BIOLOGICAL TREATABILITY OF HEXAHYDRO-1,3,5-TRINITRO-1,3,5-TRIAZINE (RDX)-CONTAMINATED SOIL

BY

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CHAPTER I

INTRODUCTION

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is a xenobiotic compound. It is a white crystalline solid that is primarily used by the military in high-impact explosives. Civilian uses of RDX include fireworks, heating fuel and rat poison (McLellan et al. 1992; Smith-Simon and Goldhaber 1995). The compound moves in surface water and through soil into groundwater. In surface water, RDX can be degraded via photolysis. However, the half-life of RDX in some natural waters is estimated to be as long as 13 days (Burton and Turley 1995). Smith-Simon and Goldhaber (1995) cites an estimated half-life of RDX in a 20 inch deep wastewater lagoon at the Louisiana Army Ammunition Plant to be as long as 2,000 days in winter and 456 days in summer.

RDX has been identified at 16 proposed National Priorities List (NPL) sites and at several sites that are currently on the NPL (Smith-Simon and Goldhaber 1995). The concentrations of RDX, found in soils or in groundwater at contaminated sites, varies from site to site. However, the RDX concentrations found at the Louisiana Army Ammunition Plant seem to be typical. The concentrations found there range from <5 to 602 mg/kg in soil and 1.3 to 14,100 µg/l in groundwater (Smith-Simon and Goldhaber 1995).

RDX is one of several nitroaromatic compounds detected in soils and groundwater at the Department of Energy (DOE) Pantex plant and in Amarillo, Texas. The compound is in a perched groundwater zone at a depth of about 230-250 feet. Also, various areas of contaminated soils including a playa lake, landfill, and ditches have been identified (U.S Army Corps of Engineers 1994). Concentrations of RDX range from 0.18 to 15,000 mg/kg in these contaminated soils. Ostrander (1994), citing various field studies at the former Pantex Ordnance Plant site (adjacent to DOE site) in Amarillo, Texas states that explosives contamination is concentrated in the upper two feet of surface soils. Also, nodules and fragments of 2,4,6-trinitrotoluene (TNT), the most common nitroaromatic compound in the Pantex soils, are found throughout the contaminated areas.

The proposed strategy to be employed in remediation of the soil at the DOE Pantex facility is to excavate and treat the soil using ex-situ bioremediation. The method of interest is a prepared-bed or a bioslurry reactor process. Therefore, this study, consisting of a limited set of preliminary bench-scale experiments, was performed to define the effect of certain operating conditions on the efficiency of such a process for the bioconversion of RDX. The aim of this research was to study the biological treatability of RDX-contaminated soil under aerobic, nitrate-reducing, sulfate-reducing, and methanogenic conditions.

The main objectives of this study were the following:

1. To study the ability of native soil bacteria to degrade RDX under various electron accepting conditions.

2. To study the use of a primary organic substrate to support cometabolic bioconversion of RDX.

3. To determine if competing substrates or sorption to soil particles limit the rate of RDX biotransformation.

4. To detect any possible inhibitory effects of RDX on sulfate-reduction and methanogenesis.

CHAPTER II

LITERATURE REVIEW

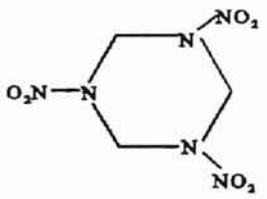
2.1 SOURCES OF RDX

RDX is a hexacyclic ring containing six nitrogen atoms. It is usually prepared by the nitration of hexamethylene tetramine ($C_6H_{12}N_4$) (McLellan et al. 1992). Production grade RDX generally contains the impurities HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) and AcRDX (1-acetylhexahydro-3,5-dinitro-1,3,5-triazine) (Binks et al. 1995).

The physical and chemical characteristics of RDX are summarized in Table I. Of particular importance for remediation of RDX-contaminated soil using prepared-bed or soil-slurry technology is the low solubility of RDX in water. Solubility in water ranges from 21.8 mg/l at 10°C to 67 mg/l at 30°C (Smith-Simon and Goldhaber 1995; McLellan et al. 1992). Another important characteristic of RDX is its low affinity for soil. Studies by Xue et al. (1995) and Haderlein et al. (1996) indicate that RDX adsorption isotherms are distinctly linear and exhibit a very low adsorption rate constant (K_d). Soil sorption coefficient (K_{oc}) of 63.1 to 270 has been reported by Smith-Simon and Goldhaber (1995), who indicate that this corresponds to medium-to-high mobility of RDX in soil.

Table I

Chemical and Physical Properties of RDX

Chemical Name	Hexahydro-1,3,5-trinitro-1,3,5-triazine
Synonyms	Cyclonite; 1,3,5-triazine-1,3,5-trinitro-cyclohexane; 1,3,5-trinitrohexahydro-1,3,5-triazine; cyclotrimethylenenitramine; hexogen; hexalite; PBX; RDX; T_4; *sym*-trimethylenetrinitramine; 1,3,5-trinitrohexahydro-s-triazine
CAS Number	121-82-4
Molecular Formula	$C_3H_6N_6O_6$
Chemical Structure	
Molecular Weight	222.26 g/mol
Physical State	White crystalline solid
Melting Point	205-206 °C
Density at 20°C	1.82 g/ml
Partition Coefficients:	
Log K_{ow}	0.87
Log K_{oc}	1.80-2.43
Vapor Pressure at 20°C	1 x 10^{-9} mm Hg
Henry's Law Constant at 25°C	1.96 x 10^{-11} atm-m^3/mol
Solubility:	
Water at 10°C	21.8 mg/l
at 20°C	38.4-42.3 mg/l
at 30°C	66.7-67 mg/l
Organic Solvent(s)	Soluble in methanol, ether, ethyl acetate, glacial acetic acid

Adapted from: Smith-Simon and Goldhaber 1995; McLellan et al. 1992

In the US, RDX production is limited to Army ammunition plants. Under SARA (Superfund Amendment and Reauthorization Act) section 313, release of RDX is not required to be reported (Smith-Simon and Goldhaber 1995). RDX enters the environment through several means. It can enter the atmosphere through contaminated particulate matter from the detonation of munitions or from the incineration of RDX-containing mixtures. It also enters the environment through wastewaters generated at munitions manufacturing and handling facilities.

RDX is commonly found in wastewaters from munitions manufacturing plants and handling facilities. McCormick et al. (1981) cites that up to 12 mg/l of RDX may be released to the environment from RDX manufacturing process waters. Process waters generally contain appreciable concentrations of TNT as well. For example, Semmens et al. (1985) found effluent from a munitions handling facility in New South Wales, Australia containing between 75 and 150 mg/l of both RDX and TNT. Improper disposal of these wastewaters can result in contamination of surface waters and soils.

RDX enters the soil by leaching from waste lagoons. Due to its low affinity for soil, RDX tends to migrate through the soil where seepage into groundwater can occur (Haderlein et al. 1996; Smith-Simon and Goldhaber 1995). Contaminated groundwater has been reported at several sites including the Milan Army Ammunition Plant in Tennessee and the Cornhusker Army Ammunition Plant in Nebraska (Smith-Simon and Goldhaber 1995).

RDX has the potential for entering the food-chain through plants grown on RDX-

contaminated soil. Harvey et al. (1991) found significant bioaccumulation of RDX in the edible tissues of bush bean plants grown in RDX-containing hydroponic solutions. Foliar concentrations of 97 ppm were found in plants grown in 10 ppm RDX hydroponic solution for 7 days. In later studies, as cited by Smith-Simon and Goldhaber (1995), plant uptake of RDX was found to be dependent on the concentration of RDX in the soil and the species of plant. Plant uptake of RDX increased as organic matter in the soil decreased. No reported cases of RDX-intoxication through this mechanism were found.

2.2 TOXIC CHARACTERISTICS OF RDX

The first reported case of RDX-poisoning was to Italian factory workers in 1939 (Testud et al. 1996). Although infrequent, RDX-poisoning is most generally related to its manufacture. Testud et al. (1996) reports a case of RDX-induced seizures and unconsciousness due to inhalation of the powdered material by a munitions factory worker. Non-occupational related RDX-poisonings have also occurred. Intentional ingestion of RDX by American soldiers during the Vietnam War has been reported (McLellan et al. 1992; Testud et al. 1996). No human fatalities have been reported involving RDX-poisoning (McLellan et al. 1992; Testud et al. 1996).

Burton et al. (1994) found RDX to be acutely toxic to fathead minnows (*Pimephales promelas*) with a 96-hr LC_{50} (lethal concentration, 50% kill) of 12.7 mg/l for 15-17 day old fathead minnows and 4.1 to 13 mg/l for juvenile channel catfish, rainbow trout and bluegill. Peters et al. (1991) found that RDX, at its solubility limit, was not

acutely toxic to three freshwater invertebrates. However, RDX was found to be chronically toxic to the daphnid *Ceriodaphnia dubia* with a lowest observed effect concentration (LOEC) and a no observed effect concentration (NOEC) of 6.01 and 3.64 mg/l , respectively. Burton et al. (1994) found RDX to be chronically toxic to fathead minnows with an LOEC of 2.4 mg/l and an NOEC of 1.4 mg/l.

McLellan et al. (1992) provide a longer-term health advisory (HA) for RDX of 0.1 mg/l for a 10 kg child and 0.4 mg/l for a 70 kg adult. These values are based on available animal data and the adverse effects on the central nervous system (CNS) of monkeys administered RDX for 90 days. A conservative estimate of the one-day and ten-day HA is the long-term HA.

Smith-Simon and Goldhaber (1995) give an acute oral MRL (minimum risk level) for RDX as 0.06 mg/kg·day. This is based on a NOAEL (no observed adverse effect limit) value of 6 mg/kg·day for seizures in rats administered RDX for 9 days. Smith-Simon and Goldhaber (1995) also give an intermediate oral MRL of 0.03 mg/kg·day and a chronic oral reference dose (RfD) of 0.003 mg/kg/day. These values are based on a NOAEL of 8 mg/kg·day and 0.3 mg/kg·day for reproductive effects in rats administered RDX for 6 months or one year, respectively.

RDX has been assigned a weight of evidence carcinogenic classification of C, which indicates that RDX is a possible human carcinogen. OSHA (Occupational Safety and Health Administration) limits the concentration of RDX in air, inside ammunition plants, at 1.5 mg/m^3. The EPA (Environmental Protection Agency) limits the

concentration of RDX in wastewater effluent at 2.0 μg per liter of water (Smith-Simon and Goldhaber 1995).

2.3 PHYSICAL/CHEMICAL TREATMENT OF RDX WASTES

Several physical and chemical treatment technologies have been evaluated for applicability to the treatment of RDX-contaminated waters. Granular activated carbon (GAC) technologies have been the primary method of treatment of these waters. Smith-Simon and Goldhaber (1995) cite a study where no residual RDX was detected in contaminated groundwater after passing through activated carbon columns. The initial concentration of RDX in the contaminated groundwater was 487 μg/l and was applied to the columns at a rate of 7.11 gpm/ft^3 with an empty bed contact time (EBCT) of 4.2 minutes. McLellan et al. (1992) also cites a study where RDX was completely removed from waters containing the contaminant at levels ranging from 1.5 to 12 mg/l. However, RDX tends to break through columns quickly, and the capacity for RDX is further reduced in the presence of other organics such as TNT (McLellan et al. 1992; Semmens et al. 1985).

Spent carbon has traditionally been disposed of through open burning. However, this practice is no longer allowed in many areas (Smith-Simon and Goldhaber 1995). Several regeneration techniques have been evaluated for the re-activation of explosive-contaminated carbon columns. The method that shows the most promise is alkaline hydrolysis. Studies by Semmens et al. (1985) indicate that the use of hot caustic is

effective in reducing RDX concentrations in process waters. Semmens et al. found that RDX (40 mg/l) was completely removed from aqueous samples that were brought to a boil then dosed with sodium hydroxide (NaOH) to a pH greater than 11. TNT (60 mg/l) was also removed using this approach. However, complete removal did not occur at a pH less than 11.6. Later studies by Heilmann et al. (1996) found that RDX and HMX could be removed from aqueous samples using similar techniques. Heilmann et al. (1996) observed complete removal of RDX (36 mg/l) from samples at 80°C with a pH of 12 after only a few minutes. HMX was much more persistent, with approximately 99.9% removal occurring after 100 minutes. Heilmann et al. (1996) applied this process to spent carbon columns and found no residual RDX on the regenerated carbon columns. This approach appears to be feasible. However, special considerations must be made due to the high temperatures and pH of the regenerant.

Other physical and chemical treatment technologies have been evaluated for use with RDX-contaminated waters including ion-exchange, chemical oxidation, chemical coagulation and enhanced photolysis (McLellan et al. 1992). Of these, enhanced photolysis, using hydrogen peroxide as a catalyst, shows the most potential. McLellan et al. (1992) cite a study where RDX (18.9 mg/l) was rapidly degraded by ultraviolet light when hydrogen peroxide was added at less than 0.01%. Hydrogen peroxide alone had no effect on RDX, but when combined with ultraviolet light, the half-life of RDX was 8.0 minutes. Semmens et al. (1985) cite a similar study using actual process waters containing TNT. Semmens et al. (1985) indicates that dilution of the process waters was necessary to reduce the intensity of its color prior to treatment.

Open incineration has been the primary method of treatment for RDX-contaminated soils and solid wastes resulting from RDX manufacturing and munitions disposal. However, this treatment method will soon be unavailable due to the potential for RDX-contaminated particulate matter to further contaminate soils and surface waters (Heilmann et al. 1996; Smith-Simon and Goldhaber 1995). Heilmann et al. (1996) cite a study that indicates combustion of RDX can also lead to the formation of large amounts of hydrocyanic acid (HCN).

Biological treatment of RDX-contaminated soils and groundwaters is an important treatment alternative to the physical and chemical process discussed previously. Biological treatment offers several advantages including the possibility of remediation of soils and groundwater in-situ.

2.4 BIOLOGICAL TREATMENT OF RDX WASTES

2.4.1 Aerobic Treatment

Early studies by McCormick et al. (1981) indicated that RDX was not amenable to microbial degradation under aerobic conditions. McCormick et al. (1981) found that concentrations of RDX (50-100 mg/l) remained unchanged in nutrient broth cultures when inoculated with aerobic activated sludge and incubated aerobically. Kitts et al. (1994) and Smith-Simon and Goldhaber (1995) cite similar results from several other studies. Manning et al. (1995) report RDX degradation in an aerobic soil-slurry of munitions-

contaminated soil from the Joliet Army Ammunition Plant in Joliet, Illinois. RDX (110 mg/kg) fell below 0.5 mg/kg after 120 days of continuous operation. However, anaerobic conditions did exist in these reactors. The reactors of Manning et al. (1995) were only aerated for 15 to 30 minutes a day. Oxygen was depleted throughout the reactor depth within 7 hours. Therefore, RDX degradation due to aerobic microbiological activity is unproven.

Smith-Simon and Goldhaber (1995) cite that aerobic microbial degradation of RDX (5.5 to 11.5 mg/l) occurred in river water samples only after the addition of river sediments. After 2-3 week lag phase, complete mineralization of RDX was observed. An estimated half-life for RDX was determined as approximately 7 days under the conditions tested. No attempt was made by Smith-Simon and Goldhaber (1995) to explain why the addition of river sediment enhanced RDX degradation in these reactors. Nor did they conclude that this is why earlier aerobic studies had failed.

Jones et al. (1995) observed RDX mineralization using [^{14}C]-labeled RDX under aerobic nitrogen-limited conditions by a bacterium cultivated from RDX-contaminated soil. Jones et al. (1995) also observed TNT mineralization under the same conditions using [^{14}C]-labeled TNT. Jones et al. (1995) comment that TNT was found to be much more recalcitrant than RDX under the test conditions. Binks et al. (1995) report RDX biotransformation under aerobic nitrogen-limited conditions by *Stenotrophomonas maltophilia* PB1. RDX , approximately 42 mg/l, completely disappeared in less than 100 hours after an initial lag period of 100 hours. A single intermediate was identified as methylene-N-(hydroxymethyl)-hydroxylamine-N'-(hydroxymethyl)nitroamine. This

molecule contains only three nitrogen atoms, suggesting that *Stenotrophomonas maltophilia* PB1 utilized only three of the nitrogen atoms available from the RDX molecule (Binks et al. 1995).

Aerobic composting of munitions-contaminated soil has been reported (Williams and Myler 1990; Bayman et al. 1995; Doyle et al. 1992). In a pilot study on soil from the Louisiana Army Ammunition Plant using an aerobic static pile process under thermophilic and mesophilic conditions, the half-life of RDX was found to be 17.3 and 30.1 days, respectively by Williams and Myler (1990). TNT and HMX were also transformed. The calculated half-lives under thermophilic and mesophilic conditions were 11.9 and 30.1 days for TNT and 22.8 and 42.0 days for HMX. Williams and Myler (1990) did not address the accumulation or disappearence of any daughter compounds. However, Bayman et al. (1995) cite that mineralization of [^{14}C]-labeled RDX occured in laboratory- and greenhouse-scale compost systems. Doyle et al. (1992) performed laboratory and pilot-scale aerobic composting studies using uncontaminated soils from the Pantex facility. The soil-compost mixtures, spiked with known concentrations of RDX (221 ppm), HMX (221 ppm), PETN (pentaeryythritol tetranitrite) (402 ppm) and TATB (triaminotrinitrobenzene) (2700 ppm), were incubated at 58-60 °C. RDX was reduced to below detection limits within 3 weeks of composting. Laboratory studies using [^{14}C]-labeled RDX indicated that RDX was degraded by ring cleavage with CO_2 as the only recognizable degradate (Doyle et al. 1992).

Fungal interactions with RDX under aerobic conditions have also been observed (Bayman et al. 1995; Gorontzy et al. 1994). Bayman et al. (1995) studied the

potential of four species of fungi to degrade RDX in laboratory tests. Loss of RDX was observered in all of the pure cultures examined. However, evidence of minerialization could not be obtained using [^{14}C]-labeled RDX (Bayman et al. 1995). Gorontzy et al. (1994) cite that mineralization of RDX occured using white rot fungus (*Phanerochaete chryssosporium)*. Only 4% of the [^{14}C]-labelled RDX was recovered in the aqueous solution, while 66.6 ± 4.1% was recovered as CO_2.

2.4.2 Anaerobic Treatment

Anaerobic biodegradation of RDX has been established (McCormick et al. 1981, 1984a, 1984b; Kitts et al. 1994; Gorontzy et al. 1994; Funk et al. 1993). McCormick et al. (1981) reported essentially complete disappearence of RDX (50-100 mg/l) after 4 days using static anaerobic batch reactors. Each reactor was inoculated with anaerobic sewage sludge and incubated at 37°C. The sequential build-up and disappearance of the mono-, di-, and trinitroso analogs of RDX were also observed. The mono-, di-, and trinitroso analogs of RDX are hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX) and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX), respectively. Sequential build-up and disappearence of these nitroso analogs was also reported by Kitts et al. (1994). McCormick et al. (1981) also detected formaldehyde (HCOH) and methanol (MeOH) in the supernatant. McCormick and coworkers proposed that stepwise reduction of the nitro groups is the first step in RDX degradation (Figure 1) (McCormick et al. 1981, 1984a, 1984b). In later studies (McCormick et al. 1984a), hydrazine, 1,1-dimethylhydrazine and 1,2-dimethylhydrazine were also identified following anaerobic degradation of RDX.

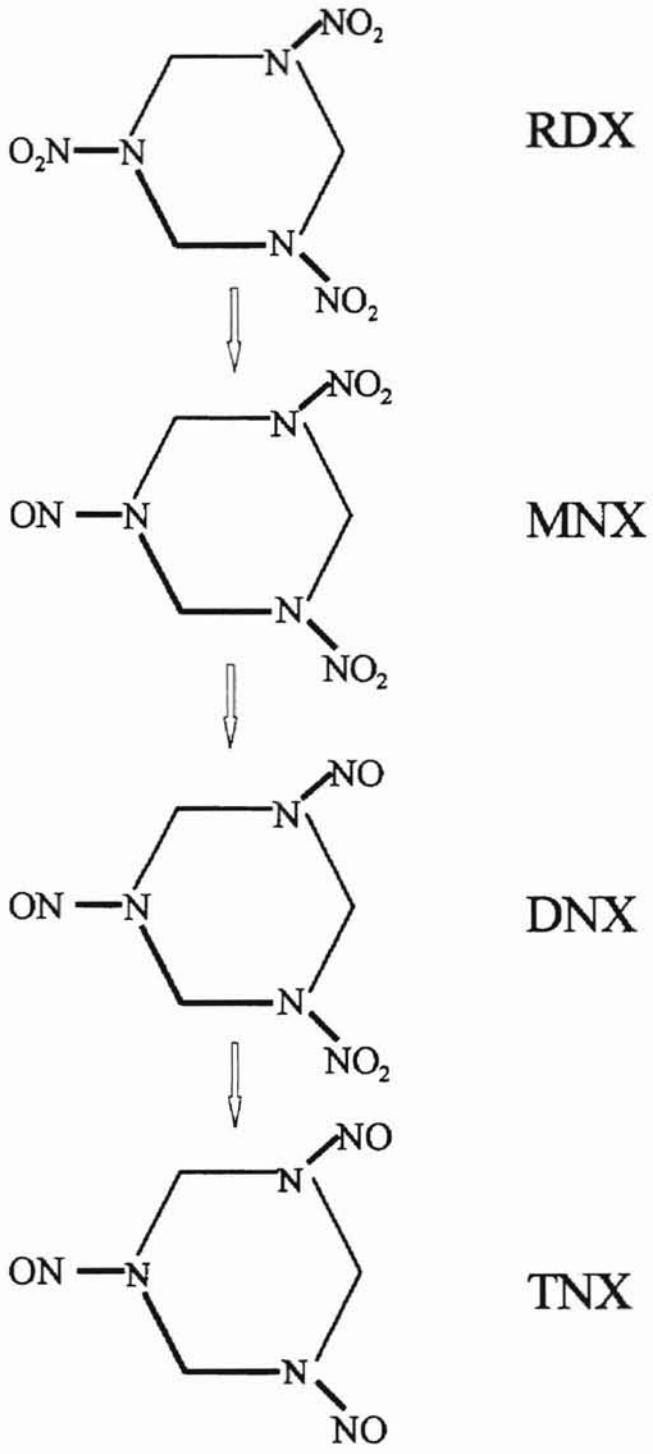


Figure 1. Stepwise reduction of nitro groups on RDX. (MNX: hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine; DNX: hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine; TNX: hexahydro-1,3,5-trinitroso-1,3,5-triazine) (McCormick et al. 1981)

Different strains of bacteria have been isolated that are capable of RDX transformation. Smith-Simon and Goldhaber (1995) cite that 97% of RDX was degraded by a mixed population of purple photosynthetic bacteria after 5 days of incubation. The mixed population included bacteria of the genera *Chromatium, Rhodospirillum* and *Rhodopseudomonas*. Gorontzy et al. (1994) cite a study where RDX was degraded by three strains of *Corynebacterium* isolated from RDX-contaminated soil. More than 90% of the 40 to 60 mg/l of RDX was degraded within 1 to 3 days. However, the addition of ammonium inhibited RDX degradation, indicating the use of RDX as a nitrogen source by these bacteria. Kitts et al. (1994) observed RDX transformation by pure cultures of *Providencia rettgeri* B1, *Morganella morganii* B2, and *Citrobacter freundii* NS2 isolated from explosives-contaminated soil.

Anaerobic degradation of RDX under denitrifying conditions has been investigated (McCormick et al. 1984b). In a continuous culture with a retention time of 10 to 14 days, RDX (30 mg/l) and related compounds (AcRDX 20 mg/l, HMX 10 mg/l, AcHMX 20 mg/l) were successfully degraded. In this study, several different enrichment media were investigated. Each of the mediums included approximately 0.04M potassium nitrate (KNO_3). Successful reduction of the target compounds did not occur in all of the media. It was determined that microbial activity increased proportionally to the amount of available carbon in the medium with 0.8% nutrient broth (TOC = 3135 mg/l) showing the most activity. The relative reaction rates for the compounds investigated were RDX>AcRDX>HMX>AcHMX.

Anaerobic soil-slurry technology has been shown to be effective in treating soils

contaminated with the nitroaromatic herbicide dinoseb (2-*sec*-butyl-4,6-dinitrophenol) (Kaake et al. 1992,1995; Roberts et al. 1993). This approach has been evaluated for use on munitions-contaminated soils (Funk et al. 1993). The primary objective was to remove TNT. However, RDX (30 mg/l) was also successfully removed from batch reactors within 24 days with no identifiable intermediates.

2.5 SUMMARY

Although biological degradation of RDX has been established, application of this approach to RDX-contaminated soil has not been fully developed. There also appear to be inconsistencies in the aforementioned studies, primarily in the ability of aerobic microorganisms to degrade RDX. Several other questions including the optimal conditions for RDX degradation and the fate of RDX and its intermediates are yet to be answered. In light of the physical and toxic characteristics of RDX and the number of contaminated sites that have already been identified, further research is needed to answer these questions. The aim of this research was to study the biological treatability of RDX-contaminated soil under aerobic, nitrate-reducing, sulfate-reducing and methanogenic conditions.

CHAPTER III

MATERIALS AND METHODS

3.1 SOIL SAMPLES

Soil samples from the DOE Pantex plant in Amarillo, Texas were provided by the U.S. Army Corps of Engineers, Tulsa District. Three samples of approximately 0.5 ft^3 each were delivered to the Environmental Engineering labs of OSU in December, 1995. The samples delivered represented three different ranges of RDX contamination: "0" mg/kg, 10-100 mg/kg, and greater than 10,000 mg/kg. The samples were collected from a landfill area at the DOE Pantex facility. All experiments were carried out using soil containing the "middle range" (10-100 mg/kg) of RDX-contamination. These soils also contained appreciable concentrations of TNT and other nitroaromatic compounds. Analysis of the "middle range" soils (as described below) indicated that the average initial concentrations of RDX, HMX, and TNT were considerably higher than expected. Average concentrations measured were approximately 5100 ppm, 2100 ppm, and 2700 ppm for RDX, HMX, and TNT, respectively. Some variations in concentrations were observed in the four samples that were analyzed and subsequently used in determining the average concentrations presented above. The ranges of RDX, HMX, and TNT in these samples were 4900-5300, 1800-2500, and 1025-5200 ppm, respectively.

3.2 REAGENT-GRADE MATERIALS

Reagent-grade RDX (1000 μg/ml in acetonitrile or methanol) was obtained from Chem Service (West Chester, PA) in 5 ml ampules or from Supelco (Bellefonte ,PA) in 1 ml ampules. Reagent-grade TNT (1000 μg/ml in acetonitrile or methanol) was obtained in 1 ml ampules from Supelco. Other chemicals were of analytical grade and, unless stated otherwise, were obtained from Fisher, Chem Service, Sigma Chemical Company (St. Louis, MO), or Supelco.

3.3 BIOLOGICAL REACTORS

Three primary series of experiments were performed, each including four sets of reactors under different electron accepting conditions (aerobic respiration, nitrate reduction, sulfate reduction and methanogenesis). All reactors were run in duplicate. The initial series of experiments used soil-water slurries of the "mid-range' Pantex soil. The purpose of these reactors was to simulate the actual operating conditions of the soil-slurry reactor with all its complexities. The second series of reactors contained no soil, but instead an aqueous extract from the same "mid-range' RDX-contaminated soil. As such, these reactors contain all of the soluble constituents initially present in the first set of reactors, but did not contain a source of continuously desorbing or dissolving nitroaromatic contaminants. The third set of reactors were fed reagent-grade RDX as the only nitroaromatic constituent, the purpose being to further simplify the initial soil-water reactors by eliminating other nitroaromatics that might compete as substrate or otherwise

inhibit RDX degradation.

All test reactors were set up using a concentrated enrichment medium and trace metal solution. The final concentrations of the enrichment medium and the trace metal solution constituents within each reactor are provided in Tables II and III, respectively. The enrichment medium was adapted from Manning et al. (1995) and Shah (1995). The trace metal solution was adapted from Shah (1995). At the beginning of each experiment, the reactors were also fed an initial dose of yeast extract to minimize the likelihood of nutrient limitations. Unless otherwise specified, the final concentration of yeast extract within each reactor was 100 mg/l. Each reactor was also dosed with two to three drops of concentrated resazurin solution to serve as a color indicator of the redox potential of the reactor.

Table II

Enrichment Medium

Compound	g/l
NH_4Cl	0.40
NaCl	0.05
$CaCl_2$	0.04
$MgCl_2$	0.01
$NaHCO_3$	0.50
K_2HPO_4	1.40
KH_2PO_4	0.60

Table III

Trace Metal Solution

Compound	mg/l
$FeSO_4$-$7H_2O$	200
$ZnSO_4$-$7H_2O$	10
$MnCl_2$-$4H_2O$	3
$CoCl_2$-$6H_2O$	20
$CuCl_2$-$2H_2O$	1
$NiCl_2$-$6H_2O$	2
Na_2MoO_4-2H2O	3

Within each set of reactors, some of the reactors were fed an additional carbon substrate to investigate the usefulness of such an amendment. Lactate was added to the sulfate-reducing reactors and glucose to the other three types. The final concentration of the additional substrate was 1 g/l. Soluble substrate concentrations were monitored (as described below) in each of the reactors were it had been amended and was reintroduced as necessary.

All the reactors within a particular set were also amended with the appropriate electron acceptor (oxygen, nitrate, sulfate or bicarbonate). The aerobic reactors were continuously aerated with compressed air throughout the duration of the experiments, which required some volume adjustment periodically using distilled water. Nitrate and sulfate were added to the appropriate reactor sets to provide an initial concentration of 660 mg/l and 1000 mg/l, respectively. The concentrations of nitrate and sulfate in these

reactors were monitored (as described below) over time and reintroduced as necessary.

Abiotic control reactors were also set up for each set of reactors and monitored for comparison to the biologically active reactors. The control reactors were identical to the active reactors except that they were dosed with sodium azide to a final concentration of 4.6 mM to prevent biological activity.

Each set of water extract and reagent-grade RDX reactors were seeded with a 2% (v/v) inoculum from the soil slurry reactors with the corresponding redox condition. A sulfate-reducing culture could not be established from the Pantex soil. Therefore, all the sulfate-reducing reactors were initially inoculated (2% v/v) with an active sulfate-reducing mixed culture established from digested sludge supernatant from the Stillwater, OK municipal wastewater treatment plant and leachate and contaminated soil from an aquifer adjacent to the landfill at Norman, OK.

The initial pH in each reactor was adjusted to 7.1 using either 10% HCl (hydrochloric acid) or 0.1 M NaOH (sodium hydroxide) as needed. Each reactor was incubated at ambient conditions (22°C) on a shaker table. Prior to being incubated, each anaerobic reactor was purged with either argon or nitrogen gas for 15-30 minutes and the headspace in the reactor was also filled with the inert gas. Aqueous samples were taken periodically from each reactor and monitored (as described below) for RDX degradation.

3.3.1 Soil-Water Reactors

Soil-water reactors were prepared using "mid-range" Pantex soil and the

aforementioned media. Aerobic reactors were prepared in 1 liter glass bottles with a liquid volume of 500 ml. The reactors were then dosed with 100 grams of "mid-range" Pantex soil. All other soil-water reactors were prepared in 500 ml glass bottles with a liquid volume of 250 ml. These reactors were dosed with 50 grams "mid-range" Pantex soil.

RDX in the aqueous-phase reached its solubility limit (38.4 to 42.3 mg/l) (Smith-Simon and Goldhaber 1995; McLellan et al. 1992) within the first few days of incubation in all soil-water reactor sets. Aqueous-phase HMX and TNT were not monitored in these reactors, although their presence was noted. Solid-phase RDX, HMX and TNT were estimated to be the average concentrations determined for the "mid-range" Pantex soil. These concentrations were 5100 ppm, 2100 ppm and 2700 ppm for RDX, HMX and TNT, respectively.

3.3.2 Water Extract Reactors

The water extract reactors were prepared using the aforementioned media and a water-extract from "mid-range" RDX-contaminated soil. The extract was prepared by adding 600 grams of "mid-range" soil to 3 liters of distilled water. The soil-water mixture was placed on a shaker table for 5 days before being allowed to settle. The supernatant was collected and filtered through 0.45 μm glass fiber filters.

The aerobic test reactors were set up in 1 liter bottles with a liquid volume of 500 ml. All other water extract (nitrate-reducing, sulfate-reducing, and methanogenic) reactors were set up in 120 ml serum bottles with liquid volumes of 100 ml.

The initial concentration of RDX in these reactors was close to its aqueous solubility of 38.4 to 42.3 mg/l (Smith-Simon and Goldhaber 1995; McLellan et al. 1992). The initial concentration of TNT in these reactors was approximately 70 mg/l. This concentration is lower than the aqueous solubility of TNT (130 mg/l at 20°C) (Shah 1995). Due to the low solubility of HMX in water (5 mg/l at 25 °C) (Gorontzy et al. 1994) and its early HPLC retention time (2-3 minutes) based on the mobile-phase used in these experiments, HMX could not be monitored successfully.

3.3.3 Reagent-Grade Reactors

Reagent-grade RDX reactors were prepared using the aforementioned media and spiked with reagent-grade RDX to a final concentration of 10 mg/l. The aerobic test reactors were set up in 1 liter bottles with a liquid volume of 500 ml. All other reagent-grade (nitrate-reducing, sulfate-reducing, and methanogenic) reactors were set up in 120 ml serum bottles with a liquid volume of 100 ml.

3.4 ADDITIONAL EXPERIMENTS

3.4.1 Abiotic Sulfide Experiment

An experiment was conducted to investigate if RDX reacts with sulfide. Active sulfate-reducing reagent-grade RDX reactors were autoclaved at 248 °F and 15 psi for 30 minutes in order to insure sterilization. The reactors contained approximately 100 mg/l total sulfide and were spiked with RDX to a final concentration of 10 mg/l. With an initial

pH of 7.1, the reactors were incubated statically at ambient conditions (22 °C). The experiment was run in duplicate and with a control reactor which contained no sulfide. These reactors were monitored for RDX transformation using procedures described below. Total sulfide measurements were made using the Iodometric Method outlined by Standard Methods (APHA 1989).

3.4.2 Experiment to Detect Inhibition to Methanogenesis

Four reactors were set up using the same enrichment medium and trace metal solution previously described. Due to the inability to establish a highly active methane-producing culture from the Pantex soil, the reactors were inoculated with 4% (v/v) anaerobic digester sludge from the Stillwater, OK municipal wastewater treatment plant. The sludge was first diluted with 2 volumes of distilled water and filtered through 4 layers of cheesecloth before being used as an inoculum.

Each reactor was fed an initial mixture of 50% glucose and 50% acetate resulting in a soluble COD (chemical oxygen demand) of approximately 1100 mg/l. The soluble COD was monitored (as described below) and maintained at this level. With an initial pH of 7.1, the reactors were incubated at ambient conditions (22 °C) on a shaker table. After one week of incubation, gas samples from the headspace of these reactors were analyzed for methane using procedures described below. After it was confirmed that the test reactors had produced methane, the headspace in each reactor was purged with nitrogen until methane was no longer detectable (15-30 minutes). Then, two of the reactors were spiked with RDX to a concentration of 10 mg/l. All the reactors were then incubated at

ambient conditions (22 °C) on a shaker table for another week. After which time, gas samples from the headspace of the reactors were again analyzed for methane.

3.4.3 Evaluation of Buffering Capacity for Nitrate-Reducing Conditions

Experiments were conducted in order to determine the optimal buffering capacity for nitrate-reducing conditions. Three sets of reactors were set up using the same enrichment medium and trace metal solution previously described. However, the concentrations of phosphate (70% K_2HPO_4 : 30% KH_2PO_4) in each set of reactors were adjusted to 12, 25 and 50 mM. Each reactor (and duplicates) was spiked with RDX to a final concentration of 10 mg/l. Each of these reactors were inoculated with a 2% (v/v) inoculum developed from previously concluded nitrate-reducing water extract reactors. Each of the reactors also contained nitrate (660 mg/l) and glucose (1 g/l). With an initial pH of 7.1, the reactors were incubated at ambient conditions (22 °C) on a shaker table. RDX transformation, nitrate reduction and pH were monitored (as described below), regularly.

3.4.4 Effect of TNT on RDX Degradation Under Nitrate-Reducing Conditions

An experiment was conducted to determine if TNT degradation must precede RDX transformation under nitrate-reducing conditions. Two sets of reactors were set up using the aforementioned media. Each reactor was fed an initial dose of glucose and nitrate of 1 g/l and 660 mg/l, respectively. The reactors were spiked with RDX to a final concentration of 10 mg/l and monitored over time until RDX was no longer detectable in the aqueous extracts. Once RDX had completely transformed in all the reactors, one set of

the reactors was then spiked with both RDX (10 mg/l) and TNT (20 mg/l) and the other with only RDX (10 mg/l). RDX, TNT, soluble substrate and nitrate concentrations were monitored (as described below) over time. Nitrate and glucose were reintroduced as necessary.

3.5 ANALYTICAL METHODS

3.5.1 Sampling and Sample Preparation

Aqueous samples (4-5 ml) were taken by introducing an equal volume of argon or nitrogen into the reactors. The aqueous samples were filtered using Gelman 25 mm Easy Pressure Syringe Filter Holders and Gelman 0.45 μm Metricel (25 mm) membrane filters. The first milliliter of filtrate was wasted and the remaining filtrate was collected for HPLC analysis. The filtered samples were not diluted prior to HPLC (high performance liquid chromatography) analysis, however the samples were diluted 10 fold before IC (ion chromatography) or GC (gas chromatography) analysis.

Several techniques have been evaluated for the extraction of munitions residues from soils (Jenkins an Grant 1987). The wrist-action shaker method selected for this study compared favorably with other methods evaluated by Jenkins and Grant (1987). In this study, soil samples were collected using an IEC (International Equipment Company) Centra-7 centrifuge. Liquid-soil samples from the soil-slurry reactors were placed in 50 ml disposable centrifuge tubes. The samples were centrifuged at 1500-2000 rpm for

approximately 20 minutes. After centrifuging, an aqueous sample was collected from the 50 ml centrifuge tubes and filtered (as described above) for analysis. The remaining supernatant was then poured off and the soil collected and dried overnight at 103 °C. After drying, 2 grams of the soil samples were suspended in 50 ml of pure methanol and placed on a shaker table for a minimum of 24 hours. At the end of the 24 hours, the samples were again centrifuged and the supernatant was collected for analysis. Samples were diluted with equal volumes of distilled water prior to HPLC analysis (described below). The methanol extract samples were not filtered using Gelman Metricel membrane filters as was the aqueous samples. The methanol extract appeared to melt the membrane filters.

3.5.2 Analysis of RDX and Other Nitroaromatic Compounds

RDX and other nitroaromatic compounds were analyzed by high performance liquid chromatography (HPLC). A number of methods have been proposed (Emmrich et al. 1993; ASTM 1990; Bauer et al. 1986; Jenkins et al. 1986). Most of these methods are similar to the method selected for this study. As such, the basic method involves the use of an HPLC with a reversed-phase C-18 column.

During this study, a Beckman liquid chromatograph equipped with two model 127 solvent pumps, a model 166 absorbance detector set at 254 nm and a System Gold controller were used. The mobile phase was methanol-water (45:55 v/v) at a flow rate of 1.5 ml/min. Sample volumes (20 µl) were injected onto a Beckman C-18 reverse-phase column (Ultrasphere ODS, 5 µm particle diameter, 4.6 mm x 25 cm). The results were

integrated on a Hewlett Packard (HP) 3396 Series II integrator. Analytical standards of reagent-grade RDX, HMX, and TNT were used for quantification of the reactor samples.

3.5.3 Measurement of Nitrate and Sulfate

Nitrate and sulfate reduction were monitored using a Dionex ion chromatograph (IC), series 2000i/sp. The procedure used in this analysis were consistent with Standard Methods (APHA 1989). Diluted aqueous samples (30 µl) were injected onto a Dionex IonPac AS4A-SC 4 mm analytical column. The system was pressurized at 50 psi using compressed nitrogen gas. The eluent consisted of 1.8 mM Na_2CO_3 (sodium carbonate) and 1.7 mM $NaHCO_3$ (sodium bicarbonate) at a flow rate of 2.0 ml/min. The column regenerant was a 25 mN H_2SO_4 (sulfuric acid) solution. The results were integrated on a HP 3380A integrator. Quantification was made using standard solutions of known concentrations of the particular anion under consideration.

3.5.4 Measurement of Methane

Methane production was monitored using a Gow-Mac Gas Chromatograph (GC) Series 350 with Thermal Conductivity Detector. Gas samples (250 µl) taken from the headspace of the reactors were injected onto a Gow-Mac Porapak column (80/100 mesh). Helium (He) at 60 ml/min was used as a carrier gas. The column temperature, detector temperature and injector temperature were set to 55, 170, and 105°C, respectively. The bridge current was set to 70 mA. The results were integrated on a HP 3380A integrator. Quantification of the gas samples was made using pure methane gas.

3.5.5 Measurement of Volatile Fatty Acids (VFA)

Volatile Fatty Acids (VFA), particularly acetic and propionic acids, were analyzed by gas chromatography. Aqueous samples (1μl) were injected on a packed glass column (Supelco 60/80 Carbopak C/0.3% Carbowax 20M/0.1% H_3PO_4, inner diameter 2mm, length 24 in.) in a model 5890 HP Series II GC equipped with a flame ionization detector (FID). Helium (He) at 50 ml/min was used as a carrier gas. The column temperature, detector temperature and the injector temperature were set to 120, 200, and 200°C, respectively. The results were integrated on a HP 3396 Series II integrator. Quantification was achieved using standard solutions of the VFA under consideration.

3.5.6 Measurement of Soluble COD

Soluble COD measurements were made using a HACH DR/3000 Spectrophotometer set to 620 nm and standard HACH 0-1500 mg/l COD tubes. The procedures used were consistent with those recommended by the HACH Instrument Company (HACH 1996).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 PRELIMINARY STUDIES

Preliminary experiments were conducted to determine the extent to which the RDX in the soil samples obtained from the DOE Pantex site could be readily desorbed into a slurry. Reactors dosed with varying amounts of soil and distilled water and placed on a shaker table yielded aqueous RDX concentrations somewhat lower than its reported aqueous solubility of 38.4 to 42.3 mg/l at 20 °C (Smith-Simon and Goldhaber 1995; McLellan et al. 1992). This was true even of those reactors dosed with relatively low soil to water ratios. These aqueous concentrations remained constant from the second day to over 50 days later, indicating that desorption and/or dissolution had reached equilibrium rapidly and would not be a rate limiting step in future soil slurry reactors.

4.2 SOIL-WATER REACTORS

4.2.1 Induction of electron accepting conditions

Four sets of soil-water reactors were set up and operated under one of four conditions: aerobic, nitrate-reducing, sulfate-reducing or methanogenic. Initial metabolic

activity in all such reactors was indicated by the production of gas: significant amounts in those reactors fed glucose or lactate, less in those fed no glucose or lactate. However, those reactors fed no additional substrate ceased producing gas after approximately three weeks of incubation. Those fed an additional substrate maintained gas production throughout the experiment.

Aerobic reactors were continuously aerated throughout the duration of these experiments. No additional testing was done to determine actual oxygen uptake rates in these reactors.

The nitrate-reducing reactors fed an additional substrate maintained active nitrate-reduction throughout the duration of the experiment. Nitrate (660 mg/l) was completely reduced in these reactors in 7 to 10 days, after which time nitrate and glucose were reintroduced. No significant reduction of nitrate was observed in those reactors fed no additional substrate during the 372 days these reactors were in operation.

Due to nitrate present in the Pantex soil samples, during the first week of incubation, the sulfate-reducing and methanogenic reactors operated under nitrate-reducing conditions. The initial concentration of aqueous-phase nitrate in these reactors was about 120 mg/l. This concentration was depleted in approximately 10 days in both reactor sets in which an additional substrate had been added. In contrast, no reduction of nitrate was observed in the reactors that were fed no glucose or lactate. This was consistent with the actual nitrate-reducing set of reactors fed no glucose.

Although nitrate was reduced quickly in the sulfate-reducing and methanogenic

reactor sets where glucose or lactate had been amended, those reactors failed in producing the desired electron-accepting conditions. The reactors intended to be methanogenic produced no measurable concentration of methane during the 337 days the reactors were in operation. No measurable reduction of sulfate was observed in the reactors intended to be sulfate-reducing during the 384 days these reactors were in operation. This was true even after being spiked several times with an active sulfate-reducing inoculum. As mentioned previously, these reactors continued to produce gas during the duration of the experiment. Besides analysis for methane, aqueous samples from the methanogenic reactors fed an additional substrate were analyzed for volatile fatty acids. Results showed the presence of both acetic and propionic acids, indicating active fermentation in these reactors. In the sulfate-reducing reactors, it is possible that something else, possibly iron or manganese, acted as a preferred electron acceptor. No data on soil composition was available to confirm the presence of these metals in the Pantex soil, although it is likely that significant concentrations are present.

4.2.2 RDX Transformation in the Aqueous-phase

Net loss of RDX from the aqueous phase was negligible in all four reactor sets, even after approximately one year of operation. In all reactors, aqueous-phase RDX concentration gradually increased over time, eventually exceeding its expected solubility. It was not determined how the reactor conditions enhanced RDX solubility. Whatever the cause, it also occurred in the abiotic control reactors.

Despite no net loss of RDX from the aqueous phase, HPLC peaks corresponding

to suspected intermediates were observed in aqueous samples from the nitrate-reducing, sulfate-reducing, and methanogenic reactors fed an additional substrate. These peaks will be discussed in following sections.

4.2.3 RDX Transformation in the Solid-phase

At the conclusion of the experiment, a solid-phase extraction was performed on soils from each of the soil-water reactors as well as on the abiotic controls. Results from the extractions are summarized in Table IV. The average initial concentrations of RDX, HMX, and TNT in the soils used in these reactors were 5100 ppm, 2100 ppm, and 2700 ppm, respectively. However, when these initial concentrations were measured, some variations in concentrations were observed in each sample. The solid-phase concentrations of RDX, HMX, and TNT from the initial soil samples ranged from 4900-5300 ppm, 1800-2500 ppm, and 1025-5200 ppm, respectively. Therefore, it is possible that the solid-phase concentrations of these chemicals in the soils-water reactors could vary significantly from the average values stated above or even from the abiotic control reactors list in Table IV.

Note that some loss of RDX and TNT present in the solid-phase occurred in each reactor due to the solubility of these compounds in water. Based on the maximum aqueous solubility of RDX (42.3 mg/l) (Smith-Simon and Goldhaber 1995; McLellan et al. 1992), the estimated loss of solid-phase RDX in these reactors due to dissolution of the compound into the aqueous media would be 212 ppm. Similarly, for TNT (130 mg/l) (Shah 1995), the estimated loss of solid-phase TNT would be 650 ppm.

Although it is possible that some solid-phase RDX was transformed in all of the

Table IV

Summary of Soil-Extraction Results Following Long-Term Soil-Water Slurry Reactor Treatment

Set	Reactor Description	RDX (ppm)	HMX (ppm)	TNT (ppm)
Aerobic	Glucose: A	1776	1455	nd
	Glucose: B	4069	1470	nd
	No Glucose: A	4663	1466	nd
	No Glucose: B	1722	1674	nd
	Abiotic	1800	1666	nd
Nitrate-reducing	Glucose: A	66	1555	nd
	Glucose: B	14	1367	nd
	No Glucose: A	3875	2074	46
	No Glucose: B	4364	2096	116
	Abiotic	3971	1635	187
Sulfate-reducing	Lactate: A	3132	2209	nd
	Lactate: B	2775	1452	nd
	No Lactate: A	4350	2110	42
	No Lactate: B	4420	1913	46
	Abiotic	4862	2560	266
Methanogenic	Glucose: A	3516	960	nd
	Glucose: B	3276	805	nd
	No Glucose: A	4394	1300	22
	No Glucose: B	4176	1661	10
	Abiotic	3742	1245	191

nd = not detectable (TNT detection limit = 5 ppm)

Note: Average initial Soil-extract concentrations prior to treatment = 5100 ppm RDX, 2100 ppm HMX, and 2700 ppm TNT.

reactors including the abiotic controls, the only reactors that showed significant loss of solid-phase RDX were the nitrate-reducing reactors fed glucose (Table IV). The loss of solid-phase RDX in these reactors based on the average initial concentration was 99%. Note that TNT was also completely transformed in these nitrate-reducing reactors as well.

By far this appears to be the most promising mode of operation for soil slurry reactor treatment of soils contaminated with RDX.

Results from the solid-phase extraction listed in Table IV also indicate that TNT was readily transformed in each of the reactors where additional carbon substrate was amended. TNT was no longer detectable in the aqueous-phase of these reactors as well. The incubation times required for the complete disappearance of TNT in the aqueous-phase of the substrate amended reactors are summarized in Table V. As mentioned previously, the sulfate-reducing and methanogenic reactors initially operated, for a period of 10 days, under nitrate-reducing conditions.

Table V

Incubation Times to Reach Non-Detectable Levels of Aqueous TNT in Soil-Water Slurry Reactors

Reactor Description	Time (days)
Aerobic	50
Nitrate-reducing	14
Sulfate-reducing	28
Methanogenic	28

No substantial reduction of solid-phase HMX was observed in these reactors even after approximately one year of operation. Due to the low solubility of HMX in water (5 mg/l at 25 °C) (Gorontzy et al. 1994) and its early HPLC retention time (2-3 minutes) based on the mobile-phase used in these experiments, the aqueous-phase HMX could not be monitored successfully.

4.3 WATER EXTRACT REACTORS

4.3.1 Aerobic Reactors

As seen in Figure 2, relatively little RDX degradation occurred in the aerobic reactors after 280 days of incubation. There appears to be little difference among those fed glucose and those that received none. However, these cultures do appear to be active as shown by the gradual removal of TNT, which is essentially gone after 45 days in those reactors supplemented with glucose (Figure 3). TNT disappearance was also observed in the reactors fed no additional substrate and the abiotic control reactor. The rates of disappearance are similar in these reactors, indicating TNT removal by some abiotic mechanisms. Possible abiotic mechanisms include reactions with the trace metal solution or UV (ultra-violet) photodegradation. Hydrolysis does not appear to be a viable explanation of TNT transformation in these reactors based on studies by Semmens et al. (1985) and Heilmann et al. (1996), which indicate that TNT, RDX, and HMX transformation by hydrolysis occurs only at elevated temperatures and pH.

4.3.2 Nitrate-Reducing Reactors

Among the water extract reactors, RDX degraded most quickly under nitrate reducing conditions. In one glucose-amended reactor, the RDX (42 mg/l) was completely transformed after about 70 days of incubation (Figure 4). The duplicate reactor started out similarly, but then RDX degradation and nitrate-reduction stalled after 25 days. Subsequent addition of buffers to raise the pH, which had fallen to near 6, resulted in renewed degradation of RDX at a slower rate, but rapid nitrate-reduction was observed

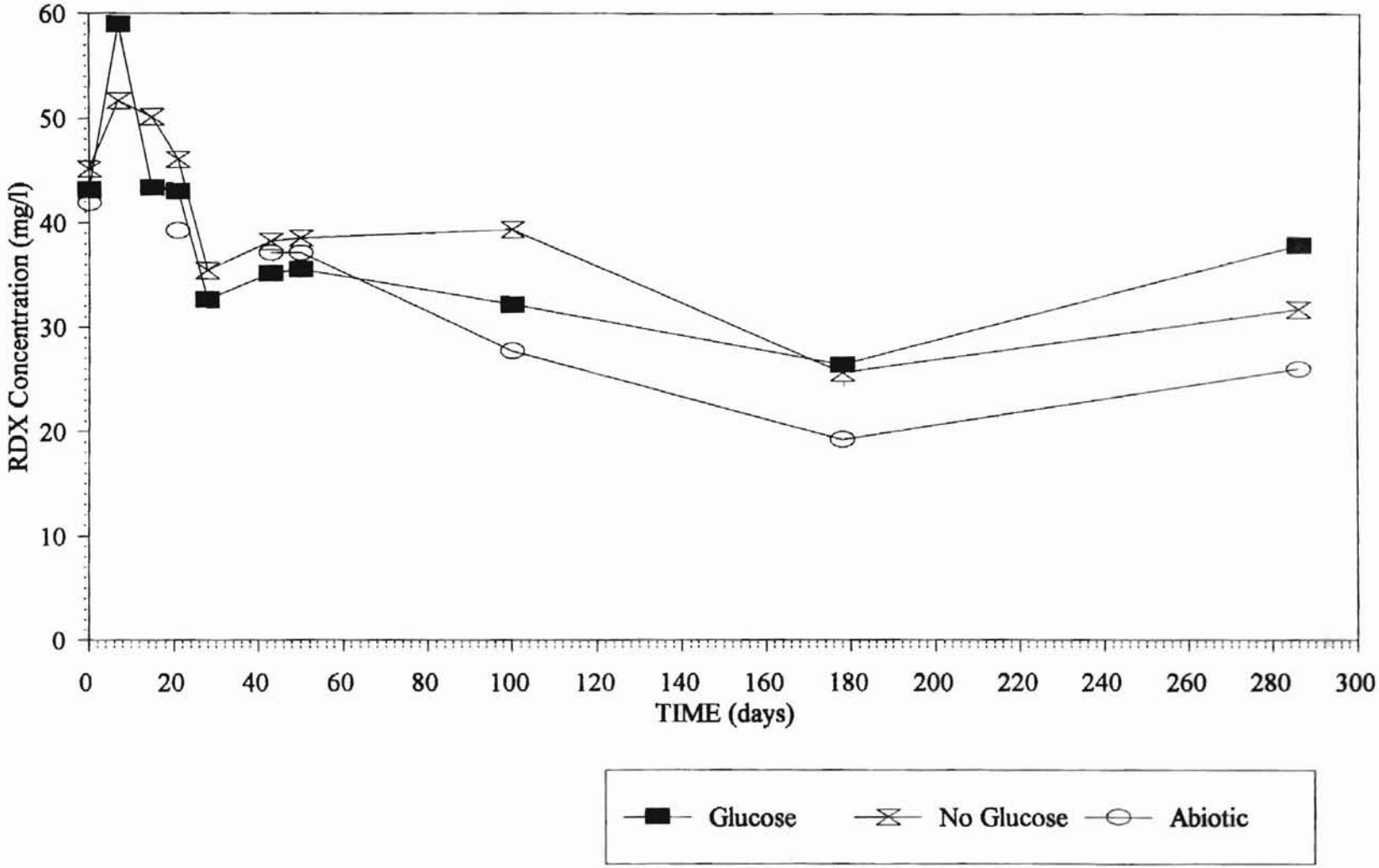


Figure 2. RDX concentrations in aerobic 'water extract' reactors.

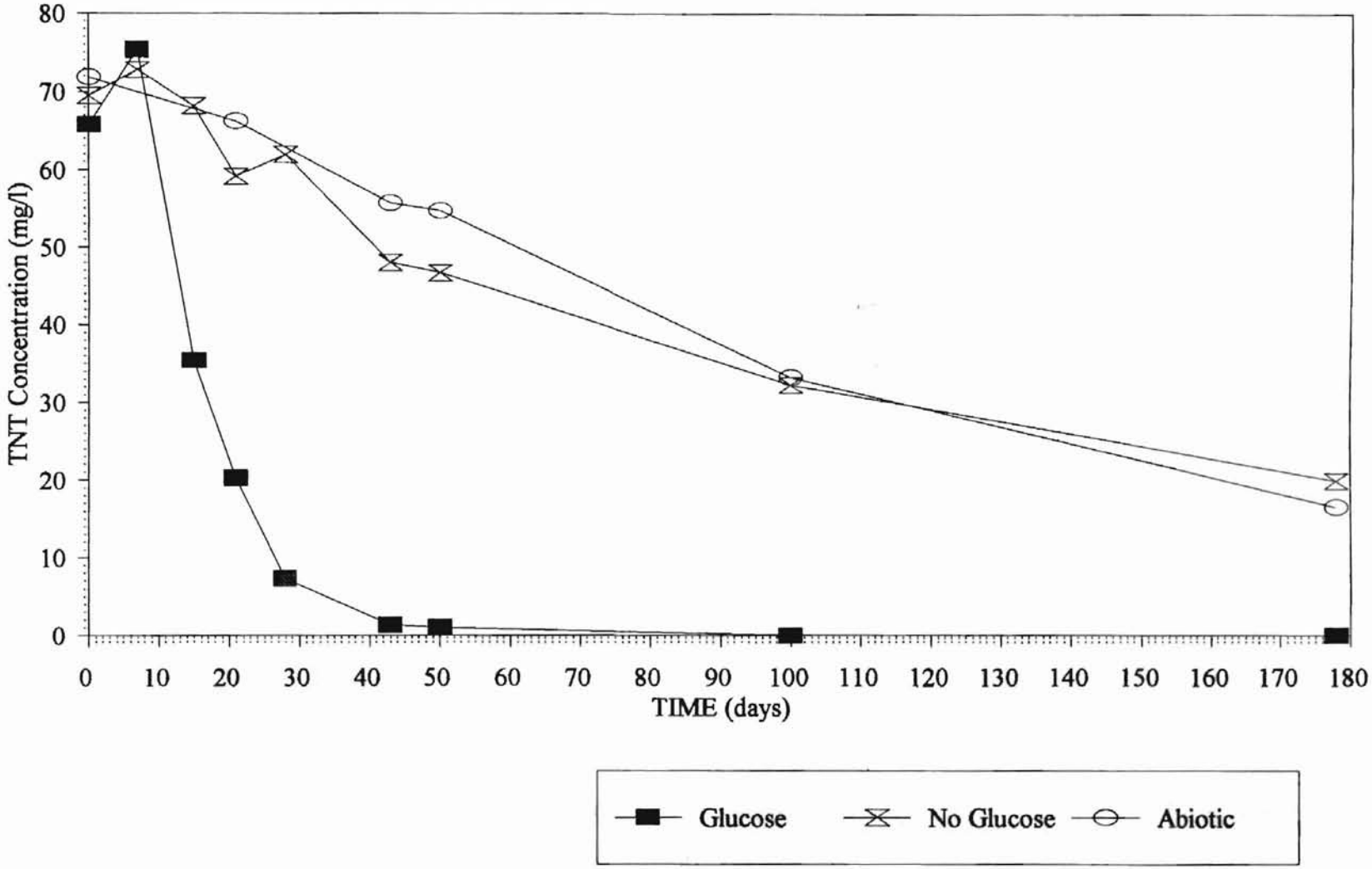


Figure 3. TNT concentrations in aerobic 'water extract' reactors.

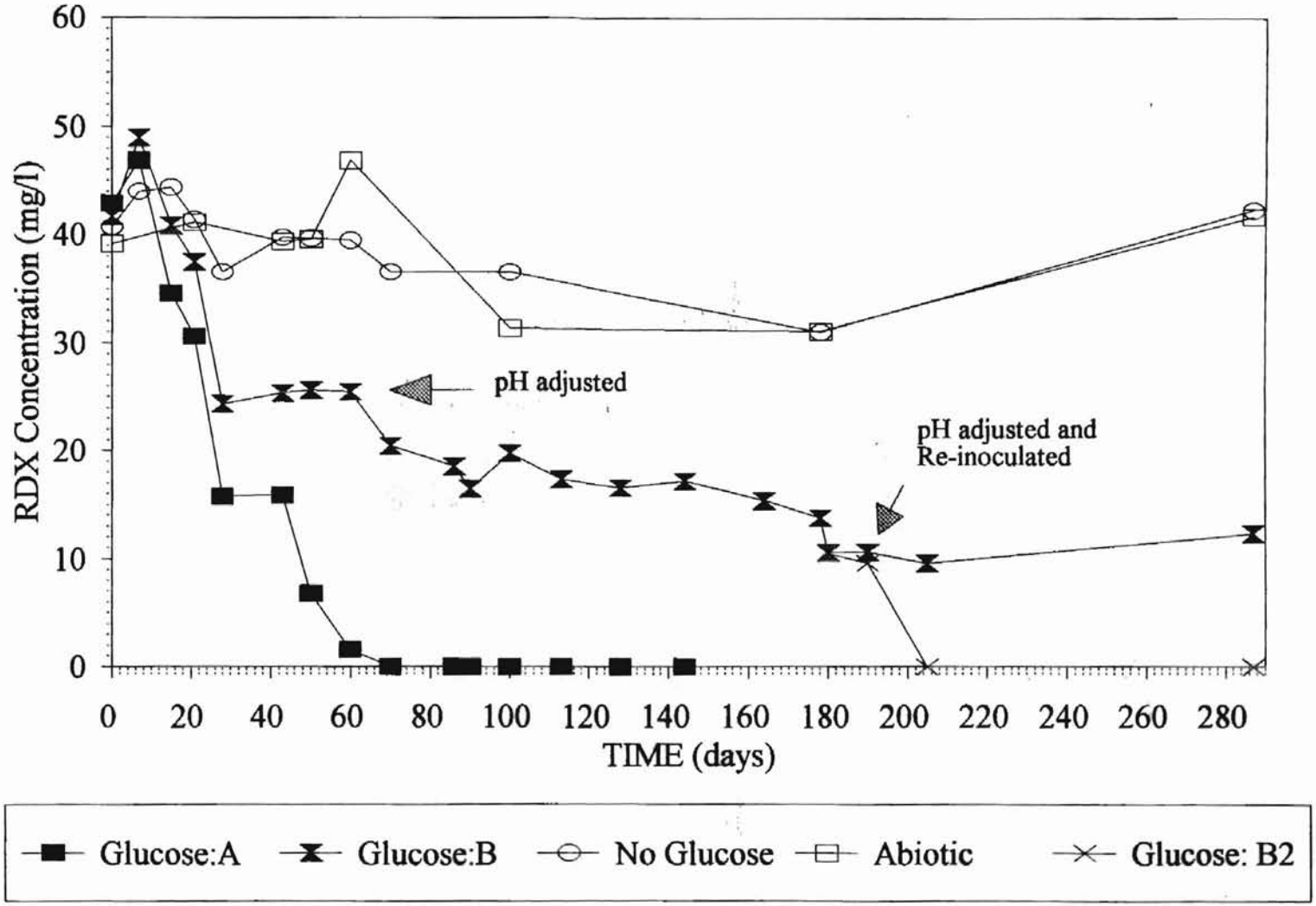


Figure 4. RDX concentrations in nitrate-reducing 'water extract' reactors.

(Figure 5). The pH of this reactor, again dropped to about 6 around day 180, at which point the pH was adjusted and then the reactor was then split in half. One of the new reactors (labeled Glucose: B2) was re-inoculated with a 2% (v/v) inoculum from the duplicate reactor that did not stall. RDX quickly disappeared in this reactor. No renewed degradation occurred in the reactor that was not re-inoculated.

Analysis of volatile fatty acids was performed on samples from the stalled reactor. Results indicated no significant build-up of acetic or propionic acid in this reactor, although some were present. Additional experiments were conducted to determine if the systems were poorly buffer. Results are discussed in subsequent sections.

As shown in Figure 6, complete transformation of TNT (62 mg/l) occurred in the glucose fed reactors after 20 days of incubation. This was true in the duplicate reactor as well. Those reactors which received no additional carbon substrate as well as the abiotic control showed some activity toward TNT. However, no significant reduction of nitrate was observed (Figure 5). The rates of TNT disappearance are similar in these reactors indicating TNT removal by some abiotic mechanisms. Possible abiotic mechanisms for loss of TNT in these reactors were described in a previous section.

4.3.3 Sulfate-Reducing Reactors

No significant reduction of sulfate was observed in any of the sulfate-reducing water extract reactors. Sulfate-reduction appears to be inhibited by the presence of the complex mixture of nitroaromatics present in the water extract. Like the soil-water reactors, the water-extract reactors were repeatedly spiked with active sulfate reducing

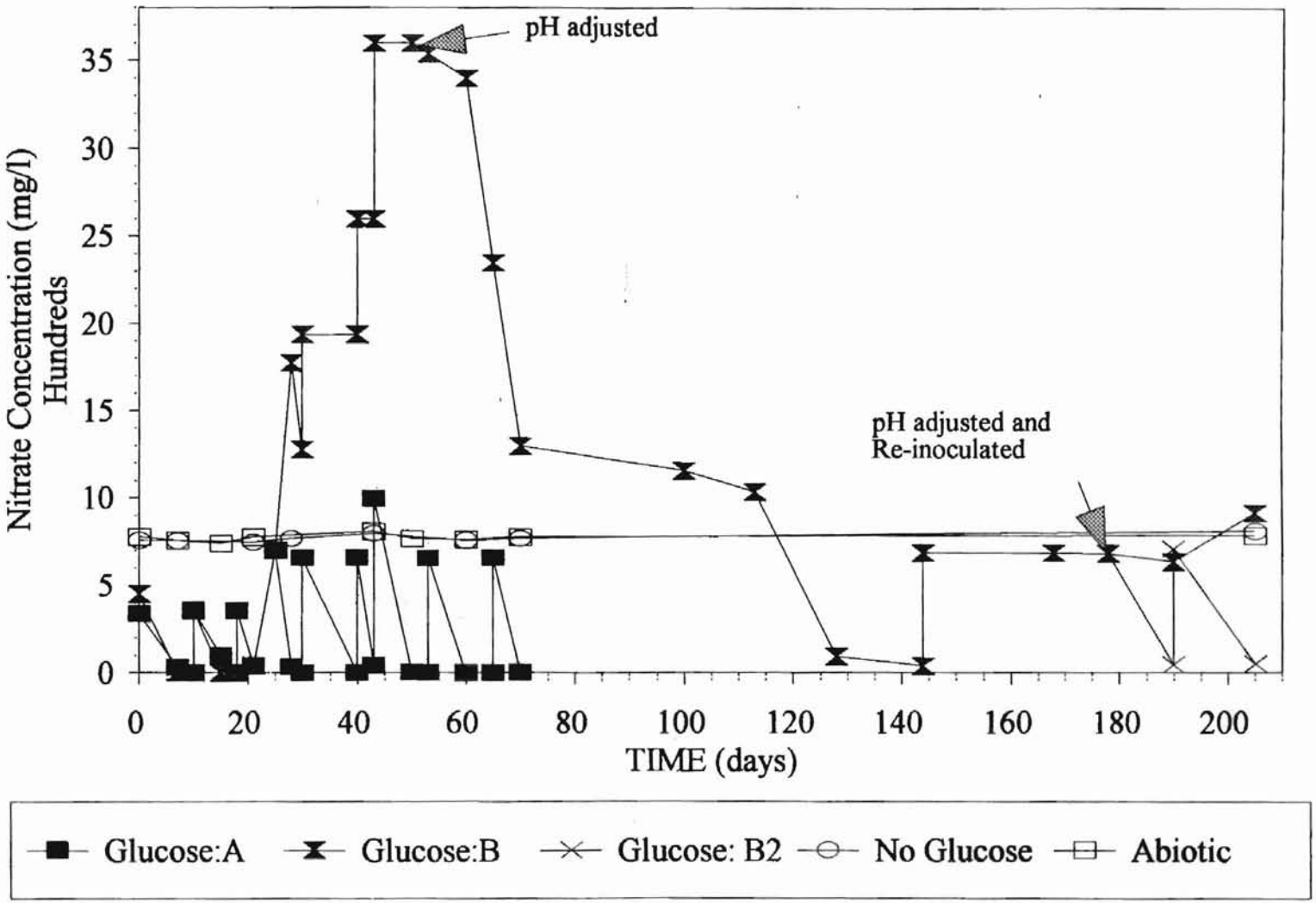


Figure 5. Nitrate concentrations in nitrate-reducing 'water extract' reactors.

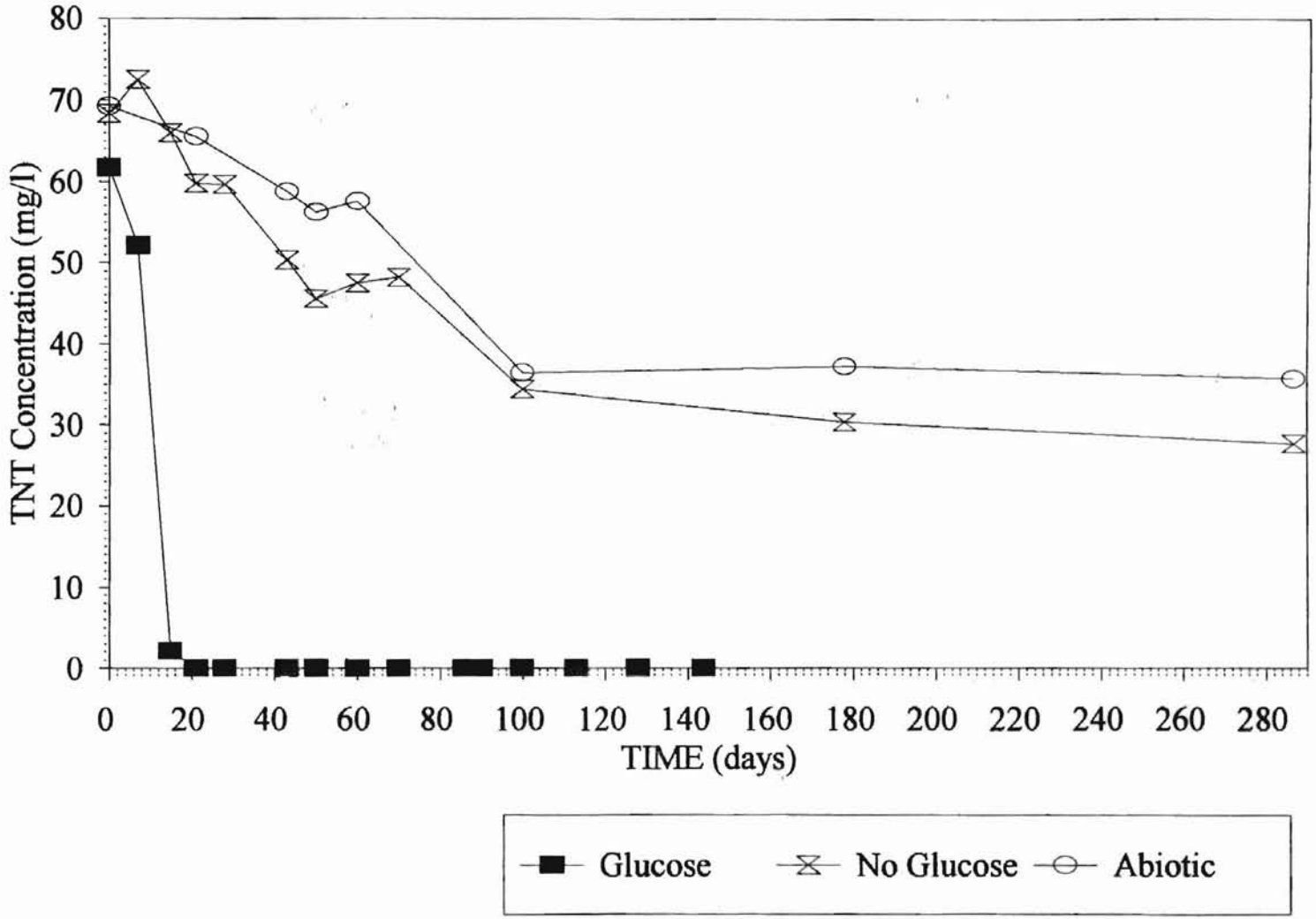


Figure 6. TNT concentrations in nitrate-reducing 'water extract' reactors.

inoculum. However, active sulfate-reduction was not established.

No reduction of RDX was observed in any of these reactors, however TNT (70 mg/l) was completely transformed in the reactors fed lactate in approximately 40 days (Figure 7). The curves shown in Figure 7 are representative of the duplicate reactors. Note that the initial reduction of TNT occurred along with the reduction of nitrate that was present in the water extract (120 mg/l). Some reduction of TNT was also observed in the reactors fed no lactate TNT (70 mg/l) was reduced to below 10 mg/l in 284 days. In the abiotic control reactor, TNT was reduced from 70 mg/l to 38 mg/l in 284 days. Two possible abiotic mechanisms that might explain the loss of TNT in the abiotic control reactor include reactions with the trace metal solution and UV photodegradation, as discussed previously. However since the rates of transformation were not similar between the control reactor and those fed no lactate, it appears that TNT may be reduced in the reactors fed no lactate by some biotic mechanism.

4.3.5 Methanogenic reactors

RDX transformation was observed in the methanogenic water extract reactors that were fed glucose. RDX was reduced from 42 mg/l to approximately 2 mg/l in 284 days (Figure 8). The curves shown in Figure 8. are representative of the duplicate reactors. Methane production was first confirmed in the glucose-fed reactors after 25 days of incubation. However, the percent of methane in the headspace never exceeded 25%, which is much lower than would be expected with a highly active methanogenic culture. Additional experiments were conducted to determine if RDX inhibits methanogenesis.

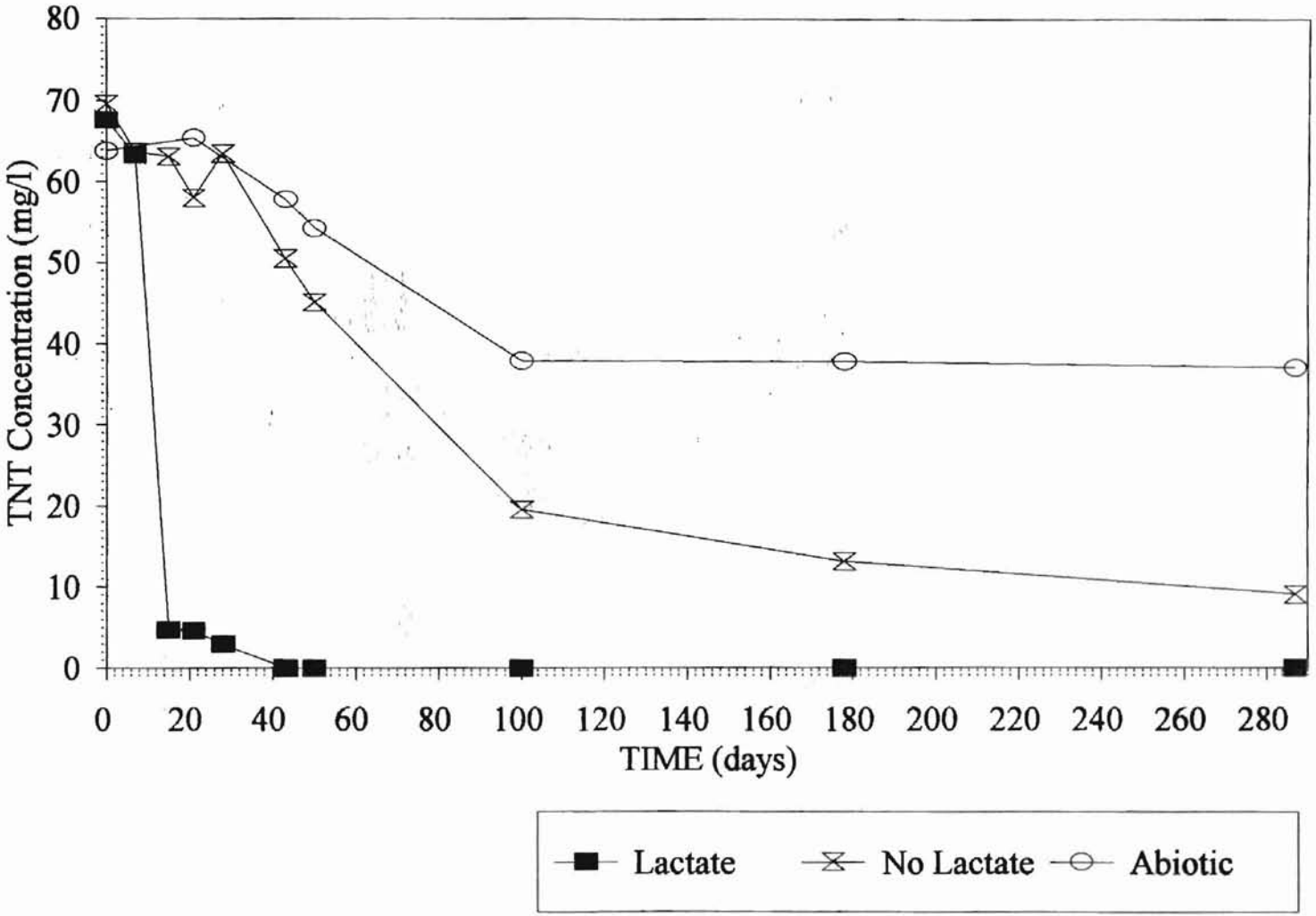


Figure 7. TNT concentration in sulfate-reducing 'water extract' reactors.

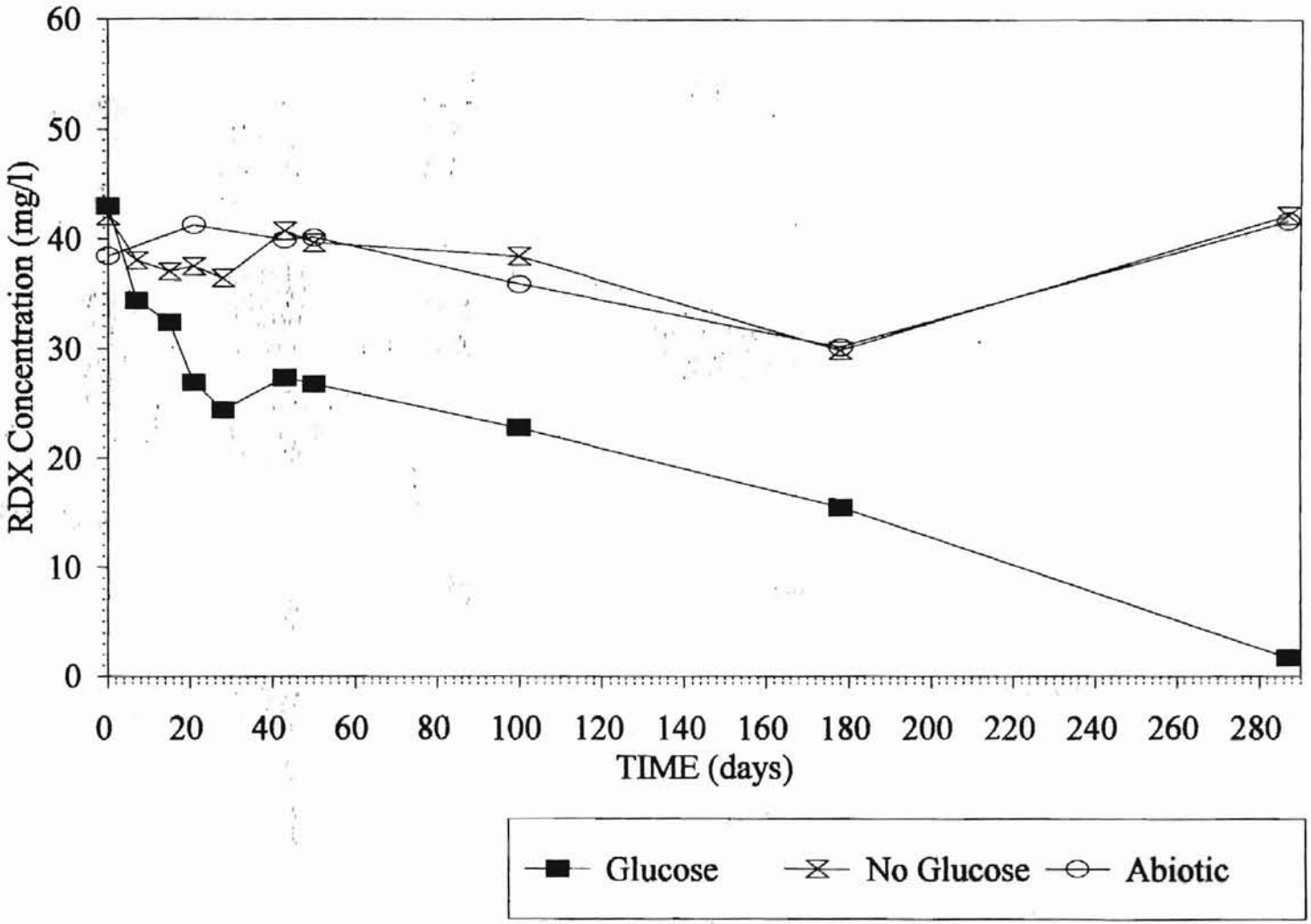


Figure 8. RDX concentrations in methanogenic 'water extract' reactors.

Results are discussed in a subsequent section. No significant reduction of RDX, nor measurable production of methane was observed in those reactors fed no glucose or the abiotic control reactor.

TNT (70 mg/l) was completely transformed in those reactors fed glucose in approximately 20 days. However, initial reduction of TNT corresponded with the reduction of nitrate that was present in the water extract. Some reduction of TNT was observed in both the reactors fed no glucose and the abiotic control. The rate of TNT transformation in these reactors is consistent with that of the sulfate-reducing reactors discussed above. As such, TNT appears to be reduced in these reactors due to a similar abiotic mechanisms.

4.4 REAGENT-GRADE REACTORS

4.4.1 Aerobic Reactors

Some loss of RDX was observed in these reactors. All the reactors, including the abiotic control, showed approximately 30% loss of RDX in 270 days. Possible explanations for this loss include, as discussed previously: reaction with the trace metal solution and UV photodegradation. However, it is also possible that these losses occurred due to scale formation on the reactors walls. Scale formation was noted in all the aerobic reactors (soil-water, water extract, and reagent-grade). This scaling was only evident in the aerobic reactors.

4.4.2 Nitrate-Reducing Reactors

RDX (10 mg/l) was completely transformed in 120 days in the nitrate-reducing reagent-grade reactors that were fed glucose (Figure 9). It is noteworthy that transformation of RDX in these reactors is slower than observed in the water extract reactors. The biomass concentration of the initial culture in the reactor was likely very small, which could explain some of the initial delay. A second possible explanation is the lack of complex substrate that was available in the water extract reactors; which in turn could limit the rate of biological growth in these reactors. However, based on the rate of gas production in these reactors, it was clear that nitrate-reducing organisms grew very rapidly. Very little change has been observed in the reactors fed no additional glucose, as well as the abiotic control (Figure 9).

Unlike the nitrate-reducing water extract reactors, both nitrate-reducing reagent-grade reactors fed glucose maintained active nitrate-reduction throughout the duration of the experiment. Nitrate (660 mg/l) was repeatedly reduced in these reactors in 7 to 10 days.

4.4.3 Sulfate-Reducing Reactors

RDX (10 mg/l) was completely transformed after 75 days in those reactors fed sulfate and lactate (Figure 10). The curves shown in Figure 10 are representative of both duplicate reactors. Approximately 20% of the RDX was transformed in 165 days in both the reactors fed no lactate and the abiotic control. Possible reasons for this loss of RDX are as previously described for TNT. In this set of experiments, the concentration of yeast

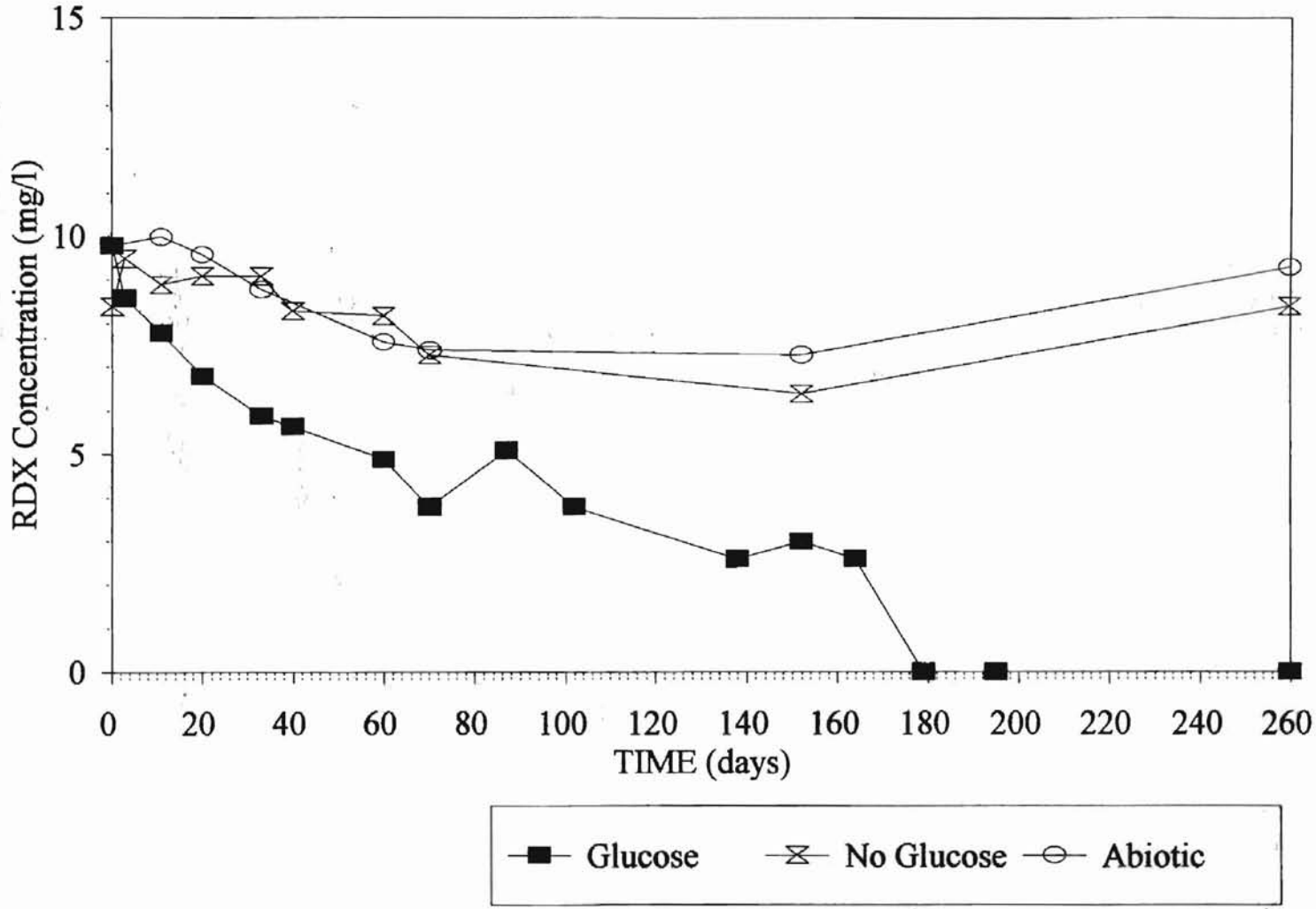


Figure 9. RDX concentrations in nitrate-reducing 'reagent-grade RDX' reactors.

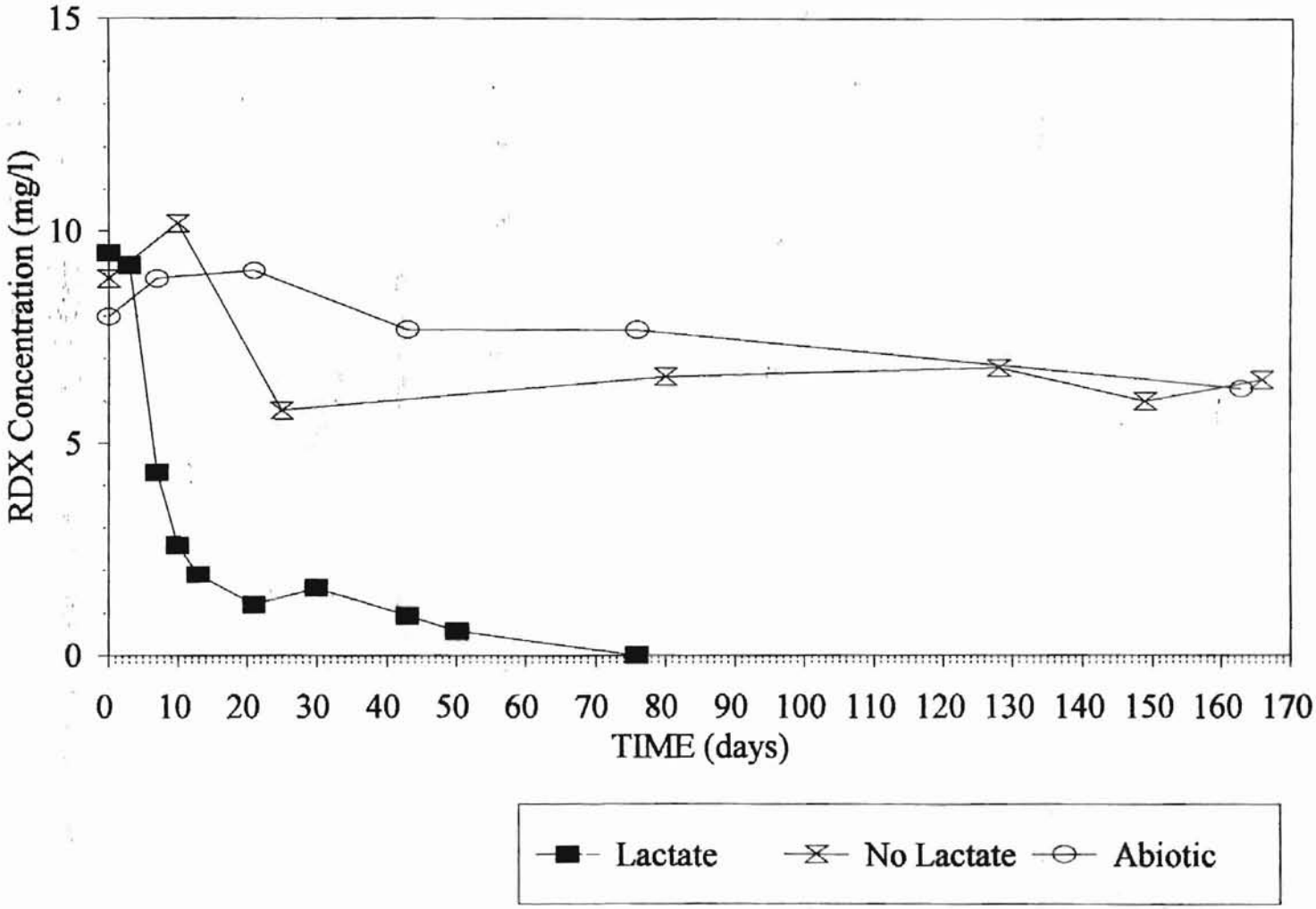


Figure 10. RDX concentrations in sulfate-reducing 'reagent-grade RDX' reactors.

extract in those reactors fed no lactate was reduced to less than 10 mg/l from 100 mg/l used in previous sulfate-reducing unamended control reactors that showed significant reduction of RDX, presumably because these organisms were using the yeast extract as primary substrate rather than as a source of minor nutrients as intended.

Although RDX was transformed in these reagent-grade reactors, no significant reduction of sulfate was observed (Figure 11). However, while the measured sulfate removal in the organic-amended reactors was small (relative to the amount of sulfate present), a definite sulfide odor was present, as was darkening of the reactors, indicating active sulfate reduction in those reactors fed lactate. A biologically active control reactor fed no RDX showed complete sulfate-reduction (at similar concentration) during this time period (Figure 11). As indicated in Figures 10 and 11, measurable sulfate reduction did not occur until RDX had been completely transformed. This indicates some inhibition of sulfate-reducing microorganisms to the presence of RDX.

In subsequent experiments using reagent-grade reactors with sulfate concentrations of 50 mg/l and spiked with a 2% (v/v) inoculum from the previous reactors, RDX (10 mg/l) was completely transformed in 5 days with no measurable reduction of sulfate, although a definite sulfide odor was present. Using this same approach, RDX (40 mg/l) was reduced by 98% after 175 days of incubation. Again, while no measurable reduction of sulfate was observed, a definite sulfide odor was present. The fact that sulfate is being slightly reduced and the RDX is degrading significantly in these reactors (in which no other nitroaromatics are present) suggests that the sulfate-reducing organisms are further inhibited in those reactors which do contain significant amounts of

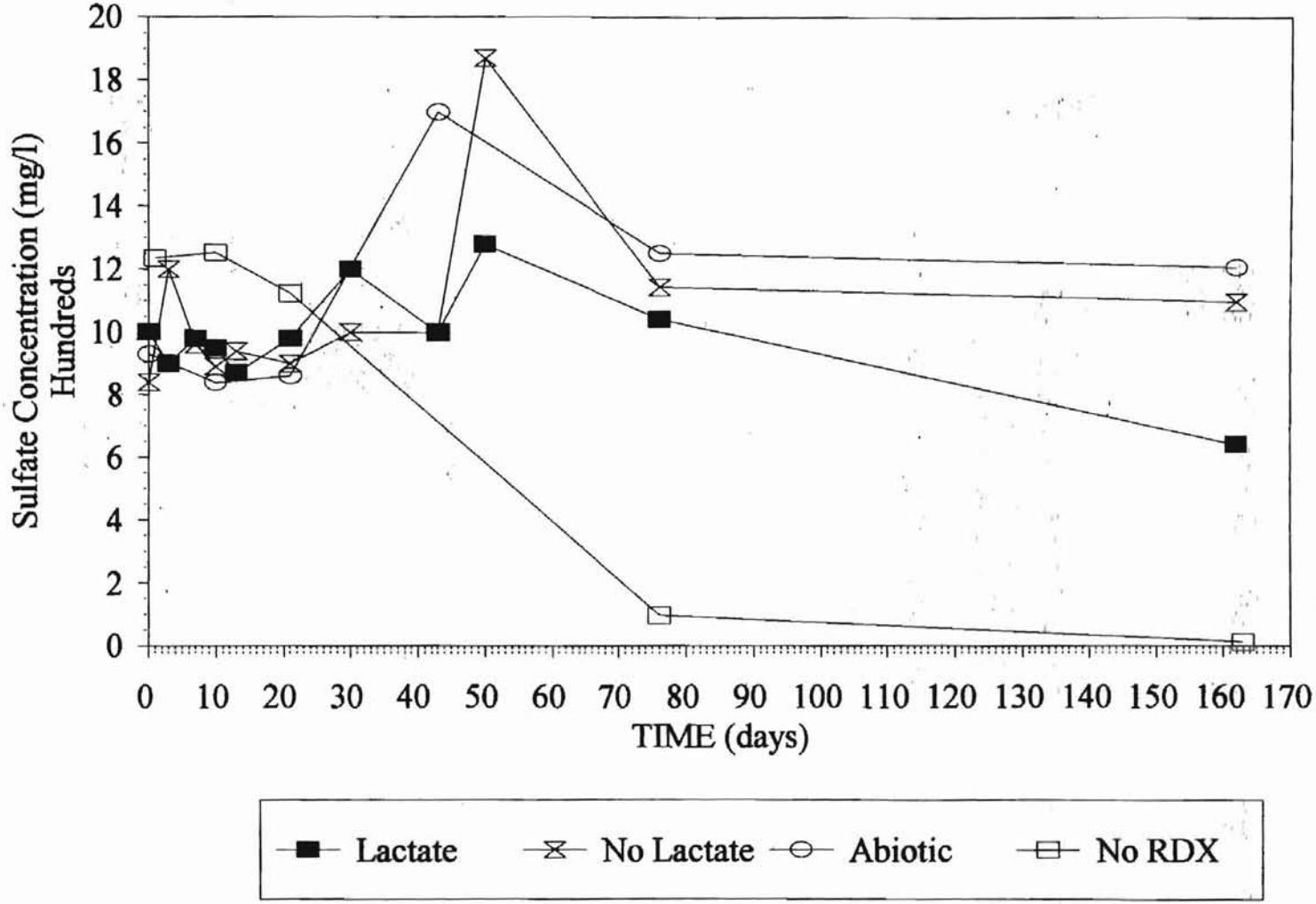


Figure 11. Sulfate concentrations in sulfate-reducing 'reagent-grade RDX' reactors.

nitroaromatics other than RDX.

An additional experiments were performed to determine if RDX reacts directly with sulfide. Results are provided in subsequent sections.

4.4.4 Methanogenic Reactors

As with the water extract reactors, complete transformation of RDX was observed in the reagent-grade glucose-fed reactors. RDX (12 mg/l) was completely transformed after approximately 160 days in these reactors (Figure 12). The curves in Figure 12 are representative of both duplicate reactors. Methane production was first confirmed in the headspace of these reactors after 50 days of incubation. Note that measurable methane production occurred in the water extract reactors after 25 days of incubation. Possible reasons for the slower production of methane in the reagent-grade reactors are as previously described for the nitrate-reducing reactors. As with the glucose-fed water extract reactors, percent methane in the headspace of these reactors did not exceed 25%. In the reactors fed no glucose, RDX (10 mg/l) was reduced by 80% in 265 days. However, no measurable amount of methane was produced. Since RDX was the only available organic substrate in these reactors and its concentration was small, measurable methane production was not expected. Some reduction of RDX (from 8.9 mg/l to 7.8 mg/l) was also observed in the control reactor. The rate of RDX transformation in the abiotic control reactor was consistent with that of sulfate-reducing reactors discussed above. As such, RDX also appears to be reduced in these reactors due to a similar, unidentified abiotic mechanisms.

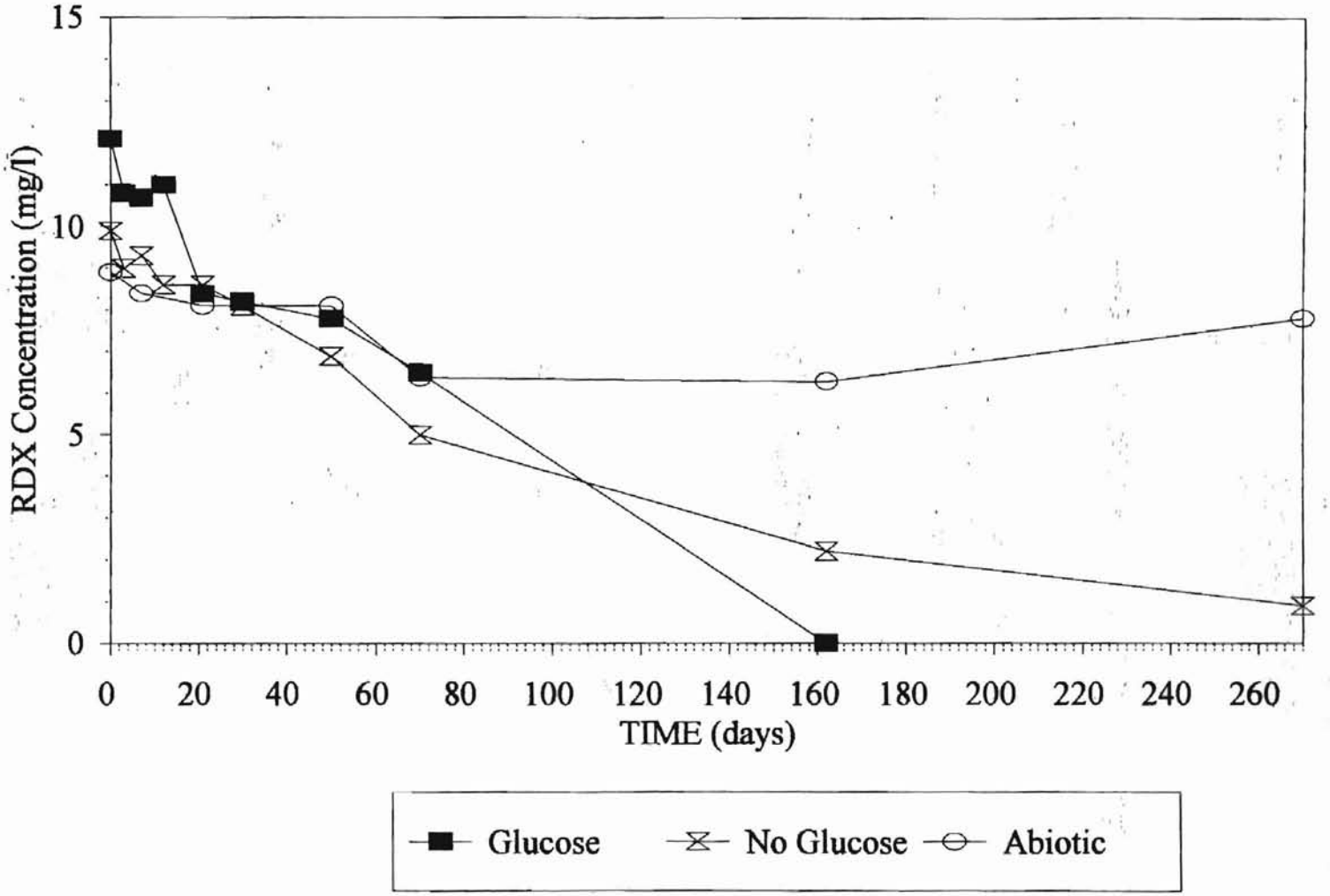


Figure12. RDX concentrations in methanogenic 'reagent-grade RDX' reactors.

Since a highly active methane producing culture was not established from the Pantex soil, additional experiments, using anaerobic digester sludge from the Stillwater, OK municipal wastewater treatment plant, were conducted to determine if RDX inhibits methanogenesis. Results are provided in subsequent sections.

4.5 ADDITIONAL EXPERIMENTS

4.5.1 Abiotic Sulfide Experiment

An experiment was conducted to investigate if RDX reacts with sulfide. Autoclaved reagent-grade RDX reactors (10 mg/l) spiked with sulfide (100 mg/l) showed some activity toward RDX. After 30 days of incubation, less than 40% of RDX had been reduced. These results indicate that RDX reacts slowly with sulfide. However, based on the rates of RDX transformation presented above, RDX reduction in these biologically active sulfate-reducing reactors must be microbially enhanced.

4.5.2 Experiment to Detect Inhibition to Methanogenesis

Since a highly active methane producing culture was not established from the Pantex soil, additional experiments were conducted to determine if RDX inhibits methanogenesis. Reactors inoculated with anaerobic digester sludge and spiked with RDX (10 mg/l) produced no measurable amount of methane after one week of incubation. Control reactors produced approximately 15 ml of 12% methane gas in the same period. Note that the percent methane in the headspace of the reactors where

RDX was not present is somewhat lower than would be expected. It is possible that some factor other than RDX also limited methane production in these reactors. Methane production appears to be not completely inhibited, but slowed in the presence of RDX, as indicated by the water extract and reagent grade reactors discussed previously.

4.5.3 Evaluation of Buffering Capacity for Nitrate-Reducing Conditions

Additional experiments were conducted to determine if previous nitrate-reducing reactors were poorly buffered. Three sets of reagent-grade reactors were set up and incubated as previously described except that the concentration of phosphate in each of the reactors was adjusted to 12, 25, or 50 mM. Each of the reactors (including the duplicates) was spiked with RDX (10 mg/l) and a 2% (v/v) active nitrate-reducing inoculum. In each of the test reactors, RDX was completely transformed in 25 days with relatively no difference in transformation rates. However, the pH of the reactor buffered with 12 mM phosphate increased from 7.1 to 7.6 in 25 days (corresponding to approximately three feeding cycles). The pH of the 25 mM and the 50 mM phosphate buffered reactors increased from 7.1 to 7.3 in the same time. Therefore, it is possible that the initial reactors (also set up with 12 mM phosphate) were poorly buffered, and it is recommended that future experiments be carried out using a minimum of 25 mM phosphate for buffering.

4.5.4 Effect of TNT on RDX Degradation Under Nitrate-Reducing Conditions

An experiment was conducted to determine if TNT degradation must precede that of RDX under nitrate-reducing conditions. Two sets of reactors were inoculated with

nitrate-reducing bacteria from an active water extract reactor, spiked with reagent-grade RDX (10 mg/l), and incubated as previously described. RDX was completely transformed in both sets within 25 days. After this initial cycle, one set of reactors was spiked with RDX (11 mg/l) and the other with RDX (11 mg/l) and TNT (20 mg/l). The set spiked with only RDX showed continued activity toward RDX, while the other set of reactors showed little activity toward RDX until TNT was completely transformed. Typical results are depicted in Figure 13 and indicate that TNT is a preferred substrate in these systems under nitrate-reducing conditions. Therefore, any system designed to biologically treat this soil under nitrate-reducing conditions must incorporate the kinetics of TNT biotransformation.

4.6 INTERMEDIATES

Several HPLC peaks were observed that are suspected to be intermediates of RDX transformation. Figure 14 shows the peaks that are in question. The particular chromatograph shown in Figure 14 was taken on day 128 from a reagent-grade sulfate-reducing reactor spiked with RDX (40 mg/l) and lactate (1 g/l). The peaks are labeled in the order of their appearance in this reactor. Peaks that are not labeled were also observed in the control reactors. Peak '1' has been the most evident in each of the reactors where RDX has been transformed. This particular peak was observed in aqueous samples from the anaerobic soil-water reactors as previously mentioned. This peak was first noted in the nitrate-reducing water-extract reactor just prior to the complete disappearance of RDX.

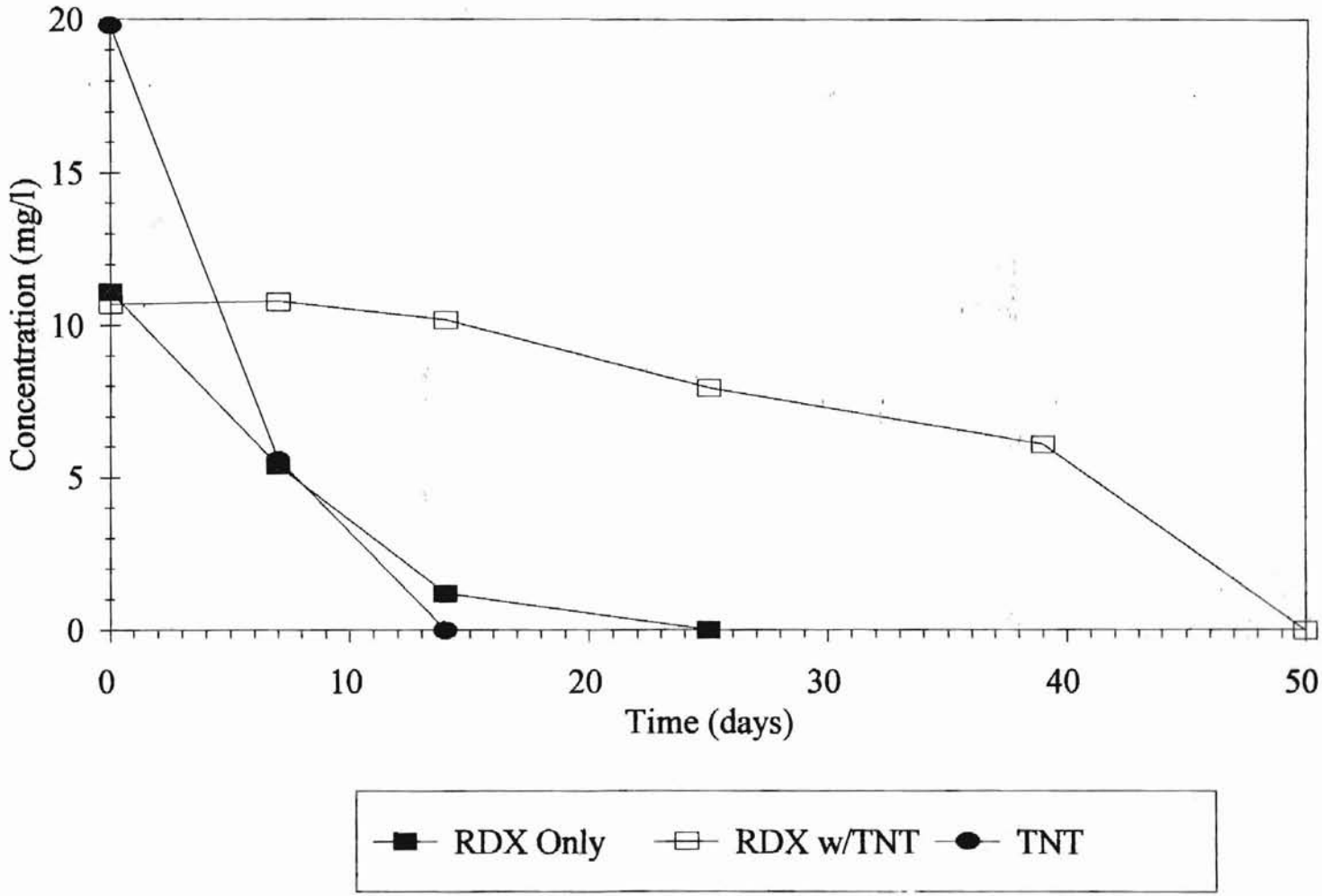


Figure 13. Effect of TNT on RDX transformation under nitrate-reducing conditions.

Although these peaks were visually noted, identification of the proposed intermediates was not vigorously addressed due to the lack of available analytical standards. These peaks did not persist in any of the reactors after RDX was completely transformed, indicating that they are also being transformed in these reactors.

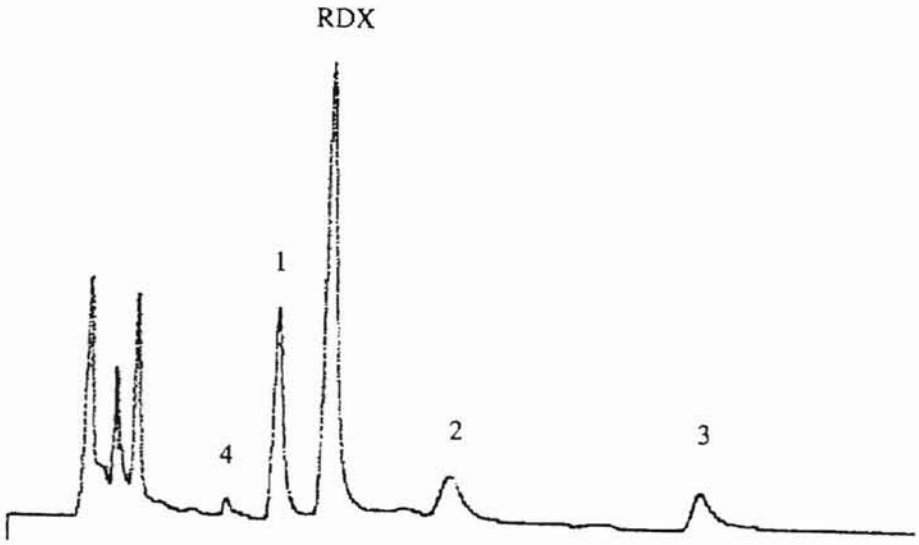


Figure 14. Suspected intermediates of RDX transformation.

CHAPTER V

CONCLUSIONS

The major aim of this study was to investigate the biological treatability of RDX-contaminated soils from the DOE Pantex plant in Amarillo, Texas. Four sets of soil-slurry reactors were operated under one of four conditions (aerobic, nitrate-reducing, sulfate-reducing, or methanogenic).

The main objectives of this study were the following:

1. To study the ability of native soil bacteria to degrade RDX under aerobic, nitrate-reducing, sulfate-reducing, and methanogenic conditions.

2. To study the use of a primary organic substrate to support cometabolic conversion of RDX under the given electron accepting condition.

3. To determine if competing substrates limit the rate of RDX transformation.

4. To detect any possible inhibitory effects of RDX on sulfate-reduction and methanogenesis.

Based on the results of this study, the following conclusions can be drawn:

- Although TNT was successfully transformed in all soil-water reactors where

additional carbon substrate was amended, significant reduction of RDX was only observed under nitrate-reducing conditions, indicating that this is the most promising mode of operation for soil slurry reactor treatment of soils contaminated with RDX.

- In the nitrate-reducing soil-slurry reactors, initial solid-phase RDX concentration of 5100 ppm (average of 4 samples) were reduced to the equivalent of aqueous concentration limits of 40 ppm (average of 2 samples) in the presence of other nitroaromatic contaminants after 372 days of incubation on a shaker table at 22°C.

- TNT acts as a preferred substrate under nitrate-reducing conditions. Significant reduction of RDX does not occur until TNT is completely transformed. Furthermore, based on the results from the solid-phase extractions, RDX must be transformed before HMX.

- Slurries of soils containing approximately 5100 ppm RDX, 2100 ppm HMX, and 2700 ppm TNT were not entirely inhibitory to biological activity. However, sulfate-reduction and methanogenic bacteria do appear to be inhibited by the mixture of nitroaromatics in the Pantex soil.

- Some loss of aqueous RDX was observed in methanogenic water extract and reagent-grade reactors where no additional carbon substrate had been amended. However, the rate of RDX transformation was significantly increased in the presence of glucose.

- RDX transformation proceeds the quickest under sulfate-reducing conditions when RDX is the only nitroaromatic present. However, reduction of sulfate appears to be somewhat inhibited by the presence of RDX. Significant reduction of sulfate does not occur until RDX is completely transformed.

- Methane production appears to be inhibited in the presence of RDX. Methanogenesis is possible at aqueous RDX concentrations of 10 mg/l. However, the rate of methane production is slower than in control reactors where no RDX is present.

Results from this study indicate that a nitrate-reducing soil-slurry reactor is a viable treatment alternative for treating RDX-contaminated soil at the Pantex facility. However, further studies are recommended. Recommendations include:

1. Monitoring of both solid-phase and aqueous-phase RDX, HMX, and TNT in bench-scale slurry reactors operated under nitrate-reducing conditions. These data would be useful in predicting required treatment times in a pilot scale system as well elucidate the relationship between desorption and biodegradation.

2. Conduct studies to investigate possible enhancement of the rate of RDX transformation by using different inexpensive carbon sources as primary substrate.

3. Identification of possible intermediates using GC-MS analysis.

4. Determine if the toxicity of the soil is substantially reduced following treatment by performing Microtox or other assays..

5. Isolation and identification of bacteria involved in the biotransformation of

RDX under nitrate-reducing conditions.

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APPENDIX

APPENDIX A

DATA FROM SOIL-WATER REACTORS

Table A1. Aerobic soil-water reactors.

Day	RDX (mg/l) Glucose (A)	RDX (mg/l) Glucose (B)	RDX (mg/l) No Glucose (A)	RDX (mg/l) No Glucose (B)	RDX (mg/l) Abiotic
1	22.6	21.8	21.3	19.4	22.3
3	32.2	36.5	38.9	33.3	
5	37.8	31.9	35.2	35.5	
7	31.0	26.6	31.4	27.6	
14	35.2	31.8	25.0	26.7	26.2
21	46.9	31.6	40.0	41.0	37.5
28	40.6	32.6	47.2	49.5	
50	60.3	58.1	61.8	58.6	73.0
100	38.9	33.0	31.6	30.7	49.5
150	32.7	30.3	35.8	40.0	42.7
228	27.2	31.5	28.0	26.9	36.9
357	51.7	53.1	62.0	62.7	68.8

Table A2. Nitrate-reducing soil-water reactors.

Day	RDX (mg/l) Glucose (A)	RDX (mg/l) Glucose (B)	RDX (mg/l) No Glucose (A)	RDX (mg/l) No Glucose (B)	RDX (mg/l) Abiotic
1	22.5	17.8	15.2	23.9	23.9
3	32.6	30.5	23.9	27.9	
5	25.4	30.5	25.5	33.1	
7	21.7	31.0	25.5	25.5	
14	35.2	31.8	25.0	26.7	26.2
21	34.1	37.8	45.8	40.9	48.0
28	41.6	37.5	45.3	46.6	
50	46.3	44.5	51.0	51.0	52.6
100	32.5	32.5	43.6	41.4	52.0
122	33.7	41.1	50.4	65.7	63.0
150	33.9	37.1	46.8	60.9	54.6
228	39.6	38.8	60.0	52.6	47.9
372	42.9	43.8	68.7	108.3	77.8

Table A3. Sulfate-reducing soil-water reactors.

Day	RDX (mg/l) Lactate (A)	RDX (mg/l) Lactate (B)	RDX (mg/l) No Lactate (A)	RDX (mg/l) No Lactate (B)	RDX (mg/l) Abiotic
1	20.5	20.2	21.4	20.7	21.2
3	25.9	26.9	24.9	27.8	
5	25.2	25.0	22.3	23.7	
7	22.8	24.9	25.6	24.8	
14	20.4	25.3	22.4	25.2	18.9
21	42.5	48.9	53.2	52.2	43.2
28	46.4	47.8	36.3	37.5	
50	56.3	57.7	62.4	63.4	62.4
100	39.9	41.8	51.0	52.3	57.9
150	33.0	38.2	35.1	39.6	48.4
228	24.4	26.2	45.9	39.6	39.4
384	34.3	41.9	63.2	61.0	56.7

Table A4. Methanogenic soil-water reactors.

Day	RDX (mg/l) Glucose (A)	RDX (mg/l) Glucose (B)	RDX (mg/l) No Glucose (A)	RDX (mg/l) No Glucose (B)	RDX (mg/l) Abiotic
1	29.7	27.4	29.8	30.5	30.5
3	17.6	33.0	28.5	32.3	35.9
5	22.1	41.2	24.7	33.8	34.3
7	20.5	32.4	47.8	32.5	33.5
14	24.4	41.9	42.0	36.5	37.4
21	26.8	44.3	57.2	36.7	42.5
28	38.6	46.7	51.4	50.7	55.5
50	53.2	38.4	34.0	32.8	49.0
80	56.8	37.0	40.4	41.7	56.0
100	37.8	36.4	43.1	62.4	58.9
130	37.6	35.8	33.0	38.9	41.2
208	31.9	48.1	44.1	34.8	43.6
337	44.7	43.0	99.5	102.0	101.4

APPENDIX B

DATA FROM WATER EXTRACT REACTORS

Table B1. Aerobic 'water extract' reactors.

Day	RDX (mg/l) Glucose (A)	RDX (mg/l) Glucose (B)	RDX (mg/l) No Glucose (A)	RDX (mg/l) No Glucose (B)	RDX (mg/l) Abiotic
0	43.2	42.9	41.6	45.2	41.9
7	59.0	57.2	45.2	51.7	
15	43.4	41.9	40.3	50.2	
21	43.1	34.5	39.2	46.1	39.3
28	32.7	31.9	36.3	35.5	
43	35.2	26.5	34.3	38.3	37.2
50	35.6	31.8	35.7	38.6	37.2
100	32.2	25.7	31.0	39.4	27.8
178	26.5	21.2	19.7	25.7	19.3
286	37.9	44.8	22.4	31.7	26.0

Table B2. Nitrate-reducing 'water extract' reactors.

Day	RDX (mg/l) Glucose (A)	RDX (mg/l) Glucose (B)	(B2)	RDX (mg/l) No Glucose (A)	RDX (mg/l) No Glucose (B)	RDX (mg/l) Abiotic
0	42.9	41.7		40.7	41.9	39.2
7	46.9	49.0		44	43.9	
15	34.6	40.9		44.4	44.1	
21	30.6	37.5		41.4	41.4	41.2
28	15.8	24.4		36.6	36.3	
43	15.9	25.4		39.8	40.3	39.4
50	6.8	25.7		39.7	39.9	39.6
60	1.6	25.5		39.5	45.8	46.9
70	0.0	20.5		36.6	40.3	
86		18.6				
90		16.5				
100		19.8		36.6	37.1	31.4
113		17.4				
128		16.6				
144		17.2				
164		15.4				
178		13.8	13.8	31.0	31.8	31.1
180		10.6	10.5			
190		10.6	9.6			
205		9.6	0.0			
287		12.3		42.3	39.2	41.7

Table B3. Sulfate-reducing 'water extract' reactors.

Day	RDX (mg/l) Lactate (A)	RDX (mg/l) Lactate (B)	RDX (mg/l) No Lactate (A)	RDX (mg/l) No Lactate (B)	RDX (mg/l) Abiotic
0	41.0	44.3	41.4	38.7	43.6
7	44.1	51.5	40.3	40.1	
15	46.7	45.4	39.5	40.2	
21	40.1	36.0	38.4	38.1	40.5
28	30.6	29.1	35.3	36.6	
43	35.8	32.2	39.1	39.2	39.3
50	34.6	31.7	39.8	39.1	38.9
100	30.9	27.3	36.9	38.1	37.8
178	23.8	22.3	28.1	25.9	27.1
287	34.6	30.8	40.9	38.0	41.7

Table B4. Methanogenic 'water extract' reactors.

Day	RDX (mg/l) Glucose (A)	RDX (mg/l) Glucose (B)	RDX (mg/l) No Glucose (A)	RDX (mg/l) No Glucose (B)	RDX (mg/l) Abiotic
0	45.3	43.0	42.1	33.2	38.5
7	34.2	34.4	38.1	41.4	
15	32.5	32.4	37.1	41.8	
21	28.6	26.9	37.6	41.0	41.3
28	25.5	24.4	36.5	36.9	
43	27.7	27.4	40.8	40.8	40.0
50	27.7	26.8	39.7	41.0	40.2
100	22.3	22.8	38.5	36.3	35.9
178	16.0	15.5	29.9	25.6	30.2
287	5.3	1.7	42.2	40.9	41.7

APPENDIX C

DATA FROM REAGENT-GRADE RDX REACTORS

Table C1. Aerobic 'reagent-grade RDX' reactors.

Day	RDX (mg/l) Glucose (A)	RDX (mg/l) Glucose (B)	RDX (mg/l) No Glucose (A)	RDX (mg/l) No Glucose (B)	RDX (mg/l) Abiotic
0	10.4	7.0	10.1	9.4	9.9
3	9.8	9.0	7.5	7.0	
7	9.4	9.1	9.1	8.5	8.6
12	8.0	8.3	8.6	7.9	
21	7.4	8.5	8.2	6.6	
30	8.6	9.1	8.4	7.6	9.0
50	8.2	8.7	8.3	6.9	9.0
70	5.8	7.6	10.2	10.9	11.0
162	5.2	4.6	6.0	8.6	5.9
270	6.4	5.3	5.2	6.2	7.1

Table C2. Nitrate-reducing 'reagent-grade RDX' reactors.

Day	RDX (mg/l) Glucose (A)	RDX (mg/l) Glucose (B)	RDX (mg/l) No Glucose (A)	RDX (mg/l) No Glucose (B)	RDX (mg/l) Abiotic
0	9.8	10.2	9.1	8.4	9.8
3	8.6	9.9	9.1	9.5	
11	7.8	8.8	9.1	8.9	10.0
20	6.8	7.6	8.7	9.1	9.6
33	5.9	6.8	8.8	9.1	8.8
40	5.65	6.2	7.8	8.3	
60	4.9	5.4	8.0	8.2	7.6
70	3.8	5.1	6.7	7.3	7.4
87	5.1	7.3			
102	3.8	4.5			
138	2.6	3.7			
152	3.0	3.9	5.9	6.4	7.3
164	2.6	3.4			
179	0.0	2.0			
195		0.0			
260			8.2	8.4	9.3

Table C3. Sulfate-reducing 'reagent-grade RDX' reactors.

Day	RDX (mg/l) Lactate (A)	RDX (mg/l) Lactate (B)	RDX (mg/l) No Lactate (A)	RDX (mg/l) No Lactate (B)	RDX (mg/l) Abiotic
0	9.7	9.5	8.6	8.9	8.0
3	9.3	9.2			
7	4.3	4.3			8.9
10	4.4	2.6	7.6	10.2	
13	3.7	1.9			
21	3.4	1.2			9.1
25			6.0	5.8	
30	2.8	1.6			
43	2.1	1.0			7.7
50	1.8	0.6			
76	0.0	0.0			7.7
80			8.7	6.6	
128			7.3	6.8	
149			6.2	6.0	
163					6.3
166			6.2	6.5	

Table C4. Methanogenic 'reagent-grade RDX' reactors.

Day	RDX (mg/l) Glucose (A)	RDX (mg/l) Glucose (B)	RDX (mg/l) No Glucose (A)	RDX (mg/l) No Glucose (B)	RDX (mg/l) Abiotic
0	10.1	12.1	11.5	9.9	8.9
3	8.6	10.8	9.7	9.0	
7	9.7	10.7	10.5	9.3	8.4
12	10.3	11.0	9.1	8.6	
21	10.1	8.4	8.3	8.6	8.1
30	8.4	8.2	8.0	8.1	
50	6.8	7.8	6.7	6.9	8.1
70	6.7	6.5	4.8	5.0	6.4
162	0.5	0.0	2.8	2.2	6.3
270			1.6	0.9	7.8

VITA

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