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Evaluating Behavior of Oxygen, Nitrate, and Sulfate during Recharge and Quantifying Reduction Rates in a Contaminated Aquifer

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This study evaluates the biogeochemical changes that occur when recharge water comes in contact with a reduced aquifer. It specifically addresses (1) which reactions occur in situ, (2) the order in which these reactions will occur if terminal electron acceptors (TEAs) are introduced simultaneously, (3) the rates of these reactions, and (4) the roles of the aqueous and solid-phase portions of the aquifer. Recharge events of waters containing various combinations of O_2, NO_3, and SO_4 were simulated at a shallow sandy aquifer contaminated with waste fuels and chlorinated solvents using modified push−pull tests to quantify rates. In situ rate constants for aerobic respiration (14.4 day $^{-1}$), denitrification (5.04−7.44 day^{-1}), and sulfate reduction (4.32−6.48 day^{-1}) were estimated. Results show that when introduced together, NO_3 and SO_4 can be consumed simultaneously at similar rates. To distinguish the role of aqueous phase from that of the solid phase of the aquifer, groundwater was extracted, amended with NO_3 and SO_4, and monitored over time. Results indicate that neither NO_3 nor SO_4 was reduced during the course of the aqueous-phase study, suggesting that NO_3 and SO_4 can behave conservatively in highly reduced water. It is clear that sediments and their associated microbial communities are important in driving redox reactions.

Introduction

Identifying the biogeochemical processes responsible for, the development of, and the transition between redox zones and quantifying the rates of these processes is crucial to understanding how many natural and anthropogenic groundwater constituents behave in the environment. Understanding the dynamics associated with the redox state of a system is important because redox controls the form, mobility, and persistence of many groundwater contaminants. Quantifying these dynamics will allow for (1) improved understanding of natural fluctuations of redox processes in pristine and contaminated aquifers, (2) the prediction of rates of natural microbial attenuation of contaminants, and (3) proper design and evaluation of monitored natural attenuation and biostimulation remediation strategies in contaminated aquifers. Although recharge events are thought to be a significant drive for redox dynamics (*1*), their exact role is not well-understood. Few studies have looked at the introduction of recharge solutions to reduced systems and none have examined the effects of introducing a multiparameter recharge solution as one might expect during a rainfall event. In addition, the roles of aqueous- and solid-phase portions of an aquifer, and their associated microbial communities, on redox reactions have not been evaluated. To further complicate our understanding of the controls on redox dynamics, laboratory and field measurements of redox reaction rates are often significantly different. This study seeks to quantify the redox reactions associated with the aqueous and solid-phase portions of a reduced aquifer during a multiparameter recharge event.

In pristine and contaminated aqueous systems, redox state is based on the activity of microorganisms, which first consume oxygen, and then a succession of alternate terminal electron acceptors to support their growth using a variety of carbon sources (*2*−*5*). The succession of terminal electron-accepting processes (TEAPs) in order of decreasing redox potential and free-energy yield is generally oxygen reduction, nitrate reduction, manganese reduction, iron reduction, sulfate reduction, and methanogenesis. In most closed, uncontaminated groundwater systems, the sequence of TEAPs follows this succession (i.e., increasingly reduced conditions occur further down the flow path) (*6*). Downgradient of contaminant plumes, the sequence of TEAPs is reversed (*2*, *3*). That is, the most reduced conditions (e.g., methanogenesis) occur near the contaminant source due to the greater availability of electron donors, while less reducing conditions (e.g., iron reduction) occur down the flow path from the contaminant source. Because hydrocarbons (which serve as electron donors) concentrate in the capillary fringe area, more reducing conditions often occur near the water-table interface, resulting in a plume that has vertical as well as horizontal redox zonation (*7*). In reduced systems, it is thought that recharge events can initiate changes in TEAPs by delivering more favorable terminal electron acceptors (TEAs) such as oxygen (O_2), nitrate (NO_3), and sulfate (SO_4) to the system (*1*). Thermodynamics would predict that when a recharge solution comes in contact with a reduced aquifer, TEAs would be consumed in the order of free-energy yield. Although thermodynamics (by evaluation of available free energies) can provide insight into the potential metabolic attractiveness of a reaction, the actual rate and extent of any given redox process is a function of other factors such as activation energy, metabolic kinetics, and the presence of biotic and abiotic catalysts. This suggests that unless competitive pressure exists (e.g., electron donor limitation), there is no reason to assume that TEAs would be consumed in the order of free-energy yield. In addition, it is unclear if other biotic and abiotic redox reactions involving TEAs may occur when recharge water comes in contact with reduced groundwater and its associated sediments.

To understand the biogeochemical processes that dominate during recharge events, it is necessary to not only identify the redox reactions which may be occurring but also to quantify these reaction rates. The determination of rates of TEA consumption is challenging. Many studies use laboratory-based methods, including microcosm experiments (*8*−*11*), direct observations (radiotracers) (*12*, *13*), molecular methods (*14*), geochemical modeling (*5*), and kinetic modeling (*15*). These studies have been performed on sediments from a wide range of aqueous environments, including aquifers, lakes, and wetlands. However, these approaches

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can be limited by possible system disturbances, contamination problems, and often unnaturally high concentrations of substrate. In situ methods to estimate rates are generally considered to be more representative of actual subsurface conditions. These methods are not as widely used and include natural gradient tracer experiments (*16*), flowline estimations (*17*), and push−pull tests (*18*).

In general, laboratory rate-estimation methods commonly yield zeroth-order rates while in situ field rate-estimation methods commonly yield first-order rates associated with the TEA concentration. None of the previous laboratory or field studies has examined the localized in situ TEA consumption rates that would result when natural recharge water, containing multiple TEAs at relatively low levels, is introduced to a reduced groundwater environment. One study by Cunningham et al. (*19*) added multiple TEAs (NO_3 and SO_4) to evaluate possible enhanced biodegradation over longer timescales (months) in a contaminant plume at Seal Beach, CA. They found that although NO_3 was utilized preferentially over SO_4, the two processes were not strictly sequential. This study utilizes a relatively new technique, in situ push−pull tests, to examine in detail the localized processes initiated during recharge events.

Push−pull tests have been shown to be useful in obtaining quantitative information on a wide range of in situ microbial processes in aquifers (*20*, *21*) and lake sediments (*22*). A test consists of three phases. In the first phase, native groundwater is extracted from the well for preliminary geochemical characterization. This information is necessary to deduce which biogeochemical reactions may already be occurring in the aquifer and to establish background concentrations of the chemicals of interest. Specifically, we wanted to ensure that the groundwater where the tests were performed was reduced. E_h measurements ranged from −150 to −200 mV, but because of the lack of specificity of E_h measurements, we chose to rely on concentrations of dissolved hydrogen (H_2) and other selected TEAP variables such as O_2, Fe^{2+}, NO_3, NO_2, and SO_4. The H_2 concentration in aquatic systems has been proposed as an indicator of in situ TEAPs (*5*). Dissolved hydrogen is known to be a fleeting but important intermediate in the decomposition of organic matter. Dissolved hydrogen is produced and consumed in anaerobic environments such that each TEAP maintains a characteristic H_2 concentration (*23*). Interpretations of TEAPs from H_2 concentrations, as measured in marine and freshwater sediments and in aquifers, are as follows: methanogenesis, greater than 5 nM; SO_4 reduction, 1−4 nM; Fe^{3+} reduction, 0.2−0.6 nM; and Mn(IV) or nitrate reduction, less than 0.05−0.1 nM (*5*). Chapelle and co-workers (*7*, *24*, *25*) demonstrated the use of H_2 concentrations in delineating redox zonation in the plume at the study site described in this paper. They concluded that delineating redox zones based on H_2 concentrations is more reliable when H_2 concentrations are interpreted in the context of other TEAP variables, a strategy we have adopted here. After the geochemical characterization has been completed to determine initial geochemical condition, the remaining phases of the test can begin. In the second phase, or “push”, a well-mixed slug of water containing conservative and reactive solutes is rapidly injected into the saturated zone of the aquifer. A conservative solute is added as a tracer to account for advection and dispersion, and the other added solute(s) are reactive. The third phase, the “pull”, begins immediately after the injection is completed. Water is slowly extracted from the well, and the concentrations of the solutes are measured over time.

These tests can provide valuable insight into the redox reactions that may be occurring in situ, but they are limited in that they cannot distinguish the roles of the aqueous and solid phases and their associated microbiological population in these reactions. The most widely accepted hypothesis for the influence of microbes on redox processes in aquifers is that microbes residing on the solid-phase portion of the aquifer are responsible for the majority of the activity (*26*). This is thought to be due to the much greater concentration of microorganisms in association with the solid phase than in the aqueous phase of the aquifer. However, it is unclear what the role of the aqueous phase is during redox reactions. On the basis of thermodynamics, abiotic geochemical reactions may occur when oxidized chemical species come in contact with a reduced environment. To determine the significance of the abiotic and biotic redox reactions associated with the aqueous phase of the aquifer, we designed surface reaction vessel (SRV) experiments to run simultaneously with the push−pull tests. The goal of the SRV experiments was to determine what reactions would occur in the aqueous phase and at what rates. Identical control SRVs were amended with formaldehyde and run simultaneously to distinguish microbially mediated redox reactions from geochemically driven reactions. The SRVs were filled with water from the aquifer where the push−pull test was conducted and then allowed to react over time to the same addition of solutes as introduced in the push−pull tests. The vessels were designed to maintain aquifer conditions as closely as possible, including maintaining the temperature and anaerobic nature of the water.

This study evaluates the biogeochemical changes that occur when a recharge solution comes in contact with reduced groundwater and sediments. It specifically addresses (1) which reactions occur in situ, (2) the order in which these reactions will occur if TEAs are introduced simultaneously, (3) the rates at which these reactions occur, and (4) the roles of the aqueous and solid-phase portions of the aquifer in these processes. Push−pull tests and surface reaction vessel experiments containing various combinations of solutes to simulate various types of recharge events were designed to address these questions.

Study Site Description

The study site is a shallow sandy aquifer contaminated with petroleum hydrocarbons and chlorinated solvents at the former Wurtsmith Air Force Base (AFB), MI. Wurtsmith AFB lies on a sandy plain of glacial lake sediment approximately 2 km west of the Lake Huron shoreline in Michigan’s Lower Peninsula. The aquifer is approximately 20 m thick and is composed of highly permeable alternating aeolian sands and glacial outwash material. Hydraulic conductivities of these deposits are on the order of 30 m/day. The unconfined aquifer is underlain by at least 30.5 m of silty clay. The water table varies from 3.5 to 5.0 m below land surface (184−186 m above sea level) and fluctuates 0.3−0.7 m annually. The water-table gradient ranges from 3 to 5 m/km. Assuming an effective porosity of 30%, average groundwater velocity is approximately 0.5 m/day. Because the direction of groundwater flow is influenced by recharge, lateral shifts in the plume direction of 7−10° have been observed.

Fire training activities performed by the Air Force from 1952 to 1986 are the source of the contamination at this site. Various compounds were used during the training exercises to ignite and extinguish the fires, including waste fuels, chlorinated solvents, aqueous film-forming foams, a multipurpose dry chemical, potassium bicarbonate-based soda, and Halon 1211. The majority of the contamination resides within the capillary fringe, and an extensive plume approximately 50 m wide and 400 m long has developed (Figure 1). The plume is complex in that it contains both BTEX compounds (benzene, toluene, ethylbenzene, and xylenes) and chlorinated solvents (e.g., dichloroethene). Concentrations of BTEX compounds in the plume range from 20 to 1000 μg/L while concentrations of dichloroethane, chloroethane, and vinyl chloride range from 2 to 1000 μg/L. Most

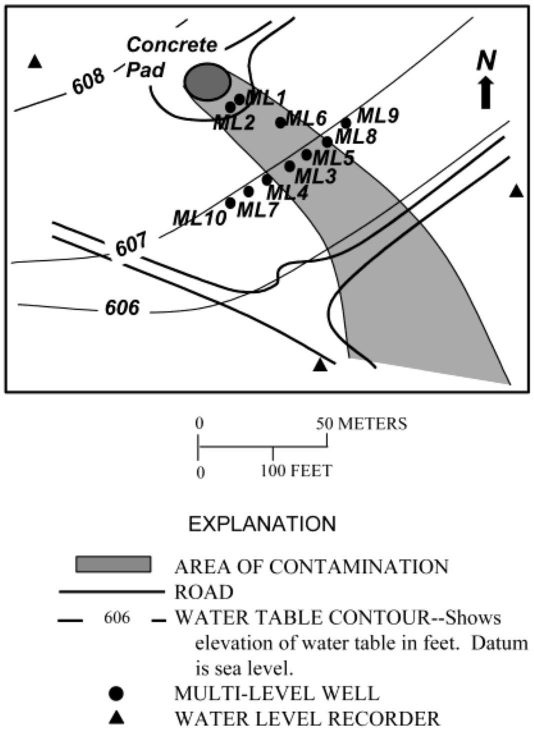

FIGURE 1. Plume map for the Fire-Training Area 2 (FTA-2) at the former Wurtsmith Air Force Base, MI. The concrete pad was the site of fire-training activities.

of the contamination is associated with aquifer solids between 4.5 and 5.7 m below land surface, which have an average concentration of total hydrocarbons of 13 650 mg/kg (*27*).

The portion of the aquifer where the push−pull experiments were performed, multilevel well 3 at the 25 ft (7.6 m) depth (ML3-25), remains in a reduced redox state throughout all groundwater flow regimes. The reducing conditions at this well vary historically from Fe(II) reducing to methanogenic (as determined by H_2 concentrations) on the timescale of months (*28*). The subject well was constructed of 2.5 cm inner diameter PVC casing, with 0.3 m long, 6.35 mm slotted PVC screens. Vertical spacing between screens is approximately 1 m. This is an ideal site to examine the effects of recharge on reduced systems because a detailed spatial and temporal geochemical, hydrogeologic, and microbiological framework for the site has been established (*28*−*31*).

Methods

Push−Pull Tests. Well selection was based on the initial complete geochemical characterization including redox-indicating parameters. A summary of the initial water chemistry from the selected wells is presented in Table 1. All activities were performed at a well with highly reduced water (ML3-25) located roughly in the center of the plume (Figure 1). Three push−pull tests were performed at this well at approximately 1-month intervals. To simulate recharge fluids for the push−pull tests, native uncontaminated groundwater from a well just outside the plume, multilevel well 10 at the 37 ft (11.3 m) depth (ML10-37), was extracted and placed in a 20-L Nalgene carboy. This fluid was chosen to represent a rain solution which had percolated through the unsaturated zone and ultimately reached the aquifer. The solution was then amended approximately 10 mg/L of the TEAs which would likely be introduced during a recharge event. Three different types of recharge waters were prepared. The first test solution represented the water resulting from a rain event containing O_2, NO_3, and SO_4. The second test solution represented water which had been depleted in O_2, perhaps upgradient but still contained NO_3 and SO_4. The third test solution represented recharge water depleted in O_2 and NO_3 but still containing SO_4. To each of these solutions, 100 mg/L chloride (as NaCl) was added as a conservative tracer to account for dilution from mixing, advection, and dispersion. Nitrate and sulfate were added as $NaNO_3$ and Na_2SO_4, respectively, and hand-shaken to ensure complete dissolution and homogenization of the solution. Oxygen was removed from test solutions 2 and 3 by stripping with inert N_2 gas for approximately 3.5 h until field test kits could no longer detect O_2 (less than 0.03 ppm). To maintain anaerobic conditions, solutions were kept in an N_2 atmosphere and monitored for O_2. Before injecting these solutions into the test well, the unscreened interval was packed off to prevent the vertical movement of water up the well casing. The packer was inserted to just above the well screen and inflated with inert N_2 gas to seal off the well. The packer remained in place throughout the test. Once the packer was in place, the anaerobic test solution was "pushed" down the well at a rate of approximately 1 L/min. This was followed by the injection of 2 L of "chaser solution" (deoxygenated, distilled deionized water) to flush the test solution from the well and force it further into the aquifer. The extraction or "pull" phase began immediately after the injection phase was completed. Effluent from the well was slowly extracted at a rate of approximately 150 mL/min and monitored in the field for Cl, O_2, NO_3, NO_2, S^{2-}, SO_4, Fe^{2+}, temperature, pH, and E_h over time. Samples were also collected for anions (Cl, NO_3, NO_2, SO_4) and NH_4^+ for analysis in the laboratory.

Surface Reaction Vessel Tests. Water was pumped from the reduced well where the push−pull tests were performed (ML3-25) into a 20-L Nalgene carboy attached to a glovebag filled with N_2 to maintain anaerobic conditions. The carboy and attached glovebag were then placed into a temperature-controlled environment (surface reaction vessel) and amended with various TEAs. Control surface reaction vessel experiments were set up in the same manner but inoculated with formaldehyde (to make a final 0.4% solution) to halt microbial activity. The water was then monitored at approximately 5-min intervals for the duration of the push−pull tests. Real-time measurements of O_2, NO_3, NO_2, S^{2-}, SO_4, and Fe^{2+} were taken in the field. Samples were also collected for anions (Cl, NO_3, NO_2, SO_4) and NH_4^+ for later analysis in the laboratory.

Geochemical Analyses. A flow cell (Yellow Springs Instruments) was used to continuously monitor temperature, pH, dissolved oxygen, E_h, and specific conductance throughout the push−pull tests. Dissolved H_2 gas samples were collected using the bubble-stripping method (*17*) and analyzed immediately in the field on a RGA3 reduction gas analyzer (Trace Analytical). In the field, NO_3, NO_2, and S^{2-} were measured by colorimetric spectroscopy using Chemetrics Vacu-Vials, Fe^{2+} by phenanthroline colorimetric analysis, and SO_4 by turbidimetric analysis using HACH Acuvacs. Samples were collected for anions (Cl, NO_3, NO_2, SO_4) and NH_4^+ analyses in the laboratory. These were preserved with formaldehyde and a flash-freezing technique, respectively, and then analyzed by capillary electrophoresis.

Rate Determination. A first-order rate equation was a better fit to the experimental data than the zeroth-order rate equation. When the experimental data was plotted over time, it formed a curve. Plotting the natural log of the data over time yielded a straighter portion of the curve. Therefore, rates were estimated assuming a first-order process. We followed a method established by Snodgrass and Kitanidis (*32*) for inferring in situ reaction rates from push−pull experiments. This analysis assumes the absence of sorption and negligible background concentrations. In our case, these are valid assumptions because NO_3 and SO_4 are soluble and relatively nonsorbing and background concentrations were below

TABLE 1. Initial Chemical Characterization of Selected Wells (mg/L)[a]

well ID	date	H_2[b]	O_2	NO_3	NO_2	Fe^{2+}	SO_4	$BTEX_{avg}$	DCE
ML3-25	6/00	3.55	<0.5	<0.05	<0.05	7.12	<0.05	17−205	5.6
ML10-37	6/00	<0.05	8.01	19.36	<0.05	<0.01	10.7	<0.9	<0.2
ML3-25	7/00	2.68	<0.5	<0.05	<0.05	13.7	<0.05	18−328	<0.2
ML10-37	7/00	<0.05	6.59	4.3	<0.05	<0.01	6.99	<0.9	<0.2
ML3-25	8/00	2.35	<0.5	0.41	<0.05	7.00	<0.05	9−314	4.87
ML10-37	8/00	<0.05	6.43	7.13	<0.05	<0.01	3.57	<0.9	<0.2

[a] DCE = cis-1,2-dichloroethene, reported in ug/L; BTEX = average benzene, toluene, ethylene, and xylene, reported in ug/L. [b] H_2 concentrations are reported in nM.

detection limits. This analysis also assumes that the solutes were injected instantaneously as a well-mixed slug. It is reasonable to assume that the injected fluids were well-mixed because the test solution was well-homogenized prior to injection. Our injection was not instantaneous, but this analysis assumes that the rapid injection was negligible as compared to the total reaction time. Our approach was modeled after single solute in situ push−pull tests performed by Haggerty et al. (*33*) and Schroth et al. (*20*). We assumed that the injected solutes were transformed within the aquifer according to a first-order rate equation $\delta C_r / \delta t = -kC_r$ so that

$$C_r(t) = C_r^\circ \, e^{-kt} \tag{1}$$

where $C_r(t)$ is the reactive solute concentration at time t, C_r° is the reactive solute concentration at time $t = 0$, and $k[t^{-1}]$ is the rate coefficient. Now, assuming that changes in saturated thickness due to injection and extraction pumping are negligible, the breakthrough curve for a reactive solute $C_r(t^*)$ is given by

$$C_r(t^*) = \frac{C_{tr}(t^*)}{kt_{inj}}[e^{-kt} - e^{-k(t_{inj} - t^*)}] \tag{2}$$

where t^* is the time elapsed since the end of the test solution injection, $C_{tr}(t^*)$ is the breakthrough curve for a co-injected tracer (Cl, in our case), and t_{inj} is the duration of the test solution injection. Equation 2 can be rewritten

$$\ln\left(\frac{C_r(t^*)}{C_{tr}(t^*)}\right) = \ln\left(\frac{1 - e^{-kt_{inj}}}{kt_{inj}}\right) - kt^* \tag{3}$$

This suggests that a plot of $\ln(C_r / C_{tr})$ versus t^* generates a straight line with a slope of $-k$. Because the determination of k is based on the evaluation of ratios of C_r / C_{tr}, complete tracer mass recovery is not necessary during tracer tests to obtain accurate estimates of k. Similarly, a portion of the breakthrough curve may be used to estimate k. A nonlinear least squares regression was used to fit eq 3 to experimental breakthrough data to obtain first-order rate coefficients for aerobic respiration, denitrification, and sulfate reduction. For this purpose, C_r in eq 3 is the relative concentration (i.e., the measured concentration divided by the concentration injected in test solution) of reactive solutes (O_2, NO_3, or SO_4) and C_{tr} is the relative Cl concentration. Because the background concentration of the reactive solutes was negligible but the background concentration of the tracer (Cl) was detectable, 1.0−3.5 ppm depending on the test, the measured values of chloride could not be used as a reliable indicator of the degree of mixing without correction. Therefore, C_{tr} was calculated as a mixing proportion between the injected Cl concentration and the background chloride concentration (eq 4) to give a more accurate description of the degree of mixing and other nonreactive processes.

$$C_{tr} = \left(\frac{Cl_m(t^*) - Cl_b}{X}\right) \tag{4}$$

Where $Cl_m(t^*)$ is the measured chloride concentration at time t, Cl_b is the measured background chloride concentration, and X is the slope of the line generated from a plot of the percent input solution (0−100%) versus concentration (background-injection values). This line represents the mixing curve between the injected solution and the background water with respect to chloride concentration. In our case, because the background concentration was very low (<3.5 ppm) as compared to the input test solution concentration (~100 ppm), the slope was near 1.

Results and Discussion

Field versus Laboratory NO_3 Measurements. A significant discrepancy was observed between the NO_3 data obtained by field analyses and the NO_3 data obtained by laboratory analyses. Field data obtained from samples collected within the plume gave much lower (often not detectable) concentrations of NO_3 than the corresponding laboratory analyses, which accurately represented the added initial concentrations. Field data obtained from samples collected from outside the contaminant plume largely agreed with corresponding laboratory analyses for NO_3. This suggests that some constituent of the contamination within the plume interfered with the analyses. This field observation was supported by laboratory experiments, which showed that when a known amount of NO_3 was added to the uncontaminated samples (collected outside the contaminant plume), the field method accurately represented the resulting concentration. However, when a known amount of NO_3 was added to the contaminated samples, the field method gave erroneously low and inconsistent readings. One possible explanation for this discrepancy is that the NO_3 field method requires cadmium to reduce NO_3 to NO_2, which is then measured colorimetrically. If the cadmium surfaces were coated with organic contaminants from the plume, they may have been unavailable to reduce the NO_3 and, as a result, were not able to be measured. For this reason, the NO_3 data presented in this paper will be from laboratory analyses only. On the basis of these observations, we conclude that caution should be used when interpreting NO_3 data from Cd-reduction field methods in contaminant plumes.

Push−Pull Tests. The first push−pull test (PP_{ONS}) evaluated oxygen, nitrate, and sulfate consumption rates at well ML3-25 (June 2000). Phase 1, the geochemical characterization, revealed that the aquifer water from that well was reduced (Table 1). PP_{ONS} involved the injection of a test solution containing approximately 100 mg/L Cl (as a conservative tracer), 6 mg/L O_2, 10 mg/L NO_3, and 10 mg/L SO_4. Concentrations of solutes were measured over time (Figure 2). As described in the rate determination section, these concentrations were then normalized and divided by the concentration of conservative tracer (Cl) at that point in time to account for nonreactive processes such as advection and dispersion. Rates were then derived from a plot of this

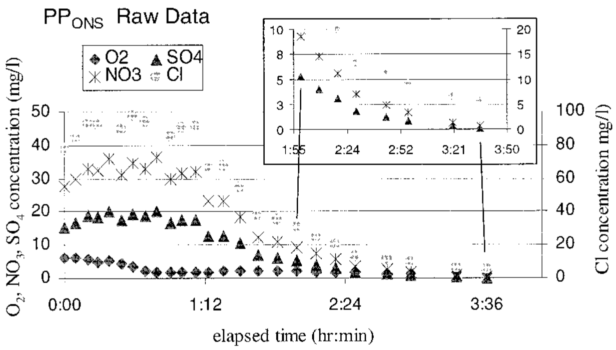

FIGURE 2. Measured concentrations of added solutes versus time. Oxygen (O_2), nitrate (NO_3), and sulfate (SO_4) concentrations are plotted on the left *y* axis and chloride (Cl) cncentrations are plotted on the right *y* axis.

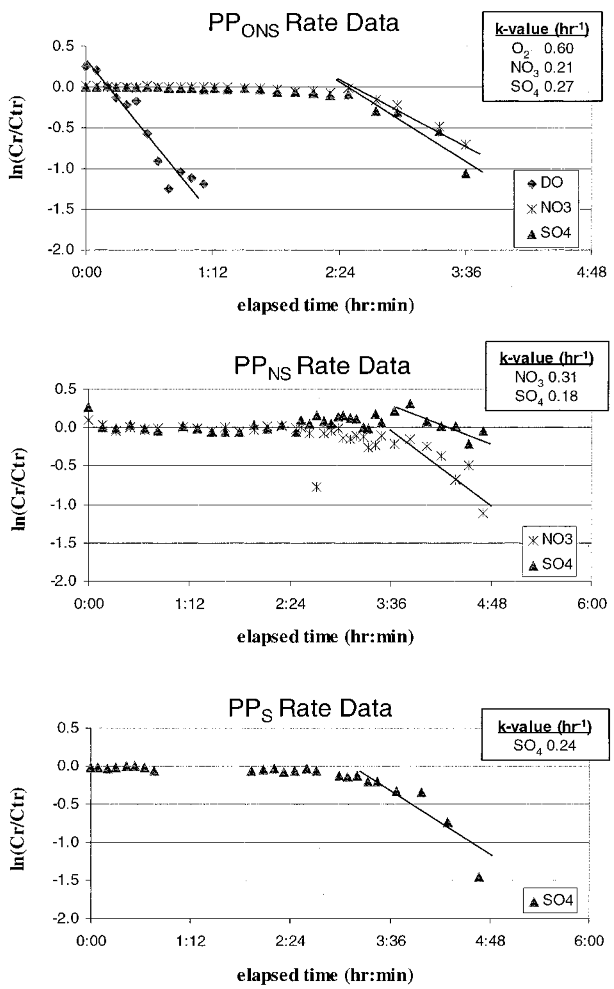

FIGURE 3. Regression plot for rate coefficient determination, obtained by fitting eq 3 to experimental data. Lines designate portion of data set used to determine rates.

ratio versus time (Figure 3). Dissolved oxygen was consumed first at a rate of approximately 0.60 h^{-1}. In an anaerobic system with excess dissolved iron, oxygen can rapidly be consumed through the abiotic oxidation of Fe(II). After the O_2 was consumed, NO_3 and SO_4 were consumed simultaneously at rates of 0.21 and 0.27 h^{-1}, respectively. The observed lag time for NO_3 and SO_4 reduction occurs because these reactions are biologically mediated. Microbiological response to system change frequently shows a lag time for microbes to adjust to new conditions (*3*). In addition, our simulated recharge water did not contain electron donors, which may also have contributed to the observed lag phase. The electron donor would have then been supplied through desorption from the solid phase and mixing with the aquifer water. PP$_{NS}$ evaluated nitrate and sulfate consumption rates at well ML3-25 (August 2000). Phase 1, the geochemical characterization, revealed that the aquifer water was reduced (Table 1). PP$_{NS}$ involved the injection of a test solution containing approximately 100 mg/L Cl (as a conservative tracer), 10 mg/L NO_3, and 10 mg/L SO_4. NO_3 and SO_4 were again consumed simultaneously at rates of 0.31 and 0.18 h^{-1}, respectively (Figure 3). PP$_S$ evaluated the rate of sulfate reduction at well ML3-25 (July 2000). Phase 1, the geochemical characterization, revealed that the well was reduced (Table 1). PP$_S$ involved the injection of a test solution containing approximately 100 mg/L Cl (as a conservative tracer) and 10 mg/L SO_4. Sulfate was consumed at a rate of 0.24 h^{-1} (Figure 3).

Obtaining representative rates of in situ processes is often a limiting factor in the development of geochemical models to evaluate processes in natural environments. Literature values for NO_3 and SO_4 consumption have been calculated based on zero- and first-order assumptions and estimated primarily by laboratory studies but also by some field experiments. Selecting a representative rate for TEA consumption is difficult because rate estimates range several orders of magnitude (Tables 2 and 3). Our field-measured first-order rates for aerobic respiration and denitrification are similar to those estimated by in situ field methods in other locations (Table 4). Estimated first-order rates of nitrate and sulfate reduction found by Chapelle et al. (*34*) and Cunningham et al. (*19*) were slower than those observed in this study, perhaps due to differences in electron-donor utilization. These studies looked at longer-term (months) consumption of NO_3 and SO_4 down the flow path, which may have allowed for utilization of a wider range of organic compounds, including more complex fuel constituents, to serve as electron donors. Our study examined the rates of TEA reduction as they would be initiated during a discrete recharge event that introduced low concentrations of electron acceptors. In this situation, the system was depleted with respect to O_2, NO_3, and SO_4 and had available microbially mediated intermediates such as H_2 and low molecular weight organic acids to serve as more attractive electron donors. The availability of these donors may have allowed for a greater rate of acceptor consumption. This hypothesis is supported by the observation that during the Cunningham et al. (*19*) flow path NO_3 augmentation study, the rate of NO_3 reduction decreased over time, presumably due to depletion of more favorable electron donors in the system. Given the nature of the zero-order laboratory-based methods, it is impossible to directly compare the rates found in laboratory studies to those found in field studies such as ours. However, our in situ rate estimates for O_2 and NO_3 consumption are similar to in situ rates estimated during push−pull tests in another petroleum contaminated aquifer (*20*), suggesting that these rates may be applicable in other reduced environments where microbial intermediates such as H_2 are available in excess of the amount of TEA introduced.

Our results show that when introduced simultaneously, NO_3 and SO_4 are consumed simultaneously at similar rates. This is contrary to the conventional idea that NO_3 will be consumed prior to SO_4 based on thermodynamic arguments. However, it is consistent with the kinetic model of terminal electron-accepting processes that suggests that, in a noncompetitive situation, there is no reason processes should follow the thermodynamic order. Simultaneous redox processes and overlapping redox zones have been observed in pristine and contaminated aquifer systems previously (*11*, *40*). It has been suggested that concentration levels of electron acceptor or donor, microniches of various TEAPs, or variability of other parameters such as pH may explain the

TABLE 2. Summary of Rate Measurements for Sulfate Reduction[a]

environment	method	rate (μM/day)	order	ref
UA: Black Creek	flux	$1.92-41.1 \times 10^{-5}$	zero	(*17*)
UA: Florida	flow path	2.74×10^{-4}	zero	(*35*)
UA: Fox Hills	flow path	5.48×10^{-4}	zero	(*36*)
UA: Fuhrberg, Germany	depth profile	3.84×10^{-2}	zero	(*37*)
UA: Sturgeon Falls, Canada	depth profile	4.38×10^{-2}	zero	(*38*)
UA: Bocholt, Germany	depth profile	6.3×10^{-2}	zero	(*13*)
UA: Tuse Naes, Denmark	radiotracer	0.41–1.0	zero	(*13*)
UA: Romo, Denmark	radiotracer	0.14–12.3	zero	(*13*)
mixed and pure culture	microcosms	3.04×10^{4}	zero	(*39*)
nearshore sediments	radiotracer	6.3×10^{2}	zero	(*12*)
UA: Denmark	depth	0.85–2.55	zero	(*40*)
UA: Denmark	direct	0.55–12.3	zero	(*40*)
CA: Norman, OK landfill	microcosms	3.5	zero	(*10*)

environment	method	rate (μmol/g·day)[b]	order	ref
Wintergreen Lake	microcosms	5.50×10^{-2}	zero	(*9*)
Wintergreen Lake	microcosms	3.60×10^{-2}	zero	(*9*)
Ocean Seds, Germany	microcosms	4.30×10^{-6}	zero	(*41*)
landfill: Norman, OK	microcosms	6.70×10^{-4}	zero	(*10*)
landfill: Norman, OK	microcosms	$0.1-1.3 \times 10^{-3}$	zero	(*10*)
landfill: Denmark	microcosms	$0.1-1.3 \times 10^{-3}$	zero	(*11*)
landfill: Denmark	microcosms	1.8×10^{-3}	zero	(*11*)
Wetland Soils	microcosms	0.1–11	zero	(*8*)
CA: petroleum	push–pull test	5.28×10^{3}	zero	(*18*)

environment	method	rate (day^{-1})	order	ref
CA: petroleum	flow path	0.02–0.08	first	(*7*)
CA: petroleum	microcosms	0.02–0.08	first	(*7*)
CA: petroleum	aug. flow path	0.1	first	(*19*)
CA: petroleum/Cl solvents	push–pull test	6.48	first	this study
CA: petroleum/Cl solvents	push–pull test	4.32	first	this study
CA: petroleum/Cl solvents	push–pull test	5.76	first	this study

[a] UA = uncontaminated aquifer; CA = contaminated aquifer. [b] μmol/gram of dry weight sediment/day.

TABLE 3. Summary of Rate Measurements for Nitrate Reduction[a]

environment	method	rate (μmol/g·day)[b]	order	ref
CA: sewage (NO_3)	microcosms	$9.6-22.4 \times 10^{-4}$	zero	(*16*)
CA: sewage (NO_3)	tracer tests	$3.1-7.8 \times 10^{-4}$	zero	(*16*)
freshwater lake sediments	microcosms	3.10×10^{-1}	zero	(*42*)
landfill: Denmark	microcosms	1.26×10^{-3} to 2.33×10^{-1}	zero	(*11*)
wetland soils: 0–2 h	microcosms	0.23	zero	(*8*)
wetland soils: 1–10 days	microcosms	0.5–9.3	zero	(*8*)
wetland soils: 10–100 days	microcosms	0.2–4.7	zero	(*8*)
forest soil	microcosms	4.8	zero	(*43*)

environment	method	rate (μM/day)	order	ref
CA: petroleum	push–pull tests	1.92×10^{3}	zero	(*18*)

environment	method	rate (day^{-1})	order	ref
CA: petroleum	push–pull tests	5.28	first	(*20*)
CA: petroleum	aug. flow path	0.1–0.6	first	(*19*)
CA: petroleum/Cl solvents	push–pull tests	5.04	first	this study
CA: petroleum/Cl solvents	push–pull tests	7.44	first	this study

[a] CA = contaminated aquifer. [b] μmol/gram of dry weight sediment/day.

complex patterns of redox processes observed. In addition to these possibilities, our results suggest that SO_4 and NO_3 may be consumed by microbially mediated reactions other than the traditional respiratory pathways since NO_3 and SO_4 were consumed simultaneously at roughly the same rates.

Four possible scenarios are proposed. One scenario assumes an abundance of electron donors in a system limited by electron acceptors, NO_3 and SO_4. This is a likely scenario both at this site and many other contaminated systems. During these tests, available H_2 concentrations were high enough (2.35–3.55 nM) to support both NO_3 and SO_4 reduction. Under these circumstances, when NO_3 and SO_4 are added simultaneously, they could be consumed simultaneously by an abundance of sulfate reducers in the system. Sulfate reducers have been shown to be ubiquitous in the environment and capable of gaining energy from O_2, Fe(III),

TABLE 4. Summary of Available First-Order Field Rate Measurements (day^{-1})

	aerobic respiration	denitrification	sulfate reduction
Chapelle et al. (*34*)			0.02−0.08
Cunningham et al. (*19*)		0.1−0.6	0.1
Schroth et al. (*20*)	3.6−40.6	2.16−10.08	
this study	14.4	5.04−7.44	4.32−6.48

and various nitrogen oxides (*8*, *44*, *45*). Similarly, a second possibility is dissimilatory reduction of NO_3 by denitrifiers under these conditions. A third possible scenario suggests that if the bacteria present are nitrogen limited, assimilatory nitrate reduction may occur simultaneously with SO_4 reduction. These processes would not necessarily follow the thermodynamic order of processes because they would not be in competition with each other. The assimilatory NO_3 reduction would provide a nitrogen source to the organism while the respiratory SO_4 reduction pathway would provide energy. A fourth possibility is the reduction of NO_3 by green rust (GR). Nitrate has been shown to be stoichiometrically reduced to ammonium while GR is oxidized to magnetite (*46*). The rate law for this reaction is first-order, dependent on Fe(II) concentration, where $k = 0.072\ h^{-1}$. This rate is slower than the observed rate for NO_3 removal at this site, however, and it is not inconsequential. It is unclear if green rust is present at the site because it is difficult to identify with standard mineralogical procedures due to its high reactivity including extreme sensitivity to oxidation by oxygen. However, reduced iron is present in the groundwater and magnetite has been detected in the aquifer material. The possibility of the activity of GR is often indicated in investigations of polluted soils, sediments, landfills, and in remediation techniques using Fe(0) (*47*, *48*). Future work needs to be conducted to determine the specific microbially mediated TEA processes being performed at this site during recharge events.

Surface Reaction Vessel Experiments. Four variations of SRV experiments were performed. This water was then amended with solutes, hand shaken to ensure it was well-mixed, and then allowed to react for the length of the corresponding push−pull test. Samples were collected at roughly 5-min intervals. SRVE 1 was performed using water from ML3-25 (June 2000). Aquifer water was pumped into the SRV and amended with 100 mg/L Cl, 10 mg/L NO_3, and 10 mg/L SO_4. The solution was allowed to react for the length of push−pull test 1, approximately 4 h. No changes in NO_3 or SO_4 concentrations were observed over this time period. SRVE 2 and SRVE 2K were performed using water from ML3-25 (July 2000). Aquifer water was pumped into two identical SRVs. Each was amended with 100 mg/L Cl, 20 mg/L NO_3, and 20 mg/L SO_4. SRVE 2K was then amended with formaldehyde (approximately 0.4% final concentration) to serve as a killed control. The solutions were hand-shaken to ensure that they were well-mixed and then allowed to react for the length of push−pull test 2, approximately 4 h. No changes in NO_3 or SO_4 concentrations were observed in either SRVE 2 or SRVE 2K over this time period. SRVE 3 and SRVE 3K were performed using water from ML3-25 (July 2000). Aquifer water was pumped into two identical SRVs. Each was amended with 100 mg/L Cl, 50 mg/L NO_3, and 50 mg/L SO_4. SRVE 3K was then amended with formaldehyde (approximately 0.4% final concentration) to serve as a killed control. The solutions were allowed to react for the length of push−pull test 2, approximately 4 h. No changes in NO_3 or SO_4 concentrations were observed in either SRVE 3 or SRVE 3K over this time period. SRVE 4 and SRVE 4K were performed using water from ML3-22, which was similar chemically to ML3-25 in all respects except it contained slightly higher DCE concentrations (4.9 ug/L) (July 2000). Aquifer water was extracted into two identical SRVs. Each was amended with 100 mg/L Cl, 50 mg/L NO_3, and 50 mg/L SO_4. SRVE 4K was then amended with formaldehyde (approximately 0.4% final concentration) to serve as a killed control. The solutions were allowed to react for approximately 4 h. No changes in NO_3 or SO_4 concentrations were observed in either SRVE 4 or SRVE 4K over this time period. Measured Fe^{2+} concentration remained consistently high (11.3−11.5 mg/L) throughout all SRVEs, and dissolved oxygen was below the detection limit of our field kit (<0.03 mg/L), suggesting that the solutions remained anaerobic.

These results indicate that any redox reactions observed in the push−pull tests do not occur in the reduced water without the influence of the sediments and their associated microbial communities. This suggests that highly oxidized compounds such as NO_3 and SO_4 may persist in reduced waters. This finding is analogous to the persistence of Cr^{3+} in oxidized water (*49*). It has been shown that reduced Cr^{3+} is oxidized to Cr^{6+} at a much greater rate by the presence of solid-phase manganese oxides than by oxygen (*50*). In the case of the persistence of NO_3 and SO_4 in reduced water, it is unclear if the absence of the solid phase or its associated microbial population is allowing these oxidized compounds to persist. However, these findings have implications for how geochemical models of reduced systems are constructed and how we interpret the results from such modeling efforts. Geochemical models are driven by free-energy yield and consider only the aqueous phase in predicting reactions, disequilibria, and other constraints. Even with thermodynamic and kinetic arguments to input into a model, the model would not predict our observed data. Our data clearly demonstrate that, in addition to a simple mixing of aqueous solutions, knowledge of the reactions being controlled by the solid phase and their associated microbial population is necessary to understand how NO_3 and SO_4 will behave in the environment.

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Literature Cited

(1) Vroblesky, D. A.; Chappelle, F. H. *Water Resour. Res.* **1994**, *30*, 1561−1570.
(2) Lovley, D. R. *Microbiol. Rev.* **1991**, *55*, 259−287.
(3) Chappelle, F. H. *Ground-Water Microbiology and Geochemistry*, 2nd ed.; John Wiley and Sons: New York, 2001.
(4) Stumm, W.; Morgan, J. J. *Aquatic Chemistry*, 2nd ed.; John Wiley and Sons: New York, 1981.
(5) Lovley, D. R.; Goodwin, S. *Geochim. Cosmochim. Acta* **1988**, *52*, 2993−3003.
(6) Champ, D. R.; Gulens, J.; Jackson, R. E. *Can. J. Earth Sci.* **1979**, *127*, 85−108.
(7) Chappelle, F. H.; Haack, S. K.; Adriaens, P.; Henry, M.; Bradley, P. M. *Environ. Sci. Technol.* **1996**, *30*, 3565−3569.
(8) D'Angelo, E. M.; Reddy, K. R. *Soil Biol. Biochem.* **1999**, *31*, 815−830.
(9) Lovley, D. R.; Dwyer, D. F.; Klug, M. J. *Appl. Environ. Microbiol.* **1982**, *43*, 1373−1379.
(10) Cozzarelli, I. M.; Suflita, J. M.; Ulrich, G. A.; Harris, S. H.; Schroll, M. A.; Schlottmann, J. L.; Christenson, S. *Environ. Sci. Technol.* **2000**, *34*, 4025−4033.
(11) Ludvigsen, L.; Albrechtsen, H.-J.; Heron, G.; Bjerg, P. L.; Christensen, T. H. *J. Contam. Hydrol.* **1998**, *33*, 273−291.

(12) Crill, P. M.; Martens, C. S. *Geochim. Cosmochim. Acta* **1987**, *51*, 1175−1186.
(13) Jakobsen, R.; Postma, D. *Geology* **1994**, *22*, 1103−1106.
(14) Bowman, J. P.; Jimenez, L.; Rosario, I.; Hazen, T. C.; Sayler, G. S. *Appl. Environ. Microbiol.* **1993**, *59*, 2380−2387.
(15) Hunter, K. S.; Wang, Y.; Van Cappellen, P. *J. Hydrol.* **1998**, *209*, 53−80.
(16) Smith, R. L.; Garabedian, S. P.; Brooks, M. H. *Environ. Sci. Technol.* **1996**, *30*, 3448−3456.
(17) Chappelle, F. H.; McMahon, P. B. *J. Hydrol.* **1991**, *127*, 85−108.
(18) Istok, J. D.; M. D., H.; Schroth, M. H.; Hyman, M. R.; O'Reilly, K. T. *Groundwater* **1997**, *35*.
(19) Cunningham, J. A.; Hopkins, G. D.; Lebron, C. A.; Reinhard, M. *Biodegradation* **2000**, *11*, 159−170.
(20) Schroth, M. H.; Istok, J. D.; Connoer, G. T.; Hyman, M. R.; Haggerty, R.; O'Reilly, K. T. *Groundwater* **1998**, *36*.
(21) Istok, J. D.; Field, J. A.; Schroth, M. H. *Groundwater* **2000**.
(22) Luthy, L.; Fritz, M.; Bachofen, R. *Appl. Environ. Microbiol.* **2000**, *66*, 712−717.
(23) Lovely, D. R.; Phillips, E. J. P. *Appl. Environ. Microbiol.* **1987**, *53*, 2636−2641.
(24) Loffler, F. E.; Tiedje, J. M.; Sanford, R. A. *Appl. Environ. Microbiol.* **1999**, *65*, 4049−4056.
(25) Chappelle, F. H.; McMahon, P. B.; Dubrovsky, N. M.; Fujii, R. F.; Oaksford, E. T.; Vroblesky, D. A. *Water Resour. Res.* **1995**, *31*, 359−371.
(26) Atlas, R. M.; Bartha, R. *Microbial Ecology: Fundamental and Applications*, 4th ed.; Benjamin/Cummings Publishing Company, Inc.: Menlo Park, CA, 1998.
(27) USGS *Installations Restoration Program: Phase II*−*Confirmation/Quantification Stage 1: Wurtsmith Air Force Base, Michigan: Investigations of groundwater and soil contamination at selected sites*; United States Geological Survey, Water Resources Division: 1991.
(28) McGuire, J. T.; Smith, E. W.; Long, D. T.; Hyndman, D. W.; Haack, S. K.; Klug, M. J.; Velbel, M. A. *Chem. Geol.* **2000**, *169*, 471−485.
(29) Reynolds, L. A.; Haack, S. K. U.S. Geological Survey toxic substances hydrology program. Proceedings from the Technical Meeting, Charleston, South Carolina, 8−12 March 1999, Morganwalp D.W., Buxton H.T. Eds. *Water*−*Resources Investigations Report (U.S. Geological Survey)* **1999**, *99-4018C*, 635−639.
(30) Dojka, M. A.; Hugenholtz, P.; Haack, S. K.; Pace, N. *Appl. Environ. Microbiol.* **1998**, *64*, 3869−3877.
(31) Haack, S. K.; Bekins, B. A. *Hydrogeol. J.* **2000**, *8*, 63−76.
(32) Snodgrass, M. F.; Kitanidis, P. K. *Groundwater* **1998**, *36*.
(33) Haggerty, R.; Schroth, M. H.; Istok, J. D. *Groundwater* **1998**, *36*.
(34) Chappelle, F. H.; Bradley, P. M.; Lovley, D. R.; Vroblesky, D. A. *Groundwater* **1996**, *34*, 691−698.
(35) Plummer, L. N. *Water Resour. Res.* **1977**, *13*, 801−812.
(36) Thorstenson, D. C.; Fisher, D. W.; Croft, M. G. *Water Resour. Res.* **1979**, *15*, 1479−1498.
(37) Bottcher, J.; Strebel, O.; Duynisveld, W. H. M. *Geol. Jahrb.* **1989**, *C51*, 3−40.
(38) Robertson, W. D.; Cherry, J. A.; Schiff, S. L. *Water Resour. Res.* **1989**, *25*, 1111−1123.
(39) Spear, J. R.; Figueroa, L. A.; Honeyman, B. D. *Appl. Environ. Microbiol.* **2000**, *66*, 3711−3721.
(40) Jakobsen, R.; Postma, D. *Geochim. Cosmochim. Acta* **1999**, *63*, 137−151.
(41) Knoblauch, C.; Jorgensen, B. B.; Harder, J. *Appl. Environ. Microbiol.* **1999**, *65*, 4230−4233.
(42) Nijburg, J. W.; Laanbroek, H. J. *Microb. Ecol.* **1997**, *34*, 254−262.
(43) Bengston, G.; Bergwall, C. *Soil Biol. Biochem.* **2000**, *32*, 545−557.
(44) Dannenberg, S.; Kroder, M.; Dilling, W.; Cypionka, H. *Arch. Microbiol.* **1992**, *158*, 93−99.
(45) Coleman, M. L.; Hedrick, D. B.; Lovely, D. R.; White, D. C.; Pye, K. *Nature* **1993**, *361*, 436−438.
(46) Hansen, H. C. B.; Guldberg, S.; Erbs, M.; Koch, C. B. *Appl. Clay Sci.* **2001**, *18*, 81−91.
(47) Gu, B.; Phelps, T. J.; Liang, L.; Dickey, M. J.; Roh, Y.; Kinsall, B. L.; Palumbo, A. V.; Jacobs, G. K. *Environ. Sci. Technol.* **1999**, *33*, 2170−2177.
(48) Fredrickson, J. K.; Zachara, J. M.; Kennedy, D. W.; Dong, H.; Onstott, T. C.; Hinman, N. W.; Li, S. M. *Geochim. Cosmochim. Acta* **1998**, *62*, 3229−3257.
(49) Eary, L. E.; Rai, D. *Environ. Sci. Technol.* **1987**, *21*, 1187−1193.
(50) Rai, D.; Eary, L. E.; Zachara, J. M. *Sci. Total Environ.* **1989**, *89*, 15−23.

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