Journal of Hazardous Materials B116 (2004) 167–171

Journal of Hazardous Materials

www.elsevier.com/locate/jhazmat

Short communication

Use of poultry litter for biodegradation of soil contaminated with 2,4- and 2,6-dinitrotoluene

Gian Gupta*, Hemalatha Bhaskaran

Department of Natural Sciences, University of Maryland Eastern Shore, Princess Anne, MD 21853, USA

Received 16 June 2004; received in revised form 9 September 2004; accepted 12 September 2004

Abstract

Pseudomonas sp. and *Pseudomonas putida* can utilize dinitrotoluene (DNT) as N-source after the enzymatic removal of nitro groups from the aromatic ring. Addition of nutrients is known to stimulate the biodegradation process. Poultry litter has consortia of microorganisms (including *Pseudomonas*) along with many nutrients. The objective of this research was to study the biodegradation of 2,4- and 2,6-DNT contaminated soil (from Badger Army Ammunition Plant) using poultry litter. Complete biodegradation of both 2,4- and 2,6-DNT in the soil was observed after 1-day interaction with poultry litter. No degradation was observed using autoclaved litter.

Keywords: 2,4-Dinitrotoluene; 2,6-Dinitrotoluene; Biodegradation; Poultry litter

1. Introduction

Nitrotoluenes have industrial applications as explosives and in the production of dyes and polyurethane foam. DNT is manufactured by mixing toluene with nitric acid in the presence of sulfuric acid resulting in the formation of 80% 2,4-DNT and 20% 2,6-DNT. 2,4-DNT is highly reactive and is an explosion hazard; 2,6-DNT does not evaporate. While the nitro-aromatic compounds are easily biodegradable, the nitrotoluenes persist for long periods in soil and water [1]. Most of the sites (including army ammunition plants) contaminated with nitrotoluenes belong to the US government. The Volunteer, Ravenna, and Badger Army Ammunition Plants have substantial amounts of 2,4- and 2,6-DNT contaminated soil (and underground water) that needs to be remediated. Contaminated soils are in most cases excavated and incinerated. New cleanup technologies at Badger Army Ammunition Plant include the use of UV radiation, steam flushing/stripping, co-solvents and chemical oxidation. Other technologies to remove the soil bound DNT include enzymatic reduction [2], reaction with cationic surfactants (cetyltrimethylammonium bromide [3]), and the use of an anaerobic fluidized bed granular activated carbon bioreactor [4]. Loss and transport of DNT in Wauconda Bay is believed to be controlled by photolysis, biotransformation and volatilization [5].

Pennington et al. [6] and Spain et al. [1] have written exhaustively on the immobilization of 2,4- and 2,6-DNT and their biodegradation. Most of the researchers have concluded that polynitroaromatic compounds can only be transformed to amino nitro compounds and cannot be mineralized. Others have reported the biodegradation of DNT [7–9] and its reduction to diamine stage under anaerobic conditions [10]. Ortega-Calvo [11] used the *Burkholderia* strain, whereas Hughes et al. [8] used *Clostridium acetobutylicum* for DNT biodegradation studies. *Pseudomonas sp*. Clone A [12] and *Pseudomonas putida* [13,14] can utilize DNT as N-source after the enzymatic removal of nitro groups from the aromatic ring. DNT degrading strains have been isolated only from industrial wastes (and not from activated sludge or other sites that have not been contaminated with DNT), which received continuous input of DNT [1].

* Corresponding author. Tel.: +1 410 651 6030; fax: +1 410 651 7579.
E-mail address: gcgupta@umes.edu (G. Gupta).

0304-3894/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2004.09.007

The microbial population of poultry litter is acidophilic bacteria, fungi, algae and aerobic heterotrophs [15]. This population includes *Pseuodomonas*, *Actinomycetes*, and *Nocardia*. Poultry litter contains a large amount of nutrients (NO_3, 4.1%; P_2O_5, 1.9%; K_2O, 2.5%) [16]. Poultry litter has been used for the biodegradation of atrazine [17], gasoline contaminated soil [18] and phenol [19]. The nutrients in the litter are needed by microorganisms for biodegradation of organic compounds [17–19]. Complete biodegradation of 10–50 ppm 2,4-DNT aqueous solution has been reported after 2 days interaction with poultry litter leachate (microorganisms) without the formation of any intermediates [20]. The objective of this research was to study the biodegradation of 2,4- and 2,6-DNT in the contaminated soil (from Badger Army Ammunition Plant) using poultry litter.

2. Materials and methods

Standard solutions of 2,4- and 2,6-DNT (97%, Aldrich Chemical Co.) were prepared in deionized water; aqueous solubility of 2,4- and 2,6-DNT is 180 and 120 ppm, respectively, at room temperature [21]. The standard solutions were analyzed by GC/ECD for calibration purposes [22]. The DNT-contaminated soil from the Badger Army Ammunition Plant was dried at room temperature and refrigerated in amber bottles. A representative sample of poultry litter was collected from the university farm, air-dried, homogenized, sieved (2 mm) and refrigerated. Soil (0.1 g) was mixed with poultry litter (0.0, 0.2, 0.4, 0.6, 0.8, 1.0 g) and 10 ml deionized water, shaken at 240 rpm (0, 6, 12, 24 h), then 10 ml of isoamyl acetate was added and shaken for 24 h to stop the reaction and extract DNT. Another 1 ml of isoamyl acetate was added to each solution to ensure the extraction of soil bound DNT, shaken again for 1 h, refrigerated for 1 h and the organic layer extracted. Using the analytical technique (GC/ECD; Agilent Technologies—Model 6890N series) employed by the USACHPPM Lab. [22] the organic layer (isoamyl acetate extract) was analyzed. The soil and poultry litter mixtures were spiked with DNT and extracted using isoamyl acetate to determine extraction efficiency. A sample of poultry litter was also autoclaved to kill the microorganisms and the experiment repeated with the autoclaved litter. All glassware used was sterilized.

3. Results and discussion

The moisture content of the contaminated soil was 3.5% and the pH was 9.27; these results compare well with the properties of the Badger Army Ammunition Plant soil reported by Zhang et al. [23]. The analytical method used for the extraction and measurement of DNT results in increased extraction of DNT, bound residue and metabolites compared with the USEPA Methods 8330 and 8095 [22].

The concentration of 2,4-DNT (Table 1) in the contaminated soil mixed with poultry litter decreased significantly both on increasing the amount of litter and the time of interaction. Complete removal of DNT took place with 1.0 g poultry litter after 12 h and with only 0.6 g litter after 24 h. The increase in the biodegradation of DNT both with an increase in the amount of litter and the time of interaction suggests the increasing role of the microorganisms. With 0.2 g litter the amount of 2,4-DNT after 24 h was <10% of the original amount (Table 1) suggesting that the mixtures were not under nutritional stress. No biodegradation of DNT was observed with autoclaved litter showing that the degradation observed with unsterilized litter was only by the microorganisms present in the litter. Based on the DNT extraction efficiency (97% and higher) from soil and litter samples spiked with DNT it is clear that adsorption of DNT was not taking place.

The amount of 2,6-DNT (Table 2) in the contaminated soil was almost one third the amount of 2,4-DNT. A decrease in the concentration of 2,6-DNT in the contaminated soil mixed with litter was also observed on increasing the amount of litter and the mixing time. In this case complete biodegradation took place on using a higher amount of litter and increased mixing time showing thereby that 2,6-DNT is more recalcitrant. Earlier research has also shown that biodegradation of 2,6-DNT is slower compared to the biodegradation of 2,4-DNT [24]. Biodegradation of 2,6-DNT is inhibited in the presence of relatively high 2,4-DNT concentrations [1].

None of the chromatograms (Fig. 1 shows only the control and the soil mixed with 0.4 g litter) showed any new peak

Table 1
Changes in 2,4-DNT concentration (ppm) in soil + poultry litter with time

Poultry litter (g)	0 h	6 h	12 h	24 h	LSD
0.0 (Control)	27.1 (0.7) a, x	27.1 (0.6) a, x	26.7 (0.5) a, x	26.9 (0.6) a, x	2.6
0.2	27.1 (0.5) a, w	24.8 (0.3) b, x	8.4 (0.6) b, y	2.3 (0.2) b, z	2.1
0.4	27.1 (0.6) a, w	23.0 (0.2) c, x	7.7 (0.3) b, y	1.5 (1.0) b, z	0.9
0.6	27.1 (0.7) a, w	20.2 (0.2) d, x	5.9 (0.2) c, y	0.0 (0.0) c, z	1.5
0.8	27.1 (0.5) a, w	18.4 (0.3) e, x	2.3 (1.2) d, y	0.0 (0.0) c, z	1.2
1.0	27.1 (0.5) a, w	9.4 (0.5) f, x	0.0 (0.0) e, y	0.0 (0.0) c, y	1.7
LSD	(0.9)	1.1	1.4	1.3	

Mean ± standard deviation in parenthesis; $n = 7$. Means followed by the same letter are not significantly different from each other ($p \leq 0.05$); a–f are column-wise comparisons and w–z are row-wise comparisons. LSD, least significant difference.

Table 2
Changes in 2,6-DNT concentration (ppm) in soil + poultry litter with time

Poultry litter (g)	0 h	6 h	12 h	24 h	LSD
0.0 (Control)	7.9 (0.1) a, x	7.9 (0.1) a, x	7.8 (0.1) a, x	7.9 (0.1) a, x	0.15
0.2	7.9 (0.1) a, x	7.8 (0.1) b, y	7.8 (0.1) a, y	7.6 (0.1) b, z	0.08
0.4	7.9 (0.1) a, w	7.1 (0.1) c, x	7.7 (0.1) b, y	7.5 (0.1) b, z	0.13
0.6	7.9 (0.1) a, w	7.1 (0.1) d, x	7.1 (0.1) c, x	6.2 (0.1) c, y	0.14
0.8	7.9 (0.1) a, w	7.0 (0.1) d, x	6.9 (0.1) d, y	2.4 (0.1) d, z	0.11
1.0	7.9 (0.1) a, w	4.9 (0.1) e, x	1.7 (0.1) d, y	0.0 (0.0) f, z	0.75
LSD	0.1	0.1	0.1	0.2	

Mean ± standard deviation in parenthesis; $n = 7$). Means followed by the same letter are not significantly different from each other ($p \leq 0.05$); a–f are column-wise comparisons and w–z are row-wise comparisons. LSD, least significant difference.

except for the peaks seen with contaminated soil mixed with poultry litter at 0 h. The absence of any new peak in the chromatograms shows that the biodegradation of 2,4- and 2,6-DNT is not accompanied with the formation of other amino or nitro organic compound intermediates. Use of isoamyl acetate has been shown to extract much higher amounts of DNT and metabolites compared with other solvents; the detection limit using toluene or benzene as solvents was around 1 ppm and nitramines could not be detected with earlier techniques [22]. A chemical kinetic study on the bio-degradation of DNT with time (Fig. 2) showed it as a pseudo-first-order rate reaction with a R^2-value and rate constant of 0.96 and $1.5 \times 10^{-1}\ h^{-1}$ for 2,4-DNT and 0.84 and $5.0 \times 10^{-2}\ h^{-1}$ for 2,6-DNT, respectively. The degradation patterns using other concentrations of both 2,4-DNT and 2,6-DNT were similar to the one shown in Fig. 2.

The mineralization of 2,4-DNT by natural river water populations collected downstream of a TNT plant showed a lag period of up to 3 week [25]. Nishino et al. reported that DNT-degrading bacteria can completely degrade mixtures of 2,4- and 2,6-DNT in soil slurries without the production of aminonitrotoluenes; adding DNT degrading strains in contaminated soil slurry resulted in the disappearance of DNT accompanied by CO_2 release [24]. Simultaneous biodegradation of 2,4- and 2,6-DNT using a mixed-culture biofilm reactor resulted in removal efficiencies over 98% [7]. Nutrient limitations control the onset of rapid DNT biodegradation; biodegradation of DNT was rapidly stimulated by the addition of a complete mineral medium but not by bicarbonate buffered deionized water or by phosphate amended tap water [26]. Using a mixed consortia (*Pseudomonas*, *Sphingomonas*, and *Stenotrophomonas*) Snellinx et al. have also

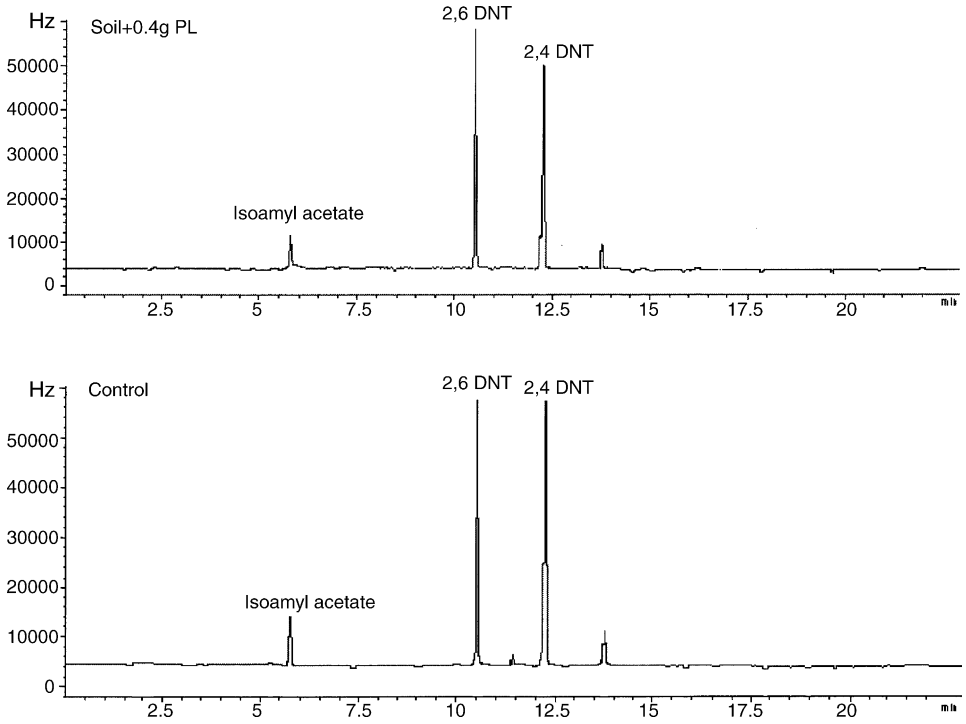

Fig. 1. Gas chromatograms of solvent extracts of DNT-contaminated soil mixed with poultry litter (*x*-axis shows time (min); *y*-axis shows abundance (Hz)).

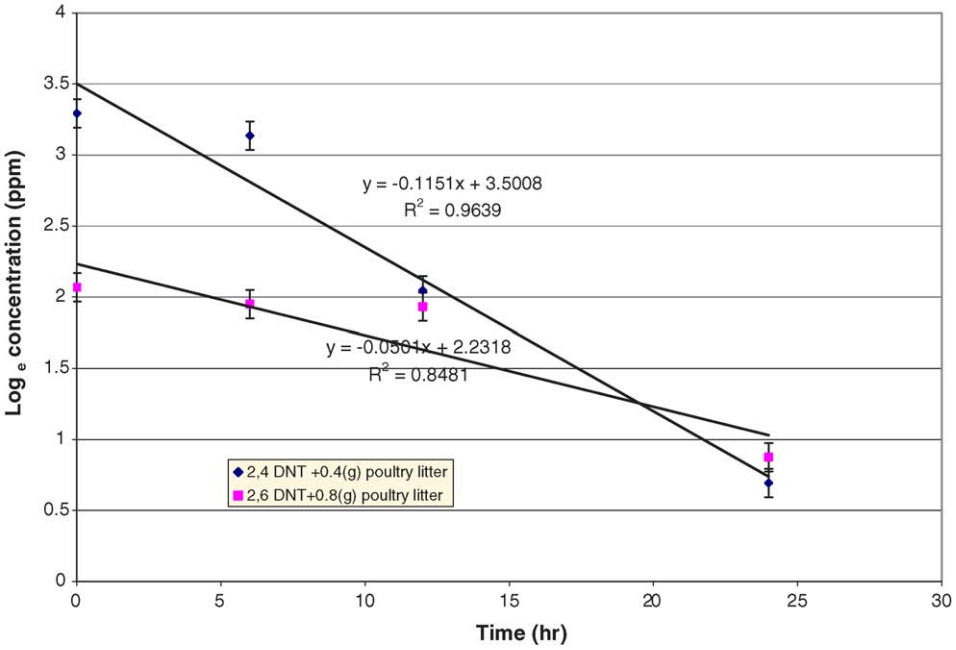

Fig. 2. Concentration of 2,4- and 2,6-DNT in solvent extracts of soil mixed with poultry litter vs. time of interaction (*x*-axis shows time (h); *y*-axis shows $\log_e$ DNT concentration (ppm) in isoamyl acetate extract of DNT-contaminated soil with poultry litter; error bars represent standard deviation).

reported the degradation of DNT without accumulation of any intermediates [27]; under aerobic conditions DNTs are degraded via oxygenase reactions [28].

From the results presented here it can be concluded that the consortia of microorganisms in the poultry litter can degrade 2,4- and 2-6-DNT without the production of other intermediates within a short period of 1 day. This is a significant finding as so far cultures from uncontaminated (without DNT) sites have not been shown to degrade DNT.

Acknowledgements

Thanks are due US Army-Waterways Experiment Station (MACERAC Grant) for partial financial support of this project.

References

[1] J.J. Spain, J.B. Hughes, H.J. Knackmuss, Biodegradation of nitroaromatic compounds and explosives, Lewis Publication, Boca Raton, FL, 2000.
[2] R.G. Riefler, B.F. Smets, Enzymatic reduction of 2,4 and 2,6-dinitrotoluene and related nitroarenes, Environ. Sci. Technol. 34 (2000) 3900–3906.
[3] R.A. Larson, C.T. Jafvert, F. Bosca, Effects of surfactants on reduction and photolysis of nitroaromatic compounds, Environ. Sci. Technol. 34 (2000) 505–508.
[4] N.R. Adrian, Pathway and controlling factors in the biodegradation of energetic wastes, 1998, www.cecer.army.mil.
[5] R.J. Spanggord, J.C. Spain, S.F. Nishino, K.E. Mortelmans, Biodegradation of DNT by *Pseudomonas sp.*, Appl. Environ. Microbiol. 57 (1991) 3200–3205.
[6] J.C. Pennington, K.A. Thorn, C.A. Hayes, B.E. Porter, K.R. Kennedy, Immobilization of 2,4- and 2,6-dinitrotoluenes in soils and composts. US Army Corps of Engineers, Waterways Experiment Station, Vicksburg, MS. ERDC/EL TR-032 (2003).
[7] U. Lendenmann, J.C. Spain, B.F. Smets, Simultaneous biodegradation of 2,4- and 2,6-dinitrotolune in an aerobic fluidized-bed biofilm reactor, Environ. Sci. Technol. (1998) 32.
[8] J.B. Hughes, C.Y. Wang, C. Zhang, Anaerobic biotransformation of 2,4- and 2,6-dinitrotoluene by Clostridium acetobutylicum: a pathway through dihydroxylamino intermediates, Environ. Sci. Technol. 33 (1999) 1065–1070.
[9] J. Cheng, Y. Kanjo, M.T. Suidan, A.D. Venosa, Anaerobic biotransformation of 2,4-dinitrotoluene with ethanol as primary substrate, Water Res. 30 (1996) 307–314.
[10] N.G. McCormick, F.F. Feeherry, H.S. Levinson, Microbial transformation of 2,4,6-trinitrotoluene and other nitroaromatic compounds, Appl. Environ. Microbiol. 31 (1976) 949–958.
[11] J.J. Ortega-Calvo, Biodegradation of sorbed 2,4-dinitrotoluene in a clay rich aggregated porous medium, Environ. Sci. Technol. 33 (1999) 3737–3742.
[12] A. Haidour, J.L. Ramos, Identification of products resulting from the biological reduction of 2,4-dinitrotoluene by *Pseudomonas sp.*, Environ. Sci. Technol. 30 (1996) 2365–2370.
[13] C. Park, T.H. Kim, S. Kim, S.W. Kim, J. Lee, S.H. Kim, Optimization for biodegradation of 2,4,6-TNT by *Pseudomonas putida*, J. Biosci. Bioeng. 95-6 (2003) 567–571.
[14] R.J. Miehr, J.Z. Bandstra, R. Po, P.G. Tratnyek, Remediation of 2,4,6-trinitrotoluene (TNT) by iron metal: kinetic controls on product distributions in batch and column experiments, in: 225th National Meeting, 23–27 March 2003, New Orleans, LA, vol. 43, No. 1, Division of Environmental Chemistry, pp. 644–648 (Preprint Extended Abstracts).
[15] R.M. Nodar, M.J. Acea, T. Carballas, Microbial populations of poultry pine-sawdust litter, Biol. Waste 3 (4) (1990) 295–306.
[16] M. North, D. Bell, Commercial Chicken Production Manual, 4th ed., Van Nostrand Reinhold, New York, 1990.
[17] G. Gupta, J. Baummer, Biodegradation of atrazine in soil using poultry litter, J. Hazard. Mater. 45 (1996) 185–192.

[18] G. Gupta, J. Tao, Bioremediation of gasoline contaminated soil using poultry litter, J. Environ. Sci. Health (Part A) 31 (9) (1996) 2395–2407.
[19] G. Gupta, V. Rao, Biodegradation of phenol with poultry litter microorganisms, J. Environ. Sci. Health (Part A) 33 (1) (1998) 83–95.
[20] G. Gupta, H. Bhaskaran, G. Kananen, J. Okoh, Biodegradation of 2,4-DNT using poultry litter leachate, J. Hazard. Mater. 113 (2004) 137–140.
[21] HSDB (Hazardous Substances Data Bank), National Library of Medicine, Bethesda, MD, 1998.
[22] R. Bishop, M. Hable, C. Oliver, R. Valis, The USACHPPM Gas Chromatographic procedure for analysis of waters and soils for energetic and related compounds, J. Chromatogr. Sci. 41 (2) (2003) 73–79.
[23] C. Zhang, J.B. Hughes, S.F. Nishino, J.C. Spain, Slurry-phase biological treatment of 2,4-DNT and 2,6-DNT, Environ. Sci. Technol. 34 (2000) 2810–2816.
[24] S.F. Nishino, J.C. Spain, H. Lenke, Mineralization of 2,4- and 2,6-dinitrotoluene in soil slurries, Environ. Sci. Technol. 33 (1999) 1060–1064.
[25] H.T. Bausum, W.R. Mitchell, M.A. Major, Biodegradation of 2,4- and 2,6-DNT by freshwater microorganisms, J. Environ. Sci. Health 27A (1992) 663–695.
[26] J.D. Fortner, C. Zhang, J.C. Spain, J.B. Hughes, Soil column evaluation of factors controlling bio-degradation of DNT in the vadose zone, Environ. Sci. Technol. 37 (2003) 3382–3391.
[27] Z. Snellinx, S. Taghavi, J. Vangronsveld, D. van der Lelie, Microbial consortia that degrade 2,4-DNT by interspecies metabolism, Biodegradation 14 (2003) 19–29.
[28] P.G. Rieger, H.J. Knackmuss, Biodegradation of 2,4,6-TNT and related nitroaromatic compounds, in: J. Spain (Ed.), Biodegradation of Nitroaromatic Compounds, Plenum Press, NY, 1995.