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Mineralization of 2,4- and 2,6-Dinitrotoluene in Soil Slurries

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DNT-degrading bacteria can completely degrade mixtures of DNT in liquid cultures without the production of aminonitrotoluenes. We determined whether specific DNT-degrading isolates could also degrade DNT from contaminated soil in the presence of indigenous microbial communities. When 2,4-DNT- and 2,6-DNT-degrading strains were added to a mixture of 2,4-DNT and 2,6-DNT in a soil slurry, disappearance of DNT was accompanied by $^{14}CO_2$ release and stoichiometric appearance of nitrite. When soil historically contaminated with mixed DNT isomers was used in slurries, the combination of DNT-degrading strains removed all of the DNT from the aqueous phase and over 99% of the initial DNT. Traces of extractable DNT remained associated with the soil; however, the toxicity of the treated soil was low. After an extended acclimation period, the 2,4-DNT, and, much later, the 2,6-DNT, was degraded in the uninoculated control. The results show that aged DNT contamination can be removed effectively from soil. The addition of specific DNT-mineralizing bacteria dramatically enhances the mineralization of DNT in soil slurries. Native bacteria do not convert DNT to aminonitrotoluenes during the short incubation times required for mineralization of DNT.

Introduction

Dinitrotoluenes (DNT) are intermediates in the manufacture of 2,4,6-trinitrotoluene (TNT), once the world's most widely used explosive, and the precursor of toluene diisocyanate used for the manufacture of polyurethane foams (*1*). Disposal practices associated with TNT manufacturing during and after World Wars I and II have resulted in an enormous contamination problem at ammunition production and handling facilities in the US and worldwide. Both 2,4- and 2,6-DNT are listed as US EPA priority pollutants in the Clean Water Act, the Safe Drinking Water Act, the RCRA, and the CERCLA.

Specific bacteria capable of using mono- and dinitroaromatic compounds as growth substrates have been detected at explosive-contaminated sites (*2*). Bacterial strains that degrade 2,4-DNT have been isolated and characterized and the degradation pathway determined (*2*−*4*). The pathway involves dioxygenation of 2,4-DNT to 4-methyl-5-nitrocatechol; monooxygenation of 4-methyl-5-nitrocatechol then yields 2-hydroxy-5-methylquinone which is enzymatically reduced to 2,4,5-trihydroxytoluene prior to ring cleavage. 2,4-DNT-degrading bacteria immobilized in bioreactors were shown to continuously degrade 2,4-DNT from aqueous solutions (*5*, *6*). Early isolates of 2,4-DNT-degrading bacteria could not, however, degrade mixtures of DNT isomers, and the presence of high concentrations of 2,6-DNT (=250 μM) inhibited 2,4-DNT degradation (*7*).

Recently, we have discovered strains able to use 2,6-DNT as the sole growth substrate (*8*). The isolation of strains able to mineralize 2,6-DNT, and strains better able to degrade 2,4-DNT, has enabled the combined use of 2,4- and 2,6-DNT-degrading bacteria for the simultaneous degradation of mixed DNT isomers. Mixtures of DNT-degrading strains effectively removed both isomers of DNT from contaminated water in a fluidized bed reactor (*9*).

Issues of bioavailability and toxicity as well as a lack of appropriate microorganisms have hindered efforts to develop biotreatment strategies for heavily contaminated soil. Although bioslurry treatment for explosive-contaminated soils has been studied by a number of groups (*10*−*12*), to date we are unaware of a successful treatment strategy for soil predominantly contaminated with DNT. We have conducted experiments to determine whether bacteria able to degrade DNT in contaminated water can be used for treatment of contaminated soil. A major goal was to determine whether bacteria can remove DNT from historically contaminated soil, and whether mineralization or reduction to amines is the predominant transformation.

Experimental Section

Bacterial Cultures. Isolation and characterization of DNT-degrading bacteria will be described elsewhere (*13*). A combination of Biolog and 16S rDNA analysis was used to identify selected isolates. The strains used for these studies were *Hydrogenophaga palleronii* JS863, a 2,6-DNT-degrading strain; *Burkholderia cepacia* JS872, a 2,4-DNT-degrading strain; and *Burkholderia cepacia* JS922, a strain capable of simultaneous degradation of 2,4- and 2,6-DNT.

Induced cultures of DNT-degrading strains were maintained on nitrogen-free minimal medium (BLKN) (*14*) solidified with 1.8% agar containing a dispersion of powdered Amberlite XAD-7 resin (2.5 g L^{-1} dry weight). The appropriate isomer of DNT was added at a concentration of 550 mg L^{-1} as the sole source of carbon, nitrogen, and energy. Medium for JS922 contained equal amounts of 2,4- and 2,6-DNT (275 mg L^{-1} each). Cells removed from plates were used as inocula for liquid cultures.

Samples of soil slurry from the final draw and fill reactor cycles (see below) were diluted and spread onto BLKN agar plates and tryptic soy agar (TSA) plates. Colony forming units (CFUs) were determined on all media. Individual colonies from selective plates were patched onto TSA plates and incubated for 48 h at 30 °C and then inoculated into individual wells of 96-well plates containing BLKN supplemented with 50 μM 2,4-DNT, 2,6-DNT, or a combination of 2,4- and 2,6-DNT. After incubation for 5 days at 30 °C, DNT degradation was assessed by nitrite release. Randomly selected nitrite-releasing strains were examined microscopically for cell morphology and Gram stain reaction and then further characterized with Biolog GN test plates.

Draw and Fill Reactor. An all-glass bioreactor with a helical ribbon impeller and a 1.2 L working volume was custom-made at the Fraunhofer Institute, IGB, Stuttgart,

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Germany. Two 1.5 cm diameter ports in the top of the reactor were left open for air exchange and sampling. Temperature was maintained at 30 °C, and reactors were stirred at 160 rpm. The reactor was filled with 600 mL of a 10% (w/v) soil slurry in phosphate buffer (20 mM, pH 7.0). The soil was from the former TNT-manufacturing plant at Hessisch Lichtenau, Germany (*10*). It consisted of equal parts by weight of the dried, sieved clay plus silt and sand fractions and contained 3.6 g of 2,4-DNT and 2.5 g of 2,6-DNT kg^{-1} soil. The organic content was 7.8% of dry weight. The soil suspension was stirred 12 h for equilibration, and a suspension of induced cells was added to initiate the experiment. The inoculum consisted of a mixture of 5 mL each of strains JS872, JS863, and JS922 (2.5, 3.4, and 3.5 mg of protein mL^{-1}, respectively). DNT concentration was monitored by high-performance liquid chromatography (HPLC). NaOH (50% w/v) was used to maintain the pH between 6.75 and 7.25. Initial DNT concentrations in the soil slurry totalled approximately 1 mM. When the concentrations of each isomer dropped below 20 μM, 90% of the slurry was drained from the bottom of the reactor, and additional contaminated soil and phosphate buffer were added to the original level. No further additions of bacteria or nutrients were made during the 600 h experiment. An identical control reactor was operated without added bacteria.

Mineralization Experiments. Experiments with pure cultures and with 10% (w/v) slurries of uncontaminated soil were conducted in duplicate 250 mL shake flasks containing 25 mL of BLKN. KOH (5 N, 200 μL) was added as a CO_2 trap to glass center wells in the bottom, and the flasks were sealed with ground glass stoppers. For experiments with single isomers, 2,4-DNT was provided at 1 mM, and 2,6-DNT at 200 μM. Cultures receiving mixtures of DNT were provided 2,4-DNT at 800 μM and 2,6-DNT at 200 μM. Controls were treated with $HgCl_2$ (2.5 μg mL^{-1}). The soil was a dried, sieved (20 mesh) composite of uncontaminated soils (organic content 2.1%) obtained from a variety of sites in the U.S. Flasks were incubated at 30 °C with shaking (100 rpm).

Mineralization experiments with aged contaminated soil were conducted in a 2 L slurry bioreactor with a 1.8 L working volume. Spent soil slurry (200 mL) from the draw and fill reactor (see above) was added as an inoculum to 1.6 L of 10% (w/v) Hessisch Lichtenau soil slurry in phosphate buffer (20 mM, pH 7.0). The reactor contained approximately 570 mg of 2,4-DNT and 390 mg of 2,6-DNT from the soil and 40 μCi of radiolabeled DNT (0.43 mg of 2,4-DNT, 0.14 mg of 2,6-DNT, each isomer added individually in separate sequential experiments). The slurry was stirred at 160 rpm with a helical ribbon impeller, and the temperature was 30 °C. The headplate and all fittings were stainless steel. Air was pumped across the surface of the slurry at 1 L h^{-1}; effluent air was sparged through a gas washing bottle containing NaOH (0.24 N).

Sampling Methods. Slurries were stirred or shaken vigorously and samples immediately withdrawn through a large-bore pipet tip to prevent settling of solids. Samples for HPLC analysis were centrifuged through a 0.22 μm filter into a microcentrifuge tube. Solids collected on the filter were washed with a volume of acetonitrile equal to the original sample volume, and the acetonitrile was combined with the aqueous phase. DNT recovery averaged 97% in spiked samples. The combined extracts were used for HPLC analysis for DNT concentration and soluble radioactivity. Total radioactivity was determined from unfiltered samples. $^{14}CO_2$ traps were changed at each sampling period. The KOH in the 250 mL shake flasks was pooled with 200 μL of rinse water and added to scintillation cocktail (Scintiverse II, Fisher) with 1 mL of MeOH. NaOH was added to scintillation cocktail (Rotiszint 2211) without further amendment.

Samples without soil were centrifuged to remove the cells. The cell pellet was used for protein determination, and the supernatant was used for nitrite analyses. When soil was present, protein determination was omitted, but samples were centrifuged through a 0.22 μm filter into a microcentrifuge tube, and the filtrate was used for nitrite analysis.

Analytical Methods. HPLC was performed with a Hypercarb porous graphite column (5 μm × 150 mm, Hypersil, U.K.) with a mobile phase of acetonitrile/water (90:10) containing trifluoroacetic acid (0.55 mL L^{-1}). Temperature was 20 °C, and flow rate was 1 mL min^{-1}. Compounds were detected by A_{230}. Under these conditions, retention times of 2,6-DNT, TNT, and 2,4-DNT were 2.3, 5.4, and 6.9 min. HPLC analyses were performed on a Waters Millenium II system equipped with a diode array detector or on a Gynkotek system equipped with a UVD340S diode array detector. Nitrite and nitrate analyses were performed using a colorimetric method (*15*) or by ion chromatography (*16*). Protein was measured using the Pierce BCA protein assay kit. The Toxicity Characteristic Leaching Procedure (TCLP) (*17*) and the luminescent bacteria test (*18*) were carried out according to standard guidelines with the exception that less than 100 g of soil was available for each of the tests.

Chemicals. 2,4-Dinitro[ring-U-^{14}C]toluene (16.6 mCi $mmol^{-1}$) was from ChemSyn (Lenaxa, KS). It was purified by HPLC before use to >98% radiochemical purity. 2,6-Dinitro-[ring-U-^{14}C]toluene (51 mCi $mmol^{-1}$) was from Amersham. It was 98% radiochemically pure as determined by HPLC and was used without further purification.

Results and Discussion

Mineralization of DNT. When pure cultures of DNT-degrading bacteria were provided either 2,4- or 2,6-DNT, the DNT disappeared from the culture fluid within 48 h (Figure 1A,B). Two moles of nitrite was released per mole of DNT consumed which indicated complete degradation of DNT. Over 40% of the initial radiolabel was trapped as $^{14}CO_2$, indicating levels of mineralization expected for growth substrates. Protein concentrations doubled twice for cultures grown with 200 μM 2,6-DNT and over 4 times for cultures grown with 800 μM 2,4-DNT. Uninoculated controls released no NO_2^- or $^{14}CO_2$, and DNT concentrations in the culture fluids remained unchanged. Trace amounts of 2-amino-6-nitrotoluene were detected in cultures provided 2,6-DNT.

Nonsterile, noncontaminated soil was added to cultures which contained strains JS872 and JS863 and both isomers of DNT. Only one isomer of DNT was radiolabeled in each flask. Nitrite accumulation was stoichiometric with DNT disappearance, and 45% of the initial radiolabel from 2,4-DNT and 43% of the initial radiolabel from 2,6-DNT were trapped as $^{14}CO_2$ (Figure 1C). No DNT reduction products were detected in the culture fluids. No DNT was detectable in the aqueous phase or in the acetonitrile extracts at the end of the experiment. The results indicate clearly that the presence of soil and indigenous bacteria did not interfere with the aerobic mineralization of low concentrations of recently added DNT.

Biodegradation of DNT in Aged Contaminated Soil in a Draw and Fill Slurry Reactor. The bioavailability of nitroaromatic compounds in historically contaminated soils can be dramatically reduced by sorption to soil (*19*). The organic extraction kinetics of explosives vary considerably between field-contaminated soils and amended soils as well as among types of soils (*20*). Organism-specific properties can make sorbed substrates more bioavailable in some instances and less bioavailable in other instances (*21*). Because of potential differences between DNT-amended soils and field-contaminated soils, a study using aged DNT-contaminated soil was conducted.

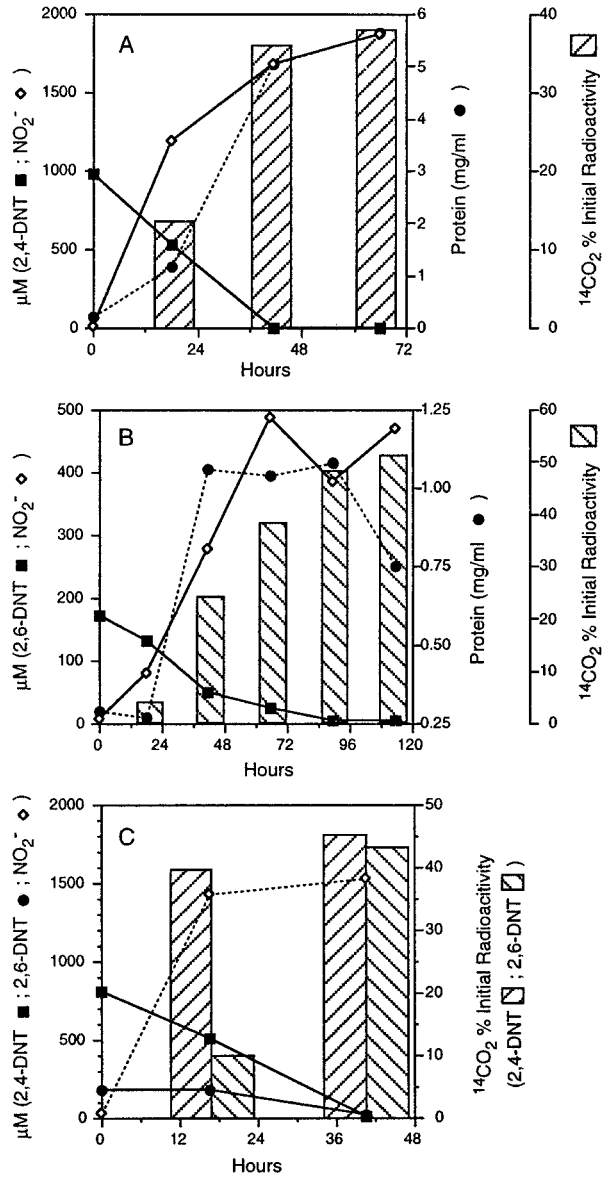

FIGURE 1. Mineralization of DNT. (A) *B. cepacia* JS872 with 2,4-DNT, average of duplicate cultures. (B) *H. palleronii* JS863 with 2,6-DNT, average of duplicate cultures. (C) Mixed culture of JS872 and JS863 in the presence of nonsterile soil. Shown is the average of four cultures: two each with 2,4-dinitro[ring-U-^{14}C]toluene and unlabeled 2,6-DNT, and two each with unlabeled 2,4-DNT and 2,6-dinitro[ring-U-^{14}C]toluene. Lines represent average of all cultures for the disappearance of DNT analyzed by HPLC. Bars represent the average of radiolabeled substrates.

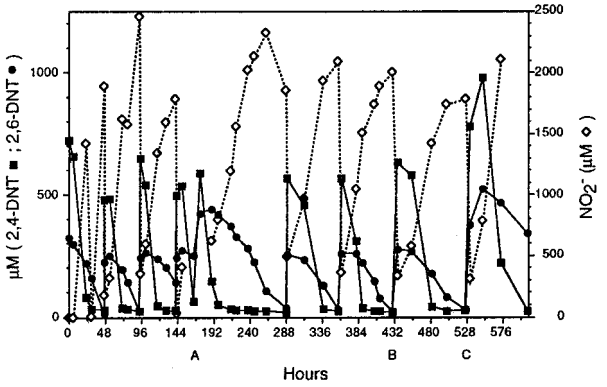

FIGURE 2. Draw and fill soil slurry reactor inoculated with DNT-degrading bacteria. (A) Additional 10% of soil (by weight) added when 2,4-DNT was gone but 2,6-DNT remained; (B) temperature of reactor reduced to 23−25 °C; (C) temperature returned to 30 °C, soil concentration doubled to 20%. Incubation was continued for 6 days past the last time point shown. DNT concentrations were reduced to residual levels, but the shape of the curve is unknown.

Experiments with aged field-contaminated soil were conducted in a bench scale slurry reactor. Within 30 h of inoculation, the 2,4-DNT was degraded to a residual concentration of approximately 20 μM; 2,6-DNT was degraded to the same concentration within 46 h (Figure 2). Two moles of nitrite was released per mole of DNT consumed which clearly indicated that the DNT was degraded by the oxidative pathway used for mineralization. The length of time for completion of each cycle remained roughly constant throughout the run. Oxygen usage at 30 °C averaged 97 μg of O_2 L^{-1} min^{-1}, and oxygen concentrations in the reactors remained above 2.5 mg L^{-1} throughout the experiment.

The time required to complete the cycles doubled when the temperature was reduced from 30 to 24 °C. Doubling the soil concentration of the slurry lengthened the time required for degradation proportionally. The fact that reinoculation was not required indicated that the bacteria grew on DNT in a self-sustaining process.

The residual levels of DNT at the end of each cycle were similar throughout the experiment regardless of the length of the cycle. Even after extended incubation a residual concentration of 20 μM total DNT in the slurry remained associated with the sediment. The residual DNT was not extractable with water and was detected only after the acetonitrile extraction. At the beginning of each cycle, 30−40% of the total DNT was in the aqueous phase. The percentage of DNT in the aqueous phase decreased to zero as total DNT levels in the slurry decreased during each cycle. The amount of DNT extractable by acetonitrile from the spent soil slurry stabilized at 35 and 30 mg kg^{-1} for 2,4- and 2,6-DNT, respectively, and did not change as the soil fraction was allowed to air-dry at room temperature over several days. Extended incubation of the soil slurry at 30 °C, with agitation for 8 days, reduced the residual concentrations of 2,4- and 2,6-DNT to 8 and 12 mg kg^{-1}. The concentration of 2,6-DNT removed from the soil slurries was approximately double the concentration that completely inhibits growth of bacteria in liquid cultures. The results suggest that partitioning of the DNT to soil reduces its toxicity without dramatically limiting its availability for biodegradation. This outcome is consistent with the prediction that DNT should not partition readily to the soil phase based on the relatively low organic carbon soil partition coefficient of DNT (*22*).

In the uninoculated reactor, the 2,4-DNT began to disappear, and nitrite was released after 118 h. The 2,4-DNT reached the residual level at 190 h. 2,6-DNT disappeared in the uninoculated reactor after 500 h with stoichiometric release of nitrite. When the uninoculated reactor was drawn down and refilled with fresh soil and buffer, 2,4-DNT reached the residual level after 78 h, and 2,6-DNT reached the residual level between 78 and 200 h. Disappearance of the DNT from the uninoculated reactor might have been the result of airborne cross-contamination by DNT-degrading bacteria; the reactors were open to the atmosphere and in close physical proximity.

Other evidence suggests, however, that the soil served as a source of indigenous bacteria capable of acclimation to degrade the contaminants. At the end of the experiment, none of the isolates recovered from either reactor appeared to be the same as those in the original inoculum by the Biolog test. Although population shifts were not quantitated, it seems likely that the bacteria in the inoculum may have been outcompeted by indigenous bacteria as the experiment progressed. Indigenous bacteria capable of specific degra-

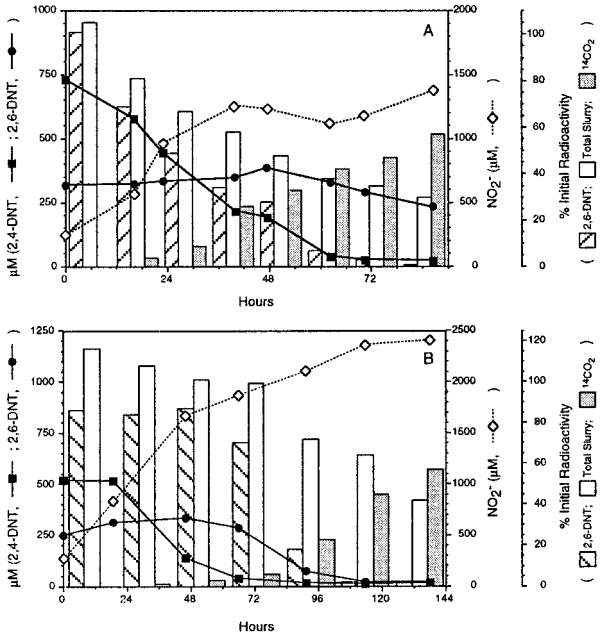

FIGURE 3. Mineralization of DNT in aged contaminated soils. Contaminated soil slurry was labeled with (A) 2,4-dinitro[ring-U-^{14}C]toluene or (B) 2,6-dinitro[ring-U-^{14}C]toluene. Striped bars represent the radioactivity of individual DNT peaks as separated by HPLC. White bars represent the radioactivity in the total slurry suspension (aqueous phase, soil solids, and cells). Recovery of radiolabel was 84% for (A) and 95% for (B).

dative activity often displace an inoculated population with the same degradative capability (*23*, *24*). Even in such cases, however, the original inoculum can significantly decrease the lag time before degradation of the contaminant begins (*25*). It is also possible that the genes that encode the ability to degrade DNT were transferred to indignenous strains during the experiment.

Mineralization of DNT in Aged Contaminated Soils. DNT disappeared almost completely from the draw and fill reactor, and extensive release of nitrite provided strong evidence of mineralization. However, in other systems containing soil (*26*), questions have been raised about the ultimate fate of the contaminants and their metabolites. We designed experiments with radiolabeled DNT to provide rigorous evidence regarding the destruction of the contaminants. In each experiment, all of the radiolabeled DNT added and 99% of the unlabeled DNT from the soil were degraded (Figure 3). Almost 60% of the initial radiolabel was recovered as $^{14}CO_2$. The high level of mineralization, taken with the stoichiometric release of nitrite, clearly indicates that undegraded byproducts do not accumulate in the aerobic soil slurry reactor. It is likely that the added radiolabeled DNT did not completely equilibrate with the less available fraction of the contaminants in the aged sediment. Small amounts of residual nonradiolabeled DNT extractable by acetonitrile remained bound to the soil. Thus, it seems likely that the radiolabel provided a good estimate of the distribution of products from the bulk of the contamination, but might have been less effective at providing insight into the behavior of the residual contamination.

Toxicity of Residual DNT in Soil. No DNT remained in the aqueous phase after treatment, and over 99% of the DNT in the soil was degraded after short (2−3 days) incubation. Extended incubation decreased the residual concentrations of DNT and significantly reduced the DNT concentration in the leachate (0.08 and 0.36 mg L^{-1} for 2,4- and 2,6-DNT) measured using TCLP Method 1311 to below the level deemed a characteristic hazardous waste (0.13 mg L^{-1} for 2,4-DNT; there is no standard for 2,6-DNT) by the RCRA standard established in the Code of Federal Regulations (section 40, paragraph 261.24). The luminescent bacteria test for ecotoxicological effects resulted in a G_L value of 4 for treated soil and a G_L value of 48 for the untreated soil. The G_L value is the lowest dilution that results in a less than 20% reduction of light emission; values of 8 or more are considered toxic (*18*).

Transformation Reactions. In experiments with specific DNT-degrading bacteria, the nonspecific transformations were insignificant as evidenced by detection of only trace amounts of aminotoluenes and complete release of the nitro groups as nitrite. The results are in marked contrast to findings from natural assemblages of DNT-degrading bacteria. Previous workers have reported that mineralization of 2,4- and 2,6-DNT by natural river water populations collected downstream of a TNT-manufacturing plant followed lag periods of up to 3 weeks (*27*). Based upon rate studies, the authors concluded that at low DNT concentrations, mineralization played only a minor role in the removal of DNT from surface waters. A series of recent papers (*28*−*30*) reported mineralization of 2,4- and 2,6-DNT by mixtures of microorganisms indigenous to an explosives-contaminated shallow aquifer at Weldon Spring Ordnance Works. DNT concentrations were less than 20 mg L^{-1}, but after 28 days only 28 and 8% of the initial 2,4- and 2,6-DNT were mineralized, with the remainder undegraded, or transformed to aminonitrotoluenes or unidentified metabolites. The picture that emerged from the Weldon Spring investigations was one of slow mineralization in competition with nonspecific transformation reactions. It appears that under optimum conditions in a bioreactor the rate of reductive transformations is negligible compared to the rate of mineralization. The lack of reductive transformations is particularly evident in the uninoculated reactor.

Situations where the degradation of a synthetic contaminant supports the growth of specific bacteria will have a high potential for successful bioremediation or natural attenuation. It is clear from previous work with contaminated water (*9*) and the present work that biodegradation is a highly effective treatment strategy for removal of DNT contamination from soil or groundwater. In situations where DNT is the predominant contaminant of soil or groundwater, the remediation strategies described here should provide substantially more cost-effective cleanup than traditional strategies.

Because bacteria able to degrade nitrotoluenes are widely distributed at contaminated sites, it is not clear why the contaminants have persisted for so long. The soil from Hessisch Lichtenau has previously been shown to contain bacteria able to degrade 2,4-DNT (unpublished results). The results raise the question of what limits natural attenuation at Hessisch Lichtenau and similar sites containing appropriate organisms and what can be done to stimulate biodegradation of DNT by indigenous DNT-degrading populations.

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Literature Cited

(1) Hartter, D. R. In *Toxicity of nitroaromatic compounds*; Rickert, D. E., Ed.; Hemisphere Publishing Corp.: Washington, D.C., 1985; pp 1−13.

(2) Spain, J. C. In *Biodegradation of nitroaromatic compounds*; Spain, J. C., Ed.; Plenum Publishing Corp.: New York, 1995; pp 19−35.

(3) Spanggord, R. J.; Spain, J. C.; Nishino, S. F.; Mortelmans, K. E. *Appl. Environ. Microbiol.* **1991**, *57*, 3200−3205.

(4) Haigler, B. E.; Nishino, S. F.; Spain, J. C. *J. Bacteriol.* **1994**, *176*, 3433−3437.

(5) Heinze, L.; Brosius, M.; Wiesmann, U. *Acta Hydrochim. Hydrobiol.* **1995**, *23*, 254−263.

(6) Reardon, K. F.; Spain, J. C. In *Abstracts of the 205th ACS National Meeting*; American Chemical Society: Washington, D.C., 1993.

(7) Nishino, S. F.; Spain, J. C. In *Abstracts, Second SETAC World Congress*; Society of Environmental Toxicology and Chemistry: Pensacola, 1995; p 277.

(8) Nishino, S. F.; Spain, J. C. In *Abstracts, 96th General Meeting of the American Society for Microbiology*; American Society for Microbiology: Washington, D.C., 1996; p 452.

(9) Lendenmann, U.; Spain, J. C.; Smets, B. F. *Environ. Sci. Technol.* **1998**, *32*, 82−87.

(10) Lenke, H.; Warrelmann, J.; Daun, G.; Hund, K.; Sieglen, U.; Walter, U.; Knackmuss, H.-J. *Environ. Sci. Technol.* **1998**, *32*, 1964−1971.

(11) Young, D. M.; Kitts, C. L.; Unkefer, P. J.; Ogden, K. L. *Biotechnol. Bioeng.* **1997**, *56*, 258−267.

(12) Shen, C. F.; Guiot, S. R.; Thiboutot, S.; Ampleman, G.; Hawari, J. *Biodegradation* **1998**, *8*, 339−347.

(13) Nishino, S. F.; Paoli, G.; Spain, J. C., in preparation.

(14) Bruhn, C.; Lenke, H.; Knackmuss, H.-J. *Appl. Environ. Microbiol.* **1987**, *53*, 208−210.

(15) Smibert, R. M.; Krieg, N. R. In *Methods for general and molecular bacteriology*; Gerhardt, P., Murray, R. G. E., Wood, W. A., Krieg, N. R., Eds.; American Society for Microbiology: Washington, D.C., 1994; pp 607−654.

(16) Vorbeck, C.; Lenke, H.; Fischer, P.; Spain, J. C.; Knackmuss, H.-J. *Appl. Environ. Microbiol.* **1998**, *64*, 246−252.

(17) US Environmental Protection Agency. Toxicity Characteristic Leaching Procedure, Test Method 1311.

(18) DECHEMA. Bioassays for soils/Ad-Hoc-Committee "Methods for Toxicological/Ecotoxicological Assessment of Soils". DECHEMA, Deutsche Gesellschaft für Chemisches Apparatewesen, Chemische Technik und Biotechnologie e.V.

(19) Grant, C. L.; Jenkins, T. F.; Myers, K. F.; McCormick, E. F. *Environ. Toxicol. Chem.* **1995**, *14*, 1865−1874.

(20) Jenkins, T. F.; Walsh, M. E.; Schumacher, P. W.; Miyares, P. H.; Bauer, C. F.; Grant, C. L. *J. Assoc. Off. Anal. Chem.* **1989**, *72*, 890−899.

(21) Guerin, W. F.; Boyd, S. A. *Appl. Environ. Microbiol.* **1992**, *58*, 1142−1152.

(22) Spanggord, R. J.; Mabey, W. R.; Chou, T. W.; Smith, J. H. In *Toxicity of nitroaromatic compounds*; Rickert, D. E., Ed.; Hemisphere Publishing Corp.: Washington, D.C., 1985; pp 15−33.

(23) Nishino, S. F.; Spain, J. C.; Pettigrew, C. A. *Environ. Toxicol. Chem.* **1994**, *13*, 871−877.

(24) Comeau, Y.; Greer, C. W.; Samson, R. *Appl. Microbiol. Biotechnol.* **1993**, *38*, 681−687.

(25) el Fantroussi, S.; Mahillon, J.; Naveau, H.; Agathos, S. N. *Appl. Environ. Microbiol.* **1997**, *63*, 806−811.

(26) Griest, W. H.; Stewart, A. J.; Tyndall, R. L.; Caton, J. E.; Ho, C.-H.; Ironside, K. S.; Caldwell, W. M.; Tan, E. *Environ. Toxicol. Chem.* **1993**, *12*, 1105−1116.

(27) Bausum, H. T.; Mitchell, W. R.; Major, M. A. *J. Environ. Sci. Health A* **1992**, *27*, 663−695.

(28) Bradley, P. M.; Chapelle, R. H.; Landmeyer, J. E.; Schumacher, J. G. *Appl. Environ. Microbiol.* **1994**, *60*, 2170−2175.

(29) Bradley, P. M.; Chapelle, F. H.; Landmeyer, J. E. In *Bioremediation of Recalcitrant Organics: International In Situ On Site Bioreclamation Symposium*; Hinchee, R. E., Anderson, D. B., Hoeppel, R. E., Eds.; Battelle Press: Columbus, OH, 1995; pp 267−271.

(30) Bradley, P. M.; Chapelle, F. H.; Landmeyer, J. E.; Schumacher, J. G. *Groundwater* **1997**, *35*, 12−17.

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