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Characterization of Anaerobic Chloroethene-Dehalogenating Activity in Several Subsurface Sediments

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Nomenclature

[A]	aqueous acetate concentration (M)
[DCE]	aqueous cis-DCE concentration (M)
ETH	moles of ethylene in a reactor (mol)
K_A	monod constant for acetate uptake (M)
K_{DCE}	monod constant for cis-DCE dechlorination (M)
k_{decay}	biomass decay coefficient (day^{-1})
K_{IVC}	inhibition coefficient for VC dechlorination (M)
K_L	monod constant for lactate uptake (M)
K_{Me}	monod constant for methanol uptake (M)
K_P	monod constant for propionate uptake (M)
K_{PCE}	monod constant for PCE dechlorination (M)
K_{TCE}	monod constant for TCE dechlorination (M)
K_{VC}	monod constant for VC dechlorination (M)
[L]	aqueous lactate concentration (M)
M	moles of methane in reactor (mol)
[Me]	aqueous methanol concentration (M)
[P]	aqueous propionate concentration (M)
[PCE]	aqueous PCE concentration (M)
[TCE]	aqueous TCE concentration (M)
V_l	liquid volume in a reactor (L)
[VC]	aqueous VC concentration (M)
[X]	aqueous biomass concentration (M)
$Y_{A/L}$	acetate to lactate yield (mol/mol)
$Y_{A/Me}$	acetate to methanol yield (mol/mol)
$Y_{A/P}$	acetate to propionate yield (mol/mol)
$Y_{A/X}$	acetate to biomass yield (mol/mol)
$Y_{B/Me}$	butyrate to methanol yield (mol/mol)
$Y_{DCE/Me}$	cis-DCE to methanol yield (L/mol-Me)
$Y_{L/X}$	lactate to biomass yield (mol/mol)
$Y_{M/A}$	methane to acetate yield (mol/mol)
$Y_{M/Me}$	methane to methanol yield (mol/mol)
$Y_{Me/X}$	methanol to biomass yield (mol/mol)
$Y_{P/L}$	propionate to lactate yield (mol/mol)
$Y_{P/Me}$	propionate to methanol yield (mol/mol)
$Y_{P/X}$	propionate to biomass yield (mol/mol)
$Y_{PCE/M}$	PCE to methane yield (mol/mol)
$Y_{PCE/Me}$	PCE to methanol yield (mol/mol)
$Y_{TCE/M}$	TCE to methane yield (mol/mol)
$Y_{TCE/Me}$	TCE to methanol yield (L/mol-Me)
$Y_{VC/Me}$	VC to methanol yield (L/mol-Me)
μ_A	maximum growth rate using acetate (day^{-1})
μ_L	maximum growth rate using lactate (day^{-1})
μ_{Me}	maximum growth rate using methanol (day^{-1})
μ_P	maximum growth rate using propionate (day^{-1})

Summary

Anaerobic microcosms of subsurface soils from four locations were used to investigate the separate effects of several electron donors on tetrachloroethylene (PCE) dechlorination activity. The substrates tested were methanol, formate, lactate, acetate, and sucrose. Various levels of sulfate-reducing, acetogenic, fermentative, and methanogenic activity were observed in all sediments. PCE dechlorination was detected in all microcosms, but the amount of dehalogenation varied by several orders of magnitude. Trichloroethylene was the primary dehalogenation product; however, small amounts of cis-1,2-dichloroethylene, 1,1-dichloroethylene, and vinyl chloride were also detected in several microcosms. Lactate-amended microcosms showed large amounts of dehalogenation in three of the four sediments. One of the two sediments which showed positive activity with lactate also had large amounts of dehalogenation with methanol. Sucrose, formate, and acetate also stimulated large amounts of dehalogenation in one sediment that showed activity with lactate. Amendment with formate, acetate, or sucrose resulted in only slight dehalogenation in the other three sediments. Elevated levels of dehalogenation were not consistently associated with any observable anaerobic metabolisms (sulfate reduction, acetogenesis, fermentation, or methanogensis). The lactate-amended cultures for two of the three sediments that showed high levels for dehalogenation were subdivided and incubated with lactate and PCE, TCE, cis-DCE, or VC. PCE and TCE were rapidly converted to TCE and cis-DCE, respectively. Neither DCE nor VC was dehalogenated; however, PCE was converted to ethylene in one sediment which was incubated with lactate, yeast extract, and vitamin B_{12}. Unfortunately, this condition was only tested with the one sediment, and further work is needed to determine if these additives will have a similar effect on the other cultures. These results suggest that lactate may be an appropriate substrate for screening sediments for PCE or TCE dehalogenation activity, but that the microbial response is not sufficient for complete in situ bioremediation. However, other cofactors may aid in stimulating the complete dehalogenation of PCE to ethylene.

Analysis of the rate of PCE and TCE dehalogenation in the sediment microcosms that showed high levels of dehalogenation products revealed similarities between all sediments. A detailed study of the Victoria activity revealed that dehalogenation rates were more similar to the Cornell culture (DiStefano et al. 1991) than to rates measured for methanogens, or a methanol-enriched sediment culture (Skeen et al. 1995; Fathepure et al. 1987). This may suggest that these sediments contain a highly efficient dehalogenation activity similar to the Cornell culture. This assertion is supported further by the fact that an average of 3% of added reducing equivalents could be diverted to dehalogenation in tests which were conducted using PCE-saturated hexadecane as a constant source of PCE during incubation. Further evidence is needed to confirm this premise. The application of these results to in situ bioremediation of highly contaminated areas are discussed.

1.0 Introduction

Chlorinated solvents, such as tetrachloroethylene (PCE) and trichloroethylene (TCE), are common groundwater contaminants (Westrick et al. 1984). Pump-and-treat systems using air stripping and adsorption onto granular-activated carbon are the primary technologies used for removing volatile organics; however, a decade of performance data has demonstrated that these systems are not as efficient as once thought (Haley et al. 1991). In situ bioremediation has been proposed as a method to reduce time and cost for site restoration of volatile organic carbon (VOC)-contaminated groundwater (Saaty et al. 1995; Skeen et al. 1993).

Both aerobic and anaerobic biological processes have been investigated for in situ remediation of chlorinated solvents. Anaerobic systems have demonstrated several potential advantages for PCE and TCE destruction. First, some anaerobic-microbial systems have the capability to destroy both PCE and TCE to nonhazardous end-products, such as ethylene (DiStefano et al. 1991), while aerobic metabolisms cannot destroy PCE (Fogel et al. 1986; Bouwer and McCarty 1985). Second, the nutrients required for anaerobic systems are extremely water soluble, whereas the destruction capacity of aerobic systems is limited by the water solubility of oxygen. Finally, laboratory tests have demonstrated that anaerobic systems have the potential to destroy orders of magnitude more contaminant than aerobic systems for the same amount of added electron donor. The maximum aerobic-degradation capacity reported by Chang and Alvarez-Cohen (1995), for example, was only 54 μmoles of TCE per gram of added COD (chemical oxygen demand). In contrast, the anaerobic culture reported by Tandoi et al. (1994) degraded 2700 μmoles of PCE or TCE to ethylene per gram of added COD.

There are several technical issues that impede widespread application of anaerobic in situ bioremediation. Little is understood about both the microorganisms that facilitate reductive dehalogenation and the environmental conditions necessary to initiate and sustain this activity in a contaminated aquifer (Maymó-Gatell et al. 1995). Only a few of the reported studies on reductive dechlorination of chloroethenes used subsurface-derived materials (Gibson et al. 1994). In addition, although many researchers have demonstrated that adding electron donors will stimulate reductive dehalogenation, there are conflicting reports on which donors are most efficient (Sholz-Muramatsu et al. 1995; Gibson et al. 1994; Neumann et al. 1994; Pavlostathis and Zhuang 1993; Gibson and Sewell 1992; Freedman and Gossett 1989). Tests using soil from a contaminated aquifer, for example, showed that complex organic substrates stimulated reductive dehalogenation of PCE while acetate and methanol did not (Gibson and Sewell 1992). In contrast, others have reported that acetate and methanol will stimulate anaerobic chloroethylene transformation by organisms from subsurface soils (Odom et al. 1995; Skeen et al. 1995); hence, it is not evident from published results whether or not similarities exist in the anaerobic PCE transformation activity that can be stimulated in subsurface materials. The purpose of this work is to compare the effects, directly, of various electron donors on reductive dehalogenation and anaerobic activity in sediments taken from a variety of contaminated sites. In addition, work was conducted to assess the utility of harnessing the resulting activity for in situ bioremediation of chloroethene dense nonaqueous phase liquids (DNAPL).

2.0 Methods

2.1 Chemicals

Tetrachloroethylene (PCE), trichloroethylene (TCE), 1,1-, 1,2-trans- and 1,2-cis-dichloroethylene (1,1-DCE, trans-DCE, and cis-DCE, respectively) and vinyl chloride (VC) were obtained from Aldrich Chemical Co. (Milwaukee, WI). Methane and ethylene were purchased from Scott Specialty Gases (Plumsteadville, PA) and Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

2.2 First-Generation PCE Microcosms

2.2.1 Soils

Four different sediments were used in this study. Two of the sediments were obtained from a jet fuel- and chlorinated ethylene-contaminated area at Tinker Air Force Base in Oklahoma City, OK. The first of these sediments (denoted Tinker VZ) was obtained at a depth of 4.57 m below ground surface (BGS) and is from a clayey/silty formation that comprises the vadose zone. The second sediment (denoted Tinker PA) was collected in the vicinity of the vadose zone sample, but at a depth of 11.28 m BGS, which is in the sandy/silty material that forms a perched aquifer. The third sediment material (denoted Victoria) was collected from a chlorinated ethylene-contaminated aquifer that underlies the DuPont Plant West Landfill near Victoria, Texas (Beeman et al. 1994). The Victoria sediment was collected at a depth of 27.4 m BGS from a confined aquifer composed of fine sand. The fourth sediment (denoted Dover) was obtained at a depth of 11.28 m BGS from a TCE-contaminated region of the upper Columbia formation at Dover Air Force Base (Dover, DE).

All materials were collected using a split-spoon sampler. Once removed from the bore hole, the material was transferred into sterile jars and shipped to our laboratory. Materials were subsequently stored at room temperature in an anaerobic glove box. Tinker soils were stored for two months and Victoria and Dover soils for one month before use. Materials were exposed to air during sampling and shipping which could result in a change in the anaerobic populations from oxygen toxicity. To minimize this effect, however, the material was purged with oxygen-free nitrogen once it was received in the laboratory.

2.2.2 Microcosm Set-Up

Microcosms were constructed in duplicate to test the capability of five substrates to stimulate PCE dehalogenation activity in the three sediments. The five substrates tested were methanol, formate, acetate, lactate, and sucrose. The initial concentration for each substrate was chosen to provide a constant number of reducing equivalents (90 meq/L) if each of the substrates were completely oxidized to carbon dioxide. This parameter was used to select substrate level, because it was desired to evaluate both the potential for dehalogenation and the relative capability of each substrate to divert electrons to dehalogenation. This approach yielded initial aqueous level of each substrate at nominally 15 mM, 45 mM, 11 mM, 7.5 mM, and 1.8 mM for methanol, formate, acetate, lactate, and sucrose, respectively.

Microcosms were constructed by placing 6 g of sediment and 60 mL of reduced anaerobic mineral medium (RAMM) into 124-mL serum bottles, and sealing the bottles with Teflon-lined septa and aluminum crimp caps (VWR Scientific, Baltimore, MD). The RAMM used in these tests was identical to that reported previously without the addition of vitamins (Skeen et al. 1995). Once the bottles were sealed, an anaerobic gas containing 10% carbon dioxide and 90% nitrogen was purged through the septa. PCE-saturated water was subsequently added to each bottle using a syringe to achieve a nominal PCE level of 0.8 μmole/bottle (aqueous concentration of 9 μM). Next, the appropriate amount of each electron donor was added. After electron donor and PCE addition, the serum bottles were mixed vigorously. All bottles were then inverted to minimize losses of volatile organics, and were quiescently incubated at 17°C. The actual amount of PCE added to each bottle was determined by sampling the aqueous phase after three days of equilibration. This value of chloroethene was used as the basis for estimating mass balances in the microcosms. Microcosms were incubated until either no substrates remained or no metabolic activity was detected for several sample periods. This resulted in an incubation period of 173 days for Victoria sediments, 297 days for both Tinker VZ and PA materials, and 292 days for Dover microcosms.

Abiotic controls were constructed using the same procedure as for the active microcosms except that autoclaved soil was used and no substrate was added. To prepare these soils, bottles with sediment were autoclaved at 121°C for 30 min.

2.2.3 Sampling and Analytical Procedures

Each microcosm bottle was sampled after three days of incubation and then every four to eight weeks for the next 11 months. The volume of liquid removed during each sampling was replaced with fresh RAMM.

Before each sampling event, the headspace pressure in the bottles was measured by puncturing the bottle septa with a 22-gauge needle connected to a Tensiometer (SMS, Tucson, AZ). Pressure measurements were necessary to calculate the total amount of gaseous species present. Next, chlorinated ethylenes were analyzed by removing 1 mL of aqueous sample with a 2.5-mL gas-tight glass syringe (Hamilton Co., Reno, NV) and extracting in 3 mL of hexane. After settling, the organic supernatant was transferred to a 2.0-mL gas chromatograph (GC) vial and sealed with a septa and aluminum crimp caps. The samples were then analyzed using an electron-capture detector (ECD) and a 30-m, 0.53-mm ID, DB-624 column (J&W Scientific, Fulsom, California) in a 5890 Series II GC (Hewlett Packard Co., Wilmington, DL). Nitrogen was used as the carrier gas at 4.2 L/min. The injector temperature was 250°C and the detector temperature was set at 325°C. The column temperature was initially 50°C and gradually increased to 100°C at a rate of 10°C per min. The hold time after the temperature ramp was 3.5 mins. Minimum detection limit was 1 μg/L for PCE, TCE, and 1,1-DCE and 50 μg/L for cis-DCE, trans-DCE, and VC. Total amounts of each compound was estimated in each bottle using the measured aqueous-phase concentration and an estimated Henry's law constant (Gossett 1987). The Henry's constants used for PCE, TCE, cis-DCE, trans-DCE, 1,1-DCE, and VC were 0.46, 0.25, 0.11, 0.26, 0.76, and 0.81 (concentration-gas/concentration-liquid), respectively.

A separate 2-mL aqueous sample was also removed from each bottle, filtered through a 0.2-μm syringe filter (Gelman Sciences, Ann Arbor, MI), and used for electron donor, volatile organic acid (VOA), and sulfate analysis. Sucrose concentration was determined by the anthrone method (Handel, 1968). For methanol, 200 μL of the filtered sample was diluted with deionized water, and analyte concentration was measured by GC-FID using a 30-m DB-Wax column (J&W Scientific, Fulsom,

California). The temperature of the column, injector, and detector was 75, 120, and 275°C, respectively. This method resulted in a minimum methanol detection limit of 30 μM.

Acetate, propionate, and butyrate concentrations were measured by diluting 200 μL of the original sample with pH 2.5 deionized water and injecting the diluted material into a GC-FID with a 30-m Nukol capillary column (Supelco Inc., Bellefonte, PA). The injector and detector temperatures were 200°C and 275°C, respectively. The column temperature was ramped from 90°C to 135°C at 5°C per min. The minimum detection limits for acetate, propionate, and butyrate were 14 μM, 6.8 μM, and 3.4 μM, respectively.

Lactate, formate, and sulfate were measured using a Dionex 4000i (Dionex, Sunnyvale, CA) ion chromatograph with a conductivity detector. For sulfate analysis, an IonPac A912A column (Dionex, Sunnyvale, CA) was used. Samples were analyzed at a flow rate of 1.5 mL/min; the eluent consisted of 2.7 mM sodium carbonate and 0.3 mM sodium bicarbonate. Lactate and formate were assayed using an IonPac ICE-AS6 column (Dionex, Sunnyvale, CA). The anion-suppression regenerant in the lactate analysis was 5.0 mM tetrabutylammonium hydroxide; the eluent was a 0.4 mM nitric-acid solution. The minimum detection limits were 5.2 μM for sulfate and 100 μM for both formate and lactate.

A 3 mL-gas sample was also taken using a 5-mL gas-tight glass syringe (Dynatech, Baton Rouge, LA). Headspace gas samples were analyzed for methane, hydrogen, ethylene, and ethane by direct injection into an HP model 5890 Series II GC (Hewlett Packard, Palo Alto, CA) that was equipped with two switching valves (Valco Instruments Co., Houston, TX), a thermal-conductivity detector (TCD), and a flame-ionization detector (FID). Helium was used as the carrier gas at a rate of 40 mL/min. The GC column was a 4.6-m Carboxen 1000 (Supelco, Inc., Bellefonte, PA) with an inside diameter (ID) of 0.318 cm. The TCD detector temperature was set at 225°C; the FID was controlled at 275°C . The column temperature was controlled at 220°C during operation. After the sample was injected on the column, the gas was diverted to the TCD for the first 12 mins to quantify hydrogen and methane; flow was then routed to the FID for detection of ethane and ethylene. Minimum detection levels were 0.319 2000 ppmv, 100 ppmv, 2 ppmv, and 0.3 ppmv for hydrogen, methane, ethylene, and ethane, respectively. Calibration standards for all analytical assays were checked daily.

2.3 Kinetics of Dehalogenation by the First Generation Victoria Culture

2.3.1 Experimental Set-Up

The bioreactor apparatus was described in detail by Skeen et al. (1994). Briefly, the reactor consists of a 1-liter stainless-steel vessel, fitted with a heat-exchange coil, gas and liquid sampling ports, a 0- to 30-psi Bourdon tube pressure gauge constructed entirely of stainless steel (McMaster-Carr, Santa Fe, CA), an in situ sterilizable pH electrode, and an in situ sterilizable redox potential electrode (Code-Palmer, Chicago, IL). An Orion 720-A dual-channel pH/mV meter (Orion, Boston, MA) was used to monitor the pH and the oxidation-reduction potential (ORP). The reactor was also equipped with an in situ YSI 416 thermocouple (YSI Inc., Yellow Springs, OH) and a five-channel Thermistor thermometer (Cole-Parmer, Niles, IL).

Triplicate sediment cultures were established in the anaerobic reactors. Before inoculation, the anaerobic reactor was sterilized in an autoclave at 121°C for 20 min and cooled to room temperature. The reactor was then transferred to an anaerobic glove box where 100 grams of Victoria sediment and 800 mL RAMM were added. After the reactor was sealed, it was purged with anaerobic gas and then

pressurized to 15 psi for overnight leak testing. Once the reactor passed the leak test, it was charged with a sterile PCE solution to give a nominal concentration of 14 μM. Reactors were mixed using magnetic stir plates for at least three days for PCE equilibration. Lactate was then added to each reactor to give a nominal concentration of 7.5 mM. After lactate was mixed with the culture medium, samples were taken for the analysis of chlorinated ethylenes, substrate, biomass, volatile organic acids (VOAs), methane, and ethylene. All reactor cultures were controlled at 18°C during the entire experimental period. The reactor was sampled twice every week.

Small volume bottle tests were used to evaluate growth rates and substrate conversion yield coefficients. Triplicate bottles were set up using lactate (4 mM) as the main substrate, while duplicate bottles were constructed using acetate (2 mM) and propionate (2 mM) as substrate. All bottles were constructed under anaerobic conditions by inoculating 10% of the reactor culture on day 328 into 124-mL serum bottles containing 60 mL RAMM. PCE was also added to these bottles with a nominal concentration of 14 μM. All the bottles were incubated at 18°C.

2.3.2 Sampling and Analysis

Anaerobic reactor tests were sampled twice every week. Before sampling, pH, ORP, headspace pressure, and culture temperature of each reactor were recorded for data analysis. For chlorinated ethylene analysis, a 1.0-mL aqueous sample was withdrawn with a 2.5-inch-long sterile needle (Gauge 22) and a 2.5-mL gas-tight glass syringe. The sample was dispensed into 3 mL hexane which was vortexed for one minute for chlorinated ethylene extraction. The organic phase was used for PCE, TCE, DCE, and VC analysis. A 3.0-mL gas sample was taken for methane and ethylene analysis. A 4.0-mL aqueous sample was taken and filtered for the analysis of lactate and VOAs. Another 4.0 mL unfiltered sample was saved for cell protein and biomass analysis. Both samples were saved in a -20°C freezer before analysis. For the bottle test, a 300-μL gas sample was taken for the analysis of methane. In addition, a 1.5-mL sample was filtered for the analysis of lactate, acetate, propionate, and butyrate. An additional 1.5-mL sample was saved for protein analysis.

PCE, TCE, DCE and VC samples were assayed by hexane extraction of aqueous samples and injection into a HP model 5890 Series II GC (Hewlett Packard, Palo Alto, CA) with an electron-capture detector (ECD). Nitrogen was used as the carrier gas at 4.2 mL/min. The separation column was a 30-m DB-624 column (J&W Scientific, Fulsom, CA) having a 0.53-mm ID. The injector temperature was set at 250°C and the detector temperature was set at 325°C. The column temperature was initially controlled at 50°C for 5 mins and then ramped up to 140°C at a rate of 10°C per min. Calibration standards were checked daily. Minimum detection limits for PCE, TCE, cis-DCE, and VC are 0.18 ppb, 0.34 ppb, 100 ppb, and 50 ppb, respectively.

Headspace gas samples were analyzed for methane and ethylene by direct sample injection into an HP model 5890 Series II GC equipped with a thermal-conductivity detector (TCD) and a flame-ionization detector (FID). The gas chromatograph column was a 4.57-m Carboxen 1000 (Supelco, Inc., Bellefonte, PA) having a .32-cm ID. The column head pressure was controlled at 40 psi and the oven temperature was set at 220°C. The temperatures of TCD and FID detectors were set at 225°C and 275°C, respectively. After the sample was injected on the column, the gas was diverted to the TCD for the first 12 mins to quantify methane; flow was then routed to the FID for detection of ethylene. The minimum detection limits were 100 ppmv and 2 ppmv for methane and ethylene, respectively.

Lactate concentration was determined in a filtered sample using a Dionex 4000i (Dionex, Sunnyvale, CA) ion chromatograph with a conductivity detector. The column used was an IonPac ICE-AS6 column (Dionex, Sunnyvale, CA). An eluent of 0.4 mM nitric acid solution was used as an eluent in the analysis.

The anion-suppression regenerant was 5.0 mM tetrabutylammonium hydroxide. The column flow rate was set at 1.5 mL eluent per min.

The concentrations of anaerobic byproducts such as acetate, propionate, and butyrate in the culture were analyzed by diluting the filtered culture sample with four parts pH 2.5 deionized water and directly injecting 1 μL of the solution into a HP model 5890 Series II GC. The water pH was adjusted with 8 N H_3PO_4 solution. The GC was equipped with a flame-ionization detector and a 30-m Nukol column (Sepelco, Inc., Bellefonte, PA) with a 0.53-mm ID. The carrier gas was helium and was controlled at 8.2 mL/min. The injector and detector temperatures were set at 200°C and 275°C, respectively. The column temperature was initially controlled at 90°C and ramped up to 130°C at a rate of 5°C per min. The GC was calibrated before sample analysis. The minimum detection limit for acetate, propionate, and butyrate is of 0.5 mg/L.

Biomass levels in the reactor were determined as protein associated with filtered solids, using the Pierce Micro BCA Protein Assay Kit (Pierce, Rockford, IL). All biomass concentrations are reported in mg-DW/L, and are calculated based on an experimentally measured protein content for this consortium of 51% of dry weight.

For protein analysis, a medium sample was separated into two parts. One part was mixed with an equal volume of 0.5 N NaOH solution. The second part of the sample was filtered through a 0.2-μm filter to remove biomass. The collected filtrate was used as a blank. As with the unfiltered sample, the filtrate solution was mixed with an equal volume of 0.5 N NaOH. For both the sample and blank, the mixture was next denatured in a water bath at 90°C for 10 mins. During this procedure, each tube was tightly capped to prevent evaporation. After cooling to room temperature, the pH in both samples was adjusted to 2.0 with 6 N HCl. The protein content for both the sample and blank was then determined using the procedures described in BCA Protein Assay. The lower detection limit for this method is 0.5 mg-protein/L.

2.3.3 Kinetic Analysis

Experimental measurements of biomass, lactate, acetate, propionate, methane, PCE, TCE, cis-DCE, VC, and ethylene were used to calculate kinetic coefficients with the SimuSolv® simulation software (Dow Chemical Company, Midland, MI). This program calculates optimal kinetic parameter values by minimizing relative errors between input experimental data sets and predicted model response curves. The goodness of fit was evaluated with parameter standard deviation values estimated by SimuSolv. The optimization algorithm used in this study was a modified generalized reduced-gradient technique. A similar approach was used by Skeen et al. (1995) to evaluate dehalogenation kinetics by a methanol-enriched methanogenic sediment consortium.

2.4 Second-Generation Microcosms

Duplicate cultures were constructed to test the capability of lactate to stimulate anaerobic-reductive dehalogenation of PCE, TCE, cis-DCE, and VC in Tinker VZ and Victoria soils. Dover material was also included in these experiments except that it was only incubated with PCE and cis-DCE. Inoculum for these tests was obtained by combining sediment material from replicate lactate-fed bottles used in the first generation PCE microcosm tests described in Section 3.2

To construct each Victoria and Tinker VZ bottle, 3 grams of the combined material was placed into 124-mL serum bottles with 60 mL of RAMM; the bottles were sealed with Teflon-lined septa and

aluminum crimp caps (VWR Scientific, Baltimore, MD). For Dover bottles, 3 g of combined material was placed into 586-mL serum bottles and 400 mL of RAMM was added. Once the bottles were sealed, an anaerobic gas containing 10% carbon dioxide and 90% nitrogen was purged through the septa. PCE-, TCE-, cis-DCE-, or VC-saturated water was added to each bottle using a syringe to achieve a nominal aqueous concentration of 10 μM. Actual initial levels were 8.3 ± 1.3, 9.5 ± 3.1, 25 ± 2, and 17 ± 2 μM (average ± standard deviation) for PCE, TCE, cis-DCE, and VC, respectively. Next, sodium lactate was added to each bottle to establish an initial aqueous concentration of 6.8 ± 1.1 mM (average ± standard deviation). After VOC and substrate addition, the serum bottles were mixed vigorously. All bottles were then inverted to minimize losses of volatile organics and quiescently incubated at 17°C. Victoria and Tinker VZ microcosms were incubated until no organic acids were detected, 121 days for Victoria sediments and 166 days for Tinker VZ materials. Dover bottles were incubated for 104 days. Abiotic controls were constructed using the same procedure as for the active microcosms except that autoclaved soil (121°C for 30 min) was used and no substrate was added.

Chloroethenes, volatile organic acids, and hydrocarbon gases were determined using identical sampling and analytical procedures as described in Section 3.2.3.

2.5 High PCE Concentration Tests

2.5.1 Tinker VZ Sediments

Duplicate microcosms were established in an anaerobic glove box by placing 100 g of Tinker VZ soil into sterile 598-mL serum bottles, adding 400 mL of RAMM, and sealing the bottles with Teflon-lined septa and aluminum crimp caps (VWR Scientific, Baltimore, MD). After the bottles were sealed and transferred out of the anaerobic glove box, anaerobic gas (10% carbon dioxide and 90% nitrogen) was purged through the headspace. At the end of purging, the headspace was pressurized to 300 mbar to keep a positive pressure in the bottles to avoid oxygen infiltration. Pure PCE was then added to each bottle to achieve a nominal aqueous concentration of 60 μM. After PCE addition, the bottles were vigorously mixed and then incubated for two days to allow PCE equilibrium. Before taking the first PCE sample, lactate and yeast extract were added to give a nominal concentration of 475 mg/L and 50 mg/L, respectively. All bottles were then inverted to minimize losses of volatile organics and quiescently incubated at 30°C. Killed controls were established in an identical manner, but autoclaved soil (121°C for 30 min) was used and no electron donor was added. In addition, 0.1% sodium azide was included in the killed controls to ensure no biological activity.

The effects of adding a constant source of PCE to the culture were also tested by adding PCE dissolved in hexadecane. These cultures were prepared as described above except that 5 ml of sterile PCE/hexadecane solution containing 1.15 g PCE was added to each bottle. Yeast extract and vitamin B_{12} were initially added to each bottle at concentrations of 400 mg/L and 0.10 mg/L, respectively. Lactate was added at a concentration of 1.0 mM once every three days. Samples were taken every month to measure PCE dechlorination activity. These methods were selected based on the work of Holliger et al. (1993).

2.5.2 Victoria Suspension Culture

Pure PCE was added to 160-mL sterile serum bottles containing 100 mL of RAMM to give nominal concentrations of 60, 300, and 600 μM. After PCE equilibrium for two days, 10 mL of Victoria enrichment culture from a soil enriched with lactate was added into each bottles. Next, lactate was added

to give a nominal concentration of 475 mg/L. All bottles were inverted to minimize losses of volatile organics and quiescently incubated at 30°C. Bottles were sampled and analyzed for chloroethenes, VOAs, and hydrocarbon gases.

Identical methods were used to establish cultures with 60 and 300 μM PCE, along with lactate, yeast extract, and vitamin B_{12}. Lactate, yeast extract and vitamin B_{12} were added to give nominal concentrations of 475, 50, and 0.10 mg/L, respectively.

Chloroethenes, VOAs, and hydrocarbon gases were determined using identical sampling and analytical procedures as described in Section 3.2.3.

2.5.3 Yakima River Delta Sediments

A local river sediment was collected in the Yakima River delta in Richland, Washington. This sediment (denoted as Yakima) was collected two inches below the river sediments. Duplicate serum bottles were set up to study the effects of adding a constant source of PCE to the culture. These cultures were prepared as described above in section 3.5.1. Sterile PCE\hexadecane solution (5 mL) containing 1.15 g PCE was added to each bottle. Yeast extract and vitamin B_{12} were initially added to each bottle at concentrations of 400 mg/L and 0.1 mg/L, respectively. Lactate was added at a concentration of 1.0 mM once every three days. Samples were taken every month to measure PCE dechlorination activity.

2.6 Kinetics of PCE Dechlorination by the Cornell Culture

2.6.1 Experimental Set-Up

Fed-batch dehalogenation experiments were performed to determine the kinetics of chlorinated ethylene transformation by the Cornell enrichment culture (DiStefano et al. 1991). Duplicate biotransformation tests were conducted for PCE and each of the chlorinated species detected as products from PCE dechlorination: TCE, cis-DCE, and VC. Methanol at 60 mg/L was used as the substrate in all eight experiments. To ensure that adequate dechlorination was achieved in each test, methanol was added to each reactor when it was depleted. The experimental apparatus used in these tests is described in detail in Section 3.3.1.

Before each experiment, the reactor was steam-sterilized at 121°C for 20 mins, charged with 850 mL of culture medium, and then pressure tested with anaerobic gas. A reduction in pressure indicated a leak in the reactor, which was corrected before proceeding. After the reactor passed the pressure test, the system was charged with an average of 513 ± 124 μmoles of chlorinated ethylene. After chloroethene addition, 12 hours were allowed to establish vapor-liquid equilibrium. Equilibrium time was determined from an abiotic-partitioning test using the reactor. Methanol was then added to the system to achieve a concentration of approximately 60 mg/L; the pH of the medium was adjusted to 7.0, using 1.0 N NaOH or 10% HCl. Finally, biomass was inoculated into the system. The initial concentration of biomass for these tests was nominally 50 mg DW/L.

During the testing period, the reactor was mixed with a magnetic stir bar and maintained at 19°C by a copper water jacket. The reactor pressure, temperature, pH, and the ORP were recorded every three days. In addition, two aqueous and one headspace sample were collected periodically to analyze for metabolic substrates, products and chlorinated ethylenes, and ethylene. For headspace gas analysis,

0.3 mL of gas were withdrawn with a 0.5-mL gas-tight, pressured-lock syringe (Dynatech, Baton·Rouge, LA) and directly injected into a GC. For chlorinated-ethylene analysis, 0.5 mL of aqueous sample was withdrawn into a clean, baked syringe. This sample was directly dispensed into a glass test tube containing 10 mL of hexane, capped with a Teflon-lined cap, and extracted by vortexing the mixture for one minute. The organic phase was used for GC analysis. For biomass, methanol, and acetate analyses, 0.4 6 mL of the media was taken from the reactor. Chloroethenes, volatile organic acids, and hydrocarbon gases were determined using identical sampling and analytical procedures as described in Section 3.2.3.

2.6.2. Kinetic Analysis

Experimental measurements of biomass, methanol, methane, PCE, TCE, cis-DCE, VC, and ethylene were used to calculate kinetic coefficients with the SimuSolv simulation software (Dow Chemical Company, Midland, MI). This program calculates optimal kinetic-parameter values by minimizing relative errors between input experimental data sets and predicted model response curves. The goodness of fit was evaluated with parameter standard deviation values estimated by SimuSolv. The optimization algorithm used in this study was a modified generalized reduced-gradient technique. A similar approach was used by Skeen et al. (1995) to evaluate dehalogenation kinetics by a methanol-enriched methanogenic sediment consortium.

2.7 DNAPL Dehalogenation by the Cornell Culture

Continuous porous media flow cell tests, using free-phase PCE and the Cornell culture, were conducted to demonstrate that chlororespiration could destroy enough contamination to keep a bulk fluid that contacted a chloroethene DANPL below the drinking water standard. The anaerobic culture medium consisted of (per liter of deionized water): NH_4Cl, 0.20 g; $K_2HPO_4 \cdot 3H_2O$, 0.10 g; KH_2PO_4, 0.055 g; $MgCl_2 \cdot 6H_2O$, 0.20 g; trace metal solution (per liter, 0.10 g of $MnCl_2 \cdot 4H_2O$; 0.17 g of $CoCl_2 \cdot 6H_2O$; 0.10 g of $ZnCl_2$; 0.20 g of $CaCl_2$; 0.019 g of H_3BO_4; 0.05 g of $NiCl_2 \cdot 6H_2O$; and 0.020 g of $NaMoO_4 \cdot 2H_2O$, adjusted to pH 7 with NaOH or HCl), 10 ml; resazurin, 0.001 g; $Na_2S \cdot 9H_2O$, 0.50 g; $FeCl_2 \cdot 4H_2O$, 0.10 g; $NaHCO_3$, 5.0 g; and yeast extract, 0.050 g. The medium preparation method followed the procedures described by Freedman and Gossett (1989).

A 2.06-liter soil-column reactor was used for the flow-cell test with PCE DNAPL. The soil column was equipped with a pH- and pressure-monitoring system and a continuous medium feeding system. Before operation, a stainless-steel cup containing 20 mL of pure PCE was placed in the bottom of two soil columns. The columns were then filled with autoclaved sand and a bacterial inoculum, and sparged with helium to remove oxygen. Anaerobic growth media (described as above) was then pumped into each column to provide an aqueous phase for the experiments. The flow rate of culture medium was controlled at 3 mL per hour. To compare the effects of microbial dechlorination, one column was inoculated with the same enriched dechlorinating culture used in the above kinetics test, while the other column was not. The effluents of both columns were sampled and assayed for biomass, substrate, anaerobic byproducts, PCE, TCE, DCE, VC, and ethylene. Chloroethenes, VOAs, and hydrocarbon gases were determined using identical sampling and analytical procedures as described in Section 3.2.3.

2.8 Reducing Equivalent Calculations

Reducing equivalents used in methanogenesis, acetogenesis, as well as VOA and hydrogen formation were calculated from measured levels of products and molar equivalents factor of 6, 8, 8, 14, 20, and

2 eq/mole for methanol, methane, acetate, propionate, butyrate, and hydrogen respectively. Similarly, the amount of reducing equivalents used for dehalogenation was determined for each microcosm using measured level of dechlorination products and a molar-equivalents factor of 2, 4, and 6 eq/mole for TCE, DCE, and VC, respectively. Reducing equivalents consumed in sulfate reduction were estimated from measured decreases in sulfate concentration, assuming all sulfate was converted to sulfide. This assumption results in a molar-equivalents factor of 8 eq/mole. A similar method for estimating electron flow was reported by DiStefano et al. (1991).

3.0 Results

3.1 First-Generation Microcosms

3.1.1 PCE Dechlorination

PCE dechlorination products were detected in all microcosms; however, there was a large difference in the amount of dehalogenation activity both between substrates and between sediments. The cumulative dehalogenation products measured at the end of the incubation period for each microcosm along with the amount of PCE that was initially added are shown in Figure 3.1. Duplicate bottles for each condition are shown separately. The average initial PCE level for Victoria, Tinker VZ, and Tinker PA were 0.80 ± 0.02, 0.64 ± 0.02, and 0.84 ± 0.03 μmole/bottle (aqueous concentrations of 9.1 ± 0.2, 7.2 ± 0.2, and 9.5 ± 0.3 μM), respectively. The average initial level for Dover was 1.8±0.2 μmole/bottle (aqueous concentration of 20±2 μM). The final mass balance for chlorinated ethenes in all bottles was between 73% and 114% (average of 100 ± 11%). The primary dehalogenation product detected in all bottles was TCE. Small amounts of cis-DCE, 1,1-DCE, and VC were also detected in both the methanol-amended and lactate-amended Tinker VZ microcosms. In each sample, these materials were present at levels less than 0.5% of that for TCE. Dehalogenation was associated with biotic activity because no dehalogenation products were detected in any of the autoclaved controls. The final mass balance for chlorinated ethenes in the autoclaved controls was between 92% and 116%.

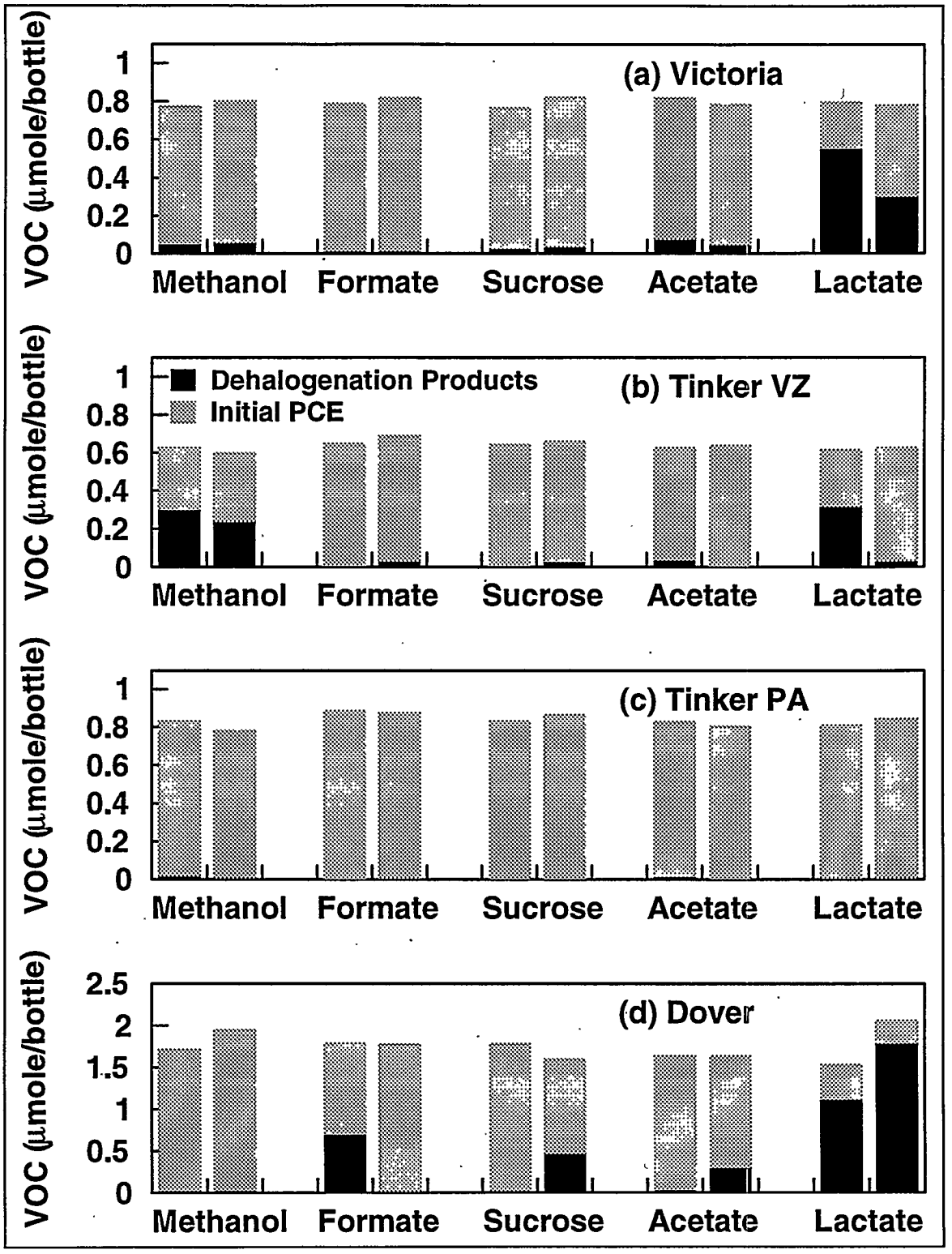

Figure 3.1. Dehalogenation Products Measured at the End of Incubation and the Initial Amount of PCE Added for All Microcosms. Duplicate bottles for each condition are shown separately.

The lactate amendment was the only condition that showed high levels of dechlorination in more than one sediment. Approximately 70% (Bottle A) and 40% (Bottle B) of the added PCE was converted to TCE in lactate-amended Victoria microcosms, and 50% of the added PCE appeared as TCE in lactate-amended Tinker VZ Bottle A. Dover lactate-amended Bottles A and B showed 73% and 86% of the added PCE as TCE. The addition of lactate, however, did not guarantee high levels of dechlorination because the remaining three lactate-amended microcosms showed at most 5% conversion of PCE to TCE.

Methanol, formate, sucrose, and acetate amendments resulted in less widespread dechlorination than the lactate amendment. In methanol-amended Tinker VZ soils, TCE and DCE production represented 50% and 40% of the added PCE for Bottles A and B, respectively. In Dover sediments, 39% of the added PCE was converted to TCE in formate-amended Bottle A. In addition, sucrose Bottle B and acetate Bottle B showed 29% and 17% of the added PCE as TCE, respectively. In contrast, the remaining bottles for all sediments showed at most 1% of the initial PCE as TCE.

There was a large difference between the Victoria and Tinker VZ microcosms in lag-time before the onset of dehalogenation that showed high levels of TCE formation, as indicated in Figures 3.2 and 3.3. Figure 3.2 shows the transient data for the lactate-amended Victoria [(a) and (b)] and Tinker VZ [(c) and (d)] microcosms, while Figure 3.3 presents the transient data for methanol-amended Tinker VZ bottles. Both lactate-amended Victoria microcosms began dechlorinating between days 40 and 75, while the onset of TCE production in both methanol-amended bottles and lactate-amended Bottle A for Tinker VZ ranged from days 85 to 125. For Dover cultures, which showed significant TCE formation, the onset of dehalogenation ranged between days 83 and 292.

Once TCE production commenced, the rate of dehalogenation was comparable in the three sediments. The apparent zero order TCE formation rates for cultures which showed significant dehalogenation are shown in Table 3.1. These values were determined based on observed TCE formation during the period of active dechlorination.

Table 3.1. Apparent Zero-Order Dehalogenation Rates for High Dechlorinating Cultures

Sediment	Condition	Zero Order Rate (nmole/Bottle/Day)
Victoria	Lactate Bottle A	7.2
Victoria	Lactate Bottle B	3.9
Tinker VZ	Methanol Bottle A	3.4
Tinker VZ	Methanol Bottle B	3.1
Tinker VZ	Lactate Bottle A	2.5
Dover	Format Bottle A	5.7
Dover	Sucrose Bottle B	2.2
Dover	Acetate Bottle B	2.4
Dover	Lactate Bottle A	7.2
Dover	Lactate Bottle B	12

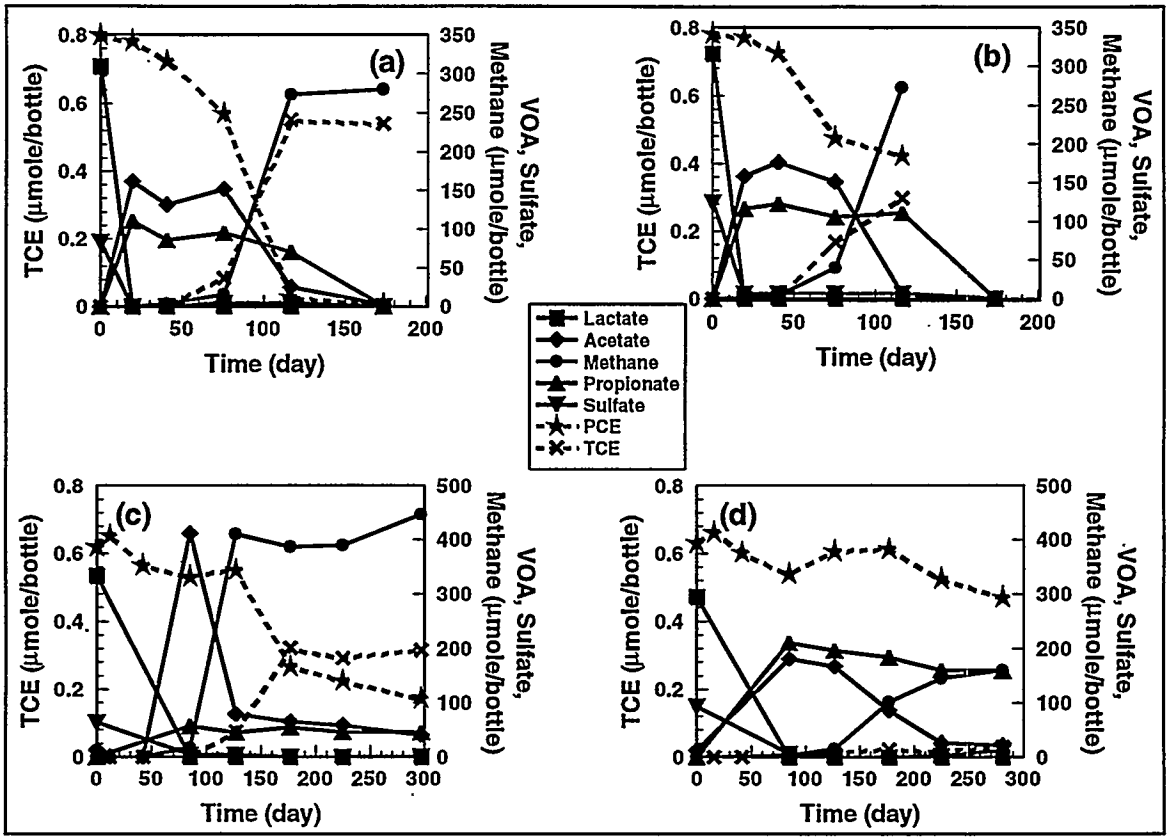

Figure 3.2. Transient Data from Lactate-Amended Microcosms: (a) Victoria Bottle A, (b) Victoria Bottle B, (c) Tinker VZ Bottle A, and (d) Tinker VZ Bottle B

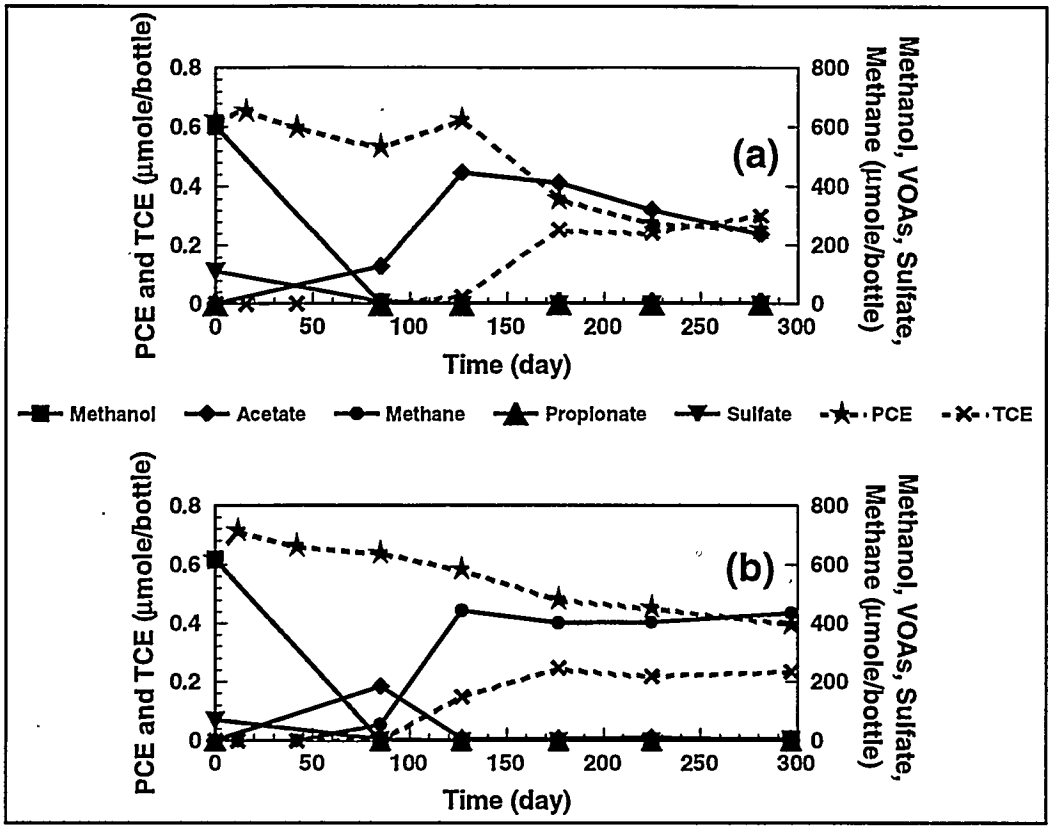

Figure 3.3. Transient Data from Methanol-Amended Tinker VZ Microcosms: (a) Bottle A and (b) Bottle B

3.1.2 Relationship Between Dechlorination and Use of Electron Donors

The fate of added reducing equivalents was examined for each microcosm to assess whether or not a consistent correlation could be established between dechlorination and the use of electron donors. The distribution of reducing equivalents at the end of the incubation period for each microcosm is shown in Figure 3.4. Cultures are presented in this figure in the same order as in Figure 3.1. Butyrate is not represented on this figure because it was not detected in any microcosm. Dehalogenation products are also not depicted on this figure because at most 0.03% of added reducing equivalents were used in this process. Arrows indicate microcosms which produced large quantities of dehalogenation products. Values in this figure have been normalized to the amount of reducing equivalents added in the electron donors. The average recovery of reducing equivalents in the indicated products was 100 ± 20% for Victoria microcosms, 90 ± 30% for Tinker VZ, 80 ± 20% for Tinker PA, and 87 ± 14% for Dover.

It is evident from Figure 3.4 that there is no correlation between the presence of any of the monitored metabolic products and the amount of dehalogenation. Similar amounts of reducing equivalents were used for sulfate reduction, methanogenesis, acetogenesis, and propionate formation in microcosms that exhibited both high and low levels of dechlorination. All four of the lactate-amended Victoria and Tinker VZ sediment microcosms, for example, showed similar amounts of sulfate reduction, lactate fermentation to acetate and propionate, and methanogenesis, yet the amount of dehalogenation products formed in these bottles varied from 0.03 to 0.55 μmole/bottle. Furthermore, both of the methanol-amended Tinker VZ microcosms formed similar high levels of TCE, but their use of reducing equivalents was quite different. In Bottle A, the added methanol was converted to acetate and this compound persisted [Figure 3.3 (a)]. TCE formation occurred in this bottle after the period of acetate formation. In Bottle B, methanol was also converted to acetate, which was subsequently consumed by methanogenesis [Figure 3.3 (b)]. Dehalogenation in this bottle occurred during, and after, the period of methane formation.

Examining the transient response of the lactate-amended Tinker VZ and Victoria microcosms (Figure 3.2) further illustrates the lack of a causal relationship between the monitored catabolic activities and dechlorination. For the three microcosms that produced large amounts of TCE [(a), (b), and (c)], dehalogenation occurred approximately at the same time as acetotrophic methanogenesis. Acetate conversion to methane, however, was not directly related to high dehalogenation activity, because the lactate-amended Tinker VZ microcosm which produced much less TCE [Figure 3.2 (d)] also produced similar amounts of methane from acetate. In addition, none of the acetate-amended or sucrose-amended Victoria microcosms produced large amounts of dechlorination products, yet these systems had transient acetate and methane profiles similar to those shown in Figure 3.2 (a) and (b).

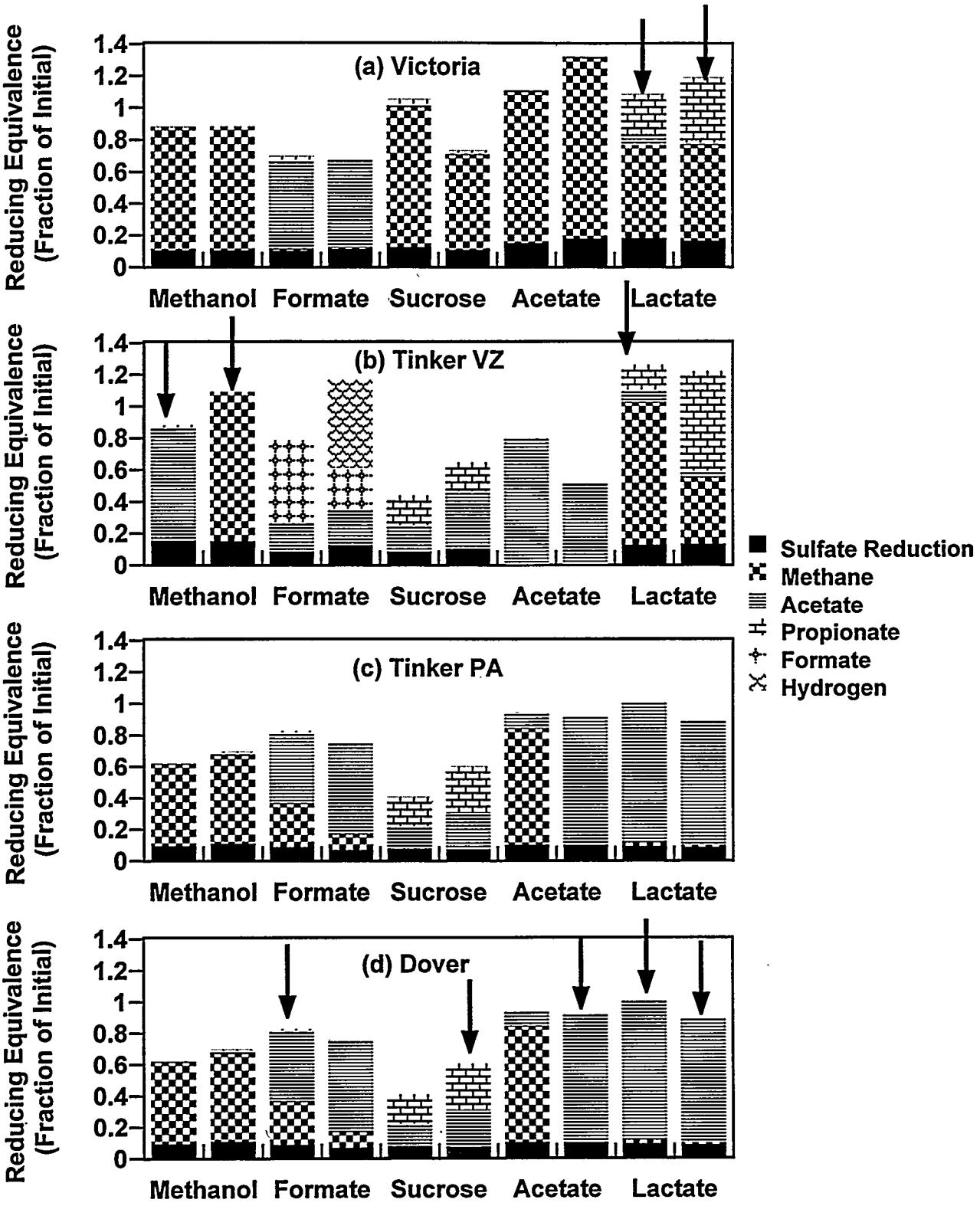

Figure 3.4 Distribution of reducing equivalents for the four sediments at the end of incubation. Duplicate bottles for each conditions are shown separately and bottles are listed in the same order as in Figure 3.1. Arrows indicate microcosms which produced large quantities of dehalogenation products.

The primary difference between the transient electron donor and product profiles for the two lactate-amended Tinker VZ microcosms was that the bottle which showed high levels of TCE formation [Figure 3.2 (c)] also produced more acetate and less propionate than did the bottle with little dechlorination [Figure 3.2 (d)]. The maximum propionate and acetate levels in the high-dehalogenating bottle were 1.2 mM and 6.8 mM, respectively. In the bottle with little TCE formation, a maximum of 4.1 mM of propionate and 3.0 mM of acetate were formed. It is possible that this level of propionate was sufficient to inhibit dehalogenation, because this was almost twice that which was measured in any other microcosm. Inhibition of dehalogenation at this level of propionate, however, would indicate that the responsible organisms are much more sensitive to the organic acid than are other anaerobes because methanogensis was not inhibited in this bottle. Furthermore, others have reported that levels of propionate between 100 and 3000 mM are required for 50% inhibition of methanogensis (Kus and Wiesmann 1995; Fukuzaki et al. 1990).

3.2 Second-Generation Microcosms

The amounts of dehalogenation products formed in second generation chloroethene-amended Victoria, Tinker VZ, and Dover sediments incubated with lactate are summarized in Table 3.2. Values in this table were obtained at the end of the incubation period for both sediments. Duplicate microcosms are reported for each condition as "Bottle A, Bottle B." The chloroethene-mass balance represented by this data was between 70% and 98% (average of 85 ± 10%) for Victoria bottles; between 85% and 111% (average of 93 ± 17%) for Tinker VZ bottles; and between 81% and 83% (average of 82 ± 1%) for Dover bottles. It is apparent from the data in Table 3.2 that between 6% and 62% of added PCE, and between 11% and 59% of added TCE, were converted to decay products. In contrast, no reductive-dehalogenation products were detected in any cis-DCE- or VC-amended culture.

TCE was the only reductive-dechlorination product detected in Dover, Victoria, or Tinker VZ bottles spiked with PCE. For TCE spiked cultures, cis-DCE and 1,1-DCE, but not t-DCE, were detected in bottles with both sediments. In each instance, the amount of 1,1-DCE was only a small fraction of the total DCEs. VC and ethylene were not detected in any of the PCE- or TCE- fed bottles. No dehalogenation products were detected in any of the autoclaved controls.

There was little difference in the flow of reducing equivalents in cultures which showed dehalogenation and those that did not, as indicated in Figures 3.5 and 3.6. Figure 3.5 shows the transient data, for example, second generation lactate-amended Victoria cultures incubated with PCE (a), TCE (b), cis-DCE (c), and VC (d). Similarly, Figure 3.6 presents the transient data, for example, second generation lactate-amended Tinker VZ bottles inoculated with PCE (a), TCE (b), cis-DCE (c), and VC (d). Duplicate bottles showed similar results for all conditions. In both sediments, with all chloroethenes, the added lactate was quickly converted to acetate and propionate, which were subsequently converted to methane. Based on the formation of decay products, it appears that dehalogenation generally occurred throughout the period of methane formation from acetate and propionate.

Table 3.2. Dehalogenation Results at the End of Incubation for Second Generation Lactate-Fed Victoria and Tinker VZ Sediment Cultures. Results for duplicate bottles are reported as Bottle A, Bottle B.

Sediment	Parent Compound	Total Decay Products (μmole/bottle)	Fraction of Parent Dechlorinated
Victoria	PCE	0.14, 0.14	0.39, 0.36
Victoria	TCE	0.29, 0.32	0.59, 0.59
Victoria	cis-DCE	ND[1]	0.0, 0.0
Victoria	VC	ND[1]	0.0, 0.0
Tinker VZ	PCE	0.41, 0.44	0.59, 0.62
Tinker VZ	TCE	0.10, 0.13	0.11, 0.14
Tinker VZ	cis-DCE	ND[1]	0.0,0.0
Tinker VZ	VC	ND[1]	0.0,0.0
Dover	PCE	0.48, 0.25	0.13, 0.06
Dover	c-DCE	ND[1]	0.0,0.0

[1] None detected in either duplicate bottle.

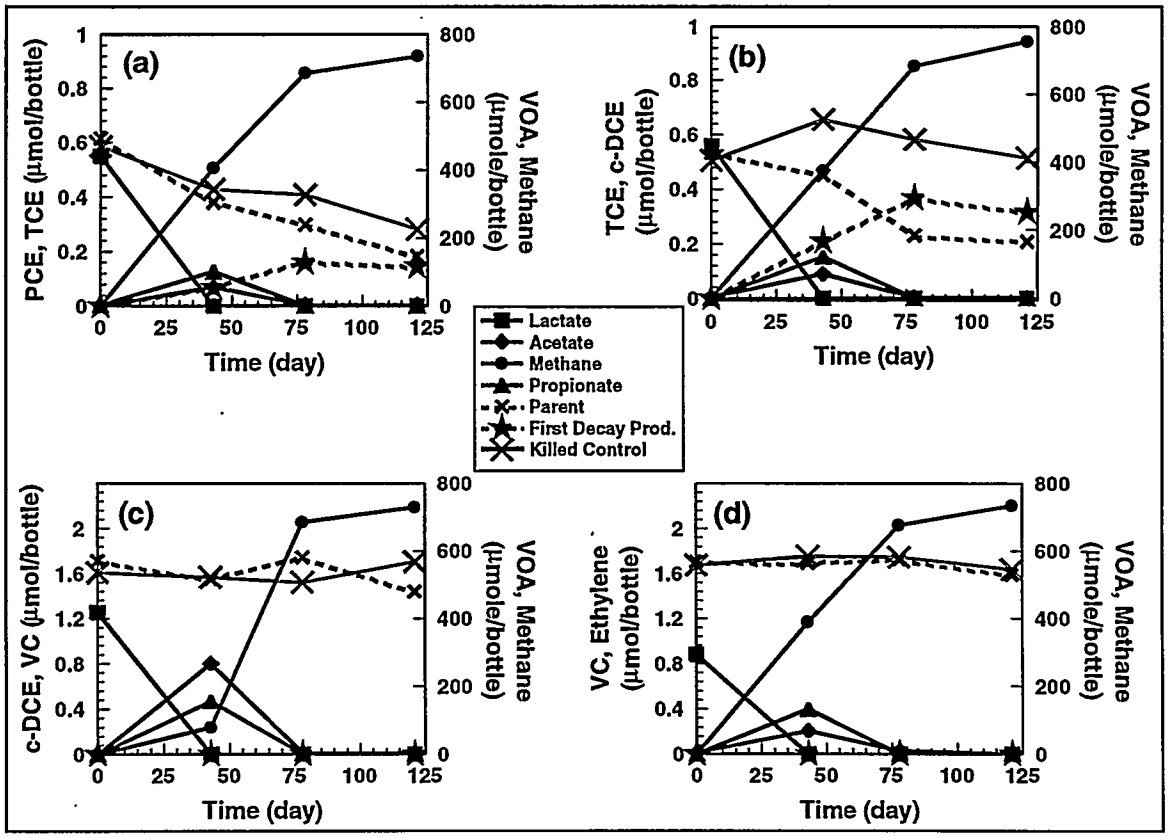

Figure 3.5. Transient Data from Victoria Second Generation Microcosms Amended with (a) PCE, (b) TCE, (c) cis-DCE, and (d) VC

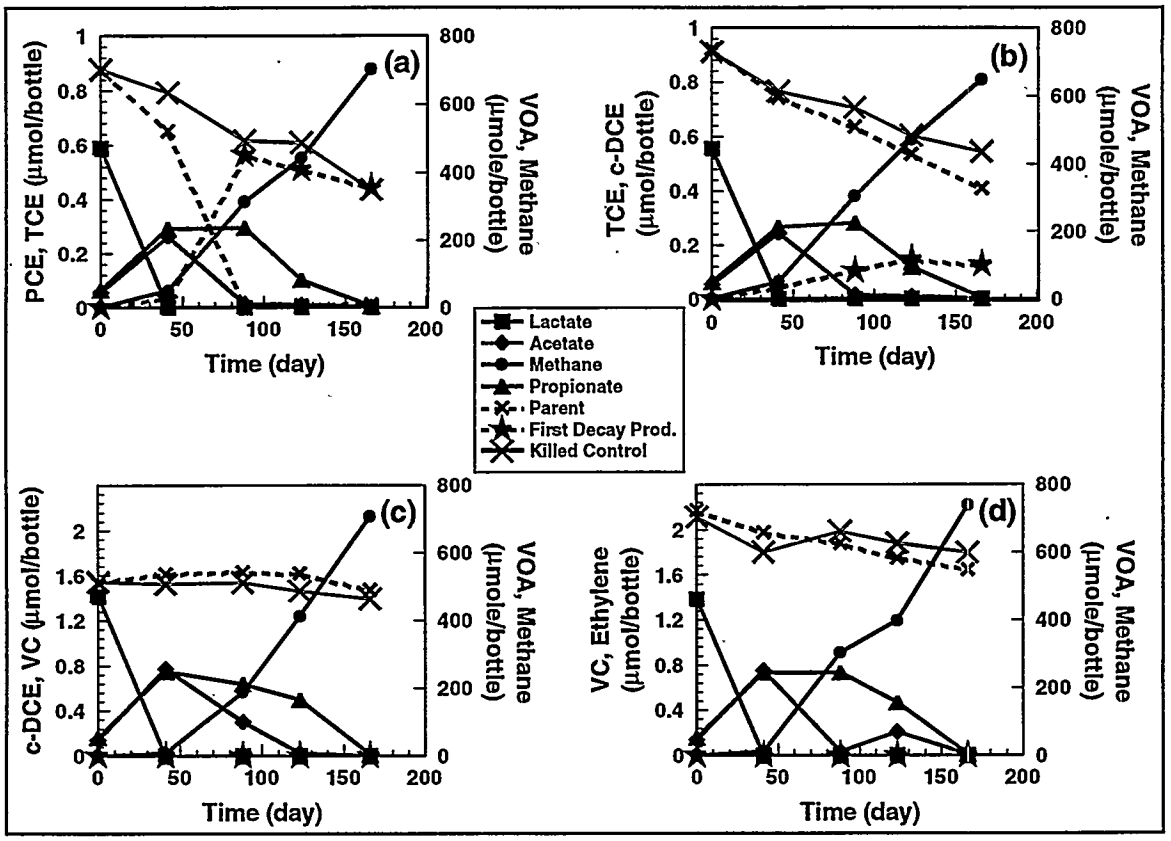

Figure 3.6. Transient Data from Tinker VZ Second Generation Microcosms Amended with (a) PCE, (b) TCE, (c) cis-DCE, and (d) VC

The efficiency of the use of added electron donor for dehalogenation in lactate-fed icrocosms was evaluated for both sediments. Table 3.3 shows the molar ratio of chlorine removed to lactate consumed ($Y_{Cl/L}$) for both initial PCE microcosms and the PCE- and TCE-fed second-generation cultures. For Victoria cultures, $Y_{Cl/L}$ varied from 3×10^{-4} to 2×10^{-3}, while a range of 2×10^{-4} to 1×10^{-3} was observed for Tinker VZ microcosms. Similarly, a range of between 1×10^{-4} and 6×10^{-3} was observed for the lactate-amended Dover bottles. Dechlorination yields appear to diminish with generation for Victoria and Dover, but increase for Tinker VZ. Within a 95% confidence interval, there was no statistically significant difference between the yield for TCE and PCE dechlorination in Victoria or Tinker VZ cultures.

Table 3.3. Dechlorination Yield: Victoria, Tinker VZ, and Dover Lactate-Fed Microcosms

Sediment	Parent VOC	Generation	Initial Aqueous VOC[1] (μM)	Bottle A $Y_{Cl/L}$ (mol/mol)	Bottle B $Y_{Cl/L}$ (mol/mol)
Victoria	PCE	First	9.0, 9.8	2×10^{-3}	9×10^{-4}
Victoria	PCE	Second	7.0, 6.9	4×10^{-4}	3×10^{-4}
Victoria	TCE	Second	6.6, 7.0	7×10^{-4}	8×10^{-4}
Tinker VZ	PCE	First	7.0, 7.1	1×10^{-3}	1×10^{-4}
Tinker VZ	PCE	Second	9.8, 9.7	1×10^{-3}	1×10^{-3}
Tinker VZ	TCE	Second	12, 12	2×10^{-4}	2×10^{-4}
Dover	PCE	First	17, 23	4×10^{-3}	6×10^{-3}
Dover	PCE	Second	7.9, 8.5	2×10^{-4}	1×10^{-4}

[1] Values are reported as Bottle A, Bottle B.

3.3 High PCE Concentration Tests

Initial screening tests were conducted to evaluate the effects of increasing PCE concentration on the dehalogenation process. Results from these experiments are shown in Table 3.4. Although these experiments were only preliminary and did not constitute a detailed study, it is evident from comparing the yield values in Tables 3.3 and 3.4 that there is an increasing trend in this parameter with PCE concentration. The average $Y_{Cl/L}$ for the low PCE Victoria cultures in Table 3.3 is $8.5 \times 10^{-4} \pm 6.1\times10^{-4}$ mol/mol, for example, while the average value for the three highest PCE concentration Victoria tests in Table 3.4 is $3.2 \times 10^{-2} \pm 3.1 \times 10^{-2}$ mol/mol. It is also evident from the data in Table 3.4 that the addition of hexadecane to a culture to provide a constant source of PCE increased the dechlorination yield. The average value of $Y_{Cl/L}$ for hexadecane cultures was 0.19 ± 0.16 mol/mol, compared to 0.032 ± 0.005 mol/mol for the high-concentration tests without hexadecane. This latter result has implications for applying this activity for DNAPL remediation, because it indicates that systems with a PCE source could show a larger fraction of added reducing equivalents used in dechlorination.

Data for two second-generation Victoria microcosms that were amended with lactate, yeast extract, and vitamin B_{12} are also shown in Table 3.4. These bottles were the only ones tested which indicated complete dechlorination to ethylene. Test are now being conducted to determine if these additives will have a similar effect on the Tinker VZ and Dover sediments.

Table 3.4. Dechlorination Yield for Victoria and Tinker VZ Lactate-Fed Microcosms

Sediment	Generation	Amendments	Initial Aqueous VOC[1] (μM)	Final Product	$Y_{Cl/L}$ (mol/mol)
Victoria	Second	Lactate	54	TCE,DCE	6.0×10^{-3}
Victoria	Second	Lactate	52	TCE,DCE	6.4×10^{-3}
Victoria	Second	Lactate	175	TCE,DCE	1.8×10^{-2}
Victoria	Second	Lactate	343	TCE,DCE	1.1×10^{-2}
Victoria	Second	Lactate,Y^1,B_{12}	25	Ethylene	5.0×10^{-3}
Victoria	Second	Lactate,Y^1,B_{12}	303	Ethylene	6.7×10^{-2}
Tinker VZ	First	Lactate,Y^1,B_{12}	472[2]	TCE,DCE	1.0×10^{-1}
Tinker VZ	First	Lactate,Y^1,B_{12}	398[2]	TCE,DCE	2.3×10^{-2}
Tinker VZ	First	Lactate,Y^1	83	TCE,DCE	1.5×10^{-2}
Tinker VZ	First	Lactate,Y^1	82	TCE,DCE	1.5×10^{-2}
Yakima	First	Lactate,Y^1,B_{12}	406[2]	TCE,DCE	3.8×10^{-1}
Yakima	First	Lactate,Y^1,B_{12}	370[2]	TCE,DCE	2.6×10^{-1}

[1] Yeast extract.
[2] Hexadecane phase was present in the bottles to provide a constant source for PCE.

3.4 Kinetics of Dehalogenation by the Victoria Sediment Culture

Equations 1 through 3 were used as the stoichiometric basis for evaluating the kinetics of lactate conversion to methane by this consortium. These equations were developed based on forms reported previously (Wagener and Schink 1988), and the observed VOA, methane, and biomass response of this culture to lactate [Figures 3.2 (a), 3.2(b), and 3.5].

$$CH_3CH_2OHCOOH + Y_{N/L}NH_3 \xrightarrow{\mu_L} Y_{A/L}CH_3COOH + Y_{P/L}CH_3CH_2COOH + Y_{X/L}C_5H_7O_2N + Y_{W/L}H_2O + Y_{CO2/L}CO_2 \quad (1)$$

$$CH_3CH_2COOH + Y_{N/P}NH_3 \xrightarrow{\mu_P} Y_{A/P}CH_3COOH + Y_{X/P}C_5H_7O_2N + Y_{W/P}H_2O + Y_{CO2/P}CO_2 \quad (2)$$

$$CH_3COOH + Y_{N/A}NH_3 \xrightarrow{\mu_A} Y_{M/A}CH_4 + Y_{CO2/A}CO_2 + Y_{X/A}C_5H_7O_2N + Y_{W/A}H_2O \quad (3)$$

The rate expressions adapted from Equations 1 through 3 to describe growth, substrate consumption, methane production, and dechlorination are shown as Equations 4 thorough 11. The form for the growth; substrate consumption; and acetate, propionate, and methane production equations were based on reported examples in literature (Yang and Okos 1991; Yang and Okos 1987). Dehalogenation reactions were linked to methane formation in a manner

similar to that used previously (Skeen et al. 1995; Baek et al. 1990). Coupling dehalogenation to methane formation may not be mechanistically correct for this culture, because similar amounts of less-chlorinated products were produced in first-generation microcosms that showed varying amounts of methane. This form of the model, however, was capable of representing the experimental data, and enables direct comparison of estimated parameters with those reported previously (Skeen et al. 1995; Baek et al. 1990).

$$\frac{d[L]}{dt} = -Y_{L/X}\frac{\mu_L[X][L]}{[L]+K_L} \tag{4}$$

$$\frac{d[P]}{dt} = Y_{P/L}Y_{L/X}\frac{\mu_L[X][L]}{[L]+K_L} - Y_{P/X}\frac{\mu_P[X][P]}{[P]+K_P} \tag{5}$$

$$\frac{d[A]}{dt} = Y_{A/L}Y_{L/X}\frac{\mu_L[X][L]}{[L]+K_L} + Y_{A/P}Y_{P/X}\frac{\mu_P[X][P]}{[P]+K_P} - Y_{A/X}\frac{\mu_A[X][A]}{[A]+K_A} \tag{6}$$

$$\frac{d[X]}{dt} = \frac{\mu_L[X][L]}{[L]+K_L} + \frac{\mu_P[X][P]}{[P]+K_P} + \frac{\mu_A[X][A]}{[A]+K_A} - k_{decay}[X] \tag{7}$$

$$\frac{dM}{dt} = Y_{M/A}Y_{A/X}\frac{\mu_A[X][A]}{[A]+K_A}V_l \tag{8}$$

$$\frac{d[PCE]}{dt} = -Y_{PCE/M}\frac{[PCE]}{[PCE]+K_{PCE}} \times \left(Y_{M/A}Y_{A/X}\frac{\mu_A[X][A]}{[A]+K_A}\right) \tag{9}$$

$$\frac{d[TCE]}{dt} = \left(Y_{PCE/M}\frac{[PCE]}{[PCE]+K_{PCE}} - Y_{TCE/M}\frac{[TCE]}{[TCE]+K_{TCE}}\right) \times \left(Y_{M/A}Y_{A/X}\frac{\mu_A[X][A]}{[A]+K_A}\right) \tag{10}$$

$$\frac{d[DCE]}{dt} = \left(Y_{TCE/M}\frac{[TCE]}{[TCE]+K_{TCE}}\right) \times \left(Y_{M/A}Y_{A/X}\frac{\mu_A[X][A]}{[A]+K_A}\right) \tag{11}$$

Table 3.5. Kinetic Parameters for the Victoria Sediment Culture

Parameter	Test 1	Test 2	Test 3	Average ± Std. Dev.[3]	Electron Donor	Test Type
μ_L (day^{-1})	0.29	0.36	0.14	0.26 ± 0.11	Lactate	I
μ_P (day^{-1})	0.082	0.27	ND[1]	0.12	Propionate	I
μ_A (day^{-1})	0.25	0.013	ND[1]	0.13	Acetate	I
k_{decay} (day^{-1})	0.036	0.016	0.032	0.028 ± 0.011	Lactate	II
$Y_{A/L}$ (mol/mol)	0.19	0.27	0.33	0.26 ± 0.07	Lactate	I
$Y_{P/L}$ (mol/mol)	0.80	0.66	0.74	0.73 ± 0.07	Lactate	I
$Y_{L/X}$ (mol/mol-DW)[5]	22	11	36	23 ± 13	Lactate	I
$Y_{A/P}$ (mol/mol)	1.3	1.1	ND[1]	1.2	Propionate	I
$Y_{P/X}$ (mol/mol-DW)[5]	15	6.0	ND[1]	11	Propionate	I
$Y_{A/X}$ (mol/mol-DW)[5]	9.3	23	ND[1]	16	Acetate	I
$Y_{M/A}$ (mol/mol)	0.95	0.90	ND[1]	0.93	Acetate	I
$Y_{PCE/M}\times10^3$ (mol/mol)	1.0,1.0[2]	1.7	1.6	1.5 ± 0.4	Lactate	II
$Y_{TCE/M}\times10^3$ (mol/mol)	0.19	0.27	0.22	0.23 ± 0.04	Lactate	II
$Y_{M/L}$[4] (mol/mol)	1.1	1.4	1.3	1.3 ± 0.1	Lactate	II
$Y_{X/L}$[4] (g-DW/mol)	1.9	3.0	2.2	2.4 ± 0.6	Lactate	II

[1] No data collected because only two bottle tests were conducted for acetate and propionate.
[2] Value measured with second addition of PCE after all TCE was converted to cis-DCE.
[3] Standard deviations are only shown for parameters that were determined in three tests.
[4] Calculated from measured changes in each parameter over full experiment rather than from a model fit.
[5] A mol-DW of biomass has the formula $C_5H_7O_2N$.

Table 3.5 summarizes the results of the kinetic evaluation for the Victoria sediment culture. Test 1, 2, and 3 in this table refer to results from replicate experiments. Values in this table were estimated using two types of tests. First, sediment-free suspension cultures were used to evaluate the maximum specific growth rate and yield parameters in Equations 4 thorough 8. These tests are labeled as Type I in Table 3.5. Inoculum for these tests were taken from fed-batch dehalogenation reactor tests (Type II tests). The two different experiments were necessary for this analysis because of the very different rates for electron-donor use and dehalogenation. Analysis of the transient response of the added electron donor required daily sampling over a period of at most two weeks. In contrast, dehalogenation occurred over the period of one year and only weekly samples were collected.

In Type I tests, the effects of acetate, propionate, and lactate on VOA production, methane formation, and biomass growth were evaluated separately. Growth rate and stoichiometric parameters related to Equation 3 (μ_A, $Y_{M/A}$, and $Y_{A/X}$) were determined exclusively using temporal data from the acetate-fed tests and SimuSolv. Parameters unique to Equation 2 (μ_P, $Y_{A/P}$, and $Y_{P/X}$) were estimated using data from the propionate-fed experiments and average values for parameters from the acetate test. Similarly, unique parameters related to Equation 1 (μ_L, $Y_{P/L}$, $Y_{A/L}$, and $Y_{L/X}$) were estimated using average values for both the acetate and propionate

Table 3.6. Half-Saturation Constants for Victoria Model

Parameter	Value	Reference
K_L (mol/L)	1.5×10^{-4}	Yang et al. 1988
K_P (mol/L)	1.0×10^{-2}	Lawrence and McCarty 1969
K_A (mol/L)	1.5×10^{-2}	Lawrence and McCarty 1969
K_{PCE} (mol/L)	1.5×10^{-6}	Chu and Jewell 1994
K_{TCE} (mol/L)	1.5×10^{-6}	Chu and Jewell 1994

parameters and data from the lactate-fed tests. Carbon balances were evaluated during the fitting process to ensure that the resulting yield estimates did not violate the law of mass conservation. The half-saturate constants, K_L, K_P, and K_A, were not estimated in this work; instead, the literature values listed in Table 3.6 were used during all simulations.

Dechlorination constants Y_{PCE} and Y_{TCE} were evaluated by optimizing the model fit to the PCE and TCE data from fed-batch reactor tests. During this evaluation the average values for parameters from the acetate-, propionate-, and lactate-fed tests were used to describe cell growth and electron-donor conversion to VOA and methane. The biomass decay parameter, k_{decay}, was also estimated in the reactor tests by optimizing the comparison of measured and predicted levels of biomass. The values for K_{PCE} and K_{TCE} shown in Table 3.6 were used in this work and correspond to that reported by Chu and Jewell (1994) for PCE dechlorination. Published values were used for these parameters because the data reported here were not sufficient to provide an accurate estimate for the half-saturation coefficients.

The model represented by Equations 4 though 11 adequately described the data from both the dehalogenation-reactor experiments and the electron-donor tests. This is demonstrated in Figures 3.7 through 3.11, which show experimental data and model predictions for acetate- (Figure 3.7), propionate- (Figure 3.8), and lactate-fed (Figure 3.9) electron-donor tests, and a dehalogenation-reactor test (Figures 3.10 and 3.11). The arrows on Figure 3.9(a) indicate the times when lactate was added to the culture. No VOA data are presented for the dehalogenation test, because the sampling frequency was not often enough to reveal transient trends. As seen in Figure 3.10 (d), losses of VOCs in the dehalogenation-reactor tests are accounted for by material removed during sampling. The average mass balance for the three tests was $114 \pm 14\%$.

Estimated values of Y_{TCE} were only approximately 15% of that for Y_{PCE}. To determine if this difference was a compound-specific response, or was a result of a loss of dehalogenation activity in the culture between the time PCE and TCE were dechlorinated, a second addition of PCE was added to one of the reactors on day 357. Before PCE addition, the reactor was purged with anaerobic gas to strip VOCs. The subsequent production of TCE was monitored over the next 50 days and used to estimate Y_{PCE}. As can be seen by the second entry for Y_{PCE} under Test 1 in Table 3.5, the resulting value was identical to the original estimate of Y_{PCE}. This result suggests that the difference in Y_{PCE} and Y_{TCE} is not a function of the culture age, but is more likely compound-specific.

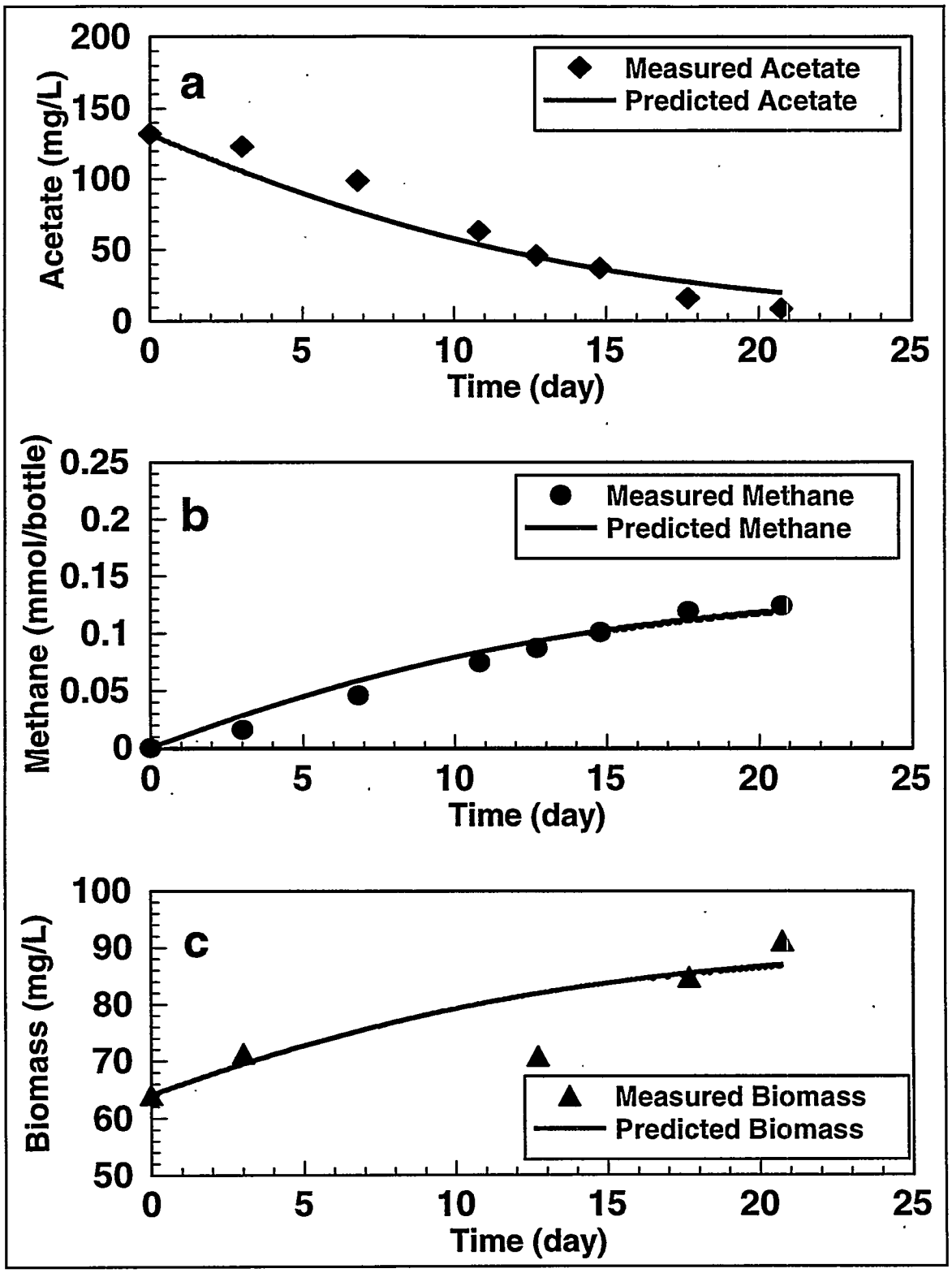

Figure 3.7. Example Experimental Data and Model Fit for an Acetate-Fed Electron-Donor Test Using the Victoria Sediment Culture: (a) Acetate, (b) Methane, and (c) Biomass

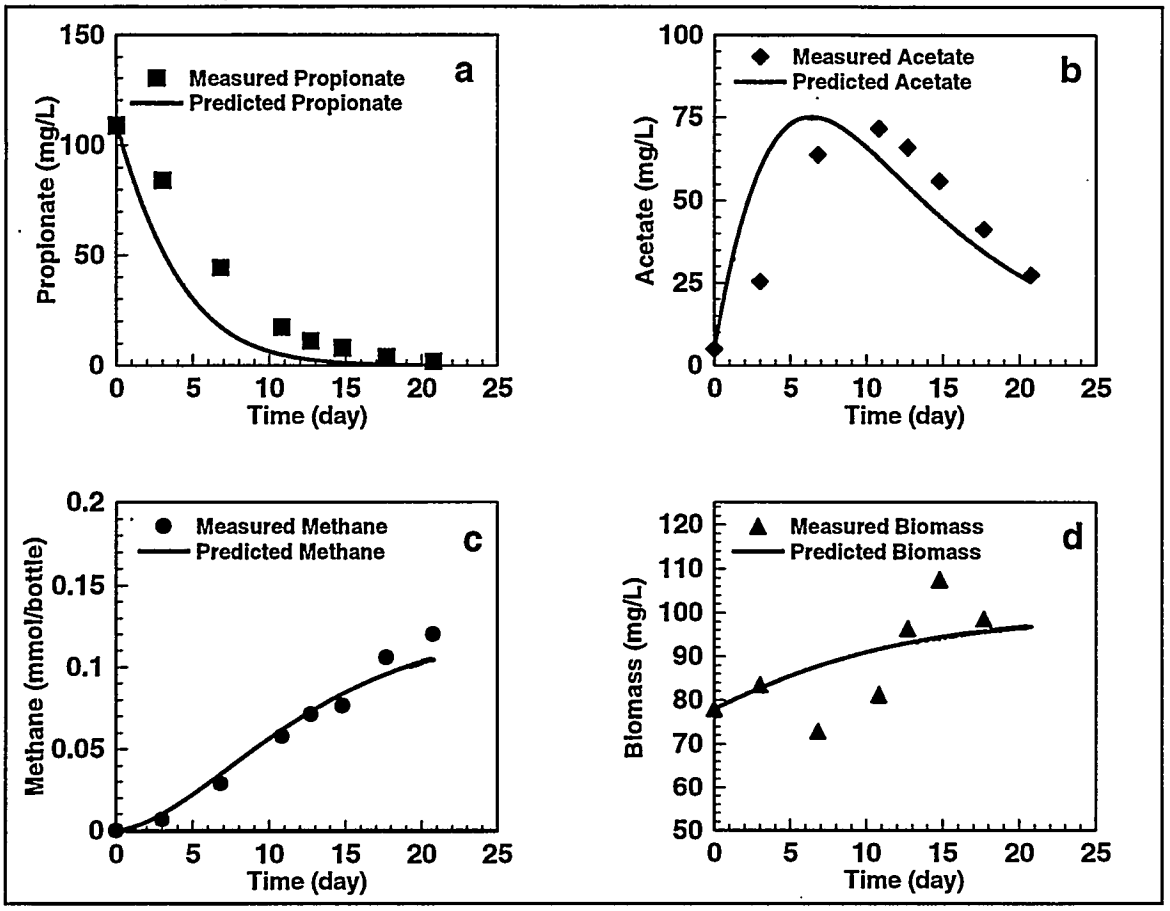

Figure 3.8. Example Experimental Data and Model Fit for a Propionate-Fed Electron-Donor Test Using the Victoria Sediment Culture: (a) Propionate, (b) Acetate, (c) Methane, and (d) Biomass

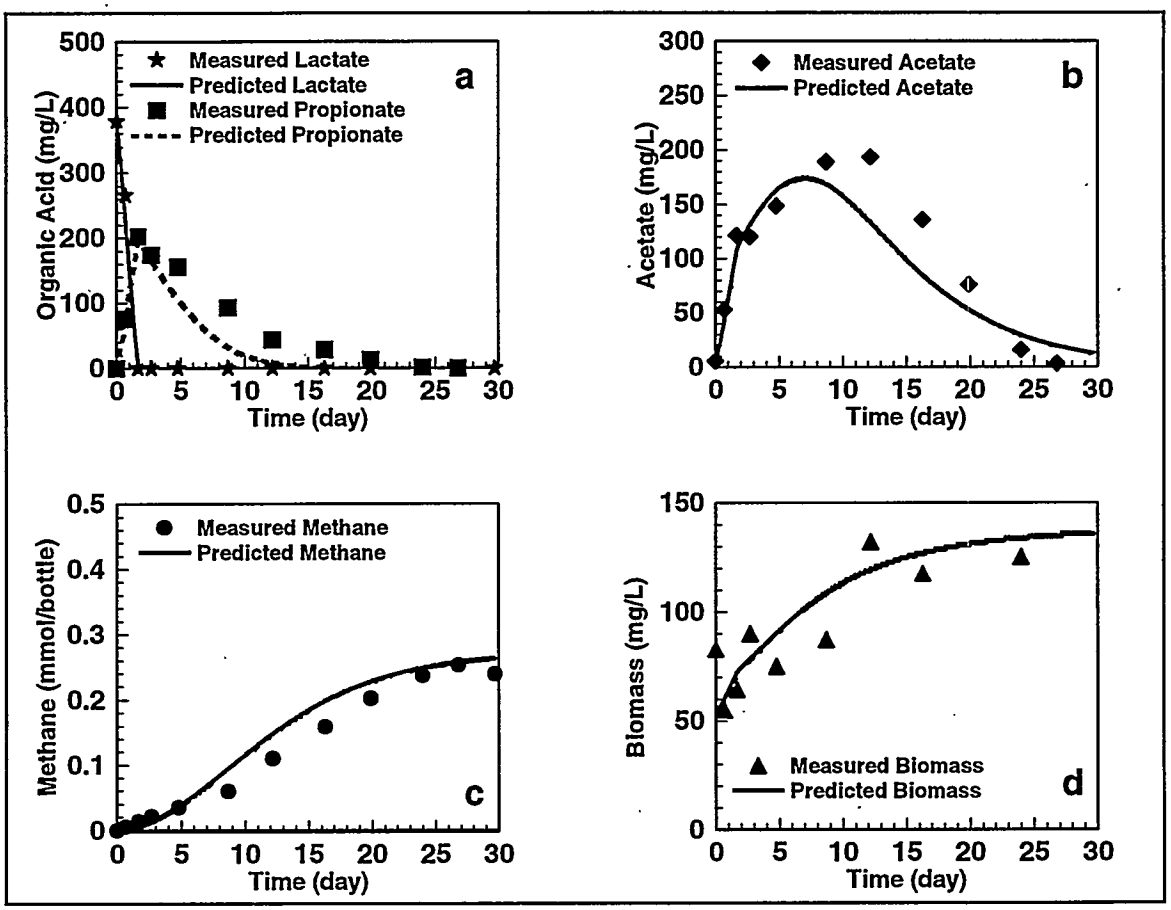

Figure 3.9. Example Experimental Data and Model Fit for a Lactate-Fed Electron-Donor Test Using the Victoria Sediment Culture: (a) Lactate and Propionate, (b) Acetate, (c) Methane, and (d) Biomass

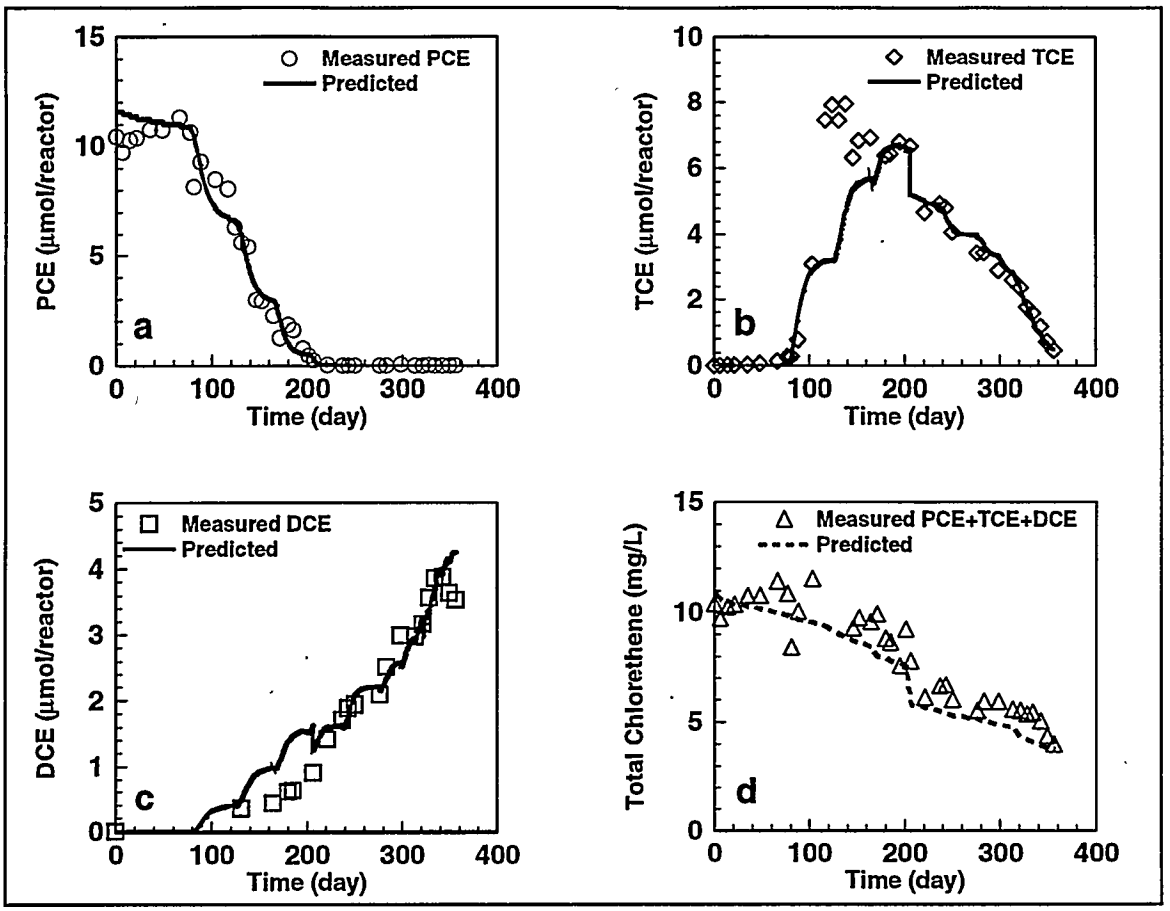

Figure 3.10. Example Experimental Data and Model Fit for a Lactate-Fed Dehalogenation-Reactor Test Using the Victoria Sediment Culture: (a) PCE, (b) TCE, (c) DCE, and (d) Total VOCs in the Reactor

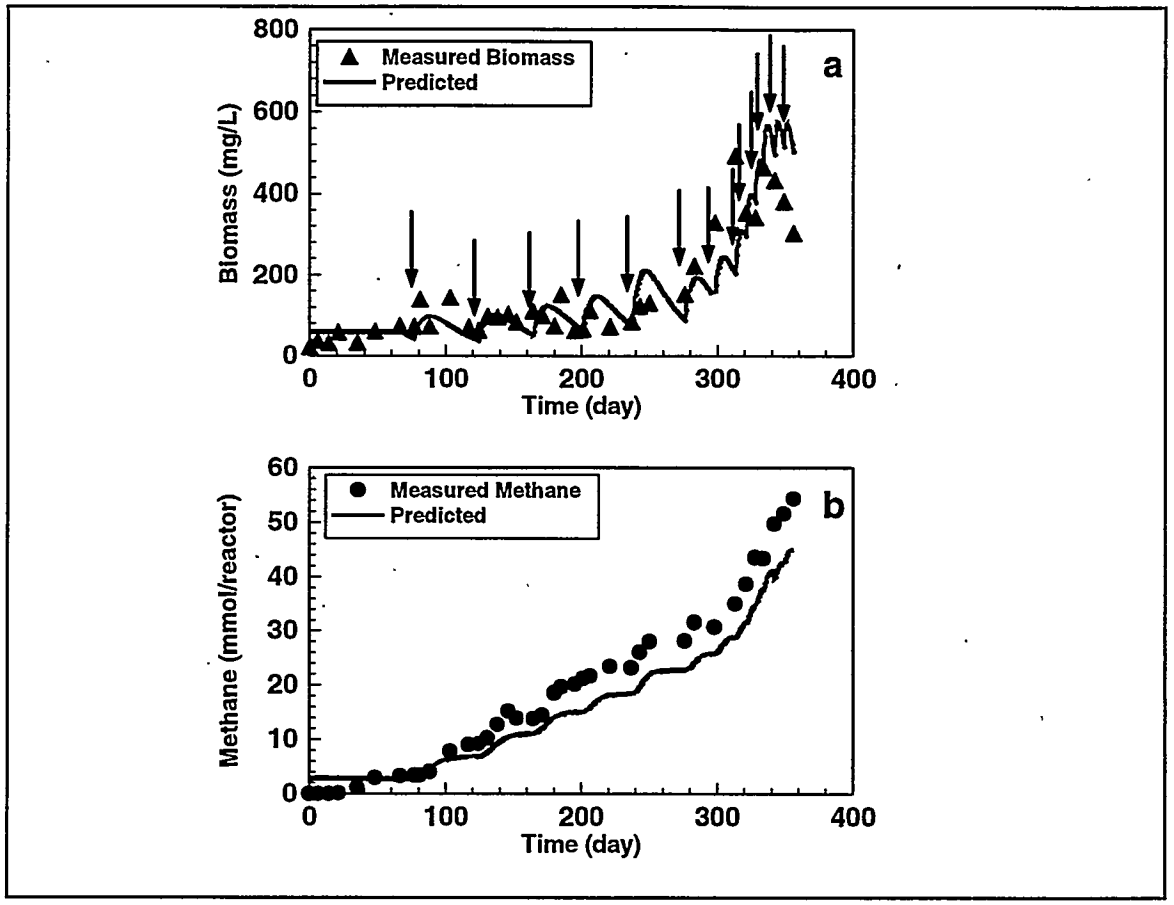

Figure 3.11. Biomass (a) and Methane (b) Data and Model Fit for the Dehalogenation-Reactor Tests Represented in Figure 3.10. Arrows on (a) indicate the time when lactate was added to the reactor.

3.5 PCE Dehalogenation by the Cornell Culture

3.5.1 Dehalogenation Kinetics

The typical PCE dechlorination response of the Cornell culture is shown in Figure 3.12. Points on this figure correspond to measured data, while the lines are simulated response using the kinetic model described below. In this test, PCE was completely dechlorinated to vinyl chloride and subsequently to ethylene. Less than 1% of the total added PCE was ever observed as either TCE or DCE. Figure 3.13 shows the methanol [Figure 3.13 (a)], biomass [Figure 3.13 (b)], methane [Figure 3.13 (c)], and organic acids [Figure 3.13 (d)] concentrations associated with the experiment represented in Figure 3.12. During the complete conversion of PCE to ethene, six additions of approximately 60 mg/L methanol were consumed, which resulted in a slight increase in both biomass and methane. In addition, acetate and propionate were produced. Similar results were also observed in dechlorination experiments using TCE, cis-DCE and VC.

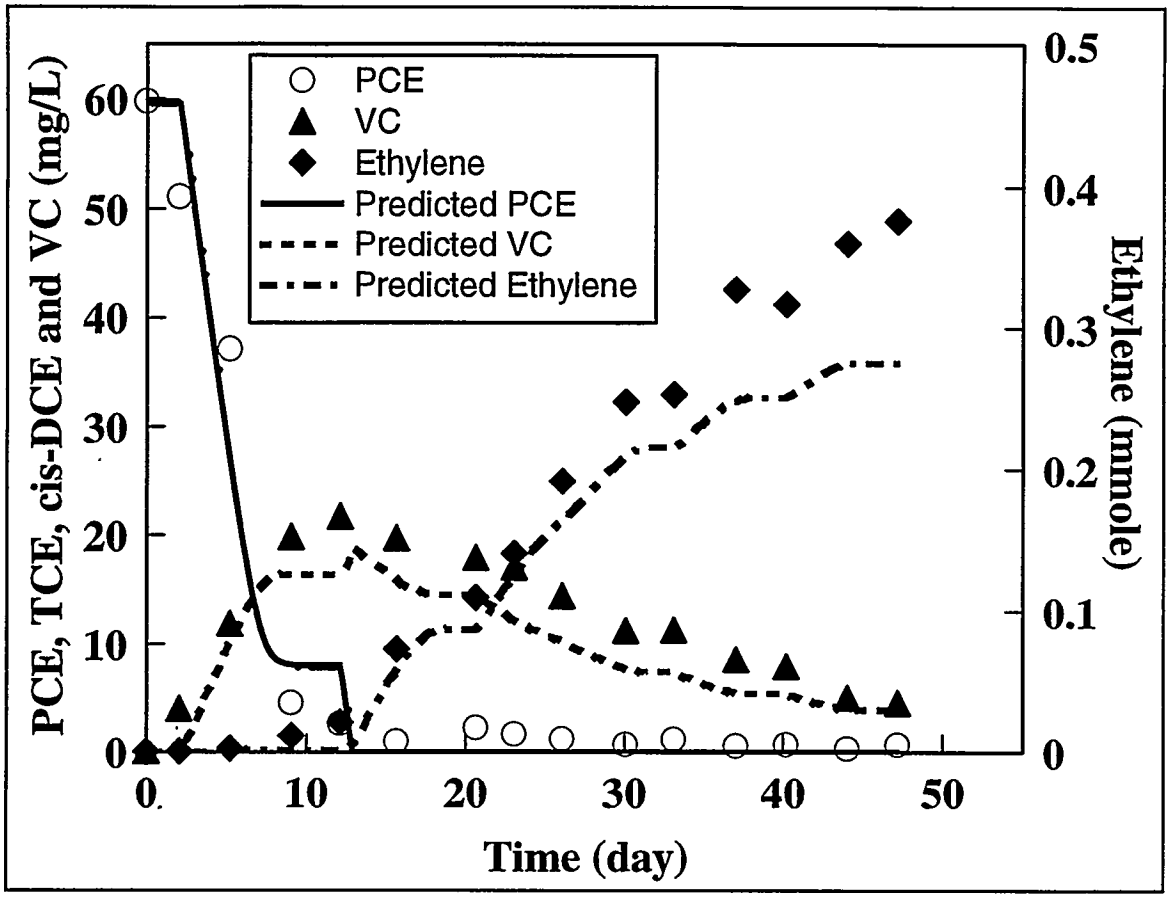

Figure 3.12. Example Data and Model Prediction for a PCE Dechlorination Test Using the Cornell Culture. TCE and DCE results are not presented, because their concentrations were near zero for the entire test.

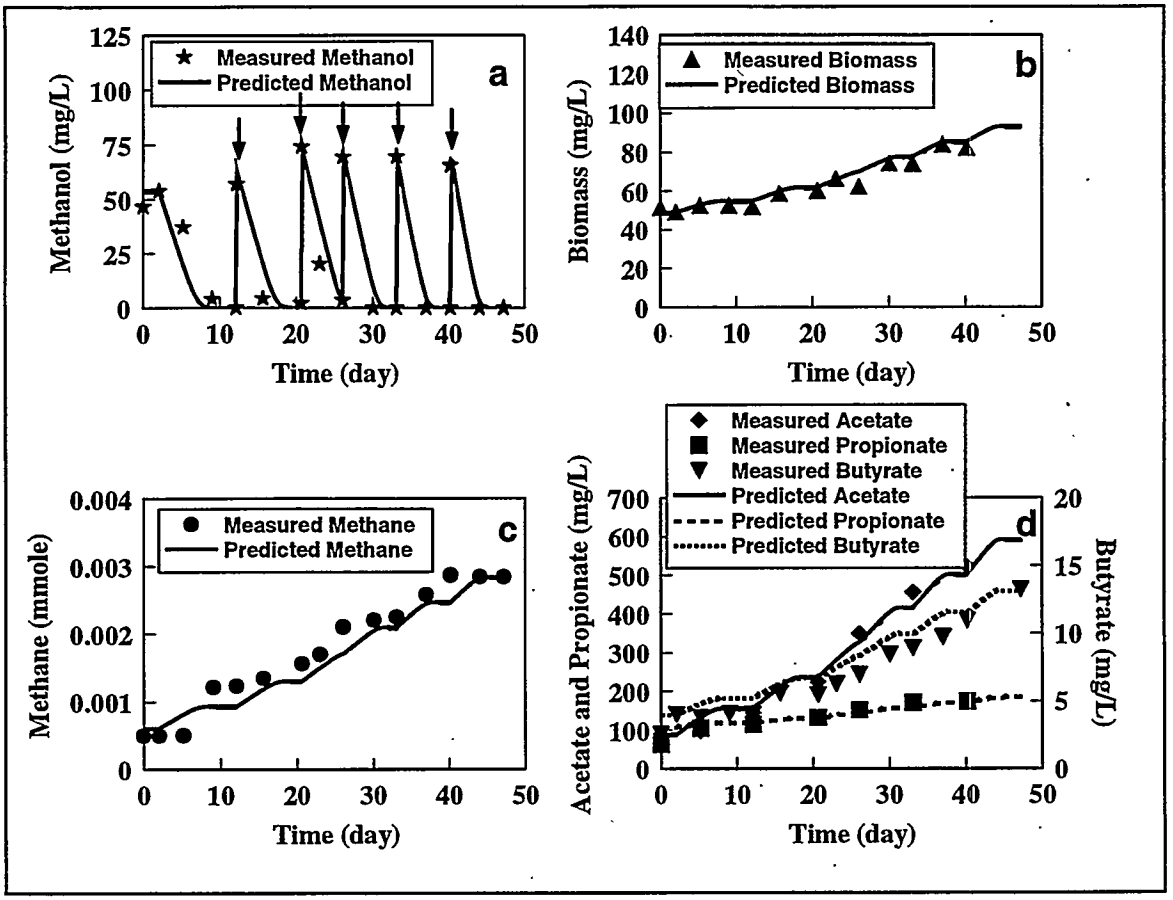

Figure 3.13. Methanol (a), Biomass (b), Methane (c), and VOAs (d) Data and Model Prediction for the PCE Dehalogenation Experiment Represented in Figure 3.12. Arrows on (a) indicate the time when methanol was added to the reactor.

A numerical model was developed to describe dehalogenation of PCE, TCE, cis-DCE, and VC by the Cornell culture. This prediction was based on a modified version of the kinetic framework published previously (Tandoi et al. 1994). The framework assumes a direct relationship between the use of added electron donor and the formation of biomass, methane, acetate, propionate, butyrate, and dechlorination products. The ordinary differential equations that describe all associated processes are given in Equations 12 through 22:

$$\frac{d[Me]}{dt} = -Y_{Me/X}\frac{\mu_{Me}[X][Me]}{[Me]+K_{Me}} \tag{12}$$

$$\frac{d[P]}{dt} = Y_{P/Me}Y_{Me/X}\frac{\mu_{Me}[X][Me]}{[Me]+K_{Me}} \tag{13}$$

$$\frac{d[B]}{dt} = Y_{B/Me}Y_{Me/X}\frac{\mu_{Me}[X][Me]}{[Me]+K_{Me}} \tag{14}$$

$$\frac{d[A]}{dt} = Y_{A/Me} Y_{Me/X} \frac{\mu_{Me}[X][Me]}{[Me] + K_{Me}} \tag{15}$$

$$\frac{d[X]}{dt} = \frac{\mu_{Me}[X][Me]}{[Me] + K_{Me}} \tag{16}$$

$$\frac{dM}{dt} = Y_{M/Me} Y_{Me/X} \frac{\mu_{Me}[X][Me]}{[Me] + K_{Me}} V_l \tag{17}$$

$$\frac{d[PCE]}{dt} = -Y_{PCE/Me} \frac{[PCE]}{[PCE] + K_{PCE}} \times (Y_{Me/X} \frac{\mu_{Me}[Me][X]}{[Me] + K_{Me}}) \tag{18}$$

$$\frac{d[TCE]}{dt} = (Y_{PCE/Me} \frac{[PCE]}{[PCE] + K_{PCE}} - Y_{TCE/Me}[TCE]) \times (Y_{Me/X} \frac{\mu_{Me}[Me][X]}{[Me] + K_{Me}}) \tag{19}$$

$$\frac{d[DCE]}{dt} = (Y_{TCE/Me}[TCE] - Y_{DCE/Me}[DCE]) \times (Y_{Me/X} \frac{\mu_{Me}[Me][X]}{[Me] + K_{Me}}) \tag{20}$$

$$\frac{d[VC]}{dt} = (Y_{DCE/Me}[DCE] - Y_{VC/Me}[VC] \frac{K_{IVC}}{K_{IVC} + [PCE] + [TCE] + [DCE]}) \times \tag{21}$$

$$(Y_{Me/X} \frac{\mu_{Me}[Me][X]}{[Me] + K_{Me}})$$

$$\frac{dETH}{dt} = (Y_{VC/Me}[VC] \frac{K_{IVC}}{K_{IVC} + [PCE] + [TCE] + [DCE]}) \times (Y_{Me/X} \frac{\mu_{Me}[Me][X]}{[Me] + K_{Me}}) \times V_l \tag{22}$$

Optimum parameters for the model are listed in Tables 3.7 and 3.8. Example comparison of predicted versus actual responses using the experimental-specific values listed in these tables is shown in Figures 3.12 and 3.13. Only values that were fit to the data for a given experiment are shown in Tables 3.7 and 3.8. Blank entries indicate that a parameter was set at the average value determined in tests with the less halogenated compounds. The Monod constants K_s, K_{TCE}, K_{DCE}, and K_{VC} were set at previously reported values (Tandoi et al. 1994; Gupta et al. 1994). The values used were 1.7×10^{-4}, 4.0×10^{-5}, 2.0×10^{-5}, and 9.1×10^{-5} mol/L for K_s, K_{TCE}, K_{DCE}, and K_{VC}, respectively. It is evident from the values shown in Table 3.7 that there is consistency in the parameters that describe substrate consumption, biomass growth, and the formation of both organic acids and methane. Similarly, values for dechlorination parameters developed in VC- and DCE-fed tests showed the transient behavior of these species in one TCE-fed test. However, significantly different kinetic parameters were necessary to describe the transient behavior for lower chlorinated compounds for the other TCE-fed test and both PCE-fed tests. This result suggests that the model is not entirely mechanistically consistent with the actual response. This is not surprising given the complex multi-species interactions that underlie the phenomena (DiStefano et al. 1992; Maymó-Gatell et al. 1995).

Table 3.7. Kinetic Parameters for Methanol Consumption, Biomass Growth, and Anaerobic By-Product Formation

Parent Compound	μ_{Me} (day^{-1})	$Y_{Me/X}$ (mol/mol)	$Y_{A/Me}$ (mol/mol)	$Y_{P/Me}$ (mol/mol)	$Y_{B/Me}$ (mol/mol)	$Y_{M/Me}$ (mol/mol)
PCE	0.020	18	10	1.5	0.27	3.0×10^{-4}
PCE	0.027	32	22	2.7	0.27	2.3×10^{-4}
TCE	0.020	24	17	2.1	0.38	2.9×10^{-4}
TCE	0.022	33	21	2.6	0.41	2.8×10^{-4}
DCE	0.015	27	16	2.4	0.10	5.6×10^{-5}
VC	0.048	15	11	0.50	0.086	4.0×10^{-5}
VC	0.13	6.0	4.6	0.33	0.051	1.0×10^{-4}

[1] ND = No data

Table 3.8. Dechlorination Kinetic Parameters

Parent Compound	Y_{VC} (L/mol-Me)	K_{IVC} (mol/L)	$Y_{DCE/Me}$ (L/mol-Me)	$Y_{TCE/Me}$ (L/mol-Me)	$Y_{PCE/Me}$ (mol/mol)
PCE	110		1000	3.7	0.070
PCE	220		1000	5.0	0.23
TCE			0.48	0.033	
TCE				0.087	
DCE		6.5×10^{-6}	2.2		
VC	600				
VC	770				

[1] ND = No data

3.5.2 Flow Cell Tests Using PCE DNAPL

Continuous porous-media flow-cell tests using free-phase PCE and the Cornell culture were conducted to demonstrate that chlororespiration could destroy enough contamination to keep a bulk fluid that contacted a chloroethene DANPL contaminant-free. The results of effluent monitoring for both biotic and abiotic columns packed with DNAPL-contaminated sand that had been inoculated with the Cornell culture are shown in Figure 3.14. Only PCE is shown in the abiotic column because less-chlorinated ethenes were not detected in the test. In the biotic column, PCE in the effluent was completely degraded within 10 days. During this period, VC was detected in the effluent, but not TCE or DCE. After day 10, reductive dehalogenated intermediates continued to decrease until, for the period between 30 to 40 days, all the PCE in

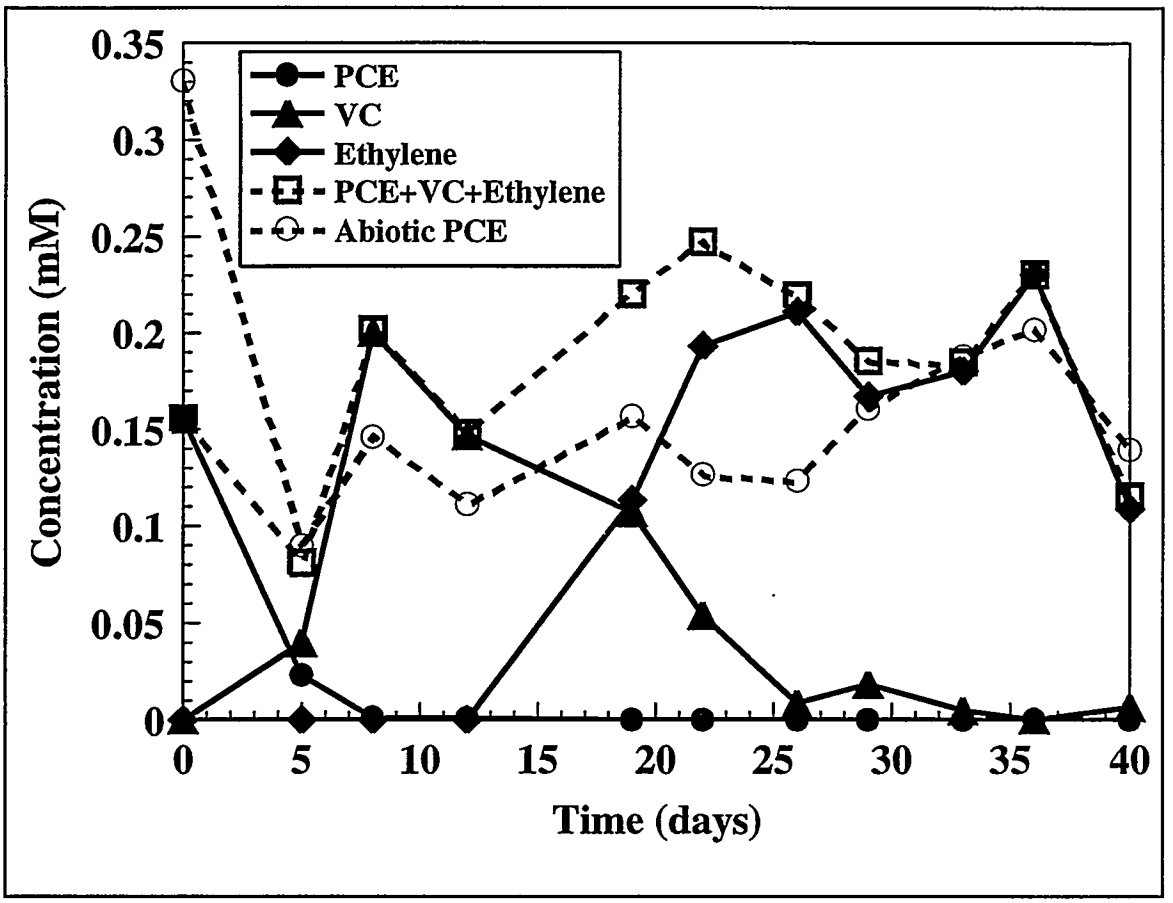

Figure 3.14. PCE and Reductive Dechlorination Products in Biotic and Abiotic Soil Columns. DCE is not shown because no significant concentrations of DCE were observed in these tests. Lines connecting the data points are not model results, but are added to help define data trends.

the effluent had been converted to ethylene. In contrast, the PCE concentrations in the abiotic column remained high for the entire experiment, and were similar to the sum of PCE, VC, and ethylene in the active column. This latter result suggests that similar amounts of PCE were dissolving from the DNAPL pool in both columns.

4.0 Discussion

4.1 PCE Dehalogenation in Anaerobic Sediments

Of the five conditions tested in the first-generation microcosms, amendment with lactate was the only one that showed significant PCE dechlorination in more than one sediment. Under all but one condition, PCE was only converted to DCE with this electron donor. Complete dehalogenation of PCE to ethylene occurred in one second-generation culture which was incubated with lactate, yeast extract, and vitamin B_{12}. Unfortunately, this condition was only tested with the one sediment, and further work is needed to determine if these additives will have a similar effect on the other cultures.

Previous studies of dehalogenation in aquifer sediments also demonstrated that several substrates could stimulate PCE dechlorination to DCE, but lactate provided the most rapid and complete response (Gibson and Sewell 1992). In addition, lactate was found to be an effective substrate for sustaining rapid dehalogenation activity in cultures developed from anaerobic digester sludge (Ballapragada et al. 1995; Fennell et al. 1995). This result suggests that lactate may be an appropriate candidate for screening sediments for PCE and TCE dehalogenation activity, but that lactate alone may not be sufficient to produce complete conversion of PCE to ethylene.

The reason the addition of lactate resulted in more dehalogenation of PCE in the first-generation cultures than did the other electron donors is not evident. Others have suggested that compounds which support dehalogenation produce hydrogen during anaerobic oxidation (DiStefano et al. 1992; Gibson and Sewell 1992). In the experiments reported here, not all substrates that produce hydrogen via fermentative processes caused high levels of dehalogenating activity. In both Victoria and Tinker VZ microcosms, lactate and sucrose were fermented to organic acids and presumably hydrogen (Wagener and Schink 1988; Thauer et al. 1977); however, only lactate supported significant dehalogenation. In addition, for all four sediments, formate was converted predominantly to acetate and levels of hydrogen ranging between 0.1 and 10 mM in the headspace. The hydrogen was subsequently consumed in Victoria, Tinker PA, and Dover sediments, but dechlorination was only observed in one Dover bottle; hence, simply forming hydrogen is not sufficient for stimulating dehalogenation.

Lactate may cause dehalogenation by both providing hydrogen and supporting the formation of other nutritional factors. Maymó-Gatell et al. (1995) observed that dehalogenation in a highly enriched anaerobic culture required the addition of hydrogen, acetate, vitamin B_{12}, and a small amount of supernate from anaerobic sludge. The necessary factors provided by the sludge supernate could not be identified. Removing any one of these components from the culture results in loss of dehalogenating activity. As indicated by these authors, such complex nutritional requirements suggest that dehalogenation in mixed cultures may be facilitated by nutritional cross-feeding. Perhaps a microbially produced micronutrient is not available in the formate- and sucrose-amended slurries while readily produced in the lactate-amended ones. However, before direct comparisons can be made between the dehalogenating activity reported here and that of Maymó-Gatell, work is needed to identify the organisms in these sediments which facilitate reductive dehalogenation.

Analysis of the dehalogenation kinetics for the Victoria sediment culture reveals activity that is very different for that reported using pure strains of methanogens or a methanol-enriched methanogenic consortium (Skeen et al. 1995; Fathepure et al. 1987). Table 4.1 provides a summary of the specific dehalogenation rates for PCE and TCE that are reported in this, and other, work. The observed maximum specific PCE dehalogenation rate for the Victoria culture of 197 ± 83 μmol/g-DW/day is over two orders of magnitude higher than that reported for either pure methanogens or the methanol-enriched methanogenic sediment culture. Similarly, the maximum specific TCE dehalogenation rate of 32 ± 14 μmol/g-DW/day is over an order of magnitude higher that that reported for the methanol-enriched methanogenic consortium. This is true, even though the Victoria kinetic parameters were measured at a lower temperature.

PCE dehalogenation rate by the Victoria culture is more similar to that reported for highly enriched anaerobic digester sludge at 15°C (Chu and Jewell 1994), or that found for the Cornell culture at 18°C. The Cornell culture is known to contain an organism that derives energy for dechlorination (chlororespiration); the same organisms may also be present in the sucrose-enriched culture, because both were derived from anaerobic digester sludge from the Ithaca wastewater treatment plant, and both cultures convert PCE to ethylene (Maymó-Gatell et al. 1996; Freedman and Gossett, 1989; Carter and Jewell 1993). The similarity in rates may suggest that the Victoria sediment culture could have chlororespiring activity similar to the Cornell culture. In addition, because PCE dechlorination rates were similar in the lactate-fed first-generation microcosms for Tinker VZ, Victoria, and Dover, all three sediments could contain this type of activity. This inference, however, must be made with caution because there are obvious differences between the lactate-fed microcosms and the Cornell culture. PCE was only converted to c-DCE, for example, in the sediment microcosms (with the one exception noted previously). In addition, the TCE dehalogenation rate for Victoria is lower than that for PCE, while the TCE conversion rate is higher than the PCE conversion rate in the Cornell culture. Further work is necessary to determine whether there is metabolic similarity in dehalogenation by these cultures.

4.2 Application of Results to In Situ Bioremediation

The possibility that activity similar to the Cornell culture is present in Victoria and other subsurface sediments opens new possibilities for in situ bioremediation. Skeen et al. (1995) demonstrated that the use of chlororespiration for in situ destruction of chloroethenes is more practical than application of anaerobic co-metabolism. Furthermore, chlororespirators have several characteristics that makes them a potential target for remediation of sites contaminated with high levels of chlorinated solvents. First, chlororespirators can tolerate high concentrations of dissolved chlorinated solvents (Ballapragada et al. 1995; DiStefano et al. 1991). This resistance was also demonstrated in the flow-cell tests reported here. Second, these

Table 4.1. Specific Dehalogenation Rate for PCE and TCE

Culture	Temperature (°C)	Parent Compound	Nominal Initial VOC Concentration (µM)	Maximum Specific Dehal. Rate (µmol/g-DW/day)	Reference
Victoria sediment culture	18	PCE	10	197±83	This study
Sucrose-enriched anaerobic digester sludge	15	PCE	60	29[3]	Carter and Jewell 1993
Cornell culture	18	PCE	360	225,1782[4]	This study
Dehalospirillum multvorans	25	PCE	300	1.1×10^5	Neumann et al. 1994
Sucrose-enriched anaerobic digester sludge	30	PCE	97	120[3]	Chu and Jewell 1994
Cornell culture	35	PCE	330	2100	Tandoi et al.1994
Methanosarcina sp.	NR[1]	PCE	6.0	0.42[2]	Fathepure et al. 1987
Methanosarcina mazei	NR[1]	PCE	6.0	0.24[2]	Fathepure et al. 1987
Methanol-enriched sediment culture	30	PCE	30	0.9±0.6	Skeen et al. 1995
Victoria sediment culture	18	TCE	10	32±14	This study
Cornell culture	18	TCE	650	980,4060[4]	This study
Cornell culture	35	TCE	390	6800	Tandoi et al. 1994
Methanol-enriched sediment culture	30	TCE	30	0.4±0.1	Skeen et al. 1995

[1] Not reported

[2] Calculated based on dry biomass consisting of 50 wt% protein.

[3] Calculated based on 0.9 g of volatile suspended solids per gram of dry biomass.

[4] Values for duplicate experiments listed individually.

organisms are capable of efficiently using reducing equivalents for dechlorination (Ballapragada et al. 1995; DiStefano et al. 1991). Third, because the organisms gain energy from the dechlorination process, they may preferentially grow near a DNAPL, thus effectively "seeking out" contamination. This last characteristic is illustrated in Figure 4.1, which shows a conceptual representation of the effects of simulating chlororespiration near a DNAPL.

The concept of using chlororespiration to remediate a DNAPL source was recently evaluated for a site where an injection well had previously been used to dispose of process wastes, including TCE. As a result, TCE DNAPL is now present in a region that extends radially for the injection well for an estimated 9.14 m, and penetrates a vertically distance of approximately 30.48 m. In addition, an $548.6 \times 121.9 \times 60.9$ m dissolved TCE plume emanates from the DNAPL hot spot. A schematic representation of the site is given in Figure 4.2.

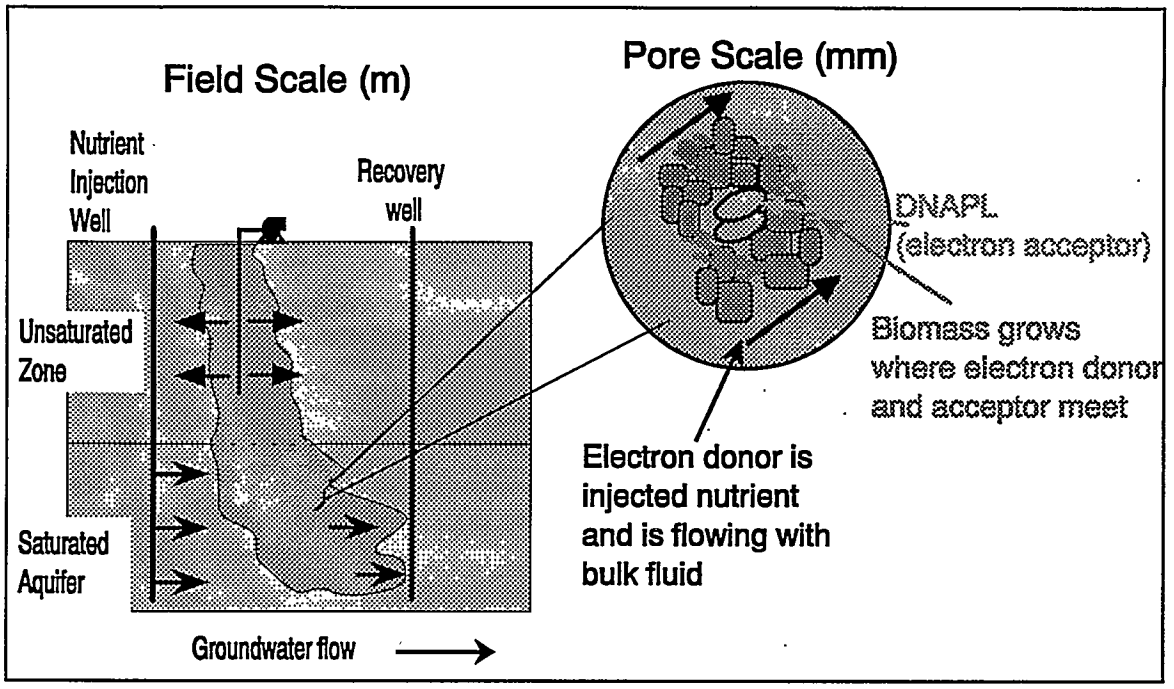

Figure 4.1. Conceptual Representation of the Subsurface Microbial Process Which Will Occur When Electron Donor is Injected Near a Chloroethene DNAPL and chlororespirators are present.

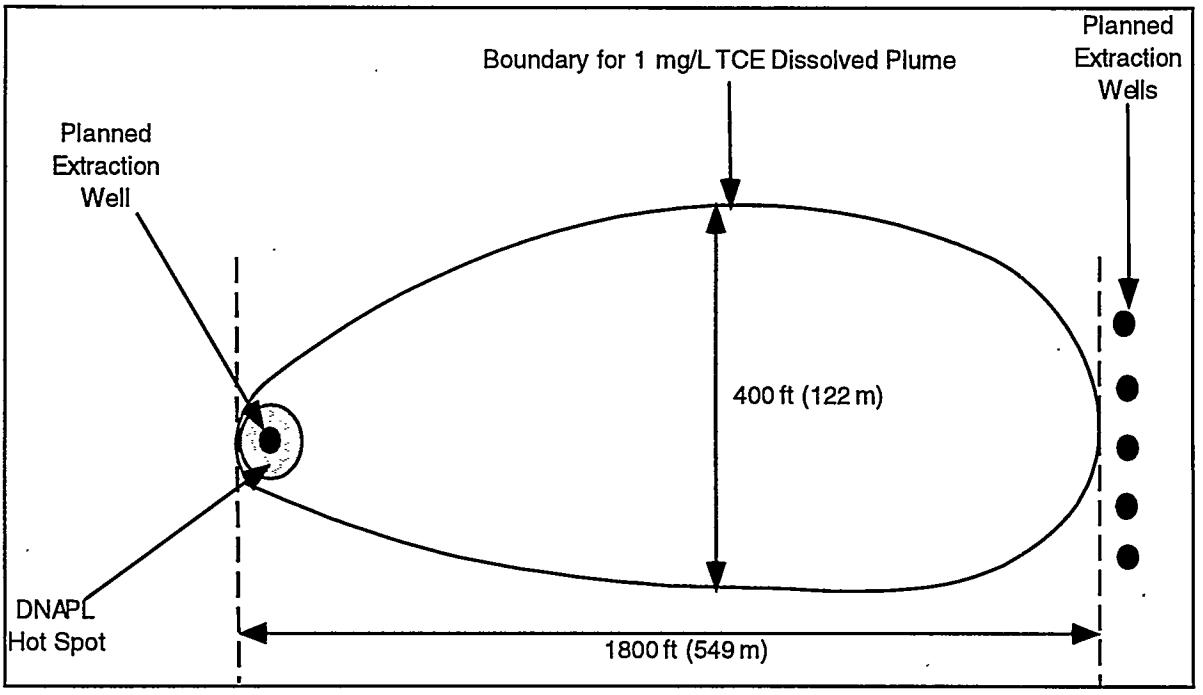

Figure 4.2. Schematic Representation of the Example TCE DNAPL Hot Spot and Associated Dissolved Plume

As shown in Figure 4.2, six extraction wells are planned for the site for a pump-and-treat system. Previous modeling for the Record of Decision had projected that 30 years of pumping at the hot spot at 50 gpm would be sufficient to remove enough mass for dissolved TCE concentrations to drop to below the drinking-water standard of 5 µg/L. Extraction at the five down-gradient wells at a total flow rate of 500 gpm would be conducted for as long as necessary to contain the 1 mg/L and greater TCE plume.

The effects of simulating chlororespiration activity on the remediation strategy were evaluated using estimates for the total mass of TCE in the hot spot and dissolved plume (Table 4.2) in conjunction with the experimentally measured microbial parameters for the Cornell culture at 18°C (first TCE-fed test in Tables 3.7 and 3.8). The resulting bioremediation system requirements are shown in Table 4.3. Anaerobic in situ bioremediation was predicted to cut the remediation time from 30 to 8 years. In addition, the in situ bioremediation process could be implemented using essentially the same well network as for the pump-and-treat system by converting the up-gradient extraction well into a nutrient-injection well and adding an additional nutrient-injection well. The cost savings associated with implementing in situ bioremediation are currently being evaluated. It is anticipated that the estimated cost savings will have a similar trend to those reported by Truex et al. (1996), who observed that the percentage reduction in remediation cost for in situ bioremediation when compared to pump-and-treat is approximately equal to the percentage reduction in remediation time.

Clearly, the results in this report do not fully confirm the presence of chlororespiring bacteria capable of converting PCE to ethylene in subsurface sediments. The results, however, do suggest that anaerobic activity with similar dehalogenation rates and chlorine-release yields for PCE conversion to cis-DCE may be stimulated in sediments from many subsurface environments. Furthermore, satisfying other nutritional limitations may further stimulate cis-DCE conversion to ethylene, as in the case of the Victoria culture incubated with lactate, yeast extract, and vitamin B_{12}. Future work to evaluate the potential for this latter reaction fully is warranted, given the potential benefits of harnessing this activity for in situ remediation of high concentration regions.

Table 4.2. System Properties

Parameter	Hot Spot	Dissolved Plume
Total volume (m^3)	7.9×10^3	4.1×10^6
Pore volume (m^3)	1.6×10^3	8.2×10^5
Initial mass of TCE (kg)	3.0×10^4	2.5×10^3

Table 4.3. Remediation System Requirements for Anaerobic Co-Metabolism of Hot Spot and Dissolved Plume

Parameter	Value
Methanol required to degrade TCE to Ethylene	7.2×10^4 kg
Methane Produced	290 kg
Biomass Produced	1.1×10^4 kg
Recirculation flow rate	50 gpm
Number of wells for nutrient delivery	7
Remediation time	8 years

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