AEROBIC BIOLOGICAL DEGRADATION OF RDX

Progress Report No. 1

by

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1. INTRODUCTION

1.1 RDX and Problems

RDX (Rapid Detonation Explosive, Research Department Explosive, or Royal Demolition Explosive) is a white crystalline high explosive compound predominantly used by the military. Its generic, other common names and trade marks are: hexahydro- 1, 3, 5- trinitro- 1, 3, 5- triazine; cyclonite; trimethylenetrinitramine; cyclotrimethylenetrinitramine; 1, 3, 5- trinitrohexahydro- s- triazine; T4; Hexogen. RDX is soluble in polar solvents, such as acetone and cyclohexanone, but is only spargingly soluble in water.

It produces convulsions and death in mice and rats (Sklyanskaya and Pozhariskii, 1944; Sunderman, 1944) and causes various toxic effects in man (Hollander and Colbach, 1969; Ketel and Hughes, 1972; Knepshield and Stone, 1972). Based on human and animal toxicity data, Etnier (1989) proposed a criterion of 105 μg/L for ingestion from drinking water. The toxicity of RDX to aquatic life has been studied and Sullivan et al. (1979) recommended a 24-h average maximum allowable concentration of 300 μg/L RDX in waters to protect aquatic life.

RDX is manufactured only at Holston, Tennessee in the U.S., but explosives and propellants which incorporate RDX are handled at several Army and Navy load, assemble and pack (LAP) facilities, and at the Lawrence Livermore National Laboratory (LLNL) and the Los Alamos Laboratory. Typical cleanup waters from the LLNL contain approximately 4 mg/L of RDX (Knezovich and Daniels, 1989) and need to be treated before discharge to the environment. Furthermore, there is a ground water contamination problem at LLNL. The focus of this study is to develop an environmentally sound, aerobic biological treatment method for waters contaminated with trace levels of RDX.

1.2 Review of Existing Treatment Methods

Various physicochemical methods have been used to treat RDX contaminated waters, such as solar-evaporation ponds and activated-carbon treatment. However, the use of activated carbon to remove RDX creates hazardous conditions when handling and thermally regenerating the RDX laden carbon. Ardren et al. (1975) found from their pilot study that the system using polymeric adsorbent and a small amount of activated carbon as polishers in series would successfully treat the RDX-rich waters. Roth and Murphy (1979) studied the destruction of RDX and TNT by ultraviolet light using ozone and hydrogen peroxide as oxidants. Others examined neutralization, chlorination and bromination (Ruchoff et al., 1943; Semmens et al., 1984). Most of these processes effectively remove RDX from the waters with relatively high concentrations of RDX. However, effectiveness or economic feasibility for using these processes to treat waters contaminated with trace levels of RDX such as the cleanup water from the LLNL is questionable. Also, the possibility of forming more toxic byproducts, such as formaldehyde, further discourages the use of the UV or other chemical processes (Hoffsommer et al., 1977; Glover and Hoffsommer, 1979).

Another possible treatment method is biological degradation. Biodegradability of RDX has been studied by McCormick et al. (1981). Rapid disappearance of 50 to 100 mg/L RDX from nutrient broth cultures inoculated with anaerobic sewage sludge during anaerobic incubation was reported. However, anaerobic degradation of RDX produced unwanted mutagenic or carcinogenic end products such as hydrazine and methylated hydrazines. McCormick et al. also reported no disappearance of RDX from cultures inoculated with aerobic, activated sludge and incubated aerobically. They concluded that the biological treatment of wastewaters contaminated with RDX must include an anaerobic stage. Bell and Burrows (1987) reported about 45 to 80% RDX removal using biological processes which included an anoxic mode, and smaller removal of RDX (27.4%) through a rotating biological contactor (RBC) system which did not

provide an anoxic condition. Removing RDX through an aerobic RBC process somewhat contradicts the finding of McCormick et al. (1981). However, nonbiological removal such as adsorption to cell surfaces or establishment of different consortium of aerobic cultures in the RBC which can degrade RDX may explain the small removal of RDX.

1.3 Development of Proposed Treatment Schemes

The possibility of aerobic biodegradation of RDX suggested by Bell and Burrows (1987) and Knezovich and Daniels (1989) encouraged us to develop an aerobic biological process to treat the LLNL cleanup waters using cultures specifically selected and acclimated for RDX degradation. Successful operation of conventional biological processes such as the activated sludge process requires relatively high concentrations of carbon sources and inorganic nutrients to maintain a flocculant biomass. The LLNL cleanup waters contain only small amounts of RDX (about 4 mg/L) and it is highly questionable whether this concentration permits successful operation of conventional biological processes, even if RDX degrading organisms are found. Therefore, additional organic substrates are required to operate conventional aerobic biological processes.

The enricher reactor process (Cardinal et al., 1990; Babcock et al., 1990) can be applied to treat the LLNL cleanup waters with slight modification. In a small bioreactor, cultures are acclimated to RDX under an optimum enrichment condition. The acclimated cultures are then either continuously or intermittently transferred into a larger reactor through which the LLNL cleanup waters are treated. In this way, cells and/or enzymes which can mineralize the RDX are manufactured in the small bioreactor and the large reactor provides the contact between the cells and the RDX chemical. This process is somewhat different from the typical enricher reactor process because the cells are not expected to grow in the large reactor but only to be retained in that reactor until this capacity to degrade RDX is lost.

A draft-tube fluidized-bed biofilm reactor (DTFBB) is an ideal candidate for the large reactor (Tang and Fan, 1987). A draft tube, which contains support particles for microorganisms attachment, is placed concentrically inside the DTFBB reactor. Figure 1 shows the reactor used in this research project. Air bubbles introduced from the bottom of the reactor rise up through the draft tube inducing liquid flow and carrying solid particles to the top of the draft tube. The bubbles also provide aeration. The liquid and the solid particles rise to the top by the air bubbles then flow down outside of the draft tube, which creates an internal circulation pattern. Internal circulation of liquid and solid particles and a vigorous mixing condition between air, liquid, and solid particles inside the DTFBB reactor promise high mass-transfer efficiency. Contrary to the conventional fluidized-bed reactors, liquid flow and fluidization of particles are carried out by buoyant force instead of upflow liquid velocity; subsequently, pumping cost is reduced.

For the purpose of treating RDX in wastewater, the DTFBB reactor also provides unique advantages over conventional mixed flow bioreactors. The acclimated microorganisms can be retained inside the large reactor for a long period of time by attaching to support particles, or by the high downward liquid current induced by heavy support particles which prevents flocs of microorganisms from escaping escape the reactor (Ro and Stenstrom, 1991). In this way, the hydraulic and solids retention times are decoupled, allowing a smaller reactor volume to be used. The ability of the DTFBB reactor to retain the microorganisms is very important in this case, because the acclimated cultures transferred from the enricher reactor are not expected to grow significantly. Retaining as many cells as possible in the DTFBB reactor will reduce the frequence and amount of cell additions from the enricher reactor.

Our goals in this study were to be achieved through two phases. The first phase is to acclimate aerobic cultures to RDX and to determine the optimum growth condition of the acclimated cultures. In the second phase, assuming success in the first phase, we would have

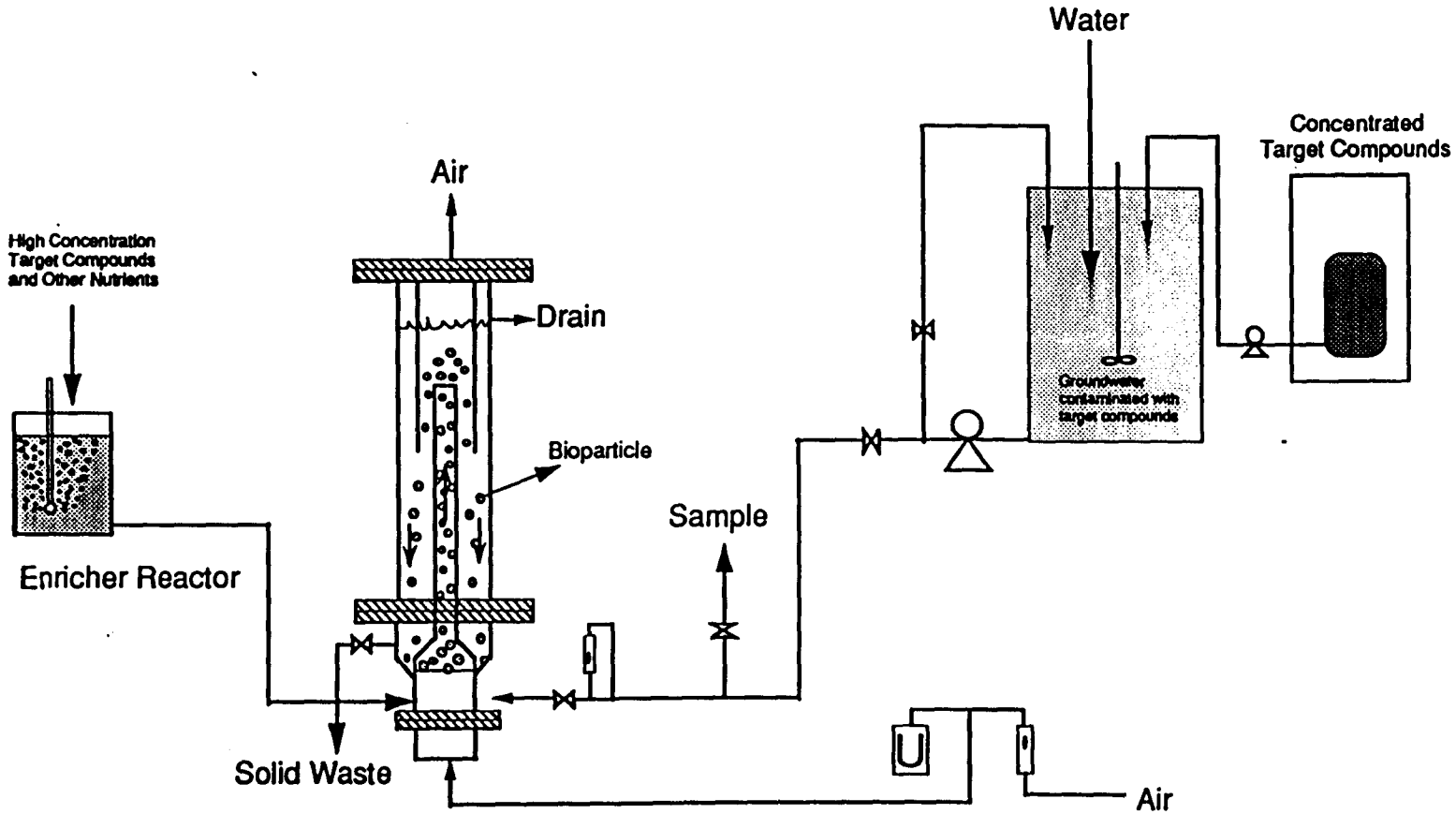


Figure 1. Schematic Diagram of Draft-Tube Fluidized-Bed Biofilm Reactor Pilot Plant

operated the modified enricher reactor system to determine optimum operating and design parameters.

2. MATERIALS AND METHODS

2.1 RDX and Chemical Nutrients Media

RDX in powder form (1 gram) enclosed in a cylindrical steel housing (6" in diameter by 14" long) was shipped from LLNL on 4/23/90, 7/2/90, 8/15/90, 10/18/90, 12/20/90, and 3/5/91. As soon as RDX arrived at the UCLA Water Quality Laboratory, it was dissolved with 25 to 100 mL of acetone and stored as a stock solution inside a refrigerator at 4° C until used. All chemicals for nutrients and solvents used in this study were obtained from Fisher Scientific Co. (Pittsburgh, PA), except yeast extract and bacto peptone media, which were obtained from Difco Laboratories (Detroit, MI), and K_2HPO_4, KH_2PO_4, and $(NH_4)_2SO_4$, which were purchased from Sigma Chemical Co. (St. Louis, MO). Complex nutrient media were used for the first phase of this study. The different inorganic, organic, and trace minerals of the complex media are shown in Tables 1, 2, and 3, respectively.

2.2 Inoculum

Various sources of inoculum were tested to find cultures that could be adapted to degrade RDX. The list of the inoculums were: water and sediment samples from the LLNL collection basin and contaminated sites (827CE, 817 Lagoon Sediments, 827E); UCLA soil samples; activated sludge from a local wastewater treatment plant (Tillman, Van Nuys CA), activated sludge acclimated to 1-amino naphthalene (Babcock et al., 1991); and resting cultures of *Pseudomonas putida* G786 capable of dehalogenation from halogenated aromatic compounds (Horowitz and Vilker, 1989). A sequencing batch reactor (5 L) was operated, starting on 5/22/90, to acclimate activated sludge cultures to RDX. The substrate composition was the same as that used by Babcock et al., except 1-amino naphthalene was replaced with an RDX concentration of 4 mg/L.

Table 1. Composition of Mineral Media*

Chemicals	Media		
	MR (g/L)	MF (g/L)	MB (g/L)
K_2HPO_4	0.8	0.4	0.125
KH_2PO_4	0.2	-	0.2
$KHCO_3$	-	10 mM	-
$NaCO_3$	-	10 mM	-
$(NH_4)_2SO_4$	12	-	0.1
NaCl	12	-	-
$MgSO_4$	0.2	-	-
$CaCl_2 \cdot 2H_2O$	0.1	0.05	0.01
$MgCl_2 \cdot 6H_2O$	-	0.1	0.0165

* All mineral media contained trace minerals with concentrations shown in Table 3.

Table 2. Organic Substrates Used For Incubation Experiments

Chemicals	Concentration (g/L)
Glucose	1.0
Peptone	0.1, 0.2, 0.5, 1.0
Pyrimidine	0.5
Tryptophan	0.5
Yeast Extracts	0.1, 0.5, 1.0
Sodium Acetate	0.1
Gentisic Acid	0.2
Protocatechuic Acid	0.2
Salicylic Acid	0.2
Catechol	0.2
Melamine	0.2
Cianuric Acid	0.2

Table 3. Composition of Trace Minerals

Chemicals	Concentration (mg/L)
Na_3 Citrate	1.8
$FeCl_3$	0.2
$MnCl_2 \cdot 2H_2O$	0.05
$ZnCl_2$	0.03
$CoCl_2 \cdot 6H_2O$	0.03
$(NH_4)Mo_7O_{24} \cdot 4H_2O$	0.02
$CuCl_2 \cdot 6H_2O$	0.02
$Na_2B_4O_7 \cdot 10H_2O$	0.01

2.3 Incubation and Microbial Growths Measurements

Typically 100 mL of a nutrient broth containing 5 to 10% (vol./vol.) inoculum and various concentrations of RDX ranging from 4 mg/L to 1000 mg/L were placed in autoclaved 250 mL Erlenmeyer flasks and incubated on an Orbit Environ-Shaker (Lab-Line Instruments, Inc., Melrose, ILL) at 30°C, and shaken at 200 rpm. The shaker flasks were covered with aluminum foil to block fluorescent light, which could have allowed phototrophs to grow. A nutrient broth without inoculum was used for control. Microbial growth was measured either by the volatile fraction of the dried mass of suspended solids (VSS) according to the Standard Methods (APAH, 1989) or by optical density at 540 nm.

2.4 DTFBB Pilot Plant

Construction of the DTFBB pilot plant began in June 1990 and finished in December 1990. A schematic diagram of the DTFBB pilot plant is shown in Figure 1. The reactor was fabricated from clear PVC with a detachable draft tube of 2 in. i.d. and 3 ft. long placed concentrically inside a 6 in. i.d. and 4 ft. long tube. At the lower end of the draft tube, inside diameter increases from 2 in. to 4 in. to trap all the air bubbles rising from an air diffuser into the draft tube. The clearance between the end of the draft tube and the bottom of the reactor is about 1/2 in. to allow unrestricted internal circulation of liquid and solids. At the top of the reactor, a 5 in. i.d. and 2 ft. long plexiglass tube is attached concentrically to a top lid. This additional tube provides a quiescent condition at the effluent region so that almost no support particles can escape to the effluent stream. Air is introduced at the bottom of the reactor and passes through a diffuser made of marbles and glass beads to provide uniform distribution of air flow before the air enters the draft tube. The support medium is 2000 g of graded sand (US mesh 50/80, average diameter of 0.25 mm) with a density of 2.69 g/cm^3.

2.5 Analytical Protocol for RDX

About 2 mL of water samples from a broth was collected through a 5 mL syringe and filtered through a series of 0.5 and 0.2 μm HPLC syringe filters (Corning) into a 4 mL sample vial. Fifty uL of this filtered sample water was injected into a high performance liquid chromatograph (Varian 5020) equipped with a reverse-phase C-18 column (5μ Adsorbosphere, Altech) and a variable UV-VIS detector. The mobile phase of 30% methanol and 70% water at a rate of 1 mL/min yielded a characteristic RDX retention time of about 17 minutes, detected at a wavelength of 236 nm. Later in the project, the composition of mobile phase was changed to 40% water, 30% methanol, and 30% acetonitrile which decreased the retention time to about 4.5 minutes. The lowest detection limit using this protocol were about 0.5 mg/L.

3. RESULTS AND DISCUSSION

3.1 Incubation I (5/8/90 to 5/17/90)

Biomass acclimated to 1-amino naphthalene and *P. putida* G786 were tested to determine if the cultures could readily degrade RDX. Types of media and results of growth and RDX removal are shown in Table 4. The *P. putida* G786 monooxygenase was found to attack halogen groups attached to aromatic rings, such as naphthalene (Horowitz and Vilker, 1989). It was speculated that this enzyme might attack the nitro groups of the trazine ring of RDX. The acclimated sewage cultures, which were believed at the time of this study to attack the amino group of the 1-amino naphthalene during the course of degradation, were also tested for their ability to attack the nitro groups of RDX.

As shown in the Table 4, broth C and G contained no additional nitrogen source. The rational behind this arrangement was to force cultures to break RDX to obtain nitrogen. Yeast extracts were added to the broth D for both C- and N-sources to compare with glucose which is very easy to degrade and might inhibit the growth of RDX utilizing microorganisms. Broth E did not contain any organic substrates in order to force cultures to utilize RDX as their only carbon source. Broth F used salicylic acid which is believed to induce enzymes to break aromatic rings (Cardinal and Stenstrom, 1990).

All the broths which had both C- and N- sources showed some growth of volatile solids. The growth of volatile solids in the presence of 100 mg/L RDX (saturated) indicated that RDX did not inhibit growth of cultures in the broths. Broths B and D showed the most growth from approximately 100 mg/L to 830 and 930 mg/L MLVSS, respectively. Broth G without any nitrogen source also showed growth from 100 mg/L to approximately 500 mg/L MLVSS, which immediately suggested the possibility of the cultures utilizing RDX as their N-source. However, none of the broths showed any decrease in RDX. The inoculum for the broth G may have con-

Table 4. Broth Composition, Growth, and Removal of RDX During Incubation 1

Broth	Organic Substrates	Mineral Media	Inoculum	Growth	RDX Removal**
A	Glucose (1 g/L)	MR	None	-	-
B	Glucose (1 g/L)	MR	1-NA*	+	-
C	Glucose (1 g/L)	MR without $(NH_4)_2SO_4$	1-NA*	-	-
D	Yeast Extract (0.01%)	MR without $(NH_4)_2SO_4$	1-NA*	+	-
E	No Organic Substrate	MR	1-NA*	+	-
F	Salicylic Acid (0.01%)	MR	1-NA*	+	-
G	Glucose (1 g/L)	MR without $(NH_4)_2SO_4$	*P.putida* G786	+	-

* Acclimated activated sludge to 1-amino naphthalene

** All broths contained 100 mg/L RDX (saturated) initially

tained some nitrogen, which would explain the growth. *P. putida* G786 monooxygenases were not effective in attacking either nitro groups, nor the triazine ring. Acetone, which was used to dissolve RDX, might be the carbon source for the E cultures, which explained the growth in those cultures.

One of reasons for non-removal of RDX by the cultures was speculated that the incubation period of 9 days might be too short for the microorganisms to adjust to RDX. Consequently, experiments with a longer incubation period were planned.

3.2 Incubation II (6/8/90 to 7/20/90)

Three types of cultures were tested for the possibility of degrading RDX; activated sludge acclimated to 1-amino naphthalene, activated sludge which had previously been exposed to 4 mg/L of RDX in the sequencing batch reactor, and the water and sediment from a contaminated site (827CE at LLNL Site 300). The media composition and the results of growth and RDX removal are shown in Table 5.

The broths B2, B3, and C2 showed growth; however, none of the broths exhibited disappearance of 40 mg/L of RDX. The B3 and B2 which contained both C- and N- sources showed the most growth as expected. Broth C2 without N sources also showed a little growth at the beginning, but it was suspected that the cultures from the enricher reactor probably contained other N sources. Both B1 and C1 did not show any growth compared with the control of which MLVSS varied from 313 to 542 mg/L. The MLVSS of the broths are shown in Figure 2.

3.3 Incubation III (8/8/90 to 9/14/90)

Three sets of broths inoculated with 827CE cultures were incubated as shown in Table 6. The carbon sources were glucose or peptone and the nitrogen sources were either $(NH_4)2SO_4$/peptone or peptone. The motivation was to duplicate the condition of Knezovich

Table 5. Broth Composition, Growth, and Removal of RDX During Incubation II

Broth	Organic Substrates	Mineral Media	Inoculum	Growth	RDX Removal**
A	Glucose (0.1%)	MR	None	-	-
B1	Glucose (0.1%)	MR	Bat***	+	-
B2	Glucose (0.1%)	MR	1-NA*	+	-
B3	Glucose (0.1%)	MR	827CE	+	-
C1	Glucose (0.1%)	MR without $(NH_4)_2SO_4$	Bat***	-	-
C2	Glucose (0.1%)	MR without $(NH_4)_2SO_4$	1-NA*	+	-
C3	Glucose (0.1%)	MR without $(NH_4)_2SO_4$	827CE	N/A	-

* Acclimated activated sludge to 1-amino naphthalene

** All broths contained 40 mg/L of RDX initially

*** Activated sludge acclimated to RDX in a sequencing batch reactor

N/A The broth dried up after 14 days of incubation.

Table 6. Broth Composition, Growth, and Removal of RDX During Incubation III

Broth	Organic Substrates	Mineral Media	Inoculum	Growth	RDX Removal*
D1	Glucose (0.1%)	MR	827CE	+	-
D2	Peptone (500 mg/L)	MR	827CE	+	-
D3	Peptone (500 mg/L)	MR without $(NH_4)_2SO_4$	827CE	+	-

* All broths contained 40 mg/L of RDX initially.

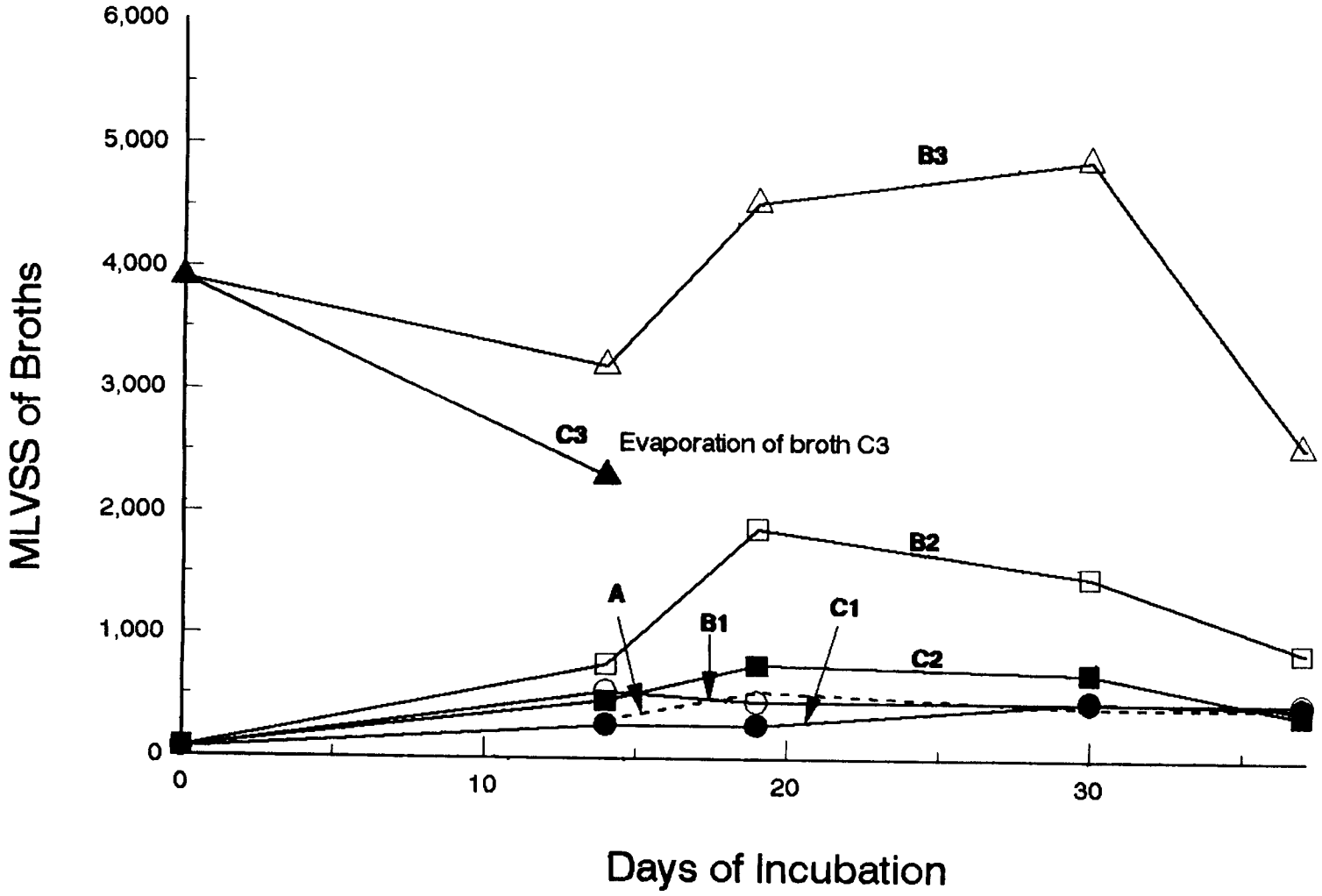


Figure 2. MLVSS of broths during Incubation II

and Daniels (1990) experiments which showed some removal of RDX. Although all the broths showed growth, none of broths showed any decrease in RDX.

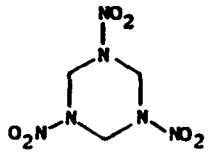
3.4 Incubation IV (9/20/90 to 10/31/90)

Two cultures obtained from 827CE and 817 sediments, freshly shipped from the LLNL were incubated in various broths, varying carbon and nitrogen sources, and RDX concentrations as shown in Table 7. All broths except A (control), D1, and D2 showed growth. D1 and D2 did not contain any C or N sources in order to force cultures to utilize RDX. Among the samples taken on 10/2/90, B1 which showed the most growth also appeared to remove RDX from 4 mg/L to non-detectable concentrations. The B1 cultures were then transferred into fresh media (10% v/v) on 10/12/90. Duplication of the previous result of RDX removal was unsuccessful. Knezovich and Daniels (1990) also encountered a similar problem and could not keep the activity of the cultures after transferring.

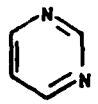
3.5 Incubation V (11/8/90 to 12/19/90)

Two cultures developed from 827CE and 817 sediments were incubated in broths containing varying concentrations of peptone as shown in Table 8. Also organic compounds with molecular structures similar to RDX were used as organic substrates in broth E and F. Both pyrimidine and tryptophane have nitrogen embedded in the ring as shown in Figure 3. The rational behind this series of experiments was to acclimate cultures to these structurally similar compounds in the hope that this procedure would eventually lead to degradation of RDX. Even though the broths containing peptone showed growth, of RDX was not removed.

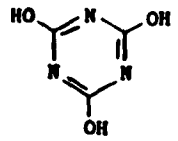
Broth C which contained 1000 mg/L RDX (saturated with residuals) and 500 mg/L peptone showed explosive growth. Five milliliters of the culture C were then transferred into two fresh broths with the same media as before, except one broth contained 25 g/L of acetone as an organic substrate and the other broth contained crystal RDX without any acetone to make an



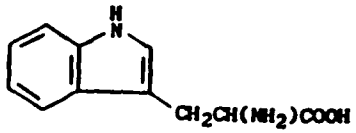
RDX



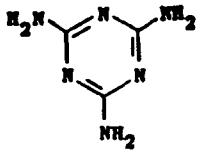
Pyrimidine



Cyanuric Acid



Tryptophane



Melamine

Figure 3. Molecular structures of compounds that are similar to RDX used in Incubation V and VI

Table 7. Broth Composition, Growth, and Removal of RDX During Incubation IV

Broth	Organic Substrates	RDX (mg/L)	Mineral Media	Inoculum	Growth	RDX Removal
A	500 ppm Peptone	4	MR	None	-	-
B1	500 ppm Peptone	4	MR	827CE	+	+
B2	500 ppm Peptone	4	MR	817 Sed.	+	-
C1	500 ppm Peptone	4	MR without $(NH_4)_2SO_4$	827CE	+	-
C2	500 ppm Peptone	4	MR without $(NH_4)_2SO_4$	817 Sed.	+	-
D1	None	40	MR without $(NH_4)_2SO_4$	827CE	-	-
D2	None	40	MR without $(NH_4)_2SO_4$	817 Sed.	-	-
H1	200 ppm Sodium Acetate and 200 ppm Yeast extracts	4	MR	827CE	+	-
H2	200 ppm Sodium Acetate and 200 ppm Yeast extracts	4	MR	817 Sed.	+	-

Table 8. Broth Composition, Growth, and Removal of RDX During Incubation V

Broth	Organic Substrates	RDX (mg/L)	Mineral Media	Inoculum	Growth	RDX Removal
A	500 ppm Peptone	4	MR	None	-	-
B	1000 ppm Peptone	4	MR	827CE	+	-
C	500 ppm Peptone	1000	MR	827CE	+	-
D	200 ppm Peptone	4	MR	827CE	+	-
E	500 ppm Pyrimidine	4	MR	827CE	-	-
F	500 ppm Tryptophan	4	MR	827CE	-	-

RDX concentration of approximately 1000 mg/L. This was to test whether the explosive growth was due to RDX or the acetone oxidation that was used to dissolve RDX. For every one gram of RDX, approximately 24 to 100 grams of acetone were used to make a stock solution. After 4 weeks of incubation (11/16/90 to 12/19/90), only the broth that contained acetone showed rapid growth as before and no growth was observed for the broth with only RDX. The broths containing pyrimidine and tryptophane did not show any growth, or decrease in RDX concentration.

3.6 Incubation VI (12/19/90 to 1/20/91)

This study was conducted by Mr. Felipe Alatriste, a Ph.D. student in Public Health (Environmental Microbiology) at UCLA, using the fresh water and sediment samples from the LLNL (827E). The types of media, substrates and the results are shown in Table 9. All broths showed no decrease in concentration. RDX.

Broth A contained acetone without any nitrogen sources and showed no growth. Broths B and C did not contain any C and N sources except RDX to force cultures to utilize RDX. This condition was similar to the broths D1 and D2 of incubation IV as shown in Table 7. As expected, the two broths did not support any growth. Broths D through K contained varying concentrations of peptone and yeast extracts without any other nitrogen sources and were similar to incubation IV and V. All supported growth but no decrease in RDX was observed. Broths L through O contained compounds known to be intermediate metabolites of aromatic compounds. It was hoped that these compounds might induce enzymes necessary to degrade RDX. Also no nitrogen sources were present. No growth was observed. Broths P through S contained compounds structurally similar to RDX. The molecular structures of these compounds are shown in Figure 3. No growth was observed from any of these broths, as was the case in the broths E and F of Incubation V.

Table 9. Broth Composition, Growth, and Removal of RDX During Incubation VI

Broth	Organic Substrates	RDX (mg/L)	Mineral Media	Inoculum	Growth	RDX Removal
A	Acetone	5	MF	827E	-	-
B	None	5	MF	827E	-	-
C	None	300	MF	827E	-	-
D	Peptone (0.1%)	5	MF	827E	+	-
E	Peptone (0.05%)	5	MF	827E	+	-
F	Peptone (0.1%)	15	MF	827E	+	-
G	Peptone (0.05%)	15	MF	827E	+	-
H	Yeast Extracts (0.1%)	5	MF	827E	+	-
I	Yeast Extracts (0.05%)	5	MF	827E	+	-
J	Yeast Extracts (0.1%)	15	MF	827E	+	-
K	Yeast Extracts (0.05%)	15	MF	827E	+	-
L	Gentisic Acid (0.02%)	30	MF	827E	-	-
M	Protocatechuic Acid (0.02%)	30	MF	827E	-	-
N	Salicylic Acid (0.02%)	30	MF	827E	-	-
O	Catechol (0.02%)	30	MF	827E	-	-
P	Tryptophan (0.02%)	5	MF	827E	-	-
Q	Pyrimidine (0.02%)	5	MF	827E	-	-
R	Melamine (0.02%)	5	MF	827E	-	-
S	Cyanuric Acid (0.02%)	5	MF	827E	+	-

3.7 Batch Reactor Operation

A sequencing batch reactor was operated from 5/22/90 to 10/2/90. The nutrient media consisted of mineral media (MB of Table 1), 100 mg/L of yeast extract and 100 mg/L of sodium acetate as carbon sources, and a daily addition of 4 mg/L of RDX. The batch reactor was originally inoculated with activated sludge acclimated to 1-amino naphthalene (Babcock et al., 1991) and later with activated sludge from a local sewage treatment plant (Tillman, Van Nuys, CA), UCLA soils, and RDX contaminated waters and sediments from the LLNL. The purpose of this set-up was to create a prolonged condition for cultures to be exposed to RDX and thereby to increase the likelihood of generating enzymes for RDX as in the case of 1-amino naphthalene. For more than 120 days of operation, no decrease in RDX was observed.

4. CONCLUSIONS

Treatability of the LLNL cleanup waters contaminated with trace level concentrations of RDX by aerobic biological sludge acclimated to RDX was tested in this study. A modified enricher reactor process with a draft-tube fluidized-bed biofilm reactor as a main reactor and a small bioreactor, a sequencing batch reactor in this case, seemed to be an ideal reactor configuration for this case. However, aerobic cultures capable of degrading RDX were needed in order to successfully operate this system.

Various cultures in many different types of broth media were tested. All showed no sign of RDX removal, except the cultures in Incubation III (B1 in Table 7). However, duplication of the results was unsuccessful. Organic substrates with their structures similar to RDX and substrates that were known either to induce enzymes for aromatic compound degradation or to be intermediate metabolites of the biodegradation of aromatic compounds all produced negative results.

Even with this extensive effort on biodegradation of RDX, aerobic degradation of RDX was not demonstrated. This result supports the finding of McCormick et al. (1981). Biological treatment of RDX with aerobic processes alone may not be successful. Processes incorporating both anaerobic and aerobic condition or a combination of chemical oxidation and biodegradation may be required to successfully treat waters contaminated with a trace level of RDX.

5. FUTURE PLANS

We plan to continue to expand this project, and to consider inclusion of an anaerobic process as well. As done in the preliminary study, systematic approaches to acclimate aerobic microorganisms to RDX will be continued. The radio-label evidence observed by Knezovich and Daniels (1990) and our brief success during Incubation III still suggests the possibility of obtaining aerobic cultures capable of biodegrading RDX. For anaerobic biodegradation, identification and treatability of end products will also be examined.

Selection of treatment processes will be dependent upon the results of the biodegradation study. If we can acclimate aerobic microorganisms to RDX, two treatment process schemes will be considered. For aerobic microorganisms with their growth inhibited by a high concentration of RDX, the process scheme will be the same as in the past study. If the microbial growth is not inhibited by high concentration of RDX and the microorganisms can utilize RDX as their sole carbon /or nitrogen sources, we can use a process that incorporates the activated carbon. In this process, the DTFBB contains granular activated carbon which adsorbs RDX from the contaminated groundwaters. A portion of the RDX-laden activated carbon is continuously removed from the DTFBB and feed into an enrichment reactor. In the enrichment reactor, acclimated microorganisms (either heterotrophs or nitrifiers) grow on high concentration of RDX adsorbed in the activated carbon and subsequently bioregenerate the activated carbon. The bioregenerated activated carbon is feed back into the DTFBB. If aerobic treatment of RDX is not possible, combinations of aerobic and anaerobic biodegradation processes will be investigated.

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