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The limited role of aquifer heterogeneity on metal reduction in an Atlantic coastal plain determined by push-pull tests

Brian J. Mailloux^{a,*}, Stephanie Devlin^a, Mark E. Fuller^b, T.C. Onstott^a,
Mary F. DeFlaun^{b,1}, Keun-Hyung Choi^c, Maria Green-Blum^c,
Donald J.P. Swift^c, John McCarthy^d, Hailiang Dong^e

^a Department of Geosciences, Princeton University, Princeton, NJ 08544, USA

^b Envirogen Inc. (Now Shaw Environmental and Infrastructure), Princeton Research Center, 4100 Quakerbridge Road, Lawrenceville, NJ 08648, USA

^c Ocean, Earth, and Atmospheric Sciences, Old Dominion University, 4600 Elkhorn Avenue, Norfolk, VA 23529, USA

^d Department of Geological Sciences, The University of Tennessee, Knoxville, TN 37996, USA

^e Miami University of Ohio, Department of Geology, Oxford, OH 45056, USA

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Abstract

Sixty push-pull experiments were conducted to determine the factors controlling Fe(III) and Mn(IV) reduction in a well-characterized, shallow, coastal plain aquifer near Oyster, VA, USA. The five multi-level samplers each equipped with 12 ports sampled a heterogeneous portion of the aquifer from 4.4 to 8 m-bgs. Each multi-level sampler (MLS) was injected with groundwater that contained NO_3^- and Br^- along with: (1) just groundwater (control treatment), (2) humics, (3) lactate (conducted twice) and (4) lactate plus humics. Microbially mediated Fe(III) reduction caused the aqueous Fe_{Tot} concentrations to increase at every depth in the lactate treatment with significant increases within 1 day even while NO_3^- was present. Little change in the Fe_{Tot} concentrations were observed in the control and humics treatment. Humics may have acted as an electron shuttle to increase Fe(III) reduction in the lactate plus humics treatment. The amount of Mn(IV) reduction was significantly lower than that of Fe(III) reduction. Geochemical modeling indicated that gas formation, sorption on reactive surfaces, and mineral precipitation were important processes and that Fe(III) and SO_4^{2-} reduction were co-occurring. Conditions were favorable for the precipitation of Fe-carbonates, Fe-sulfides and Fe-silicates. In the lactate treatment protist concentrations increased then decreased and planktonic cell concentrations steadily increased, whereas no change was observed in the control treatment. Correlations of Fe(III) reduction with physical and chemical heterogeneity were weak, probably as a result of the abundance of Fe(III) bearing minerals relative to electron donor abundance and that the push-pull test sampled a representative elemental volume that encompassed the microbial diversity within the aquifer.

* Corresponding author. Present address: Department of Environmental Sciences, Barnard College, 3009 Broadway, 404 Altschul Hall, New York, NY 10027, USA.

E-mail address: bjm2103@columbia.edu (B.J. Mailloux).

¹ Present address: GeoSyntec Consultants, Research Park, Princeton, NJ 08540, USA.

This work indicates that stimulating metal reduction in aquifer systems is a feasible method for remediating heterogeneous subsurface sites contaminated with metals and radionuclides.

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1. Introduction

Iron(III) is one of the most prevalent electron acceptors in aquifer systems (Lovley and Philips, 1986). At sites contaminated with chlorinated solvents and petroleum hydrocarbons, in situ degradation can occur under Fe(III) reducing conditions (Lovley and Anderson, 2000). At sites contaminated with radionuclides (e.g. U, Pu and Cs) and toxic metals (e.g. Cr, Hg and Pb) microbially mediated reactions are being explored as potential remedial strategies (National Research Council, 1994; Riley and Zachara, 1992; US Department of Energy, 2001). The addition of an electron donor to the subsurface at these sites would promote both direct or indirect microbial reduction and precipitation of metals and radionuclides. Despite the potential benefit of in situ metal reduction few well-constrained field-scale experiments have been conducted (Istok et al., 2004; Senko et al., 2002). The goal of this study is to examine the controls on Fe(III) reduction in a well characterized sandy aquifer by comparing Fe(III) reduction rates determined by push-pull tests to site parameters.

Laboratory microcosm studies that examine the controls on Fe(III) reduction may not capture all of the relevant subsurface processes. In microcosm studies with synthetic or aquifer sediments, only a small percentage of the available Fe(III) is usually reduced (Roden and Urrutia, 2002). The cessation of Fe(III) reduction has been linked to unfavorable thermodynamic conditions and the sorption and precipitation of Fe(II) to cell walls and mineral surfaces (Liu et al., 2001b; Roden and Urrutia, 1999, 2002; Urrutia et al., 1999). In laboratory studies, removal of Fe(II) by simulated groundwater flow enhanced the amount of Fe(III) reduction by removing Fe(II) from the system (Roden and Urrutia, 1999; Roden et al., 2000). The transport of Fe(II), however, is retarded (Martin and Kempton, 2000) and Fe(II) may remain in the zone of Fe(III) reduction for a significant amount of time even under flowing conditions. In addition the movement and amount of Fe(II) can also affect abiotic reactions controlling mineral precipitation (Benner et al., 2002; Hansel et al., 2003). Laboratory studies,

therefore, may be poor predictors of field-scale Fe(III) reduction in dynamic aquifer systems.

The effect of aquifer heterogeneity on metal reduction has not been well studied. Microcosm studies utilizing sediments from an anaerobic Atlantic Coastal Plain Aquifer have indicated that at the centimeter scale Fe(III) reduction was not ubiquitous, discrete strata existed where Fe(III) reduction was more active (Overstreet et al., 2001). These zones may be caused by localization of microbial communities, Fe(III) oxyhydroxides, or Fe(III)-bearing clays. Laboratory studies have indicated that the presence of electron shuttling compounds, such as humic acids, may enhance the amount Fe(III) reduction because the metal reducing bacteria are not required to be in direct contact with the Fe(III) bearing minerals (Fredrickson et al., 1998; Lovley et al., 1996; Zachara et al., 1998) but their role at the field-scale has not been well documented.

Push-pull experiments are a powerful method for determining in situ reaction rates within aquifers (Addy et al., 2002; Istok et al., 1997; McGuire et al., 2002; Schroth et al., 2001). During a push-pull experiment groundwater is amended, added to an aquifer (pushed), and given time to react. The groundwater is then withdrawn (pulled) from the aquifer and the concentrations of a conservative tracer are compared to the reactive species in order to study the reactions occurring in situ.

In this study push-pull tests were conducted in multi-level samplers (MLSs) with high vertical resolution to investigate the effects of small-scale, ~30 cm, physical and chemical heterogeneities in a well-characterized aquifer on microbial metal reduction. Five push-pull experiments that targeted four treatments were simultaneously performed across five 12-port MLSs that spanned a 3.6 m section of a shallow aquifer. The treatments were designed to determine the effect of an electron donor and humic acids on metal reduction. Each experiment included elevated levels of NO_3^- to simulate conditions at Department of Energy (DOE) sites and Br^- as a conservative tracer to determine reaction rates. Treatment 1 was a control treatment which received only Br^- whose goal was to determine the natural reaction rates in the aquifer. Treatment 2 added

humic acids and whose goal was to determine the effect of increased organic C concentrations on redox processes. Treatment 3 (performed twice) added lactate and whose goal was to determine the effect of an electron donor on the rates of metal reduction. Treatment 4 added lactate and humic acids and whose goal was to determine the potential of humics to act as an electron shuttle to increase the rate and amount of Fe(III) reduction in the presence of an electron donor. Rates calculated from each sampling port were compared to site parameters describing physical and chemical heterogeneity. Results indicate that aquifer heterogeneity has a small effect on Fe(III) reduction and these results have important implications for remediation of sites contaminated with metals and radionuclides.

2. Methods

2.1. Field site

Experiments were conducted at five separate wells equipped with MLSs spaced over 20 m² in the Columbia Aquifer at the South Oyster Bacterial Transport Field Site on the Southern Delmarva Peninsula (Fig. 1). The site has been extremely well

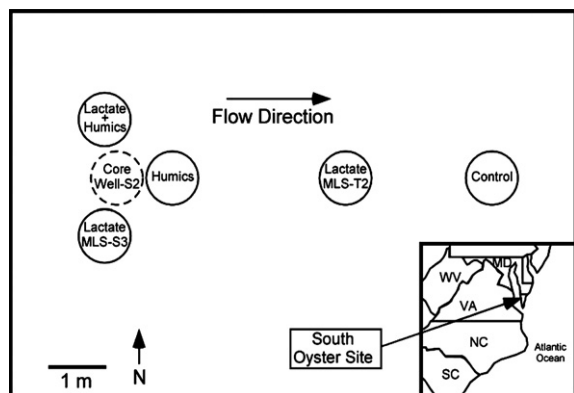


Fig. 1. Site location and map of the study area at the Oyster Site. The inset shows the location of Oyster, Virginia on the Eastern Seaboard of the United States. The map shows the relative location of the MLSs used during the push-pull experiments for each treatment. The size of circle showing the location of each MLS is approximately equal to the aerial extent of the injected water. No injection occurred at well S2 but the most complete sediment dataset was available from this MLS and was used for determining correlations (Table 5). The MLS names are shown for the two lactate treatments for clarity. Pre-experiment push-pull tests indicated that no cross-contamination of injected solutions would occur between the separate MLSs.

characterized by coupling tomographic geophysical surveys to hydrological, microbial, and sedimentological analyses (Balkwill et al., 2001; Chen et al., 2001; Hubbard et al., 2001; Overstreet et al., 2001; Scheibe et al., 2001). The site was located in a sub-oxic, heterogeneous portion of the aquifer with sediments ranging from peat and clay to sands and gravel (Green et al., 2000). The groundwater table was less than 1 m below the land surface and 3.4 m above the top sampling location. The sediments at the site contained primarily detrital grains with orange to brown coatings a few μm thick. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) analyses indicated that these coatings contained smectite, chlorite, goethite, amorphous Al, halloysite, and amorphous Fe-bearing silica (Dong et al., 2002; Penn et al., 2001). Multiple wells have been installed and sediment cores were collected with a Roto-Sonic drill rig (RD 150 Sonic Drill Rig, Boart Longyear, Little Falls, MN). Each well utilized for the push-pull experiment had core taken during installation. All wells were made from 5.1 cm (2") or 10.2 cm (4") diameter schedule 40 PVC and are 8.2 m deep with the bottom 6.1 m screened with 0.25 cm (0.10") screen with the natural formation allowed to back-collapse during installation. Two bacterial transport and two preliminary push-pull experiments were conducted at the same site prior to this experiment and monitoring indicated the aquifer had returned to in situ conditions (Fuller et al., 2004a; Mailloux, 2003).

2.2. Aquifer characterization

The hydraulic conductivities at the wells were estimated from slug and pump tests (Freeze and Cherry, 1979). Permeability and grain size for sediment cores were determined using the falling head method and sieve analysis, respectively (Green et al., 2000). For mineralogy the sediment cores were initially passed through a magnetic susceptibility bridge (MS2C, Barrington, Oxford, England) to quantify the total amount of Fe bearing minerals and then processed in an anaerobic glove bag on site, with the sediment transferred to and stored in anaerobic vials and frozen until characterized. Sediments were examined by SEM to identify minerals and to obtain crystal morphology information using a Philips XL30 field-emission gun FEG SEM fitted with backscatter and secondary electron detectors and an IMIX energy dispersive X-ray analytical

system (Dong et al., 1999). For sequential extractions sediment was dried in an anaerobic glove bag (90:10 N₂:H₂) and analyzed using a modified version of published procedures (La Force and Fendorf, 2000; Mehra and Jackson, 1960). The sediments were first shaken in degassed, deionized water at room temperature for 1 h to remove the anions and cations adsorbed to the sediment and salts that had precipitated during drying in the glove bag. The Fe and Mn present in exchangeable clay sites were extracted with 1 M MgCl₂ at room temperature for 1 h. The sediments were then extracted with 0.3 M Na citrate and 0.25 M Na dithionate at 80 °C for 2 h at pH 7 for the Fe and Mn oxyhydroxides and at pH 13 for the Al hydroxide contents. Extracts were analyzed by ICP-OES and IC (see below). The sorbed organics were extracted separately from fresh sediments with 0.1 mM NaOH and the extract analyzed using a Shimadzu TOC-V Total Organic Carbon Analyzer (Shimadzu Inc., Columbia, MD). Initial SEM analyses were used to design the extractions. HCl extractions leaching Fe and Mn out of the clays, were not specific for oxides, and therefore were not utilized. In addition, the extractions were designed to also target the Al oxyhydroxides as these may be areas of increased microbial abundance (Hall et al., 2005). Sequential extractions have received scrutiny in the literature (Martin et al., 1987; Nirel and Morel, 1990) because they are operationally defined and do not target a specific mineral phase, however, the extractions were carefully planned and enabled a large number of samples to be processed for site characterization and comparisons (La Force and Fendorf, 2000).

Cores were collected and partially analyzed from each of the five wells used for the experiments. The only complete data set was available from the core collected at MLS-S2 which was thoroughly characterized and centrally located (Fig. 1). Visual examination, magnetic susceptibility and geochemical analyses of the cores along with acoustic and radar tomographic cross-sections indicate that the physical and chemical properties of the sediments were uniform over several meters horizontally but varied markedly over ~30 cm vertically. It was therefore concluded that the data from MLS-S2 could be utilized to relate sediment properties to the rate data gathered from the push-pull experiments. Sediment cores were not collected after the push-pull experiment and therefore there are no direct measurements of mineral dissolution or precipitation during the push-pull experiment.

2.3. Push-pull experiment

Sixty push-pull experiments were simultaneously conducted in the 12 vertical ports of MLSs placed in five wells beginning September 30, 2001 (Fig. 1). The MLSs were designed to be placed in 5.1 cm and 10.2 cm wells (Mailloux, 2003). By placing the MLSs inside the well, it minimized the formation collapse that occurred during emplacement because the wells were closer in diameter to the drill bore than permanent MLSs. During design and testing of the MLSs it was shown that no vertical cross-contamination occurred between MLS levels (Mailloux, 2003). The 12 ports, with one at the top, of each MLS were located from 4.4 to 8.0 m below ground surface (m-bgs) at 0.30 m intervals with the water table approximately 1 m-bgs. The MLSs were emplaced so that the same port at each MLS was always at the same depth. The wells with the MLSs were located both cross- and down-gradient from each other and the previous Br⁻ tracer test determined that the groundwater flow rate was sufficiently low that the amended water from each well would remain in distinct zones with no cross-contamination occurring (Fig. 1).

To conduct the push-pull experiments water was extracted from well B1, located 10.5 m up gradient of MLS-S2 and pumped into a 1000 L polyethylene tank (Fig. 1). The water in the tank was bubbled with N₂:CO₂ (97:3), amendments of NaBr (~1.5 mM), humics, or lactate (NaC₃H₅O₃) (~2 mM) were added, and the tank was thoroughly mixed with a pump. Previous work at the site indicated that bubbling gas through the water minimized the chemical changes that occurred to the groundwater due to contact with the atmosphere. After mixing, the tank was emptied into 12 separate 57 L, airtight, polyethylene tanks that were completely filled and kept on ice out of direct sunlight. Peristaltic pumps were utilized to simultaneously empty each tank into one port of the MLS. This method ensured that each port received exactly the same volume of amended groundwater. The injection process took ~3 h. The tanks were rinsed and the procedure was repeated for the remaining four injections. The predicted radius of influence for each injection was ~0.45 m, assuming a porosity of 0.34 (Scheibe et al., 2001).

The humic substances were extracted from the peat-derived humic-rich groundwater from well E-0-D at the Kitty Hawk Woods Humic Reference Site using reverse osmosis (McCarthy, 2001; Sun et al.,

1995). For this study humics refer to the organic-rich water, which probably contains both humic and fulvic acids. The humic acid injection solution was highly colored and was predominantly comprised of large molecular weight, hydrophobic acids, rich in aromatic groups as determined by Huffman laboratories (Golden, CO) (Leenheer, 1981). In contrast, the South Oyster site groundwater contained trace levels of low molecular weight, hydrophilic organic matter (McCarthy et al., 1993, 1996). It was hypothesized that the humics rich in hydrophobic acids would act as an electron shuttle promoting Fe(III) reduction (Chen et al., 2003; Royer et al., 2002).

Initial aqueous samples were collected as the water was pumped into each MLS. Subsequent samples were collected daily from each sampling port for the first week, then at approximately days 10, 12 and 18. Sample collection was conducted with a peristaltic pump and consisted of a purge to remove the water in the MLS followed by collection in sample vials.

2.4. Aqueous sample collection and analysis

The water samples were collected and analyzed for temperature, pH, dissolved O₂ (DO), Eh, dissolved inorganic C (DIC), $\delta^{13}\text{C}$ of DIC, nonpurgeable organic C (TOC), lactate, acetate, anions, cations, total cell counts, and protist counts. Temperature, pH, DO, and Eh were analyzed in the field in a custom made flow through cell. DIC was analyzed for CO₂ with a Li-Cor LI-6252 (Li-Cor Inc., Lincoln, NE) after acidification, thus accounting for all inorganic C species and invariant of pH. Lactate and anions (Cl⁻, NO₂⁻, SO₄²⁻, NO₃⁻, PO₄³⁻ and Br⁻) were determined with a DX-320 ion chromatograph (IC) equipped with an AS-15 column and a limited number of samples were analyzed for acetate with an AS-11 column. Cation (Al, Ba, Co, Fe, Mn, P, Sr, Zn, Ca, Mg, Na, K, and Si) determinations were performed by Inductively Coupled Plasma Optical Emission Spectroscopy ICP-OES DV4300 (Perkin-Elmer, Shelton, CT). Comparison between Fe(II) as determined by the ferrozine method and Fe_{Tot} as determined by ICP-OES on select samples yielded similar values and only Fe_{Tot} concentrations are presented. Samples for total organic C (TOC) were analyzed using a Shimadzu TOC-V Total Organic Carbon Analyzer (Shimadzu Inc., Columbia, MD). The aromatic content of the organic C was estimated by measuring the UV-absorptivity at 270 nm (A_{270}); absorbance at 270 nm was normalized to the TOC

concentration (Gauthier et al., 1987; McCarthy, 2001; Traina et al., 1990). The samples for $\delta^{13}\text{C}$ of DIC were fixed with HgCl₂ and analyzed by the company Mountain Mass Spec (Evergreen, CO).

Total bacterial count samples were fixed with 1% (v:v) formaldehyde and sub-samples were amended with 20 mM nitrilotriacetic acid to chelate the Fe(III), stained with 5 nM Syto13 (Molecular Probes Inc., Eugene, OR) and were counted on a FacSort flow cytometer (Becton Dickson Inc., San Jose, CA) (Mailloux et al., 2003; Stewart and Steinkamp, 1982). Protist samples were fixed with 1% (v:v) glutaraldehyde buffered with 0.01 M cacodylate, at a pH of 6.0, and enumerated at 640x magnification with epifluorescence microscopy.

2.5. Data analysis

During a push-pull test, groundwater advection and dispersion dilutes the injected water with groundwater. The measured concentrations of each species were corrected, where noted, for this dilution utilizing the background and injectate concentration of the species, the injected Br⁻ concentration, and the measured Br⁻ concentrations (Haggerty et al., 1998; Snodgrass and Kitanidis, 1998). Zero order and first order reaction rates were determined by the method of Snodgrass and Kitanidis (1998) and all lines were fit by least squares.

Bacterial cells, Fe(II) and Mn(II) adsorb to sediment grain surfaces. Since the amount of adsorbed Fe(II), Mn(II) and cells could not be measured during the course of the experiment, rates of Fe(III) and Mn(IV) reduction and cell growth could not be explicitly determined. Due to this sorption the standard methods for calculating reaction rates for push-pull experiments may not always be applicable. Therefore, two methods have been utilized for examining the amount of change in the system. The first standard method is the determination of rates utilizing dilution adjusted concentrations. The second method is the comparison of the observed peak values of dissolved Fe, Mn and planktonic cell concentrations. It is hypothesized that the observed peak values may be a good indicator of the amount and rate of metal reduction and that combining these two methods should offer insights into the observed processes.

The Pearson correlation coefficient (r) was utilized to determine two-parameter linear correlations between push-pull results and the physical and geochemical aquifer properties. The rates and observed

concentrations from each port were compared to the average physical and mineralogical characteristics available for that depth. A Student's *t*-test with two tails and unequal sample variance was utilized to compare the results from two treatments. *P*-values below 0.01 were considered statistically significant, whereas results with *p*-values between 0.01 and 0.05 were considered only significant.

2.6. Mass balance considerations

Push-pull tests are normally designed to look at the soluble species. Metal reduction, however, can lead to solid or adsorbed phase formation and in this experiment it was only possible to rely on the aqueous species data to draw inferences about the in situ processes. Using the DIC concentration, assuming no losses of DIC to carbonate precipitation or methanogenesis, it may be possible to determine the main biogeochemical processes and the extent of the reactions from a mass balance perspective:

$$\text{DIC}_t = \text{DIC}_0 + \text{DIC}_{\text{O}_2} + \text{DIC}_{\text{NO}_3^-} + \text{DIC}_{\text{Mn(IV)}} + \text{DIC}_{\text{Fe(III)}} + \text{DIC}_{\text{SO}_4^{2-}} + \text{DIC}_{\text{Ferm}} \quad (1)$$

At time (*t*) the amount of (DIC_t) is a function of the initial DIC (DIC_0), and the DIC produced from the redox reactions (Table 1). During the mineralization of lactate, the production of DIC will be directly linked to the redox reactions assuming no fermentation ($\text{DIC}_{\text{Ferm}} = 0$). Since the amount of DIC produced from each reaction is not known, it can only be estimated from the change in the redox sensitive species:

$$\begin{aligned} \text{DIC}_t = & \text{DIC}_0 + 1.0\text{O}_2 + 1.25\text{NO}_3^- \\ & + 0.5\text{Mn(II)} + 0.25\text{Fe(II)} + 2.0\text{SO}_4^{2-} \\ & + \text{DIC}_{\text{Sorb}} \end{aligned} \quad (2)$$

The amount of DIC (DIC_t) is then a function of the initial DIC (DIC_0), and the amount produced from redox reactions (Eq. (2)) which can be measured over time enabling a direct mass balance. Sulfate that is adsorbed to the sediment and not represented by Eq. (2) may also be reduced and produce DIC. Fe(II) and Mn(II) may adsorb to the sediment or precipitate following reduction, and the values in Eq. (2) may underestimate the DIC produced from these reactions. Therefore, an additional DIC term that reflects production from adsorbed species that are reduced or redox products that adsorb is added to the equation (DIC_{Sorb}).

If lactate is completely oxidized, then 3 mol of DIC (DIC_{Lact}) are produced or if acetate accumulates than 1 mol of DIC is produced. Acetate can form as an intermediary during either fermentation or as a product of the redox reactions. The DIC_t can then also be estimated from the amount of lactate utilized and acetate generated.

2.7. Geochemical modeling

The presence of Fe(II) in an aquifer is not always a good indicator of the amount of Fe(III) reduction occurring in an aquifer (Albrechtsen et al., 1995), therefore, geochemical modeling was undertaken. The goal of the geochemical modeling was to determine the likelihood of Fe-bearing minerals to precipitate, the relative energies of select geochemical

Table 1
Reactions and Gibbs free energy of formations utilized in the thermodynamic calculations

Reaction	ΔG^{of} (kcal/mol of lactate or CH_4)	Chemical formulae	
Acetate + $\text{H}_2\text{O} \leftrightarrow \text{CH}_4 + \text{HCO}_3^-$	-14.4	Lactate	$\text{CH}_3\text{CHOHCOO}^-$
$4\text{H}_2 + \text{HCO}_3^- + \text{H}^+ \leftrightarrow \text{CH}_4 + 3\text{H}_2\text{O}$	-221.9	Acetate	CH_3COO^-
Lactate + 4Goethite + $7\text{H}^+ \leftrightarrow \text{Acetate} + 4\text{Fe}^{2+} + \text{HCO}_3^- + 6\text{H}_2\text{O}$	-268.7	Goethite	FeOOH
Lactate + 12Ferrihydrite + $22\text{H}^+ \leftrightarrow 12\text{Fe}^{2+} + 3\text{HCO}_3^- + 30\text{H}_2\text{O}$	-1064.8	Ferrihydrite ^a	Fe(OH) ₃
Lactate + $2\text{H}_2\text{O} \leftrightarrow \text{Acetate} + 2\text{H}_2 + \text{H}^+ + \text{HCO}_3^-$	63.9		
Lactate + 12Goethite + $22\text{H}^+ \leftrightarrow 12\text{Fe}^{2+} + 3\text{HCO}_3^- + 18\text{H}_2\text{O}$	-726.4		
Lactate + $3\text{O}_2 + \text{H}^+ \leftrightarrow 3\text{H}_2\text{O} + 3\text{CO}_2$	-1252.1		
Lactate + $2.4\text{NO}_3^- + 0.4\text{H}^+ \leftrightarrow 1.2\text{N}_2 + 3\text{HCO}_3^- + 12\text{H}_2\text{O}$	-1201.0		
Lactate + $6\text{MnO}_2(\text{gamma}) + 10\text{H}^+ \leftrightarrow 6\text{Mn}^{2+} + 3\text{HCO}_3^- + 6\text{H}_2\text{O}$	-1254.8		
Lactate + $1.5\text{SO}_4^{2-} \leftrightarrow 1.5\text{HS}^- + 0.5\text{H}^+ + 3\text{HCO}_3^-$	-109.5		

All thermodynamic calculations were performed with Geochemist's Workbench (Rockware, Inc., Golden, CO) using the Lawrence Livermore National Laboratories combined database (Delany and Lundeen, 1990).

^a The Fe(OH)₃ formula for ferrihydrite was used as a simplification of the possible true formulae, which may be closer to $\text{Fe}_2\text{O}_3 \cdot 2\text{FeOOH} \cdot 2.6\text{H}_2\text{O}$ (Russell, 1979; Zachara et al., 2002).

reactions, and the controls on the aqueous geochemistry of the system. Geochemical modeling was performed to track the saturation indices of Fe-bearing minerals and the Gibbs free energy of microbial reactions. In addition reaction path modeling was conducted to discern the minimum number of reactions and constraints required to predict the observed results. All geochemical calculations were performed using Geochemist's Workbench (Rockware, Inc., Golden, CO) with the Lawrence Livermore National Laboratories combined database (Delany and Lundeen, 1990).

3. Results

3.1. Site characterization

The average hydraulic conductivity across the site was 2.4 ± 0.7 m/day with an average grain size of 2.2 ± 0.9 phi (0.27 ± 0.16 mm). SEM analysis indicated that the aquifer is comprised mainly of

detrital quartz and feldspar with Fe oxyhydroxides predominantly in the form of goethite coating the quartz grains or present as colloids in solution (Fig. 2). Anions and cations were adsorbed to the sediment including: SO_4^{2-} 0.42 ± 0.25 mmol/kg, Fe_{Tot} 0.60 ± 1.06 mmol/kg, Mn_{Tot} 0.05 ± 0.20 mmol/kg, and Al_{Tot} 3.39 ± 7.47 mmol/kg. The average adsorbed organic C was 26.8 ± 31.5 mmol/kg. Average values of exchangeable metals were Fe_{Tot} 0.22 ± 0.22 , Mn_{Tot} 0.09 ± 0.08 , and Al_{Tot} 0.41 ± 0.27 mmol/kg (Fig. 3). Average values of metals from the oxyhydroxides were Fe_{Tot} 28.6 ± 35.9 , Mn_{Tot} 0.45 ± 0.59 and Al 17.1 ± 13.3 mmol/kg. Vertical variations in the stratigraphy, grain size, and extraction data were observed across the vertical section (Fig. 3). At the top of the experimental zone was peat-rich mudstone, as determined visually, underlain by a coarse-grained, Fe-poor zone, which became finer grained and more Fe oxyhydroxide-rich with depth. Small coarse-grained zones were interbedded with the fine sands throughout the

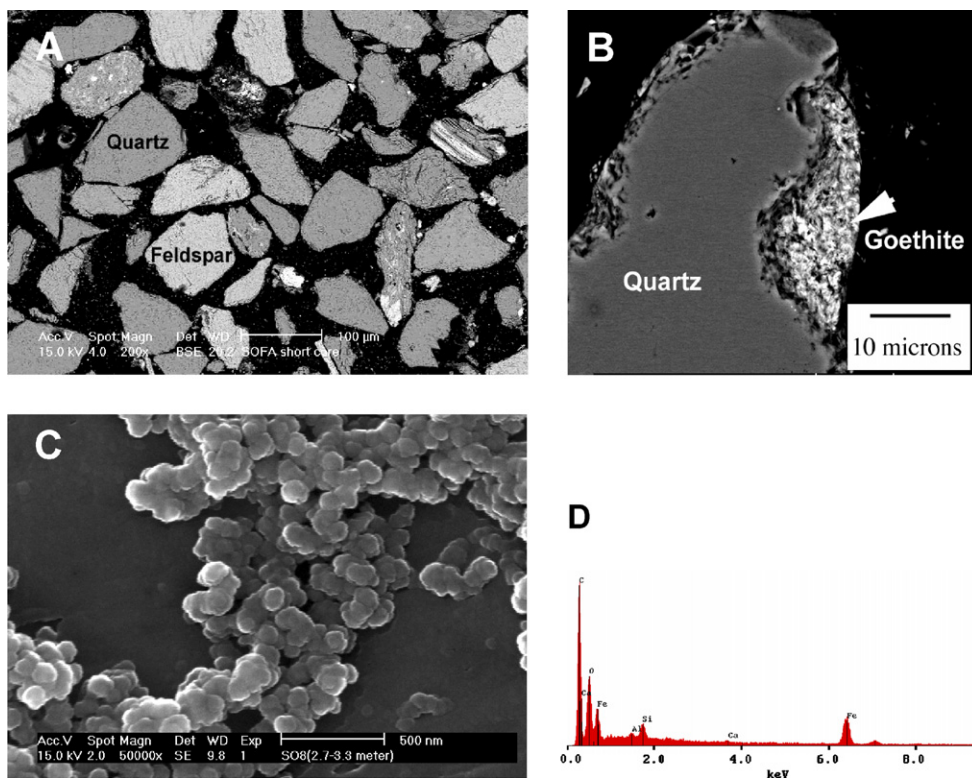


Fig. 2. SEM images and energy-dispersive spectrum of sediment collected from the site. (A) Back-scattered electron (BSE) image showing quartz and feldspar; (B) BSE image showing goethite coating quartz; (C) secondary electron image showing Fe-containing colloids collected in SOFA water. These colloids were collected by filtering the SOFA water through $0.02 \mu\text{m}$ filters; and (D) energy dispersive spectrum of the colloid grains on C showing that the colloids are composed mainly of Fe-rich oxyhydroxides, with a minor amount of Si and Al.

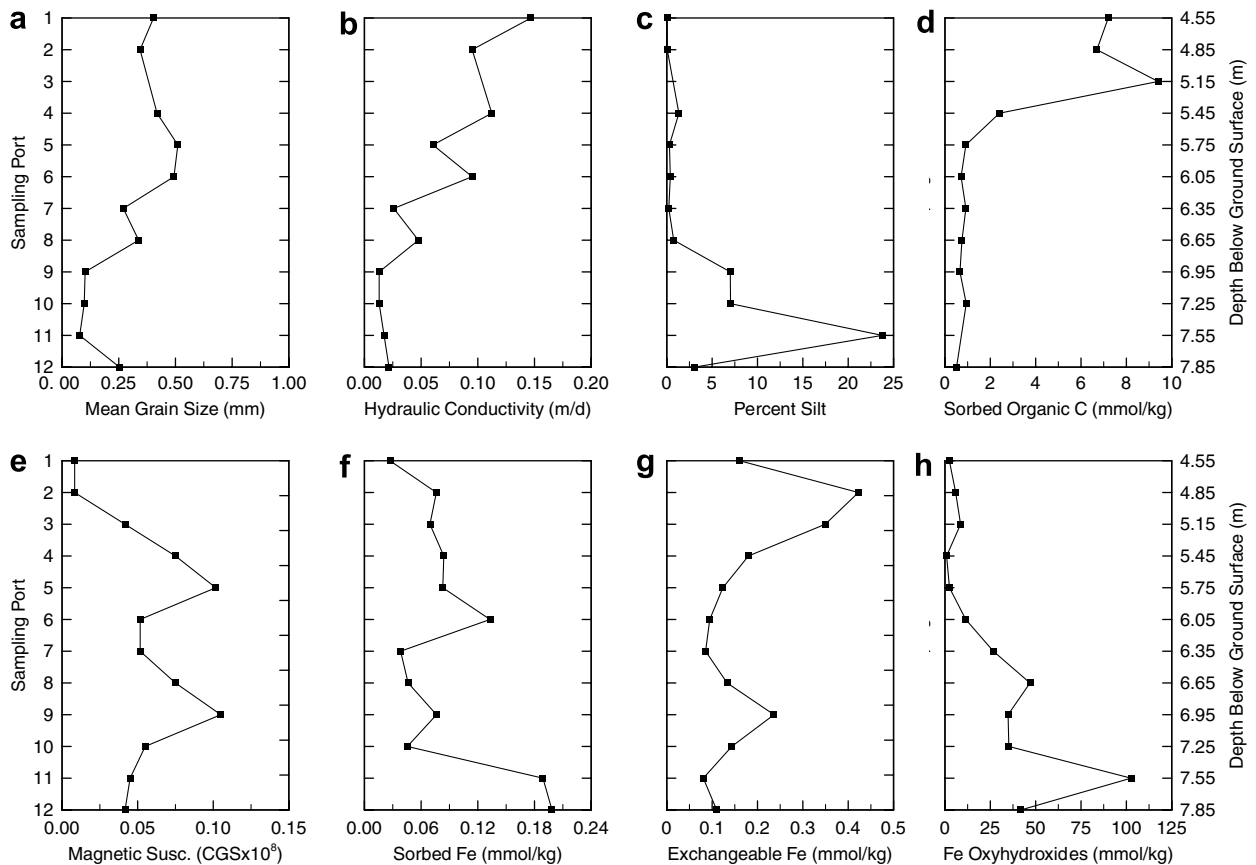


Fig. 3. Site characterization data versus depth from the core collected at well S2 (Fig. 1). Depths are presented in terms of both the corresponding port of the multilevel sampler (MLS) and the depth below ground surface. Data are shown for (a) mean grain size, (b) hydraulic conductivity, (c) % silt, (d) adsorbed organic C, (e) magnetic susceptibility, (f) adsorbed Fe, (g) exchangeable Fe, and (h) Fe oxyhydroxides.

lower ports. Contact with the underlying clay unit of the Yorktown Formation occurred below the MLSs. 2.5 cm of rain fell during the experiment and the ambient flow rate across the site during the experiment was less than 5 cm/day (Scheibe et al., 2001).

3.2. Push-pull experiment

The ground water injected into the five MLSs was compositionally identical, except for the amendments of (1) Control, (2) humics, (3) lactate, and (4) lactate and humics (Table 2; Fig. 1). In MLS-T2, two of the 12 ports were biostimulated with low-levels of *Acidovorax* sp. Strain OY107. This addition was shown to have no effect on the observed results and the results for the lactate treatment are the summation of the 24 ports from the two MLSs (Mailloux, 2003). The injected water had higher DO and NO_3^- , lower DIC, Mn_{Tot} , and Fe_{Tot} and similar levels of

SO_4^{2-} compared to that of the background ground water (Table 2), which is consistent with an upgradient source. The site's pre-injection groundwater composition also suggests that O_2 reduction, denitrification, Mn(IV) reduction, and Fe(III) reduction were probably all occurring and that the push-pull tests were therefore being conducted in a mixed redox environment. In all five treatments of the push-pull experiment, the O_2 was rapidly consumed from $85 \mu\text{M}$ to less than $30 \mu\text{M}$, near the detection limit, one day following injection, even in the control treatment (data not shown).

3.2.1. Control treatment

For the control treatment only Br^- was added to the injected water (Fig. 1) and lactate was not detected (Fig. 4d). The Br^- slowly decreased with time from 0.99 ± 0.26 to $0.17 \pm 0.12 \text{ mM}$ with an average of 21% of the injected Br^- remaining in all 12 ports after 18.9 days (Fig. 4a). The pH and

Table 2
Summary of groundwater chemistry for background and injected groundwater

Parameter	Background groundwater	Injected groundwater
pH	6.27 ± 0.07	6.15 ± 0.10
Eh (mV)	−54 ± 28	129 ± 23
Dissolved Ch (mM)	0.005 ± 0.004	0.085 ± 0.025
Dissolved inorganic carbon (mM)	0.83 ± 0.17	0.34 ± 0.04
Chloride (mM)	1.13 ± 0.36	1.40 ± 0.45
Sulfate (mM)	0.54 ± 0.10	0.44 ± 0.06
Lactate (mM)	0	Variable ^a
Br [−] (mM)	0.025 ± 0.031	1.76 ± 0.56
Nitrate (mM)	0.015 ± 0.026	0.50 ± 0.09
Nitrite (mM)	0.0005 ± 0.001	0.0004 ± 0.0007
Ammonia (mM)	0.01	0.01
Fe (mM)	0.077 ± 0.031	0.009 ± 0.009
Mn (mM)	0.0028 ± 0.0039	0.0003 ± 0.0001
Ca (mM)	0.61 ± 0.061	0.68 ± 0.08
Mg (mM)	0.36 ± 0.036	0.33 ± 0.04
Na (mM)	1.02 ± 0.28	3.78 ± 0.04
K (mM)	0.08 ± 0.01	0.12 ± 0.01
Si (mM)	0.18 ± 0.018	0.14 ± 0.03
Nonpurgeable organic carbon (TOC) (mM)	0.26 ± 0.08	Variable ^b

^a The average lactate was 0.03 ± 0.07 mM in the control treatment, 0 in the humic treatment, 1.96 ± 0.01 mM in the lactate treatment, and 1.92 ± 0.07 mM in the lactate and humics treatment.

^b The TOC was 0.12±0.02 mM C in the control treatment, 0.48 mM in the humics treatment, 4.33±0.18 mM in the lactate treatment, and 5.71 ± 1.00 mM in the lactate and humics treatment.

Eh slowly decreased with time in all ports from 6.17 ± 0.05 to 5.88 ± 0.12 and from 144 ± 32 to −64 ± 24 mV, respectively (Fig. 4b and c). The NO₃[−] slowly decreased during the experiment with an average zero order rate of 0.010 ± 0.009 (mM/day) and a first order rate of 0.046 ± 0.049 (h^{−1}). The DIC, Fe_{Tot} and Mn_{Tot} slowly increased with time in all 12 ports from 0.37 to 0.55 ± 0.25 mM for DIC, from 0.005 ± 0.008 to 0.031 ± 0.026 mM for Fe_{Tot}, and from 0.26 ± 0.03 to 0.75 ± 0.42 μM Mn_{Tot} (Fig. 4e–g). Across the depth interval sampled by the 12 ports, the peak Fe_{Tot} ranged from 0.007 to 0.105 mM, the Mn_{Tot} from 0.35 to 1.93 μM. During the first week little increase was observed in the Mn_{Tot} and Fe_{Tot} with most of the increase occurring at later times (Fig. 4f and g) with an average zero order rate of 1.6 ± 2.7 μM/day for Fe_{Tot} and 0.16 ± 0.04 μM/day for Mn_{Tot} (Table 3). The SO₄^{2−} and cell concentrations remained relatively constant (Fig. 4h and i). The δ¹³C of DIC at port 10 showed no trend with values ranging from −10.9‰ to −14.6‰ PDB (data not shown). In port

10 at a depth 7.25 m below ground surface little change in protist numbers were observed during the course of the experiment (Fig. 5).

3.2.2. Humics treatment

For the humics treatment no lactate was detected (Fig. 4d) and the Br[−] slowly decreased with time from 1.41 to 0.44 ± 0.36 mM with an average of 31% of the injected Br[−] remaining in all 12 ports after 18.1 days (Fig. 4a). The pH and Eh slowly decreased with time in all 12 ports from about 6.34 ± 0.00 to 5.80 ± 0.36, and from 91 ± 0 to 69 ± 18 mV, respectively (Fig. 4b and c). The NO₃[−] slowly decreased during the experiment with an average zero order rate of 0.034 ± 0.035 (mM/day) and a first order rate of 0.27 ± 0.22 (h^{−1}). The DIC slowly increased from 0.30 to 0.39 ± 0.13 mM (Fig. 4e). The δ¹³C of DIC at port 10 showed no trend with values ranging from −12.3‰ to −14.5‰ PDB (data not shown). The Fe_{Tot} of the injected groundwater was 0.016 mM that decreased to 0.002 ± 0.001 mM after 1 day, remained steady for the first week, and slowly increased to 0.023 ± 0.012 mM in all ports (Fig. 4f). The injected water had an Mn_{Tot} of 0.49 ± 0.51 μM that remained steady for the first week and then slowly increased to 1.86 ± 1.93 μM in all ports (Fig. 4g). Amongst the 12 ports the peak Fe_{Tot} varied from 0.016 to 0.061 mM, and the Mn_{Tot} from 0.61 to 6.06 μM (Table 3). The Fe_{Tot} increased at an average zero order rate of 2.5 ± 2.5 μM/day and the Mn_{Tot} increased at a rate of 0.12 ± 0.08 μM/day (Table 3). The SO₄^{2−} concentration remained relatively constant (Fig. 4h). The average cell concentration increased from 4.2 ± 0.4 × 10⁵ to 3.5 ± 1.0 × 10⁶ cells/mL at a zero order rate of 1.37 ± 2.1 × 10⁵ cells/mL-day with the peak cell concentration ranging from 2 × 10⁶ to 6 × 10⁶ cells/mL (Fig. 4i, Table 3). The TOC content of the injected water was elevated above the in situ concentration and decreased rapidly with time from 0.48 to 0.25 ± 0.11 mM and was relatively uniform with depth (Fig. 6a). The A₂₇₀, a measure of the aromatic content of the organic C, decreased rapidly after injection, remained steady during the main portion of the experiment, and then increased during the later sampling events (Fig. 6b).

3.2.3. Lactate treatment

The lactate treatment was conducted in two wells, MLS-S3 and MLS-T2 and the results were grouped together making it 24 ports at two MLSS

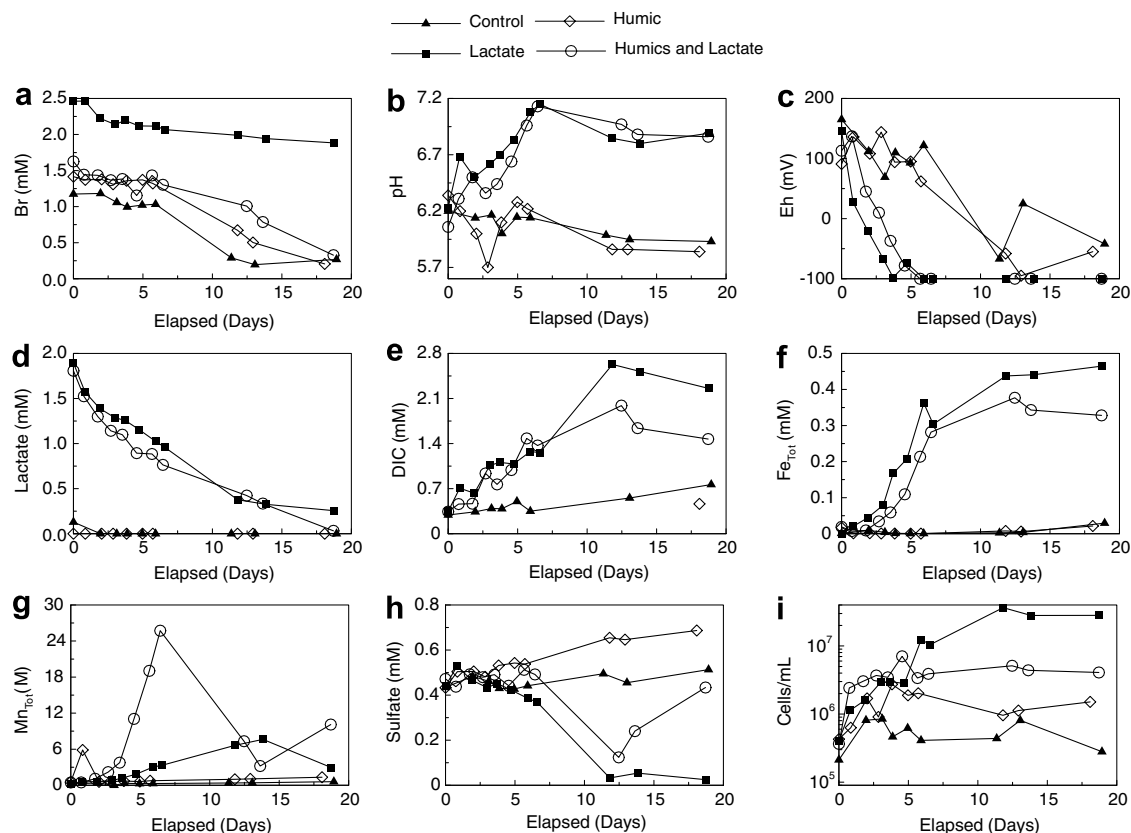


Fig. 4. Concentration versus time at the control treatment (▲), the humics treatment (◇), the lactate treatment (■), and the lactate and humics treatment (○) at port 10 at a depth of 7.25 m below ground surface. For the lactate treatment, data are shown from MSL-S3. Data are shown for (a) Br^- (mM), (b) pH, (c) Eh (mV), (d) lactate (mM), (e) DIC (mM), (f) Fe_{Tot} (mM), (g) Mn_{Tot} (μM), (h) SO_4^{2-} (mM), and (i) cells (cells/mL). The lower detection limit for Eh was -100 mV. This is the raw data and was not corrected for dilution with the background water.

that received the lactate treatment (Mailloux, 2003). The initial Br^- concentrations were slightly different between the two MLSs. The initial Br^- at MSL-S3 was 2.43 ± 0.10 and decreased to 1.37 ± 0.50 mM with an average of 70% of the injected Br^- remaining in all ports after 18.8 days. The initial Br^- at MSL-T2 was 1.07 ± 0.02 and decreased to 0.40 ± 0.26 mM with an average of 38% of the injected Br^- remaining after 16.8 days in all ports (Fig. 4a). The pH increased during the first 7 days from 6.18 ± 0.07 to 6.90 ± 0.26 and then remained steady or slowly decreased (Fig. 4b). The Eh decreased from 145 ± 12 to less than -100 mV, the detection limit (Fig. 4c). The NO_3^- rapidly decreased during the experiment with an average zero order rate of 0.24 ± 0.13 (mM/day) and a first order rate of 2.18 ± 0.86 (h^{-1}). The lactate decreased with time from 1.96 ± 0.11 to 0.12 ± 0.12 mM with only an average of 6% of the lactate remaining in all ports (Fig. 4d). The largest

acetate concentration determined from a limited number of samples was 0.3 mM with most values less than 0.04 mM. The DIC, Fe_{Tot} and Mn_{Tot} increased with time in all ports from 0.36 ± 0.04 to 2.26 ± 0.70 mM DIC, from 0.00 to 0.252 ± 0.113 mM Fe_{Tot} , and from 0.21 ± 0.01 to 17.2 ± 37.7 μM Mn_{Tot} (Fig. 4e–g). An increase in the Fe_{Tot} to 0.025 ± 0.021 mM and the Mn_{Tot} to 0.36 ± 0.21 μM occurred by the first sampling point within 1 day with NO_3^- still present (Fig. 4f and g). Across the depth interval, the peak Fe_{Tot} varied from 0.077 to 0.465 mM, the peak Mn_{Tot} varied for 0.91–130.4 μM and the peak cells varied from 0.2×10^7 to 5.9×10^7 cells/mL (Table 3; Fig. 7). The Fe_{Tot} increased at an average zero order rate of 30.8 ± 16.2 $\mu\text{M}/\text{day}$ and the Mn_{Tot} increased at a rate of 2.09 ± 4.54 $\mu\text{M}/\text{day}$ (Table 3). The amount of Fe_{Tot} in the aqueous phase indicated that $0.8 \pm 1.3\%$ of the Fe from the oxyhydroxides was reduced. The $\delta^{13}\text{C}$ of DIC at port 10 showed no

Table 3
Summary of peak concentrations, zero order, and first order rates of Fe_{Tot} , Mn_{Tot} , and cells

Treatment	Fe_{Tot} (mM)		Mn_{Tot} (nM)		Cells (cells $\times 10^7$ /mL)										
	Peak	Avg ^a	Peak	Avg	Peak	Min	Peak	Avg	Peak	Min	Peak	Zero order rate ^d (cells/ 10^7 /day)	First order rate (day ⁻¹)		
Control	0.031 \pm 0.026	0.007	0.105	1.6 \pm 2.7	0.13 \pm 0.10	0.75 \pm 0.42	0.35	1.9	0.16 \pm 0.04	0.19 \pm 0.04	0.2 \pm 0.1	0.1	0.5	-1.0 \pm 0.5	0.05 \pm 0.07
Humics	0.023 \pm 0.013	0.016	0.061	2.5 \pm 2.5	0.28 \pm 0.05	1.86 \pm 1.93	0.61	6.1	0.12 \pm 0.08	0.09 \pm 0.05	0.4 \pm 0.1	0.2	0.6	1.37 \pm 2.1	0.09 \pm 0.05
Lactate	0.25 \pm 0.11	0.077	0.465	30.8 \pm 16.2	0.25 \pm 0.13	17.24 \pm 37.67	0.91	130.4	2.09 \pm 4.54	2.24 \pm 0.09	1.4 \pm 1.3	0.5	5.9	11.3 \pm 13.4	0.19 \pm 0.11
Lactate and humics	0.33 \pm 0.09	0.191	0.440	52.0 \pm 16.5	0.32 \pm 0.05	12.28 \pm 13.31	1.36	45.00	2.44 \pm 3.72	0.15 \pm 0.05	0.8 \pm 0.2	0.5	1.0	6.33 \pm 2.45	0.17 \pm 0.04

All values are the average for the 12 or 24 ports for each amendment.

^a Average of peak observed value plus or minus one standard deviation.

^b Minimum of the peak value observed at the 12 or 24 ports of each treatment.

^c Maximum of the peak value observed at the 12 or 24 ports of each treatment.

^d Average rate was determined from the dilution adjusted concentrations. For Fe and Mn no Fe or Mn in the background water was assumed whereas for cells the initial concentrations were used. The rates for Fe and Mn were calculated from time >1.5 days to <13 days. For cells rates were calculated from 0 to <13 days.

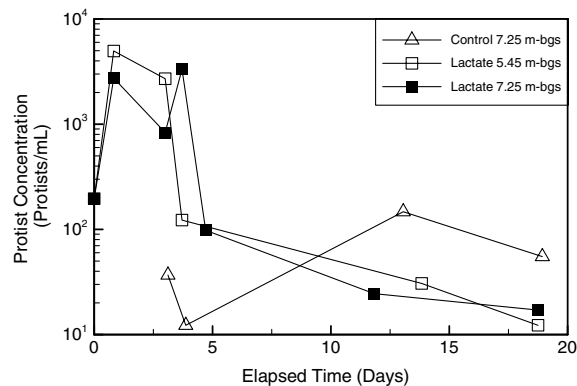


Fig. 5. Concentration versus time of protists in the control treatment and the lactate treatment. The lactate treatment results are from MLS-S3. Results are shown for port 10 located at a depth of 7.25 m below ground surface and port 4 at a depth of 5.45 m below ground surface.

trend with values ranging from -15.3‰ to -18.0‰ PDB at MLS-S3 and from 13.2‰ to -14.4‰ PDB at MLS-T2 (data not shown). The SO_4^{2-} concentration increased slightly just after injection, decreased slowly for approximately 7 days, and then in most ports it rapidly decreased from 0.49 ± 0.02 to 0.14 ± 0.13 mM (Fig. 4h). The cell concentration increased with time in all ports from $1.9 \pm 0.7 \times 10^5$ to $1.4 \pm 1.3 \times 10^7$ cells/mL (Fig. 4i). In port 4 at a depth of 5.45 m-bgs and port 10 at a depth of 7.25 m-bgs the number of protists increased by more than an order of magnitude by the first sampling event, remained constant for approximately 3 days, and then decreased to values similar to and below those of the injectate and the control treatment (Fig. 5).

3.2.4. Lactate and humics treatment

For the lactate and humics treatment the Br^- slowly decreased from 1.61 ± 0.07 to 0.38 ± 0.19 mM with an average of 24% of the injected Br^- remaining after 18.7 days in all ports (Fig. 4a). The pH increased over the first 7 days from 6.06 ± 0.02 to 6.95 ± 0.16 and then remained steady or slowly decreased (Fig. 4b). The Eh decreased with time from 113 ± 4 to less than -100 mV (Fig. 4c). The NO_3^- levels decreased with an average zero order rate of 0.18 ± 0.04 mM/day and a first order rate of 1.32 ± 0.43 h⁻¹. The lactate decreased from 1.92 ± 0.07 to 0.08 ± 0.10 with only an average of 4% of the injected lactate remaining in all ports (Fig. 4d). The DIC increased with time from 0.31 ± 0.02 to 1.89 ± 0.13 mM in all ports (Fig. 4e). The $\delta^{13}C$ of DIC at port 10 exhibited no

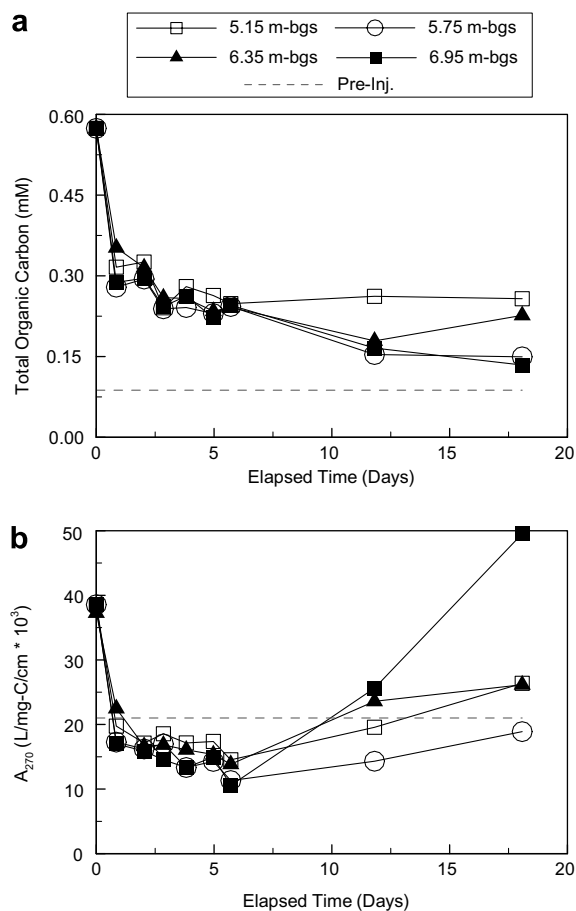


Fig. 6. (a) Concentration of total organic C versus time for select ports of the humics treatment which received humics isolated from Kitty Hawk, North Carolina. (b) A_{270} versus time for select ports of the humics treatment. A_{270} is an estimate of the aromatic content of the humic acids, based on UV absorption by π - π^* bonds at that wavelength. The dashed gray line indicates the average pre-injection values. Results are shown for port 3 at a depth of 5.15 m below ground surface (m-bgs), port 5 at 5.75 m-bgs, port 7 at 6.35 m-bgs, and port 9 at 6.95 m-bgs.

temporal trend with values ranging from -12.9% to -15.8% PDB (data not shown). The injected water had a Fe_{Tot} of 0.018 ± 0.000 mM that remained steady for the first day, then steadily increased to 0.331 ± 0.085 mM after 7 days (Fig. 4f) with a zero order rate of 52.0 ± 16.5 μ M/day (Table 3). The Mn_{Tot} increased from 0.45 ± 0.02 to 12.18 ± 13.31 μ M with a zero order rate of 2.44 ± 3.72 μ M/day (Table 3). Across the depth interval the peak Fe_{Tot} ranged from 0.19 to 0.44 mM and the Mn_{Tot} from 1.36 to 45.00 μ M. The SO_4^{2-} concentration increased slightly just after injection, slowly decreased until approximately 7 days and then rapidly decreased from an injection level of 0.46 ± 0.02

to 0.12 ± 0.05 mM in all ports (Fig. 4h). The cell concentration increased in all ports with time from $2.5 \pm 3.9 \times 10^5$ to $7.6 \pm 1.6 \times 10^6$ cells/mL (Fig. 4i) and the peak cells ranged from 0.5×10^7 to 1.0×10^7 cells/mL (Table 3). The amount of Fe_{Tot} in the aqueous phase indicated that $1.8 \pm 1.9\%$ of the Fe from the oxyhydroxides was reduced.

3.2.5. Treatment comparisons

The temporal behavior of the groundwater chemistry and cell density were distinct for the four treatments (Fig. 4; Tables 3 and 4). When only comparing port 10 at a depth of 7.25 m-bgs differences were not always observed but when comparing across all the 12 ports statistically significant variations were observed. Important differences include that the peaks of Fe_{Tot} were significantly greater at the 99% confidence level in the lactate treatment than the control treatment and that peak Fe_{Tot} concentrations in the lactate and humics treatments were greater than the lactate treatment at the 95% confidence level. Dilution adjusted zero order rates for Fe_{Tot} were greater in the lactate and humics than the lactate treatment at the 99% confidence level. Differences between treatments in the peak Mn_{Tot} and cell concentrations were observed at the 95% confidence interval but not the 99% confidence interval (Table 4C).

3.2.6. Heterogeneity

Variations in the peak concentration and the zero order rate of Fe_{Tot} , Mn_{Tot} , and cell concentrations, were observed as a function of depth for each treatment (Fig. 7; Table 5) and were compared to variations in the physical and geochemical properties of the sediments. Important correlations include that peak Fe_{Tot} concentration was positively correlated with the adsorbed Fe in the control treatment (Table 5A). In the humics treatment peak cell concentrations, however, were positively correlated with hydraulic conductivity and negatively correlated with magnetic susceptibility (Table 5B). In the lactate treatment the peak Fe_{Tot} concentrations were negatively correlated with hydraulic conductivity and adsorbed organic C and positively correlated with adsorbed Mn (Table 5C) and peak Mn_{Tot} was positively correlated with adsorbed Mn and exchangeable Mn. In the lactate and humics treatment peak Fe_{Tot} concentrations were positively correlated with adsorbed Mn and negatively correlated with adsorbed organic C (Table 5D) and peak cells were positively correlated with adsorbed organic C

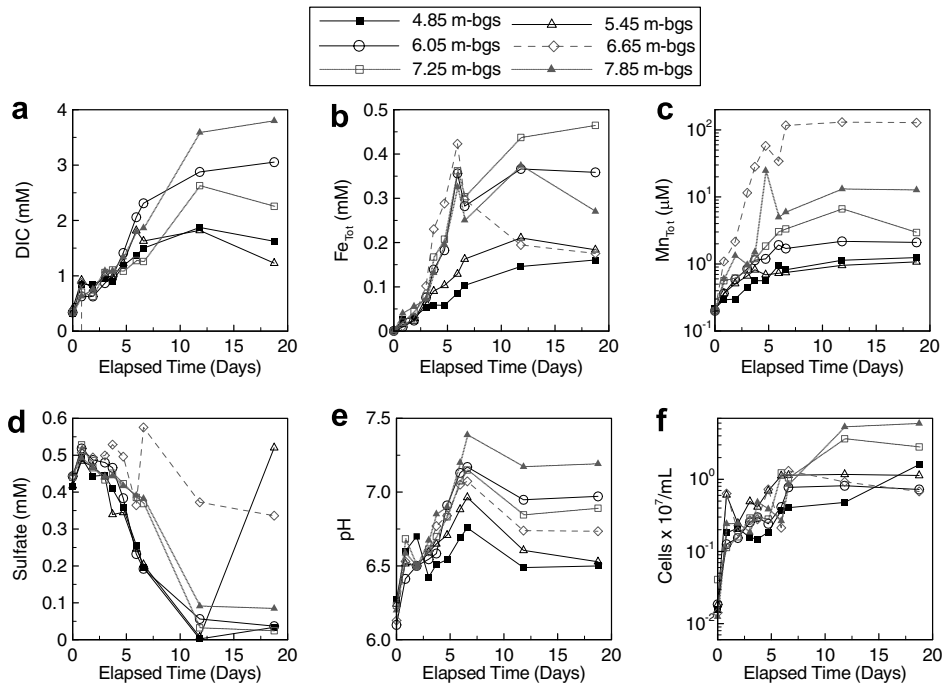


Fig. 7. Concentration versus time for even numbered ports of the lactate treatment at MLS-S3. Even numbered ports were chosen to highlight the variations with depth and the odd numbered ports had similar results. Port 2 is located at a depth of 4.85 m below ground surface (m-bgs), port 4 at 5.45 m-bgs, port 6 at 6.05 m-bgs, port 8 at 6.65 m-bgs, port 10 at 7.25 m-bgs, and port 12 at 7.85 m-bgs. The injected water was amended with lactate and Br⁻. Data are shown for (a) DIC (mM), (b) Fe_{Tot} (mM), (c) Mn_{Tot} (μM), (d) SO₄²⁻ (mM), (e) pH, and (f) cell concentration (cells × 10⁷/mL). This is the raw data and was not corrected for dilution with the background water.

(Table 5D). Correlations were also observed for zero order accumulation rates of Fe_{Tot}, Mn_{Tot}, and cells but these results from each treatment did not alter any conclusions (data not shown).

Table 4

P values determined from a Student's *t*-test with two tails and unequal variance comparing the effect of each treatment

	Humics	Lactate	Humics and lactate
<i>(A) Comparisons for peak Fe_{tot} concentrations</i>			
Control	0.383	0.000**	0.000**
Humics		0.000**	0.000**
Lactate			0.026* (0.001**)
<i>(B) Comparisons for peak Mn_{tot} concentrations</i>			
Control	0.074	0.043*	0.013* (0.06)
Humics		0.058	0.022* (0.06)
Lactate			0.561
<i>(C) Comparisons for peak cell concentrations</i>			
Control	0.009**	0.000**	0.000**
Humics		0.001**	0.000**
Lactate			0.038* (0.10)

The statistically significant variations of $p < 0.01$ are denoted by ** and the significant variations of $p < 0.05$ are denoted by *. When the dilution adjusted zero order rates produced a different result the values are shown in parentheses.

3.2.7. Mass balance

The DIC produced for the lactate and the lactate and humics treatment was balanced with the change in the redox species to determine if sorption or precipitation was occurring (Eqs. (1) and (2); Tables 1 and 6). Balancing the utilization of lactate and correcting for the presence of acetate predicted the production of 5.02 mM of DIC in the lactate treatment and 4.86 mM in the lactate and humics treatment. This is higher than the observed DIC values of 3.9 ± 1.5 mM in the lactate treatment and 3.8 ± 1.0 mM in the lactate and humics treatment after correcting for the background DIC (Table 2). This suggests that an excess DIC_{Sorb/Min} component of 1.12 mM in the lactate treatment and 1.06 mM in the lactate and humics treatment must exist assuming that methanogenesis is not occurring.

Similarly, balancing of the redox reactions based on the soluble species predicted only 2.3 mM of DIC would be produced in the lactate treatment and 2.4 mM of DIC would be produced in the lactate and humics treatment (Table 6). This indicates that the redox reactions were more extensive than predicted by balancing the aqueous redox species.

Table 5
Pearson correlation coefficient between aquifer parameters and push-pull results

	Peak Fe _{Tot}	Peak Mn _{Tot}	Peak cells
<i>(A) Control treatment</i>			
Grain size	-0.32	0.34	0.52
Hydraulic conductivity	-0.50	-0.25	0.55
Sorbed Fe	0.64*	0.25	0.08
Sorbed Mn	0.49	0.03	-0.17
Exchangeable Fe	-0.50	-0.30	0.22
Exchangeable Mn	0.20	-0.22	-0.24
Fe-oxyhydroxides	0.49	-0.10	-0.44
Mn-oxyhydroxides	0.22	-0.12	-0.22
Magnetic susceptibility	0.05	0.01	-0.35
Sorbed organic carbon	-0.49	-0.36	0.12
<i>(B) Humics treatment</i>			
Grain size	0.07	-0.41	0.22
Hydraulic conductivity	0.54	-0.49	0.61*
Sorbed Fe	-0.12	-0.33	-0.40
Sorbed Mn	-0.40	0.32	-0.16
Exchangeable Fe	0.06	-0.36	0.31
Exchangeable Mn	-0.22	0.10	-0.07
Fe-oxyhydroxides	-0.09	0.15	-0.46
Mn-oxyhydroxides	-0.21	0.03	-0.28
Magnetic susceptibility	-0.17	0.16	-0.66*
Sorbed organic carbon	0.47	-0.36	0.49
<i>(C) Lactate treatment</i>			
Grain size	-0.35	-0.27	-0.38
Hydraulic conductivity	-0.74*	-0.44	-0.31
Sorbed Fe	0.11	-0.20	0.31
Sorbed Mn	0.64*	0.66*	-0.04
Exchangeable Fe	-0.44	-0.25	-0.20
Exchangeable Mn	0.31	0.60*	-0.16
Fe-oxyhydroxides	0.37	0.30	0.28
Mn-oxyhydroxides	0.38	0.56	-0.02
Magnetic susceptibility	0.28	0.03	0.19
Sorbed organic carbon	-0.71*	-0.39	-0.27
<i>(D) Lactate and humic acid treatment</i>			
Grain size	-0.16	-0.73*	-0.30
Hydraulic conductivity	-0.31	-0.73*	0.15
Sorbed Fe	-0.32	0.13	0.02
Sorbed Mn	0.70*	0.23	-0.51
Exchangeable Fe	-0.58	-0.16	0.57
Exchangeable Mn	0.25	0.04	-0.05
Fe-oxyhydroxides	0.17	0.50	0.02
Mn-oxyhydroxides	0.39	0.25	-0.25
Magnetic susceptibility	0.02	0.46	-0.03
Sorbed organic carbon	-0.62*	-0.52	0.71*

Correlations greater than 0.6 are marked with an *. Each parameter had 12 (24 for the lactate treatment) samples except grain size and hydraulic conductivity which each had 11 samples or (22 at the lactate treatment).

In summary, if the amount of lactate utilized is examined the observed values of DIC are too low and if only the aqueous redox species is examined, the observed values of DIC are too high. Therefore, geochemical modeling was required to determine

the principal electron acceptor processes involved in mineralization of the lactate.

3.2.8. Geochemical modeling

Geochemical modeling was performed to determine the saturation indices of Fe bearing minerals over the course of the push-pull experiment. The water chemistry data from port 10 at a depth of 7.25 m below ground surface of the lactate treatment were utilized for the calculations. When O₂ values were less than 30 μM, 3.0 μM HS⁻, which is the detection limit of the Chemet kits, was included in the calculations. After approximately 3 days troilite, a Fe-sulfide, became super-saturated, after 5 days siderite, a Fe-carbonate, became super-saturated, and after 6 days greenalite and minnesotaite, Fe-silicates, became super-saturated (Fig. 8). Supersaturation of Fe-sulfides and their precipitation seems reasonable given the decrease in SO₄²⁻ corresponds to the production of several hundred μM sulfide which is not detected in the groundwater. Siderite may be a sink of DIC, however, calcite was under-saturated and was probably not a sink of DIC.

Geochemical modeling was also conducted to determine the Gibbs free energy of the major microbial reactions that might be occurring in the aquifer (Table 1; Fig. 9). The most favorable reactions were NO₃⁻ reduction, Mn(IV) reduction, and reduction of solid phase ferrihydrite. The next favorable reaction was Fe(III) reduction of goethite. As the Fe(II) and pH increased over the course of the experiment, however, SO₄²⁻ reduction becomes more favorable (Fig. 10). Fermentation and methanogenesis were estimated to be considerably less exergonic (Fig. 10).

Geochemical reaction path modeling was performed to determine the main processes occurring within the aquifer that impacted on observed results. The goal was to determine if the following processes were important: adsorption to reactive surfaces, mineral precipitation, and formation of a gas phase. The goal was to develop a simple model that captured the main processes occurring in the aquifer during the push-pull experiment. The model was constrained by visually comparing model output to the observed pH and concentrations of DIC, lactate, Fe_{Tot}, and SO₄²⁻ averaged from the lactate treatment. Modeling was performed from 0 to 12 days assuming that no mixing occurred with the background groundwater during this time and was parameterized with two distinct time periods.

Table 6

Mass balance calculations for determining the contribution of each redox species to the observed DIG

(A) The amount of O₂, nitrate and sulfate reduced, and the amount of Fe_{Tot} and Mn_{Tot} produced. These numbers were utilized to predict the DIC_{Redox} from Eq. (2). All concentrations are in mM

	Dissolved O ₂ reduced	Nitrate reduced	Fe _{Tot} produced	Mn _{Tot} produced	Sulfate reduced	Predicted DIC generated (DIC _{Redox})
Lactate	0.085	0.50	0.25	0.017	0.56	2.26
Lactate and humic acids	0.085	0.50	0.33	0.012	0.66	2.43

(B) Mass balance numbers for DIC during the push-pull experiment. Unaccounted DIC was calculated from the observed and predicted DIC values (Eq. (2)). All concentrations are in mM

	Max observed DIC (DIC _i) ^a	Initial DIC (DIC ₀)	Unaccounted DIC (DIC _{Sorb})	Lactate utilized	DIC produced by lactate (DIC _{Lact})	Unaccounted lactate DIC (DIC _{Sorb-lact})
Lactate	3.91	0.36	1.65	1.67	5.02	2.76
Lactate and humic acids	3.70	0.31	1.27	1.62	4.86	2.43

The DIG numbers were corrected for dilution.

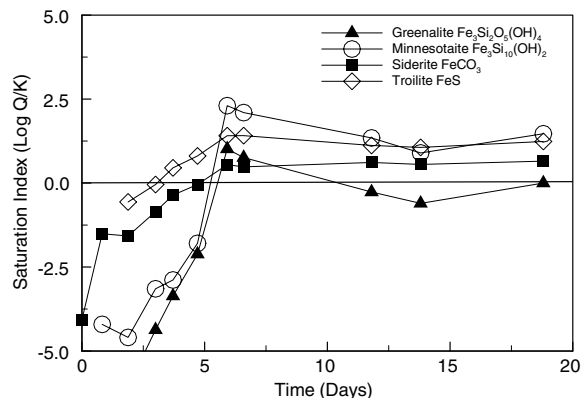
^a The maximum value was corrected for dilution by using the Br⁻ data.

Fig. 8. Mineral saturation index of selected minerals versus time. Values greater than zero indicate super-saturation and that precipitation may have occurred. All calculations were performed with GeoChemist's Workbench version 3.2 (Rockware Inc., Golden, CO). The groundwater chemistry from the lactate treatment at MLS-S3 Port 10 at a depth of 7.25 m below ground surface at each time point was utilized as input. When O₂ values were less than 30 μM, 3.0 μM HS⁻ was included for calculations. If points are not plotted they were below -5.0.

From 0 to 1.8 days O₂ reduction and denitrification were simulated with rates of 0.19 and 0.14 mM/day. From 1.8 to 12 days reduction of goethite and SO₄²⁻ were simulated (Table 2). The rate constant for Fe(III) reduction was 78 μmol/day. The SO₄²⁻ reduction rate was varied between 4 and 52 μmol/day depending on model conditions. All reactions were modeled as zero order reactions. When precipitation was modeled, the precipitation of magnetite, hematite, and C were suppressed. This is a simplistic rep-

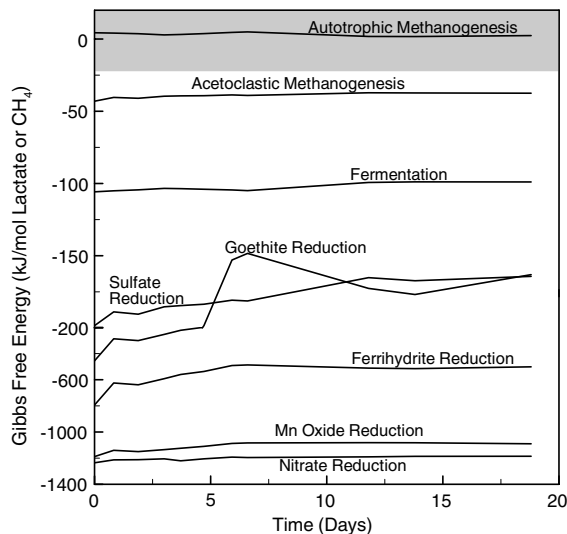


Fig. 9. Gibbs free energy of reaction for selected reactions (Table 1) over the course of the stimulation. Calculations were performed utilizing the groundwater chemistry from each sampling time point for the lactate treatment at MLS-S3 Port 10 at a depth of 7.25 m below ground surface (Fig. 2). The gray area represents the approximate minimum Gibbs free energy, -20 kJ/mol of ATP, required for the synthesis of ATP and to enable cell growth (Schink, 1997). H₂ concentrations were not measured. For fermentation H₂ was estimated to be 0.5 nM from published values (Lovley et al., 1994) and acetate was estimated to be 17 μM from pre-stimulation concentrations. Values for O₂ reduction are not shown due to its rapid removal. The scale on the y-axis is not uniform to highlight the variation.

resentation of the system but the goal was to use the minimal parameterization required to capture the main processes since the majority of reaction coefficients are poorly constrained for natural systems.

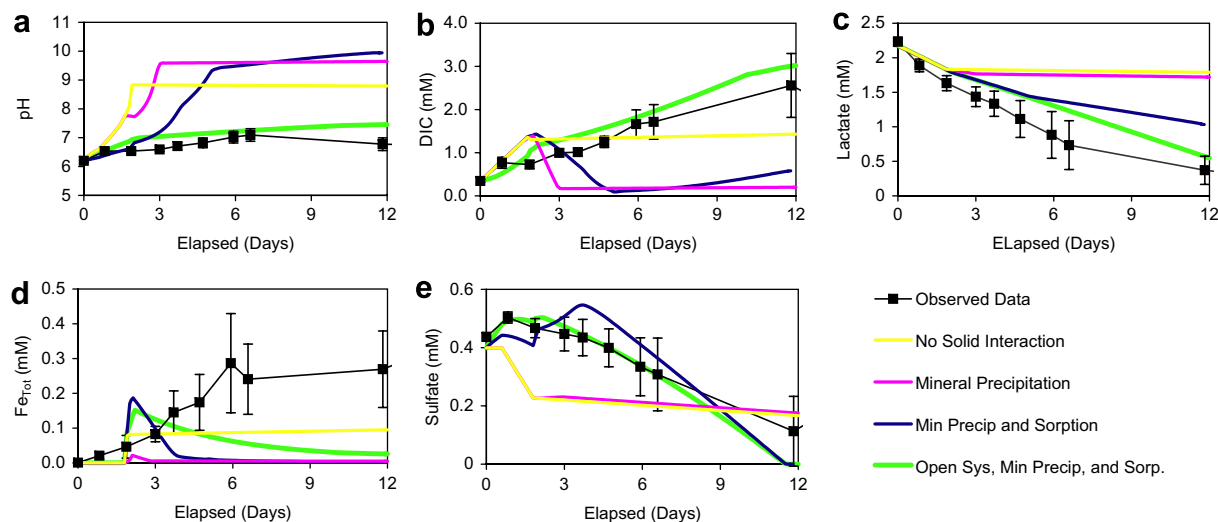


Fig. 10. Observed and modeled species for the lactate treatment at MLS-S3. Results are shown for (a) pH, (b) DIC, (c) lactate, (d) Fe_{Tot} and (e) SO_4^{2-} with four modeling scenarios; (1) only aqueous phase redox interactions, (2) reactions with mineral precipitation, (3) reactions with mineral precipitation and sorption onto reactive surfaces, and (4) reactions in an open system with mineral precipitation and sorption onto reactive surfaces. The observed results are the average and standard deviation for the 12 ports of the lactate treatment at MLS-S3.

Only considering the aqueous phase and no solid interaction poorly predicts the observed results (Fig. 10). It is predicted that 0.09 mmol of the goethite is reduced with a reduction rate of $0.1 \mu\text{mol}/\text{day}$ and the SO_4^{2-} is reduced at a rate of $4 \mu\text{mol}/\text{day}$ at 12 days. The considerable error in the observed data indicated that the solid phase would have an impact on the observed results. Adding mineral precipitation does not improve the results and worsens the observed DIC and Fe_{Tot} trends because siderite precipitation removes the aqueous phase Fe and DIC from the system. It was predicted that 2.2 mmol of the goethite was reduced with 1.3 mmol of siderite precipitating with a goethite reduction rate of $15 \mu\text{mol}/\text{day}$ and the SO_4^{2-} reduced at a rate of $22 \mu\text{mol}/\text{day}$ at 12 days. Adding adsorption to reactive surfaces improves the results; this buffers the pH producing more favorable conditions for SO_4^{2-} and Fe reduction with 4.2 mmol of the goethite reduced and 2.9 mmol of siderite precipitated with a goethite reduction rate of $37 \mu\text{mol}/\text{day}$ and SO_4^{2-} reduced at a rate of $52 \mu\text{mol}/\text{day}$ at 12 days. Treating the system as unconfined so that dissolved CO_2 exchanges with a gas phase, which may occur due to the presence of a detectable N_2 gas phase, enables a realistic representation of the system. Open system behavior is consistent with the observed values of the $\delta^{13}\text{C}$ of the DIC which are consistent with values predicted by equilibration with a CO_2 gas phase of -23.8‰ to -26.5‰ PDB

(Clark and Fritz, 1997). The trends and the magnitude of the observed data are reproduced. The fit with the Fe_{Tot} is poor but the trend of an increase followed by leveling off of the observed values is reproduced. In the open system 5.4 mmol of the goethite was reduced and the main precipitate was 4.7 mmol of siderite with a goethite reduction rate of $37 \mu\text{mol}/\text{day}$ and SO_4^{2-} reduced at a rate of $43 \mu\text{mol}/\text{day}$ at 12 days. Results from geochemical modeling indicate that SO_4^{2-} and Fe(III) reduction were co-occurring, that some DIC may be sequestered in siderite and Fe-sulfides, and that gas formation, adsorption on reactive surfaces and mineral precipitation were important processes during the experiment.

4. Discussion

Push-pull test results are usually corrected for dilution to make comparisons between tests and to calculate reaction rates (Haggerty et al., 1998; Istok et al., 1997; Snodgrass and Kitanidis, 1998). During the experiment it became evident that adsorption of Fe(II) and SO_4^{2-} along with precipitation of Fe(II) containing minerals were occurring. Under these circumstances the reactive species are not advecting at the same rate as the conservative tracer and correcting for dilution may add errors into the results as this violates the assumptions of the advection-dispersion equation (Schroth et al., 2001; Snodgrass

and Kitanidis, 1998). Fortunately, over the course of the first 12 days of the experiment, where the analyses were focused, the Br^- concentrations were relatively uniform, indicating minimal mixing within the aquifer. In addition, peak values and zero order rates for Mn, Fe, and cells gave similar results. Therefore, it is assumed that the peak values and rates estimates could be utilized to describe the changes in Fe_{Tot} with time and make comparisons to site parameters.

In the control treatment NO_3^- and pH levels slowly decreased and only small changes in the metal concentrations, cell concentration, and protist concentrations were observed. These results indicated that the rates of microbial metal reduction in the absence of an electron donor were slow.

In the humics treatment Fe and Mn concentrations were elevated during injection but then decreased to concentrations similar to the control treatment. The very rapid decrease in the TOC concentration to near background levels could be related to increased microbial utilization of the humics (Fig. 6). However, the concomitant decline in A_{270} of the recovered organics to pre-injection background levels suggests that the reduced TOC levels were largely attributable to rapid preferential adsorption of the more hydrophobic, aromatic-rich, and UV-absorbing humic components by the aquifer sediments (McCarthy et al., 1996). To test this hypothesis, humic transport experiments were conducted in the laboratory using the same source of humics and columns of repacked sediments recovered from the MLS-S10 core; the humics were strongly retarded, with preferential retention of the UV-absorbing humic components (data not shown) suggesting sorption and not microbial activity as the dominant process. The apparent effect of the humics treatment on cell concentrations may be due to desorption of cells caused by masking of positively charged sites instead of cell growth (Johnson and Logan, 1996). This is consistent with the highest concentrations of peak cells occurring in the high permeability, low magnetic susceptible zones where positively charged sites would be limited (Table 5). Humics alone did not have an effect on metal reduction, however, future experiments should utilize higher concentrations to verify these results.

In the lactate treatment, aqueous phase increases in the Fe and Mn concentrations were observed with this increase beginning within one day of the start of the experiment. The DIC increased and

the lactate decreased faster than Br^- . Since killed controls were not utilized it is not possible to definitively say the changes were microbially mediated. Bioavailable Fe(III) was present, however, within the aquifer in the form of Fe oxyhydroxides coating the quartz grains and present as colloids. From microcosm studies it is known that lactate does not abiotically reduce or chelate Fe(III) (Fredrickson et al., 1998). Therefore, in the lactate treatment, given the large increase in cell numbers, protists, and the changes in redox sensitive species it was concluded that the push-pull experiment stimulated microbial activity within the aquifer. Microbial activity occurred quickly since metal concentrations were elevated above the control treatment within one day while NO_3^- was still present. The greatest Fe(III) reducing activity occurred in the fine grained, peat poor zones with adsorbed Mn. Manganese(IV) reduction occurred in zones with adsorbed and exchangeable Mn. The peat rich zones had limited microbial activity, but, in general metal reduction occurred at all depths.

In the lactate and humics treatment, large increases in Fe and Mn concentrations were observed with the increase beginning within two days of the injection. These increases were not caused by the presence of humics alone but required an electron donor with the humics and were due to microbial activity. The peak Fe concentrations were higher than in the lactate treatment at the 95% confidence level and the rates were faster at the 99% confidence level (Table 4). This may have been caused by preferential sorption of humics causing desorption of the Fe, however, the planktonic cell concentrations did not increase and this effect was not observed in the humics treatment. The increased level of Fe(III) reduction, therefore, may have been caused by electron shuttling which has been shown to be important in laboratory environments (Lovley and Blunt-Harris, 1999; Nevin and Lovley, 2000). Further studies are needed to verify this result and the importance of electron shuttling in situ.

The increase in protist and bacterial cell concentrations in the lactate and lactate and humics treatment could be due to desorption from the sediment or growth. Extractions from core samples indicated that approximately 100 protists and 1×10^5 bacteria were associated with each gram of sediment (data not shown, K. Choi). Complete desorption of all the cells and protists from the sediment would result in approximately 500 protists/mL and 5×10^5 cells/

mL in the aqueous phase. Adding this sediment associated concentration to the initial protist and bacterial concentrations could not account for the observed concentrations in the aqueous phase (Figs. 4 and 7) and microbial growth most likely occurred. Previous studies of protist dynamics using site groundwater and sediment or conducted during bacterial transport experiments have indicated slower response times and growth rates than observed in this study (data not shown, K. Choi; Zhang et al., 2001). The only increase in protist concentrations occurred between the injection and the first sampling point of the stimulated MLSs. This indicates that the protist community preferred oxic conditions. When the system was anaerobic, the number of protists decreased but a sub-set of the protist community may be able to survive in anaerobic conditions but longer lag times may occur before growth is observed (Dawson and Pace, 2002; Kinner et al., 2002). Future experiments should be able to link the change in the microbial community to the changing redox processes and Gibbs free energy of the system.

Geochemical modeling and mass balance calculations indicated that the formation of a gas phase, adsorption to reactive surfaces, and mineral precipitation were important processes throughout the course of the push-pull experiment. The formation of a gas phase caused the system to behave as if it was open. This was observed by seeing no trend in the $\delta^{13}\text{C}$ of DIC over time and a larger than expected buffering of the pH. In addition geophysical monitoring indicated that a separate gas phase formed during the initial portions of the experiment (data not shown) (Mailloux, 2003), which is in agreement with theoretical studies indicating that during denitrification N_2 may have formed a separate gas phase (Andrews and Wilson, 1987; Heaton and Vogel, 1981; Mailloux, 2003). The formation of a gas phase may be an important but overlooked process in shallow aquifers undergoing redox reactions. Adsorption was an important process with a significant fraction of the Fe and SO_4^{2-} adsorbed to reactive sites on the mineral surfaces. Mineral precipitation was also important with Fe silicates, Fe sulfides and Fe carbonates most likely precipitating (Fig. 8). The geochemical modeling indicated that Fe(III) and SO_4^{2-} reduction were favorable and could have been co-occurring throughout the push-pull experiment and may have supported cell growth and activity (Albrechtsen et al., 1995; Liu et al., 2001a; Schink, 1997; Thauer et al., 1977).

In microcosm experiments, Fe(III) reduction usually ceases before all the solid phase Fe(III) is reduced (Zachara et al., 1998). Processes were occurring in the aquifer that should have promoted continued Fe(III) reduction. Organic C was present that may have acted as an electron shuttle especially in the lactate and humics treatment. Cell growth was occurring so new cell walls should not have been coated with Fe(II) (Roden and Urrutia, 1999) and Fe(III) reducers which are known to be present in the aquifer (Fuller et al., 2004b) could potentially dominate (Bekins et al., 1999, 2001; Snoeyenbos-West et al., 2000). In addition groundwater flow was occurring helping to flush the Fe(II) (Roden et al., 2000), however, it is not clear if abiotic transformations were occurring (Hansel et al., 2003). The conditions, therefore, existed to promote continued Fe(III) reduction even if changes in Fe(II) concentrations were not observed. However, small changes in Fe(II) concentrations may be indicative of large changes in the redox conditions of an aquifer (Cozzarelli et al., 2001). It is possible to conclude that both continued SO_4^{2-} and Fe(III) reduction were occurring and the only mechanism to observe these reactions was a decrease in lactate concentrations and an increase in DIC concentrations. Future push-pull experiments that examine Fe(III) reduction could collect and analyze sediment samples following the experiment to better constrain the adsorbed and precipitated phases. However, given aquifer heterogeneity it may be difficult to sample and constrain the sediment differences and with only a small percent of the Fe oxyhydroxides being reduced it may be difficult to quantitatively observe changes.

Examining the controls of Fe(III) reduction and alterations in mineralogy are common for both natural and synthetic sediments (Fredrickson et al., 1998; Kukkadapu et al., 2006; Roden et al., 2000) but rates are not consistently reported. Comparison of rates from the push-pull experiments with other published values indicates that some consistent trends develop (Table 7). The rates of the control and humics treatment are similar to those reported for a pristine sandy aquifer (Jakobsen and Postma, 1999). The rates from the lactate and lactate and humics treatment are similar to rates computed from aquifers contaminated from a landfill leachate (Cozzarelli et al., 2000; Ludvigsen et al., 1998). These rates are all significantly slower than soils and aquifer sediments incubated with an electron donor and a cell culture (Zachara et al., 1998).

Table 7
Summary of Fe(III) reduction rates from select laboratory and field studies

Study material	Study type	Ammendments	Rate ($\mu\text{M}/\text{day}$)	Reference
Aquifer	Field scale	Control	1.6 ± 2.7	This study
		Humics	2.5 ± 2.5	
		Lactate	30.8 ± 16.2	
		Lactate and Humics	52.0 ± 16.5	
Aquifer	Field scale	None	$1.4\text{--}3.6^a$	Jakobsen and Postma (1999)
Wetland soils	Microcosms	None	45,000–102,000	Weiss et al. (2004)
Weathered forest soil	Microcosm	None	9200	Peretyazhko and Sposito (2005)
Landfill	Microcosm	None	40–68	Ludvigsen et al. (1998)
Landfill	Microcosm	None	11–37	Cozzarelli et al. (2000)
Aquifer	Microcosm	Lactate and/or AQDS and <i>Shewanella</i>	$378\text{--}944^a$	Zachara et al. (1998)
		<i>putrefaciens</i> strain CN32 from three sites	472–3306 378–6611	

All microcosm rates were converted to $\mu\text{M}/\text{day}$ using a bulk density of 1.7 g/mL and a porosity of 0.30. Only studies using natural sediment are shown.

^a Rates were estimated by examining the change in aqueous concentrations after 30 days.

Therefore, in an aquifer system the maximum rate of Fe(III) reduction is probably significantly less than that of a shallow soil or laboratory incubations (Table 7).

In contaminant plumes it is possible to see variations in site geochemistry and microbiology that have developed over many years (Bekins et al., 1999; Bjerg et al., 1995; Cozzarelli et al., 2000; Tuccillo et al., 1999), however, push-pull experiments have only compared results across large scale variations in site geochemistry (Schroth et al., 1998) and not the smaller scale physical and chemical aquifer heterogeneities observed in coastal plain sediments. No consistent correlations were observed between push-pull results and aquifer properties. Fine-grained, Fe and Mn-rich zones may be areas of increased metal reduction, but, the correlations were weak. Instead it appeared that at all depths when an electron donor was added Fe(III) reduction coupled with cell growth occurred. This is in contrast to microcosm experiments utilizing sediments from the same aquifer which indicated that on the centimeter scale Fe(III) reduction was not ubiquitous and zones with little or no potential for Fe(III) reduction were present (Overstreet et al., 2001).

The push-pull test most likely sampled a representative elemental volume (REV) in the aquifer in which genetic variability and capabilities were captured. The idea of an REV is common in hydrology (Bear, 1972) but it is not clear at what scale the metagenome of an aquifer is captured. Even at a small scale there is a significant amount of genetic diversity within soils (Torsvik et al., 2002) but this may not capture all genetic capabilities and that

variations may be observed in microcosms. Even though aquifers are clearly heterogeneous in terms of their physical and chemical properties, microbial properties may be uniform at the scale of the push-pull experiment. It is hypothesized that the Fe(III) rates were relatively uniform because Fe(III) was present throughout the aquifer and that the push-pull tests captured the microbial REV of the aquifer. This indicates that during in situ remediation at a contaminated site it should be possible to stimulate metal reduction across a heterogeneous aquifer if the zone of interest is on the same scale as a push-pull test.

5. Conclusions

Sixty push-pull experiments were simultaneously conducted in five wells equipped with MLSs. Conducting push-pull experiments in MLSs enabled multiple hypotheses to be simultaneously tested. In the control and humics treatment rates of metal reduction were slow and similar to rates observed in another pristine aquifer. In the lactate and lactate and humics treatment O_2 and NO_3^- reduction occurred rapidly and metal reduction began within one day, even when NO_3^- was still present. Microbes capable of metal reduction and reducible metals were present at all depths studied in this aquifer. The rates of Fe(III) reduction were faster in the lactate and humics treatment than the lactate treatment indicating that the humics can act as an electron shuttle. In the lactate treatment model results indicated that the factors controlling Fe(III) reduction are the formation of a separate gas phase,

sorption of reactive species to mineral surfaces, and mineral precipitation. Physical and chemical descriptions of aquifer heterogeneity did not correlate with the observed rates of Fe(III) reduction. The observed rates in the lactate and lactate and humics treatment were similar to rates observed at landfill sites and showed only minor variations with depth. During the push-pull tests Fe(III) was not limiting and a representative elemental volume (REV) of the aquifer was sampled and encompassed enough microbial diversity that Fe(III) reduction rates were relatively uniform. This indicates Fe(III) reduction is a ubiquitous process and that stimulating metal reduction across a heterogeneous site is a feasible remedial alternative.

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