Biodegradation **8:** 339–347, 1998.

Fate of explosives and their metabolites in bioslurry treatment processes

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Accepted 18 November 1997

Key words: bioremediation, bioslurry reactor, explosive, microcosm, mineralization

Abstract

Microcosm tests simulating bioslurry reactors with 40% soil content, containing high concentrations of TNT and/or RDX, and spiked with either [^{14}C]-TNT or [^{14}C]-RDX were conducted to investigate the fate of explosives and their metabolites in bioslurry treatment processes. RDX is recalcitrant to indigenous microorganisms in soil and activated sludge under aerobic conditions. However, soil indigenous microorganisms alone were able to mineralize 15% of RDX to CO_2 under anaerobic condition, and supplementation of municipal anaerobic sludge as an exogenous source of microorganisms significantly enhanced the RDX mineralization to 60%. RDX mineralizing activity of microorganisms in soil and sludge was significantly inhibited by the presence of TNT. TNT mineralization was poor ($< 2\%$) and was not markedly improved by the supplement of aerobic or anaerobic sludge. Partitioning studies of [^{14}C]-TNT in the microcosms revealed that the removal of TNT during the bioslurry process was due mainly to the transformation of TNT and irreversible binding of TNT metabolites onto soil matrix. In the case of RDX under anaerobic conditions, a significant portion (35%) of original radioactivity was also incorporated into the biomass and bound to the soil matrix.

Introduction

Munitions compounds such as 2,4,6-trinitrotoluene (TNT) and hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) have contaminated soils in many sites where manufacturing, handling, and disposal of explosives were conducted in the past. The persistence and toxicity of these explosives represent an environmental hazard (Walker & Kaplan 1992); thus the soil from the contaminated sites must be remediated. Bioremediation holds the promise of being a cost effective and environmentally safe practice. One biological process, a bioslurry reactor system, has proven to be effective in the removal of explosives from contaminated soil (Funk et al. 1993; Manning et al. 1995; Shen et al. 1998). A complete removal of TNT and RDX has been demonstrated in these bioslurry treatment processes; however, a previous radiotracer study (Funk et al. 1993) showed that only 0.5% of the initial TNT was mineralized, while the fate of TNT and its metabolites remained unclear. To our present knowledge, no radiolabeled experiments have been addressed to the fate investigation of RDX and its metabolites during the bioslurry treatment process. Therefore, in spite of extensive studies in the biodegradation of TNT and RDX in bioslurry reactor systems, the mass balance of TNT and RDX conversion to final products is still largely missing (Crawford 1995). In this paper, microcosm tests simulating bioslurry reactor conditions using either [^{14}C]-TNT or [^{14}C]-RDX were conducted to investigate the fate of the explosive in slurry reactors, where a complete removal of TNT and RDX with respective concentrations of 1000 and 2000 mg/kg in soil was previously demonstrated (Shen et al. 1998). The partition of [^{14}C]-TNT, [^{14}C]-RDX, and their metabolites in the soil slurry over time was determined to provide some insight into the dynamic process during biodegradation, i.e., mineralization, biotransformation, and binding to soil.

Materials and methods

Sources of organisms

Microorganisms were indigenous microbes found in a sandy soil collected from munitions burning pits at Angus, Ontario. Neither TNT nor RDX was detected in this soil. Municipal activated sludge and anaerobic sludge from wastewater treatment plants of Ste-Catherine and Vaudreuil, Quebec, respectively, were also used as potential exogenous sources of explosive degrading microorganisms in this experiment.

Chemicals

Commercial grade TNT and RDX (with a purity > 99%) were provided by Defense Research Establishment Valcartier, Quebec, Canada. [^{14}C]-TNT and [^{14}C]-RDX with respective specific activities of 124 and 28.7 μCi/mmole were synthesized and recrystallized with a purity over 99% as described by Ampleman et al. (1995). 2-Amino and 4-aminodinitrotoluene, and 2,4-diamino and 2,6-diaminonitrotoluene were purchased from AccuStandard (New Haven, CT). 2,4,6-Triaminotoluene was bought from Chem Service (West Chester, PA). Mono-, and trinitroso-RDX were synthesized according to the method (Brockman et al. 1949). All other chemicals were reagent grades.

Mineralization of [^{14}C]-TNT and [^{14}C]-RDX in soil slurry microcosms

The soil slurry microcosms were prepared as follows: a series of 100-ml serum bottles was filled with 20 μl of either [^{14}C]-TNT or [^{14}C]-RDX solution (100,000 dpm), 0.80 ml of a TNT-acetone solution (20 g/L) and/or 0.80 ml of an RDX acetone solution (40 g/L). The acetone solutions were mixed and the acetone was then removed under a stream of nitrogen gas. Next, 16.0 g of the sandy soil (dry weight) and 1 ml of a molasses feed were added to the microcosms. The molasses feed was prepared as follows (g/L): 132.5 g of molasses, 29.6 g $NaHCO_3$, and 35.3 g $KHCO_3$. The molasses feed was additionally supplemented with 29.6 g of Na_2SO_4 in the case of anaerobic microcosms. Finally, 8 ml of the municipal activated sludge (with VSS concentration: 9.73 gVSS/L) or anaerobic sludge (with VSS concentration: 12.74 gVSS/L) was injected into the serum bottle as shown in Table 1, and the total weight of soil slurry in the serum bottle was completed to 40 g by distilled water, bringing the soil dry weight ratio to 40%. The added activated sludge or anaerobic sludge, which provided exogenous sources of microorganisms in the soil slurry, gave respective dry weight ratios of 0.27% and 0.45% in the soil slurry. Final TNT and RDX concentrations were 1000 and 2000 mg/kg, respectively, on the basis of soil dry weight. Before being incubated at 28 °C on a rotary shaker (150 rpm), the aerobic microcosms were flushed with pure oxygen to build strict aerobic conditions, while nitrogen gas was employed to purge the head space of anaerobic microcosms in order to maintain strict anaerobic conditions. The microcosms were sampled regularly (every two or three days), with subsequent flushing with pure oxygen or nitrogen gas to keep aerobic or anaerobic conditions. After initial incubation under anaerobic conditions for a certain period of time, some microcosms were supplemented with 4 ml of the activated sludge and switched to aerobic conditions in order to investigate the effect of sequential anaerobic-aerobic conditions on the mineralization of TNT, RDX, and their metabolites.

Distribution of [^{14}C]-TNT, [^{14}C]-RDX, and their metabolites in soil slurry

Partitioning of TNT, RDX, and their metabolites in the soil slurry was investigated in a series of microcosm bottles simulating the bioslurry reactor conditions (Shen et al. 1998). The serum bottles were filled with the soil, activated or anaerobic sludge, molasses feed, and explosives (unlabelled TNT and RDX), as described in the mineralization study. They were then spiked with 100,000 dpm of either [^{14}C]-TNT or [^{14}C]-RDX. The mineralization of TNT or RDX was measured by counting the radioactivity of $^{14}CO_2$ evolved from the serum bottles, and collected in a KOH trap. The extractable portion of TNT, RDX, and/or their metabolites in the soil slurry was assayed by extracting the soil slurry with acetonitrile and then counting the [^{14}C] radioactivity in the extract. At appropriate intervals during the incubation, one microcosm bottle was sacrificed for the extraction of TNT, RDX, and/or their metabolites by introducing 20 ml of acetonitrile. The mixture of soil slurry and acetonitrile was vigorously shaken for 5 min, left overnight on a rotary shaker at 150 rpm, and then filtered through GF/F glass microfiber filter paper (Whatman International Ltd.). The soil cake from the filtration was extracted two more times using an additional 20 ml of acetonitrile and 20 ml of distilled water, respectively. All filtrates were combined, and the total radioactivity was measured.

Table 1. Summary of microcosm conditions

[^{14}C]-TNT mineralization under aerobic conditions (Figure 2)			
Samples	Activated sludge supplement (gVSS/kg slurry)	Explosive conc. (mg/kg soil)	
		TNT	RDX
TNT*-soil	None	1000	0
TNT*-soil-sludge	2.7	1000	0
TNT*-soil-control	None	1000	0
TNT*-RDX-soil	None	1000	2000
TNT*-RDX-soil-sludge	2.7	1000	2000
TNT*-RDX-soil-control	None	1000	2000
[^{14}C]-TNT mineralization under anaerobic conditions (Figure 3)			
Samples	Anaerobic sludge supplement (gVSS/kg slurry)	Explosive conc. (mg/kg soil)	
		TNT	RDX
TNT*-soil	None	1000	0
TNT*-soil-sludge	4.5	1000	0
TNT*-soil-control	None	1000	0
TNT*-RDX-soil	None	1000	2000
TNT*-RDX-soil-sludge	4.5	1000	2000
TNT*-RDX-soil-control	None	1000	2000
[^{14}C]-RDX mineralization under aerobic conditions (Figure 4)			
Samples	Activated sludge supplement (gVSS/kg slurry)	Explosive conc. (mg/kg soil)	
		TNT	RDX
RDX*-soil	None	0	2000
RDX*-soil-sludge	2.7	0	2000
RDX*-soil-control	None	0	2000
RDX*-TNT-soil	None	1000	2000
RDX*-TNT-soil-sludge	2.7	1000	2000
RDX*-TNT-soil-control	None	1000	2000
[^{14}C]-RDX mineralization under anaerobic conditions (Figure 5)			
Samples	Anaerobic sludge supplement (gVSS/kg slurry)	Explosive conc. (mg/kg soil)	
		TNT	RDX
RDX*-soil	None	0	2000
RDX*-soil-sludge	4.5	0	2000
RDX*-soil-control	None	0	2000
RDX*-TNT-soil	None	1000	2000
RDX*-TNT-soil-sludge	4.5	1000	2000
RDX*-TNT-soil-control	None	1000	2000

* [^{14}C]-labeled compounds.

The non-extractable portion of the radiolabel, defined as incorporated into the biomass and/or irreversibly bound to the soil, was liberated by digesting the soil cake from the final filtration with potassium dichromate and a mixture containing sulfuric acid and phosphoric acid (3:2 v/v) (Nelson & Sommers 1982). $^{14}CO_2$ released from the soil digestion was absorbed in two consecutive traps each containing 20 ml of 0.1N KOH, and its radioactivity was then counted. The radioactivity associated with non-extractable metabolites in some microcosm samples (due to unavailability of the digestion set-up at the early experimental stage) was esti-

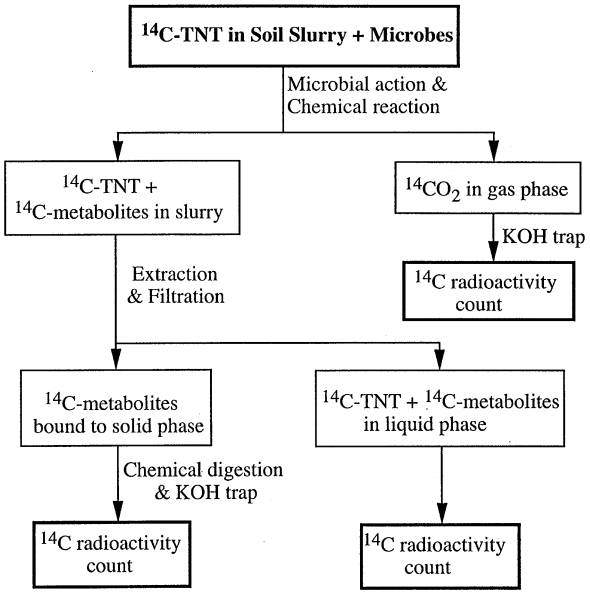

Figure 1. Flow chart of the determination of [^{14}C]-radiolabel (TNT as an example) partitioning in different phases

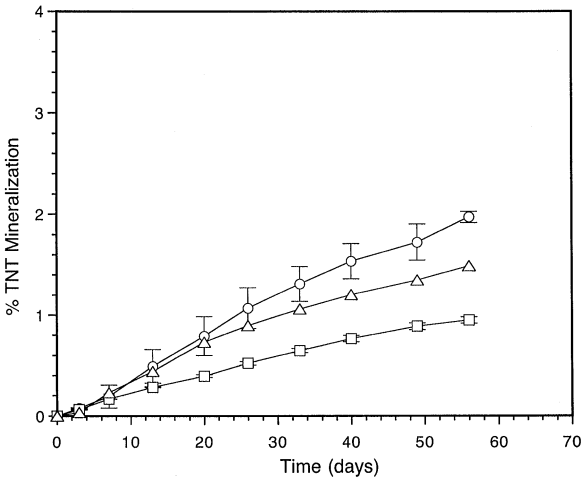

Figure 2. Mineralization of TNT in soil slurry under aerobic conditions. (○, microcosm containing TNT, RDX and soil; □, microcosm containing TNT, RDX, soil and activated sludge; △, heat killed control. TNT and RDX concentration was 1000 and 2000 mg/kg dry soil, respectively, in all microcosms).

mated by a mass balance using the initial [^{14}C]-TNT or [^{14}C]-RDX radioactivity count, the radioactivity in the CO_2, and the radioactivity count of extractable products including [^{14}C] starting material (Figure 1).

Analysis and identification of TNT and RDX metabolites

TNT, RDX, and their metabolites in the soil slurry were extracted and analyzed according to the method Bouvier and Oehrle (1995). Briefly, about 2.5 g of soil slurry was mixed with 10 ml of acetonitrile in a 15-ml glass tube with a Teflon® coated screw cap. The slurry was extracted by ultrasonicating the sample at 10 °C overnight. Five ml of the supernatant was mixed with 10 ml of $CaCl_2$ solution (10 g/L) and then analyzed by HPLC (Spectra-Physics Model SP8100, Thermo Separations Products, California Inc.) equipped with a SUPELCOSIL C8 column (25 cm × 4.6 mm, I.D.) and a 254-nm UV detector. The mobile phase consisted of 18% iso-propanol and 82% organic-free deionized water. TNT and RDX metabolites in HPLC analysis were identified by comparing their retention time and UV spectrum with pure TNT and RDX metabolites and confirmed using gas chromatography/mass spectrometry (Varian Saturn II, Varian associates, Inc., Sugar Land, TX).

Results and discussion

TNT mineralization and transformation

TNT mineralization was studied in the microcosms with and without the presence of RDX. The presence of RDX in the microcosms had no pronounced impact on the TNT mineralization. For clarity reasons, only the TNT mineralization in the microcosms containing TNT and RDX are presented in Figures 2 and 3. In general, the TNT mineralization was very poor (< 2%) under either aerobic or anaerobic conditions. A comparison of TNT mineralization in the active and control microcosms under aerobic conditions (Figure 2) indicates that the biological mineralization of TNT was less than 0.5%, and most of the TNT mineralization was due to abiotic degradation. Contrary to our expectation, supplementation of activated sludge in the microcosm resulted in a decline instead of an increase in the TNT mineralization, which may be explained by an increase in organic content in the microcosm with sludge supplement and a dilution of [^{14}C]-TNT as organic matter to be abiotically degraded. Under anaerobic conditions, TNT mineralization in all microcosms was less than 0.5% after 52 days of incubation, and the supplement of anaerobic sludge only slightly increased the TNT mineralization extent from 0.15% to 0.25%. The sequential anaerobic-aerobic processes, as shown in Figure 3, did not significantly improve the

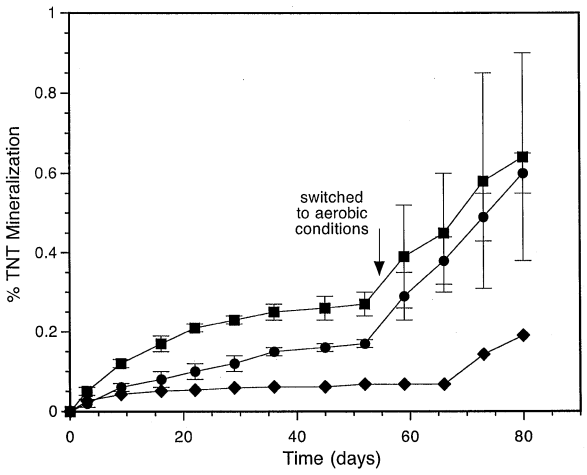

Figure 3. Mineralization of TNT in soil slurry under anaerobic-aerobic sequential conditions. (●, microcosm containing TNT, RDX and soil; ■, microcosm containing TNT, RDX, soil and anaerobic sludge; ♦, heat killed control. TNT and RDX concentration was 1000 and 2000 mg/kg dry soil, respectively, in all microcosms).

TNT mineralization, indicating that the TNT anaerobic metabolites were still recalcitrant under aerobic conditions.

In parallel to the TNT mineralization study, TNT metabolites in the soil slurry microcosms without receiving [^{14}C]-TNT were analyzed and identified over the incubation time in order to find out the fate of TNT besides mineralization. 2-Aminodinitrotoluene (2-ADNT) and 4-aminodinitrotoluene (4-ADNT) were the main identified TNT metabolites in the aerobic microcosms. However, 2-ADNT, 4-ADNT, 2,4-diaminonitrotoluene (2,4-DANT), and 2,6-diaminonitrotoluene (2,6-DANT) were observed in the anaerobic microcosms at early stages of incubation, and the total concentration of amino and diamino derivatives could make up 50% of the initial TNT concentration. In the late stage of incubation, further degraded metabolites, such as triaminotoluene and *p*-cresol, were also detected in the anaerobic soil slurry. We have never detected the above metabolites in our control microcosm (without TNT supplement), indicating these metabolites were from TNT degradation. The results of TNT mineralization and metabolite identification in the microcosm study indicate that the removal of TNT in the bioslurry reactors under either aerobic or anaerobic conditions was mainly due to biotransformation to intermediate metabolites, which are refractory to further microbial degradation and mineralization.

Our results are consistent with a recent literature survey (Marvin-Sikkema & de Bont 1994) reporting that many microorganisms are able to biotransform TNT under aerobic or anaerobic conditions. The presence of strong electron-withdrawing nitro groups on the aromatic ring causes an electron deficiency in the aromatic ring, thus prohibiting electrophilic attack by oxygenases of aerobic bacteria, but favoring the reduction of its nitro groups to amino derivatives such as aminodinitrotoluene and diaminonitrotoluene. Even under aerobic conditions, nitroaromatic compounds are reduced to corresponding metabolites (Rieger & Knackmuss 1995). The formation of azoxy compounds as a result of abiotic coupling reactions of unstable hydroxylamino metabolites under aerobic conditions poses restriction on the further mineralization of TNT metabolites (Carpenter et al. 1978; Michels & Gottschalk 1994). Although anaerobic transformation of TNT is widely reported, the partially reduced products are rarely mineralized (Preuß & Rieger 1995). To date, the most promising results of TNT mineralization were achieved in pure white rot fungus culture *Phanerochaete Chrysosporium* (Michels & Gottschalk 1994; Fernando et al. 1990), in which 40% and 15% mineralization of TNT were accomplished at a respective concentration of 0.36 and 20.4 mg/L after 3 weeks of incubation. However, due to its non-native nature in soil and its sensitivity to relatively high TNT concentrations, the application of *P. Chrysosporium* to remediate heavily contaminated soil is still questionable (Spiker et al. 1992).

RDX mineralization

The RDX mineralization in soil slurry was also investigated under conditions with and without the presence of TNT. The mineralization of RDX in all aerobic microcosms was less than 3% after 35 days of incubation under strictly aerobic conditions, as shown in Figure 4. The indigenous soil microorganisms were only able to mineralize 1.9% of RDX with an initial concentration of 2000 mg/kg dry soil when RDX was the sole explosive in the soil slurry microcosm. Supplement of activated sludge in the microcosms as an exogenous microbial sources slightly increased the RDX mineralization extent (reaching 2.2%). Our further results from the microcosms with activated sludge and RDX (data not shown here) also indicate that microorganisms in municipal activated sludge only mineralized 0.65% RDX (with a concentration of 500 mg/L sludge) after 40 days of incubation under strictly aerobic conditions. These results are consistent with those reported by Hoffsommer et al. (1978), who found no disappear-

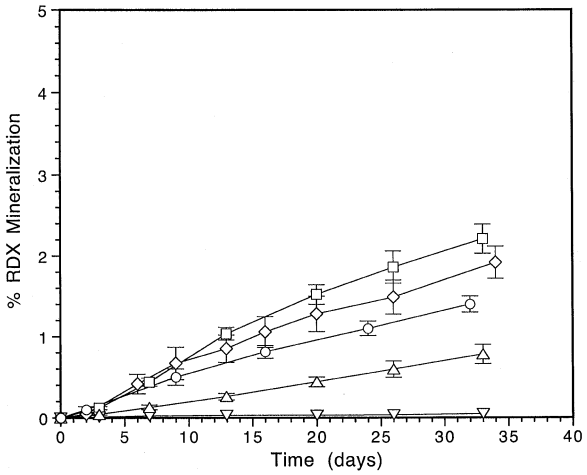

Figure 4. Mineralization of RDX in soil slurry under aerobic conditions. (◊, microcosm containing RDX and soil; □, microcosm containing RDX, soil and activated sludge; △, microcosm containing RDX, TNT and soil; ○, microcosm containing RDX, TNT, soil and activated sludge; ▽, heat killed control. TNT and RDX concentration was 1000 and 2000 mg/kg dry soil, respectively, in all microcosms).

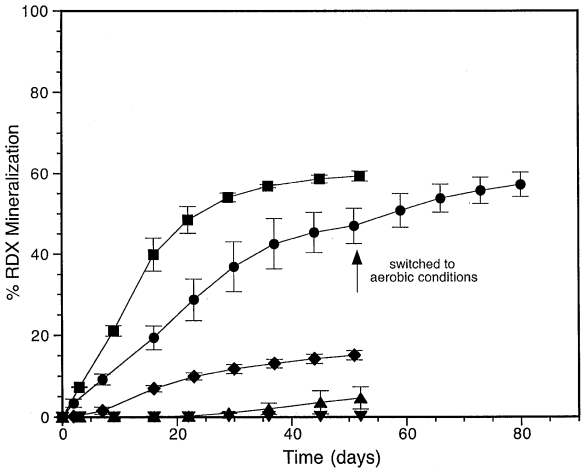

Figure 5. Mineralization of RDX in soil slurry under anaerobic or sequential anaerobic-aerobic conditions. (◆, microcosm containing RDX and soil; ■, microcosm containing RDX, soil and anaerobic sludge; ▲, microcosm containing RDX, TNT and soil; ●, microcosm containing RDX, TNT, soil and anaerobic sludge; ▼, heat killed control. TNT and RDX concentration was 1000 and 2000 mg/kg dry soil, respectively, in all microcosms).

ance of RDX in an activated sludge system. These data suggest that RDX is persistent to indigenous microorganisms in soil and activated sludge under strictly aerobic conditions. The data in Figure 4 also reveal that the RDX-mineralizing activity of indigenous microorganisms in soil and activated sludge is inhibited by the presence of TNT in the microcosms. The inhibitory effect of TNT is reflected by the significant drop in RDX mineralization from 2.2% (□) to 1.4% (○) when TNT was added to the microcosms. This antagonistic effect of TNT on the RDX mineralization may be attributed to the toxicity of TNT and its metabolites to the metabolic activity of RDX mineralizing organisms, since TNT and its metabolites are more toxic than RDX (Hawari et al. 1996).

In contrast to the mineralization of RDX under aerobic condition, soil indigenous microbes were able to mineralize RDX up to 15% under anaerobic conditions after 52 days of incubation when RDX was used as the sole explosive (Figure 5). The mineralization extent of RDX was markedly enhanced by the supplement of municipal anaerobic sludge, reaching 60% in the microcosms containing RDX, soil, and anaerobic sludge. The data in Figure 5 also illustrated that RDX mineralizing activity of indigenous microorganisms in soil and anaerobic sludge under anaerobic conditions was also inhibited by the presence of TNT, i.e. a reduction in mineralization from 60% to 47%. The reduction of RDX mineralization in aerobic and anaerobic microcosms by the presence of TNT indicates that the RDX degrading bacteria (either aerobic or anaerobic) are sensitive to toxicity of TNT and of its metabolites.

The data in Figure 5 also reveal that the supplement of activated sludge increased the mineralization rate of RDX again in the microcosms that had initially been incubated under anaerobic conditions. The improvement in RDX mineralization in the sequential anaerobic-aerobic process may be explained by enhanced degradation of RDX metabolites to CO_2 under aerobic conditions rather than additional mineralization of remaining RDX, since the RDX mineralization was very poor in the microcosm containing RDX, TNT, soil, and activated sludge under aerobic conditions (Figure 4).

In parallel to the RDX mineralization study, metabolites from RDX degradation in the anaerobic microcosms without receiving [^{14}C]-RDX were monitored by HPLC over the time course of incubation. Figure 6 shows that at least six metabolites from RDX degradation were observed in the anaerobic microcosms in the early period of incubation when comparing the HPLC chromatogram of active sample with that of control one. These metabolites were further degraded with increasing incubation times, as shown by the disappearance of their peaks in HPLC chromatograms. The formation of several RDX metabolites in the RDX

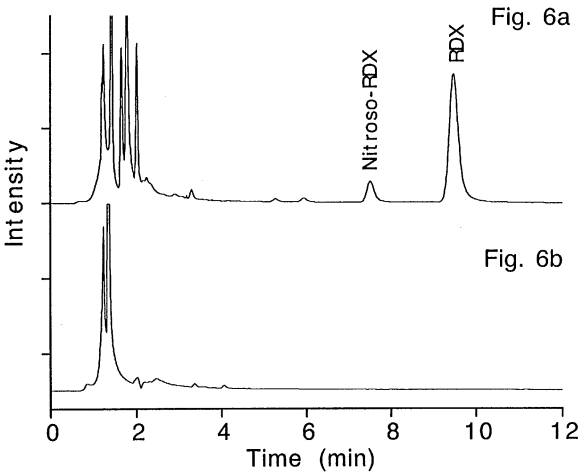

Figure 6. HPLC chromatograms of soil slurry samples from anaerobic microcosms with the supplement of RDX (Figure 6a) and without the supplement of RDX (Figure 6b).

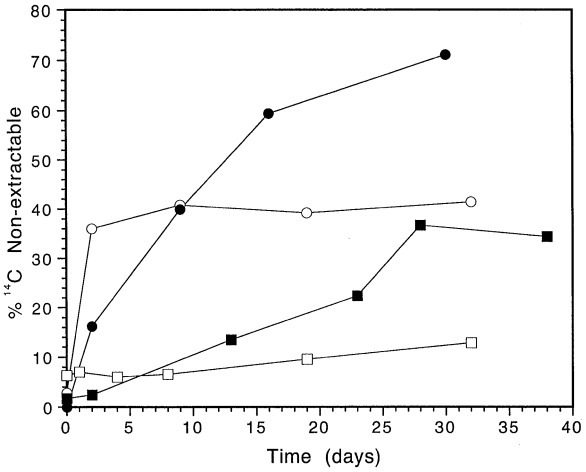

Figure 7. Non-extractable portion of explosive and its metabolites over the time course in microcosms simulating bioslurry reactors containing TNT and RDX. (○, ^{14}C-TNT under aerobic conditions; ●, ^{14}C-TNT under anaerobic conditions; □, ^{14}C-RDX under aerobic conditions and ■, ^{14}C-RDX under anaerobic conditions)

mineralization process also suggests that the RDX went through several degradation processes before the formation of CO_2. These degradation processes may be the same as those proposed by McCormick et al. (1981), however further study in the identification of these metabolites will assist the interpretation of the RDX degradation pathways.

Biological degradation of RDX has been demonstrated previously (Osmon & Klausmeier 1973; Sikka et al. 1980; McCormick et al. 1981; Yang et al. 1983). Recently, researchers from several groups (Kitts et al. 1994; Binks et al. 1995; Crawford et al. 1995) successfully isolated several microorganisms responsible for the degradation of RDX. Until now, no radiotracer study has been reported for the monitoring of the fate of RDX in bioslurry reactor processes although RDX degradation has been observed earlier (Funk et al. 1993; Manning et al. 1995; Young et al. 1997).

Fate of TNT and RDX metabolites

The data given in Figure 7 outline the dynamic processes of non-extractable portion of TNT, RDX, and their metabolites over the time course in the soil slurry microcosms simulating the bioslurry reactors containing TNT and RDX. The non-extractable portion of TNT and its metabolites, as determined by radioactivity, increased sharply (by about 35%) after 2 days of incubation under aerobic conditions, but became stable when the experiment lasted for a longer period. Unlike in the microcosms incubated under aerobic conditions, the non-extractable amounts of radioactivity from [^{14}C]-TNT elevated gradually to 72% after 30 days of incubation under anaerobic conditions. The non-extractable profile of TNT and its metabolites may be explained by the TNT transformation under different conditions, and the adsorption and interaction between TNT metabolites and the active sites of the soil matrix. The results from our previous study (Shen et al. 1998) showed that all TNT in the aerobic bioslurry reactor was transformed into aminodinitrotoluene and other metabolites in less than 10 days, and these metabolites were not readily further transformed under aerobic conditions. During this period, part of TNT metabolites might adsorb quickly with the soil matrix, resulting in a fast increase in the non-extractable amount of TNT and its metabolites. In contrast to the aerobic process, the TNT metabolites from anaerobic bioslurry reactor were further reduced over the time into diamino and triamino derivatives, which are more interactive and can be irreversibly bound to the soil matrix (Lenke et al. 1994), resulting in a decrease in the extractability of TNT metabolites over the incubation time. A detailed explanation for the mechanism of the triaminotoluene adsorption into soil matrix can be found elsewhere (Rieger & Knackmuss 1995). The non-extractable amounts of TNT and its metabolites indicate that a significant portion of TNT metabolites ended up being irreversibly bound residue in the soil matrix during the bioslurry treatment processes (Figure 7).

Table 2. Partition of [^{14}C]-RDX in the soil slurry microcosm incubated under anaerobic conditions

Incubation time (days)	^{14}C Extractable (%)	$^{14}CO_2$ (%)	^{14}C Non-extractable (%)	^{14}C Recovery (%)
0	99.9	0	1.7	101.6
2	95.4	0.6	2.4	98.4
13	75.2	6.9	13.5	95.6
23	41.9	20.4	22.4	84.7
28	17.8	33.0	36.7	87.5
38	14.9	42.2	34.4	91.5

The partitioning of ^{14}C-RDX and its metabolites in the microcosm (containing cold TNT) also is shown in Figure 7. The data reveal that less than 13% of RDX and its metabolites became non-extractable after 32 days of incubation under aerobic conditions, but the mineralization of RDX into CO_2 in this microcosm was less than 2% during this incubation period. The profile of non-extractable portion of radiolabel in this microcosm matched well with the disappearance of RDX in the aerobic slurry reactor conducted in the previous study (Shen et al. 1998), indicating that measured radioactivity in the extract was mainly from RDX or that the non-extractable portion was due to the assimilation of RDX into biomass and/or binding of its metabolites with the soil matrix.

The data in Table 2 present a mass balance and dynamic distribution of RDX and its metabolites in the soil slurry microcosms containing RDX, TNT, soil, and anaerobic sludge under anaerobic conditions. The non-extractable portion of radiolabel in the soil slurry was also presented in Figure 7 for comparison purposes. As the percentage of [^{14}C]-RDX mineralization increased over the incubation time, more RDX was transformed into its metabolites and assimilated into the biomass. Some of the amine metabolites from RDX degradation, such as dimethylhydrazine and hydroxymethylhydrazine (McCormick et al. 1981), may bind strongly to humic substances and to clay portion of the soil matrix, and consequently reduce their extractability from soil slurry. Therefore, the increase of non-extractable portion of radiolabel over the time course reflected the assimilation of RDX into biomass and the interaction (irreversibly binding) between the soil matrix and RDX metabolites. The portion of radiolabel assimilated into biomass can be theoretically estimated from the amount of evolved $^{14}CO_2$ according to the biomass yield under anaerobic conditions. Under anaerobic conditions, it is reasonable to assume that approx. 20% of the carbon consumed was used for biomass growth production (Roels 1983): With a microbial consumption of one unit of organic carbon, 0.80 unit of carbon source was evolved into CO_2, while 0.2 unit was assimilated into biomass. Based on the above assumption, the amount of [^{14}C]-RDX assimilated into biomass would be about 10.5% for 42% of evolved as $^{14}CO_2$. Therefore the portion of [^{14}C]-RDX metabolites irreversibly bound to the soil matrix could be assigned as 24%. The recovery of [^{14}C]-RDX radiolabel in this partitioning study varied from 84.7 to 101.6% for several samples incubated during a period of 38 days. Deficits in radiolabel mass balance might be attributed to incomplete chemical digestion of soil and biomass or to conversion of organic carbon (including [^{14}C]-RDX) to methane.

The results of radiotracer study in this paper provide some insight into the fate of TNT and RDX during the bioslurry treatment processes as follows: TNT was transformed with minimal mineralization during the treatment, and a significant portion of TNT metabolites bound to soil matrix. Anaerobic bioslurry reactor with the supplement of municipal anaerobic sludge provided the best conditions for the mineralization of RDX and degradation of TNT.

Acknowledgments

Authors thank Louise Paquet and Ed Zhou for excellent analytical services. This is a NRC paper numbered 41761.

References

Ampleman G, Thiboutot S, Lavigne J, Marois A, Hawari J, Jones AM and Rho D (1995). Synthesis of ^{14}C-labeled hexahydro-1,3,5-trinitro-triazine (RDX), 2,4,6-trinitrotoluene (TNT), nitrocellulose (NC) and glycidyl azide polymer (GAP) for use in assessing the biodegradation potential of these energetic compounds. J. Label. Comp. Radiopharm. 36(6): 559–577

Binks PR, Nicklin S & Bruce NC (1995) Degradation of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) by *Stenotrophomonas maltophilia* PB1. Appl. Environ. Microbiol. 61: 1318–1322

Bouvier ESP & Oehrle SA (1995) Analysis and identification of nitroaromatic and nitramine explosives in water using HPLC and photodiode-array detection. LC-GC 13: 120–130

Brockman FJ, Downing DC & Wright GF (1949) Nitrolysis of hexamethylenetetramine. III. Preparation of pure cyclonite. Can. J. Res., Section B: Chem. Sci. 27: 469–474

Carpenter DF, McCormick NG, Cornell JH & Kaplan AM (1978) Microbial transformation of ^{14}C-labeled 2,4,6-trinitrotoluene in an activated sludge system. Appl. Environ. Microbiol. 35: 949–954

Crawford RL (1995) Biodegradation of nitrated munition compounds and herbicides by obligately anaerobic bacteria. In: Spain JC (Ed) Biodegradation of nitroaromatic compounds, (pp 87–98). Plenum Press, New York.

Crawford RL, Crawford DL, Funk SB, Pumfrey LJ & Regan KM (1995) Biological isolates for degrading nitroaromatics and nitramines in water and soils. U.S. patent, 18 pp. Cont. in part of U.S. 5,387,271

Fernando T, Bumpus JA & Aust SD (1990) Biodegradation of TNT (2,4,6-trinitrotoluene) by *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 56: 1666–1671

Funk SB, Roberts DJ, Crawford DL & Crawford RL (1993) Initial-phase optimization of munition compound-contaminated soils. Appl. Environ. Microbiol. 59: 2171–2177

Hawari J, et al. (1996) Characterization and remediation of soil contaminated with TNT, RDX, GAP, and nitrocellulose towards a field trial: Phase IV–Final report. Biotechnology Research Institute, Montreal, Quebec, Canada

Hoffsommer JC, Kaplan LA, Glover DJ, Kubrose DA, Dickinson C, Goya H, Kayser EG, Groves CL & Sitzmann ME (1978) Biodegradability of TNT: A three year pilot plant study. NSWC/WOL TR 77–136. Naval Surface Weapons Center, White Oak, Silver Spring, MD.

Kitts CL, Cunningham DP & Unkefer PJ (1994) Isolation of three hexahydro-1,3,5-trinitro-1,3,5-triazine-degrading species of the family *Enterobacteriaceae* from nitramine explosive-contaminated soil. Appl. Environ. Microbiol. 60: 4608–4711

Lenke H, Wagener B, Daun G & Knackmuss H-J (1994) TNT-contaminated soil: a sequential anaerobic/aerobic process for biotransformation. abstr. Q-383, p456. Abstr. 94th Annu. Meet. Am. Soc. Microbiol. 1994

Manning JF Jr., Boopathy R & Kulpa CF (1995) A laboratory study in support of the pilot demonstration of a biological soil slurry reactor. Report No. SFIM-AEC-TS-CR-94038. Bioremediation Group, Environmental Research Division, Argonne National Laboratory, Argonne, IL 60439–4843

Marvin-Sikkema FD & de Bont JA (1994) Degradation of nitroaromatic compounds by microorganisms. Appl. Microbiol. Biotechnol. 42: 499–507

McCormick NG, Cornell JH & Kaplan AM (1981) Biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine. Appl. Environ. Microbiol. 42: 817–823

Michels J & Gottschalk G (1994) Inhibition of lignin peroxidase of *Phanerochaete chrysosporium* by hydroxylamino-dinitrotoluene, an early intermediate in the degradation of 2,4,6-trinitrotoluene. Appl. Environ. Microbiol. 60: 187–194

Nelson DW & Sommers LE (1982) Total carbon, organic carbon, and organic mater. In: Page AL, Miller RH & Keeney DR (Eds) Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties. American Society of Agronomy, Inc. and Soil Science Society of America, Inc., Madison, Wisconsin, USA

Osmon JL & Klausmeier RE (1973) The microbial degradation of explosives. Dev. Ind. Microbiol. 14: 247–252

Preuβ A & Rieger P-G (1995) Anaerobic transformation of 2,4,6-trinitrotoluene and other nitroaromatic compounds. Environ. Sci. Res. 49: 69–85

Rieger P-G & Knackmuss H-J (1995) Basic knowledge and perspectives on biodegradation of 2,4,6-trinitrotoluene and related nitroaromatic compounds in contaminated soil. Environ. Sci. Res. 49: 1–18

Roels JA (1983) Energetics and kinetics in biotechnology. Elsevier Biomedical Press BV, New York.

Shen CF, Hawari J, Thiboutot S, Ampleman G & Guiot SR (1998) Remediation of explosive contaminated soil using bio-enhanced slurry reactors. Submitted to Appl. Microbiol. Biotechnicol.

Sikka HC, Baberjie S, Pack EJ & Appleton HJ (1980) Environmental fate of RDX and TNT. Contract DAMD17-77-c-7026. Syracuse Research Corporation

Spiker JK, Crawford DL & Crawford RL (1992) Influence of 2,4,6-trinitrotoluene (TNT) concentration on the degradation of TNT in explosive-contaminated soils by white rot fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 58: 3199–3202

Walker JE & Kaplan DL (1992) Biological degradation of explosives and chemical agents. Biodegradation 3: 369–385

Yang Y, Wang X, Yin P, Li W & Zhou P (1983) Studies on three strains of *Corynebacterium* degrading cyclotrimethylene-trinitroamine (RDX). Acta Microbiol. Sin. 23(3): 251–256

Young DM, Kitts CL, Unkefer PJ & Ogden KL (1997) Biological breakdown of RDX in slurry reactor proceeds with multiple kinetically distinguishable paths. Biotech. Bioeng. 56: 258–267