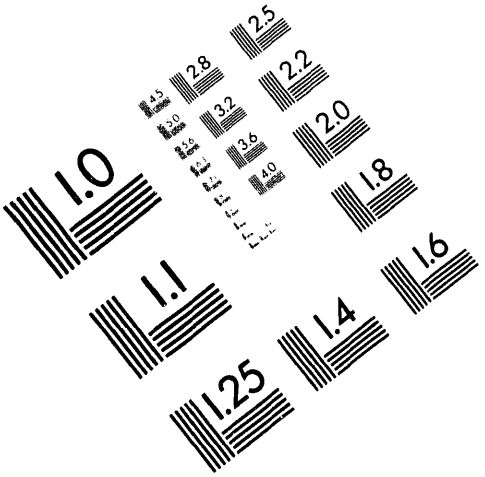

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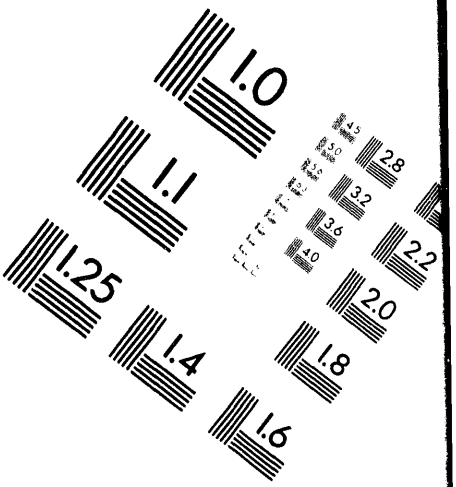

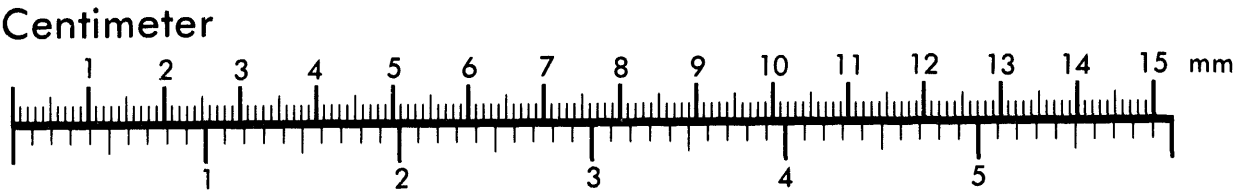

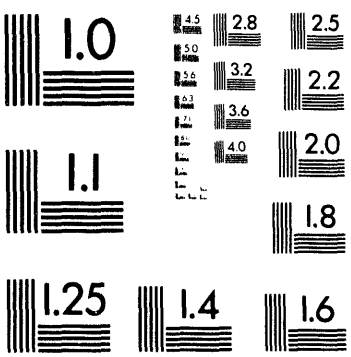

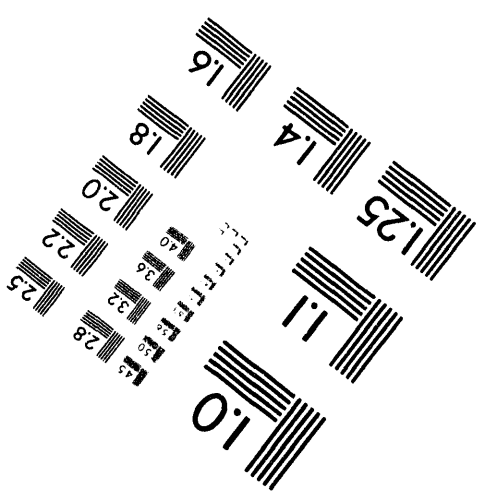

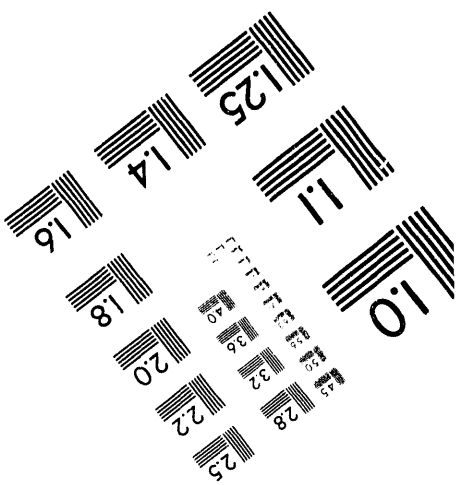

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Evaluation of a Soil Slurry Reactor System for Treating Soil Contaminated with Munitions Compounds

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ABSTRACT

Two 0.5-L semicontinuous soil slurry reactors were operated for seven months to evaluate the performance of the slurry reactor system in bioremediating soil contaminated with munitions compounds. Nitrogen and carbon were supplemented. The soil slurry was mixed continuously and aerated 10 min/day. Ten percent of the contaminated soil was replaced every week. The 2,4,6-trinitrotoluene (TNT) concentration in soil began to drop after 15 days of treatment, falling to less than 0.5 mg/kg from 7800 mg/kg. Total plate counts in both reactors indicated that the bacterial population was maintained, with an average plate count of about 10^8 CFU/mL. The soil slurry was slightly acidic. In addition to TNT, the slurry reactor also removed the other munitions compounds trinitrobenzene (TNB), 2,4-dinitrotoluene (2,4-DNT), RDX, and HMX. Radiolabeling studies on the reactor biomass showed that 23% of [$^{C}14$]TNT was mineralized, while 27% was used as biomass and 8% was adsorbed on to the soil. The rest of the [^{14}C]TNT was accounted for as TNT metabolites. Increasing the frequency of soil replacement from once to two or three times weekly did not affect the TNT removal rates. However, the slurry system showed signs of stress, with highly acidic conditions and low oxygen uptake rates.

INTRODUCTION

The major contaminant at many military sites is 2,4,6-trinitrotoluene (TNT). Contamination with munitions compounds occurs in most U.S. Army munitions facilities because of the manufacture, handling and disposal of explosives and propellants. The toxicity of TNT is well documented. Past methods for disposing of munitions wastes have included dumping in the deep sea, dumping at specified landfill areas, and incineration. All of these methods may cause serious harm to ecosystems. Biological transformation of TNT has been demonstrated previously (McCormick et al. 1976; Schackmann and Muller 1991; Boopathy et al. 1994). Recently Williams et al. (1992) studied composting as a method for removing TNT from contaminated soil and concluded that TNT, RDX, and HMX can be removed effectively. However, composting requires large amounts of additives like straw and animal feed; only a small fraction of the total volume composted is contaminated soil. Funk et al. (1993) demonstrated an anaerobic system for the bioremediation of TNT-contaminated soil, but mineralization of TNT was poor. Most of the TNT was converted to TNT metabolites.

The purposes of this study were (1) to evaluate the use of soil slurry reactor technology for removal of TNT from contaminated soil and (2) to identify the operating conditions for the large-scale treatment system. The laboratory-scale reactor system successfully removed TNT, and a significant amount of TNT was mineralized. The results of this study showed that the soil slurry reactor is an effective system for remediating soil contaminated with TNT and other munitions compounds.

MATERIALS AND METHODS

Soils: Contaminated soil was collected at the Joliet Army Ammunition Plant, Joliet, Illinois, USA. The contaminant concentrations in the soil are given in Table 1. The TNT concentration in the soil ranged from 10,000 to 20,000 mg/kg. The concentrations of the other explosives contaminants in soil were less than 300 mg/kg. The soil had a total organic matter of 4.5%, including the contaminants. The nitrogen content of the soil as ammonium ion was 7.5 mg/kg .

Soil Slurry Reactor: Two 0.5-L laboratory-scale soil slurry reactors were set up as shown in Fig. 1. The reactors were operated semicontinuously, starting with 15% (W/V) of TNT-contaminated soil. Molasses (0.3%) served as the carbon source. Nitrogen in the form of ammonium salt (0.1 g/L) was added to both reactors. Air was supplied intermittently (10 min/day) through a diffuser. The oxygen profiles of the slurry reactor at different depths are given in Fig. 2. The reactor was aerobic on the surface and up to 2.0 cm below the surface. Below 2.0 cm the reactor was anaerobic. The soil slurry was mixed continuously at the rate of 50-60 rpm with a magnetic stirrer. After a three-week stabilization period, 10% of the contaminated soil was replaced weekly, and carbon and nitrogen supplements were added at the concentrations mentioned above. After 200 days of operation, the soil replacement was increased to twice weekly (10% soil replacement each time) in one reactor and three times weekly (10% soil replacement each time) in the other reactor. The concentrations of TNT and other contaminants, the bacterial population, pH, oxygen uptake rates, nitrite and ammonium concentrations were monitored periodically.

Analyses: The TNT in the soil and soil slurry were extracted by the method recommended by the U.S. Army Environmental Research Center (Jenkins and Walsh 1987). TNT

and other munitions compounds were analyzed by using high performance liquid chromatography (HPLC) described by Boopathy et al. (1993). Bacterial activity in the reactor was monitored by total plate counts on tryptic soy agar (TSA) plates. The oxygen uptake rate in the soil slurry was monitored by using an oxygen analyzer. Nitrite and ammonium concentrations were analyzed by colorimetric methods with Hach Water Analyses reagent kits.

^{14}C Mineralization Studies: After seven months of reactor operation, 100 mL of each soil slurry was taken from the reactors. The soil slurry was incubated with uniformly labeled TNT to provide mass balance and determine metabolite production including $[^{14}C]CO_2$ (Bartha and Pramer 1965). The samples were withdrawn periodically, and the quantity of TNT converted to biomass was determined as trichloroacetic acid (TCA) precipitable material (Mans and Novelli 1961). The TNT metabolites were analyzed by collecting fractions every 30 sec after passage through the HPLC column. The radioactivity in each fraction was measured by using a liquid scintillation counter. Soil-bound radioactive TNT was analyzed by using the soil extraction procedure (Jenkins and Walsh 1987).

Chemicals: Radiolabeled TNT was purchased from Chemsyn Science Laboratories, Lenaxa, KS. The nonradioactive TNT was obtained from Chem Service Inc., West Chester, PA. TNT, 2,4-DNT, RDX and HMX were obtained from the Naval Surface Warfare Center, Indian Head, MD. The rest of the chemicals were of reagent grade.

RESULTS AND DISCUSSION

The concentrations of TNT and its metabolites 4-amino-2,6-dinitrotoluene and 2-amino-4,6-dinitrotoluene are given in Fig. 3. During the first two weeks of reactor operation, in typical batch mode, the concentration of TNT dropped constantly to less than 1000 mg/kg. After three weeks of operation, a 10% volume of contaminated soil was added to the reactor, and thereafter 10% of the soil slurry was replaced every week with a 10% of contaminated soil. The concentration of TNT fell below the detection limit (< 0.5 mg/kg) on the 95th day. After four months of continuous operation, the concentration of TNT metabolites was less than 20 mg/kg.

Figure 4 gives the concentrations of other munitions compounds in the reactor. The TNB concentration dropped to 0 mg from 300 mg in the first 50 days of the experiment. The next compound to completely disappear from the slurry system was 2,4-DNT. The RDX and HMX persisted in the soil slightly longer. After 120 days of continuous operation of the slurry reactors, the concentrations of RDX and HMX fell below 0.5 mg/kg. The removal of these compounds from the soil enhances the bioremediation potential of the soil slurry reactor system. During most of the reactor operation, the total bacterial counts were about 10^8-10^9 CFU/mL.

Figure 5 shows the pH and oxygen uptake rates in the reactor. The initial pH of the soil slurry was 7.5. During the first two weeks of operation the pH was not adjusted. Later, whenever the pH dropped below 6.5 or rose above 7.5, the pH was adjusted to 7 by using 0.1 N HCl or 0.1 N NaOH. The pH was acidic (close to 6) after 125 days, perhaps because of the accumulation of fatty acids from the metabolism of molasses by the soil microbes. The oxygen

uptake rates of the reactor biomass increased from day 0 and remained at about 7.5 mg/L throughout the experiment.

The radiolabeling study conducted on the reactor biomass showed a very reasonable mass balance of [^{14}C]TNT. Of the original [^{14}C]TNT (20,000 dpm/mL), 23% was converted to [^{14}C]CO_2, 27% was used in making cellular materials, and 8% was adsorbed onto the soil. The rest of the TNT was accounted for as TNT metabolites (Fig. 6). The major intermediates identified were 4-amino-2,6-dinitrotoluene, 2-amino-4,6-dinitrotoluene, 2,4-diamino-6-nitrotoluene and 2,3-butanediol. The mass balance was very reasonable, with recovery of 95% of the ^{14}C-TNT.

After 200 days of reactor operation, the feeding schedule was changed, and the time course of reactor operation was reset to day 0. One of the two reactors received soil and carbon additions twice weekly, the other received soil and carbon addition three times weekly. The results showed the accumulation of the 2-amino intermediate to about 200 mg/kg in the reactor that received soil addition three times per week. The pH and oxygen uptake were greatly affected by the increase in the frequency of soil addition. As Fig. 7 shows, the pH was highly acidic (4-5) in the reactor that received soil three times per week, and constant adjustment of pH in that reactor was required. The oxygen uptake rate also decreased to 4 mg/L in the reactor receiving soil addition three times weekly (Fig. 8). This result indicates that the biomass in the reactor was affected by the accumulation of metabolites either from TNT or from molasses.

SUMMARY AND CONCLUSIONS

This study showed that the natural soil bacteria present in contaminated soil can cause extensive transformation and degradation of TNT in a reasonable time under optimal conditions. Degradation was demonstrated by mineralization of [^{14}C]TNT, metabolite formation, and the presence of ^{14}C in the cell biomass as TCA-precipitable material. The soil slurry reactor system also removed the other contaminants, TNB, 2,4-DNT, RDX, and HMX. The feeding schedule experiment showed that for successful reactor operation, soil addition should not exceed twice weekly. The advantage of the slurry reactor is its simple operating conditions. The method needs only mixing, a supply of air, and a carbon source. Molasses is an inexpensive carbon source that could be used in a large-scale operation at low cost. This study indicates that this technology has great potential for the bioremediation of soils contaminated with munitions compounds.

ACKNOWLEDGMENTS

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Table 1: Explosives Concentrations in Soil

Explosive	Concentration range (mg/kg)
TNT	10,000 - 20,000
TNB	175 - 300
2,4-DNT	50 - 200
RDX	50 - 125
HMX	50 - 100
Trinitrobenzaldehyde	50 - 150

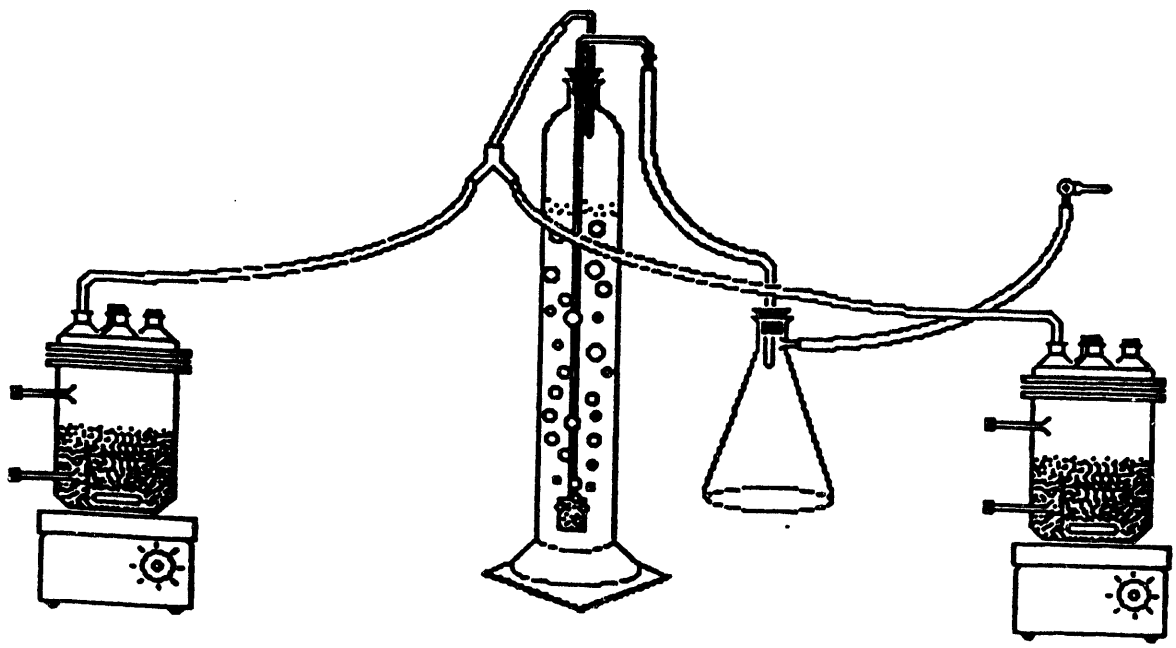
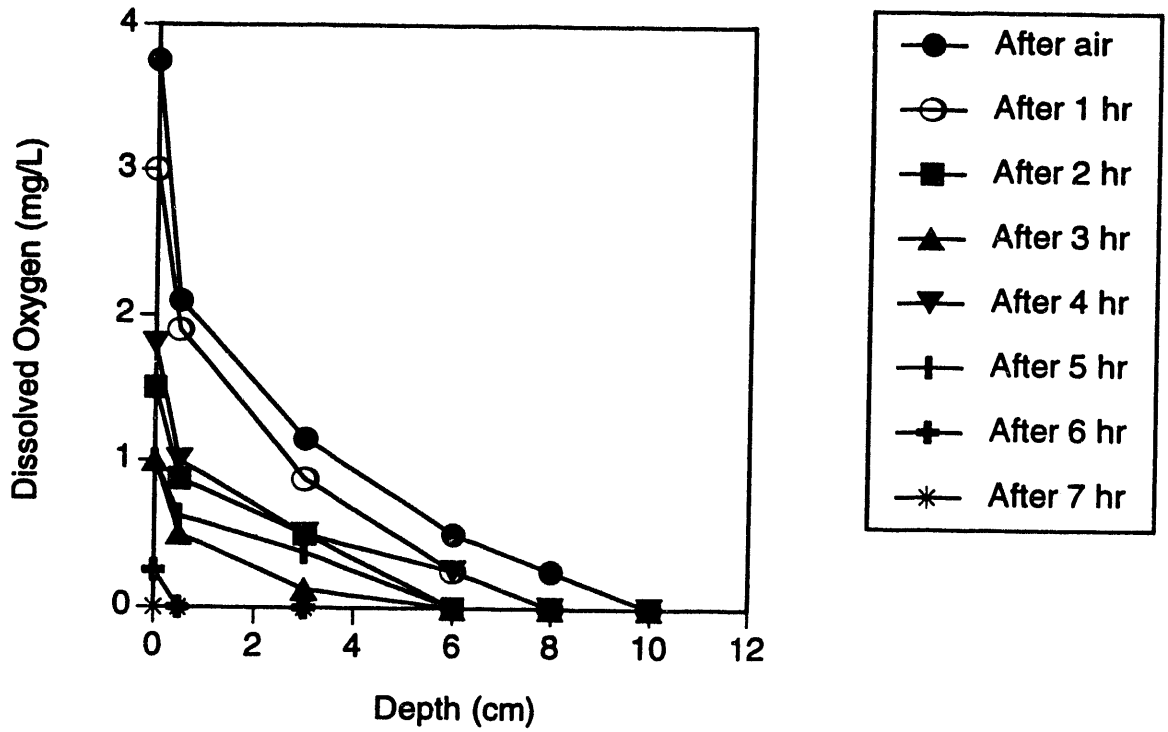


Figure 1 Reactor Setup



Figurre 2 Oxygen Profile in the Reactor

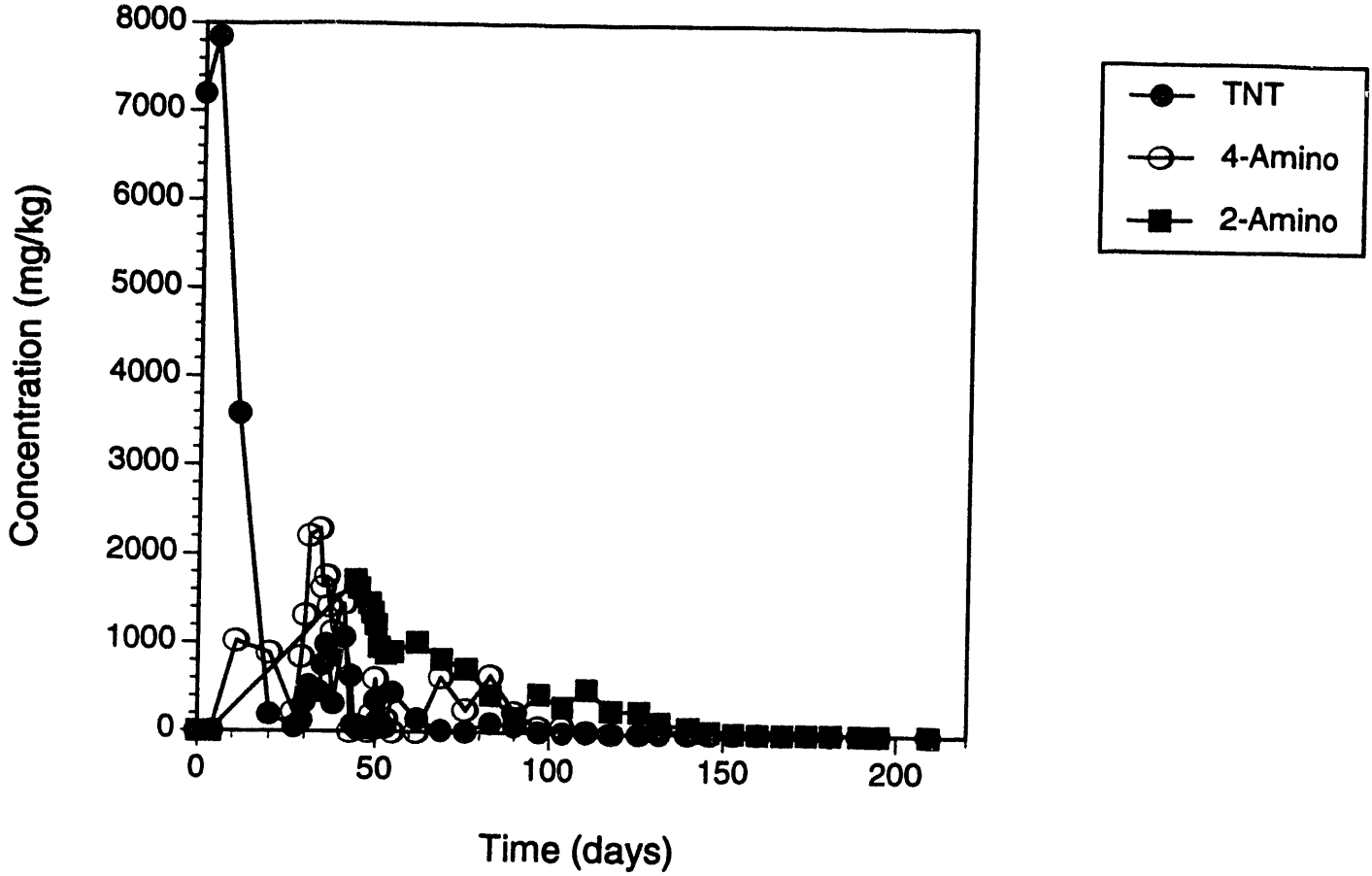


Figure 3 Concentrations of TNT and its Metabolites in the Reactor

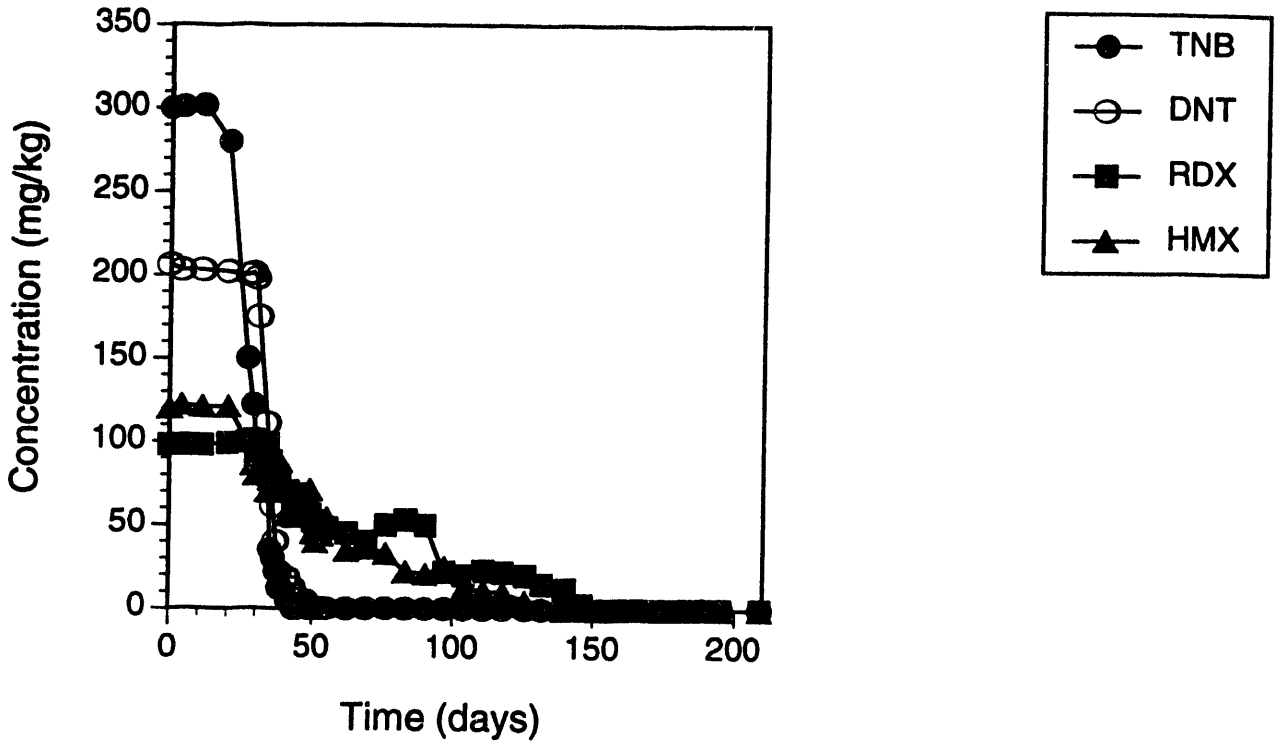


Figure 4 Concentrations of Munitions Compounds in the Reactor

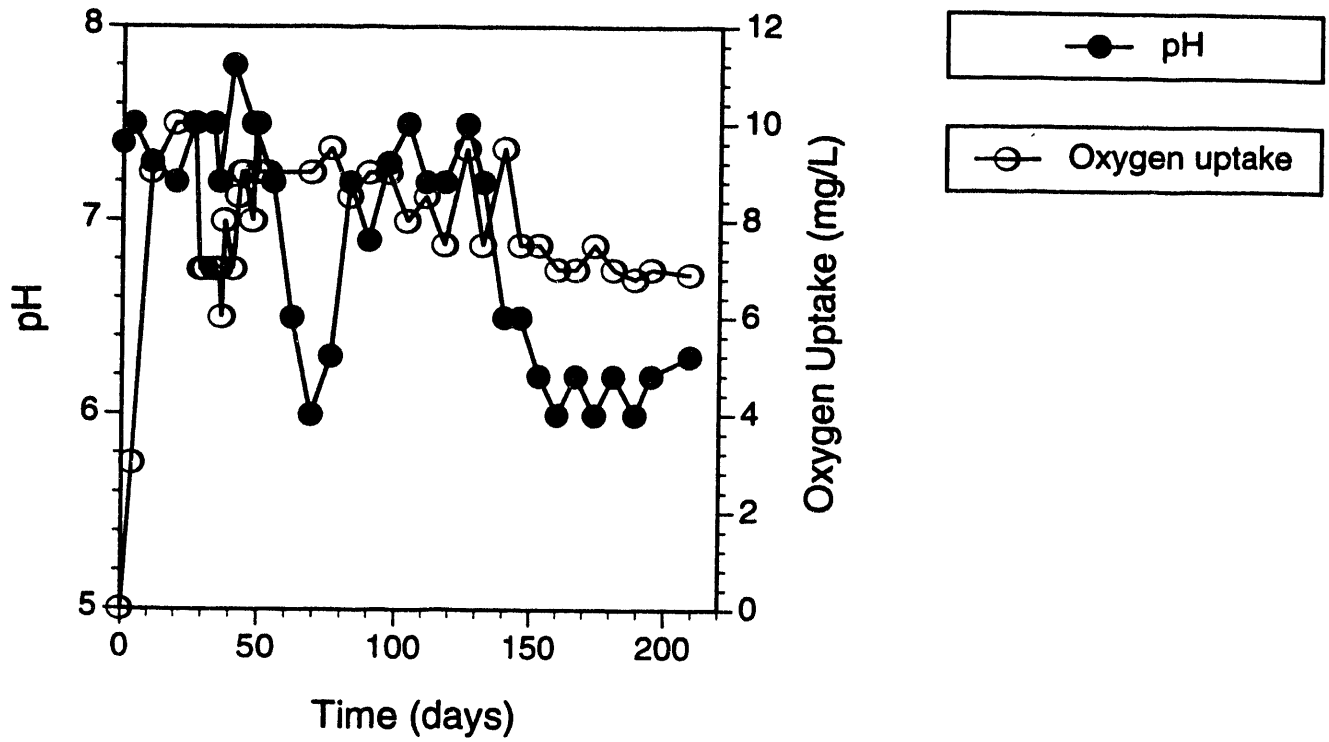


Figure 5 pH and Oxygen Uptake Rates in the Reactor

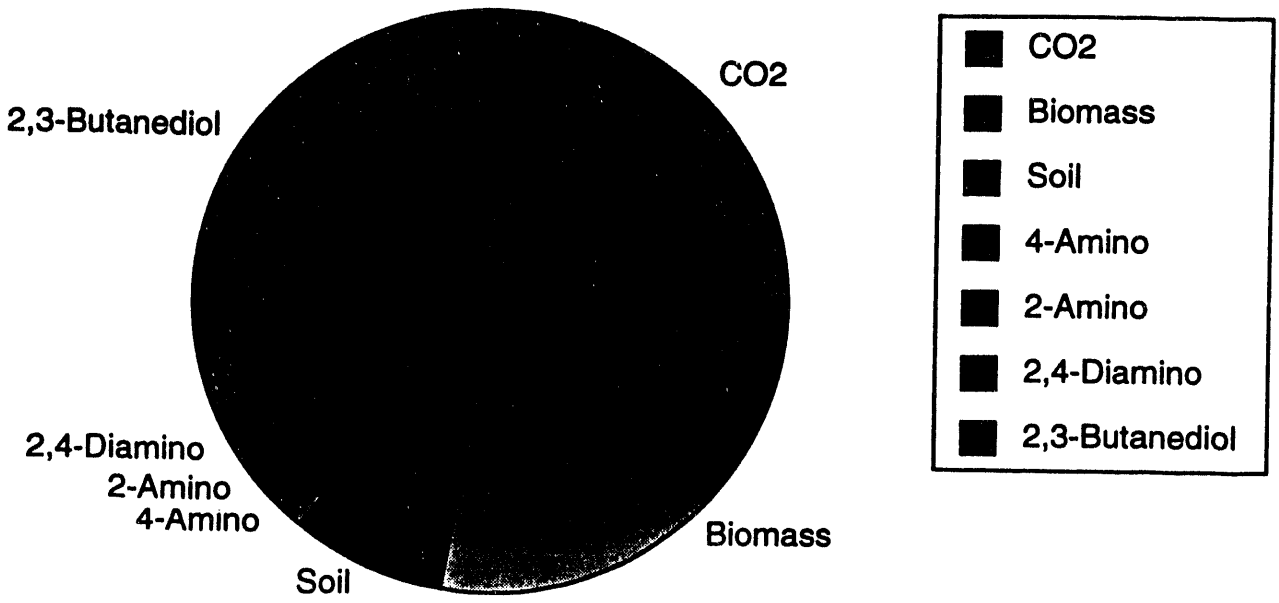


Figure 6 Mass balance of [^{14}C]TNT in the Reactor Biomass

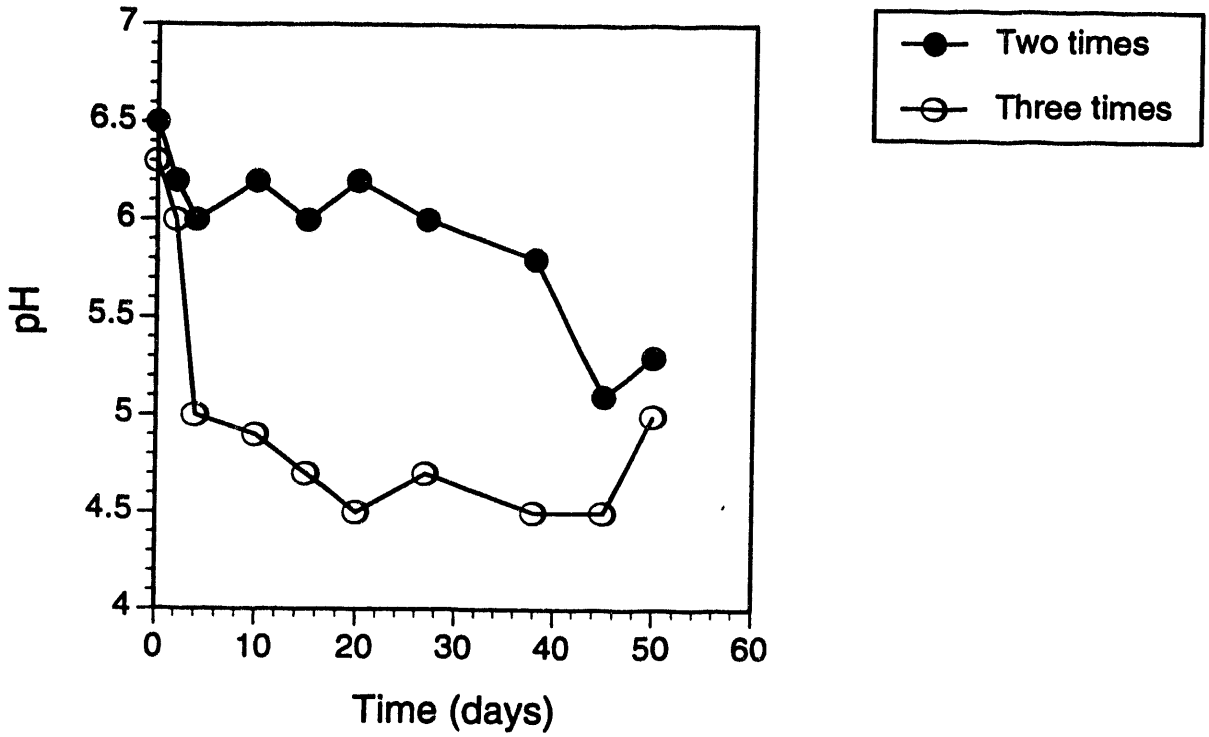


Figure 7 pH in the Reactors with More Frequent Soil Feedings

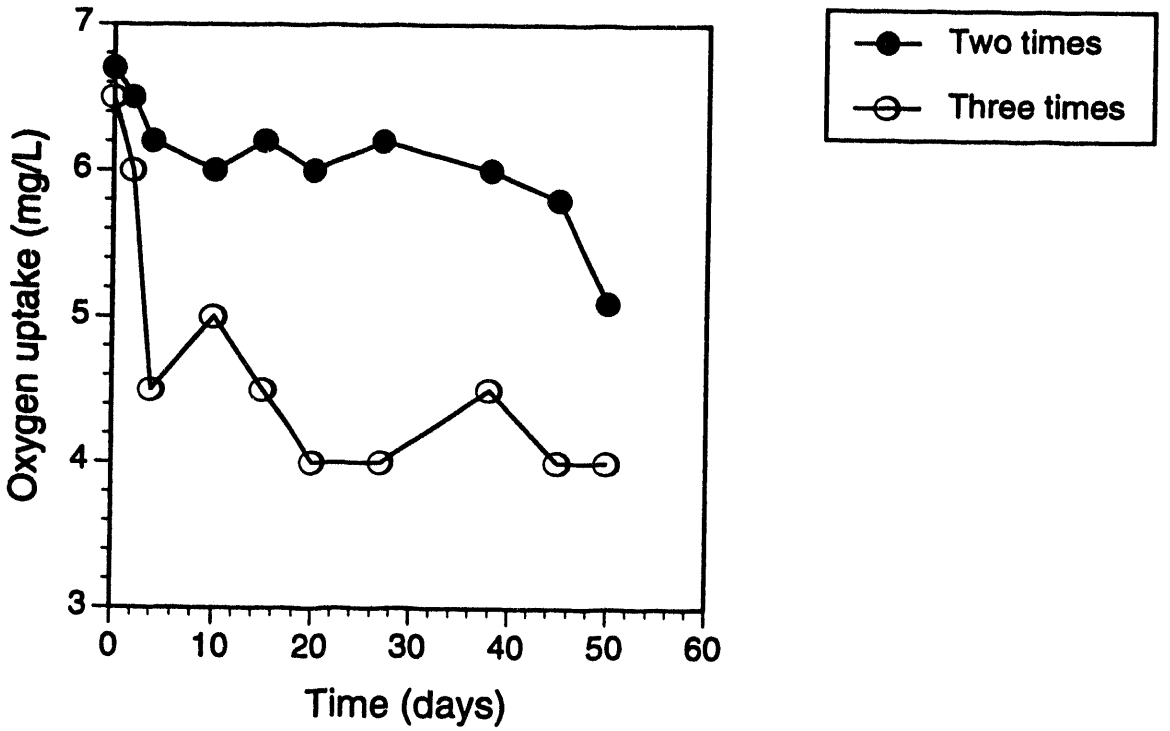


Figure 8 Oxygen Uptake Rates in the Reactors with More Frequent Soil Feedings

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