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Impact of glycerin and lignosulfonate on biodegradation of high explosives in soil

Jongho Won [a,*], Robert C. Borden [a,b]

[a] Department of Civil, Construction, and Environmental Engineering, North Carolina State University, Campus Box 7908, Raleigh, NC 27695, USA
[b] Solutions-IES, Inc., 1101 Nowell Road, Raleigh, NC 27607, USA

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ABSTRACT

Soil microcosms were constructed and monitored to evaluate the impact of substrate addition and transient aerobic and anaerobic conditions on TNT, RDX and HMX biodegradation in grenade range soils. While TNT was rapidly biodegraded under both aerobic and anaerobic conditions with and without organic substrate, substantial biodegradation of RDX, HMX, and RDX daughter products was not observed under aerobic conditions. However, RDX and HMX were significantly biodegraded under anaerobic conditions, without accumulation of TNT or RDX daughter products (2-ADNT, 4-ADNT, MNX, DNX, and TNX). In separate microcosms containing grenade range soil, glycerin and lignosulfonate addition enhanced oxygen consumption, increasing the consumption rate >200% compared to untreated soils. Mathematical model simulations indicate that oxygen consumption rates of 5 to 20 $g/m^3/d$ can be achieved with reasonable amendment loading rates. These results indicate that glycerin and lignosulfonate can be potentially used to stimulate RDX and HMX biodegradation by increasing oxygen consumption rates in soil.

1. Introduction

The high explosive (HE) compounds 2,4,6-trinitrotoluene (TNT), hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX; royal demolition explosive), and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazine (HMX; high melting explosive) are commonly used in military munitions and can be deposited on military ranges as a result of training activities. While >99.99% of the explosive load is consumed when munitions detonate as intended (high-order detonation), substantial amounts of HEs may remain on the range as unexploded ordinance (UXO) or be deposited on the range as chunks or small particles following partial or low-order detonation (Brannon and Pennington, 2002; Fuller et al., 2004; Walsh et al., 2006). Over time, the HE compounds can be dissolved by rainwater, infiltrate through the vadose zone, and potentially migrate off the range with flowing groundwater. TNT and RDX are classified as possible human carcinogens (C classification) by U.S. Environmental Protection Agency (EPA, 2014a, 2014b). HMX is not currently classified as human carcinogen, but exposure can have adverse impacts to the liver and nervous system (ATSDR, 1997).

Biodegradation can limit migration of dissolved TNT through the vadose zone. TNT can be degraded by a wide variety of microorganisms under both of aerobic and anaerobic conditions (Fuller and Manning, 1997; Singh, 2012). However, TNT degradation is often more rapid under anaerobic conditions (Pennington and Brannon, 2002). TNT is typically degraded to amino derivatives including 2-amino-4,6-dinitrotoluene (2-ADNT), 4-amino-2,6-dinitrotoluene (4-ADNT), 2,6-diamino-4-nitrotoluene (2,6-DANT), and 2,4-diamino-6-nitrotoluene (2,4-DANT) (McCormick et al., 1981; Hawari et al., 2000). These intermediate compounds can be further transformed through biotic and/or abiotic processes.

In general, RDX is more resistant to aerobic biodegradation than TNT (Bradley and Chapelle, 1995; Felt et al., 2009; Halasz et al., 2012). However, RDX can be utilized as a source of nitrogen under aerobic conditions when other nitrogen sources are limited (Binks et al., 1995; Fuller et al., 2010a). 4-Nitro-2,4-diazabutanal (4-NDAB) and methylenedinitramine (MEDINA) are typically produced in aerobic biodegradation of RDX associated with the XplA/XplB enzymes (Fournier et al., 2004; Fuller et al., 2010a; Crocker et al., 2015). Despite the potential for RDX biodegradation under aerobic conditions, several studies have reported that aerobic RDX degraders may not be widespread in soil and groundwater, resulting in little or no RDX degradation under aerobic conditions (Fuller et al., 2010b; Crocker et al., 2015; Fuller et al., 2015).

RDX is readily biodegraded under anaerobic conditions (McCormick et al., 1981; Hawari et al., 2000; Halasz et al., 2012). A common anaerobic degradation pathway for RDX involves the reduction of nitro functional groups ($-NO_2$) to nitroso groups ($-NO$) where RDX is sequentially reduced to hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), and then hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) (McCormick et al., 1981; Fuller et al., 2004; Kwon and Finneran, 2006). These

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* Corresponding author.
E-mail address: jwon@ncsu.edu (J. Won).

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intermediate nitroso compounds can then be transformed to nitrous oxide (N_2O) and carbon dioxide (CO_2) (Hawari et al., 2001). Hawari et al. also reported that RDX can also be degraded by direct ring cleavage, yielding MEDINA and bis(hydroxymethyl)nitramine. The intermediate nitramines are further degraded to nitrous oxide (N_2O) and formaldehyde (HCHO) by spontaneous chemical decomposition. However, the factors controlling these degradation pathways are not well understood.

Redox conditions and the presence of different electron acceptors and donors can influence RDX biodegradation rates. RDX biodegradation was faster under mixed electron acceptor conditions compared to single select electron acceptor conditions including sulfate reducing, fermenting, methanogenic, and nitrate reducing conditions (Boopathy, 2001). Enhanced biodegradation was observed under conditions with electron donors producing hydrogen (Adrian et al., 2003; Adrian and Arnett, 2007) and under highly reducing conditions (Price et al., 2001). However, the presence of nitrate inhibited RDX degradation due to the preferential microbial utilization of nitrate as a nitrogen source and/or an electron acceptor (Cho et al., 2015).

Biodegradation of HMX is analogous to RDX biodegradation due to its similar structure. HMX is resistant to aerobic biodegradation and its degradation is enhanced under anaerobic conditions (Boopathy, 2001; Singh, 2012). Similar to RDX, HMX can be degraded by sequential reduction of nitro groups to nitroso groups (i.e., transformation of HMX to mononitroso-, dinitroso-, trinitroso-, and tetranitroso-HMX) and by direct ring cleavage. The nitroso intermediates can be subsequently transformed to nitrous oxide (N_2O) and formaldehyde (HCHO) that are indicators of ring cleavage (Bhushan et al., 2006; Pichtel, 2012). The direct ring cleavage pathway yields 4-NDAB and MEDINA under aerobic conditions, and MEDINA under anaerobic conditions (Zhao et al., 2004; Singh, 2012). Degradation of HMX is typically slower than RDX and high concentration of RDX can inhibit biodegradation of HMX (Uchimiya et al., 2010). However, low RDX levels did not inhibit HMX degradation (Fuller et al., 2009).

Hatzinger et al. (2004) and Fuller et al. (2004) showed that addition of peat moss and soybean oil can also be effective in stimulating sorption and biodegradation to reduce explosives leaching. *Sphagnum* peat moss addition to soil slurries was effective in stimulating RDX mineralization and crude soybean oil addition stimulated TNT, RDX, and HMX mineralization (Fuller et al., 2004). The combination of peat moss and crude soybean oil (PMSO) reduced leaching of both RDX and MNX by two orders of magnitude (Schaefer et al., 2005; Fuller et al., 2009). However, application of PMSO to ranges may have some operational issues including: a) increased dust problems in arid areas; b) increased fire hazard; c) challenges in distributing the material without exposing workers to UXO hazards; and d) disturbance of the PMSO amendment by ordinance detonation, potentially reducing treatment effectiveness.

Farling (2013) proposed the use of lignosulfonate (LS) and glycerin (GL) as an alternative to peat moss and soybean oil for enhancing HEs sorption and biodegradation. The LS and GL could be mixed with water and spray applied to the range, eliminating the need to remove UXO, greatly reducing worker exposure. Once applied, natural rainfall or artificial irrigation would transport the amendments deeper into the soil. The readily biodegradable GL was intended to consume available oxygen in soil, generating anaerobic conditions and stimulating anaerobic biodegradation of the HEs. The LS was expected to biodegrade more slowly, consuming oxygen, and helping to maintain anoxic conditions. GL, produced as a byproduct of biodiesel production, is an attractive substrate due to its low cost, high solubility in water, and relatively high flash point (176 °C). LS is produced during paper production through reaction with metal bisulfites and other reagents (Pearl, 1967; Kirk et al., 1980) and is commonly used as a dust control agent, additive in concrete preparation, and dye and insecticides dispersant (Nelson and Northey, 2004; Ouyang et al., 2006; Yang et al., 2008). Laboratory soil column experiments monitored by Farling (2013) showed that LS was strongly retained by the soil, but GL rapidly migrated through the columns with 70–82% of the applied GL discharged in the column effluent. TNT leaching was significantly reduced in columns treated with GL alone and GL + LS treated columns, resulting in discharge < 5% of TNT amount applied. However, RDX degradation was limited in all treated columns. Monitoring data showed that oxygen was rapidly transported through the sandy soil, preventing establishment of anaerobic conditions required to enhance RDX degradation.

Objectives of this work are to: 1) improve our understanding of HEs biodegradation in soil at an active hand grenade range; 2) evaluate the impact of GL and LS addition to these soils on HEs biodegradation under aerobic and anaerobic conditions; and 3) evaluate the kinetics of oxygen consumption by GL and LS. This information can aid in identifying sites where natural attenuation is sufficient to prevent adverse impacts and sites where GL and LS addition might be effective at increasing oxygen consumption rates to control HEs leaching.

2. Material and methods

2.1. Chemicals

Calcium lignosulfonates (Ultrazine CA and Norlig A) were purchased from Lignotech USA (Rothschild, WI) and crude glycerin was obtained from Piedmont Biofuels (Pittsboro, NC). Acetonitrile and toluene used for soil and water extractions were HPLC grade and purchased from J.T. Baker and Fisher Scientific respectively.

Analytical standards for TNT, RDX, 2,4-DNT, 2,6-DNT, 2-ADNT, 4-ADNT, and HMX were purchased from AccuStandard, Inc. (New Haven, CT), and standards for RDX nitroso derivatives (MNX, DNX, and TNX) were purchased from SRI International (Menlo Park, CA). All standards were diluted in acetonitrile prior to use.

2.2. Active range soils used in experiments

Soils used in this work were collected from two hand grenade throwing bays at Fort Bragg, NC (Bay C and Bay T). The most recent soil survey indicates that the native soils at this site are primarily Vaucluse loamy sand. However, there has been significant re-grading of the site and much of the A soil horizon has been removed to form soil containment berms. Soil for use in the biodegradation tests was prepared by blending soil obtained from 0 to 1 m below ground surface (bgs) in the middle of each bay. Soil characterization results showed that RDX and TNT concentrations were relatively higher in Bay T than Bay C soil, and TNT concentrations were approximately an order of magnitude lower than RDX. While RDX concentrations were 0.03–0.24 mg/kg in Bay T and 0.01–0.08 mg/kg in Bay C soil, TNT concentrations were 0.008–0.012 mg/kg in Bay T and 0.005–0.008 mg/kg in Bay C soil. Both organic carbon (OC) and clay content were significantly higher ($p < 0.05$) in Bay C (OC = 0.14–0.23% and clay = 1.8–2.6%) compared to Bay T soil (OC = 0.06–0.15%, clay = 1.4–2.3%). However, Fe(II) concentrations were significantly higher ($p < 0.05$) in Bay T (1.5–2.4 g/kg) compared to Bay C soil (1.1–1.6 g/kg). Soil pH (5.4 ± 0.2), silt content (13.4 ± 2.7%), and median grain size (296 ± 70 μm) were not statistically different between Bay C and T soils. Analytical methods and result details for soil characterization are described in the companion paper (Won, 2016).

2.3. Biodegradation of HEs in soil slurry microcosms

An initial set of aerobic and anaerobic microcosms was constructed with 50 g of sand from a quarry near Fort Bragg, NC, 100 mL of groundwater, and composited effluent from laboratory columns amended with TNT, RDX, glycerin and lignosulfonates (Farling, 2013). Experimental treatments included: 1) autoclaved controls; 2) no amendment (live controls); 3) 0.256 g of glycerin (GL), 4) 0.032 g of Ultrazine CA (UCA), 5) 0.037 g of Norlig A (NA), 6) GL + UCA, and 7) GL + NA. UCA is a higher cost material where the lower molecular weight materials have been removed. NA is a relatively low cost lignosulfonate