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Development, Operation, and Long-Term Performance of a Full-Scale Biocurtain Utilizing Bioaugmentation

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A full-scale field evaluation of bioaugmentation was conducted in a carbon tetrachloride (CT)- and nitrate-impacted aquifer at Schoolcraft, MI. The added organism was *Pseudomonas stutzeri* KC (strain KC), a denitrifying bacterium that cometabolically degrades CT without producing chloroform (CF). To introduce and maintain strain KC in the aquifer, a row of closely spaced (1-m) injection/extraction wells were installed normal to the direction of groundwater flow near the leading edge of the CT plume. The resulting system of wells was used to establish and maintain a "biocurtain" for CT degradation through the intermittent addition of base to create favorable pH conditions; inoculation; and weekly addition of acetate (electron donor), alkali, and phosphorus. Although half of the test zone was inoculated twice, the long-term performance of both sections was indistinguishable: both had high CT removal efficiencies (median of 98−99.9%) and similar levels of strain KC colonization (>10^5 strain KC/g). Sustained and efficient (98%) removal of CT has now been observed over 4 yr. Transient low levels of CF (<20 ppb) and H_2S (<2 ppm) were observed, but both disappeared when the concentration of acetate in the weekly feed was reduced. Nitrate removal efficiencies ranged from 60% at low acetate concentrations to nearly 100% at high acetate concentrations. We conclude that closely spaced wells and intermittent substrate addition are effective means of delivering organisms and substrates to subsurface environments. At the Schoolcraft site, we achieved uniform removal efficiencies over a significant vertical depth (15 m), despite significant variability in hydraulic conductivity. This was accomplished by pumping 65% (v/v) of the natural gradient flow passing through the biocurtain during a given week in a single 6-h pumping event. Approximately 18,600 m^3 of contaminated groundwater was treated during the project.

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Introduction

Options for in-situ bioremediation range from monitored natural attenuation to complex engineered systems. If indigenous microflora possess the desired attributes, natural attenuation or biostimulation may be preferred. However, bioaugmentation may be necessary when the indigenous microorganisms do not degrade the contaminants at the required rates or when they generate unwanted byproducts. In-situ remediation requires effective delivery of nutrients, reactants, and potentially microbes. Innovative approaches to intercept and treat groundwater contamination include funnel and gate systems to direct groundwater and contaminant to a localized treatment zone (*1*), passive and semi-passive solute release from wells (*2*−*4*, this study), direct reactant placement (*5*), and bioscreens (combining localized sorption/ bioremediation) (*6*, *7*). In this paper, we describe the development of a large-scale *biocurtain* or zone of biodegradation activity perpendicular to groundwater flow utilizing bioaugmentation and pH control (*4*, *8*, *9*), a semi-passive operating strategy for maintenance of the biocurtain, and the 4-yr performance of the system.

In this study, bioaugmentation with *Pseudomonas stutzeri* KC (strain KC) was selected because indigenous microflora at the Schoolcraft test site did not demonstrate efficient CT degradation in bench-scale studies (*8*) and generated chloroform (CF) under some conditions (*4*, *9*). Strain KC is a denitrifying bacterium that mediates rapid CT degradation to carbon dioxide and nonvolatile products without producing chloroform under anoxic conditions (*10*, *11*). The transformation requires iron-limiting conditions (*10*−*13*) and is mediated by a secreted factor (*14*), pyridine-2,6-bis-(thiocarboxylate) (PDTC) (*15*). The genes for synthesis of PDTC are induced under iron-limiting conditions (*16*), which can be created by increasing pH to ∼8. Increasing the pH also facilitates survival and growth of strain KC (*17*). Accordingly, addition of base to increase pH is an important initial step in the development of a strain KC biocurtain (*4*, *11*). Strain KC was first used for CT remediation in a pilot-scale field experiment at Schoolcraft, MI (*4*). The pilot study demonstrated subsurface pH adjustment, transport of KC cells over a 2-m distance, assimilation of strain KC cells into the aquifer community, and CT degradation (60−65% removal efficiency). However, CF generation did occur in regions where KC activity was inadequately stimulated, and uniform CT transformation activity was not achieved because of inadequate hydraulic control.

An efficient full-scale delivery system was designed using a three-dimensional solute transport model and detailed aquifer characterization data (Table 1 and ref *18*). Design parameters included the spacing between wells, pump rates, and durations that were determined using optimization methods and experience from the pilot-scale study (*4*, *18*). This led to a design with 15 wells spaced 1 m apart (Figure 1), in which each well alternately serves as either an injection or an extraction well during different operational phases (Table 2).

Experimental Approach

The operational approach involved two distinct phases: a short *injection/extraction phase* in which groundwater is

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TABLE 1. Chemical and Physical Characteristics of Aquifer Sediments at the Schoolcraft Biocurtain Site[a]

	shallow zone	middle zone	deep zone
depth bgs (m)	9−15	15−21	21−27
sediment class[b]	fine sand	medium sand	coarse sand, gravel, coal
hydraulic conductivity[b] (cm/s)	0.0040−0.038 (*0.012*)	0.0011−0.063 (*0.027*)	0.019−0.11 (*0.046*)
velocity[c] (cm/day)	6.45	14.53	24.74
sorbed phase CT (μg/kg)	nd[d]−15.7 (*3.39*)	nd[d]−24.1 (*6.50*)	nd[d]−47.6 (*12.7*)
aqueous phase CT[e] (μg/L)	2.30−25.6 (*5.6*)	4.89−27.6 (*8.08*)	6.46−46.5 (*18.0*)
K_d (L/kg)	0.012−0.529 (*0.145*)	0.015−1.17 (*0.165*)	0.080−3.44 (*0.353*)
retardation factor[f] (CT)	1.06−3.56 (*1.70*)	1.07−6.67 (*1.80*)	1.39−17.68 (*2.70*)
aqueous phase nitrate[e] (mg/L)	13.4−57.3 (*40.2*)	22.9−58.8 (*42.0*)	1.87−63.7 (*41.0*)

[a] The range is minimum to maximum with median given in parentheses and italic type. [b] The sediment class, hydraulic conductivity, and sorbed-phase CT values are based on analysis of the cores collected from the even-numbered delivery wells (D2−D14). [c] Calculated from average K in each layer. Bulk velocity is 15 cm/day. [d] nd, below detection limit (detection limit (L_D) = 0.083 μg/kg, critical level (L_C) = 0.043 μg/kg). [e] The aqueous-phase concentration statistics are based on all aqueous phase samples collected from the observation well grid prior to system operation (November 7, 1997). [f] Calculated from K_d assuming porosity of 0.33 and bulk density of 1.6 kg/L.

circulated aboveground for nutrient and substrate addition followed by a much longer *passive phase* where contaminated water enters the treatment zone under influence of the natural hydraulic gradient. At the beginning of the passive phase, bioactivity is expected to be high, resulting in biodegradation of liquid-phase contaminant and desorption/biodegradation of solid-phase material. As activity declines due to substrate depletion, contaminant removal from the liquid phase continues due to sorption onto the now cleaned solids. Continuous cycling between the two phases results in a "trap-and-treat system", where the pumping time is only that required to restimulate a period of degradation and the interval between pumping events is a function of the sorption capacity of the solids. This system is expected to offer a significant operational advantage over traditional pump-and-treat technologies.

Site Characterization. Schoolcraft plume A is a region of CT contamination approximately 1.6 km long and 160 m wide (*8*, *18*). The aquifer consists of approximately 27 m of glacial outwash sediments, with a water table approximately 5 m below ground surface (bgs), and an average groundwater velocity is 15 cm/day. This formation is underlain by a nearly impermeable clay unit, which appears to be lacustrine in origin. Site characterization was performed during installation of the delivery well gallery, which consists of a series of 15 adjacent injection/extraction wells (details provided in ref *18*), aligned perpendicular to the natural gradient groundwater flow near the leading edge of plume A (Figure 1, wells D1−D15). The aquifer sediments can be roughly classified into three different units based on visual analysis and hydrogeologic property measurements. Characteristics of the sediment and groundwater in each level are summarized in Table 1, with the highest hydraulic conductivity sediments near the bottom of the aquifer. These deeper sediments also contain small coal particles that contribute to both higher CT sorption partition coefficients and sorbed-phase CT concentrations. Aqueous-phase CT concentrations at the biocurtain site (near the leading edge of the plume) were highest in the deep, high conductivity zone.

System Design and Operation. An aboveground chemical addition system was designed to add tracer, adjust pH using sodium hydroxide, and provide weekly delivery of substrates and nutrients to the biocurtain. During a typical delivery event, a combined flow rate of 150 L/min groundwater is extracted from alternating (odd- or even-numbered) delivery wells, circulated through the chemical addition/mixing system, and then injected into adjacent delivery wells. The mixing system consists of a static mixer and a 1140-L tank through which flow is circulated at 226 L/min to allow for equilibration. A 5-h extraction period in which flow passes from one set of delivery wells (for example, from odd-numbered wells to even-numbered wells) was followed by

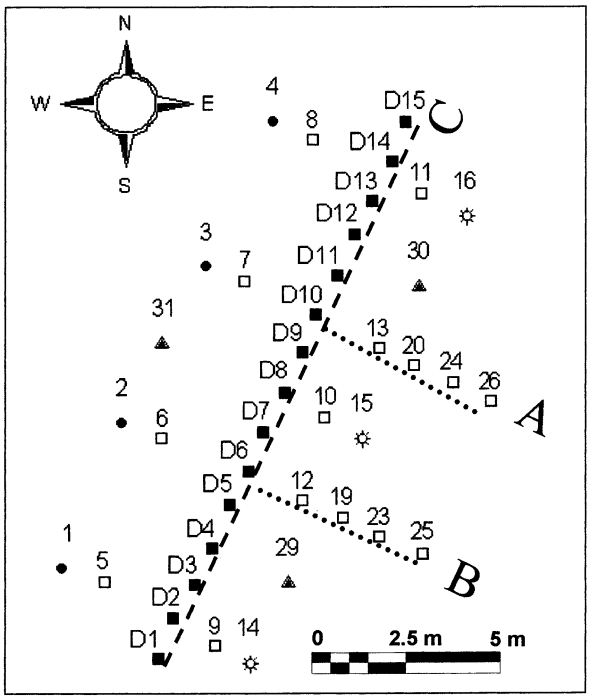

FIGURE 1. Layout of delivery and monitoring wells illustrating transects A and B (monitoring transects used in kriging).

1 h of reversed flow at the same rate. This flow reversal is used to ensure more uniform delivery of substrate around the initial extraction wells (the odd-numbered wells in this example). The 5-h pumping assignments (extraction or injection) of odd and even well sets was also switched weekly. Solute transport simulations, hydrogeologic characterization of the test zone, tracer results, and cost analysis of this system have been previously reported (*18*).

Prior to inoculation, the pH of the subsurface environment was modified to create conditions favorable for strain KC growth and CT degradation. The initial pH (7.3−7.4) was increased to pH 8.2. This pH shift decreased the stability of calcium (Langelier Index changed from 0.5 to 1.3, Ca^{2+} 168 ppm, alkalinity 347 mg/L as $CaCO_3$). Prior to inoculation, extracted groundwater was adjusted to pH 8.2 and re-injected (Table 2). Following inoculation, acetate and phosphate were added weekly along with the base. Previous studies showed effective strain KC growth using acetate as the electron donor and phosphate as a nutrient (*8*, *10*). An initial level of 100 mg/L acetate and 10 mg/L phosphate was utilized. On day 314, the acetate level was reduced to 50 ppm to limit sulfate reduction in areas of acetate excess. At approximately 6-month intervals, the aboveground components of the

TABLE 2. Major Phases of Field Activity with Day 0 Corresponding to the First Sampling Event

activity	day	composition of injected materials
baseline monitoring	0−52	no injections
tracer testing	53−72	NaOH/NaBr fluorescein (100 ppb)
pre-inoculation mixing and pH adjustment	73−116	NaOH/NaBr
inoculation	117	NaOH/NaBr NaBr (200 mg/L as Br) acetate (∼830 ppm) phosphate (10 ppm) strain KC (∼10^7 cfu/mL)
weekly feeding	118−313	phosphate (10 mg/L) NaOH/NaBr acetate (100 mg/L)
re-inoculation	200−201	NaOH/bromide acetate (∼400 ppm) phosphate (10 ppm) strain KC (∼10^7 cfu/mL) trace metals
weekly feeding	201−313	phosphate (10 mg/L) NaOH/NaBr acetate (100 mg/L)
feedings with reduced acetate concentrations	314 − present	phosphate (10 mg/L) NaOH/NaBr acetate (50 mg/L)

system were cleaned with dilute HCL. After 14 months of operation, a system rinse with municipal tap water after each run was initiated.

Experimental Plan. The full-scale demonstration of the Schoolcraft biocurtain was conducted in seven phases as described below and summarized in Table 2:

(*1*) *Aquifer Characterization and Construction*. Monitoring and delivery wells were installed; sediment cores and groundwater samples were collected to characterize aquifer properties and contaminant concentrations.

(*2*) *Tracer Testing* (*Days 53−72*). Groundwater amended with tracer was injected into the delivery well gallery to assess solute transport between delivery wells during pumping and subsequently to downgradient monitoring wells. After a 6-h pumping period, the injected tracer slug was allowed to migrate (for ∼26 days) under the influence of the natural hydraulic gradient without operation of the delivery system.

(*3*) *Pre-inoculation Mixing and pH Adjustment* (*Days 73−116*). Base addition began on day 53 (Table 2). During each base addition event, groundwater was pumped at 150 L/min from either odd- or even-numbered wells, amended with base to a pH of 8.2, and re-injected.

(*4*) *Inoculation and Feeding* (*Days 117−199*)**.** On day 117 (January 7, 1998), the delivery well grid was inoculated with 18 900 L of strain KC culture (2×10^7 cfu/ml) grown aerobically on site. For 5-h periods each week thereafter, groundwater was first extracted from either odd- or even-numbered wells; circulated through the mixing system; amended with sodium hydroxide (to pH 8.2), phosphate (10 mg/L), and acetate (100 mg/L); and then injected into the remaining wells. This 5-h period was followed by 1 h of reversed flow with pH adjustment but no acetate or phosphate addition (to reduce biofouling in the vicinity of the well screen).

(*5*) *Re-inoculation and Feeding* (*Days 200−313*). On days 200 and 201, delivery wells in the northeast half of the delivery well gallery (D8−D15) were re-inoculated with 37 000 L of strain KC culture grown with reduced acetate concentration (800 ppm) and added trace metals. The weekly feeding and mixing operations continued as described in phase 5.

(*6*) *Feeding with Reduced Acetate Concentrations* (*Days 314 to Present*). Acetate levels were reduced to 50 mg/L to prevent sulfate reduction and to establish slight nitrate-excess condition.

(*7*) *Solid-Phase Evaluation of Contaminants and Microbes* (*Days 336−342 and 1006−1013*). Borings were performed downgradient of the treatment zone for analysis of solid-phase microbial populations and contaminant levels on days 336−342 (six borings) and days 1027−1033 (eight borings).

Analytical Methods

Aquifer Sediment Sampling. Sediment cores were collected from 9 to 27 (m bgs) from the even-numbered delivery well locations as previously described (*4*). Subsamples were taken from each core for volatile organics analysis, and then cores were sectioned for analysis of hydraulic conductivity (*K*) and sorption properties (*18*).

Groundwater Sampling. The monitoring well grid consists of 96 downgradient monitoring points, 23 upgradient points, and 20 points located outside the treatment zone (Figure 1). The majority of these well clusters were screened at 3-m intervals from 10.7 to 27.4 m bgs. HDPE drop pipes were installed and sealed 150 mm above each screened interval. Above ground the drop pipes were fitted with a short (<1 m) section of Tygon fuel and lubricant tubing (to provide wide temperature flexibility) and sampled with peristaltic pumps at 200 mL/min. Sample handling and analytical procedures have been previously described (*4*).

Growth of Strain KC and Inoculation. A strain KC NB starter culture (190 L) was used to inoculate two 9400-L HDPE bioreactors. Growth media was prepared by filter sterilizing groundwater (0.2-μm filter), heating to 20 °C, adjusting the pH to 8.2, and adding acetate (1600 ppm) and phosphate (10 ppm). Cells were grown aerobically using coarse bubble aeration. The pH was maintained at 8.2 ($\pm$ 0.1) during growth. No contaminants were detected by plate count (>1000 colonies screened) or by PCR-based screening with PKC specific primers (*4*) (n = 36 colonies screened). Bromide was also added (200 mg/L) after culture growth to serve as a conservative tracer. The initial inoculation sequence proceeded in two steps. Initially, half the volume of each

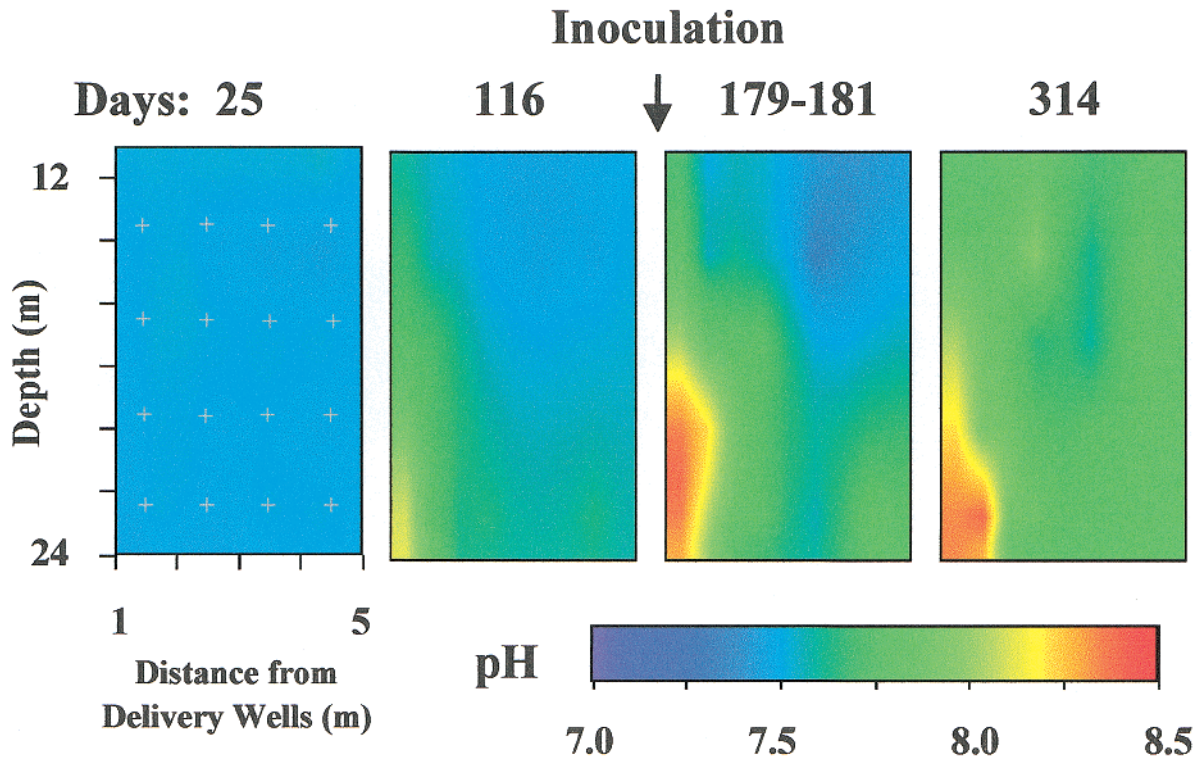

FIGURE 2. Kriged images of pH along transect A. Measured points (entire grid) used for kriging: 95 on day 25, 46 on day 116, 42 on days 179−181, and 55 on day 314. Measured points in transect A: 20 on day 25, 11 on day 116, 12 on days 179−181, and 16 on day 314. Monitoring locations indicated by (+).

bioreactor was injected into the odd-numbered wells at 150 L/min while groundwater was simultaneously extracted from the even-number wells (227 L/min) and surfaced discharged (under permit). This phase of inoculation was followed by a 2-h recirculation event at 227 L/min in which extracted groundwater was now re-injected into the odd-numbered wells to further distribute the added cells. The second half of the bioreactor volume was then injected into the even-numbered wells at an average rate of 150 L/min without extraction. This was followed by a second 2-h round of recirculation with extraction (from odd-numbered wells to even-numbered wells) at 227 L/min.

Re-inoculation of the northeast half of the delivery well gallery was performed on days 200 and 201. The decision to re-inoculate was based on uncertainties arising from the presence of flocculated cells in the initial inoculum and the limited initial detection of strain KC. Laboratory experiments performed after the inoculation demonstrated that flocculation could be prevented by adding trace nutrients ($FeSO_4$ 0.6 ppm, $CuSO_4$ 0.12 ppm, $ZnSO_4$ 0.290 ppm, NiCl 0.07 ppm, H_3BO_3 0.03 ppm, $MnCl_2 \cdot 4H_2O$ 0.4 ppm) to the filter-sterilized groundwater. The northeast half of the delivery well gallery (wells D9−D15) was re-inoculated with 37 850 L of culture nonflocculent culture. Inoculation was performed by injecting 4700 L of culture (150 L/min) into one delivery well while simultaneously extracting (110−150 L/min) from the immediately adjacent well to the northeast (example: inject D9 and extract D10) along the delivery gallery. This pattern was used for wells D9−D14; well D15 was inoculated with injection of 4700 L at 150 L/min without extraction. To detect strain KC, DNA was collected from filtered and extracted groundwater samples (1 L). The extracted DNA samples were analyzed using the previously developed PCR primer set JMT 166 and JMT 219 (*4*).

Data Analysis. Solute concentration measurements from the monitoring grid were interpolated using kriging to develop two-dimensional plots. Kriged data sets were generated using Matlab-based software developed by Kitanidis (*19*), with both exponential and linear model variograms that best fit the experimental data. Directional anisotropies were accounted for using axis scaling.

Results and Discussion

Adjustment of pH and Effect of Pumping Regime on Initial Carbon Tetrachloride Distribution. Figure 2 illustrates kriged images of pH measurements along transect A (Figure 1) for days 25, 116, 179−181 (combined to provide a more complete data set), and 314. Background levels (around 7.5) are shown for day 25. One day prior to inoculation (day 116), base addition had increased groundwater pH to 7.8−8.2 in monitoring wells 1 m downgradient from the delivery well gallery. As expected, the most rapid and pronounced response occurred in the deep, high-hydraulic conductivity zone. Upgradient wells (data not shown) were relatively unchanged. A similar response was observed in transect B (data not shown).

A three-dimensional solute transport simulation was developed to evaluate the CT distribution prior to calculating removal efficiency in the biocurtain region. This deterministic model (*18*) helped describe the redistribution of CT that occurred during the pumping required for base addition prior to inoculation. Processes included in the model were advection, dispersion, and linear equilibrium sorption/desorption. Figure 3 shows an example simulation result for well 19 at a depth interval of 19.5−19.8 m, which predicts that recirculating water from multiple zones with variable CT concentrations at the site will result in concentrations of roughly 30 ppb. This value was confirmed as the measured discharge concentration in the delivery system and corresponds to an equilibrium sediment concentration of ~60 μg/kg (calculated from K_d as described in Table 1). Prior to inoculation, modeled CT levels closely tracked the observed increase in CT, as shown in Figure 3. The spatial effect of mixing to raise pH prior to inoculation resulted in the redistribution of CT across the delivery well gallery as shown

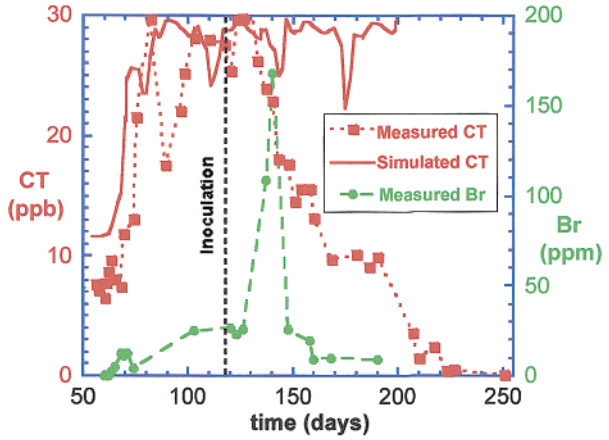

FIGURE 3. Simulated carbon tetrachloride concentrations in the absence of biodegradation vs measured concentrations of carbon tetrachloride and bromide tracer at well 9, depth 19.8 m. Bromide at high concentration (200 ppm) was injected with inoculum.

in Figures 4 and 5. CT levels increased because the high conductivity zone (22.9−25.9 m bgs) from which water was preferentially drawn was also the zone of highest CT concentrations.

Growth of Strain KC and Aquifer Inoculation. The delivery well gallery was inoculated on January 7, 1998 (day 117). Under field conditions, both of the 9.4 m^3 cultures entered the stationary phase at ~18 h and began to flocculate, generating multicell clusters 0.01−0.05 mm in diameter. The high-speed vanes of the delivery pumps appeared to effectively dispersed the floc, so inoculation proceeded despite flocculation. A total of 18 900 L of culture (2×10^7 cfu/mL) was injected, giving an average of 0.08 m^3 of inoculum/m of well screen.

Initial Colonization by Strain KC. KC-specific primers developed and tested in the pilot-scale study (*4*) were used to monitor transport of planktonic cells as summarized in Table 3. Nine days after inoculation, planktonic KC cells (Table 3) and tracer were detected in well M11, 1 m downgradient from the delivery well gallery. This indicates that some KC cells were transported as least as fast as the advective groundwater velocity (24.7 cm/day, this depth) despite the inoculum flocculation. In continuous-flow column studies (*20*), strain KC had been shown to travel 2 m at velocities up to 1.5× the advective groundwater velocity, probably as a result of chemotaxis toward nitrate. Our attempts to follow this planktonic microbial population had limited success: of the three transects of monitoring locations used to follow microbes, only the MW 12/MW 16 1- and 2-m downgradient locations tested positive for strain KC in this early phase (Table 3). Many of the wells sampled during this initial phase were negative for KC, suggesting that KC was not present in concentrations above the 10^2−10^3 cfu/mL detection limit. A possible explanation is that the bacteria initially attached to sediments close to the point of inoculation. Initial sediment sampling (day 159, 44 days after inoculation) 25 cm downgradient from delivery well D15 showed strain KC as a significant fraction of colony forming units (2.5×10^5−2.1×10^6 strain KC cfu/g vs 1.6×10^6 native flora from 13.7 to 27.9 m bgs). No strain KC cells were detected on sediment 1 and 2 m downgradient from D11 during this sediment sampling event. Upgradient wells were negative for strain KC and showed little change in native population density (10^4−10^5 cfu/mL), dominantly denitrifying microbes (data not shown).

Re-inoculation and Distribution of KC. Re-inoculation of the northeast section of the test grid was performed to determine if additional inoculation was needed to ensure colonization of the biocurtain zone. These concerns arose because of the observed flocculation in the initial inoculum and the limited initial detection of strain KC (Table 3). On day 190, just prior to re-inoculation, samples were collected that showed detection of strain KC up to 2 m from the delivery wells, suggesting that KC had begun to colonize a significant region of the subsurface. Unfortunately these data were not available prior to the decision to re-inoculate. Re-inoculation differed from the initial inoculation in three ways: trace nutrients were added to prevent flocculation, the inoculum per unit length of discharge screen was four times greater (an average of 0.31 m^3/m of well screen vs 0.08 m^3/m for inoculation), and the pumping regime utilized during bacterial addition. The average concentration of strain KC in this inoculum was 3.7×10^7 (range 1.7−6.5×10^7) cfu/mL.

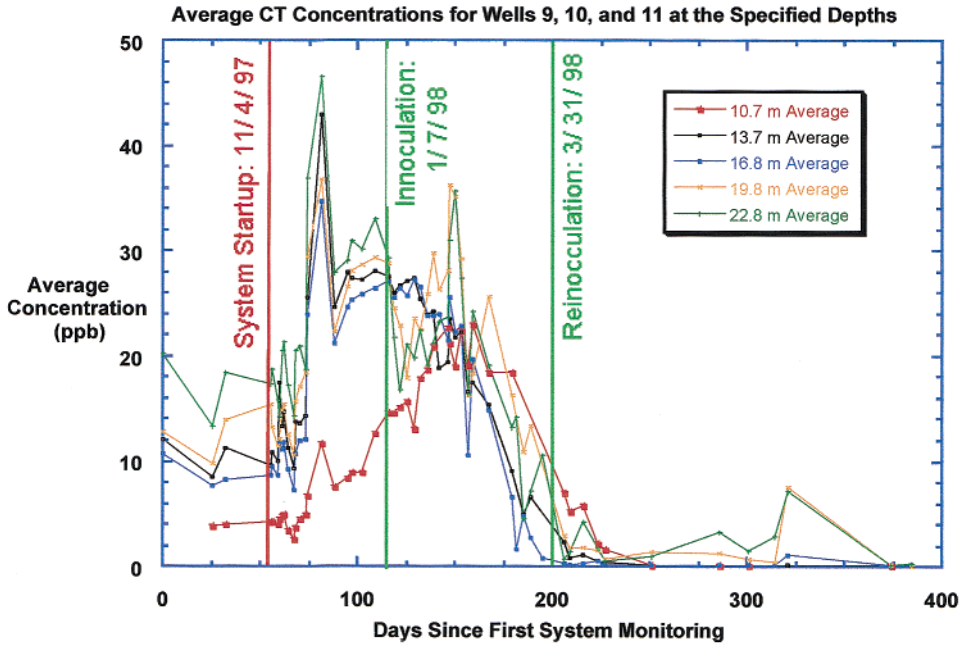

FIGURE 4. Averaged carbon tetrachloride concentrations for monitoring wells located 1 m downgradient of the delivery well gallery (wells 9−11 in Figure 2).

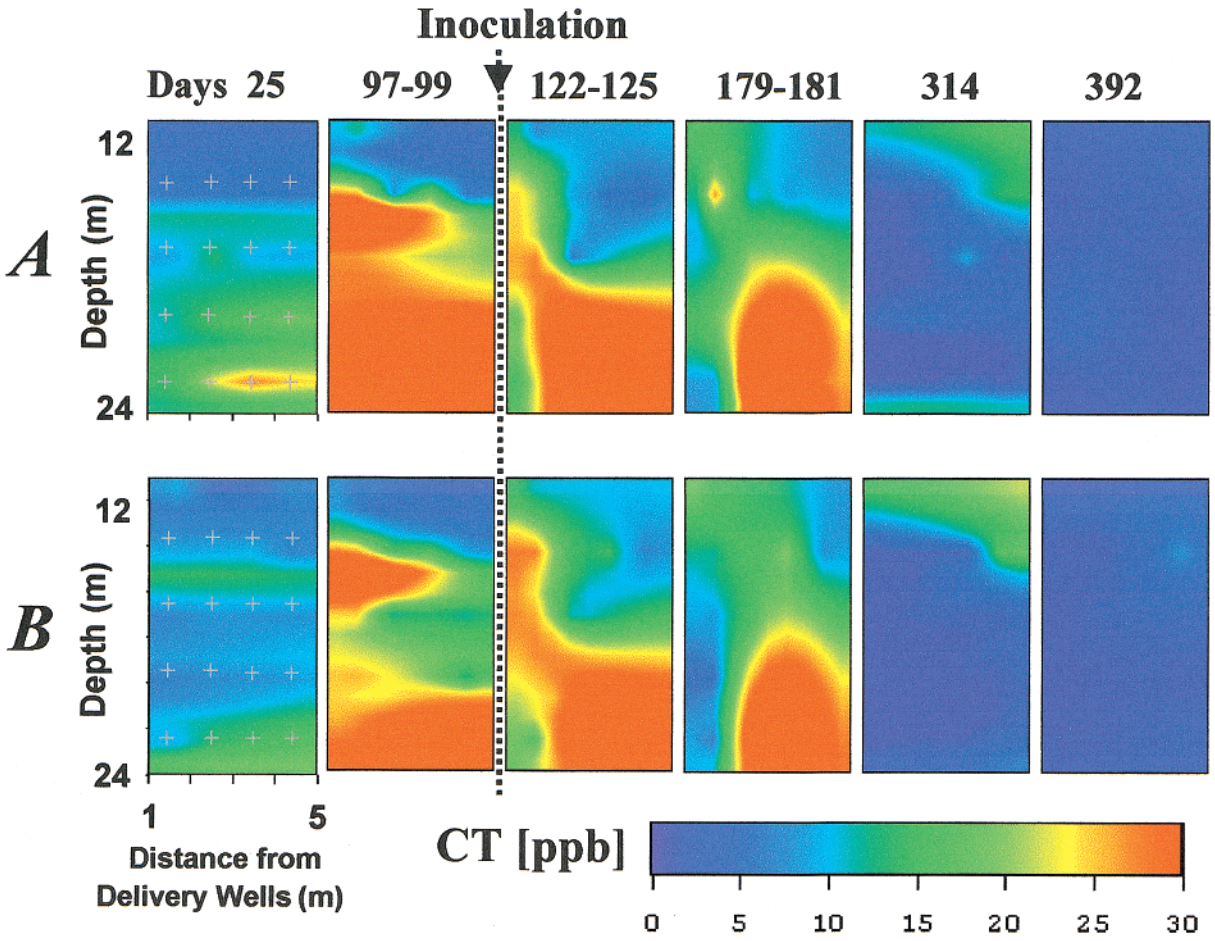

FIGURE 5. Kriged images of carbon tetrachloride concentrations along transects A and B. Day 25 represents pre-operational CT levels; days 97−99 represent the CT distribution as a result of mixing during base addition. Inoculation was on day 117. Measured points used for kriging: (transect A) 18 on day 25, 17 on days 97−99, 12 on days 122−125, 12 on days 179−181, 16 on day 314. and 8 on day 392; (transect B) 19 on day 25, 15 on days 97−99, 12 on days 122−125, 12 on days 179−181, 16 on day 314, and 12 on day 392. Monitoring locations in this image plane are indicated by (+).

TABLE 3. KC-Specific DNA Probe for Groundwater Sampled at Monitoring Wells Downstream from the Delivery Well Gallery[a]

	southwest wells				northeast wells							
	M12		M19		M13		M20		M11		M16	
distance from gallery (m)	1.5		2.5		1.5		2.5		1[b]		2[b]	
depth (m)	16.8	22.9	16.8	22.9	16.8	22.9	16.8	22.9	16.8	22.9	21.3	24.4
day 117	inoculation						inoculation					
day 126										++		
day 133									++	++		++
day 140										++		++
day 154										++		++
day 168										++		++
day 190	++	++				++			++	++		++
day 196	+	+				++			++	++	++	++
days 200−201						re-inoculation of northeast section of gallery only						
day 211	+	+			++	++			++	++		
day 218	++	++			++	++	+	++	+	++		++
day 225	++	++			++	++	++	++	++	++	+	+
day 232	++	++			++	++	++	++	++	++	++	++
day 253	++	++	+	+	++	++	++	++	++	++	++	++
day 288	++	+	+	+	++	++	++	++	++	+	+	+
day 323	++	+	++	++	++	++	+	++	++	+	++	++
day 384	+	++	++	+	++	++	+	++	++	++	+	++

[a] ++, strong signal; +, weak signal. Strong signal corresponds to over 10^3 cfu/mL. Weak signal corresponds to 10^2−10^3 cfu/mL. Blank cell represents nondetect. [b] Strain KC followed in 1 and 2 m downgradient wells in northeast section only.

Fifty-three days after re-inoculation (day 253), KC was detected in all sampled locations (Table 3), including the southwest section that only had the initial inoculation. Additional coring indicated substantial solid-phase strain

TABLE 4. Solid Phase Microbial Levels Days 336−342

soil boring location	center of grid, 1.5 m downgradient D8		center of grid, 3.0 m downgradient D8		northeast section, 1.5 m downgradient D11		southwest section, 1.5 m downgradient D3	
depth (m)	PKC[a] (cfu/g)	native flora (cfu/g)	PKC (cfu/g)	native flora (cfu/g)	PKC (cfu/g)	native flora (cfu/g)	PKC (cfu/g)	native flora (cfu/g)
10.7	2.1×10^5	5.8×10^5	9.2×10^4	1.2×10^6	8.1×10^4	4.9×10^5	8.8×10^4	4.1×10^5
13.8	nd	9.5×10^4	2.5×10^5	6.5×10^5	8.8×10^4	2.7×10^5	7.6×10^4	4.1×10^5
16.8	3.0×10^4	2.1×10^5	4.1×10^4	3.3×10^5	1.3×10^5	2.9×10^5	4.3×10^4	1.6×10^5
19.8	1.6×10^5	3.8×10^5	1.6×10^5	3.7×10^5	6.0×10^4	2.3×10^5	1.1×10^5	2.2×10^5
22.9	1.7×10^5	5.2×10^5	1.8×10^4	1.4×10^5	5.9×10^4	2.7×10^5	1.3×10^5	1.8×10^5
25.9	1.3×10^5	4.1×10^4	nd	7.1×10^4	1.5×10^4	4.4×10^4	2.2×10^4	5.4×10^4

[a] Microbes extracted and PKC identified as described in ref *20*.

KC levels (Table 4). These data and the subsequent patterns of CT removal indicate that adequate colonization was likely achieved without re-inoculation. However, due to the across-grid recirculation that occurred during feedings following re-inoculation, planktonic strain KC cells from the re-inoculated side may have been distributed throughout the delivery zone. While this detached population is a small fraction of the total strain KC (due to high attachment rate), this may have contributed to the observed degradation in both sections of the test grid.

CT Remediation and CF Production. Two similar approaches were used to evaluate the remediation performance of the biocurtain. One approach was to compare measured input concentrations in the delivery system vs downgradient CT levels, while the alternative approach compared the observed downgradient CT concentrations as compared to simulated values with no degradation term included in the model. A detailed description of the model is provided by Hyndman et al. (*18*). Both cases provided the same result because the simulation discussed earlier accurately predicted the average 30 ppb output of the delivery/mixing system. The difference between simulated and observed concentration histories (Figure 3) provides a reasonable estimate of the amount of CT that was biodegraded since biodegradation was not included in this simulation. To calculate percent removal (days 524, 995, and 1456 after inoculation), the CT levels at monitoring locations were compared to the actual system output (30 ppb CT).

Measured bromide concentrations are also illustrated in Figure 3 to indicate the time when the slug of inoculated water passed by the sampling point, which roughly corresponds to the time when CT concentrations dropped dramatically. Groundwater flow from the delivery well gallery to the first tier of downstream monitoring wells requires approximately 1 week in the high conductivity deep zone. Because sorption to sediment retards CT transport relative to groundwater (estimated retardation factor of 1.7−2.7), changes in measured CT concentrations lagged behind tracer breakthrough. Additionally, CT removal varied by depth, with the most rapid response at the highest conductivity levels and the slowest response at the lowest conductivity levels (Figure 4).

The CT degradation dynamics are illustrated in Figure 5 using a series of kriged "snapshots" of CT levels along transects A and B (locations shown in Figure 1). Initially, CT levels were highest in the conductive lower levels. Mixing during base addition caused CT levels to increase prior to inoculation (days 97−99), with the most dramatic rise in the deep high hydraulic conductivity zone. This zone was also the first to show decreases in CT concentrations, occurring approximately 1 week after inoculation (Figure 5). The influence of a reactive biocurtain is clearly visible by days 179−181. As shown for the data on day 314, the upper region was the last region to be remediated because of slower downgradient transport of substrates into these layers. The downgradient observation of these layers is also temporally delayed by the slower downgradient transport of the treated "clean" groundwater from the biocurtain region to the monitoring points. Eventually, however, uniform and essentially complete removal of CT was observed along both transects (day 392).

The entire monitoring grid was sampled on day 614. CT levels entering the biocurtain vary significantly, with highs around 50 ppb, but CT levels downgradient of the biocurtain were uniformly low, with most levels less than 5 ppb (the Michigan Department of Environmental Quality Part 201 standard for drinking water; *21*). High CT levels were observed at some lower depths where contaminated water passed below our delivery wells (screened to 24.4 m). The removal percentages for the 63 observation points downgradient of the biocurtain (laterally from well 9 to well 11, downgradient to wells 24 and 25, from depths 10.7−22.9 m; Figure 1) have a mean of 95.6% and a median of 98.5% (*12*). A similar analysis of 70 observation points spanning the same zones on day 1112 (995 days after inoculation) and 54 observation points (day 1573 or 1456 days after inoculation) demonstrated a mean CT removal of 97.3% (median = 99.9%) and 93.5% (median = 96.3), respectively.

Aqueous-phase chloroform measurements along transect A are illustrated in the snapshot kriged images of Figure 6 (a similar pattern was observed for transect B). Of significance is the absence of chloroform 1 week after inoculation on days 122−125. Measurements of CT over this same time period indicate significant CT degradation at the lower depth levels, as shown in Figure 5. The simultaneous removal of CT and absence of chloroform support the conclusion that CT removal was mediated by strain KC. A similar pattern was observed in the pilot-scale study (*4*). Following KC inoculation chloroform appeared, coinciding with detection of sulfide (1−1.4 ppm). This was attributed to acetate excess conditions created during inoculation where the spent fermentation broth introduced high acetate concentrations (1400 ppm inoculation, 800 ppm during re-inoculation). To minimize stimulation of indigenous populations, acetate levels were decreased to 50 mg/L. This created slight nitrate-excess conditions, which resulted in a substantial decline in observed chloroform concentrations. Total microbial DNA extracted from groundwater during this phase will used be in ongoing community structure analysis.

Solid-Phase Remediation of CT. Three sets of sorbed-phase CT levels were obtained to evaluate the system's efficiency at removing sorbed contaminant concentrations. Samples were collected at the locations of the delivery wells during installation to determine pre-operational levels and at post-inoculation sampling locations upgradient and downgradient of the delivery gallery. The upgradient samples did not change relative to the pre-operational samples over the multi-year period, indicating that the influent CT concentrations were relatively stable over time. All sampled distances (1, 2, 3, and 5 m) downgradient showed significant

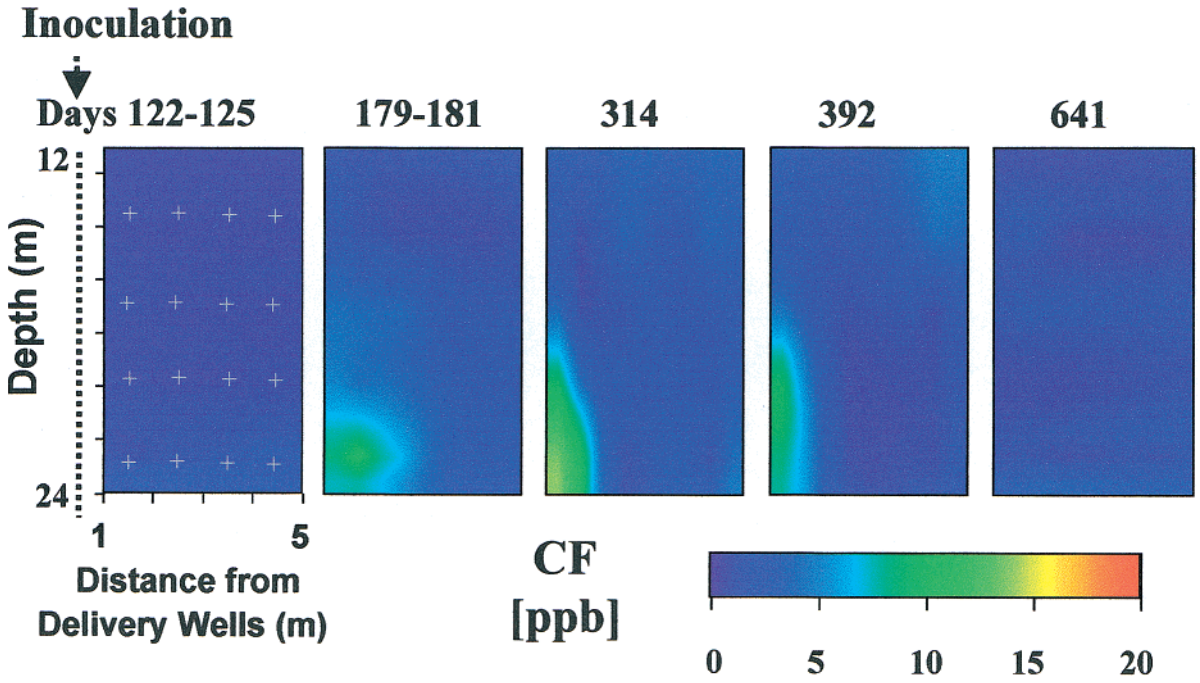

FIGURE 6. Kriged images of chloroform concentrations along transect A. Measured points used for kriging: 12 on days 122−125, 12 on days 179−181, 16 on day 314, 7 on day 392, and 16 on day 641. Monitoring locations same as in Figure 3.

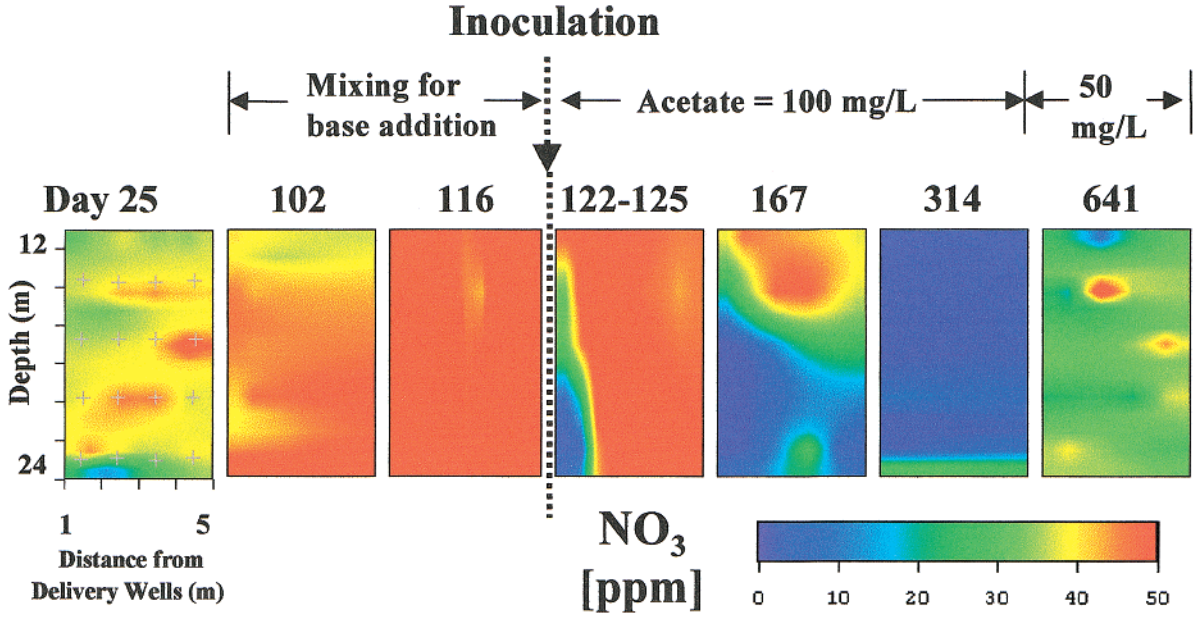

FIGURE 7. Kriged images of nitrate concentrations along transects A. Measured points used for kriging: 64 on day 25, 45 on days 122−125, 57 on day 167, 22 on day 314, and 59 on day 641. Measured points in transect A: 16 on day 25, 12 on days 122−125, 12 on day 167, 4 on day 314, and 16 on day 641. Monitoring locations are the same as in Figure 3.

reductions in sorbed CT levels from the pre-inoculation condition. Figure 8 shows the pre-operational/post-operational CT and CF levels obtained by intensive sampling (14 borings, continuous cores 9.1−27.4 m bgs) within 1 m of the delivery gallery.

Pre-operational CT levels in the vicinity of the delivery showed an increase with depth from ~3 to 13 μg/kg. Samples collected 220 days after inoculation indicated that the median sorbed CT concentration dropped 53.0−76.8% across the screened interval (Figure 8), with no increase in sorbed CF. The sediment samples collected 995 days after inoculation showed greater removal: 79.2−98.8% across the screened interval (Figure 8). Most CT levels (995 days after inoculation) over the depth interval spanned by the delivery screens were below the detection limit. The sorbed-phase concentration 1 m downgradient of the delivery system increases slightly with depth toward the base of the screened interval. Below approximately 24 m, CT levels increase dramatically (Figure 8). Within the lower screened depth intervals (21−24.4 m), high conductivity sediment layers (Table 1) rapidly convey groundwater through the biocurtain zone. CT was degraded to 0.12−0.23 μg/kg over the upper layers (9−21 m) of the screened interval (Figure 8). In the deeper layers (21−24.4 m), an average of 2.33 μg/kg CT (±3.69 μg/kg) persists 1 m downgradient of the delivery wells. Samples taken 2.5 m downgradient of the delivery wells showed nearly complete removal of CT across the entire screened interval (data not shown).

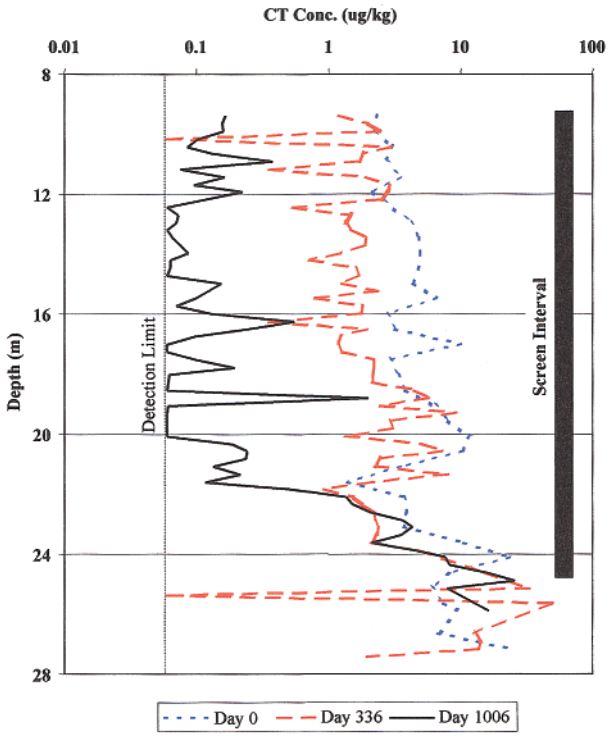

FIGURE 8. Solid-phase carbon tetrachloride concentrations in injection zone. Pre-inoculation (August 1997) levels determined during installation of delivery wells. Values represent averages of all samples taken at the depth interval shown for odd-numbered delivery wells across injection zones. Post-inoculation levels (August 1998, 8 months after inoculation, and June 2000, 30 months after inoculation) determined by coring 1 m downgradient of the delivery wells 3, 8, and 12 (southwest, center, and northeast section of delivery zone). Values represent averages of all samples taken at the depth interval shown. Samples were taken at 20 (pre) and 30 cm (post) intervals. Percent carbon tetrachloride removal (pre vs August 1998 and June 2000, respectively) are as follows: 9−15-m interval, 53% and 98%; 15−21-m interval, 60% and 96%; 21−24.4-m interval 76% and 79%; and below the screen interval (24.4−27-m interval) −32% and 24% removal.

Denitrification. An added benefit of the biocurtain is nitrate removal. In the presence of an electron donor and in the absence of oxygen, nitrate serves as the preferred terminal electron acceptor for growth of strain KC and native denitrifying microbes. Background nitrate concentrations at Schoolcraft are approximately 40−60 mg/L, and dissolved oxygen concentrations are ~2−3 mg/L. Figure 7 illustrates kriged images of nitrate concentrations along transect A (a similar profile was obtained for transect B). Prior to inoculation, nitrate levels rose over the vertical extent of the delivery well screens, as nitrate from conductive zones was redistributed (days 25, 102, and 116) similar to the CT redistribution discussed earlier. One week after inoculation (days 122−125), nitrate removal is clearly visible at the deep levels, and eventually virtually complete removal of nitrate (day 314) was observed. After decreasing acetate levels to prevent sulfate reduction, nitrate levels increased but remained at levels below the regulatory standard (10 mg/L as N, approximately 44 ppm as NO_3 (*15*)). Nitrate is required as an electron acceptor, and if absent in the groundwater would have to be added. The nitrate levels must also be evaluated to ensure that denitrification does not lead to excess N_2 formation and gas production beyond its solubility. The level of denitrification achieved in this study kept N_2 levels below 50% of solubility in groundwater.

Overall Biocurtain Performance. Aqueous data over a 4-yr period and two sets of post-inoculation solid-phase samples indicated that the strain KC biocurtain was capable of efficient long-term efficient removal of both CT and nitrate. The intermittent nature of the delivery system and closely spaced wells allowed cost-effective delivery of tracer (*18*) and delivered sufficient levels of nutrients and strain KC to support CT remediation levels of over 96%. The initial inoculumn conditions of 83 L/m of delivery screen appears to have been sufficient to support long-term colonization up to 3 m downgradient of the delivery wells (Table 4). In addition, no short- or long-term performance benefit was observed due the second inoculation cycle (Figure 5, transect A (twice inoculated) vs transect B (once inoculated). In fact, the second inoculation may have been a partial cause of the transient CF production (Figure 7, transect A) by contributing to local carbon excess in relation to the available nitrate. By balancing the levels of electron donor (acetate) to the available nitrate, effective process control was re-established and a long-term strain KC biocurtain was maintained. While at this site CT concentrations were low (average concentration less than 100 ppb; data not shown), strain KC can degrade CT up to 10 ppm (*11*) at similar cell densities, thus achieving much higher CT removal per unit acetate consumed.

The absence of CF over most of the operational cycle indicates successful bioaugmentation and colonization by strain KC: however, multiple degradation mechanisms may have contributed to the overall system performance. These include direct strain KC remediation, native flora activating PDTC to degrade CT (*22*, *23*), native flora degrading CT to CF, and possible exchange of and expression of PDTC production genes to native flora.

A significant benefit of the intermittent pumping strategy utilized in this system is the low pumping volumes and short duration of pumping. In this system, pumping to introduce and mix substrates and nutrients is followed by a natural gradient step in which transport of contaminant into the treatment zone and sorption to previously treated sediment in the biocurtain zone occur. The sorbed contaminants are then degraded when the next nutrient addition cycle stimulated degradation in the biocurtain zone. This trap-and-treat cycling may provide an efficient option for semi-passive operation of other types of bioremediation systems, where intermittent activation of degradation coupled with natural gradient contaminant delivery may be sufficient to establish a treatment biocurtain without the need for continuous nutrient delivery or hydraulic control.

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