HEXAHYDRO-1,3,5-TRINITRO-1,3,5-TRIAZINE (RDX) DEGRADATION IN BIOLOGICALLY-ACTIVE IRON COLUMNS

BYUNG-TAEK OH and PEDRO J. J. ALVAREZ*
Department of Civil and Environmental Engineering, The University of Iowa, Iowa City, Iowa U.S.A. 52242-1527
(author for correspondance, e-mail: pedro-alvarez@uiowa.edu; fax: 319/335-5660)*

Abstract. Flow-through columns were used to evaluate the efficacy of permeable reactive iron barriers to treat groundwater contamination by RDX. Three columns were packed with iron filings (Fe^0) between soil and sand layers, and were fed continuously with unlabeled plus ^{14}C-labeled RDX to characterize its removal efficiency under different microbial conditions. One column was poison-sterilized to isolate chemical degradation processes, another was not poisoned to allow colonization of the Fe^0 layer by indigenous microorganisms, and a third column was amended with anaerobic sludge to evaluate the benefits of enhancing biodegradation through bioaugmentation. Extensive RDX removal (> 99%) occurred through the Fe^0 layer of all columns for more than one year, although ^{14}C-label analysis indicated the presence of soluble byproducts such as methylenedinitramine. RDX byproducts accumulated to a lesser extent in biologically active columns, possibly due to enhanced mineralization by the cumulative action of microbial and chemical degradation processes. Denaturing gradient gel electrophoresis (DGGE) profiles and nucleotide sequencing revealed a predominance of *Acetobacterium* sp. in the iron layer of all columns after 95 days. Such homoacetogenic bacteria probably feed on hydrogen produced during Fe^0 corrosion and participate on the RDX degradation process. This notion was supported by batch experiments with a mixed homoacetogenic culture isolated from the bioaugmented column, which degraded RDX and produced acetate when H_2 was present. Overall, this work suggests that Fe^0 barriers can effectively intercept RDX plumes, and that treatment efficiency can be enhanced by biogeochemical interactions though bioaugmentation.

Keywords: bioaugmentation, explosive, permeable reactive barrier, RDX, zero-valent iron

1. Introduction

RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) is a military high explosive that is gaining notoriety as one of the most recalcitrant and toxic groundwater contaminants in the subsurface. Toxicity studies have led the U.S. Surgeon General to recommend a 24-h maximum RDX concentration of 0.3 mg L^{-1} to protect aquatic life, and the Office of Drinking Water has set a limit for lifetime exposure to RDX at 0.1 mg L^{-1}. Several *ex situ* physical-chemical and biological processes have been proposed to manage RDX contamination (e.g., incineration, composting, alkaline hydrolysis, and aqueous thermal decomposition) (Garg *et al.*, 1991). However, many of these approaches are prohibitively expensive for groundwater treatment, or are limited by the accumulation of transformation products of equal or even

Water, Air, and Soil Pollution **141:** 325–335, 2002.

greater toxicity (e.g., dimethylhydrazine, dimethylnitrosamine, and azoxymethane) (McCormick *et al*., 1981).

In the last seven years, there has been an explosion of activity directed at the development and implementation of permeable reactive iron (Fe^0) barriers that intercept and remove redox-sensitive contaminants from groundwater (Scherer *et al*., 2000). Fe^0 can be placed in the path of a contaminant plume, either on a trench (O'Hannesin and Gillham, 1998), buried as a broad continuous curtain (Blowes *et al*., 1995), or injected as colloids (Kaplan *et al*., 1996), to name a few options. Fe^0 has been used to remove waste chlorinated solvents and hexavalent chromium (Helland *et al*., 1995; Powell *et al*., 1995; Roberts *et al*., 1996; Gandhi *et al*., 2002), and recently Fe^0 was shown to react rapidly with RDX in aquifer microcosms (Singh *et al*., 1998; Oh *et al*., 2001; Wildman and Alvarez, 2001). Nevertheless, little is known about how Fe^0 interacts with indigenous aquifer microorganisms and how such biogeochemical interactions affect the long-term performance of the Fe^0 barriers.

Recent studies have reported that hydrogen gas produced from the reduction of water during Fe^0 corrosion (Equation 1) can serve as electron donor for the biotransformation of reducible contaminants (Scherer *et al*., 2000).

$$Fe^0 + 2H_2O \longrightarrow Fe^{2+} + 2OH^- + H_2 \tag{1}$$

For example, combining Fe^0 with a methanogenic consortium enhanced both the rate and extent of transformation of carbon tetrachloride and chloroform (Weathers *et al*., 1997). Similarly, combining Fe^0 and autotrophic denitrifiers significantly enhanced nitrate removal and improved the end product distribution, favoring N_2 over NH_4^+ (Till *et al*., 1998). Such experiments suggest that some biogeochemical interactions such as cathodic depolarization could enhance the efficacy of Fe^0 barriers.

This study investigated the potential benefits of combining chemical and biological degradation processes in Fe^0 barriers to enhance RDX removal from contaminated groundwater. Emphasis was placed on determining the potential benefits of adding anaerobic municipal sludge to aquifer columns simulating Fe^0 barriers, with focus on long-term RDX removal efficiency. Microbial colonization of the iron layer was also investigated.

2. Materials and Methods

Flow-through columns were used to evaluate the sustainability of RDX removal in Fe^0 barriers under different microbial conditions. Four columns (30-cm long, 2.5-cm ID) equipped with lateral sampling ports were packed with a 5-cm layer of soil (Iowa Army Ammunition Plant, Middletown, IA) followed by an 18-cm layer of Master Builder® Fe^0 filings (representing a reactive barrier) and a 7-cm sand layer (Figure 1). The aquifer material was a silt loam with 5.6% organic matter. One of

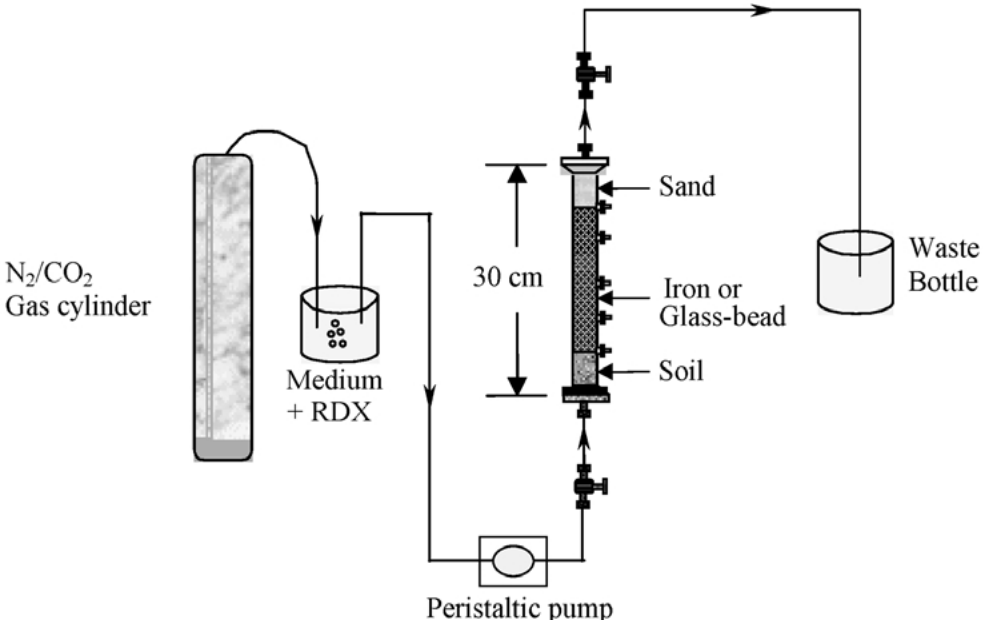

Figure 1. Flow-through columns used to mimic permeable reactive iron barriers.

the columns was poisoned with a biocide (Kathon, fed continuously at 1 mL L^{-1}) to discern degradation by Fe^0 alone. The second (non-sterile) column was used to determine if soil bacteria colonize the Fe^0 layer, presumably to feed on cathodic H_2 produced by anaerobic Fe^0 corrosion. This column also serves as a baseline to evaluate the benefits of bioaugmentation. The third column was inoculated with municipal anaerobic sludge (10 mL of stock [6.6 g-volatile suspended solids L^{-1}] added at each port) to enhance reductive treatment of RDX. The fourth column was prepared with inert glass beads instead of Fe^0 to control for the effect of indigenous microorganisms on RDX degradation. RDX (unlabeled plus ^{14}C-labeled) was dissolved in bicarbonate-buffered synthetic groundwater (von Gunten and Zobrist, 1993) at 18 mg L^{-1} (10 μCi L^{-1}) and fed continuously with a peristaltic pump. The flow rate was approximately at 6 mL hr^{-1}, resulting in a superficial velocity of 1.2 cm/hr. This velocity is within the range of reported groundwater Darcy velocities (Domenico and Schwartz, 1998). The medium was anoxic, and was stored in 2-L Pyrex bottles that were pressurized to 10 psi with a gas mixture of CO_2:N_2 (20:80, v/v). The pH of this medium was neutralized from 8.8 to 7.3 with 2 M HCl.

RDX 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 × 4.6 mm Supelcosil^{TM} 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 scintillation cocktail and analyzing by liquid scintillation counting (LSC) using a Beckman LS 6000 IC (Beckman Instrument Inc., Fullerton, CA).

Acetate concentrations were analyzed using an HP series 1100 HPLC equipped with a UV/Visible light detector operating at 210 nm. Injection of 100 μL was made by a HP series 1100 sampler. Separation was achieved with an Alltech 300 $\times$ 7.8 mm anion exchange column and an eluent of 1 mN sulfuric acid flowing at 1 mL min^{-1}. Peak areas were integrated by Hewlett Packard ChemStation software. The limit of detection (LOD) for acetate was approximately 5 mg L^{-1}.

The pH and oxidation-reduction potential (ORP) were determined after 80 days of operation using a microelectrode MI-16/800 connected to a 16-702 Flow-Thru reference and to a Fisher Scientific AB15 pH meter. A 20-gauge needle was inserted into the sampling ports through the Mininert valves to withdraw aqueous samples that were passed through the microelectrode chamber for real-time analysis of pH and ORP.

The microbial population colonizing the Fe^0 layer was analyzed after 95 days. The total viable biomass concentration was determined by phospholipid fatty acid content (PLFA analysis) (White *et al.*, 1997) and the dominant bacteria profiles were evaluated by a conserved region of the 16S rRNA gene analysis achieved by denaturing gradient gel electrophoresis (DGGE) (Muyzer *et al.*, 1993). Microbial Insights, Inc. (Rockford, TN) performed these analyses. Sequence identifications were performed using the BLASTN facility of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) and the similarity indices were obtained from the database of the Ribosomal Database Project (Maidak *et al.*, 2000).

RDX degradation assays were also conducted in batch reactors to investigate the ability of microorganisms present in the columns to degrade RDX. A mixed culture was obtained by withdrawing a 0.5-mL aqueous sample from the iron layer of the bioaugmented column after one year of operation. Based on DGGE results, this culture was enriched in ATCC *Acetobacterium* medium 1019 (Balch and Wolfe, 1976), which contains the following (in grams per liter of distilled water): yeast extract (1.0); NH_4Cl (1.0); $MgSO_4 \cdot 7H_2O$ (0.1); KH_2PO_4 (0.4); K_2HPO_4 (0.4); fructose (5.0); $NaHCO_3$ (3.0); cysteine $HCl \cdot H_2O$ (0.5); and $Na_2S \cdot 9H_2O$ (0.5). The medium also contained Wolfe's Vitamin solution (10 mL), Wolfe's Mineral solution (10 mL), and resazurin (0.01%) as a redox potential indicator; the headspace consisted of a H_2/CO_2 mixture (95/5, v/v). Biodegradation assays were conducted in anaerobic culture tubes (18 $\times$ 150 mm) amended with 20 mL of autoclaved mineral medium and RDX (2 mg L^{-1}) under the same H_2/CO_2 headspace. No-treatment controls (without RDX or bacteria addition) were also prepared to obtain a baseline for comparing RDX degradation and acetate production. Reactors were incubated in a rotary shaker (100 rpm) inside a Coy anaerobic chamber at 30

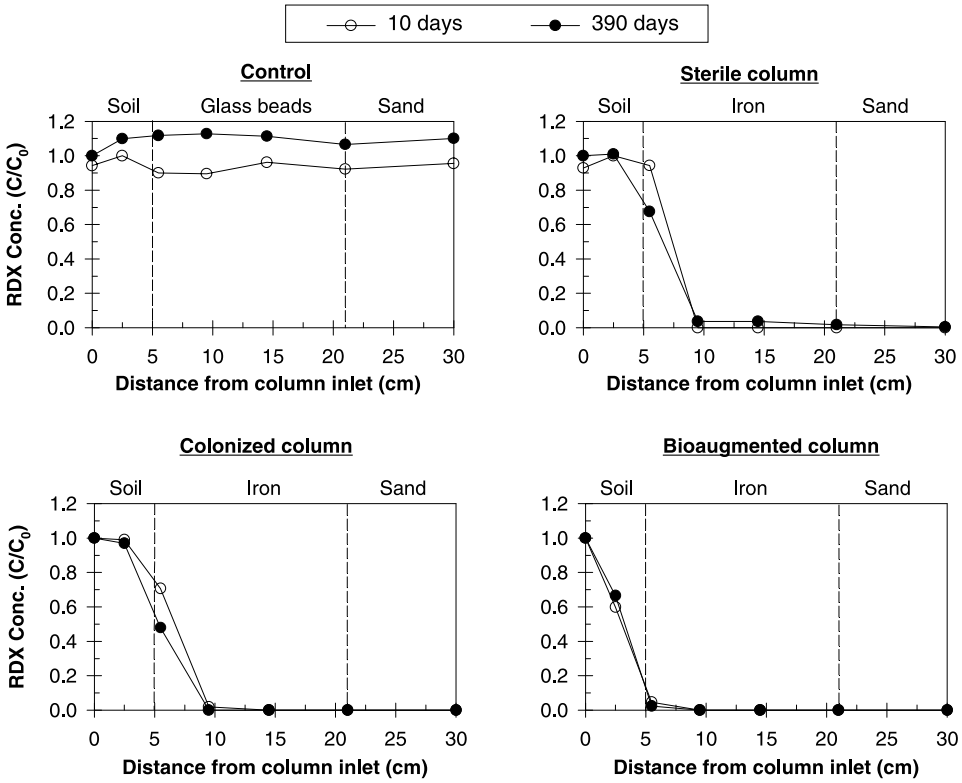

Figure 2. RDX concentration profiles along the length of various columns after 10 and 390 days of operation (20 and 764 pore volumes throughput, respectively), with C_0 = 18 mg L^{-1}.

°C. Liquid samples were analyzed periodically for RDX degradation and acetate production, as described previously.

3. Results and Discussion

RDX concentration profiles showed extensive (>99%) RDX removal in all columns with Fe^0 (Figure 2), which corroborates previous batch studies showing the high reactivity of Fe^0 with RDX (Singh *et al.*, 1998; Oh *et al.*, 2001; Wildman and Alvarez, 2001). Profiles taken after 10 and 390 days of operation (i.e., after 20 and 764 pore volumes, respectively, had passed though) showed that the high RDX removal efficiency in the Fe^0 layer was sustainable over one year. Biological activity was also responsible for significant RDX removal in the upgradient soil layer of the bioaugmented column. On the other hand, RDX concentrations remained constant through the control column packed with soil and inert glass beads, reiterating the recalcitrance of this compound under natural conditions.

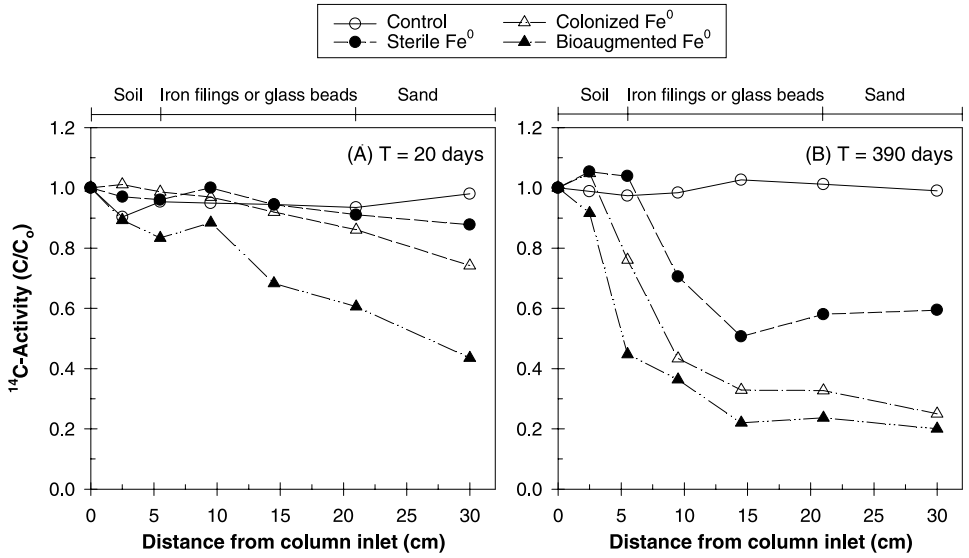

Figure 3. ^{14}C activity profiles along the length of various columns after 20 (A) and 390 (B) days of operation, with $C_0 = 10\ \mu Ci\ L^{-1}$.

The dissolved ^{14}C activity was analyzed to evaluate the fate of the added RDX. The dissolved ^{14}C label in the control column was associated with unreacted RDX, while that in the columns with Fe^0 (which removed all of the added RDX) was associated with RDX degradation products. ^{14}C concentration profiles after 20 days showed only a slight decrease in dissolved ^{14}C activity through the sterile and the viable (not bioaugmented) columns, whereas the bioaugmented column removed about 56% of the radiolabel (Figure 3A). This suggests that bioaugmentation of Fe^0 could enhance RDX mineralization, as shown previously in microcosms studies (Oh *et al.*, 2001). Interestingly, ^{14}C removal increased over time in all columns (Figure 3B), possibly due to the cumulative action of chemical and microbial degradation processes enhanced by colonization of Fe^0 by indigenous microorganisms that feed on cathodic hydrogen (Equation 1), and by acclimation of the added microorganisms. Microbial activity was detected also in the poisoned column (by plating the effluent on Difco R2A agar), which explains the increase in ^{14}C removal in this column and reflects the difficulty to keep open systems completely sterile. Microbial colonization and increased ^{14}C removal over time suggests that the benefits of bioaugmentation may be temporary, although shorter acclimation periods associated with bioaugmented iron barriers may be important for rapid RDX mineralization before breakthrough of potentially undesirable byproducts.

Attempts were made to identify the RDX degradation products. The commonly reported metabolites MNX (1,3-dinitro-5-nitroso-1,3,5-triazacyclohexane) and DNX (1,3-dinitroso-5-nitro-1,3,5-triazacyclohexane) were detected in the effluent of the columns, although these nitroso byproducts accounted for less than 2% of the in-

fluent RDX concentration on a molar basis. HPLC analysis with the radioactivity detector showed that the dissolved ^{14}C activity was primarily associated with two metabolites, one of which had the same retention time as methylenedinitramine [$(O_2NNH)_2CH_2$]. This metabolite had been previously detected using HPLC/MS in similar microcosm studies (Oh *et al*., 2001). The other metabolite was not identified. Note that the hydraulic retention time in these columns (0.54 days) was relatively short compared to that in Fe^0 barriers (e.g., 100 days for a 1-m thick barrier and a seepage velocity of 1 cm day^{-1}). Longer retention times are conducive to complete removal of the dissolved ^{14}C activity by mineralization and humification of RDX metabolites into the soil matrix (Oh *et al*., 2001). The extent to which the ^{14}C-labeled became bound residue was not determined in this work to avoid compromising the integrity of the columns.

The pH was relatively constant (7.3 to 7.5) along the length of the control (glass-bead) and sterile columns. An increase in pH (to pH 9) was observed in the iron layer of the other two columns, probably due to the corrosion of iron metal resulting from the reaction of Fe^0 with H_2O (Eq. 1). Although effluent Fe(II) concentrations were not measured, iron rust was clearly visible in the effluent collection vessel. The increase in pH did not exceed the tolerance range of common microorganisms, although a corrosion-induced increase in pH could be a (system-specific) potential concern for some iron-microbial treatment process (Till *et al*., 1998; Dejournett and Alvarez, 2000).

Recent work by Price *et al*. (2001) showed that RDX is highly unstable at low oxidation-reduction potentials (ORP), suggesting the importance to monitor this parameter. The ORP was measured (after 80 days) along the length of the columns to verify that Fe^0 induces strongly reducing conditions. While the control (glass-bead) column exhibited a constant ORP at $\pm$ 10 mV, the iron layer itself caused a steep decrease in ORP to about –250 mV. The column for bioaugmented barrier had the most reducing environment (decreasing down to –300 mV), which was consistent with the observed higher RDX removal efficiency.

Microbial colonization of the iron layer of different columns was investigated after 95 days of operation. The total viable biomass concentration determined by phospholipid fatty acid analysis (PLFA) was one order of magnitude higher (1.4×10^8 cells g^{-1} dry wt.) in bioaugmented iron samples than in samples colonized by indigenous soil microorganisms (2×10^7 cells g^{-1} dry wt). Using denaturing gradient gel electrophoresis (DGGE), we observed a relatively diverse bacterial community with several prominent bands appearing in the gel image (Figure 4). The microbial profiles revealed a predominance of Gram-positive bacteria. Iron samples from both the colonized and bioaugmented columns contained high proportions of *Acetobacterium* sp., which are strict anaerobes that use H_2 and CO_2 for growth and produce acetate:

$$4H_2 + 2CO_2 \longrightarrow CH_3COO^- + H^+ + 2H_2O \quad (2)$$

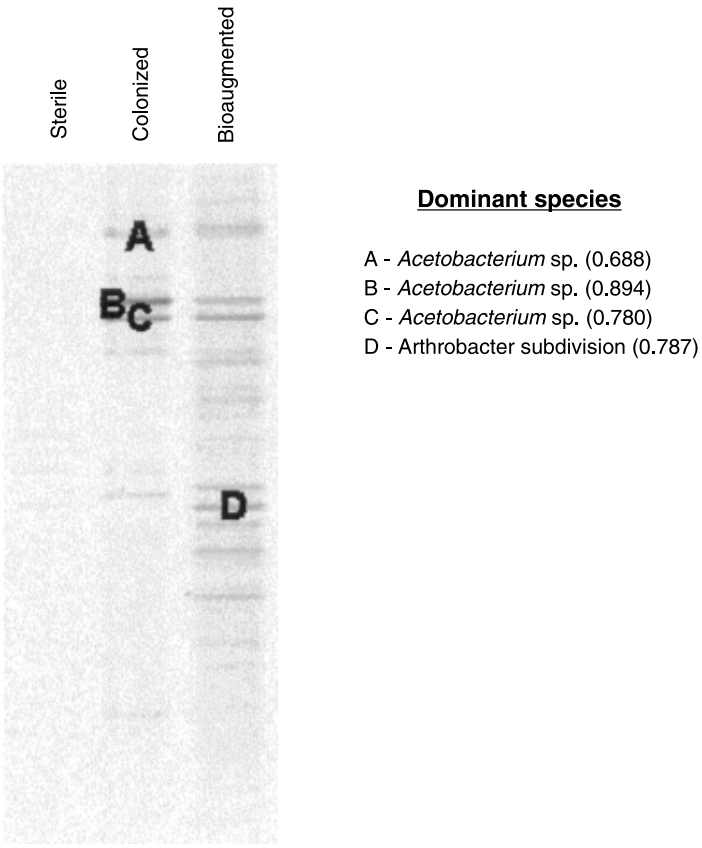

Figure 4. DGGE gel image of bacterial 16S rDNA extracted from Fe^0 samples from various columns after 95 days of operation. Dominant species must constitute at least 1–2% of the total bacterial community to form a visible band. The similarity index (SI) is shown in parenthesis.

In theory, such homoacetogens could comensalistically support heterotrophic activity in Fe^0 barriers by coupling Fe^0 corrosion (Equation 1) with acetogenesis (Equation 2):

$$4Fe^0 + 2CO_2 + 5H_2O \longrightarrow CH_3COO^- + 4Fe^{+2} + 7OH^- \quad (3)$$

These results are supported by previous reports that the homoacetogen, *Acetobacterium woodi,* can grow on cathodic hydrogen from iron corrosion (Rajagopal and LeGall, 1989). Increased availability of an organic substrate (i.e., acetate) is likely to increase heterotrophic activity, which might be beneficial for RDX biodegradation, especially if RDX is utilized as a nitrogen source by heterotrophs (Sheremata and Hawari, 2000).

Homoacetogens have also been implicated in RDX degradation by methanogenic sludge (Adrian and Lowder, 1999). Nevertheless, the ability of homoacetogens to degrade RDX under autotrophic (H_2-fed) conditions that are likely to be

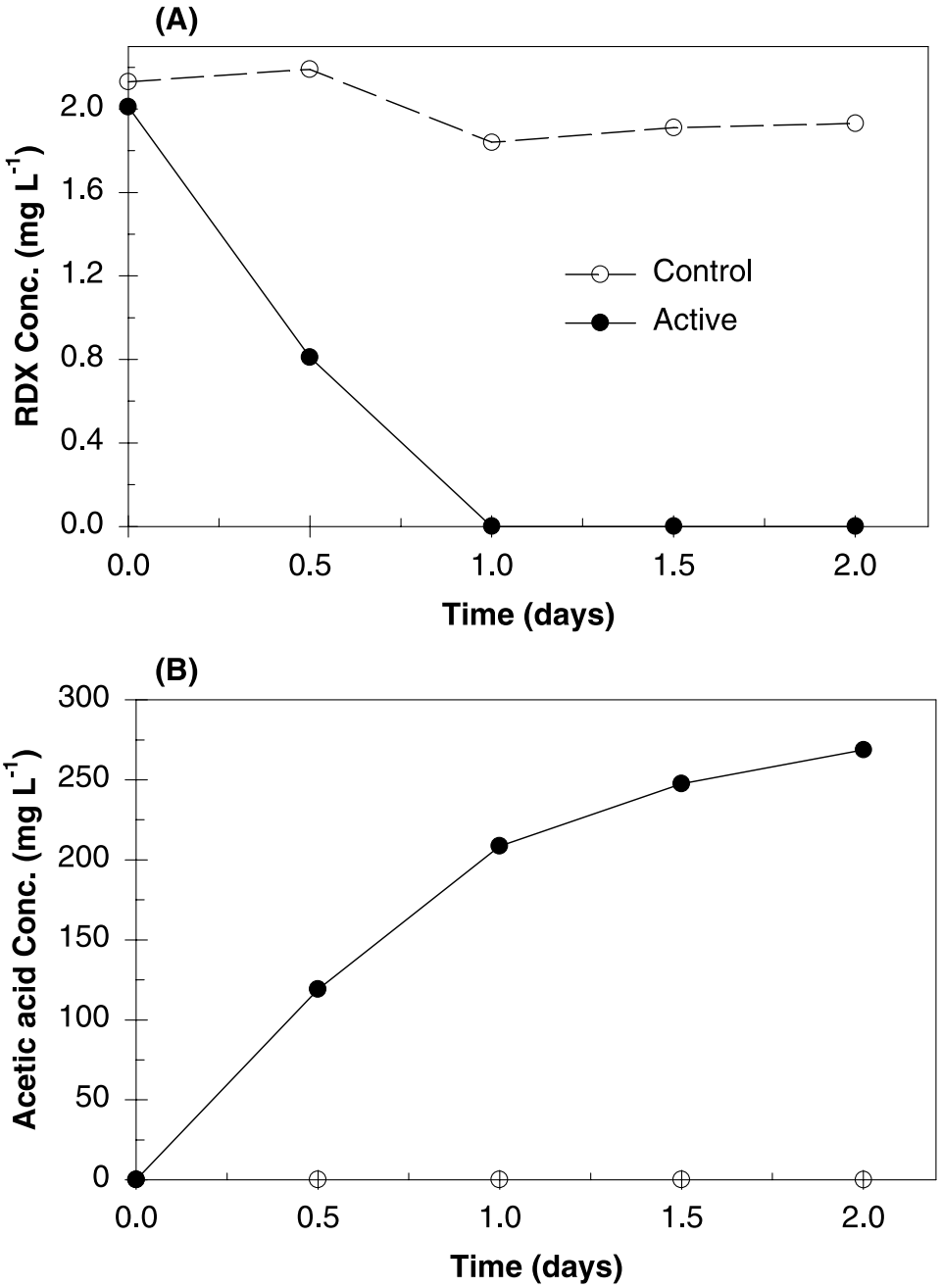

Figure 5. Changes in RDX (A) and acetic acid (B) concentrations in batch reactors seeded with a mixed homoacetogenic culture obtained from the iron layer of the bioaugmented column after 11-months of operation. The initial optical density of this culture (at 660 nm) was about 0.1.

encountered in Fe^0 barriers has not been demonstrated. Therefore, further experiments were conducted with a mixed homoacetogenic culture that was obtained from the iron layer of the bioaugmented column, as described previously. RDX was degraded by this mixed culture within 24 hours (Figure 5A), and no heterocyclic metabolites (i.e., MNX, DNX, and TNX) were detected (limit of detection = 0.1 mg L^{-1} for each), suggesting that RDX degradation proceeded beyond ring fission. The concentration of acetate increased concurrently (Figure 5B), corroborating that the mixed culture was homoacetogenic and produced acetate using hydrogen and inorganic carbon (Equation 2). Further experiments are needed to determine if homoacetogenic bacteria cometabolize RDX using H_2 as primary substrate or if they are capable mineralizing RDX to CO_2. In general, our understanding of the role of homoacetogens in bioremediation is indeed very limited.

Overall, this work suggests that Fe^0 barriers are effective tools to prevent off-site migration and remove RDX from contaminated groundwater, and that treatment efficiency might be enhanced by biogeochemical interactions through bioaugmentation.

Acknowledgements

This work was funded by SERDP (Project DACA72-00-P-0057). Partial funding was also provided by DOE (Project 0003829). We thank Craig Just for providing technical grade RDX and Michelle Scherer and Gene Parkin for valuable discussions.

References

Adrian, N. R. and Lowder, A.: 1999, 'Biodegradation of RDX and HMX by a methanogenic enrichment culture', in B.C. Alleman and A. Leeson (eds.), *Bioremediation of Nitroaromatic and Haloaromatic Compounds*, Battelle Press, Columbus, OH, USA, pp. 1–6.

Balch, W. E. and Wolfe, R. S.: 1976, 'A new approach to the cultivation of methanogenic bacteria: 2-mercaptoethanesulfonic acid (HS-CoM)-dependent growth of *Methanobacterium ruminantium* in a pressurized atmosphere', *Appl. Environ. Microbiol.* **32**, 781–791.

Blowes, D. W., Ptacek, C., Hanton-Fong, C. and Jambor, J.: 1995, 'In situ remediation of chromium contaminated groundwater using zero-valent iron', *Abstracts of the 209th American Chemical Society National Meeting*, **35**, 780–783.

Dejournett, T. and Alvarez, P. J. J.: 2000, 'Combined microbial-Fe(0) system to remove nitrate from contaminated groundwater', *Bioremed. J.* **4**, 149–154.

Domenico, P. A. and Schwartz, F. W.: 1998, *Physical and Chemical Hydrogeology*, Wiley, New York.

Gandhi, S., Oh, B. 'T., Schnoor, J. L. and Alvarez, P. J.: 2002, 'Degradation of TCE, Cr(VI), sulfate, and nitrate mixtures by granular iron in flow-through columns under different microbial conditions', *Water Res*. **36**(8), 1973–1982.

Garg, R., Grasso, D. and Hoag, G.: 1991, 'Treatment of explosives contaminated lagoon sludge', *Hazard. Waste Hazard. Mater.* **8**, 319–340.

Helland, B. R., Alvarez, P. J. J. and Schnoor, J. L.: 1995, 'Reductive dechlorination of carbon tetrachloride with elemental iron', *J. Hazard. Mater.* **41**, 205–216.

Kaplan, D. I., Cantrell, K. J., Wietsma, T. W. and Potter, M. A.: 1996, 'Retention of zero-valent iron colloids by sand columns: application to chemical barrier formation', *J. Environ. Cont*. **25**, 1086–1094.

Maidak, B. L., Cole, J. R., Lilburn, T. G., Parker, Jr, C. T., Saxman, P. R., Stredwick, J. M., Bing Li, G. M., Olsen, G. J., Pramanik, S., Schmidt, T. M. and Tiedje, J. M.: 2000, 'The RDP (Ribosomal Database Project) continues', *Nucleic Acids Res.* **28**(1), 173–174.

McCormick, N. G., Cornell, J. H. and Kaplan, A. M.: 1981, 'Biodegradation of hexahydro-1,3,5-trinitro-1,3,5-triazine', *Appl. Environ. Microbiol*. **42**(5), 817–823.

Muyzer, G., Dewaal, E. C. and Uitterlinden, A. G.: 1993, 'Profiling of complex microbial-populations by denaturating gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S ribosomal RNA', *Appl. Environ. Microbiol.* **59**(3), 695–700.

Oh, B. –T., Just, C. L. and Alvarez, P. J.: 2001, 'Hexahydro-1,3,5-trinitro-1,3,5-triazine mineralization by zerovalent iron and mixed anaerobic cultures', *Environ. Sci. Technol.* **35**(21), 4341–4346.

O'Hannesin, S. F. and Gillham, R. W.: 1998, 'Long-term performance of an in situ iron wall for remediation of VOCs', *Ground Water* **36**, 164–170.

Powell, R., Puls, R. W., Hightower, S. and Sabatini, D.: 1995, 'Coupled iron corrosion and chromate reduction: Mechanisms for subsurface remediation', *Environ. Sci. Technol.* **29**, 1913–1922.

Price, C. B., Brannon, J. M., Yost, S. L. and Hayes, C. A.: 2001, 'Relationship between redox potential and pH on RDX transformation in soil-water slurries', *J. Environ. Eng.* **127**, 26–31.

Rajagopal, B. S. and LeGall, J.: 1989, 'Utilization of cathodic hydrogen by hydrogen-oxidizing bacteria', *Appl. Microbiol. Biotechnol*. **31**, 406–412.

Roberts, A., Totten, L., Arnold, W., Burris, D. and Campbell, T.: 1996, 'Reductive elimination of chlorinated ethylenes by zero-valent metals', *Environ. Sci. Technol.* **30**, 2654–2659.

Scherer, M. M., Richter, S., Valentine, R. L. and Alvarez, P. J. J.: 2000, 'Chemistry and microbiology of permeable reactive barriers for in situ groundwater clean up', *Crit. Rev. Environ. Sci. Technol*. **30**, 363–411.

Sheremata, T. W. and Hawari, J.: 2000, 'Mineralization of RDX by the white rot fungus *Phanerochaete chrysosporium* to carbon dioxide and nitrous oxide', *Environ. Sci. Technol*. **34**, 3384–3388.

Singh, J., Comfort, S. D. and Shea, P. J.: 1998, 'Remediating RDX-contaminated water and soil using zero-valent iron', *J. Environ. Qual*. **27**, 1240–1245.

Till, B. A., Weathers, L. J. and Alvarez, P. J.: 1998, 'Fe(0)-supported autotrophic denitrification', *Environ. Sci. Technol.* **32**(5), 634–639.

von Gunten, U. and Zobrist, J.: 1993, 'Biogeochemical changes in groundwater-infiltration systems: Column studies', *Geochim. Cosmochim. Acta.* **57**, 3895–3906.

Weathers, L. J., Parkin, G. F. and Alvarez, P. J.: 1997, 'Utilization of cathodic hydrogen as electron donor for chloroform cometabolism by a mixed methanogenic culture',*Environ. Sci. Technol.* **31**(3), 880–885.

White, D. C., Pinkart, H. C. and Ringelberg, D. B.: 1997, *Manual of Environmental Microbiology*, American Society of Metals, Washington, D.C., pp. 91–101.

Wildman, M. J. and Alvarez, P. J.: 2001, 'RDX degradation using an integrated Fe(0)-microbial treatment approach', *Wat. Sci. Technol.* **43**(2), 25–33.