BIOLOGICAL DEGRADATION OF RDX USING AN ANOXIC FIXED FILM REACTOR

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PREFACE

This report is adapted from the M.S Thesis of Jennifer Alise Wilkie. It was completed and filed in 1993 in the Chemical Engineering Department at UCLA. Professor Harold Monbouquett was Ms. Wilkie's advisor. This work was supported in part from Department of Energy Grants through the Lawrence Livermore National Laboratory and the Pantex Plant, operated by Silas Mason and Hanger. The authors are grateful for the suggestions of Jeff Daniels, Philip Goodfellow, John Knezovich and Rolf Hesselmann.

ABSTRACT

The High Explosive RDX (Hexahydro-1,3,5-trinitro-1,3,5-triazine) has contaminated ground water and soil at several sites in the US and Europe. RDX is classified as a "Possible Human Carcinogen" (US EPA Group C) and has various toxic effects on mammals, fish and protozoa. In addition to the large amounts of explosive from the current stockpile, waste production from disarmament activities must also be treated. Presently, activated carbon adsorption is the most widely used method of wastewater treatment. This method, however, produces dangerous accumulations of explosives-laden carbon which then results in a waste disposal problem.

This research project investigates a novel treatment process that employs continuous flow anoxic biodegradation. In this process, RDX degradation is performed in two steps: 1) adsorption of RDX onto activated carbon with subsequent solvent desorption at elevated temperature, and 2) biological degradation of the regenerating fluid. The biodegradation process uses a mixed culture of denitrifying bacteria with nitrate as the electron acceptor and ethanol as the co-substrate.

RDX removal efficiencies up to 80% were observed for a continuous flow biological reactor operating at 35°C. The effects of temperature, retention time, co-substrate type, and co-substrate concentration on culture growth and RDX removal were examined. Adsorption isotherms were generated for several carbons. Filtrasorb-400 had the highest RDX adsorption capacity at 417 mg/g GAC; this carbon also had the greatest desorption capability. The overall degradation process can be fitted to a pseudo first-order rate equation. The rate

constants calculated for glass, silicone and Tygon packed reactors were 1.7, 4.2 and 4.2 hr^{-1}, respectively.

1. INTRODUCTION

The High Explosive RDX (Hexahydro-1,3,5-trinitro-1,3,5-triazine) is a ground water and soil contaminant at several sites in the US and Europe (Haas, 1990; Spalding, 1988). During World War II, RDX production in the US and Germany averaged 15,200,000 and 7,100,000 kg per month, respectively (Urbanski, 1967). Furthermore, during 1969 and 1970 the US produced RDX waste at a rate of 7,600,000 kg per month (Patterson, 1976). In the past, wastewater management practices at facilities producing and handling explosives used lagoons as their wastewater treatment process (Wujcik et al., 1992). Solid munitions material settled in the lagoons before the water was released to rivers and streams. This practice resulted in the contamination of groundwater via the leaching of hazardous explosives through the soil. Further soil contamination occurred at open-burning and incineration sites, and also from operational spills or seepage from landfills and wastewater holding facilities (Rosenblatt et al., 1991).

Recent increases in waste production due to the end of the cold war have augmented disposal and treatment problems. The Department of Energy (DOE) is currently processing 45,000 kg of High Explosives annually at the Pantex Plant in Texas. Moreover, the Department of Defense (DOD) has 317,500,000 kg of high explosives to treat (Byrd and Humphreys, 1993). The US Department of Energy must continue disarmament activities under the Intermediate Range Nuclear Forces Treaty and the Strategic Arms Reduction Treaties (START I and II). These activities are expected to produce an additional 50,000 kg of high explosives per year.

The dismantling process generates large volumes of extremely problematic, low concentration, wastewater. Presently, activated carbon adsorption is the most widely employed method of treatment for these wastewaters. This method, however, accumulates explosives on the surface of the carbon, and prevents its regeneration by normal means. The spent carbon must be disposed as solid hazardous waste. Some agencies, including the DOD, use open burning and open detonation of bulk quantities of high explosives; however, future air quality legislation is expected to prohibit this form of disposal (Byrd and Humphreys, 1993). Thus, it has become imperative to develop new technologies to handle this unique, explosive, hazardous waste.

This research project investigated a novel process using continuous flow biological degradation. In this process, RDX degradation occurs in two steps: 1) adsorption of RDX onto activated carbon with subsequent desorption, and 2) biological degradation of RDX in the regeneration fluid, as shown in Figure 1. Ethanol is used as both a desorption solvent and a co-substrate for metabolism. Wastewater contaminated with RDX and other high explosives pass through a series of activated carbon columns. After each carbon column becomes exhausted, the explosives are desorbed with an ethanol/water mixture. The RDX-laden desorption mixture then passes through an anaerobic biological reactor. Recirculation through the reactor is continued, with the addition of necessary nutrients, until the desired effluent concentration is reached. This process effectively treats low concentration wastewater. In addition, the process eliminates the dangerous accumulation of explosives and the need to dispose of the carbon as solid hazardous waste. Section 2.2.3 presents a more detailed description.

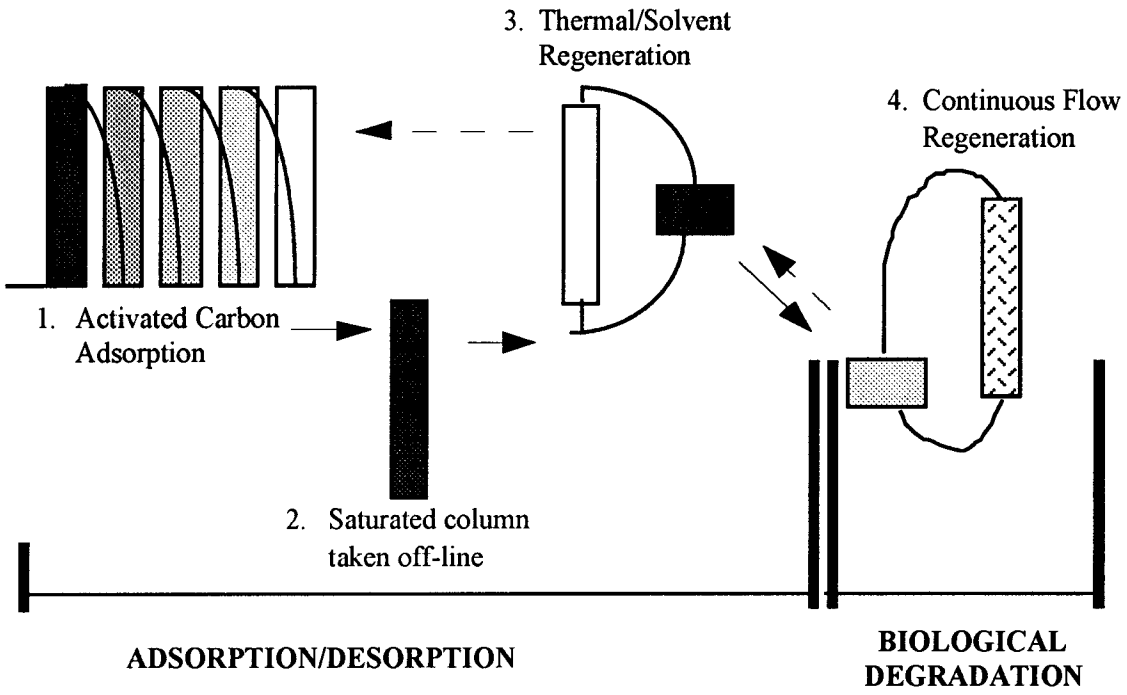


Figure 1 Schematic drawing of the proposed RDX treatment process.

2. LITERATURE REVIEW

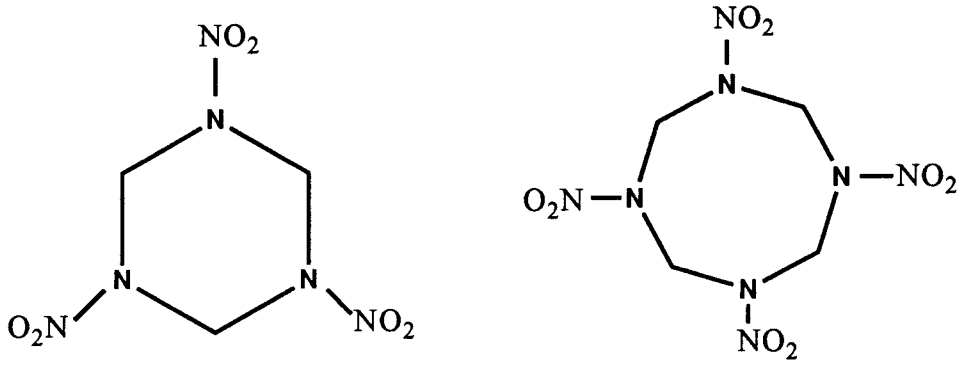
2.1 Physical Properties and Toxicity

2.1.1 Properties of RDX and HMX

RDX ($C_3H_6N_6O_6$), is a colorless polycrystalline, heterocyclic triazine, and is shown in Figure 2. It is one of the most widely used military high explosives (Rosenblatt et al., 1991). The chemical stability of RDX is similar to TNT; however, its explosive power is higher than most other explosives. In a nuclear warhead, RDX can be incorporated into a spherical layer of explosives that transform detonation waves into a uniform, inward-moving detonation wave focused on a plutonium pit.

Production grade RDX is not pure and usually contains a significant amount (~ 10%) of HMX. HMX, is an explosive byproduct of RDX synthesis, and is shown in Figure 2. It has a higher density and much higher melting point than RDX, which resulted in the name High Melting eXplosive (Gibbs and Popolato, 1980). The RDX used in this study contained an average of 10% HMX.

RDX is sparingly soluble in water; however, its solubility is greatly enhanced in the presence of polar organic solvents. Physical properties, relative to this research, are listed in Table 1; for a more extensive list see Dobratz (1981).



RDX **HMX**

Figure 2 Chemical structures of RDX and HMX.

Table 1 Physical properties of RDX and HMX (adapted from Dobratz, 1981).

Properties	RDX	HMX
CAS Reg. No.	121-82-4	2691-41-0
Synonyms	Cyclonite/Hexogen Cyclotrimethylenetrinitramine Hexahydro-1,3,5-trinitro-1,3,5-triazine	Octagen Octahydro-1,3,5,7-tetranitro-1,3,5,7-triazocine
Empirical Formula	$C_3H_6N_6O_6$	$C_4H_8N_8O_8$
Molecular Weight	222.26	296.16
Melting Point	204.1°C	276 to 280°C
Vapor Pressure (torr, 25°C)	4.03×10^{-9}	3.33×10^{-14}
Aqueous Solubility (mg/L, 25°C)	40 - 60	4 - 5

2.1.2 Toxicity

The US EPA has classified RDX as a class C carcinogen ("Possible Human Carcinogen"). Toxicity in humans is primarily manifested in the central nervous system, but gastrointestinal and renal effects have also been found (Etiner, 1990). A Lifetime Health Advisory for exposure to RDX has been set by the US EPA at 0.002 mg/L in drinking water. Acute exposure symptoms in humans include: hyper-irritability, muscle twitching, seizures, prolonged confusion and amnesia. Sunderman (1944) (as reported in Etiner, 1989) demonstrated that RDX, when applied to human skin, acts primarily as an irritant. Absorption of RDX by the skin is highly unlikely due to its lipophobic nature (Rosenblatt, 1980). RDX also has various toxic effects on mammals, fish and protozoa (Yinon, 1990). The oral lethal dose for rats and mice has been estimated to be 100 and 59 mg/kg, respectively. RDX was used as a rat poison due to its toxicity toward rodents (Merck Index, 1983). A maximum water concentration of 0.3 mg/L (24 h-average) has been suggested to protect aquatic life (Sullivan et al., 1979).

2.1.3 Environmental Fate

Significant environmental contamination has resulted from the discharge of manufacturing and processing waste streams to surface waters and lagoons. In the past, this practice was the major source of environmental entry for high explosives. As mentioned earlier, this caused the contamination of soil and groundwater at many locations in the US and Europe (Haas, 1990; Spalding, 1988). The fate and distribution of RDX is primarily affected by microbiological and photochemical transformations.

In the past, microbial degradation of RDX had not been found to occur in aerobic systems; however, a recent publication suggests aerobic degradation can occur under some conditions. Anaerobic biological reduction can occur under the appropriate environmental conditions. This subject will be more thoroughly addressed in Section 2.2.2. Although photochemical transformation reaction rates are often significantly higher than microbial reaction rates, the extent of RDX photo-degradation is minimal because the UV wavelengths found at the earth's surface are predominantly greater than 290 nm and are poorly absorbed by the molecule. Moreover, studies on the decrease of RDX photolysis with water depth have shown RDX disappearance at a depth of 4 cm to be 2200 times slower than at the surface [Kubose and Hoffsommer, 1977 as reported in Layton et al. (1987)]. Hydrolysis transformations are found only in highly alkaline waters. The pH of most natural waters is usually too low for hydrolysis to be an important environmental source of degradation. Oxidation has not been found under environmental conditions. In addition, physical transport from aqueous systems is relatively unimportant due to a low Henry's law constant which limits volatilization to the atmosphere (Rosenblatt et al., 1991). Measurements of sediment adsorption coefficients indicate that RDX does not strongly adsorb to soils and sediments (Spanggord et al., 1980; Tsai et al., 1985). The uptake and accumulation of RDX in the edible tissues of hydroponic plants suggests a possible impact on the food-chain (Harvey et al., 1991).

2.2 Treatment

2.2.1 Physiochemical

2.2.1.1 Activated Carbon Adsorption

Presently, activated carbon adsorption is the most widely employed method for treatment of wastewaters containing high explosives. The ability of activated carbon to adsorb compounds at low concentrations has made it a common sorbent in the treatment of a wide variety of contaminated waters. Although the activated carbon adsorption process is very effective, it introduces new problems as dangerous accumulations of explosives build up and result in the need to dispose of the exhausted carbon as solid hazardous waste. Open burning and open detonation are currently being used by some facilities to dispose of the explosives laden carbon; however, future legislation is expected to bar this form of disposal (Byrd and Humphreys, 1993). The operation and maintenance involved in the activated carbon treatment process is very costly. Thus methods to prolong the life/regenerability of the carbon in the adsorption column has become one of the main topics for current research in this area. Although thermal regeneration of activated carbon is a useful method for many contaminants (Chiang and Wu, 1989; Waer et al., 1992), it presents serious safety problems when applied to explosives (Andren, 1975).

Several researchers (Gitchel et al., 1980; Mundale et al., 1991; Randall, 1983) have investigated wet air oxidation as a regeneration method. This technology has also been examined for explosives wastewater treated with powdered activated carbon (Freeman, 1985). Freeman concluded that the wet air oxidation process was not an effective regeneration treatment for the removal of high explosives from activated carbon. Several

researchers (Chiang and Wu, 1989; Newcombe and Drikas, 1993; Sutikno et al., 1983) have shown that chemical/solvent regeneration is an effective method for exhausted carbon regeneration. One drawback, however, is that the mixture of solvent and desorbed chemicals still must be disposed as hazardous waste. This problem and other problems associated with activated carbon treatment are overcome in the novel regeneration process presented in Section 2.2.3.

2.2.1.2 Photolysis

Ultraviolet photolysis has been shown to degrade RDX and HMX with absorption maxima between 222 and 233 nm (Burrow et al., 1984). Smetana and Bulusu (1977, as reported in Layton, 1987) reported different reaction rates and products for RDX in distilled water with the fastest rates occurring at wavelengths below the natural sunlight spectrum. Although UV photolysis is effective in degrading high concentrations of RDX in "clean" waters, RDX production waters often contain high concentration of acetic acid, cyclohexanone, and nitrate. The presence of these strong UV adsorbing chemicals makes the process ineffective with respect to RDX degradation (Haas, 1990; McCormick et al., 1984b). Moreover, the effectiveness and economic feasibility of using UV photolysis to treat large volumes of wastewaters contaminated with RDX at low concentrations is questionable.

2.2.1.3 Oxidation

Semmens et al. (1983) attempted to treat RDX and TNT contaminated water with high dosages of ozone and hydrogen peroxide in the absence of UV light. The results

indicated that RDX remained resistant to these oxidants while TNT was slightly degraded. Similar results were obtained with respect to RDX in an additional study performed with iron catalyzed hydrogen peroxide (Fenton's Reagent). The findings for hydrogen peroxide treatment are supported by studies conducted by Burrows et al. (1984). Lastly, the resistance of RDX subjected to large concentrations of chlorine at pH 8 was observed by Semmens et al. (1983).

2.2.1.4 Hydrolysis

Several researchers (Epstein and Winkler, 1951; Jones, 1953; Croce and Okamoto, 1979) have shown that RDX can be destroyed by alkaline hydrolysis. Hoffsommer et al. (1977) identified the end products of alkaline hydrolysis of RDX and a rate determining E2-elimination as the initial step. They also confirmed the reaction to be second-order with respect to RDX and OH^- concentrations. Current research is being conducted at Los Alamos National Laboratories by Spontarelli et al. (1993). They demonstrated the hydrolysis of many explosives at temperatures ranging from 60°C to 150°C. Heilmann et al. (1996) investigated the aqueous alkaline hydrolysis of HMX and RDX for temperatures ranging from 50 to 80°C and in the pH range from 10 - 12, confirming it to be a second-order reaction.

2.2.2 Biological

2.2.2.1 Aerobic

Several researchers have investigated the possibility of aerobically degrading RDX. The predominant results from these studies conclude that aerobic biotransformation has no effect on the presence of RDX or HMX. Moreover, these results are supported by the environmental persistence of RDX in soils and wastewater lagoon sediments originating from World War II contamination (Haas, 1990). An extensive study was conducted by Ro and Stenstrom (1991) in which various cultures and broth media were tested. Inoculum were collected from several sources including contaminated lagoon sites, soils and a local wastewater treatment plant. The inocula were subjected to enrichment with and without the presence of additional carbon and nitrogen sources. All attempts to aerobically acclimate the cultures to metabolize RDX were unsuccessful. Similar studies have also concluded that aerobic cultures are ineffective in treating RDX contaminated waters. McCormick and his co-workers (1981) also investigated the prospect of utilizing aerobic cultures for RDX biodegradation. Results obtained in these studies were also negative. Hoffsommer and his co-workers (1978) investigated an aerobic activated sludge system; however, no disappearance of RDX was found. In similar studies, HMX and RDX were found to degrade solely under anaerobic conditions (Kaplan, 1989; Harvey et al., 1991; Yinon, 1990).

Most research has not observed aerobic degradation of RDX and HMX; however, a recent study conducted by Thiboutot et al. (1994) suggests that aerobic degradation is possible under certain conditions. In their study, inoculate from enrichment cultures started with RDX contaminated soils were used. Two different approaches were taken where RDX

was present, as a source of carbon or as a source of nitrogen. Approximately 38% degradation was observed after 5 days when RDX was used as a nitrogen source. There results have not been confirmed.

2.2.2.2 Anaerobic

Biodegradation of RDX can occur under anaerobic and anoxic conditions. Metabolism of RDX is facilitated through the use of organic co-substrates. The biotransformation of RDX and the corresponding N-acetylated derivative, hexahydro-1-N-acetyl-3,5-dinitro-1,3,5-tiazine (AcRDX) has been observed by several researchers (McCormick et al., 1981; McCormick et al., 1984a; Spanggord et al., 1980). In these studies, RDX was degraded only in the presence of supplemental carbon. The degradation products from the sequential reduction of the nitro groups to nitroso groups included mono-, di- and tri-nitroso derivatives. In addition to these products, formaldehyde, methanol and trace amounts of hydrazines (1,1-dimethylhydrazine and 1,2-dimethylhydrazine), were detected by GC/MS analysis. The proposed pathway for anaerobic biodegradation of RDX is shown in Figure 3 (McCormick et al., 1981). In this scheme, sequential pathways involving reduction to nitroso and hydroxylaminonitramines followed by cleavage reactions were proposed to account for the transitions. The authors postulate that the molecule becomes unstable when any one of the nitro groups is reduced beyond the nitroso level. Hydrolytic cleavage occurs at this point and the fragments may be further reduced or rearranged to give the final hypothesized products.

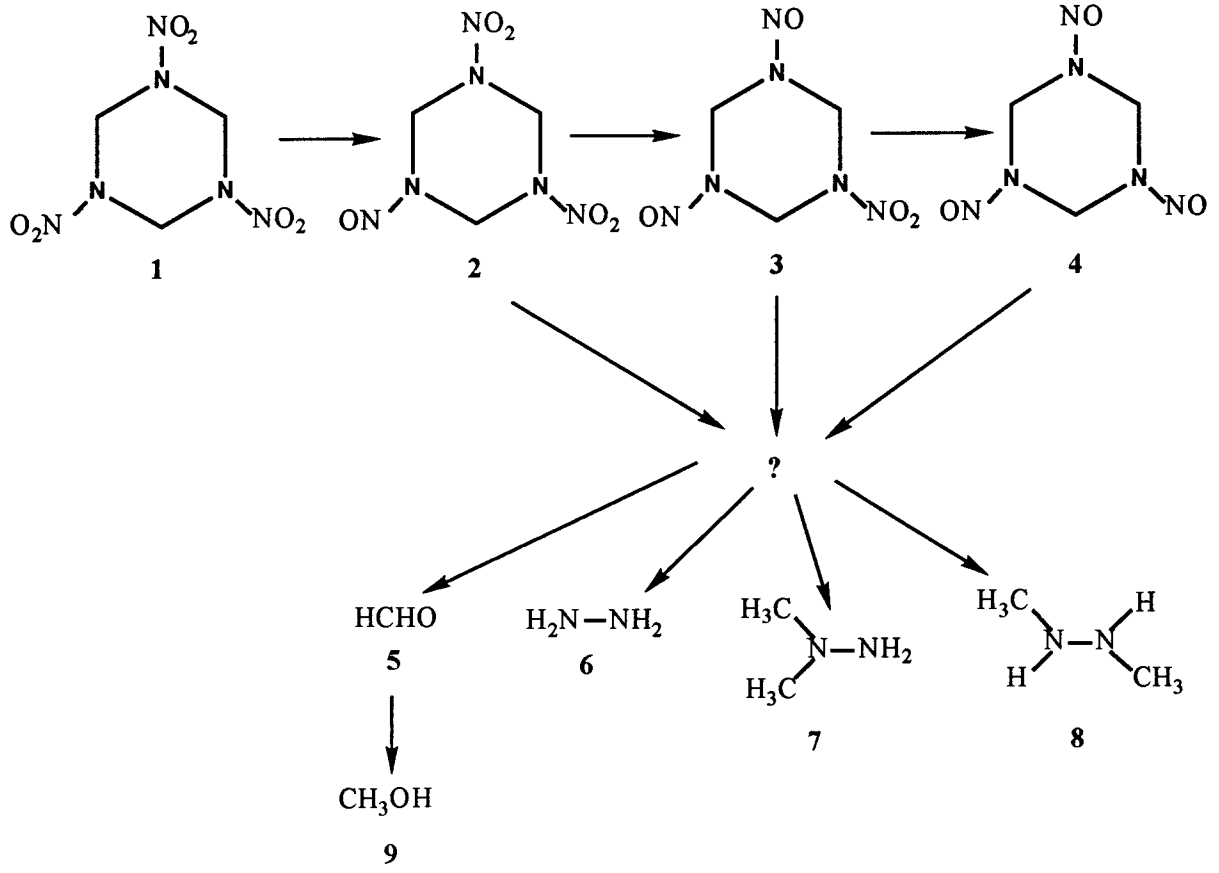


Figure 3 Proposed intermediates for the anaerobic biodegradation of RDX; 1 = RDX; 2 = MNX; 3 = DNX; 4 = TNX; 5 = formaldehyde; 6 = hydrazine; 7 = 1,1-dimethylhydrazine; 8 = 1,2-dimethylhydrazine; 9 = methanol (adapted from McCormick et al., 1981).

Hesselmann and Stenstrom. (1994) conducted extensive batch studies with cultures originating from several sites including an anaerobic digester sludge from Hyperion Waste Water Treatment Plant (Los Angeles, CA), pond sediment and a mixture collected from contaminated sites near Amarillo, Texas. He demonstrated that RDX degradation occurs under fermentative, sulfate-reducing and denitrifying redox conditions. Oxygen exposure produced inhibitory effects, however, it was not toxic to the cultures. A depletion in oxygen

was found which suggests the presence of facultative anaerobes in the mixed culture. No inhibitory effect of nitrate or sulfate on the reductive degradation was observed. Both Hesselmann and Stenstrom (1994) and McCormick (1981) confirmed that anaerobic bacterial populations are able to degrade RDX without any adaptation phase. In addition, the cultures depended on an organic co-substrate to perform the degradation reactions.

HMX degradation was shown to occur in batch after the inoculation of nutrient broth with anaerobic sewage sludge cultures (McCormick et al., 1984a). Based on the results obtained from the study, the author postulated that HMX also undergoes a stepwise reduction of nitro groups. Anaerobic incubation produced intermediates, however, they were not conclusively identified. In this study, the relative rate of HMX degradation was much slower than that of RDX when compared under similar conditions. Spanggord et al. (1980) were able to achieve microbial degradation of HMX in an anaerobic medium (as reported in Layton, 1987). They found the products corresponding to the N-nitroso intermediates of RDX degradation in addition to the isolation of 1,1-dimethylhydrazine. Bell and Burrows (1987) investigated the use of a semicontinuous activated sludge treatment system. The system incorporated conventional activated sludge treatment, nitrification, denitrification and clarification in one treatment vessel. Although RDX degradation was detected, no decrease in HMX concentration was found.

McCormick et al. (1984b) conducted continuous culture studies. The system consisted of two interconnected 1 liter flasks and a pump that was adjusted to provide the desired retention time. The retention times, typically ranging from 4 - 18 days were measured as the number of days required to collect a volume of effluent equivalent to the

total volume of the reaction vessels. Although the author describes the system as a continuous culture system, a more appropriate description would be a semibatch process since there was no flow from the reactor. Complete disappearance of RDX occurred after retention time of 10 - 14 days using peptone (4 g/L) or molasses (3 mL/L) as the organic substrate and nitrate as the terminal electron acceptor. Decreasing the peptone concentration to 0.4 g/L markedly decreased the disappearance of RDX. Hydrazines were never detected in their study.

Alatriste-Mondragon (1991) investigated the ability of methanogenic cultures to degrade RDX. He found that RDX is toxic to both pure and mixed methanogenic cultures. Currently, he is conducting studies to determine the specific enzyme(s) responsible for RDX and HMX degradation.

The ability of anaerobic and anoxic bacteria to degrade RDX and HMX make them the best candidate for the biological portion of the proposed treatment system. All biological studies presented in this paper were conducted with a mixed culture of anoxic/denitrifying bacteria.

2.2.3 Proposed Treatment Process

As stated earlier, recent increases in explosives waste production have exacerbated the disposal and treatment problems. The goal of this research is to develop a process that can effectively treat the large and increasing volumes of this extremely problematic, low concentration waste. The proposed continuous flow process involves two steps: 1)

adsorption of RDX onto activated carbon with subsequent desorption, and 2) biological degradation of RDX in the regeneration fluid .

In the first stage, RDX is adsorbed to activated carbon. This effectively removes and concentrates the explosive from the wastewater source. The influent wastewater is passed through a series of granular activated carbon (GAC) columns. As a column becomes exhausted, it is removed from the series for regeneration and the flow is re-directed through the remaining columns. This set-up allows for continuous operation.

In the second phase, the column removed from operation undergoes desorption with an organic solvent/water mixture. After complete desorption, the RDX-laden solution is transferred to a continuous flow biological reactor. The solution is recirculated through the reactor, with the addition of feed, until the desired effluent concentration is reached. After desorption, the regenerated column is returned to the series of adsorbing columns and the next saturated column is removed and placed in the desorption phase.

The system is comprised of two continuous flow processes, activated carbon adsorption and biological degradation coupled by the desorption step. The decoupling of flow between the GAC columns and the bioreactor eliminates biofouling of the activated carbon and results in extended column life.

The process will effectively treat the problematic, low concentration wastewater. In addition, the process eliminates the dangerous accumulation of explosives and the need to dispose of the carbon as solid hazardous waste. Figure 1 presents a schematic drawing of the proposed treatment process.

2.3 RDX Characterization

High Pressure Liquid Chromatography (HPLC) is the most widely employed method for RDX characterization. It is an effective technique for the separation and quantification of organic munitions including RDX, HMX and TNT. HPLC methods can vary with respect to column type and size, mobile phase composition, UV detection wavelength, injection volume and flow rate. The most common sorbents are C-8 and C-18 reversed phase columns. Several different mobile phases can effectively separate RDX and HMX: 20% methanol in water, 230 nm (McCormick, 1981); 55% methanol in DI water, 240 nm (Peters and Burton, 1991); linear gradient 15 to 40% methanol in water over 8 minutes, 229 nm (Green et al., 1985) and a biphasic linear gradient 20 to 60% acetonitrile in water over 20 minutes, solvent ramp continued from 60 to 100% acetonitrile for 10 min, 243 nm (Harvey et al., 1991). All mobile phase combinations listed above allow for direct injection of aqueous samples.

Several additional techniques for RDX analysis can be used, including gas chromatography (GC), mass spectrophotometry (MS), radio-labeling and thin layer chromatography. Semmens et al. (1984) used a gas chromatograph equipped with a flame ionization detector and carrier gas composed of nitrogen, hydrogen and air at 25, 30 and 110 mL/min, respectively. GC/MS is commonly used to facilitate identification of degradation products (McCormick et al., 1981).

3. MATERIALS AND METHODS

3.1 Materials

RDX was supplied by Lawrence Livermore National Laboratories, Livermore, CA. Shipments contained 1 gram of powdered RDX. HPLC analysis of each RDX shipment showed an average HMX impurity of 10% (w/w). Purification via recrystallization was successful (Hesselmann and Stenstrom, 1994); however, the percent yield was not high enough to justify further purification of incoming shipments. All chemicals used for nutrients (ASC Grade) and solvents (HPLC grade) were obtained from Fisher Scientific Co. (Pittsburgh, PA). Absolute grade, pure ethanol, was obtained from Quantum Chemical Co. (Tuscola, IL). A Barnstead RO/NANO pure water purification system was used to produce de-ionized (DI) water. Sterile tuberculin syringes and needles were purchased from Owens and Minor (City of Industry, CA). All samples were pre-filtered with sterile 0.2 mm ACRCODISC-13mm filters (Gelman Sciences, Ann Arbor, MI) obtained from Fisher Scientific Co. (Pittsburgh, PA).

Three different bituminous, granular activated carbons were analyzed: 1) Darco 20 X 40, Batch No. 23-91, American Norit Co., Inc. (Atlanta, GA); 2) Filtrasorb 400, Lot No. 2518-S, Calgon Carbon Co. (Pittsburgh, PA); and 3) Norit PK 1-3, Sample No. A-6512. Physical properties are listed in Table 7.

Acrylic columns with an I.D. of 25 mm and a length of 200 mm (98.17 mL volume) were used in the continuous flow biological experiments. Three different packing materials were examined: borosilicate glass beads, 3 mm (fisher Scientific Co., Pittsburgh, PA); cut

Masterflex silicone and Tygon tubing, size 13 (Cole-Parmer, Niles, IL). Glass chromatography columns (10 mm ID, 250 mm length) with PTFE end fittings were purchased from RAININ (Woburn, MA) for use in the GAC adsorption studies.

3.2 Analytical Methods

High pressure liquid chromatography was used to analyze RDX, HMX and other compounds in solution. The HPLC analysis was performed with a Hewlett Packard 1050 Series variable wavelength detector (Avondale, PA) equipped with an autosampler and a Hewlett Packard 3396 Series II integrator. The mobile phase consisted of 40% water, 30% methanol and 30% acetonitrile at a flow rate of 1 mL min^{-1}. The sample injection volume and detection wavelength were set at 10 mL and 236 nm, respectively. Separation through a 10 m, Adsorbosphere, C18 reversed phase column and corresponding 5 m guard column (Alltech, Deerfield, IL) at 25°C, resulted in retention times of 4.1 min (RDX) and 3.6 min (HMX). The following calibration standards were prepared: 1) 1, 5, 10, 20, 40 mg/L RDX in DI water; 2) 5, 10, 20, 40, 100 mg/L RDX in pure ethanol; 3) 25, 50, 100, 250, 500 mg/L RDX in acetone; and 4) 0.5, 1, 2, 4 mg/L HMX in DI water. Sample calibration curves are presented in Appendix A.

Prior to injection, samples were filtered through sterile 0.2 mm ACRCODISC-13 mm filters. A study was conducted to determine if the ACRODISC filter membrane adsorbed a detectable amount of RDX or HMX. Two filter sizes, 13 mm and 25 mm diameter, were tested over a concentration range of 0 - 40 mg/L RDX (including HMX impurity) by

comparing the HPLC output between filtered and un-filtered 1 mL aliquots. The results for this study show that a significant amount of RDX was adsorbed by the 25 mm diameter filter, while negligible adsorption occurred with the 13 mm diameter filter, see Figures 4a and 4b. To avoid correction factors, all subsequent studies were performed with the 13 mm diameter filters.

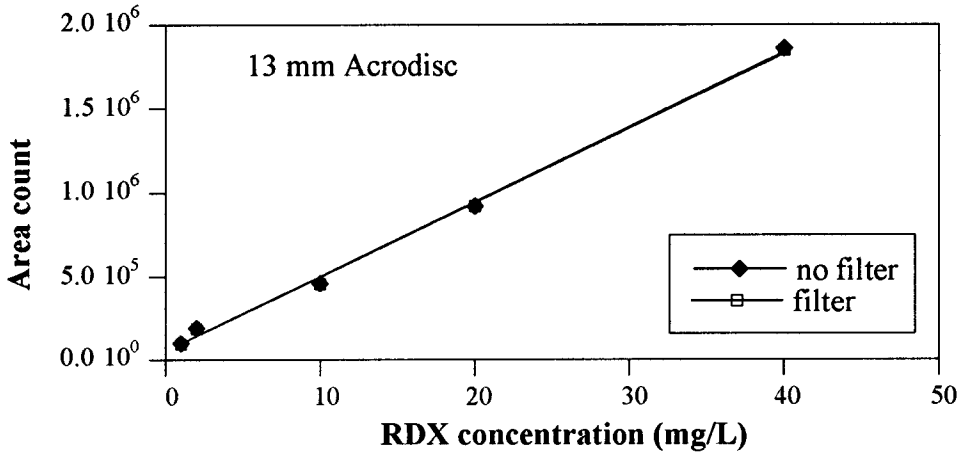


Figure 4a RDX adsorption to the 13 mm Acrodisc filters.

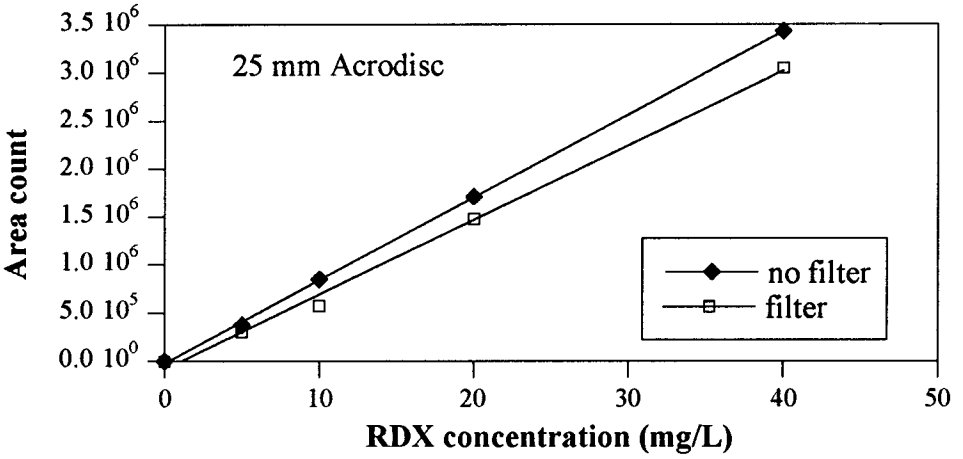


Figure 4b RDX adsorption to the 25 mm Acrodisc filters.

A Perkin Elmer Junior Spectrophotometer (Norwalk, CT) was used to quantify cell growth. The instrument was set to measure turbidity at 600 nm; the values obtained, in turbidity units, were used as a relative measurement of bacterial growth. Batch experiments were carried out in 25 mL test tubes which were directly inserted into the spectrophotometer for analysis. DI water was used to zero the instrument and the initial turbidity of each sample (prior to growth, $t = 0$) was recorded and subtracted from subsequent measurements, $t > 0$.

Continuous flow tracer studies were carried out on a Hewlett-Packard 8452 Diode Array Spectrophotometer equipped with a Hewlett-Packard 89530 MS-DOS UV/VIS software package (Avondale, PA) and a UV flow cell (Spectum, Inc, Los Angeles, CA). Potassium nitrate, $KN0_3$, at 0.01 M was used as the tracer at flowrates of 1 and 0.3 mL/min. The instrument was run in the "single wavelength" mode; the optimal wavelength of 302 nm was used.

3.3 Activated Carbon Experiments

3.3.1 Batch Adsorption Isotherms

The following procedure was used to prepare the carbon for analysis. Activated carbon of mesh size 28/30 US sieve was first washed with deionized water to remove any fines. The carbon was then air dried for 2 days at ambient conditions in order to drive off the bulk of the water. The adsorbates were subsequently dried at 105°C for 1 day and allowed to

cool to room temperature in a desiccator. The carbon was stored in a desiccator at room temperature prior to analysis.

Isotherms were developed for each of the three activated carbons using both the traditional experimental method and a novel batch method (Hesselmann and Stenstrom, 1994). The novel method was conducted as follows. A stock saturated aqueous solution of RDX in DI water was prepared (approximately 40 mg/L) and allowed to equilibrate with room temperature (25 - 26°C). Three glass vessels, one for each type of carbon, were filled with 5.5 liters of the stock solution and placed on an orbital shaker set at 90 RPM. The vessels, void of carbon, were mixed for 7 days to determine any adsorption of RDX by the glass vessel. No detectable decrease in RDX concentration was observed. Six aliquots of 0.2 g GAC were sequentially added to each vessel and allowed to equilibrate.

The following method was used to acquire adsorption isotherm data. A 0.2 g aliquot of carbon was placed into the vessel, then successive 0.5 mL samples were extracted from the vessel and analyzed on the HPLC until equilibrium was established. RDX concentration in the aqueous phase was calculated from calibration curves (peak area versus concentration in the aqueous phase) that spanned the concentration range of 1 to 40 mg/L RDX. After equilibrium was reached, the next aliquot of activated carbon was added, and the above procedure repeated. Both kinetic and equilibrium data were obtained from this novel batch method. In addition, a substantial amount of RDX-laden carbon was produced and could be used for the subsequent desorption studies. For these reasons, the novel method is more desirable than the traditional method; however, the traditional method was also conducted to confirm the results obtained in the novel method.

The traditional adsorption method was performed as follows. Precisely weighed quantities of activated carbon were added to five 4-L Erlenmeyer flasks. The flasks were then filled with the previously prepared saturated aqueous solution of RDX. One control flask containing saturated RDX solution without carbon was run simultaneously. The flasks were mixed with magnetic stirrers at ambient temperature for 7 days. Results from previous adsorption experiments indicated that a 7-day period was sufficient to ensure equilibrium.

The amount of adsorbed RDX was calculated from the following mass balance:

$$q = \frac{(C_o - C_{eq}) * V}{m} \qquad (1)$$

in which C_o and C_{eq} are the initial and final (equilibrium) concentrations (mg/L), respectively, V is the volume of the solution (L), m is the mass of the activated carbon (g), and q is the amount of RDX adsorbed (mg RDX/g GAC). The amount of RDX adsorbed to the carbon was calculated by the difference between the dried carbon weight before and after adsorption. The former method proved to be a more accurate procedure for the analysis. The activated carbon data were fit to a Freundlich isotherm and the empirical constants were calculated.

3.3.2 Continuous Flow Adsorption

A large amount of RDX-laden carbon was produced by continuous adsorption and was used in the subsequent desorption studies. Two grams of Filtrasorb-400 activated carbon were placed in a glass column (10 mm ID, 20 mm length) with adjustable PTFE end fittings. A 20 liter stock solution of 40 mg/L RDX in DI water was prepared and pumped through the

column at 1 mL/min. Both the column and stock solution were equilibrated with the ambient temperature. The effluent from the column was collected and analyzed by HPLC to detect the breakthrough of RDX. The concentration of RDX remaining in the 20 L of effluent was measured. The carbon was dried in a desiccator at room temperature for several days before the final mass was measured. Drying was continued until a constant mass was attained. The mass balance in Equation (1) was used to calculate the adsorption capacity and the value was compared to calculations based on initial and final carbon mass.

3.3.3 Desorption: Water as Solvent

RDX-laden carbon generated in the adsorption isotherm experiments was used in the first set of desorption experiments. The carbons under investigation were divided into three approximately equal portions, each of which was weighed and recorded. The experimental set-up consisted of a 4-L Erlenmeyer Flask equipped with a condenser, magnetic/stirring hotplate and a floating stirring bar. This type of stirring bar was used to minimize the grinding of carbon that frequently occurs during conventional magnetic stirring. Water at 80°C was used as the desorption solvent. Each sample was placed into 2 L of pre-heated DI water. The system was continuously mixed during which periodic 0.5 mL samples were taken for HPLC analysis. After equilibrium was established, typically two days, the final concentrations were measured and the amount of RDX desorbed was calculated.

3.3.4 Desorption: Ethanol and Water

Based on results from both the adsorption isotherm experiments and the first set of desorption experiments which used heated water as a solvent, Filtrasorb-400 was chosen for the proposed treatment process. Thus, subsequent studies and optimization concentrated on this particular carbon.

The first phase of this study was conducted at room temperature with 8 different mixtures of ethanol and water as the desorption solvents. The following ethanol/water mixtures were analyzed: 100, 50, 30, 20, 10, 5, 1, and 0% ethanol in water by volume. The RDX-laden carbon was separated into 8, 0.2 g aliquots and each was placed into a vial containing 100 mL of solvent. The vials were placed on an orbital shaker for 6 days. Several 0.5 mL samples were taken to follow the RDX in solution and confirm equilibrium. The total volume extracted from each vial for analysis did not exceed 2.5 mL. All carbon was subsequently dried and weighed to determine, by mass loss, the amount of RDX desorbed.

In the second phase of this experiment, the carbon originating from the 50% and 100% ethanol vials was placed in fresh solvent to determine if the maximum desorption at ambient conditions was obtained. The vials were mixed for several days. After equilibrium was established, the final concentration was recorded. The effect of elevated temperature was determined by placing the vials into a 70°C oven with periodic mixing. An equilibrium concentration was determined and the total desorption for each vial was calculated. Similarly, the carbon was dried and weighed to verify HPLC analysis data and assess any carbon loss.

3.3.5 RDX Solubility in Ethanol (0 - 100%)

Limited and dispersed data with respect to the solubility of RDX in absolute ethanol was found in the literature (Rodgers, 1962; Urbanski, 1967; Hesselmann and Stenstrom, 1994). In addition, only one paper reported RDX solubility at a temperature equivalent to the experimental conditions presented in this report. The following ethanol and water mixtures were used to develop the RDX solubility curve: 0, 5, 10, 15, 25, 33.3, 50, 100% (V_{EtOH}/V_{water}). A total volume of 20 mL was prepared in triplicate for each mixture. The solutions were placed in 100 mL flasks into which RDX was added in excess to pre-estimated solubility limits. All vials were mixed on a shaker table for seven days at a temperature of 26°C. The vials were periodically checked for visible precipitate to verify that each mixture was saturated. At the end of five days a 0.5 mL sample was extracted for HPLC analysis. A second set of samples was taken on the seventh day to determine if equilibrium was established. At each concentration of ethanol and water, the average of the three RDX solubilities was calculated and the corresponding solubility curve was generated.

3.4 Biological Experiments

3.4.1 Denitrifying Cultures

Anaerobic digester sludge was collected from the Hyperion wastewater treatment plant in Playa Del Rey, CA. In addition, a mixture of sludge and wastewater was collected from several different RDX-contaminated sites near Amarillo, TX. All samples were diluted with oxygen free phosphate buffer, filtered, and incubated for three weeks in a minimal

medium with ethanol, potassium nitrate and phosphate buffer. A mixture of the samples was used as inocula for subsequent batch and continuous flow experiments. All constituents of the feed are listed in Tables 2 and 3.

Table 2 Basal medium composition.

Component	Concentration (mg/L)
K_2HPO_4	5000
$NaH_2PO_4 \cdot H_2O$	2875
KNO_3	1600
NH_4Cl	200
$MgCl \cdot H_2O$	100
$CaCl_2 \cdot 2H_2O$	40
Na_2SO_3	19.7
Ethanol	20 (mL/L)

Table 3 Trace mineral composition.

Component	Concentration (mg/L)
$FeCl_3$	3.9
$MnCl_2$	0.95
$ZnCl_2$	0.66
$CoCl_2 \cdot 6\ H_2O$	0.58
$CuCl_2 \cdot 2\ H_2O$	0.30
$Na_2Mo_4 \cdot 2\ H_2O$	0.46
$Na_2B_4O_7 \cdot 10\ H_2O$	0.24

3.4.2 Batch Experiments

The following method was used to prepare the medium for the batch biological experiments. To provide constant anoxic conditions, the medium was flushed with oxygen-

free nitrogen gas throughout the duration of the preparation. In the first step, de-ionized water was heated to the point of boiling. The RDX and inorganic chemicals (non-heat sensitive) were added during the heating process. After the RDX dissolved, the flask was removed from the heating element and allowed to cool. The heat sensitive components of the medium which included the inoculum and Na_2S were subsequently added and the nitrogen flushing was continued for an additional 30 minutes. The volatile organic co-substrates were then added and the total volume was adjusted with de-oxygenated water to achieve the appropriate volume.

Experiments were conducted in 25 mL glass test tubes sealed with butyl-rubber stoppers. The test-tubes could be directly inserted into the spectrophotometer for growth measurements. Samples for RDX analysis were obtained by inserting a needle tipped Tuberculin syringe through the rubber stopper and extracting 0.5 mL aliquots.

Test tubes were flushed with oxygen-free nitrogen gas prior to being filled with 20 mL of medium. The transfer of prepared medium and any other constituents to the test tubes was done under oxygen-free conditions. Vessels were placed on a shaker table and continuously mixed at room temperature, 24 - 26°C. All experiments were conducted in triplicate with the average of the three results used for analysis.

3.4.2.1 Various Organic Solvents as Co-substrate

In this study the ability of the denitrifying cultures to utilize various organic solvents as the sole co-substrate was determined. As mentioned previously, the organic solvent will be used as both a co-substrate for metabolism and as a solvent for RDX desorption from

activated carbon. For this reason, the following seven solvents were chosen for analysis: ethanol, acetic acid, propionic acid, formic acid, ethyl acetate, acetone and methanol. In all samples, the total organic carbon was held constant. Table 4 lists the amount of each solvent added to achieve a uniform total organic carbon concentration of 1.26 g/L. An initial RDX concentration of 20 mg/L was used. Results for RDX reduction were obtained after 7 days of incubation.

Table 4 Organic substrates used in the batch co-substrate experiment.

Co-substrate	g/L	TOC (g/L)
Methanol	3.34	1.26
Ethanol	2.40	1.26
Sodium Formate	7.10	1.26
Sodium propionate	2.58	1.26
Acetone	2.57	1.26
Ethyl acetate	2.30	1.26

3.4.2.2 Effect of Ethanol Concentration

The previous experiment demonstrated the effectiveness of ethanol as a co-substrate for the RDX degrading culture. The following study was conducted to determine the effect of ethanol concentration on both growth and RDX degradation. Eight different concentrations between 0 and 200 g/L were used with corresponding total organic carbon concentrations between 0 and 104 g/L, see Table 5. A 200 mL inoculum was added to 20 mL of feed. Both RDX degradation and culture growth were followed over an 8 day period.

Table 5 Initial ethanol concentration data for the batch ethanol experiment.

Ethanol concentration (g/L)	TOC (g/L)
0.0	0.0
5.0	2.6
25.0	13.0
50.0	26.1
75.0	39.1
100.0	52.2
150.0	78.3
200.0	104.3

3.4.3 Continuous Flow Experiments

The continuous-flow experiments were conducted in anoxic reactors described in detail in the next section. The columns were operated at room temperature (26 - 27°C). Fresh feed was prepared daily using the same method as the batch experiments. The feed constituents are listed in Tables 2 and 3 unless otherwise specified. Ethanol served as the primary substrate for support of bacterial activity for degradation of the secondary or "trace" substrate RDX. Feed was continuously passed through the reactor in an upflow direction using a Masterflex Model 7524-10 pump drive equipped with a multiple cartridge pump head. Tygon tubing was used to deliver the feed from the preparation flask to the reactor. The flask and tubing were flushed with dilute acid solution every other day to prevent growth of bacteria outside the reactor.

Influent and effluent samples were analyzed every 24 hours. For this analysis, 1 mL of solution was extracted, filtered, and transferred to a 2 mL HPLC sampling vial. The

sealed vials were immediately analyzed for RDX concentration. The effluent flowrate was checked daily.

3.4.3.1 Description of Reactors

Continuous flow biological studies were conducted in 200 mm Plexiglas columns, see Figure 5. Each column had an internal diameter of 25 mm resulting in an empty bed volume of 98.17 mL. The inlet and outlet to each reactor was equipped with a stainless steel screen to retain the packing material and was sealed with Plexiglas, glide fit end caps. In addition to the ports located at the inlet and outlet of the reactor, eight sampling outlets were placed along the length of the column. Tygon or glass tubing, fittings, flasks and vials were used to minimize sorptive interactions.

Three different packing materials were tested in the continuous flow studies. Column #1 was packed with 137 grams of 3 mm borosilicate glass beads. Columns #2 and #3 were packed with 42 grams of size 13 (diam. ~ 2 mm) Silicone and Tygon tubing, cut to lengths of 1 - 2 mm. Interstitial volumes, calculated from the tracer studies are presented in Table 6. Columns were inoculated with the mixed denitrifying cultures described in Section 3.4.1. This was done by injecting 1 mL of the mixed culture into the lower quarter of the reactor (pre-filled with feed solution). The feed flow was initiated 2 weeks after inoculation to allow for culture growth and attachment to the packing material. Before experimental data was collected, each reactor was monitored to ensure steady-state conditions.

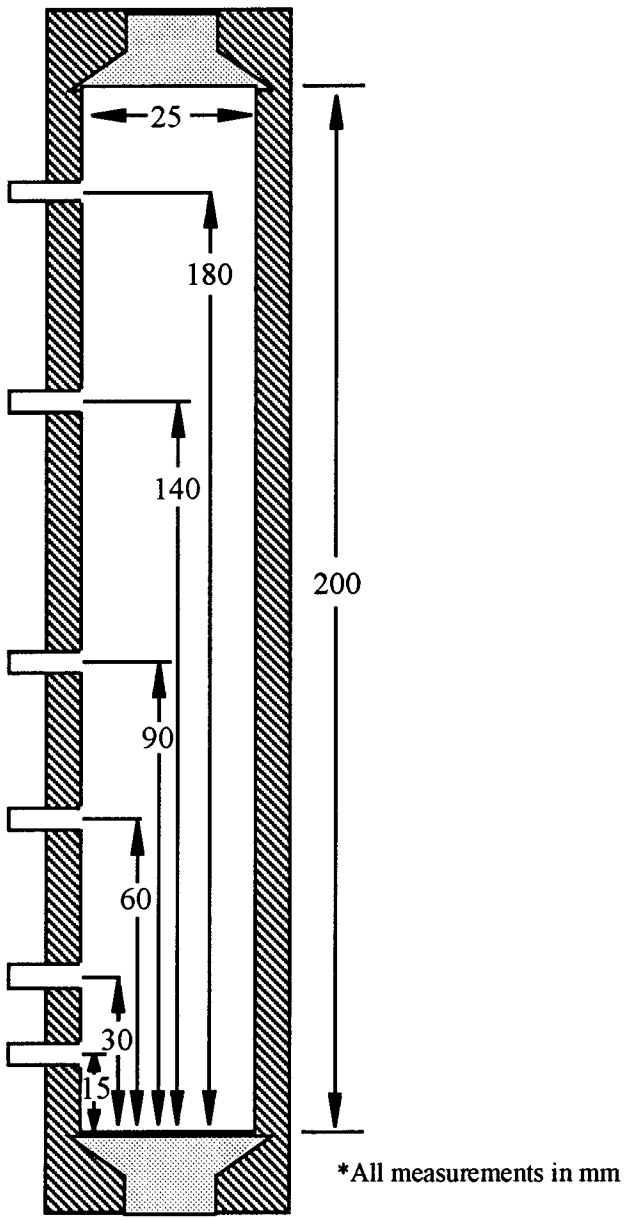


Figure 5 Biological reactor drawing (laboratory scale). Acrylic (Plexiglas) reactor operated in upflow direction. Total volume 98.2 mL.

Table 6 Continuous flow reactor characteristics.

Reactor	Glass	Silicone	Tygon
Mass of packing material (g)	137	42	42
Empty bed volume (mL)	98.17	98.17	98.17
Interstitial volume (mL)	46.3	52.7	54.13
Void fraction, θ	0.472	0.537	0.551
Hydraulic retention time (hr) for influent flowrate of:			
0.3 mL/min	2.57	2.93	3.00
0.15 mL/min	5.14	5.86	6.00

3.4.3.2 Experiment Set-up

The following experimental set-up was used for the continuous flow biological studies. As mentioned in the previous section, three reactors were analyzed, each filled with a different packing material. A single 2 L feed flask was used to supply the nutrients spiked with RDX. This allowed for uniform initial concentrations to all three reactors. Separate tubes, originating from the feed flask were passed through the Masterflex multi-cartridge pump to the inlet of their respective reactors. A rubber stopper was used to prevent the transfer of oxygen to the feed. Nitrogen gas was supplied to the flask by a fourth tube inserted through the stopper; this prevented the formation of a vacuum in the flask. The holes in the stopper through which the three feed tubes and Nitrogen gas tube were inserted were sealed with silicone rubber and the assembly was periodically checked for gas leakage.

The Masterflex pump provided for equal flowrates to all three reactors. The feed was pumped in an upflow direction through the reactors and through an outlet tube to the effluent waste collector. The influent and effluent RDX concentrations were measured daily. The

influent sample was taken directly from the feed flask and the effluent samples were collected, as the solution exited the reactor, from a sampling tube located outside of each reactor. Although the set-up was used in a one-pass mode, modifications could easily be made to recirculate the effluent. Figure 6 shows a diagram of the experimental set-up.

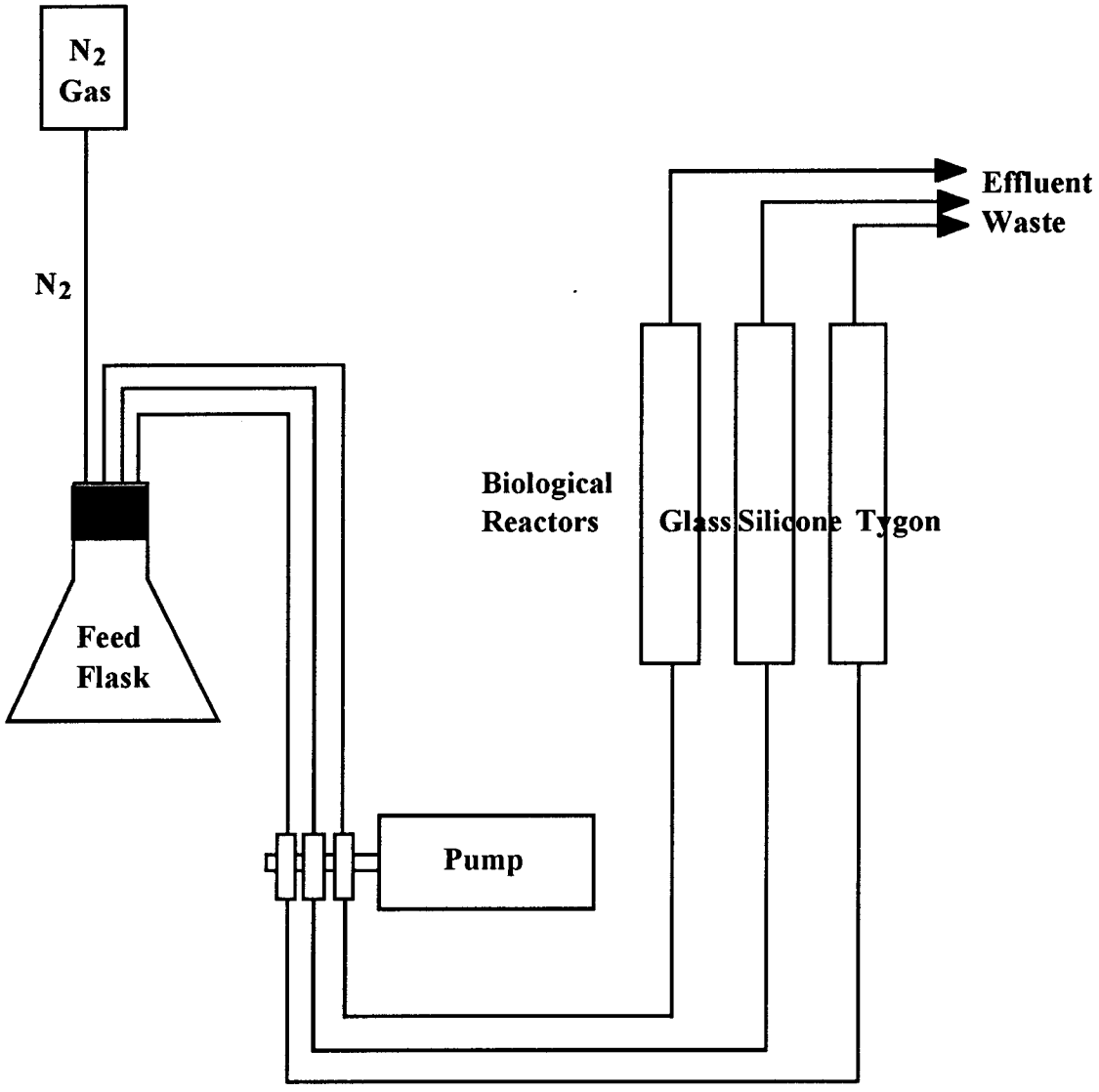


Figure 6 Schematic drawing of the experimental set-up.

3.4.3.3 *Flow Rate and Retention Time Studies*

This study was conducted to determine the effect of decreased flowrate i.e. increased retention time, on RDX degradation. In this experiment, the ethanol concentration was 1 mL/L feed. Each of the three columns were allowed to reach steady-state with respect to RDX degradation at a flowrate of 0.3 mL/min. At this point, the flowrate was decreased to 0.15 mL/min. The influent and effluent concentrations were monitored every 12 to 24 hours until the new steady-state was reached in each reactor. Several weeks later the flow was returned to its original rate of 0.3 mL/min.

3.4.3.4 *Effects of Ethanol Concentration*

A series of experiments were conducted to determine the effect of ethanol concentration on the continuous flow cultures. Preliminary information was obtained from the batch experiments with respect to the toxicity limit. Throughout the analysis, all feed components were kept constant except the ethanol concentration. The temperature of the reactors varied between 26 - 28°C. A constant flowrate of 0.3 mL/min was used.

To initiate the experiment, all reactors were allowed to reach steady state with an inlet ethanol concentration of 1 mL/L feed. After steady state was achieved, the ethanol concentration was increased to the next dosage and sequentially allowed to equilibrate. The ethanol concentrations used were 5, 10, 20 and 50 mL/L feed. RDX concentration was analyzed daily.

For the last ethanol increase (20 - 50 mL/L), only the Tygon and glass reactors were studied. The silicone reactor was kept as a control. After the toxicity limit was reached, the ethanol concentration was lowered to allow the culture to recover.

3.4.3.5 Effects of Elevated Temperature

Previous continuous flow experiments were conducted under ambient conditions, 25 - 27°C. This study was conducted to evaluate the effect of increased temperature on the degradation rate. The Tygon reactor was brought to an elevated temperature of 35°C, while the other two reactors were kept at room temperature.

A constant temperature water bath was used to maintain the elevated temperature. The reactor was wrapped with a coil of Tygon tubing. Heated water was continuously pumped from water bath, through the tubing, and then recirculated to the bath. The effluent concentration was monitored every 24 hours until steady-state was achieved.

3.4.3.6 Reactor Growth Profile

A cell growth profile was developed along the length of the glass packed reactor. This was accomplished by performing a total suspended solids (TSS) analysis on selected sections throughout the column.

Glass microfibre filters (Whatman Ltd., Maidstone, England), 5.5 cm. diameter, were prepared using the following method. All filters were washed with three successive 20-mL portions of DI water. The filters were then allowed to dry in an oven at 103-105°C for one hour and subsequently placed in a desiccator until needed. The glass packed reactor, 20 cm.

in length, was divided into five equal volumes. A TSS analysis was performed on each section, assuming constant cell density within each region. Each of the five sections was further divided into quarters, one quarter was used for TSS analysis and the other three were stored in a flask for the re-packing and startup of the reactor. Cells were removed from the packing material by placing the packing into an Erlenmeyer flask containing 100 mL of DI water. The flask was vigorously shaken for several minutes then the water was decanted into a vacuum filtering flask. This procedure was repeated until all visible growth was removed from the packing material. A last washing was done under sonication for 3 minutes. The pre-weighed filter was removed and dried for 1 hour at 105°C, cooled in a desiccator to balance the temperature and weighed. Drying was continued until a constant weight was attained. Several filters were necessary to separate the cell mass obtained from each of the five sections. The total nonfilterable residue per volume reactor was calculated using the following equation:

$$T_{nfr} = \sum_{1}^{i} (A - B) * V / V_r \qquad (2)$$

where T_{nfr} is the total nonfilterable residue per reactor volume (mg/mL), is the number of filters used, A is the weight of the filter and residue (mg), B is the weight of the filter (mg), V_r is the volume of the sample of reactor analyzed (19.64 mL) and the factor of 4 is necessary to account for 1/4 of the actual section being analyzed.

3.4.4 Kinetics

Transport of a single reactive solute species during steady flow in a one-dimensional homogeneous system may be described by the following reduced form of the general transport equation:

$$\frac{\partial C}{\partial t} = D \cdot \frac{\partial^2 C}{\partial X} - V \cdot \frac{\partial C}{\partial X} - k \cdot C \qquad (3)$$

where C is the cross section-averaged resident concentration of the solute in the liquid phase, X is the distance along the reactor, t is time, D is a dispersion coefficient combining the effects of both diffusion and hydrodynamic dispersion on transport, V is the average interstitial water velocity and k is a first order reaction coefficient (Parker and van Genuchten, 1984).

In order to represent the RDX degradation process with the above equation, several assumptions were made. Negligible mass transport limitations into the cells/biomass were assumed. Solute (RDX) adsorption to the packing material must be negligible. This assumption was validated by a batch study conducted by Hesselmann and Stenstrom (1994), in which negligible RDX adsorption was found for all three packing materials. The next assumption, a homogeneous system, cannot be verified as biological growth throughout the column is not uniform. However, this potential source of error can be quantified by testing identical columns, with and without microbial growth. The last major assumption, a pseudo-first order reaction with respect to RDX degradation, was tested by obtaining the dispersion and velocity coefficients under "no reaction" (k = 0) conditions with subsequent analysis and fitting of reaction data.

3.4.4.1 Tracer Studies

Breakthrough curves were generated under zero reaction conditions by passing a conservative tracer through "clean" columns, i.e. no microbial growth. Step-up data were collected by initially flushing the reactor with DI water in an up-flow direction. The effluent from the reactor was run directly into a diode array spectrophotometer equipped with a flow cell for continuous analysis. After a steady-state, zero absorbance reading was obtained, the inlet solution was switched to 0.01 M potassium nitrate. The pumping flow rate was monitored throughout the run and the absorbance was automatically recorded by a computer every 20 seconds. When the absorbance reached a steady-state maximum value, the inlet solution was switched back to DI water and step-down data was collected until the absorbance returned to zero.

3.4.4.2 Degradation as a Function of Column Length

The following set of experiments were conducted to obtain data that would verify or invalidate the pseudo first-order reaction assumption. The design of the biological reactors allowed for sample collection along the length of the column. One mL samples were extracted from each reactor, at steady-state, and analyzed for RDX concentration. Five separate ports on each reactor were used. At least 3 samples from each port were withdrawn on 3 separate days and averaged. The data are presented in a graph showing the normalized degradation vs. length along the reactor.

3.4.4.3 Transport Model Parameter Determination

The dispersion coefficient and average interstitial water velocity were calculated from the breakthrough data by simulating the transport equation (k = 0) using the Crank-Nicolson method to solve the partial differential equation. The curve generated for a specific D and V was compared to the experimental data in the form C(t)/Co vs. time. Adjustments in D and V were made until the curve matched the experimental data. A sensitivity analysis with respect to grid size was run to ensure accurate results.

Using the model parameters estimated above, the reaction data collected in Section 3.4.4.2 were simulated to determine the pseudo-first order reaction coefficient. The Crank-Nicolson program was used to solve the partial differential equation that included the reaction term. A constant initial condition and zero-flux exit boundary condition were used to solve the equation.

4. RESULTS AND DISCUSSION

4.1 Activated Carbon Studies

4.1.1 Batch Adsorption Isotherms

Granular activated carbon studies were conducted to evaluate the ability of 3 different activated carbons to remove RDX from aqueous solution: Darco 20X40, Norit PK 1-3 and Filtrasorb-400. A summary of the physical properties of each carbon is presented in Table 7. As a result of the relatively low solubility of RDX in water (40 mg/L) and the limited supply of RDX sent to the lab, a novel method was used to test the carbon. The novel isotherm method consisted of a series of batch adsorption experiments in which successive aliquots of GAC were added to a single vessel containing RDX in water (as described in Section 3.3.1).

Table 7 Physical properties of the activated carbon tested (from manufacturer's literature).

	Filtrasorb-400	Darco 20 x 40	Norit
Total surface area (m^2/g)	950 - 1050	665	625
Bulk density (lb/ft^3)	26 - 27	24	24
Total pore volume	0.80	0.94	1.23
-macropores (d > 50 nm)	0.21	0.27	0.74
-mesopores (2 > d < 50 nm)	0.27	0.47	0.19
-micropores (d < 2 nm)	0.32	0.20	0.30

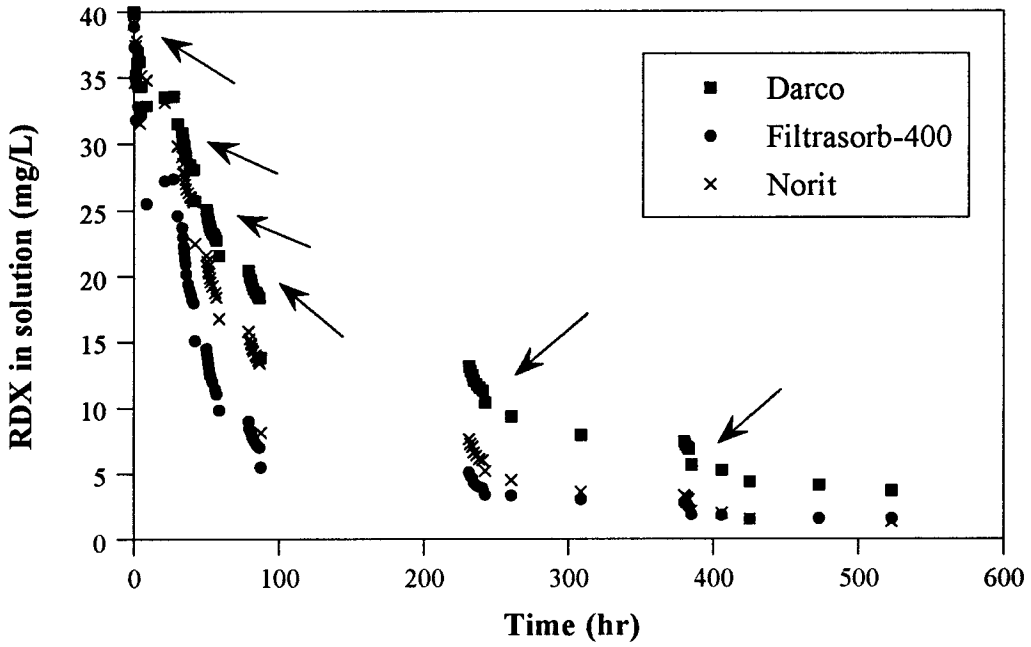


Figure 7 RDX adsorption using a novel 'batch' method. Initial condition: 5.5 L of 40 mg/L RDX. Six aliquots of 0.2 g activated carbon were sequentially added and allowed to equilibrate. Each addition is denoted by arrow.

On the basis of adsorption capacities for RDX, Filtrasorb-400 was the best performing GAC. The saturation capacities for an initial RDX concentration of 40 mg/L were 417, 272 and 227 mg/g GAC for Filtrasorb, Norit and Darco, respectively. Kinetic results, showing the decrease in RDX concentration with time, are presented in Figure 7. In this figure, the addition of each aliquot of carbon is denoted by an arrow. Data for a single addition of carbon are presented in Figure 8. Freundlich isotherms were developed by plotting the adsorption data on logarithmic coordinates as carbon loading (q) versus the equilibrium concentration (C_e) of RDX remaining in the vessel. The results are shown in Figure 9. The empirical constants for the Freundlich adsorption equation are presented in

Table 8. An additional isotherm was developed for HMX using Filtrasorb-400. The adsorption capacity, 217 mg/g GAC, was considerably lower than RDX, see Figure 10.

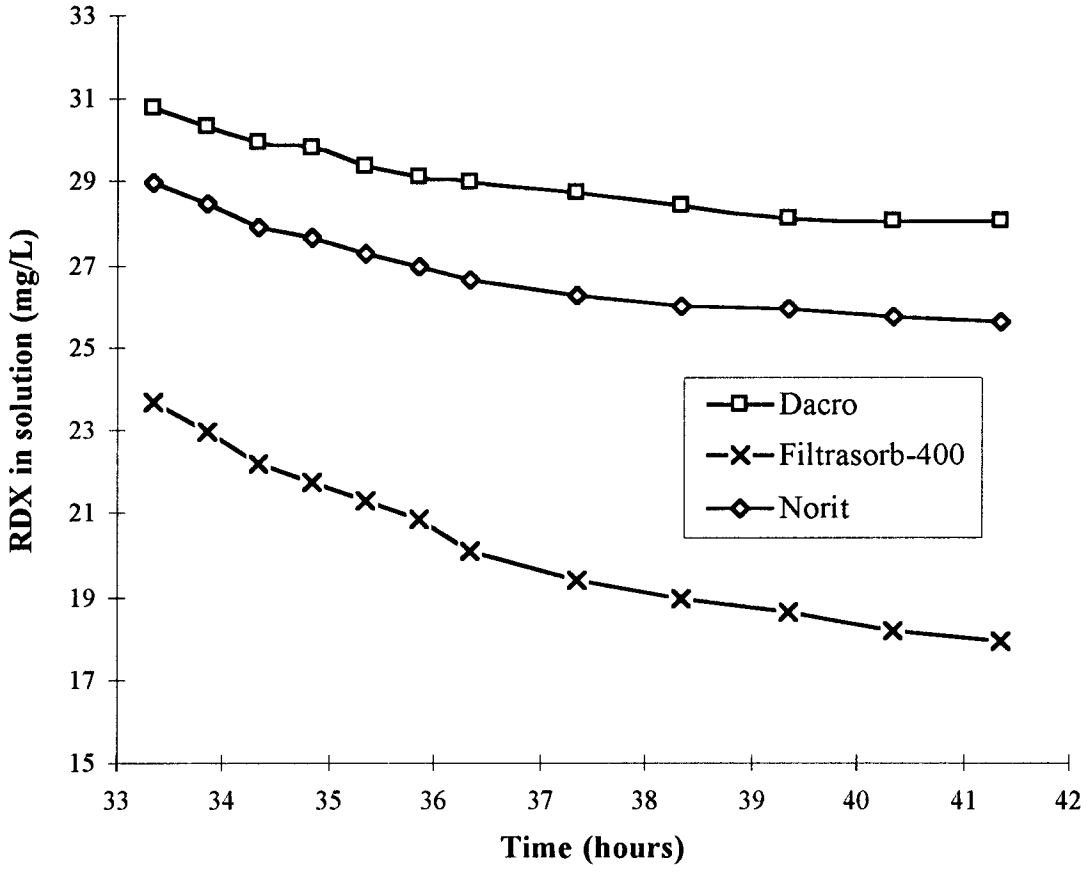


Figure 8 RDX adsorption for a single 0.2 g aliquot of activated carbon.

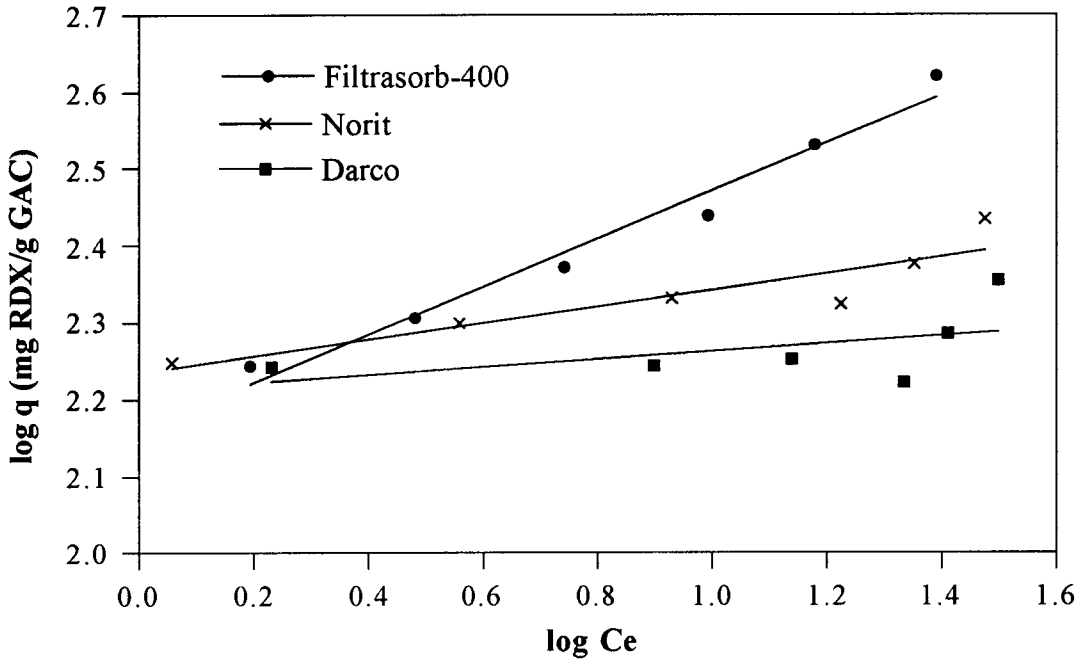


Figure 9 Freundlich isotherms for RDX adsorption to Filtrasorb, Norit and Darco.

Table 8 Empirical constants for Freundlich adsorption equation

Activated Carbon Type	RDX isotherms		Saturation Capacity
	K	1/n	q_e (mg RDX/g GAC)
Filtrasorb-400	0.334	0.31	414
Darco 20 x 40	0.350	0.11	272
Norit	0.344	0.06	227

Note:
$qe = X/M = KCe^{1/n}$
where,

q_e = Carbon loading, mg RDX/ g GAC
X = Co - Ce, the amount of RDX adsorbed for a given volume of solution, mg/L
M = Carbon dosage, mg/L
Co = Initial concentration of RDX, mg/L
Ce = Concentration of RDX remaining in solution mg/L
K = Freundlich constant $(mg/L)^{-1/n}$
n = Empirical constant, dimensionless

The novel "batch" data were compared to data obtained from a traditional adsorption isotherm determination. In this second adsorption experiment, Filtrasorb-400 had the highest adsorption capacity; however, equivalent q values were not found between the two methods. The discrepancy in values may be attributed to a difference in experimental temperatures and/or a difference in mixing methods. The batch method was agitated at 90 rpm by an orbital shaker at 25°C. The second method was conducted in Erlenmeyer flasks with magnetic stirring. Floating stir bars were used to minimize the grinding of the carbon. A small amount of heat was generated by the stirring unit and consequently raised the temperature to 30°C. Since adsorption is an exothermic process it can be expected that less adsorption would occur at the higher temperature; this is consistent with the experimental results. More controlled studies need to be performed to determine the validity of the novel "batch" method.

In the proposed treatment process, the RDX must be desorbed from the carbon prior to biological degradation. Therefore, the GAC adsorption capacity alone is not adequate to determine the optimal carbon for the process. The following section describes the results of the desorption studies.

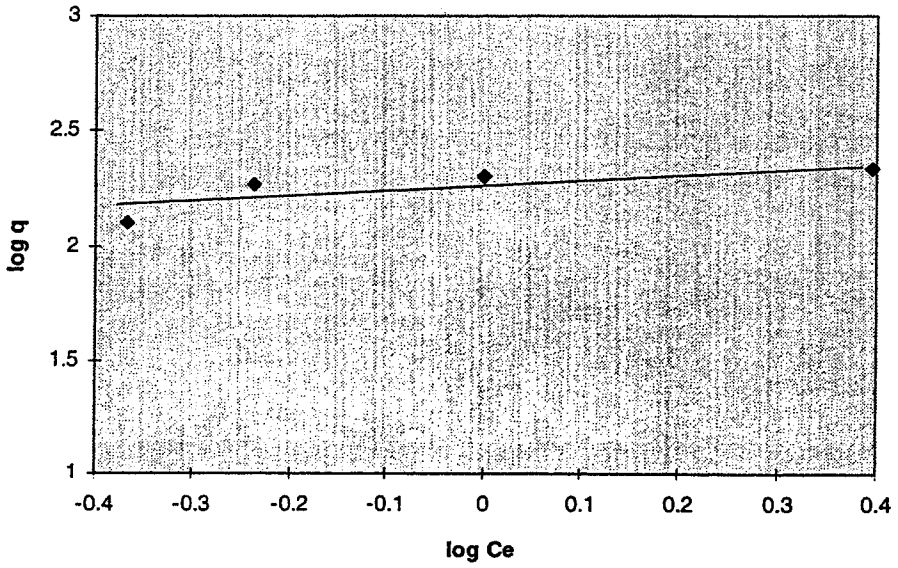


Figure 10 Langmuir isotherm for HMX adsorption to Filtrasorb-400. Adsorption capacity = 217 mg/g.

4.1.2 Desorption: Water as Solvent

Desorption experiments conducted by Hesselmann and Stenstrom (1994) concluded that water at room temperature is not an effective solvent for RDX. Since RDX solubility increases with temperature, as shown in Figure 11, a set of batch desorption experiments was conducted at 78 - 82°C to determine the extent to which elevated temperatures could enhance desorption. Even at the higher temperatures, pure water regeneration of the activated carbon was at best 40% for Filtrasorb-400 and considerably lower for the other two carbons tested. The results for pure water desorption did not look promising enough to pursue further investigation. The focus turned toward organic solvents which have much higher RDX solubilities than water.

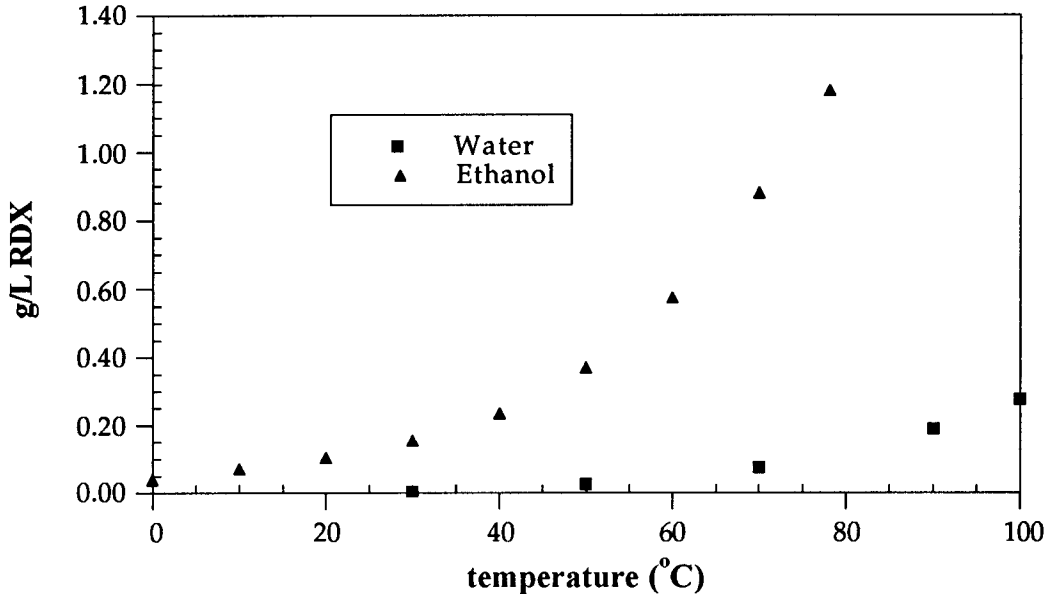


Figure 11 RDX solubility in water and 100% ethanol as a function of temperature [Adapted from Urbanski (1976)].

4.1.3 Desorption: Ethanol/Water as Solvent

Ethanol was chosen as the desorption solvent of interest because it produced both the highest RDX degradation rates and growth rates in the biological experiments, (see Section 4.2). The ultimate advantage of ethanol in the proposed treatment process is that it could be used bifunctionally as both a biological substrate and a desorption solvent. This would allow for direct transfer of the RDX-laden desorption fluid to the biological reactor. In addition, Filtrasorb-400 was chosen for further studies since it had the highest adsorption capacity and had the most promising desorption characteristics in the elevated temperature desorption studies with water.

In this experiment, a series of batch desorption experiments were conducted over a range of ethanol concentrations: 0, 1, 5, 10, 20, 30, 50 and 100% EtOH in de-ionized water. The carbon used in this series contained 354 mg RDX/g GAC. A total of 0.2 g of carbon was placed in each flask corresponding to 71.2 mg RDX. The first of three experiments was performed at room temperature over the complete range of ethanol concentrations listed above. Samples were taken from each flask over a period of 6 days to ensure equilibrium. Equilibrium was established within 1 or 2 days as shown in Figure 12. As expected, RDX desorption increased with ethanol concentration. However, concentrations from 1% to 30% ethanol only slightly enhanced the desorption relative to the pure water results. Marked effects on desorption were seen at ethanol concentrations of 50% and 100%. The amount of RDX desorbed was 23.4 mg and 44.5 mg for 50% and 100%, respectively; all other concentrations desorbed less than 6 mg.

A second desorption was run by decanting the RDX-laden solvent and replacing it with fresh solvent. As seen in Figure 12, only minimal enhancement resulted in the 100% sample while the 50% sample desorbed an additional 13.4 mg RDX. Since the proposed treatment process will be using continuous flow desorption, the amount of RDX desorbed will be higher (than the above batch results) since fresh solvent is continuously introduced into the reactor.

The last desorption experiment was conducted at 60°C with the same flasks used in the previous experiment. Very little improvement was observed in the 100% flask, however the 50% flask reached the same capacity as the 100% at this temperature. The overall RDX desorption for the two concentrations was 65%. A lack of mixing in the elevated temperature

phase may have led to lower than optimal desorption capacities. In addition, the increased concentration driving force provided by a continuous flow set-up should also result in higher desorption.

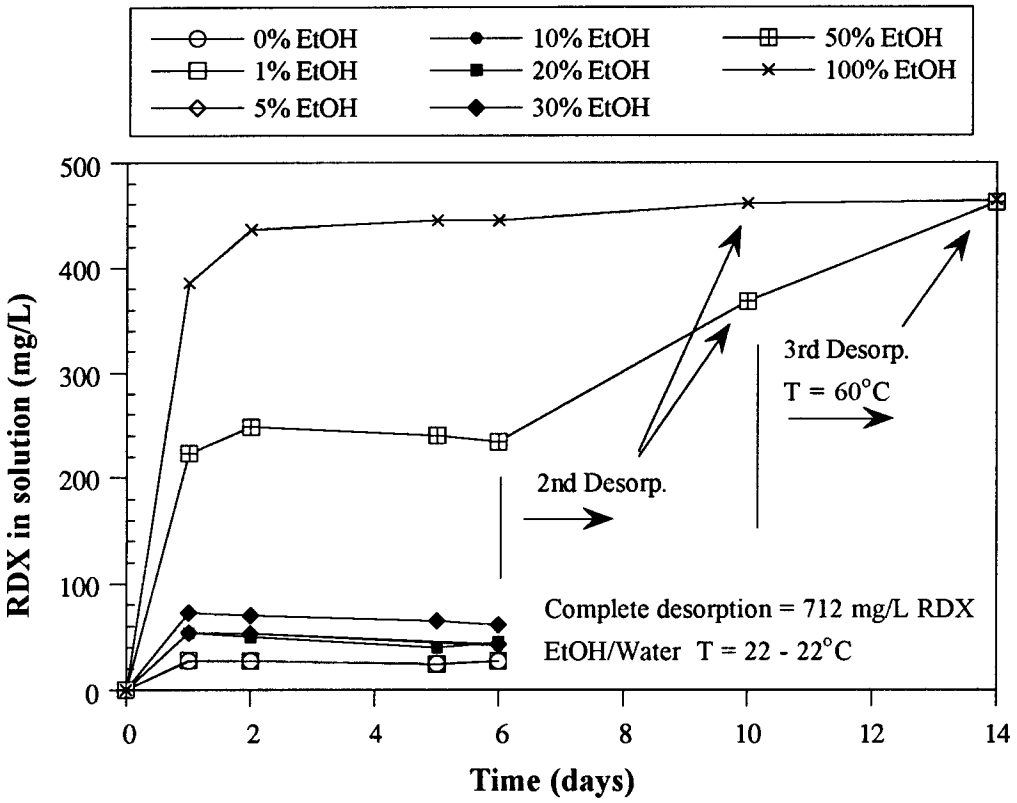


Figure 12 Effect of ethanol concentration on RDX desorption from Filtrasorb-400. Temperature = 22 - 25°C.

4.1.4 RDX Solubility in Ethanol (0 - 100%)

The solubility limit of RDX in 100% ethanol at 26°C was found to be 996 mg RDX/L. A linear relationship existed over the complete concentration range. This experiment was conducted because a review of the literature resulted in limited data with

respect to RDX solubility in organic solvents. Only two references were found for RDX in ethanol (Rogers, 1962; Urbanski, 1967). The values reported in these two studies were 1.3 g RDX/L (30°C) and 0.8 g RDX/L (25°C). Since our experimental conditions did not match either of these, a solubility experiment was conducted. Figure 13 shows the solubility of RDX in mg/L versus ethanol concentration in % (V_{EtOH}/V_{water}) over the range 0 - 100%. Each point in the curve represents an average of three data observations. The solubility of RDX is linear over the complete concentration range.

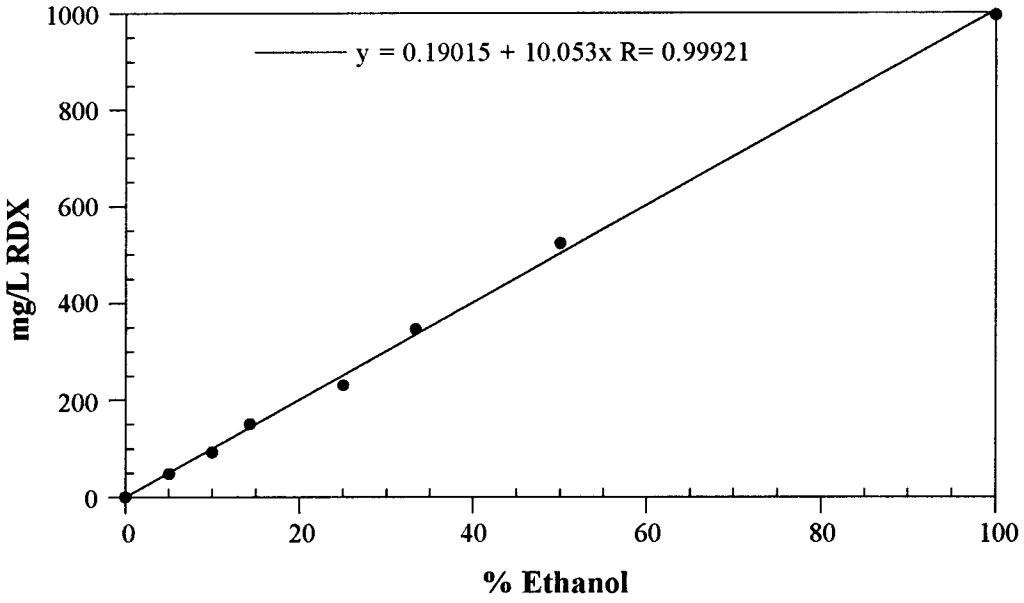


Figure 13 Solubility of RDX in ethanol (EtOH/deionized water).

4.2 Biological Studies

4.2.1 Batch

Throughout this report the general term "anaerobic" is used. A more exact description of the experimental conditions is "anoxic". Anoxic refers to the absence of oxygen but the presence of nitrate and sulfate. Fermentative conditions (absence of oxygen, nitrate and sulfate), also classified as anaerobic, were not studied in this project. Hesselmann (1992) conducted extensive batch studies under fermentative, sulfate-reducing and nitrate-reducing conditions. Results from his work served as a basis for the research continued and presented in this report. He observed RDX degradation under all three Redox conditions listed above; however, under fermentative conditions, RDX degradation required complex organic co-substrates such as peptone and molasses. These co-substrates are not suitable for the treatment process proposed in this project. The most favorable organic co-substrates, those that can be used in the RDX desorption process (simple alcohols), were successful under sulfate and nitrate-reducing conditions. The temporary presence of oxygen had an inhibitory effect on RDX degradation, but was not toxic to the culture. A depletion of oxygen demonstrates that the mixed culture contained facultative anaerobes.

4.2.1.1 Various Solvents as Co-substrates

The effects of 7 different co-substrates on RDX-reduction and culture growth are shown in Figures 14 and 15, respectively. From these results, ethanol appears to be the most promising co-substrate for the proposed treatment process. After 7 days of incubation, cultures using ethanol and acetic acid degraded 95% of the initial 20 mg/L RDX. Reduction

of RDX in the other 5 co-substrates was less than 50%. The corresponding growth measurements showed the highest rate for ethanol, although the cultures growing in acetic acid also showed substantial growth. Negligible growth occurred with methanol, acetone and formic acid. The cultures were able to adapt to ethyl acetate after 7 days as indicated by Figure 15. In addition to the biological results, ethanol was also found to be a suitable solvent for RDX desorption from activated carbon. For these two reasons, ethanol was chosen as the co-substrate for subsequent investigations.

4.2.1.2 Effect of Ethanol Concentration

Ethanol concentrations ranging from 0 to 200 g/L were investigated with an initial RDX concentration of approximately 28 mg/L. Ethanol concentrations between 5 and 25 g/L produced the highest RDX degradation rates and growth rates. Nearly 75% reduction in RDX was accomplished with the cultures in this concentration range. Figure 17 shows the toxic effects of ethanol above 50 g/L. In this study a 200 μL aliquot of inoculum was added to 20 mL of feed. Originally 500 μL was injected, however, this volume produced growth that was beyond the measurable range of the turbidity meter. The cultures formed flocks that settled to the bottom of the test tubes. Before measurements were made, each pellet of cells was resuspended by vigorous shaking. Data were collected over an 8 day period and are shown in Figures 16 and 17 for RDX degradation and culture growth, respectively. The presence of ethanol is necessary to facilitate both degradation and growth as indicated by the control sample (0% ethanol). As the ethanol concentration was increased from 5 to 50 mg/L,

a lag phase or adaptation period also increased before detectable growth began. A lag period of 1 and 2.5 days was observed for 25 and 50 g/L EtOH, respectively. These results provide a basis for the subsequent continuous flow studies.

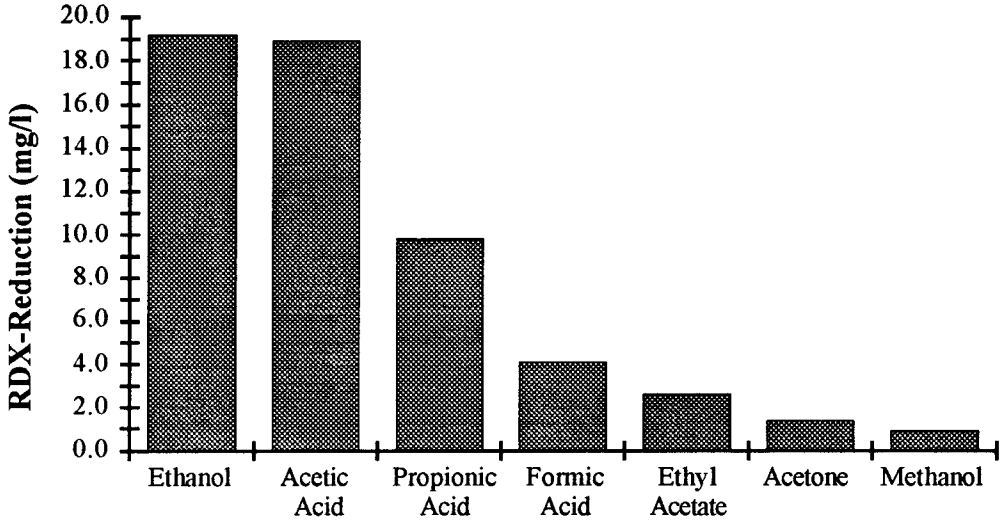


Figure 14 RDX degradation using various solvents as sole co-substrate. Values after seven days of incubation. Initial RDX concentration: 20 mg/L.

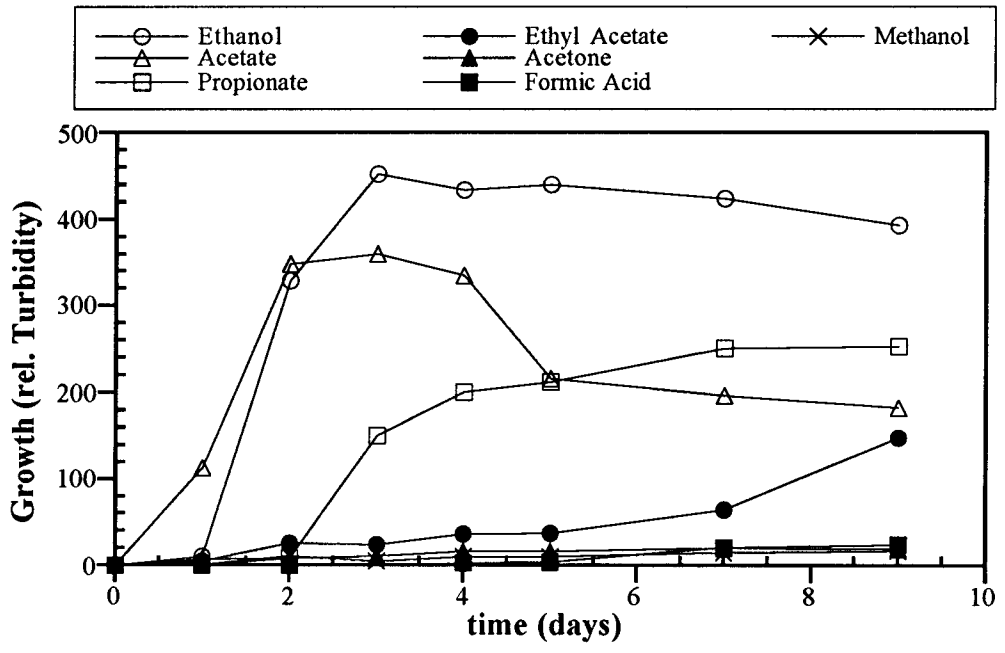


Figure 15 Culture growth using various solvents as sole co-substrate.

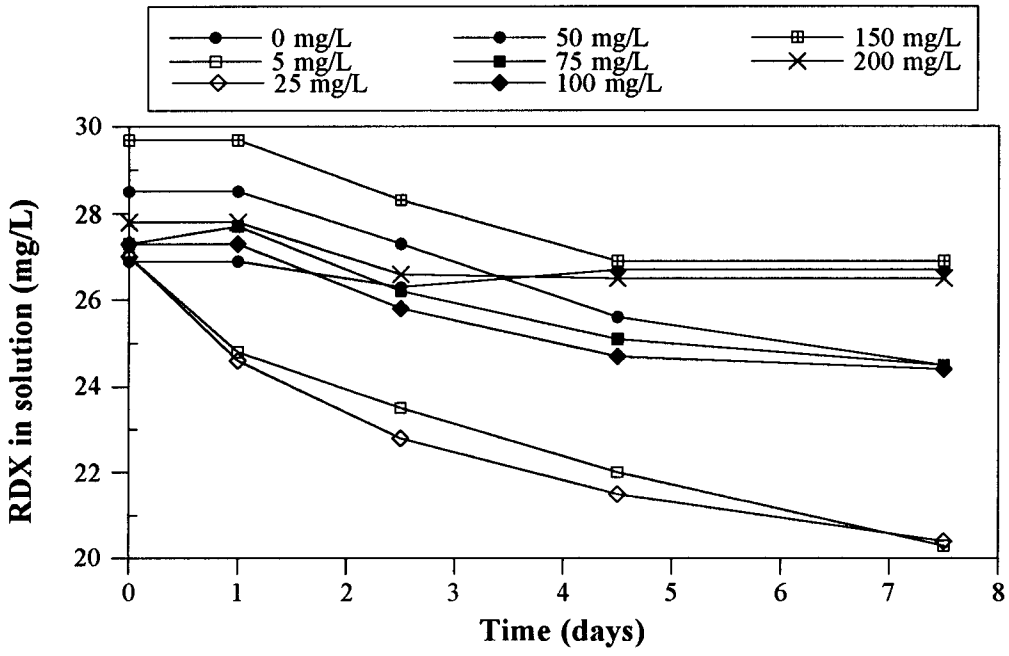


Figure 16 Effect of ethanol concentration on RDX degradation (ethanol varied from 0 - 200 g/L).

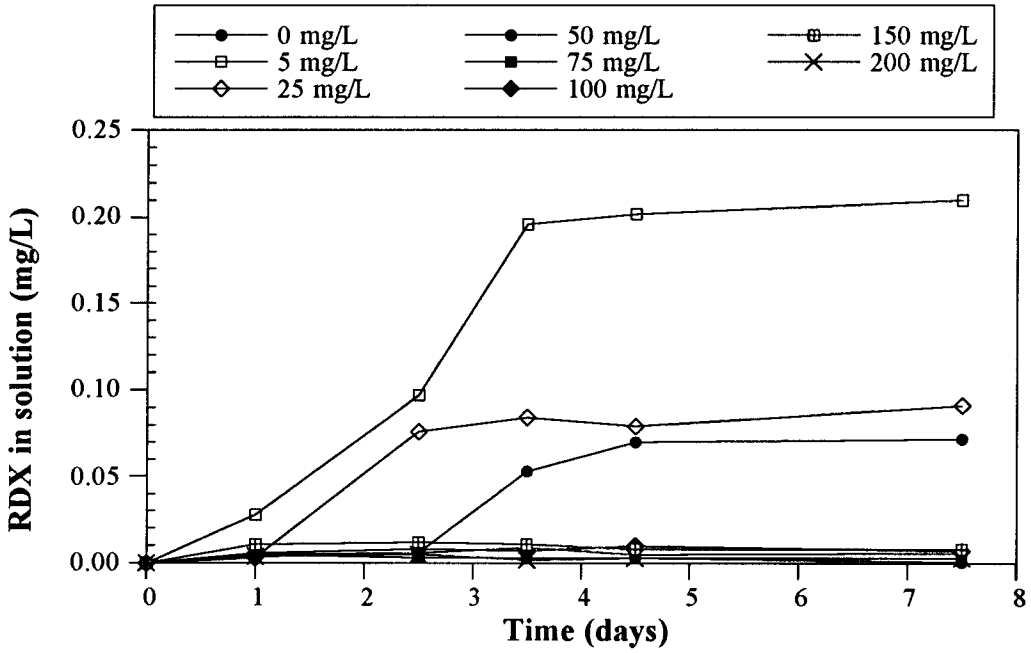


Figure 17 Effect of ethanol concentration on batch culture growth (ethanol varied from 0 - 200 g/L).

4.2.2 Continuous Flow

RDX degrading bacterial cultures from the batch experiments were used to inoculate the continuous flow reactors. Prior to inoculation, 2 L of feed solution containing 10 mg/L RDX was passed through each of the three reactors. The effluent concentration of RDX in all three reactors was equivalent to the influent feed concentration. From these results we can conclude that the packing materials, glass, Tygon and silicone, do not adsorb a detectable amount of RDX. All disappearance of RDX was attributed solely to microbial degradation.

After the removal of all air bubbles and void spaces, the pump was turned off. Two mL of inoculate was injected into the lowest port of each column. Bioreactor flow was resumed 1 week after inoculation. This initial period without influent flow provided an environment free of sheer stress to facilitate bacterial growth and attachment to the packing materials. The development of ethanol-supported bacterial growth became visible after 2 weeks of operation. At this point, the average RDX degradation in the columns was only 10%. Steady-state degradation, using 1 mL/L feed ethanol and 10 mg/L RDX, was reached in all three reactors after 2 months of continuous operation. After several months of operation, a red colored growth started to appear at the inlet and outlet of two of the reactors. The growth most likely was the result of a photosynthetic organism. To prevent further growth, the columns were covered with aluminum foil. The growth was successfully stopped and eventually diminished from the effected reactors.

The biofilm was thickest at the influent end of each reactor where the highest substrate concentration existed and decreased as substrate was consumed along the length of the reactor. Periodically the reactor inlets became clogged from either excessive bacterial

growth or precipitation from magnesium/calcium complexes. To reduce excessive pressure and clogging in the reactors, 1 to 2 mL of solution were extracted from the densely populated area (lower 1/5) of the reactor once a week or as needed. This small extraction of cells had no apparent effect on RDX degradation.

4.2.2.1 Reactor Characterization

Tracer studies were performed on all three reactors to determine the void volume or hydraulic retention time. The following interstitial volumes were calculated: 46.3, 52.7 and 54.1 mL for the glass, silicone and Tygon packing materials, respectively. Each column has an empty bed volume of 98.17 mL. Effluent tracer concentrations were recorded with respect to time and presented as C(t)/Co vs. time see Figures 18, 19, and 20. The residence time distribution curves (E(t)), obtained by differentiating the C(t)/Co are presented in Figures 29, 30 and 31. Each curve shows a Gaussian fit to the differentiated C(t)/Co experimental data. The peak of each curve represents the average retention time for a given flowrate. A summary of continuous flow reactor characteristics including the void volumes and hydraulic retention times is presented in Table 6.

4.2.2.2 Retention Time

The effect of reduced flowrate (increased retention time) was compared for two influent feed flowrates, 0.3 and 0.15 mL/min. Reducing the influent flowrate to 0.15 mL/min, produced a 60% increase in RDX degradation for all three reactors. Table 6 shows

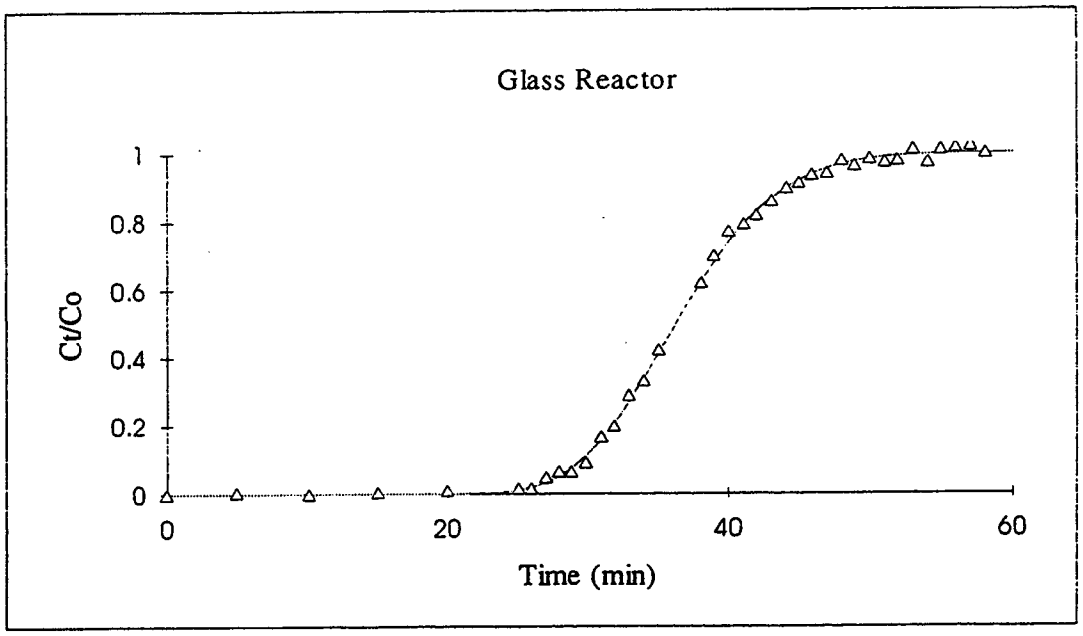


Figure 18 Glass reactor experimental breakthrough data with corresponding Crank-Nicolson simulation. Dispersion and average interstitial water velocity are 8.0 cm^2/hr and 32.8 cm/hr, respectively. Step input of 0.01 M potassium nitrate at time = 0; Q = 1.28 mL/min.

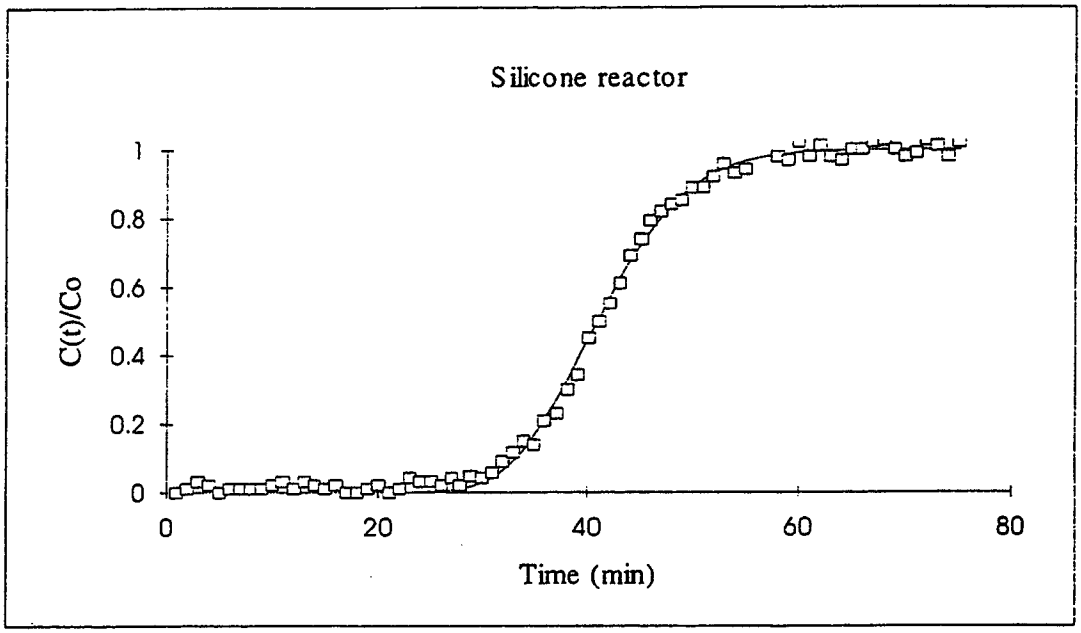


Figure 19 Silicone reactor experimental breakthrough data with corresponding Crank-Nicolson simulation. Dispersion and average interstitial water velocity are 7.9 cm^2/hr and 29.0 cm/hr, respectively. Step input of 0.01 M potassium nitrate at time = 0; Q = 1.28 mL/min.

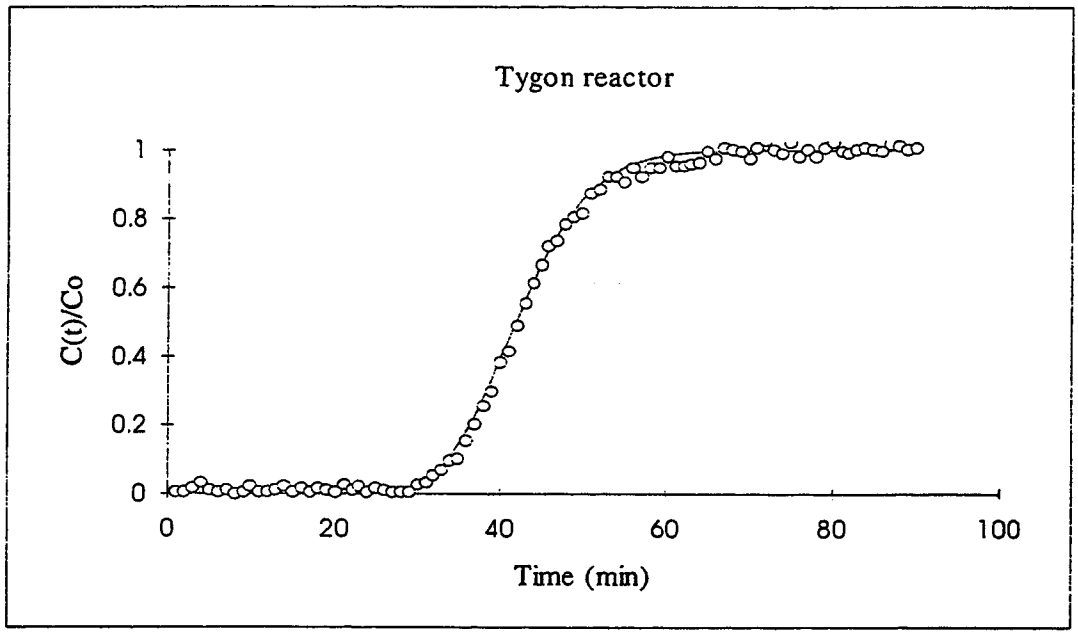


Figure 20 Tygon reactor experimental breakthrough data with corresponding Crank-Nicolson simulation. Dispersion and average interstitial water velocity are 7.9 cm^2/hr and 29.0 cm/hr, respectively. Step input of 0.01 M potassium nitrate at time = 0; Q = 1.28 mL/min.

the calculated retention times for each of the reactors at the 2 flowrates. In this experiment and all other continuous flow experiments, the effluent RDX concentrations represent one pass through the reactor, i.e. the reactor effluent was not recirculated. The influent feed contained 1 mL/L ethanol and 9 mg/L RDX. At a flowrate of 0.3 mL/min, RDX degradation for the glass, silicone and Tygon reactors was 20%, 25% and 27%, respectively. The results are presented in Figure 21.

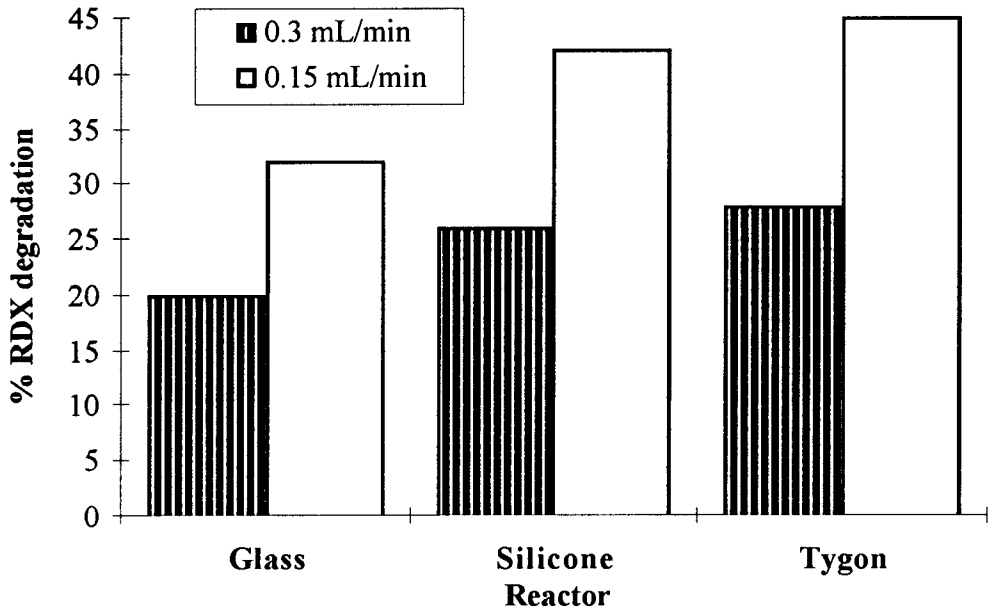


Figure 21 Effects of flowrate (retention time) on RDX degradation in the continuous flow reactors. Inlet RDX concentration of 9 mg/L.

Effects of ethanol concentration

In the following series of experiments, the effect of ethanol concentration on RDX degradation was studied. All three reactors were allowed to reach steady-state with an inlet feed concentration of 1 mL EtOH/L pumped at 0.3 mL/min. After two weeks of steady-state operation, the inlet ethanol concentration was increased to 5 mL/L feed, as shown in Figure 22. Immediately following the addition of ethanol at t = 14 days, a washout peak or sharp decrease in degradation was observed. The cultures recovered within 48 hours, showing little to no permanent effects of the increased ethanol. The spike of decreased degradation

was accompanied by an increase in cells appearing in the reactor effluent. A similar lag or adaptation period was observed in the batch ethanol studies.

The second increase in ethanol concentration was initiated at t = 51 days (see Figure 22). This increase from 5 mL/L to 10 mL/L also produced a washout spike. The cultures recovered, as before, within 48 hours. No effect on the overall RDX degradation was observed for this second increase in substrate concentration.

The third increase in ethanol concentration had a marked affect on RDX degradation. Figure 23 shows the addition of 20 mL EtOH/L at t = 60 days. A slight washout or lag effect was observed for the silicon and Tygon reactors; however, the drop was not as large as the previous ones. This particular increase in substrate was accompanied by a 1 mL/L increase in acetone due to the method of RDX dissolution. For this batch of RDX, a small amount of acetone was used to dissolve the RDX powder before the water was added. RDX degradation rated doubled for all three reactors at this ethanol concentration. Ethanol at 20 mL/L appears to be a threshold value for the mixed culture.

Studies conducted by Rittman and McCarty (1980) discuss the concept of a minimum substrate concentration, S_{min}, below which no significant biofilm activity should occur in steady-state. Their results illustrate the substrate flux and biofilm activity declined rapidly as the substrate concentration approached S_{min}. At S_{min}, the rate of energy captured through the cell growth is just equal to the rate of maintenance-energy expenditure.

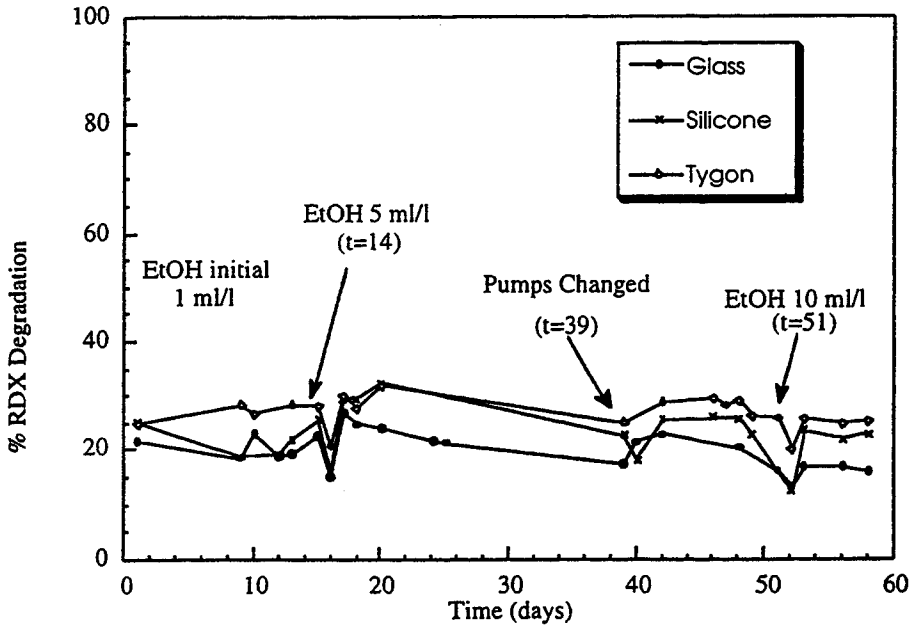


Figure 22 Effect of ethanol concentration on RDX degradation in the continuous flow reactors. No recirculation of effluent. Flowrate = 0.3 mL/min.

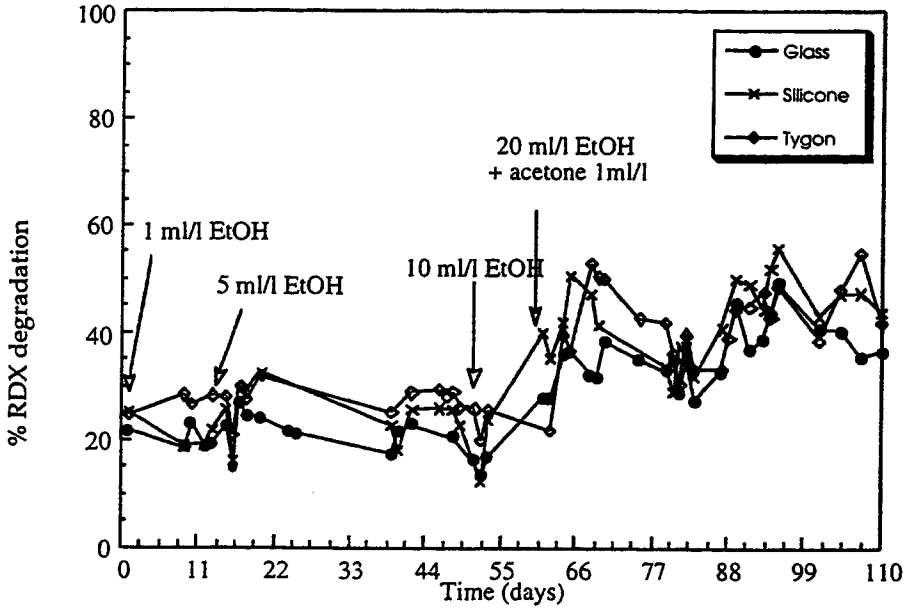


Figure 23 Effect of increased ethanol concentration on RDX degradation in the continuous flow reactors. No recirculation of effluent. Flowrate = 0.3 mL/min

The final increase in ethanol concentration to 50 mL/L was initiated at t = 168 days, as shown in Figure 24. This concentration was only fed to the glass and Tygon reactors; the silicone reactor was kept as a control at 20 mL/L. During the first three weeks of operation at 50 mL/L, sharp variations in RDX degradation were observed daily. After the third week, the reactors dropped to below 10% degradation rate. Degradation continued to drop and at t = 194 days (26 days after the increase) the ethanol concentration was decreased to 10 mL/L in an attempt to save the two reactors. Initial stages of recovery were apparent after 24 hours. The toxic/inhibitory effect of 50 mL/L ethanol was not permanent, both reactors continued to steadily recover and reached complete recovery within 10 days. The quick recovery may be an indication that 5% (v/v) ethanol was not necessarily lethal; the culture may have a dormant state that permits survival until conditions supportive of growth return. However, it is also possible that competing reactions exist between RDX and ethanol, and at the higher concentrations, the ethanol reaction takes precedence.

The maximum ethanol concentration determined from the batch studies was 5% (v/v), while the same concentration had toxic or inhibitory effects on the continuos flow reactors. Since the feed is continuously supplied to the reactors, the ethanol concentration does not decrease as it does in a batch system. Also, the inhibitory effects were not observed until three weeks of operation in the flow reactors. The batch studies were only run for one week.

The system was brought back to 20 mL/L or 2% (v/v) and allowed to reach steady-state before the next set of experiments were conducted. The effects of ethanol between 20 and 50 mL/L were not determined here but would be interesting to look at in future work.

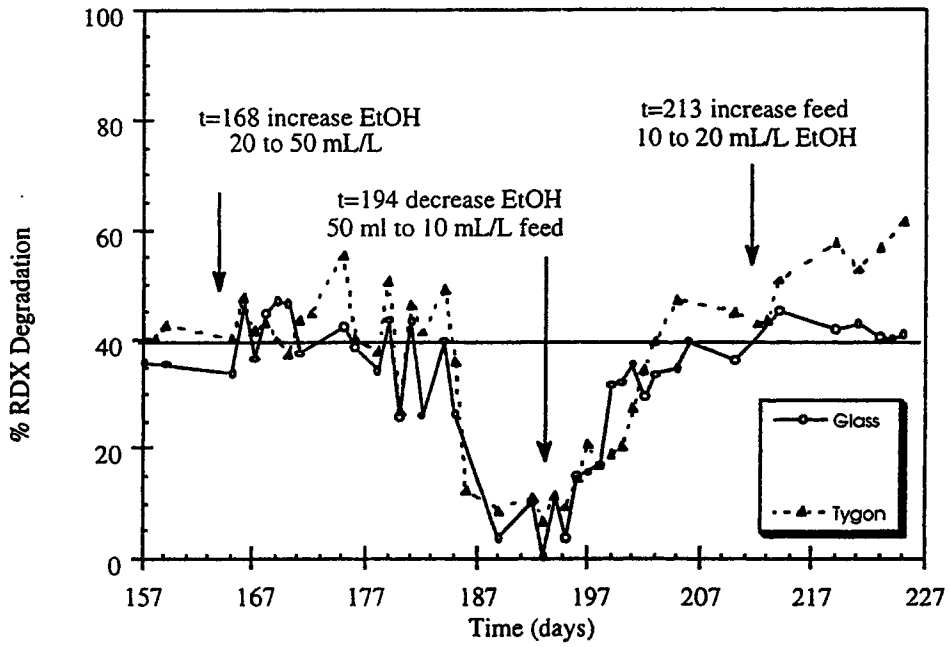


Figure 24 Inhibitory effect of ethanol at 5 mL/L.

Effects of elevated temperature

An increase in RDX degradation from 55% to 80% occurred after raising the reactor temperature from 27 to 35°C. This increase in reactor temperature resulted in the highest observed degradation rates for the continuous flow reactors. Figure 25 shows the data collected over a six month period. In this study the glass reactor was kept as a control at 27°C while the temperature of the Tygon reactor was raised to 35°C at time t = 20 days. After approximately three weeks, an increase in RDX degradation was observed. Degradation continued to increase until steady-state was reached approximately 40 days later. Data were collected for an additional 4 months, during which the percent degradation remained constant between 75% and 80%.

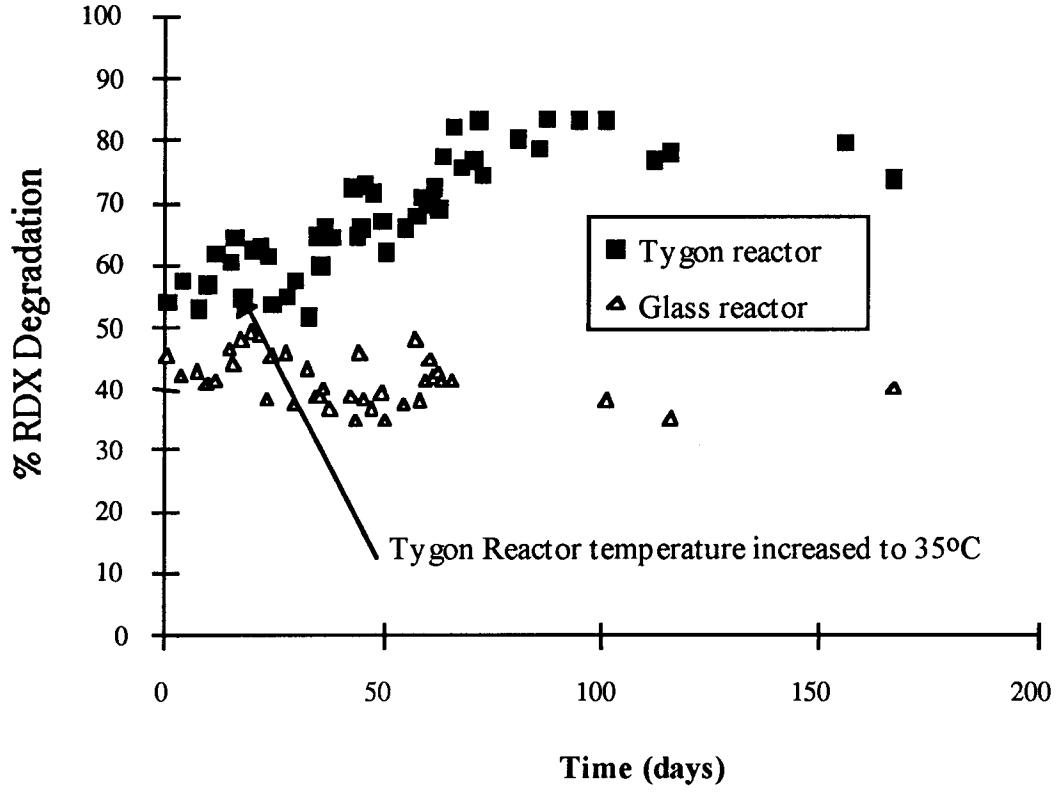


Figure 25 Effect of elevated temperature on RDX degradation. Initial temperature 25°C. Temperature of Tygon reactor raised to 35°C at time = 22 days. Glass reactor was kept as a control at 25°C.

Several variables can influence the rate of biodegradation. These factors may be substrate-related, organism-related or environment-related. Environmental variables such as temperature, control microbial metabolic activity, rather than biodegradation specifically (Lyman, 1990). Rates of biological reactions increase with increasing temperature within the range tolerated by the organism. The substrate and organism related factors were examined in previous experiments but did not result in the high degradation rates produced in this

temperature experiment. Future work should include a more detailed study of temperature and its effect on RDX degradation. Higher rates may occur if the optimal temperature is determined.

4.2.2.3 Reactor Growth Profile

A growth profile was developed for the glass reactor under the identical feed conditions used in the reactor characterization and kinetic studies. The biofilm mass was greatest at the influent end where the highest substrate concentration existed and decreased in mass as substrate was consumed to a length of about 12 cm. Beyond 12 cm., bacterial growth was small. A 20 mL/L ethanol feed was maintained until a steady-state culture, as evidenced by no change in RDX-removal, was established. Figure 26 presents the total suspended solids mass with respect to column length.

The total mass of suspended solids, represented by the area under the curve, is 10.4 grams. In the first quarter of the reactor, 53% of the total mass resided. Eighty percent of the total mass was located in the first half of the reactor. Although these results are qualitative, they support the findings in the RDX degradation profile experiments. The results for RDX degradation versus column length, Figure 32, show that 60% of the total degradation occurred in the first quarter of the reactor and approximately 80% was accomplished in the first half of the reactor.

The correlation between the growth and degradation profiles may be extrapolated to the other reactors to qualitatively compare the effects of packing material. Although there appears to be little difference between the silicone and Tygon supported growth, these

materials are more favorable than the glass supported growth. What constitutes the relevant target surface for microbial adhesion is difficult to characterize. Properties such as surface hydrophobicity, surface tension and degree of porosity may have a direct relationship the number of microbes that adhere.

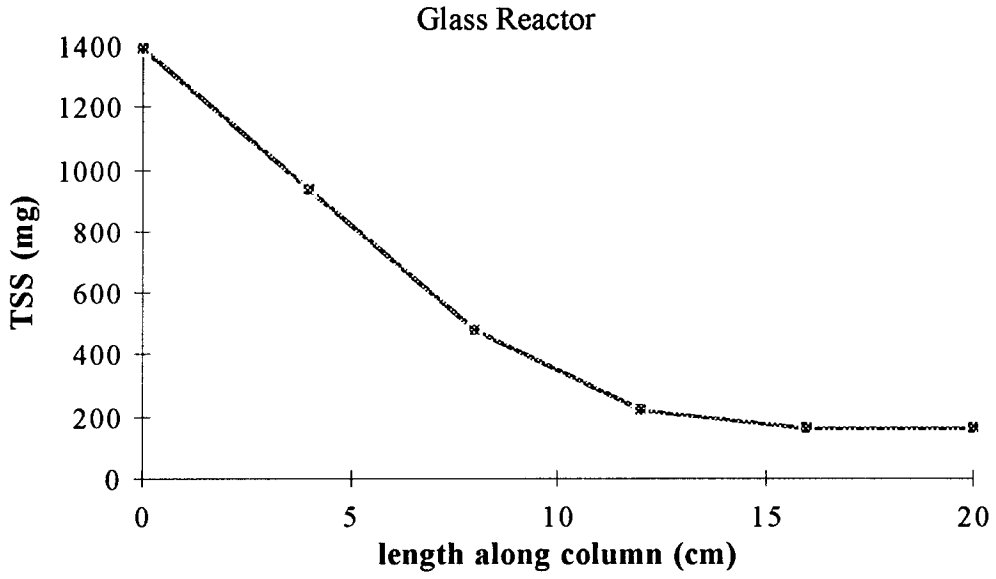


Figure 26 Total suspended solids analysis of the glass packed reactor.

4.2.2.4 Transport Model and Biodegradation Kinetics

The transport and degradation within each reactor was modeled using the following equation:

$$\frac{\partial C}{\partial t} = D \cdot \frac{\partial^2 C}{\partial X^2} - V \cdot \frac{\partial C}{\partial X} - k \cdot C \qquad (3)$$

The dispersion coefficient D and the average interstitial water velocity V were calculated from tracer study data. A conservative tracer was introduced into each reactor as a step input at t = 0 minutes. Under these conditions, the reaction term equals zero, leaving D and V as the only unknowns. Breakthrough curves for a flowrate of 1.28 mL/min are represented in Figures 18, 19 and 20, for glass, silicone and Tygon, respectively. Figure 27 shows a breakthrough curve generated for the glass reactor at a flowrate of 0.3 mL/min. Each figure presents the normalized effluent concentration data with the corresponding Crank-Nicolson (CN) simulation. The CN method was used to simulate the experimental data to the partial differential equation above with respect to D and V (r = 0). Table 8 lists the dispersion and average pore water velocity parameters calculated from the CN fit for a flowrate of 0.3 mL/min. The complete breakthrough data including both the step-up and step-down, for all three reactors can be seen in Figure 28.

Residence time distribution (RTD) curves were generated by differentiating the normalized effluent concentration data. Figures 29, 30 and 31 present the E curve or RTD curve with the corresponding area, center and width data for the glass, silicone and Tygon reactors, respectively. The maximum or peak of each curve represents the average residence time for that particular flowrate and reactor. The interstitial or pore volume can be calculated by multiplying the flowrate in mL/min by the average residence time in minutes. The average residence time for an inlet flowrate of 0.3 mL/min is 3 hours for both the silicone and Tygon reactors and 2.6 hours for the glass reactor. A summary of these parameters is presented in Table 8.

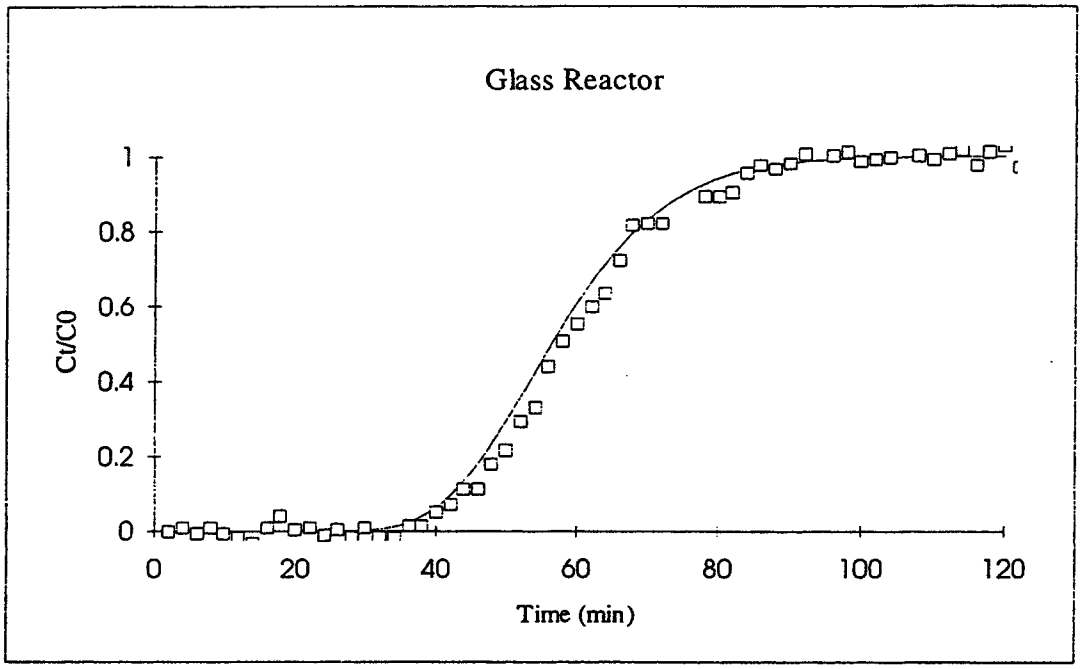


Figure 27 Glass reactor experimental breakthrough data with corresponding Crank-Nicolson PDE curve fit. Dispersion and average pore water velocity are 10.98 cm^2/hr and 20.8 cm/hr, respectively. Step input of 0.01 M potassium nitrate at time = 0; Q = 0.3 mL/min.

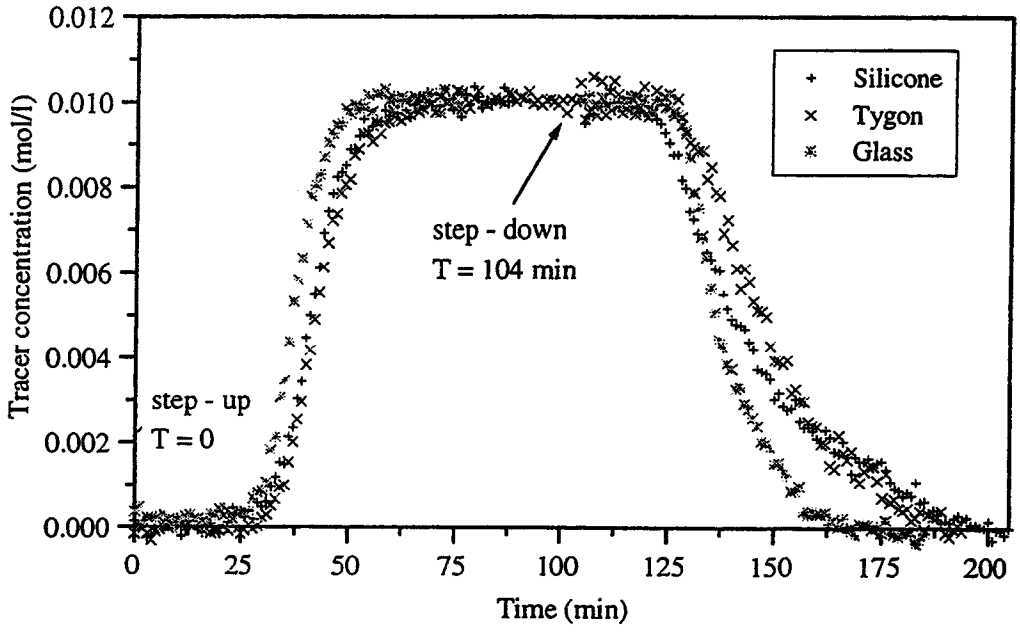


Figure 28 Experimental breakthrough data for all three reactors. Step input of 0.01 M potassium nitrate at time = 0. Step down shown at time = 104 min. Q = 0.3 mL/min.

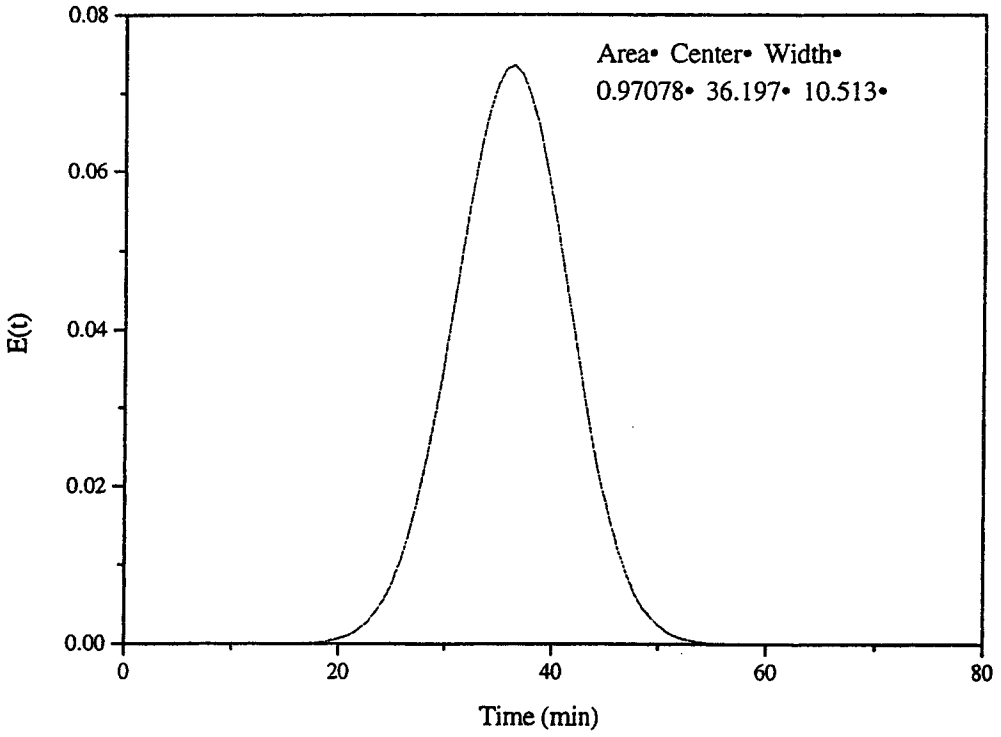


Figure 29 Glass reactor residence time distribution curve. Q = 1.28 mL/min, t_{ave} = 36.2 min, interstitial volume = 46.3 mL.

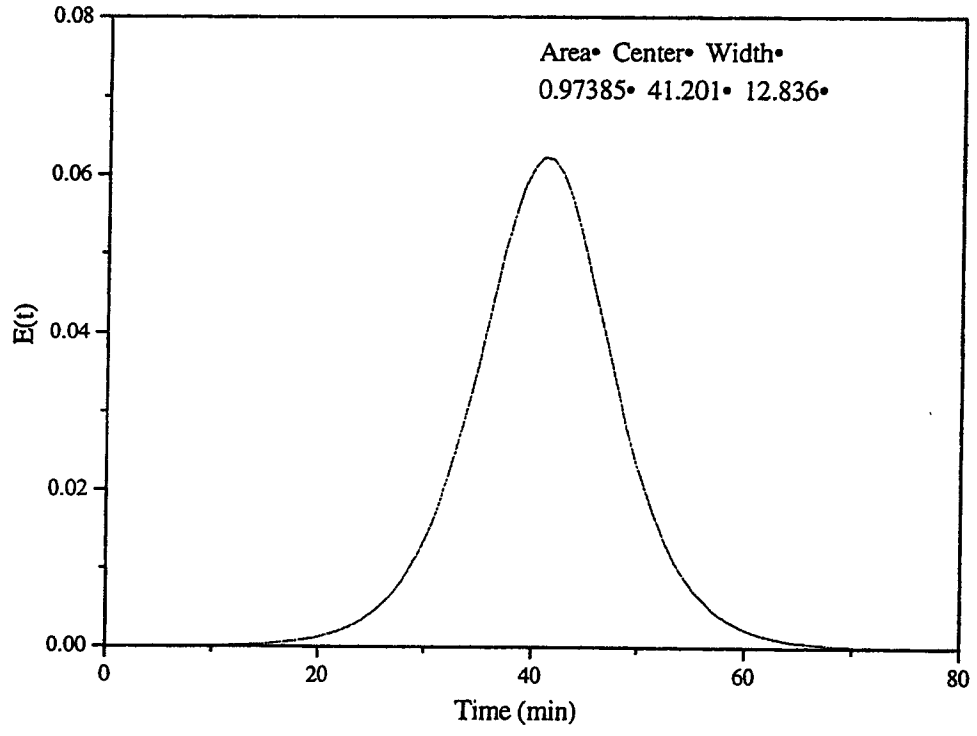


Figure 30 Silicone reactor residence time distribution curve. Q = 1.28 mL/min, t_{ave} = 41.2 min, interstitial volume = 52.7 mL.

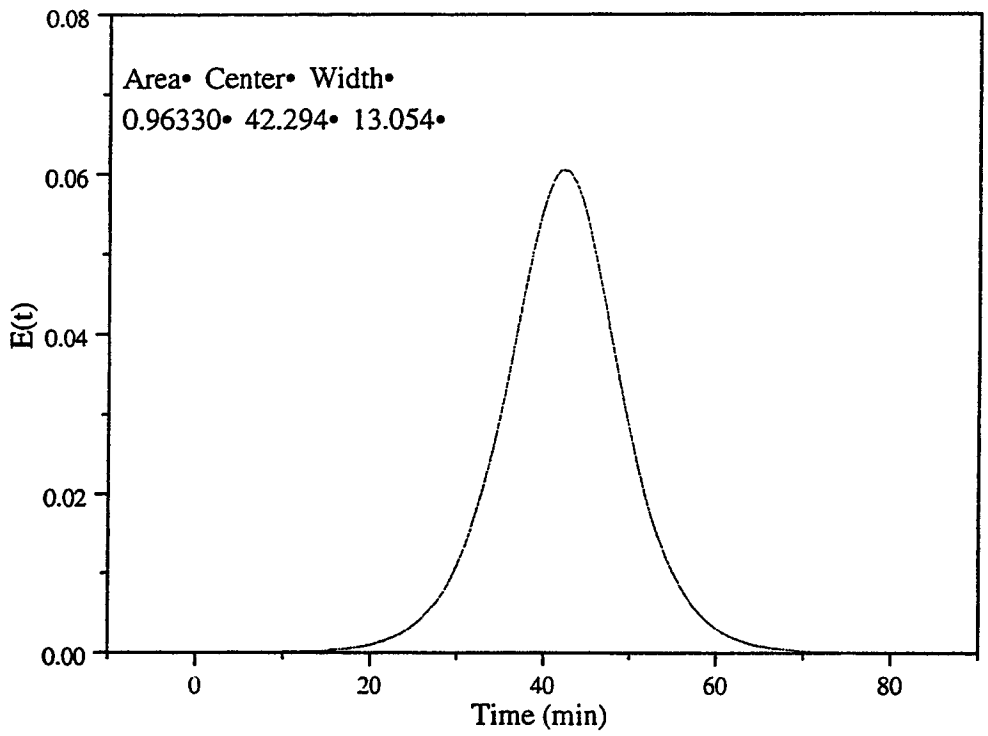


Figure 31 Tygon reactor residence time distribution curve. Q = 1.28 mL/min, t_{ave} = 42.3 min, interstitial volume = 54.13 mL.

The difference in retention time is very important when comparing degradation rates among the three reactors. Since degradation in this system has been shown to be a function of retention time, less degradation is expected in the glass reactor, if all other parameters are equal. Therefore, the effects of packing material cannot be determined directly from the percent RDX degradation data. A correction must first be made to account for the difference in retention times.

The next step in completing the overall transport equation is to determine the biodegradation rate constants. Depending on the type of degradation, extent of adsorption, existence of competing simultaneous reactions and other factors, different rate equations are

applicable for deriving the rate constant. One rate law may not adequately describe a chemical over its total degradation curve because of changes in its concentration-dependency and availability over time (Lyman et al., 1990). In the proposed system, one rate order is assumed to be in effect over the entire biodegradation curve.

The assumption of pseudo first-order is most common in homogeneous media (Hamaker, 1972) or as a first approximation when the relationship between concentration and the variables affecting it are not well understood. In order to determine the pseudo first-order rate constant k, a degradation profile was developed along the length of each reactor. The data were collected with the reactors operating at 0.3 mL/min and an inlet feed concentration of 20 mL EtOH/L. Multiple samples were taken and averaged over time from the inlet (x = 0 cm), the outlet (x = 20 cm) and 3 to 4 ports along the length. The average normalized degradations for the glass, silicone and Tygon reactors are presented in Figures 32, 33 and 34, respectively. Nearly 90% of the total degradation in both the Tygon and silicone reactors occurs within the initial 5 cm or 1/4 or the reactor. The glass reactor only reached 60% of its total degradation in the first quarter. From these results it may be suggested that Silicone or Tygon are more effective supports for microbial growth.

The concentration data collected along the length of each column was fit to the general transport equation using a nonlinear least-squares conversion method (Parker and van Genuchten, 1984). This method provides a convenient and accurate means of fitting various transport parameters to observed spatial and/or temporal concentration distributions. The

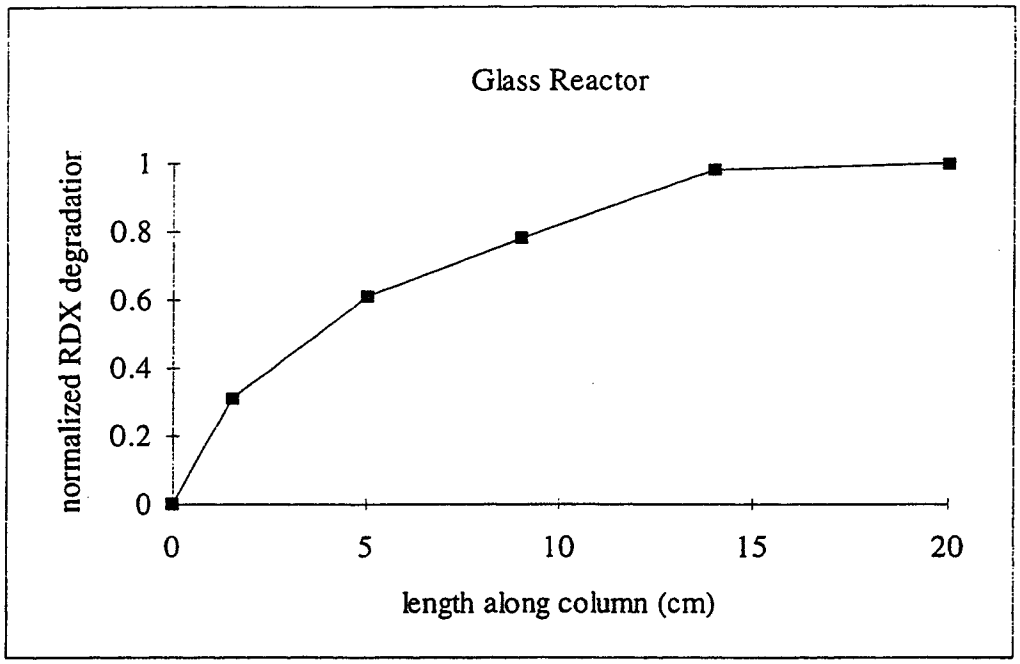


Figure 32 Glass reactor: RDX degradation as a function of reactor length. Reactor inlet located at 0 cm. Total reactor length of 20 cm (Inlet flowrate = 0.3 mL/min, 20 mL ethanol / L feed).

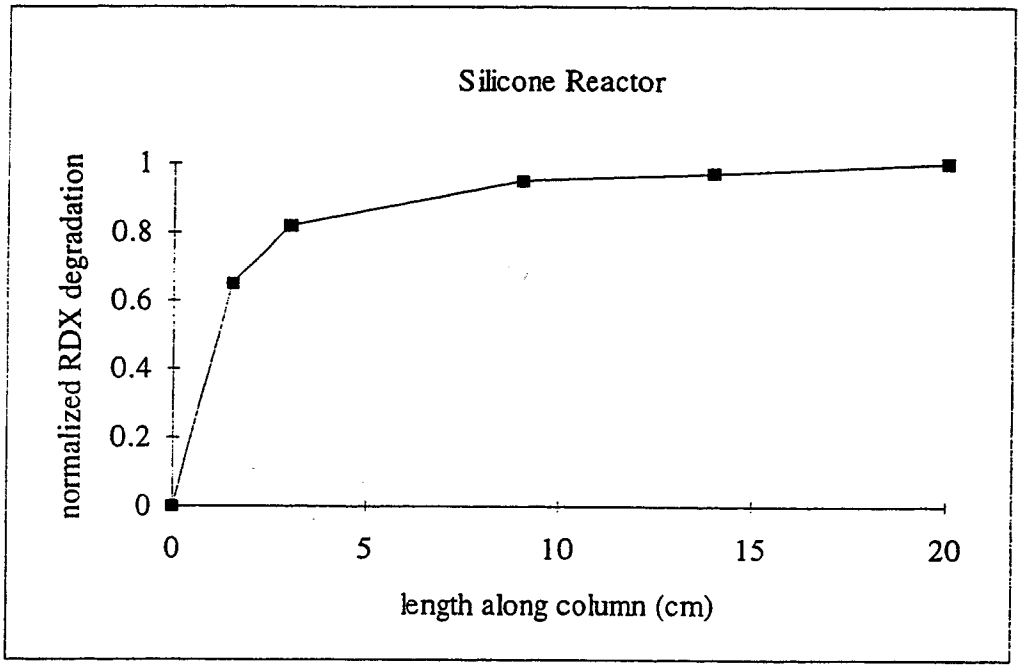


Figure 33 Silicone Reactor: RDX degradation as a function of reactor length. Reactor inlet located at 0 cm. Total reactor length of 20 cm (Inlet flowrate = 0.3 mL/min, 20 mL ethanol / L feed).

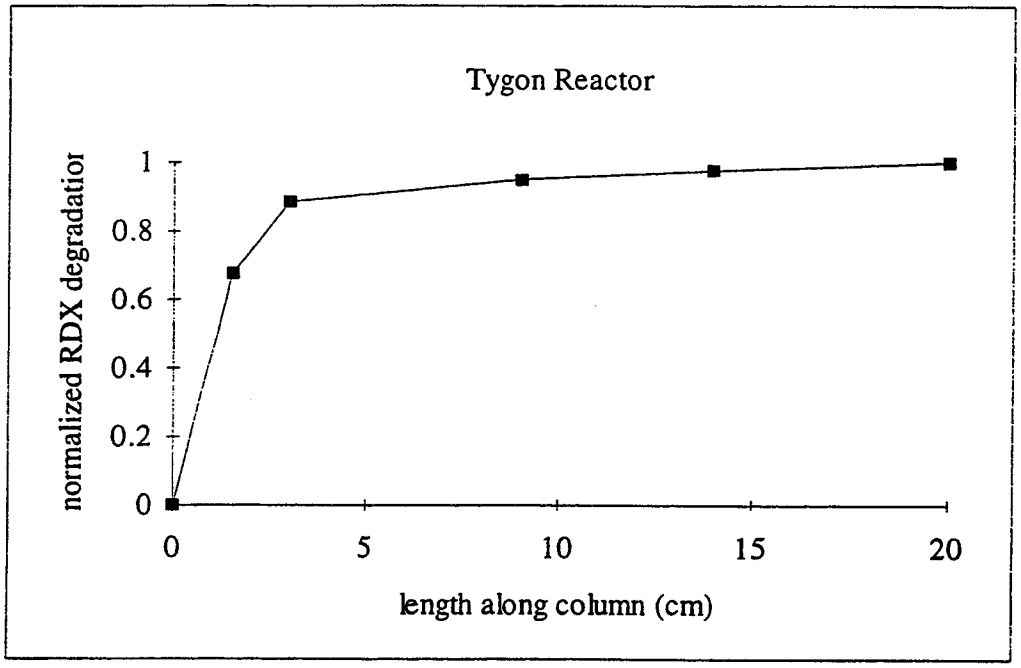


Figure 34 Tygon Reactor: RDX degradation as a function of reactor length. Reactor inlet located at 0 cm. Total reactor length of 20 cm (Inlet flowrate = 0.3 mL/min, 20 mL ethanol / L feed).

dispersion coefficient and average interstitial water velocity calculated from the tracer studies were used as constant or known parameters and the program was allowed to fit the a first-order reaction coefficient. The input files contained the data points shown in Figures 32 - 34. Representative data input and output files are presented in Appendix B. The rate constants calculated for glass, silicone and Tygon are 1.7, 4.2 and 4.2 hr^{-1}, respectively. A strong correlation between the experimental and the curve fit for a pseudo-first order reaction occurred for each reactor. The squared regression term (r^2) of observed versus predicted was 0.99 for the Tygon and glass reactors and 0.95 for the silicone reactor. Therefore, a pseudo-

first order reaction can be assumed for the overall degradation of RDX in the reactors. A summary of all three transport equation parameters (D, V and k) are listed in Table 9.

Table 9 Transport model parameters (inlet flowrate of 0.3 mL/min).

Reactor	Glass	Silicone	Tygon
Hydraulic retention time (hr)	2.57	2.93	3.00
Dispersion coefficient, D (cm^2/hr)	11.0	14.0	12.5
Average pore water velocity, V (cm/hr)	7.8	6.8	6.7
Pseudo-first order rate constant, k (hr^{-1})	1.7	4.2	4.2

5. CONCLUSIONS

RDX, a high explosive, was treated biologically in a laboratory scale investigation over a 16 month period. Three parallel columns were operated using ethanol as a co-substrate and nitrate as the electron acceptor. Results show that this continuous flow process, activated carbon adsorption followed by anoxic biological degradation, is effective in treating RDX contaminated wastewaters. The following conclusions are:

- Continuous flow, anaerobic biodegradation can effectively reduce inlet concentrations of RDX by 75 - 80% in a single pass. This rate is achieved with nitrate as the terminal electron acceptor and ethanol as a co-substrate, at a reactor temperature of 35°C;
- The co-substrate ethanol produces both the highest growth rates and the highest biodegradation rates. In addition, ethanol is an effective desorption solvent so it can be used bifunctionally in the proposed treatment process; ethanol allows for direct coupling between the activated carbon adsorption/desorption phase and the biodegradation phase;
- An ethanol concentration of 5% (V_{EtOH}/V_{feed}) produces extreme inhibition of the culture's ability to degrade RDX. This concentration, however, is not lethal as full recovery of the culture is attainable;

- Biodegradation of RDX is strongly dependent on temperature. An 80% removal efficiency can be achieved for a reactor operating at 35°C while only 40 - 50% occurs at ambient temperatures;
- RDX removal increases as a function of increased retention time. Increasing the retention time from 3 to 6 hours results in a 60% increase in degradation. In the proposed treatment process, the reactor effluent is recirculated through the column effectively increasing the retention time. Removal efficiencies for the recirculated process should exceed the results obtained for the one-pass laboratory scale reactors used in this study;
- In the first quarter of the reactor, 60 - 80% of the total degradation occurs. In addition, this is the area where more than 50% of the total biofilm mass resides. Since the highest concentration of cells are formed at the inlet, where the substrate concentration is highest, adding a second feed port midway along the length, may increase RDX removal;
- Activated carbon adsorption effectively removes RDX and HMX from wastewater. Filtrasorb-400 has the highest adsorption capacity for RDX at 417 mg/g GAC followed by Norit and Darco at 272 and 227 mg/g GAC, respectively. The adsorption capacity of Filtrasorb-400 is 217 mg/g GAC for HMX;
- Filtrasorb-400 has the greatest desorption efficiency. The desorbed concentration of RDX for Filtrasorb-400 is at least 2 times higher than the other carbons tested;

- A maximum carbon regeneration efficiency of 40% was observed using water at 80°C as the desorption solvent;
- Polar organic solvents markedly enhance the desorption of RDX from activated carbon. For mixtures of ethanol and water, at least 50% (V_{EtOH}/V_{water}) must be used to facilitate enhanced desorption;
- The overall RDX degradation process can be modeled using a pseudo first-order rate equation. The rate constants calculated for the glass, silicone and Tygon reactors are 1.7, 4.2, and 4.2 hr^{-1}, respectively;
- Microbial attachment and RDX biodegradation are favored in the Tygon and silicone reactors. However, the interaction between the packing material and the culture is not well understood and is difficult to quantify.

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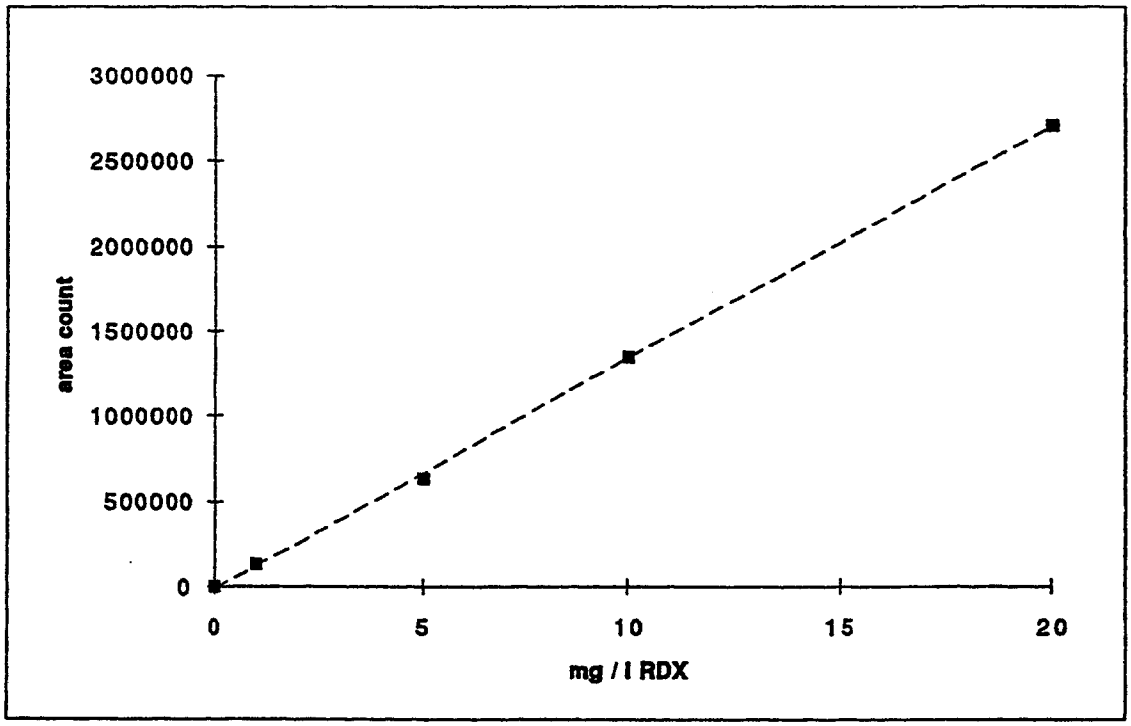
Appendix A Sample Calibration Curves



RDX conc mg/l	area 1	area 2	area 3	mean area	st.deviation	%cv
0	0	0	0	0	0	
1	131624	131022	131889	131511.6667	444.2818175	0.337826923
5	632305	634669	634502	633825.3333	1319.292361	0.208147622
10	1347712	1348450	1348678	1348280	504.9396003	0.037450648
20	2706456	2711941	2711581	2709992.667	3068.127822	0.113215355

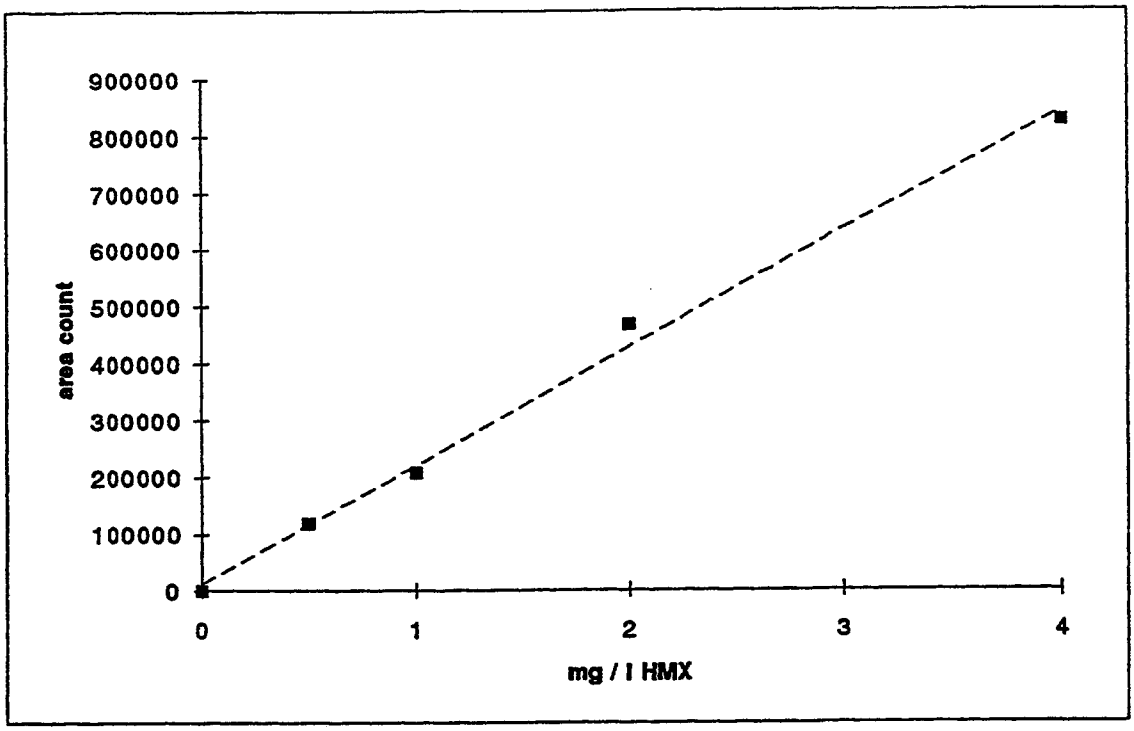
Regression Statistics

Multiple R	0.999858283
R Square	0.999716586
Adjusted R Squar	0.999622114
Standard Error	21549.46145
Observations	5

Analysis of Variance

	df	*Sum of Squares*	*Mean Square*	*F*	*Significance F*
Regression	1	4.91416E+12	4.91416E+12	10582.21468	2.02515E-06
Residual	3	1393137866	464379288.8		
Total	4	4.91555E+12			

	Coefficients	*Standard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-13537.46777	13531.66939	-1.000428504	0.373717038	-56601.31941	29526.38388
x1	135716.2316	1319.299836	102.869892	5.35457E-08	131517.6268	139914.8365



RDX conc mg/l	area 1	area 2	area 3	mean area	st.deviation	%cv
0	0	0	0	0	0	
0.5	116243	138427	100654	118441.3333	18982.21231	16.02667901
1	201597	205962	212061	206540	5255.890695	2.544732592
2	462980	463684	475340	467334.6667	6941.752324	1.485392123
4	829883	824849	827767	827499.6667	2527.625236	0.305453324

Regression Statistics

Multiple R	0.997525516
R Square	0.995057156
Adjusted R Squar	0.993409541
Standard Error	26774.06823
Observations	5

Analysis of Variance

	df	*Sum of Squares*	*Mean Square*	*F*	*Significance F*
Regression	1	4.32933E+11	4.32933E+11	603.9380044	0.000147706
Residual	3	2150552189	716850729.5		
Total	4	4.35084E+11			

	Coefficients	*Standard Error*	*t Statistic*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	11857.35833	17454.557	0.679327372	0.534207764	-43690.88421	67405.60088
x1	208070.5167	8466.703783	24.57515014	1.627E-05	181125.6612	235015.3721

Appendix B Data Input and Output Files

```
**********************************************************************
*                                                                    *
*     ONE-DIMENSIONAL CONVECTIVE-DISPERSION EQUATION                 *
*     NON-LINEAR LEAST-SQUARES ANALYSIS                              *
*                                                                    *
*     DETERMINISTIC LINEAR EQUILIBRIUM ADSORPTION FOR PULSE          *
*     INJECTION WITH FIRST- AND ZERO-ORDER PRODUCTION AND DECAY      *
*     SOLUTION FOR RESIDENT CONCENTRATIONS                           *
*                                                                    *
*       Silicone Reactor                                             *
*       (UNITS: CENTIMETERS, DAYS, MILLIGRAMS)                       *
*                                                                    *
**********************************************************************


INITIAL VALUES OF COEFFICIENTS
==============================
NAME         INITIAL VALUE
V.........     186.8000
D.........     263.5200
R.........       1.0000
PULSE.....      10.0000
RX1.......     100.0000
RX0.......        .0000
CI........        .0000
C0........       1.0000


OBSERVED DATA
=============
OBS. NO.      CONCENTRATION       DISTANCE          TIME
    1              .6900           1.5000          1.0000
    2              .3900           3.0000          1.0000
    3              .2200           9.0000          1.0000
    4              .0200          14.0000          1.0000
    5              .0000          20.0000          1.0000


ITER      SSQ        RX1...
 0       .15357   100.0000
 1       .02599    49.7380
 2       .01862    39.9480
 3       .01837    41.2706
 4       .01837    41.2622

RSQUARE FOR REGRESSION OF OBSERVED VS PREDICTED= .94839170

NON-LINEAR LEAST SQUARES ANALYSIS, FINAL RESULTS
================================================

                                                95% CONFIDENCE LIMITS
NAME          VALUE          S.E.COEFF.          LOWER           UPPER
RX1...       41.26217          5.92495        24.81281        57.71154
```

----------ORDERED BY COMPUTER INPUT----------

NO	DISTANCE	TIME	CONCENTRATION OBS	CONCENTRATION FITTED	RESI-DUAL
1	1.5000	1.0000	.6900	.6139	.0761
2	3.0000	1.0000	.3900	.4709	-.0809
3	9.0000	1.0000	.2200	.1630	.0570
4	14.0000	1.0000	.0200	.0674	-.0474
5	20.0000	1.0000	.0000	.0233	-.0233

----------ORDERED BY RESIDUAL----------

NO	DISTANCE	TIME	CONCENTRATION OBS	CONCENTRATION FITTED	RESI-DUAL
1	1.5000	1.0000	.6900	.6139	.0761
3	9.0000	1.0000	.2200	.1630	.0570
5	20.0000	1.0000	.0000	.0233	-.0233
4	14.0000	1.0000	.0200	.0674	-.0474
2	3.0000	1.0000	.3900	.4709	-.0809

```
**********************************************************************
*                                                                    *
*     ONE-DIMENSIONAL CONVECTIVE-DISPERSION EQUATION                 *
*     NON-LINEAR LEAST-SQUARES ANALYSIS                              *
*                                                                    *
*     DETERMINISTIC LINEAR EQUILIBRIUM ADSORPTION FOR PULSE          *
*     INJECTION WITH FIRST- AND ZERO-ORDER PRODUCTION AND DECAY      *
*     SOLUTION FOR RESIDENT CONCENTRATIONS                           *
*                                                                    *
*       Tygon reactor                                                *
*       (UNITS: CENTIMETERS, DAYS, MILLIGRAMS)                       *
*                                                                    *
**********************************************************************
```

INITIAL VALUES OF COEFFICIENTS

NAME	INITIAL VALUE
V.....	160.0000
D.....	300.0000
R.....	1.0000
PULSE.....	10.0000
RX1.....	100.0000
RX0.....	.0000
CI.....	.0000
C0.....	1.0000

OBSERVED DATA

OBS. NO.	CONCENTRATION	DISTANCE	TIME
1	.3500	1.5000	1.0000
2	.1800	3.0000	1.0000
3	.0300	14.0000	1.0000
4	.0000	20.0000	1.0000

ITER	SSQ	RX1...
0	.00105	100.0000
1	.00105	100.4102
2	.00105	100.4137

RSQUARE FOR REGRESSION OF OBSERVED VS PREDICTED= .98888354

NON-LINEAR LEAST SQUARES ANALYSIS, FINAL RESULTS

NAME	VALUE	S.E.COEFF.	95% CONFIDENCE LIMITS LOWER	UPPER
RX1...	100.41371	6.03597	81.20509	119.62233

----------ORDERED BY COMPUTER INPUT----------

NO	DISTANCE	TIME	CONCENTRATION OBS	FITTED	RESI-DUAL

1	1.5000	1.0000	.3500	.3386	.0114
2	3.0000	1.0000	.1800	.1943	-.0143
3	14.0000	1.0000	.0300	.0033	.0267
4	20.0000	1.0000	.0000	.0004	-.0004

----------ORDERED BY RESIDUAL----------

			CONCENTRATION		RESI-
NO	DISTANCE	TIME	OBS	FITTED	DUAL
3	14.0000	1.0000	.0300	.0033	.0267
1	1.5000	1.0000	.3500	.3386	.0114
4	20.0000	1.0000	.0000	.0004	-.0004
2	3.0000	1.0000	.1800	.1943	-.0143

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**********************************************************************
*                                                                    *
*     ONE-DIMENSIONAL CONVECTIVE-DISPERSION EQUATION                 *
*     NON-LINEAR LEAST-SQUARES ANALYSIS                              *
*                                                                    *
*     DETERMINISTIC LINEAR EQUILIBRIUM ADSORPTION FOR PULSE          *
*     INJECTION WITH FIRST- AND ZERO-ORDER PRODUCTION AND DECAY      *
*     SOLUTION FOR RESIDENT CONCENTRATIONS                           *
*                                                                    *
*       glass reactor                                                *
*       (UNITS: CENTIMETERS, DAYS, MILLIGRAMS)                       *
*                                                                    *
**********************************************************************
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INITIAL VALUES OF COEFFICIENTS

NAME	INITIAL VALUE
V.....	186.8000
D.....	263.5200
R.....	1.0000
PULSE.....	10.0000
RX1.....	100.0000
RX0.....	.0000
CI.....	.0000
C0.....	1.0000

OBSERVED DATA

OBS. NO.	CONCENTRATION	DISTANCE	TIME
1	.6900	1.5000	1.0000
2	.3900	5.0000	1.0000
3	.2200	9.0000	1.0000
4	.0200	14.0000	1.0000
5	.0000	20.0000	1.0000

ITER	SSQ	RX1...
0	.20474	100.0000
1	.01261	31.5636
2	.00879	35.3373
3	.00879	35.3253

RSQUARE FOR REGRESSION OF OBSERVED VS PREDICTED= .99123063

NON-LINEAR LEAST SQUARES ANALYSIS, FINAL RESULTS

NAME	VALUE	S.E.COEFF.	95% CONFIDENCE LIMITS LOWER	UPPER
RX1...	35.32532	3.24630	26.31264	44.33799

----------ORDERED BY COMPUTER INPUT----------

NO	DISTANCE	TIME	CONCENTRATION OBS	CONCENTRATION FITTED	RESI-DUAL
1	1.5000	1.0000	.6900	.6501	.0399
2	5.0000	1.0000	.3900	.3777	.0123
3	9.0000	1.0000	.2200	.2031	.0169
4	14.0000	1.0000	.0200	.0935	-.0735
5	20.0000	1.0000	.0000	.0368	-.0368

----------ORDERED BY RESIDUAL----------

NO	DISTANCE	TIME	CONCENTRATION OBS	CONCENTRATION FITTED	RESI-DUAL
1	1.5000	1.0000	.6900	.6501	.0399
3	9.0000	1.0000	.2200	.2031	.0169
2	5.0000	1.0000	.3900	.3777	.0123
5	20.0000	1.0000	.0000	.0368	-.0368
4	14.0000	1.0000	.0200	.0935	-.0735