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Bioslurry treatment for soils contaminated with very high concentrations of 2,4,6-trinitrophenylmethylnitramine (tetryl)

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Abstract

Past and current DoD activities have resulted in the contamination of soil, sediment and groundwater with various explosive compounds. This research was undertaken to determine the effectiveness of a soil bioslurry process for remediation of soil with very high concentrations of 2,4,6-trinitrophenylmethylnitramine (tetryl). A 99.9% reduction in tetryl concentrations (from 100,000 to below 100 mg/kg) was achieved in 180 to 200 days. A variety of process modifications (i.e. addition of fertilizer, microbial biomass, purging with nitrogen, etc.) that were performed during the course of the experiment did not increase the tetryl biodegradation rate beyond the rates of degradation without modifications. Subsequent batches of soil added as a 25% (v/v) replacement of the slurry were also degraded. These results indicate the potential for this process to remediate highly contaminated soils at many former and current ammunition manufacturing sites.

Keywords: Tetryl; Bioremediation; Biodegradation; 2,4,6-Trinitrophenylmethylnitramine; Explosives

1. Introduction

Interest in the fate of explosives and other energetic compounds in the environment has continued to grow among scientists, regulators, and the public. The major

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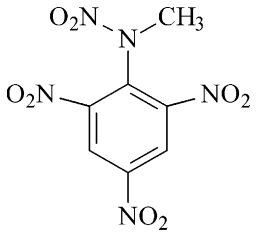


CAS No.: 479-45-8
Solubility (25°C): 80 mg/L
log K_{ow}: 1.65
Melting point: 130°C

Fig. 1. Structure and properties of tetryl.

compounds in this class of compounds include 2,4,6-trinitrotoluene, 2,4- and 2,6-dinitrotoluene (DNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX), octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), and 2,4,6-trinitrophenylmethylnitramine (tetryl, see Fig. 1). These compounds have been shown to be recalcitrant in the aerated, unsaturated soils typical at most polluted sites, leading to long-term contamination scenarios.

Chronic occupational exposure of humans and controlled exposures of laboratory animals to these compounds have resulted in several adverse effects: liver damage, blood damage (caused by methemoglobinemia and associated cyanosis), anemia, cataracts, allergenic dermatitis, discoloration of hair and skin, and nausea (Yinon [1], Goh [2], Etnier and Hartley [3]). They have also been shown to be toxic and/or mutagenic at concentrations considerably below their respective solubilities (Whong et al. [4], Vaatanen et al. [5], Lachance et al. [6], Banerjee et al. [7]). Adverse ecological effects have also been reported (Davenport et al. [8], Dodard et al. [9], Drzyzga et al. [10], Fuller and Manning [11], Gong et al. [12,13], Green et al. [14], Siciliano et al. [15], Steevens et al. [16]). Tetryl, among others, was shown to be very toxic to the bioluminescent bacterium *Vibrio fischeri* (Drzyzga et al. [10]), raising the possibility that biodegradation of this compound may be more difficult. These effects lend urgency to research efforts focused on removing these compounds from the environment.

The biodegradation of TNT, and to a lesser degree RDX and HMX, has been studied extensively (see Esteve-Nuñez et al. [17], Hawari et al. [18] for reviews), and biological treatment technologies employing composting (Bennett [19]) and bioslurry reactors (Manning et al. [20], Boopathy and Manning [21], Ederer et al. [22]) have been developed and tested for the ex situ treatment of soils contaminated with these compounds. However, literature reports specifically examining the biodegradation and bioremediation of tetryl are limited (Boopathy [23], Boopathy and Manning [24]). This research was conducted to assess the potential for the aerobic/anoxic bioslurry technology to treat soils that are contaminated with tetryl at concentrations more than an order of magnitude higher than previously examined, and to evaluate the potential for several process modifications to increase the rate of tetryl biodegradation.

2. Materials and methods

2.1. Chemicals and media

Blackstrap feedlot grade molasses was obtained from Zooks Molasses Company (Honeybrook, PA, USA). Tween 80 (polyoxyethylene (20) sorbitan monooleate) was purchased from Mallinckrodt Specialty Chemical Company (Paris, KY, USA). Solvents were HPLC grade and all other chemicals were reagent grade or better. Silica sand (NJ#80, 99% SiO_2, 157 ± 77 μm mean grain size) was a gift from US Silica Company (Berkeley Springs, WV, USA).

2.2. Soil

Contaminated soil was obtained from the Picatinny Arsenal (New Jersey, USA). A forested area downhill from the former tetryl production plant received process wastewater for approximately 15 years (1932–1945), which was directed into four shallow "pits". Soils from each hot spot were collected and portions were mixed together in equal proportions to produce a composite. Air dried portions of both the composite soil and soil from the most heavily contaminated pit (based on an initial site characterization) were separated by sieving, and the material passing the 30 mesh sieve (<600 μm) was used for the experiment. Initial tetryl concentrations were approximately 250,000 mg/kg for the composite soil and 500,000 mg/kg for the heavily contaminated pit.

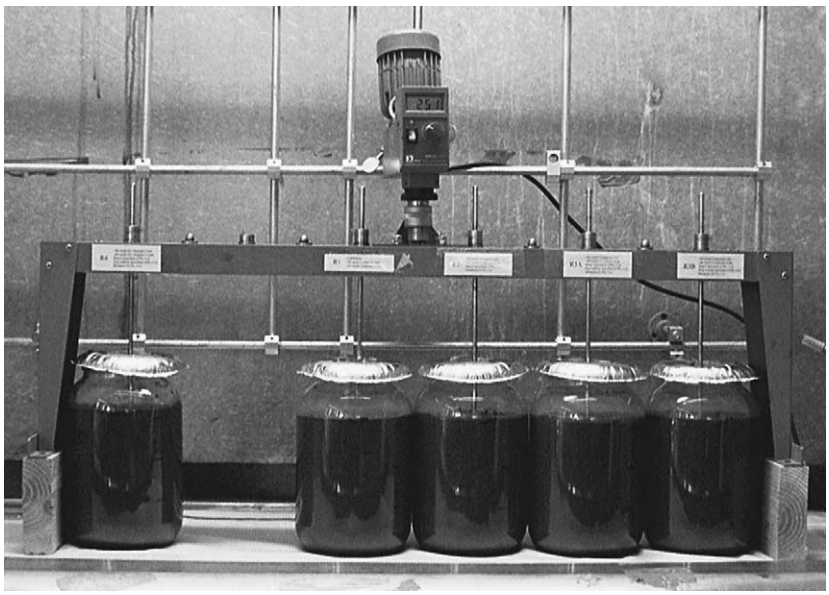


Fig. 2. Photo of the bioslurry reactor set up.

Table 1
Treatment assignment and initial slurry composition for each reactor

Reactor ID	Soil[a]	Oversize wash (ml)	Molasses[b]	Soil slurry[c]	Pure cultures[d]
Control	Composite	400	N	N	N
R2	Composite	400	Y	Y	N
R3A	Composite	400	Y	Y	Y
R3B	Composite	400	Y	Y	Y
R4	High tetryl	680	Y	Y	Y

[a] 930 g of air dry soil per reactor.
[b] Molasses (18 ml) as a 50% solution prepared with deionized distilled water.
[c] Slurry (300 ml) from a bioslurry reactor treating explosives contaminated soil from the Massachusetts Military Reservation. See text for explanation.
[d] Pure cultures (100 ml each) of *Rahnella aquitilis* BFB, *Agrobacterium* sp. 2PC, and *Sphingomonas capsulata* ATCC 14666. See text for explanation.

2.3. Reactor set up

The bioslurry reactors consisted of 4 l clear glass jars mixed with a paddle-type impeller. Five reactors were mixed at the same speed (275 rpm) using a six position gangstirrer (Fig. 2). The treatments assigned to each reactor are listed in Table 1. The aqueous phase of each reactor was a settled deionized water "wash" of the >30 mesh "oversize" soil fraction, which was used to more closely approximate employing wet-sieving soil separation methods proposed for use in the field. The oversize wash for the control reactor and R2, R3A, and R3B was derived from the oversize fraction of the composite soil, while the wash for R4 was derived from the oversize fraction of the heavily contaminated soil.

The initial composition of the slurry (wash volume, inocula, etc.) in each reactor is given in Table 1. The soil slurry added to the treatment reactors was from a bioslurry reactor treating explosives contaminated soil from the Massachusetts Military Reservation, which was known to have active RDX- and HMX-degrading microorganisms. The three pure cultures of bacteria added to the treatment reactors were shown to actively transform 2,4,6-trinitrotoluene (TNT) during previous research (Fuller and Manning [25]). Molasses was added to the treatment reactors to achieve a final concentration of 0.3% (v/v; volume of undiluted molasses to volume of slurry), while the control reactor did not receive any molasses. Deionized water was added to all the reactors to achieve an initial volume of slurry of 3 l, with a solids concentration of approximately 25% (w/v). The mouth of the jars were loosely covered with aluminum foil to reduce evaporation. Additional slurry amendments and modifications to the reactor operation are given in Table 2 and described in the Section 3.

2.4. Reactor operation and sampling

The reactors were placed in a chemical fume hood and the slurries were mixed at 275 rpm, except during monitoring and sampling events, at which time the mixing was increased to 300 rpm at least 30 min prior to performing measurements and/or taking samples. Slurry pH, dissolved oxygen (DO), redox (Eh), and temperature were monitored using appropriate

Table 2
Reactor amendments and modifications to reactor operation (see text for details)

Day	Reactor(s)	Amendment or operational modification	Event ID[a]
29	Control, R2, R3A, R3B	Diluted slurries with clean sand to reduce tetryl concentration to <100,000 mg/kg	1
63	R2, R3A, R3B, R4	Increased molasses addition from 0.3 to 0.6% (v/v)	2
77	R2, R3B, R4	Added fertilizer (N/P/K)	3[b]
78	R2, R3B, R4	Added molasses-grown biomass	3[b]
89	Control, R2, R3A, R3B, R4	Retrofit reactors to make them more "closed" vessels	NA
90	R2	Added surfactant (Tween 80)	4[c]
98	R2	Added surfactant (Tween 80)	4[c]
98	R3B	Increased molasses addition from 0.6 to 1% (v/v)	5[d]
113	R4	Increased molasses addition from 0.6 to 2% (v/v)	5[d]
151	R2	Began N_2 sparge	6[e]
162	R3A	Began N_2 sparge	6[e]
179	R2	Slurry replacement (25% (v/v))	7
207	Control, R3A, R3B	Slurry replacement (25% (v/v))	8
235	R2	Slurry replacement (25% (v/v))	9

[a] Numbers correspond to the vertical gray grid lines in the figures.
[b] Grid line is at 77 days.
[c] Grid line is at the average of the time of the two sequential additions, 94 days.
[d] Grid line in figures is at the average of the time of the additions to each reactor, 105 days.
[e] Grid line in figures is at the average of the time at which the N_2 sparge was started for each reactor, 156 days.

probes. The pH of the reactors was maintained between 7.0 and 8.5 using small additions of 10N NaOH and 6N HCl, as needed. Evaporative losses were replaced by the addition of deionized water. Molasses was added approximately weekly to all the treatment reactors; the actual amount of molasses added varied over the course of the experiment to achieve concentrations, based on the slurry volume at the time of the molasses addition. Supplemental molasses was added to individual reactors if the DO of the slurry rose to >1 mg/l between the scheduled weekly additions.

At each sampling event, 3×20 mls samples were transferred to preweighed aluminum weigh pans for determination of solids content and total explosives concentrations, and at least one 25 ml sample was transferred to a 40 ml amber glass vial and archived at $-20\,^{\circ}C$. The weight of the samples taken for solids content were recorded before and after drying at $60\,^{\circ}C$. The dried slurry was crushed, the pan was scraped, and the material was transferred to a labeled 20 ml glass vial. A 1 g subsample of this material were transferred to a 30 ml glass vial with a Teflon lined cap, ground to a powder with a glass pipet, and extracted with 10 ml acetonitrile for 18 h in a water cooled ultrasonic bath. The extract was allowed to settle for 1 h, then approximately 2 ml was removed and clarified using a 0.2 μm nylon syringe filter. Extracts were stored at $4\,^{\circ}C$ until analysis. The filtered extract was diluted further with acetonitrile prior to HPLC analysis, as needed.

Periodically, a 5 ml slurry sample was also taken for determination of culturable heterotrophs. The sample was thoroughly mixed, serially diluted in phosphate buffered saline, and plated onto triplicate R2A agar (Fisher Scientific, Fair Lawn, NJ, USA) and 0.3% (v/v) molasses solidified with 20 g/l agar. Plates were incubated at room temperature and counted until the number of colony forming units (CFU) stabilized.

2.5. Analytical

Explosives compounds and metabolite concentrations were determined using high performance liquid chromatography (HPLC) using a Hewlett-Packard Model 1050 High Performance Liquid Chromatograph (Agilent Technologies Inc., Palo Alto, CA, USA) fitted with an autosampler, quatenary pump, Pinnacle Octyl C18 reverse-phase column (Restek Corporation, Bellefonte, PA, USA), and diode array detector (peak detection at 230 nm). The mobile phase was 1:1 methanol:water (v/v), with a flow rate of 0.85 ml. EPA Method 8330 standards were purchased from Restek Corporation. Outside conformational analyses of selected samples was performed by Severn Trent Laboratories (Colchester, VT, USA).

3. Results and discussion

Reactor operational parameters are presented in Fig. 3. The temperature of the slurries decreased as colder ambient air made its way into the fume hood (Fig. 3). To maintain the slurries within the target range of 20 to 22 °C, a small electric forced-air heater was employed starting on Day 130. The pH was maintained between 7.0 and 8.5 SU, but a moderate amount of NaOH needed to be added to reactor R3B and R4 near the end of the experiment. This was likely due to the increased production of acidity in these reactors in response to the increase in molasses concentration starting around 100 days after startup. The DO of the treatment reactors was kept below 1 mg/l, whereas the control reactor remained near saturation (approximately 8 mg/l). All the reactors exhibited a slow decrease in redox for the first month. The redox of the control reactor then fluctuated around an average value of +77 mV, while the redox of treatment reactors continued to decrease until they stabilized at between −80 and −160 mV. At this redox level, the processes likely to occur are nitrate reduction, iron reduction, and sulfate reduction. This was supported by the olfactory detection on occasion of hydrogen sulfide and possibly other reduced sulfur compounds (i.e. mercaptans).

The percent solids were slightly higher than the calculated 25%, but were relatively stable over the course of the experiment. Plate counts on R2A and 0.3% molasses agar plates were always similar. During the initial 30 days of operation, culturable heterotrophs in the treatment reactors increased by approximately 1.5 orders of magnitude in response to the addition of molasses (from 3×10^7 to 1×10^9), while the unamended control reactor exhibited an approximate 30-fold decrease in culturable heterotrophs (from 3×10^7 to 1×10^6). Details regarding the culturable heterotrophs during the rest of the investigation are given below.

Fig. 4 shows an overview of tetryl concentrations in the control and treatment reactors, while Fig. 5 presents the same data at different scales in order to highlight the details at the latter times and lower tetryl concentrations for the reactors prepared with the composite soil. Tetryl concentrations in triplicate slurry samples from the same reactor at a given timepoint varied by approximately 15% over the duration of the study ($n = 65$ sets of triplicates). Key operational events (i.e. amendments, slurry replacements, etc.) are denoted on all graphs by numbered vertical gray grid lines, as defined in Table 2.

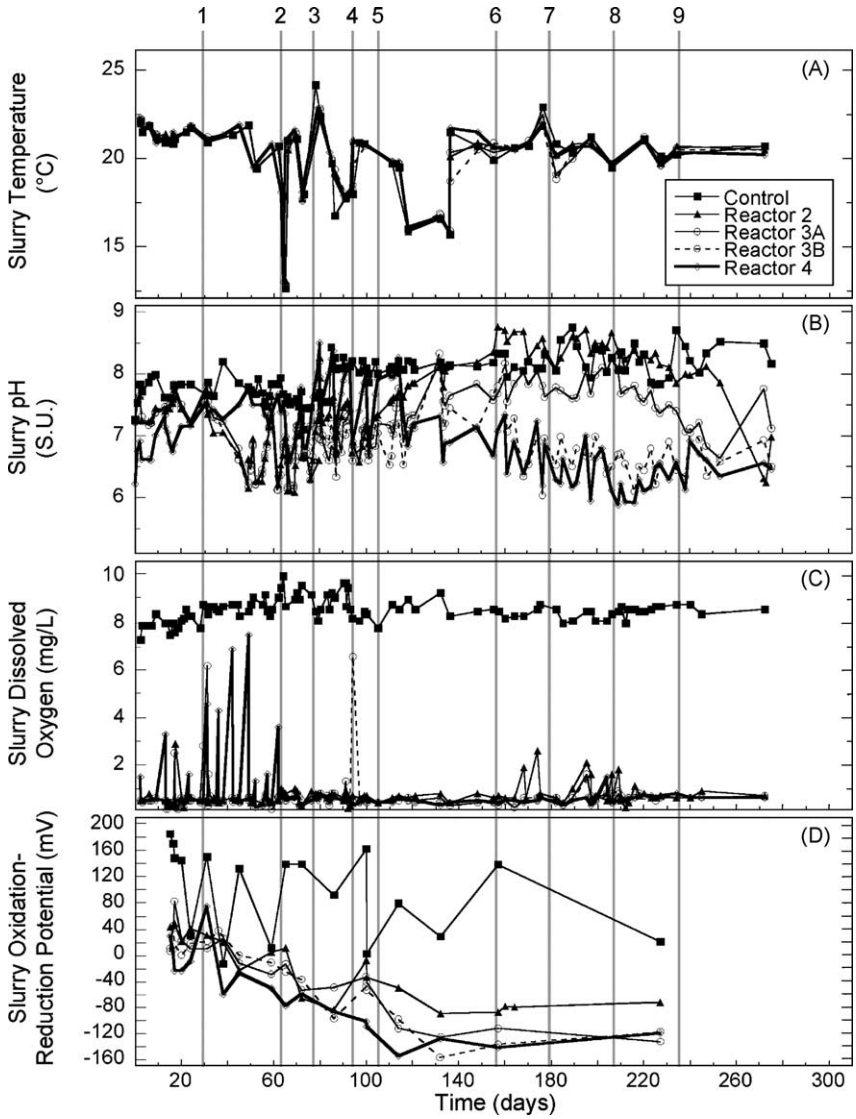


Fig. 3. Slurry parameters over the course of the experiment: (A) temperature; (B) pH; (C) dissolved oxygen; (D) oxidation–reduction potential.

The operation of the reactors was modified from what was described in the Section 2 at several points throughout the experiments. While investigating the safety hazards associated with tetryl, US Army personnel indicated that tetryl concentrations above 100,000 mg/kg were considered to be potentially unsafe in the large quantities that would need to be handled during field-scale treatment of contaminated soil. Therefore, in order to more

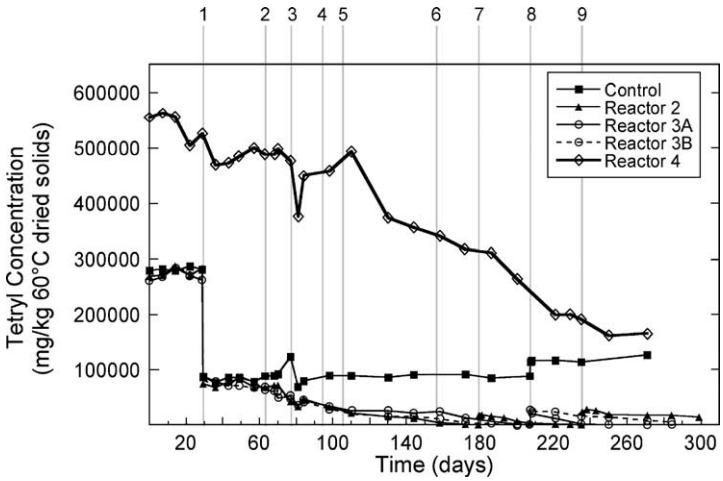


Fig. 4. Tetryl concentrations in the different reactors over the course of the experiment.

closely approximate the field-scale process, the composite soil slurries (control, R2, R3A, R3B) were diluted on Day 29 with clean quartz sand to achieve total tetryl concentrations below this threshold. This was done by removing 2/3 of the soil slurry from each reactor, and replacing it with an equivalent volume of sand in deionized water (25% (w/v)). Subsequent analyses confirmed that the tetryl concentrations were below the 100,000 mg/kg level (line 1, Fig. 4). Reactor R4 slurry, however, was not diluted since it was of scientific interest to determine if these extremely high tetryl concentrations could be biologically degraded. No marked differences between the three composite soil slurry reactors (R2, R3A, R3B) were observed over the next month with respect to tetryl concentrations. Rather, the reactors appeared to be behaving as replicates even though R2 was not inoculated with the three bacterial strains at the start of the study.

Next, an increase the molasses concentration from 0.3 to 0.6% for all the treatment reactors on Day 63, and the addition of fertilizer and molasses-grown biomass to R2, R3B, and R4 on Days 77 and 78, respectively, were performed. Reactor R3A did not receive the fertilizer or biomass additions to allow the effects of the amendments to be assessed. These modifications were performed to promote establishment and maintenance of a higher microbial population because insignificant decreases in tetryl concentrations had been observed in the month since the slurries were diluted (Fig. 5B). KGro Acid Plant Food (30-10-10 NPK, 3% NH_3–N/27% urea–N; Forkmark Corporation, Troy, MI, USA) was added in powder form to achieve a final concentration of 0.5 g N/l of reactor volume. The biomass was started from a small volume of soil-free slurry from R2, R3A, R3B, and was grown to a 10 l volume in 0.3% (v/v) molasses in a 20 l vessel at 28 °C with aeration and vigorous mixing. Biomass was concentrated to make a cell paste (2×10^{12} CFU/g wet paste), which was added to achieve CFU densities of $1 \times 10^{10}\,l^{-1}$ of reactor volume. No apparent changes in operational parameters were observed after these modifications, except less frequent increases in the DO of reactors R3B and R4 (line 2, Fig. 3). The increase in

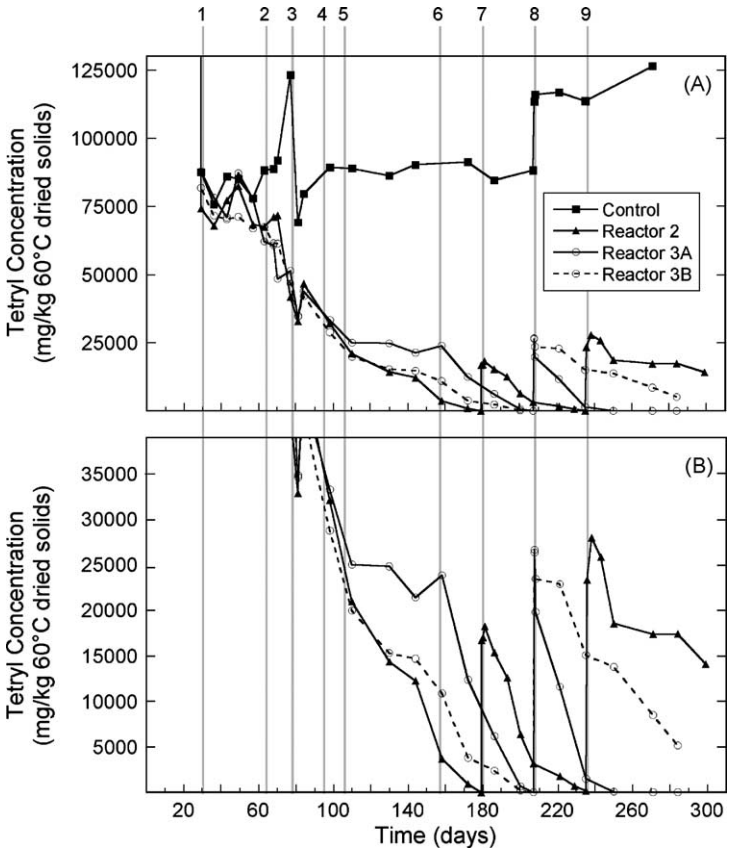


Fig. 5. (A) Tetryl concentration vs. time curves for the composite soil reactors after dilution with clean sand. (B) Tetryl concentrations vs. time curves for the composite soil reactors after 25% slurry replacement was performed.

molasses concentration may have increased the rate of tetryl degradation (line 2, Fig. 5), or this may have just corresponded with the end of the lag phase. The fertilizer and biomass additions did not lead to any apparent increases in culturable heterotrophs (data not shown) or increases in tetryl degradation rates (line 3, Fig. 5). The lack of increase in culturable heterotrophs is most likely due to inability of the added biomass to establish itself in the reactor, but may also have resulted from a change in the overall culturability of the microbial communities within the reactors. However, this observation was not examined further.

As the reactors continued to operate, the three composite soil slurry reactors appeared to be degrading tetryl almost identically. Literature reports had indicated that the addition of a surfactant (Boopathy and Manning [26], Sheremata and Hawari [27], Boopathy [28]) resulted in increased degradation rates for soils contaminated primarily with TNT and RDX. In an effort to increase the tetryl degradation rate, Tween 80 was added to reactor R2 twice (Days 90 and 98) to achieve final concentrations of 0.5% (v/v) with each addition. The additions of Tween 80 resulted in no difference in tetryl concentrations between R2 and

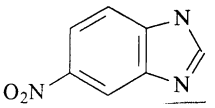
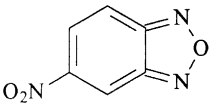
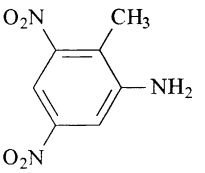
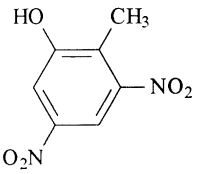
R3A/R3B (line 4, Fig. 5). However, there was some indication that the surfactant may have caused a decrease in the culturable heterotrophs in R2 compared to the other two reactors (from 1×10^9 to 2×10^7), indicating that the Tween 80 either was toxic to the culturable organisms, or that there was a shift in the microbial community towards species that were less culturable on the plate media used for enumeration. Since the degradation of tetryl did not appear to be negatively affected, the reasons underlying the change in culturable heterotrophs were not examined further.

Since the rate of tetryl degradation still appeared similar in all the reactors through Day 98, it was decided to increase the molasses concentration in R3B from 0.6 to 1%. Tetryl degradation in R4 was proceeding quite well, then appeared to slow some from the time the fertilizer and biomass was added (Days 77–113). Therefore, it was also decided to increase the molasses concentration in this reactor from 0.6 to 2%. The increase in molasses concentrations resulted in greater decreases in the pH of R3B and R4 (line 5, Fig. 3), requiring more NaOH addition. The degradation rate of tetryl appeared to increase slightly in R4, but not in R3B (line 5, Figs. 4 and 5) in response to the increased molasses.

The rate of tetryl degradation in the treatment reactors with the composite soil slurry appeared to slow around Day 100 (Fig. 5). The available literature indicated that aniline was a breakdown product of tetryl under some conditions (Boopathy and Manning [26]). With the high initial tetryl concentrations in these reactors, the aniline concentrations that could be produced, even transiently, had the potential to be inhibitory to the biological process (Osano et al. [29]). Therefore, a frozen archive slurry sample taken from reactor R3A on Day 86 (just prior to the apparent slow down) was sent to an outside laboratory (Aqua Pro-Tech Laboratories, Fairfield, NJ, USA) for a full GC/MS screen for all semivolatile compounds present. The estimated concentration of tetryl in the sample was approximately 30-fold lower than the concentration determined by Envirogen using EPA Method 8330. This likely reflects extraction efficiency differences between EPA Method 8330 and EPA Method 8270, which indicates that the results should be considered qualitative or semi-quantitative, and interpreted with caution. Additionally, the analysis yielded no peaks which matched with the library of mass spectra to a sufficient degree to allow definite identification, and aniline was not detected. The closest matches to products that could have been derived from tetryl and TNT are presented in Table 3. All the potential breakdown products were present at low estimated concentrations (<150 mg/kg, or <40 mg/l assuming all the compound was in the aqueous phase), and would not likely be inhibitory to microorganisms in the bioslurry.

The last operational change was sparging the slurry with nitrogen gas, which was started on Day 151 for R2 and Day 162 for R3A. Since tetryl degradation is a reductive process, it is desirable that more of the reducing equivalents (i.e. electrons) produced by consumption of the molasses be directed towards tetryl molecules as opposed to other electron acceptors like oxygen. It was postulated that nitrogen sparging would scrub residual oxygen, and was also expected to lead to a reduction in the Eh of the slurry, leading to increased tetryl degradation. Based on the data presented in Fig. 5, sparging with nitrogen did not result in decreased DO or Eh (line 6, Fig. 4), nor did it have any appreciable affect on the tetryl degradation rate (line 6, Fig. 5). However, the sparging did appear to lead to an increase in the pH of R2 (but not in R3A), presumably as CO_2 was purged from the slurry.

Table 3
Summary of GC/MS analysis of potential tetryl and TNT breakdown products detected in the bioslurry reactor

Name	Structure	Peak quality[a]
Potential tetryl metabolites		
5-Nitro-1H-benzimidazole		59
4-Nitrobenzofurazan		35
Potential TNT metabolites		
2-Amino-4,6-dinitrotoluene[b]		62
2-Methyl-3,5-dinitrophenol		25

[a] A higher peak quality indicates a greater match between the MS spectrum of the detected peak and the actual compound.
[b] Alternate names: 2-methyl-3,5-dinitroaniline, 2-methyl-3,5-dinitrobenzamine.

It is often the practice during field-scale bioremediation to operate reactors in sequencing batch as opposed to batch mode. During sequencing batch mode operation, and initial amount of the contaminated medium (i.e. soil, water, sediment) is loaded into the reactor and the biological process is initiated. When the concentration of the pollutant is reduced to below a given level (i.e. below the treatment goal or below the analytical detection limit), a volume of the treated material is removed, and an equivalent volume of contaminated medium is added. This allows the lag phase or acclimation period to be greatly reduced, and a large volume of active biomass to immediately start degrading the newly added contaminants. This mode of operation has proved successful during previous explosive compound bioremediation efforts (Manning et al. [30]). Therefore, after the tetryl concentrations in R2 had decreased to below the detection limit on Day 179 (10 mg/kg), a 25% (v/v) slurry replacement of the treated slurry with new contaminated soil slurry was performed (line 7, Fig. 5). Tetryl concentrations decreased from 20,000 mg/kg to below the detection limit in 45 days, at which time a second 25% slurry replacement was performed (line 9, Fig. 5). Slurry replacements for the control reactor, R3A and R3B were performed on Day 207, followed by continued tetryl degradation in R3A and R3B (line 8, Fig. 5). The slight increase in the tetryl concentration of the control reactor after slurry replacement was likely

due to higher concentrations of tetryl in the batch of soil used to prepare the slurry for the replacement, which is also reflected in the slightly higher "spike" in tetryl concentration in R3A, R3B, and the second R2 replacement (∼28,000 mg/kg) compared to the first R2 replacement (∼20,000 mg/kg).

At the time of the first slurry replacement for R3A and R3B (Day 207), the Student's *t*-test for two samples assuming unequal variances was performed pairwise on results from replicate slurry samples from each reactor. This analysis indicated that the molasses-fed reactors (R2, R3A, R3B) possessed statistically lower ($P < 0.003$, two-tail) tetryl concentrations than the control reactor (R1). At this timepoint, the tetryl concentrations in R3A and R3B were also statistically lower than in R2 ($P < 0.05$).

By the termination of the experiment on Day 299, tetryl concentrations in the control reactor were stable at approximately 125,000 mg/kg, R3A was below 20 mg tetryl/kg, and concentrations in R2 and R3B were exhibiting a downward trend from approximately 14,000 to 5000 mg/kg (Fig. 5). The tetryl in R4 had decreased from 550,000 to 165,000 mg/kg, or 70%, in 300 days (Fig. 4). A statistical analysis (Student's *t*-test on two samples assuming unequal variances) of results from R4 during the initial 50 days and the final 50 days of the experiment indicated a highly significant ($P < 3 \times 10^{-27}$, two-tail) decrease in tetryl concentrations. It is expected that had operation of reactor R4 been maintained, the concentration would likely have continued to decrease.

This last observation is a very interesting result from a scientific point of view in that it supports the idea that microbial communities can survive and thrive in the presence of extremely high concentrations of explosives. The limited microbial toxicity data using the *V. fischeri*-based Microtox® assay designated tetryl as very toxic, with an $EC_{50} < 0.5$ mg/l (Drzyzga et al. [10]). Extensive work with the related nitroaromatic TNT has been published, indicating that while some bacterial strains are also acutely sensitive to TNT, culturable bacteria could be detected in soils heavily contaminated (>25,000 mg/kg) with TNT (Fuller and Manning [11]). In these cases, as well as the experiment reported here, the most likely explanation for microbial survival in the soil (or slurry) with extremely high tetryl concentrations is that the limited solubility of the compounds maintains the aqueous bioavailable concentrations at levels that are not lethal to the organisms.

4. Conclusions

These results indicated that soils with very high concentrations of tetryl (ca. >100,000 mg/kg, and even as high as 50% (w/w)) are not inhibitory to biological degradation processes. Furthermore, soils with such high tetryl concentrations can be effectively treated using the bioslurry process, and a sequencing batch mode of operation is possible.

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