

# In-Situ Transformation of Carbon Tetrachloride and Other Halogenated Compounds Resulting from Biostimulation under Anoxic Conditions

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■ Enhanced in-situ transformation of carbon tetrachloride (CT) was observed under anoxic conditions created through biostimulation. The transformation of 1,1,1-trichloroethane (TCA), and two chlorofluorocarbons [trichlorofluoromethane (CFC-11) and 1,1,2-trichloro-1,2,2-trifluoroethane (CFC-113)], was also indicated. The evaluation was conducted in a shallow confined aquifer through controlled injection of the halogenated aliphatic hydrocarbons, acetate as a growth substrate, and nitrate and sulfate as potential electron acceptors. Biostimulation was readily accomplished, with nitrate consumption essentially complete within 100 h. CT disappearance commenced 2 weeks after active denitrification began and gradually increased over the 10-week test. Chloroform appeared as an intermediate product coincident with the disappearance of CT, representing 30–60% of the CT transformed. CFC-11, CFC-113, and TCA showed trends in concentration decrease similar to CT, but to lesser extents. When nitrate was removed from the injected fluid, the CT transformation rate accelerated. The maximum average extents of transformation within 2 m of travel in the test zone were as follows: TCA, 12%; CFC-113, 20%; CFC-11, 68%; CT, 95%. The observations indicate that the transformation may have been mediated by microorganisms other than the active denitrifiers.

## Introduction

Chlorinated aliphatic hydrocarbons (CAHs) with one or two carbon atoms are widely used as solvents, degreasing agents, and intermediates in chemical synthesis. Their widespread use and uncontrolled disposal have resulted in the contamination of groundwater supplies (1). There is an urgent need to better understand the behavior of the contaminants in the subsurface, to develop methods for monitoring the distribution and movement of the chemicals, and to clean up contamination once its extent is delineated. In-situ bioremediation of contamination by halogenated aliphatic compounds (HACs) is a promising alternative for aquifer restoration, since the process may accomplish complete mineralization to nontoxic end products and/or may create intermediate products that are less harmful, that are more easily removed from the aquifer, or that may be more readily treated by other subsequent processes.

Studies have shown that CAHs can be biologically transformed under a range of environmental conditions (2). In reviewing chemical, biological, and enzymatic studies, Vogel et al. (2) noted some general trends between the ease of aerobic or anaerobic transformation and the oxidation state and chemical structure of the chlorinated CAHs. Compounds that are more highly substituted with halides (more oxidized) are more likely to undergo reductive dehalogenation under anaerobic conditions, forming less halogenated intermediates.

Recent studies of enhanced in-situ transformation under aerobic conditions by methanotrophic bacteria (3) have shown less chlorinated ethenes such as vinyl chloride and *trans*- and *cis*-dichloroethylene (DCE) to be more rapidly transformed than trichloroethylene (TCE). Laboratory studies of Fogel et al. (4), Henson et al. (5), Nelson et al.

(6), Wackett and Gibson (7), and Tsien et al. (8) have shown that more highly chlorinated organics such as carbon tetrachloride (CT) and tetrachloroethene (PCE) are not amenable to aerobic transformations. These findings gave impetus to the investigation of anoxic bioremediation of highly halogenated compounds to nontoxic products, or less halogenated products that may be more easily degraded by aerobic bacteria or more easily removed from the aquifer by physical methods.

The occurrence of reductive transformations of HACs in groundwater was first demonstrated by Bouwer et al. (9). Several investigations have elucidated the environmental conditions required for reductive transformation, and the transformation products are somewhat known. In general, anaerobic transformations of halogenated alkanes and alkenes form a variety of less halogenated transformation products (10–18). Rates of transformation are generally faster under more reducing conditions.

CT transformation studies under conditions ranging from denitrifying to methanogenic (10, 19, 20) have indicated that CT can be assimilated into cell mass, mineralized to CO<sub>2</sub>, and transformed to chloroform (CF). Some pure cultures transformed CT to CF and dichloromethane (DM) quantitatively, under both sulfate-reducing and methane-producing conditions (21, 22). *Acetobacterium woodii* converted CT to dichloroethane and carbon dioxide (22). A *Clostridium* sp. biotransformed 1,1,1-trichloroethane (TCA), CF, and CT (23). CT transformation led to the production of CF as an intermediate, with further transformation to DM and unidentified products. A pure denitrifying culture, *Pseudomonas* sp. strain KC, degraded CT, 50% to CO<sub>2</sub> and ~40% to non-volatile compounds, with minimal CF production (24). CT was also transformed by *Escherichia coli* under fermenting conditions (25). The extent of reported mineralization of CT to CO<sub>2</sub> varies, ranging from 10 to 99% (26). Parallel pathways have been presented for the degradation of CT under anaerobic conditions, leading to potential formation of CF, DM, CO<sub>2</sub>, CO, formic acid, and hexachloroethane (26).

TCA can be reduced under anaerobic conditions, and most rapidly under reducing methanogenic conditions (2, 13, 14). TCA can also be transformed under less reducing conditions with fermentation of amino acids (23, 27). Here only about 30–40% of the TCA was observed as 1,1-dichloroethane (1,1-DCA), while the remainder appeared as different products, including acetic acid. 1,1-DCA can be reduced further to chloroethane (13); however, the rate of dechlorination of 1,1-DCA is much slower than TCA reduction to 1,1-DCA.

Wolf et al. (28) demonstrated that trichlorofluoromethane (CFC-11) is reductively changed to dichlorofluoromethane in rat liver. Disappearance of CFC-11 and dichlorofluoromethane (CFC-12) in soils (29), rice fields (30), and methanogenic freshwater sediments (31) has been reported; the latter study indicated the removal process may have been biotic. Lesage et al. (32) noted the presence of 1,2-dichloro-1,2,2-trifluoroethane (HCFC-123a) and also chlorotrifluoroethane (CFC-1113) in groundwater leachate from a municipal landfill. Since 1,1,2-trichloro-1,2,2-tri-

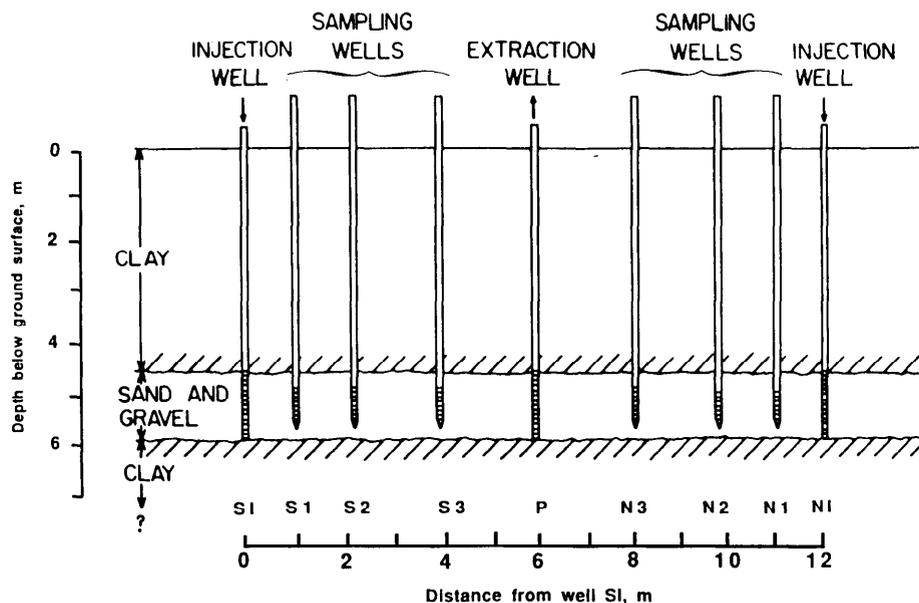


Figure 1. Schematic design of the test zone (33).

fluoroethane (CFC-113) was present, they speculated that these compounds were formed under the reductive conditions present.

This work assessed under field conditions the capacity of indigenous organisms to metabolize a suite of target HACs under anoxic conditions created by enhanced microbial growth. Since significant nitrate concentrations were already present, the ability of organisms growing under denitrifying conditions to transform highly halogenated aliphatic compounds became of interest. The test zone used in these experiments was a shallow aquifer that contained nitrate and sulfate as potential electron acceptors. The aquifer was also contaminated with TCA, CFC-11, and CFC-113. CT was chosen as the main contaminant to study since its transformation had been reported under denitrifying conditions, which was the likely condition to result upon the introduction of the appropriate electron donor. CT transformation was also shown to be greater than PCE (an alternative contaminant of interest) under denitrifying conditions (20).

The specific objectives of the field study were as follows: (1) to evaluate in a controlled field experiment the ability to biostimulate an indigenous population of denitrifying bacteria under conditions representative of groundwater environments; (2) to quantify the extent of enhanced biotransformation of CT in the biostimulated zone; (3) to track the formation of CF as an intermediate product; (4) to monitor background contaminants (TCA, CFC-11, CFC-113) to determine whether concentration decreases resulted in response to biostimulation; (5) to determine how to modify biostimulation conditions to achieve more extensive transformation and perhaps more complete mineralization of the HACs.

#### Test Zone Characterization

The Moffett Field Naval Air Station, Mountain View, CA, was chosen for this evaluation. This site was used earlier to study in-situ restoration of CAHs by an aerobic process (3, 33, 34), has been well characterized, and is typical of shallow sand-and-gravel aquifers that are commonly contaminated by CAHs (33, 35). The test site consists of a layer of sand and gravel, approximately 5 m below the surface and 1.2 m thick, well confined above and below by a silty clay layer of low permeability (Figure 1). The transmissivity of the test zone is high ( $\sim 100 \text{ m}^2/\text{day}$ ), which is favorable for enhancing microbial growth, since

biological clogging can reduce permeability.

The groundwater chemical composition was also appropriate for the field experiments. The dissolved oxygen (DO) concentration was below detection ( $0.2 \text{ mg/L}$ ), as determined using a DO probe. Nitrate and sulfate, two potential electron acceptors, were present at concentrations of 25 (as nitrate) and  $700 \text{ mg/L}$  (as sulfate) based on ion chromatographic measurements. The groundwater was contaminated with TCA ( $50 \text{ } \mu\text{g/L}$ ), CFC-113 ( $6 \text{ } \mu\text{g/L}$ ), and CFC-11 ( $3 \text{ } \mu\text{g/L}$ ). These compounds were monitored as background contaminants. No attempt was made to increase their concentration to determine transformation products formed. The main target compound, CT, was not present and therefore was continuously added in a controlled manner to the injected water. There were no appreciable amounts of toxic metals (35). With respect to nutrients for biological growth, nitrate in the subsurface groundwater served as a source of N. Phosphorus concentrations in the groundwater were low ( $0.1 \text{ mg/L}$ ); however, phosphate minerals that are present probably provided a source of P (35).

#### Experimental Methodology

The experimental methodology was similar to that used for studying aerobic methanotrophic biotransformations at the site (3, 33, 34). The methodology entailed creation of a flow field dominated by pumping from an extraction well (P), while solutes in known amounts were introduced at the SI injection well (Figure 1). Concentrations were measured regularly at the injection and extraction wells, as well as at intermediate monitoring wells S1, S2, and S3, located 1, 2.2, and 3.8 m from the injection well, respectively. Evidence of transformation was then assessed by quantitative examination of the halogenated solutes' concentration histories at the monitoring locations and comparison of the results under biostimulation conditions with results obtained under similar conditions in the absence of biostimulation. Bromide was also added as a conservative, nonsorbing, nonreacting component for comparison purposes. A specially designed automated data acquisition and control (DAC) system provided continuous records of high-accuracy data over sustained periods, which enabled mass balances to be made with relative errors of only a few percent (34-36).

Details of the analytical methods and the performance of the DAC are provided by Hopkins et al. (36) and Sem-

**Table I. Experiments Conducted and Processes Studies**

experiment	duration	chemicals injected	average concn (mg/L)	processes studied
tracer	8/9-8/31/89 (528 h)	NO <sub>3</sub> <sup>-</sup> Br <sup>-</sup> CT <sup>a</sup> CT <sup>a</sup>	25 ± 3 <sup>b</sup> 68 ± 3 0.075 ± 0.007 0.045 ± 0.003	transport of CT and bromide to evaluate advection, dispersion, retardation, and transformation in the absence of biostimulation
test 1	9/-11/15/89 (0-1260 h)	acetate nitrate Br <sup>-</sup> CT CFC-11 CFC-113 TCA	25-46 <sup>c</sup> 22 ± 3 43 ± 4 0.039 ± 0.012 <sup>d</sup> 0.0029 ± 0.0003 0.0062 ± 0.0005 0.051 ± 0.004	biostimulation of a denitrifying population to transform CT, CFC-11, CFC-113, and TCA
test 2	(1260-1585 h)	acetate nitrate all others the same as test 1	12 <sup>c</sup> 0	biotransformation in the absence of nitrate in the test zone

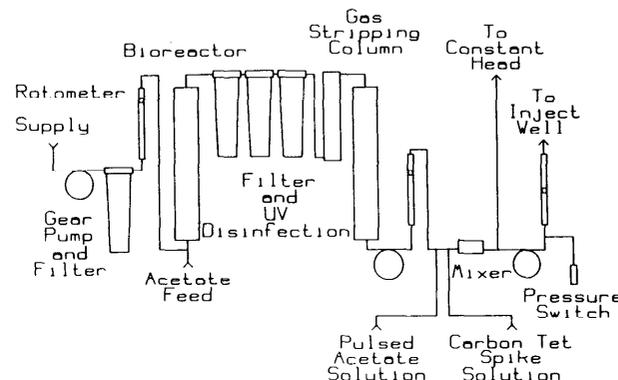
<sup>a</sup>CT injected in two concentration steps. <sup>b</sup> ± standard deviation, n = 100-175. <sup>c</sup>Pulse-averaged injection concentrations based on injecting a high acetate concentration for only 1 h of a 13-h pulse cycle period. <sup>d</sup> ± standard deviation, n = 380.

prini et al. (37). The compounds of experimental interest and their method of automated analysis by the DAC were as follows: bromide and nitrate via ion chromatography with separation on an anion column and conductivity detection; acetate via ion chromatography with separation on an ion-exclusion column and conductivity detection; CT, CF, TCA, CFC-11, and CFC-113 via chromatography with separation on Chromosorb Q with 10% squalene and detection on a electron capture detector (ECD) as the primary detector and a Hall electrolytic conductivity detector as a secondary detector.

Table I provides concentration ranges of the compounds monitored in the injected fluid along with standard deviations based on over 100 measurements, providing information on the combined performance of the DAC and the chemical injection system. The coefficient of variation (CV) of the bromide and nitrate concentration ranged from 5 to 12%. The CV of the halogenated compounds ranged from approximately 10% for TCA, CFC-11, and CFC-113 to 7-30% for CT. CT has a higher CV since it was added to the injected fluid, and variations resulted from both measurement errors and actual variations in the injection concentration. The results illustrate the ability of the DAC to provide reproducible measurements and the injection system to maintain fairly constant injection concentrations.

The experimentation comprised a sequence of field tests to provide a convincing evaluation. Table I provides the sequence of tests, the chemical injection concentrations, and a brief description of the processes studied. The transport of CT and Br<sup>-</sup> through the test zone, in the absence of biostimulation, was studied in the tracer test. The retardation of sorbing CT compared to nonsorbing Br<sup>-</sup> was determined, and the transformation of CT by biotic or abiotic processes was evaluated before active biostimulation. The test zone was saturated with CT before the start of the two biostimulation-biotransformation tests. Test 1 was performed to determine whether a denitrifying microbial population could be stimulated through acetate addition, to assess the extents of transformation of the halogenated compounds, and to determine transformation products formed. Test 2 involved a transient test in which nitrate was removed from the injected fluid, using a surface bioreactor that was fed acetate, to determine whether nitrate inhibited CT transformation.

The experiments were performed under induced-gradient conditions caused by groundwater injection and extraction. Groundwater was injected at a rate of 1.5 L/min into the SI well and extracted at a rate of 10.0



**Figure 2. Schematic design of the injection system used.**

L/min from well P. Based on past experiments (33), these rates ensured that the injected fluid completely permeated the regions around the S1 and S2 monitoring wells and that the extraction well recovered greater than 95% of the injected fluid. The injected fluid was extracted groundwater. Since the extraction rate was ~7 times the injection rate, this extracted water contained at least 85% of the nitrate, sulfate, TCA, CFC-11, and CFC-113 present in the ambient groundwater. CT and acetate, which were not present, were added to the injected fluid using the injection system shown in Figure 2 (37).

The system permitted the pulsed addition of acetate in order to prevent clogging due to biological growth and to help distribute the microbial growth through the test zone (3, 33, 38). Acetate was injected for 1 h of a 13-h pulse cycle. The bioreactor was only used during test 2 to remove nitrate from the recycled groundwater. It was seeded with groundwater-borne microbes and fed acetate for several weeks before test 2 was started.

### Experimental Results

**Tracer Test.** The concentration histories of bromide and CT at the S2 well for the first 300 h of the tracer test normalized to the injection concentration (C<sub>0</sub>) are shown in Figure 3. Hydraulic residence times between the injection and observation wells, S1, S2, and S3, ranged from 8 to 28 h based on the bromide tracer test data (Table II). The breakthrough of CT was delayed relative to bromide due to sorption onto the aquifer solids. CT retardation factors were estimated based on the ratio of the time to achieve 50% breakthrough of CT compared to bromide. Retardation factors ranged from 1.5 to 2.0, indicating CT

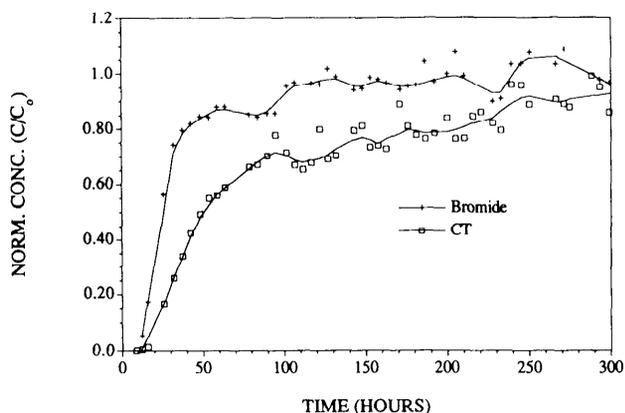


Figure 3. Normalized breakthrough curves of CT and bromide at the S2 well in the tracer 14 experiment.

Table II. Results of the Tracer Test

	well S1	well S2	well S3
normalized Br <sup>-</sup> breakthrough ( $C/C_0$ )	1.00	0.98	0.94
normalized CT breakthrough ( $C/C_0$ )	0.98	0.99	0.98
time to 50% Br <sup>-</sup> breakthrough (h)	8	24	28
time to 50% CT breakthrough (h)	12	44	57
estimated retardation factor ( $T_{CT}/T_{Br^-}$ )	1.5	1.8	2.0

was weakly sorbed onto the aquifer solids. Laboratory studies with pulverized samples from the aquifer equilibrated for 10 days yielded a retardation estimate of 6. The lower field estimate probably resulted from mass-transfer effects due to larger particle sizes in the field and short equilibration times associated with the 50% breakthrough estimate.

Normalized bromide concentrations near unity at the S1 and S2 monitoring wells (Table II) indicate the injected fluid completely permeated the test zone. In order to more quickly reach sorption equilibrium with the injected fluid, the CT injection concentration was initially raised to 75  $\mu\text{g}/\text{L}$  and then lowered to the test concentration, 45  $\mu\text{g}/\text{L}$ , after 400 h of addition. CT reached a normalized concentration near unity at all the monitoring wells (Table II), indicating minimal transformation and sorption losses with prolonged injection. A minor amount of CF production was observed soon after CT addition, with the maximum CF concentration representing 3–4% of the CT added. Thus, minor CT transformation was observed before biostimulation.

**Test 1: Biostimulation with Acetate and Nitrate.** Biostimulation of the test zone was initiated by adding acetate to the CT-amended injection water. Acetate injection concentrations ranged from 330 to 600 mg/L, representing pulsed-averaged concentrations of 25–46 mg/L. Figure 4 illustrates the subsequent concentration histories of acetate, nitrate, and nitrite at the S1 well. Biostimulation was clearly demonstrated by the decrease in nitrate concentration immediately after acetate addition. After 70 h, nitrate was only occasionally detected, during periods when acetate was absent or at low levels, as a result of the pulsed acetate addition. Nitrite appeared as a transitory intermediate of nitrate utilization, as expected in the initial stages of denitrification (39). The uptake of acetate during the first 100 h was superimposed on the cyclic concentration variations resulting from its pulsed addition. The time-averaged acetate injection concentration was reduced from 46 to 25 mg/L at 100 h, because acetate had been in stoichiometric excess of the nitrate present. The reduction caused an abrupt decrease in the acetate concentration at S1 between 100 and 120 h. The

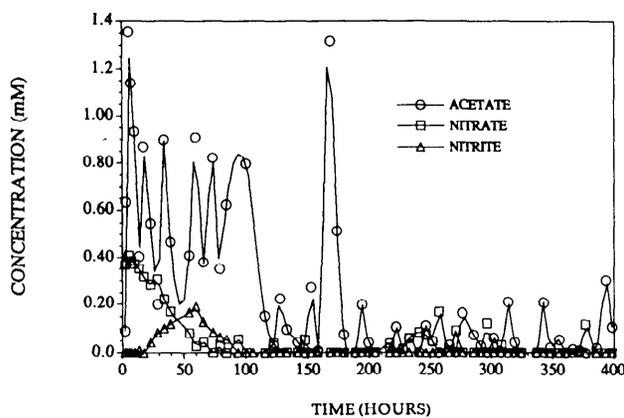


Figure 4. Response of acetate, nitrate, and nitrite at the S1 well, resulting from biostimulation with acetate.

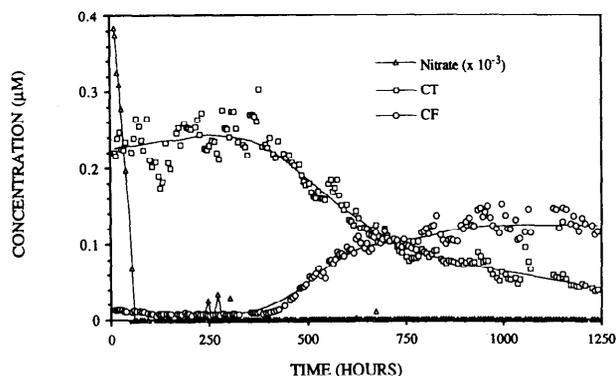


Figure 5. Response of nitrate, CT, and CF at the S2 well due to biostimulation.

spike in concentration between 160 and 180 h was due to an injection system malfunction resulting in increased acetate injection for several hours. The acetate concentration after 200 h was associated with its regular pulsed addition.

The acetate, nitrate, and nitrite responses at the S2 well were similar to those observed at the S1 well, but delayed by approximately 16 h, due to the longer transport times, consistent with the tracer tests (Table II). The oscillations in acetate concentration were greatly attenuated due to greater dispersive mixing and microbial utilization with the longer distance traveled. Nitrate was not detected, indicating essentially complete utilization within 2.2 m of transport.

After 300 h of biostimulation, greater than 80% of the acetate and 90% of the nitrate were consumed within the first meter of transport. The measured ratio of substrate consumption was approximately 1.0 mg of nitrate/mg of acetate, which is lower than the stoichiometric ratio of 1.65 for complete oxidation of the acetate consumed through nitrate reduction to nitrogen gas. The lower observed ratio suggests the incorporation of an estimated 40% of the acetate into cell mass during biostimulation, which is typical for denitrifying conditions (39).

**Biotransformation of CT.** Figure 5 shows concentration histories of nitrate, CT, and CF at the S2 well during biostimulation. The gradual decrease in CT concentration and increase in chloroform as a transformation intermediate significantly lagged behind nitrate and acetate uptake and were observed only after 400 h of acetate addition. CT concentration continuously decreased over the period shown, while the chloroform increase essentially stopped. The response indicates that CF itself may have been transformed.

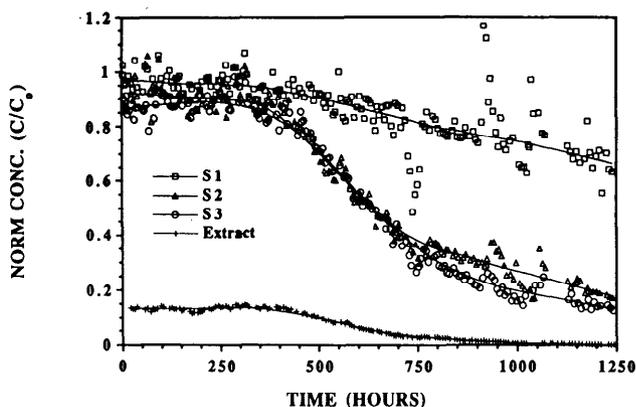


Figure 6. CT response due to biostimulation at all the observation wells.

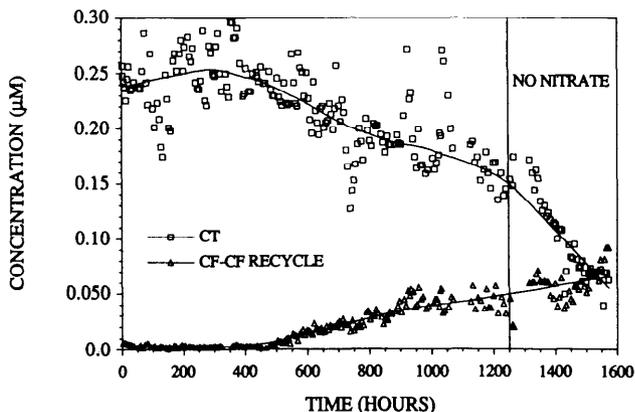


Figure 7. Response at the S1 well of CT and CF to nitrate removal from the injected fluid at 1260 h. Recycled CF in the injected fluid was subtracted from the observed concentrations.

CT transformation was faster and more complete at locations more distant from the injection well, as is illustrated in Figure 6. The responses at the S2 and S3 wells were similar, consistent with their similar hydrodynamic responses in the tracer test (Table II). The lower rates of CT transformation between the injection well and the S1 well, where most of the acetate and nitrate were consumed, suggest that the main denitrifying population did not participate in the transformation process to the same extent as microbes stimulated further away. This is also supported by the lag in transformation of CT compared to nitrate uptake illustrated in Figure 5.

The above response suggests the transformation of CT may have been strongly inhibited by the presence of nitrate in the test zone. Another possibility is that a secondary microbial population, living on decay products of the stimulated denitrifiers, grew slowly compared with the denitrifier growth and were responsible for the transformation. The growth of this population and/or its transformation of CT may have been inhibited by the presence of nitrate in the first meter of the test zone. In order to evaluate the possibility, test 2 was initiated.

**Test 2: Biotransformation with Complete Nitrate Removal.** The test 2 transient experiment was performed to study the effect that complete elimination of nitrate from the test zone would have on biotransformation. Nitrate was removed from the injected fluid through the use of a surface bioreactor fed acetate (Figure 2). No loss of the halogenated compounds was observed in the bioreactor. The transient test was initiated at 1260 h. Pulsed acetate injection was continued but at a reduced pulse-averaged concentration of 12 mg/L, since acetate was not needed for nitrate removal in the test zone. The response

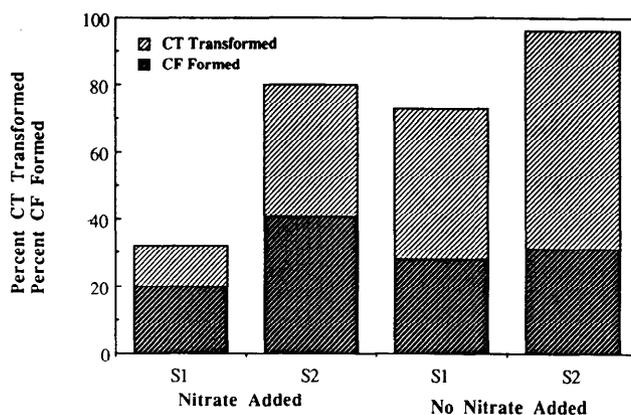


Figure 8. Bar graph showing the percentage transformation of CT and the fraction appearing as CF during periods with and without nitrate addition.

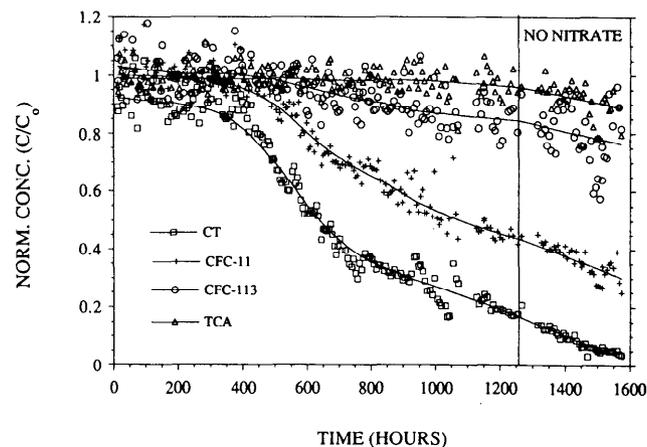


Figure 9. Response of the halogenated compounds at the S2 well due to biostimulation.

at the S1 well (Figure 7) was a significant increase in the rate of CT transformation after nitrate was removed. Enhanced CT transformation upon nitrate removal was less pronounced at the S2 well (Figure 9). Significant CT transformation was, however, already occurring here before nitrate was removed. Regression analysis of the logarithm of normalized concentration versus time strongly supports the enhancement transformation at the S2 well (Figure 10).

The CF concentration also increased, but to a lesser extent, indicating enhanced CF transformation in the absence of nitrate. The relative change in CT transformed and CF observed is indicated in Figure 8. Before nitrate was completely eliminated from the test zone, 55–67% of the transformed CT appeared as CF, whereas only 30–40% was observed after nitrate addition was terminated. CF itself may have been transformed at higher rates during this period. However, DM and chloromethane, possible intermediate products of CT and CF transformation, were not found above the detection limit of 1 µg/L. It may also be that the fraction of CT transformed directly to CO<sub>2</sub> increased following nitrate removal.

**Transformation of CFC-11, CFC-113, and TCA.** The responses of CT, CFC-11, CFC-113, and TCA at the S2 well are also shown in Figure 9 and were similar to that of CT, but with slower rates of concentration decrease. The downward inflection in the concentration trends after nitrate was removed indicates enhanced rates of transformation. The log of normalized concentration versus time at the S2 well shown in Figure 10 better illustrates the accelerated concentration decrease at the lower concentrations. Similar semilog responses were obtained for the S1 well (not shown). A significant increase in slope

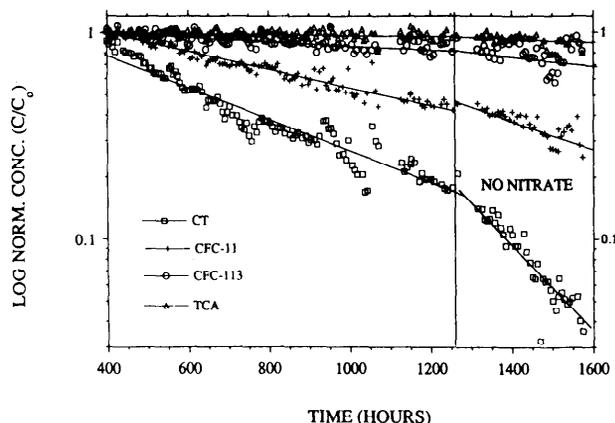


Figure 10. Semilog plot of the halogenated compounds at well S2 along with linear regression lines.

Table III. Estimates of the Average Degrees of Transformation

chemical	well	% transform. <sup>a</sup>	
		av	95% CI <sup>b</sup>
CT	S1	74	70-78
	S2	95	94-96
	S3	96	95-97
	extractn	93	89-96
CFC-11	S1	46	42-50
	S2	68	65-71
	S3	72	69-75
CFC-113	S1	8	0-16
	S2	20	10-30
	S3	18	8-27
TCA	S1	9	5-13
	S2	12	8-16
	S3	9	2-16

<sup>a</sup>Estimates based on the mean calculated values for the period between 1450 and 1550 h after commencing biostimulation.

<sup>b</sup>Confidence interval.

was observed upon nitrate removal from the injected fluid, indicating the increase in transformation rates. At the S2 well the slopes increased by a factor of 2 for both CT and CFC-11, while the slopes increased by a factor of 6-10 at the S1 well. A greater change is expected at the S1 well, since most of the nitrate was present in this region before it was removed.

The degrees of transformation quantified by normalization with bromide as a conservative tracer are presented in Table III. Estimates were made of the degree of transformation over the time period of 1450-1550 h, with over 15 observations used in the estimates. Significant increases in the degree of transformation with increased distance from the injection well were noted for all HACs with the exception of TCA. Steady-state transformation conditions had not been achieved by the end of the experiments. Thus, these transformation extents are considered as conservative estimates.

Products of CFC-11 and CFC-113 transformation were not detected with the gas chromatograph at the field site, probably because of the low concentration of the parent compounds. Also, a probable intermediate of CFC-11 is dichlorofluoromethane (HCFC-21), which elutes fast on the DAC gas chromatograph and, if present, would not have been resolved.

1,1-Dichloroethane (DCA) is an expected intermediate product of reductive TCA transformation (2, 14, 23). DCA was present as a background contaminant in the test zone at a concentration of 5  $\mu\text{g/L}$ , possibly due to the biological transformation of TCA during transport from the con-

taminant source to the test zone. DCA increases over the background levels due to 2-10  $\mu\text{g/L}$  TCA transformation would be statistically insignificant at the 95% confidence level.

There was no direct evidence for the stimulation of sulfate-reducing bacteria or methanogenic bacteria when nitrate was completely removed from the injected water. Methane was not found in groundwater extracted from the test zone, with a detection limit of 1 mg/L. No attempt was made to measure sulfate reduction, due to the high sulfate concentrations in the groundwater (>700 mg/L). A silver probe in the fluid sampling line showed no sign of discoloration, indicating sulfide was not present in high concentrations. If sulfide were produced, reactions with subsurface minerals would probably scavenge sulfide from the groundwater. Thus evidence of sulfate reduction would not be easily obtained.

#### Discussion of Results

This work indicates that CT was transformed to a significant extent and at a rapid rate under subsurface conditions in the absence of dissolved oxygen, when a native population was biostimulated by acetate addition in the presence of nitrate and sulfate. Chloroform was formed as one of the transformation products. CFC-11, CFC-113, and TCA responded like CT, but at reduced rates of transformation.

The field results indicate that the main denitrifying population may not have been responsible for the transformation of the halogenated aliphatics. A secondary microbial population that grew slowly, perhaps on the decay products of the main population, is likely to have been responsible for the transformation. The growth of such a secondary population may have been inhibited by the presence of nitrate. Supporting this hypothesis were the observations that CT transformation significantly lagged nitrate removal, zones that lacked the main population of denitrifiers showed greater degrees of transformation, and transformation was more complete after nitrate was removed from the injected water. Supporting laboratory column studies also showed delayed CT transformation compared with the onset of denitrification and the inability of isolated denitrifiers to transform CT (37). Denitrifiers isolated from Moffett test-zone groundwater samples prior to biostimulation also failed to transform CT (26). The participation of denitrifiers in the transformation, however, cannot be completely dismissed. Bae and Rittmann (40) suggested that nitrate inhibited CT transformation denitrifiers by serving as a competing electron acceptor. Further studies are needed to clarify these issues.

Approximately 8 mg/L acetate was consumed in the test zone after nitrate was removed from the injected fluid. No methane production was observed in the test zone, suggesting that a methanogen was not responsible. Sulfate reducers may have been responsible for this consumption, but with the data available this cannot be confirmed. Egli et al. (21) demonstrated CT transformation by *Desulfobacterium* with approximately 75% conversion to CF. Bouwer and Wright (19) observed faster rates of transformation in columns operated under sulfate-reducing conditions, compared to nitrate-reducing conditions. Bagley and Gossett (41) reported the reductive dechlorination of PCE to TCE and *cis*-dichloroethylene under sulfate-reducing conditions. Lesage et al. (32) observed HCFC-123a as a transformation product of CFC-113 in a sulfate-reducing zone of a landfill leachate plume.

Another possibility is that microorganisms such as *Clostridium* growing on the decay products of the stimu-

lated denitrifiers were carrying out the transformations. Gälli and McCarty (23) found that *Clostridium* sp. growing on amino acids converted CT to CF and other products, as well as converting 1,1,1-TCA to 1,1-DCA and other products.

The rates of transformation found were compound specific. The progression of the extents of transformation show a similar pattern among compounds, with greater extents of transformation the greater the distance traveled (Table III). The semilog response indicates that the same process(es) was (were) transforming the compounds, only at different rates. Based on the responses the ranked rates of transformation were CT > CFC-11 > CFC-113 > TCA.

The difference between CT and CFC-11 is consistent with that expected based on the fewer chloride atoms on the molecule. Fluoride-carbon bonds are more stable than chlorine-carbon bonds, and other evidence suggests that chlorine is the leaving group (2, 42). CFC-11 differs from CT, in that one fluoride replaces a chloride, resulting in a lower transformation rate. Of the two halogenated ethanes, CFC-113 appears to have been transformed at a higher rate than TCA; however, there is much uncertainty in the estimates. Expectations are complicated here because of the differing degrees of substitution and different substituting groups.

The more rapid rate of CT transformation compared to TCA agrees with laboratory studies. Gälli and McCarty (27) observed CT to be transformed 13 times faster than TCA by a *Clostridium* sp.; Bouwer and Wright (19) observed CT to be transformed 40 times faster than TCA under sulfate-reducing conditions; Bae et al. (20) observed much greater extents of CT transformation compared to TCA in a column stimulated with successive denitrifying zones.

The extent of complete mineralization of CT to CO<sub>2</sub> and chloride was not directly determined in the field study. Evidence for Cl<sup>-</sup> production could not be obtained due to the high background chloride concentration of 35 mg/L. However, supporting laboratory column studies with test zone core materials and [<sup>14</sup>C]CT conducted under conditions representative of the field tests (43) confirmed that a significant amount (40–50%) of the CT was completely mineralized to CO<sub>2</sub>, while chloroform production was similar to that observed in the field and represented 30–40% of the CT transformed.

A lower fraction of the CT transformed was observed as CF after more strongly reducing conditions were created by removing nitrate from the injected fluid. This finding differs from the results of Bouwer and Wright (19), who observed the opposite response: more CF was found under sulfate-reducing conditions, compared to denitrifying conditions. Also, Criddle et al. (24), using *E. coli*, observed less CF was formed under fumarate-respiring conditions than under more reducing fermenting conditions. Possibly the lower fraction of CF found in the field study was due to enhanced CF transformation in the absence of nitrate. However, available data are insufficient to confirm whether the lower fraction was due to changing pathways or greater CF transformation.

The products of the transformation of CFC-11 and CFC-113 were not identified, but based upon other studies, a likely intermediate of CFC-11 transformation is CFC-21 (28, 42), and of CFC-113 is HCFC-123a and CFC-1113 (32, 44). These products are similar to those formed from two separate parallel pathways for hexachloroethane reduction (2).

1,1-DCA is suspected, but not statistically confirmed, to be a product of TCA transformation in the field study.

*Clostridium* sp. strain TCAIIB (23) converted TCA into 1,1-DCA, acetate, and unidentified products. Under methanogenic conditions, Vogel (45) identified 1,1-dichloroethane, chloroethane, carbon dioxide, and a nonvolatile fraction as products of TCA transformation. Thus a range of products may have been formed from TCA transformation in the field test.

Abiotic processes must also be considered. Sorption of the halogenated aliphatics to the aquifer solids did not have a strong influence on the results of the field evaluation. CT and TCA were similarly but not strongly sorbed (ref 33; this study). Thus, the different responses for CT and TCA do not appear due from sorption interactions, but from differences in transformation rates.

Abiotic transformation processes may also have contributed to the overall transformation observed. Biotic reactions may have created appropriate environmental conditions for abiotic reactions. CT reduction has been observed in the presence of sulfide minerals (46) and by humic acids in the presence of Fe<sup>2+</sup> and HS<sup>-</sup> (47). Further research is needed in this complex interface between microbial and abiotic processes in the presence of aquifer solids.

The field results indicate more rapid rates of transformation under more strongly reducing conditions after nitrate was removed from the injected water, consistent with laboratory studies of Bouwer and Wright (19), Criddle (26), and Bae and Rittmann (40). Although field results are not available here to indicate whether rapid rates of transformation might have been observed if nitrate had been removed from the injected fluid at the start of the experiment, rapid halogenated compound transformation has not yet been observed under similar conditions in the absence of active stimulation with fermentable or oxidizable organic substrates. It appears that biostimulation of denitrifiers in the test zone here may have provided such a source of substrate in the form of their respiration products to be utilized by other bacteria, such as *Clostridium*, that promoted the halogenated aliphatic transformation. However, it cannot be concluded whether the enhanced biotransformation rates observed when nitrate was removed resulted from its absence as a competing electron acceptor or whether the presence of nitrate inhibited the growth of secondary microorganisms that may have been responsible for the transformation.

## Conclusions

This study provided quantitative evidence that CT was transformed when a natural bacterial population was biostimulated through the addition of acetate to an aquifer containing nitrates and sulfates. There is also evidence based on concentration decreases that two CFCs were also transformed, but to lesser extents. However, for these compounds, transformation intermediates were not identified. Minimal transformation of TCA was observed. CF was formed from CT, which is consistent with laboratory studies. The appearance of CF as a halogenated intermediate product, which is objectionable from a water quality standpoint, poses a significant obstacle to the immediate deployment of this approach for enhanced aquifer remediation for CT.

The biostimulation and transformation experiments demonstrated the following.

(1) A denitrifying population could be stimulated to remove nitrate upon the addition of acetate as a growth substrate to contaminated groundwater.

(2) CT was transformed, with a fraction (30–60%) observed as CF as an intermediate product.

(3) The transformation response supports the hypotheses that either a secondary population, rather than the main denitrifying population, was responsible for the transformation or nitrifiers may have been inhibited by nitrate and transformed CT faster in its absence.

(4) More rapid CT transformation was observed after nitrate was removed from the test zone (subsequent to the growth of an active denitrifying microbial population), and less chloroform was observed under these conditions.

(5) The same process that transformed CT likely transformed the other halogenated aliphatics, but at slower rates, with the following maximum extents transformation achieved: CT, 96%; CFC-11, 72%; CFC-113, 20%; and TCA, 12%.

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Registry No. CT, 56-23-5; TCA, 71-55-6; CFC-11, 75-69-4; CFC-113, 76-13-1; CF, 67-66-3; acetate, 64-19-7.

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