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Elliot G. Ennis for the degree of Master of Science in Chemistry presented on June 3, 2005

Title: Fluorinated and Deuterated Surrogates for Quantifying Microbial Transformation of Chlorinated Ethenes and Ethanes in Anaerobic Groundwater.

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Jennifer A. Field

Chlorinated aliphatic hydrocarbons (CAHs) such as vinyl chloride (VC) and 1,-2 dichloroethane (DCA), are wide-spread groundwater pollutants found at many contaminated field sites around the world. Quantitative tools are needed to determine the *in situ* rates of VC and DCA transformation to ethene in contaminated groundwater. The objective of this study was to evaluate the potential of E-/Z-chlorofluoroethene (E-/Z-CFE) and DCA-d_4 as surrogates for VC and DCA, respectively. Laboratory microcosm and batch reactors experiments were performed to determine the kinetics and products of VC and E-/Z-CFE transformation to ethene and fluoroethene (FE), respectively. In addition, the products and pathway of DCA and DCA-d_4 also were determined. In all microcosms and batch reactors, E-CFE was preferentially transformed over Z-CFE. For the three experimental systems, the rate of E-CFE transformation to FE was within a factor of 2.7 of that for VC to ethene, which indicates that E-CFE is suitable for use as a surrogate for VC. CFE was later

used in a separate field study as a surrogate to model *in situ* rates of VC reductive dechlorination. The rates of DCA and DCA-d_4 were statistically similar according to a t-test at the 95% confidence level, which also indicates that DCA-d_4 is an excellent surrogate for DCA. Based on this conclusion, DCA-d_4 is now ready to be used in a field test as a surrogate for DCA. This work establishes the precedence for the use of CFE and DCA-d_4 as surrogates for VC and DCA, respectively.

Fluorinated and Deuterated Surrogates for Quantifying Microbial Transformation of Chlorinated Ethenes and Ethanes in Anaerobic Groundwater

by
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Elliot G. Ennis, Author

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Fluorinated and Deuterated Surrogates for Quantifying Microbial Transformation of Chlorinated Ethenes and Ethanes in Anaerobic Groundwater

Chapter 1

Introduction

Chlorinated aliphatic hydrocarbons (CAHs), specifically chlorinated ethanes and ethenes are among the most common groundwater contaminants across the country and around the world (1). Groundwater contamination is a widely recognized problem and finding techniques to remediate contaminated groundwater is a challenge. Pump-and-treat strategies are costly and inefficient (2,3). In addition, there are microbial populations at contaminated sites that are able to convert chlorinated solvents via anaerobic degradation, making bioremediation an attractive option (4).

Vinyl chloride (VC) is a known carcinogen and neurotoxin (5) used in the production of polyvinyl chloride. Contamination of groundwater by VC can occur during accidental release, improper disposal associated with commercial or industrial use, or as a transformation product during incomplete degradation of chlorinated solvents such as trichloroethene (TCE) and perchloroethene (PCE) (1,6,7). TCE is broken down via reductive dechlorination, a process in which a carbon-halogen bond is broken and hydrogen replaces the halogen substituent. Cis-dichloroethene (DCE), trans-DCE, or 1, 1-DCE is formed first then further transformed to VC. Ethene is the end product of the pathway (1) (Figure 1.1).

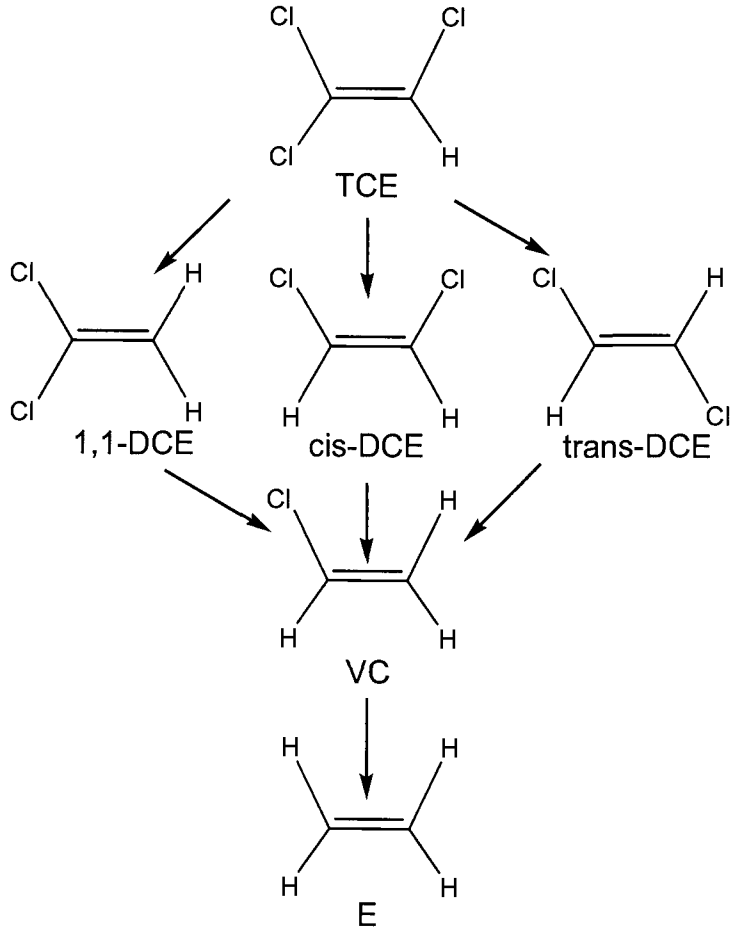

Figure 1.1. Transformation pathway of TCE to ethene.

The kinetics of the TCE degradation pathway have been well studied and are best described by the Monod equation: $-(dS/dt) = kXS/(S+K_s)$ where S is the CAH concentration, k is the maximum rate of CAH degradation per unit of biomass, X is the dechlorinating biomass concentration, K_s is the concentration of substrate that corresponds to one-half the maximum rate (3). Usually, it is assumed that dechlorinating biomass concentration remains constant over the course of the experiment in cases where biomass is not known or measured.

The transformation of VC to ethene is considered one of the more difficult steps in TCE and/or PCE mineralization (8-10). Microbes that support incomplete degradation of TCE and DCE in the field may not support the conversion of VC to ethene. For example, *Dehalococcoides* strain 195 and the Pt. Magu culture studied by Yu et al. readily dechlorinated TCE and DCE, but did not support VC to ethene transformation (9,10) and consequently, a build-up of VC was observed. On the other hand, Major et al reported a positive correlation in the field between the occurrence of *Dehalococcoides*-like microorganisms and ethene production (11). *Dehalococcoides*-like microorganisms are organisms known to transform VC and higher chlorinated ethenes to ethene (11,12). *Dehalococcoides*-like organisms in the Bachman culture, the Evanite culture, and *Dehalococcoides* strain VC1 use VC as a growth substrate (3,9,13,14). These mixed and pure cultures are characterized by Ks values of 60 μM for the Evanite enrichment culture, 5.8 μM for the Bachman culture, and 2.6 μM for strain VC1. In contrast, *Dehalococcoides*-like microorganisms such as strain 195 and the Pt. Magu culture, which cometabolically degrade VC to E, are characterized by higher Ks values of 600 μM and 602 μM, respectively (9,10).

The Evanite culture, which is a mixed culture that uses VC as a growth substrate, displayed inhibited VC transformation to ethene when in the presence of higher chlorinated ethenes such as TCE (9). Inhibition was supported by the individual Ks values determined for individual chlorinated ethenes and when used as model input, yielded kinetics for VC that was consistent with inhibition by higher chlorinated ethenes (9). Consequently, in the field, organisms that are able to transform VC may be present, but transformation of VC may not occur until the concentrations of TCE and DCE are decreased (9).

Another chlorinated solvent, 1,2 dichloroethane or DCA, is an industrial solvent and precursor for VC and considered a class II carcinogen (15,16). DCA, the most abundant chlorinated two-carbon groundwater pollutant (17), is dechlorinated via dihaloelimination (Figure 1.2) (1,15,18). Dihaloelimination is a process in which two halogen substituents on vicinal sp^3 carbon atoms are eliminated to form ethene (19). Studies to date have documented the anaerobic reductive dihaloelimination of DCA with acetogenic bacteria with ethene as the only end product (15,20-22). In contrast, a study by Maymo-Gatell et al (23) using *Dehalococcoides* strain 195 found that 99% of the DCA present was converted to ethene, but 1% was converted to VC.

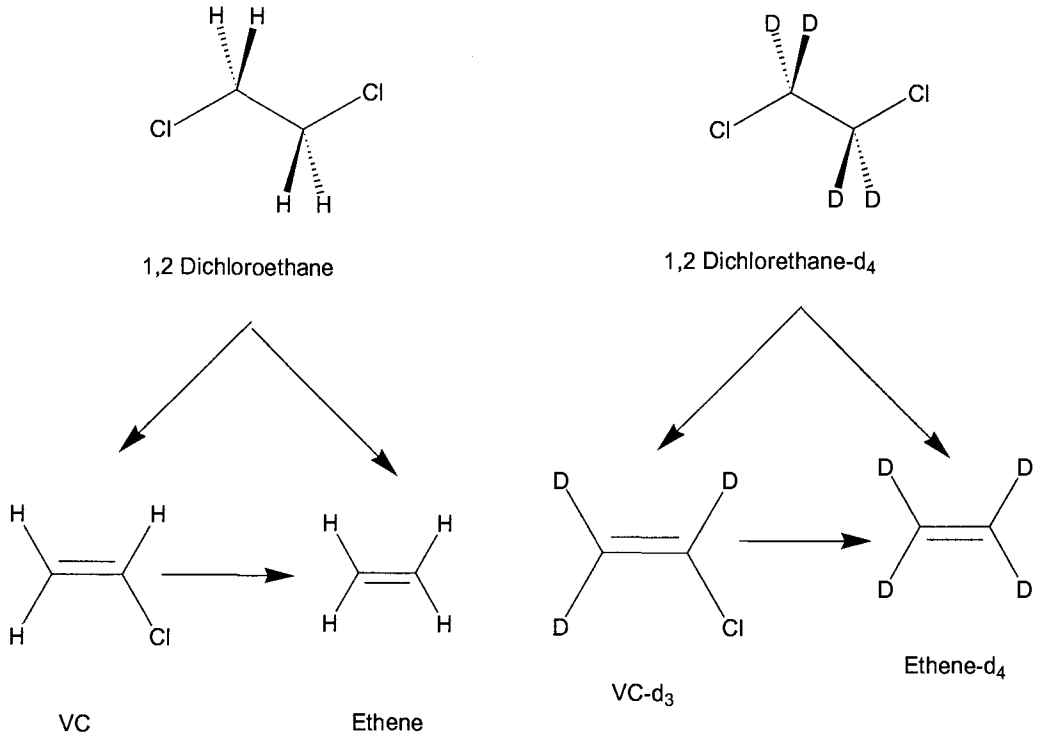

Figure 1.2. Transformation pathway of DCA and its surrogate DCA-d_4 to ethene and ethene-d_4.

Compared to TCE, not much is known about the degradation kinetics of DCA. Studies to date have focused on the types of microorganisms that will transform DCA and establishing ethene as the only end product from the reductive dihaloelimination reaction (15,20-22). To the best of my knowledge, Monod kinetic parameters are not known for DCA. Compared to the growing literature on VC, further work is needed to establish Monod kinetic parameters and to characterize cultures that are known to convert DCA to ethene. While laboratory studies with pure and mixed cultures are useful because they provide a basic understanding of pathway, products, and kinetics for CAH degradation, the application of this information to the field is problematic.

Currently, it is difficult to obtain rates for *in situ* transformations due to the variable concentrations of parent and daughter products present at many contaminated field sites. While the formation of daughter products provides qualitative evidence that a transformation is occurring, they do not give rate information. Because of pre-existing contamination, it is difficult to quantify the *in situ* rate of CAH transformation in contaminated aquifers. Conventional methods to obtain multiple converging lines of evidence to estimate rates of transformation include a) temporal and spatial monitoring of parent compounds, daughter products, 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 non-biological processes such as advection, dispersion, and sorption can obscure changes due to biological processes (7,24,25). Additional lines of evidence can be obtained by performing laboratory microcosm studies, which provide qualitative evidence that dechlorinating populations are present

but the rates obtained do not necessarily reflect *in situ* conditions. Stable carbon isotope analysis of chlorinated ethenes (natural abundance) in groundwater can provide qualitative evidence for TCE transformation to ethene (25-28). However, while stable carbon isotopes have been used to estimate the extent of transformation (25-28), they have not yet been used to quantify *in situ* rates.

A single-well push-pull test is a test where a prepared test solution containing a reactive tracer (reactant) is injected or "pushed" into an existing monitoring well and samples are "pulled" out of the monitoring well over time (29). Reactions are detected by the loss of injected reactant, the *in-situ* formation of a reaction product or both. *In-situ* rates are computed from measured reactant and product concentrations by applying a forced mass balance technique developed by Hageman et al. (30) that compensates for the effects of transport processes.

Single-well push-pull tests can provide quantitative information on transformation rates for *in-situ* processes including reductive dechlorination (30-32), denitrification (29,32), radionuclide reduction (33), anaerobic aromatic hydrocarbon degradation (2), and aerobic cometabolism (34).

One of the advantages of surrogates is that they allow for the detection of transformations in CAH-contaminated groundwater that contains high and variable concentrations of CAHs and their transformation products. It is desirable to include labeled contaminants or other surrogates in the injected test solution. 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. 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. 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. On the other hand, halogenated surrogates are inexpensive, available, and to date, have received regulatory approval for use in field tests in five states.

Chlorine 37 labeled surrogates may give rate data similar to that of ^{35}Cl, but because the natural abundance of ^{37}Cl is 32% (35), it is already present in the background at contaminated sites. In addition, when the chlorine atom is removed via reductive dehalogenation, it would be impossible to distinguish ethene formed from what is already in the background since the labeled chlorine would be lost in the reaction.

The precedence for the use of brominated surrogates was established by Gu et al. in 2003 in a laboratory microcosm experiment (36). The study demonstrated that vinyl bromide is reductively dehalogenated to ethene at rates 5-10 times faster than the dechlorination of vinyl chloride to ethene. The reaction is faster since bromine is a better leaving group than is chlorine (19), but like the labeled chlorine surrogates, the bromine is removed and the unique signature lost. Consequently, ethene formed would be impossible to distinguish from ethene already present in the background.

Iodinated surrogates would be dehalogenated even faster than either brominated or chlorinated surrogates since iodine is a better leaving group than both bromine and chlorine (19). Ethene formed from the reaction would not be able to be distinguished from that already present; its chlorinated and brominated counterparts have the same disadvantage.

Unlike the other halogens, fluorine is a poor leaving group (19). As a result, the carbon-fluorine bond would be conserved, allowing fluoroethene (FE), the fluorinated analog of ethene formed from the reaction, to be detected in the presence of background ethene. A potential limitation of fluorinated surrogates would be the inductive effects of fluorine, which may produce a faster reaction (19,37). As a practical matter, fluorine-labeled contaminants have been approved for use in five states, are easy to detect in the presence of high background concentrations of their non-fluorinated analogs, and are not regulated.

The precedence for the use of the fluorinated surrogate TCFE as a surrogate for TCE degradation was established by Vancheeswaran et al. (7) and Hageman et al. (6,30,32). Anaerobic transformation of TCFE in TCE-contaminated groundwater proceeded by a series of reductive dechlorination reactions to form Z- and E-dichlorofluoroethene (DCFE), E- and Z- and 1,1-chlorofluoroethene (CFE), and fluoroethene (FE) (Figure 1.3), analogous to the products formed from the reductive dechlorination of TCE (Figure 1.1)(6,31,32).

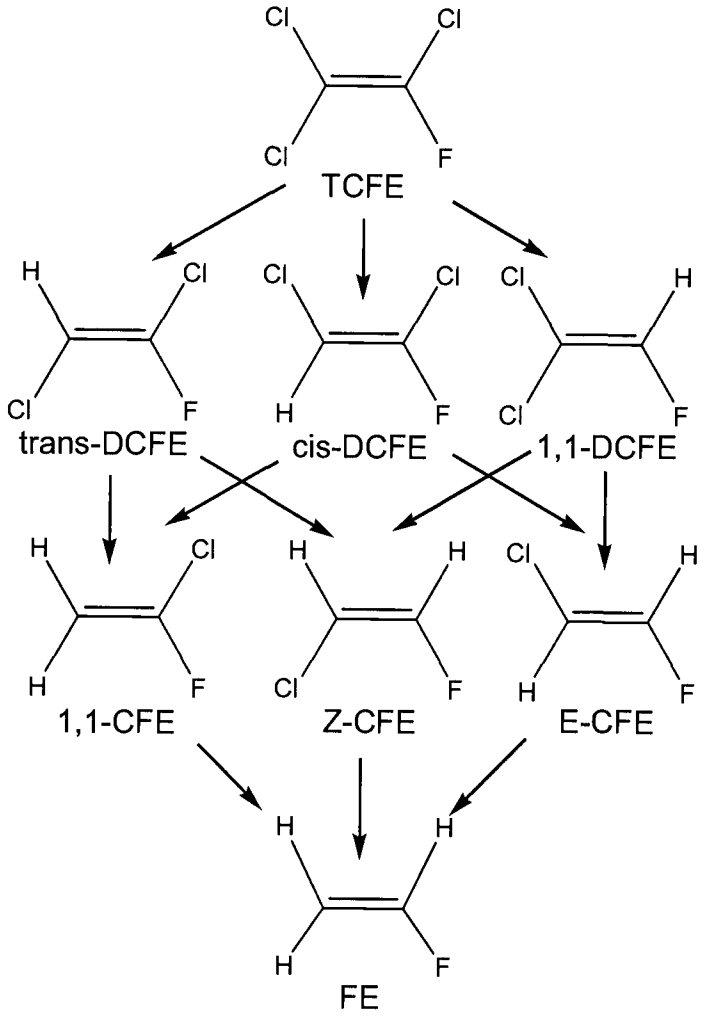

Figure 1.3. Transformation pathway of TCFE to FE.

TCFE push-pull tests were used to quantify the *in situ* rates of reductive dechlorination before and after adding fumarate to TCE-contaminated groundwater (32). 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, zero-valent iron, and hydrogen were evaluated for their effectiveness toward increasing the rate of reductive dechlorination (31). 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 (38). Kinetic studies performed with an enrichment culture containing *Dehalococcoides*-like microorganisms yielded essentially the same maximum rates (350 ±10 μM/day for CFE and 334±11 μM/day for VC) and Ks values (87±8 μM for CFE and 63±7 μM for VC) (38). Therefore, the present investigation seeks to establish the precedent for the use of an E-/Z-CFE mixture as a surrogate for VC using sediment and groundwater from two different field sites and a sediment-free enrichment culture.

An alternative to halogenated surrogates is deuterated surrogates, which are compounds that have all of their hydrogen atoms replaced by deuterium atoms. Deuterated compounds are utilized to determine pathways of contaminant degradation in the field. For example, Krieger et al. investigated the oxidation of *m*-xylene to 3-methylbenzoate with *m*-xylene-d_{10} (39). Like fluorinated surrogates, deuterated compounds do not occur in the background, allowing for unambiguous detection with GC/MS, are inexpensive, and have been approved for use in field tests in five states. Deuterated surrogates have been investigated for degradation of alkyl benzenes by Reusser et al. (2), who used toluene-d_8 and *o*-xylene-d_{10} as surrogates to monitor alkyl benzene degradation.

Limitations inherent with the use of deuterated surrogates include the potential for a kinetic isotope effect. Kinetic isotope effects can occur when the carbon-deuterium bond is involved in the rate limiting step of the enzymatic reaction (38,40-42). A deuterium kinetic isotope effect was observed by Kreiger et al. who studied *m*-xylene oxidation to 3-methyl benzoate (39). However, the applicability of deuterated surrogates is limited to compounds in which the mass difference is greater than two. In the case of TCE and possibly VC, deuterated surrogates are not appropriate due to the low number of hydrogens on each compound. However, for DCA, which has four hydrogens that can be replaced by deuterium atoms, deuterated surrogates are suitable. Since DCA is present in the background at many field sites, a deuterated surrogate is ideal for obtaining rate data. The deuterated surrogate DCA-d_4 is chemically unique which will allow separation of DCA from DCA-d_4 using mass spectrometry. The present investigation evaluates the use of DCA-d_4 as a surrogate for DCA using

sediment and groundwater microcosms from two different field sites and a sediment free enrichment culture.

The overall objective of this research was to evaluate the fluorinated surrogate E-/Z-CFE and the deuterated surrogate DCA-d_4 as surrogates for VC and DCA, respectively. The first part of chapter 2 describes experiments involving CFE and VC, and the second part describes experiments designed to evaluate DCA-d_4 as a surrogate for DCA. The objectives were undertaken by performing laboratory microcosm and batch reactor studies and comparing the kinetics of transformation of each parent compound with its potential surrogate.

Chapter 2

Microcosm and Batch Reactor Experiments

Experimental Methods

Chemicals. Chlorofluoroethene (CFE) was purchased as a 57:43 E-/Z-CFE mixture from Synquest Labs (Alachua, FL). Fluoroethene (FE) was purchased from Lancaster Synthesis (Pelham, NH). Trichloroethylene (TCE) 99.9%, sodium lactate (60% syrup) were purchased from Fisher Scientific (Fairlawn, NJ). 1,1,1-trichloroethane (TCA), 1,2-Dichloroethane (DCA), 1,2-Dichlorethane-d_4 (DCA-d_4), Vinyl Chloride (VC), ethene-d_4, and ethene were purchased from Aldrich Chemical (Milwaukee, WI).

Sites. Four sources of material were used for microcosm and batch reactor experiments. Aquifer material and groundwater were obtained from a shallow unconfined aquifer (A-zone) at a former site of a chemical plant in Richmond, CA (6). 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 (9,38,43). The third system contained sediment and groundwater from a TCE-contaminated site near Greer, SC. The fourth system contained sediment and groundwater from a TCE/TCA contaminated site near Willits, CA. The Richmond, Evanite, and Greer systems were used in the CFE/VC experiments. The DCA/DCA-d4 experiments used the Richmond, Evanite, and Willits systems.

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 glove box under an atmosphere of 90% nitrogen and 10% hydrogen gas and stored in an incubator held at 20 °C.

Aquifer material (100 mL) from the Richmond site was added to five autoclaved 320 mL serum bottles that contained 120 mL groundwater from the A-zone and 100 mL of headspace. The bottles were fitted with screw-down caps containing grey butyl rubber septa (Wheaton, Millville, NJ). Batch reactors containing the Evanite enrichment culture were constructed by adding 50 ml of media (44) and 50 mL of sediment-free enrichment culture to eight autoclaved 156 ml serum bottles with grey 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 fourth system used in this study was a set of microcosms containing sediment and groundwater material obtained from a CAH-contaminated site near Willits, CA. To the autoclaved 156 ml bottles were added 50 ml of sediment and 50 ml of site ground water. The Greer and Willits microcosms were then treated the same way as the Richmond and Evanite systems (Table 2.1).

Microcosm and Batch Reactor Kinetics. Reductive dechlorination activity was initially stimulated by incubating the microcosms and batch reactors with 115 μM

TCE in the case of the Richmond and Greer microcosms, and the Evanite batch reactors. The Willits microcosms received 115 μM each of TCE and TCA. For electron donors, either 0.2 atm of hydrogen gas (Richmond microcosms) or 1.7 mM lactate (Greer microcosm, Evanite batch reactors, and Willits Microcosms) was used. This stage was terminated by purging with nitrogen to remove all chlorinated ethenes once the microcosms and batch reactors exhibited quantitative conversion of TCE to ethene, except for the Willits microcosms, which did not transform more than 5% of the initial TCE or TCA added.

After completion of the stimulation phase, experiments were then conducted in the microcosms/batch reactors 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 (Table 2.1). The Willits microcosms were not used in the VC/CFE experiments. 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 (Table 2.1). 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 microcosms 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.

Prior to the start of the DCA experiments, the Richmond microcosms were re-homogenized and added to 156 mL bottles so the proportions were 100 ml of aquifer material and 56 mL of headspace. Batch reactors containing the Evanite enrichment culture were homogenized and 75 mL portions of culture were added to 156 mL bottles that had 81 ml of headspace. For the DCA experiments, all bottles were dosed with 110 μM DCA or DCA-d_4 for the Evanite batch reactors and 130 μM DCA or DCA- d_4 in the case of the Richmond and Willits microcosms. In this investigation, the Greer microcosm was not used and the Willits microcosms were used for the first time.

Controls. Killed controls for the Richmond microcosms (n=1), Evanite batch reactors (n=1), and Willits microcosms (n=1) consisted of bottles that were autoclaved at 120 °C for 45 min. For the CFE/VC experiments, the control bottles were dosed with either VC or CFE but no added hydrogen or lactate. For the DCA/DCA-d_4 experiments, the killed and live control bottles were dosed with either DCA or DCA-d_4 but no added hydrogen or lactate. An additional live control (n=1) was used for the Richmond microcosms in the DCA experiment.

Table 2.1. Microcosm history and experiments.

System	Initial Stimulation Phase	VC Experiment	CFE Experiment	DCA 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)	130μM DCA or DCA-d_4 0.2 atm H_2 (n=3)
Richmond control[a,d]	Killed control No TCE or H_2	80 μM VC (n=1)	80/20 μM E-/Z-CFE (n=1)	130 μM DCA (n=2)
Evanite batch reactors[b]	115 μM TCE +1.7 mM lactate	80 μM VC (n=4)	80/20 μM E-/Z-CFE (n=4)	110 μM-DCA or DCA-d_4 (n=3)
Evanite controls[b]	Killed control No TCE or lactate	80 μM VC (n=1)	80/20 μM E-/Z-CFE (n=1)	110 μM-DCA or DCA-d_4 (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)	Not used
Willits Microcosms	115 μM TCA+115 μM TCE +1.7 mM lactate	Not used	Not used	130 μM-DCA or DCA-d_4 (n=3)
Willits Controls	115 μM TCA+115 μM TCE +1.7 mM lactate	Not used	Not used	Killed 130 μM-DCA or DCA-d_4 (n=2)

[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

[d] Killed and live controls were used for the DCA experiments. Live controls received H_2 but no DCA.

This control received hydrogen, but no DCA and was used to determine if TCE present in the bottles at the start of the study formed ethene. 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.

Analytical Methods. Headspace samples from microcosm experiments were analyzed for CFE, FE, VC, and ethene by injecting 100 µl gas-phase samples into a Hewlett Packard 5890 series II gas chromatograph (GC) (Agilent Technologies, Palo Alto, CA) equipped with a flame ionization detector (FID). Separations were achieved on a 30 m x 0.32 mm x 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, DCA, DCA-d_4, ethene, and ethene-d_4 in microcosm and batch reactors were determined by external calibration using standards prepared in microcosm bottles containing known concentrations of analytes 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.

Headspace samples obtained from DCA and DCA-d_4 experiments were analyzed either by GC/FID as described above or by GC mass spectrometry (GC/MS). The selection of detector system was based on availability. For the Evanite mixed culture batch reactors, GC/MS analysis was used. Gas-phase samples were injected into a Hewlett Packard 5890 series II gas chromatograph (GC) equipped with a mass

selective detector. Separations were achieved on a J&W Gas Pro column (Agilent) 60 m × 0.32 mm x (proprietary film thickness). The instrument was operated under a thermal gradient with He as the carrier gas. Regardless of whether GC/FID or GC/MS was used, external calibration was used. External calibration standards were prepared in 156 ml serum bottles containing 75 or 100 ml of autoclaved, de-ionized water and known concentrations of DCA, DCA-d_4, VC, ethene, and ethene-d_4 ranging from 0 to 200 μM. Five-point calibration curves were linear with typical R^2 values of 0.999.

RESULTS AND DISCUSSION

Chlorofluoroethene (CFE) as a Surrogate for VC

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 2.1). The absence of a lag phase is likely due to addition of TCE and hydrogen during the initial stimulation of the Richmond microcosms. The non-constant slope of the time-course plot indicated mixed-order kinetics, which could result from the initial VC concentrations (80 μM) being above the Ks value for the dehalogenating microorganisms. For example, a Ks value of 64 μM was recently reported for the Evanite enrichment culture (43). However, for simplicity, zero-order kinetics were applied to the linear region in order to compare the behavior to the DCA experiment.

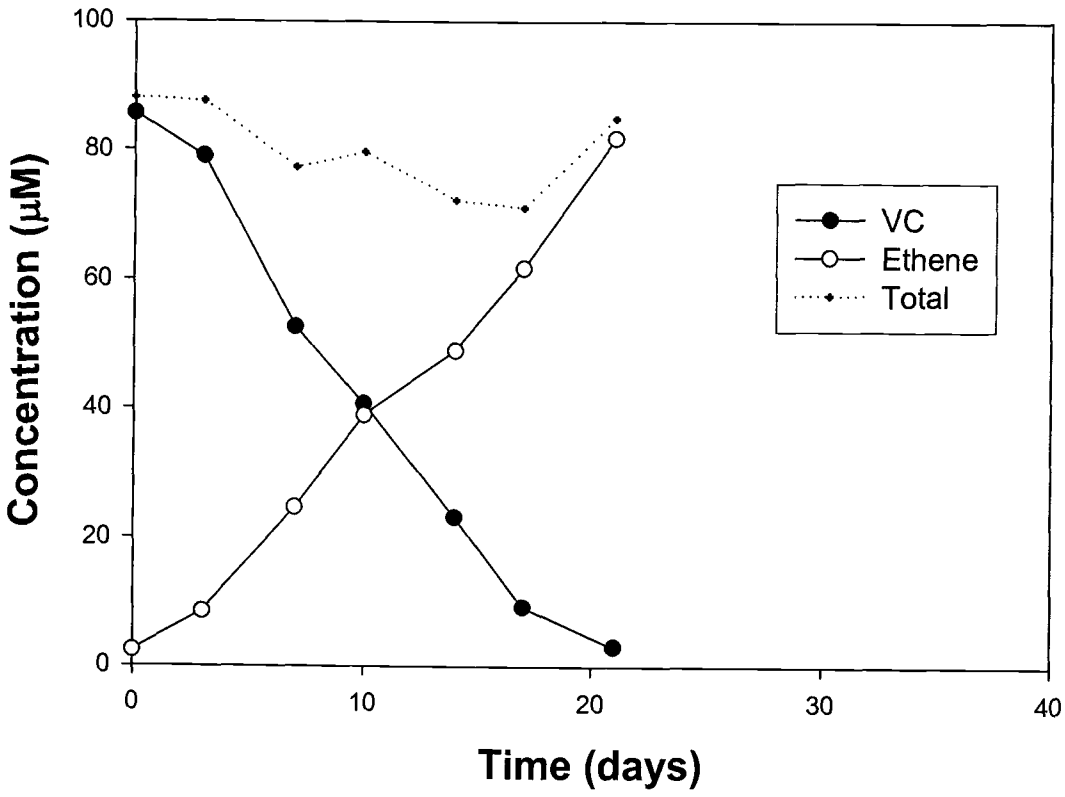

Figure 2.1. Example time progress curve for the reductive dechlorination of VC in a Richmond microcosm.

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 2.2). 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). Progress curves for the CFE experiment also indicated mixed-order kinetics for both CFE isomers (Figure 2.2). The CFE isomer concentrations of 80 and 60 μM were in the region of the Ks value determined for 1,1-CFE (87 μM) by Pon and Semprini with the Evanite enrichment culture (38). The E-CFE isomer was dechlorinated with no lag period at a maximum average rate of 6.86±1.87 μM/day (Table 2.2). 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±0.56 μM/day (Table 2.2) once the E-CFE concentration has 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 2.2; Figure 2.2). Mass balance was achieved with 93 to 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.

Table 2.2. 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	3.61±0.96	-6.86±1.87	-2.95±0.50	7.14±1.98
	(1.38)	(0.98)	(1.91)	(0.51)	(2.01)
Evanite[b]	-14.89±2.98	14.25±2.55	-9.19±0.48	ND	8.62±0.66
	(3.04)	(2.60)	(0.49)		(0.67)
Greer[c]	-2.00	2.36	-5.32	-3.27	6.42

[a] Average zero-order rate ± 95% confidence interval with the standard deviation given in parenthesis.

[b] Four microcosms each were used to determine rates.

[c] A single microcosm experiment was conducted.

ND no transformation detected

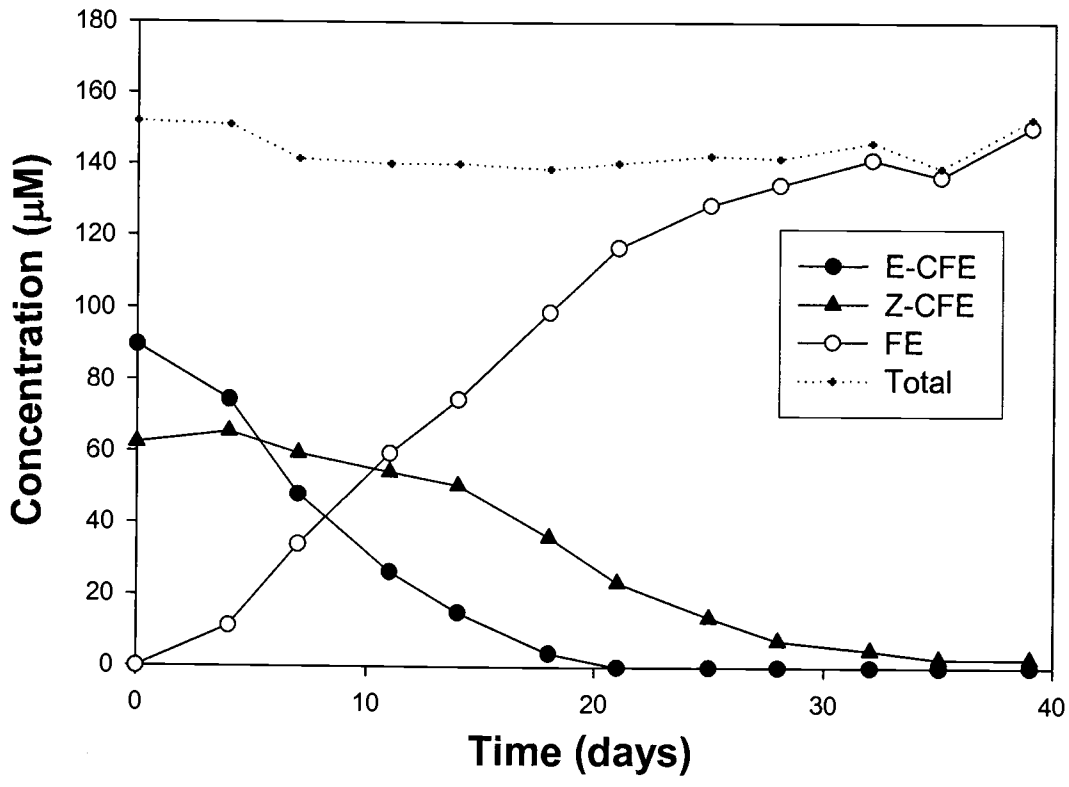

Figure 2.2. Example time progress curve for the reductive dechlorination of E-/Z-CFE to FE in a Richmond microcosm.

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 2.2). Rates of FE formation also were faster by a factor of 2 than those of ethene formation. The greater relative rate of FE formation compared to ethene formation is partially due to 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, Ks, 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 2.2 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 2.2) 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 2.3). VC conversion to ethene ranged from 84 to 102%. Mass balance was achieved with 83 to 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.

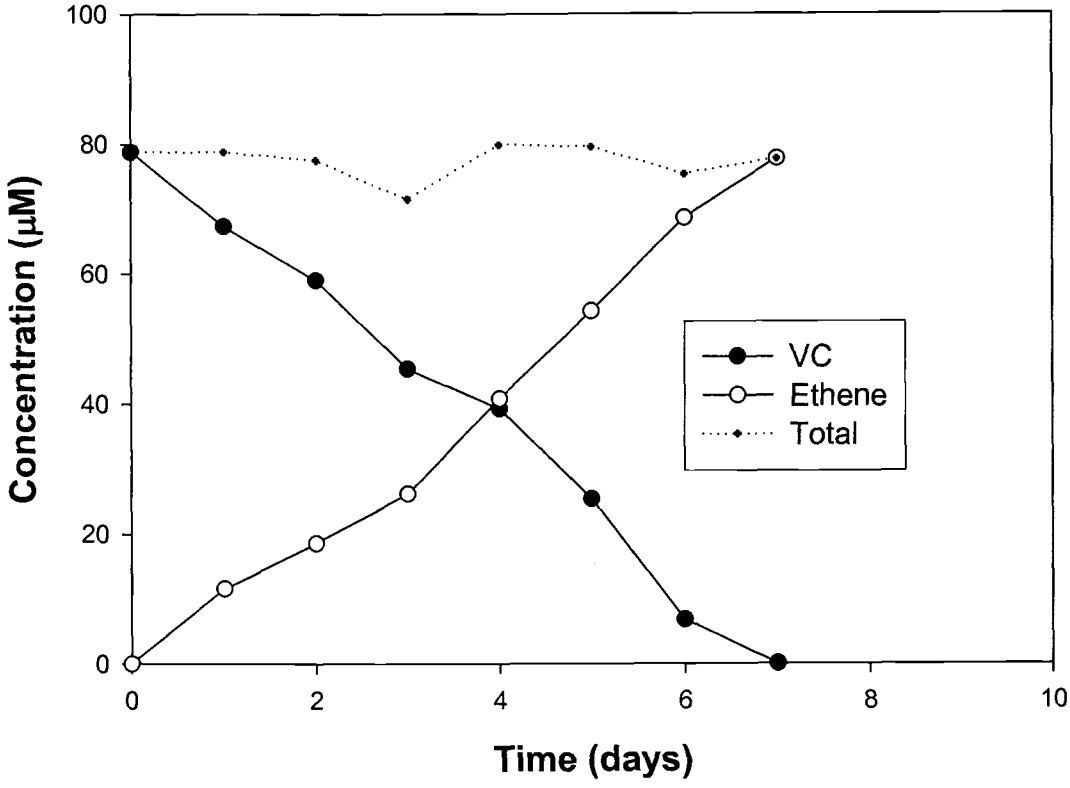

Figure 2.3. Example time progress curve for reductive dechlorination of VC in an Evanite batch reactor.

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 2.4). E-CFE was dechlorinated with no lag phase at an average rate of 9.19±0.48 μM/day (Table 2.2). Of the initial CFE added, 79 to 92% was converted to FE and no other products were detected and mass balances of 83 to 96% were achieved. The average rate of FE formation was 8.62±0.66 μM/day (Table 2.2). 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.

With the 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 (38). Although Richmond gave higher rates for E-CFE than VC degradation, the relative rates of E-CFE and VC transformation in both the 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* organisms.

Greer Microcosm. In the Greer microcosm (Figure 2.5), 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 2.2). 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.

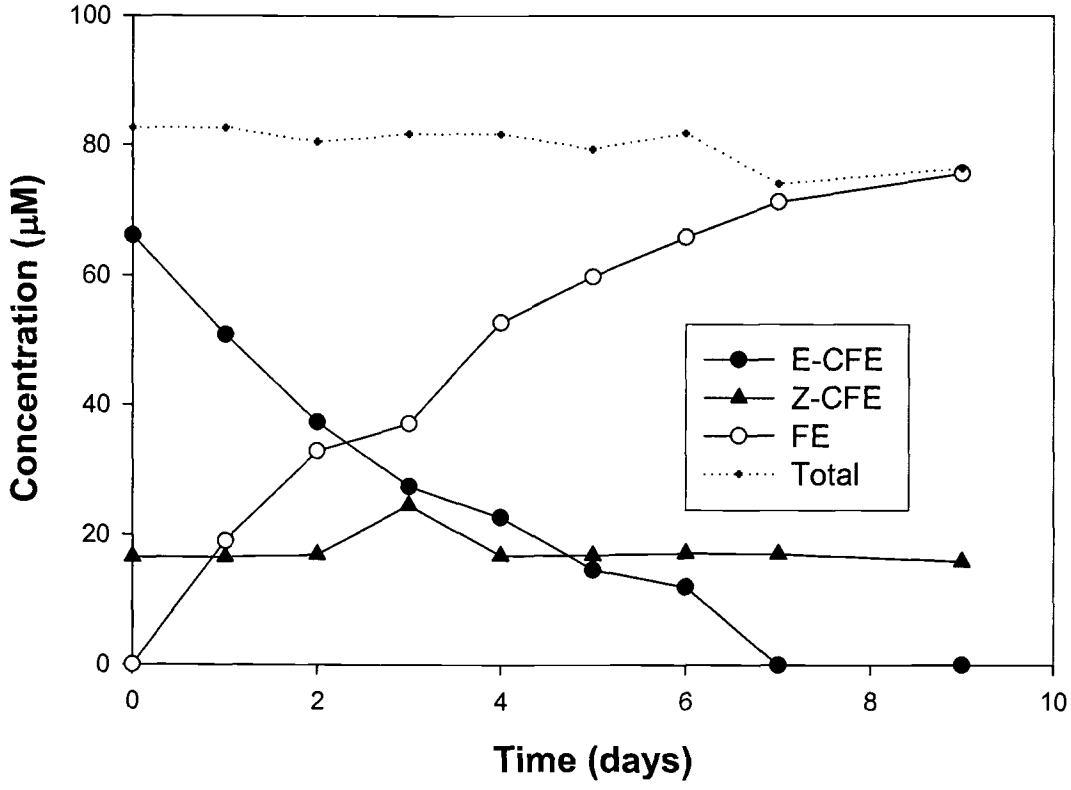

Figure 2.4. Example time progress curve for the reductive dechlorination of E-/Z-CFE to FE in an Evanite batch reactor.

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 2.6, Table 2.2) with 117% conversion of E-/Z-CFE to FE. Mass balance 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. Further work is needed to characterize the microorganisms in the Richmond and Greer microcosms.

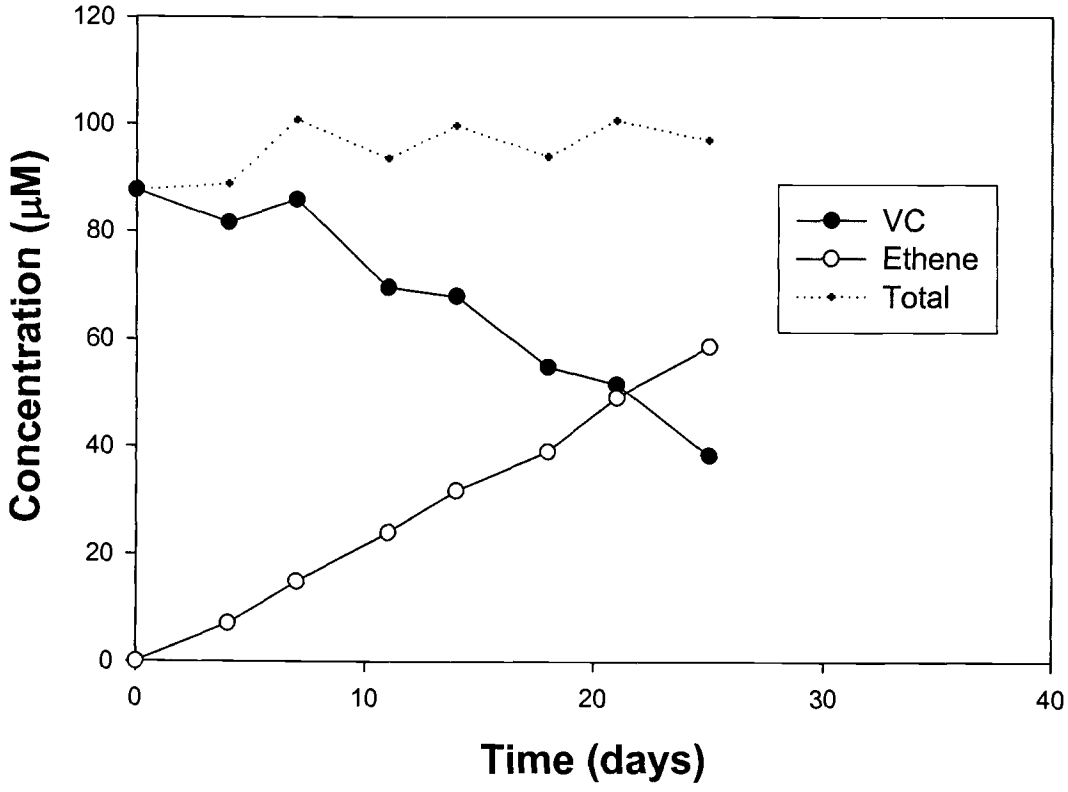

Figure 2.5. Time progress curve for reductive dechlorination of VC to ethene in the Greer microcosm.

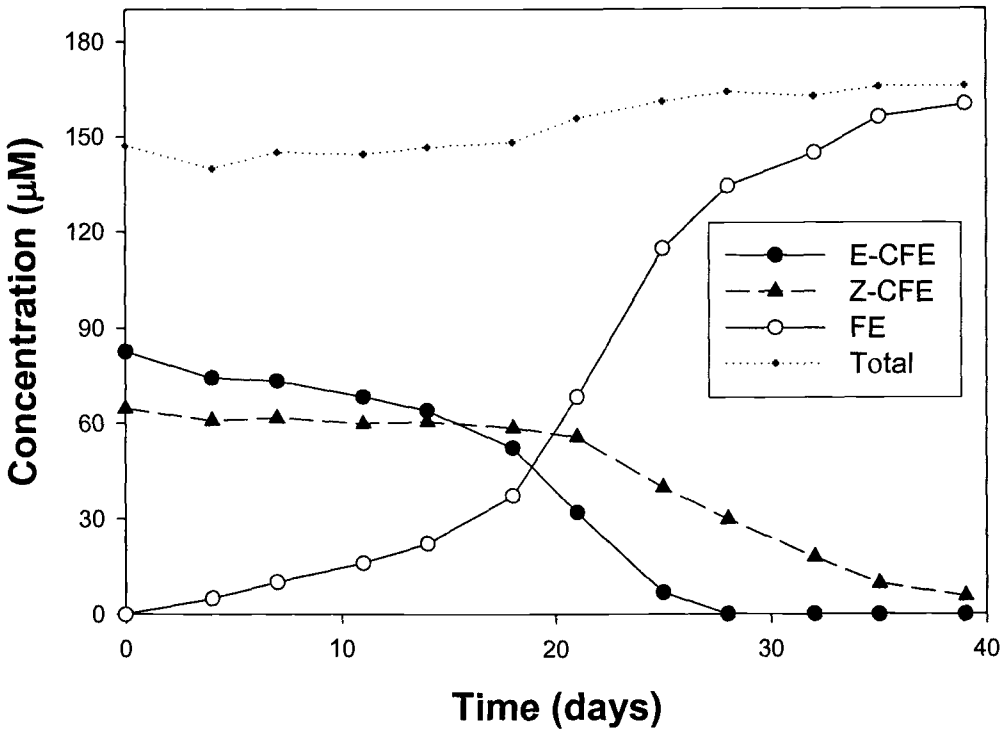

Figure 2.6. Time progress curve for the reductive dechlorination of E-/Z-CFE to FE in the Greer Microcosm.

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 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 dechlorination 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 degradation were good and indicate that E-CFE is a potential surrogate for estimating the *in situ* rates of VC transformation. Pon and Semprini found in the Evanite enrichment culture that 1,1-CFE also was readily transformed to FE at a rate that was 1.1 times that of VC to ethene (38). The Evanite enrichment culture is known to contain *Dehalococcoides*-like microorganisms. The known presence of these microorganisms (9,38,43) may explain why the Evanite enrichment culture dechlorinated VC faster than E-CFE and did not dechlorinate Z-CFE. In contrast, the Richmond and Greer microcosms dechlorinated E-CFE faster than VC. Further experimentation is needed to determine why E-CFE was dechlorinated faster than VC in the microcosms, but not in the sediment free mixed culture batch reactors. Future work could include a) determining the structure activity relationship between CFE isomers and reaction rates (e.g., why E-CFE was completely dechlorinated and Z-CFE

was not), and b) determining which enzymes in *Dehalococcoides*-like microorganisms are responsible for the transformation of VC and its fluorinated surrogates.

Alternatively, Gu et al. proposed that the brominated surrogate of VC, vinyl bromide, could be used as a surrogate in the field for VC transformation (36). They found vinyl bromide was reductively dehalogenated to ethene at rates 5-10 times faster than vinyl chloride (36) which is consistent with bromide being a better leaving group (19). Although vinyl bromide may be rapidly transformed in the field and lead to savings in time, effort, and money during field tests, the ethene produced in the reduction of vinyl bromide during field tests cannot be distinguished from the ethene that is present in the background groundwater. Application of vinyl bromide may be limited to cases where ethene concentrations are initially low. Otherwise, only vinyl bromide disappearance can be monitored. In contrast, the fluorine atom is conserved during the reductive dechlorination of E-CFE to FE, allowing both the parent and product to be detected in the presence of background ethene and VC.

A series of field tests were performed at the Richmond site where sediment for the laboratory study was obtained. Single-well push-pull tests were conducted by injecting E-/Z-CFE and monitoring for the formation of FE over a period of up to 80 days. In a field study performed in 2001 (45) the *in situ* rates of FE production from injected E-CFE ranged from 0.0018 to 1.15 μM/d while the *in situ* rates of E-CFE disappearance ranged from 0.17 to 0.99 μM/d. 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. Preferential utilization of E-CFE over Z-CFE in field tests was consistent with laboratory test results. Unequivocal detection of FE in

the field provided additional evidence of transformation. The observation of the E-CFE/Z-CFE ratio changing over the course of the experiment provided another line of evidence that a transformation was taking place.

DCA-d_4 as a Surrogate for DCA

Richmond Microcosms. Microcosm studies permitted the evaluation of DCA and DCA-d_4 transformation by dihaloelimination. Progress curves of the Richmond microcosms indicated that reductive dechlorination of DCA began almost immediately with no apparent lag after addition of the compounds (Figure 2.7). The non-constant slope of the time-course plot indicated mixed-order kinetics, which could result from the initial DCA concentrations being above the Ks value for the dehalogenating microorganisms. However, for simplicity, zero-order kinetics were applied to the linear region in order to calculate rates of degradation and formation. Further experimentation is needed to determine the Ks value of DCA for *Dehalococcoides*-like microorganisms.

Conversion of DCA to ethene was observed for all Richmond microcosms (n=3). Due to some VC in the background (0.5- 2 μM) prior to the start of the experiment, it was impossible to determine if any VC was formed during the experiment, however the amount of VC in the background remained constant over the course of the experiment.

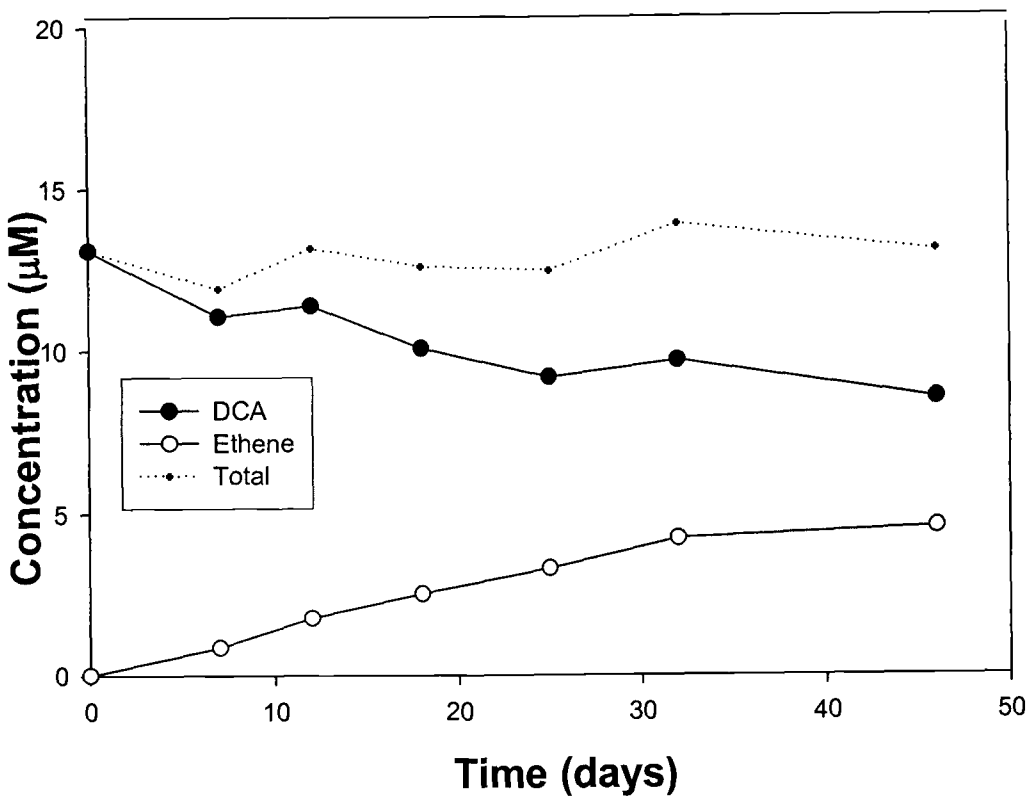

Figure 2.7. Example time progress curve for the dihaloelimination of DCA to ethene in a Richmond microcosm.

When the experiment was terminated, between 36 and 59% of the initial DCA had been metabolized. The average zero-order rate (± 95% confidence interval) for the Richmond microcosms was 1.23±0.33 μM/day for DCA disappearance and 1.47± 0.47μM/day for ethene production (Table 2.3). Between 97 and 117 % of the initial DCA mass was accounted for at the end of the experiments. In the killed control, no production of ethene or VC was detected and 101% of the initial DCA mass was accounted for at the end of the experiment. The live control showed no production of ethene from the TCE present.

A second set of Richmond microcosms (n=3) were run in parallel with the DCA experiments; DCA-d_4 was dechlorinated to ethene-d_4 (Figure 2.8). Progress curves for the DCA-d_4 experiment indicated a linear rate of degradation so zero-order kinetics were used to calculate degradation rates (Figure 2.8). However, the K_s value for DCA-d_4 in this microcosm system is not known so it is not known if the initial concentration of DCA-d_4 falls in the zero-order or first-order region of the Monod plot. DCA-d_4 dechlorination proceeded at a rate of 1.08 ± 0.33μM/day (Table 2.3). Between 32 and 53 % of the added DCA-d_4 was converted to ethene-d_4. The formation of ethene-d_4 occurred at a zero-order rate of 1.03 ± 0.07μM/day and ethene-d_4 was the only product detected (Table 2.3; Figure 2.8). Mass balance was achieved with 83 to 104 % of the initial mass accounted for at the end of the experiments. No controls were available for the DCA-d_4 experiment due to the limited amount of aquifer material.

Table 2.3. Average zero-order rate constants for DCA and DCA-d_4 degradation and ethene and ethene-d_4 formation.[a]

Microcosm	DCA (μM/day)	DCA-d_4 (μM/day)	Ethene (μM/day)	Ethene-d_4 (μM/day)
Richmond[a]	-1.23±0.33	-1.08±0.33	1.47±0.47	1.03±0.07
	(0.29)	(0.29)	(0.4)	(0.06)
Evanite[a]	-4.40±1.21	-4.44±0.63	4.40±1.52	4.71±0.61
	(1.06)	(0.56)	(1.33)	(0.54)
Willits[a]	ND	ND	ND	ND

[a] Average zero-order rate ± 95% confidence interval with the standard deviation given in parenthesis for three microcosms.

ND no transformation detected

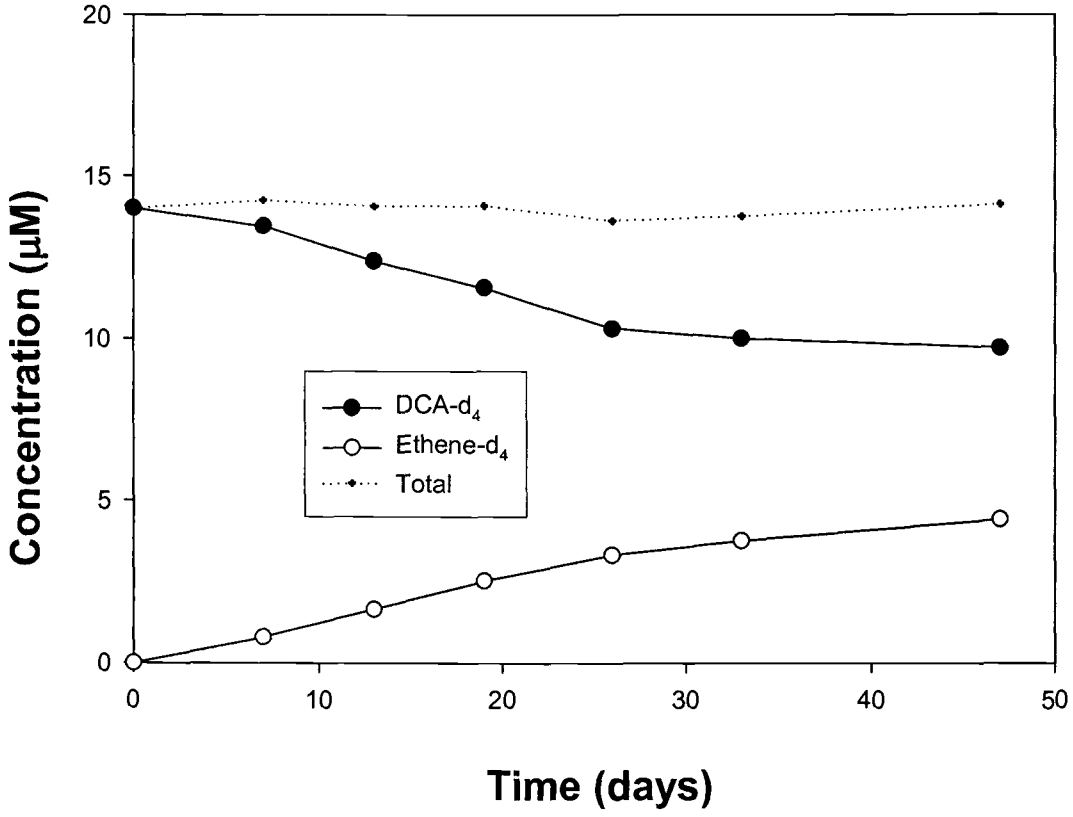

Figure 2.8. Example time progress curve for the dihaloelimination of DCA-d_4 to ethene-d_4 in a Richmond Microcosm.

Rates of DCA and DCA-d_4 were statistically similar according to a t-test at the 95% confidence level in the Richmond microcosms, indicating DCA and DCA-d4 gave similar rates. Further experimentation is needed to evaluate whether DCA and its deuterated surrogate DCA-d_4 are dehalogenated by the same enzyme system and to obtain Monod kinetic parameters for DCA and DCA-d_4

The Richmond system was able to convert DCA and VC to ethene, DCA-d_4 to ethene-d_4, and CFE to FE. The finding that the Richmond microcosms dechlorinated both VC and DCA to ethene suggests they may contain *Dehalococcoides*-like microorganisms. Maymo-Gatell et al. evaluated *Dehalococcoides* strain 195 and found that it is able to degrade both chlorinated ethenes and DCA to ethene (23).

Evanite Culture Batch Reactors. The Evanite culture batch reactors (n=3) dehalogenated DCA to ethene (Figure 2.9) from day 0 to 24 at an average zero-order rate of 4.40±1.21 μM/day while the zero-order rate of ethene production over the same time period was 4.40±1.52 μM/day (Table 2.3). Of the initial DCA added, 99% was converted to ethene, and 1% was converted to VC. Mass balance was achieved with 87 to 96% of the initial mass present accounted for at the end of the tests. For the killed control, 100% mass balance and no production of ethene or VC was detected.

A separate set of three Evanite batch reactors was run in parallel with the DCA experiments. In these experiments, DCA-d_4 was transformed to ethene-d_4 at an average zero-order rate of 4.40±1.52 μM/day (Figure 2.10) from day 0 to 24. The zero-order rate of ethene-d_4 formation over the same time period was 4.71±0.61 μM/day (Table 2.3). There was no ethene-d_4 production and 93% mass balance was

observed in the killed control. Of the initial DCA-d_4 added, 99% was converted to ethene-d_4 and 1% was converted to what is tentatively identified as VC-d_3.

During the transformation of DCA-d_4 two peaks were observed in chromatograms. Ethene-d_4 was verified by comparison with an authentic standard. However, because an authentic standard of VC-d_3 was expensive, a tentative identification was performed by comparing the full-scan mass spectrum of VC (Figure 2.9) to that of the peak believed to be VC-d_3 (Figure 2.10). The full-scan, electron impact mass spectrum of VC yielded a molecular ion $[M]^+$ at m/z 62, a $[M+2]^+$ at m/z 64, and a fragment ion at m/z 26 (Figure 2.9). The spectrum matched the library mass spectrum as well as that of an authentic standard. The full-scan, electron impact mass spectrum of VC-d_3 gave a $[M]^+$ at m/z 65, a $[M+2]^+$ at m/z 67, and a fragment ion at m/z 30 (Figure 2.10). The m/z 65 ion is consistent with the calculated mass of VC-d_3, which is 3 atomic mass units higher than that of VC (m/z of 62). In addition, VC-d_3 gave a $[M-35]^+$ fragment ion at m/z 30, corresponding to the loss of a chlorine atom. VC gave a $[M-35]^+$ at m/z 27, which corresponds to the loss of a chlorine atom. The $[M-35]^+$ ion for VC-d_3 at m/z 30 was 3 atomic mass units higher than the $[M-35]^+$ for VC at m/z 27. The ion at m/z 44 in both spectra is due to carbon dioxide present in the background. Chromatographically, the VC-d_3 peak had the same retention time as that of VC, which is consistent with only a 3 amu difference between the two compounds.

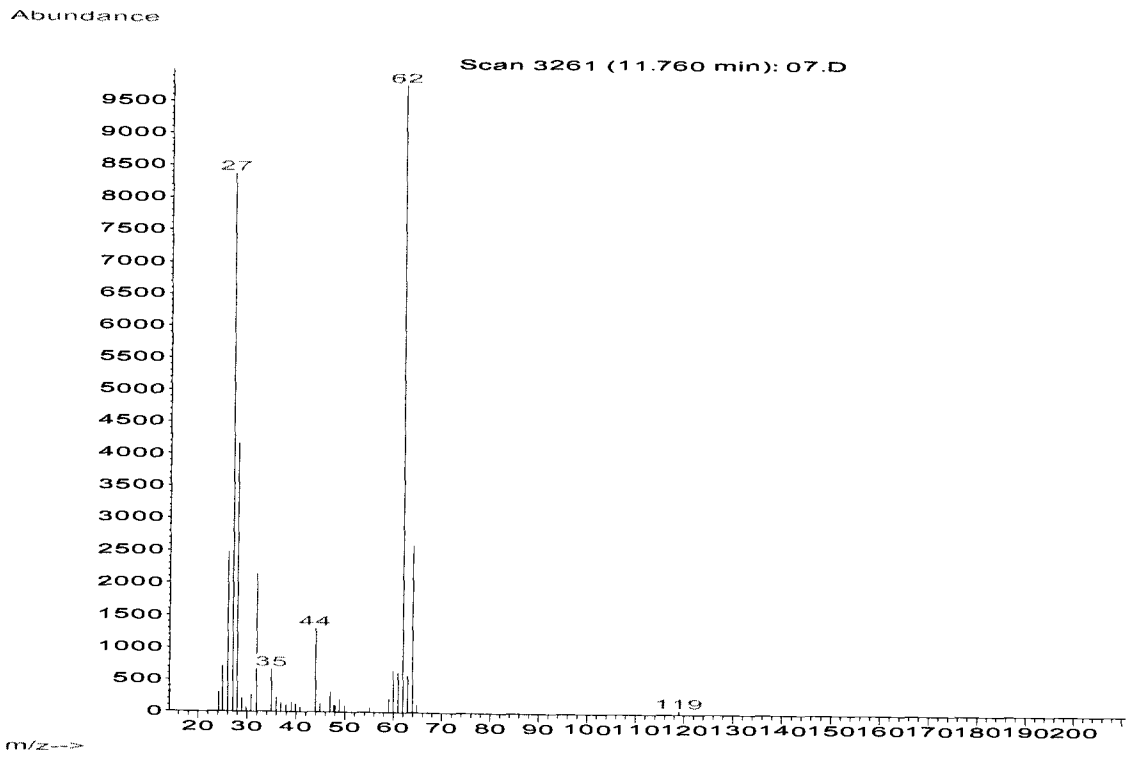

Figure 2.9. Full-scan mass spectrum of VC from incubations of DCA in an Evanite batch reactor.

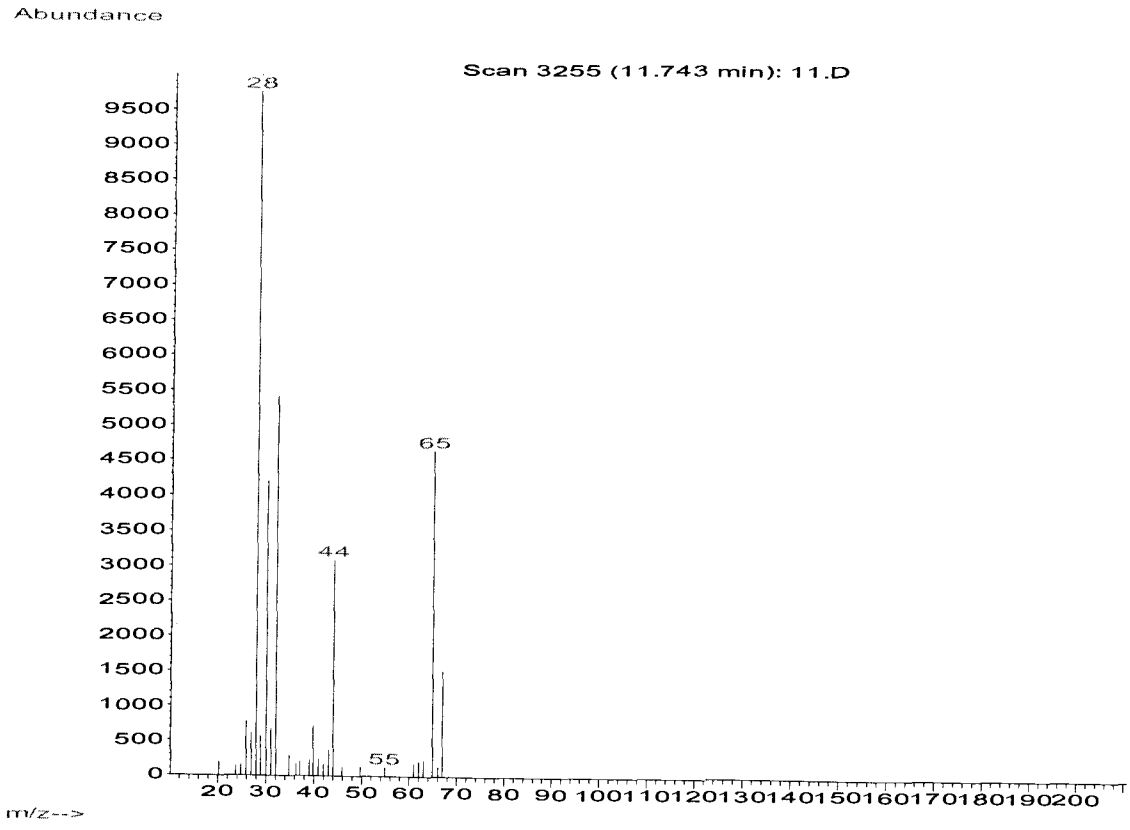

Figure 2.10. Mass spectrum of suspected VC-d_3 from incubations of Evanite batch reactors with DCA-d_4.

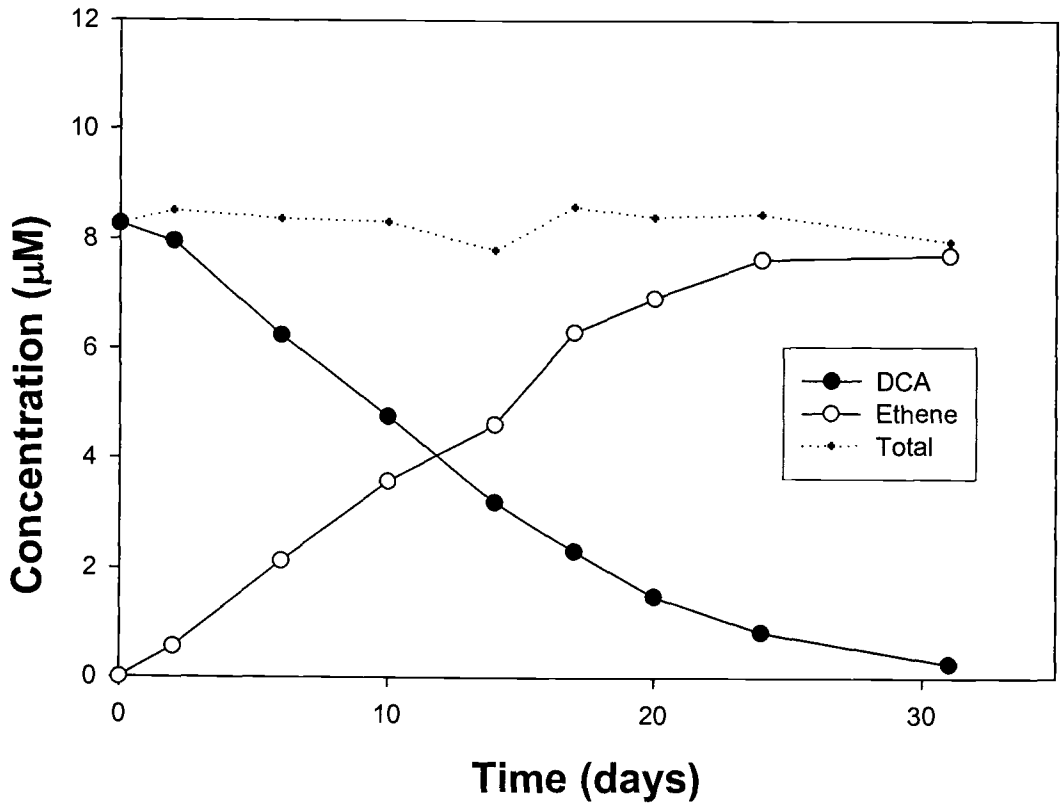

Figure 2.11. Example time progress curve for the dihaloelimination of DCA to ethene in an Evanite batch reactor.

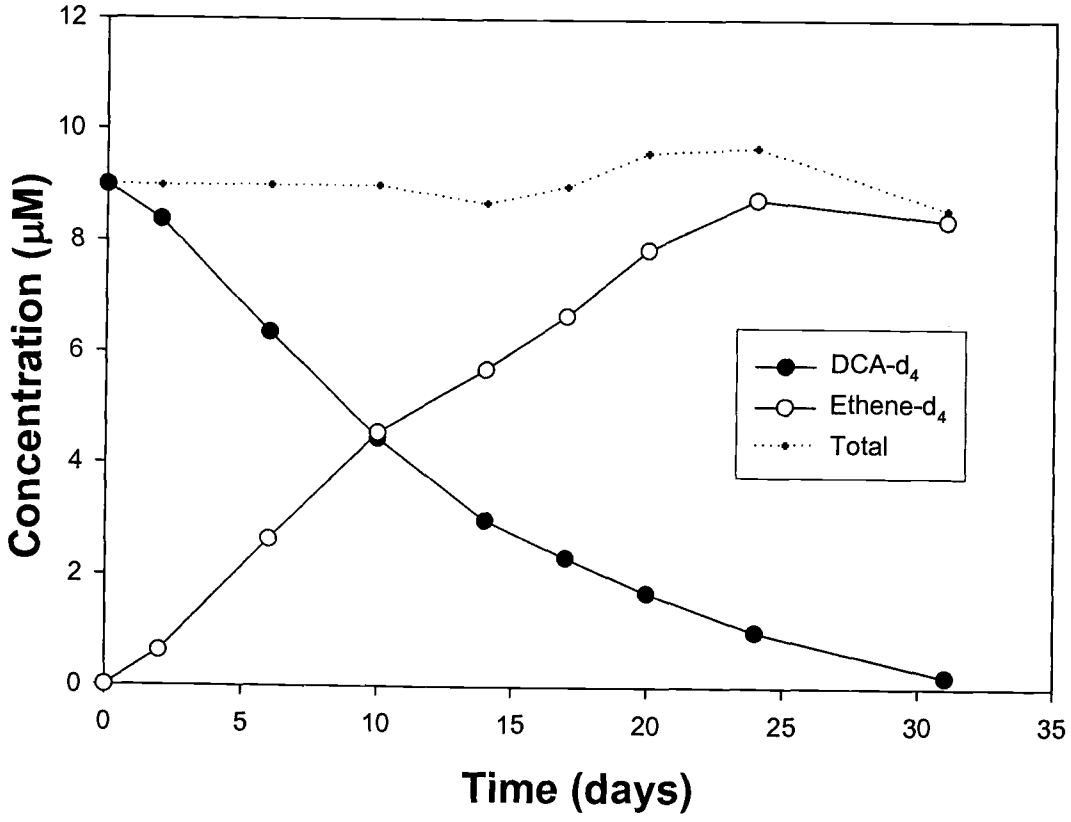

Figure 2.12. Example time progress curve for the dihaloelimination of DCA-d_4 to ethene-d_4 in an Evanite batch reactor.

The degradation rates in the Evanite batch reactors for DCA and DCA-d_4 were statistically similar at the 95% confidence level according to a t-test, which was a similar relation observed for DCA and DCA-d_4 in the Richmond microcosms. No lag phase was observed in either the DCA or DCA-d_4 experiments, which is consistent with no lag for the CFE/VC experiments. The overall lack of lag periods is attributed to the initial stimulation with TCE prior to the VC/CFE experiments.

It is interesting to note that the Evanite enrichment culture that contains *Dehalococcoides*-like organisms transformed 1% of the added DCA to VC, which suggests that Evanite culture contains a *Dehalococcoides* organism similar to strain 195. VC was observed in the Richmond microcosms at time zero and the measured concentration remained constant (data not shown), which suggests VC was not produced. The VC at time zero was due to incomplete purging of the microcosm in preparation for the DCA experiments. Although Maymo-Gattel et al. (23) found that *Dehalococcoides* strain 195 produced VC from DCA, Richmond microcosms may contain a strain of *Dehalococcoides* other than 195 that has capacity to transform VC and DCA to ethene but not DCA to VC.

Willits Microcosms. The third system used in transformation tests of DCA and DCA-d_4 was sediment and water microcosms from Willits, CA. There was no evidence of DCA or DCA-d_4 dechlorination in the three replicate microcosms (Table 2.3) over the 30 days they were monitored (data not shown). Mass balance was achieved with 82 to 117 % of the initial DCA or DCA-d_4 mass added accounted for at the end of the tests. These same microcosms transformed less than 1% of the initial TCE and TCA added during the initial stimulation phase. In addition, the lack of

DCA and DCA-d_4 transformation over the 30 day period of the study indicates that abiotic transformation in this system is not significant. It would be interesting to perform another set of DCA and DCA-d_4 experiments once the microcosms show greater reductive dechlorination activity.

The Willits microcosm did support 1% transformation of TCE and TCA to 1, 2-DCE and 1,1-DCA, respectively during the stimulation phase. These transformations are important because it shows that the Willits microcosms are active albeit at a low level.

DCA-d_4 as a Surrogate for DCA. In the two active systems (Richmond and Evanite), DCA and DCA-d_4 were transformed at similar rates as determined by the t-test at the 95% confidence level. Moreover, neither DCA nor DCA-d_4 were transformed in the Willits system. Based on these experiments, DCA-d_4 appears to be a good surrogate for DCA as it mirrors the activity of DCA in both active and inactive systems. A difference in rates due to the kinetic isotope effect between DCA and DCA-d_4 was not expected because the C-H and C-D bonds, respectively, are not involved in the transformation reaction to ethene. A kinetic isotope effect is expected when the rate-determining step involves the breaking of the C-D or C-H bond (39). The absence of a kinetic isotope effect indicates that DCA-d_4 reaction rates can be used directly to predict the in-situ rate of DCA transformation to ethene. Future work could include a) further characterization of DCA dechlorinating cultures and b) obtaining Monod kinetic parameters for DCA dechlorination.

CONCLUSIONS

Based on their persistence and toxicity, VC and DCA are significant groundwater contaminants and surrogates for these compounds are needed to obtain rate data from field experiments. Surrogates are needed to circumvent the problem of high and variable concentrations of parent and daughter products that are already present in ground water at CAH-contaminated sites. The fluorinated and deuterated surrogate compounds selected for this study are not present in groundwater at CAH-contaminated sites.

This work established precedence for the use of E-CFE and DCA-d_4 as surrogates for VC and DCA, respectively. The surrogates were selected such that their unique chemical signature was not lost during *reductive dechlorination* because the fluorine and deuterium atoms were conserved and thus transformation products were detected unambiguously. The advantages of fluorinated and deuterated surrogates selected for this study are they are not detected in CAH-contaminated groundwater. In addition, they are inexpensive relative to ^{13}C surrogates, not radioactive, and have been approved for injection at CAH-contaminated field sites in five states.

Although not identical in their physical properties, it was found that E-CFE gave reaction rates within a factor of three of that of VC. As a result, E-CFE can be used to estimate *in situ* rates of VC reductive dechlorination in CAH-contaminated ground water. Deuterated compounds are ideal choices for surrogates as indicated by the excellent agreement was obtained between the rates of DCA and DCA-d_4 transformation.

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