ERDC/EL TR-07-25

US Army Corps
of Engineers®
Engineer Research and
Development Center

Environmental Quality Technology Program

Potential for Biodegradation of the Alkaline Hydrolysis End Products of TNT and RDX

Deborah R. Felt, Catherine C. Nestler, Jeffrey L. Davis,
and Steven L. Larson

November 2007

Environmental Laboratory

Environmental Quality Technology Program

ERDC/EL TR-07-25
November 2007

Potential for Biodegradation of the Alkaline Hydrolysis End Products of TNT and RDX

Deborah R. Felt, Jeffrey L. Davis, and Steven L. Larson

Environmental Laboratory
U.S. Army Engineer Research and Development Center
3909 Halls Ferry Road
Vicksburg, MS 39180-6199

Catherine C. Nestler

Applied Research Associates, Inc., Southern Division
119 Monument Place
Vicksburg, MS 39180

Final report

Prepared for U.S. Army Corps of Engineers
Washington, DC 20314-1000

Abstract: Energetic compounds, such as 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and their degradation products, can act as a source of contamination for soil on Department of Defense testing and training ranges. Base-catalyzed hydrolysis degrades nitroaromatics and nitramines, and the potential effectiveness of this reaction in soil has been demonstrated at both bench and pilot scales. This report evaluates the potential for soil bacteria to degrade the transformation products from the alkaline hydrolysis of munitions residues. The media were obtained from the hydrolytic destruction of TNT and RDX at pH 12.5, 11.5, and 10.5. Duplicate reactors were amended with [^{14}C]-labeled explosive compounds. Bench-scale microcosms incubated aerobically and anaerobically using grenade range soil as the inoculum and reaction mixtures (quenched and neutralized) as the media showed that there is a potential for biodegradation. Nutrient analysis confirmed the presence of increased levels of nitrite and formate following both aerobic and anaerobic incubation. TNT end products from alkaline hydrolysis were aerobically mineralized, with 16% [^{14}C]-label recovered as CO_2. RDX reaction end products demonstrated much greater mineralization than TNT (roughly threefold). The use of alkaline material on training ranges has the potential to treat source-zone energetics contamination.

Contents

Figures and Tables

Figures

Tables

Preface

This report was prepared by Catherine C. Nestler, Applied Research Associates Inc., Vicksburg, MS, and Deborah R. Felt, Dr. Jeffrey L. Davis, and Dr. Steven L. Larson of the Environmental Engineering Branch (EP-E), Environmental Laboratory (EL), U.S. Army Engineer Research and Development Center (ERDC), Vicksburg, MS.

The work presented in this report was part of an effort to investigate the remediation of soils contaminated with multiple types of explosives from a variety of Department of Defense facilities. The work was conducted at ERDC. Funding for this project was provided through the Environmental Quality Technology Program.

This study was conducted under the direct supervision of Dr. Pat Deliman, Chief, EP-E, and Dr. Richard E. Price, Chief, Environmental Processes and Engineering Division, and under the general supervision of Dr. Beth Fleming, Director, EL.

COL Richard B. Jenkins was Commander and Executive Director of ERDC. Dr. James R. Houston was Director.

Acronyms

ASTM	American Society for Testing and Materials
Avg.	average
CEC	Cation exchange capacity
Da	Daltons
DAD	Diode Array Detector
DOD	Department of Defense
DDI	Distilled de-ionized water
EL	Environmental Laboratory, U.S. Army Engineer Research and Development Center
EPA	Environmental Protection Agency
ERDC	U.S. Army Engineer Research and Development Center
GPC	gel permeation chromatography
GSL	Geotechnical and Structures Laboratory, U.S. Army Engineer Research and Development Center
HPLC	high pressure liquid chromatography
IC	ion chromatography
ICP-AES	inductively couples plasma – atomic emission spectroscopy
LSC	liquid scintillation costs
mM	millimolar
N	Normality
PDA	photo diode array
PEG	polyethylene glycol
ppm	Parts per million, also expressed as mg/kg or mg/L
PSD	particle size distribution
r^2	Coefficient of determination
RDX	Hexahydro-1,3,5-trinitro-1,3,5-triazine
TNT	2,4,6-trinitrotoluene

TOC	Total organic carbon
USACE	U.S. Army Corps of Engineers
USEPA	United States Environmental Protection Agency
USGS	U.S. Geological Survey
UV/VIS	ultraviolet/visible
UXO	unexploded ordnance

1 Introduction

Alkaline Hydrolysis

This report evaluates the potential for soil bacteria to degrade the transformation products from the alkaline hydrolysis of munitions residues. Janowsky (1891) first established the transformation of TNT in basic solutions. The alkaline hydrolysis reaction and its colored intermediates now form the basis for a field test on TNT and other similar explosive compounds (Jenkins and Walsh 1992). Hydrated lime [$Ca(OH)_2$] addition is an inexpensive means to achieve the alkaline conditions required for the reaction. Arienzo (1999) reported complete removal of TNT from soil in 10 min with the application of 1 percent by weight of $Ca(OH)_2$. Emmrich (1999, 2001) also treated TNT and RDX in solution and soils with calcium hydroxide at 20°C, with nitrite and nitrate appearing as end products.

Felt et al. (2001) established that two intermediates quickly formed and were then followed by several unidentified products in a sequential manner during alkaline hydrolysis of TNT. The development of a sequential first-order rate model for the first two reactions forming these intermediates is complicated by the rapid decomposition of Intermediate 1 and the formation of multiple, visibly colored products from the further reaction of Intermediate 2. The reaction rate is slow below an initiating pH of 10.5–11, and the reaction is not quenchable with acid after initiation, suggesting that the reaction is catalytic or autocatalytic. Using the global analysis of spectral data and employing a means to slow the reaction without interfering with the formation of the intermediates, Felt et al. (2007a) identified two well-resolved and spectrally distinct reaction intermediates of the alkaline hydrolysis of TNT. Electron paramagnetic resonance (EPR) was also used in that study to determine if any reaction components exhibited unpaired electron spins, which would indicate a free radical. EPR results suggested that a single radical species was formed during the TNT–hydroxide reaction that correlated with the second reaction intermediate.

Range Remediation

The use of alkaline material has the potential to treat source zone contamination and prevent the transport of the contaminants into groundwater (Brooks et al. 2003). Soil microcosm studies conducted by Brooks et al. (2003) demonstrated that a well-mixed soil–lime system can remove explosives contaminants such as RDX and TNT from soils through alkaline hydrolysis. In addition, alkaline hydrolysis can increase the dissolution and transformation rates of solid explosives particles. TNT metabolites such as 2A- and 4A-DNT also undergo alkaline hydrolysis. Brooks et al. (2003) also used larger, mesocosm-scale studies to determine the effects of soil characteristics on the removal of explosive compounds. They confirmed that RDX and TNT were readily removed from the soil and the leachate of well-mixed systems.

Brooks et al. (2003) suggested that additional research into the alkaline hydrolysis reaction was necessary before a field demonstration. Therefore, the objectives of this research were:

- Evaluate the potential for biodegradation of the final transformation products by soil bacteria under aerobic and anaerobic environments, and
- Evaluate the contribution of different electron acceptors on the biodegradation potential.

The objectives of this work were achieved by using these methods:

- The final reaction products of alkaline hydrolysis were challenged with the inherent bacteria of a range soil using radio-labeled tracers to determine mineralization potential.
- Changes in the anion content of the culture media that occurred as a result of incubation with the soil were examined using ion chromatography.
- Changes in molecular size of the reaction end products that occurred as a result of incubation with the soil were examined using gel permeation chromatography.

Department of Defense live-fire ranges are crucial to military readiness, and the sustainability of these ranges is of paramount importance to ensure continued training at military installations. Thus, the development of effective treatment options for energetic contaminants is essential for

range management and sustainability (Borthwick and Beshore 2000, Jones et al. 2002). The research reported here answers many of the questions concerning the environmental fate of the end products of alkaline hydrolysis in soil and prepares the way for a field demonstration of the alkaline hydrolysis technology.

2 Experimental Design

Objective

The objective of this project was to evaluate the potential of inherent soil bacteria to degrade the final reaction products of the alkaline hydrolytic degradation of the munitions compounds TNT and RDX. We examined this potential in aerobic and anaerobic environments, using reaction solutions amended with different electron acceptors. In addition, this research used radio-labeled explosives to establish an activity balance for the solid, liquid, and gas phases of the bacterial cultures in order to determine the fate of the reaction products in the environment.

Experimental Design

The experimental design to evaluate the final products of the alkaline hydrolysis treatment of TNT and RDX for differences in biodegradation potential is shown in Figure 1 and detailed in Table 1. The cultures' media were the [^{14}C]-labeled and unlabeled reaction mixtures produced from the alkaline hydrolysis reactions described in Felt et al. (2007b). The soil used as the source of the microbial communities (inoculum) was an uncontaminated, unamended soil from a military training range. This soil is similar to sites where we would expect to use alkaline hydrolysis as a treatment technology. The control for the cultures' media (i.e. the reaction solutions) was an aqueous solution of the untreated explosive. Two other experimental controls were used: an abiotic control using sterilized soil and a soil control. Incubation of the HCl-neutralized reaction mixtures was performed under aerobic and anaerobic environments. The incubations using nitric- and sulfuric-acid-amended reaction mixtures were performed only under anaerobic conditions.

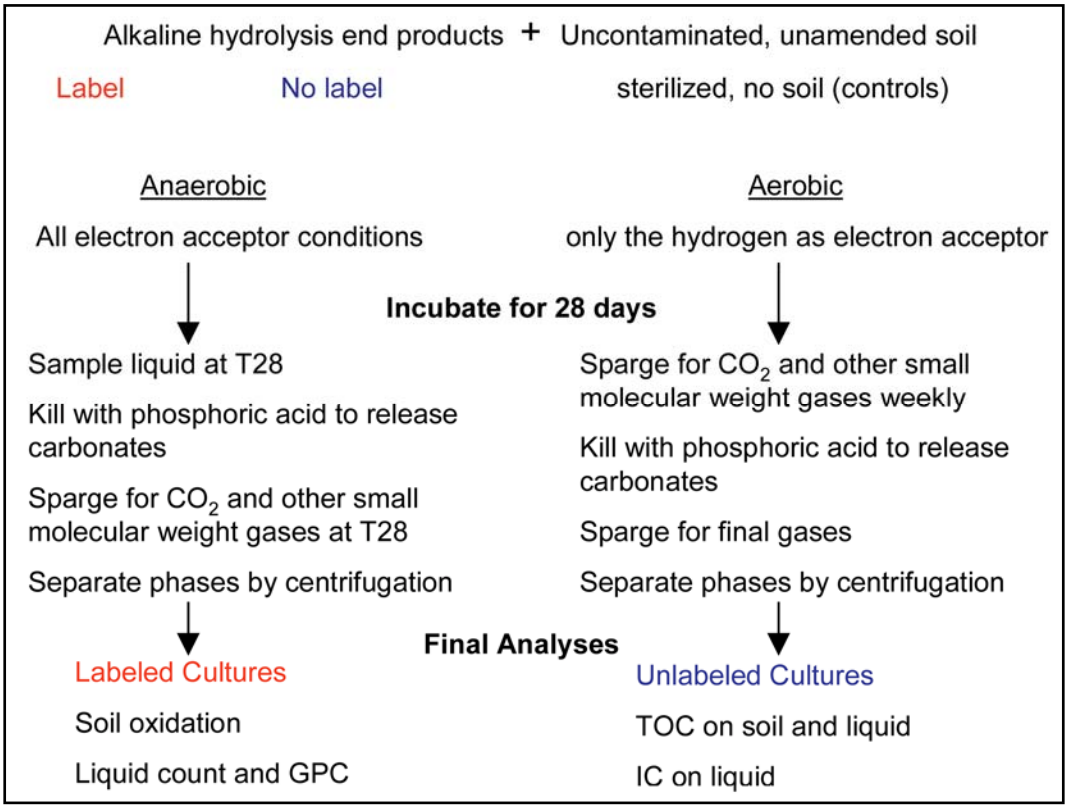


Figure 1. Flowchart of the experimental design to establish the fate and potential for biodegradation of the alkaline hydrolysis end products of TNT and RDX.

The experimental design indicating the number of replicate flasks or serum bottles for assessing the biological degradation potential of TNT and RDX alkaline hydrolysis reaction end products by range soil is outlined in Table 1. The sequence of events depicted in Figure 1 and Table 1 was repeated for each of the three pH values studied: 12.5, 11.5, and 10.5.

Table 1. Experimental design indicating the number of replicate flasks or serum bottles for assessing the biological degradation potential of TNT and RDX alkaline hydrolysis reaction end products by range soil.

	Incubation amendments and environment								
	No amendment	H^-		H^-		SO_4^{2-}		NO_3^-	
	Aerobic/Anaerobic	Aerobic		Anaerobic		Anaerobic		Anaerobic	
Culture	Untreated With Label	Label	No label	Label	No label	Label	No label	Label	No label
Active culture	2	3	3	3	3	3	3	3	3
Soil control	0	1	1	1	1	1	1	1	1
Abiotic control	0	1	1	1	1	1	1	1	1

3 Materials and Methods

Materials

Soil

The soil used to determine the biodegradation potential of the alkaline hydrolysis end products was obtained from a pristine portion of a regional hand grenade range. The soil was physically characterized by the Geotechnical and Structures Laboratory (GSL) at ERDC-Vicksburg. The Atterberg limit, specific gravity, and particle size distribution (PSD) tests were used to evaluate the physical structure of the experimental soil and provide the Uniform Soil Classification System (USCS) soil classification [U.S. Army Corps of Engineers (USACE) 1986]. The range soil is a non-plastic, fine, silty sand with a specific gravity of 2.68 g/cm^3. The particle size distribution is: gravel = 0.1 percent, sand = 72.9 percent, fines (silt, clay) = 27 percent. The soil for the abiotic control cultures was sterilized by triple autoclave procedures separated by three days each.

Table 2 lists the results of the chemical characterization of the range soil. The Environmental Chemistry Branch of the Environmental Laboratory, ERDC-Vicksburg, determined calcium and magnesium concentrations and cation exchange capacity (CEC) of the soil. Calcium and magnesium concentrations were determined using USEPA-SW 846, Method 6010B for inductively coupled plasma-atomic emission spectrometry (ICP-AES) (U.S. Environmental Protection Agency 1996). Soil CEC was determined using USEPA-SW 846, Method 9081 (U.S. Environmental Protection Agency 1986) and ICP-AES. The nitrogen and phosphate analyses were performed using a Lachat 8000 flow injection analyzer. The soil was analyzed using EPA Method 8330 (U.S. Environmental Protection Agency 1994) and found to be free of explosives contamination.

Table 2. Chemical characterization of the regional hand grenade range soil.

Test	Result	Test	Result
Initial pH	5.5	Calcium	168 mg/kg
Total organic carbon	0.1%	Iron	3.39 mg/kg
Cation exchange capacity	8 meq/100 g	Magnesium	53.8 mg/kg
Total Kjeldahl nitrogen	0.1* mg/kg	Manganese	2.13 mg/kg
Ammonia nitrogen	<1.0 mg/kg	Potassium	17.1 mg/kg
Nitrite/nitrate	1.8 mg/kg	Sodium	<4.00 mg/kg
Total phosphate	1.4 mg/kg	Sulfate	11* mg/kg
ortho-phosphate	<0.3 mg/kg	Chloride	37* mg/kg
*Values are estimates below the laboratory reporting limit but greater than the instrument detection limit.			

Liquid Media

The liquid used for the cultures' media was obtained by the alkaline hydrolysis of either TNT or RDX to a pre-determined end point. The reaction was quenched and neutralized at the conclusion of each reaction with an acid providing a specific electron acceptor. The effects of these electron acceptors were examined under both aerobic and anaerobic environments. Details of the reaction process are provided in Davis et al. (2007). As outlined in Figure 1, each of these acid-amended solutions had a replicate that had been amended with a [^{14}C]-labeled explosive. Analysis of the unlabeled cultures provided information on changes in total organic carbon and anions in the solutions. Analysis of the labeled cultures provided a means to obtain an activity balance for the treated and untreated explosive.

Incubation Setup

The aerobic incubation flasks (Figure 2) were custom manufactured by Reliance Glass, now Wilmad-Labglass (Buena, NJ). The flasks were equipped with double sidearms for simultaneous sparging and gas collection. The central column was fitted with a standard KOH trap for CO_2 collection. The flask sides had baffles for improved mixing at low shaker speeds. Serum bottles (125 mL) were used as reaction vessels for the anaerobic reactions. Stoppers for the serum bottles were purchased from Bellco Glass, Inc. (Vineland, NJ). The stainless steel, deflected point needles used in sparging (18 G, 6 in. and 12 in.) were manufactured by Popper and Sons (New Hyde Park, NY).

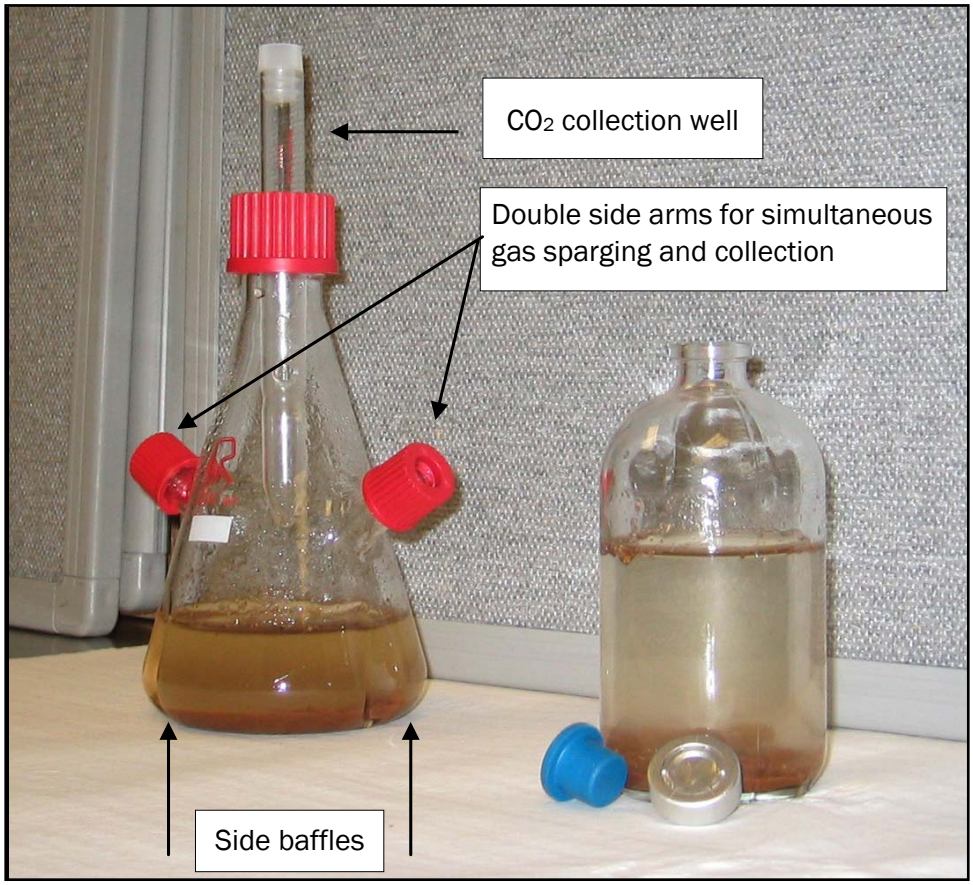


Figure 2. Custom-designed aerobic incubation flasks and serum bottles used for anaerobic incubation.

The anaerobic environmental chamber was purchased from Coy Laboratory Products, Inc. (Grass Lake, MI). The chamber's anaerobic atmosphere was maintained with a 96 percent H:N_2 mix. The pyrolysis tube used to establish the gaseous phase of the activity balance was purchased from ANTEK Corp. and used in a Lindberg/Blue M tube furnace (model TF55035A). The soil oxidizer, used to combust solid phase samples for the activity balance, was a Packard Instruments Model 307. The efficiency of the soil oxidizer was established at 98.4 percent.

Labeled samples were counted on a Packard Instruments model Tri-Carb 2900 TR liquid scintillation counter. The scintillation counter was equipped with a barium external source to enable correction for machine efficiency. The instrument protocol collected data up to 156 meqV or the maximum energy for [^{14}C]. Samples were counted twice for 2 minutes to provide a check of instrument precision. The carbon dioxide trapping solution, CarboSorb®, and the scintillation cocktails, Ultima Gold® and

Permafluor-E®, were purchased from Perkin-Elmer Life Sciences, Inc (Downers Grove, IL), as were the soil oxidation supplies.

Methods

Explosives Analysis

For pH 12.5, the explosives concentrations of the final incubation media were quantified using a Hewlett-Packard model 1090 HPLC prepared with a reverse-phase cyano column (Supelco LC-CN, 25 cm × 4 mm ID, 5 µm particle size) equipped with a diode array detector (DAD) monitored at 254, 330, and 210 nm. A mobile phase of methanol and distilled, deionized (DDI) water (50:50, v:v) was used with a flow rate of 1.0 mL/min. The run time was 25 min. Under these conditions, the retention time was 7.6 min for TNT and 10.5 min for RDX. The method detection limit was 0.02 mg/L for TNT and 0.03 mg/L for RDX. The detector response was linear from 10 mg/L down to the detection limit.

For pH 11.5 and 10.5, the explosives concentration of the final incubation media were quantified using a Dionex HPLC system consisting of a Dionex P580 pump, an ASI-100 autosampler, and a UVD 340U UV/VIS detector monitored at 254, 330, and 210 nm. The columns, mobile phase, and flow rates were the same as those used with the Hewlett-Packard HPLC system.

Molecular Weight Changes

All [^{14}C]-labeled solutions were assessed for molecular weight changes of the reaction products following soil incubation using gel permeation chromatography (GPC). GPC was performed using two systems. For pH 12.5 samples, a Waters HPLC was equipped with a Waters 600-M system controller, a Waters 991-MS photodiode array detector (PDA), and a Waters 7 Satellite WISP autosampler. A Biosep 600 × 7.8-mm column using a flow rate of 1 mL/min was used with DDI water as the aqueous mobile phase, the detector was set at 206 nm, and the run time was 40 min. Under the conditions specified above, both TNT and RDX standards had an elution time of 26 minutes. Fractions were collected at pre-determined time intervals from each of the experimental and control samples based on the retention times of molecular weight standards and the parent compound (TNT or RDX). Millenium chromatography software was used for data analysis.

The second system used for pH 11.5 and 10.5 samples was a Dionex HPLC system consisting of a Dionex P580 pump, an ASI-100 autosampler, and a UVD 340U UV/VIS detector monitored at 254, 330, and 210 nm. Separation of water-soluble products was achieved using a Bioseptcolumn (Phenomenex, 5 μm particle size). The DAD was set at 210 nm. A mobile phase of 5 percent acetonitrile in DDI water was used with a flow rate of 1 mL/min to separate water-soluble (polar) compounds. Separation of organic-soluble (non-polar) products was achieved using a Phenogel column (Phenomenex, 600 mm × 7.8 mm, ID, 50 μm and 50 Å). The guard column was a Phenomenex (50 mm × 7.8 mm ID), and the pre-column was a Phenogel (Phenomenex , 300 mm × 7.8 mm ID, 5 μm × 103 Å particle size). The mobile phase used to separate non-polar compounds was 100 per cent dichloromethane (DCM), with a flow rate of 0.8 mL/min. Chromeleon 6.40 chromatography software was used for data analysis.

The GPC analysis used polyethylene glycol (PEG) molecular weight standards to calibrate the molecular weights in the inorganic samples. The working standards were made by dissolving 20 mg of each compound in 40 mL of water. Representative samples (100 μL) of each standard (6,000–3,000; 3,000–1,500; 1,500–1,000; 1,000–750; 750–500; 500-250, 250–100, <100 Daltons (Da)) were injected onto both the water-phase and the organic-phase columns under the conditions specified above. Retention times were noted for the peak produced by each standard. A standard curve was generated for the polyethylene glycol molecular weight standards with an r^2 value of 0.9896. Fractions were collected at pre-determined time intervals from each of the experimental and control samples based on the retention times of the molecular weight standards and the parent explosive standard. Initial and final reaction mixtures were analyzed and experimental solutions compared to the untreated control.

TOC and Anion Analysis

Solid and liquid total organic carbon analysis was performed on unlabeled samples using a Shimadzu total organic carbon (TOC) -V/SSM-5000A system, according to instrument protocol. Anion analysis was performed using a Dionex ICS-2500 ion chromatograph equipped with a Dionex ASRS-ULTRA 4 mm. Chemical separation and detection was achieved using an Ionpac AS11 guard column (4 mm × 50 mm), an AS11 analytical column (4 mm × 250 mm), and Dionex CD20 conductivity detector

(1.25 µL internal volume). The gradient elution started with a concentration of 0.5 mM sodium hydroxide (NaOH), which was increased to 27 m-M NaOH over the course of 35 min using a Dionex GP40 gradient pump. The concentration was ramped back down to 0.5 mM NaOH after 2 min at 27 mM NaOH. The flow rate remained constant at 1.5 mL/min over the course of the run. The sample (100 µL) was automatically injected by a Dionex automated sampler. The instrument was calibrated using standard anionic solutions, and each sample was analyzed for formate, nitrite, nitrate, and sulfate using both DDI and tap water as controls during the analysis.

Aerobic Treatment

Representative soil samples (10 g) were placed in sterile flasks fitted with KOH traps for CO_2 collection, and 90 mL of the appropriate transformation product solution was added to serve as the cultures' medium. The flasks were incubated in the dark for 28 days at room temperature (23°C ± 1°C) on a shaker set at 100 rpm. The aerobic flasks containing unlabeled reaction mixtures were flushed with CO_2-free air weekly, for four weeks, and the KOH traps refreshed. The aerobic flasks containing [^{14}C]-labeled reaction mixtures were also sparged with CO_2-free air weekly, and any generated CO_2 was collected in CarboSorb® and counted using LSC (Fig. 4). The KOH traps were then emptied, and a sub-sample was analyzed using LSC. Fresh KOH was placed in the traps. After 28 days and the final weekly sparging, 0.5 mL of concentrated phosphoric acid was injected into each flask. Flasks were mixed gently and allowed to sit undisturbed for a minimum of 24 hr. Acidification (pH≤2) using phosphoric acid stopped biological activity and released any carbonates formed during incubation as carbon dioxide. The flasks were sparged for a final time. Gas phase recovery was determined by the addition of the weekly KOH and CarboSorb® counts and the post-acidification counts.

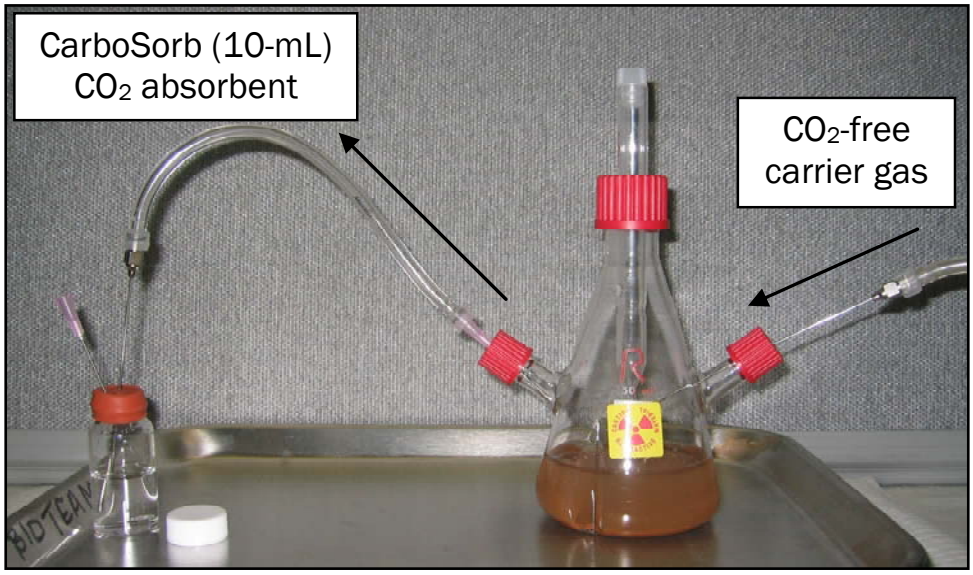


Figure 3. Gas sparging of labeled aerobic cultures.

After acidification and resting, the flasks were opened; the slurries were transferred to centrifuge tubes and spun for 50 min at 2000 rpm to separate the solid and liquid phases. The liquid was decanted and the soil was removed to a scintillation vial. Unlabeled flasks had the soil and liquid analyzed for TOC and the liquid analyzed for anion content. The liquid from the [^{14}C]-labeled flasks was sampled and counted using liquid scintillation counting (LSC). The liquid was also analyzed using GPC to assess molecular weight changes of the reaction products that may have occurred as a result of biodegradation. A sample of soil from each radio-labeled culture was dried for a moisture analysis and representative samples were oxidized. The resulting $^{14}CO_2$ was collected in Carbosorb® and counted using LSC to complete the activity balance.

Anaerobic Treatment

Range soil (10 g) was placed in sterile serum bottles and transferred to the anaerobic glove bag, where 90 mL of the appropriate transformation product solutions were added to serve as the cultures' medium. The serum bottles were capped and sealed under anaerobic conditions and incubated in the dark at room temperature for 28 days on a shaker set at 200 rpm. The anaerobic cultures were sparged for gas collection only at the end of the incubation period. The cultures were acidified by injection of 0.5 mL of phosphoric acid into each serum bottle. The bottles were stirred and replaced in the dark for a minimum of 24 hr.

After acidification, mixing, and resting, a liquid sample was taken for final explosive analysis and LSC. This sample provided the liquid-phase portion of the activity balance. The gases (carbon dioxide and others) were then trapped in a series of CarboSorb® liquid sorbent-filled serum bottles (50 mL/trap) as shown in Figure 4. Compressed air, CO_2-free, was bubbled slowly through the sample culture. The flow of the carrier gas was slowed by the use of a vacuum trap. Any carbon dioxide that had been in the sample bottle was captured in the first two traps. Other gases present in the sample bottle were forced using the carrier gas through a muffle furnace and heated to 500°C. Combustion oxidized these gases (for example, methane) into carbon dioxide that was collected in a second set of two CarboSorb®-filled serum bottles. A sample was removed from each collection bottle, amended with Permafluor-E®, the CarboSorb® scintillation cocktail, and counted using LSC. Gas-phase recovery was determined by the addition of the carbon dioxide activity and the activity found in the "other gases."

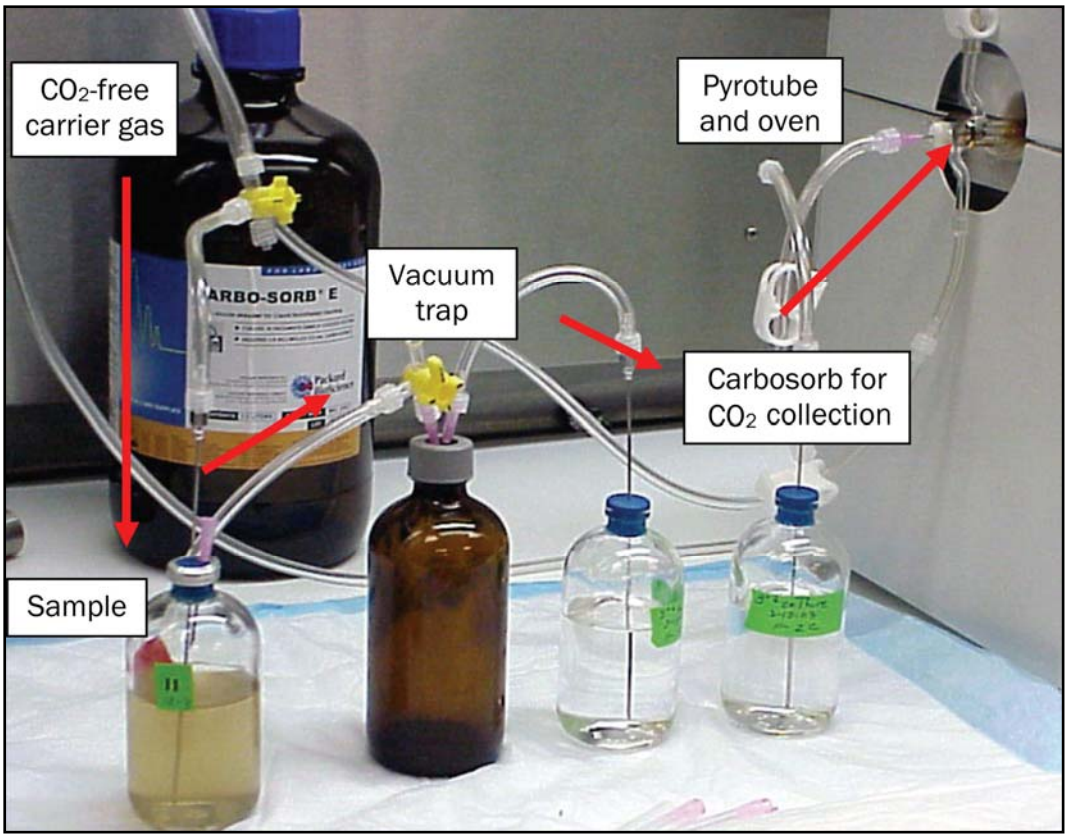


Figure 4. Gas sparging of anaerobic cultures showing the direction of flow of the CO_2-free carrier gas through the sample, vacuum trap, and CO_2 traps into the oven for oxidation of volatile organic acids. Collection of the oxidized samples occurred in Carbosorb traps at the oven outlet (not shown).

After trapping of the gas phase was performed, the serum bottles were opened and the slurries were centrifuged (50 min at 2000 rpm). The liquid was decanted from the centrifuge bottles, and the soil was removed to a scintillation vial. Soil and liquid samples from the unlabeled cultures were analyzed for TOC, and liquid samples were analyzed for anion content. Representative liquid samples from the [^{14}C]-labeled cultures were counted using LSC. The liquid was also analyzed using GPC to assess molecular weight changes of the products of alkaline hydrolysis that may have occurred during incubation. The soil from the [^{14}C]-labeled cultures was combusted using the soil oxidizer, and the resulting $^{14}CO_2$ was collected and analyzed using LSC to complete the activity balance.

4 Results and Discussion

TNT

TOC and Anion Analysis

The cultures incubated without [^{14}C] labels were used to investigate possible changes in the liquid TOC and anion concentrations as a result of interaction with soil. The culture medium that provided the Day 0 values for the incubated cultures was produced by the alkaline hydrolysis of TNT/RDX (Davis et al. 2007). The reason for the increase in TOC during the original alkaline hydrolysis reaction was not determined (Davis et al. 2007).

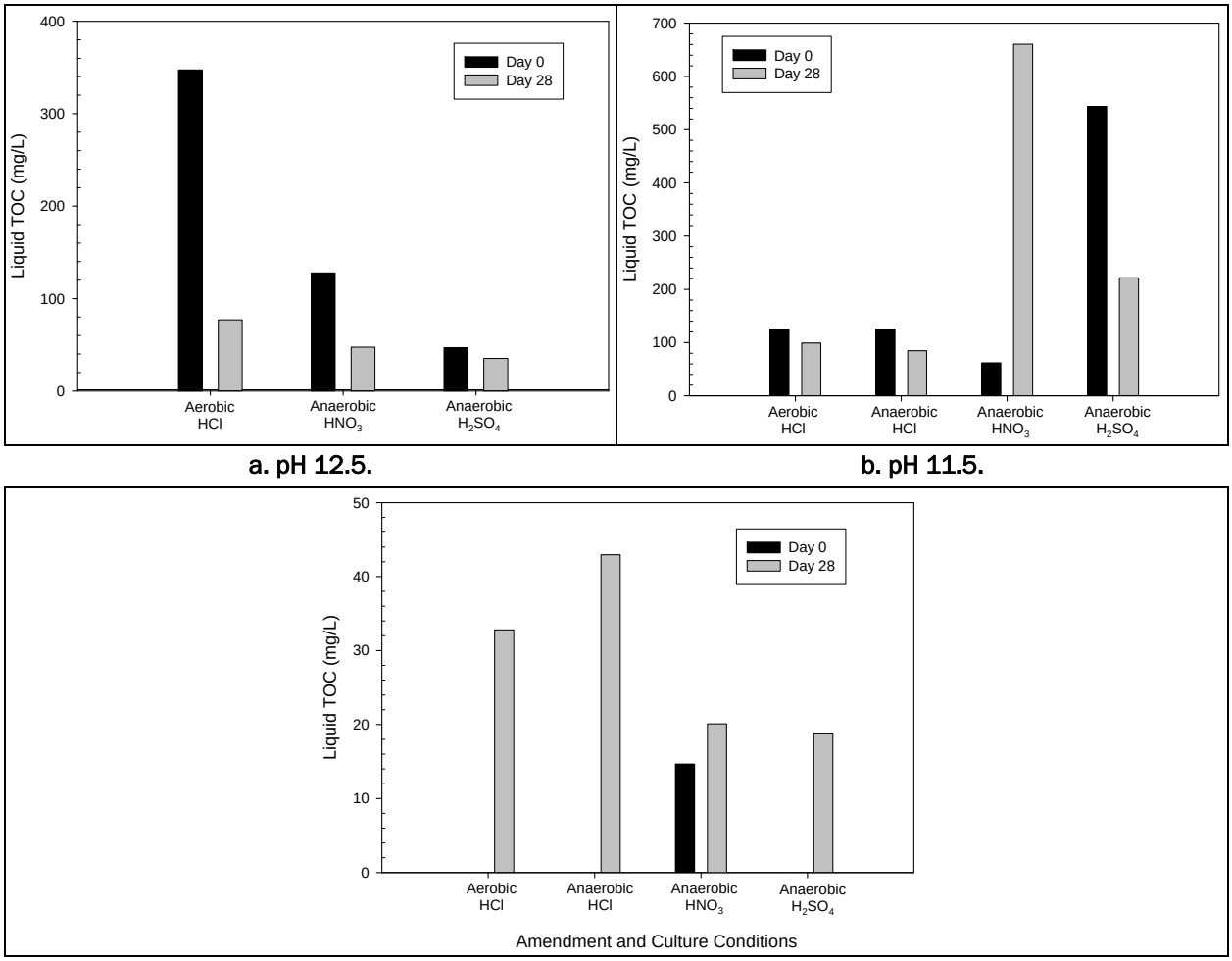
The TOC analysis of the soil from the unlabeled cultures showed no significant change from the original soil TOC of 0.13 percent. The liquid medium from the pH 12.5 reaction mixture had significant decreases in the liquid TOC, as shown in Figure 5a. The hydrogen-amended aerobic cultures showed a decrease in TOC from 350 to 95 mg/L TOC. The liquid TOC values of the nitric- and sulfuric-acid-amended cultures also decreased after anaerobic incubation.

Alkaline hydrolysis of TNT at pH 11.5 (Fig. 6b) did not produce solutions with the highly elevated liquid TOC values seen at pH 12.5, although the TOC values were much higher than the controls. Incubation with soil, under both aerobic and anaerobic conditions, reduced the liquid TOC of the cultures, except in the NO_3-amended media.

We suggest that the decrease in TOC during incubation of both the pH 12.5 and 11.5 treatment mixtures was the result of biological activity of the soil microbial communities. This is supported by mineralization of the [^{14}C]-labeled products described in the activity balance determination.

Alkaline hydrolysis of TNT at pH 10.5 produced solutions with no detectable organic carbon. In this case, aerobic and anaerobic incubation yielded increases in the liquid TOC to 30, 43, 20, and 19 mg/L for the hydrogen-amended aerobic and anaerobic cultures and nitric and sulfuric cultures, respectively (Fig. 6c). No significant difference was found between active and control cultures. The increase in TOC during

incubation supports our contention that there was no (or minimal) biological activity in these cultures. This is supported by minimal mineralization of the [^{14}C]-labeled products as described in the activity balance determination. The increase in TOC is, possibly, the addition of abiotic breakdown products from the treatment mixtures. Complete data are available in Appendix A.



a. pH 12.5.

b. pH 11.5.

c. pH 10.5.

Figure 5. Changes in liquid TOC of active cultures following incubation of the TNT alkaline hydrolysis end products with uncontaminated soil.

Changes in the anion concentration of TNT-derived pH 12.5 reaction mixtures from Day 0 to Day 28 are presented in Table 3. The concentration values for nitrate and sulfate are not included in the table where nitrate and sulfate are present in the amendment solution. Tap water and DDI water were used as controls. Further evidence of biodegradation in each one of the amendments from the pH 12.5

treatment solutions is the consumption of nitrite and the formation of formate.

Table 3. Changes in anion concentration of active cultures following incubation of the TNT alkaline hydrolysis reaction products (pH 12.5) with soil.

Amendment and Culture Conditions	Anion	Concentration (mg/L)	
		Day 0	Day 28
H^+ Aerobic Active (n=1)	Formate	nd	22.87
	Nitrite	17.61	nd
	Nitrate	nd	nd
	Sulfate	nd	24.33
NO_3^- Anaerobic Active (n = 3)	Formate	nd	19.02 ±1.99
	Nitrite	12.65	2.22 ±0.64
	Sulfate	30.80	55.50 ±1.44
SO_4^{2-} Anaerobic Active (n=3)	Formate	nd	21.33 ±6.31
	Nitrite	68.03	4.25*
	Nitrate	19.66	6.47 ±5.07
nd=non-detect * Found in just one sample			

We were unable to obtain reliable IC data from the culture media derived from the pH 11.5 and 10.5 alkaline hydrolysis reactions. The dilutions required for IC analysis yielded results too low for instrument precision (Davis et al. 2007). At these low concentrations, the nitrite/nitrate chromatographic peaks, in particular, were masked by the chloride ion peak.

Molecular Weight Changes

The liquid media of both the aerobic and anaerobic cultures were examined by GPC to assess any molecular weight changes that might have occurred as a result of biodegradation of the reaction products by soil bacteria. The conditions of the original culture media (Day 0 conditions) are outlined in Davis et al. (2007). As shown in that report, none of the treated TNT reaction mixtures contained any parent compound. This was confirmed using GPC. Only the [^{14}C]-labeled lime control, which had been maintained at a neutral pH level, still contained the parent compound and had activity in the parent fraction. GPC analysis showed that the reaction end products were polar (water soluble), in contrast to the parent compound, which is non-polar. No large polymeric compounds were

detected, as the results indicated that the reaction products were the same size or slightly smaller in molecular weight than the parent compound (TNT, 228 mw).

The GPC results for the liquid media following 28 days of incubation with soil are shown in Table 4, Table 5, and Table 6 for pH 12.5, pH 11.5, and pH 10.5-derived media, respectively. The [^{14}C]-labeled lime control of the TNT-derived aerobic and anaerobic cultures (amended with hydrogen) continued to show activity in the parent fraction (Table 4). Two of the active cultures had activity appearing in the fraction immediately preceding the parent fraction, indicating the presence of compounds with slightly larger molecular weights than the parent compound. Some activity was seen in fractions with short retention times, which is where the large polymers reported by other authors (Felt et al. 2002, 2007b, Thorn et al. 2004) would elute. Radioactive concentrations were higher in Control 1 (the soil control), reflecting the absence of the soil and soil bacteria that might react with the products of small molecular weights. Results obtained from Control 2 (the abiotic control) demonstrated both incomplete sterilization and an effect from the presence of soil. Autoclaving is not the most effective way to sterilize soil, but it was selected because of its lack of interference in other tests.

To summarize the apparent differences between:

- The aerobic and anaerobic incubations:
  - All of the aerobic cultures had [^{14}C] activity in the fraction immediately preceding the parent fraction, indicating the presence of compounds with slightly larger molecular weights than the parent compound,
  - All of the anaerobic cultures had [^{14}C] activity eluted in fractions later than the parent compound, indicative of compounds with slightly smaller molecular weights;
- The active and control cultures of the hydrogen amendments:
  - Aerobic active media had [^{14}C] activity in the parent fraction and fraction with slightly larger molecular weights,
  - No [^{14}C]-activity was obtained from the anaerobic, active culture media,
  - Aerobic controls showed [^{14}C] activity in the parent fraction and the fraction with slightly larger molecular weights (similar to the active cultures),

- Anaerobic controls had [^{14}C] activity in the parent fraction and one sample had activity in the fraction with slightly smaller molecular weights.

Table 4. Results of the GPC assessment of the TNT reaction product media (pH 12.5) following aerobic and anaerobic incubation with soil.

	Molecular weights in Daltons (as indicated by counts in fraction retention times)			
	Aerobic cultures	Anaerobic cultures		
Treatment	H^+	H^+	NO_3^-	SO_4^{2-}
Active 1	228	nc	<228	<228
Active 2	228 ≥228	nc	<228	<228
Active 3	≥228	nc	<228	228
Control 1 (no soil)	228 ≥228	228 <228	<228	<228
Control 2 (killed soil)	228 ≥228	<228	<228	<228
Lime Control	228	228	nc	nc
nc = no counts detected				

GPC analysis of the pH 11.5 reaction mixtures showed no change in the solution from the Day 0 conditions, as shown in Table 5.

Table 5. Results of the GPC assessment of the TNT reaction product media (pH 11.5) following aerobic and anaerobic incubation with soil.

	Molecular weights in Daltons (as indicated by counts in fraction retention times)							
	Aerobic cultures		Anaerobic cultures					
	H^+		H^+		NO_3^-		SO_4^{2-}	
Treatment	Water phase	Organic phase	Water phase	Organic phase	Water phase	Organic phase	Water phase	Organic phase
Active 1	<106	nc	<106	nc	<106	nc	<106	nc
Active 2	<106	nc	<106	nc	<106	nc	<106	nc
Active 3	<106	nc	<106	nc	<106	nc	<106	nc
Abiotic	<106	nc	<106	nc	<106	nc	<106	nc
Control	<106	nc	<106	nc	<106	nc	<106	nc
Lime Control		228		228				
nc = no counts detected								

To summarize:

- All [^{14}C]-activity for active and control cultures was found in the water phase, indicative of polar products,
- The [^{14}C]-activity in both the aerobic and anaerobic lime (treatment) controls (untreated TNT) remained concentrated in the organic phase (non-polar),
- The biodegradation end products were found in fractions indicative of very small molecular weight compounds, <106.

GPC analysis of the pH 10.5 reaction also indicated that the reaction end products had not changed in molecular weight (size) during soil incubation. The GPC results from the TNT (pH 10.5) aerobic and anaerobic cultures are shown in Table 6.

Table 6. Results of the GPC assessment of the TNT reaction product media (pH 10.5) following aerobic and anaerobic incubation with soil (hydrogen amendment).

	Molecular weights in Daltons (as indicated by counts in fraction retention times)							
	Aerobic cultures		Anaerobic cultures					
	H^+		H^+		NO_3^-		SO_4^{2-}	
Treatment	Water phase	Organic phase	Water phase	Organic phase	Water phase	Organic phase	Water phase	Organic phase
Active 1	<106	nc	<106 228	nc	<228	nc	<228	nc
Active 2	<106	nc	<106	nc	<228	nc	<228	nc
Active 3	<106	nc	<106	nc	<228	nc	228	nc
Soil Control	<106	nc	<106	nc	<228	nc	<228	nc
Abiotic Control	<106	nc	<106	nc	<228	nc	<228	nc
Lime Control		228		228				
nc = no counts detected								

To summarize:

- The aerobic and anaerobic hydrogen-amended cultures had the majority of [^{14}C] activity in the fraction indicative of a water-soluble compound with a molecular weight <106.
- The anaerobic NO_3- and SO_4- amended cultures had the majority of [^{14}C] activity in the fraction indicative of a water-soluble compound with a molecular weight <228.

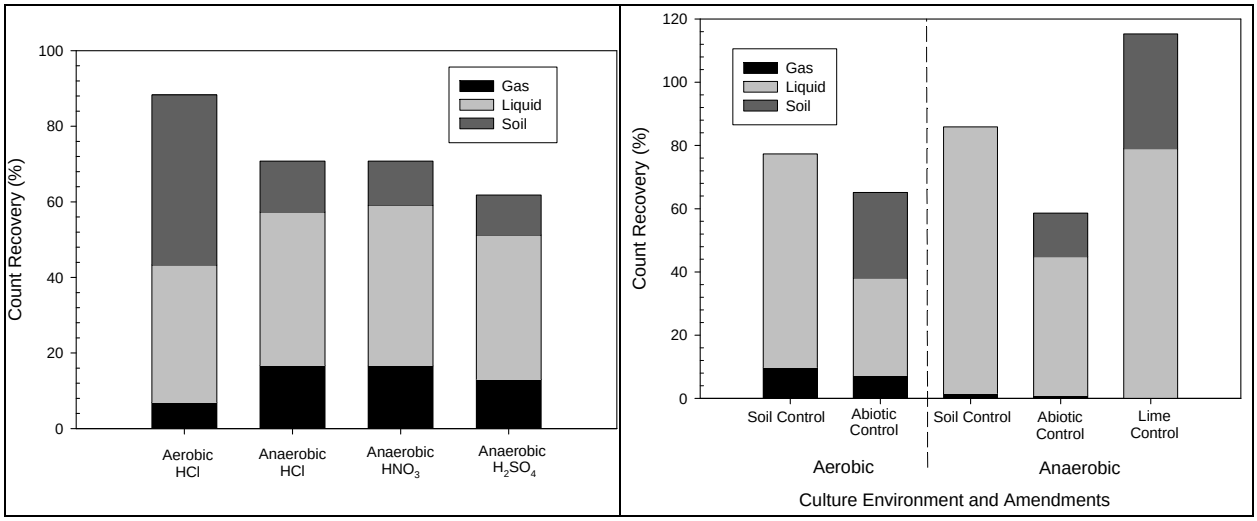
Activity Balance

Total recovery of the [^{14}C]-labeled TNT from the reaction solutions incubated with soil is detailed in Table 7 (pH 12.5), Table 8 (pH 11.5), and Table 9 (pH 10.5) and illustrated in Figure 6 (pH 12.5), Figure 7 (pH 12.5), and Figure 8 (pH 12.5). Liquid-phase recovery was determined from the final total activity of the sample taken immediately after acidification. Acidification (pH≤2) stopped biological activity and released any carbonates that were formed during incubation. Methane, and/or other volatile organic compounds, produced by the culture would be unchanged by acidification. Possible candidates for the non-CO_2 molecules, suggested by the IC analysis of the unlabeled samples, are formate and other small organic acids. Combining the radioactivity concentrations derived from the KOH traps during incubation and after acidification for the aerobic cultures, or from the CarboSorb® traps for the anaerobic cultures, determined gas-phase recovery. Solid-phase recovery was calculated from the oxidation and recovery of counts from the soil matrix. Complete activity balance data are available in Appendix A, Tables 18–32.

The activity balance obtained from the active cultures incubated with the end products of the pH 12.5 alkaline hydrolysis of TNT is shown in Figure 6a. The total recovery averaged 71 percent of the [^{14}C]-labeled TNT in the aerobic and anaerobic active samples, H^+ and NO_3^- amended. In the SO_4^{2-}-amended, anaerobic active samples, the total recovery was 62 percent of the [^{14}C]-labeled TNT. Thirty-six percent of the activity was held in the soil of the aerobic cultures, compared to less than 20 percent of the activity in the anaerobic cultures. In the anaerobic cultures, the active cultures all showed the same trend towards [^{14}C] label being held in the liquid fraction. Mineralization accounted for approximately 17 percent of the [^{14}C] label in the gas phase for the hydrogen and NO_3^- anaerobic amendments. Of this total, less than 1 percent of the counts recovered (aerobic and anaerobic) were from gases other than CO_2 (Appendix A). Results from the TNT control cultures (Figure 6b) indicated negligible CO_2 production. The majority of the [^{14}C] label in the untreated TNT system remained in the liquid fraction.

Table 7. Comparison of activity balances of aerobic and anaerobic cultures incubated with TNT alkaline hydrolysis end products at pH 12.5.

Sample		Label Recovery (%)				Active	
		CO_2/other	Liquid	Soil	Total	Avg	stdev
Aerobic							
Hydrogen	Active 1	7.47	36.83	26.76	71.06	70.50	35.29
	Active 2	6.42	35.83	63.27	105.51		
	Active 3	4.16	14.19	16.57	34.93		
No soil		9.78	67.55	0.00	77.33		
Killed soil		7.18	30.91	27.03	65.12		
Anaerobic							
Hydrogen	Active 1	18.18	39.14	17.47	74.79	70.77	3.50
	Active 2	14.39	40.58	14.15	69.12		
	Active 3	17.49	42.14	8.77	68.39		
No soil		1.59	84.28	0.00	85.86		
Killed soil		0.89	43.95	13.73	58.57		
Nitrogen	Active 1	18.25	42.38	11.79	72.42	70.75	1.47
	Active 2	14.98	44.38	10.82	70.17		
	Active 3	16.56	40.87	12.24	69.67		
No soil		12.43	73.57	0.00	85.99		
Killed soil		13.63	46.90	12.76	73.28		
Sulfur	Active 1	12.03	37.29	8.95	58.27	61.79	4.97
	Active 2	14.01	39.12	12.17	65.30		
No soil		13.89	81.65	0.00	95.54		
Killed soil		0.04	43.03	8.26	51.34		
Lime Control		0.10	78.87	36.29	115.26		



a. Aerobic and anaerobic active cultures (avg).

b. Aerobic and anaerobic control cultures (hydrogen amendment).

Figure 6. Activity balance of TNT following alkaline hydrolysis at pH 12.5 and incubation with soil.

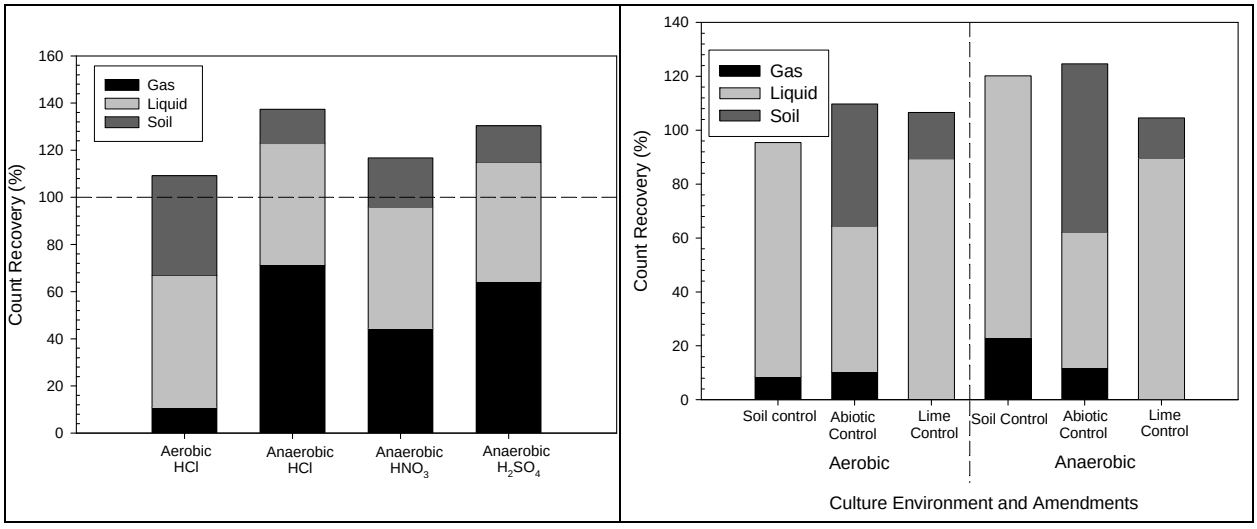
The activity balance obtained from cultures incubated with the end products of the pH 11.5 alkaline hydrolysis of TNT is shown in Figure 7 and detailed in Table 8. Recovery was roughly 100 percent of the label in both the aerobic and anaerobic active cultures. Mineralization accounted for 16 percent (avg.) of the activity recovery in the aerobic active cultures. Of this total, less than 1 percent of the counts recovered were from gases other than CO_2 (Appendix A). In the aerobic cultures, 5 percent of the total activity recovered in the gas phase (5.03±0.217) was from CO_2 released from solution by acidification of the culture media. In the aerobic cultures, the remaining activity was divided between the liquid (56.08 percent) and solid phase (42.20 percent).

The anaerobic hydrogen-amended cultures showed a slightly higher retention of radiocarbon activity on the solid phase (71 percent, avg.), compared to 51 percent in liquid phase (avg.). The trend was similar in the sulfuric-acid-amended cultures, with 64.01 percent (avg.) in the solid and 50.90 percent (avg.) in the liquid phase. The nitric-acid-amended cultures had similar recovery in the liquid phase (51 percent, avg.) but lower recovery in the solid phase (44 percent, avg.). These cultures also had the highest activity recovery in the gas phase. Greater biological activity in the soil may have contributed to the transfer of radio-labeled carbon from the solid to the gaseous phase.

Both the aerobic and anaerobic soil controls showed evidence of CO_2 production (Fig. 8b). Gas production may indicate microbial contamination or evolution of other volatile end products that were produced during incubation. The abiotic control data, when compared to the soil control results, indicated both a soil effect and potentially incomplete sterilization of the soil microbiological communities. The lime control cultures, cultures that had been maintained at neutral pH levels, showed no gas production. The majority of the [^{14}C] label in the untreated TNT system (89.5 percent) remained in the liquid fraction. Both the lack of gas production and the fact that the [^{14}C] label remained in the liquid suggest that no biodegradation had occurred. This was confirmed by HPLC analysis.

Table 8. Comparison of activity balances of aerobic and anaerobic cultures incubated with TNT alkaline hydrolysis end products at pH 11.5.

Sample		Label Recovery (%)				Active	
		CO_2/Other	Liquid	Soil	Total	Avg	St dev
Aerobic							
Hydrogen	Active 1	15.70	56.42	41.23	113.35	114.31	1.37
	Active 2	16.38	55.73	43.16	115.28		
no soil		13.51	86.84	0.00	100.35		
killed soil		14.87	54.13	45.37	114.37		
Lime control		0.56	88.97	17.18	106.71		
Anaerobic							
Hydrogen	Active 1	11.17	53.07	68.23	132.48	137.40	4.28
	Active 2	18.46	48.74	72.34	139.54		
	Active 3	13.99	52.91	73.29	140.19		
no soil		23.06	97.12	0.00	120.17		
killed soil		11.62	50.48	62.51	124.62		
Nitrogen	Active 1	17.27	50.71	43.59	111.58	116.72	7.28
	Active 2	24.60	51.96	45.30	121.86		
no soil		4.64	94.28	0.00	98.91		
killed soil		15.95	50.56	47.88	114.39		
Sulfur	Active 1	11.22	47.54	65.06	123.83	130.42	9.32
	Active 2	19.69	54.26	63.05	137.00		
no soil		7.56	96.37	0.00	103.92		
killed soil		9.86	53.80	59.78	123.45		
Lime Control		0.15	89.52	14.90	104.56		



a. Aerobic and anaerobic active cultures (avg.). b. Aerobic and anaerobic control cultures (hydrogen amendment).

Figure 7. Activity balance of TNT following alkaline hydrolysis at pH 11.5 and incubation with soil.

The activity balance obtained from cultures incubated with the end products of the pH 10.5 alkaline hydrolysis of TNT is shown in Figure 8 and detailed in Table 9. [^{14}C] label recovery from the aerobic active cultures was poor, averaging only 52 percent. [^{14}C] label recovery from the anaerobic active cultures was much better, ranging from 91 to 118 percent. Mineralization accounted for only 4.7 percent in the aerobic active cultures. Of this total, less than 1 percent of the counts recovered were from gases other than CO_2 (Appendix A). In the aerobic cultures, 2.5 percent of the total activity recovered in the gas phase was from CO_2 that had been trapped as carbonates during incubation (released during acidification). The remaining aerobic counts were divided between the liquid (25.4 percent) and solid (21.5 percent) phases. Both of the aerobic soil controls showed evidence of CO_2 production (Figure 8b). Gas production may indicate microbial contamination or evolution of other volatile end products that were produced during incubation.

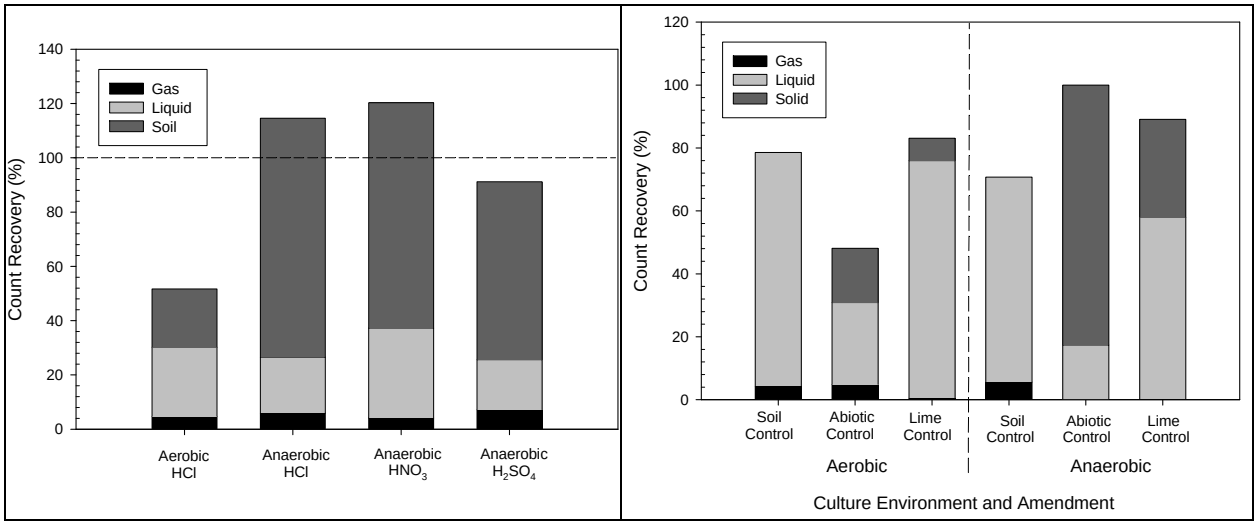
Mineralization accounted for <10 percent of the activity recovery in the anaerobic active cultures. The majority of the activity was held in the solid phase (66–89 percent, avg.). The soil control of each amended anaerobic culture also showed evidence of gas production, as did the abiotic control of the nitric acid amendment. In these cases also, gas production may indicate microbial contamination or the evolution of other volatile end products that were produced during incubation.

Neither the aerobic nor the anaerobic lime control cultures (untreated TNT) showed gas production. [^{14}C]-label recovery was 83 percent and 89 percent for the aerobic and anaerobic cultures, respectively. The majority of the [^{14}C] label in the untreated TNT system remained in the liquid fraction (was not biodegraded).

Unlike the data from pH 12.5 and 11.5 microcosms, the data from pH 10.5 microcosms indicated minimal mineralization of the [^{14}C]-labeled TNT alkaline hydrolysis products under either aerobic or anaerobic conditions. This is despite the fact that the majority of the reaction products was held by soil components and was, presumably, bioavailable.

Table 9. Comparison of activity balances of aerobic and anaerobic cultures incubated with TNT alkaline hydrolysis end products at pH 10.5.

Sample		% Recovery				Active	
		CO_2/Other	Liquid	Soil	Total	Avg	St dev
Aerobic							
Hydrogen	Active 1	4.48	24.25	18.89	47.61	51.67	5.45
	Active 2	5.04	25.95	26.88	57.86		
	Active 3	4.69	26.10	18.74	49.54		
no soil		4.21	74.34	0.00	78.55		
killed soil		4.83	26.05	17.22	48.10		
Lime control		0.47	75.52	7.09	83.08		
Anaerobic							
Hydrogen	Active 1	7.22	18.75	80.25	106.23	116.22	14.13
	Active 2	7.49	20.42	98.31	126.22		
no soil		5.61	65.14	0.00	70.74		
killed soil		0.02	17.37	82.62	100.01		
Nitrogen	Active 1	4.14	34.28	76.35	114.76	117.75	4.23
	Active 2	8.29	33.71	78.73	120.74		
no soil		6.97	69.82	0.00	76.79		
killed soil		6.29	27.74	70.16	104.19		
Sulfur	Active 1	6.07	17.84	72.07	95.98	91.20	4.38
	Active 2	7.62	18.45	64.16	90.23		
	Active 3	7.82	18.90	60.66	87.39		
no soil		1.97	33.76	0.00	35.74		
killed soil		0.01	17.39	65.36	82.75		
Lime Control		0.01	57.91	31.17	89.09		



a. Aerobic and anaerobic active cultures (avg).

b. Aerobic and anaerobic control cultures (hydrogen amendment).

Figure 8. Activity balance of TNT following alkaline hydrolysis at pH 10.5 and incubation with soil.

TNT Summary and Conclusions

There was evidence that the final reaction products of the alkaline hydrolysis of TNT could potentially biodegrade when incubated with a natural soil under both aerobic and anaerobic conditions. Biodegradation potential was gauged by the amount of [^{14}C]-- CO_2 produced during incubation. The fraction of the original tracer that had been converted to [^{14}C]-- CO_2 is said to be mineralized because it is no longer part of an organic molecule. The amounts of tracer that had been mineralized during anaerobic incubations were similar using pH 12.5 and 11.5 products (17 and 16 percent respectively), but lower using pH 10.5 products (<10 percent). The activity balance for pH 10.5 anaerobic incubations was near 100%, which validated the mineralization data. This result implies that more of the products formed at higher pH values further degrade after incubation with inherent bacteria than those formed at lower pH values.

There was other evidence that the biodegradation potential of the TNT hydrolysis products may depend on the initial reaction pH value. Along with the mineralization data, the fraction of [^{14}C] activity found in the solid phase following anaerobic incubation of TNT cultures increased with pH. This result may imply that the products had slightly different solubility and sorption properties, indicating that the products were different compounds. These data, therefore, add further evidence that more of the products formed at higher pH values were transformed after anaerobic

incubation with inherent soil bacteria than those formed at lower pH values.

Less mineralization was realized following aerobic incubation using products from reactions held at pH 12.5 and 10.5 (7 percent and 4.7 percent, respectively), although aerobic incubation using products formed at pH 11.5 was similar to the anaerobic concentration (16 percent). The activity balances for pH 12.5 and 10.5 were not as good (70 and 52 percent, respectively) as the balance for pH 11.5 (100 percent), which may imply some gases were lost to the atmosphere during the study. Therefore, no conclusions were drawn from the aerobic mineralization result because they could not be supported by a closed activity balance.

There were no major differences among the results of TNT reaction products that had been neutralized using the three different acids. Electron acceptor did not therefore appear to have an effect on TNT products' biodegradation. This would support the original hypothesis that the reaction mechanism was not dependent on the acid used to adjust the pH of the solutions.

Overall, the TNT sample results from pH 12.5 were similar to those at pH 11.5, and both were different than pH 10.5 results. This may indicate a pathway shift at lower pH values or incomplete hydrolysis at pH 10.5. There was a basic difference in the Day 0 liquid medium that reacted at pH 10.5 (Davis et al. 2007). A final reaction product, which indicated complete hydrolysis, was evidenced by a chromatographic feature at retention time 3.2 minutes at 330 nm following reactions at pH values 12.5 and 11.5. This final reaction product was not observed following the reaction at pH 10.5, as evidenced by the lack of this chromatographic feature. The reaction at pH 10.5 was stopped after 13 weeks in an effort to complete the study within a fiscal year rather than waiting for this end point.

The other experimental data from this study yielded similar results for the products from all three pH values. All GPC data implied very little change in the size of the hydrolysis products after incubation with the soil. No large biological compounds had been formed, as evidenced by the small size of the products. The actual products may have been chemically or physically altered during incubation, but their size remained relatively unchanged. TOC values were unchanged in the soil after incubation using

products from all three pH levels, and there were insufficient anion data to draw any conclusions.

RDX

TOC and Anion Analysis

The TOC analysis of the soil from the unlabeled cultures, following incubation with the hydrolysis end products, showed no significant change from the original soil TOC of 0.13 percent. The liquid media from the pH 12.5 reaction mixture, both aerobic and anaerobic incubations, had significant decreases in the liquid TOC, as shown in Figure 9a. The hydrogen-amended cultures decreased from 700 mg/L at Day 0 to 20 and 63 mg/L, aerobic and anaerobic incubations, respectively (Appendix B). The decrease was even more pronounced than that seen with the TNT-derived reaction solutions. The aerobic abiotic control had a liquid TOC value of 52 mg/L, indicating the probable involvement of soil bacteria in the TOC decrease. The pH 12.5 reaction solutions amended with nitric and sulfuric acid showed similar decreases in the liquid TOC (Figure 9a) after anaerobic incubation. We suggest that the decrease in TOC during incubation of the pH 12.5 treatment mixtures is the result of biological activity of the soil microbial communities. This is supported by mineralization of the labeled products described in the activity balance determination.

Alkaline hydrolysis of RDX at pH 11.5 did not produce solutions with the artificially elevated liquid TOC values seen at pH 12.5. Incubation with soil, under both aerobic and anaerobic conditions, increased the liquid TOC of these cultures, except in the sulfuric-acid-amended media. The increase was greatest in the hydrogen-amended anaerobic cultures (Figure 9b and Appendix B). The increase in TOC during incubation may imply that there was minimal biological activity in the HCl- and HNO3-amended cultures. However, this conclusion is not supported by the mineralization data described in the activity balance determination.

Alkaline hydrolysis of RDX at pH 10.5 produced solutions with no detectable organic carbon, except in the case of the nitric acid amendment. Figure 9c shows an increase in TOC concentration produced by active cultures, incubated either aerobically or anaerobically, from the pH 10.5 reaction solutions. All amendments yielded very similar TOC concentrations: 13, 11, 13, and 12 mg/L for the hydrogen-amended aerobic

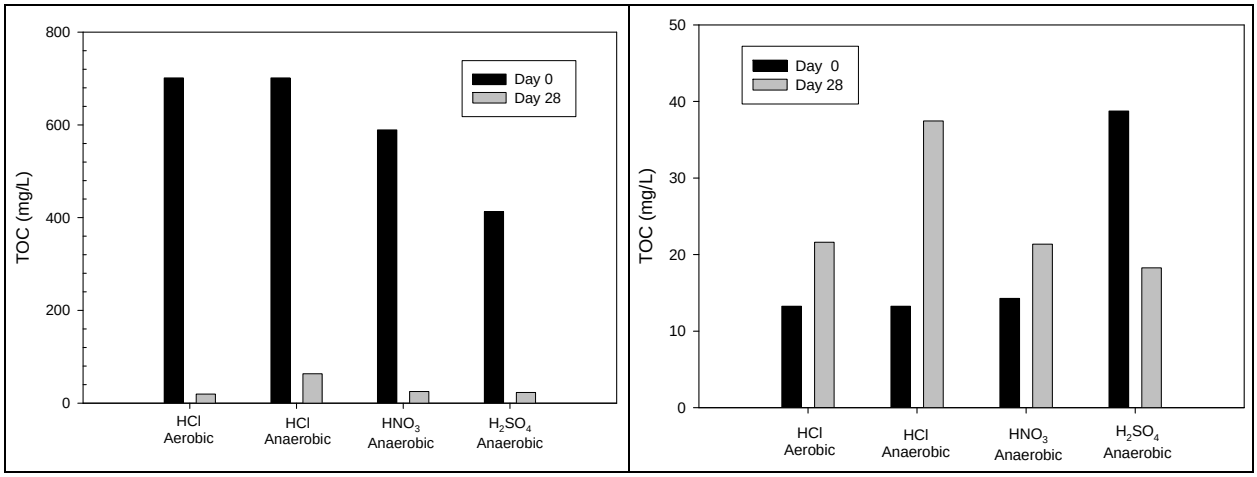
and anaerobic cultures, nitric and sulfuric cultures, respectively (Appendix B). The increase in TOC during incubation supports our contention that there was no (or minimal) biological activity in these cultures. This is supported by the absence of mineralization of the labeled products described in the activity balance determination and by the fact that there appears to be an effect from the absence of soil in the culture (except for the nitric-acid-amended cultures). The TOC was much less in the soil control.

Changes in the anion concentration of the RDX-derived reaction mixtures from initial (Day 0) to Day 28 for all three pH treatments are presented in Table 10. Nitrate and sulfate concentrations were not included in the table when nitric or sulfuric acid was used to neutralize the solution. Formate was present at Day 0 of each amendment of the pH 12.5 cultures and then increased during incubation. Formate was not detectable in any of the other Day 0 solutions and generally did not increase during incubation. Nitrite was present in each reaction mixture at Day 0 and decreased in each amendment analyzed. This provides further evidence of biodegradation activity in the RDX-derived reaction solutions.

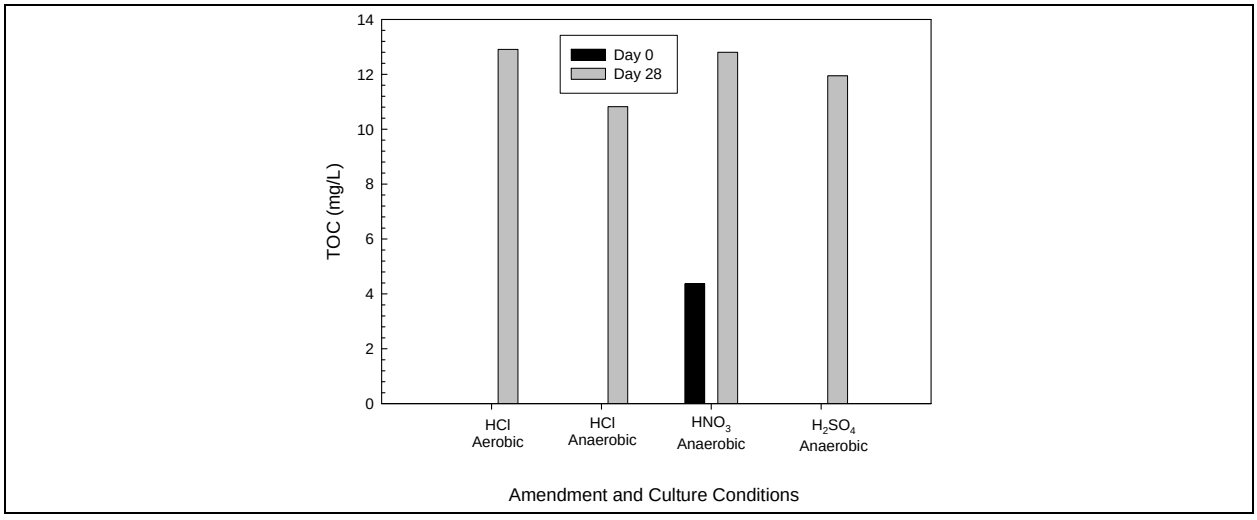
The U.S. EPA Drinking Water Standards and Health Advisories (U.S. Environmental Protection Agency 2004) list the acceptable levels of nitrite and nitrate at 1 and 10 mg/L, respectively. Before incubation (Day 0), the RDX reaction mixtures exceeded nitrite levels in all amendments of the pH 12.5- and 11.5-derived solutions and the nitric and sulfuric acid amendments of the pH 10.5-derived solutions. After both aerobic and anaerobic incubation, those concentrations were reduced to acceptable (or non-detect) levels, except in the case of the nitric acid amendment (nitrite = 4.37 mg/L). The EPA Drinking Water Standards for nitrate were exceeded at Day 0 only in the pH 12.5-derived reaction mixtures amended with HCl. Following aerobic incubation, these concentrations became non-detectable. The sulfuric acid amendment, however, showed an increase in nitrate after incubation with soil. Sulfate increased in each amendment at each pH that was analyzed.

We were unable to obtain reliable IC data for nitrate from the culture medium derived from the pH 10.5 alkaline hydrolysis reactions. The dilutions required for analysis yielded results too low for instrument precision. At these low concentrations, the nitrite/nitrate chromatographic features were masked by other ions.

The products of the alkaline hydrolysis of RDX appear to be environmentally benign, particularly for the treatments conducted at the higher pH values. The decrease in TOC and nitrite concentrations following incubation with soil are all positive indicators for the occurrence of biological activity and support field deployment of this technology.



a. pH 12.5. b. pH 11.5.



c. pH 10.5.

Figure 9. Changes in liquid TOC following incubation of the RDX alkaline hydrolysis end products with uncontaminated soil.

Table 10. Change in anion concentrations (mg/L) following incubation of the RDX alkaline hydrolysis end products with uncontaminated soil.

AmendmentCulture Conditions	Anion	pH 12.5		pH 11.5		pH 10.5	
		Day 0	Day 28	Day 0	Day 28	Day 0	Day 28
H^+ Aerobic Active	Formate	9.92	14.89±1.06	nd	nd	nd	0.43[a]
	Nitrite	5.42	nd	1.26	nd	0.87	0.47[a]
	Nitrate	10.73	nd	0.39	nd	0.38	interference
	Sulfate	2.51	23.76±0.82	0.76	17.44 ±0.48	0.23	11.40± 1.09
H^+ Anaerobic Active	Formate	9.92	nd	nd	nd	nd	nd
	Nitrite	5.42	nd	1.26	nd	0.87	nd
	Nitrate	10.73	nd	0.39	nd	0.38	interference
	Sulfate	2.51	nd	0.76	17.06 ±0.07	0.23	17.34 ±2.18
NO_3^- Anaerobic Active	Formate	10.74	18.41±2.51	nd	nd	nd	nd
	Nitrite	7.20	4.37±0.21	4.57	1.25 ±0.1	3.77	1.03[a]
	Sulfate	6.34	20.76±1.32	3.83	16.25± 0.37	0.22	interference
SO_4^{2-} Anaerobic Active	Formate	5.76	15.78	nd	nd	nd	1.17 ±0.39
	Nitrite	4.68	nd	3.97	nd	4.54	0.75 ±0.05
	Nitrate	4.21	22.25	3.32	3.25 ±0.13[b]	0.43	3.56 ±0.15

[a] The anion was found in only 1 of 3 samples
[b] The anion was found in 2 of the 3 samples
nd=non-detect

Molecular Weight Changes

The liquid media of both the aerobic and anaerobic cultures were examined using GPC to assess any molecular weight changes that might have occurred as a result of biodegradation of the reaction products by soil bacteria. The conditions of the original culture media are outlined in Davis et al. (2007). The GPC results following 28 days of aerobic and anaerobic incubation with soil are shown in Table 11. The majority of the activity of the pH 12.5-derived solutions were found in the same molecular weight fraction as the parent explosive (RDX, mw 222) and in the fraction that contained slightly smaller molecular weight compounds than the parent. The [^{14}C] activity concentrations in the active cultures were low, indicating that the products were not held in the liquid phase.

As with TNT, the soil control had [^{14}C] activity in the same fractions as the active cultures but at a much higher concentration. The high amount of [^{14}C] activity in the soil control reflects the fact that there was no soil, and therefore no soil bacteria, to react with the small-molecular-weight products. The abiotic control had [^{14}C] activity concentrations between the

active cultures and soil control, which demonstrates both an insufficient killing of soil bacteria and an effect from the presence of the soil. Unlike the aerobic TNT cultures that had a trailing of counts preceding the major peaks, RDX active cultures demonstrated a trailing of counts following the major count peaks. [^{14}C] activity in this area indicates compounds with smaller molecular weights than the parent explosive.

Table 11. Results of the GPC assessment of the RDX reaction product media (pH 12.5) following aerobic and anaerobic incubation with soil.

	Molecular weights in Daltons (as indicated by counts in fraction retention times)		
	Aerobic cultures	Anaerobic cultures	
Treatment	H^+	H^+	NO_3^-
Active 1	≤222	222	<222
Active 2	≤222	222	Not performed
Active 3	≤222	222	Not performed
Soil Control	≤222	≤222	≥222
Abiotic Control	≤222	≥222 <222	≥222

The results from the RDX anaerobic cultures (Table 11) demonstrated some differences from the aerobic cultures, primarily in [^{14}C]-activity concentration. The active anaerobic cultures amended with hydrogen and nitrogen had low [^{14}C] activities. The soil control had much higher [^{14}C]-activity concentrations in the parent fraction as well as later fractions. The abiotic control had high [^{14}C]-activity concentrations in an extended range of fractions. The results from the nitrogen control cultures were similar to those of the aerobic cultures.

The GPC results following 28 days of aerobic and anaerobic incubation of pH 11.5 RDX-derived hydrolysis solutions with soil are shown in Table 12. Unlike the pH 12.5-derived reaction mixtures, no activity was detected in any hydrogen-amended active cultures. All the hydrogen-amended control cultures had [^{14}C] activity in a fraction, indicating very small molecular weight compounds in the water-soluble phase and no [^{14}C] activity in the organic phase.

All nitric-acid-amended reaction solutions had [^{14}C] activity in both the water-soluble and organic-soluble phases. The highest radioactive concentrations were in the water-soluble phase in a fraction containing

compounds with a molecular weight of less than 106 Da. Much lower concentrations occurred in the parent fraction of the organic phase, which could indicate the presence of impurities from the original tracer.

The situation with the sulfuric-acid-amended cultures appears to fall between the other amendments. The majority of the [^{14}C] activity was in the <106 molecular weight fraction, indicating very small compounds. The lime control had [^{14}C] activity in the organic phase, as would be expected of a solution containing RDX.

Table 12. Results of the GPC assessment of the RDX (mw 222) reaction product media (pH 11.5) following aerobic and anaerobic incubation with soil.

Treatment	Molecular weights in Daltons (as indicated by counts in fraction retention times)							
	Aerobic Cultures		Anaerobic cultures					
	H^+		H^+		NO_3^-		SO_4^{2-}	
	Water phase	Organic phase	Water phase	Organic phase	Water phase	Organic phase	Water phase	Organic phase
Active 1	nc	nc	nc	nc	<106	222	nc	nc
Active 2	nc	nc	nc	nc	<106	222	≤106	nc
Active 3	nc	nc	Not performed		<106	222	<106	nc
Soil Control	<106	nc	<106	nc	<106	222	<106	nc
Abiotic Control	<106	nc	<106	nc	<106	222	nc	nc
Lime Control		222		222				
[1]nc = no counts detected								

The GPC results following 28 days of aerobic and anaerobic incubation of pH 10.5 RDX-derived hydrolysis solutions with soil are shown in Table 13. Like the pH 11.5-derived reaction mixtures, no [^{14}C] counts were detected in either the hydrochloric-acid- or nitric-acid-amended active cultures in either the water-soluble or organic phases. Only the sulfuric-acid-amended samples showed [^{14}C] activity in the water-soluble, very small molecular weight fraction. No [^{14}C] activity was seen in the samples from the aerobic soil controls, and [^{14}C] activity observed in the anaerobic soil controls was in a fraction that represented very small molecular weight, water-soluble compounds.

Table 13. Results of the GPC assessment of the RDX (222 mw) reaction product media (pH 10.5) following aerobic and anaerobic incubation with soil.

Treatment	Molecular weights in Daltons (as indicated by counts in fraction retention times)							
	Aerobic Cultures		Anaerobic cultures					
	H^-		H^-		NO_3		SO_4	
	Water phase	Organic phase	Water phase	Organic phase	Water phase	Organic phase	Water phase	Organic phase
Active 1	nc	nc	nc	nc	nc	nc	<106	222
Active 2	nc	nc	nc	nc	nc	nc	<106	nc
Active 3	nc	nc	nc	nc	<106	nc	<106	nc
Soil Control	nc	nc	<106	nc	<106	nc	<106	nc
Abiotic Control	nc	nc	nc	nc	<106	nc	nc	222
Lime Control		222		222				
[1]nc = no counts detected								

Activity balance

Total recovery of the [^{14}C]-labeled RDX from the reaction solutions incubated with soil is detailed in Table 14 (pH 12.5), Table 15 (pH 11.5), and Table 16 (pH 10.5) and illustrated in Figure 10 (pH 12.5), Figure 11 (pH 11.5), and Figure 12 (pH 10.5). Liquid phase recovery was determined from the final total count of the sample taken immediately after acidification. Combining the radioactivity concentrations derived from the KOH traps during incubation and after acidification for the aerobic cultures, or from the CarboSorb® traps for the anaerobic cultures, determined gas phase recovery. Solid phase recovery was calculated from the oxidation and recovery of counts from the soil matrix. Complete activity balance data are available in Appendix B.

The activity balance obtained from cultures incubated with the end products of the pH 12.5 alkaline hydrolysis of RDX is shown in Figure 10a and detailed in Table 14. Total recovery averaged 71 percent of the [^{14}C] labeled RDX in the aerobic active cultures. Approximately half of the [^{14}C] RDX present in the reaction medium following incubation was mineralized. Of this total, less than one percent of the [^{14}C] counts recovered were from gases other than CO_2 (Appendix B).

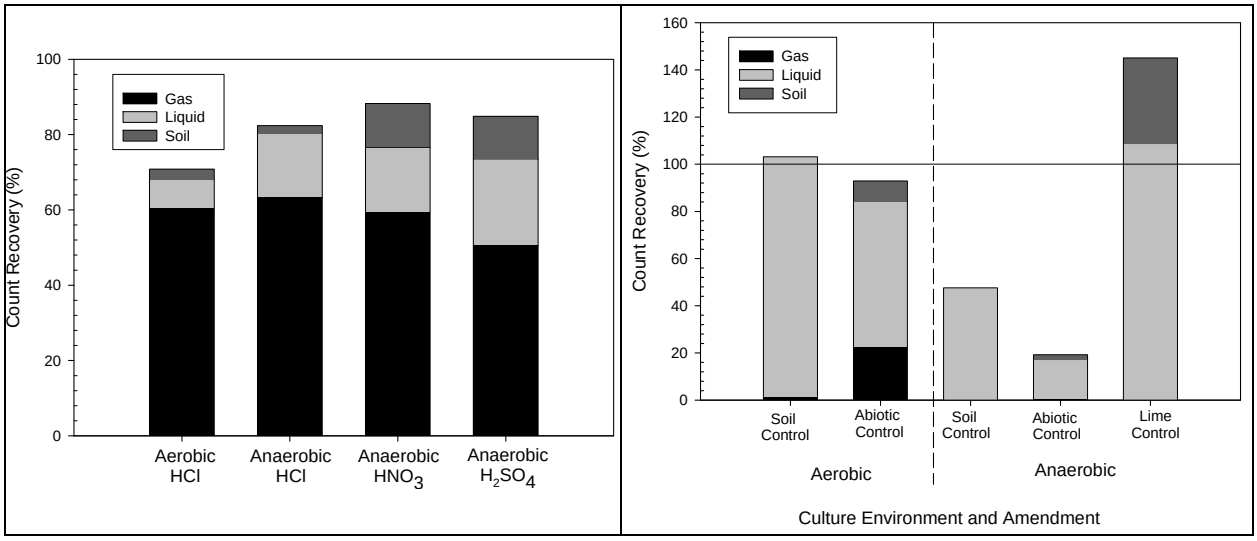
Total recovery averaged 85 percent of the [^{14}C]-labeled RDX in the anaerobic active cultures. Mineralization accounted for 51–63 percent (on average) of the recovered label. As in the aerobic cultures, of this total, less

than one percent of the [^{14}C] counts recovered were from gases other than CO_2 (Appendix B). Of the remaining [^{14}C] counts, a greater proportion was held in the liquid phase rather than in the solid phase.

As observed in the TNT-derived cultures, the abiotic control data indicated both a soil effect and incomplete killing of the soil microbiological communities. In these tests, the abiotic control did show a decrease in biological activity from the active cultures as seen by the decreased gas production. The lime control, however, had an activity balance over 100 percent with no gas production. The RDX was not mineralized in this system.

Table 14. Comparison of activity balances of aerobic and anaerobic cultures incubated with RDX alkaline hydrolysis end products at pH 12.5.

Sample		% Recovery				Active samples	
		CO_2/Other	Liquid	Soil	Total	Avg	St dev
Aerobic							
Hydrogen	Active 1	63.25	7.76	3.35	74.36	70.82	4.99
	Active 2	57.73	7.40	2.16	67.29		
Soil Control		1.52	101.64	0	103.16		
Abiotic control		22.52	61.76	8.61	92.90		
Anaerobic							
Hydrogen	Active 1	63.39	16.91	2.06	82.36	82.36	
Soil Control		0.08	47.54	0.00	47.62		
Abiotic control		0.29	16.91	2.06	19.26		
Nitrogen	Active 1	56.18	16.08	11.79	84.05	88.23	3.75
	Active 2	63.48	17.02	10.82	91.33		
	Active 3	58.73	18.34	12.24	89.30		
Soil Control		1.29	203.64	0.00	204.93		
Abiotic control		15.15	192.55	12.76	220.46		
Sulfur	Active 1	46.80	18.81	8.95	74.57	84.84	19.21
	Active 2	45.10	15.14	12.70	72.95		
	Active 3	60.05	34.78	12.17	107.01		
Soil Control		26.32	79.78	0.00	106.11		
Abiotic control		37.58	79.40	8.26	125.24		
Lime Control		0.20	108.57	36.29	145.06		



a. Aerobic and anaerobic active cultures (avg).

b. Aerobic and anaerobic control cultures (hydrogen amendment).

Figure 10. Activity balance of RDX following alkaline hydrolysis at pH 12.5 and incubation with soil.

The activity balances achieved with the cultures incubated using the end products of RDX treated at pH 11.5 is shown in Figure 11 and detailed in Table 15. The activity balance for samples treated at pH 11.5 was roughly the same as for the samples treated at pH 12.5. The total recovery was 76 percent, approximately half of the [^{14}C] RDX was mineralized (53 percent), and less than one percent of the [^{14}C] counts recovered were from gases other than CO_2 (Appendix B). Unlike the TNT-derived cultures, 27 percent of the gaseous phase recovered [^{14}C] activity in the RDX-derived aerobic cultures was from CO_2 released from solution by acidification of the culture media. Nineteen percent (average) of the recovered [^{14}C] label was held in the liquid phase versus 3.5 percent in the soil.

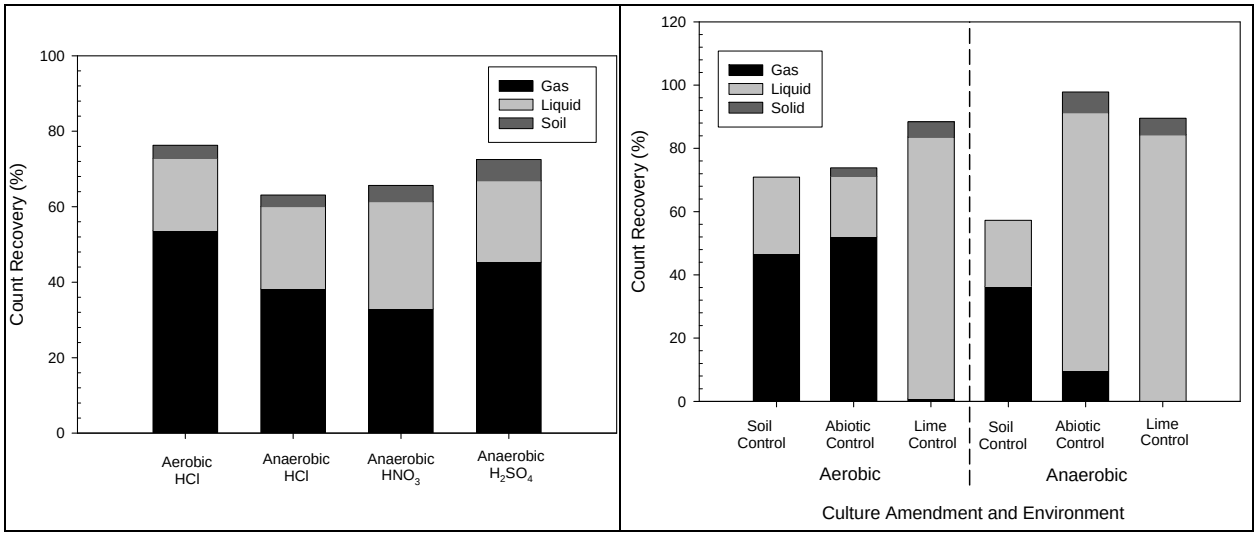
The total recovery averaged 67 percent of the [^{14}C]-labeled RDX in the anaerobic active cultures. Mineralization accounted for 33–45 percent (on average) of the recovered label. As in the aerobic cultures, of this total, less than one percent of the [^{14}C] counts recovered were from gases other than CO_2 (Appendix B). Of the remaining [^{14}C] counts, a greater proportion was held in the liquid phase rather than in the solid phase.

Figure 11b illustrates the activity balances of the aerobic and anaerobic control cultures. The aerobic and anaerobic lime controls had 88 and 90 percent label recoveries, respectively. There was no evidence of [^{14}C]

label in the gas phase (the RDX was not mineralized), and the majority of [^{14}C] counts were held in the liquid phase. All of the soil controls, except the sulfuric-acid-amended soil control, showed gas production. This may indicate microbial contamination or evolution of other volatile end products that were produced during incubation. The abiotic control data also indicated both a soil effect and potentially incomplete sterilization of the soil microbiological communities.

Table 15. Comparison of activity balances of aerobic and anaerobic cultures incubated with RDX alkaline hydrolysis end products at pH 11.5.

Sample		% Recovery				Active samples	
		CO_2/Other	Liquid	Soil	Total	avg	stdev
Aerobic							
Hydrogen	Active 1	52.91	18.70	2.69	74.30	76.28	1.95
	Active 2	54.37	18.90	3.09	76.36		
	Active 3	53.66	19.80	4.73	78.19		
Soil Control		46.69	24.24	0.00	70.93		
Abiotic Control		52.07	19.08	2.71	73.86		
Lime control		0.77	82.84	4.79	88.41		
Anaerobic							
Hydrogen	Active 1	38.19	21.82	3.07	63.07	63.07	
Soil Control		36.37	20.87	0.00	57.24		
Abiotic Control		3.29	20.10	2.27	25.66		
Nitrogen	Active 1	32.89	28.46	4.26	65.61	65.61	
Soil Control		43.46	39.57	0.00	83.03		
Abiotic Control		9.60	81.72	6.47	97.79		
Sulfur	Active 1	46.77	20.71	6.14	73.62	72.50	1.58
	Active 3	43.83	22.48	5.08	71.38		
Soil Control		0.63	53.31	0.00	53.94		
Abiotic Control		25.63	22.86	5.35	53.84		
Lime Control		0.13	84.18	5.23	89.53		



a. Aerobic and anaerobic active cultures (average).

b. Aerobic and anaerobic control cultures (hydrogen amendment).

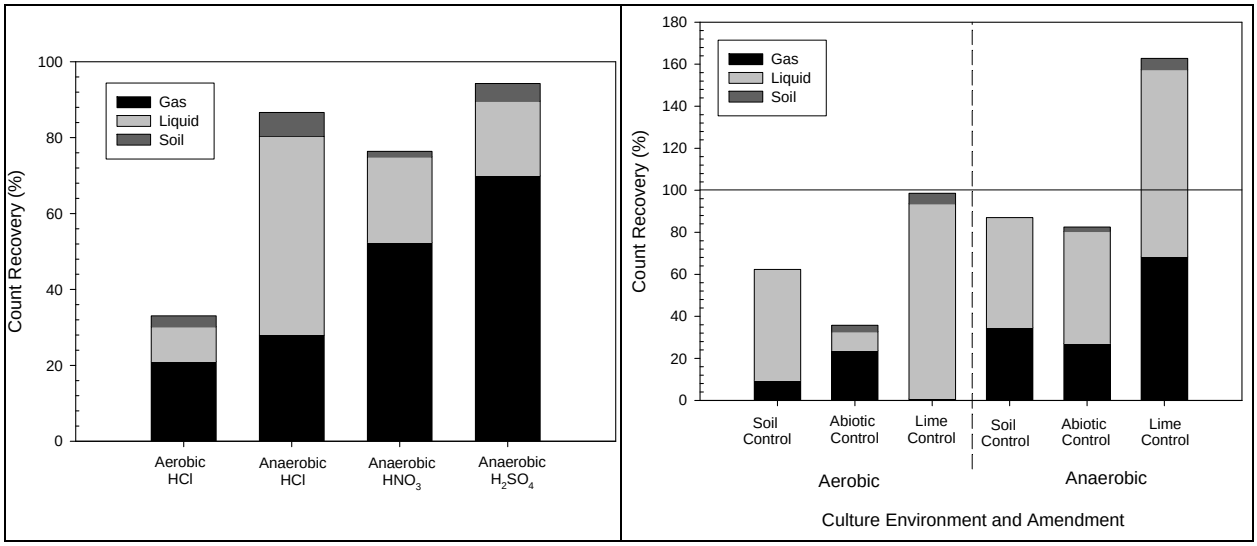
Figure 11. Activity balance of RDX following alkaline hydrolysis at pH 11.5 and incubation with soil.

The activity balance obtained from cultures incubated with the end products of the pH 10.5 alkaline hydrolysis of RDX was much less than for the other two pH values studied, as shown in Figure 12 and detailed in Table 16. Total recovery of the [^{14}C]-labeled RDX averaged only 33 percent in the aerobic active cultures. Mineralization accounted for an average 21 percent of this total. Less than one percent (0.34 percent) of the [^{14}C] counts recovered was from gases other than CO_2 (Appendix B). Of the remaining [^{14}C] counts, more were held in the liquid than in the soil. The aerobic treatment control (untreated RDX) showed no mineralization, and the total label recovery was over 98 percent.

The total recovery of the [^{14}C]-labeled RDX was much better, averaging 86 percent in the anaerobic active cultures. Mineralization ranged from 28 percent (hydrogen amendment) to 70 percent (sulfuric acid amendment) of the recovery. The majority of the recovered [^{14}C] counts were found in the liquid phase. There was CO_2 produced in all the anaerobic soil and abiotic controls except for the sulfuric-acid-amended abiotic control culture.

Table 16. Comparison of activity balances of aerobic and anaerobic cultures incubated with RDX alkaline hydrolysis end products at pH 10.5.

Sample		% Recovery				Active samples	
		CO_2/Other	Liquid	Soil	Total	Avg	St dev
Aerobic							
Hydrogen	Active 1	18.50	8.35	3.38	30.23	33.04	3.21
	Active 2	24.15	9.88	2.51	36.54		
	Active 3	20.34	9.31	2.71	32.36		
Soil Control		9.43	52.93	0.00	62.36		
Abiotic Control		23.61	9.14	2.98	35.74		
Lime control		0.71	92.93	4.90	98.54		
Anaerobic							
Hydrogen	Active 1	37.39	45.74	6.90	90.03	86.61	4.83
	Active 2	18.46	59.04	5.70	83.20		
Soil Control		34.65	52.35	0.00	89.10		
Abiotic Control		27.09	53.32	2.10	82.31		
Nitrogen	Active 1	53.56	20.83	1.90	75.43	76.48	1.47
	Active 2	51.16	24.30	1.04	77.52		
Soil Control		50.12	25.12	0.00	75.24		
Abiotic Control		53.27	26.85	1.75	81.86		
Sulfur	Active 1	69.61	16.68	4.30	90.58	94.28	5.23
	Active 2	70.25	22.80	4.93	97.98		
Soil Control		6.93	77.19	0.00	84.12		
Abiotic Control		0.03	74.34	1.98	76.36		
Lime Control		68.37	89.09	5.37	162.82		



a. Aerobic and anaerobic active cultures (average).

b. Aerobic and anaerobic control cultures (hydrogen amendment).

Figure 12. Activity balance of RDX following alkaline hydrolysis at pH 10.5 and incubation with soil.

RDX Summary and Conclusions

There was evidence of biodegradation potential of RDX hydrolysis products formed at all pH levels. The amount of aerobic mineralization decreased as the reaction pH decreased, but the activity balances were not complete for all samples. A very poor activity balance (33 percent) was realized for pH 10.5 samples, which calls the trend into question.

Anaerobic RDX cultures produced more [^{14}C] CO_2 than the other cultures in this study. This indicates that the products of RDX alkaline hydrolysis are more likely to further degrade under anaerobic conditions, such as the subsurface or flooded soils. The different electron donors supplied by the three acids did seem to have an effect on [^{14}C] CO_2 production from RDX-amended solutions. Sulfur produced the most [^{14}C] CO_2; hydrogen produced the least, with similar activity balances. This effect was more pronounced at lower pH values. This result indicates that biodegradation of the hydrolysis products may also be enhanced if sulfur- or nitrogen-containing compounds are present that can be used by sulfate and nitrate reducers in the soil.

As with the TNT products, all GPC results indicated that no significant changes in molecular weight of the RDX reaction products had occurred during incubation for all pH levels and incubation environments. All [^{14}C]

counts were associated with the same size or smaller compounds after incubation with soil as they were before incubation. The actual products' molecular size remained relatively unchanged, which does not exclude further biological degradation during incubation with soil. It does indicate that polymerization had not occurred and that the remnants of the reaction products had not been synthesized into any large biological compounds.

TOC values decreased in liquid samples of RDX products formed at pH 12.5, and increased for RDX products formed at pH 11.5 and 10.5. There is no clear explanation for this result. Formate was formed only for products formed at pH 12.5, but nitrite concentrations decreased in samples produced at all pH levels. Coupled with the other experimental data, this result indicates that the RDX reaction products had indeed been further degraded under anaerobic conditions.

5 Conclusions and Recommendations

There was evidence that the final reaction products of the alkaline hydrolysis of TNT and RDX could potentially biodegrade when incubated with a natural soil under both aerobic and anaerobic conditions. We conclude that the end products of the alkaline hydrolysis of nitroaromatics, such as TNT, and nitramines, such as RDX, will be successfully biodegraded either aerobically (surface soil contamination) or anaerobically (subsurface soil and groundwater contamination). The resulting incubation products were not incorporated into large biological compounds or polymerized, but retained their relatively small molecular size.

The end products of the alkaline hydrolysis of TNT and RDX appear to be non-toxic. The increased levels of formate and the decrease in the nitrite concentrations following incubation with soil along with the mineralization data are all positive indicators for the occurrence of biological activity. Toxicity testing of the reaction end products was not part of this study and could be completed when additional funding is available.

The different electron donors supplied by the three acids did seem to have an effect on mineralization of the RDX products, but not the TNT products. There was evidence that the biodegradation potential of the TNT hydrolysis products may depend on the initial reaction pH. Reaction pH was only relevant to RDX products in combination with the electron acceptors.

Alkaline hydrolysis of energetic contaminants in soil could be an effective treatment option for training ranges. Anaerobic incubation of RDX products is the most effective treatment and can be further enhanced when sulfur-reducing bacteria are present.

References

Arienzo, M. 1999. Degradation of 2,4,6-trinitrotoluene in water and soil slurry using a calcium peroxide compound. *Chemosphere* 40, 331-337.

Borthwick, J. O., and E. A. Beshore. 2000. Sustaining DOD ranges: A national environmental challenge. *Federal Facilities Environmental Journal*, Summer, 17-25.

Brooks, M. C., J. L. Davis, S. L. Larson, D. R. Felt, and C. C. Nestler. 2003. Topical lime treatment for containment of source zone energetics containment. ERDC/EL TR-03-19, Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Emmrich, M. 1999. Kinetics of the alkaline hydrolysis of 2,4,6-trinitrotoluene in aqueous solution and highly contaminated soils. *Environ.Sci.Technol.* 33(21): 3802-3805.

Emmrich, M. 2001. Kinetics of the alkaline hydrolysis of important nitroaromatic co-contaminants of 2,4,6-trinitrotoluene in highly contaminated soils. *Environ. Sci. Technol.* 35(5): 874-877.

Felt, D. R., S. L. Larson, and L. D. Hansen. 2001. The molecular weight distribution of the final products of the TNT-hydroxide reaction. ERDC/EL TR-01-142. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Felt, D. R., S. L. Larson, and E. J. Valente. 2002. UV-VIS spectroscopy of 2,4,6-trintrotoluene-hydroxide reaction. *Chemosphere* 49: 287-295.

Felt, D. R., E. J. Valente, and G. R. Bishop. 2007a. The alkaline hydrolysis reaction of TNT and the involvement of a long-lived organic radical. ERDC TR-07-3. Vicksburg, MS: U.S. Army Engineer Research and Development Center, Environmental Laboratory.

Felt, D. R, C. C. Nestler, J. Davis, and S. L. Larson. 2007b. Characterization of the reaction end products from alkaline hydrolysis (pH 12.5) of TNT and RDX. ERDC TR-07-3. Vicksburg, MS: U.S. Army Engineer Research and Development Center, Environmental Laboratory.

Janowsky, J. V. 1891. Ueber eine reaction der dinitrokörper. *Berichte* 24: 971.

Jenkins, T. F., and M. E. Walsh. 1992. Development of field screening methods for TNT, 2,4-DNT and RDX in soil. *Talanta* 39(4): 419-428.

Jones, D. D., M. Messenger, R. Webster, and R. Stine. 2002. Installation sustainability: Transforming – The Army's future. *Federal Facilities Environmental Journal* 13(1): 27-38.

United States Army Corps of Engineers (USACE). 1986. Laboratory testing manual, Appendix 3, III: Liquid and plastic limits. EM-1110-2-1906. Vicksburg, MS: U.S. Army Engineer Research and Development Center.

United States Environmental Protection Agency. 1986. Method 9081. Cation-exchange capacity of soils (sodium acetate). Test Methods for Evaluating Solid Wastes: Physical/Chemical Methods (USEPA SW846). Washington, DC: Office of Solid Waste Management.

United States Environmental Protection Agency. 1994. Method 8330. Nitroaromatics and nitramines by high performance liquid chromatography (HPLC). Test Methods for Evaluating Solid Wastes: Physical/Chemical Methods (USEPA SW846). Washington, DC: Office of Solid Waste Management.

United States Environmental Protection Agency. 1996. Method 6010B. Inductively coupled plasma-atomic emission spectrometry. Test Methods for Evaluating Solid Wastes: Physical/Chemical Methods (USEPA SW846). Washington, DC: Office of Solid Waste Management.

United States Environmental Protection Agency. 2004. Drinking water standards and health advisories. EPA 822-R-04-005. Washington, DC: Office of Water.

Zhao, J.-S., J. Spain, and J. Hawari. 2003. Phylogenetic and metabolic diversity of hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)-transforming bacteria in strictly anaerobic mixed cultures enriched on RDX as nitrogen source. *FEMS Microbiology Ecology* 46(2): 189-198.

Appendix A: TNT

Total Organic Carbon (TOC)

Table A1. Changes in liquid TOC following incubation of the final reaction products of alkaline hydrolysis of TNT with uncontaminated, unamended soil of an active firing range.

		TOC (mg/L)							
			pH 12.5		pH 11.5		pH 10.5	
Amendment and Environment	Sample		Day 0	Day 28	Day 0	Day 28	Day 0	Day 28
Control 1	DDI water	0.21						
Control 2	tap water	1.13						
HCl - Aerobic	Active		347.30	76.94[a]	125.20	99.19[c]	0.00	32.79[c]
	Soil Control		347.30	52.03	125.20	135.10		39.93
	Abiotic Control		347.30	46.15	125.20	129.70		29.79
HCl - Anaerobic	Active		347.30	63.37[a]	125.20	84.56[a]	0.00	42.95[b]
	Soil Control		347.30		125.20	83.79		50.46
	Abiotic Control		347.30		125.20	108.40		46.82
HNO_3 - Anaerobic	Active		127.70	47.54[c]	61.97	660.55[b]	14.65	20.09[c]
	Soil Control		127.70		61.97	625.90		23.07
	Abiotic Control		127.70		61.97	626.20		20.88
H_2SO_4 - Anaerobic	Active		46.82	35.23[c]	543.50	221.75[c]	0.00	18.73[c]
	Soil Control		46.82		543.50	218.70		20.38
	Abiotic Control		46.82		543.50	161.40		19.48

[a]average, n=1
[b]average, n=2
[c]average, n=3

Activity Balance Data, pH 12.5

Table A2. Activity balance determination. Count recovery in the gas phase from cultures incubated aerobically with media derived from the TNT titration at pH 12.5.

a. Weekly gas collection data.						
		Sample counts (dpm)[a]				
Sample day	Gas collection	Active 1	Active 2	Active 3	Soil Control	Abiotic Control
7	KOH[b]	5243	4750	8724	9394	5222
	Carbosorb	171	184	129	55	34
14	KOH[b]	7852	6269	3549	3458	3281
	Carbosorb	33	0	27	0	2
21	KOH[b]	6992	5975	1888	2395	2166
	Carbosorb	75	20	20	17	3
28	KOH[b]	3284	3990	933	1210	1224
	Carbosorb	1	26	6	30	4
Final	**Weekly total**					
CO_2	KOH	46742	41968	30188	32914	23786
Other gas	Total Carbosorb	280	230	182	102	43
[a] background = 0 [b]samples corrected for volume						

b. Summary of aerobic culture gas data.								
	Total counts (dpm)[a]				Recovery (%)			
Sample	CO_2	Other gas	PA[b]	Total	CO_2	Other gas	PA	Total gas
Active 1	46742	280	22050	69072	5.06	0.03	2.38	7.47
Active 2	41968	230	17122	59320	4.54	0.02	1.85	6.42
Active 3	30188	182	8127	38497	3.26	0.02	0.88	4.16
Soil Control	32914	102	18997	52013	6.19	0.02	3.57	9.78
Abiotic Control	23786	43	14386	38215	4.47	0.01	2.70	7.18
[a] background = 0 [b]PA=post-acidification								

Table A3. Activity balance determination. Summary of count recovery in the gas phase from cultures incubated anaerobically with media derived from the TNT titration at pH 12.5.

	Total Counts (dpm)[a]		Recovery (%)		
Sample	CO_2[b]	Other gases	CO_2	Other gases	Total gases
H - Active 1	57810	60	18.166	0.019	18.18
H - Active 2	45765	25	14.381	0.008	14.39
H - Active 3	55575	70	17.463	0.022	17.49
H – Soil Control	4965	90	1.560	0.028	1.59
H – Abiotic Control	2836	0	0.891	0.000	0.89
N - Active 1	63190	65	18.232	0.019	18.25
N - Active 2	51905	0	14.976	0.000	14.98
N - Active 3	57355	30	16.548	0.009	16.56
N – Soil Control	43040	30	12.418	0.009	12.43
N – Abiotic Control	47185	50	13.614	0.014	13.63
S - Active 1	32615	5	12.024	0.002	12.03
S - Active2	38010	5	14.012	0.002	14.01
S – Soil Control	37670	5	13.887	0.002	13.89
S – Abiotic Control	120	0	0.044	0.000	0.04
Lime Control	34	4	0.004	0.000	0.00

[a] background = 0
[b] counts are corrected for sample volume

Table A4. Activity balance determination. Summary of count recovery in the liquid phase from cultures incubated with media derived from the TNT titration at pH 12.5.

a. Aerobic incubation.					
	Counts (dpm)[a]			Active statistics	
Sample	Count/mL	Total counts[b]	Recovery (%)	Avg	St dev
Active 1	3784	340560	36.83		
Active 2	3681	331290	35.83	36.33	0.709
Active 3	1458	131220	14.19		
Soil Control	6940	624600	67.55		
Abiotic Control	3176	285840	30.91		
[a] background = 0 [b]counts adjusted for volume					
b. Anaerobic incubation.					
	Counts (dpm)[a]		Recovery	Active statistics	
Sample	Count/mL	Total counts[b]	(%)	Avg	St dev
H - Active 1	1384	124560	39.14		
H - Active 2	1435	129150	40.58		
H - Active 3	1490	134100	42.14	40.62	1.499
H – Soil Control	2980	268200	84.28		
H – Abiotic Control	1554	139860	43.95		
N - Active 1	1632	146880	42.38		
N - Active 2	1709	153810	44.38		
N - Active 3	1574	141660	40.87	42.54	1.759
N – Soil Control	2833	254970	73.57		
N – Abiotic Control	1806	162540	46.90		
S - Active 1	1124	101160	37.29		
S - Active 2	1194	107460	39.62		
S - Active 3	1179	106110	39.12	38.68	1.223
S – Soil Control	2461	221490	81.65		
S – Abiotic Control	1297	116730	43.03		
Lime Control	7313	658170	78.87		
[a] background = 0 [b]counts adjusted for volume					

Table A5. Activity balance determination. Summary of count recovery in the solid phase from cultures incubated aerobically with media derived from the TNT titration at pH 12.5.

Sample	wet weight (g)	dry weight (g)	dry wgt cone (g)	counts (dpm)	counts/ dry wgt (dpm/g)	total soil counts (dpm)	Average	Initial [a]	Recovery %
Active 1 - #1	0.208	0.79	0.164	4845	29527.739	295277.390	261723.39	978030	26.76
2	0.207		0.163	4242	25965.282	259652.817			
3	0.205		0.162	4304	26641.082	266410.820			
4	0.201		0.159	4220	26549.563	265495.634			
5	0.204		0.161	4153	25782.060	257820.600			
6	0.198		0.156	4000	25585.099	255850.992			
7	0.204		0.161	4244	26385.815	263858.148			
8	0.205		0.162	4061	25075.641	250756.406			
9	0.205		0.162	4047	24977.010	249770.103			
10	0.200		0.158	3983	25234.095	252340.949			
Active 2 - # 1	0.203	0.26	0.053	8203	155265.748	1552657.480	618769.46	978030	63.27
2	0.197		0.051	1699	33153.807	331538.071			
3	0.193		0.050	2031	40474.293	404742.925			
4	0.204		0.053	4093	77357.777	773577.774			
5	0.206		0.054	2664	49714.478	497144.777			
6	0.207		0.054	2557	47510.219	475102.192			
7	0.201		0.052	2271	43499.081	434990.806			
8	0.196		0.051	2510	49304.628	493046.280			
9	0.195		0.051	3070	60645.569	606455.691			
10	0.202		0.052	3240	61843.863	618438.633			
Active 3 - #1	0.199	0.60	0.120	1928	16131.191	161311.914	162086.74	978030	16.57
2	0.201		0.120	2174	18044.489	180444.887			
3	0.204		0.122	2158	17648.021	176480.209			
4	0.198		0.119	2409	20308.548	203085.483			
5	0.195		0.117	1589	13574.235	135742.354			
6	0.205		0.123	1985	16161.863	161618.629			
7	0.197		0.118	1505	12719.743	127197.431			
8	0.196		0.118	1569	13341.837	133418.367			
9	0.198		0.119	2149	18125.843	181258.435			
10	0.205		0.123	1967	16030.970	160309.698			
Abiotic Control - #1	0.200	0.71	0.142	5135	36216.296	362162.963	264332.42	978030	27.03
2	0.198		0.141	4763	33812.756	338127.556			
3	0.197		0.140	3148	22540.940	225409.396			
4	0.203		0.144	2737	18999.160	189991.601			
5	0.197		0.140	3233	23173.136	231731.355			
6	0.199		0.141	3379	23903.340	239033.397			
7	0.197		0.140	4361	31131.543	311315.434			
8	0.195		0.139	3745	27035.612	270356.119			
9	0.203		0.144	3169	22019.636	220196.363			
10	0.200		0.142	3621	25500.000	255000.000			

[a] initial counts in 90 ml of media

Table A6. Activity balance determination. Summary of count recovery in the solid phase from cultures incubated anaerobically with media derived from the TNT titration at pH 12.5.

Sample	wet weight (g)	dry weight (g)	dry wgt cone (g)	counts (dpm)	counts/ dry wgt (dpm/g)	total soil counts (dpm)	Average	Initial[a]	Recovery (%)
H - Active 1 - #1	0.1969	0.7001	0.1378	2238	16237.3939	162373.9389	170857.11	978030	17.47
2	0.2037		0.1426	3964	27799.9860	277999.8597			
3	0.2040		0.1428	2215	15511.2045	155112.0448			
4	0.2057		0.1440	2312	16056.6706	160566.7060			
5	0.1948		0.1364	1848	13552.3614	135523.6140			
6	0.2069		0.1448	2366	16336.3944	163363.9439			
7	0.2048		0.1434	1175	8196.1496	81961.4955			
8	0.2079		0.1455	1945	13364.9419	133649.4194			
9	0.2067		0.1447	2449	16925.8415	169258.4145			
10	0.2069		0.1448	2605	17986.6050	179866.0499			
H - Active 2 - #1	0.2057	0.7397	0.1522	1831	12028.8008	120288.0080	138356.95	978030	14.15
2	0.2021		0.1496	1493	9983.0162	99830.1617			
3	0.2098		0.1553	1530	9854.9455	98549.4551			
4	0.1943		0.1438	1868	12991.8905	129918.9050			
5	0.1944		0.1439	1769	12297.0192	122970.1924			
6	0.2077		0.1537	2721	17703.5485	177035.4852			
7	0.2020		0.1495	3306	22116.6711	221166.7113			
8	0.1966		0.1455	1872	12867.3944	128673.9435			
9	0.2000		0.1480	1831	12371.6216	123716.2162			
10	0.2077		0.1537	2481	16142.0448	161420.4479			
H - Active 3 - #1	0.2073	0.7073	0.1472	1159	7874.5507	78745.5073	85771.81	978030	8.77
2	0.1996		0.1417	1012	7141.0426	71410.4265			
3	0.1976		0.1403	952	6785.6532	67856.5319			
4	0.2084		0.1480	1201	8116.8392	81168.3923			
5	0.2047		0.1453	1109	7630.5414	76305.4143			
6	0.2057		0.1460	1324	9065.5748	90655.7478			
7	0.2083		0.1479	1300	8790.1388	87901.3882			
8	0.2046		0.1453	1781	12260.2674	122602.6737			
9	0.2007		0.1425	1358	9530.0252	95300.2519			
H – Abiotic Control - #1	0.2253	0.6102	0.1374	1421	10339.5837	103395.8365	134306.63	978030	13.73
2	0.2087		0.1273	1587	12465.9288	124659.2882			
3	0.2675		0.1632	2898	17760.0735	177600.7354			
4	0.2371		0.1446	1749	12092.8432	120928.4317			
5	0.1983		0.1210	1586	13111.4473	131114.4730			
6	0.2228		0.1359	1634	12022.8390	120228.3898			
7	0.2105		0.1284	1591	12390.4832	123904.8324			
8	0.2016		0.1230	1557	12661.0070	126610.0703			
9	0.2299		0.1402	2576	18368.6421	183686.4210			
10	0.2032		0.1240	1623	13093.7782	130937.7824			
N - Active 1 - #1	0.2020	0.7567	0.1535	1203	7836.1126	78361.1256	109727.63	930420	11.79
2	0.1989		0.1512	1877	12416.9776	124169.7759			
3	0.1981		0.1506	1710	11357.9001	113579.0005			
4	0.1981		0.1506	1199	7963.8141	79638.1413			
5	0.2072		0.1575	1338	8496.7486	84967.4863			
6	0.2000		0.1520	1191	7835.5263	78355.2632			
7	0.2012		0.1529	1967	12863.6078	128636.0783			
8	0.2013		0.1530	2785	18204.0421	182040.4215			
9	0.1962		0.1491	1692	11347.1753	113471.7528			
10	0.1975		0.1501	1712	11405.7295	114057.2951			
N - Active 2 - #1	0.2009	0.6928	0.1386	1263	9111.1736	91111.7363	100671.62	930420	10.82
2	0.2069		0.1428	1767	12377.3299	123773.2994			
3	0.2147		0.1481	1329	8971.0617	89710.6174			
4	0.1997		0.1378	904	6560.5655	65605.6549			
5	0.2079		0.1435	2163	15078.3194	150783.1943			
6	0.2080		0.1435	1378	9601.4493	96014.4928			
7	0.2032		0.1402	1278	9115.0291	91150.2910			
8	0.1999		0.1379	1457	10563.2526	105632.5264			
9	0.2097		0.1447	1460	10090.3292	100903.2918			
10	0.2022		0.1395	1284	9203.1136	92031.1358			
N - Active 3 - #1	0.2522	0.7102	0.1791	1901	10616.4345	106164.3453	113860.66	930420	12.24
2	0.1996		0.1417	1844	13011.9394	130119.3937			
3	0.2475		0.1757	1891	10761.1325	107611.3245			
4	0.2305		0.1637	1905	11640.3410	116403.4096			
5	0.2152		0.1528	1806	11819.9906	118199.9058			
6	0.2093		0.1486	1722	11587.9222	115879.2218			
7	0.2432		0.1727	2102	12173.3692	121733.6916			
8	0.1974		0.1402	1568	11187.6935	111876.9354			
9	0.1899		0.1348	1334	9894.0139	98940.1390			
10	0.2202		0.1563	1746	11167.8244	111678.2439			
N – Abiotic Control - #1	0.2040	0.7142	0.1448	892	6158.5197	61585.1975	118700.47	930420	12.76
2	0.2055		0.1459	2642	18107.6728	181076.7280			
3	0.2009		0.1426	2182	15297.3591	152973.5907			

Activity Balance Data, pH 11.5

Table A7. Activity balance determination. Count recovery in the gas phase from cultures incubated aerobically with media derived from the TNT titration at pH 11.5.

a. Weekly gas collection data.						
		Sample counts (dpm)[a]				
Sample day	Gas collection	Active 1	Active 2	Soil Control	Abiotic Control	Lime Control
7	KOH[b]	18070	17680	13580	17492	1330
	Carbosorb	769	697	157	748	0
14	KOH[b]	14942	15702	7926	14794	656
	Carbosorb	109	93	44	80	3
21	KOH[b]	8292	8140	4602	7126	458
	Carbosorb	47	33	26	35	1
28	KOH[b]	4470	4970	3286	4310	348
	Carbosorb	30	12	17	38	3
Final	Weekly total					
CO_2	KOH	45774	46492	29394	43722	2792
Other gas	Total Carbosorb	955	835	244	901	7

[a] background = 0
[b]samples corrected for volume.

b. Summary of aerobic culture gas data.								
	Total counts (dpm)[a]				Recovery (%)			
Sample	CO_2	Other gas	PA[b]	Total	CO_2	Other gas	PA	Total
Active 1	45774	955	38952	85681	10.60	0.22	4.87	15.70
Active 2	46492	835	41404	88731	11.00	0.20	5.18	16.38
Soil Control	29394	244	38658	68296	8.52	0.16	4.84	13.51
Abiotic Control	43722	901	36962	81585	10.10	0.15	4.62	14.87
Lime Control	2792	7	1173	3972	0.43	0.00	0.13	0.56

[a] background = 0
[b]PA=post-acidification

Table A8. Activity balance determination. Summary of count recovery in the gas phase from cultures incubated anaerobically with media derived from the TNT titration at pH 11.5.

Sample	Total Counts (dpm)[a]		Recovery (%)		
	CO_2[b]	Other gases	CO_2	Other gases	Total gases
H - Active 1	89265	35	11.169	0.004	11.17
H - Active 2	147440	120	18.448	0.015	18.46
H - Active 3	111200	630	13.914	0.079	13.99
H – Soil Control	184225	40	23.051	0.005	23.06
H – Abiotic Control	92855	40	11.618	0.005	11.62
N - Active 1	135360	180	17.252	0.023	17.27
N - Active 2	192935	110	24.590	0.014	24.60
N – Soil Control	36360	15	4.634	0.002	4.64
N – Abiotic Control	125060	125	15.939	0.016	15.95
S - Active 1	84160	35	11.218	0.005	11.22
S - Active 2	147535	200	19.665	0.027	19.69
S – Soil Control	56710	0	7.559	0.000	7.56
S – Abiotic Control	73920	40	9.853	0.005	9.86
Lime Control	1350	25	0.146	0.003	0.15

[a] background = 0
[b] counts are corrected for sample volume

Table A9. Activity balance determination. Summary of count recovery in the liquid phase from cultures incubated with media derived from the TNT titration at pH 11.5.

a. Aerobic incubation.					
	Counts (dpm)[a]		Recovery	Active statistics	
Sample	Count/mL	Total counts[b]	(%)	Avg	St dev
Active 1	5010	450900	56.42		
Active 2	4949	445410	55.73	55.14	1.664
Soil Control	7711	693990	86.84		
Abiotic Control	4807	432630	54.13		
Lime Control	9158	824220	88.97		
[a] background = 0 [b]counts adjusted for volume					
b. Anaerobic incubation.					
	Counts (dpm)[a]		Recovery	Active statistics	
Sample	Count/mL	Total counts[b]	(%)	Avg	St dev
H - Active 1	4713	424170	53.07		
H - Active 2	4328	389520	48.74		
H - Active 3	4698	422820	52.91	51.57	2.46
H – Soil Control	8624	776160	97.12		
H – Abiotic Control	4483	403470	50.48		
N - Active 1	4421	397890	50.71		
N - Active 2	4530	407700	51.96	51.34	0.88
N – Soil Control	8219	739710	94.28		
N – Abiotic Control	4408	396720	50.56		
S - Active 1	3963	356670	47.54		
S - Active 2	4523	407070	54.26	50.90	4.75
S – Soil Control	8033	722970	96.37		
S – Abiotic Control	4485	403650	53.80		
Lime Control	9214	829260	89.52		
[a] background = 0 [b]counts adjusted for volume					

Table A10. Activity balance determination. Summary of count recovery in the solid phase from cultures incubated aerobically with media derived from the TNT titration at pH 11.5.

Sample	wet weight (g)	dry weight (g)	dry wgt cone (g)	counts (dpm)	counts/ dry wgt (dpm/g)	total soil counts (dpm)	Average	Initial [a]	Recovery (%)
Active 1 - #1	0.246	0.77	0.188	6965	37010.468	370104.681	394523.57	799200	49.36
2	0.252		0.193	7245	37581.699	375816.993			
3	0.257		0.197	7182	36530.098	365300.984			
4	0.228		0.174	6721	38533.425	385334.251			
5	0.215		0.164	6523	39659.523	396595.227			
6	0.242		0.185	7894	42640.307	426403.068			
7	0.200		0.153	7782	50862.745	508627.451			
8	0.259		0.198	7544	38075.050	380750.498			
9	0.247		0.189	7165	37919.081	379190.813			
10	0.210		0.161	5737	35711.173	357111.734			
Active 2 - #1	0.193	0.80	0.155	5682	36754.575	367545.749	344948.77	799200	43.16
2	0.228		0.183	6396	35022.012	350220.120			
3	0.208		0.167	6463	38791.655	387916.547			
4	0.229		0.183	6450	35163.469	351634.692			
5	0.195		0.156	5218	33406.959	334069.592			
6	0.210		0.168	6209	36912.193	369121.931			
7	0.185		0.148	4536	30610.386	306103.857			
8	0.263		0.211	7697	36537.028	365370.283			
9	0.207		0.166	5119	30873.244	308732.442			
10	0.202		0.162	4996	30877.245	308772.450			
Abiotic Control - #1	0.219	0.82	0.178	6267	35112.194	351121.943	362584.50	799200	45.37
2	0.216		0.176	6623	37622.131	376221.313			
3	0.262		0.214	7868	36847.281	368472.814			
4	0.225		0.183	6713	36608.044	366080.436			
5	0.284		0.231	9487	40987.644	409876.437			
6	0.205		0.167	6142	36761.933	367619.333			
7	0.266		0.217	7320	33765.395	337653.951			
8	0.231		0.188	6744	35821.847	358218.469			
9	0.231		0.188	6429	34148.673	341486.734			
10	0.266		0.217	7568	34909.359	349093.593			
Lime Control - #1	0.230	0.75	0.173	2457	14243.478	142434.783	159118.61	926370	17.18
2	0.216		0.162	2737	16895.062	168950.617			
3	0.226		0.170	2664	15716.814	157168.142			
4	0.223		0.167	2569	15360.239	153602.392			
5	0.226		0.170	2884	17014.749	170147.493			
6	0.248		0.186	2839	15263.441	152634.409			
7	0.252		0.189	3656	19343.915	193439.153			
8	0.279		0.209	3015	14408.602	144086.022			
9	0.289		0.217	3113	14362.168	143621.684			
10	0.230		0.173	2848	16510.145	165101.449			

[a] initial counts in 90 ml of media

Table A11. Activity balance determination. Summary of count recovery in the solid phase from cultures incubated anaerobically with media derived from the TNT titration at pH 11.5.

Sample	wet weight (g)	dry weight (g)	dry wgt cone (g)	counts (dpm)	counts/ dry wgt (dpm/g)	total soil counts (dpm)	Average	Initial [a]	Recovery (%)
H - Active 1 - #1	0.2270	0.5198	0.1180	5251	44502.0365	445020.3653	489487.82	799200	61.25
2	0.2210		0.1149	5141	44752.6807	447526.8072			
3	0.2370		0.1232	6260	50814.7405	508147.4050			
4	0.2070		0.1076	5345	49675.3675	496753.6752			
5	0.2480		0.1289	6223	48273.8398	482738.3981			
6	0.2100		0.1092	5220	47820.5903	478205.9034			
7	0.2260		0.1175	5769	49108.4045	491084.0453			
8	0.2370		0.1232	6436	52243.3977	522433.9774			
9	0.2040		0.1060	5113	48218.0175	482180.1749			
10	0.2270		0.1180	6381	54078.7460	540787.4598			
H - Active 2 - #1	0.2440	0.6017	0.1468	7831	53339.3091	533393.0912	518932.06	799200	64.93
2	0.1970		0.1185	6346	53536.9752	535369.7519			
3	0.2180		0.1312	6268	47785.0982	477850.9819			
4	0.2160		0.1300	6451	49635.6004	496356.0037			
5	0.1980		0.1191	5736	48146.4134	481464.1344			
6	0.2100		0.1264	6646	52597.0069	525970.0689			
7	0.2170		0.1306	6607	50601.6364	506016.3638			
8	0.2430		0.1462	8419	57580.3399	575803.3993			
9	0.2070		0.1246	6300	50581.3239	505813.2393			
10	0.2460		0.1480	8160	55128.3558	551283.5584			
H - Active 3 - #1	0.2410	0.5082	0.1225	6569	53634.9103	536349.1029	525745.93	799200	65.78
2	0.2030		0.1032	5662	54883.1673	548831.6729			
3	0.2330		0.1184	5587	47183.2758	471832.7582			
4	0.2400		0.1220	6768	55489.9646	554899.6458			
5	0.2320		0.1179	5869	49778.4608	497784.6083			
6	0.2250		0.1143	5846	51125.9784	511259.7840			
7	0.2200		0.1118	5890	52681.4783	526814.7830			
8	0.2150		0.1093	5464	50007.7794	500077.7939			
9	0.2020		0.1027	5854	57025.1830	570251.8304			
10	0.2170		0.1103	5948	53935.7305	539357.3052			
H – Abiotic Control - #1	0.2210	0.5817	0.1286	6197	48204.7859	482047.8594	448469.18	799200	56.11
2	0.2070		0.1204	5188	43085.4426	430854.4255			
3	0.2230		0.1297	5806	44758.2507	447582.5071			
4	0.2180		0.1268	5713	45051.4389	450514.3892			
5	0.2180		0.1268	6691	52763.7280	527637.2795			
6	0.2330		0.1355	5672	41848.6293	418486.2926			
7	0.1970		0.1146	5345	46642.5644	466425.6437			
8	0.2450		0.1425	6266	43966.8389	439668.3893			
9	0.2180		0.1268	5223	41187.4086	411874.0862			
10	0.2190		0.1274	5218	40960.0894	409600.8942			
N - Active 1 - #1	0.2260	0.4871	0.1101	4167	37852.7060	378527.0601	390463.04	784620	49.76
2	0.2010		0.0979	3991	40763.1316	407631.3158			
3	0.2200		0.1072	4080	38073.1976	380731.9759			
4	0.2400		0.1169	4386	37517.9635	375179.6346			
5	0.2190		0.1067	4188	39259.4697	392594.6966			
6	0.2010		0.0979	3781	38618.2412	386182.4117			
7	0.1970		0.0960	3734	38912.5738	389125.7385			
8	0.2100		0.1023	4107	40150.1598	401501.5984			
9	0.1960		0.0955	3693	38681.6603	386816.6031			
10	0.2220		0.1081	4394	40633.9413	406339.4127			
N - Active 2 - #1	0.2830	0.5208	0.1474	5231	35491.7414	354917.4144	390854.91	784620	49.81
2	0.2310		0.1203	4702	39084.0598	390840.5982			
3	0.2630		0.1370	5178	37803.7883	378037.8826			
4	0.2100		0.1094	4352	39792.2610	397922.6099			
5	0.2170		0.1130	4214	37287.5477	372875.4769			
6	0.2110		0.1099	4373	39794.7744	397947.7435			
7	0.2150		0.1120	4587	40965.5985	409655.9854			
8	0.2060		0.1073	4283	39921.7783	399217.7829			
9	0.2090		0.1088	4265	39183.3690	391833.6898			
10	0.2290		0.1193	4953	41529.9942	415299.9416			
N – Abiotic Control - #1	0.2100	0.6177	0.1297	4632	35708.5039	357085.0390	346516.96	784620	44.16
2	0.2120		0.1310	4512	34455.2677	344552.6772			
3	0.2120		0.1310	4595	35089.0858	350890.8581			
4	0.2100		0.1297	4226	32578.6134	325786.1344			
5	0.2330		0.1439	5017	34858.6512	348586.5119			
6	0.1980		0.1223	4147	33907.1466	339071.4658			
7	0.2140		0.1322	4694	35510.0849	355100.8489			
8	0.2080		0.1285	4327	33677.9741	336779.7412			
9	0.2020		0.1248	4490	35984.6572	359846.5723			
10	0.2310		0.1427	4958	34746.9702	347469.7015			
S - Active 1 - #1	0.2020	0.5561	0.1123	5334	47484.1586	474841.5859	488137.83	750240	65.06
2	0.2510		0.1396	7455	53409.8098	534098.0978			

Activity Balance Data, pH 10.5

Table A12. Activity balance determination. Count recovery in the gas phase from cultures incubated aerobically with media derived from the TNT titration at pH 10.5.

a. Weekly gas collection data.							
		Sample counts (dpm)[a]					
Sample day	Gas collection	Active 1	Active 2	Active 3	Soil Control	Abiotic Control	Lime Control
7	KOH[b]	12186	13154	13918	12300	14464	1320
	Carbosorb	89	79	82	21	77	14
14	KOH[b]	3294	2656	1330	668	1700	366
	Carbosorb	25	22	23	11	22	10
21	KOH[b]	1450	1472	1584	866	1474	708
	Carbosorb	26	23	22	12	19	2
28	KOH[b]	1120	1072	1100	846	1028	658
	Carbosorb	7	10	14	7	17	4
Final	**Weekly total**						
CO_2	KOH	18050	18354	17932	14680	18666	3052
Other gas	Total Carbosorb	147	134	141	51	135	30

[a] background = 0
[b]samples corrected for volume

b. Summary of aerobic culture gas data.								
	Total counts (dpm)[a]				Recovery (%)			
Sample	CO_2	Other gas	PA[b]	total	CO_2	Other gas	PA	Total gas
Active 1	18050	147	18683	36880	2.19	0.02	2.27	4.48
Active 2	18354	134	23004	41492	2.23	0.02	2.79	5.04
Active 3	17932	141	20586	38659	2.18	0.02	2.50	4.69
Soil Control	14680	51	19954	34685	1.78	0.01	2.42	4.21
Abiotic Control	18666	135	21023	39824	2.27	0.02	2.55	4.83
Lime Control	3052	30	1328	4410	0.33	0.00	0.14	0.47

[a] background = 0
[b]PA = post-acidification

Table A13. Activity balance determination. Summary of count recovery in the gas phase from cultures incubated anaerobically with media derived from the TNT titration at pH 10.5

Sample	Total Counts (dpm)[a]		Recovery (%)		
	CO_2[b]	Other gases	CO_2	Other gases	Total gases
H - Active 1	59440	90	7.21	0.01	7.22
H - Active 2	61585	95	7.47	0.01	7.49
H – Soil Control	46110	80	5.60	0.01	5.61
H – Abiotic Control	100	70	0.01	0.01	0.02
N - Active 1	38015	90	4.13	0.01	4.14
N - Active 2	76185	160	8.28	0.02	8.29
N – Soil Control	63980	225	6.95	0.02	6.97
N – Abiotic Control	57835	75	6.28	0.01	6.29
S - Active 1	57340	165	6.05	0.02	6.07
S - Active 2	71970	220	7.59	0.02	7.62
S - Active 3	74020	135	7.81	0.01	7.82
S – Soil Control	18510	185	1.95	0.02	1.97
S – Abiotic Control	55	20	0.01	0.00	0.01
Lime Control	45	45	0.00	0.00	0.01

[a] background = 0
[b] counts are corrected for sample volume

Table A14. Activity balance determination. Summary of count recovery in the liquid phase from cultures incubated with media derived from the TNT titration at pH 10.5.

a. Aerobic incubation.					
	Counts (dpm)[a]			Active statistics	
Sample	Count/mL	Total counts[b]	Recovery (%)	Avg	St dev
Active 1	2220	199800	24.25		
Active 2	2376	213840	25.95		
Active 3	2390	215100	26.10	25.43	1.031
Soil Control	6807	612630	74.34		
Abiotic Control	2385	214650	26.05		
Lime Control	7844	705960	75.52		
[a] background = 0 [b]counts adjusted for volume					
b. Anaerobic incubation.					
	Counts (dpm)[a]			Active statistics	
Sample	Count/mL	Total counts[b]	Recovery (%)	Avg	St dev
H - Active 1	1717	154530	18.75		
H - Active 2	1870	168300	20.42	19.59	1.18
H – Soil Control	5964	536760	65.14		
H – Abiotic Control	1590	143100	17.37		
N - Active 1	3506	315540	34.28		
N - Active 2	3448	310320	33.71	33.99	0.40
N – Soil Control	7141	642690	69.82		
N – Abiotic Control	2837	255330	27.74		
S - Active 1	1879	169110	17.84	18.40	0.53
S - Active 2	1943	174870	18.45		
S - Active 3	1991	179190	18.90		
S – Soil Control	3556	320040	33.76		
S – Abiotic Control	1831	164790	17.39		
Lime Control	6015	541350	57.91		
[a] background = 0 [b]counts adjusted for volume					

Table A15. Activity balance determination. Summary of count recovery in the solid phase from cultures incubated aerobically with media derived from the TNT titration at pH 10.5.

Sample	wet weight (g)	dry weight (g)	dry wgt cone (g)	counts (dpm)	counts/ dry wgt (dpm/g)	total soil counts (dpm)	Average	Initial [a]	Recovery %
Active 1 - #1	0.208	2.67	0.556	9034	16262.164	162621.637	157699.02	824040	18.89
2	0.206		0.550	7832	14235.313	142353.135			
3	0.200		0.534	8301	15540.393	155403.929			
4	0.199		0.531	7624	14344.697	143446.973			
5	0.212		0.566	7361	13000.574	130005.742			
6	0.201		0.537	8785	16364.671	163646.713			
7	0.209		0.558	9292	16646.560	166465.597			
8	0.214		0.572	9161	16028.419	160284.190			
9	0.210		0.561	8440	15048.206	150482.060			
10	0.203		0.542	10967	20228.020	202280.204			
Active 2 - # 1	0.208	1.63	0.339	7641	22546.836	225468.357	221461.02	824040	26.88
2	0.215		0.350	7029	20065.677	200656.771			
3	0.214		0.349	7784	22324.811	223248.109			
4	0.200		0.326	6813	20907.742	209077.418			
5	0.202		0.329	7509	22815.473	228154.727			
6	0.215		0.350	7657	21858.428	218584.279			
7	0.207		0.337	8306	24627.499	246274.994			
8	0.204		0.332	6512	19592.189	195921.889			
9	0.215		0.350	8334	23791.059	237910.589			
10	0.221		0.360	8257	22931.304	229313.037			
Active 3 - #1	0.207	2.43	0.503	8879	17641.772	176417.719	154441.85	824040	18.74
2	0.218		0.530	8618	16259.174	162591.743			
3	0.226		0.549	8864	16131.316	161313.160			
4	0.207		0.503	7826	15549.556	155495.559			
5	0.211		0.513	8266	16112.445	161124.446			
6	0.225		0.547	8848	16173.763	161737.634			
7	0.210		0.511	7958	15585.945	155859.447			
8	0.213		0.518	7836	15130.850	151308.496			
9	0.212		0.515	6886	13359.175	133591.753			
10	0.199		0.484	6047	12497.852	124978.522			
Abiotic Control 1	0.202	2.47	0.499	6226	12473.029	124730.289	141878.02	824040	17.22
2	0.205		0.507	6488	12807.700	128077.005			
3	0.215		0.531	7085	13335.693	133356.926			
4	0.219		0.541	6918	12783.525	127835.250			
5	0.213		0.526	7961	15125.237	151252.375			
6	0.205		0.507	7578	14959.426	149594.257			
7	0.200		0.494	7412	14997.525	149975.251			
8	0.213		0.526	6801	12921.334	129213.340			
9	0.216		0.534	9053	16961.058	169610.585			
10	0.200		0.494	7667	15513.495	155134.950			
Lime Control - #1	0.212	2.03	0.431	3037	7053.135	70531.350	66241.85	934830	7.09
2	0.224		0.455	2663	5853.241	58532.411			
3	0.201		0.408	2907	7120.693	71206.927			
4	0.210		0.427	2899	6796.764	67967.640			
5	0.218		0.443	3230	7294.899	72948.992			
6	0.201		0.408	2480	6074.757	60747.567			
7	0.206		0.418	2883	6890.499	68904.992			
8	0.207		0.420	2690	6398.162	63981.616			
9	0.210		0.427	2852	6686.572	66865.715			
10	0.200		0.406	2467	6073.128	60731.278			

[a] initial counts in 90 ml of media

Table A16. Activity balance determination. Summary of count recovery in the solid phase from cultures incubated anaerobically with media derived from the TNT titration at pH 10.5.

Sample	wet weight (g)	dry weight (g)	dry wgt cone (g)	counts (dpm)	counts/ dry wgt (dpm/g)	total soil counts (dpm)	Average	Initial [a]	Recovery (%)
#1	0.2060	0.0000	0.1240	7911	63824.0196	638240.1965	661284.85	824040	80.25
2	0.2040		0.1227	8144	66347.9618	663479.6182			
3	0.2100		0.1264	8030	63550.1001	635501.0011			
4	0.2080		0.1252	8342	66654.0954	666540.9545			
5	0.2060		0.1240	7653	61742.5385	617425.3854			
6	0.2000		0.1203	8076	67109.8554	671098.5541			
7	0.1990		0.1197	7627	63697.2464	636972.4641			
8	0.1980		0.1191	8038	67468.7711	674687.7114			
9	0.2000		0.1203	8259	68630.5468	686305.4678			
10	0.2110		0.1270	9174	72259.7191	722597.1911			
H - Active 2 - #1	0.2210	0.0000	0.1123	9313	82920.6444	829206.4442	810083.86	824040	98.31
2	0.2250		0.1143	8018	70121.1247	701211.2467			
3	0.2110		0.1072	8488	79156.8047	791568.0471			
4	0.2150		0.1093	9279	84923.5331	849235.3313			
5	0.1970		0.1001	8808	87978.4728	879784.7284			
6	0.1970		0.1001	8516	85061.8386	850618.3864			
7	0.2030		0.1032	7591	73581.4417	735814.4170			
8	0.2260		0.1149	11819	102905.2739	1029052.7386			
9	0.2070		0.1052	7473	71037.8774	710378.7736			
10	0.2020		0.1027	7432	72396.8501	723968.5007			
H –Abiotic Control 1	0.2070	0.9113	0.1204	10644	88396.5787	883965.7874	680812.79	824040	82.62
2	0.2130		0.1239	8924	72024.6065	720246.0652			
3	0.2080		0.1210	8231	68028.3916	680283.9158			
4	0.2250		0.1309	8634	65967.5663	659675.6633			
5	0.2150		0.1251	8094	64718.0877	647180.8772			
6	0.2030		0.1181	6326	53571.5344	535715.3443			
7	0.2220		0.1291	8612	66688.6587	666886.5875			
8	0.2020		0.1175	8634	73478.7249	734787.2487			
9	0.2250		0.1309	8271	63194.0863	631940.8630			
10	0.2100		0.1222	7909	64744.5500	647445.5005			
N - Active 1 - #1	0.2100	0.8812	0.1094	7596	69453.5879	694535.8789	702787.89	920520	76.35
2	0.2070		0.1078	8178	75858.7680	758587.6800			
3	0.2110		0.1099	7335	66749.2957	667492.9565			
4	0.2030		0.1057	7032	66513.8135	665138.1353			
5	0.1990		0.1036	6961	67165.7056	671657.0564			
6	0.2200		0.1146	8821	76988.2000	769881.9997			
7	0.2200		0.1146	8647	75469.5573	754695.5732			
8	0.2090		0.1088	7183	65991.5919	659915.9188			
9	0.2040		0.1062	7606	71590.4641	715904.6414			
10	0.2140		0.1115	7468	67006.9053	670069.0526			
N - Active 2 - #1	0.2150	0.9115	0.1106	8209	74196.2599	741962.5991	724770.00	920520	78.73
2	0.2100		0.1081	8217	76036.8664	760368.6636			
3	0.2120		0.1091	7552	69223.9439	692239.4386			
4	0.2150		0.1106	7644	69089.5615	690895.6155			
5	0.2210		0.1137	8480	74564.7896	745647.8959			
6	0.2100		0.1081	7814	72307.6638	723076.6384			
7	0.2120		0.1091	7727	70828.0474	708280.4743			
8	0.2150		0.1106	7932	71692.6220	716926.2195			
9	0.2160		0.1112	8468	76182.8677	761828.6767			
10	0.2140		0.1101	7780	70647.3770	706473.7697			
N –Abiotic Control 1	0.2420	0.0000	0.1495	10513	70328.8793	703288.7933	645857.14	920520	70.16
2	0.2010		0.1242	8983	72351.5336	723515.3357			
3	0.2020		0.1248	7311	58593.2804	585932.8041			
4	0.1990		0.1229	8492	69084.2915	690842.9146			
5	0.2050		0.1266	8265	65269.6668	652696.6678			
6	0.2030		0.1254	8591	68512.5418	685125.4176			
7	0.2000		0.1235	8044	65112.5142	651125.1417			
8	0.2000		0.1235	6580	53262.1013	532621.0134			
9	0.2080		0.1285	7928	61705.3337	617053.3368			
10	0.2050		0.1266	7805	61636.9933	616369.9325			

Appendix B: RDX

Total Organic Carbon (TOC)

Table B1. Changes in liquid TOC following incubation of the final reaction products of alkaline hydrolysis of RDX with uncontaminated, unamended soil of an active firing range.

Amendment and Environment	Sample		TOC (mg/L)					
			pH 12.5		pH 11.5		pH 10.5	
			Day 0	Day 28	Day 0	Day 28	Day 0	Day 28
Control 1	DDI water	0.21						
Control 2	tap water	1.13						
HCl-Aerobic	Active		701.10	19.78[b]	13.24	21.61[a]	0.00	12.91[a]
	No soil		701.10		13.24	32.47	0.00	5.04
	Killed soil		701.10	51.59	13.24	25.65	0.00	17.72
HCl - Anaerobic	Active		701.10	63.37[b]	13.24	37.445[b]	0.00	10.82[a]
	No soil		701.10	59.08	13.24	21.64	0.00	4.05
	Killed soil		701.10		13.24	33.31	0.00	14.55
HNO_3 - Anaerobic	Active		589.00	25.28[b]	14.26	21.35[a]	4.37	12.80[a]
	No soil		589.00		14.26	19.29	4.37	10.14
	Killed soil		589.00	41.23	14.26	26.26	4.37	19.75
H_2SO_4 - Anaerobic	Active		412.70	23.16[b]	38.73	18.24[a]	0.00	11.95[a]
	No soil		412.70		38.73	15.55	0.00	5.52
	Killed soil		412.70	33.85	38.73	27.92	0.00	15.33

[a] average, n=3
[b] average, n=2

Activity Balance Data, pH 12.5

Table B2. Activity balance determination. Count recovery in the gas phase from cultures incubated aerobically with media derived from the RDX titration at pH 12.5.

a. Weekly gas collection data.					
		Sample counts (dpm)[a]			
Sample day	Gas collection	Active 1	Active 2	Soil Control	Abiotic Control
7	KOH[b]	34058	28872	1064	1180
	Carbosorb	4005	2431	691	667
14	KOH[b]	56832	59814	1012	5252
	Carbosorb	1697	1578	157	414
21	KOH[b]	52882	52998	984	13682
	Carbosorb	49	108	0	2
28	KOH[b]	39666	40580	988	12498
	Carbosorb	303	346	16	200
Final	Weekly total				
CO_2	KOH	183438	182264	4048	32612
Other gas	Total Carbosorb	6054	4463	864	1283

[a] background = 0
[b]samples corrected for volume

b. Summary of aerobic culture gas data.								
	Total counts (dpm)[a]				Recovery (%)			
Sample	CO_2	Other gas	PA[b]	Total	CO_2	Other gas	PA	Total gas
Active 1	183438	6054	104831	294323	18.76	0.62	10.72	30.09
Active 2	182264	4463	97057	283784	18.64	0.46	9.92	29.02
Soil Control	4048	864	2040	6952	0.41	0.09	0.21	0.71
Abiotic Control	32612	1283	48545	82440	3.33	0.13	4.96	8.43

[a] background = 0
[b]PA=post-acidification
[c]lost post-acidification KOH

Table B3. Activity balance determination. Summary of count recovery in the gas phase from cultures incubated anaerobically with media derived from the RDX titration at pH 12.5.

	Total Counts (dpm)[a]		Recovery (%)		
Sample	CO_2[b]	other gases	CO_2	other gases	total gases
H - Active 1	142540	30	14.574	0.003	14.58
H –Soil Control	233375	330	23.862	0.034	23.90
H – Abiotic Control	619775	195	63.370	0.020	63.39
N - Active 1	522740	5	56.183	0.001	56.18
N - Active 2	590615	45	63.478	0.005	63.48
N - Active 3	546370	40	58.723	0.004	58.73
N – Soil Control	12000	15	1.290	0.002	1.29
N – Abiotic Control	140920	0	15.146	0.000	15.15
S - Active 1	437290	1100	46.683	0.117	46.80
S - Active 2	422485	5	45.103	0.001	45.10
S - Active 3	561610	925	59.955	0.099	60.05
S – Soil Control	246560	10	26.322	0.001	26.32
S – Abiotic Control	351930	130	37.570	0.014	37.58
Lime Control	1170	0	0.199	0.000	0.20

[a] background = 0
[b] counts are corrected for sample volume

Table B4 Activity balance determination. Summary of count recovery in the liquid phase from cultures incubated with media derived from the RDX titration at pH 12.5.

a. Aerobic incubation.					
	Counts (dpm)[a]			Active statistics	
Sample	Count/mL	Total counts[b]	Recovery (%)	Avg	St dev
Active 1	843	75870	7.76		
Active 2	804	72360	7.40	7.58	0.254
Soil Control	11045	994050	101.64		
Abiotic Control	6712	604080	61.76		
[a] background = 0 [b]counts adjusted for volume					
b. Anaerobic incubation.					
	Counts (dpm)[a]		Recovery	Active statistics	
Sample	Count/mL	Total counts[b]	%	Avg	St dev
H - Active 1	1076	96840	9.90	9.90	
H – Soil Control	5166	464940	47.54		
H – Abiotic Control	1838	165420	16.91		
N - Active 1	1662	149580	16.08		
N - Active 2	1760	158400	17.02		
N - Active 3	1896	170640	18.34	17.15	1.14
N – Soil Control	21052	1894680	203.64		
N – Abiotic Control	19906	1791540	192.55		
S - Active 1	1958	176220	18.81		
S - Active 2	1576	141840	15.14		
S - Active 3	3620	325800	34.78	22.91	10.44
S – Soil Control	8304	747360	79.78		
S – Abiotic Control	8264	743760	79.40		
Lime Control	11300	1017000	108.57		
[a] background = 0 [b]counts adjusted for volume					

Table B5. Activity balance determination. Summary of count recovery in the solid phase from cultures incubated aerobically with media derived from the RDX titration at pH 12.5.

Sample		wet weight (g)	dry weight (g)	dry wgt cone (g)	counts (dpm)	counts/ dry wgt (dpm/g)	total soil counts (dpm)	Average	Initial [a]	Recovery %
Active 1	1	0.207	0.72	0.149	437	2929.269	29292.686	32746.26	978030	3.35
	2	0.205		0.148	514	3482.385	34823.848			
	3	0.202		0.146	532	3656.056	36560.559			
	4	0.205		0.148	448	3030.795	30307.950			
Active 2	1	0.201	0.77	0.155	328	2121.385	21213.846	21168.33	978030	2.16
	2	0.209		0.161	316	1966.409	19664.092			
	3	0.209		0.161	339	2104.492	21044.921			
	4	0.202		0.156	319	2047.878	20478.780			
	5	0.205		0.158	406	2570.808	25708.080			
	6	0.197		0.151	300	1981.742	19817.416			
	7	0.209		0.161	336	2091.868	20918.679			
	8	0.201		0.155	288	1858.053	18580.525			
	9	0.206		0.158	374	2363.573	23635.732			
	10	0.207		0.160	329	2062.127	20621.271			
Abiotic Control	1	0.203	0.40	0.081	686	8460.779	84607.795	84238.38	978030	8.61
	2	0.198		0.079	602	7604.851	76048.509			
	3	0.199		0.080	609	7646.911	76469.111			
	4	0.204		0.081	813	9982.809	99828.094			

[a] initial counts in 90 ml of media

Table B6. Activity balance determination. Summary of count recovery in the solid phase from cultures incubated anaerobically with media derived from the RDX titration at pH 12.5.

Sample	wet weight (g)	dry weight (g)	dry wgt cone (g)	counts (dpm)	counts/ dry wgt (dpm/g)	total soil counts (dpm)	Average	Initial [a]	Recovery %
H - Active 3 - #1	0.1972	0.540	0.1065	156	1464.9538	14649.5380	31809.92	978030	3.25
2	0.1981		0.1070	265	2477.2375	24772.3746			
3	0.1958		0.1057	284	2686.0364	26860.3639			
4	0.2027		0.1095	451	4120.3019	41203.0185			
5	0.2018		0.1090	295	2707.1174	27071.1742			
6	0.1999		0.1079	337	3121.9313	31219.3134			
7	0.1982		0.1070	395	3690.6230	36906.2301			
8	0.2034		0.1098	368	3350.4498	33504.4976			
9	0.2095		0.1131	460	4066.1186	40661.1862			
10	0.2074		0.1120	462	4125.1473	41251.4733			
H –Abiotic Control 1	0.2070	0.690	0.1428	177	1239.2355	12392.3545	20164.58	978030	2.06
2	0.1970		0.1359	287	2111.3809	21113.8086			
3	0.2045		0.1411	211	1495.3403	14953.4035			
4	0.2087		0.1440	252	1749.9635	17499.6354			
5	0.1978		0.1365	307	2249.3809	22493.8087			
6	0.2054		0.1417	378	2667.1182	26671.1824			
7	0.2019		0.1393	358	2569.7899	25697.8989			
8	0.1998		0.1379	226	1639.3205	16393.2048			
9	0.2026		0.1398	286	2045.8675	20458.6749			
10	0.1983		0.1368	328	2397.1877	23971.8769			
N - Active 1 - #1	0.2046	0.730	0.1494	219	1466.2757	14662.7566	15432.45	930420	1.66
2	0.2053		0.1499	230	1534.6736	15346.7361			
3	0.2047		0.1494	231	1545.8640	15458.6398			
4	0.2047		0.1494	243	1626.1686	16261.6860			
N - Active 2 - #1	0.2022	0.740	0.1496	208	1390.1141	13901.1415	13289.13	930420	1.43
2	0.1966		0.1455	169	1161.6398	11616.3977			
3	0.2082		0.1541	190	1233.2217	12332.2169			
4	0.1953		0.1445	172	1190.1302	11901.3022			
5	0.2040		0.1510	174	1152.6232	11526.2321			
6	0.2025		0.1499	205	1368.0347	13680.3470			
7	0.2004		0.1483	206	1389.1137	13891.1366			
8	0.2022		0.1496	218	1456.9466	14569.4656			
9	0.2011		0.1488	204	1370.8388	13708.3877			
10	0.2083		0.1541	243	1576.4685	15764.6845			
N - Active 3 - #1	0.2026	0.760	0.1540	201	1305.3982	13053.9824	13255.17	930420	1.42
2	0.2085		0.1585	149	940.3004	9403.0039			
3	0.2017		0.1533	269	1754.8209	17548.2086			
4	0.1951		0.1483	195	1315.1151	13151.1506			
5	0.1972		0.1499	198	1321.1274	13211.2736			
6	0.1983		0.1507	237	1572.5774	15725.7743			
7	0.2055		0.1562	167	1069.2790	10692.7904			
N – Abiotic Control #1	0.1980	0.760	0.1505	857	5695.1090	56951.0898	55362.70	930420	5.95
2	0.1982		0.1506	838	5563.2269	55632.2694			
3	0.2016		0.1532	852	5560.7769	55607.7694			
4	0.2053		0.1560	831	5325.9671	53259.6713			

(continued)

Table B6. (concluded)

S - Active 1 - #1		0.200	0.61	0.122	72	5950.819	59508.196	46833.8	93672	5.0
	2	0.194	0	0.118	68	5231.561	52315.615	1	0	0
	3	0.203		0.124	75	6432.838	64328.384			
	4	0.207		0.126	76	5704.553	57045.539			
	5	0.207		0.126	78	6630.871	66308.714			
	6	0.199		0.121	75	4600.368	46003.686			
	7	0.199		0.121	78	4763.625	47636.256			
	8	0.203		0.124	79	5622.677	56226.773			
	9	0.195		0.119	73	4518.274	45182.746			
	10	0.202		0.123	76	5378.213	53782.137			
S - Active 2 - #1		0.197	0.65	0.128	193	15072.237	150722.374	98612.7	93672	10.5
	2	0.200	0	0.130	190	14609.243	146092.430	5	0	3
	3	0.202		0.131	200	15187.473	151874.739			
	4	0.201		0.130	318	9044.381	90443.816			
	5	0.197		0.128	401	6878.976	68789.764			
	6	0.200		0.130	313	8687.963	86879.637			
	7	0.207		0.134	101	7547.450	75474.501			
	8	0.202		0.131	91	6966.044	69660.443			
	9	0.198		0.128	90	6989.477	69894.769			
	10	0.207		0.134	89	6629.505	66295.057			
S - Active 3 - #1		0.199	0.67	0.133	93	8006.092	80060.929	66256.0	93672	7.0
	2	0.203	0	0.136	80	9927.651	99276.518	9	0	7
	3	0.200		0.134	105	9835.820	78358.209			
	4	0.201		0.134	75	9624.805	96248.054			
	5	0.202		0.135	145	10697.870	106978.700			
	6	0.207		0.139	96	5916.811	59168.115			
	7	0.204		0.137	85	6255.969	62559.697			
	8	0.201		0.134	88	6061.583	60615.838			
	9	0.201		0.134	91	6324.125	63241.256			
	10	0.201		0.134	75	5605.356	56053.562			
S – Abiotic Control	1	0.200	0.61	0.122	65	2525.997	35259.978	35783.1	93672	3.8
	2	0.198	0	0.120	35	2939.228	29392.283	4	0	2
	3	0.199		0.121	52	3481.137	54811.376			
	4	0.200		0.122	39	3232.855	32328.556			
	5	0.209		0.127	50	3151.680	31516.805			
	6	0.198		0.121	40	3323.330	33233.302			
	7	0.199		0.121	44	3674.959	36749.593			
	8	0.202		0.123	47	3837.794	38377.946			
	9	0.203		0.124	57	3850.646	38506.464			
	10	0.200		0.122	46	3765.509	37655.092			
Lime Control - 1		0.198	0.73	0.144	50	3486.924	34869.240	40416.2	58707	6.8
	2	0.196	0	0.143	60	4234.248	42342.486	8	0	8
	3	0.194		0.142	54	3816.498	38164.988			
	4	0.206		0.150	55	3691.923	36919.235			
	5	0.200		0.146	60	4151.927	41519.277			
	6	0.199		0.145	72	4956.288	49562.882			
	7	0.206		0.151	65	3338.782	33387.827			
	8	0.208		0.151	61	4076.659	40766.596			
	9	0.205		0.149	94	6621.400	66214.002			
[a]initial counts in 90 ml of media		4		9	3	3	7			

Activity Balance Data, pH 11.5

Table B7. Activity balance determination. Count recovery in the gas phase from cultures incubated aerobically with media derived from the RDX titration at pH 11.5.

a. Weekly gas collection data.							
		Sample counts (dpm)[a]					
Sample day	Gas collection	Active 1	Active 2	Active 3	Soil Control	Abiotic Control	Lime Control
7	KOH[b]	173700	172714	171542	127500	170768	3964
	Carbosorb	431	408	375	335	585	17
14	KOH[b]	43266	42954	43452	29836	55982	960
	Carbosorb	197	175	174	204	243	1
21	KOH[b]	11132	19202	20178	14928	24580	384
	Carbosorb	134	94	148	169	175	7
28	KOH[b]	14782	13742	14118	11980	17184	280
	Carbosorb	51	0	3	24	15	0
Final weekly total							
CO_2	KOH	242880	248612	249290	184244	268514	5588
Other gas	Total Carbosorb	813	677	700	732	1018	25

[a] background = 0
[b]samples corrected for volume

b. Summary of aerobic culture gas data.								
	Total counts (dpm)[a]				Recovery (%)			
Sample	CO_2	Other gas	PA[b]	Total	CO_2	Other gas	PA	Total gas
Active 1	242880	813	248880	492573	26.09	0.09	26.73	52.91
Active 2	248612	677	256901	506190	26.70	0.07	27.60	54.37
Active 3	249290	700	249571	499561	26.78	0.08	26.81	53.66
Soil Control	184244	732	249692	434668	19.79	0.08	26.82	46.69
Abiotic Control	268514	1018	215183	484715	28.84	0.11	23.11	52.07
Lime Control	5588	25	1337	6950	0.62	0.00	0.15	0.77

[a] background = 0
[b]PA=post-acidification

Table B8. Activity balance determination. Summary of count recovery in the gas phase from cultures incubated anaerobically with media derived from the RDX titration at pH 11.5

Sample	Total Counts (dpm)[a]		Recovery (%)		
	CO_2[b]	Other gases	CO_2	Other gases	Total gases
H - Active 2	355495	10	38.186	0.001	38.19
H – Soil Control	338545	10	36.365	0.001	36.37
H – Abiotic Control	30630	0	3.290	0.000	3.29
N - Active 1	259005	10	32.893	0.001	32.89
N – Soil Control	341930	290	43.425	0.037	43.46
N – Abiotic Control	75530	45	9.592	0.006	9.60
S - Active 1	419310	10	46.772	0.001	46.77
S - Active 3	392905	0	43.827	0.000	43.83
S – Soil Control	5670	0	0.632	0.000	0.63
S – Abiotic Control	229785	5	25.632	0.001	25.63
Lime Control	20	1105	0.002	0.123	0.13

[a] background = 0
[b] counts are corrected for sample volume

Table B9. Activity balance determination. Summary of count recovery in the liquid phase from cultures incubated with media derived from the RDX titration at pH 11.5.

a. Aerobic incubation.					
	Counts (dpm)[a]		Recovery	Active statistics	
Sample	Count/mL	Total counts[b]	(%)	Avg	St dev
Active 1	1934	174060	18.70		
Active 2	1955	175950	18.90		
Active 3	2048	184320	19.80	19.13	0.59
Soil Control	2507	225630	24.24		
Abiotic Control	1974	177660	19.08		
Lime Control	8256	743040	82.84		
[a] background = 0 [b]counts adjusted for volume					
b. Anaerobic incubation.					
	Counts (dpm)[a]			Active statistics	
Sample	Count/mL	Total counts[b]	Recovery (%)	Avg	St dev
H - Active 2	2257	203130	21.82	21.62	
H – Soil Control	2159	194310	20.87		
H – Abiotic Control	2079	187110	20.10		
N - Active 1	2490	224100	28.46	28.46	
N – Soil Control	3462	311580	39.57		
N – Abiotic Control	7150	643500	81.72		
S - Active 1	2063	185670	20.71		
S - Active 2	2302	207180	23.11	21.91	1.70
S – Soil Control	5310	477900	53.31		
S – Abiotic Control	2277	204930	22.86		
Lime Control	8389	755010	1.11		
[a] background = 0 [b]counts adjusted for volume					

Table B10. Activity balance determination. Summary of count recovery in the solid phase from cultures incubated aerobically with media derived from the RDX titration at pH 11.5.

Sample	wet weight (g)	dry weight (g)	dry wgt cone (g)	counts (dpm)	counts/ dry wgt (dpm/g)	total soil counts (dpm)	Average	Initial[a]	Recovery %
Active 1 - #1	0.221	0.79	0.175	395	2254.308	22543.080	25032.23	930960	2.69
2	0.259		0.205	471	2293.663	22936.628			
3	0.233		0.185	462	2500.889	25008.894			
4	0.211		0.167	413	2468.744	24687.438			
5	0.238		0.189	476	2522.542	25225.421			
6	0.234		0.186	437	2355.451	23554.506			
7	0.279		0.221	536	2423.087	24230.870			
8	0.276		0.219	589	2691.626	26916.255			
9	0.209		0.166	366	2208.733	22087.330			
10	0.201		0.159	528	3313.190	33131.896			
Active 2 - # 1	0.232	0.76	0.177	496	2808.043	28080.429	28775.14	930960	3.09
2	0.208		0.158	424	2677.396	26773.958			
3	0.206		0.157	468	2983.930	29839.304			
4	0.222		0.169	533	3153.438	31534.379			
5	0.226		0.172	465	2702.431	27024.307			
6	0.210		0.160	441	2758.223	27582.228			
7	0.241		0.183	523	2850.327	28503.271			
8	0.244		0.186	544	2928.324	29283.240			
9	0.250		0.190	567	2978.881	29788.806			
10	0.218		0.166	487	2934.151	29341.514			
Active 3 - #1	0.205	0.78	0.159	779	4888.838	48888.377	44005.01	930960	4.73
2	0.234		0.182	798	4387.418	43874.185			
3	0.206		0.160	718	4484.141	44841.409			
4	0.273		0.212	1081	5094.307	50943.065			
5	0.217		0.169	716	4244.977	42449.767			
6	0.195		0.152	671	4427.004	44270.042			
7	0.272		0.211	892	4219.082	42190.821			
8	0.205		0.159	647	4060.434	40604.339			
9	0.232		0.180	771	4275.515	42755.149			
10	0.202		0.157	616	3923.299	39232.987			
Abiotic Control 1	0.221	0.77	0.171	410	2397.642	23976.420	25255.53	930960	2.71
2	0.234		0.181	412	2275.486	22754.858			
3	0.238		0.184	449	2438.160	24381.597			
4	0.225		0.174	424	2435.433	24354.325			
5	0.213		0.165	408	2475.559	24755.592			
6	0.202		0.156	386	2469.612	24696.118			
7	0.225		0.174	417	2395.225	23952.249			
8	0.224		0.173	390	2250.139	22501.390			
9	0.271		0.210	476	2270.024	22700.239			
10	0.231		0.179	438	2450.501	24505.011			
Lime Control 1	0.245	0.78	0.192	796	4151.978	41519.781	42988.87	896940	4.79
2	0.228		0.178	661	3704.885	37048.846			
3	0.221		0.173	852	4926.692	49266.918			
4	0.204		0.160	696	4360.007	43600.066			
5	0.224		0.175	778	4438.535	44385.350			
6	0.200		0.157	621	3967.982	39679.818			
7	0.238		0.186	842	4521.091	45210.906			
8	0.247		0.193	773	3999.361	39993.610			
9	0.233		0.182	839	4601.656	46016.556			
10	0.262		0.205	885	4316.682	43166.819			

[a]initial counts in 90 ml of media

Table B11. Activity balance determination. Count recovery in the gas phase from cultures incubated aerobically with media derived from the RDX titration at pH 11.5

Weekly gas collection data.							
		Sample counts (dpm)[a]					
Sample day	Gas collection	Active 1	Active 2	Active 3	Control 1	Control 2	Lime Control
7	KOH[b]	173700	172714	171542	127500	170768	3964
	Carbosorb	431	408	375	335	585	17
14	KOH[b]	43266	42954	43452	29836	55982	960
	Carbosorb	197	175	174	204	243	1
21	KOH[b]	11132	19202	20178	14928	24580	384
	Carbosorb	134	94	148	169	175	7
28	KOH[b]	14782	13742	14118	11980	17184	280
	Carbosorb	51	0	3	24	15	0
Final Weekly total							
CO_2	KOH	242880	248612	249290	184244	268514	5588
Other gas	Total Carbosorb	813	677	700	732	1018	25

[a] background = 0
[b]samples corrected for volume

Summary of aerobic culture gas data								
	Total counts (dpm)[a]				Recovery (%)			
Sample	CO_2	Other gas	PA[b]	Total	CO_2	Other gas	PA	Total gas
Active 1	242880	813	248880	492573	26.09	0.09	26.73	52.91
Active 2	248612	677	256901	506190	26.70	0.07	27.60	54.37
Active 3	249290	700	249571	499561	26.78	0.08	26.81	53.66
Control 1	184244	732	249692	434668	19.79	0.08	26.82	46.69
Control 2	268514	1018	215183	484715	28.84	0.11	23.11	52.07
Lime control	5588	25	1337	6950	0.62	0.00	0.15	0.77

[a] background = 0
[b]PA=post-acidification

Activity Balance Data, pH 10.5

Table B12. Activity balance determination. Count recovery in the gas phase from cultures incubated aerobically with media derived from the RDX titration at pH 10.5.

a. Weekly gas collection data.							
		Sample counts (dpm)[a]					
Sample day	Gas collection	Active 1	Active 2	Active 3	Soil Control	Abiotic Control	Lime Control
7	KOH[b]	23980	26038	25862	8366	18440	1906
	Carbosorb	547	2436	1870	396	1626	33
14	KOH[b]	34960	41168	33702	15656	23178	882
	Carbosorb	426	1019	735	425	515	12
21	KOH[b]	41684	43816	38374	18010	20938	892
	Carbosorb	229	516	451	209	203	10
28	KOH[b]	26562	26700	26930	12352	14246	848
	Carbosorb	87	260	286	124	141	10
Final weekly total							
CO_2	KOH	127186	137722	124868	54384	76802	4528
Other gas	Total Carbosorb	1289	4231	3342	1154	2485	65
[a] background = 0 [b]samples corrected for volume							

b. Summary of aerobic culture gas data.								
	Total counts (dpm)[a]				Recovery (%)			
Sample	CO_2	Other gas	PA[b]	Total	CO_2	Other gas	PA	Total gas
Active 1	127186	1289	41548	170023	14.69	0.15	4.80	19.64
Active 2	137722	4231	80035	221988	15.91	0.49	9.24	25.64
Active 3	124868	3342	58768	186978	14.42	0.39	6.79	21.60
Soil Control	54384	1154	31164	86702	6.28	0.13	3.60	10.01
Abiotic Control	76802	2485	137788	217075	8.87	0.29	15.91	25.07
Lime Control	4528	65	2210	6803	0.47	0.01	0.23	0.70
[a] background = 0 [b]PA=post-acidification								

Table B13. Activity balance determination. Summary of count recovery in the gas phase from cultures incubated anaerobically with media derived from the RDX titration at pH 10.5.

Sample	Total Counts (dpm)[a]		Recovery (%)		
	CO_2[b]	Other gases	CO_2	Other gases	Total gases
H - Active 1	343645	55	39.69	0.006	39.70
H - Active 2	169710	25	19.60	0.003	19.60
H - Active 3	141990	30	16.40	0.003	16.40
H – Soil Control	318460	90	36.78	0.010	36.79
H – Abiotic Control	249010	35	28.76	0.004	28.76
N - Active 1	498735	70	115.40	0.016	115.42
N - Active 2	476400	50	110.23	0.012	110.24
N - Active 3	35850	45	8.30	0.010	8.31
N – Soil Control	466770	30	108.00	0.007	108.01
N – Abiotic Control	495970	105	114.76	0.024	114.78
S - Active 1	635585	50	61.27	0.005	61.27
S - Active 2	641405	50	61.83	0.005	61.83
S - Active 3	92145	35	8.88	0.003	8.89
S – Soil Control	63185	65	6.09	0.006	6.10
S – Abiotic Control	215	65	0.02	0.006	0.03
Lime Control	651700	85	67.04	0.009	67.05

[a] background = 0
[b] counts are corrected for sample volume

Table B14. Activity balance determination. Summary of count recovery in the liquid phase from cultures incubated with media derived from the RDX titration at pH 10.5.

a. Aerobic incubation.					
	Counts (dpm)[a]		Recovery	Active statistics	
Sample	Count/mL	Total counts[b]	(%)	Avg	St dev
Active 1	853	76770	8.87		
Active 2	1009	90810	10.49		
Active 3	951	85590	9.89	9.75	0.82
Soil Control	5406	486540	56.20		
Abiotic Control	934	84060	9.71		
Lime Control	9844	885960	91.14		
[a] background = 0 [b]counts adjusted for volume					
b. Anaerobic incubation.					
	Counts (dpm)[a]			Active statistics	
Sample	Count/mL	Total counts[b]	Recovery (%)	Avg	St dev
H - Active 1	4672	420480	48.57	49.00	13.47
H - Active 2	6030	542700	62.68		
H - Active 3	3439	309510	35.75		
H – Soil Control	5347	481230	55.58		
H – Abiotic Control	5446	490140	56.61		
N - Active 1	2156	194040	44.90	38.59	17.80
N - Active 2	2515	226350	52.37		
N - Active 3	888	79920	18.49		
N – Soil Control	2599	233910	54.12		
N – Abiotic Control	2778	250020	57.85		
S - Active 1	1692	152280	14.68	18.71	3.56
S - Active 2	2313	208170	20.07		
S - Active 3	2466	221940	21.39		
S – Soil Control	7832	704880	67.94		
S – Abiotic Control	7543	678870	65.44		
Lime Control	9437	849330	87.37		
[a] background = 0 [b]counts adjusted for volume					

Table B15. Activity balance determination. Summary of count recovery in the solid phase from cultures incubated aerobically with media derived from the RDX titration at pH 10.5.

	wet weight (g)	dry weight (g)	dry wgt cone (g)	counts (dpm)	counts/ dry wgt (dpm/g)	total soil counts (dpm)	Average	Initial[a]	Recovery %
Active 1 - #1	0.240	0.78	0.187	494	2647.455	26474.549	31065.81	865800	3.59
2	0.222		0.173	463	2682.507	26825.071			
3	0.224		0.174	673	3864.381	38643.808			
4	0.207		0.161	504	3131.649	31316.488			
5	0.242		0.188	515	2737.189	27371.886			
6	0.204		0.159	692	4363.036	43630.360			
7	0.201		0.156	440	2815.590	28155.905			
8	0.238		0.185	522	2821.022	28210.216			
9	0.221		0.172	469	2729.565	27295.650			
10	0.200		0.155	509	3273.412	32734.119			
Active 2 - # 1	0.221	0.77	0.171	363	2123.852	21238.522	23068.58	865800	2.66
2	0.235		0.182	427	2349.470	23494.702			
3	0.205		0.159	576	3633.110	36331.101			
4	0.201		0.155	354	2277.284	22772.837			
5	0.228		0.176	369	2092.673	20926.734			
6	0.232		0.179	425	2368.704	23687.045			
7	0.211		0.163	364	2230.637	22306.368			
8	0.220		0.170	338	1986.570	19865.704			
9	0.239		0.185	352	1904.385	19043.848			
10	0.227		0.176	369	2101.892	21018.922			
Active 3 - #1	0.208	0.79	0.164	412	2519.334	25193.341	24883.90	865800	2.87
2	0.230		0.181	381	2106.925	21069.247			
3	0.230		0.181	572	3163.152	31631.521			
4	0.205		0.161	315	1954.378	19543.781			
5	0.219		0.172	444	2578.640	25786.401			
6	0.204		0.160	474	2955.290	29552.897			
7	0.228		0.179	428	2387.596	23875.958			
8	0.217		0.171	407	2385.539	23855.392			
9	0.214		0.168	421	2502.190	25021.896			
10	0.221		0.174	405	2330.852	23308.517			
Abiotic Control 1	0.224	0.78	0.174	433	2493.616	24936.161	27413.28	865800	3.17
2	0.226		0.175	457	2608.540	26085.398			
3	0.201		0.156	475	3048.507	30485.075			
4	0.228		0.177	569	3219.342	32193.421			
5	0.225		0.174	562	3222.133	32221.333			
6	0.231		0.179	481	2686.104	26861.039			
7	0.204		0.158	435	2750.735	27507.353			
8	0.237		0.184	491	2672.532	26725.316			
9	0.205		0.159	414	2605.171	26051.707			
10	0.205		0.159	400	2517.073	25170.732			
Lime Control - #1	0.229	0.80	0.182	803	4409.227	44092.265	46675.60	972090	4.80
2	0.232		0.185	915	4959.244	49592.438			
3	0.216		0.172	724	4214.705	42147.048			
4	0.234		0.186	887	4766.396	47663.959			
5	0.214		0.170	741	4353.984	43539.835			
6	0.214		0.170	842	4947.441	49474.415			
7	0.229		0.182	886	4864.975	48649.747			
8	0.223		0.177	941	5305.998	53059.983			
9	0.201		0.160	717	4485.444	44854.441			
10	0.230		0.183	799	4368.188	43681.877			

[a]initial counts in 90 ml of media

Table B16. Activity balance determination. Summary of count recovery in the solid phase from cultures incubated anaerobically with media derived from the RDX titration at pH 10.5.

Sample	wet weight (g)	dry weight (g)	dry wgt cone (g)	counts (dpm)	counts/ dry wgt (dpm/g)	total soil counts (dpm)	Average	Initial[a]	Recovery %
H - Active 1 - #1	0.237	0.5842	0.1385	676	4882.1281	48821.2808	63406.683	865800	7.32
2	0.258		0.1507	829	5499.7841	54997.8413			
3	0.215		0.1256	751	5978.7762	59787.7619			
4	0.22		0.1285	721	5609.4903	56094.9032			
5	0.223		0.1303	771	5917.8007	59178.0069			
6	0.201		0.1174	1394	11870.7330	118707.3296			
7	0.25		0.1461	785	5374.5297	53745.2970			
8	0.223		0.1303	773	5933.1517	59331.5166			
9	0.232		0.1355	799	5894.8073	58948.0731			
10	0.239		0.1396	900	6445.4824	64454.8241			
H - Active 2 - #1	0.206	0.5747	0.1184	651	5498.6561	54986.5610	52394.646	865800	6.05
2	0.241		0.1385	630	4548.4801	45484.8008			
3	0.216		0.1241	713	5743.5257	57435.2570			
4	0.211		0.1213	548	4518.9849	45189.8493			
5	0.204		0.1172	587	5006.6903	50066.9025			
6	0.211		0.1213	642	5294.1393	52941.3928			
7	0.222		0.1276	893	6999.0851	69990.8511			
8	0.208		0.1195	564	4718.0068	47180.0676			
9	0.205		0.1178	649	5508.5035	55085.0345			
10	0.221		0.1270	579	4558.5747	45585.7475			
H - Active 3 - #1	0.2	0.7256	0.1451	488	3362.5170	33625.1704	28976.645	865800	3.35
2	0.213		0.1546	458	2963.1971	29631.9712			
3	0.203		0.1473	461	3129.5331	31295.3314			
4	0.224		0.1625	428	2633.1186	26331.1858			
5	0.217		0.1575	433	2749.8109	27498.1091			
6	0.209		0.1517	415	2736.3804	27363.8045			
7	0.211		0.1531	429	2801.8798	28018.7983			
8	0.218		0.1582	431	2724.5542	27245.5415			
9	0.228		0.1654	498	3010.0185	30100.1849			
10	0.214		0.1553	445	2865.6352	28656.3521			
H–A. Control 1	0.211	0.8215	0.1733	320	1846.1524	18461.5243	19326.373	865800	2.23
2	0.211		0.1733	400	2307.6905	23076.9054			
3	0.212		0.1742	314	1802.9921	18029.9208			
4	0.212		0.1742	320	1837.4442	18374.4416			
5	0.215		0.1766	331	1874.0862	18740.8622			
6	0.226		0.1857	305	1642.8255	16428.2549			
7	0.21		0.1725	322	1866.5370	18665.3703			
8	0.206		0.1692	347	2050.5119	20505.1187			
9	0.221		0.1815	390	2148.1884	21481.8840			
10	0.216		0.1774	346	1949.9451	19499.4508			
N - Active 1 - #1	0.216	0.6352	0.1372	234	1705.5635	17055.6353	17715.475	432180	4.10
2	0.241		0.1531	291	1900.9984	19009.9835			
3	0.231		0.1467	262	1785.6449	17856.4493			
4	0.203		0.1289	206	1597.6328	15976.3283			
5	0.203		0.1289	223	1729.4763	17294.7631			
6	0.237		0.1505	282	1873.2966	18732.9665			
7	0.223		0.1416	256	1807.3443	18073.4431			
8	0.226		0.1435	246	1713.6908	17136.9079			
9	0.229		0.1455	269	1849.3648	18493.6482			
10	0.221		0.1404	246	1752.4621	17524.6207			
N - Active 2 - #1	0.225	0.6651	0.1496	151	1009.1058	10091.0580	9692.242	432180	2.24
2	0.23		0.1530	151	987.1687	9871.6872			
3	0.226		0.1503	162	1077.8265	10778.2648			
4	0.216		0.1437	130	904.9652	9049.6519			
5	0.236		0.1570	143	911.1006	9111.0055			
6	0.202		0.1343	133	990.0168	9900.1676			
7	0.203		0.1350	129	955.5116	9555.1156			
8	0.22		0.1463	141	963.6930	9636.9300			
9	0.239		0.1589	136	855.6246	8556.2464			
10	0.216		0.1437	149	1037.2293	10372.2934			
N - Active 3 - #1	0.24	0.6492	0.1558	262	1681.6577	16816.5772	19130.495	432180	4.43
2	0.224		0.1454	306	2104.3645	21043.6448			
3	0.248		0.1610	301	1869.6588	18696.5885			
4	0.22		0.1428	282	1974.5766	19745.7658			
5	0.218		0.1415	281	1985.6257	19856.2568			
6	0.212		0.1376	264	1918.2960	19182.9600			
7	0.199		0.1292	248	1919.7566	19197.5655			
8	0.23		0.1493	271	1815.0517	18150.5174			
9	0.233		0.1513	292	1930.5208	19305.2080			
10	0.213		0.1383	267	1930.9864	19309.8639			
N – A. Control 1	0.202	0.6988	0.1412	216	1530.1829	15301.8289	16307.756	432180	3.77
2	0.221		0.1544	239	1547.5568	15475.5676			

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
November 2007	Final report	

4. TITLE AND SUBTITLE

Potential for Biodegradation of the Alkaline Hydrolysis End Products of TNT and RDX

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Deborah R. Felt, Catherine C. Nestler, Jeffrey L. Davis, and Steven L. Larson

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Environmental Laboratory, U.S. Army Engineer Research and Development Center, 3909 Halls Ferry Road, Vicksburg, MS 39180-6199;
Applied Research Associates, Inc., Southern Division, 119 Monument Place, Vicksburg, MS 39180

8. PERFORMING ORGANIZATION REPORT NUMBER

ERDC/EL TR-07-25

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Corps of Engineers
Washington, DC 20314-1000

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Energetic compounds, such as 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), and their degradation products, can act as a source of contamination for soil on Department of Defense testing and training ranges. Base-catalyzed hydrolysis degrades nitroaromatics and nitramines, and the potential effectiveness of this reaction in soil has been demonstrated at both bench and pilot scales. This report evaluates the potential for soil bacteria to degrade the transformation products from the alkaline hydrolysis of munitions residues. The media were obtained from the hydrolytic destruction of TNT and RDX at pH 12.5, 11.5, and 10.5. Duplicate reactors were amended with [^{14}C]-labeled explosive compounds. Bench-scale microcosms incubated aerobically and anaerobically using grenade range soil as the inoculum and reaction mixtures (quenched and neutralized) as the media showed that there is a potential for biodegradation. Nutrient analysis confirmed the presence of increased levels of nitrite and formate following both aerobic and anaerobic incubation. TNT end products from alkaline hydrolysis were aerobically mineralized, with 16% [^{14}C]-label recovered as CO_2. RDX reaction end products demonstrated much greater mineralization than TNT (roughly threefold). The use of alkaline material on training ranges has the potential to treat source-zone energetics contamination.

15. SUBJECT TERMS

Biodegradation potential	Alkaline hydrolysis	Microcosms
Energetics contamination	End products	Soil bacteria

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED		88	