AN ABSTRACT OF THE DISSERTATION OF

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Title: Anaerobic Reductive Dechlorination of TCE and TCFE in TCE Contaminated Sediments: Enhanced Bioremediation and Bioaugmentation.

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Abstract approved: _____

Jonathan D. Istok

This research focused on the enhanced reductive dechlorination of trichloroethene (TCE) and its surrogate, trichlorofluoroethene (TCFE), using two bioremediation methods in anaerobic conditions. Two anaerobic bioremediation studies were conducted to investigate the effects of microbial communities in the presence of different electron acceptors and donors during anaerobic reductive dechlorination of TCE and TCFE. The first study was conducted in the groundwater microcosm bottles, filled with groundwater and sediments collected from Richmond site, CA. Parallel reductive dechlorination of TCE and TCFE was evaluated in the presence of fumarate and its product, succinate, while active reduction of high background concentrations of sulfate (2.5 mM) occurred. Because sulfate was assumed as a favorable electron acceptor during reductive dechlorination of chlorinated aliphatic hydrocarbons (CAHs), all microcosms receiving TCE and TCFE with substrates showed enhanced reductive dechlorination activity and even no substrate addition microcosms generated biotransformation products. From the electron mass balance calculations, more than 87.5% of electrons went to sulfate

reduction and less than 10% of available electrons involved in dechlorination after sulfate reductions. After amending varying concentrations of sulfate (0 – 2.5 mM), no inhibition was found between reductive dechlorination of TCE and sulfate reduction. The result indicated that reductive dechlorination could be directly competed with sulfate reduction for available electrons.

The second study investigated the effectiveness of in situ push-pull tests to evaluate bioaugmentation in physical aquifer models (PAMs) using dehalogenating strains to reductively dechlorinate TCE to ethene and TCFE to FE in the TCE contaminated sediments. Complete reduction of TCE to ethene occurred in less than 14 days with repeated additions of TCE (13.0 to 46.0 mg/L) and TCFE (15.0 mg/L) was completely transformed to FE in under 24 days. Increased rate and extent of dechlorination in the bioaugmented PAM compared to the nonaugmented control PAM indicated successful transport of the bioaugmented culture through the PAM. Similar transformation rates and time course of TCE and TCFE also indicated that TCFE was a bioprobe for reductive dechlorination of TCE. TCE and TCFE were transformed to cis-dichloroethene (c-DCE) and cis-dichlorofluoroethene (c-DCFE) respectively at two of the three sampling ports after 50 days of incubation in the nonaugmented PAM indicating reductive dechlorination activity of indigenous microorganisms. The results showed that it is possible to increase the rate and extent of reductive dechlorination of TCE and TCFE by bioaugmentation and that push-pull tests are effective tools for detecting and quantifying these processes in situ.

The third study focused on numerical modeling of the second study. The objectives of this study were (1) to evaluate a simplified method for estimating

retardation factors for injected solutes and bioaugmented microorganisms using "push-pull" test injection phase breakthrough curves, (2) to identify whether bioaugmented microorganisms have kept the same transformation capacity of Evanite culture using Michaelis-Menten kinetics by the values provided by Yu et al. (2005) and to verify *in situ* rates of TCFE reductive dechlorination rates of push-pull tests by numerical modeling, and (3) to investigate a reasonable answer for the nonuniform recovery of ethene and FE during the activity test and the push-pull test. The bioaugmented microorganisms were effectively transported through Hanford sediment. The estimated retardation factor was 1.33. A numerical simulation predicted cell transport in the PAM as far as port 5. This was qualitatively confirmed by cell counts obtained during bioaugmentation but, cells were distributed nonuniformly. The transport test indicated that TCE and TCFE transport was relatively retarded compared to coinjected bromide tracer (retardation factors ranged from 1.33-1.62 for TCE and from 1.44-1.70 for TCFE). The modeling simulation of Michaelis-Menten kinetics for the activity test was well matched for reductive dechlorination rates for TCE and less dechlorinated ethenes using the previous published values of k_{max} and K_s of chlorinated ethenes by Yu et al. (2005); the model match indicated that the bioaugmented microorganisms kept the same transformation capacity as the original source, Evanite culture (Yu et al., 2005) over 4 months in the PAM. A numerical simulation resulted in the simple first order FE production rate of ~1 day^{-1} using STOMP code (2002) and the value of FE production rate was in the range of the transformation rates of TCFE during the activity test. The bioaugmented PAM has caused slow loss of injected CAHs during the activity test and the push-pull test.

Anaerobic Reductive Dechlorination of TCE and TCFE in TCE Contaminated Sediments: Enhanced Bioremediation and Bioaugmentation

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Jae-Hyuk Lee

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APPROVED:

Signature redacted for privacy.

Major Professor, representing Civil Engineering

Signature redacted for privacy.

Head of the Department of Civil, Construction and Environmental Engineering

Signature redacted for privacy.

Dean of the Graduate School

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Jae-Hyuk Lee, Author

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To God and My Family,

CHAPTER 1

INTRODUCTION

Chlorinated aliphatic hydrocarbons (CAHs) are widely used as nonflammable solvents in large quantity by industry. The toxic solvents tetrachloroethene (PCE) and trichloroethene (TCE) have been widely used as degreasers and chemical feedstocks (1). TCE is usually released or spilled as a dense non-aqueous phase liquid (DNAPL), which can migrate deep into soils and aquifer materials forming a long-term contaminant source. The US EPA has set maximum contaminant levels (MCLs) in drinking water for PCE (5 μg/L), TCE (5 μg/L), and VC (2 μg/L) (2-4). Conventional physical and chemical treatment processes used to remediate contaminated groundwater, such as pump and treat with air stripping and carbon adsorption, are the most frequently used remediation method for TCE clean up. Unfortunately, these treatment processes are only able to transfer the chlorinated compounds from one phase of the environment to another without destroying them (5).

Biological treatment processes have received attention because it has the potential to transform chlorinated contaminants to less toxic products, such as ethene (6, 7). TCE is generally recalcitrant to biodegradation under aerobic conditions. However, a variety of microbe-mediated processes can catalyze conversion of this chlorinated solvent to harmless products under anaerobic conditions. Generally, microorganisms that catalyze these reactions use the chlorinated ethenes as electron acceptors while using a variety of substrates as electron donors (e.g., lactate, H_2, butyrate, ethanol, acetate, and pyruvate) (7, 8). Under anaerobic conditions, symbiotic relationships between anaerobic

microorganisms exist. Fermentation can transform complex organic materials to alcohols, fatty acids, and H_2. In any fermentation reaction, there must be a balance between oxidation and reduction that is maintained by molecular hydrogen (9). Reductive dechlorination of PCE and TCE by anaerobic biotransformation sequentially produce less chlorinated ethenes, such as *cis*-dichloroethene (*cis*-DCE), *trans*-dichloroethene (*trans*-DCE), 1,1-dichloroethene (1,1-DCE), vinyl chloride (VC), and finally ethene as a non-toxic end-product. In contaminated anaerobic environments, dechlorination often terminates at DCE or VC, but complete dechlorination to ethene has also been observed (10, 11). The termination at VC for reductive dechlorination is the most undesirable at CAH contaminated sites because VC is a known human carcinogen and more toxic than parent compounds (MCL at 2 μg/L). In effort to investigate the enhancement of TCE reductive dechlorination, a variety of electron donors and acceptors has been investigated in an attempt to identify substrates that lead to complete and rapid reductive dechlorination of TCE (12-14). Fumarate was also used as an alternate electron acceptor to identify the parallel and correlated reductive dechlorination of TCE when both TCE reductive dechlorination and fumarate respiration rates were enhanced (12-15).

Trichlorofluoroethene (TCFE) has been proposed as a surrogate reactive tracer to track the reductive dechlorination of in situ and microcosms with TCE contaminated sediments (16-18). To date, the only known isolates to reductively dechlorinate PCE to ethene are *Dehalococcoides ethenogenes* strain 195 and *Dehalococcoides* sp. Strain BAV1, to respire VC to ethene (20, 21). Those microorganisms are very important and popular candidates for bioaugmentation because of their ability to completely transform VC to ethene (22-29).

The main purpose of this study was to further investigate TCFE as a surrogate reactive tracer to detect and quantify reductive dechlorination of CAHs in TCE contaminated sediments. In the first manuscript (Chapter 3), fumarate was used for an electron acceptor to identify and enhance parallel TCE (~75 μM) and/or TCFE (~75 μM) reductive dechlorination for biostimulation study with indigenous microorganisms at a TCE-contaminated site in Richmond, CA. Furthermore, succinate was also examined how succinate as an electron donor after fumarate transformation could enhance TCE and TCFE reductive dechlorination in microcosms. Different Sulfate concentrations (0-2.5 mM) were used to evaluate the inhibition of TCE reductive dechlorination at varying sulfate concentrations. The specific objectives of the 1st manuscript were: (1) to investigate the effects of fumarate and succinate addition on product distribution and rates of TCE and TCFE reductive dechlorination in microcosms constructed with sediments and groundwater from the same field site used by Hageman et al. (16, 18), (2) to determine the effects of initial sulfate concentration on the rate and extent of TCE and TCFE reductive dechlorination.

In the second manuscript (Chapter 4), a dehalogenating culture was used for bioaugmention study using a laboratory scale model, physical aquifer model (PAM), to evaluate single-well, push-pull tests in TCE contaminated sediments. The mixed culture was obtained from the Evanite site in Corvallis and confirmed that *dehalococcoide*-like microorganisms were present. To our knowledge, the second study was the first approach to perform push-pull tests to evaluate bioaugmentation for TCE remediation. Different concentrations of TCE (13-46 mg/L) and/or TCFE (15 mg/L) were used to analyze reductive dechlorination and a transport test using a packed column was

investigated how the 10-fold diluted culture could transport through the Hanford formation sediment. In the control PAM without added culture, TCE and TCFE were transformed to only *cis*-DCE and *cis*-DCFE; no other transformation products were detected and transformation rates were 6-8 times smaller than in the bioaugmented PAM. The rate and extent of reductive dechlorination of TCE and TCFE by bioaugmentation and that push-pull tests are effective tools for detecting and quantifying the effects of bioaugmentation on contaminant transformation.

The specific objectives of the 2nd manuscript were: (1) to determine conditions needed to facilitate transport of the bioaugmented culture in porous media, (2) to determine the long-term viability of the bioaugmented culture, (3) to assess the effectiveness of bioaugmentation on reductive dechlorination, and (4) to develop and evaluate the single well, push-pull test as a rapid, *in situ* method for monitoring the transport, survival, and activity of bioaugmentation cultures used for reductive dechlorination of TCE.

In the third manuscript (Chapter 5), numerical simulations were used for the bioaugmentation experiment of Lee et al. (19). After bioaugmentation, the extent and reductive dechlorination rates of TCE/TCFE was stable and increased more than 3 months, but no uniform and consisted recovery of final reductive dechlorination products of TCE and TCFE, Ethene and FE, was observed. Furthermore, the push-pull test also showed smaller FE production rates for TCFE reductive dechlorination than the activity test (19). The specific objective was to investigate what was attributed to these differences between activity tests and push-pull tests for reductive dechlorination rates of chlorinated ethenes using numerical modeling.

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CHAPTER 2

Literature Review

ANAEROBIC BIOTRANSFORMATION OF CAHS

PCE and TCE are generally recalcitrant to aerobic degradation due to highly oxidized state and anaerobic reductive dechlorination is a more favorable process for the anaerobic microorganisms when oxygen is often depleted in the subsurface. In the reductive dechlorination of TCE, chlorine atoms are sequentially replaced with hydrogen to generate isomers of DCE, VC, and finally ethene as a harmless end-product (Figure 2.1). The reductive dechlorination process usually occurs in environments characterized by methanogenesis and/or acetogenesis (1). In reductive dechlorination, the chlorinated ethenes are used as electron acceptors and other organic compounds or hydrogen are used as electron donors (2, 3). Research over the last twenty years has shown that destruction of chlorinated compounds can be achieved via reductive dechlorination by stimulating indigenous microorganisms and/or adding dehalogenating microorganisms in both the laboratory and field studies (4, 5). However, incomplete reductive dechlorination of TCE to ethene has been observed at sites and this has been attributed to absence of dehalorespiring microorganisms, a suitable electron donor, or both (6, 7).

In the reductive dechlorination of PCE and TCE, many different compounds can serve as effective electron donors including methanol, ethanol, butanol, glucose, propionate, pyruvate, benzoate, lactate, formate, acetate, malate, and butyrate (3, 4, 8-11).

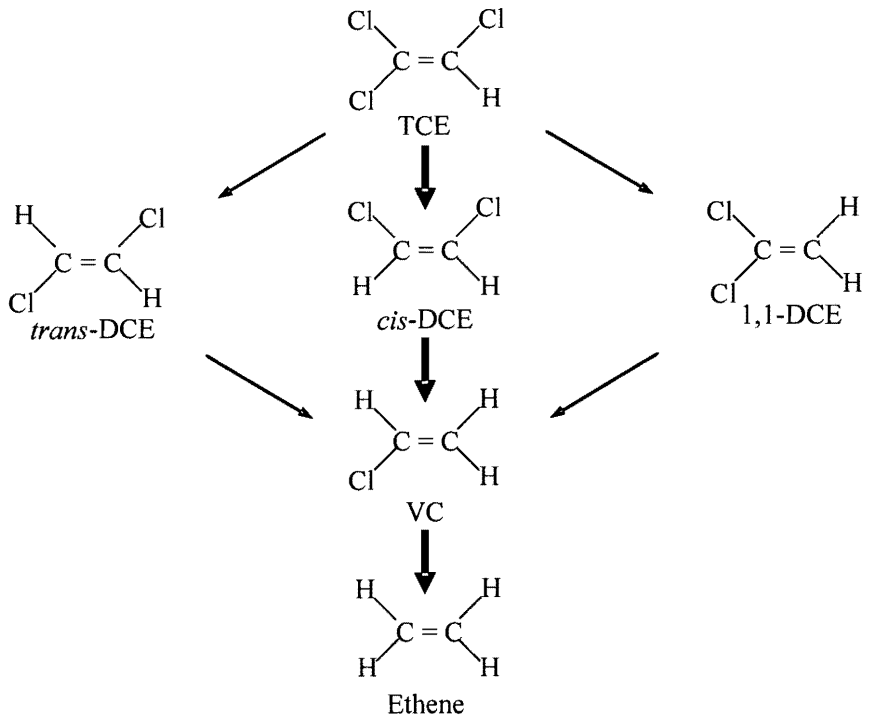

Figure 2.1. Reductive dechlorination of TCE (the bold arrows indicate main pathway)

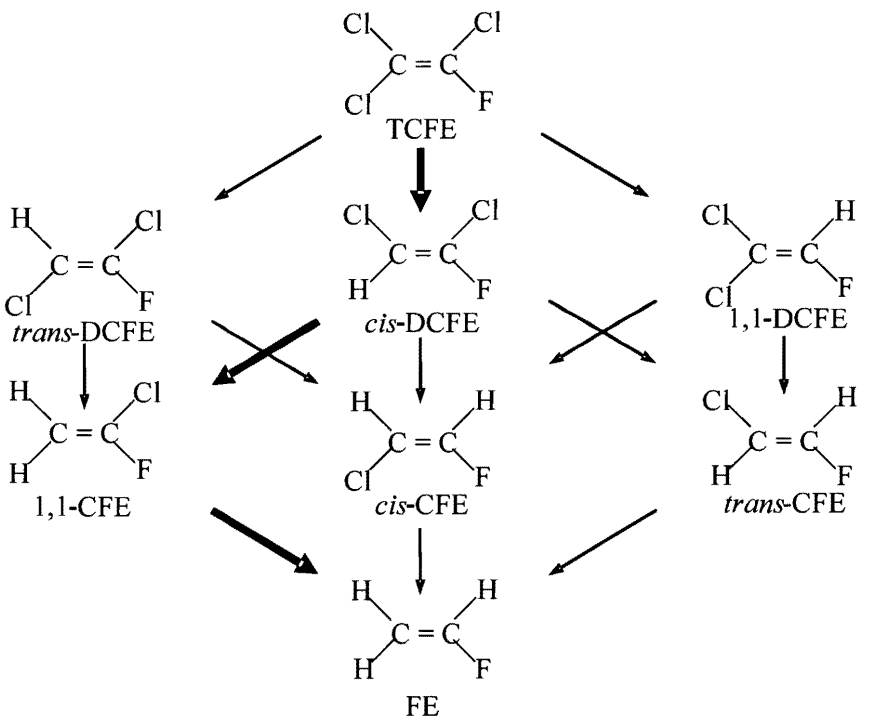

Figure 2.2. Reductive dechlorination of TCFE (the bold arrows indicate main pathway)

Furthermore, the addition of other electron acceptors has been shown to enhance dechlorination of PCE and TCE. These include sulfite, thiosulfate, fumarate, nitrate, 2-chlorophenol, 2, 4, 6-trichlorophenol, and 3-chloro-4-hydroxy-phenyl acetate (11-14). Fumarate could be effective for growth of other microorganisms and PCE was another effective and similar electron acceptor, but only *dehalospirillum multivorans* showed inhibition of PCE reductive dechlorination in the presence of fumarate (15). Recent studies have suggested that H_2 in supporting of reductive dechlorination of PCE and TCE. H_2 is the ultimate electron donor in reductive dechlorination and that dechlorinating bacteria out-compete methanogens for H_2 utilization at low H_2 concentration (3, 16-18). The selection of substrates (e.g., ethanol, lactate, propionate, and butyrate) yielding low hydrogen partial pressure is desirable therefore select organisms capable of PCE reductive dechlorination. Fennell and Gossett (3) found that butyrate and propionate amended enrichment culture generated low H_2 partial pressures that were 2-3 orders of magnitude below those generated with ethanol and lactate. However, in the long term operation, no difference was observed among electron donors and reductive dechlorination and indicated that the slowly degraded, generating low- H_2 partial pressure substrates (butyrate and propionate) could initially minimize methanogen competition (3).

COMPETITION BETWEEN DEHALOSPIRING BACTERIA AND SULFATE REDUCING BACTERIA (SRB)

Little is known about biotic interactions between dehalgenating and nondehalogenating microorganisms (i.e., SRB and methanogens). Many groundwaters contain significant concentrations of sulfate and competition can inhibit reductive

dechlorination rates and products distribution (19, 20). Bouwer and Wright (19) reported that less than 15% of initial PCE was removed in a sulfate-reducing, continuous-flow column. Best et al. also indicated that the degree of 1,1,1-trichloroethane degradation and dechlorination decreased rapidly with increase of sulfate concentrations (0.06 to 0.95 mM) in an anaerobic packed-bed reactor (20). However, Bagley and Gossett (21) reported that their sulfate-reducing enrichment culture could transform PCE to TCE and cis-DCE about 92% when using lactate as primary substrate. Using a coculture of the sulfate reducing bacterium *Desulfovibrio fructosivorans* and the dehalorespiring *Desulfitobacterium frappieri* TCE1, Drzyzga and Gottschal (22) demonstrated that complete PCE dehalogenation to cis-DCE occurred with 1 mM sulfate in the medium using 2.5 mM of fructose as the sole electron-donor. Without sulfate in the medium, PCE was also completely dehalogenated to cis-DCE and the population size of strain PCE 1 significantly increased (22). The complete transformation of PCE and TCE to ethene and ethane were also reported using the same coculture (22) maintained with lactate for a column study to demonstrate the bioremediation of chloroethene- and nickel contaminated sediment under sulfate reducing conditions (23). Recently, Hoelen and Reinhard (24) reported complete biotransformation of chlorinated ethenes to ethene in the presence of sulfate (> 100 mg/L) that fed with toluene in microcosms and monitoring slow dechlorination of TCE to ethene in live control microcosms containing only sediment without substrate addition after more than 3 yrs of incubations.

BIOAUGMENTATION USING DEHALOGENATING MICROORGANISMS

A number of in situ bioremediation approaches have been developed and tested. The first approach, natural attenuation, relies on indigenous microorganisms to destroy the contaminant of concern before it produces a significant hazard to downgradient water sources (25). This was a first alternative where applicable because no additional supplemental sources required degrading the target compounds, just monitoring of contaminant concentrations, modeling of the groundwater flow, and natural degradation rates. Another approach is biostimulation where indigenous microbial populations are stimulated to degrade the target compounds by adding growth substrate, supplemental nutrients, and/or oxygen to support their growth and degradation activity (26). Bioaugmentation is one approach that has been investigated in an effort to accelerate the reductive dechlorination ability of existing dechlorinating populations. Bioaugmentation involves injecting selected exogenous microorganisms capable of degrading the target chemicals directly into the contaminated zones. Two bioaugmentation approaches have been proposed.

The first bioaugmentation approach is to add large numbers of bacteria into an aquifer as biocatalyst that degraded a significant amount of the target contaminant before the cells becoming inactive or perished. Duba et al. (27) demonstrated a field test that an in situ biofilter created by injecting resting-phase cells (*Methylosinus trichosporium* OB3b, ~5.4 kg dry weight in 1800 L of groundwater) effectively remediated groundwater contaminated with TCE. TCE concentrations in the extracted groundwater decreased from 425 to less than 10 ppb during the first 50 hr of operation, which is equivalent to a 98% reduction (27). TCE concentrations gradually increased to background levels (~ 425 ppb) after 40 days when the test was terminated (27). The second bioaugmentation

approach is to add degradative microorganisms to subsurface to replace the native microbial population. The added microorganisms are selected for their ability to survive for a long-term period or to occupy a specific niche within the contaminated zone. Stimulants or co-substrates may be added to support prolonged survival and growth of the added microorganisms. Several laboratory scale studies of bioaugmentation for reductive dechlorination successfully demonstrated accelerated destruction of target chemicals (28-30). Dybas et al. (31) conducted field test that sustained effective removal of carbon tetrachloride (CT) for over 4 years (~ 98%). They established a row of closely spaced (1m) injection/extraction wells normal to the direction of groundwater flow near the edge of CT plume and added acetate as an electron donor, alkali, keep favorable pH and phosphorus for introduced microorganism, *Pseudomonas stutzeri* KC (31). A comparison study of bioaugmentation, biostimulation, and a recirculation-only control was performed in a chloroethene-contaminated aquifer (32). Using a *Dehalococcoides*-containing PCE to ethene dechlorinating inoculum, Lendvay et al. (32) reported successful bioaugmentation that resulted in stoichiometric dechlorination of both sorbed and dissolved chloroethenes to ethene within 6 weeks. They also performed a parallel biostimulation approach to reductively dechlorinate the chloroethenes to ethene about 76% but only following a 3-month lag period with continuous lactate and nutrient injection. This study (32) applied molecular tools targeting 16S rRNA of *Dehalococcoides* and *Desulfuromonas* spp. to identify and monitor the distribution of microorganisms and the abundance of dechlorinating populations qualitatively and quantitatively in the groundwater using real-time Polymerase chain reaction (PCR), Terminal Restriction Fragment Length Polymorphism (T-RFLP) analyses. Another

bioaugmentation study has been reported to demonstrate the accelerated PCE dense non aqueous phase liquids (DNAPL) dissolution in the intermediate laboratory scale experiment (33). In this study, dechlorination extent increased over time after establishing anaerobic conditions in a near field-scale simulated aquifer system. However, VC was detected at high concentration (11.6 μM) on day 266 and became a major product indicating incomplete degradation of PCE.

SINGLE-WELL, PUSH-PULL TESTS

Single-well, push-pull tests are used in this research. They are a method developed to determine physical characteristics of aquifers in situ, performed directly in monitoring wells. In situ test methods provide results more representative than are of actual subsurface conditions compared to laboratory methods such as microcosms. These tests are performed in situ and interrogate of the subsurface. To date push-pull tests have been used to evaluate in situ, quantitative information of a variety of aquifers for physical, chemical, microbiological characterizations including aerobic comebolism (37), reductive dechlorination of chlorinated aliphatic hydrocarbons (CAHs) (38), gas push-pull tests (39), NAPL detection (40), anaerobic transformation of deuterated benzene, toluene, ethylbenzene, xylene (BTEX) compounds (41), and radio nuclides (42). In this study, A push-pull test consists of 3 steps to determine in-situ reductive dechlorination rates and transformation products of CAHs: (1) a controlled injection ("push phase") of a prepared test solution including a non-reactive tracer and reactive solutes, (2) No pumping ("rest phase") for a certain time to insure the reaction of the test solution in the PAM, and (3) the extraction ("pull phase") of the test solution/pore water mixture from the same

location. Tracer concentrations adjust concentrations of test solution components for dilution. Mass balances are computed by integrating concentrations of CAHs during the extraction phase. Reaction rates are computed from the mass of solute consumed and/or product formed. Tests may be performed in existing monitoring wells or multilevel samplers.

TCFE

To measure *in situ* transformation rates of TCE in the presence of background concentrations of TCE in aquifers is difficult because (1) injection of TCE radiolabeled ^{14}C may not be allowed by regulation, (2) indigenous microbial transformation products of injected TCE cannot be distinguished from background transformation products, and (3) physical conditions of aquifers can be problematic that involves the dilution or unidentified sources of TCE and transformation products by advection, dispersion, sorption/desorption, and dissolution of TCE NAPL.

A novel methodology involving the application of trichlofluoroethene (TCFE) has been proposed as a TCE surrogate (43). To date two laboratory microcosm tests (43,44) and three field tests (38, 45, 46) have been performed to evaluate how TCFE reductive dechlorination rates and pathways track that of TCE. One of the abiotic studies of TCFE transformation was performed by Glod et al. (47) using metal-containing cofactors as effective electron transfer mediators for the reduction of PCE, TCE and TCFE. With respect to TCFE, only three products were detected: 1,1-DCFE, trans-DCFE, and a major product, cis-DCFE.

To track the reductive dechlorinating potentials of TCE in microcosms, Vancheeswaran et al., Pon and Semprini (43 and 44) reported that TCFE would be a good surrogate to estimate the rates of reductive dechlorination of PCE and TCE for anaerobic bioremediation. A rapid first-step dechlorination was observed of all the compounds within the first days of incubation (43). For PCE and TCE, transformation stopped at cis-DCE after 10 and 6 days of incubation respectively: while for TCFE mainly cis-DCFE was formed (87%) and a minor amount of trans-DCFE (10%). A trace amount of 2-chlorofluoroethene was observed over time and they concluded that TCFE behaves as an intermediate between PCE and TCE (43). For field studies, Hageman et al. (45) also hypothesized that TCFE reductive dechlorination rates were similar to the rates of TCE and reported a successful enhancement of TCFE reductive dechlorination at a TCE contaminated site. They also enhanced reductive dechlorination of TCFE compared to TCE about 20 times faster by addition of formate in the aquifers. The range of TCFE transformation was between 0.053 and 0.30 μM•day, while that of TCE was between 0.009 to 0.012 μM•day with an addition of formate. Without formate additions, they observed that the TCFE transformation decreased to 0.036 μM•day. Furthemore, they reported successful and enhanced reductive dechlorination of TCFE about a factor of 8.2 to 92 in three wells and the detection of FE, the final transformation product of TCFE, by five series of single-well push-pull tests with additions of fumarate as a stimulant (38). Based on these studies the reductive dechlorination pathway for TCFE is similar to that of TCE except trans- and cis- CFE isomers.

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CHAPTER 3

Fumarate- and Succinate-Enhanced Reductive Dechlorination of Trichloroethene and its Surrogate Trichlorofluoroethene

Jae-Hyuk Lee,[a] Mark Dolan,[a] Jonathan Istok,[a] Jennifer Field[b,*]

[a]Department of Civil, Construction and Environmental Engineering

[b]Department of Environmental and Molecular Toxicology

Oregon State University, Corvallis, OR 97331

*Corresponding Author: Jennifer.Field@oregonstate.edu

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ABSTRACT

The effectiveness of fumarate and its transformed product, succinate, in stimulating reductive dechlorination of trichloroethene (TCE) and trichlorofluoroethene (TCFE) was investigated in laboratory microcosm studies using sediments and groundwater from a TCE-contaminated aquifer. In live microcosms containing only sediments and groundwater, TCE and TCFE reductive dechlorination proceeded slowly to *cis*-, *trans*-DCE (DCE) and to *cis*-, *trans*-DCFE (DCFE) respectively, during 240 days of incubation. Microcosms amended with TCE and fumarate or succinate produced *cis*- and *trans*-DCE, vinyl chloride (VC), and ethene. Microcosms amended with TCFE and fumarate or succinate produced analogous reductive dechlorination products of TCE, *cis*- and *trans*-DCFE; *cis*- and *trans*-, and 1,1-chlorofluoroethene (CFE); however, fluoroethene (FE) formation was not observed. The 1st order rate increased by 4 to 8 times with added fumarate and succinate as did the extent of TCE and TCFE dechlorination. All microcosms receiving fumarate or succinate showed active sulfate reduction.

ABBREVIATIONS

Tetrachloroethene (PCE), Trichloroethene (TCE), *cis*-dichloroethene (*cis*-DCE), *trans*-dichloroethene (*trans*-DCE), 1,1-dichloroethene (1,1-DCE), Vinyl Chloride (VC), trichlorofluoroethene (TCFE), *cis*-dichlorofluoroethene (*cis*-DCFE), *trans*-dichlorofluoroethene (*trans*-DCFE), *cis*-chlorofluoroethene (cis-CFE), *trans*-chlorofluoroethene (*trans*-CFE), 1,1-chlorofluoroethene (1,1-CFE), Fluoroethene (FE), Electron Donor (ED), Electron Acceptor (EA).

INTRODUCTION

Trichloroethene (TCE) is a common industrial solvent that is the most frequently detected organic contaminant in groundwater (McCarty 1997; Kengen, et al., 1999). Previous studies have demonstrated that TCE can be degraded chemically or biologically (Maymo-Gatell et al. 1997; McCarty 1997; Glod et al. 1997; O'Loughlin et al. 1999; Arnold and Roberts, 2000). Anaerobic reductive dechlorination of TCE proceeds sequentially through less chlorinated ethenes, including *cis*-1,2-dichloroethene (*cis*-DCE), *trans*-1,2-dichloroethene (*trans*-DCE), vinyl chloride (VC) and finally to ethene, which is nontoxic (Maymo-Gatell et al. 1997; McCarty 1997; Kengen et al. 1999). The greatest concern associated with reductive dechlorination of TCE is that the process may not proceed completely, resulting in the accumulation of VC, which is a known human carcinogen or DCE.

The dechlorination of TCE to cis-DCE is a relatively fast process and can be stimulated, whereas further dechlorination of cis-DCE to VC, and especially VC to ethene is significantly slower and is more problematic to stimulate (Middeldorp et al. 1999).

A variety of electron donors and acceptors have been investigated for increasing the rate and extent of TCE reductive dechlorination including but not limited to methanol, ethanol, butanol, glucose, propionate, pyruvate, benzoate, lactate, formate, acetate, malate, and butyrate (Fennell et al.1997; Gerrste et al. 1999; Maymó-Gatell et al. 1999; Freedman and Gossett 1989; Yang and McCarty 1998; Finneran et al. 2002).

For this study, source of groundwater at a field site contained relatively high concentrations of sulfate (1-25 mM, Hageman et al. 2001, 2004) and the presence of

sulfate may inhibit reductive dechlorination. In the presence of sulfate, dechlorination has been reported to cease at TCE, *cis*-DCE or VC (Bagley and Gossett 1990; Harkness et al. 1999; Drzyzga and Gottschal 2002). To design cost-effective bioremediation strategies, it is important to know how dechlorination process is affected by sulfate concentrations.

Fumarate is reduced to succinate by dechlorinating microorganisms for a growth substrate and an alternative electron acceptor (Gerritse et al. 1996; Krumholz 1997; Madigan et al. 1997; Martinko et al. 1997; Mackiewicz and Wiegel 1998; Sung et al. 2003). *Desulfitobacterim* sp. Strain PCE1 and *Desulfuromonas chloroethenic*a sp.nov. can carry out fumarate respiration and PCE/TCE reductive dechlorination by transferring electrons from lactate or pyruvate to fumarate generating succinate (Gerristse et al. 1996; Krumholz 1997). *Desulfitobacterium dehalogenans* can also utilize fumarate and 3-chloro-4-hydroxyphenylacetate as electron acceptors with formate and pyruvate as electron donors (Mackiewicz and Wiegel 1998). *Desulfuromonas Michiganesis* sp. nov. reductively dechlorinated PCE to *cis*-DCE in the presence of free-phase of PCE, and grew best in the presence of fumarate (Sung et al. 2003). Hageman et al. (2004) reported that the addition of fumarate enhanced rates and extent of reductive dechlorination of the fluorinated TCE surrogate, trichlorofluoroethene (TCFE) at a TCE contaminated aquifer in the San Francisco bay area.

Vancheeswaran et al. (1999) suggested that TCFE can be used as a fluorinated analog of TCE to detect and quantify rates of TCE reductive dechlorination in the environment. Hageman et al. (2001) reported TCFE reductive dechlorination rates that were similar to those for TCE during *in situ* field experiments conducted in a TCE contaminated groundwater aquifer. Lee et al. (2006) also reported that enhanced TCFE

reductive dechlorination rates ranged from 8 to 21% of those of observed TCE reductive dechlorination rates for bioaugmentation in a physical aquifer model. Complete transformation of TCE to ethene and TCFE to FE within 9 days using similar concentrations indicated that TCFE would be a useful surrogate to quantify enhanced reductive dechlorination rates by bioaugmentation (Lee et al. 1996).

Hence, the first objective of this work was to quantify the transformation rates of fumarate and succinate addition on the extent and rate of TCE and TCFE reductive dechlorination in microcosms constructed with sediments and groundwater from the same field site used by Hageman et al. (2001, 2004). The second objective of this study was to investigate whether the initial sulfate concentration (0-2.5 mM) inhibit TCE and TCFE reductive dechlorination in this system. Furthermore, the third objective was to further test the hypothesis that TCFE is a surrogate for TCE reductive dechlorination in the presence of fumarate or succinate (Hageman 2004).

EXPERIMENTAL SECTION

Chemicals

Sodium sulfate (99.9%, Mallinckrodt Co., Paris, KY), sodium acetate (99+%, Aldrich Chemical, Milwaukee, WI), sodium chloride (99%, Aldrich Chemical, Milwaukee, WI), sodium lactate (60% w/w syrup, Fisher, Fair Lawn, NJ) and sodium propionate (99%, Aldrich Chemical, Milwaukee, WI) were used as substrates and/or as analytical standards. Potassium bromide (Fisher, Fair Lawn, NJ) was used as an internal standard for ion chromatography. TCE was purchased from Fisher (99.9%, Fair Lawn, NJ). *cis*-1,2-DCE (97%, Acros Organics, Pittsburgh, PA), *trans*-DCE (98%, Aldrich

Chemical, Milwaukee, WI), 1,1-DCE (99%, Aldrich Chemical, Milwaukee, WI), VC (99.5%, Aldrich, Milwaukee, WI), and Ethene (99.5%, Aldrich, Milwaukee, WI) were purchased for use as analytical standards. TCFE (97%), DCFE (98% mixture consisting of 50% of *cis* and 50% of *trans* isomers), CFE (97% mixture consisting of 69% of *cis* and 31% of *trans* isomers) were purchased from SynQuest Labs, Inc. (Alachua, FL) and FE (98%) from Lancaster synthesis (Pelham, NH).

Sediment and Groundwater

TCE-contaminated sediment and groundwater were collected from the site of a former chemical manufacturing facility in the San Francisco Bay area (Buscheck et al., 1997). This site was used in four previous field studies in which in situ rates of reductive dechlorination were determined (Hageman et al., 2001, 2004; Field et al., 2005; Ennis et al., 2005). The water table lies within 3 m of the ground surface. A bulk sediment sample was collected from the shallow A-zone. The sediments used in this study were collected with a bucket auger from the A-zone, which is an unconfined shallow layer (<5m) composed mainly of placed fill over Bay Mud. Sediments used in microcosm experiments were stored in glass bottles capped with stoppers to avoid air exposure and shipped in coolers on blue ice by overnight express. Site groundwater was collected from the same depth from well 10A in 1 gallon Nalgene bottles, with no headspace, to maintain anaerobic condition during transport and storage. The sediment and groundwater were stored at 4°C prior to microcosm preparation.

Microcosm Studies and Transformation Rate Calculation

All glass bottles (156 ml, Wheaton, Millville, NJ), butyl rubber septum, and caps were autoclaved at 140 °C for 60 min. Microcosms were constructed in an anaerobic globe box in a nitrogen atmosphere. A bulk sediment sample was mixed homogeneously before distribution into microcosms. Each microcosm contained approximately 20 g of wet sediment and 100 mL of groundwater. The total volume of groundwater and sediment was 114 ml and the headspace volume was 52 ml. After sealing, the bottles were purged with nitrogen in a fume hood for 6 or more hrs, to ensure complete removal of oxygen, residual TCE, and other volatile organic compounds. Killed control bottles were prepared by autoclaving selected microcosms >3 times (140 °C for 60 min with 6 hrs between heatings) and amending with 25 mg/L (aqueous) $HgCl_2$.

Microcosms were prepared in triplicate and included killed controls with TCE/TCFE; killed controls with fumarate or succinate, TCFE + fumarate; TCE + fumarate; TCFE + succinate; TCE + succinate; TCFE alone (live control), and TCE alone (live control) (Table 3.1). Three additions of fumarate (~750 μM) and succinate (~750 μM) added on day 0, 99, and 182.

To investigate the inhibition effects of added sulfate on reductive dechlorination, all live microcosm bottles used for the 1st objective were opened in an anaerobic glove box after complete reduction of initial sulfate, remixed together homogeneously, and used to prepare additional microcosms in triplicate to meet the range of initial sulfate concentrations (0 ~ 2.5 mM).

To compute first-order rates for TCE and TCFE transformation, linear regression was applied to each individual progress curve by natural logarithmic transformation that corresponded to the highest rate of activity.

Table 3.1. Experimental design of microcosm study to examine effects of added fumarate or succinate on TCE and TCFE reductive dechlorination

BOTTLE SET NO.	FUMARATE (750 μM)	SUCCINATE (750 μM)	TCFE (75 μM)	TCE (75 μM)	KILLED CONTROLS
1			X		X
2				X	X
3	X				X
4		X			X
5	X		X		
6	X			X	
7		X	X		
8		X		X	
9			X		
10				X	

Volatiles Analyses

Gaseous concentrations of TCE, TCFE, DCE, DCFE, CFE, VC, FE, and ethene were measured on headspace samples collected weekly using a gas-tight syringe. Samples were analyzed using a Hewlett Packard (Palo Alto, CA) 5890II gas chromatograph (GC) equipped with flame ionization detector. Separation was performed using a 30 m × 0.32 mm × 4.0 μm Supelco SPB-1 capillary column (Bellefonte, PA). The column was operated with a thermal gradient using helium as the carrier gas. The GC oven was initially set at 40°C for 4.5 min, heated at 20°C/min to 160°C, and kept at 160°C for 0.5 min.

A five-point (5-120 μM) external standard calibration curve was constructed prior to initial sampling; the detection limit was 0.5 μM ($R^2 > 0.99$). The total mass of compound in each test bottle was calculated using the headspace and liquid volumes and Henry's constants (Gosset 1987; Louie 1999). Henry's constants (H_{cc}, dimensionless) for

TCFE and its metabolites at 20°C included; 1.403, TCFE; 0.877, cis-DCFE; 0.666, trans-DCFE; 0.867, cis-CFE; 0.287, trans-CFE; 3.55, 1,1-CFE; and 2.66, FE.

Anion Sampling and Analyses

Aqueous samples (~600 μL) were collected using disposable syringes and needles. Prior to sampling, the syringe void volume was filled with deoxygenated, deionized and distilled (DI) water to prevent the introduction of air into the microcosms. Aqueous samples were centrifuged for 10 min at 14,000 rpm using a model 5415C Eppendorf microcentrifuge. The supernatant was transferred to 0.5 mL Dionex auto-sampler polyvials.

Fumarate, succinate, acetate, and propionate concentrations were determined using a Dionex model DX 500 high performance liquid chromatography (HPLC) equipped with an LC 30 chromatography enclosure, a GP 50 gradient pump, and an AD 20 UV absorbance detector with a Prevail organic acid 5 um column (250 × 4.6 mm, Alltech). The HPLC was operated with an isocratic condition of 25 mM of KH_2PO_4 eluent containing 40 mL H_3PO_4 and 75 mL acetonitrile in 2 L of DI water. A five point (5-100 mg/L) external standard calibration curve ($R^2 > 0.99$) was constructed prior to initial sampling and a one-point standard check was performed prior to every sampling analysis.

Bromide and sulfate concentrations were determined using a Dionex Series DX-320 ion chromatograph (IC) (Sunnyvale, CA) equipped with an AS40 autosampler, conductivity detector, gradient pump, eluent degassing module, and integrator, and a Dionex IonPac AS11-HC analytical column (4 × 250 mm), an IonPac AG11-HC guard

column (4 × 50 mm), and an ATC-1 trap column in series. The IC eluent was prepared by mixing 50% (w/w) sodium hydroxide (Fisher) thoroughly deaired water (by helium) to yield 5 and 100 mM sodium hydroxide solutions. The method used a 1-50 mM sodium hydroxide gradient flowing at 1.5 mL/min. A six-point (1, 5, 25, 50, 75, 100 mg/L) polynomial standard calibration curve (detection limit at 10 μM) was constructed prior to initial sampling. A one-point standard check was performed prior to every sampling analysis to determine the stability of the detector and for recalibration purposes.

RESULTS AND DISCUSSION

TCE

The maximum 1st order TCE transformation rate obtained for microcosms that received only TCE was 0.013±0.001 d^{-1} (Table 3.2). The maximum transformation rates increased by a factor of 4 for microcosms that received fumarate or succinate with rates of 0.056±0.007 d^{-1} and 0.054±0.009 d^{-1}, respectively (Fig 3.1c and e, Table 3.2). Difference in rate of TCE transformation between fumarate and succinate addition was statistically insignificant. Fumarate and succinate additions decreased the lag time by a factor of ~3 from 45 days to 15 days (Fig 3.1c and e).

Live microcosms that received only TCE produced *cis*-DCE and *trans*-DCE in a 2:1 ratio as did those that received the 1st addition of fumarate or succinate (Fig 3.1a, c, and e). After the 2nd addition of fumarate and succinate, *cis*-DCE was transformed to VC and a trace amount of ethene (Fig 3.1c and e). Concentrations of *cis*-DCE were reduced to below detection (0.5 μM) by day 182 by fumarate and by day 149 by succinate. *Trans*-DCE concentrations remained unchanged for both fumarate and succinate additions.

Table 3.2. Maximum first-order transformation rates (± 95% confidence interval) for TCE and TCFE[a]

Maximum transformation rates (day^{-1})[a]	TCE (day^{-1})	TCFE (day^{-1})
TCE or TCFE only	0.013±0.001 (0.974)[b]	0.012±0.001 (0.912)[b]
Fumarate	0.056±0.007 (0.976)*	0.092±0.023 (0.946)**
Succinate	0.054±0.009 (0.946)*	0.095±0.034 (0.927)**
Killed control	0.004±0.0004 (0.922)[b]	0.004±0.0004 (0.930)[b]

[a]First-order rate (±95% confidence interval) with correlation coefficient, r, given in parenthesis; significant at 95% confidence interval determined by student t-test.*The rates were measured from days 15-45. **The rates were measured from days 99-133. [b]The rates were measured from days 99-171.

Significant quantities (~ 15 μM) of *trans*-DCE (>20% of TCE consumed) is unusual; others (Gerritse et al. 1996; Maymo-Gatell et al. 1997) report only trace amounts (< 5% or none of PCE) of *trans*-DCE formation in microcosm studies. While most PCE and TCE dechlorinating anaerobic bacteria produce *cis*-DCE as the major DCE isomer, Griffin et al. (2004) reported that enrichment culture that produced more *trans*-DCE than *cis*-DCE in ratio of 3±0.5:1. Hageman et al. (2001) reported the formation of cis and trans-DCE (< 0.1 μM) in ratio of 13:1 in field tests that we obtained the source of sediment and groundwater for the construction of microcosms.

Decreases in TCE concentrations (~ 75 μM) were observed in killed controls and in live microcosms prior to the onset of product formation and these are attributed to diffusion of these compounds through the microcosm's butyl-rubber septas. The apparent disappearance rates (0.004±0.0004 day^{-1}) of TCE and TCFE in killed controls were almost identical statistically and the rate of loss significantly slower than that from the other microcosms (Table 3.2); these indicated that other TCE or TCFE microcosms with or without fumarate or succinate caused the biological reductive dechlorination. As much

as 75% of initial TCE was lost from the killed controls and no products were detected during the 240 day incubation (Fig. 3.1).

The 1st addition of fumarate was rapidly reduced to succinate (< 3days), which then transformed to acetate (data not shown). After the 2nd addition of fumarate, both fumarate and succinate concentrations decreased below the detection limit (10 μM) in less than 2 days (data not shown) with stoichiometric conversion to acetate (~740 μM); acetate was then degraded within 20 days. After the 3rd addition of fumarate, complete reduction of fumarate to acetate occurred within one day and acetate concentrations remained above detection for more than 20 days (data not shown).

Complete reduction of the first addition of succinate less than 6 days occurred and 100 μM of acetate was detected (data not shown). The time courses for reduction of the 2nd and 3rd additions of succinate were similar to those of fumarate additions. Hageman et al. (2004) also found increased rates of fumarate reduction to succinate and subsequent disappearance of succinate during the course of three additions of fumarate to TCE-contaminated aquifer.

From days 0 to 99, sulfate concentrations decreased from 2.5 to 1 mM and decreased from 1 mM to below detection after the 2nd addition of fumarate (data not shown). Note that sulfate also decreased similarly during succinate additions compared to fumarate additions (data not shown). Thus, sulfate reduction by fumarate or succinate occurred in parallel with TCE and TCFE reductive dechlorination in these microcosms. Sulfate concentrations (~2.5 mM) in microcosms containing only TCE did not change significantly during the test.

No statistical difference in TCE transformation rates were observed in microcosm amended with varying initial sulfate concentrations (0-2.5 mM) to investigate inhibition and competition between sulfate reduction and dechlorination of TCE. In all cases, the first addition of TCE (~ 75 μM) was rapidly transformed to *cis*-DCE (< 10μM) and VC (< 80 μM) within 20 days (data not shown) and no inhibition effects were detected for all microcosms in the presence of sulfate. In all cases, VC was a major TCE transformation product in the presence of sulfate (0~2.5 mM). The possible explanation for VC build-up with/without sulfate would be that VC dechlorinating bacteria was not fully developed in our system and might be required more time.

Fumarate reduction to succinate was observed and is attributed to the presence of uncharacterized organic compounds in sediment and groundwater from this contaminated field site (Hageman et al., 2001, 2004). Since previous studies indicate that dehalogenating microorganisms utilize fumarate as an alternative electron donor (Gerritse et al. 1996; Krumholz 1997; Martinko et al. 1997; Mackiewicz and Wiegel 1998; Sung et al. 2003), fumarate was a likely better growth substrate than either TCE or TCFE (Gerritse et al. 1999; Sung et al. 2003). Therefore, it is possible that fumarate additions stimulated the dechlonating populations that utilize both fumarate and TCE/TCFE and the stimulated microbial community was responsible for increased transformation rates of fumarate reduction and TCE/TCFE reductive dechlorination.

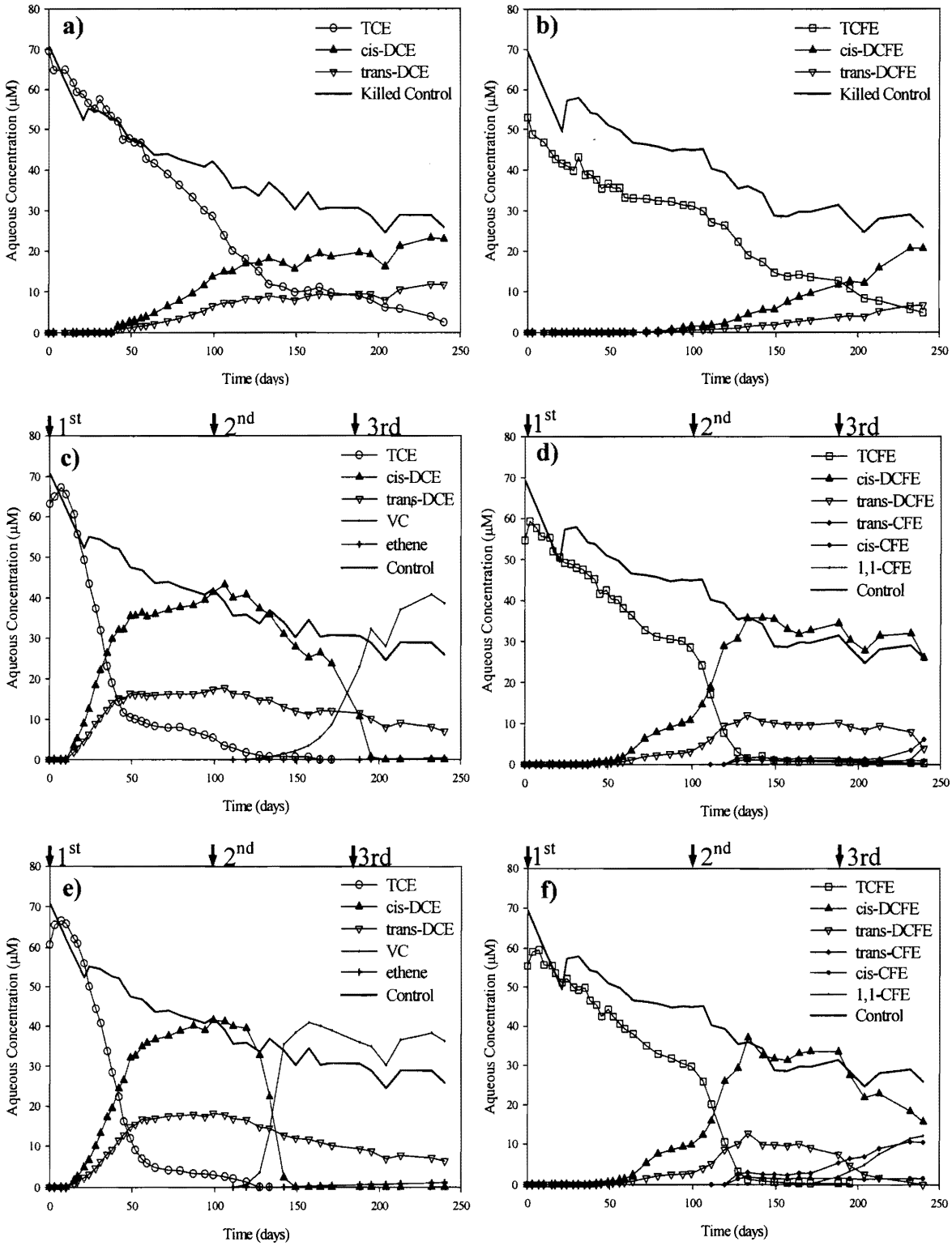

Figure 3.1. Effect of three additions of fumarate or succinate on TCE and TCFE transformation in microcosms containing: (a) TCE alone, (b) TCFE alone, (c) TCE + fumarate, (d) TCFE + fumarate, (e) TCE + succinate, and (f) TCFE + succinate. The arrows indicate each addition of fumarate or succinate.

TCFE

While the maximum 1st order TCFE transformation rate for microcosms that received only TCFE was 0.012±0.001 d^{-1} (Table 3.2), the maximum transformation rates of TCFE (days 99-133) increased by a factor of 8 for microcosms that received fumarate or succinate with rates of 0.092±0.023 d^{-1} and 0.095±0.034 d^{-1}, respectively (Table 3.2). Both fumarate and succinate decreased the lag time by a factor of ~2 (79→42, Fig 3.1b and d). The lag time for TCFE reductive dechlorination by fumarate or succinate was identical to 42 days (Fig 3.1d and f). Statistically, no significant difference in TCFE reductive dechlorination rates and products was observed between fumarate and succinate additions (Table 3.2). Our transformation rates of TCFE by additions of fumarate or succinate were in the range of the same field site by Hageman et al. (2004). For example, they reported the range of TCFE transformation rates from 1.1 to 1.6 d^{-1} and that the transformation rates increased by 8.2-92 times in the field after 3 successive additions of fumarate (Hageman et al., 2004).

The ratio of 2:1 between *cis*- and *trans*-DCFE was similar for microcosms that received TCFE those that with or without the first addition of fumarate or succinate (Fig 3.1 b, d, and f). In contrast, c*is*-, *trans*-, and 1,1-CFE were formed during the 2nd and 3rd additions of fumarate or succinate addition (Fig 3.1 d and f) while the TCFE only microcosms without fumarate or succinate couldn't produce to beyond *cis*- and *trans*-DCFE (Fig 3.1b); no FE was detected for all TCFE microcosms. It is also noted that the significant formation of *trans*-DCFE (> 20% of TCFE transformed) was observed in field tests (Hageman et al., 2004) as the source of microcosms used in this study.

Decreases in TCFE concentrations (~ 75 μM) were identical in killed controls and in live microcosms prior to the onset of product formation same as compared to those of TCE microcosms. No dechlorination products found in the killed controls and loss of TCFE (75%) was same as that from TCE killed control (Fig. 3.1 and 3.2).

The degradation of fumarate to succinate and its subsequent transformation to acetate was similar to that observed in microcosms containing TCE and fumarate. After the additions of succinate, acetate remained same as those of TCE microcosms received succinate (~0.8 mM).

The change of sulfate concentrations of TCFE microcosms that received fumarate or succinate was similar to those of TCE microcosms received fumarate or succinate. In contrast, in the absence of fumarate or succinate, sulfate concentrations (~2.5 mM) in microcosms containing TCFE only did not change significantly during the test.

TCFE as a surrogate for TCE during reductive dechlorination

No significant statistical difference was observed between TCE and TCFE reductive dechlorination rates using the 95% confidence intervals (Table 3.2) upon stimulation by fumarate or succinate with rates increasing by a factor of ~2 (Table 3.2); note that TCE were rapidly transformed after the 1st addition of fumarate or succinate while TCFE transformation rates increased after the 2nd addition of fumarate or succinate.

The lag time of TCE reductive dechlorination that received fumarate (Fig 3.1c) or succinate (Fig 3.1d) was 27 days shorter than that of TCFE that received fumarate or succinate (Fig 3.1e and f) and this might be that the dechlorinating population was not fully adjusted for TCFE because this microbial community had never been exposed to

TCFE before. Longer lag time (>30days) for TCFE reductive dechlorination in TCFE live controls also supported that the dechlorinating population was not fully developed for TCFE reductive dechlorination.

The *cis*- and *trans*- DCE and DCFE product ratios were the same (2:1) for microcosms containing TCE and TCFE only while the TCE and TCFE microcosms that received fumarate or succinate produced the same ratio between DCE and DCFE isomers before the 2nd addition of fumarate or succinate, then less-dechlorinated byproducts observed over time. The TCE product distribution for microcosms that received fumarate (Fig 3.1c) or succinate (i.e. *cis*-, *trans*-DCE, VC, and ethene, Fig 3.1e) paralleled that of the product distributions in TCFE microcosms with fumarate or succinate were similar although no FE formation occurred (i.e. *cis*-, *trans*-DCFE, and 3 CFE isomers, Fig 3.1d and f).

Vancheeswaran et al. (1999) also observed that TCE transformation stopped at *cis*-DCE after 6 days of incubation while for TCFE mainly *cis*-DCFE was formed (87%) with only a minor amount of *trans*-DCFE (10%) and a trace amount of CFE was observed over time. Pon and Semprini (2004) reported that TCFE dechlorination degree correlated with the degree of PCE dechlorination and 1,1-CFE could be a reactive surrogate to track VC dechlorination in a microcosm study. From a field test, Field et al. (2005) also suggested that TCFE was a useful surrogate for monitoring TCE remediation technologies at the TCE contaminated aquifer. For example, TCFE primarily transformed to cis-DCFE ranged from 36 to 87.4% for 5 out of 6 push-pull tests using lactate, Fe(0), hydrogen as electron donors in a TCE-contaminated aquifer; the rates of TCFE reductive dechlorination increased from 0.017 day^{-1} of the highest background transformation

(Hageman et al., 2001) to 0.02-0.2 day^{-1} after the three different treatment of electron donors (Field et al., 2005); less dechlorinated products were observed that CFE isomers confirming potential of VC formation; Only one test confirmed that FE for complete reductive dechlorination under hydrogen treatment. Hageman et al. (2001) reported higher transformation rates (6-25 times) of TCFE as compared to those of TCE transformation rates during push-pull tests, but the rate difference is likely due to higher concentration of TCFE injection (16 μM) with relatively low concentration of TCE injection (0.78 μM). Furthermore, similar transformation rates, product distributions, and time courses for TCE and TCFE transformation were observed when these compounds were added together at similar initial concentrations (TCE:171-242 μM, TCFE: 210-327 μM) to monitor activity of bioaugmented culture in a physical aquifer model; TCFE transformation rates were 8-21% faster than those of TCE rates (Lee et al., 2006). Therefore, TCFE is a good bio-surrogate to quantify and detect rates and extent of TCE reductive dechlorination. The results show that enhanced dechlorination of TCE to VC and ethene and bioremediation can successfully treat TCE-contaminated sediments and that TCFE provides information for assessing TCE reductive dechlorination activity.

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CHAPTER 4

Monitoring Bioaugmenation with Single-Well Push-Pull Tests in Sediment Systems Contaminated with Trichloroethene

Jae-Hyuk Lee,[a] Mark Dolan,[a] Jennifer Field,[b] Jonathan Istok[a,*]

[a]Department of Civil, Construction and Environmental Engineering

[b]Department of Environmental and Molecular Toxicology

Oregon State University, Corvallis, OR 97331

Corresponding Author: Jack.Istok@oregonstate.edu

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ABSTRACT

Bioaugmentation, the introduction of exogenous microorganisms to a natural environment to degrade target contaminants, was investigated in laboratory experiments to determine if this approach could be used to increase the rate and extent of reductive dechlorination of chlorinated ethenes. Bioaugmentation experiments were conducted in intermediate (~1m) scale physical aquifer models (PAMs) designed to simulate the groundwater flow field near an injection well. Push-pull tests were used to observe the reductive dechlorination of injected trichloroethene (TCE) and trichlorofluoroethene (TCFE) in prepared sediment packs with and without an added dechlorinating culture containing *Dehalococcoides*-like microorganisms. The culture was prepared and enriched by seeding TCE contaminated groundwater into anaerobic media that contained tetrachloroethene (PCE), 1-butanol, and hydrogen. During bioaugmentation, the culture was injected as a diluted (~17%) aqueous suspension in deoxygenated tap water. Successful transport of the bioaugmented culture was confirmed by microscopic observation. After bioaugmentation, injected trichloroethene (TCE) was transformed to *cis*-dichloroethene (*cis*-DCE), vinyl chloride (VC), and ethene, while injected trichlorofluroethene (TCFE) was transformed to *cis*-dichlorofluoroethene (*cis*-DCFE), *trans*-dichlorofluorethene (*trans*-DCFE), 1,1-chlorofluoroethene (1,1-CFE), 1,2-*trans*-fluoroethene (*trans*-CFE) and fluoroethene (FE). Repeated additions of TCE (13.0 to 46.0 mg/L) were completely transformed to ethene in 14 days and a following TCFE addition (15.0 mg/L) was completely transformed to FE in 24 days. Similar transformation rates, product distributions, and time courses for TCE and TCFE transformation were observed when these compounds were added together at similar initial concentrations. 1-butanol

(2-10 mM) was used as an electron donor and butyrate, propionate, and acetate production was observed during reductive dechlorination of TCE and TCFE. In the control PAM without added culture, TCE and TCFE were transformed to only *cis*-DCE and *cis*-DCFE, respectively; no other transformation products were detected and transformation rates were 6-12 times smaller than in the bioaugmented PAM. The results showed that it is possible to increase the rate and extent of reductive dechlorination of TCE and its surrogate TCFE by bioaugmentation and that push-pull tests may be effective field tools for detecting and quantifying the effects of bioaugmentation on contaminant transformation in the subsurface.

INTRODUCTION

Chlorinated aliphatic hydrocarbons (CAHs) cause environmental problems due to their recalcitrance and toxicity. As a result of spills and disposal of these compounds into the environment, trichloroethene (TCE) is one of the most common organic groundwater contaminants in the U.S. (1). Previous laboratory research and field studies (2,3) have shown that the destruction of chlorinated compounds can be achieved via reductive dechlorination by stimulating the activity of indigenous microorganisms. However, complete reductive dechlorination of TCE to ethene is not always observed and this has been attributed to the absence of dehalorespiring microorganisms, the absence of a suitable electron donor, or both (4,5). It is also known that one of dechlorination products of TCE, Vinyl Chloride (VC), is a carcinogen and a concern contaminant in the groundwater because the step for VC to ethene is cometabolic and K_s values for VC are higher relative than higher chlorinated compounds (6-8).

Bioaugmentation is defined as the introduction of exogenous microorganisms to a natural environment to degrade target contaminants (9-14). Laboratory and field studies have shown that bioaugmentation can increase the rate and the extent of reductive dechlorination of chlorinated ethenes all the way to ethene (10-14). Despite these findings, the effectiveness of bioaugmentation remains uncertain because of uncertainties about the feasibility of distributing exogenous microorganisms in the subsurface and the ability of exogenous microorganisms to survive and compete with indigenous microorganisms for nutrients and substrates and to maintain the targeted metabolic activity (14). Furthermore, the methods for assessing *in situ* rates and extent of complete reductive dechlorination of TCE to ethene after bioaugmentation are needed in the presence of background concentration of TCE in aquifers because it may or may not distinguish indigenous microbial transformation products of injected TCE background transformation products.

In situ test methods provide results more representative than are of actual subsurface conditions compared to laboratory methods such as microcosms. Most field pilot tests for bioaugmentation designed to determine *in situ* reductive dechlorination rates that involved recirculating groundwater between injection and extraction wells (11-13).

To track rate and extent of reductive dechlorination in the presence of background concentration of chlorinated ethenes, Vancheeswaran et al. reported that TCFE could be a useful surrogate for estimating rates of reductive dechlorination of PCE and TCE (15). Anaerobic transformation of TCFE proceeded by sequential reductive dechlorination to form DCFEs, CFEs, and FE, analogous to the products formed from the reductive

dechlorination of TCE (i.e. DCEs, VC, and ethene) (15-19). In two field studies, Hageman et al. (16) also demonstrated that TCFE reductive dechlorination rates were similar to those of TCE and has also reported the successful enhancement of TCFE reductive dechlorination to FE in TCE contaminated groundwater with additions of fumarate as a stimulant (18). Lee et al. (19) reported that fumarate and succinate were effective stimulants for reductive dechlorination of trichloroethene (TCE) and trichlorofluoroethene (TCFE) in the presence of high (2.5 mM) initial sulfate concentrations in laboratory microcosm studies using sediments and groundwater from the same TCE-contaminated aquifer used by Hageman et al. (16). Field et al. also used TCFE to detect, quantify, and compare TCFE reductive dechlorination activity in the presence of added Fe(0), H_2, and lactate added to large-diameter permeable columns (LDPCs) (20). Pon and Semprini (22) revealed that TCFE dechlorination degree correlated with the degree of PCE dechlorination and 1,1-CFE could be a reactive surrogate to track VC dechlorination in a microcosm study. Ennis et al. demonstrated that *trans*-CFE was a good surrogate for VC reductive dechlorination (21).

An alternative to using well-to-well tests to determine in situ transformation rates is to use single-well, push-pull tests (16-21, 24-29). This test is advantageous because one well is needed per test, *in-situ* characterization for well-to-well is allowed to compare using different wells from a single site.

Push-pull tests have been used to obtain *in situ*, quantitative information on microbial activity and contaminant transformations in the subsurface (25,26) including methane oxidation (27), aerobic cometabolism (28), anaerobic transformation of petroleum hydrocarbons (29), reductive precipitation of radionuclides (30), and anaerobic

transformations of chlorinated solvents (17-22). In this study, we used laboratory push-pull tests to evaluate the effectiveness of bioaugmentation for quantifying the rate and extent of complete reductive dechlorination of TCE to ethene and TCFE to FE using identical bioaugmented and non-bioaugmented (control) physical aquifer models (PAMs). Through a series of tests conducted in intermediate-scale PAMs, we developed procedures that should be useful for evaluation the effectiveness of bioaugmentation for assessing complete reductive dechorination of chlorinated ethenes.

The specific objectives were (a) to determine optimum growth conditions for the bioaugmented culture in a PAM, (b) to determine the emplacement of bioaugmented cells in a PAM and the long-term reductive dechlorination activity (~6 months) after bioaugmentation, and (c) to determine the utility of TCFE with push-pull tests to quantify *in-situ* reductive dechlorination rate after bioaugmentation.

MATERIALS AND METHODS

Chemicals

1-Butanol (99.5%, Aldrich Chemical, Milwaukee, WI), sodium sulfate (99.9%, Mallinckrodt Co., Paris, KY), sodium acetate (99+%, Aldrich Chemical, Milwaukee, WI), sodium chloride (99%, Aldrich Chemical, Milwaukee, WI), sodium lactate (60% w/w syrup, Fisher, Fair Lawn, NJ) and sodium propionate (99%, Aldrich Chemical, Milwaukee, WI) were used as substrates and/or as analytical standards. Sodium bromide (99+%, Acros Organics, NJ) was used as a tracer. Sodium hydroxide solution (50% w/w; Fisher Scientific, Fair Lawn, NJ) was used as an eluent for ion chromatography. Dichloromethane (DCM; Fisher Scientific Co., 99.9% HPLC Grade, Pittsburgh, PA) was

used for solvent extraction of 1-butanol. TCE was purchased from Fisher (99.9%, Fair Lawn, NJ). PCE (99.9%, HPLC grade, Sigma-Aldrich Inc., St. Louis, MO), *cis*-1,2-DCE (97%, Acros Organics, Pittsburgh, PA), *trans*-DCE (98%, Aldrich Chemical, Milwaukee, WI), 1,1-DCE (99%, Aldrich Chemical, Milwaukee, WI), VC (99.5%, Aldrich, Milwaukee, WI), and ethene (99.5%, Aldrich, Milwaukee, WI) were purchased for use as analytical standards. TCFE (97%), DCFE (98% mixture consisting of 50% of *cis* and 50% of *trans* isomers), and CFE (97% mixture consisting of 69% of *cis* and 31% of *trans* isomers) were purchased from SynQuest Labs, Inc. (Alachua, FL) while FE (98%) was purchased from Lancaster synthesis (Pelham, NH).

Culture and Growth Medium

The dehalogenating culture used in this study was harvested in a closed semi-batch reactor (Kimax, NJ) with total volume 1.2 L containing liquid volume of 1 L. The culture was obtained from a groundwater and sediment from a TCE-contaminated site at the Evanite site in Corvallis, OR (8). The Evanite culture is known to contain *Dehalococcoides*-like microorganisms and has been extensively tested for reductive dechlorination of chlorinated ethenes (8,23). The details for the Evanite culture were described elsewhere (8,23). The feed consisted of 0.54 mM 1-butanol, 10% partial pressure hydrogen headspace, 20 mg/L yeast extract, 0.98 mM PCE, and trace nutrients in basal medium (24). After the added PCE was completely converted to ethene within 7-10 days, 300 mL of culture were harvested in an anaerobic glove box from the reactor and replaced with anaerobic fresh basal medium, containing PCE, 1-butanol, and 10%

hydrogen headspace; these processes repeated to make 10 L of the culture for bioaugmentation.

Microcosm Study

A microcosm study was conducted to determine culture-dilution ratio with/without 5% fresh media to use in the bioaugmentation experiments (Table 4.1); the range of dilution factor for the Evanite culture was 0.1 - 8% and the diluted media (5%) was determined to be included or not. Glass bottles (156 ml, Wheaton, Millville, NJ), rubber septa and caps were autoclaved at 140°C for 60 minutes. Microcosms were constructed in an anaerobic globe box in a nitrogen atmosphere using 100 mL of fresh media diluted 20 fold by tap water or tap water only without media plus 40 g Hanford soil following a purge with nitrogen gas for 6 or more hours. The Evanite culture, TCE (~75 μM aqueous concentration), and 1-butanol (1-1.5 mM) were added into the microcosms to give a total liquid volume of 130 ml. 1-butanol was selected for testing since it was used as the donor in a laboratory study for the culture media (8).

Physical Aquifer Models

Experiments were conducted in two identical physical aquifer models (PAMs) constructed in a wedge-shape to approximate the radial flow field near an injection/extraction well during field push-pull tests (Fig 4.1A). The PAMs were constructed of polypropylene with interior dimensions of 5 cm (width at narrow end), 50 cm (width at wide end), 125 cm (length), and 20 cm (height) (Fig 4.1B). The PAMs were packed with sediment from the Hanford Formation, an alluvial deposit of sands and

gravels of mixed basaltic and granitic origin (19) after removing particles > 0.5 cm in diameter by sieving. The sediment contains less than 0.001 wt % organic matter and has a particle density of 2.9 g/cm^3. The porosity and bulk density of the packed sediment were 0.39 and 1.77 g/cm^3, respectively. Total internal volume was 69 L and the pore volume was 27 L. Tap water was used in all laboratory experiments. The narrow ends of the PAMs contain injection/extraction ports covered with a screen to allow it to pump water in or out during push-pull tests. After the sediment pack was water saturated, the PAMs were sealed with a lid containing sampling ports (Fig 4.1B) connected to 'well' screens that fully penetrated the sediment pack's saturated thickness. Experiments were performed under confined conditions. A schematic diagram of the experimental setup is in Figure 4.2. The lids of the PAMs were sealed with butyl rubber caulk (Sherwin-Williams, Corvallis) and all sampling ports were equipped with polyetheretherketone (PEEK) tubing (1/8″ OD, 0.08″ ID) and shut-off valves (Upchurch Scientific, WA). Injected test solutions flowed from the injection/extraction ports at the model's narrow end toward the model's wide end. A 50 L Nalgene carboy (Nalge Nunc International, NY) was connected to the model's wide end to allow pore water to leave the sediment pack during the injection phase and to allow water to enter the sediment pack during the extraction phase.

External national pipe thread (NPT) adapters for the injection line were made of PEEK: 1/8″ NPT to 1/4·28 female adaptor and 1/4″ NPT to 1/4·28 female adaptor. A 6-port manifold (Upchurch Scientific, WA) was mounted to the constant head line with PEEK tubing. The constant head reservoir in the wide end of the PAM was connected to a 50 L plastic carboy containing oxygen-free tap water. The carboy's cap was equipped with fittings for purging with nitrogen gas, ventilation, and sampling. The flow path for

the constant head reservoir was also connected with PEEK tubing and connections to deliver oxygen-free tap water into the PAM.

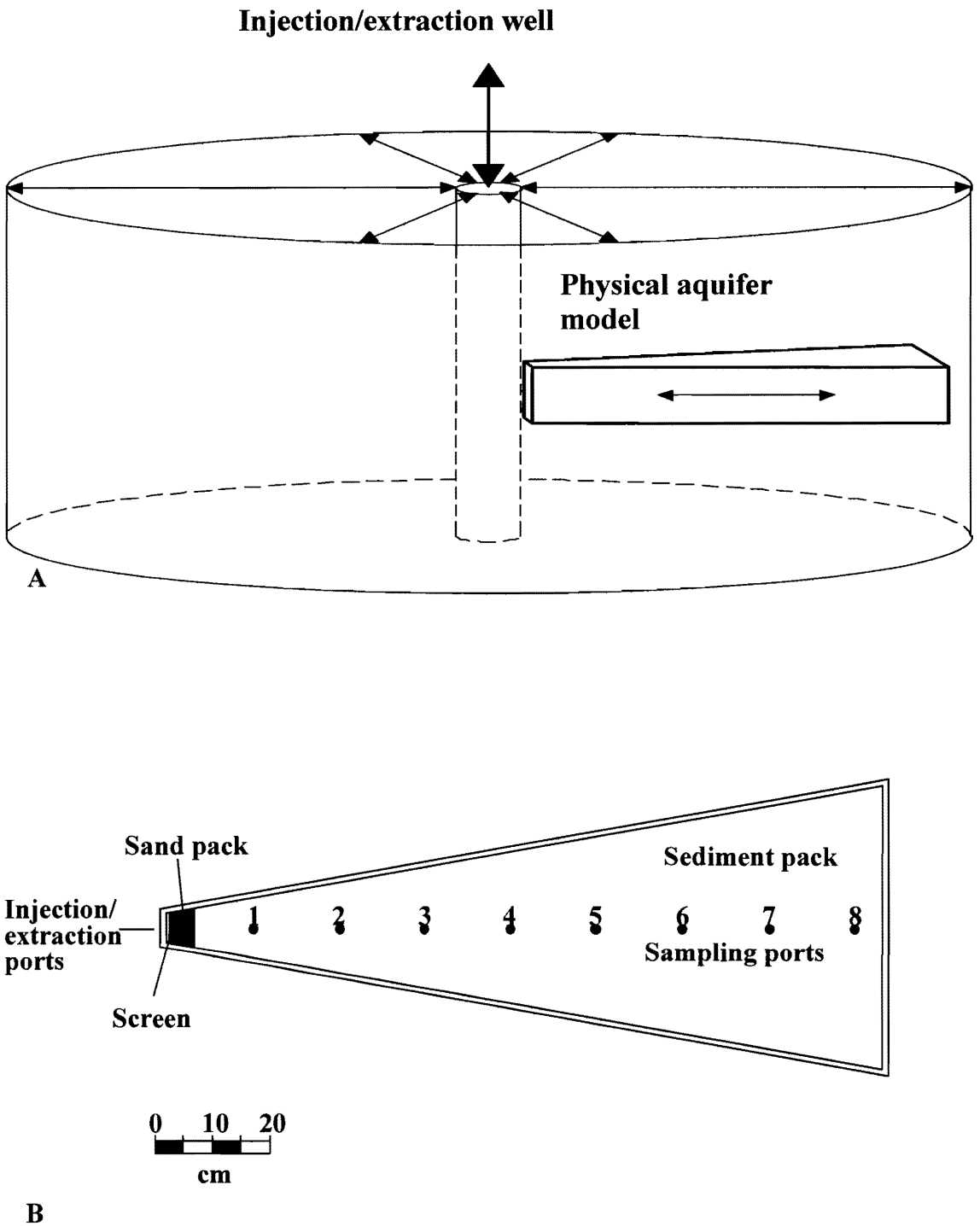

Figure 4.1. Physical aquifer models used in bioaugmentation experiments: (A) rationale and (B) plan view.

Bioaugmentation of the PAM

Six weeks prior to bioaugmentation, ~1.3 μM of titanium (III) citrate solution (30) was pumped into the PAM followed by 6 pore volumes of oxygen-free water containing sodium lactate (~1 mM) to stimulate microbial activity and create initial anaerobic and reducing conditions. Test solutions were prepared in a 48 L glass carboy (Fig 4.2). Dissolved oxygen was removed by vigorous bubbling (flowrate ~100-200 mL/min) with N_2 gas that had passed through a gas purifier (Supelco, PA) to remove trace O_2. The carboy was sealed with a black rubber stopper containing holes for sampling, sparging, and ventilation lines. PEEK tubing was used for gas and water delivery lines. A piston pump and 5-port manifold (Upchurch Scientific, WA) were used to inject test solutions into the sediment pack; injection rates varied between 40 and 100 mL/min depending on the test. The concentrated aqueous TCE and/or TCFE solutions in the bag were prepared by adding 1.5 mL neat TCE or 0.5 mL neat TCFE to 1.1 L oxygen–free tap water in 1.2 L glass bottles (Kimax, NJ). Approximately 1 L of this solution was transferred from a bottle to a bag containing 7 L oxygen free water using positive nitrogen pressure applied thorough a rubber septa.

The bioaugmentation injectate was prepared by adding sodium carbonate (37.6 mM), sodium sulfide (22 μM), and 1-butanol (10 mM) to the 48 L glass carboy and purging with carbon dioxide. The final pH was 7-8. Evanite culture (9 L) was mixed with the injectate by bubbling with N_2 and the concentration of the bioaugmented cells measured at ~1×10^7 cells/mL. During injection (~6 hrs), the bioaugmentation injectate was combined with TCE and/or TCFE by metering concentrated aqueous solutions contained in an 8 L collapsible, metalized bag (Chromatography Research Supplies, KY)

using a piston pump connected to a PEEK Y connector. Sampling for cell counts performed at ports 2,5,6, and 8 after bioaugmentation; port 5 and 8 were selected as background cell counts before bioaugmentation.

Analytical

TCE, TCFE, DCE, DCFE, CFE, VC, FE, and ethene headspace concentrations were determined on 100 μL headspace samples using a Hewlett Packard (Palo Alto, CA) 5890II GC connected to a flame ionization detector. Separation was performed using a 30 m × 0.32 mm × 4.0 μm Supelco SPB-1 capillary column (Supelco, PA). The column was operated under a thermal gradient with helium as the carrier gas. The GC oven was initially set at 40°C for 4.5 min, heated at 20°C/min to 160°C, and kept at 160°C for 0.5 min. Calibration curve consisted of a five-point (5-120 μM) external standard solution was constructed prior to initial sampling ($R^2 > 0.99$). The detection limit was about 0.5 μM. A one-point standard check was performed for every sampling activity.

Concentrations of inorganic and organic anions including bromide, chloride, acetate, butyrate, propionate, and sulfate were determined using a Dionex Series DX-320 IC (Sunnyvale, CA) equipped with a Dionex IonPac AS11-HC analytical column (4 × 250 mm), an IonPac AG11-HC guard column (4 × 50 mm), and an ATC-1 trap column in series. The gradient elution consisted of a 1-30 mM sodium hydroxide solution (50% w/w; Fisher Scientific, Fair Lawn, NJ) flowed at 1.5 mL/min. A six-point (1-100 mg/L) external standard calibration curve was used for quantification ($R^2 > 0.99$). A one-point standard check was performed for every sampling activity. 1-butanol was determined by extracting a 0.5 mL aqueous sample with 0.5 mL of DCM and vigorously mixing for 2

min on a vortex mixer. The DCM extract (10 μL) was analyzed using a HP-5890II GC connected to an FID.

Cell numbers in water samples were determined by direct counting. Cells were fixed by combining 1-1.5 ml of water sample and formaldehyde to obtain a 3-4 % final formaldehyde concentration in an autoclaved eppendorf tube. Cells were then collected, isolated from water samples on a 25 mm diameter filters (0.2-μm-pore-size black polycarbonate backed by a 0.45-μm-pore-size cellulose, Nuclepore) by vacuum filtration (< 10 kPa). The cells were stained by adding SYBR Green II (Molecular Probes Inc., Engene, OR) to the filters in the dark for 15 min using final dye concentrations between 1×10^{-4} and 5×10^{-4} units of the stocks provided by the manufacturer. The filter was then washed with 1-1.5 ml of filter-sterilized (0.2 μm) deionized water, air-dried for 1-2 minutes, placed on a clean glass microscope slide, and covered with immersion oil and a glass cover slip. Cells were enumerated under blue excitation fluorescence by using a Zeiss epifluorescence microscope with total magnification of 1000× and counted up to 300 cells from at least 10 eye fields per filter. The average number of cells per filter on the effective filter area was calculated and extrapolated to determine the number of cells per milliliter of sample as follows: Total cells/mL = (average cells/field) × 6400 (field/mL). The diluted cell concentrations in the column effluent and bioaugmentation ranged from 1 to 1.25×10^{7} cells/mL.

Activity Test at Stop-Flow

The bioaugmentation containing TCE (~50 μM) was performed; ethene only detected within 7days. After bioaugmentation with the 1st addition of TCE, 5 successive

injections of TCE, TCFE, and/or TCE/TCFE were conducted to quantify the activity of bioaugmented PAM for enhanced complete TCE/TCFE reductive dechlorination during 4 months; the 2nd and 3rd additions of TCE were to further determine mass balance of ethene production for each sampling port and also provide more favorable condition in the bioaugmented PAM. For dechlorination reaction rate analysis for complete TCE dechlorination, the 4th addition of TCE (>200μM) performed and sampled. The bioaugmented PAM was incubated under no-flow condition after TCE and/or TCFE injections stopped. After the 4th addition of TCE, the first injection of TCFE was conducted whether this compound could be a good surrogate for TCE reductive dechlorination. Co-injection of TCE/TCFE was conducted for further confirmation of feasibility of TCFE to track complete reductive dechlorination for both since then. The sampling ports (Fig 4.1) were sampled for TCE, TCFE, TCE/TCFE byproducts, and anion analysis for 2-3 times a week until complete transformation of TCE to ethene and/or TCFE to FE observed.

TCE/TCFE Transport Test

TCE/TCFE transport test was conducted after bioaugmentation to compare the transport behavior of TCE and TCFE in the bioaugmented PAM containing sediment by injecting 43 L tap water containing bromide (~90 mg/L), TCE (~155 μM), and TCFE (~30 μM) at 43 mL/min. Five samples of the injected test solution were collected during the injection phase and analyzed to determine test solution composition. Water samples were collected from sampling ports 1, 3, 5, and 8 and analyzed for bromide, TCE, TCFE, and cell numbers. Retardation factors for TCE and TCFE were calculated by comparing

injection phase breakthrough curves for bromide with those for the coinjected TCE and TCFE. Breakthrough curves are displayed as relative concentrations, C/C_0 where C is the measured solute concentration in a sample and C_0 is the average concentration of the same solute in the injected test solution; the retardation factors were measured at C/C_0 = 0.5 for bromide, TCE, and TCFE from the selected ports using the injection phase breakthrough curves.

Push-Pull Activity Test

A push-pull test consists of 3 steps to determine in-*situ* TCFE transformation rates by complete dechlorination and transformation products of TCFE to FE: (1) a controlled injection ("push phase") of a prepared test solution including a non-reactive tracer and TCFE, (2) No pumping ("rest phase") for a certain time to insure complete TCFE reductive dechlorination of the test solution in the PAM, and (3) the extraction ("pull phase") of the test solution/pore water mixture from the same location. Mass balances are computed by integrating concentrations of dechlorination products during the extraction phase. Reaction rates are computed from the mass of product formed.

For field push-pull tests, port 1 was behaving as an injection/extraction well because it was apart less than 10cm only from the injection/extraction ports (Fig 4.1B). Direct sampling from the injection/extraction ports was not performed due to a possible introduction of oxygen.

Sampling

Aqueous samples were collected during the experiments using plastic syringes connected to the sampling ports. Three mL were dispensed into 7 mL headspace vials with butyl rubber septas (Supelco, PA) for gas chromatograph (GC) headspace analysis. Samples were stored at 20 °C and analyzed within 2 days. One mL was dispensed into an eppendorf tube (1.5 mL, Brinkmann Instruments Inc.) and centrifuged for 10 minutes at 14,000 rpm (Eppendorf microcentrifuge model 5415C): 0.5 mL of the supernatant was then transferred into an auto-sampler polyvial for ion chromatography (IC; Dionex Series DX-320, Sunnyvale, CA) analysis. IC samples were stored at 4°C and analyzed within 3 days.

Push-Pull Test Data Analysis

For the push-pull tests, *in situ* rates were calculated for FE production rate by reductive dechlorination of TCFE by removing the effects of transport processes from measured aqueous concentrations using a forced mass balance (FMB) technique (17). 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 complete reductive dechlorination of TCFE, the end transformation product is FE. The FMB technique consists of first multiplying the measured aqueous phase concentrations of DCFE, CFE, and FE in extraction-phase samples by the estimated retardation factor (R) for each

analyte (17). In this manner, the analyte's total concentration (aqueous and sorbed) is obtained.

$$[DCFE]_{aq+s} = [DCFE]_{aq} R_f^{DCFE} \quad (4.1)$$

$$[CFE]_{aq+s} = [CFE]_{aq} R_f^{CFE} \quad (4.2)$$

$$[FE]_{aq+s} = [FE]_{aq} R_f^{FE} \quad (4.3)$$

The retardation factors were computed from K_{om} values estimated by the Estimations Program Interface Suite (30), the measured fraction of organic matter in the PAM (f_{om} = 0.001), and the bulk density (1.77) and porosity (0.39) of Hanford sediment: the K_{om} values for DCFE, CFE, and FE of 33.5, 12.2, and 5.99, respectively. The total concentration in aqueous and sorbed phases, $[DCFE]_{aq+s}$, $[CFE]_{aq+s}$, and $[FE]_{aq+s}$, is then divided by a transport-process adjustment factor, $\Sigma/\Sigma o$(16) to obtain an FMB-adjusted concentration for DCFE, CFE, and FE for each extraction-phase sample.

$$[DCFE]_{FMB} = \frac{[DCFE]_{aq+s}}{\Sigma / \Sigma_o} \quad (4.4)$$

$$[CFE]_{FMB} = \frac{[CFE]_{aq+s}}{\Sigma / \Sigma_o} \quad (4.5)$$

$$[FE]_{FMB} = \frac{[FE]_{aq+s}}{\Sigma / \Sigma_o} \quad (4.6)$$

The adjustment factor, Σ/Σ_o, is the summed FMB molar concentration (aqueous + sorbed) of DCFE, CFE, and FE in a sample (Σ), divided by the summed (Σ_o) FMB molar concentrations (aqueous + sorbed) for DCFE, 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 in order to obtain zero-order

transformation rates. Progress curves for FE will appear greater than the aqueous injectate concentrations as a result of the FMB data treatment, which takes into account FE in the aqueous and solid phases. Because FE is the only known end product of TCFE under anaerobic conditions, the appearance rate of FE is proportional to the disappearance rate of TCFE. An error analysis conducted by Hageman et al.(17) indicated that the actual *in situ* rates obtained using the FMB technique are within 10% of the true rates.

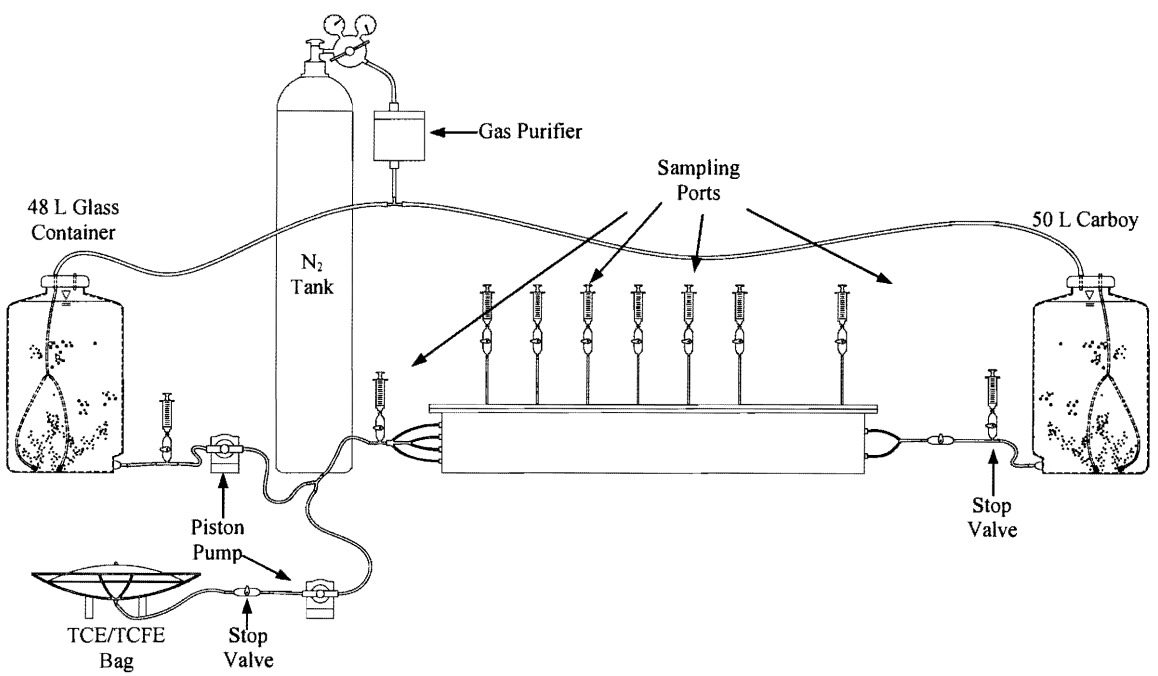

Figure 4.2. Laboratory used for physical aquifer model experiments.

RESULTS

Microcosm Study

Microcosm study was conducted to select optimum conditions for bioaugmentation: a dilution factor of the Evanite culture, an electron donor, and an addition of diluted media. Complete conversion of ~60 μM TCE to ethene was observed within 96 days in butanol fed microcosms prepared with 10 mL of cells (Fig 4.3a); the rates of TCE reductive dechlorination by addition of 10 mL (8%) of cells increased statistically by ~3.4 as compared to by addition of 1mL of cells. Lactate-fed microcosms receiving 10 ml of cells produced vinyl chloride and ethene in the same period (data not shown). *cis*-DCE (> 35 μM) was the major product in microcosms receiving 1 ml and 0.1 ml of cells in both butanol and lactate fed microcosms; slow production of VC (<10 μM) and ethene (<5 μM) were detected after the 2nd addition of electron donor (data not shown). Loss of between 20 ~ 30% of TCE mass in control bottles was attributed to loss of TCE through butyl-rubber septas (Fig 4.3A, B). Lactate was metabolized to propionate and acetate and butanol was metabolized to butyrate and acetate (data not shown). Small decreases in TCE concentration and no TCE metabolite formation were observed in non-bioaugmented microcosms (Fig 4.3A, B). The microcosms containing 5% fresh media showed relatively higher rates of reductive dechlorination of TCE compared to microcosm bottles containing no fresh media (data not shown). For optimum conditions for bioaugmentation, these factors obtained by the microcosm study: butanol for an electron donor, 5% media, and more than 8% of the Evanite culture should be injected.

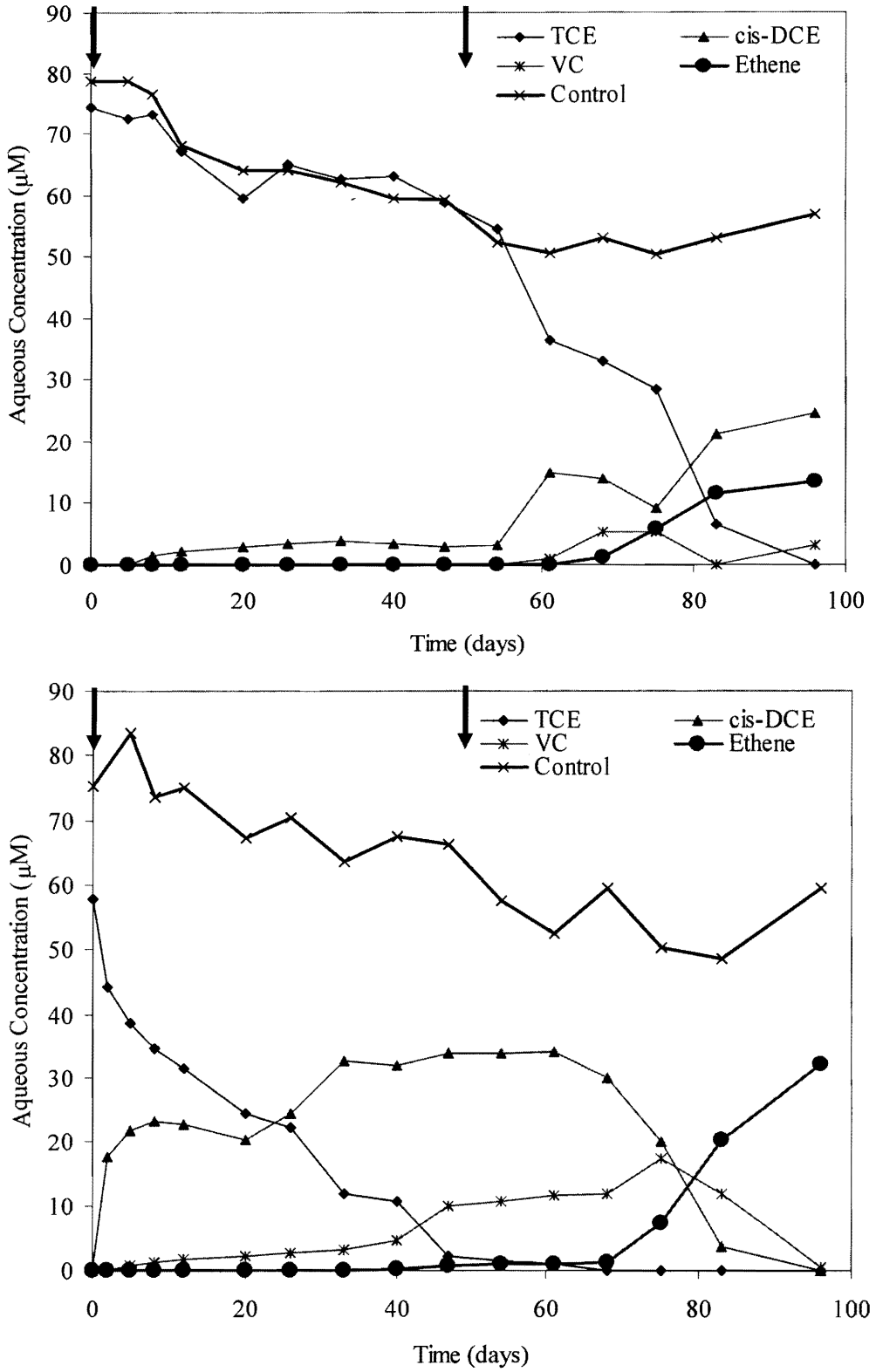

Figure 4.3. Reductive dechlorination of TCE and its metabolites in butanol fed microcosms: (A) 1 ml and (B) 10 ml of Evanite culture. Arrows show times of butanol additions.

Bioaugmentation in the PAM

Previous bioaugmentation studies (13, 14) have used cultures containing *Dehalococcoides* spp. However, these studies did not confirm cell transport during bioaugmentation by direct microscopic observations. For successful bioaugmentation, direct confirmation of cell transport must be included. Cell counting ranged 0.34-0.57 (normalized to C/C_0) of injected bioaugmented cells was observed during bioaugmentation (Fig 4.4): C, the measured cell concentration at each port, and C_0, the injected cell concentration, respectively. The background cells before the injection were less than 0.0026 compared to bioaugmented cells (data not shown). The injected bioaugmentation cells were 1.06×10^7 cells/mL and cell counts ranged from 1.65×10^4 to 2.71×10^4 cells/mL at ports 5 and 8 before bioaugmentation (data not shown); the morphology of detected cells before bioaugmentation was rod and bigger size than the bioaugmented cells. Note that the bioaugmented culture transported throughout the PAM at the sampling port 8, the farthest from the inlet port (Fig 4.4).

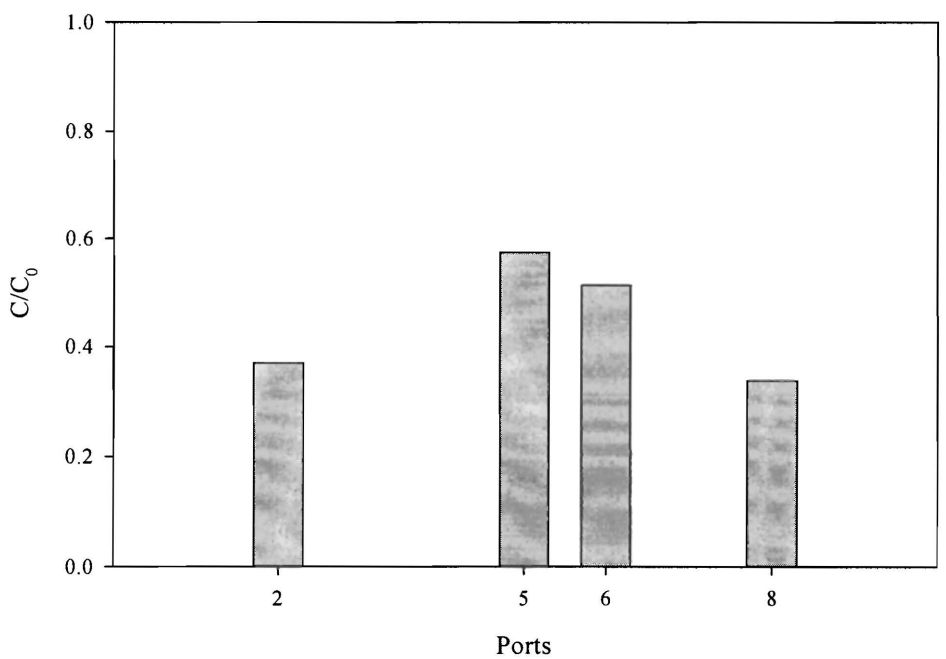

Figure 4.4. Cell counts after bioaugmentation indicating nonuniform distribution of cells.

TCE/TCFE Transport Test

Transport test of TCE/TCFE was conducted to determine the relative transport behavior of injected TCE and TCFE after bioaugmentation. TCE and TCFE transport was retarded relative to bromide as seen in the delayed arrival of these solutes relative to the coinjected bromide tracer (Fig 4.5A-D). For example, injected bromide reached C/C_0 = 0.5 at 276 minutes at port 5 compared to TCE at 378 minutes and TCFE at 411 minutes, respectively (Fig 4.5C). The calculation of retardation factor was performed by comparing the elapsed times between TCE, TCFE and bromide when $C/C_0 = 0.5$

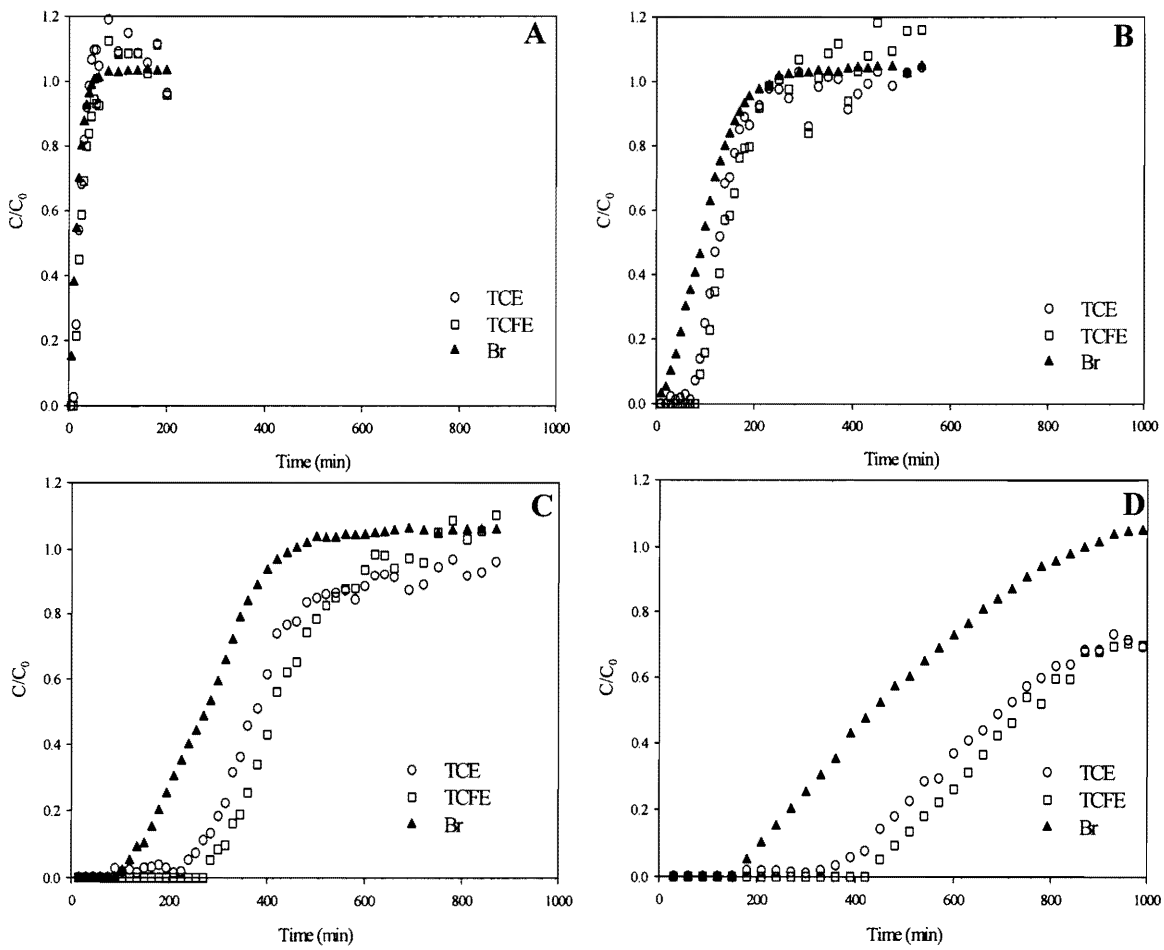

Figure 4.5. Injection phase breakthrough curves for PAM transport test showing retarded transport of TCE and TCFE relative to coinjected bromide tracer: (A) port 1, (B) port 3, (C) port 5, and (D) port 8.

The computed retardation factors were 1.42 for TCE and 1.59 for TCFE compared to the bromide tracer at port 1. It is noted that TCFE is more retarded than TCE during the injection phase and should be considered about its dechlorination products for retardation factors during push-pull test using the equations 4.1-4.6.

Activity Test at Stop-Flow

TCE Increased rates and extent of TCE and TCFE reductive dechlorination during the activity test indicated that the PAM maintained the optimum conditions for enhanced complete dechlorination of chlorinated ethenes after bioaugmentation. Ethene and FE production were observed in all ports indicating survival and activity of the bioaugmented culture in the PAM. The injections followed by stop-flow of TCE/TCFE were conducted to check reductive dechlorination activity under stop-flow condition in the bioaugmented PAM; 4 injections of TCE alone, an injection of TCFE alone, and an injection of TCE/TCFE together resulted in all CAHs being completely transformed at all ports. Data for port 1 and 8 are shown as examples (Fig 4.6A,B). The 1st TCE (24-56 μM) injection was completely transformed to ethene in 7 days at all ports (Fig 4.6A,B). The 2nd (33-165 μM) and 3rd (18-64 μM) injections of TCE were transformed to *cis*-DCE, VC, and ethene within 8 days at all ports (Fig 4.6A,B). Transformation rates ranged from 10.5 to 31.8 μM•day^{-1} and ethene production rates from 0.5 to 17.3 μM•day^{-1} (2nd injection, Table 4.2 and Fig 4.6A,B); coversion calculations of TCE to ethene were likely inaccurate due to partitioning of less-dechlorinated products, VC and ethene, into possible gas pockets within the PAM. The calculations were not performed (ND) due to varying TCE concentrations of during the incubation at several ports (2, 3, 6, and 8,

Table 4.2). The rate calculations for TCE transformation was not included for the 1st and 3rd injections due to no data points obtained under fast transformation with low TCE concentration (<50 μM); no TCE was detected after the injections and observed main transformation products were ethene for these injections (Fig 4.6A,B). Conversion of the 2nd injection of TCE to ethene was varied at each port (data not shown). The 4th injection of TCE (174-505 μM) was completely transformed within 9 days at all ports and transformation rates ranged from 32 to 85 $\mu M \bullet day^{-1}$ (Table 4.2). Ethene production rates ranged from 0.4 to 10 $\mu M \bullet day^{-1}$ (Table 4.2). The transformation rates for TCE for each addition (2nd, 4th, and TCE/TCFE coinjection) were increased during the activity test (Table 4.2). The transformation rates of TCE in the control PAM followed by stop-flow mode were 5-7 times smaller than in the bioaugmented PAM.

Injected butanol (2-10 mM) was completely transformed first to butyrate, then to propionate, and finally to acetate (Fig 4.7A,B) during the activity test. Similar products were observed at all ports. Sulfate was present in the injected media and sulfate reduction was observed (13-75 μM) in all ports. From the other studies, Adamson et al. (14) monitored activity using microcosms of indigenous sediment (controls) and effluent of the experimental setup after bioaugmentation and observed no reductive dechlorination products of PCE for the controls and *cis*-DCE (96%) and VC (4%) after 240 days of bioaugmentation for the effluent microcosms. They reported a lag time of 2 weeks with VC as the most abundant transformation product for the final 2 months of the experiment (14) while we observed ethene and FE production by complete TCE and TCFE reductive dechlorination. Lendvay et al. (13) observed complete transformation of PCE to ethene within 6 weeks after bioaugmentation in field tests, but our system indicated no lag time

for reductive dechlorination of TCE to ethene. They (13) also reported that a parallel biostimulation plot resulted in PCE to ethene conversion after a 3 month lag period compared to our control PAM that produced from TCE to cis-DCE.

Table 4.1. Summary of computed maximum zero-order transformation rates and conversion for activity tests with a stop-flow in bioaugmented PAM (mean± 95% confidence interval)

TCE or TCFE injections		Port 1	Port 2	Port 3	Port 4	Port 5	Port 6	Port 8
TCE 2nd injection	TCE transformation rate (μM•day^{-1})	10.5 ± 36.7 (0.60)	19.8 ± 23.3 (0.79)	ND	23.3 ± 27.0 (0.66)	29.8 ±15.3 (0.86)	ND	ND
	Ethene production rate (μM•day^{-1})	0.5 ± 0.6 (0.92)	ND	0.5 ± 0.4 (0.86)	4.0 ± 0.7 (0.98)	17.3 ± 11.9 (0.84)	8.2 ± 12.2 (0.70)	20.8 ± 13.2 (0.87)
TCE 4th injection	TCE transformation rate (μM•day^{-1})	32.0 ± 41.2 (0.75)	38.6 ± 54.0 (0.72)	23.7 ± 64.0 (0.61)	32.5 ± 33.7 (0.67)	84.7 ± 23.5 (0.94)	76.8 ± 30.8 (0.88)	56.3 ± 40.2 (0.80)
	Ethene production rate (μM•day^{-1})	0.4 ± 0.2 (0.94)	4.6 ± 5.2 (0.80)	1.3 ± 0.6 (0.96)	3.0 ± 1.4 (0.93)	8.1 ± 5.4 (0.85)	3.3 ± 0.6 (0.96)	10.0 ± 3.2 (0.94)
TCFE 1st injection	TCFE transformation rate (μM•day^{-1})	20.6 ± 49.7 (0.76)	6.3 ± 19.2 (0.66)	8.5 ± 26.2 (0.66)	9.0 ± 9.0 (0.83)	8.3 ± 8.0 (0.85)	7.5 ± 6.8 (0.81)	6.2 ± 3.4 (0.90)
	FE production rate (μM•day^{-1})	3.3 ± 0.4 (0.99)	5.9 ± 4.2 (0.91)	6.1 ± 4.5 (0.90)	8.3 ± 6.3 (0.90)	7.0 ± 4.3 (0.93)	6.3 ± 2.9 (0.93)	6.5 ± 1.2 (0.98)
TCE/ TCFE injection	TCE transformation rate (μM•day^{-1})	30.5 ± 6.3 (0.95)	30.2 ± 6.8 (0.94)	34.0 ± 9.5 (0.92)	36.8 ± 10.5 (0.94)	30.8 ± 8.0 (0.93)	27.3 ± 5.0 (0.96)	26.2 ± 4.8 (0.97)
	Ethene Production rate (μM•day^{-1})	4.9 ± 1.1 (0.97)	5.2 ± 3.0 (0.89)	5.8 ± 3.6 (0.87)	3.3 ± 0.6 (0.96)	6.5 ± 1.0 (0.95)	3.5 ± 0.3 (0.99)	5.7 ± 0.4 (0.99)
	TCFE transformation rate (μM•day^{-1})	38.6 ± 9.1 (0.93)	38.1 ± 8.7 (0.94)	37.0 ± 10.0 (0.90)	44.8 ± 13.9 (0.91)	36.4 ± 11.0 (0.89)	33.0 ± 6.8 (0.94)	29.0 ± 5.1 (0.97)
	FE Production rate (μM•day^{-1})	7.3 ± 0.7 (0.99)	7.1 ± 3.6 (0.91)	7.7 ± 4.2 (0.90)	4.7 ± 1.3 (0.93)	5.2 ± 0.9 (0.95)	5.0 ± 0.6 (0.97)	7.1 ± 1.2 (0.94)

ND: No calculations were obtained due to R^2 <0.6

The 1st and 3rd injections were not included because TCE aqueous concentration was below detection during the 2nd or 3rd sampling period after the injections.

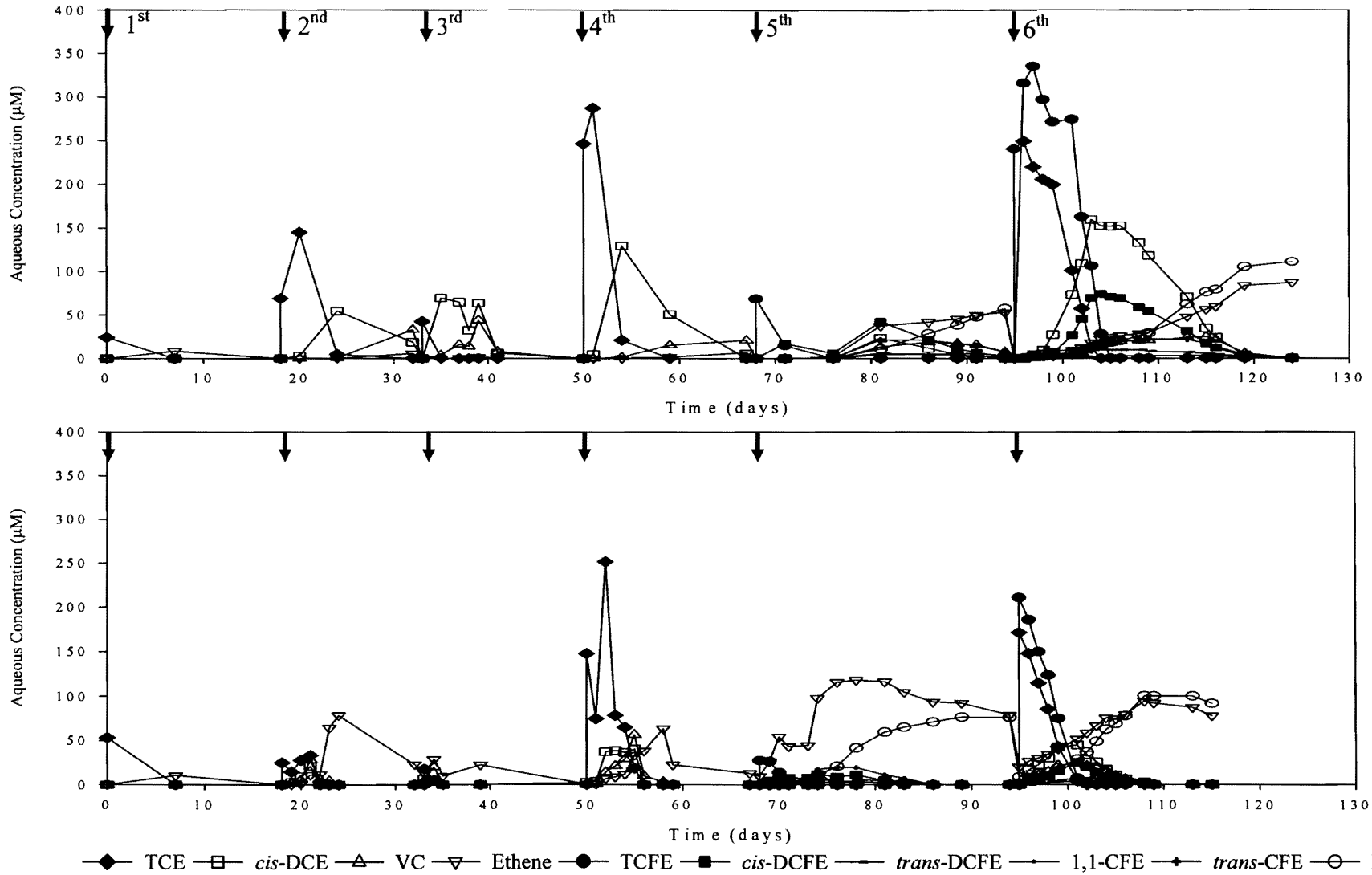

Figure 4.6. Reductive dechlorination of TCE and TCFE in bioaugmented PAM for (A) port 1 and (B) port 8. Arrows indicate additions of TCE (1st to 4th), TCFE (5th), or both (6th)

TCFE The 1st injection of TCFE (27-75 μM) was completely transformed to FE in 24 days at all ports (Fig 4.6A,B). Transformation rates ranged from 6.2 to 20.6 $\mu M \bullet day^{-1}$. The 1st TCFE injection was transformed to *cis*-, *trans*-DCFE, 1,1-, and *trans*-CFE, and FE. TCFE transformation rates were 2-10 times smaller than the TCE transformation rates for the 4th TCE injection (Table 4.2) and it might be related to adjustment of the bioaugmented culture to TCFE because the bioaugmented PAM has never exposed to TCFE before. A microcosm study (Lee et al. 2006) also reported that transformation rates of TCFE initially slower than those of TCE although the rates of TCFE were faster after more substrate additions. TCFE transformation rates increased after the injection of TCE/TCFE together as compared to the 1st injection of TCFE indicating dechlorinating population adjusted for TCFE (Table 4.2).

Both TCE and TCFE disappeared after 9 days and complete transformations were observed TCE and TCFE to ethene and FE, respectively. Transformation rates for TCE and coinjected TCFE were increased as compared to those of the previous injections and calculated at 26-37 $\mu M \bullet day^{-1}$ for TCE and 29-45 $\mu M \bullet day^{-1}$ for TCFE (Table 4.2). The rates of TCE and TCFE transformation were similar (within 8 to 21%) for the simultaneous addition of similar concentrations for the two compounds (Table 4.2, Fig 4.6A,B). The results indicated that TCFE could be useful to assessing *in situ* rates of TCE transformation and agreed with the other references (15-21)

Sulfate reduction also was observed in the control (non-bioaugmented) PAM (Fig 4.8C,D) along with transformation of injected butanol to butyrate, acetate, and some propionate within 35 days (Fig 4.8E, F).

TCFE transformation rates were 50-80 times smaller in the control PAM as compared to the bioaugmented PAM (data not shown). No TCE or TCFE transformations were observed at port 2, but transformation to *cis*-DCE and *cis*-DCFE and associated chloride release were observed at ports 1 and 3 (Fig 4.8A). A longer incubation time would be required to more fully assess CAHs transformations in the control PAM (Fig 4.8B). Complete degradation of TCE to ethene in all ports within 7 days was observed in the bioaugmented PAM but not in the control indicating successful distribution of active dehalogenating microorganisms.

To sustain anaerobic dechlorinating activity in our study, addition of an electron donor was essential following bioaugmentation and butyrate, propionate, acetate production was observed. The non-bioaugmented control PAM exhibited sulfate reducing conditions, but showed significantly slower TCE and TCFE transformation ability with only *cis*-DCE and *cis*-DCFE produced at port 1 and 3, suggesting that sulfate reducing microorganisms co-existed with the dechlorinators in the control PAM.

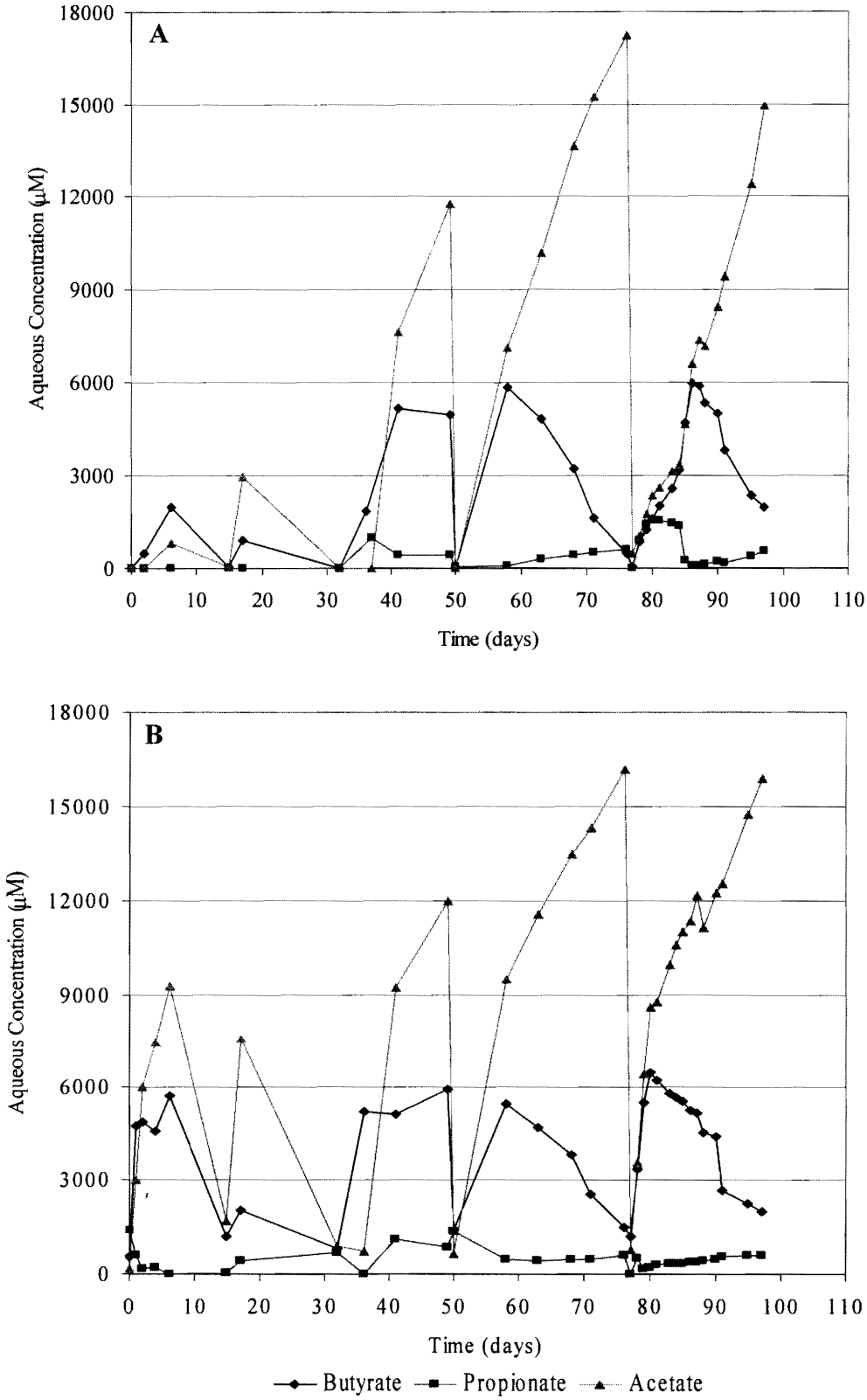

Figure 4.7. Fermentation products of butanol detected at (A) port 1 and (B) port 8.

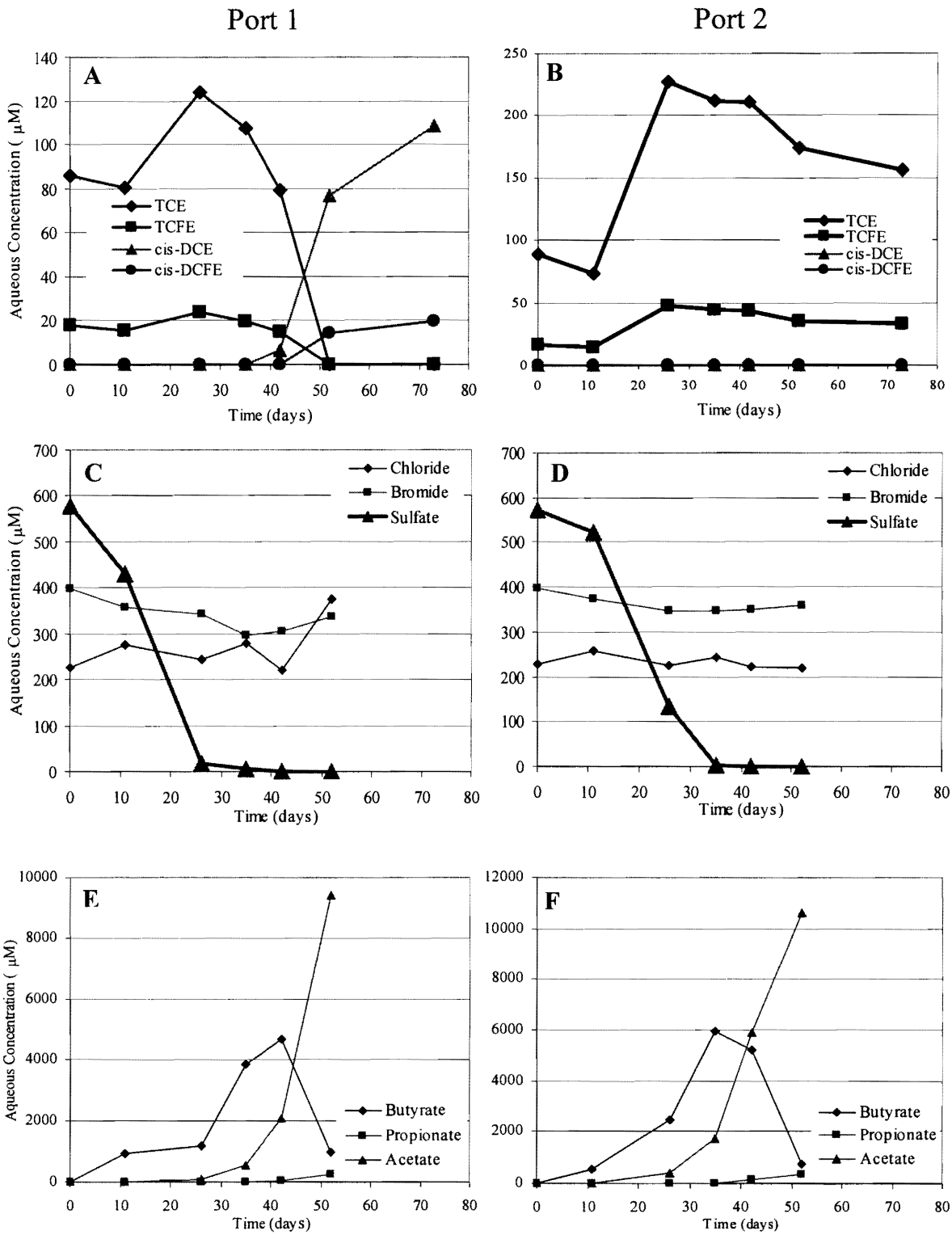

Figure 4.8. Results of activity test in non-bioaugmented PAM for port 1 (left) and port 2 (right): TCE and TCFE (A, B), inorganic anions (C, D), and fermentation products of butanol (E, F).

Push-Pull Activity Test

Push-pull test was conducted to (a) determine *in situ* transformation rate of TCFE to FE, and (b) compare the in situ TCFE transformation rate of push-pull test with those rates obtained from the activity test at stop-flow. Mass balance calculation was performed by integrating injection and extraction flow with measured concentrations of chlorinated ethenes during the push-pull test. Injected TCFE was completely transformed to FE at all ports during a push-pull test conducted after the activity tests (Fig 4.9A,B). TCFE transformation products detected at port 1 including: *cis*-DCFE, *trans*-DCFE, 1,1-CFE, *trans*-CFE, and FE (Fig 4.9A,B). Computed FE production rates by forced mass balance (FMB) was 60±6 $\mu M \bullet day^{-1}$. Computed mass balances for TCFE and its transformed products were obtained at 47%. The value of 47% indicated possible partitioning of TCFE dechlorination products such as FE gas by partitioning or diffusion out of the bioaugmented PAM during the push-pull test. Computed mass balances for bromide was 85%.

Increased FE production rate was obtained as compared to the activity test while the value of 60±6 $\mu M \bullet day^{-1}$ was in the range of TCFE transformation rates (Table 4.2). The FE production rate (60±6 $\mu M \bullet day^{-1}$) was relatively high compared to the values obtained by the activity test at stop-flow for FE production rate (4.7-7.7 $\mu M \bullet day^{-1}$), but the activity test involved in longer test period (>3 weeks) with a stop-flow as compared by push-pull test (< 4days). Thus, transformation rates of TCFE by push-pull test is more accurate than the activity test because of a short test period while results in less FE loss (< 4days). From figure 4.9B, TCFE was not included because TCFE was transformed completely and not detected after 32.5 hrs of the rest phase. To compare FE rates with

another field test, Ennis et al. (21) reported that the value of FE production rate at 1.15±0.27 $\mu M \bullet day^{-1}$ in the period of 20 days at a TCE-contaminated aquifer. Our result (60 ± 6 $\mu M \bullet day^{-1}$) demonstrated that bioaugmentation could enhance the rate of complete dechlorination of chlorinated ethenes for the slow dechlorination of Ennis et al. (21).

To compare the other bioaugmentation studies, computed mass recoveries (14) resulted in less than 1% of ethene formation indicating incomplete transformation of chlorinated ethenes to ethene. Contrary in our study, TCFE transformation products observed during the push-pull test and FE was the major product indicating complete transformation of TCFE to FE.

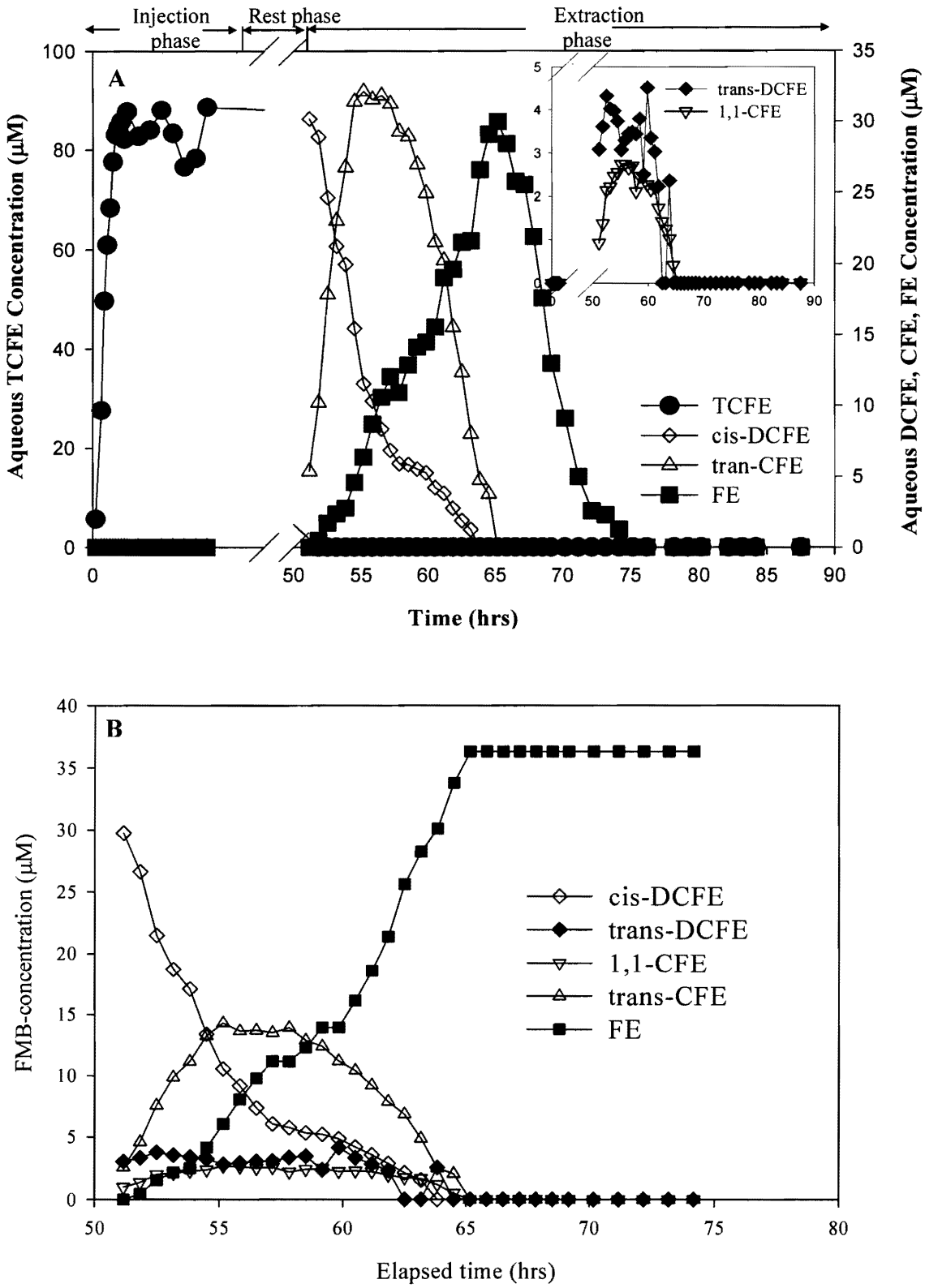

Figure 4.9. Results of push-pull test conducted in bioaugmented PAM at (A) port 1 and (B) transformed view by FMB during the extraction phase. The breaks indicated the 32.5 hrs of rest phase.

To our knowledge, this is the first study to demonstrate the capability of the single-well push-pull test as a tool to evaluate the effectiveness of bioaugmentation for remediating TCE contaminated sediments under laboratory conditions.

In CAH contaminated aquifer systems in the subsurface, this test has important implications with respect to the feasibility of *in situ* anaerobic bioremediation technology. The addition of dehalogenating culture to establish a treatment zone near sources of CAHs reduces start-up time and eliminates costs associated with repeated inoculations of new cultures.

The push-pull tests have the benefit of providing *in situ* information on microbial activity that can be specifically related to TCE transformation. These methods are developed to determine the rates *in situ* and are obtained from a larger volume of aquifers. Thus, the rates obtained are likely more representative than those obtained from sediment microcosm experiments. Field testing with push-pull test is needed to assess the effectiveness of bioaugmentation at CAH contaminated sites in future.

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CHAPTER 5

Evaluation of Bioaugmentation to Remediate a TCE Contaminated Physical Aquifer Model: Numerical Modeling

ABSTRACT

Reductive dechlorination of trichloroethene (TCE) and trichlorofluoroethene (TCFE) at aqueous concentration of ~ 300 μM was enhanced by adding dehalogenating microorganisms (bioaugmentation) to a saturated sediment system in a small scale physical aquifer model (PAM). The objectives of this study were (1) to evaluate a simplified method for estimating retardation factors for injected solutes and bioaugmented microorganisms using "push-pull" test injection phase breakthrough curves and (2) to identify factors that may have caused observed differences in transformation rates during an activity test using Michaelis-Menten kinetics and a push-pull test. The bioaugmented microorganisms were effectively transported through Hanford sediment. The estimated retardation factor was 1.33. A numerical simulation predicted cell transport in the PAM as far as ~ 60 cm from the injection/extraction ports. This was qualitatively confirmed by cell counts obtained during bioaugmentation, but cells were distributed nonuniformly. The transport test indicated that TCE and TCFE transport was relatively retarded compared to coinjected bromide tracer, with retardation factors ranging from 1.33-1.62 for TCE and from 1.44-1.70 for TCFE.

The modeling simulation of Michaelis-Menten kinetics for the activity test was well matched for reductive dechlorination rates for TCE and less dechlorinated ethenes using the previous published values of k_{max} and K_s of chlorinated ethenes by Yu et al. (2005); the model match indicated that the bioaugmented microorganisms kept the same transformation capacity as the original source, Evanite culture (Yu et al., 2005) over 4 months in the PAM. A numerical simulation resulted in the simple first order TCFE reductive dechlorination rate to FE production (~1 day^{-1}) using STOMP code (2002) and

the value of FE production rate was in the range of the transformation rates of TCFE during the activity test. The model simulations indicated that partitioning of chlorinated ethenes into trapped gas pocket might be one possible reason for the mass loss of ~50% of chlorinated ethenes during the push-pull test and the estimated value of ~30% of gas saturation in the PAM by models could be feasible because water still flows with one third of the pore space with gas saturated. Furthermore, the PAM itself involved in slow release of the chlorinated ethenes during the activity test and the push-pull test.

INTRODUCTION

Contamination of soil and groundwater with chlorinated ethenes is a widespread and serious environmental problem. Several laboratory and field studies have demonstrated that destruction of chlorinated ethenes via reductive dechlorination can be achieved by stimulating indigenous microorganisms and/or by adding dehalogenating microorganisms (Bagley and Gossett, 1990; Ellis et al., 2000; Maymó-Gatell et al., 1999). Reductive dechlorination processes require or are favored by the following: (1) microorganisms specific to the desired degradation pathway, (2) absence of dissolved oxygen, (3) low redox potential, (4) near neutral pH, (5) presence of sufficient electron donors, (6) low to moderate dissolved hydrogen levels (higher hydrogen levels may favor methanogenesis and sulfidogenesis), and (7) low nitrate and sulfate levels. Enhanced bioremediation of chlorinated compounds is based on creating or enhancing these conditions.

Recently, bioaugmentation has been investigated in an effort to increase the extent and rate of reductive dechlorination ability of existing dechlorinating populations (Dybas

et al., 2002; Ellis et al., 2000; Harkness et al., 1999; Lendvay et al., 2003; Major et al., 2002). Bioaugmentation involves injecting selected exogenous microorganisms capable of degrading the target chemicals directly into the contaminated zones. For example, Lendvay et al. (2003) reported successful bioaugmentation with a *Dehalococcoides*-containing PCE-to-ethene dechlorinating inoculum that resulted in stoichiometric dechlorination of both sorbed and dissolved chloroethenes to ethene using a recirculating groundwater system. Dybas et al. (2002) developed a three-dimensional solute transport simulation to evaluate carbon tetrachloride distribution prior to calculating removal efficiency in a field study. The simulation included advection, dispersion, and linear equilibrium sorption/desorption to help calculation of base addition prior to bioaugmentation. Duba et al. (1996) performed a computer simulation to compare with a tracer test and found the aquifer was highly heterogeneous.

Push-pull tests have been used to obtain *in situ*, quantitative information on microbial activity and contaminant transformations in the subsurface (Istok et al., 1997) including methane oxidation (Urmann et al., 2005), aerobic cometabolism (Kim et al., 2004), anaerobic transformations of petroleum hydrocarbons (Reusser et al., 2002), reductive precipitation of radionuclides (Senko et al., 2002), and anaerobic transformations of chlorinated solvents (Hageman et al., 2001, 2004; Field et al., 2005; Ennis et al., 2005). Furthermore, push-pull tests to evaluate first-order and zero order *in situ* reaction rates using experimental data with/without a numerical model have been presented (Snodgrass and Kitanidis, 1998; Haggerty et al., 1998; Schroth et al., 1998; Istok et al., 1997; Reinhard et al., 1997).

For examples of model simulations, Reinhard et al. (1997) demonstrated that push-pull tests to investigate *in situ* anaerobic biotransformation of BTEX under enhanced nitrate- and sulfate reducing conditions by using field data and numerical modeling. A numerical solute transport model was used to interpret data from a push-pull test conducted in a gasoline-contaminated aquifer and first-order and zero-order degradation rates of BTEX were obtained (Snodgrass and Kitanidis, 1998). Haggerty et al. (1998) described a simplified method of push-pull test data analysis and used it to estimate first-order rates of aerobic respiration and denitrification. Schroth et al. (1998) used push-pull tests to quantify spatial variability in rates of aerobic respiration and denitrification *in situ* in a gasoline-contaminated aquifer. Hageman et al. (2003) used field data and numerical modeling to demonstrate that the push-pull test with a forced mass balance technique for dilution-adjustment was a valid method for estimating transformation rates of sorbing solutes (e.g., chlorinated ethenes).

Lee et al. (chapter 4, 2006) conducted laboratory push-pull tests in a physical aquifer model (PAM) to study the effects of bioaugmentation with a dechlorinating culture on rates of TCE and TCFE transformation. The culture was enriched from groundwater and sediment aseptically obtained from a TCE-contaminated site, the Evanite site, in Corvallis, Oregon (Yu et al., 2005). The Evanite culture, containing *Dehalococcoides*-like microorganisms, has been extensively studied and described elsewhere (Pon and Semprini 2005, Yu et al., 2005). After bioaugmentation, monitoring repeated TCE and/or TCFE injections following a stop-flow ("the activity test") were performed for complete TCE and TCFE transformation. The push-pull test followed to quantify *in situ* rates of TCFE reductive dechlorination after the activity test. The extent

and reductive dechlorination rates of TCE/TCFE increased over 4 months, but nonuniform recovery of final reductive dechlorination products of TCE and TCFE, Ethene and FE, was observed. Furthermore, the push-pull test resulted in greater FE production rates during TCFE reductive dechlorination than were observed during a "stop-flow" activity test (chapter 4, 2006) conducted in the same PAM.

In this study, we conducted numerical simulations of the activity test for stop-flow and the push-pull test with flow conditions. The objectives were (1) to evaluate a simplified method for estimating retardation factors for injected solutes and bioaugmented microorganisms using "push-pull" test injection phase breakthrough curves, (2) to identify whether bioaugmented Evanite culture kept the same transformation rate constants (Michaelis-Menten kinetics) as determined by Yu et al. (2005), to verify *in situ* rates of TCFE reductive dechlorination rates of push-pull tests by numerical modeling, and (3) to investigate possible reasons for the nonuniform recovery of ethene and FE during the activity test and the push-pull test.

METHODS

Microbial Transport Test in a Sediment Packed Column

A column test (5cm diameter × 34 cm length) was performed using Hanford sediments to assess the transportability of the bioaugmentation culture. Air-dried sediment was packed into the glass column; Hanford sediment was used for packing. Cell suspensions were injected at the same flow rate as the bioaugmentation PAM test (Chapter 4, Lee, 2006) to simulate the pore water velocities in the PAM (11 cm/min). The flow rate to achieve this pore water velocity was 85 mL/min. The mathematical model

which describes the transport of a solute in an incompressible medium is usually referred to as the advection-dispersion equation; the transport of bioaugmented cells in the porous medium was described by the advection-dispersion equation of 5.1 (Yates and Yates, 1988). For one-dimensional transport, the equation is

$$\frac{\partial C}{\partial t} = \frac{D_x}{R}\frac{\partial^2 C}{\partial x^2} - \frac{v_x}{R}\frac{\partial C}{\partial x} \tag{5.1}$$

where C is the microorganism or bromide concentration, (cells/mL) or (μM) respectively, t is time (min) from start of flow, v_x is the pore water velocity (cm/min), R is the retardation factor interpreted as the effect of slowing down the entire process of bioaugmented cells migration, and D_x is the dispersion coefficient in the x-direction which is defined as

$$D_x = \alpha_x v_x + D^* \tag{5.2}$$

where α_x is a characteristic property of the porous medium known as the dynamic dispersivity or simply as dispersivity (cm), $\alpha_x v_x$ is the mechanical dispersion, and D^* is the molecular diffusion for the solute in the porous medium (cm^2/min).

For the bromide tracer, R = 1, normalized concentrations (C/C_0) for the bromide tracer were fit to the advection-dispersion equation using CXTFIT 2.1 (Toride et al., 1999) to estimate the effective dispersivity of the packed sediment. CXTFIT 2.1 minimizes the sum of the squared differences between observed and fitted concentrations using the one-dimensional form of the advection-dispersion equation 5.1. Using the dispersivity determined from the bromide tracer test, an estimate of the retardation factor for bioaugmented cells was found by fitting the normalized cell concentration breakthrough curve for the bioaugmented cells using CXTFIT 2.1.

The Physical Aquifer Models

The PAMs were constructed of polypropylene with interior dimensions of 5 cm (width at narrow end), 50 cm (width at wide end), 125 cm (length), and 20 cm (height) in a wedge-shape to approximate the radial flow field near an injection/extraction well during field push-pull tests (Figure 5.1). The PAMs were packed with sediment from the Hanford Formation, an alluvial deposit of sands and gravels of mixed basaltic and granitic origin after removing particles > 0.5 cm in diameter by sieving. The sediment contains less than 0.001 wt % organic matter and has a particle density of 2.9 g/cm^3. The porosity and bulk density of the packed sediment were 0.39 and 1.77 g/cm^3, respectively. Total internal volume was 69 L and the pore volume was 27 L. Further details about the PAM setup, experimental design, and analytical methods are provided elsewhere (Chapter 4, Lee, 2006).

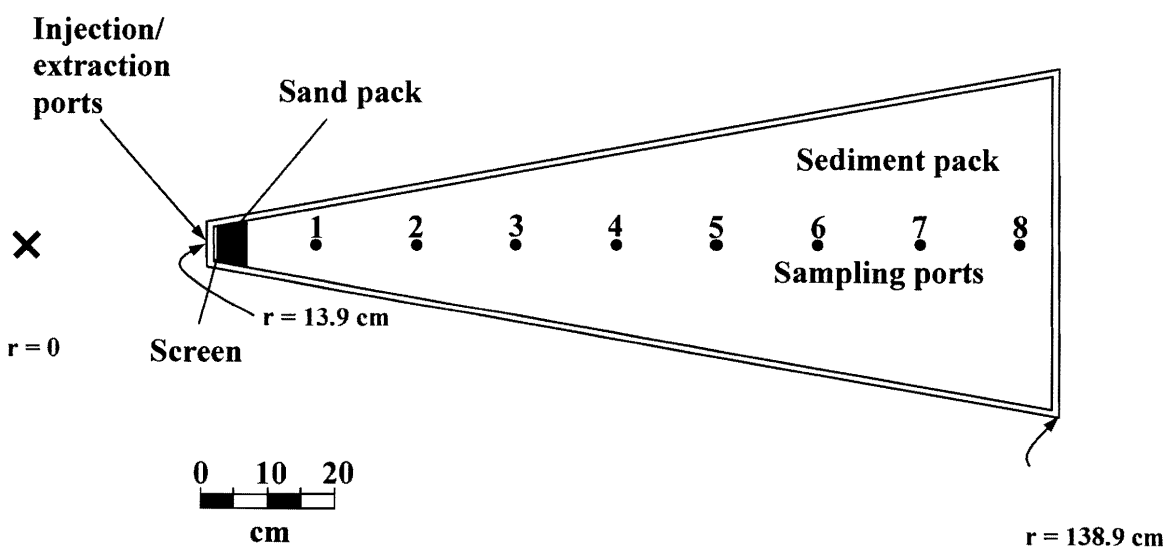

Figure 5.1. Physical aquifer models used in bioaugmentation experiments (plan view)

Transport in the PAM

A transport test was conducted to quantify TCE and TCFE transport behavior in the physical aquifer model (PAM). The test was conducted by injecting 43 L of tap water containing bromide (~1100 μM), TCE (~155 μM), and TCFE (~30 μM) (Fig 5.1). The test solution was injected at 43 mL/min. Water samples were collected from sampling ports 1, 3, 5, and 8 and analyzed for bromide, TCE, and TCFE concentrations (Fig 5.1).

The one-dimensional (radial) form of the advection-dispersion equation in the vicinity of an injection/extraction well can be written as (e.g., Hoopes and Harleman 1967)

$$\frac{\partial C}{\partial t} = \alpha_L |v| \frac{\partial^2 C}{\partial r^2} - v \frac{\partial C}{\partial r} \tag{5.3}$$

where α_L is longitudinal dispersivity, v is the pore water velocity, and r is radial distance.

PAM transport test data were interpreted using an approximate analytical solution to equation 5.3 (Schroth et al., 2001). The solution gives normalized concentration (C/C_0) as a function of time and radial distance for the injection phase, the solution is

$$\frac{C}{C_0} = \frac{1}{2} erfc\left\{ \left(r^2 - \hat{r}_{inj}^2\right) \Big/ \left[\frac{16}{3} \alpha_L \left(\hat{r}_{inj}^3 - r_w^3\right) \right]^{1/2} \right\} \tag{5.4}$$

where r_w is the injection/extraction well radius and $\hat{r}_{inj}$ (defined here as the location where $C/C_0 = 0.5$) is obtained by

$$\hat{r}_{inj} = \sqrt{\frac{Q_{inj} t_{inj}}{\pi b n R} + r_w^2} \tag{5.5}$$

where Q_{inj} is the pumping rate during the injection phase, t_{inj} is the time since injection began, b is the saturated thickness, n is the porosity, and R is the retardation factor. At

the end of the injection phase, the frontal position attains a maximum value $\hat{r}_{max}$ which is the maximum radial distance traveled by the $C/C_0 = 0.5$ tracer front at the end of the injection phase (referred as the radius of influence of the tracer) by

$$\hat{r}_{max} = \sqrt{\frac{V_{inj}}{\pi bnR} + r_w^2} \tag{5.6}$$

where the total volume injected $V_{inj} = Q_{inj}T_{inj}$ and T_{inj} is the duration of the injection phase.

Sampling port data from the injection phase were analyzed by using a minimized least-squares procedure to fit equation 5.4 to the normalized bromide data to obtain an estimate for α_L. Then another minimized least-squares procedure was used to fit equation 5.4 to the normalized TCE and TCFE concentrations to obtain estimates for R using the value of α_L estimated from the bromide data. The retardation factor was then computed using

$$R^* = \left(\frac{\hat{r}_{max,\ Bromide}}{\hat{r}_{max,\ TCE}} \right)^2 \tag{5.7a}$$

and

$$R^* = \left(\frac{\hat{r}_{max,\ Bromide}}{\hat{r}_{max,\ TCFE}} \right)^2 \tag{5.7b}$$

Dehalogenation Activity Tests

The dehalogenating activity of halogenated ethenes was monitored in the PAM after bioaugmentation over ~ 4 months. The transformation rate of a given chlorinated ethenes can be described using Michalis-Menton kinetics (Eq 5.8-11, Fennell and Gossett 1998, Yu et al., 2005). The change in concentration of chlorinated ethenes and ethene for

ports 1-6, and 8 from day 95 to 124 after bioaugmentation was used for reductive dechlorination rates using Michaelis-Menten kinetics. The injection of TCE was followed by a period of no-flow to check the dechlorinating activity of the bioaugmented PAM. Port 7 was not sampled because the sampling line on port 7 was removed during the PAM construction and the port 7 was 15 cm apart from ports 6 and 8 (Figure 5.1). A numerical model of subsequential transformation and production of chlorinated ethenes was performed using Michaelis-Menten kinetics and kinetic parameters previously determined for the bioaugmentation culture (Pon and Semprini, 2004, Yu et al., 2005). The following equations were used to model chlorinated ethene transformation in the PAM;

$$\frac{dC_{TCE}}{dt} = \frac{-k_{\max,TCE} XC_{TCE}}{K_{S,TCE} + C_{TCE}} \tag{5.8}$$

$$\frac{dC_{DCE}}{dt} = \frac{k_{\max,TCE} XC_{TCE}}{K_{S,TCE} + C_{TCE}} - \frac{k_{\max,cis-DCE} XC_{cis-DCE}}{K_{S,cis-DCE} + C_{cis-DCE}} \tag{5.9}$$

$$\frac{dC_{VC}}{dt} = \frac{k_{\max,cis-DCE} XC_{cis-DCE}}{K_{s,cis-DCE} + C_{DCE}} - \frac{k_{\max,VC} XC_{VC}}{K_{S,VC} + C_{VC}} \tag{5.10}$$

$$\frac{dC_{Ethene}}{dt} = \frac{k_{\max,VC} XC_{VC}}{K_{S,VC} + C_{VC}} \tag{5.11}$$

where C_{TCE}, $C_{cis\text{-}DCE}$, C_{VC}, C_{Ethene} are the aqueous concentration (μmol/L) of TCE, *cis*-DCE, VC, and Ethene, respectively. $k_{max,TCE}$, $k_{max,cis\text{-}DCE}$, and $k_{max,VC}$ are the maximum

specific dechlorination rate (μmol/mg of protein/day) of TCE, *cis*-DCE, and VC, respectively. X is the dehalogenating biomass concentration (mg of protein/L), and $K_{s,TCE}$, $K_{s,cis\text{-}DCE}$, $K_{s,VC}$ are the half-velocity coefficients (μmol/L) for TCE, *cis*-DCE, and VC, respectively. The model included cis-DCE and VC as intermediates in TCE dechlorination to ethene; 1,1-DCE and *trans*-DCE were excluded because 1,1-DCE and *trans*-DCE were not detected during the activity tests. The biomass was calculated by using the k_{max} value for TCE (=125 μmol/mg of protein/day from Yu et al., 2005) and matching the TCE transformation rate by adjusting initial biomass values. Furthermore, the values of initial biomass at each port (0.005-0.05 mg of protein) were used to simulation for TCE transformation using k_{max} values: 13.8, *cis*-DCE, 8.1, VC, respectively, values obtained from Yu et al. (2005). The k_{max} value of *cis*-DCE was adjusted from 13.8 (the original value of Yu et al., 2005) to 6.0 in order for model fit the simulations to fit the data obtained at each sampling port. The K_s values used were those obtained from Yu et al. (2005): 1.8, TCE, 1.8, cis-DCE, and 62.6, VC, respectively. The model simulation was performed using the k_{max} and K_s values (Yu et al., 2005) by fitting the equations 5.8-11 to measured concentrations using STELLA Research 5.0 (High Performance Systems, Lebanon, NH). Note that the only changed input value for the model simulation was the adjustment of k_{max} of *cis*-DCE from 13.8 (Yu et al., 2005) to 6.0.

Since the activity test for TCE reductive dechlorination lasted about 30 days, cell growth was included in the model as follows:

$$\frac{dX}{dt} = Y\frac{k_{max}C}{K_S + C} - k_d X \tag{5.12}$$

where Y is the growth yield of dechlorinating microorganisms and k_d is the decay constant of the dechlorinating microorganisms (day^{-1}). The cell growth involved in only the utilization of chlorinated ethenes at these steps: TCE to cis-DCE, cis-DCE to VC, and VC to ethene, respectively. For the simulation, values of Y (0.006 mg of protein/μmol of Cl^- dechlorinated) and k_d (0.024 day^{-1}) were obtained from the literature (Fennell and Gossett, 1998). Ethene was assumed as the final product of reductive dechlorination of TCE. Further simulations were conducted including a headspace term to account for potential partitioning of the CAHs into gas-filled pore spaces. Total mass of chlorinated ethenes in the PAM to include gas-filled pores were computed using published Henry's Law constants (Gossett, 1987).

Push-Pull Test

Numerical simulations of the push-pull tests were performed with the Subsurface Transport Over Multiple Phases (STOMP) code, a fully implicit volume-integrated finite difference simulator (White and Oostrom, 2000). STOMP has been extensively tested and validated against published analytical solutions as well as other numerical codes (Nichols et al., 1997). A modeling exercise was used to quantitatively evaluate the transportability of the bioaugmentation culture using the same input values for ρ_b, n, α_L, and water velocity from the PAM (Chapter 4, Lee, 2006); the retardation factor was obtained from the column test. The governing equation for the model (one-dimensional radial form) is equation 5.3. The computational domain consisted of a line of 250 nodes with a uniform radial node spacing of Δr = 1.0 cm. Constant head and zero solute flux boundary conditions were used to represent PAM conditions beyond the radius of

influence in the PAM. Initial conditions were a constant hydraulic head for the aqueous phase and absence of CAHs for all solutes. Furthermore, solute transport, distribution, and the extent of TCFE reductive dechlorination were simulated using bioaugmented PAM sediment pack properties (presented in the Transport in the Physical Aquifer Model section in this chapter) for a range of retardation factors (R) for TCFE and its transformation products using the dispersivity from the previous transport test; these were then used in the simulation of a push-pull test for TCFE reductive dechlorination after bioaugmentation using STOMP, thus producing a series of simulated breakthrough curves. For the reductive dechlorination of the CAHs in the simulation, simple first-order kinetics used: $dC/dt = k_1 C$. The k_1 was first-order rate constants. The reductive dechlorination reactions were conducted in series with two steps: A→B→C. Note that A→B considers TCFE to DCFE and B→C equals DCFE to FE at the end.

The bioaugmented PAM was a closed system, but chlorinated ethene recovery (~50%) was relatively poor compared to a tracer recovery (~85%) at port 1, the closest sampling port to the injection/extraction port (Fig 5.1). In addition, the use of dilution-adjusted concentrations based on concentrations of a conservative tracer like bromide (Kim et al., 2004, Haggerty et al., 1998) was not appropriate for this study because dilution by recharge water flow was not a factor. The interpretation for TCE and TCFE metabolite losses was not resolved in the previous study of Lee et al. (Chapter 4, 2006), so addition of a numerical simulation was used as an approach to investigate the mass losses. Further investigation was conducted for resolving the mass losses of chlorinated ethenes during a push-pull test after bioaugmentation by numerical modeling, gas partitioning included into Equation 5.13 for a retardation factor.

Partitioning of chlorinated ethenes between the pore water and possible trapped gas pockets present in the sediment pack was simulated for the activity test and the extraction phase of the push-pull test, by assuming equilibrium partitioning between the trapped gas and the aqueous phases. The retardation factor for a dissolved gas can be written and modified as (Fry et al., 1995 and Davis et al., 2002)

$$R_{total} = 1 + R_{partitioning} + R_{sorb} = 1 + H_{cc}\frac{\theta_g}{\theta_w} + \frac{\rho_b K_{oc} f_{oc}}{n} \tag{5.13}$$

where $R_{partitioning}$ is a retardation factor caused by partitioning of chlorinated ethenes into a gas headspace, R_{sorb} is a retardation factor caused by adsorbed species relative to the advective velocity of the water: H_{cc} is the dimensionless Henry's constant and θ_g, θ_w are respective gas and water fractions of the pore space.

The Estimations Programs Interface Suites (Syracuse Research Corporation, 2004) was used to assign K_{oc} values of 33.5, 12.2, 6.0 L/kg to DCFE, CFE, and FE, respectively. Values of f_{oc} (0.001), ρ_b (1.77 kg/L), and n (0.39) for use in equation 5.13 were selected based on measurements from previous study (chapter 4, Lee, 2006). In equation 5.13, a value of H_{cc} = 2.66 for FE was used (Pon, 2000). In order to estimate the portion of FE retardation due to FE partitioning into the trapped gas headspace, best fit R values were adjusted to match the pull phase extraction curve at port 1 using the STOMP code. Adjusted retardation factors were used to estimate the trapped gas headspace (θ_g) required to cause the retardation effect.

Force Mass Balanced Data Analysis for Ethene and FE Production Rates

For the activity test and the push-pull test, *in situ* rates of Ethene and FE production were calculated for the reductive dechlorination of TCE and TCFE to form Ethene and FE by removing the effects of transport processes from measured aqueous concentrations using a force mass balance (FMB) technique (Hageman et al., 2003). Normalizing solutes to a conservative tracer to account for test solution dilution cannot be applied to solutes whose transport may be retarded due to sorption or partitioning in the bioaugmented PAM. The result of the push-pull test for bioaugmentation (chapter 4, Lee, 2006) indicated FE retarded (R = ~2.4 by numerical modeling) compared to the bromide tracer. The bromide mass recovery of 85% indicated that the dilution is minimal. For this reason, the FMB technique was used to account for difference between reactants and products when all reactants and products are known and can be accounted for. In the case of TCE and TCFE reductive dechlorination, the final transformation products are Ethene and FE, respectively. The FMB technique consists of first multiplying the measured aqueous phase concentrations of Ethene and FE either in the activity test samples or in extraction-phase samples by the estimated retardation factor (R_{total}) for each analyte (Hageman et al., 2003) using the equation 5.13. In this manner, the analyte's total concentration (aqueous and sorbed plus partitioning) is obtained.

$$[\text{Ethene}]_{aq+s+p} = [\text{Ethene}]_{aq} R_{total}^{\text{Ethene}} \quad (5.14)$$

$$[\text{FE}]_{aq+s+p} = [\text{FE}]_{aq} R_{total}^{\text{FE}} \quad (5.15)$$

The retardation factors were computed from K_{oc} values estimated by the Estimations Program Interface Suite (2004) and partitioning by the Henry's Law constants for the equation 5.13, the measured fraction of organic matter in the PAM (f_{oc} = 0.001), and the bulk density (1.77) and porosity (0.39) of Hanford sediment: the K_{oc} values for Ethene and FE of 5.99. The production of Ethene and FE followed the changes of retardation factor of each chlorinated ethenes of interest by sorption and partitioning: the Henry's law constants for Ethene and FE at 20°C, 2.7 and 4.7, respectively. The total concentration in aqueous and sorbed plus partitioning phases, $[Ethene]_{aq+s+p}$ and $[FE]_{aq+s+p}$, is then adjusted by R_{total} (= R for Ethene or FE) using the equation 5.13. The estimates of R_{total} for Ethene and FE increased from 2.6 and 1.9 to 3.2 and 2.4, respectively using the equation 5.13 after the modeling resulted in 2.4 for R_{total} of FE; the increased value of ethene was estimated with the same ratio of FE increase.

The FMB concentrations were plotted with time to create progress curves and the linear portion of the curves was fitted using linear regression in order to obtain zero-order production rates; the zero-order curve was best fitted for the FE production during the push-pull test and this was an example for the FMB technique for sorbing/partitioning solutes. Progress curves for Ethene and FE will appear greater than the aqueous extract concentrations as a result of the FMB data treatment, which takes into account Ethene and FE in the aqueous and the solid plus partitioning phases. Because Ethene and FE is the only known end product of TCE and TCFE respectively under anaerobic conditions, the appearance rate of Ethene and FE is proportional to the disappearance rate of TCE and TCFE. An error analysis conducted by Hageman et al.(2003) indicated that the actual *in situ* rates obtained using the FMB technique are within 10% of the true rates.

RESULTS & DISCUSSION

Microbial Transport in a Sediment Packed Column

The cell suspension was effectively transported through the Hanford sediment (Fig 5.2) at a pore water velocity of 11 cm/min (= flow rate/area of column/porosity). The modeling exercise was performed using the values, ρ_b (1.77 kg/L) and n (0.39) in the method section. The estimated effective dispersivity of the sediment was 2.4 cm and the estimated retardation factor for the cell suspension was 1.33 based on the simulation of CXTFIT 2.1. The result was associated with a sample collection of the pore volumes as follows: 1, 4, and 7 pore volumes (= time at 4, 17, and 30 min in Figure 5.2).

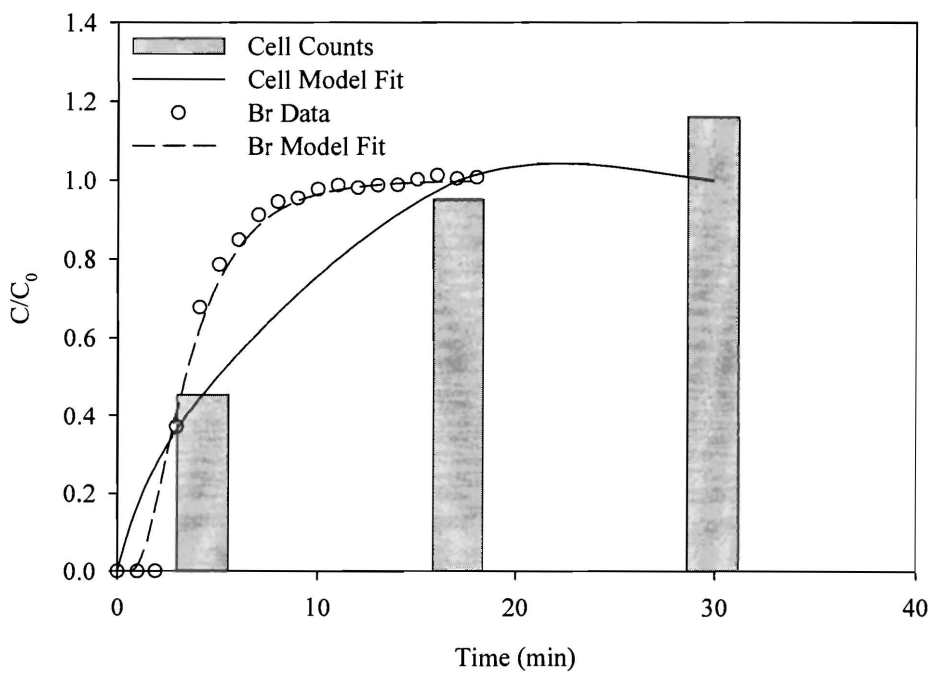

Figure 5.2. Injection phase breakthrough curves showing retarded cell transport in a laboratory column experiment.

Bioaugmentation

Using the estimated dispersivity from the PAM and the estimated cell retardation factor from the column test, cell transport during bioaugmentation was simulated using STOMP. The simulation results suggest (Fig 5.3B) that the bioaugmented cells would be transported 100% to port 5 (~60 cm from the injection/extraction port in Figure 5.1); C/C_0 would be less than 10% at port 8 after 360 minutes of injection. As a result of the previous chapter 4, measured cell concentrations of bioaugmentatation at sampling ports ranged from 4.6×10^6 to 5.1×10^6 cells/mL (34-57% of bioaugmentation at flow rate = 85 mL/min) at ports 2, 5, 6, and 8 (Fig 5.3A). TCE (24-53 μM), 1-butanol (2mM), and 10% diluted anaerobic media were combined for an injectate at 85 mL/min for bioaugmentation and no additional bioaugmentataion was conducted. The model simulation predicted successful bioaugmentation and decreased bioaugmented cells in the PAM beyond port 5, but the data after bioaugmentation indicated nonuniform distribution of cells at ports 2,5,6, and 8 (34 to 57%, Figure 5.3A).

Transport in the PAM

The flow rate of injectate decreased from 85 to 43 mL/min due to leaking at the side of the lid of the bioaugmented PAM near port 8 and the flow rate after the bioaugmentation was fixed at 43 mL/min. The estimated longitudinal dispersivity (= a flow rate 43 mL/min) values based on bromide data for the sediment pack increased with increasing travel distance (Table 5.1, Fig 5.4A-D). An injectate contained TCE (~155 μM) , TCFE (~ 30μM), and bromide (~ 1mM) and the injection time was 16.5 hrs. Estimated dispersivities were all less than the 2.4 cm value obtained from the column test,

but the answer for increased dispersivities along with the sampling ports was not certain. The increased values of dispersivity may have been related to partitioning of TCE/TCFE into trapped gas pockets or to loss of chlorinated ethenes through the PAM seals. TCE and TCFE transport was retarded relative to bromide (Fig 5.4A-D). TCE and TCFE transport was retarded relative to bromide (Fig 5.4A-D).

Computed retardation factors (Table 5.1) ranged from 1.33 to 1.62 for TCE and from 1.44 to 1.70 for TCFE (Fig 5.4A-D) based on co-injected bromide transport and these values were used for the simulation of the push-pull test.

Table 5.1 Computed retardation factors and longitudinal dispersivity for ports 1-8.

Port	R (TCE)	R (TCFE)	α_L (cm)
1	1.43	1.59	0.23
3	1.33	1.44	0.68
5	1.37	1.49	0.64
8	1.62	1.70	1.60
Average	1.44	1.55	0.79

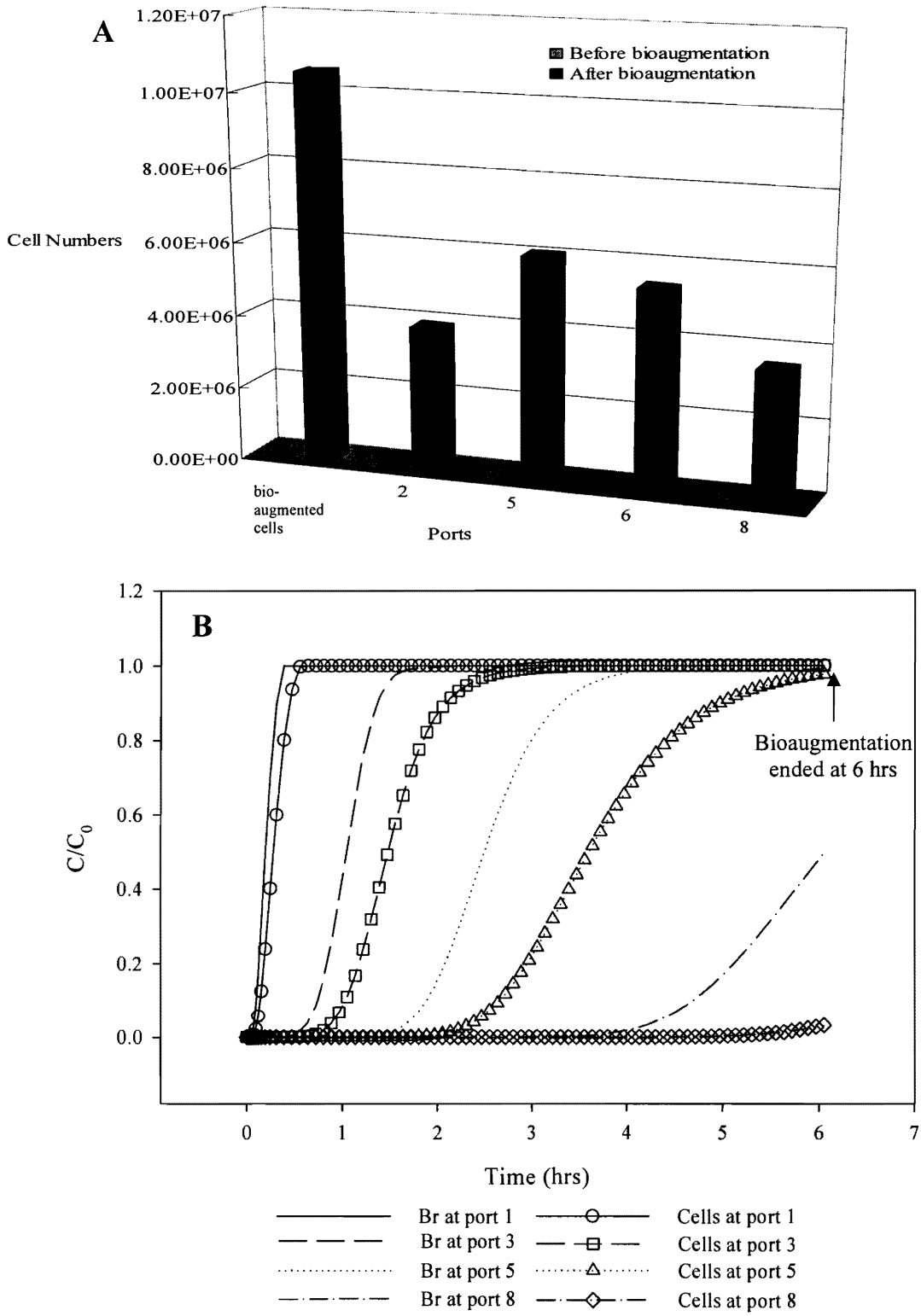

Figure 5.3. Cell transport during bioaugmentation: (A) cell counts after bioaugmentation and (B) simulations. Before bioaugmentation, injectate of cell counts ranged from 1.7 to 2.7×10^4 cells/mL.

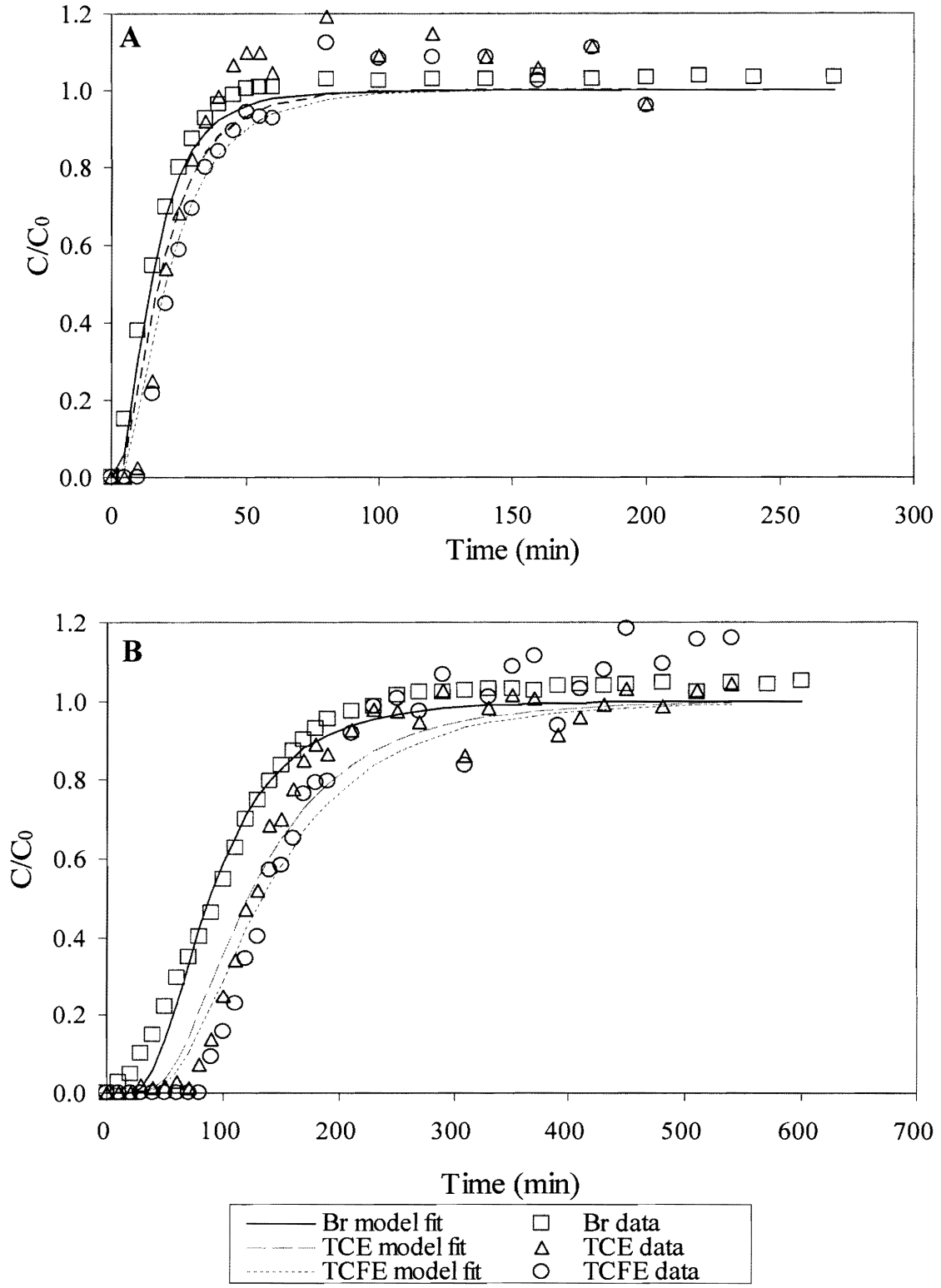

Figure 5.4. Injection phase breakthrough curves with model fits for transport test in bioaugmented PAM showing retarded transport of TCE and TCFE relative to bromide tracer: (A) port 1 and (B) port 3

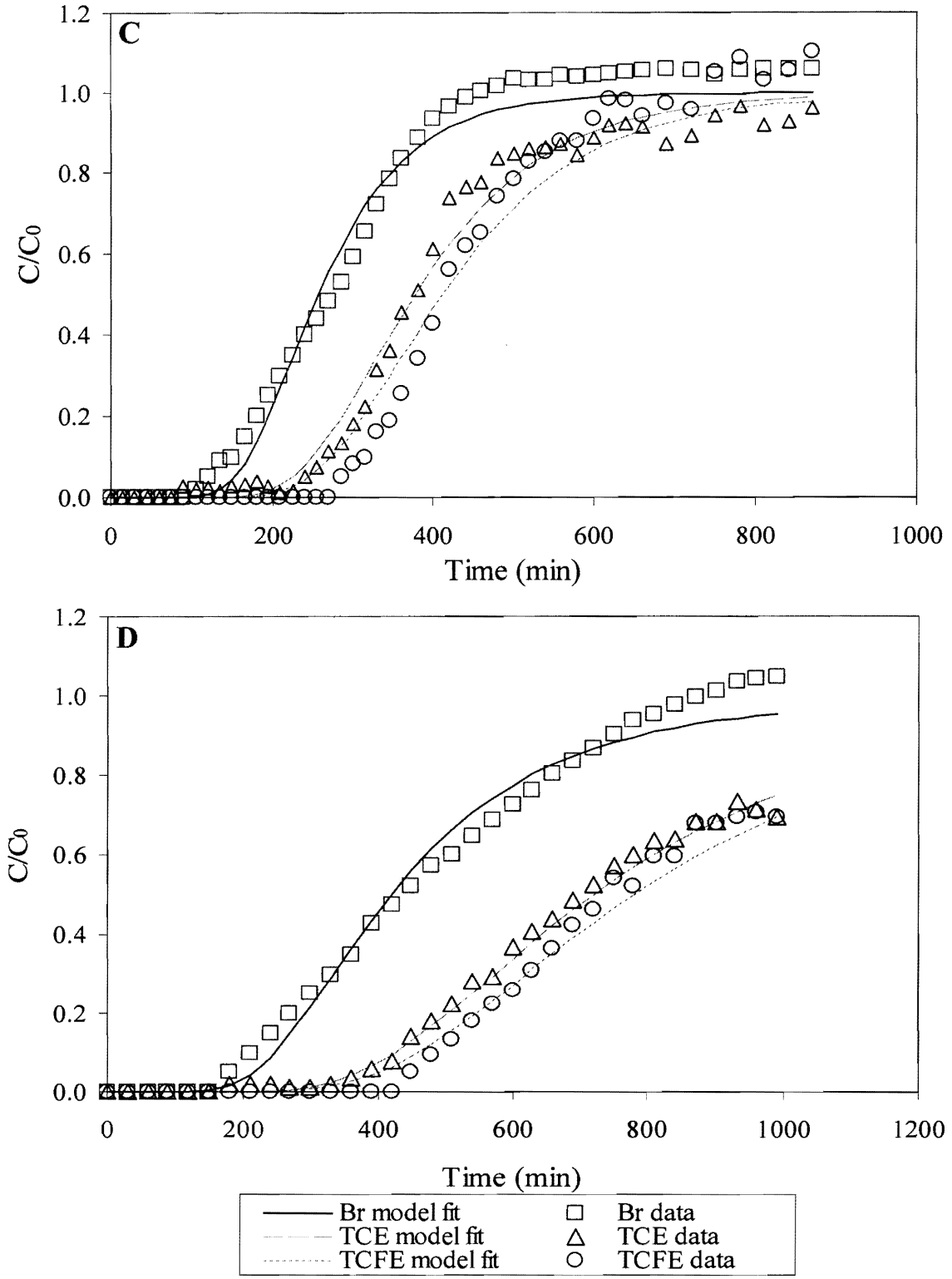

Figure 5.4 (continued). Injection phase breakthrough curves with model fits for transport test in bioaugmented PAM showing retarded transport of TCE and TCFE relative to bromide tracer: (C) port 5 and (D) port 8

Dehalogenation Activity Tests

Michaelis-Menten kinetic simulations were conducted by using the values of k_{max} and K_S of Yu et al. (2005) for TCE, *cis*-DCE, and VC transformation under assumed initial biomass, then the selected value of k_{max} of the chlorinated ethene (*cis*-DCE in this study) was adjusted to achieve the best match to the observed concentrations of each compound at each port; the initial cell mass (0.005-0.05 mg of protein) was calculated for the modeling because actual dechlorinating cell mass in the PAM after bioaugmentation was not known. The best matches with the Michaelis-Menten curves are shown as examples for each port except port 7 (Fig 5.5-11). The rate estimates give a reasonable match to the observed values of aqueous concentration of each compound except for VC and Ethene (Fig 5.5A) when no partitioning term was included. From the simulation of Fig 5.5-11, TCE transformation was initially slower but increased (Fig 5.5-11) over time. The simulations of Fig 5.5-11 indicated that ethene production more closely matched by the ratio of gas headspace (= trapped gas pockets) and liquid phase increased as follows: none (= no headspace), 0.1 (= 10% of headspace), and 0.3 (= 30% headspace), respectively.

The mass balance (referred to as Total mass of CAHs in Fig 5.5-11) indicated significant CAH loss during the test period from day 95 to 124. A likely explanation involves the partitioning of VC and Ethene between the aqueous phase and possible trapped gas pockets in the PAM. VC and Ethene are relatively volatile with Henry's constants (H_{cc}) at 20°C of 0.98 and 4.7, respectively. The bioaugmented PAM may have resulted in slow loss of chlorinated ethenes during the test period (~30 days) because the PAM was made of polypropylene and had many plastic joints and tubing attached.

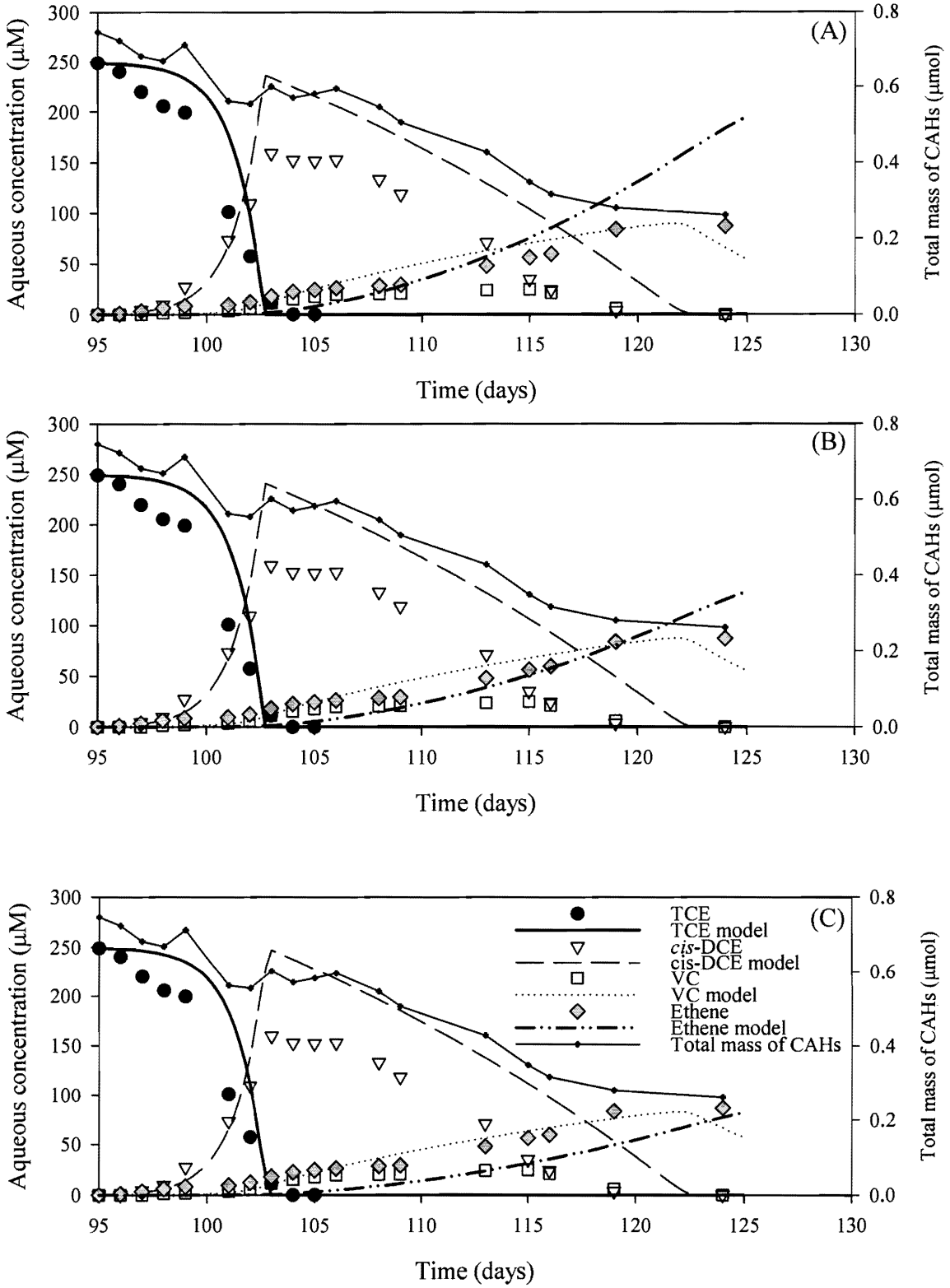

Figure 5.5. Observed and model chlorinated ethenes and ethene concentration during PAM activity test at port 1: (A) none, (B) 0.1, and (c) 0.3 of gas/water volume ratio, respectively.

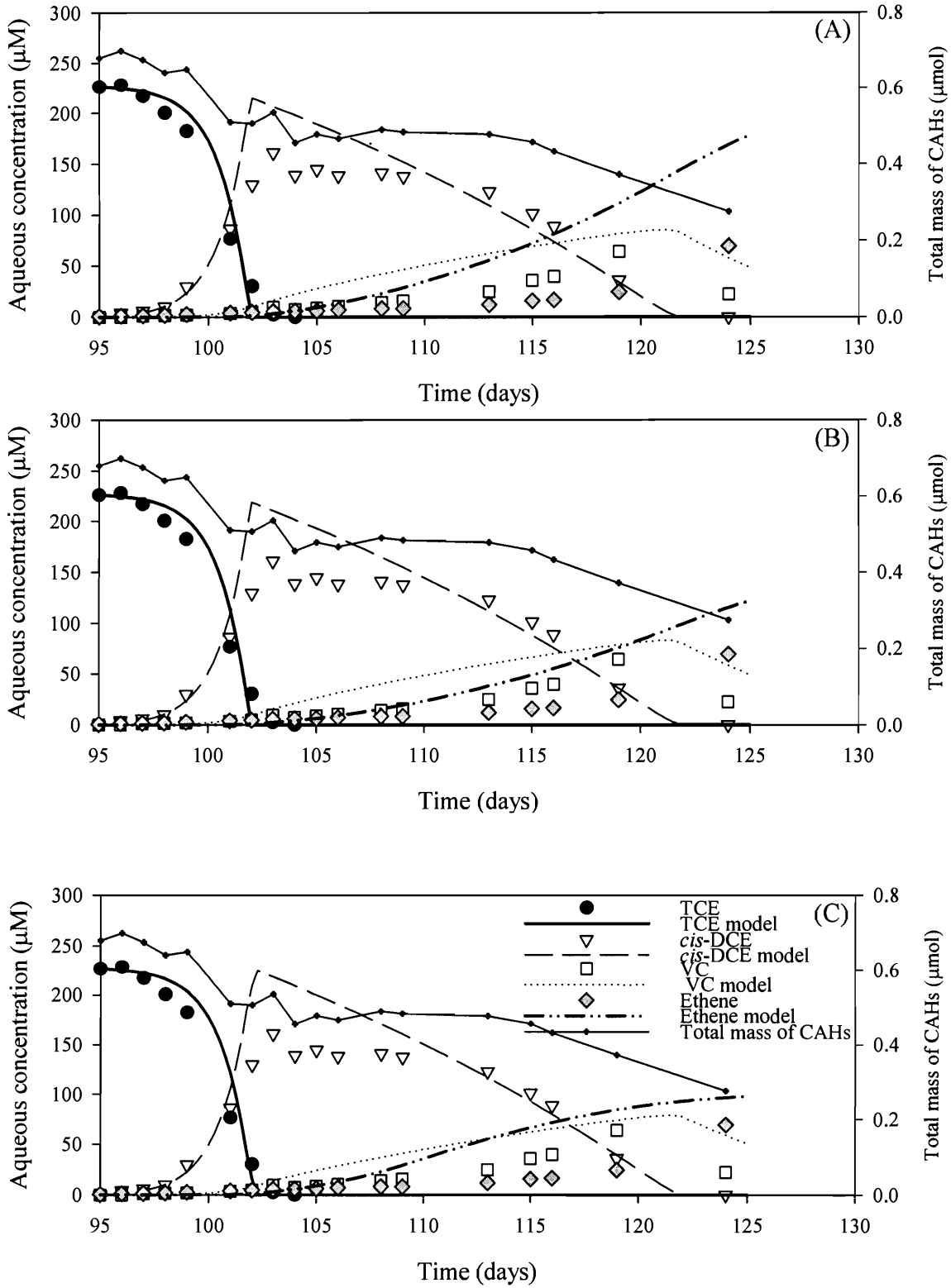

Figure 5.6. Observed and model chlorinated ethenes and ethene concentration during PAM activity test at port 2: (A) none, (B) 0.1 and (c) 0.3 of gas/water volume ratio, respectively.

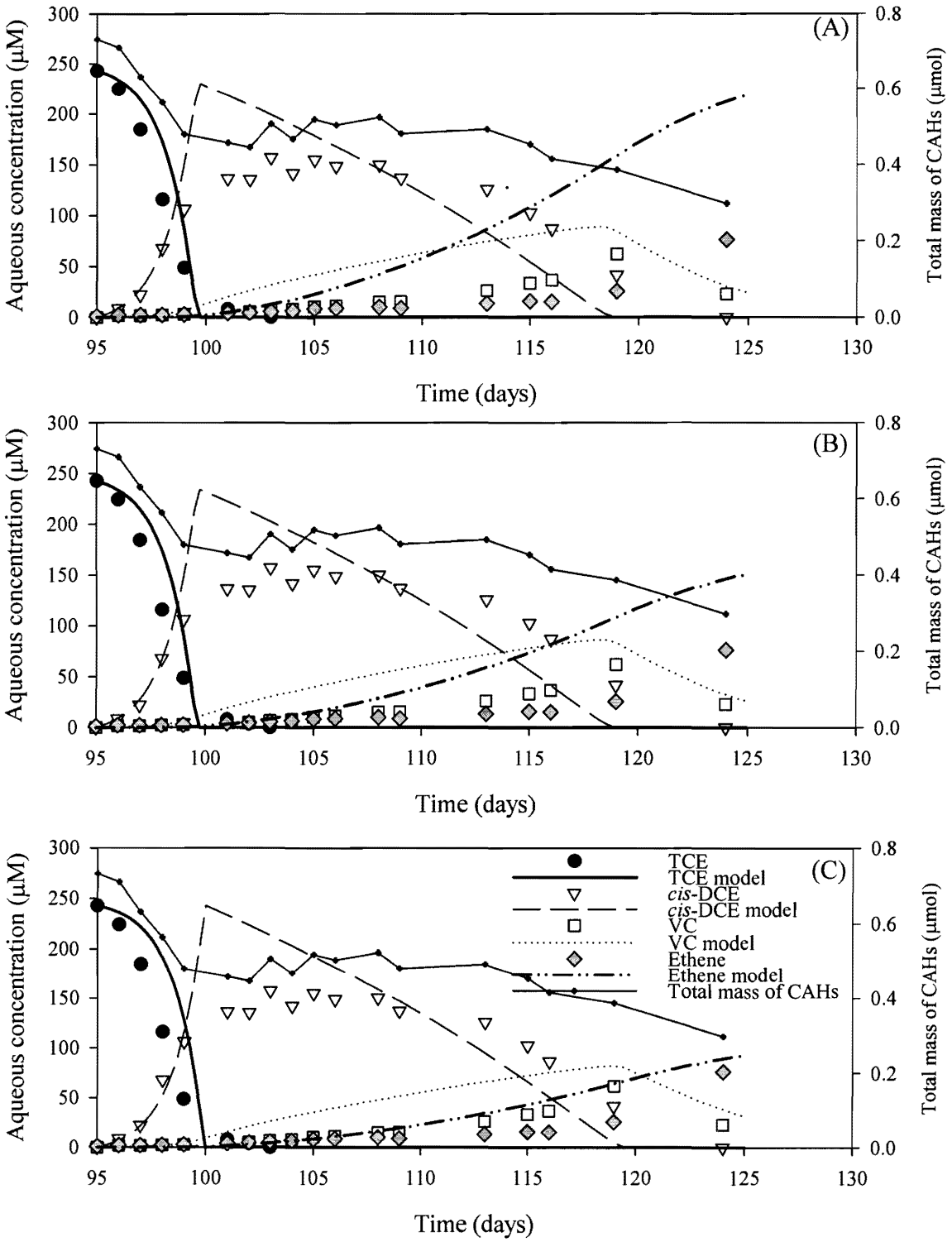

Figure 5.7. Observed and model chlorinated ethenes and ethene concentration during PAM activity test at port 3: (A) none, (B) 0.1, and (c) 0.3 of gas/water volume ratio, respectively.

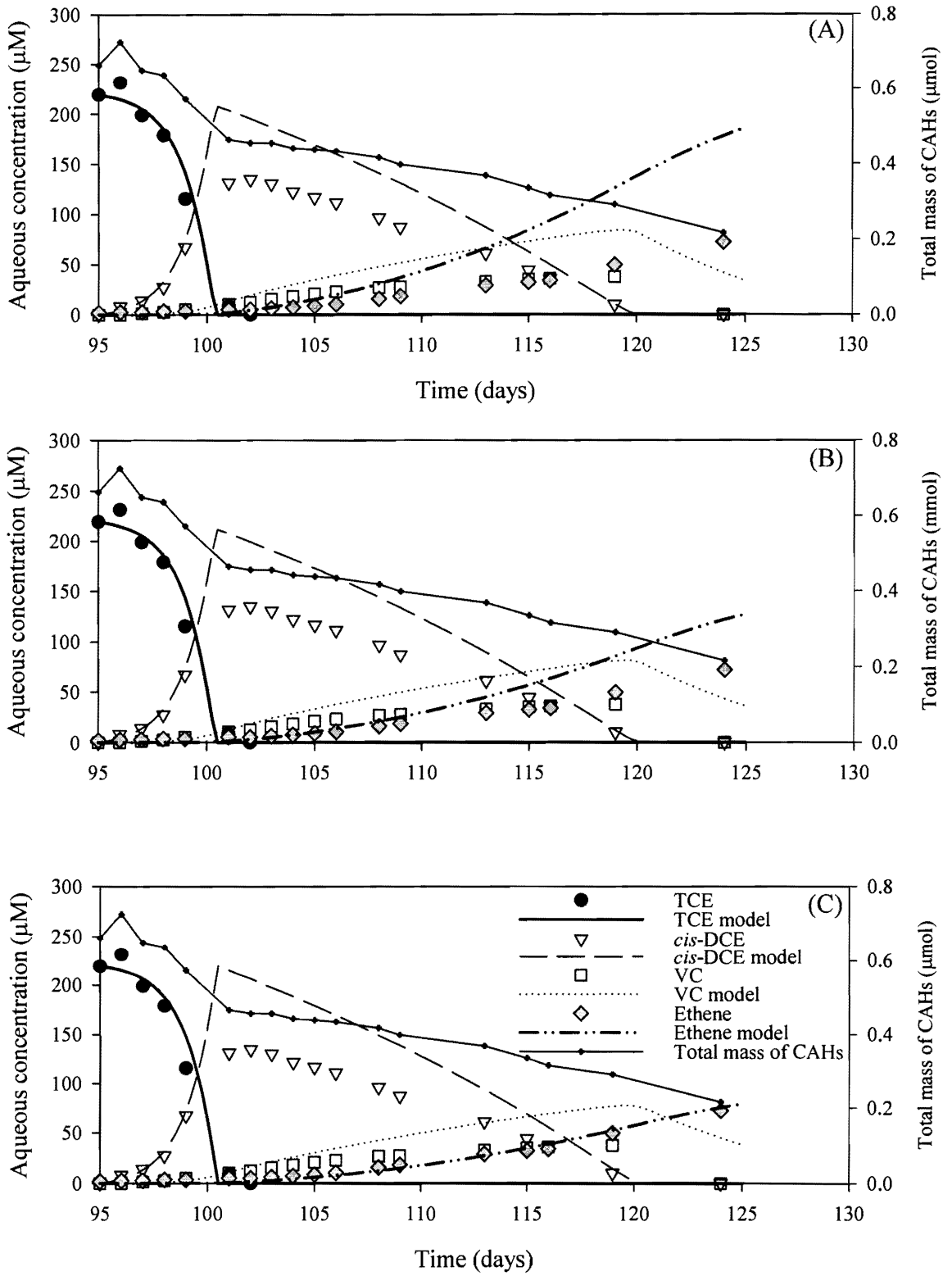

Figure 5.8. Observed and model chlorinated ethenes and ethene concentration during PAM activity test at port 4: (A) none, (B) 0.1, and (c) 0.3 of gas/water volume ratio, respectively.

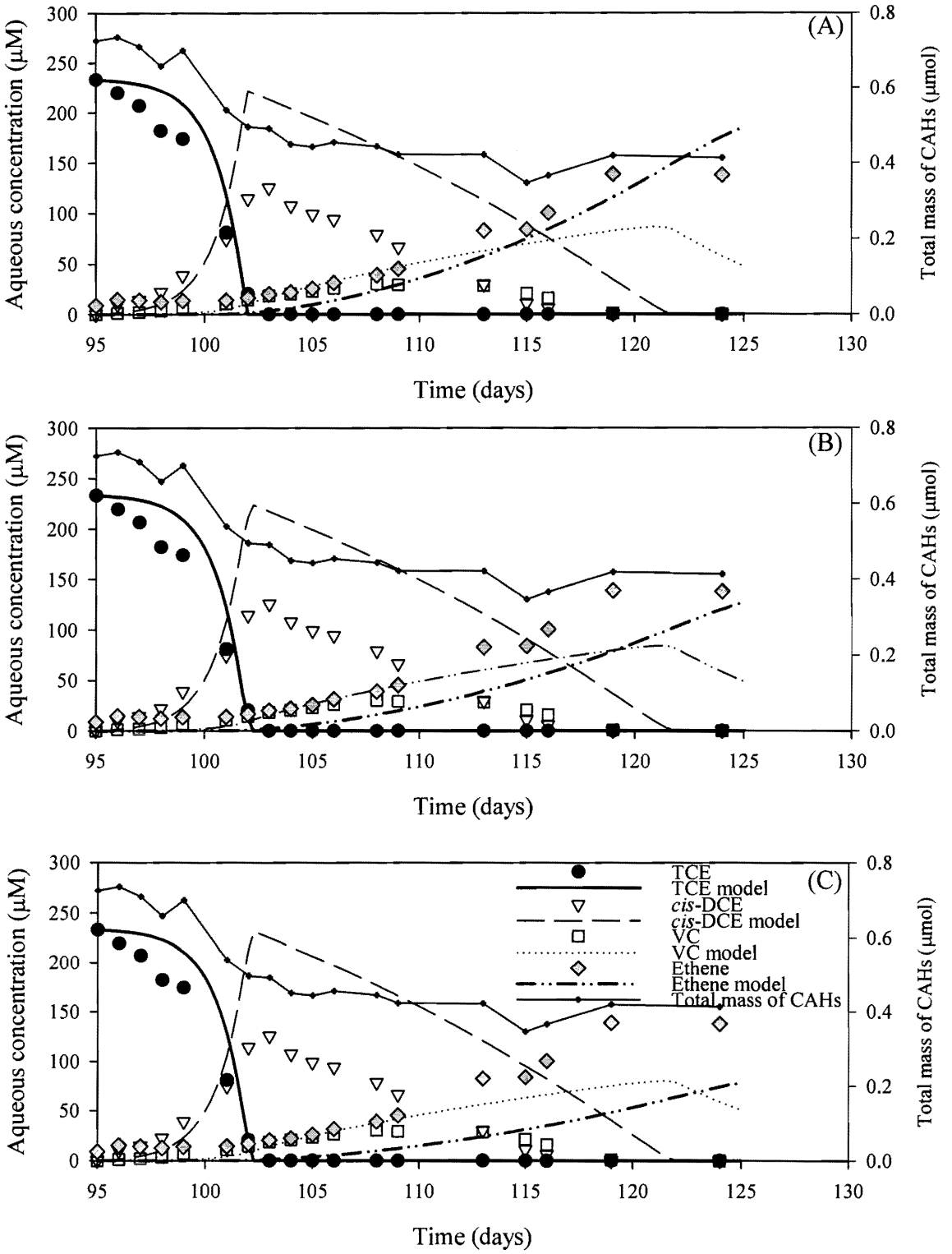

Figure 5.9. Observed and model chlorinated ethenes and ethene concentration during PAM activity test at port 5: (A) none, (B) 0.1, and (c) 0.3 of gas/water volume ratio, respectively.

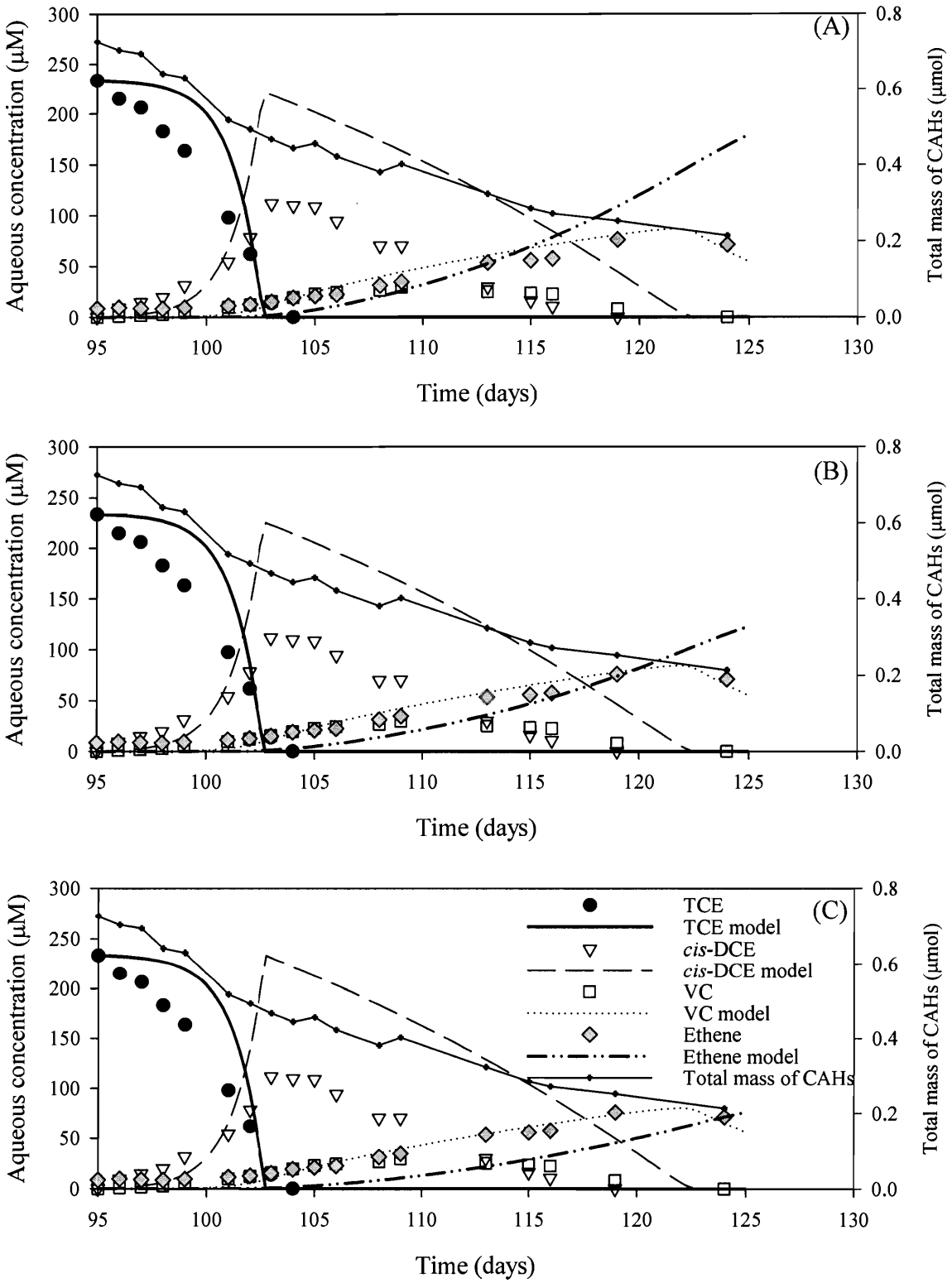

Figure 5.10. Observed and model chlorinated ethenes and ethene concentration during PAM activity test at port 6: (A) none, (B) 0.1, and (c) 0.3 of gas/water volume ratio, respectively.

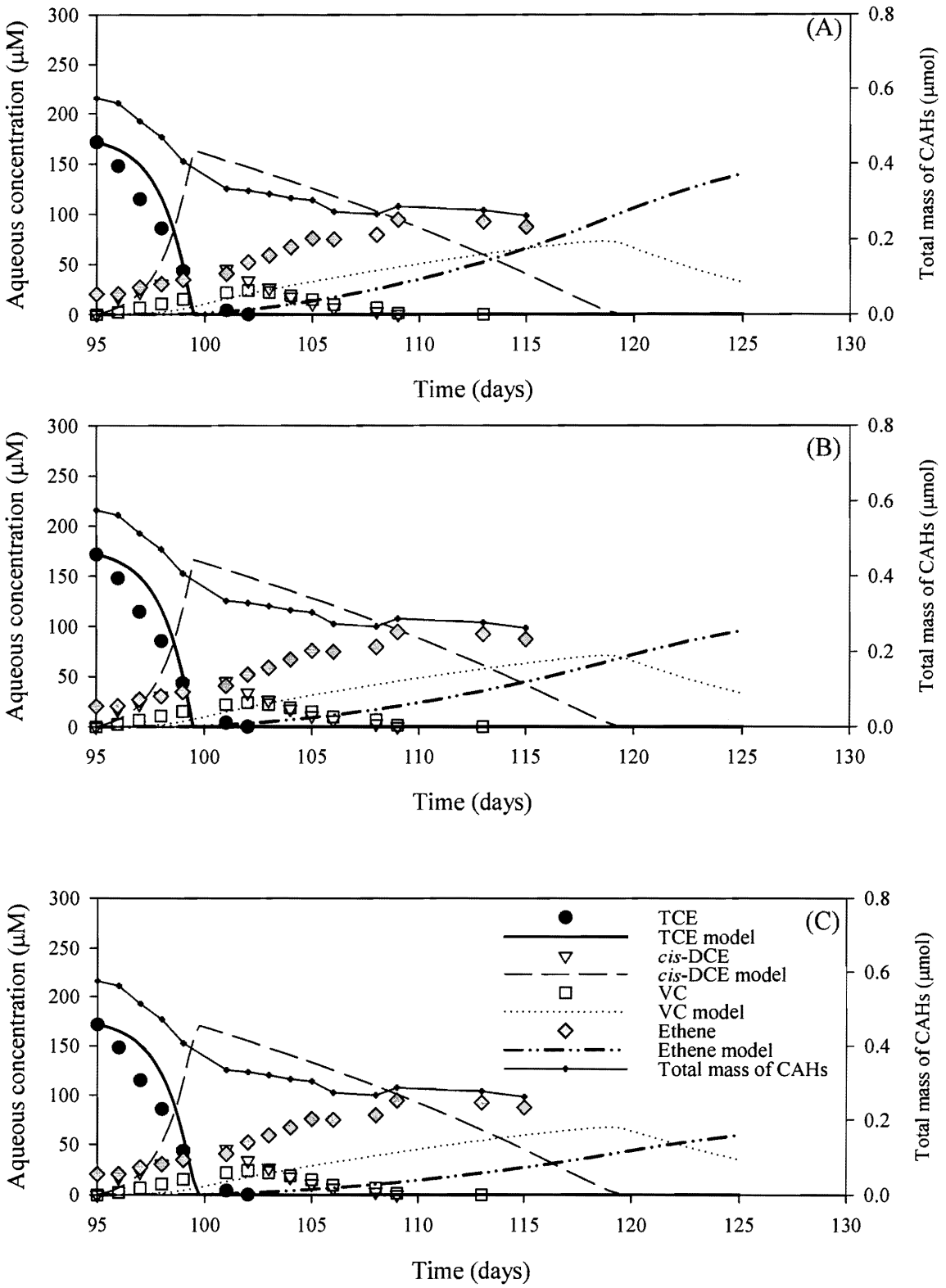

Figure 5.11. Observed and model chlorinated ethenes and ethene concentration during PAM activity test at port 8: (A) none, (B) 0.1, and (c) 0.3 of gas/water volume ratio, respectively.

From the modeling results in Fig 5.5-11, increasing the ratio of gas pockets (0.1→0.3) reasonably matched PAM results compared to no partitioning especially for the production of ethene.

The increased ratio of trapped gas pocket of 0.3 may not be feasible because the value of 0.3 means that 30% of pore space could be gas saturated. Water flow might be transported with this amount of gas in the PAM. Another possibility is slow mass loss of chlorinated ethenes by the PAM itself. The PAM consists of interior dimensions of 5 cm (width at narrow end), 50 cm (width at wide end), 125 cm (length), and 20 cm (height). The internal volume is 69 L and the pore volume is 27 L. The calculation indicated that either 2 cm (= 10% gas pocket) or 6 cm (= 30% gas pocket) of the PAM thickness was gas saturated based on the calculation of gas pocket. It was apparent that ethene and FE observation during the activity test was consistent and the PAM established increased reductive dechlorination rates of chlorinated ethenes (data not shown).

Push-Pull Test

Numerical modeling was conducted to match injection and extraction phase of the push-pull test of Lee et al. (chapter 4, 2006) to verify that the activity tests were correlated to the push-pull test. Breakthrough curves for TCFE and a bromide tracer were identical for an injection phase and FE was retarded relative to bromide during an extraction phase at port 1 (Fig 5.12A,B). Numerical simulations using STOMP were also conducted for the injection phase data using R = 1.7 for TCFE and for the extraction phase data R = 2.4 for FE at port 1. Data shown for examples are port 1, 3 and 5. Longitudinal dispersivity (cm) of each port increased during injection phase for a best match to bromide tracer for

numerical simulations: 1.22 for port 1, 2.22 for port 3 and 5, and 2.4 for port 8. Transformation rate of TCFE (~ 80 μM of aqueous phase) was 2.7 day^{-1} at all ports for the numerical simulation under first-order transformation. During the push phase, no metabolites of TCFE were detected the data, but the model produced small amount of FE (<0.1 of C/C_0) at port 5 (Fig. 5.12E). Retardation factors (R) for a match of TCFE was also performed for simulations: 1.2 for port 3, 5, and 1.4 for port 8 (Figure 5.12C,E). The result of numerical simulation for push phase was not fit well. After a rest phase of 32.5 hrs, an extraction began and all metabolites of TCFE and residual TCFE were collected to estimate TCFE transformation rate. No TCFE was detected during the extraction phase. A best match for transformation rates of TCFE to FE using STOMP was conducted and resulted in transformation rate of TCFE is not equal to FE production rate. Thus, FE production rate was adjusted to 0.9 day^{-1} and a model simulation using the value of 0.9 day^{-1} was well matched at port 1 (Figure 5.12B); the FE production rate of 0.9 day^{-1} was in the range of transformation rates of TCFE during the activity test (data not shown). A model simulation resulted in DCFE and CFE transformation roughly matched for the PAM results, but about ~ 50% of FE was not detected during the extraction phase (Figure 5.12B). Numerical simulations for port 3 and 5 were not fit well for the chlorinated ethenes (Figure 5.12D,F). The longitudinal dispersivity (α_L, range from 0.6 to 1.0 cm) for pull phase of each port were adjusted for the model simulation because the previously obtained values (Table 5.1) were not matched well for the bromide data during the extraction phase.

The best fit R = 2.4 for the pull phase breakthrough curves was adjusted to estimate the trapped gas headspace. Using equation 5.13, the calculated amount of the

trapped gas headspace is ~33%. A possible scenario was that water could still flow with 30% of gas pockets in the bioaugmented PAM. The loss of chlorinated ethenes was likely form the leaking for the PAM. Other possibilities such as nonuniform reductive dechlorination activity or sampling procedure errors would be source of error. In addition, push-pull test conducted in the PAM sampling wells penetrated up to 10 cm and did not fully penetrated the entire thickness of the PAM (20 cm).

The results of the modeling studies for the push-pull tests show that the extent and reductive dechlorination rates of chlorinated ethenes are larger than undergoing reductive dechlorination of metabolites. These methods can be applied at contaminated field sites using bioaugmentation to achieve complete and faster degradation of chlorinated ethenes.

Test design should be considered carefully for push-pull tests for bioaugmentation if selected monitoring wells are highly heterogeneous. Tests conducted over fully penetrated wells on the homogeneous aquifer could yield a consistent data. The further studies of bioaugmentation in the field sites could provide more applicable information for enhanced and complete cleanup of chlorinated ethenes. However, the numerical modeling for push-pull tests provided quantitative information on changes for bioaugmentation.

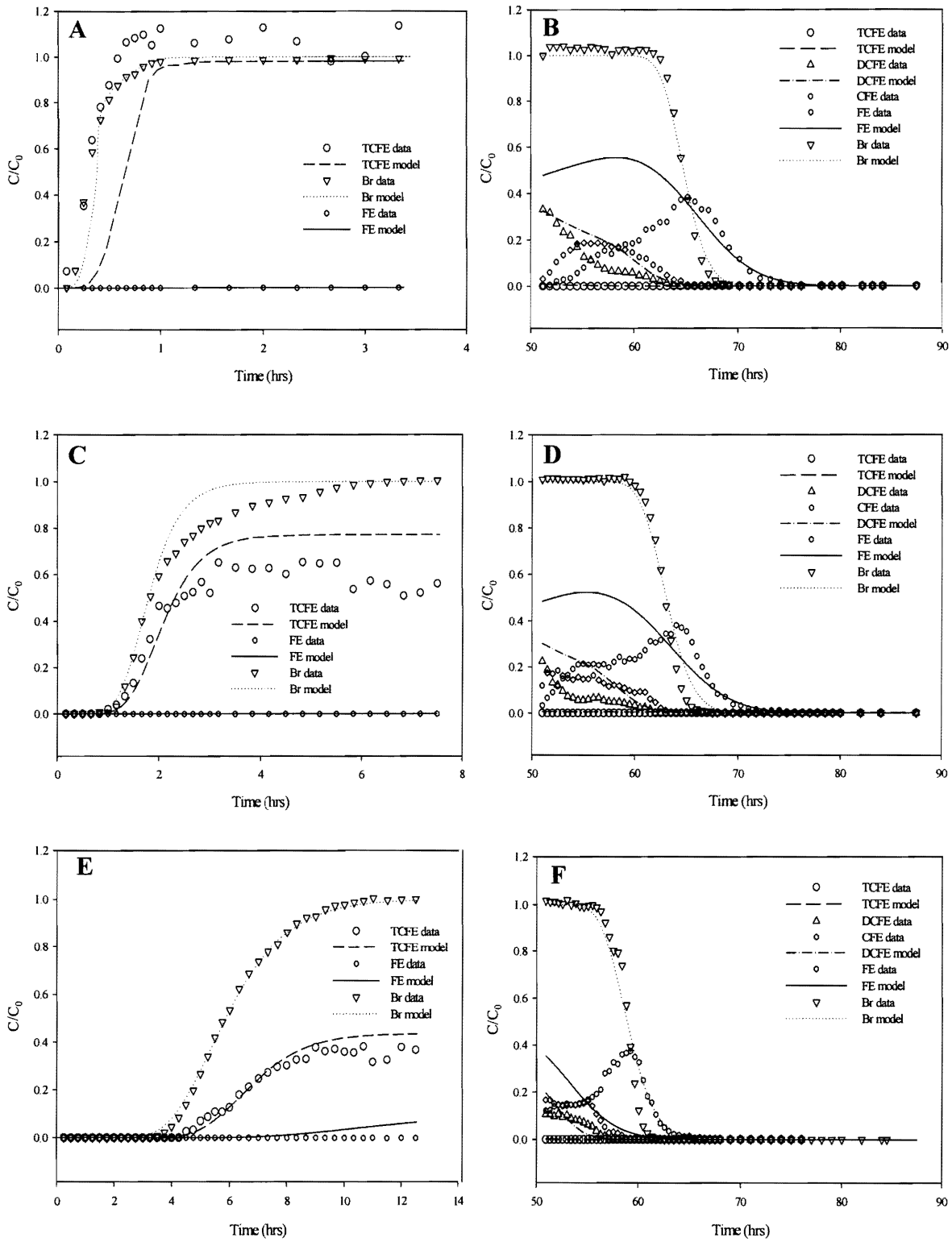

Figure 5.12. Push-pull test and a modeled push-pull test of TCFE: A,B, an injection phase and an extraction phase at port 1, C,D, an injection phase and an extraction phase at port 3, and E, F, an injection phase and an extraction phase at port 5

Forced Mass Balance Data Analysis

In situ rates for the reductive dechlorination of TCE and TCFE (here referred to as Ethene and FE production rates) were determined by removing the effects of transport process. The examples of Ethene and FE production rates are present in figure 5.13 (b) and 5.14 after adjustment by the FMB technique. It is apparent the FMB technique removes that transport behavior including sorption and partitioning during the activity test and the push-pull test. Before the FMB, the production rates for the activity test of Ethene and FE were 3.2 and 4.2 μM/day, respectively (Fig 5.13A). The production rates of Ethene and FE were 10.2 μM/day after the FMB. The dehalogenating microorganisms are responsible for the transformation of CAHs and the production of the final products, Ethene and FE in the bioaugmented PAM. Figure 5.14 indicated that the production rates of ethene and FE are correlated to retardation factor similar to the activity test after using the FMB technique. The difference of production rates of FE was that the value of the push-pull test was ~10-fold increased and it is related to the elapsed time between the activity test (~30 days) and the push-pull test (~3.5 days). Using R_{total} for CAHs is an appropriate method for the FMB when push-pull tests are applied to sorbing plus partitioning solutes.

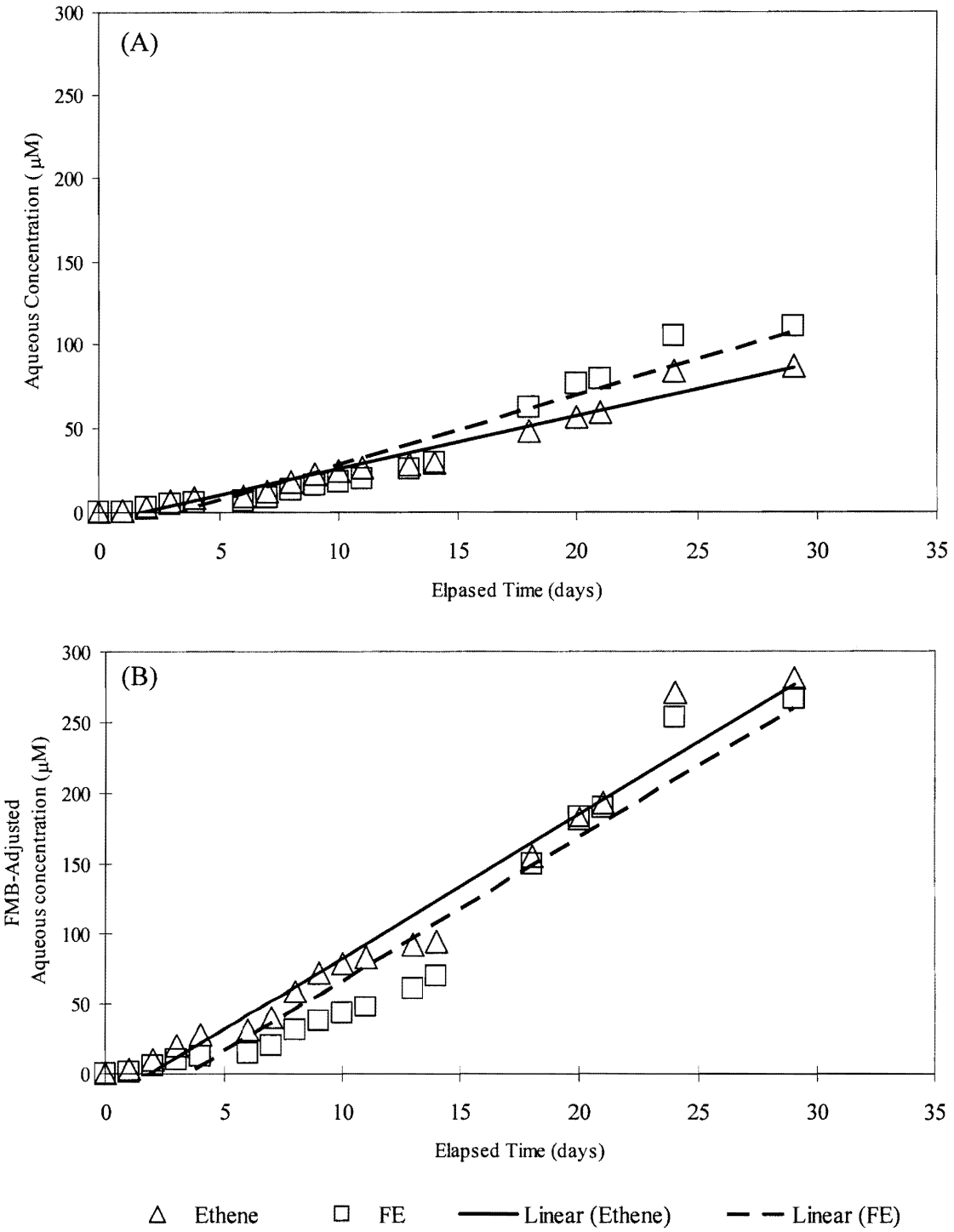

Figure 5.13. Example time progress curves for reductive dechlorination of TCE and TCFE: (A) the production of Ethene and FE for the activity test and (B) FMB-adjusted concentrations of Ethene and FE

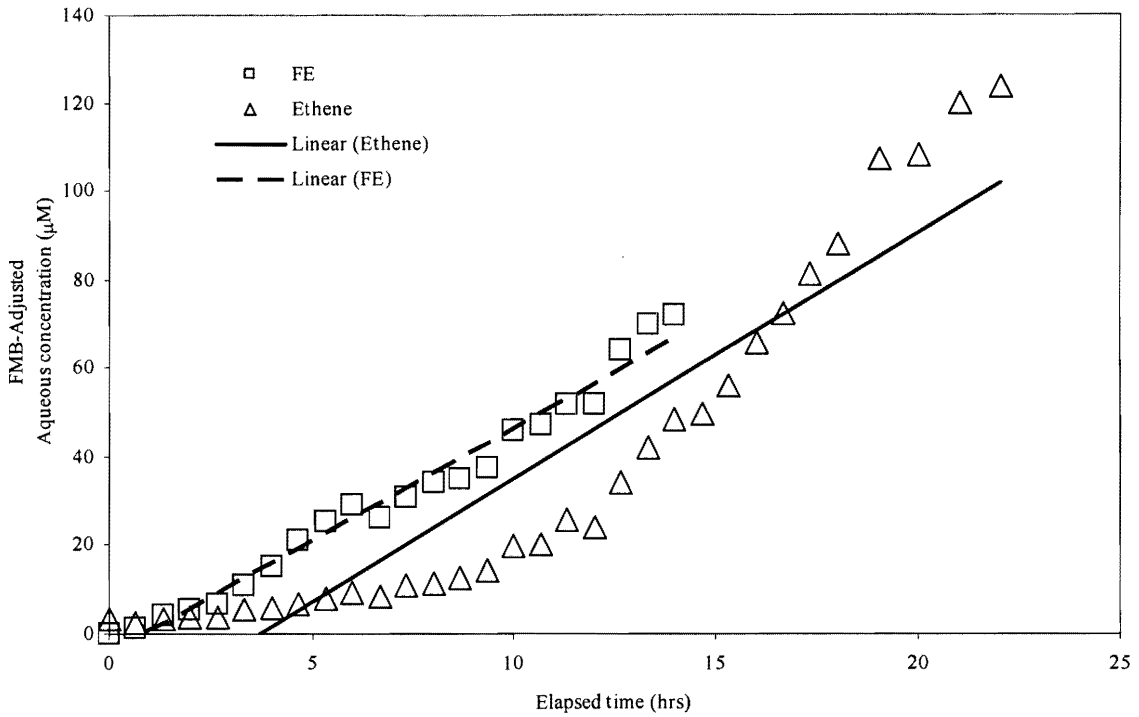

Figure 5.14. Example time progress curves for production of FMB-adjusted concentrations of Ethene and FE for the extraction phase: the production rates for Ethene and FE, 133 and 121 μM/day, respectively

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SUPPORTING INFORMATION: THE INPUT CODE FOR STOMP

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Jason Lee,
Oregon State University,
24 Oct. 2005,
5:00 pm PDT,
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PAM simulation,
simulate tracer, TCE, TCFE
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flow rate Q/A set to 0.371,
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diffusion set to 10^-30,
use TVD transport option, time step of 10s,
logintudinal dispersivity set to 1.60,
transverse dispersivity set to 0.004,
porosity set to 0.392,
Half-life for A = 6 hrs,


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# ----------------------------------------
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Cylindrical,
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13.9,cm,250@1.0,cm,
0,deg,20.4,deg,
0,cm,20.0,cm,


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# PNNL_Soil numbers are fake so far, but not important
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PNNL_Soil,Mualem,,



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B,Conventional,1.0E-9,m^2/s,Continuous,18.0,hr,
C,Conventional,1.0E-9,m^2/s,Continuous,1.0E10,yr,
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B,C,1,

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T,0.0000,cm^3/g,
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3030,min,-0.371,cm/min,,,,,,,,
East,Dirichlet Aqueous,Zero Flux,Zero Flux,Zero Flux,Zero Flux,
250,250,1,1,1,1,1,
3030,min,102450.9,Pa,,,,,,,,


# ----------------------------------------
~Output Control Card
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4,
14,1,1,
44,1,1,
74,1,1,
104,1,1,
1,1,min,cm,,4,6,6,
4,
Solute Aqueous Conc.,T,1/cm^3,
Solute Aqueous Conc.,A,1/cm^3,
Solute Aqueous Conc.,B,1/cm^3,
Solute Aqueous Conc.,C,1/cm^3,
1,
1080,min,
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4,
Solute Aqueous Conc.,T,1/cm^3,
Solute Aqueous Conc.,A,1/cm^3,
Solute Aqueous Conc.,B,1/cm^3,
Solute Aqueous Conc.,C,1/cm^3,


# ----------------------------------------
~Surface Flux Card
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6,
Aqueous Volumetric Flux,cm^3/hr,cm^3,West,1,1,1,1,1,1,
Solute Flux,T,1/hr,,West,1,1,1,1,1,1,
Solute Flux,T,1/hr,,East,250,250,1,1,1,1,
Solute Flux,A,1/hr,,West,1,1,1,1,1,1,
Solute Flux,B,1/hr,,West,1,1,1,1,1,1,
Solute Flux,C,1/hr,,West,1,1,1,1,1,1,
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CHAPTER 6

Engineering Significance and Conclusion

ENGINEERING SIGNIFICANCE

Pump-and-treat remediation is a frequently used remediation method for groundwater contaminated with high concentrations of CAHs. The high operation and maintenance costs for pump-and-treat systems and the long time required will be a big concern when prolonged time to reach concentrations are major limitations of pump-and-treat. Biostimulation and bioaugmentation are potentially more cost-effective alternatives to pump-and-treat.

Methods for detecting and quantifying any reductive dechlorination approach of CAHs are needed to assess the feasibility of using in situ bioremediation to clean up TCE contaminated sites. This study focused on developing and monitoring a TCE surrogate, TCFE, which can help tracking TCE reductive dechlorination rates on both biostimulation and bioaugmentation methods and monitoring that surrogate to investigate the effectiveness of bioaugmentation with *in situ* push-pull tests. Microcosms were constructed to evaluate TCE and TCFE reductive dechlorination rates in the presence of high background sulfate (2.5 mM) concentrations from a Richmond site, CA. Additions of fumarate and succinate enhanced reductive dechlorination of TCE and TCFE with sulfate reduction, while unfed microcosms also exhibited only slow dechlorination and no

sulfate reduction. Many TCE contaminated sites contain high background concentrations of sulfate and this sulfate can compete with CAHs for available electron donors. Once dechlorinating populations are stimulated after additions of favorable low-hydrogen partial-pressure producing electron donors, they can out-compete sulfate reducing bacteria. Bioaugmentation is a potential approach when indigenous microorganisms only slowly or incompletely transform CAHs.

A bioaugmented culture was harvested using a source from a TCE contaminated site in Corvallis, OR. The added PCE about 0.98 mM usually completely dechlorinated within 10 days in a 1 L mother reactor, then the enriched culture diluted 10 fold and introduced into a PAM. After the emplacement of dechlorinating microorganisms, no lag time observed for TCE reductive dechlorination. Repeated additions of increased TCE concentrations up to 46.0 mg/L were completely degraded to ethene in less than 14 days and 15.0 mg/L of TCFE was completely transformed to FE within 24 days. Moreover, similar transformation rates and time courses for TCE degradation and TCFE degradation were observed when added at similar initial concentrations. The result showed that the extent and rates of reductive dechlorination increased over time using TCE/TCFE with push-pull tests after bioaugmentation, so push-pull tests were very effective for assessing and monitoring tools about bioaugmentation in situ at TCE contaminated aquifers.

CONCLUSIONS

This 1st study was performed to investigate the activities of indigenous microbial populations towards the anaerobic transformation of TCFE, and TCE in presence of

different electron donor and acceptor, especially fumarate and different concentrations of sulfate. The following summaries the key findings of 1st study:

- All substrates (fumarate and succinate) added to the microcosm bottles helped to transform TCE and TCFE to its less-chlorinated compounds. The addition of fumarate did not enhance TCE and TCFE reductive dechlorination rates compared to the addition of succinate.
- The transformation rates of TCE were larger than those of TCFE. The analogue of ethene, FE, was not formed from TCFE for all fumarate and succinate fed microcosms.
- No influence of sulfate concentrations on TCE reductive dechlorination rates was observed.
- The unamended TCE microcosms produced only *cis*- and *trans*-DCE when fumarate- and succinate-fed microcosms produced *cis*-, *trans*-DCE, VC, and ethene.
- The unamended TCFE microcosms produced only *cis*- and *trans*-DCFE when fumarate- and succinate-fed microcosms produced *cis*-, *trans*-DCFE, 1,1-CFE, *cis*-, and *trans*-CFE.

The 2nd study was focused on the activities of bioaugmented microbial populations for enhanced reductive dechlorination of TCE and/or TCFE compared to non-bioaugmented control using push-pull tests. The following summaries the key findings of 2nd study:

- The complete transformation rates of TCE were larger than that of TCFE by comparison of 7 out of 8 sampling ports, but almost identical at port #8.
- The successful transport of dechlorinating culture was achieved for the bioaugmented PAM by comparison of transformation rate of TCE and TCFE with high flow rate, Q = 85mL/min, at each port and the column test.
- The analogues of ethene, FE, was formed for all sampling ports after addition of TCFE.
- All TCE transformed to ethene in less than 2 weeks and all TCFE also transformed to FE in less than 24 days.
- A series of TCE and TCFE additions transformed to ethene and FE in less than 3 or 4 weeks for each port, showing good survival condition of bioaugmented strains after emplacement.
- The non-bioaugmented PAM had capability to reduce sulfate (~550 μM) less than 35 days showing a good reducing condition.
- The analysis and mass balance of TCE, TCFE, and all intermediate metabolites was not successful, but chloride release was a good indication for different kinetics between TCE and TCFE reductive dechlorination.
- Mass balance calculations were performed that the bromide mass was recovered about only 12.3 % due to the largest volume to the outlet (Table 3). The other ports indicated that the increased mass recovery of bromide from 74.7, 81.1, and 85 % in a series of port 5, 3, and 1 close to the inlet during the extraction about 2 pore volumes compared to injection volume of the test solution.

The 3rd study was focused on identifying what factors may have caused the differences in transformation rates observed during two types of activity test and push-pull tests using numerical method for the result of the bioaugmentation. The following summaries the key findings of 3rd study:

- The bioaugmented microorganisms were effectively transported through Hanford sediment and the estimated retardation factor was 1.4 in a column experiment.
- A numerical simulation predicted cell transport in the PAM as far as port 5. This was confirmed by cell counts obtained during bioaugmentation, but cells were distributed nonuniformly.
- The transport test indicated that TCE and TCFE were relatively retarded compared to coinjected bromide tracer (retardation factors ranged from 1.33-1.62 for TCE and from 1.44-1.70 for TCFE).
- Michaeles-Menten kinetics for the activity test indicated that transformation capacity was well maintained from the original source, Evanite culture.
- The rates of TCE and TCFE reductive dechlorination were larger than those rates of Vinyl Chloride (VC) and Chlorofluoroethene (CFE) reductive dechlorination.
- FE production rate during the push-pull test was in the range of TCFE transformation rates during the activity test and a best fit for a numerical simulation resulted in TCFE reductive dechlorination to FE production (3:1 ratio).
- The bioaugmented PAM likely contributed to slow loss of chlorinated ethenes during the activity test and the push-pull test.

The possibility of treating hazardous or other contaminated sites by injecting favorable electron acceptor and donor together (fumarate and lactate) to stimulate in-situ

bioremediation could be a cost-effective treatment. Push-pull tests can be easily extended and examined for monitoring of bioaugmentation to clean up CAH contaminated aquifers.

FUTURE WORK

Future work should concentrate on field and modeling investigations of the bioaugmentation to remediate a TCE contaminated aquifer. The goal of future research should be an acceptable and useful method as a valid technique for quantifying *in situ* microbial activity that can be specially related to TCE transformation. To that end, additional studies are needed at field sites that are characterized for non- or slow TCE reductive dechlorination sites needed for bioaugmentation. Furthermore, studies could be performed at sites where TCE reductive dechlorination are being stopped at the final step, VC dechlorination, such as a known carcinogen to be needed complete detoxification. For VC dechlorination study in fields, CFE reductive dechlorination by bioaugmentation could be feasible for push-pull tests. Additional modeling studies of the push-pull tests are needed, for both hypothetical and field trials of the tests. The incorporation of 2-D and 3-D simulations could allow for better interpretation of TCE reductive dechlorination by bioaugmentation, and may result in more accurate estimates of TCE reductive dechlorination rates.

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