In Situ Bioremediation of Carbon Tetrachloride: Field Test Results

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Abstract: Results of a 7-month field test of in situ bioremediation of carbon tetrachloride (CT) under denitrifying conditions are reported. The demonstration was conducted in a portion of a several-square-mile CT and nitrate plume. Pretest CT and nitrate levels were 12.5 ± 0.14 μM and 3.87 ± 0.26 mM, respectively. During the test, the CT concentration dropped by 3.71 ± μM, representing an estimated total of 1.42 kg of CT destroyed. The total quantities of acetate and nitrate injected during the demonstration were 221 and 300 kg, respectively. Nitrate injection was composed of short-duration, high-concentration pulses added with acetate pulses, and continuously injected nitrate that was present in the surrounding groundwater. Biomass was distributed successfully within the flow field without fouling the injection well. Levels of planktonic denitrifiers increased 10- and 5-fold in monitoring wells 3 and 6 m downstream from the injection well, respectively. A distributed growth pattern was indicated through reductions in the concentrations of acetate, nitrate, and nitrite between these wells. Chloroform (CF) production was controlled by adjusting acetate and nitrate pulsing to keep low levels of nitrate in most of the flow field. Under this regime only 1 mol% of transformed CT appeared as CF. In contrast, approximately 33 mol% of CT transformed to appear as CF when nutrient-feeding conditions were adjusted so that nitrate was consistently absent.

Keywords: in situ bioremediation, carbon tetrachloride, field scale, dechlorination, denitrification.

Introduction

Past operations of the U.S. Department of Energy (DOE) at the Hanford Site in southeastern Washington State have resulted in carbon tetrachloride (CT) contamination in both the aquifer and vadose zone. Bioremediation is one of the technologies currently being developed by the DOE to meet the need for cost-effective methods to remove this contaminant. Transformation of CT has been demonstrated previously with a number of different bacteria. The conditions that favor biodegradation of CT are predominantly anaerobic. For example, methanogenic (Bouwer and McCarty, 1983a; Bouwer and Wright, 1988; Liang and Grbic-Galic, 1993), fermentative (Egli et al., 1988; Galli and McCarty, 1989), sulfate-reducing (Cobb and Bouwer, 1991), and nitrate-reducing (Bae et al., 1990, Bae and Rittmann, 1990; Bouwer and McCarty, 1983b; Criddle et al., 1990; Lewis and Crawford, 1993; Petersen et al., 1994; Semprini et al., 1992; Tatara et al., 1993) organisms have shown CT transformation capability. This information, coupled with results for CT destruction by subsurface denitrifying microbes obtained from the Hanford Site, led to the hypothesis that native denitrifiers may be stimulated to biodegrade CT in situ.

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1058-8337/98/$.50

In a previous field demonstration effort at Moffett Field Naval Air Station, Semprini et al. (1992) showed that denitrifying bacteria could be stimulated to degrade CT. In this test, continuous nitrate and pulsed acetate addition were used to enhance microbial activity in a 1.2-m-thick, confined aquifer. Groundwater was extracted continuously 6 m downgradient from the nutrient injection well. CT and nutrients were added to a portion of the extracted water that was recirculated into the formation. This resulted in nominal CT levels of 0.49 μM. Results showed that, although there was an 80% reduction in CT concentration across the flow field, between 30 and 60% of the lost CT was converted to chloroform (CF). Others have reported that CF is recalcitrant under denitrifying conditions (Bouwer and McCarty, 1983b; Sherwood et al., 1996).

Several researchers have proposed that CT transformation can proceed by at least two routes, one producing CF and the other producing carbon dioxide (Criddle et al., 1990; Egli et al., 1988; Hansen et al., 1994). Radiolabeled CT experiments using a Hanford denitrifying consortium (HDC) also revealed the formation of carbon dioxide and CF (Hansen et al., 1994). Sherwood et al. (1996) found that CF production by the HDC could be controlled by manipulating the ratio of added electron donor to added electron acceptor. Under acetate (electron donor)-limited conditions, the HDC converted only 4% of degraded CT to CF, whereas nitrate-limited conditions resulted in approximately 50% conversion to CF.

These results suggest that it is feasible to develop in situ nutrient addition strategies to minimize CF production by operating under acetate-limited conditions. However, the level of excess nitrate needs to be controlled carefully because nitrate inhibits CT destruction (Hooker et al., 1994; Petersen et al., 1994; Skeen et al., 1995). In addition, nutrient addition strategies should be formulated to prevent preferential growth of microorganisms at the injection well, leading to both poor contaminant destruction characteristics and wellbore biofouling. Previous results have shown rapid aquifer plugging using denitrifiers under continuous electron donor and acceptor feeding conditions (Jennings et al., 1995). Conversely, a variety of nutrient addition strategies involving discrete pulses of substrate have been reported to maintain recirculation conditions without significant biofouling (Peyton, 1996; Shouche et al., 1993).

This paper reports the results of a field test of in situ bioremediation of CT at the Hanford Site through stimulation of indigenous denitrifiers. Objectives of this demonstration were to maintain CT destruction conditions while avoiding both CF accumulation and injection wellbore biofouling.

Methods

Experiment Design

Field Operations. A schematic cross-section of the well network used in the demonstration is shown in Figure 1. Groundwater was extracted from well E1 and reinjected 12 m upgradient into well I1. Monitoring wells M1 and M2 were located on the centerline between the recirculation wells at distances of 3 m and 6 m from the injection well, respectively. The process control trailer shown in Figure 1 housed a computer system that controlled nutrient injection, sampling operations, and data collection. Concentrated nutrient stock solutions were stored in polypropylene tanks located in a separate section of the trailer. Sodium acetate and sodium nitrate stock solutions were maintained at nominal anion concentrations of 3.14 and 3.95 M, respectively. To achieve appropriate nutrient injection concentrations, stock solutions were mixed with recirculation flow in well I1.

The field test was conducted in four distinct phases. In the first, or control phase, groundwater was recirculated for 3 months without nutrient addition to determine intrinsic changes in CT and CF concentrations. In addition, the stability of other groundwater properties, including the concentrations of nitrate, nitrite, oxygen, sulfate, and planktonic bacteria, was determined. Groundwater samples were collected from all wells and used for weekly anion analysis and biweekly volatile organic compound (VOC) analysis. Planktonic biomass was measured in all wells at both the start and end of this phase of operation. The recirculation flowrate during this period was 109 m^3/day.

The second (startup), third (continuous operation), and fourth phases (chloroform test) comprised the biostimulation operations. Both the startup and continuous operation phases were conducted under electron donor-limited conditions, whereas the chloroform test phase was operated under electron acceptor-limited conditions. Details of nutrient addition at the injection well are depicted in Figure 2. Data in this figure are calculated from the total mass of nutrient added per pulse and the average recirculation flowrate during injection. These data account for nutrient addition only and do not reflect levels of previous nitrate contaminant. Nutrients were introduced in temporally separated pulses. Except where noted, pulses were 1 hour in duration, separated by 2 hours, and injected on a 24-hour frequency. The level of hydraulic control maintained during recirculation in all three phases was between 60 and 70%. This parameter was estimated by comparing nitrate concentrations at wells M2 and E1 and from the bromide tracer data.

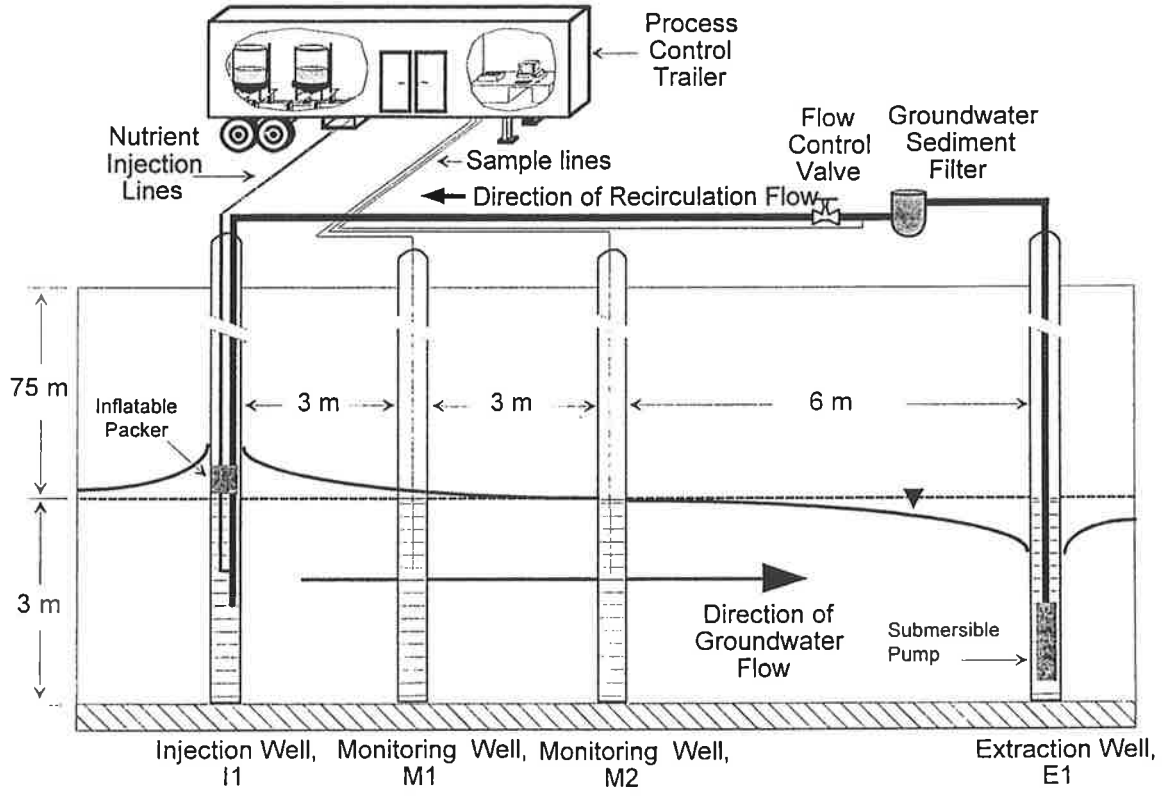

Figure 1. Layout of field test site including a cross-section of the well network used for recirculation and monitoring (not to scale).

During startup, acetate and nitrate were added to the flow field over a period of 27 days to develop a stable population of denitrifiers and to reduce test region nitrate and nitrite concentrations to levels amenable to CT biodegradation. As shown on Figure 2, 1-hour pulses of acetate only were added for the first 6 days at a frequency of one per day. Once nitrate was maintained consistently below 1.6 mM and nitrite below 0.54 mM (data not shown), operating conditions shifted to maintain a stoichiometric ratio of 0.75 mol-acetate/mol-nitrate in the field, based on previous work reported by Hooker et al. (1994). Groundwater samples were collected at least twice a week from all wells and used for anion analyses. The nominal flowrate used during this phase was 65 m^3/day.

During continuous operation, pulsed nutrient addition with continuous groundwater recirculation was maintained in the flow field for 78 days. The groundwater recirculation rate was nominally 65 m^3/day. Acetate and nitrate were added in separate pulses every 24 hours, skewed by 2 hours. Concentrations of acetate and nitrate in the pulses were generally 17 mM and 5.6 mM, respectively. Groundwater samples were collected from all wells and used for weekly anion analysis and biweekly VOC and planktonic biomass analyses.

The final operating phase focused on confirming control of CF production through appropriate nutrient addition. In this phase, nitrate-limiting conditions were established in the flow field by injecting a single 5-hour, 14-mM acetate pulse and discontinuing groundwater recirculation. Aqueous samples were collected from all wells and used for daily anion and VOC analyses.

Tracer Tests. Nonreactive tracer release tests were completed periodically to estimate changes in flow properties. Separate tests were conducted during the control phase, as well as at the beginning and end of the continuous operation phase. In each test, a 500-mg/L, 2-hour bromide pulse was introduced at the injection well. Groundwater samples were collected at monitoring and extraction wells as the pulse migrated through the formation. The MODFLOW and MT3D codes were used to simulate tracer release and estimate field permeability (McDonald and Harbaugh, 1988; Zheng, 1988).

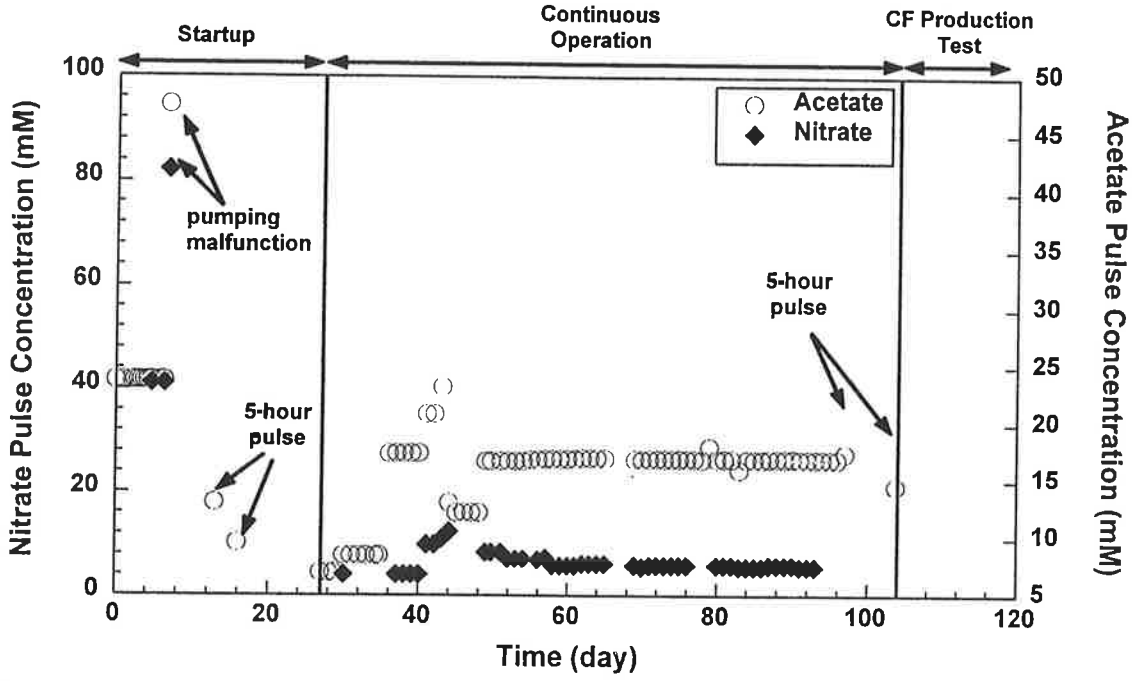

Figure 2. Concentrations of pulse nutrient additions at well I1 during the final three phases of the field demonstration. These data account for nutrient addition only and do not reflect levels of previous nitrate contaminant. Nutrients were introduced in temporally separated pulses. Except where noted, pulses were 1 hour in duration, separated by 2 hours, and injected on a 24-hour frequency.

Sediment and Water Sampling

Groundwater samples were collected from each well using Redi-Flo™ (Grundfos, Clovis, CA) pumps and Teflon™-lined polypropylene sampling lines. The lines were flushed with 3 volumes of pore water prior to sampling. Between sampling events, the lines were purged with air to avoid biomass growth in the system. For anion measurements, 10 mL of groundwater was collected, sterilized, and frozen for later analysis. Additional 10-mL samples for VOC analysis were collected in a 25-mL Gastight® syringe (Hamilton, Reno, NV) containing 10 mL of hexane. The hexane and water were mixed in the syringe, dispensed to a 25-mL glass screw-top centrifuge tube, and stored at –4°C until analysis. All VOC and anion samples were analyzed within 24 hours of collection. The 5-mL groundwater samples for planktonic bacteria enumeration were withdrawn using a 10-mL syringe, transferred to a nitrogen-purged, sterile Balsch™ tube, and placed in ice prior to analysis. Sediment cores were collected aseptically during drilling using a percussion-driven split-spoon sampler with an autoclaved Lexan™ liner.

Analytical Methods

Site Physical and Hydraulic Parameters. The total porosity of the aquifer sediment samples was calculated from soil bulk and particle density measurements. Porosity was determined for at least two core samples collected from the upper aquifer regions of each well. Vertical hydraulic conductivity was measured in these cores using a falling-head permeameter (Klute and Dirksen, 1986). Transmissivity of the upper permeable zone was determined directly from flow and drawdown data taken during constant-rate pumping tests as reported by Newcomer et al. (1996). Horizontal hydraulic conductivity was estimated directly from these values.

Sediment and Groundwater Chemistry. Acetate, nitrate, nitrite, sulfate, chloride, and bromide concentrations were determined with a Dionex 500 ion chromatograph (Dionex, Sunnyvale, CA), using published methods (Petersen et al., 1994). CT, CF, and dichloromethane concentrations were measured on a HP5890 Series II gas chromatograph (Hewlett Packard, Palo Alto, CA). Methods for VOC analysis are reported in Petersen et al. (1994).

Sediment carbon and phosphorous analyses were conducted by Huffman Laboratories, Inc. (Golden, CO). Phosphorous levels were determined using an inductively coupled plasma atomic emission spectrophotometer (ThermoJarrellAsh, Menlo Park, CA). The total carbon concentration was determined using a

Leco CR 12 carbon analyzer (Leco, St. Joseph, MI). Inorganic carbon levels were determined using a UIC/ Coulometrics System 140 carbonate carbon analyzer (UIC, Joliet, IL). Organic carbon was calculated as the difference between total and inorganic carbon.

Groundwater pH was monitored both during site characterization and throughout operations using a HydroLab™ in situ multiprobe (Austin, TX). Probe calibration was completed intermittently throughout all phases of operation.

Sediment and Groundwater Microbiology. Aerobic heterotrophic bacteria were enumerated in triplicate serial dilutions of sediment and groundwater spread on optimal plate count agar plates (Stevens et al., 1992). Most probable number (MPN) enumeration was completed for denitrifying, sulfate-reducing, and iron-reducing organisms present in sediment and groundwater using modifications of methods reported by Tiedje (1982), Stevens et al. (1992), and Postgate (1984), respectively.

Site Characteristics

The DOE's Hanford Site in the 200 West area plateau was selected for this demonstration. Between 1955 and 1973, a minimum of 637 tons of CT was discharged to the subsurface. The location for this demonstration was approximately 1,700 m downgradient from the point of CT disposal. Groundwater samples indicated that the CT concentration of 13 μM was uniform over at least 50,000 m^2 in this region. In addition, the site has a low regional gradient of 0.001. Both features were advantageous because they aided accurate estimation of CT destruction rates during the test.

Consistent with the rest of the 200 West area plateau, the aquifer at the test site is unconfined and extends from approximately 75 m to 185 m below ground surface. This portion of the formation is approximately 5% clay, 20% silt, and 75% sand and gravel. The distribution of permeability is highly stratified with depth due to cementation by carbonate deposits. Permeability in transmissive layers is approximately 1×10^{-3} cm/s, whereas permeability in cemented layers typically is between 1×10^{-5} and 1×10^{-6} cm/s. The groundwater flowrate at the site is low, less than 1 m/year.

The field test reported here was conducted in the upper 3 m of the aquifer. This region has a fairly uniform permeability between 7 and 22 m/d and a vertical anisotropy ratio of approximately 10, and is underlain by a 10-m-thick aquitard with a vertical permeability of 0.005 m/d. The average porosity in the upper permeable zone is 0.27 ± 0.05. An extensive discussion of the lithology and hydrology of this aquifer region is given by Newcomer et al. (1996).

Sediment and groundwater chemical analyses conducted during pretest site characterization are reported on Table 1. The presence of low levels of CF in both

Table 1. Pretest characteristics at the demonstration site.

Parameter	Value
pH	7.4 ± 0.2[a]
Carbon tetrachloride	12.5 ± 0.9 μM[a]
Chloroform	0.10 ± 0.02 μM[a]
Oxygen	5.6 ± 1.4 mg/L[a]
Nitrate	3.87 ± 0.29 mM[a]
Nitrite	Less than 0.02 mM
Sulfate	0.56 ± 0.16 mM[a]
Carbon tetrachloride	Between 0.0 and 0.286 mg/kg-soil
Chloroform	Between 0.0 and 0.103 mg/kg-soil
Organic carbon	253 ± 14 mg/kg-soil
Aerobic heterotrophs[b]	700 ± 500 CFU/mL
Denitrifiers[b]	800 ± 500 CFU/mL
Sulfate reducers[b]	None detected 100 CFU/mL
Iron reducers[b]	None detected 100 CFU/mL
Aerobic heterotrophs[c]	$1.2 \times 10^6 \pm 2.8 \times 10^5$ CFU/g-soil[a]
Denitrifiers[c]	Greater than 2.4×10^5 CFU/g-soil
Sulfate reducers[c]	None detected (detection limit of 100 CFU/g)
Iron reducers[c]	None detected (detection limit of 100 CFU/g)

[a] ± Indicates one standard deviation above and below the mean.
[b] Measured from groundwater samples.
[c] Measured from sediment materials.

groundwater and sediments suggest past intrinsic dechlorination activity, because there is no record of CF disposal. Soil sorption of both CT and CF appears to be insignificant as evidenced by low soil concentrations as well as the negligible amount of organic carbon in the sediments.

Pretest chemical analyses indicate an aerobic aquifer with neutral pH and high nitrate and sulfate levels. Bacterial enumeration data, as shown in Table 1, indicate the presence of both aerobic and denitrifying microbial activity, which is consistent with the predominant electron acceptors present in the groundwater. No strictly anaerobic activity was found in the groundwater or sediment samples.

Results

Continuous Operations

Contaminant Destruction. Figure 3 shows aqueous concentrations of CT and CF at the extraction well for the startup and continuous operation phases. Each value plotted in this figure represents the average and standard deviation of concentrations measured in a minimum of 17 separate samples taken over 4 hours. CT and CF levels measured at the extraction well E1 are consistent with concentrations observed at monitoring well M1 (data not shown). The overall CT concentration was reduced from 12.5 μM to 8.8 μM (95% confidence intervals ±0.14 μM and ±0.26 μM, respectively) after 95 days of nutrient injection. During this time, CF levels rose from 0.10 μM to 0.14 μM (95% confidence intervals ±0.004 μM and ±0.007 μM, respectively), representing approximately 1 mol% of the transformed CT. Prior to startup, CT and CF remained constant at prebiostimulation levels. Baseline concentrations of contaminant were established by collecting and analyzing 149 CT and 110 CF samples during the recirculation control phase (data not shown).

Numerically integrating measured CT levels observed during startup and continuous operations, accounting for the influent of CT from far-field regions, yields an estimate of 1.42 kg of CT destroyed over the duration of the test. The total quantities of acetate and nitrate injected during the demonstration were 221 and 300 kg, respectively. The injected nitrate was composed of short-duration, high-concentration pulses added with the acetate pulses and continuously injected nitrate that was present in the surrounding groundwater. The total inventory of injected nutrients would produce approximately 37 kg-dry weight (DW) of biomass, based on a denitrification yield of 0.17

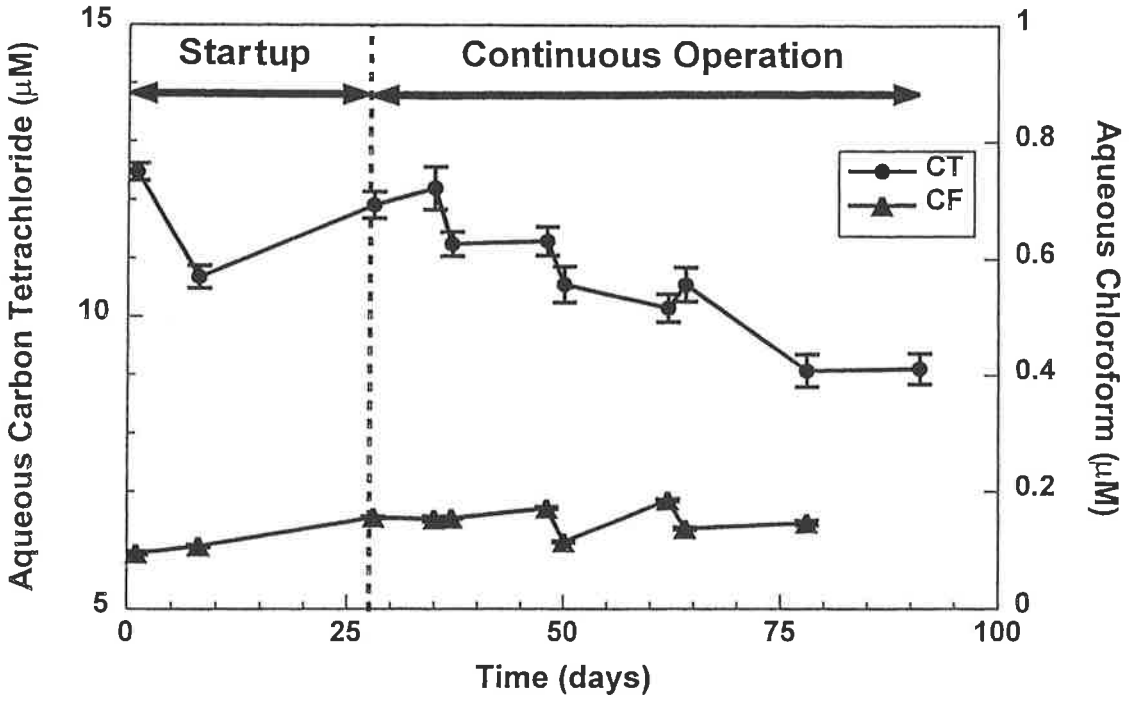

Figure 3. CT and CF concentrations measured at well E1 during the startup and continuous operation phases of the field demonstration. Error bars represent 95% confidence intervals.

g-DW/g-acetate, estimated from the kinetics reported by Hooker et al. (1994).

Denitrification and Nutrient Uptake. During startup operations, the objective of the nutrient feeding strategy was to reduce nitrate concentrations to levels amenable to CT destruction. Based on the kinetics of Skeen et al. (1995), this required reductions in nitrate concentration from the background level of approximately 3.9 mM to below 0.8 mM. Results of denitrification during startup are shown on Figure 4. Values represented in this figure were measured at well M1. Vertical arrows correspond to the times when acetate pulses were introduced to the flow field. Horizontal arrows show intervals when recirculation was discontinued.

For the first 7 days, nitrate levels were reduced from approximately 3.5 mM to 0.8 mM. Nitrite increased during this period to concentrations over 2.4 mM. Comparable nitrite levels had been demonstrated to inhibit metabolic activity in laboratory studies (Hooker et al., 1994). Hence, the nitrite concentration was lowered by first recirculating groundwater between days 7 and 13 with no acetate addition, thereby diluting the flow field with nitrite-free water.

Once the nitrite level was sufficiently low to allow for biostimulation, 5-hour-duration acetate pulses were introduced on days 13 and 16 to stimulate denitrification. After each pulse was added, pumping was stopped to stimulate growth of a nitrite-consuming population. This method of removing inhibitory levels of nitrite was effective, as demonstrated by the reduction in nitrite concentration from 2.4 mM on day 6 to below 0.7 mM after day 18. Nitrite buildup was not encountered for the remainder of the test (data not shown).

As shown in Figure 2, steady acetate and nitrate pulsing began on day 27 and continued until day 95. The typical anion response to 1-hour additions of nitrate and acetate is shown Figure 5. The time axis in this figure is referenced to the addition of the acetate pulse on day 42. Background levels of nitrate are a result of continuous reinjection of far-field groundwater. The peak in nitrate at 5 hours in well M1 and at 14 hours in well M2 is the result of a 1-hour nitrate pulse added 2 hours prior to the acetate pulse to maintain stoichiometry between the electron donor and acceptor. Appearance of the acetate pulse at well M1 between 6 and 12 hours is accompanied by a reduction in nitrate and a corresponding increase in the concentration of nitrite from 0.3 mM to above 0.8 mM. A similar anion response is seen in well M2 between 17 and 24 hours. However, the modest increases in nitrite coinciding with the appearance of the acetate pulse at this

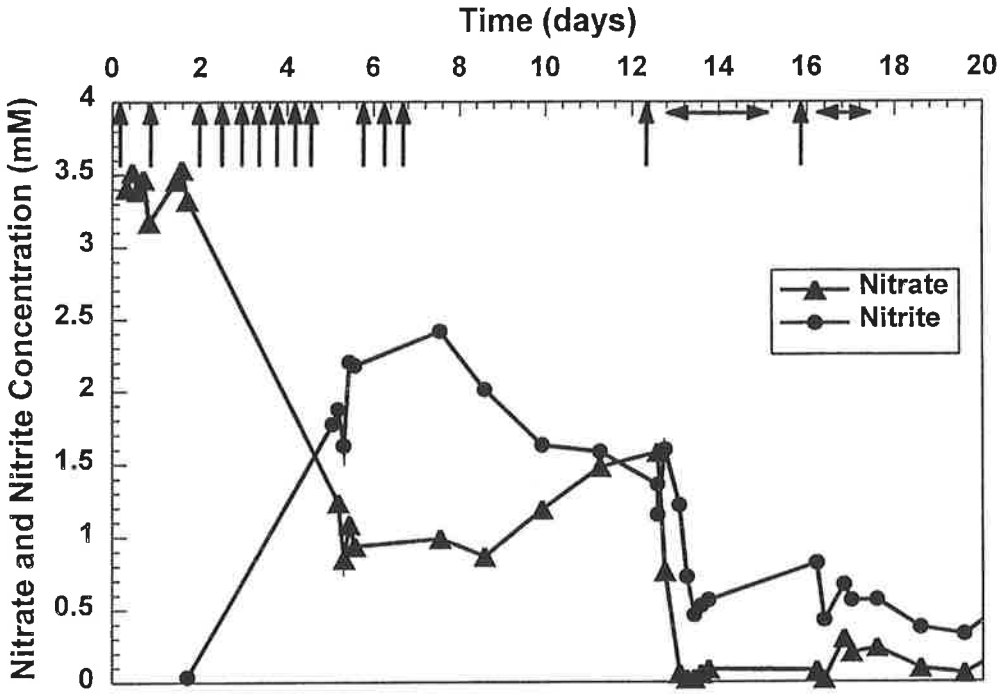

Figure 4. Nitrate and nitrite concentrations measured at well M1 during the startup phase of the field test. Vertical arrows indicate the time when an acetate pulse was added to the flow field, and horizontal arrows show intervals where recirculation was discontinued.

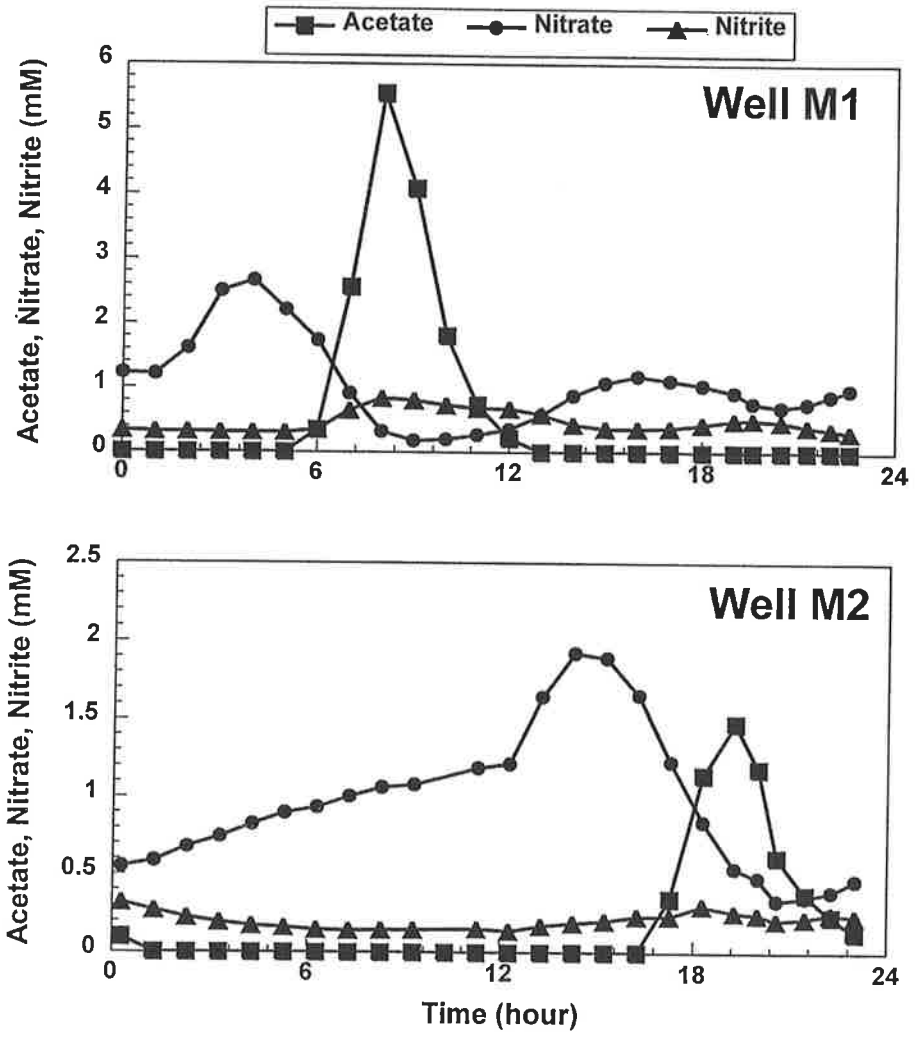

Figure 5. Anion response at wells M1 and M2 after a pulse addition of acetate and nitrate on day 42. The 1-hour acetate and nitrate pulses were added at the injection well at time 0 and time –2, respectively.

well suggest that denitritification and physical dispersion have attenuated the larger nitrite peak seen in M1.

Biostimulation and Biomass Accumulation. To demonstrate that the destruction and denitrification of CT were biologically mediated, counts of planktonic denitrifiers were measured at both monitoring wells. The average pretest level of planktonic denitrifiers was 800 ± 500 colony-forming units (CFU)/mL (average (standard deviation). After 91 days of nutrient injection, denitrifier counts had risen to 7800 ± 4500 CFU/mL at M1 and 4000 ± 920 CFU/mL at M2. In contrast, the numbers of other anaerobic organisms remained unchanged.

The increase in biomass is further demonstrated by comparing results for tracer release experiments conducted prior to nutrient addition and on days 28 and 85. For each tracer experiment, a 500-mg/L bromide solution was introduced at the injection well for 2 hours. The recirculation flowrates for the first, second, and third tracer tests were 109, 65, and 44 m^3/day, respectively. Results of these tests were simulated using MODFLOW and MT3D groundwater flow-and-transport codes. Simulation of the first test was completed using hydraulic properties obtained during characterization efforts. Values of permeability and porosity were calibrated to measured tracer data. This resulted in the use of a uniform permeability of 9 m/day and an effective porosity of 0.12, lowered from estimates obtained from core samples to reflect deviations from true porous media flow. A similar approximation was made by Yeh et al. (1993). Goodness of fit

was determined visually with emphasis on matching breakthrough times rather than pulse amplitudes, because simulated pulse amplitudes may be subject to numerical dispersion and do not reflect any field heterogeneities.

Figures 6a, b, and c show measured and simulated bromide concentrations at wells M1 and M2 for the tracer tests conducted prior to nutrient injection and 28 and 85 days after nutrient injection started, respectively. Breakthrough times for the peak concentrations are correctly reflected for the first two tests, but not for the third test. For this experiment, the simulated bromide breakthrough times at wells M1 and M2 occur at 0.2 and 0.6 day, respectively. In contrast, measured results show well M1 breakthrough at approximately 1.0 day and no apparent breakthrough at well M2. The shift between simulated and actual tracer responses suggests that biomass accumulation had produced a heterogeneous low-permeability zone between injection and extraction wells. Because the monitoring wells were located directly between I1 and E1, they were in the region of high microbial activity. Hence, a reduction in permeability would result in the bulk of the injected fluid no longer passing directly by the monitor-

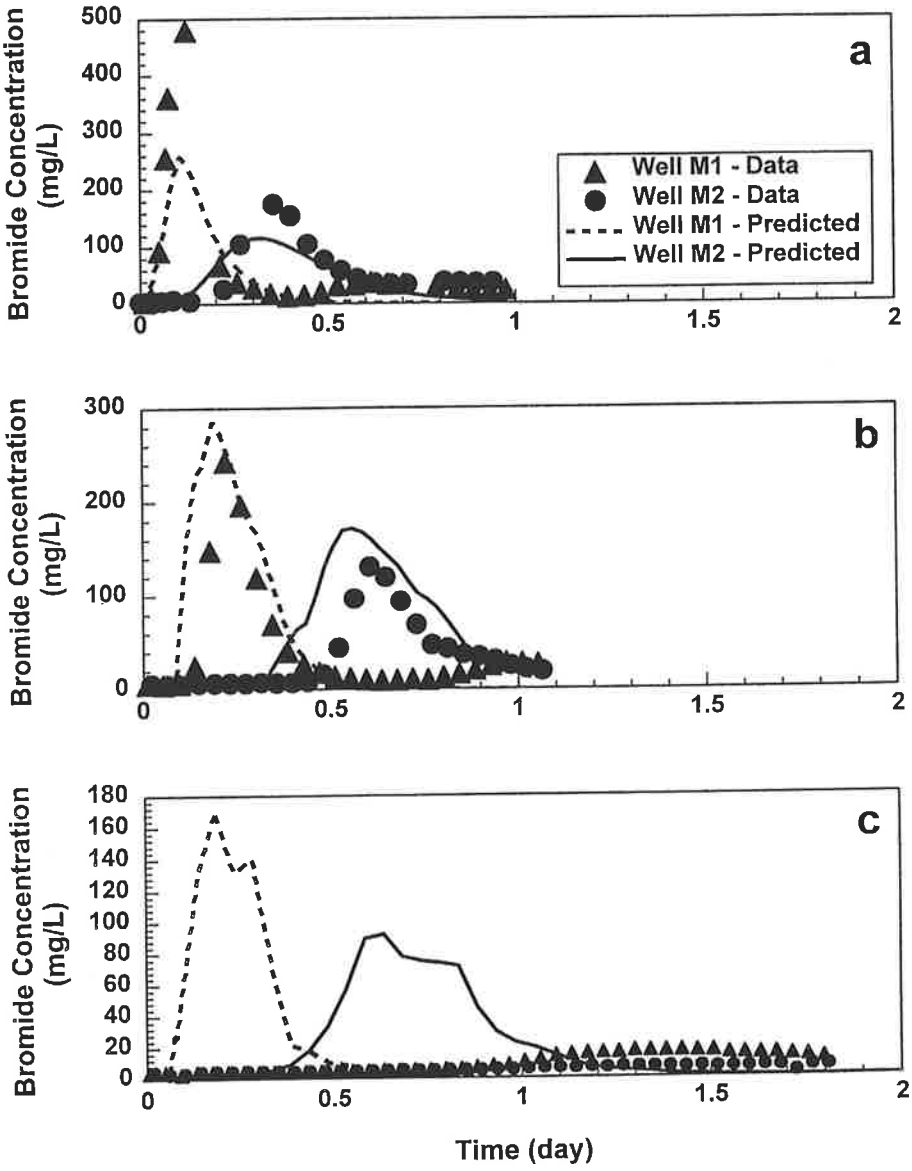

Figure 6. Measured and predicted bromide concentrations at wells M1 and M2 for the first (a), second (b), and third (c) tracer test.

capable of reducing the CT levels to below detectable levels. The feasibility of achieving these low concentrations using the HDC has been has been demonstrated previously (Hooker et al., 1994). Work is currently underway to design such a treatment system for the Hanford Site.

One of the primary constraints to the choice of the nutrient feeding strategy for this demonstration was avoiding biofouling of the nutrient injection well. Previous simulations (Clement et al., 1996; Hooker et al., 1994; Shouche et al., 1993), laboratory experiments (Jennings et al., 1995; Peyton, 1996), and field tests (Semprini et al., 1991) have shown that improper addition of electron donor and acceptor will result in preferential growth at the injection point. However, the use of temporally separated pulses of electron donor and acceptor has been shown to form a more even distribution of biomass throughout a flow field (Clement et al., 1996; Hooker et al., 1994).

During the continuous operation phase of this demonstration, acetate and nitrate were added daily in 1-hour pulses, separated by 2 hours. Subsurface transport simulations of pulse addition of conservative tracer indicate that a 2-hour pulse skew would result in dispersive nutrient mixing between 1 and 2 m from the injection well. Pumping and drawdown data, coupled with nutrient losses between monitoring locations and the fact that nutrient injection was maintained continually over 3 months, suggest that biomass was accumulating throughout the formation and at the extraction well, but not preferentially at the injection well. This growth pattern is further evidenced by Figures 5 and 7 which show significant denitrification occurring between wells M1 and M2 as well as rapid CT destruction activity at well M1.

Acknowledgments

This project was supported by the U.S. Department of Energy Office of Technology Development. The Pacific Northwest National Laboratory is operated by Battelle Memorial Institute for the U.S. Department of Energy under contract DE-AC-6-76RLO 1830.

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