ENVIRONMENTAL FATE OF TRINITROTOLUENE AND RELATED COMPOUNDS UNDER ANAEROBIC SUBSURFACE CONDITIONS

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CHAPTER I

INTRODUCTION

§1.1 Background

Nitroaromatic compounds are introduced into the environment mainly from anthropogenic activities. Polynitroaromatic compounds such as 2,4,6-trinitrotoluene (TNT) are commonly used military explosives, whereas many other nitro compounds are widely used in production of pesticides, solvents, dyes, and pharmaceuticals (Rieger and Knackmuss, 1995). Nitroaromatic compounds represent an environmental hazard because of their relatively recalcitrant nature to biodegradation by microorganisms and their toxicological and potentially mutagenic effects on a number of organisms (Spanggord et al., 1982; Tan et al., 1992; Won et al., 1976). Hartter (1985) estimated that TNT was produced in amounts of 2 million pounds per year in the 1980s. TNT and its transformation intermediates are commonly found as contaminants in soils and subsurface environments, mostly due to leaching, leakage, and discharge of waste from facilities for manufacturing, processing and disposing of explosives. There is a growing interest in the fate of these compounds in soils and groundwater aquifers because of their complicated physical, chemical and biological characteristics, their adverse health effects,

and their extensive and persistent existence in subsurface environments around numerous military locations.

A number of factors and processes determine the fate of dissolved contaminants in aquifers. Of the physical processes governing the migration of a chemical in aquifers, Bonazountas (1983) cited hydraulic transport (advection and dispersion/diffusion), adsorption/desorption, and volatilization as being important in investigation of both small, chronic chemical releases and large spills. In general, important chemical processes may include oxidation/reduction, photolysis, hydrolysis, complexation, polymerization, and ionization, while biological processes include microbial biotransformation and biodegradation (Samiullah, 1990). Among the physical, chemical, and biological processes and phenomena listed above, some are essential and must be considered in studying the fate of TNT and related compounds under subsurface conditions. These essential mechanisms include, but are not limited to, adsorption/ desorption, chemical oxidation/reduction, and microbial transformation and degradation. It has been reported that TNT is subject to photolysis (Tsai, 1991), but this is not likely to occur in subsurface environments. Complexation, polymerization, and ionization may occur to some of the products of TNT transformation, but have not been reported to be significant for TNT itself under natural conditions, probably because of the un-ionizable nature of TNT molecules.

Considerable research has been performed investigating various aspects of TNT fate in the environment. For example, a few research groups have reported studies on transport and adsorption/ desorption of TNT in soils and aquifers (Pennington and

Patrick, 1990; Selim et al., 1995; Comfort et al., 1995; Haderlein et al., 1996). Adsorption and desorption of TNT and its intermediates in soils and aquifer materials seem to vary largely under different conditions, especially when long-term effects are considered. Surprisingly little can be found in the scientific literature about the characteristics of chemical/abiotic transformations of TNT, although it is somewhat arbitrary to classify TNT reactions into strictly abiotic and biological processes since many of these two types of processes are involved in continuous biogeochemical reaction systems (Haderlein and Schwarzenbach, 1995). Abiotic transformations of other nitroaromatic compounds such as nitrobenzenes and nitroaromatic pesticides were reported to occur commonly and sometimes very rapidly under various conditions (Glaus et al., 1992; Schwarzenbach et al., 1990; Dunnivant et al., 1992b; Tratnyek and Macalady, 1989). Microbial transformation and degradation of TNT and other nitro compounds have been investigated by numerous researchers (Boopathy et al., 1993; Bradley et al., 1994; Heijman et al., 1995; Preuss et al., 1993; Roberts et al., 1996), and under proper conditions, it is believed that this approach may represent an economical alternative of remediating TNT-contaminated soils and groundwater.

Details of the current literature appear in the following chapter. Despite this wealth of information, the fate of TNT and related compounds under subsurface conditions is far from well understood. More notably, relatively few attempts have been found in the literature to investigate the environmental fate of TNT and its transformation intermediates which take into account the co-existence and interactions of various physical, chemical, and biological environmental factors and processes.

In this study, the overall objective was to comprehensively as well as separately examine three categories of environmental fate processes which were considered to dominate the fate of TNT in aquifer environments. Batch reactor techniques were used to provide a well-controlled environment to isolate individual environmental factors and separately characterize physical adsorption/desorption of TNT in aquifer materials, abiotic TNT reactions with naturally occurring reductants, and microbial transformation of TNT under different conditions. Mathematical models were identified to describe the adsorption equilibrium of TNT and its intermediates in aquifer materials. The adsorption kinetics of TNT were studied with short- and long-term adsorption experiments. Bisulfide, which may be present in significant amounts in sulfate-reducing environments, was investigated as an important reductant reacting with TNT abiotically. The effects of aquifer materials on the rate of the abiotic reactions were also investigated. TNT biotransformation was examined under three types of electron accepting conditions, including denitrifying, sulfate-reducing, and methanogenic, since these metabolic regimes commonly occur in subsurface environments and play important roles in determining the rate of biodegradation of xenobiotic compounds (Berry et al., 1987; Kuhn and Suflita, 1989). Quantitative parameters and reaction rate constants were obtained from these batch reactor experiments to characterize the physical, chemical, and biological processes affecting TNT fate. In addition, aquifer column reactors were used as microcosms in which more than one category of the above-mentioned environmental processes were taking place under dynamic (flowing) conditions. Quantitative parameters were

introduced to described the over-all effects of these environmental processes on TNT removal in aquifer columns.

§1.2 Objectives of the Study

In light of the above concerns, the specific objectives of this study were set as follows:

1) To examine the equilibrium and kinetics of adsorption of TNT and several of its reaction products on aquifer materials;

2) To examine the kinetics of the abiotic conversions of TNT and the effects of aquifer materials on these reactions;

3) To identify electron accepting conditions favorable for TNT biotransformation by aquifer microflora;

4) To characterize the patterns of production and disappearance of major TNT metabolites under different electron accepting conditions; and

5) To evaluate the environmental fate of TNT in aquifer column studies.

CHAPTER II

LITERATURE REVIEW

§2.1 Introduction

Hazardous explosives, or energetic organo-nitro compounds, are found as contaminants in many environments. Organo-nitro compounds can be divided into at least three categories: (1) nitroaromatic compounds (e.g., 2,4,6-trinitrotoluene, 2,4-dinitrotoluene), (2) nitramines [e.g., hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine(HMX)], and (3) nitrate esters (e.g., nitrocellulose) (Walker and Kaplan, 1992). Nitroaromatic compounds (NACs), especially 2,4,6-trinitrotoluene (TNT), are the main concerns of this study.

TNT is the most widely used military explosive because of its desirable properties including stability and relatively safe methods of manufacture (Boopathy et al., 1993). Soil and water contamination with TNT and other explosives compounds has resulted from munitions manufacturing, loading, assembling, handling, packing, and disposal operations. Disposal of waste TNT has become a particularly difficult problem in operations such as shell loading, which use large volumes of hot water to wash off residual explosives. Relatively large volumes of water are required because TNT is only slightly soluble in water. An important source of TNT-containing waste is "red water", a

red colored waste stream generated from TNT manufacture and purification (Tsai, 1991). It was a common practice to discharge wastewater which was saturated with TNT into drainage ditches; this water might then flow into local streams or infiltrate into soils and aquifers (Won et al., 1974). These waste streams may also be contaminated with other explosives, such as hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), another important conventional explosive used by military forces (McCormick et al., 1981).

This literature review focuses on major research into the environmental fate, including physical, chemical, and biological aspects, of nitroaromatic compounds (NACs) in subsurface environments, with particular emphasis on biological and abiotic transformation and degradation of TNT. It presents a general description of research findings and conclusions on physical adsorption/desorption and abiotic and biological transformations of these compounds, followed by a review of the environmental fate of TNT in soils and aquifers. The anaerobic biotransformation and biodegradation of nitroaromatic compounds deserve particular attention for several reasons. First, anaerobic/anoxic conditions commonly exist in subsurface environments and anaerobic transformations of these compounds by indigenous microorganisms occur extensively. Secondly, reductive transformation of anthropogenic organic chemicals in aquifers may lead to intermediates and products that can be highly recalcitrant and/or of considerable toxicological concern. Thirdly, biodegradation of xenobiotics catalyzed by the indigenous aquifer microflora has proven one of the essential factors responsible for pollution abatement in aquifers (Bradley et al., 1994; Heijman et al., 1995). Microbial transformations are discussed with respect to different electron accepting conditions,

since alternate electron acceptors play an important role in soils and aquifers where molecular oxygen becomes insufficient or unavailable. Finally, a review is given on the recent development of technologies applied to treatment and remediation of NAC-contaminated water and soils, with the intention to lead to thoughts on the further development of remediation technologies driven by recent research findings, including those in this study.

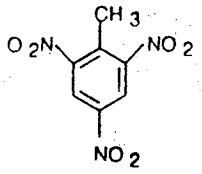
§2.2 General Properties and Toxic Effects of TNT

General physical and chemical characteristics of TNT are presented in Table 2-1. Crude TNT usually contains the meta, or unsymmetrical, isomers, dinitrotoluenes, and oxidation products. Trinitrotoluene is one of the least impact- and friction-sensitive high explosives (US EPA, 1992). This desirable property contributes to its large-scale manufacture and use. TNT can be dissolved in water with a relatively low solubility, about 130 mg/L at 20 °C.

TNT is believed to be toxic to certain fresh water life forms at concentrations greater than 2 mg/L. Toxic effects have been noted for bluegills at 2.3 to 2.8 mg TNT/L, and a mean tolerance limit of 2.0 to 3.0 mg TNT/L for a number of fresh water fish has been reported (Osman and Klausmeier, 1972). In humans, TNT has been shown to cause liver injury. Exposure to TNT is also known to cause pancytopenia, a disorder of the blood-forming tissues characterized by a pronounced decrease in the number of leukocytes, erythrocytoes, and reticulocytes in humans and other mammals (Harris and Killermeyer, 1970). It is also reportedly mutagenic (Kaplan and Kaplan, 1982a). Other

health effects in humans, including skin problems, cataracts, and male reproductive disorders, have been reported by several researchers (US Dept. of Health and Human Services, 1995). The Drinking Water Equivalent Level (DWEL), a life time exposure at which adverse health effects would not be expected to occur, is 20 μg/L as suggested by EPA (US EPA, 1989).

Table 2-1 Physical and Chemical Properties of 2,4,6-Trinitrotoluene

Characteristic	Information
CAS number	118-96-7
Chemical formula	$C_7H_5N_3O_6$
Molecular weight	227.13
Structure	
Specific gravity	1.65
Color	Yellow to white
Physical state	Monoclinic rhombohedral crystals
Specific gravity	1.654
Vapor pressure	0.000199 mmHg at 20 °C 0.106 mmHg at 100 °C
Solubility Water Carbon tetrachloride Acetone	 130 mg/L (20 °C) 0.65 g/L (20° °C) 109 g/L (20 °C)
Partition coefficient $LogK_{ow}$	1.60 - 2.7
Melting point	80.1 to 80.6 °C
Boiling point	210 °C (10 mmHg) to 212 °C (12 mmHg)
Explosive temperature	240 °C

Sources: (1) U.S. Department of Health and Human Services, 1995

(2) HSDB, 1994.

§2.3 Adsorption/desorption of TNT in Soils and Aquifer Materials

The impact of adsorption/desorption processes of nitroaromatic compounds is very important not only to the mobility and transport of the chemicals but also to other aspects of the fate of the compounds. The distribution of a contaminant between aqueous and solid phases may determine its availability and reactivity for abiotic and microbial transformation reactions (Haderlein and Schwarzenbach, 1995). In the case of neutral nitroaromatic compounds like TNT, two types of adsorption mechanisms have been found to be predominant in most subsurface environments: (1) hydrophobic partitioning into the organic fraction of soils or aquifer materials, and (2) adsorption resulting from complex formation on clay mineral surfaces that bear exchangeable cations (Spanggord et al., 1985; Haderlein et al., 1996). TNT is a slightly polar compound with a small dipole momentum of 1.37 debye (Xue et al, 1995). This may explain to some extent the adsorption/desorption behaviors of TNT on soils. In research by Pennington and Patrick (1990), batch adsorption and sequential desorption studies were conducted with uncontaminated surface soils collected from 13 Army Ammunition Plants (AAP). It was found that steady state adsorption of TNT was reached within 2 hours and that adsorption isotherms were best fit by the Langmuir adsorption model. The results showed that oxidized conditions consistently decreased adsorption compared to reduced conditions and that microbial transformation appeared to be greater, or perhaps faster, under reduced conditions. TNT retention was found to be more dependent on soil cation exchange capacity (CEC) than on fraction of organic carbon (FOC), a fact that might be attributed to the slight polarity and the presence of $-NO_2$ groups of TNT. The authors indicated that

TNT was only slightly resistant to desorption following the batch adsorption experiments. For the soil with the highest fraction of organic carbon (FOC = 0.036) and most recalcitrant to desorption among the 13 AAP soils, about 20% of adsorbed TNT was retained after three sequential desorption cycles using water as desorbing agent. Lack of hysteresis meant that adsorption and desorption occurred to the same extent. These results indicated that soil sorption would not effectively prevent mobility of TNT through surface soils into the solution phase. Tucker et al. (1985) found that the TNT distribution coefficient values for water/soil phases were largely accounted for by soil CEC and FOC, with the CEC being more responsible. TNT desorption from soils is considered readily achievable, and irreversible behavior is not significant in short-term experiments (Pennington and Patrick, 1990; Leggett, 1985).

Haderlein and co-workers (1996) conducted an extensive study on specific adsorption of 31 nitroaromatic explosives and pesticides, including TNT, RDX, 2-amino-4,6-dinitrotoluene (2-ADNT), and 4-amino-2,6-dinitrotoluene (4-ADNT), to clay minerals. Three types of clay minerals, kaolinite, illite, and montmorillonite, were chosen as model minerals with representative properties. They found that the adsorption equilibrium for most of the investigated nitroaromatic compounds was essentially established within as short a time as a few minutes. The adsorption isotherms of all NACs investigated could be approximated by the Langmuir equation, although the authors indicated that the Langmuir fit might underestimate the extent of the adsorption at low sorbed-phase concentrations and that a linear isotherm with a slope, K_d (the adsorption constant), might better describe the adsorption equilibrium in these cases. For

neutral or non-ionizable NACs, K_d values remained constant between pH 3 and 9. It was shown that adsorption of NACs was high when exchangeable cations on the clays were K^+ and NH_4^+, but was negligible for Na^+-, Ca^+-, Mg^{2+}-, and Al^{3+}-clays. The authors suggested that the mechanisms of specific adsorption of NACs on clay minerals could be described by the formation of an electron donor-acceptor complex with the oxygen atoms on the external siloxane surfaces of clay minerals. Therefore, NACs such as TNT, which have a relatively strong electron-accepting tendency due to the electron-withdrawing nature of the nitro groups, can be relatively strongly adsorbed on highly exchangeable, highly charged minerals such as K^+-montmorillinite. Adsorption of the NACs on the clay minerals was found to be reversible. Therefore, the mobility, or adsorption/desorption, of NACs such as TNT and transformation intermediates in soils rich with these minerals may be manipulated by changing the degree of K^+ saturation of the minerals. This may have useful implications for remediation practices (Haderlein et al., 1996).

The equilibrium of sorption or exchange of solutes present in the soil solution has been mostly described by linear, Freundlich and Langmuir models. Among the kinetic models for adsorption and desorption, first- and *n*th- order kinetic forms are perhaps the most common. Multi-site and/or multi-reaction models are also used to deal with the multiple interactions of one solute in the soil, where two or more different types of reaction/retention sites are considered (Xue et al., 1995).

§2.4 Abiotic Transformations

It has been observed that many nitroaromatic compounds are susceptible to various abiotic transformation reactions (Glaus et al., 1992; Macalady et al., 1986). Because of the electron-withdrawing nature of nitro groups, reducing, nucleophilic, and/or electron-donating compounds are likely to induce the reductive transformation of TNT and other nitroaromatics. The reduction of nitro groups to amines is a widely observed transformation pathway for nitroaromatic compounds in anaerobic environments. Besides biological electron donating mechanisms, the most abundant abiotic and naturally occurring reductants include reduced inorganic forms of iron and sulfur, such as iron (II) and bisulfide (Stumm and Morgan, 1981). It has been reported that total reduced sulfur concentrations as high as 10^{-4} M (3.2 mg/L as sulfide) have been detected in envrionments with microbial sulfate-reducing activities, such as the bottom sediments of lakes, wells and groundwater (Dohnalek and Fizpatrick, 1983; Chen and Morris, 1972; O'Brien and Birkner, 1977).

Many researchers believe that naturally occurring organic chemicals such as quinone, iron porphyrin, and extracellular biomolecules play an important role in mediating reduction of nitroaromatic compounds (Schwarzenbach et al., 1990; Dunnivant et al., 1992b; Tratnyek and Macalady, 1989). It has been assumed that hydroquinone-like sub-units are the reducing moieties that make up part of the humic material, a commonly ocurring form of natural organic matter (Wolfe and Macalady, 1992). Schwarzenbach et al. (1990) investigated the reaction of nitrobenzenes with sulfide mediated by quinone

and iron porphyrin, two naturally occurring electron carriers found in biological systems (Lehninger, 1970; Buffle and Altmann, 1987; Thurman, 1985). These electron-transfer mediators effectively increased the reaction rate, and it was concluded that the reactivity of such mediators might depend on pH in a rather complex way. Tratnyek and Macalady (1989) reported rapid abiotic reduction of nitroaromatic pesticides with quinone-hydroquinone redox couples, which were selected to model the redox-labile functional groups in natural organic matter. Their experiments showed that observed rate constants increased as model system redox potential, E_h, became more negative. It was also noted that the observed reaction rate constant had a maximum value around pH 7.1. The kinetics of the pesticide (methyl parathion) were first order in methyl parathion and first order in the monophenolate form of the hydroquinone.

Besides quinone-like natural organic matter, other proteins, enzymes, or bacterial cell exudates were also reported to mediate abiotic reactions of NACs. Glaus et al. (1992) found that the reaction of 4-chloronitrobenzene with hydrogen sulfide alone was very slow but that the presence of cell exudates of a *Streptomyces* strain significantly increased the reaction rate. It should be indicated that in their experiments, very high buffer concentrations (50 to 100 mM), compared with the concentration of total sulfide (5 mM) and of the nitro compound (0.1 mM), were used to maintain a constant pH. They observed that the pH values affected the reaction rate dramatically and that the reaction rate increased with increasing time in some cases, especially when the initial pH was greater than 7. Pseudo-first order rate constants were used to quantify the reaction kinetics in this study and no attempt to monitor the sulfide consumption over time was

mentioned. Van Beelen and Burris (1995) reported that a catalyst contained in a crude protein extract from aquatic sediments mediated the reduction of TNT by cysteine and that this catalyst might be Fe^{2+}. It may be interesting to note that Schwarzenbach and co-workers (1990) observed the reductive transformation of 4-chloronitrobenzene in a cysteine solution mediated by iron porphyrin. Van Beelen and Burris (1995) further indicated that while strongly reducing chemicals such as cysteine could reduce TNT without a catalyst or non-enzymatically, less strong reductants such as nicotinamide adenosine dinucleotide phosphate (NADPH) were also capable of reducing TNT in the presence of enzymes extracted from aquatic sediments. These enzymes may be originated from aquatic plants and are ubiquitous in aquatic systems. The reduction products include 2-ADNT, 4-ADNT, 2,4-diamino-6-nitrotoluene (2,4-DANT), and 2,6-diamino-4-nitrotoluene (2,6-DANT).

The environmental processes and factors influencing the rate of abiotic reduction of nitroaromatic compounds have been reviewed by Haderlein and Schwarzenbach (1995). They indicate that naturally occurring abiotic electron donors, such as reduced iron species, reduced sulfur species, and organic carbon constituents, are all intimately coupled to and continuously influenced by microbial activities. Various processes and reaction steps, such as regeneration of reactive species, formation of a precursor complex, or actual transfer of electrons, may be rate-limiting. While phosphate buffer is widely used in reaction systems to stabilize the pH, Barbash and Reinhard (1989) found, when they investigated the abiotic reactions of 1,2-dichloroethane and 1,2-dibromoethane with H_2O and bisulfide, that phosphate buffer accelerated the nucleophilic substitution of both

halogenated compounds by H_2O. The authors did not present a full explanation for the catalysis, although it was proposed that increases in ionic strength due to the presence of phosphate buffer could only account for a relatively minor proportion (<10%) of the catalytic effect observed.

§2.5 Microbial Transformations

The vast majority of nitroaromatic compounds are anthropogenic and considered xenobiotic because of the inclusion of unusual chemical bonds and/or substitutions resistant to enzyme systems of microorganisms, which take geological time periods to evolve the abilities to exploit certain compounds as sources of carbon and energy (Spain, 1995). Research in the past two decades, however, has revealed a number of microbial systems capable of biotransforming or biodegrading nitroaromatic compounds. Species of fungi have been found to degrade and mineralize such compounds as 2,4-dinitrotoluene and TNT. Anaerobic bacteria, including some pseudomonads under denitrifying conditions, sulfate-reducing and methanogenic bacteria, and clostridia, are able to reduce the nitro group via nitroso and hydroxylamino intermediates to the corresponding amines, which have shown promise for further degradation and mineralization under appropriate conditions. However, deep biodegradation (e.g. ring cleavage leading to mineralization) and large-scale decontamination of nitroaromatic compounds are still scarce, mainly because of the demanding and poorly understood biodegradation/mineralization pathways of these compounds. When biotransformation and biodegradation of nitroaromatic compounds are studied, the difficulties of this task

lie in the fact that some compounds are highly resistant to microbial attack while others may be partially broken down to unknown, undetected, or nondegradable (dead-end) intermediates or even transformed to more toxic products (Gorontzy et al., 1994). Recent research on TNT biotransformation under various electron accepting conditions is reviewed as follows.

§2.5.1 Aerobic Conditions

Many researchers have found that the reduction of the first nitro group in the TNT molecule can be catalyzed by many aerobic and anaerobic bacteria (Amerkhanova and Naumova, 1978; Kaplan and Kaplan, 1982c; Schackmann and Muller, 1991). However, further biodegradation of TNT under aerobic conditions is considered difficult because TNT is usually resistant to conversion by oxygenase enzymes due to the presence of the electron-withdrawing nitro groups on the ring (Walker and Kaplan, 1992). Another concern is the formation of dead-end metabolites (azoxy compounds) resulting from polymerization of intermediates by abiotic coupling reactions under aerobic conditions (Schackmann and Muller, 1991). The formation of azoxy compounds under aerobic conditions, which appear to be resistant to further biodegradation, was also reported by earlier researchers (Won et al., 1974; Carpenter et al., 1978).

It has been reported that several pseudomonads, fungi, and yeasts transformed TNT, leaving the aromatic ring intact. The transformation products identified were 2-ADNT, 4-ADNT, 4-hydroxylamino-2,6-dinitrotoluene, 2,2',6,6'-tetranitro-4,4'-azoxytoluene, and 2,2',4,4'-tetranitro-6,6'-azoxytoluene (Won et al., 1974; Parrish,

1977; Naumova et al., 1982). Some research has given indications of mineralization of [^{14}C]TNT by some species of *Pseudomonas* under aerobic conditions, although the recovery of $^{14}CO_2$ is as low as 0.02 to 3% (Traxler, 1974; Boopathy et al., 1994). However, some studies with fungal systems have provided substantive evidence for mineralization of the aromatic ring of TNT, as discussed below, although the details of the mechanism and pathway remain to be shown.

Fernando et al. (1990) investigated the biodegradation of TNT by the wood-rotting (white rot) fungus *Phanerochaete chrysosporium* in soil and liquid cultures. This fungus is one of the relatively few microorganisms known to be able to degrade lignin, a naturally occurring and recalcitrant biopolymer, to carbon dioxide (Bumpus, 1989). *Phanerochaete chrysosporium* is also able to degrade a wide variety of environmentally persistent xenobiotics to carbon dioxide, including a number of chlorinated hydrocarbons such as DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl) ethane], chloroanilines, and polychlorinated biphenyls (Bumpus, 1989; Eaton, 1985). It is suggested that the ability to degrade such a diverse group of compounds is dependent on the nonspecific and nonstereoselective lignin-degrading system which is expressed by this organism under nutrient (nitrogen, carbon, or sulfur)-limiting conditions. Fernando et al. (1990) reported that about 20% of [^{14}C]TNT was converted to [^{14}C]CO_2 at an initial concentration of 100 mg/L in liquid cultures, while 18.4% of initial TNT (10,000 mg/kg) was converted to [^{14}C]CO_2 in soil cultures after a 90-day incubation period. Glucose at a concentration of 10 g/L (56 mM) was used as the primary substrate in the incubation. In another study by Spiker and co-workers (1992) using the same fungus, however, no significant

mineralization was observed at TNT concentrations greater than 15 ppm. Consequently, the utility of *P. chrysosporium* as an agent for bioremediation of TNT contamination was questioned. Studies have shown that the lignin-degrading system of *P. chrysosporium* functions only in the presence of a hydrogen peroxide generating system, as hydrogen peroxide is required as a co-substrate for lignin peroxidase (Fernando and Aust, 1994). The production of H_2O_2 is affected by inhibitors, O_2 concentration, and nutritional parameters such as nitrogen and carbon starvation. A study by Michels and Gottschalk (1994) showed that during the mineralization of TNT (at about 20 mg/L), the lignin peroxidase of *P. chrysosporium* was inhibited by the transient accumulation of 2-hydroxylamino-4,6-dinitrotoluene and 4-hydroxylamino-2,6-dinitrotoluene, whereas such a pronounced inhibition was not observed at lower TNT concentrations. Bumpus and Tatarko (1994) also found that 4-hydroxylamino-2,6-dinitrotoluene was a potent lignin peroxidase inhibitor.

A thermophilic compost system has also transformed ring-[^{14}C]-labeled TNT to ADNTs and diaminonitrotoluene compounds (Kaplan and Kaplan, 1982c). In another investigation using compost systems, however, only very minor amounts of these intermediates (<2%) were found. The major part of the applied radioactive material was detected in insoluble polymerized macromolecules (Isbister et al., 1984). The formation of insoluble products was probably due to the polymerization of TNT metabolites under aerobic conditions, as mentioned above.

In a recent study, Bruns-Nagel et al. (1996) used aerobic soil columns to evaluate the remediation of TNT-contaminated soils. A percolating fluid, containing glucose and

phosphate buffer at pH 7, was added into soils with 70 to 2100 mg of TNT per kg (dry weight), resulting in a TNT removal of over 90% in 19 days. The major TNT metabolites identified were 2,4-DANT and 4-*N*-acetylamino-2-amino-6-nitrotoluene. Azoxy derivatives were not detectable. After 19 days of aerobic percolation of the soil, the numbers of viable cells grown on soil extracts increased by one order of magnitude and the inhibition of the bioluminescence of *Vibrio fischeri* by aqueous soil extracts decreased by one order of magnitude, indicating a significant decrease in toxicity of the soil.

§2.5.2 Denitrifying Conditions

Denitrification is a potentially important mechanism for biotransformation of nitroaromatic compounds in aquifers due to the widespread occurrence of anoxic zones in this environment, especially when it is taken into account that nitrate and nitrite are commonly occurring components in munitions wastewaters (Tsai, 1991). Nitrate-reducing bacteria are ubiquitous in soils. While a variety of facultatively anaerobic bacteria, including *Alcaligenes*, *Escherichia*, and *Bacillus*, reduce nitrate to nitrite, various *Pseudomonas* species have a more complete reduction pathway, converting nitrate through nitrite to nitric oxide (NO), and nitrous oxide (N_2O) to molecular nitrogen (Atlas and Bartha, 1993).

TNT biotransformation under denitrifying and other anaerobic conditions has been of interest for many researchers. Batch bottle tests by Boopathy et al. (1993) showed that TNT was subject to anaerobic biotransformation under various electron accepting conditions, including denitrifying, sulfate-reducing, and CO_2-reducing

conditions. The best growth and the fastest TNT removal were seen under denitrifying conditions. The main intermediates of TNT transformation were 2-ADNT and its isomer 4-ADNT. The TNT removal in this study appeared to have been accomplished by co-metabolic processes because the reactors containing TNT as the sole source of carbon and energy under different electron accepting conditions showed no growth or TNT removal. The authors suggested that under nitrate-reducing conditions the main enzyme responsible for nitrate reduction was nitrate reductase, which could have acted on the nitro group of TNT and reduced it to an amino group. Similar results were also reported by Shah (1995), with denitrifying conditions inducing the fastest TNT transformation when compared with sulfate-reducing and methanogenic conditions. Han (1993) found that denitrifying conditions made possible the complete transformation of the intermediates produced in anaerobic TNT biotransformation.

In the aquifer slurry reactor studies reported by Krumholz et al. (1997), however, nitrate-reducing conditions yielded a TNT removal rate lower than those under methanogenic and sulfate-reducing conditions. This observation, different from the above-mentioned conclusions, may be at least partially due to a different procedure that Krumholz and co-workers used. Their reactors were run for two weeks prior to the addition of TNT to obtain better developed methanogenic and sulfate-reducing activities. Also, aquifer materials contained in their reactors might have imposed a reaction environment different from those in other researchers' experiments in which no aquifer materials were involved.

Preuss et al. (1993) found that triaminotoluene (TAT), an intermediate of reductive TNT transformation, was converted to unknown products by a *Pseudomonas* strain isolated from an anaerobic biofilm under denitrifying conditions. No TAT conversion was observed with killed controls, indicating that the conversion was due to a biological rather than a chemical process. The TAT concentration remained essentially constant in the absence of nitrate. However, unspecific chemical conversion of TAT did occur rapidly under other conditions where pH was lower than 6 or molecular oxygen was present.

Braun and Gibson (1984) investigated anaerobic degradation of 2-aminobenzoate (anthranilic acid) by denitrifying bacteria. They found that 2-aminobenzoate was used as a growth substrate by some *Pseudomonas* strains under nitrate reducing conditions. One mole of 2-aminobenzoate was converted to 0.4 mol of NH_4^+ and 5 mol of CO_2, indicating a large extent of mineralization. Another interesting finding was that the second stage in denitrification (nitrite to nitrogen) never occurred before the medium was totally depleted of nitrate. If excess nitrate was added to the growth medium, the cells grew by NO_3^- /NO_2^- respiration with little or no production of nitrogen.

Freedman et al. (1994) examined biotransformation of 2,4-dinitrotoluene (DNT) under nitrate reducing conditions. The presence of an electron donor (ethanol) was necessary for DNT to be biotransformed. DNT was stoichiometrically reduced to aminonitrotoluenes and 2,4-diaminotoluene, which disappeared slowly to unknown products.

§2.5.3 Sulfate-reducing Conditions

Sulfate-reducing bacteria are found in diverse environments and are of great application as well as academic interest. Two strains of *Desulfovibrio* have been studied extensively because of their ability to transform nitroaromatic compounds (Spain, 1995). Boopathy and Kulpa (1992) studied a sulfate-reducing bacterium, *Desulfovibrio* sp. strain B, which was capable of using TNT as a sole nitrogen source. For this bacterium, nitrate, nitrite, and TNT could all serve as electron acceptors in the absence of sulfate. The major intermediate of TNT transformation by this bacterium was identified as a diaminonitrotoluene, which was presumably converted to toluene via triaminotoluene. This tentatively proposed pathway would be very promising if confirmed, because the pathway of toluene mineralization was already established (Shelley et al., 1996). The authors did not mention whether or not active sulfate reduction and bisulfide production occurred in their system, which could be an interesting aspect worthy of studying because high TNT concentrations (e.g. 100 ppm) might inhibit sulfate reduction (as discussed in Chapter IV).

In another study, Preuss et al. (1993) examined another strain of *Desulfovibrio* sp. using TNT as the sole nitrogen source and pyruvate and sulfate as the carbon and energy sources. The organism was able to reduce TNT to TAT via 2,4-DANT and 2,4-diamino-6-hydroxylaminotoluene (2,4-DAHAT). A significant part of the TNT added to the medium was chemically reduced via ADNTs to 2,4-DANT by sulfide, which was applied as an oxygen scavenger. The authors indicated that the conversion of 2,4-DANT to TAT

was achieved by the growing bacteria and was the rate-limiting step in microbial TNT reduction, and could not be catalyzed by aerobic or facultative microorganisms. On the other hand, the reduction of TNT to DANT was significantly faster and mediated by non-specific enzymes. The rate of reduction of each successive nitro group is reported to decrease dramatically because amino groups deactivate the molecule for further reaction. When DANT is converted to TAT, DAHAT may accumulate as an intermediate. The authors suggest that the reduction of DANT and/or DAHAT to TAT involves a dissimilatory sulfite reductase, which converts sulfite to sulfide and can be significantly inhibited by CO, NH_2OH, DANT and DAHAT. This finding is significant because if the reduction of sulfite, which is one of the intermediates in the reduction from sulfate to sulfide (Singleton, 1993), is stopped, the whole process of sulfate reduction might be inhibited, which, in turn, might inhibit the growth of sulfate reducers. TAT is a compound which may be converted or "degraded" by trace elements (e.g. Mn^{2+}) as well as by cellular components, probably abiotically, due to the chemical instability of this compound. The products of TAT conversion are poorly understood, although it has been demonstrated by Preuss and co-workers (1993) that approximately one third of the amino groups can be released as ammonia.

Many sulfate-reducing bacteria, especially so-called "classical sulfate-reducing bacteria" which mainly utilize hydrogen, formate, lactate, pyruvate, some dicarboxylic acids, or a few alcohols as energy substrates, cannot oxidize organic multicarbon substrates beyond the level of acetate. This metabolic limitation usually reflects the absence of a biochemical pathway for oxidation of acetyl-CoA to CO_2. In the past two

decades, however, some species or genera of sulfate reducers have been found able to use acetate as the primary substrate by a modified citric acid cycle or an oxidative carbon monoxide dehydrogenase pathway, but grow more slowly and require more carefully controlled conditions than "classical sulfate-reducing bacteria" (Hansen, 1993).

§2.5.4 Methanogenic Conditions

Relatively little information is found in the scentific literature on the capability of methanogenic bacteria to transform or degrade TNT. Boopathy and Kulpa (1994) isolated a methanogen, *Methanococcus* sp. strain B, which could transform 100 ppm TNT to 2,4-DANT. The TNT transformation rates were faster with cells growing on H_2/CO_2 than with cells growing on formate. This bacterium did not use acetate or methanol as sole source of carbon and energy. A nearly stoichiometric (97 ppm) amount of 2,4-DANT was produced from 100 ppm TNT, and 2,4-DANT was not further transformed by this isolate. This study showed that TNT could be used as an electron sink under anaerobic conditions by methanogenic bacteria. It is not clear whether or not methane production was observed in this study. In another study by Boopathy et. al. (1993) using a mixed culture obtained from a TNT-contaminated soil, acetotrophic (acetate as carbon source, no external electron acceptor) conditions, which were expected to be methane-producing, did not result in microbial growth or TNT removal when the TNT concentration was 100 ppm. Under similar conditions except that the gas phase was H_2/CO_2 rather than N_2, however, microbial growth and TNT removal were observed.

Gorontzy et al. (1993) examined the biotransformations of nitrophenols, *p*-nitroaniline, and *p*-nitrobenzoic acid by several strains of methanogenic bacteria, including strains of *Methanobacterium*, *Methanosarcina*, *Methanospirillum*, *Methanogenium*, and *Methanoculleus*. All these bacteria were able to completely transform the investigated NACs to corresponding amino derivatives. However, it was necessary to pre-grow the cells to a certain density before adding the nitroaromatics. The authors further investigated the biotransformation of *p*-nitrophenol by *Methanosarcina frisia* and observed that as long as *p*-nitrophenol was present, methane production ceased entirely. When transformation had been completed, bacterial growth and methane production recovered. However, these and other researchers (Fedorak et al., 1990) did not observe inhibitory effects of anilines on methanogenesis. Therefore, it is assumed that nitroaromatics and their early-stage intermediates like nitroso- and/or hydroxyl-amines are the real inhibitors. They may react with the unique membrane components of the methanogens and cause cell lysis, ceasing the methane production. The authors also suggested other toxic effects of NACs on methanogens, including that these compounds might act as an "electron trap" leading to the breakdown of ATP synthesis.

§2.5.5 Other Anaerobic Regimes

McCormick et al. (1976) investigated the microbial transformation of TNT by *Clostridium pasteurianum*, *Veillonella alkalescens*, and *Escherichia coli* under anaerobic conditions. They found that cell-free extracts of these organisms, utilizing molecular H_2, reduced the three nitro groups of TNT to the corresponding amino groups. Resting cells

of the strict anaerobes (the former two) also reduced all three nitro groups, whereas resting cells of anaerobically grown *E. coli* reduced only two of the nitro groups. In the absence of added hydrogen, none of these organisms reduced the nitro groups. Several other strains of clostridia have been studied because of their ability to reduce nitroaromatic compounds. It was reported that hydrogenase and carbon monoxide dehydrogenase contained in two *Clostridium* species converted DANT to DAHAT when ferrodoxin was included in the reaction mixture (Preuss et al., 1993). Regan and Crawford (1994) found that pure cultures of *Clostridium bifermentans* and similar strains degraded RDX and TNT. Gorontzy et al. (1993) also used two *Clostridium* strains in their study of NAC biotransformations. In contrast to the methanogens, these bacteria were less sensitive to the presence of nitroaromatics and able to transform these compounds without cell lysis. The cells of these bacteria, different from methanogen cells, might be protected by the presence of a murein-containing cell wall and a different composition of the cell membrane.

Heijman and co-workers (1995) studied reductive biotransformation of ten monosubstituted nitrobenzenes by iron-reducing anaerobes in aquifer columns. The nitro group in the compounds was believed to be reduced to the amino group via nitroso and hydroxylamino groups, receiving six electrons in total. The authors indicated that the major electron donor in their system was Fe(II) which, after conversion to Fe(III), was regenerated by iron-reducing bacteria. The evidence included that the microbial activity, and hence the nitrobenzene reduction and the Fe(II) production, was enhanced by increasing the carbon source, acetate, in the column influent.

Funk and coworkers (1993) investigated the biotransformation of TNT in explosives-contaminated soils using an anaerobic mixed culture. It was found that the first stage of TNT metabolism, in which TNT was anaerobically reduced to its amino derivatives, could be optimized by employing pH 6.5 ~ 7.0, temperatures around 30 °C, and an added NH_4Cl level of 1.33 g/L for anaerobic soil cultures. The formation of recalcitrant polymers could be minimized and the completion of the reductive reactions in the first stage could be enhanced under these conditions. TAT formation, which indicated the completion of the first stage, was indirectly demonstrated by the presence of its transformation products, methyl phloroglucinol (MPG) and *p*-cresol. They suggested a second, aerobic stage after the completion of the first stage to degrade the products produced under anaerobic conditions.

§2.5.6 Transformation and Mineralization Pathways

Under both aerobic and anaerobic conditions, the initial steps in TNT biotransformation typically involve reducing the nitro groups to amino groups. It is indicated that the *para* nitro group is usually the first to be reduced, followed by reduction of one of the *ortho* groups, producing DANT isomers (Funk et al., 1993). The transformation of TNT to DANTs via ADNTs can also be completed by abiotic reactions. However, the reduction of the third nitro group, or the conversion of DANTs to TAT, is believed to be achieved only biologically under strict anaerobic conditions (Preuss et al. 1993). It seems that a commonly accepted reductive biotransformation pathway of TNT

can be expressed as follows (Rieger, P.-G. and H.-J. Knackmuss, 1995; Gorontzy et al., 1994; Preuss et al. 1993; Han, 1993; Shah, 1995; see also Figure 2-1):

TNT ==> 4-hydroxylamino-2,6-dinitrotoluene (4-HADNT, or its isomer 2-HADNT) ==> 4-ADNT (or its isomer 2-ADNT) ==> 2,4-DANT ==> 2,4-DAHAT TAT ==> poorly characterized products.

Boopathy et al. (1993) proposed that the bacterium *Desulfovibrio* sp. strain B metabolized TNT via TAT to toluene (Pathway A). On the other hand, Duque et al. (1993) reported the isolation of two *Pseudomonas* hybrid strains that metabolized TNT through dinitrotoluene and nitrotoluene to toluene by removing the three nitro groups on TNT and releasing them as nitrite ions (Pathway B). In these reactions, nitro group removal involves the formation of a hydride-Meisenheimer complex (Lenke and Knackmuss, 1992). Vobeck et al. (1994) indicate that the formation of a Meisenheimer complex (a dark red-brown colored H^--TNT complex) is the initial metabolic step of TNT biotransformation under aerobic conditions.

Both proposed pathways mentioned above end with the formation of toluene. Many microorganisms are able to transform toluene into TCA cycle intermediates under both aerobic and anaerobic conditions. Shelley et al. (1996) summarized three toluene degradation pathways, two aerobic and one anaerobic. All three toluene pathways can lead to ring cleavage and further catabolism.

Funk et al. (1993) proposed a third TNT degradation pathway beyond TAT (Pathway C, see Figure 2-1). With the stepwise transformations from TNT to TAT the same as the first pathway described above, this new pathway proposed TAT

biodegradation proceeding through methyl phloroglucinol (MPG) and *p*-cresol. *p*-Cresol is known to be degraded under both aerobic and anaerobic conditions by various microorganisms. Ring cleavage and mineralization pathways of *p*-cresol under aerobic conditions have already been established (Bayly and Barbour, 1984; Hopper and Taylor, 1975; Joback and Reid, 1987), involving either direct ring attack by oxygen-dependent enzymes or hydroxylation of the methyl group.

In addition to research on the pathways shown in Figure 2-1, an earlier work on biodegradation of TNT was reported by Naumova et al. (1988). The authors found that 2,4-DANT, a TNT metabolite, was used as the sole nitrogen source by *Pseudomonas florescence* and transformed to nitrogen-free products phloroglucine and pyrogallol, the latter also being a conversion product of phloroglucine. It was assumed that pyrogallol was the last aromatic intermediate of TNT and the starting point of ring cleavage.

Shelley and co-workers (1996) have used a thermodynamic approach to analyzing these three TNT biodegradation and mineralization pathways. It is indicated, from a thermodynamic perspective, that the third pathway mentioned earlier (TNT => TAT => *p*-cresol) is a favorable one and should be the focus of future research because there is a relatively large total change of Gibbs free energy (-288 kcal/mol) in this pathway and because the stepwise free energy changes are relatively small and easy to achieve.

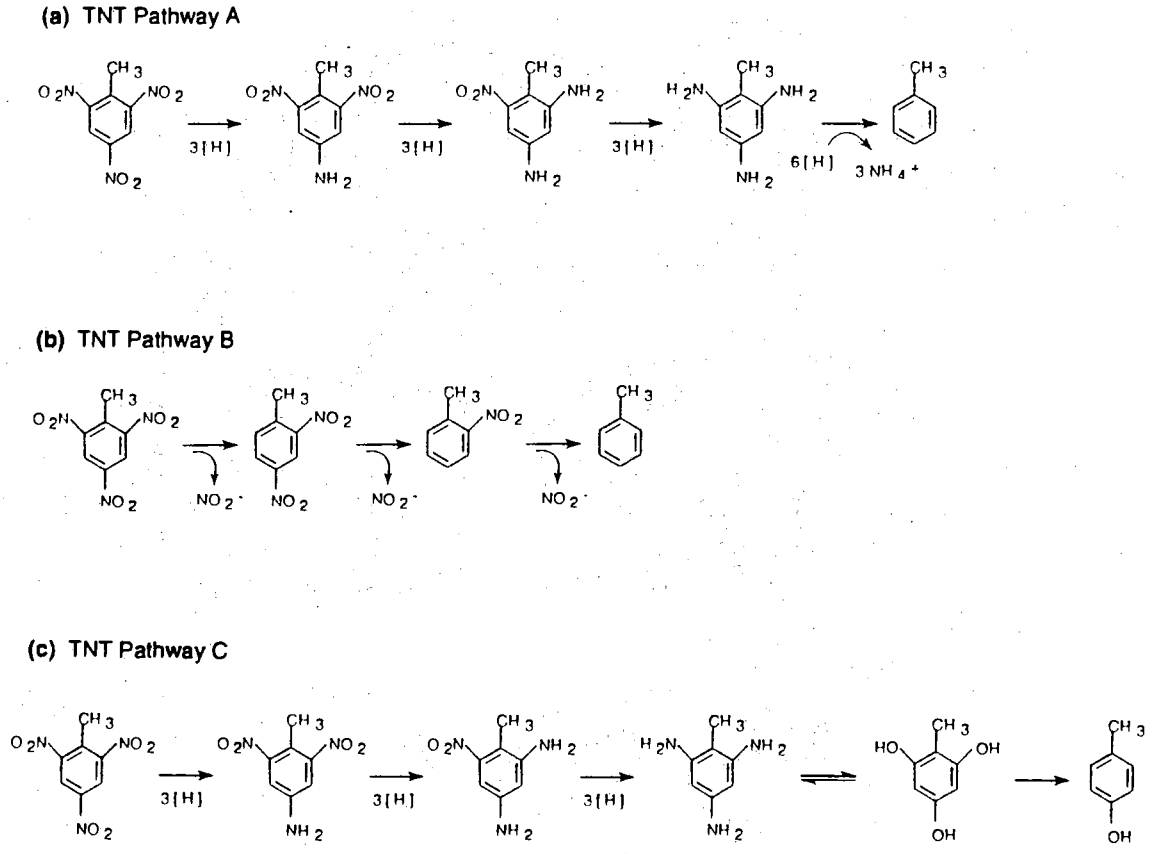


Figure 2-1 Proposed TNT biodegradation pathways

(Source: Shelley et al., 1996)

Bradley and Chapelle (1995) have studied the environmental factors affecting microbial TNT mineralization. It was observed that TNT mineralization by indigenous soil microorganisms was inhibited by addition of cellobiose and syringate because the indigenous bacteria, although capable of metabolizing TNT, preferentially utilized less recalcitrant substrates when available. Compared with strictly aerobic or anaerobic conditions, the authors indicated that a mosaic of aerobic and anaerobic micro-environments, i.e. heterogeneous micro-aerobic conditions, may be optimal for TNT biodegradation and mineralization.

§2.6 Fate of TNT in Soils and Aquifers

TNT migration and transport, long-term adsorption and desorption, and abiotic and microbial transformations are the major aspects contributing to the fate of TNT in subsurface environments, mainly soils and aquifers. Potential migration and transport of TNT from contaminated soils, as well as from waste disposal lagoons, is of great concern. Selim et al. (1995) used clay (bentonite/sand) columns and soil columns to investigate the transport of TNT and RDX. The TNT mobility varied largely, depending on clay and soils and on the composition of the background solution introduced into the column. Major transformation products of TNT were the ADNTs. A flow interruption resulted in significant TNT decrease and corresponding ADNT increase in the column effluent, indicating enhanced TNT transformation due to longer retention time. For RDX, only limited retardation was observed under all conditions. The authors used a nonlinear

multi-reaction and transport model, based on the classical convection-dispersion model, to describe the transport and transformation of the investigated compounds.

Another attempt to comprehensively examine the TNT transport, transformation, and adsorption in soils was made by Comfort and co-workers (1995) using column techniques. The authors reported that TNT breakthrough curves based on the column experiments never reached initial solute pulse concentrations, probably due to adsorption and/or transformation of TNT. ADNTs were identified as major transformation intermediates. A nonlinear adsorption isotherm (Freundlich) was employed to predict the mobility and retardation of TNT in the soil columns. In their study, the sorbed TNT was not completely extractable, possibly due to the relatively high organic matter content and CEC of the soils.

Although numerous investigators, as mentioned previously, have examined microbial transformation of TNT, these studies usually utilized artificially enriched or isolated cultures of bacteria. Surprisingly little is known about the capability of native microbial communities to transform and/or degrade TNT *in-situ*. However, this type of study may be very valuable for evaluation of the TNT fate in natural environments. One of few studies of biotransformation of TNT by indigenous microorganisms in aquifer materials was reported by Bradley et al. (1994). Their results indicated that the microbial communities associated with surface soils and aquifer materials were capable of completely transforming TNT, 2,4-DNT, and 2,6-DNT in 20 to 70 days. Microcosms created to simulate the anaerobic conditions in sediments showed amino-nitro compounds as major intermediates. Tests with uniformly labeled [^{14}C]TNT indicated that the

indigenous aquifer microorganisms were also capable of partial mineralization of TNT at a concentration of 100 μM (22.7 mg/L). The activities of the white rot fungus *P. chrysosporium*, which was considered one of the most promising microbial species for mineralizing TNT, were reportedly completely inhibited by TNT concentrations greater than 66 μM.

Many researchers have examined the fate of reduction products of TNT and other nitroaromatic compounds in soils, aquifers and sediments (Delgado and Wolfe, 1992; Dunnivant et al., 1992b; Pillai et al., 1982; Somasundram and Coats, 1991). Under anaerobic conditions, anilines produced from transformation of NACs are relatively stable. In aerobic or sub-oxidative environments, however, the anilines are subject to rapid further transformation to form polymers, bound residues, and other unknown or poorly characterized products. Haderlein and Schwarzenbach (1995) indicate that the fate of many of the oxidation products of aromatic amines can be better understood by examining the formation and conversion of the aryl-amino radical, $ArNH^{\bullet}$. Delocalization of the charged site of this radical can make the aromatic ring negatively charged and subject to further reactions. These radicals may combine with each other to form coupling products or, more likely, react with numerous substances in soils and aquifers to yield a variety of unknown products. It is believed that natural organic matter in soils and aquifers can bind these transformation products irreversibly and may be the most important sink of aromatic amines (Haderlein and Schwarzenbach, 1995).

§2.7 Recent Developments and Application of Treatment Technologies

Various treatment and remediation technologies, including incineration, carbon adsorption, photolysis, chemical treatment, composting, and biotreatment, have been tested for their applicability to TNT-contaminated water and soils.

A conventional and proven technology of disposal of TNT-containing wastes is incineration, an expensive and energy intensive process. Furthermore, the ash accumulated from incineration can cause a leachate problem when it is landfilled (Tsai, 1991).

Wujcik et al. (1992) described a technology of explosives removal using granular activated carbon adsorption. However, regeneration of spent carbon was hazardous thermally and difficult to achieve chemically.

Hao and co-workers (1993) examined the feasibility of wet air oxidation of red water, a TNT-containing wastewater produced in manufature of explosives. The treated water had adverse effects on the efficiency of *Nitrosomonas* in converting ammonia to nitrite, indicating residual toxicity. Another chemical treatment method was reported by Semmens et al. (1985). The TNT- and RDX-containing wastewater from a munitions handling facility was treated with hot caustic dose and calcium hypochlorite. It was believed that TNT could be effectively decomposed if desirable pH (~11) and temperatures (~100 ^{o}C) were maintained for 10 to 15 minutes. However, the toxicological and chemical characteristics of the treated water were not addressed in the article.

Tsai (1991) studied the biotreatment of a TNT waste stream (red water) with extracts from fungal systems. The enzyme complex secreted by the white rot fungus *P. chrysosporium* was extracted, concentrated, and added to red water samples. Results showed that the fungal extracts were effective in causing transformations of components in the red water during a one-week laboratory incubation. The red color intensity and the acute cytotoxicity were reduced after the treatment. Pretreatment of the water with UV seemed to make the water more sensitive to this form of biotreatment. The author did not report whether or not the TNT had been mineralized to any extent, although the enzyme systems of white rot fungus *P. chrysosporium* were expected to be capable of TNT mineralization, as discussed earlier.

Composting of explosives-contaminated soils has been examined by some researchers. Full-scale composting was conducted at the Louisiana Army Ammunition Plant (Williams et al., 1989). The test sediments contained approximately 76,000 ppm of total explosives, including TNT (66%), RDX (25%), and HMX (9%). The results showed that total explosives were reduced by 99% in the thermophilic pile (55 °C) after 22 weeks. In the chemical and toxicological testing of the composted explosives-contaminated soil at the Umatilla Army Depot Activity (UMDA, Umatilla, OR), Griest et al. (1993) found that the toxicity, mutagenicity, and concentrations of explosives decreased more than 90% in some cases after 44 days (in a mechanical composter) or 90 days (in static piles) of composting. However, low levels of explosives and metabolites, bacterial mutagenicity, and leachable toxicity remained after composting. Caton and co-workers (1994) indicate that the bulk of the transformed products of TNT may

accumulate as an acetonitrile-nonextractable, but hydrolyzable, fraction after static pile composting. Their experiments showed that the insoluble fraction of transformed TNT would not be released appreciably by the action of acid rain and sunlight. These observations seem to be consistent with the earlier discussion (Haderlein and Schwarzenbach, 1995) about the fate of the TNT transformation products under aerobic conditions.

In another development, the U.S. EPA participated in a technology demonstration which tested a bio-enhancement procedure treating soils contaminated with nitroaromatic compounds (US EPA, 1993). This technology utilized an anaerobic bioreactor amended with nutrients and pH buffers. Preliminary data collected during a demonstration test with dinoseb (a teratogenic, nitrophenolic herbicide) indicated that the dinoseb in the soil was reduced to below the analytical detection limit (0.15 mg/kg) in less than 25 days. The biodegradation of TNT was anticipated to take approximately the same length of time. Roberts et al. (1993) indicated that the use of an acclimated inoculum was an effective way to complete rapid large-scale anaerobic treatment of dinoseb-contaminated soil and that it could take a much longer time period to remove the transformation intermediates than to remove the parent compound itself.

Funk et al. (1995b) conducted a full-scale demonstration of anaerobic bioremediation of TNT-contaminated soils. A 50/50 soil/water slurry, amended with phosphate buffer and 1~2% starch, was mixed and incubated in an anaerobic bioreactor for about 5 months. It has been shown that nearly complete TNT and ADNT removal occurred after the redox potential was lowered to about -400 mV, which was driven by

the microbial utilization of starch. Low levels of 2,4-DANT were found in the treated slurry at the termination date, and *p*-cresol transiently appeared throughout the incubation process. This demonstration perhaps showed the promise of the feasibility of bioremediating TNT-contaminated soils on a large scale.

Another recent development is a study performed in support of the pilot demonstration of a biological soil slurry reactor (Manning et al., 1995). The investigators in this study used an aerobic/anoxic soil slurry reactor operated in batches or semicontinuously, in which 100% TNT was removed and 23% was recovered as CO_2. A rarely reported intermediate, 2,3-butanediol, was identified in this system. This study showed that the natural soil bacteria present in contaminated soils were able to cause extensive transformation and degradation of TNT under aerobic/anoxic conditions and that molasses, compared with other carbon sources, was an ideal substrate for large-scale TNT removal.

It is interesting to notice that the aerobic/anoxic regime in the above study might have something in common with or similar to the concept of the heterogeneous micro-aerobic conditions recommended by Bradley and Chapelle (1995) or the process of two-stage (anaerobic/aerobic) bioremediation recommended by many others (Funk et al., 1995a; Dickel et al.; Han, 1993; Roberts et al., 1996). These researchers reported successful TNT biodegradation when a second aerobic stage was introduced to degrade the intermediates, mostly 2,4-DANT and TAT, produced in the anaerobic stage of TNT biotransformation. Nevertheless, it is felt that the aerobic transformation and mineralization pathways beyond TAT are still not fully revealed and that the extent of

ring cleavage and mineralization, especially at high initial TNT concentrations, is not well demonstrated in most cases.

§2.8 Summary

Many studies have been conducted and significant findings have been made in the fields of physical adsorption and desorption, abiotic conversion, and microbial transformation of TNT and other nitroaromatic compounds. These sources of information provide the basis for better understanding of TNT environmental fate and further development of related pollution-abating strategies. On the other hand, the above review of the current literature has also revealed a number of questions which are essential in investigation of the fate of TNT and related compounds in subsurface environments, but have not been answered sufficiently. These questions include, but are not limited to, the following: How do the characteristics of long-term adsorption of TNT on aquifer materials differ from those of short-term adsorption? How do the presence of reductants and aquifer materials induce abiotic transformation of TNT? What are the effects of such factors as the primary substrate concentration or the initial TNT concentration on TNT biotransformation under different electron accepting conditions? What are the effects of aquifer materials on TNT biotransformation? How can we predict TNT environmental fate taking into account the dynamic conditions in aquifers and the co-existence of several different environmental processes? Attempts are made in this dissertation to at least partially answer these and some other questions, as listed in the study objectives in Chapter I.

CHAPTER III

EXPERIMENTAL MATERIALS, PROCEDURES, AND METHODS

§3.1 Materials

§3.1.1 Chemicals

2,4,6-Trinitrotoluene (TNT) was purchased from Chem Service (West Chester, PA). About 10~20% of water was added to the crystallized TNT product (99% purity) by the manufacturer in consideration of safe shipping and handling. Before use, therefore, the wet TNT crystals were placed in a desiccator at room temperature for at least 5 days to remove the moisture. Dried TNT solids were then sealed in a glass vial at room temperature until use. 2-Amino-4,6-dinitrotoluene (2-ADNT) and 4-amino-2,6-dinitrotoluene (4-ADNT), purchased from Supelco (Bellefonte, PA), were in the form of liquid standards dissolved in acetonitrile with a concentration of 1000 μg/ml. These reagents were preserved at 4 °C. All other chemicals were of the highest purity available and were used as received.

Deionized water was used for the preparation of all growth media, nutrient solutions, and reactor contents. Milli-Q water (≥18mΩ·cm) produced by a Milli-Q purification system (Millipore Co., Molsheim, France) via deionization and reverse osmosis was used for all chemical analyses, standard preparation, and sample treatment.

§3.1.2 Inocula and Seed Reactors

The original bacterial inocula were denitrifying, sulfate-reducing, and methanogenic mixed cultures used in previous experiments on TNT biotransformations (Han, 1993; Shah, 1995). These cultures, amended with aquifer materials, landfill leachate (described below), and TNT, were used as inocula to set up 160 ml seed reactors operated under denitrifying, sulfate-reducing, or methanogenic conditions. The methods of setting up these initial seed reactors were described in detail by Shah (1995). The TNT concentration in the reactors was increased gradually from 5 mg/L to higher levels to acclimate the bacteria, and maintained at 80 to 100 mg/L for denitrifying reactors, 30 to 80 mg/L for sulfate-reducing reactors, and 20 to 60 mg/L for methanogenic reactors. Lower TNT concentrations were used for the sulfate-reducing and methanogenic seed reactors because, as discussed in the literature review, sulfate reducers and methanogens were more sensitive to the inhibitory effects of TNT and its metabolites. Cultures from these seed reactors were used as inocula for the test reactors operated under their respective electron accepting conditions in later experiments.

§3.1.3 Aquifer Materials

The aquifer materials used in this study were collected from a methanogenic site located within the aquifer adjacent to the municipal landfill in Norman, Oklahoma. Landfill leachate was also collected at this site. The aquifer site has been characterized in detail elsewhere (Beeman and Suflita, 1987). The aquifer materials were very sandy and

had been polluted by municipal landfill leachate, with volatile solids content of about 3 g/kg dry wt. (i.e. 0.3%). Samples of aquifer solids and leachate were collected in August, 1994, by digging to the top of the ground water table (4 m depth) and collecting the solids and the leachate separately into glass or plastic vessels. Samples were then stored at 4 °C until use.

§3.2 Experimental Methods

§3.2.1 Batch Experiments of TNT Adsorption

§3.2.1.1 Kinetics Kinetics experiments were conducted to determine the time period required for the tested compounds to reach adsorption equilibrium. The aquifer material was dried at 103 °C for about 24 hours. The solutions of tested compounds, including 2-ADNT, 4-ADNT, and TAT, were mixed with aquifer material samples in 250 ml flasks and sampled at certain time intervals. The solution concentrations used for kinetics tests were 30 mg/L for 2-ADNT and 4-ADNT, and 20 mg/L for TAT. A lower concentration was used for TAT because dissolution of this chemical was difficult. The ratio of aquifer materials to compound solution (soil/solution ratio) was 15 g soil/75 ml. The flasks were covered with parafilm and wrapped with aluminum foil. All reactors were duplicated and incubated on a shaker table at room temperature.

To inhibit possible biotransformation, solutions of the compounds were amended with sodium azide to achieve a final concentration of 0.3 g/L. Because preliminary experiments showed that TAT tended to react significantly with biocides such as sodium

azide and mercuric chloride, sodium azide was not added to the TAT reactors. For other compounds, no significant interaction with azide was observed. Experiments with different sodium azide concentrations (0, 0.3 and 2 g/L) indicated that azide addition did not interfere or compete with the adsorption of the tested compounds. Because TAT is chemically unstable and sensitive to oxygen, especially in solutions with pH less than 6 (Preuss et al., 1993), it was dissolved in boiled, degassed, and slightly basic (pH 8.5) water and handled under an argon atmosphere to minimize the oxygen exposure and chemical conversion. After the aquifer material, solution, and sodium azide were mixed in the flask, the pH value was adjusted to 7.5, which was considered typical under natural conditions.

§3.2.1.2 Isotherm Experiments In these experiments, the aquifer material sample was placed in a 20 ml glass vial to which was added a 15 ml solution of the tested compound. The soil/solution ratio was the same as described in the kinetics test for each compound. The concentration levels used for each compound were 5, 10, 15, 20, and 30 mg/L for 2-ADNT and 4-ADNT, and 2, 5, 10, 15, and 20 for TAT. In the TNT isotherm test, the soil/solution ratio was 1 g/5 ml and the concentration levels were 5, 10, 20, 50, and 100 mg/L. Such a concentration range was chosen for TNT mainly because the concentration of 100 mg/L was a frequently used level in many other experiments of this research. Azide addition, TAT handling, and pH adjustment were as described above. The glass vials were capped, wrapped with aluminum foil, and equilibrated at room temperature on a shaker table for 4 hours (2 hours for TAT, because of its instability).

Pennington and Patrick (1990) reported that TNT adsorption on a soil reached steady state in 2 hours when the initial concentration was 16 mg/L, the soil FOC (fraction of organic carbon) was 0.0037, and the soil to solution ratio was 1:20. In light of this information, 4 hours of equilibrating time for TNT was estimated to be enough in this study and could be appropriate for the sake of consistency with other compounds. After the equilibrating procedure, the compound concentration in each vial was analyzed to determine the loss due to adsorption.

§3.2.1.3 Desorption After the adsorption process of the isotherm experiments was finished, desorption experiments were conducted on the samples with the initial concentration of 20 mg/L. The solution phase was removed from the glass vial, and the solid phase was extracted by adding 5 to 7 ml methanol, shaking the vial manually for about 1 minute, centrifuging (IEC Centra-7, Damon/IEC, Needham Hts., MA) the vial at 2000 rpm for 15 minutes, and collecting the extract. Extraction was performed three times sequentially for each sample. The 3 extracts from each sample were then combined and concentrated under an argon stream in the dark.

§3.2.1.4 Sampling When samples were taken from the flasks in the adsorption kinetics experiments, the mixed suspensions, rather than the supernatants, were sampled. The sample volume was small (1.5 to 2 ml each time) so as to minimize the possible change of soil/solution ratios. Several samples were taken from each flask over a 24-hour time period in the kinetics experiments.

§3.2.2 Abiotic Reaction with Bisulfide

Abiotic reactions of TNT with bisulfide were examined in this set of experiments. Since commercially obtained sodium sulfide products are not stable in air and tend to contain various oxidation products and impurities, the following procedures have been conducted to make bisulfide stock solution as pure as possible (Qin, 1995). Individual crystals of $Na_2S \cdot 9H_2O$ were rinsed with deoxygenated (alternately evacuated and argon-purged) Milli-Q water to remove the oxidized surface on the crystals and wiped dry with paper tissue inside a gasbag filled with argon. The rinsed crystals were then dissolved with deoxygenated Milli-Q water to prepare a stock solution of about 4000 mg/L (as total sulfide). The solution was standardized using the method described in Standard Methods (APHA et al., 1985), Section 427D, and stored at 4 °C until use.

The experimental reactors were prepared by mixing TNT stock solution, buffer stock (phosphate or bicarbonate), and water in a flask, adjusting the pH to 7.0, and deoxygenating the mixture in a manner described by Glaus et al. (1992). The initial TNT concentration was 30 mg/L (0.132 mM) in all abiotic reaction experiments. The mixture in the flask was alternately (three times) evacuated with a vacuum pump for five minutes and purged with argon for five minutes. The liquid was then quickly distributed into a series of 60 or 120 ml serum bottles, with reactor content of 50 or 100 ml respectively. The bottles were further purged with argon for 20 minutes, then sealed with Teflon-faced rubber septa and aluminum caps, and autoclaved at 248 °F and 15 psi for 20 minutes. After the bottles were cooled to room temperature, aliquots of bisulfide stock solution

were injected into the serum bottles to achieve the desired initial bisulfide concentraion followed by immediate monitoring of TNT and bisulfide concentrations.

In order to test the effects of aquifer materials on the abiotic reactions, one set of serum bottles was set up as described above and amended with autoclaved and dried aquifer materials before adding bisulfide. After the aquifer materials were added, the bottles were purged with argon for 20 minutes, sealed with rubber stoppers, and then amended with bisulfide stock solution. The effects of pH buffer were examined by setting up reaction bottles with 4 mM phosphate buffer, 4 mM bicarbonate buffer, or no buffer. The contents in all bottles had an initial pH of 7.0 before bisulfide was added.

The experimental conditions used in this study are summarized in Table 3-1.

Table 3-1 Experimental Parameters Tested in the Sulfide Study

Tested Parameter	Values	Other Conditions
Total sulfide conc.	Set 1: 30 mg/L	TNT = 30 mg/L
	50 mg/L	PO_4^{3-} buffer = 4 mM
		No aquifer materials
	Set 2: 15 mg/L	TNT = 30 mg/L
	30 mg/L	No buffer
	50 mg/L	No aquifer materials
Aquifer material conc.	0	TNT = 30 mg/L
	3 g/100 ml	Total sulfide = 30 mg/L
	10 g/100 ml	PO_4^{3-} buffer = 4 mM
Buffer	0	TNT = 30 mg/L
	4 mM PO_4^{3-}	Total sulfide = 30 mg/L
	50 mM PO_4^{3-}	No aquifer materials
	4 mM HCO_3^-	

The abiotic batch reactors with bisulfide were sampled with time intervals from 20 minutes to 1 hour in most cases. If bisulfide concentrations were to be measured over time for a set of reactors, a series of identical serum bottle reactors was set up and two bottles (as duplicates) were opened each time for the bisulfide concentration measurement. This is necessary because bisulfide measurement (iodometric method) often requires a relatively large sample volume, 20 to 80 ml in this study.

§3.2.3 Batch Reactors for TNT Biotransformation

Batch reactors, divided into two sets, were set up to examine TNT biotransformations under various conditions. These reactors were not amended with aquifer materials so as to facilitate isolating the effects of microbial activities and monitoring the appearance and disappearance of TNT metabolites. Set One was designed to have reaction conditions (i.e. primary substrate concentrations, initial TNT concentrations, etc.) close to those in the column reactors, while Set Two was under more nutrient-rich conditions.

§3.2.3.1 Set One of Batch Reactors Serum bottles of 160 ml served as the reactors in this set. Three types of electron accepting conditions, denitrifying, sulfate-reducing, and methanogenic, were employed for these reactors, which also included abiotic controls. All the reactors were duplicated. The volume of liquid culture in each reactor was 120 ml. The recipes of the media used for the reactors are presented in Table 3-2. The nutrient concentrations, which were the same for the three types of electron

accepting conditions, are shown in Table 3-3. Table 3-4 shows the recipe of the trace metal solution used in the reactor media (Vishniac and Santer, 1957). These recipes were adopted by modifying the medium recipes reported by other researchers (Boopathy et al., 1993; Han, 1993; Shah, 1995). TNT and carbon source concentrations in these reactors were chosen to be close to those in aquifer column reactors, so that the comparison of the batch and the column results could be made as straightforward as possible.

Table 3-2. Medium Recipes for Batch Reactor Set One (Room Temperature)

Denitrifying Reactors		Sulfate-reducing Reactors		Methanogenic Reactors	
Ac^- / NO_3^- (mg/L)	180/360 or 1000/2000	Lactate / SO_4^{2-} (mg/L)	300/450 or 1000/1500	Ac^- (mg/L)	180 or 1000
NaAc / KNO_3 (mg/L)	250/586 or 1390/3260	Na lactate / Na_2SO_4 (mg/L)	380/665 or 1260/2220	NaAc (mg/L)	250 or 1390
Ac^- / NO_3^- as mM	3.05/5.81 or 16.9/32.3	Lactate / SO_4^{2-} as mM	3.37/4.69 or 11.2/15.6	Ac^- as mM	3.05 or 16.9
TNT (mg/L)	60 or 100	TNT (mg/L)	30 or 60	TNT (mg/L)	30 or 60
TNT as mM	0.264 or 0.44	TNT as mM	0.132 or 0.264	TNT as mM	0.132 or 0.264
Na_2SO_4 (mg/L)	15			Na_2S (mg/L)	5
pH	7.3	pH	6.9	pH	7.0
Nutrients : see Table 3-3					

Table 3-3. Nutrient Concentrations in Batch Reactor Set One

Yeast extract	10 mg/L
NH_4Cl	0.15 g/L
NaCl	0.025 g/L
$CaCl_2$	0.02 g/L
$MgCl_2$	0.005 g/L
$NaHCO_3$	0.1 g/L
Na_2HPO_4	0.355 g/L
KH_2PO_4	0.34 g/L
Trace metal solution	1 ml/100 ml

Table 3-4 Trace Metal Solution

Compounds	Concentration (mg/L)
$FeSO_4 \cdot 7H_2O$	200
$ZnSO_4 \cdot 7H_2O$	10
$MnCl_2 \cdot 4H_2O$	3
$CoCl_2 \cdot 6H_2O$	20
$CuCl_2 \cdot 2H_2O$	1
$NiCl_2 \cdot 6H_2O$	2
$Na_2MoO_4 \cdot 2H_2O$	3

Before TNT was added into the batch reactors, TNT-free cultures were grown to obtain active microbial cultures. Serum bottles were filled with stock solutions of the primary substrate, the electron acceptor, and other additives and nutrients listed in Tables

3-2 and 3-3. Water was added to bring the reactor content to the 120 ml mark, and the pH was adjusted using HCl or NaOH solution. The bottles were then purged with argon gas for 20 minutes before they were sealed with rubber sleeve stoppers. A stock solution of Na_2S was added to yield a concentration of 5 mg/L in the methanogenic bottles as oxygen scavenger. All the reactors under each type of electron accepting conditions were first set up identically. The bottles were then inoculated with 2 ml of culture from the seed reactor under each of the respective electron accepting conditions and incubated at room temperature (22 ± 2 °C) for 6 to 10 days until the culture turned cloudy and significant gas production was observed. At this point, the biomass was harvested by centrifuging the culture at 2000 rpm for 15 minutes and discarding the supernatant. The harvested cells were transferred to fresh TNT-free media in test reactors which were set up in the same manner described above.

After several days of incubation of these bottles at room temperature, active microbial activities were observed and confirmed by measuring the gas production, biomass concentration, substrate utilization, and electron acceptor consumption. Since biomass (approximated as cell dry mass, or volatile suspended solids) measurement required relatively large sample volumes and there was only 120 ml of culture in each reactor, two extra bottles were set up under each type of electron accepting conditions, identical to others and dedicated to biomass determination. The mean value of biomass concentrations of these duplicates was used to estimate the average biomass concentration in all other bottles under the same electron accepting conditions. After these measurements, concentrations of primary substrates and electron acceptors were brought

to the desired initial levels (in Table 3-2) by injecting stock solutions, and aliquots of TNT stock solution (12 g/L in acetonitrile) were spiked into the reactors to obtain desired initial TNT concentrations. The abiotic control bottles were amended with sodium azide to produce a concentration of 0.3 mg/L, sealed with Teflon-faced rubber septa and aluminum caps, and autoclaved at 248 °F and 15 psi for 20 minutes before adding TNT. Blank reactors, receiving a representative volume of acetonitrile (0.5 ml) without TNT, were also set up to examine whether or not the acetonitrile would affect the microbial activities significantly. All the reactors were then incubated at room temperature in the dark.

§3.2.3.2 Set Two of Batch Reactors This set of reactors was set up mainly to observe the patterns of appearance and disappearance of TNT metabolites under three types of electron accepting conditions within a reasonably short time period. Therefore, these reactors, compared with those in Set One, were amended with significantly higher concentrations of primary substrates including considerable amounts of yeast extract and peptone and incubated at 37 °C in the dark. Tables 3-5 and 3-6 show the recipes of the media used for these reactors.

Table 3-5. Medium Recipes for Batch Reactor Set Two (T = 37°C)

Denitrifying Reactors		Sulfate-reducing reactors		Methanogenic Reactors	
Na acetate	2.87 g/L	Na lactate	3.92 g/L	Na acetate	2.87 g/L
KNO_3	2.02 g/L	Na_2SO_4	2.48 g/L	Na_2S	0.01 g/L
Na_2SO_4	0.04 g/L				
TNT	100 mg/L	TNT	100 mg/L	TNT	100 mg/L
Inoculum	1 ml/100 ml	Inoculum	1 ml/100 ml	Inoculum	1 ml/100 ml
pH	7.3	pH	6.9	pH	7.0
Nutrients: See Table 3-6					

Table 3-6 Nutrients Concentrations in Batch Reactor Set Two

Yeast extract	0.3 g/L
Peptone	0.1 g/L
NH_4Cl	0.4 g/L
NaCl	0.05 g/L
$CaCl_2$	0.04 g/L
$MgCl_2$	0.01 g/L
$NaHCO_3$	0.2 g/L
Na_2HPO_4	0.71 g/L
KH_2PO_4	0.68 g/L
Trace metal solution	1 ml/100 ml

Set Two reactors were set up using 500 ml glass flasks or bottles with culture volume of 400 ml. The procedures of setting up these reactors were similar to those used for the reactor Set One except that all reactor ingredients, including TNT and inocula,

were added at the starting point. Therefore, these reactors did not have a TNT-free growth phase to accumulate a relatively high biomass concentration prior to TNT addition. The inocula were also from the same seed reactors described earlier.

§3.2.3.3 Sampling Biological batch reactors were sampled by using a 5-ml plastic syringe with a stainless steel needle to withdraw 1.5 to 2 ml samples each time and replacing the reactor head space with an equal volume of argon to prevent negative pressure. Samples were taken at short time intervals (1 to 3 days) in the early stage of the experiments and at longer intervals thereafter. It was ensured that the total volume loss of the culture in a reactor due to sample withdrawing was less than 15% of the initial culture volume during the life time of the reactor.

§3.2.4 Aquifer Column Reactors

§3.2.4.1 Reactor Set-up The procedures of preparing aquifer columns were similar to those described by Siegrist and McCarty (1987). The glass columns (Corning Incorporated, Corning, NY) used in this study were 40 cm long and 2 cm in inner diameter, with a narrowed bottom and a glass micropore filter fixed near the bottom (Figure 3-1). The depth of aquifer materials filled in each column was approximately 35 cm, corresponding to a volume of about 110 ml. A layer of glass wool was placed both underneath and atop the aquifer material to obtain better hydraulic distribution and minimize the turbulence in the column. The upper opening of the column was sealed by a

rubber stopper, which was penetrated with a stainless steel needle connecting to Teflon tubing. The bottom of the column was connected to glass tubing.

During filling, the aquifer material was added with a spoon through the top of the column while argon-purged landfill leachate was pumped into the column through the bottom at 4 ml/min by a Masterflex tubing pump (Cole-Parmer Instrument Co., Chicago, IL). An argon stream, provided via tubing placed into the top of the column, was maintained in the column head space throughout the process of column filling to help provide an anaerobic atmosphere. To obtain even settling of the aquifer material, sometimes the column slurry was tapped periodically with a plastic rod and bubbled with argon gas for a short time period during filling. Gravel and debris above 5 mm in diameter were excluded manually from the aquifer material. When the desired depth of aquifer materials was obtained, the top layer of glass wool was placed, the column head space was filled with landfill leachate, and the column was sealed with the rubber stopper. Then the column was allowed to stand for at least two days for further settling and stabilization of the aquifer material layer. Columns to be used as abiotic controls, after being filled with aquifer materials, were autoclaved at 248 °F and 15 psi for 3 hours prior to the beginning of routine operation.

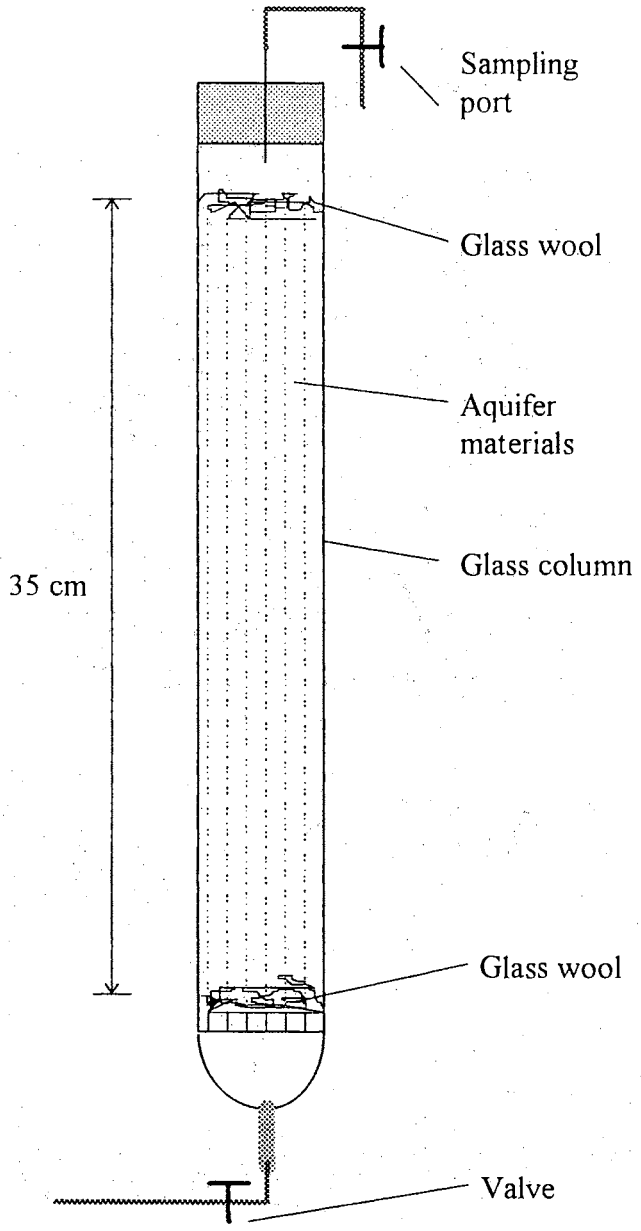


Figure 3-1 Glass column with aquifer materials

During column conditioning and operation, the column reactors were fed in upflow mode by a Harvard 22 syringe pump (Harvard Apparatus, South Natick, MA) equipped with 140 cc Monoject polypropylene plastic syringes (Sherwood Medical, Ireland) as shown in Figure 3-2. It was found that the plastic syringe, when filled with 100 mg/L TNT solution, would adsorb TNT slightly in the first 3 to 4 days, resulting in a decrease in TNT concentration of 5 to 10%. After two to three cycles of refilling the syringe with fresh TNT solution and equilibrating the syringe for 3 days with each refilling, the syringe wall became saturated with TNT and no significant adsorption would be detected. In order to minimize possible adsorption by the tubing, Teflon and glass tubing with 3.2 mm inner diameter were used in the column reactor set-up and the tubing length was minimized, with about 8 inches between the pump and the column inlet and about 2 inches between the column outlet and the sampling port. Because of the rigidity of the Teflon and glass tubings, short sections of silicon tubing were used as connections where necessary. The columns and attached tubing were wrapped with aluminum foil to prevent light penetration. Before operation, the column was conditioned by injecting a medium using the syringe pump at 0.028 ml/min (40 ml/day). The medium used to condition the column was the same as the medium which was to be used in the experiments immediately after conditioning, except that TNT, carbon source, and electron acceptors were omitted from the conditioning medium. Four pore volumes of conditioning medium were pumped through before the routine operation of each column.

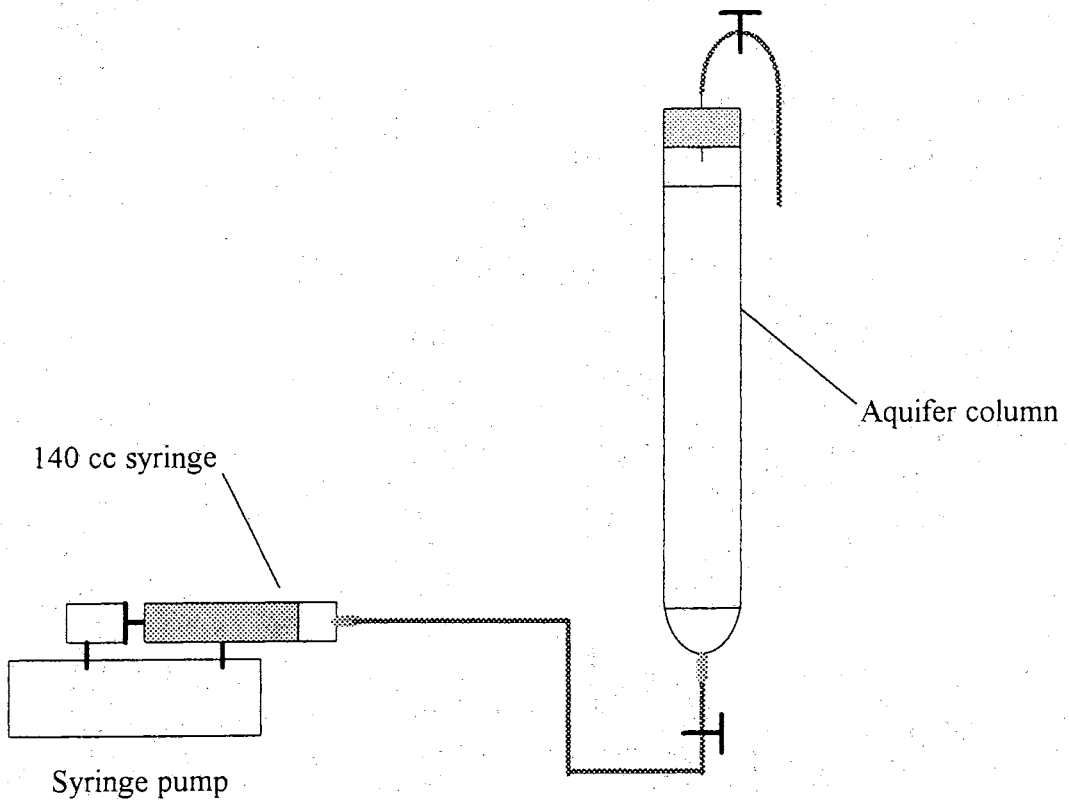


Figure 3-2 Aquifer column and syringe pump set-up

§3.2.4.2 Column Media Table 3-7 illustrates the recipes of various column media. Different levels of TNT, carbon source, and electron acceptor concentrations were used to obtain various combinations of operating conditions in order to test their effects on TNT transformation. Specific concentrations of carbon sources used in each operation period for each column are illustrated in Section 4.4. Based on stoichiometry and preliminary experiments, appropriate electron acceptor concentrations were chosen to ensure that the columns were not electron-acceptor limited. Yeast extract was used to supply micronutrients and organic growth factors and was in most cases kept at a significantly low concentration compared with the concentration of the primary carbon source (acetate or lactate) used in the medium.

Before column medium preparation, 150 mg/L or 120 mg/L aqueous TNT stock solution was made by adding TNT crystals into water, and gently heating (50 ~ 60 °C) and stirring the liquid overnight. To prepare a column medium, appropriate amounts of stock solutions of TNT, the primary substrate and the electron acceptor, stock solution containing yeast extract and phosphate buffer, trace metal solution, and water were mixed together to achieve desired concentrations. The pH of the medium was adjusted using 10% HCl or 1 N NaOH. The medium was then boiled in a flask for about 2 minutes, transferred into 60 ml serum bottles, and purged with argon gas for 20 minutes to remove dissolved oxygen. The serum bottles were then sealed with Teflon-faced rubber septa and crimped with aluminum caps, and autoclaved at 248 °F and 15 psi for 20 minutes. 50 ml of the medium was contained in each 60 ml serum bottle. A biocide, 0.3 g/L sodium azide, was added to the media for abiotic columns in order to inhibit microbial growth

due to possible contamination (Anid et al., 1993). The column medium was transferred from the serum bottle, in which it was sterilized, into an autoclaved 140 cc syringe aseptically and anaerobically.

Table 3-7 Recipes of Media for Aquifer Column Reactors

	Denitrifying	Sulfate-reducing	Methanogenic
TNT (mg/L)	60 or 100	30, 60, or 100	30 or 60
Primary Substrate and Electron Acceptor (mg/L)	Ac^- 30 / NO_3^- 80, Ac^- 90 / NO_3^- 250, Ac^- 180 / NO_3^- 400, or Ac^- 300 / NO_3^- 600	Ac^- 30 / SO_4^{2-} 80, Ac^- 90 / SO_4^{2-} 250, Ac^- 180 / SO_4^{2-} 500, Ac^- 300 / SO_4^{2-} 600, or Lactate 90 / SO_4^{2-} 250	Ac^- 30, Ac^- 90, or Ac^- 180
Yeast Extract (mg/L)	10 or 100	10 or 100	10
Buffer	4 mM Na_2HPO_4 + 4 mM KH_2PO_4	4 mM Na_2HPO_4 + 4 mM KH_2PO_4	4 mM Na_2HPO_4 + 4 mM KH_2PO_4
Trace Metal Sol'n	0.2 ml/100 ml	0.2 ml/100 ml	0.2 ml/100 ml
pH	7.3	6.9	7.0

§3.2.4.3 Tracer Experiments Tracer experiments were conducted to determine the flow characteristics of the aquifer columns. Two columns received a tracer solution, 50 mg Br^-/L, injected using the syringe pump at flow rate of 4.0 ml/min. Samples of the column effluent were taken at intervals of 2 minutes until the column breakthrough was established and the column effluent concentration reached the influent concentration. After the breakthrough of bromide, the column was flushed with TNT-free medium with

a few pore volumes. Then, a TNT solution of 100 mg/L was injected at the same flow rate used for bromide to obtain a TNT breakthrough curve under this condition.

Two other columns, also abiotic, were used for a long-term adsorption and breakthrough experiment at the flow rate of 0.007 ml/min. This flow rate, corresponding to a retention time of 4 days, was used as the routine operating condition for all other columns. One of these two columns was fed with an aqueous solution of 50 mg/L bromide. A sulfate-reducing medium with 100 mg/L TNT, 90 mg/L acetate, and 250 mg/L sulfate (see Table 3-7) was continuously injected into the other abiotic column. The pH of column feeds was 7.0. Therefore, the bromide breakthrough curve obtained here can be used to compare with TNT breakthrough under the same condition, which may reflect the effects of long-term adsorption or retardation of TNT in aquifer materials when the retention time and the column operation period are long.

§3.2.4.4 Column Operation Nine aquifer column reactors were set up and operated at room temperature (22 ± 2 °C) over the course of this study. Two modes of column operation were employed: continuous for some columns and batch-fed for others (Table 3-8). For continuous operation, the column was continuously fed with the medium from the syringe pushed by the syringe pump with a constant flow rate. For batch-fed columns, the column fluids were exchanged at an interval of several days (usually 4 days). When a column's medium was exchanged, fresh medium was injected into the column using syringe pump at the flow rate of 4.0 ml/min to replace the medium in the column. Based on the results of tracer experiments (discussed in Section 4.4.2) on

columns D3 and S3, the fluid in the pore volume in a column could be completely exchanged in about 45 minutes when the exchange flow rate was 4.0 ml/min, corresponding to a required exchange medium volume of 180 ml. According to the tracer study, the first 20 to 30 ml of the column effluent during each exchange accurately represented the old column fluid from the previous exchange and was not contaminated by the fresh column feed. Therefore, this part of the column effluent was collected for analysis, such as for changes in TNT concentrations and other parameters during the period since the previous exchange (Siegrist and McCarty, 1987). The last 20 to 30 ml of the column effluent represented the newly injected feed itself, which had completely replaced the old column fluid and could be considered as the starting conditions of the new period of column reactions.

Table 3-8 Description of Aquifer Column Reactors

Column	Metabolic Regime	Hydraulic Mode
D1	Denitrifying	Continuous
D2	Denitrifying	Batch-fed
D3	Abiotic, nitrate-amended	Continuous
S1	Sulfate-reducing	Continuous
S2	Sulfate-reducing	Batch-fed
S3	Abiotic, sulfate-amended	Continuous
S4	Abiotic, sulfate-amended	Continuous
M	Methanogenic	Batch-fed
B	Abiotic, bromide-amended	Continuous

To investigate TNT transformation under different conditions (TNT concentrations, primary substrate concentrations, etc.), several sets of different operation conditions were employed for each column (see Section 4.4 and Table 3-7). Every time a new set of conditions was introduced to a column, the column was run for at least two "pore volume retention times" (or 8 to 10 days) for the column effluent to reach a steady state before representative samples were taken for this set of conditions.

§3.2.4.5 Desorption of TNT in Columns TNT desorption experiments were conducted on columns D3, S3, and S4 after the TNT concentration in the column effluent reached a steady state. Sterilized media, the same as previously used for these columns except that TNT was omitted, were injected into columns D3 and S3 at a flow rate of 0.007 ml/min (R.T. = 4 days) to examine the long-term desorption of TNT in aquifer materials. For column S4, a desorption experiment was conducted by sequentially extracting the column aquifer materials with acetonitrile for three times. About 80 ml of acetonitrile was used each time, and the three extracts were combined to determine the TNT concentration. This method provided a strong desorbing condition to estimate the amount of TNT recovered in desorption.

§3.2.4.6 Porosity of Aquifer Material Columns Two glass graduated cylinders, used for column porosity measurement, were filled with aquifer materials in the same manner as in setting up aquifer columns. After the cylinders were filled with aquifer materials to the desired volume and the pore space was filled with water (rather than

landfill leachate), the cylinders were allowed to stand for two days to ensure that the aquifer particle surfaces were fully wetted with water. Then the water table in each cylinder was adjusted so that the aquifer materials were fully soaked in the water but with no excess water volume above the upper surface of the aquifer material layer. The cylinders were weighed to obtain the weight of the aquifer material with water filled in pore space. Then the cylinders were emptied, and the aquifer material in each cylinder was collected in a glass beaker and dried at 103 °C for 24 hours to obtain the dry weight. The difference of the weight of wet aquifer materials filled with water and the weight of dried aquifer materials yielded the weight of the water filled in the pore space. This weight, divided by the density of water, gave the volume of the pore space, which in turn gave the value of column porosity.

§3.2.4.7 Sampling Continuous column reactors were sampled every 2 to 8 days, depending on the retention time of the column reactors. The column influent samples were taken from the 140 cc plastic syringe feeding the column. When samples were taken for the effluents of continuous columns, a small glass vial was attached to the sample port on the tubing connected to the top of the column (Figure 3-1). 1.5 to 2 ml of sample was collected each time. For batch-fed columns, samples of column influents and effluents were taken when the column fluid was exchanged with fresh medium, as described earlier.

§3.3 Analytical Methods

3.3.1 Sample Treatment

All samples were filtered using a Gelman Syringe Type Filter Holder assembly with a 0.2 μm pore size, 25 mm diameter Supor-200 membrane filter. The first few drops (about 0.5 ml) of the filtrate were discarded and the remaining filtrate was collected. Samples were diluted with Milli-Q water so that the concentrations of chemicals to be analyzed would fall within the range of calibration, and kept frozen until analysis was performed. However, samples from abiotic batch reactors with bisulfide were analyzed immediately after sampling because of the instability of the reaction mixture.

§3.3.2 TNT and Its Transformation Intermediates

TNT and its transformation intermediates, including 2-ADNT and 4-ADNT, were separated and analyzed by high performance liquid chromatography (HPLC) with a Beckman liquid chromatograph (Beckman Instruments, Inc., Fullerton, CA) equipped with two model 127s solvent pumps, a model 166 absorbance detector, and a Beckman C18 reversed phase column (5 μm particle diameter, 4.6 mm x 25 cm). Aliquots of 20 μL were injected onto the reversed phase column. Quantification was achieved with a Hewlett Packard 3396II integrator based on the peak response factor (peak area). The separated peaks were identified based upon retention times matching with those of TNT

and ADNT standards. The isocratic HPLC method for the analysis of each compound is described as follows:

(1) 2-ADNT, 4-ADNT, and TNT

Mobile phase: 45% methanol/55% 10 mM phosphate buffer (pH = 5.0) (v/v); flow rate: 1.5 ml/min; and wavelength: 254 nm.

(2) TAT

Mobile phase: 8% methanol/92% 10 mM phosphate buffer (pH = 6.5) (v/v); flow rate: 1.5 ml/min; and wavelength: 230 nm.

Calibrations curves of TNT were obtained from the HPLC peak areas of a TNT standard series, which consisted of an initial TNT standard solution and its further dilutions. At least four concentration levels of standard solutions, usually 5, 10 15 and 20 mg/L, were used for a calibration curve. 2-ADNT and 4-ADNT calibration curves were obtained with the same method except that the initial standards of ADNTs were acetonitrile-dissolved liquid rather than crystals. These curves were updated every three to six months and always had an R-squared value greater than 0.99 in linear regression, indicating very good linearity in this concentration range. In routine measurement of samples, one standard (e.g. 10 mg/L) was run twice along with each set of samples being analyzed. The peak area of this standard was used in a correction factor which accounted for possible fluctuations in the HPLC performance and peak responses among different runs, as described in the following formula:

Sample Conc. = [(Peak area)/(Slope of calibration curve)](Correction factor)

$$\text{Correction factor} = \frac{\text{(Peak area of 10 mg/L std. when calibration curve was made)}}{\text{(Peak area of 10 mg/L std. when samples were measured)}}$$

This correction procedure proved to be reasonable and relatively simple. Only one standard was used for the correction in routine analysis because it was already ensured that the calibration curve had a good linearity in the concentration range of interest. Since resolution of 2-ADNT and 4-ADNT isomers could not be well achieved under the HPLC operation conditions used in this study, the observed ADNT peak in the reactor samples was actually the combination of both isomers and expressed as 2-ADNT equivalent.

Similar procedures were followed in the diode-array HPLC analysis, which is further described in Appendix F.

§3.3.3 Sulfate, Nitrate, Nitrite, Acetate, Lactate and Bromide

These anions were measured with a Dionex (series 2000i/sp) ion exchange chromatograph equipped with an IonPac AS4A-SC 4 mm analytical column. Nitrogen gas was used to pressurize the ion chromatograph system. The eluent consisted of 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$ under N_2 gas pressure of 5 psi, and the flow rate was maintained at 2.0 ml/min. A 25 mM H_2SO_4 solution under pressure of 10 psi was used as column regenerant with a flow rate of 5 ml/min. The peak areas representing individual anions were integrated on a Hewlett Packard 3380A integrator. Gravimetrically prepared standard solutions of sodium sulfate, potassium nitrate, sodium nitrite, sodium acetate, sodium lactate and sodium bromide with known concentrations were used for calibration of each anion, respectively. The calibration and correction procedures were similar to

those used for TNT analysis discussed earlier. When analyzed, 0.4 ml sample was injected into the instrument with a 1-ml glass syringe.

§3.3.4 Sulfide

Total sulfide was measured in samples from abiotic batch reactors added with sodium sulfide using the iodometric method described in Standard Methods (APHA et al., 1985), Section 427D. In this method, excess iodine is added to the sample to react with sulfide and the remaining iodine is back-titrated with sodium thiosulfate.

§3.3.5 Methane

Methane produced in methanogenic aquifer column and batch bottle reactors was measured with either of the two instruments described below.

(1) Gow Mac model 350 gas chromatograph (GC) with thermal conductivity detector (TCD). This chromatograph was fitted with a 6-foot stainless steel column (I.D.=1/4 in.) packed with Porapak Q, 60/80 mesh. Helium was used as carrier gas at a flow rate of 60 ml/min. The column temperature was maintained at 55 °C, with the detector temperature of 170 °C and the injection port temperature of 105 °C. The bridge current of the TCD was 70 mA and the attenuation was adjusted to full scale. A Hewlett Packard model 3380A integrator was connected to this GC.

(2) HP GC model 5890II equipped with a Carbopack C column (60/80 mesh, 30% Carbowax) and a flame ionization detector (FID). Helium was used as carrier gas

and the instrument was operated at oven temperature of 55 °C, detector temperature of 250 °C, and injection temperature of 200 °C. The integrator used was an HP 3396II.

The first GC method has the advantage that it can detect not only methane but also CO_2, giving better information about methanogenesis. However, the sensitivity of methane detection of this instrument was relatively low, with a lower limit of detection of about 1~2% methane. The second method was used when the first one was not able to detect methane in samples.

Methane produced in batch bottles was measured by sampling the head space of the bottles. Methane produced in the methanogenic aquifer column was determined using the method of head space analysis (Heijman et al., 1995; Siegrist and McCarty, 1987). Samples of the column effluent containing dissolved methane was carefully collected in a 7-ml glass vial, closed with a rubber stopper and vacuumed slightly with a syringe. Shaking the bottle for 1 to 2 minutes at room temperature would equilibrate the methane between the gaseous and the liquid phases. Samples of the head space in the vial was then injected into the GC.

§3.3.6 Volatile Solids

The volatile solids content of the aquifer material was measured by igniting the samples at 550 °C, as described in Standard Methods (APHA et al., 1985), Section 209D. This analysis was used to estimate the organic matter content in the aquifer material. Before ignition, the samples were dried at 103 °C for two hours. Sample size for volatile solids measurements was between 10 to 30 grams.

§3.3.7 Biomass

Biomass concentrations in batch reactors were approximated by volatile suspended solids content, which was measured using the procedures in Standard Methods (APHA et al., 1985), Sections 209C and 209D. Sample size was between 20.0 ml to 80.0 ml.

§3.3.8 pH

A glass combination electrode in conjunction with an Accumet model 900 pH meter (Fisher Scientific Co.) was used for pH measurement. Standard buffer solutions (HACH Co.) with pH values of 4.0, 7.0, and 10.0 were used to calibrate the meter before sample determination.

CHAPTER IV

RESULTS AND DISCUSSION

This chapter presents the results and discussion of the adsorption/desorption experiments, abiotic reactions of TNT with bisulfide, biological batch reactor experiments, and aquifer column reactor experiemnts, followed by comparisons for results of TNT biotransformation under different conditions.

§4.1 Adsorption/desorption of TNT and Related Aminotoluenes

§4.1.1 Kinetics of Batch Adsorption

Figures 4-1 and 4-2 show that the adsorption process of 2-ADNT and 4-ADNT reached steady state in about 4 hours under the soil-to-solution ratios used in this study. No significant changes in solution concentrations occurred between 4 and 24 hours when the initial concentration was about 30 mg/L. Therefore, the time period required to reach steady state should be equal to or less than 4 hours if the initial concentrations are equal to or lower than 30 mg/L (Pennington and Patrick, 1990), as are those employed in the isotherm tests. TAT concentration did not reach a steady state but decreased to zero in 24 hours, probably due to chemical reactions (Figure 4-3) as discussed in Section 4.1.3.

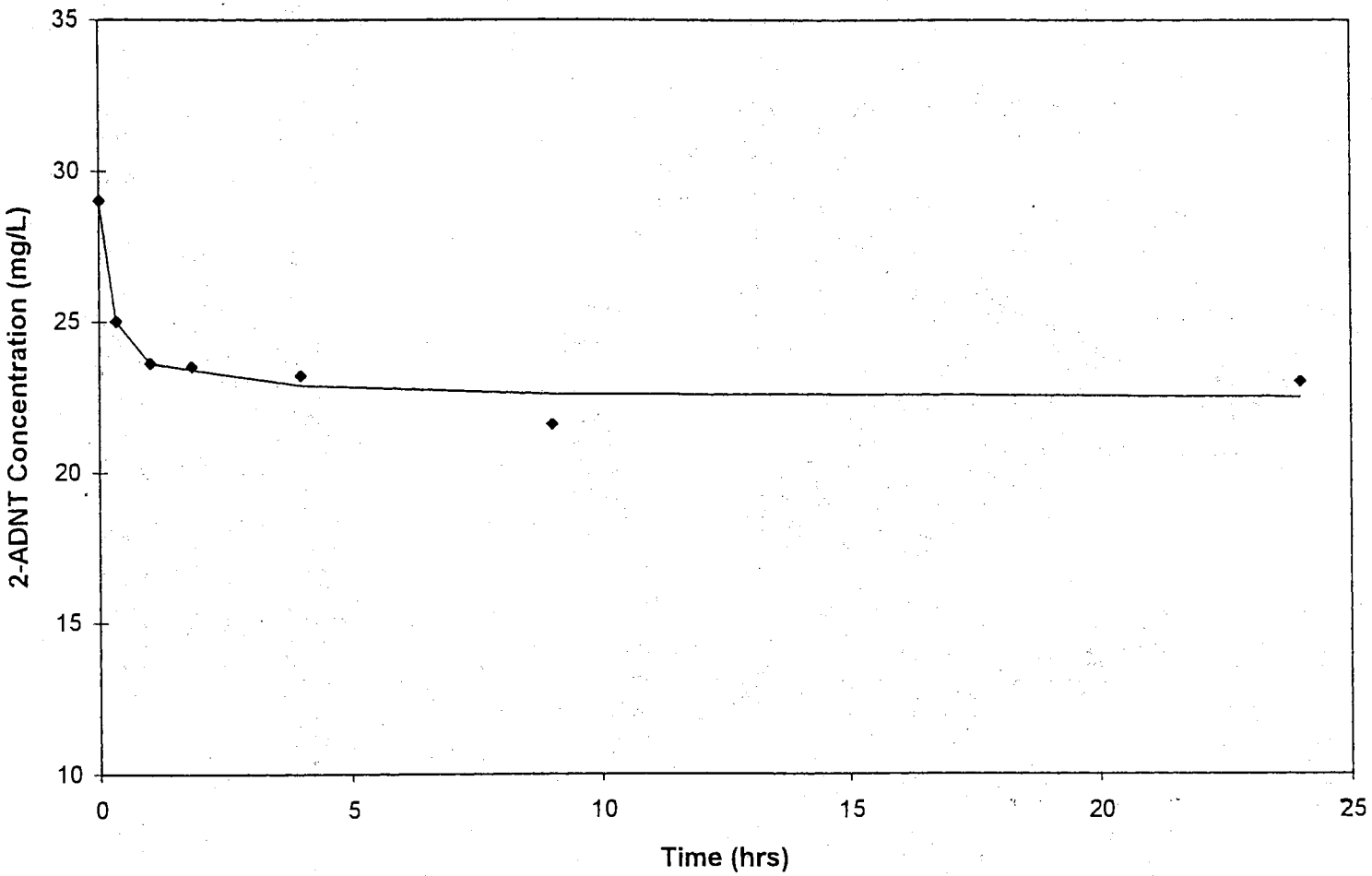


Figure 4-1 Adsorption kinetics of 2-amino-4,6-dinitrotoluene

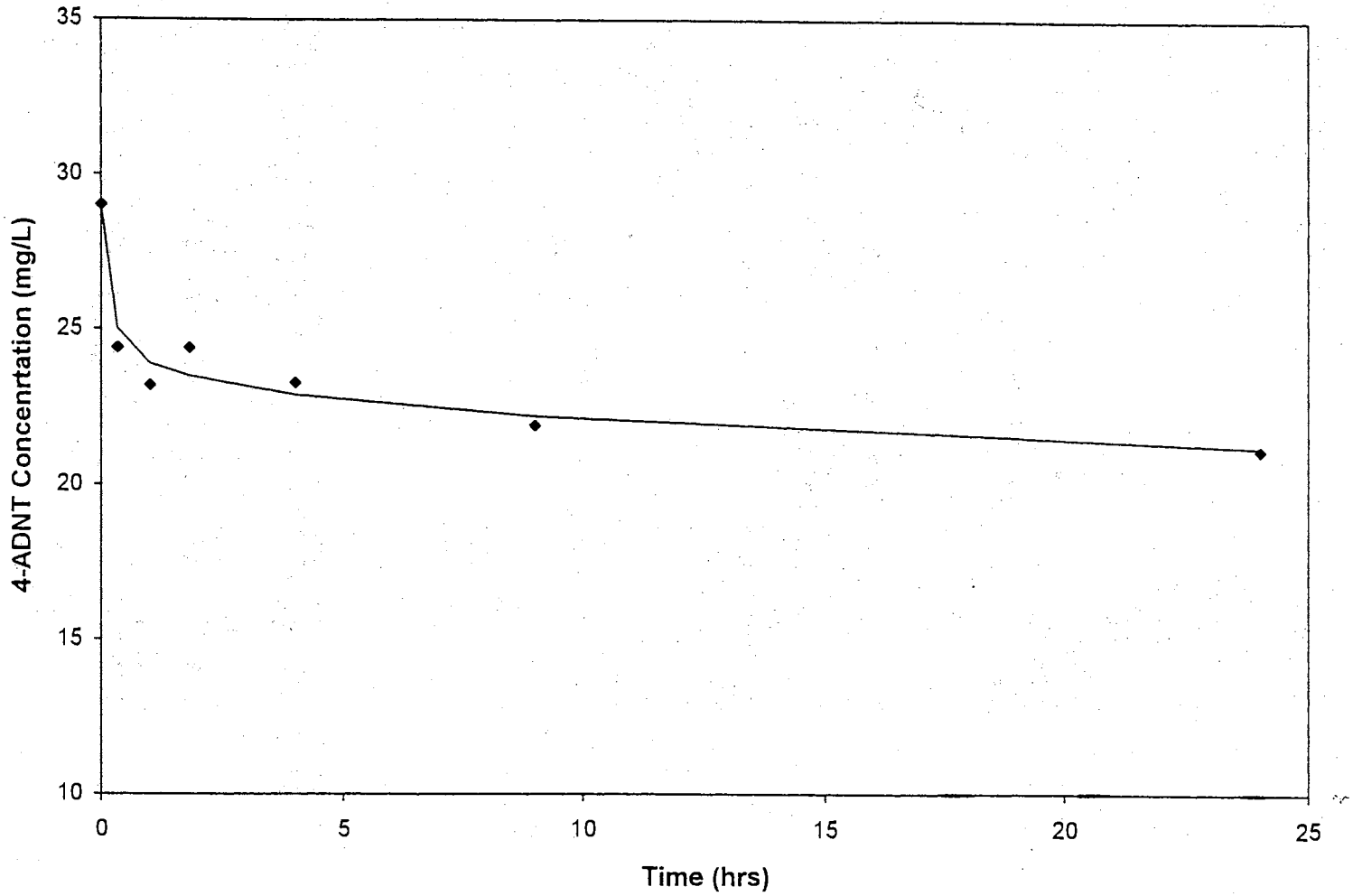


Figure 4-2 Adsorption kinetics of 4-amino-2,6-dinitrotoluene

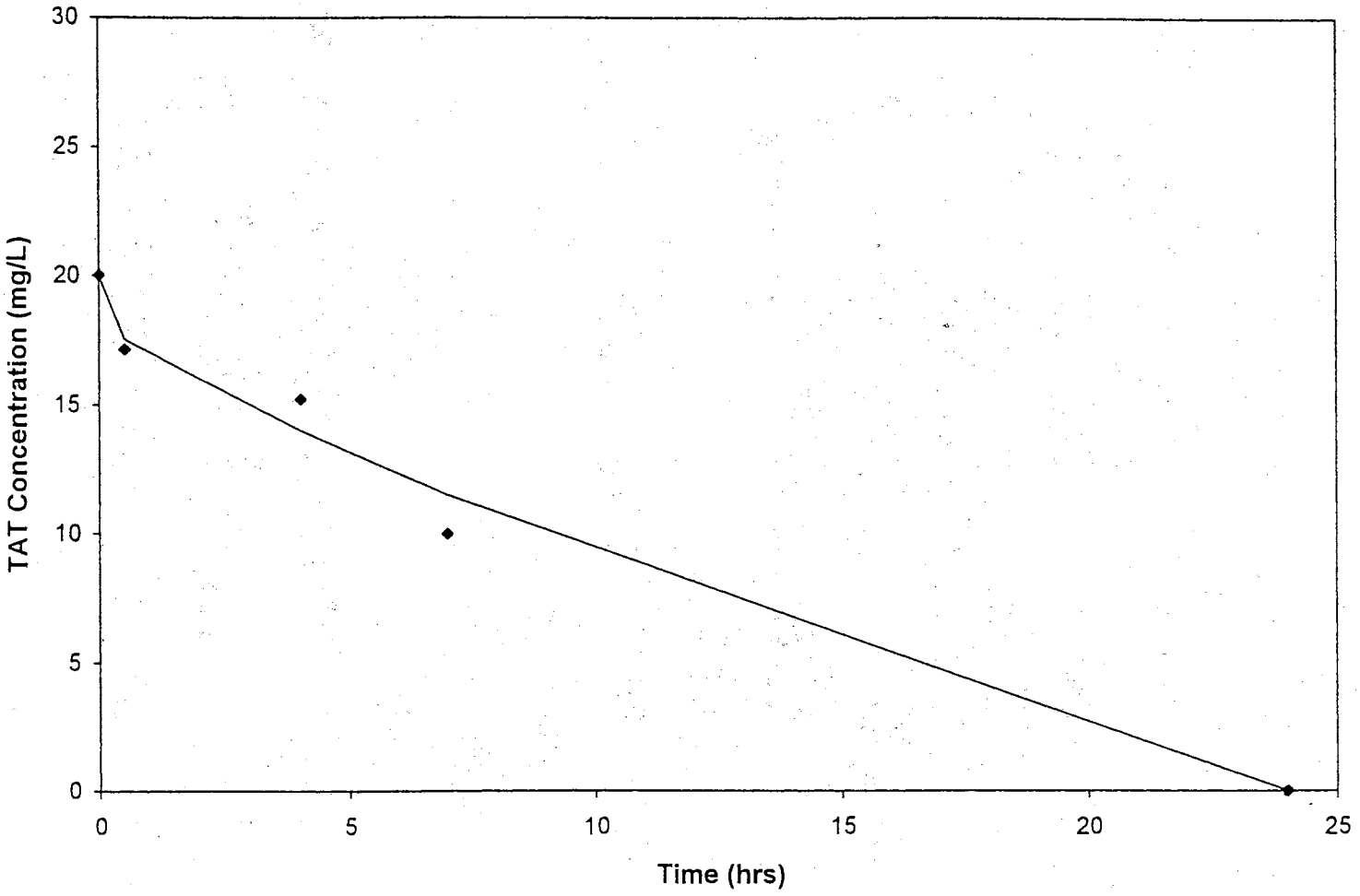


Figure 4-3 Adsorption kinetics of 2,4,6-triaminotoluene

§4.1.2 Isotherms of Batch Adsorption

The amounts of adsorbed mass were calculated based on the solution volumes and the decreases in solution concentrations. Adsorption data were fit to the linearized forms of the Langmuir and Freundlich models as given below (Pennington and Patrick, 1990) .

$$\text{Langmuir:} \quad 1/q = (1/Q) + (1/bQ)(1/C) \tag{4.1}$$

$$\text{Freundlich:} \quad \ln(q) = \ln(K) + (1/n)\ln(C) \tag{4.2}$$

where q is the solid phase concentration of the tested compound (μg/g); C is the equilibrium solution concentration (mg/L); Q is the monolayer sorption capacity (μg/g); b is the Langmuir constant related to entropy; K is the Freundlich adsorption coefficient; and n is the Freundlich characteristic constant. The results of the regression calculations are listed in Table 4-1. The linearized Langmuir isotherm curves for the tested compounds in Table 4-1 are shown in Figures 4-4 through 4-7.

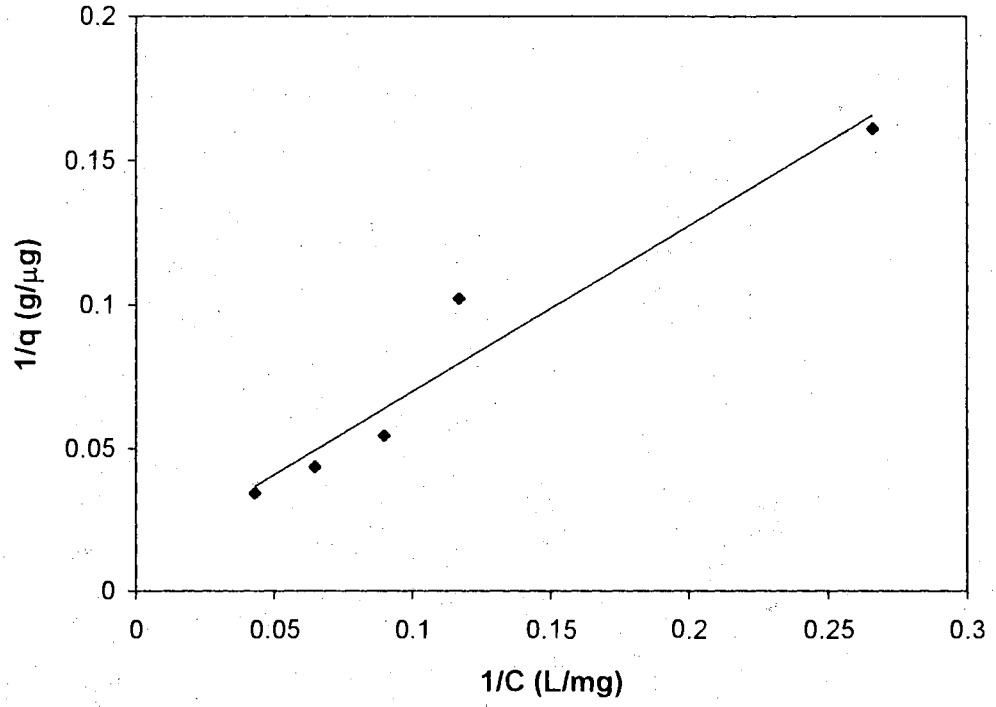


Figure 4-4 Linearized Langmuir isotherm for 2-ADNT

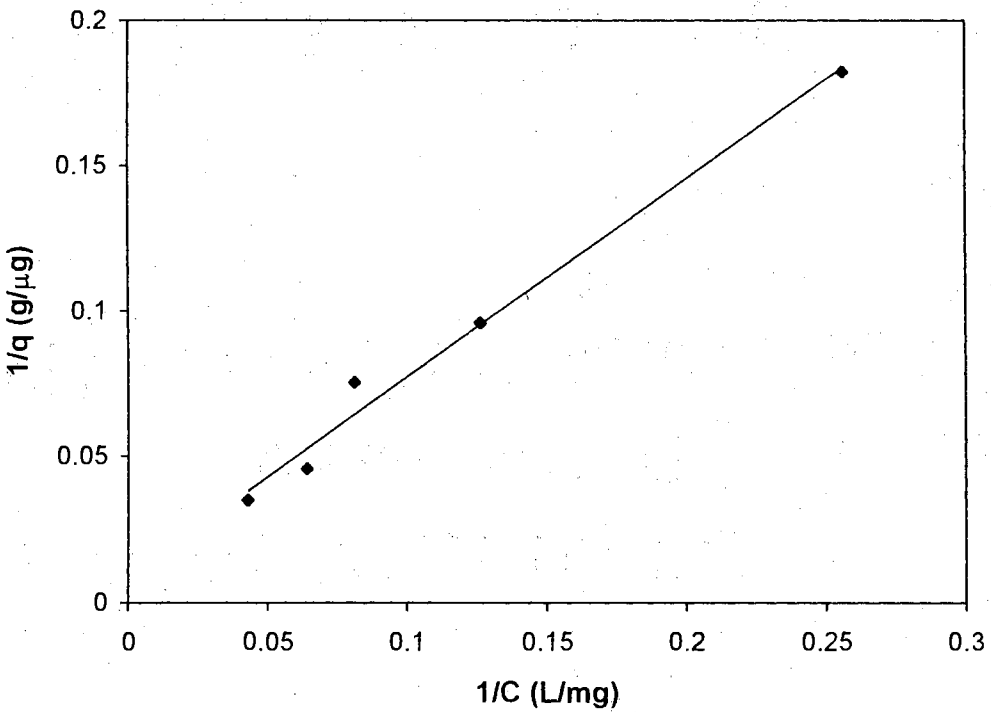


Figure 4-5 Linearized Langmuir isotherm for 4-ADNT

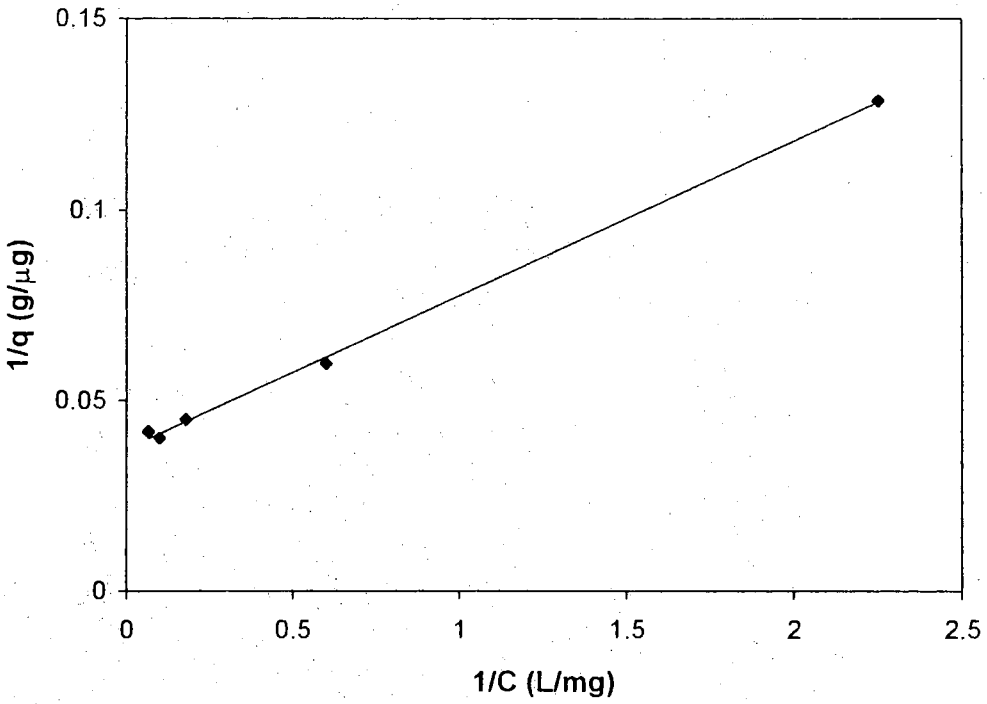


Figure 4-6 Linearized Langmuir isotherm for TAT

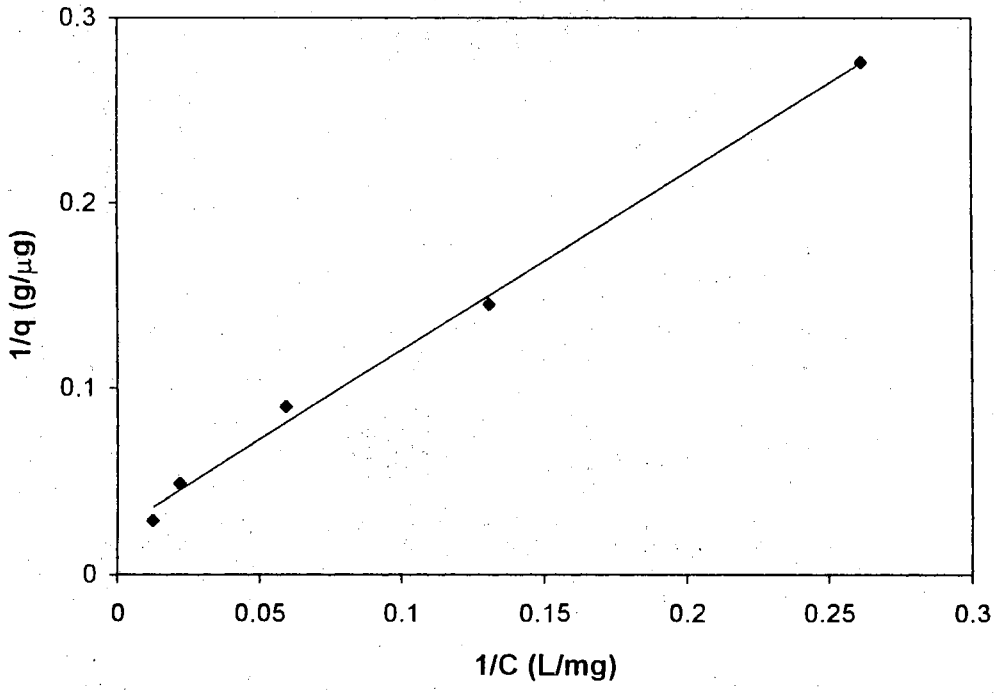


Figure 4-7 Linearized Langmuir isotherm for TNT

Table 4-1 Regression Results of Isotherm Experiments

Compound	Langmuir			Freundlich		
	R square	Q (μg/g)	b	R square	K	n
2-ADNT	0.941	84	0.020	0.943	1.8	1.1
4-ADNT	0.987	112	0.013	0.975	1.5	1.1
TAT	0.999	27	0.93	0.902	12	3.1
TNT	0.996	41	0.026	0.993	1.5	1.4

For all the four chemicals, both the Langmuir model and the Freundlich model seemed to be accurate for description of the adsorption of the compounds in this experiment, according to the R-squared values (square of error between the experimental data point and the fitting curve) in Table 4-1. The adsorption behaviors of 2-ADNT and 4-ADNT were similar to each other and both compounds were significantly more sorptive than TNT, as suggested by comparison of Langmuir Q values. Isotherm results for TAT are questionable due to the lack of steady state kinetic data for this compound, which is further discussed below.

§4.1.3 Extraction in Batch Experiment

The extraction recovery was calculated on the basis of the following formula:

$$\text{Recovery} = \frac{\text{Desorbed mass}}{\text{Adsorbed mass}} = \frac{\text{(Conc. of extract)(Vol. of extract)}}{\text{(Decrease in sol'n conc.)(Vol. of sol'n)}} \qquad (4.3)$$

The extraction recovery of each compound is shown in Table 4-2. The high percentages of the recovery of 2-ADNT, 4-ADNT, and TNT confirmed the physical adsorption of these compounds, although minor losses could occur due to chemical reactions. This might account for slight concentration decreases which may be observed between 4 and 24 hours in the kinetics curves (Figures 4-1 and 4-2). However, the TAT concentration changes appear to be mostly due to chemical mechanisms rather than adsorption, as several (3 or 4) unknown HPLC peaks were detected in the TAT samples immediately after the 2-hour equilibrating process in the isotherm test. Presumably, these peaks represented transformation products of TAT. The TAT disappearance in the kinetics test is another indication of chemical conversion. The zero recovery of TAT after desorption also implied that this chemical had been converted to unknown products before or during the desorption treatment. Therefore, the TAT parameters presented in Table 4-1 could be merely viewed as "apparent" parameter equivalents for a "pseudo-adsorption" process. It has been reported that TAT is very unstable and subject to rapid chemical conversion in the presence of oxygen and/or trace elements, which are very likely to occur in aquifer materials (Preuss et al., 1993).

Table 4-2 Extraction Recovery Following Isotherms

2-ADNT	99%
4-ADNT	75%
TAT	0%
TNT	87%

§4.1.4 Column Breakthrough Curves at Low Flow Rate

Two abiotic columns, B and S4 (see Table 3-8), were used for a long-term adsorption and breakthrough experiment at a relatively low flow rate, 0.007 ml/min, the flow rate at which all other continuous columns were operated. The pore space retention time was 4 days at this flow rate since the pore volume in a column was 40.3 ml, as calculated in Section 4.4.1. Column B was fed an aqueous solution containing 50 mg/L bromide. A sulfate-reducing medium with 100 mg/L TNT was amended with 0.3 g/L sodium azide and continuously injected into column S4. The pH of both column feeds was 7.0.

Figure 4-8 presents the breakthrough curves of both bromide and TNT under the long-term conditions. The TNT breakthrough curve at this slow flow rate (different from that at high flow rate as discussed in Section 4.4.2) lagged significantly behind the tracer breakthrough curve, indicating significant adsorption/retardation and abiotic transformation at longer retention time. Dunnivant and co-workers (1992a), when investigating the long-term adsorption of dissolved organic carbon (DOC) in aquifer columns, also observed the extensive tailing (retardation) of the breakthrough curves and believed that this phenomenon was attributed primarily to the slow adsorption kinetics of DOC to the aquifer material and the nonlinear nature of the adsorption isotherms. Jardine et al. (1992) found that the initial adsorption rate coefficient obtained from batch reactors was significantly larger than that observed for aquifer column displacement experiments. It was indicated that the extended tailing of the observed DOC breakthrough curves was mainly influenced by the slow, time-dependent adsorption of DOC during transport. This

appears true for the TNT breakthrough curves observed in this study. Since the long-term adsorption in aquifer columns, without vigorous hydraulic agitation as in short-term batch reactors, is slow and time-dependent, it is understandable that longer retention times resulted in more significant adsorption of TNT and lagging of the breakthrough curve. Abiotic transformation may also be quite considerable here because longer retention time meant more sorbed-phase TNT available to react with various reactants in aquifer materials.

A calculation of TNT mass balance can be performed on the basis of the breakthrough curves in Figure 4-8, as illustrated below.

$$M_L = M_{in} - M_{out} - M_{ac} \qquad (4.4)$$

where M_L is the TNT mass loss due to physical adsorption and chemical (abiotic) transformation (biological transformation is believed negligible because the column was maintained under sterilized conditions); M_{in} is the TNT mass injected into the column; M_{out} is the TNT mass exiting the column; and M_{ac} is the TNT mass accumulated in the aqueous phase stored in the column pore space (and the column "head space" and other related space, e.g. tubing and the column bottom space, etc.).

According to the breakthrough curves, we have

$M_{in} - M_{ac}$ = (Area under bromide breakthrough curve)(100 mg/L)(0.01008 L/day)

M_{out} = (Area under TNT breakthrough curve)(100 mg/L)(0.01008 L/day)

Assuming that the adsorption process reached saturation by day 75 (based on Figure 4-8), then M_L can be calculated by integrating the areas under these two breakthrough curves. The detailed calculation is presented in Appendix B. It is found that M_L equals **13.6 mg**.

Since M_{in} is known and equal to 75.6 mg in the first 75 days, M_L accounts for 18% of the TNT mass injected into the column.

According to the results of desorption recovery for column S4 (discussed further in Section 4.4.6 and Appendix B), 5.8 mg TNT was recovered from the column aquifer material after the adsorption was finished. The unaccounted-for TNT was therefore 7.8 mg, which was about 10% of M_{in} and might have undergone abiotic transformations and/or been irreversibly adsorbed on the aquifer material. Small amounts of ADNTs (about 1 mg/L) were detected in the desorption extract, an indication of the occurrence of abiotic transformations. Since column S4 was also fed sodium azide, reductive microbial activity (such as sulfate reduction) was absent and the redox condition in the column was likely to be one of "suboxic" or microaerobic conditions (as indicated by the pink color of the redox indicator, resazurin). Under this type of redox condition, Haderlein and Schwarzenbach (1995) reported that anilines, produced from transformations of nitroaromatic compounds in aquifers, tended to react with natural organic matter, clay minerals, and iron and manganese oxides in aquifer materials to form "bound residues" that were difficult to recover and analyze. The authors indicated that this binding process was mostly irreversible and played an important role in the long-term fate of nitroaromatics and aromatic amines.

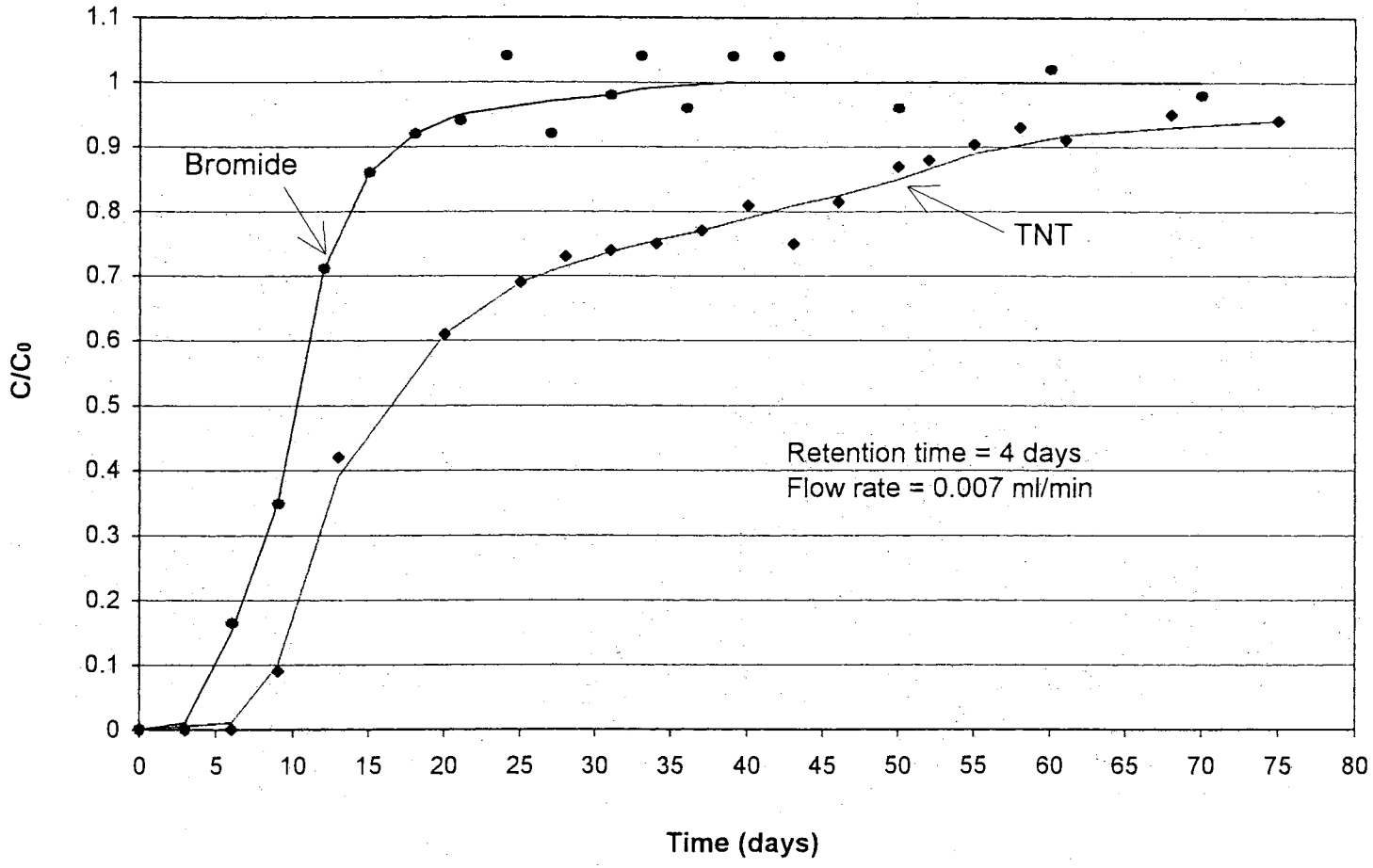


Figure 4-8 Column breakthrough curves at low flow rate

Since the mass of the aquifer material in the column was 205.5 g (dry weight), the physically, reversibly adsorbed TNT on the aquifer materials was about 5.8 g/205.5 g = **28 μg/g**. Notice that the maximum TNT adsorption capacity of the aquifer materials was 41 μg/g as determined in batch adsorption experiments (Table 4-1). Given the amount of data available, it is difficult to determine if the difference between these two numbers represents a statistically significant difference between the maximum TNT adsorption capacity of the aquifer materials in column and in batch reactors. There were no duplicated column data for a statistical analysis to confirm the significance of the difference. As indicated earlier in this section, however, aquifer column conditions do impose influence that makes the initial adsorption rate coefficient significantly lower than that found in batch reactors (Dunnivant et al., 1992a; Jardine et al., 1992).

In many cases, the long-term adsorption rate is described by a first-order rate equation (Chen and McTernan, 1992),

$$(dq)/(dt) = r(q^* - q)\,, \tag{4.5}$$

where q is the actual solid-phase concentration of the compound of interest at time t, and the equilibrated solid-phase concentration, q*, is described by the Langmuir or Freundlich isotherm in most situations, as indicated earlier. Therefore, the procedure described below can be used to estimate the observed first-order adsorption mass transfer rate coefficient, r, in aquifer columns.

$$\text{Since} \quad r = \frac{(dq/dt)}{(q^* - q)} \approx \frac{(\Delta q/\Delta t)}{(q^* - q)} \tag{4.6}$$

r can be determined on the basis of long-term breakthrough curves shown in Figure 4-9. Curve 1 is the breakthrough curve of an inert tracer (e.g. bromide) obtained under the flow rate of interest, and curve 2, under the same flow rate, is the TNT breakthrough curve which lags behind the tracer curve due to adsorption. At any time point t, there is a corresponding V which is the cumulative volume of TNT solution injected into or discharged from the column during time t. From these curves, $\Delta t = \Delta V/F$ (F is flow rate), Δq = (area II)/(mass of aquifer material), and q = (area I)/(mass of aquifer material). If the Langmuir isotherm holds, then $q^* = QbC/(1 + bC)$ where C is the average concentration of TNT in the column at time t. The value of C can be approximated as $C \approx (C_0 + C_t)/2$ where C_t is the TNT concentration in the column effluent at time t. Alternatively, C could be estimated by a logarithmic average because the TNT concentration may not be linearly distributed along the length of the column. Using this method and assuming that the ratio of the physically adsorbed TNT to the total TNT loss was 0.43 (i.e. 5.8 g/13.6 g as mentioned earlier), we obtained that the adsorption mass transfer rate coefficient, r, was **0.04 day^{-1}** under the conditions in this experiment (Appendix B). This parameter will be useful in developing a mathematical model describing the environmental fate of TNT, as discussed in Section 5.2. It should be noted that the adsorption mass transfer rate coefficient determined by this method is the average rate coefficient in the entire mass transfer (non-saturated) zone. Actually, the local mass transfer rate will vary along the length of the aquifer column and depends on the the solid and aqueous phase TNT concentrations at a given position in the column (Geankoplis, 1993).

In this experiment, TNT loss due to biological transformation was inhibited by adding a biocide, sodium azide, into the column feed. However, chemical/abiotic reactions in aquifer materials did occur and consume some of the TNT in the column feed. These reactions will be discussed further below.

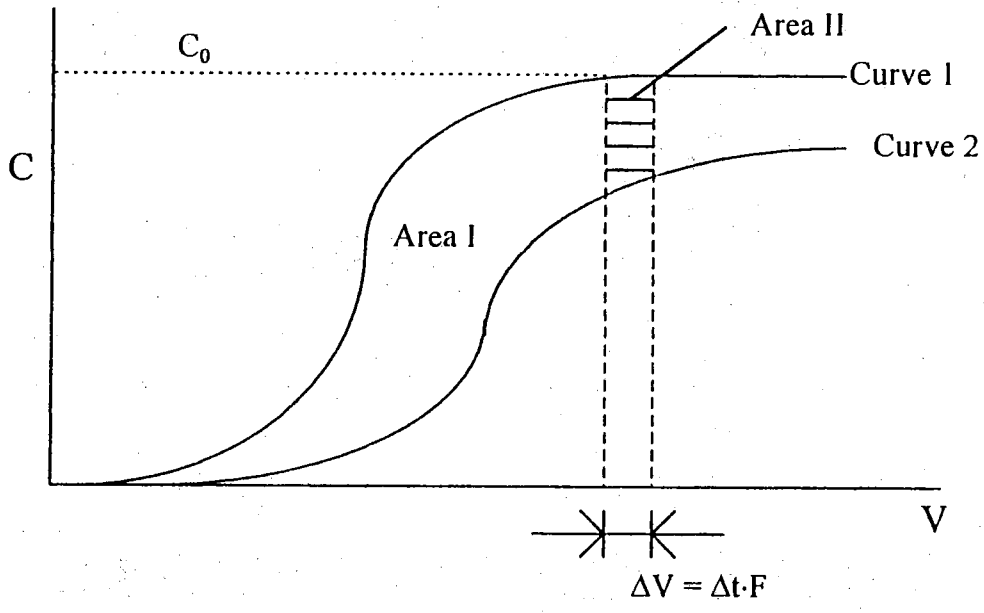


Figure 4-9 Determination of adsorption mass transfer coefficient

§4.2 Abiotic Reactions of TNT with Bisulfide

The batch serum bottle experiments presented here were conducted to examine the abiotic reactions of TNT with bisulfide, taking into account the effects of bisulfide concentrations, the presence of aquifer materials, and pH buffer conditions. These experiments were essentially performed to serve as abiotic controls for the study of microbial transformation of TNT under anaerobic conditions because (1) significant amount of bisulfide can be produced under sulfate-reducing conditions, and (2) sulfide was often used as an oxygen scavenger for anaerobic microcosms in many cases. Therefore, the conditions (pH, concentrations of TNT, sulfide, and buffer) in the experiments were chosen in such a way that they were rendered similar to those used in the microbial TNT transformation experiments.

§4.2.1 Lag Phase in Reaction Kinetics

Under most conditions examined in this study, an initial lag phase was observed in TNT transformation (Figures 4-10, 4-11, 4-14, and 4-16). This slow-reaction phase may last 0.5 to 2 hours, followed by significantly faster TNT transformations. Glaus et al. (1992) also reported this phenomenon when they investigated the abiotic reactions of various substituted nitrobenzenes with bisulfide mediated by *Streptomyces* sp. exudates. They found that no lag phase was observed when the reaction solution was re-spiked with the nitro compound after complete reduction of the initially added reactant. The same was true for TNT in this study. This phenomenon of accelerated reactions following a

slow initial phase might be explained by one or more of the following phenomena: (1) residual dissolved oxygen in the reaction system has to be consumed by bisulfide before TNT reactions become significant; (2) surfaces of particulate substances such as elementary sulfur, which might be produced in the reaction, catalyze the TNT/bisulfide reactions; and (3) accumulated TNT transformation products/intermediates catalyze the reactions.

The first suggested phenomenon, which meant the O_2/bisulfide reaction was favored over TNT/bisulfide reaction, does not seem very likely because the TNT concentration in the reaction system was about 30 mg/L (0.132 mM) while the residual DO was estimated to be lower than 1 mg/L (0.031 mM, as indicated by the pink color of resazurin). Furthermore, in the experiments by Glaus et al. (1992), the bisulfide stock solution was added into the reactor before adding the nitro compounds. Therefore, the residual DO, if any, should have mostly been depleted before the nitro compound was added. However, they still observed the lag phenomenon for some compounds. The second proposed phenomenon is not likely to be a major explanation either. In most reactors, no precipitate formation or cloudiness was observed, indicating no significant formation of sulfur particles. Also, the addition of aquifer materials, which provided large amounts of surface areas, did not eliminate the lag phase, although this stage was shortened and the over-all reaction rate was increased (discussed below). Therefore, the third process, catalysis by TNT intermediates, is more likely to be a reasonable explanation of this observation, considering the fact that the lag phase was not observed when TNT was re-spiked.

§4.2.2 Effects of Bisulfide Concentrations on Reaction Rates

§4.2.2.1 Reaction Rates in Presence of Phosphate Buffer Two levels of total sulfide concentrations (30 and 50 mg/L) were used to examine the effects of sulfide concentrations on TNT reaction rates. These concentrations of sulfide were of interest because they were encountered in other sulfate-reducing reactors tested in this study. Because of the apparent existence of the initial lag phase in the reactions, the reaction kinetics were determined by two stages: (1) the duration and the reaction rate in the lag phase, and (2) the final reaction rate after the lag phase. Figure 4-10 shows the TNT concentration change over time when different total sulfide concentrations were applied. Because sulfide is a relatively strong base, the pH value of the reaction system increased from 7.0 to 8.4 ~ 9.5 (depending on the sulfide concentration) immediately after the sulfide stock solution was added, although 4 mM phosphate buffer was present.

Since it is known that the ionization constants for H_2S are $pK_{a1} = 7.0$ and $pK_{a2} = 13.9$ (Morel, 1983), the speciation of sulfide can be calculated on the basis of the pH value and the initial total sulfide concentration (see Appendix C). According to the calculation, the concentration of bisulfide species, $[HS^-]$, is 96 to 100% of the total sulfide concentration when the pH is in the range of 8.4 ~ 12.5. Therefore, almost all the effective species that reacted with TNT was HS^- and the concentration of this species was essentially constant within the pH range tested.

Based on observations of the data, it was found that the initial lag phase ended when the TNT concentration decreased to approximately 2/3 the initial concentration (as observed for most reactors). The rate of TNT removal after the lag phase could be expressed by a pseudo-first-order model defined as follows:

$$\text{Rate} = d[TNT]/dt = -k_{obs}[TNT] \tag{4.7}$$

Thus,

$$\ln(C/C_0) = -k_{obs} \cdot t \tag{4.8}$$

where C is the TNT concentration at time t, k_{obs} is the observed pseudo-first-order reaction rate constant, and C_0 is the initial TNT concentration.

In this experiment, k_{obs} equaled 1.70 hr^{-1} when the initial total sulfide was 30 mg/L and 1.73 hr^{-1} when the initial total sulfide was 50 mg/L. The difference between these two k_{obs} values is not considered significant. However, the duration of the initial lag phase was significantly affected by the sulfide concentration, as illustrated in Figures 4-10 and 4-11. If we assume that the lag phase ended when the TNT concentration was decreased to 20 mg/L, then the lag phase was about 2 hours when the initial sulfide concentration was 30 mg/L and about 1.2 hours when the initial sulfide concentration was 50 mg/L.

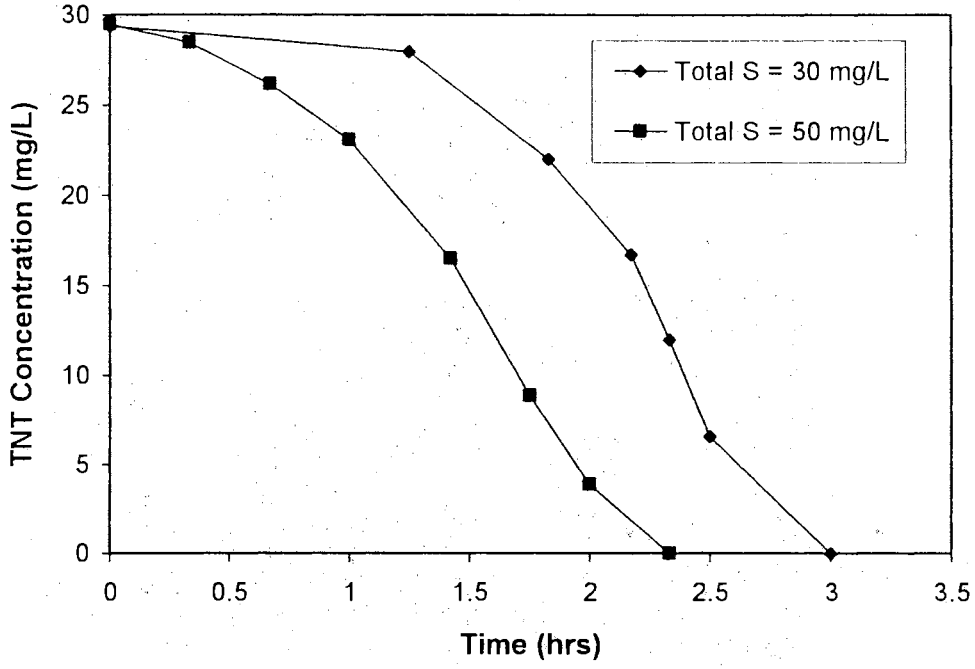


Figure 4-10 Effects of sulfide concentration on TNT transformation (4 mM phosphate, no aquifer material)

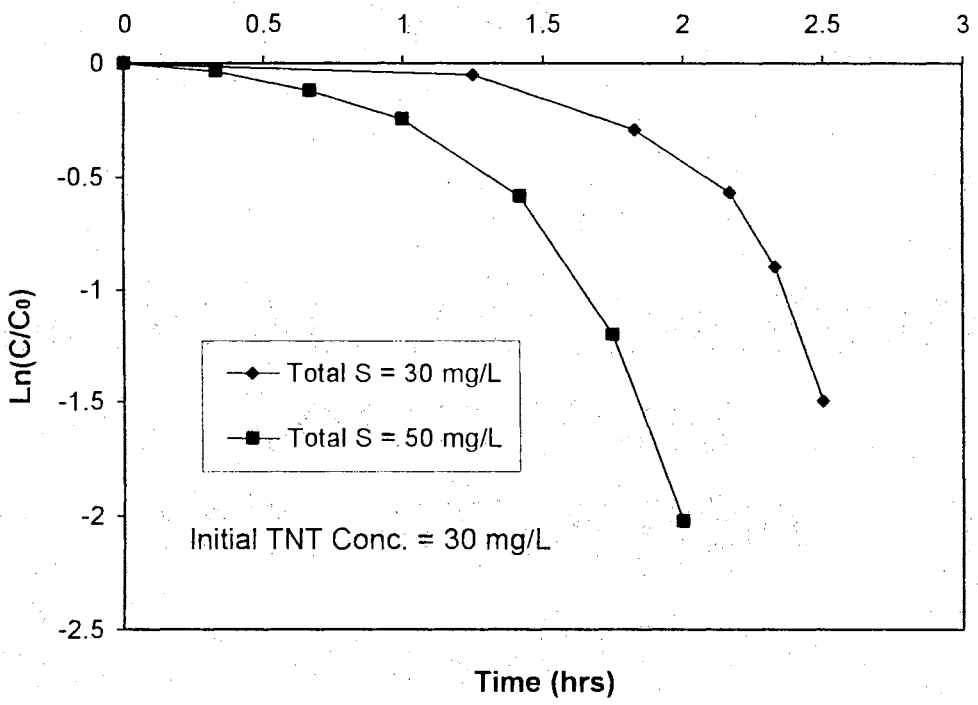


Figure 4-11 Lag phase in TNT abiotic reactions

§4.2.2.2 Reaction Rates in Absence of pH Buffer The same approach as discussed above can be applied to the experimental results in this experiment. Since no pH buffer was used here, the pH increased from 7 to about 10.8 after adding sodium sulfide. Nevertheless, the pH was still in the range where almost all the effective sulfide species that reacted with TNT was HS^-. It is interesting to notice that the initial lag phase, in the absence of pH buffer, was not very significant, especially when the initial sulfide concentration was relatively high (Figure 4-12). A possibility might be that higher pH in this experiment changed the speciation status of the TNT transformation intermediates, i.e. amino compounds, which could affect the reaction rate, as discussed in Section 4.2.1.

The observed pseudo-first-order reaction rate constants are presented in Figure 4-13. It seems that a linear relationship existed between the k_{obs} value and the initial sulfide concentration under the conditions in this experiment.

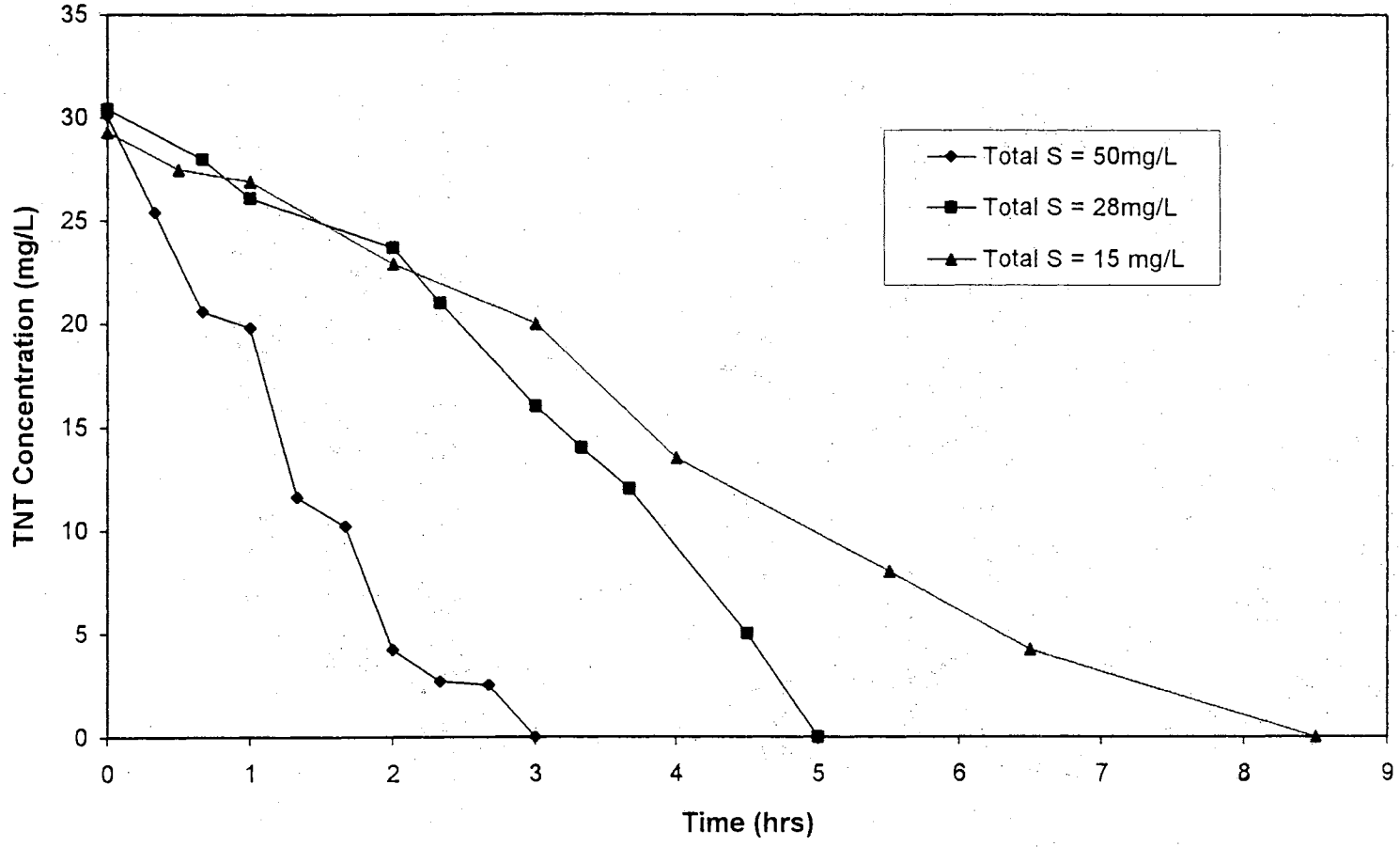


Figure 4-12 Effects of sulfide concentration on TNT transformation (No buffer, no aquifer materials)

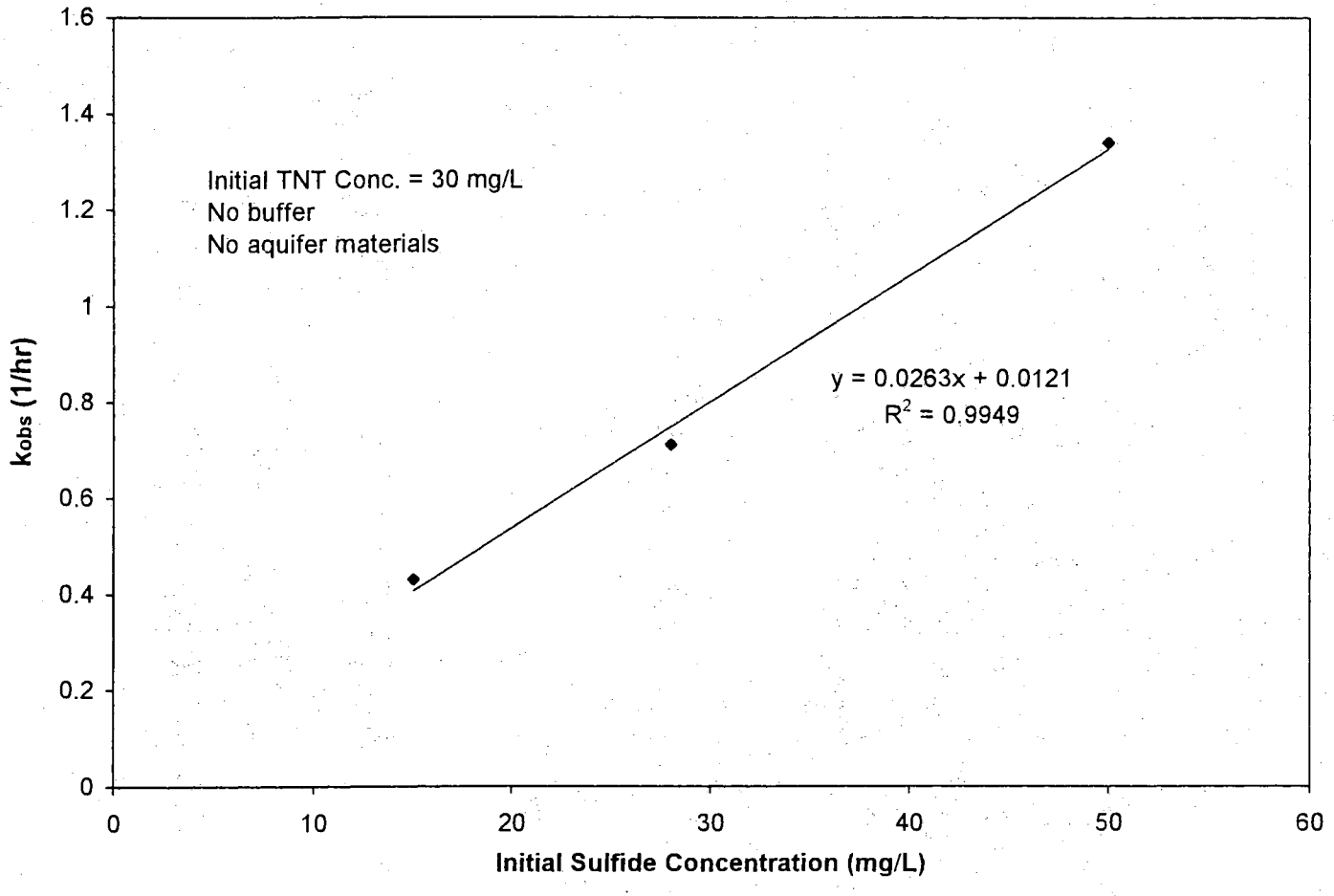


Figure 4-13 Observed abiotic reaction rate constant, k_{obs}, after lag phase

§4.2.3 Catalytic Effects of Aquifer Materials

The possible catalytic effects of naturally occurring organic matter in soils or aquifer materials on abiotic transformation of nitroaromatic compounds have been reported by many researchers, as discussed earlier. The surface of aquifer material particles may also change the thermodynamics and kinetics of abiotic reactions (Morel, 1983). Figure 4-14 shows the effects of varying the ratio of aquifer materials to liquid volume (soil/solution ratio) on the rate of TNT reaction with bisulfide. Addition of aquifer materials significantly accelerated the transformation of TNT, indicating the influence of either or both types of catalytic mechanisms (organic matter and particle surfaces) mentioned above.

Figure 4-15 shows the k_{obs} values after the lag phase varying with the soil/solution ratio. The curve seems to be close to a linear relationship but begins to level off as the soil/solution ratio increases. It is possible that when the soil/solution ratio was relatively high, the catalytic substances and/or particle surfaces were no longer the rate-limiting factor and the reaction rate was mainly controlled by other factors such as the bisulfide concentration. However, it is difficult to confirm this trend here because of the limited number of data points.

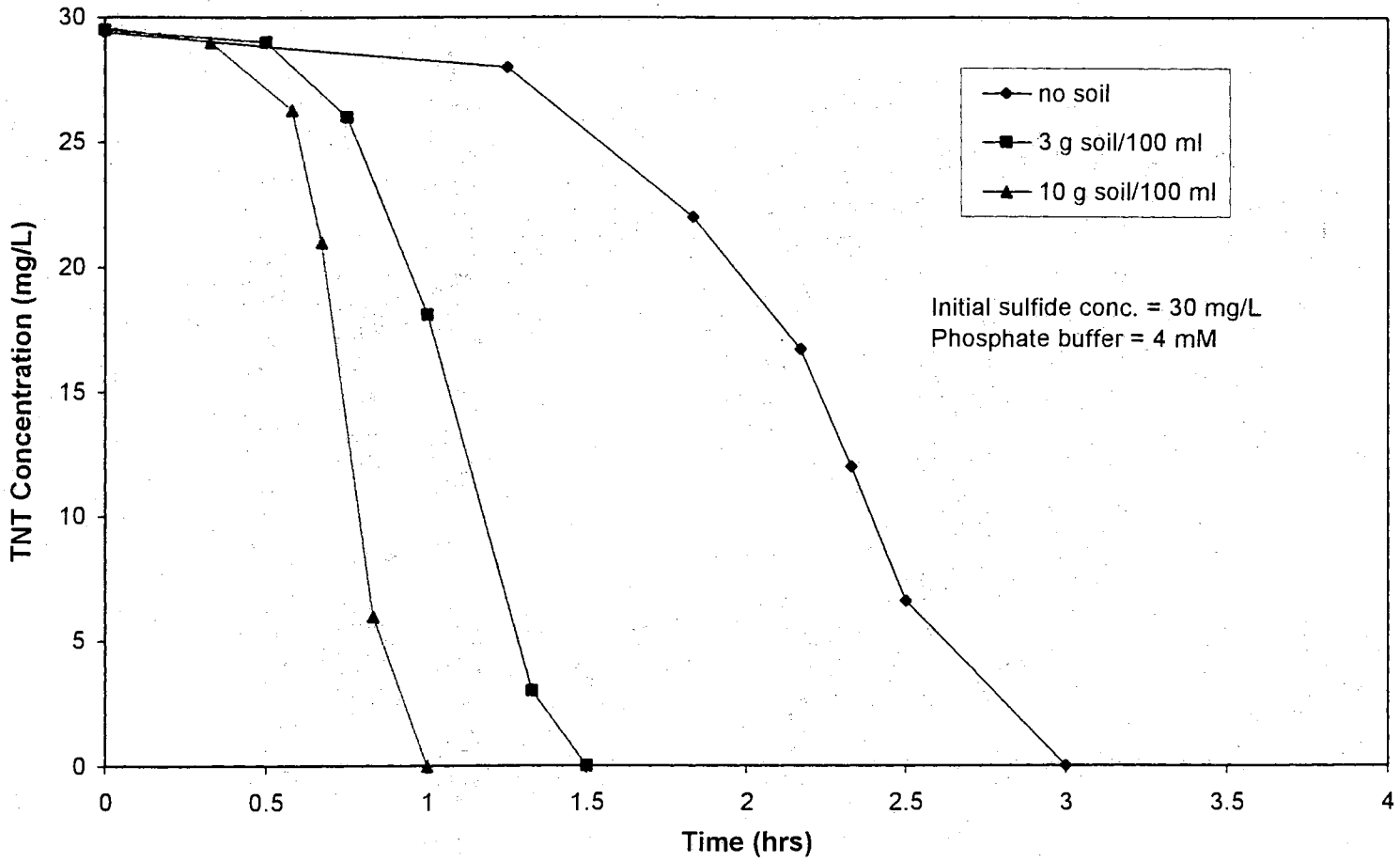


Figure 4-14 Effects of aquifer materials on TNT reaction with sulfide

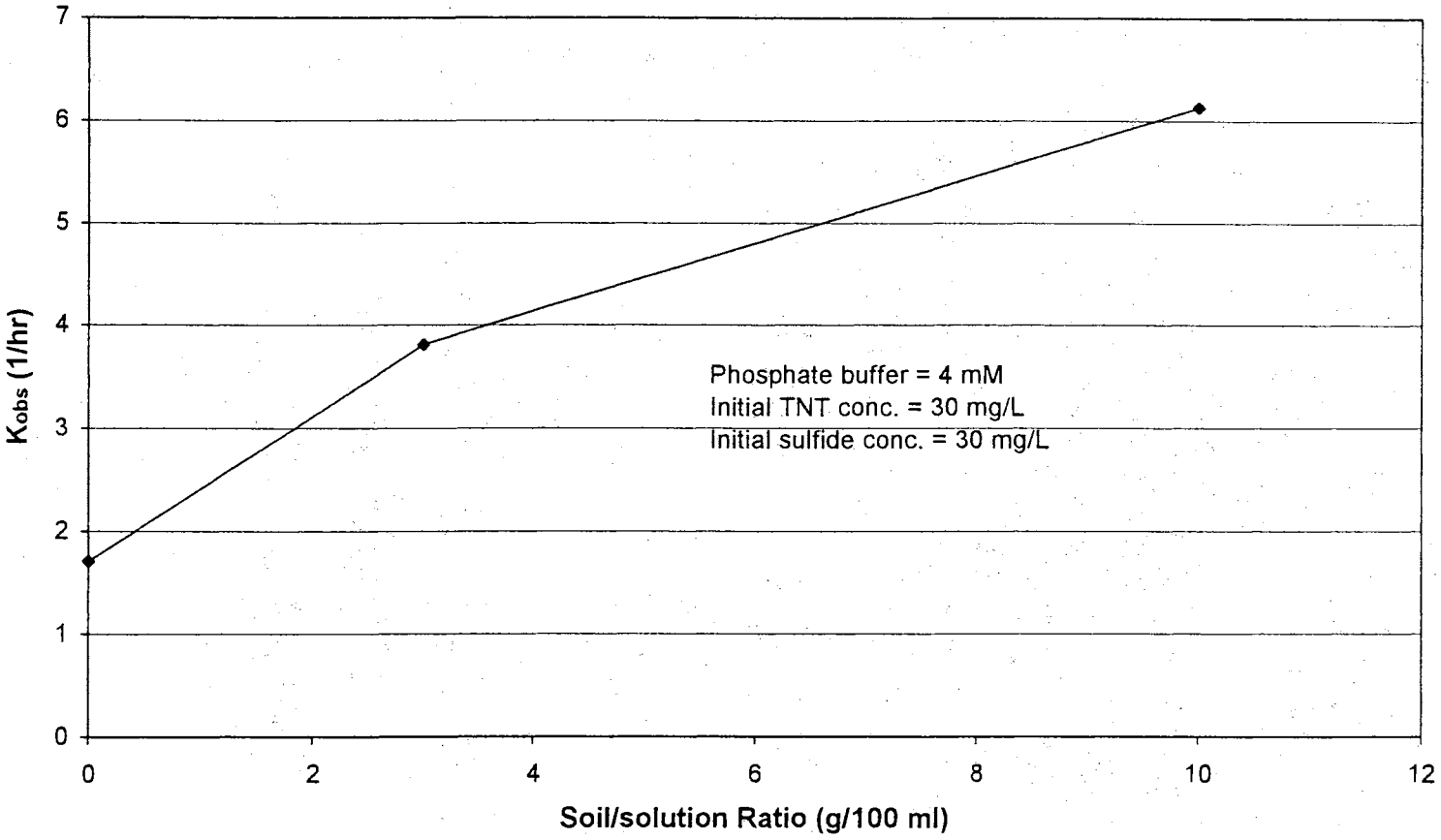


Figure 4-15 Effects of aquifer materials on k_{obs}

§4.2.4 Catalytic Effects of pH Buffer

Since a phosphate buffer of 4 to 8 mM was used in all biological batch and column reactors, 4 mM phosphate buffer was used in this study as a representative buffer concentration. The phosphate buffer seems to have dual effects on the rate of TNT/bisulfide reaction. On one hand, it may regulate the reaction rate by buffering the pH and stabilizing the speciation of HS^- and other ionizable chemicals such as amino compounds in the reaction system. On the other hand, it may increase the reaction rate by catalyzing the reaction. Barbash and Reinhard (1989) found, when they investigated the abiotic reactions of 1,2-dichloroethane and 1,2-dibromoethane with H_2O and bisulfide, that phosphate buffer accelerated the nucleophilic substitution of both halogenated compounds by H_2O, increasing the hydrolytic process.

Bicarbonate buffer was used in another set of reactors to examine its effects on the reaction rate. It took a much longer time period (about 11 hours) for the TNT concentration to reach zero with 4 mM bicarbonate present than with no buffer (data not shown). Apparently, bicarbonate did not accelerate the reaction. Table 4-3 presents the effects of buffering practice on the reaction rate after the lag phase.

Table 4-3 Effects of pH Buffer on Abiotic TNT Reaction Rate Constant

pH Buffer	k_{obs} after lag phase (1/h)	pH
4 mM bicarbonate	0.5	9.0
No buffer	0.7	10.8
4 mM phosphate	1.7	9.0

§4.2.5 Abiotic Transformation Intermediates and Stoichiometry

The HPLC peaks of major intermediates observed in abiotic TNT reactions were very similar to those observed in biological reactors. These peaks included (1) ADNT peaks, (2) two peaks around 12.0 minutes and 12.4 minutes respectively, and (3) a DANT peak. The two early-stage peaks around 12.0 and 12.4 minutes have been tentatively identified as 2-HADNT and 4-HADNT isomers, as explained in Section 4.3.2.

Glaus et al. (1992) indicate that nitroaromatic compounds ($Ar\text{-}NO_2$) are usually reduced to anilines ($Ar\text{-}NH_2$) in three steps, with nitroso (Ar-NO) and hydroxylamino (Ar-NHOH) species as intermediates:

$$Ar\text{-}NO_2 + 2e^- \Longrightarrow Ar\text{-}NO + 2e^- \Longrightarrow Ar\text{-}NHOH + 2e^- \Longrightarrow Ar\text{-}NH_2$$

Therefore, it requires six electrons in total for a nitro compound to be reduced to the corresponding amino compound. If this is true and if the bivalent S atom in HS^- is oxidized to S^0, then it would take 3 moles of HS^- to meet the stiochiometric demand of 1 mole of TNT, assuming complete reaction. Figure 4-16 shows the sulfide consumption along with TNT transformation in one of the abiotic experiments in this study. The observed stoichiometry here was: Bisulfide : TNT = 2.84 : 1 (mM : mM). A ratio approximately equal to this value held in all abiotic TNT/bisulfide reactions in which the sulfide concentration was monitored. However, strict stoichiometric calculations are very difficult to achieve here when one realizes that the TNT reduction consists of a series of stepwise reactions, that conversions from TNT to ADNTs and from ADNTs to DANTs may proceed simultaneously, and that branch reactions may occur before a nitro group is completely reduced to corresponding amino group (Spain, 1995). In this study, the

ADNTs detected by HPLC usually accounted for less than one third of the originally added TNT.

Since the concentrations of TNT and bisulfide were of the same order of magnitude in these experiments and both were significantly decreased during the reaction, it seems reasonable to use a second-order reaction rate model to describe the reaction kinetics, according to general kinetic theories (Moore and Pearson, 1981; Brezonik, 1994). However, a pseudo-first-order expression fit the experimental data (after the lag phase) better than a second-order one. An explanation could be that there was an autocatalytic mechanism (possibly associated with TNT intermediates) that altered the reaction kinetics. Other observations, as indicated earlier, support this assumption.

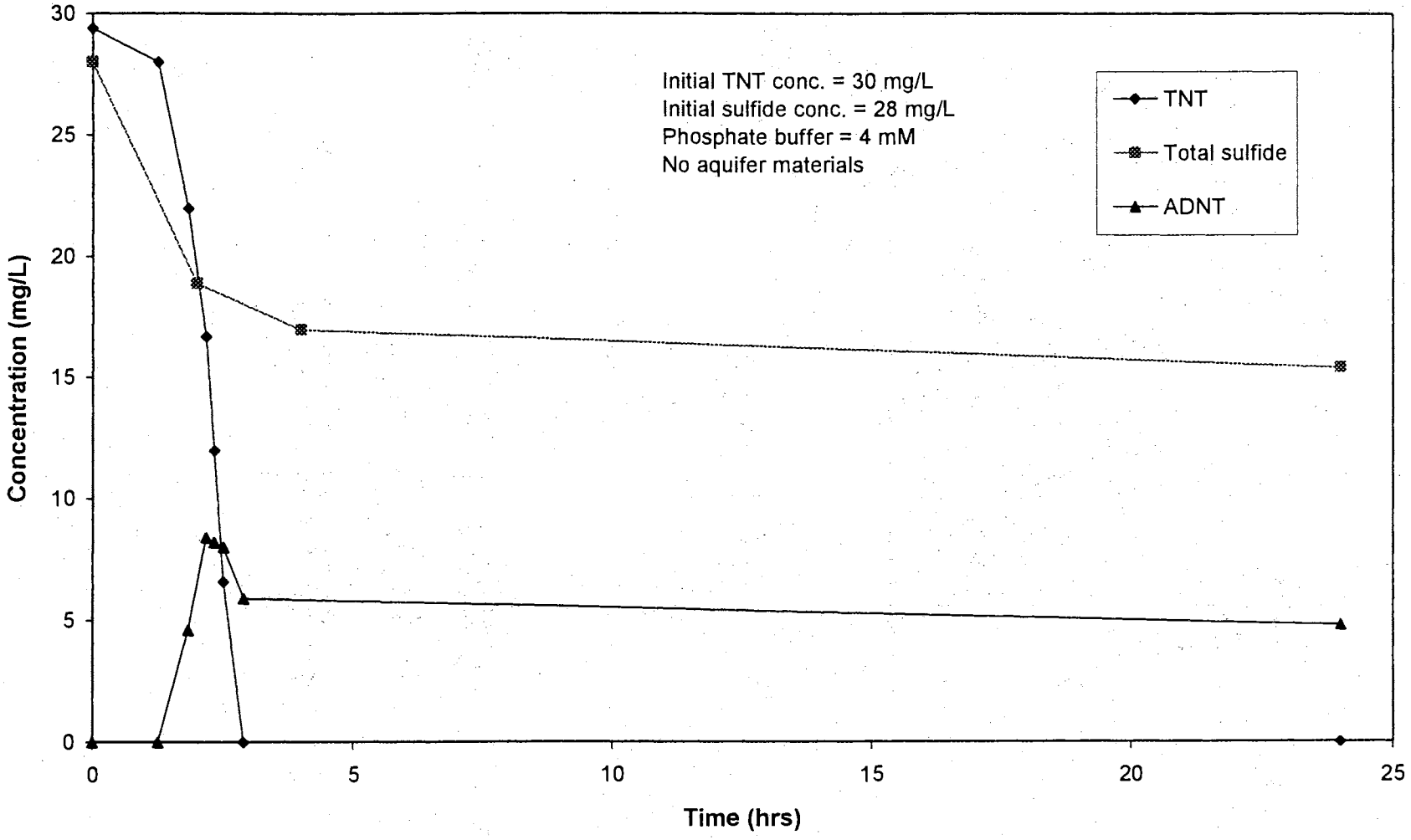


Figure 4-16 Sulfide consumption in TNT-bisulfide reaction

§4.3 Biological Batch Experiments

Two sets of batch reactors were set up and tested to examine biotransformations of TNT under denitrifyting, sulfate-reducing, and methanogenic conditions. Reactor set one was amended with relatively lower concentrations of carbon and energy sources and incubated at room temperature, while reactor set two had relatively higher concentrations of carbon and energy sources and incubated at 37 °C (see Section 3.2.3).

§4.3.1 Batch Reactor Set One

§4.3.1.1 Denitrifying Reactors Observations in these reactors suggested that the primary substrate concentration and the initial TNT concentration had significant impact on the rate of TNT biotransformation. Figures 4-17 and 4-18 show the TNT removal in denitrifying batch reactors with the initial TNT concentration of about 60 mg/L. From the slopes of the linearized curves in Figure 4-18, it is obvious that the concentration of the primary substrate (acetate) largely influenced the rate of TNT biotransformation. If the pseudo-first-order model is used to describe the reaction rate, the reaction rate constant, k, can be expressed in the following equation:

$$\ln(C/C_0) = -kt \tag{4.9}$$

where C is the TNT concentration at time t.

Since the initial concentration of the primary substrate is known and other conditions are comparable, a normalized initial reaction rate constant, k_N, can be defined as follows:

$$k_N = k/(\text{initial acetate concentration}) \quad (4.10)$$

The values of k and k_N for this set of reactors (initial TNT concentration = 60 mg/L) are listed in Table 4-4. The fact that the k_N values under both conditions are close to each other seems to imply that the reaction rate constant k is essentially proportional to the initial primary substrate concentration.

Table 4-4 Rate Constants of Denitrifying Reactors (TNT = 60 mg/L)

Initial Acetate (mg/L)	180	1000
k (day^{-1})	0.0091	0.0474
k_N (L/day ·mg)	5.05×10^{-5}	4.74×10^{-5}

The performance of reactors with initial TNT concentration of 100 mg/L is presented in Figure 4-19a. According to these data, the abiotic loss of TNT in the reactors was less than 15%. Under this set of conditions, Figure 4-19b shows that the TNT transformation intermediates, ADNT isomers, did not accumulate. The highest concentration of ADNTs detected was 5.8 mg/L, corresponding to 6.7% of the initially added TNT, on a molar basis.

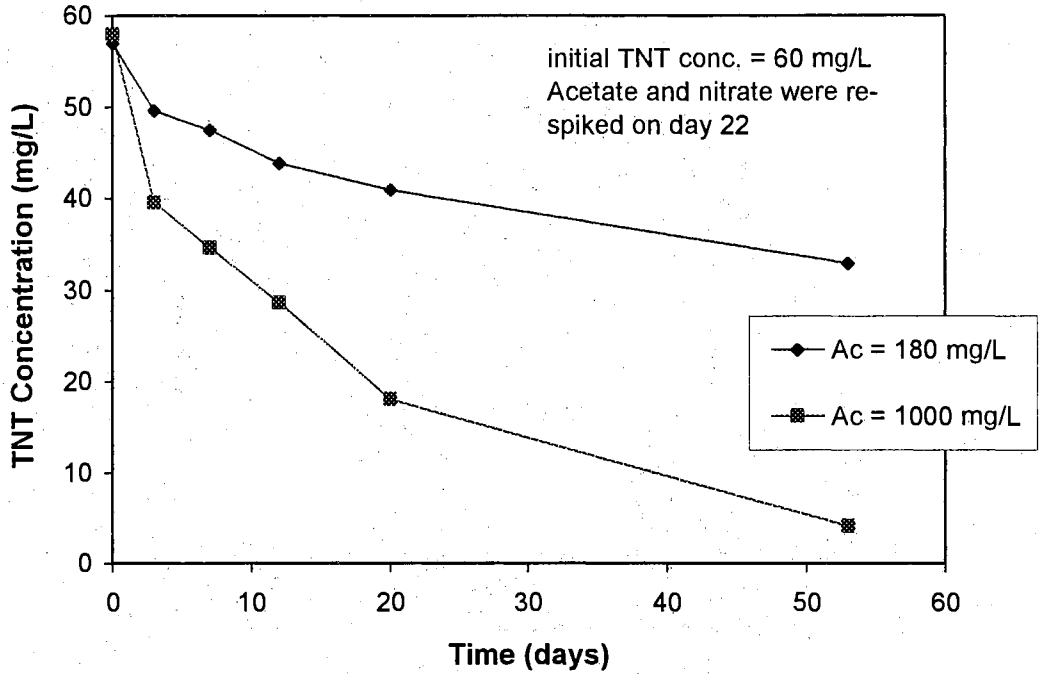


Figure 4-17 TNT Biotransformation in Denitrifying Batch Reactors (Set One, TNT = 60 mg/L)

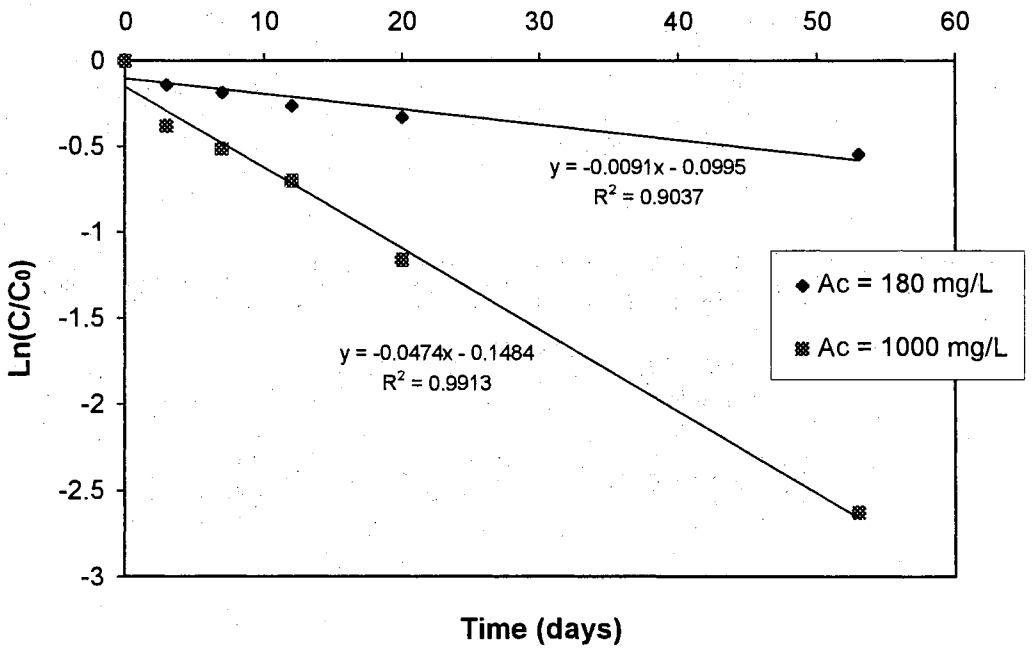
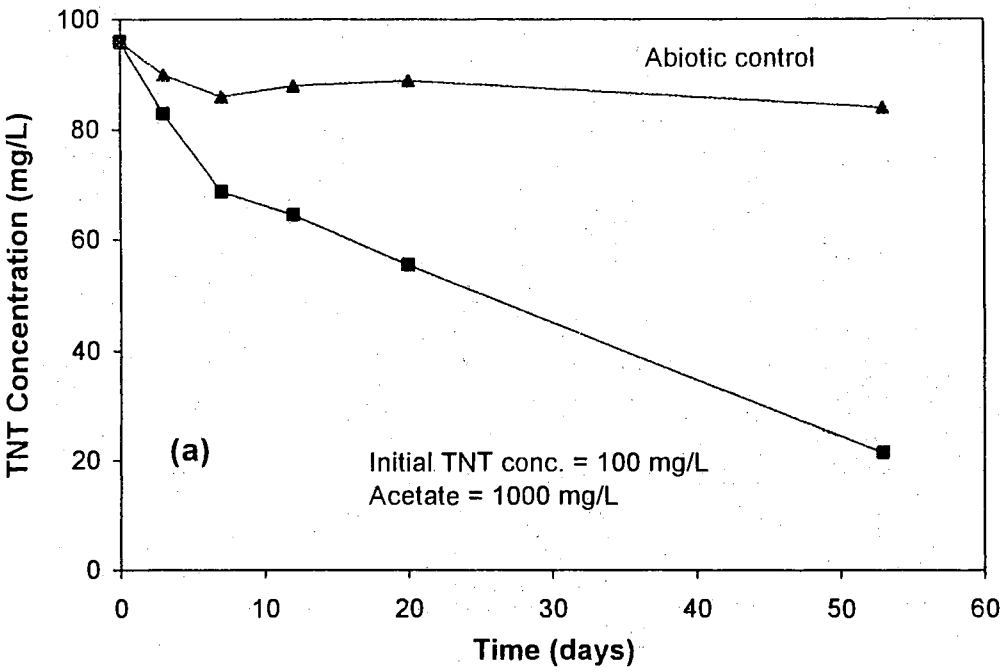


Figure 4-18 Pseudo-first-order Fitting of TNT Biotransformation Kinetics in Denitrifying Batch Reactors (Set One)



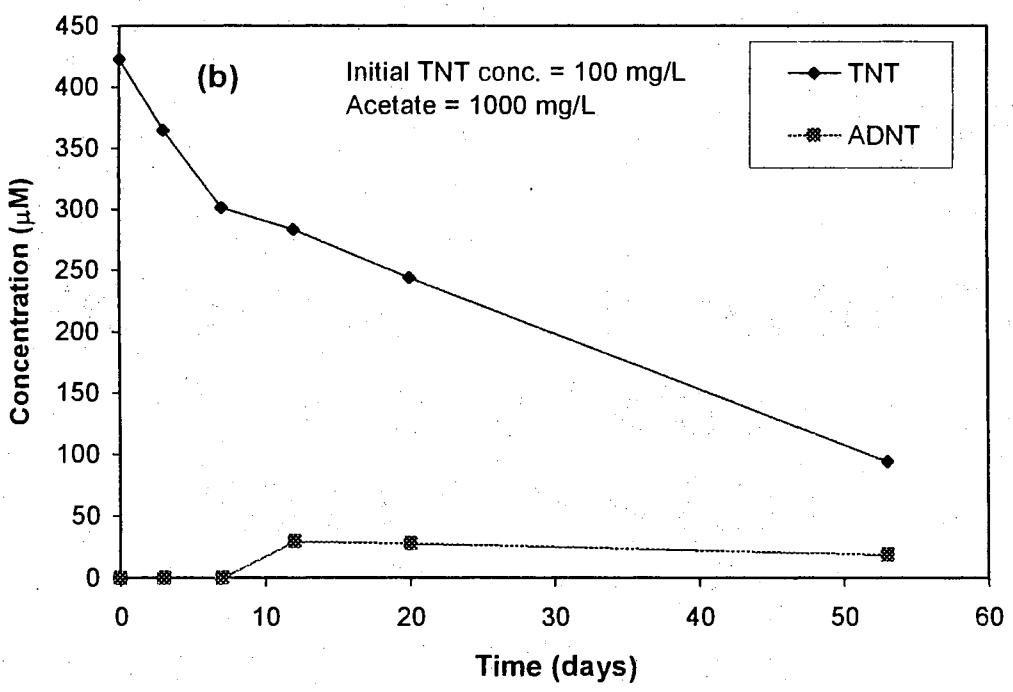


Figure 4-19 TNT biotransformation in denitrifying batch reactors (Set One)
(a) Biological vs. abiotic reactors when TNT was 100 mg/L
(b) TNT removal and ADNT evolution in biological reactors

To examine the effects of the initial TNT concentration on the TNT removal rate, the linearized TNT concentration curves with the initial TNT concentration of both 60 mg/L and 100 mg/L are plotted in Figure 4-20. Based on Table 4-5, it is interesting to notice that the ratio [$k_{TNT=60\ mg/L}$] / [$k_{TNT=100\ mg/L}$] is 1.75, which is close to the ratio of initial TNT concentrations, (100 mg/L) / (60 mg/L) = 1.67. This nearly reversely-proportional relationship between k and the initial TNT concentration may suggest that the TNT transformation reaction rate was accordingly decreased as the initial TNT concentration increased.

Table 4-5 Rate Constants of Denitrifying Reactors (Acetate = 1000 mg/L)

$k\ (day^{-1})\ \vert_{TNT=60\ mg/L}$	0.0474
$k\ (day^{-1})\ \vert_{TNT=100\ mg/L}$	0.0271
$[k_{TNT=60\ mg/L}] / [k_{TNT=100\ mg/L}]$	1.75
(100 mg/L) / (60 mg/L)	1.67

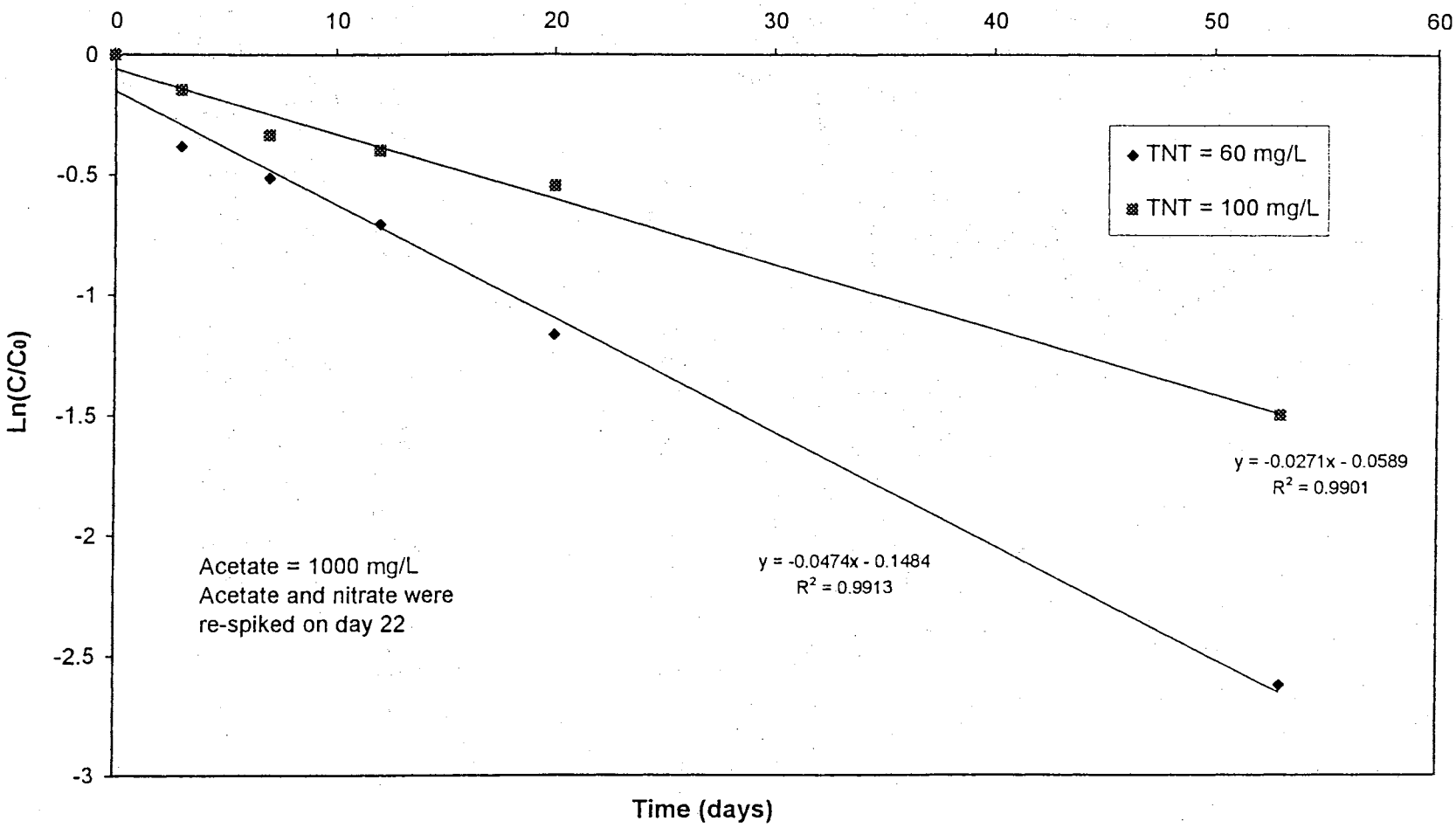


Figure 4-20 Denitrifying Batch Reactors (Set One) with different initial TNT concentrations

§4.3.1.2 Sulfate-reducing Reactors Identical reactors were first set up without TNT in order to obtain active sulfate-reducing conditions. Lactate and sulfate concentrations were then adjusted to desired levels and the abiotic controls were autoclaved before the TNT stock solution was spiked into the reactors (described in Section 3.2.3). The biomass concentration was about 20 mg/L in all the reactors. It was observed that TNT, following addition to the reactors, disappeared in about 2 hours. This rapid removal of TNT was attributed to abiotic reactions with bisulfide, which had been produced from sulfate reduction and reached a concentration of about 30 mg/L as total sulfide. The abiotic TNT transformation was discussed in Section 4.2. Since TNT was rapidly removed, the later monitoring of the reactors was focused on its transformation intermediates, mainly ADNTs.

Figures 4-21 and 4-22 show the removal of ADNTs, produced from abiotic transformation of TNT as described above, under different conditions. It can be seen from Figure 4-21 that ADNTs could be removed abiotically as they reacted with bisulfide. This was also observed in the abiotic reactors discussed in Section 4.2. The abiotic transformation of ADNTs in the presence of sulfide was much slower than that of TNT, taking weeks rather than hours. Figure 4-21 indicates that it took about 25 days for the ADNT to decrease from its highest concentration, 9.4 mg/L, to half of this value, 4.7 mg/L, under the abiotic conditions. In the presence of microbial activity, ADNT removal was considerably faster, indicating that ADNTs were transformed partially biologically and partially abiotically. These figures also illustrate the effects of primary substrate

concentrations on the transformation of ADNTs, with faster transformation in the presence of higher lactate concentrations.

In a parallel experiment, the reactors were set up without pre-growing the culture before adding TNT. Instead, the 30 mg/L TNT was added at the beginning along with lactate, sulfate, and the inoculum. Under this condition, the sulfate reduction was inhibited, as shown in Figure 4-23, although TNT was still transformed. By day 31, the TNT concentration reached below detection limits and the concentrations of ADNTs and two other early-stage intermediates, tentatively identified as 2-HADNT and 4-HADNT (discussed in Section 4.3.2 below), were decreased to negligible levels. It was after this point of time that sulfate reduction became active, as shown in Figure 4-23. On the other hand, significant sulfate reduction and primary substrate utilization were observed on day 24 after 30 mg/L TNT was added in the reactors with pre-grown biomass, as shown in Table 4-6.

Table 4-6 Sulfate Reduction in Reactors with Pre-grown Biomass
(Initial TNT: 30 mg/L, lactate and sulfate in mg/L)

	Biological (I)		Biological (II)		Abiotic	
Day	Lactate	Sulfate	Lactate	Sulfate	Lactate	Sulfate
0	313	443	975	1430	978	1365
24	223	394	628	1100	960	1347

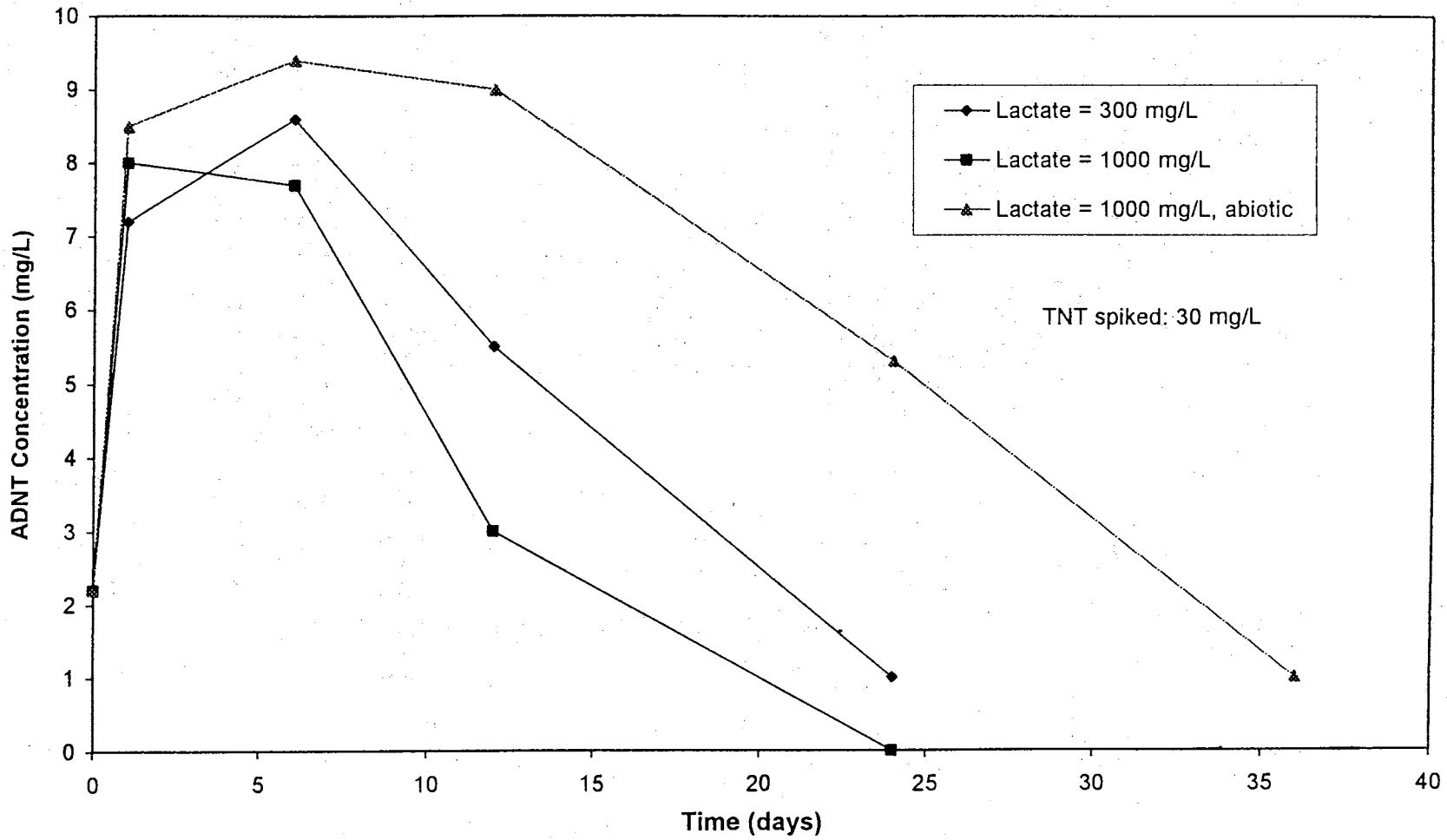


Figure 4-21 ADNT in sulfate-reducing batch reactors (Set One, TNT spiked: 30 mg/L)

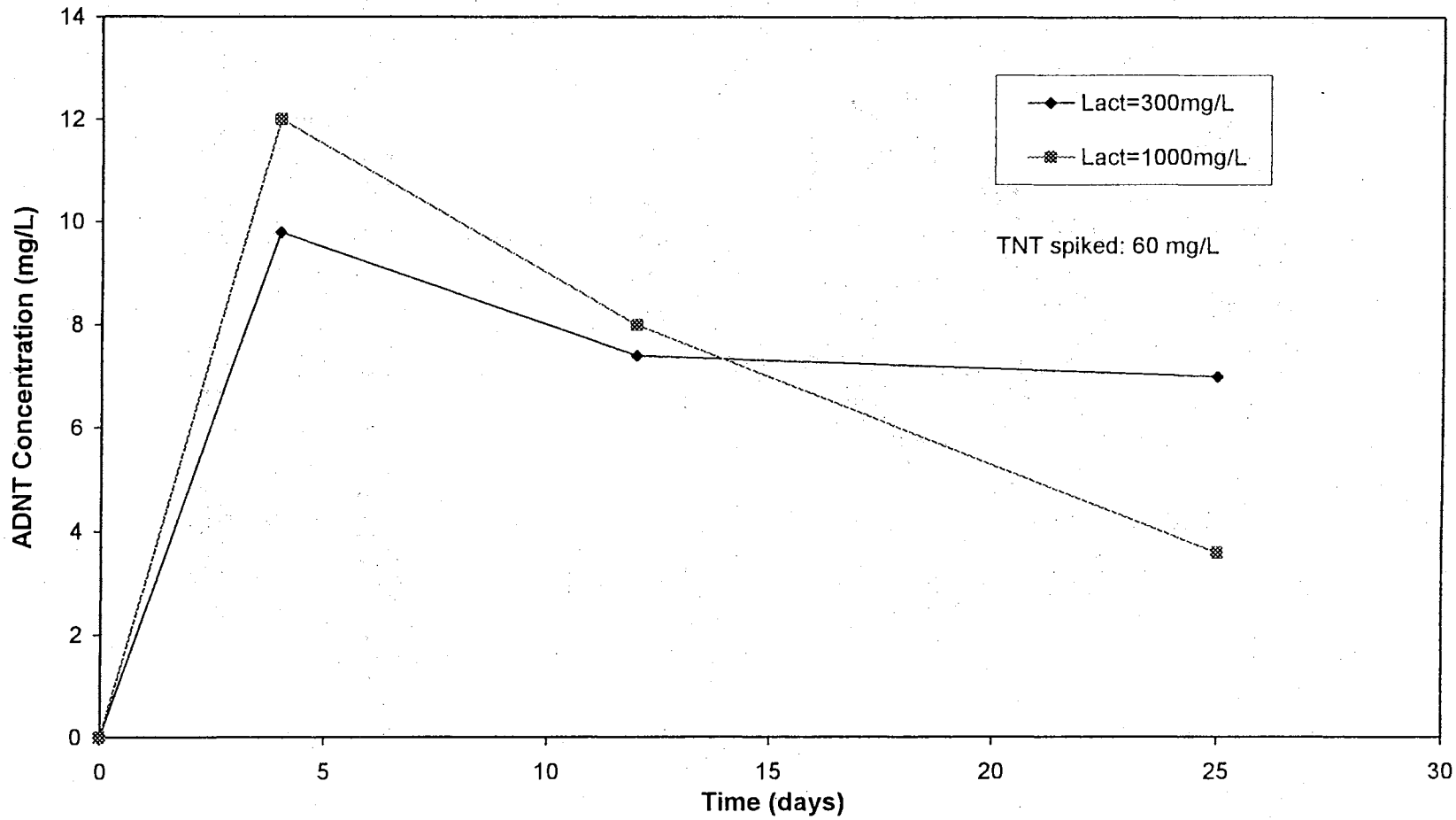


Figure 4-22 ADNT in sulfate-reducing batch reactors
(Set One, TNT spiked: 60 mg/L)

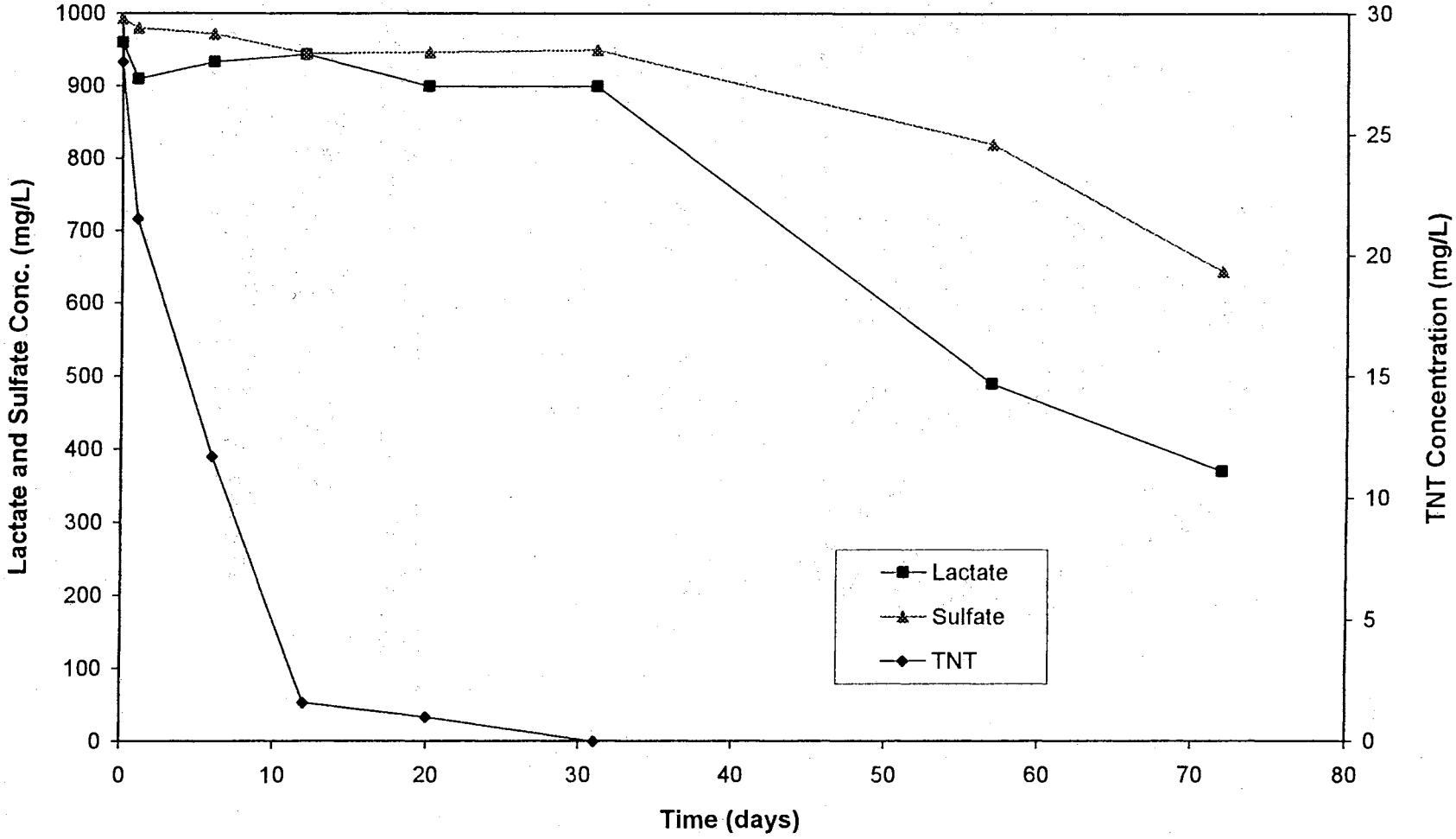


Figure 4-23 Inhibition of sulfate reduction by TNT presence

§4.3.1.3 Methanogenic Reactors Similar to sulfate-reducing reactors described earlier, identical methanogenic reactors were first set up without TNT to obtain active methanogenic conditions. Acetate concentrations were then adjusted to desired levels before the TNT stock solution was spiked into the reactors. Figures 4-24a, 4-24b, and 4-25 show the TNT removal in methanogenic reactors. Although the reactors were actively methanogenic before TNT was spiked, methanogenesis ceased in all reactors after TNT was added and never recovered over the time period the reactors were monitored. Gorontzy et al. (1993) found that nitroaromatics and their early-stage intermediates like nitroso- and/or hydroxyl-amines inhibited methanogenic bacteria. These compounds could react with the unique membrane components of the methanogens, cause cell lysis, and cease the methane production. Therefore, the authors indicated that it was necessary to pre-grow the cells to a certain density before adding the nitroaromatics. In this study, the pre-grown biomass density in the methanogenic reactors was about 20 mg/L (as VSS). Probably this initial biomass concentration was not sufficiently high and most of the cells lysed, or at least were inhibited, upon TNT addition, because the TNT transformation seemed to be limited by biomass concentrations. With largely different primary substrate concentrations (Ac = 180 mg/L and 1000 mg/L), the pseudo-first-order TNT transformation rates (0.0366 day^{-1} and 0.0648 day^{-1}, respectively, in Figure 4-24b) did not show a correspondingly large difference, indicating that the biomass concentration, rather than the primary substrate concentration, was likely to be the rate-limiting factor in these reactors.

Not only was TNT removal slower under methanogenic conditions than under denitrifying and sulfate-reducing conditions, the removal of ADNTs, produced from TNT transformation, exhibited a much lower transformation rate in the methanogenic reactors. Figure 4-26 shows the ADNT appearance and disappearance in methanogenic and sulfate-reducing reactors, which had an initial TNT concentration of 30 mg/L and initial primary substrate concentration of 1000 mg/L. As indicated earlier, ADNT removal in sulfate-reducing systems was enhanced due to the abiotic reactions with bisulfide.

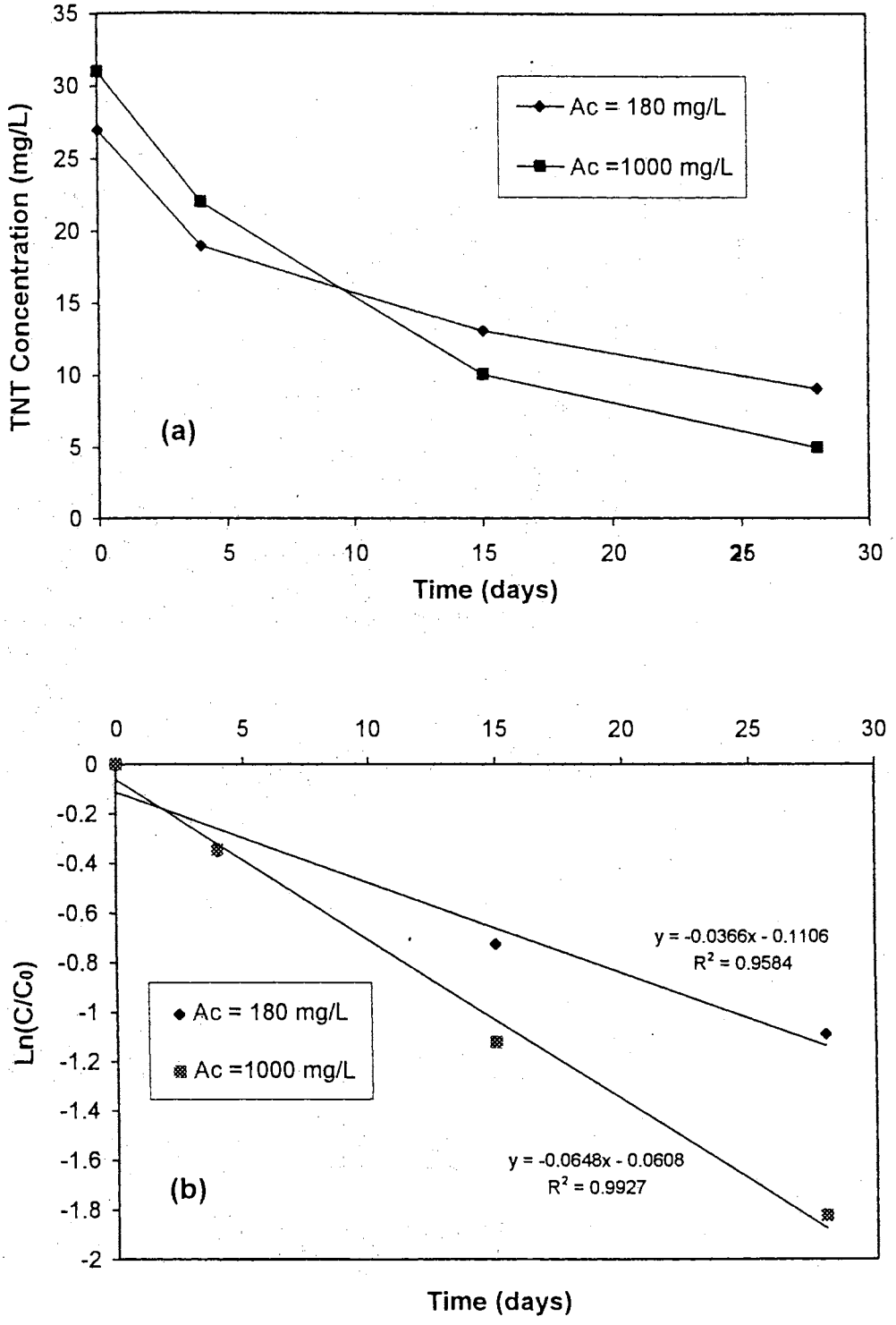


Figure 4-24 TNT biotransformation in methanogenic batch reactors (Set One) (a) TNT concentration over time (b) Pseudo-first-order fitting of TNT transformation kinetics

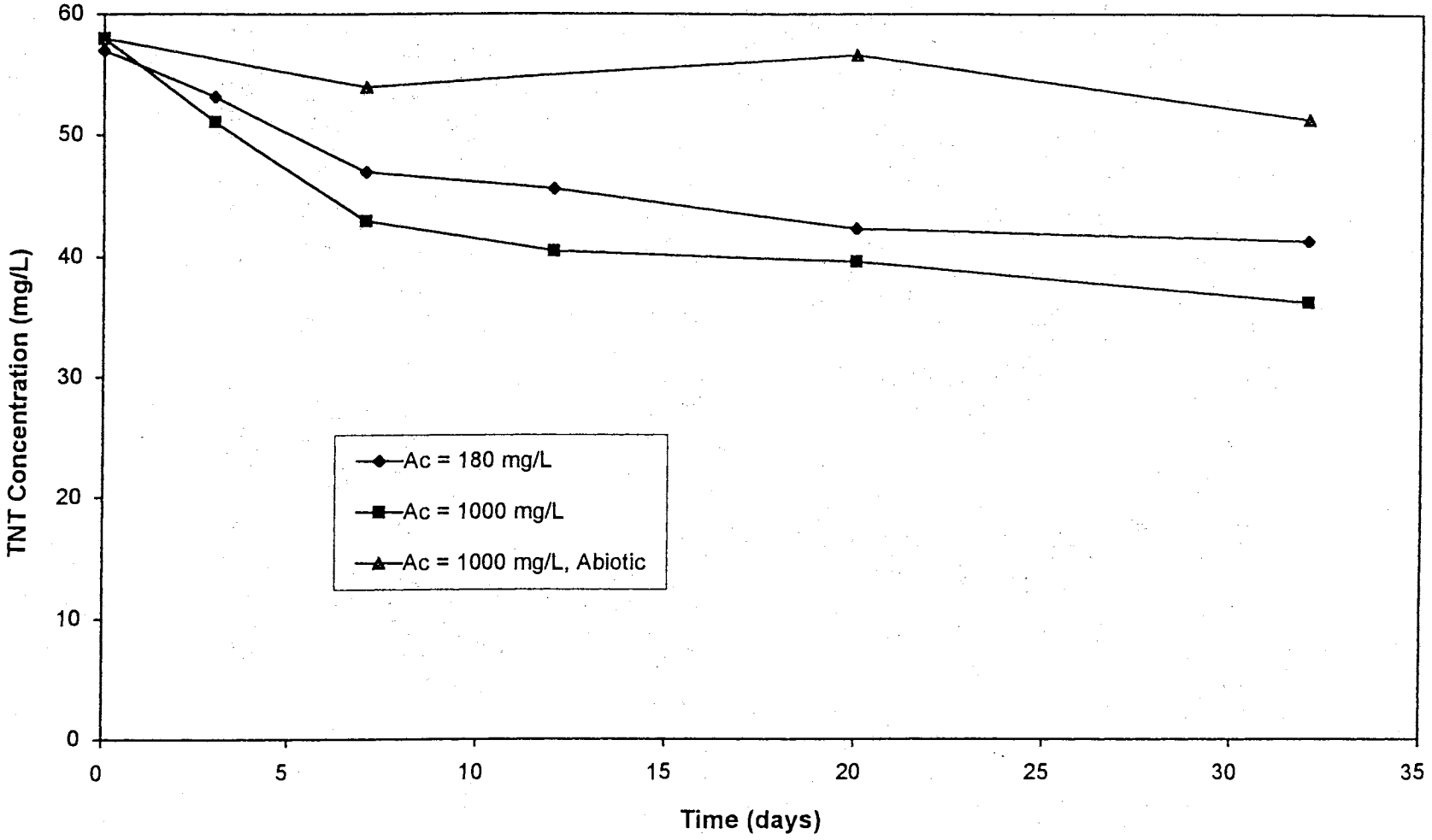


Figure 4-25 TNT biotransformation in methanogenic batch reactors
(Set One, TNT conc. = 60 mg/L)

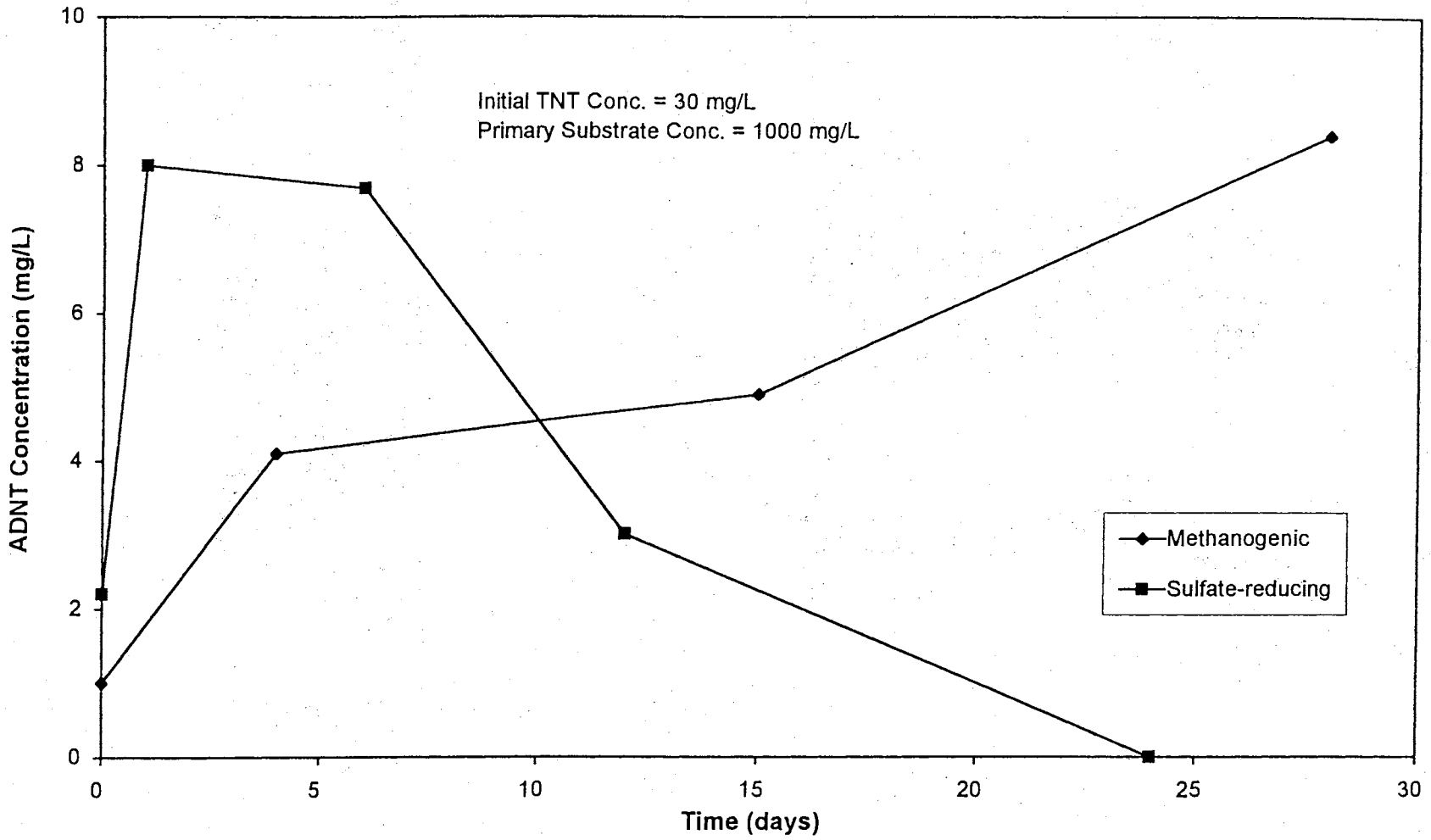


Figure 4-26 Comparison of ADNT in methanogenic and sulfate-reducing reactors

§4.3.2 Batch Reactor Set Two

TNT removal of this set of reactors is shown in Figure 4-27. The fastest TNT removal was observed in the denitrifying reactors. TNT transformation was moderately fast in the reactors with sulfate as external electron acceptors while considerably slower in the reactors with no external electron acceptors. Many researchers have indicated that TNT biotransformation, especially the reduction of the first nitro group, can be achieved under various different redox conditions, as discussed in the literature review. Since correlating relationships exist between redox potentials and electron accepting conditions, the results obtained in this study that TNT biotransformation occurred under various electron accepting conditions further confirmed the observations of those researchers. However, the electron accepting conditions did significantly affect the rate of TNT removal and the fate of TNT metabolites. Table 4-7 shows the TNT biotransformation intermediates detected in this experiment. Intermediates are numbered in the order of their appearance during TNT transformation. "ADNT" is the combination of both 2-ADNT and 4-ADNT isomers and expressed as 2-ADNT equivalent. "DANT" has been identified as 2,4-DANT by using diode-array HPLC (Appendix E).

Under the three electron accepting conditions examined, the intermediates that first appeared during TNT metabolism, i.e. Int-D1 under denitrifying conditions, Int-S1 and Int-S2 under sulfate-reducing conditions, and Int-M1 and Int-M2 under methanogenic conditions, seemed to be two distinct compounds according to their HPLC peak retention times (Table 4-7). These two compounds exhibited very similar characteristics. Their HPLC retention times were close to each other (12.0 min and 12.4 min). They presented

similar concentrations (or at least similar peak sizes) during TNT metabolism, and their appearance and disappearance occurred almost concurrently. According to other investigations discussed in Chapter II, 4-hydroxylamino-2,6-dinitrotoluene (4-HADNT) and its isomer 2-hydroxylamino-4,6-dinitrotoluene (2-HADNT) are often believed to be the first intermediates appearing in TNT biotransformation. Therefore, the two early-stage intermediates observed in this study, behaving very similarly, are hypothesized to be HADNT isomers. No analytical grade HADNTs were available for confirmation. Under denitrifying conditions, Int-D1 never accumulated to a significantly high concentration (Figure 4-28a). Therefore, this small peak might represent either of the HADNT isomers or a combination of small amounts of both. Denitrifying conditions favored rapid removal of these two isomers and prevented them from building up to high concentrations. On the other hand, these compounds accumulated to considerable concentrations and existed for about 40 days in methanogenic reactors (Figure 4-30a). Gorontzy et al. (1993) and Fedorak et al. (1990) indicated that early-stage intermediates of nitroaromatic compounds such as hydroxyl-amines were inhibitors of methanogenic bacteria. This could perhaps explain the fact that the two intermediates discussed above prevailed in the methanogenic reactors much longer than in other reactors and that methanogenesis was inhibited.

The appearance and disappearance of ADNTs were observed in all three types of reactors, with the fastest removal under denitrifying conditions and slowest removal under methanogenic conditions. Since resolution of 2-ADNT and 4-ADNT isomers could not be achieved under the HPLC operation conditions used in this study, the

observed ADNT peak was actually the combination of both isomers and expressed as 2-ADNT equivalent. The total ADNT concentrations observed in these reactors at most accounted for 20 to 30% of the original TNT added into the systems (Figures 4-28b, 4-29b, and 4-30b). This observation meant that while ADNTs were produced in TNT metabolism, they were transformed at the same time, with comparable reaction rates, to other intermediates, mainly DANTs as discussed below.

While ADNT isomers could be further transformed to 2,4-DANT and 2,6-DANT theoretically (see Chapter II), only one major intermediate peak was observed after the completion of ADNT transformation. This peak was positively identified as 2,4-DANT by comparing the diode-array spectrum of the peak and that of the known 2,4-DANT standard (see Appendix F). Like its precursors, DANT was transformed the fastest in the denitrifying reactors and the most slowly in the methanogenic reactors. By day 27 and day 50, DANT was removed to negligible levels in the denitrifying and the sulfate-reducing reactors, respectively (Figures 4-28 and 4-29). Following this, no major intermediate peaks were detected by HPLC with the system parameters used in this study. In the methanogenic reactors, however, DANT still presented a considerable concentration of about 162 μM on day 50 (Figure 4-30), accounting for 35% of the originally added TNT, although the DANT concentration was in a decreasing trend at that point of time.

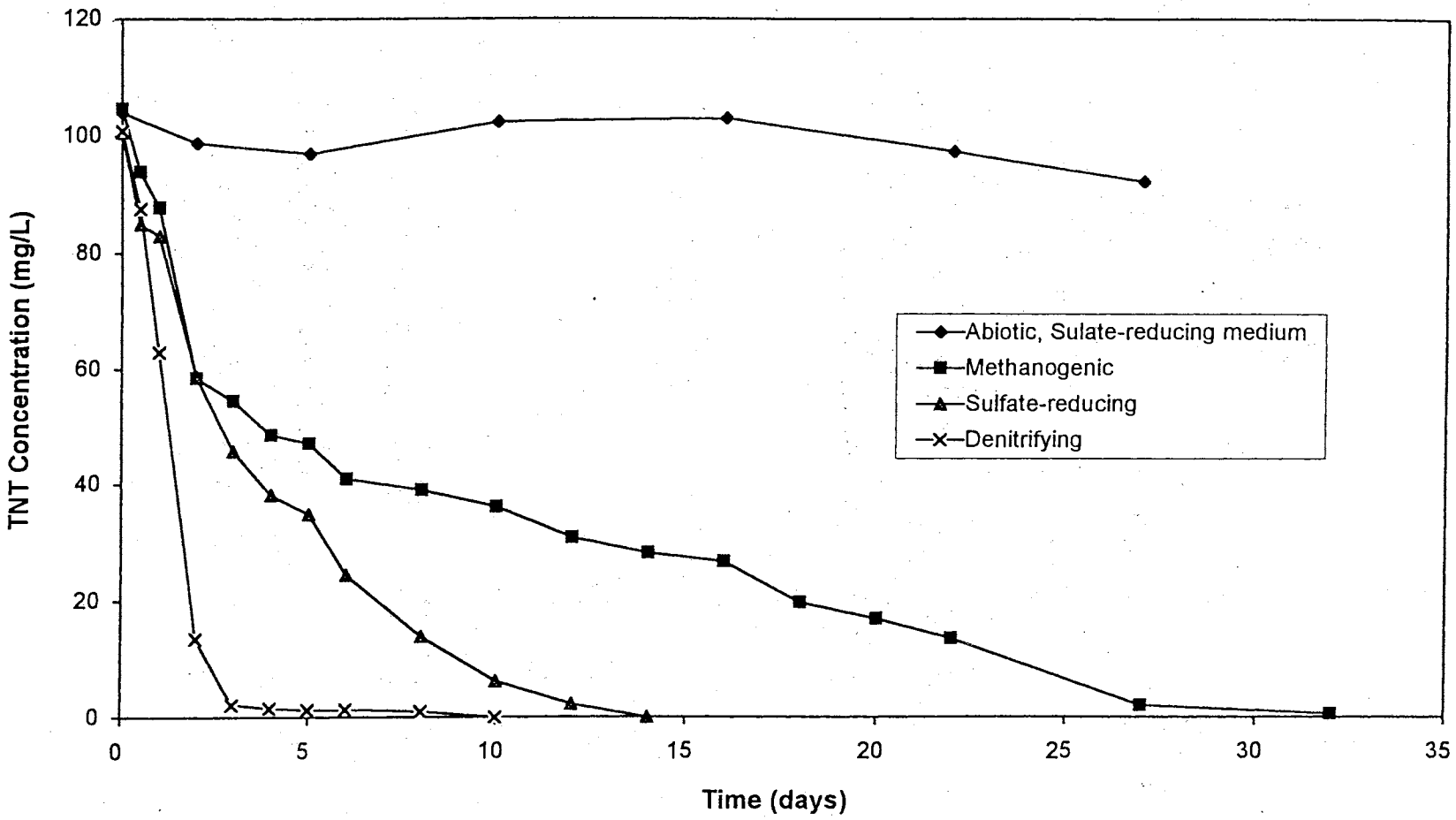


Figure 4-27 Comparison of different electron accepting conditions for batch reactor Set Two

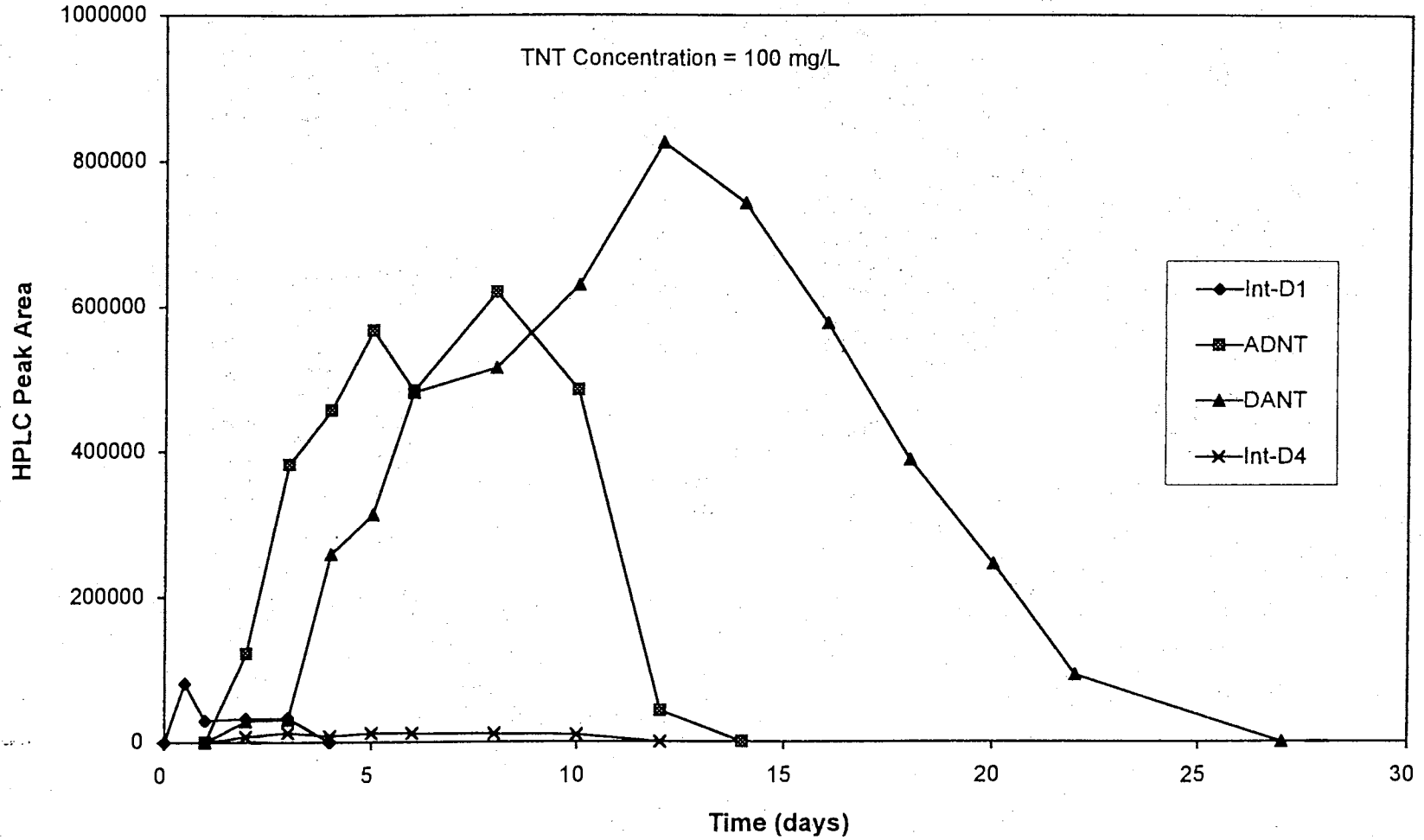


Figure 4-28a TNT intermediates under denitrifying conditions (Set Two)

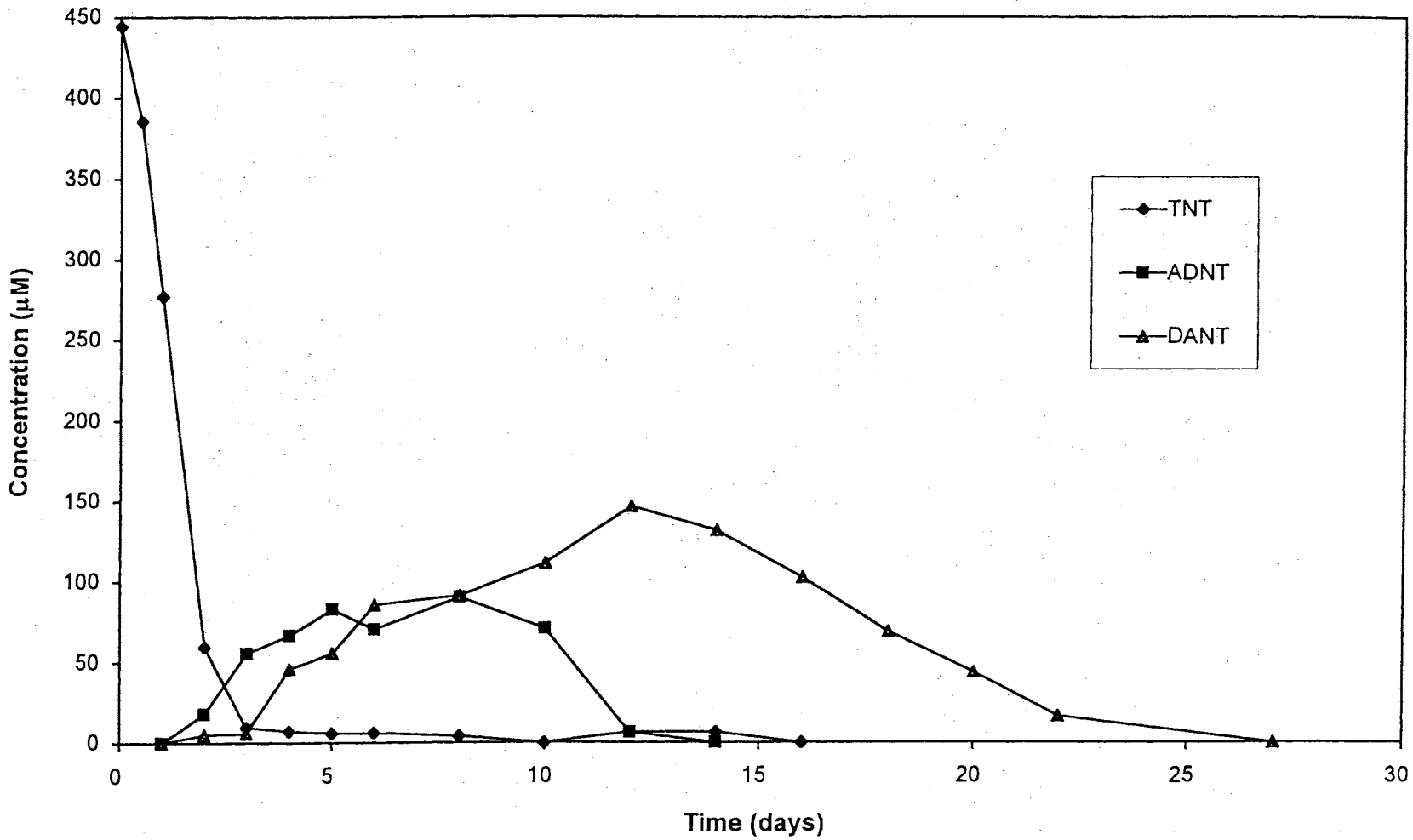


Figure 4-28b Identified TNT intermediates --- Denitrifying conditions

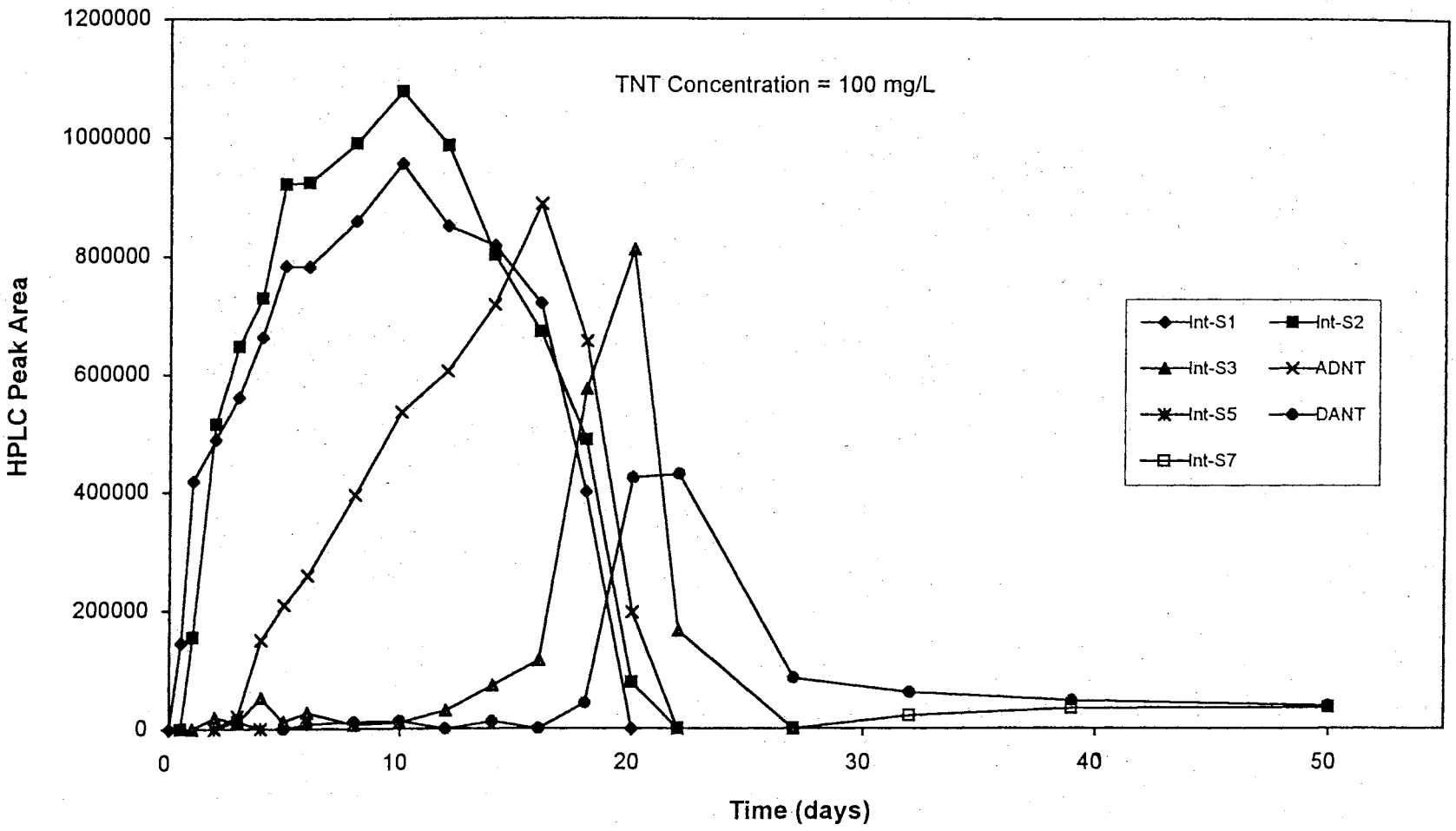


Figure 4-29a TNT intermediates under sulfate-reducing conditions (Set Two)

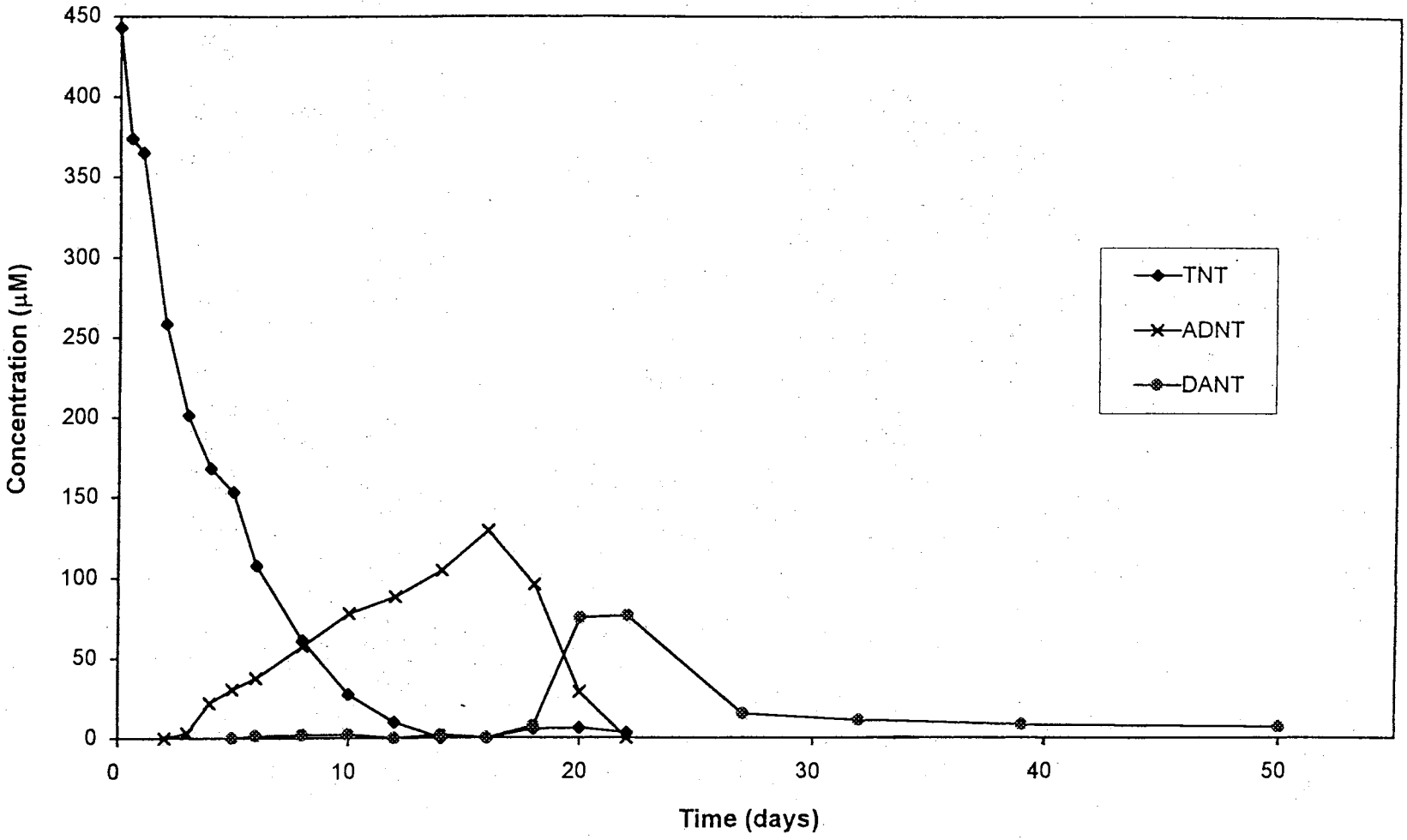


Figure 4-29b Identified TNT intermediates --- Sulfate-reducing conditions

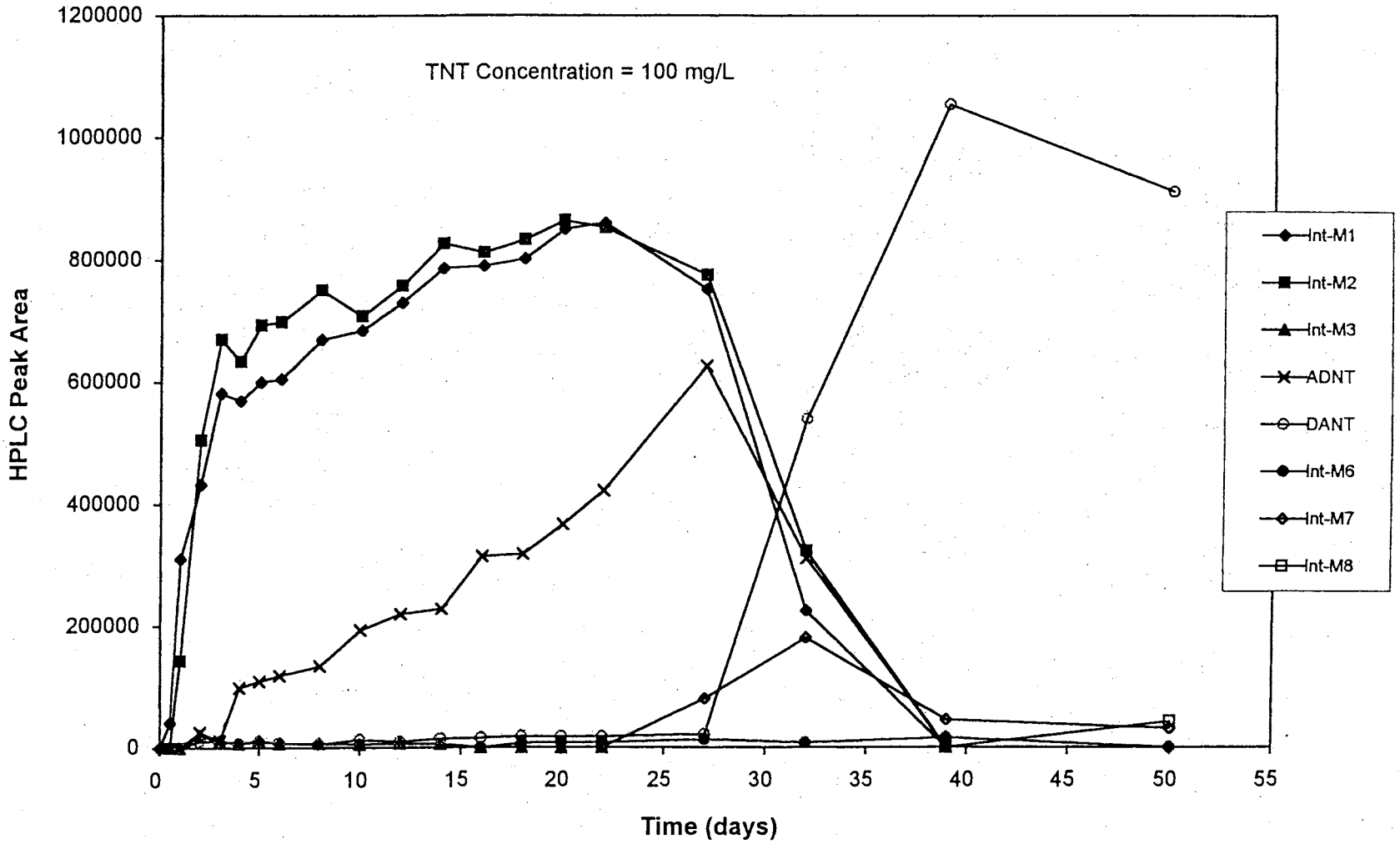


Figure 4-30a TNT intermediates under methanogenic conditions (Set Two)

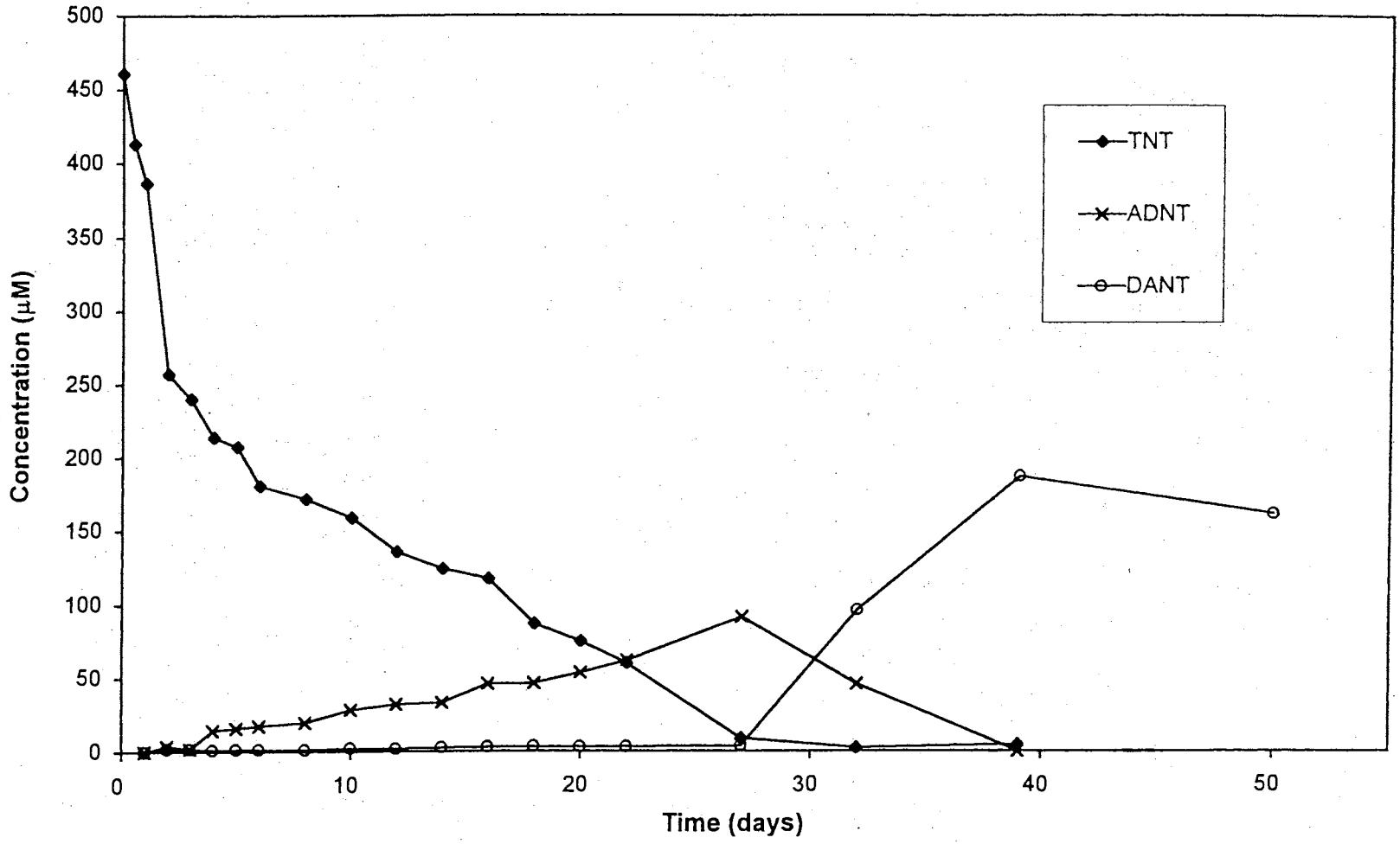


Figure 4-30b Identified TNT intermediates --- Methanogenic conditions

Table 4-7 HPLC Retention Times of TNT and Metabolites

Retention Time (min)	Denitrifying	Sulfate-reducing	Methanogenic
2.7		Int-S3	Int-M3
3.1			Int-M7
3.4	DANT (Int-D2)	DANT (Int-S6)	DANT (Int-M5)
4.3	Int-D4	Int-S5	Int-M6
5.4		Int-S7	Int-M8
12.0	Int-D1	Int-S1	Int-M1
12.4		Int-S2	Int-M2
13.4	TNT	TNT	TNT
14.6	ADNT (Int-D3)	ADNT (Int-S4)	ADNT (Int-M4)

§4.4 Aquifer Column Experiments

This section presents the experimental results of the aquifer column reactors, including the column porosity measurement, breakthrough curves, TNT removal, and primary substrate utilization. Kinetic constants are introduced to help describe the TNT removal rate and to aid in comparing the effects of different factors (initial TNT concentration, primary substrate concentration, retention time, and electron accepting conditions) on TNT transformation in aquifer materials.

§4.4.1 Column Porosity

Two glass graduated cylinders filled with aquifer materials were used to measure the porosity of aquifer columns. Table 4-8 shows the measurement results. From the two porosity values shown in this table, an average column porosity of **36.7%** was obtained. Since the aquifer columns had an inner diameter of 2 cm and an aquifer material depth of 35 cm, the volume of aquifer materials in each column was about 110 ml. Therefore, the pore volume in each column was (110 ml)(36.7%) = **40.3 ml**. This value was used in determining "pore replacement" volumes for subsequent experiments.

Table 4-8. Aquifer Column Porosity

	Cylinder 1	Cylinder 2
Tare Wt. (g)	100.66	49.10
Total Wt. (g)	305.40	99.72
Packing Vol. (ml)	100.0	25.0
Wet Packing (g)	204.74	50.62
Dry Packing (g)	168.10	41.44
Pore Water (g)	36.64	9.18
Pore Vol. (ml)	36.64	9.18
Porosity (%)	36.64	36.72

§4.4.2 Breakthrough Curves at High Flow Rate

Tracer experiments were conducted to characterize the aquifer column breakthrough at a relatively high flow rate, 4 ml/min. This flow rate was to be used in the column fluid exchange for the batch-fed columns. The results obtained here, therefore, were used to determine the media volume required for a complete column fluid exchange. Columns D3 and S3, prior to routine operation, were utilized in the tracer study. Solutions used in this experiment were an aqueous sodium bromide solution as 50 mg Br^-/L and a 100 mg/L aqueous TNT solution. Figure 4-31a shows that the bromide breakthrough curves obtained from these two columns were close to each other with a maximum error of about 10% in C/C_0 values at any given point of time, indicating that the hydraulic conditions among different columns were reasonably similar. The breakthrough curve of TNT, under the conditions in this experiment, only slightly lagged behind that of the tracer material, bromide, as shown in Figure 4-31b. Because the columns were autoclaved and the pore space retention time (10 minutes) and the duration of the breakthrough experiment (60 minutes) were relatively short, the TNT loss due to microbial and abiotic transformations was negligible and the TNT level in the column effluent did finally reach the influent level. The lag in the TNT breakthrough curve might indicate slight adsorption/retardation of TNT in the aquifer materials, which had not been exposed to TNT before. From Figure 4-31b, it was estimated that the column fluid in a batch-fed column could be completely exchanged in 45 to 55 minutes, corresponding to a medium volume of 180 to 220 ml, when the exchange flow rate was 4.0 ml/min. TNT

adsorption in the aquifer materials was minimal at this flow rate even when the column was new and had not been saturated with TNT.

In order to examine the effects of the flow rate and the retention time on TNT adsorption in aquifer materials, a long-term adsorption experiment with a relatively low flow rate was conducted, as presented in Section 4.1.4.

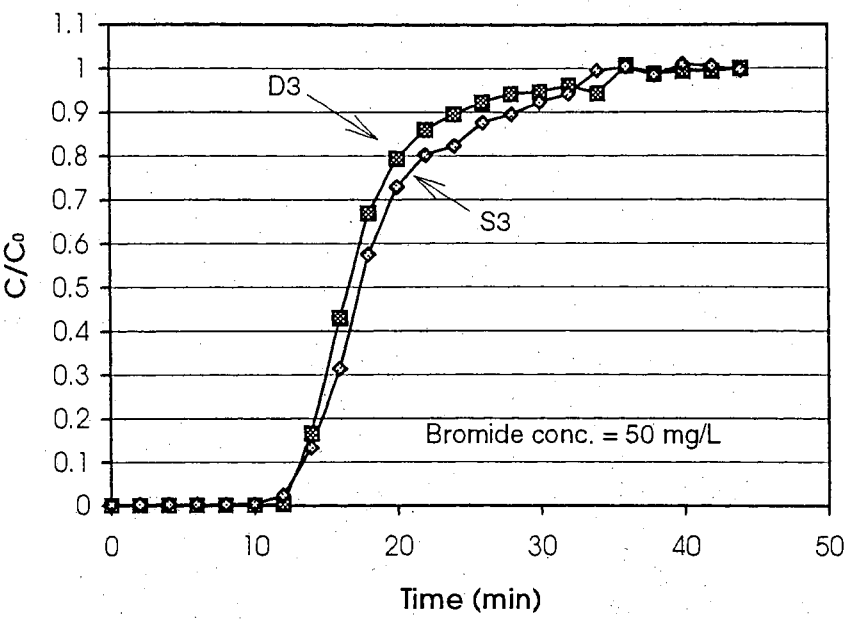
§4.4.3 Continuous Flow Columns D1 and D3

These two columns, with an influent medium amended with nitrate, had a pore space retention time of 1 day in the first 58 days and then 4 days in the rest of column operation. Several sets of different column operation conditions, as shown in Table 4-9, were employed during the life time of the columns. The raw data of influent and effluent TNT concentrations are illustrated in Figures 4-32 and 4-33.

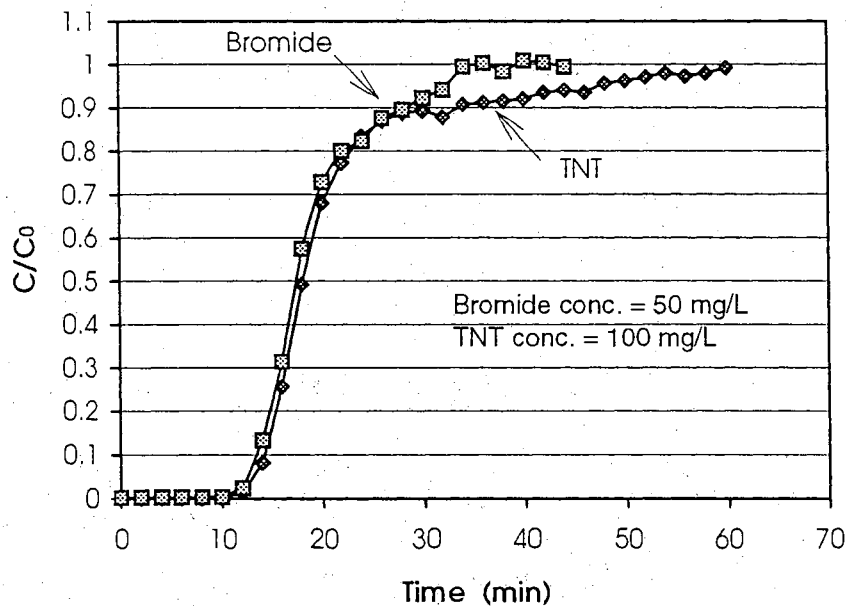
Table 4-9. Operation History of Columns D1 and D3

Phase No.	Time (day)	Retention Time (day)	Initial TNT Conc. (mg/L)	Primary Substrate Conc. (mg/L)
1	0 - 58	1	100	0
2	58 - 106	4	100	0
3	106 - 168	4	100	Ac = 30, Y.E. = 30
4	168 - 325	4	100	Ac = 300, Y.E. = 100
5	325 - 352	4	100	Ac = 90, Y.E. = 100
6	352 - 387	4	60	Ac = 90, Y.E. = 10
7	387 - 470	4	60	Ac = 180, Y.E. = 10
8	470 - 500	4	60	Ac = 30, Y.E. = 10

* Desorption of Column D3 was started on Day 343.



(a) Bromide breakthrough curves for columns D3 and S3



(b) Bromide and TNT breakthrough curves for column S3

Figure 4-31 Bromide and TNT breakthrough curves at flow rate of 4.0 ml/min

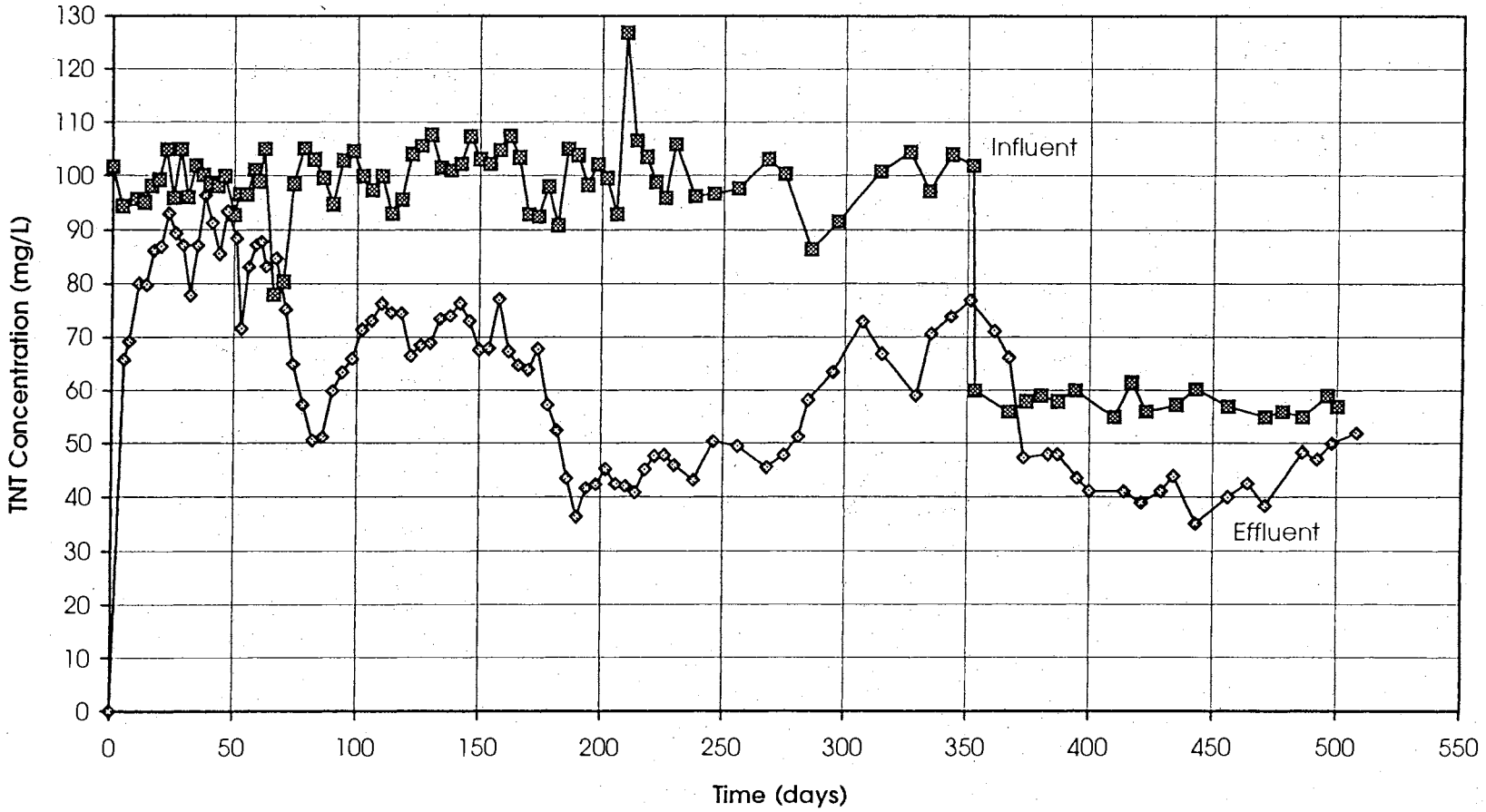


Figure 4-32 TNT concentration in influent and effluent of column D1

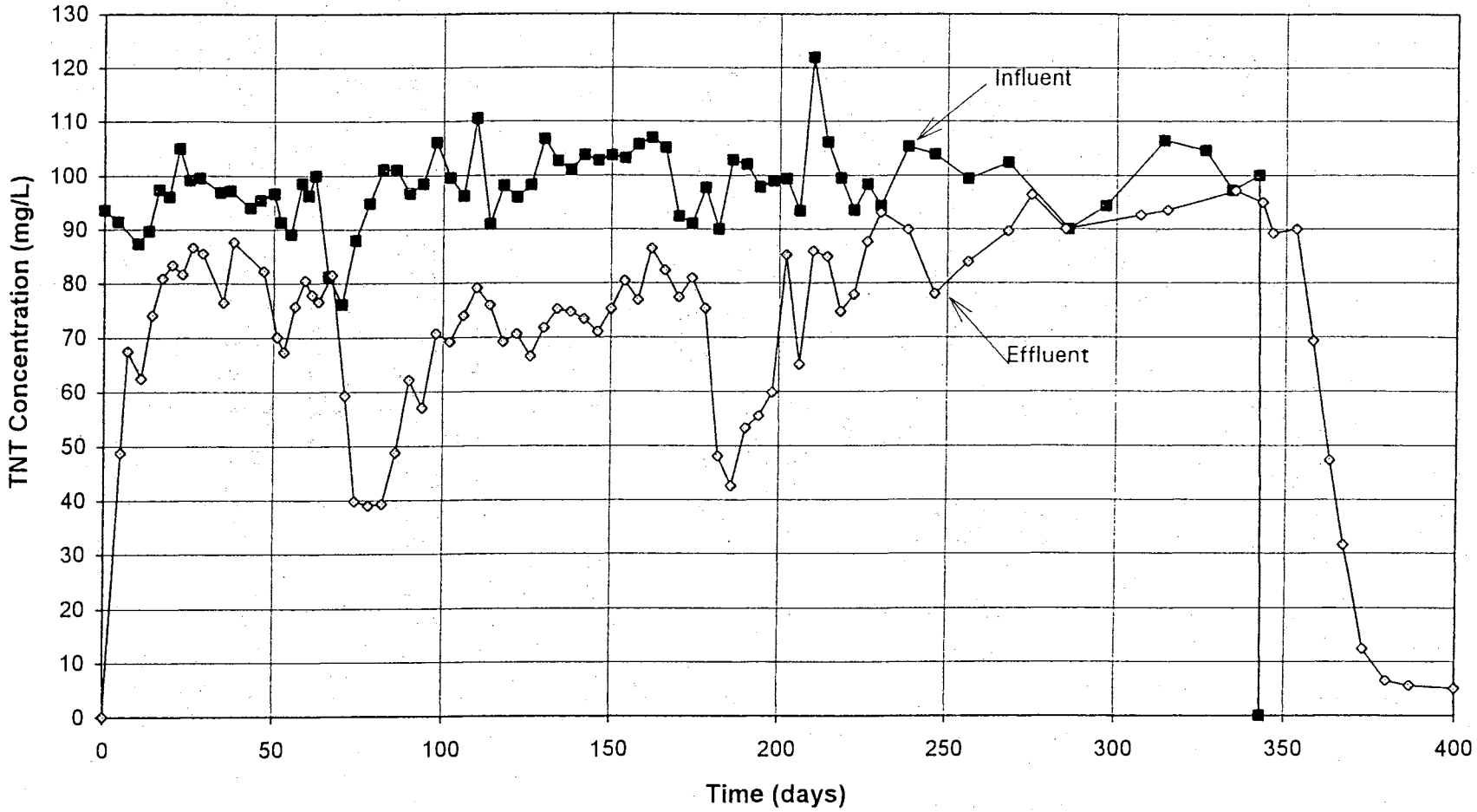


Figure 4-33 TNT concentration in influent and effluent of column D3

In the first, second, and third phases (day 0 - 168), there were no significant differences in TNT removal between the living column D1 and the abiotic column D3 (Figures 4-32 and 4-33), indicating that microbial TNT transformation was limited, likely due to the limited primary substrates in the column media. 30 mg/L acetate was added in the third phase and small amounts of organic matter might also exist in the aquifer materials, but these organic substances did not seem to be enough to support active microbial growth and significant TNT removal. In the second phase, the effluent TNT concentrations of both columns exhibited a big drop (day 70 - 90) followed by gradual recovery. This could be mainly attributed to two causes. First, increasing retention time from 1 day to 4 days resulted in significant increases in TNT loss, hence decreases in effluent concentrations, mainly due to adsorption and abiotic transformation. In aquifer columns, unlike in agitated batch reactors, adsorption and desorption tend to be a non-equilibrium process (Selim et al., 1995) and largely dependent on the flow rate or the retention time (Dunnivant et al., 1992; Jardine et al., 1992). Figure 4-31b shows negligible TNT adsorption at the flow rate of 4.0 ml/min, whereas Figure 4-8 indicates extensive adsorption at the flow rate of 0.007 ml/min with a time period of about 75 days to reach saturation. The gradual increases following the drop in effluent TNT concentrations in Figures 4-32 and 4-33 were indications that the TNT adsorption in aquifer materials was gradually reaching equilibrium. Secondly, a 20% decrease in column influent concentrations occurred around day 70, probably because of some mishandling in medium preparation. Note that the influent concentration then recovered, and this was reflected in the effluent concentration also.

In the first phase of column D3, adsorption equilibrium was nearly reached by day 20 (Figure 4-34), faster in comparison with column S4 because here the retention time was 1 day. By using the mass balance method as used for column S4 (see Section 4.1.4 and Appendix B) and assuming M_{ac} (the TNT mass in the aqueous phase accumulated in the column pore volume) was the same as in column S4, it is obtained that the total TNT loss due to adsorption and abiotic transformation in the first 20 days was about **15.7 mg** in column D3. This value is reasonably close to the total TNT mass loss in column S4 (13.6 mg). This is understandable because column D3 also experienced a "long-term" adsorption in the first 20 days of operation.

In phase 4, up to 60% of TNT was removed in column D1 in the presence of relatively high concentrations of primary substrates, as shown in Figure 4-35a. Here, 3 to 10 mg/L of ADNTs were detected in the effluent of this column. It should be noted of Figure 4-35 that although the theoretical hydraulic retention time was 4 days in this phase, it actually took 6 to 10 days for the column effluent to exhibit a response corresponding to a given change in the influent because the column "head space" and the void space in the bottom portion of the column and in tubing delayed the response in the effluent. This delay of response in column effluent can be comfirmed in abiotic columns D3 and S3 when these columns underwent desorption operations, as shown in Figure 4-45. This figure indicates that after the influent TNT concentration was shifted to zero abruptly, the effluent TNT concentration remained unchanged for 6 to 10 days before significant concentration decrease was detected. In following discussions on column

results, therefore, for a given data point of influent conditions, the corresponding effluent data point is considered 6 to 10 days behind when the theoretical retention time is 4 days.

During the end of phase 4 (day 280 - 325), the effluent TNT concentrations of this column exhibited a gradual increase, probably due to declining microbial activities which were partially inhibited by the relatively high pH resulting from denitrification. The pH values of the column effluent were about 9 in this period. For column D3, the abiotic control, an unusual decrease in effluent TNT concentrations was observed around day 180 - 200. Microbial activity might have been initiated in this column to some extent during this period since the abiotic column medium was only periodically amended with the biocide, sodium azide, until day 250. Before this day, the abiotic column was fed alternately with azide-amended medium for 4 - 6 days and then azide-free medium for 8 - 10 days, in consideration that sodium azide might interfere with TNT adsorption. After day 250, the medium for column D3 was always amended with 0.3 mg/L sodium azide and the abiotic condition was well maintained.

The fifth phase in column D1 exhibited a significant increase in effluent TNT concentrations (Figure 4-35b), resulting from a cut in primary substrate supplies.

In phases 6, 7, and 8, column D1 was operated under a lower influent TNT concentration, 60 mg/L. Changing the concentration of the primary substrate, acetate, resulted in corresponding changes in effluent TNT concentrations (Figures 4-36a, 4-36b, and 4-36c). These results are further discussed in Section 4.5.

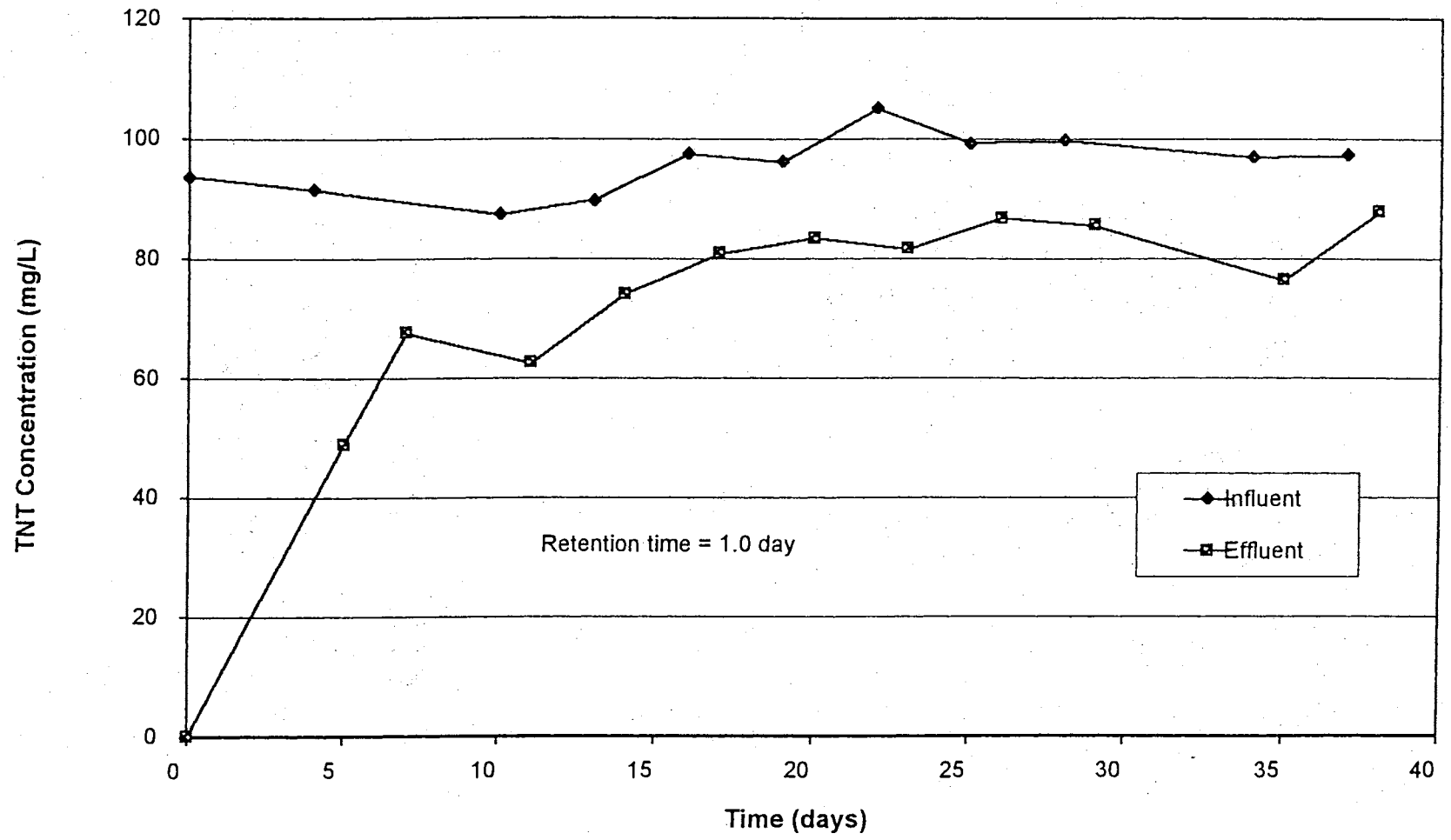
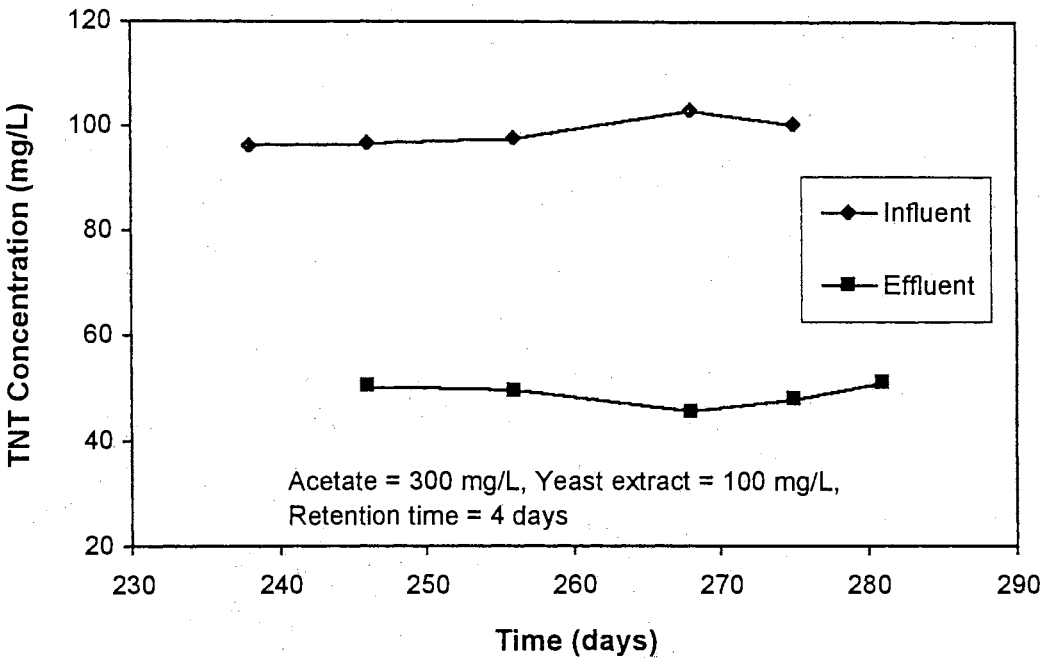
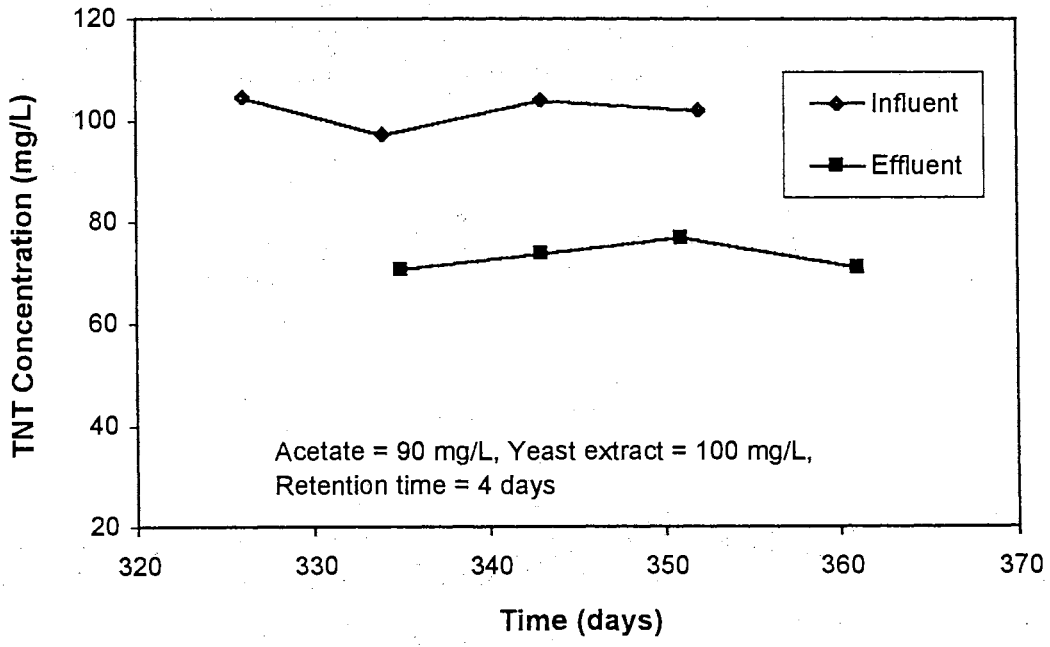


Figure 4-34 TNT concentration data in early stage of column D3



(a) TNT data in phase 4



(b) TNT data in phase 5

Figure 4-35 Effects of primary substrate concentrations on TNT removal in column D1 (initial TNT concentration = 100 mg/L)

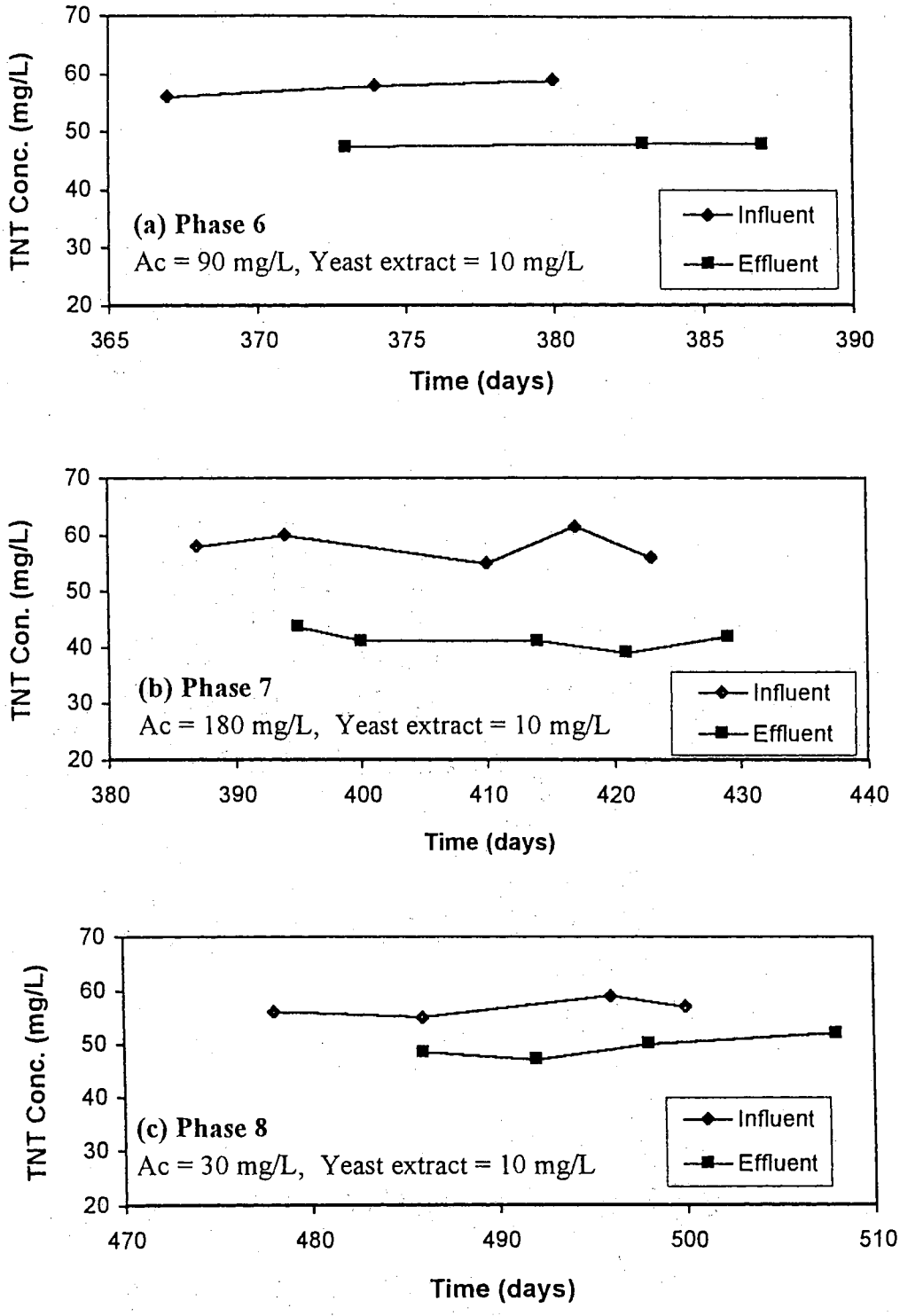


Figure 4-36 Effects of primary substrate concentrations on TNT removal in column D1 (TNT conc. = 60 mg/L, retention time = 4 days)

§4.4.4 Continuous Flow Columns S1 and S3

These two columns were amended with sulfate, with a pore volume retention time of 1 day in the first 15 days and 4 days thereafter. The column operation conditions, divided in several phases, are shown in Table 4-10. The raw data of influent and effluent TNT concentrations are illustrated in Figures 4-37 and 4-38.

Table 4-10. Operation History of Columns S1 and S3

Phase No.	Time (day)	Retention Time (day)	Initial TNT Conc. (mg/L)	Primary Substrate Conc. (mg/L)
1	0 - 15	1	100	0
2	15 - 63	4	100	0
3	63 - 125	4	100	Ac = 30, Y.E. = 30
4	125 - 282	4	100	Ac = 300, Y.E. = 100
5	282 - 309	4	100	Ac = 90, Y.E. = 100
6	309 - 344	4	60	Ac = 90, Y.E. = 10
7	344 - 378	4	60	Ac = 180, Y.E. = 10
8	378 - 427	4	60	Lact. = 90, Y.E. = 10
9	427 - 458	4	60	Ac = 30, Y.E. = 10

* Desorption of Column S3 was started on Day 300.

Like columns D1 and D3, columns S1 and S3 did not show significant differences in terms of TNT removal in the first three operation phases due to insufficient primary substrates in column S1 to support active microbial activities. The considerable drops in effluent concentration in abiotic column S3 observed in two periods (day 27 - 47 and day 137 - 157) could be attributed to the same causes as discussed earlier for columns D1 and

D3 because (1) these four columns were subject to the same manner of maintenance, and (2) the sulfate-amended columns were started 43 days later than the nitrate-amended columns so that the effluent concentration fluctuations in D3 and S3 actually occurred during the same time periods. Abiotic conditions were well maintained for column S3 after day 200 as described earlier.

During day 225 to 231, a stock solution of sodium sulfide was injected into column S3 to produce a total sulfide concentration of 50 mg/L in the column aqueous phase in order to test abiotic reactions in aquifer columns. This was done by injecting the sodium sulfide stock solution through a small plastic syringe installed on the syringe pump and letting the solution and the column medium mix at the entrance (bottom) of the column. This practice resulted in a sharp decrease in the column effluent concentrations during day 235 to 250 (Figure 4-38), indicating that TNT was significantly transformed by abiotic reactions in the presence of sulfide, as observed in the abiotic reaction experiments discussed in Section 4.2. ADNT concentrations of up to 12 mg/L were detected during this period.

The fourth phase of column S1 (day 125 - 282) saw a large removal of TNT because of the high concentrations of primary substrates. Decreased concentrations of acetate in the fifth phase resulted in decreased TNT removal and increased effluent concentrations.

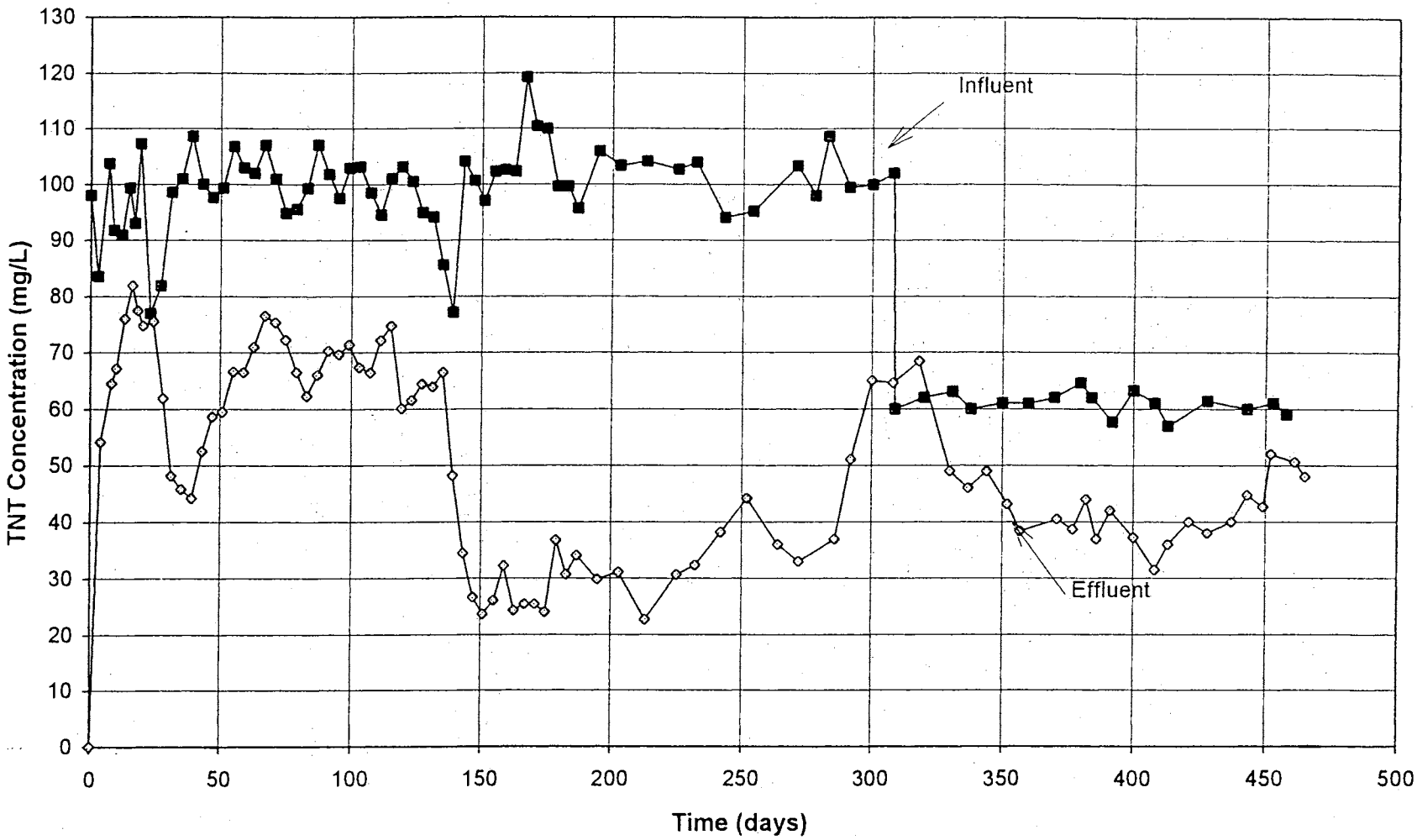


Figure 4-37 TNT concentration in influent and effluent of column S1

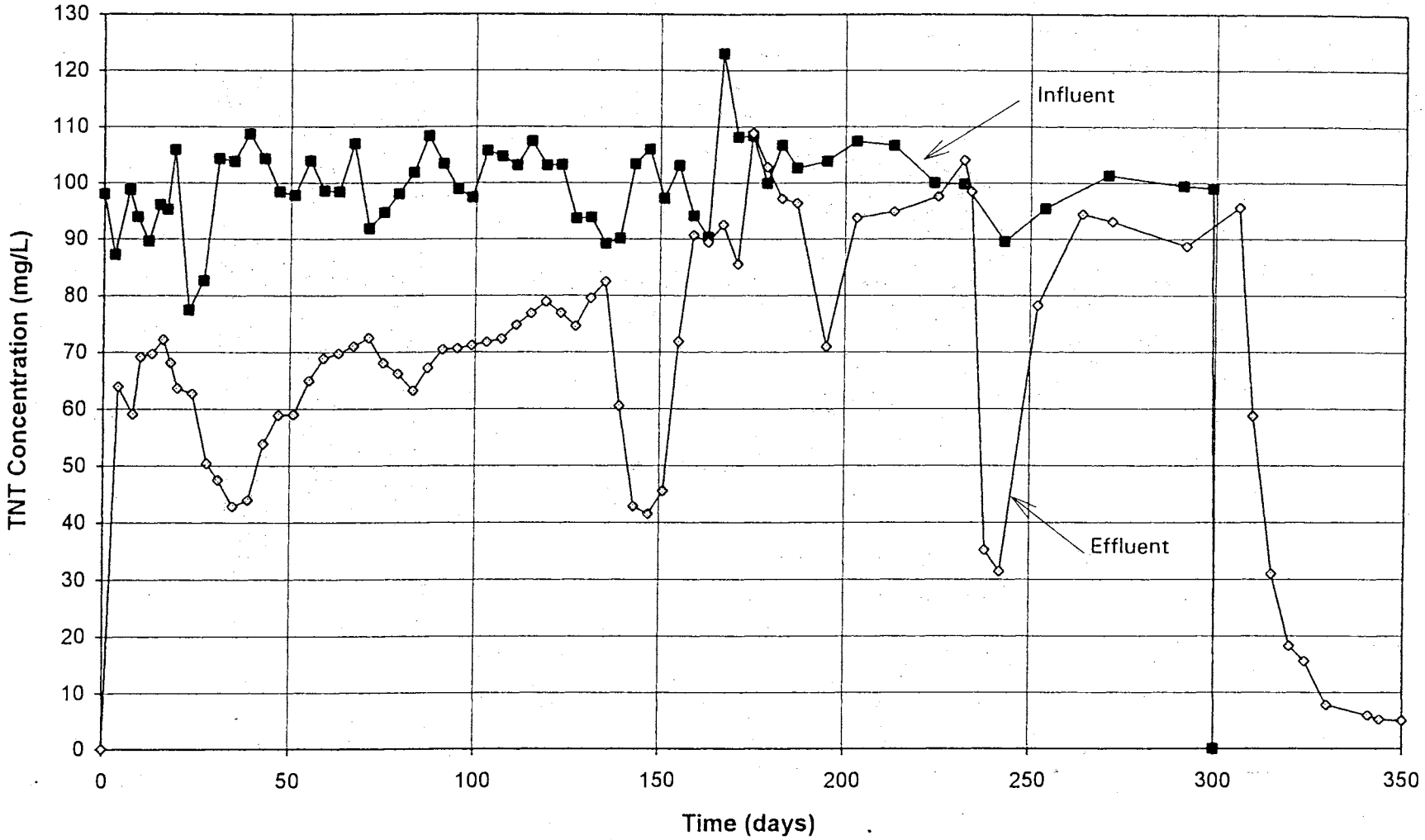


Figure 4-38 TNT concentration in influent and effluent of column S3

Phases 6, 7, 8, and 9 had a constant influent TNT concentration of 60 mg/L and varied concentrations of primary substrates, with corresponding changes in the effluent TNT concentrations. Phase 8, with 90 mg/L lactate, gave lower effluent concentrations than phase 6 in which 90 mg/L acetate was used as the primary substrate. The carbon content in the lactate molecule (40.4%) is almost the same as in the acetate molecule (40.7%), yet TNT removal was enhanced by using lactate as the carbon source. The reason may be that more sulfate-reducing bacteria tend to use lactate rather than acetate as the primary substrate, as discussed in Section 2.4.3.

In column S1, which was expected to produce a sulfate-reducing environment, sulfate reduction was never significant enough to be confirmed by IC measurement of sulfate. Because the sulfate concentration in column media was relatively high (80 to 500 mg/L) and a 10- or 20-fold dilution was often required before it was measured by IC, and because the lower limit of sulfate detection on IC was about 1 mg/L, even a sulfate concentration change of up to 20 mg/L in original samples might fail to be detected in 20-fold dilutions. Therefore, occurrence of sulfate reduction in column S1 could not be totally excluded although it was, if any, clearly very minor. The inhibition of sulfate reduction, which was also observed in batch reactors, was likely to be the consequence of high TNT concentrations, as discussed in the literature review.

Figure 4-39, derived from data in Figure 4-37, shows the effluent TNT concentrations of column S1 responding to different primary substrate concentrations in the influent. It is clear in this figure that TNT biotransformation was enhanced by higher primary substrate concentrations. These results will be further discussed in Section 4.5.

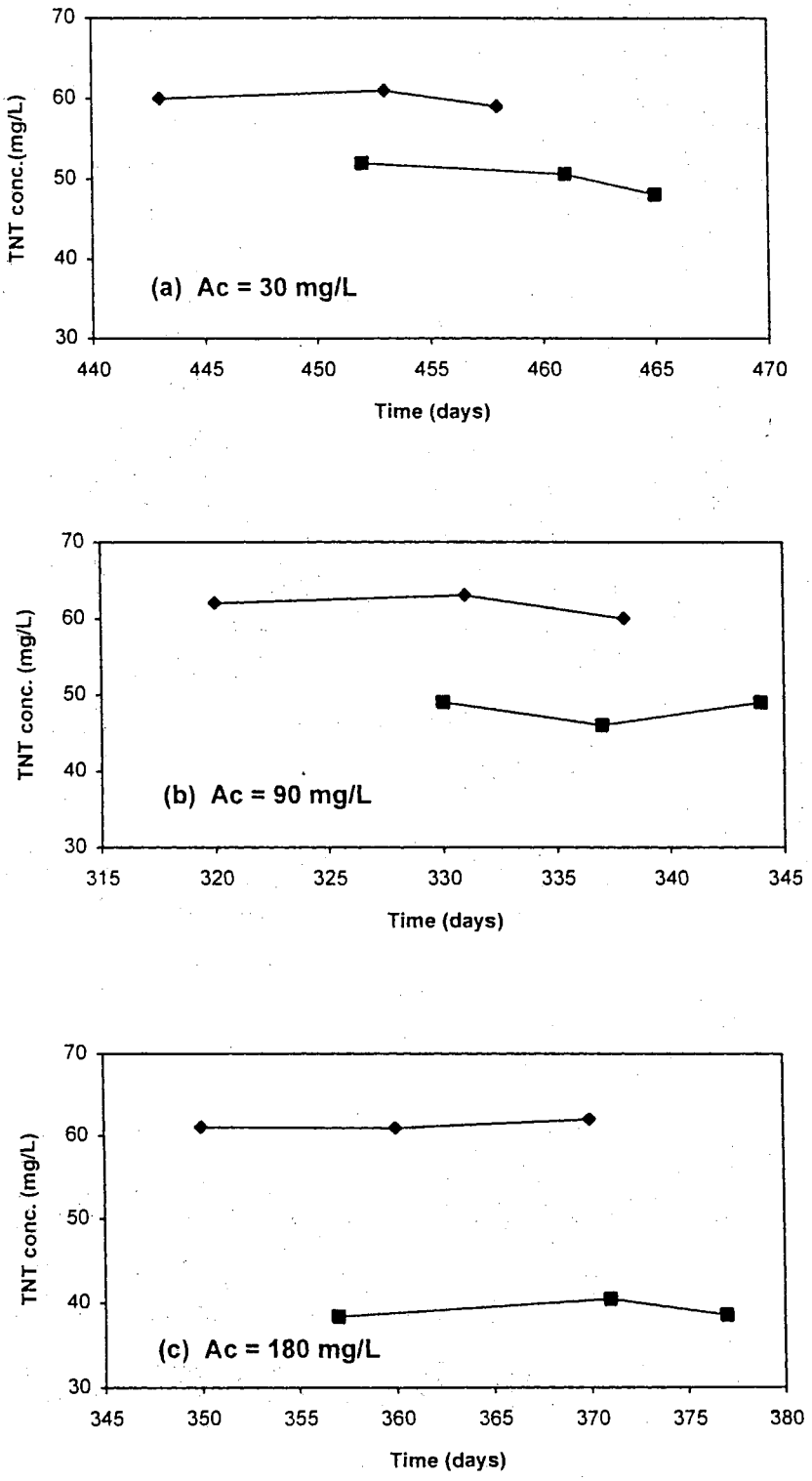


Figure 4-39 Effects of primary substrate concentrations on TNT removal in column S1 (TNT conc. = 60 mg/L, retention time = 4 days)

§4.4.5 Batch-fed Columns D2, S2, and M

Several aquifer column reactors were operated under batch-fed mode, as indicated in Section 3.2.4.4. The operation history of batch-fed column D2 is presented in Table 4-11, and the TNT measurement is shown in Figure 4-40.

Table 4-11. Operation History of Column D2

Phase No.	Time (day)	Retention Time (day)	Initial TNT Conc. (mg/L)	Primary Substrate Conc. (mg/L)
1	0 - 16	4	30	Ac = 300, Y.E. = 10
2	16 - 88	4	100	Ac = 300, Y.E. = 10
3	88 - 100	4	100	Ac = 90, Y.E. = 10
4	100 - 121	7	100	Ac = 90, Y.E. = 10
5	135 - 156	7	60	Ac = 90, Y.E. = 10
6	156 - 180	12	60	Ac = 90, Y.E. = 10
7	180 - 192	4	60	Ac = 90, Y.E. = 10
8	192 - 196	2	60	Ac = 90, Y.E. = 10
9	196 - 212	4	60	Ac = 30, Y.E. = 10
10	212 - 228	4	60	Ac = 180, Y.E. = 10

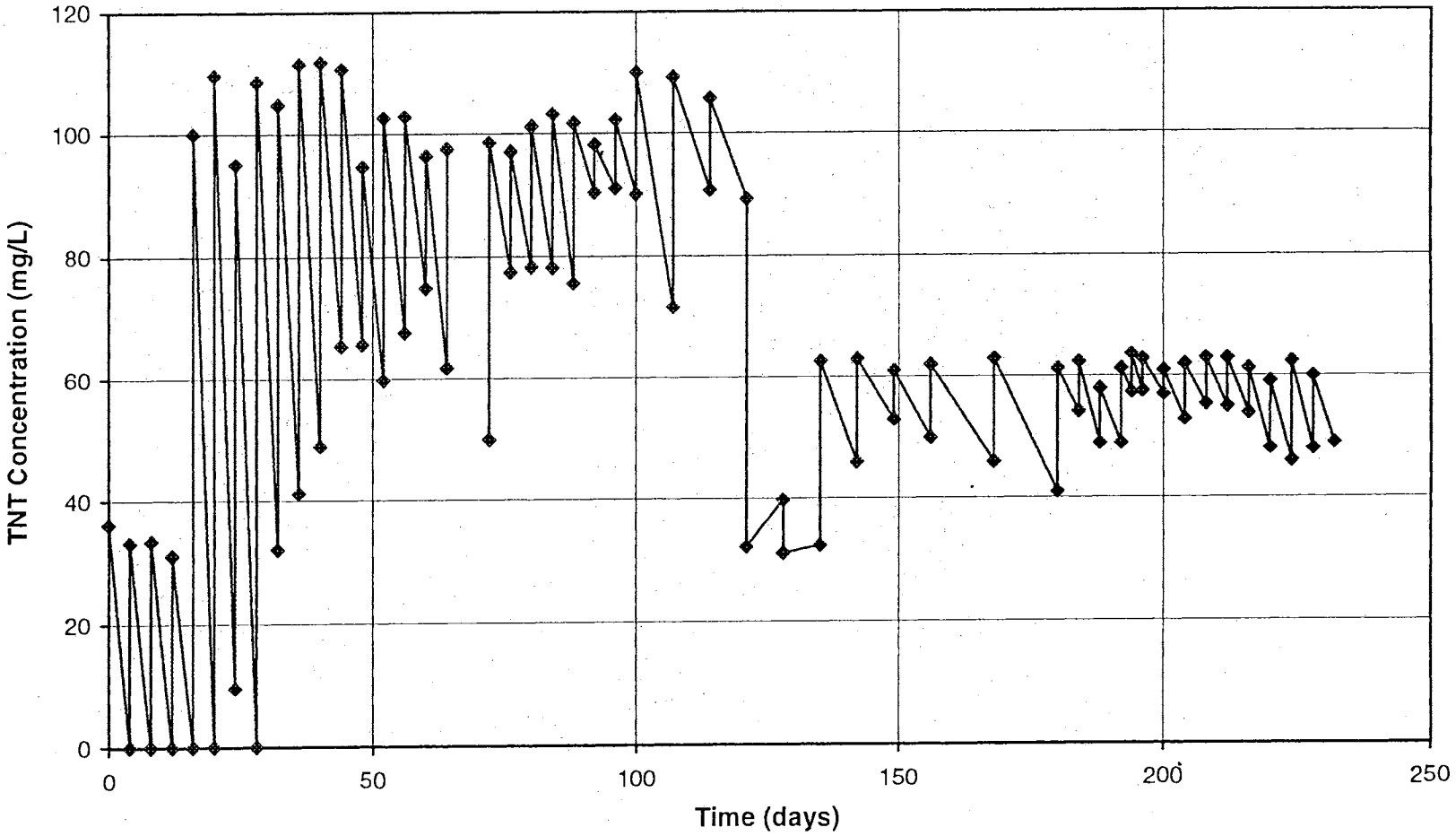


Figure 4-40 TNT concentration changes in column D2

In the first 16 days, column D2 was fed with a medium with relatively low TNT concentrations to acclimate the microorganisms. In the second phase it took a long time period (until day 76) for the column effluent to reach a steady state, possibly indicating the process of reaching the equilibrium of long-term TNT adsorption, as discussed in Section 4.1.4. Various operation conditions, as shown in Table 4-11, were employed to examine the effects of different factors, including primary substrate concentration, retention time, and initial TNT concentration, on TNT transformations. Figure 4-41 shows the TNT removal in column D2 under different influent conditions.

Column S2 was started with a TNT concentration of 30 mg/L in the early stage followed by higher initial TNT concentrations, as illustrated in Table 4-12 and Figure 4-42.

Table 4-12. Operation History of Column S2

Phase No.	Time (day)	Retention Time (day)	Initial TNT Conc. (mg/L)	Primary Substrate Conc. (mg/L)
1	0 - 16	4	30	Ac = 300, Y.E. = 100
2	16 - 44	4	100	Ac = 300, Y.E. = 100
3	44 - 76	4	30	Ac = 300, Y.E. = 100

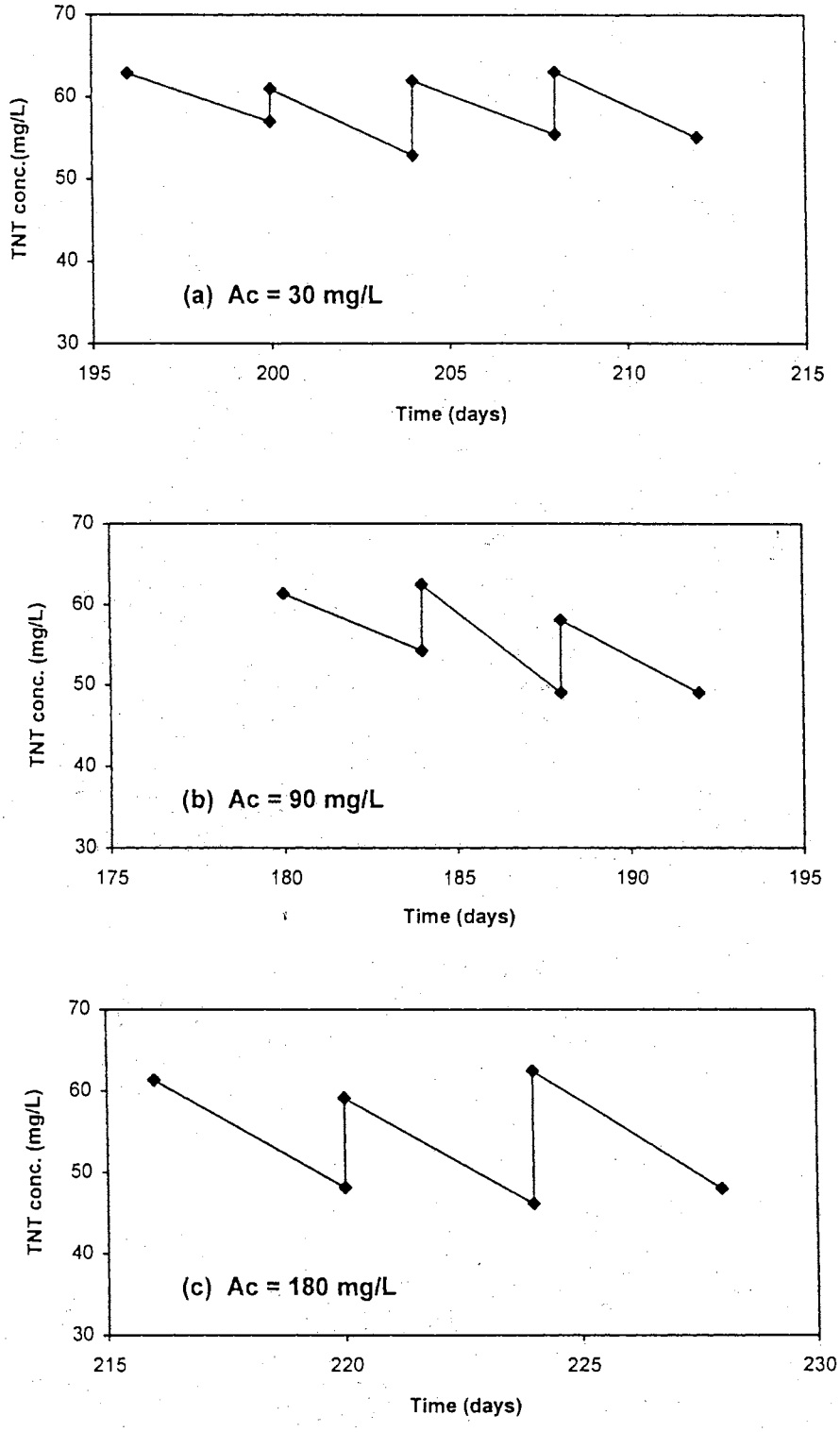


Figure 4-41 Effects of primary substrate concentrations on TNT removal in column D2 (TNT conc. = 60 mg/L, retention time = 4 days)

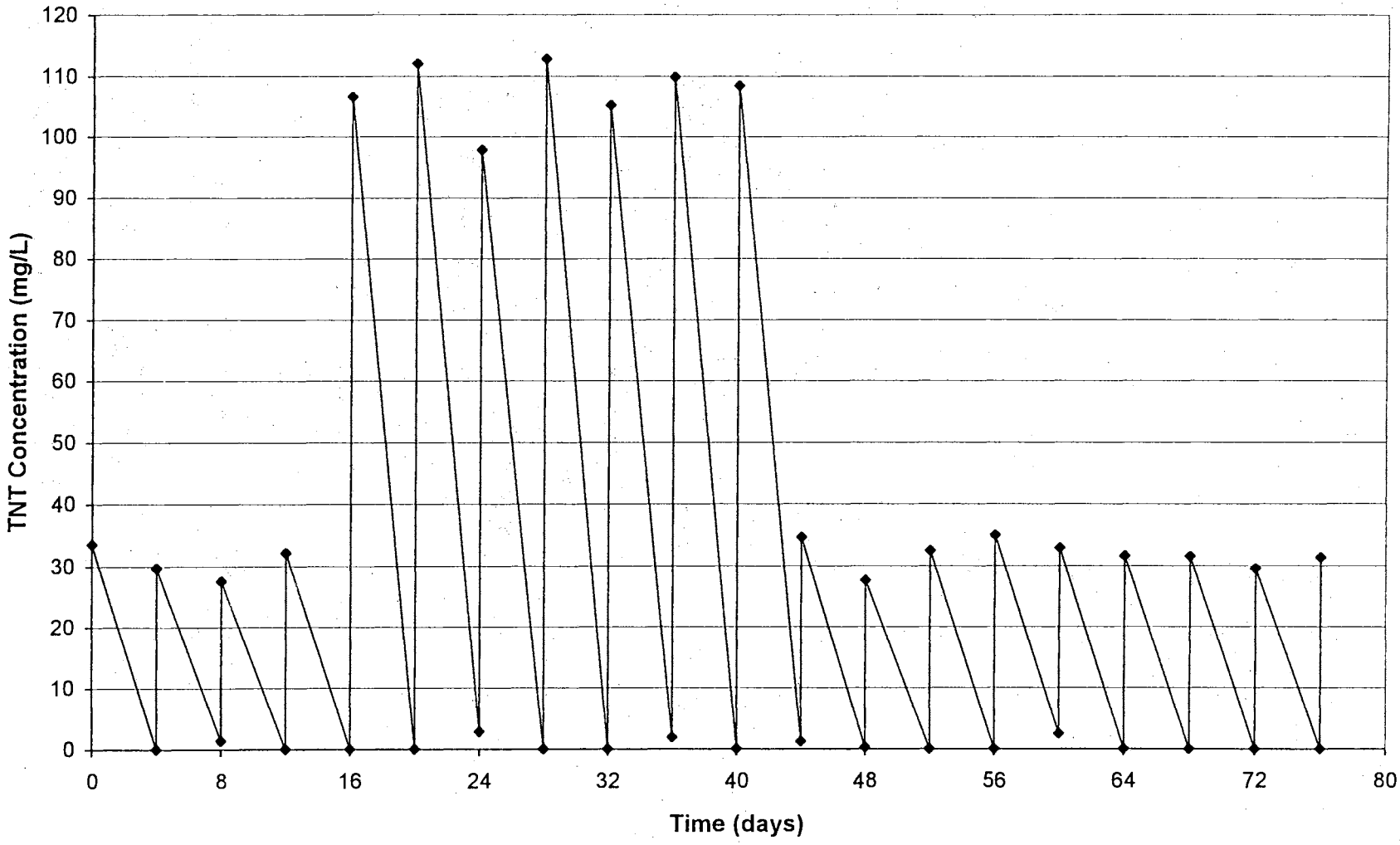


Figure 4-42 TNT concentration changes in column S2

In phase 1, TNT was 100% removed in every operation cycle (4 days). Unlike in column S1 where sulfate reduction was inhibited, most likely by high concentrations of TNT, here active sulfate reduction was observed in the first phase in which the initial TNT concentration was 30 mg/L. Figure 4-43 shows the consumption of electron acceptors (sulfate) and utilization of carbon sources (acetate). By day 8, visual observation showed that the aquifer material in the column had turned dark, an indication of precipitation of metal sulfides in the aquifer material. The TNT removal in the second and the third phases remained as high as 100% and ADNT concentrations of up to 29 mg/L were detected. However, neither substrate (acetate) utilization nor electron acceptor consumption was observed in any significant amount in these phases. TNT transformations in this period were considered mainly the results of abiotic reactions of TNT with sulfide. Batch experiments showed that this type of abiotic reaction could occur rapidly and totally remove 100 mg/L TNT in a few hours. Microbial sulfate reduction did not recover in the third phase even when the initial TNT concentration was decreased back to 30 mg/L. The reasons may include (1) relatively large amounts of TNT and its intermediates were adsorbed in the aquifer material during the second phase and some of them remained in the column through the third phase, and (2) sulfide toxicity could inhibit the microorganisms responsible for sulfate reduction. It should be noted that the TNT removal in this column was partly due to adsorption since the results of column D2 showed that it took about 70 days for the column effluents to reach adsorption equilibrium.

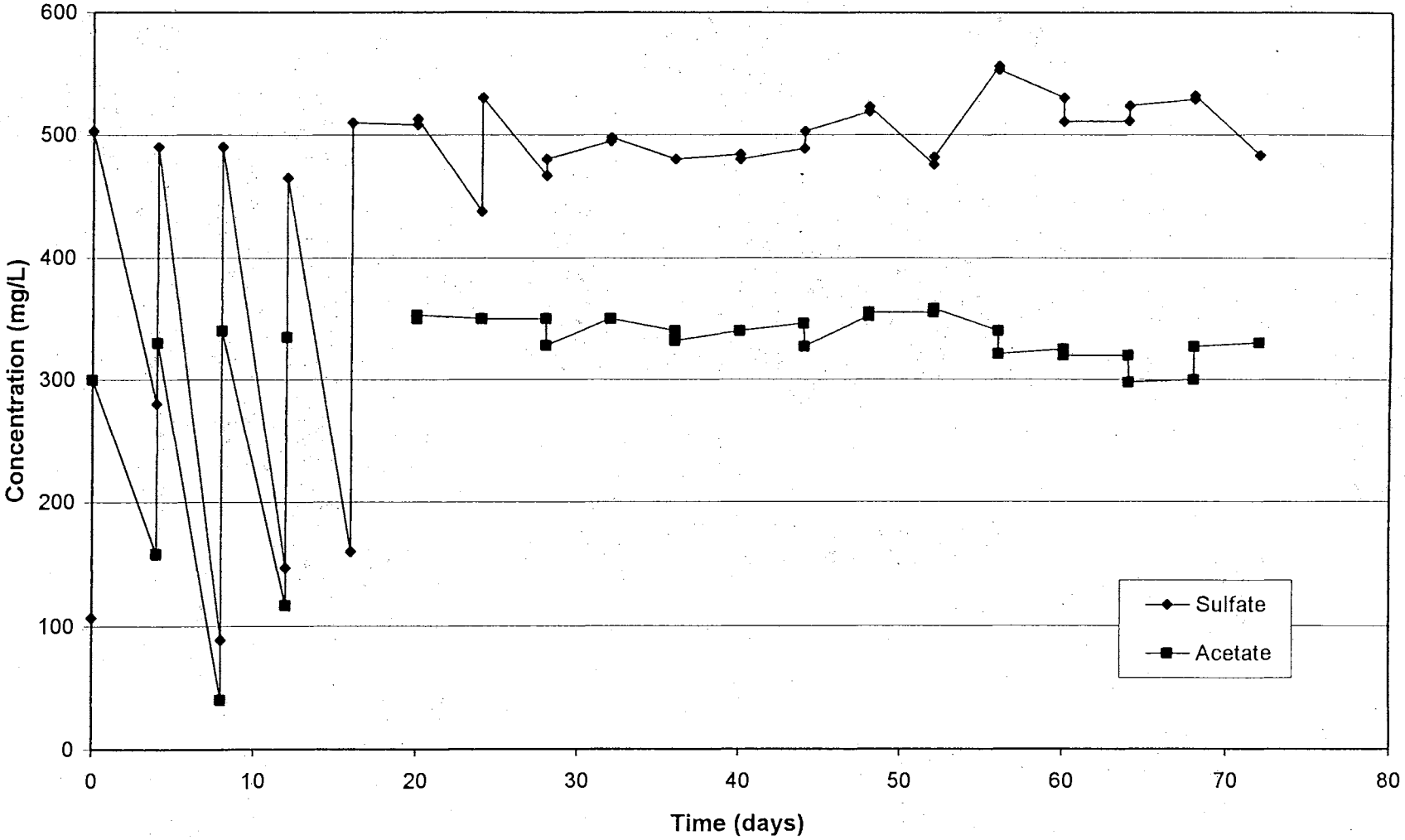


Figure 4-43 Changes in sulfate and acetate concentrations in column S2

Table 4-13 and Figure 4-44 illustrate the operation of Column M, the batch-fed methanogenic column.

Table 4-13. Operation History of Column M

Phase No.	Time (day)	Retention Time (day)	Initial TNT Conc. (mg/L)	Primary Substrate Conc. (mg/L)
1	0 - 20	7	10	Ac = 90, Y.E. = 10
2	20 - 35	7	20	Ac = 90, Y.E. = 10
3	35 - 56	7	30	Ac = 90, Y.E. = 10
4	56 - 80	12	30	Ac = 90, Y.E. = 10
5	80 - 92	4	30	Ac = 90, Y.E. = 10
6	92 - 96	2	30	Ac = 90, Y.E. = 10
7	96 - 112	4	30	Ac = 30, Y.E. = 10
8	112 - 164	4	60	Ac = 180, Y.E. = 10

In the early stage (phases 1, 2, 3, and 4) of this column, relatively low concentrations of TNT and relatively long retention times were employed in order to acclimate the microorganisms. In the later stages, the TNT removal was characterized by increasing effluent concentrations. This observation indicated that the TNT removal was partially due to adsorption and that gradually saturated adsorption sites in aquifer materials resulted in the decrease of TNT removal and increase of effluent TNT concentrations. Nevertheless, TNT transformation in this column was also partially attributed to microbial activity which was indicated by substrate utilization and methane production, as discussed in Section 4.5.1.

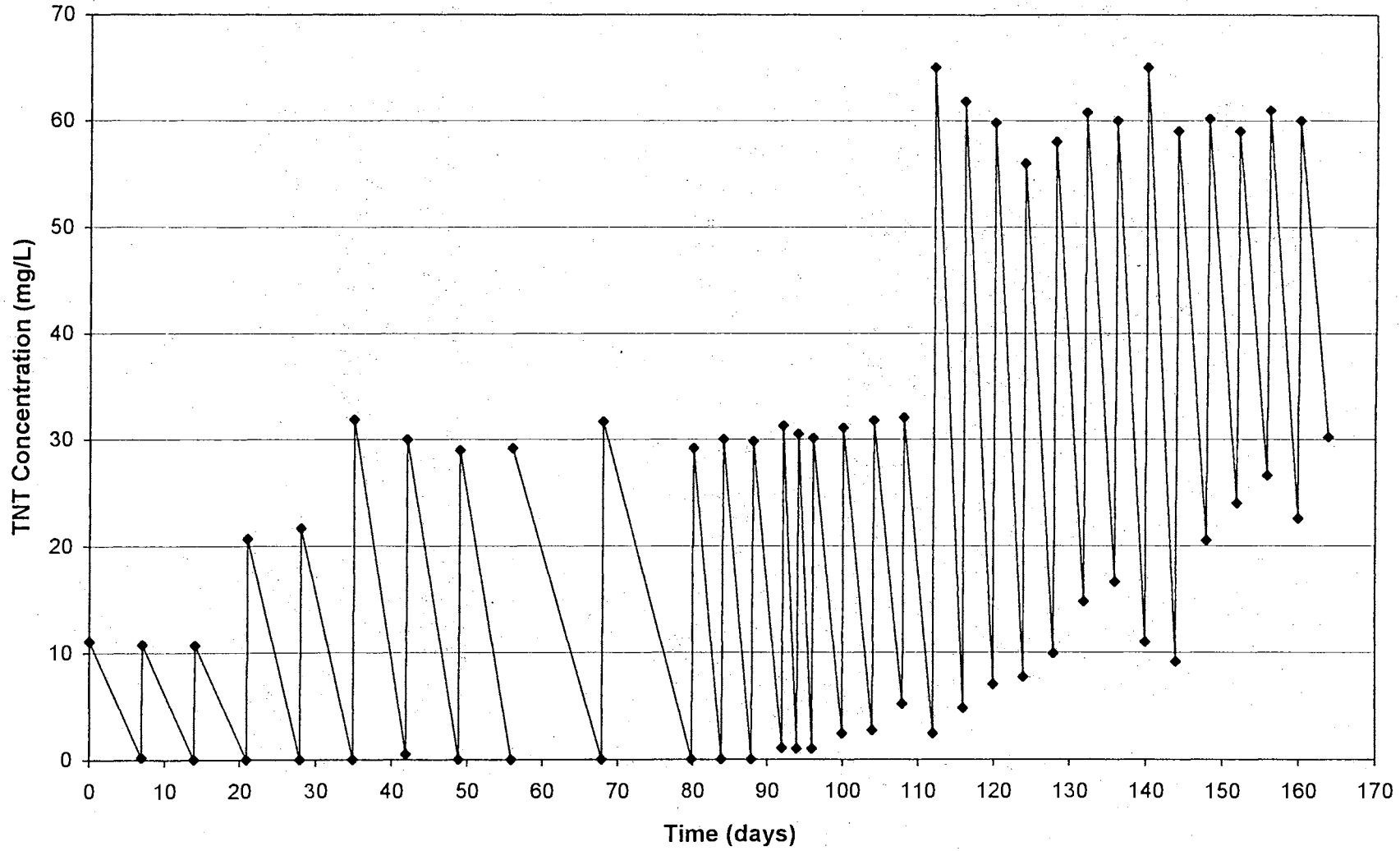
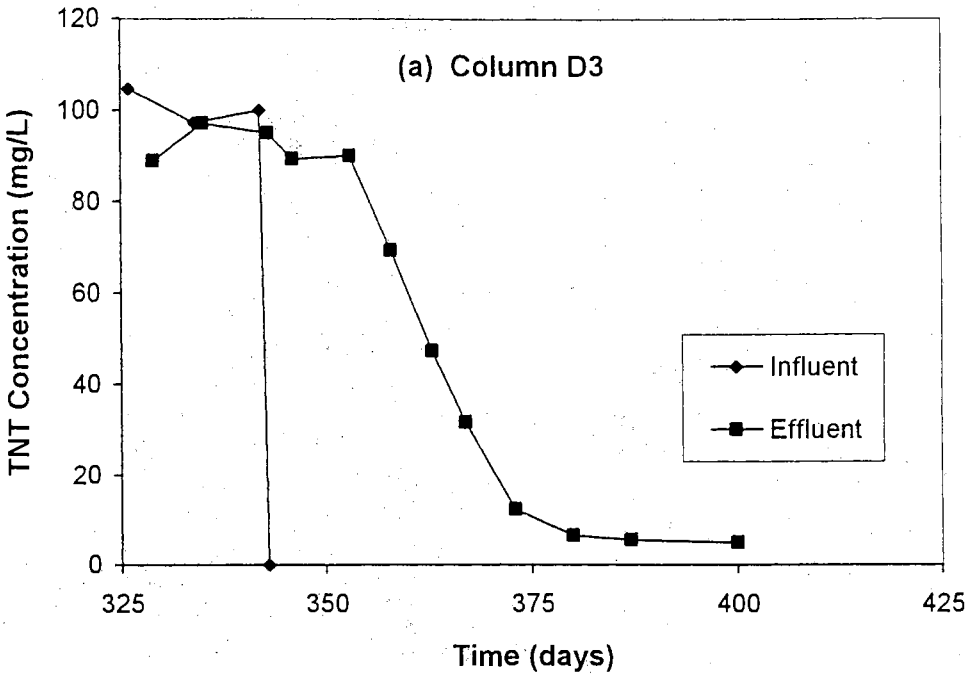


Figure 4-44 TNT concentration changes in column M

§4.4.6 Desorption of TNT in Aquifer Columns

Figure 4-45 shows the long-term process of TNT desorption from columns D3 and S3. After the aquifer materials had been desorbed with TNT-free media for 57 days, the TNT concentration in column effluents reached below 6 mg/L. By integrating the area under the curve of the TNT effluent concentration (Figure 4-45), it was estimated that about 7 mg TNT was desorbed from each column (data corrected with the TNT mass accumulated in the aqueous phase in columns, which was about 10.1 mg according to calculations in Appendix B). In the short-term desorption for column S4, on the other hand, measuring the TNT concentration in the acetonitrile extracts revealed that 5.8 mg TNT was recovered from the column aquifer material. Therefore, a significant amount of TNT was not recovered in each of these three columns since the total TNT sink in a column was about 13.6 mg, according to the mass balance calculation described earlier. Prior to desorption, all these three columns underwent a process considered "long-term adsorption", i.e. an adsorption process that occurred in a relatively long operation period (75 to 343 days) and a relatively long pore volume retention time (4 days). Some of the TNT loss in this process may not be recoverable because of irreversible adsorption and/or abiotic conversion, as indicated earlier.



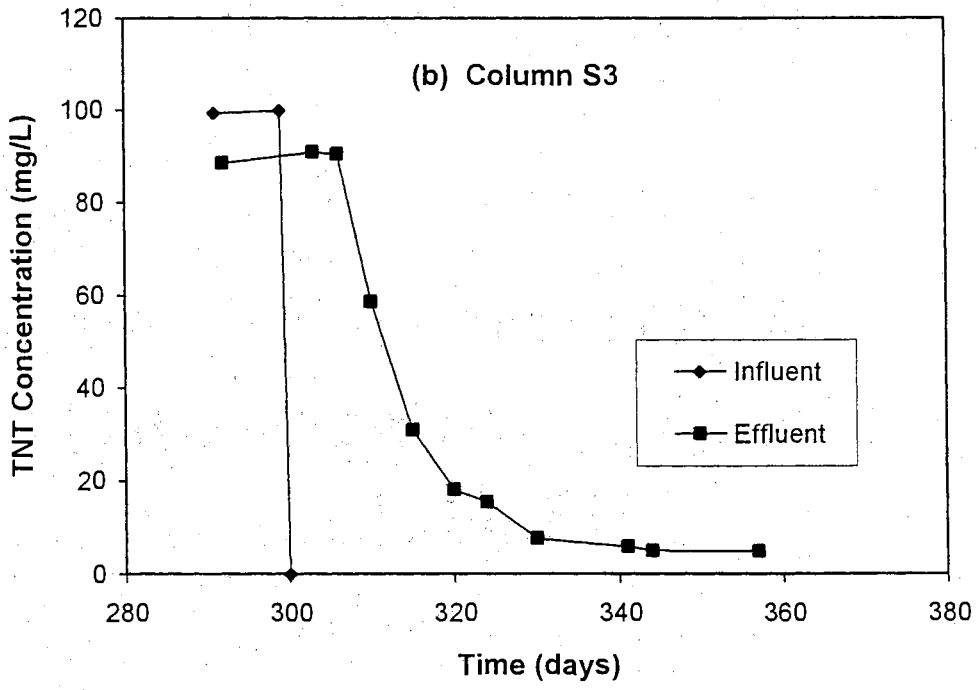


Figure 4-45 Aquifer column desorption (flow rate = 0.007 ml/min)

§4.5 Comparison of Column Results under Different Conditions

This section includes comparisons among TNT removal results derived from aquifer columns operated under different conditions in order to examine the effects of electron accepting conditions and primary substrate concentrations on TNT transformations in aquifer materials. Also, a comparison between continuous and batch-fed columns is made to present a discussion on column methodology.

§4.5.1 Aquifer Columns with Different Electron-accepting Conditions

Figures 4-36 and 4-39 show the TNT removal in columns D1 and S1, respectively, under various conditions. TNT removal under a given set of operation conditions, after reaching steady state, is calculated as follows:

$$\text{TNT removal} = \text{Avg}[(\text{TNT conc. in influent}) - (\text{TNT conc. in effluent})] \qquad (4.11)$$

The average removal is obtained from several pairs of influent/effluent data points. Note that the effluent concentraion data usually lagged 6 to 10 days behind the corresponding influent in the continuous columns because of the hydraulic delay, as indicated earlier.

Based on the data in Figures 4-36 and 4-39 and the above equation, a comparison of TNT removal for columns D1 and S1 can be made, as presented in Figure 4-46. A statistical comparison (Student t-test) was conducted to examine the significance of the difference between TNT removal data of these two columns (Table 4-14 and Appendix J).

Table 4-14. TNT Removal in Columns D1 and S1 with Retention Time of 4 Days

Substrates (mg/L)	TNT: 60 Ac: 30 Y.E.: 10	TNT: 60 Ac: 90 Y.E.: 10	TNT: 60 Ac: 180 Y.E.: 10	TNT:100 Ac: 90 Y.E.: 100	TNT:100 Ac: 300 Y.E.: 100
Denitrifying column D1:					
TNT removal (mg/L)	7.4	9.9	16.8	28.8	49.9
No. of inf./eff. data pairs *	4	3	5	4	5
Sulfate-reducing column S1:					
TNT removal (mg/L)	9.8	13.7	22.1	34.4	61.2
No. of inf./eff. data pairs	3	3	3	3	5
Comparison of TNT removal					
Statistic t value	1.92	2.04	2.27	2.05	2.94
t with 95% confidence	2.571	2.776	2.447	2.571	2.306
Significant difference ?	No	No	No	No	Yes

* An inf./eff. data pair refers to an influent TNT concentration and the corresponding effluent TNT concentration used to calculate the TNT removal.

From this table, it can be seen that although the TNT removal in column S1 seemed to be about 20% higher than that in column D1 (Figure 4-46), the difference was not statistically significant except for one set of substrate conditions (TNT = 100 mg/L, Ac = 300 mg/L). Factors that could contribute to the similarity of these two columns may include the following. First, active sulfate reduction, which could have produced significant amounts of bisulfide and resulted in rapid abiotic transformations of TNT, was never detected in column S1. However, the primary substrate was still utilized to the same extent as in column D1. This may indicate that other microorganisms, rather than sulfate reducers, played important roles in TNT transformation in column S1. Among the possible candidates of these organisms are iron-reducing bacteria and clostridia. These

bacteria have been demonstrated to be capable of transforming various nitroaromatic compounds (Heijman et al., 1995; Gorontzy et al., 1993). Heijman and co-workers (1995) reported that microbial iron-reducing activity in aquifer columns was able to completely transform nitrobenzenes to corresponding amino compounds in as short as 15 hours when the parent compound concentration was 250 μM and acetate concentration was about 10 mg/L. Secondly, the TNT removals in column D1 and in column S1 appear to be similar because only the column effluents were monitored. Before the primary substrate was depleted at some point in the column, the TNT transformation rate might have been different in these two columns. However, this possible difference was not confirmed because there were no sampling ports along the length of the glass columns.

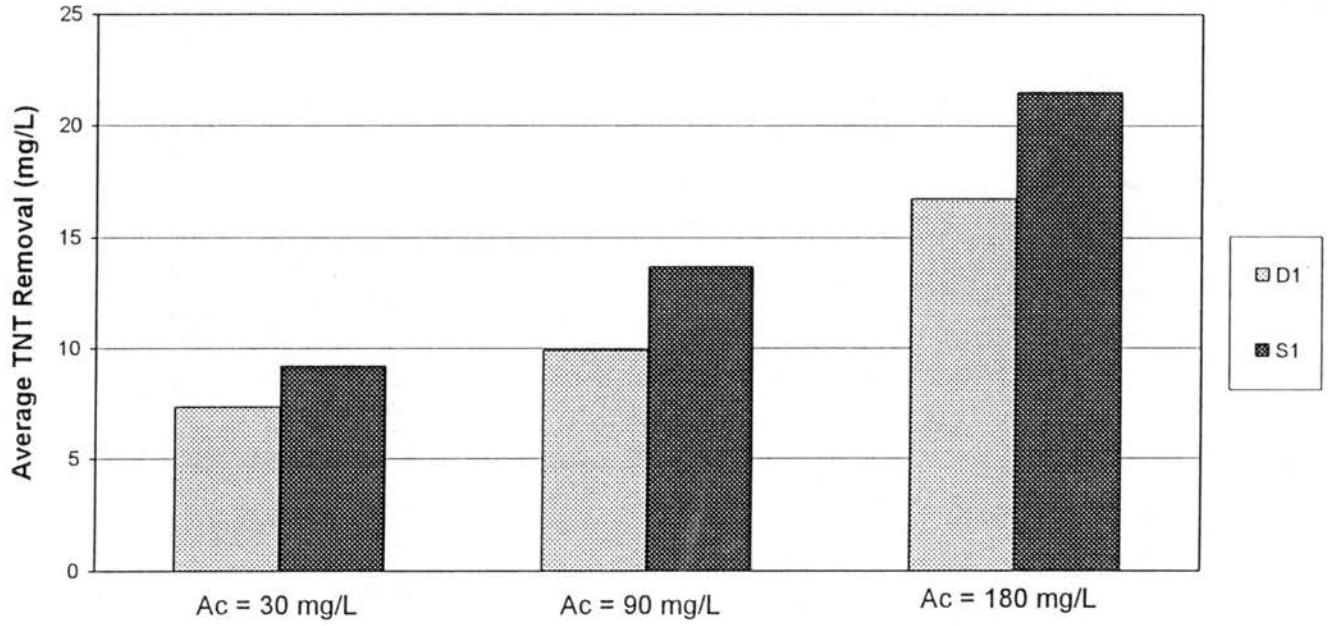


Figure 4-46 Comparison of TNT removal in columns D1 and S1
(TNT conc. = 60 mg/L, retention time = 4 days)

Columns M and D2, both batch-fed, were operated under methanogenic and denitrifying conditions, respectively. It should be noted that there were a few inconsistencies between the operation conditions of these two columns that made their comparison somewhat difficult. Based on the effluent data points in Figure 4-44, it can be understood that the TNT adsorption in column M had not reached equilibrium when the column operation ceased. Therefore, TNT removal in this column can be accounted for by adsorption to a significant extent. On the other hand, it appeared that TNT adsorption was in equilibrium most of the time in column D2. The substrate conditions for these two columns, as shown in Table 4-15, were not exactly identical either. Nevertheless, a preliminary comparison for TNT removal in columns M and D2 can be performed on the basis of mass balance calculations as follows.

From day 112 to day 164 in column M when the influent TNT concentration was 60 mg/L, the total TNT mass injected into and discharged from the column was 31.7 mg and 8.3 mg, respectively, according to Figure 4-44. On the basis of long-term adsorption experiments (Appendix B), the total TNT loss due to adsorption and abiotic reactions, before equilibrium was reached, was approximately 13.6 mg (or about 18.8% of the total input of TNT) in an aquifer column when the influent TNT concentration was 100 mg/L. This value (13.6 mg) can be used as an overstated or conservative estimate for column M because the influent TNT concentration for this column was 10, 20, 30, or 60 mg/L rather that 100 mg/L and because the column did not reach adsorption equilibrium. If the percentage 18.8% was used for estimation, then the total TNT loss in non-biological processes in column M was 6.0 mg from day 112 through day 164. From day 168 to day

232 in column D2 when the influent TNT concentration was also 60 mg/L, on the other hand, the total TNT mass injected into and discharged from the column was 37.3 mg and 31.2 mg, respectively, according to Figure 4-40. The column was apparently in adsorption equilibrium in this time period, and TNT was likely not being removed by physical adsorption. The TNT loss due to abiotic reactions, according to data from abiotic columns D3, S3, and S4, might be in the range of 4 to 10% of the total TNT input, or 1.5 to 3.9 mg. Therefore, microbial TNT removal data can be derived from the above mass balance procedures. Detailed calculations of mass balance are presented in Appendix J and the results are shown in Table 4-15.

Table 4-15. TNT Removal in Columns M and D2

Column	M	D2
Time period	day112 - day 164 (52 days)	day 168 - day 232 (64 days)
Influent TNT conc.	60 mg/L	60 mg/L
Hydraulic retention time	4 days	2 to 12 days average 5.37 days
Total acetate input *	96.2 mg	63.3 mg
Total acetate utilization	15.7 mg	57.0 mg
Influent acetate conc.	180 mg/L	30, 90, or 180 mg/L
Total TNT input	31.7 mg	37.3 mg
Total TNT discharged	8.3 mg	31.2 mg
Adsorption and abiotic removal of TNT	6.0 to 13.6 mg	1.5 to 3.9 mg
Microbial removal of TNT	9.8 to 17.4 mg	2.2 to 4.6 mg
Percentage of microbial TNT removal **	31 to 55%	6 to 12%

* A parameter referred to as "total" represents the cumulative total mass during the indicated time period.
** This percentage is defined as [microbial TNT removal/total TNT input]×100%.

The data in Table 4-15 show that the total input and the influent concentration of acetate were higher for column M while the total acetate utilization was significantly higher in column D2. Other operation conditions were reasonably similar in both columns during the indicated time periods. Under these conditions, the percentage of microbial TNT transformation in column M was several times higher than that in column D2 even though the TNT loss due to non-biological processes in column M may have been overestimated. This result means that the TNT biotransformation rate was significantly higher in column M than in column D2 if we consider the fact that the average hydraulic retention time was even slightly longer for D2 than for M.

This finding is very different from the observations derived from the previously discussed batch reactors (Section 4.3) in which no aquifer materials were present, but it is suprisingly consistent with what Krumholz and co-workers (1997) observed in batch reactors containing aquifer materials. These batch studies revealed a TNT removal rate of 27 μM/day under methanogenic conditions and of 5.9 μM/day under nitrate-reducing conditions. In batch reactors with no aquifer materials (Section 4.3), denitrifying conditions were characterized by the highest TNT removal rate while methanoganic conditions exhibited the lowest. The column results indicate that the aquifer material may support growth of certain species of microorganisms which, in turn, accelerated biotransformations of TNT. As discussd earlier, iron-reducing bacteria may be among these microorganisms. Methane production did occur in column M, indicating the existence of methanogenic activity. But the trace amount of methane detected in the column effluent was not enough to account for the acetate utilization in the column. The

dissolved methane concentration in the column effluent was at most 1.5 mg/L, corresponding to acetate utilization of about 5.8 mg/L (see Appendix K for stoichiometric calculations). However, the measured acetate utilization in this time period was 20 to 40 mg/L. Furthermore, 10 mg/L yeast extract and other organic matter in the aquifer materials were also available as primary substrates. Utilization of these carbon sources must be accounted for by some non-methanogenic microorganisms. Therefore, it was very likely that other microbial activities, besides methanogenesis, were greatly contributing to the TNT biotransformations in column M.

Although TNT removal was enhanced in column M, it was doubtful that the metabolic regimes in column M were also very favorable for biotransformation of ADNTs. ADNT concentrations as high as 17 mg/L (86 μM) were detected in the column effluent when the TNT concentraion was decreased from 64 (282 μM) mg/L to 31 mg/L (137 μM). For column D2, however, no ADNT was detected in the effluent when the TNT removal was above 30 mg/L, indicating minimal accumulation of ADNTs. This is consistent with the observations from batch reactors.

In column D2, it is not clear whether or not the reactions of TNT transformation proceeded to TAT and further. By assuming that acetate was stoichiometrically converted to CO_2, TNT to TAT, and NO_3^- to N_2, an electron balance calculation can be conducted to estimate the extent of the TNT transformation preliminarily. The detailed calculation is shown in Appendix J. The results show that the total supply of electrons from acetate was approximately equal to the amount of electrons accepted by nitrate and TNT, revealing no indication of conversion of TAT to more oxidized intermediates and

eventually CO_2. If TAT was further converted to more oxidized products, it would donate electrons, resulting in the likely consumption of more of the terminal electron acceptor, NO_3^-. However, this is not confirmed by the electron balance calculation. The same is true for the denitrifying reactors in batch reactor Set Two. It should be noted that the concentrations of acetate and of nitrate in these reactors (both column and batch) were close to each other and much higher than that of TNT. This situation made it difficult to observe the significance of the electron transfer originating from TNT.

§4.5.2 Effects of Primary Substrate Concentrations

The effects of primary substrate concentrations on TNT removal can be examined in Figure 4-46. In column D1, for example, the average TNT removal was increased as acetate concentrations in the influent increased. If we define an observed pseudo-zero-order TNT removal rate constant as follows,

$$k_0 = \frac{\text{Average TNT removal}}{\text{Retention time}}, \qquad (4.12)$$

then the relationship between k_0 and acetate utilization is basically a linear one, as shown in Figure 4-47. This linear relationship supports the argument that TNT biotransformation is a co-metabolic process and is dependent upon the utilization of primary substrates (Boopathy et al., 1993). Similar results were found for column S1.

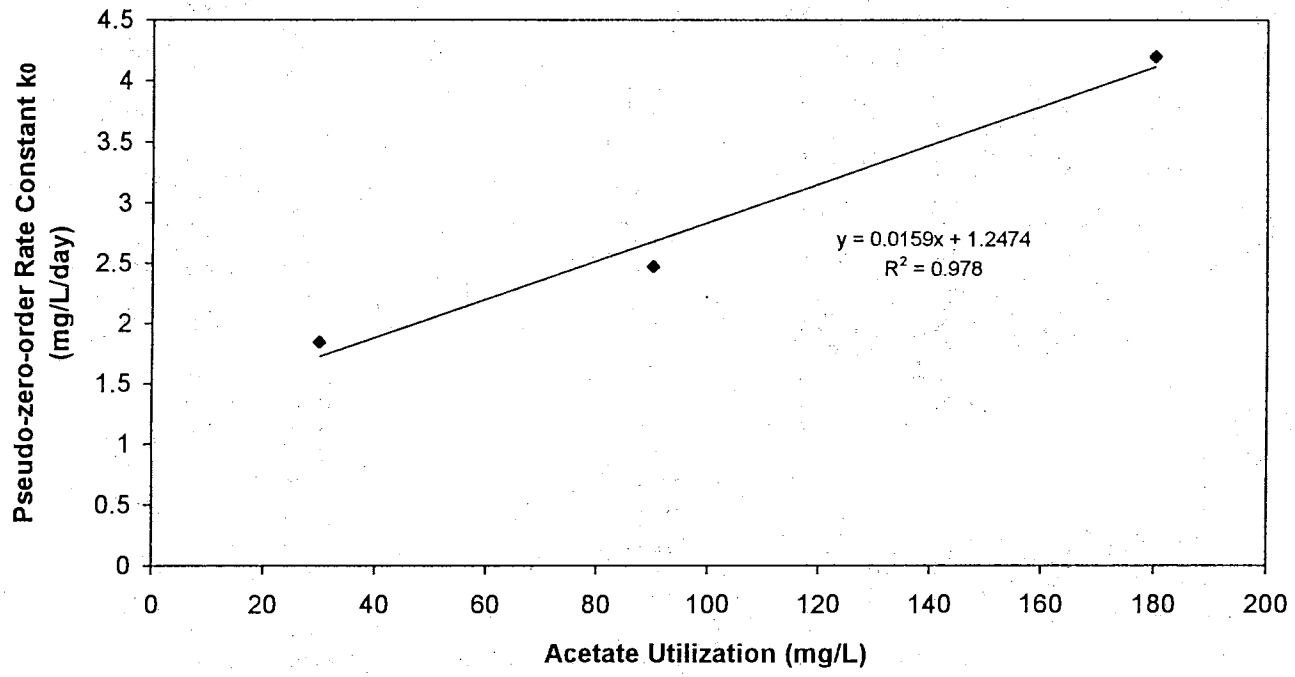


Figure 4-47 Observed pseudo-zero-order rate constants for TNT removal in column D1 (TNT conc. = 60 mg/L, retention time = 4 days)

§4.5.3 Comparison between Continuous and Batch-fed Columns

Continuous column D1 and batch-fed column D2 were both under denitrifying conditions. Based on data in Figures 4-36 and 4-41 and the method of calculating average TNT removal discussed in Section 4.5.1, the TNT removal in columns D1 and D2 has been calculated and shown in Table 4-16. The detailed calculation procedures are presented in Appendix J.

Table 4-16. TNT Removal in Columns D1 and D2 with Retention Time of 4 Days

Substrates (mg/L)	TNT: 60 Ac: 30 Y.E.: 10	TNT: 60 Ac: 90 Y.E.: 10	TNT: 60 Ac: 180 Y.E.: 10
Continuous column D1:			
TNT removal (mg/L)	7.4	9.9	16.8
No. of inf./eff. data pairs *	4	3	5
Batch-fed column D2:			
TNT removal (mg/L)	6.4	9.8	13.5
No. of inf./eff. data pairs	4	3	3
Comparison of TNT removal			
Statistic t value	0.93	0.02	1.40
t with 95% confidence	2.447	2.776	2.447
Significant difference ?	No	No	No

* An inf./eff. data pair refers to an influent TNT concentration and the corresponding effluent TNT concentration used to calculate the TNT removal.

The statistics in this table show that TNT removal in continuous column D1 and batch-fed column D2 did not exhibit significant difference under comparable conditions.

In theory, a batch-fed column is, in fact, a batch reactor because the column fluid resides stagnantly in the column for a certain time period after each column fluid exchange. A continuous column, however, is actually more like a plug-flow reactor. Nevertheless, the efficiency of an ideal plug-flow reactor without recycle is equal to that of a batch reactor, provided that both reactors have the same reaction kinetics and the same hydraulic residence time (Metcalf and Eddy, 1991). Therefore, it is not suprising that columns D1 and D2 had very similar TNT removal characteristics. Even though a plug-flow reactor can be as efficient as a corresponding batch reactor, the spatial distribution of substrates and biomass in it is different from that in a batch reactor. In batch reactors, the substrates, biomass, and other items or parameters are relatively evenly distributed in the reactor space. In plug-flow reactors, however, the concentration of substrates and biomass are higher near the entrance (Metcalf and Eddy, 1991). Siegrist and McCarty (1987) and Miller et al. (1985) observed that continuous feed of a primary substrate into a column reactor would stimulate growth at the entrance to the column and that microorganisms in periodically exchanged columns would tend to grow more evenly throughout the column. While a nearly-ideal plug-flow reactor (continuous column) and a batch reactor (batch-fed column) do not significantly differ from each other in TNT removal, batch-fed columns may be more desirable in some circumstances. For relatively slow processes such as biotransformations of TNT or other xenobiotic compounds, continuous feed of a column reactor means that very small volumes of column medium are injected into the column continuously at low flow rates (e.g. 10 or 20 ml/day). This, in turn, means either that some of the medium may have to stay in the syringe (assuming

a syringe pump is used as in this study) for a long time before it enters the column or that one has to frequently install a new syringe filled with small amounts of fresh column medium. The former increases the risk of letting the chemicals in the medium undergo possible reactions in the syringe before they enter the column, and the latter requires intense maintenance and increases the chance of exposure to air (for anaerobic columns) and of microbial contamination (for abiotic columns). These difficulties can be eliminated or minimized by using batch-fed columns. Also, more evenly distributed substrates and biomass in batch-fed columns are desirable for data interpretation. Of course, continuous aquifer columns with sampling ports along the length should be used if one is to examine dynamic conditions and related phenomena within the aquifer column.

CHAPTER V

CONCLUSIONS

§5.1 Conclusions

This study made an attempt to comprehensively investigate the environmental fate of TNT and its transformation intermediates in subsurface environments, including physical, chemical, and biological aspects. Physical adsorption and desorption of TNT in aquifer materials were examined on long-term as well as short-term bases. Abiotic reactions of TNT with bisulfide were investigated under different conditions, taking into account the effects of the presence of aquifer materials and pH buffer. TNT biotransformation was studied under three types of electron accepting conditions, including denitrifying, sulfate-reducing, and methanogenic, by using aquifer column as well as batch reactor techniques. The main findings of this study are summarized as follows:

1. Under the conditions of short-term batch experiments, the equilibrium of TNT adsorption on the aquifer materials could be described by a Langmuir isotherm with a maximum adsorption capacity of 41 μg/g, indicating that TNT was considerably less sorptive than its two important transformation intermediates, 2-ADNT and 4-ADNT.

2. The adsorption of 2-ADNT and 4-ADNT on aquifer materials reached steady state in about 4 hours in the short-term batch experiments. The TAT concentration did not reach a steady state but decreased to below detection limits in 24 hours, probably due to chemical reactions. TAT was considered unstable and subject to rapid chemical conversion in the presence of trace elements, which are very likely to occur in aquifer materials.

3. Desorption experiments following the short-term adsorption showed that the TNT, 2-ADNT, and 4-ADNT sorbed on aquifer materials were extracted with reasonably high recovery, indicating that physical adsorption was the predominant mechanism in the short-term adsorption.

4. Under the conditions of aquifer column operation with a relatively short retention time (2.5 minutes), TNT adsorption on aquifer materials was negligible and the breakthrough curve only slightly lagged behind that of bromide tracer.

5. Long-term TNT adsorption in aquifer columns (retention time: 4 days, column operation period: over 75 days) revealed that the TNT breakthrough curve was significantly retarded and that about 57% of the TNT loss was irreversible, implying the existence of either or both of the following processes: (a) irreversible adsorption of TNT on aquifer materials, or more likely, (b) abiotic transformation of TNT by various substances in aquifer materials.

6. Comparing the observations in (4) and (5) with each other resulted in the conclusion that TNT adsorption on aquifer materials under dynamic (flowing) conditions

involved a non-equilibrium process in which the un-recoverable TNT loss was dependent on the retention time.

7. Abiotic reaction of TNT with bisulfide occurred rapidly in comparison with abiotic reactions of other nitroaromatic compounds (such as nitrobenzenes) with bisulfide reported by other researchers. TNT could be completely transformed by bisulfide in several hours in the absence of any additional mediators, catalysts, or electron carriers.

8. The kinetics of abiotic reaction of TNT with bisulfide was characterized by an initial lag (slow) phase followed by significantly faster transformations. The increased reaction rate following the lag phase indicated a possible autocatalytic mechanism associated with TNT transformation intermediates.

9. The presence of aquifer materials shortened the duration of the lag phase and accelerated the abiotic reactions of TNT with bisulfide, indicating the catalytic or mediating effects of aquifer materials.

10. Phosphate buffer exhibited a catalytic effect that increased the TNT-bisulfide reaction rate after the initial lag phase while bicarbonate buffer did not show such an effect.

11. The identified intermediates in the abiotic TNT reactions, which appeared within 24 hours, included ADNT isomers and 2,4-DANT.

12. Of the three types of electron accepting conditions examined in the biological batch experiments, denitrifying conditions promoted the fastest biotransformation of TNT while methanogenic conditions exhibited the slowest. This was observed in the batch

reactors where sulfate reduction and methanogenesis were inhibited by high TNT concentrations (about 100 mg/L).

13. When pre-grown sulfate-reducing microorganisms were present and active sulfate-reducing activities were established, TNT was completely transformed in a few hours due to the abiotic reaction with bisulfide. Under this condition, the inhibition of sulfate reduction by added TNT was less severe than in reactors without pre-established sulfate-reducing activity.

14. In batch reactors with and without pre-established methanogenic activities, the addition of TNT into the system could totally cease the methane production. Although methanogenic conditions might be considered favorable for TNT biodegradation because of the low redox potential associated with methanogenesis, this type of conditions was unlikely to be readily achievable and feasible for TNT biotransformation in consideration of the high sensitivity of methanogens to the presence of TNT, especially when relatively high TNT concentrations were present.

15. Under the three types of electron accepting conditions, the sequence of appearance and disappearance of major TNT metabolites, including two early-stage intermediates (tentatively identified as 2-HADNT and 4-HADNT), ADNTs, and 2-DANT, was basically the same. Each of these compounds was transformed the fastest in the denitrifying reactors and the most slowly in the methanogenic reactors.

16. Significant TNT transformations could occur, both biologically and abiotically, in aquifer materials under different electron accepting conditions. When there

was no significant amount of strong reducing agents such as bisulfide present, biological transformations could account for up to 90% of the total TNT transformations.

17. As in batch reactors, sulfate reduction may also be inhibited in aquifer columns. This was shown in the sulfate-amended column when the influent TNT concentration was 60 to 100 mg/L. When the initial TNT concentration was relatively low (30 mg/L), active sulfate reduction was observed, which resulted in complete TNT transformation in 4 days.

18. The TNT biotransformation rate in the methanogenic column was significantly higher than that in the denitrifying column. Stoichiometric calculations based on methane production suggested that this fast TNT removal was mostly due to other microorganisms in aquifer materials rather than methanogens. The biotransformation of TNT metabolites was not favored as well by the metabolic regime in this column. The least accumulation of major TNT metabolites, ADNTs and 2,4-DANT, was observed in denitrifying columns.

19. TNT biotransformations were largely affected by the primary substrate concentration. In nitrate- and sulfate-amended aquifer columns, a nearly linear relationship existed between the observed pseudo-zero-order TNT removal rate constant and acetate utilization, indicating the co-metabolic nature of TNT biotransformation.

20. Continuous flow and batch-fed denitrifying columns, when the hydraulic retention time and other operation conditions were the same, exhibited very similar characteristics in TNT transformation. However, when low flow rates are used, or for

relatively slow processes such as TNT biotransformation, batch-fed columns are more desirable in terms of column operation/maintenance and data interpretation.

§5.2 Recommendations for Future Research

From the results and conclusions in this study, it can be seen that many questions are still unanswered and require further study in order to better understand the environmental fate of TNT and related compounds. The following topics are recommended for future research.

1. In long-term TNT adsorption on aquifer materials, there may be more than one mechanism that results in non-biological loss of TNT. These mechanisms may include abiotic reactions of TNT with mineral surfaces in aquifer materials as well as irreversible or specific adsorption with different equilibrium and kinetics. These processes seem to be relatively slow and very important to the environmental fate of TNT and its transformation intermediates.

2. While TNT can be abiotically transformed by bisulfide within hours, the transformation intermediates, such as ADNTs, react with bisulfide much more slowly. The abiotic reactions of these intermediates deserve further investigation if we are to fully understand the long-term impact of these compounds.

3. The inhibition of sulfate reduction and methanogenesis, possibly by TNT and/or its intermediates, needs to be better understood. Under certain conditions, it is desirable to know what the major inhibitors are, whether or not an inhibition threshold exists, and if it is possible to overcome the inhibition.

4. Besides the three types of electron accepting conditions examined in this study, other metabolic regimes, such as iron-reducing conditions, may be interesting for future research on TNT biotransformation because (1) Fe species exist in aquifer environments extensively and may be significant; and (2) the microbial iron-reducing process has been demonstrated to be important in transformation of other nitroaromatic compounds (Heijman et al., 1995).

5. On the basis of further predicting TNT fate in the subsurface, it is valuable to develop a mathematical model describing the environmental fate of TNT and, possibly, some of its important intermediates. This model may have a form similar to those proposed by other researchers (Wilber, 1991; Chen and McTernan, 1992) and take into account hydraulic transport, physical (short- and long-term) adsorption/desorption, abiotic reactions, and biotransformations of interested compounds in aquifer materials. Parameters derived in this study, such as the adsorption mass transfer rate coefficient (Section 4.1.4) and biotransformation rate constants (Section 4.3.1), will be helpful in the development of this mathematical model.

6. In order to better test a dynamic model of TNT fate in aquifer materials, further column studies are recommended using columns with sampling ports along the length. It would be valuble to quantify the microbial biomass in aquifer columns using a biochemical marker such as ATP, phospholipids, or cellular protein (Findlay et al., 1989).

REFERENCES

Amerkhanova, N. N. and R. P. Naumova, 1978. 2,4,6-Trinitrotoluene as a source of nutrition for bacteria. Translated from *Microbiologiya* 47:393-395.

Anid, P. L., P. J. J. Alvarez and T. M. Vogel, 1993. Biodegradation of monoaromatic hydrocarbons in aquifer columns amended with hydrogen peroxide and nitrate. *Water Research* 27:685-691.

APHA (American Public Health Association), AWWA and WPCF, 1985. *Standard Methods for the Examination of Water and Wastewater*, 16th Edition. APHA, Washington, DC.

Atlas, R. M. and R. Bartha, 1993. *Microbial Ecology*, 3rd ed. 323, The Benjamin/Cummings Publishing Company Inc., Redwood City, CA.

Barbash, J. E. and M. Reinhard, 1989. Abiotic dehalogenation of 1,2-dichloroethane and 1,2-dibromoethane in aqueous solution containing hydrogen sulfide. *Environ. Sci. Technol.* 23:1349-1357.

Bayly, R. C. and M. G. Barbour, 1984. Degradation of aromatic compounds by the meta and gentisate pathways. In: D. T. Gibson (ed.) *Microbial Degradation of Organic Compounds*, 253-286, Marcel Dekker, New York.

Beeman, R. E. and J. M. Suflita, 1987. Microbial ecology of a shallow unconfined ground water aquifer polluted by municipal landfill leachate. *Microb. Ecol.* 14:39-54.

Berry D. F., A. J. Francis and J.-M. Bollag, 1987. Microbial metabolism of homocyclic and heterocyclic aromatic compounds under anaerobic conditions. *Microbiological Reviews* 51:43-59.

Bonazountas, M., 1983. Soil and ground water fate modeling. In: R. L. Swann and A. Eschenroeder (eds.) *Fate of Chemicals in the Environment.* ACS Symp. Ser. 225 (Chapter 3), 41-65.

Boopathy, R. and C. F. Kulpa, 1992. Trinitrotoluene (TNT) as a sole nitrogen source for a sulfate reducing bacterium *Desulfovibrio* sp. (B strain) isolated from an anaerobic digester. *Current Microbiology* 25:235-241.

Boopathy, R. and C. F. Kulpa, 1994. Biotransformation of 2,4,6-trinitrotoluene (TNT) by a *Methanococcus* sp. (strain B) isolated from a lake sediment. *Canadian Journal of Microbiology* 40:273-278.

Boopathy, R., J. Manning, C. Montemagno, and C. Kulpa, 1994. Metabolism of 2,4,6-trinitrotoluene by a *Pseudomonas* consortium under aerobic conditions. *Current Microbiology* 28:131-137.

Boopathy, R., M. Wilson, and C. F. Kulpa, 1993. Anaerobic removal of 2,4,6-trinitrotoluene (TNT) under different electron accepting conditions: laboratory study. *Water Environment Research* 65:271-275.

Bradley, P. M. and F. H. Chapelle, 1995. Factors affecting microbial 2,4,6-trinitrotoluene mineralization in contaminated soil. *Environ. Sci. Technol.* 29:802-806.

Bradley, P. M., F. H. Chapelle, J. E. Landmeyer, and J. G. Schumacher, 1994. Microbial transformation of nitroaromatics in surface soils and aquifer materials. *Appl. Environ. Microbiol.* 60:2170-2175.

Braun, K. and D. T. Gibson, 1984. Anaerobic degradation of 2-aminobenzoate (anthranilic acid) by denitrifying bacteria. *Appl. Environ. Microbiol.* 48:102-107.

Brezonik, P. L., 1994. *Chemical Kinetics and Process Dynamics in Aquatic Systems*, Lewis Publishers, Boca Raton, FL.

Bruns-Nagel, D., J. Breitung, E. von Low, K. Steinbach, T. Gorontzy, M. Kahl, K.-H. Blotevogel and D. Gemsa, 1996. Microbial transformation of 2,4,6-trinitrotoluene in aerobic soil columns. *Appl. Environ. Microbiol.* 62:2651-2656.

Buffle, A. J. and R. S. Altmann, 1987. In: W. Stumm (ed.) *Aquatic Surface Chemistry*, Wiley Interscience, New York.

Bumpus, J. A., 1989. Biodegradation of polycyclic aromatic hydrocarbons by *Phanerochaete chrysosporium*. *Appl. Environ. Microbiol.* 55:154-158.

Bumpus, J. A. and M. Tatarko, 1994. Bioremediation of 2,4,6-trinitrotoluene by *Phanerochaete chrysosporium*: Identification of initial degradation products and the discovery of a TNT metabolite that inhibits lignin peroxidases. *Current Microbiology* 28:185-190.

Carpenter, D. F., N. C. McCormick and J. H. Cornell, 1978. Microbial transformation of ^{14}C-labeled 2,4,6-trinitrotoluene in an activated-sludge system. *Appl. Environ. Microbiol.* 35:949-954.

Caton, J. E., C. H. Ho, R. T. Williams and W. H. Griest, 1994. Characterization of insoluble fractions of TNT transformed by composting. *Journal of Environmental Science and Health. Part A: Environmental Science and Engineering* 29(4):659-670.

Chen, K. Y. and J. C. Morris, 1972. Kinetics of oxidation of aqueous sulfide by O_2. *Environ. Sci. Technol.* 6:529-539.

Chen, Z. and W. F. McTernan, 1992. Multi-substrate, multi-option groundwater transport model. *J. Contam. Hydrol.* 11:215-244.

Comfort, S. D., P. J. Shea, L. S. Hundal, Z. Li, B. L. Woodbury, J. L. Martin and W. L. Powers, 1995. TNT transport and fate in contaminated soil. *J. Environ. Qual.* 24:1174-1182.

Delgado, M. C. and N. L. Wolfe, 1992. Structure-activity relationships for the reduction of *p*-substituted nitrobenzenes in anaerobic sediments. In *ACS, Extended Abstract Books*, American Chemical Society, San Francisco, CA.

Dickel, O., W. Haug and H.-J. Knackmuss, 1993. Biodegradation of nitrobenzene by a sequential anaerobic-aerobic process. *Biodegradation* 4:187-193.

Dohnalek, D. A. and J. A. Fizpatrick, 1983. The chemistry of reduced sulfur species and their removal from ground water supplies. *American Water Works Association Journal* 75:298-308.

Dunnivant, F. M., P. M. Jardine, D. L. Taylor and J. F. McCarthy, 1992a. Transport of naturally occurring dissolved organic carbon in laboratory columns containing aquifer material. *Soil Sci. Soc. Am. J.* 56:437-444.

Dunnivant, F. M., R. P. Schwarzenbach and D. L. Macalady, 1992b. Reduction of substituted nitrobenzenes in aqueous solutions containing natural organic matter. *Environ. Sci. Technol.* 26:2133-2141.

Duque, E., A. Haidour, F. Godoy and J. L. Ramos, 1993. Construction of a *Pseudomonas* hybrid strain that mineralizes TNT. *J. Bacteriol.* 175:2278-2283.

Eaton, D. C., 1985. Mineralization of polychlorinated biphenyls by *Phanerochaete chrysosporium*, a ligninolytic fungus. *Enzyme Microbial. Technol.* 7:194-196.

Fedorak, P. M., W. B. Kindzierski and S. E. Hurdfy, 1990. Effects of anilines and hydantoins on the methanogenic degradation of selected phenols. *Water Research* 24:921-925.

Fernando, T. and S. D. Aust, 1994. Biodegradation of toxic chemicals by white rot fungi. In: G. R. Chaudhry (ed.) *Biological Degradation and Bioremediation of Toxic Chemicals*, 386-402, Dioscorides Press, Portland, Oregon

Fernando, T., J. A. Bumpers and S. D. Aust, 1990. Biodegradation of TNT (2,4,6-trinitrotoluene) by *Phanerochaete chrysosporium. Appl. Environ. Microbiol.* 56:1666-1671.

Findlay, R. H., G. M. King and L. Watling, 1989. Efficacy of phospholipid analysis in determining microbial biomass in sediments. *Appl. Environ. Microbiol.* 55:2888-2893.

Freedman, D. L., F. Huang, D. Noguera, R. Shanley, J. T. Pfeffer and R. Scholze, 1994. Biotransformation of 2,4-dinitrotoluene and aminonitrotoluenes. ASCE National Conference on Environmental Engineering, Boulder, Colorado.

Funk, S. B., D. J. Roberts, D. L. Crawford and R. L. Crawford, 1993. Initial-phase optimization for biodegradation of munition compound-contaminated soils. *Appl. Environ. Microbiol.* 59:2171-2177.

Funk, S. B., D. L. Crawford, D. J. Roberts and R. L. Crawford, 1995a. Two-stage bioremediation of TNT contaminated soils. In: B. S. Schepart (ed.) *Bioremediation of Pollutants in Soil and Water, ASTM STP 1235.* American Society for Testing and Materials, Philadelphia.

Funk, S. B., D. L. Crawford, R. L. Crawford, G. Mead and W. Davis-Hoover, 1995b. Full-scale anaerobic bioremediation of trinitrotoluene (TNT) contaminated soil: A US EPA site program demonstration. *Applied Biochemistry and Biotechnology* 51/52:625-633.

Geankoplis, C. J., 1993. *Transport Processes and Unit Operations*, 3rd edition. PTR Prentice-Hall, Inc., Englewood Cliffs, New Jersey.

Glaus M. A., C. G. Heijman, R. P. Schwarzenbach and J. Zeyer, 1992. Reduction of nitroaromatic compounds mediated by *Streptomyces* sp. exudates. *Appl. Environ. Microbiol.* 58:1945-1951.

Gorontzy, T., J. Kuver and K.-H. Blotevogel, 1993. Microbial transformation of nitroaromatic compounds under anaerobic conditions. *Journal of General Microbiology* 139:1331-1336.

Gorontzy, T., O. Drzyzga, M. Kahl, D. Bruns-Nagel, J. Breitung, E. von Loew, and K. Blotevoge, 1994. Microbial degradation of explosives and related compounds. *Critical Reviews in Microbiology* 20:265-284.

Griest, W. H., A. Steward, R. Tyndall, J. Caton, C. Ho, K. Ironside, W. Caldwell and E. Tan, 1993. Chemical and toxicological testing of composted explosives-contaminated soil. *Environmental Toxicology and Chemistry* 12(6):1105-1116.

Haderlein, S. B. and R. P. Schwarzenbach, 1995. Environmental processes influencing the rate of abiotic reduction of nitroaromatic compounds in the subsurface. In: J. C. Spain (ed.) *Biodegradation of Nitroaromatic Compounds*, 199-225, Plenum Press, New York.

Haderlein, S. B., K. W. Weissmahr and R. P. Schwarzenbach, 1996. Specific adsorption of nitroaromatic explosives and pesticides to clay minerals. *Environ. Sci. Technol.* 30:612-622.

Han, G. B., 1993. Biodegradation of nitroaromatic compounds in TNT munitions wastes under different metabolic regimes. Ph.D. dissertation, Oklahoma State University.

Hansen, T. A., 1993. Carbon metabolism of sulfate-reducing bacteria. In: J. M. Odom and R. Singleton, Jr. (eds.) *The Sulfate-reducing Bacteria: Contemporary Perspectives*, Springer-Verlag, New York.

Hao, O. J., K. K. Phull, A. P. Davis, J. M. Chen and S. W. Maloney, 1993. Wet-air oxidation of trinitrotoluene manufacturing red water. *Water Environment Research* 65:213-220.

Harris, J. W. and R. W. Killermeyer, 1970. *The Red Cell Production, Metabolism, Destruction: Normal and Abnormal.* Harvard University Press, Cambridge, Massachusetts.

Hartter, D. R., 1985. The use and importance of nitroaromatic chemicals in the chemical industry. In: D. E. Rickert (ed.) *Toxicity of Nitroaromatic Compounds*, 1-14. Chemical Industry Institute of Toxicology Series. Hemisphere Publishing Corp., New York.

Heijman, C. G., E. Grieder, C. Holliger, and R. P. Schwarzenbach, 1995. Reduction of nitroaromatic compounds coupled to microbial iron reduction in laboratory aquifer columns. *Environ. Sci. Technol.* 29:775-783.

Hopper, D. J. and D. G. Taylor, 1975. Pathways for the degradation of *m*-cresol and *p*-cresol by *Pseudomonas putida*. *J. Bacteriol.* 122:1-6.

HSDB, 1994. *TNT. Hazardous Substances Data Bank.* National Library of Medicine, National Toxicology Information Program, Bethesda, MD.

Isbister, J. D., G. L. Anspach, J. F. Kitchens and R. C. Doyle, 1984. Composting for decontamination of soils containing explosives. *Microbiology* 7:47-73.

Jardine, P. M., F. M. Dunnivant, H. M. Selim and J. F. McCarthy, 1992. Comparison of models for describing the transport of dissolved organic carbon in aquifer columns. *Soil Sci. Soc. Am. J.* 56:393-401.

Joback, K. G. and R. C. Reid, 1987. Estimation of pure-component properties from group contributions. *Chem. Eng. Commun.* 57:233-243.

Kaplan, D. L. and A. M. Kaplan, 1982a. 2,4,6-Trinitrotoluene-Surfactant complexes: decomposition, mutagenicity, and soil leaching studies. *Environ. Sci. Technol.* 16:566-571.

Kaplan, D. L. and A. M. Kaplan, 1982b. Separation of mixtures of 2,4,6-trinitrotoluene reduction products with liquid chromatography. *Analytica Chimica Acta* 136:425-428.

Kaplan, D. L. and A. M. Kaplan, 1982c. Thermophilic biotransformations of 2,4,6-trinitrotoluene under stimulated composting conditions. *Appl. Environ. Microbiol.* 44:757-760.

Krumholz, L. R., J. Li, W. W. Clarkson, G. G. Wilber and J. M. Suflita, 1997. Transformations of TNT and related aminotoluenes in groundwater aquifer slurries under different electron-accepting conditions. *Journal of Industrial Microbiology and Biotechnology* 18:161-169.

Kuhn, E. P. and J. M. Suflita, 1989. Microbial degradation of nitrogen, oxygen, and sulfur heterocyclic compounds under anaerobic conditions: Studies with aquifer samples. *Environmental Toxicology and Chemistry* 8:1149-1158.

Lebron, C. A., L. A. Karr, T. Fernando and S. D. Aust, 1992. Biodegradation of 2,4,6-trinitrotoluene by white-rot fungus. US Patent 5,085,998.

Leggett, D. C., 1985. Sorption of military explosive contaminants on bentonite drilling mud. U. S. Army Cold Regions Research and Engineering Laboratory, CRREL Report 85-18.

Lehninger, A. L., 1970. *Biochemistry*, Worth Publishers Inc., New York,

Lenke, H. and H.-J. Knackmuss, 1992. Initial hydrogenation during catabolism of picric acid by *Rhodococcus erythropolis* HL 24-2. *Appl. Environ. Microbiol.* 58:2933-2937.

Macalady, D. L., P. G. Tratnyek and T. J. Grundl, 1986. Abiotic reduction reactions of anthropogenic organic chemicals in anaerobic systems: a critical review. *J. Contam. Hydrol.* 1:1-28.

Manning, Jr., J. F., R. Boopathy and C. F. Kulpa, 1995. A laboratory study in support of the pilot demonstration of a biological soil slurry reactor (Report No. SFIM-AEC-TS-CR-94038). U.S. Army Environmental Center, Aberdeen Proving Ground, Maryland.

McCormick, N. G., F. E. Feeherry and H. S. Levinson, 1976. Microbial transformation of 2,4,6-trinitrotoluene and other nitroaromatic compounds. *Appl. Environ. Microbiol.* 31:949-958.

McCormick, N. G., J. H. Cornell, and A. M. Kaplan, 1981. Biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine. *Appl. Environ. Microbiol.* 42:817-823.

Metcalf and Eddy, Inc., 1991. *Wastewater Engineering: Treatment, Disposal, and Reuse.* McGraw-Hill, Inc., New York.

Michels, J. and G. Gottschalk, 1994. Inhibition of the lignin peroxidase of *Phanerochaete chrysosporium* by hydroxylamino-dinitrotoluene. *Appl. Environ. Microbiol.* 60:187-194.

Miller, G. D., G. M. Deeley, J. C. Chang and J. T. Wilson, 1985. Minimizing problems caused by unequal development of biological activity within column microcosms used to predict the fate of pollutants in subsurface materials. In: N. N. Durham and A. E. Redelfs (Editors), *Proceedings, Second International Conference on Ground Water Quality Research.* Oklahoma State University Printing Services, Stillwater, OK, 197-198.

Moore, J. W. and R. G. Pearson, 1981. *Kinetics and Mechanisms.* John Wiley ans Sons, Inc., New York.

Morel, F. M. M., 1983. *Principles of Aquqtic Chemistry*, p96-105. John Wiley and Sons, Inc., New York.

Naumova, R. P., S. Y. Selivanovskaya and F. A. Mingatina, 1988. Possibility of deep bacterial destruction of 2,4,6-trinitrotoluene. Translated from *Microbiologiya* 57:218-222.

Naumova, R. P., T. O. Belousova and R. M. Gilyazova, 1982. Microbial transformation of trinitrotoluene. Translated from *Prikl. Biokhim. Mikrobiol.* 18(1):85-90.

O'Brien, D. J. and F. B. Birkner, 1977. Kinetics of oxygenation of reduced sulfur species in aqueous solution. *Environ. Sci. Technol.* 11:1114-1120.

Osman, J. L. and R. E. Klausmeier, 1972. The microbial degradation of explosives. *Devel. Ind. Microbiol.* 14:247-252.

Parrish, F. W., 1977. Fungul transformation of 2,4-dinitrotoluene and 2,4,6-trinitrotoluene. *Appl. Environ. Microbiol.* 34(2):232-233.

Pennington, J. C. and W. H. Patrick, Jr., 1990. Adsorption and desorption of 2,4,6-trinitrotoluene by soils. *Journal of Environmental Quality* 19:559-567.

Pillai, P., C. S. Helling and J. Dragun, 1982. Soil-catalyzed oxidation of aniline. *Chemosphere* 11:299-317.

Preuss, A., J. Fimpel, and G. Diekert, 1993. Anaerobic transformation of 2,4,6-trinitrotoluene (TNT). *Archieves of Microbiology* 159:345-353.

Qin, Y., 1995. Abiotic reactions of acetanilide herbicides with bisulfide. Master's thesis, Oklahoma State University.

Regan, K. M. and R. L. Crawford, 1994. Characterization of *Clostridium bifermentans* and its biotransformation of 2,4,6-trinitrotoluene (TNT) and 1,3,5-triaza-1,3,5-trinitrocyclohexane (RDX). *Biotechnol. Lett.* 16:1081-1086.

Rieger, P.-G. and H.-J. Knackmuss, 1995. Basic knowledge and perspectives on biodegradation of 2,4,6-trinitrotoluene and related nitroaromatic compounds in contaminated soil. In: J. C. Spain (ed.) *Biodegradation of Nitroaromatic Compounds*, 1-18, Plenum Press, New York.

Roberts, D. J., F. Ahmad and S. Pendharkar, 1996. Optimization of an aerobic polishing stage to complete the anaerobic treatment of munitions-contaminated soils. *Environ. Sci. Technol.* 30:2021-2026.

Roberts, D. J., R. H. Kaake, S. B. Funk, D. L. Crawford and R. L. Crawford, 1993. Anaerobic remediation of dinoseb from contaminated soil: An on-site demonstration (scientific note). *Applied Biochemistry and Biotechnology* 39/40: 781-789.

Samiullah, Y., 1990. *Prediction of the Environmental Fate of Chemicals*. Elsevier Applied Science Publishers Ltd., New York.

Schackmann, A. and R. Muller, 1991. Reduction of nitroaromatic compounds by different *Pseudomonas* species under aerobic conditions. *Appl. Microbiol. Biotechnol.* 34:809-813.

Schwarzenbach, R. P., R. Stierli, K. Lanz and J. Zeyer, 1990. Quinone and iron porphyrin mediated reduction of nitroaromatic compounds in homogeneous aqueous solution. *Environ. Sci. Technol.* 24:1566-1574.

Selim, H. M., S. K. Xue and I K. Iskandar, 1995. Transport of 2,4,6-trinitrotoluene and hexahydro-1,3,5-trinitro-1,3,5-triazine in soils. *Soil Science* 160:328-339.

Semmens, M. J., D. Barns and M. O'Hara, 1985. Treatment of an RDX-TNT waste from a munitions factory. In: *Proceedings of the 39th Industrial Waste Conference, Purdue University*, 837-842. Butterworth Publishers, Boston, MA.

Shah, S. R., 1995. Anaerobic degradation intermediates of 2,4,6-trinitrotoluene (TNT) under different electron accepting conditions. Master's thesis, Oklahoma State University.

Shelley, M. D., R. L. Autenrieth, J. R. Wild and B. E. Dale, 1996. Thermodynamic analysis of trinitrotoluene biodegradation and mineralization pathways. *Biotechnology and Bioengineering* 50:198-205.

Siegrist, H. and P. L. McCarty, 1987. Column methodologies for determining sorption and biotransformation potential for chlorinated aliphatic compounds in aquifers. *J. Contam. Hydrol.* 2:31-50.

Singleton, Jr., R., 1993. The sulfate-reducing bacteria: an overview. In: J. M. Odom and R. Singleton, Jr. (eds.) *The Sulfate-reducing Bacteria: Contemporary Perspectives*, Springer-Verlag, New York.

Somasundram, L. and J. R. Coats (eds.), 1991. *Pesticide Transformation Products - Fate and Significance in the Environment.* American Chemical Society, Washington, DC.

Spain, J. C., 1995. Biodegradation of nitroaromatic compounds. *Annual Review of Microbiology* 49:523-555.

Spanggord, R. J., K. E. Mortelmans, A. F. Griffing and V. F. Simmon, 1982. Mutagenicity in *Salminella typhimurium* and structure activity relationships of waste water components emanating from the manufacture of trinitrotoluene. *Environ. Mutagen.* 4:163-179.

Spanggord, R. J., W. R. Mabey, T. W. Chou and J. H. Smith, 1985. Environmental fate of selected nitroaromatic compounds in aquatic environments. In: D. E. Rickert (ed.) *Toxicity of Nitroaromatic Compounds*, Hemisphere Publishing Corporation, Washington.

Spiker, J. K., D. L. Crawford and R. L. Crawford, 1992. Influence of 2,4,6-trinitrotoluene (TNT) concentration on the degradation of TNT in explosive-contaminated soils by the white rot fungus *Phanerochaete chrysosporium*. *Appl. Environ. Microbiol.* 58:3199-3202.

Stumm, W. and J. J. Morgan, 1981. *Aquatic Chemistry*, 2nd ed., 442, Wiley Interscience, New York.

Tan, E. L., C. H. Ho, W. H. Griest and R. L. Tyndall, 1992. Mutagenicity of trinitrotoluene and its metabolites formed during composting. *J. Toxicol. Environ. Health* 36:165-175.

Thurman, E. M., 1985. *Organic Geochemistry of Natural Waters*, Nijhff-Junk Publishers, Boston, MA.

Tratnyek, P. G. and D. L. Macalady, 1989. Abiotic reduction of nitro aromatic pesticides in anaerobic laboratory systems. *J. Agric. Food Chem.* 37:248-254.

Traxler, R. W., 1974. Biodegradation of trinitrotoluene (α-TNT) and its production isomers. AD-A 029346, Department of Plant Pathology-Entomology, University of Rhode Island, Kinhston.

Tsai, T. S., 1991. Biotreatment of red water -- a hazardous waste stream from explosive manufacture -- with fungal systems. *Hazardous Waste and Hazardous Materials* 8:231-144.

Tucker, W. A., E. V. Dose, G. J. Gensheimer, R. E. Hall, C. D. Pollman and D. H. Powell, 1985. Evaluation of critical parameters affecting contaminant migration through soils. Final Report for the U. S. Army Toxic and Hazardous Materials Agency. ESE, Gainseville, FL.

U.S. Department of Health and Human Services, 1995. *Toxicological Profile for 2,4,6-Trinitrotoluene*. Agency for Toxic Substances and Disease Registry, Atlanta, GA.

US EPA, 1989. *Drinking Water Health Advisory of 2,4,6-trinitrotoluene*. Washington, DC: Office of Drinking Water.

US EPA, 1992. *Drinking Water Health Advisory: Munitions*, 237-238. Lewis Publishers, Boca Raton, FL.

US EPA, 1993. Demonstration of the J. R. Simplot ex-situ bioremediation technology for treatment of nitroaromatic contaminants at the Bowers Field site in Ellensburg, Washington. *EPA Fact Sheet*, August.

Van Beelen, P. and D. R. Burris, 1995. Reduction of the explosive 2,4,6-trinitrotoluene by enzymes from aquatic sediments. *Environmental Toxicology and Chemistry* 14:2115-2123.

Vishniac, W. and M. Santer, 1957. The Thiobacilli. *Bacteriol. Rev.* 21:195-213.

Vobeck, C., H. Lenke, P. Fischer and H.-J. Knackmuss, 1994. Identification of a hydride-Meisenheimer complex as a metabolite of 2,4,6-trinitrotoluene by a *Mycobacterium* strain. *J. Bacteriol.* 176:932-934.

Walker, J. E. and D. L. Kaplan, 1992. Biological degradation of explosives and chemical agents. *Bioremediation* 3:369-385.

Wilber, G. G., 1991. Kinetics of alachlor, atrazine and chloroform transformation under various electron acceptor conditions. Ph.D. dissertation, the University of Iowa.

Williams, R. T., P. S. Ziegenfuss, G. B. Mohrman and W. E. Sisk, 1989. Composting of explosives and propellant contaminated sediments. In: *Hazardous and Industrial Wastes: Proceedings of the 21st Mid-Atlantic Industrial Waste Conference*, 599-611. Technomic Publishing Co., Inc., Lancaster, PA.

Wolfe, N. L. and D. L. Macalady, 1992. New perspectives in aquatic redox chemistry: abiotic transformations of pollutants in groundwater and sediments. *J. of Contam. Hydrol.* 9:17-34.

Won, W. D., L. H. Di Salvo and J. Ng, 1976. Toxicity and mutagenicity of 2,4,6-trinitrotoluene and its microbial metabolites. *Appl. Environ. Microbiol.* 31:576-580.

Won, W. D., R. J. Heckly, D. J. Glover, and J. C. Hoffsommer, 1974. Metabolic Disposition of 2,4,6-trinitrotoluene. *Applied Microbiology* 27:513-516.

Wujcik, W. J., W. L. Lowe, P. L. Marks and W. E. Sisk, 1992. Granular activated carbon pilot treatment studies for explosives removal from contaminated groundwater. *Environmental Progress* 11:178-189.

Xue S. K, I. K. Iskandar and H. M. Selim, 1995. Adsorption-desorption of 2,4,6-trinitrotoluene and hexahydro-1,3,5-trinitro-1,3,5-triazine in soils. *Soil Science* 160:317-327.

APPENDICES

APPENDIX A

RAW DATA OF BATCH ADSORPTION EXPERIMENTS

A) Kinetics Data

2-ADNT		4-ADNT		TAT	
Time (hrs)	Conc. (mg/L)	Time (hrs)	Conc. (mg/L)	Time (hrs)	Conc. (mg/L)
0	29	0	29	0	20
0.33	25	0.33	24.4	0.5	17.1
1	23.6	1	23.2	4	15.2
1.83	23.5	1.83	24.4	7	10
4	23.2	4	23.3	24	0
9	21.6	9	21.9		
24	23	24	21.1		

B) Isotherm Data

1) Isotherm of TNT

Initial conc. (mg/L)	Final conc. (C) (mg/L)	Conc. in solid (q) (ug/g)	1/C	1/q	ln(C)	ln(q)
5.27	3.82185	3.62038	0.26165	0.27621	1.34073	1.28658
10.39	7.63779	6.88053	0.13093	0.14534	2.03311	1.9287
21.29	16.8369	11.1329	0.05939	0.08982	2.82357	2.4099
54.1	45.943	20.3926	0.02177	0.04904	3.8274	3.01517
95	81.0654	34.8364	0.01234	0.02871	4.39526	3.55066

Regression as Langmuir :

R square:	0.9958
Slope:	0.9596
Intercept:	0.02454

2) Isotherm of 2-A-4,6-DNT

Initial conc. (mg/L)	Final conc. (C) (mg/L)	Adsorbed amount (mg)	Conc. in solid (q) (ug/g)	1/C	1/q	ln(C)	ln(q)
5	3.7579	0.018632	6.2105	0.052423	31.72047	2.948412	-3.456962
10.5	8.5409	0.029387	9.7955	0.023065	20.11128	3.769418	-3.001281
14.8	11.1202	0.055197	18.399	0.017716	10.7071	4.033315	-2.370907
20	15.4223	0.068666	22.8885	0.012774	8.606942	4.360366	-2.152569
29	23.2	0.087	29	0.008491	6.793103	4.768704	-1.915908

3) Isotherm of 4-A-2,6-DNT

Initial conc. (mg/L)	Final conc. (C) (mg/L)	Adsorbed amount (mg)	Conc. in solid (q) (ug/g)	1/C	1/q	ln(C)	ln(q)
5	3.9026	0.016461	5.487	0.050479	35.90304	2.986195	-3.580822
10	7.911	0.031335	10.445	0.024902	18.8607	3.692806	-2.93708
15	12.3418	0.039873	13.291	0.015962	14.82206	4.137543	-2.696117
20	15.6186	0.065721	21.907	0.012613	8.992559	4.373014	-2.196398
29	23.3	0.0855	28.5	0.008455	6.912281	4.773005	-1.9333

4) Isotherm of TAT

Initial conc. (mg/L)	Final conc. (C) (mg/L)	Adsorbed amount (mg)	Conc. in solid (q) (ug/g)	1/C	1/q	ln(C)	ln(q)
2	0.4438	0.023343	7.781	0.308698	17.60699	1.175393	-2.868296
5	1.6626	0.050061	16.687	0.082401	8.209984	2.496157	-2.105351
10	5.5765	0.066353	22.1175	0.024567	6.19419	3.706336	-1.823612
15	10.0231	0.074654	24.8845	0.013668	5.505435	4.292667	-1.705736
20	15.2231	0.071654	23.8845	0.008999	5.735938	4.710588	-1.746751

APPENDIX B

LONG-TERM ADSORPTION IN AQUIFER COLUMNS

B.1 Breakthrough Curves at Low Flow Rate

Breakthrough curves at a relatively low flow rate were obtained from columns S4 and B, which had the following operation conditions:

Flow rate = 0.007 ml/min = 0.01008 L/day, Retention time = 4.0 days
Influent of column S4: TNT conc. = 100 mg/L, Ac = 90 mg/L
SO_4^{2-} = 250 mg/L, Nutrients: the same as in other columns (Table 3-7)
NaN_3 = 0.3 g/L, autoclaved, pH = 7.0
Influent of column B: Br^- = 50 mg/L, pH = 7.0

The effluent data for both columns are shown in the following table.

Time (days)	(TNT)eff (mg/L)	$(C/C0)_{TNT}$	Time (days)	(Br)eff (mg/L)	$(C/C0)_{Br}$
0	0	0	0	0	0
6	0	0	3	0	0
9	9	0.09	6	8.2	0.164
13	42	0.42	9	17.4	0.348
20	61	0.61	12	35.6	0.712
25	69	0.69	15	43	0.86
28	73	0.73	18	46	0.92
31	74	0.74	21	47	0.94
34	75	0.75	24	52	1.04
37	77	0.77	27	46	0.92
40	81	0.81	31	49	0.98
43	75	0.75	33	52	1.04
46	81.4	0.814	36	48	0.96
50	86.9	0.869	39	52	1.04
52	88	0.88	42	52	1.04
55	90.4	0.904	50	48	0.96
58	93	0.93	60	51	1.02
61	91	0.91	70	49	0.98
68	95	0.95			
75	94	0.94			

B.2 Mass Balance for Column S4

A calculation of TNT mass balance can be performed on the basis of the breakthrough curves in Figure 4-8, as illustrated below.

$$M_L = M_{in} - M_{out} - M_{ac}$$

where M_L is the TNT mass loss due to physical adsorption and chemical/abiotic transformation (biological transformation is negligible because the column was maintained under sterilized conditions);

M_{in} is the TNT mass injected into the column;

M_{out} is the TNT mass exiting the column; and

M_{ac} is the TNT mass accumulated in the aqueous phase stored in the column pore space (and the column "head space" and other related space).

According to the breakthrough curves,

$M_{in} - M_{ac}$ = (Area under bromide breakthrough curve)(100 mg/L)(0.01008 L/day)

M_{out} = (Area under TNT breakthrough curve)(100 mg/L)(0.01008 L/day)

Assume that the adsorption process reached saturation by day 75, then the values of ($M_{in} - M_{ac}$) and M_{out} can be calculated by integrating the areas under these two breakthrough curves in the range from day 0 through day 75.

Since $M_L = M_{in} - M_{out} - M_{ac} = (M_{in} - M_{ac}) - M_{out}$,

Then M_L = (Area under bromide breakthrough curve)(100 mg/L)(0.01008 L/day)
- (Area under TNT breakthrough curve)(100 mg/L)(0.01008 L/day)

= (Area between two breakthrough curves)(100 mg/L)(0.01008 L/day)

= **13.6 mg/L**

M_{ac} can be represented by the area between the straight line $C/C_0 = 1$ and the bromide breakthrough curve. This value is estimated to be **10.1 mg** according to Figure 4-8.

The data for desorption of column S4 are as follows:

3 times of sequential extraction with acetonitrile

Total volume of the extract = 240 ml

TNT conc. in the extract = 40.1 mg/L

Total TNT recovered = (0.24 L)(40.1 mg/L) = 9.62 mg/L

Aquifer materials in column = 205.5 g

This recovered TNT mass included the TNT desorbed from the solid phase and the TNT from the aqueous phase remaining in the pore space of the aquifer material. The latter should be subtracted from the total recovered TNT to yield the TNT mass desorbed from the solid phase.

Column pore space = 40.3 ml. The TNT concentration of the aqueous phase remaining in the pore space was about 94 mg/L. Therefore,

TNT mass in aqueous phase = (40.3 ml)(94 mg/L) = 3.8 mg

TNT desorbed from the aquifer material = 9.62 mg - 3.8 mg = **5.8 mg**

Unaccounted-for TNT = M_L - 5.8 mg = 13.6 mg -5.8 mg = **7.8 mg**

B.3 Mass Balance for the First Phase of Column D3

According to Figure 4-34, assume that the TNT adsorption was close to equilibrium by day 20 in the first phase of column D3, which was under the following conditions:

Flow rate = 0.028 ml/min = 0.0403 L/day, Retention time = 1.0 days

Influent of column D3 in the 1st phase:

TNT conc. = 100 mg/L, Ac = 0, NO_3^- = 80 mg/L

NaN_3 = 0.3 g/L, autoclaved, pH = 7.3

Since all of the aquifer column reactors were set up in the same manner and had approximately the same porosity, it is assumed that the accumulated TNT mass M_{ac} in column D3 was the same as that in column S4, which was 10.1 mg based on the tracer study. In column D3, therefore, we have

$M_L = M_{in} - M_{out} - M_{ac}$

= (Area under influent TNT conc. curve through day 20)(0.0403 L/day)
- (Area under effluent TNT conc. curve through day 20)(0.0403 L/day) - M_{ac}

= 74.15 mg - 48.36 mg - 10.1 mg

= **15.7 mg**

This value is reasonably close to the M_L value for column S4 (13.6 mg).

B.4 Long-term Adsorption Rate Coefficient for Column S4

In many cases, the long-term adsorption mass transfer rate coefficient, r, is expressed in a first-order rate equation (Chen and McTernan, 1992)

$$(dq)/(dt) = r(q^* - q) .$$

where q* is the equilibrated solid-phase concentration which can be described by Langmuir or Freundlich isotherm in many situations, and q is the actual solid-phase concentration at time t. Therefore,

$$r = \frac{(dq/dt)}{(q^* - q)} \approx \frac{(\Delta q/\Delta t)}{(q^* - q)}$$

For column S4, r can be determined on the basis of long-term breakthrough curves shown in Figure 4-8.

Theoretically, r can be obtained at any time point t if the kinetics is strict first-order. Assume t = 15 days, Δt = 2 days (day 15 - day 17). Then we have

$$q_{obs} = m_T / m_a$$

where q_{obs} is the observed, actual TNT loss per unit weight of aquifer materials, m_T is the total observed TNT loss by day 15, and m_a is the mass of aquifer materials in the column. m_T can be calculated from the area between bromide and TNT breakthrough curves. From Figure 4-8, q_{obs} = (2.85 mg)/(205.5 g) = 13.9 μg/g

The observed TNT loss was attributed to both reversible adsorption and irreversible loss (including abiotic transformation and irreversible adsorption) as discussed earlier. According to the results in Section B.2, the ratio of reversibly adsorbed

TNT to total TNT loss was (5.8 mg)/(13.6 mg) = 42.6%. For a tentative estimate, assume this ratio held in column S4 throughout the column operation. Then the reversibly adsorbed solid-phase TNT concentration, q, can be estimated as:

$$q = 42.6\%\ q_{obs} = 42.6\%(13.9\ \mu g/g) = 5.9\ \mu g/g$$

Suppose the Langmuir isotherm held for adsorption equilibrium (as shown in §4.1.2), then $q^* = QbC/(1 + bC)$

where C is the average concentration of TNT in the column at time t (day 15), parameters Q (41 μg/g) and b (0.026) were obtained in the batch adsorption experiment (§4.1.2). The value of C can be estimated as $C \approx (C_0 + C_t)/2$ where C_0 is the influent TNT concentration (about 100 mg/L) and C_t is the TNT concentration in the column effluent at time t. Alternatively, C can be estimated by a logarithmic average as follows, because the TNT concentration may not be linearly, but exponentially, distributed along the length of the column.

$$C \approx (C_0 - C_t)/\ln(C_0/C_t) = (100 - 46)/\ln(100/46)$$
$$= 69.5\ (mg/L)$$

Therefore, $q^* = (41)(0.026)(69.5)/(1 + (0.026)(69.5))$

$$= 26.4\ \mu g/g\ .$$

Consider $\Delta q_{obs} = \Delta m_T / m_a$

where Δq_{obs} and Δm_T are the observed TNT loss per unit weight of aquifer materials and the total observed TNT loss, respectively, in time interval Δt (2 days). Thus,

$$\Delta q_{obs} = (0.8\ mg)/(205.5\ g) = 3.9\ \mu g/g \quad \text{based on Figure 4-8,}$$

and $$\Delta q = (42.6\%)\Delta q_{obs} = (42.6\%)(3.9\ \mu g/g) = 1.7\ \mu g/g$$

So we have $$r \approx \frac{(\Delta q/\Delta t)}{(q^* - q)} = \frac{1.7/2}{(26.4 - 5.9)} = \mathbf{0.041\ day^{-1}}$$

The same procedure can be repeated at time point t = day 30, where it is estimated that $q^* = 29.5\ \mu g/g$, $q = 15.7\ \mu g/g$, and $\Delta q = 1.04\ \mu g/g$. Thus, $r = 0.038\ day^{-1}$.

APPENDIX C

SPECIATION OF H_2S IN WATER

The total concentration of sulfide species, S_{tot}, in an aqueous solution can be expressed as follows (Morel, 1983):

$$S_{tot} = [H_2S] + [HS^-] + [S^{2-}] \qquad C.1$$

According to the Mass Reaction Law, we have

$$[H_2S]\, K_{a1} = [HS^-]\, [H^+] \qquad C.2$$

and $$[HS^-]\, K_{a2} = [S^{2-}]\, [H^+] \qquad C.3$$

where the equilibrium constant K_{a1} equals 10^{-7} and K_{a2} equals $10^{-13.9}$.

Combining Equations C.1, C.2, and C.3 yields

$$[HS^-] = S_{tot} - [H_2S] - [S^{2-}]$$

$$= S_{tot} - 10^{7}\, [HS^-]\, [H+] - 10^{-13.9}\, [HS^-]/[H^+]$$

Therefore $$[HS^-]\, \{1 + 10^{7}\, [H^+] + 10^{-13.9}/[H^+]\} = S_{tot}$$

$$\%[HS^-] \text{ in solution} = [HS^-]/\, S_{tot}$$

$$= 1 / \{1 + 10^{7}\, [H^+] + 10^{-13.9}/[H^+]\} \qquad C.4$$

When pH = 8.4, $[H^+] = 10^{-8.4}$, $\%[HS^-]$ in solution = 96%.

When pH = 11.0, $[H^+] = 10^{-11}$, $\%[HS^-]$ in solution = 100%

APPENDIX D

RAW DATA OF BIOLOGICAL BATCH REACTORS: SET ONE

D.1 Denitrifying Reactors

Reactors:

D11 and D12: TNT = 60 mg/L, Ac = 180 mg/L

D21 and D22: TNT = 60 mg/L, Ac = 1000 mg/L

D31 and D32: TNT = 100 mg/L, Ac = 1000 mg/L

D41 and D42: TNT = 100 mg/L, Ac = 1000 mg/L, abiotic controls

TNT concentration data (mg/L):

Time (day)	0	3	7	12	20	53
D11	57	49.7	43.9	41.0	40	36.2
D12	57	49.5	51.0	46.8	42	29.7
D21	58	39.2	36.4	27.7	15.4	4.4
D22	58	40	33	29.7	20.8	4.0
Time (day)	0	3	7	12	20	53
D31	96	80.8	71	65	46.9	12.8
D32	96	85	66.3	64	64	30
D41	96	92	87	90	90	87
D42	96	88	85	86	88	81

ADNT concentrations in reactors D31 and D32:

Time(day)	0	3	7	12	20	53
D31	0	0	0	4.6	5	3.4
D32	0	0	0	7	6	4

Acetate and nitrate concentrations (mg/L):

Time (day)	0		7		20	
	Acetate	Nitrate	Acetate	Nitrate	Acetate	Nitrate
D11	175	360	5	0	3	0
D12	170	362	5	0	0	0
D21	900	1800	98	626	5	600
D22	945	1760	68	382	4	375
Time (day)	0		7		20	
	Acetate	Nitrate	Acetate	Nitrate	Acetate	Nitrate
D31	900	1800	240	839	2	488
D32	960	1820	180	684	3	475
D41	980	1800	960	1760	954	1790
D42	960	1860	985	1790	976	1786

Acetate and nitrate were re-spiked on day 22

D.2 Sulfate-reducing Reactors

Reactors:

S11 and S12: TNT = 30 mg/L, Lactate = 300 mg/L

S21 and S22: TNT = 30 mg/L, Lactate = 1000 mg/L

S31 and S32: TNT = 30 mg/L, Lactate = 1000 mg/L, abiotic controls

TNT and ADNT concentrations (mg/L):

	TNT		ADNT		TNT		ADNT		TNT		ADNT	
Reactor	S11	S12	S11	S12	S21	S22	S21	S22	S31	S32	S31	S32
Day 0	30	30	2.2	2.2	30	30	2.1	2.3	30	30	2.2	2.2
Day 0.1	0	0	6.3	7	0	0	6.7	6.5	0	0	6.0	6.4
Day 1	0	0	6.9	7.5	0	0	9.5	6.5	0	0	9.0	8.0
Day 6			9.0	8.2			8	7.4			8.5	10.3
Day 12			4.5	6.5			4	2			9.5	8.5
Day 24			1	1			0	0			6	4.6

Lactate and sulfate concentrations (mg/L):

	Lactate		Sulfate		Lactate		Sulfate		Lactate		Sulfate	
Reactor	S11	S12	S11	S12	S21	S22	S21	S22	S31	S32	S31	S32
Day 0	314	312	450	436	981	969	1438	1422	983	973	1370	1360
Day 24	236	210	406	382	645	611	1200	1000	965	955	1353	1341

Reactors:

S41 and S42: TNT = 60 mg/L, Lactate = 300 mg/L

S51 and S52: TNT = 60 mg/L, Lactate = 1000 mg/L

TNT and ADNT concentrations (mg/L):

	TNT		ADNT		TNT		ADNT	
Reactor	S41	S42	S41	S42	S51	S52	S51	S52
Day 0	60	57	0	0	58	57	0	0
Day 4	0	0	9.1	10.5	0	0	14.5	9.5
Day 12	0	0	8.0	6.8	0	0	9.0	7.0
Day 25			7.0	6.0			3	4.2

Reactors S61 and S62 (without pre-grown cells):

TNT = 30 mg/L, Lactate = 1000 mg/L

Time (day)	TNT (mg/L)		Lactate (mg/L)		Sulfate (mg/L)	
	S61	S62	S61	S62	S61	S62
0	28	28	955	965	984	1002
1	20.4	22.6	900	920	973	987
6	11.4	12.0	920	946	969	975
12	1.2	2.0	925	963	933	957
20	1	1	900	900	930	964
31	0	0	910	890	943	957
57			525	455	803	837
72			395	345	621	669

D.3 Methanogenic Reactors

Reactors:

M11 and M12: TNT = 30 mg/L, Acetate = 180 mg/L

M21 and M22: TNT = 30 mg/L, Acetate = 1000 mg/L

TNT and ADNT concentrations (mg/L):

	TNT		ADNT		TNT		ADNT	
Reactor	M11	M12	M11	M12	M21	M22	M21	M22
Day 0	28	26	0	0	30	32	2	0
Day 4	20	18	4	3.6	20	24	4.3	3.9
Day 15	13.5	12.7	6.4	5.4	9.9	10.3	4.6	5.2
Day 28	9.8	8.4	8.2	9.0	4	6	8.1	8.7

Reactors:

M31 and M32: TNT = 60 mg/L, Acetate = 180 mg/L

M41 and M32: TNT = 60 mg/L, Acetate = 1000 mg/L

M51 and M52: TNT = 60 mg/L, Acetate = 1000 mg/L, abiotic controls

TNT concentrations (mg/L):

Time (day)	M31	M32	M41	M42	M51	M52
0	58	56	58	58	59	57
3	54.2	52.2	51.7	50.5		
7	48.7	45.3	43.9	42	53	55
12	46.5	44.7	41.3	39.7		
20	43	41.6	40	39.2	55.9	57.3
32	42.2	40.4	36.8	35.6	50.1	52.5

APPENDIX E

RAW DATA OF BIOLOGICAL BATCH REACTORS: SET TWO

E.1 Denitrifying Reactors

	TNT (mg/L)		Acetate (mg/L)		Nitrate (mg/L)	
Time (days)	RD1	RD2	RD1	RD2	RD1	RD2
0	99.9	101.8	1935	1865	1207	1181
0.5	92.2	82.9				
1	61.4	64.4				
2	12.7	14.4	1318	1356	0	14
3	2.3	1.9				
4	2.1	1.1			500	500
5	0.6	2	971	1001	7.4	12.9
6	1.4	1.4				
8	1.9	0				
10	0	0			500	500
12	1.9	1				
14	2.1	0.8				
16	0	0				
18	0	1				
20	0	0	380	440	6.3	9.4
22	1	1.2				
27	0	1.5	400	400	4.3	4.8
32	1.5	0				
39	0	0	400	400	2.6	3.4

500 mg/L nitrate was re-spiked on Days 4,10, and 47.

HPLC Peak Area of Intermediates in Reactors RD1 and RD2

Time	Int #1	Int #2	Int #3	Int #4
(days)				
	Int-D1	ADNT	DANT	Int-D4
0	0			
0.5	81295			
1	29810	0	0	0
2	32805	122660	28563	7573
3	33094	382571	31403	12725
4	0	457652	259134	8220
5		568223	312992	12234
6		484151	482284	12160
8		620292	515904	11679
10		485025	630078	9699
12		42568	826001	0
14		0	742838	
16			577190	
18			387738	
20			244838	
22			91835	
27			0	

E.2 Sulfate-reducing Reactors

	TNT (mg/L)		Lactate (mg/L)		Sulfate (mg/L)	
Time (days)	RS1	RS2	RS1	RS2	RS1	RS2
0	100.7	100.5	3300	3250	1526	1447
0.5	80.2	89.7				
1	81.2	84.5				
2	54.8	62.5	3300	3300	1500	1400
3	43	48.5				
4	40	36.3				
5	41	28.7				
6	29.9	19				
8	21.8	6.1				
10	12.5	0				
12	4.5	0				
14	0	0				
16	0	0				
18	2.5	0				
20	1.5	1.2	3300	3039	1560	1562
22	0	1.4				
27	2.0	1.5	1225	1123	1549	1457
32	0	0	1236	1155	1600	1500
39	0	0	1250	1100	1500	1500
50	0	0	927.6	1080	1355	1500

HPLC Peak Area of Intermediates in Reactors RS1 and RS2:

Time		Int #2	Int #3	Int #4	Int #5	Int #6	Int #7
(days)							
	Int-S1	Int-S2	Int-S3	ADNT	Time	Int #1	Int-S7
0	0						
0.5	145085	0					
1	418230	154340	0				
2	489118	515108	19605	0	0		
3	560833	647000	9905	21858	13470		
4	663560	729895	52275	150535	0		
5	784715	921875	12603	209323		0	
6	783422	925163	26825	258590		7363	
8	859978	990495	6445	394883		11533	
10	957400	1077975	10528	535323		13505	
12	852005	987273	30750	604785		0	
14	818465	802038	73588	718158		12380	
16	720560	671615	115020	888163		0	
18	399738	487823	574553	656200		42848	
20	0	77840	811868	196215		423000	
22		0	165240	0		429408	
27			0			85775	0
32						60883	21163
39						47238	34220
50						38710	35690

E.3 Methanogenic Reactors and Abiotic Controls

TNT concentrations (mg/L):

Time	Abiotic controls		Methanogenic	
(days)	C1	C2	RM1	RM2
0	102.7	105.3	103.9	105.3
0.5			89.1	98.6
1			88	87.5
2	96.2	101.3	58.3	58.5
3			52	57.1
4			49	48.1
5	96.3	97.7	48	46.3
6			42.4	39.7
8			41	37.1
10	100.8	104	35.7	36.5
12			28.1	33.5
14			26.8	29.6
16	102.8	103.2	24.8	28.6
18			18.3	21.1
20			16.9	17
22	99.8	94.8	14	13.1
27	94.7	89.6	4	0
32			0	1.1
39	92.8	94.1	1	1.1

HPLC Peak Area of Intermediates in Reactors RM1 and RM2:

Time	Int #1	Int #2	Int #3	Int #4	Int #5	Int #6	Int #7	Int #8
(days)								
	Int-M1	Int-M2	Int-M3	ADNT	DANT	Int-M6	Int-M7	Int-M8
0	0							
0.5	41210	0						
1	310940	142265	0	0	0			
2	431978	504695	19945	25000	10468			
3	581810	669740	11135	11670	10073			
4	569080	634358	7393	97705	6938			
5	599835	693130	11578	108078	9220			
6	605533	698803	6715	117958	7630			
8	670255	750693	6565	133265	5485			
10	684458	707955	5655	192815	12780			
12	730048	757775	7225	219513	9925			
14	786815	827135	6412	227978	15003			
16	790093	812483	0	314675	16130	0		
18	802088	833230		317705	18480	7005		
20	850223	864693		366930	17580	8388		
22	860193	852483		421720	17322	7768	0	
27	752023	775190		626088	21445	13235	79783	
32	224765	323050		311185	538973	8188	180188	
39	0	0		0	1054300	15885	44453	0
50					911293	0	30898	41805

APPENDIX F

IDENTIFICATION OF A TNT INTERMEDIATE USING DIODE-ARRAY HPLC

Samples taken from most batch and column reactors consistently contained an unknown chemical which had a peak retention time of about 3.4 minutes on the currently used HPLC system (Section 3.3.2). This chemical was considered a TNT transformation intermediate and identified by using a diode-array HPLC system at the University of Oklahoma. The model and parameters of the diode-array equipment are as follows.

Beckman HPLC pump: programmable solvent Module 126

Detector: model 168

Column: Econosphere C18 5μ column, length: 250 mm

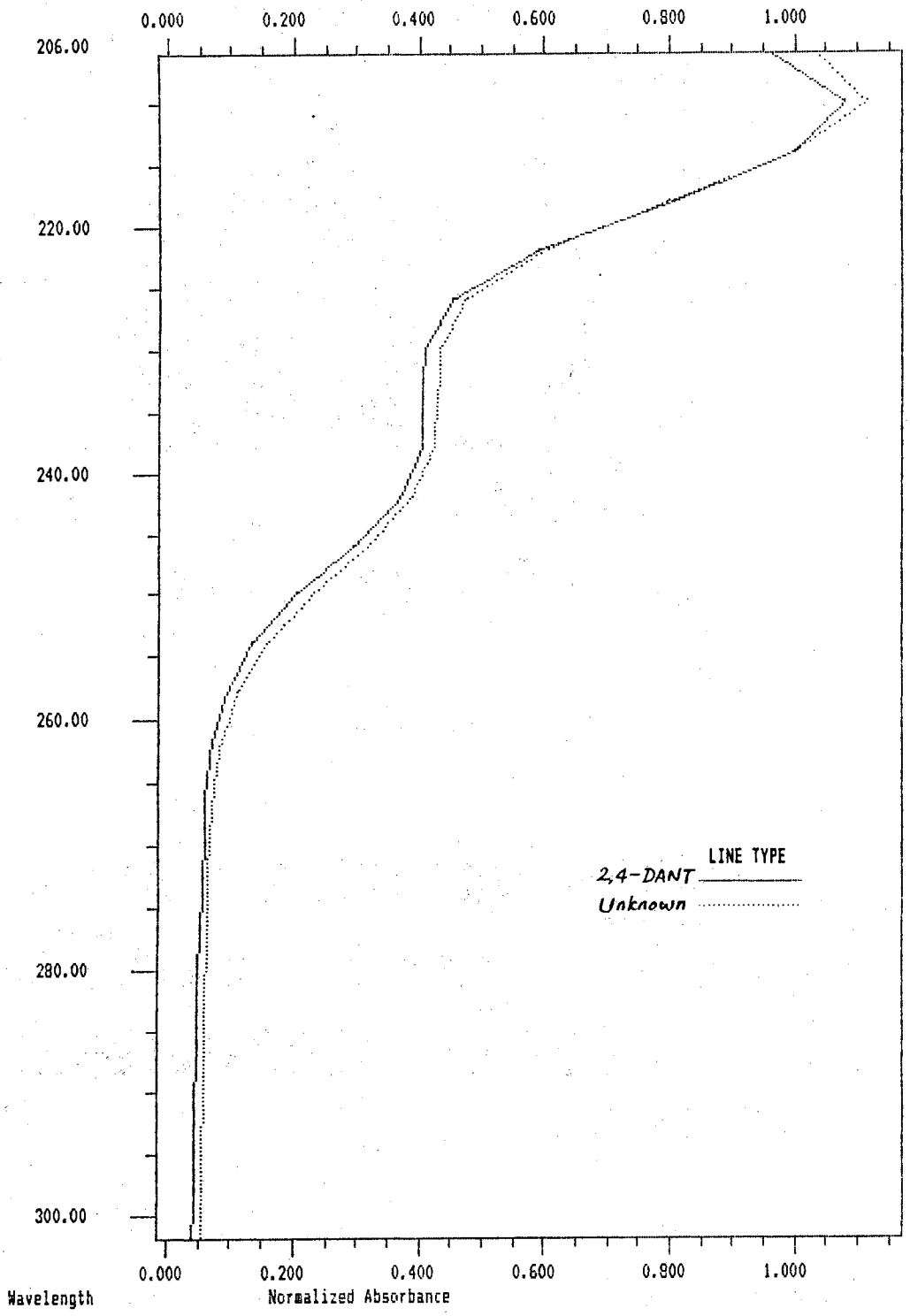
Flowrate: 1.0 ml/min.

Wavelength of diode-array scanning: 206 - 302 nm

Mobile phase: acetonitrile 35%, 10 mM PO_4^{3-} (pH 6.0) 65%

A 50 μM solution of analytical grade 2,4-diamino-6-nitrotoluene (2,4-DANT) was used as identification standard. The following figure shows the normalized absorbance of diode-array scanning of the standard and the unknown peak in one of the samples. This sample was taken from one of the sulfate-reducing batch reactors in reactor Set Two. The absorbance spectra of the standard and the unknown peak matched to each other very closely. Several other samples were analyzed using this method and the scanning results

were the same. Therefore, it was concluded that the unknown peak in these samples represented the same chemical, which was positively identified as 2,4-DANT.



APPENDIX G

RAW DATA OF CONTINUOUS COLUMNS D1 AND D3

TNT Concentration in Influent and Effluent of Column D1

Time (days)	TNT Inf. (mg/L)	Time (days)	TNT Inf. (mg/L)	Time (days)	TNT Inf. (mg/L)
0	101.7	106	97.4	230	106
4	94.5	110	100	238	96.3
10	95.8	114	93	246	96.7
13	95.1	118	95.6	256	97.7
16	98.2	122	104.1	268	103.1
19	99.4	126	105.6	275	100.4
22	105	130	107.7	286	86.4
25	96	134	101.6	297	91.6
28	105	138	101.1	314	100.9
31	96.1	142	102.3	326	104.5
34	101.9	146	107.4	334	97.2
37	100.2	150	103.1	343	104
40	98.6	154	102.3	352	102
43	98.1	158	104.9	353	60
46	100	162	107.5	367	56
50	92.8	166	103.5	374	58
52	96.7	170	92.9	380	59
55	96.5	174	92.5	387	58
58	101.2	178	98	394	60
60	99	182	90.9	410	55
62	105.1	186	105.1	417	61.5
66	78	190	103.9	423	56
70	80.3	194	98.4	435	57.3
74	98.6	198	102.2	443	60.3
78	105.2	202	99.7	456	57
82	103.2	206	92.9	471	55
86	99.7	210	126.8	478	56
90	94.8	214	106.6	486	55
94	102.9	218	103.6	496	59
98	104.7	222	98.9	500	57
102	100	226	96		

Time (days)	TNT Eff. (mg/L)	Time (days)	TNT Eff. (mg/L)	Time (days)	TNT Eff. (mg/L)
0	0	110	76.3	256	49.5
5	65.8	114	74.6	268	45.6
7	69.2	118	74.5	275	47.9
11	80	122	66.6	281	51.3
14	79.7	126	68.5	285	58.2
17	86.1	130	68.9	295	63.5
20	86.9	134	73.4	307	72.9
23	93.1	138	74	315	66.9
26	89.4	142	76.3	329	59.1
29	87.1	146	73	335	70.6
32	77.8	150	67.6	343	73.8
35	87.1	154	67.9	351	76.9
38	96.4	158	77.1	361	71.1
41	91.3	162	67.4	367	66.2
44	85.5	166	64.8	373	47.4
47	93.5	170	63.9	383	48
51	88.5	174	67.8	387	48
53	71.6	178	57.4	395	43.6
56	83.1	182	52.5	400	41.1
59	87.2	186	43.6	414	41.1
61	87.8	190	36.4	421	39
63	83.2	194	41.7	429	41.1
67	84.7	198	42.4	434	44
71	75.2	202	45.3	443	35.1
74	65	206	42.5	456	40
78	57.4	210	42.1	464	42.6
82	50.6	214	40.9	471	38.4
86	51.2	218	45.2	486	48.4
90	59.9	222	47.7	492	47.1
94	63.4	226	47.9	498	50
98	66	230	46	508	52
102	71.4	238	43.3		
106	73.1	246	50.4		

TNT Concentration in Influent and Effluent of Column D3:

Time (days)	TNT Inf. (mg/L)	Time (days)	TNT Inf. (mg/L)
0	93.6	134	102.6
4	91.5	138	101
10	87.4	142	103.8
13	89.7	146	102.8
16	97.4	150	103.7
19	96.1	154	103.2
22	105	158	105.7
25	99.2	162	107
28	99.6	166	105.1
34	96.9	170	92.4
37	97.2	174	91.1
43	94	178	97.7
46	95.5	182	90
50	96.7	186	102.9
52	91.4	190	102
55	89.1	194	97.8
58	98.5	198	99
60	96.3	202	99.4
62	100	206	93.4
66	81.2	210	121.9
70	76.1	214	106.1
74	88	218	99.5
78	94.9	222	93.5
82	101.1	226	98.3
86	101	230	94.3
90	96.7	238	105.3
94	98.4	246	103.9
98	106.1	256	99.4
102	99.6	268	102.3
106	96.2	286	90
110	110.5	297	94.4
114	91	314	106.4
118	98.1	326	104.6
122	96	334	97.2
126	98.2	342	100
130	106.7	343	0

Time (days)	TNT Eff. (mg/L)	Time (days)	TNT Eff. (mg/L)
0	0	150	75.2
5	48.8	154	80.4
7	67.5	158	76.8
11	62.5	162	86.5
14	74.1	166	82.4
17	80.9	170	77.3
20	83.4	174	80.9
23	81.6	178	75.3
26	86.7	182	48
29	85.5	186	42.5
35	76.4	190	53.3
38	87.7	194	55.5
47	82.3	198	59.8
51	70.1	202	85.2
53	67.3	206	64.9
56	75.7	210	86
59	80.5	214	84.9
61	77.9	218	74.7
63	76.5	222	77.9
67	81.5	226	87.7
71	59.3	230	93.1
74	39.7	238	89.9
78	38.9	246	78
82	39.2	256	83.9
86	48.8	268	89.6
90	62.2	275	96.4
94	57.1	285	90
98	70.7	307	92.6
102	69.1	315	93.5
106	74	335	97.1
110	79.1	343	95
114	75.9	346	89.2
118	69.1	353	90
122	70.6	358	69.3
126	66.5	363	47.2
130	71.7	367	31.6
134	75.2	373	12.4
138	74.7	380	6.5
142	73.3	387	5.5
146	71	400	5

APPENDIX H

RAW DATA OF CONTINUOUS COLUMNS S1 AND S3

TNT Concentration in Influent and Effluent of Column S1:

Time (days)	TNT Inf. (mg/L)	Time (days)	TNT Inf. (mg/L)	Time (days)	TNT Inf. (mg/L)
0	98	99	102.9	232	103.9
3	83.5	103	103.1	243	94
7	103.7	107	98.4	254	95.2
9	91.7	111	94.5	271	103.3
12	90.9	115	101	278	98
15	99.3	119	103.1	283	108.6
17	93	123	100.4	291	99.4
19	107.2	127	94.9	300	99.9
23	76.9	131	94.1	308	102
27	81.9	135	85.6	309	60
31	98.5	139	77.1	320	62
35	100.9	143	104.1	331	63
39	108.6	147	100.6	338	60
43	100	151	97	350	61
47	97.6	155	102.3	360	60.9
51	99.3	159	102.5	370	62
55	106.8	163	102.3	380	64.6
59	102.9	167	119.2	384	62
63	102	171	110.4	392	57.7
67	107	175	109.9	400	63.2
71	100.9	179	99.6	408	61
75	94.8	183	99.6	413	57
79	95.6	187	95.7	428	61.4
83	99.3	195	105.9	443	60
87	107	203	103.3	453	61
91	101.8	213	104.1	458	59
95	97.5	225	102.6		

Time (days)	TNT Eff. (mg/L)	Time (days)	TNT Eff. (mg/L)	Time (days)	TNT Eff. (mg/L)
0	0	107	66.4	272	33
4	54.1	111	72.1	286	36.9
8	64.5	115	74.7	292	51.1
10	67.2	119	60.1	300	65
13	76	123	61.5	308	64.6
16	81.9	127	64.4	318	68.4
18	77.4	131	63.9	330	49
20	74.7	135	66.5	337	46
24	75.5	139	48.2	344	49
28	61.9	143	34.4	352	43.1
31	48.2	147	26.7	357	38.4
35	45.8	151	23.6	371	40.5
39	44.2	155	26.1	377	38.6
43	52.5	159	32.2	382	44
47	58.6	163	24.3	386	36.9
51	59.5	167	25.4	391	42
55	66.6	171	25.4	400	37.2
59	66.5	175	24	408	31.5
63	71	179	36.7	413	36
67	76.5	183	30.7	421	40
71	75.3	187	34.1	428	38
75	72.2	195	29.8	437	40
79	66.5	203	31.1	443	44.8
83	62.3	213	22.7	449	42.7
87	66	225	30.7	452	52
91	70.3	232	32.3	461	50.6
95	69.6	242	38.2	465	48
99	71.4	252	44.2		
103	67.3	264	36		

TNT Concentration in Influent and Effluent of Column S3:

Time (days)	TNT Inf. (mg/L)	Time (days)	TNT Inf. (mg/L)
0	98	115	107.4
3	87.2	119	103.1
7	98.8	123	103.2
9	93.9	127	93.6
12	89.6	131	93.8
15	96.1	135	89.2
17	95.2	139	90.1
19	105.9	143	103.4
23	77.5	147	106
27	82.6	151	97.2
31	104.3	155	103.1
35	103.8	159	94.1
39	108.7	163	90.3
43	104.3	167	123
47	98.4	171	108.1
51	97.8	175	108.3
55	103.9	179	99.9
59	98.6	183	106.7
63	98.4	187	102.6
67	107	195	103.8
71	91.9	203	107.4
75	94.7	213	106.6
79	98	224	100
83	101.9	232	99.8
87	108.3	243	89.5
91	103.4	254	95.4
95	98.9	271	101.3
99	97.4	291	99.4
103	105.7	299	99
107	104.7	300	0
111	103.1		

Time (days)	TNT Eff. (mg/L)	Time (days)	TNT Eff. (mg/L)
0	0	135	82.4
4	63.9	139	60.5
8	59.1	143	42.8
10	69.2	147	41.5
13	69.7	151	45.5
16	72.2	155	71.9
18	68.1	159	90.7
20	63.6	163	89.3
24	62.7	167	92.5
28	50.4	171	85.5
31	47.5	175	108.9
35	42.8	179	102.8
39	43.9	183	97.2
43	53.8	187	96.4
47	58.9	195	70.9
51	59	203	93.7
55	65	213	94.9
59	68.9	225	97.6
63	69.7	232	104
67	71	234	98.4
71	72.5	238	35.1
75	68	242	31.4
79	66.2	252	78.2
83	63.2	264	94.4
87	67.2	272	93.1
91	70.5	292	88.7
95	70.7	306	95.6
99	71.2	310	58.7
103	71.8	315	30.9
107	72.3	320	18.2
111	74.8	324	15.5
115	76.8	330	7.8
119	78.9	341	5.9
123	76.9	344	5.1
127	74.6	350	5
131	79.5		

APPENDIX I

RAW DATA OF BATCH-FED COLUMNS

TNT Concentration Changes in Column D2:

Time (days)	TNT Inf. (mg/L)	TNT Eff. (mg/L)	Time (days)	TNT Inf. (mg/L)	TNT Eff. (mg/L)
0	32		100	110	90.1
4	33.2	0	107	109.2	71.6
8	33.5	0	114	105.7	90.7
12	31.1	0	121	32.2	89.3
16	100	0	128	31.1	39.8
20	109.6	0	135	62.6	32.4
24	95.1	9.6	142	63	46
28	108.6	0	149	61	53
32	104.8	32.1	156	62	50
36	111.5	41.2	168	63	46
40	111.8	49	180	61.3	41
44	110.6	65.4	184	62.4	54.2
48	94.6	65.7	188	58	49
52	102.6	59.8	192	61.3	49
56	102.8	67.6	194	63.6	57.4
60	96.4	74.9	196	62.9	57.6
64	97.5	61.8	200	61	57
68			204	62	52.9
72	98.5	50	208	63	55.4
76	97.1	77.5	212	63	55
80	101.2	78.3	216	61.3	53.9
84	103.2	78.2	220	59.1	48.1
88	101.7	75.6	224	62.4	46.1
92	98.2	90.4	228	60	48
96	102.2	91.1			

Raw data for column S2:

Time (day)	TNTin/eff (mg/L)	Time (day)	SO4in/eff (mg/L)	Ac in/eff (mg/L)
0		0	107	
0.01	33.5	0.01	503	300
3.99	0	3.99	280	158
4	29.7	4	490	330
7.99	1.4	7.99	88.7	40.3
8	27.6	8	490	340
11.99	0	11.99	147	117
12	32.1	12	465	335
15.99	0	15.99	160	
16	106.6	16	510	
19.99	0	19.99	508	350
20	112	20	513	353
23.99	2.9	23.99	438	350
24	97.8	24	530	350
27.99	0	27.99	467	350
28	112.8	28	480	328
31.99	0	31.99	495	350
32	105.2	32	498	350
35.99	1.9	35.99	480	340
36	109.9	36	480	332
39.99	0	39.99	484	340
40	108.4	40	480	340
43.99	1.2	43.99	489	346
44	34.6	44	503	327
47.99	0.3	47.99	519	352
48	27.7	48	523	355
51.99	0	51.99	476	355
52	32.5	52	482	358
55.99	0	55.99	556	340
56	35	56	553	321
59.99	2.5	59.99	530	325
60	32.9	60	510.9	320
63.99	0	63.99	511	320
64	31.6	64	523.5	298
67.99	0	67.99	529	300
68	31.5	68	532	327
71.99	0	71.99	483	330
72	29.5			
75.99	0			
76	31.3			

Raw data for column M:

Time (day)	TNTin/eff (mg/L)	Time (day)	TNTin/eff (mg/L)	Time (day)	TNTin/eff (mg/L)
0	11.1	84	30	128	58
6.9	0.2	87.9	0	131.9	14.8
7	10.8	88	29.8	132	60.8
13.9	0	91.9	1.1	135.9	16.6
14	10.7	92	31.3	136	60
20.9	0	93.9	1	139.9	11
21	20.7	94	30.5	140	65
27.9	0	95.9	1	143.9	9.1
28	21.7	96	30.1	144	59
34.9	0	99.9	2.4	147.9	20.5
35	31.9	100	31.1	148	60.2
41.9	0.5	103.9	2.7	151.9	24
42	30	104	31.8	152	59
48.9	0	107.9	5.2	155.9	26.6
49	29	108	32.1	156	61
55.9	0	111.9	2.4	159.9	22.6
		112	65	160	60
56	29.2	115.9	4.8	163.9	30.2
67.9	0	116	61.8		
68	31.7	119.9	7		
79.9	0	120	59.8		
80	29.2	123.9	7.7		
83.9	0	124	56		
		127.9	9.9		

APPENDIX J

COMPARISONS OF COLUMN RESULTS UNDER DIFFERENT CONDITIONS

J.1 Column D1 vs. Column S1

(1) TNT = 60 mg/L, Ac = 30 mg/L, Yeast extract = 10 mg/L

	Time (day)	TNTinf. (mg/L)	Time (day)	TNTeff. (mg/L)	Removal (mg/L)
D1:	478	56	486	48.4	7.6
	486	55	492	47.1	7.9
	496	59	498	50	9
	500	57	508	52	5
					Mean = 7.38 mg/L
S1:	443	60	452	52	8
	453	61	461	50.6	10.4
	458	59	465	48	11
					Mean = 9.8 mg/L

Statistic $t = 1.92 < t_{(0.025,5)} = 2.571$

(2) TNT = 60 mg/L, Ac = 90 mg/L, Yeast extract = 10 mg/L

	Time (day)	TNTinf. (mg/L)	Time (day)	TNTeff. (mg/L)	Removal (mg/L)
D1:	367	56	373	47.4	8.6
	374	58	383	48	10
	380	59	387	48	11
					Mean = 9.87 mg/L
S1:	320	62	330	49	13
	331	63	337	46	17
	338	60	344	49	11
					Mean = 13.67 mg/L

Statistic $t = 2.04 < t_{(0.025,4)} = 2.776$

(3) TNT = 60 mg/L, Ac = 180 mg/L, Yeast extract = 10 mg/L

	Time (day)	TNTinf. (mg/L)	Time (day)	TNTeff. (mg/L)	Removal (mg/L)
D1:	387	58	395	43.6	14.4
	394	60	400	41.1	18.9
	410	55	414	41.1	13.9
	417	61.5	421	39	22.5
	423	56	429	41.9	14.1
					Mean = 16.76 mg/L
S1:	350	61	357	38.4	22.6
	360	60.9	371	40.5	20.4
	370	62	377	38.6	23.4
					Mean = 22.13 mg/L

Statistic $t = 2.27 < t_{(0.025,6)} = 2.447$

(4) TNT = 100 mg/L, Ac = 90 mg/L, Yeast extract = 100 mg/L

	Time (day)	TNTinf. (mg/L)	Time (day)	TNTeff. (mg/L)	Removal (mg/L)
D1:	326	104.5	335	70.6	33.9
	334	97.2	343	73.8	23.4
	343	104	351	76.9	27.1
	352	102	361	71.1	30.9
					Mean = 28.83 mg/L
S1:	291	99.4	300	65	34.4
	300	99.9	308	64.6	35.3
	308	102	318	68.4	33.6
					Mean = 34.43 mg/L

Statistic $t = 2.05 < t_{(0.025,5)} = 2.571$

(5) TNT = 100 mg/L, Ac = 300 mg/L, Yeast extract = 100 mg/L

	Time (day)	TNTinf. (mg/L)	Time (day)	TNTeff. (mg/L)	Removal (mg/L)
D1:	238	96.3	246	50.4	45.9
	246	96.7	256	49.5	47.2
	256	97.7	268	45.6	52.1
	268	103.1	275	47.9	55.2
	275	100.4	281	51.1	49.3
					Mean = 49.94 mg/L
S1:	232	103.9	242	38.2	65.7
	243	94	252	44.2	49.8
	254	95.2	264	36	59.2
	271	103.3	272	33	70.3
	278	98	286	36.9	61.1
					Mean = 61.22 mg/L

Statistic $t = 2.94 > t_{(0.025,8)} = 2.306$

J.2 Column D1 vs. Column D2

(6) TNT = 60 mg/L, Ac = 30 mg/L, Yeast extract = 10 mg/L

	Time (day)	TNTinf. (mg/L)	Time (day)	TNTeff. (mg/L)	Removal (mg/L)
D1:	478	56	486	48.4	7.6
	486	55	492	47.1	7.9
	496	59	498	50	9
	500	57	508	52	5
					Mean = 7.38 mg/L
D2:		62.9		57	5.9
		61		55.9	5.1
		62		55.4	6.6
		63		55	8
					Mean = 6.4 mg/L

Statistic $t = 0.93 < t_{(0.025,6)} = 2.447$

(7) TNT = 60 mg/L, Ac = 90 mg/L, Yeast extract = 10 mg/L

	Time (day)	TNTinf. (mg/L)	Time (day)	TNTeff. (mg/L)	Removal (mg/L)
D1:	367	56	373	47.4	8.6
	374	58	383	48	10
	380	59	387	48	11
					Mean = 9.87 mg/L
D2:		61.3		54.2	7.1
		62.4		49	13.4
		58		49	9
					Mean = 9.83 mg/L

Statistic $t = 0.02 < t_{(0.025,4)} = 2.776$

(8) TNT = 60 mg/L, Ac = 180 mg/L, Yeast extract = 10 mg/L

	Time (day)	TNTinf. (mg/L)	Time (day)	TNTeff. (mg/L)	Removal (mg/L)
D1:	387	58	395	43.6	14.4
	394	60	400	41.1	18.9
	410	55	414	41.1	13.9
	417	61.5	421	39	22.5
	423	56	429	41.9	14.1
					Mean = 16.76 mg/L
D2:		61.3		48.1	13.2
		59.1		46.1	13
		62.4		48	14.4
					Mean = 13.53 mg/L

Statistic $t = 1.40 < t_{(0.025,6)} = 2.447$

J.3 Column D2 vs. Column M

Column D2 (day 168 - day 232)

1) TNT injected: 37.25 mg based on influent TNT data in Appendix I.

2) TNT discharged: 31.18 mg based on effluent data in Appendix I.

3) Abiotic loss: 4% to 10.4% according to data from columns D3 and S3. Thus, abiotic loss = 1.49 to 3.87 mg.

4) Adsorption: zero (the column was in status of adsorption equilibrium).

5) Accumulation of TNT the aqueous phase in the column pore volume: zero (because the column fluid was periodically replaced, and the TNT accumulation was accounted for in discharged TNT in the last cycle of column replacement).

6) Acetate injected: 63.3 mg.

7) Acetate discharged: 6.4 mg.

Therefore, the biological TNT removal can be calculated as follows.

Biological removal = Injected - Discharged - Abiotic loss - Adsorption - Accumulation

= 37.25 - 31.18 - (1.49 ~ 3.87)

= 2.2 ~ 4.6 (mg)

Percentage of biological removal = (2.2 ~ 4.6)/37.25 = 6 ~ 12%

Acetate utilization = 63.3 - 6.4 = 57 (mg)

Column M (day 112 - day 164)

1) TNT injected: 31.66 mg based on influent TNT data in Appendix I.

2) TNT discharged: 8.25 mg based on effluent data in Appendix I.

3) Loss due to adsorption and abiotic reactions:

a) At most 13.6 mg according to data in Appendix B; or

b) Approximately 18.9% of the total injected TNT according to data in Appendix B. Thus, TNT loss = (18.9%)31.66 = 5.97 (mg).

4) Accumulation: zero.

5) Acetate injected: 96.2 mg.

6) Acetate discharged: 80.5 mg.

Biological removal = Injected - Discharged - Abiotic loss - Adsorption - Accumulation

= 31.66 - 8.25 - (5.97 ~ 13.6)

= 9.8 ~ 17.4 (mg)

Percentage of biological removal = (9.8 ~ 17.4)/31.66 = 31 ~ 55%

Acetate utilization = 96.2 - 80.5 = 15.7 (mg)

J.4 Electron Balance in Denitrifying Reactors

1) The Stoichiometry of Electron Transfer

a) $CH_3COO^- + 3H_2O == CO_2 + HCO_3^- + 8H^+ + 8e^-$

b) $TNT + 18e^- ==> TAT$

c) $NO_3^- + 6H^+ + 5e^- == 0.5N_2 + 3H_2O$

2) The Observed Data

a) Column D2 (Day 80 - 88, TNT = 100 mg/L, Ac ≅ 300 mg/L)

Ac utilization = 21.2 mg = 0.36 mM, equivalent to 2.9 mM e^-

TNT conversion = 2.04 mg = 0.009 mM, equivalent to 0.16 mM e^-

NO_3^- consumption = 33 mg = 0.53 mM, equivalent to 2.7 mM e^-

Electron supply = **2.9 mM**

Electron sink = 0.16 mM + 2.7 mM = **2.86 mM**

b) Denitrifying reactors (duplicates) of batch reactor Set Two (TNT = 100 mg/L)

Ac utilization = 2789 mg = 47.3 mM, equivalent to 378 mM e^-

TNT conversion = 80 mg = 0.35 mM, equivalent to 6.3 mM e^-

NO_3^- consumption = 4283 mg = 69.1 mM, equivalent to 345 mM e^-

Electron supply = **378 mM**

Electron sink = 6.3 mM + 345 mM = **351.3 mM**

APPENDIX K

STOICHIOMETRIC CALCULATIONS FOR METHANOGENESIS

Assuming the chemical composition of bacterial cells is $C_5H_7O_2N$ and the substrate is acetate, we have the following equations for methanogenesis.

R_c: $0.05C_5H_7O_2N + 0.45H_2O = 0.2CO_2 + 0.05HCO_3^- + 0.05NH_4^+ + H^+ + e^-$

R_a: $0.125CH_4 + 0.25H_2O = 0.125CO_2 + H^+ + e^-$

R_d: $0.125CH_3COO^- + 0.375H_2O = 0.125CO_2 + 0.125HCO_3^- + H^+ + e^-$

Assume that the cell yield, Y, is 0.03 mg VSS/mg HAc (Wilber, 1991). Thus,

$f_s = 1.42Y = 0.043$, $f_e = 1 - f_s = 0.957$.

The final reaction equation R equals ($R_d - f_sR_c - f_eR_a$). Therefore,

$$CH_3COO^- + 0.0256\ CO_2 + 0.0172NH_4^+ + 0.928\ H_2O = 0.0172\ C_5H_7O_2N + 0.957\ CH_4 + 0.984HCO_3^-$$

From the above equation, the ratio of acetate utilization to methane production is as follows.

$$(CH_3COO^-)/(0.957\ CH_4) = 59/(0.957\times16) = 3.85/1$$

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