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ABSTRACT

DETERMINATION OF ELECTRON DONORS IN THE REDUCTIVE DECHLORINATON OF TETRACHLOROETHENE

by
Samantha L. Marasigan Bernal

Several substrates, namely fatty acids and alcohols, were used to enhance the reductive microbial dechlorination of PCE to ethene. All of the microcosms amended with the volatile fatty acids (butyrate, succinate, lactate, formate, butyrate/formate mixture and butyrate/succinate mixture) demonstrated complete reductive dechlorination of PCE to ethane. The cultures amended with the butyrate/succinate mixture was the quickest to completely dechlorinate PCE to ethene (49 days). Those amended with butyrate, succinate, formate and the butyrate/formate mixture exhibited complete reductive dechlorination at 77 days. Microcosms amended with lactate exhibited complete reductive dechlorination at 160 days.

The microcosms amended with the alcohols (ethanol, propanol, propanol/ethanol mixture, ethylene glycol and ethylene glycol/butanol mixture) demonstrated less activity than those amended with the volatile fatty acids. Those amended with propanol demonstrated complete reductive dechlorination of PCE to ethene. Those amended with ethanol, ethylene glycol, butanol/propanol mixture and propanol/ethanol mixture produced incomplete PCE degradation, resulting in the accumulation of cis-DCE and VC. Those amended with ethylene glycol/butanol mixture showed no activity.

DETERMINATION OF ELECTRON DONORS IN THE REDUCTIVE DECHLORINATION OF TETRACHLOROETHENE

by
Samantha L. Marasigan Bernal

A Thesis
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APPROVAL PAGE

DETERMINATION OF ELECTRON DONORS IN THE REDUCTIVE DECHLORINATION OF TETRACHLOROETHENE

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To my beloved husband and parents

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CHAPTER 1

INTRODUCTION

1.1 Objective

The objective of this work is to determine the effect of substrates or mixtures of substrates on the reductive dechlorination of tetrachloroethene (PCE) to ethene using soil microcosms that simulate conditions normally found in nature.

1.2 Background Information

Tetrachloroethene (perchloroethene, PCE) is a synthetic chlorinated solvent commonly used in dry-cleaning, degreasing and fumigating operations (1). It is a contaminant frequently found in groundwater (2). PCE is one of the 14 volatile organic compounds regulated under the Safe Drinking Water Act Amendments of 1986 (2).

PCE is a pollutant of major concern. In addition to being a suspected carcinogen, it is also resistant to degradation under aerobic conditions (3, 4, 5). Studies have shown, however, that PCE, under anaerobic conditions, are biodegraded by microorganisms through a process known as reductive dechlorination (6, 7, 8, 9, 10, 11, 12) (Figure 1.1). PCE has been reported to be sequentially biotransformed to trichloroethene (TCE) (13, 14), dichloroethene (DCE) (14, 15) and vinyl chloride (VC) (6, 16). Furthermore, several researchers have reported a complete dehalogenation of PCE to ethene as the final product.

1. $Cl_2C{=}CCl_2 + H_2$ ----------> $ClCH{=}CCl_2 + H^+ + Cl^-$
2. $ClCH{=}CCl_2 + H_2$ ----------> $ClCH{=}CHCl + H^+ + Cl^-$
3. $CHCl{=}CHCl + H_2$ ----------> $CH_2{=}CHCl + H^+ + Cl^-$
4. $CH_2{=}CHCl + H_2$ ----------> $CH_2{=}CH_2 + H^+ + Cl^-$

Figure 1.1 Sequential reductive dechlorination of PCE to ethene

High concentrations of PCE were reduced to ethene using an methanol-fed anaerobic enrichment culture (7, 17). A complete reductive dehlogenation was also reported using a mixed PCE-methanol methanogenic enrichment culture (8, 18, 19). More importantly, PCE has been reductively transformed to ethane (20, 21). Consequently, focus on PCE treatment has shifted away from physical and chemical processes, such as air-stripping and activated-carbon adsorption, and into the biological processes (19).

Biological treatment processes seem more favorable than chemical and physical treatment technologies. One advantage is the transformation of toxic chemicals into non-hazardous compounds rather than merely transferring the pollutant from one medium to another. Another advantage may be a more cost effective and less time consuming process (17).

Further studies, however, need to be conducted in order to better understand the microorganisms responsible for the biotransformation of PCE to ethene and the environmental conditions necessary for complete dahalogenation (17). In addition, conflicting results on which electron donors work best need to be further examined. Despite several demonstrations on the stimulating effect of electron donor addition on

reductive dehalogenation, conflicting reports still remain on which electron donors work best in aiding the dechlorination of PCE to ethene (8, 10, 15, 22, 23).

CHAPTER 2

LITERATURE REVIEW

2.1 Methanogens

Methanogens are strict anaerobes that belong to the kingdom archaebacteria (24). They are the largest and most diverse group within the kingdom. They derive energy by metabolizing acetic acid to carbon dioxide (CO_2) and methane (CH_4), or by reducing CO_2 to CH_4 (25, 29). Carbon monoxide, methanol and formic acid are also metabolized by methanogens (25) (see Figure 2-2). In sediments, about 40% of methane produced comes from H_2 and CO_2 and about 60% comes from acetic acid (26).

(a) $4\ H_2 + CO_2 \text{ --------} \rightarrow CH_4 + 2\ H_2O$
(b) $4H^+ + HCOO^- \text{ --------} \rightarrow CH_4 + 2\ H_2O + 3\ CO_2$
(c) $CH_3OH + H_2 \text{ --------} \rightarrow CH_4 + H_2O$
(d) $CH_3COOH + 4H_2 \text{ --------} \rightarrow 2CH_4 + 2H_2O$

Figure 2.1 Methanogenesis when metabolizing (a) CO_2 and H_2, (b) formic acid, (c) methanol and (d) acetic acid.

Habitats of methanogens are found throughout nature. They include the following: rice paddies, landfills, marshes, sediments, tundra, sewage sludge digesters, termite hindgut, wetwood of trees, rumens of ruminant animals, human large intestine, cecum, protozoa, hydrothermal vent, sediments of freshwater lakes and rivers and anaerobic oceans (11, 26, 29). The combined global production of methane annually is estimated to be 10^9 metric tons (9).

Methanogens play a vital role in the anaerobic food chain, an important part of the carbon cycle. In the methanogenic decomposition of organic matter, methanogens is one of at least three anaerobic groups that are required to interact. The fermentative bacteria degrade polymers, such as amino acids, carbohydrates and pyrimidines to organic acids, alcohols, H_2 gas and carbon dioxide. The acetogenic bacteria oxidize the fermentation end products to carbon dioxide, H_2 and acetic acid. Then, the methanogenic bacteria utilize acetic acid, H_2 and formic acid to produce methane and carbon dioxide (29, 30) (see Figure 2.2).

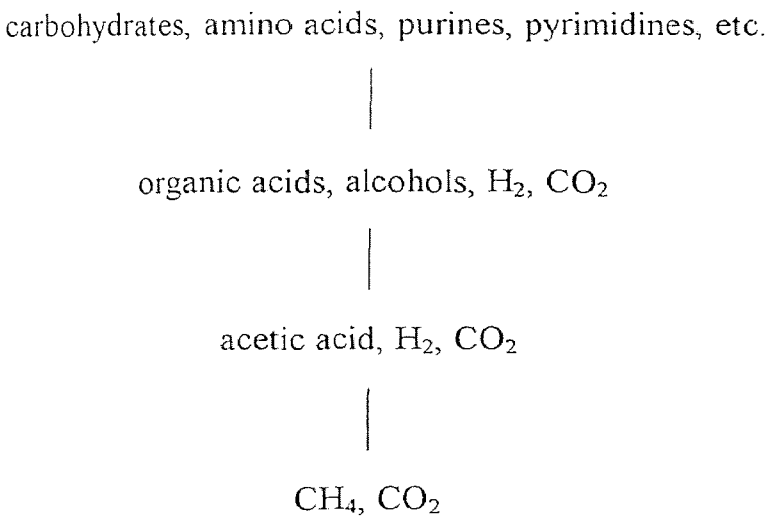


Figure 2.2 The anaerobic food chain.

2.2 Methane Formation from CO_2 and H_2

The CO_2 reduction pathway begins with the reduction of CO_2 to a formyl group. The formyl group is transferred to a methanofuran (MFR) to form HCO-MFR. Ferredoxin, an

iron-sulfur protein, is the most probable electron donor. Tetrahydromethanopterin (H4MPT) is the next C1 carrier. A series of enzyme-catalyzed reactions precedes the final step in the reduction of CO_2 to CH_4. The formyl group is transferred from HCO-MFR to HCO-H4MPT by a formyl-transferase enzyme. The next enzyme cyclohydrolase then forms a methenyl-H4MPT. Methylene-H4MPT dehydrogenase aids the electron carrier $F_{420}H_2$ to form a methylene-H4MPT, followed by a further reduction of the methylene reductase and the electron carrier $F_{420}H_2$ to form methyl-H4MPT (26, 29, 30). The methyl group is transferred from methyl-H4MPT to CoMSH ($HS\text{-}CH_2CH_2SO_3^-$) to form methyl-coenzyme M (SCoM). This is then further reduced by the methyl reductase system, which has two components. One component is a methylreductase that reduces CH3-SCoM to methane and CoM-S-S-CoB. HS-CoB, formerly known as HS-HTP, serves as the electron donor for the methylreductase. A tetrapyrrole (F_{430}) containing a nickel serves as the electron carrier. The other component is a heterodisulfide reductase containing FAD that reduces CoM –S-S-CoB to CoMSH and HS-CoB (29).

2.3 Methane Formation from Acetic acid

The electron derived from the oxidation of the carboxyl group of the acetic acid drives the reduction of the methyl group to methane (26). Acetic acid is first converted to acetyl-CoA, the substrate for carbon monoxide dehydrogenase (CODH), by the actions of acetic acid kinase (reaction 1) and phosphotransacetylase (reaction 2) (29, 30).

Acetic acid kinase:

Acetic acid + ATP --------→ acetyl-phosphate + ADP (1)

Phosphotransacetylase:

Acetyl-phosphate + CoA --------→ actyl-CoA + inorganic phosphate (2)

The methyl group binds to the nickel atom in the Ni-FeS cluster of the acetyl-CoA synthase site, whereas the carboxyl portion binds to the iron atom in the Fe-S cluster. The carbon-carbon bond is then cleaved. The methyl group moves to the cobalt atom in the corrinoid-iron-sulfur protein. It is then transferred to tetrahydromethanopterin (H4MPT), and finally to CoMSH, where it is reduced to methane using the electron derived from the oxidation of the carboxyl group (29). The carboxyl group is transferred to the iron atom in the CODH iron-sulfur cluster, where it is oxidized to CO_2 (26, 29).

Methanogenesis from acetic acid:

(1) $CH_3CO\text{-}CoA$ --------→ $[CH_3] + [CO] + CoA$

(2) $[CO] + H_2O$ --------→ $CO_2 + 2[H]^+$

(3) $[CH_3] + 2[H] + ADP + Pi$ --------→ $CH_4 + ATP$

2.4 Role of Methanogens in Reductive Dechlorination

Several researchers have shown that H_2 serves as a direct electron donor in the reductive dechlorination of PCE. DiStefano et al. demonstrated that H_2 was able to serve as the direct electron donor in the reductive dechlorination of PCE to VC and ethene using a methanol-fed enrichment culture (7, 13). Maymo-Gatell et al. also demonstrated results consistent with those of DiStefano et al. using a H_2-PCE culture produced from a 10^{-6}

dilution of a methanol-PCE culture (19). H_2 has also been shown to serve as the direct electron donor for the growth of strain PER-K23 (20, 27) and *Dehalobacter multivorans* (28).

Because methanogens metabolize H_2 along with CO_2 to derive energy during the production of CH_4, dechlorinators must compete with the methanogens for any available H_2. Smatlak et al. (17) reported inhibitory effects of high levels of PCE on methanogens. However, in the presence of noninhibitory levels of PCE (110 uM), dechlorination activity slowed down as competition for electron donor became greater. The methanol-using acetogens and methanogens competed for methanol, while the hydrogenotrophic methanogens and H_2-using dechlorinators competed for the little H_2 availble (17, 19). Fennell and Gossett also reported the activity of both dechlorinators and methanogens at high levels of H_2. However, at low H_2 levels, dechlorination continued at a slower pace while methanogenesis stopped completely (22).

This suggests the importance of selectively adding substrates (electron donors) that would degrade slowly, and therefore, provide a slow and steady release of low levels of H_2 in order to provide a more advantageous environment to the dechlorinators (22).

CHAPTER III

MATERIALS AND METHODS

3.1 Chemicals

The following were used for the preparation of analytical standards: TCE (99%, anhydrous, Aldrich Chemical Co.), cis-1,2 DCE (97%, Aldrich Chemical Co.), trans-1,2 DCE (1000mg Neat, Supelco), VC (200ug/ml in methanol, Supelco), isobutyric acid (99%, Sigma Chemical Co.), propionic acid (sodium salt, Sigma Chemical Co.), methane (1050 ppm balance of Helium, Scotty I Analyzed Gases), ethene (1000 ppm balance of Helium, Scotty I Analyzed Gases) and ethane (102 ppm balance of Helium, Scotty I Analyzed Gases). The following were used for the preparation of analytical standards and as culture amendments: formic acid (sodium salt, Sigma Chemical Co.), succinic acid (ACS Reagent Grade, Sigma Chemical Co.), n-butyric acid (Sigma Chemical Co.), L(+) lactic acid (98%, sodium salt, Sigma Chemical Co.), acetic acid (2.0N, Sigma Chemical Co.), methanol (HPLC Grade, Fisher Scientific Co.), ethylene glycol (99+% Spectrophotometric Grade, Aldrich Chemical Co.), ethanol (Dehydrated 200 proof, Pharmco), 1-propanol (99+% Spectrophotometric Grade, Aldrich Chemical Co.), 1-butanol (HPLC Grade, Fisher Scientific Co.) and PCE (99+% anhydrous, Aldrich Chemical Co.). Na_2S (3% APHA, Lab Chem Inc.) and 0.10% Resazurin (Fisher Scientific Co.) were used for the preparation of media. The water used in the experiment was 18-megaohm Milli-Q water.

3.2 Media Preparation

The medium used to make the soil microcosm was prepared from the following stock solutions: solution A, solution B, trace element solution and vitamin solution. Solution A (non-sterile) consisted of the following (in g/L): 1.0 KH_2PO_4, 1.0 K_2HPO_4, 2.0 NaCl and 1.0 NH_4Cl. Solution B (non-sterile) consisted of the following in (g/L): 0.10 $MgSO_4$ and 0.10 $CaCl_2$. The trace element solution consisted of the following in (g/L): 2.0 disodium nitriloacetate, 0.8 $Fe(NH_4)_2(SO_4)_2.6H_2O$, 1.0 $MnSO_4.H_2O$, 0.2 $CoCl_2.6H_2O$, 0.2 $ZnSO_4.7H_2O$, 0.02 $CuCl_2.2H_2O$, 0.02 $Na_2MoO_4.2H_2O$, 0.02 $NiCl_2.6H_2O$, 0.02 Na_2WO_4 and 0.02 Na_2SeO_3. The vitamin solution contained the following in (mg/L): 10.0 pyridoxine.HCl, 5.0 riboflavin, 5.0 thiamine.HCl, 2.0 biotin, 5.0 vitamine B_{12}, 10.0 mercaptoethanesulfonic acid and 2.0 folic acid.

In a 2-L flask, 100 mL of solution A were added to 800 mL of autoclaved deionized water, which was autoclaved at 120°C and 15 psi for 20 minutes. It was then purged at 5 psig with N_2:CO_2 mixed gas (80:20) which had been passed through a column of hot reduced copper fillings. While being purged, the solution was heated and maintained at 80°C for one hour. Once the solution cooled down to room temperature, 100 ml of solution B were added. The solution was then purged for an additional 30 minutes, followed by the addition of the following reagents: 0.60 g $NaHCO_3$, 10 ml of 3% Na_2S, 0.10 ml of 0.10% resazurin, 1.0 ml of trace element solution and 1.0 ml of vitamin solution. The volume was then adjusted to 1.0 liter. The flask was promptly sealed with a butyl rubber stopper and transferred to the anaerobic chamber (70 N_2: 30 H_2).

3.3 Sediment Slurry Preparation

Sediment was collected from the Arthur Kill with a hollow, metal cylindrical device attached to a reel on the boat that was dropped into the water. With the sediment packed inside the metal cylinder, it was then retrieved and immediately captured by plastic cylinders placed under the metal cylinder to catch the falling sediment. The sediment from the plastic cylinders was transferred into a wide mouth Ball™ 2-quart mason jar. It was then stored at 4°C in a BBL Gas Pack™ jar containing a BBL Gas Generator Envelope™, which generates CO_2 and H_2, to maintain anaerobic conditions.

In an anaerobic chamber (70:30; N_2:H_2), 100 g of the sediment was dispensed into a 1-L flask and diluted to the 500-mL mark with the media. The soil slurry was stirred and incubated for 24 hours. Prior to sample analysis, the soil was screened for any chlorinated ethene with the purge and trap. No measurable amounts of chlorinated ethene were detected.

3.4 Reagent Preparation

A 712.5 uM PCE solution was prepared in the anaerobic glove box. In a 160-ml serum bottle, 11 uL of PCE was dispensed into 150 ml of prepurged [N_2:CO_2 mixed gas (80:20)] autoclaved deionized water. A teflon-coated magnetic stir bar was added to ensure proper mixing. The bottle was immediately sealed with a teflon-coated rubber stopper and an aluminum crimp seal. The solution was stirred on a magnetic hot plate at room temperature for 24 hours. All preparations were performed inside the anaerobic chamber.

57mM electron donor solutions were prepared by dispensing a suitable amount of the appropriate electron donor into 200 mL of prepurged [N_2:CO_2 mixed gas (80:20)] autoclaved deionized water in a 250 mL flask (see Table 3.1). The pH was adjusted with NaOH solution to about 7.5 with a Fisher Scientific Accumet™ digital pH/mV meter. The flask was diluted quantitatively to mark. The solutions were transferred into a 160 mL bottle and sealed with a teflon-coated rubber stopper and an aluminum crimp seal.

Table 3.1 Amount of electron donor added per 250 mL of autoclaced DI H_2O

Electron donor	Mass added in grams	Volume added in mL
Lactic Acid	1.5974	---------
Succinic Acid	3.8489	---------
Formic Acid	0.9691	---------
Butyric Acid	---------	1.3024
Ethanol	0.8360	---------
Propanol	1.0650	---------
Ethylene Glycol	0.7950	---------
Butanol	1.3040	---------

3.5 Microcosm Preparation

Microcosms were prepared in a 38-ml serum bottle inside the anaerobic glove box. Two variations of microcosms were prepared: one was made with the addition of one electron donor, and another with the addition of two electron donors. The first variation was prepared by adding the following: 20 ml of media, 1.0 ml of 712.5 uM PCE, 2.5 ml of the 57 mM electron donor and 5.0 ml of soil slurry. There was a total volume of 28.5 mL and a 9.5 mL headspace. The second variation was prepared by adding the following: 17.5 ml of media, 1.0 ml of 712.5 uM PCE, 2.5 ml of each 57mM electron donor, and 5.0 ml of

soil slurry. There was a total volume of 28.5 mL and a headspace of 9.8 mL. The serum bottles were immediately sealed with a teflon-coated rubber stopper and an aluminum crimp seal after all additions were made. Nineteen bottles were prepared for each electron donor for a total of six sampling periods (see Table 3.2). Triplicates were sacrificed every sampling period. They were incubated in the dark at 25°C until point of analysis.

Table 3.2 Acid and alcohol microcosms

Lactic Acid
Formic Acid
Succinic Acid
n-Butyric Acid
n-Butyric Acid/Succinic Acid
n-Butyric Acid/Formic Acid
Ethanol
1-Propanol
Ethylene Glycol
1-Propanol/Ethanol
1-Butanol/1-Propanol
Ethylene Glycol/1-Butanol

Negative controls were set up in the anaerobic glove box. 5 mL of the soil slurry, 2.5 mL of the 57 mM electron donor solution and 20 mL of the media were dispensed into a 38 mL serum bottle. The serum bottles were sealed with a teflon-coated rubber stopper and an aluminum crimp seal. After autoclaving the bottles for one hour at 120°C and 15 psi, the bottles were cooled to room temperature. It was brought back inside the anaerobic glove box, where 1 mL of 712.5 uM PCE was added. The bottles were resealed with a teflon-coated rubber stopper and aluminum crimp seal. Six negative controls were prepared for each electron donor for a total of six sampling periods (see Table 3.3). The negative controls were incubated in the dark at 25°C until point of analysis.

Table 3.3 Negative controls

n-Butyric Acid
Succinic Acid
Lactic Acid
Methanol
Ethanol
1-Propanol
1-Butanol

3.6 Headspace Gas Analysis

Ethene, ethane and methane were analyzed by performing a headspace gas analysis on a Varian 3600TM Gas Chromatograph (GC) equipped with a flame ionization detector (FID). Air was used as the carrier gas at a flow rate of 300mL/min and a pressure of 36 psi at 50°C. The H_2 rate was set at 30mL/min to keep the flame lit. A stainless steel column (Hayesep D, 10' x 1/8" i.d. x 0.085" df, mesh 80/100, Alltech Co.) was used. The column temperature was set at 50°C; the injector temperature was set at 100°C; the detector was set at 200°C. The GC was programmed to have an attenuation of 8 and a range of 12. The GC was hooked up to a computer, which used a Hewlett Packard Minichrom Chromatography Data System™ version 1.62 software to process the data. The duration of the sampling time was 9 minutes per sample.

The analysis was performed by injecting a Pressure Lok™ gas-tight glass syringe into the serum bottle. The valve on the syringe was opened and then 1 mL of the gas from the headspace was withdrawn. After 15 seconds, the valve was closed. Precautions were taken to ensure no liquid was withdrawn with the gas. The syringe was then removed from the bottle, and then injected into the GC

3.7 Alcohol Analysis

Short-chained alcohols were analyzed using a Hewlett Packard Series II 5890™ GC equipped with an FID and a Hewlett Packard GC System Auto Injector™, which injects 1 uL into the GC. Air was the primary component of the carrier gas at a pressure of 50 psi and a flow rate of 426 mL/min. Helium was used as the auxiliary gas and was set at a flow rate of 15.5 mL/min. H_2 was used to keep the flame lit in the FID and was set at a flow rate of 29.5mL/min. A guard column (deactivated phenyl-methyl, 5 m x 0.32 mm i.d.) and a Restek RTX-200™ column (30 m x 0.32" i.d. x 1.0 df) were used to separate the alcohols. The GC oven temperature was set at 55°C for ten minutes and then raised to a final temperature of 150°C for ten minutes at a rate of 25°C/min. The injector temperature was set at 200°C; the detector temperature was set at 250°C, and the column flow was set at 31.8 mL/sec with the column feature enabled. A split injection ratio of 9.4:1 was set to prevent the FID flame from extinguishing due to the water in the samples. The duration of the sampling time was 23.80 minutes per sample. The GC was hooked up to a computer, which used a Hewlett Packard Minichrom Chromatography Data System™ version 1.62 software to process the data.

With a 3.0 ml Becton Dickinson™ syringe fitted with a 21-gauge needle, 2 mL of the liquid sample was withdrawn and filtered with a non-sterile 0.22 um nylon syringe filter (Micron Separations Inc.) into a Target DP™ vial (National Scientific Company) fitted with a cap that contained a teflon/silicone septum. The vials were then loaded into the autosampler.

3.8 Fatty Acid Analysis

Fatty acids were analyzed with a Waters High Performance Liquid Chromatography (HPLC) equipped with a Waters 484™ Tunable Absorbance Detector set at a wavelength of 210 nm, a Waters 600E™ System Controller and a Waters 715™ Ultra Wisp Sample Processor. The eluent used for the HPLC was a 0.1% H_3PO_4 set at an isocratic flow rate of 0.50 mL/min with a somewhat stable pressure on the column of 522 psi. Prior to addition to the reservoir bottle, the 0.1% H_3PO_4 solution was sonicated for 30 minutes. To further ensure the absence of air bubbles, the solution was continuously sparged with He at a flow rate of 20 mL/min. A guard column (Supelcogel C610H 5.0 cm x 4.6 mm i.d.) was installed to catch material that woud otherwise bind permanently to the column. The Supelcogel C-610HTM carbohydrate column with a polystyrene divinylbenzene support (30 cm x 7.8 mm i.d.) was used to separate the acids. It was maintained at 30°C by a Waters Temperature Control Module. The duration of the sampling time was 60 minutes. The GC was hooked up to a computer, which used a Hewlett Packard Minichrom Chromatography Data System™ version 1.62 software to process the data.

With a 3.0 mL Becton Dickinson syringe fitted with a 21-gauge needle, 2.0 mL of the liquid sample was withdrawn from the 38.0 mL serum bottle and filtered through a non-sterile 0.22 um nylon syringe filter (Micron Separations Inc.). 0.70 mL of the filtrate was dispensed into a 0.75 mL HPLC vial (Kimble Glass Inc.), acidified by adding 10 uL of 85.0% H_3PO_4., and then capped. The samples were loaded into the autosampler, which was set to inject a volume of 200 uL.

3.9 Chloroethene Analysis

Chloroethenes were analyzed by a Tekmar LCS 2000™ Purge and Trap controller equipped with an ALS 2016™ autosampler. The purge and trap was programmed to purge the sample with He with a flow rate of 40 mL/min at 20 psi for 12 minutes and then desorb the chloroethenes from the Tenax K adsorbent by heating the adsorbent to 250°C for 6 minutes. With a heated transfer line, the desorbed chloroethenes were transported and injected into the GC.

The GC was a Varian 3400™ equipped with an electrolytic conductivity detector (ELCD, model 4430, OI Corporation). Helium was the carrier and makeup gas set at a flow rate of 20 mL/min. The column flow rate for Helium was set at 10 mL/min with a pressure of 20 psi at 22°C. N-propanol was the solvent used for the ELCD. The reaction chamber was set at 850°C. To separate the chlorinated ethene, a Restek Rtx-624™ capillary column (105m x 0.53 mm ID x 3.0 um df) was used. The GC was programmed to maintain the oven temperature at 35°C for 10 minutes before raising it to a final temperature of 200°C for 1.5 minutes at a rate of 7.0°C/min. The injector temperature was set at 150°C, and the detector was set at 200°C. The duration of the sampling time was 35.07 minutes. The Purge and Trap GC was hooked up to a computer, which used a Hewlett Packard Minichrom Chromatography Data System™ version 1.62 software to process the data.

The samples were vigorously shaken before 1.0 mL of sample was withdrawn from a 38.0 mL serum bottle using a 1-mL Gastight™ Syringe (model 1001 Hamilton Co.) fitted with a 22-gauge needle (8.0 cm). The samples were then dispensed into purge

and trap test tubes containing 4.0 mL of DI water preloaded into the Purge and Trap autosampler.

3.10 Mass Balance Analysis

In order to determine the mass balance of the microcosms, several preliminary calculations had to be performed:

(1) To determine the total number of moles in a 25 uM PCE solution:

Concentration of PCE (mol/L) * volume of liquid (L) = # of moles of PCE

2.5×10^{-5} mol/L * 0.0285 L = 7.125×10^{-7} mol PCE

(2) To determine the number of moles of chlorinated ethene in the liquid phase:

Concentration of chlorinated ethene (moles/L) * volume of liquid (L) = # of moles

1.558×10^{-5} mol/L * 0.0285 L = 4.441×10^{-7} mol PCE

(3) To determine the number of moles of chlorinated ethene in the gas phase:

Concentration of chlorinated ethene (moles/L) * Henry's constant (31) (Table 3.4) = concentration (moles/L) of chlorinated ethene in gas phase

1.558×10^{-5} mol/L * 0.723 = 1.126×10^{-5} mol/L PCE

then, value from above * volume of headspace (L) = # of moles in gas phase

1.126×10^{-5} mol/L * 0.0095 L = 1.070×10^{-7} mol PCE

(4) To determine the total number of moles of chlorinated ethene in the sample:

Moles in liquid phase + moles in gas phase = total # of moles

4.441×10^{-7} mol + 1.070×10^{-7} mol = 5.480×10^{-7} mol PCE

2.5×10^{-5} mol/L * 0.0285 L = 7.125×10^{-7} mol PCE

(5) To determine the number of moles of gas in a 1 mL sample at 1 atm:

PV = nRT

Therefore, n = PV/RT

1 atm * 10^{-3} L / 0.0821 L atm $mol^{-1}K^{-1}$ * 298 K = 4.2 x 10^{-5} mol

(6) To determine the number of moles of gas in a 1000 ppm 1 mL sample:

1000 ppm = 10^{-3} mol / total mol

which can be represented as, n_{gas} = 10^{-3} * total mol in 1 mL

10^{-3} * 4.2 x 10^{-5} mol = 4.2 x10^{-8} mol in a 1000 ppm 1 mL sample

(7) To determine the number of moles of ethene or ethane gas in the headspace:

Moles in 1000 ppm / 1000 arbitrary units = number of moles / arbitrary units

Number of moles in 1 mL = 4.2 x 10^{-11} * arbitrary units

Then, number of moles in 1 mL * volume of headspace (L)

Number of moles in 0.001 L * 0.0105 L = number of moles of gas in headspace

(8) To determine the total number of moles present in the sample:

Total number of moles of chlorinated ethene + total number of moles of gas in the headspace = total number of moles in the sample

(9) To determine the mass balance of a sample:

(Total number of moles in the sample / total number of moles of PCE in a 25 uM solution) * 100 = mass balance

(5.463 x 10^{-7} mol / 7.125 x 10^{-7} mol) * 100 = 76.7 %

Theoretically, a 25 uM PCE solution should yield a total of 7.125 x 10^{-7} moles of PCE

per bottle. However, experimentally when analyzed with a Purge and Trap, the 25 uM PCE standard solution prepared with a plastic pipette yielded only a total of 3.380×10^{-7} moles of PCE per bottle. Therefore, under these circumstances, the mass balance was calculated based on the initial (t=0) calculated number of moles of PCE rather than the theoretical value of 7.125×10^{-7} moles of PCE.

Table 3. 4 Values of Henry's Constant vs. Temperature (31)

compound	temp. (°C)	Hc
tetrachloroethene	9.6	0.294
	17.5	0.492
	24.8	0.723
	34.6	1.116
trichloroethene	9.6	0.163
	17.5	0.265
	24.8	0.392
	34.6	0.591
cis-1,2 dichloroethene	10.3	0.0741
	17.5	0.111
	24.8	0.167
	34.6	0.216
trans-1,2 dichloroethene	10.0	0.181
	17.5	0.277
	24.8	0.384
	34 6	0.545
vinyl chloride	10.3	0.631
	17.5	0.811
	24.8	1.137
	34.6	1.420

CHAPTER 4

RESULTS AND DISCUSSION

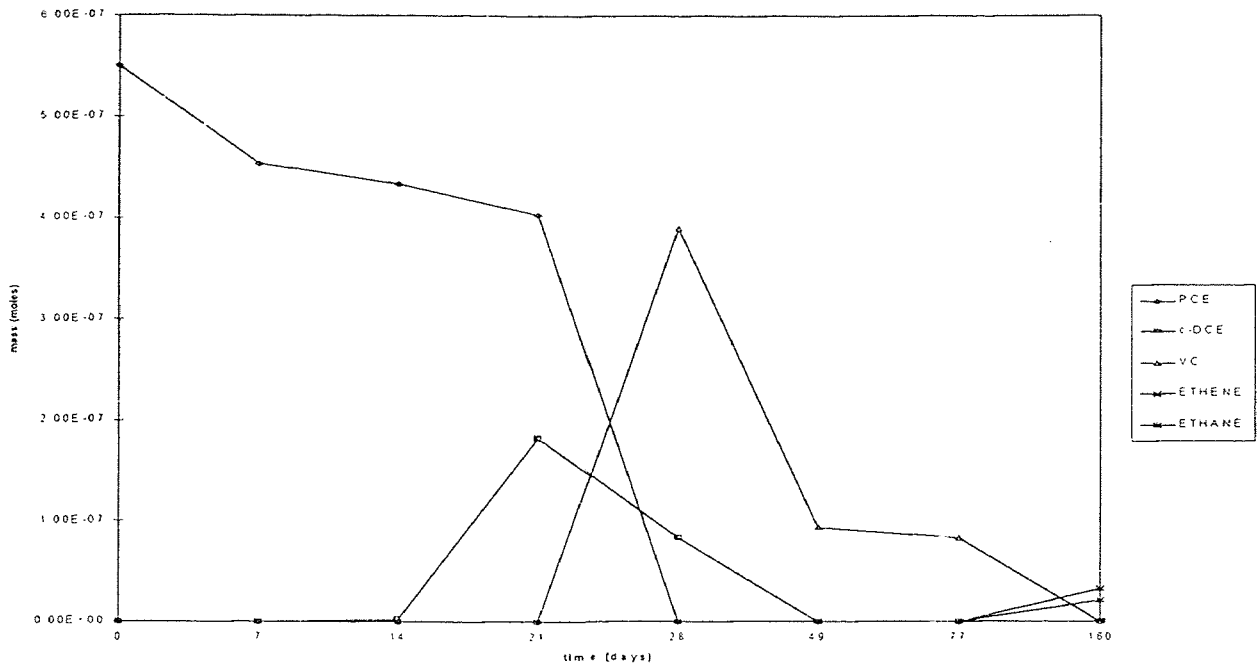
4.1 Effects of Volatile Fatty Acids

All of the microcosms exhibited a complete reductive dechlorination of PCE to ethene; however, the rate at which dehalogenation occurred varied significantly among the different microcosms (Figures 4.1, 4.2, A-1 – A-4 and Tables B-1 – B-9). Each point in these figures and tables is the average of three measurements taken from the three bottles sacrificed at each sampling point.

The average starting PCE level for these microcosms was 0.502 $\pm$ 0.54 umol/bottle, a value equivalent to 70% of the theoretical PCE level of 0.7125 umol/bottle. Due to the inability to quantitatively measure the gases, ethene and ethane, the mass balance reported for the chlorinated ethenes are only partially accurate. For the initial sampling period, the calculated mass balance for the chlorinated ethenes fell between 63 and 82% when compared against the theoretical PCE level of 0.7125 mol/bottle. Two probable conclusions could be drawn from these values: (1) PCE immediately binds to the sediment in the microcosms (2 hours passed before the microcosms were ananlyzed), or (2) the starting PCE levels are those measured at t = 0. The latter seems more reasonable since a PCE standard solution prepared with plastic pipette yielded a total of 0.338 mol/bottle rather than the expected 0.7125 mol/bottle, which would explain the low initial PCE level for the microcosms.

The average starting PCE level for the negative controls was 0.460 $\pm$ 0.17

(a)



(b)

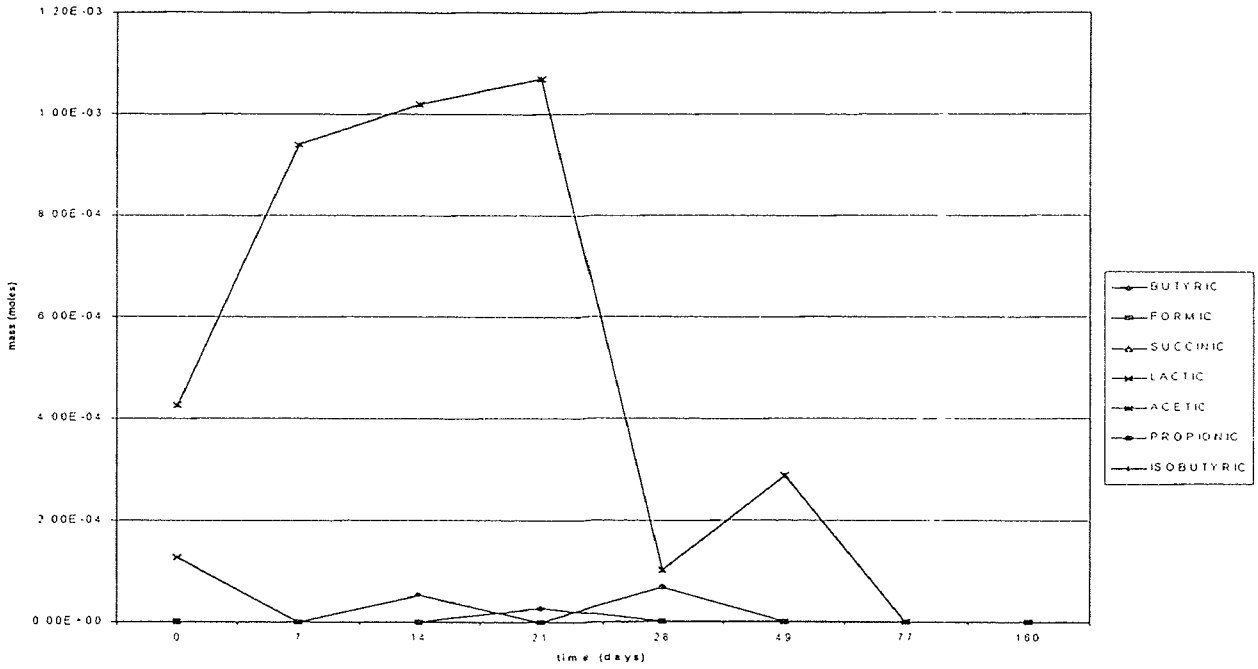


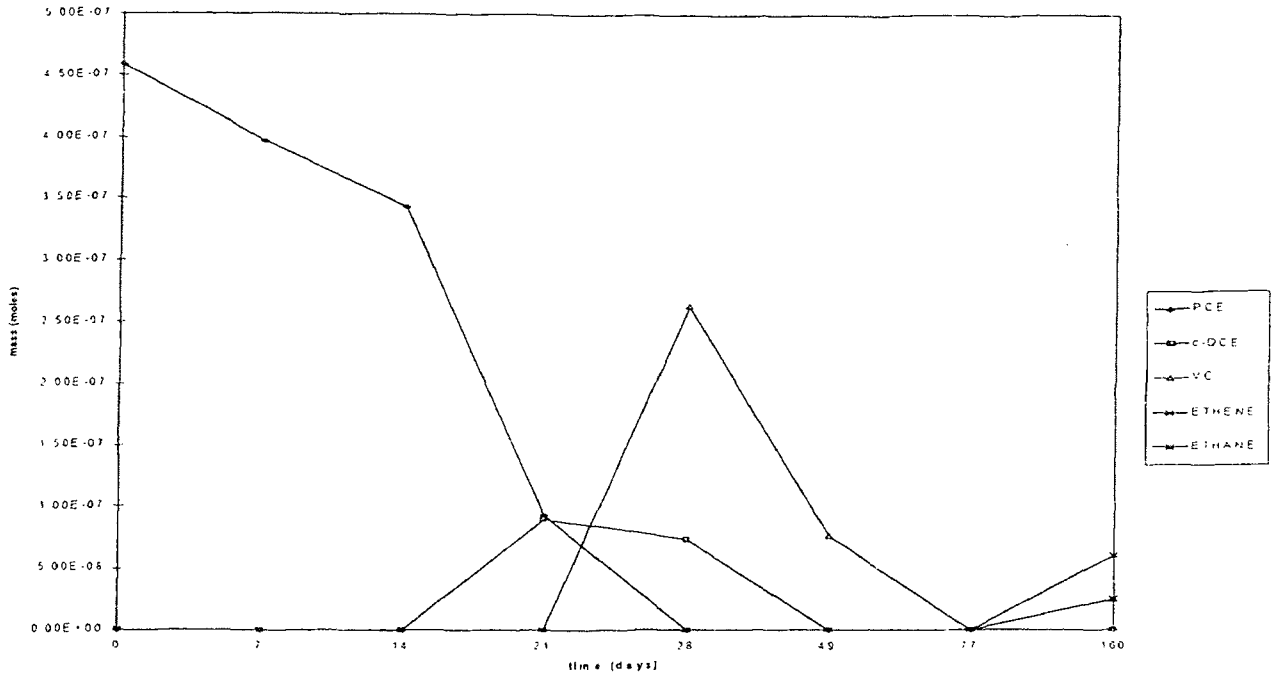
Figure 4.1 Mass in moles of (a) chlorinated ethene and (b) acid byproducts as a function of time for microcosms amended with lactic acid.

umol/bottle (Figures C-1 –C-3). For the final sampling period, the calculated mass balance for the chlorinated ethenes fell between 33.7 and 63.6 % when compared against the initial calculated value of PCE. A loss of almost 50% of the initial PCE level and the absence of dechlorination products may be due to any of the three possible explanations: (1) PCE was absorbed by the sediment in the microcosms over the course of the experiment; (2) PCE escaped from underneath the teflon stopper; or (3) a systematic sampling error occurred throughout the course of the experiment. The second explanation is not likely the cause of the loss of PCE over time since those bottles which showed activity produced and exerted greater pressure than those that showed less activity. The final explanation is also not likely since prior applications of the method have demonstrated consistent mass balance calculations.

All of the microcosms except for those amended with butyric acid, demonstrated dehalogenation activity within 14 days. Butyric acid, which is more difficult to degrade under anaerobic conditions (28), had a longer lag period (21 days). All cultures exhibited further dehalogenation activity. Accumulation of c-DCE up until 28 days was consistent throughout all six conditions. Then, VC became the predominant species.

Of all the conditions, those amended with the butyric acid/succinic acid mixture supported the fastest reductive dechlorination of PCE to ethene. No traces of any kind of chlorinated ethene were detected at 49 days. Those amended with succinic acid, butyric acid, formic acid and the butyric acid/formic acid mixture took 77 days to exhibit complete reductive dechlorination, while those amended with lactic acid took 160 days. Only ethene and ethane were detected at 160 days.

(a)



(b)

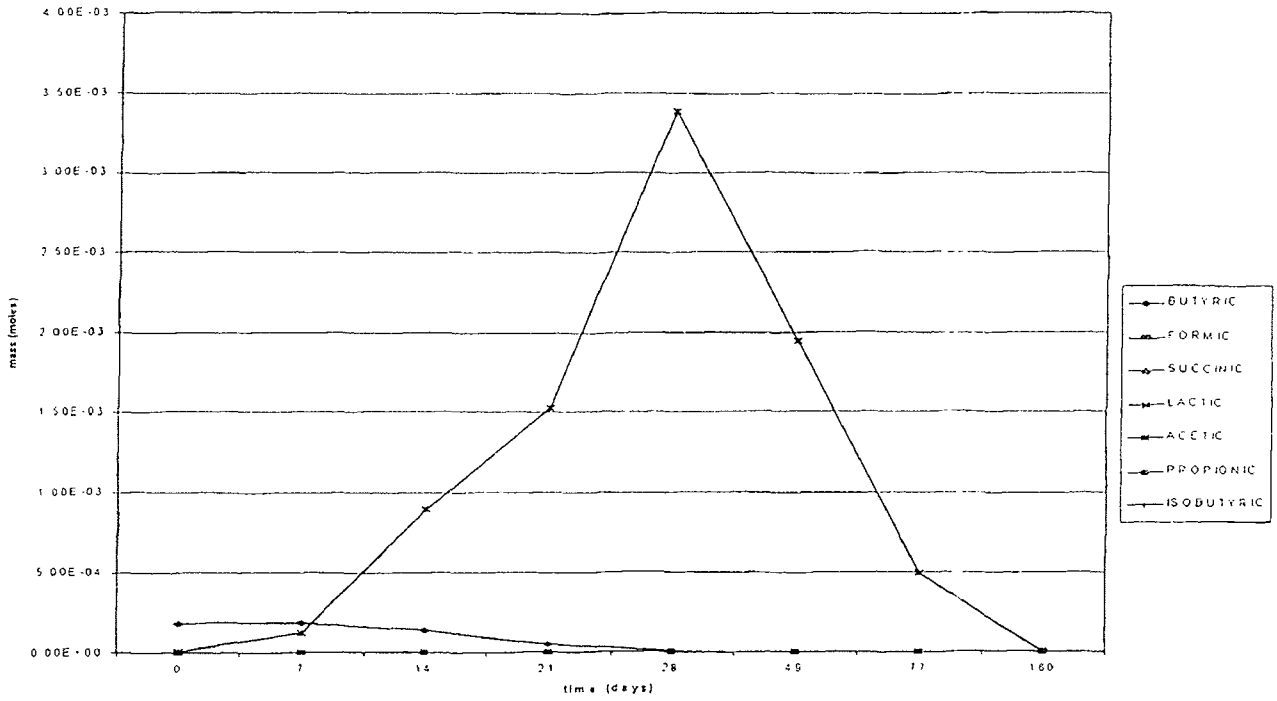


Figure 4.2 Mass in moles of (a) chlorinated ethene and (b) acid byproducts as a function of time for microcosms amended with butyric acid.

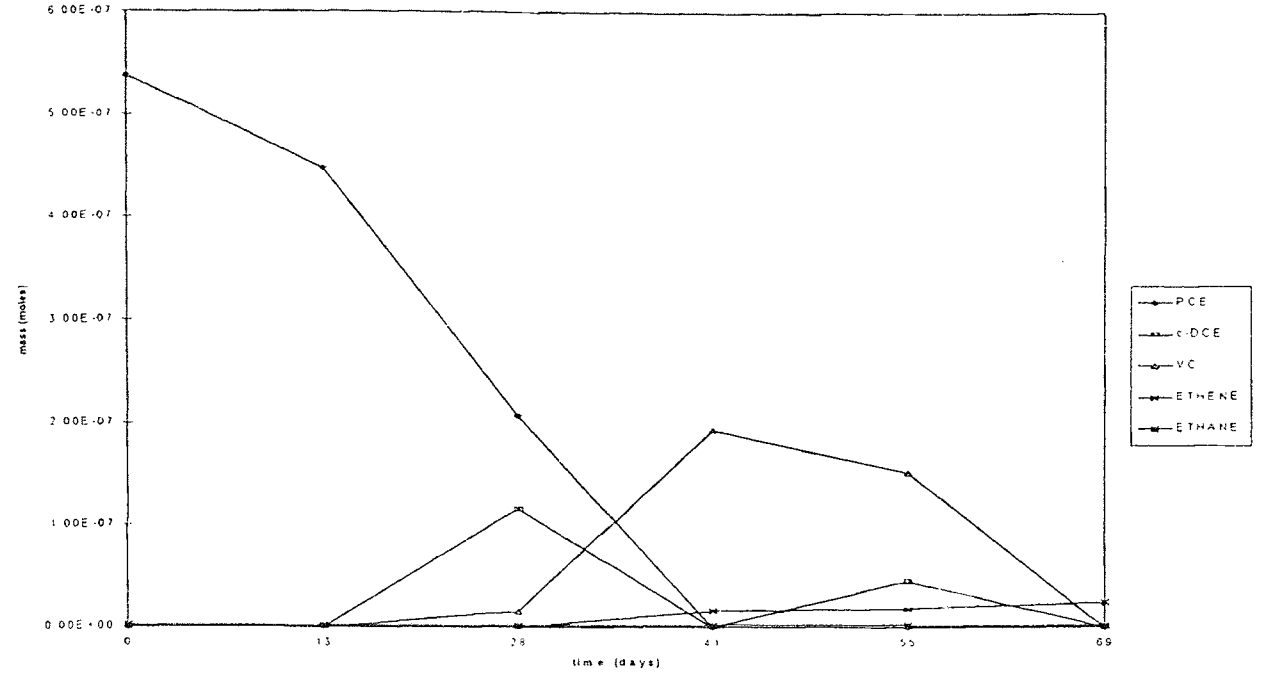
4.2 Effects of Alcohols

Five out of six alcohols used as electron donors resulted in dehalogenation activity. However, the rate at which dechlorination occurred varied significantly among the different microcosms (Figures 4.3, 4.4, A-5 – A-8 and Tables B-10 –B-18). Each point in these figures and tables is the average of three measurements taken from the three bottles sacrificed at each sampling point.

The average starting PCE level for these microcosms was 0.422 $\pm$ 1.41 mol/bottle, a value equivalent to 59% of the theoretical PCE level of 0.7125 mol/bottle. For the final sampling period, the calculated mass balance fell between 3.7 and 78% when compared against the theoretical PCE level of 0.7125 mol/bottle. Two probable conclusions could be drawn from these values: (1) PCE immediately binds to the sediment in the microcosms (2 hours passed before the microcosms were ananlyzed), or (2) the starting PCE levels are those measured at t = 0. The latter seems more reasonable since a PCE standard solution prepared with plastic pipette yielded a total of 0.338 mol/bottle rather than the expected 0.7125 mol/bottle, which would explain the low initial PCE level for the microcosms, but not the mass balance for the chlorinated ethene which fell between 4.83 and 62.0%

The average starting PCE level for the negative controls was 0.261 $\pm$ 0.07 umol/bottle (Figures C-4 –C-6). For the final sampling period, the calculated mass balance for the chlorinated ethenes fell between 36.0 and 67.9 % when compared against the initial calculated value of PCE. A loss of almost 50% of the initial PCE level and the absence of dechlorination products may be due to any of the three possible explanations: (1) PCE was absorbed by the sediment in the microcosms over the course of the

(a)



(b)

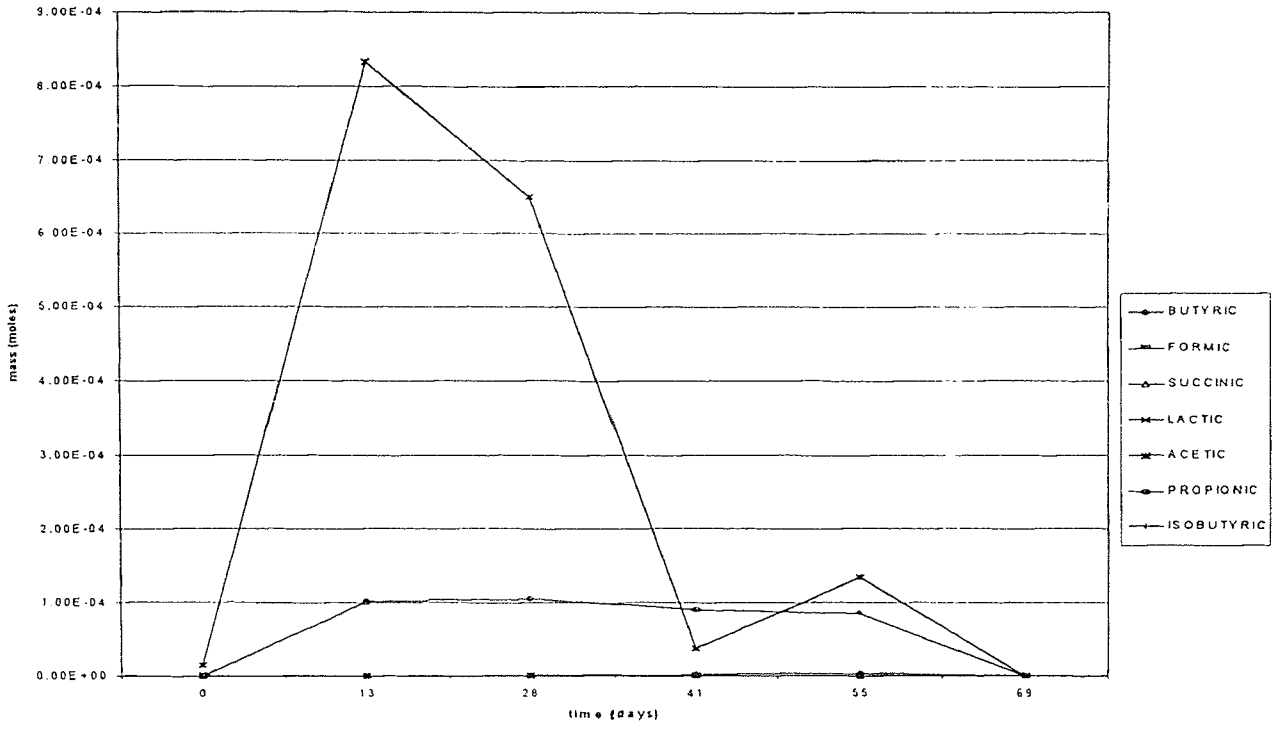


Figure 4.3 Mass in moles of (a) chlorinated ethene and (b) acid byproducts as a function of time for microcosms amended with propanol.

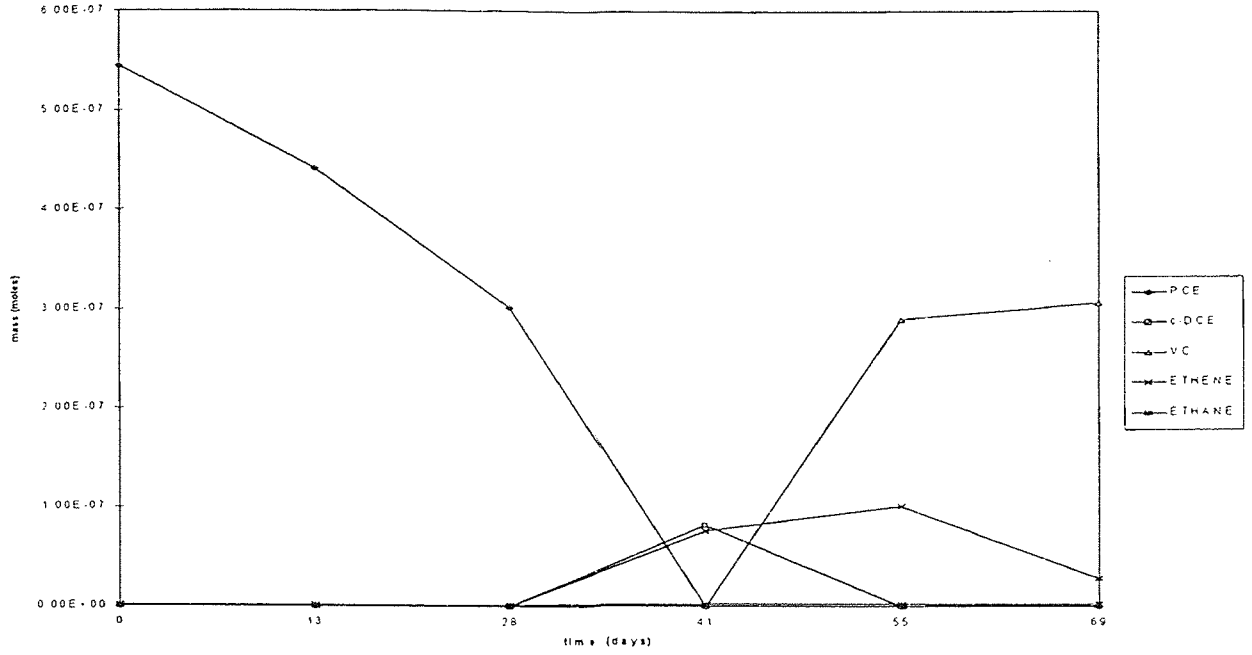
experiment; (2) PCE escaped from underneath the teflon stopper; or (3) a systematic sampling error occurred throughout the course of the experiment. The second explanation is not likely the cause of the loss of PCE over time since those bottles which showed activity produced and exerted greater pressure than those that showed less activity. The final explanation is also not likely since prior applications of the method have demonstrated consistent mass balance calculations.

Of the six conditions studied, only the microcosms amended with the ethylene glycol/butanol mixture demonstrated no dehalogenation activity. PCE was consistently detected throughout all six sampling times. Those amended with the butanol/propanol mixture showed dehalogenation activity at 13 days. Those amended with propanol, ethylene glycol and the propanol/ethanol mixture showed signs of dehalogenation activity at 27 days, while those amended with ethanol took 41 days to show any sign of activity.

The late onset of dehalogenation activity in the microcosms amended with ethanol may be due to its ability to be rapidly degraded under anaerobic conditions, and therefore, result in the production of higher levels of H_2. Because excess H_2 are made easily available to both methanogens and dechlorinators, competition between the two may have persisted until lower levels of H_2 were left over. Low levels of H_2 are less available to methanogens, and consequently, would result in the predominance of dechlorinators (17).

Despite the longer lag period of ethanol, the microcosms amended with ethanol demonstrated greater dehalogenation activity than every other microcosms amended with alcohol except for those amended with propanol. Other than those amended with propanol, none exhibited a complete reductive dechlorination of PCE to ethene. No traces

(a)



(b)

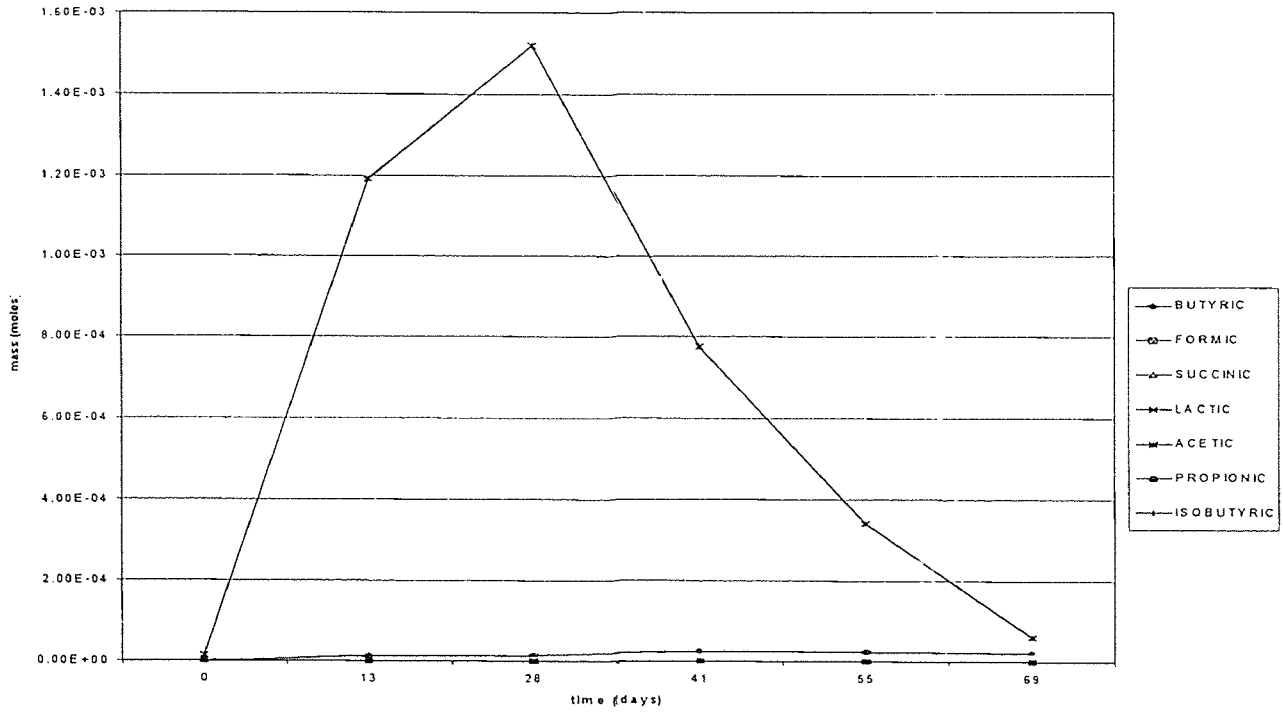


Figure 4.4 Mass in moles of (a) chlorinated ethene and (b) acid byproducts as a function of time for microcosms amended with ethanol.

of any kind of chlorinated ethenes were detected at 69 days. Only ethene and some traces of ethane were detected. The rest continually exhibited significant quantities of PCE, c-DCE and VC throughout the entire course of the experiment.

CHAPTER 5

CONCLUSION

The experiments carried out in this work demonstrated that a complete biotransformation of PCE to ethene can be observed when the cultures are supplemented with one of the following electron donors: succinic acid, lactic acid, butyric acid, formic acid, propanol, butyric acid/succinic acid mixture and butyric acid/formic acid mixture. Although the microcosms amended with butyric acid and succinic acid by themselves were able to reductively dechlorinate PCE to ethene, the combination of the two seems to have a positive effect on the rate of dechlorination. However, only 32% of the initial PCE level could be accounted for as ethene or ethane. The remainder was either lost or absorbed by the sediment in the microcosm.

On the contrary, the microcosms amended with the butyric acid/formic acid and propanol/ethanol mixtures seem to demonstrate slower dehalogenation activity than their individually amended counterparts. Microcosms amended with butyric acid and formic acid by themselves demonstrated complete reductive dehalogenation at 77 days. Those amended with the butyric acid/formic acid mixture still showed levels of VC at 77 days. Similarly, those amended with propanol completely biotransformed PCE to ethene. Those amended with ethanol stopped dehalogenation activity at VC at 69 days. While those microcosms amended with the propanol/ethanol mixture continually demonstrated levels of PCE, c-DCE and VC for the most part of the experiment.

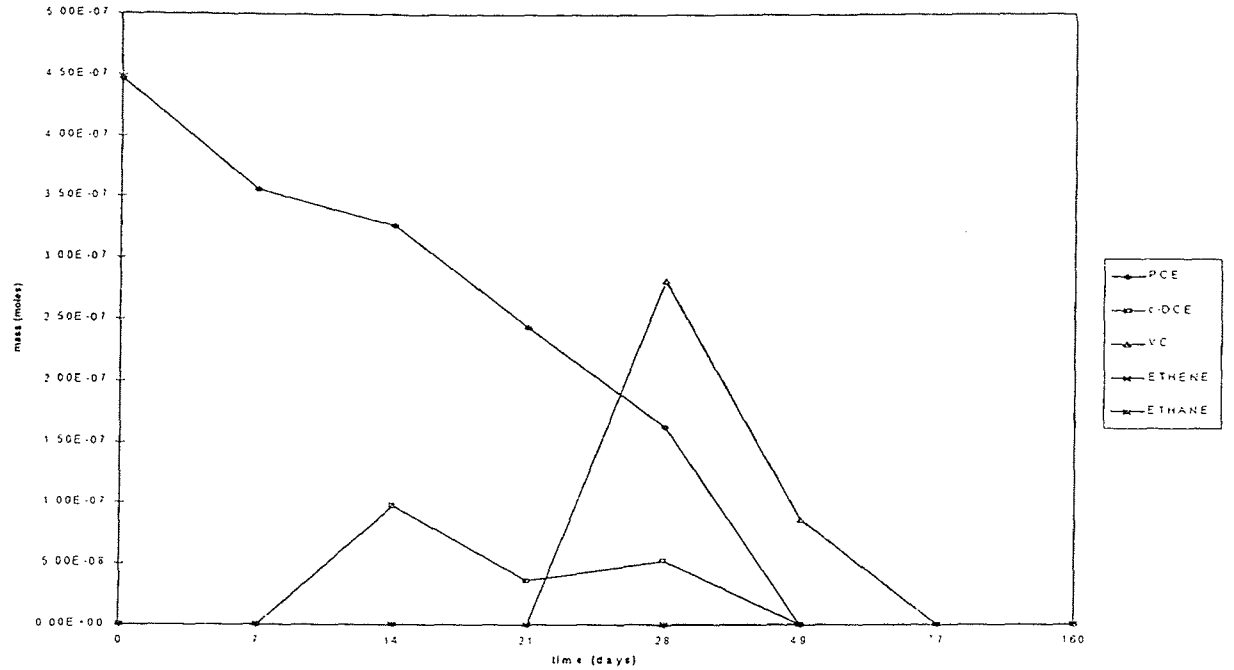
Although no evident explanation can be given as to which electron donor works

the best, it appears that the volatile fatty acids, specifically the butyric acid/succinic acid mixture, work better than the alcohols chosen in this experiment. More research needs to be done on various electron donors as well as their optimal concentrations necessary to provide the maximum results in the initiation and enhancement of reductive dechlorination.

APPENDIX A

ACID AND ALCOHOL GRAPHS

(a)



(b)

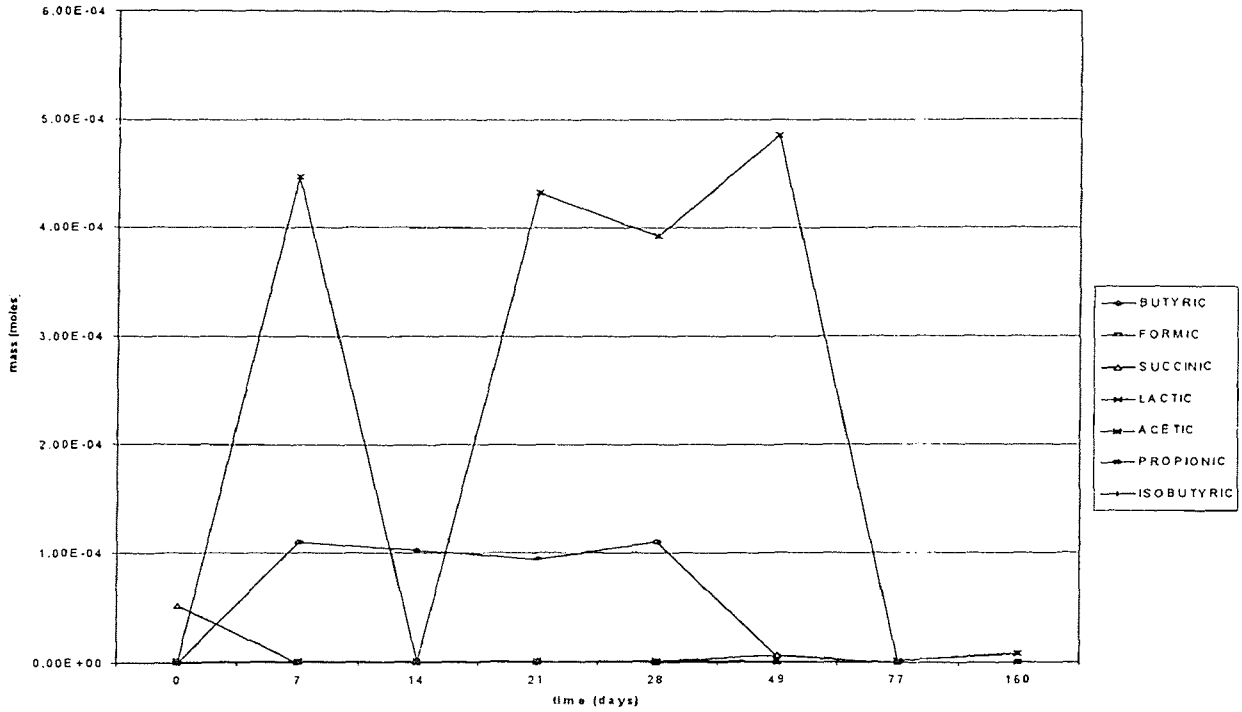


Figure A-1 Mass in moles of (a) chlorinated ethene and (b) acid byproducts as a function of time for microcosms amended with succinic acid.

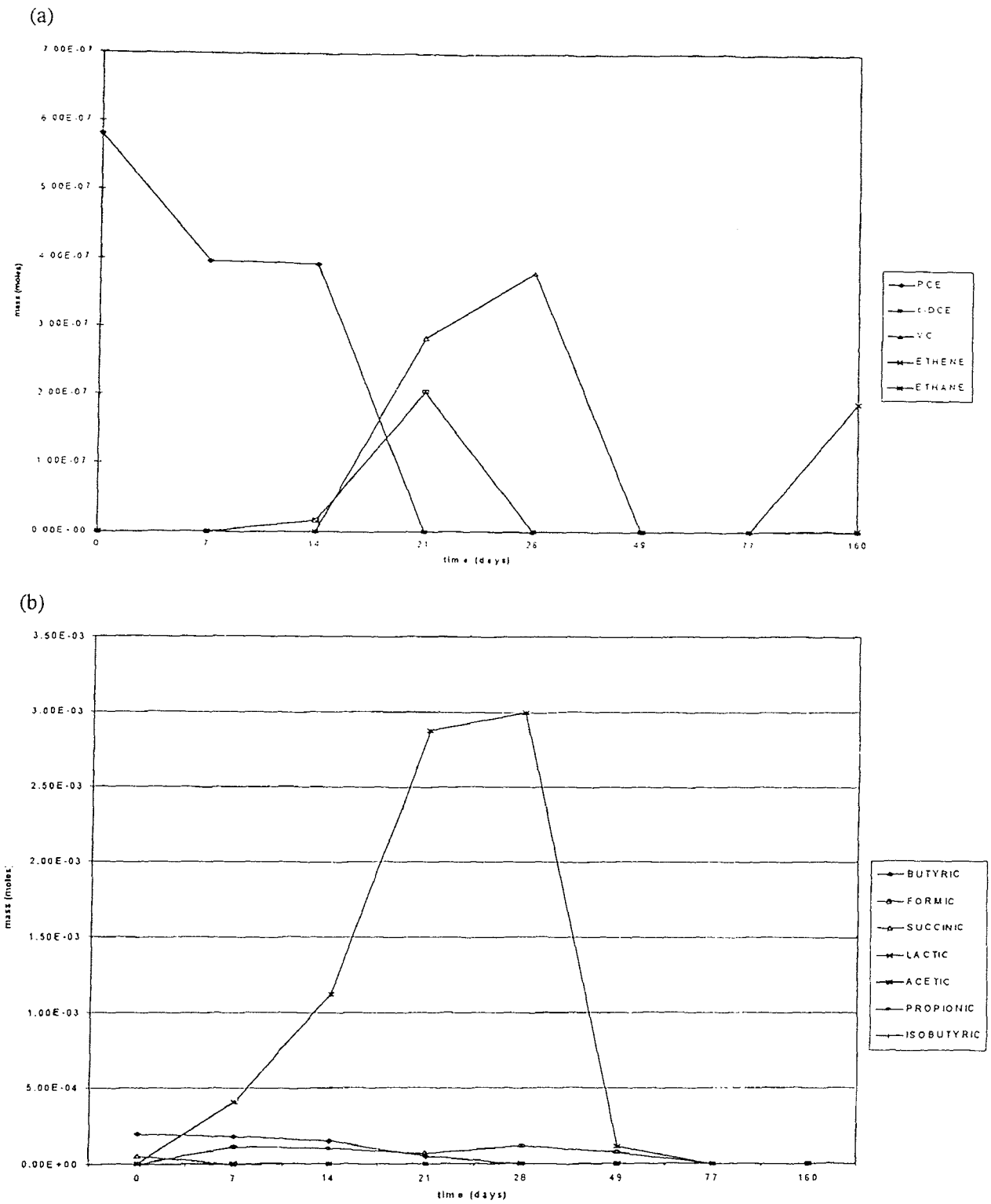
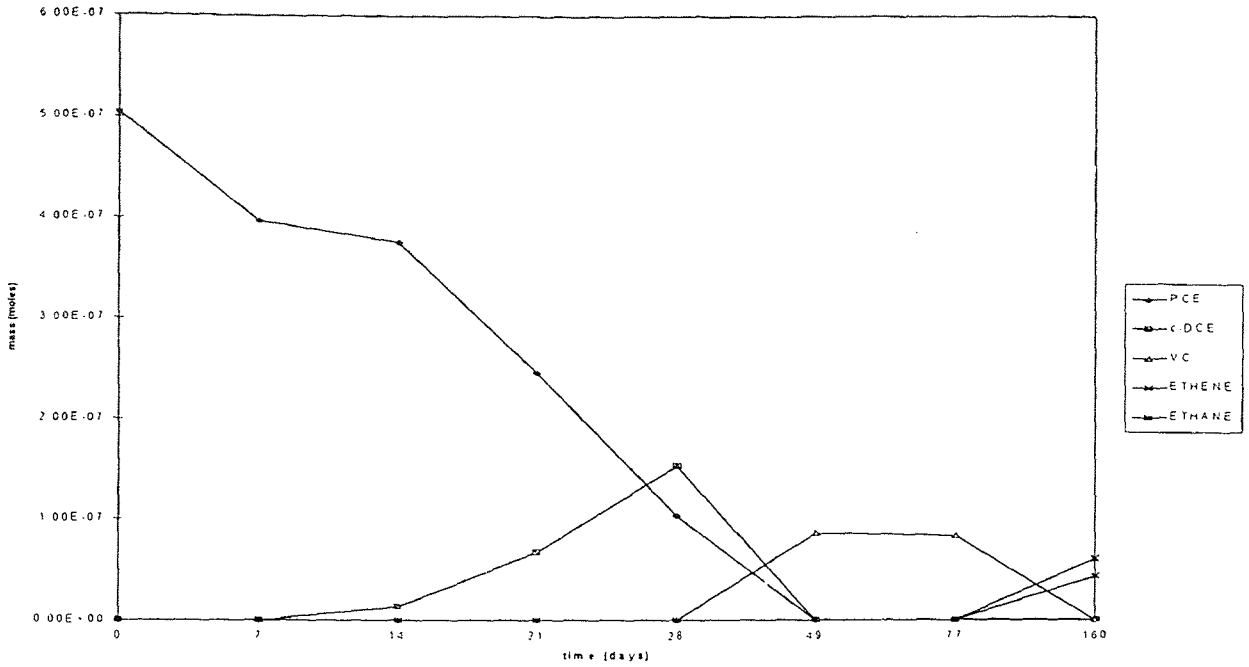


Figure A-2 Mass in moles of (a) chlorinated ethene and (b) acid byproducts as a function of time for microcosms amended with butyric acid/succinic acid mixture.

(a)



(b)

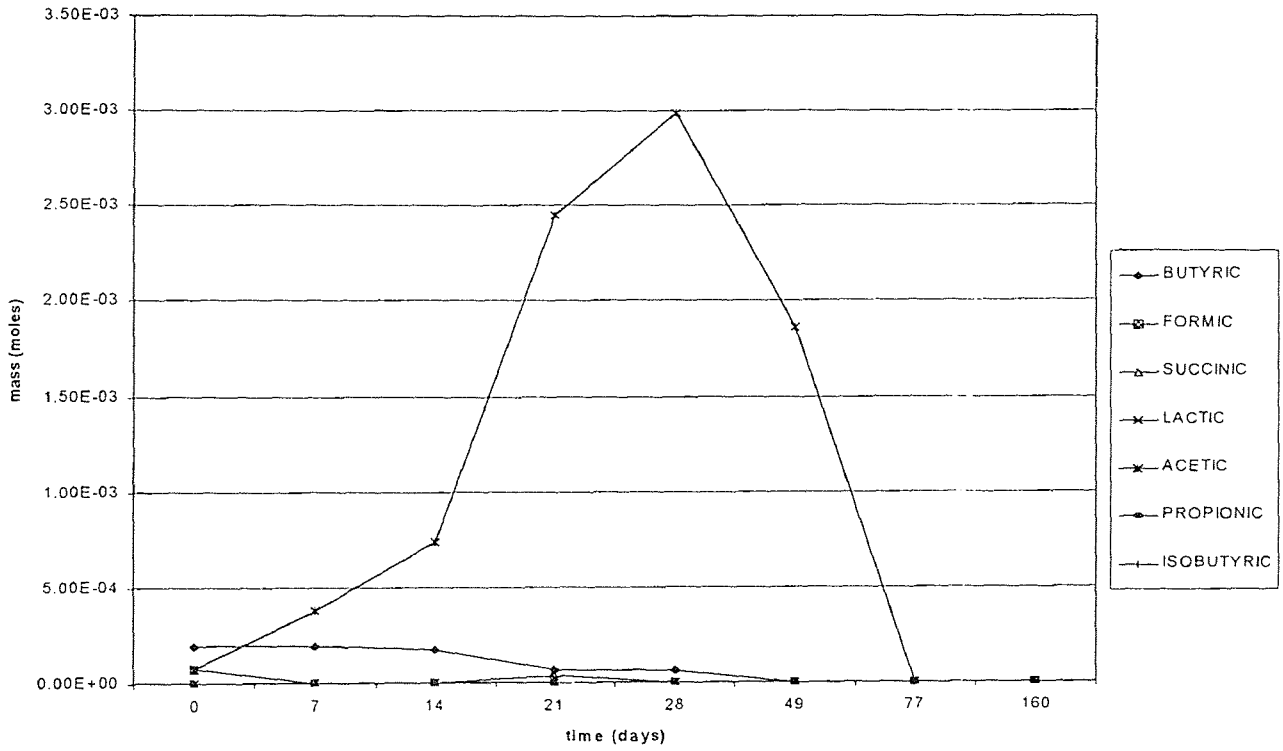
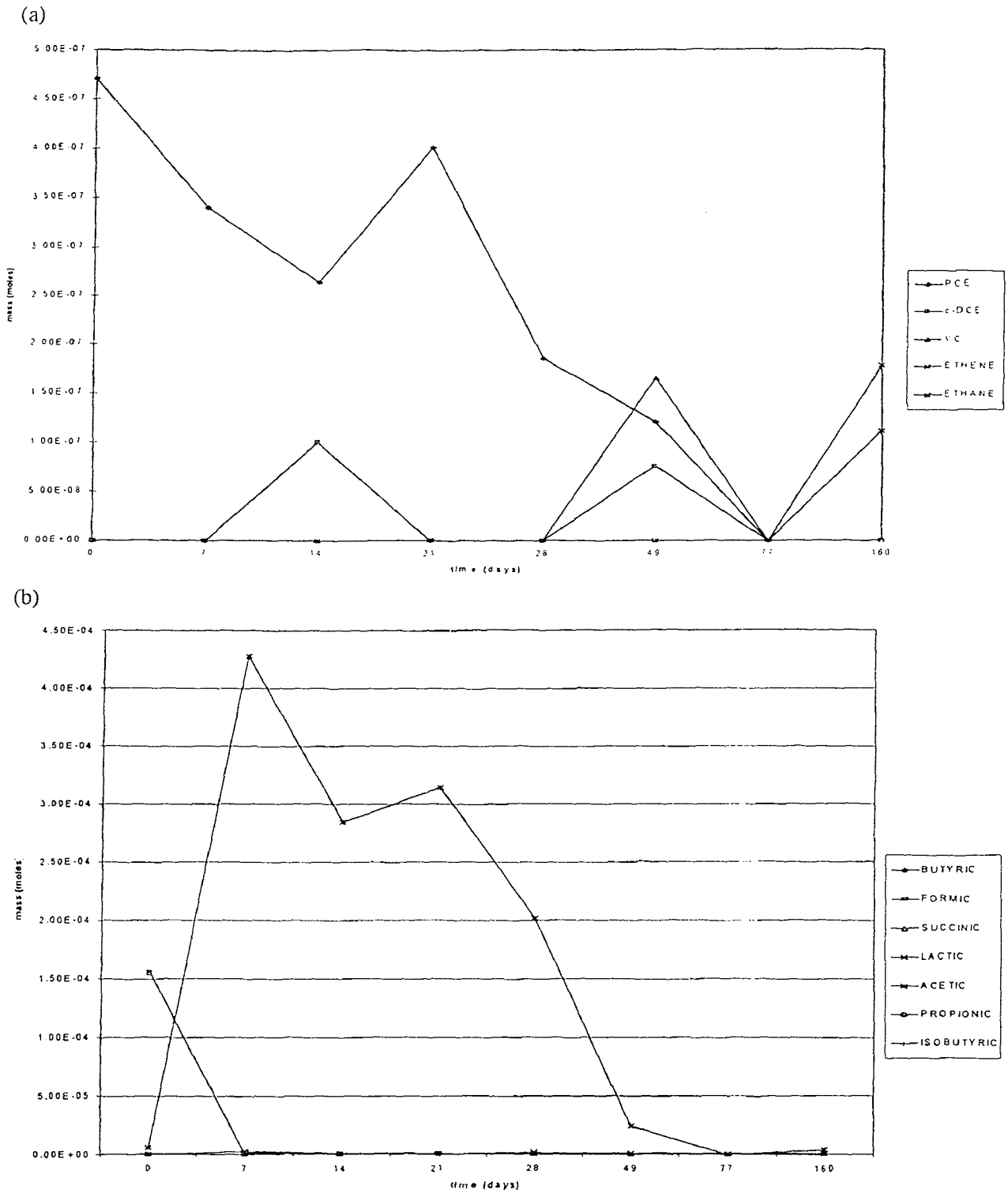
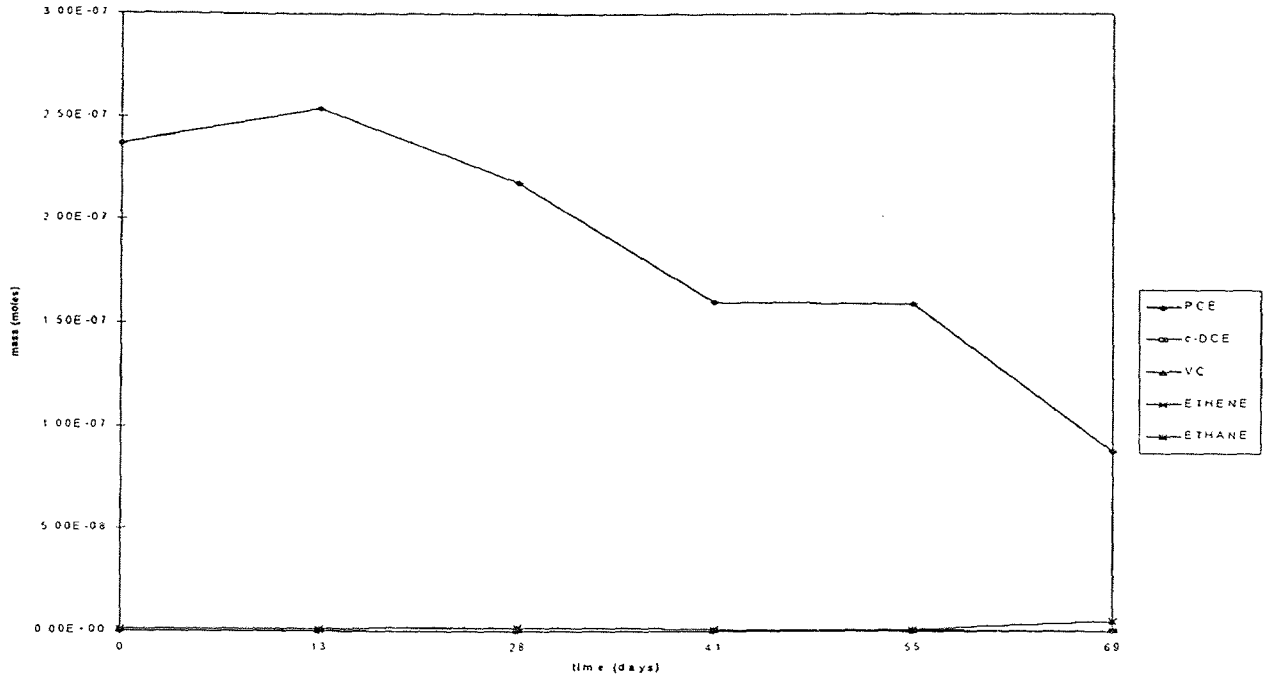


Figure A-3 Mass in moles of (a) chlorinated ethene and (b) acid byproducts as a function of time for microcosms amended with butyric acid/formic acid mixture.



FigureA-4 Mass in moles of (a) chlorinated ethene and (b) acid byproducts as a function of time for microcosms amended with formic acid.

(a)



(b)

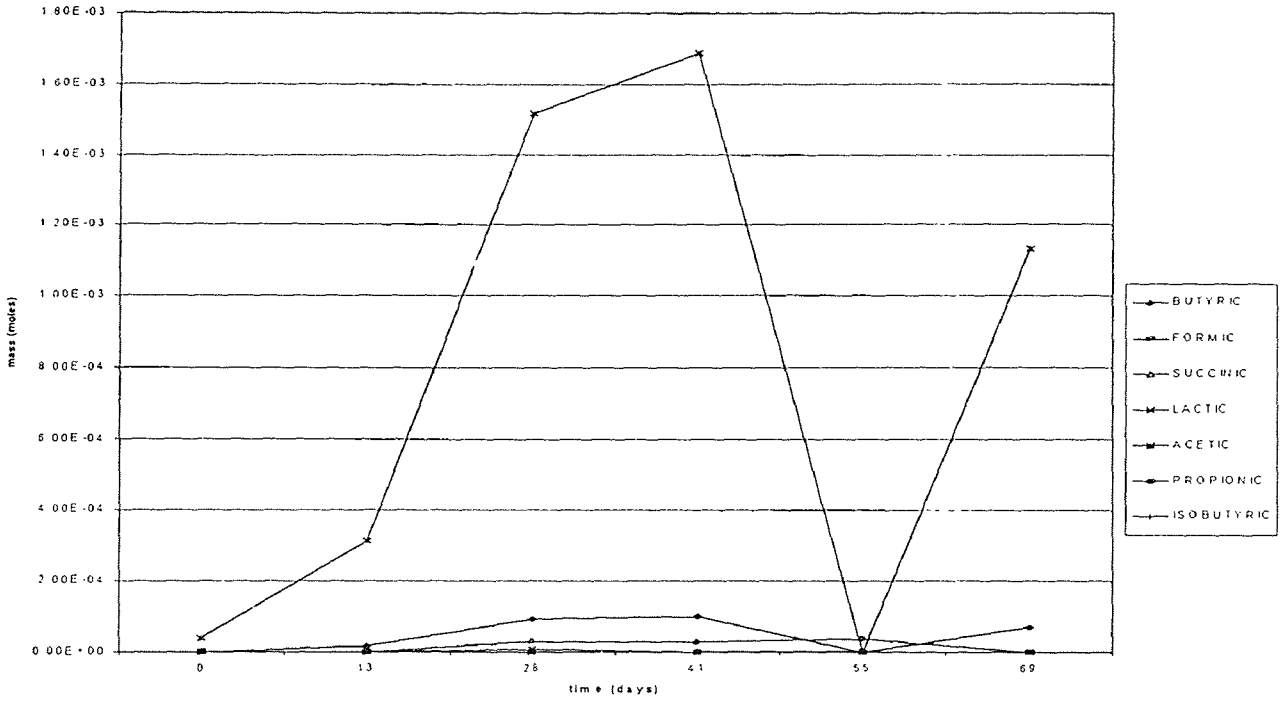


Figure A-5 Mass in moles of (a) chlorinated ethene and (b) acid byproducts as a function of time for microcosms amended with ethylene glycol/butanol mixture.

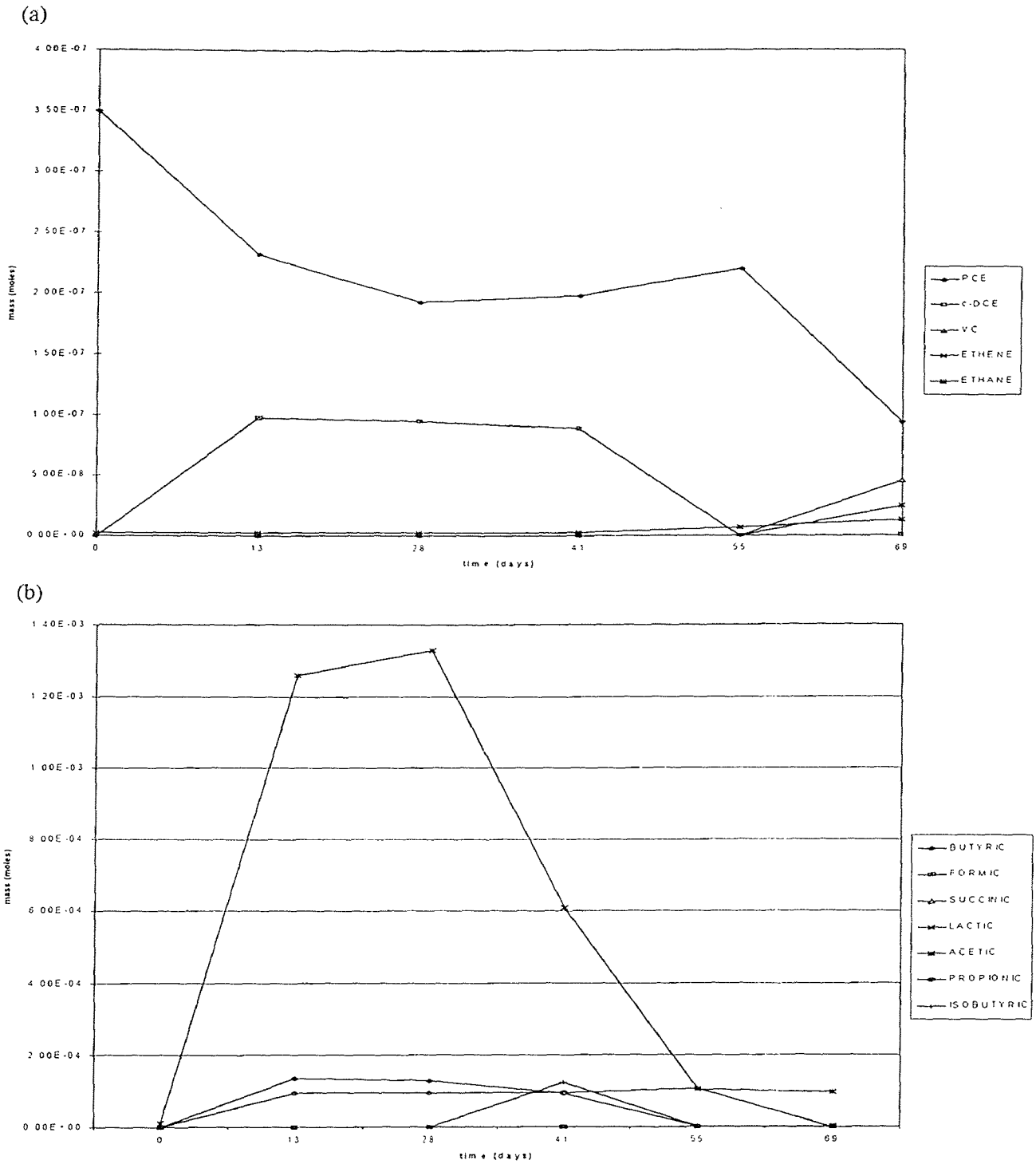
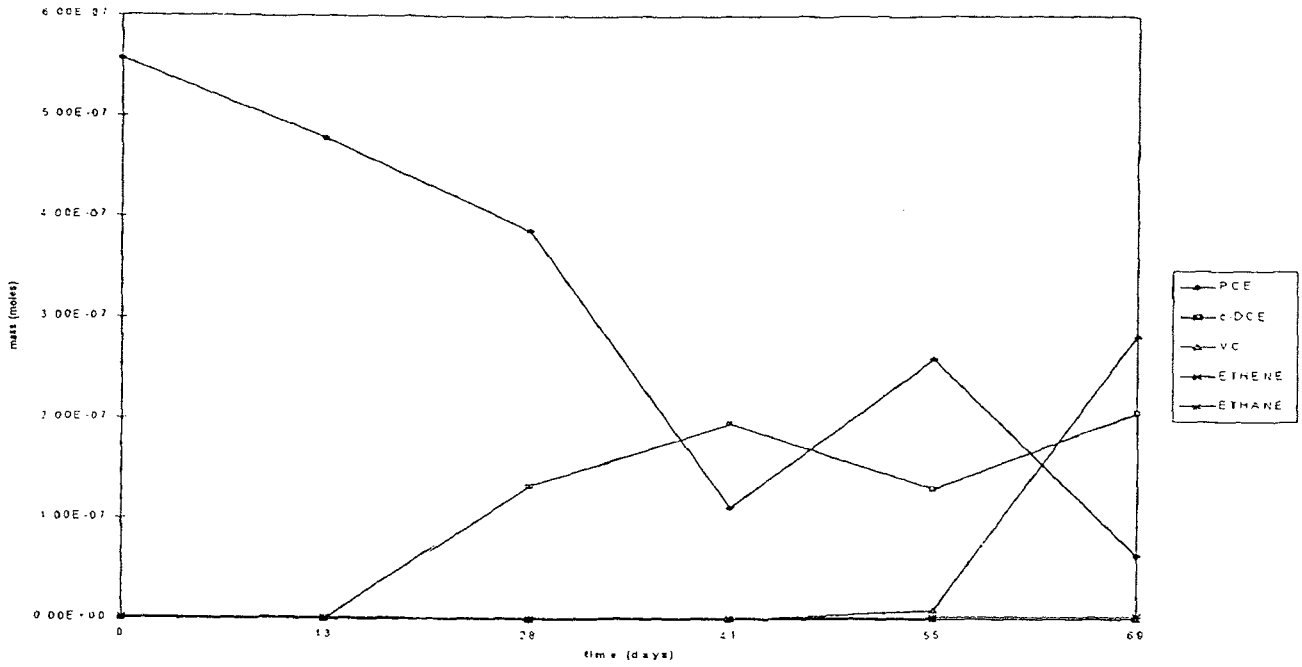


Figure A-6 Mass in moles of (a) chlorinated ethene and (b) acid byproducts as a function of time for microcosms amended with butanol/proopanol mixture.

(a)



(b)

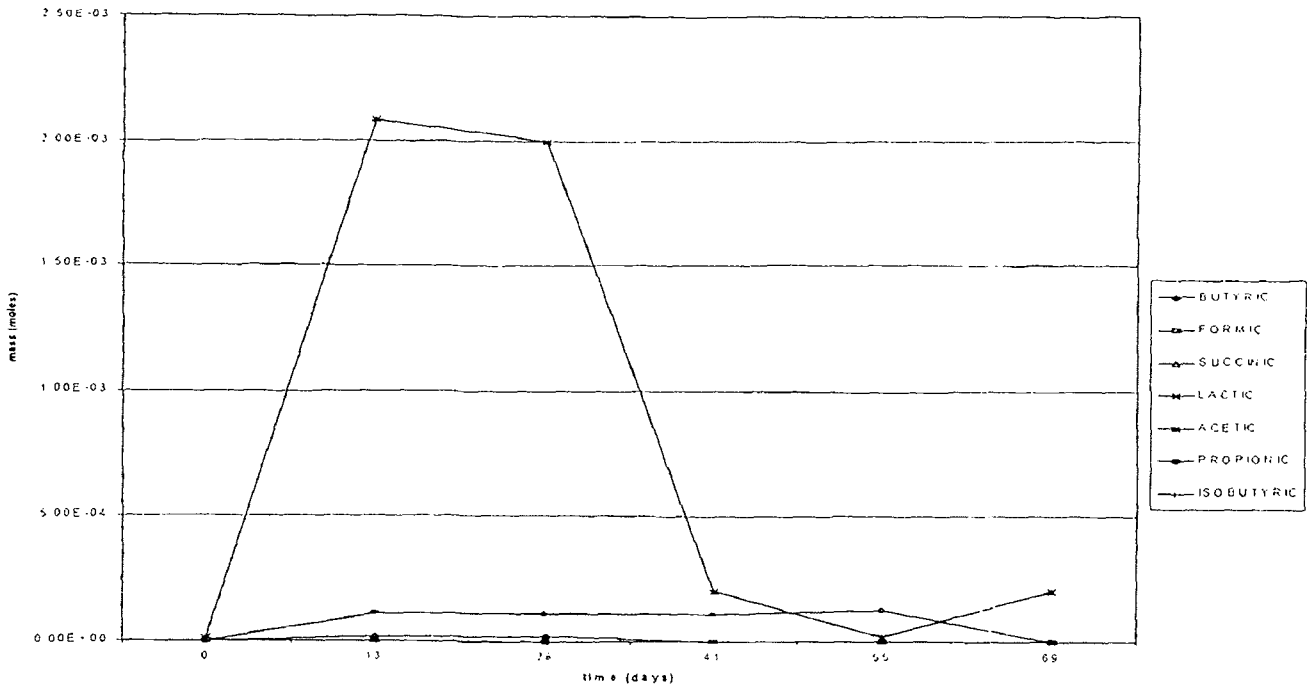
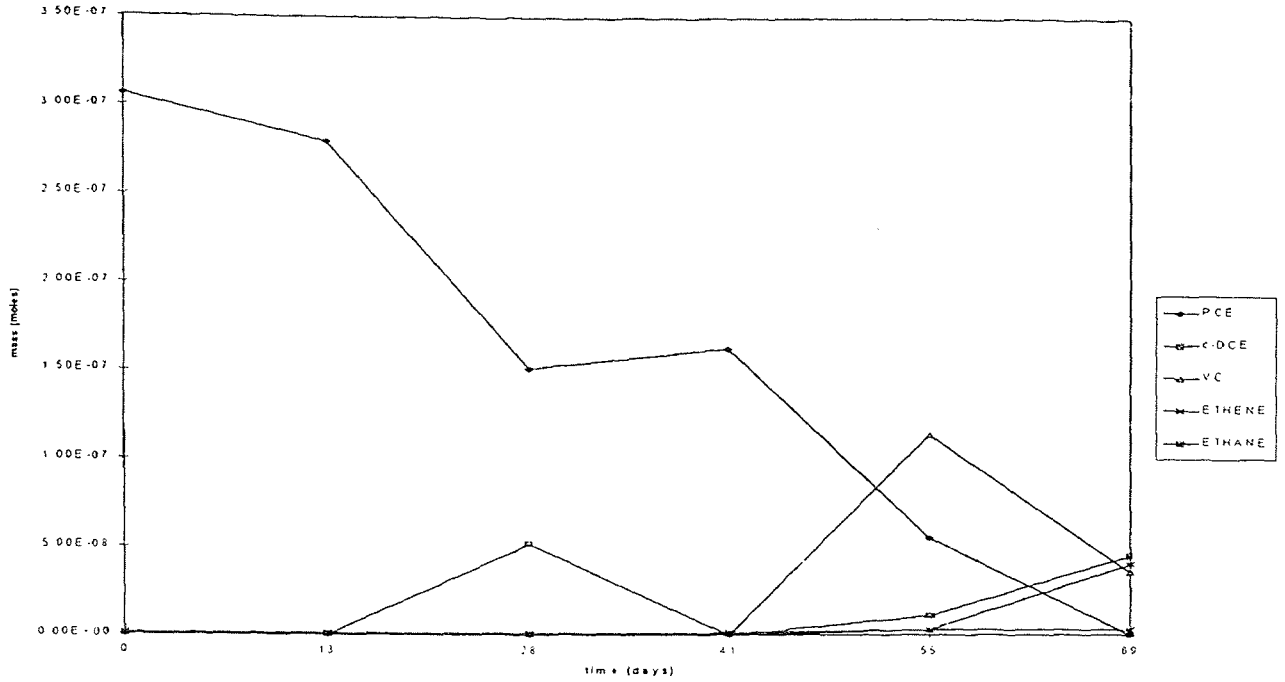


Figure A-7 Mass in moles of (a) chlorinated ethene and (b) acid byproducts as a function of time for microcosms amended with propanol/ethanol mixture.

(a)



(b)

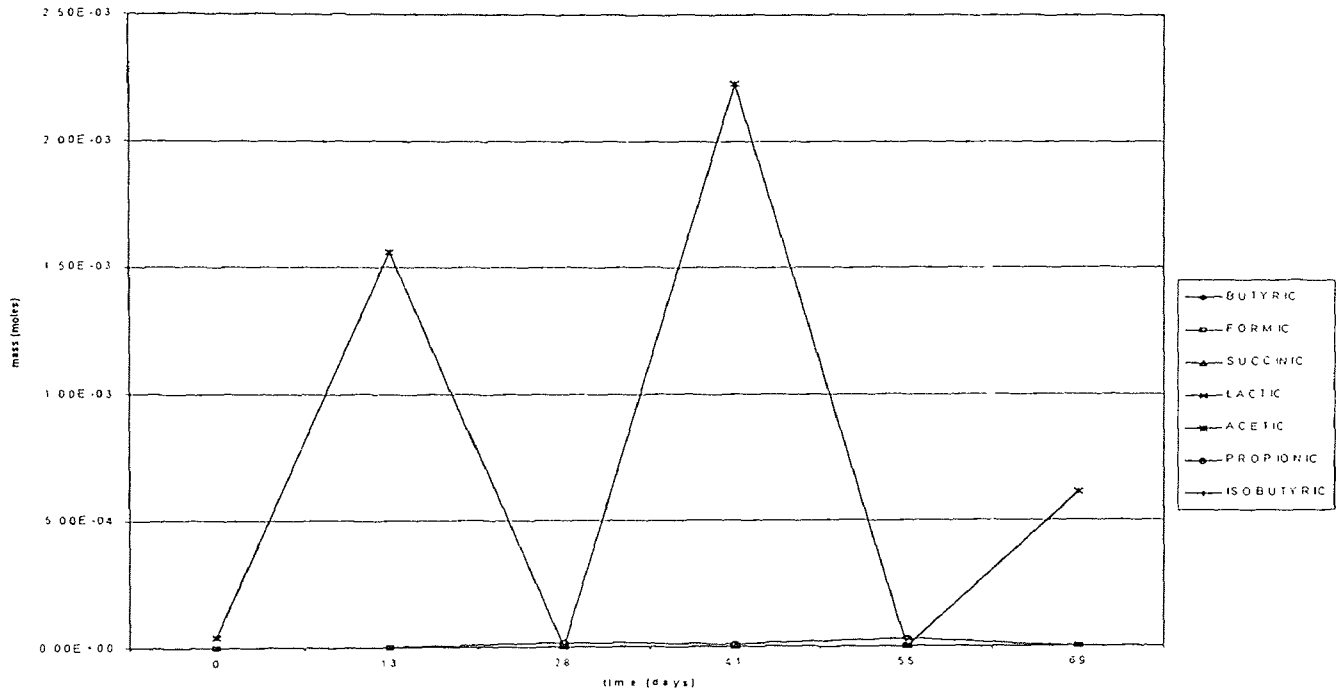


Figure A-8 Mass in moles of (a) chlorinated ethene and (b) acid byproducts as a function of time for microcosms amended with ethylene glycol.

APPENDIX B

TABLES FOR MICROCOSMS AND NEGATIVE CONTROLS

Table B-1 Microcosms amended with Lactic aci

DAY	PCE	TCE	c-DCE	t-DCE	VC	ETHENE	ETHANE	MOLES	MB	BUTYRIC	FORMIC	SUCCINIC	LACTIC	ACETIC	PROPIONIC	ISOBUTYRI
0	5.51E-07	ND	ND	ND	ND	ND	ND	5.51E-07	100	ND	1.43E-06	ND	1.27E-04	4.25E-04	ND	ND
7	4.53E-07	ND	ND	ND	ND	ND	ND	4.53E-07	82.2	ND	5.04E-07	ND	5.51E-07	9.40E-04	4.77E-05	ND
14	4.33E-07	ND	2.89E-09	ND	ND	ND	ND	4.36E-07	79.1	1.25E-06	ND	ND	4.85E-07	1.02E-03	5.34E-05	ND
21	4.03E-07	ND	1.83E-07	ND	ND	ND	ND	5.86E-07	106	2.62E-05	ND	ND	ND	1.07E-03	ND	ND
28	ND	ND	8.34E-08	ND	3.90E-07	ND	ND	4.73E-07	85.9	1.06E-06	5.05E-07	ND	2.08E-06	1.02E-04	6.81E-05	ND
49	ND	ND	ND	ND	9.35E-08	ND	ND	9.35E-08	17.0	1.64E-06	4.76E-07	ND	1.60E-06	2.89E-04	1.78E-06	ND
77	ND	ND	ND	ND	8.22E-08	ND	ND	8.22E-08	14.9	ND	ND	ND	1.18E-06	ND	ND	ND
160	ND	ND	ND	ND	0	2.11E-08	3.25E-08	5.36E-08	9.7	ND	ND	ND	ND	ND	ND	ND

Table B-2 Negative controls amended with Lactic acid

DAY	PCE	TCE	c-DCE	t-DCE	VC	ETHENE	ETHANE	MOLES	MB	BUTYRIC	FORMIC	SUCCINIC	LACTIC	ACETIC	PROPIONIC	ISOBUTYRI
0	4.28E-07	ND	ND	ND	ND	ND	1.64E-09	4.30E-07	99.9	ND	1.37E-06	ND	1.67E-04	1.65E-05	ND	ND
13	3.68E-07	ND	ND	ND	ND	ND	1.58E-09	3.70E-07	85.9	ND	1.45E-06	ND	1.66E-04	1.12E-05	ND	ND
28	3.01E-07	ND	ND	ND	ND	ND	1.59E-09	3.03E-07	70.4	ND	1.45E-06	ND	1.66E-04	2.82E-03	ND	ND
41	2.86E-07	ND	ND	ND	ND	ND	ND	2.86E-07	66.5	ND	ND	ND	1.68E-04	ND	ND	ND
55	1.59E-07	ND	ND	ND	ND	ND	1.49E-09	1.60E-07	37.3	ND	ND	ND	1.68E-04	1.72E-05	ND	ND
69	1.45E-07	ND	ND	ND	ND	ND	ND	1.45E-07	33.7	ND	3.34E-06	ND	1.73E-04	2.03E-05	ND	ND

Table B-3 Microcosms amended with Butyric acid

DAY	PCE	TCE	c-DCE	t-DCE	VC	ETHENE	ETHANE	MOLES	MB	BUTYRIC	FORMIC	SUCCINIC	LACTIC	ACETIC	PROPIONIC	ISOBUTYRIC
0	4.59E-07	ND	ND	ND	ND	ND	ND	4.59E-07	100	1.85E-04	ND	ND	1.37E-06	1.00E-05	ND	ND
7	3.56E-07	ND	ND	ND	ND	ND	ND	3.56E-07	77.6	1.89E-04	1.01E-06	ND	1.08E-06	1.28E-04	ND	ND
14	3.27E-07	ND	9.78E-08	ND	ND	ND	ND	4.25E-07	92.5	1.37E-04	1.45E-06	ND	5.70E-07	8.94E-04	ND	ND
21	2.44E-07	ND	3.59E-08	ND	ND	ND	ND	2.80E-07	61.0	8.08E-05	ND	ND	ND	1.53E-03	ND	ND
28	1.62E-08	ND	5.21E-08	ND	2.82E-07	ND	ND	3.50E-07	76.3	7.70E-06	4.85E-07	ND	1.17E-06	3.39E-03	ND	ND
49	ND	ND	ND	ND	8.63E-08	ND	ND	8.63E-08	18.8	7.22E-07	1.10E-06	ND	1.74E-06	1.95E-03	ND	ND
77	ND	ND	ND	ND	ND	ND	ND	0.00E+00	0.0	ND	ND	ND	1.57E-06	4.94E-04	ND	ND
160	ND	ND	ND	ND	ND	2.47E-08	6.07E-08	8.54E-08	18.6	ND	5.61E-07	ND	ND	4.17E-06	ND	ND

Table B-4 Negative controls amended with Butyric acid

DAY	PCE	TCE	c-DCE	t-DCE	VC	ETHENE	ETHANE	MOLES	MB	BUTYRIC	FORMIC	SUCCINIC	LACTIC	ACETIC	PROPIONIC	ISOBUTYRIC
0	4.23E-07	ND	ND	ND	ND	ND	1.63E-09	4.25E-07	99.9	1.72E-04	1.54E-06	ND	1.45E-06	1.93E-05	ND	ND
13	3.14E-07	ND	ND	ND	ND	ND	1.59E-09	3.16E-07	74.3	1.80E-04	1.45E-06	ND	ND	1.37E-05	ND	ND
28	2.74E-07	ND	ND	ND	ND	ND	1.62E-09	2.76E-07	64.9	1.90E-04	ND	ND	1.91E-06	2.69E-04	ND	ND
55	3.10E-07	ND	ND	ND	ND	ND	7.25E-10	3.11E-07	73.1	1.82E-04	1.74E-06	ND	ND	1.80E-05	ND	ND
69	2.63E-07	ND	ND	ND	ND	ND	1.38E-09	2.64E-07	62.2	1.91E-04	1.85E-06	ND	1.37E-06	1.85E-05	ND	ND

Table B-5 Microcosms amended with Succinic acid

DAY	PCE	TCE	c-DCE	t-DCE	VC	ETHENE	ETHANE	MOLES	MB	BUTYRIC	FORMIC	SUCCINIC	LACTIC	ACETIC	PROPIONIC	ISOBUTYRIC
0	4.47E-07	ND	ND	ND	ND	ND	ND	4.47E-07	100	ND	1.45E-06	5.27E-05	ND	ND	ND	ND
7	3.56E-07	ND	ND	ND	ND	ND	ND	3.56E-07	79.6	ND	4.85E-07	ND	1.11E-06	4.47E-04	3.8751.10	ND
14	3.27E-07	ND	9.78E-08	ND	ND	ND	ND	4.25E-07	95.0	ND	9.69E-07	3.52E-07	4.75E-07	4.71E-04	1.03E-04	ND
21	2.44E-07	ND	3.59E-08	ND	ND	ND	ND	2.80E-07	62.6	ND	9.88E-07	ND	ND	4.32E-04	9.46E-05	ND
28	1.62E-07	ND	5.21E-08	ND	2.82E-07	ND	ND	4.96E-07	111	6.08E-07	ND	ND	1.80E-06	3.92E-04	1.10E-04	ND
49	ND	ND	ND	ND	8.63E-08	ND	ND	8.63E-08	19.3	5.63E-06	1.24E-06	ND	1.10E-06	4.86E-04	4.99E-06	ND
77	ND	ND	ND	ND	ND	ND	ND	ND	0.0	ND	ND	ND	ND	1.90E-06	ND	ND
160	ND	ND	ND	ND	ND	ND	ND	ND	0.0	ND	ND	ND	ND	7.11E-06	ND	ND

Table B-6 Negative controls amended with Succinic acid

DAY	PCE	TCE	c-DCE	t-DCE	VC	ETHENE	ETHANE	MOLES	MB	BUTYRIC	FORMIC	SUCCINIC	LACTIC	ACETIC	PROPIONIC	ISOBUTYRIC
0	3.97E-07	ND	ND	ND	ND	ND	1.61E-09	3.99E-07	99.9	ND	1.34E-06	1.49E-04	ND	1.45E-05	ND	ND
13	3.79E-07	ND	ND	ND	ND	ND	1.59E-09	3.81E-07	95.4	ND	ND	1.54E-04	ND	1.07E-05	ND	ND
28	3.54E-07	ND	ND	ND	ND	ND	1.59E-09	3.56E-07	89.1	ND	ND	1.51E-04	ND	ND	ND	ND
41	3.50E-07	ND	ND	ND	ND	ND	1.53E-09	3.52E-07	88.1	ND	ND	1.51E-04	ND	2.15E-05	ND	ND
55	2.51E-07	ND	ND	ND	ND	ND	1.51E-09	2.53E-07	63.3	ND	ND	1.53E-04	ND	1.72E-05	ND	ND
69	2.53E-07	ND	ND	ND	ND	ND	1.05E-09	2.54E-07	63.7	ND	ND	1.52E-04	ND	1.96E-05	ND	ND

Table B-7 Microcosms amended with Butyric acid/Succinic acid mixture

DAY	PCE	TCE	c-DCE	t-DCE	VC	ETHENE	ETHANE	MOLES	MB	BUTYRIC	FORMIC	SUCCINIC	LACTIC	ACETIC	PROPIONI	ISOBUTYRI
0	5.81E-07	ND	ND	ND	ND	ND	ND	5.81E-07	100	2.00E-04	1.45E-06	5.36E-05	ND	3.19E-06	ND	ND
7	3.96E-07	ND	ND	ND	ND	ND	ND	3.96E-07	68.2	1.82E-04	9.88E-07	ND	2.62E-06	4.11E-04	1.10E-04	ND
14	3.92E-07	ND	1.68E-08	ND	ND	ND	ND	4.09E-07	70.4	1.53E-04	9.79E-07	ND	ND	1.12E-03	1.01E-04	ND
21	ND	ND	2.04E-07	ND	2.83E-07	ND	ND	4.87E-07	83.8	4.75E-05	ND	ND	ND	2.87E-03	9.88E-05	ND
28	ND	ND	ND	ND	3.79E-07	ND	ND	3.79E-07	65.2	7.03E-07	ND	ND	1.16E-06	3.00E-03	1.19E-04	ND
49	ND	ND	ND	ND	ND	ND	ND	ND	0.0	ND	1.06E-06	ND	1.65E-06	1.14E-04	7.22E-05	ND
77	ND	ND	ND	ND	ND	ND	ND	ND	0.0	ND	ND	ND	1.71E-06	ND	ND	ND
160	ND	ND	ND	ND	ND	1.87E-07	2.08E-09	1.89E-07	32.6	ND	1.06E-06	ND	9.50E-07	ND	ND	ND

Table B-8 Microcosms amended with Butyric acid/Formic acid mixture

DAY	PCE	TCE	c-DCE	t-DCE	VC	ETHENE	ETHANE	MOLES	MB	BUTYRIC	FORMIC	SUCCINIC	LACTIC	ACETIC	PROPIONI	ISOBUTYRI
0	5.04E-07	ND	ND	ND	ND	ND	ND	5.04E-07	100	1.96E-04	7.27E-05	ND	4.75E-07	7.65E-05	ND	ND
7	3.97E-07	ND	ND	ND	ND	ND	ND	3.97E-07	78.8	1.913-4	1.06E-06	ND	1.88E-06	3.79E-04	ND	ND
14	3.76E-07	ND	1.37E-08	ND	ND	ND	ND	3.90E-07	77.3	1.76E-04	2.02E-06	ND	ND	7.37E-04	ND	ND
21	2.46E-07	ND	6.73E-08	ND	ND	ND	ND	3.13E-07	62.2	6.67E-05	9.69E-07	ND	ND	2.45E-03		ND
28	1.04E-07	ND	1.53E-07	ND	ND	ND	ND	2.57E-07	51.0	5.67E-05	ND	ND	9.79E-07	2.99E-03	ND	ND
49	ND	ND	ND	ND	8.61E-08	ND	ND	8.61E-08	17.1	5.32E-07	4.85E-07	ND	1.60E-06	1.86E-03	ND	ND

Table B-9 Microcosms amended with Formic acid

DAY	PCE	TCE	c-DCE	t-DCE	VC	ETHENE	ETHANE	MOLES	MB	BUTYRIC	FORMIC	SUCCINIC	LACTIC	ACETIC	PROPIONIC	ISOBUTYRIC
0	4.71E-07	ND	ND	ND	ND	ND	ND	4.71E-07	100	ND	1.56E-04	ND	ND	5.56E-06	ND	ND
7	3.40E-07	ND	ND	ND	ND	ND	ND	3.40E-07	72.2	ND	9.69E-07	ND	2.51E-06	4.29E-04	ND	ND
14	2.64E-07	ND	1.00E-07	ND	ND	ND	ND	3.64E-07	77.3	ND	4.85E-07	ND	4.56E-07	2.84E-04	ND	ND
21	4.01E-07	ND	ND	ND	ND	ND	ND	4.01E-07	85.1	ND	4.85E-07	ND	ND	3.14E-04	ND	ND
28	1.85E-07	ND	ND	ND	ND	ND	ND	1.85E-07	39.3	ND	ND	ND	1.71E-06	2.02E-04	ND	ND
49	1.21E-07	ND	7.60E-08	ND	1.65E-07	ND	ND	3.62E-07	76.9	ND	ND	ND	1.54E-06	2.41E-05	ND	ND
77	ND	ND	ND	ND	ND	ND	ND	ND	0.0	ND	ND	ND	5.51E-07	ND	ND	ND
160	ND	ND	ND	ND	ND	1.77E-07	1.11E-07	2.88E-07	61.1	ND	ND	ND	9.98E-07	3.72E-06	ND	ND

Table B-10 Microcosms amended with Propanol

DAY	PCE	TCE	c-DCE	t-DCE	VC	ETHENE	ETHANE	MOLES	MB	BUTYRIC	FORMIC	SUCCINI	LACTIC	ACETIC	PROPIONIC	ISOBUTY
0	5.38E-07	ND	ND	ND	ND	ND	2.32E-09	5.40E-07	100	6.27E-07	1.75E-06	ND	1.81E-06	1.46E-05	ND	ND
13	4.48E-07	ND	ND	ND	ND	ND	2.06E-09	4.50E-07	83.3	ND	ND	ND	ND	8.34E-04	1.02E-04	ND
28	2.08E-07	ND	1.16E-07	ND	1.54E-08	ND	1.36E-09	3.41E-07	63.1	6.27E-07	ND	3.52E-07	1.47E-06	6.50E-04	1.05E-04	ND
41	ND	ND	ND	ND	1.94E-07	1.55E-08	2.36E-09	2.12E-07	39.2	2.39E-06	ND	ND	ND	3.70E-05	8.97E-05	ND
55	ND	ND	4.48E-08	ND	1.52E-07	1.73E-08	2.34E-09	2.16E-07	40.1	4.02E-06	ND	ND	ND	1.34E-04	8.49E-05	ND
69	ND	ND	ND	ND	ND	2.43E-08	1.81E-09	2.61E-08	4.83	ND	5.80E-07	ND	5.04E-07	ND	ND	ND

Table B-11 Negative controls amended with Propanol

DAY	PCE	TCE	c-DCE	t-DCE	VC	ETHENE	ETHANE	MOLES	MB	BUTYRIC	FORMIC	SUCCINI	LACTIC	ACETIC	PROPIONIC	ISOBUTY
0	2.67E-07	ND	ND	ND	ND	ND	1.90E-09	2.69E-07	100	ND	1.45E-06	ND	1.74E-06	ND	ND	ND
13	2.03E-07	ND	ND	ND	ND	ND	1.81E-09	2.05E-07	76.3	ND	1.45E-06	ND	ND	ND	ND	ND
28	2.09E-07	ND	ND	ND	ND	ND	1.86E-09	2.10E-07	78.2	ND	2.08E-06	ND	1.54E-06	1.95E-05	ND	ND
41	1.26E-07	ND	ND	ND	ND	ND	1.86E-09	1.27E-07	47.4	ND	ND	ND	ND	ND	ND	ND
55	1.27E-07	ND	ND	ND	ND	ND	1.83E-09	1.29E-07	47.9	ND	3.28E-06	ND	1.43E-06	1.79E-05	ND	ND
69	9.51E-08	ND	ND	ND	ND	ND	1.77E-09	9.69E-08	36	ND	2.08E-06	ND	ND	1.92E-05	ND	ND

Table B-12 Microcosms amended with Ethanol

DAYS	PCE	TCE	c-DCE	t-DCE	VC	ETHENE	ETHANE	MOLES	MB	BUTYRIC	FORMIC	SUCCINIC	LACTIC	ACETIC	PROPIONIC	ISOBUTYRI
0	5.44E-07	ND	ND	ND	ND	ND	2.08E-09	5.46E-07	100	ND	1.19E-06	ND	1.54E-06	1.56E-05	ND	ND
13	4.41E-07	ND	ND	ND	ND	ND	1.99E-09	4.43E-07	81.1	1.88E-06	ND	ND	ND	1.19E-03	1.41E-05	ND
28	3.02E-07	ND	ND	ND	ND	ND	1.52E-09	3.04E-07	55.6	ND	ND	ND	1.57E-06	1.52E-03	1.59E-05	ND
41	ND	ND	8.18E-08	ND	ND	7.59E-08	2.12E-09	1.60E-07	29.3	1.82E-06	ND	ND	9.69E-07	7.75E-04	2.73E-05	ND
55	ND	ND	ND	ND	2.90E-07	1.01E-07	2.21E-09	3.93E-07	72.0	6.84E-07	ND	ND	1.10E-06	3.41E-04	2.52E-05	ND
69	ND	ND	2.10E-09	ND	3.06E-07	2.82E-08	2.19E-09	3.38E-07	62.0	ND	ND	ND	ND	6.25E-05	2.22E-05	ND

Table B-13 Negative controls amended with Ethanol

DAYS	PCE	TCE	c-DCE	t-DCE	VC	ETHENE	ETHANE	MOLES	MB	BUTYRIC	FORMIC	SUCCINIC	LACTIC	ACETIC	PROPIONIC	ISOBUTYRI
0	2.53E-07	ND	ND	ND	ND	ND	1.85E-09	2.55E-07	99.9	ND	1.43E-06	ND	1.74E-06	1.45E-05	ND	ND
13	2.26E-07	ND	ND	ND	ND	ND	1.82E-09	2.28E-07	89.3	ND	ND	ND	ND	2.74E-04	ND	ND
28	1.83E-07	ND	ND	ND	ND	ND	1.93E-09	1.85E-07	72.4	ND	1.97E-06	ND	1.68E-06	1.91E-05	ND	ND
41	1.70E-07	ND	ND	ND	ND	ND	ND	1.70E-07	66.8	ND	2.02E-06	ND	ND	1.78E-05	ND	ND
55	7.12E-08	ND	ND	ND	ND	ND	1.85E-09	7.31E-08	28.7	ND	1.82E-06	ND	1.48E-06	3.16E-04	ND	ND
69	1.71E-07	ND	ND	ND	ND	ND	1.79E-09	1.73E-07	67.9	ND	2.11E-06	ND	1.43E-06	1.79E-05	ND	ND

Table B-14 Microcosms amended with Ethylene glycol/Butanol mixture

DAY	PCE	TCE	c-DCE	t-DCE	VC	ETHENE	ETHANE	MOLES	MB	BUTYRIC	FORMIC	SUCCINIC	LACTIC	ACETIC	PROPIONIC	ISOBUTYRIC
0	3.06E-07	ND	ND	ND	ND	ND	1.17E-09	3.07E-07	100	ND	1.94E-06	ND	ND	4.01E-05	ND	ND
13	2.80E-07	ND	ND	ND	ND	ND	1.04E-09	2.81E-07	91.5	1.65E-05	4.85E-07	ND	4.94E-07	3.14E-04	2.00E-07	ND
28	1.51E-07	ND	5.15E-08	ND	ND	ND	1.06E-09	2.04E-07	66.3	9.33E-05	ND	ND	1.06E-05	1.52E-03	3.12E-05	1.43E-07
41	1.63E-07	ND	ND	ND	ND	ND	1.61E-09	1.65E-07	53.6	1.00E-04	ND	ND	ND	1.69E-03	2.82E-05	1.26E-06
55	5.54E-08	ND	1.06E-08	ND	1.14E-07	3.14E-09	3.14E-09	1.86E-07	60.7	ND	ND	ND	ND	ND	3.65E-05	1.88E-06
69	ND	ND	4.54E-08	ND	3.57E-08	4.04E-08	2.88E-09	1.24E-07	40.5	7.09E-05	ND	ND	4.75E-07	1.13E-03	ND	ND

Table B-15 Negative controls amended with Butanol

DAY	PCE	TCE	c-DCE	t-DCE	VC	ETHENE	ETHANE	MOLES	MB	BUTYRIC	FORMIC	SUCCINIC	LACTIC	ACETIC	PROPIONIC	ISOBUTYRIC
0	2.62E-07	ND	ND	ND	ND	ND	1.86E-09	2.64E-07	99.9	ND	1.60E-06	ND	1.43E-06	1.15E-05	ND	ND
13	2.32E-07	ND	ND	ND	ND	ND	1.88E-09	2.34E-07	88.5	ND	1.45E-06	ND	ND	1.45E-05	ND	ND
28	2.57E-07	ND	ND	ND	ND	ND	1.91E-09	2.59E-07	98.0	ND	2.91E-06	ND	ND	1.97E-05	ND	ND
41	1.32E-07	ND	ND	ND	ND	ND	8.87E-09	1.41E-07	53.5	ND	1.45E-06	ND	1.60E-06	9.78E-06	ND	ND
55	1.29E-07	ND	ND	ND	ND	ND	1.76E-09	1.31E-07	49.7	ND	2.05E-06	ND	ND	1.85E-05	ND	ND
69	1.78E-07	ND	ND	ND	ND	ND	1.91E-09	1.78E-07	67.3	ND	2.05E-06	ND	ND	1.93E-05	ND	ND

Table B-16 Microcosms amended with Butanol/Propanol mixture

DAY	PCE	TCE	c-DCE	t-DCE	VC	ETHENE	ETHANE	MOLES	MB	BUTYRIC	FORMIC	SUCCINIC	LACTIC	ACETIC	PROPIONIC	ISOBUTYRI
0	3.50E-07	ND	ND	ND	ND	ND	2.04E-09	3.52E-07	100	ND	1.57E-06	ND	1.80E-06	1.08E-05		ND
13	2.32E-07	ND	9.68E-08	ND	ND	ND	2.02E-09	3.31E-07	94.0	1.36E-04	ND	ND	5.80E-07	1.26E-03	9.61E-05	ND
28	1.93E-07	ND	9.48E-08	ND	ND	ND	2.02E-09	2.90E-07	82.3	1.29E-04	5.04E-07	ND	8.47E-07	1.33E-03	9.46E-05	ND
41	1.98E-07	ND	8.78E-08	ND	ND	ND	2.00E-09	2.88E-07	81.8	9.42E-05	ND	ND	ND	6.08E-04	9.46E-05	1.24E-04
55	2.20E-07	ND	ND	ND	ND	ND	6.86E-09	2.27E-07	64.4	1.50E-06	4.56E-07	ND	ND	1.06E-04	1.08E-04	1.97E-06
69	9.32E-08	ND	ND	ND	4.51E-08	2.46E-08	1.30E-08	1.76E-07	50.0	1.19E-06	1.04E-06	ND	4.94E-07	9.73E-05	ND	ND

Table B-17 Microcosms amended with Propanol/Ethanol mixture

DAY	PCE	TCE	c-DCE	t-DCE	VC	ETHENE	ETHANE	MOLES	MB	BUTYRIC	FORMIC	SUCCINIC	LACTIC	ACETIC	PROPIONIC	ISOBUTYRI
0	5.57E-07	ND	ND	ND	ND	ND	2.07E-09	5.59E-07	100	ND	1.49E-06	3.52E-07	4.66E-07	1.42E-05	ND	ND
13	4.79E-07	ND	ND	ND	ND	ND	1.91E-09	4.81E-07	86.0	1.61E-05	ND	ND	9.60E-07	2.09E-03	1.09E-04	ND
28	3.87E-07	ND	1.33E-07	ND	ND	ND	1.31E-09	5.21E-07	93.3	1.97E-05	ND	ND	1.45E-06	2.00E-03	1.12E-04	ND
41	1.12E-07	ND	1.96E-07	ND	ND	ND	2.01E-09	3.10E-07	55.5	6.13E-06	ND	ND	ND	1.97E-04	1.07E-04	ND
55	2.60E-07	ND	1.30E-07	ND	9.43E-09	ND	2.77E-09	4.02E-07	71.9	ND	ND	ND	1.54E-06	1.94E-05	1.27E-04	ND
69	6.33E-08	ND	2.06E-07	ND	2.83E-07	ND	2.99E-09	5.55E-07	99.3	2.48E-06	ND	ND	5.23E-07	1.05E-07	ND	ND

Table B-18 Microcosms amended with Ethylene glycol

DAY	PCE	TCE	c-DCE	t-DCE	VC	ETHENE	ETHANE	MOLES	MB	BUTYRIC	FORMIC	SUCCINI	LACTIC	ACETIC	PROPIONIC	ISOBUTYRIC
0	3.06E-07	ND	ND	ND	ND	ND	1.17E-09	3.07E-07	100	ND	1.21E+06	ND	ND	4.07E-05	ND	ND
13	2.80E-07	ND	ND	ND	ND	ND	1.04E-09	2.81E-07	91.5	ND	6.75E-07	ND	ND	1.56E-03	3.78E-06	ND
28	1.51E-07	ND	5.15E-08	ND	ND	ND	1.06E-09	2.04E-07	66.3	4.99E-06	ND	ND	ND	2.14E-06	1.62E-05	ND
41	1.63E-07	ND	ND	ND	ND	ND	1.61E-09	1.65E-07	53.6	1.24E-07	ND	ND	ND	2.23E-03	1.18E-05	ND
55	5.54E-08	ND	1.06E-08	ND	1.14E-07	3.14E-09	3.14E-09	1.86E-07	60.7	ND	ND	ND	ND	ND	3.12E-05	ND
69	ND	ND	4.54E-08	ND	3.57E-08	4.04E-08	2.88E-09	1.24E-07	40.5	1.17E-06	1.01E-06	ND	ND	6.13E-04	ND	ND

APPENDIX C

NEGATIVE CONTROL GRAPHS

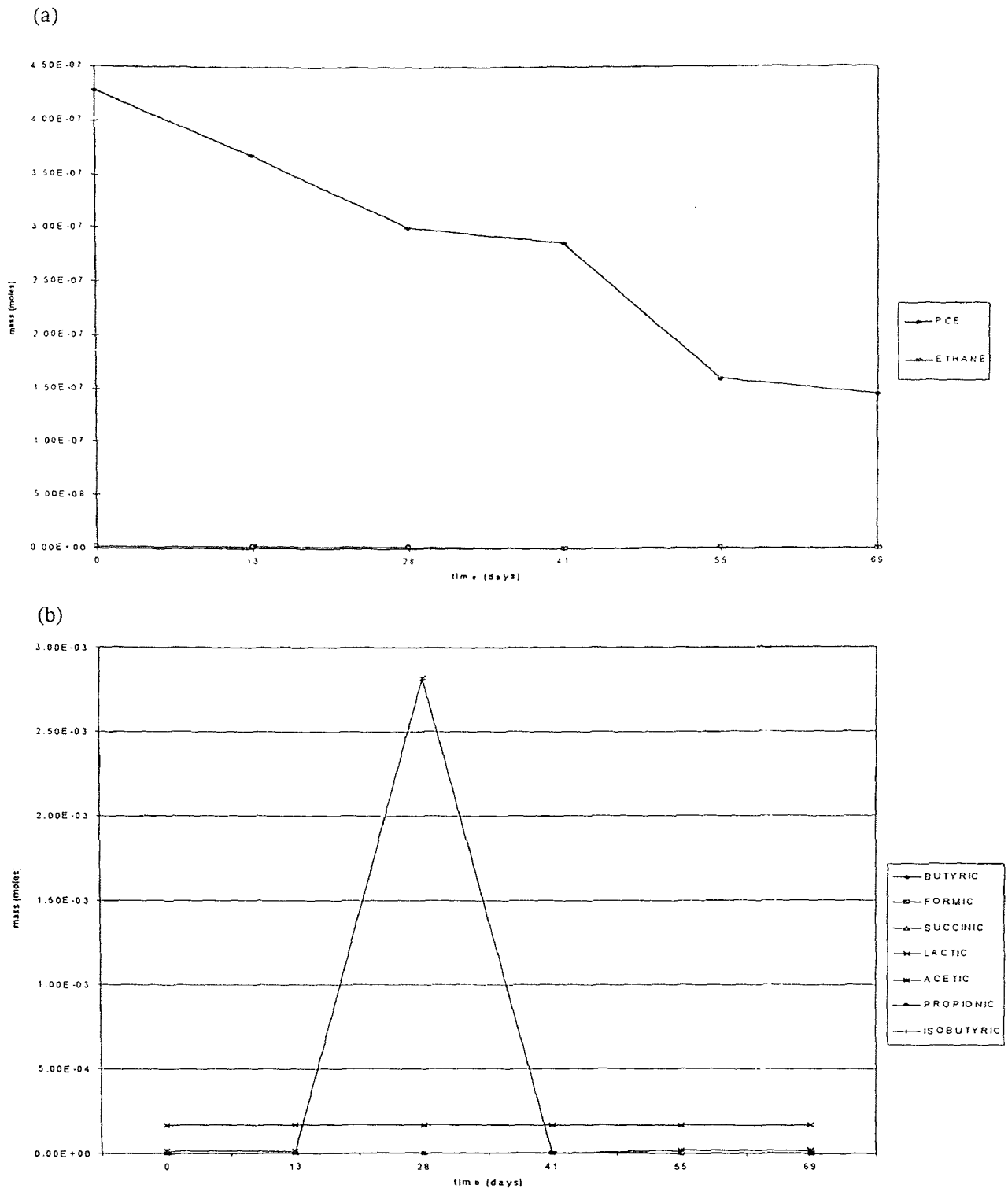


Figure C-1 Mass in moles of (a) chlorinated ethene and (b) acid byproducts as a function of time for negative controls amended with lactic acid.

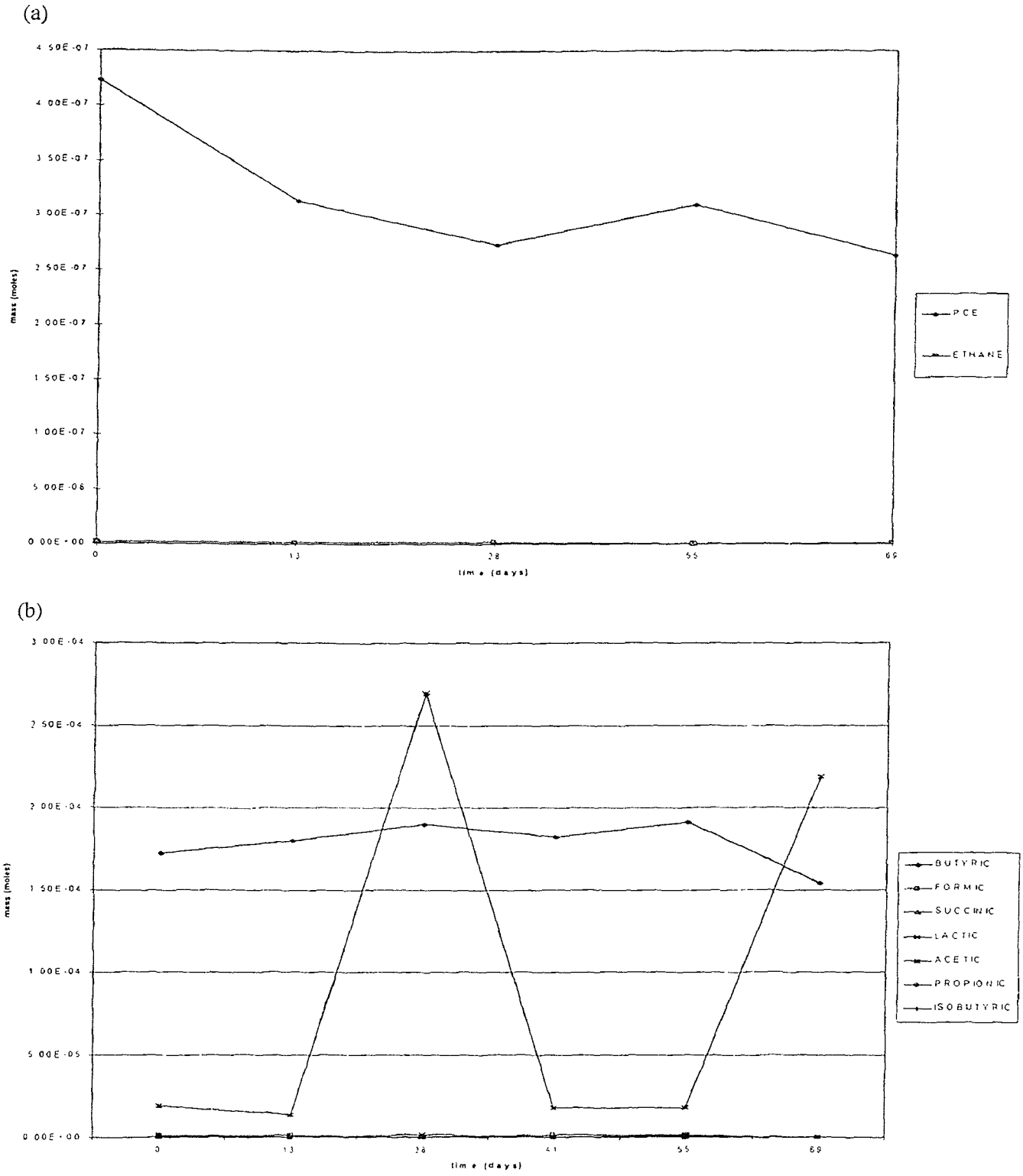
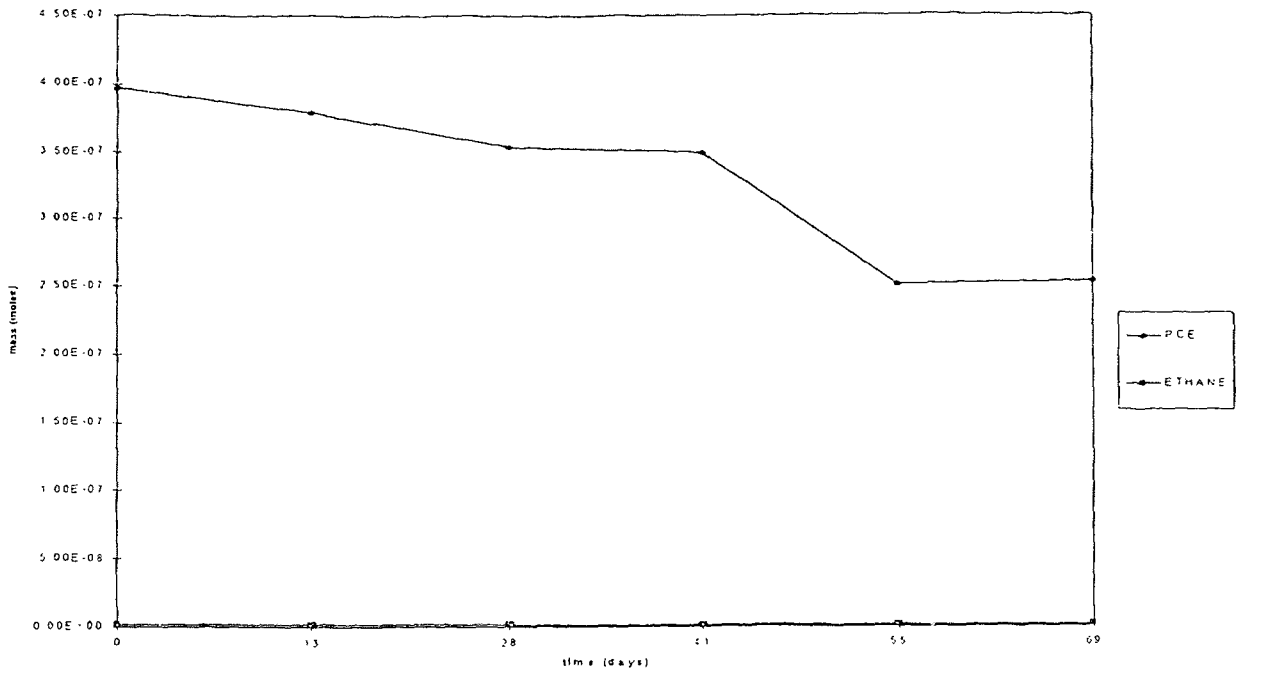


Figure C-2 Mass in moles of (a) chlorinated ethene and (b) acid byproducts as a function of time for negative controls amended with butyric acid.

(a)



(b)

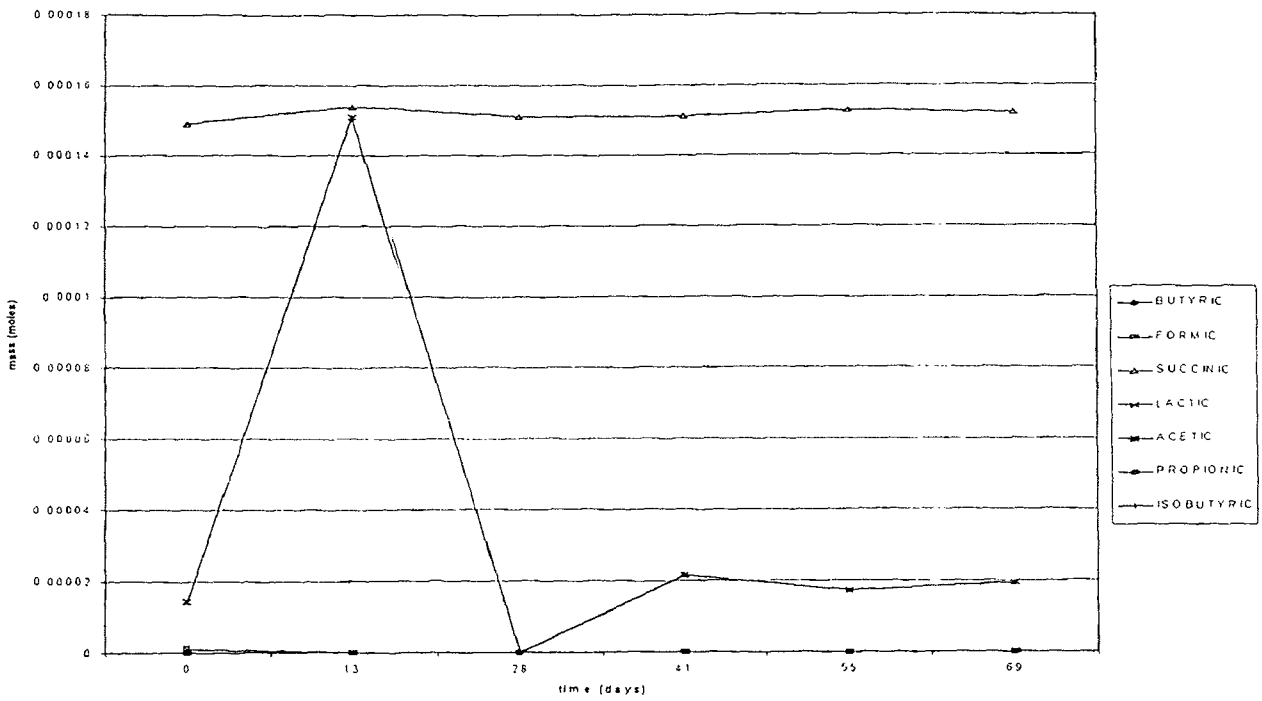


Figure C-3 Mass in moles of (a) chlorinated ethene and (b) acid byproducts as a function of time for negative controls amended with succinic acid.

(a)

(b)

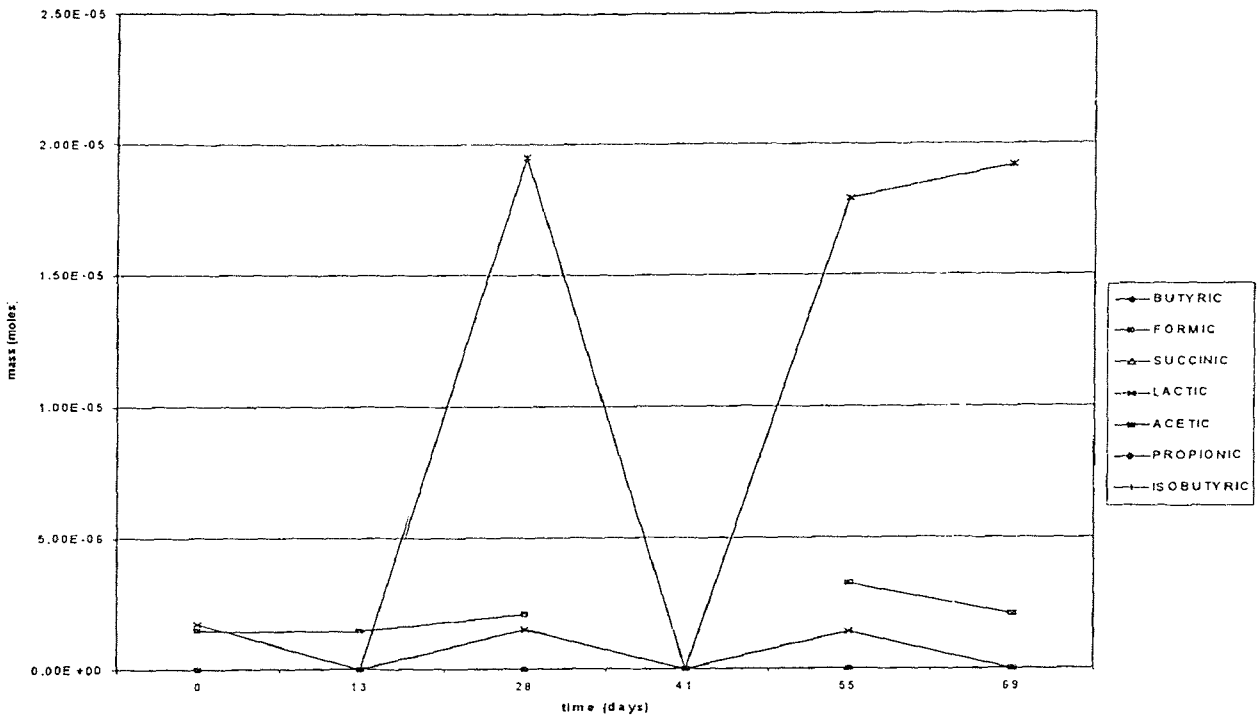
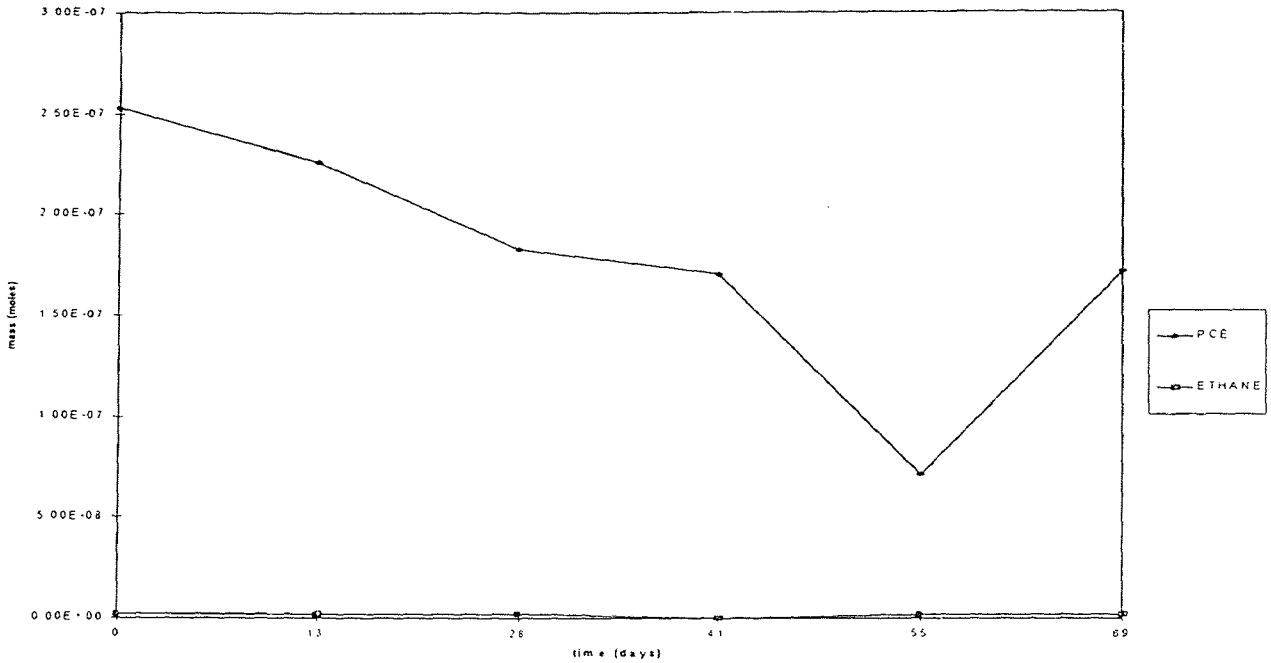


Figure C-4 Mass in moles of (a) chlorinated ethene and (b) acid byproducts as a function of time for negative controls amended with propanol.

(a)



(b)

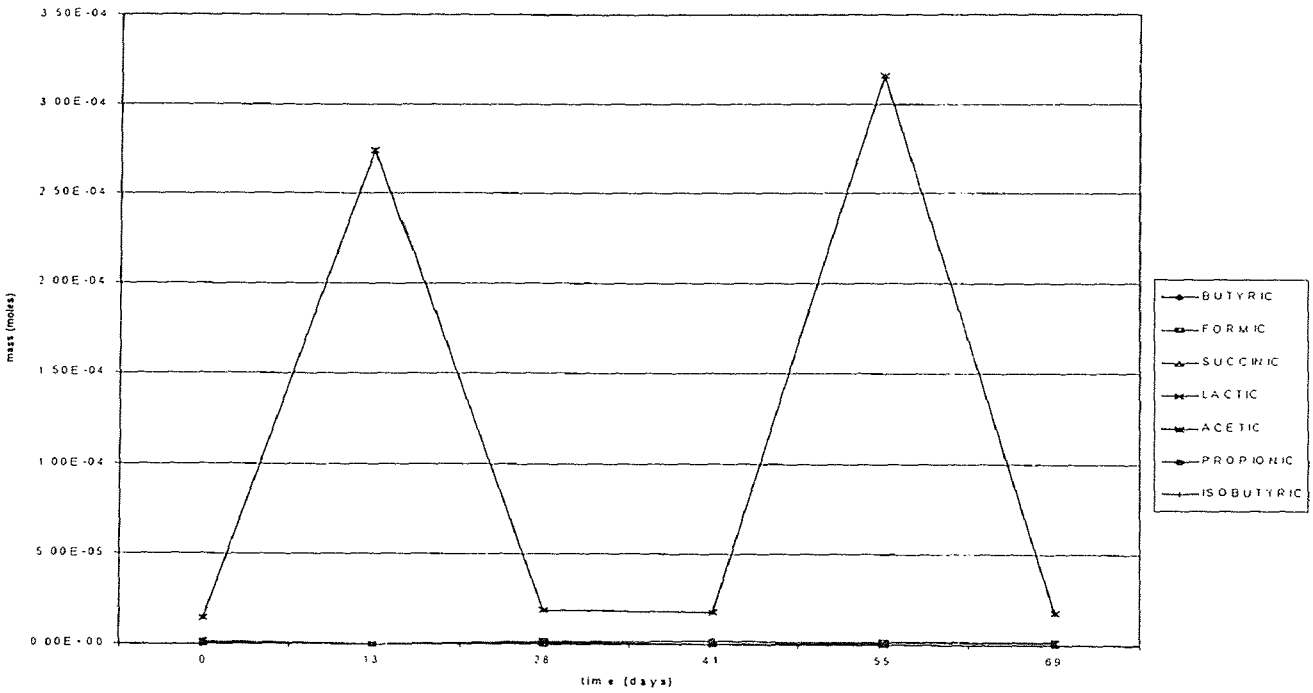
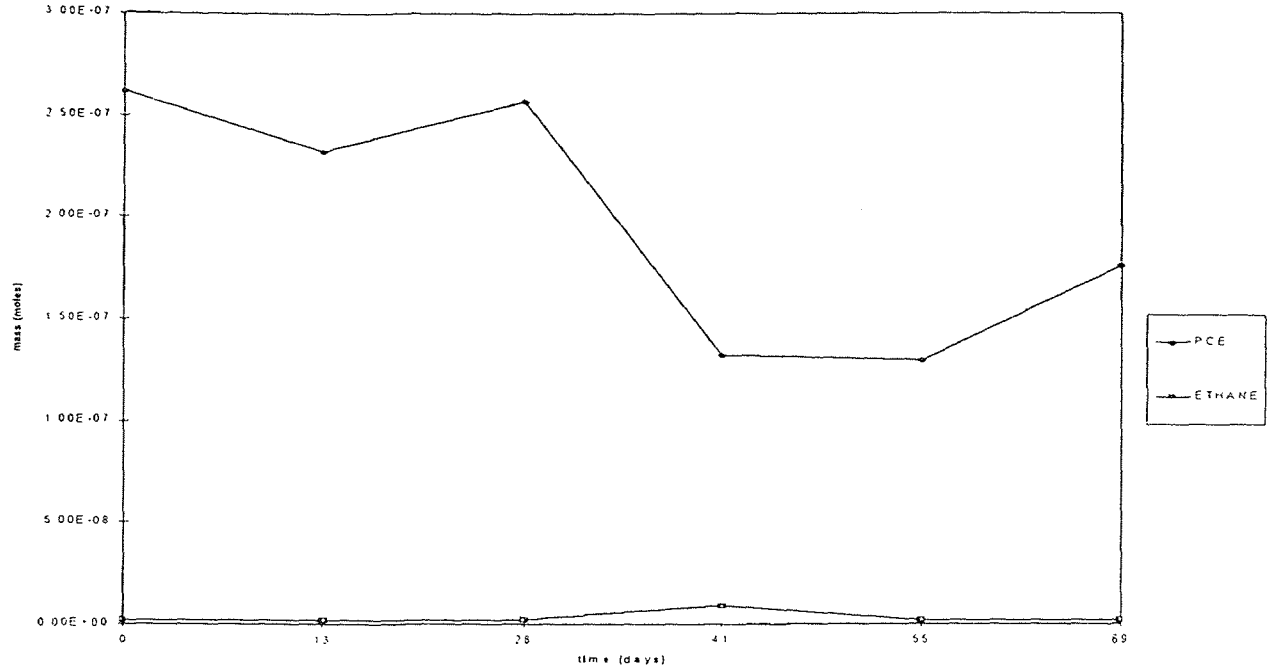


Figure C-5 Mass in moles of (a) chlorinated ethene and (b) acid byproducts as a function of time for negative controls amended with ethanol.

(a)



(b)

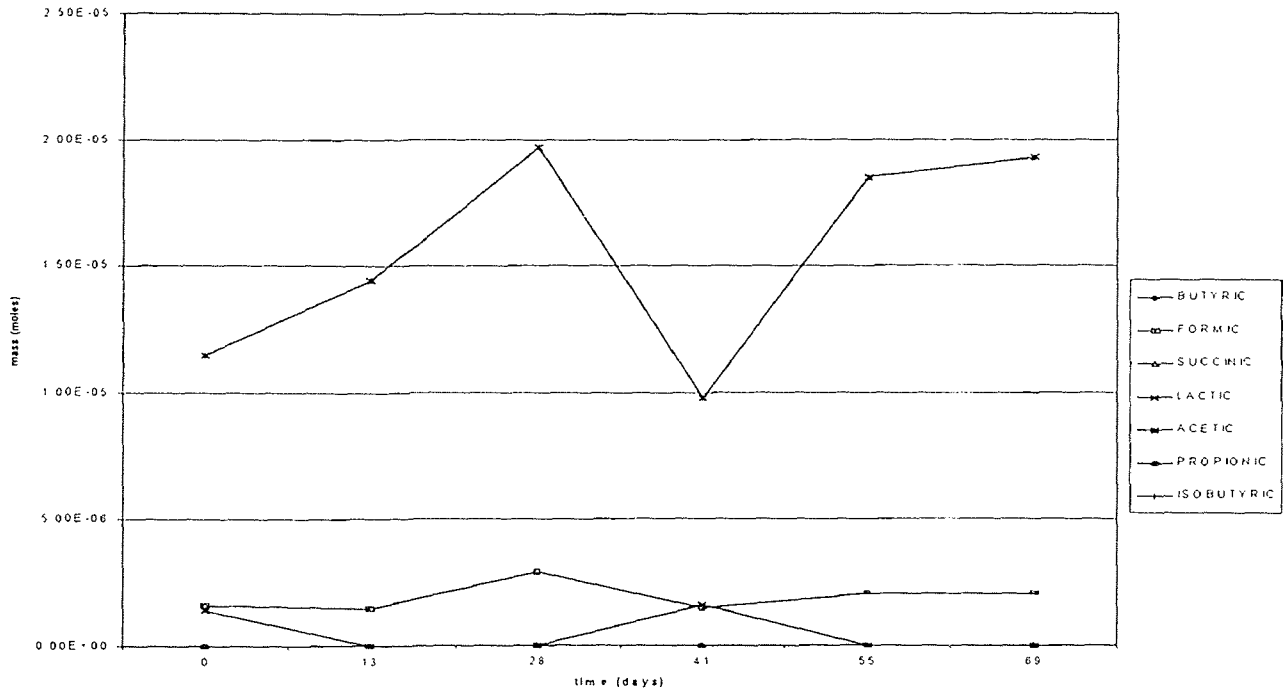


Figure C-6 Mass in moles of (a) chlorinated ethene and (b) acid byproducts as a function of time for negative controls amended with butanol.

APPENDIX D

RAW DATA FOR MICROCOSMS

Table D-1 Data for microcosms amended with **Lactic Acid** (moles)

	T=0	T=0	T=0	MEAN	SD
PCE	4.57E-07	4.60E-07	4.16E-07	4.44E-07	0.863
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=7	T=7	T=7	MEAN	SD
PCE	3.35E-07	3.84E-07	3.77E-07	3.65E-07	0.94
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=14	T=14	T=14	MEAN	SD
PCE	3.23E-07	3.27E-07	3.96E-07	3.49E-07	1.43
cis-DCE	ND	8.24E-09	ND	2.74E-09	0.167
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=21	T=21	T=21	MEAN	SD
PCE	ND	ND	9.74E-08	3.25E-08	1.97
cis-DCE	1.92E-07	2.25E-71.	1.01E-07	1.73E-07	2.25
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=28	T=28	T=28	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	1.07E-07	ND	1.30E-07	7.90E-08	2.44
VC	2.96E-07	3.59E-07	1.92E-07	2.83E-07	2.96
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=49	T=49	T=49	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	9.99E-08	ND	1.04E-07	6.78E-08	2.06
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=77	T=77	T=77	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	1.79E-07	5.96E-08	3.62
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=160	T=160	T=160	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	6.66E-08	ND	2.22E-08	87.2
Ethane	ND	1.02E-07	ND	3.42E-08	134

Table D-2 Data for microcosms amended with Butyric Acid (moles)

	T=0	T=0	T=0	MEAN	SD
PCE	3.91E-07	3.25E-07	3.72E-07	3.63E-07	1.18
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=7	T=7	T=7	MEAN	SD
PCE	2.93E-07	3.12E-07	3.33E-07	3.13E-07	0.7
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=14	T=14	T=14	MEAN	SD
PCE	2.88E-07	2.90E-07	2.34E-07	2.71E-07	1.22
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=21	T=21	T=21	MEAN	SD
PCE	ND	ND	2.21E-07	7.35E-08	4.47
cis-DCE	1.94E-08	2.17E-07	1.82E-08	8.49E-08	4.01
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=28	T=28	T=28	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	2.06E-07	ND	ND	6.88E-08	4.18
VC	ND	3.72E-07	1.84E-07	1.85E-07	6.52
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=49	T=49	T=49	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	ND	1.62E-07	ND	5.41E-08	3.29
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=77	T=77	T=77	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=160	T=160	T=160	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	1.20E-07	4.01E-08	2.44
Ethene	ND	4.59E-08	3.19E-08	2.47E-08	53.4
Ethane	ND	1.90E-07	1.01E-09	6.07E-08	248

Table D-3 Microcosms amended with **Succinic acid** (moles)

	T=0	T=0	T=0	MEAN	SD
PCE	3.41E-07	3.62E-07	3.57E-07	3.53E-07	0.39
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=7	T=7	T=7	MEAN	SD
PCE	2.53E-07	2.82E-07	3.08E-07	2.81E-07	0.96
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=14	T=14	T=14	MEAN	SD
PCE	2.56E-07	2.61E-07	2.57E-07	2.58E-07	0.09
cis-DCE	2.93E-10	ND	ND	9.79E-11	0.01
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=21	T=21	T=21	MEAN	SD
PCE	1.75E-07	1.56E-07	2.51E-07	1.93E-07	1.78
cis-DCE	3.29E-08	5.79E-08	1.06E-08	3.38E-08	0.83
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=28	T=28	T=28	MEAN	SD
PCE	ND	3.85E-08	ND	1.28E-08	0.78
cis-DCE	2.62E-08	1.21E-07	ND	4.91E-08	2.24
VC	2.71E-07	ND	3.25E-07	1.99E-07	6.11
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=49	T=49	T=49	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	ND	1.82E-07	ND	6.08E-08	3.7
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=77	T=77	T=77	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=160	T=160	T=160	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

Table D-4 Microcosms amended with Butyric acid/Succinic acid mixture (moles)

	T=0	T=0	T=0	MEAN	SD
PCE	5.04E-07	4.32E-07	4.40E-07	4.58E-07	1.78
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=7	T=7	T=7	MEAN	SD
PCE	3.12E-07	3.23E-07	3.03E-07	3.13E-07	0.27
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=14	T=14	T=14	MEAN	SD
PCE	2.66E-07	3.46E-07	3.16E-07	3.09E-07	1.98
cis-DCE	4.42E-09	ND	4.29E-08	1.58E-08	0.11
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=21	T=21	T=21	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	2.24E-07	5.41E-08	2.98E-07	1.92E-07	4.22
VC	2.06E-07	3.51E-07	4.06E-08	1.99E-07	3.61
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=28	T=28	T=28	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	4.05E-07	3.34E-07	3.98E-07	3.79E-07	1.76
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=49	T=49	T=49	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=77	T=77	T=77	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=160	T=160	T=160	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	2.87E-07	2.99E-07	3.34E-09	1.87E-07	19.7
Ethane	4.54E-09	1.40E-09	ND	2.08E-09	5.55

Table D-5 Microcosms amended with **Butyric acid/Formic acid mixture** (moles)

	T=0	T=0	T=0	MEAN	SD
PCE	3.82E-07	3.95E-07	4.15E-07	3.97E-07	0.59
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=7	T=7	T=7	MEAN	SD
PCE	3.14E-07	3.33E-07	2.93E-07	3.13E-07	0.7
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=14	T=14	T=14	MEAN	SD
PCE	3.06E-07	2.91E-07	2.94E-07	2.97E-07	0.27
cis-DCE	4.70E-09	2.47E-08	9.41E-09	1.29E-08	0.37
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=21	T=21	T=21	MEAN	SD
PCE	2.67E-07	3.11E-09	3.11E-07	1.94E-07	5.85
cis-DCE	ND	1.90E-07	ND	6.34E-08	3.85
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=28	T=28	T=28	MEAN	SD
PCE	ND	2.47E-07	ND	8.23E-08	5
cis-DCE	2.74E-07	3.53E-09	1.53E-07	1.44E-07	4.75
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=49	T=49	T=49	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	1.82E-07	ND	ND	6.06E-08	3.69
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=77	T=77	T=77	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	ND	1.77E-07	ND	5.90E-08	3.58
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=160	T=160	T=160	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	1.36E-07	4.32E-08	0.79
Ethane	ND	ND	1.82E-07	6.09E-08	1.05

Table D-6 Microcosms amended with **Formic acid** (moles)

	T=0	T=0	T=0	MEAN	SD
PCE	3.60E-07	3.60E-07	4.20E-07	3.80E-07	1.22
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=7	T=7	T=7	MEAN	SD
PCE	2.84E-07	2.26E-07	3.12E-07	2.74E-07	1.53
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=14	T=14	T=14	MEAN	SD
PCE	3.17E-07	ND	3.21E-07	2.13E-07	6.47
cis-DCE	ND	2.84E-07	ND	9.47E-08	5.75
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=21	T=21	T=21	MEAN	SD
PCE	2.90E-07	3.14E-07	3.65E-07	3.25E-07	1.11
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=28	T=28	T=28	MEAN	SD
PCE	1.20E-07	1.70E-07	1.56E-07	1.49E-07	0.9
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=49	T=49	T=49	MEAN	SD
PCE	ND	2.93E-07	ND	9.78E-08	5.94
cis-DCE	2.73E-08	1.67E-07	2.12E-08	7.20E-08	2.91
VC	1.12E-07	ND	2.48E-07	1.20E-07	4.35
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=77	T=77	T=77	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	ND	ND	ND

	T=160	T=160	T=160	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	1.00E-07	2.27E-07	1.77E-07	1.77E-07	0.65
Ethane	1.50E-07	8.62E-08	7.90E-08	1.11E-07	0.4

Table D-7 Data for microcosms amended with Propanol (moles)

	T=0	T=0	T=0	MEAN	SD
PCE	4.46E-07	3.88E-07	4.38E-07	4.24E-04	1.11
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	2.41E-09	2.28E-09	2.62E-09	2.44E-09	0.389

	T=13	T=13	T=13	MEAN	SD
PCE	3.63E-07	3.73E-07	3.29E-07	3.54E-07	0.802
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	2.11E-09	2.18E-09	2.21E-09	2.17E-09	0.118

	T=28	T=28	T=28	MEAN	SD
PCE	2.52E-07	3.95E-10	2.38E-07	1.64E-07	4.96
cis-DCE	3.85E-08	2.39E-07	5.14E-08	1.10E-07	3.94
VC	ND	3.19E-08	ND	1.06E-08	0.647
Ethene	ND	ND	ND	ND	ND
Ethane	2.30E-09	1.98E-09	ND	1.43	2.83

	T=41	T=41	T=41	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	1.60E-07	ND	1.44E-07	4.33E-08	3.09
Ethene	7.48E-09	2.68E-08	1.44E-08	1.62E-08	22.2
Ethane	2.44E-09	2.55E-09	2.44E-09	2.48E-09	0.13

	T=55	T=55	T=55	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	1.88E-08	6.27E-09	0.38
VC	ND	ND	3.21E-07	1.07E-07	6.5
Ethene	2.56E-08	2.89E-08	ND	1.82E-08	35.9
Ethane	2.53E-09	2.51E-09	2.33E-09	2.46E-09	0.25

	T=69	T=69	T=69	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	2.88E-08	1.75E-08	3.04E-08	2.55E-08	16
Ethane	2.83E-09	ND	2.86E-09	1.90E-09	3.73

Table D-8 Data for microcosms amended with **Ethanol** (moles)

	T=0	T=0	T=0	MEAN	SD
PCE	4.45E-07	4.23E-07	4.21E-07	4.30E-07	0.45
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	1.91E-09	2.22E-09	2.42E-09	2.25E-09	0.83

	T=13	T=13	T=13	MEAN	SD
PCE	3.61E-07	3.50E-07	3.32E-07	3.48E-07	0.51
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	2.12E-09	1.97E-09	2.17E-09	2.09E-09	0.24

	T=28	T=28	T=28	MEAN	SD
PCE	8.46E-08	3.55E-07	2.75E-07	2.38E-07	4.87
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	ND	ND	4.80E-09	1.60E-09	6.29

	T=41	T=41	T=41	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	1.03E-08	4.35E-08	9.82E-08	5.07E-08	1.56
VC	ND	ND	ND	ND	ND
Ethene		ND	ND	6.41E-09	24.9
Ethane	2.23E-09	2.17E-09	2.27E-09	2.26E-09	0.25

	T=55	T=55	T=55	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	ND	2.91E-07	3.21E-07	2.04E-07	6.23
Ethene		ND	ND	8.93E-09	35.1
Ethane	2.32E-09	2.40E-09	2.23E-09	2.32E-09	0.19

	T=69	T=69	T=69	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	9.12E-09	ND	3.05E-09	0.19
VC	1.81E-07	2.65E-07	2.01E-07	2.15E-07	1.53
Ethene		ND	ND	6.35E-10	2.5
Ethane	2.69E-09	2.18E-09	2.02E-09	2.30E-09	0.79

Table D-9 Microcosms amended with **Ethylene glycol/Butanol mixture** (moles)

	T=0	T=0	T=0	MEAN	SD
PCE	1.52E-07	1.95E-07	2.18E-07	1.88E-07	1.18
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	1.18E-09	1.19E-09	1.15E-09	1.17E-09	0.05

	T=41	T=41	T=41	MEAN	SD
PCE	1.51E-07	1.54E-07	1.75E-07	1.60E-07	0.45
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	1.47E-09	1.44E-09	1.43E-09	1.44E-09	0.05

	T=13	T=13	T=13	MEAN	SD
PCE	1.78E-07	1.95E-07	2.29E-07	2.01E-07	0.91
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	1.37E-09	1.36E-09	1.38E-09	1.37E-09	0.02

	T=55	T=55	T=55	MEAN	SD
PCE	1.21E-07	1.28E-07	1.28E-07	1.25E-07	0.14
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	1.06E-09	7.45E-10	1.11E-09	9.70E-10	0.45

	T=28	T=28	T=28	MEAN	SD
PCE	1.73E-07	1.88E-07	1.56E-07	1.72E-07	0.56
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	3.41E-09	3.02E-08	1.70E-08	1.69E-08	30.4

	T=69	T=69	T=69	MEAN	SD
PCE	4.39E-08	5.81E-08	1.06E-07	6.93E-08	0.14
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	2.06E-09	5.49E-09	7.27E-09	4.94E-09	6

Table D-10 Microcosms amended with **Butanol/Propanol mixture** (moles)

	T=0	T=0	T=0	MEAN	SD
PCE	2.89E-07	3.00E-07	2.57E-07	2.82E-07	0.19
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	2.18E-09	2.09E-09	2.15E-09	2.14E-09	0.1

	T=41	T=41	T=41	MEAN	SD
PCE	2.58E-07	ND	2.20E-07	1.59E-07	1.39
cis-DCE	ND	2.50E-07	ND	8.32E-08	1.44
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	2.07E-09	2.17E-09	2.07E-09	2.10E-09	0.14

	T=13	T=13	T=13	MEAN	SD
PCE	2.73E-07	2.88E-07	ND	1.87E-07	1.62
cis-DCE	ND	ND	2.76E-07	9.17E-08	1.59
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	2.01E-09	2.17E-07	2.17E-09	2.12E-09	0.21

	T=55	T=55	T=55	MEAN	SD
PCE	1.84E-07	1.85E-07	1.63E-07	1.78E-07	0.12
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	1.49E-08	3.88E-09	2.81E-09	7.20E-09	15.2

	T=28	T=28	T=28	MEAN	SD
PCE	2.35E-07	2.31E-07	ND	1.56E-07	1.35
cis-DCE	ND	ND	2.69E-07	8.98E-08	1.55
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	2.30E-09	2.14E-09	1.93E-09	2.13E-09	0.43

	T=69	T=69	T=69	MEAN	SD
PCE	1.11E-07	ND	1.15E-07	7.51E-08	0.65
cis-DCE	ND	ND	ND	ND	ND
VC	9.81E-08	ND	ND	3.27E-08	5.66
Ethene	ND	4.10E-11	ND	1.37E-11	0.05
Ethane	ND	2.39E-08	1.72E-08	1.37E-08	28

Table D-11 Microcosms amended with **Propanol/Ethanol mixture** (moles)

	T=0	T=0	T=0	MEAN	SD
PCE	4.59E-07	4.57E-07	4.28E-07	4.48E-07	0.608
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	2.17E-09	2.10E-09	2.25E-09	2.18E-09	0.173

	T=13	T=13	T=13	MEAN	SD
PCE	3.78E-07	4.00E-07	3.79E-07	3.86E-07	0.446
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	2.15E-09	1.97E-09	1.89E-09	2.00E-09	0.293

	T=28	T=28	T=28	MEAN	SD
PCE	3.32E-07	3.35E-07	ND	2.22E-07	6.76
cis-DCE	ND	ND	3.77E-07	1.26E-07	7.63
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	2.12E-09	2.01E-09	ND	1.38E-09	2.7

	T=41	T=41	T=41	MEAN	SD
PCE	ND	ND	2.71E-07	9.01E-08	5.73
cis-DCE	2.78E-07	2.78E-07	ND	1.85E-07	5.63
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	2.17E-09	2.24E-09	1.93E-09	2.12E-09	0.36

	T=55	T=55	T=55	MEAN	SD
PCE	2.01E-07	2.18E-07	2.09E-07	2.09E-07	0.3
cis-DCE	2.08E-08	3.48E-07	ND	1.23E-07	6.86
VC	ND	2.05E-08	ND	6.84E-09	0.42
Ethene	ND	ND	ND	ND	ND
Ethane	2.63E-09	3.63E-09	2.60E-09	2.91E-09	1.43

	T=69	T=69	T=69	MEAN	SD
PCE	1.53E-07	ND	ND	5.10E-08	3.1
cis-DCE	2.97E-08	ND	5.99E-10	1.01E-08	0.58
VC	ND	3.36E-07	2.80E-07	2.05E-07	6.32
Ethene	ND	ND	ND	ND	ND
Ethane	2.54E-09	3.24E-09	3.64E-09	3.14E-09	1.27

Table D-12 Microcosms amended with **Ethylene glycol** (moles)

	T=0	T=0	T=0	MEAN	SD
PCE	2.36E-07	2.46E-07	2.44E-07	2.41E-07	0.186
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	1.29E-09	1.18E-09	1.23E-09	1.23E-09	0.117

	T=13	T=13	T=13	MEAN	SD
PCE	2.33E-07	2.57E-07	1.73E-07	2.21E-07	1.51
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	9.94E-10	1.22E-09	1.07E-09	1.09E-09	0.26

	T=28	T=28	T=28	MEAN	SD
PCE	1.75E-07	1.84E-07	ND	1.20E-07	3.64
cis-DCE	ND	ND	1.46E-07	4.85E-08	2.95
VC	ND	ND	ND	ND	ND
Ethene	ND	ND	ND	ND	ND
Ethane	1.14E-09	1.11E-09	1.08E-09	1.11E-09	0.074

	T=41	T=41	T=41	MEAN	SD
PCE	1.31E-07	1.20E-07	1.36E-07	1.29E-07	0.29
cis-DCE	ND	ND	ND	ND	ND
VC	ND	ND	ND	ND	ND
Ethene	2.78E-08	ND	ND	9.26E-09	36.4
Ethane	1.62E-09	1.69E-09	1.76E-09	1.69E-09	0.15

	T=55	T=55	T=55	MEAN	SD
PCE	1.31E-07	ND	ND	4.38E-08	2.66
cis-DCE	ND	2.79E-08	1.95E-09	9.95E-09	0.55
VC	ND	1.06E-07	1.35E-07	8.02E-08	2.49
Ethene	ND	ND	ND	ND	ND
Ethane	6.70E-10	1.16E-09	8.07E-09	3.30E-09	9.38

	T=69	T=69	T=69	MEAN	SD
PCE	ND	ND	ND	ND	ND
cis-DCE	ND	ND	ND	ND	ND
VC	ND	7.55E-08	ND	2.52E-08	1.53
Ethene	7.79E-09	ND	ND	2.60E-09	10.2
Ethane	7.90E-09	1.18E-09	ND	3.03E-09	9.67

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