Anaerobic Biotransformation of Trichlorofluoroethene in Groundwater Microcosms

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The biological reduction of trichlorofluoroethene (TCFE) was investigated in anaerobic groundwater microcosms. TCFE was reductively dehalogenated by microorganisms to produce three dichlorofluoroethene isomers, with cis-1,2-dichlorofluoroethene (c-DCF) being the main isomer formed. Further sequential biological transformation of these compounds to mono-chlorofluoroethene isomers was incomplete and occurred at much slower rates. The rates of TCFE reduction were compared to the rates of reduction of two common chlorinated solvents, perchloroethylene (PCE) and trichloroethylene (TCE), when present at similar concentrations. Aqueous concentrations ranged from 7.0 to 14.0 mg/L for TCFE and from 7.5 to 15.0 mg/L for PCE and TCE. Similar rates of PCE and TCE transformation relative to TCFE were observed in single-compound tests (PCE, TCE, and TCFE in separate microcosms) and when the contaminants were present together as mixtures in the microcosms. The close similarities between the time course and kinetics of TCFE degradation and the degradation of both PCE and TCE, when present at comparable initial concentrations, suggest that TCFE could potentially be used as a benign reactive tracer to measure in-situ rates of PCE and TCE transformation in contaminated environments.

Introduction

Chlorinated ethenes such as perchloroethylene (PCE) and trichloroethylene (TCE) are common groundwater contaminants in aquifers throughout the United States (1) and rank among the top priority pollutants listed by the U.S. EPA (2). In view of the widespread distribution of these compounds, there is considerable interest in the potential use of aquifer microorganisms for remediation purposes. It has been reported that reductive dechlorination under anaerobic conditions is a predominant pathway for their biotransformation (3–5). Under anaerobic conditions, chlorinated ethenes such as PCE and TCE are transformed through reductive dechlorination reactions that involve the sequential replacement of the chlorine atoms by hydrogen (3–11). In these instances, the chlorinated ethenes serve as alternate electron acceptors for microbial electron-transfer reactions that involve metal-containing cofactors such as hemes, corrinoids, and other macrocyclics.

Anaerobic biotransformation is now recognized as an important if not dominant factor in the natural attenuation of PCE and TCE (7). It is also a promising technology for enhanced bioremediation (5). Accurate estimates of in-situ biodegradation rates are needed for effective planning, design, and monitoring of bioremediation processes. These rates are usually either indirectly estimated from geochemical data and modeling approaches or directly estimated by following the kinetics of biodegradation by monitoring (a) the rates of disappearance of parent compounds or (b) the accumulation of reduction daughter products (7). Frequently, the direct estimation approach provides inaccurate and obscure results because of one or more of the following reasons: (a) it is generally difficult to distinguish small changes in parent compound concentrations against a large background contamination, (b) it is also difficult to discriminate between preexisting and newly generated daughter products, (c) sorption and desorption processes complicate evaluation of results, and (d) the presence of the contaminants as dense nonaqueous phase liquids (DNAPLs) that slowly dissolve can affect the aqueous phase concentration, which may provide misleading results.

An alternative approach is the indirect measurement of CAH transformation rates using nonregulated analogues of these compounds. Ideally a chlorinated ethene analogue would have similar physical, structural, and chemical properties as PCE and TCE, which would enable these compounds to be analyzed and quantified using previously established and verified approaches. Furthermore, the potential analogue should undergo the same biological transformation as the compound of interest while retaining a distinctive chemical feature or signature throughout the biotransformation process. A review of recent literature indicates that many of these features could be found in fluorinated analogues of TCE and PCE (12–15).

One such compound is trichlorofluoroethene (TCFE), which has transformation properties that are of interest as an analogue for TCE and PCE. TCFE reduction was compared to that of PCE and TCE in corrinoid-mediated reduction reactions (12). Second-order rate coefficients for TCFE transformation fell between those of PCE and TCE, with PCE rates being a factor of 4 greater than TCFE and TCE rates being a factor of 12 greater than TCE. The corrinoid-mediated transformation of TCE and TCFE also produced mainly the cis isomers upon the removal of one chloride ion. The cis-1,2-dichlorofluoroethene (c-DCE) isomer is the common DCE anaerobic transformation product of PCE and TCE in the subsurface (6, 16).

The objective of this research was to evaluate TCFE for estimating the rates of reductive dehalogenation of PCE and TCE. Anaerobic groundwater microcosm studies were performed, and the products of TCFE transformation were identified and monitored along with the disappearance of TCFE. The rates of TCFE transformation and product formation were compared to that of TCE and PCE, both in the presence and in absence of these compounds. Analytical methods were also developed to determine the simultaneous presence of all parent compounds and transformation intermediates.

Materials and Methods

Chemicals and Stock Solutions. Trichloroethene (TCE) (99.9% purity), cis-1,2-dichloroethene (c-DCE) (99.9%), and PCE (99.9%) were obtained from Aldrich Chemical Co. (Milwaukee, WI). Trichlorofluoroethene (TCFE) (97%) (Lancaster Synthesis, Inc., Windham, NH) and dichlorofluoro-
ethene (DFCE) (98% pure mixture consisting of 50% cis and 50% trans isomers) were obtained from ABCR GmbH (Karlsruhe, Germany).

**Microcosm Studies.** Microcosms were constructed with groundwater from well D3 at Site-300, Lawrence Livermore National Laboratory (LLNL), CA. LLNL Site-300 is a TCE-contaminated site with indigenous anaerobic microorganisms capable of dechlorinating high concentrations of TCE (100–200 mg/L) to c-DCE (17). The co-contaminants 2-ethylbutanol (110 mg/L), 2-ethylbutyric acid (410 mg/L), acetic acid (60 mg/L), and tetrakis(2-ethylhexoxy)silane (TKEBS) (75 mg/L) present in the groundwater served as the electron donors for supporting the dechlorination reaction (17). The pH of the groundwater was 6.8. Prior to microcosm construction, the groundwater was stored at 30 °C for approximately 1 month.

The microcosms were constructed with glass serum bottles (310 mL) (Wheaton Industries, Seattle, WA) sealed using screw caps containing butyl rubber septa. The microcosms contained 225 mL of groundwater and 85 mL of headspace and were operated in an anaerobic glovebox (Coy, Grasslake, MI) with an atmosphere of 10% H2 and 90% N2. To prepare the microcosms for the TCFE degradation studies, H2 and the residual TCE and c-DCE in the groundwater were then purged with nitrogen that was treated in a tube furnace (Thermolyne, Inc., Dubuque, IA) to remove any traces of O2. Neat PCE, TCDE, and TCE were added to achieve the desired mass in the microcosms. Microcosms were incubated with either single compounds or a mixture of TCFE and PCE or TCDE and TCE. Control microcosms were prepared by chemical poisioning with mercuric chloride (HgCl2) (25 mg/L). The microcosms were incubated at 30 °C, and the contents were mixed periodically in a gyraatory shaker at 150 rpm about 1–2 h prior to sampling. Before each sampling, the pressure within the microcosms was equilibrated to atmospheric pressure by adding pure N2 gas.

**Analytical Methods.** Gas-phase PCE, TCDE, cis-1,2-dichlorofluoroethene (c-DFCE), trans-1,2-dichlorofluorothene (t-DFCE), TCE, and c-DCE concentrations were determined by gas chromatography. Samples from the microcosm headspace (50 μL) were injected into a HP-5890 GC equipped with a photoionization detector (PID) followed by a flame ionization detector (FID). Chromatographic separation was achieved with a 30-m megabore GSQ-PLOT column (J&W Scientific, Folsom, CA). The concentrations of H2 and CO2 in the microcosm headspace were measured with a HP-5890 GC equipped with a thermal conductivity detector. Chromatographic separation was achieved with a Carboxen 1000 packed column (15 ft × 0.25 mm, 1.0 μm film) (Restek, Inc., Bellefonte, PA). The electron ionization (EI) mass spectral data provide evidence for the dechlorination of TCFE to DFCE and further to chlorofluorothene. However, it was difficult to distinguish between the three isomeric forms of DFCE because they had similar mass spectra.

The qualitative verification of c-DFCE, t-DFCE, and 1,1-dichlorofluoroethene (1,1-DFCE) was performed by proton nuclear magnetic resonance (1H NMR) spectroscopy (19, 20, 21). Samples for NMR analysis were prepared by stripping liquid samples (20 mL) from the microcosms (at the end of the incubation period) with helium in a purge-and-trap apparatus (OI Analytical, College Station, TX). The volatiles were then trapped in a Tenax trap that was subsequently thermally desorbed and collected in a vial containing CC14 with tetramethysilane (1 ppm TMS). The same procedure was repeated with a total of 200 mL of liquid to yield a detectable amount of the analytes of interest. The 1H NMR spectra were obtained with a Bruker AM-400 spectrometer operated at 400 MHz. The approximate chemical shifts are reported in a δ scale (in ppm) and were expected to be 6.06, 5.49, and 7.1 for c-DFCE, t-DFCE, and 1,1-DFCE, respectively, based on theoretical calculations (21). An equimolar mixture of c-DFCE and t-DFCE (CDCl3 with 1 ppm TMS) was used to obtain the reference spectrum. A standard for 1,1-DFCE was not available.

The aqueous-phase concentrations and total mass balances of PCE, TCE, c-DCE, and H2 in the microcosms were determined by measuring gas-phase concentrations and applying appropriate Henry’s law constants (22). The dimensionless Henry’s constants for TCFE, c-DFCE, and t-DFCE are 1.39, 0.88, and 0.67, respectively, at 20 °C (23).

The GC was calibrated using external standards. The gas and liquid volumes in the standard bottles and microcosms were also kept constant.

**Results**

**Reductive Dechlorination in Anaerobic Microcosms.** Initial experiments focused on examining whether TCFE underwent reductive dehalogenation reactions in the anaerobic groundwater microcosms. The microcosms were constructed as described in the Materials and Methods section and were incubated with TCFE (32 μmol) for 10 days. The contaminants 2-ethylbutanol, 2-ethylbutyric acid, and TKEBS present inherently in the groundwater served as precursors to H2, which likely served as the ultimate electron donor for the dehalogenation reactions (17, 18).

The transformation of TCFE to three isomers of DFCE (three peaks with relative percent areas of 87%, 10%, and 3%) and subsequently to 2-chlorofluorooethene was qualitatively verified by GC/MS analysis (Figure 1b–d). No further dechlorination to fluoroethene was observed in these microcosms. The isomeric composition of dichlorofluorothenes was further investigated by 1H NMR analysis. Two peaks at chemical shifts of 6.2 and 5.61 ppm were observed that confirmed the presence of c-DFCE (large peak with a relative area of 0.043) and t-DFCE (small peak with a relative area of 0.004). 1,1-DFCE was observed in trace quantities with GC/MS analysis (by SPME analysis on liquid samples). c-DFCE was the major transformation product, and t-DFCE was the next major intermediate. Defluorinated products were not observed, which suggests that TCFE was only transformed through dechlorination reactions under the experimental conditions studied. The pathway showing the possible products of TCFE dechlorination and the likely dominant pathway is shown in Figure 1a.

The validity of the proposed pathway of TCFE degradation was subsequently examined by following the time course of TCFE reduction and by conducting a mass balance in the microcosms to establish that the earlier qualitative analyses had identified all reactants and reaction products. Rapid dechlorination of TCFE to c-DFCE, t-DFCE, and 1,1-DFCE was observed within 10 days of incubation (Figure 2). c-DFCE accounted for 85–90% of the total TCFE transformed, while t-DFCE accounted for about 10–5% of the total TCFE transformed. Due to the unavailability of authentic 1,1-DFCE standards, the exact concentration of 1,1-DFCE formed could not be determined. After 8 days of incubation, approximately 5% of the TCFE was dechlorinated to 2-chlorofluorooethene. This reaction occurred at much slower rates than the initial dechlorination of TCFE (data not shown).

To determine whether TCFE was transformed at similar rates as TCE and PCE, the rates of dehalogenation were
examined in separate microcosms containing equal moles (32 μmol) of each compound. This permitted investigation of the transformation rates in the absence of potential competitive inhibition among the chlorinated electron acceptors.

Dechlorination of TCE, PCE, and TCFE was observed in all the microcosms over 10 days of incubation (Figure 3a). HgCl₂ completely inhibited the dechlorination of TCFE, PCE, and TCE indicating that the transformations were biologically mediated. PCE was transformed to TCE, which was further transformed to c-DCE (data not shown). During the first 6 days of incubation, TCE concentration increased, after which PCE and TCE were transformed at approximately the same rate (data not shown). PCE was completely dechlorinated to c-DCE within 10 days. In the TCE-amended microcosm, TCE was dechlorinated to c-DCE within 6 days. c-DCE persisted in both the PCE- and TCE-spiked microcosms, and no further transformation to vinyl chloride and ethene was observed after 1 month of incubation. In both the TCE and PCE microcosms, the rate of removal followed zero-order kinetics over most of the concentration range studied. In the TCFE-amended microcosm, dechlorination of TCFE to c-DCFE and t-DCFE was rapid for the first 2 days, after which it slowed considerably. Thus, zero-order removal kinetics did not fit the TCFE response well.

Hydrogen concentrations in the headspace of the microcosms are shown in Figure 3b. Hydrogen accumulated in the microcosms in response to the fermentation processes,
with the most rapid accumulation occurring during the initial first 2 days of incubation. The responses were similar in the microcosms; however, some differences were observed. For example, the slowing of dehalogenation of TCFE after 2 days appears to be associated with acceleration in the rate of hydrogen accumulation. The headspace hydrogen concentrations in the microcosms reach pseudo-steady-state levels of approximately 0.008 atm. This hydrogen level is in the range that is energetically favorable when alcohols are fermented \( (\text{10}) \). The fermentation of ethylbutanol, which was present in the microcosm groundwater as a result of TKEB hydrolysis \( (\text{18}) \), was likely supporting these hydrogen tensions. Differences in the fermentation among the microcosms, as indicated by the hydrogen responses, cannot be ruled out as potentially causing some differences in the dehalogenation rates among the microcosms. Methanogenesis was not occurring in any of the microcosms since no methane production was observed.

The transformation rates during the single-compound tests were obtained from linear regression fits of the parent compound and daughter product concentration histories. Over the first 2 days of the test when the maximum zero-order rates were expressed, rates of PCE and TCE transformation were approximately 50% and 75% of the TCFE rates, respectively. During the period of 3–5 days, PCE and TCE were transformed at 2.4–3.0 times the rate of TCFE as a result of the slowing of the TCFE rate.

The potential competitive interactions between TCFE and both PCE and TCE were examined when equal moles of each compound were incubated as mixtures in the microcosms. These tests were performed in the same microcosms as the previous single-contaminant tests. The microcosms were purged with furnace-treated nitrogen to remove the transformation products and hydrogen from the previous stimulation. The groundwater containing the fermenting organics was not exchanged. In addition, the total amount of the chlorinated ethenes added (32 Mmol) was similar to that used for the single-compound tests.

The results of the tests with TCFE and PCE are shown in Figure 4. After TCFE and PCE addition, there was approximately a 5-day lag period before observable transformation of either PCE or TCFE occurred, based on the detection of transformation products. During this lag period, \( \text{H}_2 \) accumulated at about 50% of the rate observed in the initial incubation (Figure 3). During the period of 5–20 days, PCE was transformed at a rate about a factor of 2 greater than TCFE, while during the period from 20 to 30 days, TCFE and PCE were transformed at similar rates. The reason for these differences are not known, but PCE inhibition of TCFE reduction at the higher initial PCE concentrations is a possibility. After 48 days of incubation, PCE was completely transformed to c-DCE, while about 85% of TCFE was transformed to both c-DCF and t-DCF. Traces of c-DCF and 2-chlorofluoroethene were also observed. Microcosm mass balances found that DCFE and c-DCE accounted for 78% and 80% of the TCFE and PCE transformed, respectively.

In the microcosm containing TCE and TCFE, there was also an approximate 5-day lag period before transformation products of TCFE and TCE were detected (Figure 5). During the lag period, \( \text{H}_2 \) accumulated at a rate similar to that observed with the PCE and TCFE mixture. During the period from 5 to 15 days, TCE was transformed at a rate a factor of...
2 greater than TCFE based on the decrease in the parent compound concentration and the production of daughter products. During the period of 15–25 days, TCE and TCFE were transformed at similar rates. Both TCE and TCFE transformation stopped after about 30 days of incubation, in contrast to continued transformation of TCFE and TCE in the microcosm originally fed PCE and TCFE (Figure 4). Hydrogen partial pressures were similar in both microcosms; thus, H₂ availability does not appear to have caused the loss of transformation in the TCE/TCFE microcosm. But the response indicates that even transient changes in the transformation behavior of TCE and PCE were reflected in the rate of TCFE reduction. The absolute rates of TCE, PCE, and TCFE transformation obtained in mixture tests were an order of magnitude lower than those obtained with the single-compound tests. The lower rates may have been due to changes in dechlorinating activity of the microorganisms with time. The slower accumulation of hydrogen in the later tests may be associated with the slower rates of dehalogenation. Simultaneous work with microcosms containing only TCFE during the same time period also showed lower transformation rates, which indicated that the reduction was not likely the result of competitive inhibition among the compounds.

Discussion

This study investigated both the pathway and the rates of TCFE reductive dehalogenation in groundwater microcosms. TCFE was degraded through a consistent and predictable pathway, and the rates of this reaction were comparable to those observed with TCE and PCE for the specific concentration and experimental conditions tested. These results indicate that TCFE is a potentially useful analogue of these common groundwater contaminants and that TCFE reduction could be used to estimate the rates of reduction of these compounds in the environment.

Using mass spectrometry and ¹H NMR analysis c-DCFE (85–99%), t-DCFE (10%), and 2,2-DCFE (less than 5%) were identified as the primary transformation products in these anaerobic microcosms. Glod and co-workers (12) reported a slightly different product distribution of 73%, 15%, and 12% for c-DCFE, t-DCFE, and 1,1-DCFE, respectively, by corrinoid-mediated reduction of TCFE. Notably, in both the studies, c-DCFE was observed as the major product.

Further transformation to 2-chlorofluoroethene was observed in our studies, but to a very small extent. This is consistent with other results in this study and previous results obtained in our laboratory (17) where both PCE and TCE transformation stopped at c-DCE in microcosms fabricated with this site’s groundwater. Under the conditions of these tests, TCFE behaves as an intermediate between PCE and TCE with respect to reductive dechlorination reactions. Dechlorination of TCFE to the DCFE isomers occurs more rapidly than the subsequent step to chlorofluoro isomers. Slower transformation of DCFE may be a good indicator of TCE dehalogenating systems that stop at c-DCE. More work is needed to verify this in systems where PCE or TCE transformation stops at c-DCE and those that transform beyond c-DCE to VC and ethene.

The single-compound and the mixture tests demonstrated similar rates for the transformation of TCFE, PCE, and TCE as indicated by the relative rate comparisons. The relative dechlorination rates of TCFE to PCE and TCE ranged from approximately 0.3 to 2.0 depending on the data used for the estimate. It is interesting that the corrinoid-based second-order rate constants varied over a greater range, with the PCE rate coefficient being a factor of 4 greater than TCFE and a factor of 50 greater than TCE (12). Although much more kinetic data are needed on the biotic reactions presented here, the differences in rates among these compounds appears to be less variable. For example, similar zero-order transformation rates of PCE and TCE were observed by an anaerobic enrichment in studies conducted over a similar concentration range as these tests (24). It would be of interest to determine how rates of TCFE transformation compared with PCE and TCE with different dechlorinating cultures.

The changes in transformation rates of PCE or TCE among the microcosm studies were closely tracked by TCFE. The actual rates of TCE, PCE, and TCFE transformation were about an order of magnitude lower in the mixture tests when compared to the single-compound tests; however, the relative rates did not change significantly. Similar lags and acceleration or slowing in transformation rates were observed, suggesting that both PCE and TCE transformation rates were tracked using TCFE as a surrogate compound.

This study provides an initial evaluation of how TCFE might be used as a reactive tracer to study PCE and TCE transformation. TCFE or similar analogues could prove to be extremely useful to demonstrate active dehalogenation reactions in situ. It would be potentially useful when direct estimation of rates from contaminant disappearance or daughter product formation is complicated by sorption–desorption processes, the presence of a DNAPL phase, and transport processes in heterogeneous aquifers. The fact that TCFE and other chlorofluoroethenes are not listed in the Toxic Substances Control Act (TSCA) may result in regulatory approval. The persistence of fluorine on the daughter products also serves as a marker to trace and discriminate against background contaminants.

Further studies are needed to develop field-scale techniques to apply this methodology. One possible approach is the single-well, “push–pull” test (25, 26). The test would consist of the controlled injection of a prepared test solution with TCFE into a single well. The test could be performed with or without anaerobic substrates present depending on whether intrinsic or enhanced transformation is being studied. The solution would be given sufficient reaction time in the aquifer for transformation processes to occur. The test solution/groundwater would then be extracted from the same well. By measuring the concentrations of TCFE and its transformation products during the extraction phase, reaction rates may be computed using analytical methods (27).

The methodology requires extensive monitoring of TCFE and its reduction products. Fortunately, no special instrumentation is required, and analysis of these compounds can be easily incorporated into routine VOC analysis techniques because the chromatographic peaks for PCE, TCE, TCFE, and their reduction products are well resolved. The results of gas chromatographic analyses of headspace samples illustrated high resolution and separation between the chlorinated ethenes that were monitored (data not shown). The relative retention times obtained were 10.5 (PCE), 9.2 (TCFE), 8.2 (TCE), 8.0 (c-DCE), 7.2 (t-DCFE), and 7.0 min (c-DCFE) with the GC temperature program used. This suggests that the analysis of TCFE and its transformation products can easily be incorporated into existing volatiles analysis techniques.

More kinetic studies need to be conducted under varying concentration and environmental conditions with groundwater from other sites in order to determine the generality of this approach. Studies discussed here are at elevated TCFE concentrations as compared to those likely to be used in practice. More studies are needed where TCFE concentrations are reduced as compared to the TCE and PCE concentrations that are present. Here competitive inhibition could impact the results. Studies are also needed at groundwater temperatures as compared to the 30 °C temperature used in these tests. The research also needs to be extended to systems...
that transform PCE to VC and ethene to determine how well TCFE tracks more complete dehalogenation. Potentially, this application could be extended to predict rates of other chlorinated hydrocarbons by using comparable fluorinated analogues.

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Literature Cited

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