Bench-Scale Evaluation of Bioaugmentation to Remediate Carbon Tetrachloride-Contaminated Aquifer Materials

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Abstract

Pseudomonas sp. strain KC is a denitrifying bacterium that, under iron-limited conditions and in the presence of sufficient quantities of growth substrate, rapidly transforms carbon tetrachloride (CCl_4) to carbon dioxide and nonvolatile products without production of chloroform. Bench-scale laboratory methods were used to evaluate the feasibility of bioaugmentation with strain KC to remediate aquifer materials from an aquifer of documented CCl_4 contamination at Schoolcraft, Michigan. Nine glass columns packed with uncontaminated aquifer materials from the site were used to simulate aquifer conditions. Columns were alternately exchanged with ground water from the site in a rapid (20-30 minute) displacement of the pore fluid, then incubated under static conditions for a period of days to weeks. The exchange and incubation procedure continued until CCl_4 removal began to plateau, indicating equilibration of the sorbed CCl_4 with CCl_4 in the pore fluid. Information obtained during this period was used to evaluate porosity (total and effective) and CCl_4 sorption. In subsequent exchanges, base, acetate, and phosphorus were added to the exchange fluids to create conditions favorable for growth of strain KC and expression of its CCl_4 transformation activity (niche adjustment). Three columns were inoculated with strain KC; three were not inoculated; and three were chemically disinfected with thimersol. Strain KC was transported more rapidly than the average linear velocity of the exchange fluids in the inoculated columns. Protein levels measured in the effluent of the inoculated columns during subsequent exchanges indicated that niche adjustment enabled rapid growth and colonization of the aquifer solids by strain KC. Little or no protein was detected in the effluent of uninoculated columns. CCl_4 mass balances on the inoculated, noninoculated, and chemically disinfected columns indicated that niche adjustment and inoculation with strain KC created conditions favorable for CCl_4 removal from the aquifer solids. Up to 70% removal of soluble CCl_4 (30-50 μg/l) occurred in inoculated columns over a period of seven to nine days.

Introduction

Restoration of aquifer materials contaminated by chlorinated aliphatic hydrocarbons is a difficult endeavor, owing to the mobility and persistence of these solutes in subsurface environments. Bioremediation offers the potential for removing at least some of these compounds. Many chlorinated hydrocarbons can be transformed and/or completely mineralized by native aquifer flora under optimized growth conditions. In addition, organisms capable of degrading many chlorinated hydrocarbons have now been isolated, and these organisms can be introduced into contaminated aquifers for remediation. For this study, we used bench-scale methods to evaluate the feasibility of altering conditions within an aquifer to favor growth and colonization of an introduced organism (niche adjustment).

Pseudomonas sp. strain KC is a denitrifying bacterium that was isolated from aquifer materials collected from Orange County Water District Well #7 of the Naval Weapons Station, Seal Beach, California (Criddle et al., 1990). Studies conducted at Stanford University, the University of Idaho, and Michigan State University have shown that strain KC rapidly transforms carbon tetrachloride (CCl_4) to carbon dioxide and nonvolatile compounds without production of chloroform (Criddle et al., 1992; Lewis and Crawford, 1993; Tatara et al., 1993). Soluble (i.e., bioavailable) iron is inhibitory to CCl_4 transformation (Criddle et al., 1990; Lewis and Crawford, 1993; Tatara et al., 1993). Optimal CCl_4 transformation is obtained under denitrifying, iron-limited conditions. Iron-limited conditions can be obtained by increasing the medium pH to 8.0-8.2, a range of minimum solubility for ferric iron (Stumm and Morgan, 1981). Under these conditions, iron salts are precipitated, and iron inhibition of CCl_4 transformation is negligible (Tatara et al., 1993).

Criddle et al. (1990) and Tatara et al. (1993) postulated

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that CCl_4 transformation by strain KC is linked to a trace metal scavenging mechanism induced under iron-limited conditions. Many microorganisms secrete biomolecules, such as siderophores, to bind iron and transport it back to the cell membrane for assimilation. For strain KC, one of these secreted biomolecules reacts with CCl_4.

To assess the feasibility of niche adjustment under field conditions, ground water and aquifer materials were obtained from an aquifer of documented CCl_4 contamination at Schoolcraft, Michigan. The site has been under investigation by the Michigan Department of Natural Resources (MDNR) since 1987, and is referred to as "Plume A" in associated characterization reports. Plume A is defined as a region of ground-water contamination believed to be originating from an abandoned grain elevator located near the center of the Village (Figure 1). The primary contaminant characterizing Plume A is CCl_4, which has been detected in ground-water samples collected from monitoring wells positioned near the plume's center of mass at concentrations as high as 400 μg/l (HALLIBURTON NUS Environmental Corporation, 1991). Other contaminants are present locally within the plume at significantly lower concentrations including trichloroethene, chloroform, and 1,1,1-trichloroethane. Ground water in the Schoolcraft area also contains elevated concentrations of nitrate (up to 40 mg/l) and exhibits pH levels ranging from 7 to 8. The results of the MDNR-sponsored investigations reveal that Plume A extends approximately 3,700 feet downgradient from its source and impacts over 2 million cubic yards of aquifer material (HALLIBURTON NUS Environmental Corporation, 1991).

The aquifer impacted by Plume A is unconfined and consists of highly stratified glacial outwash deposits possessing prolific hydraulic characteristics. The depth to the water table in the vicinity of Plume A is approximately 15 feet below grade. The saturated thickness of the aquifer averages between 70 feet and 90 feet. Ground-water flow is essentially unidirectional, with an average linear velocity of 15 cm/day which is uniform throughout the aquifer in the Schoolcraft area (HALLIBURTON NUS Environmental Corporation, 1991). Hydraulic conductivities have been measured within the range of 10^{-1} to 10^{-2} cm/s.

A plan to remediate Plume A was put forth by the MDNR in the Winter of 1993. The primary objective of the

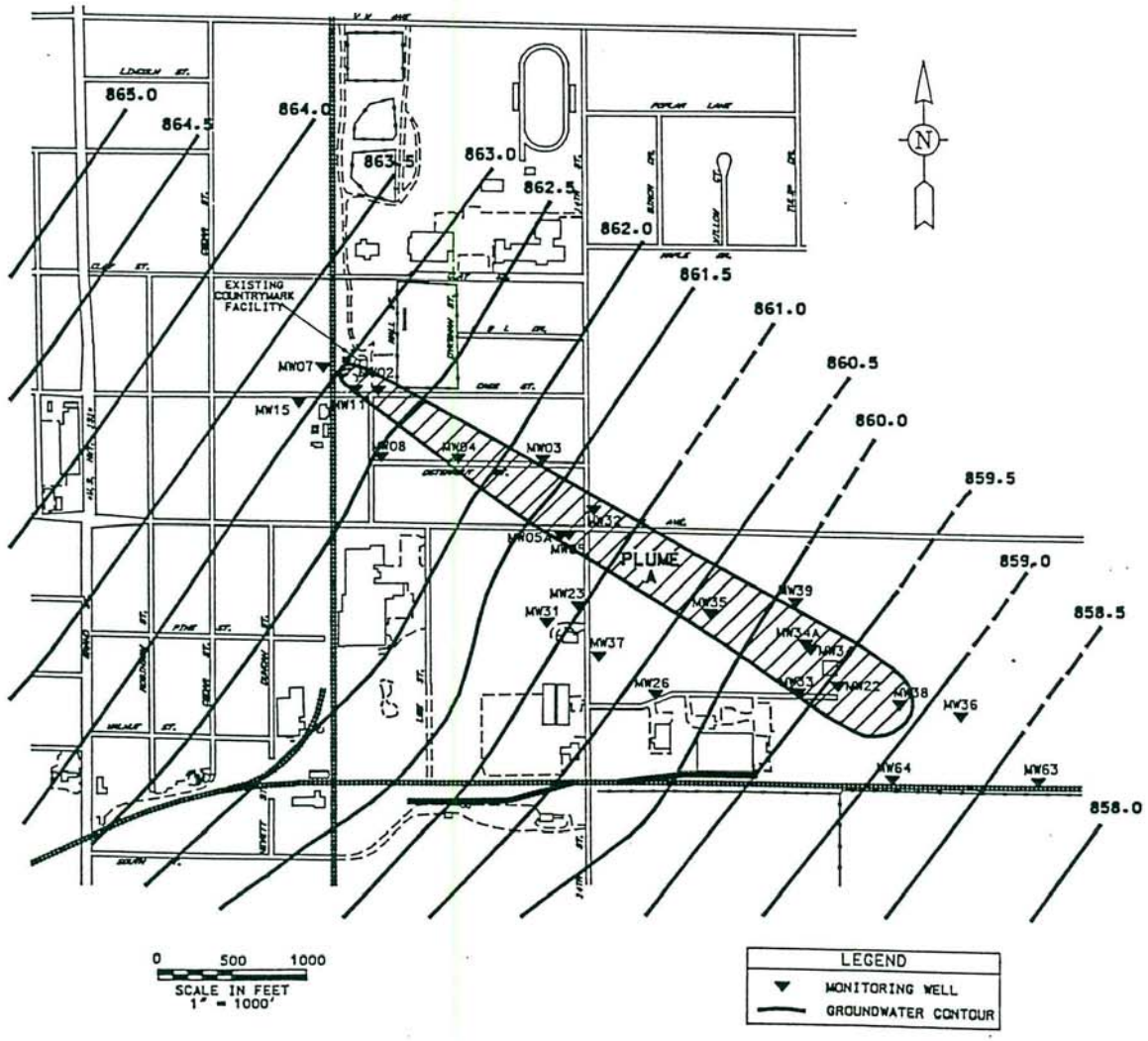

Fig. 1. Outline of the plume of CT (Plume A) in Schoolcraft, Michigan.

proposed remedial action is to constrain additional contaminant migration, and will entail the extraction of the impacted ground water using a single recovery well positioned a short distance beyond the downgradient limit of the plume. The extracted ground water is to be treated on-site by air stripping. Modeling efforts performed during the MDNR-sponsored investigation suggest that due to the highly transmissive character of, and CCl_4 sorption capacity within, the aquifer, the proposed remedial action may require up to 25 years to complete (HALLIBURTON NUS Environmental Corporation, 1991). Because Plume A is an orphan site of the State of Michigan, the cost to taxpayers to clean up the plume under the existing plan may exceed $5 million (HALLIBURTON NUS Environmental Corporation, 1991).

As a matter of policy, the MDNR recognizes the technical and economic benefits potentially afforded by implementing certain innovative treatment technologies, including bioenhancement and bioaugmentation, at sites like Plume A to reduce the duration of remedial action. Accordingly, the MDNR has endorsed plans to study the applicability of bioaugmenting the Schoolcraft aquifer with strain KC to remediate CCl_4.

Dybas et al. (1995) demonstrated that strain KC was capable of transforming CCl_4 in Plume A ground water if the water was supplemented with acetate and phosphorus and the pH was adjusted to about 8.2. Schoolcraft ground water was pumped continuously into a model aquifer column packed with CCl_4-saturated Ottawa sand to determine whether a CCl_4-transforming zone could be established and maintained by addition of base, acetate, and phosphorus. A region of strain KC colonization was obtained, and sustained removal of 20-30 μg/l CCl_4 was observed. Based on these and other studies, Dybas et al. (1995) concluded that the same conditions that are required for CCl_4 removal (i.e., addition of suitable electron donor, denitrifying conditions, and iron-limitation by base addition) simultaneously create a niche favorable for the growth and maintenance of strain KC in several nonsterile environments, including soils, ground water, and model aquifer systems. In the present study, the concept of niche adjustment is further extended to aquifer materials from the Schoolcraft site. The goal is to determine whether conditions in the aquifer materials can be modified to favor growth of strain KC and expression of its CCl_4-degrading capability, which, if successful, would justify field-scale demonstration of the bioaugmentation technique at the site.

In the present work, experiments were conducted using nine columns packed with Schoolcraft aquifer solids saturated with Plume A ground water. The use of columns enabled laboratory-scale simulation of Schoolcraft aquifer conditions. It also permitted parallel experiments to assess the fate of CCl_4 under niche-adjusted conditions within aquifer materials that were: (1) inoculated with strain KC; (2) noninoculated; and (3) chemically treated to suppress microbial activity. By measuring cumulative CCl_4 removal and protein production, we were able to assess sorption on Schoolcraft aquifer solids and CCl_4 transformation by strain KC.

Experimental Procedures

The experimental method used for this study is an extension of the method originally developed by Siegrist and McCarty (1987). Nine columns packed with Schoolcraft aquifer solids and saturated with ground water from the site were prepared. These columns were exchanged with ground water from the site in a rapid (20-30 minute) displacement of the pore fluid and then incubated for a period of days to weeks under static conditions. This exchange and incubation procedure was repeated until CCl_4 removal began to plateau, indicating equilibration of the sorbed CCl_4 with CCl_4 in the pore fluid (at least two successive exchange events were executed without significant differences between influent and effluent CCl_4 concentrations). Information obtained during this period was used to evaluate porosity and CCl_4 sorption. In subsequent exchanges, the niche was adjusted by addition of base, acetate, and phosphorus to create conditions favorable for growth of strain KC and expression of its CCl_4 transformation activity. Three columns (column set 1) were inoculated with strain KC; three (column set 2) were not inoculated; and three (column set 3) were chemically disinfected with thimersol.

The experiment was conducted in three phases: (1) determination of effective porosity by tracking bromide and CCl_4 breakthrough curves for each of the nine columns during the initial exchanges, (2) evaluation of sorption during initial incubation periods, and (3) assessment of niche adjustment and bioaugmentation with strain KC. In the first exchange event of the final phase, each column received ground water that was adjusted to pH 8.1 and supplemented with phosphate. Subsequently, in the second exchange of the final phase, an inoculum containing strain KC was introduced into column set 1. At the same time, the ground water used for exchanges of all three column sets was supplemented with acetate. Acetate, base, and phosphate supplements continued throughout the remainder of the experiment.

Aquifer Material

Aquifer material for column preparation was obtained by Brown & Root Environmental through hollow stem augers following Standard Penetration Test procedures (ASTM-D-1586-84). The boring from which the aquifer samples were collected was located approximately 50 feet downgradient of Michigan Department of Natural Resources (MDNR) monitoring well VS-MW-35, near the inferred center of mass of Plume A (Figure 1). At this location, the augers were advanced to a depth of 23 feet below grade (approximately 8 feet below the water table). Samples of aquifer solids then were acquired using split-barrel core sampling devices (18 inches in length, 2 inches in diameter) inserted through, and driven beyond, the terminal depth of the augers. A total of three samples were collected from a continuous interval between 25 and 29.5 feet below grade. Upon acquisition, cubic centimeter samples of soil and associated pore fluids were extracted from each core using sterilized 10 ml syringes that had been cut to remove their needle adapters so as to create a larger opening through which to draw the sample. The remaining mass of soil from

the cores was transferred to sterile glass mason jars and packed on ice for transport. Prior to drilling and sample acquisition, the hollow stem augers and split-barrel sampling devices were sterilized by high-pressure steam.

Ground Water

Ground water for column exchanges was obtained from monitoring well VS-MW-05, located within, and approximately 800 feet upgradient of the center of mass of Plume A (Figure 1). Before sample acquisition, approximately 50 gallons (approximately six well volumes) of ground water were purged from the well using a suction pump to ensure that the ground water sampled was representative of ambient aquifer conditions. Sample collection consisted of filling as many as two 5 gallon Nalgene® carboys with water withdrawn from the well using Teflon® bailers. Each carboy was filled completely to eliminate headspace within the container. The carboys were then placed on ice for transport to the laboratory where they were stored at 4° C. Periodically, an aliquot of ground water was removed from the carboy and used to measure concentrations of nitrate, nitrite, iron, and CCl_4.

Column Preparation

Nine Kontes® glass columns (30 cm length, 2 cm inside diameter) fitted with Teflon® leur lock stopcocks were sanitized by soaking in a solution of 0.06% hypochlorite, rinsed with sterile distilled water, and packed aseptically in a laminar hood with a slurry of Schoolcraft aquifer solids in degassed (e.g., CCl_4-free) Plume A ground water. The columns were periodically tapped during the filling process to enhance packing. Each column was connected to external Teflon® plumbing consisting of influent and effluent liquid transfer tubing and appurtenances necessary to make direct connections to a syringe pump. Once packed, columns were refitted with external plumbing (rendering the assemblage air-tight) and placed in a chamber at 10° C to simulate aquifer conditions. The columns were then connected to a Harvard® syringe pump. For a period of several hours, CCl_4-free, Plume A ground water was exchanged through each column to remove small bubbles and stabilize dissolved oxygen concentrations to the levels present in the ground water.

Preparation of Exchange Fluids and Calibration Standards

Effluent fractions generated during each exchange of pore fluid were collected in 28 ml glass Balch tubes sealed with Teflon®-lined butyl rubber septa. By inserting a hypodermic needle through the septa and withdrawing 5 ml of air, a vacuum was created in each tube prior to sample collection preventing pressurization of the tubes during sample collection. Creating the vacuum also served to promote volatilization of CCl_4 from the sampled ground water into the headspace of the Balch tube.

To prepare calibration standards for each exchange event, five Balch tubes were injected with 5 ml of CCl_4-free (air stripped) Schoolcraft ground water. Standard stock solution (8.3 ηg CCl_4 / ml methanol) was then added to four of the five tubes in 5, 10, 20, and 30 μl aliquots, respectively. The fifth tube served as a CCl_4-free blank.

After preparation of calibration standards, approximately 300 ml of Plume A ground water was transferred from sample carboys to a sterilized glass beaker. Niche adjustment and substrate/nutrient addition was accomplished by transferring 0.55 ml of 0.1 M NaOH, 1.8 ml of 0.1 M sodium acetate, and 0.3 ml of 0.1 M KH_2PO_4 solution from sterile serum bottles to the beaker. The resulting solutions (pH 8.1, 0.59 mM acetate, 0.099 mM phosphate phosphorus) were then mixed aseptically and transferred to two 250 ml Teflon® syringes. Column set 3 (chemically inhibited control columns) also received 200 mg/l of Thimersol.

Exchange Protocol

Syringes were loaded into the syringe pump and connected to the external plumbing of the column set to be exchanged. The pump was then activated and Plume A ground water pumped through the influent transfer lines at a rate of 2.5 ml/min. To ensure that these lines were adequately flushed of prior exchange fluids, and to quantify the initial concentration of CCl_4 in the influent ground water, up to three 5 ml samples of the ground water were collected prior to initiating the pore fluid exchange within the first column of a set. This was accomplished by disconnecting the influent transfer line from the third column of the set and attaching a hypodermic needle to facilitate injecting the ground water through the septa of the sample tube. After sampling, the influent line was reconnected. The stopcocks on the top and bottom of the first of the three columns in the set were then opened, and the exchange of fluid initiated.

During exchanges, 60 to 65 ml of ground water were pumped through each column at a rate of 2.5 ml/min. Effluent samples were collected in 5 ml fractions in the sealed, evacuated Balch tubes.

Inoculation

Inoculation of column set 1 was performed in a single exchange event. Column set 2 was "mock inoculated" following similar procedures. Inoculation entailed injecting a 1 ml suspension of strain KC cells directly into each of the three columns prior to the exchange of pore fluids. This was accomplished by attaching a sterile 1 ml syringe containing the suspension (4.7×10^{10} cells of strain KC in 100 mM KH_2PO_4 buffer, pH 8.0) to the influent transfer line for each column, and quickly dispensing the inoculum. Immediately following inoculation, the transfer lines were reattached and exchange of ground water commenced. "Mock inoculation" of column set 2 was accomplished in the same manner as inoculation in column set 1, but the inoculum consisted only of 100 mM KH_2PO_4 buffer, pH 8.0. Effluent fractions were acquired aseptically by sterilizing with ethanol swab the septa on each Balch tube and the hypodermic needles used to transfer pore fluids from the columns to the tubes.

Analyses and Enumeration

CCl_4 and chloroform concentrations were measured in all effluent fractions collected during this study. Initially, bromide was also measured to determine effective porosity

and breakthrough characteristics. After niche adjustment, additional effluent analyses included pH, phosphate, protein, and strain KC cell numbers.

CCl_4 and chloroform were quantified by withdrawing a 0.01 ml aliquot of gas from the headspace of each fraction and calibration standard and injecting the gas sample into a Perkin Elmir model 8500 gas chromatograph equipped with a 100/120-mesh column (10% Alltech CS-10 on Chromsorb W-AW; Alltech catalog no. 12009 PC). A complete description is provided by Tatara et al. (1993). When possible, headspace analyses were performed within a half hour of sample acquisition. Occasionally, circumstances required overnight storage (at 4° C) before analysis. Of these samples, all were analyzed within 24 hours of collection.

Bromide and phosphate were measured by ion chromatography (Dionex model 2000i/SP ion chromatograph with suppressed conductivity detection equipped with a Sarsep AN 300 anion exchange column and utilizing a 1.8 mM bicarbonate/1.7 mM carbonate mobile phase at 1 ml/min). Chromatographs were recorded and data integrated using a Spectra Physics model SP 4270 integrator. External standard calibration curves were prepared by diluting primary ion standards into secondary water standards with the same ionic composition as the test samples.

Measurements of pH were obtained with an Orion model 720A pH meter. Protein was determined by the modified Lowry method, with bovine serum albumin as the standard (Markwell et al., 1981). Cell numbers were estimated by serial dilution/standard plate count methods and verified by most probable number analysis (MPN), as per Dybas et al. (1995).

Mass Balances

In order to evaluate the fate of CCl_4, mass balances were performed using effluent concentrations for each exchange event. Our analysis follows the protocol of Siegrist and McCarty (1987), with minor modifications.

Figure 2 illustrates key concentration and volume relationships important in this analysis. C_1 is the CCl_4 in the column pore fluid prior to exchange. C_2 is the concentration of CCl_4 in the pore volume at the conclusion of the exchange, following breakthrough. C_0 is the concentration of the influent for each exchange.

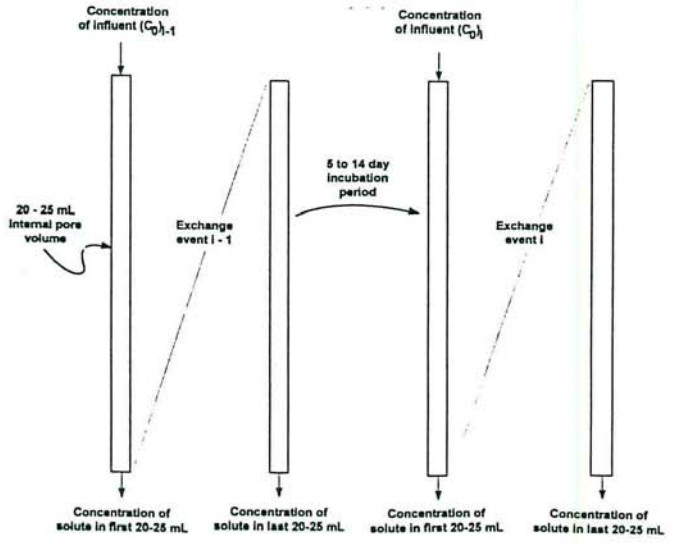

Fig. 2. Column exchange schematic.

During the initial exchange of fluids in each column, breakthrough of both bromide and CCl_4 were evaluated. For this first exchange, $C_1 = 0$. Using the bromide data, the effective porosity, ϵ, of the aquifer media within each column was calculated by:

$$\epsilon = \frac{M}{(C_2 - C_1)}\left(\frac{1}{V_{column}}\right) = \frac{V_{pore}}{V_{column}} \qquad (1)$$

when $C_2 = C_0 = C_{column}$, and $M = C_{column} V_{column} \epsilon = C_2 V_{pore}$.

Unlike bromide, CCl_4 was sorbed to the aquifer solids during exchange. CCl_4 also underwent more dispersion than bromide. These two mechanisms were reflected in the shape of the CCl_4 breakthrough profile, which lagged behind and sloped less than the profile for bromide (Figure 3). Analysis of the CCl_4 data indicated that a minimum exchange volume of 45-50 ml was required for complete breakthrough.

Mass removal between exchange events was assumed to be the result of either sorption or biotransformation. Thus, to determine the cumulative mass removed between exchange events, it was only necessary to know the concentration in the effluent at the end of an exchange event $(C_2)_{i-1}$ and the concentration exiting the column at the beginning of the next exchange event $(C_1)_i$. These data enabled estimation of cumulative mass removal between exchange events M_r:

$$M_r = V_{column}\epsilon \sum_{i=1}^{n} (C_2^{i-1} - C_1^i) \qquad (2)$$

Prior to inoculation and niche adjustment, information on cumulative mass removal was used to quantify sorption. Sorption was estimated by comparing the total mass of CCl_4 removed to the liquid phase concentration of CCl_4 present in the pore fluid once the solids were saturated. This ratio is defined as the dimensionless equilibrium partition coefficient, R_p, where:

$$R_p = \frac{M_r}{M_{dissolved}} = \frac{V_{column}\epsilon \sum_{i=1}^{n} (C_2^{i-1} - C_1^i)}{V_{column}\epsilon C_1^i} \qquad (3)$$

With knowledge of R_p, the retardation factor R_t and distribution coefficient K_d (cm^3/g) for CCl_4 in the Schoolcraft aquifer materials could be estimated from:

$$R_t = \frac{V_{eff}}{V_{contaminant}} = R_p + 1 \qquad (4)$$

$$K_d = \frac{R_p \epsilon}{\rho_b} \qquad (5)$$

where v_{eff} = average linear velocity of ground-water flow through the aquifer solids within the column (cm/hr); $v_{contam.}$ = the average linear velocity of the solute front in the aquifer

Table 1. Characteristics of Schoolcraft Aquifer Materials

Parameter	Media	
	Soil	Ground water
pH	8.8	7.04-7.5
Alkalinity (as $CaCO_3$)	—	331 mg/l[1]
Hardness (as $CaCO_3$)	—	410 mg/l[1]
Fraction of organic carbon	0.1%	—
Iron	37 mg/Kg	11 mg/l
Copper	—	.03 mg/l[1]
Nitrate	<1 mg/Kg	39 mg/l
Phosphate	—	60 mg/l[1]
Sulfate	—	16 mg/l[1]
Carbon tetrachloride	<1 μg/Kg	30-50 μg/l
Microbial population	2.3×10^7 CFU/g	1×10^6 CFU/ml

[1]Brown & Root Environmental.
CFU — cell forming unit.

solids within the column (cm/hr); and ρ_b = soil bulk density (g/cm^3).

Kinetics of Biotransformation

Tatara et al. (1993) showed that the transformation of CCl_4 by strain KC is first order with respect to the solute concentrations within the range evaluated during this study (30-50 μg/l). Assuming a first-order kinetic expression and equilibrium between the sorbed and dissolved phases, the following mass balance can be obtained:

$$-\frac{dM_{CCl_4}}{dt} = k'' C_{liquid} V_{column} \epsilon = k'' \left(\frac{M_{CCl_4}}{V_{liquid} + K_d M_{soil}} \right) V_{liquid} \quad (6)$$

$$\ln\left(\frac{M^i_{CCl_4}}{M^{i-1}_{CCl_4}} \right) = -\left(\frac{k'' V_{column} \epsilon}{V_{column} \epsilon + K_d M_{soil}} \right) t \quad (7)$$

where k'' = apparent first-order rate coefficient (day^{-1}); C_{liquid} = concentration of CCl_4 in liquid-flled volume of the column (μg/l); M_{CCl_4} = mass of CCl_4 within column at (i − 1) and i (μg); M_{soil} = mass of soil in column (g); and t = time interval between (i − 1) and i (days).

Results and Discussion

Characterization of Aquifer Materials

Table 1 summarizes selected physical and chemical characteristics of the Plume A ground water and Schoolcraft aquifer solids, as determined from analyses performed during this study and previous MDNR-sponsored investigations. These data indicate that the Schoolcraft aquifer materials currently support a significant microflora (measured in numbers of cell forming units per gram of soil or ml of ground water in Table 1). In fact, it is possible that the chloroform detected in ground-water samples from one of the monitoring wells (well number VS-MW-05, HALLIBURTON NUS Environmental Corporation, 1991) may have originated from the biotransformation of CCl_4 by indigenous microorganisms since alternative sources of chloroform are unknown.

Over the ground-water pH range reported in Table 1 (7.04-7.5), copper is toxic to strain KC (Tatara et al., 1993). At higher pH levels, copper is not inhibitory to cell growth and, in fact, is required for CCl_4 transformation (Tatara et al., 1993). In addition, total iron concentrations in the ground water (11 mg/l) are at levels likely to be inhibitory to CCl_4 transformation. As a result, growth of strain KC and associated transformation of CCl_4 required pH adjustment to reduce the solubility of copper and iron.

The concentrations of nitrate (39 mg/l) in the Schoolcraft ground water are sufficient to sustain denitrification by strain KC and support CCl_4 transformation (Dybas et al., 1995). Although the phosphate data reported in Table 1 suggest that phosphorus is present, batch growth experiments and analysis of the ground water used for the exchanges indicated that phosphorus was limiting microbial growth.

CCl_4 concentrations in the ground water used for the column exchanges were consistently within the range of 30 μg/l and 50 μg/l. No detectable levels of CCl_4 were measured (via thermal desorption and gas chromatography) on the aquifer materials used for column preparation.

Flow-Through Properties

Results of the bromide tracer experiments are presented in Table 2. Composite bromide breakthrough profiles for each of the three column sets are presented in Figure 3. The flow-through characteristics for each of the nine columns are similar. In general, the effective porosities of the re-packed aquifer solids within the columns were 44% to 52%, indicating internal pore volumes of 22-26 ml. The average linear velocities, v_{eff}, of ground-water flow during each exchange were determined from the porosity data and the superficial velocity, v (1.41 cm/min), to range from 2.7-3.2 cm/min. The Reynolds number ($R_e = vd/\upsilon$) was 0.235 assuming an average soil grain diameter, d, of 0.1 cm, and kinematic viscosity, υ, of 36 cm^2/hr at 20° C (Siegrist and McCarty, 1987). A value of R_e less than unity indicates that intertial forces dominate. Therefore, flow was essentially laminar during the exchanges (Freeze and Cherry, 1979).

The movement of ground water through each column deviated slightly from ideal plug flow. A composite breakthrough profile for the nine sets of tracer experiments (Figure 4) was used to evaluate the dispersion characteristics of the aquifer solids within the columns. The first 15 ml of pore fluids exchanged was essentially free of bromide, and a total throughput of 35 ml to 40 ml of ground water was necessary to achieve complete breakthrough, or saturation. The approximate spreading of the breakthrough front (as defined by the dimensionless quantity $D_{dis}/v_{eff}L$, where L is the column length) was approximately 0.01, giving a dispersion coefficient, D_{dis}, of less than 58 cm^2/hr.

Sorption Characteristics

Composite breakthrough profiles were developed for CCl_4 and bromide for each of the three column sets (Figure

Table 2. Column Specifications, Flow-Through Properties, and CCl_4 Sorption Characteristics

Parameter	Column set 1 (Inoculated) Mean	± Std. dev.	2 (Noninoculated) Mean	± Std. dev.	3 (Thimersol treated) Mean	± Std. dev.
Column specifications:						
Length (cm)	30	—	30	—	30	—
Diameter (cm)	1.5	—	1.5	—	1.5	—
Empty volume (ml)	50	—	50	—	50	—
Flow-through properties:						
Effective porosity, ϵ	.47	.01	.46	.01	.52	.03
Pore volume, V_0 (ml)	23.7	.6	23	.4	24.3	1.7
Soil bulk density, ρ_b (g/cm^3)	1.4	0	1.4	0	1.4	0
Superficial velocity, v (cm/min)	1.4	0	1.4	0	1.4	0
Average linear velocity, v_{eff} (cm/min)	3.0	0.09	3.0	0.03	2.7	0.19
Dispersion coefficient D_{dis} (cm^2/hr)	54.0	1.62	54.0	0.63	48.6	3.42
Reynolds number, R_e	.23	—	.23	—	.23	—
CCl_4 sorption characteristics:						
Dispersion coefficient, D_{dis} (cm^2/hr)	270.0	8.1	270.0	3.15	243.0	17.1
Equilibrium partition coefficient, R_p	.82	.34	1.02	.11	.45	.12
Retardation coefficient, R_t	1.82	.34	2.02	.11	1.45	.12
Distribution coefficient, K_d (cm^3/g)	.28	.11	.33	.04	.16	.05
CCl_4 biotransformation characteristics:						
Apparent first-order rate coefficient, k'' (day^{-1})	4.14	.32	—	—	—	—

3) and the complete set of nine columns (Figure 4). As illustrated in the figures, CCl_4 was retarded in comparison to bromide. CCl_4 concentrations first were observed in the columns after 20 ml of pore fluids had been exchanged. Saturation of a column during an exchange event was achieved after exchange of 45-50 ml of CCl_4 ground water. Concentrations within the first four and last five 5 ml fractions of fluids emerging from the columns during exchanges were averaged to determine C_1 and C_2, respectively. The CCl_4 breakthrough profile composited from the complete set of nine columns was typical of flow through a closed vessel (Levenspiel, 1979) with a dispersion coefficient, D_{dis}, of less than 160 cm^2/hr (Table 2).

The ratio of solute mass sorbed to aquifer solids in pore fluids defines the equilibrium partition coefficient R_p. A summary of the change in the magnitude of R_p for each column set is presented on Figure 5. After five to six exchanges in each column set, R_p values stabilized, indicating that sorption was essentially complete. The resulting "equilibrium" R_p values range from a low of 0.3 (Column 9) to a high of about 1.3 (Column 1) (Table 2). Therefore, retardation factors of 1.3 to 2.3 characterize the movement of CCl_4 relative to the rate of ground-water flow in the columns of repacked aquifer solids.

From the values of R_p reported in Table 2, a range of distribution coefficients were calculated. K_d values between 0.1 cm^3/g to 0.43 cm^3/g were estimated (Table 2). This indicates that 33% to 70% of the total CCl_4 mass in the columns was retained on the solid matrix following the completion of sorption. In general, the magnitude of sorption was most significant and comparable in column sets 1 and 2.

Transport of Strain KC and Colonization of Aquifer Materials

As illustrated in Figure 6, strain KC is readily transported through re-packed Schoolcraft aquifer solids. In fact, breakthrough was more rapid than the average linear velocity of the exchange fluids (as defined by bromide breakthrough), probably owing to charge and/or pore size exclusion and the motility of the organism. Only about 40% of the mass of cells introduced were washed from the inoculated columns during the first exchange following inoculation. The remaining 60% were retained on the column. The mechanism of retention is unknown.

Figure 7 illustrates the incremental growth of strain KC as estimated from protein levels measured during each exchange following inoculation. Strain KC cells remaining in each column after inoculation (i.e., about 2.6×10^{10} cells/ml) provided an ample inoculum for the aquifer materials. Growth occurred rapidly within the first 15 days following inoculation. Protein levels essentially stabilized after 22 to 25 days, suggesting a balance between growth and decay. The results from standard plate counts and MPN analyses (data not shown) reveal that the overwhelming majority of suspended growth cell forming units (CFUs) in column set 1 exhibited a colony morphology distinctive of strain KC. No net accumulations of protein were observed in the effluent samples from either column set 2 or column set 3 following niche-adjustment.

Biotransformation of CCl_4

Prior to niche adjustment, sorption was virtually complete in each of the three column sets, as evidenced by the lack of significant removal of CCl_4 between exchanges.

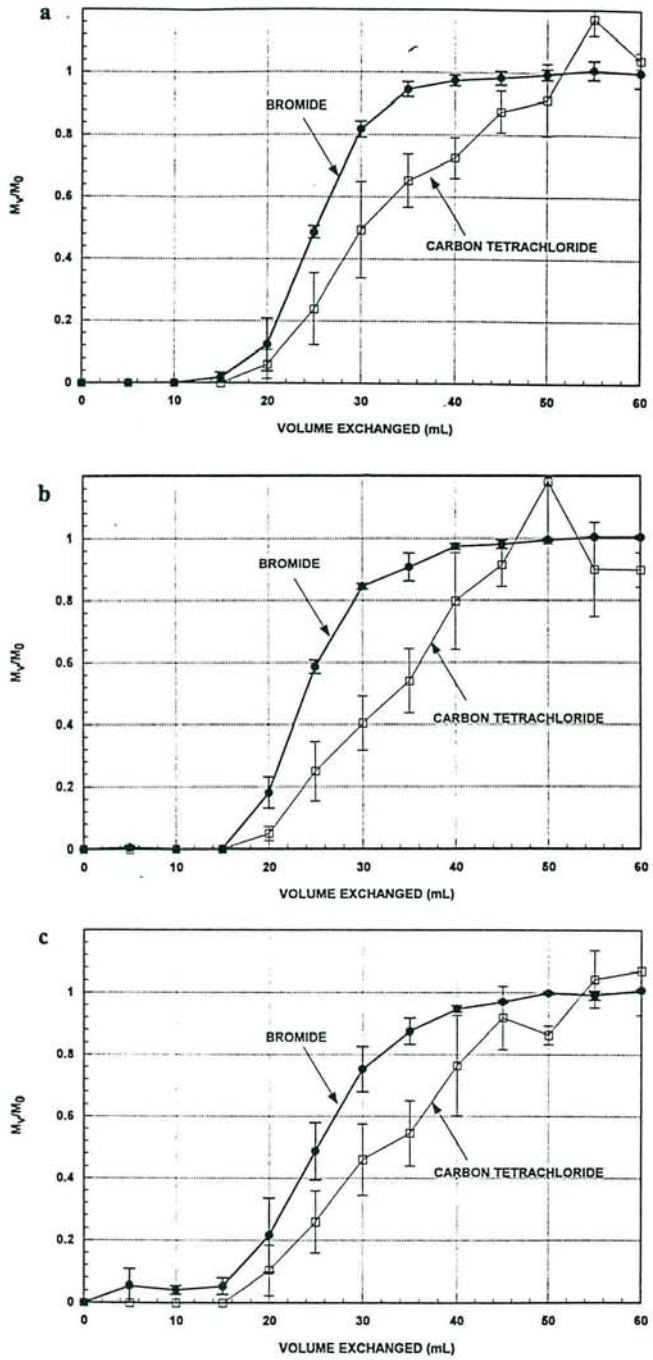

Fig. 3. Bromide and CT breakthrough profiles; a) column set 1 (columns 1-3); b) column set 2 (columns 4-6); and c) column set 3 (columns 7-9).

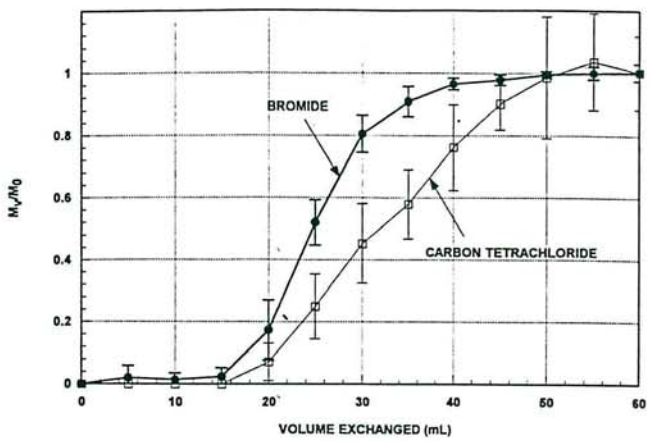

Fig. 4. Breakthrough profile for bromide and CT composited from columns 1-9.

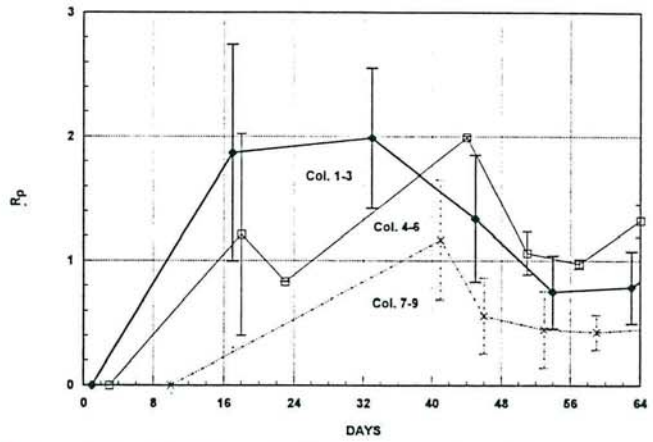

Fig. 5. Dimensionless equilibrium coefficient, R_p, measured during column exchanges through saturation of each column with CT. Saturation is indicated by stabilization of R_p values which generally occurred after four to five exchange events.

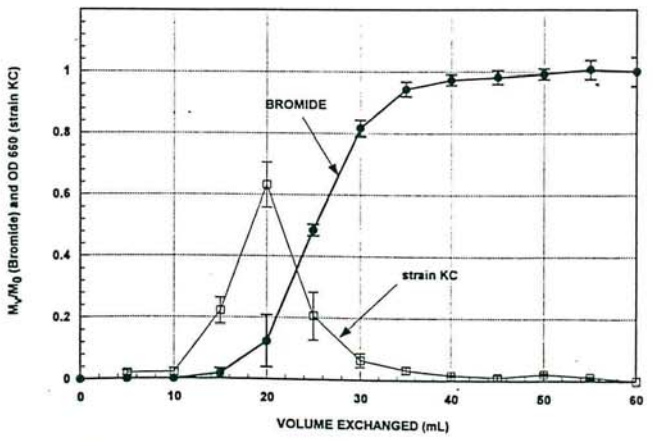

Fig. 6. Breakthrough of strain KC in column set 1 (columns 1-3) as compared to the breakthrough of bromide for that column set.

After niche adjustment, CCl_4 removal resumed in the inoculated column set (columns 1-3), indicating biotransformation of CCl_4.

Figure 8 illustrates the cumulative removal of CCl_4 within each of the three column sets over the duration of the study. CCl_4 removal increased sharply in column set 1 following inoculation with strain KC. No chloroform was detected in effluent samples from column set 1. No distinct increase in CCl_4 uptake was observed in the other two column sets. For column set 3, this observation was expected. However, there was some expectation that niche

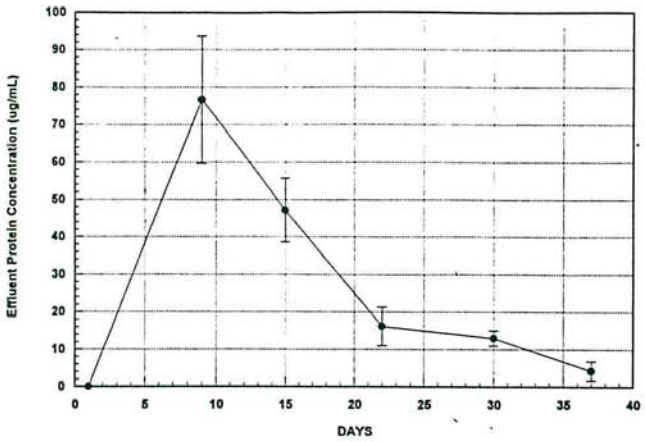

Fig. 7. Effluent protein concentrations as measured in column set 1 (columns 1-3) during exchange events following inoculation.

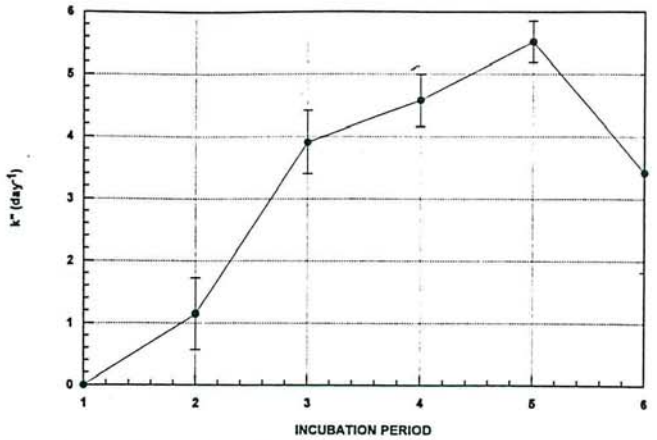

Fig. 9. Apparent first-order rate coefficient as measured in column set 1 (columns 1-3) during exchange events inoculation.

adjustment within the nonsterilized column set (column set 2) would result in stimulation of indigenous microorganisms capable of transforming CCl_4. Although there was some evidence of limited CCl_4 removal in column set 2 following niche adjustment, the trend of CCl_4 uptake was neither distinct nor consistent.

Figure 8 reveals a trend that was common to each of the three inoculated columns. After inoculation, CCl_4 levels steadily decreased. With the exception of the period prior to the last exchange event (which coincided with a sharp increase in mass loading), CCl_4 levels decreased at a relatively consistent rate. Further, CCl_4 removal during this period was equal to or exceeded the mass of CCl_4 loaded into the columns during an exchange, indicating removal of sorbed contaminant.

Figure 9 illustrates changes in the apparent first-order rate, k″, of CCl_4 mass removal in column set 1 during the period of niche adjustment (e.g., days 72 through conclusion of study). From these data, an average k″ of 4.14 per day was obtained. Laboratory studies have shown that the rate of CCl_4 transformation in batch experiments is a function of culture age, and decay of transformation activity occurs as cells enter a stationary phase (Tatara et al., 1993). The k″ value reported herein reflects this loss of activity. Approximately 50% to 70% of the mass of soluble CCl_4 input into column set 1 was removed during the incubation periods between exchanges.

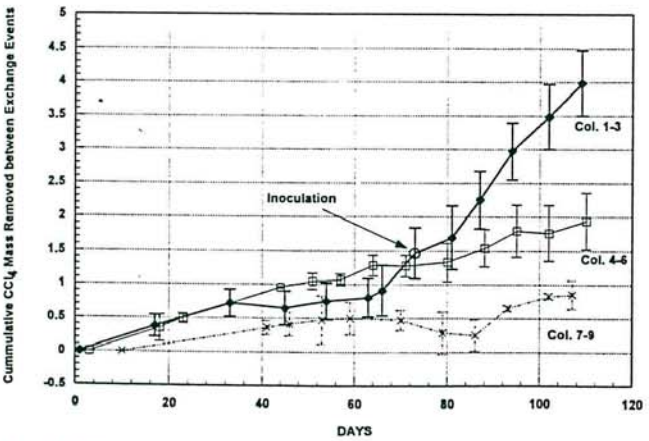

Fig. 8. CT mass removal measured over the duration of the experiment.

The cumulative mass removal data from the column experiments indicate that strain KC will co-metabolize CCl_4 in niche-adjusted Schoolcraft aquifer materials. The apparent first-order rate coefficient reported here, although slightly lower than anticipated, is high enough to expect that favorable rates of CCl_4 transformation will be attainable from engineered bioaugmentation of Plume A.

The results suggest that bioaugmentation of the Schoolcraft aquifer with strain KC is feasible. However, caution is warranted when extrapolating to field conditions. Repacking of the Schoolcraft aquifer solids resulted in a significant increase in effective porosity. Typical values of effective porosity for close-packed, medium-grained, alluvial sands are 15-30% (Bear, 1979). Data collected during aquifer performance tests in the vicinity of Plume A revealed that specific yield values of 10-20% are typical of the Schoolcraft aquifer materials (Brown & Root Environmental). Ground-water models developed during previous MDNR-sponsored investigations have assumed effective porosities of 20-25%. Thus, column packing may have resulted in a 50-100% increase in effective porosity. As a result of the increase in effective porosity from re-packing, the magnitude of CCl_4 sorption that was quantified during Phase II likely was less than would be expected in the field. However, the distribution coefficients estimated from the sorption experiments were consistent with fitted values obtained by contaminant transport modeling efforts at the Schoolcraft site (HALLIBURTON NUS Environmental Corporation, 1991). Since the apparent first-order rate coefficient reported herein was corrected for sorption, it is likely to be similar to the removal rate expected in the field.

The discrepancy between laboratory and field estimates of effective porosity must also be considered when evaluating the transport characteristics of strain KC. It is probable that strain KC will be transported somewhat less effectively under field conditions because of cell straining in materials of lower porosity.

Conclusions

Column experiments appear to be an effective means for evaluating the potential for bioaugmentation. The experimental protocols were simple, and results were produced in a time-frame typical of bench-scale treatability studies of soil and ground-water remediation technologies. In addition, the use of columns enabled simulation of the anticipated field-scale sequence of chemical additions and inoculation.

The results indicate that strain KC is readily transported through repacked Schoolcraft aquifer materials. After inoculation, strain KC rapidly grew and colonized the aquifer materials. The expression of CCl_4 tranformation activity was immediate and significant. Up to 70% removal of soluble CCl_4 was observed over periods of approximately seven to nine days. Bioaugmentation of the Schoolcraft aquifer with strain KC appears feasible and merits further evaluation at the field scale.

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