AN ABSTRACT OF THE THESIS OF

Incheol Jonathan Pang for the degree of Master of Science in Civil Engineering presented on September 25, 2000. Title: Microcosm Study of Enhanced Biotransformation of Vinyl Chloride to Ethylene with TCE Additions Under Anaerobic Conditions from Point Mugu, California.

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This microcosm study demonstrated the enhanced anaerobic transformation of vinyl chloride (VC) to ethylene. A previous microcosm study from Point Mugu site showed the accumulation of VC due to the slow transformation step of VC to ethylene. To overcome the rate-limiting step, two laboratory experiments tested the effect of trichloroethylene (TCE) additions on the rate enhancement, repeated low TCE additions and high TCE concentration additions.

TCE (2 μmol) was repeatedly added over a two week interval. In a parallel study, an equal amount of VC was added to another set of microcosms. TCE addition increased VC transformation to ethylene, with nearly 19% VC conversion to ethylene compared to 4% VC conversion in the VC added controls. However, the increased VC transformation rates were not sufficient enough to avoid VC accumulation. Rate of VC transformation decreased once TCE addition was

stopped. This indicated the mixed culture required the transformation of TCE to maintain VC transformation rates.

With TCE added at high concentrations (100 mg/L and 200 mg/L), nearly complete transformation of TCE to ethylene was observed. After the addition of high TCE concentrations, low concentration TCE (3 μmol) was added and near 95% transformed to ethylene in 45 days. Two different low hydrogen yielding substrates, butyrate and propionate, were tested. Both were equally effective in promoting TCE dechlorination. Methanogenesis was inhibited at high TCE concentration with both substrates. Kinetic analysis of VC transformation data showed VC transformation followed the first order kinetics with respect to concentrations using a modified Monod equation. First-order kinetic constants increased after the addition of high TCE concentrations. After 200 mg/L of TCE addition, the first-order kinetic constant increased by factor of six compared to the rate obtained from the earlier low TCE concentration addition. However, reintroduction of TCE at low concentration maintained similar enhanced kinetic constants, as achieved at high concentration. This indicated the enhancement of VC transformation to ethylene was likely due to the growth of microorganisms using TCE as a terminal electron acceptor. These microorganisms were likely responsible for the transformation of VC to ethylene.

Microcosm Study of Enhanced Biotransformation of Vinyl Chloride to Ethylene with TCE Additions Under Anaerobic Conditions from Point Mugu, California.

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Microcosm Study of Enhanced Biotransformation of Vinyl Chloride to Ethylene with TCE Additions Under Anaerobic Conditions from Point Mugu, California.

CHAPTER 1
INTRODUCTION

1.1 TCE and Its Contamination in Environment

Trichloroethylene (TCE) has been used as a chlorinated solvent since the 1940s. TCE applications continued for several decades until the 1970s. TCE has been utilized as a splendid degreaser in dry-cleaning operations, an extraction agent in decaffeinating coffee, and even as a general anesthetic in medical communities (Vogel, 1987 & Schaumburg, 1989). This wide range of applications increased the demand of TCE, and during those years the average production was over 200 million pounds per year (Vogel et al., 1987). However, the usage of TCE has been tremendously restricted after a study from the National Cancer Institute concluded that TCE was a suspected human carcinogen. The US Environmental Protection Agency included TCE on the list of hazardous substance in 1976 (Schaumburg, 1989).

Due to no federal, state or local laws and regulations on handling, storing and disposal, TCE had been discharged to the surface waters and groundwater through industrial, military and used by general public. Military installations practiced direct disposal of TCE onto the land, which caused major groundwater

contaminations at military installations. Chlorinated solvents, such as TCE, also penetrated into the subsurface environment through landfill leachates, leakage from underground storage tanks as well as transporting purpose piping systems (Westrick et al., 1984).

TCE was first detected in a groundwater aquifer in Sacramento, California in 1979 (Munter and DeVries, 1987). The investigation revealed that the aquifer had been contaminated with TCE for number of years. This study indicated the fate of TCE in the subsurface environment. TCE was found to be generally recalcitrance to biodegradation under aerobic conditions. TCE forms a dense non-aqueous liquid phase (DNAPL). Because TCE has relatively high water solubility (1100 mg/L), this regulated chlorinated compound is highly mobile in soils and aquifer materials (US EPA, 1985).

The public was alarmed by carcinogenic nature of TCE and the presence of its anaerobic transformation products including the isomers of dichloroethylene (DCE) and vinyl chloride (VC), in the groundwater, which much of US water supplies rely on (Schaumburg, 1989). TCE is among 14 volatile organic compounds regulated under the Safe Drinking Water Act Amendments of 1986 with the maximum contaminants level (MCL) of 5 μg/L (Sayre, 1988).

Physical and chemical treatment processes, such as conventional pump and treat with air stripping and carbon adsorption were the most frequently used remediation method for TCE clean up. Unfortunately, these treatment processes were only able to transfer the chlorinated compounds from one phase of

environment to another without destroying them (Cartwright, 1991). Biological treatment processes received attention due to the fact of converting the chlorinated contaminants to environmentally acceptable ethylene is a better alternative, instead of physically transferring TCE from one phase to another.

Since poly chlorinated solvents such as PCE and TCE are highly oxidized nature, biological transformation as electron donor is rare (Vogel et al., 1987). This recalcitrant nature of PCE and TCE allows them to be transformed under mostly anoxic conditions in subsurface. A field study reported biotransformation of PCE to TCE under anaerobic conditions (Roberts et al., 1982). Number of other column studies (Vogel and McCarty, 1985) and microcosm studies (Parsons et al., 1984; Wilson et al., 1986) observed biological transformation of PCE and TCE by sequential removal of chloride under anaerobic conditions. Most these studies showed incomplete reductive dechlorination of PCE and TCE under anaerobic conditions with accumulation of equal or more toxic compounds.

In an effort to investigate the enhancement of VC transformation to ethylene, Freedman and Gossett (1989) reported a long-term study of PCE transformation to ethylene with PCE additions. DiStefano (1999) reported that presence of PCE is required to maintain VC transformation to ethylene. The anaerobic reductive dechlorination of TCE was also studied here to determine if VC transformation could be enhanced.

1.2 Site Overview

This study was conducted with soil and groundwater samples from Point Mugu Naval Air Weapon Station, CA, that has experienced groundwater contamination with chlorinated solvents and petroleum hydrocarbons. This military facility is located along the California coastline between Ventura and the Santa Monica Mountains. The facility was used for testing and evaluating of weapon systems by US Navy.

At the site, chlorinated solvents were released from underground storage tanks (UST) and piping systems. According to a report from OHM Remediation Services, two adjacent USTs had been installed in western area of the facility designed Sites 23 and 55 in the Installation Restoration Program (IRP). A 23,550 gallon of concrete oil/water separator from Site 23 was installed in 1970 and replaced in 1989. A second UST from Site 55, a 500 gallon of steel tank for waste etching and cleaning solutions from circuit board etching and washing operations, was used from the late 1950s to the early 1960s. OHM Remediation Services reported detection of tetrachloroethylene (PCE), trichloroethylene (TCE), isomers of DCEs (1,1-DCE and 1,2-DCE), vinyl chloride (VC), isomers of dichloroethane (1,1-DCA and 1,2-DCA), isomers of trichloroethane (1,1,1-TCA and 1,1,2-TCA), chloroform (CF), carbon tetrachloride (CT), BTEX (benzene, toluene, ethylbenzene, and xylenes), and total recoverable petroleum hydrocarbons in the groundwater at the two sites. Although their contamination levels varied, all the

chlorinated solvents detected from the groundwater investigation were well above the maximum contamination levels (MCLs).

The site has a complex subsurface hydrogeology with a six-layer sedimentary aquifer system. This results from a complex deposition environment of fluvial, alluvial, dune, and shoreline tidal deposition from the Oxnard Plain and Mugu Lagoon. Groundwater contamination has been reported in the upper part of a semi-perched aquifer of 5 to 30 feet below the ground surface. Site investigation revealed that the groundwater has high total dissolved solids (500 to 60,000 mg/L), has substantial amount of sulfate concentrations (34 to 5,500 mg/L), and low dissolved oxygen (0.1 to 3.7 mg/L), due to coastal influences and seawater penetration.

The site investigation by OHM also reported that there were signs of possible intrinsic remediation with TCE anaerobic transformation products (DCEs and VC) being detected, which had never been used in the history of the facility.

1.3 Previous Study

An initial microcosm study was conducted with site-specific groundwater and aquifer soilds to investigate the feasibility of stimulating indigenous microorganisms to biotransformation of TCE and its dechlorinated products to harmless ethylene (Keeling, 1999). The study focused on stimulation of anaerobic reductive dechlorination of TCE with three different potential substrates, lactate,

benzoate, and methanol. From this 302 day study, both lactate and benzoate served as anaerobic substrates to support sulfate reduction, followed by partial TCE dechlorination to cis-DCE, VC and ethylene. Similar TCE dechlorination, results were achieved from both substrates. TCE was transformed to VC and ethylene. VC accumulated in the microcosms due to the slow rate of VC transformation to ethylene. The addition of TCE into both substrates amended microcosms appeared to enhance the rate of transformation of TCE to VC. The microcosms amended with trace nutrient and substrate showed slightly better performance in TCE dechlorination than those only supplemented with substrates.

Based on the results of this previous microcosm study, lactate was injected into the subsurface at the site to stimulate the biological transformation of TCE under anaerobic conditions. Lactate was selected and applied in the field application due to the operational convenience of handling a syrup form of lactate at the site. A similar pattern resulted from the field demonstration with accumulation of VC in subsurface because of the slow transformation of VC to ethylene. The research project on the anaerobic microcosm study was extended to investigate the enhancement of biological reductive dechlorination of VC to ethylene.

1.4 Objectives

The current microcosm study focused on the stimulation of dechlorinating microorganisms with repeated additions of TCE to enhance VC transformation to ethylene. In the previous study (Keeling, 1999), TCE transformation to VC was substantially faster than the dechlorinating step of VC to ethylene. With array of microcosms, this study focused on two main issues related to biological transformation of TCE under anaerobic conditions. The main hypothesis of entire experiment is that TCE additions support enhancement of both TCE and VC transformation rates by either stimulating enzymatic activity or supporting microbial population growth as an electron acceptor for the reductive dechlorination process.

1. The effect of the stepwise TCE addition was studied as a mean of enhancing the anaerobic transformation of VC to ethylene. The previous microcosm study concluded that VC concentration might have effect on its dechlorination to harmless ethylene, and that TCE transformation might enhance the rate of VC transformation. To test the hypothesis, equal amounts of TCE and VC were added to different microcosms and the rates of VC dechlorination to ethylene were determined. The effects of VC concentration of and the potential stimulatory effect of TCE on VC dechlorination rates were compared.
2. The effect of high TCE concentration additions (from 30 mg/L to 200 mg/L), into nutrient amended microcosms was tested to study the change of

VC dechlorination rate and the inhibition of methanogenesis. In the high TCE concentration experiments, the microcosms were amended with two different substrates, propionate and butyrate. The differences between these two electron donors were also compared in terms of complete TCE transformation to ethylene. Repeated additions of high TCE concentrations into the microcosms attempted to evaluate the stimulatory role of TCE addition and/or microbial population growth.

CHAPTER 2
LITERATURE REVIEW

2.1 Microbial Transformation of CAHs

Microorganisms break down of organic compounds for carbon and energy through complex series of coupled oxidation-reduction reactions is termed catabolism. In this process, electrons are removed and added along the pathway. The energy released in these reactions is conserved in the form of the high-energy phosphate bond of adenosine triphosphate (ATP) for using in number of different biochemical processes including biosynthetic reactions (Baker and Herson, 1994). The biochemical processes central to this process can be distinguished into two different classes: aerobic and anaerobic respiration. These groups of reactions can be differentiated by different terminal electron acceptors. For aerobic respiration, the terminal electron acceptor is molecular oxygen. Oxidized inorganic or organic compounds are used as electron acceptors for the case of anaerobic respiration (Cookson, 1995) (Table 2.1).

Under anaerobic respiration, a special case called fermentation can be sub classified where organic compounds serve both as electron donors and acceptors. Since the final oxidation potential must be identical with the initial oxidation potential of the substrate, the mixture of end products, some of which more oxidized and some more reduced normally include acids, alcohols, ketones and gases (Madigan et al., 1997).

Table 2.1 Microbial metabolisms and their electron acceptors (Cookson, 1995)

Mode	Electron Acceptors
Aerobic Respiration	Oxygen (O_2)
Anaerobic Respiration	
1. Denitrification	Nitrate (NO_3^-)
2. Nitrate reduction	Nitrate (NO3-)
3. Sulfate reduction	Sulfate (SO_4^{2-})
4. Ferric iron reduction	Iron (Fe^{3+})
5.Organic respiration	Fumerate

Another very important mode of metabolism in biological remediation is cometabolism. Cometabolism, or co-oxidation, is defined as the degradation of a compound only in the presence of other organic material that serves as the primary energy source (McCarty, 1987). For example, microorganisms, which are not capable of growing on chlorinated aliphatic hydrocarbons, such as TCE and TCA, may be able to carry out limited transformation of these CAHs in the presence of non-chlorinated compounds as primary carbon and energy sources (Cookson, 1995, Vogel, 1987). This metabolic mechanism can be explained as competition for degradative enzyme between CAHs and the primary substrate. Cometabolic reactions are particularly important in the transformation and degradation of CAHs.

2.2 Aerobic Metabolism

With molecular oxygen as terminal electron acceptor, one to three atoms substituted chlorinated compounds can be transformed by three different types of

enzymes: oxygenase, dehalogenases, and hydrolytic dehalogenases (Semprini el at, 1992). With oxygenase enzymes the transformation products are alcohols, aldehydes, or epoxides. Dehalogenase transformation products are an aldehyde and glutathione. Hydrolytic dehalogenases will hydrolyze the aliphatic halogenated compounds to produce alcohols.

Chlorinated compounds with two carbons were considered as non-biodegradable until the early 1980s. Chlorinated ethylenes such as tetrachloroethylene (PCE) and trichloroethylene (TCE) were found anaerobically degradable (Bouwer et al., 1981). Later, a number of different studies showed that TCE could be transformed under aerobic condition via cometabolism (Wilson et al., 1985; Fogel et al., 1986). Wilson and Wilson (1985) found that TCE could be degraded aerobically to carbon dioxide in a soil column fed mixture of natural gas and air (0.6%). In their soil column test, less than 5% of the initially fed TCE passed through the soil column. These experiments led researchers to focus on the degradation of chlorinated ethylenes via aerobic cometabolism. Under aerobic metabolism supported conditions, some CAHs are degraded via cometabolic processes. These aerobic microorganisms produce oxygenase enzymes with broad substrate specificity that can initiate the oxidation of CAHs. Little et al. (1988) successfully isolated a methanotrophic bacterium. That bacterium degraded TCE. Besides methanotrophs, microorganisms that can degrade TCE when grown on phenol or toluene have been identified as *Pseudomonas* (*G-4*) (Nelson et al., 1986).

The initial step of TCE oxidation produces an epoxide mediated by a monooxygenase or dioxygenase enzyme. Because the epoxide is unstable, it further transformations to produce acids (Figure 2.1). These end products under different pH conditions are further metabolized by different microorganisms (Vogel et al., 1987).

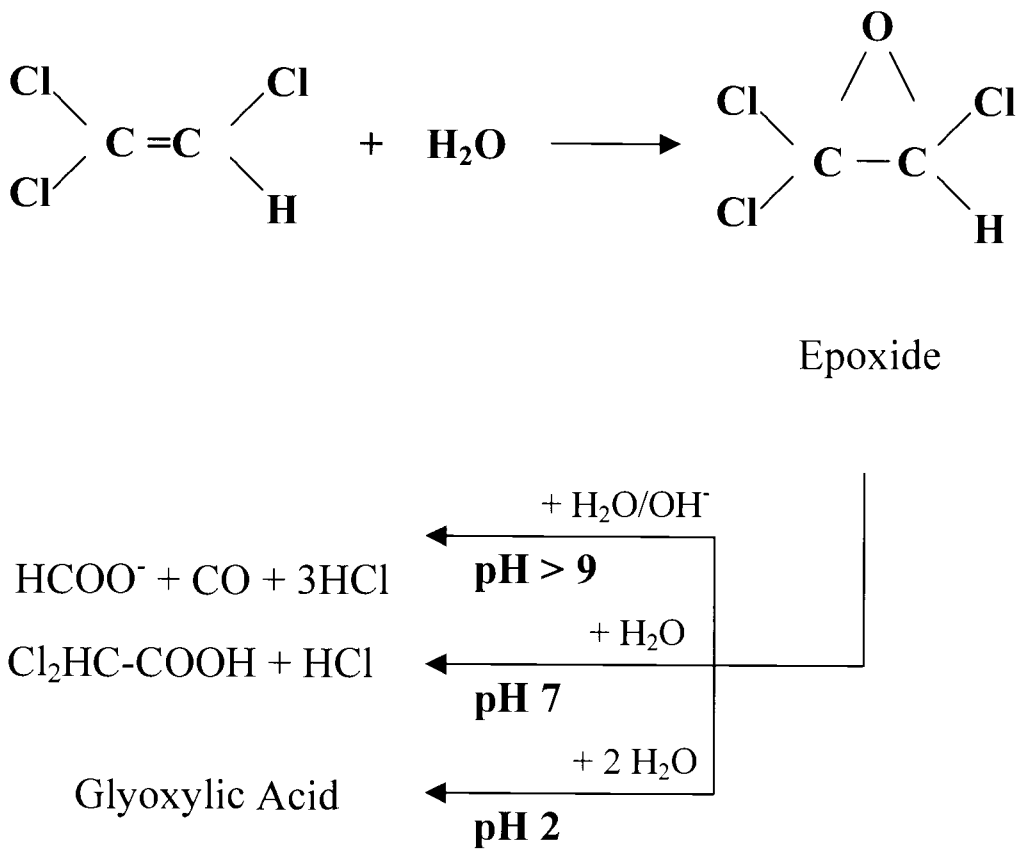

Figure 2.1 Transformation of TCE under aerobic conditions (Vogel, et al., 1987)

Vannelli et al. (1990) have reported isolation of an ammonia oxidizing microorganism, *Nitrosomonas europaea* that can transform chlorinated aliphatic compounds. A study by Ensign et al. (1992) found that *Xanthobacter* when grown on propylene degraded TCE, isomers of DCE, VC, and dichloropropylenes, but were unable to degrade PCE. Semprini et al. (1994) in a field study demonstrated TCE transformation with both methane and phenol utilizers. In general, the aerobic transformation of CAHs decreases as number of chlorine atoms on the compounds increase (Vogel, el at, 1987). Thermodynamically, polychlorinated compounds with higher oxidation state are less susceptible to oxidation than less chlorinated compounds with a lower oxidation state. Therefore, PCE and TCE are generally more difficult to transform under aerobic system than less chlorinated ethylenes, such as vinyl chloride.

2.3 Anaerobic Respiration

Since polychlorinated PCE and TCE are recalcitrant to aerobic degradation due to their higher oxidation state, anaerobic reductive chlorination has great potential for transforming these highly chlorinated compounds. In the reductive dechlorination of TCE, chlorine atoms are sequentially replaced with hydrogen to produce isomers of DCE, VC, and ethylene (Figure 2.2).

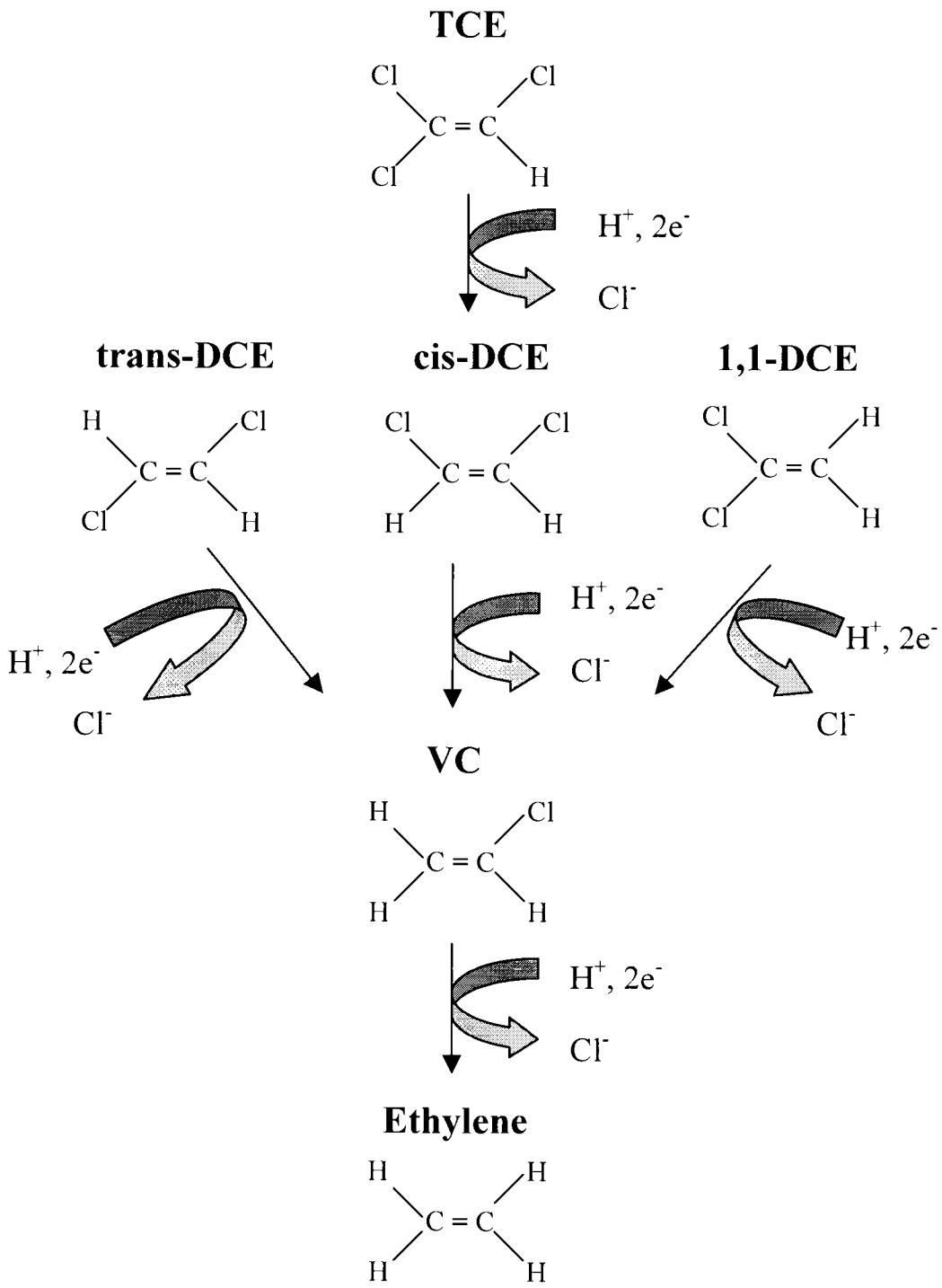

Figure 2.2 Reductive dechlorination of TCE pathway (Vogel et al., 1987)

From the reductive dechlorination process, the chlorinated ethylenes are used as electron acceptors. Organic compounds or hydrogen are used as electron donors.

The organic substrate is a key factor in determining the rate of dechlorination under anaerobic conditions (Cookson, 1995). The understanding of redox conditions and the availability of certain electron donors and acceptors in the environment are very important to control the bioremediation of targeted chlorinated compounds (Baker and Herson, 1994). Since different available electron acceptors compete with chlorinate compounds for available electrons, their presence affects the rates of reductive dechlorination. For instance, the presence of sulfate can inhibit the dechlorination of TCE due to thermodynamically favorable reduction of sulfate over the reduction of TCE. Sulfide a product of sulfate reduction can be toxic to some microorganisms, such as methanogens (Li et al, 1996). Because many groundwaters contain a significant concentration of sulfate, there is concern as to whether PCE and TCE transformation can take place in sulfate-reducing environments. Bouwer and Wright (1988) showed that only 15% of initial amount of PCE was removed under sulfate-reducing conditions in their continuous-flow column test. However, Bagley and Gossett (1990) reported that their sulfate-reducing enrichment culture when using lactate as primary substrate could transform PCE to TCE and cis-DCE. They also showed that sulfate-reducing enrichment culture inhibited with fluoroacetate degraded PCE with overall transformation performance of up to 92%, with low residual acetate and low

methane production accounting for only 2% of the electron equivalents measured. Based on their results, Bagley and Gossett (1990) suspected another class of organisms was responsible for the dechlorination instead of methanogens.

Methanogens are known to reduce PCE and TCE when fed different primary substrates, such as lactate, acetate, methanol, and ethanol. (Bouwer and McCarty, 1983: Parsons et al., 1984: Freedman and Gossett, 1989: DiStefano et al., 1991) In the early 1980s, Bouwer and McCarty (1983) reported biotransformation of PCE by a mixed methanogenic culture. Vogel and McCarty (1985) confirmed the quantitative conversion of PCE to TCE, DCE, VC and carbon dioxide by the reductive dechlorination process under methanogenic condition. Fathepure and Boyd (1988) showed that PCE dechlorination was correlated with methanogenesis. Dechlorination stopped when the methane production ceased. All these results supported the importance of methanogenesis on reductive dechlorination, but the biological mechanism remained unclear. Reductive dechlorination of PCE and TCE can result in the accumulation of less chlorinated compounds from incomplete dechlorination (Bouwer and McCarty, 1983: Freedman and Gossett, 1989: Keeling, 1999). Accumulation of VC is of concern because it is a human carcinogenic. The last step of sequential reductive dechlorination, VC to ethylene, is often the rate limiting step resulting the accumulation of VC in environmental systems. However, laboratory studies have shown and confirmed that PCE and TCE can be completely reduced to ethylene without a substantial amount of VC build up. DiStefano et al. (1991) observed over 80% conversion of PCE to ethylene in 2 days

with an anaerobic enrichment culture. de Bruin et al. (1992) reported that complete biological reductive dechlorination of 9μM of PCE to ethylene with less than 5% PCE residual in their fixed bed column with sediment from Rhine River.

In the reductive dechlorination of PCE and TCE, many different compounds can serve as effective electron donors. Some of the electron donors that support anaerobic dechlorination are methanol, ethanol, glucose, benzoate, lactate, formate, acetate, and butyrate (Nielsen and Keasling, 1999: Yang and McCarty, 1998:, Carr and Huges, 1998). Several recent studies have focused on the role of H_2 as the terminal and ultimate electron donor to drive the anaerobic reductive dechlorination process for PCE and TCE. H_2 was believed to be the ultimate electron donor to enhance the reductive dechlorination in organic rich environment undergoing fermentation reactions (Ballapragada et al., 1988). Freedman and Gossett (1989) showed that their methanol enriched culture was able to dechlorinate PCE and TCE using hydrogen as an effective electron donor. A study of PCE dechlorination to ethylene was conducted with methanol and other substrates (e.g., ethanol, lactate, and butyrate), which can be fermented directly to produce H_2 at relatively low hydrogen partial pressures. The maximum H_2 partial pressure obtainable by fermentation of lactate/ethanol and butyrate/propionate are 10^{-3} atm and 10^{-4} atm respectively (Fennell et al., 1997). Methanol supported only methanogenesis with insignificant dechlorination. However, nonmethanogenic substrate fed systems showed the complete dechlorination of PCE. This result suggested the presence of microorganisms responsible only for dechlorination, and the dechlorinator could

use H_2 as an ultimate electron donor at a lower level than methanogens. This indicated that dechlorinators and methanogens compete for the electron donor, H_2 (Smatlak, 1996). Fennell and Gossett (1997) compared those low H_2 yielding substrates' ability to enhance PCE reductive dechlorination. They found that butyrate and propionate amended systems performed better in promoting reductive dechlorination of PCE than lactate/ethanol system, which were fermented at higher H_2 partial pressures. While supporting reductive dechlorination, butyrate and propionate amended systems showed little or the absence of methanogenesis. However, ethanol also supported electron for reductive dechlorination in long-term study of over 120 days (Fennell and Gossett, 1997). Fennell and Gossett (1997) indicated that some portion of ethanol was converted to propionate to support transformation of PCE with low hydrogen partial pressures. The results indicated the importance of selection for electron donor in biological remediation of chlorinated solvents.

2.4 High Concentrations of PCE and TCE Transformations

The study for biological transformations of high concentrations of PCE and TCE has received relatively less attention. TCE with a higher density (1.46 mg/L) than water tends to travel downward forming a separate dense non-aqueous phase liquid (DNAPL). This DNAPL phase can slowly dissolve. High concentrations of TCE contamination can be detected around the source of the DNAPL TCE (Nielsen and Keasling, 1999). DiStefano et al. (1991) initiated an early study of the

anaerobic transformation of high concentrations of chlorinated compounds. The anaerobic transformation of PCE as 550 μM (approximately 55 mg/L concentration) was observed using methanol as substrate. Freedman and Gossett (1989) showed PCE and TCE could be transformed under anaerobic conditions using number of different substrates (methanol, formate, acetate, and glucose). Methanol was found to be the most effective electron donor among the substrates tested. However, they observed the slow transformation of VC to ethylene as the rate limiting step, under methanogenic conditions. In batch experiments, PCE that was repeatedly added immediately transformed to VC. Accumulated VC transformation to ethylene followed after PCE addition was stopped. Even though this methanogenic mixed culture showed its ability to transform PCE to VC and ethylene, the complete transformation of PCE to ethylene was not achieved. DiStefano et al. (1991) studied the transformation of PCE at high concentrations. Using methanol as substrate and with repeated PCE additions. The PCE injection dose had been increased from 25 to 55 μmol. At the initial PCE dose of 0.6 to 12 μmol, two-thirds of the PCE was converted to VC and rest for ethylene with, stoichiometric conversion of methanol to methane. When PCE dose was increased to 25 to 55 μmol, rapid transformation of VC to ethylene was observed with little methanogenesis occurring. PCE transformation to ethylene continued without methanogenesis throughout the course of the study. This indicated high PCE concentration inhibits methanogenesis, which was to be a key process for reductive transformation of PCE. This indicated the possibility of the presence of another

microorganism responsible for PCE dechlorination, instead of methanogens (DiStefano et al., 1991).

Nielson and Keasling (1999) reported the reductive dechlorination of PCE, TCE and VC at water saturated concentrations levels by a TCE enriched culture grown on glucose as substrate. Dechlorination at concentrations at and below the aqueous solubility limit showed PCE transformation followed first-order rates at low concentration and zero-order rate at high concentration. Meanwhile, both TCE and VC transformation rates were first-order with respect to concentration for concentrations up to their solubility limits in water. Assuming the dechlorination rate can be modeled by Monod equation, K_s, and V_{max}/K_s were determined for PCE, TCE and VC based on their experimental data. The Monod equation expressed by

$$\frac{d[S]}{dt} = -V_{max} \frac{[S]}{K_S + [S]}[X]$$

where [S] = concentration of growth-limiting substrate, chlorinated ethylenes for this study
[X] = the concentration of biomass
K_s = half-velocity constant (substrate concentration at one-half of the maximum growth rate)
V_{mas} = maximum rate of substrate utilization per unit mass of microorganism

Table 2.2. Kinetic parameters associated with reductive dechlorination of PCE and TCE under saturated conditions of Nielsen and Keasling (1999).

Substrate	V_{max}	K_S	V_{mas}/K_S
	($nmol*mg^{-1}*h^{-1}$)	(μM)	($nmol*mg^{-1}*h^{-1}*\mu M^{-1}$)
PCE	1150	252	4.56
TCE	-	-	2.24
VC	-	-	1.61

Nielson and Keasling (1999) found that high concentrations of PCE and TCE did not have an inhibitory effect on reductive dechlorination. Methanogenesis was apparently inhibited under both saturated and unsaturated conditions, which coincided with result from DiStefano et al. (1991). The rate of conversion of PCE and TCE to ethylene was substantially higher under saturated conditions (Table 2.3).

Table 2.3 Ethylene production rate under high concentration of PCE and TCE

	Production Rate ($nmol*h^{-}$)	
	Unsaturated	Saturated
PCE	0.12	3.40
TCE	0.16	19.30

2.5 Summary

The current study focused on testing the enhancement of VC transformation with TCE additions and on transformation of high concentrations of TCE with two different low hydrogen yielding substrates, butyrate and propionate. Freedman and Gossett (1989) tested the enhancement of PCE transformation under methanogenic conditions with PCE additions. Distefano et al. (1991) also showed high concentrations of PCE could be transformed in their long term study (195 days). DiStefano (1999) recently reported that PCE presence is necessary to maintain VC transformation with demonstrating decreasing rate of VC transformation to ethylene in the absence of PCE.

Instead of PCE additions, the current microcosm study, however, tested the effect of TCE additions on enhancement of TCE and VC transformation. Nielsen and Keasling (1999) studied on transformation of saturated PCE and TCE transformations, but had never tested on TCE additions under methanogenic conditions. The current study is also being performed in site microcosms containing groundwater and aquifer solids, to determine if enhanced rates can be achieved under conditions more representation of the subsurface.

CHAPTER 3
MATERIALS AND METHODS

3.1 Chemicals and Stock Solutions

TCE (99.9%; Fisher Scientific, Pittsburgh, PA), cis-1,2 dichloroethylene (97%; Acros, Pittsburgh, PA), VC (99.5%; Aldrich Chemical Co., Milwaukee, WI), ethylene (98%; Alltech, Deerfield, IL) and methane (grade 2.0 from Airco, Inc., Albany, OR) were purchased for making GC standard and for additions to the microcosms. Also 1000 ppm VC balanced with nitrogen gas was obtained from Alltech (Deerfield, IL) for use as external standards of gas chromatography calibration of the photo ionization detector (PID) and flame ionization detectors (FID). For organic acids, lactate (sodium lactate) in form of syrup (60% w/w) was obtained from Fisher Scientific Co. (Pittsburgh, PA). The sodium forms of propionate (99%), butyrate (98%), and acetate (99%) were purchased from Aldrich Chemical Co. (Milwaukee, WI).

3.2 Microcosm Preparation

Inoculum source: The inoculum source was obtained from the anaerobic microcosms operated in previous study from Point Mugu Site (Keeling, 1999). These microcosms consisted of 200 ml of site groundwater with an average sulfate concentration of 1300 mg/L and amended with Owen's nutrient media plus vitamin

B12 and 50 ml of aquifer solids. Microcosm LTCEM previously fed lactate was selected for preparation of the new microcosms. This microcosm was incubated for one year with TCE, from 1997 to 1998.

Microcosm Preparation: The new microcosms were prepared in 310 ml transparent clear Wheaton media bottles with black phenolic screw caps and butyl rubber septa. The bottles were wrapped with aluminum foil to omit light. Site groundwater used in preparation of the microcosms was collected from the Point Mugu Site monitoring wells in 1 gallon Nalgene bottles without any headspace to maintain the anaerobic condition during transport and storage. Aquifer soil samples were also collected from site soil borings and were stored in glass bottles capped with stoppers to prevent exposure to oxygen. Both soil and groundwater samples were stored at 5 ^{o}C prior to microcosm preparation. The groundwater sample was collected from extraction well #1 of the field demonstration and the aquifer solids were from a core obtained during the construction of the extract well at a depth of 20 to 23 ft. Each microcosm was constructed to contain approximately 100 ml of aquifer solids and 150 ml of groundwater, and 60 ml of headspace for gas sampling. Residual TCE and other volatile organic compounds were purged out of the groundwater with pure nitrogen gas, treated with tube furnace to eradicate any trace of oxygen. Actual construction of microcosms was conducted in an anaerobic globe box, which containing roughly 10 % of hydrogen. Each bottle contained 110 ml of fresh groundwater and 90 ml of new aquifer

material. 50 ml (40 ml of groundwater and 10 ml of soil) from inoculum source microcosm was added to each newly constructed microcosm. Five microcosms were prepared. Table 3.1 indicates the composition of those microcosms.

Table 3.1. Matrix for new microcosms constructed for anaerobic TCE transformation study.

microcosms	initial TCE	initial amount of Lactate	sulfate concentration
	(μmol)	(mmol)	(mg/L)
M1	3.64	2.77	560
M2	3.64	2.88	623
M3	3.90	2.81	518
M4	5.44	2.9	600
M5	5.32	2.81	596
LT1	4.53	2.77	577
LT2	4.26	3.14	635
LT3	4.86	2.74	617
LT4	4.75	2.73	598
LT5	4.33	2.61	603
T1	4.85	2.41	646
T2	4.92	2.44	610
T3	4.57	2.54	672
T4	3.73	2.56	658
T5	3.57	2.4	647

M series microcosms are amended with Owen's nutrient media.
LT and T series microcosms were prepared with site groundwater and aquifer solids without nutrient addition.

Each microcosm received lactate as substrate in an amount equal to 3 times electron equivalent required to complete the reduction of the sulfate present. TCE added to the microcosm was 4 μmol to mimic the actual site groundwater contamination level. This corresponds to an aqueous concentration of 3 mg/L

based on liquid and gas partitioning under the assumption of no TCE sorption to the aquifer solids. The microcosms were stored at 20 ^{o}C, and were wrapped in aluminum foil to prevent exposure to light.

Since the microcosms have liquid and gas phases, the mass of volatile organic compounds were estimated using partition coefficient (Henry's constants) by measuring the gas phase concentration and performing mass balance by assuming Henry's Law equilibrium. Control results indicated minimal sorption of TCE onto the aquifer solids.

3.3 Analytical Methods

Lactate, acetate, butyrate, and propionate were measured by high performance liquid chromatography (HPLC) using Dionex DX 500 model. Dionex DX 500 system consisted of LC 20 chromatography enclosure, GP 50 gradient pump, and AD 20 UV absorbance detector with Phenomenex Rezex 8μ 8% H. organic acid column (300 * 7.8 mm) along with Phenomenex Rezex (50 * 7.8 mm) column. The high performance liquid chromatography was operated with an isocratic pump with eluent of 0.013 N of sulfuric acid as a carrier flow through the column. A five point external standard calibration curve was constructed before actual sampling for the organic acids. Prior to any sampling event, a one point standard check was performed.

The sulfate concentration was determined using ion chromatography (IC) with a Dionex Series 4000i IC. The IC unit was operated with conductivity detector, elluent degassing module, and a Dionex 4270 integrator with Dionex IonPac AS4A 4mm and IonPac C6I2 (10 – 32) columns in series. Eluent was prepared in 0.371 g/L of Na_3CO_3 and 0.084 g/L of $NaHCO_3$. A seven point linear standard calibration curve was constructed prior to initial sampling for sulfate concentration. And one point standard check performed for every sampling and analysis event. Peak areas were determined using Peaknet Software (Dionex, Sunnyvale, CA).

Carbon dioxide and hydrogen were analyzed with a Hewlett Packard (HP) 5890 Series II gas chromatography (GC) equipped with a thermal conductivity detector (TCD) and a Supelco 60/80 Carboxen 1000 packed column. Grade 4.5 helium gas was used as a carrier gas for operation of HP GC. A headspace gas sample of 100 µl was injected for determining the headspace concentration. A HP Chemstation software version B.02.04 was used to integrate peak in the microcosms headspace.

For quantitative analysis of TCE, cis-DCE, ethylene, and methane, a HP 6890 Series GC equipped with photo ionization detector (PID) and a flame ionization detector (FID) was used. Chromatographs separation was achieved using GS-Q PoraPlot Mega Bore column (J&W Scientific, Folsom, CA). Headspace gas sample (100 µl) using Hamilton 100 µ gas-tight syringe was injected into the GC. A HP Chemstation (version a. 04.02) was used to integrate

chromatogram peak area. A seven point external standard calibration curve was generated for the compounds of interest before actual sampling event. For each sampling event, a one point standard check was performed to determine the stability of the detectors and for recalibration purposes.

Headspace gas samples (100 µl) for a gas chromatography were obtained using Hamilton gas-tight syringe for all volatile organic compounds including TCE, cis-DCE, ethylene as well as methane, carbon dioxide and hydrogen. Before the headspace gas sampled, the microcosms were vigorously shaken for 2 minutes.

Liquid samples (0.1 ml) were collected from a microcosm and diluted with 0.9 ml of DI water in 1.5 ml of micro-centrifuge tube. The micro-centrifuge tube was mixed with VWR vortex mixer for 30 seconds and was centrifuged at 14,000 rpm for 5 minutes using an Effendorf centrifuge. 0.5 ml of the diluted liquid sample was transferred to 0.5 ml Dionex auto-sampler polyvial for HPLC analysis, or 0.5 ml of diluted liquid sample were directly injected into IC injection port for sulfate concentration analysis.

CHAPTER 4
ENHANCED VC TRANSFORMATION WITH TCE ADDITIONS UNDER METHANOGENIC CONDITIONS

4.1 Low Concentration of TCE Transformation Under Sulfate Reducing Conditions.

Two sets of duplicate microcosms were tested for TCE transformation at low TCE concentration under sulfate reducing conditions. These microcosms were all inoculated with previous simulated microcosms as described in Chapter 3. None of the microcosms were amended with supplemental nutrients except receiving lactate as primary substrate. Each microcosm set was named LT and T.

Shown in the Table 3.1, the average initial TCE concentrations added into the microcosms were 3.5 mg/L, and 3.3 mg/L, while average initial sulfate concentrations were 646 mg/L, and 605 mg/L in average for LT and T sets respectively. Lactate was added to all the microcosms at 3 times of electron equivalents required to reduce the initial sulfate present. Propionate was fed as substrate for the latter part of the experiment.

At the beginning of the experiment, the reduction of sulfate lagged the instantaneous fermentation of lactate to propionate and acetate (Figure 4.1). However, relatively faster reduction of sulfate was observed after propionate and acetate accumulated in the microcosms. This response was observed in every microcosm. The initial sulfate was essentially completely reduced in less than 30 days of incubation (Figure 4.1). In the earlier study, the fermentation to propionate

and acetate also preceded the reduction of the sulfate (Keeling, 1999). The reduction of the initial sulfate (approximately over 1,000 mg/L) was completed in that study between 130 and 160 days in lactate fed microcosms (Keeling, 1999). Under the similar sulfate reduction conditions, the current microcosm study experienced sulfate reduction prior to fermentation of propionate and acetate (Figure 4.1 a). As a result of sulfate reduction, a blackish sulfide precipitate was observed in all microcosms. In the LT set of microcosms, near stoichiometric conversion of lactate to propionate and acetate was observed. The maximum amount of acetate produced was 2.7 mmol from addition of 2.7 mmol of lactate. Qatibi et al. (1990) observed acetate accumulation after lactate fermentation under sulfate reducing conditions.

Active methanogenesis in response to acetate consumption was observed after approximately 30 days incubation of microcosm LT1 (Figure 4.1 b). However, the T set of microcosms showed almost no accumulation of acetate. Acetate consumption was likely linked to methanogenesis that occurred earlier in the T set of microcosms. For T1 microcosm (Figure 4.2), methanogenesis became active while sulfate reduction was still occurring. Methane production started on day 13, and a significant amount of methane production (0.3 mmol) was observed by day 20.

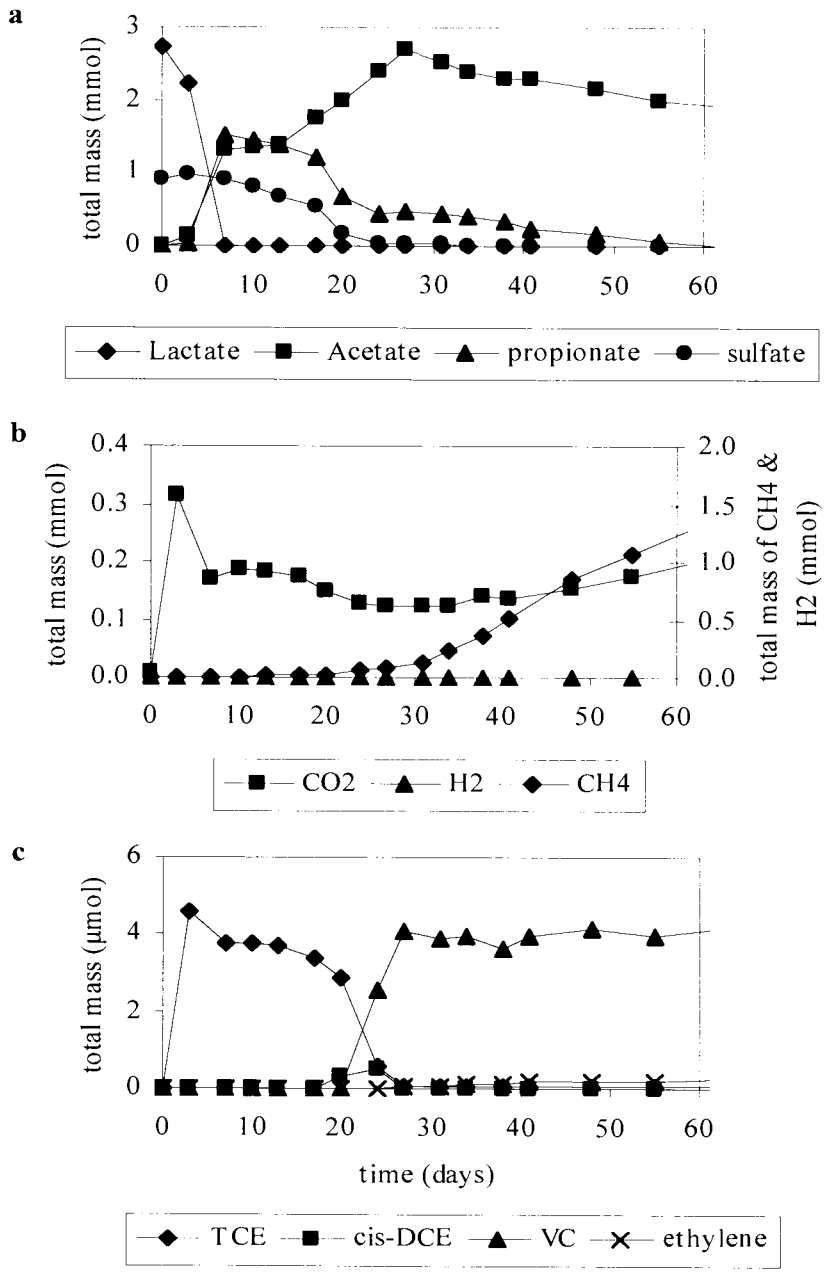

Figure 4.1. Reductive dechlorination of TCE under sulfate reducing conditions from the microcosm LT1. Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

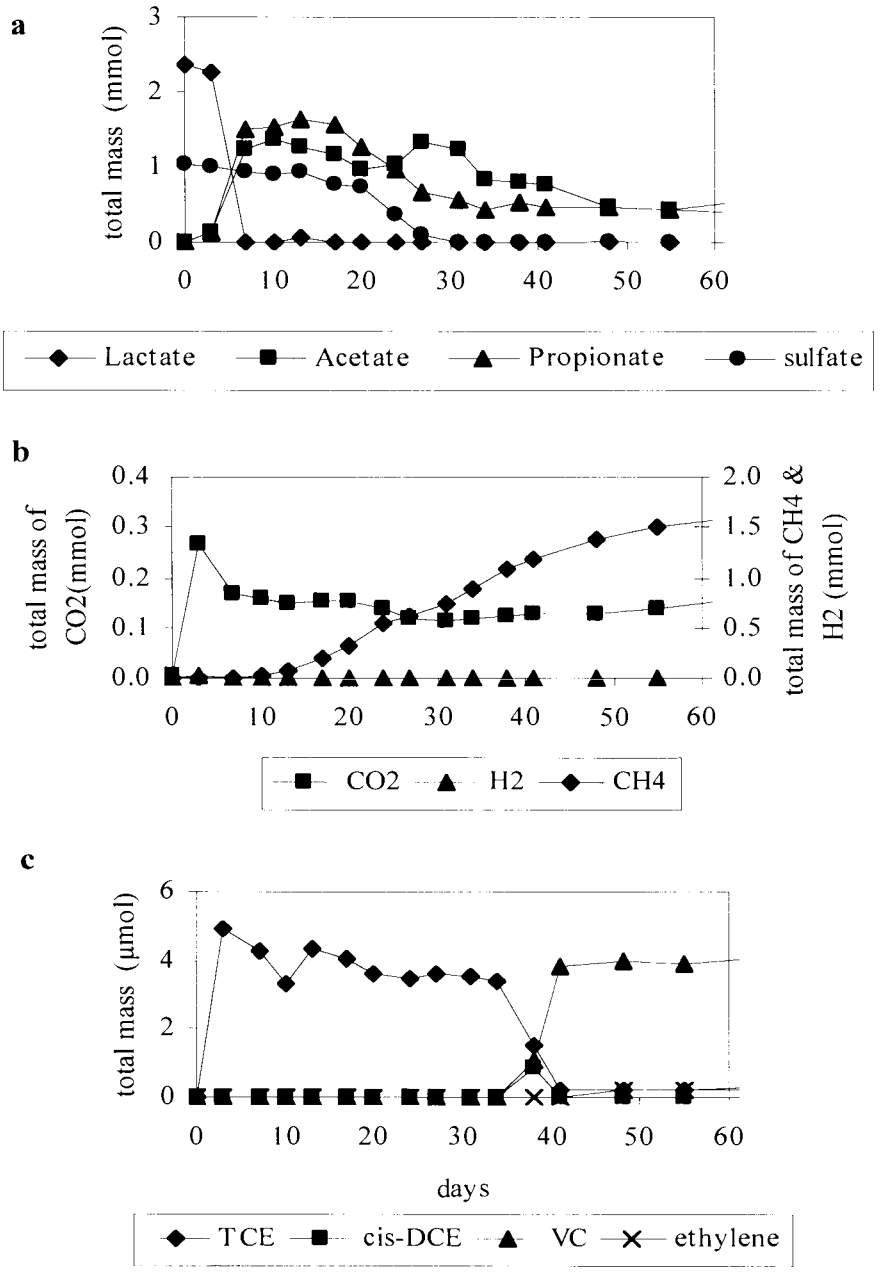

Figure 4.2. Reductive dechlorination of TCE under sulfate reducing conditions from the microcosm T1. Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

TCE transformation was not initiated until all sulfate was reduced (Figure 4.1 c). By day 24, sulfate was completely reduced, and TCE was starting to be transformed to cis-DCE and VC. Both microcosm sets showed that TCE transformation to VC instantaneously took place after essentially complete reduction of the sulfate. The major product of TCE transformation was VC from both sets of microcosms. cis-DCE was short-lived and was only observed while TCE transformation progressed. After approximately 30 and 40 days of incubation in microcosms LT1 and T1 respectively, the entire amount of initial TCE was transformed to VC. Under the assumption that hydrogen utilization is required for dechlorination, one possible reasoning of delayed dechlorination for T1 bottles is that early active methanogenesis consumed hydrogen pool generated from lactate and propionate fermentation. Despite to the rapid transformation of TCE to cis-DCE and VC, the subsequent transformation of VC to ethylene was extremely slow. Even though VC transformation to ethylene was initiated right after TCE transformation to VC, the rate of transformation of VC was very slow. This slow transformation is shown in microcosm T1 (Figure 4.3) and LT5 (Figure 4.4). On day 250, approximately 22% of the initial TCE added in the microcosm was converted to ethylene. The incomplete TCE transformation has been observed in number of different studies, including previous microcosm study for this site conducted by Keeling (1999). Active methanogenesis was observed throughout the time course along with dechlorination of TCE to ethylene. Rapid transformation to

VC was observed from all other duplicate microcosms, with very slow transformation of VC to ethylene.

Table 4.1. History of substrates and TCE/VC additions.

	Addition days and amounts of			
	Lactate (day : mmol)	Propionate (day : mmol)	TCE (day : μmol)	VC (day : μmol)
LT1	(1 : 2.72)	(67 : 0.79) (112 : 1.16) (187 : 1.08)	(3 : 4.61) (67 : 2.63) (83 : 2.46) (112 : 3.28) (122 : 2.42) (133 : 3.01) (143 : 2.98)	
LT2	(1 : 3.09)	(67 : 0.84) (112 : 1.52) (187 : 1.10)	(3 : 4.34) (67 : 1.99) (83 : 2.32) (97 : 3.14) (112 : 3.51) (122 : 2.52) (133 : 3.39) (143 : 2.69)	
LT3	(1 : 2.69)	(67 : 0.93) (112 : 1.30) (187 : 1.02)		(67 : 0.78) (83 : 1.37) (97 : 2.19) (112 : 1.81) (122 : 1.64) (133 : 1.58) (143 : 2.78)
LT4	(1 : 2.68)	(67 : 0.89) (112 : 1.29) (187 : 0.78)		(67 : 0.82) (83 : 2.12) (97 : 2.22) (112 : 1.89) (122 : 1.82) (133 : 1.43) (143 : 3.02)

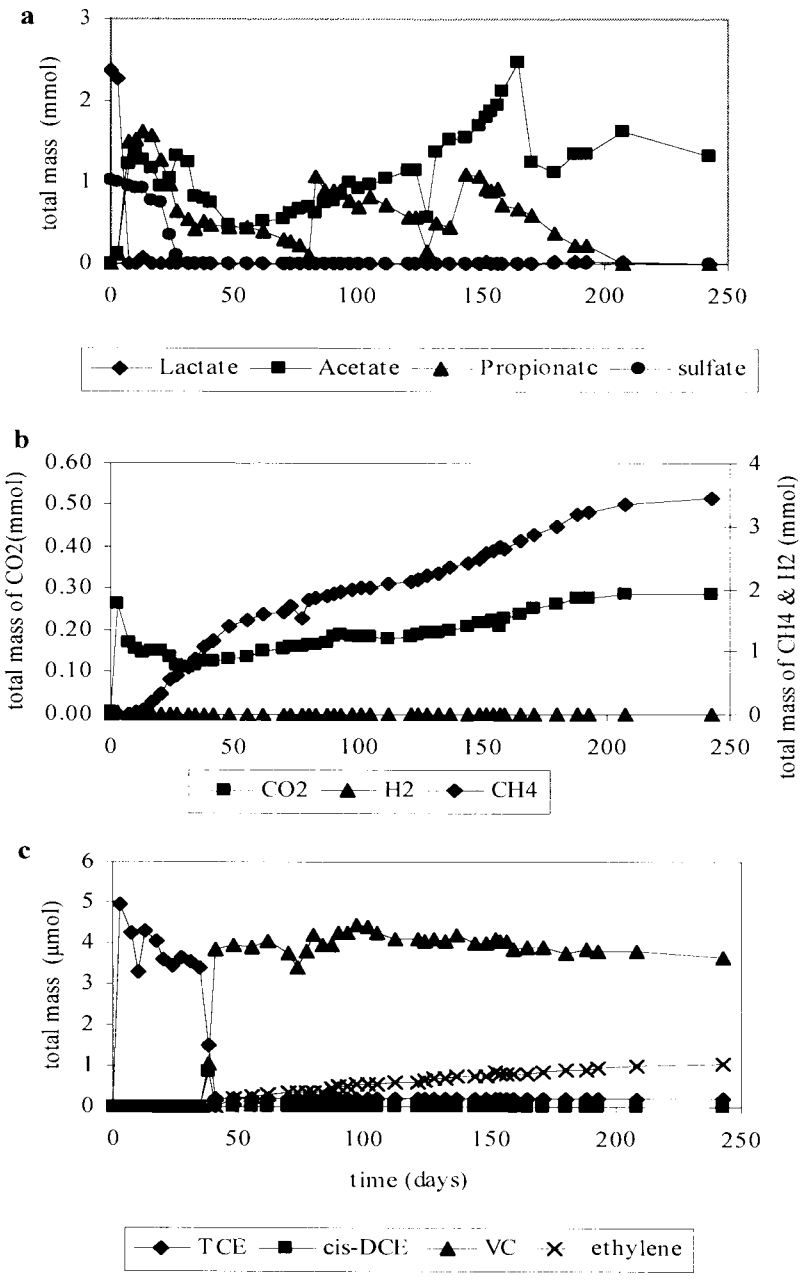

Figure 4.3. Rate-limiting step, VC transformation to ethylene in long term study of reductive dechlorination (T1) of TCE under methanogenic conditions. Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

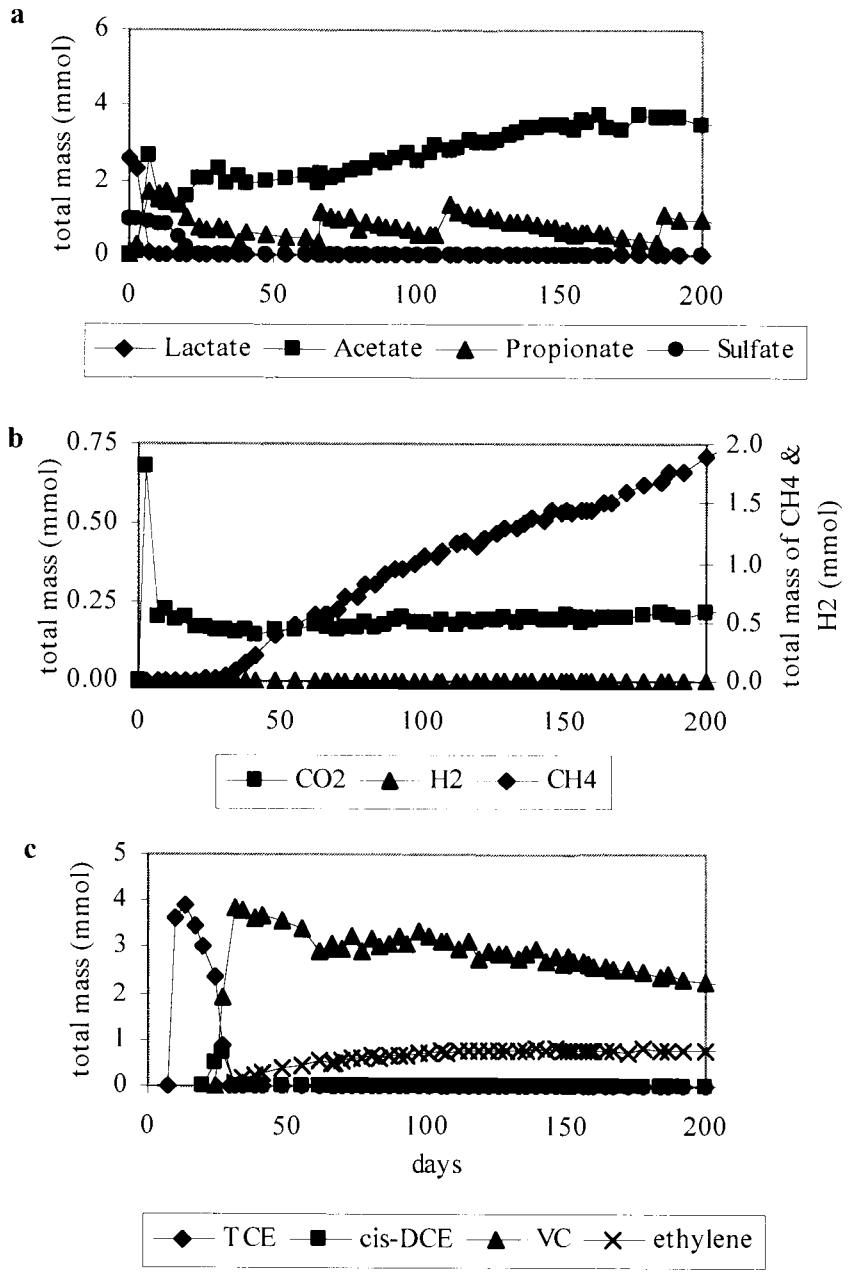

Figure 4.4. Microcosm (LT5) without TCE/VC additions. Lactate was added day 1, and propionate was added day 67, 112, and 187. Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

4.2 Step-wise TCE & VC Additions

The bench scale microcosm study conducted by Keeling (1999) showed the addition of TCE increased the rate of VC transformation rate to ethylene. Freedman and Gossett (1989) reported that their mixed culture could transform PCE to VC and ethylene, but incomplete transformation of VC to ethylene was observed due to the slow transformation rate. DeStafano et al. (1992) conducted experiments using the same methanogenic mixed culture as Freedman and Gossett (1989) with repeated additions of PCE. An enhanced rate of VC transformation resulted and methanogenesis was inhibited due to the high concentration of PCE.

A similar method of repeated additions of the chlorinated solvent was evaluated in this study to determine if enhanced rates of dechlorination of VC to ethylene could be achieved. Tests were performed in microcosms that lacked minor nutrient additions. Two microcosms (LT1 & LT2) were selected for repeated TCE additions. Into another pair of microcosms (LT3 & LT4), VC was repeatedly added in equivalent molar amounts to the TCE added. These microcosms would serve as controls to determine if enhanced rate results from an increase in concentration of VC alone, or as a result of TCE transformation.

Figure 4.5, 4.6, 4.7, and 4.8 present data for LT1, LT2, LT3, and LT4, respectively. For LT1 and LT2 microcosms, TCE was added seven times in amounts ranging from 2 to 3.5 μmol total over two week intervals. Fermentation of lactate produced acetate and propionate while sulfate reduction occurred. Propionate was further fermented to acetate, which accumulated in the microcosm

until methanogenic activity was initiated. When propionate was added as a substrate on day 67, TCE additions were started. Propionate was fermented to accumulate acetate and to support the transformation of TCE. Methane production was maintained as long as acetate was remained in the microcosms (Figure 4.4 and 4.5).

The addition of TCE resulted in rapid transformation to VC, without the accumulation of cis-DCE. Prior to the repeated TCE additions, TCE transformation was delayed by presence of sulfate. Since the entire sulfate was reduced prior to TCE additions, rapid and immediate transformation of TCE to VC was observed. Stoichiometric conversion of added TCE to VC was observed from both LT1 and LT2 bottles. After the last dose of TCE, the microcosm total mass of VC were 21 and 22 μmol for LT1 and LT2 respectively.

During the repeated addition of TCE to these duplicate bottles, two major observations were noted. As expected, both bottles accumulated VC from TCE transformation.

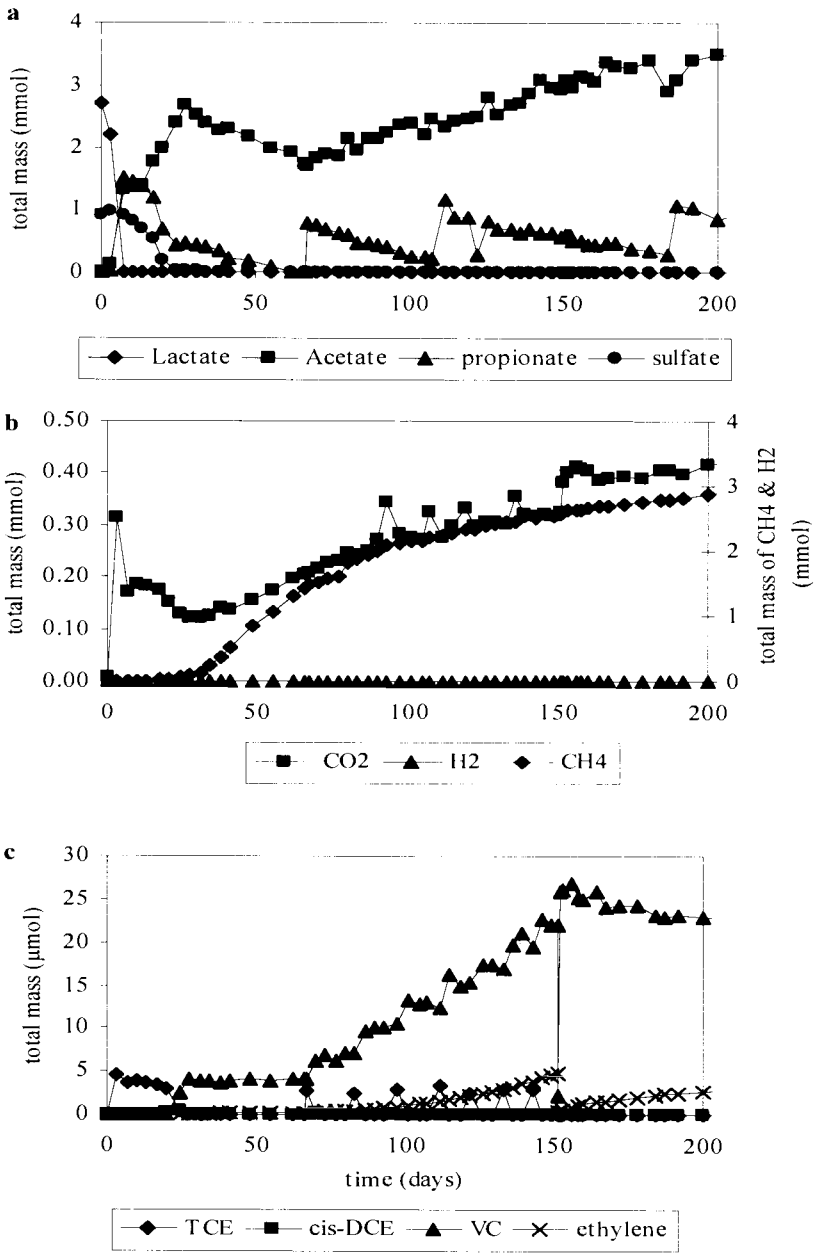

Figure 4.5. Step-wise TCE additions to enhance VC transformation to ethylene (LT1). TCE was added at the following time: 67, 83, 97, 112, 122, 133, and 143 days. VC was reintroduced at 150 days after purging after TCE addition was stopped. Concentrations of (a) substrates and sulfate, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

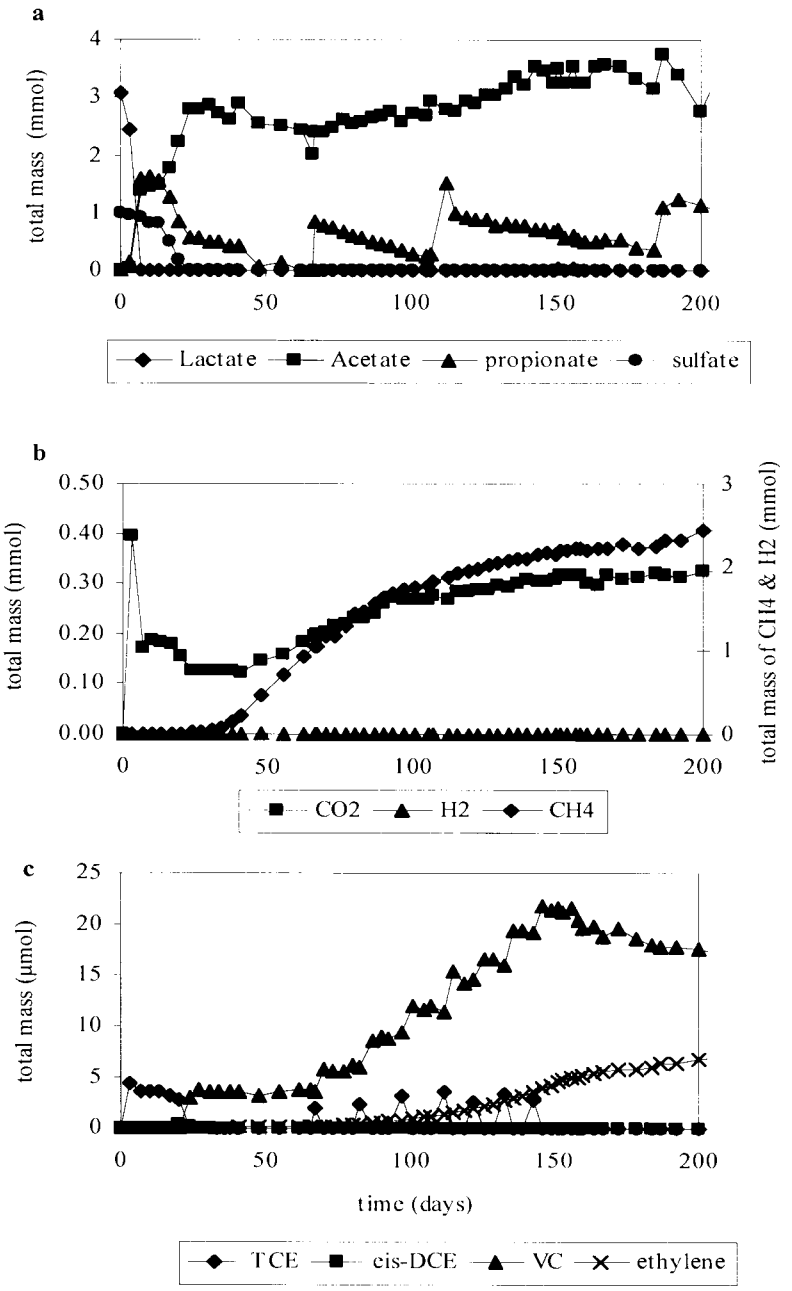

Figure 4.6. Duplicate of LT1, step-wise TCE addition for enhancing VC transformation (LT2). This microcosm was not purged after TCE addition was stopped at 143 day. TCE was added on the following days: 67, 83, 97, 112, 122, 133, and 143 days. Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

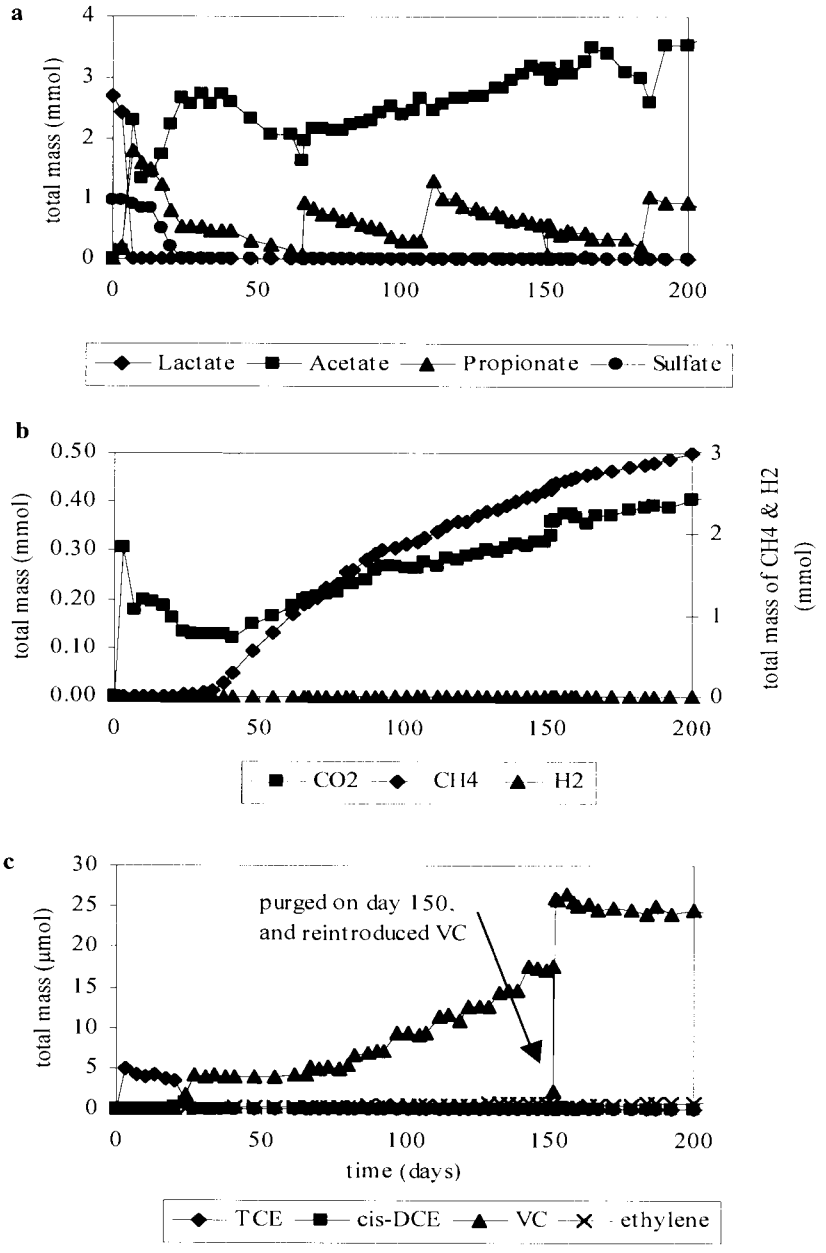

Figure 4.7. LT3 microcosm with step-wise VC additions. VC was added on the following days: 67, 83, 97, 112, 122, 133, and 143. After purging, VC was added on day 150. Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

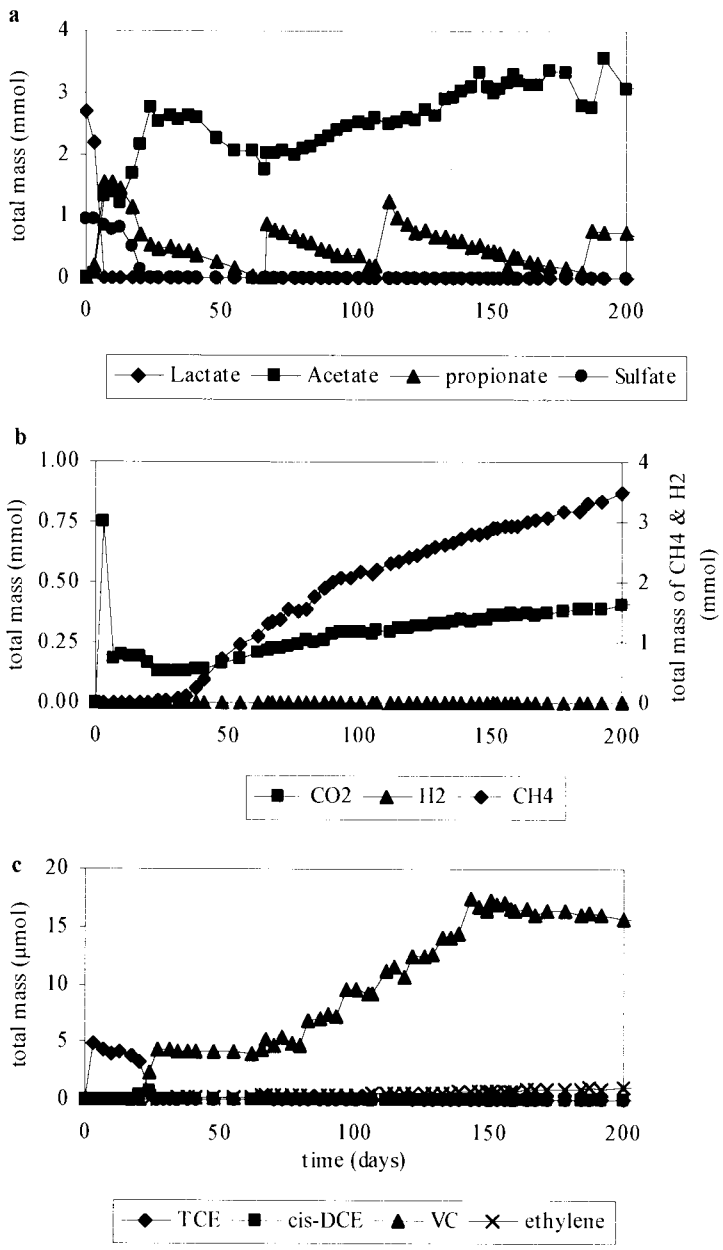

Figure 4.8. VC addition microcosm (LT4) without purging out at the end of step-wise VC additions. Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

Despite to accumulation of VC in those microcosms, the rate of ethylene production dramatically increased. Similar results were observed for substrates utilization and methanogenic activity from LT3 and LT4 (Figure 4.7 and 4.8). Added propionate was fermented to acetate, which accumulated in the microcosms to support methanogenic activity. From Figure 4.9, total mass of ethylene produced from adding TCE increased nearly exponentially until day 150 when TCE addition was stopped. Rate of ethylene production also increased with the repeated additions of TCE. Based on the data presented, both total mass and rate of ethylene production dramatically increased compared to initial long term TCE transformation to ethylene and the microcosm LT3 and LT4 to which only VC was repeatedly added.

Table 4.2 summarizes the rates of ethylene production prior to and during the additions of TCE. The rate increased by almost a factor of 10 after TCE additions than prior to the repeated addition of TCE. The final fractional conversion to ethylene was closed to 20% of the TCE added. However, microcosms to which VC was added showed little changes in ethylene production rates (Table 4.2). The rate of ethylene production was more than 15 times greater with TCE addition than the initial low concentration of TCE transformation. However, VC additions had a little effect on ethylene production rate.

Table 4.2. Rates of ethylene production prior to TCE addition and during TCE addition period.

	rate of ethylene production prior to TCE/VC addition	Normalized rate of ethylene production to TCE/VC addition	total amount of TCE/VC added	% of Ethylene conversion	rate of ethylene production during TCE/VC addition	Normalized rate of ethylene production during TCE/VC addition *
	(µmol/day) Day 27-66	(µmol/day/µmol)	(µmol)	Day 151	(µmol/day) Day 130-151	(µmol/day/µmol)
LT1	(TCE) 0.006	1.30E-03	23.6	19.1%	0.10	0.016
LT2	(TCE) 0.0051	1.18E-03	23.1	18.9%	0.10	0.017
LT3	(VC) 0.0065		16.4	3.38%	0.01	
LT4	(VC) 0.0066		17.7	4.7%	0.01	

Normalized rate* was normalized rate of ethylene produced with amount of TCE added.

The result clearly demonstrated that the additions of TCE and not just an increase in VC concentration resulted in the enhanced rates of transformation. After 7th TCE addition, one microcosm with TCE and VC added was purged out, and same amount of VC before purging was reintroduced. LT1 and LT3 were selected for purging out to eliminate residual ethylene, which might be inhibitor of VC transformation. When TCE addition was stopped, the rate of ethylene production slowed in both the purged and the microcosms that were not purged.

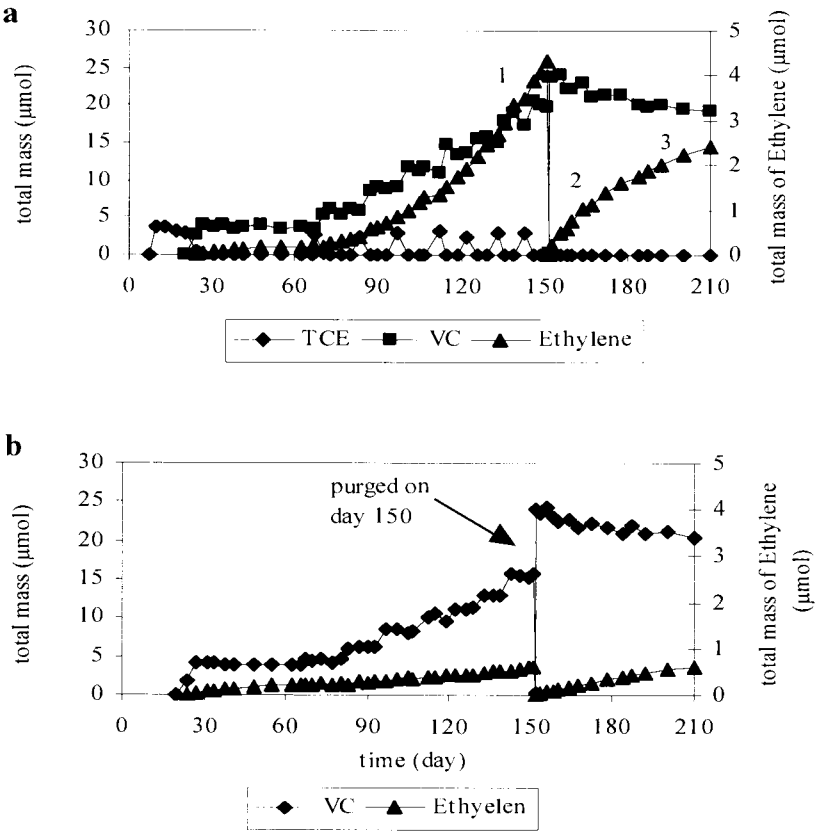

Figure 4.9. Step-wise TCE (LT1) (a) and VC (LT3) (b) additions from duplicated microcosms. The microcosms were purged at 150 days. Ethylene production rates for periods 1, 2, and 3 from (a) were 0.10, 0.06, and 0.03 µmol/day.

From Figure 4.9, the rates of VC transformation from day 150 to 200 were 0.0639 and 0.031 µmol/day for LT1 and LT3 respectively while ethylene production rates were 0.048 and 0.012 µmol/day. The ethylene production rates for corresponding period of time were decreased compared to the rates during the addition of TCE. Before purging on day 150, the total amount of ethylene produced in the microcosms with TCE additions showed factor of five times more total mass of

ethylene than the microcosm with VC additions. Similar greater amount of ethylene production in the TCE stimulated microcosm was observed after purging. Shown in Figure 4.9 (a), three ethylene production rates were determined in three different time periods. The ethylene production rate was highest during period 1 (0.10 μmol/day) when last TCE addition was injected. The rates were decreased for period 2 (0.06 μmol/day) and 3 (0.03 μmol/day) after TCE addition was stopped. DiStefano (1999) reported similar results of decreased ethylene production rate when PCE was not present. His study concluded that PCE is needed to be present in order to maintain rate of VC transformation to ethylene. This current study showed the correlation of TCE presence to sustain VC transformation to ethylene.

Table 4.3. Rate of VC transformation and ethylene production rates: before and after TCE and VC additions were stopped.

	VC transformation rate (initial/final)*	Ethylene production rate (initial/final)*	Ethylene production rate when TCE/VC stopped=
	(μmol/day)	(μmol/day)	(μmol/day)
LT1	0.075 / 0.014	0.063 / 0.035	0.10
LT2 (purged)	0.12 / 0.032	0.045 / 0.035	0.10
LT3	0.049 / 0.002	0.012 / 0.010	0.01
LT4 (purged)	0.021 / 0.016	0.0079 / 0.0068	0.01

*Estimated rates initial rate on 150-180 day and final rate on 190-210 day.
=Estimated ethylene production rates on 130-150 day during TCE/VC stopped

Table 4.3 summarizes the rates after TCE/VC addition was stopped. Ethylene production rates slowed after TCE addition to the microcosm was stopped. The initial and final rate indicated that both VC transformation rates and ethylene production rates decreased after TCE/VC additions were stopped. The ethylene production rates from purged microcosms showed higher ethylene production rates as well as VC transformation rates than non-purged microcosms (Table 4.3). This data supported an ethylene inhibitory effect on VC transformation.

During the course of study, the effect of TCE on ethylene production rate was apparent. However, the increased rate of ethylene production rate was not sufficient enough to overcome the accumulation of VC due to the transformation of TCE. During TCE addition period, only fraction of total TCE added (over 20% in average) was converted into ethylene with leaving substantial amount of more toxic VC in the system. Despite of high residual fraction of VC left in the system, ethylene production rate during TCE addition averaged 0.051 μmol/day, which is 10 times greater than the ethylene production rate from the initial TCE transformation rate of 0.005 μmol/day. The VC transformation rate after TCE addition was stopped was enhanced to an average value of 0.066 μmol/day, which is three times greater than VC transformation from microcosms receiving an equal amount of VC, instead of TCE.

The ethylene production rates were lower than VC transformation rate by a factor of two or more. The rate difference shown in Table 4.3 might possibly result

from VC being transformed to something other than ethylene in the microcosms. This would cause a slower transformation rate to ethylene than to VC transformation rate. The discrepancy in those corresponding rates was greater with lower rates. Error in measurement of the high concentration of VC and determining rates from small differences in concentration of VC may be another reason for the discrepancy.

Electron mass balance showed that the dechlorination utilized less than 1% of total recovered electrons from 200 days of incubation (Table 4.4). Over 50% of electrons remained in forms of acetate and propionate. Methane production and sulfate reduction accounted the remaining electron transferred.

Table 4.4. Electron mass balance after 120 days of incubation.

	Lactate, propionate & butyrate*	Acetate & Propionate	Sulfate reduction	Methane	Dechlorination	Total recovery of electron at day 120	Electron efficiency %
LT1	100%	53%	10%	30%	0.2%	93%	<1
LT2	100%	50%	10%	26%	0.2%	86%	<1
LT3	100%	53%	10%	31%	0.1%	94%	<1
LT4	100%	48%	10%	38%	0.1%	96%	<1

Electron mass balance at initial 0 day *

4.3 Fumarate as An Alternative Terminal Electron Acceptor

Even though TCE additions stimulated the rate of VC transformation to ethylene, VC accumulation resulted in the microcosms due to the slow rate of

ethylene production rate. Under the assumption of selectivity for terminal electron acceptor by the indigenous anaerobic microorganism from the site subsurface, another alternative terminal acceptor was tested for this part of the experiment. A study by Gerritse et al. (1996) showed strain PCE1 grew on reductive dechlorination of PCE as an electron acceptor under anaerobic conditions. Gerrites et al. (1996) reported that same strain PCE1 could also utilize fumarate as an alternative electron acceptor for cell growth. Their study reported that the presence of these electron acceptors is coupled to ATP synthesis for cell growth. Loffler et al. (1999) reported that fumarate reduction (Figure 4.8) is one of the terminal electron-accepting processes (TEAP). The hydrogen threshold model indicated that fumarate reduction is energetically favorable TEAP than acetogenesis, methanogenesis and sulfate reduction (Loffler et al., 1999). This indicated that fumarate addition as an alternative electron acceptor might support microbial growth of a dehalogenating population without accumulating more toxic compounds.

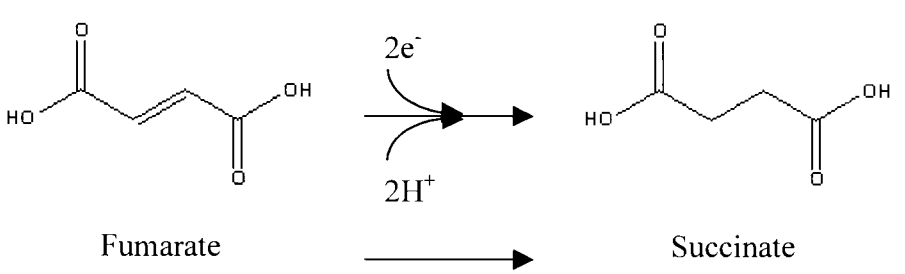

Figure 4.10. Fumarate reduction to succinate as an alternative terminal electron accepting process.

An average 0.038 mmol of fumarate was added in duplicated microcosms on day 121. As shown in Figure 4.11, fumarate was transformed to succinate in 20 days. Succinate disappeared without being accumulated. However, there was no sign of enhancement on VC transformation to ethylene during and after fumarate was reduced in the duplicate microcosms. Even though alternative electron acceptor was present and being reduced, this redox reaction was not able to help to enhance or induce VC transformation to ethylene

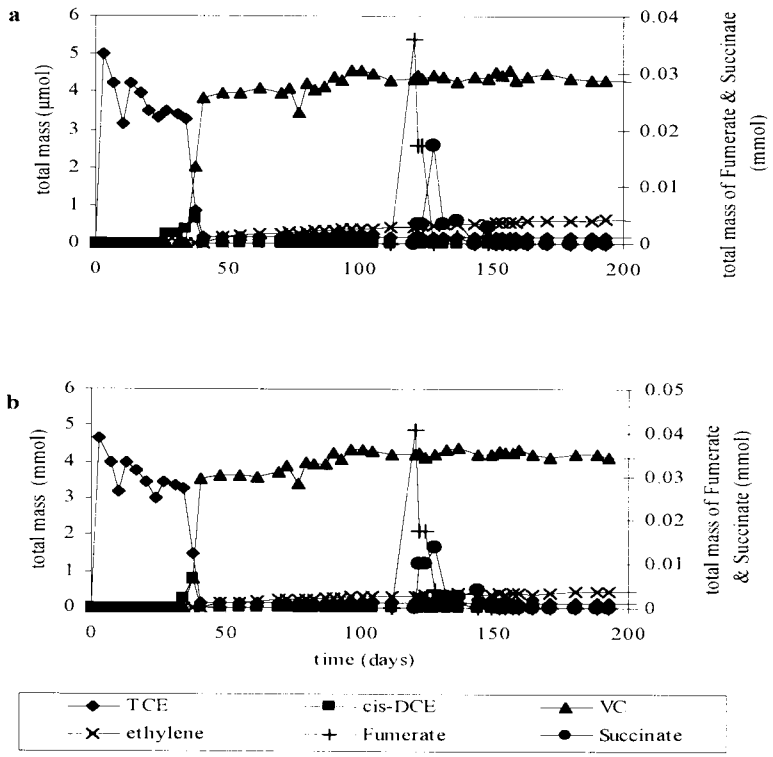

Figure 4.11. Fumarate additions on T2 (a) and T3 (b) on day 120. These duplicated microcosms indicated transformation of fumarate into succinate with little changes of chlorinated ethylenes.

4.4 Conclusion/Discussion

This part of the study focused on TCE additions for the enhancement of VC transformation to ethylene. The results are summarized below.

1. TCE additions stimulated the rate of VC transformation to ethylene under methanogenic conditions. TCE additions increased the ethylene production rate by factor of ten or greater compared to rates before TCE was added. This was supported by a parallel study with VC additions that showed little or no enhancement of VC transformation to ethylene. Freedman and Gossett (1989) and DiStefano et al. (1991) showed the similar results of increased ethylene production rate with PCE additions under methanogenic conditions.

2. The ethylene production rate slowed down when TCE addition was stopped (Figure 4.9 and Table 4.3). The rate decreased from 0.1 μmol/day to 0.03 μmol/day in 50 days. This demonstrated stimulatory effect of TCE addition on VC transformation to ethylene under the site conditions. DiStefano (1999) also observed that ethylene production rates slowed without presence of PCE and concluded that PCE presence is required to maintain rate of VC transformation to ethylene.

3. Since TCE addition showed the effect on stimulation of VC transformation as a terminal electron acceptor, fumarate was tested as an alternative non-toxic terminal electron acceptor to stimulate the growth of microorganism population. Gerritse et al. (1996) showed their strain PCE1 could grow on fumarate, and Loffler et al. (1999) defined fumarate reduction as another possible terminal

electron accepting process. The result of fumarate addition as an alternative terminal electron acceptor showed no enhancement on VC transformation to ethylene. Even though fumarate reduction to succinate was observed in the microcosms, this terminal electron accepting process was not able to promote the VC transformation to ethylene.

4. Based on the results from this low TCE concentration addition, both TCE and VC transformation enhancement can be achieved in in-situ treatment at a site by recirculating groundwater contaminated with low concentration of TCE. The VC transformation to ethylene would be obtainable by low residual TCE concentration from the extracting wells.

CHAPTER 5
MICROCOSM STUDY OF HIGH CONCENTRATION OF TCE ADDITIONS FOR ENHANCEMENT OF VC TRANSFORMATION

5.1 Introduction

Chlorinated aliphatic hydrocarbons (CAHs) such as trichloroethylene (TCE), dichloroethylene (DCE) isomers, and vinyl chloride (VC) are the most common ground water contaminants in the United States (Westrick, 1984). Due to suspected or known human carcinogenic nature of these chlorinated solvents, these compounds are regulated under the Safe Drinking Water Act of 1986. Previous studies (DiStefano et al., 1991 and De Bruin et al., 1992) on chlorinated alkenes have shown that the PCE and TCE can be completely dechlorinated to harmless ethylene under anaerobic reductive dechlorination when utilizing different electron acceptors. Investigations on isolation of microorganisms causing dechlorination have performed and successfully identified number of different dechlorinators (Maymo-Gatell, et al., 1997). However, many studies reported that reduction step from DCE or VC to ethylene is slow. Often these rate-limiting steps cause partial dechlorination with leaving more toxic intermediates, such as VC in the subsurface.

A number of studies has been performed to investigate the effect of different electron donors in promoting the complete dechlorination. Fennell et al. (1997) compared four different electron donors, butyric acid, lactic acid, propionic acid and ethanol for the reductive dechlorination of tetrachloroethylene (PCE) with an anaerobic mixed culture. Their study showed that butyric and propionic acid

can be fermented under low H_2 partial pressures, between $10^{-3.5}$ and $10^{-4.4}$ atm. Under such low H_2 partial pressures, dechlorinators can effectively consume electrons for dechlorination over methanogenesis (Smatlak et al., 1996). Fennell and Gossett (1997) observed butyric and propionic acid supporting dechlorination with suppressed methanogenesis during short-term incubations. However, ethanol equally supported the dechlorination during a long-term study. Even though a variety of electron donors are effectively utilized for dechlorination of TCE and its less chlorinated compounds, TCE, cis-DCE and VC have accumulated at many contaminated sites.

When a TCE DNAPL phase is present in groundwater, TCE can slowly dissolve via diffusion resulting in high TCE concentration around the DNAPL source zone. A number of laboratory studies has been conducted on anaerobic dechlorination at high concentrations of PCE and TCE. DiStefano et al. (1992) studied the anaerobic reductive dechlorination of PCE at high concentrations (approximately 550 μM) under methanogenic conditions with methanol as a substrate. Repeated additions of PCE to their batch reactor caused rapid transformation of VC to ethylene, with inhibition of methanogenesis. Their study indicated that high PCE concentrations enhanced the transformation rate of VC dechlorination to ethylene, while inhibiting methanogenesis. Nielson and Keasling (1999) work supported DisStefano's results. At aqueous concentrations below and at their solubility limits in water, Neilson and Keasling (1999) reported that their methanogenic mixed culture was able to perform complete dechlorination of PCE

and TCE to ethylene with no methane production. Under subsaturating concentration of both TCE and PCE, VC and ethylene production rates showed not much of differences between PCE and TCE. However, ethylene production rate under saturated TCE concentrations was a factor of six times greater with no methane production. This indicated that presence of high TCE concentration promoted the VC transformation to ethylene.

The current microcosms study focused on biological transformation of high TCE concentration under anaerobic conditions. In this study, we have conducted three laboratory experiments regarding anaerobic TCE transformation under methanogenic conditions. The first experiment focused on dechlorination of low TCE concentration under sulfate reducing conditions with three different substrates: lactate, butyrate and propionate. This study helped identifying a substrate promoting the dechlorination at the low TCE concentration and initial rates of transformation of VC to ethylene. In the second series of tests we evaluated the effect of high concentrations of TCE on VC transformation to ethylene under methanogenic conditions with two low hydrogen yielding substrates, butyrate and propionate. Remediation of DNAPL and high concentration of chlorinated compounds is one of the most problematic groundwater contamination situations. The third experiment focused on determination of enhanced TCE and VC transformation at low TCE concentration after the high TCE concentration additions. The results from the study indicated that enhanced anaerobic transformation of high TCE concentrations to ethylene is

possible in microcosms that are representative of the subsurface. The results also provide evidence for the growth of dehalogenating microorganisms on TCE in the microcosms.

5.2 Low Concentration TCE Transformation Under Methanogenic Conditions with Three Different Substrates

Five nutrient amended microcosms (Table 5.1) were selected to perform this experiment. At the start of the experiment, all the microcosms were fed with lactate. Propionate and butyrate were fed as substrates following the initial stimulation on lactate. Two microcosms (M1 & M2) were fed with propionate, and two microcosms labeled M3 & M4 were fed with butyrate. A microcosm fed with lactate was labeled as M5.

Table 5.1. History of TCE and substrate additions for M series microcosms.

Day and amount of TCE and substrate additions					
	M1	M2		M3	M4
Lactate (day : mmol)	(1 : 2.72)	(1 : 2.84)	Lactate (day : mmol)	(1 : 2.76)	(1 : 2.86)
Propionate (day : mmol)	(80 : 0.75) (101: 0.88) (115 : 1.14) (127 : 0.79) (142 : 0.88) (160 : 0.24) (185 : 0.18) (202 : 0.72) (249 : 0.69) (277 : 0.85) (287 : 1.22) (314 : 3.70)	(80 : 0.94) (101: 0.79) (115 : 1.08) (142 : 0.77) (170 : 0.76) (187 : 0.47) (202 : 0.74) (229 : 0.20) (249 :0.70) (277 : 0.81) (287 : 1.30) (314 : 1.50)	Butyrate (day : mmol)	(80 : 0.55) (115 : 0.72) (127 : 0.55) (142 : 0.57) (156 : 0.53) (170 : 0.51) (202 : 0.51) (223 : 0.37) (249 : 0.51) (272 : 0.32) (277 : 0.92) (287 : 1.26) (314 : 0.85) (326 : 0.48) (395 : 0.33)	(80 : 0.65) (115 : 0.74) (127 : 0.55) (142 : 0.68) (156 : 0.52) (170 : 0.53) (202 : 0.52) (223 : 0.36) (249 : 0.51) (272 : 0.17) (277 : 1.02) (287 : 1.36) (314 : 0.88) (326 : 0.51)
TCE (day : µmol)	(3 : 3.69) (121 : 5.79) (156 : 6.98) (201 : 16.58) (259 : 175) (357 : 260) (445 : 3.88)	(3 : 3.69) (121 : 1.70) (127 : 1.6) (142 : 2.30) (156 : 2.00) (170 : 3.20) (185 : 1.60) (201 : 18.2) (259 : 176) (375 : 102)	TCE (day : µmol)	(3 : 3.96) (121 : 6.51) (156 : 6.65) (201 : 14.58) (259 : 57.99) (326 : 275) (395 : 74.65) (445 : 4.48)	(3 : 5.56) (121 : 3.09) (127 : 1.77) (142 : 2.14) (156 : 2.07) (170 : 3.41) (186 : 1.79) (201 : 5.79) (224 : 8.79) (259 : 44.9) (326 : 150)

The initial study of low TCE concentration transformation was performed with an average initial TCE concentration of 3.37 mg/L (total mass of 4.66 μmol). Due to the presence of a fairly high sulfate (average of 560 mg/L) content in the groundwater, the initial TCE transformation proceeded sulfate reduction (Figure 5.1 and Figure 5.2). Lactate utilization showed similar pattern in all the microcosms before adding propionate or butyrate on day 80. It took less than one week to ferment the lactate to acetate and propionate, which then fermented slowly in the microcosms. Acetate from these microcosms completely disappeared in 40 days, while propionate was slowly utilized until day 60. During the acetate utilization period, methanogenic activity was observed. Methane production slowed down after depletion of acetate in 30 days. Lactate fed microcosm (Figure 5.3) showed higher methane production than butyrate and propionate fed microcosms. Between day 60 and 80, acetate accumulation was observed with complete depletion of the remaining propionate. As propionate and acetate were further utilized, the initial sulfate was completely reduced in approximately 30 days. During the same period of time, TCE transformation began, and most of TCE was transformed to VC by day 40. Utilization of acetate and propionate, active methanogenesis and TCE transformation were also observed during the sulfate reduction as well as. TCE transformation accumulated VC in these microcosms with having propionate likely being the primary substrate. The concentration of VC stayed constant up to 80 days of incubation before

reintroduction of propionate (M1 and M2) and butyrate (M3 and M4) to the microcosms.

The reintroduction of propionate (0.75 mmol) and butyrate (0.55 mmol) at day 80 supported very slow dechlorination of VC to ethylene as well as maintain of methanogenic activity. From both M1 and M3 (Figure 5.1 and Figure 5.2) microcosms, very active methane production occurred between days 10 and 25 when acetate was still present in the system with propionate. Between days 25 and 70, methane concentration slowly increased when propionate was the only substrate in the microcosms. Subsequently propionate and butyrate were rapidly fermented to acetate and the methanogenic activity increased. The results indicated that methanogenesis mainly resulted from acetate utilization. More rapid transformation of VC to ethylene did not result from increased rates of methanogenesis.

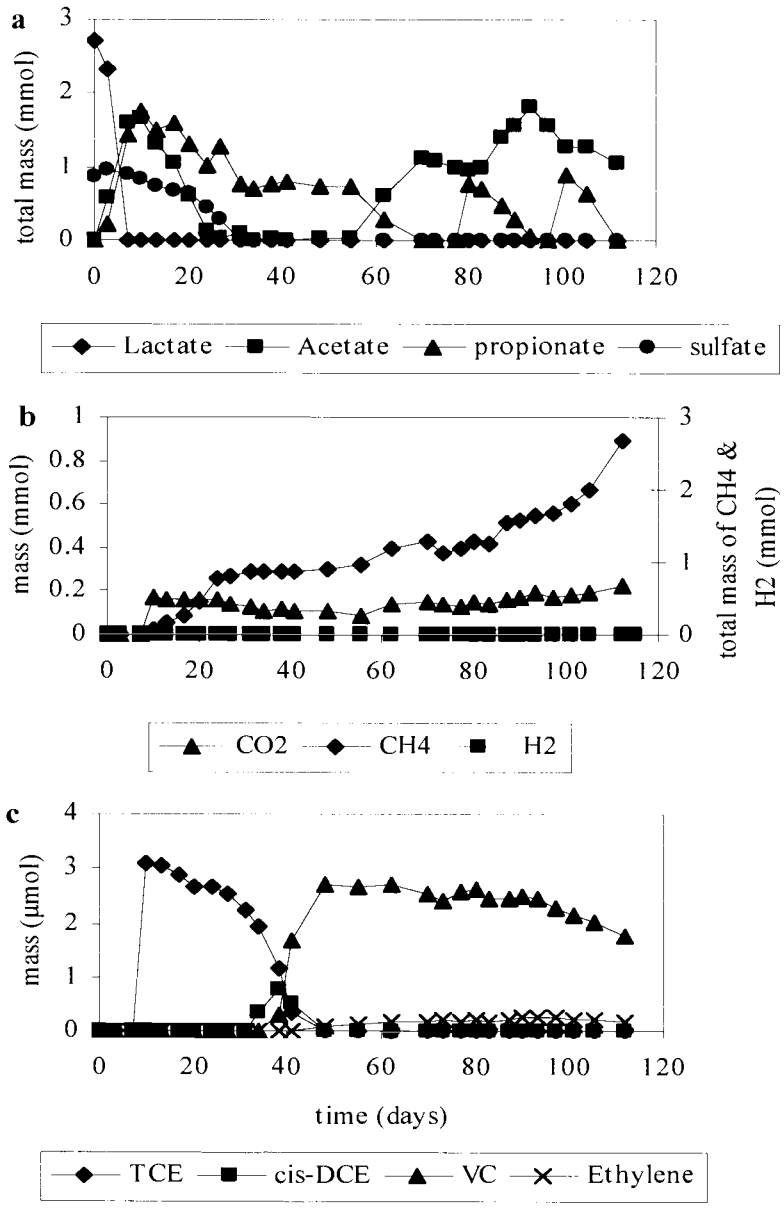

Figure 5.1. Low concentration of TCE transformation under sulfate reducing conditions (M1). The microcosm was initially fed lactate, and then was switched to propionate at 80 days. Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

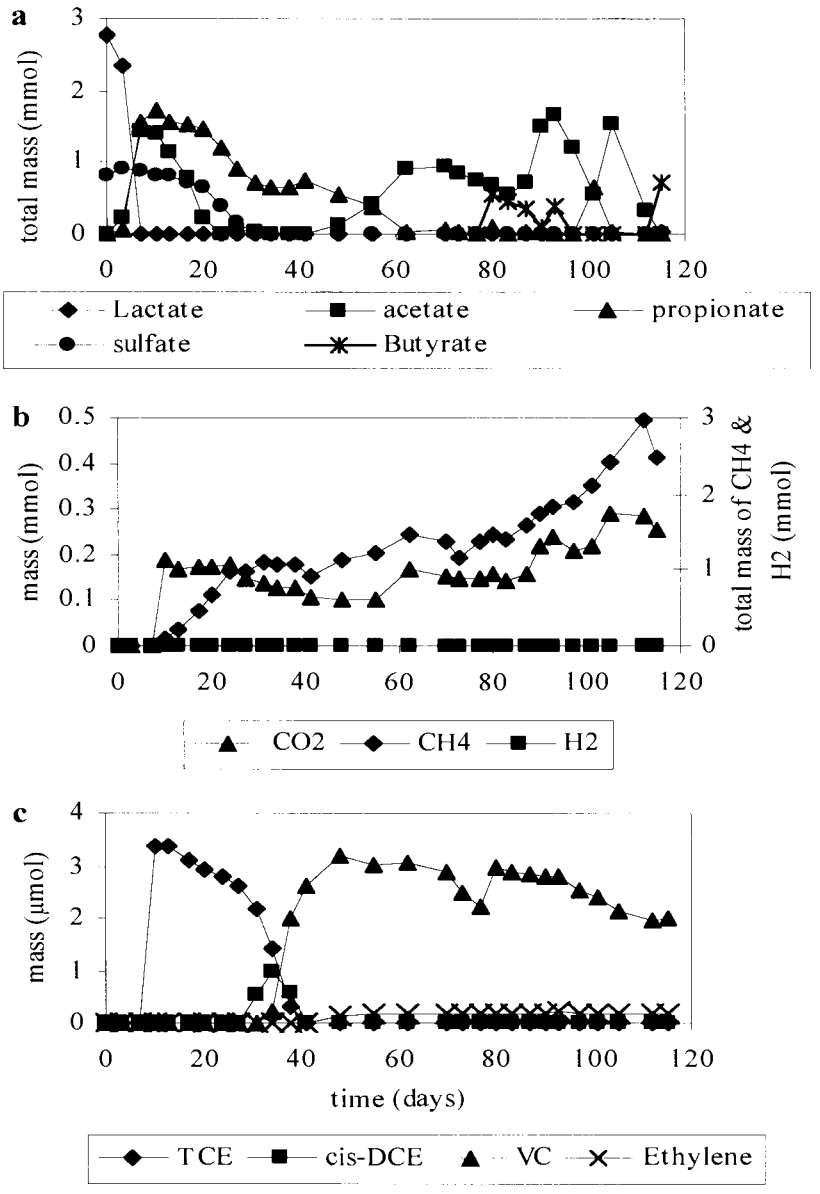

Figure 5.2. Low concentration of TCE transformation under sulfate reducing conditions (M3). The microcosm was initially fed lactate, and then was switched to butyrate at 80 days. Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

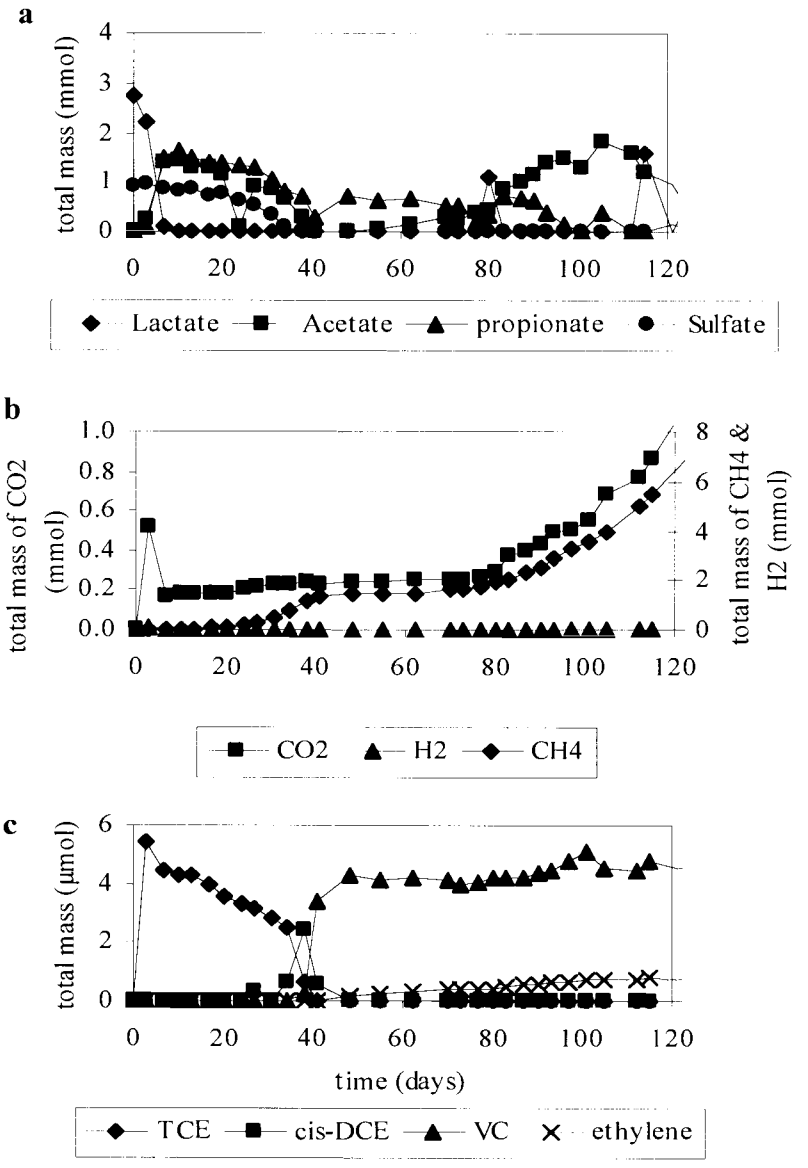

Figure 5.3. Microcosm (M5) without TCE additions. Lactate was added day 1 (2.76 mmol), and 80 (1.1 mmol). Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

The performance for each different substrate in terms of VC transformation to ethylene was compared after 120 days of incubation. Figure 5.4 represents the total amount of ethylene produced in all five different microcosms. The results showed that the rate of VC transformation to ethylene did not significantly depend on substrates added. Table 5.2 summarizes the ethylene production rates and normalized rates based on amount of TCE added to each microcosm and transformed to VC. The normalized ethylene production rates indicated that each different substrate fed into microcosms resulted in similar rates of VC dechlorination to ethylene.

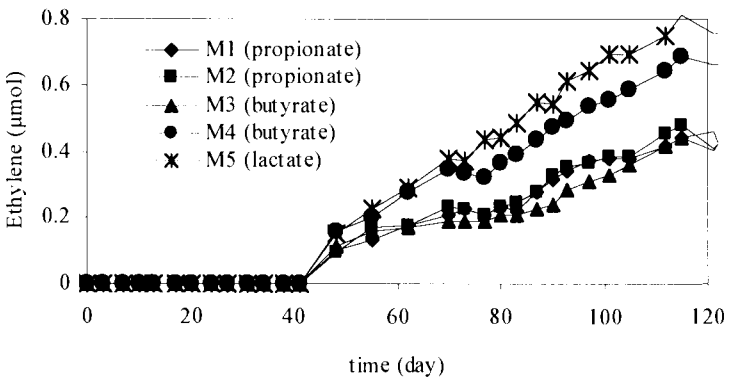

Figure 5.4 Comparison of ethylene production with the different substrate tested. Butyrate and propionate was added on day 80.

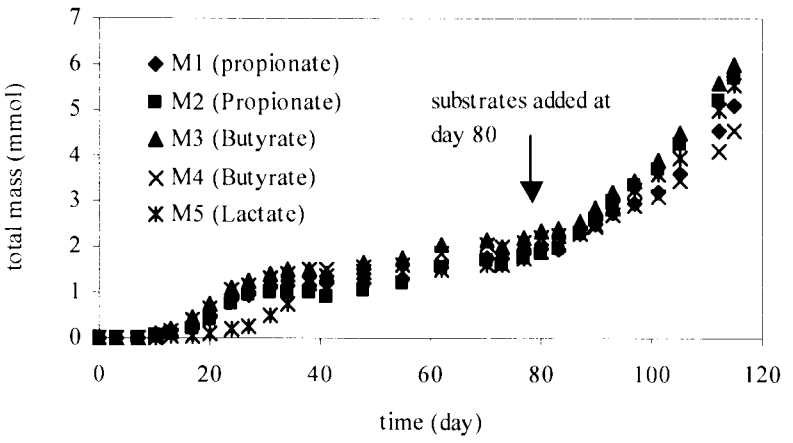

Figure 5.5. The rate of methanogenesis after receiving more substrates after day 70. Butyrate and propionate was added at day 80.

All substrates produced acetate upon fermentation, and acetate utilization was correlated with methane production. Note that the increase in rate of methanogenesis (Figure 5.5) after 80 day does not result in an increase in the rate of VC transformation to ethylene (Figure 5.4). All three organic substrates (lactate, butyrate and propionate) supported low concentration of TCE degradation in similar level under active methanogenesis.

Table 5.2. Initial amount of TCE for each microcosm and rate of ethylene production.

	Initial TCE (μmol) on day 3	Total mass of ethylene produced (μmol)	Rate of ethylene produced (μmol) day 41-115	Normalized rate of ethylene production (μmol/day/μmol)
M1 (propionate)	3.69	0.41	0.0056	0.0015
M2 (propionate)	3.96	0.48	0.0065	0.0017
M3 (butyrate)	3.96	0.44	0.0059	0.0015
M4 (butyrate)	5.55	0.67	0.0093	0.0017
M5 (lactate)	5.43	0.75	0.0100	0.0019

Normalized to amount of TCE transformation to VC.

5.3 High Concentration of TCE Additions

From the previous tests with different substrates, butyrate and propionate fed microcosms showed similar rate of dechlorination of TCE to VC, and VC to ethylene. For the high concentration of TCE transformation experiment, four microcosms were selected, the two propionate fed microcosms (M1 & M2) and two butyrate fed microcosms (M3 & M4). All the microcosms were purged with pure nitrogen gas before each TCE addition. Microcosm M5 fed lactate was maintained as a control microcosm.

Between 120 days and 260 days, TCE addition tests were performed for these microcosms (Appendix). TCE (6 μmol) was added two times to M1 and M3 on day 121 and 156, while 2 μmol of TCE was added six times to M2 and M4 during the same time period. On day 201, 15 μmol of TCE was added to all four microcosms. This experiment was performed based on the results from Chapter 4, to stimulate VC transformation to ethylene with TCE additions. The bulk amount

of TCE addition was compared to small dose of TCE additions in terms of ethylene production rate enhancement. Each microcosm showed similar ethylene production rates whether TCE was added in bulk or small doses in equal total mass added. The rate of VC transformation to ethylene increased by both bulk and step-wise TCE additions. Similar pattern of ethylene production rate increase was observed as the repeated TCE additions with non-nutrient amended microcosms. Higher TCE concentration additions followed in this section of the report.

5.3.1 First high concentration of TCE addition

Approximately 100 and 30 mg/L of TCE were added to propionate fed and butyrate fed microcosms (Figure 5.6 and Figure 5.7) respectively. TCE was transformed within 4 weeks (28 days) in all the microcosms. VC was the major product of this rapid TCE transformation, and cis-DCE was short-lived and never accumulated, but was rapidly transformed to VC. An insignificant amount of ethylene was generated during the period when TCE was being transformed to VC.

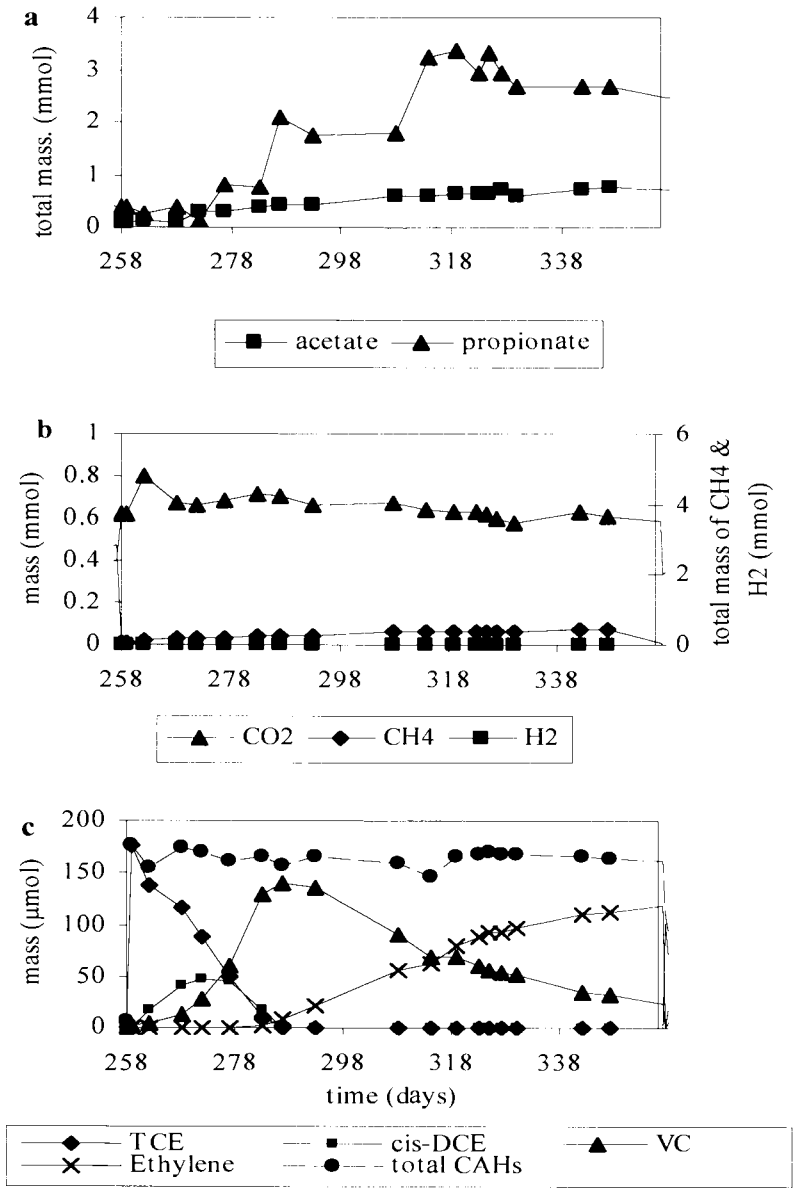

Figure 5.6. TCE addition to microcosm M2 (propionate, 100 mg/L of TCE addition). Total 5.2 mmol of propionate was added on day 277 (0.81 mmol), 287 (1.3 mmol), and 314 (1.5 mmol). Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

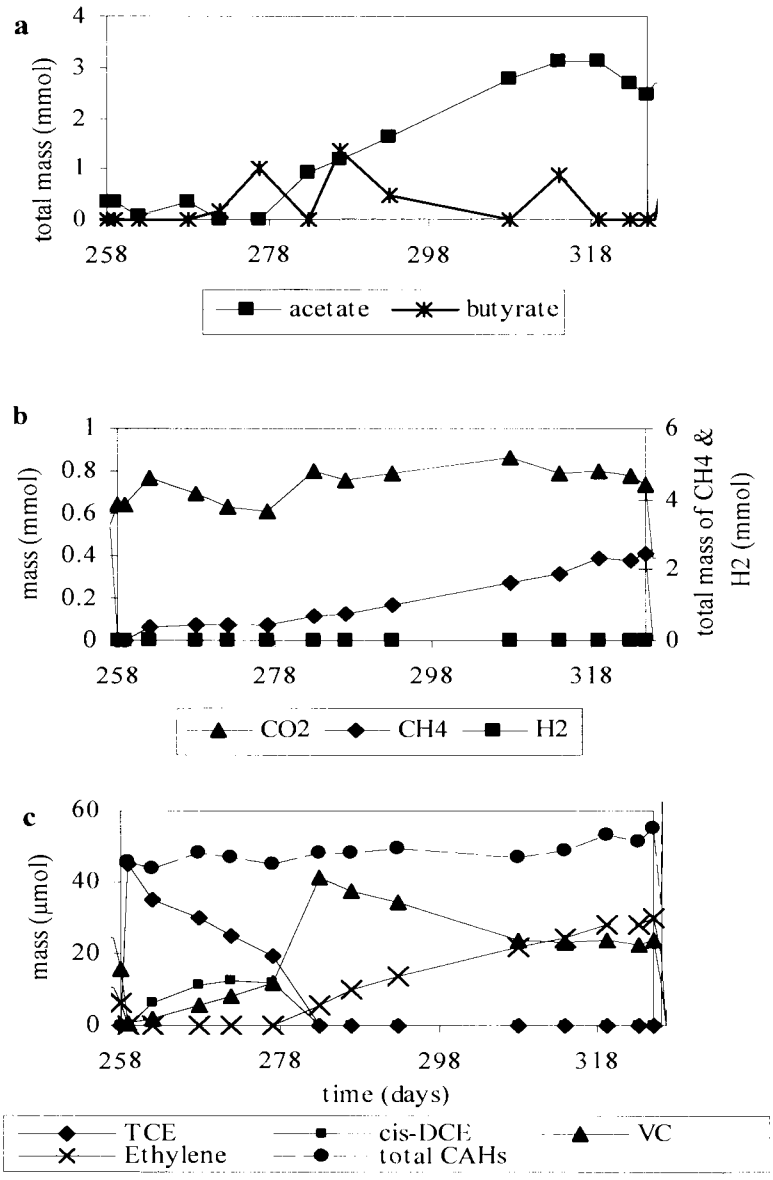

Figure 5.7. TCE addition to microcosm M4 (butyrate, 30 mg/L of TCE addition). Total 3.4 mmol of butyrate was added on day 277 (1.02 mmol), 287 (1.36 mmol) and 314 (0.88). Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

This was observed for all four microcosms regardless of added TCE concentrations and substrates. With the greater amount of TCE added microcosms faster rates that TCE transformed were observed. For instance, the TCE transformation rate at an aqueous concentration at 100 mg/L of TCE was 6.25 μmol/day, while 30 mg/L microcosms produced one third of the rate (2 μmol/L). Thus the rates of TCE transformation were proportional to TCE concentrations.

VC transformation rates were also dependent on the initial concentrations of TCE and subsequently VC. However, normalized VC transformation rates with respect to the amount of TCE added indicated that the VC transformation rates depended on the initial concentration of TCE. VC transformation rates also are correlated with the rates of TCE transformation. These microcosms showed similar ratio of VC transformation rate to TCE transformation rate. However, there was no significant evidence of dependence of TCE and VC transformation with different substrates at this particular range of TCE concentration.

There was less than a 20% of difference between VC transformation rates and ethylene production rates (Table 5.3). The earlier low concentration study showed factor of six differences between these two rates. The similar transformation rates indicated near stoichiometric conversion of VC to ethylene in the range of TCE concentrations between 30 mg/L and 100 mg/L.

The fraction of VC conversion to ethylene increased to range between 55% and 82%. This is a much higher conversion rate compared with the low TCE concentration study, which showed 3.3% to 19.1%.

Table 5.3. TCE and VC transformation rates resulting from the first addition of high TCE concentration.

		100 mg/L of TCE (propionate fed)		30 mg/L of TCE (butyrate fed)	
		M1	M2	M3	M4
TCE transformation rates	(μmol/day)	7.01 (day 259-287)	6.67 (259-283)	2.07 (259-277)	1.34 (259-277)
VC transformation rates	(μmol/day)	1.45 (day 283-357)	1.60 (287-357)	1.04 (283-326)	0.68 (283-326)
Eth. Production rates	(μmol/day)	1.25 (day 262-357)	1.32 (262-357)	0.66 (262-325)	0.48 (262-325)
Normalized VC transformation rate with mass of TCE added	(μmol/day/μmol)	8.29E-03	9.14E-03	1.22E-02	1.07E-02
Ratio of VC rate/TCE rate		0.21	0.24	0.50	0.51
Fraction of TCE converted to ethylene	%	82	78	60	55

5.2 mmol of propionate was added in M2, but very small fraction was converted to acetate, and rest of the propionate remained in the microcosm. Methanogenic activity was apparently inhibited by the 100 mg/L TCE concentration (Figure 5.6). In contrast, the added butyrate was instantaneously fermented to acetate. Methane production was activated when butyrate was added on 278. The methanogenic activity was relatively active compare to the microcosm with 100 mg/L of TCE and propionate (Figure 5.7). In the earlier low TCE transformation experiment, methane was actively produced as TCE transformation to VC and ethylene occurred in the microcosms, regardless of the substrates added. The average methane production rates from the earlier study were 0.051 and 0.055 mmol/day for propionate fed microcosms while 0.058 and 0.046 mmol/day for

butyrate fed microcosms. As the TCE concentration was increased to 100 mg/L, methanogenic activity was suppressed. The average methane production rates were 0.004 mmol/day (100 mg/L of TCE) and 0.06 mmol/day (30 mg/L of TCE). These methane production rates indicated that microcosms with lower TCE concentration range (30 mg/L) showed less inhibition on of methanogenic activity. Meanwhile, higher concentration of TCE (100 mg/L) added microcosms showed distinctively lower methane production rate. However, the substrates also differed in these tests, so other factors may be important.

To better determine the effect of different substrates on TCE and VC transformation rates, as well as methanogenesis, second time TCE addition experiment has been conducted at even higher TCE concentrations.

5.3.2 Second high concentration of TCE addition

Prior to the second addition of TCE, the microcosms were purged with pure nitrogen gas. After purging the residual VC, ethylene and methane, higher TCE concentrations were introduced. M1 (propionate amended) and M3 (butyrate amended) microcosms were selected to test TCE concentrations of approximately 200 mg/L (260 μmol of TCE in total mass). To M2 (propionate amended) and M4 (butyrate amended) microcosms, 100 mg/L of TCE was introduced.

TCE transformation proceeded immediately upon TCE addition at a faster rate than in the previous high concentration of TCE addition test (Figure 5.8 and

Figure 5.9). Within a period of two weeks, 200 mg/L of TCE concentration was transformed to VC and ethylene. As observed in the initial TCE addition test, a limited amount of cis-DCE accumulated as TCE was transformed. VC was the TCE dechlorination product that accumulated. Regardless of the amended substrates, TCE was completely transformed to VC and some ethylene in less than 2 weeks. For the microcosms with 100 mg/L of TCE added, TCE was transformed to VC and ethylene, similar to 200 mg/L test.

Faster TCE transformation rates were observed in the 200 mg/L TCE microcosm (14.4 and 17.2 μmol/day for M1 and M3, respectively) than the microcosms fed 100 mg/L of TCE (9.18 and 9.38 μmol/day for M2 and M4, respectively). Based on the rates the different substrates had minimal impact on TCE transformation (Table 5.4). The initial concentration of TCE was a key affecting the transformation rates.

VC accumulated at both concentrations as a major product of dechlorination of TCE, and was then effectively transformed to ethylene. Transformation of VC to ethylene proceeded in same manner as in the first high TCE concentration addition. Approximately 30 days after TCE addition, nearly 90 % of the TCE added (200 mg/L) was converted to ethylene and 10% remained as VC.

Table 5.4 presents TCE and VC transformation rates during the addition of high TCE concentrations.

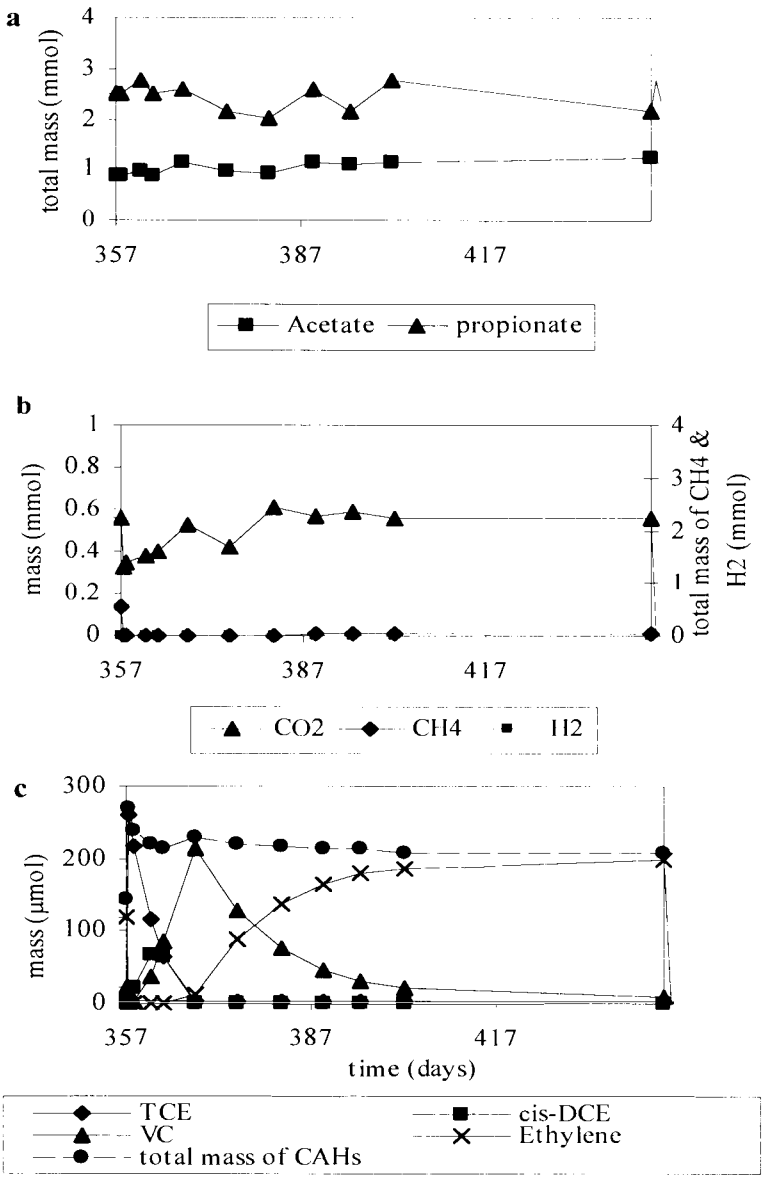

Figure 5.8. TCE addition to microcosm M1 (propionate, 200mg/L after 100 mg/L) (refer Table 5.1 for time and amount). Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

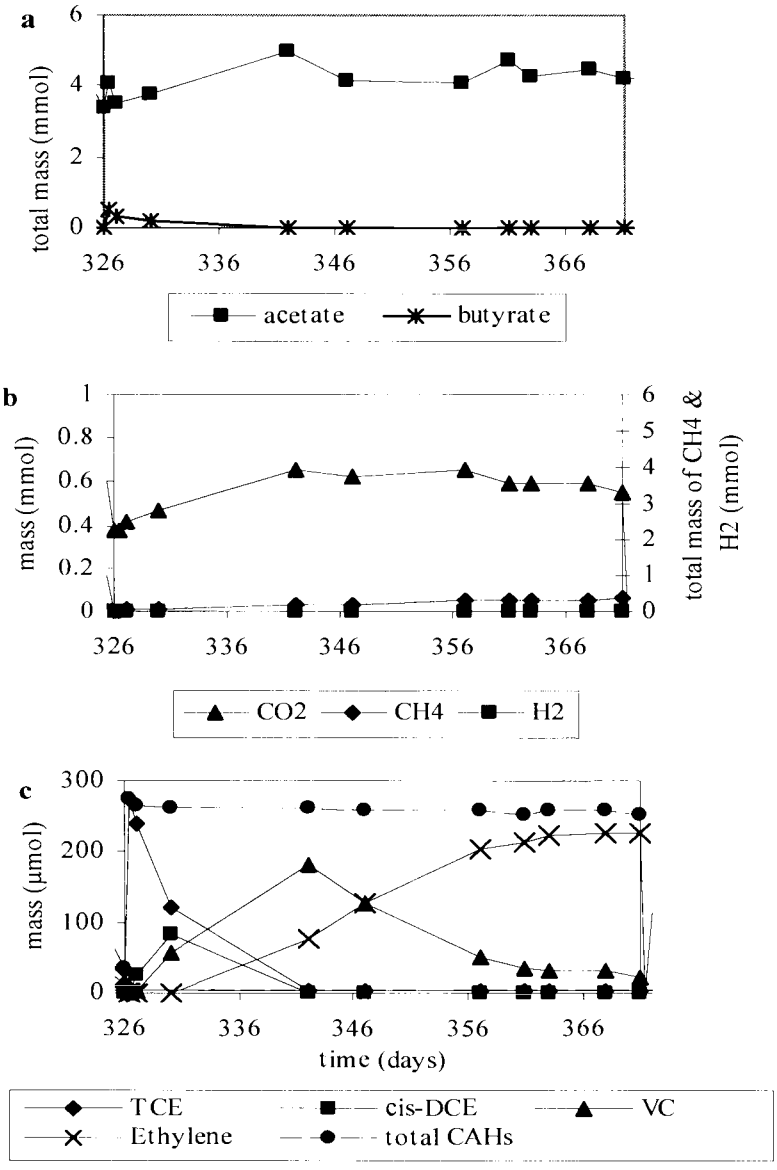

Figure 5.9. TCE addition to microcosm M3 (butyrate, 200mg/L after 30 mg/L) (refer Table 5.1 for time and amount). Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

Microcosms with 200 mg/L of TCE added showed 1.5 to 2 times faster TCE transformation rates than the 100 mg/L microcosms. Bottles with 200 mg/L showed approximately 3 times faster VC transformation rates than with the 100 mg/L. However, after normalizing VC transformation rates respect to the amount of TCE added showed that transformation rates were very closed for both 100 and 200 mg/L of initial TCE concentrations. Similar normalized rates were observed from the lower concentration additions the first high TCE addition, as well as the low 3 mg/L of TCE concentration as an indication of VC transformation dependency on TCE concentration.

Table 5.4. Transformation rates for the second TCE addition.

		200 mg/L of TCE		100 mg/L of TCE	
		M1 (propionate)	M3 (butyrate)	M2 (propionate)	M4 (butyrate)
TCE transformation rate	(μmol/day)	24.54 (day 357-368)	17.59 (326-342)	6.67 (259-277)	9.27 (326-342)
VC transformation rate	(μmol/day)	5.73 (day 368-402)	5.52 (342-371)	1.84 (287-357)	3.19 (342-371)
Eth. Production rate	(μmol/day)	4.78 (day 363-402)	5.16 (327-371)	1.27 (259-357)	2.76 (327-371)
Ratio of VC/TCE rates		0.23	0.31	0.28	0.34
Normalized VC transformation rate to mass TCE added	(μmol/day/μmol)	0.022	0.021	0.018	0.015
Fraction of TCE converted to ethylene		95%	87%	91%	76%

M2 (propionate fed) bottle received two additions of 100 mg/L of TCE. Both TCE and VC transformation rates increased with the second addition of 100 mg/L of TCE. The normalized VC transformation respect to initial amount of TCE added increased from first time to second time 100 mg/L of TCE addition. Microcosms with 200 mg/L TCE added showed higher ratio of VC to TCE transformation rates. This higher TCE concentration addition continued to the VC transformation rates. This indicated that the increase in TCE and VC transformation rates were caused by factor other than concentration alone. The other factor is likely growth of microorganisms that utilize TCE, and possible cis-DCE and VC, as electron acceptors via haloresipratory.

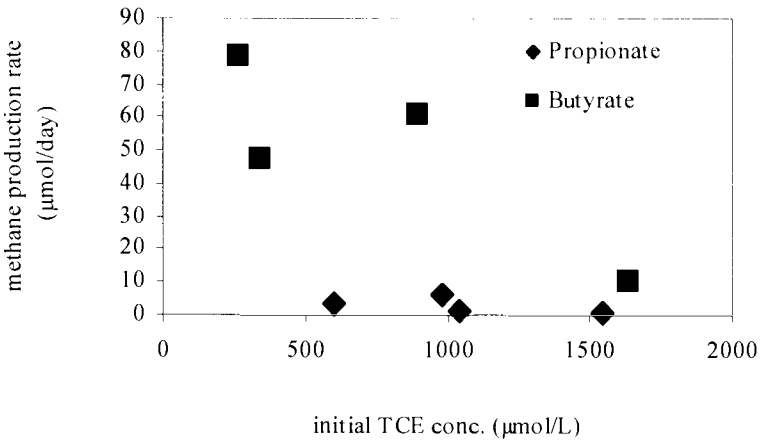

Figure 5.10. Methane production rate along initial TCE concentrations.

Methane production rates decreased at the higher concentrations (200 mg/L, 1500 μM) of TCE. Figure 5.10 presented the inhibition of methanogenesis at the high concentrations of TCE. The butyrate amended microcosms supported methanogenesis at a relatively higher concentration range of TCE compared to the propionate amended microcosms. However, methanogenesis was inhibited at TCE concentrations above 200 mg/L, regardless of the substrates fed.

Electron mass balance (Table 5.5) on substrates, methane, and chlorinated ethylene showed an electrons efficiency for dehalogenation of up to 26 % of when 200 mg/L of TCE was added. This higher electron efficiency likely resulted from methanogenesis inhibition at high TCE concentrations, and the growth of a halorespiring microbial population. The higher electron efficiency is correlated with higher TCE and VC transformation rates. Shown in Table 5.5, electron efficiency for methanogenesis decreased for the second TCE addition when higher TCE was added, indicating the inhibition of methanogenic activity.

Table 5.5. Electron mass balance for first and second TCE additions.

	first TCE addition			Second TCE addition		
	% recovered	% used for methanogenesis	% used for dechlorination	% recovered	% used for methanogenesis	% used for dechlorination
M1	98.9	41.8	6.6	99.7	12.6	26.3
M2	95.9	41.8	8.6	105.1	29.2	10.7
M3	99.3	45.2	0.7	99.3	41.3	19.5
M4	96.9	70.8	0.5	99.9	0	8.2

5.4 Reintroduction of Low Concentration of TCE

After M2 (propionate fed) bottle received 100 mg/L of TCE twice, TCE and VC transformation rates increased (Table 5.3 and 5.4). The increase of rates likely resulted from the growth of a halorespiring population on TCE and possible cis-DCE. This part of the experiment conducted to verify possible microbial population growth by adding low TCE concentrations after the high concentration additions.

M1 and M3 bottles were selected and 3.0 μmol/L and 3.4 μmol/L of TCE was reintroduced respectively (Figure 5.11). TCE was completely transformed after 3 days. VC transformation occurred over the next 40 days. The fraction of VC converted to ethylene increased to 94% and 95% for M1 and M3 respectively. The conversion increased by a factor of 8 compared to the earlier low concentration tests prior to the addition of high TCE concentrations. The transformation of VC to ethylene was completed in 40 days compared to 120 days for earlier low concentration test that yield a much lower fraction al conversion of only 20%. The kinetic test demonstrated the enhancement of the transformation is not only due to the concentration effects alone, but likely due to the growth of a halorespiring population.

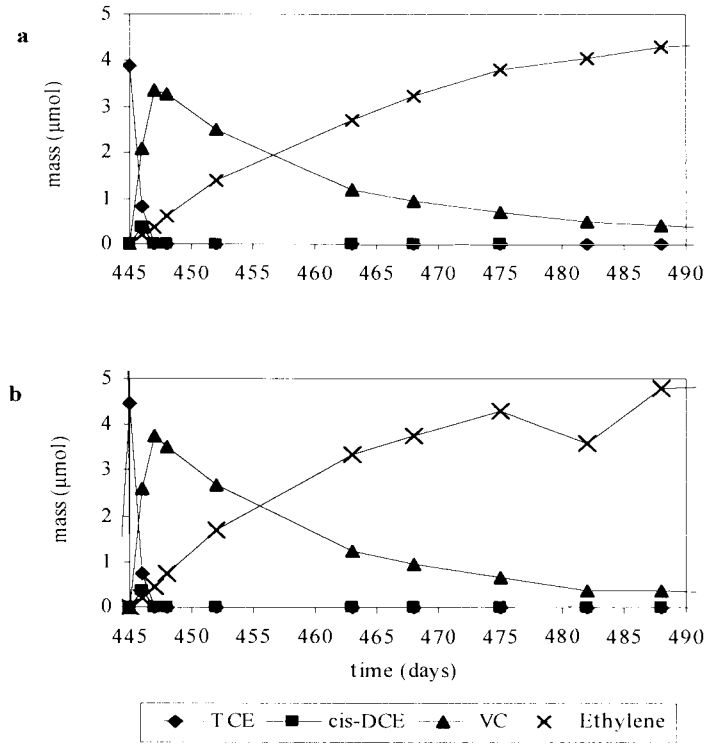

Figure 5.11. Reintroduction of low TCE concentrations in to (a) microcosm M1 (propionate), and (b) microcosm M3 (butyrate).

5.5 Kinetics of TCE and VC Transformation Rates

Both TCE and VC transformation rates increased with increasing TCE concentrations with both first and second TCE additions. Figure 5.12 presents TCE and VC transformation rates and ethylene production rate as a function of the initial concentration of TCE. Whether the microcosms were butyrate or propionate amended, increasing TCE concentration enhanced the rates of TCE transformation.

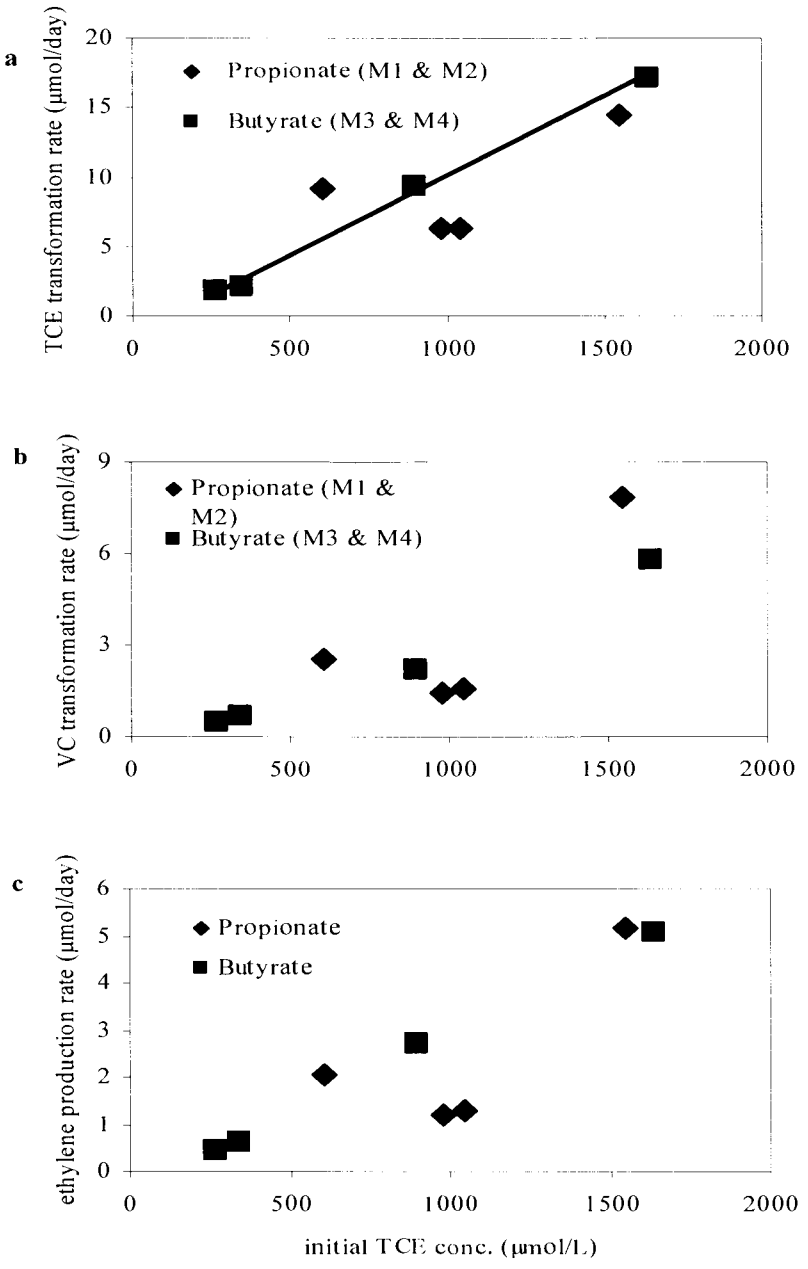

Figure 5.12. TCE and VC transformation rates with initial TCE concentrations. (a) TCE dechlorination rate, (b) VC transformation rate, and (c) ethylene production rate.

A similar phenomenon was observed for the rates of VC transformation and ethylene production. Rates of ethylene production also increased when normalized to the amount of TCE added. Additionally normalized TCE and VC transformation rates (Table 5.3 and 5.4) and normalized ethylene production rates support effect of TCE concentration on enhancing the rates of transformation.

VC transformation and ethylene production data shown in Figures 5.8 to 5.9 appear to follow first-order kinetic trends. The rate data were fit to the first-order reaction kinetic model for different TCE transformation tests. The first-order kinetics are given as:

$$\frac{dS}{dt} = \frac{-xKS}{K_S + S} \tag{1}$$

$$\frac{dS}{dt} = \frac{-xKS}{K_S} \; where \; k' = \frac{-xK}{K_S} \tag{2}$$

$$\frac{dS}{dt} = k'S \tag{3}$$

$$\ln \frac{S}{S_O} = -k't \tag{4}$$

where S is concentration of VC and ethylene and t is time. K_S is half-velocity constant, x is concentration of biomass and K is maximum rate of substrate utilization per unit mass of microorganism. (1) full Monod equation, (2) modified

Monod equation for the VC transformation rate. Replacement of the equation with positive sign would represent the rate of ethylene production.

Using Monod kinetics in a batch reactor, a relatively high K_S compared to S was assumed to modify from equation (1) equation (2) and (3) to yield first-order kinetic model. For this kinetic study, we simply implied the first-order kinetic model to fit the VC transformation data as well as production rate of ethylene under the assumption of k' term represents $\frac{xK}{K_S}$ with unknown biomass concentration in the microcosm system. The model assumes the change of x, (biomass) is small during the time course of the analysis.

The microcosms data was tested for fit to the first-order kinetic model by plotting ln (Ca/Cao) vs. time using equation (4). After verifying the laboratory data following the first-order kinetic trend by determining linearity of the data, kinetic rate constant, representing $\frac{xK}{K_S}$, was determined by measuring the slope of the ln (Ca/Cao) vs. time plot. Model generated data was created using the kinetic constant to show the deviation of the microcosm data to the model data. Using the same kinetic constant, ethylene production model was also created. Smooth line representing the model generated data nearly followed the microcosm data (Figure 5.13 and Figure 5.14) both figures. Great degree of VC transformation data fit to the model generated data demonstrated the first-order kinetic for VC transformation. The deviation of ethylene production data to the model was greater. Results are presented for the first addition of high TCE concentration in

Figure 5.13 and the second addition in Figure 5.14. This is consistent with the difference between the VC transformation rate and ethylene production rate (Table 5.3 and Table 5.4). The earlier low TCE concentration study showed greater rate difference between VC transformation and ethylene production than high TCE addition studies. Model fitting also showed the greater deviation from earlier low concentration of TCE study than the later high TCE concentration study. Kinetic constants were compared for any correlation between TCE additions and VC transformation rates. Table 5.6 presents those constants. The deviation of the microcosm data to the model is very minimal for the VC data from all of the microcosms.

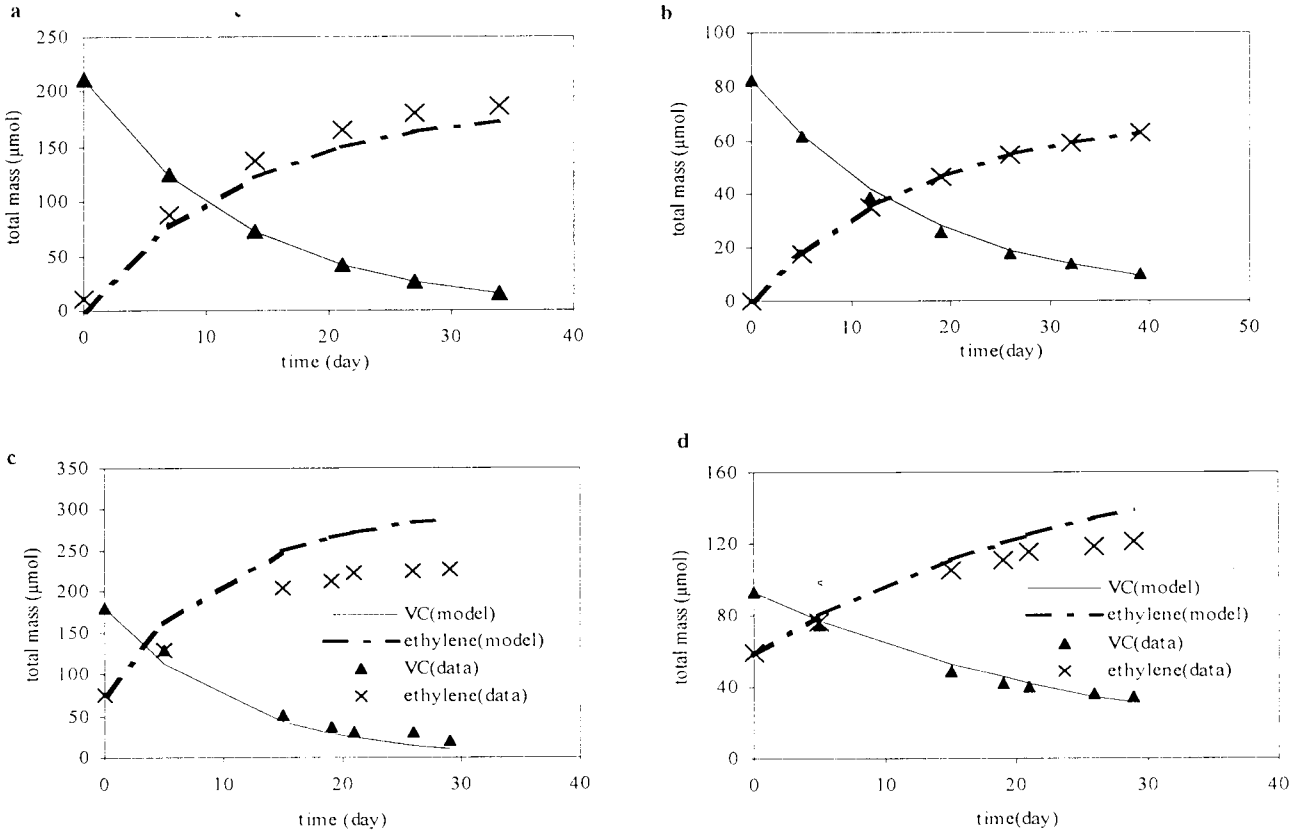

Figure 5.14. Data fitting to the first-order kinetics for second high TCE concentration. (a) M1, k'=0.077, (b) M2, k'=0.059, (c) M3, k'=0.096, and (d) M4, k'=0.060.

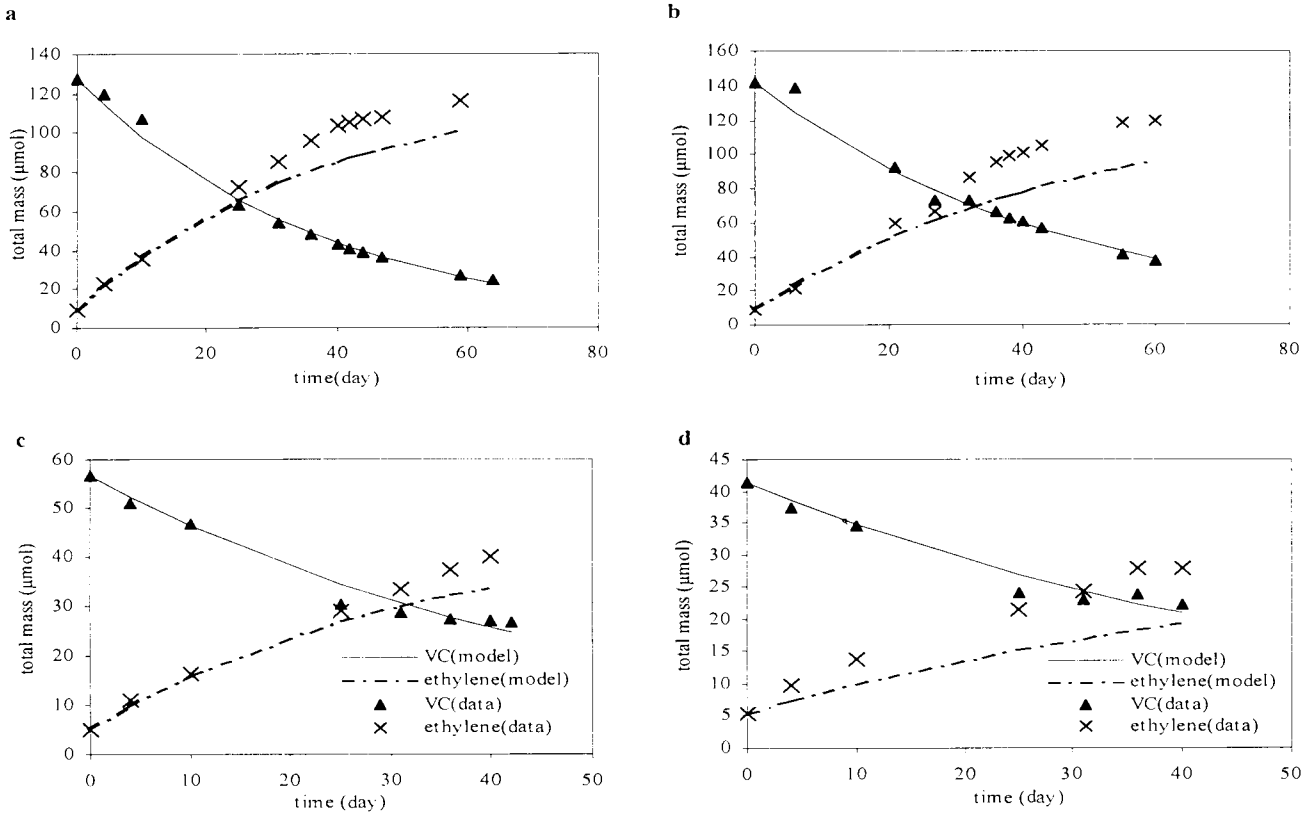

Figure 5.13. Data fitting to the first-order kinetics for first high TCE concentration. (a) M1, k'=0.027, (b) M2, k'=0.022, (c) M3, k'=0.20, and (d) M4, k'=0.020.

Table 5.6. First-order kinetic constants for VC transformation to ethylene.

	First-order Kinetic Constant, k' for VC transformation (1/day)					
	Early Low TCE	First High TCE		Second High TCE		Low TCE
	(3.3 mg/L)	TCE (mg/L)	k'	TCE (mg/L)	k'	(3.3 mg/L)
M1	0.010	100	0.027	200	0.077	0.062
M2	0.019	100	0.022	100	0.060	ND
M3	0.0071	30	0.020	200	0.096	0.067
M4	0.012	30	0.020	100	0.060	ND

The results of all the kinetic analyses are reported on Table 5.6. The rate constant increased by a factor of three or four between the first and second TCE additions, even if same TCE concentration was added twice (M2). Although low concentration of TCE was reintroduced, first-order kinetic constants of VC transformation definitely increased by factor of six compared to a similar concentration before the high TCE additions. The kinetic constants compare very well with those values obtained from the second high TCE concentration addition.

This kinetic study showed VC transformation followed the first-order kinetics over broad range of VC concentrations. Along with the VC transformation, ethylene formation from the final step of TCE dechlorination also followed the first-order kinetics with similar rate kinetic constants. The results indicated a relatively high K_s value for Monod kinetics for the transformation of VC to ethylene. The results also indicated an increase in first-order-rates that are likely associated with the growth of a dehalogenating culture with high TCE concentration transformation. The results also indicated the growth of dehalogenating population happened during high TCE concentration

transformation. Thus the assumption of little biomass change to modify the Monod equations was held for the first-order kinetic study for VC transformation to ethylene. Shown in Figure 5.8 and Figure 5.9, TCE transformation completed within two weeks. Dechlorinating microorganisms likely grew on TCE and increased in population using TCE as terminal electron acceptor during this short time period. Figure 5.15 illustrates that biomass growth occurred during TCE transformation period using TCE as a terminal electron acceptor. The assumed first-order rate model indicates little changes in biomass during VC transformation period. This is consistent with observations of Distefano (1999), who indicates little or no growth on VC as an electron acceptor.

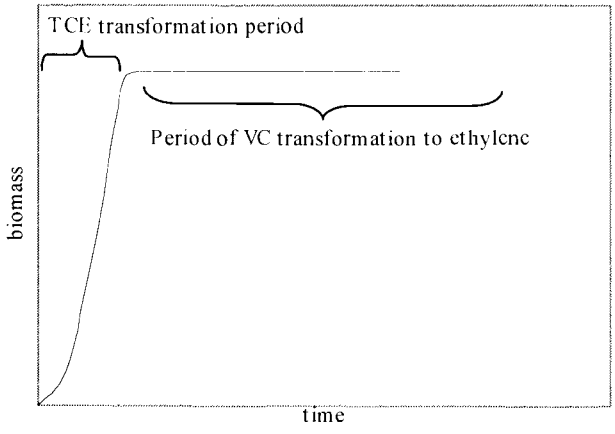

Figure 5.15. Dechlorinating population growth during TCE transformation period.

5.6 Discussion/Conclusion

The following summarizes the key findings of this study.

1. Essentially complete dechlorination of TCE to VC and to ethylene was achieved after two additions of high TCE concentration. DiStefano et al. (1991) a similar result was reported. Ethanol enriched mixed culture showed slow rate of VC transformation to ethylene (Freedman and Gossett, 1989). DiStefano et al. (1991) added high concentrations of PCE to achieve complete anaerobic PCE transformation with the same mixed culture.

In my study TCE and VC transformation rates were enhanced regardless of the choice between low hydrogen yielding substrates, butyrate and propionate. This indicated the dependence of dehalogenation on substrates was very minimal between these two particular substrates. Hydrogen is believed to be the primary source for electrons in reductive dechlorination. Hydrogen was seldom detected, at out of detection limit.

2. Our system initially showed active methanogenesis utilizing both butyrate and propionate at low TCE concentrations. At high concentrations of TCE, methane production dramatically decreased with active dechlorination progressing in the system over the same period. Previous studies (DiStefano at el., 1991 & Nielsen and Keasling, 1999) indicated that high concentration PCE or TCE could inhibit methanogenesis without suppressing dechlorination. This suppression of methanogenesis would allow dechlorinator to utilize hydrogen without competition with methanogens. Comparison between two different substrates tested indicated

that butyrate supported methanogenesis below TCE concentrations of 200 mg/L. When the initial TCE concentration exceeded 200 mg/L, microcosms with either substrates showed minimal methane production. This little difference in methane production with less than 200 mg/L of TCE concentration can be reasoned by the fermentation pattern of both substrates. Propionate got slowly fermented to acetate to yield very limited amount of hydrogen pool in the system. In competition of hydrogen, dechlorinator out compete suppressed methanogen. Meanwhile butyrate tended to be fermented acetate very rapidly. This may have generated burst of hydrogen supporting both methanogenesis and dechlorination.

The higher electron transfer efficiency during the high TCE concentration additions would be correlated with inhibition of methanogenic activity. Shown in Table 5.4, the electron efficiency for dechlorination was over 20% after second high TCE concentration addition compared to less than 10 % from the first high TCE concentration.

3. Fitting the transformation trends to a first-order kinetic model showed 5 to 6 fold increase in the pseudo first-order kinetic constant, compared to the earlier results at low TCE concentration. Nielsen and Keasling (1999) reported results for TCE and PCE dechlorination under both saturating and under saturating conditions. The study also showed TCE and VC transformation following the first-order kinetics at every concentration level. The use of first-order rate model eliminates rate effect that result from concentration alone. The model also assumes a high Ks value. The kinetic study supported the assumption of a high K_S by fitting the

microcosm data to the first-order kinetic model at both low and high TCE concentrations (Figure 5.12).

4. This apparent TCE and VC degradation rate enhancement suggested two possibilities. The first possibility is a stimulatory effect of high TCE concentration. Transformation of high TCE concentration definitely supported the rate increase of TCE transformation to ethylene. However, reintroduction of low concentration of TCE maintained the same first-order rates as high TCE concentration. The results indicated a microbial population was growing that utilized TCE as a terminal electron acceptor for growth. These microorganisms were also capable of transforming VC to ethylene. With this culture the last step of VC transformation to ethylene is likely cometabolic and nature, and rates must be enhanced through growth on TCE and possible cis-DCE to get effective transformation of VC to ethylene.

CHAPTER 6
ENGINEERING SIGNIFICANCE

The primary objective of this study was to study the effect of TCE addition on the enhancement of slow VC transformation rate to ethylene. The Point Mugu site experienced VC accumulation after addition of lactate as an electron donor for TCE dechlorination. The results of this study showed two significant findings applicable to the bioattenuation and enhanced remedial processes. First part of the results showed definite dependence of VC transformation enhancement on TCE additions, under anaerobic conditions. In the groundwater recirculating system with injection and extraction wells, groundwater with low TCE concentration extracted from extraction wells can be introduced to injection wells where biological transformation of TCE to VC is active. The low TCE content in the extracted groundwater could have a stimulatory effect on enhancing accumulated VC transformation to ethylene in subsurface. From an operational point of view, the recirculation of TCE contaminated groundwater into bio active injection well would minimize the cost on remediation of TCE contaminated groundwater, as well as promoting VC transformation to ethylene. Even if there are microorganism(s) capable transforming TCE to ethylene, microbes appear to require a certain amount of TCE for grow to provide for effective rates of VC transformation.

Thus at many sites the natural accumulation of VC with high concentration of TCE additions, our study observed near complete dechlorination of TCE to

ethylene without a significant accumulation of more toxic VC in the microcosms. This indicates potential for remediation of high TCE concentrations near contaminated source zone with these particular consortia of microorganisms. The kinetic study indicated the possible microbial population growth during high concentration TCE transformation. This enhancement on rate of TCE and VC transformation was due to effective electron utilization by dechlorinator with inhibition of methanogenesis. Growth on TCE would result in an efficient process for the remediation of high concentration source zones. This is supported by the electron transfer efficiency of up to 26%, at high TCE concentration observed in the microcosm studies.

The most difficult situation for chlorinated solvent remediation is DNAPL contaminated sites. Since DNAPL becomes a source of chlorinated compounds contamination in groundwater, direct remediation of DNAPL would reduce both time and cost of decontamination. Our mixed culture showed the capability of degrading up to 200 mg/L concentration of TCE to ethylene within 70 days of incubation in batch reactor. The solubility of PCE and TCE are 200 mg/L and 1100 mg/L, respectively. Thus these microorganisms can degrade concentration associated with NAPL contamination. Transforming DNAPL contamination under biological treatment was troublesome task that most remediation engineers encounter with. However, the result of this microcosms study can serve as a good sign for stimulating indigenous microorganisms to treat DNAPL contamination.

BIBLIOGRAPHY

Bagley, D.M., and J.M. Gossett. 1990. Tetrachloroethene Transformation to Trichloroethene and cis-1,2-Dichloroethene by Sulfate-Reducing Enrichment Cultures. *Applied and Environmental Microbiology. 56:*2511-2516.

Baker, K.H., and D.S. Herson. 1994. Microbiology and Biodegradation. In: K.H. Baker, and D.S. Herson, *Bioremediation*, pp. 9-60. McGrawHill, Inc., New York, NY.

Ballapragada, B. S., H.D. Stensel, J.A. Puhakka, and J.F. Ferguson. 1997. Effect of hydrogen on reductive dechlorination of chlorinated ethenes. *Environmental Science and Technology. 31*:1728-1734.

Bouwer, E. J., and P.L. McCarty. 1983. Transformation of 1- and 2-carbon halogenated aliphatic organic compounds under methanogenic conditions. *Environmental Science and Technology. 45*: 1286-1294.

Bouwer, E. J., B.E. Rittmann, and P.L. McCarty. 1981. Anaerobic degradation of halogenated 1- and 2-carbon organic compounds. *Environmental Science and Technology. 15*:596-599.

Bouwer, E. J., and, J.P. Wright. 1988. Transformation of trace halogenated aliphatics in anoxic biofilm columns. *Journal of. Contamination. Hydrology.* 2:155-169.

Carr, C.S., and J.B. Hughes. 1998. Enrichment of High-Rate PCE Dechlorination and Compariative Study of Lactate, Methanol, and Hydrogen as Electron Donors To Sustain Activity. *Environmental Science and Technology. 32*:1817-1824.

Cartwright, G.C. 1991. Limitation of Pump and Treat Technology. *Pollution Engineering. Nov.*:64-68

Cookson, Jr. J.T., 1995. Microbial Systems of Bioremediation. In J.T. Kookson, Jr., *Bioremediation Engineering: Design and Application*, pp. 51-94. McGrawHill, Inc., New York, NY.

De Bruin, W.P., M.J.J. Kotterman, M.A. Posthumus, G. Schraa, and A.J.B. Zehnder. 1992. Complete Biological Reductive Transformation of Tetrachloroethene to Ethane. *Applied and Environmental Microbiology. 58:*1996-2000.

DiStefano, T.D., J.M. Gossett, and S.H. Zinder. 1992. Gydrogen as an Electron Donor for Dechlorination of Tetrachloroethene by an Anaerobic Mixed Cultre. *Applied and Environmental Microbiology. 58*:3622-3629.

DiStefano, T.D. 1999. The Effect of Tetrachloroethene on Biological Dechlorination of Vinyl Chloride: Potential Implication for Natural Bioattenuation. *Water Research. 33*:1688-1694.

Ensign, S. A., M.R.Hyman, and D.J. Arp, 1992. Cometabolic degradation of chlorinated alkenes by alkene monooxygenase in a propylene grown *Xanthobacter* strain. *Applied and Environmental Microbiology. 53*:3038-3046.

Fathepure, B. Z., and S.A. Boyd.1988. Dependence of tetrachloroethylene dechlorination on methanogenic substrate consumption by *Methanosarcina* sp. Strain DCM. *Applied and Environmental Microbiology. 54*:2976-2980.

Federal Register. 1989. National Primary and Secondary Drinking Water Regulations: Proposed Rule. *54*:22062-22160.

Fennell, D.E., and J.M. Gossett. 1997. Comparison of Butyric Acid, Ethanol, Lactic Acid, and Propionic Acid as Hydrogen Donors for the Reductive Dechlorination of Tetrachloroethene. *Environmental Science and Technology. 31*:918-926.

Freedman, D.L., and J.M. Gossett. 1989. Biological Reductive Dechlorination of Tetrachloroethylene and Trichloroethylene to Ethylene under Methanogenic Conditions. *Applied and Environmental Microbiology. 55*:2144-2151.

Gerritse, J., Renard, V., Gomes, T.M. P., Lawson, P.A., Collins, M.D. and Gottschal, J.C. 1996. Desulfitobacterium sp. Strain PCE1, and anaerobic bacterium that can grow by reductive dechlorination of tetrachloroethene or orthro-chlorinated phenols. Arch. Microbiol. 132-140.

Keeling, M. T., 1999, MS Dissertation, Bench-scale for the bioremediation of chlorinated ethylenes at Point Mugu Naval Air Weapons Station, Point Mugu California, IRP Site 24.

Loffler, 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. Microbiology. 65: 4049-4056

Li, Y., S. Lam, and H.H. Fang. 1996."Interactions between methanogenic sulfate reducing and syntrophic acetogenic bacteria in the anaerobic degradation of benzoate." *Water Research. 30*:1555-1562.

Little, C. D., A.V. Palumbo, S.E. Herbes, M.E. Lidstrom, R.L. Tyndall, and P.J. Gilmer. 1988. "Trichloroethylene biodegradation by a methane-oxidizing bacterium." *Applied and Environmental Microbiology*. 54:951-956.

Madigan, M.T., J.M. Martinko, and J. Paker. (8^{th} Ed.). 1997. *Biology of Microorganisms,* 126 pp. Prentice Hall International, Inc., Upper Saddle River, New Jersey.

Maymo-Gatell, X., Y. Chien, J.M. Gossett, and S.H. Zinder. 1997. Isolation of a Bacterium That Reductively Dechlorinates Tetrachloroethen to Ethene. *Science. 276:*1568-1571.

McCarty, P.L., *Environmental Biotechnology*, 1987,Bioengineering Issues Related to In-Situ Remediation of Contaminate Soils and Groundwater. Omenn, G.S. (Ed), pp. 143-162. Plenum Press, New York,.

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

Munter, J.E., and S.P. DeVries. 1987 *Toxic Law Reporter. 14*:874

Nelson, M. J. K., E.J. Montgomery, E.J. O'Neill, and P.H. Pritchard. Aerobic metabolism of trichloroethylene by a bacterial isolate. *Applied and Environmental Microbiology. 52*:383-384.

Nielson, R.B., and J.D. Keasling. 1999. Reductive Dechlorination of Chlorinated Ethene DNAPLs by a Culture Enriched from Contaminated Groundwater." *Biotechnology and Bioengineering. 62*:160-165.

OHM Remediation Services. 1998. Site Cleanup at 14 Former Underground Storage Tanks Sites, Naval Weapons Station, Point Mugu, California – Draft Technical Memorandum: IRP Site 24.

Parsons, F., Wood, P.R., and Demarco, J. 1984. Transformations of Tetrachloroethene and Trichloroethene in Microcosms and Groundwater. *Journal of American Water Works Association. 76*:56-59.

Roberts, P.V., Schreiner, J., and Hopkins, G.D. 1982. Field Study of Organic Water Quality Changes During Groundwater Recharge in the Palo Alto Baylands. *Water Resources. 16*:1025-1035.

Sayre, I.M. 1988. International Standards for Drinking Water. *Journal of America Water Works Association. 80*:53-60

Schaumburg, F.D. 1990. Banning Trichloroethylene: Responsible Reaction or Overkill? *Environmental Science and Technology. 24:*17-22

Semprini, L., D. Grbic-Galic, P.L. McCarty. and P.V. Roberts. 1992. Methodologies for Evaluating In-Situ Bioremediation of Chlorinated Solvents. U.S. Environmental Protection Agency EPA/600/R-92/042, March 1992.

Semprini, L., G.D. Hopkins, and P.L. McCarty. 1994. A field and modeling comparison of in-situ transformation of trichloroethylene by methane utilizers and phenol utilizers. In bioremedication of chlorinated and polycyclic aromatic hydrocarbon compounds. CRC Press, 248-254.

Smatlak, C.R., and J.M. Gossett. 1996. Comparative Kinetics of Hydrogen Utilization for Reductive Dechlorination of Tetrachloroethene and Methanogenesis in an Anaerobic Enrichment Culture. *Environmental Science and Technology. 30*:2850-2858.

US Environmental Protection Agency. 1985. Substances found at Proposed and Final NPL Sites through Update Number Three. Document NPL-U3-6-3, US EPA. Washington, DC.

Vanelli, T., M. Logan, D.M. Arciero, and A.B. Hooper. 1990. Degradation of halogenated aliphatic compounds by the ammonia-oxidizing bacterium *Nitrosomonas europaea. Applied and Environmental Microbiology.56*:1169-1171.

Vogel, T.M., C.S. Criddle, and P.L. McCarty. 1987. Transformations of Halogenated Aliphatic Compounds. *Environmental Science and Technology. 21*:722-736.

Vogel, T.M., and P.L. McCarty. 1985. Biotransformation of Tetrachloroethylene to Trichloroethylene, Dichloroethylene, Vinyl Chloride, and Carbon Dioxide under Methanogenic Conditions. *Applied and Environmental Microbiology. 49*:1080-1083.

Yang, Y. and P.L. McCarty. 1998. Comepetition for Hydrogen within a Chlorinate Solvent Dehalogenating Anaerobic Mixed Culture. *Applied and Environmental Microbiology. 32*:3591-3597.

Westrick, J.J., J.W. Mello, and R.F. Thomas. 1984. The Groundwater Supply Survey. *Journal of American Water Works Association. 76:*52-59.

Wilson, B. H. 1985, Biotransformation of Trichloroethylene in Soil. *Applied and Environmental Microbiology. 49*:242-243.

Wilson, J.T., and B.H. Wilson. 1985. Biotransformation of Trichloroethlyene in Soil. *Applied and Environmental Microbiology. 49*:242-242.

Wilson, B.H., Smith, G.B., and Rees, J.F. 1986. Biotransformations of Selected Alkylbenzenes and halogenated Aliphatic Hydrocarbons in methanogenic Aquifer Material: a Microcosm Study. *Environmental Science and Technology. 20*:997-1002.

APPENDIX

Table A.1. Electron mass balance from early TCE/VC addition study

	LT1				LT3			
	initial	e- equv.	final	e- equiv	initial	e- equv.	final	e- equiv
	day 0		day 120		day 0		day 120	
Lactate	2.72	3.26E+04			2.69	3.23E+04		
propionate	3.023	4.23E+04	0.85	1.19E+04	3.25	4.55E+04	0.92	1.29E+04
acetate			3.49	2.79E+04			3.54	2.83E+04
TCE								
cis								
VC			22.79	91.16			5	20
ethy			2.82	16.92			0.81	4.86
CH4			2.89	2.31E+04			3	2.40E+04
SO4=			0.9	7.20E+03			0.96	7.68E+03
total		7.50E+04		7.02E+04		7.78E+04		7.29E+04

	LT2				LT4			
	initial	e- equv.	final	e- equiv	initial	e- equv.	final	e- equiv
	day 0		day 120		day 0		day 120	
Lactate	3.09	3.71E+04			2.68	3.22E+04		
propionate	2.76	3.86E+04	1.13	1.58E+04	2.9	4.06E+04	0.74	1.04E+04
acetate			2.76	2.21E+04			3.04	2.43E+04
TCE								
cis								
VC			17.63	70.52			4	16
ethy			6.81	40.86			1.08	6.48
CH4			2.44	1.95E+04			3.49	2.79E+04
SO4=			0.99	7.92E+03			0.93	7.44E+03
total		7.57E+04		6.55E+04		7.28E+04		7.01E+04

Initial and final number of lactate, propionate, acetate, butyrate, methane, and sulfate are in unit of mmol. Chlorinated ethylene are denoted in unit of μmol. e^- equivalent for each mole: lactate (8), propionate (14), butyrate (20), acetate (12), methane (8), sulfate (8), cis-DCE (2), VC (4), and ethylene (6).

Table A.2. Electron mass balance for M1 and M2.

M1

	First TCE addition				Second TCE addition			
	initial day 259	e- equv.	final day 357	e- equiv	initial day 357	e- equv.	final day 444	e- equiv
propionate	3.5	4.90E+04	2.5	3.50E+04	2.5	3.50E+04	2.15	3.01E+04
acetate	0.102	816	0.88	7040	0.88	7.04E+03	1.22	9.76E+03
TCE								
cis								
VC			20.64	82.56			8.73	34.92
ethy			118.65	7.12E+02			189.6	1.14E+03
CH4	16.85	1.35E+05	17.48	1.40E+05	16.92	1.35E+05	16.99	1.36E+05
total		1.85E+05		1.83E+05		1.77E+05		1.77E+05
recovered %				99				100

M2

	First TCE addition				Second TCE addition			
	initial day 259	e- equv.	final day 347	e- equiv	initial day 357	e- equv.	final day 402	e- equiv
propionate	3.76	5.26E+04	2.47	3.46E+04	2.48	3.47E+04	2.34	3.28E+04
acetate	0.08	6.40E+02	0.71	5.68E+03	0.82	6.56E+03	1.15	9.20E+03
TCE								
cis								
VC			29.8	119.2			10.3	41.2
ethy			125	7.50E+02			71.12	4.27E+02
CH4	17.44	1.40E+05	17.97	1.44E+05	17.52	1.40E+05	17.68	1.41E+05
total		1.93E+05		1.85E+05		1.81E+05		1.84E+05
recovered %				96				101

Initial and final number of lactate, propionate, acetate, butyrate, methane, and sulfate are in unit of mmol. Chlorinated ethylene are denoted in unit of μmol. e^- equivalent for each mole: lactate (8), propionate (14), butyrate (20), acetate (12), methane (8), sulfate (8), cis-DCE (2), VC (4), and ethylene (6).

Table A.3. Electron mass balance for M3 and M4.

M3

	First TCE addition				Second TCE addition			
	initial day 259	e- equv.	final day 325	e- equiv	initial day 325	e- equv.	final day 368	e- equiv
butyrate	2.83	5.66E+04	0	0	0.48	9.60E+03	0	0
acetate	0.31	2480	4.05	3.24E+04	4.07	3.26E+04	4.44	35520
TCE								
cis								
VC			26.69	106.76			31.16	124.64
ethy			41.65	2.50E+02			225	1.35E+03
CH4	18.05	1.44E+05	21.17	1.69E+05	19.79	1.58E+05	20.18	1.61E+05
total		2.03E+05		2.02E+05		2.00E+05		198434.64
recovered %				99				99

M4

	First TCE addition				Second TCE addition			
	initial day 259	e- equv.	final day 325	e- equiv	initial day 325	e- equv.	final day 371	e- equiv
butyrate	3.26	6.52E+04	0	0	0.51	1.02E+04	0	0
acetate	0.34	2.72E+03	2.45	1.96E+04	2.69	2.15E+04	1.09	8720
TCE								
cis								
VC			23.44	93.76			23.44	93.76
ethy			30.1	1.81E+02			121.53	7.29E+02
CH4	17.65	1.41E+05	22.84	1.83E+05	21	1.68E+05	23.75	1.90E+05
total		2.09E+05		2.03E+05		2.00E+05		2.00E+05
recovered %				97				100

Initial and final concentrations of lactate, propionate, acetate, butyrate, methane, and sulfate are in unit of mmol. Chlorinated ethylene are denoted in unit of μmol. e^- equivalent for each mole: lactate (8), propionate (14), butyrate (20), acetate (12), methane (8), sulfate (8), cis-DCE (2), VC (4), and ethylene (6).

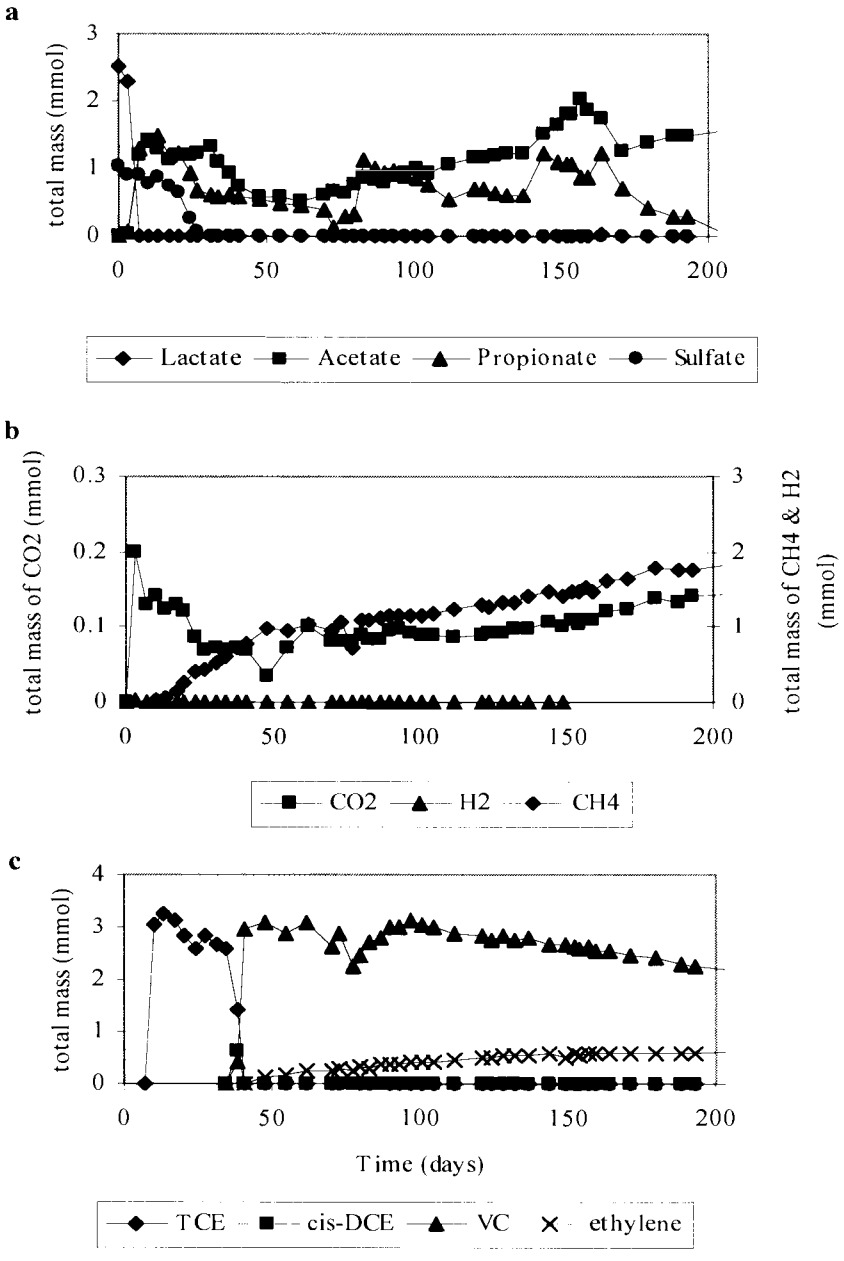

Figure A.1. Microcosm (T4) without TCE/VC additions. Lactate was added day 1, and propionate was added day 67, 112, and 187. Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

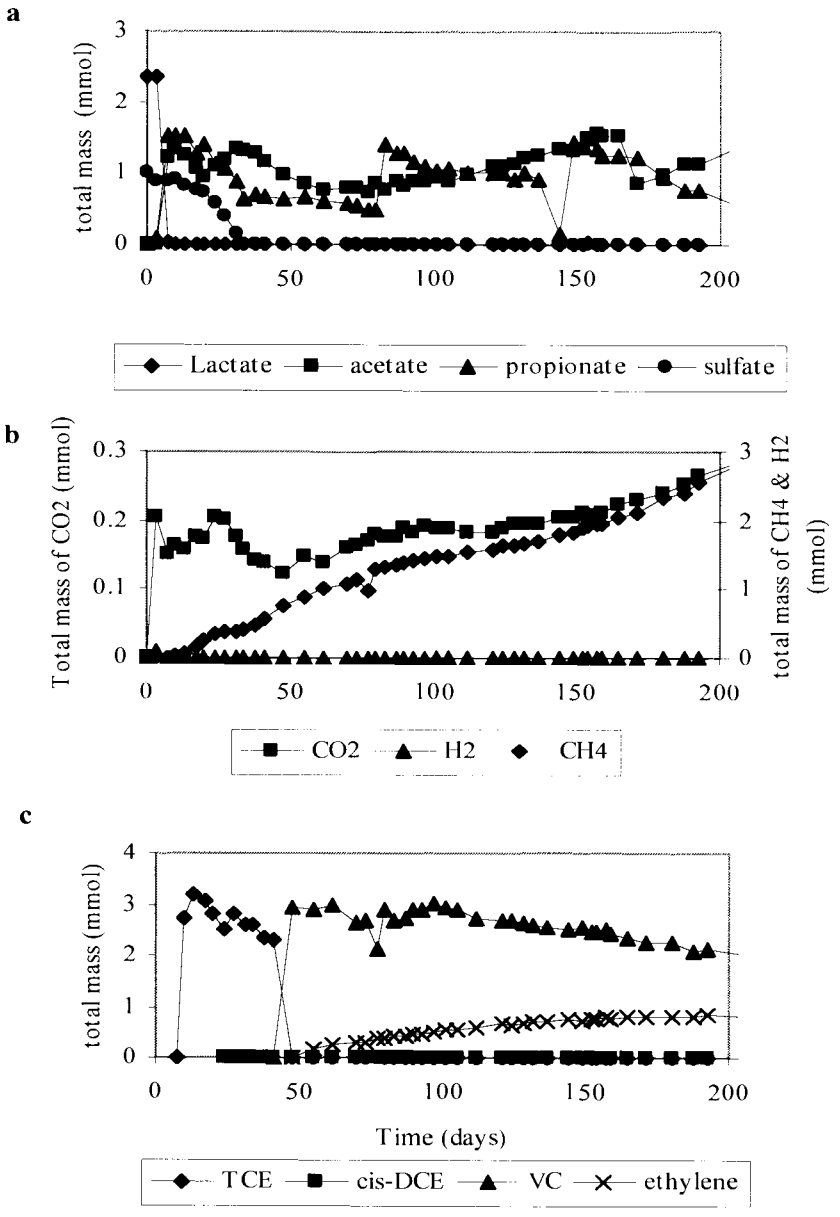

Figure A.2. Microcosm (T5) without TCE/VC additions. Lactate was added day 1, and propionate was added day 67, 112, and 187. Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

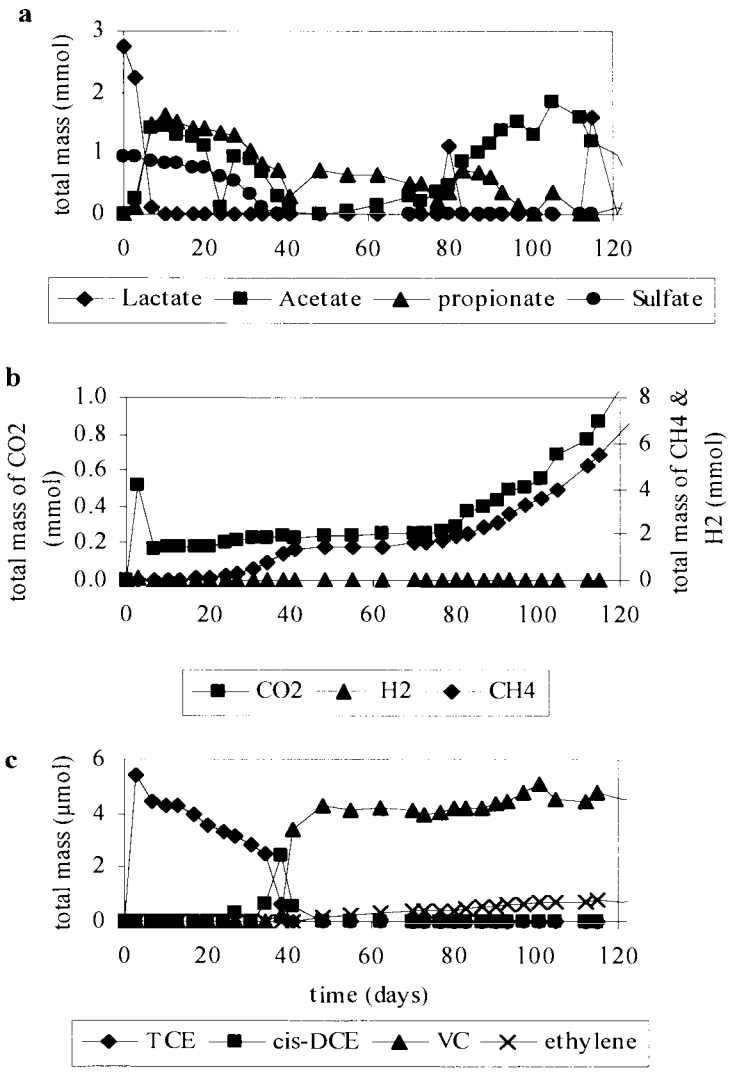

Figure A.3. Microcosm (M5) without TCE additions. Lactate was added on day 1 (2.76 mmol), and 80 (1.1 mmol). Concentration of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

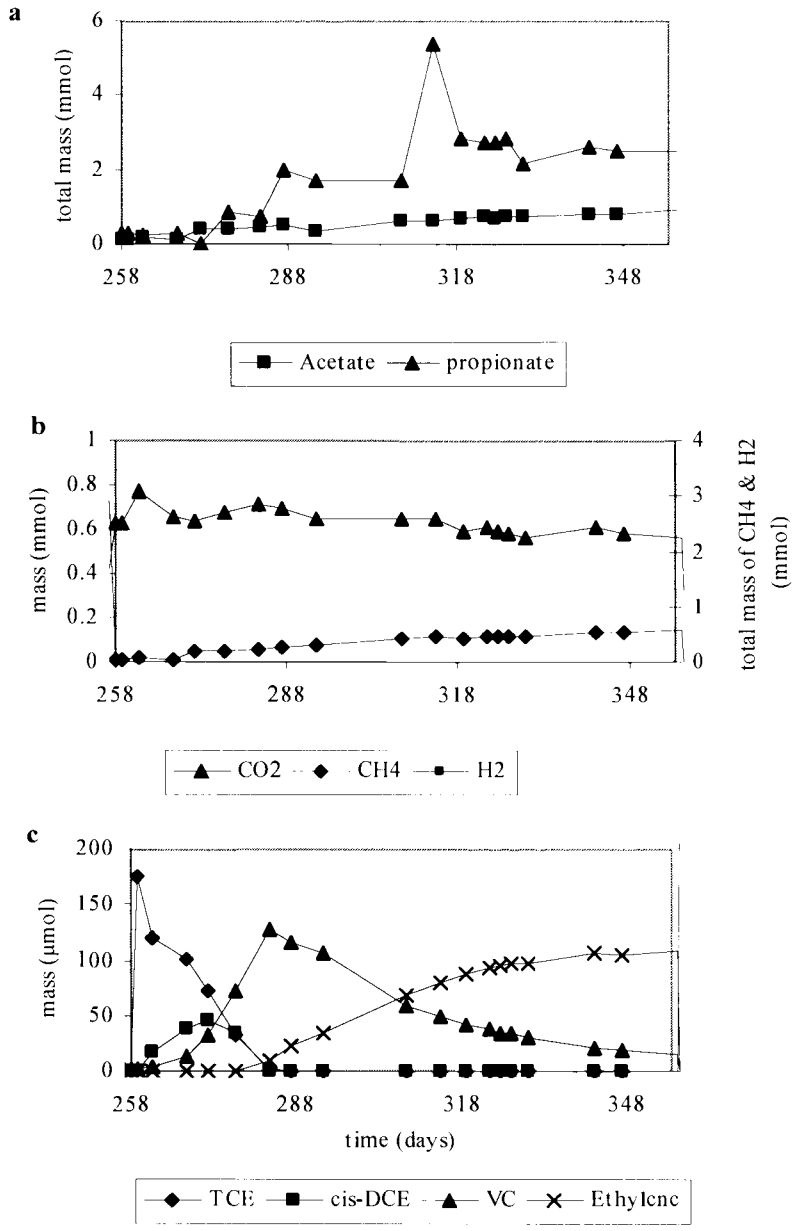

Figure A.4. First high TCE addition of 100 mg/L (M1). Propionate was added on day 277 (0.85 mmol), 287 (1.22 mmol) and 314 (3.70 mmol). Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

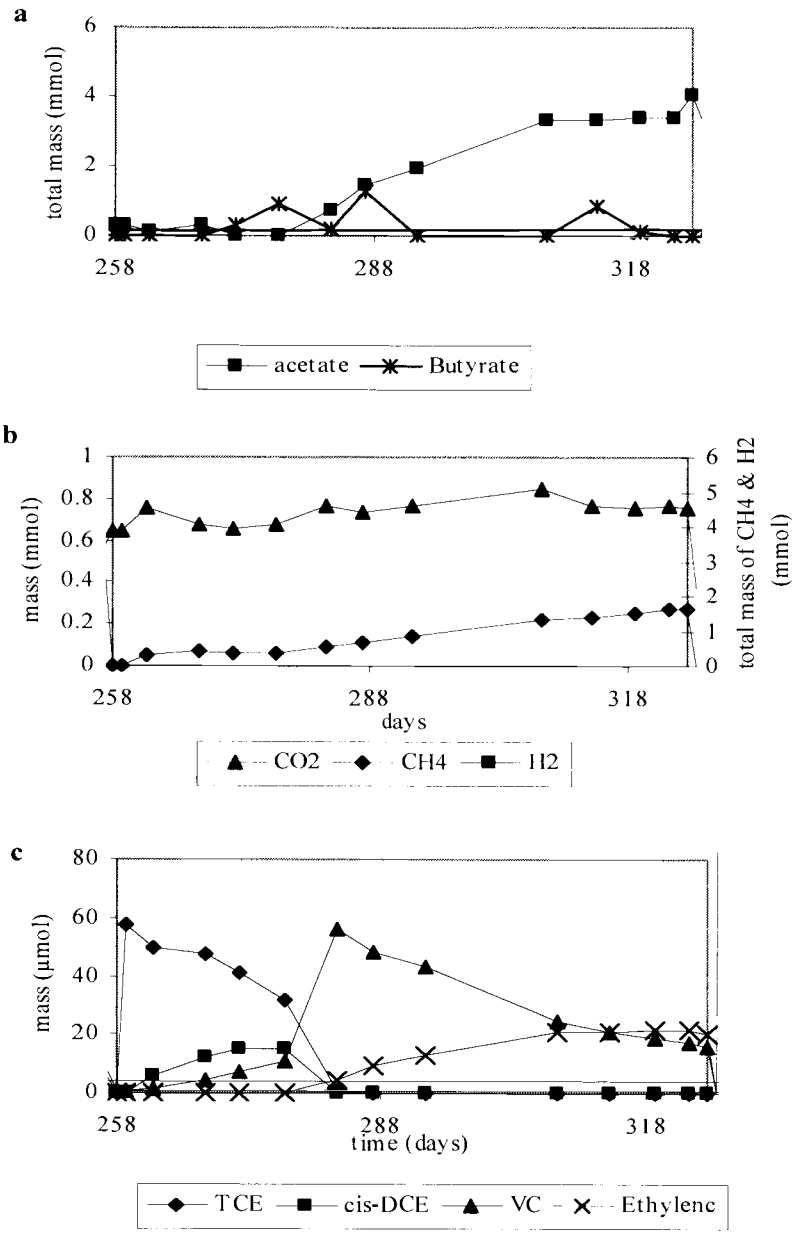

Figure A.5. First high TCE addition of 30 mg/L (M3). Butyrate was added on day 277 (0.92 mmol), 287 (1.26 mmol) and 314 (0.85 mmol). Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

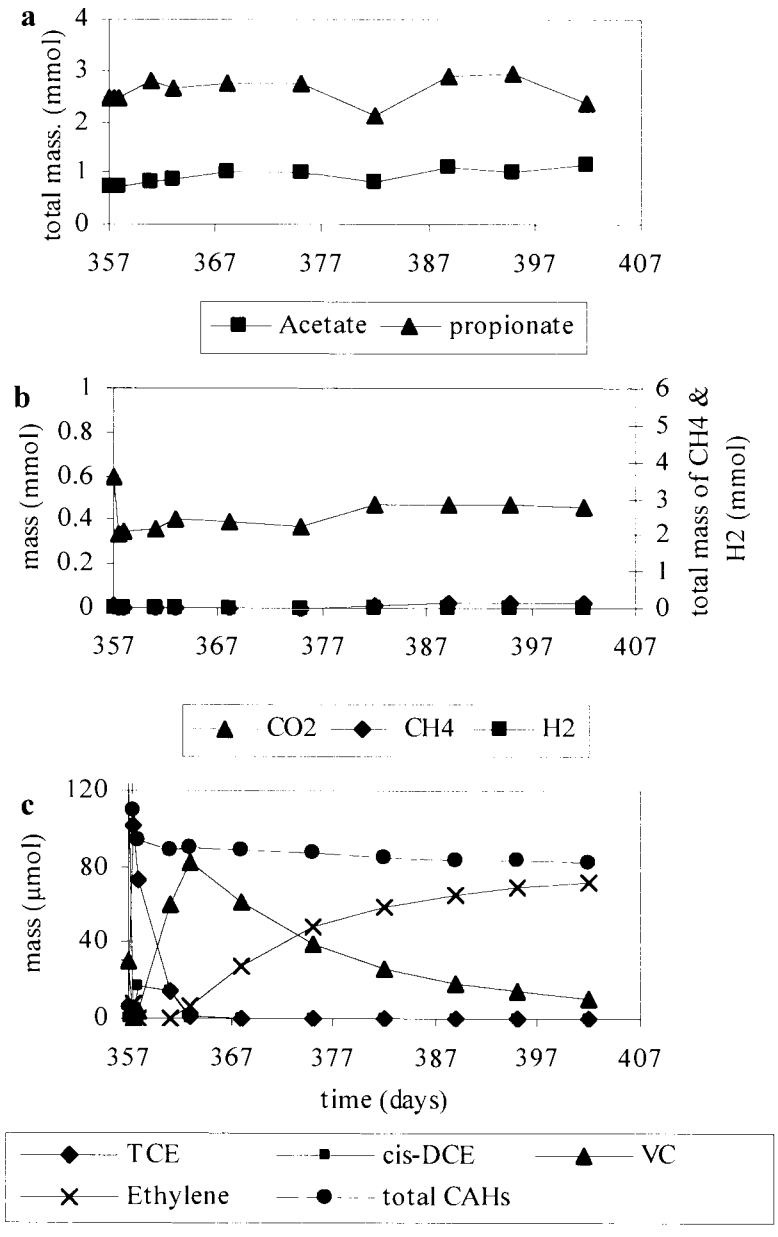

Figure A.6. Second TCE addition of 100 mg/L (M2). Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

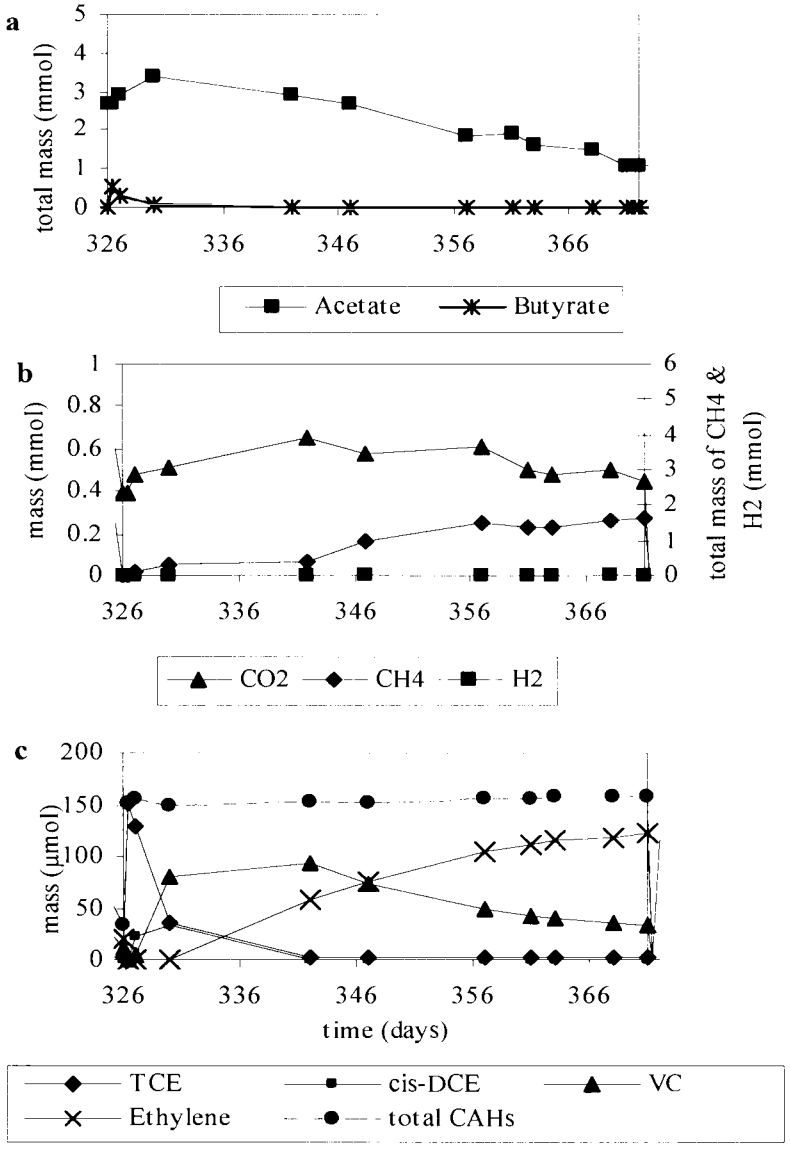

Figure A.7. Second TCE addition of 100 mg/L (M4). Concentrations of (a) sulfate and substrates, (b) methane and carbon dioxide, and (c) chlorinated ethylenes.

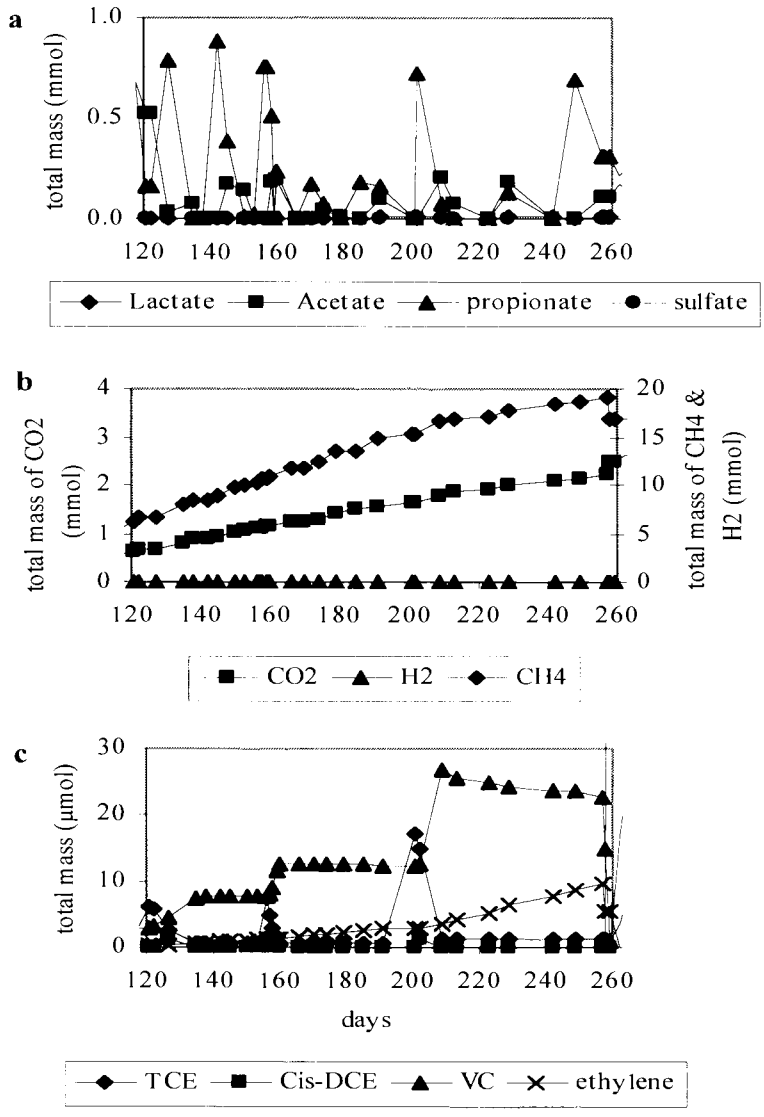

Figure A.8. Bulk amount of TCE addition test before high TCE concentration addition (M1). Propionate was added on day 127 (0.79 mmol), 142 (0.88 mmol), 160 (0.24 mmol), 185 (0.18 mmol), 202 (0.72 mmol), and 249 (0.69 mmol). TCE was added on day 156 (6.98 µmol), and 201 (16.58 µmol).

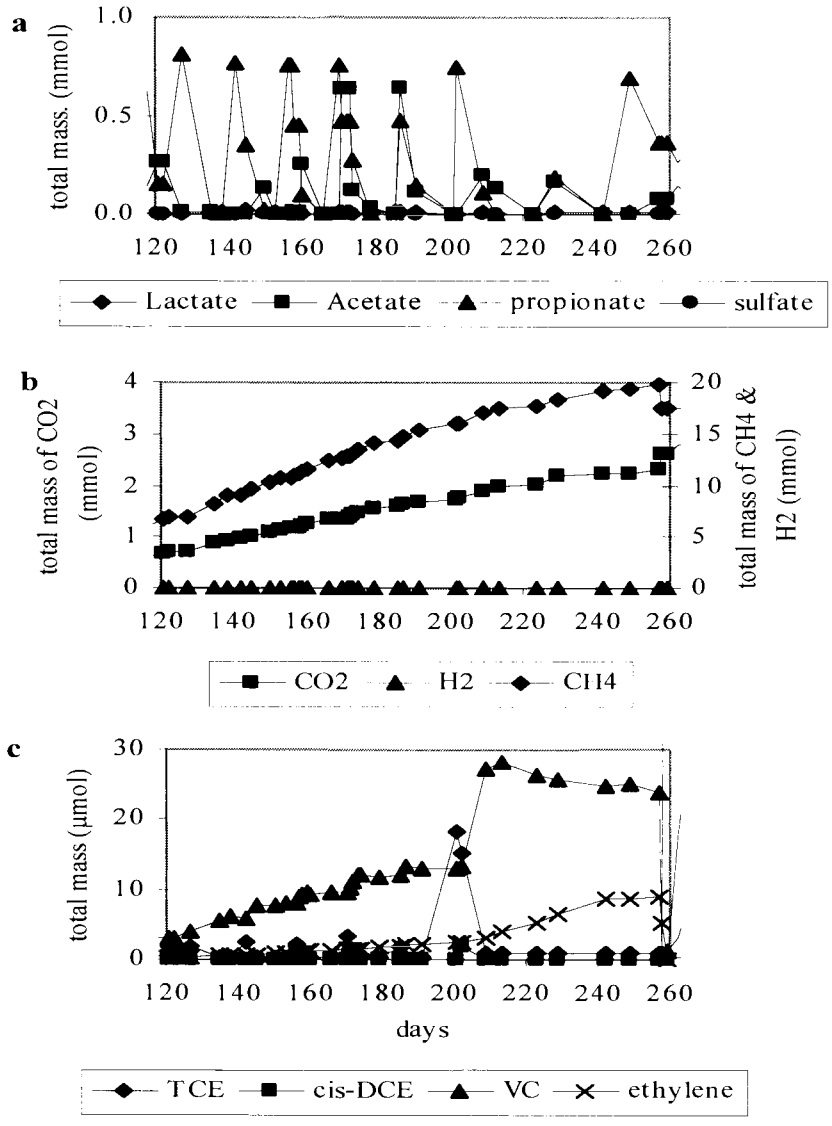

Figure A.9. Step-wise TCE addition test before high TCE concentration addition (M2). Propionate was added on day 127 (0.81 mmol), 142 (0.77 mmol), 157 (0.76 mmol), 170 (0.76 mmol), 187 (0.47 mmol), 202 (0.74 mmol), 229 (0.20 mmol) and 249 (0.7 mmol). TCE was added on day 142 (2.30 µmol), 156 (2.00 µmol), 170 (3.20 µmol), 185 (1.60 µmol), and 201 (18.2 µmol).

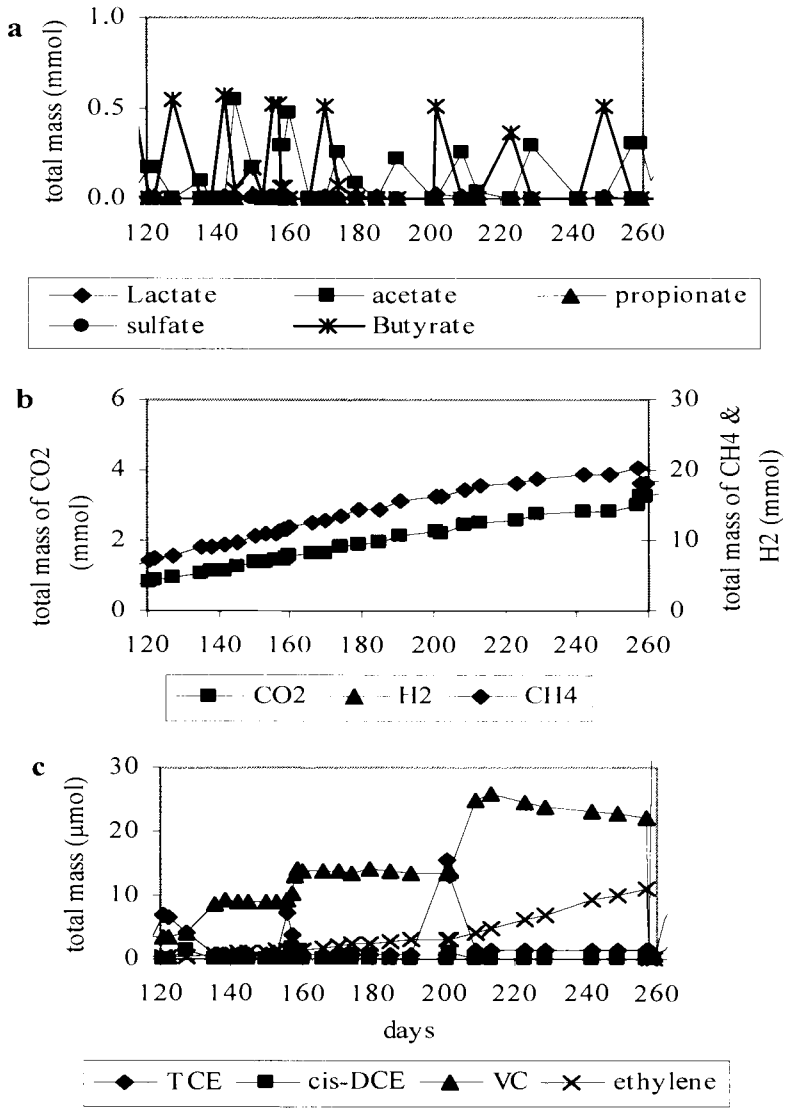

Figure A.10. Bulk amount of TCE addition test before high TCE concentration addition (M3). Butyrate was added on day 127 (0.55 mmol), 142 (0.57 mmol), 156 (0.53 mmol), 170 (0.51 mmol), 202 (0.51 mmol), 223 (0.37 mmol), and 249 (0.7 mmol). TCE was added on day 121 (6.51 µmol), 156 (6.65 µmol), and 201 (14.58 µmol).

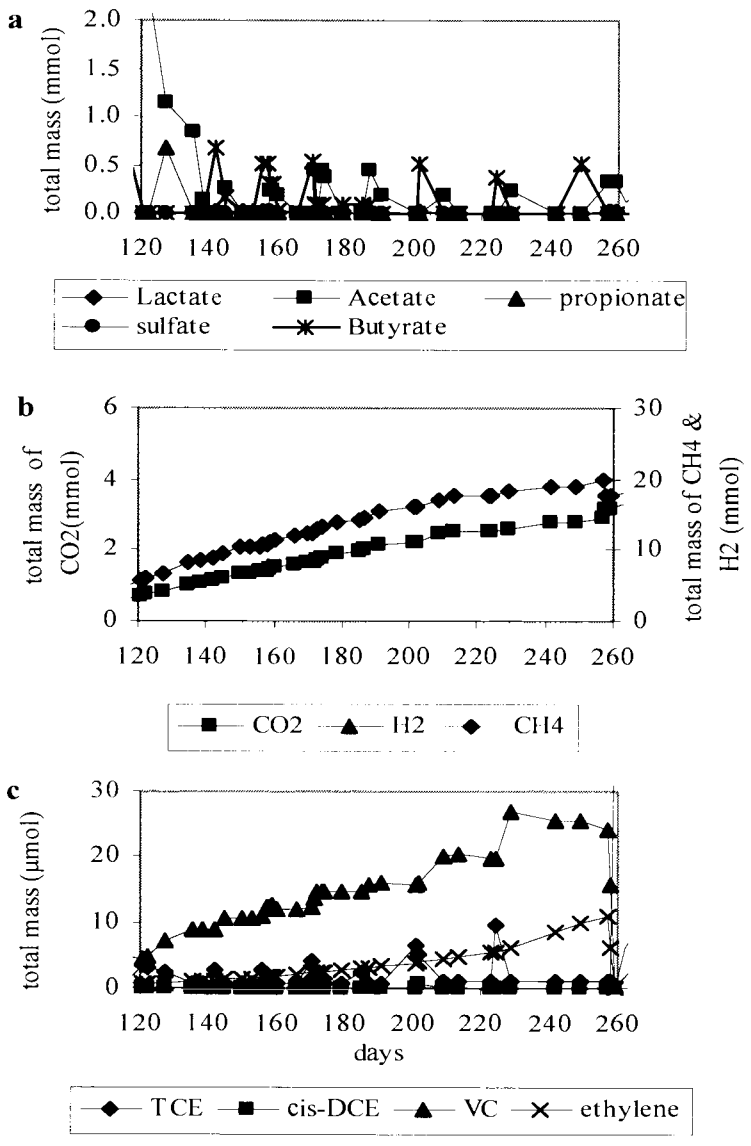

Figure A.11. Step-wise TCE addition before high TCE concentration addition (M4). Butyrate was added on day 127 (0.55 mmol), 142 (0.68 mmol), 156 (0.52 mmol), 170 (0.53 mmol), 202 (0.52 mmol), 223 (0.36 mmol) and 249 (0.51 mmol). TCE was added on day 127 (1.77 µmol), 142 (2.14 µmol), 156 (2.07 µmol), 170 (3.41 µmol), 186 (1.79 µmol), 201 (5.79 µmol), and 224 (8.79).