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Anaerobic biotransformation of explosives in aquifer slurries amended with ethanol and propylene glycol

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Abstract

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-trinitrotoluene (TNT) are explosives that are frequently found as environmental contaminants on military installations. Hydrogen has been shown to support the anaerobic transformation of these explosives. We investigated ethanol and propylene glycol as electron donors for providing syntrophically produced H_2 for stimulating the anaerobic biodegradation of explosives in contaminated soil. The study was conducted using anoxic microcosms constructed with slurries of the contaminated soil and groundwater. The addition of 5 mM ethanol and propylene glycol enhanced the biodegradation of RDX and HMX relative to the control bottles. Ethanol was depleted within about 20 days, resulting in the transient formation of hydrogen, acetate, and methane. The hydrogen headspace concentration increased from 8 ppm to 1838 ppm before decreasing to background concentrations. Propylene glycol was completely degraded after 15 days, forming hydrogen, propionate, and acetate as end-products. The hydrogen headspace concentrations increased from 56 ppm to 628 ppm before decreasing to background concentrations. No methane formation was observed during the incubation period of 48 days. Our findings indicate the addition of ethanol and propylene to the aquifer slurries increased the hydrogen concentrations and enhanced the biotransformation of RDX and HMX in the explosive-contaminated soil.

Keywords: Explosives; Anaerobic biodegradation; RDX; HMX; TNT

1. Introduction

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-trinitrotoluene (TNT) are high explosives used extensively by the United States military (Gorontzy et al., 1994). Past handling practices of contaminated wastewater has resulted in soil and groundwater contamination at Army sites throughout the United States (Funk et al., 1993). The Department of Defense has identified more than 1200 explosive-contaminated sites in the United States (Scmelling et al., 1997). Due to the potential adverse effects of these compounds to human health and to the environment (Talmage et al., 1999; US Department of Health and Human Services, 1995), contaminated environments must be remediated (Hawari et al., 2000b). However, appropriate bioremediation strategies are lacking for treating soils *in situ*, a strategy that would be particularly cost effective and useful, especially in circumstances where excavation of soil is not possible.

Developing an anaerobic approach for *in situ* bioremediation of contaminated sites appears to be warranted because explosives are more readily transformed under these conditions compared to oxic environments (Hawari et al., 2000a). Because explosives do not serve as growth substrates for microbial growth, an external cosubstrate must be added for the explosive-degrading bacteria (Young et al., 1997; Kitts et al., 2000). For example, nutrient broth

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(McCormick et al., 1981), yeast extract (Kitts et al., 1994; Young et al., 1997), glucose (Hawari et al., 2000b), and starch (Funk et al., 1993) have been used as a carbon source for the anaerobic transformation of RDX. Starch has also been used to support the transformation of TNT and HMX (Funk et al., 1993).

Introducing these substrates into a groundwater aquifer, however, is not very practical. These compounds serve as carbon and energy sources for a variety of heterotrophs. Most of these compounds listed above have a high growth yield, resulting in larger amounts of biomass being produced. This increases the probability of plugging the formation, especially around the injection wells. The use of some of these compounds would also not be expected to be very cost effective. Before appropriate in situ bioremediation strategies can be devised, consideration needs to be given to the type of electron donors that would be appropriate for use in the field to simulate the explosive-degrading bacteria.

Our research has demonstrated that the addition of reduced electron donors supports the transformation of explosives by a methanogenic mixed culture (Adrian et al., 2003). The culture transforms RDX, HMX, and TNT when hydrogen, 1,2-propanediol (propylene glycol) or ethanol are added as the sole electron donor. We believe the syntrophic hydrogen produced during the metabolism of ethanol or propylene glycol was serving as the immediate electron donor for the explosive-degrading bacteria. We hypothesized that the homoacetogens may be responsible for the explosives transformation. In support of this, *Acetobacterium malicum* strain HAAP-1 was isolated from the mixed culture (Adrian and Arnett, 2004). It degrades RDX when grown in a mineral medium using H_2 gas as the sole electron donor.

The primary objective of this study was to examine whether a strategy of adding ethanol or propylene glycol to contaminated soils would result in their metabolism by the indigenous bacteria and result in an increase in the H_2 concentration, with the latter then serving as an electron donor for the explosive-degrading bacteria. We first determined the presence of the homoacetogens and other relevant anaerobic bacteria in the contaminated soil. Once their presence was confirmed, we evaluated ethanol and propylene glycol as sources of reducing power for stimulating explosives transformation in aquifer slurries. In the present study, we show that the addition of ethanol or propylene glycol to the aquifer slurries resulted in significantly greater H_2 concentrations and a more rapid loss of the explosives compared to the control microcosms. We believe adding these electron donors to subsurface environments is a practical strategy for increasing the H_2 concentration and thereby the metabolic activity of the explosive-degrading bacteria. Further research in this area should lead to developing an innovative and more cost-effective strategy for the *in situ* bioremediation of explosive-contaminated soils and groundwater.

2. Materials and methods

2.1. Chemicals

RDX, HMX, and TNT used in this study were obtained from the Holston Army Ammunition Plant and had purities greater than 99%. 2,4,6-Triaminotoluene (TAT) was obtained from Chem Service (Chem Service, West Chester, PA) and was of the highest purity obtainable. 2-Amino-4,6-dinitrotoluene (2A46DNT), 4-amino-2,6-dinitrotoluene (4A26DNT), 2,4-diamino-6-nitrotoluene (24DA6NT), and 2,6-diamino-4-nitrotoluene (26DA4NT) were obtained from AccuStandard, Inc. (AccuStandard, New Haven, CT) or Supelco (Supelco, Bellefonte, PA) and were greater than 99% pure. All other chemicals were of the highest purity obtainable.

2.2. Soil and groundwater

Explosive-contaminated soil was obtained from the Milan Army Ammunition Plant (Milan, TN). The soil, a medium loam, contained on average the following concentrations of explosives: RDX, 2.4 mg/kg soil; HMX, 1.5 mg/kg soil; and TNT, 3.8 mg/kg soil. Groundwater obtained from the area was not contaminated with explosives.

2.3. Bacterial enumerations

A five-tube most probable number (MPN) procedure was used to estimate total aerobes and anaerobes, heterotrophic and autotrophic methanogens, acetogens, ethanol-oxidizers, and propylene glycol-oxidizers in soil samples. Microcosms were prepared by adding 1 g of soil to 9 ml of basal medium consisting of an appropriate electron donor and the following (g/l): NaCl, 0.8; NH_4Cl, 1.0; KCl, 0.1; $MgSO_4 \cdot 7H_2O$, 0.02; KH_2PO_4, 1.35; K_2HPO_4, 1.75; $NaHCO_3$, 1.5; TES buffer, 4.6; resazurin, 0.001. Trace metal and vitamin solutions were made as described by Tanner (1989) and added at a concentration of 10 ml/l. These microcosms were subsequently serially diluted by a factor of 10 to a final dilution of 10^{-7}. Five replicate tubes were made for each concentration. All incubations were carried out in the dark at 28 °C.

Autotrophic and heterotrophic methanogens were enumerated by adding H_2:CO_2 (80:20, 1.7 atm) and 10 mM acetate to tubes as sole electron donors respectively. Tubes were analyzed for CH_4 production using gas chromatography (GC) and scored positive if concentrations increased greater than 0.1% after 6 weeks of incubation. Ethanol fermenters were assayed by amending tubes with 10 mM ethanol and monitoring for ethanol depletion by GC analysis. Tubes were scored positive after 6 weeks of incubation if more than 1.0 mM ethanol was depleted. The bacteria capable of fermenting propylene glycol were enumerated by amending tubes with 10 mM propylene glycol and analyzing for propionate formation by reverse phase high-pressure liquid chromatography (HPLC). Tubes were