TREATMENT CONCEPT FOR RDX-CONTAINING WASTEWATERS USING ACTIVATED CARBON WITH OFFLINE SOLVENT BIOLOGICAL REGENERATION

by

Rolf M.X. Hesselmann
and
Michael K. Stenstrom

Civil and Environmental Engineering Department
University of California, Los Angeles
Los Angeles, CA 90095

1994

School of Engineering and Applied Science
Report Eng. 94-23

TABLE OF CONTENTS

TABLE OF CONTENTS (Cont'd)

LIST OF FIGURES

LIST OF FIGURES (Cont'd)

LIST OF TABLES

PREFACE

This report was funded in part by Lawrence Livermore National Laboratory. The report is adapted from Diploma Thesis of Rolf M.X. Hesselmann, who was a student at the Technical Institute of Berlin and a visiting scholar in Civil and Environmental Engineering Department at UCLA, at the time this work was performed. The research was conducted at UCLA. The authors are grateful for the assistance of John Knezovich and Jeff Daniels.

ABSTRACT

The explosive Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) is one of the most important explosive compounds. It is classified as a "Possible Human Carcinogen" (US EPA Group C) and has various toxic effects on mammals, fish and protozoa. At several sites in the United States and Europe, RDX has contaminated groundwater and soil. It is not subject to aerobic biodegradation but undergoes anaerobic transformations in the presence of other organic substrates (co-substrates). Potentially hazardous intermediates are further degraded but mineralization has not yet been demonstrated.

Large amounts of RDX-containing wastewater are treated with activated carbon, but adsorption of the explosive causes serious safety problems. Exhausted carbon must be disposed of as hazardous waste or in some cases may be burned in open pits. Upcoming environmental regulations will prohibit open burning and open detonations due to harmful side effects.

In this report we propose a treatment concept for waters contaminated low concentrations of RDX. The RDX is first adsorbed onto activated. The carbon is regenerated in a two step process. RDX is first desorbed using a solvent at elevated temperatures. The RDX-containing solvent is then treated with an anaerobic biological process that transforms the RDX to non-explosive byproducts. The solvent can be reused if the cells are separated, which prevents biofouling of the adsorbent. The advantage of this process is that only a small volume of fluid needs to be treated, and the large wastewater stream does not have to be deoxygenated or contaminated with organisms and nutrients required for biological growth. Anaerobic biotreatment is required for therecirculated process water, which is small in volume compared to the original wastewater volume.

Desorption of RDX from activated carbon is the rate limiting step in the process. Desorption using water at room temperature is unsatisfactory. We examined the effects of elevated temperature and organic solvents to increase the RDX desorption in batch experiments. The desorbed RDX concentration increased exponentially with increasing water temperature. Raising the water temperature from 29°C to 90°C enhanced the desorption fifteen times. Using various organic solvents increased the desorption of RDX between 480 times (propanol) and 830 times (ethyl acetate), as compared to water.

Microbiological experiments were conducted to find the best organic co-substrates and bacteria populations, and to examine the influence of oxygen, nitrate and sulfate as terminal electron acceptors. Anaerobic transformation was observed using various inocula and incubation conditions, which confirms results reported in the literature. Anaerobic fortuitous cometabolism seems to be the only possibility to biologically transform RDX. We found significant and interesting differences in degradation rates using various organic substrates and different redox-conditions. Under fermentative conditions, the RDX degradation is very sensitive to the type of co-substrate. Sugars were utilized as growth substrates but did not support RDX transformation. Even between different peptone types significant differences in RDX transformation could be observed.

Denitrifying cultures were obtained which were able to utilize ethanol and acetic acid as co-substrates for RDX transformation. Both ethanol and acetate are good RDX solvents

and do not adsorb strongly to activated carbon, which indicates that they can be used to increase RDX desorption and to serve as the co-substrate.

1.0 INTRODUCTION

The manufacturing of high explosives (HE) has long been an important segment of the chemical industry. Munitions production became the fourth largest industry in the United States during full mobilization (Walsh et al., 1973). Production, usage, and demilitarization of HEs have caused the release of various organic nitro compounds into the environment. Disposal of contaminated wash-down water is one of the most serious problems; up to 2 million liters per day can be generated at a single plant (Kooke, 1981; Jenkins, 1986). This type of water usually contains low HE concentrations (e.g.: 1 - 20 mg/L of RDX; Patterson, 1976a). The combination of large volumes and low concentrations is specifically problematic. Current treatment practice for those aqueous HE wastes is filtration followed by lagoon treatment, or filtration and adsorption onto granular carbon. Future environmental regulations will impose stricter cleanup criteria and prohibit open-burning of HE containing wastes, such as exhausted activated carbon. Moreover, environmental laws and regulations will require detoxification of existing sites contaminated soil and groundwater. Thus, treatment technologies for HE waste and wastewater that are efficient, economical, and meet environmental regulations are urgently needed.

This study proposes a new treatment scheme for Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), one of the most important High Explosives. The treatment process involves adsorption onto activated carbon and a novel method for regenerating the carbon which uses biodegradation of RDX. Such a combination is especially suitable to treat low RDX concentrations in large volumes, e.g. contaminated groundwater or wash-down water.

The following parts of the introduction provide background information about the chemical compound, environmental problems associated with RDX, physicochemical waste treatment options, and biodegradation and biotransformation techniques for RDX. Chapter 2 introduces the new treatment process and its rationale in detail. The experimental part of the study (Chapters 3 and 4) reports on the experimental investigation of adsorption and desorption of RDX onto activated carbon, and biotransformation of RDX under various conditions. The experimental results are discussed in Chapter 5, with specific reference to their significance for the proposed treatment process.

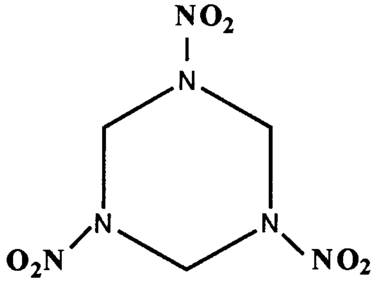
We used industrial RDX in most experiments which usually contains 8 to 15% of Octahydro--1,3,5,7-tetranitro-1,3,5,7-tetraocine (HMX). HMX is usually produced in the production of RDX, as an unwanted byproduct. Consequently, this nitramine is almost always a co-contaminant in RDX-containing wastewaters. This report also contains information about HMX wherever it seemed necessary, or whenever we obtained meaningful experimental results regarding HMX.

1.1 RDX - Properties, Importance, Toxicity, and Environmental Fate

Hexahydro-1,3,5-trinitro-1,3,5-triazine, most commonly referred to as RDX (Rapid Detonation Explosive), is the most important high explosive for military applications in the United States (Rosenblatt et al., 1991). The chemical stability is similar to TNT but the explosive power and sensitivity to mechanical impact are higher than TNT and most other explosives. Its structure, physical properties and other names are shown in Figure 1.1. Mass production of RDX started in the USA (15.2 x 10^6 kg per month), Great Britain, and Germany (7.1 x 10^6 kg per month) during World War II (Urbanski, 1964). Averaged over 1969 - 1970, Approximately 7.6 x 10^6 kg per month were produced in the United States over the 1969-1970 period (Patterson et al. 1976a).

Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)

Empirical formula = $C_3H_6N_6O_6$
Molecular weight = 222.15
Melting point (°C) = 205-207
CAS Reg. No. = 121-82-4
(more data in Rosenblatt, 1991)



also known as: Cyclonite (British), Hexogen (German), Hexogene (French), T4 (Italian) cyclo-trimethylenetrinitramine, 1,3,5-triaza-1,3,5-trinitrocyclohexan, Chemical 506

Figure 1.1 Structure, properties, and names of RDX.

Toxicity: RDX has long been known to have various toxic effects on humans, mammals, fish and protozoa (Yinon, 1990). It has been used as a rat poison (Merck Index, 1989). The primary toxic effect in humans is on the central nervous system. It can cause convulsions, loss of consciousness, vomiting, skin lesions, or even death. The US EPA (1988) recommends a limit of 0.002 mg/L in drinking water as "Lifetime Health Advisory" and classifies it as "Possible Human Carcinogen" (US EPA Group C).Sullivan (1979) proposed a maximum concentration of 0.3 mg/L (24 h-average) to protect aquatic life.

Furthermore, it is known that RDX can be reduced biologically to its trinitroso-derivate, hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX). The latter has been used as an experimental tumorigen (Urban, 1976). There is concern that this strong carcinogen may be produced in the mammal gastrointestinal tract after ingestion of RDX.

Environmental fate: In the past large amounts of RDX wastes were released into the environment at production, handling and research facilities. Patterson et al. (1976b) estimated that one specific ammunition plant wasted 7,300 kg RDX during the Korean war, and another plant discharged up to 450 kg per 24 hours into a river. The practice of discharging aqueous wastes into rivers and lagoons has caused contamination of soil and groundwater at different locations in the US (Spalding and Fulton, 1988). Contamination has also been reported for an old WW II production site in Germany (Haas et al., 1990). Volatilization from soil or water is negligible, as indicated by a vapor pressure of only 3.06×10^{-6} atm (25°C) and a Henry's law constant of $1.96 \cdot\times 10^{-11}$ atm·m^3/mole (25°C) (Rosenblatt et al., 1991). Photolysis in sunlight should not play an important role in RDX destruction, because wavelengths above 290 nm are only poorly absorbed by the molecule. RDX is not immobilized by soil or sediment (Spanggord et al., 1980) due to its low sorptivity to sand ($K_{d(sand)} = 1.6$, Tsai et al., 1980) and to soil organic carbon ($\log K_{OC} = 2.00$, Rosenblatt et al., 1991). Only the low solubility in water (40 - 60 mg/L) limited RDX migration rates in lysimeter studies (Hale, 1979). Consequently, Spalding (1988) found RDX to spread out with almost no retention in a groundwater aquifer. From his field monitoring data, Spalding also concluded that RDX is much more persistent than TNT. Recently, it was reported that RDX accumulates in hydroponic plants raising concern that this could be an entry into the

Furthermore, it is known that RDX can be reduced biologically to its trinitroso-derivate, hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX). The latter has been used as an experimental tumorigen (Urban, 1976). There is concern that this strong carcinogen may be produced in the mammal gastrointestinal tract after ingestion of RDX.

Environmental fate: In the past large amounts of RDX wastes were released into the environment at production, handling and research facilities. Patterson et al. (1976b) estimated that one specific ammunition plant wasted 7,300 kg RDX during the Korean war, and another plant discharged up to 450 kg per 24 hours into a river. The practice of discharging aqueous wastes into rivers and lagoons has caused contamination of soil and groundwater at different locations in the US (Spalding and Fulton, 1988). Contamination has also been reported for an old WW II production site in Germany (Haas et al., 1990). Volatilization from soil or water is negligible, as indicated by a vapor pressure of only 3.06×10^{-6} atm (25°C) and a Henry's law constant of $1.96 \cdot\times 10^{-11}$ atm·m^3/mole (25°C) (Rosenblatt et al., 1991). Photolysis in sunlight should not play an important role in RDX destruction, because wavelengths above 290 nm are only poorly absorbed by the molecule. RDX is not immobilized by soil or sediment (Spanggord et al., 1980) due to its low sorptivity to sand ($K_{d(sand)} = 1.6$, Tsai et al., 1980) and to soil organic carbon ($\log K_{OC} = 2.00$, Rosenblatt et al., 1991). Only the low solubility in water (40 - 60 mg/L) limited RDX migration rates in lysimeter studies (Hale, 1979). Consequently, Spalding (1988) found RDX to spread out with almost no retention in a groundwater aquifer. From his field monitoring data, Spalding also concluded that RDX is much more persistent than TNT. Recently, it was reported that RDX accumulates in hydroponic plants raising concern that this could be an entry into the food-chain (Harvey, 1991). The roles of biodegradation and transformation in the environmental fate of RDX are reviewed in Section 1.3.

1.2 Physico-Chemical Treatment of RDX Wastes

Adsorption: Activated carbon is often used for treating RDX-containing wastewaters (Patterson et al., 1976a). Burrows et al. (1984) determined the Freundlich isotherm constants K and n as 0.1118 and 2.938, respectively (for Filtrasorb 300, Calgon Carbon Co.). The treatment of mixed HE wastewaters is problematic, because RDX is competitively adsorbed with TNT and HMX. Even more problematic is the fact that exhausted carbon must be handled as hazardous waste. The common disposal practice for the spent carbon is open-burning and disposal of the ash as a Class I hazardous waste, disposal to a licensed hazardous waste disposer (Goodfellow, 1991). Thermal regeneration is not feasible due to safety problems when the explosives adsorbed onto the carbon exceeds 8% (w/w) (Walsh et al., 1973; Andren et al., 1975). Treatment with activated carbon is expensive and creates a different problem: disposal of activated carbon, laden with HE. Current spent carbon disposal techniques will not meet the future environmental regulations (Knezovich, 1991). Nevertheless, it is still the "state-of-the-art treatment" for wastewaters from munition production, blending, loading and packing facilities.

There were some efforts in the past to find physicochemical treatment alternatives. Although none of these alternatives are widely used, they are briefly reviewed in the following section with specific reference to wastewaters or ground waters with trace to low concentrations.

One direct alternative to activated carbon adsorption is adsorption onto synthetic polymeric adsorbents. They were found to be less efficient in removing RDX than activated carbon (Patterson et al., 1976a). Solvent regeneration with acetone was demonstrated (Andren et al., 1975); however, the solvent-RDX mixture is still a hazardous waste. This waste problem is probably the main reason that polymeric adsorbents are not widely used for this application (Semmens et al., 1984).

UV-Radiation: Photolytic decomposition of RDX with ultraviolet-radiation (UV 254 nm) is rapid (First-Half-Life-Time: 3.7 min.) in water free of turbidity and free of other UV-absorbing substances (Burrows et al., 1984). RDX production wastewaters often carry high concentrations of acetic acid, cyclo-hexanone, and nitrate (Haas, 1990; McCormick et al., 1984b) which are all strong UV-absorbents. Therefore, UV-treatment is not suitable in these cases. Its application was estimated to be economical for treating small volumes of "clean" water with low RDX-concentrations (< 20 mg/L) (Fisher et al., 1982). Nevertheless, using UV-radiation for groundwater remediation is critical because of hazardous decomposition intermediates (e.g. N-nitroso-methylenediamine, formaldehyde, formamide) (Glover and Hoffsommer, 1979; Rosenblatt et al., 1991; Yinon, 1990). Furthermore, the costs to treat large water volumes with UV-radiation might be prohibitive.

Oxidative treatment: Ozone, hydrogen peroxide, iron catalyzed hydrogen peroxide (Fenton's Reagent), or chlorine are reported to be ineffective in RDX oxidation (Semmens et al., 1984).

Chemical hydrolysis: Alkaline hydrolysis of RDX has been used to desensitize highly concentrated RDX wastes (Shelby, 1984). Freeman (1985) applied surfactants to accelerate RDX hydrolysis in wastewater. He also increased the reaction rates using high pH values (10 - 12) and elevated temperatures (> 50°C). It should be noted that the enhancing effect of the surfactants was more and more reduced with increasing temperatures and pHs. Products, reaction pathways, and the kinetics of alkaline RDX-hydrolysis were studied intensively by Jones (1953) and Hoffsommer et al. (1977). They found evidence for a bimolecular elimination of a hydrogen-cation and nitrous-anion from adjacent ring atoms. The alkaline proton abstraction was the rate-limiting step. The intermediate 1,3,5-triazine-3,5-dinitrocyclohex-1-ene (RDX-h-5) disappeared about five magnitudes faster than RDX, and thus was not accumulated. After the complete disappearance of RDX the hydrolysis products identified were NO_2^-, N_2, NH_3, N_2O, $HCOO^-$, CH_2O, and H_2. The quantities of these products varied with initial hydroxide concentration and complete material balances for RDX-nitrogen and -carbon were not observed. Treating small amounts of RDX in large volumes of groundwater by alkaline hydrolysis should not be economical, considering the available data for reaction kinetics and its dependency on temperature and pH.

1.3 Biodegradation of RDX

Aerobic: The persistence of RDX in soil and groundwater for more than forty years (Haas, 1990) strongly suggests that this substance is nondegradable in aerobic, natural environments. Correspondingly, several laboratory studies with mixed bacteria cultures failed to find a metabolic destruction under aerobic conditions. Osmon and Klausmeier (1973) started enrichment cultures with RDX as sole carbon source and with additional organic co-substrates. McCormick (1981) used a peptone medium for his aerobic incubations. Both could not find any transformation or degradation of RDX. In a 3-year pilot plant study with aerobic activated sludge no bioconversion of 7.3 mg/L RDX was observed (Hoffsommer, 1977; Yinon, 1990). Small amounts of volatilized radioactivity was found while incubating [^{14}C]-RDX aerobically by Knezovich and Daniels (1991), but the measured values were too small and too unreproducible to be evidence for aerobic metabolism of RDX. Another intensive examination of aerobic RDX degradation was conducted by Ro and Stenstrom (1991). They tried enrichment with and without additional carbon sources, used several inocula from sites contaminated with RDX, and applied the strategy to supply RDX as sole nitrogen source (Bruhn et al., 1987). No significant and reproducible reduction of RDX occurred under any conditions. Toxicity of RDX was not a limiting factor, because cultures with organic co-substrates did grow well. No RDX transformation was found in a two-month study with incubated soil (Harvey et al., 1991). The resistance to an oxidative attack is also demonstrated by the chemical inertness in the presence of strong oxidants (discussed earlier in this chapter). We conclude that aerobic biodegradation is not an option for treating RDX-containing wastewaters.

Anaerobic: In contrast to the obvious recalcitrance in aerobic environments, RDX is readily degraded anaerobically in the presence of suitable organic co-substrates. A digester

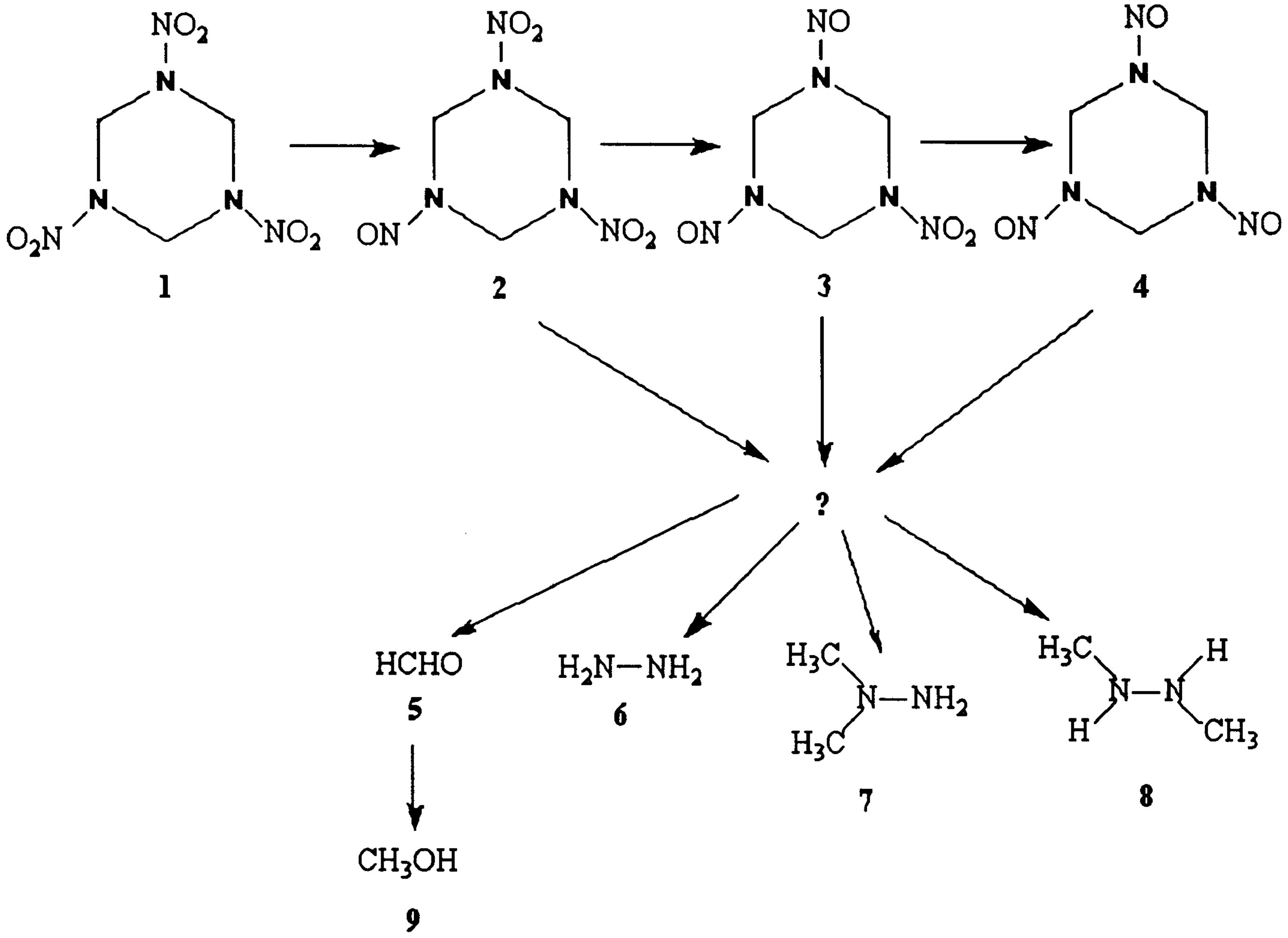


Figure 1.2 Proposed intermediates for the anaerobic biodegradation of RDX.

inoculum metabolized 50 mg/L RDX in four days when incubated in peptone medium at 37°C (McCormick et al., 1981). They observed the production and subsequent disappearance of the mono-, di-, and trinitroso derivatives (MNX, DNX, and TNX, see Figure 1.2). The last detectable traces of TNX and DNX disappeared between the 7th and 18th days. This indicates a reduction of nitro- to nitroso-groups as the first degradation step. The authors postulated that the ring structure becomes unstable when one nitroso group is further reduced to a hydroxylamine group, and they proposed a subsequent, spontaneous hydrolytic ring cleavage. It should be noted that non-aromatic nitroso compounds are also subject to spontaneous hydrolytic reactions themselves. This is described qualitatively and semi-quantitatively for TNX (Urbanski, 1964; Druckrey, 1967). Formaldehyde and traces of dimethylhydrazines and hydrazine were found in McCormick's batch experiments and were claimed to be intermediates arising from RDX. Methanol was also detected and proposed as a final product of RDX metabolism. The fate of the RDX-carbon was also studied with [^{14}C]-RDX. Interestingly, almost no radioactivity was ever adsorbed at or incorporated in the biomass. This confirmed that the disappearance of RDX was not due to adsorption and it could mean that an exoenzymatic degradation was taking place. The final radioactive products were volatile, neutral, organic substances, such as MeOH and HCOH, but were not specifically identified. Mineralization to CO_2 or CH_4 did not occur. The assumed origins of dimethylhydrazines, hydrazine, HCOH, and MeOH from RDX were not controlled by in an experiment without RDX. Aerobic and anaerobic biodegradation of hydrazine and 1,1-dimethylhydrazine was demonstrated in subsequent experiments (McCormick et al., 1984a).

The same authors studied also continuous culture systems under deni

conditions (McCormick et al., 1984b). These experiments are discussed here in some detail, not only because they are important to us but also because they are not the published in a readily available journal. Bench-scale, mixed reactors were used without any bacteria immobilization or recycling. The reactor temperature was not reported, but was most probably room temperature. Degradation of RDX (30 mg/L) was tested using a digester sludge inoculum and various organic co-substrates with nitrate as terminal electron acceptor. Using a peptone (4 g/L) or a molasses (3 mL/l) medium and retention times of 10 - 14 days, 100% disappearance of RDX could be observed after reaching steady state. After reducing the peptone concentration to 0.4 g/L, the rate of disappearance of RDX decreased rapidly; the co-substrate concentration was changed back to 8 g/L before a new steady state was reached. An RDX disappearance of 100% could also be achieved using 10% acid-hydrolyzed sludge or 20% alkaline-hydrolyzed sludge with retention times between 14 - 18 days. Unhydrolyzed sludge was totally unsuccessful as co-substrate. Hydrazines could never be detected in the reactor effluents. Analytical results for MNX, DNX, or TNX are not reported. The gas phase analysis with GC revealed CO_2 and N_2 as the major components; CH_4 and N_2O were never found.

Because the authors concluded that peptones, hydrolyzed sludge and molasses are unrealistic co-substrates for wastewater treatment, they also tried methanol, acetate and glucose. None of those co-substrates were able to support a stable RDX degradation under denitrifying conditions. The authors hypothesized that the rate of RDX disappearance is correlated with the amount of total organic carbon (TOC) in the medium, independently of the type of co-substrate. However, the reported results are not suitable to prove this hypothesis; in fact, they indicate a strong dependency on the type of co-substrate.

McCormick et al. (1981) did not detect methane gas in his RDX degrading cultures, although a digester sludge inoculum and a rich organic substrate were used in his incubations. The production of methane should have been expected. This absence of methane was confirmed by Alatriste-Mondragon (1991) in experiments similar to McCormick's. Subsequently, he was able to demonstrate that RDX is highly toxic to methanogens in mixed as well as pure cultures. Consequently, no significant degradation of RDX occurred in pure methanogenic cultures. These findings were the reasons to exclude methanogenic conditions from the experimental biodegradation studies presented in this report.

2.0 PROPOSED TREATMENT CONCEPT AND RESEARCH REQUIREMENTS

2.1 Treatment Concept

RDX removal from groundwater with activated carbon is a reliable primary treatment step, but it produces a new hazardous waste. Anaerobic biodegradation could be used to transform the RDX and would not accumulate it on the surface of activated carbon; however, treating low concentrations of RDX in otherwise relatively "clean" water would contaminate it with high amounts of organic co-substrates, bacteria, and oxygen scavengers. If the two techniques could be combined by first adsorbing the RDX onto activated carbon, and then regenerating the carbon with anaerobic treatment, a useful process for disposing of RDX containing waters could be developed. Therefore, we propose a treatment concept for low concentrations of RDX in water that uses adsorption on activated carbon and indirect "bioregeneration" of exhausted adsorber columns. The process is shown schematically in Figure 2.1.

Carbon regeneration involves two steps: RDX is first desorbed with a fluid such as hot water; the RDX in the fluid is then treated in an external anaerobic bioreactor, such as an anaerobic fixed-film process. After cell separation, the regenerating fluid can be recirculated for a new desorption cycle. Alternatively, the regenerating fluid could be disposed if this were economical and environmentally sound. The desorbing fluid should be heated before flushing the carbon column to increase the RDX desorption rate. Desorption should be enhanced by the increased RDX solubility at higher temperatures as well as the less favorable

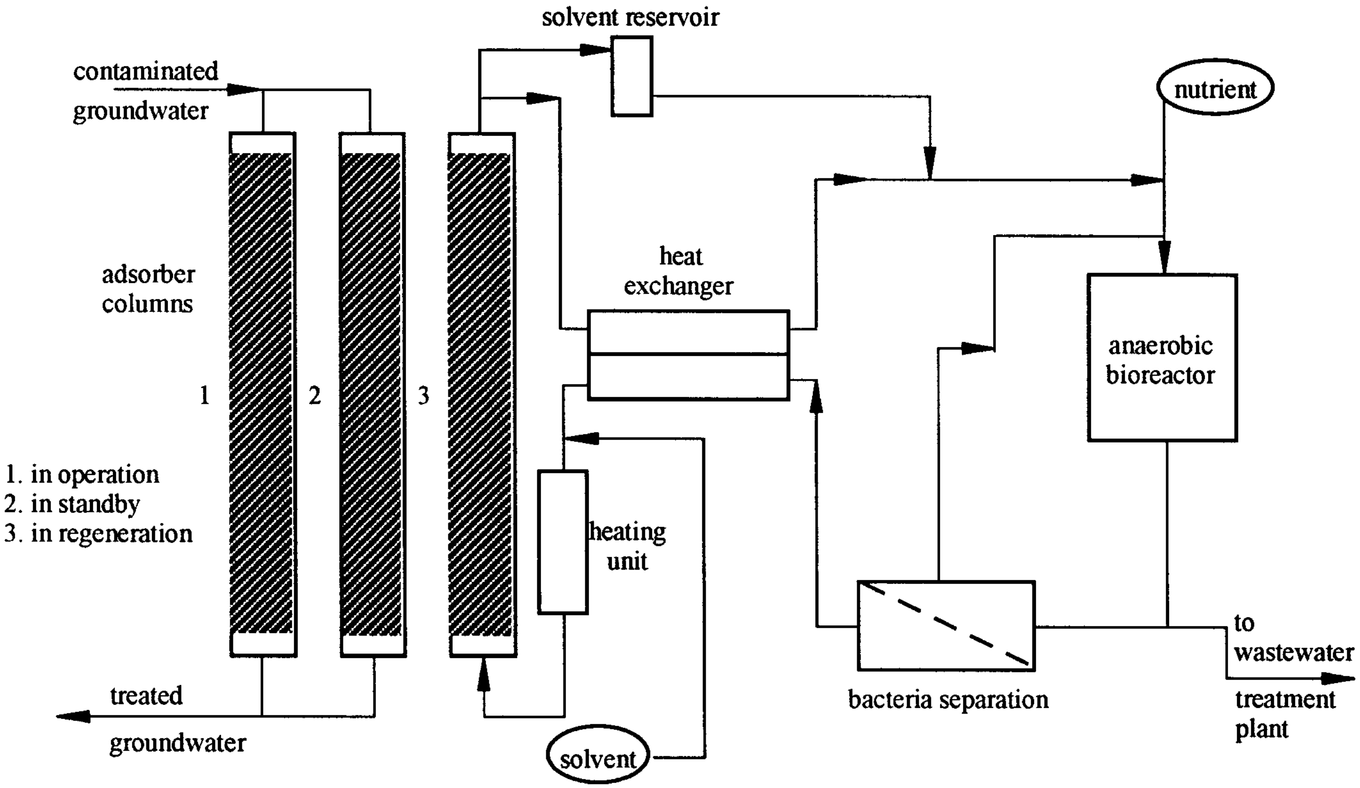


Figure 2.1 Proposed treatment scheme using an indirect-offline-bioregeneration (IOBR) of activated carbon.

isotherm at elevated temperatures. The column effluent is cooled before being reused. An organic RDX solvent or a solvent-water mixture could be used as desorbing fluid. The most desirable solvent should render RDX very soluble and should function as the organic co-substrate for the anaerobic biodegradation of RDX. The amount of solvent used in the process will be determined by the demand on co-substrate to complete one regeneration. At the end of the regeneration cycle the carbon is flushed with new, hot water which replaces the fluid in the system. The anaerobically treated desorbing fluid will still have high concentrations of solvent, and if not reused, must be further treated as a wastewater. It will be free of HE, and may be suitable for discharged to a conventional wastewater treatment plant. Alternatively, an aerobic final biological treatment process could be added. This regeneration process will be described as an "Indirect-Off-line-Bioregeneration" (IOBR).

The advantage of this process is that we do not have to contaminate the bulk groundwater stream with organic co-substrates, oxygen scavengers and bacteria. Therefore the carbon adsorber effluent should be suitable for reinjection without further treatment. The RDX is degraded or transformed to non-hazardous byproducts and does not accumulate to create a second disposal problem, as with carbon adsorption alone. There should be no HE containing byproducts. The carbon could be reused and would not have to be incinerated or disposed off as hazardous waste. Finally, such a remediation technique should be able to meet upcoming environmental laws and regulations, since OB/OD is not required.

2.2 Detail Considerations and Research Requirements

In the following sections, important considerations for the process design are addressed in detail, and the corresponding research requirements are identified. At the end of this chapter, the experiments which are described in this report are summarized to provide an overview.

Bioregeneration: Most researchers consider the desorption of adsorbate molecules to be the crucial, initial part in bioregeneration of activated carbon (Hutchinson and Robinson, 1990a, 1990b; Goeddertz et al., 1988; Kim et al., 1986; Schultz and Keinath, 1984). The build-up of a concentration gradient caused by adsorbate biodegradation in the bulk fluid is understood to be the driving force for adsorbate desorption and transport out of the particle. Still in question is the role of exoenzymes in bioregeneration. Dobrevski (1989) found that activated carbons with many pores in the range of 5 - 50 nm are more suitable to bioregeneration than more microporous carbons. He assumed this to be due to exoenzymes which can enter these mesopores. The smallest bacteriological exoenzymes (diameter = 3 - 4 nm) are only able to enter pores with diameters greater than 5 nm; however, the greatest part of the adsorbate is adsorbed in micropores (< 2 nm, Xiaojia et al., 1991). Consequently, only a small portion of adsorbed molecules can be reached by exoenzymes. Still, desorption in and transport out of the micropores must be the initial step for most of the adsorbed molecules. The better bioregeneration results might be caused by better desorption or transportation characteristics of macroporous carbons.

It will be most important to increase desorption and intra-particle transport of RDX during regeneration. This could be achieved using an organic RDX solvent as desorbing fluid and by heating the column influent, both discussed in the following two paragraphs. Additionally, different kinds of activated carbons should be tested; microporous and macroporous carbons will be compared. The three commercial carbons that will be tested later in this project are listed in Appendix B.

The increased temperature of the desorbing fluid shifts the dynamic equilibrium of the adsorption/desorption reaction towards desorption. Intra-particle transport should be accelerated, because of higher diffusion rates. Additionally, the transport into the bulk fluid is enhanced, because the decreased fluid viscosity of the solvent/water mixture decreases the hydro-dynamic boundary layer. A closed system with pressure build-up should be used, to avoid wasting energy on a phase transformation, instead of temperature increase. This is also the only way to study temperature levels above 100°C. Furthermore, RDX is a solid with a melting point of 205°C, thus, desorption with water steam should be less efficient than desorption with water at the same temperature. Consequently, bench-scale set-up and pilot plant should be constructed to withstand temperatures up to 150°C and appropriate pressures, in order to study the effect of increased temperature on the desorption rate.

The same three advantages - shifted adsorption equilibrium, increased RDX diffusion, and decreased hydro-dynamic boundary resistance - can be reached using the right organic solvent. Fortunately, organic compounds with nitro-groups, like RDX, are well suited to desorption with organic solvents (Tamon et al., 1990). Additional considerations for solvent choice exist. The solvent must be suitable as co-substrate, and it should not be highly toxic or dangerous. Secondly, it must have a low adsorbtivity to activated carbon. Table 4.1 (see: Results) shows the RDX solvents with a reasonably low adsorbtivity. Another consideration is the way to use the solvent in order to desorb the most RDX using a fixed, limited amount of solvent. Kookana et al. (1990) has suggested that it might bemore effective to a solvent/water mixture. Also, optimal flow rates exist for desorption (Shorten et al., 1990; Sutikno et al., 1983).

Co-substrate: As discussed previously the co-substrate of choice should be an RDX solvent that does not strongly adsorb to activated carbon. Furthermore, it should be inexpensive and readily available in standardized quality. McCormick and his coworkers were only successful using peptone or molasses media. Extended experiments to reach RDX degradation in denitrifying conditions with glucose, methanol, and acetate did not work well. Molasses is clearly not recommended for use in the proposed system, because some of its components adsorb strongly to activated carbon. Peptones and amino may be good candidates; peptones are too large to enter most of carbon pores and amino acids adsorb only weakly (Faust and Aly, 1987). However, they are expensive and may not be RDX solventsAn important goal of this project is to develop a bacteria culture which can use one of the suitable solvents as co-substrate to degrade RDX. Another goal will be to minimize co-substrate usage. This will be important not only to minimize cost but also to reduce bacteria growth and bacterial lysis products in the bioreactor effluent. It is believed that lysis products adsorb strongly to activated carbon, causing a slow fouling of the carbon (Schultz and Keinath, 1984). More growth also means more lysis products and more operational problems in the fixed-film reactor. Minimizing co-substrate utilization will have several important advantages.

Bacteria immobilization: A fixed-bed bioreactor seems to be the best choice for this treatment requirement. The process water will be completely exchanged after each regeneration cycle, and it will be advantageous to retain biomass using biofilm growth on packing material. Fixed-film reactors are also known to work well with low growth substrate concentrations. Therefore, this type of reactor will be choosen to minimize co-substrate utilization. Bacteria immobilization helps also to relieve the strain on the cell separation unit, a great economical and operational advantage. Finally, the plug flow character of a fixed bed design will help to secure a RDX-free effluent. This is important to avoid new RDX adsorption at the influent side during recirculation.

Cell separation: A cell separation process (e.g. cross-flow-filtration) will prevent biofouling of the adsorbent and an important part of the concept that we call "indirect bioregeneration". In "direct bioregeneration" the exhausted carbon is directly contacted with the degrading biomass. However, there are examples in the literature showing that bacteriological growth on the carbon particles can decrease the regeneration efficiency (Hutchinson and Robinson, 1990b; Koganovskii et al., 1981). The advantages of close contact between bacteria and carbon are unknown; bacteria cells are too large to enter the carbon pores.

Metabolites: The impacts of intermediates or endproducts of anaerobic RDX transformation must be considered with great care in the process design. Considerable amounts of carcinogenic nitroso-derivatives were regularly observed by previous biologcial treatment researchers; in one case traces of carcinogenic hydrazines were detected (see Section 1.2). Fortunately, all of those detected nitrosamines and hydrazines are unstable substances.

Their decomposition under anaerobic conditions was demonstrated. Both substance classes are biodegraded and also chemically decay under environmental conditions. Nitrosamines undergo hydrolytic decomposition (Urbanski, 1964) and are readily reduced to unsymmetrical hydrazines (Druckerey, 1967). TNX, for example, disappeared at rates greater than 10% per day in distilled water at 22°C (Druckerey, 1967). Hydrazine is rapidly oxidized to N_2 by molecular oxygen in neutral or alkaline conditions; autooxidation to N_2, H_2O_2 and NH_4^+ occurs in alkaline solutions and is catalyzed by metal- and phosphate-ions (Moliner and Street, 1989). Build-up of stable cyclic amines and their subsequent polymerization products is not a concern, because the non-aromatic ring structure of RDX becomes unstable after reduction of the adjacent nitro groups (cf.: McCormick et al., 1981; Barnes and Eagon, 1986; Urbanski, 1964). For example, hexahydro-1,3,5-triethyl-1,3,5-triazine (nitro groups substituted by ethyl groups) decomposes in environmental conditions and is also subject to biodegradation (Barnes and Eagon, 1986).

Another concern is that metabolites might adsorb to the activated carbon during recirculation of bioreactor effluent; fortunately, this is not likely. We do not have adsorption data for MNX, DNX or TNX but they are much more soluble in water than RDX, and should adsorb less. Hydrazines are relatively small, highly polar, weak bases (hydrazine is partly ionized at pH 7). Therefore, adsorption of hydrazines should be small. The high temperatures in the carbon column during recirculation will accelerate the hydrolyzation of nitrosamines (Urbanski, 1964) and the autooxidation of hydrazines, which should also reduce the probability of adsorbing to the carbon surface.

The experiments described in the following chapters of the report are preliminary adsorption and desorption studies and detailed experiments on anaerobic biodegradation of RDX. Adsorption and desorption of RDX onto activated carbon was first studied in a fixed-bed column using continuous flow and water as a solvent. Thereafter, desorption experiments in batch mode were conducted to study the influence of water temperature and the desorbing abilities of several RDX solvents. In biodegradation experiments, we examined a broad spectrum of different physiological conditions with mixed cultures. We started with conditions that were shown to be successful by previous research, but quickly changed to conditions most promising for achieving rapid RDX degradation with simple organic co-substrates that are compatible with the Indirect-Off-line-Bioregeneration process. Different terminal electron acceptors were also evaluated in our experiments.

3.0 MATERIALS AND METHODS

3.1 Chemicals

Powdered RDX and HMX (Octahydro-1,3,5,7-tetranitro-1,3,5,7-tetraocine) were supplied by Lawrence Livermore National Laboratories (LLNL) in 1 g shipments. Both substances were dissolved in acetone as stock solutions and stored in glass flasks at 4°C. All RDX shipments were from the same production batch. The RDX had an HMX content of 10.9% (w/w) as production related impurity, according to determinations with HPLC (as described later). A portion of 2 g RDX was twice recrystallized from acetone to examine the possibility for reducing the HMX-content. The recrystallization product contained only 4.3% (w/w) HMX but 25% of the original mass was wasted. This loss was too high to continue the purification efforts. The purified batch was used in experiments B-8 and B-9; the original quality was used in all other experiments. The HMX contained less than 0.1% (w/w) RDX as impurity. The activated carbon used in adsorption and desorption experiments was Filtrasorb 400 (Calgon Carbon Co., Pittsburgh, PA), a bituminous coal with a high surface area (1000 m^2/g) and microporous structure. Complex nutrient sources were used in the biological experiments: bacto peptone, beef extract, and casamino acids (Type I) from Sigma Co. (St. Louis, MO), soytone from Difco Laboratories (Detroit, MI), and pharmamedia (cotton seed flour) from Traders Protein (Memphis, TN). Pure ethanol (Absolute-Grade) was supplied by Goldman (Sacramento, CA). All other solvents were HPLC grade and all other chemicals - were ACS reagent grade. They were purchased from Fisher Scientific Co. (Pittsburgh, PA).

3.2 Adsorption and Desorption Experiments

3.2.1 Continuous flow experiments in column reactors

The continuous adsorption (AC-1) and desorption (DC-1) experiments were conducted in a Plexiglas column with an of internal diameter (I.D.) of 25 mm and length of 200 mm (volume: 98.17 mL). The column and two Plexiglas head-pieces were connected using slip fit; no sealing compound was necessary. The column was equipped with eight sampling-outlets (threaded barb fittings) over the length of the column. Packing material was retained at both ends by stainless steel screens. The column was filled with 25 g of Filtrasorb 400 producing a bed length of 110 mm (bed volume: 52.8 mL). The space below and above was filled with 3 mm borosilicate glass beads (Fisher Scientific Co., Pittsburgh, PA). Glass beads and activated carbon granules were separated by stainless steel screens. Fluids were pumped into a standpipe (PVC, I.D.: 10 mm, length: 2000 mm) using a Masterflex peristaltic-pump (1 - 100 rpm) and Masterflex silicone tubing of size 14 (both, Cole-Parmer, Chicago, IL). The standpipe prevented air from entering the carbon column and helped to equalize the flow rate before passing through the packed carbon in an upflow mode. The flow rate of 30 mL/min (in AC-1 and DC-1) was measured at the effluent with a graduated cylinder (100 mL) and a stop-watch two times per day. The room temperature varied between 23 - 26°C.

The granular carbon was washed with deionized water to remove fines, dried (24 hr at 103°C), weighed, and then resuspended in deionized water before pouring it into the column. After completing the filling procedure, hot water was pumped through the column for six hours to remove entrapped gas. Water solutions of RDX for the adsorption experiment (AC-

1) were prepared in 18 L glass flasks by adding acetone stock solutions into the empty flask. The acetone was allowed to evaporate and tap water was added. The vessels were stirred and slightly heated (ca. 50°C) to increase the solvation rate.

At the conclusion of experiment AC-1, the column was turned upside down and flushed with RDX-free tap water to study RDX desorption, which was called experiment DC-1. The effluent was collected in Nalgene tanks (200 L each).

3.2.2 Desorption experiments in batch reactors

Desorption experiments DB-1 and DB-2 were conducted in 250-mL Erlenmeyer flasks filled with 100 mL water (DB-1) or 100 mL organic solvents (DB-1). A fixed amount of wet carbon was added and the flasks were stoppered. The carbon used in DB-1 was obtained from the adsorber column after experiment DC-1 ended. The carbon was thoroughly mixed and afterwards stored on a nylon screen to let the water drain. After DB-1 ended, the carbon from the four flasks was combined, mixed and allowed to drip off. This batch supplied the carbon for experiment DB-2. In both cases the dry weight percent for each carbon was determined using a small portion from each batch. In DB-1, magnetic stirrers with integrated hotplates were used to study desorption of RDX in water at different temperatures. In experiment DB-2, the flasks with solvent-carbon suspensions were kept in a shaker (60 rpm) at room temperature (25°C). Samples from DB-1 were filtered and directly measured, samples from DB-2 were diluted (100 µL in 1 mL) prior to filtration with a methanol/propanol solution (50:50). Dilutions were made in two replications.

3.2.3 Solubility determination

At the beginning of the study, we had quantitative RDX-solubility data only for acetone and water. Therefore, we determined the solubility of RDX in eight organic solvents, using 0.1 g RDX and 2 mL of solvent in 4-mL glass vials. The vials were closed with a gas - tight Teflon-coated screw caps and stored five days at room temperature in the dark. At the sixth day, the vials were shaken for five hours in a water bath controlled 22°C. Then 100 µl of supernatant was transferred into 2 mL of methanol. The methanol solution was immediately filtered and used for HPLC-determination of the RDX concentration.

3.3 Inocula

The different inocula are presented here in relation to the experiments where they were used. The experiments themselves are listed in chronological order. The acronym BB refers to Biodegradation in Batch reactors. The chronological order is indicated by a adjacent Arabic number. The same acronyms are also used in the results and discussion sections of the report.

BB-1: anaerobic digester sludge from Hyperion Waste Water Treatment Plant (Los Angeles, CA), 1:4 diluted with phosphate-buffer and filtered through glass wool.

BB-2: from the culture in BB-1, which was incubated with Bacto Peptone (0.5 g/L) and Beef Extract (0.3 g/L).

BB-3: from the culture in BB-2, which was incubated with Bacto Peptone (2 g/L).

BB-4: *Digester Sludge (precultivated):* from the culture in BB-3, which was incubated with Sodium Sulfide.

Pond Sediment (precultivated): enriched from a pond in the Ornamental Garden at UCLA by Felipe Alatriste-Mondragon, School of Public Health, UCLA; grown in peptone medium containing RDX.

Digester Sludge (not precultivated): anaerobic digester sludge (source as described for BB-1), 1:4 diluted with oxygen-free phosphate-buffer, filtered through glass wool and paper, dilution and filtration under H_2/CO_2-atmosphere.

Sites Mix (not precultivated): mixture of sediment and wastewater from different sites at the Pantex Plant in Amarillo, Texas, diluted and filtered as described for *Digester Sludge (not precultivated)*. These sites were believed to be exposed to RDX.

BB-5: same as BB-4.

BB-6: from BB-5 cultures, corresponding to the organic solvent used as co-substrate and the originating inocula [*Digester Sludge (not precultivated) or Sites Mix (not precultivated)*] in BB-5.

BB-7: from BB-6 cultures, corresponding to organic solvent used as co-substrate and originating inoculum in BB-5.

BB-8: derived from BB-7, by incubation in a minimal medium with EtOH, KNO_3, and phosphate buffer solved in tap water for three weeks.

BB-9: from the culture in BB-8, which was incubated with 6.4 g/L of nitrate.

3.4 Incubation Methods and Media

The vessels for biodegradation experiments were 115-mL glass flasks or 25-mL glass tubes closed with butyl-rubber stoppers, as commonly used for anaerobic incubation techniques. The stoppers were mechanically secured with aluminum caps. To prepare media, deionized water was heated to the boiling point. The water was flushed with oxygen-free nitrogen gas all the time during medium preparation.

The inorganic chemicals of the basal medium were added during the heating. After, the medium cooled down, the heat sensitive components (inoculum, organic substrates, vitamins, RDX, Na_2S) were added, and the flushing with nitrogen gas was continued for 30 minutes. Depending on the experiment, some components were added to individual vessels rather than to the total medium volume. The composition of the standard basal medium including trace minerals and vitamins is shown in Table 3.1. Deviations of the basal medium are stated for every individual experiment in the results section. However, Table 3.2 gives an overview about the organic co-substrates tested and Table 3.3 contains information about the terminal electron acceptor and amount of sodium sulfide used in each experiment.

The flasks or tubes were flushed with oxygen-free nitrogen gas prior to filling with 100 mL or 20 mL medium, respectively. The prepared medium and all individual components were transferred to the growth vessels under oxygen-free conditions. The vessels were incubated in a temperature-controlled shaker at 35°C, except for experiments BB-4 to BB-7 where they were shaken at room temperature (28 - 30°C). Materials and methods which were only used in a single experiment are reported together with the results of this experiment.

Table 3.1: Composition of the standard basal medium

Chemicals	Concentration (mg/L)
Minerals:	
$K_2HPO_4.3H_2O$	4176
$NaH_2PO_4.H_2O$	1614
NH_4Cl	418
$MgCl_2.6H_2O$	200
$CaCl_2.2H_2O$	100
Trace minerals:	
$FeCl_3$	3.90
$MnCl_2.4H_2O$	0.95
$ZnCl_2$	0.66
$CoCl_2.6H_2O$	0.58
$CuCl_2.2H_2O$	0.30
$Na_2Mo_4.2H_2O$	0.46
$Na_2B_4O_7.10H_2O$	0.24
Vitamins:	
Thiamine	0.50
Nicotinic acid	0.50
Pyridoxine	1.00
Vitamin B12	0.05
Riboflavin	0.50
Biotin	0.20
Thioetic acid	0.10
Folic acid	0.20
Ca-Panthothenate	0.05

Table 3.2: Organic substrates in biodegradation experiments

Experiment	RDX (mg/L)	Organic Co-substrates	(g/L)	TOC*[1] (g/L)
BB-1	50	Bacto Peptone (62.5%) and Beef Extract (37.5%)	8.0 or 0.8	3.14 or 0.31
BB-2	30	Bacto Peptone	none or 2.0	0.78
BB-3	30	Bacto Peptone	1.0	0.39
BB-4	30	Bacto Peptone or	1.60	0.63 and
		Soytone or	1.60	0.63
		Cotton Seed Meal or	1.60	0.63
		Molasses or	1.72	0.63
		Glucose or	1.57	0.63
		Sucrose	1.49	0.63
BB-5	30	Bacto Peptone and	1.60	0.31 and
		Methanol or	1.67	0.63
		Ethanol or	1.20	0.63
		Propanol or	1.05	0.63
		Na-Acetate or	2.14	0.63
		Na-Propionate or	1.67	0.63
		Ethyl Acetate	1.51	0.63
BB-6	30	Casamino Acids and	0.80	0.31 and
		Methanol or	1.67	0.63
		Ethanol or	1.20	0.63
		Propanol or	1.05	0.63
		Na-Acetate or	2.14	0.63
		Na-Propionate	1.67	0.63
BB-7	30	Casamino Acids and	0.80	0.31 and
		Ethanol or	1.20	0.63
		Propanol	1.05	0.63
BB-8	35	Ethanol	0.0	-
			1.2	0.63
			2.4	1.26
			4.8	2.52
			9.6	5.04
BB-9	20	Methanol or	3.34	1.26
		Ethanol or	2.40	1.26
		Na-Formate or	7.10	1.26
		Na-Acetate or	3.13	1.26
		Na-Propionate or	2.58	1.26
		Acetone or	2.57	1.26
		Ethyl Acetate	2.30	1.26

---

*[1] Total organic carbon: values for peptone, casamino acids and molasses were derived from McCormick et al. (1984), other values are calculated.

Table 3.3: Electron acceptors, sulfide, and redox-conditions in biodegradation experiments.

Experiment	Co-substrate^{*1}	Na_2SO_4 (g/L)	KNO_3 (mg/L)	Na_2S (mg/L)	Redox^{*2}	Comments
BB-1		-	-	81.3	**f**^{*R}	
BB-2		-	-	81.3	**f**^{*R}	
BB-3				none or 81.3	**f**^{*R}	tested with Na_2S, w/o Na_2S, and w/o deoxygenation
BB-4		-	-	14.6	**f**	
BB-5	Methanol	4.95	1.62	14.6	**s + n**	SO_4 & NO_3 additive
	Ethanol	4.95	1.62	14.6		SO_4 & NO_3 additive
	Propanol	4.95	1.62	14.6	**s**	only SO4
	Na-Acetate	4.95	1.62	14.6		only SO4
	Na-Propionate	4.95	1.62	14.6		only SO4
	Ethyl Acetate	4.95	1.62	14.6		only SO4
BB-6		3.59	1.21	9.1	**f, s, s + n**	all tested with SO_4 & NO_3, with SO_4 only, and w/o both (fermentative)
BB-7	Ethanol	3.59	1.21	9.1	**s, n**	either SO_4 or NO_3
	Propanol	"	-	"	**s**	
BB-8		-	0.0 0.8 1.6 3.2 6.4	none or 9.1	**n**	Na_2SO_4 and Na_2SO_3 were tested as sulfur-source alternative to Na_2S
BB-9		-	4.8	-	**n**	Na_2SO_3 as sulfur-source

^{*1} listed when experimental differences depending on co-substrate; ^{*2} Redox-conditions stated as, **f** = fermentative, **s** = sulfate-reducing, **n** = nitrate-reducing; ^{*R} Resazurine added: 1 mg/L.

3.5 Analytical Methods

Growth measurement: The culture growth was measured as turbidity at 600 nm with a Perkin Elmer Junior Spectrophotometer. The anaerobic tubes remained closed during turbidity measurements, because the anaerobic tubes could be directly inserted into the photometer. The instrument was adjusted to zero with a reference tube filled with deionized water. The turbidity of each growth vessel was determined at the beginning of each biodegradation experiment. The results presented later are the extinction values, corrected by subtraction of this start value (zeroing of each individual tube), and multiplied with 1000 (for convenience).

Determinations of organic substances with liquid chromatography: The HPLC-method employed a mobile phase of water/methanol/acetonitrile 40%/35%/35% (v/v/v) at a flow rate of 1 mL/min to separate RDX, HMX, and intermediates. The column was a C18 Reversed Phase (particle size 10 μm) for biodegradation studies and a C8 Reversed Phase, (particle size 5 μm) for adsorption/desorption studies (both: "Adsorbosphere" series from Alltech). The substances were detected with an Fixed-Wavelength-Detector at 254 nm (experiments BB-1 and BB-2) or at 236 nm with an Diode-Array-Detector (all other experiments). Peak spectra were routinely checked for consistency to control peak purity.

Samples from biodegradation experiments were filtered with sterile filters (0.2 μm, ACRODISC from Gelman). Samples from adsorption and desorption experiments and undiluted organic solvents were filtered using Nylon-66 membrane filters (0.2 μm). The filters were tested for loss of RDX as described by Jenkins et al. (1986). The test method compares RDX determinations from directly filtered samples and from samples pretreated by 1:2 dilution with methanol and ultrasonic prior to filtration. The tests revealed no detectable loss using Nylon filters. Filtration with ACRODISC filters caused ca. 10% loss at concentrations above 5 mg/L, and ca. 20% for concentrations under 1 mg/L. These results were adequate for our purposes and we decided to work without sample extraction. It should be noted that filters with PVDF membrane, which were also tested, adsorbed up to 80% of the RDX, at concentrations of 20 mg/L. More information about other kinds of filters are published by Jenkins et al. (1986).

Injection volumes of the filtered samples were 10 or 15 μl for biodegradation samples and 25 μl for samples from adsorption and desorption experiments. Calibration standards were 1, 5, 10, 20 and 40 mg/L for RDX and 1, 2, 4 and 5 mg/L for HMX; both standard series were dissolved in water. HMX was quantitatively analyzed only in experiments BB-8 and BB-9. In earlier experiments we determined its peak-retention time, -spectra, and -area, but did not run HMX standards. To determine RDX concentrations below 5 mg/L we used an injection volume of 25 μl and a standard series of 0.1, 1, 2, 4, and 8 mg/L. Detection limits for RDX were 0.2 mg/L and 0.1 mg/L for the methods with 10 μl and 25 μl sample injection volume, respectively. These estimations are three times the standard deviation of the y-axis-intersection after linear regression of three data series.

4.0 RESULTS

4.1 Adsorption and Desorption of RDX

Experiment AC-1: The loading of 25 g Filtrasorb 400 with RDX at concentrations between 3.7 - 6.8 mg/L was stopped when the first, detectable traces of RDX appeared in the column effluent (Table A.1, Appendix A). The throughput at this time was 205 L water with an average RDX concentration of 5.02 mg/L. The average load (41 mg RDX per gram activated carbon) reached 21% of the equilibrium capacity as calculated from isotherm data (Burrows, 1984). The breakthrough observed at 60 mm bed length was very flat indicating a long mass-transfer zone. The original plan to produce RDX-saturated carbon was abandoned because to much RDX was already consumed.

Experiment DC-1: The flushing of the RDX-laden carbon with tap water was stopped after approximately 50 % of the RDX was desorbed (Table A.2, Appendix A). A volume of 961 L tap water had flown through the column; the effluent concentration was ca. 0.3 mg/L at that point. Compared with the bed volumes to produce the corresponding load (520 mg RDX), the desorption had taken 10.7 times more water (Figure 4.1). The values for RDX-Load and -Recovery in Figure 4.1 were calculated with the measured concentrations at the influent (AC-1) and effluent (DC-1), respectively. The concentration average of two subsequent samples was multiplied with the throughput volume between those two samples. This calculated load can be compared with the RDX amount derived from the total stock solution volume that we used to prepare the influent charges. Both values of 1139 mg and 1025 mg differ quite a bit. The latter should be closer to the true value, but the other method is more comparable with the recovery calculation, and for this reason was used in Figure 4.1. The calculated recovery values were measured twice by determining the total effluent volume concentration of the effluent, which was collected in the storage tanks. The throughput volumes of 190 L and 382 L agree reasonably well (163 vs. 164, and 271 vs. 265 mg; cf. Table A.2, Appendix A).

Experiment DB-1: Using the activated carbon from DC-1 with its remaining RDX-load, we found a strong temperature influence on the equilibrium concentration in water (Figure 4.2). The 60-minute value for 90°C could not be determined, because the flask stopper became loose and a considerable amount of water was lost. The desorbed RDX concentration was as much as 15 times higher at 90°C than at 29°C after 45 minutes. The data sets can be approximated by e-functions with correlation coefficients between 0.9962 (45 min.) and 0.9990 (60 min.). To compare the curve shapes, literature data for the temperature dependency of the RDX-solubility was included into Figure 4.2 .

Solubility determination: Literature values of RDX solubility could be obtained for five organic solvents examined here (Table 4.1), and serve as a comparison to estimate the accuracy of our experimental method. However, literature values for solubility often differ substantially. Our method produced similar variability. Literature data for RDX solubility in formic acid, propionic acid, and propanol were not available. It must be noted that a metabolite peak was observed in the formic acid/RDX solutions. This peak appeared also after addition of formic acid to a RDX-methanol solution in a matter of hours. The

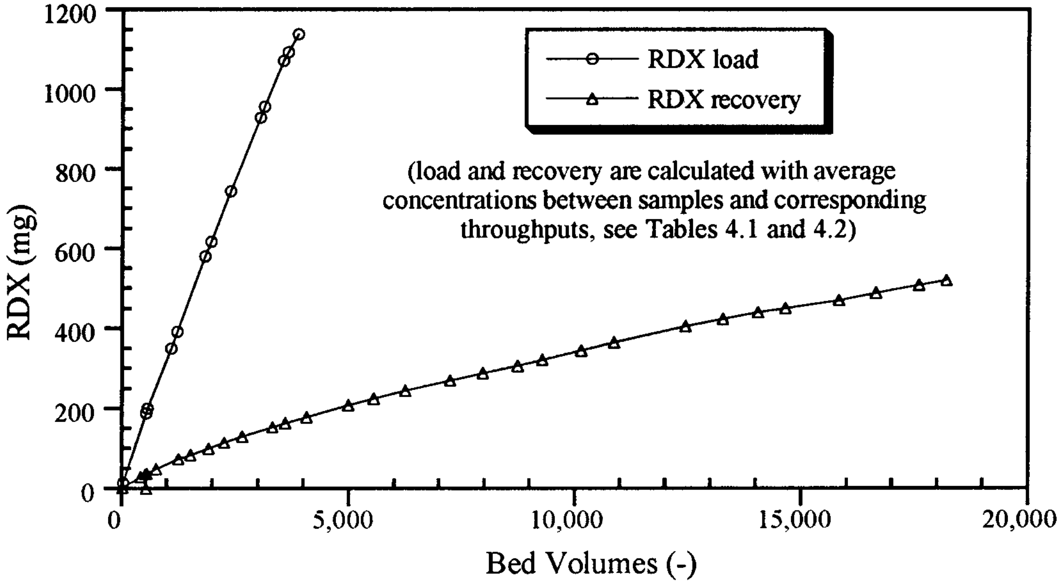


Figure 4.1 Adsorption and desorption of RDX in continuous flow {DC-1}.

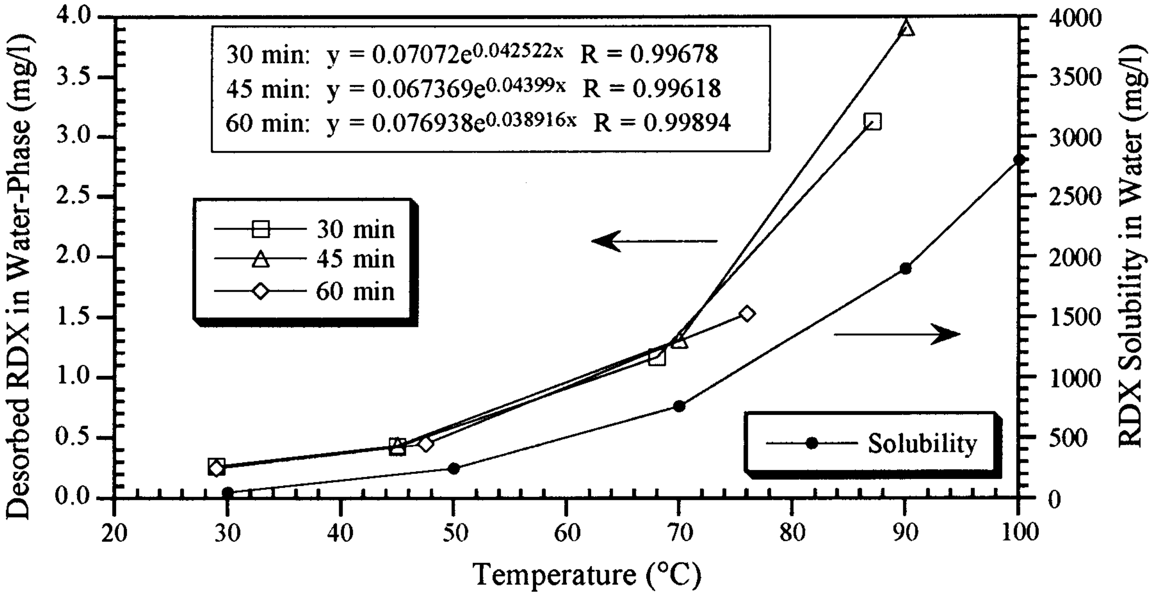


Figure 4.2 Temperature influence on RDX desorption {DB-1}.

Table 4.1: Solubility of RDX in organic solvents.

Solvent	RDX Solubility (g/L)				
	experimental	Literature			
	(22°C)	Ref. [1]	Ref. [2]	Ref. [3]	Ref. [4]
Acetone	43.6	69.0 (30°C)	53.7 (20°C)	57.5 (20°C)	40.0 (20°C)
Ethyl Acetate	11.4	16.0 (30°C)	4.6 (20°C)	25.9 (28°C)	
Formic Acid	6.9				
Acetic Acid	3.7	4.0 (30°C)			
Methanol	1.9	3.0 (20°C)	1.9 (20°C)	1.8 (20°C)	
Propionic Acid	1.0				
Ethanol	0.9	1.3 (30°C)	0.8 (20°C)		
Propanol	0.5				

References:
[1] = Rodgers (1962); [2] = Urbanski (1964); [3] = US Army Material Command; [4] = Merck & Co. (1989)

metabolite was not identified. The estimated solubility (6.9 g/L) in for formic acid might be inaccurate. However, we refer always to our own experimental solubility data (Table 4.1, second column) in the remainder of the report.

The RDX-solubility decreases with respect to the chemical classification of the solvent in the order of ketone > carbonic acid > alcohol. With respect to the length of the alkyl chain, the solubility decreases in the order $C_1 > C_2 > C_3$. The order of solubility shown in Table 4.1 can be explained by a superposition of both rules.

Experiment DB-2: Using the remaining RDX-load on the activated carbon from DB-1, we studied desorption using six organic solvents. Acetone was not included in this experiment because we knew from previous biodegradation experiments that acetone is not a suitable co-substrate for anaerobic RDX degradation. Formic acid was not studied, because of the observed metabolite.

The reproducibility of the sample dilution was satisfactory and the replicates varied less than 4%; the averages are presented in Table A.5 (Appendix A) and Figures 4.3a and 4.3b. Figure 4.3a shows the time axis to 800 minutes; the time scale is shown only to 70 minutes in Figure 4.3b to better illustrate the early part of the test. The desorption of RDX reached an equilibrium after 13 hr, which can be concluded by comparing the concentrations after 13 hours and 30 days (Table A.5). The 30 days values are therefore not included in Figure 4.3.

Apparently, RDX desorption by the solvents follows the same order as the RDX solubility in the solvents (Figure 4.3). Also, the solvents with higher solubility desorb faster; the RDX concentration in ethyl acetate after 60 min. was already 88% of its value after 13 hours; the corresponding value for propanol was only 51%. The exceptions from the rules are methanol and propionic acid. At the beginning, methanol was the fastest desorbing solvent (51% of the equilibrium value after only 15 min.), although, its RDX solubility is not the highest. At the conclusion of the experiment (after 13 hr), the RDX concentration in methanol is less than in propionic acid, although, the RDX-solubility is higher in methanol than in propionic acid.

The differences between the solvents in RDX desorption are much smaller than their differences in solubility. For example, RDX in acetic acid is only 33% as soluble as in ethyl acetate, but the desorbed RDX concentration in acetic acid is 95% of its value in ethyl acetate (Table A.6, Appendix A).

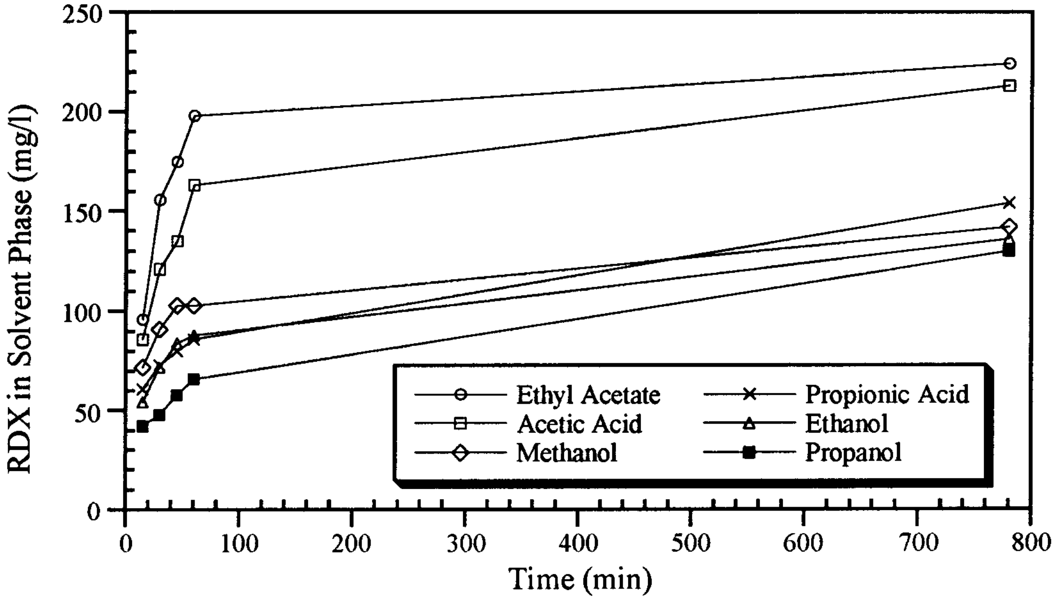


Figure 4.3a RDX desorption from activated carbon using different solvents {DB-2}.

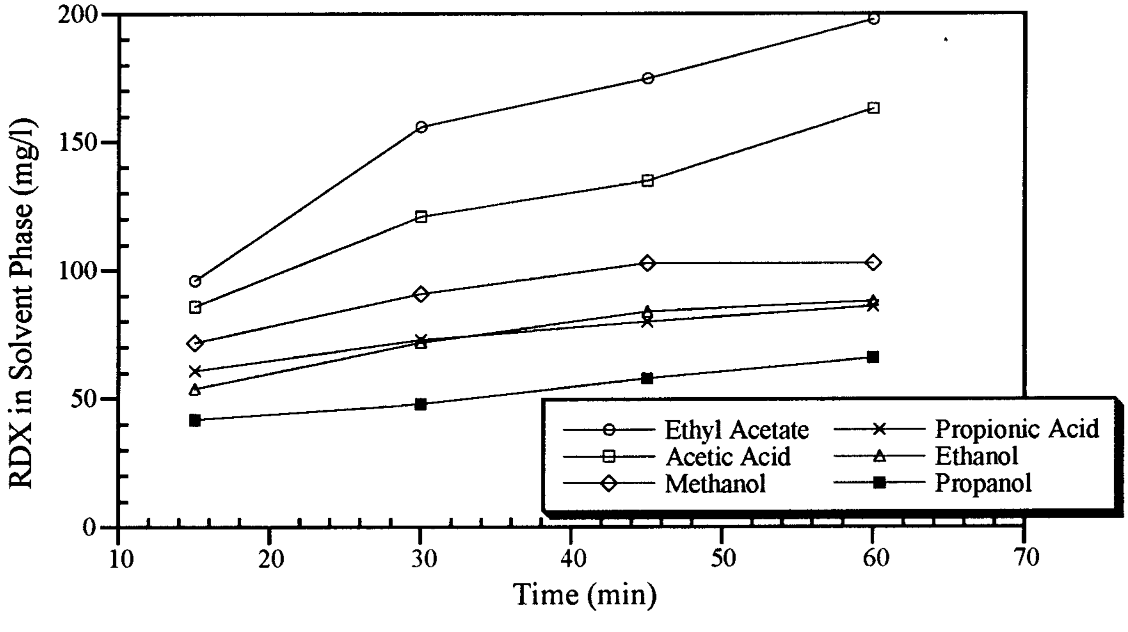


Figure 4.3b RDX desorption from activated carbon using different solvents for t < 70 min {DB-2}.

4.2 Anaerobic Transformation of RDX

In the following sections we describe the anaerobic transformation experiments. The first paragraph of the experimental description discusses the special methods used only in that experiment. The goals of the experiment, deviations from the standard basal medium and the most important parameters are discussed. The actual results are reported in subsequent paragraphs. Additional results are reported in tables in Appendix A instead of in the text. The term "fermentive conditions" refers to the absence of oxygen, nitrate, and sulfate, whereas the term "anoxic conditions" means the absence of oxygen but presence of nitrate or sulfate. "Anaerobic conditions" is the generic term for both.

Experiment BB-1: Using a fresh digester sludge as inoculum and nutrient broth (62.5% bacto peptone, 37.5% beef extract) as co-substrate, we found that RDX disappeared rapidly (Figure 4.4). Using 8 g/L peptone the initial 50 mg/L concentration of RDX disappeared in five days or less. The experiment confirmed McCormick's results (1981) who used the same conditions and kind of inoculum. The culture with only 0.8 g/L of the organic co-substrate was less efficient in RDX degradation.

Tests with a methanol extraction prior to sample filtration, as described in Chapter 3.5, revealed a 10 - 20% loss of RDX due to direct filtration (data not shown). The loss was not clearly correlated with the amount of biomass in the samples or with the absolute RDX concentration.

Experiment BB-2: In this experiment the basal medium did not contain NH_4Cl or any other inorganic nitrogen source. Abiotic decay was tested without peptone in the basal medium but with 81.3 mg/L Na_2S, to check the possibility of reductive reactions. The abiotic control received no inoculum but was not sterilized. Two more cultures ("*only Inoculum*" and "*Hydrogen*") did not receive the bacto peptone. In the remaining two cultures the concentration of the organic substrate was reduced to 25% compared to experiment BB-1. Two cultures were supplied with hydrogen by flushing the gas space in the bottles (ca. 15 mL) with hydrogen before closing.

As expected no abiotic disappearance was observed (Figure 4.5). No bacterial RDX metabolism occurred with RDX as sole organic carbon source ("*only Inoculum*"). Also, the addition of hydrogen as inorganic electron donator did not enable the bacteria to metabolize RDX as sole organic substrate ("*Hydrogen*"). A slight rise in the RDX concentration was observed in all three cases that showed no RDX transformation. Degradation occurred in the cultures containing the organic co-substrate. Considering the results obtained here (using 2 g/L bacto peptone) and the results from experiment BB-1 (using 0.8 and 8 g/L nutrient broth), a correlation between the co-substrate concentration and the RDX degradation rate became obvious. The rate of RDX degradation was slightly higher in the culture with hydrogen and peptone, but because of missing replications we cannot determine the statistical significance.

Experiment BB-3: The main purpose of this experiment was to study the effect of different redox-conditions on the RDX degradation. As in experiment BB-2, the NH_4Cl was not added to the basal medium. The peptone concentration in the medium was reduced to 1 g/L, because bacto peptone contains reducing agents itself. Consequently, large concentrations of this complex substrate would tend to equalize the different redox-conditions studied in this experiment. Moreover, the bacteria themselves are may reduce the

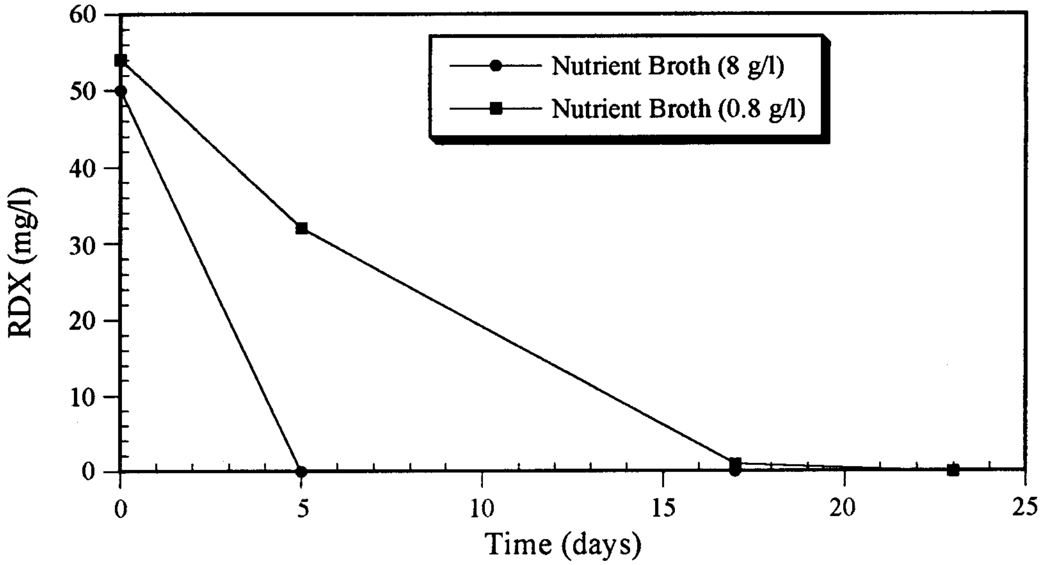


Figure 4.4 Anaerobic RDX degradation using fresh digester sludge as inoculum and nutrient broth as medium {BB-1}.

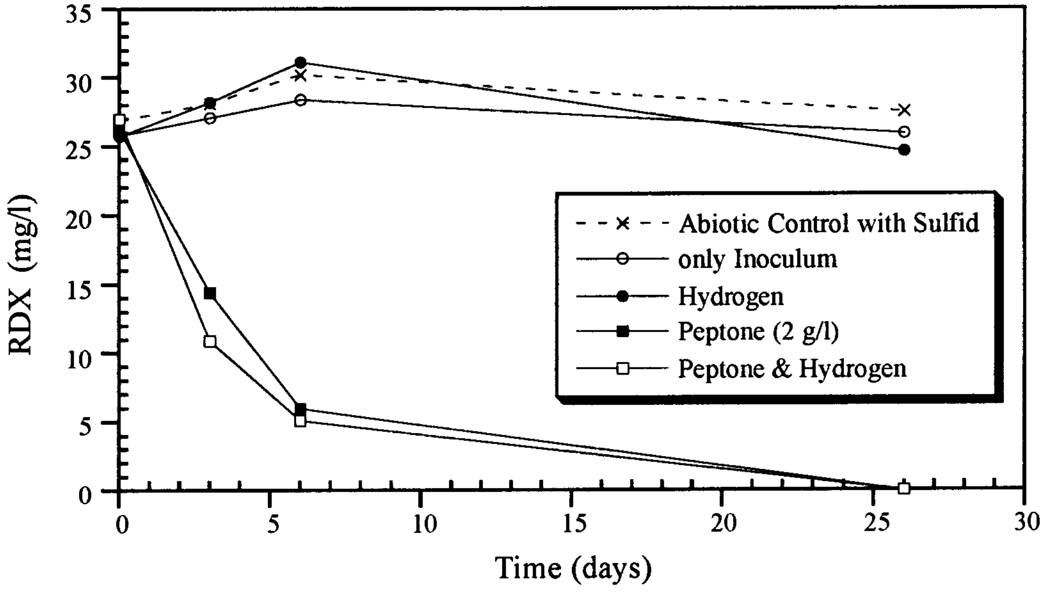


Figure 4.5 Anaerobic RDX degradation: abiotic, without co-substrate, with hydrogen, with peptone, or with hydrogen and peptone {BB-2}.

redox-potential as more peptone is metabolized. The medium preparation followed the standard procedure except that Na_2S was only added to one third of the individual culture vessels (labeled "*Nitrogen Flush + Sodium Sulfide*" in Figure 4.6). The medium for another third of the cultures (labeled "*Oxygen Added*") was filled into a 1000-mL beaker and allowed to equilibrate with normal air for 30 min.; occasionally, the beaker was shaken by hand. This procedure re-introduced oxygen into the medium. Then the incubation vessels were filled and stoppered with gas tight stoppers leaving a 15-mL headspace filled with air. On the 3rd and 4th day 250 mL air was flushed through the closed bottles using syringe and needles. The third part of the culture was started oxygen-free and without Na_2S (labeled "*Nitrogen Flush*"). The conditions were tested in two replicates; Figure 4.6 shows the averages and standard deviations.

The addition of 81.3 mg/L Na_2S increased the RDX degradation by 126% compared to the cultures only flushed with nitrogen gas (values after 7 days). The oxygen pool in the *"Oxygen Added"* bottles was depleted in less than 12 hours, as indicated by the reduction of resazurine to its colorless species. After the last air flush it took between 12 and 24 hours to decolorize the resazurine. The degradation rate (slope in Figure 4.6) increased in the *"Oxygen Added"* cultures between two and three days after the initial oxygen was depleted. The subsequent oxygen additions clearly decreased the degradation rate again. Nevertheless, the totally degraded amount still reached 80% of the *"Nitrogen Flush"* cultures at day seven.

Conditions in Experiments BB-1, BB-2, and BB-3 were similar in that all cultures were derived from the initial digester sludge inoculum using a peptone medium. One important result obtained while working with these cultures was that the RDX degradation rate did not increase during repeated transfers into flasks under the same conditions (data not shown).

Experiment BB-4: Efforts to achieve RDX degradation failed using glucose or methanol as sole co-substrate and the peptone grown cultures from BB-2 and BB-3 as inocula (data not shown). We decided, therefore, to test four different inocula in their ability to use various organic co-substrates. The inocula were incubated with peptones and sugars under fermentative conditions (BB-4), and with organic solvents under sulfate- and nitrate-reducing conditions (BB-5). Two of those inocula were fresh, i.e., they were not precultivated in peptone media (see Section 3.3). We hoped the fresh cultures would have greater physiological variability.

In experiment BB-4, the basal medium contained double phosphate-buffer concentration of the standard basal medium. The amount of organic growth substances added was calculated to produce the same total organic carbon (TOC) concentration. The calculation for the peptones and molasses was based on DOC determinations by McCormick et al. (1984b). All different combinations of the four inocula and six co-substrates were tested in two replications, except for the cotton seed-culture.

Figure 4.7 compares the six different co-substrates (results averaged over all inocula) and Figure 4.8 compares the four different inocula (results averaged over all co-substrates); the original data are presented in Table A.8 in Appendix A. Careful comparison of the original data and the calculated averages confirms that this undifferentiated averaging reproduces the general trends quite correctly. Significant differences in RDX degradation

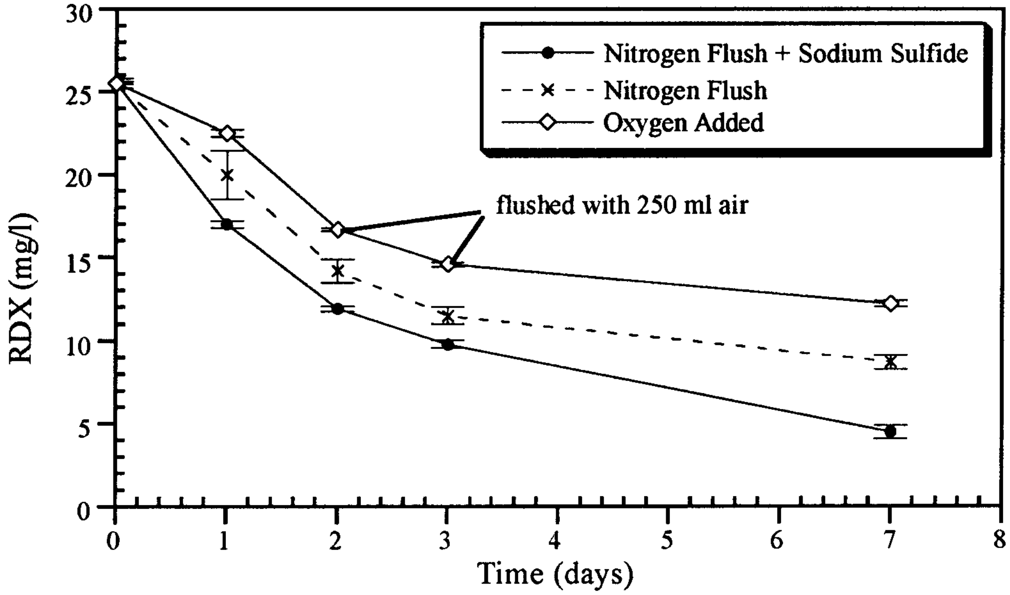


Figure 4.6 Influence of Na_2S and O_2 on anaerobic RDX degradation under fermentative conditions {BB-4}.

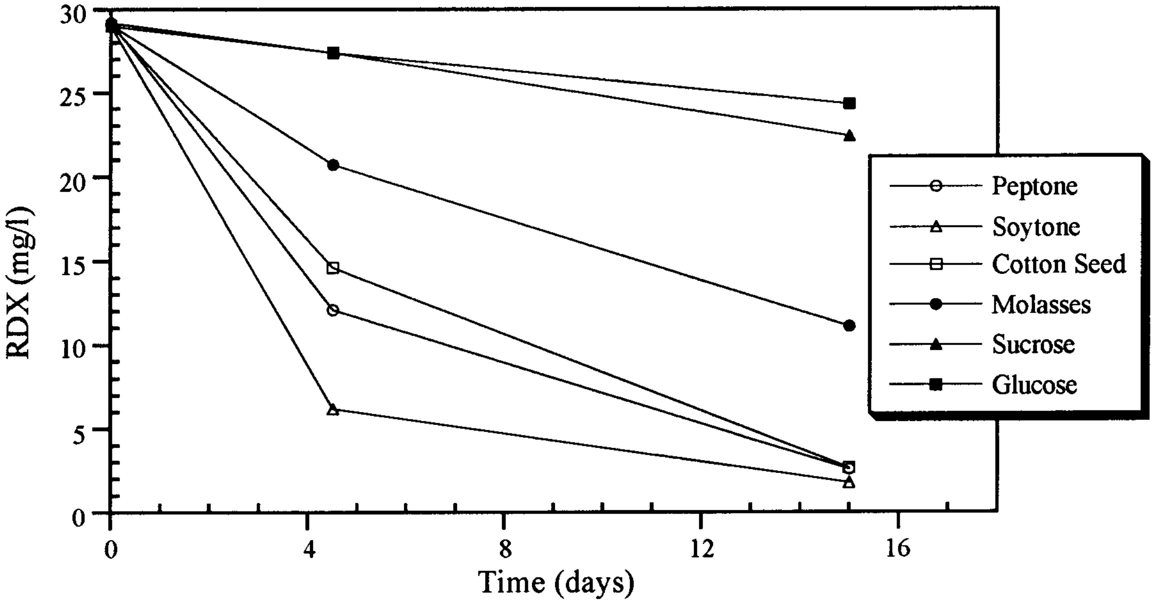


Figure 4.7 Biodegradation of RDX using sugars and peptones as co-substrates under fermentative conditions {BB-4}.

rates exist even between the peptones or protein mixtures; Soytone - a pancreatic digest of soybean - best supports RDX degradation (Figure 4.7). Second, pure sugars are poor co-substrates for RDX degradation; molasses - a complex mixture of different sugars, proteins and other substances - is a suitable co-substrate, but it is less efficient than peptones. Third, a clear difference between the inocula regarding RDX degradation could not be observed under this condition (Figure 4.8). The culture growth rate (Figure 4.9) does not correlate with the RDX degrading activity. Turbidity measurements for cotton seed as co-substrate was not determined because the substrate was not totally soluble.

The disappearance of RDX was accompanied by the appearance of two metabolite peaks in the chromatogram at 0.2 and 0.4 minutes before the RDX peak. These peaks were also observed in experiments BB-1, BB-2, and BB-3. Their origin from RDX was confirmed by incubating cultures with identical conditions but without RDX. In those controls no peaks were present near the retention times of the RDX, HMX or RDX-metabolite peaks (data not shown). A third metabolite peak could not be separated from the HMX peak, but its appearance and subsequent disappearance could be followed by spectra analysis of the mixed peak (data not shown). The observation of these intermediates peaks helped confirm that the disappearance of RDX was due to biological transformation or degradation and not only by adsorption or precipitation.

Experiment BB-5: The same inocula as in experiment BB-4 were incubated with six organic solvents as co-substrates to degrade RDX under sulfate-reducing conditions. Nitrate was applied as additional electron acceptor only in the case of methanol and ethanol. The

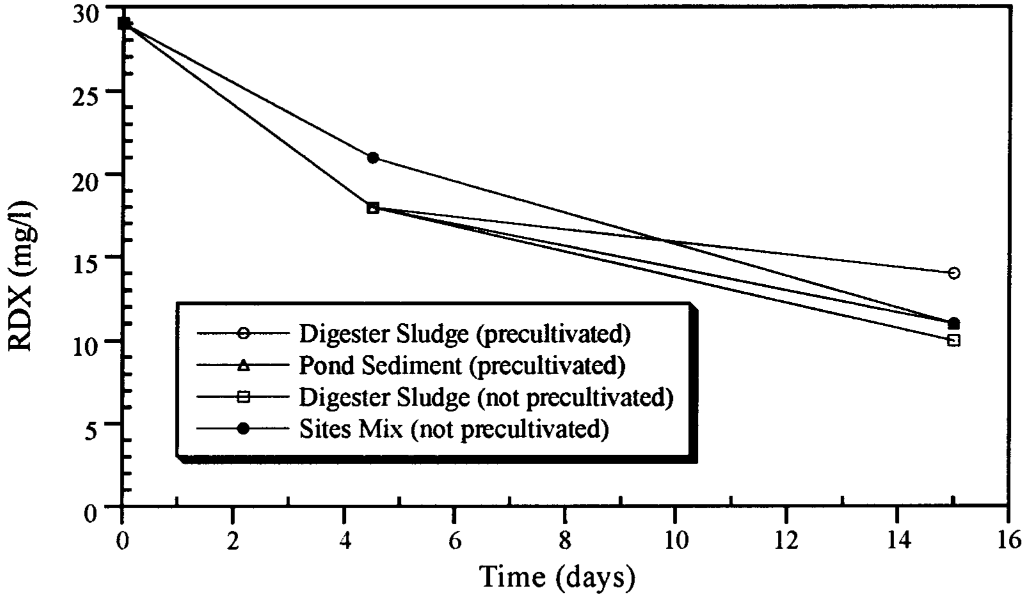


Figure 4.8 Biodegradation of RDX using different inocula under fermentative conditions {BB-4}.

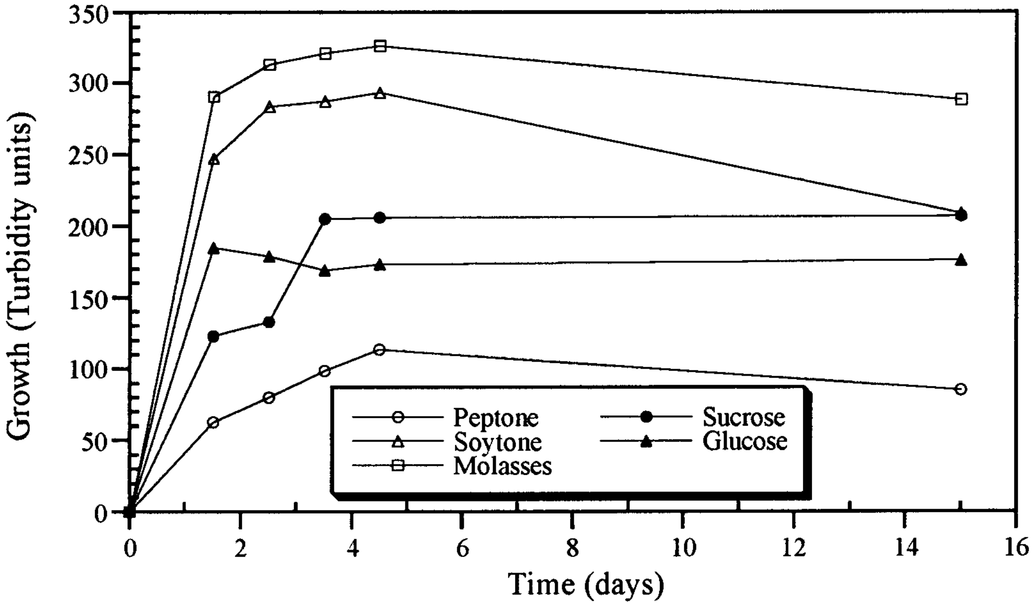


Figure 4.9 Anaerobic biomass growth using peptones and sugars as co-substrates {BB-4}.

corresponding solvents were already tested for their ability to desorb RDX from activated carbon in experiment DB-2 (Table A.4, Appendix A). The solvents supplied 50% of the total TOC, bacto peptone supplied the remaining 50%. The corresponding reference cultures were incubated containing only 1.6 g/L of bacto peptone. Every combination was tested in two replicates. Just as in BB-4, the results were averaged either over all inocula, or over all co-substrates.

A clear enhancement in RDX degradation rate could not be observed by comparing the RDX disappearance in the solvent containing cultures and the peptone reference cultures (Figure 4.10). Cultures with methanol as an additional co-substrate transformed RDX more slowly than other cultures and less than the reference culture. The pond sediment culture, which had been precultured longer in peptone than the other cultures, was clearly less effective in transforming RDX (Figure 4.11).

Performance differences become somewhat clearer by including the amount of accumulated intermediates into the results consideration. This was done by summing the peak areas with retention times between 3.9 and 4.8 min.; those include RDX (4.7 min.), HMX (4.1 min.), and two or three intermediates peaks which arose from RDX or HMX transformation (Table 4.2). The sumations show an advantage for ethanol over the other co-substrates, and advantages for inocula that were not precultivated (*"Digester Sludge"* and *"Sites Mix"*) against the precultivated inocula. Methanol appears as the second best co-substrate even though it showed the least RDX transformation; it had the second lowest intermediate accumulation. Generally, the denitrifying cultures accumulated fewer RDX intermediates at lower concentrations.

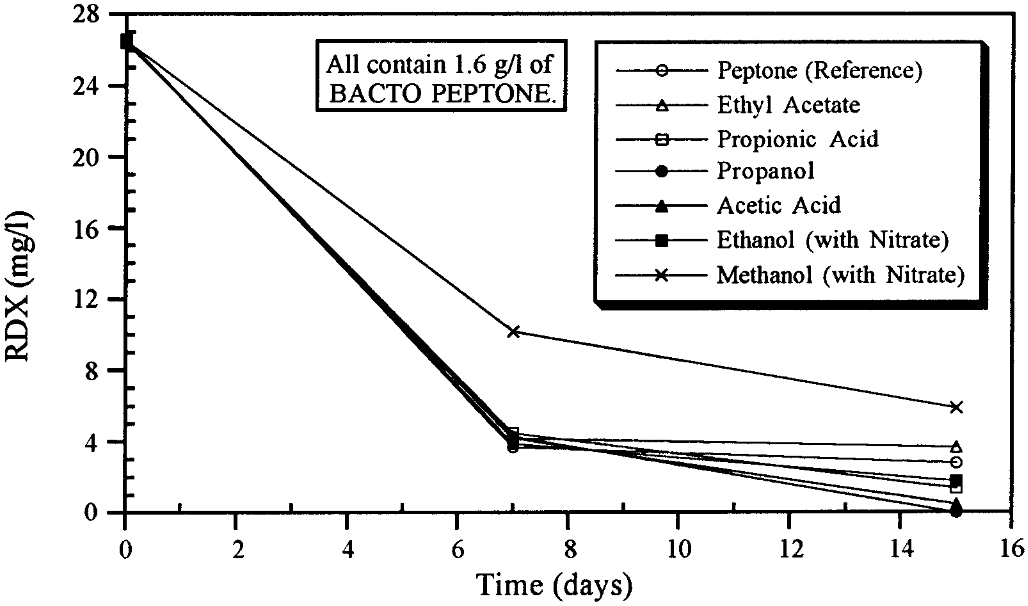


Figure 4.10 Anoxic RDX degradation using various organic solvents as co-substrates {BB-5}.

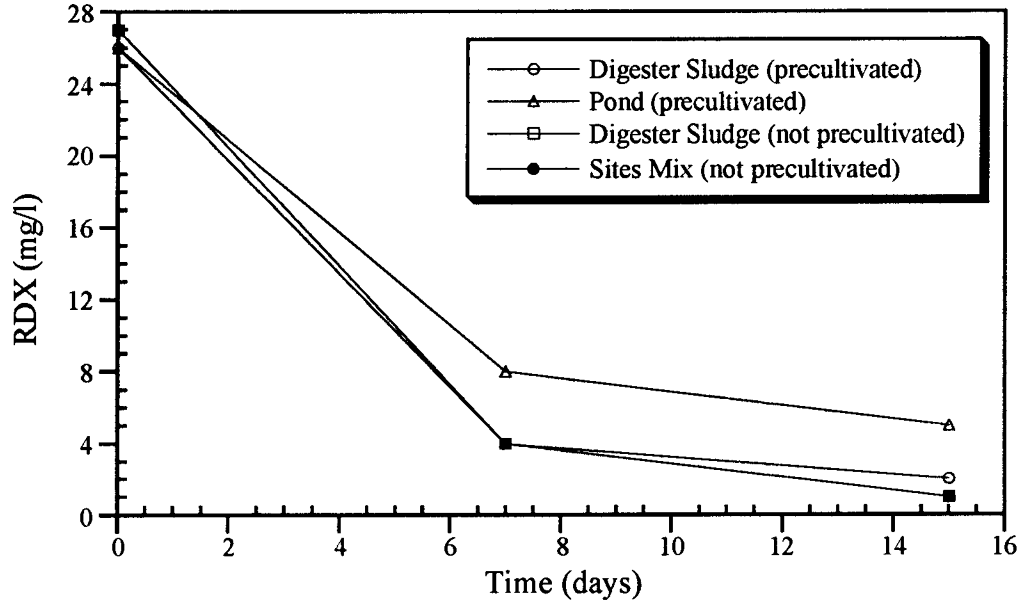


Figure 4.11 Anoxic RDX degradation using four different inocula {BB-5}.

Table 4.2: Sum of RDX-, HMX-, and intermediates' peak areas {BB-5}.

	Σ Peak Area (RDX, HMX, Intermediates)*1								
Inoculum*2	R		F		D		S		Co-substrate
Replication	R1	R2	F1	F2	D1	D2	S1	S2	Average
Peptone (Ref.)	143	116	189	124	78	90	168	251	145
Ethyl Acetate	229	260	186	207	124	140	165	167	185
Propionic Acid	250	293	255	180	105	80	132	85	173
Propanol	209	176	179	126	77	87	95	105	132
Acetic Acid	141	136	234	171	114	141	102	124	146
Ethanol	146	92	122	81	65	45	42		85
Methanol	78	63	421	91	82	48	68		121
Inoculum Avg	167		183		91.1		125		

*1 Peaks with retention times between 3.9 and 4.7 min. at the 15th day; *2 R = precultivated Digester Sludge; F = precultivated Pond Sediment, D = Digester Sludge, S = Sites Mix.

The cultures containing ethyl acetate and sodium propionate grew at lower rates than the reference culture (Figure 4.12). This indicates a toxic effect of the solvents. As expected, biomass production was greater under denitrifying conditions than in sulfate-reducing conditions. A correlation between growth rate and RDX degradation does not exist, which is similar to the results for sugars and peptones {BB-4}.

Experiment BB-6: This experiment was designed to confirm the trends observed in BB-5. Instead of bacto peptone, the less complex casamino acids were used as a second co-substrate with concentrations adjusted to provide 1/3 of the total TOC concentration. The best "Digester Sludge" and "Sites Mix" cultures from BB-5 were used as inocula. Propionic acid, propanol, and ethanol were tested with the *"Digester Sludge"* and a *"Sites Mix"* inocula. Acetic acid and methanol were tested with the *"Sites Mix"* inoculum only. Ethyl acetate was not studied in this experiment. As in BB-4, reference cultures were incubated containing the casamino acids but no solvent. All cultures contained sulfate as electron acceptor; acetic acid, ethanol, and methanol cultures were also tested with nitrate as additional electron acceptor. Every combination was tested in two replicates; results are presented as averages.

No difference between *"Digester Sludge"* and *"Sites Mix"* inocula could be observed. The results for propionic acid, propanol, and ethanol are presented as averages of both inocula (Figure 4.13). Most cultures transformed RDX similarly; however, there were important differences. Ethanol and propanol addition increased the rate of RDX transformation under sulfate-reducing conditions. Adding nitrate with the sulfate decreased the rate of RDX transformation when ethanol was used as the co-solvent. The other solvents - propionic acid, acetic acid, and methanol - were not effective in increasing the rate of RDX degradation. Methanol and propionate actually decreased the RDX degradation compared to their corresponding reference cultures without the solvents.

Experiment BB-7: In this experiment we examined how nitrate and sulfate influence RDX transformation with propanol and ethanol as primary co-substrates. The amount of casamino acids as second co-substrate was chosen as in experiment BB-6. The best *"Digester"* and *"Sites"* cultures from BB-6 (already precultivated with ethanol and propanol) were used as inocula. Every culture was tested with and without sulfate or nitrate, in two replicates.

The addition of sulfate and nitrate had positive effects on RDX transformation in all

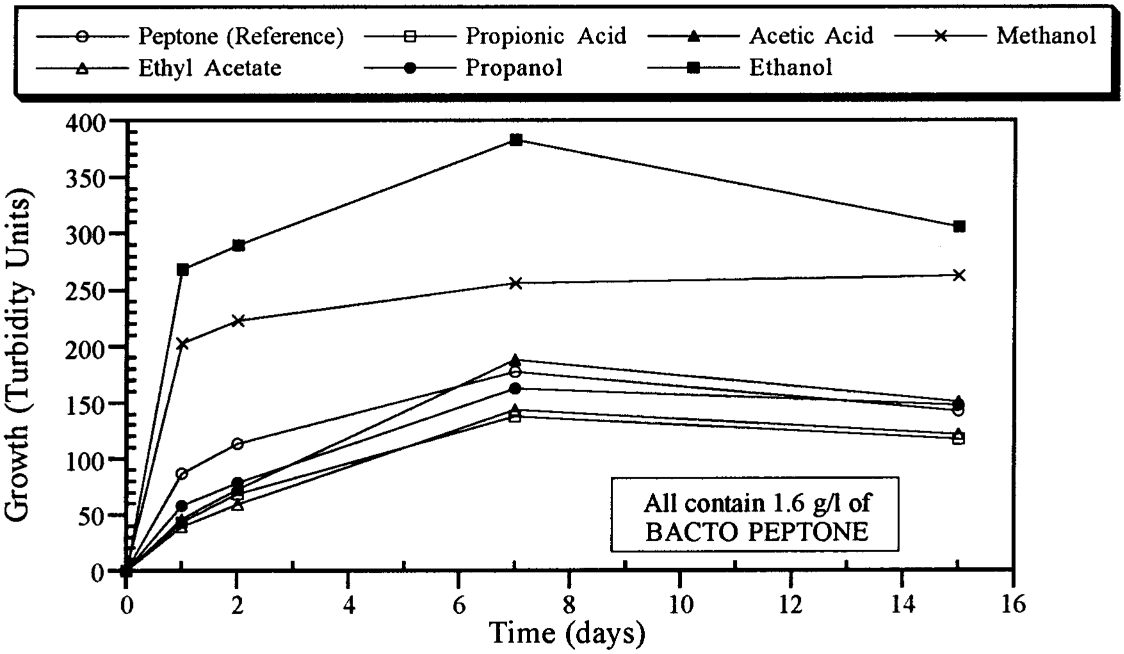


Figure 4.12 Anoxic growth using various organic solvents as co-substrate {BB-5}.

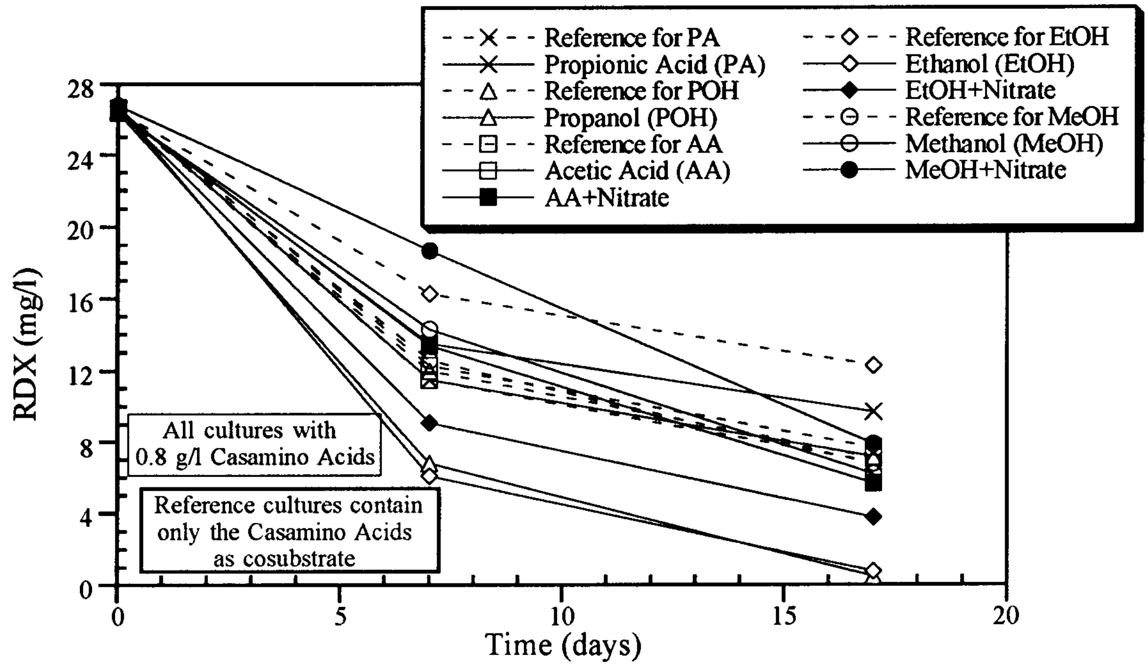


Figure 4.13 Anoxic RDX degradation using various solvents as co-substrates {BB-6}.

instances (Table 4.3). The ethanol culture with and without nitrate showed the greatest rates of best transformation; the nitrate-containing culture showed the highest growth rate. The addition of both electron acceptors clearly increased the biomass growth; nitrate showed greater increase than sulfate (Figure 4.15).

Experiment BB-8: The inoculum was derived from the denitrifying, ethanol-culture in BB-7. It was cultivated for three weeks prior to the beginning of this experiment in tap water containing only nitrate, ethanol and phosphate-buffer. This culture was tested to determine its dependency on ethanol and nitrate concentration. The possibility of substituting sulfate

Table 4.3: Beneficial effect of nitrate and sulfate on RDX transformation using ethanol and propanol as the primary co-substrate.

Culture*[1]	RDX (mg/L)	
	starting value	value after 7 days
D-Propanol-1*[2]	33.5	15.8
D-Propanol-2	34.1	16.6
D-Propanol + SO_4-1	33.2	10.2
D-Propanol + SO_4-2	32.5	7.1
S-Propanol-1	32.9	14.7
S-Propanol-2	33.6	15.7
S-Propanol + SO_4-1	31.6	4.1
S-Propanol + SO_4-2	32.5	5.2
S-Ethanol-1	32.4	6.5
S-Ethanol-2	32.8	3.2
S-Ethanol + NO_3-1	33.0	1.4
S-Ethanol + NO_3-2	33.2	0.2

*[1] all with 0.8 g/L Casamino Acids, *[2] D- = Digester Inoculum, S- = Sites Inoculum, -1 and -2 = replicate number.

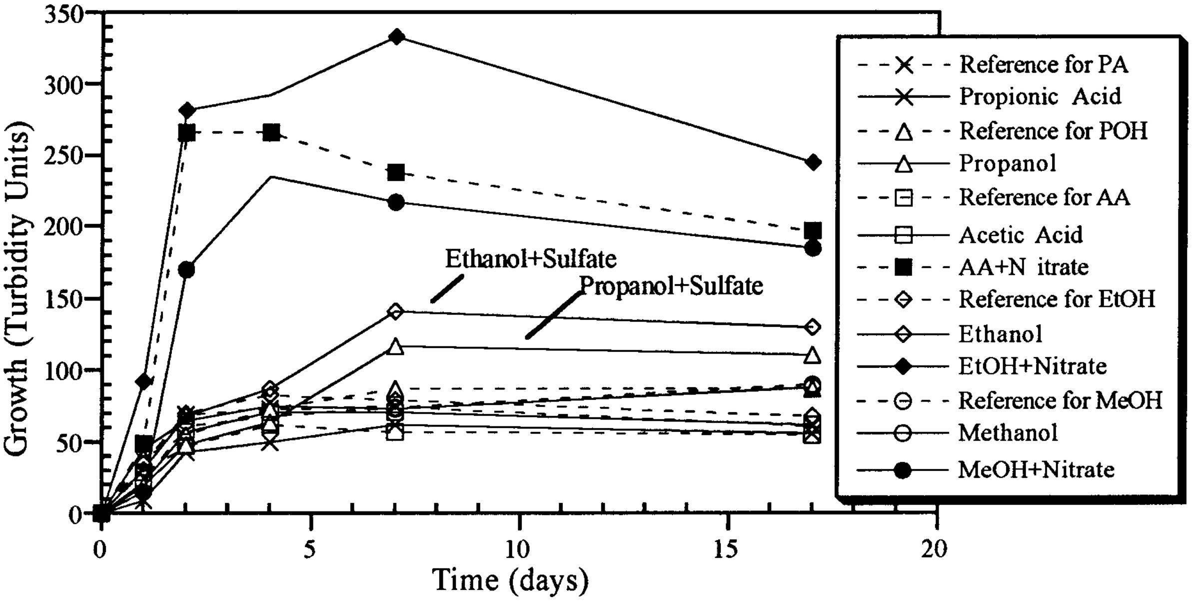


Figure 4.14 Anoxic growth using various solvents as co-substrate {BB-6}.

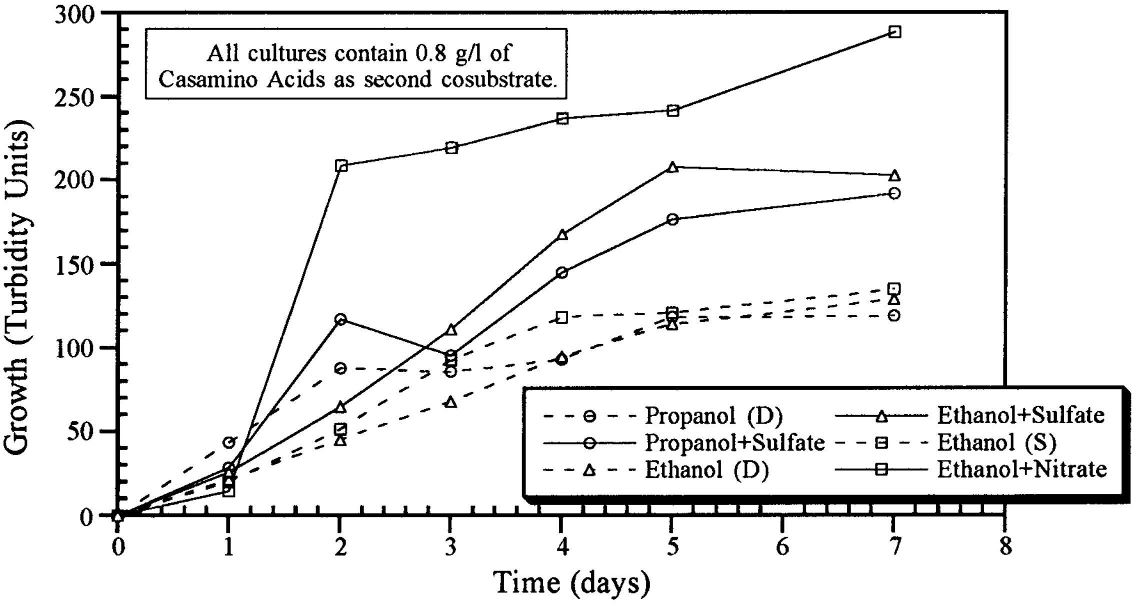


Figure 4.15 Anaerobic growth with and without sulfate or nitrate {BB-7}.

or sulfite for sulfide as the sulfur-source was also studied. In cultures containing ethanol as the co-substrate, 2.4 g/L ethanol was used with 1.6 g/L KNO_3 concentration. All cultures contained sulfide as sulfur source. The cultures with sulfur source variations contained 1.6 g/L KNO_3 and 2.4 g/L ethanol. The results are presented as averages of three replicates; the error bars in Figures 4.18, 4.19, 4.20, and 4.22 represent the corresponding standard deviations.

Variations of the ethanol concentration between 1.2 and 9.6 g/L did not effect the RDX transformation or the biomass growth rate (Figures 4.16 and 4.17). Without ethanol no reduction of RDX occurred, and biomass growth stayed under 40 turbidity units.

The RDX transformation and culture growth were positively correlated with increasing nitrate concentration (Figures 4.18 and 4.19). In case of RDX transformation the correlation can be approximated with a linear function, whereas the growth dependency is linear at lower nitrate concentrations but saturates at higher concentrations (Figure 4.20). The maximum turbidity was obtained after approximately 24 hours; the biomass decay was greater at higher biomass concentrations, as expected. Normalization of RDX transformation with the biomass concentration (measured as turbidity) shows that the RDX transformation rate increased more than 100% when the nitrate concentration was increased from 3.2 g/L to 6.4 g/L (Figure 4.21). The cultures with 6.4 g/L of nitrate were the first to show the total disappearance of HMX. Samples from the cultures with 6.4 g/L nitrate, which were extracted with methanol to test for adsorption losses, did not show any more RDX or HMX than samples without this methanol extraction.

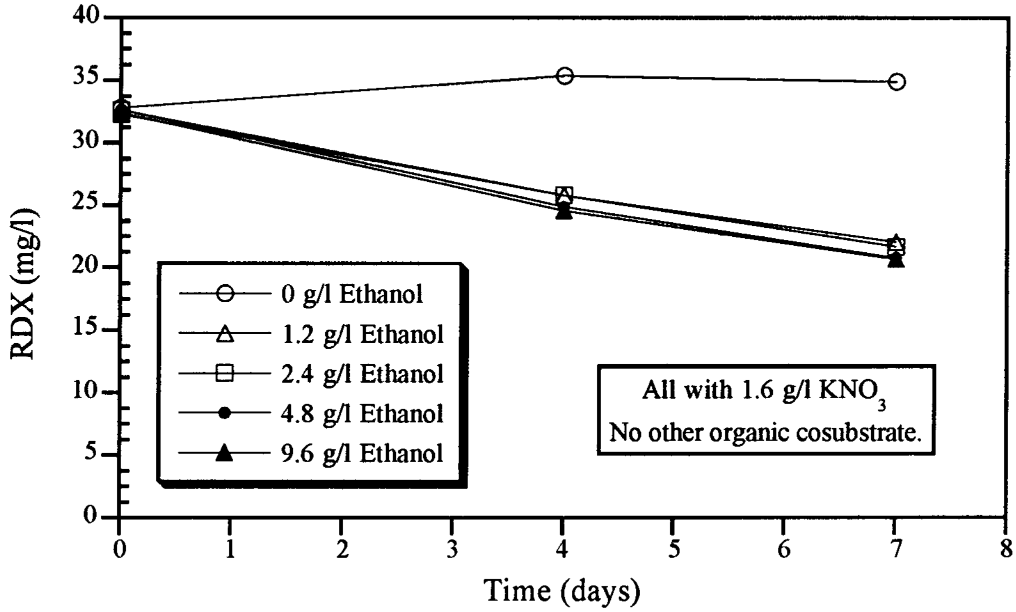


Figure 4.16 Dependency of denitrifying RDX degradation on ethanol concentration {BB-8}.

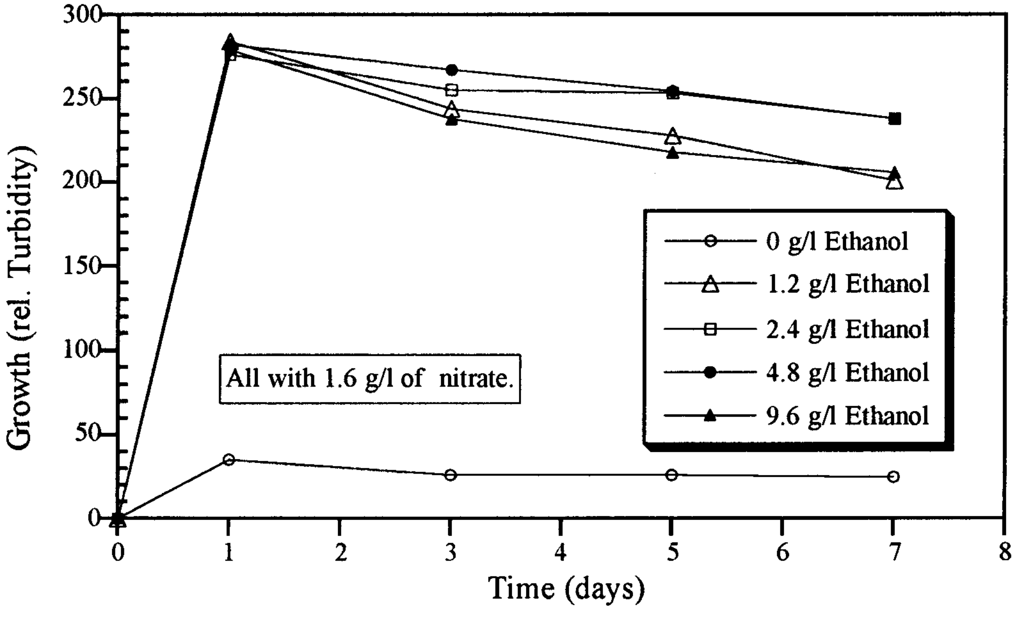


Figure 4.17 Dependency of denitrifying growth on ethanol concentration {BB-8}.

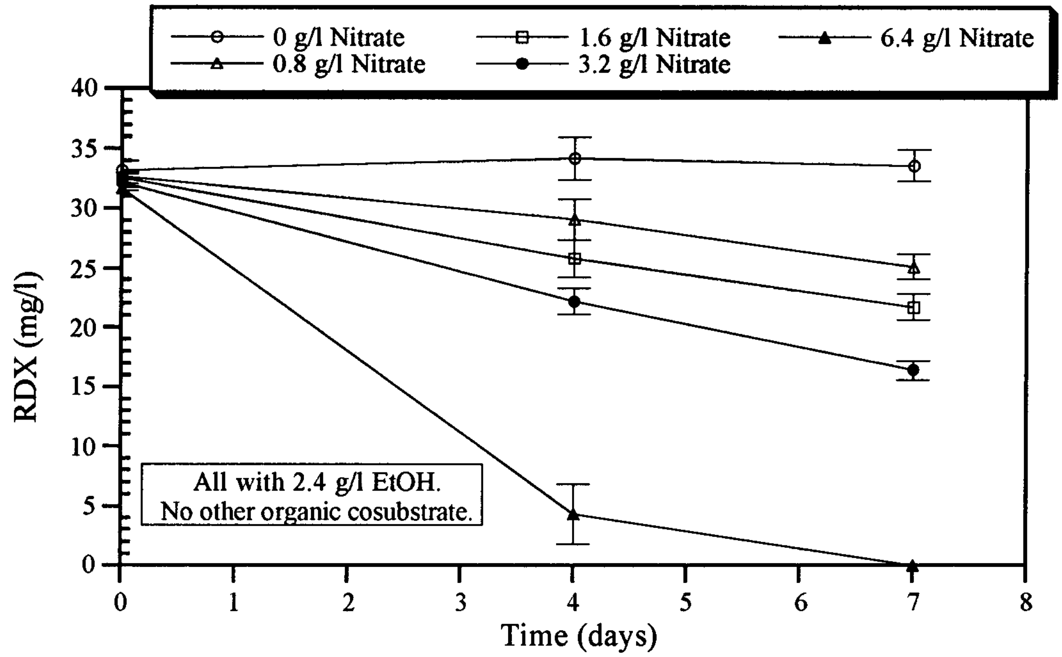


Figure 4.18 Dependency of denitrifying RDX degradation on nitrate concentration {BB-8}.

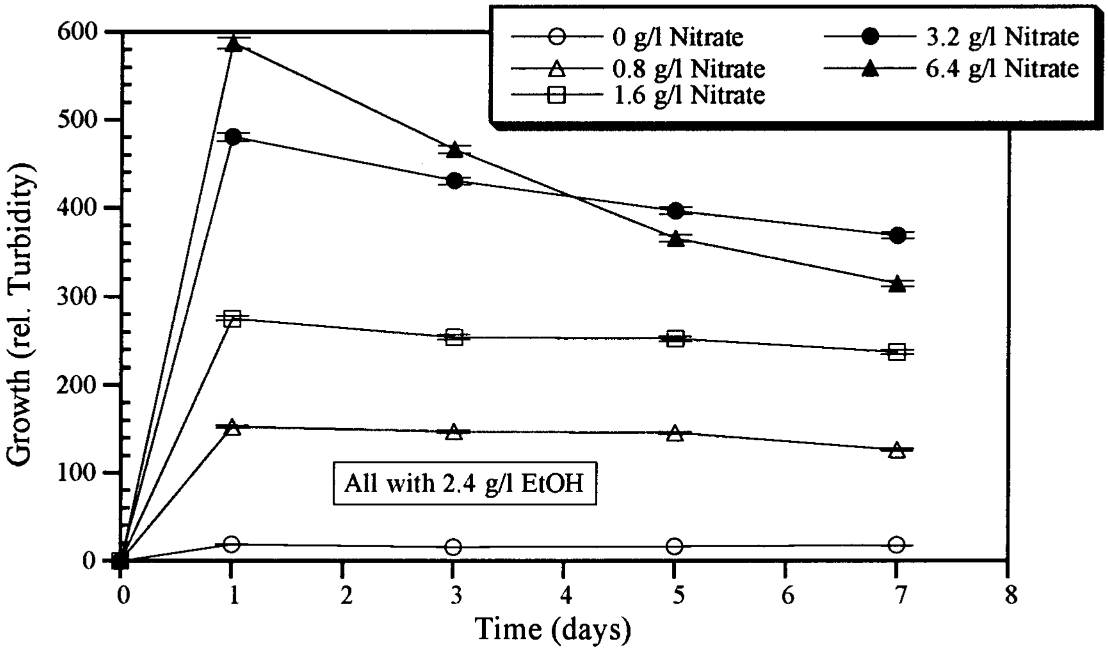


Figure 4.19 Dependency of denitrifying growth on nitrate concentration {BB-8}.

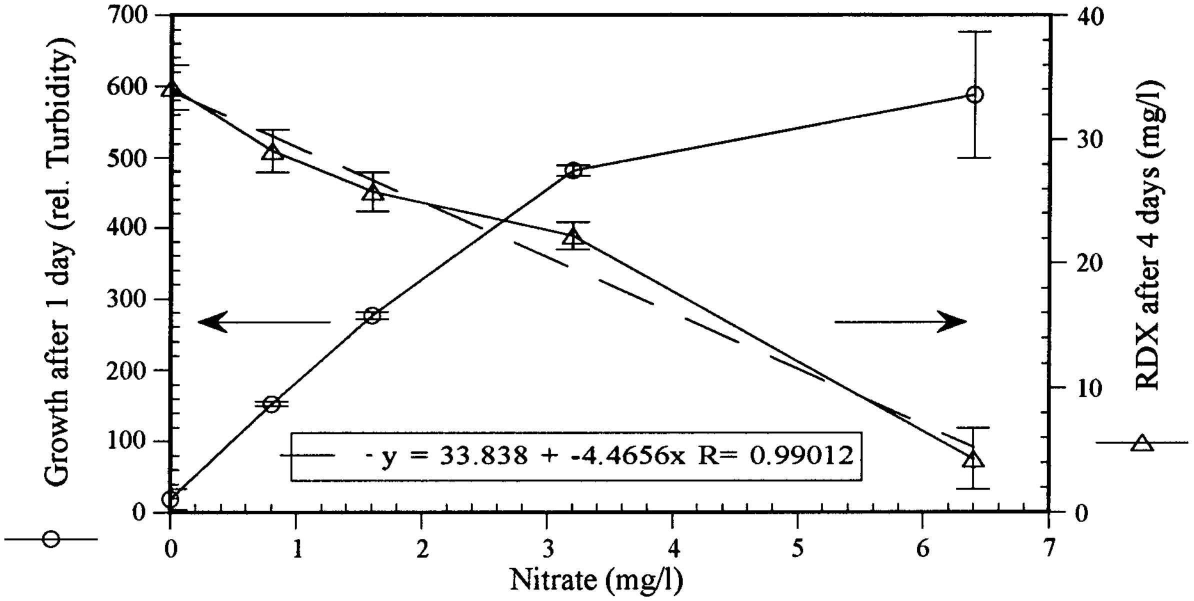
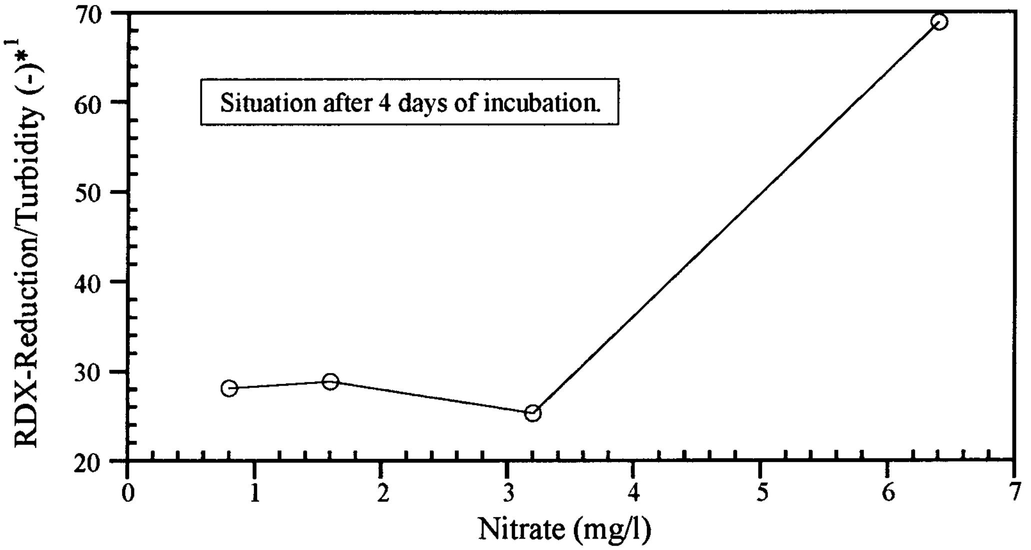


Figure 4.20 RDX degradation and growth versus nitrate concentration {BB-8}.



*[1] RDX-Reduction/Turbidity = [reduced RDX-concn. (mg/l)*1000 (l/mg)]/Turbidity units (-)*[2]
*2 Turbidity at 4th day calculated as average from 3rd and 5th day.

Figure 4.21 Dependency of RDX degradation on nitrate concentration after normalization with biomass {BB-8}.

RDX transformation rate was similar when using sulfite and sulfate as sulfur source and approximately two times better than using sulfide (70% reduction versus 35%) (Figure 4.22). Growth was similar for all three sulfur salts; at the conclusion of the experiment, the turbidity was slightly higher in sulfate containing cultures (Figure 4.23).

Experiment BB-9: This experiment evaluated the ability of the denitrifying culture from experiment BB-8 to use other organic solvents than ethanol. It was feared that the culture may have lost its ability to transform RDX with other co-substrates due to its long exposure to ethanol. Transformation results are presented as the average and standard deviation of RDX reduction in three replicates, after 7 days of incubation. Growth results are presented as averages.

Acetic acid was as good for RDX degradation as ethanol, whereas the culture containing propionic acid showed approximately 50% of the transformation rate of the ethanol reference culture (Figure 4.24). These three substrates best supported bacteria growth (Figure 4.25). The RDX reduction of 4 mg/L in cultures containing formic acid was not accompanied by the anticipated cell growth. The transformation rate of the ethyl acetate cultures did correlate with growth, at the end of the incubation period. Methanol and acetone could neither be used as co-substrate for RDX degradation nor as growth substrate.

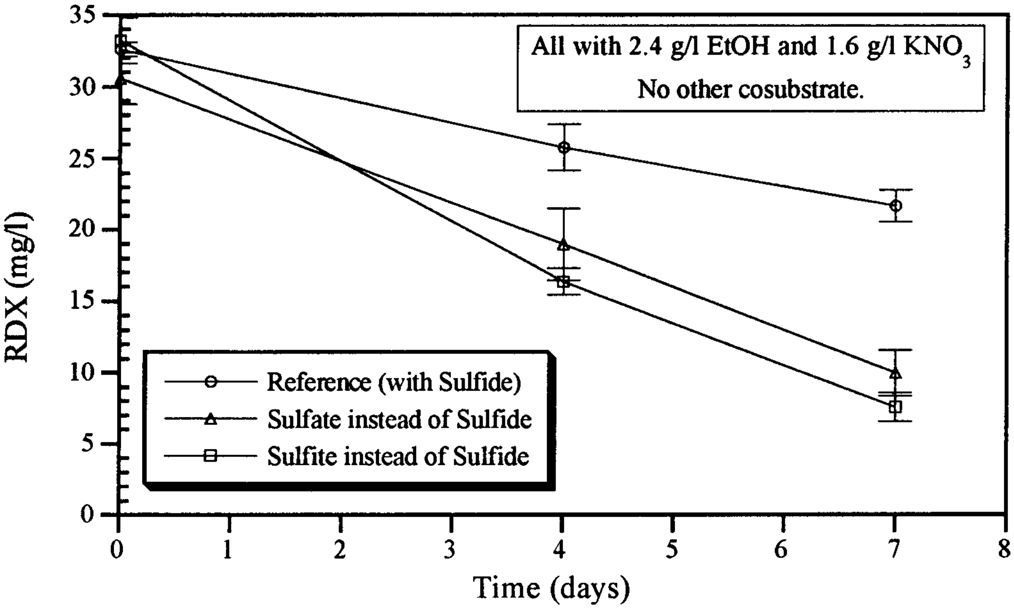


Figure 4.22 Dependency of denitrifying RDX degradation on sulfur-source {BB-8}.

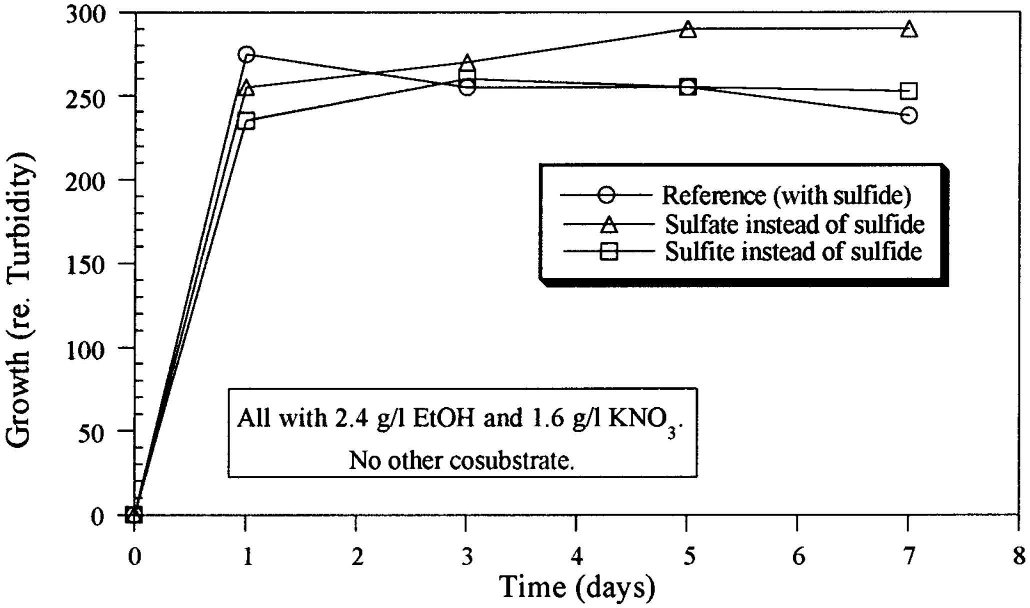


Figure 4.23 Dependency of denitrifying growth on sulfur-source {BB-8}.

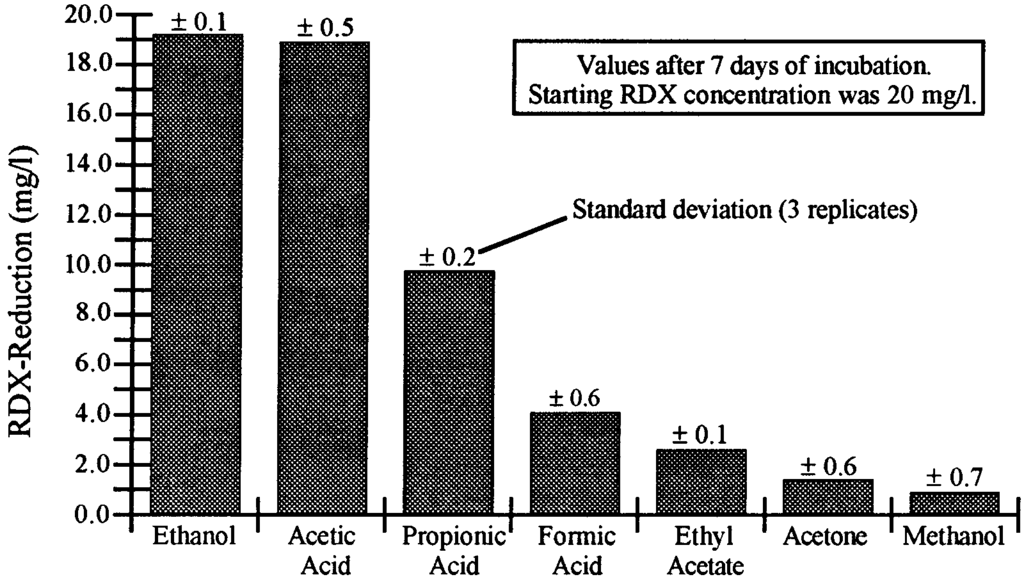


Figure 4.24 Denitrifying RDX degradation using various solvents as sole co-substrate {BB-9}.

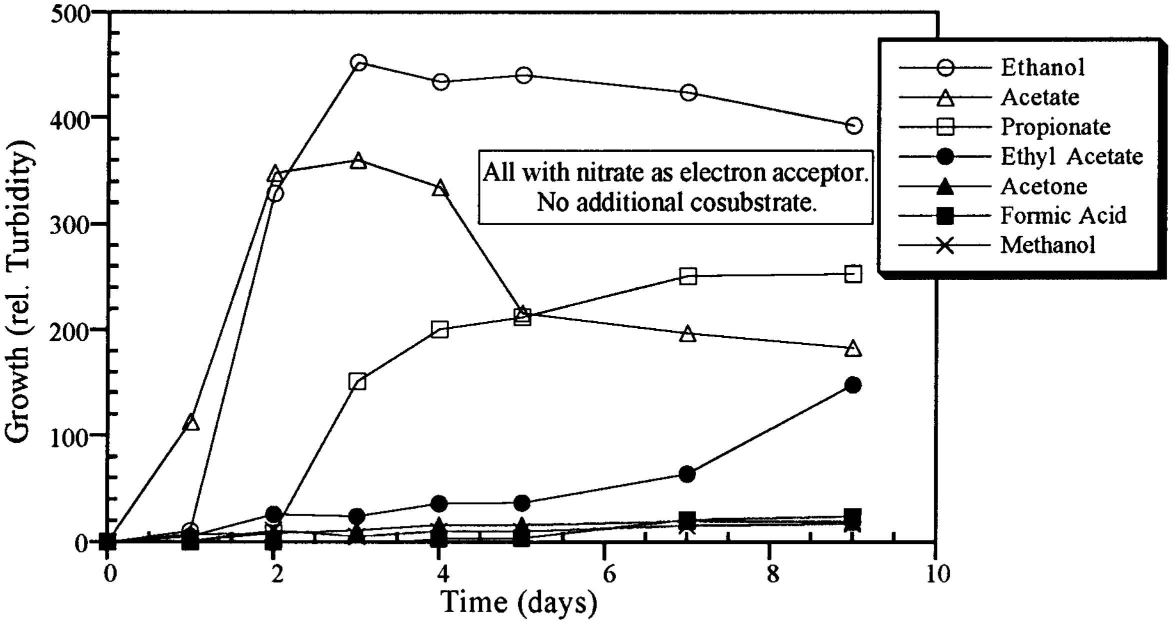


Figure 4.25 Denitrifying growth using various solvents as sole co-substrate {BB-9}.

5.0 DISCUSSION

5.1 Adsorption and Desorption of RDX

Continuous adsorption: The primary purpose of the continuous adsorption experiment AC-1 was to create RDX-laden carbon; however, it also became clear that the experimental method should be improved, because the consistency of the influent concentration was unsatisfactory. The varying concentrations were most probably caused by the difficulties in achieving reproducible RDX-solvation in the 20-L glass containers used to prepare the influent. To improve this situation, larger batch volumes and longer stirring intervals should be used in the future.

The flat shape of the breakthrough curve was most likely caused by the short empty-bed-contact-time (EBCT) of 1.76 min. The EBCT was chosen so short in order to obtain breakthrough more quickly and with less RDX. Nevertheless, the amount of required RDX was still too high, considering the problems of supplying RDX (our laboratory was limited to 1 g shipments approximately one every two weeks). The consumption of RDX could be reduced by using only 4 g or less of activated carbon in small chromatographic glass columns (I.D. 10 mm, Rainin). These columns are also temperature, pressure, and solvent resistant.

Continuous desorption: As expected, the regeneration of the activated carbon by desorption of RDX using water at room temperature needed more water than treated in the adsorption step. This is problematic for the proposed treatment concept, even though, the regenerating throughput might be supplied from a relatively small volume of recirculating process water. Using a Freundlich-isotherm-model, the dilution ratio between ad- and desorption is solely determined by the recovery ratio and the parameter "n" from the Freundlich equation:

$$\frac{V_d}{V_a} = \left[1 - \frac{Q_d}{Q_a}\right]^{-n}$$

where:

$\frac{V_d}{V_a}$ = dilution ratio;

V_d = volume of water to desorb Q_d;

V_a = volume of water to adsorb Q_a;

$\frac{Q_d}{Q_a}$ = recovery ratio;

Q_d = desorbed amount of RDX per mass of activated carbon;

Q_a = adsorbed amount of RDX per mass of activated carbon, and

n = reciprocal exponent of the equilibrium concentration in the Freundlich equation.

To derive this relationship, the following assumptions were made:

1) The same solvent and the same temperature are used for adsorption and desorption.
2) An identical Freundlich isotherm validly describes adsorption <u>and</u> desorption.
3) RDX is homogeneous distributed at the carbon surface.
4) Compared are equilibrium situations.
5) $Q_a = \frac{V_a \cdot C_e}{\text{total mass of activated carbon}}$; C_e = RDX concentration in the water volume V_a.to be used as equilibrium concentration in the Freundlich equation.

Applying these assumptions, the dilution ratio is independent of the Freundlich parameter K and the concentration C_e; increasing values of n raise the dilution ratio exponentially. It should be noted that K and n are influenced by the temperature, solvent, and the carbon type.

It is crucial for process performance to increase the desorption rates. In addition to the possibilities of increasing desorption rates with increased temperature or organic solvents, different types of activated carbons could be compared. The influence of the Freundlich parameter n was derived above. The influence of the carbon pore structure was already discussed in Section 2.2. Inorganic salts and bacterial excretions in the recirculated water could also influence the desorption behavior. For example, high concentrations of nitrate ions could be advantageous, because RDX is very soluble in concentrated nitric acid (Urbanski, 1964). Furthermore, it should be noted that the carbon in experiment AC-1 lightly loaded when the experiment was terminated. In a full scale application the carbon columns would be mostly likely operated to higher loads. The higher loads will create higher RDX effluent concentrations at the beginning of the regeneration.

These limited range of conditions and objectives in our desorption experiments (continuous and batch type) limits the applicability of the results. Experiments were performed for one specific RDX-loading, one specific carbon type, and one specific solvent composition. We know of no model or procedure to evaluate and extend the results of our desorption experiments. Therefore the quantitative influence of the factors mentioned above cannot be theoretically predicted. Nevertheless, we believe our preliminary results are useful as a first approximation of the impact of temperature and solvents on RDX desorption.

Temperature effect on desorption: RDX saturation concentrations in water are three orders of magnitude higher than the desorbed concentrations in the Water-RDX-Carbon system (Figure 4.2). Nevertheless, a comparison of the curve shapes (legitimate because of equal y-axis subdivision) suggests a similar temperature dependency in the saturation and desorbed RDX concentrations. The discrepancy in results at higher temperatures might be due to the increased laboratory temperature, which may have other effects in addition to increasing RDX-solubility.

The data suggest that desorption at higher temperatures than tested may produce even better desorption rates. Extrapolation of the data results suggests desorbed RDX concentrations in the range of 8.2 - 13.2 mg/L. The upper and lower estimates are calculated with the empirical e-functions for the data after 60 and 45 min., respectively. Hydrolysis at higher temperatures will get important and reduce liquid phase concentration. This may further increase RDX desorption rates.

We assume that the desorption into an organic solvent will also increase exponentially with the temperature. As in the case of water (Figure 4.2), an almost proportional relation should exist between RDX solubility and RDX desorption. If so, the possible enhancement could be roughly predicted by the temperature-solubility functions (Figure 5.1).

Organic solvents as desorbing fluids: The strong enhancement in RDX desorption rates using polar organic solvents suggests that RDX adsorption is caused by its hydrophobicity than by its affinity to activated carbon (Hering, 1991). The observed differences between the solvents in RDX desorption are much smaller than the differences in RDX-solubility (Table A.5, Appendix A). This may be because the solvent-phase concentrations were much less than the saturation concentrations (Table A.6, Appendix A). Desorptoin at higher carbon loads may be impacted by the different RDX solubilities of the solvents. The quantitative influence of this factor should be determined experimentally in future research.

In the following paragraphs, we discuss the utility of an adding a solvent distillation step in the proposed process process (Figure 2.1). Although the results are discussed only for ethanol, the principal conclusions should be the same for the other solvents.

The maximum value of the RDX concentration in the ethanol regeneration fluid would be the solubility limit. This limit will be approximately 10 g/L in ethanol at 80°C (boiling

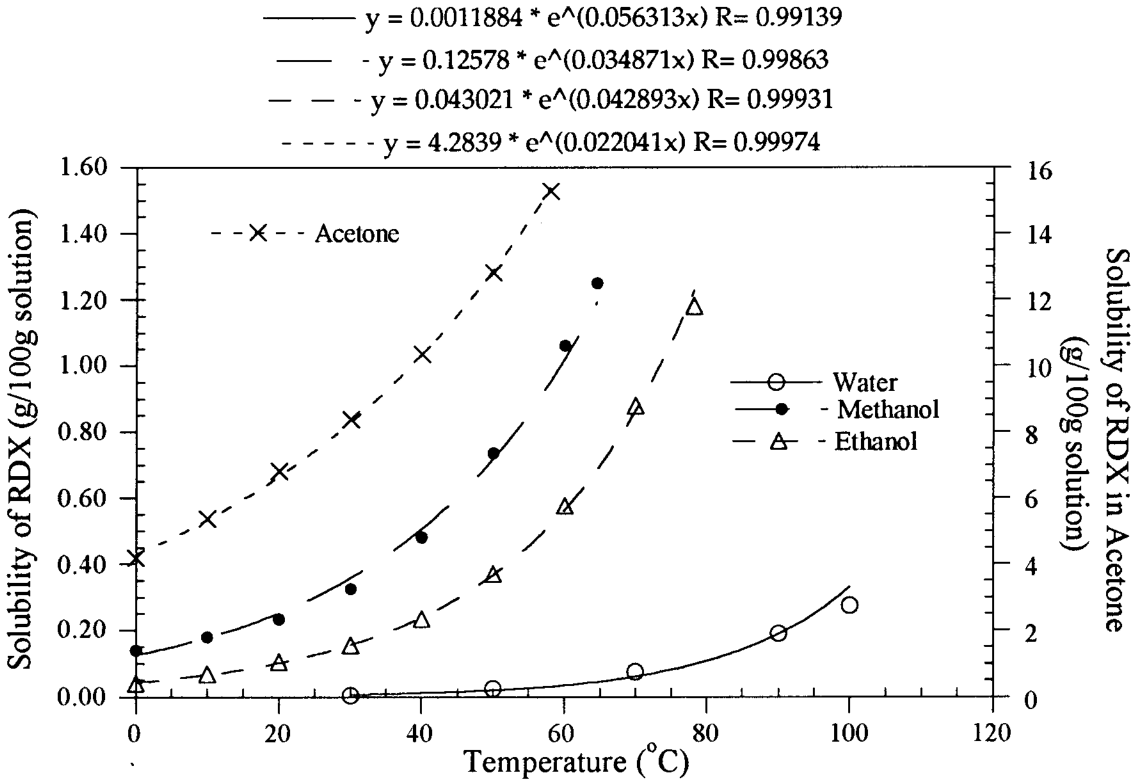


Figure 5.1 Solubility-temperature functions for RDX-solvents (literature data: Urbanski, 1964 and 1983).

point) (cf. Figure 5.1). If we assume that the ethanol concentration in the bioreactor influent is 2.4 g/L (3.0 mL/L, which was the maximum value used in our biological transformation experiments), the RDX concentration in the reactor influent should be approximately 30 mg/L. The water itself will contain less than 10 mg/L after passing through the carbon column. These RDX concentrations should be favorable for anaerobic transformation. If a more pessimistic assumption is made for the maximum attainable RDX concentration in ethanol regenerating fluid, such as 1.5 g/L, distilling the RDX-laden solvent in order to concentrate it up to its solubility limit at 80°C might be advisable. Fortunately, the separation of RDX and ethanol is not difficult because both have widely separated boiling points. The concentrate would be treated by the bioreactor, and the distillate could be reused to desorb more RDX.

5.2 Anaerobic Biodegradation of RDX

Fortuitous cometabolism: Our experiments BB-1, BB-4, and BB-5 confirmed McCormick's (1981) results that ordinary anaerobic bacteria populations are able to transform RDX without an adaptation phase. This ability is obviously coupled to constitutive enzyme systems. Moreover, this enzymes appear to be widely distributed in natural anaerobic environments. Such a phenomenon is commonly described as a "fortuitous metabolism" (Knackmuss, 1981; Janke, 1985). The strict dependency on an organic co-substrate to perform RDX transformation (as confirmed in experiments BB-2 and BB-8) is a strong suggestion for a cometabolic action. {Note: The terms cometabolism and co-substrate are used here as defined by Dalton (1982).} The anaerobic RDX transformation might therefore be classified as a "fortuitous cometabolism".

A logical consequence of such metabolism is that no selection pressure exists to increase the efficiency of RDX degradation. Therefore, we should not observe accelerated degradation when transferring cultures several times because there is no selection pressure to increase transformation. This was observed in our results. Cultivating peptone-growing and RDX-degrading cultures for several months did not increase RDX transformation rates. In all culture transfers the transformation rate remained relatively constant. The only way to increase RDX transformation rate is to change the environmental conditions, i.e., co-substrate type and concentration, electron acceptor, pH, temperature, and perhaps other medium factors.

Redox-conditions: We were able to demonstrate that RDX degradation occurs under fermentative, sulfate-reducing, and denitrifying redox-conditions. The reduction in transformation rates after the introduction of oxygen into the cultures shows the inhibitory effect of oxygen. The inhibition of the introduced oxygen in experiment BB-3 was not permanent, which suggests that it is not highly toxic to RDX-degrading bacteria. A pure culture of methanogenic bacteria, for example, would have been killed or at least totally inhibited for several days. The rapid depletion of oxygen demonstrates that there are facultative anaerobes in the cultures. The positive effect of sulfide addition under fermentative conditions is probably due to a rapidly decreased redox-potential, which supports the activity of reducing enzymes. When sulfide is missing, the bacteria have to lower the redox-potential by themselves. An inhibitory effect of nitrate or sulfate on the reductive degradation could not be observed. The requirement for a low Redox-Potential to perform the reductive reactions is not very strict under these conditions. The use of nitrate as electron acceptor has important results and is discussed later.

Organic co-substrates: McCormick's hypothesis (1984b) that RDX degradation is correlated to the concentration of organic carbon (TOC) in the growth medium should be repostulated more specifically. For example, the TOC values in experiment BB-4 were the same for all sugars and peptone which were tested as co-substrates, but the rates of RDX transformation were dramatically different. The type of co-substrate is as important in defining the RDX transformation rate is as important as the TOC concentration. Therefore, McCormick's hypothesis is restricted to comparing concentrations of the same organic substrate. Another restriction is that the organic co-substrate must be the limiting substrate; this was demonstrated by the independence of RDX transformation rate from the ethanol concentration in experiment BB-8.

Our results showed that a greater variety of organic substrates can be utilized under denitrifying conditions than under sulfate-reducing conditions. The choice of co-substrate is even more restrictive in fermentative conditions. Low molecular weight carbohydrates were utilized under denitrifying conditions, to a lesser extend also under sulfate-reducing conditions, and not at all under fermentative conditions. The results of experiment BB-7 sulfate or nitrate suggests that both electron acceptors, nitrate and sulfate, are mandatory in order to use ethanol and propanol as co-substrates. Table 5.1 summarizes the successful co-substrates in the presence of different electron acceptors and redox-conditions. It must be noted that we did not rigorously tested all co-substrates under all three redox-conditions; we focused on the most promising combinations. Consequently, the greater number of successful co-substrates under denitrifying conditions compared to sulfate-reducing or fermentative conditions is only a preliminary result.

The apparent existence of a metabolite peak during solubility determinations in formic acid should be studied further. Formic acid is a strong reducing agent. A chemical reaction could explain why we found disappearance of RDX but no bacteriological growth in cultures containing formic acid {BB-8}.

For all co-substrates evaluated, ethanol and acetic acid are the most favorable for RDX transformation. Both supported RDX degradation under denitrifying conditions and both are fair RDX solvents. Both fulfill all criteria required for a suitable solvent (see Section 2.1). Acetic acid has higher RDX solubility (3.7 vs. 0.9 g/L), but it is also more aggressive and has the higher heat capacity. Consequently, it will be more difficult to handle, heat, and distill as compared to ethanol. Both solvents should be studied more in greater detailed in desorption and biotransformation experiments.

Table 5.1: Successful co-substrates and the corresponding redox-conditions.

	Redox-Conditions	
Fermentative	Sulfate-reducing	Nitrate-reducing
Peptones and Protein (BB-1 - BB-4)*1	Peptones (BB-5)	Peptones (BB-5)
	Casamino acids (BB-6) Ethanol (BB-6, BB-7) Propanol (BB-5 to BB-7)	Casamino acids (BB-6) Ethanol (BB-5 - BB-9) Propanol (BB-6 - BB-7) Na-Acetate (BB-9) Na-Propionate (BB-9)

*1 listed are only experiments in which the substrate successfully supported RDX degradation

Nitrate as electron acceptor: Theoretically, denitrifying bacteria should be the best candidates for use in our treatment concept. There growth efficiency is the best of all anaerobes. They are able to mineralized organic substances, and they do not produce toxic and corrosive H_2S. Alternatively, it is known that reductive degradation processes can be inhibited by nitrate; for example, the reductive dechlorination of chlorinated aromatics is suppressed by high nitrate concentrations. Fortunately, we found that denitrifying conditions were suitable to support RDX transformation. Furthermore, denitrifying cultures accumulated the smallest concentrations of RDX intermediates (BB-5), and they were the only cultures to show significant HMX disappearance (BB-8). An inhibitory effect of nitrate on RDX degradation was not observed (BB-7, BB-8). In fact, increasing nitrate concentration enhanced the RDX degradation over the entire range of tested concentrations (BB-8). The results in BB-8 indicate that nitrate was limiting under these conditions. The dependency between growth rate (measured herein as turbidity after the first day) and nitrate concentration fits a Monod-kinetic reasonably well (Figure 5.2).

The nitrate limitation substrate in experiment BB-8 is also in accordance with theoretical considerations. One mole of ethanol can supply five hydrogen equivalents, which exactly equals the theoretical demand of reducing one mole nitrate to N_2. The molar ratio of ethanol to nitrate was always greater than 1, except for the cultures with the highest nitrate concentration (6.4 g/L), where the ratio was 0.82 mole EtOH/mole NO_3^-. Consequently, we could expect nitrate to be the limiting substance in this experiment.

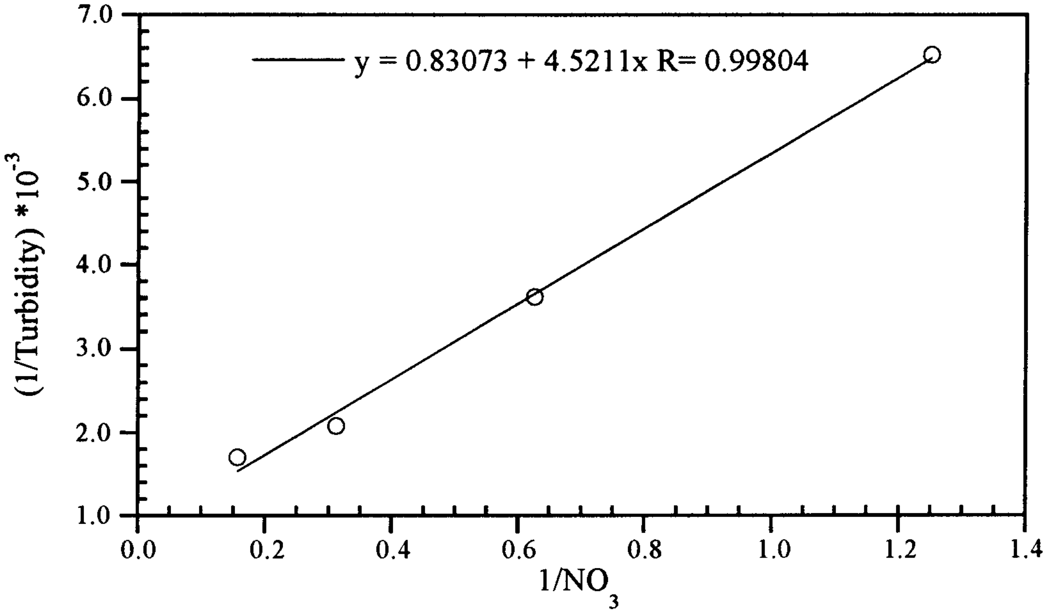


Figure 5.2 Lineweaver-Burk diagram of turbidity (after one day) over nitrate concentration in {BB-8}.

There are some reasons to hypothesize that denitrifying enzymes are directly involved in the reduction of the RDX nitro-groups. For example, the more than proportional increase in RDX degradation when increasing nitrate concentration from 3.2 g/L to 6.4 g/L (Figure 4.21) supports this speculation. Furthermore, it is known that some denitrifying enzyme systems are able to reduce the nitro-groups in other nitro-organics such as nitrobenzene (Yamashina et al., 1954 and Yamashina, 1954). This interesting question should be studied more thoroughly in future work, because it could help to optimize RDX treatment.

Comparison RDX vs. HMX: In all experiments, HMX was more resistant to transformation than RDX, which is in accordance to McCormick's results (1984a and b). Complete disappearance of HMX was observed only in experiment BB-8 at the highest nitrate concentration. McCormick proposed that HMX undergoes a similar biodegradation pathway as RDX, because he detected traces of Mononitroso- and Dinitroso-HMX as biotransformation products. The investigators did not offer an explanation for the slower transformation rates for HMX. To understand the reasons for this difference, we should consider the other differences between the two compounds. HMX is also more resistant to alkaline hydrolysis (Croce and Okamoto, 1979). It adsorbs more strongly to activated carbon (Burrows et al., 1984) and to biomass (McCormick et al., 1984b), which could be due to the larger molecule size. Alternatively HMX elutes earlier from a chromatographic column (C18-Reversed Phase), even though it is larger. All these differences cannot be explained by constitutional differences, because the Lewis-structure for every functional group and its neighborhood is the same in both molecules. Consequently, the differences should be caused by their molecule conformations or their size. We are unable to analyze the differences between RDX and HMX more thoroughly at this point; however, it should be noted that conformational differences are discussed in the literature (Lehn et al., 1967; Iqbal et al., 1974).

6.0 CONCLUSIONS

The following conclusions and recommendations for future research are made:

- Literature results indicated that biological RDX degradation or transformation requires complex organic co-substrates (peptones or molasses) and anaerobic conditions. All previous efforts to use more simple and less expensive co-substrates failed. Temporary accumulation of hazardous intermediates was observed in some cases. Biological treatment of low RDX concentrations in relatively clean groundwater or wash-down wastewater is therefore problematic. The addition of organic substances and bacteria growth would decrease the overall water quality and defeat the reasons for treatment. An expensive secondary clean-up would be necessary, including COD- and BOD-reduction, as well as bacteria removal.
- Removal of RDX from the wastewaters or groundwaters by adsorption onto activated carbon and subsequent, separated biodegradation of RDX will be advantageous, especially, when RDX concentrations are lowest. We therefore propose a process combination which includes a "indirect-off-line-bioregeneration" of exhausted activated carbon.
- Desorption of RDX from activated carbon and the transformation of RDX will be the limiting steps in the process. Consequently, the preliminary experiments presented in this report focused on possibilities to increase desorption and to optimize transformation of RDX.

- Desorption of RDX from activated carbon is exponentially increased by raising water temperatures. For example, the concentration of desorbed RDX was approximately 15 times greater at 90°C than at 29°C. The increase is approximately equal to the increase in solubility at the elevated temperature.
- Desorption of RDX from activated carbon is dramatically increased using polar organic solvents instead of water. For example, the concentration of desorbed RDX was between 480 (propanol) and 830 (ethyl acetate) times higher than the concentration obtained using water. The desorption of RDX will be further increased by increasing solvent temperature; we assume that the increase will be exponential and that it can be predicted from the increase in solubility, as observed in the case of water.
- Evidence supporting the belief that anaerobic biodegradation of RDX is fortuitous cometabolism was found. The involved enzyme systems are constitutional and widely distributed in regular anaerobic bacteria populations, like lake sediments and digester sludge (fortuitous metabolism). The mixed bacteria cultures were not able to use RDX as growth substrate (cometabolism). Consequently, enhanced biodegradation of RDX should not obtained through bacteria adaptation to RDX.
- Biodegradation of RDX occurred under fermentative, sulfate- and nitrate-reducing conditions. The presence of oxygen decreased the RDX degradation somewhat, but was not strongly toxic to the responsible bacteria.

- Under fermentative conditions, peptones and proteins were successfully used as co-substrates for RDX degradation. Sugars did not support RDX degradation, although they were used as growth substrate.
- Under sulfate-reducing conditions, peptones and amino acids from casein were successfully used as co-substrates for RDX degradation. Ethanol and Propanol supported RDX degradation in a mixture with amino acids or peptones. The alcohols were not tested as a sole co-substrate.
- Under nitrate-reducing conditions, the same co-substrates were successful, as in the case of sulfate-reducing conditions. Additionally, ethanol, propanol, sodium acetate, and sodium propionate were able to serve as sole co-substrates. Experiments using methanol, Na-formiate, acetone, and acetone were less encouraging.
- A temporary accumulation of RDX metabolites was observed mainly under fermentative and sulfate-reducing conditions. Most probably, those metabolites were nitroso-derivatives of RDX (McCormick et al., 1981).
- HMX, the important impurity in industrial RDX and an explosive itself, was more resistant to biodegradation than RDX. Considerable disappearance of HMX was found only under nitrate-reducing conditions.
- The experimental results are sufficiently encouraging to continue the development of the proposed treatment process.
- Denitrifying cultures using ethanol or acetic acid as co-substrates are the most promising for use in the proposed treatment concept. Further studies should compare both organic

solvents in their ability to desorb RDX from activated carbon and as co-substrates for biodegradation of RDX.

- Efforts should be made to obtain denitrifying cultures which are able to use acetone as a co-substrate for RDX degradation, because acetone is the best possible RDX solvent.
- After using the organic solvent (e.g. ethanol) to desorb RDX, and before injecting it into the bioreactor, it might be advantageous to increase the RDX concentration by distilling a portion of the solvent. This will provide a more favorable RDX concentration for biodegradation, and a portion of the organic solvent can be reused.

7.0 REFERENCES

Andren, R. K., Nystron, J.M., McDonnell, R.P. and Stevens, B.W. (1975). "Explosives Removal from Munitions Wastewater," *Proceedings of the 30th Industrial Waste Conference*, Purdue University, West Lafayette, IN, pp. 816-825.

Banerjee, S.,Yalkowsky, S.H. and Valvani, C. (1980). "Water Solubility and Octanol/Water Partition Coefficients of Organics: Limitations of the Solubility-Partition Coefficient Correlation," *Environmental Science and Technology*, Vol. 14, No. 10, pp. 1227-1229.

Barnes, C.P. and Eagon, R.G. (1986). "The Mechanism of Action of Hexahydro-1,3,5-triethyl-s-triazine," *Journal of Industrial Microbiology*, Vol. 1, pp. 105-112.

Bell, B.A. and Burrows, W.D. (1987). "Biological Treatment of Explosive Bearing Wastewaters," Mid-Atlantic 19th Industrial Waste Conference, June 21-23, Bucknell University, New Jersey.

Boyd, S.A., Shelton, D.R., Berry, D. and Tiedje, J.M. (1983). "Anaerobic Biodegradation of Phenolic Compounds in Digested Sludge," *Applied and Environmental Mircobiology*, Vol. 46, No. 1, pp. 50-54.

Brockman, F.J., Downing, D.C. and Wright, G.F. (1948). "Nitrolysis of Hexamethylenetetramine," *Canadian Journal of Research*, Vol. 27, Section B, Part 1, pp. 469-474.

Bruhn, C., Lenke, H. and Knackmuss, H.-J. (1987). "Nitrosubstituted Aromatic Compounds as Nitrogen Source for Bacteria," *Applied and Environmental Mircobiology*, Vol 53, No. 1, pp. 208-210.

Burrows, W.D., Chyrek, R.H., Noss, C.I., Small, M.J. and Kobylinski, E.A. (1984). "Treatment for Removal of Munition Chemicals from Army Industrial Wastewaters," *Proceedings of the 16th Mid-Atlantic Industrial Waste Conference*, pp. 331-342.

Croce, M. and Okamoto, Y. (1979). "Cationic Micellar Catalysis of the Aqueous Alkaline Hydrolyses of 1,3,5-triaza-1,3,5-trinitrocyclohexane and 1,3,5,7-tetraaza-1,3,5,7-tetranitrocyclooctane," *Journal of Organic Chemistry*, Vol. 44, No. 13, pp. 2100-2103.

Dalton, H. and Stirling, D.I. (1982). "Co-Metabolism," *Philosophical Transactions of the Royal Society of Landon*, Vol. 297, pp. 481-496.

Dobrevski, I. and Zvezdava, L. (1989). "Biological Regeneration of Activated Carbon," *Water Science and Technology*, Vol. 21, No. 1, pp. 141-143.

Druckrey, H., Preusssman, R., Ivankovice, S. and Schmahl, D. (1967). "Organotrope Carcinogene Wirkungen bei 65 Verschieden N-Nitroso-Verbindungen an BD-Ratten," *Zeitschrift fur Krebsforschung*, Vol. 69, pp 103-201.

Faust, S.D. and Aly, O.M. (1987). *Adsorption Processes for Water Treatmentt*, Butterworth Publishers.

Fisher, G., Jackson, B. Jr. and Jachowski, J.N. (1982). *Ultraviolet-ozone Treatment of RDX (Cyclonite) Contaminated Wastewater*, Contractor Report AR LCD-CR-82024, U.S. Army Armament Research and Development Command, Dover, NJ.

Freeman, D. J. (1985). "Continuous Fixation and Removal of Explosive Wastes from Pink Water using Surfactant Technology," *Proceedings of the 40th Industrial Waste Conference* , Purdue University, West Lafayette, IN, pp. 659-675.

Glover, D.J. and Hoffsommer, J.C. (1979). *Photolysis of RDX. Identification and Reactions of Products,* Technical Report NSWC TR-79-349, Naval Surface Weapons Center, Silver Spring, Md.

Goeddertz, J.G., Matsumoto, M.R. and Weber, A.S. (1988). "Off-line Bioregeneration of Granular Activated Carbon," *Journal of the Environmental Engineering Division,* ASCE., Vol. 114, No. 5, pp. 1063-1076.

Greene, B., Kaplan, D. L. and Kaplan, A.M. (1985). *Degradation of Pink Water Compounds in Soil - TNT, RDX, HMX,* Technical Report/TR-85-046, U.S. Army Natick Research and Development Center, Natick, MA.

Goodfellow, P. (1991). Mason & Hanger-Silas Mason Co. Inc., Explosives Technology Division, Pantex Plant, Amarillo, Texas: Personal Communication

Haas, R., Schreiber, I. Low, E.U. and Stork, G. (1990). "Conception for the Investigation of Contaminated Munition Plants," *Fresenius 'Journal of Analytical Chemistry.*, Vol. 338, pp. 41-45.

Harvey, S.D., Fellows, R.J, Cataldo, D.A. and Bean, R.J. (1991). "Fate of the Explosive Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) in Soil and Bioaccumulation in Bush Bean Hydroponic Plants," *Environmental Toxicology and Chemistry* , Vol. 10, pp. 845-855.

Hering, J. (1991). Civil and Environmental Engineering, University of California at Los Angeles: Personal Communication.

Hoffsommer, J.C., Kubose, D.A. and Glover, D.J. (1977). "Kinetic Isotope Effects and Intermediate Formation for the Aqueous Alkaline Homogeneous Hydrolysis of 1,3,5-triaza-1,3,5-trinitrocyclohexane (RDX)," *Journal of Physical Chemisty*, Vol. 81, No. 5, pp. 380-385.

Hoffsommer, J.C., Kaplan, L.A., Glover, D.J., Kubose, D.A., Dickenson, C.D., Goya, H., Kayser, E.G., Groves, C.L. and Sitzmann, M.E. (1978). "Biodegradability of TNT: A Three year Pilot Plant Study," AD-061144. Naval Surface Weapon Center, White Oak, Silver Spring, MD, Report AMXTH-TE-GR87111.

Holst, J., Martens, B., Gulyas, M. Greiser, N. and Sekoulov, I. (1991). "Aerobic Biological Regeneration of Dichloromethane-Loaded Activated Carbon," *Journal of the Environmental Engineering Division,* ASCE, Vol. 117, No. 2, pp. 194-208.

Hutchinson, D.H. and Robinson, C.W. (1990a). "A Microbial Regeneration Process for Granular Activated Carbon - I. Process Modelling," *Water Research*, Vol. 24, No. 10, pp. 1209-1215.

Hutchinson, D.H. and Robinson, C.W. (1990b). "A Microbial Regeneration Process for Granular Activated Carbon - II. Regeneration Stuides," *Water Research*, Vol. 24, No. 10, pp. 1217-1223.

Iqbal, Z., Bulusu, S. and Autera, J. R. (1974). "Vibrational Spectra of beta-Cyclotetramethylene Tetranitramine and Some of its Isotopic Isomers," *Journal of Chemical Physics*, Vol. 60, No. 1, pp. 221-230.

Janke, D. and Fritsche, W. (1985). "Nature and Significance of Microbial Cometabolism of Xenobiotics," *Basic Microbiology*, Vol 25, No. 9, pp. 603-619.

Jenkins, T.F., Leggett, D.C., Grant, C.L. and Bauer, C.F. (1986). "Reversed-Phase High-Performance Liquid Chromatographic Determination of Nitroorganics in Munitions Wastewater," *Analytical Chemistry*, Vol. 58, No. 1, pp. 170-175.

Jones, W.H. (1953). "Mechanism of the Homogeneous Alkaline Decomposition of Cyclotrimethylenetrinitramine: Kinetics of Consecutive Second- and First-order Reactions. A Polarographic Analysis for Cyclotrimethylenetrinitramine," *Journal of American Chemical Society,* Vol. 76, pp. 829-835.

Kaplan, D.L. and Kaplan, A.M. (1982). "2,4,6-Trinitrotoluene - Surfactant Complexes: Decompostion, Mutagenicity, and Soil Leaching Studies," *Environmental Science and Technology*, Vol. 16, No. 9, pp. 565-571.

Kaplan, D.L. (1989). "Biotransformation Pathways of Hazardous Energetic Organo-Nitro Compounds," *Biotechnology and Biodegradation, Advances in Applied Biotechnology Series,* Vol.4, 155 p., Portfolio Publishing Co., Houston.

Kim, B.R., Chian, E.S.K., Cross, W.H. and Gheng, S.S. (1986). "Adsorption, Desorption, and Bioregeneration in an Anaerobic, Granular Activated Carbon Reactor for the Removal of Phenol," *Journal of the Water Pollution Control Federation*, Vol. 58, No. 1, pp. 35-40.

Knackmuss, H.-J. (1981). "Degradation of Halogenated and Sulfonated Hydrocarbons," In: *Microbial Degradation of Xenobiotics and Recalcitrant Compounds.* T. Leisinger, et al. (editors), Academic Press, New York and London, pp. 189-212.

Knezovich, J.P. and Daniels, J.L. (1991). Environmental Science Division, Lawrence Livermore National Laboratory, Livermore, CA: Personal communication.

Koganovskii, K.A., Vdod, V.M. and Lysenko, V.V. (1981). "Biological Regeneration of Activated Carbon After the Adsorption of the Reactive Brilliant Red 5SKH from an Aqueous Solution," *Khimiya i Tekhologiya Vody*, Vol. 3, No. 1, pp. 81-82.

Kookana, R.S., Gerritse, R.G. and Aylmore, L.A.G. (1990). "Effect of Organic Cosolvent on Adsorption and Desorption of Linuron and Simazine in Soil," *Australian Journal of Soil Research.*, Vol. 28, No. 5, pp. 717-725.

Kooke, R.M.M., Lustenhouwer, J.W.A., Olie, K. and Huntzinger, O. (1981). "Extraction Efficiencies of Polychlorinated Dibenzo-p-dioxins and Polychlroinated Dibenzofurans from Fly Ash," *Anayltical Chemistry*, Vol. 53, pp. 461-463.

Lehn, J.M., Riddel, F.G., Price, B.J. and Sutherland, I.O. (1967). "NMR Studies of Rate Processes and Conformations. Part VI. Ring Inversion on Hexahydro-1,3,5-triazines," *Journal of the Chemical Society, Section B,:Physical Organic Chemistry*, pp. 387-390.

McCormick, N.G., Feeburg, F.E. and Levinson, H.S. (1976). "Microbial Transformation of 2.4.6-Trinitrotoluene and Other Nitroaromatic Compounds," *Applied and Environmental Microbiology*, Vol. 31, No. 6, pp. 949-995.

McCormick, N.G., Cornell, J.H. and Kaplan, H.S. (1981). "Biodegradation of Hexadro-1,3,5-trinitro-1,3,5-triazine," *Applied and Environmental Microbiology*, Vol. 42, No. 5, pp. 817-823.

McCormick, N.G., Cornell, J.H. and Kaplan, A.M. (1984a). *The Anaearobic Biotransformation of RDX, HMX, and Their Acetylated Derivatives,* Technical Report Natick/TR-85-007, U.S. Army Natick Research & Development Center, Natick, MA.

McCormick, N.G., Cornell, J.H. and Kaplan, A.M. (1984b). *The Fate of Hexahydro-1,2,3-trinitro-1,3,5-triazine (RDX) and Related Compounds in Anaerobic Denitrifying Continuous Culture Systems Using Simulated Waste Water.* Technical Report Natick/TR-85-008, U.S. Army Natick Research & Development Center, Natick, MA.

Merck & Co., Inc. (1989). *The Merck Index,* 11th Edition, The Merck Chemical Company, Rathway, NJ.

Molnier, A.M. and Street, J.J. (1989). "Decomposition of Hydrazine in Aqueous Solutions," *Journal of Environmental Quality,* Vol. 18, pp. 483-487.

Osmon, J.L. and Klausmeier, R.E. (1973). "The Microbial Degradation of Explosives," *Developments in Industrial Microbiology,* Vol. 14, pp. 247-252.

Patterson, J.W., Shapira, N.I. and Brown, J. (1976a). "Pollution Abatement in the Military Explosives Industry," *Proceedings of the 31st Industrial Waste Conference.,* Purdue University, West Lafayette, IN

Patterson, J.W., Shapira, N.I., Brown, J., Duckert, W. and Polson, J. (1976b). *State-of-the-Art: Military Explosives and Propellants Production Industry. Vol. II,* Report No. EPA-600/2-76-213b (PB-260918), American Defense Preparedness Association, Washington, D.C.

Ro, K.S. and Stenstrom, M.K. (1991). *Aerobic Biological Degradation of RDX- Progress Report No. 1,* UCLA-ENG 91-17, University of California at Los Angeles, Los Angeles, CA.

Rodgers, J.T. (1962). *Physical and Chemical Properties of RDX and HMX.* Holston Defense Corporation, 50 p.

Rosenblatt, D.H., Burrows, E.P., Mitchell, W.R. and Parmer, D.L. (1991). "Organic Eplosives and Related Compounds," In: *The Handbook of Environmental Chemistry,* Vol. 3, Part G, Springer-Verlag, Berlin.

Schultz, J.R. and Keinath, T.M. (1984). "Powdered Activated Carbon Treatment Process Mechanisms," *Journal of the Water Pollution Control Federation,* Vol. 56, No. 2, pp. 143-151.

Semmens, M.J., Barnes, D. and O' Hara, M. (1984). "Treatment of an RDX-TNT Waste form a Munitions Factory," *Proceedings of the 39th Industrial Waste Conference*, Purdue University, West Lafayette, IN, pp. 837-842.

Shelby, S.E., Lankford, P.W. and McCollum, R.W. (1984). "A Case Study for the Treatment of an Explosives Wastewater from an Army Munitions Plant," *Proceedings of the 39th Industrial Waste Conference*, Purdue University, West Lafayette, IN, pp. 821-

Shorten, C.V., Elzerman, A.W. and Mills, A.G. (1990). "Methods for the Determination of PAH Desorption Kinetics in Coal Fines and Coal Contaminated Sediments," *Chemosphere*, Vol. 20, No. 1-2, pp. 137-159.

Spalding, R.F. and Fulton, J.W. (1988). "Groundwater Munition Residues and Nitrate Near Grand Island, Nebraska, USA," *Journal of Contaminant Hydrology*, Vol. 2, pp. 139-153.

Spanggord, R.J., Mill, T., Chou, T.W., Mabey, W.R. Smith, J.H. and Lee, S. (1980). *Environmental Fate Studies on Certain Munition Wastewater Constituents, Phase II - Laboratory Studies.* Report DAMD-17-78-C-808. U.S. Army Medical Research and Development Command, Fort Detrick, Md.

Sullivan, J.H., Putnam, H.D., Kerrn, M.A., Nicholas, J.C. and McClave, J.T. (1979). *A Summary and Evaluation of Aquatic Environmental Data in Relation to Establishing Water Quality Criteria for Munitions Unique Compounds. Part 4: RDX and HMX,* Report DAMD-17-77-C-7027. U.S. Army Medical Research and Development Command, Fort Detrick, Md.

Sutikno, T. and Himmelstein, K.J. (1983). "Desorption of Phenol from Activated Carbon by Solvent Regeneration," *Industrial and Engineering Chemistry Fundamentals*, Vol. 22, No. 4, pp. 420-425.

Tamon, H., Saito, T., Kishimura, M., Okazaki, M. and Toei, R. (1990). "Solvent Regeneration of Spent Activated Carbon in Wastewater Treatment," *Journal of Chemical Engineering of Japan*, Vol. 23, No. 4, pp. 426-432.

Tsai, S.Y., Davis, M.J. and Benioff, P.A. (1985). *Cornhusker Army Ammunition Plant Aquifer Restoration Alternatives Study,* U.S. Army Toxic and Hazardous Materials Agency, Report AMXTH-AS-CR-850132, Aberdeen Proving Ground, Md, 134 pp.

Urban, H. and Danz, M. (1976). "Tumorinduzierende Wirkung von Trinitroso-trimethylentriamin in Verbindung mit Dimethylsulfoxid," *Archiv Geschwulstforschung,* Vol. 46, No. 8, pp. 657-662.

Urbanski, T. (1964). *Chemistry and Technology of Explosives,* Vol. 3, Pergamon Press, Oxford.

Urbanski, T. (1983). *Chemistry and Technology of Explosives,* Vol. 3, Pergamon Press, Oxford.

U.S. Army Material Command. *Engineering Design Handbook: Explosives Series.* AMCP 706-177; Headquarter U.S. Army Material Command.

U.S. Environmental Protection Agency (1988). *Health Advisory for Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX),* Criteria and Standards Division, Office of Drinking Water, Washington, DC.

Walsh, J.T., Chalk, R.C. and Merritt, Jr., C. (1973). "Application of Liquid Chromatography to Pollution Abatement Studies of Munition Wastes," *Analytical Chemistry*, Vol. 45, No. 7, pp. 1215-1220.

Yamashina, I., Shikata, S. and Egami, F. (1954). "Enzymatic Reduction of Aromatic Nitro, Nitroso and Hydroxyl Amino Compounds," *Bulletin of the Chemical Society of Japan*, Vol. 27, No. 1, pp. 42-45.

Yamashina, I. (1954). "Enzymatic Reduction of Aromatic Nitro Compounds," *Bulletin of the Chemical Society of Japan*, Vol. 27, No. 2, pp. 85-89.

Yinon, J. (1990). *Toxicity and Metabolism of Explosives.* CRC Press Inc., Boco Raton, FL.

Zhang, X., Wang, Z. and Gu, X. (1991). "Simple Combination of Biodegradation and Carbon Adsorption - The Mechanism of the Biological Activated Carbon Process," *Water Research*, Vol. 25, No. 2, pp. 165-172.

APPENDIX A: TABLES

Table A.1: Continuous column adsorption of RDX*[1]

Throughput (l)	Bed Volumes (-)*[2]	RDX concentration (mg/L)					RDX load*[3] (mg)
		position of sampling points					
		influent	15 mm	60 mm	105 mm	effluent	
2	38	6.72			< 0.1	< 0.1	13
28	530	6.77			< 0.1	< 0.1	189
30	568	5.51	2.78	< 0.1	< 0.1	< 0.1	201
57	1080	5.53	3.31	0.16	< 0.1	< 0.1	350
64	1212	6.59	3.77	0.18	< 0.1	< 0.1	393
96	1818	5.15	3.58	0.32	< 0.1	< 0.1	580
103	1951	5.51	3.82	0.36	< 0.1	< 0.1	618
126	2386	5.47	4.07	0.56	< 0.1	< 0.1	744
134	2538			0.54	< 0.1	< 0.1	
160	3030	5.45	4.13	0.74	< 0.1	< 0.1	930
165	3125	5.27	4.07	0.87	< 0.1	< 0.1	956
187	3542	5.25	4.36	0.99	< 0.1	< 0.1	1072
192	3636	3.76	3.33	0.90	< 0.1	< 0.1	1095
204	3864	3.67	3.19	0.94	< 0.1	< 0.1	1139

*[1] 25 g of Filtrasorb 400 in column as described in 3.1.2; *[2] bed volume = 52.8 mL, flow rate = 30 mL/min; *[3] calculated with average influent concentrations and corresponding throughputs.

Medium influent concentration = 1025 mg/204 l = 5.02 mg/L

Medium carbon load = 1025 mg/25 g = 41 mg/g = 4.1% (w/w)

Table A.2: Continuous column desorption of RDX*[1].

Throughput*[2] (l)	Bed volume*[3] (-)	RDX effluent concentration (mg/L)	RDX recovery*[4] (mg)
1	17	1.48	1
22	415	1.15	29
27	523	1.09	35
29	552	1.01	37
40	758	1.02	48
66	1250	0.89	73
80	1515	0.75	84
101	1913	0.82	101
119	2254	0.79	115
140	2652	0.72	131
175	3314	0.59	154
190	3598	0.63	163
215	4072	0.64	179
263	4981	0.60	209
293	5549	0.56	226
330	6250	0.50	246
382	7235	0.48	271
420	7955	0.43	289
461	8731	0.49	308
490	9280	0.50	322
535	10133	0.53	345
573	10852	0.51	365
657	12443	0.44	405
701	13277	0.43	424
741	14034	0.38	440
773	14640	0.30	451
836	15833	0.34	471
879	16648	0.43	488
929	17595	0.41	509
961	18201	0.32	520

*[1] Activated carbon loaded with RDX in Exp. AC-1; *[2] tap water (without RDX); *[3] bed volume = 52.8 mL, flow rate = 30 mL/min; *[4] calculated with average influent concentrations and corresponding throughputs.

Table A.3: RDX desorption using organic solvents.

Solvent	RDX in solvent phase (mg/L)					
	15 min.	30 min.	45 min.	60 min.	13 hr	30 days
Ethyl Acetate	96	156	175	198	224	221
Acetic Acid	86	121	135	163	213	209
Methanol	72	91	103	103	142	138
Propionic acid	61	73	80	86	154	151
Ethanol	54	72	84	88	136	131
Propanol	42	48	58	66	130	122

2.12 gram Filtrasorb 400 (dry weight) from DB-1 (loaded with ca. 42 mg RDX) suspended in 100 mL solvent and shaken at room temperature in stoppered 250-mL flask.

Table A.4: Normalized RDX concentration in solvent phase.

Solvent	RDX in solvent phase (% from ethyl acetate value)						Solubility
	15 min.	30 min.	45 min.	60 min.	13 hr	30 days	(%)*[1]
Ethyl Acetate	100	100	100	100	100	100	100
Acetic Acid	90	78	77	82	95	95	33
Methanol	75	58	59	52	63	62	17
Propionic Acid	64	47	46	43	69	68	11
Ethanol	56	46	48	44	61	59	8
Propanol	44	31	33	33	58	55	4

*[1] values from Table A.3, normalized with ethyl acetate values.

Table A.5: Grade of RDX saturation in solvent phase.

Solvent	RDX in solvent phase (% from solubility)*1					
	15 min.	30 min.	45 min.	60 min.	13 hr	30 days
Ethyl Acetate	0.8421	1.3684	1.5351	1.7368	1.9649	1.9386
Acetic Acid	2.3248	3.2703	3.6486	4.4054	5.7568	5.6486
Methanol	3.7895	4.7895	5.4211	5.4211	7.4737	7.2632
Propionic Acid	6.1000	7.3000	8.0000	8.6000	15.4000	15.1000
Ethanol	6.0000	8.0000	9.3333	9.7778	15.1111	14.5556
Propanol	8.4000	9.6000	11.6000	13.2000	26.0000	24.4000

*1 solubility values from Table A.3.

Table A.6: Kinetics of RDX desorption.

Solvent	RDX in solvent phase (% from 30 days value)					
	15 min.	30 min.	45 min.	60 min.	13 hr	30 days
Ethyl Acetate	43	71	79	90	101	100
Acetic Acid	41	58	65	78	102	100
Methanol	52	66	75	75	103	100
Propionic Acid	40	48	53	57	102	100
Ethanol	41	55	64	67	104	100
Propanol	34	39	48	54	107	100

Table A.7: Turbidity and RDX concentration data from experiment BB-4.

Culture*1	Turbidity (Extinction*1000)						RDX (mg/L)	
	start	1 day	2 days	3 days	4 days	15 days	4 days	5 days
PepR1	17	106	130	165	181	119	8.2	3.3
PepR2	13	97	123	159	181	116	2.6	1.8
PepF1	7	106	133	139	155	100	14.6	4.1
PepF2	8	66	76	92	110	97	14.3	4.2
PepD1	21	58	68	88	110	86	15.0	2.1
PepD2	19	99	110	117	132	95	16.3	2.0
PepS1	24	53	67	88	93	110	12.6	0.0
PepS2	11	36	53	64	72	77	13.4	3.1
SoyR1	20	285	348	356	365	290	5.8	2.1
SoyR2	51	295	350	359	372	296	5.5	1.9
SoyF1	17	310	318	323	322	210	5.4	2.1
SoyF2	29	314	339	349	359	242	6.9	2.2
SoyD1	29	296	347	345	360	273	6.3	0.0
SoyD2	39	309	366	380	376	255	7.3	2.1
SoyS1	20	209	225	216	216	204	6.1	1.9
SoyS2	36	202	209	210	215	143	5.9	2.2
CotR							15.2	3.3
CotF							8.0	1.9
CotD							16.9	3.0
CotS							18.2	2.4
MolR1	91	388	369	353	351	379	25.0	15.5
MolR2	95	399	399	399	405	332	24.1	15.5
MolF1	92	362	387	458	454	410	12.1	6.5
MolF2	96	344	363	383	414	425	24.0	12.2
MolD1	105	386	406	400	409	340	14.6	6.1
MolD2	100	389	404	408	404	336	15.7	6.5
MolS1	90	405	462	450	453	414	24.6	9.9
MolS2	69	388	452	454	459	408	25.3	16.4
SucR1	9	74	109	95	83	89	28.5	28.1
SucR2	8	81	109	95	87	101	28.5	18.5
SucF1	18	32	40	348	363	413	27.5	20.3
SucF2	9	48	55	369	379	382	27.4	23.0
SucD1	10	195	185	174	177	160	26.1	19.2
SucD2	12	236	207	203	202	170	25.9	20.2

Table A.7 (Cont'd)

Culture*[1]	Turbidity (Extinction*1000)						RDX (mg/L)	
	start	1 day	2 days	3 days	4 days	15 days	4 days	5 days
SucS1	4	187	211	212	211	201	27.8	12.8
SucS2	5	202	221	220	222	212	27.7	26.8
GluR1	4	81	103	86	86	90	28.2	28.8
GluR2	8	109	99	84	96	99	28.2	28.1
GluF1	5	344	313	301	303	383	27.0	24.2
GluF2	4	338	340	342	352	329	26.4	23.8
GluD1	9	155	143	126	129	105	27.3	22.2
GluD2	9	171	157	142	142	113	27.1	22.5
GluS1	4	161	156	153	153	158	27.5	27.3
GluS2	2	167	162	164	164	179	27.6	17.1

*[1] Pep = Peptone, Soy = Soytone, Cot = Cotton Seed, Mol = Molasses, Suc = Sucrose, Glu = Glucose, R = Digester sludge (precultivated), F = Pond Sediment (precultivated), D = Digester Sludge (fresh), S = Sites Mix (fresh).

APPENDIX B

Table B-1: Parameters of activated carbons which should be used in future work.

		Activated Carbon Type		
		Norit PK 1-3	Filtrasorb 400	Darco 20 X 40
Total Pore Volume	(mL/g)	1.23	0.80	0.94
Micropores (d < 2 nm)	(mL/g)	0.30	0.32	0.20
Mesopores (2 > d < 50 nm)	(mL/g)	0.19	0.27	0.47
Macropores (d > 50 nm)	(mL/g)	0.74	0.21	0.27
Surface area	(m^2/g)	625	1000	665
Surface area/mesopores vol.	(m^2/mL)	3289	3703	2771

Note: Norit PK 1-3 is a typical macroporous carbon, Filtrasorb 400 has a large portion in the microporus range, and Darco 20 X 40 is a more microporous coal. Comparision of the three activated carbons in adsorption, desorption, and bioregeneration will reveal the influence of the pore distribution. However, it must be noted that the carbons also differ in their basic material and manufacturing processes. Commercial products with different pore structures but identical basic material and manufacturing are not avaiable.