7   0.672  0.50839
mag         -1299.9      842.6  -1.543  0.13654
Fe2         -5531.6     6093.8  -0.908  0.37343
pH            -457.4      393.3  -1.163  0.25675
mag:Fe2      911.5      710.4   1.283  0.21229
mag:pH      297.2      102.6   2.896  0.00815 **
Fe2:pH       679.8      216.2   3.148  0.00285 **
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Residual standard error: 1332 on 23 degrees of freedom
Multiple R-squared:  0.793,  Adjusted R-squared:  0.739
F-statistic: 14.69 on 6 and 23 DF,  p-value: 7.517e-07
```
k_{\text{magnetite}}

> kmineral.reg <- lm((kmineral ~ (mag + Fe2 + pH)^2), data=magnetite)
> summary(kmineral.reg)

Call:
lm(formula = (kmineral ~ (mag + Fe2 + pH)^2), data = magnetite)

Residuals:

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Coefficients:

|            | Estimate | Std. Error | t value | Pr(>|t|) |
|------------|----------|------------|---------|---------|
| (Intercept)| 76.82    | 832.27     | 0.092   | 0.927   |
| mag        | -134.76  | 214.99     | -0.627  | 0.537   |
| Fe2        | -1997.35 | 1554.92    | -1.285  | 0.212   |
| pH         | -17.66   | 100.35     | -0.176  | 0.862   |

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mag:Fe2   98.54   181.28  0.544   0.592
mag:pH    37.96   26.19   1.450   0.161
Fe2:pH    318.74  175.87  1.812   0.083 .
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
Residual standard error: 339.8 on 23 degrees of freedom
Multiple R-squared: 0.6979,  Adjusted R-squared: 0.6191
F-statistic: 8.857 on 6 and 23 DF,  p-value: 4.626e-05

![Graph 1](image1)

![Graph 2](image2)
\( K_{\text{TotalFe(II)}} \)

> `ktotFe2.reg <- lm((ktotFe2 ~ (mag + Fe2 + pH)^2), data=magnetite)`
> `summary(ktotFe2.reg)`

Call:
`lm(formula = (ktotFe2 ~ (mag + Fe2 + pH)^2), data = magnetite)`

Residuals:

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<tr>
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<th>Min</th>
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</table>

Coefficients:

|              | Estimate | Std. Error | t value | Pr(>|t|) |
|--------------|----------|------------|---------|---------|
| (Intercept)  | 98.97    | 734.43     | 0.135   | 0.8940  |
| mag          | -135.30  | 189.71     | -0.713  | 0.4829  |
| Fe2          | -1545.10 | 1372.11    | -1.126  | 0.2717  |
| pH           | -22.37   | 88.56      | -0.253  | 0.8028  |
| mag:Fe2      | 77.11    | 159.97     | 0.482   | 0.6343  |
| mag:pH       | 39.78    | 23.11      | 1.721   | 0.0986  |
| Fe2:pH       | 231.23   | 155.19     | 1.490   | 0.1498  |

---

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 299.9 on 23 degrees of freedom
Multiple R-squared: 0.7068, Adjusted R-squared: 0.6303
F-statistic: 9.24 on 6 and 23 DF, p-value: 3.36e-05

---
$k_{\text{obs2}}$

```r
kobs2.reg <- lm((kobs2 ~ (mag + Fe2 + pH)^2), data=magnetite)
> summary(kobs2.reg)
Call:
  lm(formula = (kobs2 ~ (mag + Fe2 + pH)^2), data = magnetite)
Residuals:
     Min      1Q  Median      3Q     Max
-198.544 -34.208   2.615  18.356  268.554
Coefficients:
                     Estimate Std. Error t value Pr(>|t|)
(Intercept)         -256.711    205.748   -1.248  0.224696
mag                   241.052     53.148    4.535 0.000148 ***
Fe2                   -237.188    384.395   -0.617  0.543266
pH                    19.206     24.809    0.774  0.446723
mag:Fe2               -107.938     44.814    -2.409  0.024426 *
mag:pH                -21.812      6.474    -3.369 0.002649 **
Fe2:pH                53.389     43.477     1.228 0.231875
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 .’ 0.1 ‘ ’ 1
Residual standard error: 84 on 23 degrees of freedom
Multiple R-squared: 0.6799, Adjusted R-squared: 0.5964
F-statistic: 8.141 on 6 and 23 DF, p-value: 8.607e-05
```
DNAN

> DNAN.reg <- lm((DNAN ~ (mag + Fe2 + pH)^2), data=magnetite)
> summary(DNAN.reg)

Call:
lm(formula = (DNAN ~ (mag + Fe2 + pH)^2), data = magnetite)

Residuals:

       Min        1Q      Median        3Q       Max
-0.12423  -0.03570   0.01327   0.03406   0.12397

Coefficients:

                      Estimate Std. Error t value Pr(>|t|)
(Intercept)          0.681333   0.150477  4.528 0.000151 ***
mag                 -0.144965   0.038871  -3.729 0.001099 **
Fe2                 -0.523656   0.281133  -1.863 0.075332 .
pH                  -0.036692   0.018144  -2.022 0.054933 .
mag:Fe2             -0.125940   0.032775  -3.843 0.000831 ***
mag:pH               0.125940   0.032775   3.843 0.000831 ***
Fe2:pH              -0.005174   0.031798  -0.163 0.872159

---

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.06144 on 23 degrees of freedom
Multiple R-squared: 0.7971,  Adjusted R-squared: 0.7441
F-statistic: 15.06 on 6 and 23 DF,  p-value: 6.043e-07
2-HA-NAN

> HANAN.reg <- lm((HANAN ~ (mag + Fe2 + pH)^2), data=magnetite)
> summary(HANAN.reg)

Call:
  lm(formula = (HANAN ~ (mag + Fe2 + pH)^2), data = magnetite)

Residuals:
   Min     1Q   Median     3Q    Max
-0.068154 -0.045595  0.008658  0.026940  0.127529

Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.327502   0.130303   2.513   0.0194 *
mag       -0.055270   0.033659  -1.642   0.1142
Fe2        -0.136326   0.243442  -0.560   0.5809
pH         -0.020417   0.015712  -1.299   0.2067
mag:Fe2    -0.004685   0.028381  -0.165   0.8703
mag:pH      0.002423   0.004100   0.591   0.5602
Fe2:pH     0.018454   0.027535   0.670   0.5094

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.0532 on 23 degrees of freedom
Multiple R-squared:  0.604,   Adjusted R-squared:  0.5007
F-statistic: 5.846 on 6 and 23 DF,  p-value: 0.0008078
2-ANAN
> ANAN.reg <- lm((ANAN ~ (mag + Fe2 + pH)^2), data=magnetite)
> summary(ANAN.reg)

Call:
  lm(formula = (ANAN ~ (mag + Fe2 + pH)^2), data = magnetite)
Residuals:
   Min     1Q   Median     3Q    Max
-0.17591 -0.09452 -0.04102  0.09479  0.33377
Coefficients:
            Estimate Std. Error t value Pr(>|t|)
(Intercept)  0.623275   0.354203   1.760   0.091     .
mag          -0.026488   0.091497  -0.289   0.7748
Fe2           0.653051   0.661749   0.987   0.3340
pH           -0.054728   0.042709  -1.281   0.2128
mag:Fe2       -0.200445   0.077149  -2.598   0.0161    *
mag:pH         0.007508   0.011145   0.674   0.5072
Fe2:pH       -0.020159   0.074847  -0.269   0.7901
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 .’ 0.1  ‘ ’ 1
Residual standard error: 0.1446 on 23 degrees of freedom
Multiple R-squared:  0.3735,  Adjusted R-squared:  0.21
F-statistic: 2.285 on 6 and 23 DF,  p-value: 0.07079
DAAN

> DAAN.reg <- lm((DAAN ~ (mag + Fe2 + pH)^2), data=magnetite)
> summary(DAAN.reg)

Call:
  lm(formula = (DAAN ~ (mag + Fe2 + pH)^2), data = magnetite)

Residuals:
  Min       1Q   Median       3Q      Max
-0.218585 -0.055715 -0.000386  0.049342  0.268188

Coefficients:
                         Estimate  Std. Error    t value  Pr(>|t|)
(Intercept)               -0.496876   0.333884   -1.488    0.1503
mag                      0.240918   0.086248    2.793    0.0103 *
Fe2                      -0.723630   0.623789   -1.160    0.2579
pH                       0.033552   0.040259    0.833    0.4132
mag:Fe2                  0.067958   0.072724    0.934    0.3598
mag:pH                   -0.006596   0.010506   -0.628    0.5363
Fe2:pH                   0.107469   0.070554    1.523    0.1413

---

Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.1363 on 23 degrees of freedom
Multiple R-squared:  0.8819,  Adjusted R-squared:  0.8511
F-statistic: 28.62 on 6 and 23 DF,  p-value: 1.44e-09
Mass Balance

```r
> MassBalance.reg <- lm((MassBalance ~ (mag + Fe2 + pH)^2), data=magnetite)
> summary(MassBalance.reg)

Call:
  lm(formula = (MassBalance ~ (mag + Fe2 + pH)^2), data = magnetite)

Residuals:
    Min       1Q   Median       3Q      Max
-0.20620  -0.09008  -0.00212  0.04304  0.31717

Coefficients:
                      Estimate Std. Error t value Pr(>|t|)
(Intercept)         1.170910   0.325711   3.595  0.00153 **
mag               -0.006562   0.084136  -0.078  0.93851
Fe2                -0.733863   0.608518  -1.206  0.24008
pH                 -0.080718   0.039274  -2.055  0.05138 .
mag:Fe2             -0.006456   0.070943  -0.091  0.92828
mag:pH              0.012306   0.010249   1.201  0.24207
Fe2:pH             0.099538   0.068827   1.446  0.16161

---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Residual standard error: 0.133 on 23 degrees of freedom
Multiple R-squared:  0.6064,  Adjusted R-squared:  0.5038
F-statistic: 5.906 on 6 and 23 DF,  p-value: 0.0007576
```
> cor(magnetite)

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ktotFe2 DNAN HANAN ANAN DAAN NONAN MassBalance

> MassBalance 0.72357936 0.055368427 -0.200093079 0.58545754 0.4397528

ktotFe2 kobs2 DNAN HANAN ANAN DAAN NONAN MassBalance

> DAAN           NONAN      MassBalance

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