Remediation of Pentaerythritol Tetranitrate (PETN) Contaminated Water and Soil

by

Li Zhuang

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Pentaerythritol tetranitrate (PETN), a nitrate ester, is widely used as a powerful explosive and is classified as a munitions constituent of great concern by DoD in U.S.A. It is an environmental concern and poses a threat to ecosystem and human health. Our objective was to examine potential remediation strategies for both PETN-contaminated water and soil.

Flow-through iron columns were used to determine the potential for using granular iron to degrade PETN in aqueous phase. PETN transformation in both a 100% iron column and a 30% iron and 70% silica sand column followed pseudo-first-order kinetics, with average half-lives of 0.26 and 1.58 minutes, respectively. Based on the identified intermediates and products, the reaction pathway was proposed to be a sequential denitration process, in which PETN was stepwisely reduced to pentaerythritol with the formation of pentaerythritol trinitrate (PETriN) and pentaerythritol dinitrate (PEDN). Although pentaerythrito mononitrate was not detected, an approximately 100% nitrogen mass recovery indicated that all nitro groups were removed from PETN. Nitrite was released in each denitration step and subsequently reduced to NH_4^+ by iron. Nitrate was not detected during the experiment, suggesting that hydrolysis was not involved in PETN degradation. Furthermore, batch experiments showed that PETN dissolution was likely a rate-limiting factor for PETN degradation, especially in the case with high amount of iron. Using 50% methanol as a representative co-solvent, PETN solubility was greatly enhanced and thus the removal efficiency was improved. The results demonstrate the use of granular iron to remediate PETN-contaminated water.

The biodegradability of aqueous PETN was examined with a mixed microbial culture from a site contaminated with PETN. The mixed culture was enriched and selected using a mineral medium containing acetate and yeast extract as carbon and nutrient sources in the presence of nitrate or sulfate. The final enrichment cultures were used as inocula for studying PETN biodegradation under nitrate-reducing and sulfate-reducing conditions. In addition, PETN degradation was tested using the original microbial culture under the mixed electron

acceptor conditions of nitrate and sulfate. The results showed that under all conditions tested, PETN was sequentially reduced, apparently following the same pathway as the abiotic reduction by granular iron. Pentaerythritol mononitrate, a suspected intermediate in the abiotic degradation by iron, was identified in this experiment. The presence of nitrate seemed not to affect the kinetics of PETN degradation, with both PETN and nitrate degrading simultaneously. However, the rate of nitrate reduction was much faster than PETN degradation. With respect to sulfate, its presence did not have an adverse effect on PETN degradation, indicated by the very similar degradation rates of PETN in the presence and absence of sulfate. Under all conditions, PETN appeared to act as a terminal electron acceptor for energy generation during biodegradation. A utilization sequence by bacteria in the order of nitrate, PETN, PETriN, PEDN and sulfate was clearly observed. The study in this phase demonstrated that under anaerobic conditions, with carbon sources provided, PETN can be effectively biodegraded by indigenous bacteria in contaminated soil, most likely by denitrifying bacteria.

Based on the successful demonstration of abiotic and biotic degradation of PETN in the aqueous phase, both methods were further tested for remediating PETN-contaminated soil in both laboratory and pilot scale. In the laboratory, a systematic soil microcosm experiment was conducted using soil from a contaminated site and additions of either granular iron or organic materials, with deoxygenated Millipore water. Because of the high concentration in the contaminated soil, solid-phase of PETN was initially present in the microcosms. Two types of DARAMEND products, D6390Fe20 (containing 20% iron + 80% botanical materials) and ADM-298500 (100% botanical materials), were used as sources of carbon and other nutrients. During the 84-day incubation period, more than 98% was removed in all DARAMEND treatments, following pseudo-first-order kinetics with half-lives ranging between 8 and 18 days. The results clearly demonstrated that PETN can be effectively degraded under anaerobic conditions with the addition of carbon and possibly nutrients. As in the aqueous tests, the sequence of microbial utilization was nitrate followed by PETN and sulfate. In contrast to the tests with aqueous PETN, iron was not effective in removing PETN

in the contaminated soil, due to iron passiviation caused by the presence of high levels of nitrate in the soil. In addition, a slight enhancement was observed in a combined system of iron and biodegradation over biodegradation only. However, the extent of enhancement is not believed to be significant relative to the extra cost for iron addition.

A pilot scale test was conducted at a PETN-contaminated site at Louviers, CO, a waste pond which had received waste water from PETN manufacture for over 20 years. The test involved 10 treatments, one control without amendment, one amended with iron (10%), eight with different types and amounts of organic carbon (1%, 2% and 4% of D6390Fe20; 2% and 4% of ADM-298500 and 1%, 2% and 4% of brewers grain). Each treatment was performed in a plastic tub (45 cm wide × 90 cm long × 25 cm deep), containing approximately 18 cm thick layer of soil and 6-8 cm of standing water. Over 74 days, very little consistent reduction of PETN was found in the iron treatment, which was also due to iron passivation in the presence of nitrate in the soil. In contrast, significant removal of PETN (11,200 to 33,400 mg/kg) was observed in the treatments amended with organic materials, and the extent of removal increased with increasing amounts of organic materials. The pilot test was consistent with the results of the laboratory experiments for iron and biodegradation with carbon addition. For biological treatment, the stoichiometric estimation suggests that the complete remediation in many of the treatments will be ultimately limited by carbon sources.

Results of this study showed the great potentials of using granular iron to degrade PETN in solution and using indigenous bacteria present in contaminated soils to biodegrade PETN in both the solution and soil phase. Both iron and biodegradation with carbon addition represent viable approaches for remediation of PETN-contaminated water and soil, though iron may not be appropriate in the presence of high concentration of nitrate.

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Dedication

I dedicate this work to my mother, who did not have her opportunity to pursue education.

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Chapter 1

Introduction

1.1 Background

1.1.1 Contamination of Organic Nitrate Explosives

As a result of intensive military activities over the past century, contamination of soil and water with residues of explosives and related compounds is a widespread environmental concern (Gorontzy et al., 1994). Contamination sources are mainly associated with their manufacture, use, loading, storage and disposal processes. Their occurrence in the environment not only poses the risk of detonation but also a hazard to biological systems and human health because of their toxic and mutagenic effects (Bruns-Nagel et al., 1999; Honeycutt et al., 1996; Steevens et al., 2002). Depending on the organic structures to which the nitro groups are attached, organic nitrate explosives are categorized into three groups: nitroaromatics with NO_2 groups bonded to carbon atoms on an aromatic ring (e.g. dinitrotoluene (DNT) and 2,4,6-trinitroluene (TNT)), nitramines with NO_2 groups bonded to a nitrogen atom that is present within an alicyclic ring (e.g. hexahydro-1,3,5-trinitro-1,3,5 triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7 tetrazocine (HMX)) and nitrate esters in which the NO_2 groups are bonded to an oxygen atom attached to an aliphatic carbon (e.g. 1,2,3-propanetriol trinitrate (NG) and pentaerythritol tetranitrate (PETN)). The estimation of suspect explosives-contaminated sites is in the thousands in the United States, and a much greater number in Europe and the former Soviet Union (Major et al., 1997). Under ambient environmental conditions, most explosives exhibit a resistance to natural attenuation processes including volatilization, biodegradation and hydrolysis, resulting in persistence in soil and groundwater (Twibell et al., 1984). An increased public awareness of the toxicity and risk associated with these compounds has stimulated research activities on the development of remediation technologies for cleaning up explosives-contaminated water and soil.

1.1.2 Nitrate Esters and PETN

Nitrate esters are widely manufactured for two major industrial applications; primarily as secondary explosives in blasting caps and detonators. In addition, it is used as a coronary vasodilator in the treatment of heart disease. Nitrate esters commonly used for these two applications include pentaerythritol tetranitrate (PETN; 2,2-bis[(nitrooxy)methyl]-1,3-propanediol dinitrate) and glycerol trinitrate (GTN; propane-1,2,3,-triyl trinitrate). PETN is synthesized by direct nitration of pentaerythritol with nitric acid using sulphuric acid as a catalyst. Current production data is not available, but the estimation by the NCI/SRI (National Cancer Institute/Socially Responsible Investing) shows that in 1973, $1.8 \sim 2.3 \times 10^9$ kg of PETN were used for explosives and 2.3×10^4 kg were used for production of pharmaceuticals (Bucher, 1989). As for the environmental concern, GTN is toxic to mammals at levels of 30 to 1300 mg/kg (Wendt et al., 1978) and LC_{50} for aquatic organisms is between 1 and 3 mg/L (Urbanski, 1984). Currently, PETN is not vigorously regulated and the threshold limit value (TLV) and maximum workplace concentration (MAK) have not been established. However, short-term exposure may affect the cardiovascular system, resulting in lowering blood pressure, as indicated by medical observation (IPCS & CEC, 2005). It is categorized as "toxic to aquatic organisms" (Drzyzga et al., 1995) and is classified by the U.S. DoD as a munitions constituent of great concern because of the widespread use and the potential environmental impact (DoD, 2002).

Due to its low vapor pressure (1.035×10^{-10} mmHg) and low Henry's law constant (1.2×10^{-11} atm·m^3/mole), PETN is unlikely to disperse in the ambient air, and as a highly hydrophobic compound, it has very low water solubility (6 mg/L). PETN also has a low $logK_{ow}$ of 1.61 (Chemical properties from Hazardous Substances Data Bank (HSDB), 2003), suggesting that it sorbs weakly to organic materials in the soil and sediment and therefore once dissolved it can be readily transported by groundwater. The structure of PETN (C-O-NO_2) is analogous to sulfate esters (C-O-SO_3^-) and phosphate esters (C-O-PO_3^{2-}), which are ubiquitous in the natural environment. However, nitrate esters have not been detected as naturally-occurring compounds and have only been introduced to the environment by human

activities. They appear to be recalcitrant to natural breakdown and pose a xenobiotic challenge to biological systems (White and Snape, 1993). For instance, in a PETN waste disposal pond in Louviers, Colorado, U.S.A., the PETN concentration has been consistently high over the past 20 years, indicating that no significant natural attenuation has occurred. Such persistence in soil also represents a potential source for groundwater contamination. There is an urgent need for developing effective remediation methods for such contaminated sites due to public health concerns and more stringent environmental regulations.

1.1.3 Treatment Methods for Explosives

The conventional treatment method for remediating explosives-contaminated soil is incineration. Because of the high cost and the associated disadvantages such as production of large volumes of unusable ash, increased attention has turned to alternative remediation methods, such as chemical reduction with metallic iron and bioremediation including composting, bioslurry and landfarming. Based on exhaustive review, Craig et al. (1995) and Lewis et al. (2004) concluded that composting and bioslurry are the most affordable and acceptable biological treatment methods for explosives-contaminated soil.

1.1.3.1 Degradation of Explosives by Iron

Granular iron is being used increasingly in permeable reactive barriers (PRBs) as a cost-effective groundwater remediation technology because it is capable of reducing a variety of important pollutants. The contaminants examined most extensively for degradation by granular iron include chlorinated solvents (Gillham and O'Hanneisn, 1994; Matheson and Tratnyek, 1994), azo dyes (Weber, 1996; Cao et al., 1999; Nam and Tratnyek, 2000), nitroaromatic pesticides and explosives (Agrawal and Tratnyek, 1996; Hundal et al., 1997; Devlin et al., 1998; Keum and Li, 2004), nitrate (Huang et al., 1998; Zawaideh and Zhang, 1998; Alowitz and Scherer, 2002) and high-valency toxic metals (Gould, 1982; Powell, 1995; Blowes et al., 1997).

The nitro group is a facile electron acceptor and previous studies have shown that iron is capable of reducing organic nitro compounds. Nitrobezene, a nitroaromatic compound, has

received a great deal of research interest in past years because of its high toxicity and structural simplicity (Agrawal and Tratnyek, 1996; Lavine et al., 2001; Scherer et al., 2001; Mu et al., 2004). Agrawal and Tratnyek (1996) reported pseudo-first-order kinetics for nitrobenzene reduction by iron under anaerobic conditions. Nitrobenzene underwent sequential nitro reduction to aniline, producing intermediates of nitroso and hydroxylamine compounds. The potential use of iron for degrading nitramine explosives has also been demonstrated (Hundal et al., 1997; Singh et al., 1998a; Oh et al., 2005). In a cast iron-water system (Oh et al., 2005), both RDX and HMX were transformed to formaldehyde, NH_4^+, N_2O and other soluble products. Methylenedinitramine (MDNA), an intermediate of both RDX and HMX reduction by iron, was also degraded to formaldehyde with iron. Glycerol trinitrate (GTN), a structurally related compound to PETN, has recently been demonstrated to undergo sequential denitration with concomitant release of nitrite in a batch experiment with cast iron (Oh et al., 2004). 1,2-dinitroglycerins, 1,3-dinitroglycerins, 1-mononitroglycerins and 2-mononitroglycerins were detected as the reduction intermediates in the experiment. Based on these, and particularly the findings on GTN, one would expect iron to have a high potential to remediate PETN-contaminated water.

More recently, the use of iron has also been successfully extended to remediate contaminated soil. Under laboratory conditions, the ability of iron to remediate soil contaminated with TNT, RDX (Hundal et al., 1997; Singh et al., 1998a and 1999) and atrazine (Singh et al., 1998b) was reported. These findings suggest considerable potential for using granular iron as a practical method for in situ remediation of contaminated soil. In a pesticide spill site contaminated with metolachlor, the 5% addition of iron decreased metolachlor from 1789 to 504 mg/kg in 90 days. More effective reduction, 1402 to 13 mg/kg, was observed by using 5% iron with $Al_2(SO_4)_3$ and CH_3COOH. In addition to the direct reaction between iron and metolachlor, the presence of $Al_2(SO_4)_3$ and CH_3COOH altered the pH, redox potential and soil solution composition during corrosion of the iron surface, in turn facilitating reductive transformation (Comfort et al., 2001). In another field trial involving 70 kg soil from a munitions wastewater disposal site, using iron (5%, w/w), RDX concentration

decreased to 540 mg/kg, resulting in a 96% removal from the average initial concentration of 12,100 mg/kg within 120 d (Comfort et al., 2003).

The reductive transformation of contaminants in Fe^0-H_2O systems has been demonstrated to be a surface-mediated process (Weber, 1996). Typically, five steps are involved in such surface reactions (Matheson and Tratnyek, 1994): (i) mass transport of reactant to the reactive surface from bulk solution; (ii) adsorption of reactant to the surface; (iii) chemical reaction at the surface; (iv) desorption of products from the surface; and (v) mass transfer of products back to the bulk solution. Any one or a combination of these steps may affect the overall kinetics. For compounds with fast reaction rates, the likelihood of mass transfer processes affecting the overall degradation kinetics is increased. The correlation between reaction rate and square root of mixing rate is commonly used to evaluate the influence of mass transport on degradation rate. The reduction of RDX (Fenton, 2001) and nitrobenzene (Agrawal and Tratnyek, 1996) by iron both were demonstrated to be mass transport limited reactions since the rate coefficient exhibited a linear relationship with respect to the square root of mixing rate. Further, Scherer et al. (2001) separated external mass transport rate from surface reaction rate by using a rotating disk electrode. For nitrobenzene, the measured rate coefficient for surface reaction (1.7×10^{-3} cm s^{-1}) was almost 10 times faster than the mass transport rate coefficient (2×10^{-4} cm s^{-1}), indicating a significant mass transport limitation on degradation kinetics for nitrobenzene. Of the many classes of compounds degraded by iron, compounds containing nitro groups such as RDX, TNT and nitrobenzene, generally exhibit much faster rates of reduction by iron than chlorinated solvents such as TCE and VC (Scherer et al., 2001), implying the increased potential for mass transport to limit the overall kinetics of contaminant reduction. The mass transfer limitation may be the case for PETN, being a similar compound to other organic nitrate contaminants, containing readily reducable nitro groups.

1.1.3.2 Bioremediation of Explosives

The persistence of explosives in the environment is associated with the fact that they are anthropogenic compounds and the occurrence of biologically-derived nitro-organic

compounds of similar structure is very rare in nature. This reduces the possibility for indigenous bacterial populations to have the capability of degrading these compounds. The most promising bacterial communities that could provide biotransformation pathways are the acclimated indigenous microbial communities which have been exposed to contaminants for a long period of time. Natural attenuation, the use of naturally selected microbial populations to incorporate contaminants into their primary metabolism as a source of carbon and energy, is an attractive model for remediation of xenobiotics. This scheme motivated many studies on isolating pure strains of bacteria which can derive energy and/or nutrients needed for microbial growth from organic contaminants. Other studies have focused on identifying necessary parameters for stimulating the activity.

TNT is the most extensively studied nitrogen-containing explosive. Several reviews have been published on aerobic and anaerobic degradation of TNT (Ahmad and Hughes, 2000; Hawari et al., 2000; Esteve-Nuñez et al., 2001). Under aerobic conditions, microorganisms tend to transform TNT by reducing one or two nitro groups to hydroxylamino or amino groups. Generally, anaerobic bacterial strains are capable of reducing TNT to TAT, and some strains can use TNT as a sole nitrogen source. To date, no microorganism has been identified that is capable of using TNT as both a carbon and energy source. RDX and HMX, typical compounds of nitramines, have been demonstrated to resist aerobic degradation (McCormick et al., 1981). An exception was the finding of bacterial populations isolated from contaminated soil which were capable of using RDX as a sole source of nitrogen for growth under aerobic conditions (Binks et al., 1995; Coleman et al., 1998). Under anaerobic conditions, *C. acetobutylicum* was able to transform RDX into hydroxylamino and amino derivatives (Zhang and Hughes, 2003). Similarly, *K. pneumoniae* can degrade RDX to HCHO, CH_3OH, CO_2 and N_2O through intermediary formation of methylenedinitramine ($O_2NNHCH_2NHNO_2$) (Zhao et al., 2002). Three species of the family *Enterobacteriaceae*, isolated from nitramines explosives-contaminated soil, can reduce both RDX and HMX under anaerobic conditions (Kitts et al., 1994). For nitrate esters, Marshall and White (2001) isolated four anaerobic bacterial species of *P. putida*, *Arthrobacter*, *Klebsiella* and

Rhodococcus from GTN-contaminated soil, all of which can use GTN as the sole nitrogen source. Significantly, *Rhodococcus* sp. was capable of complete denitration of GTN.

Microbial co-metabolism is frequently observed in transformation of xenobiotic non-growth substrates and serves as a potential tool for remediation of recalcitrant synthetic compounds (Janke and Fritsche, 1985). Related studies have been performed on bioremediation of explosives using carbon amendments (e.g., starch, glucose, sucrose, or molasses). In anaerobic processes, the presence of additional substrate facilitates oxygen removal by growing aerobes and also serves as the electron donor for reduction of nitro groups. Roberts et al. (1996) examined the ability of glucose, soluble starch, insoluble starch and acetate as external carbon sources to create anaerobic conditions for TNT removal. The addition of glucose induced the fastest decline and lowest redox potential and resulted in rapid and complete removal of TNT in the contaminated soil. In a soil slurry reactor, contaminants such as TNT, HMX and tetryl can all be effectively removed from contaminated soil by indigenous bacteria through co-metabolic processes with the addition of molasses for bacterial growth (Boopathy, 2000, 2001b and 2005).

Most of the work has focused on nitroaromatics and nitramines, particularly on TNT and RDX. To date, no work has been reported on the target explosive of this study, PETN, regarding biodegradation as a remediation method.

1.1.3.3 Iron-Microbial Treatment Approach

Previous studies found that a Fe^0-microbial treatment system may offer significant advantage over treatment with Fe^0 alone or anaerobic bacteria alone for remediation of contaminated groundwater. Such synergistic effects to enhance treatment efficiency were reported for chlorinated solvents (Weathers et al., 1997; Gregory et al., 2000; Lampron et al., 2001), nitrate (Till et al., 1998; Kielemoes et al., 2000), chromium (Gandhi et al., 2002) and explosives (Oh et al., 2001; Wildman and Alvarez, 2001; Oh and Alvarez, 2002 and 2004).

For nitrate explosives, the benefits of an integrated system with iron and microorganisms may be a consequence of the following factors. First, iron corrosion can rapidly create anaerobic conditions that favor biotransformation of explosives. Second, the water-derived hydrogen gas, produced during anoxic iron corrosion, can serve as an electron donor to support biodegradation of explosives. In addition, reduction or removal of nitro groups by iron may result in the formation of products that are persistent with respect to iron, such as amino-derivatives from reduction of nitroaromatics, whereas these may be more susceptible to biotransformation, leading to a higher potential for complete mineralization of contaminants. Hundal et al. (1997) observed that iron pretreatment increased TNT mineralization, evidenced by greater CO_2 production than microbial degradation processes alone. In addition, the presence of iron-reducing bacteria may reduce $Fe(III)$ oxides that passivate iron surfaces, thus enhancing the reactivity of iron and the treatment efficiency.

1.1.4 Previous Studies of PETN

Bacterial metabolism of PETN was first recognized through medical applications. King and Fung (1984) found that PETN degradation in rat urine and feces was primarily of bacterial origin. PETN rapidly decayed, following pseudo-first-order kinetics, with a half-life of 20 min after a short lag phase at 25ºC. The PETN appeared to undergo sequential denitration, as indicated by the formation of metabolites including tri-, di-, and mononitrated pentaerythritol (PETriN, PEDN and PEMN) and pentaerythritol during the 24 h period.

Enterobacter cloacae PB2 (Binks et al., 1996) was isolated from a mixed microbial culture obtained from soil enrichments under aerobic and nitrogen-limiting conditions and was shown to be capable of metabolizing PETN. The pure culture was capable of aerobic growth with PETN as the only nitrogen source and utilized 2 atoms of nitrogen per mol of PETN, producing metabolites of pentaerythritol dinitrate, 3-hydroxy-2,2-bis-[(nitrooxy)methyl] propanal and 2,2-bis-[(nitrooxy)methyl]-propanedial. The responsible enzyme, an NADPH-dependent PETN reductase, was isolated from cell extract and found to reductively release nitrite from PETN producing tri-, and dinitrate pentaerythritol. *Enterobacter cloacae PB2* was also capable of slow aerobic growth using TNT as the sole

nitrogen source (French et al., 1998). The results suggest that the *Enterobacter cloacae PB2* and the PETN reductase could be useful for bioremediation of both nitrate esters and nitroaromatics contaminated soil and water.

Phytoremediation is an alternative in-situ bioremediation technology used for cleaning up contaminated sites by green plants. Vanek et al. (2003) studied the phytoremediation of PETN in model systems of plant tissue cultures (*Rheum palmatum*, *Saponaria officinalis and Populus simonii*). The analysis of products suggested that PETN was fully degraded to penterythritol as an end product via sequential denitration in 10 days. The application, however, may be limited by the fact that the indigenous biodegradability of plants is less effective than those of adapted bacteria. This limitation might be overcome by incorporating bacterial nitroreductase genes into the plant genomes (French et al., 1999; Hannink et al., 2001; Rosser et al., 2001). However, this in-situ technology is still in the demonstration stage, and has many limitations such as dependence on location and season, soil depth and toxic effects caused by high concentrations of contaminants. The effectiveness would be less for contaminants with low solubility and weak adsorption to the soil sediments such as PETN.

In summary, even though PETN is of environmental concern and may pose a threat to ecosystem and human health, it has received very little attention with respect to its fate in the environment and very limited information is available regarding remediation technologies for contaminated water and soil. Like many other explosives (Isayev et al., 2006), the electron-withdrawing character of nitro groups on PETN would be responsible for the low susceptibility to typically advanced oxidative catabolism; therefore anaerobic conditions hold greater potential for remediation of explosives-contaminated sites. Thus, the potential remediation methods were tested under anaerobic conditions in this study.

1.2 Goal and Objectives

The goal of this study was to develop an effective method to remediate PETN-contaminated water and soil. To achieve this goal, the following specific objectives were identified:

1) Evaluate the potential use of granular iron for remediating PETN-contaminated water and determine its transformation kinetics;
2) Determine intermediate products and thus elucidate the reaction pathways in the presence of granular iron;
3) Investigate the potential rate-limiting factors in PETN degradation by iron and explore means of enhancement;
4) Evaluate the biodegradability of PETN in aqueous phase under anaerobic conditions;
5) Identify intermediate products and elucidate the pathway of PETN biodegradation;
6) Determine the effects of other electron acceptors on PETN biodegradation;
7) Evaluate the potential use of granular iron for remediating PETN-contaminated soil;
8) Evaluate bioremediation of PETN-contaminated soil under anaerobic conditions, including the possible effects of carbon source amendments;
9) Evaluate remediation of PETN-contaminated soil in an iron-microbial combined system;
10) Test the effectiveness of laboratory-tested remediation methods in a pilot-scale study at a contaminated site.

1.3 Scope of Research

This study was conducted in four phases:

Phase-I: Abiotic degradation of aqueous PETN by granular iron

This phase was designed to fulfill objectives 1 to 3, including studies of degradation kinetics, identification of intermediate products, elucidation of the reaction pathways, and determining the rate-limiting factor. Laboratory iron column and batch experiments were used.

Phase-II: Biotic degradation of PETN in aqueous phase under anaerobic conditions

This phase addressed objectives 4 to 6, to explore the biodegradability of aqueous PETN under anaerobic conditions, to elucidate the degradation pathway and further examine the effects of other electron acceptors such as nitrate and sulfate on PETN biodegradation. This phase involved the series enrichment procedures and aqueous microcosm batch tests.

Phase-III: Remediation of PETN in contaminated soil in laboratory-scale experiments

To achieve objectives 7 to 9, systematic laboratory soil microcosm experiments were conducted to evaluate the potential of using granular iron, biodegradation and iron-microbial integrated methods for remediation of PETN-contaminated soil.

Phase-IV: Field demonstration at Louvier, Colorado

Based on the laboratory results, a range of treatment options was proposed and tested in a field trial at a PETN-contaminated site.

Chapter 2

Degradation of PETN by Granular Iron

The objective of this study was to evaluate the use of granular iron for remediating PETN-contaminated water. Using column procedures, we determined PETN reduction kinetics with iron, identified the intermediates produced by PETN degradation and proposed the reaction pathways. In additional batch experiments, the effect of mass transfer limitation on PETN degradation kinetics was examined and methods for enhancement were explored.

2.1 Materials and Methods

2.1.1 PETN Synthesis

PETN used to conduct the experiments was synthesized in the laboratory by adding pentaerythritol to an excess nitrating solution consisting of concentrated HNO_3 and H_2SO_4 in a ratio of 2:1. The solution was kept on ice to maintain the temperature below 25~30 ºC and was stirred occasionally over a 30 min period. The raw PETN was filtered, washed with DI water, and neutralized with sodium carbonate solution. PETN was purified by recrystallization with acetone and air-dried before use. The synthesized PETN was verified using an analytical standard (0.1 mg/mL in methanol) purchased from AccuStandard Inc. (New Haven, Connecticut). The analytical standard was also used for external calibration.

2.1.2 Iron and Silica sand

Granular iron was obtained from Connelly-GPM Inc. (Chicago, Illinois). It was used without pretreatment for the column experiments, but was immersed in either Millipore water or co-solvent solution for 10 days before being used in the batch experiments. The iron material was characterized previously, containing 89.8% metallic iron and the surface was covered with various forms of iron oxide (data provided by Connelly-GPM Inc., 1998). The specific surface area of iron used in this study was 1.02 m^2/g, measured by the BET (Brunauer-Emmett-Teller) method using a Micrometrics Gemini III 2375 Surface Area Analyzer.

ASTM 20-30 sand was purchased from US Silica Company (Ottawa, Illinois). The sand was washed with 10% nitric acid, rinsed three times with Millipore water and oven dried before use.

2.1.3 Column Experiments

The configuration of the two columns used in this study was similar to that described in Gillham and O'Hannesin (1994). Each Plexiglas column was 30 cm long by 2.54 cm I.D. Along the column, 16 sampling ports were located at distances of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 17, 21, 25 and 29 cm from the influent end. Each sampling port consisted of a nylon Swagelok$^{®}$ fitting (0.16cm O.D.) with a 16G Luer-Lok$^{®}$ syringe needle. One column was packed with 100% Connelly iron (porosity = 0.59), and the other was packed with a mixture of 30% Connelly iron and 70% ASTM 20-30 silica sand by weight (porosity = 0.42). The ratios of surface area of iron to solution volume for the 100% and 30% iron columns were 4.70 and 1.37 m^2/mL, respectively.

PETN solution was prepared by adding crystalline PETN to Millipore water, stirring for 48 h followed by filtration. The concentration of PETN solution was therefore at its water solubility (6 mg/L). The solution was deoxygenated by sparging with nitrogen gas for approximately 2 h before being delivered to the column. During the entire period of the experiment, the solution was stored in a carboy with a headspace of nitrogen gas to prevent oxygen invasion. Flow rates, determined by measuring discharge over a period of time, were 8.90 mL/min and 1.34 mL/min for the 100% and 30% iron columns, respectively. Aqueous samples were collected by clamping the effluent line to allow water to flow from the sampling port to a glass syringe. The solution was then transferred from the syringe to analytical vials for both organic (PETN and potential intermediates) and inorganic (NO_3^-, NO_2^- and NH_4^+) analyses. Samples were collected periodically over time and data analysis was performed with only the concentration profiles obtained after the steady state had been achieved.

2.1.4 Batch Experiments

Two batch experiments were conducted in this study. The first set (Fe^0-H_2O) was to examine the effect of PETN dissolution rate on PETN reduction kinetics by iron. The second set (Fe^0-co-solvent) was to examine the potential for using a co-solvent to enhance PETN solubility and thus to increase the rate of PETN reduction. The co-solvent used in this study was 50% water and 50% methanol. The set-up, pretreatment method and sampling procedures were identical for both batch experiments. Before initiating the degradation experiments, each batch vial was prepared in the sequence of weighing the empty vial, adding a specific amount of iron, filling the vial with deoxygenated Millipore water (first set) or co-solvent solution (second set), crimp-sealing with Teflon-lined silicon septum and cap leaving no headspace, and re-weighing. The vials were stored for 10 days to remove most of the pre-existing passive surface oxides by the auto-reduction processes (Odziemkowski et al., 2000; Ritter et al., 2002). In order to reduce abrasion of the iron surface during rotation, the iron particles were held in place by magnets, attached on opposite sides of the vials.

The Fe^0-H_2O experiment consisted of 4 sets of vials in duplicate, each set with a different Fe/H_2O ratio and 8 mg of PETN. Connelly iron in amounts of 20, 10, 5, or 1 g were added to 60 mL glass vials, resulting in solution/iron ratios (g/g) of approximately 3, 6, 12, and 60. The Fe^0-co-solvent experiment consisted of 5 sets of 40 mL glass vials in duplicate. Each pair contained 10 g of Connelly iron and 20, 35, 50, 65 or 80 mg/L of PETN.

The reduction reaction was initiated by addition of PETN stock solution in acetonitrile (10,000 mg/L) via needle injection through the septum into the individual vials. In the first batch experiment, 8 mg PETN was introduced to all vials by spiking PETN stock solution and allowing it to recrystallize. In the second batch experiment, a specific amount of PETN stock solution was injected into each set of vials to achieve the desired initial concentrations (20, 35, 50, 65 and 80 mg/L). Because of the co-solvent effect, PETN remained in solution. All vials were loaded onto an orbital rotator at 50 rpm. At particular elapsed times, duplicates

were sacrificed for organic and inorganic analyses. PETN were analyzed for both batch tests and NO_3^-, NO_2^- and NH_4^+ were analyzed only for the first set.

2.1.5 Analytical Methods

Concentrations of nitrate and nitrite were analyzed using a Dionex ion chromatograph (Dionex ICS 2000) equipped with an Ion-Eluent Generator and conductivity detector and a Dionex AS-40 autosampler. A Dionex IonPac AS18 column (4×250 mm) was used. The injection volume was 25 μL and the mobile phase was 30 mM KOH at a flow rate of 1.2 mL/min. The detection limit for both nitrite and nitrate was 0.5 mg/L.

Ammonium (NH_4^+-N) analysis was performed using the phenate method, following the procedures described in Standard methods for the examination of water and wastewater (American Public Health Association, 1985). The absorbance at a wavelength of 630 nm was measured using a Beckman DU 530 UV/VIS spectrophotometer with a light path of 1cm. The detection limit was 0.1 mg/L.

Analyses for PETN and intermediate products were performed using a series 1100 Hewlett-Packard high performance liquid chromatograph (HPLC) equipped with a UV visible diode array detector, a quaternary pump, an autosampler and a degasser. A Zorbax SB-C18 column (3.5 μm particles, 4.6×150 mm) and a Zorbax guard column (5 μm particles, 4.6×12.5 mm) were used. Aqueous samples were centrifuged in 1.5 mL vials for 5 min at 10,000 rpm prior to being loaded onto the atuosampler. A water-methanol-acetonitrile mixture (40:50:10, v/v/v) was used as the mobile phase at a flow rate of 1.0 mL/min. The injection volume was 100 μL and the absorbance was measured at a wavelength of 210 nm. The detection limit for PETN was 0.1 mg/L. A series of PETN external standards was prepared using the analytical standard and analyzed with the samples for PETN quantification. Because standards for the intermediates were not commercially available, the intermediates were not quantified.

To identify unknown intermediates, fractions of unknown peaks appearing on the HPLC chromatogram were collected at the corresponding retention times through repeated injections and concentrated by air-drying. The samples were redissolved in acetonitrile and analyzed using the positive ion ammonia chemical ionization method, with a JEOL HX110 double focusing mass spectrometer operated at a mass resolution of ~1000. The source temperature was 200°C with an electron energy of 200 eV. All samples were introduced by direct injection and heated when necessary.

2.2 Results and Discussion

2.2.1 PETN Degradation Kinetics

The kinetics of PETN degradation was studied using the iron column tests. Distances along the column were converted to residence time, using the porosity and measured flow rate and assuming the columns were saturated and homogeneous. The measured PETN concentrations were normalized and plotted as C/C_0 versus residence time. Figure 2.1 shows three concentration profiles for the 100% iron column, all measured after steady-state conditions had been reached. Fitting the first-order kinetic model to the data gave an average R^2 value (correlation coefficient of a least-squares fit) of 0.96±0.005, indicating the kinetics of PETN degradation to be pseudo-first-order. The average rate constant was 2.70±0.22 min^{-1}, giving a half-life of 0.26±0.02 min. Due to the extremely fast degradation rate in the 100% iron column, there may be a significant level of uncertainty in the measured rate constant. Consequently, the amount of iron was reduced to 30% in the second column. Figure 2.2 shows four representative PETN concentration profiles in the second column, measured between 380 and 650 PV after the achievement of steady state. The pseudo-first-order model also fit the data well (R^2 = 0.97±0.028). The average rate constant was 0.44±0.029 min^{-1}, giving a half-life of 1.58±0.10 min. The corresponding surface area-normalized rate constants (k_{SA}) for the 100% and 30% iron columns were (3.44±0.28) ×10^{-2} and (1.92±0.13) ×10^{-2} L·m^{-2}·h^{-1}, respectively. These values are similar and in reasonable agreement with k_{SA} values reported in the literatures for other explosives that had been tested with similar iron materials. For example, Oh et al. (2004, 2005) reported values of 1.65±0.30 ×10^{-2} L·m^{-2}·h^{-1},

$3.60 \pm 0.64 \times 10^{-2}$ $L \cdot m^{-2} \cdot h^{-1}$ and $7.72 \pm 0.81 \times 10^{-2}$ $L \cdot m^{-2} \cdot h^{-1}$ for nitroglycerin, RDX and HMX reduction by granular iron, respectively. These reported average rate constants for nitro-compounds, including PETN, were almost 2 orders of magnitude faster than rate constants of dehalogenation of most chlorinated compounds by iron ($\sim 10^{-4}$ $L \cdot m^{-2} \cdot h^{-1}$) (Johnson et al., 1996). Previous studies (Agrawal and Tratnyek, 1996; Burris, 1996; Hung et al., 2000; Scherer et al., 2001) have shown that the reduction of nitrobenzene, highly reactive in the presence of iron ($k_{SA} \sim 1$ $L \cdot m^{-2} \cdot h^{-1}$), was mass transport limited. The fact that the k_{SA} values for a wide range of compounds falls within a narrow range further suggests the rate of degradation for these compounds to be limited by mass transport rather than by degradation rate. Thus, it is likely that the rate of degradation of PETN in the iron columns were mass transfer limited. The potential rate limiting factor will be considered further in section 2.2.5.

2.2.2 Nitrogen Mass Balance

In addition to PETN, NH_4^+, NO_2^- and NO_3^- were measured to establish the nitrogen mass balance during the column experiments. No NO_3^- was detected over the entire experiment. Figure 2.3 is typical of the concentration profiles of PETN, NH_4^+ and NO_2^- (expressed in relative moles of N) along the column, taken from the 30% iron column after 420 PV. PETN concentration decreased to the detection limit (100 µg/L) within 10 min. Concurrently, the concentration of NO_2^- reached a maximum of 49% of the initial N and began to decline. Meanwhile, NH_4^+ continued to accumulate over time. NO_2^- gradually decreased to below the detection limit and the amount of NH_4^+ approached a maximum towards the end of the column. Throughout the column, the nitrogen mass recovery, the ratio of the sum of the N in nitrogenous compounds including PETN, NH_4^+ and NO_2^- to the initial N in PETN, expressed in percentage, ranged from 84% to 104%. In particular, towards the end of the column, all of the N in the initial PETN was present as NH_4^+, suggesting that all the nitro groups on PETN were reductively removed by the release of nitrite, which was subsequently reduced to NH_4^+, as the final nitrogen-bearing product. The reduction of NO_2^- by iron, producing NH_4^+, was previously reported (Hu et al., 2001; Alowitz and Scherer, 2002).

A reproducible dip in the nitrogen mass balance occurred at about 4 to 5 min (Figure 2.3). The deficit was attributed to unidentified nitrogen-bearing intermediates. Three unknown peaks were observed on the HPLC chromatograms during PETN analyses, appeared at retention times of 1.3 (peak-1), 2.3 (peak-2) and 3.9 (peak-3) min (cf. 6.5 min for PETN) (Figure 2.4). All unknown peaks show similar trends of an initial increase to a maximum followed by a decrease, with eventual disappearance for peak-2 and 3 (Figures 2.5) and a plateau for peak-1 before exiting the column (Figure 2.6). The pattern of the appearance and disappearance of the unknown peaks suggests that they are intermediate products of PETN degradation. In addition, peak-3, 2 and 1 reached their maximum at 4.1, 5.8 and 8.5 min, suggesting sequential formation of intermediates. We noted that the area of peak-1 was apparently greater relative to peak-2 and 3. This was a consequence of the interference from nitrite, which eluted at the same time as peak-1. The similar profile of nitrite measurement by IC to peak-1 by HPLC indicates the presence of nitrite in the HPLC analysis; on the other hand, the dissimilarity at maximum and aftermost phase suggests the coexistent of peak-1 rather than nitrite itself (Figure 2.6). Though a larger residence time would be helpful, the data suggests that unknown-1 may have approached a constant value towards the end of the column where the nitrite concentration was below detection limit.

2.2.3 Identification of Intermediate Products

Sample fractions of the three unknown peaks separated by the HPLC column were manually collected at the corresponding retention times in clean vials, air dried, re-dissolved in acetonitrile and analyzed by positive NH_3-CI mass spectrometry. When ammonia is used as the reagent gas in chemical ionization, compounds react with ionized ammonia molecules to form $[M+H]^+$ and /or $[M+NH_4]^+$ ions (van Leuken and Kwakkenbos, 1992). When pure PETN was analyzed by this method, five fragment ions at m/z of 334, 289, 244, 199 and 154 appeared in the spectrum (Figure 2.7). The equidifferent value in this progression is 45, suggesting a stepwise replacement of nitrite by hydrogen on PETN. The molecular weights of the five fragments were consistent with PETN (MW=316), pentaerythritol trinitrate (MW=271), pentaerythritol dinitrate (MW=226), pentaerythritol mononitrate (MW=181) and pentaerythritol (MW=136) in $[M+NH_4]^+$ form. The mass spectrum of PETN suggests that the

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molecular ion $[M+NH_4]^+$ form of PETN was not stable at the high temperature and lost nitrite groups during LC/MS analysis. When pure pentaerythritol of analytical grade was analyzed using this method, two fragment ions at m/z of 154 and 137 occurred in the spectrum (Figure 2.8), corresponding to the ionization products of pentaerythritol in both $[M+H]^+$ and $[M+NH_4]^+$ form. The spectrum showed that the $[M+H]^+$ ion was only about 10% of the quantity of $[M+NH_4]^+$ ion. These fragment ions of pure compounds were used to tentatively identify the reaction intermediates.

Figures 2.9, 2.10 and 2.11 show the mass spectra of the three unknown peaks-1, 2, 3, analyzed by the same method. Figure 2.9 contains two major ions at m/z of 154 and 137, identical to the spectrum of pure pentaerythritol, indicating that unknown peak-1 was pentaerythritol. In Figure 2.10, the spectrum contains fragment ions at 154, 199 and 244, corresponding to the m/z of pentaerythritol, mononitrate and dinitrate pentaerythritol in $[M+NH_4]^+$ form. In Figure 2.11, the spectrum includes fragment ions at 154, 199, 244 and 289, corresponding to the m/z of pentaerythritol, mononitrate, dinitrate, and tirinitrate pentaerythritol in $[M+NH_4]^+$ form. Since the reaction intermediates bear structural features in common with the parent compound PETN, they would behave consistently in LC/MS analysis with PETN. Therefore, by comparing these spectra with the PETN spectrum, the two unknown chemicals in Figures 2.10 and 2.11 were identified as pentaerythritol dinitrate (peak-2) and pentaerythritol trinitrate (peak-3), which lost nitro groups stepwise during LC/MS analysis, similar to PETN. Because of the unavailability of analytical standards for pentaerythritol dinitrate and pentaerythritol trinitrate and the interference of nitrite with pentaerythritol, the identified intermediates could not be quantified in this study. Instead of concentration, the variation in peak size with reaction time was used to describe the behavior of the breakdown products (Figures 2.6 and 2.7). The production of identified pentaerythritol dinitrate and pentaerythritol trinitrate as transient intermediate products explains the reproducible early-time deficit in the nitrogen mass balance because their maximum peak areas coincide with the lowest range in the nitrogen mass recovery curve.

2.2.4 Degradation Pathway

Based on the identified intermediate breakdown products, tri- and dinitrate pentaerythritol, and the final carbon-bearing end product, pentaerythritol, as well as the near 100% nitrogen mass balance, it is proposed that PETN degradation in the presence of iron follows a sequential reductive degradation pathway with the release of NO_2^- in each denitration step (Figure 2.12), as shown in eq 2.1 to eq 2.4. The nitrite liberated from the stepwise reductive reactions was further reduced by iron, as in eq 2.5. Eq 2.6 indicates that stoichiometrically a total of 32 moles of electrons are required to degrade 1 mol of PETN to NH_4^+ and pentaerythritol as the final reduction products. Thus, considering Fe^{2+} to be the final iron product, 16 moles of Fe^0 would be required. In the iron column tests, the amount of iron significantly exceeded the stoichiometric requirement for complete reduction of PETN, and thus resulted in the pseudo-first-order kinetics of PETN degradation with respect to PETN concentration.

$$C_5H_8(ONO_2)_4 + 2e^- + H_2O \rightarrow C_5H_8(ONO_2)_3(OH) + NO_2^- + OH^- \qquad \text{(eq 2.1)}$$

$$C_5H_8(ONO_2)_3(OH) + 2e^- + H_2O \rightarrow C_5H_8(ONO_2)_2(OH)_2 + NO_2^- + OH^- \qquad \text{(eq 2.2)}$$

$$C_5H_8(ONO_2)_2(OH)_2 + 2e^- + H_2O \rightarrow C_5H_8(ONO_2)(OH)_3 + NO_2^- + OH^- \qquad \text{(eq 2.3)}$$

$$C_5H_8(ONO_2)(OH)_3 + 2e^- + H_2O \rightarrow C_5H_8(OH)_4 + NO_2^- + OH^- \qquad \text{(eq 2.4)}$$

$$NO_2^- + 6e^- + 6H_2O \rightarrow NH_4^+ + 8OH^- \qquad \text{(eq 2.5)}$$

$$C_5H_8(ONO_2)_4 + 32e^- + 28H_2O \rightarrow C_5H_8(OH)_4 + 4NH_4^+ + 36OH^- \qquad \text{(eq 2.6)}$$
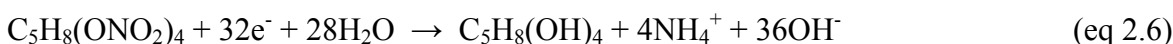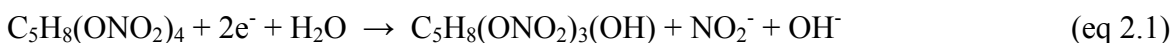
The apparent absence of pentaerythritol mononitrate may be due to (i) the rate of conversion of pentaerythritol mononitrate to pentaerythritol was faster than its formation from pentaerythritol dinitrate; (ii) the amount of pentaerythritol mononitrate was very small, particularly with the low initial PETN concentration, resulting in the concentration being below the detection limit of HPLC analysis; or (iii) the analytical method used in this study was not sensitive to pentaerythritol mononitrate. Even though the lack of mononitrate pentaerythritol made the last denitration step in the degradation pathway somewhat uncertain, the complete nitrogen recovery indicated that all nitrite groups were removed.

Hydrolysis did not appear to be involved since NO_3^- was not detected. If NO_3^- were released, its slower reduction rate with iron compared to NO_2^- would have made it detectable (Rahman et al, 1997; Alowitz and Scherer 2002). Nitrate was also not detected in the later batch experiment conducted at an initial concentration of 200 mg/L PETN (data not shown), which precludes the possibility of production of NO_3^- below the detection limit (0.5 mg/L) caused by the low initial PETN concentration in the column experiments.

The proposed pathway for PETN degradation is similar to that of glycerol trinitrate, another nitrate ester compound. Oh et al. (2004) showed that in the presence of cast iron, glycerol trinitrate was stepwise reduced to 1,2- and 1,3-dinitroglycerin and then 1- and 2-mononitroglycerin and finally to benign products of glycerol and NH_4^+. The reduction process was proposed to be reductive rather than hydrolytic because of the absence of NO_3^- during the experiment, consistent with the observations of this study.

2.2.5 Mass Transfer Considerations

As described in section 1.1.3.1, the rate-limiting factor for PETN degradation could be any one of the five steps involved in the reduction of contaminants on the surface of iron. However, it is difficult to determine the relative rate of each step because many of the parameters can not be measured directly. As an alternative, we used the method of Arnold et al. (1999), in which the observed rate of reduction can be represented as the sum of contributions of mass transfer and surface reaction (eq 2.7 or eq 2.8), in which the mass transfer coefficient can be estimated independently.

$$\frac{1}{k_{obs}} = \frac{1}{\alpha}(\frac{1}{k_{mt}} + \frac{1}{k_{SA-geom}}) \qquad \text{eq 2.7}$$

$$k_{obs} = \frac{\alpha \bullet k_{mt} \bullet k_{SA-geom}}{k_{mt} + k_{SA-geom}} \qquad \text{eq 2.8}$$

where k_{obs} is the overall reaction rate constant (s^{-1}), observed by batch or column experiments; k_{mt} ($m^.s^{-1}$) is the mass transfer rate coefficient; $k_{SA-geom}$ ($m^.s^{-1}$) is the surface-area-normalized rate constant in which the geometric (external) specific surface area of the

particles is used rather than the BET specific surface area; α is the ratio of iron geometric surface area to solution volume (m^{-1}).

The difference between the rate of mass transfer (k_{mt}) and the rate of surface reaction ($k_{SA\text{-}geom}$) can determine three potential kinetic domains: (i) mass transfer limited kinetics, where the mass transfer of contaminant to the iron surface is much slower than the reaction rate at the iron surface ($k_{mt} \ll k_{SA\text{-}geom}$, $k_{obs} \approx \alpha k_{mt}$); (ii) reaction limited kinetics, where the reaction of contaminant with iron at the surface is much slower than the rate of mass transfer ($k_{SA\text{-}geom} \ll k_{mt}$, $k_{obs} \approx \alpha k_{SA\text{-}geom}$); (iii) intermediate kinetics, where the mass transfer and reaction rates are similar ($k_{SA\text{-}geom} \approx k_{mt}$, $k_{obs} \approx 0.5\ \alpha k_{SA\text{-}geom}$ or $0.5\ \alpha k_{mt}$).

In this study, an empirical correlation (eq 2.9) developed to represent mass transport in packed-bed reactors (Scherer et al., 2001) was used to estimate the mass transfer rate coefficient (k_{mt}) for PETN in the iron column.

$$Sh = \frac{k_{mt} \bullet d_P}{D} = 1.09\varepsilon^{-2/3}\,\mathrm{Re}^{1/3}\,Sc^{1/3} \qquad\qquad \text{eq 2.9}$$

where Sh is the Sherwood number, d_P is the particle diameter (m), D is the diffusion coefficient of the reactant in water (m^2/s). Reynolds number ($\mathrm{Re} = ud_P/\nu$) and Schmidt number ($Sc = \nu/D$) are independent variables in the correlation, in which u is velocity (m/s), ν is kinematic viscosity of the fluid (m^2/s) and ε is the porosity. For the 100% iron column, with fluid velocity of 4.96×10^{-4} m/s, particle diameter of 0.65 mm, porosity of 0.59, and a PETN diffusion coefficient of 5.65×10^{-6} cm^2/s, the k_{mt} value was computed using eq 2.9 to be 6.72×10^{-4} $m^{\cdot}min^{-1}$.

$k_{SA\text{-}geom}$ was not determined, but as indicated by eq 2.8, a comparison of αk_{mt} with k_{obs} can be used as an indication of the relative magnitude of the mass-transfer limitation term. Assuming ideal spherical geometry, α, the ratio of iron geometric surface area to solution volume, was estimated to be 3.34×10^{3} m^{-1} for the 100% iron column. With the calculated k_{mt} value, the αk_{mt} value is 2.24 min^{-1} (calculation shown in Table 2.1). From the kinetics

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experiment, the average value of k_{obs} for the 100% iron columns was 2.70 min^{-1}. Therefore, in the 100% iron column, αk_{mt} contributes 83% of k_{obs}. It is clear that the contribution of αk_{mt} to k_{obs} is substantive, suggesting that the reduction of PETN could be at least partially limited by external mass transfer.

2.2.6 Effect of Dissolution on Mass Depletion and Enhancement

In the case of dissolved PETN, though the rate of degradation may be limited by mass-transfer processes, the rate is nevertheless very high. However, in spite of the high rate constant, if solid-phase PETN is present, rates of mass depletion may be limited by the relatively low solubility of PETN (6 mg/L). In such cases, the mass depletion is likely to be controlled by the rate of PETN dissolution. The first batch experiment described in the method section, in which the initial PETN mass exceeded its solubility, was performed to evaluate this possibility. Figures 2.13 and 2.14 summarize the concentrations of PETN in the solution phase and the masses of nitrogenous reduction products (sum of N in NO_2^- and NH_4^+ divided by N in initial PETN) during PETN reduction processes for the four tests conducted with different loadings of iron at 20, 10, 5 and 1 g.

In Figure 2.13, the data show a relatively slow decline in the aqueous concentration of PETN for all cases and an apparent inconsistency with the first-order kinetic model. In this test, when crystal PETN is present, the concentration of PETN in solution is a consequence of depletion by degradation and addition by dissolution. The decrease in concentration within the first 3 days suggests that dissolution of PETN was likely to be a limiting factor on its reductive transformation; if not so, the aqueous concentration should tend to remain constant or achieve stable at the level of its solubility. As a common trend for all cases, following the initially rapid decline in PETN concentration, the aqueous concentrations approached steady values (below solubility), suggesting equilibrium between the rates of degradation and dissolution. It is noted that the "equilibrium" concentration of PETN in solution over the entire experiment decreased with increasing iron loading. This is undoubtedly a consequence of a higher rate of depletion in the solution phase by the greater surface area concentration of iron. The consequently increased concentration gradient should enhance the rates of

dissolution and thus the rates of mass depletion. This is clearly reflected in the accumulated masses of the nitrogenous reduction products (Figure 2.14) over time, an indicator of mass depletion of PETN. For the 20 g iron test, with the lowest PETN concentration in solution, complete removal of PETN was achieved in 5 days. Progressively lower iron loadings resulted in progressively higher aqueous concentrations and thus lower rates of PETN removal. By day 8, the nitrogen mass recovery reached only 44% in the test with 1 g iron. A linear increase in the nitrogen mass balance was observed after 2 or 3 d, especially in the tests with 10, 5, and 1 g iron, corresponding with the period during which the PETN concentration in solution was constant. This trend in the test with 20 g iron was not distinctive, which was likely due to the rapid and complete reduction of PETN before achieving steady state.

Results suggest that the more iron present and thus the faster degradation rate, will result in the lower concentration of PETN at steady state, the more likely that the dissolution of PETN will limit the degradation process. Following this trend, one would expect the more significant limitation of dissolution on PETN mass removal when PETN degradation was conducted with more iron, such as the cases of column experiments.

Under conditions where crystalline PETN is present, the primary limiting factor for PETN mass depletion is the dissolution process, with iron concentration being of secondary importance. Therefore, the potential for improving the rate of degradation by means of increasing PETN solubility was examined. Methanol (50%), as a co-solvent, was used for this purpose in the second batch experiment. Very similar pseudo-first-order disappearance of PETN was observed in the five batches (Figure 2.15), which were initiated with different PETN concentrations at 20, 35, 50, 65 and 80 mg/L. PETN was degraded to below detection limit after 12 h in all batches at an average half-life of 1.68±0.38 h. The results show that with the loading of iron used in this study, and within the concentration range from 20 to 80 mg/L, the rate of PETN mass removal from solution can be increased when the aqueous PETN concentration increases.

Co-solvents, though effective in increasing mobility and solubility of hydrophobic contaminants, may also pose negative effects on degradation rates. To explore this potential effect, a supplemental parallel batch experiment using 10 g of iron and aqueous PETN (6 mg/L), in the presence and absence of co-solvent, was conducted. The k_{SA} was 8.81×10^{-3} and 2.90×10^{-3} $L \cdot m^{-2} \cdot h^{-1}$ for PETN reduction in the water and co-solvent systems, respectively. Apparently, the degradation rate in the water system was about 3 times greater than that in the presence of co-solvent. The difference suggests a decrease in degradation rate caused by the presence of co-solvent. In a similar experiment, Clark II et al. (2003) found that the dechlorination rate by granular iron decreased with increasing co-solvent fractions. The decrease in degradation rate was attributed to a reduction in organic sorption on the iron surface.

Despite the somewhat negative effect of the co-solvent, the rate of mass removal can still be enhanced by using a co-solvent. Given the k_{SA} values of 1.60×10^{-3} and 8.81×10^{-3} $L \cdot m^{-2} \cdot h^{-1}$ in the Fe^0-co-solvent and Fe^0-H_2O system, 1.60×10^{-3} L and 8.81×10^{-3} L of PETN-contaminated water can be completely treated in unit time with unit iron surface in the Fe^0-co-solvent and Fe^0-H_2O system, respectively. As a consequence, PETN mass removal in the Fe^0-co-solvent system would be 0.13 mg (1.60×10^{-3} L of 80 mg/L PETN-contaminated water) whereas the mass depleted in the Fe^0-H_2O system was 0.05 mg (8.81×10^{-3} L of 6 mg/L PETN-contaminated water). Therefore, using a co-solvent to increase PETN solubility can be an effective means to improve treatment efficiency when crystalline PETN is present.

2.3 Conclusion

This study demonstrated that granular iron can degrade PETN in the aqueous phase rapidly and effectively. The reductive reaction followed pseudo-first-order kinetics with a rate constant of 2.70 ± 0.22 min^{-1} in the 100% iron column and 0.44 ± 0.029 min^{-1} in the 30% iron column. In the presence of iron, PETN degradation proceeds through a reductive denitration process. Based on the detection of tri- and dinitrate pentaerythritol and pentaerythritol, as well as NO_2^- and NH_4^+, it appeared that PETN underwent sequential denitration with the

transient formation of trinitrate pentaerythritol and dinitrate pentaerythritol, releasing NO_2^-, which was further reduced to NH_4^+. Mononitrate pentaerythritol was not detected, leaving the last step of denitration unconfirmed. However, the nearly 100% nitrogen mass balance suggests that all nitro groups were removed. The kinetics of degradation in the column tests is, in part, mass-transfer limited. When solid PETN is present, the process of dissolution is likely to be the rate-limiting factor in mass depletion. Using a co-solvent, such as methanol, PETN solubility can be enhanced thus increasing the rates of mass removal. This study shows that granular iron is a viable and effective treatment method for remediating water containing PETN and when present in crystalline form the efficiency of removal can be greatly enhanced by introducing a co-solvent.
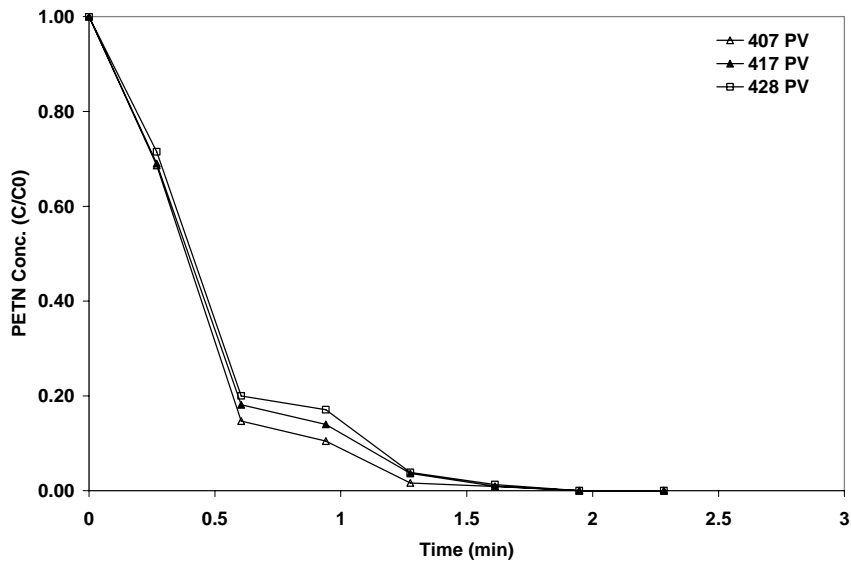
Figure 2.1: Normalized PETN concentration with time in the 100% iron column
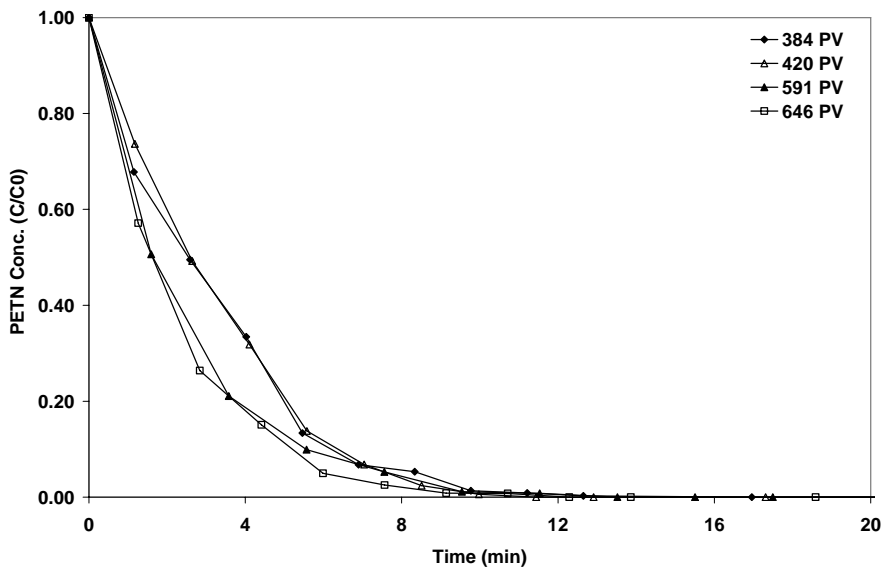
Figure 2.2: Normalized PETN concentration with time in the 30% iron column
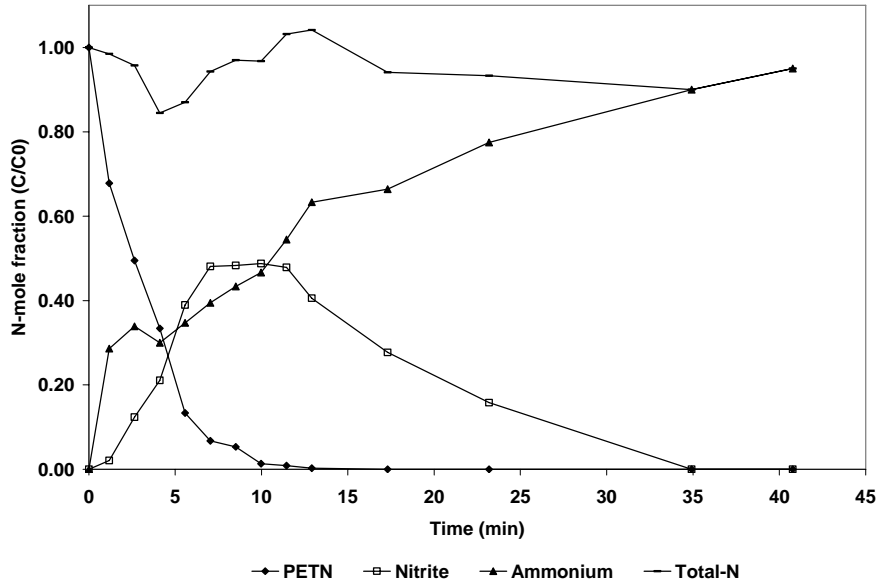
Figure 2.3: Masses of nitrogenous compounds (PETN, nitrite and ammonium) versus time in the 30% iron column after 420 PV
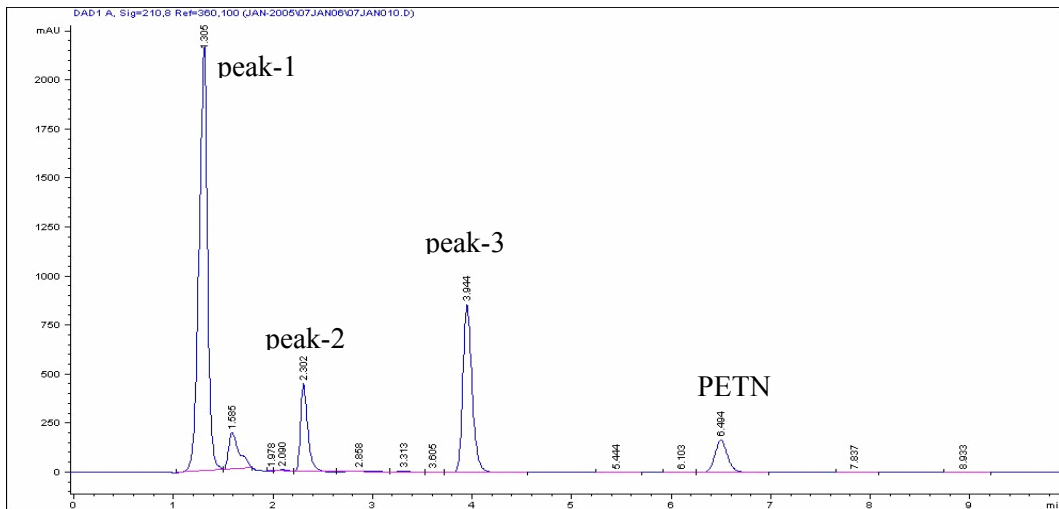
Figure 2.4: HPLC chromatogram of PETN (at 6.5 min) and unknown intermediate products (peak-1 at 1.3 min, peak-2 at 2.3 min and peak-3 at 3.9 min)
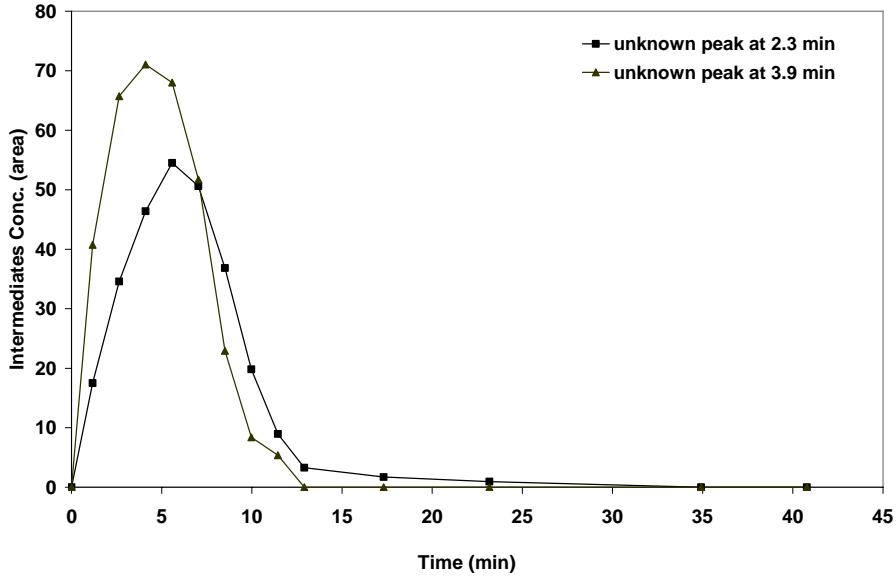
Figure 2.5: Changes in peak size (area) of unknown peak-2 (retention time = 2.3 min) and peak-3 (retention time = 3.9 min) versus time in the 30% iron column after 420 PV
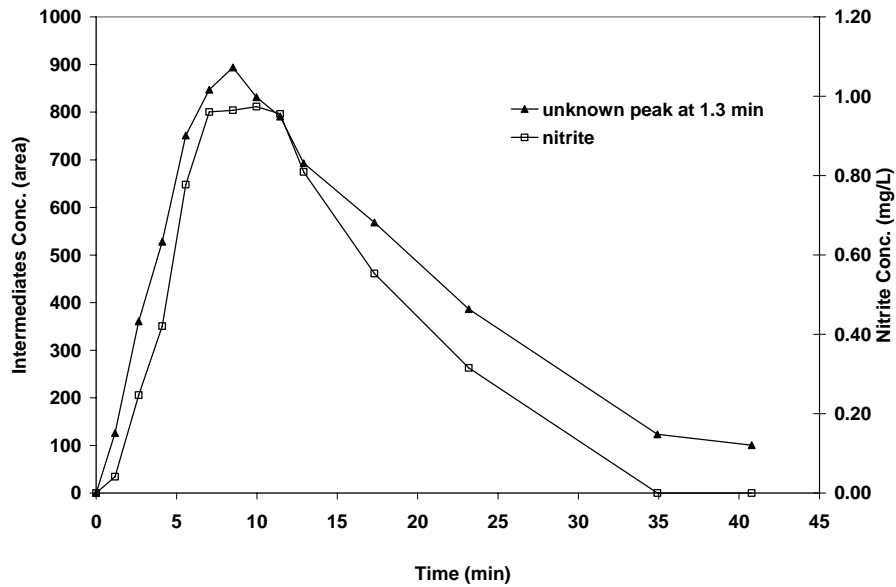
Figure 2.6: Changes in peak size (area) of unknown peak-1 (retention time = 1.3 min) and nitrite concentration versus time in the 30% iron column after 420 PV

```
[ Mass Spectrum ]
Data  : MS123                          Date : 08-Dec-2005 11:42
Sample: PETN mw=316 +NH3CI
Note : Lai - Earth Sciences
Inlet : Direct                        Ion Mode : EI+
Spectrum Type : Normal Ion [MF-Linear]
RT : 0.38 min          Scan# : 8
BP : m/z 289.0874      Int.  : 26.03
Output m/z range : 50.2930 to 349.9795      Cut Level : 0.00 %
```
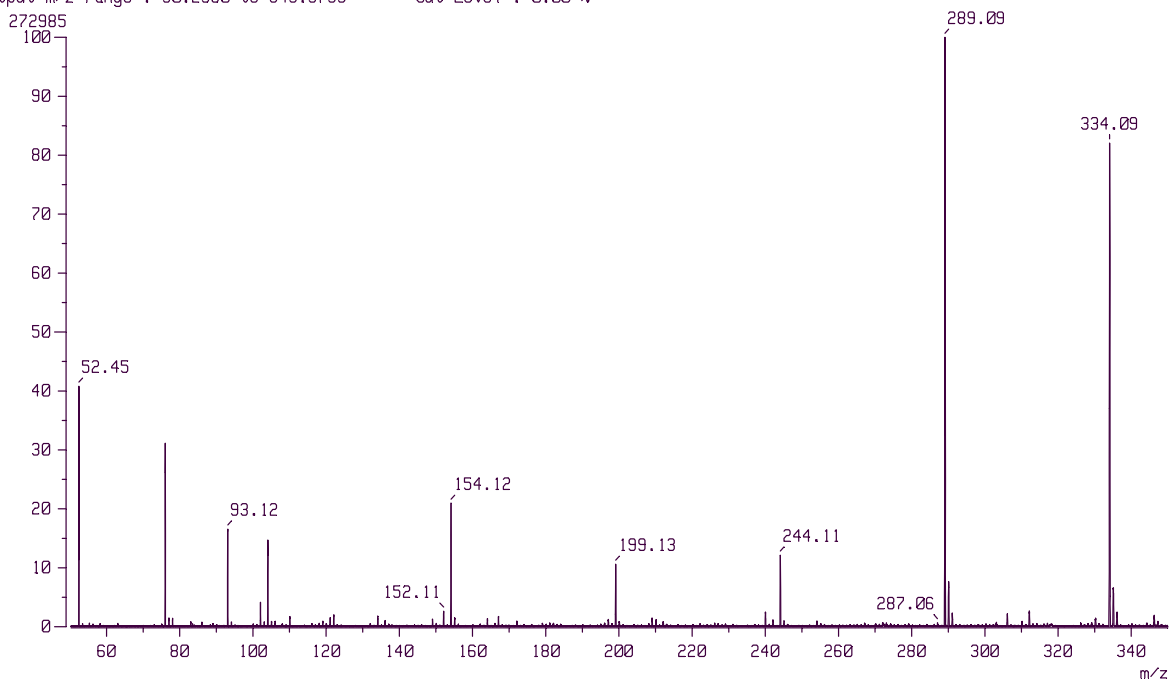
Figure 2.7: Positive ion NH₃ CI mass spectrum of PETN (analytical standard)

```
[ Mass Spectrum ]
Data  : MS122                          Date : 08-Dec-2005 11:36
Sample: Pentaerythritol mw=136 +NH3CI
Note : Lai — Earth Sciences
Inlet : Direct                         Ion Mode : EI+
Spectrum Type : Normal Ion [MF-Linear]
RT : 1.13 min          Scan# : 22
BP : m/z 154.1194      Int.  : 54.78
Output m/z range : 50.0000 to 350.4451        Cut Level : 0.00 %
```
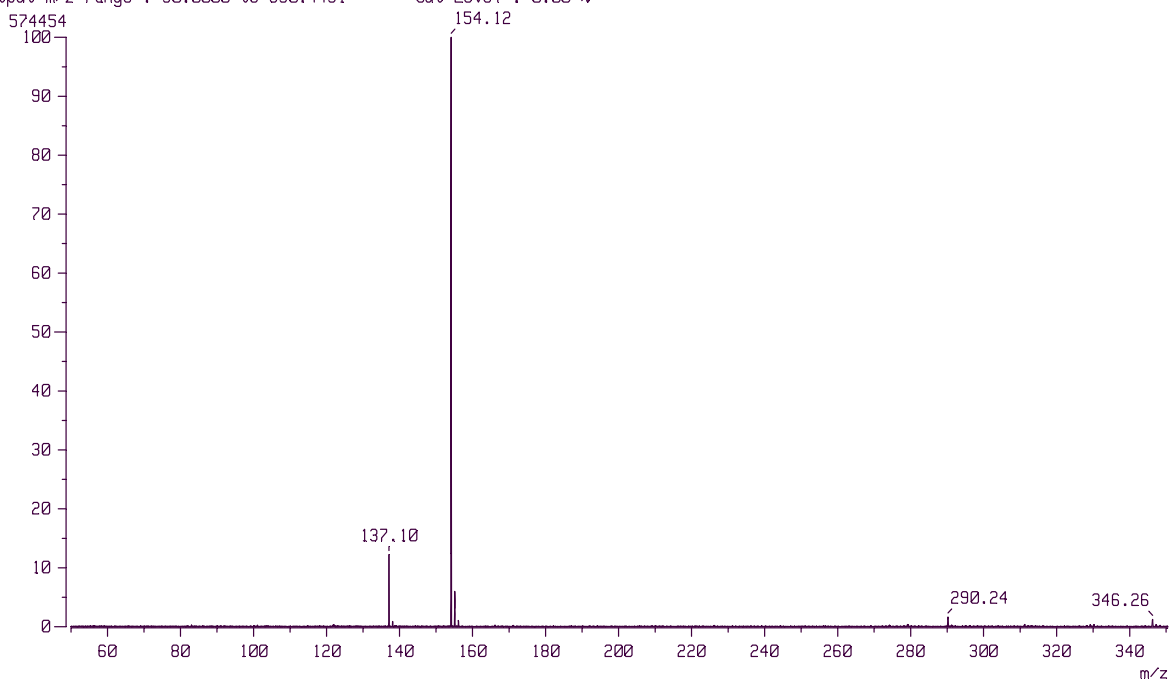
Figure 2.8: Positive ion NH₃ CI mass spectrum of pentaerythritol (analytical standard)

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[ Mass Spectrum ]
Data  : MS174                          Date : 18-Jan-2006 09:14
Sample: X-1 mw=136? +NH3CI
Note : Lai - Earth Sciences
Inlet : Direct                         Ion Mode : EI+
Spectrum Type : Normal Ion [MF-Linear]
RT : 1.45 min          Scan# : 28
BP : m/z 154.1271      Int.  : 26.81
Output m/z range : 50.0000 to 350.4451      Cut Level : 0.00 %
```
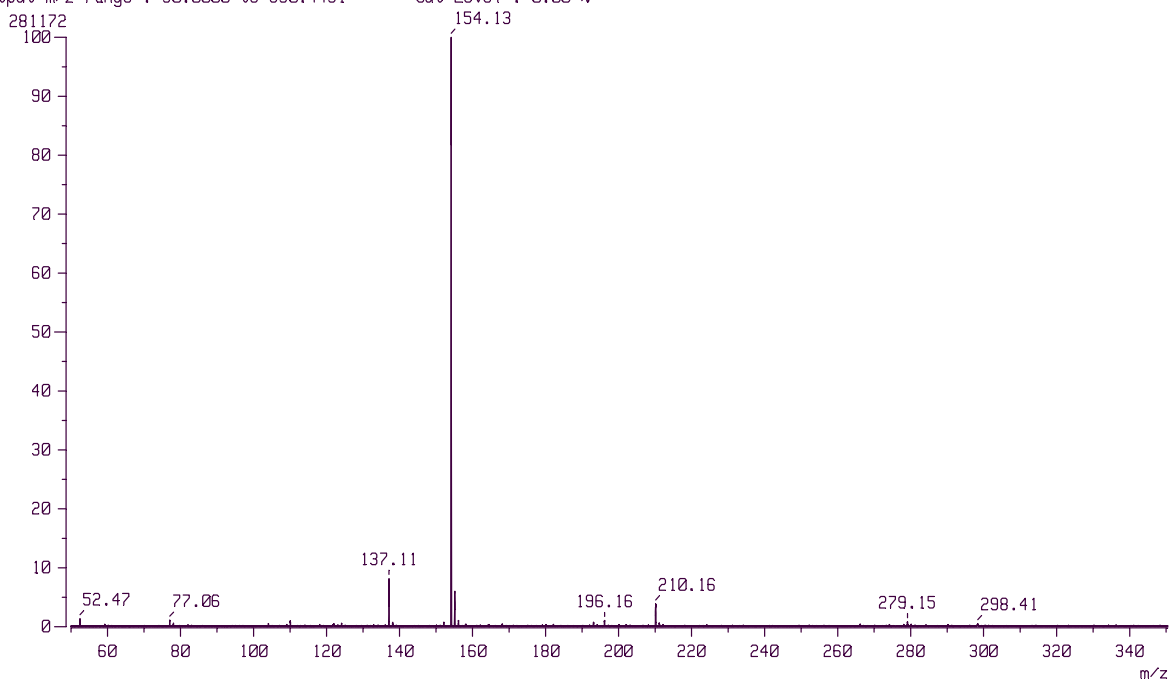
Figure 2.9: Positive ion NH$_3$ CI mass spectrum of unknown peak-1 (retention time = 1.3 min in HPLC Chromatography)

[Mass Spectrum]
Data : MS144   Date : 06-Jan-2006 12:24
Sample: X2 mw=226? +NH3CI
Note : Lai — Earth Sciences
Inlet : Direct   Ion Mode : EI+
Spectrum Type : Normal Ion [MF-Linear]
RT : 0.32 min   Scan# : 7
BP : m/z 244.1340   Int. : 8.63
Output m/z range : 50.0000 to 350.4451   Cut Level : 0.00 %
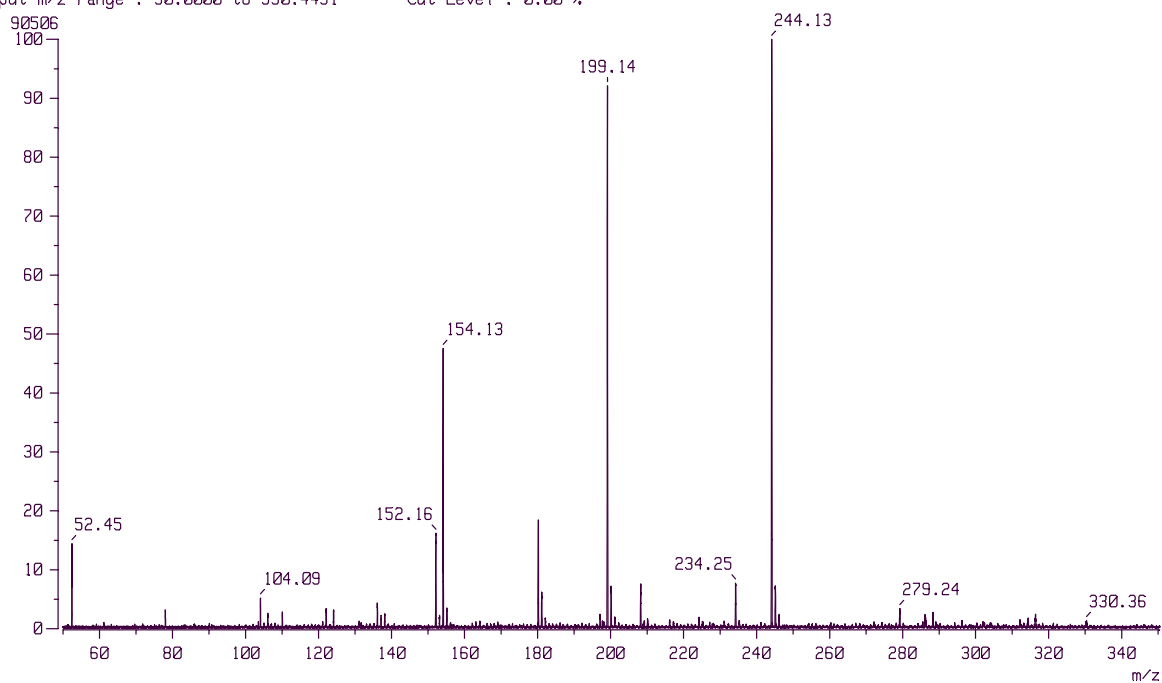
Figure 2.10: Positive ion NH₃ CI mass spectrum of unknown peak-2 (retention time = 2.3 min in HPLC Chromatography)
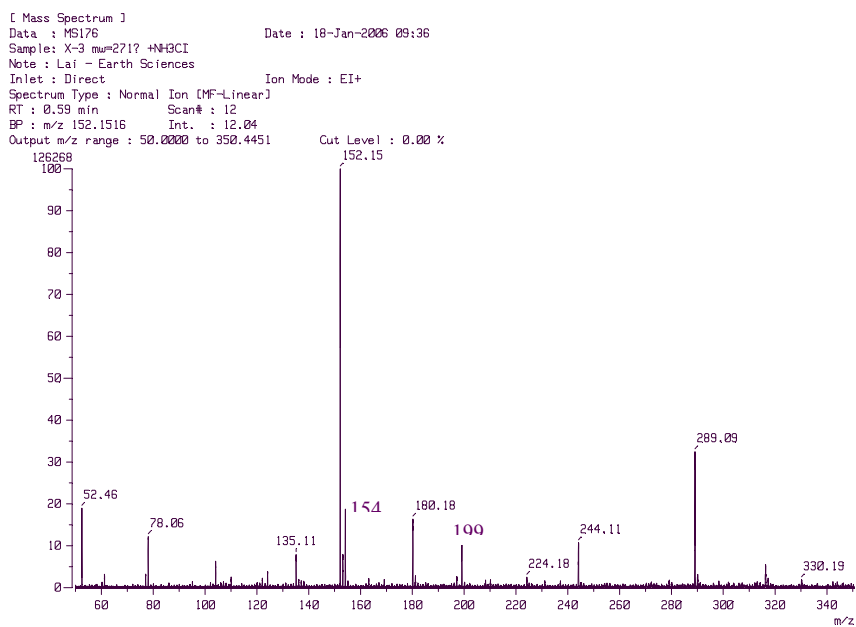
Figure 2.11: Positive ion NH₃ CI mass spectrum of unknown peak-3 (retention time = 3.9 min in HPLC Chromatography)
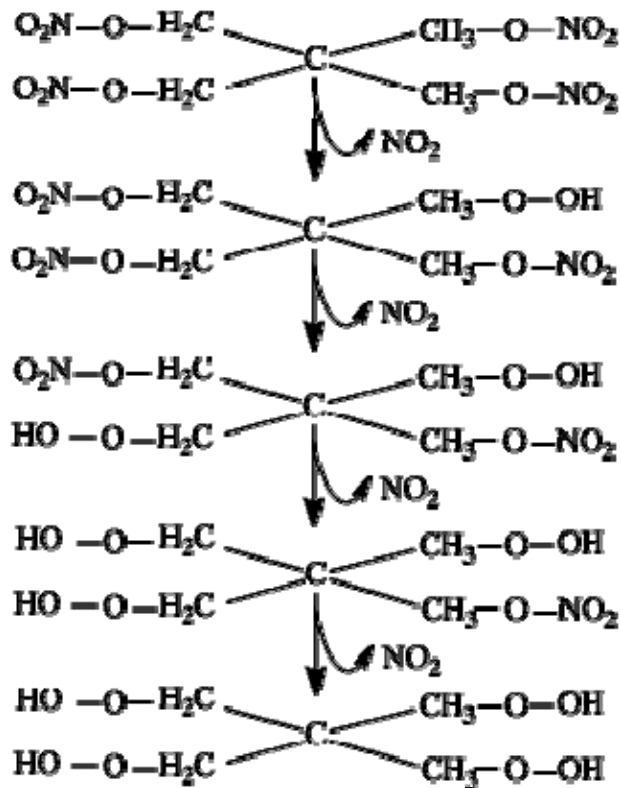
Figure 2.12: Proposed pathway for degradation of PETN by granular iron
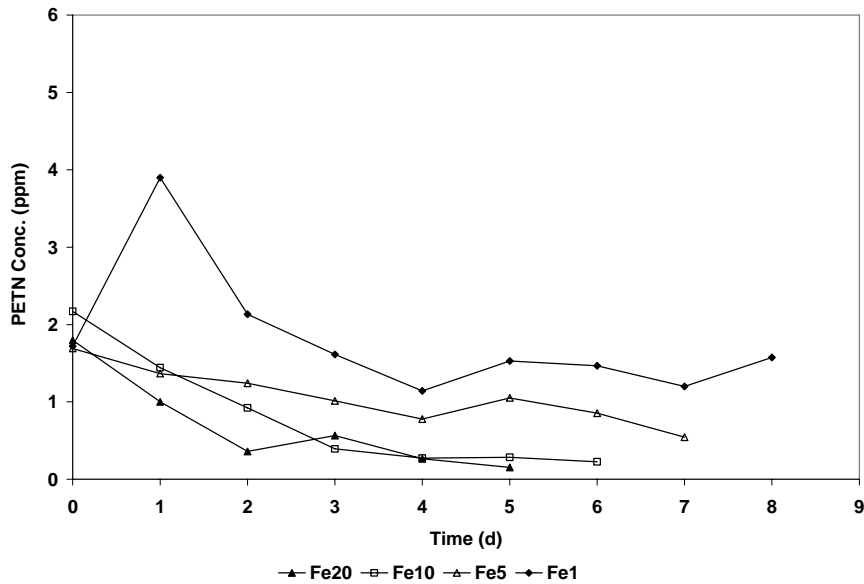
Figure 2.13: Changes in aqueous PETN concentrations with time in batch tests with solid-phase PETN (200 mg/L) and 20, 10, 5 and 1 g iron
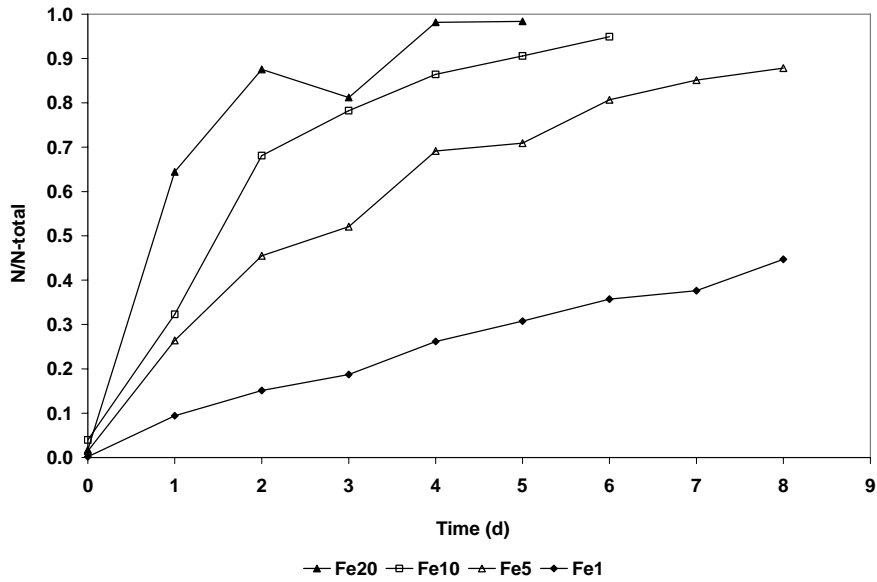
Figure 2.14: Masses of nitrogenous compounds (nitrite and ammonium) over time in batch tests with solid-phase PETN (200 mg/L) and 20, 10, 5 and 1 g iron
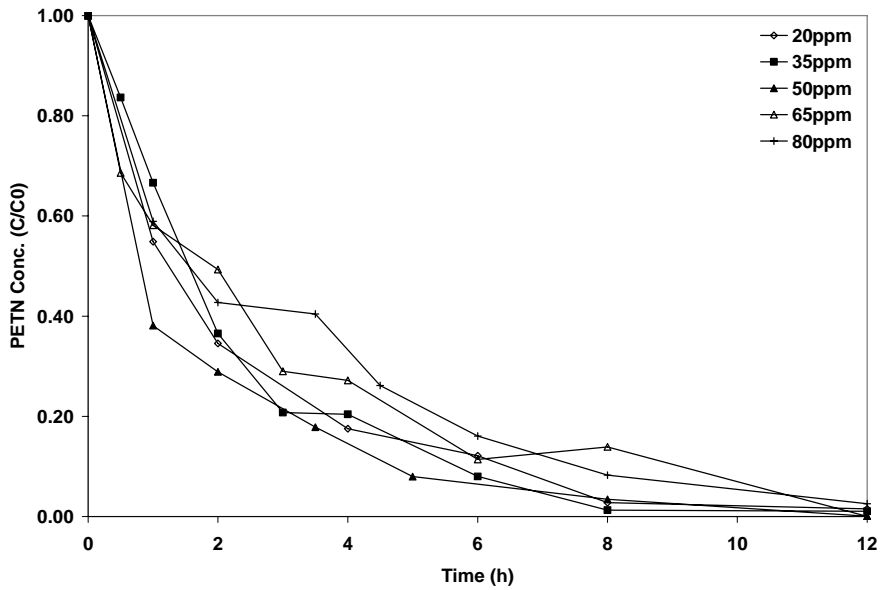
Figure 2.15: Changes in PETN concentrations in batch tests using methanol as a co-solvent to increase initial PETN concentration to 20, 35, 50, 65 and 80 mg/L

Table 2.1: Calculation of mass transfer rate coefficient for 100% iron column

	Column-1
Packing material	100% Connelly iron
Porosity (ε)	0.59
Particle diameter (d_P) -m	6.5×10^{-4}
Flow rate (q) - ml/min	8.9
Fluid velocity (u) - m/s	$\dfrac{q}{A \bullet \varepsilon} = \dfrac{8.9 ml / \min}{\pi (1.27 cm)^2 \bullet 0.59} = 2.98 cm / \min = 4.96 \times 10^{-4} m / s$
Diffusion coefficient (D) - m²/s	5.65×10^{-10}
Kinematic visocity (V) - m²/s	1.004×10^{-6}
$Re = u \bullet d_P / v$	$\dfrac{u \bullet d_p}{v} = \dfrac{4.96 \times 10^{-4} m / s \bullet 6.5 \times 10^{-4} m}{1.004 \times 10^{-6} m^2 / s} = 0.32$
$Sc = v / D$	$\dfrac{v}{D} = \dfrac{1.004 \times 10^{-6} m^2 / s}{5.65 \times 10^{-10} m^2 / s} = 1.78 \times 10^3$
$Sh = 1.09 \bullet \varepsilon^{-2/3} \bullet Re^{1/3} \bullet Sc^{1/3}$	$= 1.09 \times (0.59)^{-2/3} \times (0.32)^{1/3} \times (1.78 \times 10^3)^{1/3} = 12.84$
$K_{mt} = Sh \bullet D / d_p$ - m/s	$\dfrac{Sh \bullet D}{d_P} = \dfrac{12.84 \times 5.65 \times 10^{-10} m^2 / s}{6.5 \times 10^{-4} m} = 1.12 \times 10^{-5} m / s$ $= 6.72 \times 10^{-4} m / \min$
Geometric surface area - m²/g	$\dfrac{4\pi \bullet r^2}{\dfrac{4}{3}\pi \bullet r^3 \bullet \rho} = \dfrac{3}{6.5 \times 10^{-4} m \times 6.37 \times 10^3 kg / m^3} = 7.24 \times 10^{-4} m^2 / g$
Iron weight - g	412.9
Solution volume - ml	89.5
$\alpha - m^{-1}$	$= \dfrac{7.24 \times 10^{-4} m^2 / g \times 412.9 g}{89.5 ml} = 3.34 \times 10^3 m^{-1}$
$\alpha \bullet K_{mt}$ - min⁻¹	$= 3.34 \times 10^3 m^{-1} \times 6.72 \times 10^{-4} m / \min = 2.24 \min^{-1}$
K_{obs} - min⁻¹	2.96

Chapter 3

PETN Biodegradation by Anaerobic Consortia in Liquid Culture

The previous chapter demonstrated the abiotic degradation of PETN by granular iron. This chapter examined the potential for biotic degradation of PETN by using enrichment cultures prepared from PETN-contaminated soil. Generally, high levels of nitrate and sulfate are found in PETN-contaminated sites associated with its manufacture, as a consequence of the use of nitric acid and sulfuric acid in the process of PETN synthesis. Therefore this study specifically examined the biodegradability of PETN in the presence of nitrate, sulfate and mixture of nitrate and sulfate. The metabolites formed from biotransformation of PETN were characterized and a degradation pathway was proposed. Determination of the microbial population that degrades PETN and appropriate electron acceptor conditions can offer the prospect of optimizing bioremediation systems for PETN and other nitrate-ester contaminated sites.

3.1 Materials and Methods

3.1.1 Mineral Media

The biodegradability of PETN under anaerobic conditions was tested in liquid culture. The mineral salt medium used for microbial enrichment in this study was modified from Cobb and Bouwer (1991), consisting of the following components (in mg/L): $MgCl_2 \cdot 6H_2O$ (21.2), NH_4Cl (74.6), $CaCl_2 \cdot 2H_2O$ (36.7), KH_2PO_4 (24.6), K_2HPO_4 (25.3), $Na_2HPO_4 \cdot 7H_2O$ (34.1), and $NaHCO_3$ (27.1). A 100-fold concentrated trace elements solution was added to this medium to give the following final concentrations (in mg/L): $FeCl_2 \cdot 4H_2O$ (1.0), $FeCl_3 \cdot 6H_2O$ (0.26), $MnSO_4 \cdot H_2O$ (0.13), H_3BO_3 (0.029), $ZnCl_2$ (0.031), $NiCl_2$ (0.025), $Cu(NO_3)_2 \cdot 2.5H_2O$ (0.025), $CoCl_2 \cdot 6H_2O$ (0.032), $AlCl_3 \cdot 6H_2O$ (0.022), $Na_2MoO_4 \cdot 2H_2O$ (0.034) and Na_2SeO_3 (0.013).

In a preliminary test, acetate, as a common carbon source for most bacteria, was added to the above medium; however, the bacterial growth was not significant, especially for sulfate-reducing enrichment. Therefore, yeast extract, rich in various nutrients as well as carbon, was added at 0.01% and 0.1% (w/w) for nitrate-reducing and sulfate-reducing enrichment, respectively, and a substantial enhancement in microbial activity and growth was observed. Therefore, for nitrate-reducing condition, the mineral medium was used with the addition of 0.01% yeast extract, 300 mg/L acetate and 150 mg/L sodium nitrate. For sulfate-reducing condition, the mineral medium was amended with 0.1% yeast extract, 300 mg/L acetate and 150 mg/L sodium sulfate. The medium used for mixed electron acceptor conditions was added with 0.1% yeast extract, 300 mg/L acetate, 150 mg/L sodium nitrate and 150 mg/L sodium sulfate. In each medium, acetate was stoichiometrically in excess for complete reduction of the electron acceptor(s) in the medium.

Each type of medium was autoclaved at 121ºC for 1h, and deoxygenated with nitrogen gas using a sterilized sparger and tubing system to a DO level of < 0.2 mg/L. Since PETN decomposes during autoclaving, it was added to the media via a sterile syringe in the presence of a nearby open flame to a final concentration of 6 mg/L at the end of preparation. The final pH values of the media were 6.4~6.5.

3.1.2 Enrichment Procedure

The initial enrichment was prepared in a 160 mL serum bottle with 5 g PETN-contaminated soil from a site at Louviers, CO, U.S.A, which had received PETN wastewater for over 20 years. Since the concentrations of nitrate and sulfate were high in the contaminated soil, the initial inoculation was conducted with sterile mineral medium containing 300 mg/L acetate in the absence of nitrate and sulfate. The bottle was sealed with autoclaved Teflon®-coated septa and aluminum crimp caps and incubated at room temperature. After 60 days, apparent turbidity and black precipitation were observed in the culture, and complete depletions of nitrate and sulfate were achieved. Then, two aliquots of 2 mL inocula were transferred to 60 mL sterile serum bottles containing fresh nitrate-reducing medium and sulfate-reducing medium and PETN, respectively. The bottles were incubated in a 37ºC constant temperature

room and the bacterial growth was monitored by measuring the depletion of electron acceptors. The transfer process was repeated seven times, with each transfer occurring once a substantial growth of culture was evident and at least 50% electron acceptor in the medium was depleted. The finally enriched denitrifying and sulfate-reducing cultures were used as inocula for the study of PETN biotransformation under nitrate-reducing and sulfate-reducing conditions, respectively. The experiment under mixed electron acceptor conditions was performed with the inoculum from the initial liquid culture.

3.1.3 Batch Experiments

For each electron acceptor condition, two controls and two active cultures were established. Each treatment was conducted in a 250 mL sterile glass bottle with a screw cap fitted with a Mininert Valve™ (Precision Sampling Corporation, Baton Rouge, Louisiana). The controls included an uninoculated medium (set-1) and an inoculated medium sterilized by the addition of mercuric chloride ($HgCl_2$) at a concentration of 100 mg/L (set-2). The active treatments were inoculated by the finally enriched cultures (10 mL) in the presence of and the absence of the electron acceptor(s) (set-3 and set-4). Initially, the bottles were filled with media leaving no headspace. The bottles were incubated in an anaerobic glovebox (5% H_2 + 5% CO_2 + 90% N_2) for the entire experiment. Periodically, approximately 1 mL of aqueous sample was withdrawn from each bottle for inorganic and organic analyses.

3.1.4 Analytical Methods

Inorganic analyses of nitrate, nitrite and sulfate and organic analyses of PETN and possible intermediate products were performed using the same analytical methods described in Chapter 2. As well, the same LC/MS method was used for identification of intermediate products.

3.2 Results and Discussion

3.2.1 PETN Biodegradation in the Presence of Nitrate

The concentrations of nitrate and PETN over time in the nitrate-reducing medium are shown in Figures 3.1 and 3.2, respectively. In both controls (set-1 and set-2), concentrations of

PETN and nitrate remained constant over time. The unchanged concentration in the uninoculated control (set-1) indicated that PETN was persistent in the absence of microbial activity and the sterilization procedure for media was effective. The constant concentration in the inoculated control sterilized by $HgCl_2$ (set-2) suggested that no abiotic factor in the inoculated medium would affect the reduction of PETN. In the active culture with nitrate addition (set-3), nitrate was reduced to below the detection limit within the first 36 h. As nitrate decreased, nitrite accumulated to a maximum of about 79 mg/L at 36 h and then declined to the detection limit by 121 h. No nitrate or nitrite was observed in the test without nitrate addition (set-4). As seen in Figure 3.2, PETN gradually degraded in both active treatments (set-3 and 4) without a lag phase. Initially, PETN degraded at a similar rate in both active cultures but began to diverge after 61 h, with the "nitrate added" treatment (set-3) showing a higher rate of degradation. At the conclusion of the experiment (1240 h), 98% and 59% of the initial PETN had been transformed in the tests with and without nitrate addition, respectively.

It is clear that the rate of PETN degradation was significantly increased by the presence of nitrate in the medium (Figure 3.2). If PETN were degraded by the various denitrifying bacterial species in the enrichment, it is reasonable to propose that the enhancement in PETN removal is a consequence of a larger population of bacteria, stimulated by the greater amount of electron acceptors in set-3 containing 150 mg/L of nitrate and 6 mg/L of PETN, relative to the sole presence of PETN in set-4. With the greater amount of electron acceptor, particularly with nitrate, more energy would be produced, which would induce faster and larger bacterial growth. The more extensive microbial growth was noticeable in the visual observation of turbidity in the culture and also evidenced by the consumption of acetate (Figure 3.3). It is apparent that more acetate was consumed in the treatment with nitrate addition (set-3) than in the absence of nitrate (set-4) during the first 121 h, the time for complete denitrification. Following that, acetate was used at a similar rate in both treatments. The enhanced degradation rate by nitrate addition is consistent with the principle of enhanced

biodegradation by providing alternative electron acceptors to increase bacterial growth and consequently yield faster degradation rates for contaminants.

The presence of nitrate does not appear to affect the kinetics of PETN degradation based on the shape of the PETN curves at the initial period (Figure 3.2). However, in the above experiment, nitrate only presented for a short period of time relative to the degradation of PETN. Thus, a supplemental experiment was conducted, in which nitrate concentration was maintained above 50 mg/L for a period of over 130 h by multiple additions (Figure 3.4). In this case, PETN initially degraded at the same rate as in the treatment with single addition of nitrate (set-3). However, after 121 h, when the majority of nitrate had been removed, PETN degradation was halted. Due to the multiple additions of nitrate, nitrite accumulated to a maximum of 350 mg/L after nitrate depletion, from then on no further decrease in nitrite concentration was observed. In contrast, nitrite decreased relatively quickly (about 100 h) in the culture with single nitrate addition (set-3, Figure 3.1). The temporary plateau for both PETN and nitrite reduction was believed to be a consequence of nitrite inhibition on denitrifying rather than carbon limitation since the concentration of acetate remained above 300 mg/L by multiple additions. After a lag phase of 450 h, both nitrite and PETN showed significant declines in concentration, indicating the restored activity of bacteria. The concurrent onset of PETN and nitrite reduction suggests the degradation of nitrite and PETN to be undertaken by the same microbial consortium, likely to be denitrifying bacteria. This supports the explanation for the enhancement in the rate of PETN degradation by the extra presence of nitrate, which induced greater population of denitrifying bacteria to degrade PETN. Our results agree with previous research concerning the biotransformation of TNT, DNT and HMX by denitrifying enrichment cultures (Boopathy et al., 1998a and 2001a; Freedman et al., 1998). In this experiment, with multiple additions of nitrate, consistent with previous findings, the presence of nitrate, over an extended period of time, did not appear to affect PETN degradation; however, the consequent production of nitrite, particularly in large quantity, may temporarily delay PETN degradation.

In set-3, with the coexistence of nitrate and PETN, complete removal of the initial nitrate, at 160 mg/L, occurred within 36 h while it took 1240 h to achieve 98% removal of 6 mg/L PETN (Figure 3.5), indicating a much faster rate of nitrate reduction than PETN degradation. The faster degradation of nitrate was also observed in the experiment with multiple additions of nitrate. With multiple additions of nitrate, more than 1,000 mg/L was removed within 187 h. After the inhibition phase, approximately 300 mg/L nitrite (41% of nitrate added) was reduced within 232 h while it took 667 h for 3.8 mg/L of PETN to be completely removed (Figure 3.4). Without knowledge of the redox potential for PETN, we can not define the thermodynamic favorability of PETN relative to nitrate/nitrite. However, nitrate/nitrite is a more structurally favorable molecule for microorganisms relative to PETN, which has a structure that does not occur naturally. This characteristic may reduce enzyme accessibility to PETN, explaining the much slower process of PETN degradation than nitrate/nitrite reduction.

In both active treatments, regardless of fast or slow rate of degradation, as PETN degraded, the reduction products of trinitrate pentaerythritol and dinitrate pentaerythritol were detected, with a typical pattern of appearance and disappearance for being intermediates.

3.2.2 PETN Biodegradation in the Presence of Sulfate

Figures 3.6 and 3.7 show the concentrations of sulfate and PETN over the incubation period in the sulfate-reducing enrichment media. Sulfate remained constant in the two controls throughout the entire experiment. In the active culture with sulfate present (set-3), the onset of sulfate reduction occurred after 983 h and complete removal was achieved within the following 800 h (Figure 3.6). Figure 3.7 shows that relative to no decline in PETN concentration in the control treatments, PETN was completely degraded in both active treatments in the presence of (set-3) and absence of sulfate (set-4) within 800 h. Data shows insignificant difference between the active treatments with respect to the rate of degradation, suggesting that the presence of sulfate has no adverse effect on PETN degradation.

As PETN degradation in the nitrate-reducing media, pentaerythritol dinitrate and pentaerythritol trinitrate were observed during PETN degradation in both active cultures (set-3 and 4). Figure 3.8 plots the performances of these intermediates along with PETN and sulfate over time in the medium in the presence of sulfate (set-3). Sulfate shows a significant decrease after 1512 h, when both PETN and its intermediates were completely removed, suggesting that the presence of PETN and its intermediates may have temporarily delayed the onset of sulfate reduction. Similar to our results, Wani and Davis (2003) observed no sulfate reduction by biological activity during RDX removal, proposing an inhibition from RDX and its metabolic products. In addition, they observed similar RDX transformation rates in the presence and the absence of 100 mg/L sulfate in soil column studies.

The occurrence of sulfate reduction in set-3, though delayed, suggests the presence of sulfate-reducing bacteria in all media. Most sulfate-reducing bacteria are also capable of utilizing nitrate or similar organic compounds as terminal electron acceptors for energy generation; it offers the possibility that PETN in the medium without sulfate (set-4) was degraded by sulfate-reducing bacteria. In the presence of sulfate (set-3), the observed degradation of PETN was completely independent of sulfate reduction, showing a predominant preference over sulfate reduction. This does not contradict with the presumption that sulfate-reducing bacteria may be responsible for degradation of PETN, because in the presence of mixed electron acceptors either sulfate or other electron acceptors may be the preferred electron acceptor depending on the species of sulfate-reducing bacteria. To further clarify the capability of sulfate-reducing bacteria to degrade PETN and examine the biodegradability of PETN under sulfate-reducing conditions, a supplementary experiment was conducted. It used the same medium and inoculum without initial addition of PETN. As seen in Figure 3.9, after 168 h lag phase, sulfate concentration in the culture was decreased rapidly from 168 to 11 mg/L within the following 90 h, indicating that sulfate-reducing bacteria was in its exponential growth phase. At this time, PETN (6 mg/L) and more sulfate (~100 mg/L) were added into the medium. Data show that sulfate reduction was immediately ceased and PETN was converted to an unknown product, differing from all those

intermediates observed in the previous experiments. The peak area of the unknown, more sensitive than PETN and intermediates in same HPLC analysis, persisted over time. Even though the new chemical is not identified, the results clearly suggest the inhibition effect of PETN on sulfate reduction, in other words, sulfate-reducing bacteria can not grow in the presence of PETN, and consequently are not capable of degrading PETN.

Given the above evidence, it is conclusive to say that PETN can not be biodegraded under sulfate-reducing conditions and its degradation in the sulfate-reducing medium is a consequence of other bacterial species than sulfate-reducing bacteria. Since PETN was present during the enrichment procedures, PETN-acclimated bacteria were also stepwisely enriched with sulfate-reducing bacteria and would therefore be present in the inoculum, providing an explanation to independent degradation rate of PETN in the presence of sulfate, and further evidence for the proposal that the PETN-degrading bacteria belong to the denitrifying community.

3.2.3 PETN Biodegradation in the Presence of Nitrate and Sulfate

To better represent the conditions at contaminated sites, a bacterial liquid culture was used in this experiment that was derived from PETN-contaminated soil without enrichment and selection. Concentrations of nitrate, sulfate and PETN during the incubation period are presented in Figures 3.10, 3.11 and 3.12, respectively. In the two control treatments (set-1 and 2), the concentrations of nitrate, sulfate and PETN all remained unchanged during the experiment. In contrast, all three underwent complete reduction in the active treatments. Nitrate was rapidly depleted within the first 37 h, at this point nitrite reached a maximum concentration of 72 mg/L then decreased to below the detection limit during the following 12 h (Figure 3.10). Sulfate remained relatively constant during the initial 160 h, and the majority of sulfate was reduced between 160 and 306 h (Figure 3.11). Complete removal of PETN occurred in both active treatments, with an apparent faster rate of degradation in the treatment with addition of mixed electron acceptors (set-3) than in their absence (set-4) (Figure 3.12). It took 88 h and 614 h to achieve 100% removal of PETN for the treatments

with and without the addition of mixed electron acceptors, respectively. Since the presence of sulfate was demonstrated not to affect the processes of PETN degradation in the prior test (section 3.2.2), the improved degradation rate was attributed to the presence of nitrate. As discussed in section 3.2.1, the presence of nitrate led to a faster and greater microbial growth of denitrifying bacteria, resulting in an increase in the rate of PETN degradation. This may similarly explain the enhancement in PETN removal when mixed electron acceptors were present in the culture.

Figure 3.13 summarizes the performances of nitrate, sulfate, PETN and observed intermediates of pentaerythritol dinitrate and pentaerythritol trinitrate in the culture amended with mixed electron acceptors (set-3). Nitrate was firstly fully reduced, followed by compete removal of PETN. As PETN degraded, pentaerythritol dinitrate and pentaerythritol trinitrate were sequentially produced and degraded with complete disappearance after 112 and 180 h, respectively. At the end of the sequence, sulfate reduction took place. This sequence of degradation highly suggests the utilization preference by the bacterial consortium in the order of nitrate, followed by PETN and its intermediate products, and finally sulfate. The delay in sulfate reduction by the presence of PETN and its metabolites in this experiment is consistent with the observations under sulfate-reducing conditions, indicating the involvement of denitrifying bacteria rather than sulfate-reducing bacteria in PETN degradation.

3.2.4 Metabolic Processes of PETN Degradation by Anaerobic Bacteria

The biodegradability of PETN was demonstrated under the electron acceptor conditions of nitrate or/and sulfate and three different processes may be responsible for its degradation: (i) PETN may serve as a primary substrate for bacterial growth. The carbon and nitrogen atoms in the PETN structure may support microbial growth as carbon and/or nitrogen sources. (ii) PETN may serve as an electron acceptor. The electron-deficient character of nitro groups on PETN makes it a good candidate as an electron acceptor. In this case, a carbon source (electron donor) is required for bacterial metabolic growth. (iii) PETN may be used via co-metabolic processes. PETN may be reduced by a non-specific enzyme or co-factor from metabolism of primary substrates, providing no energy benefit to the microorganisms.

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The results of this study suggest that PETN served as a terminal electron acceptor. In the inoculated set-4 for all conditions in which either nitrate or sulfate was absent in the culture, PETN was the only potential electron acceptor for microorganisms to gain energy from. If PETN can only be co-metabolized, no PETN degradation would be expected in set-4 and no further degradation of PETN would continue in set-3 at times when the reductions of other electron acceptors was not proceeding. Therefore, the observed microbial growth and PETN reduction in the above situations suggest that PETN functioned as an electron acceptor to provide energy for microbial growth and activity. This is in agreement with other aromatic compounds such as TNT, which can serve as a terminal electron acceptor in respiratory chains by *Pseudomonas* sp. strain JLR11 under anaerobic conditions (Esteve-Nunez et al., 2000).

Acetate was present in all enrichment media and the concentration was always much greater than PETN over the entire experiment. Therefore, it is reasonable to assume that the anaerobic bacteria would prefer to use easier and more available carbon sources for their growth, reducing the possibility of using PETN as a source of carbon. This is also consistent with no degradation of PETN in a supplemental test in which acetate and yeast extract were absent in the medium. To date, no microorganism has been identified that is capable of using PETN as a carbon source. NH_4Cl and yeast extract, as the most favorable nitrogen sources for bacterial growth, were present in all media, therefore, their preference over PETN as nitrogen sources greatly reduced the possibility of using PETN as a nitrogen source. In general, the reported bacteria, which can use explosives as sole nitrogen source, are pure strain cultures isolated from enrichment under nitrogen-limiting conditions. As an example, a strain of *Enterobacter* cloacae, isolated under aerobic and nitrogen-limiting conditions, can utilize PETN as a sole source of nitrogen for bacterial growth (Binks et al., 1996). RDX and HMX can be used as the sole sources of nitrogen for growth by *Desulfovibrio* spp. in the absence of other nitrogen sources (Boopathy et al., 1998b).

3.2.5 Biodegradation Pathway

Regardless the different electron acceptor conditions, the same intermediate products, pentaerythritol dinitrate and pentaerythritol trinitrate were observed during PETN degradation for all conditions. Both of the denitrated metabolites followed a trend of appearance and disappearance, consistent with sequential denitration (see Figures 3.8 and 3.13). Besides the distinct peaks of di- and trinitrate pentaerythritol, an unknown peak occurred at similar retention time to pentaerythritol, with a much greater peak area than in the iron experiments. The peak was collected and analyzed by LC/MS using the same method as described in Chapter 2. The spectrum shows that the peak contains a mixture of various compounds, however, fragment ions at m/z of 154 and 199 were observed in the spectrum, corresponding to the ionization products of pentaerythritol and mononitrate pentaerythritol in $[M+NH_4]^+$ form. Thus the results suggest the presence of mononitrate pentaerythritol, as an intermediate product during PETN biotransformation. The fragment ion at m/z of 154 is also one of ionization products of pentaerythritol, the other fragment ion at 137 for pentaerythritol was observed with very small amount in the spectrum, indicating the potential presence of pentaerythritol during PETN biodegradation. Unfortunately, separation of mononitrate pentaerythritol and pentaerythritol from the mixed peak was not successful; therefore the dynamic trend for these two compounds can not be delineated. The presence of mono-, di- and trinitrate pentaerythritol and the potential presence of pentaerythritol suggest that three or four nitro groups are sequentially removed from PETN via biological reactions.

Di- and trinitrate pentaerythritol and pentaerythritol were observed during abiotic PETN degradation by granular iron (chapter 2). The similar pattern of intermediates suggests an identical reaction pathway for both abiotic and biotic systems. The positive identification of mononitrate pentaerythritol in this study supports a complete denitration pathway for PETN degradation in both systems, in which PETN is sequentially reduced to pentaerythritol, with the formation of denitrated intermediates of tri-, di- and mononitrate pentaerythritol. A similar denitration pathway was found for the reduction of glycerol trinitrate (GTN), an analogous compound to PETN, by granular iron and anaerobic biodegradation.

Christodoulatos et al. (1997) reported that by using a mixed culture from an anaerobic digester, GTN was completely denitrated to glycerol via successive removal of nitrite from the parent compound, forming GDN (glycerol dinitrate) and GMN (glycerol mononitrate) as intermediates. Further, Oh et al. (2004) showed that in the presence of cast iron, GTN was stepwisely reduced to 1,2- and 1,3-dinitroglycerin and then 1- and 2-mononitroglycerin and finally to glycerol.

3.3 Conclusion

This study demonstrated that PETN can be biodegraded by indigenous bacteria present in contaminated soil and the results suggest that PETN was reduced by denitrifying bacteria, provided the medium was amended with organic carbon. PETN degradation under mixed electron acceptor conditions provided evidence for PETN biotransformation under anaerobic conditions in a mixed microbial population system, similar to the conditions that might be expected at contaminated field sites.

The presence of nitrate did not appear to affect PETN degradation; however, addition of nitrate can stimulate faster and greater microbial growth of denitrifying bacteria, subsequently enhancing the rate of PETN degradation. The high level of nitrite, accumulated from nitrate reduction, may temporarily delay PETN degradation. Similar rates of PETN degradation were observed in the presence and the absence of sulfate, but the presence of PETN inhibited sulfate reduction. The results suggest that PETN served as a terminal electron acceptor for bacterial growth under all the conditions tested. Preferential utilization by microorganism was observed in the order of nitrate followed by PETN and its intermediates, and finally sulfate. Since nitrate is more readily reduced than PETN, the available carbon source (electron donor) is preferentially consumed by nitrate over PETN. Thus, in order to ensure efficient biotransformation of PETN, the supply of electron donor should be sufficient for both nitrate and PETN reduction.

Mono-, di- and trinitrate pentaerythritol were detected as intermediate products under the various electron acceptor conditions, suggesting that PETN was sequentially denitrated,

liberating nitrite, which was further reduced to nitrogen. The degradation pathway in biological system is believed to be the same as in the abiotic degradation of PETN by granular iron.

To date, the literature on the metabolism of PETN is very limited. I believe this is the first study of PETN biotransformation under various electron acceptor conditions. Although the study is not exhaustive with respect to the range in anaerobic conditions, the investigation under nitrate-reducing and sulfate-reducing conditions, in particular, will provide some basis for better understanding of field performance if the sites are also contaminated by nitrate and sulfate.
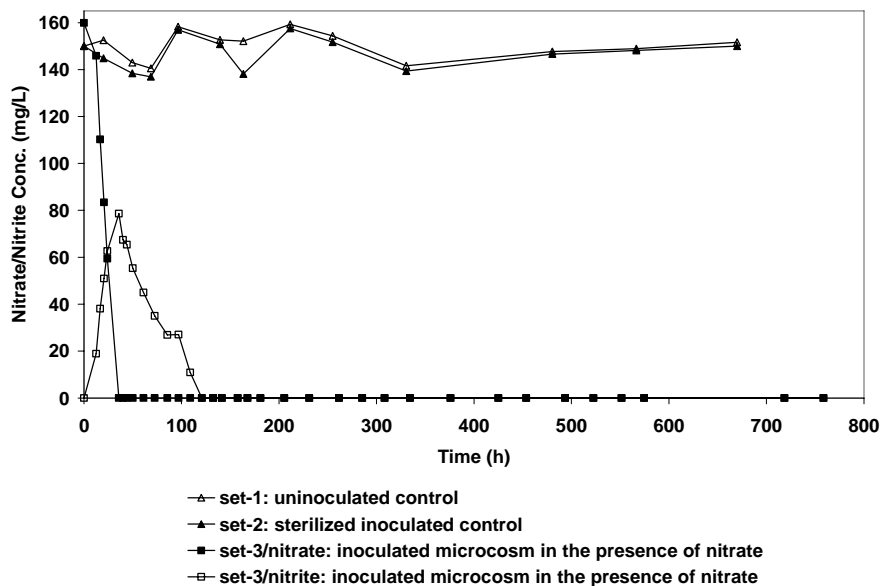
Figure 3.1: Changes in nitrate concentration in the control treatments (set-1 and set-2) and nitrate/nitrite concentration in the active medium in the presence of nitrate (set-3)
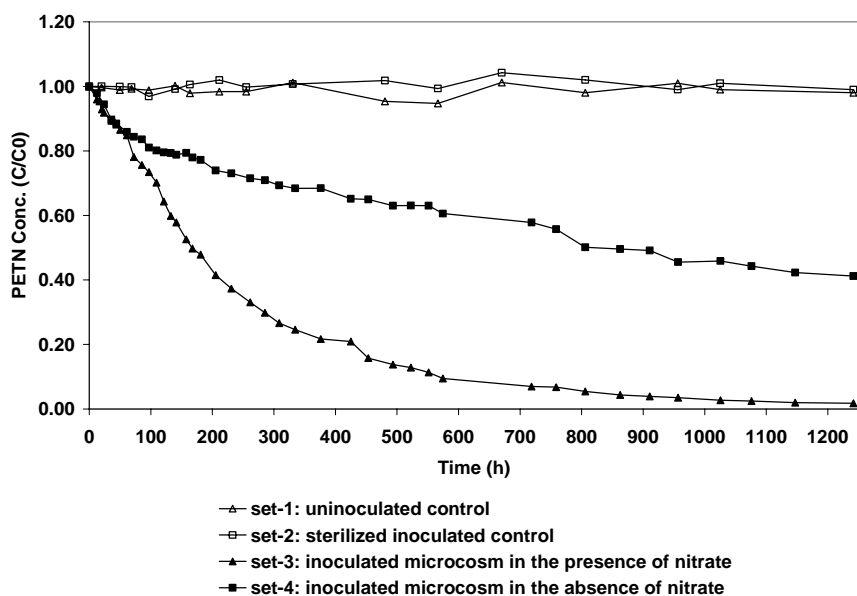
Figure 3.2: Changes in PETN concentration in the control treatments (set-1 and set-2) and the active media in the presence and absence of nitrate (set-3 and set-4)
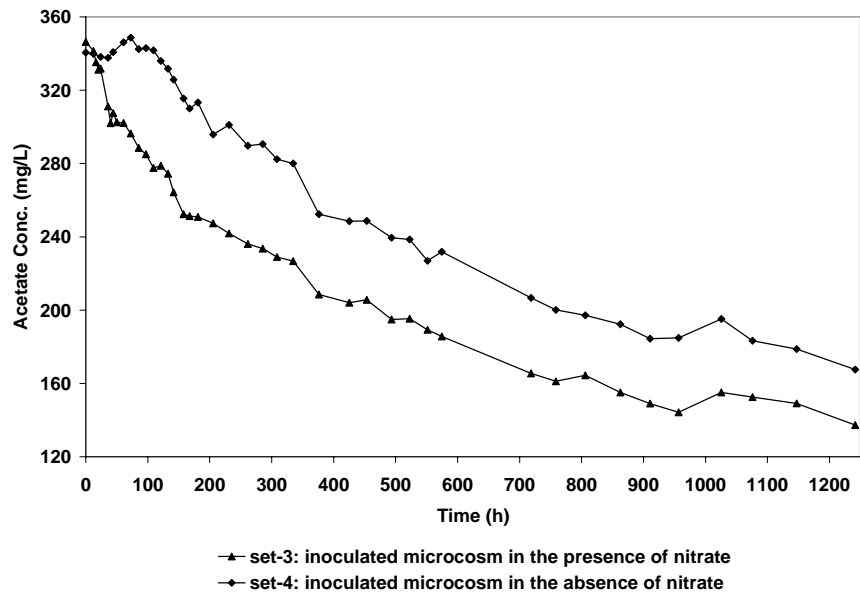
Figure 3.3: Changes in acetate concentration in the active media in the presence of nitrate (set-3) and the absence of nitrate (set-4)
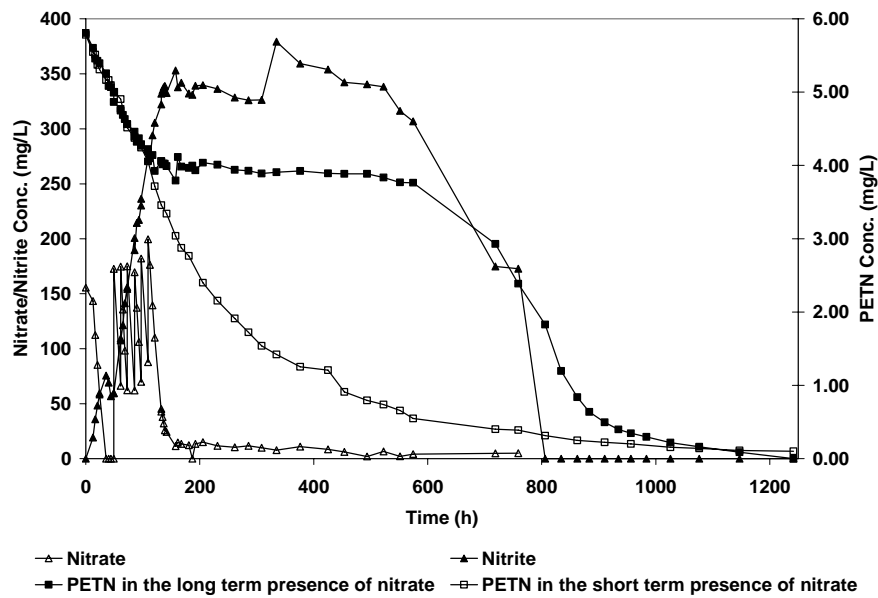
Figure 3.4: Changes in concentrations of nitrate, nitrite and PETN in the medium received multiple additions of nitrate, compared to PETN concentration in the medium with single nitrate addition (set-3)
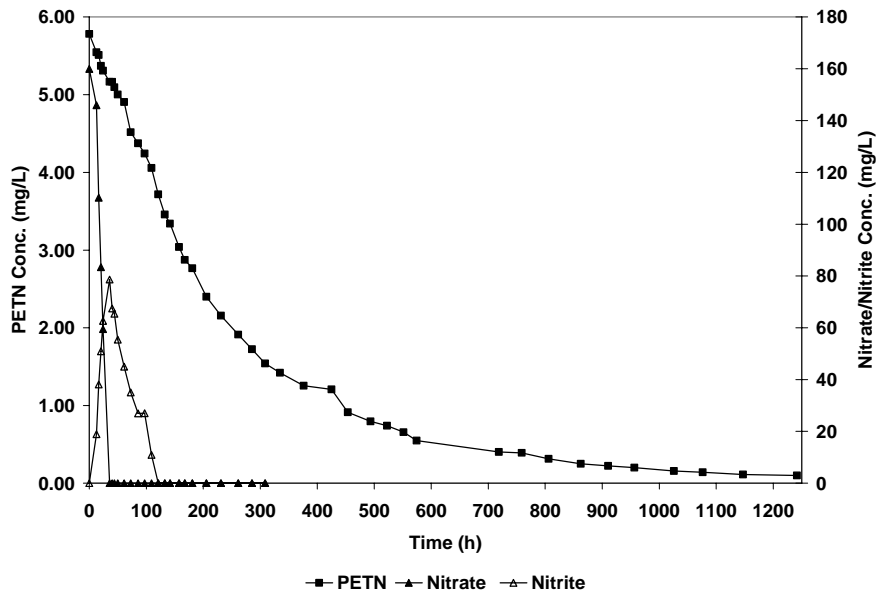
Figure 3.5: Changes in concentrations of nitrate, nitrite and PETN in nitrate-reducing medium (set-3)
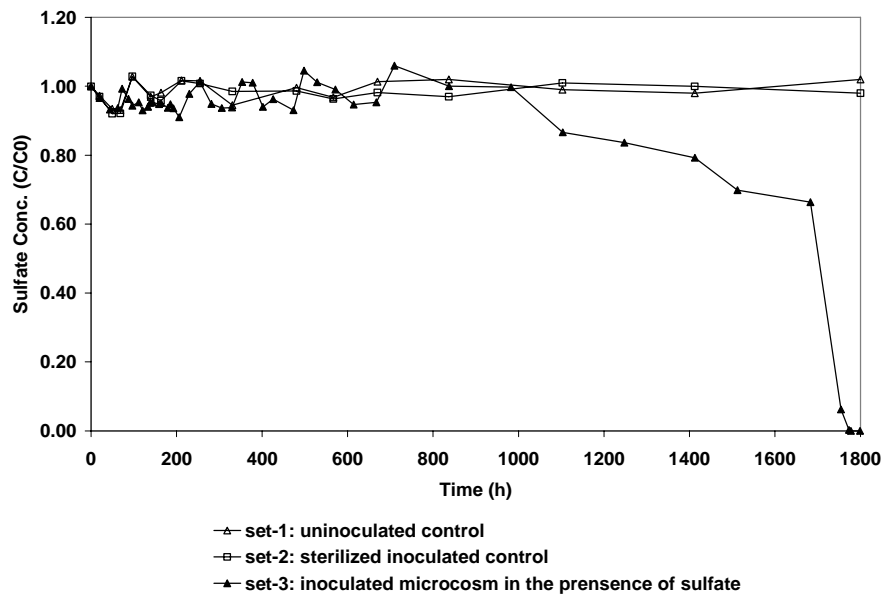
Figure 3.6: Changes in sulfate concentration in the control treatments (set-1 and set-2) and in the active medium in the presence of sulfate (set-3)
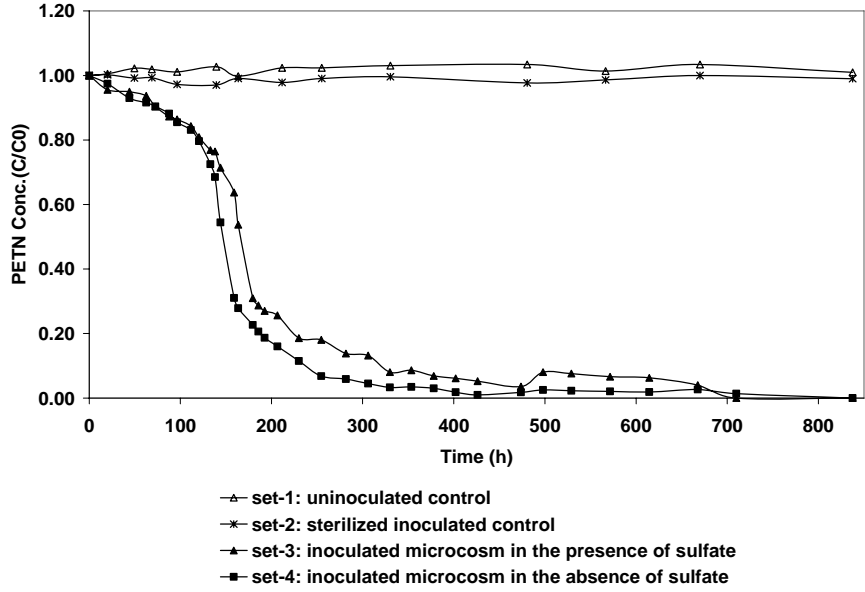
Figure 3.7: Changes in PETN concentration in the control treatments (set-1 and set-2) and the active media in the presence and absence of sulfate (set-3 and set-4)
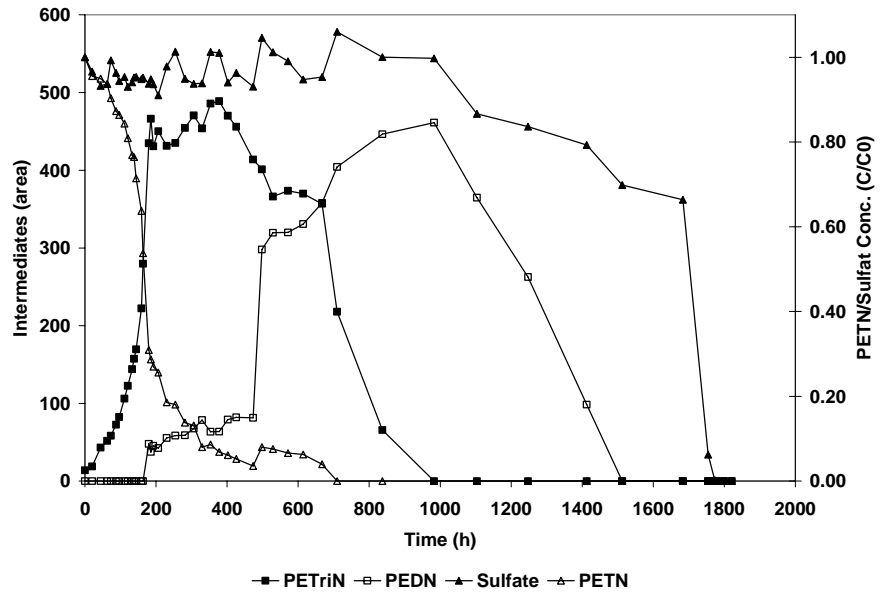
Figure 3.8: Changes in concentrations of PETN, PETriN, PEDN and sulfate in the active medium in the presence of sulfate (set-3)
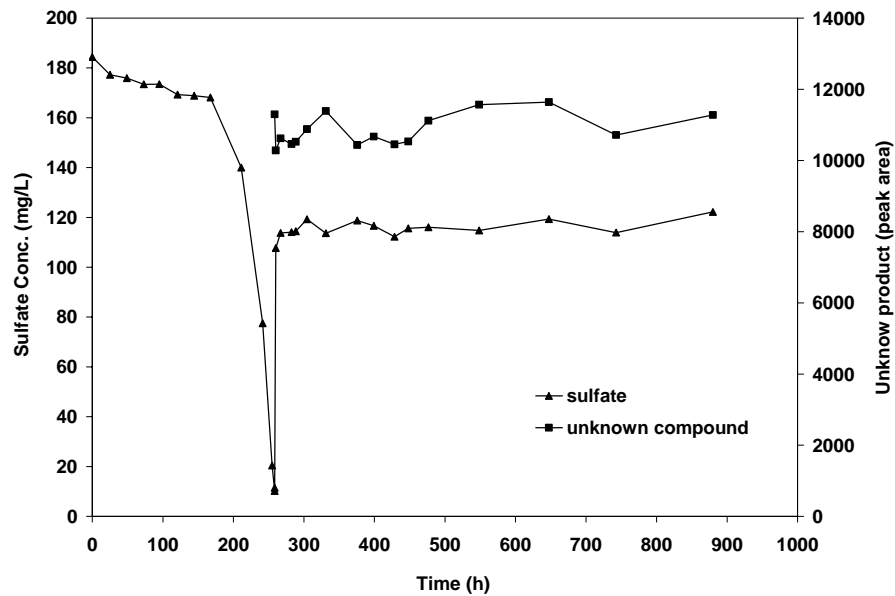
Figure 3.9: Changes in concentrations of sulfate and new produced unknown compound in the supplementary experiment
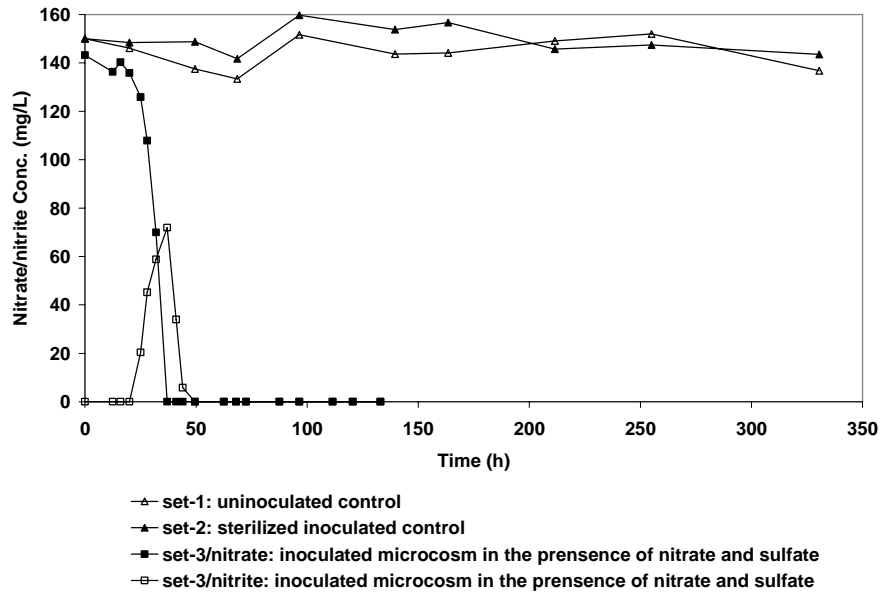
Figure 3.10: Changes in nitrate concentration in the control treatments (set-1 and set-2) and nitrate/nitrite in the active medium in the presence of nitrate and sulfate (set-3)
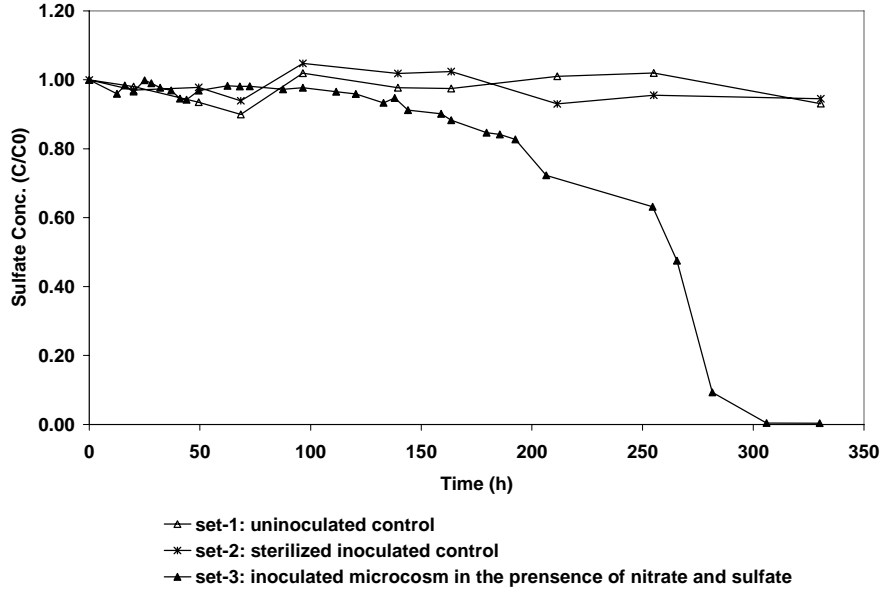
Figure 3.11: Changes in sulfate concentration in the control treatments (set-1 and set-2) and in the active medium in the presence of nitrate and sulfate (set-3)
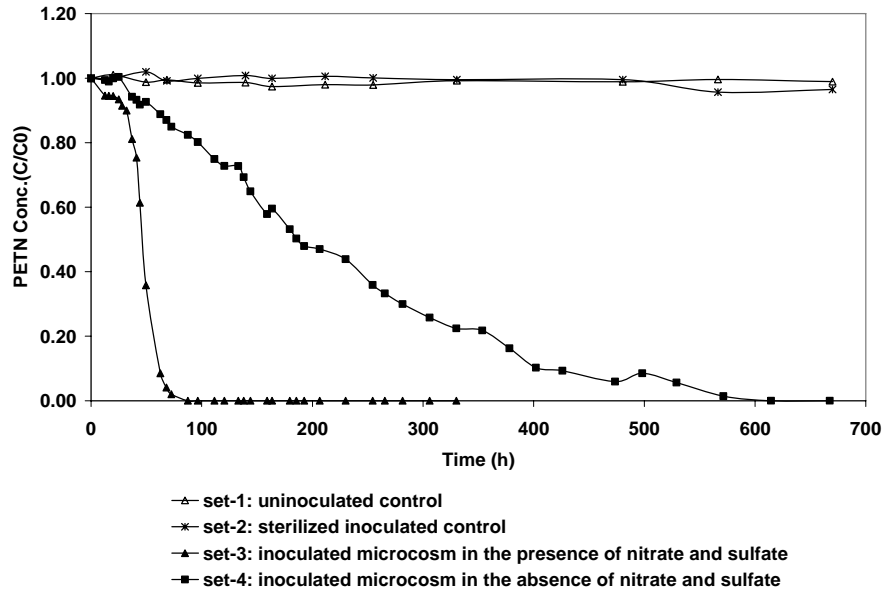
Figure 3.12: Changes in PETN concentration in the control treatments (set-1 and set-2) and the active media in the presence and absence of nitrate and sulfate (set-3 and set-4)
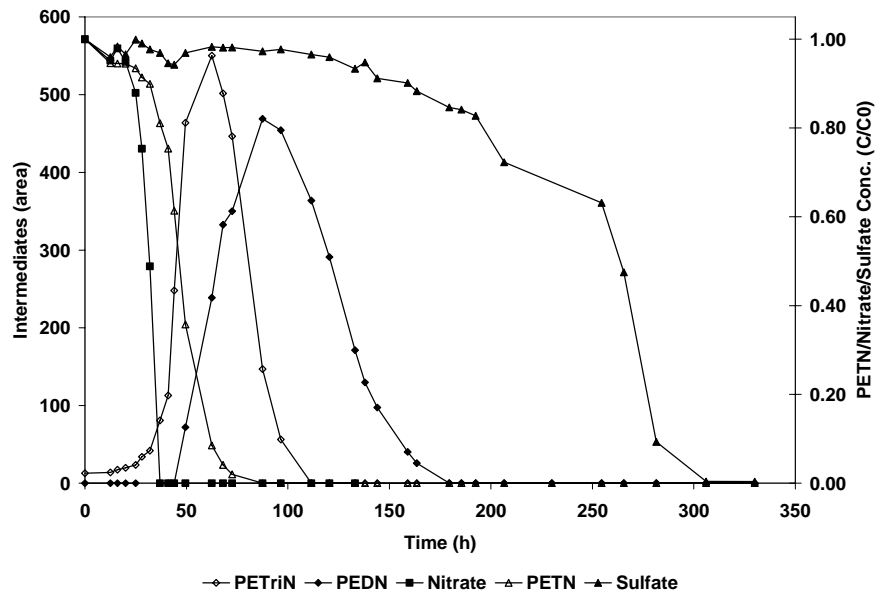
Figure 3.13: Changes in concentrations of nitrate, sulfate, PETN, PETriN and PEDN in the active medium in presence of nitrate and sulfate (set-3)

Chapter 4

Remediation Strategies for PETN-Contaminated Soil:
Laboratory Studies

Chapters 2 and 3 demonstrated that both abiotic and biotic degradation of PETN can proceed in the aqueous phase. However, due to the low solubility, PETN tends to exist primarily in the solid phase at contaminated sites. Thus this chapter addressed remediation of PETN in contaminated soils. Iron may be an applicable remediation method based on the rapid rate of degradation in the solution phase; however, the rate of mass removal and thus the time to clean up is likely to be determined by the rate of PETN dissolution. The demonstrated PETN biodegradability in the aqueous phase suggests that bioremediation also has potential for remediating PETN-contaminated soil. Therefore, the goal of this study was to explore remediation strategies for PETN-contaminated soil. Three strategies were tested: (1) reductive transformation using granular iron, (2) anaerobic biodegradation stimulated by amendment with organic materials, and (3) a combination of iron and microbial processes.

The findings should provide support for selecting a practical treatment method for PETN or other nitrate ester-contaminated sites and provide a basis for larger-scale field testing.

4.1 Materials and Method

4.1.1 PETN-Contaminated Soil

The soil used in this study was obtained from a settling pond that had received waste water from a PETN manufacturing facility for more than 20 years. The inorganic and organic contamination was heterogeneously distributed in the soil at the base of the pond. The soil contained PETN concentrations ranging from 65 to 600 mg/kg and high levels of nitrate and sulfate, with each varying from 8,000~10,000 mg/kg. The presence of nitrate and sulfate in the soil was due to the use of nitric acid and sulfuric acid in the process of PETN synthesis. For the purpose of this study, the PETN concentration was increased to 4,500~5,000 mg/kg

by spiking with pure PETN powder. A portion of the soil was leached several times with Millipore water to reduce the level of nitrate and sulfate in order to minimize the accumulation of analytical errors from multiple dilutions. On average, the concentrations of nitrate and sulfate were decreased to 1,500 and 2,500 mg/kg, respectively. The soil was air dried and ground to pass a 2 mm sieve before use. Soil used in the sterile controls was triple-autoclaved for 1 h at 121ºC on three consecutive days. The soil had a total organic carbon content of 0.41%, including PETN (0.12%).

4.1.2 Iron and Organic Materials

The granular iron was obtained from Connelly-GPM Inc. (Chicago, Illinois) and used without pretreatment (iron parameters same as in Chapter 2). DARAMEND materials (D6390 Fe20 and ADM-298500) were used as carbon amendments in the experiment and were provided by ADVENTUS Remediation Technologies (Mississauga, Ontario). The DARAMEND products are manufactured from naturally occurring plant materials, rich in carbon and nutrients. The precise composition is proprietary, but D6390 Fe20 has 20% (wt) fine-granular metallic iron while ADM-298500 has no metallic iron.

4.1.3 Experimental Procedures

A total of 15 treatments were tested: set-1 to set-6 contained different percentages of granular iron ranging from 2 to 10% (by weight), set-7 to set-14 tested the two types of organic materials (D6390 Fe20 and ADM-298500) at 1% and 2% levels, and one treatment, set-15, had a combination of 5% iron and 2% ADM-298500. The composition of each treatment is given in Table 4.1. Each treatment involved the same laboratory conditions with identical set-up and sampling procedures.

Tests were conducted in 40 mL glass vials with screw caps fitted with Teflon-lined septa. The set-up procedure included the steps of: weigh empty vial, add 15 g PETN-contaminated soil, add desired amount of amendments (granular iron or DARAMEND materials), fill with deoxygenated Millipore water, create 10 mL headspace (5% H_2 + 5% CO_2 + 90% N_2) by removing 10 mL of water in the anaerobic glovebox, cap and re-weigh the vial. The filled

vials were vortexed for 1 min and then kept in the dark at room temperature (25ºC) during the experiment. Triplicate vials were sacrificed for chemical analysis at each of the eight sampling times, requiring the preparation of 24 vials for each treatment. Before analysis, the vials were centrifuged for 15 min at 1,500 rpm. The aqueous solution was removed for inorganic analyses including nitrite, nitrate and sulfate. The soil was analyzed for PETN following the acetonitrile-sonication extraction method (US EPA Method 8330). Briefly, soil samples were first dried in air at room temperature to a constant weight, then ground and homogenized thoroughly in an acetonitrile-rinsed mortar to pass a 30 mesh sieve (0.6 mm). A 2 g sample of soil was placed in a 15 mL glass vial and 10 mL of acetonitrile was added. The vial was capped with a Teflon-lined cap, vortexed for 1 min and placed in a cooled ultrasonic bath for 18 h. After sonication and settlement, 5 mL of the supernatant was removed and well mixed with 5 mL $CaCl_2$ solution (5 g/L). The supernatant of the mixture was transferred to a 1.5 mL HPLC vial for analysis.

4.1.4 Analytical Methods

Inorganic analyses for nitrate, nitrite and sulfate and organic analysis of PETN were performed using the same analytical methods described in Chapter 2.

4.2 Results and Discussion

4.2.1 Iron Treatments

The iron treatments were all conducted with the leached soil, including sterilized and unsterilized controls without iron addition (set-1 and set-2), a treatment using autoclaved soil amended with 10% iron (set-3), and three treatments using unautoclaved soil amended with 2%, 5% and 10% (w/w) iron (set-4 to set-6). The changes in PETN concentrations in the soil over the 93-day treatment period are shown in Figure 4.1. There was little or no PETN removal in the two controls, while 17%, 19% and 26% reductions were observed in the unsterilized soils containing 2%, 5% and 10% iron, respectively. A 19% removal was also observed in the autoclaved soil amended with 10% iron. Removal of PETN in the soils amended with iron (set-3 to set-6) relative to the persistence of PETN in the controls without

iron (set-1 and set-2) suggests that iron is capable of removing PETN from contaminated soil. However, while the degree of PETN removal in the soil was in the order of increasing iron content, it was not proportional. Furthermore, the rate of removal declined rapidly over time, with most of the removal occurring within the first 20 days of the experiment. While it is clear that granular iron can degrade PETN in the soil, the rates and degrees of removal are substantially lower than would be expected, based on the previous results of PETN degradation in aqueous solution. The results, with rapidly declining rates of removal at early time, suggest that the iron was being passivated.

Although the leaching procedure reduced nitrate to less than 20% of the initial value, the concentration in the soil was still approximately 1,500 mg/kg. Even though nitrate can be reduced by granular iron to ammonia (Cheng et al., 1997; Huang et al., 1998), as an oxidizer, nitrate causes the formation of passive iron oxide films on the iron surfaces (Schlicker et al., 2000; Ritter et al., 2003). Though iron was found to be very effective in degrading PETN in the aqueous phase (chapter 2), no nitrate was present in those tests. The relatively poor performance in the present tests is believed to be a consequence of passivation of the iron by the high nitrate concentration in the soil.

The trends in nitrate concentration over time for the various treatments are shown in Figure 4.2. For the autoclaved treatments, there was a gradual but slight decline in nitrate concentration in the autoclaved soil without iron (set-1); however, in the autoclaved soil containing 10% iron (set-3), there was a 14% decline in the nitrate concentration over the first 10 days, followed by little or no further decline over the remainder of the test period. Though the data is sparse, this is consistent with reduction of nitrate at early time by the iron, resulting in passivation of the iron and thus no further reductive degradation by the iron. Comparing to Figure 4.1, this agrees with the trend in PETN in the autoclaved iron treatment, with an early and rapid decline in concentration followed by a much slower rate of PETN removal over the remainder of the test period. For the unautoclaved treatment without iron, nitrate did not decrease within the initial 10 days but showed a relatively steady decrease

over the remainder of the experiment. In all treatments with iron, both autoclaved and unautoclaved, there was a similar decline in nitrate concentration during the first 10 days of about 14 to 19%. In the autoclaved sample there was only a minor decline in nitrate concentration over the remainder of the experiment, while in all unautoclaved treatments with iron, there was a continuing decline in nitrate, with almost total consumption by day 93. The continued removal of nitrate following passivation of the iron suggests that nitrate is being consumed by denitrification. This has important consequences in that it indicates that the soil materials are biologically active, and that some portion of the organic carbon fraction of the soil is labile. Thus the generally low rates of PETN removal at late time (Figure 4.1) may also be a consequence of either biological activity or residual activity of the iron. The greater reduction in the PETN concentration in the soil with 10% iron compared to the 10% iron autoclaved treatment, suggests that PETN removal at late time is more likely a consequence of biological processes. From a comparison of Figures 4.1 and 4.2, denitrification appears to be the more competitive processes relative to PETN degradation. This is consistent with our previous findings (chapter 3) that rate of nitrate reduction exceeds the rate of PETN degradation.

Based on the results of this study, granular iron did not prove to be an effective amendment for PETN removal due to passivation of the iron in the presence of high concentration of nitrate in the soil. Granular iron may however be effective for PETN contaminated soil if nitrate or other competing oxidants are not present. Furthermore, Lu (2005) showed that the change in the iron surface and the loss of iron reactivity due to nitrate is a reversible process. Thus, at some time after the nitrate is depleted, there is reason to expect that more effective degradation of PETN by the iron would proceed. The current study did not proceed for a sufficient period of time to explore this possibility.

4.2.2 Enhanced Biodegradation

The test of biodegradation with amendment of organic materials involved 8 treatments (set-7 to set-14), as described in Table 4.1. During incubation, some characteristic biological phenomena were observed in the treatments with DARAMEND amendments. Within the

first 5 days, large quantities of gas were generated. Subsequently, the formation of black precipitates was observed in set-11 and set-12 after 12 days, and appeared in set-13 and set-14 after 43 and 24 days, respectively. The distinctive odor of hydrogen sulfide was noticeable in the samples with black precipitates. None of the above biological phenomena was observed in the control treatments. The observation in the treatments amended with DARAMEND materials suggests intense microbial activity in the soil, and is consistent with denitrification and sulfate reduction processes.

The concentrations of both nitrate and sulfate in the unautoclaved treatments are plotted in the Figures 4.3 and 4.4. In the unautoclaved controls without amendment, 894 of 1058 mg/kg of nitrate was removed in the leached soil and 1341 of 9296 mg/kg in the unleached soil by the end of the incubation period (93 days). In contrast, regardless of whether the soil was leached or unleached, nitrate concentrations in the treatments with both types of DARAMEND materials decreased to below the detection limit within the first 5 days (Figure 4.3), which is consistent with the period of observed gas production. Due to the frequency of sampling and high rate of nitrate removal, it is not possible to relate the rate of nitrate removal to the particular treatments. Nevertheless, nitrate in the leached soil is expected to be removed earlier than in the unleached soil because of the lower initial nitrate concentration. Though denitrification occurred in the controls without amendment for both leached and unleached soil, the rate and extent of nitrate removal was much lower than in the treatments with DARAMEND materials. The remarkable contrast indicates that though the organic carbon fraction in the soil is bioavailable to certain microbial activity and growth, it is not sufficient to support high rates of anaerobic activity, even for the energetically favorable reaction of nitrate reduction. Thus, the availability of carbon in the contaminated soil appears to be a limiting factor for anaerobic microbial activity.

There was no decrease in the sulfate concentration in the control treatments without amendment. This is not surprising because the carbon source in the soil was not sufficient for complete nitrate reduction and thus other anaerobic processes, which are less

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thermodynamically favorable, such as sulfate reduction, would not be expected to proceed. In contrast, appreciable declines in sulfate concentration were observed in the treatments amended with both types of DARAMEND materials. For all treatments, an unexpected rise in sulfate concentration occurred over the early time (Figure 4.4). A subsequent test confirmed this to be a consequence of the rate of dissolution of the sulfate-containing minerals in the soil phase (data not shown). The onset of sulfate reduction took place after 12 days in the leached soil, while the lag phase appeared to be much longer in the unleached soil, but in both cases, the onset of sulfate reduction was consistent with the time of appearance of black precipitates in the soils. In the treatments that used the unleached soil, after nitrate removal and a lag period, there was a relatively rapid decline in sulfate concentration, over a period of about 20 days, followed by a flattening of the curves, suggesting little or no further removal. In the leached soil, though the onset of sulfate reduction occurred earlier, the rate of reduction appears slower than in the unleached soil, and the plateau at later times of incubation also suggests little further reduction, particularly in the treatment containing 1% D6390Fe20. The trends in sulfate concentration, in particular the plateau observed in the unleached soil, suggest carbon limitation since much of the available carbon had been consumed by denitrification prior to the onset of sulfate reduction.

PETN concentrations for all treatments are plotted in Figure 4.5. Little or no reduction in PETN concentration was observed in the sterilized and unsterilized controls without amendment (set-7 to set-9). A 20% PETN removal was observed in the sterile treatment with DARAMEND amendment (set-10). Based on the shape of the curve, and particularly the significant delay in PETN removal, it is likely that the removal was a consequence of incomplete sterilization. In contrast, PETN was almost completely removed in all treatments in which DARAMEND materials were added (set-11 to set-14). The apparent difference indicates the requirement for a carbon source amendment in order to stimulate PETN degradation. This is consistent with the results of the aqueous PETN tests of Chapter 3.

As seen in Figures 4.3, 4.4 and 4.5, removal of nitrate, PETN and sulfate occurred predominantly during the early (0 to 5 d), middle (5 to 36 d) and late (36 to 105 d) phases, respectively, of the incubation period, suggesting that the majority of PETN degradation was not concurrent with other energy-yielding reactions (such as nitrate and sulfate reduction in this case), which would be a requirement for co-metabolic processes. The previous study on biodegradation of aqueous PETN suggests that PETN serves as a terminal electron acceptor (Chapter 3). Though the soil microcosms amended with DARAMEND materials represent a more complex system than the liquid culture, PETN is believed to function in the same way in both systems, i.e., acts as an electron acceptor during its biotransformation processes. The presence of DARAMEND materials in the soil, rich in carbon and nutrients, also greatly reduced the chance for PETN being used as a source of carbon and nitrogen.

The addition of DARAMEND materials stimulated high levels of biological activity, including reductions of nitrate, PETN and sulfate. Figures 4.6 and 4.7 summarize the consumption of nitrate, PETN and sulfate in the leached and unleached soil amended with 2% D6390Fe20. Although there was an overlap between PETN and sulfate reduction in both cases, particularly in the leached soil, a sequence of nitrate over PETN followed by sulfate reduction is consistent with that observed in the previous mineral medium experiment under mixed electron acceptors condition (refer to Figure 3.12).

In this study, since sulfate concentration was still high in the soil by the conclusion of the test period, methanegenesis was not expected to be involved in PETN biodegradation.

4.2.3 Kinetics and Controlling Parameters

Generally, the kinetics of biodegradation processes can be described by empirical Monod equation involving variables of substrate concentration and bacterial growth rates and microbial population. However, the kinetics of PETN degradation in the treatments amended with DARAMEND materials all fit the pseudo-first-order kinetic model. This suggests that with respect PETN, the substrate concentration may be much smaller than the K_s in the Monod equation, which is the coefficient constant of half-saturation of the enzyme sites. The

estimated first-order half-lives were: 17.5 d (R^2=0.984) for leached soil with 1% D6390Fe20, 8.9 d (R^2=0.960) for leached soil with 2% D6390Fe20, 14.4 d (R^2=0.986) for original soil with 2% D6390Fe20 and 15.8 d (R^2=0.984) for original soil with 2% ADM-298500.

Referring to the half-lives, the degradation rate in the leached soil amended with 2% D6390Fe20 (set-11) was twice as fast as in the same soil with 1% D6390Fe20 (set-12). Though the evidence is sparse, it appears that the rate of PETN removal increases with increasing amount of amendment. This is not unexpected since increasing the organic amendment could induce greater microbial growth and activity, enhancing the rate of PETN reduction.

Set-12 and set-13 are parallel treatments amended with the same type and amount of organic materials but were conducted with leached and unleached soil, respectively. Because of the leaching procedure, nitrate and sulfate concentrations in the leached soil were reduced to 1,500 mg/kg and 2,500 mg/kg from 8,000 mg/kg and 10,000 mg/kg in the original soil, respectively. The estimated half-lives for the leached soil treatment (set-12) and unleached soil treatment (set-13) were 8.9 d and 14.4 d, respectively. The appreciable difference reflects the effect of competing electron acceptors on PETN removal. That is, the lower concentration of competing electron acceptors leads to faster PETN biodegradation rates. The results in the mineral medium experiments and the evidence in this soil test both suggest that PETN serves as an electron acceptor during PETN biotransformation and its competitive capability for carbon appears to be intermediate between nitrate and sulfate. Since nitrate is more readily degraded than PETN, and thus a greater amount of the carbon sources would be used to complete denitrification in the unleached soil which contains higher concentration of nitrate. Consequently, less carbon was available for PETN degradation in the unleached soil compared with the leached soil, contributing to the slower degradation rate in the unleached soil. In fact, the result is equivalent to increasing the amount of amendment, which as discussed above, resulting in higher rate of PETN removal.

Two different types of DARAMEND products (D6390Fe20 and ADM-298500) were tested using the same soil in set-13 and set-14. The results show only a small difference in degradation rates, with half-lives of 15.8 d and 14.4 d in the material with and without iron respectively. The addition of iron provided little or no benefit, possibly as a consequence of passivation of the iron.

4.2.4 Iron-Microbial Combined Method

In order to enhance the efficiency of treatment, the third strategy for remediation of PETN-contaminated soil was to combine two potential remediation methods. The combined treatment (set-15) was conducted with the leached soil, containing 5% granular iron and 2% ADM298500. Similar to the other four treatments with DARAMEND materials (set-10 to set-14), odorless gas was produced at early time, followed by the formation of black precipitates and the odour of hydrogen sulfide. Figure 4.8 includes the changes in concentrations of PETN, nitrate and sulfate over the incubation period. Complete nitrate removal was achieved within the first 5 days. The degradation of PETN followed pseudo-first-order kinetics (R^2=0.972), with an estimated half-life of 8.4 d. A substantial decrease in sulfate concentration occurred after 12 days. Though there was overlap, as noted previously, the results showed a similar trend in the order of nitrate, PETN and sulfate removal.

The combined system is clearly superior to the iron treatment alone, which only achieved 19% removal within the same period. A direct comparison with the bioremediation method can not be made since ADM298500 was not tested in the leached soil. However a reasonable comparison can be made with the results of leached soil with 2% D6390Fe20. The combined system showed a small (6%) improvement over the treatment without iron addition.

The synergistic effect of an iron-microbial integrated system for explosive remediation was reported in previous studies (Oh et al., 2001; Wildman and Alvarez, 2001). In the combined system, microorganisms can use hydrogen gas derived from anoxic iron corrosion as an electron donor to support biotransformation of contaminants in soil. However, this benefit is not substantial in our case given that the production of hydrogen was dramatically

reduced due to iron passivation in the presence of high levels of nitrate. Relative to the long period of nitrate presence in the iron treatments, nitrate was rapidly removed within the first 5 days in the combined system. Though the recovery of iron reactivity may occur, the recovery process is slow. For example, Lu (2005) observed early signs of recovery of iron reactivity for TCE degradation after 40 days of nitrate removal, and significant recovery was achieved after 140 days. Thus, the function of iron in the combined system is dramatically reduced by the presence of nitrate in the soil.

In sum, considering the slight improvement in performance versus the added cost and complexity, for the soil of this study (contaminated with high concentration of nitrate), the use of the combined system does not appear to be warranted.

4.3 Conclusion

Three potential remediation methods for PETN-contaminated soil were tested: granular iron, bioremediation with addition of organic materials and a combination of the iron and microbial methods. Of these, the effectiveness of the iron treatments was seriously compromised by iron passiviation caused by the presence of high levels of nitrate in the soil. In both bioremediation and the combined methods, PETN at between 4,500 and 5,000 mg/kg was effectively removed by indigenous soil bacteria within 84 days. Though the iron-microbial integrated method showed a slight enhancement in the rate of PETN removal compared with the organic amendment method, the combined method is not encouraged considering the balance between the small improvement in performance and high cost.

In biological treatment, PETN biodegradation was substantially enhanced by the addition of organic carbon and the rate of PETN removal increased with greater amount of amendment. The results in this study also suggest that PETN serves as an electron acceptor during biotransformation and shows a utilization sequence by microorganism in the order of nitrate, PETN and sulfate, consistent with the previous findings in the liquid media. Since the co-contaminants of nitrate and sulfate in the soil also underwent biological reduction, consuming significant amount of the carbon amendment, thus the consumption of the carbon

amendment by co-contaminants should be considered in determining the amount of amendment required for complete PETN degradation.

In summary, biological treatment with organic amendment appears to be the most effective strategy for remediation of PETN-contaminated soil, particularly in situation where high concentrations of nitrate are present. Though the experiment showed DARAMEND materials to be effective amending materials, other carbon sources, though not tested, may be equally effective.
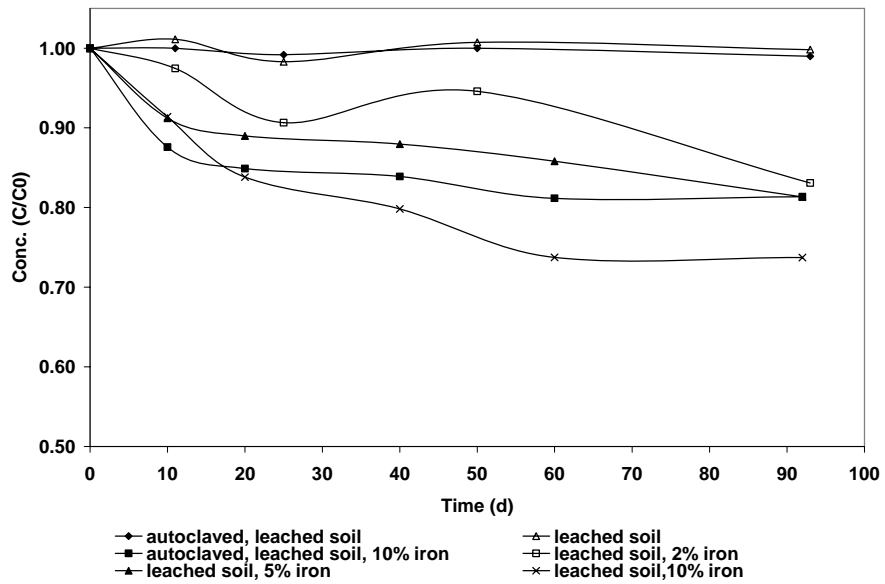
Figure 4.1: Changes in PETN concentration over time in the presence of iron at 0, 2, 5 and 10% in the soil microcosm experiments
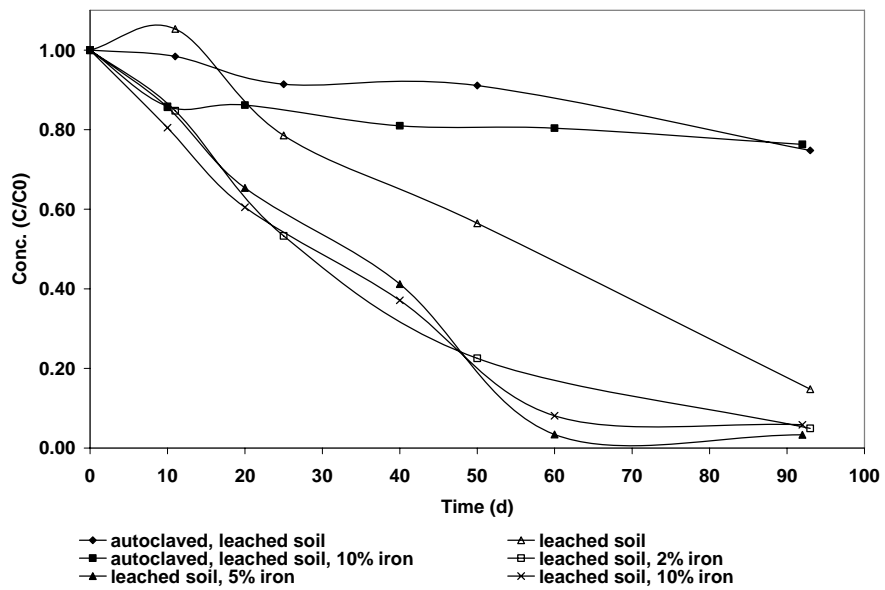
Figure 4.2: Changes in nitrate concentration over time in the presence of iron at 0, 2, 5 and 10% in the soil microcosm experiments
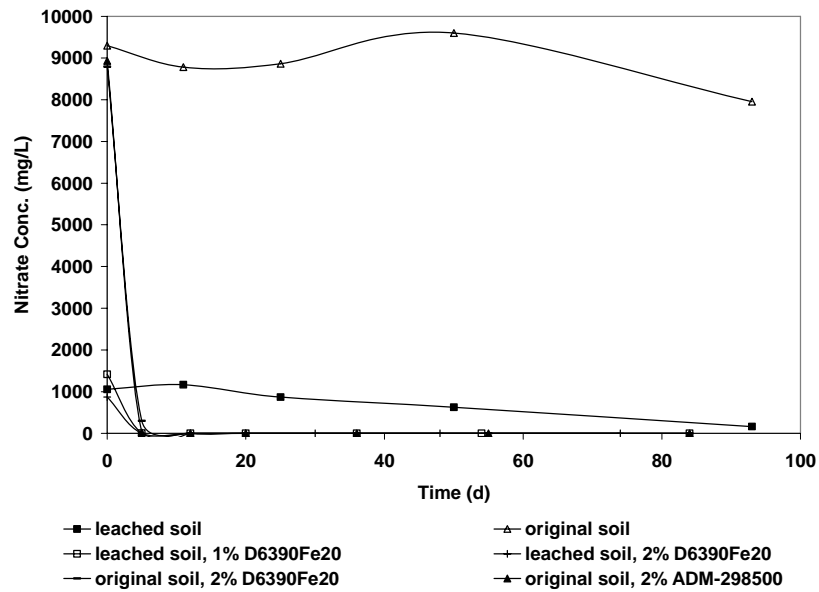
Figure 4.3: Changes in nitrate concentration over time in the soil microcosms with carbon amendments
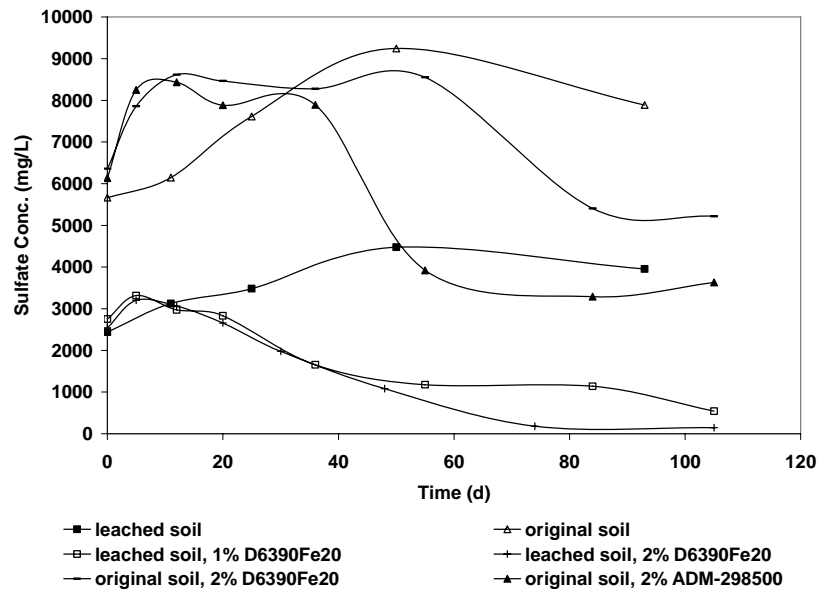
Figure 4.4: Changes in sulfate concentration over time in the soil microcosms with carbon amendments
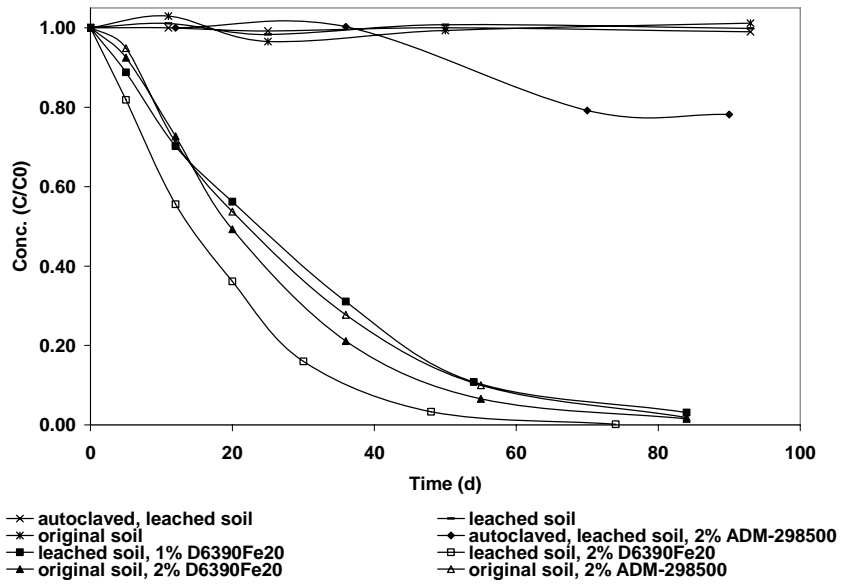
Figure 4.5: Changes in PETN concentration over time in the soil microcosms with carbon amendments
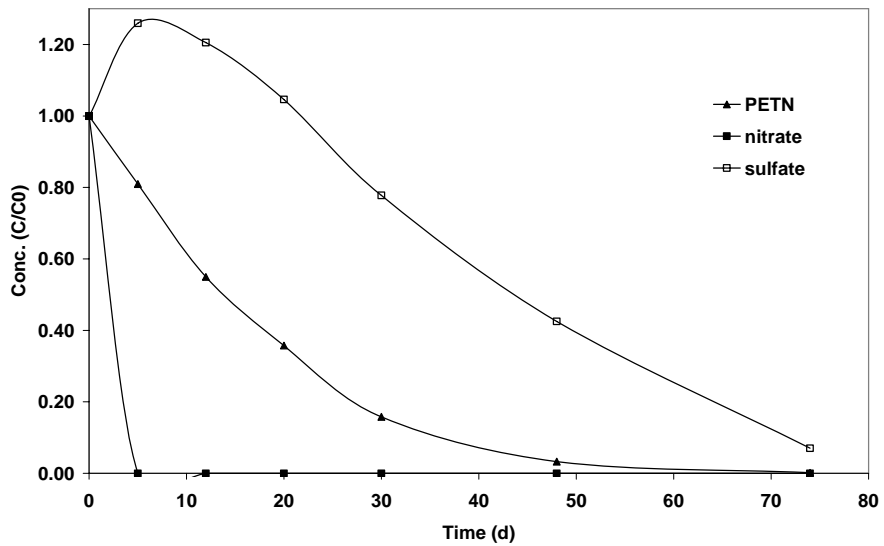
Figure 4.6: Changes in concentrations of PETN, nitrate and sulfate in the treatment using leached soil containing 2% D6390Fe20 (set-12)
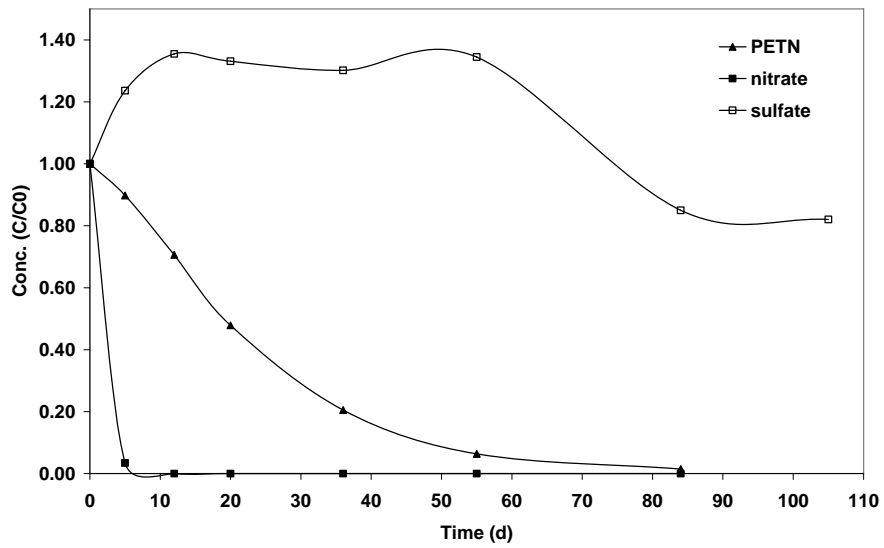
Figure 4.7: Changes in concentrations of PETN, nitrate and sulfate in the treatment using unleached soil containing 2% D6390Fe20 (set-13)
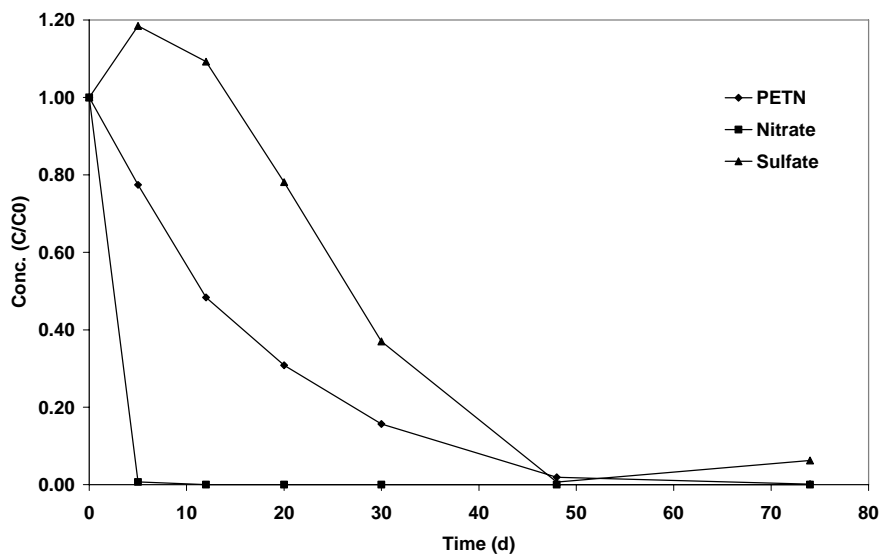
Figure 4.8: Changes in concentrations of PETN, nitrate and sulfate in the treatment using leached soil containing 2% ADM298500 and 5% granular iron (set-15)

Table-4.1: Composition of the anaerobic microcosm experiments

Method	Treatment No.	Soil/Pretreatment	Amendment
Iron Amendment	1	Autoclaved, leached soil	None
	2	Leached soil	None
	3	Leached autoclaved soil	10% Connelly iron
	4	Leached soil	2% Connelly iron
	5	Leached soil	5% Connelly iron
	6	Leached soil	10% Connelly iron
Organic Amendment	7	Autoclaved, leached soil	None
	8	Leached soil	None
	9	Original soil	None
	10	Autoclaved, leached soil	2% ADM-298500
	11	Leached soil	1% D6390Fe20
	12	Leached soil	2% D6390Fe20
	13	Original soil	2% D6390Fe20
	14	Original soil	2% ADM-298500
Iron-Organic Amendment	15	Leached soil	2% ADM-298500 and 5% Connelly iron

Chapter 5

In Situ Anaerobic Bioremediation of PETN: A Pilot Study at Louviers, CO, U.S.A.

The previous laboratory study demonstrated that PETN in soil can be biodegraded anaerobically by bacteria indigenous to the contaminated site, provided readily usable organic matter is added as an amendment. However, the laboratory tests can not simulate the heterogeneous conditions in the field and may not recognize other potential issues that could arise in the field. A pilot test, to serve as a small-scale field demonstration, is considered to be an essential step in proceeding full-scale remediation. This chapter described a scale-up pilot experiment based on the results of the laboratory tests. The objectives were to test the feasibility of removing PETN from the contaminated soil using iron and bioremediation methods under field conditions, and to evaluate the applicability of laboratory results to the field conditions. The results of this pilot test should indicate the degree to which the laboratory results are transferable to the field, the effect of environmental conditions on PETN removal and provide further evidence concerning remediation effectiveness for full-scale treatment.

The pilot test was conducted at a contaminated site at Louviers, CO, U.S.A. This site is believed to be typical of sites that have received long-term discharge of wastewater from the manufacture of PETN. Currently, the site is regulated under the Resource Conservation and Recovery Act (RCRA) solid waste management units (SWMUs) and is required to be decontaminated.

5.1 Materials and Methods

5.1.1 PETN-Contaminated Site
The site is located approximately 40 km south of Denver in Louviers, Colorado. The region is underlain by alluvial terrace deposits (unconsolidated gravel, sand, silt and clay deposited

primarily by running water). The climate in the region is typical of the high range and foothill areas of the Rocky Mountains, with precipitation averaging approximately 40.9 cm per year and daytime temperatures ranging from -7ºC to 41ºC.

The site consists of two solar ponds near a currently inactive explosives manufacturing facility, which had received wastewater from PETN production for over 20 years (between 1967 and 1989). As part of the manufacturing process, wastewater effluent contaminated with PETN was discharged into two surface impoundments (settling ponds). The ponds, each 119 m by 119 m, are lined with high-density PVC sheet with approximately 22 to 36 cm of clayey soil on top of the liner. The PETN concentration in the soil is highly heterogeneous, ranging from 1 to 200,000 mg/kg. Because of the use of nitric acid and sulfuric acid in the process of PETN synthesis, nitrate and sulfate also vary from about 1 to 10,000 mg/kg in the soil. (Site information provided by James L. Beck and Mark Vetter of DuPont)

5.1.2 Materials

The granular iron (from Connelly) and DARAMEND materials (D6390 Fe20 and ADM-298500) used in this pilot experiment are the same as those used in the laboratory microcosm study (Chapter 4). In addition, brewers grain was tested as an alternative carbon source, and was obtained from Teague Diversified, Inc (Ft. Morgan, CO. U.S.A.). It is a by-product from brewing, with a moisture content of 65% and is fibrous with high protein content.

5.1.3 Experimental Approaches

The trial consisted of the following 10 treatments (% by dry weight): a control (no amendment), 10% iron, 1%, 2% and 4% of D6390Fe20, 2% and 4% of ADM-298500, and 1%, 2% and 4% of brewers grain. The final amendment percentages in the treatments were slightly different from the objective values, at 1.19%, 2.63% and 4.37% for D6390Fe20, 2.32% and 5.81% for ADM-298500, and 1.33%, 2.99% and 6.38% for brewers grain (Table 5.2). For convenience, the text will continue to use the objective concentrations (1%, 2% and 4%), though the actual values are used in all calculation.

For each treatment, contaminated soil was first dug from the pond, and then thoroughly mixed with the desired amount of amendment material in a cement mixer. The original soil from the pond was under unsaturated condition, and thus water was added to improve mixing. The moisture contents for each treatment after mixing were 42.9% (control), 51.4% (10% iron), 45.5% (1% D6390Fe20), 51.2% (2% D6390Fe20), 40.2% (4% D6390Fe20), 41.8% (2% ADM-298500), 42.0% (4% ADM-298500), 43.9% (1% brewers grain), 51.0% (2% brewers grain) and 61.2% (4% brewers grain). The mixture was transferred to a plastic tub (45 cm wide × 90 cm long × 25 cm deep) equipped with a float and valve to control the water level. Each tub contained an approximately 18 cm layer of soil with 6~8 cm of standing water (see Picture 5.1). The purpose of the water layer was to reduce oxygen invasion and thus assist in maintaining anaerobic conditions within the soil. The tubs were finally buried in the soil in the solar pond and the floats were connected by hoses to a nearby water source (tap water) (see Picture 5.2).

At each sampling time, Eh and pH in the surface soil (depth between 5~8 cm) were first measured before sampling. Triplicate aliquots of sediment samples were collected at random locations in each tub at depths between 0~10 cm and 15~25 cm using a soil probe. The soil samples from each depth were mixed and subsampled in duplicate into plastic bottles with screw caps. Samples were stored in a freezer prior to shipping to the University of Waterloo. In the laboratory, each soil sample was first air-dried in a fume hood to a constant weight, and then PETN was extracted following the acetonitrile-sonication extraction method (US EPA method 8330) for PETN analysis (see details in section 4.1.3). PETN quantification was determined by HPLC. The remaining soil (air-dried soil passed through a 30 mesh sieve) was used for inorganic analyses including nitrite, nitrate and sulfate. For this, approximately 1 g dry soil and 20 mL Millipore water were added to a 25 mL HDPE vial with a screw cap and the vial was placed on a mechanical shaker at 300 rpm for 1 h. Then the soil-water mixture was allowed to settle until the supernatant liquid was clear and IC analysis was performed on aliquots of this liquid.

5.1.4 Analytical Method

Inorganic analyses of nitrate, nitrite and sulfate and organic analysis of PETN were performed using the same analytical methods as described in Chapter 2.

5.2 Results and Discussion

5.2.1 PETN Removal

The distribution of PETN contamination at the site was very heterogeneous, resulting in different initial concentrations for the different treatments, ranging from 60,000 to 170,000 mg/kg. The large variation in initial concentrations makes data interpretation less straight forward and thus the results are summarized in two ways. Figure 5.1 shows the decline in relative PETN concentration, which is the measured residual PETN concentration in the soil (C) normalized by its initial concentration (C_0). Figure 5.2 shows the PETN mass removal, which is the cumulative PETN mass removed (mg/kg) over the test period. In both figures, the data represent the average of the PETN concentrations in the upper and lower soil samples, which individually, is the average of duplicate samples. Table 5.1 summarizes the final removal results for both the upper and lower samples in the treatments with organic amendments (tub 3 to tub 10) and includes the initial concentrations of PETN, nitrate and sulfate for the treatments.

As indicated in Figures 5.1 and 5.2, there is a great deal of variation over time in the data for the control treatment, and also, to a lesser degree, for the iron treatment. These were the first two treatments that were prepared (control followed by iron treatment), and at the time the procedure for mixing the materials was not well established. We believe the variability in the control and iron treatments to be a consequence of high variability of the PETN concentration in the soil material and inadequate mixing in the initial stages of the preparation processes in the field.

The data suggest a minor decrease of PETN in the control treatment; however, because of the high degree of variability, this is uncertain. It can nevertheless be said with a reasonable

degree of confidence, that if there were losses in the control, the losses were not substantial. Similar statement can be made with respect to the iron treatment. As indicated by the laboratory microcosm tests, the lack of decline in the PETN concentration in the iron treatments is undoubtedly a consequence of passivation of the iron by the presence of nitrate in the soil. With improvements in the mixing process (different mixer, addition of more water and longer mixing time), the trends in the organic amendment treatments (tub 3 to tub 10) were considerably more consistent.

Appreciable declines in PETN concentration were observed in the treatments amended with organic materials (tub 3 to tub 10). The percentage removal at the end of the 74-day test period varied from 10% to 42%, with the greatest decline in concentration in the treatment containing 4% D6390Fe20. In terms of PETN mass removal, 11,195 to 33,397 mg/kg PETN were removed within 74 days, with the greatest mass removal in the treatment amended with 4% brewers grain. In tubs 5, 7 and 10, D6390Fe20, ADM-298500 and brewers grain were added as carbon sources at the same percentage (4%). In terms of decline in PETN concentration, D6390Fe20 was the most favorable (42%), followed by brewers grain (20%) and ADM-298500 (17%) (Figure 5.1). The order changes to brewers grain, D6390Fe20 and ADM-298500 in terms of mass removal (Table 5.1, Figure 5.2). The assessment of performance is complicated by the great variability in initial concentrations in the treatments and thus it is difficult to define the best substrate for PETN remediation in the field. Indeed, all three carbon sources appeared to be effective in stimulating PETN bioremediation. Previous studies have demonstrated that DARAMEND® is an excellent carbon and nutrient source for stimulation of both aerobic and anaerobic biodegradation in soils contaminated with persistent organics such as chlorinated pesticides (toxaphene, DDT, dieldrin), wood preservatives (PAH, CP) and organic explosives (TNT, DNT, RDX, HMX) (Bucens et al., 1996; Seech et al., 2002; EPA Evaluation Report, 1996). In this particular case, it is not clear that DARAMEND has any particular advantage over brewers grain, a readily available product from the local brewering industry. Regardless of how one expresses PETN removal and the types of organic material, the results clearly show that PETN can be effectively

degraded under anaerobic conditions through the addition of organic carbon and possible nutrients, provided by DARAMEND material or brewers grain. This observation is consistent with the previous laboratory results, which demonstrated the requirement of organic materials for PETN biodegradation in contaminated soil (chapter 4). The results suggest that the persistence of PETN in the waste pond is a consequence of an insufficient supply of labile organic carbon, necessary for PETN degradation, even though the indigenous bacteria in the contaminated soil had clearly acclimated to PETN.

In addition, the trend of greater PETN removal with higher loading of carbon sources was observed for all three types of organic materials. For instant, approximately 16417, 21388 and 27644 mg/kg of PETN were removed in the treatments amended with 1%, 2% and 4% D6390Fe20 over the test period, respectively (Figure 5.3). This suggests that increasing the amount of organic amendment would result in greater microbial growth and activity, leading to greater PETN removal. This observation is also consistent with the findings of the laboratory microcosm tests where the PETN degradation rate was twice as fast in the 2% D6390Fe20-fed microcosm as in the 1% D6390Fe20-fed microcosm. Based on the similarity in the trends of PETN removal, it is concluded that the degradation processes are similar between the laboratory microcosms and field tests. Previous laboratory results (chapters 3 and 4) both indicate that PETN serves as an electron acceptor during degradation. Thus in the field test, consistent with laboratory results, the organic amendments served as electron donors to stimulate reductive degradation of PETN, which served as an electron acceptor, and PETN degradation followed a sequential denitration pathway. The most striking difference between the laboratory and the field results is the rate and degree of PETN removal. In the laboratory, with initial PETN concentration of 4,500 to 5,000 mg/kg in the treatments with organic amendments, nearly 100% removal occurred in 84 days following pseudo-first-order kinetics. In the field however, generally there was rapid removal during the first 40 days, but by the end of the experiment, the rates of removal decreased and deviated from the initial pseudo-first-order behavior (Figure 5.1). The decline in degradation rates might be a result of variability in biodegradability of various components in the carbon

sources, which originate from plant materials. Generally, the easiest degradable components were quickly used first.

5.2.2 Inorganic Parameters and Other Factors

Soil used in this pilot test contained 89 to 254 mg/kg of nitrate and 898 to 2399 mg/kg of sulfate. Their concentrations in the soil were monitored over the test period and are plotted in Figures 5.4 and 5.5. The data present the average of nitrate or sulfate concentrations in the upper and lower soil samples. Nitrate reduction appeared to occur in all the treatments and the extent of nitrate depletion increased with the loading of carbon sources. The control and iron treatments both showed a significant decline in initial concentration over the first 10 days; however, because of the improper mixing, it is not certain that these trends are meaningful. Furthermore, in both cases, the concentrations increased at later time, adding to the possibility that the data is significantly affected by sample variability. As for sulfate concentration, no significant reduction was observed in the control and iron treatments whereas gradual and appreciable sulfate reduction was observed in the treatments amended with organic materials (tub 3 to tub 10). Unlike nitrate reduction, the extent of sulfate consumption did not increase significantly with increasing amounts of amendments.

It is clear that the sequence of reduction of nitrate, PETN and sulfate in the pilot test is much less distinct than in the previous laboratory studies. Figure 5.6, as an example, presents the performance of PETN, nitrate and sulfate in the treatment amended with 4% D6390Fe20. Though nitrate is reduced most rapidly, there is essentially concurrent reduction of PETN, nitrate and sulfate. This considerable overlap in the reduction sequence is likely a consequence of the presence of microenvironment and the heterogeneity in the field soil.

Besides the observed denitrification and sulfate reduction processes, algal growth was clearly observed in the treatments with organic amendments, with the greatest algal production in the treatments receiving the largest amendments (4% D6390Fe20, 4% ADM298500 and 4% brewers grain). There was no algal growth in the control and iron treatments. The difference suggests that the algal bloom was caused by the large addition of

nutrients (such as P and N) provided by DARAMEND materials and brewers grain. Interestingly, we found greater contrast in PETN removal between upper and lower soil samples in the treatments with higher loading of organic amendment (Table 5.1). Taking D6390Fe20 as an example, the removals for surface and subsurface soil were quite similar at low concentrations of amendment (s:b (surface: bottom) = 26%:27% for 1% D6390Fe20, s:b = 28%:25% for 2% D6390Fe20). A more significant contrast was observed in the treatment with 4% D6390Fe20 (s:b = 52%:31%). A similar trend was observed in the other two types of carbon amendment, ADM298500 and brewers grain. This difference in effectiveness as a function of soil depth in the treatments with greater amendment (tubs 5, 7 and 10) is proposed to be a result of the extensive algal growth in the water layer. Since the algae growth will produce exudates and dead algae cells, which are highly labile carbon sources for bacteria, this may result in greater microbial activity and growth in the upper soil than in the lower soil, and thus explains the greater PETN removal in the surface soil.

Besides PETN, the variation of concentration between the upper and lower samples was also observed for nitrate and sulfate. However, the variation for nitrate is not very significant (data not shown), while a more noticeable difference was observed for sulfate concentration, especially in the treatments with greater amounts of amendments. Similar to PETN degradation, more sulfate was reduced in upper soil than the lower soil in the high amendment treatments. For instance, at 1% brewers grain, 41% and 33% sulfate removal was observed in the upper and lower soil, while at higher amendment, 4%, for example, 71% and 9% removal was observed for the upper and lower soil samples, respectively. This is believed to be a consequence of intensive algal growth in the upper water layer, same as explained for PETN.

The field test proceeded from early August to mid October in 2006. The average ambient daytime temperature was 20ºC, varying from a high of 32ºC to a low of 16ºC. The temperature variation was not substantial over the period of the pilot experiment and is expected to be less in the soil. Thus temperature does not appear to be a factor in the

observed degradation of PETN. The pH in the soil was monitored throughout the test period for all the treatments and is plotted in Figure 5.9. Except for the control and iron treatments, where pH remained above 9, pH generally ranged between 6.3 and 8.4, an optimal range for microbial activity and growth. Therefore, pH at the contaminated site of this study was not a sensitive factor and adjustment with respect to pH was not required for promoting bioremediation.

Besides pH, Eh was also measured as an indicator of redox potential in the soil. The data (not shown) showed an average range of 0 to -115 mV for the treatments with organic amendments, and a slightly lower potential of -65 to -130 mV for the iron treatment. Since the measurement was taken at a depth of approximately 5 cm in the 18 cm thick soil layer, lower redox potential may have occurred in the deeper soils. The Eh values and observed nitrate and sulfate reduction indicate anaerobic conditions for the entire period of the experiment.

5.2.3 Practical Consideration of Carbon Amendment

As shown in the previous chapters, PETN is used as an electron acceptor during biodegradation, and requires sufficient supply of electron donors to achieve complete PETN removal. For complete denitration of PETN, the theoretical requirement of organic material (electron donor) for each treatment can be estimated from the following stoichiometric equation:

$$5\ C_6H_{10}O_5 + 6\ C_5H_8(ONO_2)_4 = 6\ C_5H_8(OH)_4 + 12\ N_2\ (g) + 30\ CO_2 + 13\ H_2O \qquad \text{eq 5.1}$$
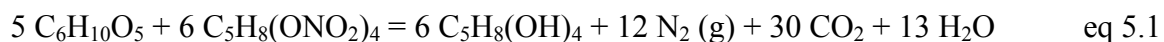
$C_6H_{10}O_5$, a representative formula for cellulose, is assumed to represent DARAMEND materials and brewers grain, based on their original composition of plant materials. They serve as electron donors during the process of PETN ($C_5H_8(ONO_2)_4$) degradation, in which PETN is completely denitrated to pentaerythritol ($C_5H_8(OH)_4$) and the liberated nitrite is further reduced to nitrogen gas.

The "organic carbon availability index" is introduced in this study to quantify the adequacy of the carbon source in each treatment. This dimensionless number is the ratio of the actual addition of carbon sources and the stoichiometric estimation of organic carbon requirement. Theoretically, the complete removal of PETN can be achieved if the carbon availability index is greater than 1; otherwise, the carbon source is not sufficient and will limit PETN degradation. For instance, the initial PETN concentration in the treatment containing 4% D6390Fe20 was 66,438 mg/kg based on the analytical results using the acetonitrile-sonication extraction method. Using equation 5.1, the required amount of organic amendment was estimated to be 28,383 mg/kg or 2.8%. However, the value was based on the soil used in the standard analytical method, in which the soil was ground to pass through a 30 mesh sieve (0.6 mm). Generally, the mass of the coarse particles, larger than 0.6 mm, in the soil sample accounts for half of the total mass of sample. Therefore, the number of 2.8% will be halved to 1.4% on the basis of dried field soil without sieving. As stated in the method section, the actual dose of D6390Fe20 in this treatment was 4.4% based on the dried untreated field soil. Therefore the organic carbon availability index for this treatment is 3.1 (4.4% divided by 1.4%). Using the same method of estimation, Table 5.2 shows that the supply of organic carbon is sufficient for the treatments containing higher concentration of amendments at 2% or 4%, and is in deficit for the treatments amended with 1% D6390Fe20, 2%ADM298500 and 1% brewers grain.

Besides PETN, nitrate and sulfate are also effective electron acceptors, consuming electron donors in the soil. The preference for nitrate over PETN by microorganisms requires that sufficient carbon be added for complete denitrification, as well as for PETN degradation. Though the results in the mineral medium experiments clearly demonstrated that sulfate will not be reduced until PETN is fully removed, sulfate reduction was observed before complete PETN removal in both the laboratory microcosms and pilot scale tests conducted with soil. Thus, to be conservative, the organic carbon availability index for each treatment was reevaluated, taking the carbon requirement for both nitrate and sulfate into consideration (Table 5.3). The values are slightly less than the previous ratios in Table 5.2 because nitrate

and sulfate are both present in small amounts relative to the high concentrations of PETN in the soil. In practice, the need for carbon is likely to be greater than the stoichiometric values, due to the incomplete utilization of carbon sources. In addition, although the soil in each tub was covered with 6~8 cm of standing water in order to diminish oxygen invasion, the water layer was open to the atmosphere, allowing oxygen diffusion into the water, particularly during the occasional precipitation events. Therefore, the presence of oxygen, as a more energetic terminal electron acceptor, will also consume the added carbon sources, increasing the possibility of carbon limitation in many of the treatments. Based on the stoichiometric calculation, the "carbon bioavailability index" in the treatments amended with 1% or 2% organic material are smaller or slightly greater than 1, in order to achieve complete removal of PETN in those treatments, resupply of carbon sources may be required. Only the three treatments amended at 4%, whose carbon bioavailability index are much greater than 1, hold the potential for complete removal without reapplication of carbon sources.

The stoichiometric estimation from the microbial-mediated redox reaction between organic carbon (electron donors) and PETN (electron acceptor) (eq 5.1) provides a theoretical minimum requirement of carbon sources for PETN degradation. Because high levels of nitrate are common at many sites contaminated with PETN and it is a more competitive electron acceptor than PETN, depletion of carbon by nitrate needs to be considered. Thus, knowledge of the distribution of PETN and other co-contaminants is necessary prior to successful implementation of field-scale remediation. In practice, the organic carbon sources need to be added in larger amount than the stiochiometric values, taking the utilization efficiency of organic carbon and additional consumption from oxygen into account. Based on the results in this study, DARAMEND materials and brewers grain all seem to be effective organic carbon sources for stimulating PETN degradation. Brewers grain may be the more cost-effective alternative due to its lower cost. Other sources of organic carbon were not tested but may be equally effective.

5.3 Conclusion

This study involved pilot tests of PETN biodegradation at a PETN-contaminated site at Louvier, Colorado, U.S.A., building on the earlier laboratory experiments. Two remediation alternatives, granular iron and bioremediation, were evaluated for further scale-up tests. The iron-mediated method was not effective because of iron passivation by the presence of nitrate in the soil. Although the rates of PETN biodegradation in the pilot tests were slower than in the laboratory experiments, they were qualitatively similar. The stoichiometric estimation of carbon requirement suggests that PETN degradation in most treatments will be ultimately limited by carbon sources. More carbon sources are required to complete 100 % removal of PETN in the tests.

In summary, the pilot tests clearly showed that PETN can be effectively biodegraded under anaerobic conditions provided with sufficient carbon sources. DARAMEND materials and brewers grain appears to be equally effective, suggesting other carbon sources may also be used for PETN remediation. The promising results in this study provide support for larger-scale field test or full-scale treatment.
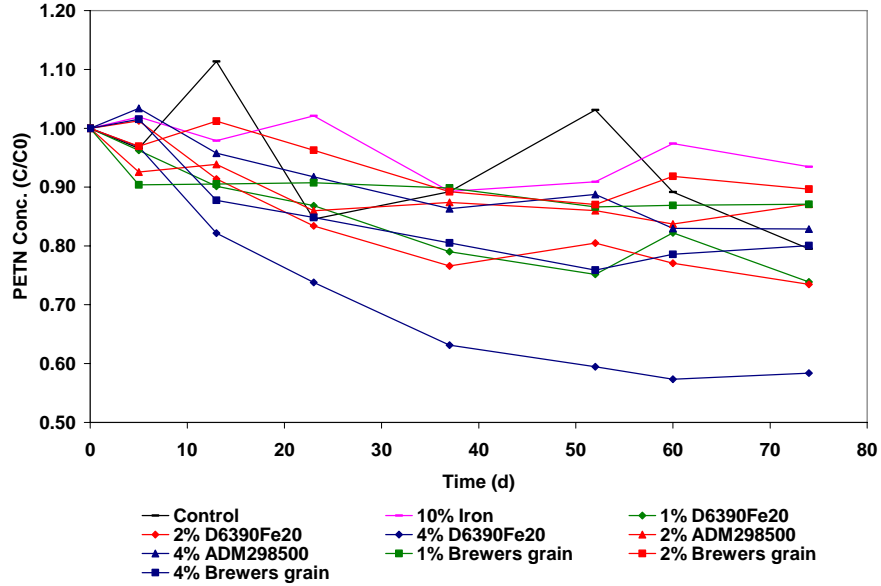
Figure 5.1: Average PETN percent removal in all treatments during the 74-day test. Data represents the average of removal in the upper and lower soil samples
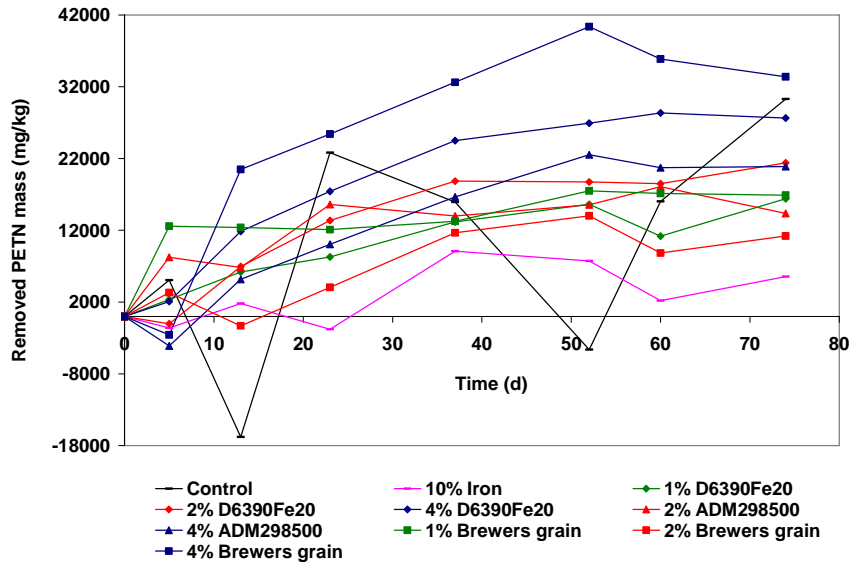
Figure 5.2: Average PETN mass removal (mg/kg) in all treatments during the 74-day test. Data represents the average of removal in the upper and lower soil samples

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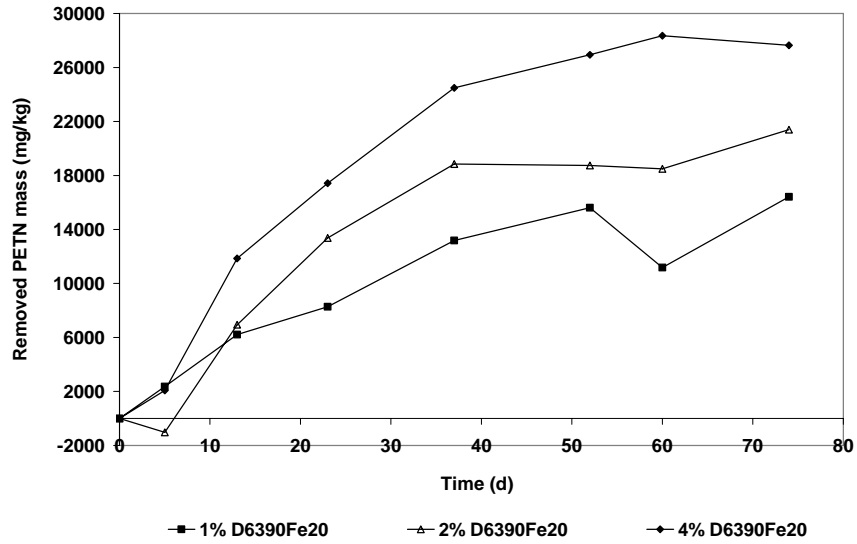
Figure 5.3: Average PETN mass removal (mg/kg) in the treatments amended with 1%, 2% and 4% D6390 Fe20 during the 74-day test
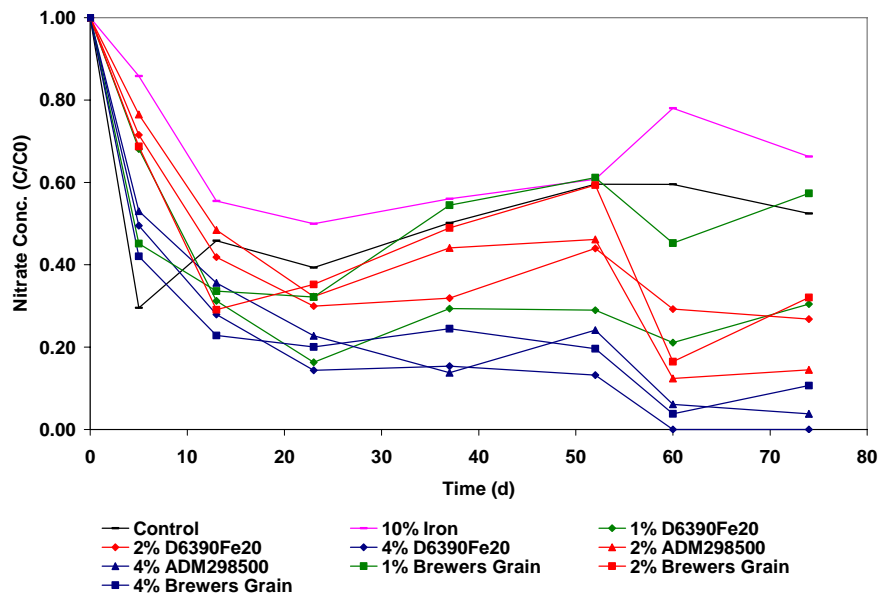
Figure 5.4: Changes in average nitrate concentration in all treatments during the 74-day test. Data represents the average of removal in the upper and lower soil samples
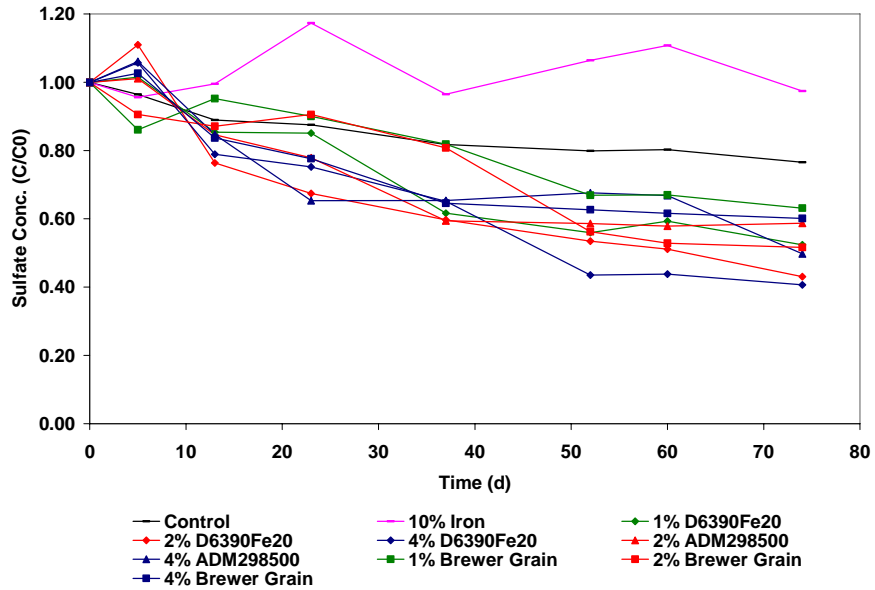
Figure 5.5: Changes in average sulfate concentration in all treatments during the 74-day test. Data represents the average of removal in the upper and lower soil samples
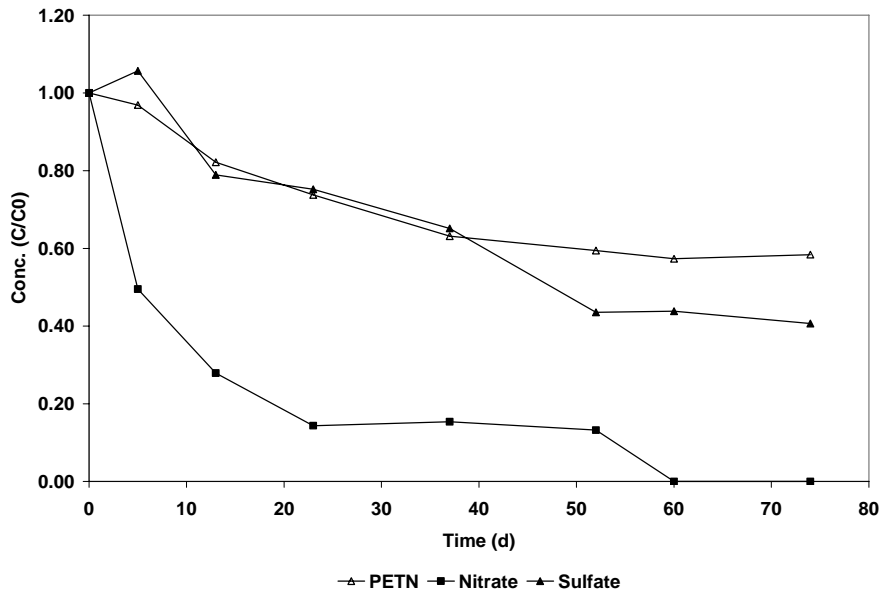
Figure 5.6: Changes in average concentrations of PETN, nitrate and sulfate in the treatment amended with 4% D6390Fe20 during the 74-day test. Data represents the average of removal in the upper and lower soil samples
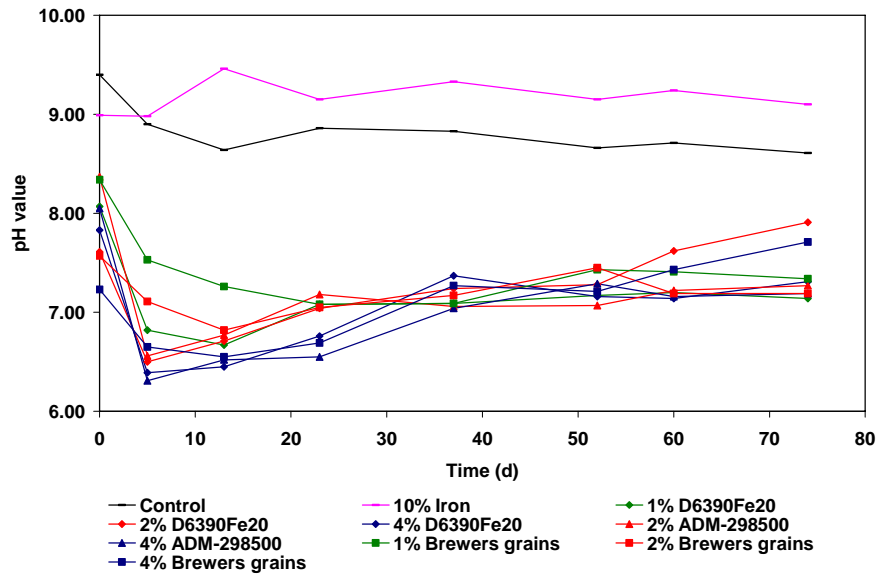
Figure 5.7: pH measurements in all treatments during 74-day test (the measurements taken at a depth of ~5 cm in the 18 cm thick soil layer)

Table 5.1: PETN removal in the treatments amended with organic materials

Tub #	Amendments	Nitrate (mg/kg)	Sulfate (mg/kg)	PETN Conc. (mg/kg)	Removal (%)		Removed Mass (mg/kg)	
					Surface	Bottom	Surface	Bottom
3	1% D6390Fe20	114	898	62837	26	27	16111	16724
4	2% D6390Fe20	130	1232	80591	28	25	22898	19879
5	4% D6390Fe20	254	1225	66438	52	31	34655	20634
6	2% ADM298500	118	1452	111044	15	11	16209	12492
7	4% ADM298500	218	1622	121808	25	9	30830	10940
8	1% brewers grain	107	1741	130602	17	15	22202	19089
9	2% brewers grain	89	1890	108177	18	9	19540	9770
10	4% brewers grain	180	2399	167407	26	14	43198	23597

Table 5.2: Stoichiometric carbon requirement for PETN denitration

$C_6H_{10}O_5$, representing amended organic materials in the pilot tests, serves as electron donor,

PETN ($C_5H_8(ONO_2)_4$) serves as electron acceptors.

$$5\ C_6H_{10}O_5 + 6\ C_5H_8(ONO_2)_4 = 6\ C_5H_8(OH)_4 + 12\ N_2\ (g) + 30\ CO_2 + 13\ H_2O$$
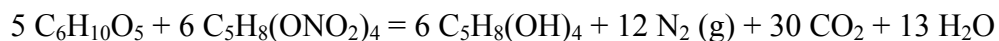
Tub #	Amendments	PETN Initial Conc. (mg/kg)	Requirement for carbon source (mg/kg)	Total need for carbon sources basis on fine soil (%)	Total need for carbon sources basis on field soil (%)	Actual addition of organic material (%)	Carbon bioavailability index
1	Control (none)	147835	63157	6.32	3.16	0.00	0.00
2	10% Connelly iron	84820	36236	3.62	1.81	0.00	0.00
3	1% D6390Fe20	62837	26845	2.68	1.34	1.19	0.89
4	2% D6390Fe20	80591	34430	3.44	1.72	2.63	1.53
5	4% D6390Fe20	66438	28383	2.84	1.42	4.37	3.08
6	2% ADM298500	111044	47440	4.74	2.37	2.32	0.98
7	4% ADM298500	121808	52038	5.20	2.60	5.81	2.23
8	1% Brewer grain	130602	55795	5.58	2.79	1.33	0.48
9	2% Brewer grain	108177	46215	4.62	2.31	2.99	1.29
10	4% Brewer grain	167407	71519	7.15	3.58	6.38	1.78

Table 5.3: Stoichiometric carbon requirement for nitrate, sulfate and PETN

$C_6H_{10}O_5$, representing amended organic materials in the pilot tests, serves as electron donor, nitrate, sulfate and PETN serve as electron acceptors.

$$5\ C_6H_{10}O_5 + 24\ NO_3^- = 12\ N_2\ (g) + 30\ HCO_3^- + 6\ H^+ + 7\ H_2O$$

$$C_6H_{10}O_5 + 3\ SO_4^{2-} + H_2O = 3\ HS^- + 6\ HCO_3^- + 3\ H^+$$

$$5\ C_6H_{10}O_5 + 6\ C_5H_8(ONO_2)_4 = 6\ C_5H_8(OH)_4 + 12\ N_2\ (g) + 30\ CO_2 + 13\ H_2O$$

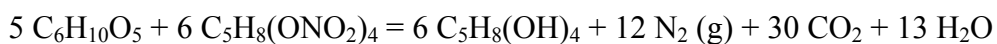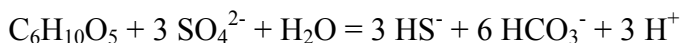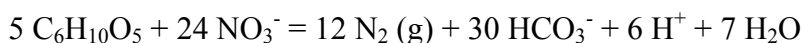| Tub #	Amendments	Initial Conc. (mg/kg)			Requirement for carbon source (mg/kg)			
		PETN	Sulfate	Nitrate	PETN	sulfate	nitrate	Total
1	Control (none)	147835	1926	223	63157	1083	121	64362
2	10% Connelly iron	84820	1019	172	36236	573	94	36903
3	1% D6390Fe20	62837	898	114	26845	505	62	27412
4	2% D6390Fe20	80591	1232	130	34430	693	71	35193
5	4% D6390Fe20	66438	1225	254	28383	689	138	29211
6	2% ADM298500	111044	1452	118	47440	817	64	48321
7	4% ADM298500	121808	1622	218	52038	912	119	53069
8	1% Brewer grain	130602	1741	107	55795	979	58	56833
9	2% Brewer grain	108177	1890	89	46215	1063	48	47326
10	4% Brewer grain	167407	2399	180	71519	1349	98	72966

Tub #	Amendments	Total need for carbon source (%) basis on dry, fine soil	Total need for carbon source (%) basis on dry, site soil	Actual addition of organic material (%)	Carbon bioavailability index
1	Control (none)	6.44	3.22	0.00	0.00
2	10% Connelly iron	3.69	1.85	0.00	0.00
3	1% D6390Fe20	2.74	1.37	1.19	0.87
4	2% D6390Fe20	3.52	1.76	2.63	1.49
5	4% D6390Fe20	2.92	1.46	4.37	2.99
6	2% ADM298500	4.83	2.42	2.32	0.96
7	4% ADM298500	5.31	2.65	5.81	2.19
8	1% Brewer grain	5.68	2.84	1.33	0.47
9	2% Brewer grain	4.73	2.37	2.99	1.26
10	4% Brewer grain	7.30	3.65	6.38	1.75
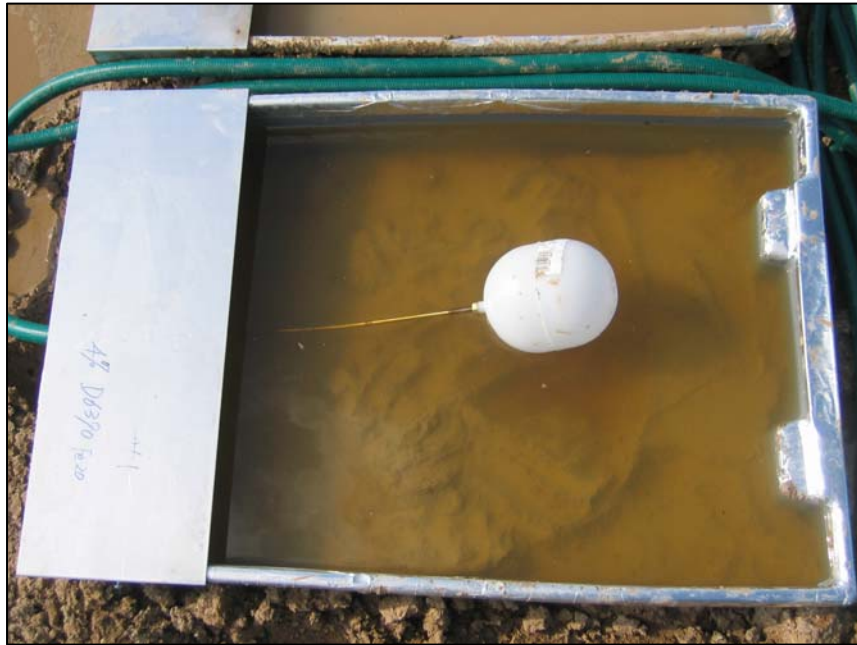
Picture 5.1: Configuration of each treatment in the field

Picture 5.2: In situ pilot test at Louviers, CO, U.S.A.

Chapter 6

Conclusions and Recommendations

This study, four experimental steps were undertaken to evaluate the potential remediation methods for PETN-contaminated water and soil.

The potential of using iron to degrade PETN in the aqueous phase was demonstrated in the flow-through iron column experiments. In the 100% iron and 30% iron columns, aqueous PETN degraded with rate constants of $3.44\pm0.28 \times10^{-2}$ and $1.92\pm0.13 \times10^{-2}$ $L \cdot m^{-2} \cdot h^{-1}$, respectively, and the degradation profiles were highly consistent with pseudo-first-order kinetics. The observed intermediate and end products during PETN degradation were identified to be pentaerythritol trinitrate, pentaerythritol dinitrate and pentaerythritol. The suspected intermediate product of the last step of denitration, pentaerythritol mononitrate, was identified in the subsequent biotic tests. Based on the identified intermediates and nearly 100% nitrogen mass balance, it is proposed that PETN was sequentially reduced to pentaerythritol, releasing NO_2^- in each denitration step, which was further reduced to NH_4^+ by iron. Though the degradation rate of aqueous PETN by iron is very high, PETN removal rate is limited by dissolution when present as solid phase. However, using a co-solvent, such as methanol, the solubility can be enhanced, thus the mass removal rate by iron can be increased accordingly.

For PETN in the solution phase, the study also demonstrated the effectiveness of biological degradation of PETN under anaerobic conditions, provided a carbon amendment is used. The microbial culture used in this study, derived from a PETN-contaminated site, has been acclimated to degrade PETN. The commonly found electron acceptors, nitrate and sulfate, did not show any significant adverse effect on PETN degradation when excluding their consumption for carbon sources. PETN can be degraded simultaneously with nitrate, but at a much lower rate. PETN degraded at approximately equal rates regardless of the presence and absence of sulfate. Pentaerythritol trinitrate, pentaerythritol dinitrate and

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pentaerythritol mononitrate were identified as intermediates of degradation under all conditions tested. We propose that PETN followed the same degradation pathway in both the abiotic and biotic systems. The results suggested that PETN was consumed as an electron acceptor during its degradation. Although the energetic favorability of PETN is unknown, the data indicate a utilization order for microorganisms of nitrate followed by PETN and its intermediate products and finally sulfate. The preference of nitrate over PETN suggests that a sufficient amount of carbon source for both reductions is a requirement for complete degradation of PETN.

The effective remediation methods for PETN in aqueous phase were further applied to PETN-contaminated soil in both laboratory and pilot scale experiments. The potential for granular iron to degrade aqueous PETN was not effectively transferred to the soil phase, mainly due to iron passivation caused by the presence of nitrate in the contaminated soil. However, the application of biodegradation in the soil was successful. In the laboratory tests, PETN at 4,500 mg/kg was removed by indigenous soil bacteria within 84 days in the microcosms amended with DARAMEND materials, serving as sources of carbon and other nutrients. In the pilot test conducted at Louvier, Colorado, U.S.A., significant removals of PETN were observed in the treatments in which a source of labile organic carbon, such as DARAMEND materials or brewers grain, was provided. Due to the unexpectedly high initial concentration of PETN in the field, the effectiveness of PETN remediation in the pilot tests will be ultimately reduced by carbon limitation. Complete removal could be expected when more organic material is added. The results of the laboratory tests and pilot tests are qualitatively similar, providing evidence and support for further larger-scale field tests and full-scale treatment. In addition, though an iron-microbial integrated method showed a slight enhancement in degradation rate relative to the biodegradation method alone, it is not encouraged considering the small improvement in performance and higher cost for iron.

This research suggests that in the absence of nitrate, abiotic degradation by granular iron should be a viable and effective remediation method for PETN-contaminated water. Biotic

degradation by the addition of organic carbon is a better alternative for PETN-contaminated soil. The presence of nitrate at the contaminated sites requires particular consideration in both cases due to the iron passivation and its more competitive consumption of carbon sources relative to PETN. The availability of carbon sources is a key factor in designing efficient biological treatment system, the minimum amendment of organic carbon can be calculated from stoichiometry, as in this study. A more detailed investigation for the extent and variability of contaminations (PETN and nitrate) is strongly recommended in order to estimate a suitable addition of carbon sources. Based on the results of this study, a full-scale treatment should be considered.

References

Agrawal, A. and Tratnyek, P. G. (1996) Reduction of Nitro Aromatic Compounds by Zero-Valent Iron Metal. *Environmental Science and Technology* **30** (1), 153-160.

Ahmad, F. and Hughes, J. B. (2000) Anaerobic Transformation of TNT by Clostridium. In: Spain, J. C., Hughes, J. B., and Knackmuss, H. J. (eds), *Biodegradation of nitroaromatic compounds and explosives.* 185-212, Lewis, Boca Raton, Florida.

Alowitz,M.J. and Scherer,M.M. (2002) Kinetics of Nitrate, Nitrite, and Cr(VI) Reduction by Iron Metal. *Environmental Science and Technology* **36** (3), 299-306.

Arnold, W. A., Ball, W. P., and Roberts, A. L. (1999) Polychlorinated Ethane Reaction with Zero-Valent Zinc: Pathways and Rate Control. *Journal of Contaminant Hydrology* **40** (2), 183-200.

Binks, P. R., Nicklin, S., and Bruce, N. C. (1995) Degradation of Hexahydro-1,3,5-Trinitro-1,3,5-Triazine (RDX) by Stenotrophomonas Maltophilia PB1. *Applied and Environmental Microbiology* **61** (4), 1318-1322.

Binks, P. R., French, C. E., Nicklin, S., and Bruce, N. C. (1996) Degradation of Pentaerythritol Tetranitrate by Enterobacter Cloacae PB2. *Applied and Environmental Microbiology* **62** (4), 1214-1219.

Blowes, D. W., Ptacek, C. J., and Jambor, J. L. (1997) In-situ Remediation of Cr(VI)-Contaminated Groundwater Using Permeable Reactive Walls: Laboratory Studies. *Environmental Science and Technology* **31** (12), 3348-3357.

Boopathy, R., Kulpa, C. F., Manning, J., and Montemagno, C. D. (1994) Biotransformation of 2,4,6-trinitrotoluene (TNT) by Co-metabolism with Various Co-substrate: A Laboratory-scale Study. *Bioresource Technology* **47** (3), 205-208.

Boopathy, R., Manning, J., and Kulpa, C. F. (1998a) Biotransformation of Explosives by Anaerobic Consortia in Liquid Culture and in Soil Slurry. *International Biodeterioration and Biodegradation* **41** (1), 67-74.

Boopathy, R., Gurgas, M., Ullian, J., and Manning, J. F. (1998b) Metabolism of Explosive Compounds by Sulfate-Reducing Bacteria. *Current Microbiology* **37** (2), 127-131.

Boopathy, R. (2000) Bioremediation of Explosives Contaminated Soil. *International Biodeterioration & Biodegradation* **46** (1), 29-36.

Boopathy, R. (2001a) Enhanced Biodegradation of Cyclotetramethylenetetranitramine (HMX) under Mixed Electron-Acceptor Condition. *Bioresource Technology* **76** (3), 241-244.

Boopathy, R. (2001b) Bioremediation of HMX-Contaminated Soil Using Soil Slurry Reactors. *Soil and Sediment Contamination* **10** (3), 269-283.

Boopathy, R. (2005) Bioremediation of Tetryl-Contaminated Soil Using Sequencing Batch Soil Slurry Reactor. *International Biodeterioration & Biodegradation* **55** (4), 293-297.

Brock, T. D. and Madigan, M. T. (1988) *Biology of Microorganisms*. Prentice Hall, Englewood Cliffs, New Jersey.

Bruns-Nagel, D., Scheffer, S., Casper, B., Garn, H., Drzyzga, O., Von Löw, E., and Gemsa, D. (1999) Effect of 2,4,6-trinitrotoluene and its Metabolites on Human Monocytes. *Environmental Science and Technology* **33** (15), 2566-2570.

Bucens, P., Seech, A., and Marvan, I. (1996) Pilot-scale demonstration of DARAMEND enhanced bioremediation of sediment contaminated with polycyclic aromatic hydrocarbons in Hamilton Harbor. *Water Quality Research Journal of Canada* **31** (3), 433-451.

Bucher, J. R. (1989) NTP Toxicology and Carcinogenesis Studies of Pentaerythritol Tetranitrate (CAS No. 78-11-5) with 80% D-Lactose Monohydrate (PETN, NF) in F344/N

Rats and B6C3F1 Mice (Feed Studies). Environmental Health Information Service. National Toxicology Program Technical Report Series. 365, 1-192.

Burris, D. R. (1996) Laboratory Experiments with Heterogeneous Reactions in Mixed Porous Media. *Journal of Environmental Engineering* **122** (8), 685-691.

Cao, J., Wei, L., Huang, Q., Wang, L., and Han, S. (1999) Reducing Degradation of Azo Dye by Zero-Valent Iron in Aqueous Solution. *Chemosphere* **38** (3), 565-571.

Cheng, I.F., Muftikian, R., Fernando, Q., and Korte, N. (1997) Reduction of Nitrate to Ammonia by Zero-Valent Iron. *Chemosphere* **35** (11), 2689-2695.

Christodoulatos, C., Bhaumik, S., and Brodman, B. W. (1997) Anaerobic Biodegradation of Nitroglycerin. *Water Research* **31** (6), 1462-1470.

Clark II, C. J., Rao, P. S. C., and Annable, M. D. (2003) Degradation of Perchloroethylene in Cosolvent Solution by Zero-Valent Iron. *Journal of Hazardous Material* **2003** (96), 1-65.

Cobb, G. D. and Bouwer, E. J. (1991) Effects of Electron Acceptors on Halogenated Organic Compound Biotransformations in a Biofilm Column. *Environmental Science and Technology* **25** (6), 1068-1074.

Coleman, N. V., Nelson, D. R., and Duxbury, T. (1998) Aerobic Biodegradation of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) as a Nitrogen Source by a Rhodococcus sp., Strain DN22. *Soil Biology & Biochemistry* **30** (8/9), 1159-1167.

Comfort, S. D., Shea, P. J., Machacek, T. A., Gaber, H., and Oh, B. T. (2001) Field-Scale Remediation of a Metolachlor-Contaminated Spill Site. *Journal of Environmental Quality* **30** (5), 1636-1643.

Comfort, S. D., Shea, P. J., Machacek, T. A., and Satapanajaru, T. (2003) Pilot-Scale Treatment of RDX-Contaminated Soil with Zerovalent Iron. *Journal of Environmental Quality* **32** (5), 1717-1725.

Craig, H. D., Sisk, W. E., Nelson, M. D., and Dana, W. H. Bioremediation of Explosives-Contaminated Soils: A Status Review. Proceedings of the 10th Annual Conference on Hazardous Waste Research, 164-179. 1995.

Devlin, J. F., Klausen, J., and Schwarzenbach, R. P. (1998) Kinetics of Nitroaromatic Reduction on Granular Iron in Recirculating Batch Experiments. *Environmental Science and Technology* **32** (13), 1941-1947.

Drzyzga, O., Gorontzy, T., Schmidt, A., and Blotevogel, K. H. (1995) Toxicity of Explosives and Related Compounds to the Luminescent Bacterium Vibrio fischeri NRRL-B-11177. *Archives of Environmental Contamination and Toxicology* **28** (2), 229-235.

Esteve-Nuñez, A., Lucchesi, G., Philipp, B., Schink, B., and Ramos, J. L. (2000) Respiration of 2,4,6-Trinitrotoluene by *Pseudomonas* sp. Strain JLR11. *Journal of Bacteriology* **182** (5), 1352-1355.

Esteve-Nuñez, A., Caballero, A., and Ramos, J. L. (2001) Biological Degradation of 2,4,6-trinitrotoluene. *Microbiology and Molecular Biology Reviews* **65** (3), 335-352.

Fenton, H. L. R. (2001) Degradation of RDX Using Granular Iron and Nickel-Enhanced Granular Iron. M.Sc. Thesis University of Waterloo, Ontario, Canada.

Freedman, D. L. and Sutherland, K. W. (1998) Biodegradation of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) under Nitrate-Reducing Conditions. *Water Science and Technology* **38** (7), 33-40.

French, C. E., Rosser, S. J., Davies G.J., Nicklin, S., and Bruce, N. C. (1999) Biodegradation of Explosives by Transgenic Plants Expressing Pentaerythritol Tetranitrate Reductase. *Nature Biotechnology* **17** (5), 491-494.

French, C. E., Nicklin, S., and Bruce, N. C. (1998) Aerobic Degradation of 2,4,6-Trinitrotoluene by Enterobacter Cloacae PB2 and by Pentaerythritol Tetranitrate Reductase. *Applied and Environmental Microbiology* **64** (8), 2864-2868.

Gandhi, S., Oh, B. T., Schnoor, J. L., and Alvarez, P. J. J. (2002) Degradation of TCE, Cr(VI), Sulfate, and Nitrate Mixtures by Granular Iron in Flow-Through Columns under Different Microbial Conditions. *Water Research* **36** (8), 1973-1982.

Gillham, R. W. and O'Hannesin, S. F. (1994) Enhanced Degradation of Halogenated Aliphatics by Zero-Valent Iron. *Ground Water* **32** (6), 958-967.

Gorontzy, T., Drzyzga, O., Kahl, M. W., Bruns-Nagel, D., Breitung, J., von Loew, E., and Blotevogel, K. H. (1994) Microbial Degradation of Explosives and Related Compounds. *Critical Reviews in Microbiology* **20** (4), 265-284.

Gould J.P. (1982) The Kinetics of Hexavalent Chromium Reduction by Metallic Iron. *Water Research* **16** (6), 871-877.

Gregory, K. B., Mason, M. G., Picken, H. D., Weathers, L. J., and Parkin, G. P. (2000) Bioaugmentation of Fe(0) for the Remediation of Chorinated Aliphatic Hydrocarbons. *Environmental Engineering Science* **17** (3), 169-181.

Hannink, N., Rosser, S. J., French, C. E., Basran, A., Murray, J. A. H., Nicklin, S., and Bruce, N. C. (2001) Phytodetoxification of TNT by Transgenic Plants Expressing a Bacterial Nitroreductase. *Nature Biotechnology* **19** (12), 1168-1172.

Hawari, J., Beaudet, S., Halasz, A., Thiboutot, S., and Ampleman, G. (2000) Microbial Degradation of Explosives: Biotransformation versus Mineralization. *Applied Microbiology Biotechnology* **54** (5), 605-618.

Honeycutt, M. E., Jarvis, A. S., and McFarland, V. A. (1996) Cytotoxicity and Mutagenicity of 2,4,6-trinitrotoluene and its Metabolites. *Ecotoxicology and Environmental Safety* **35** (3), 282-287.

Hu, H., Goto, N., and Fujie, K. (2001) Effect of pH on the Reduction of Nitrite in Water by Metallic Iron. *Water Research* **35** (11), 2789-2793.

Huang, C. P., Wang, H. W., and Chiu, P. C. (1998) Nitrate Reduction by Metallic Iron. *Water Research* **32** (8), 2257-2264.

Hundal, L. S., Singh, J., Bier, E. L., Shea, P. J., Comfort, S. D., and Powers, W. L. (1997) Removal of TNT and RDX from Water and Soil Using Iron Metal. *Environmental Pollution* **97** (1/2), 55-64.

Hung, H. M., Ling, F. H., and Hoffmann, M. R. (2000) Kinetics and Mechanism of the Enhanced Reductive Degradation of Nitrobenzene by Elemental Iron in the Presence of Ultrasound. *Environmental Science and Technology* **34** (9), 1758-1763.

Isayev, O., Rasulev, B., Gorb, L., and Leszczynski, J. (2006) Structure-Toxicity Relationships of Nitroaromatic Compounds. *Molecular Diversity* **10** (2), 233-245.

Janke, D. and Fritsche, W. (1985) Nature and Significance of Microbial Cometabolism of Xenobiotics. *Journal of Basic Microbiology* **25** (9), 603-619.

Johnson, T. L., Scherer, M. M., and Tratnyek, P. G. (1996) Kinetics of Halogenated Organic Compound Degradation by Iron Metal. *Environmental Science and Technology* **30** (8), 2634-2640.

Keum, Y. S. and Li, Q. X. (2004) Reduction of Nitroaromatic Pesticides with Zero-Valent Iron. *Chemosphere* **54** (3), 255-263.

Kielemoes, J., De Boever, P., and Verstraete, W. (2000) Infuence of Denitrification on the Corrosion of Iron and Stainless Steel Power. *Environmental Science Technology* **34** (4), 663-671.

King, S. Y. P. and Fung, H. L. (1984) Rapid Microbial Degradation of Organic Nitrates in Rat Excreta: Re-examination of the Urinary and Fecal Metabolite Profiles of Pentaerythritol Tetranitrate in the Rat. *Drug Metabolism and Disposition* **12** (3), 353-357.

Kitts, C. L., Cunningham, D. P., and Unkefer, P. J. (1994) Isolation of Three Hexahydro-1,3,5-Trinitro-1,3,5-Triazine-Degrading Species of the Family Enterobacteriaceae from Nitramine Explosive-Contaminated Soil. *Applied and Environmental Microbiology* **60** (12), 4608-4711.

Lampron, K. J., Chiu, P. C., and Cha, D. K. (2001) Reductive Dehalogenation of Chlorinated Ethenes with Elemental Iron: The Role of Microorganisms. *Water Research* **35** (13), 3077-3084.

Lavine, B. K., Auslander, G., and Ritter, J. (2001) Polarographic Studies of Zero Valent Iron as a Reductant for Remediation of Nitroaromatics in the Environment. *Microchemical Journal* **70** (2), 69-83.

Lewis, T. A., Newcombe, D. A., and Crawford, R. L. (2004) Bioremediation of Soils Contaminated with Explosives. *Journal of Environmental Management* **70** (4), 291-307.

Lu, Q. (2005) Effect of Oxidant (nitrate) on TCE Degradation by Granular Iron. M.Sc. Thesis University of Waterloo, Ontario, Canada.

Major, M. A., Griest, W. H., Amos, J. C., and Palmer, W. G. (1997) Evidence for the Chemical Reduction and Binding of TNT During Composting of Contaminated Soils. Toxicological Study No. 87-3012-95, 1-25.

Marshall, S. J. and White, G. F. (2001) Complete Denitration of Nitroglycerin by Bacteria Isolated from a Washwater Soakaway. *Applied and Environmental Microbiology* **67** (6), 2622-2626.

Matheson, L. J. and Tratnyek, P. G. (1994) Reductive Dehalogenation of Chlorinated Methanes by Iron Metal. *Environmental Science and Technology* **28** (12), 2045-2053.

McCormick, N. G., Cornell, J. H., and Kaplan, A. M. (1981) Biodegradation of Hexahydro-1,3,5-Trinitro-1,3,5-Triazine. *Applied and Environmental Microbiology* **42** (5), 817-823.

Mu, Y., Yu, H. Q., Zheng, J. C., Zhang, S. J., and Sheng, G. P. (2004) Reductive Degradation of Nitrobenzene in Aqueous Solution by Zero-Valent Iron. *Chemosphere* **54** (7), 789-794.

Nam, S. and Tratnyek, P. G. (2000) Reduction of Azo Dyes with Zero-Valent Iron. *Water Research* **34** (6), 1837-1845.

Odziemkowski, M.S., Gui, L., Gillham, R.W., Irish, D.E. (2000) The role of oxide films in the reduction of N-nitrosodimethylamine with reference to the iron groundwater remediation technology. In: Hebert, K.R., Lillard, R.S., MacDougall, B.R. (Eds.), Oxide Films, Proceedings of the International Symposium. The Electrochemical Society, Pennington, NJ, 2000-4, 357-368.

Oh, B. T., Just, C. L., and Alvarez, P. J. J. (2001) Hexahydro-1,3,5-trinitro-1,3,5-triazine Mineralization by Zerovalent Iron and Mixed Anaerobic Cultures. *Environmental Science and Technology* **35** (21), 4341-4346.

Oh, B. T. and Alvarez, P. J. J. (2002) Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) Degradation in Biologically Active Iron Columns. *Water, Air, and Soil Pollution* **141** (1-4), 325-335.

Oh, B. T. and Alvarez, P. J. J. (2004) Removal of Explosives Using an Integrated Iron-Microbial Treatment in Flow-Through Columns. *Bulletin of Environmental Contamination and Toxicology* **73** (1), 1-8.

Oh, S. Y., Cha, D. K., Kim, B. J., and Chiu, P. C. (2004) Reduction of Nitroglycerin with Elemental Iron: Pathway, Kinetics, and Mechanisms. *Environmental Science and Technology* **38** (13), 3723-3730.

Oh, S. Y., Cha, D. K., Kim, B. J., and Chiu, P. C. (2005) Reductive Transformation of Hexahydro-1,3,5-Trinitro-1,3,5-Triazine, Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine,

and Methylenedinitramine with Elemental Iron. *Environmental Toxicology and Chemistry* **24** (11), 2812-2819.

Powell, R. M., Puls, R. W., Hightower, S. K., and Sabatini, D. A. (1995) Coupled Iron Corrosion and Chromate Reduction: Mechanisms for Subsurface Remediation. *Environmental Science and Technology* **29** (8), 1913-1922.

Rahman, A. and Agrawal, A. (1997) Reduction of Nitrate and Nitrite by Iron Metal: Implications for Ground Water Remediation. American Chemical Society Division of Environmental Chemistry Preprints of Papers, 213th National Meeting. San Francisco, CA. 37(1), 157-160.

Ritter, K., Odziemkowski, M. S., and Gillham, R. W. (2002) An in situ Study of the Role of Surface Films on Granular Iron in the Permeable Iron Wall Technology. *Journal of Contaminant Hydrology* **55** (1/2), 87-111.

Ritter, K., Odziemkowski, M. S., Simpgraga, R., Gillham, R. W., and Irish, D. E. (2003) An in situ study of the Effect of Nitrate on the Reduction of Trichloroethylene by Granular Iron. *Journal of Contaminant Hydrology* **65** (1/2), 121-136.

Roberts, D. J., Ahmad, F., and Pendharkar, S. (1996) Optimization of an Aerobic Polishing Stage To Complete the Anaerobic Treatment of Munitions-Contaminated Soils. *Environmental Science and Technology* **30** (6), 2021-2026.

Rosser, S. J., French, C. E., and Bruce, N. C. (2001) Engineering Plants for the Phytodetoxification of Explosives. *In Vitro Cellular and Development Biology-Plant* **37** (3), 330-333(4).

Scherer, M. M., Johnson, K. M., Westall, J. C., and Tratnyek, P. G. (2001) Mass Transport Effects on the Kinetics of Nitrobenzene Reduction by Iron Metal. *Environmental Science and Technology* **35** (13), 2804-2811.

Schlicker, O., Ebert, M., Fruth, M., Weidner, M., Wüst, W., and Dahmke, A. (2000) Degradation of TCE with Iron: The Role of Competing Chormate and Nitrate Reduction. *Ground Water* **38** (3), 403-409.

Seech, A., Bell, G., Raymond, D., and Slater, J. T. (2002) In-situ DARAMEND bioremediation of chlorinated pesticides in soil. Gavaskar, Arun R. and Chen, Abraham S. C. Proceedings of the International Conference on Remediation of Chlorinated and Recalcitrant Compounds. Battelle Press, Columbus, Ohio. 599-603.

Singh, J., Comfort, S. D., and Shea, P. J. (1998a) Remediating RDX-Contaminated Water and Soil Using Zero-Valent Iron. *Journal of Environmental Quality* **27** (5), 1240-1245.

Singh, J., Shea, P. J., Hundal, L. S., Comfort, S. D., Zhang, T. C., and Hage, D. S. (1998b) Iron-Enhanced Remediation of Water and Soil Containing Atrazine. *Weed Science* **46** 381-388.

Singh, J., Comfort, S. D., and Shea, P. J. (1999) Iron-Mediated Remediation of RDX-Contaminated Water and Soil under Controlled Eh/pH. *Environmental Science and Technology* **33** (9), 1488-1494.

Steevens, J. A., Duke, B. M., Lotufo, G. R., and Bridges, T. S. (2002) Toxicity of the explosives 2,4,6-trinitrotoluene, hexahydro-1,3,5-trinitro-1,3,5-triazine, and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine in sediments to Chironomus tentans and Hyalella azteca: low-dose hormesis and high-dose mortality. *Environmental Toxicology and Chemistry* **21** (7), 1475-1482.

Till, B. A., Weathers, L. J., and Alvarez, P. J. J. (1998) Fe0-Supported Autotrophic Denitrification. *Environmental Science and Technology* **32** (5), 634-639.

Twibell, J. D., Turner, S. L., and Smalldon, K. W. (1984) The Persistence of Military Explosives on Hands. *Journal of Forensic Sciences* **29** (1), 284-290.

Urbañski, T. (1984) *Chemistry and Technology of Explosives*., Pergamon Press, Toronto.

van Leuken, R. G. J. and Kwakkenbos, G. T. C. (1992) Chemical Ionization with Gaseous Ammonia for Normal-Phase Liquid Chromatographic-Thermospray Mass Spectrometric Applications. *Journal of Chromatography A* **626** (1), 81-86.

Vanek, T., Nepovím, A., Podlipná, R., Zeman, S., and Vágner, M. (2003) Phytoremediation of Selective Explosives. *Water, Air, and Soil Pollution: Focus* **3** (3), 259-267.

Wani, A. H. and Davis, J. L. (2003) RDX Biodegradation Column Study: Influence of Ubiquitous Electron Acceptors on Anaerobic Biotransformation of RDX. *Journal of Chemical Technology and Biotechnology* **78** (10), 1082-1092.

Weathers, L. J., Parkin, G. F., and Alvarez, P. J. (1997) Utilization of Cathodic Hydrogen as Electron Donor for Chloroform Cometabolism by a Mixed Methanogenic Culture. *Environmental Science and Technology* **31** (3), 880-885.

Weber, E. J. (1996) Iron-Mediated Reductive Transformations: Investigation of Reaction Mechanism. *Environmental Science and Technology* **30** (2), 716-719.

Wendt, T. M., Cornell, J. H., and Kaplan, A. M. (1978) Microbial Degradation of Glycerol Nitrates. *Applied and Environmental Microbiology* **36** (5), 693-699.

White G.F. and Snape, J. R. (1993) Microbial Cleavage of Nitrate Esters: Defusing the Environment. *Journal of General Microbiology* **139** (9), 1947-1957.

Wildman, M. J. and Alvarez, P. J. J. (2001) RDX Degradation Using an Integrated Fe(0)-Microbial Treatment Approach. *Water Science and Technology* **43** (2), 25-33.

Zawaideh, L. L. and Zhang, T. C. (1998) The Effects of pH and Addition of an Organic Buffer (HEPES) on Nitrate Transformation in Fe0-Water Systems. *Water Science and Technology* **38** (7), 107-115.

Zhang, C. and Hughes, J. B. (2003) Biodegradation Pathways of Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by Clostridium Acetobutylicum Cell-Free Extract. *Chemosphere* **50** (5), 665-671.

Zhao, J. S., Halasz, A., Paquet, L., Beaulieu, C., and Hawari, J. (2002) Biotransformation of Hexahydro-1,3,5-trnitro-1,3,5-triazine and its Mononitroso Derivative Hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine by Klebsiella Pneumoniae Strain SCZ-1 Isolated from an Anaerobic Sludge. *Applied and Environmental Microbiology* **68** (11), 5336-5341.

EPA/540/R-95/536. (1996) GRACE Bioremediation Technologies Daramend^{TM} Bioremediation Technology: Innovative Technology Evaluation Report.

IPCS, CEC. Pentaerythritol Tetranitrate (ICSC: 1576). http://www.inchem.org/documents/icsc/icsc/eics1576.htm. 2005.

U.S.National Library of Medicine. Hazardous Substances Data Bank (HSDB). http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB. 2003.