AN ABSTRACT OF THE DISSERTATION OF

Seungho Yu for the degree of Doctor of Philosophy in Civil Engineering presented on December 16, 2003.

Title: Kinetic and Modeling Investigations of the Anaerobic Reductive Dechlorination of Chlorinated Ethylenes Using Single and Binary Mixed Cultures and Silicon-based Organic Compounds as Slow-release Substrates.

Abstract approved:

Redacted for Privacy

Lewis Semprini

This study investigated complete reductive dechlorination of chlorinated aliphatic hydrocarbons (CAHs) over a broad range of concentrations. Tetrabutoxysilane (TBOS), as a slow-release anaerobic substrate, was studied for enhanced reductive dechlorination of tetrachloroethylene (PCE) present as a dense non-aqueous liquid (DNAPL). Four different site-mixed cultures were used in the study: Site-300 Lawrence Livermore National Laboratory, CA (LLNL), Point Mugu, CA (PM), the Evanite site in Corvallis, OR (EV), and a binary mixed culture of the PM and EV cultures (BM). Batch studies showed that one mol of TBOS abiotically and slowly hydrolyzed to 4 mol of 1-butanol, which fermented to butyrate and/or acetate, producing H_2 during fermentation. The produced H_2 as a direct electron

donor was shown to effectively promote the reductive dechlorination of PCE and trichloroethylene (TCE).

A simple kinetic method was developed for determining maximum utilization rates (k_{max}) and half-velocity coefficients (K_S) that well characterize the dechlorinating microorganisms. Batch inhibition studies indicated that the more chlorinated ethylenes inhibited dechlorination of the less chlorinated ethylenes. Competitive inhibition models simulated well the inhibition experimental data. Inhibition constants of chlorinated ethylenes, K_{CI} (μM), were comparable to their respective half-velocity coefficients, K_S (μM).

Two kinetic models fitted the sequential transformation experimental data over a wide range of PCE and TCE concentrations (~ PCE 1000 μM and TCE 4000 μM): one that included competitive inhibition kinetics and the other that included both competitive and Haldane inhibitions. Both kinetic models captured the data well with up to PCE concentrations of 300-400 μM. The kinetic model with competitive and Haldane inhibitions better fit the higher PCE and TCE concentration tests. The PM culture had Haldane inhibition constants of 900, 6000, and 7000 μM for TCE, *cis*-1,2-dichloroethylene (*c*-DCE), and vinyl chloride (VC), respectively, indicating slight Haldane inhibition for *c*-DCE and VC. The EV culture showed significant Haldane inhibition for TCE, *c*-DCE, and VC (Haldane inhibition constants of 900, 750, and 750 μM, respectively). The BM culture showed better dechlorination ability over a broad range of PCE and TCE concentrations, and more complete dechlorination for remediating PCE DNAPL with TBOS than either of the single mixed cultures.

Kinetic and Modeling Investigations of the Anaerobic Reductive Dechlorination of Chlorinated Ethylenes Using Single and Binary Mixed Cultures and Silicon-based Organic Compounds as Slow-release Substrates

By

Seungho Yu

A DISSERTATION

Submitted to

Oregon State University

in partial fulfillment of

the requirement for the

degree of

Doctor of Philosophy

Presented December 16, 2003

Commencement June 2004

Doctor of Philosophy dissertation of Seungho Yu presented on December 16, 2003.

APPROVED:

Redacted for Privacy

Major Professor, representing Civil Engineering

Redacted for Privacy

Head of the Department of Civil, Construction, and Environmental Engineering

Redacted for Privacy

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Redacted for Privacy

Seungho Yu, Author

ACKNOWLEDGEMENTS

First, I would like to express my sincerest appreciation to my major professor, Dr. Lewis Semprini, whose excellent example of a disciplined and systematic approach to research and scholarship is one for which I strive. In addition, I'd like to thank my committee members Dr. Charles Brunner, Dr Mark Dolan, Dr. Jack Istok, and Dr Kenneth Williamson for their guidance and encouragement during my studies.

An additional thanks should be given for the EPA-sponsored Western Region Hazardous Substance Research Center. The center has been a great support for my research here.

I owe all of my lab and officemates thanks for their encouragement and assistance during my study. Their warmth and friendship made the rainy winter days and nights in Oregon a lot happier. In particular, I would also like to thank Andy Sabalowsky for his proofreading.

I would like to thank my best friend Jeong-Hoon Kim in Korea who showed me the true meaning of friendship and deserves a special thanks. When I left Korea, he gave me a fountain pen which has not been used since that time. However, I used the pen today for the signature on my dissertation.

I don't know how to thank my family in Korea. I confess that I couldn't finish my study without their support and patience. Special thanks go to my parents and parents-in-law for their endless love and support which are impossible to pay off

in my lifetime. I believe that my grandmother and father-in-law, who passed away during my study, are seeing me and are very as happy as I am.

To my wife, JungA Lee, I can never adequately express my love and gratitude. Thank you for taking care of me and for loving me through all of the hard time during my study at Corvallis. And of course, thank you for the lovely son, Taewook, you've given me. Taewook, thank you for your giving up your time to play and talk with me for these last years. You've given Mom and me invaluable joy in our life. JungA, thank you for every single thing. We have pushed through. Now, finally, it is time to go forth. I love you.

CONTRIBUTION OF AUTHORS

Dr Dolan provided helpful comments and guidance on this dissertation.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF FIGURES (Continued)

LIST OF TABLES

Dedicated to my God and ancestors.

Kinetic and Modeling Investigations of the Anaerobic Reductive Dechlorination of Chlorinated Ethylenes Using Single and Binary Mixed Cultures and Silicon-based Organic Compounds as Slow-release Substrates

CHAPTER 1

INTRODUCTION

Tetrachloroethylene (PCE) and trichloroethylene (TCE) are suspected human carcinogens that are among the most commonly observed groundwater contaminants (National Research Council, 1994). The Environmental Protection Agency (EPA) reported that TCE was found in over 700 Superfund sites, and estimated that 9-34% of the United States groundwater may be contaminated with TCE (U.S. EPA, 2003). Chlorinated ethylenes are effective solvents in dry-cleaning operations, machine cleaning process, and semiconductor manufacture (McCarty, 1997; Vogel *et al.*, 1987). Due to their physical properties, such as being dense non-aqueous phase liquids (DNAPLs), and low solubility in water, they can potential migrate deep into the subsurface (Mackay and Cherry, 1989), leading to difficulties in locating and removing sources (Mackay and Cherry, 1989).

Many organic compounds, such as organic acids and alcohols, have been studied as potential electron donors to promote anaerobic dechlorination (Fennell *et al.*, 1997). Complex substrates are fermented to less complex substrates, producing H_2, which can serve directly as an electron donor for reductive dechlorination (Maymo-Gatell *et al.*, 1995). However, other microorganisms including methanogens, homoacetogens, and sulfidogens compete for H_2 as an electron donor

in the subsurface environment (McCarty, 1997). Enhanced bioremediation may require the use of substrates that are efficiently used for anaerobic dechlorination (Yang and McCarty, 1998). Many researchers have studied hydrogen competition for a variety of hydrogenotrophic microbial consortiums. Dechlorinators were shown to have a lower hydrogen threshold concentration of 0.3 nM compared to acetogens (336~3640 nM), methanogens (5~95 nM), and sulfidogens (1~15 nM) (Löffler *et al.,* 1999). Other studies reported a hydrogen threshold on the range of 0.05~11 nM for dechlorinators (Smatlak *et al.,* 1996; Yang and McCarty, 1998), suggesting that anaerobic dechlorinators can outcompete the other hydrogenotrophs at low hydrogen concentrations. In addition to the physiological hydrogen threshold, free energy calculations indicate a lower hydrogen threshold for dechlorinators than for other hydrogenotrophs (Smatlak *et al.,* 1996). Several studies revealed that an electron donor that is slowly and steadily transformed to maintain low H_2 concentrations could optimize anaerobic dechlorination and limit methanogenesis (Ballapragada *et al.,* 1997; Fennell *et al.,* 1997; Fennell and Gossett, 1998; Smatlak *et al.,* 1996; Yang and McCarty, 1998).

Tetrabutoxysilane (TBOS, $[Si(CH_3CH_2CH_2CH_2O)_4]$) and tetrakis(2-ethylbutoxy)silane (TKEBS, $[Si(CH_3CH_2CH(CH_2CH_3)CH_2O)_4]$) are silicon-based compounds having four oxygen branches with butyl and 2-ethylbutyl groups, respectively. These compounds were observed to act as LNAPLs and mix well with CAHs, as observed in our laboratory. They have a low solubility in water and thus slowly dissolve. The silicon-based compounds have been shown to support

anaerobic dechlorination of TCE at chlorinated solvent-contaminated sites (Vancheeswaran *et al.*, 1998; Vancheeswaran *et al.*, 1999; Vancheeswaran *et al.*, 2003) and with laboratory cultures (Yang and McCarty, 2000a). Evidence for the transformation pathway of alkoxysilanes, mainly TKEBS, and their potential to serve as substrates for promoting TCE transformation was discovered through analysis of groundwater from Site 300 at Lawrence Livermore National Laboratory, CA (LLNL) (Vancheeswaran, 1998). Based on the field monitoring data, TBOS and TKEBS were observed to slowly hydrolyze to 1-butanol and 2-ethylbutanol, respectively, and ferment to butyrate and 2-ethylbutyrate (Vancheeswaran, 1998; Vancheeswaran *et al.*, 1999; Vancheeswaran *et al.*, 2003). However, the abiotic hydrolysis of TBOS and the biotic fermentation of the hydrolysis products needed additional study, as well as the effect of TBOS addition on reductive dehalogenation. The effectiveness of TBOS addition as a slow-release substrate with microbial communities from different sites was also needed to be evaluated. During the fermentation of the alcohols and acids, H_2 is produced and becomes a potential electron donor for the dechlorinators (McCarty, 1997; Löffler *et al.*, 1999). Butyrate, one potential fermentation product, has been shown to be an excellent slow fermenting substrate among other fatty acids to produce hydrogen needed for dehalogenation reactions (Fennell *et al.*, 1997).

The use of a binary mixed culture of two different aerobic pure cultures has been reported to show interspecies interactions and changes in the rate and extent of biodegradation (Fairlee *et al.*, 1997; Rogers *et al.*, 2000). The studies revealed that

the utilization of a binary mixed culture could enhance the biodegradation of mixed pollutants such as toluene, phenol, and benzene. Previously, we reported (Yu and Semprini, 2002a) that a binary anaerobic mixed culture isolated from two different contaminated groundwaters was more effective in reductively dechlorinating PCE to ethylene (ETH). Thus, studies were performed here to investigate potential and effectiveness of a binary mixed culture.

Various models have been developed that describe reductive dechlorination of chlorinated ethylenes in the subsurface. The H_2 competition of dechlorinating microorganisms with nitrate and sulfate reducers, methanogens, and acetogens has been reported (McCarty, 1997; Yang and McCarty, 1998; Löffler *et al.*, 1999). However, few studies have been performed on the inhibition among chlorinated ethylenes during reductive dechlorination. During complete reductive dechlorination of PCE to ETH, competitive inhibition of vinyl chloride (VC) dechlorination by the other chlorinated ethylenes was assumed and the simulation model was compared with the experimental data (Tandoi *et al.*, 1994). Fennell and Gossett (1998) simulated both fermentation of electron donors and competition for the evolved H_2 between hydrogenotrophic tetrachloroethylene dechlorinators and methanogens, but the comprehensive biokinetic model employing Michalis-Menten-type kinetics did not consider inhibition between chlorinated ethylenes. Another model was developed that can depict and predict reductive dechlorination of PCE by incorporating competitive dechlorination between PCE and TCE (Tonnaer *et al.*, 1997). Garant and Lynd (1998) showed that competitive kinetics for complete

reductive dechlorination of PCE afford better chi-squared and visual fits of the experimental data than noncompetitive inhibition. Haston (1999) reported the competitive inhibition between *c*-DCE and VC, and showed the inhibition constants were comparable to their respective half-velocity coefficients.

OBJECTIVES

In spite of possible toxicity of high PCE and TCE concentrations, recent studies reported that anaerobic reductive dechlorination is a promising technology for remediation of high concentrations of PCE and TCE associated with dissolution of the DNAPL source zone (Sharma and McCarty, 1996; Nielsen and Keasling, 1999). Other research has shown that the enhancement of PCE DNAPL dissolution was observed due to biotransformation processes (Carr *et al.*, 2000; Yang and McCarty, 2000b; Cope and Hughes, 2002). These reports suggest the potential for biological dechlorination of PCE or TCE DNAPL. The main objective of this study was to evaluate the potential and effectiveness of anaerobic reductive dechlorination of CAH DNAPLs as well as high concentrations, where two different mixed cultures isolated from CAH-contaminated sites. TBOS was evaluated as a slow-release anaerobic substrate to investigate the bioremediation of the PCE and TCE DNAPLs. In this study, three different anaerobic mixed cultures were used: Site-300 Lawrence Livermore National Laboratory (LLNL), Point Mugu Naval Weapons Facility, CA (PM), and the Evanite site in Corvallis, OR (EV). Detailed kinetic and modeling

studies were performed with the PM, EV, and a binary mixed culture representing a mixture of the two cultures. The specific objectives of this study were:

1) To investigate the effectiveness of TBOS as a slowly hydrolyzing substrate to drive the enhanced anaerobic transformation of TCE using different anaerobic mixed cultures.
2) To study the effectiveness of TBOS using a binary mixed culture for biological dechlorination of PCE DNAPL.
3) To kinetically characterize two different mixed cultures that completely dechlorinate PCE to ETH. A simple multi-equilibration method was developed to determine kinetic parameters (k_{max} and K_S values), and batch experiments were also conducted to study the inhibitions among chlorinated ethylenes.
4) To conduct batch experiments of sequential transformation of PCE and TCE to ETH over a wide range of concentrations up to the solubility limit and to model the results using the independently measured kinetic parameters.

REFERENCES

Ballapragada, B. S., Stensel, H. D., Puhakka, J. A. and Ferguson, J. F. (1997). Effect of Hydrogen on Reductive Dechlorination of Chlorinated Ethenes. *Environ. Sci. Technol.* **31**, 1728-1734.

Carr, C. S., Garg, S., and Hughes, J. B. (2000) Effect of Dechlorinating Bacteria on the longevity and Composition of PCE-Containing Nonaqueous Phase Liquids under Equilibrium Dissolution Conditions. *Environ. Sci. Technol.* **34**, 1088-1094.

Cope, N. and Hughes, J. B. (2001) Biologically-Enhanced Removal of PCE from NAPL Source Zones. *Environ. Sci. Technol.* **35**, 2014-2021.

Fairlee, J. R., Burback, B. L., and Perry, J. J. (1997) Biodegradation of Groundwater Pollutants by a Combined Culture of *Mycobacterium vaccae* and a *Rhodococcus sp. Can. J. MIcrobiol.* **43**, 841-846.

Fennell, D. E. (1998). Comparison of Alternative Hydrogen Donors for Anaerobic Reductive Dechlorination of Tetrachloroethene. Ph.D. Dissertation, Cornell University, Ithaca, NY.

Fennell, D. E., Gossett, J. M. and Zinder, S. H. (1997) Comparison of Butyric Acid, Ethanol, Lactic Acid, and Propionic Acid as Hydrogen Donors for the Reductive Dechlorination of Tetrachloroethene. *Environ. Sci. Technol.* **31**, 918-926.

Garant, H. and Lynd, L. (1998) Applicability of Competitive and Noncompetitive Kinetics to the Reductive Dechlorination of Chlorinated Ethenes. *Biotechnol. Bioeng.* **57**, 751-755.

Löffler, F. E., Tiedje, J. M. and Sanford, R. A. (1999). Fraction of Electrons Consumed in Electron Acceptor Reduction and Hydrogen Thresholds as Indicators of Halorespiratory Physiology. *Appl. Environ. Microbiol.* **65**, 4049-4056.

Mackay, D. M. and Cherry, J. A. (1989) Groundwater Contamination: Pump-and-Treat Remediation. *Environ. Sci. Technol.* **23**, 630-636.

Maymo-Gatell, X., Tandoi, V., Gossett, J. M., and Zinder, S. H. (1995) Characterization of an H_2-Utilizing Enrichment Culture that Reductively Dechlorinates Tetrachloroethene to Vinyl Chloride and Ethene in the Absence of Methanogenesis and Acetogenesis. *Appl. Environ. Microbiol.* **61**, 3928-3933.

McCarty, P. L. (1997) Breathing with Chlorinated Solvents. *Science* **276**, 1521-1522.

National Research Council (1994) *Alternatives for Ground Water Cleanup*, National Academy Press, Washington DC.

Nielsen, R. B. and Keasling, J. D. (1999) Reductive Dechlorination of Chlorinated Ethene DNAPLs by a Culture Enriched from Contaminated Groundwater. *Biotechnol. Bioeng.* **62**, 160-165.

Rogers, J. B., DuTeau, N. M., and Reardon, K. F. (2000) Use of 16S-rRNA to Investigate Microbial Population Dynamics During Biodegradation of Toluene and Phenol by a Binary Culture *Biotechnol. Bioeng.* **70**, 436-445.

Sharma, P. K. and McCarty, P. L. (1996) Isolation and Characterization of a Facultatively Aerobic Bacterium That Reductively Dehalogenates Tetrachloroethene to cis-1,2-Dichloroethene *Appl. Eniron. Microbiol.* **62**, 761 – 765.

Smatlak, C. R., Gossett, J. M., and Zinder, S. H. (1996). Comparative Kinetics of Hydrogen Utilization for Reductive Dechlorination of Tetrachloroethene and Methanogenesis in an Anaerobic Enrichment culture. *Environ. Sci. Technol.* **30**, 2850-2858.

Tandoi, V., DiStefano, T. D., Bowser, P. A., Gossett, J. M., and Zinder, S. H. (1994) Reductive Dehalogenation of Chlorinated Ethenes and Halogenated Ethanes by a High-Rate Anaerobic Enrichment Culture. *Environ. Sci. Technol.* **28**, 973-979.

Tonnaer H., Alphenaar, A., Wit, H. D., Grutters, M., Spuij, F., Gerritse, J., and Gottschal, J. C. (1997) Modelling of Anaerobic Dechlorination of Chloroethenes for In-Situ Bioremediation. In *In Situ and On-Site Bioremediation*, papers from the Fourth International In Situ and On-Site Bioremediation Symposium, Ed. Alleman, B. C. and Leeson, A., Battelle Press, Columbus, OH, pp. 591-596.

U.S. EPA (2003) Consumer Factsheet on: Trichlorpethylene. U.S. Environmental Protection Agency, http://www.epa.gov/safewater/dwh/c-voc/trichlor.html.

Vancheeswaran, S. (1998) Abiotic and Biological Transformation of TBOS and TKEBS, and Their Role in the Biological Transformation of TCE and *c*-DCE. Master's Thesis, Oregon State University.

Vancheeswaran, S., Halden, R. U., Williamson, K. J., Ingle, J. D., and Semprini, L. (1999) Abiotic and Biological Transformation of Tetraalkoxysilanes and Trichloroethene/cis-Dichloroethene Cometabolism Driven by Tetrabutoxysilane-Degrading Microorganisms. *Environ. Sci. Technol.* **33**, 1077-1085.

Vancheeswaran, S., Yu, S., Daley, P., Halden, R. U., Williamson, K. J., Ingle, J. D., and Semprini, L. (2003) Intrinsic Remediation of Trichloroethene Driven by Tetraalkoxysilanes as Co-contaminants: Results of Microcosm and Field Studies. *Remediation*. **13**, 7-25.

Vogel, T. M., Criddle, C. S., and McCarty, P. L. (1987). Transformations of Halogenated Aliphatic Compounds. *Environ. Sci. Technol.* **21**, 722-736.

Yang, Y. and McCarty, P. L. (1998). Competition for Hydrogen within a Chlorinated Solvent Dehalogenating Anaerobic Mixed Culture. *Environ. Sci. Technol.* **32**, 3591-3597.

Yang, Y. and McCarty, P. L. (2000a) Biomass, Oleate, and Other Possible Substrates for Chloroethene Reductive Dehalogenation. *Biorem. J.* **4**, 125-133.

Yang, Y. and McCarty, P. L. (2000b) Biologically Enhanced Dissolution of Tetrachloroethene DNAPL. *Environ. Sci. Technol.* **34**, 2979-2984.

Yu, S. and Semprini, L. (2002a) Dechlorination of PCE DNAPL with TBOS Using a Binary Mixed Culture. In *Remediation of Chlorinated and Recalcitrant Compounds*, Battelle Press, Columbus, OH, Paper 2B-49.

CHAPTER 2

LITERATURE REVIEW

REDUCTIVE DECHLORINATION OF CHLORINATED ETHYLENES.

Reductive dechlorination is an important process for the microbial transformation of CAHs under anaerobic conditions. Especially for the transformation of highly chlorinated compounds like PCE and carbon tetrachloride (CT) which are inert to hydrolytic and oxidative reactions (Rittmann *et al.*, 1994). The CAHs are reduced by the process of hydrogenolysis with a two-electron transfer to the CAH and the replacement of a chlorine atom with a hydrogen atom (Assaf-Anid *et al.*, 1994; Vogel *et al.*, 1987). As shown in Figure 2-1, the reductive transformation of PCE proceeds via sequential dechlorination steps to replace each chlorine atom with a hydrogen atom resulting in TCE, DCE isomers (mainly *c*-DCE), VC and finally ETH as transformation intermediates and end products with hydrogen as an electron donor. The energy for this reductive mechanism is derived from the oxidation of other organic compounds/substrates that serve as electron donors for the reduction of transition metal ions which exist as coordination complexes at the active site of various enzymes and cofactors (Rittmann *et al.*, 1994). Once reduced, the transition metal ions are then available to catalyze the reduction of CAHs.

Many electron donors have been reported to stimulate reductive dechlorination in anaerobic mixed culture studies, such as sucrose (Carter and Jewell,

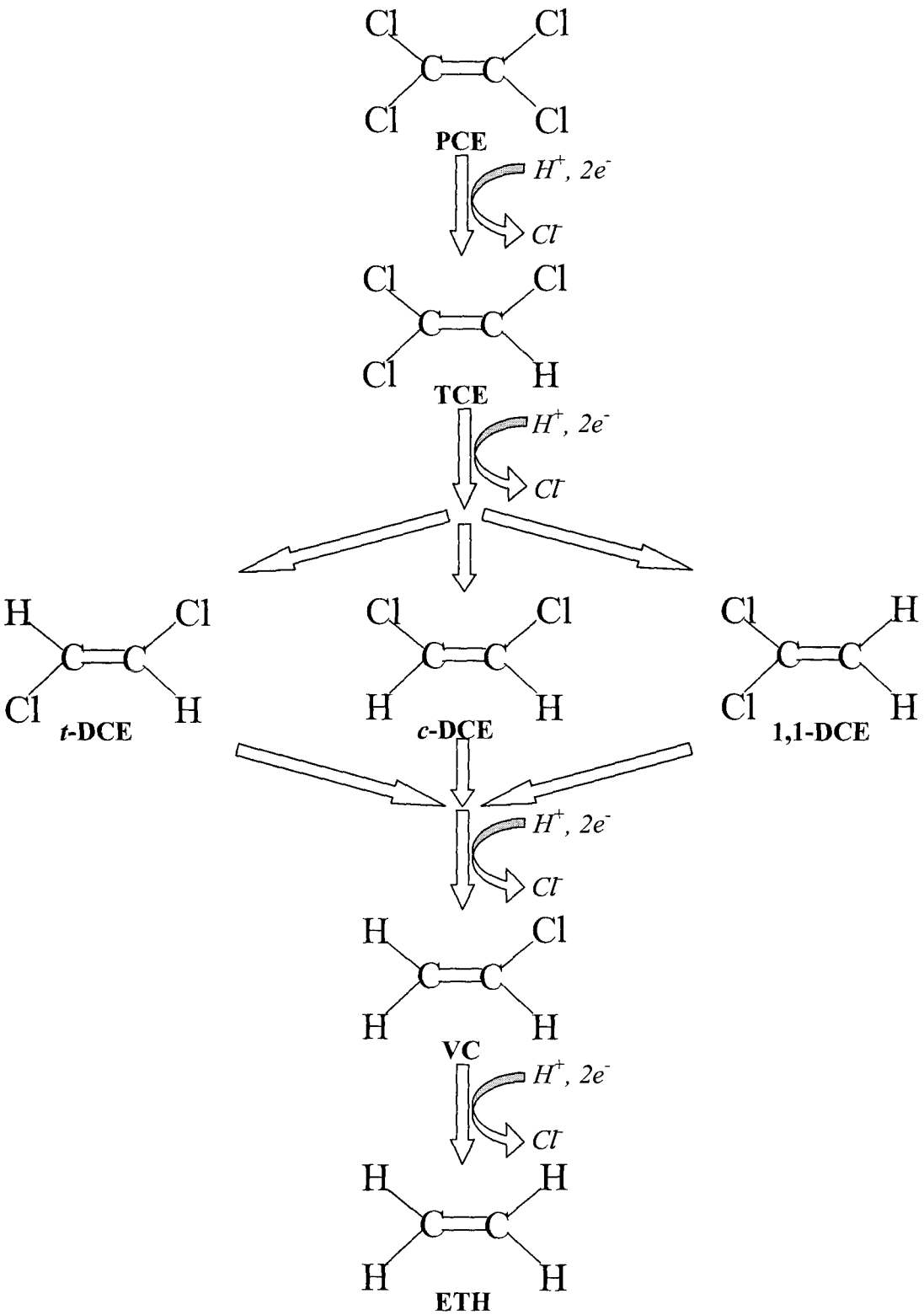

Figure 2-1. Pathway for anaerobic reductive dechlorination of PCE to ETH.

1993; Christiansen *et al.*, 1997), glucose (Bouwer and McCarty, 1983b; Freedman and Gossett, 1989), acetate (Bouwer and McCarty, 1983b; Freedman and Gossett, 1989; Vogel and McCarty, 1985), toluene (Liang and Grbic-Galic, 1993; Sewell and Gibson, 1991), benzoate (Scholz-Muramatsu *et al.*, 1990; Yang and McCarty, 1998), butyrate (Gibson *et al.*, 1994; Gibson and Sewell, 1992; Smatlak et al., 1996), lactate, propionate, ethanol (Gibson *et al.*, 1994; Gibson and Sewell, 1992; Fennell and Gossett, 1997; Ballapragada et al., 1997), methanol (Freedman and Gossett, 1989), H_2 (DiStefano *et al.*, 1992; Löffler *et al.*, 1999), slow-release organic substrates (Yang and McCarty, 2000; Yu and Semprini, 2002; Yang and McCarty, 2002), and the natural organic carbon present in aquifer material (Lyon, 1995).

Early reports indicated that PCE and TCE reductively dechlorinate to the DCE isomers and VC by mixed cultures under different anaerobic conditions (Bouwer *et al.*, 1981; Bouwer and McCarty, 1981a and 1981b; Vogel and McCarty, 1985; Vogel *et al.*, 1987). Freedman and Gossett (1989) provided the first evidence that PCE or TCE were completely transformed to non-toxic ETH by reductive dechlorination. It was later reported that ETH was transformed to ethane by dechlorinating cultures under anaerobic conditions (de Bruin *et al.*, 1992).

HYDROGEN AS THE ELECTRON DONOR FOR REDUCTIVE DECHLORINATION.

Even though a wide range of electron donors support dechlorination especially with mixed consortia, it has been shown that hydrogen produced via microbial fermentation activities is a key electron donor used in the reductive

dechlorination of PCE (DiStefano *et al.*, 1992; Fennell *et al.*, 1997). Freedman and Gossett (1989) showed that their methanol-enriched culture was able to dechlorinate PCE and TCE using hydrogen as an effective electron donor. Therefore, H_2 production and subsequent competition for its use are important issues to consider in selecting an electron donor for dechlorination. The reports concerning microorganisms that produce H_2 through breakdown activities and the competition for H_2, primarily between sulfate-reducing bacteria and methanogens are extensive. Fermentations of alcohols and short-chain volatile fatty acids (VFAs) to H_2 are carried out by syntrophic, obligate proton-reducing organisms and, under sulfate-depleted conditions, by sulfate-reducing bacteria (Fennell, 1998). H_2 is commonly utilized under anaerobic conditions for the reduction of CO_2 to CH_4 by methanogens, the reduction of sulfate to sulfides by sulfate reducers and the production of acetate by homoacetogenesis bacteria (Uberoi and Bhattacharya, 1995; Cord-Ruwisch, 1988).

Interspecies Hydrogen Transfer and Syntrophy.

As an important intermediate in the degradation of complex organic matter in anaerobic systems, H_2 plays a crucial role in the complete biotic transformation of organic compounds by anaerobic microorganisms including fermenters, methanogens, acetogens, and sulfate reducers. This is especially the case for the fermentation of alcohols and higher chained fatty acids such as propionate, butyrate, benzoate, and amino acids to acetate and hydrogen. These transformations are

generally endergonic under standard conditions and are not thermodynamically favorable (Zehnder and Stumn, 1988; Madigan *et al.*, 1997). The transformation of these compounds is not possible by fermenters alone. In the presence of hydrogenotrophic microorganisms (hydrogen consumers) like methanogens, homoacetogens, and sulfate-reducers, the overall transformation of higher chained fatty acids and amino acids becomes thermodynamically favorable. This syntrophic relationship between hydrogen producers and hydrogen consumers is called interspecies hydrogen transfer (Zehnder and Stumn, 1988; Madigan *et al.*, 1997). By utilizing the available hydrogen in a given system, hydrogenotrophs remove hydrogen from the system and drive down its partial pressure to levels which make the fermentation reactions thermodynamically favorable (exergonic). For example, the ethanol fermenter produces hydrogen and acetate, but this reaction has an unfavorable (positive) standard free-energy balance (+ 19.36 kJ/reaction). However, the hydrogen produced by the ethanol fermenter is consumed by the methanogen in an energetically favorable reaction. When the energies of these two reactions are summed, the overall reaction is favorable energetically (- 111.33 kJ/reaction) (Madigan *et al.*, 1997).

Hydrogen Threshold and Competition between Hydrogenotrophs.

Many organic compounds, such as organic acids and alcohols, have been studied as potential electron donors to promote anaerobic dechlorination (Fennell *et al.*, 1997). Complex organic materials are fermented to less complex substrates,

producing H_2, which can serve directly as an electron donor for reductive dechlorination (Maymo-Gatell *et al.*, 1995). However, other microorganisms including methanogens, homoacetogens, and sulfidogens compete for H_2 as an electron donor in the subsurface environments (McCarty, 1997). Enhanced bioremediation may require the use of electron donors that are efficiently used for anaerobic dechlorination (Yang and McCarty, 1998). Many researchers have physiologically and thermodynamically studied hydrogen competition for a variety of hydrogenotrophic microbial consortiums. Dechlorinators were shown to have a lower hydrogen threshold concentration of 0.3 nM (Löffler *et al.*, 1999) than acetogens (336-3640 nM) (Breznak, 1994; Cord-Ruwisch *et al.*, 1988), methanogens (5-95 nM) (Conrad, 1996; Cord-Ruwisch *et al.*, 1988; Lovely, 1985; Lovely and Goodwin, 1988), and sulfidogens (1-15 nM) (Conrad, 1996; Cord-Ruwisch *et al.*, 1988; Lovely and Goodwin, 1988). Other studies reported a hydrogen threshold in the range of 0.05-11 nM for dechlorinators (Smatlak *et al.*, 1996; Yang and McCarty, 1998), suggesting that anaerobic dechlorinators can outcompete the other hydrogenotrophs at low hydrogen concentrations. In addition to the physiological hydrogen threshold, free energy calculations indicate a lower hydrogen threshold for dechlorinators than for other hydrogenotrophs (Smatlak *et al.*, 1996). Several studies revealed that an electron donor that is slowly and steadily transformed to maintain low H_2 concentrations could optimize anaerobic dechlorination and limit methanogenesis (Smatlak *et al.*, 1996; Yang and McCarty, 1998; Fennell *et al.*, 1997; Ballapragada *et al.*, 1997; Fennell and Gossett, 1998).

REDUCTIVE DECHLORINATION OF PCE AND TCE DNAPLs.

PCE and TCE are common and recalcitrant contaminants in soil and groundwater. PCE and TCE with densities of 1.63 and 1.46 g/cm^3, respectively being denser than water, tend to sink downward in subsurface forming a separate dense non-aqueous phase liquid (DNAPL). Contaminated sites with DNAPLs are among the most difficult to remediate, and PCE and TCE in a DNAPL phase can be long-term sources of soil and groundwater contamination due to their relatively low solubilities (Rittmann *et al.*, 1994).

DiStefano *et al.* (1991) initiated an early study of the anaerobic transformation of high concentrations of chlorinated ethylenes. Introduced PCE concentrations as high as 550 μM (approximately 55 mg/L aqueous concentration) were routinely dechlorinated to 80% ETH and 20% VC within 2 days at 35°C. In the study, anaerobic methanol-PCE enrichment cultures which proved capable of dechlorinating high concentrations PCE to ETH were developed.

Nielsen and Keasling (1999) reported the reductive dechlorination of PCE and TCE at their solubility limits by a TCE enriched mixed culture grown on glucose. The results from the initial dechlorination rate studies indicated that both PCE and TCE dechlorination rates increased with increasing concentration, and that no decrease in rate was found even near their respective solubility limits. The studies of the initial dechlorination rate also showed that the presence of VC had little or no effect on the TCE dechlorination for the range of VC concentrations used. The results suggested that the accumulation of VC that might occur during the anaerobic

dechlorination of a PCE or TCE contaminated aquifer should not inhibit the dechlorination activity to the point were it would cease. The study by Nielsen and Keasling (1999) implied that reductive PCE and TCE dechlorination could be used in sites contaminated with high PCE and TCE concentrations that would exist near PCE and TCE DNAPLs.

An early modeling study suggested that biodegradation can increase the dissolution flux from DNAPL contamination source (Seagren *et al.*, 1994). Recent studies reported that the enhancement of PCE DNAPL dissolution can be achieved by biotransformation process (Carr et al., 2000; Yang and McCarty, 2000; Cope and Hughes, 2001). Experiments in continuous-flow stirred tank reactors (CFSTRs) demonstrated that microbial reductive dechlorination resulted in a factor of 14 increase in PCE removal rates from the NAPL (Carr et al., 2000). Yang and McCarty (2000b) showed that the dissolution rate from PCE DNAPL was substantially enhanced by up to a factor of 5 in continuously fed column when directly coupled with biological dechlorination. Another column study demonstrated that biological dechlorination led to enhanced PCE removal, by up to a factor of 16 (Cope and Hughes, 2001). These recent studies for biological enhancement of PCE DNAPL dissolution suggested that biological dechlorination can significantly increase removal rates of CAHs from DNAPLs. This process may be a key factor to determine the duration and effectiveness of bioremediation strategies for DNAPL zone treatment.

KINETICS AND MODELING STUDY OF REDUCTIVE DECHLORINATION.

The model developed in this study encompasses the kinetics of reductive dechlorination over a wide range of PCE and TCE concentrations, inhibitions among chlorinated ethylenes, and the growth of microorganisms. Kinetics of dechlorination were of Michaelis-Menten form wherein the rate of dechlorination was described by the CAH concentrations, but not by the H_2 or electron donor concentrations, since the electron donors were maintained at sufficient levels to avoid concentration limitations during the experiments. A review of previous modeling work is provided below.

Kinetic Models for Reductive Dechlorination.

A number of models have been developed to describe anaerobic reductive dechlorination (Fennell and Gossett, 1998; Tandoi *et al.*, 1994; Tonnaer *et al.*, 1997; Garant and Lynd, 1998; Bagley, 1998; Haston, 1999). Tandoi *et al.* (1994) assumed that VC transformation is competitively inhibited by the other chlorinated ethylenes during complete reductive dechlorination of PCE to ETH, and the simulation model was compared with experimental data. Fennell and Gossett (1998) simulated both fermentation of electron donors and competition for the evolved H_2 between hydrogenotrophic tetrachloroethylene dechlorinators and methanogens, but the comprehensive biokinetic model employing Michaelis-Menten-type kinetics did not consider inhibition between chlorinated ethylenes. Another model was developed that can depict and predict reductive dechlorination of PCE by incorporating

competitive dechlorination between PCE and TCE (Tonnaer *et al.*, 1997). Garant and Lynd (1998) showed that competitive kinetics for complete reductive dechlorination of PCE achieved better chi-squared and visual fits of the experimental data than noncompetitive inhibition. Haston (1999) reported competitive inhibition between *c*-DCE and VC, and developed a competitive inhibition model to explain his experimental data. However, the above models simulated reductive dechlorination of PCE concentrations typically lower than 300-400 μM. The following sections discuss inhibition models for reductive dechlorination over a wide range of PCE and TCE concentrations up to solubility limit.

Competitive Inhibition Model.

Many researchers have found that competitive inhibition model predicts reductive dechlorination of CAHs better than non-competitive inhibition (Garant and Lynd, 1998; Haston, 1999; Tonnaer *et al.*, 1997). If a dechlorinating enzyme is responsible for dechlorination of two different CAHs, they may affect degradation of each other in a variety of ways. The two CAHs can compete for the same dechlorinating enzyme. Then, the simple Michaelis-Menten equation 2-1 for dechlorination of one CAH can be modified to include the effect of competition of the other CAH as shown in Equation 2-2 (Bailey and Ollis, 1986).

$$\frac{dC}{dt} = \frac{-k_{\max} XC}{K_S + C} \tag{2-1}$$

$$\frac{dC}{dt} = \frac{-k_{\max} XC}{K_S\left(1+\frac{C_I}{K_{CI}}\right)+C} \tag{2-2}$$

$$\frac{dX}{dt} = Y\frac{dC}{dt} - k_d X \tag{2-3}$$

where C is the CAH aqueous concentration (μM), k_{max} is the maximum specific CAH dechlorination rate (μmol/mg of protein/day), K_S is the half-velocity coefficient (μM), and competitive inhibition constants of each chlorinated ethylene, K_{CI} (μM). The K_{CI} value might be set equal to their respective half-velocity coefficients (K_S) based on previously reported results (Haston, 1999). X is total cell concentration (mg of protein/L), and microbial growth is calculated with respect to the transformation rate of each chlorinated ethylene. In the model for biomass growth, values for growth yield, Y (=0.006 mg of protein/μmol of Cl^- dechlorinated), and decay constant, k_d (=0.024 day^{-1}), can be obtained from the literature (Maymo-Gatell *et al.*, 1997; Fennell and Gossett, 1998).

Haldane Inhibition Model.

Haldane inhibition (Bailey and Ollis, 1986) might be used to model toxicity resulting from high CAH concentrations. The biomass growth rate is proportional to the degradation rate of CAHs. However, growth may be limited by inhibition or toxicity of CAHs. A Haldane kinetic model (Bailey and Ollis, 1986) is generally applied:

$$\frac{dC}{dt} = \frac{-k_{max} XC}{K_S + C\left(1 + \frac{C}{K_{HI}}\right)} \quad (2\text{-}4)$$

where K_{HI} is the Haldane inhibition constant (μM). Maillard *et al.* (2003) observed that the degradation rates of PCE and TCE were inhibited at high concentrations (~1000 μM).

SLOW-RELEASE ANAEROBIC SUBSTRATE FOR REDUCTIVE DECHLORINATION.

Gibson *et al.* (1994) reported that lactate was quickly fermented and probably did not persist long enough to support dechlorination, while butyrate persisted for a longer period of time and was a better electron donor to stimulate reductive dechlorination. Fennell *et al.* (1997) also observed that amendment with butyrate and propionate led to less methanogenesis than did amendment with ethanol or lactate, which generated much higher H_2 concentrations. These results suggested approaches that may be used to impart a competitive advantage to dechlorinating microorganisms in bioremediation. Efficient dechlorination to outcompete other hydrogenotrophs can be achieved through the use of an appropriate hydrogen precursor that is slowly degradable so that it can release hydrogen in a slow manner to maintain the ideal hydrogen concentration.

Two different types of slow-release or -fermenting anaerobic substrates have been studied and implemented at CAH-contaminated sites. One type includes butyrate, propionate, and benzoate, which are useful with a continuous injection

system (Fennell *et al.*, 1997; Yang and McCarty, 1998). The other is edible oils, such as vegetable oil or olive oil, and silicon-based organic compounds such as tetrabutoxysilane (TBOS) and tetrakis(2-ethylbutoxy)silane (TKEBS) (Yu and Semprini, 2002; Yang and McCarty, 2000a and 2002; Bell *et al.*, 2001; Wiedemeier and Henry 2001), which potentially have an advantage in that chlorinated solvents could easily partition into the slow-release substrates injected near a DNAPL zone, thus reducing the aqueous concentrations of PCE or TCE, and potentially reducing toxicity and inhibition. Therefore, these concentration reductions may actually enhance rates of reductive dechlorination (Yu and Semprini, 2002).

GENERAL CHARACTERISTICS AND TRANSFORMATIONS OF SILICON-BASED ORGANIC COMPOUNDS.

TBOS ($Si(CH_3CH_2CH_2CH_2O)_4$) and TKEBS ($Si(CH_3CH_2CH(CH_2CH_3)CH_2O)_4$) are silicon-based compounds having four oxygen branches with butyl and 2-ethylbutyl groups, respectively, that are primarily used as heat-transfer fluids, hydraulic fluids, and lubricants. No report is available about the contamination of subsurface by these compounds, and Site-300 at Lawrence Livermore National Laboratory, CA (LLNL) is probably unique as a TBOS and TKEBS-contaminated site. These compounds were observed to act as light non-aqueous phase liquids (LNAPLs) and mix well with CAHs (Table 2-1). They have a low solubility in water and thus slowly dissolve. The silicon-based compounds have been shown to support anaerobic dechlorination of TCE at chlorinated solvent-

Table 2-1. Physical properties of tetraalkoxysilanes and the corresponding alcohols (adapted from Vancheeswaran, 1998).

Property	Compound			
	TBOS ($C_{16}H_{36}O_4Si$)	TKEBS ($C_{24}H_{52}O_4Si$)	1-Butanol (C_4H_9OH)	2-Ethyl butanol ($C_6H_{13}OH$)
Mol. Wt. (g/mol)	320.5	432.8	74	102
Boiling Pt. (°C)	115	166	117.7	146
Melting Pt. (°C)	-145	N/A	-90	-15
Density (g/mL)	0.899	0.892	0.81	0.83
Refractive Index	1.41	1.43	1.399	1.422
Solubility (mg/L) @ 20°C	< 1	< 1	74,000	N/A
Viscosity @ 38°C (cSt)[a]	2.33	4.35	N/A	N/A
Surface Tension (dyn/cm)	22.8	22.8	N/A	N/A

[a]cSt: centistokes = 0.01 cm2/sec.

contaminated sites and with laboratory cultures (Vancheeswaran *et al.*, 1999; Yang and McCarty, 2000a; Yu and Semprini, 2002; Vancheeswaran *et al.*, 2003). Evidence for the transformation pathway of alkoxysilanes, mainly TKEBS, and their potential to serve as substrates for promoting anaerobic TCE transformation was discovered through analysis of groundwater from LLNL (Vancheeswaran et al., 2003). Based on the field-monitoring data, TKEBS was observed to slowly hydrolyze to 2-ethylbutanol (Figure 2-2) and ferment to 2-ethylbutyrate (Vancheeswaran *et al.*, 1999; Vancheeswaran et al., 2003). TBOS abiotically and slowly hydrolyze to 1-butanol (Figure 2-2), which ferments to butyrate and/or acetate, producing H_2 during the fermentation process. Butyrate could then serve as a slowly fermenting substrate to drive reductive dechlorination (Yu and Semprini, 2002). Butyrate and propionate have also been reported as low concentration level H_2-producing substrates favored by dechlorinators rather than methanogens (Fennell *et al.*, 1997).

Dehalococcoids-LIKE MICROORGANISMS.

Chlorinated ethylenes were previously believed to be recalcitrant to degradation by microbial processes. There now is rapidly accumulating laboratory and field evidence that microorganisms can transform chlorinated ethylenes to nontoxic products under a variety of environments (Bolesch *et al.*, 1997; de Bruin *et al.*, 1992; Deweerd *et al.*, 1998; DiStefano *et al.*, 1991; Ellis *et al.*, 2000; Freedman and Gossett, 1989; Holliger *et al.*, 1998; Lee *et al.*, 1998; Maymo-Gatell *et al.*, 1995;

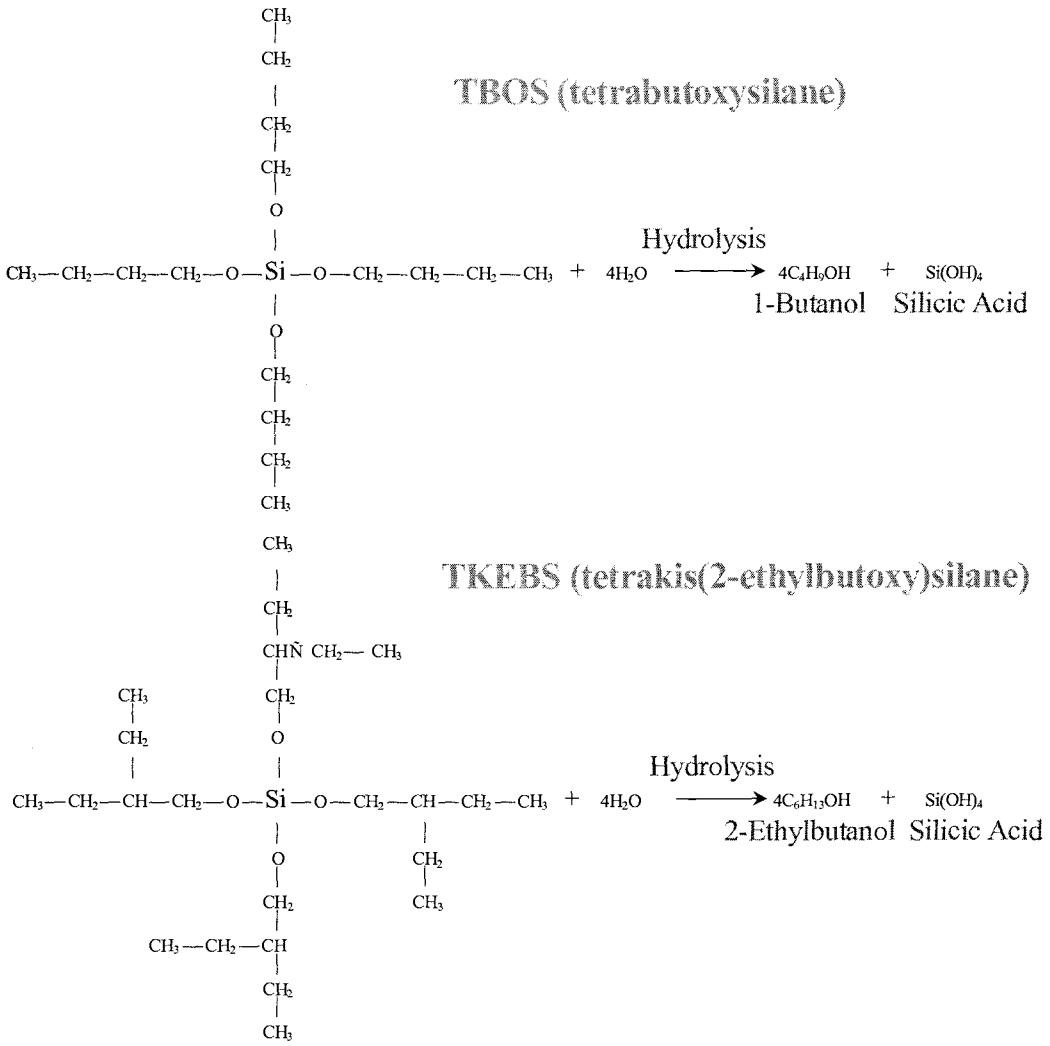

Figure 2-2. Structures of TBOS and TKEBS and their hydrolysis reactions.

Nielsen and Keasling, 1999; Odom *et al.*, 1995; Scholz-Muramatsu *et al.*, 1995).

Dehalococcoides-like microorganisms are listed in Table 2-2, which were divided into three subgroups based on specific base substitution patterns in variable regions 2 and 6 of the *Dehalococcoides* 16S rDNA sequences: Cornell, Victoria, and Pinellas cultures (Hendrickson *et al.*, 2002). Maymo-Gatell *et al.* (1997 and 1999) isolated the first pure culture from an enrichment dechlorinating culture inoculated with municipal sewage sludge, which completely dechlorinates chlorinated ethenes. This pure culture, called *Dehalococcoides ethenogenes* strain 195, was grown on PCE, TCE, and *c*-DCE with H_2 as an electron donor (Maymo-Gatell, 1997). A *Dehalococcoides*-like microorganism, *Dhc.* sp. strain VS, was identified in a mixed enrichment culture initially obtained from a PCE-contaminated site in Victoria, TX. The growth of bacterium VS was found to be coupled to the dehalogenation of *c*-DCE and VC, but this bacterium showed little TCE dechlorination and no PCE dechlorination (Cupples *et al.*, 2003). *Dhc.* sp. strain FL2, one of the Pinellas group, exhibited dechlorination of TCE and *c*-DCE with cometabolic transformation of PCE and VC (Löffler *et al.*, 2003), whereas BAV1 is the first isolate capable of the metabolic dechlorination of all DCE isomers and VC. PCE and TCE were cometabolized in the presence of a growth-supporting chlorinated ethylenes and ETH was produced (He *et al.*, 2003a and 2003b).

It is very important to determine kinetic parameter for characterizing dechlorinating microorganisms and predicting CAH dechlorination rate in the CAH-contaminated sites. Kinetic parameters such as k_{max} and K_S of several

Dehalococcoides-like microorganisms are listed in Table 2-3. *Dhc. Ethenogenes* strain 195 showed relatively high K_S value compared to those of other dechlorinating bacteria, since VC dechlorination to ETH by the dechlorinator is observed to be cometabolic (Maymo-Gatell *et al.*, 1997), while the other dechlorinators growing on VC show much lower K_S values. For site cleanup of CAH-contaminated groundwater, when the CAH concentrations become close to K_S value, the dechlorination rates can decrease significantly from maximum dechlorination rates. Thus, K_S values as well as k_{max} could have a great impact on overall dechlorination rate in intrinsic or engineered bioremediation.

Table 2-2. Defined *Dehalococcoides*-like populations (adapted from He *et al.*, 2003a).

Dehalococcoides sp.	Electron Acceptor for Cell Growth
Dhc. Ethenogenes strain 195 (C, AF004928.2)[a]	PCE, TCE, *c*-DCE, 1,1-DCE, 1,2-DCA, 1,2-dibromoethane
Dhc. sp. strain VS (V, AF388550)[b]	*c*-DCE, VC
Dhc. sp. strain FL2 (P, AF357918.2)[c]	TCE, *c*-DCE
Dhc. sp. strain BAV1 (P, AY165308)[d]	*c*-DCE, *trans*-DCE, 1,1-DCE, VC, vinyl bromide, 1,2-DCA
Dhc. sp. strain CBDB1 (P, AF230641)[e]	1,2,3-TCB, 1,2,4-TCB, 1,2,3,4-TeCB, 1,2,4,5-TeCB, PCDD

[a]Maymo-Gatell *et al.*, 1997. [b]Cupples *et al.*, 2003. [c]Löffler *et al.* 2003. [d]He *et al.* 2003a. [e]Adrian *et al.*, 2000 and Bunge *et al.*, 2003.

Table 2-3. Maximum specific dechlorination rates (k_{max}) and half-velocity coefficients (K_S) for different *Dehalococcoides*-like populations.

		PCE	TCE	*c*-DCE	VC
Dhc. Ethenogenes strain 195	k_{max} (μmol/mg-VSS/day)	43.2[a]	72.0[a]	72.0[a]	72.0[a]
	K_S (μM)	0.54[a]	0.54[a]	0.54[a]	290[a]
Dhc. sp. strain VS	k_{max} (μmol/mg-VSS/day)	2.0[b]	1.6[b]	0.37[b]	0.34[b]
	K_S (μM)	0.11[b]	1.4[b]	3.3[b]	2.6[b]
Dhc. sp. strain BAV1	$k_{max}X$ (μM/day)			23.1[c]	54.4[c]
	K_S (μM)			8.9[c]	5.8[c]

[a]Fennell and Gossett (1998). [b]Haston and McCarty (1999). [c]He *et al.* (2003b)

REFERENCES

Adrian, L., Szewzyk, U., Wecke, J., and Görisch, H. (2000) Bacterial Dehalorespiration with Chlorinated Benzenes. *Nature* **408**, 580-583.

Assaf-Anid, N., Hayes, K. F., and Vogel, T. M. (1994). Reductive dechlorination of carbon tetrachloride by cobalamin(II) in the presence of dithiothreitol: Mechanistic study, effect of redox potential and pH. *Environ. Sci. Technol.* **28**, 246-252.

Bagley, D. M. (1998) Systematic Approach for Modeling Tetrachloroethene Biodegradation. *J. Environ. Eng.* **124**, 1076-1086.

Bailey, J.and Ollis, D. (1986) *Biochemical Engineering Fundamentals*, McGraw-Hill, New York, NY.

Ballapragada, B. S., Stensel, H. D., Puhakka, J. A., and Ferguson, J. F. (1997). Effect of Hydrogen on Reductive Dechlorination of Chlorinated Ethenes. *Environ. Sci. Technol.* **31**, 1728-1734.

Bell, M., Brown, D., Burns, D. A., and Lee, M. D. (2001) Biologically Enhanced Reductive Dechlorination. Platform presentation. In Sixth In Situ and On-Site Bioremediation Symposium, San Diego, CA.

Bolesch, D., Nielsen, R., and Keasling, J. (1997) Complete Reductive Dechlorination of Trichloroethene by a Groundwater Microbial Consortium. Ann. N. Y. Acad. Sci., **829**, 97-102.

Bouwer, E. J. and McCarty, P. L. (1983a). Transformations of 1- and 2-Carbon Halogenated Aliphatic Organic Compounds under Methanogenic Conditions. *Appl. Environ. Microbiol.* **45**, 1286-1294.

Bouwer, E. J. and McCarty, P. L. (1983b). Transformations of Halogenated Organic Compounds under Denitrification Conditions. *Appl. Environ. Microbiol.* **45**, 1295-1299.

Bouwer, E. J., Rittmann, B. E., and McCarty, P. L. (1981). Anaerobic Degradation of Halogenated 1- and 2-Carbon Organic Compounds. *Environ. Sci. Technol.* **15**, 596-599.

Breznak, J. A. (1994) Acetogenesis from Carbon Dioxide in Termite Guts. In *Acetogenesis*. Ed. Drake, H. L. Chapman and Hall, Inc., New York, NY, pp. 303-330.

Bunge, M., Adrian, L., Kraus, A., Opel, M., Lorenz, W. G., Andreesen, I. R., Gorisch, H., and Lechner, U. (2003) Reductive Dehalogenation of Chlorinated Dioxins by an Anaerobic Bacterium. *Nature* **421**, 357-360.

Carr, C. S., Garg, S., and Hughes, J. B. (2000) Effect of Dechlorinating Bacteria on the longevity and Composition of PCE-Containing Nonaqueous Phase Liquids under Equilibrium Dissolution Conditions. *Environ. Sci. Technol.* **34**, 1088-1094.

Carter, S. R. and Jewell, W. J. (1993). Biotransformation of Tetrachloroethylene by Anaerobic Attached-Films at Low Temperatures. *Water Res.* **27**, 607-615.

Christiansen, N., Christensen, S. R., Arvin, E., and Ahring, B. K. (1997). Transformation of Tetrachloroethene in an Upflow Anaerobic Sludgeblanket Reactor. *Appl. Environ. Microbiol.* **47**, 91-94.

Conrad, R. (1996) Soil Microorganisms as Controllers of Atmospheric Trace Gases (H_2, CO, CH_4, OCS, N_2O, and NO). *Microbiol. Rev.* **60**, 609-640.

Cope, N. and Hughes, J. B. (2001) Biologically-Enhanced Removal of PCE from NAPL Source Zones. *Environ. Sci. Technol.* **35**, 2014-2021.

Cord-Ruwisch, R., Steitz, H. J., and Conrad, R. (1988) The Capacity for Hydrogenotrophic Anaerobic Bacteria to Compete for Traces of Hydrogen Depends on the Redox Potential of the Terminal Electron Acceptor. *Arch. Microbiol.* **149**, 350-357.

Cupples, A. M., Spormann, A. M., and McCarty, P. L. (2003) Growth of a Dehalococcoides-Like Microorganism on Vinyl Chloride and cis-dichloroethene as Electron Acceptors as Determined by Competitive PCR. *Appl. Environ. Microbiol.* **69**, 953-959.

de Bruin, W. P., Kotterman, J. J. J., Posthumus, M. A., Schraa, G., and Zehnder, A. J. B. (1992). Complete Biological Reductive Transformation of Tetrachloroethylene to Ethane. *Appl. Environ. Microbiol.* **58**, 1996-2000.

DeWeerd, K. A., Flanagan, W. P., Brennan, M. J., Principe, J. M., and Spivack, J. L. (1998) Biodegradation of Trichloroethylene and Dichloroethylene in Contaminated Soil and Groundwater. *Biorem. J.* **2**, 29-42.

DiStefano, T. D., Gossett, J. M., and Zinder, S. H. (1992). Hydrogen as an Electron Donor for the Dechlorination of Tetrachloroethene by an Anaerobic Mixed Culture. *Appl. Environ. Microbiol.* **58**, 3622-3629.

DiStefano, T. D., Gossett, J. M., and Zinder, S. H. (1991) Reductive Dechlorination of High Concentrations of Tetrachloroethene to Ethene by an Anaerobic Enrichment Culture in the Absence of Methanogenesis. *Appl. Environ. Microbiol.* **57**, 2287-2292.

Ellis, D. E., Lutz, E. J., Odom, J. M., Buchanan, Jr., R. J., Lee, M. D., Bartlett, C. L., Harkness, M. R., and Deweerd, K. A. (2000) Bioaugmentation for Accelerated In Situ Anaerobic Bioremediation. *Environ. Sci. Technol.* **34**, 2254-2260.

Fennell, D. E. (1998). Comparison of Alternative Hydrogen Donors for Anaerobic Reductive Dechlorination of Tetrachloroethene. Ph.D. Dissertation, Cornell University, Ithaca, NY.

Fennell, D. E. and Gossett, J. M. (1998) Modeling the Production of and Competition for Hydrogen in a Dechlorinating Culture. *Environ. Sci. Technol.* **32**, 2450-2460.

Fennell, D. E., Gossett, J. M., and Zinder, S. H. (1997) Comparison of Butyric Acid, Ethanol, Lactic Acid, and Propionic Acid as Hydrogen Donors for the Reductive Dechlorination of Tetrachloroethene. *Environ. Sci. Technol.* **31**, 918-926.

Freedman, D. L. and Gossett, J. M. (1989). Biological Reductive Dechlorination of Tetrachloroethylene and Trichloroethylene to Ethylene under Methanogenic Conditions. *Appl. Environ. Microbiol.* **55**, 2144-2151.

Garant, H. and Lynd, L. (1998) Applicability of Competitive and Noncompetitive Kinetics to the Reductive Dechlorination of Chlorinated Ethenes. *Biotechnol. Bioeng.* **57**, 751-755.

Gibson, S. A. and Sewell, G. W. (1992). Stimulation of Reductive Dechlorination of Tetrachloroethene in Anaerobic Aquifer Microcosms by Addition of Short-Chain Organic or Alcohols. *Appl. Environ. Microbiol.* **58**, 1392-1393.

Gibson, S. A., Roberson, D. S., Russell, H. H., and Sewell, G. W. (1994). Effects of Three Concentrations of Mixed Fatty Acids on Dechlorination of Tetrachloroethene in Aquifer Microcosms. *Environ. Toxicol. Chem.* **13**, 453-460.

Haston, Z. C. (1999). Factors Affecting Growth and Utilization in the Anaerobic Dehalogenation of Chlorinated Ethenes. Ph.D. Dissertation, Stanford University, Standord, CA.

He, J., Ritalahti, K. M., Aiello, M. R., and Löffler, F. E. (2003b) Complete Detoxification of Vinyl Chloride by an Anaerobic Enrichment Culture and Identification of the Reductively Dechlorinating Population as a *Dehalococcoides* Species. *Appl. Environ. Microbiol* **69** (2), 996-1003.

He, J., Ritalahti, K. M., Yang, K.-L., Koenigsberg, S. S., and Löffler, F. E. (2003a) Detoxification of Vinyl Chloride to Ethene Coupled to Growth of an Anaerobic Bacterium. *Nature* **424**, 62-65.

Hendrickson, E. R., Payne, J. A., Young, R. M., Starr, M. G., Perry, M. P., Fahnestock, S., Ellis, D. E., and Ebersole, R. C. (2002) Molecular Analysis of Dehalococcoides 16S Ribosomal DNA form Chloroethene-Contaminated Sites through North America and Europe. *Appl. Environ. Microbiol.* **68**, 485-495.

Holliger, C., Hahn, D., Harmsen, H., Ludwig, W., Schumacher, W., Tindall, B., Vazquez, F., Weiss, N., and Zehnder, A. J. B. (1998) *Dehalobacter restrictus* gen. nov. and sp. nov., a Strictly Anaerobic Bacterium that Reductively Dechlorinates Tetra- and Trichloroethene in Anaerobic Respiration. *Arch. Microbiol.* **169**, 313-321.

Lee, M. D., Odom, J. M., and Buchanan, Jr., R. J. (1998) New Perspectives on Microbial Dehalogenation of Chlorinated Solvents: Insights from the Field. *Annu. Rev. Microbiol.* **52**, 423-452.

Liang, L.-N. and Grbic-Galic, D. (1993). Biotransformation of Chlorinated Aliphatic Solvents in the Presence of Aromatic Compounds under Methanogenic Conditions. *Environ. Toxicol. Chem.* **12**, 1377-1393.

Löffler, F. E., Cole, J. R., Ritalahti, K. M., and Tiedje, J. M. (2003) In *Dehalogenation: Microbial Processes and Environmental Applications*, Ed. Hägbblom, M. M. and Bossert, I. D., Kluwer Academic, New York, NY., pp. 53-87.

Löffler, F. E., Tiedje, J. M., and Sanford, R. A. (1999). Fraction of Electrons Consumed in Electron Acceptor Reduction and Hydrogen Thresholds as Indicators of Halorespiratory Physiology. *Appl. Environ. Microbiol.* **65**, 4049-4056.

Lovely, D. R. (1985) Minimum Threshold for Hydrogen Metabolism in Methanogenic Bacteria. *Appl. Environ. Microbiol.* **49**, 1530-1531.

Lovely, D. R. and Goodwin, S. (1988) Hydrogen Concentrations as an Indicator of the Predominant Terminal Electron-Accepting Reactions in Aquatic Sediments. *Geochim. Cosmochim. Acta.* **52**, 2993-3003.

Lyon, W. G., West, C. C., Osborn, M. L., and Sewell, G. W. (1995) Microbial Utilization of Vadose Zone Organic Carbon for Reductive Dechlorination of Tetrachloroethene. *J. Environ. Sci. Health., Part A* **A30**, 1627-1639.

Madigan, M. T., Martinko, J. M., and Parker. J. (1997) *Brock, Biology of Microorganisms*. Eight Edition, Prentice Hall, Upper Saddle River, NJ.

Maymo-Gatell, X., Anguish, T., Gossett, J. M., and Zinder, S. H. (1999) Reductive Dechlorination of Chlorinated Ethene and 1,2-Dichloroethane by "*Dehalococcoides Ethenogenes*" 195. *Appl. Environ. Microbiol.* **65**, 3108-3113.

Maymo-Gatell, X., Chien, Y.-T., Gossett, J. M., and Zinder, S. H. (1997) Isolation of Bacterium that Reductively Dechlorinates Tetrachloroethene to Ethene. *Science* **276**, 1568-1571.

Maymo-Gatell, X., Tandoi, V., Gossett, J. M., and Zinder, S. H. (1995) Characterization of an H_2-Utilizing Enrichment Culture that Reductively Dechlorinates Tetrachloroethene to Vinyl Chloride and Ethene in the Absence of Methanogenesis and Acetogenesis. *Appl. Environ. Microbiol.* **61**, 3928-3933.

McCarty, P. L. (1997) Breathing with Chlorinated Solvents. *Science* **276**, 1521-1522.

Nielsen, R. B. and Keasling, J. D. (1999) Reductive Dechlorination of Chlorinated Ethene DNAPLs by a Culture Enriched from Contaminated Groundwater. *Biotechnol. Bioeng.* **62**, 160-165.

Odom, J. M., Tabinowski, J. A., Lee, M. D., and Fathepure, B. Z. (1995) Anaerobic Biodegradation of Chlorinated Solvents: Comparative Laboratory Study of Aquifer Microcosms, In *Bioremediation of Chlorinated Solvents*, Ed. Hinchee, R. E., Leeson, A. and Semprini, L., Battelle Press, Columbus, OH., **1**, 17-24.

Rittmann, B. E., Seagren, E., and Wrenn, B. A. (1994). In *In-Situ Bioremediation*. Second Edition. Parkridge, New Jersey, USA: Noyes Publications, pp. 37-50.

Scholz-Muramatsu, H., Neumann, A., Messmer, M., Moore, E., and Diekert, G. (1995) Isolation and Characterization of *Dehalospirillum multivorans* gen.

nov., sp. nov., a Tetrachloroethene-Utilizing, Strictly Anaerobic Bacterium. *Arch. Microbiol.* **163**, 48-56.

Scholz-Muramatsu, H., Szewzyk, R., Szewzyk, U., and Gaiser, S. (1990). Tetrachloroethylene as Electron Acceptor for the Anaerobic Degradation of Benzoate. *FEMS Microbiol. Lett.* **66**, 81-86.

Seagren, E. A., Rittmann, B. E., and Valocchi, A. J. (1994) Quantitative Evaluation of the Enhancement of NAPL-Pool Dissolution by Flushing and Biodegradation. *Environ. Sci. Technol.* **28**, 833-839.

Sewell, G. W. and Gibson, S. A. (1991). Stimulation of the Reductive Dechlorination of Tetrachloroethene in Anaerobic Aquifer Microcosms by the Addition of Toluene. *Environ. Sci. Technol.* **25**, 982-984.

Smatlak, C. R., Gossett, J. M., and Zinder, S. H. (1996). Comparative Kinetics of Hydrogen Utilization for Reductive Dechlorination of Tetrachloroethene and Methanogenesis in an Anaerobic Enrichment culture. *Environ. Sci. Technol.* **30**, 2850-2858.

Tandoi, V., DiStefano, T. D., Bowser, P. A., Gossett, J. M., and Zinder, S. H. (1994) Reductive Dehalogenation of Chlorinated Ethenes and Halogenated Ethanes by a High-Rate Anaerobic Enrichment Culture. *Environ. Sci. Technol.* **28**, 973-979.

Tonnaer H., Alphenaar, A., Wit, H. D., Grutters, M., Spuij, F., Gerritse, J., and Gottschal, J. C. (1997) Modelling of Anaerobic Dechlorination of Chloroethenes for In-Situ Bioremediation. In *In Situ and On-Site Bioremediation*, papers from the Fourth International In Situ and On-Site Bioremediation Symposium, Ed. Alleman, B. C. and Leeson, A., Battelle Press, Columbus, OH, pp. 591-596.

Uberoi, V. and Bhattacharya, S. K. (1995) Interactions among sulfate reducers, acetogens, and methanogens in anaerobic propionate systems. *Water Environ. Res.* **67**, 330-339.

Vancheeswaran, S., Halden, R. U., Williamson, K. J., Ingle, J. D., and Semprini, L. (1999) Abiotic and Biological Transformation of Tetraalkoxysilanes and Trichloroethene/cis-Dichloroethene Cometabolism Driven by Tetrabutoxysilane-Degrading Microorganisms. *Environ. Sci. Technol.* **33**, 1077-1085.

Vancheeswaran, S., Yu, S., Daley, P., Halden, R. U., Williamson, K. J., Ingle, J. D., and Semprini, L. (2003) Intrinsic Remediation of Trichloroethene Driven by

Tetraalkoxysilanes as Co-contaminants: Results of Microcosm and Field Studies. *Remediation*. **13**, 7-25.

Vogel, T. M and McCarty, P. L. (1985). Biotransformation of Tetrachloroethylene to Trichloroethylene, Dichloroethylene, Vinyl Chloride, and Carbon Dioxide under Methanogenic Conditions. *Appl. Environ. Microbiol.* **49**, 1080-1083.

Vogel, T. M., Criddle, C. S., and McCarty, P. L. (1987). Transformations of Halogenated Aliphatic Compounds. *Environ. Sci. Technol.* **21**, 722-736.

Wiedemeier, T. and Henry, B. M. (2001) Technical Protocol for Enhanced Reductive Dechlorination via Vegetable Oil Injection. In Sixth In Situ and On-Site Bioremediation Symposium, San Diego, CA.

Yang, Y. and McCarty, P. L. (1998). Competition for Hydrogen within a Chlorinated Solvent Dehalogenating Anaerobic Mixed Culture. *Environ. Sci. Technol.* **32**, 3591-3597.

Yang, Y. and McCarty, P. L. (2000a) Biomass, Oleate, and Other Possible Substrates for Chloroethene Reductive Dehalogenation. *Biorem. J.* **4**, 125-133.

Yang, Y. and McCarty, P. L. (2000b) Biologically Enhanced Dissolution of Tetrachloroethene DNAPL. *Environ. Sci. Technol.* **34**, 2979-2984.

Yang, Y. and McCarty, P. L. (2002). Comparison between Donor Substrate for Biologically Enhanced Tetrachloroethene DNAPL Dissolution. *Environ. Sci. Technol.* **36**, 3400-3404.

Yu, S. and Semprini, L. (2002b). Comparison of Trichloroethylene Reductive Dehalogenation by Microbial Communities Stimulated on Silicon-Based Organic Compounds as Slow-Release Anaerobic Substrate. *Water Res.* **36**, 4985-4996.

Zehnder, A. J. B. and Stumm, W. (1988) Geochemistry and Biogeochemistry of Anaerobic Habitats. In *Biology of Anaerobic Microorganism*. Ed. Zehnder, A. J. B. John Wiley & Sons, Inc., pp. 29-33.

CHAPTER 3

COMPARISON OF TRICHLOROETHYLENE REDUCTIVE DEHALOGENATION BY MICROBIAL COMMUNITIES STIMULATED ON SILICON-BASED ORGANIC COMPOUNDS AS SLOW-RELEASE ANAEROBIC SUBSTRATES

Seungho Yu and Lewis Semprini

Water Research

International Association on Water Quality, London, U.K.

Vol. 36 (2002) pp. 4985-4996

ABSTRACT

Microcosm studies were conducted to demonstrate the effectiveness of tetrabutoxysilane (TBOS) as a slow-release anaerobic substrate to promote reductive dehalogenation of trichloroethylene (TCE). The abiotic hydrolysis of TBOS and tetrakis(2-ethylbutoxy)silane (TKEBS), and the biotic transformations of the hydrolysis products from both were also investigated. Comparison of TCE reductive dehalogenation was performed with microbial communities stimulated from three different sites: Site-300 Lawrence Livermore National Laboratory (LLNL), CA, Point Mugu Naval Weapons Facility, CA, and the Evanite site in Corvallis, OR. Poisoned microcosms showed that 1 mol of TBOS slowly and abiotically hydrolyzes to 4 mol of 1-butanol, while the live microcosms showed the 1-butanol ferments to butyrate and/or acetate, producing H_2. The hydrolysis of TBOS and TKEBS was abiotic and not enhanced by biotic processes under the anaerobic conditions of these tests. Hydrogen consumption was correlated with reductive dehalogenation, indicating it served as an electron donor for reductive dehalogenation. TBOS was found to be a slow-release anaerobic substrate to support long-term dechlorination of TCE to ethylene in Pt. Mugu microcosms, and in the LLNL microcosm bioaugmented with the Evanite culture. Electron mass balances showed most of the electron flow went into the creation of organic acids, especially acetate, and the production of methane. Electron efficiencies for reductive dechlorination were as high as 14% based on the electrons used for dechlorination to the total electrons associated with the mass of TBOS and TKEBS hydrolyzed. Rates of TBOS

hydrolysis increased with greater TBOS concentrations as a light nonaqueous-phase liquids (LNAPL). These results indicate that TBOS has promise as an effective anaerobic substrate for remediating a wide range of CAH concentrations at different CAH contaminated sites.

Key Words: reductive dehalogenation, trichloroethylene, tetrabutoxysilane, hydrolysis

INTRODUCTION

Trichloroethylene (TCE) is one of the most common groundwater contaminants in the United States (Westrick *et al.*, 1984). Under anaerobic conditions, TCE can be reductively dechlorinated to cis-1,2-dichloroethylene (c-DCE), vinyl chloride (VC), and finally ethylene or ethane (Freedman and Gossett, 1989; McCarty, 1997). However, the extent of dechlorination differs among contaminated sites. Anaerobic dechlorination has been drawing attention for the in-situ bioremediation of PCE and TCE (Smatlak *et al.*, 1996; Yang and McCarty, 1998). Furthermore, anaerobic dechlorination has potential for remediating high concentrations of TCE associated with dense nonaqueous-phase liquids (DNAPLs) contamination (Nielsen and Keasling, 1999) and for enhancement of DNAPL dissolution (Carr *et al.*, 2000; Yang and McCarty, 2000b).

Many organic compounds, such as organic acids and alcohols, have been studied as potential electron donors to promote anaerobic dechlorination (Fennell *et*

al., 1997). Complex substrates are fermented to less complex substrates, producing H_2, which can serve directly as an electron donor for reductive dechlorination (Maymo-Gatell *et al.*, 1995). However, other microorganisms including methanogens, homoacetogens, and sulfidogens compete for H_2 as an electron donor in the subsurface environment (McCarty, 1997). Enhanced bioremediation may require the use of substrates that are efficiently used for anaerobic dechlorination (Yang and McCarty, 1998). Many researchers have physiologically and thermodynamically studied hydrogen competition for a variety of hydrogenotrophic microbial consortiums. Dechlorinators were shown to have a lower hydrogen threshold concentration of 0.3 nM compared to acetogens (336~3640 nM), methanogens (5~95 nM), and sulfidogens (1~15 nM) (Löffler *et al.*, 1999). Other studies reported a hydrogen threshold on the range of 0.05~11 nM for dechlorinators (Smatlak *et al.*, 1996; Yang and McCarty, 1998), suggesting that anaerobic dechlorinators can outcompete the other hydrogenotrophs at low hydrogen concentrations. In addition to the physiological hydrogen threshold, free energy calculations indicate a lower hydrogen threshold for dechlorinators than for other hydrogenotrophs (Smatlak *et al.*, 1996). Several studies revealed that an electron donor that is slowly and steadily transformed to maintain low H_2 concentrations could optimize anaerobic dechlorination and limit methanogenesis (Ballapragada *et al.*, 1997; Fennell *et al.*, 1997; Fennell and Gossett, 1998; Smatlak *et al.*, 1996; Yang and McCarty, 1998).

Table 3-1. Physical properties of tetraalkoxysilanes (adapted from Vancheeswaran, 1997).

Compound / Property	TBOS ($C_{16}H_{36}O_4Si$)	TKBS ($C_{24}H_{52}O_4Si$)
Molecular Weight (g/mol)	320.5	432.8
Boiling Point (°C)	115	166
Density (g/ml)	0.899	0.892
Refractive Index	1.41	1.43
Solubility (mg/l at 20°C)	< 1	< 1
Viscosity (cSt* at 38°C)	2.33	4.35
Surface Tension (dynes/cm)	22.8	22.8

* cSt = centistoke = 0.01 cm^2/sec.

Tetrabutoxysilane (TBOS, $[Si(CH_3CH_2CH_2CH_2O)_4]$) and tetrakis(2-ethylbutoxy)silane (TKEBS, $[Si(CH_3CH_2CH(CH_2CH_3)CH_2O)_4]$) are silicon-based compounds having four oxygen branches with butyl and 2-ethylbutyl groups, respectively. These compounds were observed to act as LNAPLs and mix well with CAHs (Table 1) (Yu and Semprini, 2001). They have a low solubility in water and thus slowly dissolve. The silicon-based compounds have been shown to support anaerobic dechlorination of TCE at chlorinated solvent-contaminated sites (Vancheeswaran *et al.,* 1998; Vancheeswaran *et al.,* 1999) and with laboratory cultures (Yang and McCarty, 2000a). Evidence for the transformation pathway of alkoxysilanes, mainly TKEBS, and their potential to serve as substrates for promoting TCE transformation was discovered through analysis of groundwater from Site 300 at Lawrence Livermore National Laboratory, CA (LLNL)

(Vancheeswaran, 1997). Based on the field monitoring data, TBOS and TKEBS were observed to slowly hydrolyze to 1-butanol and 2-ethylbutanol, respectively, and ferment to butyrate and 2-ethylbutyrate (Vancheeswaran, 1997; Vancheeswaran *et al.,* 1998; Vancheeswaran *et al.,* 1999). However, the abiotic hydrolysis of TBOS and the biotic fermentation of the hydrolysis products needed additional study, as well as the effect of TBOS addition on reductive dehalogenation. The effectiveness of TBOS addition as a slow-release substrate with microbial communities from different sites also needed to be evaluated. During the fermentation of the alcohols and acids, H_2 is produced and becomes a potential electron donor for the dechlorinators (McCarty, 1997; Löffler *et al.,* 1999). Butyrate, one potential fermentation product, has been shown to be an excellent slow fermenting substrate among other fatty acids to produce hydrogen needed for dehalogenation reactions (Fennell *et al.,* 1997).

The objectives of this study were: to investigate the effectiveness of TBOS as a slowly hydrolyzing substrate to drive the enhanced anaerobic transformation of TCE; to evaluate the abiotic hydrolysis of TBOS; and to determine the biotic transformation of the hydrolysis products from TBOS. This study focused on TBOS addition as a slow release substrate, since it hydrolyzes more rapidly than TKEBS. Butyrate, as a potential fermentation product of TBOS, has been studied in greater detail than ethylbutyrate produced from TKEBS. Some TKEBS was present in groundwater from Site-300 LLNL, so its fate was also monitored.

Comparison of TCE reductive dehalogenation was performed with microbial communities stimulated from three different sites; Site-300 LLNL, CA, Point Mugu Naval Weapons Facility, CA, and the Evanite site in Corvallis, OR, and stimulated with the silicon-based organic compounds. The microbial community stimulated from LLNL Site-300 groundwater showed incomplete dechlorination of TCE to c-DCE without methanogenesis. The communities stimulated from the two other sites had the ability to dechlorinate TCE to ethylene. In the Point Mugu microcosms, a very slow dechlorination rate from VC to ethylene was observed.

MATERIALS AND METHODS

Chemicals

TKEBS (United Chemical Technologies Inc., 97%, Bristol, PA) and TBOS (Aldrich Chemical, 97%, Milwaukee, WI) were used as substrates and in the preparation of analytical standards. Dichloromethane (DCM) (Fisher Scientific Co., 99.9% HPLC Grade, Pittsburgh, PA) was used for solvent extraction of TBOS, TKEBS, and alcohols prior to gas chromatographic (GC) analysis. 2-ethylbutanol (Aldrich Chemical, 98%, Milwaukee, WI), 1-butanol (Aldrich Chemical, 99.8%, HPLC grade, Milwaukee, WI), 2-ethylbutyric acid (Aldrich Chemical, 99%, Milwaukee, WI), sodium butyrate (Aldrich Chemical, 98%, Milwaukee, WI), and sodium acetate (Aldrich Chemical, 99+%, Milwaukee, WI) were used for preparing

analytical standards. Sodium carbonate (99%) was obtained from Mallinckrodt Co. (Paris, KY) for preparing buffer solution (pH 7.2) in abiotic hydrolysis experiments.

Analytical Methods

TKEBS, TBOS, 2-ethylbutanol, and 1-butanol were determined by GC analysis after solvent extraction. An aqueous sample (0.5 ml) was taken after actively shaking the microcosms. The aqueous samples were extracted with 1ml of DCM with vigorously mixing for 10 min on a vortex mixer and centrifuged at 10,000 rpm for 5 min. The extract (1 µl) was introduced into a HP-5890 gas chromatograph equipped with a flame ionization detector (FID) and Rtx-5 column (30m x 0.32 mm, 0.25-µm film) from Restek, Inc. (Bellefonte, PA). The oven temperature program was set as follows: 35°C for 5 min; increased at 40°C/min to 300°C; kept at 300°C for 4 min. Helium served as the carrier gas (30 ml/min) for the column.

TCE, c-DCE, vinyl chloride, ethylene, and methane were measured by GC analysis via separation on a 30 m × 0.53 mm GS-Q column (J&W Scientific, Folsom, CA). A microcosm headspace sample (20~100 µl) was injected into an HP-6890 GC equipped with a photoionization detector (PID) and flame ionization detector (FID) connected in series. The GC oven was initially set at 40°C for 2 min, heated at 25°C/min to 160°C and 15°C/min to 220°C, and kept at 220°C for 1 min. Hydrogen and carbon dioxide concentrations in microcosm headspace gas samples (200 µl) were determined using an HP-5890 GC with a thermal conductivity detector (TCD), operated isothermally at 220°C. The headspace gas samples were

chromatographically separated with a Carboxen 1000 column (15 ft × 1/8 in, Supelco, Bellefonte, PA). Argon gas was used as a carrier gas at 15 ml/min for operation of HP GC. The hydrogen detection limit was 4 nM (liquid concentration basis) with the TCD measurement. Volatile acids in aqueous samples were determined using high performance liquid chromatography (HPLC). A Dionex DX 500 HPLC (Dionex, Sunnyvale, CA) equipped with a UV absorbance detector operated at 210nm. Separation of acids was made using a Phenomenex Rezex ROA-Organic Acid column (300 × 7.8 mm) and 0.013 N H_2SO_4 (pH 3.0) as an eluent at the flow rate of 0.5 ml/min.

A six-point linear standard calibration curve was constructed with triplicate samples prior to initial sampling for all chromatographic analyses. For each sampling event, one point check was carried to determine the stability of the detectors and for recalibration purposes.

Culture Enrichment and Growth

The culture used for bioaugmentation was enriched from groundwater from the Evanite site in Corvallis, OR. The enrichment culture was grown in a batch reactor bottle on butanol in groundwater (no addition of nutrients or salts) at 30°C with continuous shaking at 200 rpm over the six month incubation period, with repeated additions of TCE. After each addition of TCE was converted completely to ethylene, the batch bottle was purged with the mixed gas of N_2 (90%) and CO_2

(10%) and neat TCE of 6 ~ 8 mg/l was added to achieve an aqueous concentration. Neat butanol was also added to maintain aqueous concentration of 100 ~ 300 mg/l. A fairly constant TCE dechlorination rate was obtained after 150 days of incubation. Additional tests showed the enrichment culture was capable of completely transforming TCE to ethylene with active methanogenesis with lactate, propionate, butyrate, and butanol added as electron donors.

Microcosm Preparation

Batch microcosm bottles were constructed with serum bottles (Wheaton Industries, Millville, NJ) fitted with rubber-lined caps and butyl rubber septa (Wheaton Industries, Millville, NJ) (Table 2). The microcosms were constructed in an anaerobic glove box filled with 10% H_2 and 90% N_2. The site and microcosm volumes were as follows: LLNL Site-300 (316 ml), Pt. Mugu (1060 ml), and the LLNL bioaugmentation study (155 ml). LLNL microcosms consisted of 250 ml of groundwater (well D3) and 66 ml of headspace. No solids were added due to the unavailability of core material. The bioaugmentation microcosms contained 70 ml of LLNL groundwater, 5 ml of an enriched liquid culture from the Evanite site, and 80 ml of headspace. Pt. Mugu microcosms contained 750 ml of groundwater, 200 ml of aquifer solids, and 110 ml of headspace. After construction in the glove box, the microcosm headspace hydrogen was removed by purging with the mixed gas of N_2 (90%) and CO_2 (10%) that was treated in a tube furnace to remove trace oxygen. TBOS and TCE were added to each microcosm bottle as neat liquid or diluted

aqueous solution (Table 2). TBOS was added at a high concentration to the Pt. Mugu microcosm since high sulfate concentrations (~ 1000 mg/l) were initially present in the groundwater. TKEBS was detected in the ambient groundwater used to construct LLNL microcosms, but was not present in groundwater later used to construct LLNL microcosms bioaugmented with the Evanite culture. Duplicate batch microcosms were incubated for each experiment. Control microcosms were constructed in the same manner as the live microcosms, but were poisoned with 25 mg/l mercuric chloride ($HgCl_2$). All microcosms were incubated with continuous shaking at 200 rpm in a 30°C environmental chamber.

Table 3-2. Initial experimental conditions of microcosm bottles.

	LLNL (At day 0)	LLNL with the Evanite Culture (At day 0)	Pt. Mugu (At day 0)
TCE, µM (mg/l)	381 (50)[a]	15 (2)[b]	91 (12)[a]
TBOS, µM (mg/l)	156 (50)[a]	281 (90)[a]	3121 (1000)[a]
Methanogenesis	No	Yes[c]	Yes
Groundwater (ml)	266	75[d]	750
Headspace (ml)	66	80	110
Aquifer Solids (ml)	-	-	200

[a] Neat liquid added. [b] Stock solution added. [c] Methanogenesis from bioaugmentation of the Evanite culture. [d] 70 ml of LLNL groundwater + 5 ml of the Evanite enrichment culture.

For abiotic hydrolysis experiments, 0.5 g of Ottawa sand (sieved between #30 and # 50) was placed in 2 ml glass vials, and 0.2 ml carbonate buffer solution (pH 7.2) was added with different TBOS concentrations. These glass vials were capped

with Teflon-coated butyl rubber septa (Wheaton Industries, Millville, NJ) sealed in place with an aluminum crimp cap. A total of 24 bottles were prepared for each concentration, and incubated at 20°C for 80 days with continuous shaking at 200 rpm. Three vials from each concentration were sacrificed for every analysis of TBOS and 1-butanol. The abiotic hydrolysis rates of TBOS were obtained by a simple linear regression fit of 8 data points (each data point was the average of the triplicates).

RESULTS AND DISCUSSION

Enhanced TCE Reduction Driven by TBOS in LLNL Microcosms

The poisoned microcosms were used to track the abiotic hydrolysis of TBOS and TKEBS. The background site groundwater initially had several fermentation products present. The hydrolysis of TBOS and the accumulation of its product, 1-butanol, are shown in Fig. 1 (a). 2-ethylbutanol was also produced from the slow hydrolysis of TKEBS present in the site groundwater. The abiotic hydrolysis rate of TBOS is about 10 times faster than that of TKEBS, consistent with previous laboratory studies (Vancheeswaran, 1997). Over 100 days of incubation, 35~40 μmol (11.2 ~ 12.8 mg) of TBOS disappeared and 140~160 μmol (10.4 ~ 11.8 mg) of 1-butanol was produced, while 4~5 μmol (1.7 ~ 2.2 mg) TKEBS disappeared, resulting in the production of 15~16 μmol (1.5 ~ 1.6 mg) of 2-ethylbutanol. These

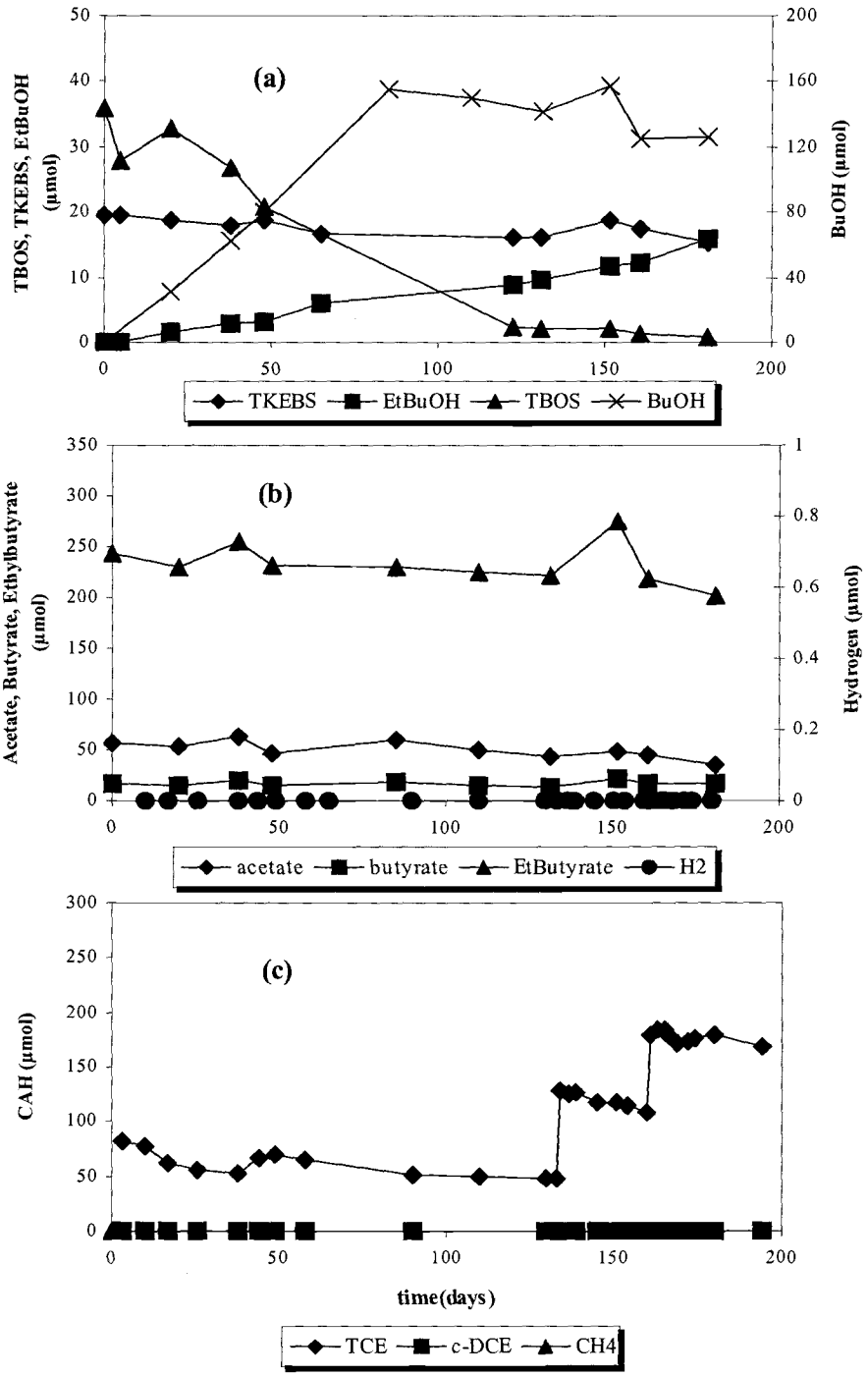

Figure 3-1. Poison control microcosm containing groundwater from Site 300 of LLNL: (a) TBOS, TKEBS, and their hydrolysis products, (b) acids from fermentation process and hydrogen, and (c) CAHs. The initial TCE mass of 80 µmol is equal to an aqueous concentration of 290 µM. The hydrogen detection limit of 4 nM represents a total mass of 0.015 µmol.

results are consistent with a stoichiometric ratio of 4 moles of alcohol formed for each mole of alkoxysilanes hydrolyzed. The acids in the background site groundwater (acetate, butyrate, and 2-ethylbutyrate) and hydrogen were constant over the 180-day incubation period of the poisoned control. The stepwise additions of TCE at 0, 133, and 160 days show no dechlorination to c-DCE (Fig 1 (c)), indicating $HgCl_2$ poisoning inhibited the biological fermentation reactions and the dechlorination process. The duplicate control showed near identical results.

Fig. 2 shows the fermentation of the alcohols and organic acids produced from the hydrolysis of TBOS and TKEBS, resulting in H_2 production in a biologically active LLNL microcosm. Dechlorination of TCE to c-DCE was correlated with hydrogen consumption. TCE was transformed to c-DCE within 25 days of incubation, and hydrogen began to accumulate after TCE was transformed to c-DCE. 1-butanol, butyrate, and acetate concentrations started to accumulate and increased until the second addition of TCE at 133 days, while little change in 2-ethylbutanol concentration was observed. Aqueous hydrogen concentrations (600 to 1500 nM from 44 to 134 day, total H_2 masses of 2 to 6 μmol, respectively) were maintained at levels consistent with H_2 threshold concentrations for acetogenesis (Löffler *et al.,* 1999). After the second addition of TCE, the concentrations of butyrate and H_2 decreased, and acetate increased. Decreases in hydrogen concentration occurred during the period when TCE was transformed to c-DCE, indicating hydrogen was used as an electron donor to support TCE dechlorination. Aqueous hydrogen concentration levels decreased to below the detection limit of 4

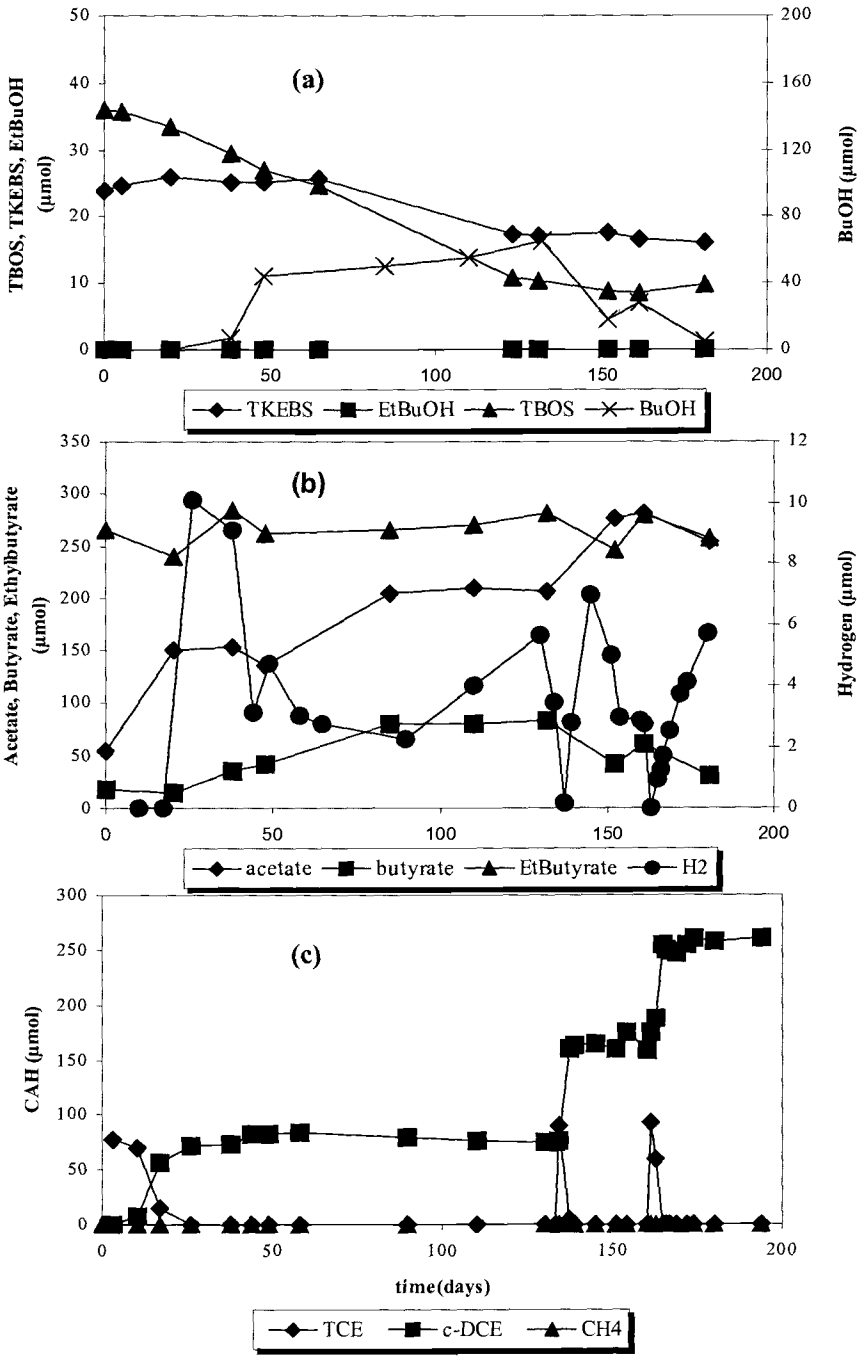

Figure 3-2. Live microcosm amended with TBOS to groundwater from Site 300 of LLNL: (a) TBOS, TKEBS, and their hydrolysis products, (b) fermentation products and hydrogen, and (c) CAHs. The initial TCE mass of 80 µmol is equal to an aqueous concentration of 290 µM. The hydrogen detection limit of 4 nM represents a total mass of 0.015 µmol.

nM, consistent with dehalogenation reactions. Hydrogen concentrations increased after TCE was transformed to c-DCE as a result of butanol and butyrate fermentation. The third addition of TCE showed similar results as the second, with respect to hydrogen consumption and accumulation. The rates of TCE dechlorination at 133 and 160 days were much faster than the initial transformation at 20 day, but the transformation did not proceed past c-DCE, which is consistent with the field monitoring results. No production of methane was observed during the course of the experiment.

Bioaugmentation Microcosm Study with TBOS Addition

TBOS was also evaluated as an anaerobic substrate in a LLNL microcosm that was bioaugmented with the Evanite culture that can completely dechlorinate TCE to ethylene. The slow hydrolysis of TBOS in the bioaugmented microcosm is shown in Fig. 3(a). TKEBS was not present in the site groundwater used to construct these microcosms. Butanol was not detected, but butyrate and acetate were observed as fermentation products of butanol. In the beginning of the experiment, butyrate increased along with an increase in H_2 concentration. When c-DCE was transformed to VC, butyrate and H_2 rapidly decreased and were maintained at decreased levels during the slow transformation of VC to ethylene. The concentration of acetate increased throughout most of the study. The decrease in acetate concentration observed after 150 days coincided with accelerated methanogenesis and was likely associated with acetotrophic methanogenesis. The bioaugmentation resulted in

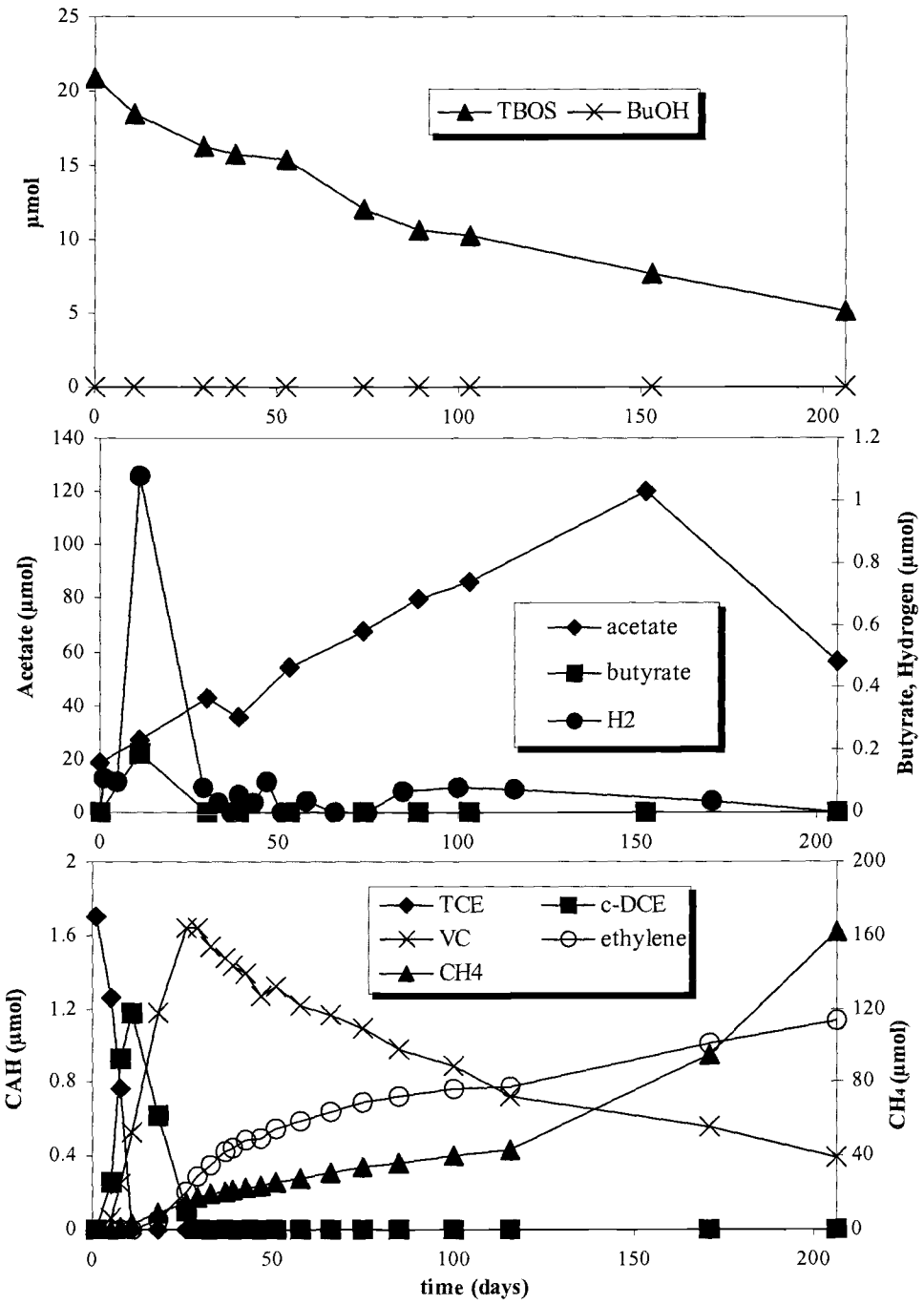

Figure 3-3. Groundwater microcosm from the LLNL Site 300 bioaugmented with the enriched mixed culture from the Evanite site. The initial TCE mass of 1.7 µmol is equal to an aqueous concentration of 17.2 µM in the microcosm. The hydrogen detection limit of 4 nM represents a total mass of 0.017 µmol.

methanogenesis that was absent in the LLNL microcosms (Figure 2(c)).

As shown in Fig. 2 (c), the groundwater microcosm from LLNL shows limited dechlorination of TCE to c-DCE over a prolonged incubation period. However, when LLNL groundwater was bioaugmented with the Evanite culture grown on butanol, dechlorination to ethylene was observed as a result of a single addition of TBOS (Fig. 3 (c)). c-DCE was rapidly dechlorinated to VC, and VC was slowly dechlorinated to ethylene. Ethylene continuously accumulated in the microcosm bottle even though the production rate was very slow compared to the dechlorination of TCE and c-DCE. Aqueous hydrogen concentrations were also maintained at levels of 8.5 nM (a total mass of 0.04 μmol; average value between 29 and 116 day) in the bioaugmented microcosm.

Pt. Mugu Microcosms Amended with TBOS as a Sole Anaerobic Substrate

The Pt. Mugu microcosms were initially amended with TBOS of 2341 μmol (1000 mg/l = 3121 μM) as a sole substrate. Higher TBOS concentrations were used than in the LLNL microcosms since sulfate (~ 1000 mg/l) was present in the groundwater. The microcosms were operated over a period of 900 days, generating effective anaerobic conditions with only the initial addition of TBOS.

Fig. 4 shows the dechlorination of TCE to VC and a slow rate of ethylene formation. Dechlorination was delayed for about 60 to 70 days while the background sulfate (~1000 mg/l) and nitrate (trace amounts) were reduced to low concentration levels (data not shown). One period of incubation (505 to 575 days)

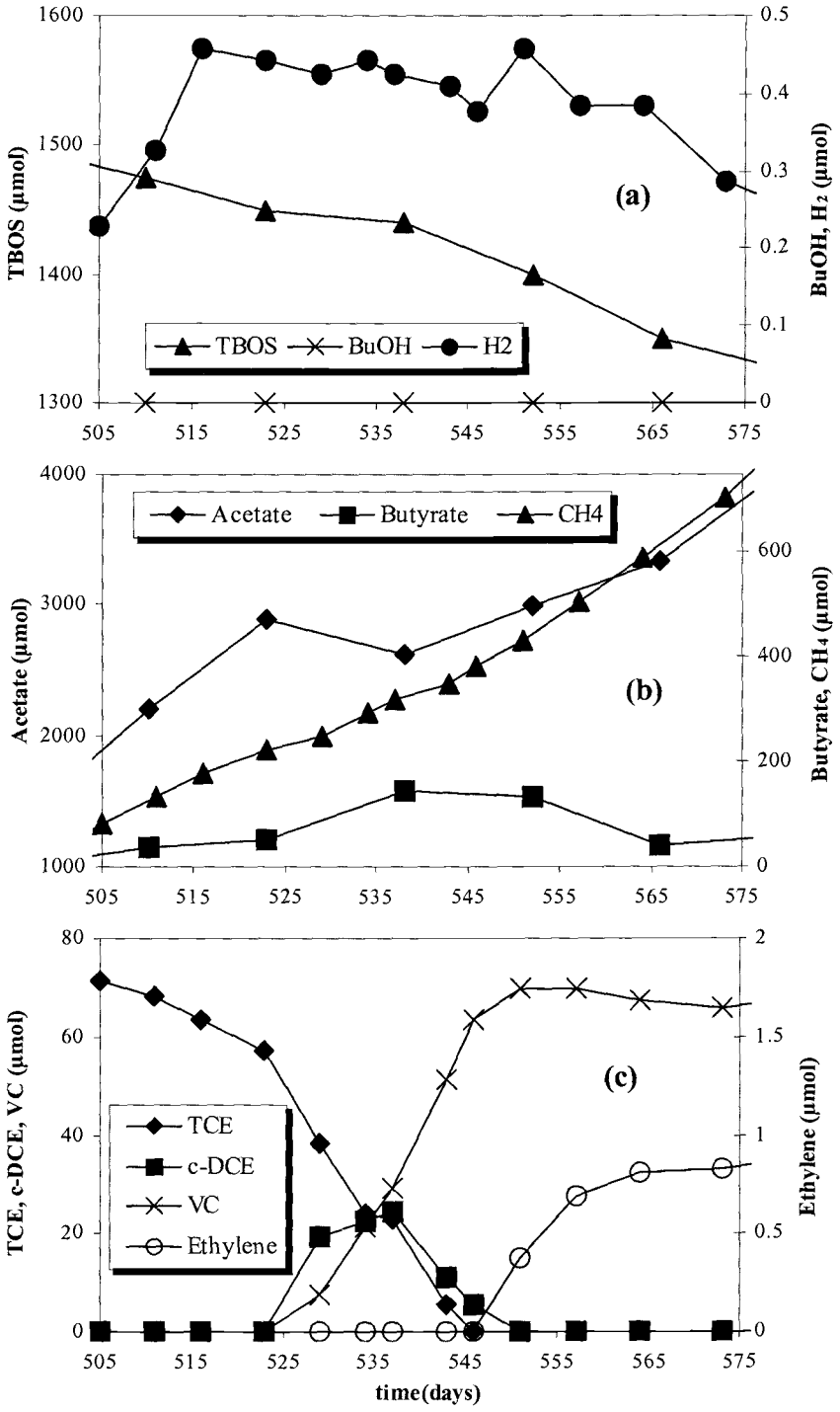

Figure 3-4. Pt. Mugu microcosm bottle amended with TBOS as an anaerobic substrate. The initial TCE mass of 71 µmol is equal to an aqueous concentration of 89 µM. The hydrogen detection limit of 4 nM represents a total mass of 0.025 µmol.

was chosen to show how TBOS supports the reductive dechlorination of TCE to ethylene (Figure 4). At 505 days, the microcosm headspace was purged and TCE was added. The abiotic hydrolysis of TBOS, the production of acetate and butyrate from butanol, and the maintenance of hydrogen concentrations are shown. The accumulation of butanol was not observed over the period, indicating it was rapidly fermented to butyrate and potentially acetate. Aqueous hydrogen concentrations remained relatively constant (36 to 72 nM) as TCE was actively transformed. The fermentation of butanol and butyrate likely maintained the constant and high hydrogen concentrations. Acetate concentration continuously increased over the time course of the experiment, while butyrate increased from 525 to 535 days and then started to decrease. VC was dechlorinated to ethylene at a very slow rate. Previous microcosm studies for Pt. Mugu have also shown slow transformation of VC to ethylene (Keeling, 1998).

TBOS Transformation Pathway

Based on the results of this study, the pathway for the abiotic and biotic transformation of TBOS is presented in Fig. 5. TBOS abiotically hydrolyzes to 1-butanol, which ferments to butyrate and/or acetate producing H_2 during the fermentation process. One mol of TBOS was shown to produce 4 mol of 1-butanol through abiotic hydrolysis, suggesting that butanol is the main hydrolysis product. Butanol ferments to butyrate and/or acetate, consistent with the observations of Eichler and Schink (1984). Rapid fermentation of butanol to butyrate was observed,

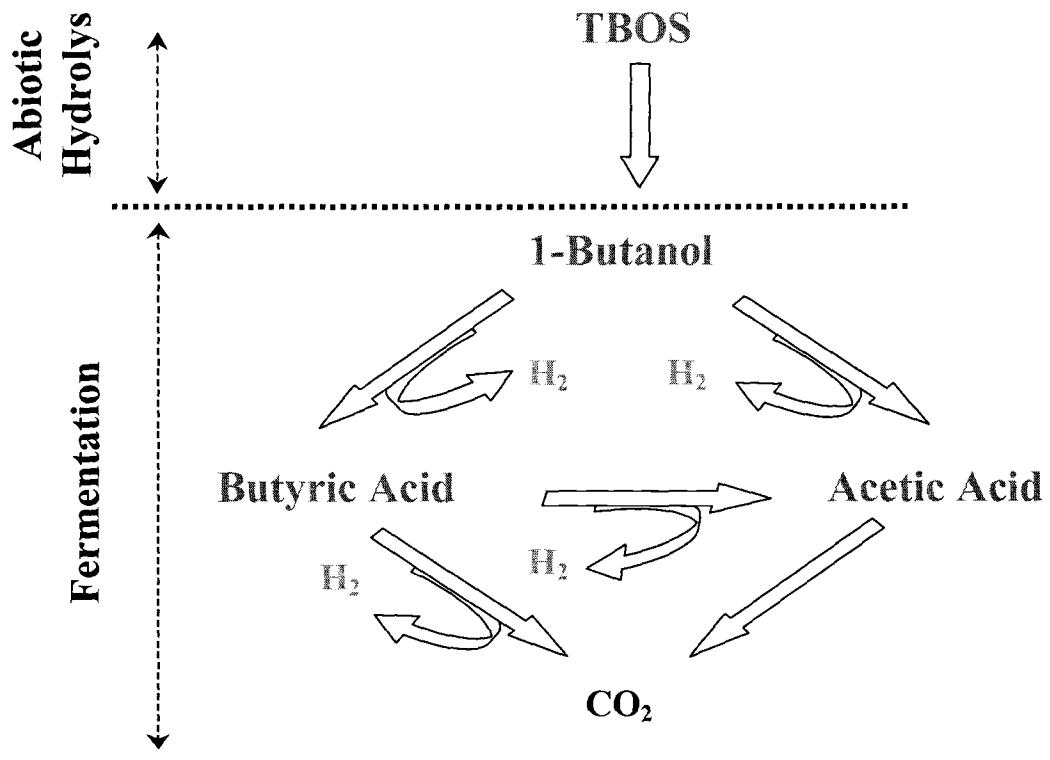

Figure 3-5. Proposed anaerobic transformation pathway of TBOS. Adapted from Vancheeswaren (1997). Possible fermentation of butanol to butyrate and acetate: $2C_4H_9OH + 2HCO_3^- \rightarrow 2C_3H_7COO^- + CH_3COO^- + H^+ + 2H_2O$ (Eichler and Schink, 1984), and butyrate to acetate: $C_3H_7COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$ (Thauer *et al.*, 1977).

since no butanol accumulated in the microcosms. Butyrate could then serve as a slowly fermenting substrate to stimulate reductive dechlorination. Butyrate has been reported as a low-H_2-producing substrate that favors dechlorination rather than methanogenesis (Fennell *et al.,* 1997). However, the H_2-forming fermentation of butanol to butyrate will produce higher H_2 levels as shown in the standard free energy change ($\Delta G° = 16.5$ KJ/mol compared to 48.1 KJ/mol for the fermentation of butyrate to acetate and H_2). For anaerobic bioremediation of CAHs, the control of TBOS hydrolysis rates would be important to achieve a high efficiency of utilization for dechlorination.

Rates of TBOS Hydrolysis

The abiotic hydrolysis rate of TBOS governed the rate of butanol production. Several factors for the abiotic hydrolysis of alkoxysilanes and other silicon polymers (*i.e.*, pH, moisture content in soil, and exchangeable cations) have been considered by other researchers (Chu, 1999; Vancheeswaran, 1997; Vancheeswaran *et al.,* 1999; Xu, 1998) in the abiotic hydrolysis of different types of alkoxysilanes. In this study, several factors related to TBOS hydrolysis to butanol were monitored and compared with experiment data obtained here and other data obtained with batch vials with Ottawa sand (Table 3).

At initial concentrations of 50 mg/l (156 μM), TBOS hydrolyzes at zero order rates of 0.87 and 0.49 μM/day, in LLNL groundwater and Ottawa sand,

respectively (Table 3 (a) and (b)). The hydrolysis rate of TBOS in the bioaugmentation microcosm at an initial concentration of 90 mg/l (278 μM) (Fig 3

Table 3-3. Hydrolysis rates of TBOS based on disappearance of TBOS.

	Environmental Conditions				Rate of Hydrolysis (μM/day) based on disappearance of TBOS
	Temp (°C)	Initial concentration μM (mg/l)	Solids	Water	
(a)	30	156 (50)	N/A	LLNL Site-300	0.87[a]
(b)	20	156 (50)	Ottawa sand	Carbonate buffer	0.49*
(c)	30	156 (50)	N/A	LLNL Site-300	1.27[b]
(d)	20	278 (90)	N/A	LLNL Site-300	1.2[c]
(e)	20	278 (90)	Ottawa sand	Carbonate buffer	0.87*

All rates were obtained from a simple linear regression fit. * From our hydrolysis tests. [a] from Figure 2 (a). [b] from Figure 1 (a) ($HgCl_2$ poisoned). [c] from Figure 3 (a).

(a)) was 1.2 μM/day (Table 3 (d)). This rate was slightly higher than the rate of 0.87 μM/day (Table 3 (e)) obtained in the sterile control with Ottawa sand and the carbonate buffer (pH 7.2). Increasing TBOS concentrations resulted in more rapid abiotic hydrolysis rates (Table 3 (d) and (e)) as compared with the LLNL groundwater or Ottawa sand and carbonate buffer (Table 3 (a) and (b)). Hydrolysis of TBOS as an LNAPL phase could be controlled by direct hydrolysis on the surface of suspended TBOS droplets (Vancheeswaran *et al.,* 1999). Accordingly, the increases of zero order rates with the higher TBOS concentrations could result from the greater surface area of TBOS LNAPL. Thus, the concentration of TBOS as a LNAPL phase is likely one factor controlling the rates of hydrolysis. The highest

rate of TBOS hydrolysis was achieved in the $HgCl_2$ poisoned microcosm. It is possible that Hg^{2+} ion helped catalyze the hydrolysis of TBOS. Liu *et al.* (1999), for example, found Hg^{2+} enhanced the hydrolysis of a herbicidal additive (Irgarol 1051). The results of our studies indicate that the hydrolysis observed in the anaerobic microcosms was abiotic and not biologically mediated, since rates of TBOS utilization were not faster in the microcosms that were biologically active.

Electron Mass Balances

Electron mass balances were calculated for the three different microcosm studies by comparing the concentrations of TBOS, TKEBS, alcohols, acids, CAHs, and methane before and after dechlorination (Table 4). The data in Table 4 represents the averages of the duplicate microcosms. Excellent electron mass balances were achieved at the end of the incubations, ranging from 91 to 102 %. The electron flow into dechlorination was low, ranging from 1 to 14%. These dechlorination estimates were based on the electron equivalents utilized in the dechlorination of TCE to the amounts of TBOS and TKEBS hydrolyzed. This is a conservative estimate since the electron donor potential in the organic acids and alcohols formed was not considered. In our study, most of the electron flow went into the creation of organic acids, especially acetate, which ranged from 44 to 82%. Electron flow to methanogenesis increased from 0% in the LLNL microcosms that were not bioaugmented to 31% in the bioaugmented microcosms. Also, CAH concentrations were maintained at low levels in these microcosm tests, which likely

lowered the efficiency of the electron transfer to dehalogenation and heightened that to acetogenesis.

Table 3-4. Electron balances for the three different microcosms.

	e^- equiv. Per mole	e^- µequivalents					
		LLNL		LLNL with the Evanite Culture		Pt. Mugu	
		Day 0	Day180	Day 0	Day 100	Day 511	Day 511
TKEBS	**144**[a]	3427	2304	0	0	0	0
TBOS	**96**[a]	3446	950	2006	989	142080	134400
Ethylbutanol	**36**[c]	0	0	0	0	0	0
Butanol	**24**[c]	0	115	0	0	0	0
Ethylbutyrate	**32**[c]	8467	8233	0	0	0	0
Butyrate	**20**[c]	356	602	0	0	720	2654
Acetate	**8**[b]	436	2037	149	690	17600	23893
c-DCE	**2**	0	514	0	0	0	0
VC	**4**	0	0	0	4	0	279
Ethylene	**6**	0	0	0	5	0	2
CH_4	**8**[b]	0	0	0	321	1076	3432
Total		16132	14755	2155	2009	161476	164660
Recovery (%)			91		93		102

[a] Based on the assumption that 1 mol of TBOS/TKEBS hydrolyzes to 4 mol of butanol/ethylbutanol.
[b] Sawyer et al. (1994).
[c] Based on the following equations:
Ethylbutanol: $C_6H_{13}OH + 11H_2O \rightarrow 6CO_2 + 36H^+ + 36e^-$
Butanol: $C_4H_9OH + 7H_2O \rightarrow 4CO_2 + 24H^+ + 24e^-$
Ethylbutyrate: $C_5H_{11}COO^- + 11H_2O \rightarrow HCO_3^- + 5CO_2 + 32H^+ + 32e^-$
Butyrate: $C_3H_7COO^- + 7H_2O \rightarrow HCO_3^- + 3CO_2 + 20H^+ + 20e^-$

CONCLUSIONS

TBOS abiotically and slowly hydrolyzes to 1-butanol, which ferments to butyrate and/or acetate, producing H_2 during the fermentation process. One mol of TBOS was shown to produce 4 mol of 1-butanol through abiotic hydrolysis. Butanol ferments to butyrate and/or acetate, consistent with the observations of Eichler and Schink (1984). Butyrate could then serve as a slowly fermenting substrate to drive

reductive dechlorination. Butyrate has also been reported as a low-H_2-producing substrate favored by dechlorinators rather than methanogens (Fennell *et al.,* 1997).

Based on the results of microcosm study, TBOS was found to be a slow-release anaerobic substrate to support long-term dechlorination in LLNL microcosms, Pt. Mugu microcosms, and the LLNL microcosm bioaugmented with the Evanite culture. These results indicate that TBOS has promise as an effective anaerobic substrate for remediating a wide range of CAH concentrations at many contaminated sites. First, it may be possible to maintain a rate of TBOS abiotic hydrolysis that matches that required to achieve effective in-situ bioremediation, if the initial TBOS amount introduced is carefully controlled according to the mass of CAH contaminants. This could provide a better environment for dechlorinators to outcompete the other hydrogenotrophs such as methanogens, sulfidogens, and acetogens. Second, high operational costs resulting from repeated or continuous injections of commonly used anaerobic substrates such as lactate, butyrate, propionate, and benzoate might be significantly reduced through the use of a slow release substrate, such as TBOS. TBOS as a LNAPL with a low solubility in water might be injected into the subsurface to create a barrier that slowly releases butanol. The results of these microcosm studies show a single addition of TBOS will produce an electron donor at a slow rate to drive anaerobic transformations for extended periods of time. The commercial availability of TBOS at a low cost may limit its use. Analytical grade TBOS costs about $17 per pound, however the commercial cost should be lower. Since it is not a food or food additive, obtaining regulatory

approval for its addition may prove more difficult than other slow release substrates such as vegetable oil. TBOS might be effectively used to treat a DNAPL zone through a single addition. Anaerobic substrates have been widely used for in-situ CAH bioremediation, including lactate, butyrate, propionate, benzoate, and hydrogen (Fennell *et al.*, 1997; Smatlak *et al.*, 1996; Yang and McCarty, 1998), but these substrates need continuous injection. Slowly fermentating vegetable oil, for example, has been implemented at several CAH-contaminated sites (Bell *et al.*, 2001; Wiedemeier and Henry, 2001). As observed in the vegetable oil studies, chlorinated solvents could easily partition into TBOS injected near a DNAPL zone (Yu and Semprini, 2001), thus reducing the aqueous concentrations of PCE or TCE, and potentially reducing toxicity and inhibition. Thus, these concentration reductions may actually enhance rates of reductive dehalogenation. This potential treatment of NAPL phases using TBOS needs to be studied in future work, as well as treatments under conditions that are more representative of in-situ bioremediation.

Acknowledgements

The research was supported by a research grant from the U.S. Environmental Protection Agency-sponsored Western Region Hazardous Substance Research Center under agreement R-815738. This article has not been reviewed by the agency, and no official endorsement should be inferred.

REFERENCES

Ballapragada, B. S., Stensel, H. D., Puhakka, J. A., and Ferguson, J. F. (1997) Effect of Hydrogen on Reductive Dechlorination of Chlorinated Ethenes. *Environ. Sci. Technol.* **31**, 1728~1734.

Bell, M., Brown, D., Burns, D. A., and Lee, M. D. (2001) Biologically-Enhanced Reductive Dechlorination. Platform Presentation, In *Sixth In Situ and On-Site Bioremediation Symposium*, San Diego, CA.

Carr, C. S., Garg, S., and Hughes, J. B. (2000) Effect of Dechlorinating Bacteria on the Longevity and Composition of PCE-Containing Nonaqueous Phase Liquids under Equilibrium Dissolution Conditions. *Environ. Sci. Technol.* **34**, 1088~1094.

Chu, M. (1999) Abiotic Transformation of TKEBS and Other Lower Homologues of Tetraalkoxysilanes by Subsurface Solids from a Contaminated Site. Master's Thesis, Oregon State University.

Eichler, B. and Schink, B. (1984) Oxidation of Primary Aliphatic Alcohols by Acetobacterium carbinolicum sp. Nov., a Homoacetogenic Anaerobe. *Arch. Microbiol.* **140,** 147~152.

Fennell, D. E., Gossett, J. M., and Zinder, S. H. (1997) Comparison of Butyric Acid, Ethanol, Lactic Acid, and Propionic Acid as Hydrogen Donors for the Reductive Dechlorination of Tetrachloroethene. *Environ. Sci. Technol.* **31**, 918~926.

Fennell, D. E. and Gossett, J. M. (1998) Modeling the Production of and Competition for Hydrogen in a Dechlorinating Culture. *Environ. Sci. Technol.* **32**, 2450~2460.

Freedman, D. L. and Gossett, J. M. (1989) Biological Reductive Dechlorination of Tetrachloroethylene and Trichloroethylene to Ethylene under Methanogenic Conditions. *Appl. Environ. Microbiol.* **55**, 2144~2151.

Keeling, M. T. (1998) Bench-Scale Study for the Bioremediation of Chlorinated Ethylenes at Point Mugu Naval Air Weapons Station, Point Mugu California, IRP Site 24. Master's Thesis, Oregon State University.

Liu, D., Pacepavicious, G. J., Maguire, R. J., Lau, Y. L., Okamura, H., and Aoyama, I. (1999) Mercuric Chloride-Catalyzed Hydrolysis of the New Antifouling Compound Irgarol 1051. *Wat. Res.* **33**, 155~163.

Löffler, F. E., Tiedje, J. M., and Sanford, R. A. (1999) Fraction of Electrons Consumed in Electron Acceptor Reduction and Hydrogen Thresholds as Indicators of Halorespiratory Physiology. *Appl. Environ. Microbiol.* **65**, 4049~4056.

Maymo-Gatell, X., Tandoi, V., Gossett, J. M., and Zinder, S. H. (1995) Characterization of an H_2-Utilizing Enrichment Culture That Reductively Dechlorinates Tetrachloroethene to Vinyl Chloride and Ethene in the Absence of Methanogenesis and Acetogenesis. *Appl. Environ. Microbiol.* **61**, 3928~3933.

McCarty, P. L. (1997) Breathing with Chlorinated Solvents. *Science* **276**, 1521~1522.

Nielsen, R. B. and Keasling J. D. (1999) Reductive Dechlorination of Chlorinated Ethene DNAPLs by a Culture Enriched from Contaminated Groundwater. *Biotechnol. Bioeng.* **62**, 160~165.

Sawyer, C. N., McCarty, P. L., and Parkin, G. F. (1994) *Chemistry for Environmental Engineering*, 4th ed. McGraw-Hill, New York.

Smatlak, C. R., Gossett, J. M., and Zinder, S. H. (1996) Comparative Kinetics of Hydrogen Utilization for Reductive Dechlorination of Tetrachloroethene and Methanogenesis in an Anaerobic Enrichment Culture. *Environ. Sci. Technol.* **30**, 2850~2858.

Thauer, R. K., Jungermann, K., and Decker K. (1977) Energy Conservation in Chemotrophic Anaerobic Bacteria. *Bacteriol. Rev.* **41**, 100~180.

Vancheeswaran, S. (1997) Abiotic and Biological Transformation of TBOS and TKEBS, and Their Role in the Biological Transformation of TCE and c-DCE. Master's Thesis, Oregon State University.

Vancheeswaran, S., Halden, R. U., Williamson, K. J., Ingle, J. D., and Semprini, L. (1999) Abiotic and Biological Transformation of Tetraalkoxysilanes and Trichloroethene/cis-1,2-Dichloroethene Cometabolism Driven by Tetrabutoxysilane-Degrading Microorganisms. *Environ. Sci. Technol.* **33**, 1077~1085.

Vancheeswaran, S., Semprini, L., Pon, G., Williamson, K. J., Ingle, J. D., and Daley, P. (1998) Anaerobic Transformation of TCE Driven by Organo-Silicon Compounds. In *1st International Conference on Remediation of Chlorinated and Recalcitrant Compounds*, Monterey, CA, pp. 57~62.

Westrick, J. J., Mello, J. W., and Thomas, R. F. (1984) The Groundwater Supply Survey. *J. Am. Water Works Assoc.* **76**, 52~59.

Wiedemeier, T. and Henry, B. M. (2001) Technical Protocol for Enhanced Reductive Dechlorination via Vegetable Oil Injection. Platform Presentation, In *Sixth In Situ and On-Site Bioremediation Symposium*, San Diego, CA.

Xu, S. (1998) Hydrolysis of Poly(dimethylsiloxanes) on Clay Minerals as Influenced by Exchangeable Cations and Moisture. *Envron. Sci. Technol.* **32**, 3162~3168.

Yang, Y. and McCarty, P. L. (1998) Competition for Hydrogen within a Chlorinated Solvent Dehalogenating Anaerobic Mixed Culture. *Environ. Sci. Technol.* **32**, 3591~3597.

Yang, Y. and McCarty, P. L. (2000a) Biomass, Oleate, and Other Possible Substrates for Chloroethene Reductive Dehalogenation. *Bioremediation Journal* **4**, 125~133.

Yang, Y. and McCarty, P. L. (2000b) Biologically Enhanced Dissolution of Tetrachloroethene DNAPL. *Envron. Sci. Technol.* **34**, 2979~2984.

Yu, S and Semprini, L. (2001) Oregon State University, unpublished results.

CHAPTER 4

ENHANCED REDUCTIVE DECHLORINATION OF PCE DNAPL WITH TBOS AS A SLOW-RELEASE SUBSTRATE

Seungho Yu and Lewis Semprini

To be submitted to:

Water Research

ABSTRACT

Tetrabutoxysilane (TBOS) was evaluated as a slow-release anaerobic substrate for reductive dechlorination of tetrachloroethylene (PCE) as a dense non-aqueous-phase liquid (DNAPL) with an anaerobic binary mixed culture (BM), which consisted of the Pt. Mugu (PM) and the Evanite (EV) mixed cultures. The BM culture showed more enhanced and complete reductive dechlorination of PCE DNAPL mixed with TBOS, as a slow-release anaerobic substrate than either of the single mixed cultures. Minimal PCE dechlorination was observed in the batch reactors with the PM culture, and the EV culture showed the accumulation of *cis*-1,2-dichloroethylene (*c*-DCE) as a dominant dechlorination intermediate. The BM culture dechlorinated PCE to vinyl chloride (VC) and ethylene (ETH). In the batch reactor experiments, TBOS was mixed with PCE DNAPL to achieve different PCE mol fractions. Mixing with TBOS lowered PCE aqueous concentrations by Raoult's Law, which resulted in further reductive dechlorination and more dechlorination activity based on the amount of chloride ion released, presumably due to the reduction of inhibition and/or toxicity of PCE DNAPL to dechlorinating microbes. Good mass balances of total chlorinated aliphatic hydrocarbons (CAHs) were achieved using independently measured partition coefficients. The measurements of chloride released showed good agreement with the total chloride calculated from the dechlorination products. Total amounts of PCE DNAPL and TBOS were important factors for reductive dechlorination of PCE DNAPL in the experiments. Direct contact of the PCE/TBOS mixture may have caused toxicity to dechlorinating bacteria. PCE may have also

inhibited the rates of reductive dechlorination. Decreases in pH lowered microbial activity for reductive dechlorination due to the accumulation of acetate and/or butyrate. Despite these problems in the batch reactors, for bioremediation in real contaminated sites, the application of TBOS as a slow-release substrate has potential for enhancing bioremediation of DNAPL contamination. The experiments also showed the potential of developing a mixed culture composed of different contaminated site-cultures to achieve more effective CAH reductive dechlorination.

Key words: reductive dechlorination, PCE, DNAPL, Tetrabutoxysilane, slow-release substrate.

INTRODUCTION

PCE and TCE are common and recalcitrant contaminants in soil and groundwater. Contaminated sites with DNAPLs are among the most difficult to remediate, and PCE and TCE DNAPLs can be long-term sources of soil and groundwater contamination (Rittmann *et al.*, 1994). For the clean-up of DNAPL contaminants, bioremediation technologies are being considered (Yang and McCarty, 2000b; Carr *et al.*, 2000; Cope and Hughes; 2001).

Recent studies reported that anaerobic reductive dechlorination is a promising technology for remediation of high concentrations of PCE and TCE associated with dissolution of the DNAPL source zone (Sharma and McCarty, 1996; Nielsen and Keasling, 1999). The enhancement of PCE DNAPL dissolution was observed due to

biotransformation processes (Carr *et al.*, 2000; Yang and McCarty, 2000b; Cope and Hughes, 2002). These reports suggest the potential for biological dechlorination of PCE or TCE DNAPL.

For enhanced anaerobic biodegradation of a DNAPL source zone, slow-release anaerobic substrates have recently drawn attention (Yu and Semprini, 2002b; Yang and McCarty, 2000a and 2002; Bell *et al.*, 2001; Wiedemeier and Henry 2001; Adamson *et al.*, 2003). The use of slow-release substrates can potentially reduce operational costs resulting from repeated or continuous injection of soluble anaerobic substrates commonly used in contaminated sites, such as lactate, butyrate, propionate, and benzoate (Yu and Semprini, 2002b). Another important advantage of slow-release anaerobic substrates, such as vegetable oil, and TBOS (silicon-based organic compound), is the partitioning of chlorinated solvents into insoluble or semi soluble substrates injected near a DNAPL zone. The partitioning may possibly reduce toxicity of high PCE and TCE concentrations (Yu and Semprini, 2002b). The treatment of DNAPL contaminants with a slow-release anaerobic substrate may enhance rates of biological reductive dechlorination.

Intrinsic biostimulation has been a remedial option for biotransfomation of chlorinated solvent-contaminated groundwater. However, many contaminated sites showed incomplete reductive dechlorination with *c*-DCE or VC accumulation due to an absence of appropriate dechlorinating bacteria, such as *Dehalococcoids ethenogenes* strain 195, *Dehalococcoids* sp. strain FL2, and *Dehalococcoids* sp. strain BAV1. *Dehalococcoids ethenogenes* strain 195 can grow on PCE, TCE, and

c-DCE, and cometabolically dechlorinates VC to ETH (Maymo-Gatell *et al.*, 1997). *Dehalococcoids* sp. strain FL2 reductively dechlorinates TCE and *c*-DCE with cometabolic PCE and VC transformations (Löffler *et al.*, 2003), while *Dehalococcoids* sp. strain BAV1 grows on *c*-DCE and VC, but can cometabolize PCE and TCE in the presence of a growth-supporting chlorinated ethylene (He *et al.*, 2003a and 2003b). To overcome the situation where an appropriate dechlorinating microorganism is missing for complete reductive dechlorination, bioaugmentation can be an important strategy (Harkness *et al.*, 1999; Ellis *et al.*, 2000)). The pure cultures isolated to date do not grow on all of the chlorinated ethylenes, and a cometabolic process is necessary to achieve complete reductive dechlorination. This indicates that a mixed culture of two or more different types of dechlorinating microorganisms with growth on each step is a promising candidate for use in engineered bioremediation approaches.

In this study batch reactors were constructed with different mol fractions of PCE/TBOS NAPL mixtures. Two different mixed cultures enriched from two different contaminated sites (Pt. Mugu, CA (PM) and the Evanite site, Corvallis, OR (EV)), and a binary mixed culture (BM), which is the mixture of these two mixed cultures, were evaluated. TBOS as a slow-release anaerobic substrate was observed to slowly and abiotically hydrolyze to 1-butanol, which ferments to butyrate and/or acetate with hydrogen production (Yu and Semprini, 2002b). Yu and Semprini (2002b) reported that TBOS was found to be an effective slow-release anaerobic substrate for remediating TCE at low aqueous concentrations (15-381 μM). The

objectives of this research were to: 1) evaluate the enhancement of reductive dechlorination of NAPL PCE by mixing PCE with TBOS, 2) demonstrate mass balances with partitioning between NAPL, aqueous, and headspace of the parent and daughter products, and compare mass balances with chloride release measurements, 3) study fermentation reactions that occur during the incubations, and make mass balances on electron transfer efficiency for reductive dechlorination, and 4) determine whether a binary mixed culture was more effective for complete reductive dechlorination of PCE DNAPLs than the single mixed cultures used to make the binary mixed culture.

MATERIALS AND METHODS

Chemicals

PCE (99%, spectrophotometric grade), TCE (99.9%), and c-1,2-DCE (97%) were purchased from Acros Organics (Pittsburgh, PA), and VC and ETH (both 99.5%) from Aldrich Chemical Co. (Milwaukee, WI). 1-Butanol (99.8%, HPLC grade), sodium butyrate (98%), and sodium acetate (99+%), were purchased from Aldrich Chemical (Milwaukee, WI) and used for preparing analytical standards. TBOS (97%) used as a slow-release substrate was purchased from Aldrich Chemical (Milwaukee, WI). Dichloromethane (DCM) (99.9 %, HPLC Grade, Fisher Scientific Co., Pittsburgh, PA) was used for solvent extraction of 1-butanol prior to gas chromatographic (GC) analysis.

Analytical Methods

PCE, TCE, *c*-DCE, VC, ETH, and methane were measured with a Hewlett-Packard model 6890 gas chromatograph equipped with a 30 m (0.53 mm GS-Q column (J&W Scientific, Folsom, CA). A microcosm headspace sample (20~100 µl) was injected into the GC equipped with a photoionization detector (PID) and flame ionization detector (FID) connected in series. The GC oven was initially set at 80°C for 1.5 min, heated at 65°C/min to 170°C and 40°C/min to 220°C, and kept at 220°C for 2.7 min. Hydrogen concentrations in headspace gas samples were determined using an HP-5890 GC with a thermal conductivity detector (TCD), operated isothermally at 220°C. The chloride ion concentration was measured by ion chromatography (IC) using Dionex DX 500 chromatography system. Dionex DX 500 system consists of C25 chromatography oven, CD20 conductivity detector, and Dionex IonPac AS14 4 mm column. Eluent was prepared in 0.371 g/L of Na_2CO_3 and 0.084 g/L of $NaHCO_3$. 1-butanol extracted with DCM was determined by a GC equipped with a FID and Rtx-5 column (30 m $\times$ 0.32 mm, 0.25 µm film) from Restek, Inc. (Yu and Semprini. 2002b). Fatty acids were measured using high performance liquid chromatography (HPLC) with an UV absorbance detector at 210 nm (Dionex, Sunnyvale, CA) (Yu and Semprini, 2002b).

Culture Enrichment and Growth Condition

Two different mixed cultures from Point Mugu, CA and the Evanite site in Corvallis, OR were enriched and maintained in two separate batch reactors (total 1.2

L with 1 L liquid) at 20°C with continuous shaking at 200 rpm for about 1 year. PCE (900 μM) was repeatedly added to the EV culture, and TCE (1500 μM) to the PM culture. After most of the PCE or TCE was biotransformed to ETH (50 – 100% of the initial PCE or TCE), the batch reactors were purged with a mixed gas (10 % CO_2 and balanced with N_2). Liquid from each reactor (300 mL) was replaced with fresh anaerobic medium in an anaerobic glove box, and neat PCE (100 μL) or TCE (100 μL) was added along with neat 1-butanol of 60 μL to achieve butanol electron donor concentrations of 50-60 mg/L. Both cultures were grown in sterile basal medium with trace nutrients, which was modified from Yang and McCarty (1998). Chemical salts containing Cl^- were replaced with salts containing Br^- to make low chloride content medium, to permit chloride release measurements.

The PM culture was observed to rapidly biotransform high concentrations of TCE to VC, and to slowly degrade VC to ETH. The EV culture showed the ability to transform PCE to ETH, with rapid transformation kinetics of VC to ETH, but slowly dechlorinated *c*-DCE compared to the PM culture. Each mother reactor was observed to reach steady-state biomass concentration of about 40 mg/L, on a protein basis.

Batch Experiments

All batch reactor experiments (except PM, EV, BM experiments) were performed in serum bottles (total volume 156 mL) fitted with rubber-lined caps and butyl rubber septa (Wheaton Industries, Millville, NJ). 100 mL of the liquid culture

harvested from the mother reactor and 25 mL fresh medium were added to the serum bottles in an anaerobic glove box filled with 10% H_2 and 90% N_2. For the PM, EV, and BM experiments, 316 mL serum bottles were used, with 200 mL of liquid culture and 50 mL of fresh anaerobic media added. After construction in the glove box, the headspace of each reactor bottle was purged with the mixed gas (10 % CO_2 and 90% N_2) which was treated in a tube furnace to remove trace oxygen. At the onset of the experiment, PCE/TBOS NAPL mixtures were added to create different NAPL conditions in the bottles, as shown in Table 4-1. The reactors were incubated at 20°C on a shaker table operated at 200 rpm to enhance mass transfer between headspace, aqueous, and NAPL phases.

In the initial study of the reductive dechlorination of PCE DNAPLs, the three different mixed cultures were compared to investigate the effectiveness of the PM and the EV cultures, and the BM representing the mixture of the two (50 ml of each liquid culture). Each of the reactor bottles was fed a mixture of PCE (2120 μmol)/TBOS (17300 μmol) NAPLs representing PCE mol fraction of 0.11. The mol fraction yielded an aqueous PCE concentrations of 99 μM according to Raoult's Law (PCE solubility = 900 μM) (Yaws, 1999). The experimental conditions represented by PM, EV, and BM batch reactors in Table 4-1 were performed in duplicate. In a second set of experiments the BP-1, BP-2, and BP-3 batch reactors shown in Table 4-1 contained different PCE mol fractions in the PCE/TBOS NAPL mixture (0.02, 0.11, and 0.5) to investigate the effect of PCE aqueous concentrations on reductive dechlorination of PCE/TBOS NAPL. PCE aqueous concentrations

Table 4-1. Experimental conditions for PCE/TBOS Mixtures in batch microcosm bottles. All reactor studies were conducted in duplicate and poisoned controls were constructed for each set.

Batch Reactors	PCE (μmol)	TBOS (μmol)	PCE mol fraction (mol-PCE/mol-NAPL)	Resulting aqueous concentration (μM)*	Total volume of NAPL (mL)	Culture used	Total Cl mass balance
PM	2120	17300	0.11	99	6.39	PM	8480
EV	2120	17300	0.11	99	6.39	EV	8480
BM	2120	17300	0.11	99	6.39	BM	8480
BP-1	182	8660	0.02	18	3.20	BM	728
BP-2	1060	8660	0.11	99	3.30	BM	4240
BP-3	8660	8660	0.50	450	4.10	BM	34640
BP-4	182	1490	0.11	99	0.57	BM	728
BP-5	424	3460	0.11	99	1.32	BM	1696
BP-6	741	6060	0.11	99	2.30	BM	2964
BP-7	200	200	0.50	450	0.09	BM	800
BP-8	200	467	0.30	270	0.19	BM	800
BP-9	200	667	0.23	207	0.27	BM	800
BP-10	200	1333	0.13	117	0.51	BM	800
BP-11	200	2000	0.09	81	0.76	BM	800

*Based on Raoult's Law. BM was the mixture (1:1 volume ratio) of the PM and the EV cultures. All batch reactor sets were duplicates and had killed control bottles.

ranged from 18, 99, and 450 μM. The effect of PCE mass in the NAPL mixture was investigated in the third set of experiments with different total volumes of the PCE/TBOS mixture (0.57 – 3.30 mL), but the same PCE mol fraction of 0.11 (BP-4, BP-5, BP-6, and BP-2). The batch bottles had the same PCE aqueous concentration of 99 μM, but the total PCE amounts were increased from 182 to 1060 μmol. A final experiment was designed to study the effect of the amounts of TBOS on reductive dechlorination of PCE DNAPLs. Every PCE/TBOS NAPL mixture had the same PCE amount (200 μmol), but the mass of TBOS mixed with the PCE was increased from 200 to 2000 μmol, which resulted in different PCE mol fractions and PCE aqueous concentrations (BP-7, BP-8, BP-9, BP-10, and BP-11). The batch bottles

from BP-1 to BP-11 used the BM culture, representing a mixture of the PM and the EV cultures (1:1 volume ratio). For all the experimental sets, control bottles were constructed in the same manner as the live batch reactors, but were poisoned with 50 mg/L formaldehyde. All of the reactor tests were conducted in duplicate.

Total Mass Balance for Partitioning into the NAPL Phase

The partitioning of the chlorinated ethylenes and ETH between gas, aqueous, and NAPL phases was determined to complete reaction mass balances. Headspace samples for all chlorinated ethylenes and ETH were analyzed by gas chromatography and the total mass was calculated using partitioning coefficients assuming equilibrium between three different phases. Total mass balances for each chlorinated ethylenes and ETH in batch microcosm bottles were calculated using Equation 4-1.

$$M_T^i = C_W^i V_W + C_G^i V_G + C_N^i V_N = \frac{C_G^i}{H_{CC}^i}\left(V_W + H_{CC}^i V_G + K_{N-W}^i V_N\right) \qquad (4-1)$$

where M_T^i (μmol) represents total mass of a chlorinated ethylene; C_W^i, C_G^i, and C_N^i (μmol/L) are the concentrations of a chlorinated ethylene (i) in aqueous, headspace, and NAPL phases, respectively; V_W, V_G, and V_N (L) are the volumes of the aqueous, headspace, and NAPL phases; H_{CC}^i ($= C_G^i / C_W^i$) represents the dimensionless Henry's constant for partitioning between the aqueous and gas phases; K_{N-W}^i ($= C_N^i / C_W^i$) is the dimensionless partition coefficient between the aqueous and NAPL phases.

Since TBOS is the dominant phase for most of the tests, partition coefficients between aqueous and TBOS NAPL phases were determined independently for PCE, TCE, *c*-DCE, VC, and ETH. For PCE, TCE, and *c*-DCE, TBOS NAPL was mixed with a neat chlorinated ethylene, and the mixture was added to batch bottles completely filled with anaerobic media (no headspace) under the same conditions as the batch reactor experiments. A known amount of TBOS NAPL and gaseous VC and ETH were separately added to batch bottles filled with anaerobic media. The bottles with TBOS NAPL and a chlorinated ethylene or ETH were agitated on a wrist-action shaker at 20°C for 24 hours and allowed to sit quiescently for another 24 hours for complete phase separation. Aqueous phase samples were taken and analyzed by GC equipped with a purge-and-trap (HP purge & trap concentrator with autosampler). Partitioning coefficients were determined using mass balances between NAPL and aqueous phase. Triplicate batch experiments were performed with different mol fractions of CAH or ETH/TBOS mixtures (0.1, 0.3, and 0.5), in which TBOS of 2000 μmol was used with the different amounts of CAHs or ETH.

RESULTS

Partitioning of Chlorinated Ethylenes between Three Different Phases

Dimensionless partition coefficients for PCE, TCE, *c*-DCE, VC, and ETH between TBOS NAPL and aqueous phases were determined as described in the methods section. The average partition coefficients from triplicates are shown in

Table 4-2. These experimental values (K^i_{N-W}) have the same trend as the octanol-water partition coefficients (K^i_{OW}) for these compounds. Some variation in the partition coefficients may exist due to the presence of PCE. However, in most tests, TBOS represented greater than 80% of the NAPL on a mole fraction basis, thus the partition coefficient should be representative of experimental conditions. Henry's constants used for partitioning between gas and aqueous phases were those reported by Gossett (1987) and Perry *et al.*(1997).

Table 4-2. List of partition coefficients between three phases. All values are based on 20°C incubation.

	Between	PCE	TCE	*c*-DCE	VC	ETH
H^i_{CC}	Gas/Aqueous	0.545 [a]	0.296 [a]	0.122 [a]	0.903 [a]	7.636 [b]
K^i_{N-W}	TBOS NAPL/Aqueous	2860 [c]	450 [c]	72 [c]	27 [c]	1.8 [c]
K^i_{O-W}	Octanol/Aqueous	2510 [d]	263 [d]	72 [d]	42 [d]	13 [d]

[a]Gossett, 1987, [b]Perry *et al.*, 1997, [c]Experimentally measured values, [d]Yaws, 1999.

Reductive Dechlorination of PCE DNAPL by three different mixed cultures

The batch experiments were designed to compare reductive dechlorination of PCE DNAPL by the PM and EV cultures, and the BM culture (Figure 4-1). As shown in Table 4-1, the experiments with the three cultures were performed with PCE/TBOS NAPL mixtures with a PCE mol fraction of 0.11 (PCE = 2120 μmol, TBOS = 17300 μmol). Total mass balances were performed by applying Equation 4-1 with the measured headspace concentrations. The initial concentration

measurements were made 1 day after the addition of PCE to permit phase equilibration.

Presented in Figure 4-1 are the cumulative mass balances of the chlorinated ethylenes and ETH for the PM, EV, and BM cultures, and the estimated Cl^- based on the mass balances. The amount of chloride released was calculated from a mass balance of the dechlorination products (TCE, *c*-DCE, VC, and ETH). Throughout the 65 day incubation good mass balances were achieved. The PM culture showed almost no PCE reductive dechlorination ability. This culture was previously shown to reductively dechlorinate TCE to VC, which was slowly dechlorinated to ETH (Yu and Semprini, 2002b). This result indicates that the PM culture is able to reductively dechlorinate TCE, but shows very little PCE dechlorination ability. The EV culture was observed to reductively dechlorinate PCE, but *c*-DCE continuously accumulated up to 1322 μM over the course of the experiment (Figure 4-1). Lower concentrations of TCE (25-76 μM) and trace amounts of VC (16 μM) and ETH (less than 2 μM) were observed in the batch reactor.

The BM culture clearly showed more complete reductive dechlorination of PCE DNAPL than either of the PM or the EV culture, alone. After 65 days of incubation, VC (972 μM) accumulated to the greatest extent and a greater mass fraction of the initial PCE was transformed to ETH (95 μM), compared to the single mixed cultures. Very little TCE or *c*-DCE was detected over most of the incubation, but some TCE (50 μM) and *c*-DCE (175 μM) in the BM culture accumulated toward the end of the experiment. After 65 days of incubation, the EV culture had a

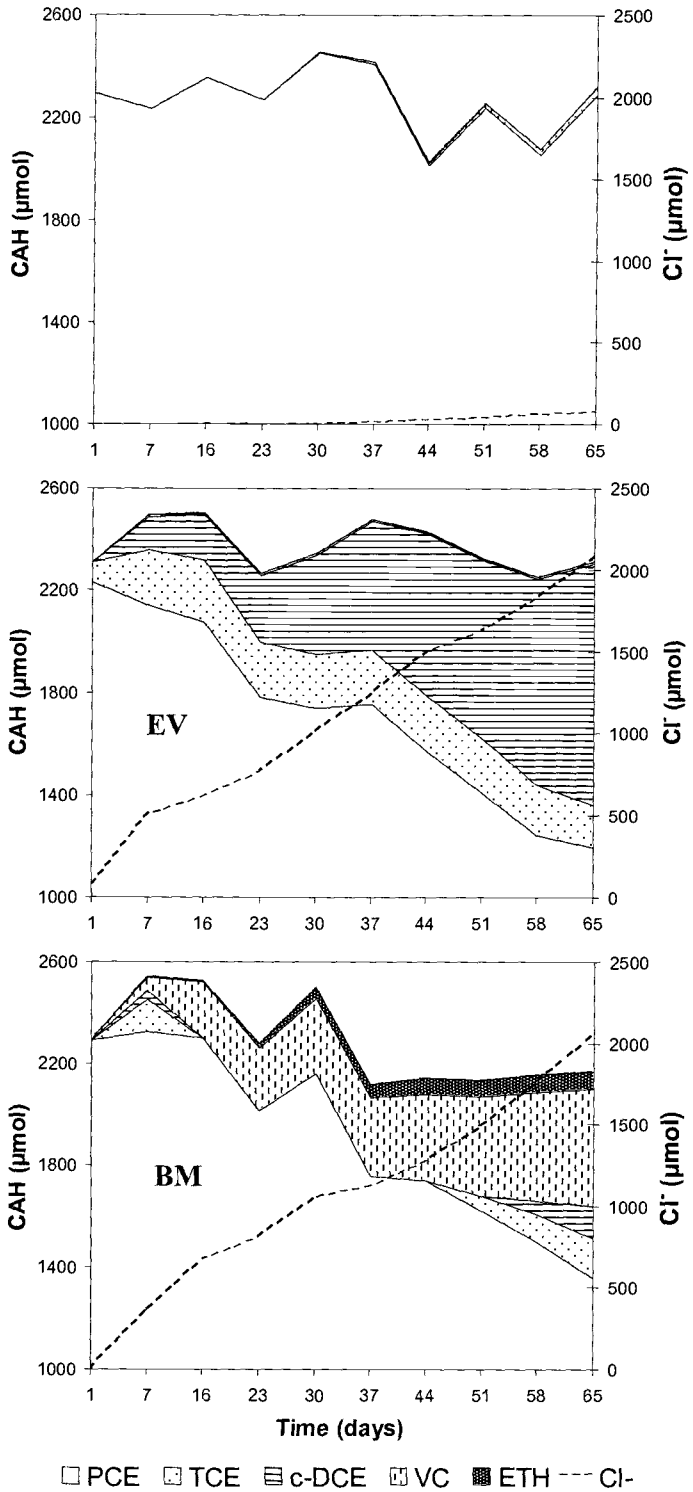

Figure 4-1. Comparison of reductive dechlorination of PCE/TBOS NAPL mixture by the PM culture, the EV culture, and the BM culture.

calculated chloride release of 2085 μmol, similar to the total dechlorination of the BM culture (2065 μmol as chloride). Thus, despite the different distribution of products, a similar degree of dechlorination was achieved by the two cultures. In all the systems very low concentrations of methane were observed. This result is consistent with those of Yang and McCarty (2000a) who found that 0.1 mM of PCE inhibited methanogenesis.

Effect of PCE Aqueous Concentrations on Reductive Dechlorination

The effect of PCE aqueous concentrations on reductive dechlorination of PCE DNAPL mixed with TBOS was investigated using different PCE mol fractions (0.02, 0.11, and 0.5), yielding different PCE aqueous concentrations (Table 4-1). The BM culture was used for these experiments (BP-1, BP-2, and BP-3), since the BM culture was shown to more efficiently dechlorinate PCE DNAPLs to ETH.

As shown in Figure 4-2, a greater mass of ETH was produced at a lower aqueous PCE concentration of BP-1 (ETH mass = 4.3 μmol) compared to BP-2 (ETH mass = 1.8 μmol) and BP-3 (ETH mass = 0 μmol). The total chloride released estimated in BP-1 (average mass = 115 μmol) was slightly higher than BP-2 (average mass = 103 μmol). The higher PCE aqueous concentration resulted in a slightly lower transformation to ETH in BP-2 compared to BP-1. Almost no TCE or *c*-DCE was detected in the BP-1 and BP-2 reactors during the experiments, showing that VC dechlorination to ETH was the slowest dechlorination step. No reductive dechlorination of PCE occurred in BP-3, and continuous butanol accumulation was

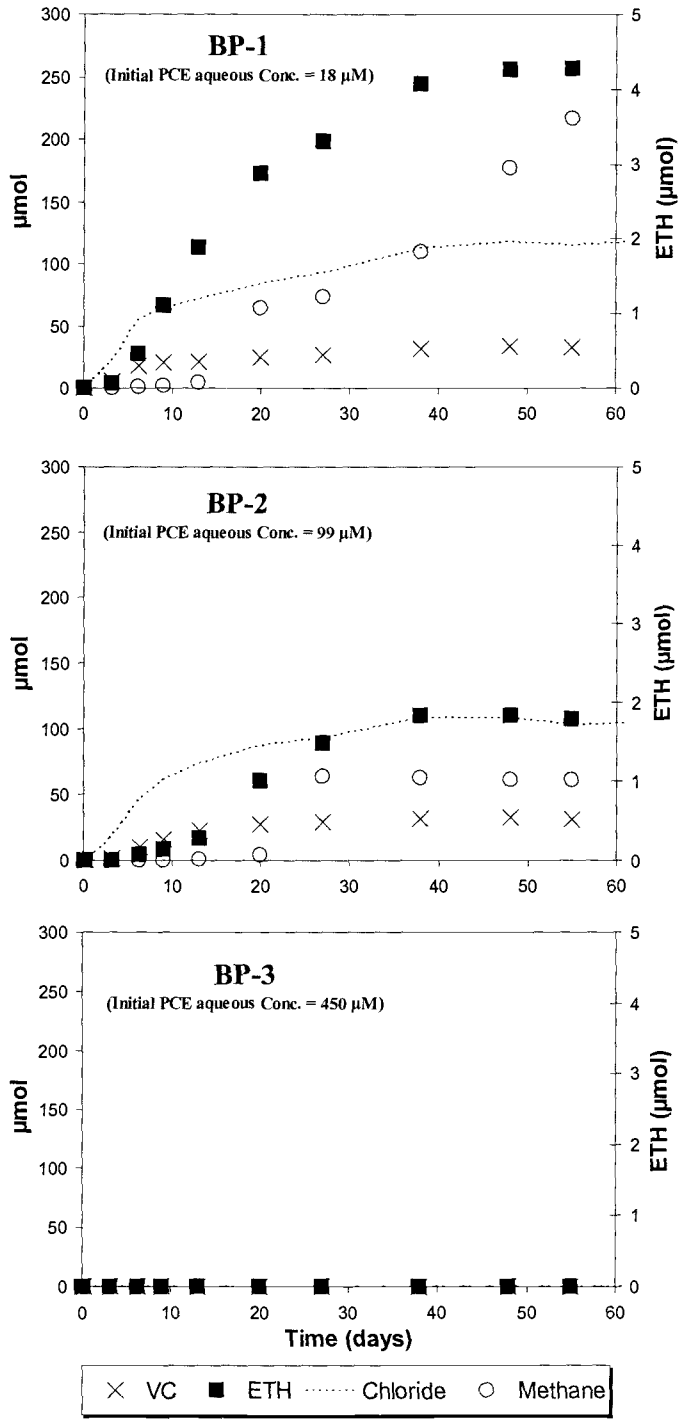

Figure 4-2. Effect of PCE aqueous concentrations on reductive dechlorination for the BM culture. Chloride ions were calculated from the concentrations of the dechlorination products. Almost no TCE or *c*-DCE were detected in the batch reactors. The results show average values in duplicate. The range of duplicate values are generally smaller than the size of the symbols.

observed (data not shown). This result indicates that high PCE aqueous concentrations likely inhibited the fermentation process of butanol to butyrate and/or acetate producing H_2. Butanol fermentation to butyrate and/or acetate with H_2 production and consumption was observed in BP-1 and BP-2. Methane production in BP-1 increased between 15 – 20 days, and started to increase in BP-2 between 20-25 days started to increase. After 30 days, methane production rate of BP-1 was much higher than BP-2, while BP-3 showed no methane production. These results indicate that PCE had an inhibitory effect on methanogenesis. Reductive dechlorination activities in BP-1 and BP-2 were observed to be slow down and finally stop after 40 days. The reason is not clear, but the toxicity or inhibition from PCE mixed with TBOS NAPL maybe related to the dechlorination inactivity (Yang and McCarty, 2000b). BP-3 with the greatest PCE mass of 8660 μmol showed no dechlorination ability during the experiment.

Effect of PCE Mass in NAPL on Reductive Dechlorination

Since the highest PCE aqueous concentrations showed no reductive dechlorination of PCE, possibly indicating inhibition or toxicity, the effect of PCE mass in the PCE/TBOS NAPL mixture was evaluated for the BM culture at the same PCE aqueous concentration, but with different PCE masses of 182, 424, 741, and 1060 μmol, respectively, in the NAPL mixture. PCE mol fractions in PCE/TBOS mixtures were all set to 0.11, resulting in a PCE aqueous concentration of 99 μM, but

the total volume of the NAPL mixture increased from 0.57 to 3.30 mL (BP-4, BP-5, BP-6, and BP-2 (Table 4-1)).

All experiments were performed in duplicate, and showed very reproducible results and good CAH mass balances (Figure 4-3). All of the PCE was transformed after 40 days in BP-4 that had the lowest mass of PCE. The other batch reactors (BP-5, BP-6, and BP-2) that had greater mass of PCE NAPL had PCE transformations of 121, 80, and 35 μmol, respectively, by day 40, which were measured using the partition coefficients among gas, aqueous, and NAPL phases. These results indicate that PCE dechlorination rate is likely affected by PCE mass in a PCE/TBOS NAPL mixture.

As depicted in Figure 4-3, very little accumulation of TCE and *c*-DCE was observed during the experiments. Higher ETH mass production was observed in the reactors with lower volumes of PCE/TBOS NAPL mixtures (BP-4 and BP-5), while BP-6 and BP-2 with larger volumes of the NAPL mixtures produced only trace amounts of ETH. BP-4 produced three times more ETH than BP-5, and the VC production of BP-6 was about twice that of BP-2. The batch systems showed different amounts of ETH production, even though they had the same initial 99 μM PCE aqueous concentration. The best performance in BP-4 was achieved with the least amount of TBOS and PCE present.

During reductive dechlorination of PCE NAPL, the concentrations of butanol and fatty acids were monitored along with the concentrations of hydrogen and methane. Butanol was produced from the hydrolysis of TBOS. As shown in Figure

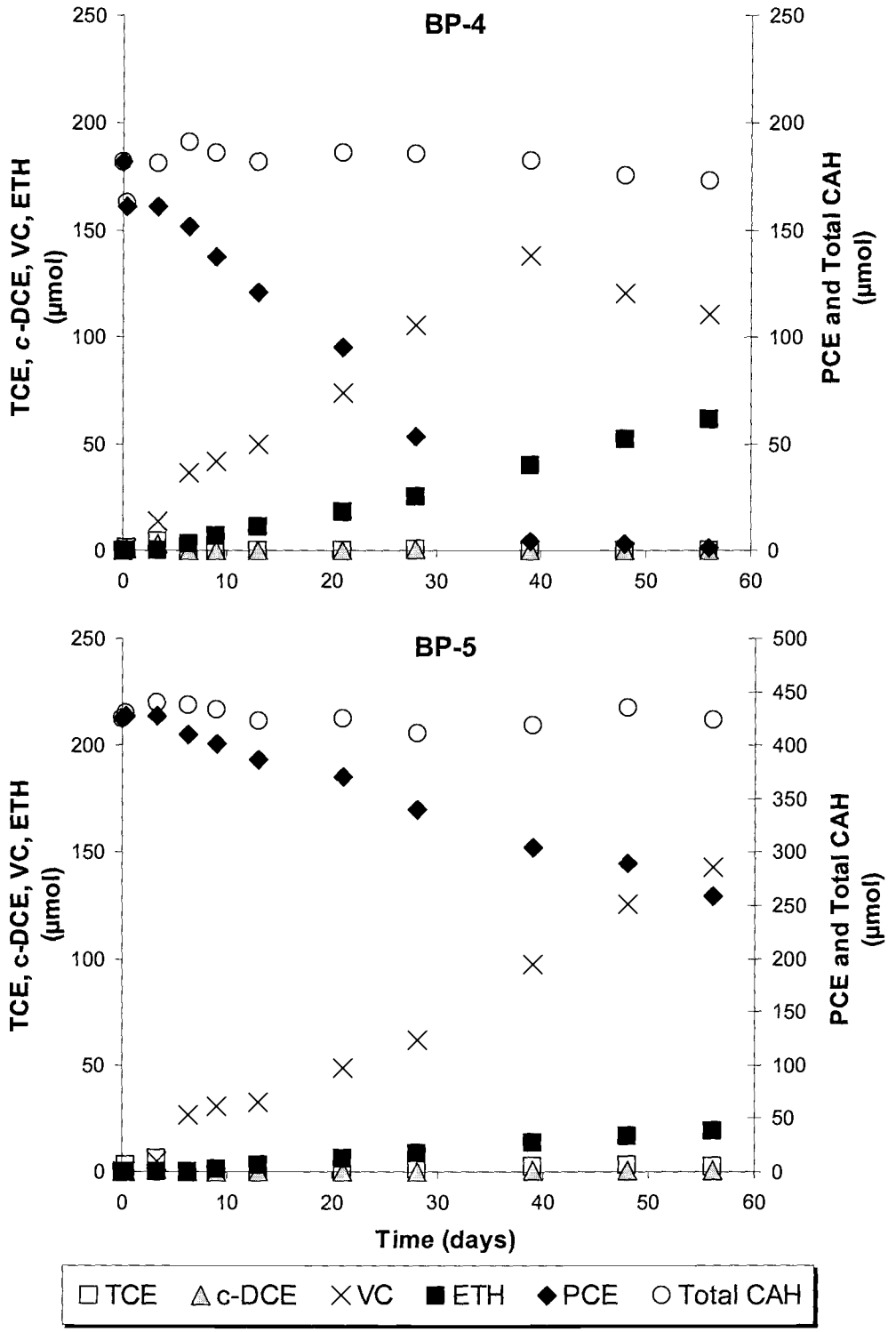

Figure 4-3. Effect of PCE mass in NAPL on reductive dechlorination for the BM culture. The results show average values of the duplicates. The range of duplicate values are generally smaller than the size of the symbols.

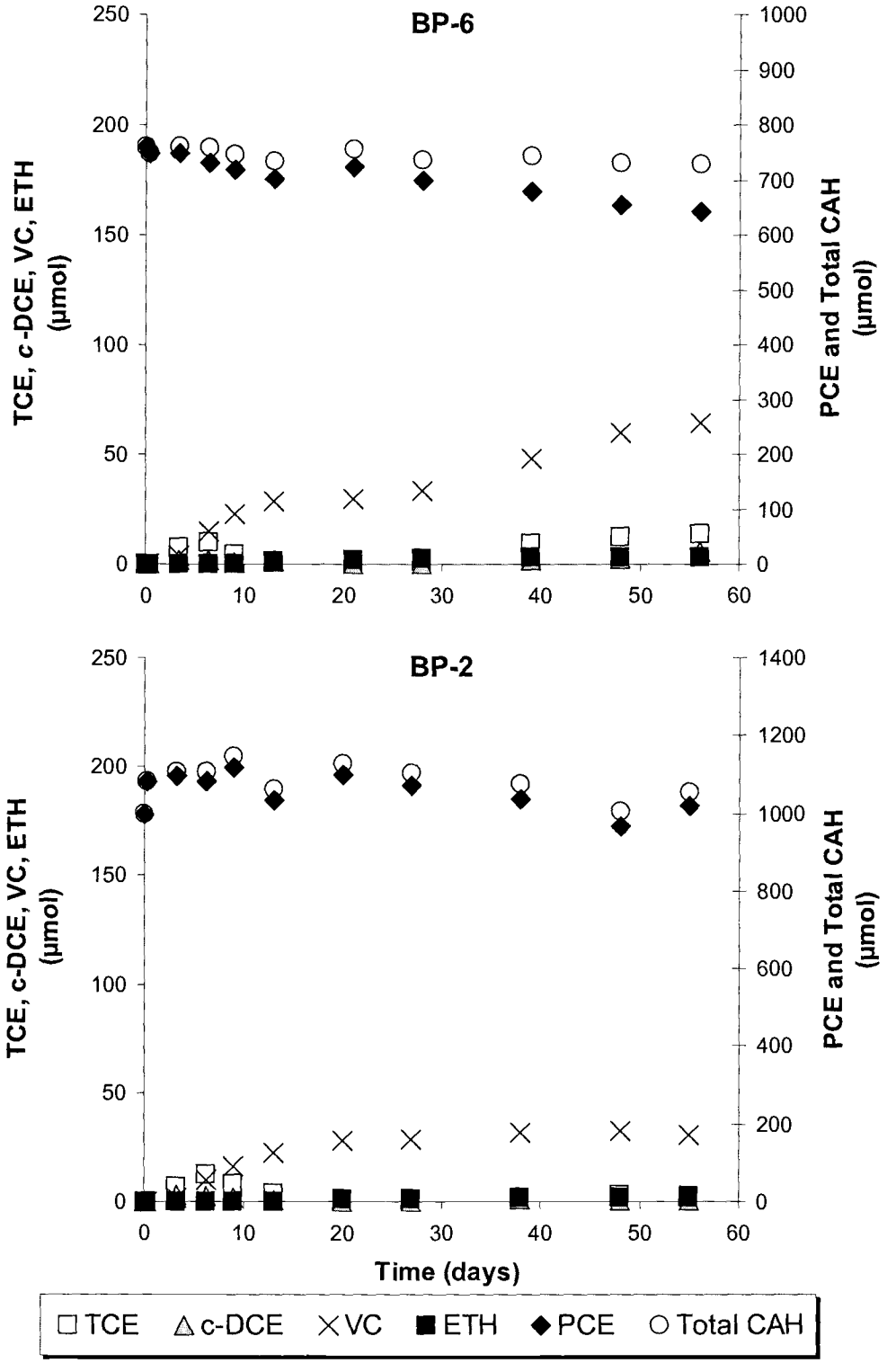

Figure 4-3. (continued)

4-4, early in the incubation most of the butanol was fermented to butyrate and/or acetate. Hydrogen concentrations accumulated at early time. Butanol was observed in BP-6 and BP-2 over the course of the experiments, whereas almost no butanol was detected in BP-4 and BP-5. Butyrate, a fermentation product of butanol, accumulated in BP-5, BP-6, and BP-2, but no significant increase in butyrate concentration occurred in BP-4. Acetate concentrations in BP-4 continuously increased during the experiments, indicating butyrate fermented to acetate. Acetate concentrations in the other batch reactors (BP-5, BP-6, and BP-2) increased during the first 10 days. Very little methane production was observed in all the batch reactors. The average hydrogen concentrations were also 263, 386, 1698, and 2333 nM between day 10 and 40 for BP-4, BP-5, BP-6, and BP-2, respectively. The lower hydrogen concentrations were associated with reactors that were most actively dechlorinating PCE to ETH.

The chloride ion concentrations calculated from the concentrations of reductive dechlorination products (TCE, *c*-DCE, VC, and ETH) were compared with measured chloride ion concentrations of aqueous samples (Figure 4-5). Very good agreement was observed between the calculated and measured amounts of Cl^-. The calculated amounts of Cl^- were determined from a mass balance of the dechlorination products (TCE, *c*-DCE, VC, and ETH) using the independently measured partitioning coefficients. Throughout the 55 day incubation, good mass balances were achieved.

The highest rate of estimated chloride ion production was observed in BP-4,

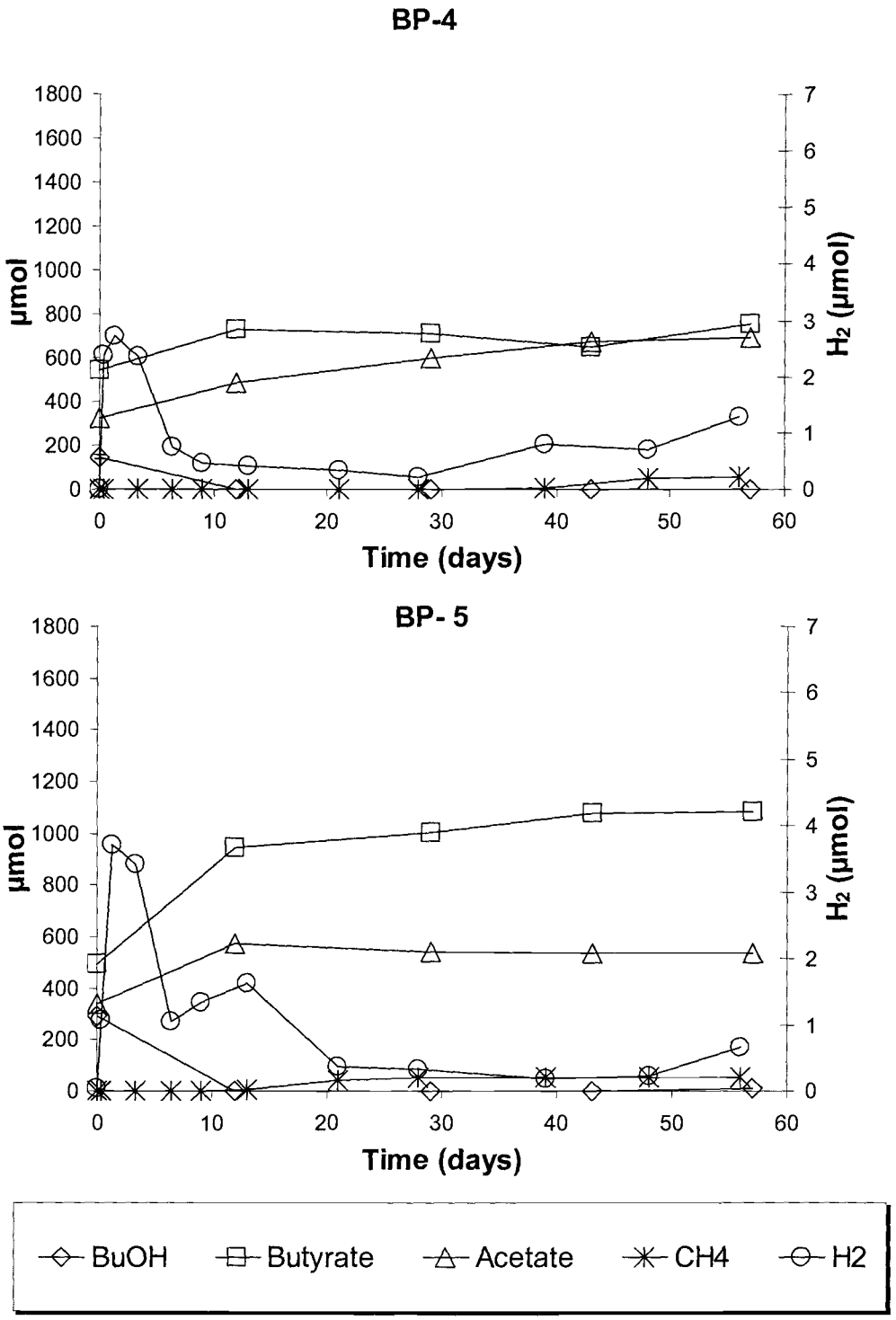

Figure 4-4. Butanol, acids, CH_4, and H_2 productions during reductive dechlorination for the BM culture. The results show average values in duplicate.

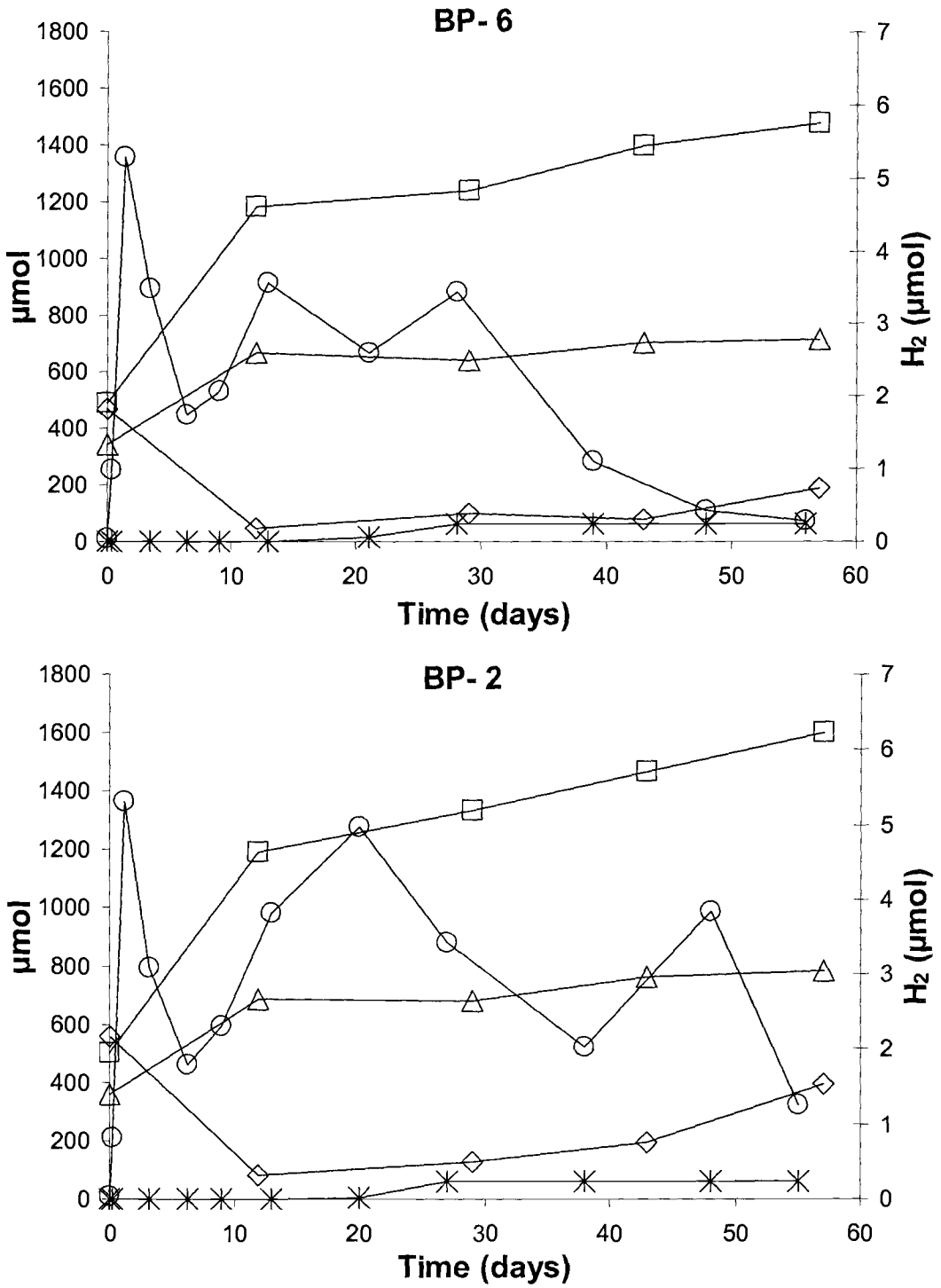

Figure 4-4. (continued)

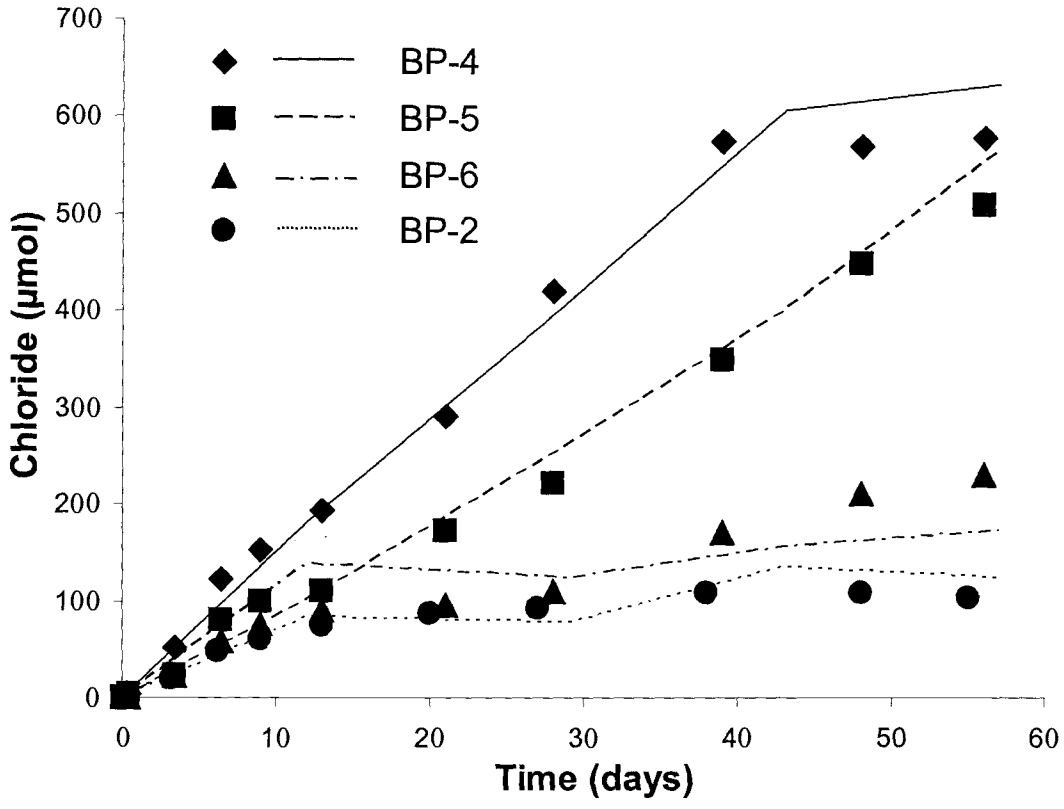

Figure 4-5. Total reductive dechlorination activity based on chloride ion released as calculated from dechlorination products, and chloride ion measured from aqueous samples in the batch reactors. Calculated from dechlorination products (◆, ■, ▲, ●) and ion chromatography-measured from aqueous samples (solid and dashed lines). The results show average values of duplicates.

which had the lowest PCE mass and volume of NAPL mixture. In contrast, BP-2 with the greatest PCE/TBOS NAPL volume had the lowest production rate of chloride ion. This comparison indicates that dechlorination process was inhibited by mass of PCE/TBOS. There are several possible causes for this inhibition. One possibility is the direct contact of dechlorinating microorganisms with the NAPL, resulting in toxicity. Yang and McCarty (2000a) observed similar results, and proposed direct contact of PCE DNAPL with dechlorinating microorganisms might cause inhibition or toxicity. The second possible reason for decreased dechlorination with greater PCE/TBOS NAPL mass is the reduction in pH in the batch reactors. After the experiments, the pH measurements in the batch reactors were conducted using pH indicator paper, and showed pH values were lower than 5. As the predominant products were acetate and butyrate, and H^+ liberated from the dechlorination reactions, the reduction in pH would occur. The greatest amount of TBOS NAPL released more butanol via abiotic hydrolysis, followed by the fermentation of the butanol to butyrate and/or acetate with H_2 production (Yu and Semprini, 2002b). It is interesting to note that once the PCE NAPL was completely utilized in BP-4 after 40 days, rates of dechlorination greatly slowed. This was likely caused by the reduction in pH and/or the inherently slow VC degradation as indicated by the k_{max} and K_S of VC. It is also interesting to note the greatest accumulation of butyrate and H_2 occurred in the reactors with the poorest dechlorination (BP-6 and BP-2).

Comparison of Electron Mass Balances

Stoichiometric electron mass balances were conducted for the electron donors and the acceptors. Table 4-3 gives the overall electron equivalence mass balances for the active batch reactors (BP-1 through BP-6) on day 0 and day 56. TBOS concentration was not measurable due to NAPL phase of TBOS mixed with PCE, but the abiotic hydrolysis rate of TBOS can be estimated based on the production of butanol from the control bottles under the same conditions. The abiotic production of butanol was used for the calculation of TBOS transformation to butanol in Table 4-3. The batch reactor bottles listed in Table 4-3 had good electron equivalence mass balances at the end of the experiments, varying from 98% to 105% electron recovery. Most of electron equivalent flow was channeled to the formation of butyrate, and a much lower electron flow went into the creation of acetate. This observation suggests that the fermentation of butanol to butyrate is more preferred by the fermenting microorganisms than that of butyrate to acetate. This can be explained by the standard free energy change (ΔG^{o} = 16.3 kJ/mol for the fermentation of butanol to butyrate with H_2 production, compared to 48.1 kJ/mol for butyrate to acetate) (Thauer *et al.*, 1977). Dechlorination efficiency was calculated from the total electron equivalence change for the fermentation of butanol and butyrate between day 0 and day 56 (assumption of no further fermentation of acetate). This calculation shows 8 to 10% for BP-1 and BP-2, but 0% for BP-3. For the other set of experiments, the calculation of dechlorination efficiencies was 23, 45, 14, and 10% for BP-4, BP-5, BP-6, and BP-2, respectively. The efficiency in BP-4 was

Table 4-3. Electron balances from different microbial processes in batch reactors. The values are represented as average in duplicates.

	e^- equiv per mol	BP-1 (e- μ equiv.)		BP-2 (e- μ equiv.)		BP-3 (e- μ equiv.)		BP-4 (e- μ equiv.)		BP-5 (e- μ equiv.)		BP-6 (e- μ equiv.)	
		Day 0	Day 56	Day 0	Day 56	Day 0	Day 56	Day 0	Day 56	Day 0	Day 56	Day 0	Day 56
TBOS[a]	**96**	20064	0	20064	0	20064	0	5760	0	9312	0	16224	0
Butanol	**24**	11496	4284	13428	9504	6876	25956	3492	0	6948	276	11232	4512
Butyrate	**20**	10280	34680	10060	31990	9820	10820	10870	15100	9890	23630	9820	29580
Acetate	**8**	2812	5948	2864	6260	2884	3044	2580	5576	2732	4284	2724	5728
TCE	**2**	0	0	0	0	0	0	0	0	0	0	0	27
***c*-DCE**	**4**	0	0	0	0	0	0	0	0	0	0	0	22
VC	**6**	0	195	0	186	0	0	0	663	0	858	0	384
ETH	**8**	0	34	0	14	0	0	0	492	0	152	0	26
CH_4	**8**	0	1736	0	492	0	0	0	440	0	420	0	524
H_2	**2**	0	0.2	0	2.5	0	4.7	0	2.5	0	1.3	0	0.6
Total		44652	46877	46416	48449	39644	39825	22702	22274	28882	29621	40000	40804
Recovery %			105		104		100		98		103		102

	e^- μ equiv. used for					
Dechlorination	229	200	0	1155	1010	459
Methanogenesis	1736	492	0	440	420	524

[a]based on the amounts of TBOS hydrolyzed to BuOH, calculated from control bottle for the same period of the experiment. [b]Yu and Semprini (2002b). TBOS hydrolysis rates measured from the control bottles; BP-1, BP-2, BP-3 (3.8 μmol TBOS/day), BP-4 (1.1 μmol TBOS/day), BP-5 (1.7 μmol TBOS/day), BP-6 (3.0 μmol TBOS/day).

lower than that in BP-5 for 56 day incubation. This is likely attributed to the complete depletion of PCE between day 30 and 40 in BP-4, while BP-5 had PCE NAPL present during the whole incubation period.

DISCUSSION

This study investigated the reductive dechlorination of PCE DNAPL mixed with TBOS as a slow-release anaerobic substrate. Different mixed cultures and batch reactor conditions were tested. Experimental results indicated that TBOS as a slow-release anaerobic substrate promoted dechlorination of PCE DNAPL. In addition, the BM culture, a combination of a mixture of two single mixed cultures, was able to more completely dechlorinate PCE DNAPLs with a higher ETH production rate than either of the single mixed cultures (the PM and the EV cultures). Many CAH-contaminated sites showed incomplete reductive dechlorination leading to the accumulation of *c*-DCE and VC. The BM culture showed almost no accumulation of TCE or *c*-DCE during the reductive dechlorination of PCE NAPL. The BM culture showed better and enhanced dechlorinating ability, even though the single mixed cultures are able to reductively dechlorinate PCE or TCE to ETH. The PM culture with a TCE/TBOS NAPL mixture (data not shown) rapidly biotransformed TCE to VC, which was slowly dechlorinated to ETH, consistent with the previous observation for reductive dechlorination of TCE below saturation concentration (Yu and Semprini, 2002b). This result indicates that the PM culture is able to reductively dechlorinate TCE, but very little PCE. The EV culture more

slowly transforms *c*-DCE to VC than the PM culture, but more rapidly dechlorinates VC to ETH. However, when two single mixed cultures were added together (the BM culture), less *c*-DCE accumulation and higher ETH production was observed. The binary mixed culture likely has different dechlorinating microorganisms with varying dechlorination abilities to achieve more efficient and enhanced *in situ* bioremediation. Thus, bioaugmentation with a binary mixed culture could be an attractive strategy when completely dechlorinating bacteria can not be found at the contaminated sites.

TBOS could be an effective substrate for remediating CAH DNAPL contamination and enhancing rates of reductive dechlorination. TBOS injected into the DNAPL zone and co-mixing with the NAPL would reduce the aqueous concentrations of PCE and TCE, and potentially decreases toxicity or inhibition. This could enhance the overall rates of reductive dechlorination (Yu and Semprini, 2002b). In these batch systems, different amounts of NAPL PCE were mixed with the same amount of TBOS (Figure 4-2). A lower PCE mol fraction in PCE/TBOS NAPL resulted in a lower aqueous PCE concentration, and greater extents of transformation were achieved. At day 56, BP-1 with the lowest PCE aqueous concentration showed the highest chloride concentration and ETH production compared to BP-2 and BP-3. High concentrations of aqueous PCE or a high PCE mol fraction in PCE/TBOS NAPL (for example, 0.5 in BP-3) also inhibited fermenting microorganisms, since only abiotic hydrolysis of TBOS occurred with almost no fermentation of the butanol. The butanol accumulation (19080 e^- μ-

equivalent) from abiotic hydrolysis of TBOS in BP-3 was very close to that in the control bottle (20064 e^- μ-equivalent) without biological activity (Table 4-3).

The total chloride mass balances based on dechlorination products were very consistent with the measured chloride in aqueous samples (Figure 4-5), indicating the measurement of chloride released from dechlorination activity is a good indicator for reductive dechlorination of PCE NAPL and good mass balances for dechlorination products can be achieved based on partitioning between gas, aqueous, and NAPL phases. Figure 4-3 and 4-5 indicated that better extents of dechlorination were observed in the batch reactor with lower amount of PCE/TBOS NAPL even at the same PCE aqueous concentration.

Figure 4-6 summarizes total chloride release concentrations as calculated from the dechlorination products as a function of the amount of PCE and TBOS for all experiments. Generally, the volume or amount of PCE and TBOS NAPL are key factors affecting reductive dechlorination of PCE DNAPL. Lower degrees of reductive dechlorination are associated with higher amounts of PCE and TBOS. Additionally, at a given PCE mol fraction of 0.11 (BP-4, BP-5, BP-6, and BP-2), PCE dechlorination decreases as amount of PCE and TBOS increases. This indicates greater amounts of PCE/TBOS NAPL likely are toxic to dechlorinating microorganisms and reduce the dechlorination activity. PCE mol fractions are also an important consideration for promoting better reductive dechlorination of PCE DNAPL. The best dechlorination performance was obtained with a PCE mol fraction of around 0.1 to 0.13 (BP-4 and BP-10), and dechlorination ability decreases

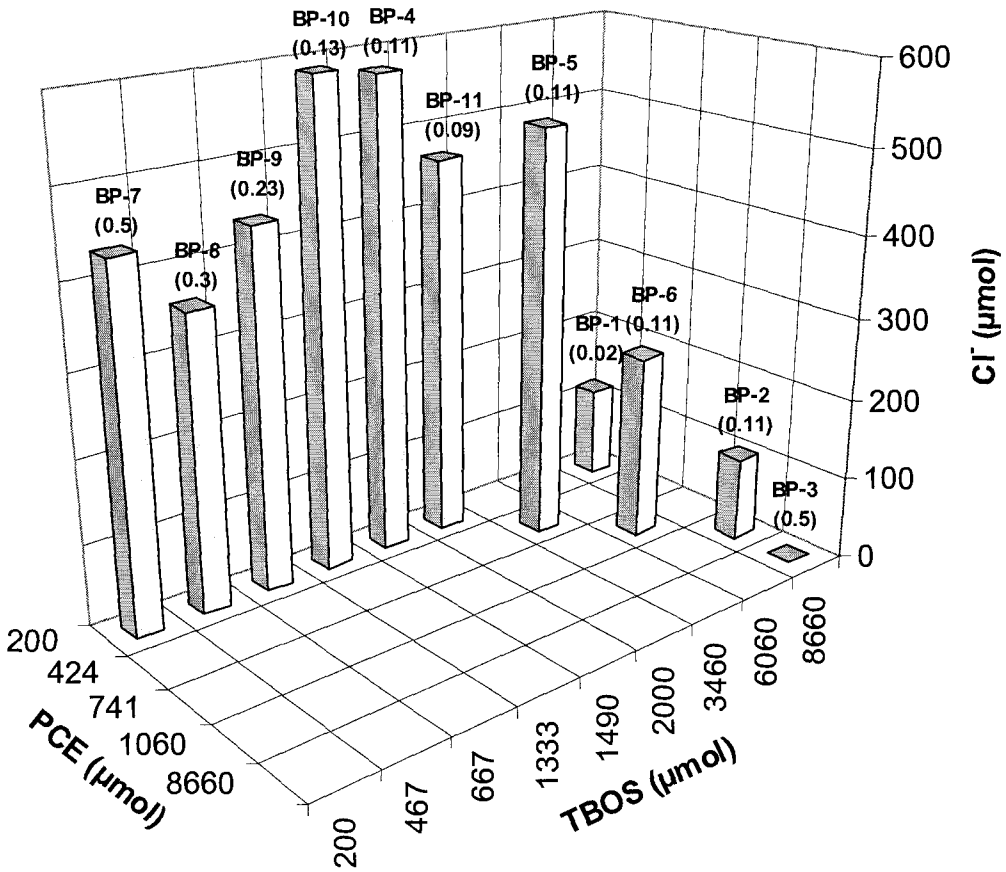

Figure 4-6. Total dechlorination in chloride as calculated from dechlorination daughter products at different PCE/TBOS NAPL conditions. PCE mol fractions in NAPL mixtures are represented in parentheses.

with decrease of mol fraction to 0.02.

Through the all batch experiments, one key consideration for remediating PCE NAPL should be toxicity or inhibition resulting from the direct contact of dechlorinating microorganisms to PCE/TBOS NAPL. Another factor to be carefully considered is the decrease in pH resulting from the accumulation of fatty acids as well as hydrogen ion released during dechlorination, likely resulting in decreases in dechlorination activity.

The difficulties (NAPL toxicity and pH drop) encountered in batch systems might be overcome in a groundwater aquifer setting with continuous flow. First, the possibility of the continuous direct contact of PCE NAPL with dechlorinating bacteria would be minimized in subsurface systems, resulting in less toxicity or inhibition effects compared to our batch reactors. Second, the dilution effect in flow-through systems would likely prevent fatty acids from accumulating, which might limit pH decreases. Overall, the results obtained here suggest that the application of TBOS as a slow-release substrate has potential for remediating CAH DNAPL as well as aqueous CAHs (Yu and Semprini, 2002b). TBOS could be added to a DNAPL zone to create a reactive barrier system (Yang and McCarty, 2000a). Butanol released from TBOS hydrolysis was found to ferment mainly to butyrate. Butanol with a high aqueous solubility (60 to 80 g/L) may be beneficial in supporting reductive dechlorination of high CAH concentrations, since the fermentation of butanol could possibly maintain high hydrogen tensions. In addition, the butyrate produced is a slow fermenting fatty acid (Fennell *et al.*, 1997), and would be

effective for promoting reductive dechlorination in the down-gradient plume containing chlorinated products.

In our study, a binary mixed culture composed of two mixed cultures was more effective for complete reductive dechlorination of CAH DNAPLs than either of the single mixed cultures. However, in real contaminated aquifers, more complex interaction between different cultures will occur and microbial growth will vary spatially on the basis of preferred electron. To better understand these interactions, more research is needed on microbial interactions and community shifts using molecular techniques. Modeling studies will allow us to understand and predict the fate and biotransformation of chlorinated ethylene DNAPL by a binary mixed culture in real contaminated aquifers.

Acknowledgements

The research was supported by a research grant from the U.S. Environmental Protection Agency-sponsored Western Region Hazardous Substance Research Center under agreement R-815738. This article has not been reviewed by the agency, and no official endorsement should be inferred.

REFERENCES

Adamson, D. T., Mcdade, J. M., and Hughes, J. B. (2003) Inoculation of a DNAPL Source Zone to Initiate Reductive Dechlorination of PCE. *Environ. Sci. Technol.* **37**, 2525-2533.

Bell, M., Brown, D., Burns, D. A., and Lee, M. D. (2001) Biologically Enhanced Reductive Dechlorination. Platform presentation. In Sixth In Situ and On-Site Bioremediation Symposium, San Diego, CA.

Carr, C. S., Garg, S., and Hughes, J. B. (2000) Effect of Dechlorinating Bacteria on the longevity and Composition of PCE-Containing Nonaqueous Phase Liquids under Equilibrium Dissolution Conditions. *Environ. Sci. Technol.* **34**, 1088-1094.

Cope, N. and Hughes, J. B. (2001) Biologically-Enhanced Removal of PCE from NAPL Source Zones. *Environ. Sci. Technol.* **35**, 2014-2021.

Ellis, D. E., Lutz, E. J., Odom, J. M., Buchanan, Jr., R. J., Lee, M. D., Bartlett, C. L., Harkness, M. R., and Deweerd, K. A. (2000) Bioaugmentation for Accelerated In Situ Anaerobic Bioremediation. *Environ. Sci. Technol.* **34**, 2254-2260.

Fennell, D. E. and Gossett, J. M. (1998) Modeling the Production of and Competition for Hydrogen in a Dechlorinating Culture. *Environ. Sci. Technol.* **32**, 2450-2460.

Fennell, D. E., Gossett, J. M., and Zinder, S. H. (1997) Comparison of Butyric Acid, Ethanol, Lactic Acid, and Propionic Acid as Hydrogen Donors for the Reductive Dechlorination of Tetrachloroethene. *Environ. Sci. Technol.* **31**, 918-926.

Gossett, J. M. (1987) Measurement of Henry's Law Constants for C1 and C2 Chlorinated Hydrocarbons. *Environ. Sci. Technol.* **21** (2), 202 - 208.

Harkness, M. R., Bracco, A. A., Brennan, Jr., M. J., Deweerd, K. A., and Spivack, J. L. (1999) Use of Bioaugmentation To Stimulate Complete Reductive Dechlorination of Trichloroethene in Dover Soil Columns. *Environ. Sci. Technol.* **33** (7), 1100 - 1109.

He, J., Ritalahti, K. M., Aiello, M. R., and Löffler, F. E. (2003b) Complete Detoxification of Vinyl Chloride by an Anaerobic Enrichment Culture and Identification of the Reductively Dechlorinating Population as a *Dehalococcoides* Species. *Appl. Environ. Microbiol* **69** (2), 996-1003.

He, J., Ritalahti, K. M., Yang, K.-L., Koenigsberg, S. S., and Löffler, F. E. (2003a) Detoxification of Vinyl Chloride to Ethene Coupled to Growth of an Anaerobic Bacterium. *Nature* **424**, 62-65.

Löffler, F. E., Cole, J. R., Ritalahti, K. M., and Tiedje, J. M. (2003) In *Dehalogenation: Microbial Processes and Environmental Applications*, Ed. Hägbblom, M. M. and Bossert, I. D., Kluwer Academic, New York, NY., pp. 53-87.

Maymo-Gatell, X., Chien, Y.-T., Gossett, J. M., and Zinder, S. H. (1997) Isolation of Bacterium that Reductively Dechlorinates Tetrachloroethene to Ethene. *Science* **276**, 1568-1571.

Nielsen, R. B. and Keasling, J. D. (1999) Reductive Dechlorination of Chlorinated Ethene DNAPLs by a Culture Enriched from Contaminated Groundwater. *Biotechnol. Bioeng.* **62**, 160-165.

Perry, R. H., Green, D. W., and Maloney, J. O. (1997) *Perry's Chemical Engineers Handbook*, 7th Ed., McGraw-Hill, New York, NY.

Rittmann, B. E., Seagren, E., and Wrenn, B. A. (1994). In *In-Situ Bioremediation*. Second Edition. Parkridge, New Jersey, USA: Noyes Publications, pp. 37-50.

Sharma, P. K. and McCarty, P. L. (1996) Isolation and Characterization of a Facultatively Aerobic Bacterium That Reductively Dehalogenates Tetrachloroethene to cis-1,2-Dichlorroethene. *Appl. Eniron. Microbiol.* **62** (3), 761 – 765.

Thauer, R. K., Jungermann, K., and Decker K. (1977) Energy Conservation in Chemotrophic Anaerobic Bacteria. *Bacteriol. Rev.* **41**, 100~180.

Wiedemeier, T. and Henry, B. M. (2001) Technical Protocol for Enhanced Reductive Dechlorination via Vegetable Oil Injection. In Sixth In Situ and On-Site Bioremediation Symposium, San Diego, CA.

Yang, Y. and McCarty, P. L. (1998). Competition for Hydrogen within a Chlorinated Solvent Dehalogenating Anaerobic Mixed Culture. *Environ. Sci. Technol.* **32**, 3591-3597.

Yang, Y. and McCarty, P. L. (2000a) Biomass, Oleate, and Other Possible Substrates for Chloroethene Reductive Dehalogenation. *Biorem. J.* **4**, 125-133.

Yang, Y. and McCarty, P. L. (2000b) Biologically Enhanced Dissolution of Tetrachloroethene DNAPL. *Environ. Sci. Technol.* **34**, 2979-2984.

Yang, Y. and McCarty, P. L. (2002). Comparison between Donor Substrate for Biologically Enhanced Tetrachloroethene DNAPL Dissolution. *Environ. Sci. Technol.* **36**, 3400-3404.

Yaws, C. L. (1999) *Chemical properties handbook: physical, thermodynamics, environmental, transport, safety, and health related properties for organic and inorganic chemicals*. McGraw-Hill: New York, NY.

Yu, S. and Semprini, L. (2002b). Comparison of Trichloroethylene Reductive Dehalogenation by Microbial Communities Stimulated on Silicon-Based Organic Compounds as Slow-Release Anaerobic Substrate. *Water Res.* **36**, 4985-4996.

Yu, S.and Semprini, L. (2003) Kinetic Studies and Comparisons for Reductive Dechlorination by a Binary Mixed Culture. Platform presentation, In: In Situ and On-Site Bioremediation: from the Seventh International Symposium, Orlando, FL.

CHAPTER 5

KINETICS AND INHIBITION OF REDUCTIVE DECHLORINATION OF CHLORINATED ETHYLENES BY TWO DIFFERENT MIXED CULTURES

Seungho Yu, Mark E. Dolan, and Lewis Semprini

To be submitted to:

Environmental Science and Technology

ABSTRACT

A simple kinetic method was developed to obtain reproducible kinetic values of k_{max} and K_S for anaerobic dechlorination reactions of chlorinated ethylenes. Kinetic parameters were determined for two different mixed cultures (the PM and the EV cultures) from different sites. The k_{max} values obtained for tetrachloroethylene (perchloroethylene, PCE) and trichloroethylene (TCE) for the two cultures were very similar, but almost two-fold differences were observed for K_S values between the PM and EV cultures. The k_{max} for *cis*-1,2-dichloroethylene (*c*-DCE) of the EV culture was about two times lower than that of the PM culture, reflecting the slower *c*-DCE biotransformation of the EV culture. Vinyl chloride (VC) was transformed more slowly by the PM culture (k_{max} and K_S values of 2.44 ± 0.36 μmol/mg of protein/day and 602 ± 7.06 μM, respectively) compared to the EV culture (8.08 ± 0.94 μmol/mg of protein/day and 62.6 ± 2.37 μM). Inhibition studies with both cultures showed more chlorinated ethylenes inhibited the reductive dechlorination of the less chlorinated ethylenes. PCE inhibited reductive TCE dechlorination, but not *c*-DCE dechlorination, while TCE strongly inhibited *c*-DCE and VC dechlorination. *c*-DCE also inhibited VC transformation to ethylene (ETH). When a competitive inhibition model was applied, inhibition constants of each chlorinated ethylene, K_I (μmol/L), were comparable to their respective K_S values. In contrast, less chlorinated ethylenes weakly inhibited the dechlorination of the more chlorinated ethylenes, indicating inhibition constants are greater than their K_S values. Model simulations with competitive inhibition using the independently derived kinetic parameters matched

well the experiments results. The results indicate that the model has potential for predicting reductive CAH dechlorination rates, and the simple kinetic method provided good estimates of k_{max} and K_S values.

Key words: reductive dechlorination, PCE, TCE, DNAPL, competitive inhibition, K_S values.

INTRODUCTION

PCE and TCE are among the most commonly observed groundwater contaminants (Westrick *et al.*, 1984; Rittmann *et al.*, 1994). Many laboratory studies have shown that PCE acting as an electron acceptor can be reductively dechlorinated to TCE, *c*-DCE, VC and finally to ETH using hydrogen as an electron donor (Freedman and Gossett, 1989; Maymo-Gatell *et al.*, 1995). However, the accumulation and persistence of intermediate products like *c*-DCE and VC is of concern, since VC is a known carcinogen.

The competition for H_2 by dechlorinating microorganism, nitrate and sulfate reducers, methanogens, and acetogens has previously been reported (McCarty, 1997; Yang and McCarty, 1998; Löffler *et al.*, 1999). However, few studies have been performed on the inhibition among chlorinated ethylenes during reductive dechlorination. Inhibition of VC dechlorination by other chlorinated ethylenes was indicated by modeling studies of Tandoi *et al.* (1994). Haston (1999) reported the competitive inhibition between *c*-DCE and VC, and showed the inhibition constants

were comparable to their respective half-velocity coefficients. Garant and Lynd (1998) showed that a competitive inhibition model fit experimental data better than the noncompetitive for a culture capable of complete dechlorination of PCE to ETH. The kinetic parameters used in their study were obtained from statistical fits of the model to the experimental data, not independently determined.

Reported here are the results of the kinetic characterization of two different mixed cultures that completely dechlorinate PCE to ETH. A simple multi-equilibration method was developed to determine k_{max} and K_S values using a single batch reactor. Batch kinetic experiments were also conducted to study the inhibitory effects of chlorinated ethylene mixtures on reductive dechlorination. A model was constructed including terms for transformation inhibition and compared with the experimental results using independently determined kinetic parameters.

MATERALS AND METHODS

Chemicals

PCE (99.9%, spectrophotometric grade), TCE (99.9%) and *c*-1,2-DCE (97%) were obtained from Acros Organics (Pittsburgh, PA), and VC and ETH (both 99.5%, Aldrich Chemical, Milwaukee, WI) were used in the preparation of analytical standards and for addition to the batch reactors. 1-Butanol (99.8 %, HPLC grade, Aldrich Chemical, Milwaukee, WI) and hydrogen (99%, Airco, Inc., Albany, OR) were used as the electron donors.

Analytical Methods

PCE, TCE, *c*-DCE, VC, and ETH were measured with an HP-6890 gas chromatograph (GC) equipped with a photoionization detector (PID) and flame ionization detector (FID) connected in series. Chromatographic separation was achieved using a 30 m × 0.53 mm GS-Q column (J&W Scientific, Folsom, CA) with helium as the carrier gas (15 ml/min). 100-200 µL reactor headspace samples were introduced into the GC. The GC oven was initially set at 80°C for 1.5 min, heated at 65°C/min to 170°C and 40°C/min to 220°C, and kept at 220°C for 2.7 min. Hydrogen concentrations in headspace gas samples (200 µl) were determined using an HP-5890 GC with a thermal conductivity detector (TCD), operated isothermally at 220°C. The hydrogen samples were chromatographically separated with a Carboxen 1000 column (15 ft × 1/8 in, Supelco, Bellefonte, PA) using argon gas as a carrier gas at 15 ml/min. The hydrogen detection limit was 4 nM (aqueous concentration).

Culture Enrichment and Growth

Two different mixed cultures were enriched and used in this study. Microbial mixed cultures obtained from Point Mugu Naval Weapon Facility, CA (PM) and the Evanite site in Corvallis, OR (EV) were enriched in two separate batch reactors (total volume 1.2 L with liquid volume of 1 L). The PM culture was originally enriched under anaerobic conditions with the soil and the groundwater from the site (Yu and Semprini, 2002b). After 1.5 years, supernatant (300 mL) from the shaken bottle was

transferred into sterile basal medium with trace nutrients, which was modified from Yang and McCarty (1998). The modification included a two-fold increase of buffer capacity, and sodium chloride and ammonium chloride were replaced with sodium bromide and ammonium bromide, respectively. TCE concentrations when increased in steps up to 100 mg/L over a 1 year period showed complete reductive dechlorination to ETH. After each addition of TCE was reductively dechlorinated to ETH (50 to 100%), the batch reactor was purged with a furnace-treated mixed gas consisting of N_2 (90%) and CO_2 (10%). Fresh anaerobic medium was used to replace 300 ml of the liquid culture, followed by the addition of neat TCE and butanol to achieve aqueous concentrations of 100 mg/L and 40 mg/L, respectively. The batch reactor was maintained at 20°C with continuous shaking at 200 rpm. Oreginally, the PM culture was fed TCE as the electron acceptor because the culture was incapable of PCE dechlorination. However, after about 3 years of enrichment with nutrient media, the PM mixed culture started to show PCE dechlorination ability.

The EV culture was enriched from groundwater obtained from the Evanite site in Corvallis, OR (Yu and Semprini, 2002b; Pon *et al.*, 2003). A batch reactor was fed repeated neat PCE to achieve an aqueous concentration of 10 mg/L, and neat butanol was added to yield an aqueous concentration of 100-300mg/L. After one year of enrichment, the groundwater (300 mL) from the reactor was transferred into a batch reactor containing sterile basal media of the same formulation used for growing the PM culture. The batch reactor of the EV culture was maintained in the

same manner of the PM culture, but PCE was added as the electron acceptor at 100 mg/L, since the EV culture had the ability to dechlorinate PCE. The EV culture was enriched at 20°C with continuous shaking at 200 rpm over 3 years.

Both of the cultures initially showed very active methanogenesis with fatty acids and alcohols as electron donors (Yu and Semprini, 2002b). However, there has been no evidence of methanogenesis after 2-3 years of enrichment with repeated additions of high PCE or TCE concentrations.

A PCR assay targeting 16S ribosomal DNA was performed to identify the microbial population catalyzing the reductive dechlorination of chlorinated ethylenes. DNA was extracted from the PM and the EV mixed cultures with a FastDNA spin kit for soil (Qbiogene, Inc., Carlsbad, CA). For each culture, the amplification reaction was performed using 5 μL of extracted DNA with three different pairs of primers. One primer pair was specific for the *Desulfuromonas* dechlorinator group consisting of the forward primer 5'AACCTTCGGGTCCTACTGTC3' (DSM-205F, *Escherichia coli* 16S rRNA positions 205 to 222) and the reverse primer 5'GCCGAACTGACCCCTATGTT3' (DSM-1015R, 1033 to 1015) (Löffler *et al.*, 2000). The other two primer pairs were specific to the *Dehalococcoides* group (*D. ethenogenes* and FL2). One primer pair consisted of the forward primer 5'AAGGCGGTTTTCTAGGTTGTCAC3' (DHG-728F, 728 to 750) and the reverse primer 5'CGTTTCGCGGGGCAGTCT3' (DHG-1155R, 1172 to 1155) (Löffler *et al.*, 2000). The other primer pair was 5'GATGAACGCTAGCGGCG3' (DHC-1F, 1 to 17) and 5'GGTTGGCACATCGACTTCAA3' (DHC-1377, 1385 to 1366)

(Hendrickson *et al.*, 2002). Both the PM and the EV mixed cultures yielded bright positive PCR signals with both DHG and DHC primers, but negative with the DSM primers. This PCR assay indicated that the PM and EV cultures contain *Dehalococcoides*-like microorganisms that may be responsible for the complete reductive dechlorination to ETH.

Determination of Cell Concentration

The total cell concentration, X, in the study was determined by protein analysis using the Pierce Micro BCA™ Protein Assay Reagent Kit (Pierce, Rockford, IL). All samples were pretreated with the Compat-Able™ Protein Assay Preparation Reagent Set (Pierce, Rockford, IL) to eliminate potentially interfering substances prior to total protein quantitation. The protein concentration detection limit was 0.5 mg/L.

Kinetic Study for Determination of k_{max} and K_S

An experimental procedure was developed to determine k_{max} and K_S values for PCE, TCE, *c*-DCE, and VC dechlorination using sequential equilibrations in batch kinetic reactors. The method involved stepwise increases of the CAH concentration over a short time interval and measuring parent compound disappearance and daughter product production at each concentration step (Figure 5-1). The batch kinetic studies were conducted in 150 mL serum bottles containing 125 mL of liquid media and culture. The culture added to the reactor was harvested

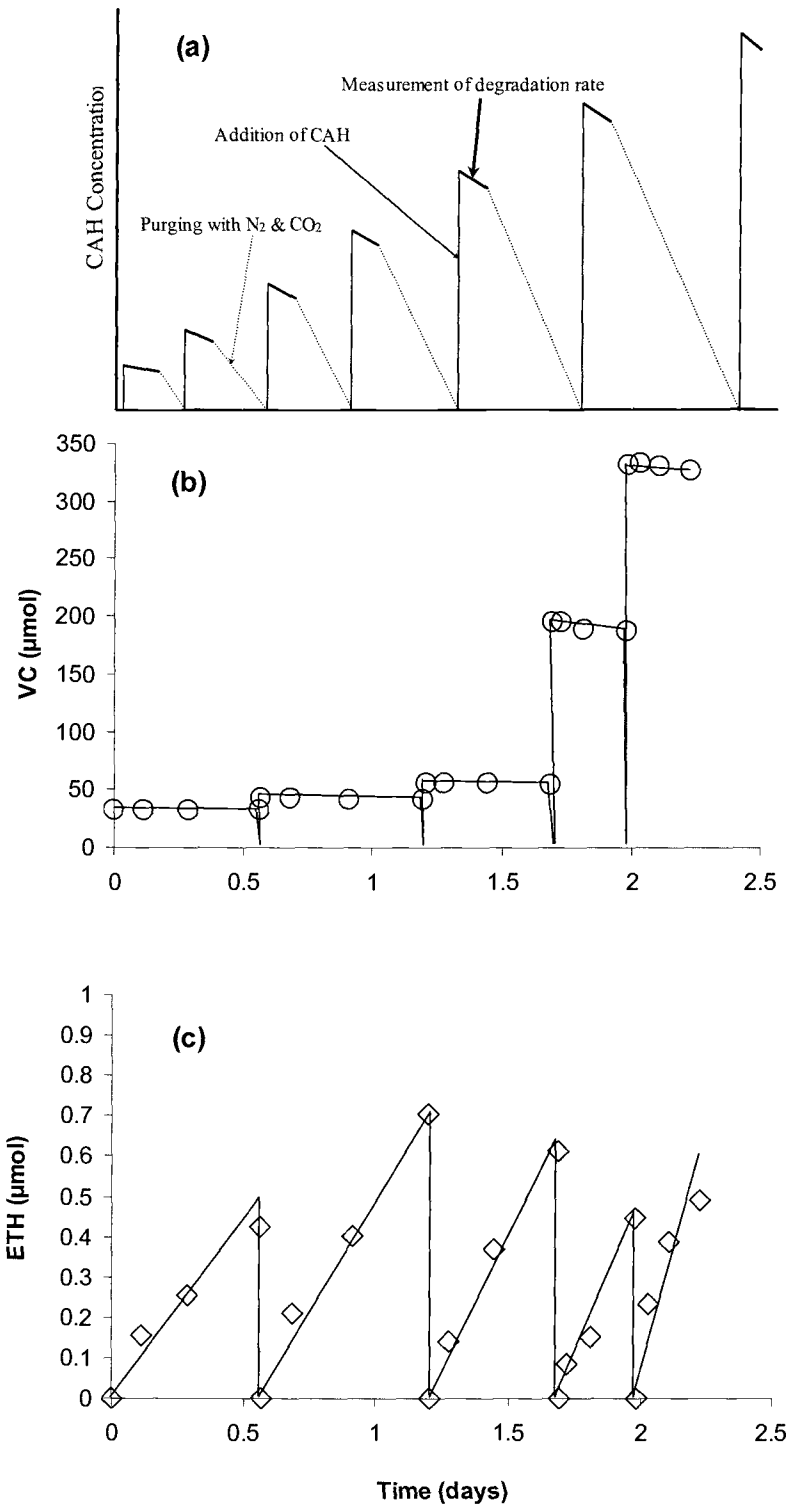

Figure 5-1. (a) Measurement method of CAH degradation rates with increasing concentrations. (b) and (c) Measurements of VC transformation and ETH production by the PM mixed culture.

from the enrichment mother batch reactor, 10 to 14 days after the batch feeding of PCE or TCE (50 to 100% converted to ETH). The amount of culture used was varied depending on the test to yield protein concentrations ranging from 2 to 50 mg/L. The batch reactors were constructed in an anaerobic glove box and then purged with a furnace-treated mixed gas of N_2 (90%) and CO_2 (10%), followed by the addition of 10 µL of neat butanol (99.8%) and 0.5–2.0 mL of H_2 gas (99%) as electron donors. Initially, the reactors were amended with the lowest concentration of a chlorinated ethylene to be tested, and shaken at 300 rpm at 20°C. The rates of parent compound disappearance and daughter product production were generally measured over a period of less than 1 hr by headspace sampling and analysis of the reaction (except VC transformation). Total CAH mass in the reaction was computed using published Henry's Law constants (Gossett, 1987; Perry *et al.*, 1997), V_L (volume of liquid), and V_G (volume of headspace). All kinetic parameters in this experiment were obtained based on the measurements of dechlorination products, instead of reactants, due to the greater sensitivity in measuring product production rate. After measuring the dechlorination rate at the lowest CAH concentration, the reactor was purged with the mixed gas (90 % N_2 and 10% CO_2) for 5 - 10 min to remove all the dechlorination products from the reactor. H_2 was added again, along with a higher concentration of the chlorinated ethylene, and the transformation rate was measured using the procedures previously described.

Factors that could affect rates in a multiple-step kinetic test, such as changes in biomass and redox conditions were minimized. The set of kinetic experiments

with a particular chlorinated ethylene were usually finished within 10 hrs. Changes in total cell concentrations as measured by the protein content before and after the experiments were indistinguishable. Doubling times for dechlorinating cultures have been reported to be approximately 2 days (He *et al.*, 2003a; Cupples *et al.*, 2003), thus, the 10 hour time period to complete the test was relatively short compared to the doubling time. The kinetic batch experiments were conducted in duplicate or triplicate to ensure that kinetic parameters were reproducible. Commercially available PCE, TCE, and VC (99% no stabilizers) were used in the kinetic experiments. Biogenic *c*-DCE was used for *c*-DCE dechlorination rate measurements, since chloroform is present in commercial *c*-DCE and has inhibitory effects on CAH utilization (Haston, 1999; Maymo-Gatell *et al.*, 2001). Biogenic *c*-DCE was produced from TCE dechlorination by the mixed anaerobic culture from Site 300 at Lawrence Livermore National Laboratory, CA (LLNL), which dechlorinates TCE to *c*-DCE (Yu and Semprini, 2002b). An enrichment reactor of the LLNL culture was fed TCE and allowed to fully transform it to *c*-DCE. *c*-DCE dissolved in liquid was obtained from centrifuging the reactor at 8000 rpm and filtering the liquid suspension through 0.2 μm syringe filter. The filtrate was used as the biogenic source of *c*-DCE in the kinetic experiments.

Inhibition Study

Inhibition of chlorinated ethylenes on transformation of each other was also studied. For example, PCE and TCE were tested for inhibitory effects on each other.

The same aqueous concentration of TCE was added to 3 to 4 batch reactors with different concentrations of PCE for the evaluation of PCE inhibition of TCE transformation. Similarly, inhibition of TCE on the reductive PCE dechlorination was evaluated at the same aqueous PCE concentration with different concentrations of TCE. For the experiments of PCE inhibition on TCE dechlorination, the production rates of daughter products were measured (*c*-DCE, VC, and ETH). However, decreases in PCE concentrations were used to quantify TCE inhibition on PCE dechlorination because high concentrations of TCE as an inhibitor were already present. Mass balances showed that there was less than a 20% difference in rates determined by parent compound disappearance and daughter product production. For these inhibition studies, several sets of chlorinated ethylenes were chosen including PCE ↔ TCE, PCE ↔ *c*-DCE, TCE ↔ *c*-DCE, TCE ↔ VC, and *c*-DCE ↔ VC. PCE inhibition of VC transformation was not studied after it was established that PCE did not inhibit *c*-DCE transformation and thus would not likely inhibit a further step in the transformation process.

MODEL DEVELOPMENT

Kinetic Model for Reductive Dechlorination

The dechlorination rate of a given chlorinated aliphatic hydrocarbon (CAH) was described using Michaelis-Menten kinetics. Reductive dechlorination of CAHs can be affected by several factors, including inhibition among chlorinated ethylenes,

electron donor concentrations, and CAH toxicity (Haston, 1999). However, for these kinetic experiments the chlorinated ethylene concentration was considered to only influence the kinetic parameters, k_{max} and K_S. This was regarded as a valid assumption because the initial dechlorination rates were measured before concentrations of the product CAHs could build to inhibitory levels, and to avoid potential toxicity (Haston and McCarty, 1997). Electron donor limitations were also excluded in the experiments by adding H_2 and butanol in excess. Thus, a simple Michaelis-Menten model based on electron acceptor concentration was used:

$$\frac{dC_L}{dt} = -\frac{k_{\max} X C_L}{K_S + C_L}\left(\frac{1}{1+\frac{V_G}{V_L}H_{CC}}\right) \tag{5-1}$$

where C_L is the CAH aqueous concentration (μmol/L), $k_{\max}$ is the maximum specific CAH dechlorination rate (μmol/mg of protein/day), X is the biomass concentration (mg of protein/L), and K_S is the half-velocity coefficient (μmol/L). Since the kinetic experiments were conducted with batch bottles consisting of gas and aqueous phases, the Monod equation was modified (Chang *et al.*, 1993), where V_G and V_L are the volumes of gas and aqueous phases, respectively, and the dimensionless Henry's constant (H_{CC}) was used for each chlorinated ethylene (Gossett, 1987; Perry *et al.*, 1997). Initial dechlorination rates were measured at different CAH concentrations and the first measured CAH concentration was regarded as the initial concentration for the dechlorination rate. For measurement of the dechlorination rates, total CAH mass in the bottle was computed from headspace measurement using Henry's

constants. Aqueous CAH concentration in Equation 5-1 is related to the total mass of each CAH ($C_L = M / (V_L + V_G H_{CC})$). All kinetic experiments conducted to determine kinetic parameters were based on CAH production rates instead of the disappearance rates of reactants because measuring changes in product concentration was more precise. The values of k_{max} and K_S were estimated from a nonlinear least squares method based on Equation 5-1. Standard deviations in the kinetic parameters were determined from a nonlinear least square regression fitting program (Solver in Excel and S-PLUS).

Competitive Inhibition among Chlorinated Ethylenes

Competitive inhibition among chlorinated ethylenes was included in the modeling analysis (Haston, 1999; Garant and Lynd, 1998). The reductive dechlorination rate of a CAH was modeled with competitive inhibition kinetics using Equation 5-2.

$$\frac{dC_L}{dt} = \frac{k_{max} X C_L}{K_S\left(1 + \frac{C_{I,1}}{K_{I,1}} + \frac{C_{I,2}}{K_{I,2}}\right) + C_L} \qquad (5\text{-}2)$$

where inhibition constants of each chlorinated ethylene are expressed as $K_{I,1}$ and $K_{I,2}$ (μmol/L). As will be described in the results, the respective half-velocity coefficients were used for the inhibition constants. This model included TCE, *c*-DCE, and VC as the major intermediates in PCE dechlorination to ETH, not 1,1-DCE or *trans*-DCE.

Based on the results of the inhibition experiments that will be presented, an

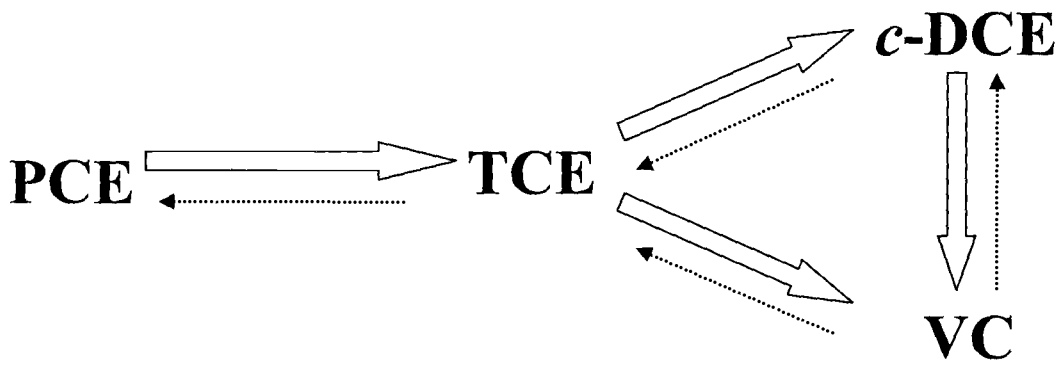

Figure 5-2. Proposed Inhibition Model between chlorinated ethylenes for reductive dechlorination based on the inhibition studies of Figures. 5-4 through 5-8. Arrows represent inhibition directions. Dashed arrows indicate very weak inhibition effects.

inhibition model was developed (Figure 5-2). A competitive inhibition model was chosen based on the model analysis of Garant and Lynd (1998) and Haston (1999). The kinetic equations for reductive dechlorination of the mixed cultures are presented Equations 5-3, 5-4, 5-5, and 5-6. The proposed inhibition model (Figure 5-2) and equations assume PCE inhibits TCE transformation, TCE inhibits both *c*-DCE and VC transformation, and *c*-DCE inhibits VC transformation. As the inhibition results will show, the less chlorinated ethylene inhibition on more chlorinated ethylenes were not significant and therefore were not included in the model formulation.

$$\frac{dC_{PCE}}{dt} = \frac{-k_{\max,PCE} X C_{PCE}}{K_{S,PCE} + C_{TCE}} \tag{5-3}$$

$$\frac{dC_{TCE}}{dt} = \frac{-k_{\max,TCE} X C_{TCE}}{K_{S,TCE}\left(1 + \frac{C_{PCE}}{K_{I,PCE}}\right) + C_{TCE}} \tag{5-4}$$

$$\frac{dC_{c-DCE}}{dt} = \frac{-k_{\max,c-DCE} X C_{c-DCE}}{K_{S,c-DCE}\left(1+\frac{C_{TCE}}{K_{I,TCE}}\right)+C_{c-DCE}} \quad (5\text{-}5)$$

$$\frac{dC_{VC}}{dt} = \frac{-k_{\max,VC} X C_{VC}}{K_{S,VC}\left(1+\frac{C_{TCE}}{K_{I,TCE}}+\frac{C_{c-DCE}}{K_{I,c-DCE}}\right)+C_{VC}} \quad (5\text{-}6)$$

The above equations were combined to model the results of the inhibition experiments using the independently determined parameters shown in Table 5-1. For the modeling analysis, inhibition constants of each chlorinated ethylene, K_I (μmol/L), were set equal to their respective half-velocity coefficients (K_S) given in Table 5-1.

Table 5-1. Kinetic parameters obtained from the experiments and used in the model.

	PM		EV	
	k_{max} (μmol/mg of protein/day)	K_S (μmol/L)	k_{max} (μmol/mg of protein/day)	K_S (μmol/L)
PCE	13.3 ± 1.80	3.86 ± 1.40	12.4 ± 0.72	1.63 ± 0.16
TCE	124 ± 17.4	2.76 ± 0.25	125 ± 14.0	1.80 ± 0.43
c-DCE	22.0 ± 2.02	1.90 ± 0.50	13.8 ± 1.09	1.76 ± 0.34
VC	2.44 ± 0.36	602 ± 7.06	8.08 ± 0.94	62.6 ± 2.37

All k_{max} and K_S values were determined from the simple multiple equilibration method in a single bottle.

Kinetic Model for Biomass Growth

Since the inhibition experiments were of longer duration, the potential growth of culture was included in the model using Equation 5-7. Microbial growth was

calculated with respect to the transformation rate of each chlorinated ethylene. The PM culture was assumed to grow on PCE, TCE, and *c*-DCE. VC transformation to ETH was assumed to be cometabolic, and thus, no biomass growth was coupled with kinetic modeling (Maymo-Gatell *et al.*, 1997). Unlike the PM culture, microbial growth on each dechlorination step was considered for the EV culture (Pon *et al.*, 2003).

$$\frac{dX}{dt} = Y\frac{dC}{dt} - k_d X \tag{5-7}$$

where Y is the growth yield of dechlorinating microorganisms, dC/dt is the rate of concentration change of each chlorinated ethylenes over time (μmol/L/day), and k_d is the decay constant of the dechlorinating microorganisms (day^{-1}). Values for growth yield, Y (= 0.006 mg of protein/μmol of Cl^- dechlorinated), and decay constant, k_d (= 0.024 day^{-1}), were obtained from the literature (Maymo-Gatell *et al.*, 1997; Fennell and Gossett, 1998). For our tests, we do not know the actual biomass, X, of the dechlorinating microorganisms, and whether different dechlorinators are active at different steps of the process. Since k_{max} values were determined on a total protein basis, the values were normalized to the total mixed culture biomass.

RESULTS AND DISCUSSION

Determinations of Kinetic Parameters

The Monod curve for VC transformation by the PM culture using the multi-equilibration kinetic method that was developed is shown in Figure 5-3. The rates for one set of data determined in the kinetic test are shown in Figure 5-1. The triplicated determinations show that very reproducible results were obtained. The triplicated determinations yield an average k_{max} value of 2.44 ± 0.36 μmol/mg of protein/day, while an average value of K_S was 602 ± 7.06 μmol/L. The kinetic parameters were obtained by nonlinear least-squares fitting using Equation 5-1 for each set of the experimental data, and the mean k_{max} and K_S values and a 95% confidence interval results from the triplicate determinations. Table 5-1 shows k_{max} and K_S values for both the PM and EV cultures for each step of the dechlorination process. The average standard deviation in the k_{max} values was approximately 11% of the average value, while the average standard deviation of the K_S was about 16%. For comparison, Haston and McCarty (1999) reported standard deviations in the k_{max} and K_S of 17% and 60% of the average values, respectively, using single equilibrations with multiple reactors. The results and comparison indicate that very reproducible k_{max} and K_S values can be obtained using a simple multiple equilibration method in a single reactor.

Previous observations showed that the PM culture rapidly dechlorinated TCE to VC, and very slowly biotransformed the VC to ETH. The EV culture reductively

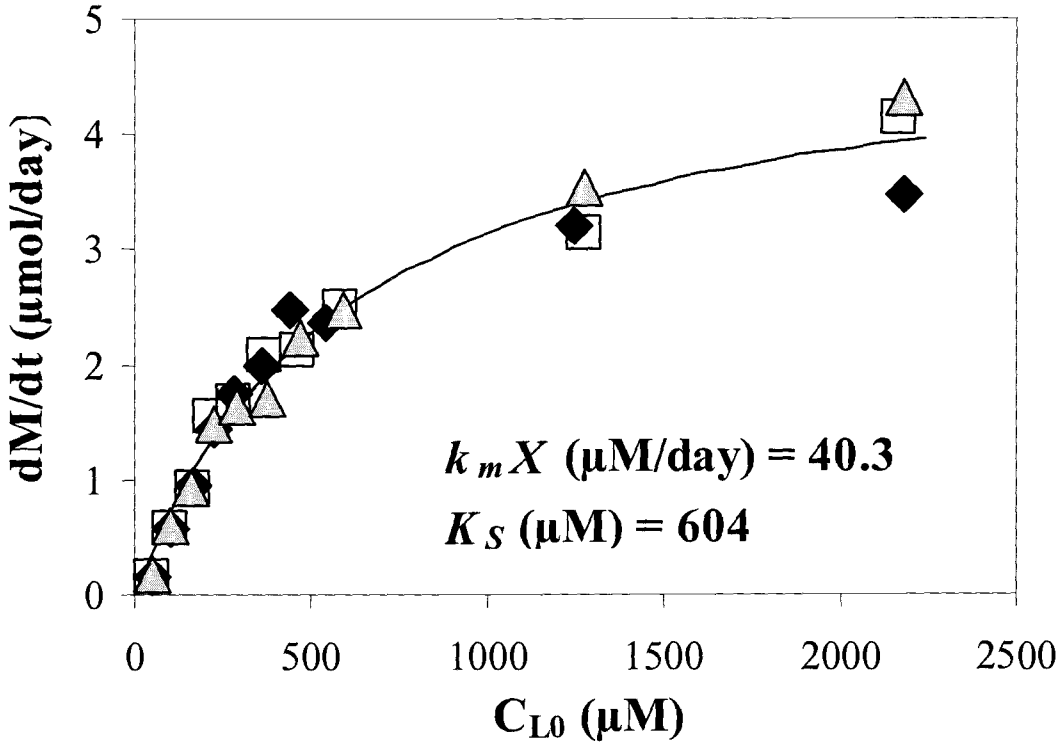

Figure 5-3. Initial reductive dechlorination rates of VC by the PM culture. A simple multi equilibration method was used to determine $k_{max}X$ and K_S values.

dechlorinated PCE to ETH, with rapid transformation kinetics of VC to ETH, but *c*-DCE accumulated and more slowly transformed *c*-DCE to VC than the PM culture (Yu and Semprini, 2002b). Table 5-1 shows that both cultures have comparable k_{max} and K_S values for PCE and TCE. The TCE k_{max} values for both cultures are a factor of 10 greater than PCE. The K_S value for PCE of the PM culture is a factor of two greater than the EV culture, while the k_{max} values are essentially equal. The results indicate that the EV culture is more efficient for PCE dechlorination when PCE concentrations are below the K_S values. The k_{max} and K_S values for *c*-DCE and VC show significant differences in transformation rates of the two cultures. The k_{max} for

c-DCE of the EV culture was 40% lower than that of the PM culture, while the K_S values were almost the same, reflecting the slower *c*-DCE biotransformation of the EV culture. Another very significant difference is in the k_{max} and K_S values for VC. The k_{max} of the EV culture was a factor of 3 greater, while the K_S was a factor of 10 lower. Thus, much more rapid VC transformation is observed with the EV culture. This result is consistent with the potential growth of the EV culture on VC as an electron acceptor as discussed by Pon and *et al.* (2003). Recently, VC-grown dechlorinating cultures (*Dehalococcoides* sp. strain BAV1 and *Dehalococcoides* sp. strain VS) were reported by He *et al.* (2003a and 2003b) and Cupples *et al.* (2003). The K_S value (62.6 μM) of the EV culture is closer to those of the two cultures (5.8 μM and 2.6 μM, respectively) than that of *Dehalococcoides ethenogenes* strain 195 (290 μM). The slower transformation and high K_S values for VC of the PM culture is consistent with the cometabolic VC transformation. The transformation characteristics of the PM culture are similar to *Dehalococcoides ethenogenes* strain 195, since the K_S value of the mixed culture having strain 195 was reported to be 290 μM, close to the K_S value of the PM culture (Fennell and Gossett, 1998; Maymo-Gatell *et al.*, 1997).

The protein tests showed no appreciable change in protein level (detection limit 0.5 mg/L) during the course of the kinetic tests. Protein concentrations used in the test ranged from 15 to 35 mg/L.

Competitive Inhibition among Chlorinated Ethylenes

Inhibition of one chlorinated ethylene on the dechlorination rate of another has been observed in several studies (Tandoi *et al.*, 1994; Haston 1999; Tonnaer 1997). VC dechlorination was inhibited by PCE and other chlorinated ethylenes, except *trans*-DCE (Tandoi *et al.*, 1994). Competitive inhibition between *c*-DCE and VC was also found with inhibition constants similar to the respective half-velocity coefficients and the competitive model showed good agreement with experimental results (Haston 1999).

In our study, competitive inhibition between pairs of chlorinated ethylenes was examined for both the PM and EV cultures. Results of inhibition studies are shown in Figures 5-4, 5-5, 5-6, 5-7, and 5-8. The lines shown in each Figure represent model simulations using the equations previously described in the Model Development section. The model used independently determined kinetic parameters listed in Table 5-1, and initial biomass was measured by protein analysis. Figures 5-4(a) and (b) present PCE inhibition on TCE transformation at initial aqueous TCE concentrations of 38 and 31 μM for the PM and EV cultures, respectively. Initial protein concentrations were 1.5–1.95 mg/L for the PM culture, and 1.26–1.36 mg/L for the EV culture. Rates of TCE transformation are presented as rates of *c*-DCE formation. As PCE concentrations increased, rates of *c*-DCE production decreased for both cultures, indicating that TCE dechlorination is inhibited by PCE. Both cultures showed almost the same extent of PCE inhibition on TCE dechlorination even at a narrower range of PCE concentrations for the EV culture, resulting from a

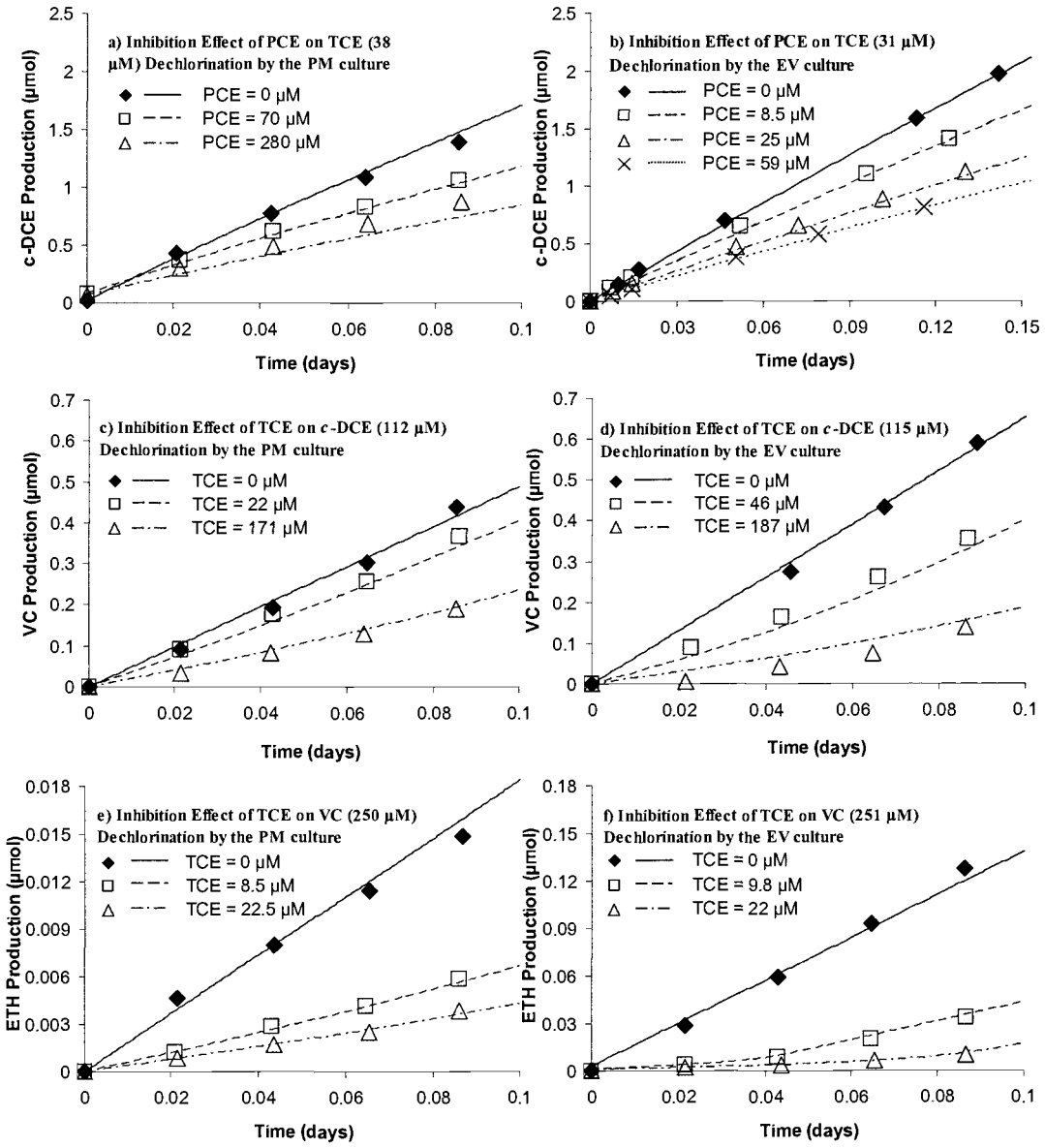

Figure 5-4. Competitive inhibition studies between CAHs. Lines represent the model simulations. Average initial cell concentrations for the model simulations are 1.7 (a), 1.3 (b), 2.3 (c), 4.0 (d), 2.3 (e), and 1.5 mg/L (f).

factor of 2.3 lower $K_{S,PCE}$ of the EV culture. The stronger PCE inhibition effect on the EV culture was confirmed in the model simulations which showed good fits of the experimental data for both cultures. The effect of TCE on PCE dechlorination was also evaluated (data not shown). PCE dechlorination was not clearly inhibited by TCE, suggesting there is very weak inhibition of TCE on PCE dechlorination.

Inhibition of TCE on *c*-DCE dechlorination is presented in Figures 5-4 (c) and (d). Initial protein levels measured at the start of the experiment were 2.0–2.5 mg/L and 3.9-4.2 mg/L for the PM and EV cultures, respectively. TCE inhibition of *c*-DCE transformation was observed with both cultures, with the EV culture being more strongly inhibited. The K_S value for TCE of the EV culture was a factor of 1.5 lower than the PM culture, which likely causes the greater TCE inhibition with the EV culture. The results of modeling based on the inhibition model given in Equation 5-5 showed good agreement with those of the inhibition experiments. TCE inhibition on VC transformation was examined as shown in Figures 5-4 (e) and (f) for the PM and EV cultures, respectively. TCE strongly inhibited VC transformation to ETH with both cultures. The EV culture more rapidly transformed VC by a factor of 10 than the PM culture (note the different time scales), even though the difference between protein concentrations used in the batch test (1.5 mg/L for the EV and 2.3 mg/L for the PM) was small. This result is consistent with the EV's k_{max} value being a factor of 3 higher and K_S value being a factor of approximately 10 lower compared to the PM (Table 5-1). The strong inhibition of TCE on VC transformation is consistent with the much lower K_S of TCE compared to the K_S values of VC for both

the PM and EV cultures. The modeling results using Equations 5-4, 5-5, and 5-6 provide an excellent match to the experimental data using the independently derived kinetic values given in Table 5-1, and the K_S values representing the inhibition constants. In contrast, both cultures showed no clear VC inhibition on TCE dechlorination (data not shown), consistent with the previous study (Nielsen and Keasling, 1999).

VC dechlorination rates to ETH in the presence of *c*-DCE were measured for a longer time period compared to other inhibition experiments, due to the slower rates of VC transformation (Figure 5-5). *c*-DCE concentrations for the PM and EV cultures increased from 0 μM to 271 μM and 181 μM, respectively, at an initial aqueous VC concentration of 290 μM. It should be noted that the initial protein content of the EV culture was about a factor of 24 greater than the PM culture for the tests. This results in much shorter time for *c*-DCE to be transformed as predicted by the model simulations. As shown in Figure 5-5 (a), (b), and (c), ETH production rates for the PM culture increased (except with no initial addition of *c*-DCE), after all *c*-DCE completely transformed to VC. After complete transformation of *c*-DCE to VC, ETH production rates were faster with the *c*-DCE initially added. The higher concentrations of VC, as a result of the addition and transformation of *c*-DCE, led to these greater rates of ETH production, as were confirmed in model simulations. VC transformations were very well predicted, based on the proposed inhibition model. The PM culture has a much higher K_S value of VC, indicating that the VC dechlorination rate to ETH increases along with higher VC concentrations. The

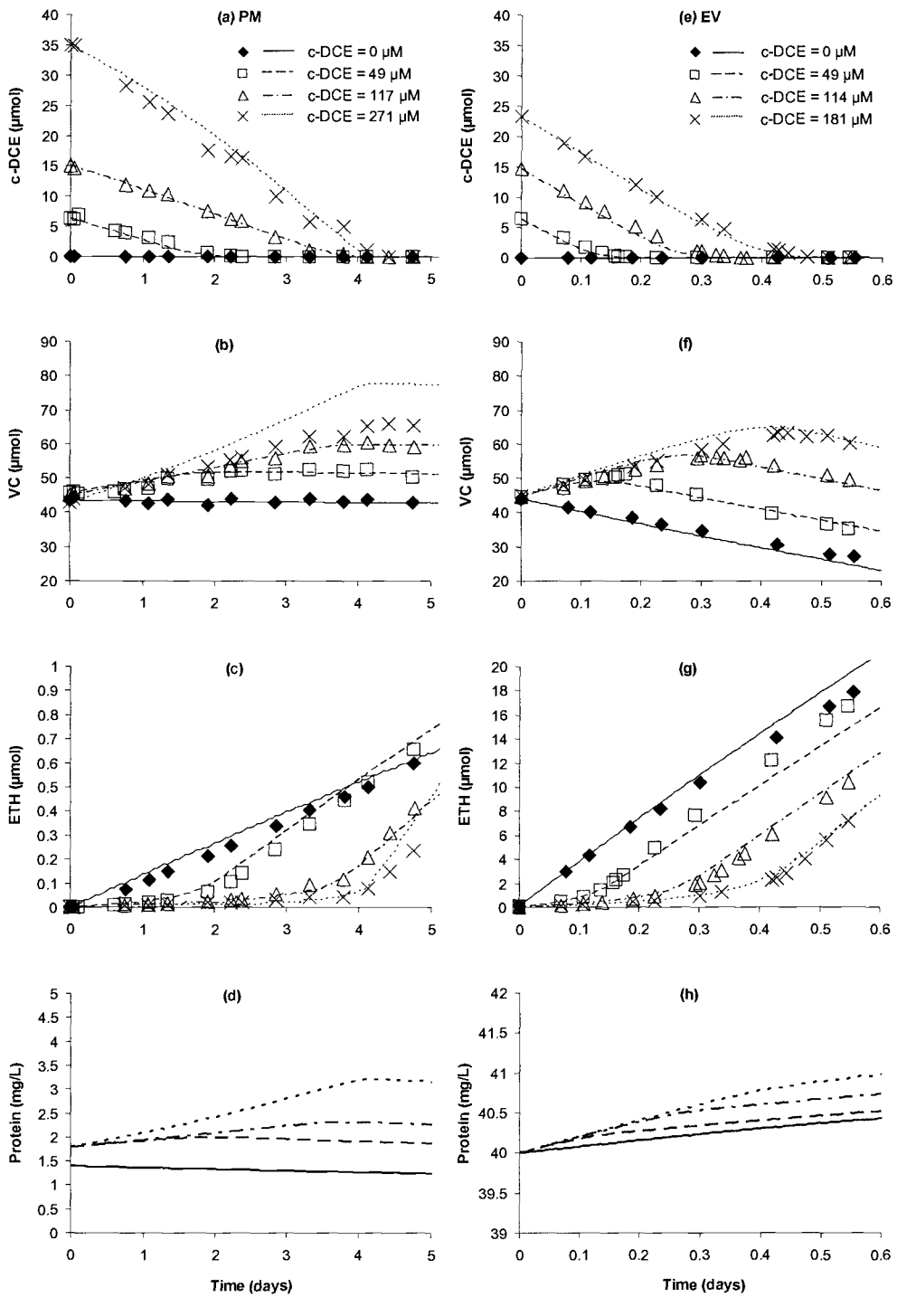

Figure 5-5. Long-term competitive inhibition effects of c-DCE on VC dechlorination by the PM culture (a, b, c, d) and the EV culture (e, f, g, h). Lines represent model simulations. Biomass of model simulation was shown in (d) and (h) with initial measurement of protein.

other reason for the increased rate is biomass growth over the several day-period of the tests with the PM culture. The simulated increase in biomass concentrations are presented in Figure 5-5 (d). A greater relative increase in biomass is predicted for the PM culture, which would cause an increase in the rate of ETH production.

The EV culture was also investigated for inhibition of *c*-DCE on VC dechlorination (Figure 5-5 (e), (f), (g), and (h)). Initial biomass concentration on a protein basis of the EV culture was 40 mg/L. Over the time scale of these tests, the change in cell mass should be minimal. Although *c*-DCE was added as an inhibitor, *c*-DCE was much more rapidly transformed than VC. VC concentrations were also observed to increase in reactor with *c*-DCE added. Figure 5-5 (e) to (h) show that VC transformation to ETH is clearly inhibited with an increase in initial aqueous *c*-DCE concentrations. Until *c*-DCE is removed to significantly low concentrations, ETH production is not observed. The lags in ETH production are clearly associated with the higher concentrations of *c*-DCE, with ETH production initiated when *c*-DCE concentrations are reduced to low values. Unlike the PM culture, after complete transformation of initial *c*-DCE to VC, VC dechlorination to ETH showed almost the same rates even at different initial *c*-DCE concentrations. One reason is that the lower K_S of VC for the EV culture and increase in VC concentrations have little affected the transformation rate, since the initial VC concentration (290 μM) was much higher than the K_S value (62.6 μM). Also, the initial higher cell mass and short duration of the tests result in less of a biomass change, as predicted by the model simulations. The model simulations do a very good job capturing the

temporal observations of *c*-DCE, VC, and ETH concentrations. The models here include the sequential transformation of *c*-DCE to VC and ETH. The potential for microbial growth of dechlorinating microorganisms is also included.

An experiment was performed with both cultures to study PCE inhibition on *c*-DCE dechlorination (Figure 5-6). Although the PCE concentration was increased up to 395 μM and 491 μM for the PM and EV cultures, respectively, almost no difference was observed on the rate of *c*-DCE transformation to VC. Two different models with and without PCE inhibition on *c*-DCE transformation were tested and compared with the experimental results. The model without PCE inhibition simulated the experimental data more closely than that with PCE inhibition, where inhibition was represented by its measured K_S value. The experimental results and model simulations indicate that reductive *c*-DCE dechlorination is very weakly or not inhibited by PCE. The results shown in Figure 5-6 showed the difference in *c*-DCE dechlorination rate of about a factor of 7 between the PM and the EV cultures. This resulted from the higher initial protein content of the PM reactor, and the higher k_{max} value.

Several batch reactors were established to investigate VC inhibition on *c*-DCE dechlorination. Haston (1999) reported that competitive inhibition was observed between *c*-DCE and VC dechlorination. The two CAH compounds competitively inhibit each other, and the inhibition constants were found comparable to their respective half-velocity coefficients. As shown in Figure 5-7, the experimental results were compared with two different models with and without VC

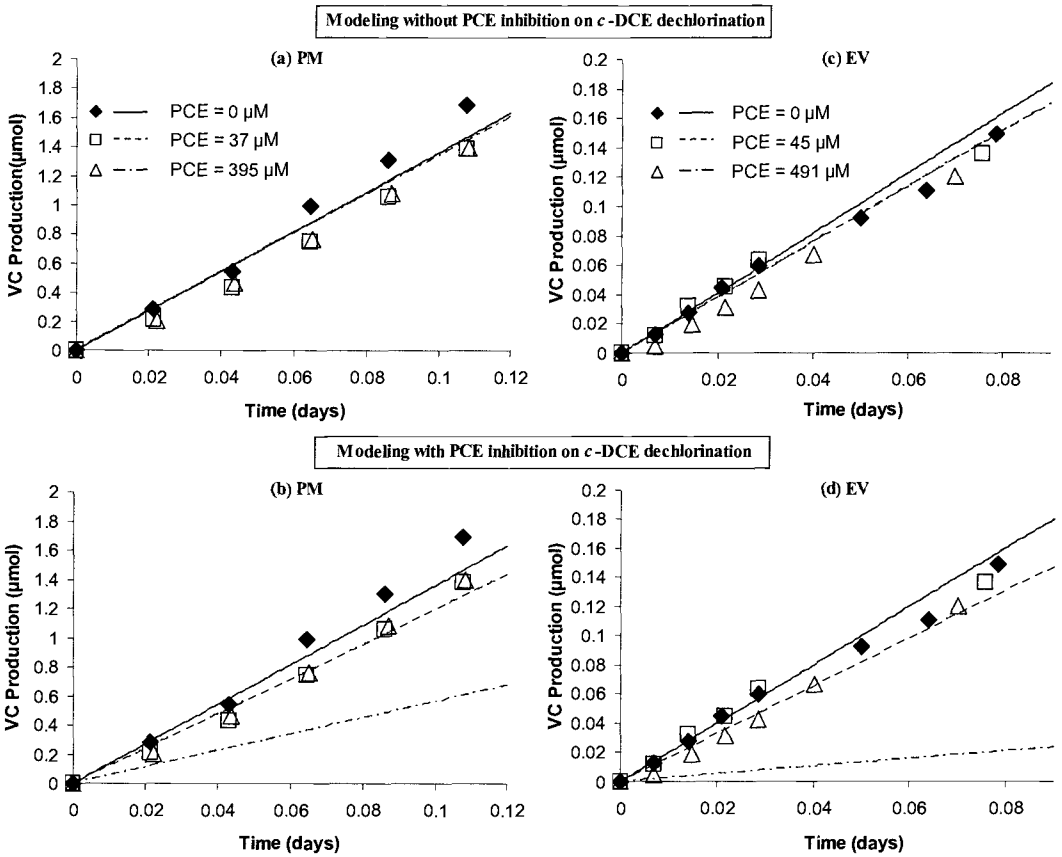

Figure 5-6. Comparison of modeling with and without PCE inhibition on experimental data of *c*-DCE dechlorination by the PM culture (a and b) and the EV culture (c and d). Initial *c*-DCE concentrations were 124 and 107 µM with cell concentrations of 5.0 and 1.3 mg/L for the PM and EV cultures, respectively.

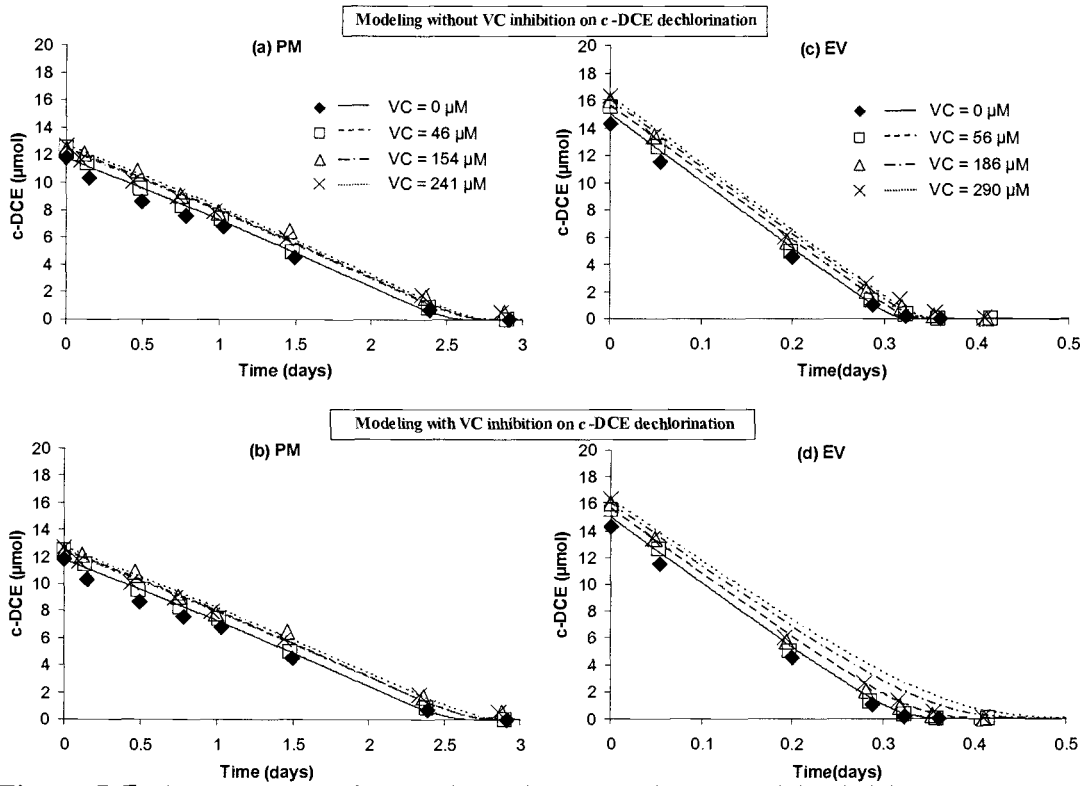

Figure 5-7. Comparison of modeling with and without VC inhibition on experimental data of *c*-DCE dechlorination by the PM culture (a and b) and the EV culture (c and d). Initial *c*-DCE concentrations were 96 and 121 µM with cell concentrations of 1.6 and 29 mg/L for the PM and EV cultures, respectively.

inhibition. For both cultures, the experimental observations showed no apparent VC inhibition on *c*-DCE dechlorination, and there was also no clear difference between the two different model simulations. For the EV culture, VC concentrations about a factor of about 5 greater than the K_S value were investigated. These results indicated that VC has a very weak inhibitory effect on *c*-DCE transformation for both cultures even at up to the VC concentration of 290 μM. This likely results from the high VC K_S values of the PM and EV cultures of 602 and 62.6 μM, respectively, compared to the low K_S value for *c*-DCE of 1.9 and 1.76. Thus, VC inhibition effect was excluded in our model as represented in Figure 5-2.

As shown in Figure 5-8, the batch kinetic experiments were performed to study *c*-DCE inhibition on TCE dechlorination for both cultures. Interestingly, although K_S values of *c*-DCE were little lower than those of TCE, rates of TCE dechlorination showed no clear inhibition of *c*-DCE as an inhibitor on reductive TCE dechlorination. The model without *c*-DCE inhibition simulated the experimental data more closely than that with inhibition. Some inhibition was observed at the highest *c*-DCE concentration tested, but it was a very weak. This suggests that K_S values as inhibition constants are not effective for less chlorinated ethylene inhibition on more chlorinated dechlorination.

Several pairs of chlorinated ethylenes (PCE ↔ TCE, PCE ↔ *c*-DCE, TCE ↔ *c*-DCE, TCE ↔ VC, and c-DCE ↔ VC) were investigated for the inhibition effects. All observations in the inhibition studies (Figures 5-4, 5-5, 5-6, 5-7, and 5-8) indicate that more chlorinated ethylenes inhibited reductive dechlorination of the less

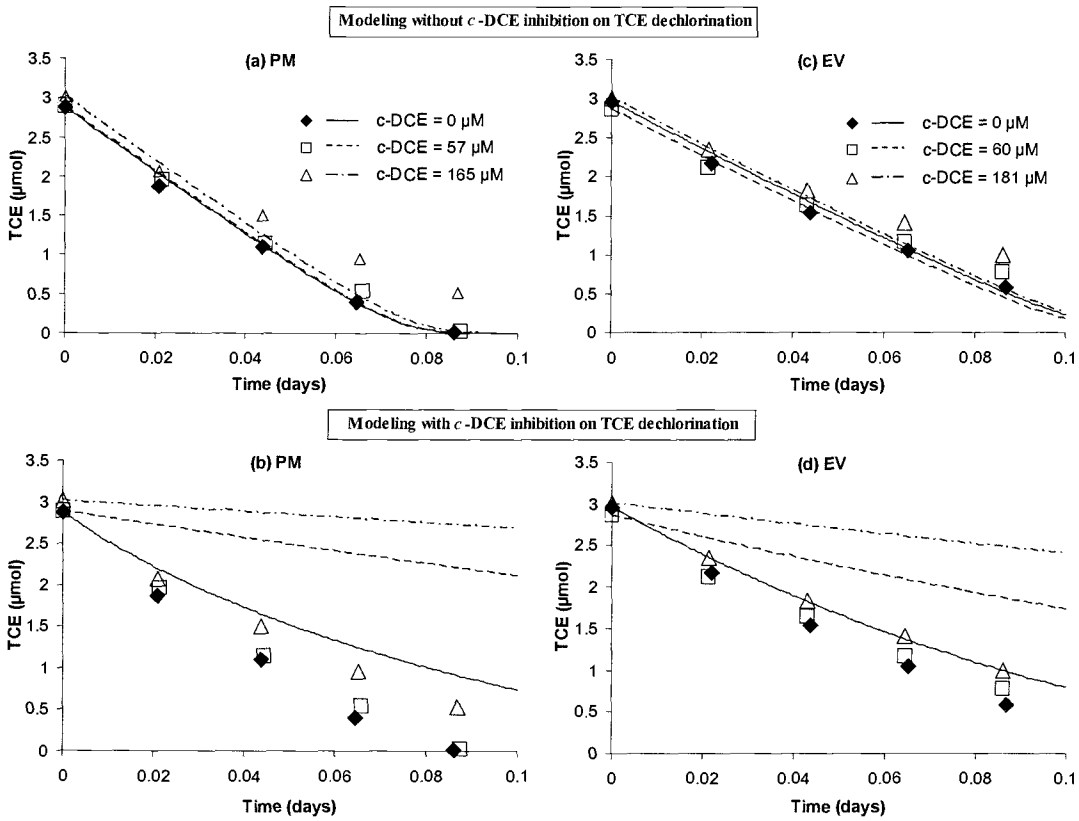

Figure 5-8. Comparison of modeling with and without c-DCE inhibition on experimental data of TCE dechlorination by the PM culture (a and b) and the EV culture (c and d). Initial TCE concentrations were 21.8 and 21.9 μM with cell concentrations of 12 and 10.3 mg/L for the PM and EV cultures, respectively.

chlorinated products, except PCE did not inhibit *c*-DCE dechlorination. Inhibition of less chlorinated ethylenes on more chlorinated parent compound dechlorination was also tested for the chlorinated ethylene pairs used in this study. The experimental results showed that less chlorinated ethylenes had very weak inhibitory effects on more chlorinated ethylenes. The K_S of VC for the PM and EV cultures were much higher (602 μM and 62.6 μM, respectively) than the parent compounds. Thus, if inhibition were related to the K_S values, weaker inhibition would be expected. For VC inhibition on *c*-DCE, the inhibitory effect of VC was also very weak for both cultures (Figure 5-7). Very weak inhibition of *c*-DCE on TCE transformation was also observed. Model simulation with much higher inhibition constant than the K_S value would be required to simulate the less chlorinated ethylene inhibition on the more chlorinated ethylene. Haston (1999) reported that VC inhibited *c*-DCE dechlorination, but a VC inhibition constant higher than K_S value was also used in the model simulation, whereas *c*-DCE inhibition constant was the same as the K_S value. However, in our study, the inhibition effect of less chlorinated ethylene was observed to be very minimal, and could be ignored and model simulations still obtained good fitting at the concentrations tested.

Inhibition of PCE on VC transformation was not tested, because PCE did not inhibit *c*-DCE. It was reported that reductive dechlorination of *c*-DCE and VC was different from reductive dechlorination of PCE and TCE (Rosner *et al.*, 1997. The growth of *Dehalococcoides* sp. strain VS and *Dehalococcoides* sp. strain BAV1 were reported to be coupled to the dechlorination of *c*-DCE and VC, but not PCE and TCE

(Cupples *et al.*, 2003; He *et al.*, 2003a, 2003b). Another completely dechlorinating bacterium that cometabolically dechlorinates PCE and VC (*Dehalococcoides* sp. strain FL2) uses only TCE and *c*-DCE as electron acceptors (Löffler *et al.*, 2003). Other groups of bacteria, like *Dehalospirillum multivorans*, *Dehalobacter restrictus* strains PER-K23A, and *Desulfuromonas* sp., can reduce only PCE and TCE in terminal electron accepting processes (Löffler *et al.*, 2000; Hollinger *et al.*, 1998). Another incompletely dechlorinating bacterium (*Desulfitobacterium* sp. strain PCE1) reductively dechlorinates only PCE to TCE (Gerritse *et al.*, 1996). These reports would support there being no PCE inhibition on *c*-DCE and VC dechlorination. Since both cultures used in this study were mixed culture isolated from CAH contaminated sites, several different microorganisms might be involved in the PCE dechlorination to ETH.

A simple multiple equilibration method in a single bottle was developed to obtain kinetic values like k_{max} and K_S. The kinetic parameters measured here are comparable to the values reported by Fennell and Gossett (1998) and Haston and McCarty (1999). However, a simple multiple equilibration method might provide more reproducible kinetic values than a typical single equilibration method in multiple bottles. Compared with values reported by Haston and McCarty (1999), the standard deviations of the kinetic parameters reported here are lower. However, with the multiple equilibration model, the time scale must be short enough so significant growth does not occur.

All model simulations shown in Figures 5-4, 5-5, 5-6, 5-7, and 5-8 were in good agreement with the inhibition experimental results. Inhibition of the more chlorinated ethylenes on the less chlorinated appears to be well represented by K_S value being used as the inhibition constant. Through the experimental results and model simulations, low K_S values corresponded to efficient reductive dechlorination for each of chlorinated ethylenes, but caused stronger inhibition on daughter product dechlorination. Simulations performed with independently determined kinetic parameters and K_S values used for their inhibition constants match the experimental observations well. Thus, this study demonstrated that K_S values as well as k_{max} have a great impact on overall dechlorination rates, suggesting that accurate and reproducible measurements of kinetic parameters are critical for designing intrinsic or engineered bioremediation.

A limitation of the current study is that the actual concentration of the dechlorinating cultures is not known. k_{max} values were based on the total protein content. However, when the initial protein content of the reactor was measured and used as an input value, model simulations match the experimental data very well. This likely resulted from the short time scale of these tests, where potential for changes in the dechlorinating population were limited.

Acknowledgements

The research was supported by a research grant from the U.S. Environmental Protection Agency-sponsored Western Region Hazardous Substance Research

Center under agreement R-815738. This article has not been reviewed by the agency, and no official endorsement should be inferred.

REFERENCES

Chang, M.-K., Voice, T. C., and Criddle, C. S. (1993) Kinetics of Competitive Inhibition and Cometabolism in the Biodegradation of Benzene, Toluene, and p-Xylene by Two Pseudomonas Isolates. *Biotechnol. Bioeng.* **41**, 1057-1065.

Cupples, A. M., Spormann, A. M., and McCarty, P. L. (2003) Growth of a Dehalococcoides-Like Microorganism on Vinyl Chloride and cis-dichloroethene as Electron Acceptors as Determined by Competitive PCR. *Appl. Environ. Microbiol.* **69**, 953-959.

Fennell, D. E. and Gossett, J. M. (1998) Modeling the Production of and Competition for Hydrogen in a Dechlorinating Culture. *Environ. Sci. Technol.* **32**, 2450-2460.

Freedman, D. L. and Gossett, J. M. (1989). Biological Reductive Dechlorination of Tetrachloroethylene and Trichloroethylene to Ethylene under Methanogenic Conditions. *Appl. Environ. Microbiol.* **55**, 2144-2151.

Garant, H. and Lynd, L. (1998) Applicability of Competitive and Noncompetitive Kinetics to the Reductive Dechlorination of Chlorinated Ethenes. *Biotechnol. Bioeng.* **57**, 751-755.

Gerritse, J., Renard, V., Pedro-Gomes, T. M., Lawson, P. A., Collins, M. D., and Gottschal, J. C. (1996) *Desulfitobacterium* sp. strain PCE1, an Anaerobic Bacterium that can Grow by Reductive Dechlorination of Tetrachloroethene or *ortho*-Chlorinated Phenols. *Arch. Microbiol.* **165**, 132-140.

Gossett, J. M. (1987) Measurement of Henry's Llaw Constants for C1 and C2 Chlorinated Hydrocarbons. *Environ. Sci. Technol.* **21** (2), 202 - 208.

Haston, Z. C. (1999). Factors Affecting Growth and Utilization in the Anaerobic Dehalogenation of Chlorinated Ethenes. Ph.D. Dissertation, Stanford University, Standord, CA.

Haston, Z. C. and McCarty, P. L. (1999) Chlorinated Ethene Half-Velocity Coefficients (K_S) for Reductive Dehalogenation. *Environ. Sci. Technol.* **33** (2), 223-226.

Haston, Z. and McCarty, P. L. (1997) In *In situ and On-Site Bioremediation, Battelle Press*, Columbus, OH, **4** (3), 31.

He, J., Ritalahti, K. M., Aiello, M. R., and Löffler, F. E. (2003b) Complete Detoxification of Vinyl Chloride by an Anaerobic Enrichment Culture and

Identification of the Reductively Dechlorinating Population as a *Dehalococcoides* Species. *Appl. Environ. Microbiol* **69** (2), 996-1003.

He, J., Ritalahti, K. M., Yang, K.-L., Koenigsberg, S. S., and Löffler, F. E. (2003a) Detoxification of Vinyl Chloride to Ethene Coupled to Growth of an Anaerobic Bacterium. *Nature* **424**, 62-65.

Hendrickson, E. R., Payne, J. A., Young, R. M., Starr, M. G., Perry, M. P., Fahnestock, S., Ellis, D. E., and Ebersole, R. C. (2002) Molecular Analysis of Dehalococcoides 16S Ribosomal DNA form Chloroethene-Contaminated Sites through North America and Europe. *Appl. Environ. Microbiol.* **68**, 485-495.

Holliger, C., Hahn, D., Harmsen, H., Ludwig, W., Schumacher, W., Tindall, B., Vazquez, F., Weiss, N., and Zehnder, A. J. B. (1998) *Dehalobacter restrictus* gen. nov. and sp. nov., a Strictly Anaerobic Bacterium that Reductively Dechlorinates Tetra- and Trichloroethene in Anaerobic Respiration. *Arch. Microbiol.* **169**, 313-321.

Löffler, F. E., Cole, J. R., Ritalahti, K. M., and Tiedje, J. M. (2003) In *Dehalogenation: Microbial Processes and Environmental Applications*, Ed. Hägbblom, M. M. and Bossert, I. D., Kluwer Academic, New York, NY., pp. 53-87.

Löffler, F. E., Tiedje, J. M., and Sanford, R. A. (1999). Fraction of Electrons Consumed in Electron Acceptor Reduction and Hydrogen Thresholds as Indicators of Halorespiratory Physiology. *Appl. Environ. Microbiol.* **65**, 4049-4056.

Löffler, F. E., Sun, Q., Li, J., and Tiedje, J. M. (2000) Microbial Ecology - 16S rRNA Gene-Based Detection of Tetrachloroethene-Dechlorinating Desulfuromonas and Dehalococcoides Species. *Appl. Environ. Microbiol.* **66** (4), 1369-1374.

Maymo-Gatell, X., Chien, Y.-T., Gossett, J. M., and Zinder, S. H. (1997) Isolation of Bacterium that Reductively Dechlorinates Tetrachloroethene to Ethene. *Science* **276**, 1568-1571.

Maymo-Gatell, X., Tandoi, V., Gossett, J. M., and Zinder, S. H. (1995) Characterization of an H_2-Utilizing Enrichment Culture that Reductively Dechlorinates Tetrachloroethene to Vinyl Chloride and Ethene in the Absence of Methanogenesis and Acetogenesis. *Appl. Environ. Microbiol.* **61**, 3928-3933.

Maymo-Gatell, X., Nijenhuis, I., and Zinder, S. H. (2001) Reductive Dechlorination of *cis*-1,2-Dichloroethene and Vinyl Chloride by *"Dehalococcoides ethenogenes" Environ. Sci. Technol.* **35** (3), 516-521.

McCarty, P. L. (1997) Breathing with Chlorinated Solvents. *Science* **276**, 1521-1522.

Nielsen, R. B. and Keasling, J. D. (1999) Reductive Dechlorination of Chlorinated Ethene DNAPLs by a Culture Enriched from Contaminated Groundwater. *Biotechnol. Bioeng.* **62**, 160-165.

Perry, R. H., Green, D. W., and Maloney, J. O. (1997) *Perry's Chemical Engineers Handbook*, 7th Ed., McGraw-Hill, New York, NY.

Pon, G., Hyman, M. R., and Semprini, L. (2003) Acetylene Inhibition of Trichloroethene and Vinyl Chloride Reductive Dechlorination *Environ. Sci. Technol.* **2003**, 37 (14), 3181-3188.

Rittmann, B. E., Seagren, E., and Wrenn, B. A. (1994). In *In-Situ Bioremediation*. Second Edition. Parkridge, New Jersey, USA: Noyes Publications, pp. 37-50.

Rosner, B. M., McCarty, P. L., and Spormann, A. M. (1997) In Vitro Studies on Reductive Vinyl Chloride Dehalogenation by an Anaerobic Mixed Culture. *Appl. Environ. Microbiol.* **63** (11), 4139-4144.

Tandoi, V., DiStefano, T. D., Bowser, P. A., Gossett, J. M., and Zinder, S. H. (1994) Reductive Dehalogenation of Chlorinated Ethenes and Halogenated Ethanes by a High-Rate Anaerobic Enrichment Culture. *Environ. Sci. Technol.* **28**, 973-979.

Tonnaer H., Alphenaar, A., Wit, H. D., Grutters, M., Spuij, F., Gerritse, J., and Gottschal, J. C. (1997) Modelling of Anaerobic Dechlorination of Chloroethenes for In-Situ Bioremediation. In *In Situ and On-Site Bioremediation*, papers from the Fourth International In Situ and On-Site Bioremediation Symposium, Ed. Alleman, B. C. and Leeson, A., Battelle Press, Columbus, OH, pp. 591-596.

Westrick, J. J., Mello, J. W., and Thomas, R. F. (1984) The Groundwater Supply Survey. *J. Am. Water Works Assoc.* **76** (5), 52-59.

Yang, Y. and McCarty, P. L. (1998). Competition for Hydrogen within a Chlorinated Solvent Dehalogenating Anaerobic Mixed Culture. *Environ. Sci. Technol.* **32**, 3591-3597.

Yu, S. and Semprini, L. (2002b). Comparison of Trichloroethylene Reductive Dehalogenation by Microbial Communities Stimulated on Silicon-Based Organic Compounds as Slow-Release Anaerobic Substrate. *Water Res.* **36**, 4985-4996.

CHAPTER 6

KINETICS AND MODELING OF REDUCTIVE DECHLORINATION AT HIGH PCE AND TCE CONCENTRATIONS

Seungho Yu and Lewis Semprini

To be submitted to:

XXXX

XXXX

ABSTRACT

Two biokinetic models employing the Michaelis-Menten equation for anaerobic reductive dechlorination of tetrachloroethylene (PCE) and trichloroethylene (TCE) were developed and compared with results of batch kinetic tests conducted over a wide range of PCE and TCE concentrations. One model applies Michaelis-Menten kinetics with competitive inhibition among chlorinated aliphatic hydrocarbons (CAHs), and the other model includes both competitive inhibition and Haldane inhibition at high CAH concentrations. Model simulations with competitive inhibition simulated the experimental results well for PCE concentrations lower than 300 μM. However, simulations deviated from the experimental observations for PCE or TCE concentrations greater than 300-400 μM. A kinetic model that incorporated both competitive and Haldane inhibitions better simulated experimental data for PCE concentrations near the solubility limit (1000 μM), and TCE concentrations half its solubility limit (4000 μM). Based on the experimental results and modeling studies, the PM culture (Point Mugu, CA) has very high Haldane inhibition constants for *cis*-1,2-dichlororethylene (*c*-DCE) and vinyl chloride (VC) (6000 and 7000 μM, respectively), indicating very weak Haldane inhibition, while the EV culture (the Evanite site in Corvallis, OR) had significant Haldane inhibition for TCE, *c*-DCE, and VC with Haldane inhibition constants of 900, 750, and 750 μM, respectively. For the reductive dechlorination of a broad range of PCE and TCE concentrations, the PM and BM (a binary mixed culture of the PM and EV cultures) dechlorinating cultures showed better dechlorination abilities at a high TCE

concentration (4073 μM) than the EV culture. The EV and BM cultures more effectively transformed a wide range of PCE concentrations than the PM culture. These results indicate that a binary mixed culture system has better transformation abilities than the individual cultures. Our kinetic and modeling studies also indicated that a model with the combination of competitive and Haldane inhibitions is of potential use to predict dechlorination over a broad range of concentrations up to the solubility limits of PCE and TCE.

Key words: reductive dechlorination, PCE, TCE, DNAPL, competitive inhibition, Haldane inhibition.

INTRODUCTION

Chlorinated ethylenes are ubiquitous soil and groundwater contaminants (Westrick *et al.*, 1984), for which bioremediation is a promising technology. Anaerobic reductive dechlorination is an important process for the transformation of CAHs in anaerobic subsurface, especially for the dechlorination of highly chlorinated compounds such PCE and TCE that can be present at high concentrations due to the presence of dense non-aqueous phase liquid (DNAPL) that are slowly dissolving (Rittmann *et al.*, 1994).

Although intrinsic biostimulation has been a remedial alternative for biotransfomation of chlorinated solvent-contaminated groundwater, observations at many contaminated sites have shown that reductive dechlorination of PCE and TCE

stalls at *c*-DCE and VC (Major *et al.*, 2002). This may be due to an absence of appropriate dechlorinating bacteria. A recent study of 24 chlorinated ethylene-contaminated sites reported that *Dehalococcoides* spp. were not detected at incomplete dechlorinating sites (Hendrickson *et al.*, 2002). In these cases, bioaugmentation with complete dechlorinating bacteria could be an appropriate strategy to initiate and enhance the reductive dechlorination of PCE or TCE (Harkness *et al.*, 1999; Ellis *et al.*, 2000)).

The use of a binary mixed culture of two different aerobic pure cultures has been reported to show interspecies interactions and changes in the rate and extent of biodegradation (Fairlee *et al.*, 1997; Rogers *et al.*, 2000). The studies revealed that the utilization of a binary mixed culture could enhance the biodegradation of mixed pollutants such as toluene, phenol, and benzene. Previously, we reported (Yu and Semprini, 2002a) that a binary anaerobic mixed culture isolated from two different contaminated groundwaters was more effective in reductively dechlorinating PCE to ethylene (ETH).

Various models have been developed that describe reductive dechlorination of chlorinated ethylenes in the subsurface. Inhibition among the CAHs has been a process that has been considered in these models. During complete reductive dechlorination of PCE to ETH, competitive inhibition of vinyl chloride (VC) dechlorination by the other chlorinated ethylenes was assumed and the simulation model was compared with the experimental data (Tandoi *et al.*, 1994). Fennell and Gossett (1998) simulated both fermentation of electron donors and competition for

the evolved H_2 between hydrogenotrophic tetrachloroethylene dechlorinators and methanogens, but the comprehensive biokinetic model employing Michaelis-Menten-type kinetics did not consider inhibition between the chlorinated ethylenes. Another model was developed to predict PCE reductive dechlorination by incorporating competitive dechlorination between PCE and TCE (Tonnaer *et al.*, 1997). Garant and Lynd (1998) showed that competitive kinetics for complete reductive dechlorination of PCE achieved better chi-squared and visual fits of the experimental data than noncompetitive inhibition.

Recently, we experimentally determined maximum rates (k_{max}) and half-velocity coefficients (K_S) for each step of the dechlorination of PCE to ETH for two different mixed cultures. We also investigated the inhibitions between chlorinated ethylenes (Yu *et al.*, 2003). This study aims to further simulate the sequential transformation of PCE and TCE to ETH over a broad range of concentrations up to the PCE solubility limit and half the TCE solubility limit with independently measured kinetic parameters. The kinetic models developed for each culture were also combined to investigate the transformation ability of a binary mixed culture.

MATERIALS AND METHOD

Chemicals

Liquid chlorinated ethylenes such as PCE (99.9%, spectrophotometric grade), TCE (99.9%) and *c*-1,2-dichloroethylene (*c*-DCE) (97%) were obtained from Acros

Organics (Pittsburgh, PA) for preparing stock feed solutions and analytical standards. Gaseous VC and ETH (both 99.5%, Aldrich Chemical, Milwaukee, WI) were used in the preparation of analytical standards. Hydrogen gas (99%, Airco, Inc., Albany, OR) and 1-butanol (99.8 %, HPLC grade, Aldrich Chemical, Milwaukee, WI) were used as the electron donors.

Analytical Methods

Gas chromatography was used to determine the gas sample concentrations of all chlorinated ethylenes, ETH, and hydrogen. PCE, TCE, *c*-DCE, VC, and ETH were quantified with an HP-6890 gas chromatograph (GC) equipped with a photoionization detector (PID) and flame ionization detector (FID) connected in series. The GC was fitted with a 30 m × 0.53 mm GS-Q column (J&W Scientific, Folsom, CA), and helium was used as the carrier gas (15 ml/min). 100-200 µL reactor headspace samples were introduced into the GC. The GC oven was initially set at 80°C for 1.5 min, heated at 65°C/min to 170°C and 40°C/min to 220°C, and kept at 220°C for 2.7 min. Quantification of hydrogen concentrations in headspace gas samples (200 µl) was accomplished using an HP-5890 GC with a thermal conductivity detector (TCD), operated isothermally at 220°C. The gas samples were chromatographically separated with a Carboxen 1000 column (15 ft × 1/8 in, Supelco, Bellefonte, PA). Argon gas was the carrier gas at 15 ml/min for operation of HP GC. The hydrogen detection limit was 4 nM (aqueous concentration) with the TCD.

Enrichment Cultures and Kinetic Batch Reactors

This study used two anaerobic mixed cultures isolated from Point Mugu Naval Weapon Facility, CA (PM) and the Evanite site in Corvallis, OR (EV), and a binary mixed culture (BM), which was a mixture of the PM and EV cultures (1:1 volume ratio). PCR assay targeting 16S ribosomal DNA indicated that the PM and EV cultures had *Dehalococcoides*-like microorganisms present with the ability of complete reductive dechlorination to ETH (Yu *et al.*, 2003). The two site cultures were grown in separate batch mother reactors (total volume 1.2 L with liquid volume of 1 L) as described in Yu and Semprini (2002b) and Yu *et al.* (2003). The mother reactors were added with an aqueous TCE or PCE concentration of 100 mg/L for the PM and EV cultures, respectively. 1-butanol of 40-60 mg/L and 10% hydrogen were also added as electron donors. The liquid cultures for kinetic studies were obtained from the batch mother reactor after PCE or TCE added was converted to ETH (about 50 to 100% conversion).

Batch kinetic reactors were constructed in an anaerobic glove box with serum bottles (156 mL total volume with 125 mL liquid) fitted with rubber-lined caps and butyl rubber septa (Wheaton Industries, Millville, NJ). The batch reactors contained 125 mL enrichment liquid culture and 31 mL anaerobic gas headspace. After construction in the glove box, the kinetic batch reactors were purged with a mixed gas mixture of N_2 (70%), CO_2 (20%), and H_2 (10%) that was treated in a tube furnace to remove trace oxygen. At the onset of the experiment, neat PCE or TCE and butanol were added to each purged batch reactors to achieve the desired initial

concentrations. As listed in Table 6-1, the kinetic reactors were purged with the mixed gas just after each experimental run, and higher PCE concentrations were introduced with butanol as an electron donor. All kinetic batch reactors were shaken at 200 rpm and incubated at 20°C in an environmental chamber throughout the experiments. The total cell concentration, X, was determined by a protein analysis just before each experimental run, as previously described (Yu *et al.*, 2003).

Table 6-1. Experimental conditions of kinetic batch reactors.

Run	CAH added	PM	EV	BM
1	PCE (μM)	92	39	43
2	PCE (μM)	282	282	317
3	PCE (μM)	1128	1057	1128
4	TCE (μM)	3875	4173	4173

MODEL DEVELOPMENT

A number of models have been proposed and utilized to describe anaerobic reductive dechlorination for different cultures (Fennell and Gossett, 1998; Tandoi *et al.*, 1994; Tonnaer *et al.*, 1997; Garant and Lynd, 1998; Bagley, 1998; Haston, 1999). One model that has been studied and commonly modeled for reductive dechlorination of PCE and TCE is competitive Michaelis-Menten kinetics. Tonnaer *et al.*(1997) developed the competitive inhibition model for reductive dechlorination of PCE and TCE. Competitive inhibition was observed and modeled for *c*-DCE and VC dechlorination (Haston, 1999). It was also found that inhibition constants of each chlorinated ethylene were comparable to their respective half-velocity

coefficients, but for VC inhibition a higher value than its half-velocity coefficient was used (Haston, 1999). Our previous studies with two anaerobic mixed cultures (PM and EV) indicated that the more chlorinated ethylenes inhibited reductive dechlorination of the less chlorinated ethylenes (Yu *et al.*, 2003). However, the less chlorinated ethylenes had very weak inhibitory effects on the more chlorinated ethylenes. PCE inhibits reductive TCE dechlorination, but not *c*-DCE or VC dechlorination, while TCE strongly inhibits *c*-DCE and VC dechlorination. *c*-DCE also inhibits VC transformation to ETH. However, the comparison of the experimental results and model simulation was achieved with comparably low CAH concentrations. A recent kinetic study using purified PCE-RDase from *Dehalobacter restrictus* indicated that CAH itself can be inhibitory or toxic at high concentrations of PCE and TCE (up to 1000 μM) (Maillard *et al.*, 2003).

Two models were developed here; one with Michaelis-Menten kinetics and competitive inhibition as previously described by Yu *et al.* (2003), and the other model includes both competitive inhibition and Haldane inhibition at high CAH concentrations (Bailey and Ollis, 1986). The models using independently measured kinetic parameters were implemented in STELLA (High Performance Systems) and compared with the experimental data. Haldane inhibition constants were fit to results presented here. As previously reported by Yu *et al.* (2003), the PM culture is assumed to grow on PCE, TCE, and *c*-DCE, and the EV culture on PCE, TCE, *c*-DCE, and VC (Pon *et al.*, 2003).

Competitive Inhibition Model

Based on observation from the previous studies (Yu *et al.*, 2003), a competitive inhibition model was developed. The kinetic equations for reductive dechlorination of mixed cultures are presented as Equations 6-1, 6-2, 6-3, 6-4, and 6-5, with both competitive and Haldane inhibition included. When the Haldane inhibition constant (K_{HI}, μM) is very high (∞), the equation reduce to competitive inhibition only. Electron donor limitations were not included in the model equations by providing H_2 and 1-butanol in excess in the experiments. These equations are linked together and simultaneously solved for the simulation of reductive dechlorination.

$$\frac{dC_{PCE}}{dt} = \frac{-k_{\max,PCE} XC_{PCE}}{K_{S,PCE} + C_{PCE}} \tag{6-1}$$

$$\frac{dC_{TCE}}{dt} = \frac{-k_{\max,TCE} XC_{TCE}}{K_{S,TCE}\left(1+\frac{C_{PCE}}{K_{CI,PCE}}\right) + C_{TCE}\left(1+\frac{C_{TCE}}{K_{HI,TCE}}\right)} + \frac{k_{\max,PCE} XC_{PCE}}{K_{S,PCE} + C_{PCE}} \tag{6-2}$$

$$\frac{dC_{c-DCE}}{dt} = \frac{-k_{\max,c-DCE} XC_{c-DCE}}{K_{S,c-DCE}\left(1+\frac{C_{TCE}}{K_{CI,TCE}}\right) + C_{c-DCE}\left(1+\frac{C_{c-DCE}}{K_{HI,c-DCE}}\right)} + \frac{k_{\max,TCE} XC_{TCE}}{K_{S,TCE}\left(1+\frac{C_{PCE}}{K_{CI,PCE}}\right) + C_{TCE}\left(1+\frac{C_{TCE}}{K_{HI,TCE}}\right)} \tag{6-3}$$

$$\frac{dC_{VC}}{dt} = \frac{-k_{\max,VC} X C_{VC}}{K_{S,VC}\left(1+\frac{C_{TCE}}{K_{CI,TCE}}+\frac{C_{c-DCE}}{K_{CI,c-DCE}}\right)+C_{VC}\left(1+\frac{C_{VC}}{K_{HI,VC}}\right)} + \frac{k_{\max,c-DCE} X C_{c-DCE}}{K_{S,c-DCE}\left(1+\frac{C_{TCE}}{K_{CI,TCE}}\right)+C_{c-DCE}\left(1+\frac{C_{c-DCE}}{K_{HI,c-DCE}}\right)} \quad (6\text{-}4)$$

$$\frac{dC_{ETH}}{dt} = \frac{k_{\max,VC} X C_{VC}}{K_{S,VC}\left(1+\frac{C_{TCE}}{K_{CI,TCE}}+\frac{C_{c-DCE}}{K_{CI,c-DCE}}\right)+C_{VC}\left(1+\frac{C_{VC}}{K_{HI,VC}}\right)} \quad (6\text{-}5)$$

$$\frac{dX}{dt} = Y\frac{dC}{dt} - k_d X \quad (6\text{-}6)$$

where C is the CAH aqueous concentration (μM), k_{max} is the maximum specific CAH dechlorination rate (μmol/mg of protein/day), K_S is the half-velocity coefficient (μM), and inhibition constants of each chlorinated ethylene, K_{CI} (μM), were set equal to their respective half-velocity coefficients (K_S) as previously reported (Yu *et al.*, 2003). X is total cell concentration (mg of protein/L), and microbial growth was calculated with respect to the transformation rate of each chlorinated ethylene. In the model for biomass growth, values for growth yield, Y (= 0.006 mg of protein/μmol of Cl^- dechlorinated), and decay constant, k_d (= 0.024 day^{-1}), were obtained from the literature (Maymo-Gatell *et al.*, 1997; Fennell and Gossett, 1998).

Two separate models for the PM and the EV cultures were incorporated to depict PCE dechlorination to ETH and microbial growth of the BM culture. The CAH dechlorination models (Equations 6-1 through 6-5) and microbial growth rates (Equation 6-6) were solved separately to simulate the performance of the BM culture with the kinetic values obtained from kinetic studies with the single mixed cultures.

Haldane Inhibition Model

Haldane inhibition (Bailey and Ollis, 1986) is used to model toxicity or inhibition resulting from high CAH concentrations. The biomass growth rate is proportional to the degradation rate of CAHs. However, growth may be limited by inhibition or toxicity of CAHs. Maillard *et al.* (2003) also observed that the degradation rates of PCE and TCE were inhibited by themselves at high concentrations. In this study, a Haldane kinetic model incorporating competitive inhibition among chlorinated ethylenes is applied and compared with the experimental data (Equations 6-1 through 6-5). Haldane inhibition by high PCE concentrations was not included in this study, since no significant inhibition was observed at PCE concentrations near its solubility limit in Maillard *et al.*'s study (2003) or in our study.

RESULTS

Batch kinetic tests of the sequential transformation of PCE and TCE were conducted in duplicate at different initial PCE concentrations and at a very high initial TCE concentration for the PM, EV, and BM cultures as represented in Table 6-1. PCE was step-wisely increased from 39–92 μM to 1057–1128 μM in succession. In addition, a very high TCE concentration of about 4000 μM was added into each reactor with the different mixed cultures. The experimental data are represented by the average of the duplicate reactors for each kinetic test. Differences between duplicate reactors were usually smaller than the size of the symbols shown

in the figures. During the experiments, hydrogen concentrations were kept higher than 0.03 atm in the headspace of batch reactors.

Reductive Dechlorination of PCE by the PM culture

Shown in Figures 6-1 and 6-2 are results for PCE transformation test with the PM culture for PCE aqueous concentrations ranging from 92 to 1128 μM, and corresponding model simulations with competitive inhibition. The transformation rates of VC to ETH by the PM culture were very slow compared to those of other CAHs (Figures 6-1 and 6-2). PCE was transformed rapidly, resulting in the accumulation of VC with no significant concentrations of TCE or *c*-DCE observed. With an initial PCE concentration of 92 μM, TCE and *c*-DCE were not detected, and VC was very slowly transformed to ETH. The rate of VC transformation to ETH increased with higher initial PCE concentrations, resulting from a high K_S value of 602 μM previously reported (Yu *et al.*, 2003). The PM culture was fed neat PCE of 160 μmol (1128 μM aqueous concentration calculated based on the complete dissolution of all PCE added), which is a higher concentration than PCE solubility of 900 μM (Yaws, 1999). For the first 2–3 days, total CAH mass balances were slightly lower than the 160 μmol added (Figures 6-1(c) and 6-2(c)), because the total mass values obtained from headspace and liquid masses were calculated according to the headspace concentration measured using Henry's law constants, without considering the amount of PCE DNAPL present.

Two different Michaelis-Menten models were tested and compared with the

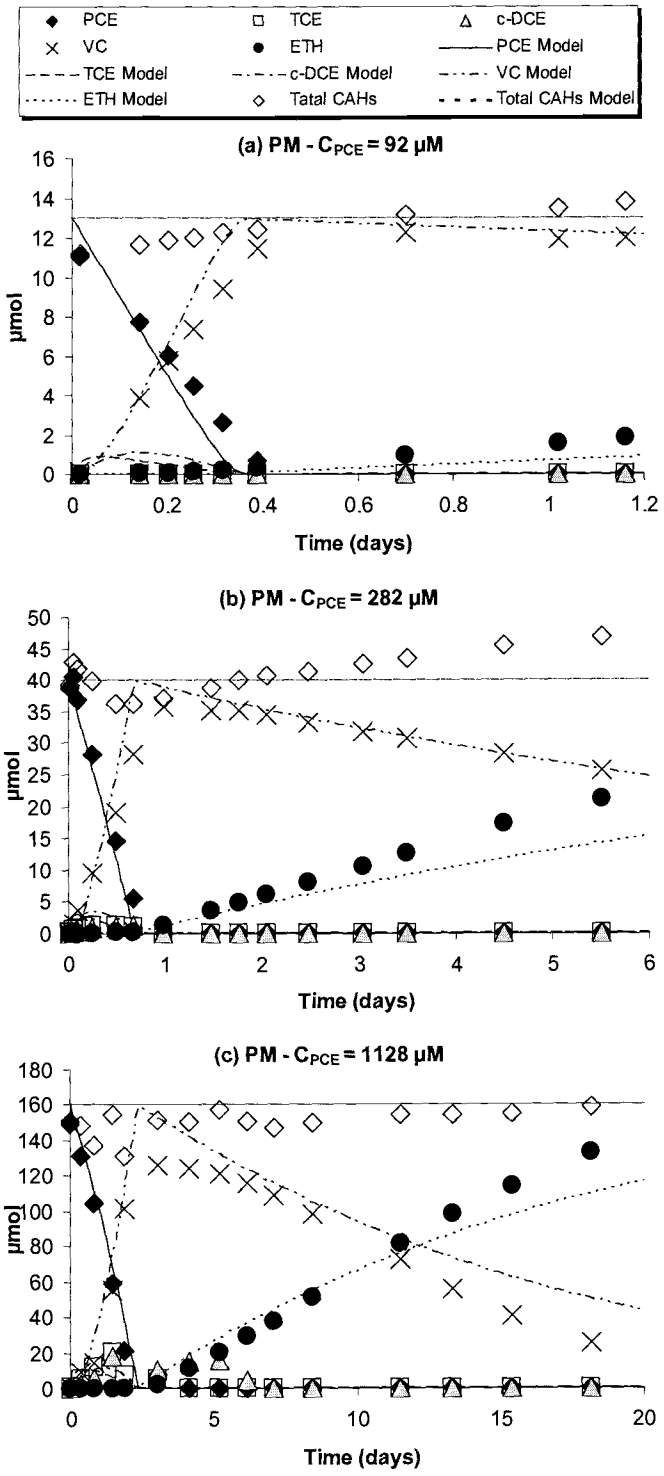

Figure 6-1. Reductive dechlorination kinetics at different initial PCE concentrations by the PM culture. Lines represent model simulations with competitive inhibition kinetics.

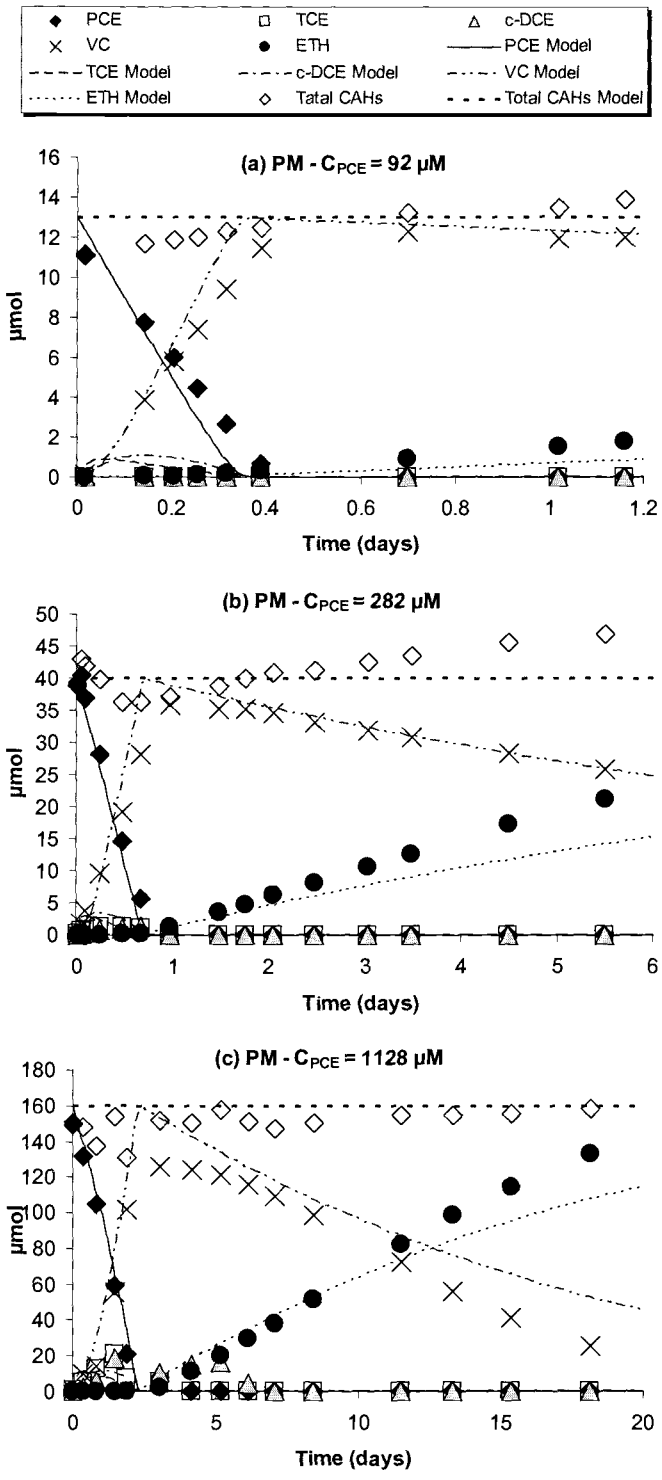

Figure 6-2. Reductive dechlorination kinetics at different initial PCE concentrations by the PM culture. Lines represent model simulations with both Haldane and competitive inhibition kinetics.

experimental data of sequential PCE dechlorination with the PM culture (Figures 6-1 and 6-2). All models used the same independently measured kinetic parameters reported in our previous study, as listed in Table 6-2 (Yu *et al.*, 2003). Haldane inhibition constants, K_{HI} (μM), for TCE, *c*-DCE, and VC were obtained from heristic fitting all of the experimental data. The models were simulated with measured total cell concentrations of 25, 34, 34 mg of protein/L, respectively. No clear differences were observed between the two model simulations with and without Haldane kinetics included. The model fits for different initial PCE concentrations captured the overall trends in the experimental data well. The K_{HI} values for TCE, *c*-DCE, and VC were obtained from fits of both PCE dechlorination (Figures 6-1 and 6-2) and TCE dechlorination data (Figures 6-7(a) and 6-8(a) as will be discussed). TCE and *c*-DCE as transformation products (Figures 6-1 and 6-2) did not accumulate sufficiently to differentiate between the two model simulations. K_{HI} values for TCE, *c*-DCE, and VC of 900, 6000, and 7000 μM (Table 6-2) were obtained by heristic fitting of all experimental data obtained for PCE and TCE experiments (Figures 6-1, 6-2, 6-7(a), and 6-8(a)). The results show that the models simulate very well the temporal histories of chlorinated ethylenes, as well as the effects of changes in initial PCE concentrations.

Table 6-2. Kinetic parameters used in the model simulations.

	PM				EV			
	PCE	TCE	c-DCE	VC	PCE	TCE	*c*-DCE	VC
K_{max}[a] (μmol/mg of protein/day)	13.3	124	22.0	2.44	12.4	125	13.8	8.08
K_S[a] (μM)	3.86	2.76	1.90	602	1.63	1.80	1.76	62.6
K_{CI} (μM)	3.86	2.76	1.90	602	1.63	1.80	1.76	62.6
K_{HI}[b] (μM)		900	6000	7000		900	750	750
Y[c] (mg-protein/ μmol of Cl^-	0.006	0.006	0.006		0.006	0.006	0.006	0.006
k_d[c] (day^{-1})		0.024				0.024		

[a] Yu *et al.* (2003). [b] obtained from the experimental data fitting (Figures 6-2, 6-4, 6-6, and 6-8). [c] Maymo-Gatell *et al.* (1997); Fennell and Gossett (1998).

Reductive Dechlorination of PCE by the EV culture

The same procedures of incremental addition of PCE in succession were used with the EV culture (Table 6-1). As shown in Figures 6-3 and 6-4, almost complete reductive dechlorination to ETH was obtained with all PCE concentrations within 1.2, 3.5, and 15 days, respectively. No lag time to initiate PCE dechlorination was observed even at the initial near-solubility PCE concentration of 1057 μM (Figures 6-3(c) and 6-4(c)), where no significant toxicity of the PCE concentration is indicated. Unlike the PM culture, *c*-DCE accumulated at all PCE concentrations tested. With the highest concentration of 1057 μM, a significantly high *c*-DCE concentration of 700 μM was observed, possibly resulting from a lower $k_{max,cDCE}$ value of 13.8 compared to 22.0 μmol/mg-protein/day of the PM culture (Yu *et al.*, 2003). For all PCE concentrations, ETH production occurred after most of *c*-DCE was transformed to VC, indicating *c*-DCE strongly inhibits VC dechlorination to ETH, as previously reported (Yu *et al.*, 2003).

Model results shown in Figures 6-3 and 6-4 were run with initial total cell concentrations for each test of 30, 30, 27 mg-protein/L, respectively. The fits of the kinetic model with competitive inhibition to the experimental data are shown in Figure 6-3. The simulations fit the data well for initial PCE concentrations of 39 and 282 μM (Figures 6-3(a) and 6-3(b)). At the highest PCE concentration of 1057 μM, however, the model did not simulate sequential PCE dechlorination as well (Figure 6-3 (c)). Simulated *c*-DCE concentrations were much lower than those experimentally observed, and the experimental production and transformation rates

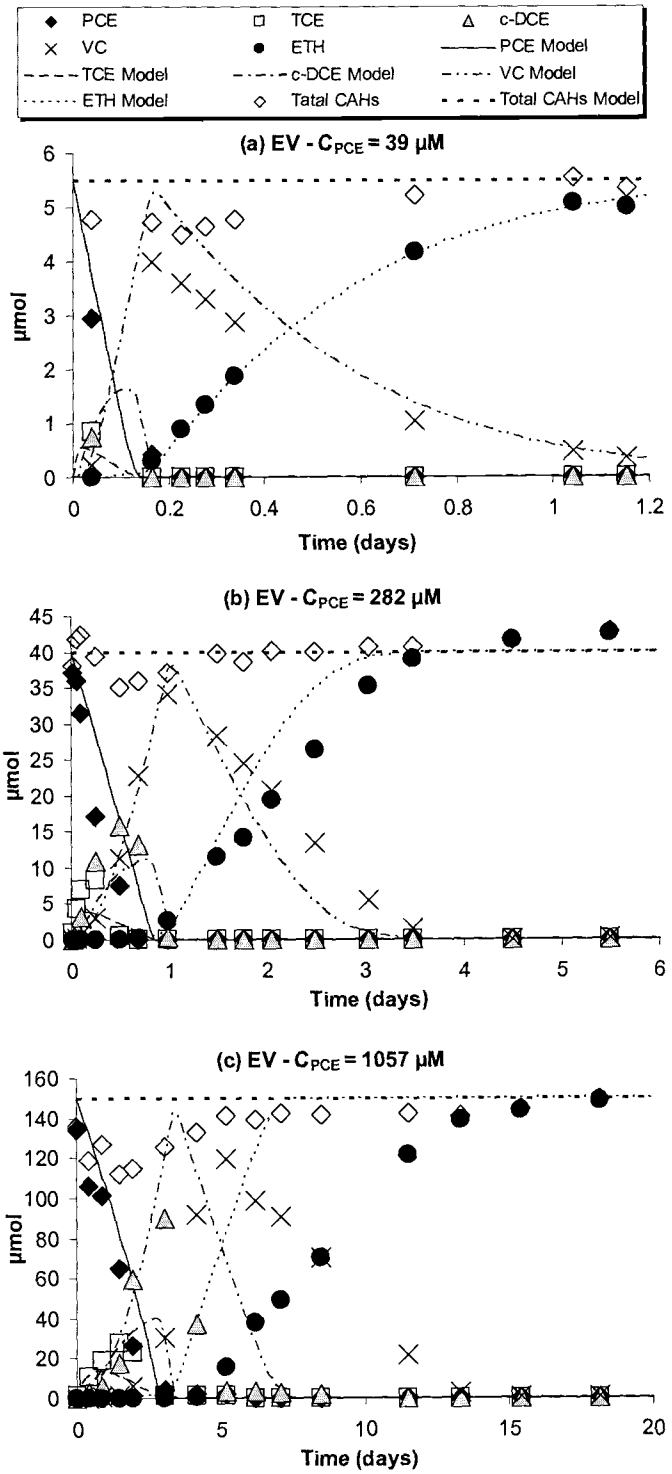

Figure 6-3. Reductive dechlorination kinetics at different initial PCE concentrations by the EV culture. Lines represent model simulations with competitive inhibition kinetics.

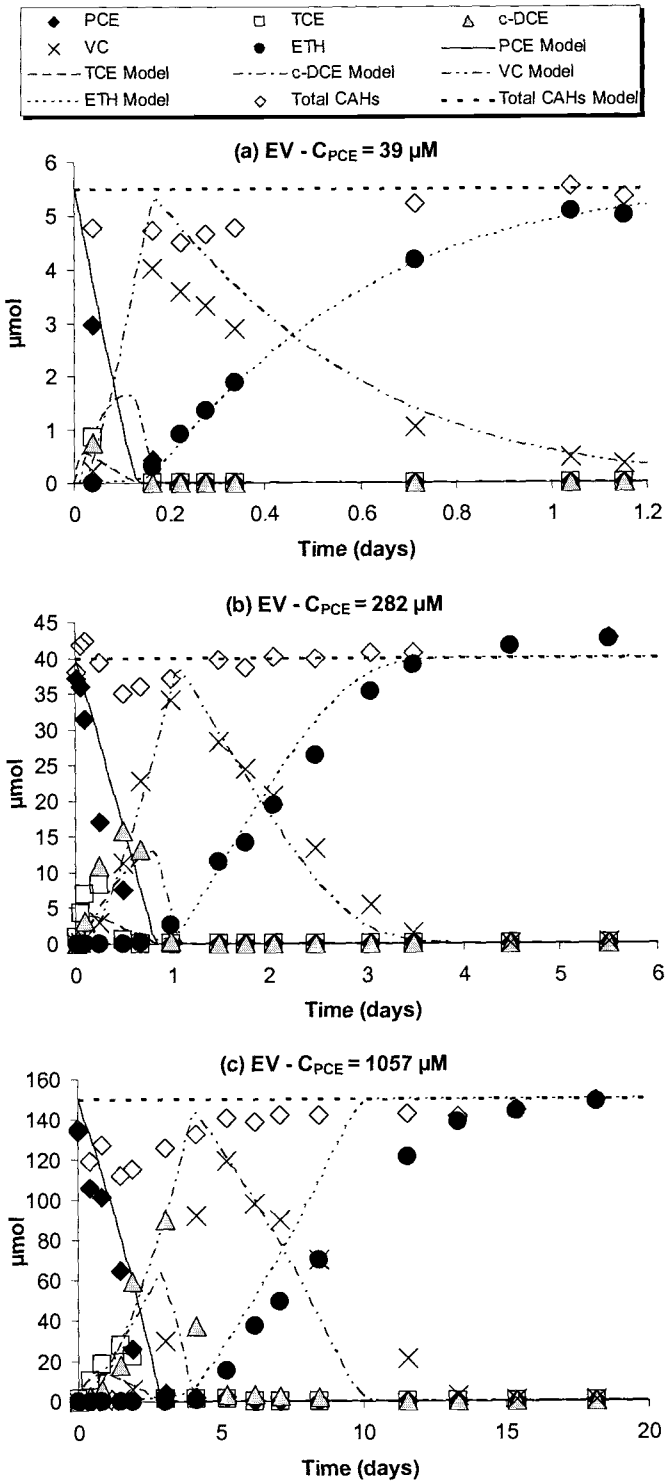

Figure 6-4. Reductive dechlorination kinetics at different initial PCE concentrations by the EV culture. Lines represent model simulations with both Haldane and competitive inhibition kinetics.

of VC and ETH were much slower than the model simulations (Figure 6-3(c)).

Figure 6-4 showed the Michaelis-Menten kinetic model simulation incorporating the combination of competitive and Haldane inhibition kinetics. The K_{HI} values for TCE, *c*-DCE, and VC of 900, 750, and 750 μM were obtained by fitting the PCE results (Figure 6-4) and TCE results (Figure 6-8(b)). The simulation presented in Figure 6-4(a) shows good agreement with the experimental data. Similar results shown in Figure 6-3(a) were obtained, indicating little effect of Haldane kinetics at the low initial PCE concentration of 39 μM. With the highest PCE concentration of 1057 μM (Figure 6-4(c)), the model with both competitive and Haldane inhibition kinetics fits the experimental data much better than that with only competitive inhibition shown in Figure 6-3(c). Figure 6-4(b) also showed that the simulation with both competitive and Haldane inhibition kinetics better reproduced the experimental results for the EV culture dechlorination at an initial PCE concentration of 282 μM.

Reductive Dechlorination of PCE by the BM culture

The batch kinetic experiments were also performed to study reductive dechlorination of the BM culture at different initial PCE concentrations. With the lowest PCE concentration of 43 μM, 80% of the initial PCE was converted to ETH within 1.2 days (Figures 6-5(a)). The ETH production rate of the BM culture was slightly lower than that of the EV culture for all PCE concentrations tested, but much more enhanced than that of the PM culture. Complete reductive dechlorination at a

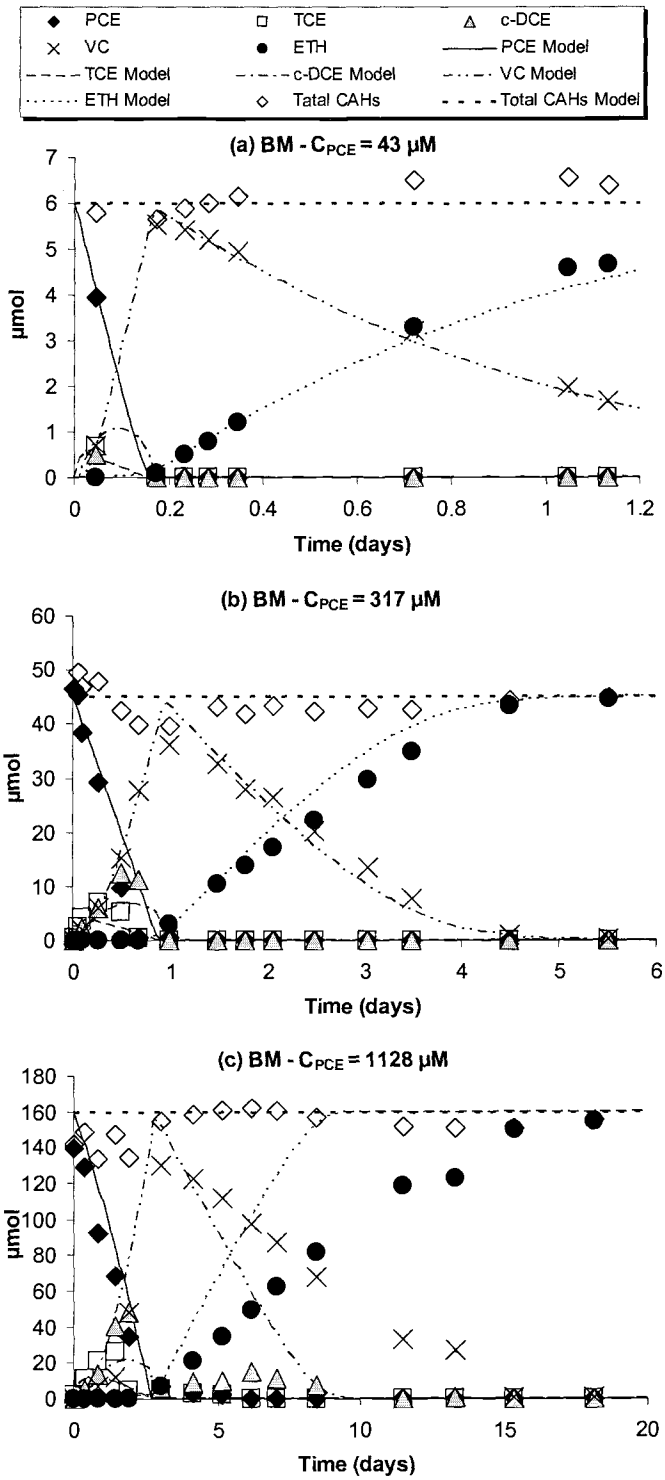

Figure 6-5. Reductive dechlorination kinetics at different initial PCE concentrations by the BM culture. Lines represent model simulations with competitive inhibition kinetics.

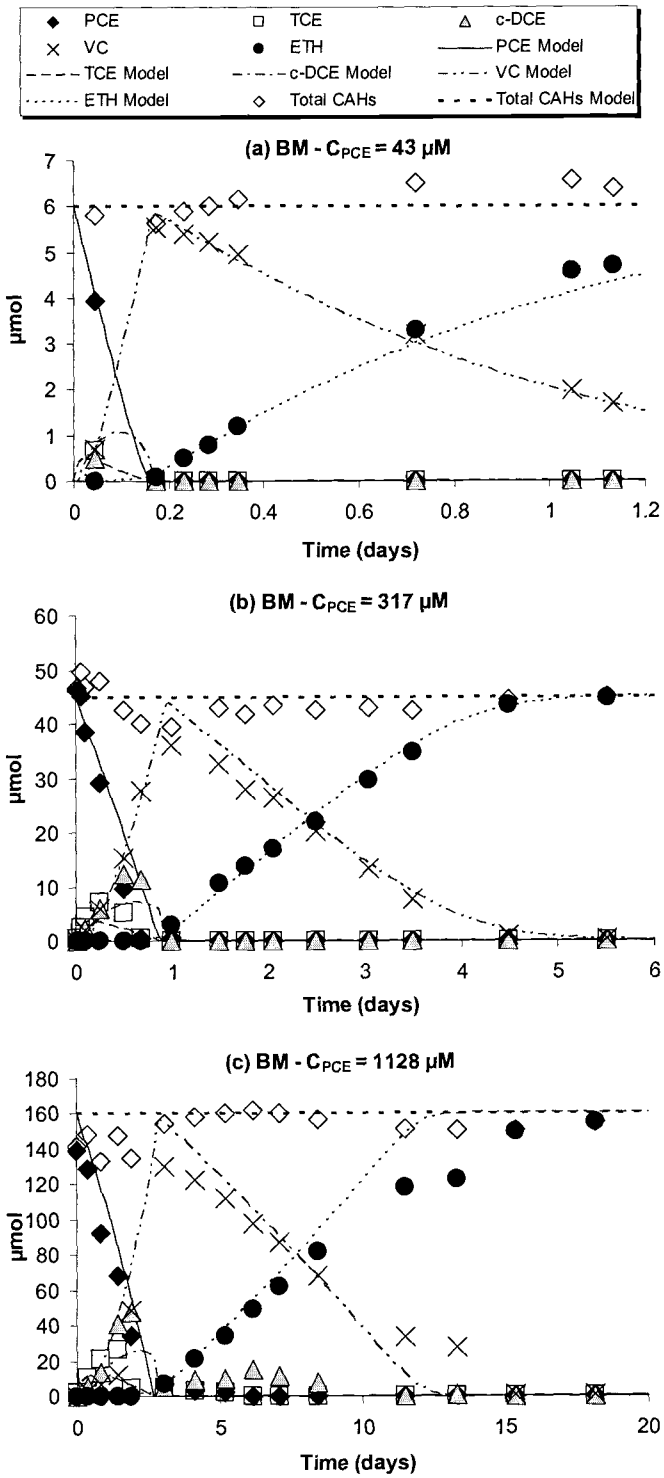

Figure 6-6. Reductive dechlorination kinetics at different initial PCE concentrations by the BM culture. Lines represent model simulations with both Haldane and competitive inhibition kinetics.

PCE concentration of 317 μM was achieved on day 4.5, which is slower than 3.5 days for the EV culture. However, with the highest PCE concentration of 1128 μM, 100% transformation to ETH was observed within 15-20 days for both the EV and BM cultures. The maximum *c*-DCE concentration was measured around day 2, yielding an aqueous concentration of 310 μM (about 50% maximum concentration observed with the EV culture). No significant difference was found in PCE transformation rates among the PM, EV, and BM cultures except at the lowest initial PCE concentration of 92 μM with the PM culture, which resulted from a higher K_S value of PCE (3.86 μM) than that of the EV culture (1.63 μM).

Using the determined initial cell concentrations of 13, 14, and 14 mg-protein/L for the PM culture, and 15, 17, and 16 mg-protein/L for the EV culture, respectively, the kinetic models were compared with the data (Figures 6-5 and 6-6). Total cell concentrations of the BM culture for the model inputs were measured based on protein analysis when each concentration of PCE was incrementally added in succession. It was impossible to obtain the separate cell concentrations of the PM and EV cultures in the BM culture. Thus, the measured total cell concentrations of the BM culture were separated into the PM and EV cultures for model inputs in proportional to the initial cell concentration ratio of 25 and 30 mg/L, respectively. The model simulation of the BM culture used the K_{HI} values obtained from the data fitting of the PM and EV experiments. Similar to the model fits for the PM and EV cultures, the rate of PCE transformation was simulated well by both models at all initial PCE concentrations, suggesting that even high PCE concentrations have no or

very weak Haldane inhibition effects. Similarly, both models showed good agreement with the sequential transformation data at the lowest PCE concentration (43 μM) as represented in Figures 6-5(a) and 6-6(a). At the higher PCE concentrations of 317 and 1128 μM, the model with competitive and Haldane inhibition kinetics provided a better fit of the data than the model with only competitive inhibition kinetics.

Reductive Dechlorination of a High TCE concentration

Initial TCE concentrations shown in Figures 6-7 and 6-8 were near half saturation concentrations (TCE solubility = 8.4 mM, (Yaws, 1999)). TCE was readily dechlorinated within 4 days without any lag time with the three mixed cultures. The highest *c*-DCE aqueous concentration of 3930 μM was observed on day 2-3 with the EV culture, while the PM culture showed the lowest concentration of 2480 μM. Different patterns of ETH production were observed with the high TCE concentrations of average 4073 μM compared to those of PCE dechlorination. On day 18, the PM culture produced the highest ETH concentration (383 μM) among the three different cultures, which is close to that of the BM culture (323 μM). The lowest ETH production was observed with the EV culture. Much slower transformation of *c*-DCE to VC was observed with the EV culture than with the PM and BM cultures. This leads to an apparently slow rate of ETH production, suggesting toxicity or inhibition at high *c*-DCE concentrations.

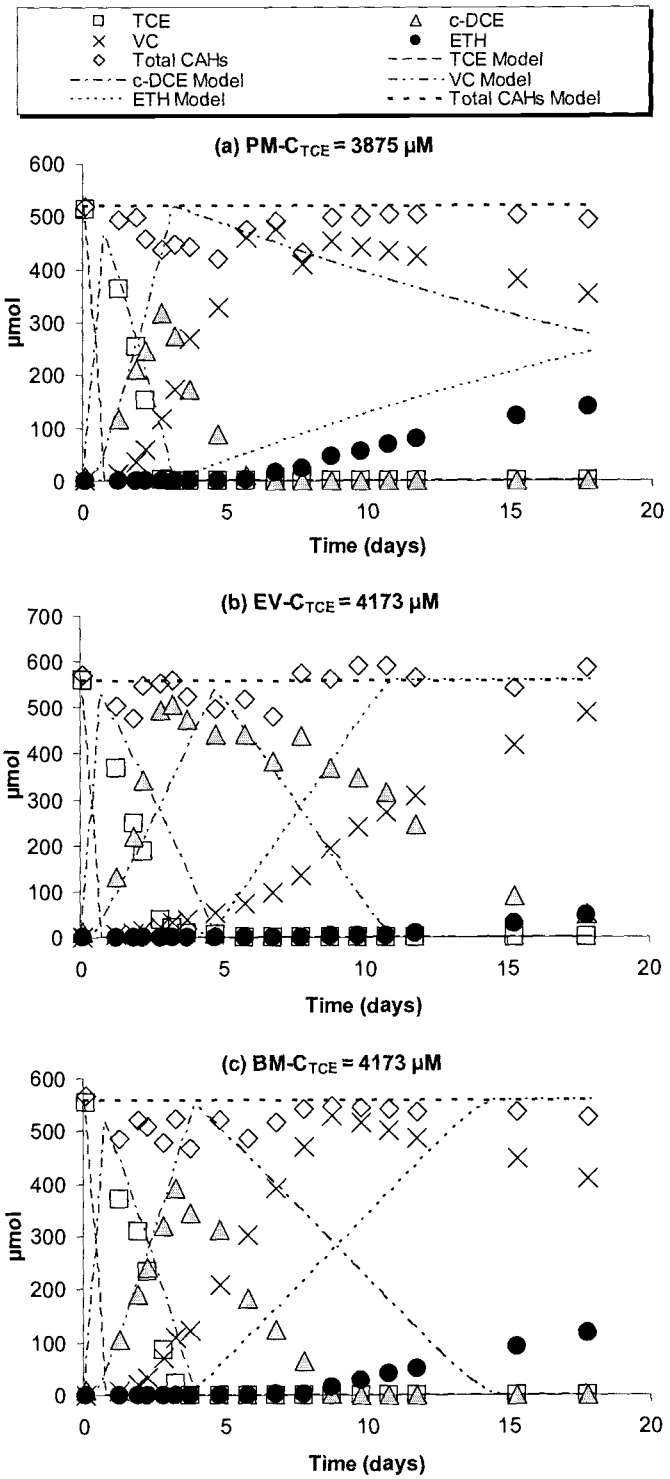

Figure 6-7. Reductive dechlorination kinetics at high TCE concentrations by the PM, EV and BM cultures. Lines represent model simulations with competitive inhibition kinetics.

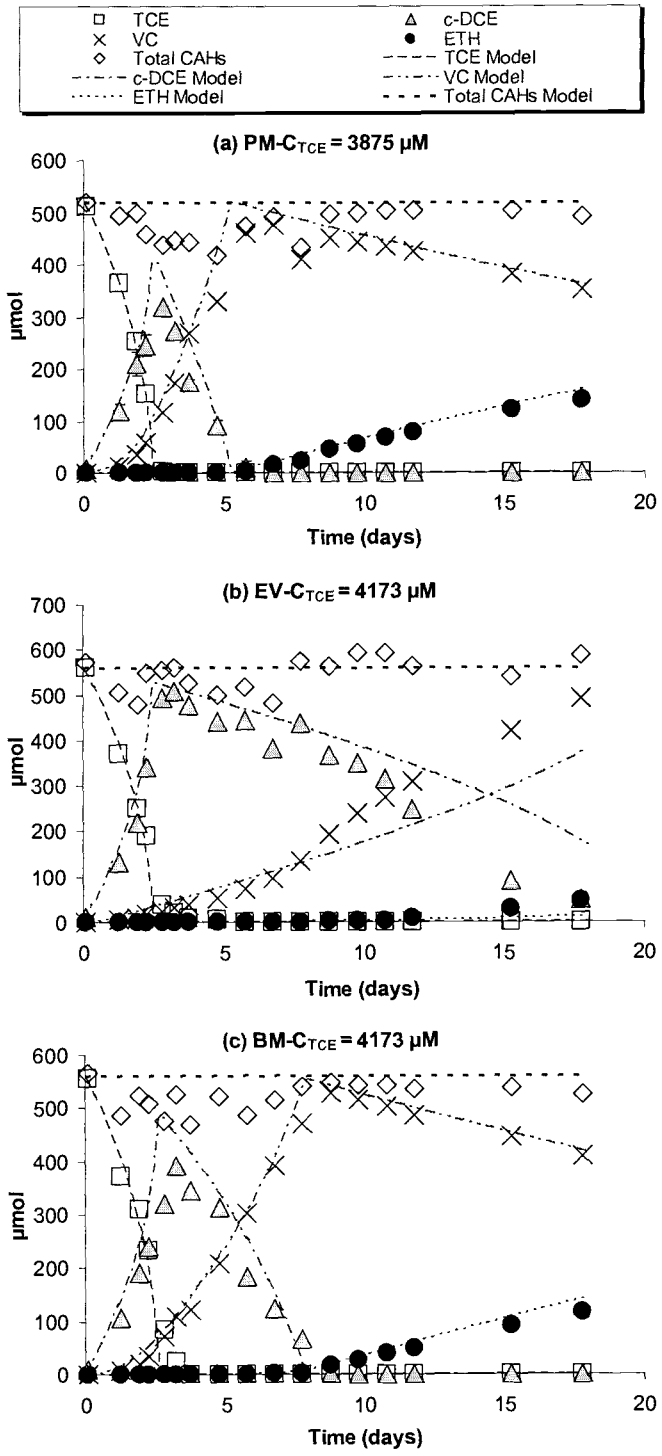

Figure 6-8. Reductive dechlorination kinetics at high TCE concentrations by the PM, EV and BM cultures. Lines represent model simulations with Haldane and competitive inhibition kinetics.

Simulations using competitive inhibition kinetics for these tests are shown in Figure 6-7. Model simulations with competitive and Haldane inhibition are shown in Figure 6-8. The initial measured total cell concentrations for the model simulations were 35, 40, and 37 mg-protein/L for the PM, EV, and BM cultures, respectively. The 37 mg-protein/L for the BM culture consists of 17 mg-protein/L of the PM culture and 20 mg-protein/L of the EV culture, calculated as previously described. The dechlorination of TCE, *c*-DCE, and VC by all three cultures was predicted to occur more rapidly than was actually observed (Figure 6-7). Model simulations with both competitive and Haldane inhibitions (Figure 6-8) more accurately captured the experimental data. For the PM culture, the main differences between the model prediction and the experimental data appeared to result from including Haldane inhibition of TCE (Figure 6-8(a)). With a Haldane inhibition constant of 900 μM, the model with both competitive and Haldane inhibitions agreed much better with the experimental results (Figure 6-8(a)). *c*-DCE and VC transformations had weak Haldane inhibitory effects with Haldane inhibition constants of 6000 and 7000 μM. Comparison of the two model simulations for the EV culture showed that TCE, *c*-DCE, and VC have significant Haldane inhibition. The model fit of the experimental data gave Haldane inhibition constants of 900, 750, and 750 μM, respectively. The transformations of *c*-DCE and VC were much better predicted by the Haldane inhibition model simulations. With Haldane inhibition constants obtained from fitting the data with the PM and EV cultures, the model simulations for the BM culture showed very good agreement with the experimental results (5-8(c)).

DISCUSSION

A kinetic model employing Michaelis-Menten equations with a combination of competitive and Haldane inhibition kinetics simulated well the sequential transformation of PCE and TCE over a very wide range of concentrations, for the three different mixed anaerobic cultures. The competitive inhibition kinetic model with inhibition constants set equal to the measured K_S values (Yu *et al.*, 2003) resulted in simulations that well matched experimental data for PCE concentrations lower than 300 μM. However, simulations did not match experimental data as well for PCE or TCE concentrations higher than 300 - 400 μM. The kinetic model that included both competitive and Haldane inhibition kinetics provided much better fits to the experimental results, especially for the EV and BM cultures.

A number of modeling studies described reductive dechlorination of PCE using competitive inhibition (Tandoi *et al.*, 1994; Tonnaer *et al.*, 1997; Haston, 1999; Garant and Lynd, 1998). These studies were all conducted at aqueous PCE concentrations lower than 300 μM. Our modeling and experimental results indicate that a kinetic model that incorporates both competitive and Haldane inhibition kinetics results in a better match to experimental results near the saturation PCE concentration (1000 μM), and for a TCE concentration of 4000 μM that is 50% of its solubility limit. Maillard *et al.* (2003) observed and reported kinetics for cell free extracts that exhibited Haldane type kinetics for TCE concentrations higher than approximately 300-400 μM. The results also showed limited inhibition of PCE transformations, consistent with the results of this study.

Additional model simulations, including only competitive inhibition, were run in our study for high concentrations of PCE (1000 μM) and TCE (4000 μM). The cell decay constant, k_d (0.024 day^{-1}) (Fennell and Gossett, 1998) that was used in all the simulations, was increased up to 0.09 day^{-1} as reported by Cupples *et al.* (2003). The model with a cell decay constant of 0.09 day^{-1} slightly improved the agreement with the experimental data, but the model predicted much faster production and transformation rates of CAHs than were observed experimentally. A change in the cell decay constant did not result in the improvement in the simulations as achieved by invoking Haldane kinetics.

The total cell concentrations during PCE dechlorination experiments were simulated, as shown in Figure 6-9. PCE kinetic experiments were conducted with the incremental addition of PCE in succession (Table 6-1). Total cell concentrations were measured by protein analysis at the beginning of each run, and the measured initial cell concentrations of each PCE experiment were used as the model simulation inputs. As shown in Figure 6-9, simulated cell concentrations for the PM, EV, and BM cultures were reasonably consistent with the protein concentrations measured at the beginning of each PCE experiment.

Based Haldane kinetic parameters obtained from our model simulations, Figures 6-10 and 6-11 represent comparison of simple Michaelis-Menten and Haldane kinetics for TCE, *c*-DCE and VC for the PM and EV cultures. The PM and EV cultures showed different Haldane inhibition patterns. According to the experimental results and modeling studies, the PM culture has very high Haldane

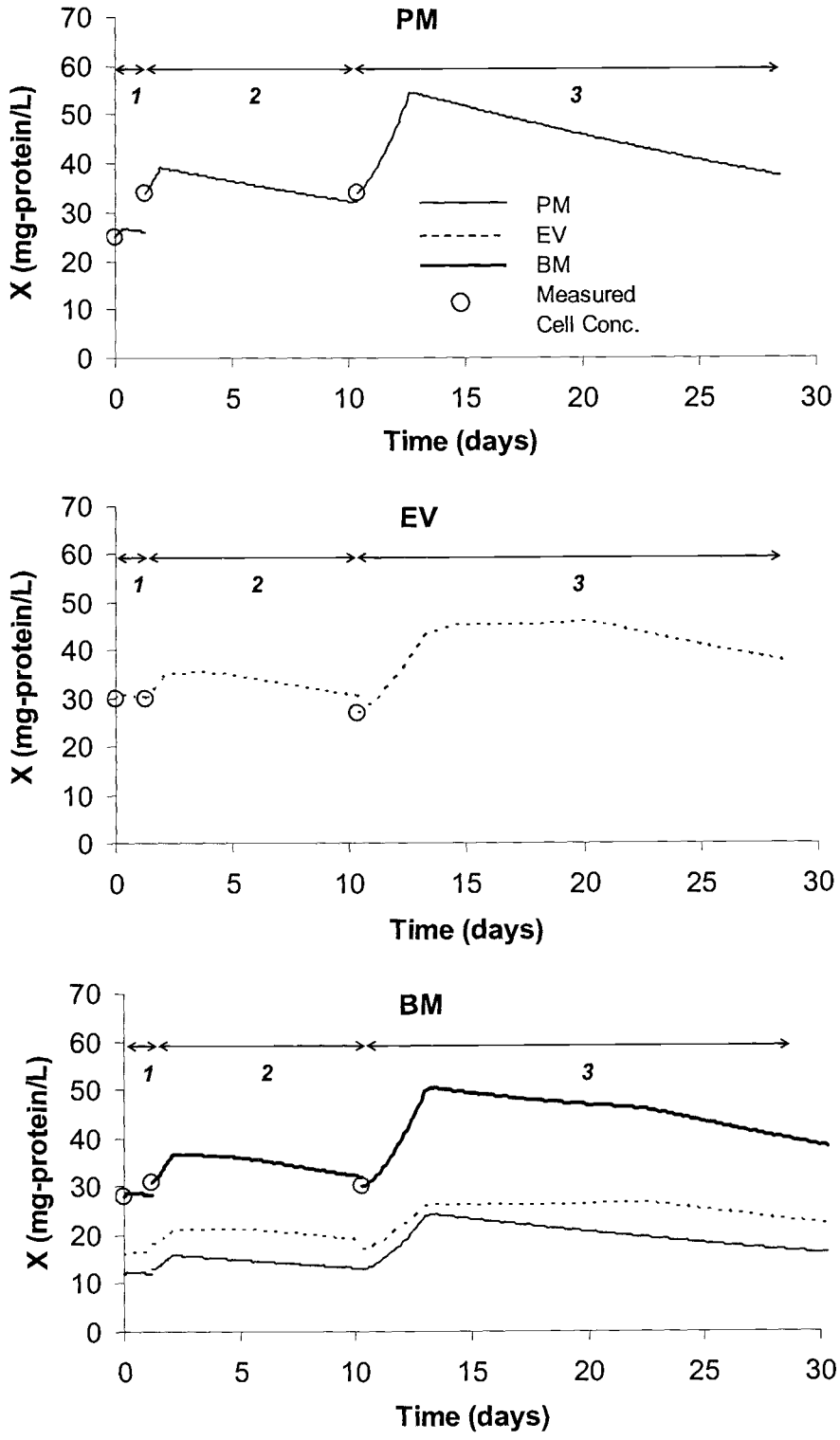

Figure 6-9. Model simulations of total cell concentrations during PCE reductive dechlorination (Run 1, 2, and 3) as listed in Table 6-1.

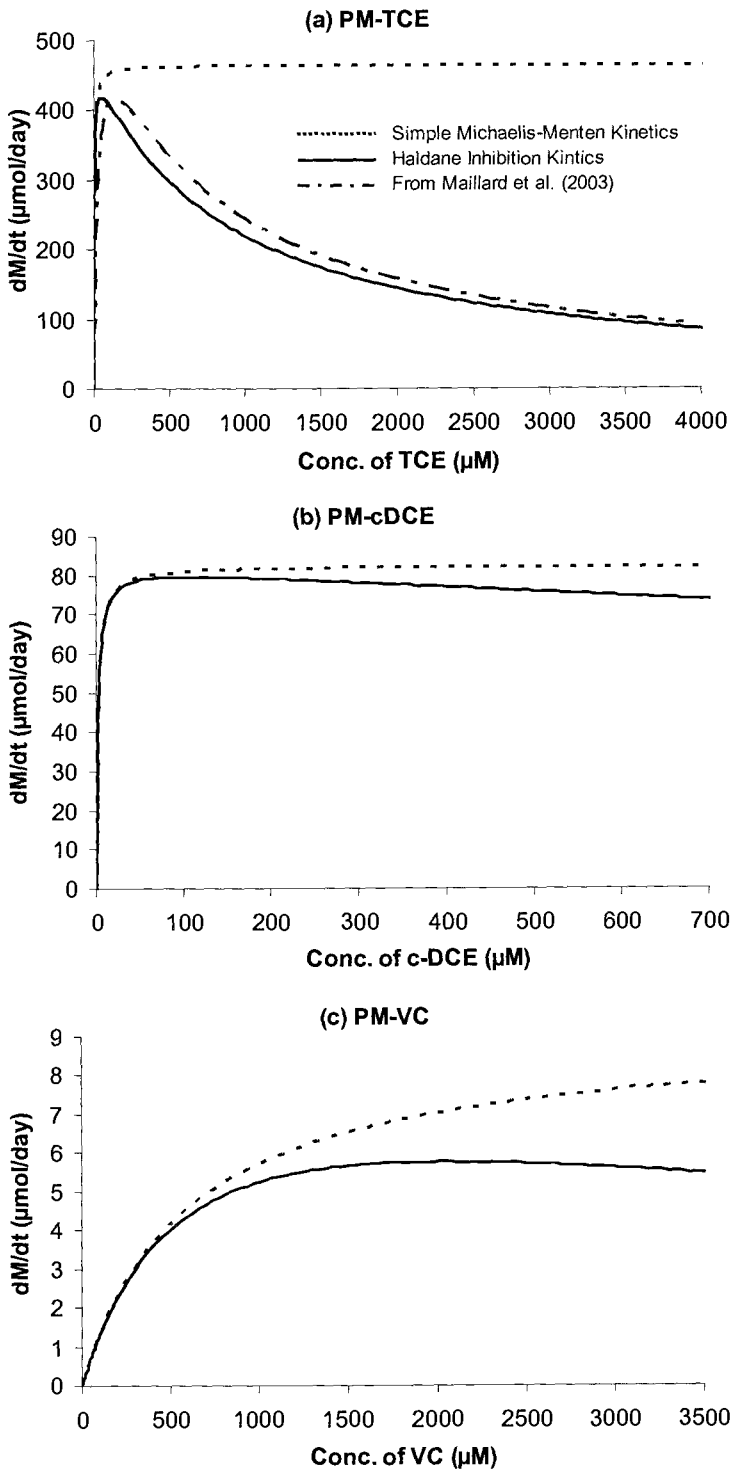

Figure 6-10. Michaelis-Menten kinetics with and without Haldane inhibition for the PM culture. Both kinetic curves are simulated with total cell concentration of 30 mg/L.

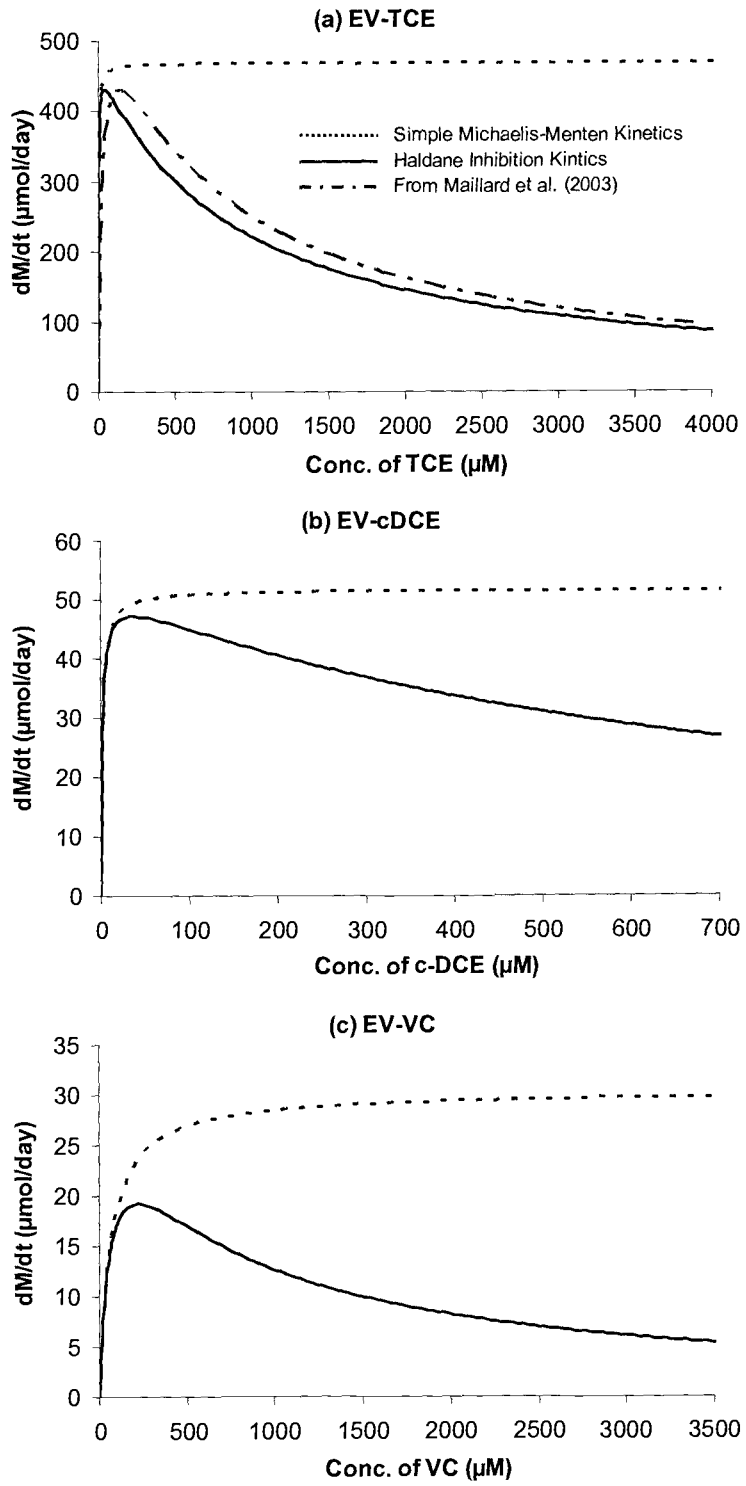

Figure 6-11. Michaelis-Menten kinetics with and without Haldane inhibition for the EV culture. Both kinetic curves are simulated with total cell concentration of 30 mg/L.

inhibition constants for *c*-DCE and VC (6000 and 7000 μM, respectively), indicating very slight Haldane inhibition effects, while the EV culture showed significant Haldane inhibition for TCE, *c*-DCE, and VC. Figures 6-10(b) and (c) indicate that little reduction in dechlorination rates occurs at relatively high *c*-DCE and VC concentrations for the PM culture. However, significant decreases in dechlorination rates are expected with increases of TCE concentration for the PM culture (Figure 6-10(a)), and TCE, *c*-DCE, and VC concentrations with the EV culture (Figure 6-11 (a), (b), and (c)). TCE kinetics using a model with noncompetitive inhibition reported by Maillard *et al.* (2003) was compared in Figures 6-9(a) and 6-10(a). An inhibition constant of 760 μM (close to the K_{HI} value of 900 μM observed in our study) and a half-velocity coefficient of 23.7 μM were used in the model kinetics. Since Maillard *et al.* (2003) purified PCE-RDase, the cell concentrations were normalized to compare the kinetic patterns with ours. It is very interesting that our Haldane model fit to our experimental data with whole cell is very close to that observed by Maillard *et al.* (2003). Thus, our observation of Haldane type kinetics at high TCE concentration is supported by their observations.

As shown in Figures 6-1 through 6-6, the BM and EV cultures more effectively transformed PCE to ETH. However, PCE solubility is much lower than the other chlorinated ethylenes. TCE has a solubility of 8400 μM and high concentrations may be found near DNAPL source zones in soil and groundwater. The experimental and modeling results shown in Figures 6-7 and 6-8 show sequential dechlorination of TCE at an average initial concentration of 4073 μM.

The pattern of reductive dechlorination between three different mixed cultures differed from that of PCE dechlorination in Figures 6-1 through 6-6. The PM and BM dechlorinating cultures showed greater transformations of high TCE concentrations to ETH than the EV culture. The EV and BM cultures, however, show better transformation of PCE to ETH than the PM culture. The results of our modeling analysis indicate factors causing this response. One possible explanation for the difference at higher TCE concentrations is that the EV culture is experiencing greater toxicity and inhibition at the higher CAH concentrations. The experimental results in Figure 6-8 showed that TCE depletion rates of the PM, EV, and BM cultures are almost the same, but differences for *c*-DCE and VC transformations are observed between the PM and EV cultures. Our modeling studies also revealed that the EV culture possibly has lower K_{HI} values of 750 μM for both *c*-DCE and VC compared to those of 6000 and 7000 μM of the PM, respectively, indicating higher *c*-DCE and VC concentrations to the EV culture are potential toxic or inhibitory. Strong inhibition of c-DCE on VC transformation limits ETH production in all the cultures, as previously observed (Yu *et al.*, 2003). The modeling presented here suggests that the modeling of *c*-DCE transformation with Haldane kinetics is an important process to predict reductive dechlorination of PCE to ETH at high concentrations. From the reductive dechlorination results with a broad range of PCE and TCE concentrations, the BM culture showed more complete dechlorination to ETH than either of the single mixed cultures. Bioaugmentation with a binary mixed culture system might be more effective at real contaminated sites, where wide ranges

in concentrations and contaminant mixture are present. Further investigations utilizing more complex microbial multi-systems of defined cultures with different degradation properties need to be performed to better utilize and understand their potential for application in the polluted environments as suggested by Fairlee *et al.* (1997) and Rogers *et al.* (2000).

A variety of reductive CAH dechlorination models have been suggested (Fennell and Gossett, 1998; Tonnaer *et al.*, 1997; Garant and Lynd, 1998; Haston, 1999; Bagley, 1998). Fennell and Gossett (1998) developed a model for complete PCE reductive dechlorination to ETH, in which PCE concentration was a 70 μM PCE aqueous concentration and no inhibition between chlorinated ethylenes was considered. Models suggested by Tonnaer *et al.* (1997) and Haston (1999) focused on competitive inhibitions between PCE and TCE, and *c*-DCE and VC, respectively. To the best of knowledge, experimental data sets and the model here are the first one describing the sequential transformation of PCE and TCE over a wide range of concentrations. Both competitive and Haldane kinetics were required to model the experimental observations. Our kinetic and modeling studies indicate that a model with the combination of competitive and Haldane inhibitions is of potential use to predict the fate and bioremediation of CAHs as pollutants with both high concentrations possibly produced from DNAPL source zone, as well as comparably low concentrations. Kinetic studies are now needed with these cultures at high concentrations of TCE, *c*-DCE, and VC, to confirm the Haldane kinetics obtained from our fitting the high concentration transient results.

Acknowledgements

The research was supported by a research grant from the U.S. Environmental Protection Agency-sponsored Western Region Hazardous Substance Research Center under agreement R-815738. This article has not been reviewed by the agency, and no official endorsement should be inferred.

REFERENCES

Bagley, D. M. (1998) Systematic Approach for Modeling Tetrachloroethene Biodegradation. *J. Environ. Eng.* **124**, 1076-1086.

Bailey, J.and Ollis, D. (1986) *Biochemical Engineering Fundamentals*, McGraw-Hill, New York, NY.

Cupples, A. M., Spormann, A. M., and McCarty, P. L. (2003) Growth of a Dehalococcoides-Like Microorganism on Vinyl Chloride and cis-dichloroethene as Electron Acceptors as Determined by Competitive PCR. *Appl. Environ. Microbiol.* **69**, 953-959.

Ellis, D. E., Lutz, E. J., Odom, J. M., Buchanan, Jr., R. J., Lee, M. D., Bartlett, C. L., Harkness, M. R., and Deweerd, K. A. (2000) Bioaugmentation for Accelerated In Situ Anaerobic Bioremediation. *Environ. Sci. Technol.* **34**, 2254-2260.

Fairlee, J. R., Burback, B. L., and Perry, J. J. (1997) Biodegradation of Groundwater Pollutants by a Combined Culture of *Mycobacterium vaccae* and a *Rhodococcus sp. Can. J. MIcrobiol.* **43**, 841-846.

Fennell, D. E. and Gossett, J. M. (1998) Modeling the Production of and Competition for Hydrogen in a Dechlorinating Culture. *Environ. Sci. Technol.* **32**, 2450-2460.

Garant, H. and Lynd, L. (1998) Applicability of Competitive and Noncompetitive Kinetics to the Reductive Dechlorination of Chlorinated Ethenes. *Biotechnol. Bioeng.* **57**, 751-755.

Harkness, M. R., Bracco, A. A., Brennan, Jr., M. J., Deweerd, K. A., and Spivack, J. L. (1999) Use of Bioaugmentation To Stimulate Complete Reductive Dechlorination of Trichloroethene in Dover Soil Columns. *Environ. Sci. Technol.* **33** (7), 1100 - 1109.

Haston, Z. C. (1999). Factors Affecting Growth and Utilization in the Anaerobic Dehalogenation of Chlorinated Ethenes. Ph.D. Dissertation, Stanford University, Standord, CA.

Hendrickson, E. R., Payne, J. A., Young, R. M., Starr, M. G., Perry, M. P., Fahnestock, S., Ellis, D. E., and Ebersole, R. C. (2002) Molecular Analysis of Dehalococcoides 16S Ribosomal DNA form Chloroethene-Contaminated Sites through North America and Europe. *Appl. Environ. Microbiol.* **68**, 485-495.

Maillard, J., Schumacher, W., Vazquez, F., Regeard, C., Hagen, W. R., and Hollinger, C. (2003) Characterization of the Corrinoid Iron-Sulfur Protein Tetrachloroethene Reductive Dehalogenase of *Dehalobacter restrictus*. *Appl. Environ. Microbiol.* **69** (8), 4628-4638.

Major, D. M., McMaster, M. L., Cox, E. E., Edwards, E. A., Dworatzek, S. M., Hendrickson, E. R., Starr, M. G., Payne, J. A., and Buonamici, L. W. (2002) Field Demonstration of Successful Bioaugmentation To Achieve Dechlorination of Tetrachloroethene To Ethene. *Environ. Sci. Technol.* **36**, 5106-5116.

Maymo-Gatell, X., Chien, Y.-T., Gossett, J. M., and Zinder, S. H. (1997) Isolation of Bacterium that Reductively Dechlorinates Tetrachloroethene to Ethene. *Science* **276**, 1568-1571.

Pon, G., Hyman, M. R., and Semprini, L. (2003) Acetylene Inhibition of Trichloroethene and Vinyl Chloride Reductive Dechlorination Environ. *Sci. Technol.* **37** (14), 3181-3188.

Rittmann, B. E., Seagren, E., and Wrenn, B. A. (1994). In *In-Situ Bioremediation*. Second Edition. Parkridge, New Jersey, USA: Noyes Publications, pp. 37-50.

Rogers, J. B., DuTeau, N. M., and Reardon, K. F. (2000) Use of 16S-rRNA to Investigate Microbial Population Dynamics During Biodegradation of Toluene and Phenol by a Binary Culture *Biotechnol. Bioeng.* **70**, 436-445.

Tandoi, V., DiStefano, T. D., Bowser, P. A., Gossett, J. M., and Zinder, S. H. (1994) Reductive Dehalogenation of Chlorinated Ethenes and Halogenated Ethanes by a High-Rate Anaerobic Enrichment Culture. *Environ. Sci. Technol.* **28**, 973-979.

Tonnaer H., Alphenaar, A., Wit, H. D., Grutters, M., Spuij, F., Gerritse, J., and Gottschal, J. C. (1997) Modelling of Anaerobic Dechlorination of Chloroethenes for In-Situ Bioremediation. In *In Situ and On-Site Bioremediation*, papers from the Fourth International In Situ and On-Site Bioremediation Symposium, Ed. Alleman, B. C. and Leeson, A., Battelle Press, Columbus, OH, pp. 591-596.

Westrick, J. J., Mello, J. W., and Thomas, R. F. (1984) The Groundwater Supply Survey. *J. Am. Water Works Assoc.* **76** (5), 52-59.

Yaws, C. L. (1999) *Chemical properties handbook: physical, thermodynamics, environmental, transport, safety, and health related properties for organic and inorganic chemicals*. McGraw-Hill: New York, NY.

Yu, S. and Semprini, L. (2002a) Dechlorination of PCE DNAPL with TBOS Using a Binary Mixed Culture. In *Remediation of Chlorinated and Recalcitrant Compounds*, Battelle Press, Columbus, OH, **2002**, Paper 2B-49.

Yu, S. and Semprini, L. (2002b). Comparison of Trichloroethylene Reductive Dehalogenation by Microbial Communities Stimulated on Silicon-Based Organic Compounds as Slow-Release Anaerobic Substrate. *Water Res.* **36**, 4985-4996.

Yu, S, Dolan, M. E., and Semprini, L. (2003) Unpublished data.

CHAPTER 7

CONCLUSIONS AND ENGINEERING SIGNIFICANCE

CONCLUSIONS

This study used three different anaerobic mixed cultures obtained from CAH-contaminated sites for reductive dechlorination of PCE and TCE. TBOS as a slow-release anaerobic substrate was demonstrated to support and enhance reductive dechlorination of PCE DNAPL as well as aqueous PCE and TCE concentrations. Detailed kinetic and modeling studies were performed with the PM, EV, and a binary mixed culture representing a mixture of the two cultures. The following conclusions can be drawn from this study of PCE and TCE reductive dechlorination over a wide range of the concentrations:

1) TBOS abiotically and slowly hydrolyze to 1-butanol, which ferments to butyrate and/or acetate, producing H_2 during the fermentation process. One mol of TBOS was shown to produce 4 mol of 1-butanol through abiotic hydrolysis.
2) TBOS was effective as a slow-release anaerobic substrate to promote dechlorination reactions for PCE DNAPL and support long-term reductive dechlorination of TCE (about 600 days), and has promise as an effective anaerobic substrate for remediating a wide range of CAH concentrations and PCE DNAPL at many contaminated sites.

3) A simple kinetic method developed was very effective in determining reproducible and reliable kinetic parameters that well characterize the dechlorinating mixed cultures.

4) The more chlorinated ethylenes inhibit reductive dechlorination of the less chlorinated ethylenes. PCE inhibited reductive TCE dechlorination, but not *c*-DCE dechlorination, while TCE strongly inhibited *c*-DCE and VC dechlorination. *c*-DCE also inhibits VC transformation to ETH. Inhibition constants of each chlorinated ethylene, K_I (μmol/L), were comparable to their respective half-velocity coefficients, when a competitive inhibition model was applied.

5) The Michaelis-Menten kinetics incorporating both competitive and Haldane inhibitions showed good agreement with the experimental data of sequential transformation of PCE and TCE over a wide range of concentrations (up to near-saturation PCE concentration and a half-saturation TCE concentration).

6) The PM and BM dechlorinating cultures showed better dechlorination abilities at a high TCE concentration (4073 μM) than the EV, while the EV and BM cultures were better for reductive PCE dechlorination than the PM culture.

7) A binary mixed culture resulted in more complete dechlorination than either of the single mixed cultures for remediating PCE DNAPL.

ENGINEERING SIGNIFICANCE

Anaerobic substrates have been widely used for in situ CAH bioremediation, including lactate, butyrate, propionate, benzoate, and hydrogen (Smatlak *et al.*, 1996; Yang and McCarty, 1998; Fennell *et al.*, 1997). High operational costs resulting from repeated or continuous injections of the commonly used anaerobic substrates might be significantly reduced through the use of a slow-release substrate, such as TBOS. As observed in the vegetable oil studies, chlorinated solvents could easily partition into TBOS injected near a DNAPL zone, thus reducing the aqueous concentrations of PCE or TCE, and potentially reducing toxicity and inhibition. Thus, these concentration reductions may actually enhance rates of reductive dechlorination.

A variety of reductive CAH dechlorination models have been proposed. However, the models simulated reductive PCE and TCE dechlorination over a limited range of concentrations. A kinetic model was developed incorporating the combination of competitive and Haldane inhibitions, which showed good agreement with the experimental data over a wide range of PCE and TCE concentrations. Thus, the model is of potential use to predict the fate and bioremediation of CAHs as pollutants with both high CAH concentrations produced from DNAPL source zone as well as comparably low concentrations.

For bioremediation in real contaminated sites, a binary mixed culture of different dechlorinating microorganisms with varying dechlorination abilities can achieve more efficient and enhanced in situ bioremediation. Thus, bioaugmentation

with a binary mixed culture could be an attractive strategy when completely dechlorinating bacteria can not be found at contaminated sites.

REFERENCES

Smatlak, C. R., Gossett, J. M., and Zinder, S. H. (1996). Comparative Kinetics of Hydrogen Utilization for Reductive Dechlorination of Tetrachloroethene and Methanogenesis in an Anaerobic Enrichment culture. *Environ. Sci. Technol.* **30**, 2850-2858.

Yang, Y. and McCarty, P. L. (1998). Competition for Hydrogen within a Chlorinated Solvent Dehalogenating Anaerobic Mixed Culture. *Environ. Sci. Technol.* **32**, 3591-3597.

Fennell, D. E., Gossett, J. M., and Zinder, S. H. (1997) Comparison of Butyric Acid, Ethanol, Lactic Acid, and Propionic Acid as Hydrogen Donors for the Reductive Dechlorination of Tetrachloroethene. *Environ. Sci. Technol.* **31**, 918-926.

BIBLIOGRAPHY

Adamson, D. T., Mcdade, J. M., and Hughes, J. B. (2003) Inoculation of a DNAPL Source Zone to Initiate Reductive Dechlorination of PCE. *Environ. Sci. Technol.* **37**, 2525-2533.

Adrian, L., Szewzyk, U., Wecke, J., and Görisch, H. (2000) Bacterial Dehalorespiration with Chlorinated Benzenes. *Nature* **408**, 580-583.

Assaf-Anid, N., Hayes, K. F., and Vogel, T. M. (1994). Reductive dechlorination of carbon tetrachloride by cobalamin(II) in the presence of dithiothreitol: Mechanistic study, effect of redox potential and pH. *Environ. Sci. Technol.* **28**, 246-252.

Bagley, D. M. (1998) Systematic Approach for Modeling Tetrachloroethene Biodegradation. *J. Environ. Eng.* **124**, 1076-1086.

Bailey, J.and Ollis, D. (1986) *Biochemical Engineering Fundamentals*, McGraw-Hill, New York, NY.

Ballapragada, B. S., Stensel, H. D., Puhakka, J. A. and Ferguson, J. F. (1997). Effect of Hydrogen on Reductive Dechlorination of Chlorinated Ethenes. *Environ. Sci. Technol.* **31**, 1728-1734.

Bell, M., Brown, D., Burns, D. A., and Lee, M. D. (2001) Biologically Enhanced Reductive Dechlorination. Platform presentation. In Sixth In Situ and On-Site Bioremediation Symposium, San Diego, CA.

Bolesch, D., Nielsen, R., and Keasling, J. (1997) Complete Reductive Dechlorination of Trichloroethene by a Groundwater Microbial Consortium. Ann. N. Y. Acad. Sci., **829**, 97-102.

Bouwer, E. J. and McCarty, P. L. (1983a). Transformations of 1- and 2-Carbon Halogenated Aliphatic Organic Compounds under Methanogenic Conditions. *Appl. Environ. Microbiol.* **45**, 1286-1294.

Bouwer, E. J. and McCarty, P. L. (1983b). Transformations of Halogenated Organic Compounds under Denitrification Conditions. *Appl. Environ. Microbiol.* **45**, 1295-1299.

Bouwer, E. J., Rittmann, B. E., and McCarty, P. L. (1981). Anaerobic Degradation of Halogenated 1- and 2-Carbon Organic Compounds. *Environ. Sci. Technol.* **15**, 596-599.

Breznak, J. A. (1994) Acetogenesis from Carbon Dioxide in Termite Guts. In *Acetogenesis*. Ed. Drake, H. L. Chapman and Hall, Inc., New York, NY, pp. 303-330.

Bunge, M., Adrian, L., Kraus, A., Opel, M., Lorenz, W. G., Andreesen, I. R., Gorisch, H., and Lechner, U. (2003) Reductive Dehalogenation of Chlorinated Dioxins by an Anaerobic Bacterium. *Nature* **421**, 357-360.

Carr, C. S., Garg, S., and Hughes, J. B. (2000) Effect of Dechlorinating Bacteria on the longevity and Composition of PCE-Containing Nonaqueous Phase Liquids under Equilibrium Dissolution Conditions. *Environ. Sci. Technol.* **34**, 1088-1094.

Carter, S. R. and Jewell, W. J. (1993). Biotransformation of Tetrachloroethylene by Anaerobic Attached-Films at Low Temperatures. *Water Res.* **27**, 607-615.

Chang, M.-K., Voice, T. C., and Criddle, C. S. (1993) Kinetics of Competitive Inhibition and Cometabolism in the Biodegradation of Benzene, Toluene, and p-Xylene by Two Pseudomonas Isolates. *Biotechnol. Bioeng.* **41**, 1057-1065.

Christiansen, N., Christensen, S. R., Arvin, E., and Ahring, B. K. (1997). Transformation of Tetrachloroethene in an Upflow Anaerobic Sludgeblanket Reactor. *Appl. Environ. Microbiol.* **47**, 91-94.

Chu, M. (1999) Abiotic Transformation of TKEBS and Other Lower Homologues of Tetraalkoxysilanes by Subsurface Solids from a Contaminated Site. Master's Thesis, Oregon State University.

Conrad, R. (1996) Soil Microorganisms as Controllers of Atmospheric Trace Gases (H_2, CO, CH_4, OCS, N_2O, and NO). *Microbiol. Rev.* 60, 609-640.

Cope, N. and Hughes, J. B. (2001) Biologically-Enhanced Removal of PCE from NAPL Source Zones. *Environ. Sci. Technol.* **35**, 2014-2021.

Cord-Ruwisch, R., Steitz, H. J., and Conrad, R. (1988) The Capacity for Hydrogenotrophic Anaerobic Bacteria to Compete for Traces of Hydrogen Depends on the Redox Potential of the Terminal Electron Acceptor. *Arch. Microbiol.* **149**, 350-357.

Cupples, A. M., Spormann, A. M., and McCarty, P. L. (2003) Growth of a Dehalococcoides-Like Microorganism on Vinyl Chloride and cis-dichloroethene as Electron Acceptors as Determined by Competitive PCR. *Appl. Environ. Microbiol.* **69**, 953-959.

de Bruin, W. P., Kotterman, J. J. J., Posthumus, M. A., Schraa, G., and Zehnder, A. J. B. (1992). Complete Biological Reductive Transformation of Tetrachloroethylene to Ethane. *Appl. Environ. Microbiol.* **58**, 1996-2000.

DeWeerd, K. A., Flanagan, W. P., Brennan, M. J., Principe, J. M., and Spivack, J. L. (1998) Biodegradation of Trichloroethylene and Dichloroethylene in Contaminated Soil and Groundwater. *Biorem. J.* **2**, 29-42.

DiStefano, T. D., Gossett, J. M., and Zinder, S. H. (1991) Reductive Dechlorination of High Concentrations of Tetrachloroethene to Ethene by an Anaerobic Enrichment Culture in the Absence of Methanogenesis. *Appl. Environ. Microbiol.* **57**, 2287-2292.

DiStefano, T. D., Gossett, J. M., and Zinder, S. H. (1992). Hydrogen as an Electron Donor for the Dechlorination of Tetrachloroethene by an Anaerobic Mixed Culture. *Appl. Environ. Microbiol.* **58**, 3622-3629.

Eichler, B. and Schink, B. (1984) Oxidation of Primary Aliphatic Alcohols by Acetobacterium carbinolicum sp. Nov., a Homoacetogenic Anaerobe. *Arch. Microbiol.* **140,** 147~152.

Ellis, D. E., Lutz, E. J., Odom, J. M., Buchanan, Jr., R. J., Lee, M. D., Bartlett, C. L., Harkness, M. R., and Deweerd, K. A. (2000) Bioaugmentation for Accelerated In Situ Anaerobic Bioremediation. *Environ. Sci. Technol.* **34**, 2254-2260.

Fairlee, J. R., Burback, B. L., and Perry, J. J. (1997) Biodegradation of Groundwater Pollutants by a Combined Culture of *Mycobacterium vaccae* and a *Rhodococcus sp. Can. J. MIcrobiol.* **43**, 841-846.

Fennell, D. E. (1998). Comparison of Alternative Hydrogen Donors for Anaerobic Reductive Dechlorination of Tetrachloroethene. Ph.D. Dissertation, Cornell University, Ithaca, NY.

Fennell, D. E. and Gossett, J. M. (1998) Modeling the Production of and Competition for Hydrogen in a Dechlorinating Culture. *Environ. Sci. Technol.* **32**, 2450-2460.

Fennell, D. E., Gossett, J. M. and Zinder, S. H. (1997) Comparison of Butyric Acid, Ethanol, Lactic Acid, and Propionic Acid as Hydrogen Donors for the Reductive Dechlorination of Tetrachloroethene. *Environ. Sci. Technol.* **31**, 918-926.

Freedman, D. L. and Gossett, J. M. (1989) Biological Reductive Dechlorination of Tetrachloroethylene and Trichloroethylene to Ethylene under Methanogenic Conditions. *Appl. Environ. Microbiol.* **55**, 2144~2151.

Garant, H. and Lynd, L. (1998) Applicability of Competitive and Noncompetitive Kinetics to the Reductive Dechlorination of Chlorinated Ethenes. *Biotechnol. Bioeng.* **57**, 751-755.

Gerritse, J., Renard, V., Pedro-Gomes, T. M., Lawson, P. A., Collins, M. D., and Gottschal, J. C. (1996) *Desulfitobacterium* sp. strain PCE1, an Anaerobic Bacterium that can Grow by Reductive Dechlorination of Tetrachloroethene or *ortho*-Chlorinated Phenols. *Arch. Microbiol.* **165**, 132-140.

Gibson, S. A. and Sewell, G. W. (1992). Stimulation of Reductive Dechlorination of Tetrachloroethene in Anaerobic Aquifer Microcosms by Addition of Short-Chain Organic or Alcohols. *Appl. Environ. Microbiol.* **58**, 1392-1393.

Gibson, S. A., Roberson, D. S., Russell, H. H., and Sewell, G. W. (1994). Effects of Three Concentrations of Mixed Fatty Acids on Dechlorination of Tetrachloroethene in Aquifer Microcosms. *Environ. Toxicol. Chem.* **13**, 453-460.

Gossett, J. M. (1987) Measurement of Henry's Law Constants for C1 and C2 Chlorinated Hydrocarbons. *Environ. Sci. Technol.* **21** (2), 202 - 208.

Harkness, M. R., Bracco, A. A., Brennan, Jr., M. J., Deweerd, K. A., and Spivack, J. L. (1999) Use of Bioaugmentation To Stimulate Complete Reductive Dechlorination of Trichloroethene in Dover Soil Columns. *Environ. Sci. Technol.* **33** (7), 1100 - 1109.

Haston, Z. and McCarty, P. L. (1997) In *In situ and On-Site Bioremediation, Battelle Press*, Columbus, OH, **4** (3), 31.

Haston, Z. C. (1999). Factors Affecting Growth and Utilization in the Anaerobic Dehalogenation of Chlorinated Ethenes. Ph.D. Dissertation, Stanford University, Standord, CA.

Haston, Z. C. and McCarty, P. L. (1999) Chlorinated Ethene Half-Velocity Coefficients (K_S) for Reductive Dehalogenation. *Environ. Sci. Technol.* **33** (2), 223-226.

He, J., Ritalahti, K. M., Aiello, M. R., and Löffler, F. E. (2003b) Complete Detoxification of Vinyl Chloride by an Anaerobic Enrichment Culture and

Identification of the Reductively Dechlorinating Population as a *Dehalococcoides* Species. *Appl. Environ. Microbiol* **69** (2), 996-1003.

He, J., Ritalahti, K. M., Yang, K.-L., Koenigsberg, S. S., and Löffler, F. E. (2003a) Detoxification of Vinyl Chloride to Ethene Coupled to Growth of an Anaerobic Bacterium. *Nature* **424**, 62-65.

Hendrickson, E. R., Payne, J. A., Young, R. M., Starr, M. G., Perry, M. P., Fahnestock, S., Ellis, D. E., and Ebersole, R. C. (2002) Molecular Analysis of Dehalococcoides 16S Ribosomal DNA form Chloroethene-Contaminated Sites through North America and Europe. *Appl. Environ. Microbiol.* **68**, 485-495.

Holliger, C., Hahn, D., Harmsen, H., Ludwig, W., Schumacher, W., Tindall, B., Vazquez, F., Weiss, N., and Zehnder, A. J. B. (1998) *Dehalobacter restrictus* gen. nov. and sp. nov., a Strictly Anaerobic Bacterium that Reductively Dechlorinates Tetra- and Trichloroethene in Anaerobic Respiration. *Arch. Microbiol.* **169**, 313-321.

Keeling, M. T. (1998) Bench-Scale Study for the Bioremediation of Chlorinated Ethylenes at Point Mugu Naval Air Weapons Station, Point Mugu California, IRP Site 24. Master's Thesis, Oregon State University.

Lee, M. D., Odom, J. M., and Buchanan, Jr., R. J. (1998) New Perspectives on Microbial Dehalogenation of Chlorinated Solvents: Insights from the Field. *Annu. Rev. Microbiol.* **52**, 423-452.

Liang, L.-N. and Grbic-Galic, D. (1993). Biotransformation of Chlorinated Aliphatic Solvents in the Presence of Aromatic Compounds under Methanogenic Conditions. *Environ. Toxicol. Chem.* **12**, 1377-1393.

Liu, D., Pacepavicious, G. J., Maguire, R. J., Lau, Y. L., Okamura, H., and Aoyama, I. (1999) Mercuric Chloride-Catalyzed Hydrolysis of the New Antifouling Compound Irgarol 1051. *Wat. Res.* **33**, 155~163.

Löffler, F. E., Cole, J. R., Ritalahti, K. M., and Tiedje, J. M. (2003) In *Dehalogenation: Microbial Processes and Environmental Applications*, Ed. Hägbblom, M. M. and Bossert, I. D., Kluwer Academic, New York, NY., pp. 53-87.

Löffler, F. E., Sun, Q., Li, J., and Tiedje, J. M. (2000) Microbial Ecology - 16S rRNA Gene-Based Detection of Tetrachloroethene-Dechlorinating Desulfuromonas and Dehalococcoides Species. *Appl. Environ. Microbiol.* **66** (4), 1369-1374.

Löffler, F. E., Tiedje, J. M. and Sanford, R. A. (1999). Fraction of Electrons Consumed in Electron Acceptor Reduction and Hydrogen Thresholds as Indicators of Halorespiratory Physiology. *Appl. Environ. Microbiol.* **65**, 4049-4056.

Lovely, D. R. (1985) Minimum Threshold for Hydrogen Metabolism in Methanogenic Bacteria. *Appl. Environ. Microbiol.* **49**, 1530-1531.

Lovely, D. R. and Goodwin, S. (1988) Hydrogen Concentrations as an Indicator of the Predominant Terminal Electron-Accepting Reactions in Aquatic Sediments. *Geochim. Cosmochim. Acta.* **52**, 2993-3003.

Lyon, W. G., West, C. C., Osborn, M. L., and Sewell, G. W. (1995) Microbial Utilization of Vadose Zone Organic Carbon for Reductive Dechlorination of Tetrachloroethene. *J. Environ. Sci. Health., Part A* **A30**, 1627-1639.

Mackay, D. M. and Cherry, J. A. (1989) Groundwater Contamination: Pump-and-Treat Remediation. *Environ. Sci. Technol.* **23**, 630-636.

Madigan, M. T., Martinko, J. M., and Parker. J. (1997) *Brock, Biology of Microorganisms*. Eight Edition, Prentice Hall, Upper Saddle River, NJ.

Maillard, J., Schumacher, W., Vazquez, F., Regeard, C., Hagen, W. R., and Hollinger, C. (2003) Characterization of the Corrinoid Iron-Sulfur Protein Tetrachloroethene Reductive Dehalogenase of *Dehalobacter restrictus*. *Appl. Environ. Microbiol.* **69** (8), 4628-4638.

Major, D. M., McMaster, M. L., Cox, E. E., Edwards, E. A., Dworatzek, S. M., Hendrickson, E. R., Starr, M. G., Payne, J. A., and Buonamici, L. W. (2002) Field Demonstration of Successful Bioaugmentation To Achieve Dechlorination of Tetrachloroethene To Ethene. *Environ. Sci. Technol.* **36**, 5106-5116.

Maymo-Gatell, X., Anguish, T., Gossett, J. M., and Zinder, S. H. (1999) Reductive Dechlorination of Chlorinated Ethene and 1,2-Dichloroethane by "*Dehalococcoides Ethenogenes*" 195. *Appl. Environ. Microbiol.* **65**, 3108-3113.

Maymo-Gatell, X., Chien, Y.-T., Gossett, J. M., and Zinder, S. H. (1997) Isolation of Bacterium that Reductively Dechlorinates Tetrachloroethene to Ethene. *Science* **276**, 1568-1571.

Maymo-Gatell, X., Nijenhuis, I., and Zinder, S. H. (2001) Reductive Dechlorination of *cis*-1,2-Dichloroethene and Vinyl Chloride by "*Dehalococcoides ethenogenes*" *Environ. Sci. Technol.* **35** (3), 516-521.

Maymo-Gatell, X., Tandoi, V., Gossett, J. M., and Zinder, S. H. (1995) Characterization of an H_2-Utilizing Enrichment Culture that Reductively Dechlorinates Tetrachloroethene to Vinyl Chloride and Ethene in the Absence of Methanogenesis and Acetogenesis. *Appl. Environ. Microbiol.* **61**, 3928-3933.

McCarty, P. L. (1997) Breathing with Chlorinated Solvents. *Science* **276**, 1521-1522.

National Research Council (1994) *Alternatives for Ground Water Cleanup*, National Academy Press, Washington DC.

Nielsen, R. B. and Keasling J. D. (1999) Reductive Dechlorination of Chlorinated Ethene DNAPLs by a Culture Enriched from Contaminated Groundwater. *Biotechnol. Bioeng.* **62**, 160~165.

Odom, J. M., Tabinowski, J. A., Lee, M. D., and Fathepure, B. Z. (1995) Anaerobic Biodegradation of Chlorinated Solvents: Comparative Laboratory Study of Aquifer Microcosms, In *Bioremediation of Chlorinated Solvents*, Ed. Hinchee, R. E., Leeson, A. and Semprini, L., Battelle Press, Columbus, OH., **1**, 17-24.

Perry, R. H., Green, D. W., and Maloney, J. O. (1997) *Perry's Chemical Engineers Handbook*, 7th Ed., McGraw-Hill, New York, NY.

Pon, G., Hyman, M. R., and Semprini, L. (2003) Acetylene Inhibition of Trichloroethene and Vinyl Chloride Reductive Dechlorination Environ. *Sci. Technol.* **37** (14), 3181-3188.

Rittmann, B. E., Seagren, E., and Wrenn, B. A. (1994). In *In-Situ Bioremediation*. Second Edition. Parkridge, New Jersey, USA: Noyes Publications, pp. 37-50.

Rogers, J. B., DuTeau, N. M., and Reardon, K. F. (2000) Use of 16S-rRNA to Investigate Microbial Population Dynamics During Biodegradation of Toluene and Phenol by a Binary Culture *Biotechnol. Bioeng.* **70**, 436-445.

Rosner, B. M., McCarty, P. L., and Spormann, A. M. (1997) In Vitro Studies on Reductive Vinyl Chloride Dehalogenation by an Anaerobic Mixed Culture. *Appl. Environ. Microbiol.* **63** (11), 4139-4144.

Sawyer, C. N., McCarty, P. L., and Parkin, G. F. (1994) *Chemistry for Environmental Engineering*, 4th ed. McGraw-Hill, New York.

Scholz-Muramatsu, H., Neumann, A., Messmer, M., Moore, E., and Diekert, G. (1995) Isolation and Characterization of *Dehalospirillum multivorans* gen. nov., sp. nov., a Tetrachloroethene-Utilizing, Strictly Anaerobic Bacterium. *Arch. Microbiol.* **163**, 48-56.

Scholz-Muramatsu, H., Szewzyk, R., Szewzyk, U., and Gaiser, S. (1990). Tetrachloroethylene as Electron Acceptor for the Anaerobic Degradation of Benzoate. *FEMS Microbiol. Lett.* **66**, 81-86.

Seagren, E. A., Rittmann, B. E., and Valocchi, A. J. (1994) Quantitative Evaluation of the Enhancement of NAPL-Pool Dissolution by Flushing and Biodegradation. *Environ. Sci. Technol.* **28**, 833-839.

Sewell, G. W. and Gibson, S. A. (1991). Stimulation of the Reductive Dechlorination of Tetrachloroethene in Anaerobic Aquifer Microcosms by the Addition of Toluene. *Environ. Sci. Technol.* **25**, 982-984.

Sharma, P. K. and McCarty, P. L. (1996) Isolation and Characterization of a Facultatively Aerobic Bacterium That Reductively Dehalogenates Tetrachloroethene to cis-1,2-Dichlorroethene. *Appl. Eniron. Microbiol.* **62** (3), 761 – 765.

Smatlak, C. R., Gossett, J. M., and Zinder, S. H. (1996) Comparative Kinetics of Hydrogen Utilization for Reductive Dechlorination of Tetrachloroethene and Methanogenesis in an Anaerobic Enrichment Culture. *Environ. Sci. Technol.* **30**, 2850-2858.

Tandoi, V., DiStefano, T. D., Bowser, P. A., Gossett, J. M., and Zinder, S. H. (1994) Reductive Dehalogenation of Chlorinated Ethenes and Halogenated Ethanes by a High-Rate Anaerobic Enrichment Culture. *Environ. Sci. Technol.* **28**, 973-979.

Thauer, R. K., Jungermann, K., and Decker K. (1977) Energy Conservation in Chemotrophic Anaerobic Bacteria. *Bacteriol. Rev.* **41**, 100~180.

Tonnaer H., Alphenaar, A., Wit, H. D., Grutters, M., Spuij, F., Gerritse, J., and Gottschal, J. C. (1997) Modelling of Anaerobic Dechlorination of Chloroethenes for In-Situ Bioremediation. In *In Situ and On-Site Bioremediation*, papers from the Fourth International In Situ and On-Site Bioremediation Symposium, Ed. Alleman, B. C. and Leeson, A., Battelle Press, Columbus, OH, pp. 591-596.

U.S. EPA (2003) Consumer Factsheet on: Trichlorpethylene. U.S. Environmental Protection Agency, http://www.epa.gov/safewater/dwh/c-voc/trichlor.html.

Uberoi, V. and Bhattacharya, S. K. (1995) Interactions among sulfate reducers, acetogens, and methanogens in anaerobic propionate systems. *Water Environ. Res.* **67**, 330-339.

Vancheeswaran, S. (1998) Abiotic and Biological Transformation of TBOS and TKEBS, and Their Role in the Biological Transformation of TCE and *c*-DCE. Master's Thesis, Oregon State University.

Vancheeswaran, S., Halden, R. U., Williamson, K. J., Ingle, J. D., and Semprini, L. (1999) Abiotic and Biological Transformation of Tetraalkoxysilanes and Trichloroethene/cis-Dichloroethene Cometabolism Driven by Tetrabutoxysilane-Degrading Microorganisms. *Environ. Sci. Technol.* **33**, 1077-1085.

Vancheeswaran, S., Semprini, L., Pon, G., Williamson, K. J., Ingle, J. D., and Daley, P. (1998) Anaerobic Transformation of TCE Driven by Organo-Silicon Compounds. In *1st International Conference on Remediation of Chlorinated and Recalcitrant Compounds*, Monterey, CA, pp. 57~62.

Vancheeswaran, S., Yu, S., Daley, P., Halden, R. U., Williamson, K. J., Ingle, J. D., and Semprini, L. (2003) Intrinsic Remediation of Trichloroethene Driven by Tetraalkoxysilanes as Co-contaminants: Results of Microcosm and Field Studies. *Remediation.* **13**, 7-25.

Vogel, T. M and McCarty, P. L. (1985). Biotransformation of Tetrachloroethylene to Trichloroethylene, Dichloroethylene, Vinyl Chloride, and Carbon Dioxide under Methanogenic Conditions. *Appl. Environ. Microbiol.* **49**, 1080-1083.

Vogel, T. M., Criddle, C. S., and McCarty, P. L. (1987). Transformations of Halogenated Aliphatic Compounds. *Environ. Sci. Technol.* **21**, 722-736.

Westrick, J. J., Mello, J. W., and Thomas, R. F. (1984) The Groundwater Supply Survey. *J. Am. Water Works Assoc.* **76**, 52~59.

Wiedemeier, T. and Henry, B. M. (2001) Technical Protocol for Enhanced Reductive Dechlorination via Vegetable Oil Injection. Platform Presentation, In *Sixth In Situ and On-Site Bioremediation Symposium*, San Diego, CA.

Xu, S. (1998) Hydrolysis of Poly(dimethylsiloxanes) on Clay Minerals as Influenced by Exchangeable Cations and Moisture. *Envron. Sci. Technol.* **32**, 3162~3168.

Yang, Y. and McCarty, P. L. (1998) Competition for Hydrogen within a Chlorinated Solvent Dehalogenating Anaerobic Mixed Culture. *Environ. Sci. Technol.* **32**, 3591~3597.

Yang, Y. and McCarty, P. L. (2000a) Biomass, Oleate, and Other Possible Substrates for Chloroethene Reductive Dehalogenation. *Biorem. J.* **4**, 125-133.

Yang, Y. and McCarty, P. L. (2000b) Biologically Enhanced Dissolution of Tetrachloroethene DNAPL. *Environ. Sci. Technol.* **34**, 2979-2984.

Yang, Y. and McCarty, P. L. (2002). Comparison between Donor Substrate for Biologically Enhanced Tetrachloroethene DNAPL Dissolution. *Environ. Sci. Technol.* **36**, 3400-3404.

Yaws, C. L. (1999) *Chemical properties handbook: physical, thermodynamics, environmental, transport, safety, and health related properties for organic and inorganic chemicals*. McGraw-Hill: New York, NY.

Yu, S and Semprini, L. (2001) Oregon State University, unpublished results.

Yu, S, Dolan, M. E., and Semprini, L. (2003) Unpublished data.

Yu, S. and Semprini, L. (2002a) Dechlorination of PCE DNAPL with TBOS Using a Binary Mixed Culture. In *Remediation of Chlorinated and Recalcitrant Compounds*, Battelle Press, Columbus, OH, Paper 2B-49.

Yu, S. and Semprini, L. (2002b). Comparison of Trichloroethylene Reductive Dehalogenation by Microbial Communities Stimulated on Silicon-Based Organic Compounds as Slow-Release Anaerobic Substrate. *Water Res.* **36**, 4985-4996.

Yu, S.and Semprini, L. (2003) Kinetic Studies and Comparisons for Reductive Dechlorination by a Binary Mixed Culture. Platform presentation, In: In Situ and On-Site Bioremediation: from the Seventh International Symposium, Orlando, FL.

Zehnder, A. J. B. and Stumm, W. (1988) Geochemistry and Biogeochemistry of Anaerobic Habitats. In *Biology of Anaerobic Microorganism*. Ed. Zehnder, A. J. B. John Wiley & Sons, Inc., pp. 29-33.