



Comparison of biotic and abiotic treatment approaches for co-mingled perchlorate, nitrate, and nitramine explosives in groundwater

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Abstract

Biological and abiotic approaches for treating co-mingled perchlorate, nitrate, and nitramine explosives in groundwater were compared in microcosm and column studies. In microcosms, microscale zero-valent iron (mZVI), nanoscale zero-valent iron (nZVI), and nickel catalyzed the reduction of RDX and HMX from initial concentrations of 9 and 1 mg/L, respectively, to below detection (0.02 mg/L), within 2 h. The mZVI and nZVI also degraded nitrate (3 mg/L) to below 0.4 mg/L, but none of the metal catalysts were observed to appreciably reduce perchlorate (~5 mg/L) in microcosms. Perchlorate losses were observed after approximately 2 months in columns of aquifer solids treated with mZVI, but this decline appears to be the result of biodegradation rather than abiotic reduction. An emulsified vegetable oil substrate was observed to effectively promote the biological reduction of nitrate, RDX and perchlorate in microcosms, and all four target contaminants in the flow-through columns. Nitrate and perchlorate were biodegraded most rapidly, followed by RDX and then HMX, although the rates of biological reduction for the nitramine explosives were appreciably slower than observed for mZVI or nickel. A model was developed to compare contaminant degradation mechanisms and rates between the biotic and abiotic treatments.

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1. Introduction

Explosive compounds, including hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX) and octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX), are widespread soil contaminants at many

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current and former military facilities. Perchlorate, which is used as an energetics booster and oxidant in a variety of military rockets and munitions, is also present in soils at many sites. Because both explosives and perchlorate can be transported through soils to the subsurface, these compounds are now impacting groundwater and drinking water at numerous locations across the country (Yamamoto et al., 2004; Hatzinger, 2005). For example, according to a recent report from the US Army Corps of Engineers, the US Army has 583 sites at 82 installations that have explosives in groundwater, and 87 additional locations with suspected contamination (Wani et al., 2003). Some of these sites, such as those used as live-fire ranges, testing facilities for weapons systems with solid rocket propulsion, and open-burn/open-detonation areas, have perchlorate co-occurring with explosives (Parette et al., 2005; ITRC, 2005).

Health advisory levels have been established by the US Environmental Protection Agency (USEPA) for several explosives, including HMX, RDX, 2,4,6-trinitrotoluene (TNT), 2,4-dinitrotoluene (2,4-DNT) and 2,6-dinitrotoluene (2,6-DNT) (USEPA, 2004). The Drinking Water Equivalent Levels (DWELs) for the latter four compounds range from 20 µg/L for TNT to 100 µg/L for RDX and 2,4-DNT. The DWEL for HMX is significantly higher at 2 mg/L. The USEPA also recently established an official reference dose for perchlorate that equates to a DWEL of 24.5 µg/L, and advisory levels ranging from 1 to 18 µg/L have been established by several US states (Hatzinger, 2005).

Techniques to remove explosives from surface soils, including soil washing, soil bioreactors, iron addition, and enhanced in situ bioremediation, are well established (e.g., Griest et al., 1993; Zhang et al., 2001; Fuller et al., 2003; Comfort et al., 2003), but there are presently few proven methods to treat explosive compounds in groundwater. In addition, although bioremediation technologies for perchlorate in water have been developed (Hatzinger, 2005), there is relatively little information concerning the joint treatment of perchlorate and explosives (Weeks et al., 2003; Parette et al., 2005). Nitrate was also included as a target contaminant in these studies due to its ubiquity in groundwater from agriculture and other sources (Spalding and Exner, 1993), and recent studies suggesting that nitrate can influence the rates of biodegradation of both perchlorate (Hatzinger et al., 2002; Tan et al., 2004) and nitramine explosives (Freedman and Sutherland, 1998; Wani et al., 2003).

The capacity to metabolize nitramine and nitroaromatic explosives appears to be reasonably widespread across bacterial genera (Fuller and Manning, 1997), and pathways for reductive degradation of the major classes of explosives have been the subject of extensive study during the past several years (Spain, 1995; Esteve-Nuñez et al., 2000; Hawari et al., 2000; Shen et al., 2000). A diverse group of perchlorate-reducing bacteria have also been isolated, and these organisms appear to be ubiquitous in many environments, including groundwater aquifers (Coates and Achenbach, 2004). Moreover, although explosives and perchlorate are likely to be degraded by different bacteria in most environments, both groups of bacteria (i.e., explosives and perchlorate degraders) require a suitable organic or inorganic substrate to perform their respective degradative processes. The same is true for the dissimilative reduction of nitrate by bacteria. Select substrates, including various fatty acids, sugars, hydrogen, and vegetable oil are likely to promote all three processes (Boopathy et al., 1994; Fuller et al., 2004; Gayle et al., 1989; Hatzinger, 2005).

Zero-valent iron (ZVI) has been applied in the field for more than a decade to mediate the reductive dechlorination of chlorinated solvents. Laboratory research also shows that ZVI is effective for the reductive treatment of nitramine and nitroaromatic explosives (Hundal et al., 1997; Oh and Alvarez, 2002; Comfort et al., 2003; Johnson et al., 2005). A few recent studies suggest that perchlorate is also susceptible to reduction by ZVI particles, although the rates of this process are considerably slower than for many other contaminants (Moore et al., 2003; Cao et al.,

2005). Precious metal catalysts, either alone or in combination with ZVI, have also been shown to promote the reduction of a number of pollutants, including solvents, and explosives (Li and Klabunde, 1998; Lowry and Reinhard, 1999; Schrick et al., 2002; Fuller et al., in press).

The objective of this research was to compare the effectiveness of biological and abiotic treatment approaches for removing co-mingled explosives, perchlorate, and nitrate in groundwater. Biological treatment consisted of adding organic substrates to promote bioreduction of the target compounds, and abiotic approaches included amending aquifer solids with micron-sized ZVI, nanometer-sized ZVI, or nickel as catalysts for chemical reduction of the contaminants. A model was subsequently developed to compare degradation rates and mechanisms among the target compounds and between the biotic and abiotic treatments.

2. Model development

2.1. Biodegradation

Biodegradation of perchlorate, RDX and HMX is based on a pseudo first-order kinetic model that includes first-order microbial growth as follows:

$$r = [-MKC] \div R \quad (1)$$

and

$$M = M_0 \exp(\alpha t) \quad (2)$$

where r is the biodegradation rate (mg/cm³/day), K is the pseudo first-order rate constant (cm³/mg/day), C is the contaminant aqueous concentration (mg/cm³), M is the concentration of contaminant-degrading microorganisms (mg/cm³), M_0 is the initial microorganism concentration (mg/cm³), α is the microbial growth rate constant (day⁻¹), t is the time (day), and R is the dimensionless retardation factor that accounts for contaminant partitioning between the soil and aqueous phases. R is defined as

$$R = 1 + \frac{\rho K_d}{\theta} \quad (3)$$

where ρ is the bulk density of the soil in the batch microcosms or the columns (kg soil per cm³ volume), K_d is the linear soil–water sorption coefficient (cm³/kg, regressed from the column data), and θ is the water filled porosity.

Eq. (2) assumes that microbial growth is a first-order process that occurs via metabolism of the electron donor, and that the electron donor is present in excess. Combining Eqs. (1) and (2), the following expression is obtained:

$$r = [-\beta \exp(\alpha t) C] \div R \quad (4)$$

where β is a constant that is specific to the rate of contaminant biodegradation and is equal to the product $M_0 K$. For the batch experiments, Eq. (4) is solved analytically as follows:

$$C = C_0 \exp \left[\frac{-\beta}{R\alpha} \exp(\alpha t) \right] \quad (5)$$

where C_0 is the initial contaminant aqueous concentration in mg/cm³.

Contaminant fate and transport through the laboratory columns is described by the following equation, which incorporates the same biodegradation model presented in Eq. (4):

$$R \frac{dC}{dt} = D \frac{d^2C}{dx^2} - v \frac{dC}{dx} - \beta \exp(\alpha t) C \quad (6)$$

where D is the dispersion coefficient (cm²/day) and v is the linear velocity (cm/day) of the groundwater through the column. Values of D and v were determined by performing bromide tracer experiments in each of the columns (described in the Materials and methods section). For the bromide experiments, the reaction term in Eq. (6) is neglected, allowing Eq. (6) to be solved analytically for the bromide elution curve assuming a continuous bromide input (Schnoor, 1996). Values of D and v were regressed to the bromide elution curves using a non-linear least squares regression. Values of R for each contaminant were determined in a similar manner (using the same analytical solution) for the contaminant elution curves in non-reactive control columns (described in the Materials and methods section).

For contaminant fate and transport in the columns amended with biotic or abiotic reactants, Eq. (6) was solved numerically using commercially available MODFLOW and SEAM3D software (GMS, 2003). Values of D , v , and R regressed from the bromide tracer and control columns were used in the numerical simulation, so α and β were the only parameters that needed to be determined in the numerical model. The numerical model was constructed using a simple one-dimensional grid that consisted of ten cells to simulate the length of the aquifer column. Values for α and β were regressed using the model by employing a trial- and-error method to minimize residual error between the model prediction and experimental data. The biodegradation simulation was performed using a large excess of electron donor, such that the electron donor concentration remained essentially constant throughout the duration of the column study.

2.2. Abiotic reduction via ZVI and nickel catalyst

Reduction of target contaminants via ZVI and nickel catalysts is modeled as described for biodegradation, except that the bracketed term in Eq. (4) is substituted with a pseudo first-order kinetic expression (i.e., kC), where k is the regressed first-order rate constant (day⁻¹). First-order kinetic models have been shown to reasonably describe these reactions (Johnson et al., 2005; Schaefer et al., in press). Both mass-based and surface area-based first-order rate constants are defined as follows:

$$k_m = \frac{k}{\rho} \quad (7)$$

$$k_a = \frac{k}{\rho a} \quad (8)$$

where k_m is the first-order mass-based rate constant (Lg⁻¹day⁻¹), k_a is the first-order surface area-based rate constant (Lm⁻²day⁻¹), ρ is the metal (ZVI or nickel) dosage (g/L), and a is the surface area of the metal, as measured by BET-nitrogen adsorption (m²/g).

3. Materials and methods

Saturated aquifer solids and groundwater were obtained from a military facility in Maryland at a location where RDX and HMX were historically discharged in wastewater. The aquifer solids were collected in 1.2 m (4-ft) soil cores from a depth of approximately 1.8 to 4.3 m (6 to 14 ft) below

ground surface using a direct push (Geoprobe) rig. Core samples were obtained in acetate sleeves, which were sealed with end caps in the field. The cores were shipped to the laboratory on ice, then the solids were removed from the acetate sleeves and thoroughly homogenized prior to use. The texture of the aquifer materials consisted of silty sand with trace amounts of clay. Groundwater was collected from a temporary well set in the Geoprobe boring using a peristaltic pump. Although the environmental samples were obtained from an area in which explosives were discharged in the past, the aquifer sediments and groundwater did not contain measurable concentrations of target explosives, perchlorate, or nitrate. These contaminants were added in the laboratory.

Several substrates were evaluated for their potential to promote biological reduction of nitramine explosives, perchlorate, and nitrate in site samples. These substrates included lactate (60% sodium lactate solution), ethanol, hydrogen gas, crude soybean oil (Cargill, Minneapolis, MN, USA), refined vegetable oil (Wesson oil), and an emulsified vegetable oil substrate, which also includes lactate and trace nutrients (EOS, EOS Remediation, Inc., Raleigh, NC, USA). These materials were selected based on previous laboratory and field studies conducted to evaluate the degradation of explosives, nitrate, and perchlorate in various environments (Hatzinger et al., 2002; Fuller et al., 2004).

Abiotic amendments consisted of a micron-sized ZVI (mZVI), a nanometer-sized ZVI (nZVI), and a nickel catalyst. The H-200 mZVI was supplied by ARS Technologies, Inc. (New Brunswick, NJ, USA), and had a mean particle diameter of approximately 850 μm and a surface area of approximately 0.1 m^2/g . The nZVI was supplied by Pars Environmental, Inc. (Robbinsville, NJ, USA), and had a mean particle diameter of 70 nm and a surface area of 12 m^2/g . The nickel catalyst was purchased from Sigma–Aldrich and had a mean particle size <60 μm and a surface area of 190 m^2/g . The nickel particles consisted of a reduced and stabilized nickel (65% wt/wt; supported on silica/alumina).

All solvents were of high performance liquid chromatography (HPLC) grade and chemicals were of reagent grade or better. A research quantity of RDX was provided by Jim Phelan at Sandia National Laboratories (Albuquerque, NM). HMX was a gift from Herb Fredrickson at the US Army Engineer Research and Development Center (Vicksburg, MS). RDX breakdown products were obtained from SRI International (Menlo Park, CA, USA). Sodium perchlorate and sodium nitrate were purchased from Sigma–Aldrich.

3.1. Microcosm experiments

Laboratory microcosms to assess biodegradation were prepared by spiking site groundwater with RDX (5 mg/L), HMX (1 mg/L), perchlorate (5 mg/L) and nitrate (4 mg/L as nitrate-N). A 100-mL volume of the spiked groundwater was then added to 160-mL serum bottles, along with 50 g of homogenized aquifer material. Triplicate bottles were then amended with one of the selected electron donors. Lactate and ethanol were added to provide an initial concentration of 200 mg/L for each treatment, the various oils were each applied in a 1-mL volume (approximately 0.75% (vol/vol) as carbon in the groundwater), and the hydrogen gas was added in a 10-mL volume to the bottle headspace. In addition to these treatments, triplicate bottles were prepared without electron donor (live control), and triplicates were prepared using a 0.1% (vol/vol) formaldehyde solution to inhibit microbial activity (killed control). All microcosms were sealed with Teflon-lined butyl rubber septa and incubated with shaking at 15 °C to simulate typical *in situ* groundwater temperatures.

The groundwater in each bottle was sub-sampled using a needle and syringe after various incubation times and analyzed for nitrate (EPA Method 300.0), perchlorate (EPA Method 314.0), explosives and explosive breakdown products (modified EPA Method 8330), and short chain

fatty acids (modified EPA Method 8015). To insure that electron donor concentrations were not limiting biodegradation, each bottle received an additional amendment of electron donor (equal to the original amount) on Day 66.

Microcosms to evaluate abiotic treatment using micron-sized and nanoscale ZVI and the nickel catalyst were prepared and monitored similarly to the biotic treatments. Triplicate treatments were prepared containing 0.5 g of the nickel catalyst, 0.1 g of nZVI, or 1 g of the mZVI. The initial zero-valent metal dosages were based on preliminary estimates of reactivity. Control bottles without ZVI or nickel catalyst were prepared, but no killed control bottles were prepared during this experiment. The bottles were purged for 20 s with nitrogen gas and then sealed with Teflon-lined butyl rubber stoppers. During sampling, 10-mL of nitrogen gas was injected into each bottle with a 20-mL syringe, and then 11.5 mL of liquid was withdrawn. A 1.5 mL volume of the sample was centrifuged at 14,000 rpm for 2 min, and then passed through a 0.45 μm glass microfiber syringe filter unit to remove the metal catalyst and other particulates before analysis for explosives. The remaining 10 mL was centrifuged at 3400 rpm for 10 min, and the supernatant was passed through a 0.2 μm pore-size cellulose acetate filter. These samples were then analyzed for nitrate and perchlorate.

3.2. Column experiments

Laboratory column studies were performed to evaluate biotic and abiotic treatment of the target contaminants in a flow-through system. Columns were constructed from PVC pipe (2.5 cm I.D. \times 15 cm length) with threaded ends and caps (schematic and photo in Fig. 1). Column experiments were performed in an environmental chamber at 15 °C. The columns received homogenized aquifer sediments as described for the microcosm studies. However, due to the volume of water required for the studies and the absence of a readily available source at the site, an artificial groundwater was prepared to simulate the site water geochemistry. Table 1 describes the composition of the artificial groundwater.

Five soil columns were prepared based on the data obtained from microcosms. One column received no amendments, and served as a live control, while a second received 0.1% formaldehyde to inhibit biological activity (i.e., killed control). The remaining three columns received emulsified vegetable oil substrate (EOS), mZVI, and nickel catalyst, respectively. Approximately 120 g of soil was firmly packed into each column. The mZVI and nickel catalyst were mixed with the aquifer solids by weight until roughly 0.008 g was added per g wet solids. The metals were added and homogenized with the aquifer materials prior to packing the columns. Emulsified vegetable oil was delivered by injecting 33 mL of a 1:4 dilution of concentrated emulsion through the column after it was packed, and then passing 330 mL of the artificial groundwater through the column to remove excess organic material (i.e., leaving the adsorbed oil behind).

A parallel column test was performed to assess the quantity of emulsified oil present in the solids. For this test, the oil substrate was added as described above, then the sediments were removed and placed in 6 tubes (\sim 20 g solids ea.) based on distance from the column inlet. The samples were analyzed for total organic carbon (TOC) according to EPA Method 415.1. An unamended sample was also analyzed to determine the background level of TOC. Based on this study, the EOS delivery procedure provided an average TOC concentration of approximately 7500 mg/kg (or, approximately 0.02 cm^3 EOS per cm^3 of soil bed) across the length of the column. There was no measurable gradient between the influent and effluent ends of the column.

A syringe pump was used to inject artificial groundwater water into each column. The pump was connected to the inlet of each column using Teflon tubing, and the water exited each column

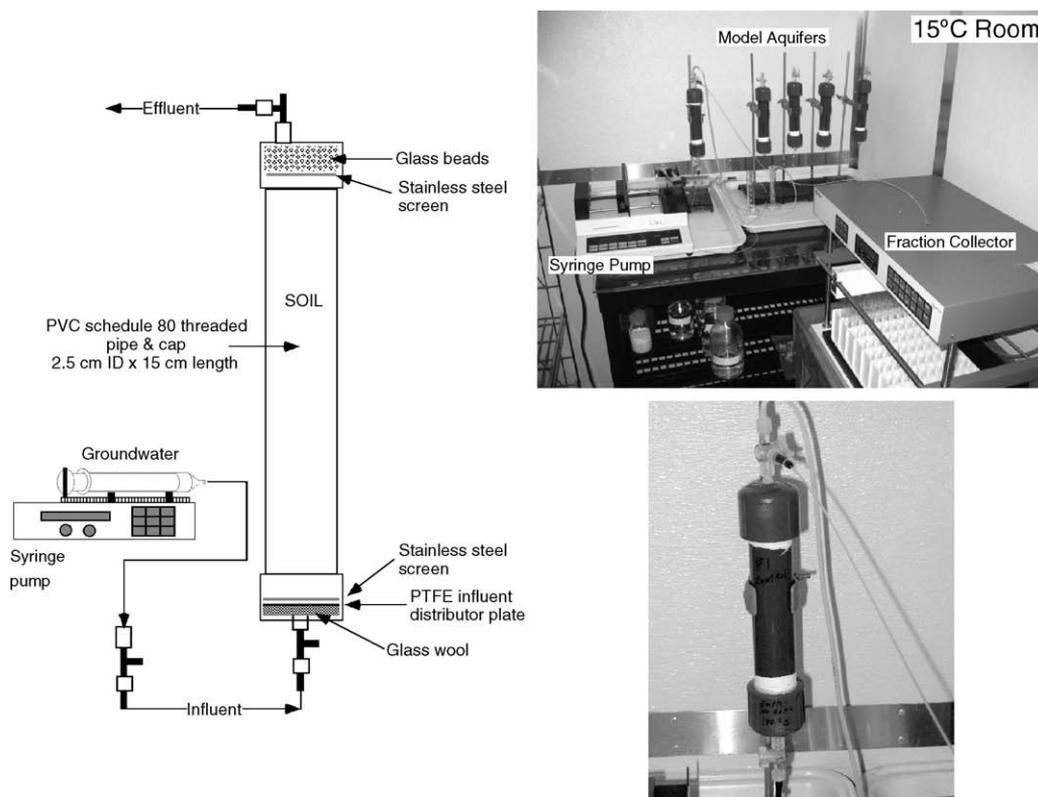


Fig. 1. Schematic and photographs of the flow-through columns.

to a fraction collector. All five columns were initially saturated in an upflow direction with the artificial groundwater. A bromide tracer test was performed with each column to determine the groundwater velocity and dispersion coefficient, and to evaluate any potential impacts of the mZVI, nickel, and emulsified oil amendments on these parameters.

Following the tracer tests, artificial groundwater for each column was spiked with the target contaminants. The concentrations of HMX, RDX, perchlorate and nitrate (as nitrate-N) were 1, 5, 5, and 5 mg/L, respectively. The influent contained HMX and RDX from Day 0, but nitrate and perchlorate addition was not started until Day 6. Therefore, no samples of influent or effluent

Table 1
Composition of the artificial groundwater

Component	Concentration (g/L)
NaNO ₃ as N	0.10
MnSO ₄ ·H ₂ O	0.001
Na ₂ SO ₄	0.180
NaCl	0.113
NaHCO ₃	0.040
HCl*	0.011

*The pH of the water was 6.5.

were analyzed for nitrate or perchlorate until Day 7. The influent water for the killed control column was amended with formaldehyde (0.1% vol/vol) and mercuric chloride (750 mg/L) as microbial inhibitors. Influent artificial groundwater passed through the columns at a rate of approximately 0.9 mL/h for the duration of the study, which, based on bromide tracer results, equates to a residence time of approximately 24 h.

Samples of influent and effluent from each column were collected at regular intervals throughout the study, and analyzed for explosive compounds, perchlorate, and nitrate as described above. The pH was also measured using a meter.

4. Results and discussion

4.1. Microcosm experiments — biotic treatment

Nitrate was biodegraded from the initial concentration of ~4 mg/L (as nitrate-N) to below detection (MDL=0.4 mg/L) within 4 days in all microcosms receiving an organic electron donor (Fig. 2). No accumulation of nitrite was observed. Nitrate was also biodegraded in the microcosms receiving hydrogen as an electron donor, although the rate of loss was appreciably slower than for the bottles receiving organic electron donors. Degradation of nitrate was not observed in the microcosm that received formaldehyde as a killing agent or in the controls that did not receive an electron donor.

Biodegradation of perchlorate to below detection (MDL=0.004 mg/L) was observed in microcosms receiving lactate, refined vegetable oil, and emulsified vegetable oil during the 73-day study, with the most rapid loss occurring in the bottles receiving the emulsified oil (Fig. 2). The lag period preceding perchlorate biodegradation in each of these treatments was significantly longer than that observed for nitrate. This lag period may represent biochemical inhibition of perchlorate reduction by nitrate, as previously reported (Hatzinger et al., 2002; Coates and Achenbach, 2004) or may reflect differences in the relative biomass or degradation kinetics of perchlorate- and nitrate-reducing organisms in the samples. Approximately 50% of the added perchlorate was also biodegraded in microcosms amended with crude soybean oil during the experiment. Unlike nitrate, there was no apparent degradation of perchlorate in bottles receiving hydrogen or ethanol as electron donors. Perchlorate was also not degraded in the live unamended samples or in those receiving formaldehyde as a killing agent.

Soluble RDX concentrations declined by approximately 20% (from ~4.5 to 3.5 mg/L) in microcosms receiving crude and refined soybean oil during 102 days of incubation (Fig. 3). In the samples receiving lactate and ethanol, RDX concentrations declined from 4.7 mg/L at the beginning of the study to 2.8 and 2.5 mg/L, respectively, after 102 days. Much of the loss was observed during the last month of incubation. Emulsified vegetable oil was the most effective substrate for promoting RDX biodegradation. RDX concentrations declined by an average of 95% (to 0.20 mg/L) during the 102-day study in samples initially amended with emulsified oil. RDX concentrations did not decline appreciably in the samples receiving hydrogen, or in the live or killed control samples. Besides parent RDX, the three initial nitroso-breakdown products of RDX; hexahydro-1-nitroso-3,5-dinitro-1,3,5-triazine (MNX), hexahydro-1,3-dinitroso-5-nitro-1,3,5-triazine (DNX), and hexahydro-1,3,5-trinitroso-1,3,5-triazine (TNX) were observed in the samples receiving the emulsified oil, confirming that biodegradation accounted for the decline in RDX concentrations in these samples.

Parallel experiments showed that partitioning of RDX into the vegetable oil phase was negligible (data not shown). This result is consistent with the fact that the RDX concentrations in

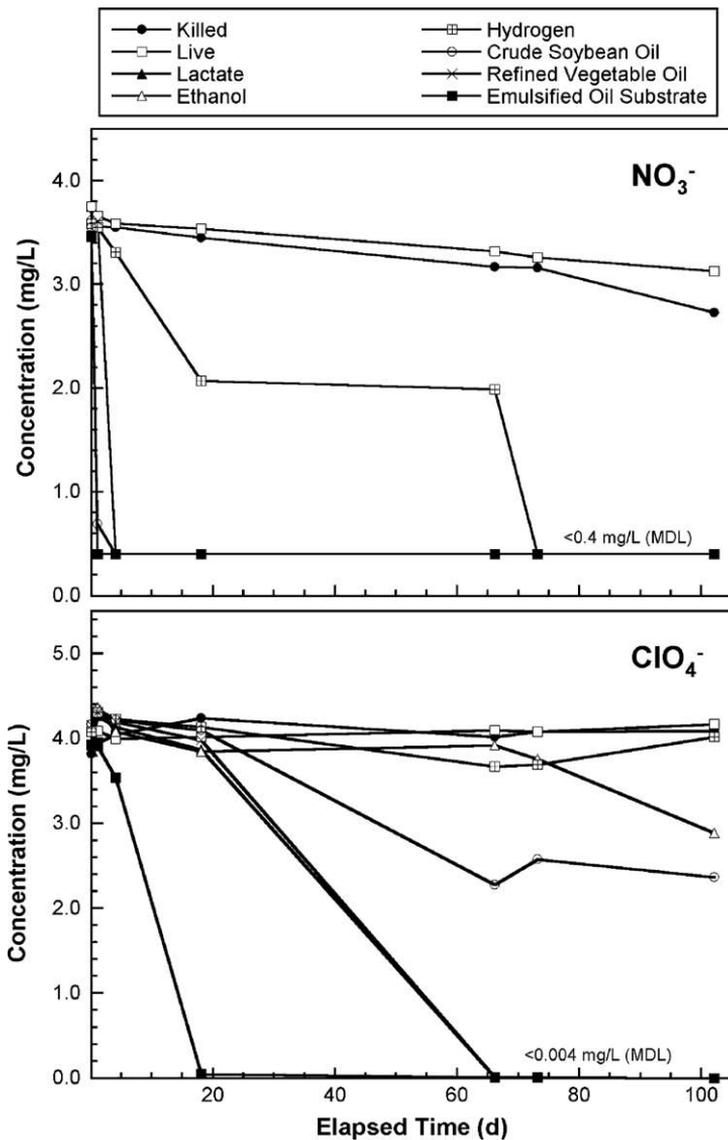


Fig. 2. Nitrate and perchlorate levels in microcosms amended with different substrates. Mean values from triplicate samples are shown.

the controls and emulsified oil treatments were identical through the first 4 days. If a significant amount of RDX partitioned into the oil phase, measurable decreases in RDX concentration relative to the controls would have been expected to occur during this initial 4-day period.

Unlike RDX, biodegradation of HMX was not observed in any of the microcosms during the study. This result is somewhat unexpected since HMX is structurally similar to RDX, and the mechanism of biological degradation of these two nitramine explosives is generally assumed to be the same (Hawari, 2000). However, previous results from soil bioslurry reactors show that HMX biodegradation can be delayed by RDX when the two are present in a mixture (Shen et al., 2000).

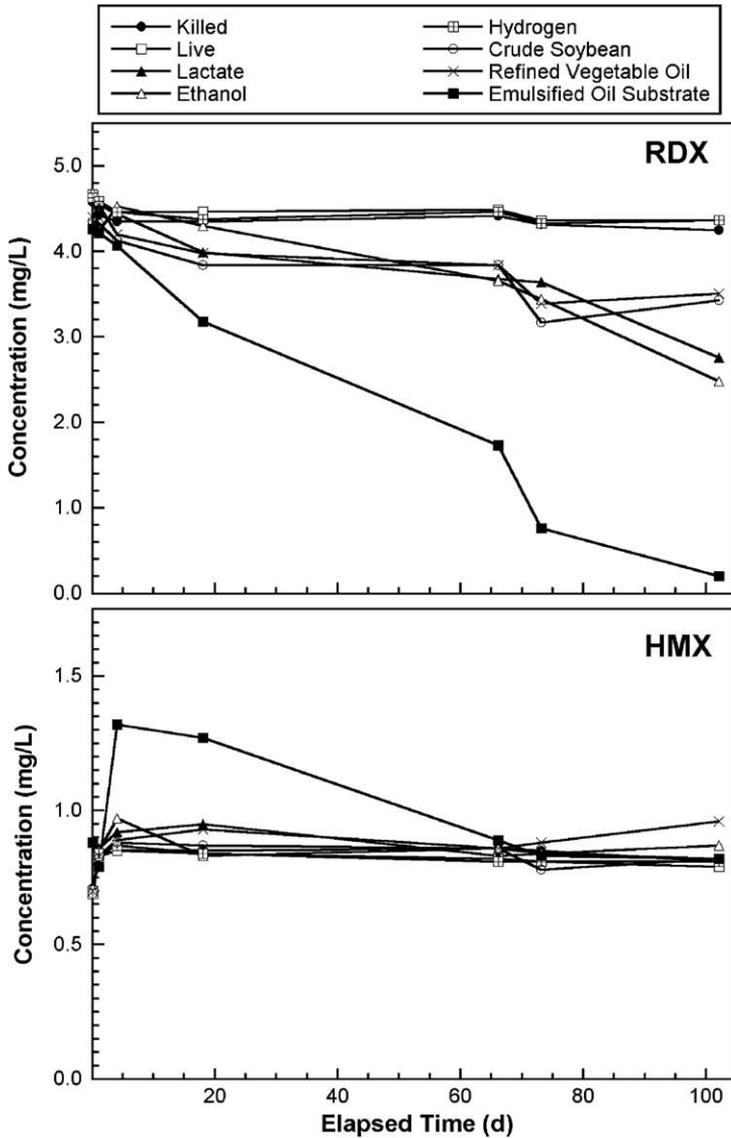


Fig. 3. RDX and HMX levels in microcosms amended with different substrates. Mean values from triplicate samples are shown.

Thus, the fact that RDX was present in the microcosms, albeit at declining concentrations, during the study may explain the observed recalcitrance of HMX to biodegradation in this study. This hypothesis is supported by the data from flow-through columns, in which HMX biodegradation was observed, but only after RDX was near or below detection in column effluent (see Section 4.3).

4.2. Microcosm experiments — abiotic treatment

RDX and HMX were rapidly degraded in microcosms receiving nickel and each of the iron catalysts; concentrations of each explosive were reduced to below detection (MDL=0.02 mg/L) by the

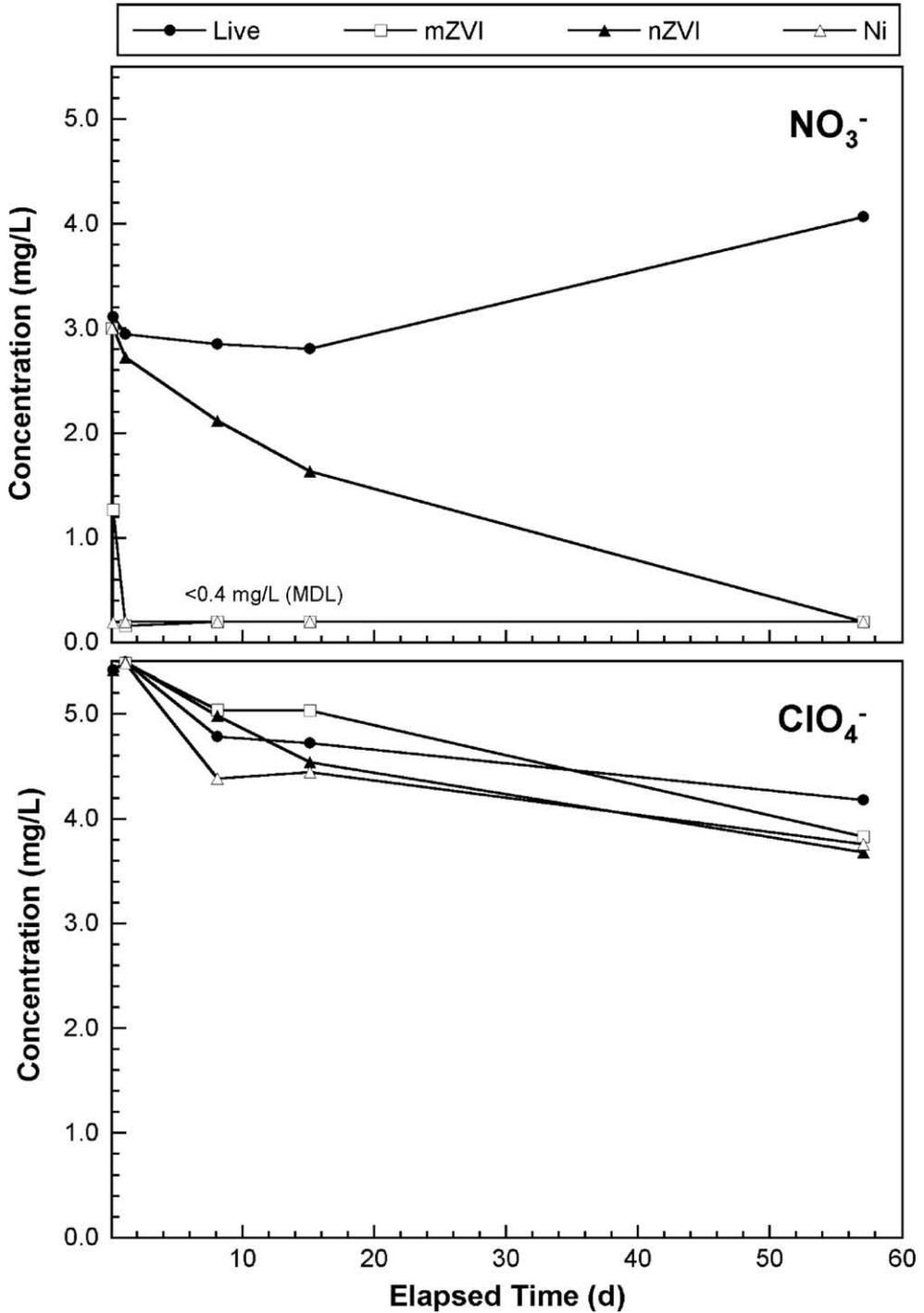


Fig. 4. Nitrate and perchlorate levels in microcosms amended mZVI, nZVI, nickel, or no catalyst (live). Mean values from triplicate samples are shown.

initial sampling point at 2 h (data not shown). Because degradation was so rapid, meaningful rate constants could not be obtained for these compounds. Nitrate was degraded more slowly, particularly in samples treated with nZVI (Fig. 4), with resulting first-order mass-based rate constants of $0.26 \text{ L day}^{-1} \text{ g}^{-1}$, $0.040 \text{ L day}^{-1} \text{ g}^{-1}$, and $6.6 \text{ L day}^{-1} \text{ g}^{-1}$ for the mZVI, nZVI, and nickel catalyst, respectively. Corresponding surface area based rate constants were $2.6 \text{ L day}^{-1} \text{ m}^{-2}$, $0.0033 \text{ L day}^{-1} \text{ m}^{-2}$, and $0.035 \text{ L day}^{-1} \text{ m}^{-2}$ for the mZVI, nZVI, and nickel catalyst, respectively. The comparatively slow rate of nitrate reduction by the nZVI is not readily explained, as nanoscale ZVI has previously been reported to be more reactive towards nitrate than micron-sized ZVI (Choe et al., 2000).

In contrast to the nitramine explosives and nitrate, little or no abiotic degradation of perchlorate was observed. Perchlorate concentrations did decline somewhat during the 57-day study in each of the treatments, but concentrations were not appreciably lower than those in a live unamended control (Fig. 4). Thus, rate constants were not obtained. Other researchers have reported perchlorate reduction by iron particles, but the kinetics of the reductive process at ambient temperature or in the absence of large quantities of zero-valent iron are exceedingly slow (Moore et al., 2003; Cao et al., 2005). Moreover, although perchlorate reduction can be catalyzed by some transition metal catalysts (Amadei and Earley, 2001), our data suggest that reaction rates with nickel in groundwater are too slow to be of practical importance. Similar results were recently reported for palladium-catalyzed destruction of perchlorate in a reactor system (Barney, 2005).

4.3. Column experiments

The bulk density of the aquifer solids in each column was approximately 1.6 g/cm^3 . Assuming a solids density of 2.5 g/cm^3 , the calculated porosity in each column was 0.36. The elution of bromide during tracer tests was similar for each column. The average regressed values for D and v were 10.3 cm/day and $13.7 \text{ cm}^2/\text{day}$, respectively. Regressed values for each column were within 20% of these average values. These results indicate that the amendments (e.g., emulsified oil, mZVI, or nickel) did not have a significant impact on groundwater flow through the columns.

Regressed retardation factors for RDX and HMX were 2.1 and 3.8, respectively. Using Eq. (3), along with the soil bulk density of 1.6 g/cm^3 and a porosity of 0.36, the sorption coefficient for RDX was calculated to be $0.25 \text{ cm}^3/\text{kg}$ and that for HMX was calculated as $0.63 \text{ cm}^3/\text{kg}$. No retardation or sorption was observed for nitrate or perchlorate.

The most rapid loss of perchlorate was observed in the column amended with emulsified vegetable oil (Fig. 5). Effluent perchlorate concentrations from this column were below detection (MDL = 0.004 mg/L) on Day 22 and remained non-detect for the duration of the study. Perchlorate degradation was not observed in the unamended live column, the killed control column, or the nickel-treated column. As previously noted, perchlorate was observed to elute at the same rate as the bromide tracer in the live and killed control columns, indicating negligible sorption of the anion to the aquifer solids.

Similar to the nickel-treated column, perchlorate degradation was not initially observed in the column that received mZVI. However, after approximately 55 days of operation, perchlorate effluent concentrations from this column began to decrease (Fig. 5). This reduction most likely reflects enhanced perchlorate biodegradation by native microflora. The corrosion of the mZVI would be expected to create anoxic conditions in the column and to produce dissolved hydrogen, which could subsequently serve as an electron donor for autotrophic perchlorate-reducing bacteria (Zhang et al., 2002; Logan and LaPoint, 2002; Yu et al., 2006). Hydrogen was observed

to promote denitrification but not perchlorate reduction in microcosm studies, but this experiment may not have been conducted long enough to observe losses of perchlorate. Alternatively, enhanced reactivity of ZVI toward perchlorate due to the formation of iron oxide coatings may account for the reduction in effluent perchlorate concentrations. Although the catalytic reduction of organic pollutants by ZVI typically decreases over time (Klausen et al., 2001; Bandstra et al., 2005; Johnson et al., 2005), enhanced activity due to magnetite formation has been reported for nitrate (Huang et al., 2003). Thus, this possibility can not be ruled out for perchlorate. Additional studies in which ZVI is added to live and killed samples are required to better assess the mechanism of enhanced perchlorate reduction.

Like perchlorate, nitrate concentrations in the emulsified oil treated columns were below detection (<0.5 mg/L) for much of the column study (Fig. 5). In contrast to perchlorate, however, nitrate concentrations were also reduced to below detection in the column receiving mZVI. Nitrate was not degraded during transport through the killed column, but losses were observed during the initial 60 days of transport through the live unamended column. This loss probably reflects biodegradation supported by natural organic matter present in the aquifer materials. These results are interesting in that nitrate degradation was not observed in static microcosms that did not receive an exogenous substrate.

Nitrate concentrations in the effluent from the nickel-treated column were similar to those from the live control during the first 3 months of the study. However, the effluent nitrate concentrations declined to below detection after this time. This reduction occurred approximately 2 weeks after hydrogen gas was introduced to the column in the influent groundwater as a source of reducing equivalents. Hydrogen, which is adsorbed on the surface of the fresh nickel catalyst, is expected to become depleted during reductive reactions. The influent water stream to the nickel column was subsequently amended with hydrogen gas in order to provide additional reducing equivalents. From Day 82 through Day 89, 1 mL of hydrogen gas was injected daily into the influent water immediately upgradient of the column. Hydrogen addition was increased to 5 mL every 2 days from Day 90 through Day 118. It is unclear from the data whether the reduction of nitrate was mediated catalytically by the nickel or whether the results represent biodegradation with hydrogen as the microbial electron donor.

RDX was not degraded in the unamended live column or in the killed control during the course of the study (Fig. 6). In contrast, RDX was below detection (MDL=0.02 mg/L) in the effluent of the column treated with mZVI during the entire 120-day study. Low concentrations (<0.5 mg/L) of the RDX daughter products MNX, DNX, TNX were observed in the effluent from this column at periodic intervals (data not shown).

The effluent concentrations of RDX in the nickel-treated column were similar to those observed for mZVI through the first 10 weeks of the study. After this period, however, the RDX concentration in the effluent slowly began to increase. This increase was hypothesized to reflect a gradual decrease in the catalytic degradation rate caused by depletion of hydrogen that was initially present on the catalyst surface. As previously noted, hydrogen was added to the column beginning on Day 82. A decline in effluent RDX concentrations was observed after the initial hydrogen addition indicating that the hydrogen increased the rate of contaminant treatment.

Effluent concentrations of RDX in the column receiving emulsified oil showed an initial increase through the first 15 days of the study, but then decreased to non-detect concentrations by approximately 65 days. The effluent concentrations then remained below detection for the duration of the study. The increase in the biodegradation rate for RDX during the first few months of the study (as evidenced by the declining effluent concentrations under steady flow) probably reflects an increase in the biomass of the nitramine-degrading microbial population. The degradation kinetics

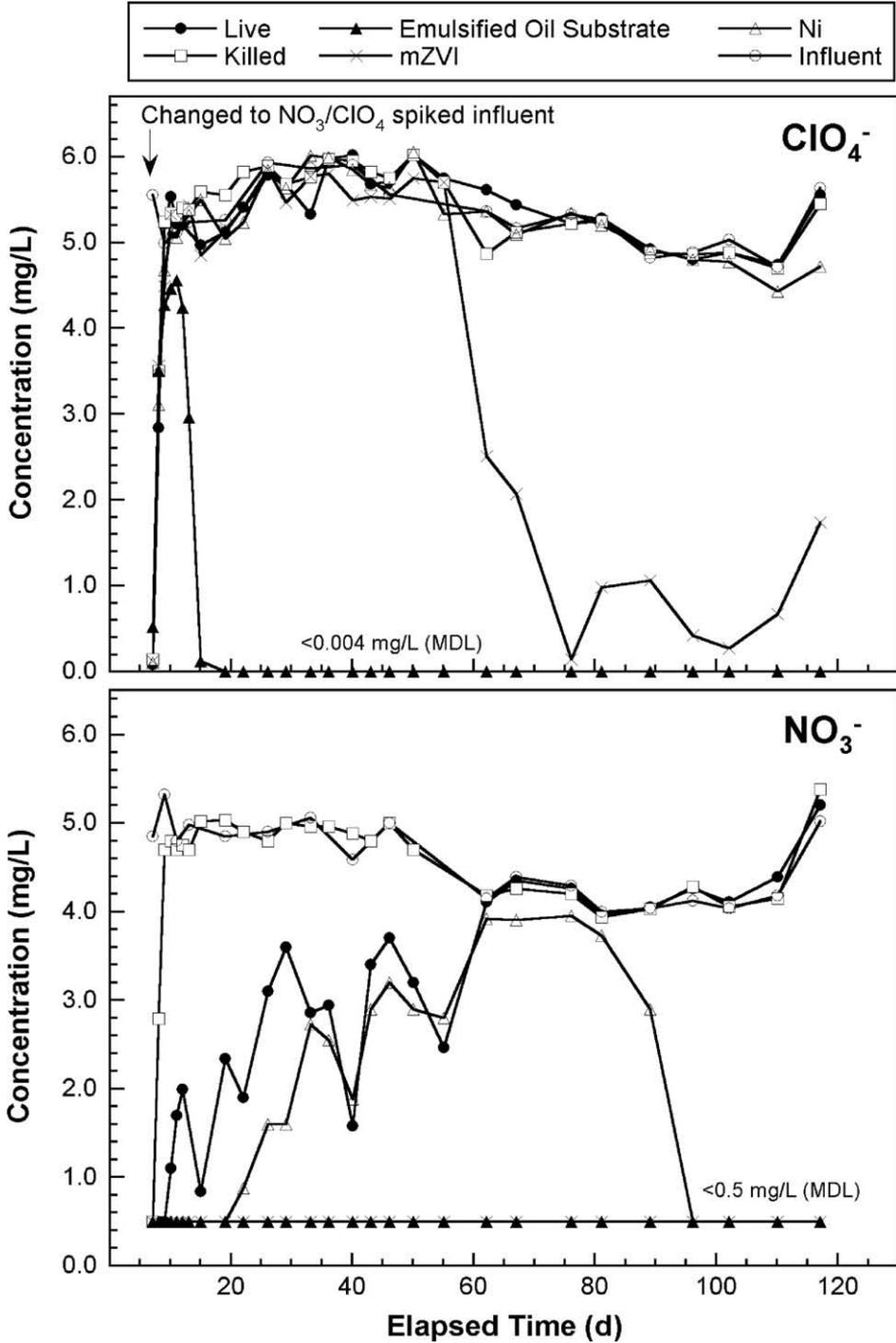


Fig. 5. Influent and effluent concentrations of nitrate and perchlorate in flow-through columns that received no amendment (live), mZVI, nickel, emulsified oil substrate, or killing agents (killed).

for the emulsified oil amended columns differ appreciably from those treated with mZVI or nickel, for which RDX concentrations were below detection at the first sampling point.

The RDX daughter products MNX, DNX, and TNX appeared in the column effluent of the emulsified oil treated column. Although trace levels of these compounds also appeared in the columns receiving nickel and mZVI, concentrations were appreciably higher in the column treated with emulsified oil. Evaluation of contaminant mass balance indicated that roughly 40% of the influent RDX eluted from the emulsified oil-amended columns as MNX, DNX, or TNX. Based on previous RDX degradation studies (Hawari et al., 2000; Zhao et al., 2004), the remaining mass of RDX was likely mineralized and/or converted to smaller products resulting from cleavage of the heterocyclic ring (e.g., methylene dinitramine, formaldehyde, etc). It is also possible that a fraction of the remaining mass was irreversibly bound to the soil matrix. Based on the measured partition coefficient between RDX and vegetable oil (Schaefer et al., 2005), as well as the low concentrations of emulsified vegetable oil in the columns, uptake of RDX into the oil phase was considered negligible.

HMX concentrations in the effluent of the column receiving mZVI were below detection throughout the course of the study (Fig. 6). These data are similar to those observed for both RDX and nitrate in this column. HMX was also initially below detection in the nickel-treated column, but as with RDX, effluent concentrations began to increase after approximately 65 days. The addition of hydrogen beginning at Day 82 appeared to slow the rate of increase, but influent HMX concentrations were only slightly below those observed in the killed column by Day 90. HMX was not degraded in either the unamended column or in the killed control.

The degradation pattern for HMX in the emulsified oil treated column was similar to that observed for RDX. The HMX concentrations in the effluent slowly increased through the first 35 days, then decreased through the end of the experiment. However, unlike RDX, HMX concentrations in the effluent reached those in the unamended and killed columns prior to their subsequent decline. In addition, the decline in HMX in the column effluent occurred much more slowly than for RDX, and occurred most significantly only after the RDX concentrations in the effluent were near or below detection.

4.4. Model results

Model results from the mZVI-amended column show that the regressed first-order rate constants (k) are greater than 2.0/day and 1.7/day for RDX and HMX, respectively; the first-order rate constant for nitrate was also greater than 2.0/day. Identical values of the regressed first-order rate constants to those in the mZVI-amended column were attained for RDX and HMX in the nickel catalyst-amended column, but only for the first 9 to 10 weeks of the study. No abiotic degradation of nitrate was observed in the nickel catalyst-amended column. These rates are consistent with those measured in previous studies (Fuller et al., in press).

Comparison of RDX, HMX, and perchlorate elution data to the biodegradation model (Eq. (6)) for the emulsified oil amended column are shown in Fig. 7. Nitrate degradation occurred too rapidly for evaluation by the model developed herein. Simulation results indicate that contaminant elution is reasonably described by a pseudo first-order biodegradation rate constant, accompanied by first-order microbial growth. Regressed biodegradation parameters for the oil amended column and microcosms are listed in Table 2. Results from the column studies show that the value of α , which is the microbial growth rate constant, is the same for both RDX and HMX. This indicates that the microbial consortia responsible for degrading both of these compounds in the columns grew at approximately the same rate. One can infer from this

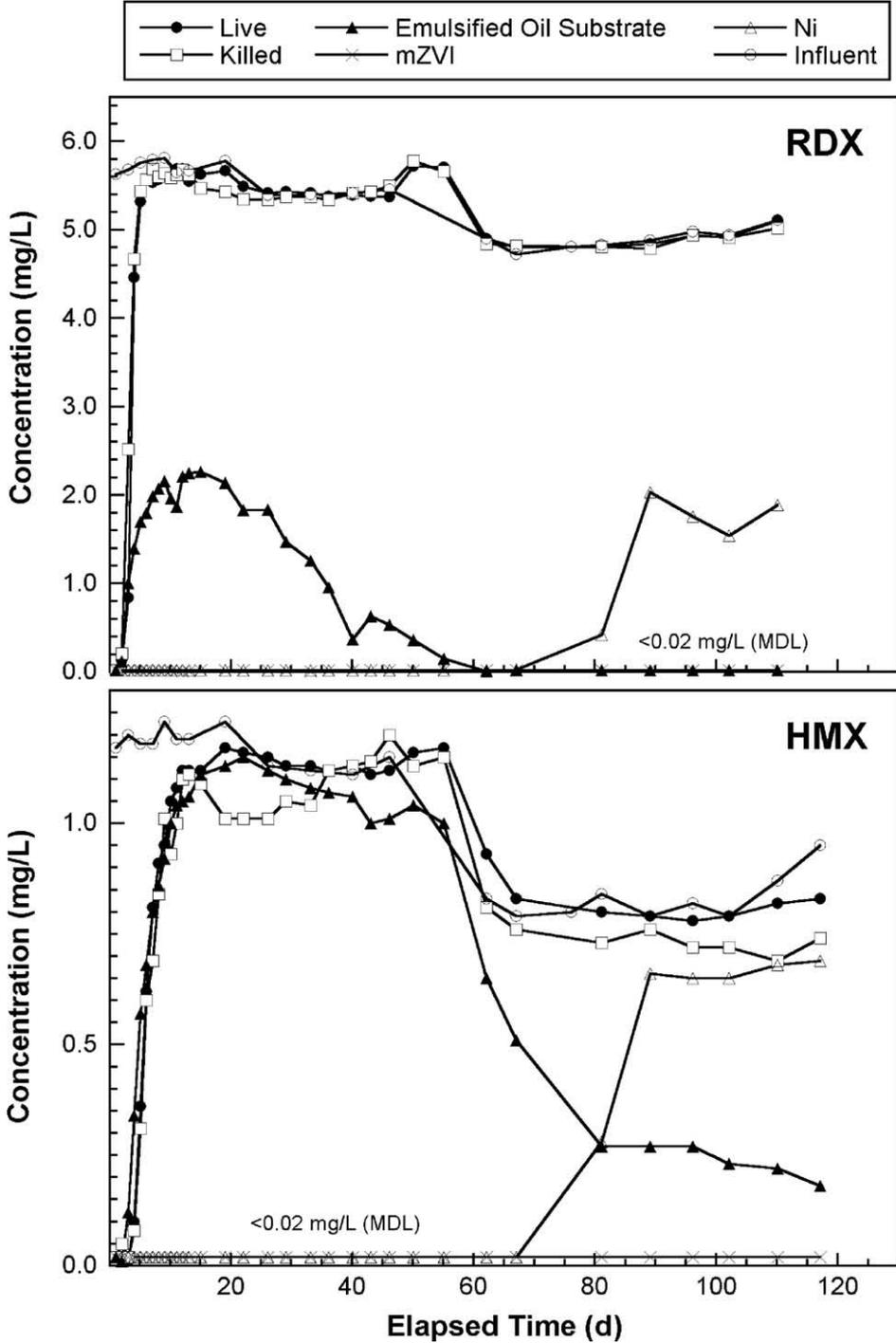


Fig. 6. Influent and effluent concentrations of RDX and HMX in flow-through columns that received no amendment (live), mZVI, nickel, emulsified oil substrate, or killing agents (killed).

observation that the same microbial consortia is responsible for both RDX and HMX biodegradation. However, a more detailed microbial analysis would be necessary to confirm this conclusion.

In contrast, the regressed microbial growth rate constant for perchlorate in the column experiment is roughly ten-times greater than for RDX and HMX. Thus, by analogy, a different microorganisms and/or microbial consortia appear to be responsible for perchlorate degradation. This is a reasonable conclusion based on current knowledge of nitramine degraders and perchlorate-reducing strains. To our knowledge, no cultures have been described that perform both activities. It is feasible that the same microorganisms are degrading both the explosives and perchlorate, but degradation of these electron acceptors support growth at different rates. However, modifying the model such that the microbial growth rate was dependent upon the contaminant concentration did not provide a reasonable prediction of the experimental data, thus this alternative explanation is not likely.

The regressed value of β , the biodegradation rate constant, in the column experiments is roughly ten-times greater for RDX and perchlorate than for HMX, suggesting that RDX and perchlorate are more readily biodegradable than HMX. This observation is consistent with the microcosm studies described above, as well as results published by other researchers (Price et al., 1998; Shen et al., 2000; Zhao et al., 2004). The relatively low value of β for HMX may also reflect inhibition of HMX biodegradation by RDX. Such inhibition may be the cause for the lack

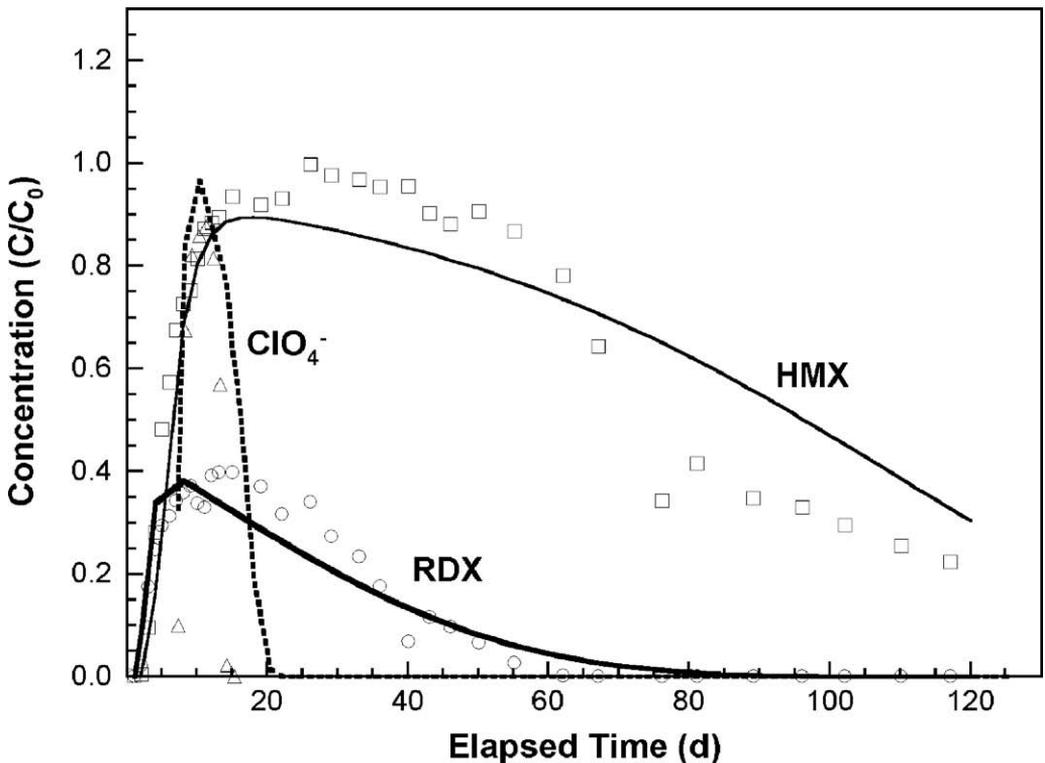


Fig. 7. Comparison of simulated effluent concentrations to measured effluent concentrations in the column treated with emulsified oil substrate. Effluent concentrations are plotted relative to the influent concentration of each contaminant.

Table 2
Regressed model parameters \pm the standard error

Experiment	Parameter	RDX	HMX	Perchlorate
Microcosms	α	0.037 \pm 0.005	No degradation	0.61 \pm 0.41
	β	0.0048 \pm 0.0010	No degradation	0.0085 \pm 0.0080
Columns*	α	0.025	0.025	0.500
	β	0.180	0.015	0.120

* A standard error could not be calculated for the column experiments because a numerical solution was employed.

of HMX degradation in the emulsified oil treated microcosm. The similar structure of these compounds, and the likelihood that each would be degraded by the same organism or consortia of microorganisms (and thus by the same enzymes), make inhibitory interactions possible. Results from the biodegradation model support this hypothesis. Slow kinetics of HMX degradation in the presence of RDX were also reported by Shen et al. (2000) in bioslurry reactors. Additional studies are required to firmly establish the cause of the observed differences in degradation rate between the two compounds.

The regressed microbial growth rate constant for RDX was similar (within a factor of two) in the microcosm and column experiments. This observation indicates that the rate of growth of the microbial consortia responsible for RDX degradation was nearly identical in both sets of experiments. Similar results were observed for perchlorate. However, the regressed biodegradation rate constant for RDX and perchlorate in the column experiments is approximately 15- to 40-times greater than in the corresponding microcosms. This may be due to accumulation of toxic or inhibitory RDX daughter products in the closed microcosm system.

5. Conclusions

This study indicates that bioremediation of co-mingled perchlorate, RDX, HMX, and nitrate in groundwater aquifers is feasible through the addition of emulsified vegetable oil as a microbial growth substrate. Emulsified oils have been successfully applied at field scale for treatment of perchlorate and chlorinated solvents, so this approach is anticipated to be a viable option for full-scale applications where mixed explosives, perchlorate, and/or nitrate are present (Zawtocki et al., 2004). Several other substrates were also effective as electron donors for perchlorate and nitrate reduction, but did not support the reduction of RDX and HMX in the samples studied. Additional studies in which microbial populations (particularly nitramine degraders) are monitored in conjunction with substrate and contaminant degradation would be useful for interpreting the substrate specificity observed during this investigation.

In addition to enhanced bioremediation, mZVI was also shown to effectively treat all four contaminants in column experiments, with RDX, HMX, and nitrate degradation occurring quickly and simultaneously, presumably through abiotic interactions. There was a long lag period prior to perchlorate reduction in aquifer columns (and no reduction was observed in microcosms), suggesting that perchlorate treatment by mZVI was the result of secondary biotic processes. Hydrogen generated by the oxidation of ZVI likely served as the electron donor for perchlorate biodegradation in this case. The nickel catalyst also rapidly degraded RDX and HMX, but this metal was ineffective for treating perchlorate. Moreover, the degradative capacity of nickel was limited, presumably by the supply of available reducing equivalents on the nickel surface. The addition of exogenous hydrogen as an electron donor appeared to regenerate catalytic activity to some extent.

Model results suggest that similar microbial consortia are likely responsible for both RDX and HMX biodegradation, while a separate consortia are responsible for perchlorate degradation. Model results are also consistent with inhibited biodegradation of HMX in the presence of RDX, as regressed values of the HMX biodegradation rate constant are substantially lower than for RDX.

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