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Reductive Dechlorination of the Vinyl Chloride Surrogate Chlorofluoroethene in TCE-Contaminated Groundwater

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At many trichloroethene (TCE)-contaminated field sites, microbial transformation of TCE results in the accumulation of vinyl chloride (VC), a known carcinogen and neurotoxin. Quantitative tools are needed to determine the in situ rates of VC transformation to ethene in contaminated groundwater. For this study, *E*-/*Z*-chlorofluoroethene (*E*-/*Z*-CFE) was evaluated as a surrogate for VC in laboratory microcosm and field push−pull tests. Single-well push−pull tests were conducted at a TCE-contaminated field site by injecting *E*-/*Z*-CFE and monitoring for the formation of fluoroethene (FE) over a period of up to 80 days. The rates for VC transformation to ethene and *E*-CFE transformation to FE were within a factor of 2.7 for laboratory microcosm systems and all preferentially transformed *E*-CFE over *Z*-CFE. In the field, the in situ rates of FE production from injected *E*-CFE ranged from 0.0018 to 1.15 μM/day, while the in situ rates of *E*-CFE disappearance ranged from 0.17 to 0.99 μM/day. No significant *Z*-CFE transformation was observed in field tests, which indicated preferential utilization of *E*-CFE over *Z*-CFE under in situ field conditions. The results of this study indicate *E*-CFE as a potential surrogate for estimating the in situ rates of VC transformation.

Introduction

Vinyl chloride (VC) is used by the plastics industry to produce poly(vinyl chloride) (PVC) and copolymers. In 1995, the U.S. production of VC was 6.8 billion kilograms, placing it among the top 50 high volume chemicals produced in the U.S. (*1*). VC contamination of groundwater can occur during accidental release or improper disposal associated with PVC manufacturing. In addition, accumulation of VC, a known carcinogen (*2*), is sometimes observed during reductive dehalogenation of TCE and/or PCE (*3*). The transformation of VC to ethene under anaerobic conditions is considered to be one of the more difficult steps in TCE and/or PCE mineralization, especially at sites where *Dehalococcoides* organisms, which are organisms that are known to transform DCE and VC to ethene, are not present (*4*). Incomplete transformation of TCE under anaerobic conditions may result when the last transformation step is cometabolic in nature and because K_s values for VC are higher relative to the higher chlorinated compounds (*5*−*7*).

Currently, it is difficult to quantify the in situ rate of VC transformation to ethene in contaminated aquifers. Conventional methods for measuring rates of VC transformation to ethene include (a) temporal and spatial monitoring of VC, ethene, ethane, and electron donors and acceptors and (b) laboratory microcosm tests. The temporal and spatial monitoring approach is problematic for chlorinated solvents and their transformation products because changes in concentration due to nonbiological processes such as advection, dispersion, and sorption can obscure changes due to biological processes (*8*−*10*). Laboratory microcosms provide qualitative evidence that dechlorinating populations are present, but the rates obtained do not necessarily reflect in situ conditions. Although stable carbon isotope analysis of chlorinated ethenes (natural abundance) in groundwater has been used to estimate the extent of TCE degradation (*10*−*13*), stable carbon isotopes for quantifying in situ rates has only recently been investigated in the vadose zone (*14*) and under controlled hydraulic conditions in the saturated zone (*15*).

Single-well push−pull tests can provide quantitative information on transformation rates for in situ processes including reductive dechlorination (*16*−*18*), denitrification (*18*, *19*), radionuclide reduction (*20*), anaerobic aromatic hydrocarbon degradation (*21*), and aerobic cometabolism (*22*). A single-well push−pull test consists of injecting or pushing a test solution containing a reactive tracer (reactant) into an existing monitoring well and pulling samples of the test solution/groundwater mixture from the same well over time. Reactions are detected by the loss of injected reactant, the in situ formation of a reaction product, or both. The length of the extraction phase is tailored in accordance with the objectives of the test. For example, fast reactions such as aerobic respiration and denitrification can be quantified when the extraction phase immediately follows injection and consists of continuously withdrawing at least 3 times the injected volume over a period of hours (*19*). In the case where the extraction phase occurs over a period of hours, transformation rates can be obtained for reactants whose reactions are rapid enough to occur over the time scale of the test (*23*). Alternatively, rates can be obtained for slower reactions such as reductive dechlorination (*17*, *18*, *23*), anaerobic alkylbenzene transformation (*21*), and uranium or technicium reduction (*20*, *24*), if the extraction phase consists of periodic sampling over an extended period of time (e.g., days to months). For slow reactions, the volume of injected test solution may need to be increased for field sites characterized by higher groundwater velocities. In situ rates for chlorinated solvents and their transformation products are computed from measured reactant and product concentrations by applying a forced mass balance technique developed by Hageman et al. (*16*) that compensates for the effects of dilution and sorption as a result of transport processes.

To detect microbial transformations in contaminated groundwater that contains high and variable concentrations of contaminants and their transformation products, it is desirable to include labeled contaminants or other surrogates in the injected test solution. For example, deuterium-labeled forms of aromatic hydrocarbons (e.g., toluene-d_5) and a fluorine-labeled form of TCE, trichlorofluoroethene (TCFE), were used to detect anaerobic transformations of petroleum

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hydrocarbons and TCE, respectively, in contaminated groundwater (*16*, *18*, *21*, *23*). These labeled compounds and surrogates do not occur in contaminated groundwater, and their unique chemical signature allows for the unambiguous and sensitive detection (by gas chromatography/mass spectrometry) of the targeted compound and its transformation products. Thus, the detection of diagnostic transformation products from injected labeled contaminants or surrogates provides unambiguous evidence for microbial transformation. Moreover, progress curves (concentration vs time) may be used to estimate in situ transformation rates, which are needed for site risk assessment and remedial design. As a practical matter, deuterium- and fluorine-labeled contaminants are inexpensive and, to date, have received regulatory approval for use in push−pull tests in five states. In contrast, injection of contaminants enriched in carbon isotopes (e.g., ^{13}C and ^{14}C) may be expensive, and the use of ^{14}C substrates may be unacceptable at many field sites.

Vancheeswaran et al. (*8*) and Hageman et al. (*16*, *18*, *23*) studied TCFE as a surrogate for TCE. Anaerobic transformation of TCFE in TCE-contaminated groundwater proceeded by a series of reductive dechlorination reactions to form *Z*- and *E*-dichlorofluoroethene (DCFE); *E*-, *Z*-, and 1,1-chlorofluoroethene (CFE); and fluoroethene (FE), analogous to the products formed from the reductive dechlorination of TCE (*17*, *18*, *23*). TCFE push−pull tests were used to quantify the in situ rates of reductive dechlorination before and after adding fumarate to TCE-contaminated groundwater (*18*). In addition, TCFE push−pull tests were conducted as part of a pilot study conducted in large diameter permeable columns in which three remedial treatments including lactate, zerovalent iron, and hydrogen were evaluated for their effectiveness toward increasing the rate of reductive dechlorination (*17*). In all these previous studies, the most abundant CFE isomer to form typically was *E*-CFE followed by 1,1-CFE and *Z*-CFE.

At many TCE-contaminated sites, interest is focused on the final dechlorination step in which VC is transformed to ethene. When TCFE is added as a reactive tracer, CFE is observed late in the test (~40 days) and at low concentrations due to dilution of the test solution by background groundwater. Furthermore, CFE is simultaneously being formed and transformed. Thus, rates of CFE transformation to FE are difficult to quantify from injected TCFE. Pon and Semprini demonstrated the potential for using 1,1-CFE as a surrogate for vinyl chloride in laboratory microcosm tests (*25*). Kinetic studies performed with an enrichment culture containing *Dehalococcoides*-like microorganisms yielded essentially the same maximum rates and K_s values for 1,1-CFE and VC (*25*). Therefore, the injection of CFE as a tracer into TCE-contaminated groundwater is proposed as a means for directly quantifying its rate of transformation to FE. Alternatively, Gu et al. demonstrated in laboratory microcosm experiments that vinyl bromide is reductively dehalogenated at rates 5−10 times faster than vinyl chloride and could potentially be used in field experiments to obtain evidence of in situ reductive dechlorination quickly (*26*).

The objective of this study was to determine the suitability of *E*-/*Z*-CFE for use as a fluorine-labeled surrogate in field push−pull tests to evaluate the in situ rate of VC reductive dechlorination to ethene. *E*-/*Z*-CFE was selected for this study based on its formation in previous field studies where TCFE was added as a surrogate for TCE. To compare the relative kinetics of VC and CFE reductive dechlorination, experiments were conducted in microcosms prepared using aquifer material and groundwater from two field sites and in batch reactors containing an enriched, mixed-microbial culture isolated from a third TCE-contaminated field site. In addition, push−pull tests were conducted by injecting *E*-/*Z*-CFE into existing wells at a TCE-contaminated site to estimate the in situ rates of *E*-/*Z*-CFE reductive dechlorination to FE, a step that is analogous to the transformation of VC to ethene.

Experimental Procedures

Chemicals. Chlorofluoroethene (CFE) was purchased as a 57:43 (*E*)/(*Z*) mixture from Synquest Labs (Alachua, FL). Fluoroethene (FE) was purchased from Lancaster Synthesis (Pelham, NH). Trichloroethylene (TCE) 99.9% and sodium lactate (60% syrup) were purchased from Fisher Scientific (Fairlawn, NJ). Vinyl chloride (VC) and ethene were purchased from Aldrich Chemical (Milwaukee, WI). Potassium bromide (99.7%) was obtained from Fisher Scientific and used as a conservative tracer in the field push−pull tests. Standards of 1-chloropropane and 1-chlorobutane were obtained from Matheson Company (Cincinnati, OH) and Mallinckrodt (St. Louis, MO), respectively, for use as internal standards for purge-and-trap analyses.

Microcosm and Batch Reactor Preparation. All bottles (Wheaton, Millville, NJ) for the microcosm and batch reactors were initially autoclaved at 120 °C for 30 min. Microcosms were assembled in an anaerobic glovebox under an atmosphere of 90% nitrogen 10% hydrogen gas and stored in an incubator held at 20 °C.

Three sources of material were used for microcosm and batch reactor experiments. Aquifer materials and groundwater were obtained from a shallow unconfined aquifer (A-zone) at a former site of a chemical plant in Richmond, CA. (*23*). The push−pull tests conducted for this study (described next) were conducted in wells at this site. Aquifer material (100 mL) from the Richmond site was added to five autoclaved 320 mL serum bottles that contained 120 mL of groundwater from the A-zone and 100 mL of headspace. The bottles were fitted with screw-down caps containing gray butyl rubber septa (Wheaton, Millville, NJ). The second system was a sediment-free mixed (Evanite) culture enriched from a TCE-contaminated site in Corvallis, OR and is known to contain *Dehalococcoides*-like microorganisms (*7*, *25*, *27*). Microcosms were constructed by adding 50 mL of media (*28*) and 50 mL of sediment-free enrichment culture to eight autoclaved 156 mL serum bottles with gray butyl rubber septa (Wheaton, Millville, NJ). The bottles contained 100 mL of liquid media and 56 mL of headspace. The third system was a single microcosm constructed with sediment and groundwater obtained from a TCE-contaminated site near Greer, SC. Sediment core material (25 mL) and groundwater (75 mL) were added to one autoclaved 156 mL serum bottle with 56 mL headspace. The Greer microcosms were then treated in an identical manner as the Richmond and Evanite systems.

Microcosm and Batch Reactor Kinetics. Reductive dechlorination activity was initially stimulated by incubating the microcosms and batch reactors with 115 μM TCE from a saturated stock solution and either 0.2 atm (20 mL) of hydrogen gas (Richmond microcosms) or 1.7 mM lactate (Greer microcosm and Evanite batch reactors) as the electron donor. This stage was terminated by purging with nitrogen to remove all chlorinated ethenes once the microcosms exhibited quantitative conversion of TCE to ethene.

After completion of the stimulation phase, experiments were then conducted in the microcosms to determine the kinetics of VC transformation to ethene. Four Richmond microcosms, four Evanite batch reactors, and the single Greer microcosm were amended to give an initial aqueous concentration of 80 μM VC (added as a neat gas and allowed to equilibrate) (Table 1). In addition, hydrogen (0.2 atm) was added to the Richmond microcosms, and 1.7 mM lactate was added to the Evanite batch reactors and Greer microcosm. The same four Richmond microcosms, a second set of four Evanite microcosms that had not received VC, and the Greer microcosm were then amended with *E*-/*Z*-CFE (added as a neat gas and allowed to equilibrate) (Table 1).

TABLE 1. Microcosm and Batch Reactor Composition and History

system	initial stimulation phase	VC experiment	CFE experiment
Richmond microcosms[a]	115 μM TCE + 0.2 atm H_2	80 μM VC ($n = 4$)	80:60 μM *E*-/*Z*-CFE ($n = 4$)
Richmond control[a]	killed control; no TCE or H_2	80 μM VC ($n = 1$)	80:20 μM *E*-/*Z*-CFE ($n = 1$)
Evanite batch reactors[b]	115 μM TCE + 1.7 mM lactate	80 μM VC ($n = 4$)	80:20 μM *E*-/*Z*-CFE ($n = 4$)
Evanite controls[b]	killed control; no TCE or lactate	80 μM VC ($n = 1$)	80:20 μM *E*-/*Z*-CFE ($n = 1$)
Greer microcosm[a,c]	115 μM TCE + 1.7 mM lactate	80 μM VC ($n = 1$)	80:60 μM *E*-/*Z*-CFE ($n = 1$)

[a] The same microcosm bottles were used for all the experiments (conducted in sequence). [b] Experiments with VC and CFE were conducted in parallel in separate batch reactors for VC and CFE. [c] No control was available.

TABLE 2. Background Groundwater Constituent Concentrations Determined Prior to the CFE Push−Pull Tests

	9A	10A	15C	16C	17C	21C
TCE (μM)[a]	1	0.02	49	2553	345	ND[b]
DCE (μM)[a]	879	0.2	0.6	16	33	ND
VC (μM)[a]	69	0.005	0.9	0.5	21	ND
nitrate (mM)[c]	ND	NS[d]	ND	ND	ND	ND
sulfate (mM)[c]	28.4	NS	0.35	3.8	4.2	0.15

[a] Concentrations determined February 2001, 15 months prior to CFE tests. [b] ND: not detected. [c] Concentrations determined May 2002. [d] NS: no sample available.

TABLE 3. Injectate Concentrations and Volume for Each Well

	9A	10A	15C	16C	17C	21C
E-CFE (μM)[a]	80.3	5.5	34.5	37.0	34.9	45.8
Z-CFE (μM)[a]	32.0	2.7	16.7	15.4	13.3	18.9
E-CFE/*Z*-CFE[a]	2.5	2.0	2.1	2.4	2.6	2.4
Br (mM)	1.3	1.4	1.3	1.3	1.4	1.5
sulfate (mM)	0.2	0.2	0.2	0.1	0.1	0.16
injection volume (L)	50	50	250	250	250	250

[a] Determined from the first extraction phase sample.

Over the course of the study, different lots of *E*-/*Z*-CFE were used, and the ratios of *E*-/*Z*-CFE ranged from 1.3 to 4. Prior to conducting the CFE tests, the Richmond microcosms were purged with nitrogen to remove all volatile organics. The headspace of the microcosm and batch reactors was sampled twice each week for volatile organics. To compute zero-order rates for *E*-CFE, *Z*-CFE, VC, FE, and ethene, linear regression was applied to the linear portion of each individual progress curve that corresponded to the highest rate of activity.

Controls. Killed controls for the Richmond microcosms ($n = 1$) and for the Evanite batch reactors ($n = 2$) consisted of bottles that were autoclaved at 120 °C for 45 min and then dosed with either VC or CFE but no added hydrogen or lactate. No control microcosms were available for the Greer site microcosm. All controls were stored and sampled in the same manner as that of the live microcosm bottles.

Push−Pull Tests. Field push−pull tests were conducted at the site of a former chemical manufacturing facility near Richmond, CA where in situ reductive dechlorination was previously quantified by push−pull tests conducted with TCFE (*16*, *17*, *23*). The water table lies within 3 m of the ground surface, and groundwater velocities range from 1.5 to 6 m/year. Two water-bearing zones are present at the site including the A-zone (3−6 m below land surface), which is an unconfined shallow layer composed mainly of placed fill over Bay Mud, and the C-zone (6−23 m below land surface), which underlies the Bay Mud and is characterized by alluvial fan deposits (*29*).

Six push−pull tests were conducted in May 2002 in two A-zone wells (9A and 10A) and in four C-zone wells (15C, 16C, 17C, and 21C). Background concentrations of chlorinated ethenes (i.e., TCE, *cis*-DCE, and VC) and electron acceptors (nitrate and sulfate) for the test wells are in Table 2. Background samples also were analyzed for chlorofluoroethenes; however, none were above the quantification limit of the method (0.005 μM). This same set of wells was last used for TCFE push−pull tests in February 2001. The substrate (fumarate) added during the tests conducted in February 2001 was quickly utilized and was therefore not present at the time of the CFE push−pull tests.

Field push−pull test procedures were similar to those described in Hageman et al. (*23*). Briefly, test solutions were prepared from tap water sparged with compressed Ar gas to reduce dissolved oxygen concentrations to <1 mg/L. Tap water was selected at this site due to the limited availability of site groundwater. Concentrated aqueous stock solutions were prepared in metalized-film gas-sampling bags that contained *E*-/*Z*-CFE (30−129 μM with an *E*-/*Z*-CFE ratio that ranged from 2 to 2.6) and bromide (1.3 mM) to serve as a conservative tracer (Table 3). Calibrated peristaltic and piston pumps were used to combine the tap water and stock solutions to achieve the targeted concentrations in the injected test solutions. For the 2.5 cm diameter A-zone wells, 9A and 10A, 50 L of test solution was injected into the bottom of the well at a rate of ∼0.2 L/min. For the 10 cm diameter C-zone wells including 15C, 16C, 17C, and 21C, 250 L of test solution was injected at a rate of ∼2 L/min between a pair of inflatable packers used to isolate a meter-long section of the well screen.

After injection, samples of the test solution/groundwater mixture were collected from the test wells approximately once per week for up to 84 days. Prior to sample collection, the wells were purged by pumping with a peristaltic pump (0.3 L for the shallow A-zone wells and 12 L for the deeper C-zone wells, respectively). Samples were collected in duplicate or triplicate in 40 mL glass vials without headspace, preserved in 0.75% (v/v) concentrated HCl, shipped on ice, and stored at 4 °C until analysis. Samples for bromide analysis were collected in 7 mL glass vials with no preservation.

Analytical Methods. Headspace samples from microcosm experiments were analyzed for CFE, FE, VC, and ethene by injecting gas-phase samples into a Hewlett-Packard 5890 series II gas chromatograph (GC) (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector. Separations were achieved on a 30 m × 0.32 mm × 4 μm Supelco SPB-1 (Supleco, Bellfonte, PA) column. The instrument was operated under a thermal gradient with He as the carrier gas. The quantification limit was 0.5 μM for all volatile organics. The precision of the GC/FID method, as indicated by the relative standard deviation of replicate analyses, was ±1.1%.

The concentrations of VC, CFE, FE, and ethene in microcosm and batch reactors were determined by external calibration using standards prepared in microcosm bottles containing known concentrations of VC, CFE, FE, and ethene ranging from 0 to 200 μM in deionized water. Five 156 or 320 mL bottles were used to construct five-point calibration curves that were linear with typical R^2 values of 0.999.

Extraction-phase samples from the push−pull tests were analyzed with a purge-and-trap system composed of a

Tekmar-Dohrmann (Cincinnati, OH) 3100 sample concentrator and an AQUA Tek 70 liquid analyzer. The purge-and-trap was interfaced with a Hewlett-Packard model 5890 GC (Palo Alto, CA) and a Hewlett-Packard 5972 mass selective detector (MSD). Chromatographic separations were performed on a J&W Gas Pro (Agilent) 60 m × 0.32 mm × (proprietary film thickness) column. For quantification of analytes in field samples, the MSD was operated in selected ion monitoring mode. 1-Chloropropane and 1-chlorobutane were used as internal standards. The quantification limit of the purge-and-trap GC/MSD method was 0.005 μM for all analytes. The precision of the GC/MS method, as indicated by the relative standard deviation of replicate analyses, was ±1.02%.

Push−Pull Test Data Analysis. For the push−pull tests, in situ rates were calculated for the reductive dechlorination of the CFE isomers to form FE by removing the effects of transport processes from measured aqueous concentrations using a forced mass balance (FMB) technique (*16*). Conventional methods that normalize solutes to nonsorbing (conservative) tracers to account for test solution dilution cannot be applied to solutes whose transport may be retarded due to sorption. For this reason, the FMB technique was developed to account for differences in retardation between reactants and products when all reactants and products are known and can be accounted for. In the case of reductive dechlorination of VC and CFE, the only known transformation products are ethene and FE, respectively. The FMB technique consists of first multiplying the measured aqueous phase concentrations of CFE and FE in extraction-phase samples by the estimated retardation factor (R) for each analyte (*16*). In this manner, the analyte's total concentration (aqueous and sorbed) is obtained.

$$[\mathrm{CFE}]_{\mathrm{aq+s}} = [\mathrm{CFE}]_{\mathrm{aq}} R_{\mathrm{f}}^{\mathrm{CFE}} \quad (1)$$

$$[\mathrm{FE}]_{\mathrm{aq+s}} = [\mathrm{FE}]_{\mathrm{aq}} R_{\mathrm{f}}^{\mathrm{FE}} \quad (2)$$

The retardation factors were computed from K_{om} values estimated by the Estimations Program Interface Suite (*29*), the measured fraction of organic matter in the A- ($f_{\mathrm{om}} = 0.02$) and C-zones ($f_{\mathrm{om}} = 0.001$), and the bulk density (2.12) and porosity (0.2) of site sediments. The total concentration in aqueous and sorbed phases, $[\mathrm{CFE}]_{\mathrm{aq+s}}$ and $[\mathrm{FE}]_{\mathrm{aq+s}}$, is then divided by a transport-process adjustment factor, Σ/Σ_o (*15*), to obtain an FMB-adjusted concentration for CFE and FE for each extraction-phase sample.

$$[\mathrm{CFE}]_{\mathrm{FMB}} = \frac{[\mathrm{CFE}]_{\mathrm{aq+s}}}{\Sigma/\Sigma_o} \quad (3)$$

$$[\mathrm{FE}]_{\mathrm{FMB}} = \frac{[\mathrm{FE}]_{\mathrm{aq+s}}}{\Sigma/\Sigma_o} \quad (4)$$

The adjustment factor, Σ/Σ_o, is the summed FMB molar concentration (aqueous + sorbed) of CFE and FE in a sample (Σ), divided by the summed (Σ_o) FMB molar concentrations (aqueous + sorbed) for CFE and FE in the injected test solution at the end of the injection phase, which is taken as the first extraction-phase sample. The FMB concentrations were plotted with time to create progress curves, and the linear portion of the curves was fitted using linear regression to obtain zero-order transformation rates. Progress curves for CFE will appear greater than the aqueous injectate concentrations as a result of the FMB data treatment, which takes into account CFE in the aqueous and solid phases. Because FE is the only known degradation product of CFE under anaerobic conditions, the appearance rate of FE is proportional to the disappearance rate of *E*-/*Z*-CFE. An error analysis conducted by Hageman et al. (*18*) indicated that the actual in situ rates obtained using the FMB technique are within 10% of the true rates.

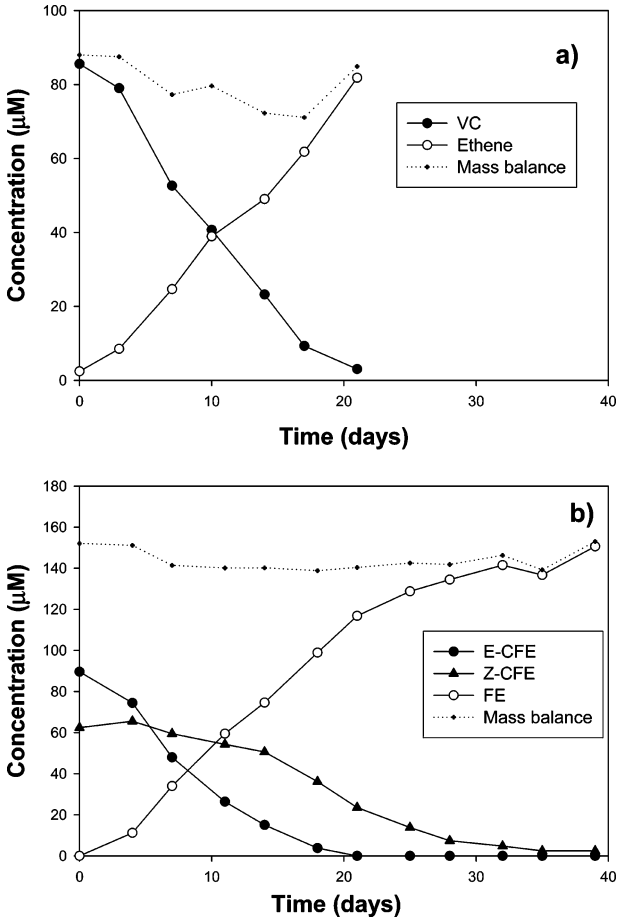

FIGURE 1. Example time progress curves for reductive dechlorination of (a) vinyl chloride and (b) chlorofluoroethene in a Richmond microcosm.

Results and Discussion

Richmond Microcosms. Microcosm studies permitted the evaluation of VC and CFE transformation kinetics. Progress curves of the Richmond microcosms indicated that reductive dechlorination of VC began almost immediately, with no apparent lag after addition of the compounds (Figure 1a). The absence of a lag phase is likely due to the addition of TCE and hydrogen during the initial stimulation of the Richmond microcosms. The nonconstant slope of the time-course plot indicated mixed-order kinetics, which could result from the initial VC concentrations (80 μM) being above the K_{s} value for the dehalogenating microorganisms. For example, a K_{s} value of 64 μM was recently reported for the Evanite enrichment culture (*27*). However, for simplicity, zero-order kinetics was applied to the linear region to facilitate comparison of the laboratory and field data.

Conversion of VC to ethene (60−95%) was observed for all Richmond microcosms, and no other products were detected. The average zero-order rate (±95% confidence interval) for the Richmond microcosms was 4.80 ± 1.35 μM/day for VC disappearance and 3.61 ± 0.96 μM/day for ethene production (Table 4). Between 84 and 96% of the initial VC mass was accounted for at the end of the experiments. In the control, no production of ethene was detected, and 85% of the initial VC mass was accounted for at the end of the experiment.

Subsequently, the *E*- and *Z*-CFE isomers were dechlorinated to FE in the Richmond microcosms ($n = 4$) (Figure 1b).

TABLE 4. Average Zero-Order Rate Constants for VC, E-CFE, and Z-CFE Transformation and the Formation of FE and Ethene[a]

microcosm	VC (μM/day)	ethene (μM/day)	E-CFE (μM/day)	Z-CFE (μM/day)	FE (μM/day)
Richmond[b]	−4.80 ± 1.35 (1.38)	3.61 ± 0.96 (0.98)	−6.86 ± 1.87 (1.91)	−2.95 ± 0.50 (0.51)	7.14 ± 1.98 (2.01)
Evanite[b]	−14.89 ± 2.98 (3.04)	14.25 ± 2.55 (2.60)	−9.19 ± 0.48 (0.49)	ND[c]	8.62 ± 0.66 (0.67)
Greer[d]	−2.00	2.36	−5.32	−3.27	6.42

[a] The average zero-order rate is reported along with ± 95% confidence interval, which is a measure of the accuracy of the reported average rate. Precision, as indicated by the standard deviation, is given in parentheses and is a measure of the variability between the replicate microcosms. [b] Four microcosms each were used to determine rates. [c] ND: no transformation detected. [d] A single microcosm experiment was conducted.

Progress curves for the CFE experiment also indicated mixed-order kinetics for both CFE isomers (Figure 1b). The CFE isomer concentrations of 80 and 60 μM were in the region of the K_s value determined for 1,1-CFE (87 μM) by Pon and Semprini with the Evanite enrichment culture (*24*). The *E*-CFE isomer was dechlorinated with no lag period at a maximum average rate of 6.86 ± 1.87 μM/day (Table 4). In contrast, the dechlorination of *Z*-CFE began after a lag period of 7 days during which substantial *E*-CFE was transformed. After the lag phase, *Z*-CFE dechlorination proceeded at a rate of 2.95 ± 0.50 μM/day (Table 4) once the *E*-CFE concentration had decreased to 20−50 μM. Between 82 and 99% of the added CFE was converted to FE. The formation of FE occurred at a rate of 7.14 ± 1.98 μM/day, and FE was the only product detected (Table 4 and Figure 1b). Mass balance was achieved with 93−101% of the initial mass accounted for at the end of the experiments. No FE production was detected in the killed control, and 89% of the initial CFE mass was accounted for at the end of the experiment.

The average rate of *E*-CFE transformation to FE was faster by a factor of 1.4 than those of VC transformation to ethene (Table 4). Rates of FE formation also were faster by a factor of 2 than those of ethene formation. The greater relative rate of FE formation as compared to ethene formation is the result of the *Z*-CFE transformation to FE that occurred. The behavior of *E*-CFE and *Z*-CFE indicates that the presence of *E*-CFE may be inhibitory to *Z*-CFE dechlorination, which suggests a higher Monod half-saturation coefficient, K_s, for *Z*-CFE than for *E*-CFE. However, more detailed kinetic tests are required to determine K_s values for the individual CFE isomers. The preferential and faster utilization of *E*-CFE relative to *Z*-CFE can be seen in Figure 1b and also was indicated by a decrease in the *E*-CFE/*Z*-CFE ratio, which decreased from an initial value of 1.5 to 0 over the course of the experiment.

Evanite Culture Batch Reactors. The Evanite culture batch reactors (n = 4) dechlorinated VC to ethene (Figure 2a) with no lag phase at an average zero-order rate of 14.89 ± 2.98 μM/day, while the rate of ethene production was 14.25 ± 2.55 μM/day (Table 4). VC conversion to ethene ranged from 84 to 102%. Mass balance was achieved with 83−102% of the initial mass present accounted for by the end of the tests. No production of ethene was detected, and 99% mass balance was attained for the killed control.

A separate set of four Evanite batch reactors was run in parallel with the VC experiments. In these experiments, *E*-CFE was preferentially transformed relative to *Z*-CFE as indicated by the complete removal of *E*-CFE without any evidence of *Z*-CFE transformation (Figure 2b). *E*-CFE was dechlorinated with no lag phase at an average rate of 9.19 ± 0.48 μM/day (Table 4). Of the initial CFE added, 79−92% was converted to FE, no other products were detected, and mass balances of 83−96% were achieved. The average rate of FE formation was 8.62 ± 0.66 μM/day (Table 4). The ratio of *E*-CFE/*Z*-CFE decreased over the experiment from an initial value of 4 to 0. No FE production and 87% mass balance was observed in the killed control.

In the batch reactors containing the Evanite enrichment culture, both VC and *E*-CFE disappearance occurred without a lag phase. The absence of a lag phase is consistent with the observed complete dechlorination of TCE to ethene during the initial stimulation phase. Although *E*-CFE was preferentially utilized relative to *Z*-CFE in both Richmond microcosms and Evanite batch reactors, no detectable utilization of *Z*-CFE occurred for the Evanite batch reactors after *E*-CFE had completely transformed to FE. The rate of VC transformation to ethene was a factor of 1.6 greater than the rate of *E*-CFE to FE in the Evanite batch reactors. With Evanite enrichment culture, Pon and Semprini found that 1,1-CFE also was readily transformed to FE at a rate that was 1.1 times that of VC to ethene (*25*). Although Richmond gave higher rates for *E*-CFE than VC, the relative rates of *E*-CFE and VC transformation in both Richmond and Evanite systems were within a factor of 2. The fact that the Evanite enrichment culture contains *Dehalococcoides*-like microorganisms indicates that further research is needed to determine if correlations exist between the relative rates of VC and *E*-CFE transformation and the presence of *Dehalococcoides*-like organisms.

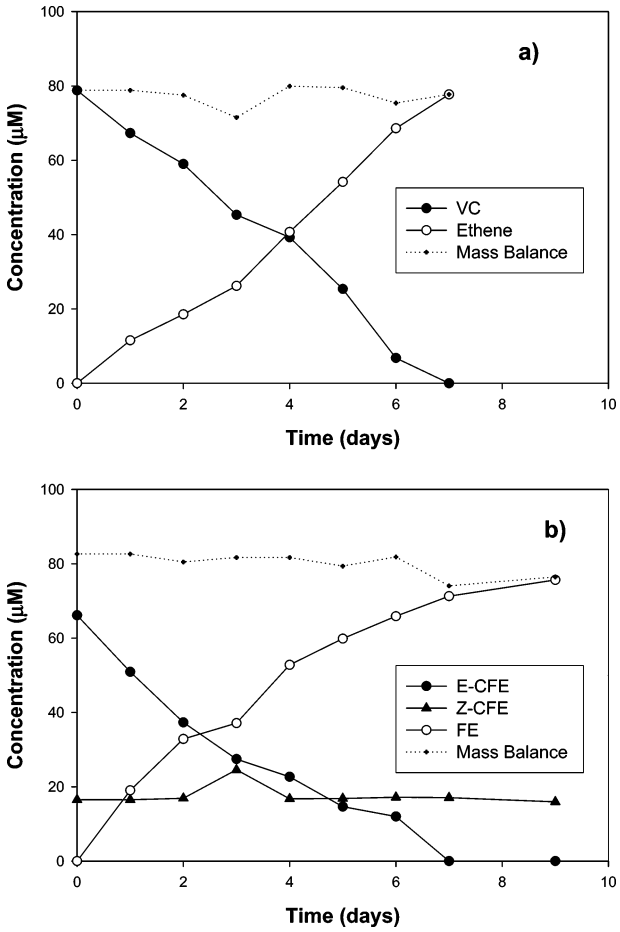

FIGURE 2. Example progress curves for reductive dechlorination of (a) vinyl chloride and (b) chlorofluoroethene in an Evanite enrichment culture batch reactor.

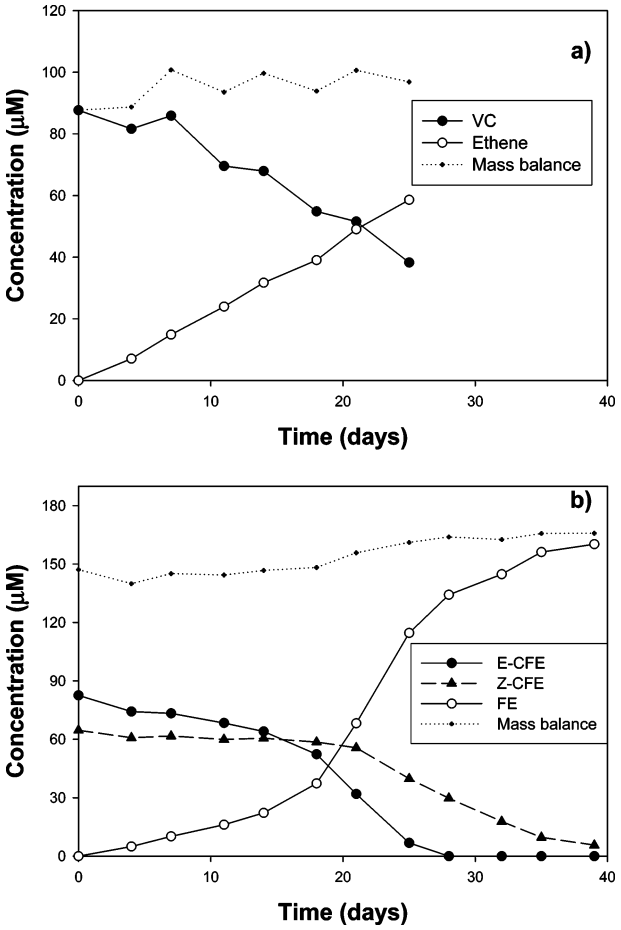

FIGURE 3. Progress curves for reductive dechlorination of (a) vinyl chloride and (b) chlorofluoroethene in a Greer microcosm.

Greer Microcosm. In the Greer microcosm (Figure 3a), 67% of the VC was transformed to ethene at a maximum zero-order rate of 2.00 μM/day, while the rate of ethene production (measured between days 0 and 25) was 2.36 μM/day (Table 4). The absence of a lag phase is consistent with the observed transformation of 70 μM TCE to ethene in a 50 day period prior to the VC experiment. Mass balance was achieved with 110% of the initial mass accounted for at the end of the experiment.

The rate of *E*-CFE dechlorination (5.32 μM/day) was 1.6 times greater than the rate of *Z*-CFE dechlorination (3.27 μM/day), and the rate of FE production was 6.42 μM/day (Figure 3b and Table 4) with 117% conversion of *E*-/*Z*-CFE to FE. Mass balance also was achieved with 116% of the initial mass accounted for. The utilization of *Z*-CFE began on day 21 once the aqueous concentration of *E*-CFE decreased to ~30 μM, which suggests inhibition of *Z*-CFE by *E*-CFE as suggested by data from the Richmond microcosms. This preferential utilization is illustrated by the decrease in the *E*-/*Z*-CFE ratio from 1.3 to 0 over the course of the experiment.

The Greer microcosm was similar to the other two microcosm systems in that it transformed VC to ethene and *E*-CFE to FE. The rate of *E*-CFE transformation to FE in this single microcosm (no replicates) was a factor of 2.7 times faster than the rate of VC to ethene transformation. The behavior of CFE and VC in the Greer microcosms was similar to that of the Richmond microcosms. The Greer and Richmond microcosms containing site sediment and groundwater gave contrasting results to that of the Evanite enrichment culture batch reactors that contain *Dehalococcoides*-like microorganisms for which VC transformation was faster than that of *E*-CFE.

Field Push−Pull Tests. In situ transformation of injected *E*-/*Z*-CFE to FE was observed, and rates were quantified in both A-zone wells (9A and 10A) and in two (15C and 16C) out of the four C-zone wells tested. Background samples showed no *E*-CFE, *Z*-CFE, or FE above the quantification limit of the analytical method in wells 9A, 15C, 16C, 17C, and 21C (0.005 μM). Background samples could not be obtained prior to testing of well 10A.

Well 9A. The CFE experiment in well 9A provided a quantitative measure of the in situ rate of CFE transformation to FE. Progress curves for each push−pull test display the FMB concentrations for each CFE isomer and FE, as determined by eqs 3 and 4 (see Experimental Procedures), against elapsed time in days. In well 9A, a zero-order rate (±95% confidence interval) of transformation of 0.17 ± 0.06 μM/day (Table 5) was obtained for *E*-CFE by linear regression of the FMB *E*-CFE concentrations over days 0−56 (Figure 4a). The corresponding rate (±95% confidence interval) of FE formation was 0.11 ±0.01 μM/day (Table 5). Linear regression of the *Z*-CFE isomer data gave a rate with a confidence interval that was not different from zero, indicating no significant removal of that isomer (Table 5). The preferential removal of the *E*-CFE isomer relative to the Z-isomer was supported by the ratio of *E*-CFE/*Z*-CFE, which declined from a value of 2.5 to 2.1 over 56 days. The removal of *E*-CFE relative to *Z*-CFE was consistent with the microcosm and batch reactor experiments that indicated inhibition of *Z*-CFE reductive dechlorination by *E*-CFE.

The actual measured aqueous concentrations of *E*-CFE ranged from 80 to 9 μM, *Z*-CFE concentrations ranged from 30 to 5 μM, and the aqueous FE concentrations ranged from below detection to 0.7 μM over the 84 day test period (data not shown). The test solution was diluted by 90% with groundwater at the end of the extraction phase as determined by the relative concentrations of the co-injected bromide tracer (data not shown).

FE formation was unambiguously detected in this CFE push−pull test conducted in well 9A. In prior tests when TCFE was added, no FE formation was detected before or after three successive additions of fumarate (*18*). The lack of FE detection during the earlier TCFE tests may have resulted from the formation of CFE late in the test (~40 days) at concentrations near the quantification limit (0.005 μM) due to dilution of the test solutions by background groundwater.

Well 10A. This test yielded an in situ rate of *E*-CFE transformation (Figure 4b) of 0.99 ± 0.33 μM/day, between days 21 and 40 (Table 5). The *Z*-CFE isomer was not significantly transformed (Table 5). The corresponding rate (±95% confidence interval) of FE formation was 1.15 ± 0.27 μM/day (Table 5). Over days 21−40, the ratio of *E*-CFE/*Z*-CFE decreased from 2.6 to 0.4. During the test, the actual measured aqueous concentrations of *E*-CFE varied from 5.5 to 0.09 μM, *Z*-CFE concentrations ranged from 2.7 to 0.15, and FE concentrations ranged from below the quantification limit (0.005 μM) to 3.7 μM (data not shown). The test solution was diluted by 80% at the end of the test period as determined by the bromide tracer (data not shown).

A higher rate of CFE transformation to FE was expected for well 10A because FE was detected in this well during a TCFE push−pull test conducted 15 months prior to the CFE test (*17*). The higher rate of *E*-CFE transformation to FE in well 10A as compared to 9A is consistent with the background contaminant profile in well 10A (Table 2). The higher rate of CFE transformation in well 10A relative to well 9A may be due, in part, to the lower total concentration of the higher-chlorinated ethenes (e.g., TCE, DCE, and VC) in well 10A (0.2 μM) as compared to well 9A (949 μM) (Table 2). Others have found that TCE and *cis*-DCE have lower K_s values as compared to VC and may therefore may be inhibitory to VC reductive dechlorination (*7*, *31*).

TABLE 5. Rates (±95% Confidence Interval) of CFE Disappearance and FE Formation Obtained from Push−Pull Tests Conducted in Each A- and C-Zone Well

rate (μM/day)[a]	9A (days 0−56)	10A (days 21−40)	15C (days 26−82)	16C (days 26−82)	17C	21C
E-CFE	−0.17 ± 0.06 (0.6926)[b]	−0.99 ± 0.33 (0.94)[b]	−0.47 ± 0.08 (0.9771)[b]	NS[c]	ND[d]	ND
Z-CFE	NS	NS	NS	NS	ND	ND
FE	0.11 ± 0.01 (0.9833)[b]	1.15 ± 0.27 (0.9857)[e]	0.48 ± 0.07 (0.9811)[b]	0.0018 ± 0.0006 (0.9082)[b]	ND	ND

[a] Rate (± 95% confidence interval) with correlation coefficients, *r*, given in parentheses. [b] Significant at the 99% confidence interval determined by two-tailed significance test. [c] NS: rate determined from linear regression is not significant. [d] ND: no rate determined since no FE was formed. [e] Insufficient degrees of freedom to evaluate significance by two-tailed significance test.

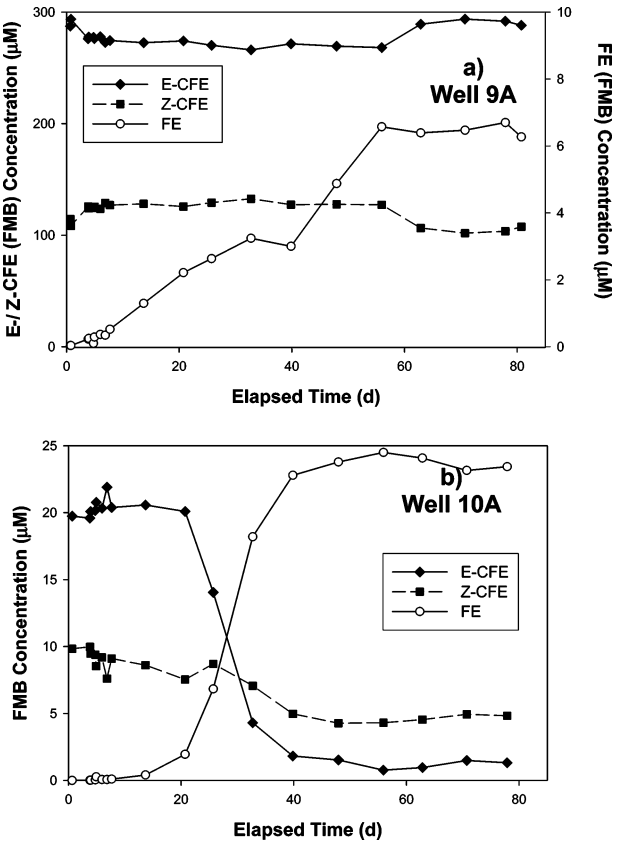

FIGURE 4. Progress curves for reductive dechlorination of *E*-/*Z*-chlorofluoroethene (CFE) to fluoroethene (FE) during push−pull tests conducted in (a) well 9A and (b) well 10A.

Well 15C. Unequivocal evidence of CFE transformation to FE was obtained (Figure 5a). The *E*-CFE isomer was transformed at a rate of 0.47 ± 0.08 μM/day between days 26 and 82, but no significant transformation of *Z*-CFE was observed (Table 5). The maximum rate of FE formation over days 26−82 was 0.48 ± 0.07 μM/day (Table 5). The *E*-CFE/*Z*-CFE ratio decreased from 1.7 to 0.4 over the period of maximum transformation, which is consistent with inhibition of *Z*-CFE by *E*-CFE suggested by the data from the microcosm and batch reactor systems and push−pull tests in wells 9A and 10A. However, if inhibition is occurring in the field, it is occurring at much lower *E*-CFE concentrations than were present in the microcosm and batch reactor systems.

Measured aqueous *E*-CFE concentrations decreased from 34 to 0.02 μM during the test, while FE concentrations increased from below the quantification limit (0.005 μM) to 0.06 μM. Overall, the test solution was diluted to 20% by the end of the test period as indicated by bromide concentrations (data not shown).

As indicated, FE formation was observed when CFE was injected into well 15C. In contrast, when TCFE was injected into this well in an earlier test, no FE formation was detected (*17*). The rate of *E*-CFE transformation in well 15C was

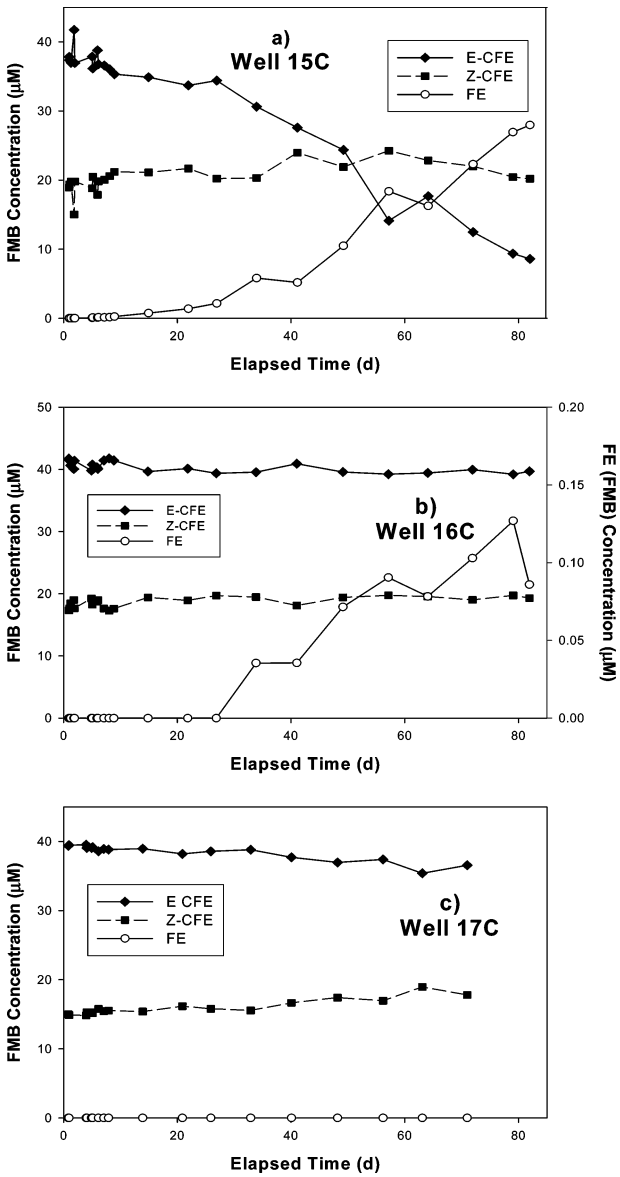

FIGURE 5. Progress curves for reductive dechlorination of *E*-/*Z*-chlorofluoroethene (CFE) to fluoroethene (FE) during push−pull tests conducted in (a) well 15C, (b) well 16C, and (c) well 17C.

intermediate between that for wells 9A and 10A (Table 5) as was the total concentration (51 μM) of the higher-chlorinated ethenes (Table 2).

Well 16C. The rate of FE formation over days 26−82 was 0.0018 ± 0.0006 μM/day (Table 5). No significant removal of *E*-CFE and *Z*-CFE occurred in this well, and the *E*-CFE/*Z*-CFE ratio did not change significantly over the time period of FE production. The measured aqueous concentrations of *E*-CFE decreased from 37 to 2 μM, while the measured FE concentrations ranged from below quantification (0.005 μM)

to 0.01 μM. While FE production was unambiguously detected, the small rate of FE formation may be due to the relatively high total concentration of the higher chlorinated ethenes (2570 μM) in this well as compared to well 15C and A-zone wells 9A and 10A. Previous TCFE push−pull tests conducted before and after the addition of fumarate did not detect the formation of CFE or FE (*18*).

Well 17C and Well 21C. Although both wells are in the C-zone, TCE contamination impacts only well 17C (Table 2) (*23*). No FE was detected during push−pull tests conducted in wells 17C (Figure 5c) and 21C (data not shown), and the *E*-CFE/*Z*-CFE ratio remained constant during these tests. The lack of activity detected during the CFE push−pull tests indicates that the aquifer in the vicinity of these two wells does not support CFE to FE transformation activity. Background groundwater in well 17C contains a relatively high total concentration of the higher-chlorinated ethenes (399 μM), while well 21C does not have detectable background concentrations of these compounds (Table 2).

Advantages of CFE Push−Pull Tests. In this study, reductive dechlorination of CFE in the field was detected by (a) the production of FE and (b) a shift in *E*-CFE/*Z*-CFE isomer ratios. Moreover, in situ rates of CFE transformation and FE production were quantified. The highest rate of CFE transformation was observed in well 10A, which is consistent with the observed production of FE from injected TCFE in earlier tests (*18*).

CFE was selected as a surrogate for VC in field tests because it allowed us to target the specific transformation reaction of interest, namely, the reductive dechlorination of VC. Previous research has shown that it may be difficult to detect CFE transformation by injecting TCFE due to the large number of transformation products that form and because injected test solutions may be diluted by regional flow before FE forms, unless transformation rates are sufficiently large. In previous tests, CFE production was not detected until after 40 days, when CFE and FE, if present, may be close to analytical detection limits.

When TCFE is injected, it may not be possible to quantify rates of formation of transformation products because they are simultaneously being formed and transformed. For example, during TCFE push−pull tests, sufficient quantities of CFE must first form and then be transformed to produce detectable concentrations of FE. To determine rates of CFE to FE transformation from TCFE experiments, the rate constants for each step must be known. In contrast, in CFE tests, FE was the only transformation product, and it is detected earlier in the test (e.g., <40 days) and at higher concentrations. In addition, injection of the *E*-CFE/*Z*-CFE mixture has the additional benefit in that the isomer ratio provides additional evidence of CFE transformation.

The in situ field rates reported reflect rates obtained 15 months after the last addition of substrate (fumarate) at this site (*18*). As a result, the in situ rates determined in this study likely reflect intrinsic rates. The method also has potential applicability for evaluating remedial strategies. Once background or intrinsic rates of in situ CFE transformation to FE are established, changes in activity from test to test can be used to identify amendments that successfully increase in situ rates. Likewise, estimates of push−pull test precision can be potentially determined from repeated tests but without the addition of amendments that may enhance the in situ rates.

Unlike other methods for estimating in situ rates, CFE push−pull tests do not require hydraulic control, which typically requires the installation of multiple injection and extraction wells. The uncertainty associated with using CFE to estimate in situ VC rates is similar to the uncertainty between estimating in situ rates from stable carbon isotope data and field concentration data obtained under hydraulically controlled conditions at Kelley AFB, Texas (*14*). At this site, a controlled hydraulic recirculation system was used to assess the efficacy of bioaugmentation of a dehalogenating culture for remediation of chlorinated ethenes. In this system with a known hydraulic residence time, dehalogenation rates were independently estimated from concentration profiles and from stable-carbon isotope data that was combined with the Rayleigh equation and literature-reported fractionation factors for the various chlorinated ethenes (*4*, *15*). The estimated isotope-based rate was a factor of 2−4 times lower than the concentration-based estimate. In situ reductive dechlorination rates also are estimated using models that are calibrated to site concentration data (*32*). However, high uncertainty is associated with this type of rate estimate because the reaction rate and reactive-zone length are varied to best fit the concentration data. In addition, the model neglected microbial growth and decay and possible nutrient limitations. In situ reductive dehalogenation rates also were estimated at a field site in Michigan from extensive analysis of spatial and temporal concentration data and estimation of mass fluxes that were input into a reactive transport model to calculate reaction rates (*33*). The intensive sampling approach used in this study required significant knowledge of site hydraulics and potential mass flux and resulted in high rate estimates as compared to other literature values.

CFE as a Surrogate. In the three laboratory test systems, no lag period was observed prior to the dechlorination of VC and CFE. Each system consistently indicated the preferential utilization of *E*-CFE relative to *Z*-CFE. In the microcosms containing site groundwater and sediment, the relative rates of *E*-CFE and VC transformation were within a factor of 2.7, and the rates of *E*-CFE were faster than those of VC. In contrast, in the Evanite batch reactors containing an enrichment culture with *Dehalococcoides*-like microorganisms, the relative rates for *E*-CFE and VC were within a factor of 2 but with the rates of VC transformation faster than those of *E*-CFE. Overall, the agreement between the relative rates of *E*-CFE and VC were good and indicate that CFE is a potential surrogate for estimating the in situ rates of VC transformation.

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