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Biodegradation of 2,4-dinitrotoluene using poultry litter leachate

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Abstract

Various micro-organisms are known to degrade 2,4-dinitrotoluene (DNT) through pathways involving intermediates. Addition of nutrients stimulates the biodegradation process. Poultry litter has a consortia of micro-organisms along with many nutrients. The objective was to study the DNT biodegradation using poultry litter in an aqueous medium. Complete biodegradation of 10–50 ppm 2,4-DNT solutions was observed after two days interaction with poultry litter leachate without the formation of any intermediates. No degradation was observed using autoclaved litter leachate.

Keywords: Dinitrotoluene; Biodegradation; Poultry litter

1. Introduction

Dinitrotoluenes are a component of propellants for artillery shells and are found in soils on firing ranges. DNT is also present, as an impurity, in soils contaminated by trinitrotoluenes (TNT). The Radford Army Ammunition Plant was a major production site of DNT. The Volunteer, The Ravenna, and the Badger Army Ammunition Plants have a substantial amount of DNT contaminated soil that needs to be treated. Various technologies to remove the soil bound DNT and TNT include alkaline hydrolysis [1], enzymatic reduction [2], and reaction with cationic surfactants (cetyltrimethylammonium bromide [3]). Pennington et al. [4] and Spain et al. [5] have written exhaustively on the immobilization of 2,4- and 2,6-DNT and their biodegradation. Most of the explosive nitroaromatic compounds including DNT are subject to transformation by micro-organisms. Nitroaromatic compounds are in general more recalcitrant to biodegradation. Most of the researchers have concluded that polynitroaromatic compounds can only be transformed to aminonitro compounds and can not be mineralized. Many others have investigated the biodegradation of DNT [6–8]; reduction to diamine stage has been reported [9] under anaerobic conditions.

Kinetics of adsorption and desorption of DNT to soil and organic matter determine the degree of cleanup that can be achieved. The unavailable DNT (soil bound) persists in soils even when no DNT can be detected in the aqueous leachate. This portion of the DNT is soluble in acetonitrile [10]; these authors used the *Burkholderia* sp. strain DNT using column experiments whereas Hughes et al. [7] used *Clostridium acetobutylicum* for bio-degradation studies. Haidour and Ramos [11] observed that *Pseudomonas* sp. Clone A was able to use DNT as N-source after the enzymatic removal of nitro groups from the aromatic ring. Optimum conditions for complete biodegradation of TNT by *Pseudomonas putida* have been reported that result in complete mineralization [12,13].

The microbial population of poultry litter is acidophilic bacteria, fungi, algae and aerobic heterotrophs [14]. This population includes *Pseuodomonas*, *Actinomycetes*, and *Nocardia*. Poultry litter contains a large amount of nutrients (NO_3, P_2O_5, K_2O) [15]. These nutrients are needed by microorganisms for bioremediation [16–18] of organic compounds. The objective of this research was to study the biodegradation of 2,4-DNT in aqueous medium using poultry litter leachate.

2. Methods and materials

A stock solution of 2,4-DNT (97%, Aldrich Chemical Co.) was prepared (50 mg in 500 ml of deionized water);

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Table 1
Changes in 2,4-DNT concentrations (ppm) on mixing with poultry litter leachate (1 ml leachate; $n = 7$; standard deviation in parentheses)

Initial concentration	Final concentration	
	1 day	2 days
10	0.18 (0.1)	0.0
20	4.84 (1.0)	0.0
30	14.72 (2.5)	0.0
40	17.96 (3.0)	0.0
50	37.41 (5.8)	3.6 (1.1)
100	66.51 (6.3)	55.1(2.6)

water solubility of 2,4-DNT is around 180 ppm [19]. A representative sample of poultry litter was collected from the university farm, air dried, homogenized and sieved (2 mm). Five grams of litter was mixed with 50 ml deionized water, shaken at 240 rpm for 1 h, centrifuged at 3000 rpm for 30 min and the leachate separated. Standard solutions of 0, 10, 20, 30, 40, 50 and 100 ppm DNT were each mixed with 1 ml of poultry litter leachate. Solutions both with and without litter leachate were shaken on an orbital shaker for 24 h at 240 rpm. Then 1 ml of isomyl acetate was added to each solution, 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. [20] the organic layer was analyzed (Table 1). A sample of poultry litter was also autoclaved to kill the micro-organisms; the leachate from autoclaved litter was prepared as before and used as above. Glassware used was also sterilized.

3. Results and discussion

Almost complete degradation took place with 10 ppm 2,4-DNT (Table 1) on the addition of 1 ml poultry litter leachate after 1 day; the percentage degradation decreased significantly with increasing concentrations of DNT; 100 ppm DNT was degraded by only 34%. None of the chromatograms (Fig. 1) showed any extra peak except for those (isomyl acetate and 2,6-DNT) seen with the standard solutions without the poultry litter leachate. No degradation or change in initial concentration of 2,4-DNT was observed with autoclaved litter leachate showing that the degradation observed with un-sterilized litter was only by the micro-organisms present in the litter.

On increasing the mixing time, between 2,4-DNT and un-sterilized leachate (1 ml) to 2 days complete degradation was seen with 10–40 ppm DNT but only 45% degradation with 100 ppm DNT. Increasing the amount of leachate to 2 ml resulted in 100% degradation of 10–50 ppm DNT after 1 day; increasing the mixing time to 2 days resulted in 92% degradation with 100 ppm DNT (data not shown in Table 1). A chemical kinetic study on the bio-degradation of 20 ppm DNT with time (Fig. 2) showed it as a first-order rate reaction with an R^2 value of 0.98. Absence of any new peak in the chromatogram (Fig. 1) shows that the biodegradation of 2,4-DNT is not accompanied with the formation of other amino or nitro organic compounds.

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 weeks [21]. Nishino et al. [22] reported that DNT-degrading bacteria can completely degrade mixtures of DNT in liquid cultures without the production of aminon-

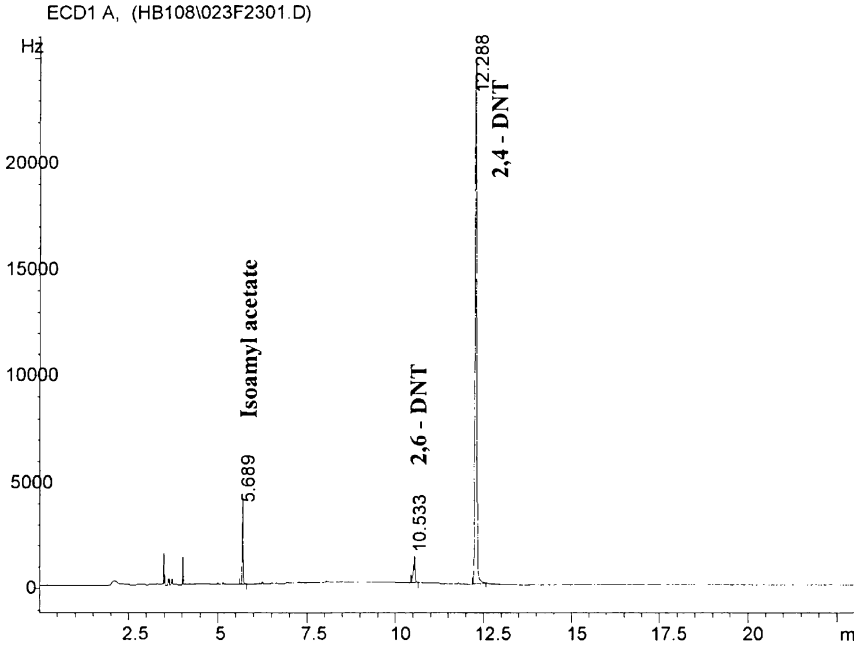

Fig. 1. Gas chromatogram of 20 ppm DNT after reaction with poultry litter organisms.

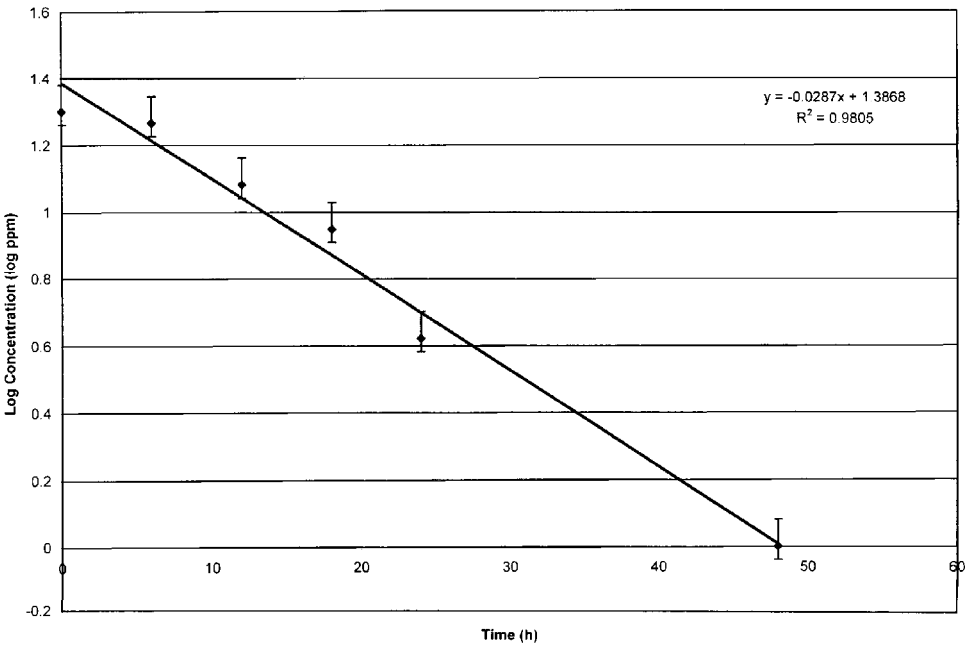

Fig. 2. Log concentration of DNT (20 ppm) mixed with poultry litter leachate vs. time of interaction.

itrotoluenes; adding DNT degrading strains in contaminated soil slurry resulted in the disappearance of DNT accompanied by CO_2 release. Nutrient limitations control the onset of rapid 2,4-DNT biodegradation; biodegradation of DNT was rapidly stimulated by the addition of a complete mineral medium but not by bi-carbonate buffered deionized water or by phosphate amended tap water [23]. Using a mixed consortia (*Pseudomonas*, *Sphingomonas*, and *Stenotrophomonas*) Snellinx et al. [24] have also reported the degradation of 2,4-DNT without accumulation of any intermediates.

From the results presented here it can be concluded that the micro-organisms in the poultry litter leachate can degrade 2,4-DNT (up to 100 ppm) without the production of other intermediates and possibly at a faster rate. Research work on the biodegradation of DNT in contaminated soil from the Badger Army Ammunition Plant is in progress; preliminary data suggest that the use of poultry litter is more effective in biodegradation of DNT than the use of litter leachate.

Acknowledgements

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References

[1] M. Emmrich, Kinetics of the alkaline hydrolysis of important nitroaromatic co-contaminants of 2,4,6-trinitrotolune in highly contaminated soils, Environ. Sci. Technol. 35 (2001) 874–877.

[2] R.G. Riefler, B.F. Smets, Enzymatic reduction of 2,4,6-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] 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, ERDC/EL TR-032, Waterways Experiment Station, Vicksburg, MS, 2003.

[5] J.J. Spain, J.B. Hughes, H.J. Knackmuss, Biodegradation of Nitroaromatic Compounds and Explosives, Lewis Publishers, Boca Raton, FL, 2000.

[6] 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. 32 (1998) 82–87.

[7] 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.

[8] 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.

[9] N.G. McCormick, F.F. Feeherry, H.S. Levinson, Microbial transformation of 2,4,6 trinitrotolune and other nitroaromatic compounds, Appl. Environ. Microbiol. 31 (1976) 949–958.

[10] J.J. Ortega-Calvo, Biodegradation of sorbed 2,4-dinitrotoluene in a clay rich aggregated porous medium, Environ. Sci. Technol. 33 (1999) 3737–3742.

[11] 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.

[12] 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 (96) (2003) 567–571.

[13] R. Miehr, J.Z. Bandstra, R. Po, P.G. Tratnyek, Remediation of 2,4,6-TNT by iron metal: kinetic controls on product distributions in batch and column experiments, Am. Chem. Soc., National Meeting, Preprint 43 (1) (2003) 644–648.

[14] R.M. Nodar, M.J. Acea, T. Carballas, Microbial populations of poultry pine-sawdust litter, Biol. Waste 3 (4) (1990) 295–306.
[15] M. North, D. Bell, Commercial Chicken Production Manual, 4th ed., Van Nostrand Reinhold, New York, NY, 1990.
[16] G. Gupta, J. Baummer, Biodegradation of atrazine in soil using poultry litter, J. Haz. Mater. 45 (1996) 185–192.
[17] G. Gupta, J. Tao, Bioremediation of gasoline contaminated soil using poultry litter, J. Environ. Sci. Hlth. A 31 (9) (1996) 2395–2407.
[18] G. Gupta, V. Rao, Biodegradation of phenol with poultry litter microorganisms, J. Environ. Sci. Hlth. A 33 (1) (1998) 83–95.
[19] HSDB (Hazardous Substances Data Bank), National Library of Medicine, Bethesda, MD, 1998.
[20] 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. Chromat. Sci. 41 (2) (2003) 73–79.
[21] H.T. Bausum, W.R. Mitchell, M.A. Major, Biodegradation of 2,4 and 2,6 DNT by freshwater microorganisms, J. Environ. Sci. Hlth. 27A (1992) 663–695.
[22] 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.
[23] 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.
[24] 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.