Behaviour and Treatment of Nitroaromatics in Groundwater

by

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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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Albanie J.T Tremblay
Abstract

The purpose of this study was to determine the chemical and/or biological factors that cause 2,4-dinitrotoluene (2,4-DNT), 2,6-dinitrotoluene (2,6-DNT) and nitrobenzene (NB) to transform to their respective aromatic amines in the Borden aquifer, and to investigate the biodegradation of 2,4-diaminotoluene (2,4-DAT) and 2,6-diaminoluene (2,6-DAT) under aerobic conditions. In situ microcosms (ISM) and laboratory microcosm experiments were used in the investigation. In addition, a sequential treatment system was tested in which columns containing granular iron were followed by either an anaerobic or aerobic soil column. Both 2,4- and 2,6-DNT were used to determine if competitive effects exist between the two.

The ISM isolates a volume of the aquifer material and allows for in situ solute loading and sampling in order to characterize chemical or biological reactions. Four ISMs were installed below the water table at CFB Borden. Each ISM was injected with 10 mg/L of either 2,4-DNT, 2,6-DNT, NB, or 2,4-DNT + 2,6-DNT, in two repetitions. In all cases, chloride was also injected as a conservative tracer to monitor for dilution. The results indicated transformation of nitroaromatics via nitro-reduction to their intermediate products, mainly as 2,4-DAT, 2,6-DAT, and aniline. Within 20 days, a loss of up to 92% of 2,4-DNT was observed with the formation of 2,4-DAT. Minor amounts of 2-amino-4-nitrotoluene (2-A-4-NT) and 4-amino-2-nitrotoluene (4-A-2-NT) were also observed. Similarly, up to a 96% loss of 2,6-DNT was seen after 29 days, with degradation products including 2-amino-6-nitrotoluene (2-A-6-NT) and 2,6-DAT. When 2,4- and 2,6-DNT were present in combination, 99% loss of both compounds at similar rates was observed over 20 days following the injections, with degradation products including aminonitrotoluenes and diaminotoluenes. Finally, when nitrobenzene was injected, degradation of up to 99% was observed by day 29, with the formation of aniline as the primary product.
To determine the cause of the nitro-reduction, laboratory microcosm experiments were conducted using soil from within the chamber of the ISM’s. Duplicate microcosms were prepared with Borden groundwater and spiked with 2,4- and 2,6-DNT in an anaerobic glovebox. Microcosms were incubated and sampled periodically for approximately 3 months. Several different conditions, including: groundwater and soil, autoclaved groundwater and soil, soil taken at ground surface and groundwater, and autoclaved silica sand and groundwater were created for microcosm experiments to determine whether abiotic or biotic factors caused the reduction of 2,4- and 2,6-DNT. Microcosms which duplicated field conditions in the laboratory had average half-lives of 4.2 days and 5.1 days for 2,4- and 2,6-DNT, respectively, compared to the field result with average half-lives between 3.9 days (2,4-DNT) and 3.5 days (2,6-DNT). Subsequently, a nutrient medium was added to each repetition. The behaviour of DNT degradation did not change significantly, suggesting minimal involvement of biological processes. Furthermore soil analysis showed relatively high concentrations of extractable iron and the presence of magnetite, which are species capable of reducing nitroaromatics. Therefore, it is concluded that nitro-reduction in Borden soil is likely a result of abiotic surface mediated processes.

The competitive behaviour of 2,4- and 2,6-DNT was studied in a sequential treatment system which consisted of an anaerobic iron column, followed by either an anaerobic or aerobic soil column. Results showed the same rate of transformation from 2,4- and 2,6-DNT within the iron column, with 100% conversion to 2,4- and 2,6-DAT, respectively. Within the anaerobic and aerobic soil columns, the DATs were highly persistent. When a nutrient solution was added only to the aerobic soil column with DNTs as the initial compounds, results showed a reduction of 2,4-DNT of 17%, with an increase in 2,6-DNT of 22%. The increase in 2,6-DNT may have been a result of differing influent concentrations at earlier pore volumes. When stock solutions in the aerobic column were altered to only include DATs, a reduction of 2,4- and 2,6-DAT was observed at 17% and 18%, respectively. It would appear that an acclimated bacterial community able to transform DNT and DAT was present in the aerobic Borden
sand column. Degradation of 2,4- and 2,6-DAT was dependant on the degree of nutrients supplied to indigenous bacterial communities under aerobic conditions.
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Chapter 1 Introduction

1.1 Background

1.1.1 Nitroaromatics

Nitroaromatic compounds are widely used around the world. In this study the compounds of interest are nitrobenzene (NB) and dinitrotoluenes (DNT). Primary uses of nitrobenzene are in the manufacture of aniline, lubricating oils, dyes, and synthetic rubber. Dinitrotoluenes are predominantly used as intermediates in the production of explosives, polyurethanes and smokeless gun powder. In 2000, 2 billion pounds of NB were produced, and in 1999, 2.3 billion pounds of DNTs were produced in the United States [Nishino et al., 2000a]. Dinitrotoluenes are formed by the sequential nitration of toluene where 2,4- and 2,6-DNT form in a ratio of 4:1 [Smets and Mueller, 2001; Sponza and Atalay, 2003]. Nitroaromatic compounds have moderate to low water solubilities at 25°C, 2090 mg/L for nitrobenzene [USEPA, 1995], 166 mg/L and 145 mg/L for 2,4-DNT and 2,6-DNT, respectively [OECD Screening Information DataSet, 2005]. NB and DNTs are not known to readily sorb to organic material in soil, though the degree of sorption is believed to be directly related to the amount of clay materials present [OECD Screening Information DataSet, 2005]. Therefore, they are known to be ubiquitous in the environment.

During the last decade attention has been drawn to NB and DNT in remediation of contaminated sites due to the potential for adverse human health effects. NB has serious chronic health effects causing methemoglobinemia, as well as adverse effects on reproductive systems [USEPA, 1995]. DNT has been associated with anemia, disorders of the central nervous system, heart disease, cyanosis, leucopenia, liver necrosis amongst other health concerns [USEPA, 2006]. These nitroaromatic compounds are toxic and mutagenic to many life forms, and therefore of environmental concern [Razo-Flores et al., 1999]. DNT and nitrobenzene are listed as priority pollutants by the USEPA [USEPA, 2005].
1.1.2 Behaviour of Nitrobenzene and 2,4- & 2,6-Dinitrotoluene in the subsurface

Both nitrobenzene and dinitrotoluenes are highly persistent in the subsurface environment and in some areas have been present for upwards of 50 years [Darrach et al., 1998]. The majority of contaminated sites contain several nitroaromatics in one area as a result of previous TNT manufacturing plants and demilitarizing activities [Bradley et al., 1997; Lendenmann and Spain, 1998; Rodgers and Bunce, 2001]. The persistence of nitroaromatics in the subsurface is a result of the electron withdrawing nitro groups that are resistant to electrophilic attack by oxygenases and hydrolysis [Boopathy and Kulpa, 1993; Dickel et al., 1993; Hailgley and Spain, 1991; Razo-Flores et al., 1999; Rodgers and Bunce, 2001]. Chemical reduction of both NB and DNTs are potentially possible as a result of the net positive charge on the nitrogen atom of the nitro groups. Final reduction products of NB and DNTs are however, dependent on the reductants present in the subsurface [Vanderloop et al., 1999].

The products of NB and DNT reduction are primarily their respective aromatic amines. The most commonly identified product for NB reduction is aniline, although nitrosobenzene and phenylhydroxylamine may accumulate as incomplete reduction products from biodegradation [Peres et al., 1998]. Biodegradation products of DNT include diaminotoluene (DAT) and aminonitrotoluenes [Bradley et al., 1995; Hughes et al., 1999; Johnson and Spain, 2003; Liu et al., 1984]. Hydroxylaminotoluenes are biodegradation intermediates but are often not identified because of their highly unstable nature [Hughes et al., 1999]. The products resulting from incomplete mineralization of NB and DNTs, i.e. aniline and DATs, are considered harmful to humans and the environment. In humans aniline has been found to increase methaoglobin and decrease hemoglobin as well as damage the spleen through chronic exposure [USEPA, 1994]. DATs are classified as probable carcinogens to humans and wildlife.
Reduction of NB and DNT by both biological and abiotic means, have been documented under a variety of conditions. Microbial strains able to degrade NB, 2,4-DNT or 2,6-DNT have only been isolated at contaminated industrial waste sites, which at some point had received NB or DNT [Nishino et al., 2000a]. Furthermore, most studies where biodegradation of DNT and NB have been shown to occur involved the introduction of indigenous microbial strains in soil slurries or sludges, or primary substrates were added. Many researchers suggest that the availability of nutrient sources to bacteria present in the subsurface has an important role in the biodegradation of NB, 2,4- and 2,6-DNT [Fortner et al., 2003; Lendenmann and Spain, 1998; Nishino et al., 2000b; Peres et al., 1998; Smets and Mueller, 2001; Zhang et al., 2000]. Microbes that reduce both NB and DNT isomers under aerobic conditions do so by using nitrogen and carbon in the nitroaromatics as energy sources, i.e. NB and DNTs are used as primary substrates [Johnson and Spain, 2003; Nishino et al., 2000; Spangsgord et al., 1991]. In contrast, under anaerobic conditions these compounds are degraded through co-metabolic processes which require excess of carbon or other electron source for anaerobic biotransformation of nitroaromatics to occur [Cao et al., 2004; Lui et al., 1984; Razo-Flores et al., 1999; Berchtold et al., 1995]

NB has been found to biodegrade under anaerobic [Cao et al., 2004; Hailgley and Spain, 1991; Majumder and Gupta, 2003] and aerobic [Hallas and Alexander, 1983; Johnson and Spain, 2003; Peres et al., 1998] conditions. However aerobic or anaerobic biodegradation of NB is highly uncommon at most contaminated sites. Degradation products of both NB and DNT, including DATs and aniline are less degradable under anaerobic conditions [Hallas and Alexander, 1983]. One study found that reduction of NB to aniline occurred in an iron-reducing column by surface-bound iron species originating from microbial oxidation of organic matter by iron-reducing bacteria [Heijman et al., 1995]. Abiotic reduction of NB to aniline has also been documented in the presence of smectite and other phyllosilicates, with the reduction being dependant on concentrations of the electron acceptor (NB) and accessible Fe(II) [Yan and Bailey, 2001]. Similar results were observed in the presence of Fe(II) at the surface of Fe(III) (hydr)oxides [Hofstetter et al., 1999]. NB transformation has also been
found to occur under methanogenic and Fe$^{3+}$-reducing conditions in an anaerobic landfill leachate plume, with the use of in situ microcosms and laboratory microcosm experiments. The reduction was believed to be caused by abiotic transformation, though reductants for this study were not identified \cite{Nielsen1995}.

Both biodegradation and abiotic reduction of DNTs have been observed. Biodegradation of DNTs has been identified under aerobic \cite{Bradley1994, Bradley1994a, Christopher2000, Freedman1996, Lendenmann1998, Smets2001, Spanggord1991, Zhang2000}, and anaerobic conditions \cite{Dutta2003, Hallas1983, Hughes1999, Razo-Flores1999, Smets2001}, including methanogenic \cite{Berchtold1995} sulfate-reducing \cite{Boopathy1993}, nitrate-reducing \cite{Noguera1996} and iron-reducing \cite{Hiejman1995} conditions. 2,4-DNT was also found to be transformed to aminonitrotoluenes and diaminotoluene (DAT) using either methanol, acetic acid or hydrogen as a primary substrate \cite{Cheng1996, Cheng1997}. When DNT is present in the subsurface for ‘long’ periods of time, oxidative microbial populations may result in the degradation of DNT, though it is very uncommon \cite{Nishino1999}. It is more common that microbial strains are added to soil slurry reactors that completely degrade 2,4- and 2,6-DNT, rather than occurring in nature \cite{Nishino2000a}. Common reduction products are aminonitrotoluenes and diaminotoluene (DATs) \cite{Bradley1994, Nishino2000b}. In most situations DNT is relatively persistent under anaerobic environments. When either 2,4- or 2,6-DNT are present in high concentrations, the isomer at the highest concentration will inhibit the biodegradation of the other \cite{Nishino2000b}. Surface mediated abiotic reduction of DNTs has been sparsely documented. One study concluded that in the presence of high concentrations of sulfide, abiotic transformation of 2,4-DNT occurred to form 2-A-4-NT and 4-A-2-NT, resulting in a ratio of 2:1 of 2-A-4-NT: 4-A-2-NT \cite{Cheng1996}. To my knowledge abiotic reduction of 2,6-DNT has not been cited in the literature, although it is expected to behave in a similar manner as 2,4-DNT. The reduced sulfur and iron species are the most important reductants for abiotic degradation of
nitroaromatic compound in the subsurface [Hofstetter et al., 1999], particularly in the presence of electron transfer mediators, such as organic matter.

Behaviour of Aromatic Amines in Groundwater

DATs [Berchtold et al., 1994] and aniline [De et al., 1994; Lyons et al., 1984] are known to degrade under aerobic conditions through acclimated microbes present in the subsurface [Krumholz et al., 1997; Pesce and Wunderlin, 1997; Vanderloop et al., 1999]. Mineralization of DAT under anaerobic conditions has also been found to occur under sulfate- and nitrate-reducing conditions [Krumholz et al., 1997; Noguera and Freedman, 1997; Razo-Flores et al., 1999]. Aniline has been shown to degrade under anaerobic [Schnell and Schink, 1991], methanogenic [De et al., 1994], sulfate-reducing conditions [Schnell and Schink, 1991] and denitrifying conditions [De et al., 1994; Khang et al., 2000]. Furthermore binding of aromatic amines to humic substances in soil has been observed in aerobic environments [Eriksson et al., 2004; Li and Lee, 1999]. Although degradation of aniline and DATs has been reported to occur under anaerobic conditions, their transformation in oxidative environments occurs much faster and is thus more favorable [Bell et al., 2003]. Therefore, remediation strategies which completely mineralize aromatic amines usually rely on aerobic conditions.

1.1.3 Remediation of Nitroaromatics

Many treatment strategies for nitroaromatics in groundwater have been explored over the past decade. Perhaps the most common means of treatment is through adsorption by granular activated carbon (GAC) [Nishino et al., 2000a; Rajagopal and Kapoor, 2001]. Though activated carbon is widely used, it is quite expensive and the adsorbed nitroaromatics must be disposed of appropriately. Another successful remediation strategy for nitrobenzene is air stripping, though this contributed to pollution of air and is an odor nuisance [Dickel et al., 1993]. Degradation of NB has also occurred in bioreactors or activated sludges, though these have primarily been in laboratory-scale experiments [Cao et al., 2004; Dickel et al., 1993; Majumder and Gupta, 2003]. Dinitrotoluene on the other hand, can be removed by using
above ground treatments using steam flushing, co-solvent extraction and chemical oxidation, as well as UV radiation [Gupta and Bhaskaran, 2004]. Another study found, through a column experiment, that bioventing using hydrogen as an electron donor was successful in completely removing 2,4-DNT [Shah et al., 2001]. Furthermore iron has shown to reduce NB and 2,4-DNT [Bell et al., 2003; Kim, 2006]. Most remediation technologies, which remove DNT and NB, including iron PRBs, often result in aromatic amines as end products.

Reduction of Nitroaromatics by Granular Iron
Degradation of several organic compounds, including nitroaromatics, has been shown to occur through reduction by granular iron. Catalyzed metallic iron powder was first shown to reduce chlorinated organic compounds from aqueous solutions in wastewater by Sweeny and Fisher (1972). Gillham and O’Hannesin (1994) used this concept to study the ability of granular iron to degrade 14 halogenated aliphatics in aqueous solution. Subsequently, they developed the iron PRB technology for in situ treatment of a wide variety of compounds as a means of groundwater remediation [ETI, 2007]. The widespread use of iron permeable reactive barriers (PRB) to reduce groundwater contaminants is related to its effectiveness, availability of the material and relatively low cost. Furthermore, reduction reactions by iron occur faster than most biological or other abiotic processes.

Iron is an effective reductant. In the absence of oxygen, it reacts with water and produces H₂ and OH⁻, thus causing an increase in pH and a decrease in Eh. In the case of nitroaromatics, nitro groups of NB and 2,4- DNT are reduced to amino groups, forming end products of aniline [Agrawal and Tratnyek, 1996; Agrawal, 1995; Bell et al., 2003; Devlin et al., 2004; Mantha et al., 2001] and 2,4-DAT [Kim, 2006; Oh et al., 2002], respectively. Reactions for the reduction of NB to aniline are shown in the following reaction sequence, where aniline is formed through nitrosobenzene and hydroxylamine. The overall reaction is shown in equation (4) [Agrawal and Tratnyek, 1996; Devlin et al., 2004; Mantha et al., 2001].
Similarly, 2,4-DNT was found to be reduced through 2-amino-4-nitrotoluene and 4-amino-2-nitrotoluene to form 2,4-DAT \cite{Oh et al., 2002}. To my knowledge there is no published literature showing the reduced species of 2,6-DNT in the presence of granular iron, though it is assumed, based on its similar chemical structure, that the reduction will proceed via 2-amino-6-nitrotoluene (2-A-6-NT), to a final product of 2,6-DAT. Competition between 2,4- and 2,6-DNT has been observed in biodegradation studies \cite{Nishino et al., 2000a}, though studies of the competitive effects of 2,4- and 2,6-DNT in the presence of granular iron have yet to be undertaken.

Following degradation of NB and DNT by iron, amino products have been found to weakly sorb to the iron surface \cite{Agrawal and Tratnyek, 1996; Bell et al., 2003; Oh et al., 2002}, with the degree of sorption being dependent on pH \cite{Mantha et al., 2001}. They also adsorb to the organic matter in aquifers thus limiting the mobility in groundwater under anaerobic conditions \cite{Eriksson et al., 2004}. Because of the highly toxic nature and low mobility of aromatic amines including aniline \cite{USEPA, 1994} and both 2,4- and 2,6-DAT \cite{Rodgers and Bunce, 2001}, these compounds must be degraded to achieve an effective remediation strategy.

\begin{align*}
C_6H_5NO_2 + Fe^0 + 2H^+ \rightarrow C_6H_5NO + Fe^{2+} + H_2O & \quad (1) \\
C_6H_5NO + Fe^0 + 2H^+ \rightarrow C_6H_5NHOH + Fe^{2+} & \quad (2) \\
C_6H_5NHOH + Fe^0 + 2H^+ \rightarrow C_6H_5NH_2 + Fe^{2+} + H_2O & \quad (3) \\
C_6H_5NO_2 + 3Fe^0 + 6H^+ \rightarrow C_6H_5NH_2 + 3Fe^{2+} + 2H_2O & \quad (4)
\end{align*}
Sequential Treatment of Nitrobenzene and Dinitrotoluenes

It is clear that degradation of nitroaromatic compounds occurs more readily in anaerobic environments, though aromatic amines often result as the persistent products from anaerobic treatment of NB and DNT. The resulting aromatic amines are often degraded in oxidative environments by acclimated microbial populations present at contaminated sites. Thus, sequential treatment of NB and DNT is desirable when designing remediation strategies.

Sequential treatment ending in complete mineralization of nitroaromatic compounds has recently been demonstrated in several studies [Bell et al., 2003; Kim, 2006; Mantha et al., 2001; Zang et al., 2001]. One study showed complete mineralization of nitroaromatics using a sequential treatment system where an anaerobic biofilm supported on GAC in a fluidized bed reactor reduced the nitroaromatics to amines, followed by an aerobic activated sludge to completely mineralize the resulting amines [Maloney et al., 1998]. Another study used a sequential treatment system which included an anaerobic portion using glucose, and mixtures of solvents acting as a cosubstrate, reducing NB to aniline, followed by an aerobic activated sewage sludge for complete mineralization of aniline [Dickel et al., 1993].

More recently, some researchers have focused on sequential treatment of NB and 2,4-DNT using granular iron, followed by aerobic biodegradation in an oxidized zone [Bell et al., 2003; Kim, 2006; Mantha et al., 2001]. Degradation of dissolved NB [Bell et al., 2003] and 2,4-DNT [Kim, 2006] have been successfully demonstrated in the laboratory using an anaerobic granular iron column, followed by an oxygen diffusion column for the addition of oxygen, and finally an aerobic soil column. The experiment involving treatment of 2,4-DNT [Kim, 2006] showed that in the granular iron zone, 2,4-DNT was reduced to 2,4-DAT with surface normalized first order kinetic rate constants ranging from $2.22 \times 10^{-5}$ L/m$^2$/min to $1.05 \times 10^{-4}$ L/m$^2$/min. The resulting DAT was mineralized in the subsequent soil column. A higher DAT degradation rate was observed in the soil column that contained soil from a DNT contaminated site with a high $f_{oc}$. Approximately 12% of the initial 2,4-DAT was
mineralized to $^{14}\text{CO}_2$ within the first 5 cm of the column where oxygen concentration was high. Similar results are given in Bell et al (2003) where reduction of NB to aniline and complete removal of aniline across the soil column was observed, although mineralization of aniline was not confirmed.

Following the laboratory experiments of Bell et al. (2003) and Kim (2006), a pilot-scale sequential treatment system for NB and 2,4-DNT was conducted in an anaerobic zone of the aquifer at Canadian Forces Base (CFB) Borden [Robinson, 2006]. The sequential treatment system included an iron permeable reactive barrier (PRB) (50% iron and 50% sand), followed by an oxygen addition zone 17 m down gradient of the iron zone (Figure 1). Following the injection of NB and 2,4-DNT, concentrations of aniline and 2,4-DAT were detected after 4 and 2 days, respectively, with the transformations occurring 4.5 m up gradient of the iron PRB. The results were unexpected in that they suggested transformation of the compounds in the natural Borden aquifer material. However, using Borden aquifer material and groundwater, Bell et al. (2003) found that nitrobenzene was persistent in the microcosm experiments. Results from the aerobic zone of the sequential treatment system showed decreases in 2,4-DAT and aniline concentrations by 46% and 52%, respectively. The declines in Robinsons study were believed to be a consequence of aerobic biodegradation, which is in agreement with the results of microcosm studies of Bell et al. (2003) who reported rapid degradation of aniline under aerobic conditions. Following the unexpected results from the field, laboratory microcosm tests were conducted in an attempt to determine whether biological or surface mediated abiotic processes caused the reduction of NB and 2,4-DNT. The results from the laboratory study did not reproduce field observations (Robinson, 2006).
1.2 Objectives

The goals of this research were to identify the reducing properties causing the transformation of selected nitroaromatics including NB, 2,4-DNT and 2,6-DNT, in the Borden aquifer and to evaluate whether competitive effects exist between 2,4- and 2,6-DNT. Specific research objectives were:

- To confirm the reduction of NB and 2,4-DNT observed in the absence of iron in the field test of Robinson (2006)
- To determine the causes of 2,4-DNT, 2,6-DNT and NB transformation in the Borden aquifer (biotic or abiotic)
- To determine factors controlling DAT degradation
- To explore the competitive effects of degradation of 2,4-DNT and 2,6-DNT when present in combination

To address these objectives, several experimental approaches were used:

- *in situ* microcosms were used to determine degradation rates of NB, 2,4-DNT, 2,6-DNT, aniline, 2,4-DAT, and 2,6-DAT in the field
- Laboratory microcosm tests were used to further assist in delineating the biotic and abiotic processes involved in 2,4-DNT and 2,6-DNT transformation
- A sequential treatment system was used to evaluate the kinetics of degradation of 2,4-DNT and 2,6-DNT in combination and to assess the ability of indigenous microorganisms to degrade 2,4 DAT and 2,6-DAT under anaerobic and aerobic conditions
Chapter 2 Methods

2.1 In situ Microcosm Tests

2.1.1 Site Description

The site selected for this study was located at CFB Borden, Ontario. The aquifer is glaciolacustrine in origin and is relatively homogeneous, consisting primarily of fine sand with a minor silt fraction. Discontinuous bedding of silty sand exists in fine layers throughout the thickness of the aquifer, as well as an occasional peat layer. The composition of the sand includes primarily quartz and feldspar, though carbonates, amphiboles and magnetite are also present [Mackay et al., 1986]. Porosity of the Borden aquifer is around 0.35 [MacFarlane et al., 1983]. The aquitard is located approximately 3.5 meters below ground surface [Brown et al., 1997].

The in situ microcosm experiment was conducted within an enclosed section of the aquifer, in “gate 3” as shown in Figure 1. The “gates” are sections of the aquifer surrounded on three sides by sealable-joint sheet piling. The gates are oriented in such a way that groundwater enters the gates at the open end. The installation of the steel sheet piling is described in detail by Katic (1999). The water table elevation varies seasonally to a maximum depth of 1.6 meters below ground surface in 2006. Gate 3 was selected for this study as a result of the previous experiment in 2005, where transformation of NB and 2,4-DNT occurred upgradient of the iron PRB [Robinson, 2006]. The only other study involving the injection of organics in gate 3 was in 1999, in which tetrachloroethene, tetrachloromethane and toluene were injected to study the effectiveness of a sequential treatment system that included an anaerobic nutrient injection wall followed by an oxygen addition zone [Devlin et al., 2004].

2.1.2 In situ Microcosm description

The in situ microcosm (ISM) was introduced by Gillham et al. (1990a, 1990b) as a means of conducting small-scale microcosm tests in the field, thus minimizing the physical and
geochemical disturbances normally caused during the setup and operation of laboratory experiments.

The device was initially used for the in situ measurement of transformation rates of benzene and nitrate. Other studies have used the ISM for bioremediation experiments [Mandelbaum et al., 1997], or monitoring of biodegradation rates [Acton and Barker, 1992; Bjerg et al., 1999]. Kinetics of sorption [Bjerg et al., 1996] and degradation rate constants have also been determined using ISMs [Nielson et al., 1996].

The ISMs used for this study consist of a cylindrical test chamber which holds approximately 3 liters of aquifer material (chamber has an ID of 6.5cm, length of 91.5cm), and is open at the bottom. A stainless steel screen is located at the top of the test chamber which allows for the extraction and reinjection of groundwater. A sampling spike, 10 cm in length and screened over the bottom (1 cm) protrudes from the center of the ‘main’ screen. The main screen is attached to 6.5 mm stainless steel tubing, and the sampling spike is attached to 3.2 mm Teflon® tubing, both of which, when installed, reach to ground surface. A schematic of the ISM is presented in Figure 2. The main screen and tubing are used to purge groundwater from the ISM. Contaminants or other amendments are added at ground surface and the water is reinjected back into the ISM test chamber. 3.2 mm Teflon® tubing in this particular experiment was used for sampling over time for organics, Eh, pH, anions and a tracer (chloride).

2.1.3 ISM Procedures

Four ISMs were installed down gradient from the contaminant injection wells used by Robinson (2006). The location of each ISM (1, 2, 3, 4) is shown in Figure 1 & Figure 3. A borehole was hand augured to the water table. A casing was installed and the ISM (which was attached to drill rods) was lowered into the casing. A vibratory hammer was attached to the drill rods which gradually advanced the ISM and casing, while water was jetted into the casing surrounding the ISM to loosen the surrounding aquifer material. This process continued until the ISM reached the desired depth of two meters (at the main screen), and the
test chamber was filled with aquifer material from the saturated zone. A depth of two meters at the main screen was necessary to ensure that the sampling spike would be below the water table during seasonally low water table depths. Once the installation was completed the casing was removed but the drill rods were left attached to the ISM to aid in its removal at the end of the experiment. Following the installation, the ISMs were developed by drawing water from the sampling spike and the main screen as well as re-injecting water back into the test chamber; this was done repeatedly until the withdrawn water contained little or no sediment.

Teflon® tubing (6.4 mm) connected to the main screen passed through the drill rods to ground surface where a low-speed peristaltic pump purged groundwater from the main screen. 2.5 L of groundwater was purged, approximately three times the pore volume of the test chamber, into a glass bottle. \( \text{N}_2(g) \) was then bubbled through the withdrawn groundwater to remove any oxygen that may have been added during the purging process (Figure 4). Nitroaromatics were then injected into the groundwater withdrawn from each ISM. In all tests, once groundwater was amended with the selected nitroaromatics, a low speed peristaltic pump was used to re-inject the purged and spiked groundwater back into the respective ISMs through the stainless steel tubing connected to the main screen. During injection the 3.2 mm tubing was clamped off to prevent spiked groundwater from coming back up to the surface via the sampling spike. Between injections, the glass bottles were rinsed three times with DI water to avoid cross contamination.

The ISMs were amended with nitroaromatics for three separate experiments. Compounds used were 2,4-DNT (97%), 2,6-DNT (98%), 2,4-DAT (98%), 2,6-DAT(97%), NB (99%), and aniline (99.5+%), obtained from Aldrich Chemical Company Inc. (Milwaukee, WI). Stock solutions prepared in pure methanol were used for each injection. Stock concentrations were 61525 mg/L of 2,4-DNT, 64913 mg/L of 2,6-DNT, 138713 mg/L of nitrobenzene, 1395 mg/L of 2,6-DAT and 2,4-DAT, and 1655 mg/L of aniline. In addition, NaCl was used as a
conservative tracer using a stock solution of 10,000 mg/L, and was added to the solution being injected for the purpose of monitoring for dilution. The ISM treatments were as follows:

**Test 1:** ISMs were amended with nitroaromatics at an initial concentration of approximately 10 mg/L, where ISM 1 contained 2,4-DNT, ISM 2 contained 2,6-DNT, ISM 3 contained NB, and ISM 4 was amended with 2,4-DNT and 2,6-DNT at 10 mg/L each. The chloride concentration in each injection solution was approximately 60 mg/L Cl\(^-\).

**Test 2:** Replicate of Test 1

**Test 3:** Nitro-reduction products of 2,4- and 2,6-DNT and NB, ie. 2,4-and 2,6-DAT and aniline, were injected, with target concentrations of 10 mg/L each. ISM 3 was amended with aniline, and ISM 4 with aniline, 2,6-DAT and 2,4-DAT. NaCl was included at a target concentration of 80 mg/L Cl\(^-\), in both ISMs.

In all ISMs, concentrations of methanol, as a carrier solvent, ranged between 0.04 and 0.39 mg/L.

Samples were withdrawn from the sampling spike through 3.2 mm Teflon® tubing to ground surface. Prior to sampling, 15 mL of groundwater was purged (with a plastic syringe) from each of the ISMs (approximately one tubing volume). An air-tight glass on Teflon® syringe was then attached to the tubing, and different volumes of water were extracted, depending on the analyses required for the specific sampling event. Samples for organic analysis were collected at every sampling event. Sampling frequency was dependent on the behavior of the compounds, where sampling was most frequent (every other day) when organic concentrations were changing significantly between sampling events. For these analyses, 2 mL autosampler vials were filled, then capped and crimped leaving no head space.

Additional analyses were conducted on a biweekly basis, including alkalinity, total dissolved iron, anions, Eh and pH. Between samplings, syringes were cleaned once with methanol and three times with DI water. Equipment blanks were taken between every sampling event and trip blanks were occasionally taken to monitor for cross contamination during sampling and transportation.
2.1.4 Removal and Recovery of ISMs and Aquifer Material

The objective of this procedure was to retrieve the aquifer material in the ISM chamber for subsequent use in laboratory microcosm tests and sequential treatment of 2,4- and 2,6-DNT. The ISMs were removed using a hydraulic jack and winch system attached to the drill rods on the ISMs. Tubing attached to the main screen was connected to a peristaltic pump to create suction for the purpose of reducing the amount of soil falling out of the test chamber during the removal process. Once the ISM chamber was removed, \( \text{N}_2\text{(g)} \) was vented over the bottom and a Ziploc® bag was placed underneath. Suction was removed from the tubing and the test chamber was hit with a hammer until all the aquifer material fell into the bag. The bag of soil was continuously vented with \( \text{N}_2\text{(g)} \) while removing the soil from the test chamber. This was done to reduce exposure of soil to oxygen during the removal process. Excess \( \text{N}_2\text{(g)} \) was then removed from the bag, which was sealed and transported in coolers filled with ice to the laboratory. Upon arrival at the laboratory, the soil was immediately placed in an anaerobic glove-box. The soil remained in the glove-box for two days for the purpose of removing any oxygen that may have penetrated the Ziploc® bags during transport. The soil was then transferred to glass jars, sealed and stored in a refrigerator at 2°C.

2.2 Laboratory Microcosms

2.2.1 Materials

Stock solutions of 2,4- and 2,6-DNT were prepared in methanol at concentrations of 92,491 mg/L and 92,557 mg/L, respectively. Three different soil materials were used in the microcosm tests, including soil collected from within the ISM test chamber, soil collected from the surface near the ISM site at CFB Borden and silica sand. The silica sand was washed with 5% nitric acid, rinsed with deionized water to a pH of 7, and autoclaved three times for one hour prior to use. Groundwater collected from CW1 (Figure 3) following the removal of the ISMs, was stored below 2°C and used in all microcosm tests.
Modified Bushnell Haas (MBH) medium stock solution was prepared using $K_2HPO_4$, $NH_4NO_3$, $MgSO_4\cdot7H_2O$, $CaCl_2\cdot2H_2O$, $K_2HPO_4$ and $FeCl_3\cdot6H_2O$ at the concentrations listed in Appendix A.

2.2.2 Procedures

The microcosm tests were conducted using 250 mL bottles, filled with 50 g of soil and approximately 230 mL of groundwater.

Duplicates of each of the following were prepared:

**Micro 1**: Anaerobic groundwater and soil from the ISM test chamber, for the purpose of duplicating the field results.

**Micro 2**: Autoclaved groundwater and soil were used to differentiate between biotic and abiotic reduction processes. Soil was autoclaved three times for one hour, and groundwater was autoclaved for one hour, each with 24 h interval.

**Micro 3**: Anaerobic groundwater and soil from the ground surface at CFB Borden were used to determine if the reducing potential present within the subsurface is also present in oxidized soil at ground surface.

**Micro 4**: Autoclaved silica sand and anaerobic Millipore water to act as a control.

Bottles (250 mL) were filled with water and soil, then capped, and spiked with the stock solution of 2,4-DNT and 2,6-DNT to concentrations of 10 mg/L each and methanol concentrations ranged around $9.3 \times 10^{-4}$ mg/L. The microcosms were incubated in an anaerobic glove-box.

Microcosms were sampled each week over a total period of eight months. A glass on glass syringe was used to withdraw 0.5 mL samples which were transferred to 2.0 mL autosampler vials. Between samples, syringes were rinsed once with methanol and then three times with Millipore water. Following each sampling event the microcosms were shaken for 10 minutes, and then stored on their sides.
Following approximately three months, bottles from Micro 1 and Micro 2 were modified by adding an MBH nutrient stock solution of 5% of the volume remaining in the bottles. The nutrient solution was added to determine if microbial populations present in the microcosm bottles would be stimulated by the nutrients resulting in faster degradation of DNTs.

2.3 Sequential Treatment of 2,4-DNT & 2,6-DNT

Two sequential treatment systems were established for the purpose of determining if competitive effects of 2,4- and 2,6-DNT would occur. The sequential treatment systems were setup similar to those described in Kim (2006) and Bell et al. (2003). The first system, referred to as ‘anaerobic treatment’, included an anaerobic granular iron column followed by an anaerobic soil column (Figure 5). The second system referred to as ‘aerobic treatment’, consisted of an anaerobic granular iron column, an oxygen diffusion column, and an aerobic soil column (Figure 5).

2.3.1 Column Design and Source Solution

All columns used in the sequential treatment experiments were made of Plexiglas®, with screw-on end caps and Viton® O-ring seals. The influent and effluent ends of the columns were fitted with nylon screen (#11) and nylon mesh (#91) to ensure minimal loss of iron or sediment at each end of the column and to insure uniform flow across the column. Prior to packing the columns, each was cleaned with commercial bleach and autoclaved water. Tubing used in the anaerobic portions of the systems was 3.2 mm (ID) stainless steel, for the purpose of reducing oxygen diffusion that might otherwise occur through the tubing. All connectors between tubing, and tubing in the peristaltic pump were made of Viton®.

The granular iron columns were 14 cm (L) x 2.5 cm (ID) with sampling ports along the column at every 1.25 cm. Granular iron used in the column was from Connelly (Chicago,
IL), batch UW-297. Surface area of the iron was determined to be 1.37 m$^2$/g using the Brunauer, Emmit, Teller (BET) method. Iron columns were packed with 30% iron and 70% silica sand. Silica sand was washed with dilute nitric acid and thoroughly rinsed with deionized water. Packing of the columns occurred with the gradual addition of small quantities of iron sand mixtures. During packing, the surface was roughened between the additions of iron-sand mixture in order to achieve a homogenous packing. The procedure was repeated until the columns were completely filled. The columns were then flushed with CO$_2$ to remove any oxygen and clamped off until they were used.

An oxygen diffusion emitter, similar to that of Bell et al. (2003), was used in the aerobic sequential treatment system following the granular iron column. The oxygen emitter consisted of a Plexiglas® column, 20 cm (L) x 3.8 cm (ID). Three ports were located in the column at 2.5 cm, 15 cm and 17.5 cm from the influent end. A 20 cm length of silicone tubing ran between the sampling ports at 2.5 cm and 17.5 cm from the influent end, and was held in place by 3.2 mm stainless steel Swagelock® fittings installed in the column wall. The silicone tubing was attached to an oxygen tank and pressurized to 20 psi, to facilitate continuous diffusion of oxygen into solution.

Prior to packing the soil columns, all instruments and columns were cleaned with diluted commercial bleach and Millipore water. Soil columns were packed using the same method as described for the granular iron columns. Soil was at residual saturation, and originated from the test chambers of ISM 3 and ISM 4. The length of both soil columns was 50 cm with sampling ports at 9, 19.5, 24, 29, 34, 41.5, 44.5, 45.4, and 46.5 cm from the influent end. Columns were then saturated with autoclaved Borden groundwater, though the anaerobic treatment water was first sparged with N$_2$(g) to remove any oxygen present prior to saturating the column.
Source solutions prepared for the sequential treatment systems were contained in 9L Pyrex® carboys, Borden groundwater was autoclaved (for one hour) and deoxygenated with N$_2$(g) to DO levels below 0.1 mg/L. Solid phase 2,4-DNT and 2,6-DNT were added to the carboys, following deoxygenation of the water, to concentrations of 10 mg/L and mixed until dissolved. In order to ensure stock solutions remained anaerobic, a N$_2$(g) filled foil balloon was attached to the stopper which allowed for the headspace to be filled with N$_2$(g) when solution was lowered in the carboy.

2.3.2 Operation

Operation of the column experiments included 2 phases. A log of the operations is listed in Appendix B. Columns were operated in each phase until steady state concentration profiles were achieved.

Samples were taken at one-week intervals from sampling ports in the iron and soil columns, as well as from the influent and effluent ends of the columns. A glass on glass syringe was used, which was cleaned with methanol followed by rinsing three times with autoclaved Millipore water. The syringe was screwed onto sampling ports for collection of appropriate sample volumes, based on the types of analyses required for the particular sampling event. Volumes of samples were as follows: organics (1 mL), Eh/pH (2 mL), dissolved oxygen (DO) (2 mL), NH$_4^+$ (2 mL). A needle tip was added to a syringe following collection of the sample and was then placed at the bottom of sample vial and appropriate volumes of water were released into each vial as required for the analyses. During column operation, waste was collected and weighed to determine the number of pore volumes that had passed through the systems, from which the average flow rates were calculated.

In Phase I, sequential treatment systems were in operation for 91 days. Samples for nitroaromatic analyses were collected every seven days, and samples for Eh, pH, DO, NH$_4^+$...
were collected bimonthly. One pore volume was considered to be the sum of all pore volumes of the individual columns in the system.

In Phase II, the iron columns and the oxygen emitter were disconnected from the soil columns. Source solutions were amended with 10 mg/L each of 2,4-DNT, 2,6-DNT, 2,4-DAT and 2,6-DAT and MBH nutrient solution was added to the columns at a T connector in the influent line (Figure 6). MBH solutions were prepared in 9L Pyrex® carboys, and diluted by 50% from the prepared stock solution (Appendix A), and were stored in a refrigerator during this phase of the experiment.

Initial flow rates through the columns were 0.25 mL/min and 0.30 mL/min for the anaerobic and aerobic treatments, respectively, and were gradually decreased to 0.10 mL/min and 0.14 mL/min, respectively, over the course of three weeks prior to commencing sampling. Sampling frequency was the same as in Phase I, and until steady state was achieved. Pore volumes of solution that passed through the soil column in Phase II began at zero.

During the later stages of Phase II, in the aerobic soil column, MBH and nitroaromatics were combined in one source solution bottle and contained only 2,4-DAT and 2,6-DAT. Aerobic solutions were bubbled with pure oxygen which was filtered with a 0.45 micron sterile filter, at 20 psi for approximately 3 hours daily. A 0.45 micron filter was also added to the stopper to eliminate bacterial contamination when air entered the bottle while the stock solution levels lowered in the carboy. Anaerobic solutions were prepared as described earlier, though \( \text{N}_2(\text{g}) \) was filtered with a 0.45 micron filter prior to entering the sterile carboy. Solutions were again kept in a refrigerator. Source solution with DATs was flushed through the column until 2,4- and 2,6-DNT could no longer be detected in the aerobic soil column. Source solutions were adjusted to accurately determine the amounts of DAT that were degrading. This could not be calculated in the early stages of Phase II because DNT was degrading and forming
DAT, while at the same time DAT was being reduced. Sampling procedures were as described earlier for Phase I.

2.4 Analytical Methods

Organic samples in all experiments were centrifuged at 10,000 rpm for 5 minutes following collection, for the purpose of removing suspended particulates. Analyses included 2,4-DNT, 2,6-DNT, 2-A-4-NT, 4-A-2-NT, 2-A-6-NT, 2,4-DAT and 2,6-DAT for the microcosm and sequential column experiments. For the ISM experiment, NB and aniline were also analyzed in each sample. A Hewlett Packard 1100 Quatetnary Pump High Performance Liquid Chromatograph (HPLC) equipped with a Diode Array Detector was used for the analyses. A Zorbax® SB-C18 column with a Zorbax® guard column was used to separate and identify target compounds. The injection volume was 50 µL. For NB, aniline, DNTs and aminonitrotoluens, the mobile phase consisted of 55% methanol and 45% Millipore water, with a flow rate of 1 mL/min. For DATs a mobile phase of 95% of 0.1% phosphoric acid and 5% methanol solution, at a flow rate of 1 mL/min was used. A wavelength of 254 nm was used. Standards were included each time samples were analyzed for organics. Standards were prepared at concentrations of 10 mg/L, 5 mg/L, 3 mg/L, 1.5 mg/L, 0.5 mg/L and 0.1 mg/L using pure nitroaromatics and Millipore Water for each.

Eh and pH measurements in all experiments were conducted using a portable meter. pH measurements were taken with an Orion Model 9107 triode and Orion model 250A meter. The electrode used for Eh measurements was VWR Ag/AgCl used on an Orion model 250A meter. For ISM tests, Eh and pH measurements were made using a flow through cell while pumping water at a low rate. Following the collection of each sample in the sequential treatment experiments, Eh and pH samples were injected into a glass vial where the needle tip was placed at the bottom and the electrode was placed near the bottom of the vial.
Dissolved oxygen (DO) was determined using a colourmetric method using CHEMets Kits (model K-7512 or K-7540) from the manufacturer CHEMetrics. If dissolved oxygen levels were above 1 mg/L kit model K-7512 was used, and below 1 mg/L kit K-7540 was used. An Oakton DO 100 series hand-held DO meter and probe was used for verification of the DO concentrations. The CHEMets kits tended to give higher DO concentrations than the DO meter. This is believed to be a consequence of interference from ‘elevated’ concentrations of DAT in solution, though the threshold of the ‘elevated’ concentration was not determined.

Total dissolved iron was determined using a CHEMet kit (K-62909) in the ISM experiments.

Anion analyses included fluoride, chloride, nitrite, sulphate, and nitrate. All samples were placed into plastic 0.5 mL Bionex IC autosampler vials. Samples (25 μL) were injected into a Dionex ICS-2000 Ion Chromatograph (IC), equipped with an Ion-Eluent Generator and conductivity detector. The column was a Dionex IonPac AS18 (4 x 250 mm), and the mobile phase was 30 mM KOH at a flow rate of 1.2 mL/min.

Unfiltered samples for full cation analyses were acidified to a pH less than 1 following collection and were shipped to Maxxam Analytics Inc, in Waterloo, ON, for quantification of iron, manganese, and calcium.

Total organic carbon (TOC), extractible iron and manganese were determined in soil samples collected from the test chamber of ISM 3 and ISM 4. Soil samples were sent to Guelph Chemicals, Guelph ON, for analysis.
Alkalinity samples were collected during the ISM field experiments and filtered through a 0.45µ filter. Concentrations in the form of CaCO$_3$ were determined using a HACH Alkalinity Test Kit (Model AL-DT).

Ammonium (NH$_4^+$-N) concentrations for the laboratory sequential treatment tests were determined using the phenate method as described in Standard Methods for the Examination of Water and Wasterwater [American Public Health Association, 1985]. A wavelength of 630 nm was set on a Beckman BU530 UV/VIS spectrophotometer for quantification of ammonium.

Water level measurements were taken throughout the summer months, while the ISM experiments were being conducted. The purpose of these measurements was to determine where the water table was relative to the main screen of the ISM. Water levels were taken from wells CW1, CW2, CW3, CW4, and averaged to a more accurate representation of where the water table was likely located at each of the ISMs. A Solinst model 101 water level meter was used for the measurement of the water table elevation relative to ground surface.
Chapter 3 Results and Discussion

3.1 Geochemical Environment at CFB Borden

Background water samples were collected prior to the ISM experiments on June 10, 2006, from the ISMs and CW2. CW2 was selected in addition to the ISMs because this well is located at the center of the gate (Figure 3) and allows for comparison between geochemical conditions near the contaminant injection wells and the ISMs. All samples were analyzed for anions and cations. Only samples collected from CW2 were analyzed for pH and DO.

Because of the small degree of variability between sampling locations, the results as given in Table 1 are averaged values. The results showed low levels of nitrate (0.82 mg/L), high amounts of nitrite (5.9 mg/L), low sulphate (22.4 mg/L) and moderate concentrations of total dissolved iron (3.19 mg/L) across all sampling locations. Dissolved oxygen was 1 mg/L in CW2 indicating that the study area was close to anaerobic. The pH in CW2 was 7.13. Very low concentrations of 2,4-DNT, 2,4-DAT, NB and aniline were detected, and were all below 0.410 mg/L. These are assumed to be a consequence of residuals from the previous study of Robinson (2006).

Water levels, pH, DO, Eh and alkalinity measurements were taken throughout the summer months to monitor for changes in the geochemical environment. Water levels gradually lowered to a depth of 1.6 meters below ground surface (mbgs) between June and September of 2006 (Figure 7) and pH values were relatively constant at about 8 over the course of the study (Figure 7). Table 2 summarizes DO and alkalinity measurements during the ISM experiments. Measured DO concentrations ranged between 0.5 mg/L and 3 mg/L.
Soil samples obtained from the test chamber of the ISMs were analyzed for extractable iron and manganese. Results were averaged across ISM 2, ISM 3, and ISM 4, for total iron and total manganese and were 2928 μg/g and 62 μg/g, respectively.

Table 1: Results of background water samples at CFB Borden (June 10, 2006)

<table>
<thead>
<tr>
<th>Cations</th>
<th>Concentration (mg/L)</th>
<th>Anions</th>
<th>Concentration (mg/L)</th>
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<table>
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ND = Non detected, -- = Data not available
Note: All samples were averaged across ISM1, ISM 2, ISM 3, ISM 4, and CW 2
* Sample collected June 13, 2006
Table 2: Summary of alkalinity and DO results in ISMs

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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25/9/6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>140</td>
<td>1</td>
<td>84</td>
<td>1</td>
</tr>
</tbody>
</table>

3.2 ISM Experiments

As described in Section 2.1, three tests were completed using four ISMs. The second test was a duplicate of the first and Test 3 included injection of the degradation products of the DNTs and NB. ISM 1 received 2,4-DNT and chloride; duplicate results (Test 1 and Test 2) are shown in Figures 8a & 8b. A decrease in chloride concentrations over time was observed in both tests, and was attributed to dilution within the test chamber as a consequence of sampling. In all tests, when chloride concentrations were below 50% of the injected value, the experiments were terminated as a result of uncertainty in the data due to dilution. Therefore, in ISM 1 both tests were terminated 20 days after the initial injection.

Over the 20-day period of the tests, the concentration of 2,4-DNT decreased by 87% and 92% in Test 1 and Test 2, respectively, with the formation of degradation products including 2-A-4-NT, 4-A-2-NT, and 2,4-DAT. The trends in 2,4-DNT were very similar in the duplicate tests, though the pattern of the aminonitrotoluenes and DATs differed. In Test 1, the 2-A-4-NT and 4-A-2-NT were observed at a later time, and at lower concentrations than in Test 2. In Test 1, transformation of 2,4-DNT may have formed unidentified products prior
to formation of 2,4-DAT in the reduction pathway, resulting in DAT appearing at a later
time. During the reduction of 2,4-DNT there did not appear to be a favored direction in the 2
or 4 position of aminonitrotoluenes, though the trends in both tests suggest 2,4-DAT to be the
final product. The formation of aminonitrotoluenes and 2,4-DAT clearly indicates the decline
in 2,4-DNT to be a consequence of degradation rather than sorption.

Mass balances for all ISM tests were calculated by converting all measured concentrations to
DNT (or NB) equivalents and adding. For complete mass balances, the trends in mass
balance should reflect the dilution that has occurred within the ISM and thus should follow
the trend of the chloride concentration. Mass balance results for ISM 1 duplicates follow the
chloride trends moderately well, considering the natural heterogeneity of the soil within the
ISM. Of particular note, both tests showed a depression in the mass balance during the first
five days, possibly indicating the presence of unidentified intermediate products of 2,4-DNT
degradation.

First order kinetic rate constants were calculated and adjusted to account for the soil to
solution ratios in the ISM tests. A volume ratio of soil to solution was calculated for the ISM
test chambers in order to compare the observed pseudo first order rate constants ($k_{\text{obs}}$) in
various experiments. Calculations are as follows:

$$k_{\text{obs}} = \frac{\ln \left( \frac{C}{C_0} \right)}{t}$$

Volume of Solids = 1.00 – 0.33 (porosity)

= 0.67

Soil/Solution = 0.67 [(volume of solid)/ 0.33 (porosity)]

Soil/ Solution = 2.03

$k_N = \frac{k}{2.03}$

$t_{1/2} = \frac{\ln(2)}{k_N}$
Half lives for all ISM tests were calculated using the $k_N$ values, and are included in Table 3. The normalized half lives for 2,4-DNT were 15.6 days and 10.1 days in tests 1 and 2, respectively. These results are similar to those reported by Robinson (2006). Though Robinson (2006) could not duplicate the field results in the laboratory, they were reproduced with considerable confidence using the ISM. The unexpected results of Robinson (2006) are confirmed, and as a secondary result, the utility of the ISM has been demonstrated.

In ISM 2, duplicate tests included 2,6-DNT and chloride. The results are shown in Figure 9a & 9b. Chloride concentrations gradually decreased over time in both tests, resulting in termination at 29 days and 28 days in Test 1 and Test 2, respectively. Reduction of 2,6-DNT was similar to that of 2,4-DNT, with decline in 2,6-DNT concentrations in Test 1 and Test 2 of 96% and 87%, respectively. Transformation products included 2-A-6-NT, and 2,6-DAT with similar patterns of appearance in the duplicate tests. Mass balance calculations for 2,6-DNT followed the chloride concentrations closely, in Test 1 and moderately well in Test 2. In both tests a depression was observed in mass balances, possibly indicating the presence of unidentified intermediates of 2,6-DNT transformation. Calculated normalized half lives, for Test 1 and Test 2 were 13.9 days and 1.9 days, respectively. Overall, 2,4- and 2,6-DNT behaved in a very similar manner in the ISM tests, with similar product distributions, similar normalized half lives and no apparent lag phase.

ISM 3 included duplicate tests, with the addition of NB and chloride. The results are shown in Figure 10a and 10b. The NB experiments continued for periods of 29 days for each test. Chloride trends in both tests were relatively consistent over the duration of the experiments. Reduction of NB was observed in both tests at 99% over 29 days. Aniline was the only detected product of transformation. Tests showed nearly complete transformation of the initial NB concentrations to aniline, though in Test 2, aniline concentrations exceeded NB injected concentrations by up to 64%. Though all reasonable causes have been considered the reason for the apparent excess of aniline is currently unknown. None the less, it is clear that
transformation of NB to aniline occurs in the Borden aquifer. In Test 2 NB had a normalized half life of 2.1 days, Test 1 produced a poor fit to the kinetic model and thus a half life was not calculated. Maximum mass loss of NB concentrations occurred within approximately 20 days of contaminant injection, similar to Robinsons (2006) results (14 days), though Robinson did not observe aniline concentrations that exceeded initial NB concentrations.

In duplicate tests, ISM 4 was injected with equal concentrations of 2,4- and 2,6-DNT (10 mg/L of each). The results are shown in Figure 11a and 11b. In Test 1 chloride concentrations gradually decreased over time, though chloride levels in Test 2 were relatively steady until late times. Dilution below 50% of the injected chloride concentration and subsequent termination of the experiment occurred at 20 days and 16 days for Test 1 and Test 2, respectively. Complete transformation of 2,4- and 2,6-DNT occurred in both tests. Observed transformation products included respective aminonitrotoluenes and DATs as seen in ISM 1 and ISM 2. In Test 1, fluctuations of 2,4- and 2,6-DNT exceeded initial injected concentrations, and occurred at day 1, which is believed to be a consequence of poor mixing prior to the injection into the ISM. 2,4- and 2,6-DAT formed at concentrations close to the injected DNT values in Test 1, 2,4-DAT formed at slightly higher concentrations than 2,6-DAT. In Test 2, formation of 2,6-DAT slightly exceeded that of 2,4-DAT. Normalized rate constants were also calculated until days 11 and 8 (Test 1 and Test 2) and showed normalized half lives for 2,4-DNT to be 4.8 days and 3.0 days in Test 1 and Test 2, respectively. Similar half lives were observed for 2,6-DNT of 4.3 days and 2.7 days, respectively. Normalized rates of transformation of 2,4- and 2,6-DNT were higher when present together compared to when present individually, as shown in Table 3.

The concentration of methanol present in the initial stock solutions of all microcosm tests exceeded 0.30 mg/L, providing additional carbon source and may have stimulated bacterial growth. Therefore, even though results from the ISM tests seem to suggest that DNTs and NB were transformed by abiotic processes, further tests are needed to confirm this.
In the previous tests, dilution in the ISM occurred before there was sufficient time to observe the behaviour of the aromatic amines. For this reason, in Test 3, aniline and DATs (2,4 and 2,6) were injected in ISM 3 and ISM 4, respectively. The results are shown in Figure 12a and 12b. The experiments continued for 25 days and 28 days, in ISM 3 and ISM 4, respectively. Changes in aniline concentrations followed the chloride concentrations very closely in both ISMs, indicating the absence of degradation. Slight decreases of 2,4- and 2,6-DAT concentrations were apparent in ISM 4 indicating that degradation or sorption likely occurred due to heterogeneities between ISMs across the gate. The presence of methanol may have stimulated bioactivity in ISM 4, the observed difference between DATs and aniline is inconsistent with biodegradation. Thus the cause will be further determined in subsequent laboratory tests.

Table 3: Summary of kinetic rate constants for ISM experiments

<table>
<thead>
<tr>
<th>ISM</th>
<th>Rep</th>
<th>(K_{\text{obs}}) (Days(^{-1}))</th>
<th>(R^2)</th>
<th>(K_N) (Days(^{-1}))</th>
<th>(t_{1/2}) (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ISM 1</td>
<td>1</td>
<td>0.09</td>
<td>0.90</td>
<td>0.04</td>
<td>15.6</td>
</tr>
<tr>
<td>ISM 1</td>
<td>2</td>
<td>0.14</td>
<td>0.98</td>
<td>0.69</td>
<td>10.1</td>
</tr>
<tr>
<td>ISM 2</td>
<td>1</td>
<td>0.10</td>
<td>0.99</td>
<td>0.05</td>
<td>13.9</td>
</tr>
<tr>
<td>ISM 2</td>
<td>2</td>
<td>0.73</td>
<td>0.95</td>
<td>0.36</td>
<td>1.9</td>
</tr>
<tr>
<td>ISM 3</td>
<td>1</td>
<td>0.13</td>
<td>0.73</td>
<td>0.06</td>
<td>---</td>
</tr>
<tr>
<td>ISM 3</td>
<td>2</td>
<td>0.17</td>
<td>0.96</td>
<td>0.08</td>
<td>8.3</td>
</tr>
<tr>
<td>ISM 4</td>
<td>1</td>
<td>0.29</td>
<td>0.89</td>
<td>0.14</td>
<td>4.8</td>
</tr>
<tr>
<td>ISM 4</td>
<td>2</td>
<td>0.47</td>
<td>0.98</td>
<td>0.23</td>
<td>3.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(K_{\text{obs}}) (Days(^{-1}))</th>
<th>(R^2)</th>
<th>(K_N) (Days(^{-1}))</th>
<th>(t_{1/2}) (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2,4-DNT</td>
<td>2,6-DNT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ISM 4</td>
<td>1</td>
<td>0.33</td>
<td>0.90</td>
</tr>
<tr>
<td>ISM 4</td>
<td>2</td>
<td>0.52</td>
<td>1.00</td>
</tr>
</tbody>
</table>
3.3 Laboratory Microcosms

Laboratory microcosm tests were conducted to determine the extent to which the ISM results could be duplicated in the laboratory. Specific tests were conducted to determine if reduction of DNTs was a result of biotic or abiotic processes. Microcosms included both 2,4- and 2,6-DNT, as there is a lack of literature concerning the behaviour of 2,4- and 2,6-DNT when present in combination in aqueous solution. Since, the behaviour of NB and aniline has been documented in several studies, it was not included in the laboratory microcosm experiments [Agrawal et al., 2002; Bell et al., 2003; Eriksson et al., 2004; Hiejman et al., 1995; Klausen et al., 1995].

Descriptions of all laboratory microcosms using 2,4- and 2,6-DNT are given in Section 2.3. Micro 1 tests were designed to duplicate field observations in the lab. The results are shown in Figure 13a. In Figure 13 (and all other microcosm results), the data points represent the average of duplicate microcosms. Over the 87-day period of the test, 2,4- and 2,6-DNT showed reductions in concentrations of 73% and 64%, respectively. Transformation of 2,4- and 2,6-DNT was confirmed by the formation of aminonitrotoluenes and DATs. The normalized half lives of 2,4- and 2,6-DNT were estimated by adjusting for the soil to solution ratios (0.08) to allow for comparison to field results, and were 3.9 days and 5.5 days, respectively. Normalized half lives for both 2,4- and 2,6-DNT in Micro 1 were slower than the normalized half lives in the ISM tests (Table 4). Results from laboratory microcosms were very similar to ISM results when considering heterogeneities in geochemical conditions in soils. Overall, duplication of field results showing reductions of 2,4- and 2,6-DNT in the lab was successful.
Table 4: Summary of kinetic rate constants for Micro 1 and Micro 2

<table>
<thead>
<tr>
<th>Micro</th>
<th>2,4-DNT</th>
<th>2,6-DNT</th>
<th>t_{1/2}</th>
<th>t_{1/2}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K ( (\text{Days}^{-1}) )</td>
<td>R^2</td>
<td>K_{obs}</td>
<td>R^2</td>
</tr>
<tr>
<td>Micro 1</td>
<td>0.014</td>
<td>0.99</td>
<td>0.18</td>
<td>3.9</td>
</tr>
<tr>
<td>Micro 2</td>
<td>0.012</td>
<td>0.90</td>
<td>0.15</td>
<td>4.5</td>
</tr>
</tbody>
</table>

Subsequent microcosm tests were conducted to assist in determining whether surface mediated abiotic or biological processes were responsible for the observed reduction of 2,4- and 2,6-DNT. Figure 13b shows results from the Micro 2 tests, which used autoclaved groundwater and soil. Over 60 days a reduction of 58% for both 2,4- and 2,6-DNT was observed. Observed kinetic rate constants were calculated and adjusted for soil to solution ratios (0.08), as presented in Table 4. Normalized half lives for 2,4 and 2,6-DNT in Micro 2 were very similar to Micro 1, with differences of approximately 0.5 days between the two microcosm tests. The pattern and rates of removal in the unsterilized (Micro 1) and sterilized (Micro 2) tests were very similar. Anaerobic biodegradation of nitroaromatics has been identified in several studies [Berchtold et al., 1999; Cao et al., 2004; Lui et al., 1984; Razo-Flores et al., 1995], and in all cases an additional primary substrate was required for the reduction of DNT to DAT to occur. The methanol used in the microcosm experiment could serve as a primary substrate, though it is unlikely when considering that reduction rates of DNT in the unsterilized (Micro 1) and sterilized (Micro 2) microcosms were very similar. This further suggests that the transformation of the nitroaromatics was caused by abiotic processes.

Figure 14a shows the results from Micro 3 which included soil taken at ground surface and groundwater from CFB Borden. Between day 4 and day 70, a reduction of 2,4- and 2,6-DNT of 18% and 13%, was observed. Initial concentrations (Day 0) were lower than subsequent data and were attributed to poor mixing. Observed degradation products included 4-A-2-NT,
2-A-4-NT, 2-A-6-NT, and 2,6-DAT. 2,4-DAT was not observed. DNT reduction in the oxidized surface soil (Micro 3) was substantially slower than in the material collected from the anaerobic zone below the water table (Micro 1 and Micro 2). This further supports the conclusion that the reduction of 2,4- and 2,6-DNT was caused by surface mediated abiotic processes that were not active in the oxidized surface soil.

Figure 14b shows the results from the control microcosms (Micro 4), which included autoclaved silica sand and Millipore water. Over 85 days, there was no significant decline in the 2,4- and 2,6-DNT concentrations. The appearance of trace concentrations of aminonitrotoluenes suggests that a very small degree of degradation occurred.

A further test to investigate the biotic versus abiotic hypothesis was conducted, which included the addition of a nutrient solution (Appendix A) to Micro 1 and Micro 2. The nutrient solution (MBH) was added to determine if a bacterial community would be enhanced, and subsequently the rate of DNTs. Results from Micro 1 and Micro 2 prior to and following the addition of nutrients are shown in Figure 13a and 13b, respectively. When comparing trends before and after the addition of nutrients, both Micro 1 and Micro 2 sets showed that reduction of 2,4- and 2,6-DNT remained relatively unchanged following the addition of nutrients. Biologically mediated processes can usually be stimulated by the addition of nutrients.

Further evidence supporting the view that abiotic transformation of DNTs to DATs is occurring is seen in the results from extractible iron and manganese concentrations. Extractible iron concentrations were particularly high compared to the lower manganese concentrations, suggesting Fe^{2+} is likely an important reductant in the Borden aquifer material. DNT is reduced when ferrous iron (Fe^{2+}) losses one electron, becoming ferric iron (Fe^{3+}), and six electrons are required to reduce one nitro group. Therefore, stoichiometrically, 12 Fe^{2+} are needed to reduce one DNT molecule. When considering 20
mg/L (109 µmol/L) of DNTs are present in 2928 µg/g (20.9 µmol/g) of Fe_{Total}, there is potentially a sufficient amount of Fe^{2+} present at the soil surface to reduce the DNTs. Previous studies have shown that high levels of ferrous iron present at the surface of soil grains can reduce nitroaromatics [Klausen et al., 1995; Nefso et al., 2005]. Another study found that monosubstituted nitrobenzenes were reduced to anilines, and such reductions were a result of the presence of magnetite which contains ferrous iron [Heijman et al., 1995]. This is significant because Borden soil has been shown to contain a mixture of amphiboles and carbonates, which include magnetite [Ball et al., 1990]. Evidence therefore suggests that under anaerobic condition, the reduction of nitroaromatics in Borden soil could be the result of magnetite and possibly other minerals containing ferrous iron.

### 3.4 Sequential Treatment of 2,4-DNT and 2,6-DNT

The possibility of competitive effects between 2,4- and 2,6-DNT transformation was investigated in a sequential treatment system. The sequential treatment system included an iron column followed by a soil column. The iron column was designed to reduce DNTs to DATs and the soil column was used to determine if indigenous bacteria capable of degrading DAT were present in Borden soil.

**Anaerobic Treatment**

Phase I of the anaerobic treatment of 2,4- and 2,6-DNT included anaerobic iron and soil columns. A total of 26.1 pore volumes passed through the sequential treatment system which included both iron and soil columns. The average flow rate was 0.010 mL/min, resulting in residence times of 298 min in the iron zone and 3427 min in the soil zone. DO levels were below 1 mg/L (Figure 15a), and pH was about 8 across the iron and soil columns (Figure 16a & b). At a pore volume of 16.6, Eh decreased from 79 mV to -524 mV across the iron column (Figure 16a), indicating that the iron was active. Eh measurements in the soil column
did not vary to the same degree, generally ranging from -26 mV to 109 mV, at a pore volume of 16.6 (Figure 16b). Similar results and trends were observed at subsequent pore volumes.

Complete removal of both 2,4- and 2,6-DNT occurred very quickly in the iron column at a residence time of 159 min during Phase I of the study, as represented in the typical concentration profile in Figure 17a. Degradation kinetics of 2,4- and 2,6-DNT in the iron column could not be calculated as the rates of degradation were very high, resulting in low $R^2$ values, even when the iron columns included only 30% iron to slow degradation of DNTs across the column. Degradation products of DNT were first identified within 19 min along the iron column, including 2-A-4-NT, 4-A-2-NT, 2-A-6-NT, 2,4- and 2,6-DAT, with a mass balance of 100% (not shown in Figure 17a). No evidence of competitive effects or passivation of the iron during Phase I was observed. As shown in Figure 17b, DAT concentrations varied slightly across the soil column during a residence time of 872 min, with an indication of persistence in the anaerobic soil column. Small decreases of DATs across the soil column may have been a result of sorption. Total organic carbon was measured to be 0.573%, indicating that sorption was possible. Sorption of nitroaromatics has been observed in some studies, although it was particularly dependent on factors such as organic carbon content and soil type [Cowen et al., 1998; Krumholz et al., 1997]. Furthermore, $\text{NH}_4^+$ concentrations were constant throughout Phase I (Figure 15a), suggesting that DATs were not degrading.

In Phase II of the anaerobic treatment, the feed solution contained 2,4-DNT, 2,6-DNT, 2,4-DAT, and 2,6-DAT entered the soil column directly (Figure 6). A nutrient solution was also added to the influent at a T connector to mix with the feed solution prior to entering the soil column. The nutrient solution was added to determine if a bacterial community able to degrade DNTs and/or DATs would be enhanced thus increasing degradation. Pore volumes in Phase II for anaerobic treatment began at zero, and only included the volume of the soil column. Steady state concentrations were achieved after 25.9 pore volumes, with an average
flow rate of 0.14 mL/min during Phase II. DO, Eh and pH measurements in Phase II were consistent with the results of Phase I (Figure 19).

In order to calculate the amount of DAT being reduced in the soil column during Phase II, relative DAT concentrations were adjusted to account for the reduction of DNT to DAT. C/C₀ values for DAT were calculated as follows:

$$C/C_0,_{DAT} = \frac{\text{DAT Concentration at time } X \ (\mu\text{mol/L})}{\text{(Initial DAT Concentration + Initial DNT Concentration) (\mu\text{mol/L})}}$$

Upon the addition of nutrients to the feed solution, DNT and DAT concentrations remained fairly consistent across the soil column with small variations (Figure 18) even after nutrient addition, suggesting that biological processes able to degrade DNTs and DATs did not exist within the soil column. A summary of relative concentrations removed in the anaerobic soil column during Phase I and Phase II is given in Appendix D and Appendix E.

**Aerobic Treatment**

Phase I of the aerobic treatment of 2,4- and 2,6-DNT included an anaerobic iron column, followed by an oxygen addition column and finally an aerobic soil column. A total of 24.3 pore volumes flowed through the iron and soil columns, at an average flow rate of 0.13 mL/min. DO concentrations throughout the study were steady, at <1.0 mg/L in the iron column and 10 mg/L in the soil column (Figure 15b). pH ranged from 7.7 to 9.0, as shown in Figure 19. Eh measurements in the iron column decreased across the column from 79 mV to -181 mV, at a pore volume of 17.5 (Figure 19a). Eh measurements in the soil column had no consistent trend with values ranging between 193 mV and 4.5 mV (Figure 19b).
Complete reduction of 2,4- and 2,6-DNT was observed in the iron column, similar to the anaerobic sequential treatment system, and is shown in Figure 20a. 2,4- and 2,6-DAT concentrations appeared to be consistent throughout the soil column (Figure 20b). Even though several studies have shown that the reduction of DATs commonly occurs under aerobic conditions where previous contamination of DAT occurred [Berchtold et al., 1999; Freedman et al., 1996; Krumholz et al., 1997; Pesce and Wunderlin, 1997; Vanderloop et al., 1999]. The lack of bioactivity in the aerobic soil column was possibly due to the limitation of nutrients. Researchers have attributed the ability of bacterial cultures to degrade nitroaromatics as being directly related to the ‘sufficient’ supply of nutrients at a site [Fortner et al., 2003; Lendenmann and Spain, 1998; Nishino et al., 2000b; Peres et al., 1998; Smets and Mueller, 2001; Zhang et al., 2000]. It has also been suggested that the onset of biodegradation in 2,4-DNT contaminated soils is directly related to nutrients supplied [Fortner et al., 2003]. Furthermore, NH$_4^+$ measurements shown in Figure 15b revealed that concentrations slightly increased across the soil column, suggesting that very small amounts of DATs may have been degraded.

Following the assumption that the lack of reduction of DNTs and DATs was due to insufficient supply of nutrients, a nutrient solution was subsequently added following the same procedure as Phase II of the anaerobic sequential treatment system. A total of 27.4 pore volumes flowed through the soil column, with an average flow rate of 0.14 mL/min. Reduction of 2,4- and 2,6-DNT was observed at 42% and 22%, respectively, across the soil column (Figure 21a). The increase in 2,6-DNT concentrations may be a result of differing influent concentrations at earlier pore volumes, persistence of 2,6-DNT is possible though an overall increase in 2,6-DAT is observed. Relative DAT concentrations during Phase II were adjusted as described in Phase II of the anaerobic sequential treatment system. The behaviour of 2,4-DAT showed an increase of 75% from the influent end to the first sampling port. The increase in 2,4-DAT at a residence time of 536 min was attributed to the reduction of 2,4-DNT that occurred between the influent end and the first sampling port. Strong evidence for the reduction of 2,4-DAT is realized when considering that while there is a decrease in 2,4-
DAT, simultaneous reduction of 2,4-DNT and formation of 2,4-DAT is also occurring. 2,6-DAT increased until a residence time of 2026 min and then decreased slightly until the effluent end of the column.

In order to accurately determine the behaviour of DATs, feed solutions were altered to only include 2,4- and 2,6-DAT, and the nutrient stock solution, at a pore volume of 21.2. Small decreases of 2,4- and 2,6-DAT were observed at 17% and 18%, respectively, and are shown in Figure 21b. Upon these results it would appear that the transformation of DAT under aerobic conditions is likely enhanced in the presence of DNT and a sufficient supply of nutrients. One study showed that biodegradation of DAT occurred under aerobic conditions when organic substrates and excess nitrogen was supplied [Freedman et al., 1996]. In 2006 a sequential treatment system including iron followed by an aerobic soil zone confirmed complete degradation of 2,4-DNT with $^{14}$C analysis in a soil with a higher organic carbon content and nutrients than Borden sand [Kim, 2006].

In summary sequential treatment of 2,4- and 2,6-DNT including an iron zone followed by an aerobic soil zone was only moderately successful. While the DNTs degraded rapidly in the iron column, aerobic biodegradation of the DATs was much slower. The supply of nutrients and presence of organic carbon seemed to be important in supporting the growth of DAT-degrading bacteria in Borden soil.
Chapter 4 Conclusions

Because NB and DNTs are frequently found to be persistent in the subsurface environment, the rapid degradation of these compounds observed by Robinson (2006) in the Borden aquifer was particularly surprising, prompting questions concerning the reliability of the results. The ISM tests of this study were highly effective in duplicating the results of Robinson (2006). DATs and aniline were persistent when present as degradation products from the injected DNTs and NB. Though, aniline was persistent and DAT concentrations decreased somewhat over time when injected together in a separate injection. Also, no competitive effects were observed between 2,4- and 2,6-DNT when present together at similar concentrations.

Laboratory microcosm experiments gave results that were highly consistent with the ISM field results. Subsequent addition of a nutrient medium to laboratory microcosms showed no change in the transformation rates of 2,4- and 2,6-DNT, suggesting that the transformation process was not biological. This was supported by the similar rates of transformation in the autoclaved controls (Micro 2 and Micro 4). Soil analyses showed high concentration of total extractable iron, suggesting that ferrous iron species present in the form of magnetite and possibly other minerals are likely the reducing agent in Borden soil, though further tests are needed to confirm this. The structure and behaviour of NB is similar to DNT, thus it can be assumed that the same conclusions can be made from the transformation of DNT as for the reduction of NB.

Sequential treatment of 2,4- and 2,6-DNT was partially successful. Complete removal of 2,4- and 2,6-DNT was observed in the iron column. DATs decreased by approximately 20% across the aerobic soil column, suggesting that a bacterial community able to degrade DATs was likely present. The reduction of 2,4- and 2,6-DAT seemed to be dependent on the degree
of nutrients supplied to indigenous bacterial communities under aerobic conditions. A sufficient supply of nutrients in soils is required for a microbial population able to degrade 2,4- and 2,6-DAT to grow. This study also showed that under anaerobic conditions 2,4- and 2,6-DAT were persistent.
Chapter 5 Recommendations

Following this study, several recommendations for future studies involving *in situ* microcosms, the behaviour and treatment of 2,4- and 2,6-DNT should be considered.

1) One should strongly consider altering the slot size of the main screen of the *in situ* microcosm in soils with porosities lower than 0.33. This alteration would reduce the likelihood of clogging the main screen during installation and development. The specific selection of screen size for the ISM would allow for a broader range of soil types to utilize the tool for biological and chemical monitoring.

2) Confirm the presence of abiotic reducing agents in the Borden soil, including minerals which cause the reduction of NB and DNTs.

3) The bacterial community able to transform 2,4- and 2,6-DAT under aerobic conditions should be identified and quantified.

4) Degradation pathways for 2,4-and 2,6-DAT should be investigated. The confirmation of whether complete mineralization of DATs, under aerobic conditions occurred in Borden soil should also be investigated.

5) Pilot scale sequential treatment of 2,4- and 2,6-DNT system, including an anaerobic iron zone (PRB) followed by an aerobic soil zone should be investigated in other soils apart from Borden sand. The contaminated site selected for pilot scale sequential treatment should contain a variety of nutrients and organic matter in the soil.
Figures
Figure 1: Map view of Gate 3, in the ‘Barker Barn’ at CFB Borden, Ontario

(After Robinson, 2006)
Figure 2: Schematic of an in situ microcosm
Figure 3: Location of previous contaminant wells (CWs) and in situ microcosms (ISMs)
Figure 4: Representation of withdrawal and injection of contaminants into ISMs

- Peristaltic pump
- Beaker containing water
- Stir plate
- Nitrogen tank
- Glass mixing bottle with stopper
- Sandy Aquifer
- Clay Aquitard
Figure 5: Setup of sequential treatment systems during Phase I
Figure 6: Representation of Phase II, nutrient addition to soil columns

10mg/L of 2.4-DNT & 2.6-DNT

MBH Nutrient Solution

Anaerobic Soil Column

Aerobic Soil Column

Wast Wast
Figure 7: a) Average water levels over CW1, CW2, CW3, CW4  b) Average pH readings over ISM 1, ISM 2, ISM 3, and ISM 4
Figure 8: Results from ISM experiments: a) Test 1, ISM1, b) Test 2, ISM 1
Figure 9: Results from ISM experiment: a) Test 1, ISM 2, b) Test 2, ISM 2
Figure 10: Results from ISM experiments: a) Test 1, ISM 3 b) Test 2, ISM 3
Figure 11: Results from ISM experiments: a) Test 1, ISM 4  b) Test 2, ISM 4
Figure 12: Results from ISM experiments: a) Test 3, ISM 3 b) Test 3, ISM 4
Figure 13: Microcosm results in the anaerobic glovebox: a) Micro 1 (groundwater and soil), b) Micro 2 (autoclaved groundwater and soil)
Figure 14: Microcosm results in anaerobic glovebox: a) Micro 3 (soil from ground surface) b) Micro 4 (control)
Figure 15: Sequential treatment systems, $\text{NH}_4^+$ and DO concentrations at selected pore volumes: a) anaerobic treatment, b) aerobic treatment.
Figure 16: Results of pH and Eh in the anaerobic sequential treatment system: a) Iron column, b) Soil Column
Figure 17: Results of 2,4- and 2,6-DNT in the anaerobic sequential treatment at pore volume 23.7: a) Iron column b) anaerobic soil column
Figure 18: Soil column results of the addition of nutrient solution to the anaerobic sequential treatment system at pore volume 25.9
Figure 19: Results of pH and Eh in the aerobic sequential treatment: a) Iron column, b) Soil Column
Figure 20: Results of 2,4- and 2,6-DNT in the aerobic sequential treatment at a pore volume of 21.9: a) Iron Column b) Soil Column
Figure 21: Soil column results of the addition of nutrient solution to the aerobic sequential treatment system: a) 18.4 pore volumes b) 27.4 pore volumes
Appendices
Appendix A- Recipe for MBH solution

Preparation of Modified Bushnell Hass Medium Stock Solution
(Modified from Mueller et al., 1981)

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Manufacturer</th>
<th>Concentration (mg/L)</th>
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</thead>
<tbody>
<tr>
<td>K₂HPO₄</td>
<td>BDH</td>
<td>1000</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>Aldrich</td>
<td>1000</td>
</tr>
<tr>
<td>NH₄NO₃</td>
<td>Aldrich</td>
<td>1000</td>
</tr>
<tr>
<td>MgSO₄*7H₂O</td>
<td>BDH</td>
<td>200</td>
</tr>
<tr>
<td>CaCl₂*2H₂O</td>
<td>BDH</td>
<td>20</td>
</tr>
<tr>
<td>FeCl₃*6H₂O</td>
<td>Aldrich</td>
<td>8.3</td>
</tr>
</tbody>
</table>

Dilution water: Millipore

Sterilize no more than 2 hours following preparation.

Autoclave time: 1 hour
Appendix B- Operating Schedule of Sequential Treatment Columns

Dec 6, 2006  Initial set-up of aerobic and anaerobic sequential treatment columns. Begin flushing columns with DI water.

Dec 12, 2006  Phase I begins, CO₂ is flushed through the iron column only and 10 mg/L 2,4-DNT & 2,6-DNT solutions commence being flushed through the sequential treatment columns at a flow rate of 0.166 mL/min.

Dec 20, 2007  Aerobic and anaerobic treatment columns are sampled for organics, Eh and pH at a flow rate of 0.14 mL/min and 0.15 mL/min respectively.

Feb 21, 2007  Pump tubing for both aerobic and anaerobic treatment are changed.

Mar 13, 2007  Shut off O₂(g) to oxygen diffusion column.

Mar 15, 2007  Disconnected iron columns from sequential treatment systems and Phase I ends.

Mar 24, 2007  Phase II of aerobic soil column begins, 10 mg/L of 2,4-DNT, 2,6-DNT, 2,4-DAT, 2,6-DAT and MBH solution are combined at a T-junction prior to entering the soil column.

Mar 27, 2007  Aerobic column becomes unsaturated to residual saturation levels, caused by not having enough tension in pump tubing. Pump tubing is tightened and soil column is re-wetted with solutions.

April 6, 2007  Phase II begins for anaerobic soil column, where 10 mg/L of 2,4-DNT, 2,6-DNT, 2,4-DAT, 2,6-DAT and MBH solution are combined at a T-junction prior to entering soil columns. Flow rate for columns begin at 0.25 mL/min and 0.297 mL/min, for anaerobic and aerobic treatment.

May 21, 2007  Anaerobic soil columns are disconnected and completed. Aerobic columns change source solutions where DATs are mixed with MBH, resulting in the removal of T-junction.

June 6, 2007  Phase II is completed for aerobic treatment.
Appendix C - Summary of phase I results before adding nutrients in anaerobic treatment

<table>
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<td>20-Dec-06</td>
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<td>1.149</td>
<td>0.944</td>
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<td>28-Dec-06</td>
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<td>0.716</td>
<td>0.091</td>
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<td>5-Jan-07</td>
<td>5.9</td>
<td>1.171</td>
<td>0.854</td>
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<td>13-Jan-07</td>
<td>8.6</td>
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<td>11.4</td>
<td>1.165</td>
<td>0.889</td>
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<td>0.011</td>
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<td>30-Jan-07</td>
<td>14.2</td>
<td>1.150</td>
<td>0.900</td>
<td>0.081</td>
<td>0.007</td>
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<tr>
<td>6-Feb-07</td>
<td>16.6</td>
<td>1.372</td>
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</tr>
<tr>
<td>13-Feb-07</td>
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<tr>
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<td>21.0</td>
<td>1.125</td>
<td>0.897</td>
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<td>0.093</td>
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<tr>
<td>27-Feb-07</td>
<td>23.7</td>
<td>1.382</td>
<td>0.872</td>
<td>0.149</td>
<td>0.022</td>
</tr>
</tbody>
</table>

Note: +: Maximum C/Co value
-: Minimum C/Co value
Φ: Standard deviation
Φ²: Variance
Appendix D- Summary of anaerobic treatment results following the addition of nutrients

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<th>φ2</th>
<th>+</th>
<th>-</th>
<th>φ</th>
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<tbody>
<tr>
<td>25-Apr-07</td>
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<td>0.004</td>
</tr>
<tr>
<td>16-May-07</td>
<td>25.9</td>
<td>1.000</td>
<td>0.848</td>
<td>0.097</td>
<td>0.009</td>
<td>0.414</td>
<td>0.211</td>
<td>0.058</td>
<td>0.003</td>
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</table>

<table>
<thead>
<tr>
<th>Date</th>
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<th>-</th>
<th>φ</th>
<th>φ2</th>
<th>+</th>
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<th>φ</th>
<th>φ2</th>
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<td>19.8</td>
<td>1.000</td>
<td>0.921</td>
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<td>0.001</td>
<td>0.558</td>
<td>0.398</td>
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<tr>
<td>16-May-07</td>
<td>25.9</td>
<td>1.000</td>
<td>0.658</td>
<td>0.056</td>
<td>0.003</td>
<td>0.624</td>
<td>0.427</td>
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Note: +: Maximum C/Co value
   -: Minimum C/Co value
   Φ: Standard deviation
   Φ²: Variance
Appendix E- Summary of aerobic treatment results prior to adding nutrients

<table>
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<th>-</th>
<th>φ</th>
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<td>20-Dec-06</td>
<td>2.6</td>
<td>0.923</td>
<td>0.522</td>
<td>0.132</td>
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<td>0.872</td>
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<td>0.646</td>
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<td>1.152</td>
<td>0.903</td>
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<td>5-Jan-07</td>
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<td>1.049</td>
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<td>0.093</td>
<td>0.009</td>
<td>1.035</td>
<td>0.891</td>
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<td>0.063</td>
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<td>0.776</td>
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<td>21-Jan-07</td>
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<td>30-Jan-07</td>
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<td>1.063</td>
<td>0.771</td>
<td>0.082</td>
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<td>6-Feb-07</td>
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<td>0.110</td>
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<td>0.075</td>
<td>0.006</td>
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<tr>
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<td>0.665</td>
<td>0.126</td>
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<tr>
<td>20-Feb-07</td>
<td>21.9</td>
<td>0.922</td>
<td>0.828</td>
<td>0.030</td>
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<td>27-Feb-07</td>
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<td>0.004</td>
<td>1.039</td>
<td>0.862</td>
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</tbody>
</table>

Note: +: Maximum C/Co value  
-: Minimum C/Co value  
Φ: Standard deviation  
Φ²: Variance
### Appendix F - Summary of aerobic treatment results following the addition of nutrients

<table>
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<tr>
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<th>2,4-DAT-φ</th>
<th>2,4-DATφ²</th>
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<tr>
<td>14-Apr-07</td>
<td>7.9</td>
<td>1.000</td>
<td>0.558</td>
<td>0.348</td>
<td>0.121</td>
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<tr>
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<td>1.000</td>
<td>0.348</td>
<td>0.207</td>
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<th>2,6-DAT-φ</th>
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<td>1.139</td>
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<tr>
<td>28-May-07</td>
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<td>7-Jun-07</td>
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<td>11-Jun-07</td>
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Note: +: Maximum C/Co value  
 -: Minimum C/Co value  
 Φ: Standard deviation  
 Φ²: Variance
References


Agrawal, A. (1995), Reduction of nitro aromatic compounds in Fe(0)-CO₂-H₂O systems; implications for groundwater remediation with iron metal, Master's thesis, Oregon Graduate Institute of Science and Technology, Portland, OR, United States (USA), United States (USA).

Agrawal, A., S. Bose, and M. Shelley (2002), Simultaneous biodegradation of nitrobenzene and nitrate by naturally occurring microbes in wetland soils; Geological Society of America, Southeastern Section, 51st annual meeting; Geological Society of America, North-Central Section, 36th annual meeting, *Abstracts with Programs - Geological Society of America*, 34, 106.


Katic, D. J. (1999), Field trial of an in situ anaerobic/aerobic bioremediation sequence. M.Sc. University of Waterloo, Ontario, Canada


Lendenmann, U. and J. C. Spain (1998), Simultaneous biodegradation of 2,4-dinitrotoluene and 2,6-dinitrotoluene in an aerobic fluidized-bed biofilm reactor, Environmental Science & Technology, 32, 82-87.


Lui, D., K. Thomson, and A. C. Anderson (1984), Identification of nitroso compounds from biotransformation of 2,4-dinitrotoluene, Applied & Environmental Microbiology, 47, 1295-1298.


Mandelbaum, P. T., M. Rietti Shati, and D. Ronen (1997), In situ microcosm in aquifer bioremediation studies, FEMS Microbiology Reviews, 20, 489-502.


OECD Screening Information Data Set (2005), Dinitrotoluene (isomers mixtures) CAS: 25321-14-6, 1-236.


Razo-Flores, E., G. Lettinga, and J. A. Field (1999), Biotransformation and biodegradation of selected nitroaromatics under anaerobic conditions, Biotechnology Progress, 15, 358-365.


Schnell, S. and B. Schink (1991), Anaerobic aniline degradation via reductive deamination of 4-aminobenzoyl-CoA in Desulfbacterium anilini, Archives of Microbiology, 155, 183-190.


Smets, B. and R. J. Mueller (2001), Metabolism of 2,4-dinitrotoluene (2,4-DNT) by Alcaligenes sp. JS867 under oxygen limited conditions, Biodegradation, 12, 217.


USEPA, Toluene-2, 4-diamine 95-80-7, 2007, 1.


USEPA (2006), 2,4-Dinitrotoluene, 121-14-2.

