ENHANCEMENT OF RDX REMOVAL BY IRON-REDUCING BACTERIA IN FLOW-THROUGH COLUMN

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Abstract : Flow-through column apparatus were used to examine the reactivity of permeable reactive rusted iron barriers to clean up RDX-contaminated groundwater. Three columns were comprised of soil, rusted iron, and sand layers, and were fed with unlabeled plus ^{14}C-labeled RDX to verify its removal efficiency under different conditions. One column was prepared with inert glass beads instead of rusted iron to serve as baseline to evaluate the benefits of rusted iron. The second column was used to determine if soil bacteria colonize the rusted iron layer. The third column was inoculated with iron-reducing bacteria, *Shewanella algae* BRY, to evaluate the reactivity of iron oxides, which participate in the RDX degradation through bioaugmentation. RDX was completely removed in rusted iron layer of all columns, and bioaugmented column showed highest ^{14}C removal efficiency (approx. 90%) for 65 days. Dissimilatory iron-reducing bacteria such as *Shewanella algae* BRY may contribute to the reactivity of permeable reactive barrier through reductive dissolution and transforming oxides to more reactive forms.

Key Words : Bioaugmentation, Permeable Reactive Barriers, Zerovalent Iron, RDX, *Shewanella algae* BRY

INTRODUCTION

RDX (hexahydro-1,3,5-trinitro-1,3,5-triazine) is the British code name for Research Department Explosive.[1] RDX is toxic to humans and a variety of organisms, and is classified as a Class C (possible human) carcinogen by the US EPA.[2] Toxicity studies have led the Surgeon General to recommend a 24-hr maximum RDX concentration of 0.3 mg/L to protect aquatic life.

Because of its recalcitrance to microbial degradation, low tendency to volatilize (dimensionless Henry's constant, $H' = 2 \times 10^{-11}$) and high mobility in aquifers (log $K_{ow} = 0.8$)[3], clean-up of contaminated sites with RDX is a challenging problem. Treatment strategies to remove RDX from contaminated soil include physical-chemical processes[4], bioremediation[5], chemical degradation[6,7], photodegradation[8], and phytoremediation.[9,10] However, some of *ex situ* approaches are not cost-effective to treat large volumes of contaminated groundwater. In addition, complete destruction of RDX is not always accomplished, giving rise to the possibility that products of equal or greater toxicity accumulate.[5]

Many studies have reported that permeable reactive barriers using zero-valent iron (Fe^0) can remediate groundwater contaminated with chlorinated solvents, chromium, nitrate, and RDX.[11,12] In theory, iron-reducing bacteria could also enhance the reactivity of iron barriers by reductive dissolution of iron oxides (i.e., depassivation), or by formation of highly reactive surface-associated Fe(II) with semi-conductor

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properties (e.g., green rust and magnetite).[13] Our preliminary work also shows that reductive treatment of RDX with Fe^0 reduces its toxicity and enhances its subsequent biodegradability under either aerobic or anaerobic conditions[14], which suggests a high potential for natural attenuation of any byproducts that could break through an iron barrier. Consequently, an integrated microbial-Fe^0 system holds great promise to intercept and degrade numerous RDX plumes.

Although the technology of permeable reactive Fe^0 barriers (PRBs) is well developed, the complex mineralogy of these barriers is only now beginning to be uncovered. The oxides formed in these environments are a function of groundwater composition, type of Fe^0 used, target contaminant, and time. Field samples from active barriers have been identified as magnetite[15], goethite[16], maghemite[17], and green rust.[18] Apparently, the hydrogenotrophic consortium that exploits Fe^0 corrosion as a metabolic niche seems to enhance the further degradation of intermediates produced by the reaction of RDX with Fe^0. In batch experiments, we also found that iron-reducing bacteria such as *Shewanella alga* BRY and *Geobacter metallireducens* GS-15 could produce biogenic reactive iron-oxide species, resulting in degrading RDX.[13] This represents an additional biogeochemical interaction that could be exploited for enhanced RDX degradation in iron barriers.

This study was conducted to determine if dissimilatory iron-reducing bacteria (DIRB) enhance barrier reactivity, resulting in higher RDX removal, by reductive dissolution of Fe(III) oxides or by formation of more reactive oxides in PRBs.

MATERIALS AND METHODS

Batch Studies

In PRB performance, passivating Fe^0 surface and hindering its reactivity are concerned due to the formation of oxides. Iron oxides are known to hinder Fe^0 reactivity.[19] The oxides can also serve as a strong reducing agent where surface-

associated Fe(II) is present (e.g., green rust).[20] Thus, in order to estimate the reactivity of rusted iron, batch reactor studies were conducted. The rusted iron was obtained from columns that had been exposed to TCE, Cr(VI), sulfate, and nitrate mixtures for 1 yr.[11] This iron was used to mimic iron that has been aged in a reactive barrier over time, which could be amenable for reactivation by DIRB. We investigated the reactivity of this rusted iron towards RDX and compared it with that of "new" Master Builder® Fe^0 filings in batch studies. This "new" iron was used as shipped, and was not acid-washed. Iron samples (1 g) were added to serum bottles containing 26 mL of RDX solution (20 mg/L) and shaken on the reciprocating shaker for 2 hr. Liquid samples were withdrawn every 20 min, filtered, and analyzed by HPLC. In addition, the rusted iron surface was investigated using X-ray diffraction (XRD) and compared with new Fe^0 filings using scanning electron microscope (SEM).

Column Studies

Regarding this additional column experiment, three columns (30-cm long, 2.5-cm ID) equipped with lateral sampling ports were packed with a 5-cm layer of soil followed by an 18-cm layer of the rusted iron described in batch experiments (representing a passivated iron barrier) and a 7-cm sand layer as described in Oh and Alvarez.[12] Uncontaminated soil was obtained from the field in Iowa City, IA. Physicochemical properties of the soil were determined by Western Laboratories Inc. (Parma, ID) (Table 1). One of the columns was used to determine if soil bacteria colonize the iron layer, presumably to feed on cathodic H_2 produced by the anaerobic iron corrosion (Eq. $Fe^0 + 2H_2O \rightarrow Fe^{2+} + 2OH^- + H_2$). This column also serves as a baseline to evaluate the benefits of bioaugmentation. The second column was inoculated with the iron-reducing bacterium, *Shewanella algae* BRY (ATCC 51181, 10 mL of stock (52.6 mg protein/L) added at each port). This strain is facultative and can tolerate oxygen. It can feed

Table 1. Characteristics of the uncontaminated soil obtained from the field in Iowa City, IA

soil pH with H_2O	8.3
organic matter	2.3%
cation exchange capacity	22 meq
sand	39%
silt	50%
clay	11%

on H_2[21], which makes it attractive for use in PRBs. Thus, this strain was inoculated to determine if DIRB enhance barrier reactivity by reductive dissolution of Fe(III) oxides or by formation of reactive oxides. The third column was prepared with inert glass beads instead of the rusted iron to control for the effect of iron oxides on RDX degradation. RDX (unlabeled plus ^{14}C-labeled) was dissolved in bicarbonate-buffered synthetic groundwater[22] at 18 mg/L (10 μCi/L) and fed continuously at 2.3 mL/hr (about 0.5 ft/day superficial velocity) using peristaltic pump.

Analytical Methods

The rusted iron filings were also characterized by various techniques to obtain a baseline for comparing future changes in surface chemistry associated with DIRB activity. The first characterization technique was SEM. The air-dried Fe^0 samples were mounted on a sample stub and sputter coated with gold/palladium in an Emitech K550 sputter coater (Ashford, Kent, UK). The samples were imaged in a Hitachi S-4000 field emission scanning electron microscope (Tokyo, Japan). Digital images were collected using a Kevex digital beam control interface and Kevex Ultrascan software (Thermo Noran, Middleton, WI).

We also characterized the surface precipitate of oxidized iron by XRD. Identification of the surface precipitate removed from oxidized iron granules was accomplished with powder XRD on a Siemens D-5000 Diffractometer utilizing CuK α radiation. The surface precipitate was ground to a fine powder (<325 mesh) and

scanned between 15° and 80° with a step of 0.02° every two seconds.

RDX 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™ 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 and spectrophotometric detection at 240 nm (limit of detection = 0.1 mg/L). ^{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 liquid scintillation counting (LSC) using a Beckman LS 6000 IC (Beckman Instrument Inc., Fullerton, CA).

RESULTS AND DISCUSSION

Many studies have reported that Fe^0 can remove RDX in batch reactors.[23-25] Oh et al.[25] reported that Fe^0 could be used to remediate RDX-contaminated aquifers. Figure 1 verifies previous studies and shows that RDX was removed with Fe^0 faster than rusted iron in batch studies. For 120 min, the "new" Fe^0 transformed RDX by 95% of the initial RDX, whereas rusted iron transformed only 29% (Figure 1). The rusted iron used in this study was even less reactive than that of other study[26] on RDX removal, which was prepared in deionized water through air flushing for 2 weeks. Therefore, we concluded that the rusted iron used had been passivated and was suitable for the column study below investigating potential reactivation by DIRB.

The rusted iron surface was investigated and determined using SEM and XRD (Figure 2 and 3). SEM analysis showed that "rusted" iron had iron oxides on the surface (Figure 2b) whereas the "new" Fe^0 surface was clean (Figure 2a).
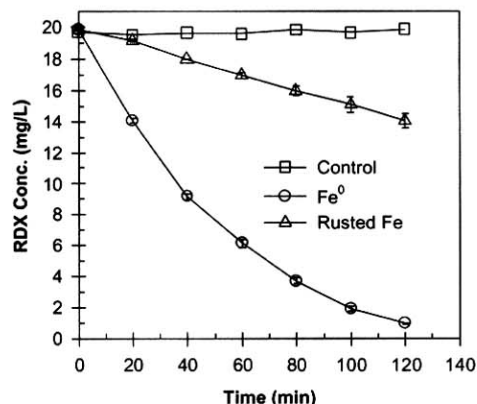
Figure 1. Reactivity of new versus rusted (i.e., passivated) iron for RDX removal.
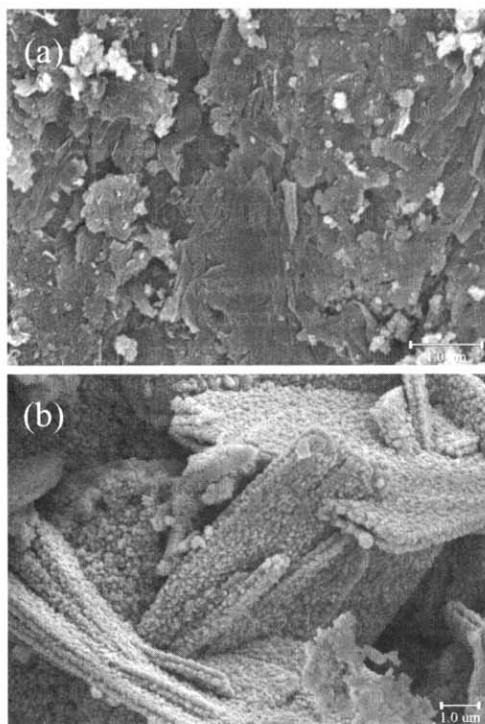

Figure 2. SEM pictures of "new" Fe^0 and "rusted" iron samples: (a) new Master Builder® Fe^0 filings and (b) rusted iron obtained from columns exposed to TCE, Cr(VI), sulfate, and nitrate mixtures for 1 yr.

The spectrum for surface precipitate of oxidized iron matched reference spectrum for goethite (α-FeOOH) (Figure 3). The only crystalline phase present on the rusted iron as observed with
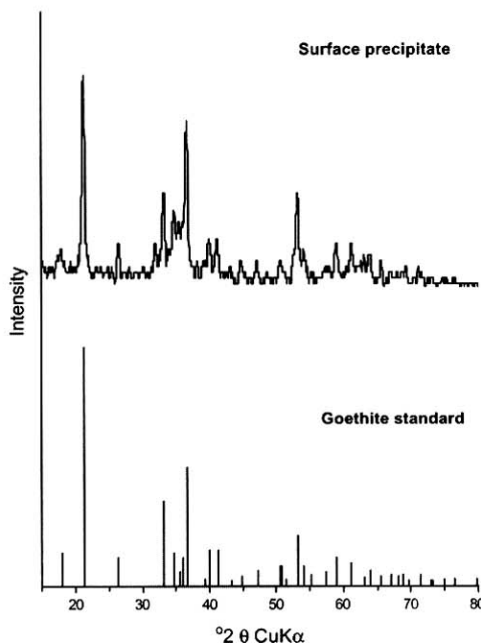

Figure 3. XRD pattern for surface precipitate removed from rusted iron sample. XRD scans were made between 15° and 80° with a step of 0.02° per two seconds.

XRD was goethite. Pratt et al.[16] reported goethite coated the iron filings after reducing chromate. This oxide might be slightly reactive towards RDX (Figure 1). Other studies indicated that DIRB respiration on Fe(III) bearing oxides produces ferrous iron oxides that are reactive towards redox-sensitive compounds.[27-29]

We have monitored removal of RDX and dissolved ^{14}C activity along the length of the columns by sampling the side ports and analyzing the samples by HPLC and LSC (Figure 4). RDX concentration profiles for 65 days showed extensive RDX removal in all columns with rusted iron filings (Figure 4). For the control column packing with glass beads, RDX concentration and ^{14}C activity remained constant through column. The colonized column showed decrease in dissolved ^{14}C activity along the length of the column. The column inoculated with *Shewanella algae* BRY exhibited a higher removal of dissolved ^{14}C activity than the colonized column. Dissolved ^{14}C removal might
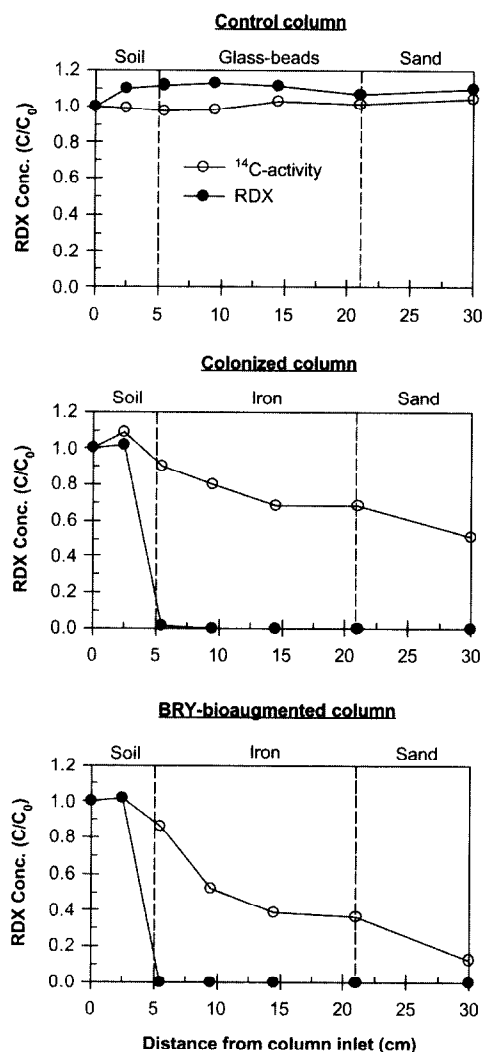
Figure 4. Change of RDX concentration and soluble ^{14}C activity using rusted iron after 65 days of operation. (C_0 = 18 mg/L and 10 μ Ci/L).

be contributed to mineralization and polymerization of RDX metabolites into the soil and iron oxides. This result suggests that *Shewanella algae* BRY might be able to enhance barrier reactivity through de-passivating iron oxides and transforming oxides to more reactive forms (e.g., magnetite, green rust, and other forms of surface-bound Fe(II)). Main soluble metabolite of the radiolabel had the same retention time as methylenedinitramine on HPLC analyses with radio-chromatographic detector.[25]

SEM analysis confirmed that *Shewanella algae* BRY colonized on the rusted iron surface (data not shown). Samples from bioaugmented column showed rod-shaped bacteria on the surface of iron oxides. This result suggests that iron-reducing bacteria might use cathodic hydrogen as an electron donor through respiring iron oxides.[11]

In preliminary experiments, *Shewanella algae* BRY could degrade RDX (3.5 mg/L) for 10 days of incubation. However, we observed a fast disappearance of RDX in batch reactors with bacteria plus hydrous ferric oxide within 1 day (data not shown). In another study, biological iron oxides were produced through the respiration of the iron(III)-reducing bacterium, *Geobacter metallireducens* GS-15, in batch cultures containing hydrous ferric oxide.[28] X-ray diffraction patterns confirmed the presence of magnetite (Fe_3O_4) and siderite ($FeCO_3$) in the biological oxides. These biogenic oxides contributed to the abiotic removal of RDX. Similar to *Geobacter metallireducens* GS-15, *Shewanella algae* BRY can also produce oxides that are reactive towards redox-sensitive compounds (i.e., RDX).

CONCLUSIONS

The surface precipitate of rusted iron was determined as goethite that has slight activity for RDX removal. The bioaugmented column inoculated with *Shewanella algae* BRY exhibited highest ^{14}C removal efficiency, suggesting that DIRB might be able to enhance reactivity of iron oxides.

Overall, this study suggests that the rusted iron barrier can be reactive by DIRB to remove RDX from contaminated groundwater, and that treatment efficiency might be enhanced by biogeochemical interactions through bioaugmentation.

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