

Removal of Explosives Using an Integrated Iron–Microbial Treatment in Flow-Through Columns

B.-T. Oh,¹ P. J. Alvarez²

¹ School of Chemical Engineering, BK21, Seoul National University, San 56-1, Shinlim-dong, Gwanak-gu, Seoul 151-744, Korea

² Department of Civil and Environmental Engineering, The University of Iowa, Iowa City, IA 52242-1527, USA

Received: 1 September 2003/Accepted: 20 April 2004

Soils and groundwater at military sites in many countries are often contaminated with RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) and other toxic compounds as a result of wastewater disposal from munitions production, handling, and testing operations (Higson 1992; Jenkins and Walsh 1992). RDX is toxic to humans and a variety of organisms, and is classified as a Class C (possible human) carcinogen by the U.S. Environmental Protection Agency (McLellan et al. 1988). Several *ex situ* physical-chemical and biological processes, such as incineration, composting, alkaline hydrolysis/oxidation, and aqueous thermal decomposition, have been practiced to manage RDX contamination (Garg et al. 1991). However, many of these approaches are not cost-effective to treat large volumes of contaminated groundwater. In addition, complete RDX destruction is not always achieved, which can result in the accumulation of transformation products of equal or even greater toxicity (McCormick et al. 1981).

Previous studies have shown that permeable reactive barriers (PRBs) using zero-valent iron (ZVI) can remove redox-sensitive groundwater pollutants (e.g., chlorinated solvents and Cr(VI)) (Gandhi et al. 2002; Scherer et al. 2000). Recent studies have also shown that reductive treatment with ZVI might also be a viable remediation alternative for RDX-contaminated groundwater (Wildman and Alvarez, 2001), and that RDX mineralization can be significantly enhanced by some anaerobic bacteria that feed on cathodic hydrogen-produced by the reduction of water during ZVI corrosion (Equation 1) (Oh et al., 2001):



Eq. 1 proceeds under anaerobic conditions that are invariably found in PRBs due to the rapid reduction of dissolved O₂ by iron. H₂ gas production by this reaction is a common phenomenon (Reardon, 1985; Sorel et al., 2001; Vikesland et al., 2003), and it has been shown to stimulate the growth of a wide variety of microorganisms (Daniels et al., 1987; Oh and Alvarez, 2002; Rajagopal and LeGall, 1989) as well as to enhance the rate and extent of biotransformation of redox-sensitive pollutants in ZVI treatment systems (Till et al., 1998; Weathers et al., 1997). Whereas other alternatives exist to introduce H₂, RDX is toxic to bacteria at the ppm level (Adrian and Lowder, 1999; Wildman and Alvarez, 2001), and iron may degrade RDX below inhibitory levels that facilitate microbial participation in the clean-up process.

High and sustainable RDX removal efficiencies (> 99% for 1 year) were previously observed in flow-through ZVI columns bioaugmented with municipal anaerobic sludge, and *Acetobacterium* spp. were isolated from the iron layer (Oh and Alvarez 2002). Presumably these homoacetogenic bacteria colonized the iron layer to feed on H₂ produced by the anaerobic corrosion reaction. These bacteria were also found to transform RDX when fed H₂. However, it is unknown if RDX removal would be hindered by likely co-occurring pollutants associated with munitions wastes, and if such other compounds could also be removed in bioaugmented ZVI barriers.

This study investigated the potential benefits of combining chemical and biological degradation processes in ZVI-PRBs to enhance the removal of RDX and other explosives from contaminated groundwater. Emphasis was placed on determining if likely co-occurring explosives, such as TNT (2,4,6-trinitrotoluene), 2,6DNT (2,6-dinitrotoluene), 2,4DNT (2,4-dinitrotoluene), and HMX (octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine) would hinder RDX removal in iron-packed columns simulating bioaugmented PRBs.

MATERIALS AND METHODS

One concern about PRB performance is passivation and loss of reactivity due to the formation of oxide layers. Many types of oxides are known to passivate the iron surface and hinder iron reactivity (Phillips et al., 2000). However, some oxide layers could also serve as a semi-conductor to transfer the electrons donated by the inner ZVI core (Scherer et al., 1998), or as a powerful reductant if surface-associated Fe(II) is present, as is the case with green rust (Scherer et al., 2000). Thus, we investigated if oxide formation is a potential significant limitation for using PRB to treat RDX. Specifically, oxidation of granular ZVI (0.5 mm diameter) was accomplished by adding the iron to 400 mL of deionized water and shaking for 2 weeks while flushing with air. The rusted iron was then filtered and dried. Batch degradation assays were subsequently run to test the reactivity of the rusted granular iron towards RDX and other common pollutants that are commonly treated by PRBs. For this experiment, rusted iron (1g, 4%, w/v) was added to 35-mL serum bottles containing 25-mL of deionized water amended with RDX, trichloroethene (TCE), Cr(VI), NO₂⁻, or NO₃⁻ at 10 mg/L each. The reactors were prepared in an anaerobic chamber and sealed with Teflon-lined, gray-butyl septa and aluminum crimps. Reactors were shaken on a reciprocating shaker (150 rpm) and periodically analyzed for each pollutant. RDX and Cr(VI) were quantified using HPLC, TCE was by GC, NO₂⁻ and NO₃⁻ were by ion chromatography as described in Gandhi et al. (2002).

In separate experiments, flow-through columns were used to evaluate the potential for an integrated ZVI-microbial system to remove RDX. Four columns (30-cm long, 2.5-cm ID) with lateral sampling ports were packed with a 5-cm layer of soil (from the Iowa Army Ammunition Plant) followed by an 18-cm layer of Master Builder® ZVI filings (representing a PRB) and a 7-cm sand layer (Oh and Alvarez, 2002). One column was prepared with inert glass beads instead of ZVI to evaluate potential RDX losses due to hydrolysis (control). The second column was sterilized with a biocide (Kathon, 1 mL/L) to discern degradation by iron alone. The third column was not poisoned to determine if soil bacteria colonize the iron layer, presumably to feed on cathodic H₂ produced during corrosion (Eq. 1). This column also served as a

baseline to discern the benefits of bioaugmentation. The fourth column was inoculated with municipal anaerobic sludge (10 mL, 6.6 g-volatile suspended solids/L added at each port) to enhance reductive treatment of RDX. RDX (cold plus ^{14}C -labeled) was dissolved in bicarbonate-buffered synthetic groundwater (von Gunten and Zobrist, 1993) at 19 mg/L (10 $\mu\text{Ci/L}$) and fed continuously with a peristaltic pump at 2.3 mL/hr. All columns were covered with aluminum foil to preclude potential photocatalytic reactions. Although H_2 gas production rates were not measured in these experiments, gas bubbles were observed in all columns with ZVI.

Three additional columns were prepared as described above (i.e., naturally colonized and bioaugmented columns) to determine how potential co-contaminants might affect RDX removal efficiency in PRBs under different microbial conditions. HMX (5 mg/L), TNT, 2,4DNT, 2,6DNT, and RDX (10 mg/L) were dissolved in the bicarbonate-buffered synthetic groundwater and fed continuously at 2.1 mL/hr (about 0.5 ft/day superficial velocity) using a peristaltic pump. The third column was inoculated with municipal anaerobic sludge (10 mL of stock (10 g-VSS/L) added at each port) and fed with RDX (19 mg/L). Every two months, a ^{14}C -labeled slug (200 mL, 10 $\mu\text{Ci/L}$) of RDX was fed to evaluate its fate through the columns. The medium was anoxic, and was stored in 2-L Pyrex bottles that were pressurized to 10 psi with a gas mixture of $\text{CO}_2:\text{N}_2$ (20:80, v/v). The pH of this medium was adjusted to 7.3 with 2 M HCl.

Further experiments were conducted to analyze the effluent ^{14}C and determine what percent of this was accounted for by dissolved inorganic carbon (DIC) derived from $^{14}[\text{RDX}]$ mineralization to $^{14}\text{CO}_2$ (which can become ^{14}C -labeled dissolved inorganic carbon, such as bicarbonate). Approximately 3 pore volumes (200 mL) of ^{14}C -RDX (19 mg/L, 10 $\mu\text{Ci/L}$) flowed through the columns before 10 mL of effluent was collected into sealed 30-mL serum bottles that were capped under a negative pressure. Each serum bottle contained three 1-mL test tubes containing 0.75 mL of 0.5 M NaOH (to trap $^{14}\text{CO}_2$), liquid scintillation counting (LSC) cocktail (also to trap any $^{14}\text{CO}_2$), or ethylene glycol (to trap any ^{14}C -volatile organic compounds from ^{14}C -RDX). The serum bottles were equilibrated on a reciprocating shaker (90 rpm) at 30°C for 1.5 days. The partitioning of ^{14}C into each trap was determined by LSC.

RDX and co-contaminants removal was monitored along the length of the columns by sampling the side ports and analyzing the samples by high performance liquid chromatography (HPLC). This analysis was conducted using a 250 $\times$ 4.6 mm SupelcosilTM LC-18 column with an isocratic mobile phase of deionized water and methanol (4:6, v/v) at a flow rate of 1.0 mL min⁻¹ and spectrophotometric detection at 240 nm (limit of detection = 0.1 mg L⁻¹). ^{14}C -labeled RDX and its ^{14}C -metabolites were quantified by HPLC using a radioactivity detector (Radiomatic, Series A-500, Packard Instrumental Co., Downers Grove, IL). Dissolved ^{14}C activity was determined by mixing 0.5 mL of sample with 10 mL of Ultima Gold counting solution (Packard, Meriden, CT) and analyzing by LSC using a Beckman LS 6000 IC (Beckman Instrument Inc., Fullerton, CA).

RESULTS AND DISCUSSION

Previous studies have reported that ZVI can remove RDX in batch reactors. Hundal et al. (1997) and Singh et al. (1998) suggested that ZVI could be used to remediate RDX-contaminated water and soil. Figure 1 corroborates these previous findings and shows that RDX was removed in batch studies with rusted granular iron faster than other pollutants that are commonly treated with iron barriers. Among the tested redox-sensitive pollutants, RDX was the most reactive, followed closely by hexavalent chromium (Cr(VI)) while TCE and nitrate reduction by iron was relatively slow. For example, RDX was completely removed within 2 hours, whereas only 70% of the added TCE was removed in 5 days. These results support the notion that iron barriers might effectively intercept and degrade RDX plumes, and that the RDX removal process is not likely to be significantly hindered by iron oxidation.

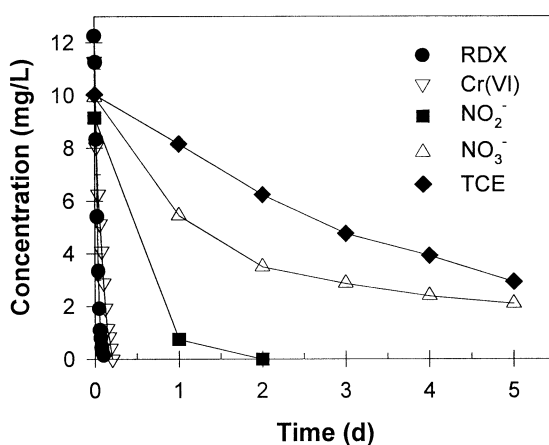


Figure 1. Degradation of redox-sensitive pollutants in separate batch reactors containing rusted iron (4%, w/v).

We also monitored RDX removal in flow-through columns by sampling the side ports and analyzing the aqueous samples by HPLC and LSC. RDX concentration profiles after 16 months showed extensive RDX removal (possibly abiotic) in all columns with ZVI. Significant RDX removal was also observed in the bioaugmented soil upgradient of the ZVI layer, due to biological activity (Figure 2). The poisoned column removed RDX, but in the absence of microbial activity, it showed little decrease in dissolved ¹⁴C levels. This was likely due to the abiotic transformation of RDX into soluble ¹⁴C-labeled byproducts that accumulated, possibly including methylenedinitramine (Oh et al., 2001) and formaldehyde (Gregory et al., 2004). The column colonized by indigenous microorganisms and the bioaugmented column showed extensive decrease in dissolved (effluent) ¹⁴C activity, which was likely due to microbial mineralization of RDX and its byproducts to ¹⁴CO₂ (which is subject to volatilization losses) as well as polymerization of RDX metabolites into the soil and ZVI matrices (Oh et al., 2001). The bioaugmented column exhibited the highest ¹⁴C removal efficiency, and no clogging problems were experienced during two years of operation.

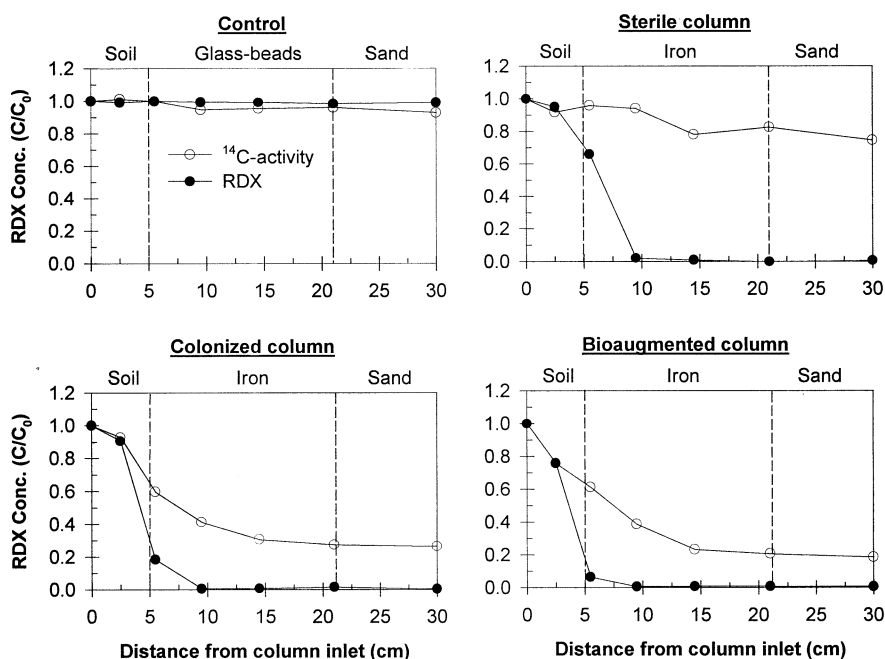


Figure 2. Change of RDX concentration and soluble ^{14}C activity after 16 months of operation. ($C_0 = 19 \text{ mg/L}$ and $10 \mu\text{Ci/L}$). The hydraulic retention time through the columns was 1.71 days.

Table 1. Composition of ^{14}C in effluent of columns after 16 months of operation.

Columns	% of effluent ^{14}C			Total (%)
	DIC (trapped in NaOH)	VOC (trapped in EG + SC)	DOC (remaining in solution)	
Control column	0.2 ± 0.0	0.2 ± 0.0	99.6 ± 0.0	100.0
Sterile column	2.3 ± 0.1	0.8 ± 0.0	96.3 ± 0.1	99.4
Colonized column	17.0 ± 1.9	1.6 ± 0.2	84.3 ± 0.8	102.9
Bioaugmented column	11.8 ± 0.7	0.8 ± 0.2	92.1 ± 3.2	104.7

DIC: Dissolved Inorganic Carbon

EG: Ethylene glycol

DOC: Dissolved Organic Compound

VOC: Volatile Organic Compound

SC: Scintillation cocktail

For the control column (packed with inert glass beads), all of the ^{14}C activity remained in solution (Table 1), and corresponded to unreacted RDX. Bioaugmented and naturally colonized columns showed that 12-17% of the soluble ^{14}C was dissolved inorganic carbon derived from ^{14}C -RDX mineralization. The poisoned column showed only 2.3% mineralization. Most of the dissolved ^{14}C ($> 84\%$) did

not partition into the $^{14}\text{CO}_2$ or VOC traps (Table 1). This corroborates previous microcosm studies indicating that RDX can be transformed by granular iron to soluble metabolites that accumulate transiently, or it can be mineralized to CO_2 if appropriate microorganisms are present (Oh et al. 2001). HPLC analyses with radio-chromatographic detector showed that some of the radiolabel had the same retention time as methylenedinitramine (Oh et al. 2001). Heterocyclic reduction products such as MNX, DNX and TNX were not detected in significant amounts (total less than 2% on a molar basis).

Additional columns were run to investigate the efficacy of biologically-active iron barriers to treat groundwater contamination by munitions waste mixtures. After 8 months of operation, we began to monitor HMX, TNT, 2,4DNT, 2,6DNT, and RDX removal along the length of the columns by sampling the side ports and analyzing the samples by HPLC (Figure 3). These compounds, which are likely to co-exist with RDX in contaminated military sites, were extensively removed in the colonized and bioaugmented ZVI columns. This corroborates previous batch studies showing the high reactivity of ZVI with RDX and TNT (Agrawal and Tratnyek, 1996; Hundal et al. 1997; Singh et al. 1998; Oh et al. 2001). The bioaugmented column inoculated with municipal anaerobic sludge exhibited complete removal of HMX. Because of biological activity, significant removal of TNT, 2,4DNT, 2,6DNT and RDX was also observed in the bioaugmented soil upgradient of the ZVI layer. Both columns showed complete removal of 2,4DNT, 2,6DNT, TNT, and RDX. We did not investigate the metabolites and fate of the co-contaminants in this study.

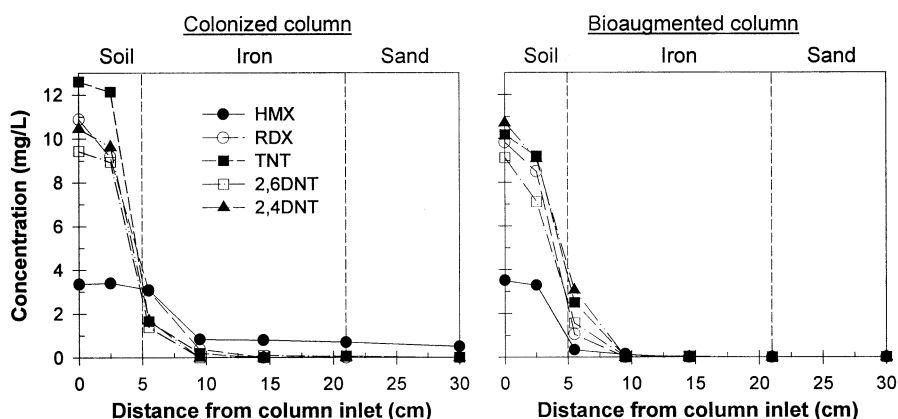


Figure 3. Removal of RDX and likely co-contaminants in flow-through columns after 8 months of operation.

To investigate how the presence of potential co-contaminants affects RDX removal efficiency, we also compared RDX removal profiles along the length of the bioaugmented columns fed with a mixture of co-contaminants versus RDX alone (Figure 4). The addition of TNT, HMX, 2,4DNT, and 2,6DNT at 5 to 10 mg/L each did not hinder RDX removal efficiency in flow-through columns. The apparently higher RDX removal efficiency with co-contaminants is misleading due to the slightly higher hydraulic retention time in this column (i.e., 1.6 versus 1.9 days).

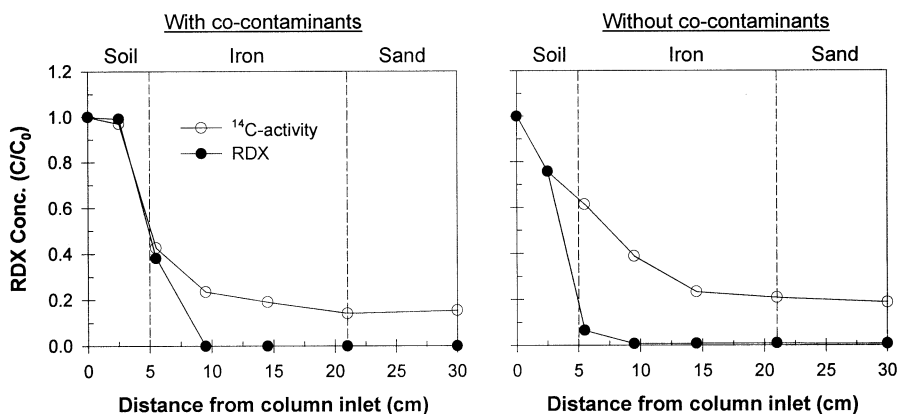


Figure 4. Change of RDX concentration and soluble ^{14}C activity in bioaugmented columns fed with a mixture of co-contaminants ($C_0 = 10 \text{ mg/L}$ for RDX, $10 \mu\text{Ci/L}$, $Q = 2.1 \text{ mL/hr}$) and with RDX alone ($C_0 = 19 \text{ mg/L}$, $10 \mu\text{Ci/L}$, $Q = 2.5 \text{ mL/hr}$) after 6 months of operation.

Overall, this work suggests that permeable reactive ZVI barriers can effectively clean up groundwater contaminated with varying military explosives, and that treatment efficiency can be enhanced by biogeochemical interactions through bioaugmentation.

Acknowledgments. This project was funded by SERDP (Project CU1231). We thank Craig Just for providing technical grade RDX and Michelle Scherer and Gene Parkin for valuable discussions. The preparation of this manuscript was supported by the Brain Korea 21 Project in 2003 to B.-T. Oh through the School of Chemical Engineering, Seoul National University, Korea.

REFERENCES

- Adrian NR, Lowder A (1999) Biodegradation of RDX and HMX by a methanogenic enrichment culture. In: Bioremediation of nitroaromatic and haloaromatic compounds. Alleman B (ed.), Battelle Press: 1-6
- Agrawal A, Tratnyek PG (1996) Reduction of nitro aromatic compounds by zero-valent iron metal. *Environ Sci Technol* 30:153-160
- Daniels L, Belay N, Rajagopal BS, Weimer PJ (1987) Bacterial methanogenesis and growth from CO_2 with elemental iron as the sole source of electrons. *Sci* 237:509-511
- Gandhi S, Oh B-T, Schnoor JL, Alvarez PJ (2002) Degradation of TCE, Cr(VI), sulfate, and nitrate mixtures by granular iron in flow-through columns under different microbial conditions. *Water Res* 36:1973-1982
- Garg R, Grasso D, Hoag G (1991) Treatment of explosives contaminated lagoon sludge. *Hazard Waste Hazard Mater* 8:319-340

- Gregory KB, Larese-Casanova P, Parkin GF, Scherer MM (2004) Abiotic transformation of hexahydro-1,3,5-trinitro-1,3,5-triazine by Fe^{II} bound to magnetite. *Environ Sci Technol* 38:1408-1414
- Higson FK (1992) Microbial degradation of nitroaromatic compounds. *Adv Appl Microbiol* 37:1-19
- Hundal LS, Singh J, Bier EL, Shea PJ, Comfort SD, Powers WL (1997) Removal of TNT and RDX from water and soil using iron metal. *Environ Pollut* 97:55-64
- Jenkins TF, Walsh ME (1992) Development of field screening methods for TNT, 2,4DNT, and RDX in soil. *Talanta* 39:419-428
- McCormick NG, Cornell JH, Kaplan AM (1981) Biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine. *Appl Environ Microbiol* 42:817-823
- McLellan W, Hartley WR, Brower M (1988) Health advisory for hexahydro-1,3,5-trinitro-1,3,5-triazine. Technical Report PB90-273533. Office of Drinking Water, U.S. EPA, Washington, DC.
- Oh B-T, Just CL, Alvarez PJ (2001) Hexahydro-1,3,5-trinitro-1,3,5-triazine mineralization by zerovalent iron and mixed anaerobic cultures. *Environ Sci Technol* 35:4341-4346
- Oh B-T, Alvarez PJ (2002) Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) degradation in biologically-active iron columns. *Wat Air Soil Pollut* 141:325-335
- Phillips DH, Gu B, Watson DB, Roh Y, Liang L, Lee SY (2000) Performance evaluation of a zero-valent iron reactive barrier: mineralogical characteristics. *Environ Sci Technol* 34:4169-4176
- Rajagopal B, LeGall J (1989) Utilization of cathodic hydrogen by hydrogen-oxidizing bacteria. *Appl Microbiol Biotechnol* 31:406-412
- Reardon EJ (1995) Anaerobic corrosion of granular iron: measurement and interpretation of hydrogen evolution rates. *Environ Sci Technol* 29:2936-2945
- Scherer MM, Balko BA, Tratnyek PG (1998) The role of oxides in reduction reactions at the metal-water interface. In: *Mineral-Water Interfacial Reactions: Kinetics and Mechanisms*. Sparks DL and Grundl TJ (eds.). ACS Symposium Series 715. pp. 301-332
- Scherer MM, Richter S, Valentine RL, Alvarez PJ (2000) Chemistry and microbiology of permeable reactive barriers for in situ groundwater clean up. *Crit Rev Environ Sci Technol* 30:363-411
- Singh J, Comfort SD, Shea PJ (1998) Remediating RDX-contaminated water and soil using zero-valent iron. *J Environ Qual* 27:1240-1245
- Till BA, Weathers LJ, Alvarez PJ (1998) $\text{Fe}(0)$ -supported autotrophic denitrification. *Environ Sci Technol* 32:634-639
- von Gunten U, Zobrist J (1993) Biogeochemical changes in groundwater-infiltration systems: Column studies. *Geochim Cosmochim Acta* 57:3895-3906
- Wildman MJ, Alvarez PJ (2001) RDX degradation using an integrated $\text{Fe}(0)$ -microbial treatment approach. *Wat Sci Technol* 43:25-33