Ground Water Monitoring&Remediation

Column Studies of Anaerobic Carbon Tetrachloride Biotransformation with Hanford Aquifer Material

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Abstract

Continuous-flow and batch experiments were conducted using a column reactor system containing Hanford Aquifer material in order to assess the potential of in situ bioremediation of carbon tetrachloride (CT) at the Hanford site in south-central Washington state. Benzoate and acetate were evaluated as primary substrates both in the presence and absence of nitrate as a potential electron acceptor. Each of the four resulting test conditions was first run under continuous-flow mode until a pseudo–steady state was achieved and then switched to batch mode. The longer residence time of the batch portion of the test resulted in more complete transformations and helped elucidate zones of variable activity within the column. Reductions in CT concentration and chloroform (CF) production were observed in all test conditions. Benzoate generally supported faster and more complete CT transformations than acetate, even though substrate use and denitrification was more rapid for acetate. Sulfate was present in all cases; yet, sulfate reduction was never observed, even during extended absences of nitrate and nitrite. CT transformation was most rapid near the point of injection, with rates decreasing toward the effluent end of the column. These results indicate that the microbial population at Hanford is capable of transforming CT in the subsurface. However, methods to control the production of CF may be necessary before this technology can be successfully implemented in the field.

Introduction

The Hanford Nuclear Reservation located in south-central Washington state has been a defense materials production complex since 1943. Carbon tetrachloride (CT, CCl_4) was used extensively at Hanford in organic solvent mixtures designed to recover plutonium from irradiated uranium fuel rods. The waste CT was disposed of in three unlined, subsurface liquid-disposal facilities that permitted direct infiltration into the underlying soil column. Up to 580,000 L were discharged to the soil between 1955 and 1973. Although <2% of this has been accounted for in the ground water, ~75 m below the land surface, a plume of ~13 km^2 has been observed (Last et al. 1991). The feasibility of in situ biotransformation as a means to remediate the CT-contaminated soil and ground water at Hanford has been an area of active research. However, the potential of this technology to succeed at the Hanford site remains in question. Of primary concern is the production of chloroform (CF, $CHCl_3$) as a toxic by-product of the biotransformation process by the microbial consortia indigenous to Hanford (Sherwood et al. 1999).

CT is a known carcinogen with a drinking water maximum contaminant level of 5 μg/L designated by the U.S. EPA. Although CT is classed as a dense nonaqueous phase liquid, its aqueous solubility of ~800 mg/L (Montgomery 1991) greatly exceeds the 5 μg/L federally allowable level in drinking water. Little potential exists to transform CT under aerobic conditions as a result of its fully oxidized nature (Vogel et al. 1987). The most commonly observed biological transformation process has been reductive dechlorination. Considerable variability has been observed in the extent of lesser-chlorinated intermediates resulting from the biotransformation of CT. Some degree of CF production has been documented in the majority of CT biotransformation studies. Dichloromethane (DCM, CH_2Cl_2) has been observed under some highly reducing conditions (Semprini et al. 1991). These lesser-chlorinated by-products are more amenable to biotransformation under aerobic conditions (Alvarez-Cohen and McCarty 1991; Kim et al. 2000) and can tend to accumulate under the anaerobic conditions conducive to CT transformation.

Biological mineralization of CT (complete transformation to CO_2) by *Pseudomonas stutzeri* KC has been observed (Criddle et al. 1990). KC has been found to perform optimally in a pH range of 8.0 to 8.9 under denitrifying conditions and with sufficient concentrations of nitrate and electron donor (Lewis and Crawford 1993; Tatara et al. 1993; Dybas et al. 1995; Witt et al. 1999). Recent work with KC has been primarily involved with the Schoolcraft,

Michigan site. In column experiments, ~70% removal of CT was achieved in Schoolcraft sediments without the production of CF (Mayotte et al. 1996). In a pilot field study, bioaugmentation with KC resulted in CT transformation; however, some CF was observed after 1 month (Dybas et al. 1998). It was speculated that the CF production was related to CT transformation by native microorganisms instead of, or in conjunction with, KC after an extended period. The results of a multiyear, full-scale biocurtain experiment with KC showed nearly full removal of CT with little or no CF production at the Schoolcraft site (Hyndman et al. 2000; Dybas et al. 2002). While the KC culture has been highly successful in these experiments, long-term performance remains to be demonstrated since bioaugmentation, pH adjustment, and excess nitrate are required. Also, prolonged treatment may result in the indigenous microbes becoming the dominant CT transformers, which lack KC's transformation abilities.

Past research in support of CT remediation at the Hanford site has involved denitrifying cultures isolated from Hanford soil and grown externally (Skeen et al. 1993; Stensel and DeJong 1994; Truex et al. 1994; Petersen et al. 1994). Nitrate reduction has particular appeal in this case due to significant nitrate contamination in Hanford ground water (Table 1). Interestingly, the results of Sherwood et al. (1996, 1999) showed that relatively greater rates of CT transformation were achieved under nitrate-limiting conditions. CT transformation continued in the absence of a primary substrate, although at a lesser rate, with less CF production on a relative basis.

Although the majority of CT biotransformation studies have been performed under denitrifying conditions, successful results have been achieved under sulfate-reducing (Bouwer and Wright 1988; Egli et al. 1988, 1987; Devlin and Müller 1999) and methanogenic (Bouwer and McCarty 1983; Galli and McCarty 1989; Mikesell and Boyd 1990) conditions. Zones of progressively reducing conditions have been observed as a result of the continuous flow of substrate through a single column (Cobb and Bouwer 1991). Since transformation rates for chlorinated aliphatic hydrocarbons (CAHs) are generally greatest under highly reducing conditions (Vogel et al. 1987; Bouwer and Wright 1988) and sulfate is present in Hanford ground water (Table 1), it is of interest to determine the effect of more reducing conditions on CT transformations in Hanford soil.

Table 1
Comparison of Hanford Ground Water Chemistry to Synthetic Ground Water

	Observed Range[1]	Synthetic Ground Water
K^+ (mg/L)	3–6	5.1
Na^+ (mg/L)	21–52	0
Ca^{2+} (mg/L)	17–59	17 or 0[2]
Mg^{2+} (mg/L)	6–17	9.7
Cl^- (mg/L)	4–22	4.6
NO_3^- (mg/L)	27–162	53 or 0[2]
SO_4^{2-} (mg/L)	17–47	38
HCO_3^- (mg/L)	76–155	0
pH	7.6–7.9	7

[1]From Last et al. (1991).
[2]The addition of calcium nitrate was used for the test conditions with nitrate.

This work assessed the potential for in situ CT biotransformation under anaerobic conditions that mimicked the environmental conditions at the Hanford field site. Benzoate ($C_6H_5COO^-$) and acetate (CH_3COO^-) were evaluated as primary substrates for inducing the transformation of CT. Benzoate has been shown to be an effective substrate for inducing the anaerobic transformation of perchloroethylene (C_2Cl_4) (Beeman et al. 1994) and may ferment to produce hydrogen as a potential electron acceptor for dehalogenation reactions (Yang and McCarty 1998). Conversely, acetate has been used extensively in CT biotransformation studies (Stensel and Dejong 1994; Truex et al 1994; Petersen et al. 1994; Doong and Wu 1996, among others). The specific objectives of this study were to (1) evaluate the effect that different primary electron acceptors present in Hanford ground water had on CT transformation; (2) compare the effectiveness of benzoate to that of acetate as the primary substrate driving CT transformation; (3) observe the extent of CF production as a result of CT biotransformation under the different electron donor/acceptor conditions; (4) detect possible problems resulting from microbial stimulation such as flow impediment due to excessive biological growth; (5) evaluate a flow/batch scenario as a means to increase the effective residence time in small-scale column experiments; and (6) determine spatial variability in CT transformation rates.

Materials and Methods

The experiments used Hanford Aquifer material and were performed in a column-type bioreactor system run in both continuous-flow and batch modes. A schematic of the column reactor system is shown in Figure 1. The reactor was constructed of stainless steel and had the following internal dimensions: length = 30 cm, diameter = 5.4 cm, and volume = 690 mL. The ends were sealed with removable end caps equipped with dual Viton O-rings. Stainless steel mesh was placed on the inside of the end caps. Four sample port lugs were installed at distances of 3.5, 7.6, 14.0, and 23.0 cm from the base with short pieces of 3.2-mm stainless steel tubing extending to the column center. The sample ports were sealed with 3.2-mm ball valves.

The core sample was obtained from a CT-contaminated location at the 200 West area of the Hanford site. The sample was obtained from the saturated zone, ~75 m below the ground surface, that lacked measurable dissolved oxygen. It was shipped from the site in a sealed acrylic sleeve that was purged with argon gas in order to maintain anaerobic conditions. The core was stored, as received, at 4°C for ~7 months prior to use, while the experimental system was being constructed. The sealed container was opened in an anaerobic glove box under a nitrogen/hydrogen atmosphere and the soil was transferred to the reactor column, while continuously maintaining an upward flow of deaired synthetic ground water to maintain saturated conditions. The

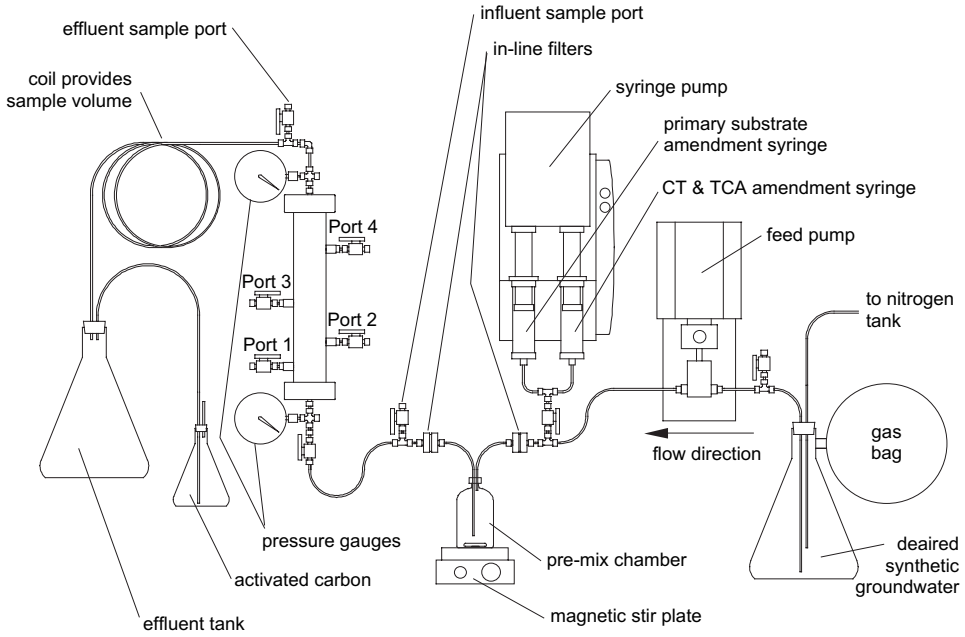

Figure 1. Column configuration and supply system.

column, glove box, synthetic ground water, and all utensils used in packing were autoclaved or methanol rinsed to minimize microbial contamination.

The soil from the core was poorly sorted and consisted mostly of wet silt, coarse sand, and fine gravel, with cobble-sized stones accounting for 20% to 40% of the core. In order to maintain a fairly homogenous soil pack within the column, rocks larger than ~1 cm in diameter were excluded.

Due to regulatory difficulties, Hanford site ground water was not available. Therefore, synthetic ground water was prepared to approximate the chemistry of Hanford ground water as reported by Last et al. (1991) (Table 1). Dissolved oxygen was removed from the synthetic ground water solution by sparging with nitrogen gas prior to use. No trace nutrients or growth medium was added to the synthetic ground water.

Under flow conditions, two separate amendment solutions were added to the synthetic ground water shortly before entry into the soil column. One contained CT and 1,1,1,-trichloroethane (TCA, $C_2H_3Cl_3$), and the other contained benzoate and/or acetate. TCA was selected as a CAH tracer since it has been shown to be resistant to biotransformation under denitrifying conditions (Semprini et al. 1992). The amendment solutions were prepared from dilutions of saturated aqueous stock solutions made from autoclaved deionized water, which were free of organic solvent carriers. The amendment solutions were drawn into separate autoclaved, 50-mL gastight syringes for use in the supply system.

The column supply network is shown in Figure 1. The column was fed in an up-flow fashion by two low-flow pumps. The synthetic positive-displacement feed pump supplied ~85% of the combined flow and had stainless steel and ceramic wetted parts. A syringe pump provided the remainder of the flow with the CT and TCA, and benzoate and/or acetate amendments. After the influent streams were combined, they were mixed in a 125-mL serum bottle on a magnetic stir plate.

Pressure gauges on both ends of the column were used to indicate clogging within the column. The gauges contained stainless steel wetted parts and were capable of measuring between 0 and 100 kPa. To minimize dissolved oxygen in the influent, a rubber gasbag was attached to the influent flask to maintain a positive pressure of nitrogen. The effluent flask was attached to a separate vessel containing activated carbon to minimize any gaseous CAH emissions into the laboratory.

Two 0.2-µm Teflon® in-line filters were installed on either side of the 125-mL premix chamber to prevent biological growth within the chamber. The filter membranes were changed weekly or when a drop in flow rate was observed. All wetted components in the supply network were autoclaved, soaked in a bleach solution, soaked in methanol, or flamed prior to assembly.

Experimental Procedure

The retardation of CT and TCA was evaluated in a column transport experiment performed prior to the addition of primary substrate. Synthetic ground water amended with bromide (25 mg/L, as a conservative tracer) and CT and TCA at target concentrations of 250 µg/L were pumped through the column at a rate of 340 mL/d. Bromide was added in the synthetic ground water solution, while CT and TCA were amended using the syringe pump.

After the transport study, four different primary substrate and electron acceptor combinations were established within the column reactor in the following sequence: benzoate with nitrate, benzoate without nitrate, acetate without nitrate, and acetate with nitrate. Sulfate was present in all cases. The column alternated between continuous-flow and

batch modes as follows: (1) a substrate and electron acceptor condition was established in the column influent under continuous-flow mode; (2) sufficient time was allowed to establish pseudo–steady state conditions (~3 weeks); (3) flow was stopped and the column was operated in batch mode for ~3 weeks; and (4) the sequence was repeated for a different condition.

The influent flow rate (combined synthetic ground water and amendment streams) was 100 mL/d, with the exception of benzoate with nitrate where the flow rate was 340 mL/d. The experimental condition of benzoate with nitrate was the first to be investigated. Following that test, it became evident that a longer hydraulic residence time (HRT) would allow for more complete transformations across the column, and the flow rate was reduced from 340 to 100 mL/d (0.5-d vs. 2-d HRT, respectively) for the remaining three conditions. In all cases, the target influent concentration of CT and TCA was ~250 µg/L. The concentration of benzoate and acetate in the influent varied from 20 to 50 mg/L depending on the experiment. The conditions of each experiment are summarized in Tables 2 and 3.

For the batch experiments, the flow was stopped and the valve just upstream of the influent pressure gauge was closed (Figure 1). The effluent end of the column was fitted with a 50-mL gastight syringe containing synthetic ground water amended with CT and TCA at target concentration of 250 µg/L as a reservoir to compensate for sample volume. Due to the limited volume of the closed batch system, samples were taken from only one or two ports, typically port 3 (Figure 1), throughout the batch experiments to determine temporal changes in concentration. At the conclusion of the batch experiments, samples were collected

Table 2
Continuous-Flow Results

	Benzoate							
	With Nitrate, Flow = 340 mL/d (HRT = 0.5 d)				Without Nitrate, Flow = 100 mL/d (HRT = 2 d)			
	Influent	Effluent	Change	k (d^{-1})[1]	Influent	Effluent	Change	k (d^{-1})[1]
CT	202 µg/L (1.31 µM)	176 µg/L (1.14 µM)	−13%	0.28	247 µg/L (1.61 µM)	172 µg/L (1.12 µM)	−30%	0.18
CF	0	2 µg/L (0.017 µM)	10%[2]	—	0	14 µg/L (0.12 µM)	24%[2]	—
TCA	221 µg/L (1.66 µM)	199 µg/L (1.49 µM)	−10%	—	214 µg/L (1.60 µM)	208 µg/L (1.56 µM)	−3%	—
Benzoate	25 mg/L (0.21 mM)	16 mg/L (0.13 mM)	−36%	—	22 mg/L (0.18 mM)	20 mg/L (0.17 mM)	−12%	—
Nitrate	50 mg/L (0.94 mM)	45 mg/L (0.85 mM)	−10%	—	0	0	0	—
Nitrite	0	4 mg/L (0.087 mM)	100%[3]	—	0	0	0	—
Sulfate)	38 mg/L (0.40 mM)	38 mg/L (0.40 mM)	0	—	37 mg/L (0.39 mM)	38 mg/L (0.40 mM)	1%	—
	Acetate							
	With Nitrate, Flow = 100 mL/d (HRT = 2 d)				Without Nitrate, Flow = 100 mL/d (HRT = 2 d)			
	Influent	Effluent	Change	k (d^{-1})[1]	Influent	Effluent	Change	k (d^{-1})[1]
CT	180 µg/L (1.17 µM)	146 µg/L (0.95 µM)	−19%	0.10	186 µg/L (1.21 µM)	175 µg/L (1.14 µM)	−6%	0.030
CF	0	9 µg/L (0.075 µM)	34%[2]	—	0	10 µg/L (0.084 µM)	117%[2]	—
TCA	153 µg/L (1.14 µM)	143 µg/L (1.07 µM)	−7%	—	110 µg/L (0.82 µM)	121 µg/L (0.91 µM)	10%	—
Acetate	57 mg/L (0.97 mM)	23 mg/L (0.34 mM)	−59%	—	60 mg/L (1.02 mM)	61 mg/L (1.03 mM)	2%	—
Nitrate	24 mg/L (0.45 mM)	0	−100%	—	0	0	0	—
Nitrite	11 mg/L (0.24 mM)	0	0[3]	—	0	0	0	—
Sulfate	32 mg/L (0.33 mM)	30 mg/L (0.31 mM)	−4%	—	31 mg/L (0.32 mM)	30 mg/L (0.31 mM)	−1%	—

[1]The first-order rate constant, k, was determined by $-\ln(C/C_0)/t$; where C = effluent concentration, C_0 = influent concentration, and t = HRT.
[2]Percentage of CT reduction transformed to CF on a molar basis.
[3]Percentage of nitrate reduction transformed to nitrite on a molar basis.

Table 3
Batch Incubation Results

	Benzoate							
	With Nitrate, after 12 d (average of ports 1 and 4)[1]				Without Nitrate, after 12 d (port 3)			
	Start	Finish	Change	k (d^{-1})[2]	Start	Finish	Change	k (d^{-1})[2]
CT	189 µg/L (1.23 µM)	48 µg/L (0.31 µM)	−75%	0.11	193 µg/L (1.25 µM)	17 µg/L (0.11 µM)	−91%	0.20
CF)	1 µg/L (0.008 µM)	22 µg/L (0.18 µM)	19%[3]	—	10 µg/L (0.084 µM)	70 µg/L (0.57 µM)	44%[3]	—
TCA	210 µg/L (1.57 µM)	170 µg/L (1.27 µM)	−19%	—	211 µg/L (1.58 µM)	180 µg/L (1.35 µM)	−15%	—
Benzoate	21 mg/L (0.17 mM)	1 mg/L (0.008 mM)	−95%	—	20 mg/L (0.17 mM)	12 mg/L (0.099 mM)	−43%	—
Nitrate	48 mg/L (0.91 mM)	1 mg/L (0.019 mM)	−98%	—	0	0	0	—
Nitrite	2 mg/L (0.043 mM)	2 mg/L (0.043 mM)	0	—	0	0	0	—
Sulfate	38 mg/L (0.40 mM)	39 mg/L (0.41 mM)	3%	—	38 mg/L (0.40 mM)	39 mg/L (0.41 mM)	2%	—
	Acetate							
	With Nitrate, after 10 d (port 3)				Without Nitrate, after 12 d (port 3)			
	Start	Finish	Change	k (d^{-1})[2]	Start	Finish	Change	k (d^{-1})[2]
CT	151 µg/L (0.98 µM)	80 µg/L (0.52 µM)	−47%	0.064	161 µg/L (1.05 µM)	75 µg/L (0.49 µM)	−53%	0.063
CF	7 µg/L (0.059 µM)	25 µg/L (0.21 µM)	33%[3]	—	9 µg/L (0.075 µM)	35 µg/L (0.29 µM)	39%[3]	—
TCA	146 µg/L (1.09 µM)	132 µg/L (0.99 µM)	−10%	—	114 µg/L (0.85 µM)	117 µg/L (0.88 µM)	3%	—
Acetate	31 mg/L (0.53 mM)	25 mg/L (0.42 mM)	−18%	—	63 mg/L (1.07 mM)	57 mg/L (0.97 mM)	−9%	—
Nitrate	0[4]	0	0	—	0	0	0	—
Nitrite	0	0	0	—	0	0	0	—
Sulfate	31 mg/L (0.32 mM)	31 mg/L (0.32 mM)	0	—	30 mg/L (0.31 mM)	30 mg/L (0.31 mM)	0	—

[1]The sampling procedure was modified from two samples at the extreme ports to one sample from the center port following the batch experiment for benzoate with nitrate. The average of the results from ports 1 and 4 was used for comparison to the results from port 3 for the other three batch experiments.
[2]The first-order rate constant, k, was determined by $-\ln(C/C_0)/t$; where C = concentration at finish, C_0 = concentration at start, and t = duration of batch test.
[3]Percentage of CT reduction transformed to CF on a molar basis.
[4]Nitrate was degraded prior to entry into the column.

from all four ports along the column to determine spatial changes in concentration. In order to prevent fluid from the compensation syringe from influencing the results, the column had a 7-cm segment of media between port 4 and the end of the column. The pore space of this section of the column was at least 40% greater than the total amount of sample volume drawn from the column throughout the batch experiments. The entire laboratory system was operated at room temperature (~22°C) throughout the study.

Analytical

Samples were drawn from the various valved sample ports shown in Figure 1. Typically, two 1-mL samples were drawn from each port, the first for anion analysis and the second for CAH analysis. During flow experiments, column profiles were frequently obtained in which case all four sample ports plus the influent and effluent ports were sampled. The effluent coil (Figure 1) provided ~4 mL of fluid volume for sampling, thus preventing air from being drawn into the back of the column. The sampling syringe was rinsed three times in both methanol and deionized water between samplings.

Anion concentrations (nitrate, nitrite, chloride, bromide, sulfate, benzoate, and acetate) were quantified on a Dionex 4000i ion chromatograph with a Dionex Ionpac AS4A column. The method for CAH analysis was adapted from the method for trihalomethane analysis presented in *Standard Methods for the Examination of Water and Wastewater* (Clesceri et al. 1998). The 1-mL aqueous samples were extracted into 2 mL of pentane. Concentrations (CT, CF, TCA) were quantified on a Hewlett Packard 5890 gas chromatograph with a ^{63}Ni electron-capture detector and a

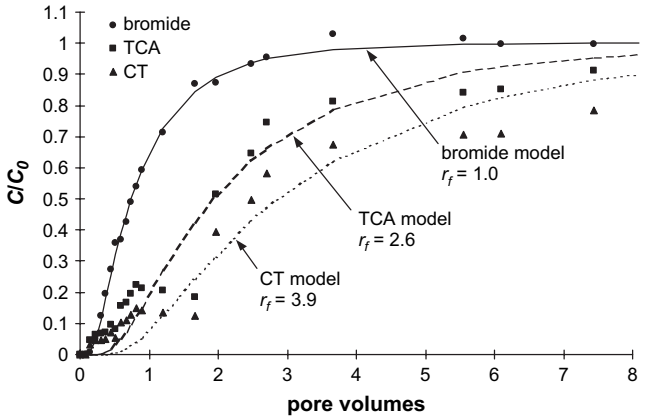

Figure 2. Results of breakthrough experiments, flow rate = 340 mL/d. The lines show the best fit obtained with the equilibrium sorption CFITM model.

stainless steel–packed column. DCM could not be detected in the concentration range of interest using this method. All calibration curves for the CAHs were developed using standard stock solutions prepared in methanol.

Results

Transport Study

The normalized breakthrough curves for bromide, CT, and TCA are shown in Figure 2. A one-dimensional advection-dispersion equation computer model (CFITM) was used to solve for the porosity and Peclet number via a nonlinear least-squares technique (van Genuchten 1981). The bromide data was modeled first. The best fit was obtained with a porosity of 27%, a retardation factor of 1.0, and a Peclet number of 2.7. The retardation factor of 1.0 is consistent with bromide being a conservative tracer. The flow rate of 340 mL/d for the transport experiments resulted in a 0.5-d HRT and an average linear velocity of ~60 cm/d through the column. These results indicate that dispersion was significant within the column, which is reasonable considering the heterogeneous nature of the aquifer solids.

The retardation factors for CT and TCA were found to be 3.9 and 2.6, respectively, based on the porosity and Peclet number determined from the bromide data. These retardation factors assume equilibrium linear partitioning. The model fit the bromide data better than the CT and TCA, due to the CT and TCA tailing at later times, which is likely indicative of rate-limited sorption. The relatively lower retardation factor for TCA is consistent with its lower octanol-water partition coefficient relative to CT; $\log(K_{ow}) = 2.34$ vs. 2.78, respectively (Montgomery 1991).

Continuous Flow

Table 2 summarizes the changes observed in all of the major components between the influent and effluent sampling ports (across the column) under continuous-flow conditions. It is important to note that the experiment for benzoate with nitrate was conducted at a higher flow rate than the others, 340 mL/d (0.5-d HRT) vs. 100 mL/d (2-d HRT). Although not shown in the table, no significant changes in chloride concentration were observed (e.g., Figure 3).

A net reduction in CT concentration, and CF production, was consistently observed across the column, indicating that CT transformation was proceeding in all cases. The greatest CT reduction across the column (30%) was achieved with benzoate in the absence of nitrate with an HRT of 2 d. However, based on estimated first-order rate constants, the greatest rate of CT transformation across the column length occurred for benzoate with nitrate. Also depicted are the percentages of the transformations resulting in CF, on a molar basis. The largest concentration of CF in the effluent was observed for benzoate without nitrate. However, for acetate without nitrate, the CF produced accounted for approximately all of the CT transformed on a molar basis. The least CF observed was for benzoate with nitrate, where the retention time was ~3.4 times less than for the other conditions.

A regular pattern in TCA behavior was not observed; TCA both decreased and increased slightly in these tests. It is believed that this variability was due to the use of saturated aqueous stock solutions, which resulted in inconsistencies in the influent CAH concentrations. Because the supply syringe had to be refilled during the experiments, these fluctuations were likely to have propagated at times through the column as well. Since TCA and CT were added

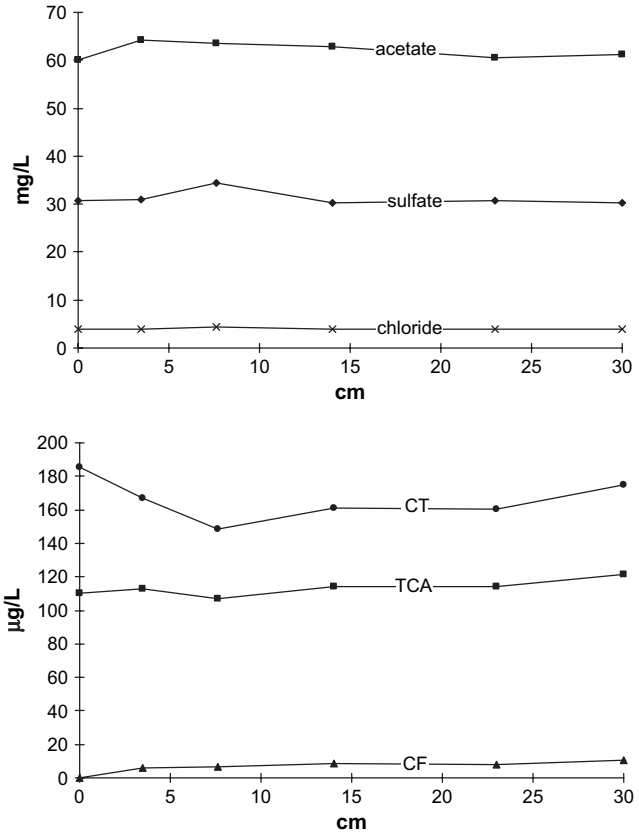

Figure 3. Continuous-flow column profiles 3 weeks after removal of nitrate from the synthetic ground water with acetate as the primary substrate, retention time = 2 d.

from the same saturated stock and TCA is expected to act as a recalcitrant CAH tracer, some information about the CT variability can be inferred from the TCA observations.

Benzoate as a primary substrate resulted in relatively greater overall CT transformations than acetate. Also, benzoate produced less CF, on a percentage basis, from the transformations. However, acetate was far better at inducing denitrification; 100% of the nitrate was removed when acetate was the primary substrate. With acetate, it appeared that microbial growth occurred in the feed line, as indicated by the appearance of nitrite in the column influent (Table 2), since nitrite was not present in the synthetic ground water supply. Growth from the feed line into the column was prevented through the use of in-line filters (Figure 1). This was not a problem when benzoate was the substrate.

The absence of nitrate was not found to result in sulfate reduction, with no decrease in sulfate observed in any of the tests. Nitrate removal increased CT transformation with benzoate as a substrate, but the relative degree of CT transformation decreased when nitrate was removed with acetate as the substrate. For both substrates, the relative percentage of CF produced increased in the absence of nitrate. Benzoate use proceeded in the absence of nitrate, but acetate use was nonexistent when nitrate was removed.

The CAH and anion concentrations, along the column length, for the continuous-flow condition of acetate without nitrate are shown in Figure 3. The profiles showed fairly insignificant changes in CAHs and anions across the column. The most substantial evidence in support of CT transformation within the column was the appearance of CF, particularly within the first 5 cm. These results indicated that more complete transformations would likely be observed, given longer residence times within the column. However, an HRT of 2 d was about the maximum that could be obtained with the given system. In order to evaluate the effect of additional residence time, the technique of switching to batch mode was developed following the establishment of steady-state continuous-flow conditions. The results of the batch-mode tests are described in the following section.

Throughout the flow experiments, the microbial mass within the column never grew to a sufficient density to impede flow through the column. The pressure drop across the column remained consistently at ~2.8 kPa over all conditions.

Batch

Throughout the continuous-flow experiments, the percent reduction in CT concentration ranged from 6% to 30%, which at times was not significantly greater than the −3% to 10% variability in TCA concentration observed across the column (Table 2). In order to determine if lengthening the residence time could increase the overall percentage of CT transformation, column operation was switched to batch mode following each of the continuous-flow experiments. Switching to batch mode also allowed for the evaluation of transformation kinetics spatially within the column, as opposed to across the whole column. The concentration history at port 3 throughout the 3-week batch experiment for acetate without nitrate is shown in Figure 4. Periodic sampling from a single port

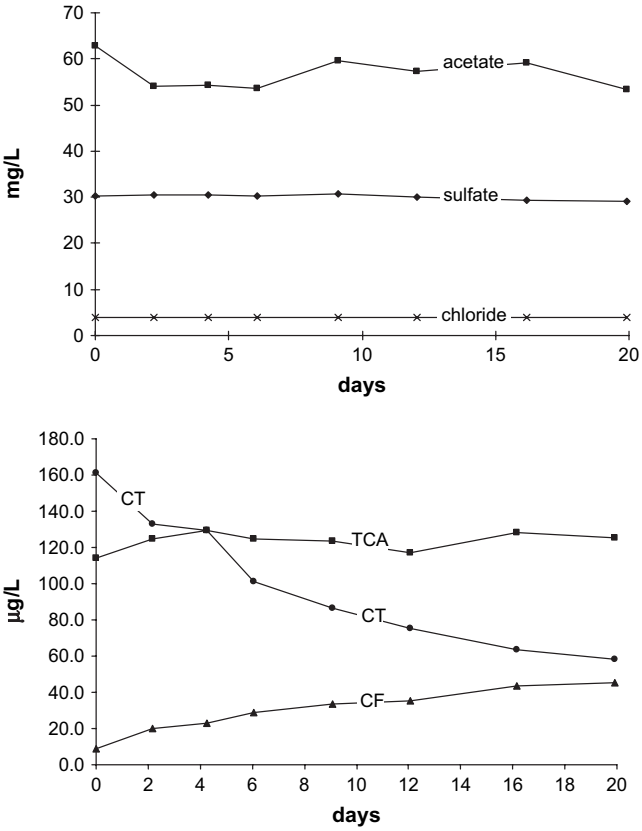

Figure 4. Concentrations at port 3 during the batch experiment with acetate in the absence of nitrate. Nitrate was removed from the synthetic ground water 3 weeks prior to initialization.

(port 3) was performed in order to minimize the volume of water extracted from the column over the course of the batch experiment. After 12 d, the concentration of TCA was virtually unchanged, while the concentration of CT was reduced by 53%, relative to the influent concentration at the time of batch-mode initialization. Throughout the test, CF accounted for ~39% to 53% of the CT transformed. The acetate, sulfate, and chloride concentrations remained virtually unchanged throughout the experiment. The concentration histories in the batch-phase systems for the other electron donor/acceptor conditions followed similar trends and are not shown.

At the end of the batch experiments all four column ports were sampled. Figure 5 shows the concentration of anions and CAHs along the column length at the end of the 3-week batch experiment for acetate without nitrate. The sulfate and chloride concentrations remained unchanged, while acetate decreased toward the effluent end. The concentrations of acetate at the first two ports were greater than the concentration of acetate in the influent solution, possibly indicative of the production of a metabolic by-product that coeluted with acetate. CT increased along the column length, with the concentration at port 4 approximately four times that at port 1. Conversely, the CF concentration decreased by ~26% from port 1 to port 4. This indicates that CT transformation was most complete near the influent end of the column, with greater CF

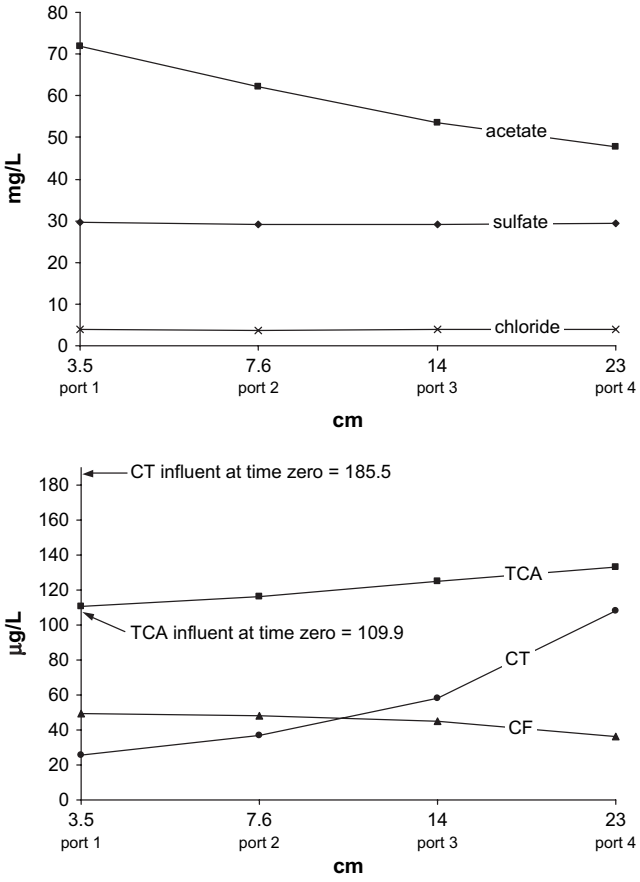

Figure 5. Concentrations throughout the column at the end of the 3-week batch test for acetate without nitrate.

concentrations associated with areas of greatest CT reduction. TCA increased by ~21% between ports 1 and 4.

Table 3 summarizes the changes observed for all batch experiments at port 3 (average of ports 1 and 4 for benzoate with nitrate) after 12 d (10 d for acetate with nitrate). Note that the test for acetate without nitrate corresponds to Figure 4; figures of the other three batch conditions are not included here. The greatest percentages of CT reduction occurred with benzoate as the primary substrate, with 91% reduction achieved after 12 d in the absence of nitrate and 75% with nitrate present. CT reductions with acetate were 47% and 53% with and without nitrate, respectively. The largest relative fraction of CF produced (44% of the CT reduced) occurred for the case of benzoate without nitrate. The condition with the least relative amount of CF produced (19% of the CT reduced) was benzoate with nitrate. The CF produced with acetate as the primary substrate represented 33% and 39% of the CT reduced with and without nitrate, respectively. Overall, the batch experiments increased the fraction of CT transformed three to nine times over that observed in the continuous-flow experiments, as a result of longer effective residence time within the column.

Discussion

CF was produced as a product of CT transformation in all cases, accounting for 10% to 100% of the CT transformed on a molar basis. This is contrary to the results of Petersen et al. (1994) who reported that a denitrifying culture, isolated from Hanford sediments, transformed CT without CF production. However, in other cases pertaining to Hanford Aquifer materials, CF production was observed to some extent (Skeen et al. 1993; Truex et al. 1994).

With benzoate as the primary substrate, the presence of nitrate resulted in less extensive CT transformations than without nitrate, consistent with the findings of Semprini et al. (1992), Stensel and DeJong (1994), Petersen et al. (1994), and Sherwood et al. (1996, 1999). However, when acetate was the primary substrate, equal or slightly greater CT transformation was observed in the presence of nitrate. The absence of nitrate for 3 weeks prior to the experiment with acetate may have negatively affected the microbial population toward CT transformation. Thus, the sequence of the experiments could have impacted the results. Acetate was far more effective at inducing denitrification than benzoate. In the continuous-flow experiments with acetate as the primary substrate, denitrification was complete (Table 2). Complete denitrification was not observed with benzoate as the primary substrate.

Semprini et al. (1992) suggested that the inhibitory effect of nitrate could be caused by (1) CT and nitrate competing as electron acceptors; (2) another microbial population living off the decay products of the denitrifiers; and (3) the presence of nitrate preventing the stimulation of sulfate reducers. However, here, sulfate reduction was never observed, even when nitrate was absent for extended periods in the batch experiments. Another possibility is that iron-reducing microorganisms were using iron contained in the aquifer solids to cometabolically transform CT in the cases where nitrate was absent (Workman et al. 1997; Gerlach et al. 2000; McCormick et al. 2002). However, the concentration of ferrous iron was not determined in these tests.

Both continuous-flow and batch tests indicate more effective CT transformation with benzoate as a substrate than acetate. Benzoate was also more effective in promoting transformation in the absence of nitrate. Benzoate differs from acetate in that it can potentially ferment. Yang and McCarty (1998) showed that slow fermentation of benzoate to hydrogen gas and acetate was supportive of trichloroethylene (C_2HCl_3)–reductive dehalogenation, with hydrogen used as the electron donor. Similarly, in this study, it is possible that the fermentation of benzoate was responsible for the continued use of benzoate in the absence of nitrate, but the production of acetate was not confirmed. Benzoate might have also been used as an electron donor for iron reduction. Additionally, when nitrate was present, the stoichiometric equivalent of benzoate required to achieve the observed reduction of nitrate to nitrogen gas accounted for <7% of the benzoate removed. This indicates that other processes, including fermentation and iron reduction, were likely occurring. With acetate as the primary substrate in the presence of nitrate, denitrification accounted for ~56% of the acetate removed. In the absence of nitrate, no removal of acetate was observed. Therefore, much of the acetate removal was associated with denitrification.

Switching from continuous flow to batch mode provided a novel method to investigate the kinetics of CT transformation within the column, based on conditions

developed under the continuous-flow regime. Analysis of port 3 observations over time showed that decreases in CT concentration followed first-order kinetics (Figure 4). Thus, first-order rate constants were determined for the various conditions after ~12 d and are shown in Table 3. The highest rate was achieved for benzoate without nitrate.

Sample port 3 was the only port to be sampled periodically throughout the batch tests in order to minimize the amount of fluid extracted from the column. However, at the end of three of the four batch experiments, all ports were sampled and rate constants were calculated along the column length based on the initial and final concentrations. The batch experiments varied in duration, and the first-order rate constants along the column at the end of the batch experiments are shown in Figure 6. Overall, the rates decreased with distance along the column and were fairly consistent between the different conditions.

While CT has been shown to be susceptible to abiotic transformation, particularly under iron-reducing conditions (Butler and Hayes 2000; McCormick et al. 2002), the observed transformation trends are supportive of biological processes. The rates of CT transformation in this study were higher near the influent end of the column and decrease in a consistent pattern along the column. The decreasing CT transformation rates with respect to distance along the column length were observed for benzoate and acetate as substrates, both with and without nitrate present. This indicates that the CT transformation process was mainly microbial, and the decreased rates near the effluent end of the column is likely due to less biomass in these areas. Truex et al. (1994) used a first-order process to model CT transformation by Hanford denitrifiers, using glycerol as a primary substrate. A first-order rate constant of 0.25 d^{-1} can be estimated from figure 1A of their work, which is within the range observed in this study.

Conclusions

Overall, it appeared that the Hanford subsurface possesses a microbial population able to transform CT and remove nitrate from the ground water. The production of CF from these transformations ranged between 10% and 100% of the CT reduced. Both benzoate and acetate were capable of inducing denitrification and CT transformation. However, denitrification was much faster with acetate as the primary substrate, while benzoate generally produced more rapid and slightly more efficient transformation of CT. Under continuous-flow conditions, CT transformation rates were highest when nitrate was present in the column influent (Table 2). However, 12 d after switching to batch mode, CT transformation rates in the absence of nitrate met (acetate) or exceeded (benzoate) those in the presence of nitrate (Table 3). By the end of the batch experiments, the greatest rates were observed in the absence of nitrate for both benzoate and acetate (Figure 6). The increased transformation rates at later times in the absence of nitrate may have been due to iron reduction, or in the case of benzoate possibly fermentation, which were relatively slow in becoming established. Sulfate reduction was never observed in any of the experiments.

Benzoate may be the preferred substrate in a field demonstration since its relatively slow use, and fermentation potential, would permit benzoate to travel a greater distance through the subsurface, presumably resulting in remediation of a larger volume. These results suggest that greatest rates of CT transformation can be expected nearest to the point of injection and once the denitrification process is complete. Rapid growth on benzoate and subsequent biofouling was not a problem in these experiments and can likely be controlled in the field as well. However, before a successful field demonstration of CT biotransformation can be implemented at the Hanford site, further research is needed to investigate the role of iron reduction in this process, as well as the lack of sulfate reduction. Also, more work is needed to determine the appropriate conditions necessary to minimize the production of CF.

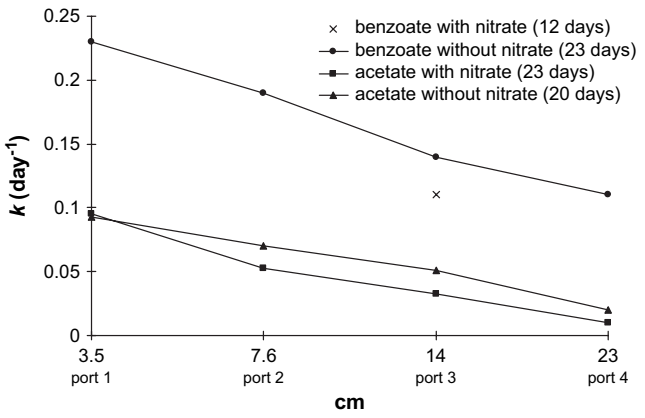

Figure 6. First-order rate constant estimates for the reduction in CT concentrations observed throughout the column for the batch experiments.

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