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Evaluation of bioremediation methods for the treatment of soil contaminated with explosives in Louisiana Army Ammunition Plant, Minden, Louisiana

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Abstract

Two bioremediation methods, namely, soil slurry reactor and land farming technique were evaluated for the treatment of soil contaminated with explosives in Louisiana Army Ammunition Plant, Minden, Louisiana. The soil had a high concentration of 2,4,6-trinitrotoluene (TNT) of 10,000 mg/kg of soil and medium level contamination of RDX 1900 mg/kg and HMX 900 mg/kg of soil. The results indicated that soil slurry reactor under co-metabolic condition with molasses as co-substrate removed TNT more efficiently than land farming method. TNT removal efficiency was 99% in soil slurry reactor compared to 82% in land farming after 182 days. HMX and RDX were also removed from the soil in both methods, but the removal efficiency was low. The radiolabeled study showed that soil microbes mineralize TNT. The mass-balance of TNT indicated 23.5% of TNT was mineralized to CO_2, 22.6% was converted to biomass, and 52.3% was converted to various TNT intermediates in the soil slurry reactor. Both methods maintained high bacterial population fairly well. The results of this bench-scale study are promising with regard to transferring the technology to full-scale application at this site.

Keywords: TNT; Soil slurry; Land farming; Bioremediation; Co-metabolism; Co-substrate

1. Introduction

By the late nineteenth century, nitroaromatic explosives such as 2,4,6-trinitrotoluene (TNT) had been synthesized [1]. The manufacturing and handling of these explosives and propellants at many army industrial sites has resulted in contamination of soils and sediments. In the US, the army has estimated over 1.2 million tonnes of soil have been contaminated with explosives [2]. According to the US Environmental Protection Agency [3], TNT is the most frequently encountered explosive at former munitions handling facilities.

Soil in many parts of the Louisiana Army Ammunition Plant (LAAP) in Minden, LA is contaminated with explosive compounds such as 2,4,6-trinitrotoluene (TNT), octahydro-1, 3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX). Past methods for disposal of munitions wastes have included dumping at sea, dumping at specified landfill areas [4], and incineration [5]. These methods of disposal have a negative impact on the environment. The dumping of the contaminants causes groundwater and soil contamination, while the incineration of the contaminants causes air and soil contamination.

Currently, incineration is the most effective and widely used remediation alternative, but this method is expensive because of the costs involved in soil excavation, transport, and energy for incineration. Over the years, many new biological methods of bioremediation for explosive contaminated soil have been developed [6]. These methods include soil slurry reactors, composting, land farming, and phytoremediation. Each biologically based technology has comparative advantages and disadvantages. Soil slurry reactors are designed to optimize mass transfer of nutrients and electron acceptors by using mechanical mixing and aeration [7]. Land farming is a solid-phase treatment process in which contaminated soil is mixed with nutrients and moisture, using periodic mechanical turning of the soil to increase aeration [6]. Composting is similar to land farming but includes additional significant amendments of organic substrates, which are primarily used as the carbon source to promote increased bacterial activity and higher degradation rates [6]. The use of composting for explosive remediation was reported by Williams

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et al. [8]. Phytoremediation uses plants to mobilize the contaminant into plant biomass and the concentrated contaminants are later harvested and disposed in incinerators [9].

The biodegradation rates are much faster in soil slurry reactors compared with composting, land farming, and phytoremediation [8]. Soil slurry reactor technology is capital-intensive and has relatively high operating costs. Full-scale land farming and composting operations have the potential for significant savings in both capital and operating costs. Although composting is effective for biodegrading TNT, several concerns are associated with its use on a large scale. Conventional composting methods require a large input of natural organic substrates such as straw, woodchips, and livestock manure and relatively small amount of soil is treated. Land farming is a slow process [6,10]. Each method is site specific and has to be evaluated for each site prior to full-scale remediation.

This research was conducted mainly to see the site specific response of explosives-contaminated soil present in LAAP, Minden, LA to two bioremediation methods, namely, soil slurry reactor and land farming techniques. In our previous studies, we found that molasses is the best co-substrate that enhances the bacterial population and TNT degradation rates in a soil slurry reactor [5]. The optimum concentration of molasses was 0.3% (v/v) [5]. The TNT degradation is accomplished by mixed soil bacteria under anoxic/microaerophilic conditions [5]. The objective of this study was to evaluate the best bioremediation method for the treatment of TNT in soil in LAAP, Minden, LA.

2. Materials and methods

2.1. Soil

The contaminated soil was collected from the LAAP in Minden, Louisiana, USA. The contaminant concentrations in the soil are given in Table 1. The TNT concentration in the soil ranged from 4000 to 10,000 mg/kg. The RDX concentration in the soil ranged from 800 to 1900 mg/kg. The HMX concentration ranged from 600 to 900 mg/kg. The soil had a total organic matter content of 4–5%, which included the contaminants. The soil had an average pH of 6.5.

2.2. Soil slurry reactor

Four 2 L laboratory-scale soil slurry reactors were set up and were operated at ambient temperature (20–22 °C). The batch reactors were operated starting with 20% (w/v) slurry of explosive contaminated soil obtained from the Louisiana Army Ammunition Plant (Minden, LA) in water. The first two reactors were the control, which received no molasses as a co-substrate. The second two reactors received 0.3% (v/v) molasses (Grandma's molasses, Mott's, USA, Cadbury Beverages Inc., Stanford, CT) as a co-substrate once every week. Air was supplied through a diffuser once a day for 10 min using a timer. The soil slurry was mixed continuously at an average rate of 100 rpm by using a stirring motor (model RW 20 DZM, Tekmar Company, Cincinnati, OH). The explosive concentrations, bacterial growth, pH, and dissolved oxygen were monitored weekly in the reactors, and the results were averaged for the two sets of reactors.

Table 1
Explosive concentrations in the contaminated soil

Explosive	Concentration range (mg/kg of soil)
TNT	4000–10,000
RDX	800–1900
HMX	600–900

2.3. Land farming

Four sets of pans were used for this experiment. A set consisted of a small aluminum pan (8 in. × 3.75 in. × 2.375 in.) placed in a larger steel pan (9.5 in. × 5.2 in. × 2.7 in). The bottoms of the smaller pans were perforated with 2-mm-diameter holes spaced 4 cm apart to allow for the drainage of fluids during and after flooding cycles. Each small pan contained 300 g of contaminated soil obtained from the LAAP (Minden, LA) that was placed in a larger pan. Water (500 mL) was added every 2 weeks during the wet cycle. After 2 weeks of flooding, the water was emptied from the larger pans to allow the soil to dry for a 2-week dry cycle. The first two sets of pans served as the control in which no molasses was added as a co-substrate. The second sets of pans received 0.3% (w/v) molasses (Grandma's molasses, Mott's, USA, Cadbury Beverages Inc., Stanford, CT) as a co-substrate once a week for 2 weeks during the flooded cycle. Each pan received tillage once a week. The explosive concentrations and bacterial growth were monitored weekly in the pans, and the results were averaged for the control and treatment sets of pans.

2.4. Analyses

The explosives in the soil were extracted by the method recommended by the US Army environmental Research Center [11]. The soil slurry was dried in an oven at 100 °C. Then 1 g of soil was transferred into a 15-mL serum vial and extracted with 9 mL of acetonitrile. A Teflon-lined septum cap was placed on the vial and the suspension was subjected to vortex mixing for 1 min. The mixture was sonicated for 18 h. After sonication, the sample was allowed to settle for 30 min, and then 1.0 mL of the supernatant was removed and combined with 1.0 mL of aqueous $CaCl_2$ solution (5 g/L) in a glass scintillation vial. The vial was hand-shaken, allowed to stand for 15 min, and then centrifuged in a microcentrifuge at 10,000 rpm for 5 min. The supernatant was removed and stored in a vial to be analyzed by high-performance liquid chromatography (HPLC) using a liquid chromatograph equipped with two Model 210 solvent pumps, a Model 320 programmable multi-wavelength ultraviolet (UV) detector set at 254 nm, a Model 410 system auto sampler (Varian, Walnut Creek, CA), and an LC-CN 4.6-mm-i.d. × 25-cm HPLC column (C-18 Supelco column) with a particle size of 5–6 μm. The mobile phase was methanol:water (50:50, v/v) at a flow rate of 1.5 mL/min with an injection volume of 50 μL.

Dissolved oxygen in the soil slurry was monitored weekly using an oxygen analyzer before the aeration event (YSI 5000,

Yellow Springs, OH). The probe of the analyzer was placed directly in the reactor, and the dissolved oxygen concentration in the reactor was measured and expressed as mg/L. The pH of the slurry was also measured weekly with a pH meter (UltraBasic UB-10, Denver Instrument) by placing the probe directly in the reactor.

Bacterial activity in the reactors was monitored weekly. Slurry samples (1 mL from each reactor) were serial diluted with a phosphate buffer solution. Standard methods for total plate counts were followed with tryptic soy agar plates [12].

2.5. [^{14}C]-TNT mineralization studies

After 49 days of operation, 20 mL of slurry was taken from each soil slurry reactor and 10 g of dry soil was taken from each land-farming pan. The samples were incubated with uniformly labeled TNT to provide mass balance and determine metabolite production, including [^{14}C]CO_2. The [^{14}C]TNT was added to the soil slurry at the level of 20,000 disintegrations per minute (dpm)/mL in a respirometer flask. Samples were withdrawn periodically, and the quantity of TNT converted to biomass was determined as trichloroacetic acid (TCA) precipitable material [13] by using a Beckman (Palo Alto, California) model LS5000TD liquid scintillation spectrometer.

Respirometer flasks [13] containing [^{14}C]TNT were used to monitor the carbon dioxide evolved by the soil bacteria. This experiment was conducted anaerobically with a modified respirometer. Potassium hydroxide (KOH) (0.5 N) was added to the side arms. The flasks were incubated at ambient temperature in a shaker at 50 rpm. The respirometers were sampled periodically by withdrawing the potassium hydroxide, measuring the radioactivity with a liquid scintillation spectrometer, and replacing the potassium hydroxide. The percentage of [^{14}C]TNT mineralized as [^{14}C]CO_2 was calculated.

The TNT metabolites were analyzed by collecting the fractions every 30 s after passage through the HPLC column. The radioactivity in each fraction was measured using a liquid scintillation counter. Soil-bound radioactive TNT was analyzed using the soil extraction procedure described above, and the radioactivity in the soil was measured using a liquid scintillation spectrometer.

2.6. Chemicals

Radiolabeled TNT (uniformly labeled, specific activity 21.5 mCi/mM, 98% pure) was purchased from Chemsyn Science Laboratories, Lenexa, KS. The non-radioactive TNT was obtained from Chem Service Inc., West Chester, PA. Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetraazocine (HMX) were obtained from the Naval Surface Warfare Center, Indian Head, MD.

3. Results and discussion

3.1. Soil slurry reactor

The concentrations of TNT in the slurry reactors are given in Fig. 1. The soil-TNT concentration in the no carbon control

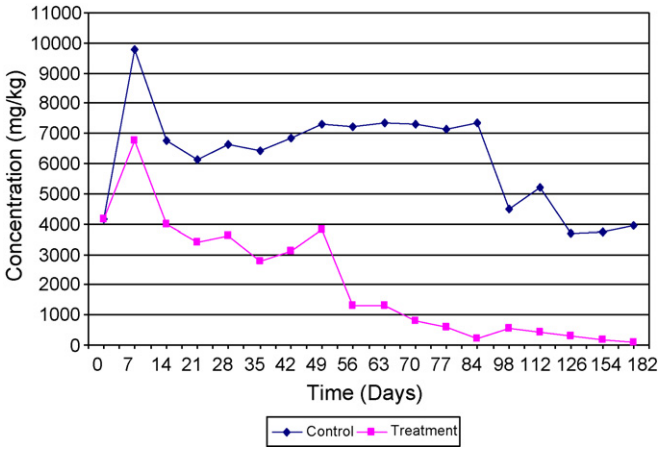

Fig. 1. Concentrations of TNT in the soil slurry reactors.

reactor remained high around 7500 mg/kg of soil throughout the experiment. This observation suggests that the indigenous microflora from the contaminated site would not degrade TNT without the addition of nutrients or co-substrates. The soil-TNT concentration in the reactor that received molasses as co-substrate dropped gradually and fell below 50 mg/kg of soil on day 182 of the study.

Our previous study demonstrated that TNT removal in the soil slurry system was accomplished by a co-metabolic process that required an additional carbon source such as molasses or succinate [5]. Molasses is a very effective carbon source that enhances the TNT degradation rate significantly over other carbon sources [14]. This study showed that the soil slurry reactors can effectively remediate TNT in the contaminated soil. The operation of laboratory-scale soil slurry reactors over 182 days showed that 99% removal of TNT can be achieved. The soil slurry reactor also removed other explosives, namely, HMX and RDX (Figs. 2 and 3). However, the removal efficiency was not as high as TNT. This could be due to the complexity of molecules. HMX and RDX degradation can be achieved, but it will take longer period of time as indicated by many other studies [5,8,15].

The addition of radiolabeled TNT to the reactor biomass provided evidence for the mineralization of TNT (Fig. 4). Of the original radiolabeled TNT (20,000 dpm/mL), 23% was converted to CO_2 and 24% was used in making cellular materi-

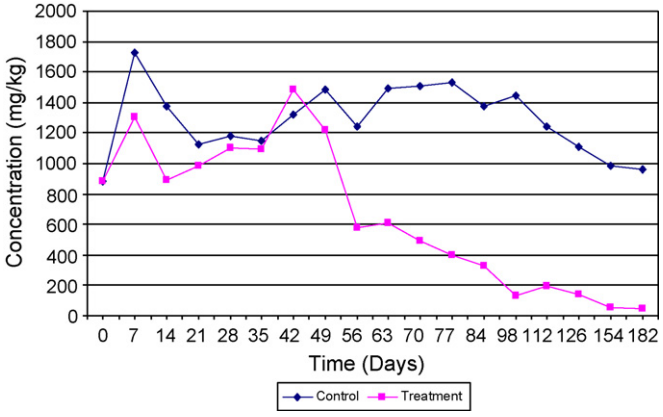

Fig. 2. Concentrations of RDX in the soil slurry reactors.

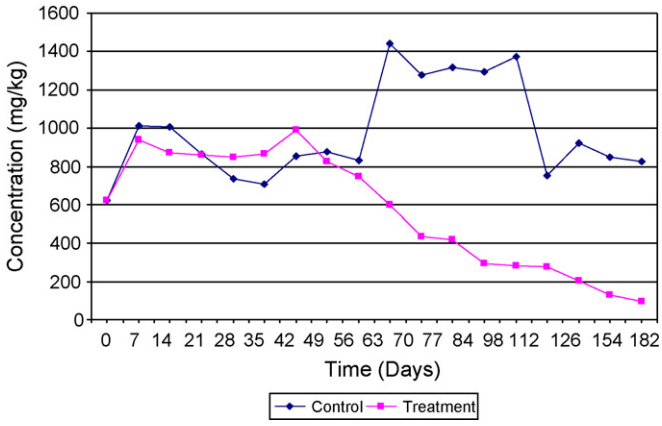

Fig. 3. Concentrations of HMX in the soil slurry reactors.

als. Radiotracer studies with the reactor biomass also revealed various intermediates, including 4-amino-2,6-dinitrotoluene, 2-amino-4,6-dinitrotoluene, 2,4-diamino-6-nitrotoluene, fatty acids, and an unidentified metabolite after 3 weeks of starting the radiolabeled studies. Extraction of soil with acetonitrile showed that 2% of the TNT was adsorbed on to the soil. The rest of the TNT was accounted for as intermediates. The mass balance was reasonable with the recovery of 98% of radiolabeled TNT. In the no carbon control, 78% of radiolabeled TNT was recovered as TNT, which did not undergo degradation. This radiolabeling study showed that the natural soil bacteria present in the contaminated soil can cause extensive degradation of TNT in a reasonable time under optimum conditions. Degradation was demonstrated by mineralization of radiolabeled TNT, metabolite formation, and the presence of radioactivity in the cell biomass as TCA-precipitable material.

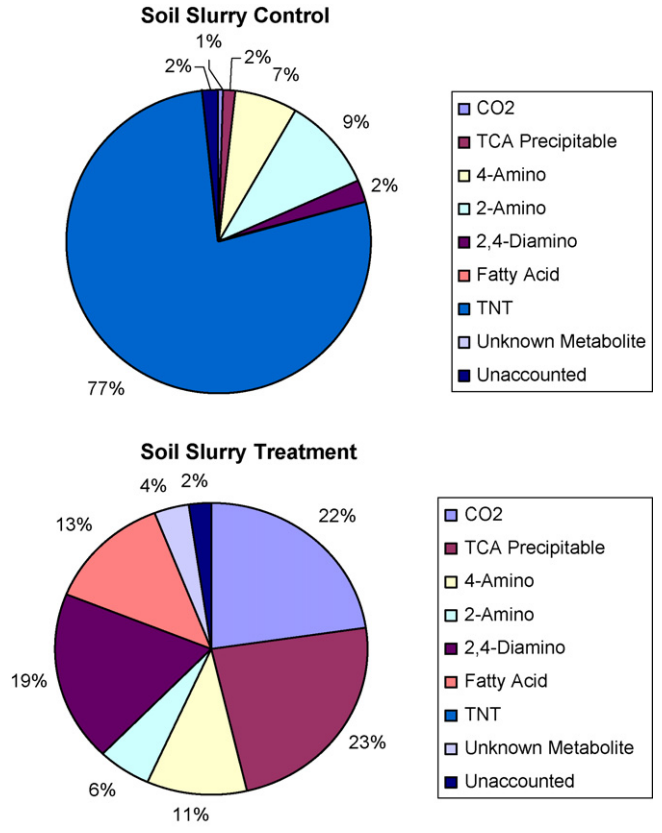

Fig. 4. Mass balance of radiolabeled TNT in soil slurry reactor biomass.

The pH in the reactors was monitored throughout the experiment. The pH remained approximately neutral in the no carbon control reactor. However, the molasses contained reactor tended to be acidic with pH value of 5 (data not shown). Dissolved oxygen (DO) concentrations were monitored weekly in the soil slurry reactors. The DO concentration remained around 6.5 mg/L in the no carbon control and in the reactors with molasses the DO was less than 1 mg/L (data not shown).

Bacterial plate counts were performed several times over the course of the experiment. The bacterial plate counts in the reactor receiving molasses were consistently higher than those in the no carbon control reactor (Table 2). This result also shows the value of molasses addition, which helps to increase the population of soil bacteria in the reactor. Molasses is the best among various substrates studied, such as succinate, glucose, acetate, and citrate [14], it is well balanced with nutrients including carbon, nitrogen, phosphorous, vitamins, and minerals for bacterial activity [14].

3.2. *Land farming method*

The TNT concentration in soil samples taken from pans 1 to 4 during the 182-day study is shown in Fig. 5. For each sampling date, three soil samples were collected from each pan, analyzed, and the mean concentrations were plotted. The TNT concentrations in the control pan remained at high levels over the course of the experiment. The treatment that included molasses solution was biologically active and showed removal of TNT. Starting from a high concentration of approximately 7000 mg/kg of soil, the average concentration of TNT after 182 days of treatment was less than 1250 mg/kg of soil, which was equaled to 82% removal of TNT. Very little RDX and HMX were removed in soil in both the control and treatment pans (data not shown). The degradation rates of RDX and HMX are extremely slow [5,8,15] and continuation of the experiment over a 300-day period might show significant reduction in HMX and RDX [15].

Table 2
Bacterial counts in the soil slurry reactors (CFU/mL of soil slurry)[a]

Day	Control	Treatment
Bacterial count (colony forming unit/mL of slurry) in the reactor		
0	72×10^2	81×10^2
14	33×10^7	53×10^7
28	46×10^5	167×10^6
42	60×10^5	235×10^6
56	47×10^5	55×10^6
70	123×10^4	36×10^6
84	187×10^4	103×10^6
98	93×10^4	59×10^6
112	173×10^4	105×10^6
126	88×10^4	78×10^6
154	67×10^4	104×10^6
182	72×10^4	121×10^6

[a] The data represent an average of two plates.

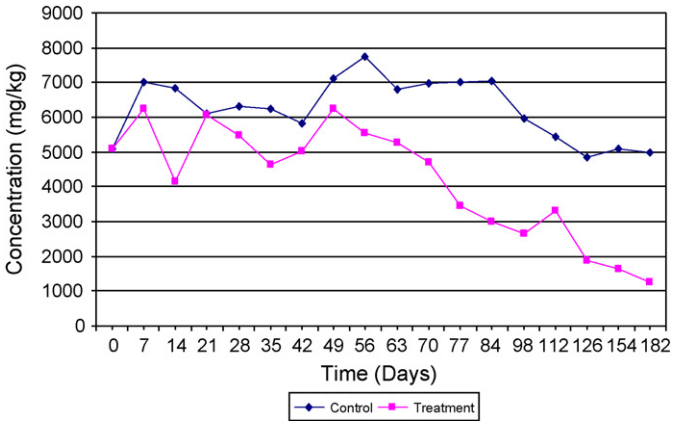

Fig. 5. Concentrations of TNT in the land farming pans.

The radiolabeled study used biomass taken from the pans and provided evidence for the mineralization of TNT. In pan biomass samples that received molasses, the proportion of the initial radiolabeled spike that was transformed to radiolabeled CO_2 was 6.5%. In the control pans, the radiolabeled CO_2 was 1.2% (Fig. 6). This result clearly demonstrates that TNT was mineralized by the soil bacteria in the treatment that received molasses. The analysis of TCA-precipitable material showed that a significant amount of radiolabeled TNT was converted to cell biomass. There are various TNT metabolites present in varying degrees (Fig. 6). The calculated mass balance was very

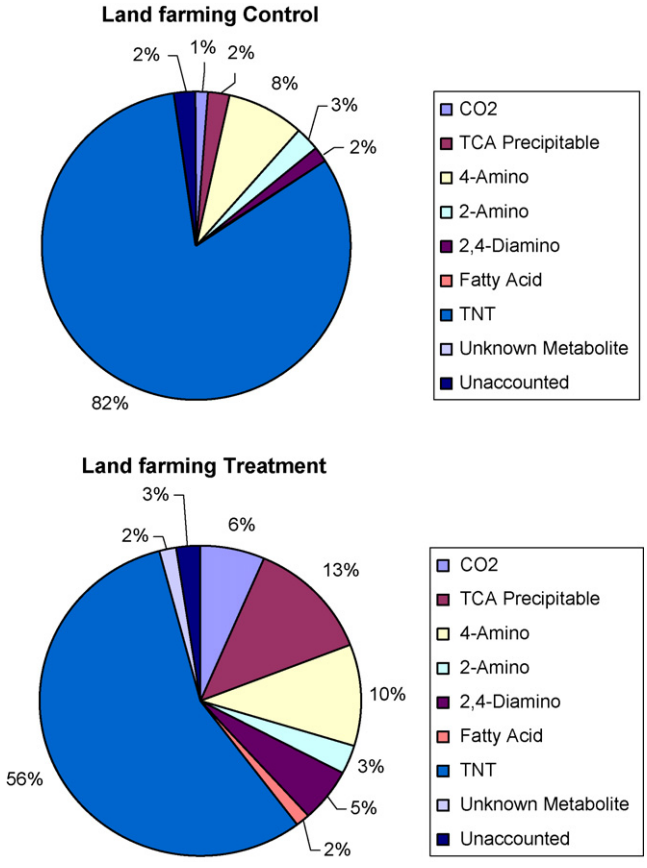

Fig. 6. Mass balance of radiolabeled TNT in land farming soil biomass.

Table 3
Bacterial counts in the land farming pans (CFU/g of soil slurry)[a]

Day	Control	Treatment
Bacterial count (colony forming unit/g of soil) in the reactor		
0	63×10^2	74×10^2
14	58×10^6	146×10^6
28	41×10^5	44×10^6
42	40×10^5	88×10^6
56	32×10^4	158×10^5
70	63×10^4	60×10^6
84	273×10^4	129×10^6
98	73×10^4	112×10^6
112	220×10^4	162×10^6
126	188×10^4	119×10^6
154	112×10^4	67×10^6
182	123×10^4	87×10^6

[a] The data represent an average of two plates.

reasonable, with recovery of 98% of radiolabeled TNT in this study. This experiment showed that the control pans did not have an active biomass to convert TNT to CO_2 due to the lack of molasses as co-substrate.

Bacterial plate counts were performed several times over the course of the experiment on soil samples taken from each pan. The bacterial population densities in the soils receiving molasses solutions were consistently higher than those in the control pans (Table 3). This result also shows that the control pans consistently exhibited plate counts on the order of 10^4 colony forming units/g of soil and thus were not strictly abiotic controls; however, negligible biodegradation of TNT occurred in the control as TNT concentration in control remained high throughout the study.

Between the two different bioremediation methods, the soil slurry reactor system showed efficient removal of TNT for the LAAP soil in Minden, LA. The land farming method also removed TNT, but the removal rate was very slow. The land farming in the field should be done in a constructed cell with liner to prevent any leachate migrating to ground water. Both methods showed that the native soil bacteria present at the contaminated site are capable of mineralizing TNT as demonstrated in the radiolabeled study. The advantage of soil slurry rector is its simple operating conditions. The method needs only mixing, 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. Based on this study, the soil slurry reactor can be used for effective and fast remediation of TNT at LAAP, Minden, LA. The removal of HMX and RDX in the soil can also be achieved with prolonged incubation.

Acknowledgement

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