Copyright Warning & Restrictions

The copyright law of the United States (Title 17, United States Code) governs the making of photocopies or other reproductions of copyrighted material.

Under certain conditions specified in the law, libraries and archives are authorized to furnish a photocopy or other reproduction. One of these specified conditions is that the photocopy or reproduction is not to be "used for any purpose other than private study, scholarship, or research." If a, user makes a request for, or later uses, a photocopy or reproduction for purposes in excess of "fair use" that user may be liable for copyright infringement,

This institution reserves the right to refuse to accept a copying order if, in its judgment, fulfillment of the order would involve violation of copyright law.

Please Note: The author retains the copyright while the New Jersey Institute of Technology reserves the right to distribute this thesis or dissertation

Printing note: If you do not wish to print this page, then select "Pages from: first page # to: last page #" on the print dialog screen

ABSTRACT

A MICROCOSM STUDY IN THE REDUCTIVE DECHLORINATION OF TETRACHLOROETHYLENE USING FERMENTATION ACIDS AND ALCOHOLS AS ELECTRON DONORS

by
Anthony Siccardi III

Anaerobic microcosms of Arthur Kill (New Jersey) sediment were used to investigate the effects of several electron donors on tetrachloroethylene (PCE) dechlorination activity. The substates tested were methanol, butanol, butyrate, lactate and succinate both by themselves and in various combinations. Different levels of PCE dehalogenation were noticed in all of the microcosms regardless of the electron donor used. Vinyl chloride was the major dehalogenation product detected in the majority of the microcosms. The causitive organism or group that carried out the reductive dehalogenation was not identified. Only the microcosms admended with a mixture of butanol/methanol were able to fully reduce the PCE to ethene and ethane. No correlations could be drawn between the added electron donors and their metabolic products with the reductive dechlorination process.

A MICROCOSM STUDY IN THE REDUCTIVE DEHALOGENATION OF TETRACHLOROETHYLENE USING FERMENTATION ACIDS AND ALCOHOLS AS ELECTRON DONORS

by
Anthony Siccardi III

A Thesis
Submitted to the Faculty of
New Jersey Institute of Technology
in Partial Fulfillment of the Requirements for the Degree of
Master of Science in Environmental Science

Department of Chemical Engineering,
Chemistry, and Environmental Science

May 1998

APPROVAL PAGE

A MICROCOSM STUDY IN THE REDUCTIVE DEHALOGENATION OF TETRACHLOROETHYLENE USING FERMENTATION ACIDS AND ALCOHOLS AS ELECTRON DONORS

Anthony Siccardi III

Dr. Piero Armenante, Thesis Advisor — Date
Professor of Chemical Engineering, NJIT

Dr. David Kafkewitz, Thesis Advisor — Date
Professor of Microbiology, Rutgers University-Newark

Dr. Richard Trattner, Committee Member — Date
Associate Chairperson for Environmental Science and Professor of Chemical Engineering, Chemistry, and Environmental Science, NJIT

BIOGRAPHICAL SKETCH

Author: Anthony Siccardi III

Degree: Master of Science in Environmental Science

Date: May 1998

Undergraduate and Graduate Education:

- Master of Science in Environmental Science,
  New Jersey Institute of Technology, Newark, NJ, 1998

- Bachelor of Science in Marine Biology,
  Long Island University-Southampton Campus, Long Island, NY, 1995

Major: Environmental Science

This thesis is dedicated to
My Parents

ACKNOWLEDGMENT

I would like to express gratitude to Dr. Piero Armenante and Dr. David Kafkewitz for serving as advisors on this study and for providing their guidance throughout the project. Special thanks are given to Dr. Richard Trattner for serving as a committee member. Special thanks are also given to Clint Brockway and Gwendolyn San Augustin for sharing their expertise on operating the instruments used in the study and their help in designing the G.C. method used to measure the alcohols.

I am very grateful to Sheng-Yih-Lee and Andrea Giorgioni for sharing their past experiences on the project and for providing guidance on the set up of the microcosms.

I would also like to thank Dr. Monica Tonga for supplying the sediment used in this study and my brother, Matthew Siccardi, for his help in proofreading the document.

Last, but definitely not least, I would like to thank Samantha Marasigan for working as my partner on the design, set up, and sampling of this study.

TABLE OF CONTENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF TABLES

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Scope of the Problem

Tetrachloroethene (perchloroethylene; PCE) and trichloroethene (TCE) are synthetic chlorinated solvents that belong to a diverse group of industrial chemicals known as chlorinated aliphatic compounds. These chlorinated aliphatics react like an alkene and have gained prominence through industrial use, environmental persistence, toxicity, and their potential carcinogenicity.

PCE and TCE are used in many different industrial processes because of their low flammability and explosive potential, which makes them ideal solvents in many different processes. Through years of careless use, handling, storage, and disposal PCE and TCE have found their way into the environment, specifically groundwater, where they persist due to their high chemical stability. This persistence along with their heavy use have contributed to their status as being the most frequently encountered groundwater pollutants (1). A survey conducted in the United States in 1984 revealed that over 8% of the groundwater used as drinking water showed measurable levels (0.2 μg/liter) of PCE (2). Although the nation wide median was low (0.6 μg/liter) some groundwater was found to contain PCE levels up to 69 μg/liter (3).

PCE and TCE are among the 14 volatile organic compounds regulated under the Safe Drinking Water Act Amendments of 1986 and must be effectively removed to insure the quality of the groundwater for potable use. Until recently the primary technologies

used for removing volatile organics involved pump-and-treat systems using air stripping and adsorption onto granular activated carbon because these technologies were believed to be highly efficient (4). However, a decade of performance data has demonstrated that these systems are not as efficient as once thought and they mearly transfer the pollutants from one environment to another (5). This has prompted researchers to propose in-situ bioremediation as a method to reduce time and cost for site restoration of groundwater contaminated with PCE and TCE and also because bioremediation offers the prospect of converting the contaminants to harmless products.

1.2 Bioremediations Potential

Based on the current knowledge of bacterial degradation potential, PCE contaminated sites can be treated in two ways. The process can either be carried out under a solely anaerobic process or an anaerobic process and aerobic process can be employed sequentially (1). The success of both processes depends on the efficient performance during the anaerobic stage to reductivley dechlorinate the PCE and TCE to less or non chlorinated ethenes (1). Merely converting PCE and TCE to less chlorinated alkenes is of little or no benefit. *cis*-1,2-dichloroethylene (*cis*-1,2 DCE), *trans*-1,2-dichloroethylene (*trans*-1,2 DCE), and vinyl chloride (VC) are also regulated under the 1986 Safe Drinking Water Act because they also pose a threat to public health. In fact VC is considered to be a greater health concern than PCE or TCE and its accumulation must be prevented during the bioremediation process. For the bioremediation to be successful the final end product should be ethene or ethane because these products are sparingly soluble

in water and have not been associated with any long-term toxicological problems, making them environmentally acceptable biotransformation products (6).

1.3 The Process of Reductive Dehalogenation

The vast majority of the studies reported in the literature have been carried out under anaerobic conditions using continuous flow fixed-film reactors (7,8,9), in soil (10), aquifer microcosms (11), sediment (12,13), and even with pure cultures (1,14-20). Under anaerobic conditions PCE undergoes a process of sequential reductive dehalogenation to TCE, DCE, VC, and ethene (Figure 1.1). During reductive dehalogenation chlorine atoms are replaced with hydrogen atoms in either a co-metabolic process or in a repiratory process (19,20).

PCE------>TCE------>1,2-DCEs------>VC------>Ethene

Figure 1.1 The process of sequential reductive dehalogenation of PCE to ethene

In a co-metabolic process, the organism gains no benefit in the reductive dehalogenation because the dehalogenations are not coupled to energy conservation. This process is unspecifically carried out in a bypass reaction by certain enzyme systems (19). Methanogens, acetogens, sulfate-reducers, and iron-reducers have been shown to carry out reductive dehalogenation utilizing this process (19). In a respiratory process, the organism gains a direct benefit in the reductive dehalogenation because the dehalogenations are coupled to electrogenic energy conservation. In this process

molecular hydrogen, formate, and acetate serve as the electron donors while a halogenated compound, such as PCE, serves as the terminal electron acceptor. Two examples of respiratory-dehalogenating bacteria are *Dehalobacter restrictus* and *Dehalospirillum multivorans* which have been isolated in pure cultures and are known to use PCE as the terminal electron acceptor (19). For PCE dehalogenation, it has been determined that the respiratory process is several orders of magnitude higher than the co-metabolic process (20). This greater dehalogenation potential, along with the broader substrate acceptance of the co-metabolic bacteria makes them more suitable to treat waste streams with a mixture of chlorinated compounds (20).

1.4 The Search for the Perfect Electron Donor

Since reductive dehalogenation consumes electrons and aquifers are usually oligotrophic a great deal of research has been done on finding suitable electron donors (4,6,21,22). Gao et al. (4) tested the effects of several electron donors on the dechlorination of PCE in anaerobic soil microcosms. The electron donors they tested were methanol, formate, acetate, lactate, and sucrose at initial concentrations to provide a constant number of reducing equivalents (90meq/liter) if each of the substrates was completely oxidized to carbon dioxide. Of these five electron donors the microcosms that were amended with lactate showed the greatest dechlorinating activity converting 40% of the added PCE to TCE and *cis*-DCE in two of the sediments (4). Methanol was only able to stimulate TCE production in one sediment and formate, acetate, and sucrose additions only resulted in at most 1% reductive dehalogenation of PCE (4).

Gibson and Sewell (21) conducted a study to determine if the addition of short-chain organic acids or alcohols would stimulate the reductive dechlorination of PCE. They set up microcosms using acetate, lactate, propionate, butyrate, crotonate, methanol, ethanol, and isopropanol to act as the source for the reducing equivalents for PCE dehalogenation. The microcosms to which lactate or ethanol were added had TCE present by day six and while butyrate, crotonate, and propionate also supported dehalogenation the lag time was longer (21). In this study acetate, methanol, and isopropanol were not observed to support dehalogenation to any degree above that noticed in the negative controls (21).

Although in the above study methanol was unable to support significant dehalogenation activity Freedman and Gosset (6) determined that methanol was the most effective electron donor in their studies in terms of both maintaining the rate at which repetitive additions of PCE and TCE were degraded and the extent to which VC was converted to ethene. They were unable to determine why methanol was the most effective source of reducing equivalents but they proposed that it might have something to do with the metabolism of methanol by the methanogens (6). These studies clearly demonstrate one of the problems with the reductive dehalogenation of PCE in different environments. All this research has shown that there is no single electron donor, or even combination, that is able to function efficiently in every type of environment in which PCE is a contaminant.

1.5 The Role of Hydrogen as an Electron Donor

Previous studies have also indicated that H_2 is one of the substrates (and in some cases, the only one) that can serve as a direct electron donor in the reductive dechlorination of PCE in many environments (23-25). This is an important finding because studies have indicated that dechlorinators can utilize H_2 at lower concentrations than can methanogens (23). Fennel et al. (23) has suggested that by managing H_2 delivery, through the addition of electron donors that are fermented only under low H_2 partial pressures, one may be able to impart a competitive advantage to dechlorinators. This suggestion was further strengthened when Smatlak (26) determined that the half-velocity constant with respect to H_2 for this dechlorinator was one-tenth that of the methanogenic organisms in the culture. These finding all suggest that one may be able to selectively enhance dechlorination by managing H_2 delivery.

DiStefano et al. (24) determined that hydrogen was able to function as the sole electron donor in the reductive dechlorination of PCE to VC and ethene over a period of 14 to 40 days in anaerobic mixed PCE-methanol (MeOH) methanogenic enrichment cultures. PCE at an initial concentration of 550μM was routinely dechlorinated to 80% ethene and 20% VC within 2 days at 35°C (24). The dehalogenation process however, declined dramatically after 40 days unless the cultures were amended with filtered culture supernatant from MeOH-fed bottles (24). This demonstrated that the organisms responsible for the PCE dehalogenation suffered from a nutritional deficiency that was supplied from a presumably more diverse, MeOH-fed system (24). This possible dependency of the hydrogen-utilizing dechlorinators on the activities of other organisms

makes the search for a pure culture that can degrade PCE to ethene much more difficult and may explain why the bacteria isolated in section 1.6.1 can only degrade PCE to DCE. DiStefano et al. (24) hypothesized that for bioremediation of high levels of PCE, electron donors that cause the production of a large hydrogen pool should be selected or methods that directly use H_2 should be devised.

Fennel et al. (23) performed an extensive study on the comparison of butyric acid, ethanol, lactic acid, and propionic acid as hydrogen donors for the reductive dechlorination of PCE and found that all donors facilitated dechlorination to VC and ethene in comparable amounts during the long term study. Differences among the electron donors was however observed during the short-term, time-intensive tests (23). Butyric acid and propionic acid, which are slowly degraded and produce low concentrations of H_2, supported the dechlorinators while minimizing, and in the case of propionic acid essentially excluding, methanogenic competition (23). They also observed that at a 1:1 donor:PCE ratio lactic acid degradation also produced a much lower H_2 peak than ethanol and resulted in less competition from methanogens (23). When using ethanol, both at 1:1 and 1:2 ratios and lactic acid at a 2:1 ratio the amount of H_2 produced was orders of magnitude higher than when using the electron donors mentioned previously (23). This high H_2 production fueled initial rapid dechlorination and methanogenesis but as the donor and H_2 were depleted the dechlorination slowed drastically and there was often a significant amount of PCE that remained which was degraded only slowly over time (23). This study showed that the fate of electron donors

and their fermentation products, including not only H_2 but also other intermediates as well, is of critical importance in order to understand the dechlorinating communities (23).

1.6 The Bacteria Involved in Reductive Dehalogenation

Even with all the research that has been performed on the reductive dehalogenation of PCE to ethene relatively little is known about the bacteria and the environmental conditions necessary to start and maintain the process. It has been assumed that methanogens are involved in these dechlorination reactions mainly because most studies reporting PCE dechlorination have been carried out under methanogenic conditions (4,6,8,11,14,15,21). Fathepure and Boyd (14) have been able to show that methanogens isolated from a methanogenic enrichment growing on chlorophenol are indeed able to dechlorinate PCE to TCE. Fathepure and Boyd (14) also observed that the dechlorination rate was parallel to the methane production rate in that 52μmol TCE was formed per mole of methane produced. They proposed that electrons transferred during methanogenesis are diverted to PCE by a reduced electron carrier involved in the methane production but was unable to achieve a reduction of the PCE beyond TCE (14).

Freedman and Gosset (6) were also able to show that PCE and TCE could be dechlorinated to ethene using a mixed culture under methanogenic conditions. In this study they observed that when 2-Bromoethanesulfonic acid, BES, (a selective inhibitor of methyl-coenzyme M reductase, the enzyme which catalyzes the final step in methanogenesis) was added to a bottle in which PCE degradation was taking place the degradation and methane production ceased (6). This finding, along with other research

being performed at the time, strongly suggested that methanogens played a key role in the dechlorination of PCE.

It is however, believed that neither methanogens nor acetogens are the primary bacteria responsible for the reductive dehalogenation of PCE (1,19,23-25,27). Both the methanogens and acetogens are only able to dechlorinate the PCE to TCE and only at rates much slower than those observed in previous studies (1,14,28). DiStefano et al. (24) have also questioned the effectiveness of using BES in determining the role methanogens have on dechlorination. BES is a brominated alkane and bears structural similarity to PCE and its reduced products which makes it conceivable that it would inhibit reductive dehalogenation regardless of whether the dechlorinating organism was a methanogen (24). Finally Schink (29) determined that ethene was a potent selective inhibitor of methanogenesis, in sewage sludge and anaerobic sediments, at aqueous concentrations greater than 36μm but studies have shown that it is possible to dechlorinate PCE to ethene (6,24,25,27,30).

In a study performed by DiStefano et al. (27) high concentrations of PCE were dechlorinated to ethene in the absence of methanogenesis which further questions the role methanogens play in the dechlorination process. When the dose of PCE was increased to 55μm per bottle the methane production in the bottles essentially ceased (27). For the remainder of the experiment the PCE transformation was sustained in the absence of methanogenesis and an increase in vinyl chloride conversion to ethene was noticed (27). DiStefano et al. (27) believed that methanogenesis was inhibited by the high concentrations of PCE and/or its reduction products. They were also able to show

through microbiological studies that there was a major decrease in the number of methanol-utilizing methanogens after the PCE dose was increased in the cultures (27).

1.6.1 Isolation of Pure Cultures

With the recent isolation of two strains of bacteria that are able to utilize PCE as an electron acceptor the belief that many of the reductive dehalogenations in the environment are catalyzed by specific bacteria is strengthened. These bacteria, *Dehalobacter restrictus* and *Dehalospirillum multivorans*, are able to dechlorinate PCE stoichiometrically to *cis*-1,2-DCE and couple this reaction to growth on molecular hydrogen (1). These bacteria, as with all the presently available pure cultures that grow with a halogenated compound as an electron acceptor, are members of a new genera (19).

D. restrictus, formerly PER-K23, has a very narrow substrate range, with hydrogen and PCE as the sole electron donor/acceptor pair supporting growth (19). *D. restrictus* was isolated from an anaerobic packed-bed column in which PCE was reductively transformed to ethane (31). This anaerobic bacterium is a gram-negative rod and requires fermented yeast extract for growth (31). It is unique because it needs a chlorinated hydrocarbon, such as PCE, as an electron acceptor to grow (31). This is an interesting dependence because chlorinated ethenes have no natural origin and were not present in the environment in large concentrations until 50 years ago (31). These unique features prevented its classification until complete purification and cytochemical and molecular tests had been conducted (31).

D. multivorans is able to utilize several electron donors such as pyruvate, lactate, ethanol, formate, glycerol, and hydrogen while PCE, fumarate, and nitrate act as electron acceptors (19). By obtaining pure cultures Hollinger and Schumacher (19) were able to carry out experiments with intact and lysed cells to determine where specific dehalogenation processes take place in the cells. They were also able to show that in *D. restrictus* the hydrogenase is located on the outside and PCE reductase is located on the inside of the cytoplasmic membrane (19). These results along with results from other experiments allowed Hollinger and Schumacher (19) to present a model for the respiratory system of *D. restrictus* which has greatly enhanced ones knowledge of this process in dechlorinating bacteria.

1.7 The Future of PCE Bioremediation

As more and more research is performed the picture becomes clearer on the processes involved in the dehalogenation of PCE to ethene. With each gain of knowledge the chances in the future for the successful bioremediation of PCE contaminated sites becomes more practical. Recent advances have been made in isolating a bacterium, *Dehalococcoides ethenogenes* 195, which is able to dechlorinate chloroethenes to ethene (32). This is the first pure isolate of a bacterium that can completely reductively dechlorinate PCE to ethene and is a major advancement in this field. In another recent study two membrane bound, reductive dehalogenases that constitute a novel pathway for the complete dechlorination of PCE to ethene were partially purified from an anaerobic microbial culture containing *Dehalococcoides ethenogenes* 195 (33). This research

should provide a better understanding of the catalytic mechanisms involved in biological reductive dehalogenation. Much more research however, still needs to be performed to isolate these bacteria and to determine the types and optimum concentrations of substrates which will give maximum results in the field.

1.8 Objectives of this Study

The main objective of this study was to compare the effects different fermentation acids and alcohols would have on the reductive dehalogenation of PCE in a sediment sample taken from the Arthur Kill. Other objectives included measuring metabolism products generated in the microcosms to determine if a correlation could be drawn to the dechlorination of the PCE and also to provide insight as to what microorganisms may have dominated in these microcosms.

CHAPTER 2

LITERATURE REVIEW

2.1 Anaerobes

Obligate anaerobic microorganisms have adapted to life without the presence of O_2 and therefore must generate energy by using electron acceptors other than oxygen. Anaerobes commonly use ferric iron, nitrate, sulfate, carbonate, and organic compounds derived from the original substrate undergoing oxidation as terminal electron acceptors. Less energy is released when these electron acceptors are used instead of oxygen because of their relative position on the electron tower in respect to the highly electropositve Eo' of the O_2/H_2O couple. Even though the process of anaerobic respiration is not as efficient as aerobic respiration this hardly means that anaerobic bacteria are hard to find.

Anaerobic microorganisms can be found in bogs, water logged soils, the sediments of oceans, lakes, and rivers, and many other types of anoxic environments (34-36). Since oxygen is not very soluble in water (9.6 mg/l in distilled water at equilibrium with air at 25°C) the respiratory activities of organisms can rapidly consume the oxygen in environments, such as the ones listed above, that do not allow for the replacement of oxygen. These environments then become ideal habitats for anaerobic microorganisms because of their anoxic conditions and also because they have low reduction potentials which are produced when O_2 consuming organisms release reducing substances such as H_2 and H_2S during respiration.

The anaerobic microorganisms are able to gain energy through either fermentations or respiratory processes which involve the chemical transformations of organic or inorganic compounds (37,38). In anaerobic respiration microorganisms are able to generate energy in the form of adenosine triphosphate (ATP) which functions as the principle energy carrier of the cell. The microorganisms then use the ATP for life sustaining processes and their growth

Four major metabolic groups of bacteria are involved in the complete anaerobic degradation of organic matter (39). The first group is comprised of the hydrolytic fermentative bacteria which hydrolyze complex organic polymers (such as fats, lipids, and proteins) and then ferment the products to CO_2, volatile fatty acids and alcohols. The second and third groups are comprised of the hydrogen-producing and hydrogen-consuming acetogenic bacteria while the fourth group includes the hydrogen-using methanogenic and sulfate reducing bacteria. Of these four metabolic groups of bacteria three have been associated with the reductive dehalogenation of PCE to some extent and are described in more detail in sections 2.2-2.4.

2.2 Acetogenesis

Acetogenesis is the process whereby homoacetogenic bacteria produce acetate from CO_2 and H_2 under anaerobic conditions. The overall reaction of acetogenesis is shown in Figure 2.1. It should however, be stated that most homoacetogenic bacteria can also grow chemoorganotrophically by the fermentation of sugars and a wide variety of other organic compounds (40). Acetogens have a typical ester-linked alkyl lipids and a

peptidoglycan wall constructed from polymerized N-acetylmuramic acid and N-acetylglucosamine residues. However, the cross-linking bridge peptides contain unusual sequences (41). The acetogens also contain unusual complements of enzymes and coenzymes in which nickel, cobalt, iron, tungsten, molybdenum, selenium, and zinc are present either singly or in various combinations (41).

$$4\ H_2 + H^+ + 2\ HCO_3^- \text{------>}\ CH_3COO^- + 4\ H_2O$$

Gibbs Free Energy of -104.6 kJ/reaction

Figure 2.1 The overall reaction of acetogenesis when utilizing CO_2 and H_2

Homoacetogens include a wide variety of organisms, such as gram-positive and spore forming bacteria which has prevented them from being assigned a taxonomic group. Although they are diverse all homoacetogens convert CO_2 to acetate by the acetyl-CoA pathway and in many this pathway accounts for autotrophic growth. Since homoacetogens utilize the acetyl-CoA pathway for growth they must be able to extract energy from the process to synthesize ATP. One potential site in the pathway for such an energy yielding reaction is at the terminal step when acetyl-CoA is converted to acetate and ATP via acetyl-P (40). However, because an Na^+ gradient is established across the cytoplasmic membrane during growth of the acetogen other energy-linked steps occur (40). This Na^+ gradient is responsible for ATP synthesis through a Na^+-driven ATPase (40).

Although it has been suggested that homoacetogens are not the primary bacteria responsible for the reductive dehalogenation of PCE to ethene is has been shown that they

are able to dechlorinate PCE to TCE (1,28). DiStefano et al. (24) proposed that acetogens might serve two roles in the PCE dehalogenation process in MeOH-fed cultures. They believed that the acetogens might provide a source of reducing equivalents in the form of hydrogen and also produce unknown nutritional factors apparently required by H_2-utilizing dechlorinators (24). Other researchers have also stated that further studies are needed to determine the interaction of acetogens with other trophic groups (41). Several studies have shown that pure cultures of acetogens are able to dechlorinate dichloromethane to CO_2 (16,42). Egli et al. (16) was able to show that the bacteria used in his study that possessed the acetyl-CoA pathway were able to transform tetrachloromethane to dichloromethane and CO_2 while *Desulfobacter hydrogenophilus*, which has all the enzymes of a complete citric acid cycle, was unable to metabolize the tetrachloromethane. This is an important finding because it shows that acetogens possess the necessary systems to dehalogenate compounds although it does not prove that these same cultures could be used to dehalogenate PCE. They also observed that *Acetobacterium woodii* was able to degrade 90% of the tetrachloromethane added to the culture directly to nonchlorinated products such as CO_2 and acetate bypassing the usual formation of the less chlorinated intermediates trichloromethane and dichloromethane (16). This is important because by skipping the intermediates of the dehalogenation process one is able to avoid generating products, such as VC, which are more toxic than its predecessors.

Magli et al. (42) also determined that acetogenesis from dichloromethane was catalyzed by a single bacterium but attempts to isolate the strain in pure culture, either

with dichloromethane or with another compound as the substrate, have failed. One component of the mixed culture, named strain DMB, was identified by a 16S ribosomal DNA analysis as a *Desulfovibrio sp.* while the other component, a gram-positive organism, strain DMC, could not be isolated (42). This obligatory dependence of strain DCM on a partner during growth with dichloromethane is believed to arise from the need for a growth factor produced by the associated organism (42). This type of obligatory dependence may indeed be why a pure strain of dehalogenating bacteria has been difficult to isolate that dechlorinates PCE to ethene.

2.3 Methanogenesis

Methanogenesis is the process whereby methanogens (a major group of Archaea) produce methane. Methanogens are nutritionally diverse and display autotrophic, heterotrophic, or methylotrophic growth modes (43). Many methanogens produce methane from CO_2 and H_2 but are also able to use methanol, methylamines, and acetate to produce methane (Figure 2.2). A few methanogens are also able to grow on alcohols other than methanol such as ethanol, 1-propanol, 2-propanol, and 1-butanol producing acetate, propionate, or butyrate, and CH_4 from the reduction of CO_2 (40). In sediments, about 60% of methane comes from acetic acid and about 40% comes from hydrogen and carbon dioxide (44). Methanogens are perhaps the most strictly anaerobic bacteria known, are structurally diverse, and display no unique features by which all species can be characterized (45). Through the use of 16S ribosomal RNA sequence analysis methanogens have been classified into seven major groups containing a total of 17 genera.

Methanogens are primarily found in organotrophic ecosystems such as the rumen and gastrointestinal tract of animals, mud, sediment, marshes, landfills, flooded soil of marine and freshwater environments, and sewage sludge digestors. In the oceans methanogenesis is not as extensive as seen in freshwater and terrestrial environments due to the composition of marine waters and sediments. Many marine waters and sediments contain high levels of sulfate which are utilized by sulfate reducing bacteria which are able to out compete the methanogens for acetate and H_2. When H_2 levels get below 1-μM, as they often do in these environments, the methanogens H_2 uptake systems function less efficiently and they are easily out competed by the sulfate reducing bacteria (40). Some sulfate reducers also have over 10 times a greater affinity for acetate than methanogens which further complicates the growth of methanogens in marine sediments. To overcome this methanogens utilize methylated substrates, such as methylamines and methanol, to produce methane because these substances are poorly utilized by sulfate reducing bacteria (40).

(a) $4\ H_2 + CO_2$ ------> $CH_4 + 2\ H_2O$
Gibbs Free Energy of -130.7 kJ/reaction

(b) $4\ CH_3OH$ ------> $3\ CH_4 + CO_2 + 2\ H_2O$
Gibbs Free Energy of -319 kJ/reaction

(c) $4\ CH_3NH_3Cl + 2\ H_2O$ ------> $3\ CH_4 + CO_2 + 4\ NH_4Cl$
Gibbs Free Energy of -230 kJ/reaction

(d) $4\ HCOO^- + 4\ H^+$ ------> $CH_4 + 3\ CO_2 + 2\ H_2O$
Gibbs Free Energy of -145 kJ/reaction

Figure 2.2 The overall reaction of methanogenesis when utilizing (a) CO_2 and H_2, (b) methanol, (c) methylamines, and (d) formate.

Methanogens are able to derive energy in the form of ATP from the reduction of CO_2 to CH_4. In the terminal step (the CH_3 to CH_4 conversion) of this reduction a proton motive force is generated whose energy is captured by membrane-bound ATPases. As seen in the homoacetogenic bacteria methanogens utilize the Acetyl-CoA pathway to convert CO_2 to its organic form however, methanogens growing on $CO_2 + H_2$ integrate their biosynthetic and bioenergetic pathways because common intermediates are shared (40). The merging of these two pathways most likely allows the methanogens to save energy because the synthesis of additional enzymes is not necessary in order to make the CH_3 group of acetate for biosynthesis (40). The role by which methanogens actually produce methane is extremely complex and research is still being undertaken to understand the process in full detail (44).

Research has shown that methanogens are not likely responsible for the complete reductive dehalogenation of PCE to ethene but it still has not been conclusively determined if they play a role in mediating the reductive dehalogenation process (24,27). Fatherpure and Boyd (14) were able to isolate an aceticlastic methanogen that was able to dehalogenate PCE to TCE when growing on methanol, acetate, methylamine, and trimethylamine. The methanogen they used was isolated from a chlorophenol degrading enrichment culture and on the basis of distinct morphological and physical characteristics has been partially identified as belonging to the genus *Methanosarcina* (14). The bacterium, *Methanosarcina sp.* strain DCM, displays unique characteristics different from those of other methanogens (14). During the incubation period, methane and TCE accumulated simultaneously and when the methane production ceased no dehalogenation

was noted (14). Fatherpure and Boyd (14) concluded that there must be a linkage between CH_4 production and TCE formation and proposed that electrons transferred during methanogenesis are diverted to PCE by a reduced electron carrier involved in methane formation. They were able to show that at all levels of added electron donor, the TCE formed per millimole of CH_4 was constant at approximately 50nmol of TCE mmol of CH_4^{-1} which was used to establish a relationship between the added electron donor and the total CH_4 and TCE accumulation (14).

Vogel and McCarty (8) observed the dehalogenation of PCE to TCE, DCE, VC and carbon dioxide under methanogenic conditions using a continuous flow fixed film column. In their small column study, which had a 2-day retention time, they observed a 99.98% reduction of PCE to TCE, DCE, and VC but were unable to account for all of the PCE initially added because the concentrations of TCE, DCE, and VC were not quantified (8). In their large column study they also observed a significant dehalogenation of PCE and TCE from an initial influent concentration of about 300μg/liter to 5μg/liter at a sampling port 10-cm away from the influent port (8). After 22 days of column operation, the highest concentration of VC found was 57ug/liter which represented only 23% of the influent PCE and TCE (8). From this data they concluded that VC was also dehalogenated but they could not confirm this (8).

Belay and Daniels (46) were able to prove that methanogenic bacteria were able to produce ethene when they were exposed to DCE. This is a significant finding because it shows that methanogenic bacteria may be involved in the process of PCE dehalogenation beyond its reduction to TCE. They also determined that halogenated hydrocarbons, such

as *cis*-DCE and *trans*-DCE, were able to inhibit the methanogens growth at different concentration depending on the organism and halogenated hydrocarbon tested (46). *Trans*-DCE inhibition of *Methanobacterium thermoautotrophicum* was complete at 270-μM, but 1,081-μM only slightly inhibited *Methanococcus thermolithotrophicus* (46). This suggests that treatment of highly contaminated sites by methanogens may not be possible unless the halogenated compounds are first diluted to an acceptable concentration to support bacterial growth.

2.4 Sulfate Reduction

Most sulfate reducing bacteria are found in shallow sediments of marine environments where neither organic matter nor sulfate is limiting and play an important role in the degradation of organic matter under anaerobic conditions (47). Sulfate reducing bacteria are obligate anaerobes and utilize sulfate as an electron acceptor. Sulfate reducing bacteria display a large range of morphological and physiological characteristics and are commonly isolated from marine sediments, soil, sewage systems, animal guts, and in subsurface oil and gas deposits. Sulfate reducers are able to utilize a variety of electron donors but the most widely utilized electron donors are H_2, lactate, and pyruvate. Ethanol and other alcohols, acetate, propionate, butyrate, and long-chain fatty acids can also be utilized but have a more restricted use (48). The overall process of sulfate reduction is shown in Figure 2.3 which clearly shows that hydrogen sulfide (the end product of sulfate reduction) is excreted into the environment. This is important because HS^- has been shown to participate in many biogeochemical processes.

Several studies involving sulfate reducing bacteria and the reductive dehalogenation of PCE have been reported (3,15,17,49). Bagley and Gosset (3) observed a transformation of PCE to TCE and *cis*-1,2-DCE by sulfate reducing enrichment cultures. In all the studies they performed methane production was less than 2% of the total measured electron equivalent which indicates that there was very little methanogenic activity in the bottles (3). They were also able to measure PCE dechlorination in cultures inhibited with 50mM BES that were equivalent to the noninhibited bottles and even witnessed enhanced PCE dechlorination in the cultures which were inhibited with 3mM fluoroacetate over the non inhibited bottles (3). They also observed less PCE dechlorinating capability and slower dehalogenation rates than were seen in previous studies which were carried out under methanogenic conditions (3). These finding strongly suggest that the PCE dehalogenation in this study was not a result of methanogenic bacteria but they also do not conclusively prove that the dehalogenation was a result of sulfate reducing bacteria (3).

$$4\ H_2 + SO_4^{2-} + H^+ \text{------>} HS^- + 4\ H_2O$$

Figure 2.3 The overall process of sulfate reduction

Fatherpure et al. (15) worked with an obligate anaerobic bacterium which was able to dechlorinate PCE to TCE. This bacterium, strain DCB-1, was originally isolated from a methanogenic consortium which utilized 3-chlorobenzoic acid as its sole carbon and energy source (49). Using 0.2% pyruvate as a carbon and energy source, DCB-1

was able to dechlorinate PCE to 2.34nmol of TCE per mg of protein per day (15). In a pure culture the DCB-1 was able to degrade approximately 180nmol of PCE per 50-ml in six weeks and when grown in a methanogenic consortium the DCB-1 was able to degrade TCE at a significant rate (15). The dehalogenation of the PCE was also observed to occur at a faster rate when grown in the methanogenic consortion (15).

Stevens et al. (17) worked with the same DCB-1 strain and was able to conclude that it is a sulfidogenic bacterium. This bacterium is a very slow growing, gram-negative, non-sporeforming, obligatory anaerobic bacillus which developed an unusual morphological feature (a collar) and had an extremely restricted substrate range (49). They concluded that this bacterium was a sulfidogen because its growth was stimulated in the presence of sulfite and thiosulfate, it produced sulfide from thiosulfate, and spectral evidence indicated the presence of a c-type cytochrome and sulfite reductase (desulfoviridin)(17). They however, have not decided to name this bacterium until molecular taxonomic studies conclusively prove its relationship to other strains of bacteria(17). If strain DCB-1 is conclusively proven to be a sulfidogenic bacterium it would show that sulfate reducing bacteria have the capabilities to reductively dechlorinate PCE to a certain extent.

CHAPTER 3

MATERIALS AND EXPERIMENTAL METHODS

3.1 Chemicals

Formic acid (sodium salt, Sigma Chemical Co.), n-butyric acid (Sigma Chemical Co.), succinic acid (ACS Reagent Grade, Sigma Chemical Co.),L(+) lactic acid (98%,sodium salt, Sigma Chemical Co.), acetic acid (2.0N, Sigma Chemical Co.), ethylene glycol (99+% Spectrophotometric Grade, Aldrich Chemical Co.), methanol (HPLC Grade, Fisher Scientific 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.) were used as direct culture amendments and for the preparation of analytical standards. Isobutyric acid (99%, Sigma Chemical Co.), propionic acid (sodium salt, Sigma Chemical Co.), 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), methane (1050 ppm, by mole, balance of Helium, Scotty I Analyzed Gases), ethene (1000 ppm by mole, balance of Helium, Scotty I Analyzed Gases), and ethane (102 ppm, by mole, balance of Helium, Scotty I Analyzed Gases) were used for the preparation of analytical standards. A 3% APHA Na_2S solution (Lab Chem Inc.), and a resazurin solution (Fisher Chemical Company) were used in the preparation of the media. All water used in the experiment was 18-megaohm Milli-Q water.

3.2 Medium Preparation

The medium used to construct the microcosms was made from stock solutions. Solution A consisted of the following salts (in g/L): K_2HPO_4, 1.0; KH_2PO_4, 1.0; NaCl, 2.0; and NH_4Cl, 1.0. Solution B consisted of the following salts (in g/L): $MgSO_4$, 0.1; and $CaCl_2$, 0.1. The trace element solution consisted of (in g/L): nitriloacetic acid, 2.0; $MnSO_4.H_2O$, 1.0; $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 0.8; $CoCl_2 \cdot 6H_2O$, 0.2; $ZnSO_4 \cdot 7H_2O$, 0.2; $CuCl_2 \cdot 2H_2O$, 0.2; $NiCl_2 \cdot 6H_2O$, 0.02; $Na_2MoO_4 \cdot 2H_2O$, 0.02; Na_2SeO_3, 0.02; Na_2WO_4, 0.02. The vitamin solution consisted of (in mg/L): pyridoxine•HCL, 10.0; thiamine•HCL, 5.0; riboflaven, 5.0; calcium pantothenate, 5.0; thioctic acid, 5.0; p-aminobenzoic acid, 5.0; nicotinic acid, 5.0; vitamin B12, 5.0; biotin, 2.0; folic acid, 2.0; and mercaptoethanesulfonic acid, 10.0.

The medium was prepared as follows: in a 2-L flask 100-ml of solution A was added to 800-ml of deionized (DI) water. The solution was then maintained at 80°C while being purged at 5-psig with N_2:CO_2 mixed gas (80:20) which had been passed through a column of hot reduced copper filings to remove oxygen. After one hour, the solution was allowed to cool to room temperature (25°C) and then 100-ml of solution B was added. The solution was purged for an additional 30-minutes and then the following reagents were added: 0.60g $NaHCO_3$, 10.0-ml of the 3% Na_2S solution, 0.10-ml of the 0.10% resazurin, 1.00-ml of the trace element solution and 1.00-ml of the vitamin solution. The volume of the solution was then adjusted to one liter with DI water that had been purged with the mixed gas above for 1-hour. The flask was then sealed with parafilm and immediately transferred to the anaerobic glove box.

3.3 Sediment Slurry Preparation

Sediment was collected from the Arthur Kill in about 5 meters of water by lowering a weighted 1 meter long plastic tube (5 cm diameter) until it struck the sediment. The plastic tube was then carefully raised through the water column and then brought aboard the research vessel. The sediment was then transferred to a wide mouth Ball™ 2-quart mason jar. The jar was filled to the top with this sediment and then sealed. Back in the laboratory the sediment was stored at 4°C in the sealed wide mouth Ball™ 2-quart mason jar which was overpacked in a BBL Gas Pack™ jar. To maintain anaerobic conditions a BBL Gas Generator Envelope™, which generated CO_2 and H_2, was placed into the BBL Gas Pack™ jar. In an anaerobic glove box (which contained an atmosphere of 70% N_2, and 30% H_2) the BBL Gas Pack™ jar was opened and then a sample of the sediment from the mason jar weighing 100 grams was dispensed into a 1-L bottle and diluted to the 500-ml mark with media. This slurry was stirred manually to disperse the soil particles and incubated for 24-hours before it was used to set up the microcosms. A preliminary screening of the sediment was under taken by transferring 5-ml of the sediment slurry to a Purge and Trap tube for analysis on the Purge and Trap G.C. (see section 3.9 for use of Purge and Trap G.C.). This analysis indicated that the sediment slurry did not contain any measurable concentrations of PCE or its dehalogenation products.

3.4 Reagent Preparation

A stock solution of 712.5μM PCE was prepared in an anaerobic glove box by transferring 11-μl of PCE into a 160-ml serum bottle which contained 150-ml of DI water which had

been previously purged with a N_2:CO_2 mixed gas (80:20). A teflon-coated magnetic stirrer was added and the bottle was then immediately sealed with a teflon-coated gray butyl serum stopper and a aluminum crimp seal. The solution was stirred at room temperature on a magnetic hot plate for 24-hours before it was used in the microcosms. This PCE stock solution was freshly prepared prior to use in the microcosms.

Solutions of the electron donors were prepared separately to a final concentration of 57mM by dispensing a suitable amount of alcohol or acid into a 250-ml volumetric flask which contained 200-ml of autoclaved DI water which had been previously purged with a N_2:CO_2 mixed gas (Table 3.1). The pH was adjusted to 7.5 with a Fisher Scientific Accumet™ 10 digital pH/mVmeter using a 2.0N NaOH solution and then the volumetric flasks were diluted to the 250-ml mark with purged DI water. The volumetric flasks were transferred to an anaerobic glove box and then approximately 125-ml of the solutions were dispensed into two labeled 160-ml serum bottles. The bottles were immediately sealed with a teflon-coated gray butyl serum stopper and an aluminum crimp seal. All solutions were prepared 24-hours prior to their expected use in the microcosms.

Table 3.1 Amount of electron donor added per 250-ml of autoclaved DI water which had been previously purged with a N_2:CO_2 mixed gas

Electron donor	Amount added in grams	Volume added in ml
Lactic acid	1.5974	----------
Succinic acid	3.8489	----------
n-Butyric acid	----------	1.302
Methanol	----------	0.577
Ethanol	----------	0.836
1-Propanol	----------	1.065
1-Butanol	----------	1.304

3.5 Microcosm Preparation

All microcosms were prepared in the anaerobic glove box in 38-ml serum bottles. The microcosms which contained only one electron donor were prepared by pipetting 5-ml of the shaken sediment slurry, 2.5-ml of the 57mM electron donor, 20-ml of the media, and 1-ml of the 712.5uM PCE solution to give a total volume in the serum bottles of 28.5-ml and a total headspace volume of 9.5-ml. This gave a final nominal concentration in the liquid of 5mM for the electron donor and 25μM for the PCE. After the last addition was made the serum bottles were immediately sealed with a teflon-coated gray butyl serum stopper and an aluminum crimp seal.

The microcosms which contained two electron donors were prepared by adding 5-ml of the shaken sediment slurry, 2.5-ml of each 57mM electron donor, 17.5-ml of the media, and 1-ml of the 712.5μM PCE solution to give a total volume in the serum bottles of 28.5-ml and a total headspace volume of 9.5-ml. This gave a final concentration of 5mM for the sum of the electron donors and 25μM for the PCE. After the last addition was made the serum bottles were immediately sealed with a teflon-coated gray butyl serum stopper and an aluminum crimp seal.

Microcosms were set up for the electron donors listed in Table 3.2. Each microcosm which contained a different set of electron donors was set up in nineteen 38-ml serum bottles to allow for 6 sampling periods in triplicate with one spare bottle. All microcosms were incubated in the dark at 25°C until they were sacrificed in the sampling procedure.

Table 3.2 Format by which electron donors were added to each microcosm

Methanol
1-Butanol
Methanol/Ethanol
1-Butanol/Methanol
1-Propanol/Methanol
1-Butanol/Ethanol
Succinic acid
Lactic acid
n-Butyric acid
n-Butyric acid/Succinic acid
Succinic acid/Lactic acid
n-Butyric acid/Lactic acid

Negative controls were prepared in the anaerobic glove box by dispensing 5-ml of sediment slurry, 2.5-ml of 57mM electron donor, and 20-ml of media into 38-ml serum bottles. The serum bottles were sealed with a teflon-coated gray butyl serum stopper and an aluminum crimp seal and autoclaved for one hour at 120°C and 15 psi. The bottles were allowed to cool and then they were brought back into the anaerobic glove box and opened. One milliliter of 712.5μM PCE was added to the bottles and they were then sealed with a teflon-coated gray butyl serum stopper and a aluminum crimp seal. Negative controls were set up for the electron donors listed in Table 3.3. Each negative control which contained a different electron donor was set up in six 38-ml serum bottles to allow for 6 sampling periods. The negative control microcosms were incubated in the dark at 25°C until they were sacrificed in the sampling process.

Table 3.3 Format by which electron donors were added to the negative controls

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

3.6 Microcosm Analysis for Methane, Ethene, and Ethane

Samples of the headspace in the serum bottles were analyzed on a Varian 3600™ 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. To maintain the flame in the FID the H_2 rate was set at 30ml/min. An Alltech stainless steel column (Hayesep D, 10' long x 1/8" i.d. x 0.085" df with a 80/100 mesh) was used to separate the gases. The GC was programmed to maintain the column temperature at 50°C, the injector temperature at 100°C, and the detector at 200°C. The GC was also adjusted to have an attenuation of 8 and a range of 12 (the most sensitive setting for this machine). The above conditions provided a good separation of the gases with a analysis time of 9-minutes per sample.

The GC was connected to a computer which utilized Hewlett Packards Minichrom Chromatography Data System™ version 1.62 software. This program collected the data from the GC and stored it on the computers hard drive for retrieval when needed. This program also could be utilized to prepare calibration curves from the data acquired from

injected standard samples. Calibration curves were prepared by injecting 1-ml, 0.8-ml, 0.6-ml, 0.4-ml, and 0.2-ml of the ethene or ethane gas standard at atmospheric pressure into the GC using a 1-ml Pressure Lok™ gas tight syringe (VICI Precision Sampling Company) (see mass balance calculations in Appendix C) Calibration curves are reported in Appendix A.

For gas analysis, a 1-ml sample was removed from the headspace in the serum bottles by inserting the needle of the Pressure Lok™ gas tight syringe through the teflon-coated gray butyl serum stopper until the tip of the needle was approximately halfway between the serum stopper and the surface of the liquid. The valve on the syringe was then opened and 1-ml of gas was removed at the ambient pressure in the bottle. Approximately 15-seconds were allowed to elapse before the syringe valve was closed and the needle removed in an effort to allow the pressure in the syringe and the microcosm to reach equilibrium. The 1-ml sample still at the bottle pressure was then injected into the GC and the procedure was repeated for all subsequent samples throughout the duration of the experiment. The calculations of the concentration mass were carried out according to the procedure listed in Appendix C.

3.7 Microcosm Analysis for Alcohols

Samples were analyzed on a Hewlett Packard Series II 5890™ GC equipped with a FID. Air was the main component of the carrier gas at a pressure of 50-psi and a flow rate of 426ml/min while helium was used as the auxiliary carrier gas and maintained at a flow rate of 15.5ml/min. Hydrogen was used to maintain the flame in the FID and set at a

flow rate of 29.5ml/min. A guard column (deactivated phenyl-methyl, 5m long x 0.32 mm i.d.) was installed prior to a Restek RTX-200™ column (30m long x 0.32" i.d. x 1.0 df) which was used to separate the alcohols. The GC was programmed to hold the oven temp at 55°C for 10-minutes before it ramped at 25°C/minute to a final temperature of 150°C. This final temperature was held for 10-minutes before the program was reset. The injector temperature was maintained at 200°C, the detector temperature at 250°C, and the column flow at 31.8cm/sec (1.92ml/min) with the constant flow feature enabled. To prevent the FID flame from being extinguished from the water in the samples the GC was set up with split injection (18ml/min) at a ratio of 9.4:1. The GC was also fitted with a Hewlett Packard GC System Auto Injector™ which injected 1-ul into the GC through a Merlin Microseal™ septum. The above conditions provided good separation for the alcohols with a analysis time of 23.80-minutes per sample.

The GC was connected to a computer which utilized Hewlett Packards Minichrom Chromatography Data System™ version 1.62 software. This program collected the data from the GC and stored it on the computers hard drive for retrieval when needed. This program also could be utilized to prepare calibration curves from the data acquired from injected standard samples. Calibration curves were prepared by injecting varying concentrations of alcohol standards in triplicate (Appendix A).

For alcohol analysis, a 2-ml sample of the liquid was removed from the serum bottles using a 3cc-Becton Dickinson™ syringe fitted with a 21-gauge needle. The needle was inserted through the teflon-coated gray butyl serum stopper until the tip of the needle was approximately halfway between the bottom of the serum bottle and the top of

the liquid. The 2-ml sample was then filtered through a non-sterile 0.22um nylon syringe filter (Micron Separations Inc.) into Target DP™ Vials (National Scientific Company) and fitted with caps which contained teflon/silicone septa. The vials were then loaded into the autosampler. This procedure was repeated for all subsequent samples throughout the duration of the experiment. The calculations of the concentration mass were carried out according to the procedure listed in Appendix C.

3.8 Microcosm Analysis for Fatty Acids

Samples were analyzed on a Waters High Performance Liquid Chromatography (HPLC) equipped with a Water 484™ Tunable Absorbance Detector set to a wavelength of 210 nanometers, a Waters 600E™ System Controller, and a Waters 715™ Ultra Wisp Sample Processor. The HPLC used a 0.1% H_3PO_4 (pH 2.3) eluent set at an isocratic flow rate of 0.50ml/min which maintained a fairly constant pressure on the column of 522-psi. To prevent air bubbles from entering the column the eluent was sonicated for 30-minutes before it was used and was also continuously sparged with helium at a flow rate of 20ml/min. A Supelcogel C-610H™ carbohydrate column with a polystyrene divinylbenzene support (30cm long x 7.8mm i.d.) was used to separate the acids. The column was maintained at 30°C using a Waters Temperature Control Module and a guard column (Supelcogel C-610H™, 5.0cm long x 4.6mm i.d.) was installed prior to the main column in an effort to trap materials that would bind irreversibly to the main column. The conditions stated above provided a good separation of the acids with a analysis time of 60-minutes per sample.

The HPLC was connected to a computer which utilized Hewlett Packards Minichrom Chromatography Data System™ version 1.62 software. This program collected the data from the GC and stored it on the computers hard drive for retrieval when needed. This program also could be utilized to prepare calibration curves from the data acquired from injected standard samples. Calibration curves were prepared by injecting varying concentrations of acid standards in triplicate (Appendix A).

For the acid analysis, a 0.70-ml sample was removed from the serum bottles using a 3cc-Becton Dickinson™ syringe fitted with a 21-gauge needle. The needle was inserted through the teflon-coated gray butyl serum stopper until the tip of the needle was approximately halfway between the bottom of the serum bottle and the top of the liquid. The 0.70-ml sample was then filtered through a non-sterile 0.22um nylon syringe filter (Micron Separations Inc.) into a 0.75-ml HPLC autosampler vial (Kimble Glass Inc.). The sample was acidified by adding 10-uL of 85% H_3PO_4, capped and loaded into the autosampler which was set up to inject a volume of 200-uL. This procedure was repeated for all subsequent samples throughout the duration of the experiment. The calculations of the concentration mass were carried out according to the procedure listed in Appendix C.

3.9 Microcosm Analysis for PCE, TCE, *cis*-1,2 DCE, *trans*-1,2 DCE, and VC

For chloroethylene analysis, a 1.00-ml sample was removed from the serum bottles using a 1-ml Gastight™ Syringe (model 1001 Hamilton Co.) fitted with a 22-gauge needle (8.0 cm long). The serum bottles were vigorously shaken for approximately 30-seconds and then the needle was inserted through the teflon-coated gray butyl serum stopper until the

tip of the needle was approximately halfway between the bottom of the serum bottle and the top of the liquid. The 1-ml sample was then dispensed into a Purge and Trap tube which contained 4-ml of DI water and the tube was loaded into the Purge and Trap autosampler. This procedure was repeated for all subsequent samples throughout the duration of the experiment.

The Purge and Trap used in the experiment was a Tekmar LCS 2000™ Purge and Trap controller which was equipped with a Tekmar ALS 2016™ sixteen position autosampler. The Purge and Trap was programmed to purge the sample with helium (40.0ml/min at 20-psi) for 12-minutes and then desorb the chloroethylenes from the Tenax K adsorbent by heating the adsorbent at 250°C for 6-minutes. The desorbed chloroethylenes were then transferred through a heated transfer line and injected into the GC.

Samples were analyzed on a Varian 3400™ GC equipped with a electrolytic conductivity detector (ELCD, model 4430, OI Corporation). Helium was used as the carrier and makeup gas at flow rate of 20ml/min, the column flow rate was 10ml/min (helium), and the column pressure was set at 20-psi at 22°C. The ELCD was set up in mode P/T and used n-propanol at a flow rate of 50ul/min as its solvent. The reaction chamber was set to a temperature of 850°C with an attenuation of 1 and used hydrogen (90ml/min) as its reaction gas. A Restek RTX-624™ column (105m long x 0.53mm i.d. x 3um df) was used to separate the chloroethylenes. The GC was programmed to hold the oven temp at 35°C for 10-minutes before it ramped at 7.0°C/minute to a final temperature of 200°C. This final temperature was held for 1.5-minutes before the program was reset.

The injector temperature was maintained at 150°C, and the detector temperature at 200°C. The conditions stated above provided a good separation of the chloroethylenes with a analysis time of 35.07-minutes per sample.

The Purge and Trap GC was connected to a computer which utilized Hewlett Packards Minichrom Chromatography Data System™ version 1.62 software. This program collected the data from the GC and stored it on the computers hard drive for retrieval when needed. This program also could be utilized to prepare calibration curves from the data acquired from injected standard samples. Calibration curves were prepared by injecting varying concentrations of chloroethylene standards in triplicate (Appendix A). The calculations of the concentration mass were carried out according to the procedure listed in Appendix C.

3.10 Mass Balance Calculations

Henry's Constant is the term commonly given to the partition coefficient relating air and aqueous concentrations of a volatile substance. Values of Henry's constant are required by transport models that attempt to describe the movement of volatile pollutants in the unsaturated zone of subsurface environments and also by design and performance models of air-stripping processes for the renovation of organic contaminated water (50). These values are also necessary for the calculations in this experiment. Since a headspace volume of 9.5-ml was left in each microcosm bottle a certain amount of the chlorinated ethenes would partition into the headspace. The amount of chlorinated ethene in the headspace would eventually be expected to reach a equilibrium based on the

concentration of the chloroethene in the liquid phase and also on the temperature of the bottles. Unfortunately reliable values of Henry's constant are often not available for solutes of environmental concern (50).

Gosset (50) however, was able to measure Henry's constant for 13 volatile organic compounds by using a modification in the Equilibrium Partitioning in Closed Systems, (EPICS), procedure. The EPICS procedure is based on the addition of equal masses of a volatile solute to two sealed serum bottles that are identical in all respects except they possess different solvent (water) volumes (50). The resulting ratio of the two headspace concentrations is used to compute Henry's constant. This method is capable of producing values of Henry's constant with a precision (coefficient of variation, CV) averaging 4-5% for compounds with "dimensionless" Henry's constants, Hc [(mol/L gas concentration) / (mol/L aqueous concentration)], between 0.06 and 0.9---the full range previously evaluated (50). The limiting precision with the EPICS procedure was said to be associated with the attempted delivery of equal solute masses to the bottle pairs (50).

Gosset (50) modified the EPICS procedure by eliminating the assumption of equal solute masses in the individual bottles comprising the original EPICS system. Instead, differences in mass due to imperfect, volumetric additions are accounted for through gravimetric means. If one attempts to inject equal volumes of a stock solution to two bottles, gravimetric analysis of the stock masses injected (via weighing of a syringe or bottle just before and just after injection) will, in general, detect differences (50). Gosset was able to determine the mean CV in Henry's constant was 3-4% as compared to the

original EPICS procedures 4-5%. Henry's constants used in this experiment are listed in Table 3.4 and were obtained at a temperature of 24.8°C.

Table 3.4 Henry's constants (measured at 24.8°C) used in this experiment to calculate the mass of chlorinated ethenes in the 9.5-ml headspace of the microcosm bottles

Chlorinated Ethene	PCE	TCE	*trans*-1,2 DCE	*cis*-1,2 DCE	VC
Henry's Constant (H_c)	0.723	0.392	0.384	0.167	1.137

In order for the above constants to be used in this study one has to first agree that the values determined by Gosset are indeed true. Some reported values of VC are at least an order of magnitude higher than the measured Henry's constant in his study. Second one would have to assume that the conditions in the microcosms in this experiment followed the conditions in the bottles set up by Gosset. Due to the lack of any substantial evidence against Gosset's results the first assumption was made. The second assumption was made because the temperature dependence of Henry's constant has been well modeled with the classical, van't Hoff equation for temperature's effect on an equilibrium constant (50).

In this experiment the concentration of chlorinated ethenes in a 1-ml sample were measured using a Purge and Trap GC as described in section 3.9. This GC reading, in mM, was was then multiplied by 0.0285 L, which was the volume of the liquid in the microcosms, to obtain the mass of the chlorinated ethenes in the bottle for the *liquid phase*. To determine the concentration of a particular chlorinated ethene in the *gas phase*

the GC reading in mM for that particular chlorinated ethene was multiplied by Henry's constant listed in Table 3.4. This value was then multiplied by 0.0105 L, which is the volume of the headspace (0.0095 L) plus the volume of the sample removed for testing (0.001L), to obtain the mass of that particular chlorinated ethene in the headspace. Adding the total mass obtained for the chlorinated ethene in the liquid phase to the total mass obtained in the gas phase provides the total mass of that particulr chlorinated ethene in the bottle. This procedure was repeated for each chlorinated ethene detected by the GC.

Ethene and ethane were both detected in the headspace by the procedure outlined in section 3.6. No attempt was made to determine the concentration of these compounds in the liquid phase because past research (6) has shown that ethene and ethane are sparingly soluble in water. Also there is no reliable Henry's constant currently available for ethene and ethane (50).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 The Effect of Adding Alcohols as Electron Donors

During the course of the experiment PCE and/or its dechlorination products were detected in all the microcosms. However, the rate at which the dehalogenation proceeded and the concentration of intermediates formed varied considerably among the different electron donors (Tables C1,C5,C13,C15-C17 Figures 4.1, 4.2, B5-B8). Each point in these figures represents the average of three measurements from the three bottles sacrificed at each sampling period. The average starting PCE level for these microcosms was 0.454 ±0.082 μmol/bottle which represented only 64% of the initial target level of 0.7125 μmol/bottle. These values suggest that either the PCE was rapidly (and permanently) bound to the sediment in the microcosms (there was a 2-h period between the PCE addition and sampling at t = 0) or that the starting values for the microcosms were those measured at t = 0. This second scenario is more likely correct because when the PCE standard solution was prepared a plastic pipette tip was used to transfer the 11-ul of PCE into the serum bottle. A subsequent experiment revealed that when a plastic pipette tip was used to make a standard solution of PCE under the identical conditions the standard solution only had an initial concentration of 338.0 ± 6.04 μM as opposed to 712.5 μM.

The mass balance for the chlorinated ethenes for the final sampling period in all the bottles was between 28 and 86% (average of 48.35% ± 22.21%) when compared

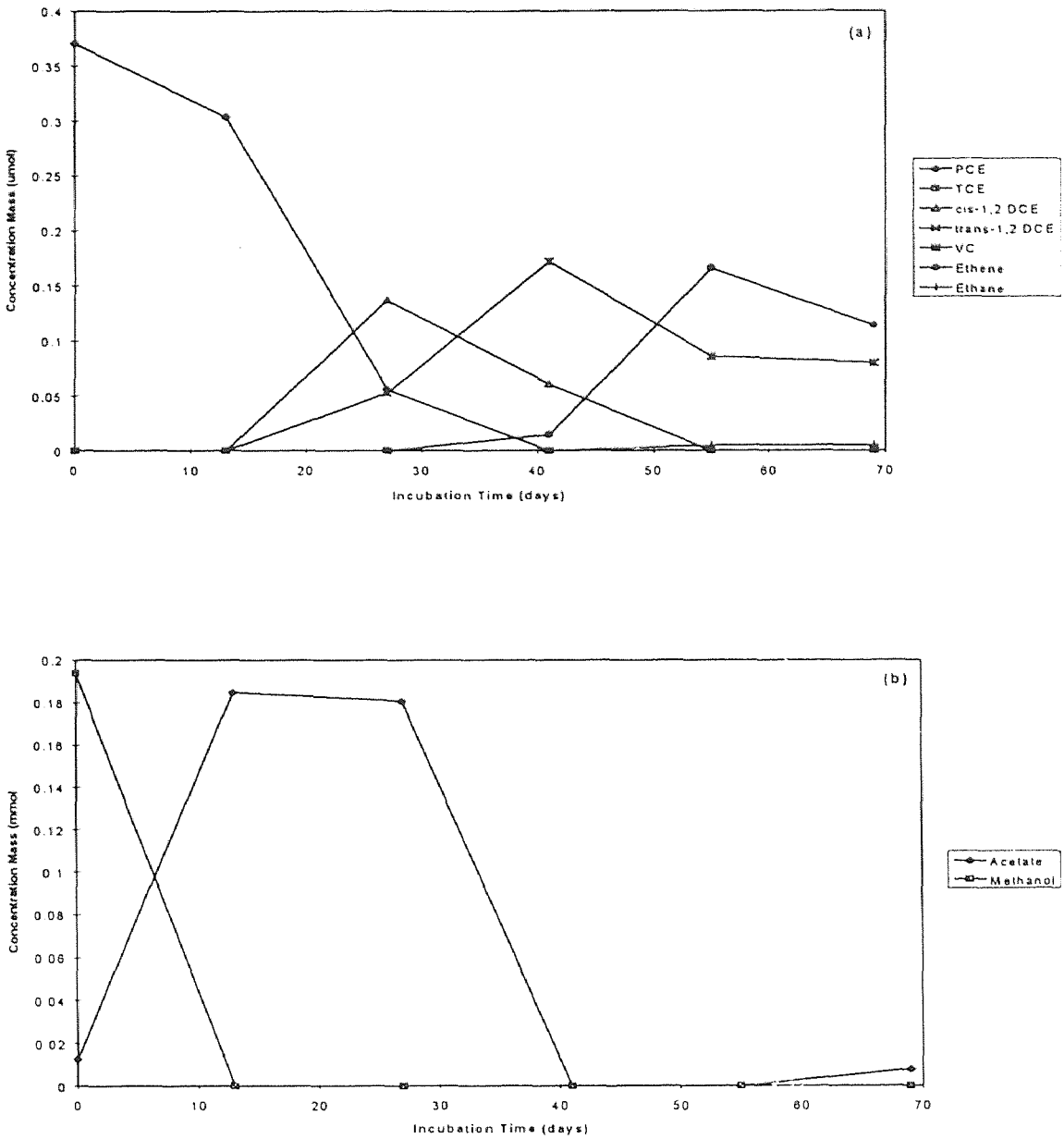


Figure 4.1 Concentration of chloroethenes (a) and electron donors (b) as a function of time for the microcosm supplemented with methanol

against the initial target level of 0.7125 μmol/bottle and only improved to an average of 57.41% ± 16.95% when compared against the starting concentration measured at t = 0. Similar low mass balances were noticed in the negative-control bottles. The mass balance for the chlorinated ethenes for the final sampling period in all the negative-control bottles was between 33.9 and 64% (average of 53.266 ± 16.79%) when compared against the starting concentration measured at t = 0 days.

The average loss of almost 50% of the initial PCE added to these negative-control bottles without the presence of dehalogenation products can be explained by three theories. First one can conclude that the PCE was absorbed by the soil over the period of the experiment and therefore was not detected by the Purge and Trap. The second explanation is that the PCE slowly leaked out from underneath the teflon serum bottle stoppers. This theory is harder to believe because if the PCE leaked out then the bottles which showed activity (and therefore produced a greater pressure in the headspace from methane production) should have had a lower mass balance than witnessed in the negative controls. The lower mass balance however, was not noticed and in fact the overall mass balance for the active microcosms was 48.35 ± 22.21% which is very close to the average mass balance of 53.266 ± 16.79% detected in the negative-controls. The third explanation is that there was a systematic sampling error which accounted for the low mass balance. This theory is also hard to believe because the method used to sample these microcosms had been used before without any significant loss in mass balance.

The variability between the individual bottles showed no pattern between a particular chlorinated ethene and a large standard deviation (Tables E 1, E3, E9-E12).

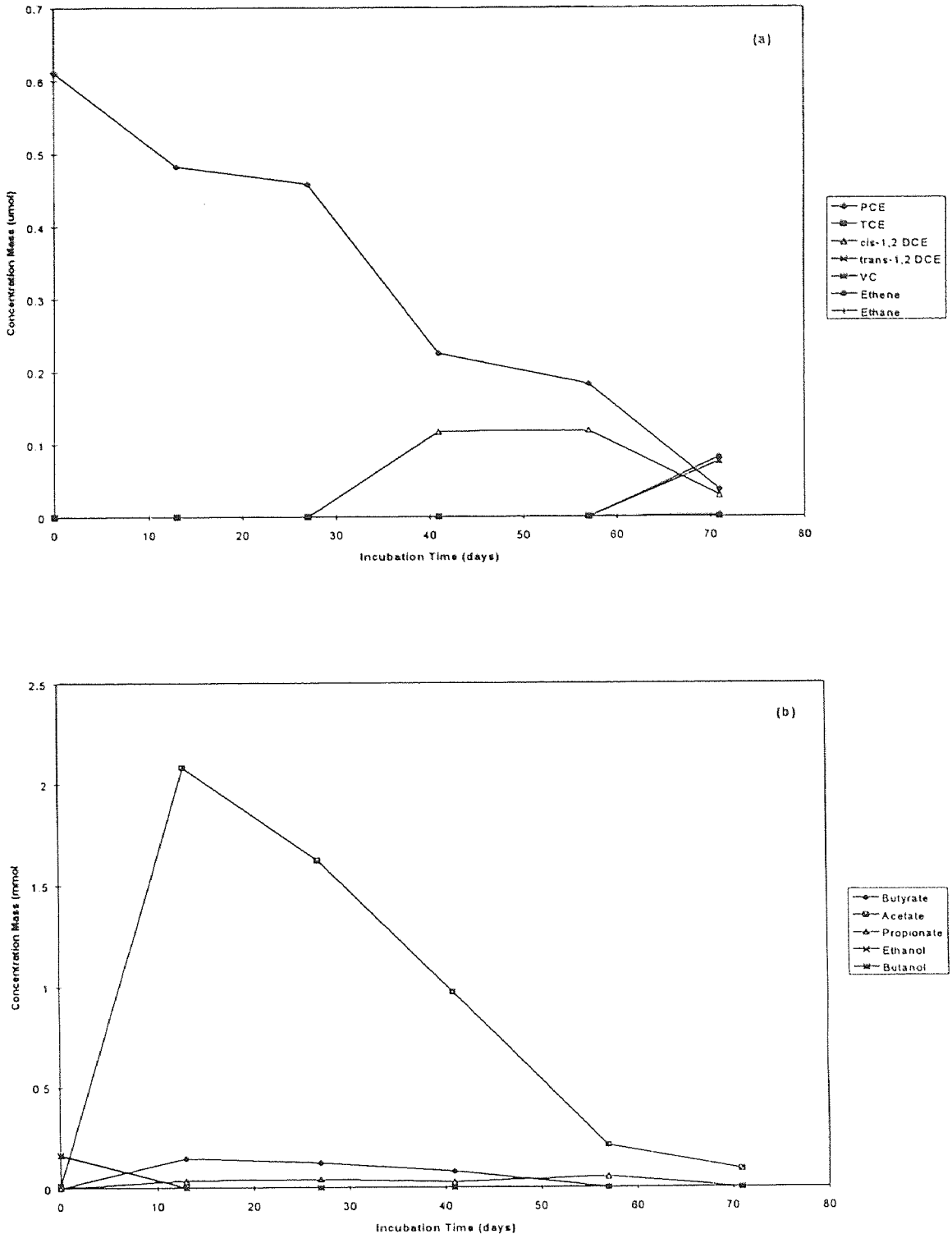


Figure 4.2 Concentration of chloroethenes (a) and electron donors (b) as a function of time in the microcosm supplemented with a mixture of butanol/ethanol

The sampling points for which the chlorinated ethenes were detected in all three bottles produced standard deviations which were usually small compared to the average concentration. The largest deviations occurred when a particular chlorinated ethene was only present in one or two of the bottles. No particular PCE dechlorination product was consistently missing in only one or two of the bottles. The individual microcosms seemed to have displayed a true randomness which is common among mixed cultures.

The primary dehalogenation product for the microcosms supplemented with butanol and methanol/ethanol was VC at final values of 0.353 and 0.18 μm/bottle respectively. The rest of the PCE was either dehalogenated to *cis*-1,2 DCE, ethene, ethane, or unaccounted for. Ethene was the primary dehalogenation product for the microcosms supplemented with propanol/methanol, methanol, butanol/ethanol, and butanol/methanol.

The microcosm supplemented with butanol/methanol was the only microcosm which contained no VC at the end of the experiment and the microcosm supplemented with butanol/ethanol was the only one to still have PCE at the final sampling point. The lag time before the onset of PCE dehalogenation in the microcosms varied between 27 and 41 days before cis-1,2 DCE was produced. TCE and *trans*-1,2 DCE were measured only in a few of the microcosms in small amounts.

In all of the microcosms the added electron donor was completely degraded by t = 28 which was well before any PCE dehalogenation took place (Figures 4.1, 4.2, B5-B8). All of the microcosms produced acetate and the microcosms supplemented with butanol/ethanol and propanol/methanol produced propionate.

The dehalogenation of the PCE could only be accounted for from bacterial action as the autoclaved negative-controls only contained PCE at the final sampling time (Tables C2, C14, C18, C19 Figures D1-D4).

It has been suggested that since butyrate and propionate are slowly fermentable and produce low levels of H_2 a selective advantage would be afforded to the dechlorinating microorganisms over the methanogens (23). If this is true then the microcosm supplemented with butanol/ethanol should have displayed a greater reductive dechlorination than the other microcosms since the butanol was rapidly converted to its acid equivalent butyrate and the ethanol was rapidly converted to propionate. This however, was far from what was noticed under the conditions in this experiment. These microcosms showed the least ability to dechlorinate PCE and actually had 0.138 um of PCE in the bottles at t = 57.

Methanol has also been shown to produce some hydrogen under certain circumstances (50) and therefore should afford a selective advantage to the dechlorinators (23). In this experiment the microcosms supplemented with methanol were able to dechlorinate PCE to VC, ethene, and ethane and in fact appears to be the best alcohol substrate. When butanol was used as the sole electron donor the microcosm was only able to dechlorinate the PCE to VC and trace amounts of ethene and ethane but in the presence of methanol the microcosm was able to fully dechlorinate the PCE to ethene and ethane.

Freedman and Gosset (6) also determined that methanol was the most efficient electron donor in their studies in terms of both maintaining the rate at which repetitive

additions of PCE and TCE were degraded and also the extent to which VC was converted to ethene. DiStefano et al. (24) were also able to show that cultures amended with methanol were able to dechlorinate 550μM PCE to 80% ethene and 20% VC within 2 days. However, Gibson and Sewell (21) reported that cultures amended with methanol were not able to support dehalogenation and even suggested that a substrate that produces more H_2 during anaerobic metabolism be selected instead of methanol.

4.2 The Effect of Adding Acids as Electron Donors

During the course of the experiment PCE dechlorination products were detected in all the microcosms. However, the rate at which the dehalogenation proceeded and the intermediates formed varied considerably between the different electron donors (Tables C3, C6, C7, C9, C11, C12 Figures 4.3, 4.4, B1-B4). Each point in these figures represents the average of three measurements from the three bottles sacrificed at each sampling period. The average starting PCE level for these microcosms was 0.409 ±0.080 μmol/bottle which represented only 57% of the initial target level of 0.7125 μmol/bottle. These values suggest that either the PCE was rapidly (and permanently) bound to the sediment in the microcosms (there was a 2-h period between the PCE addition and sampling at t = 0) or that the starting values for the microcosms were those measured at t = 0 days. This second scenario is more likely the case as was discussed above in section 4.1.

The mass balance for the chlorinated ethenes for the final sampling period in all the bottles was between 8 and 42% (average of 30.18% ± 11.75%) when compared

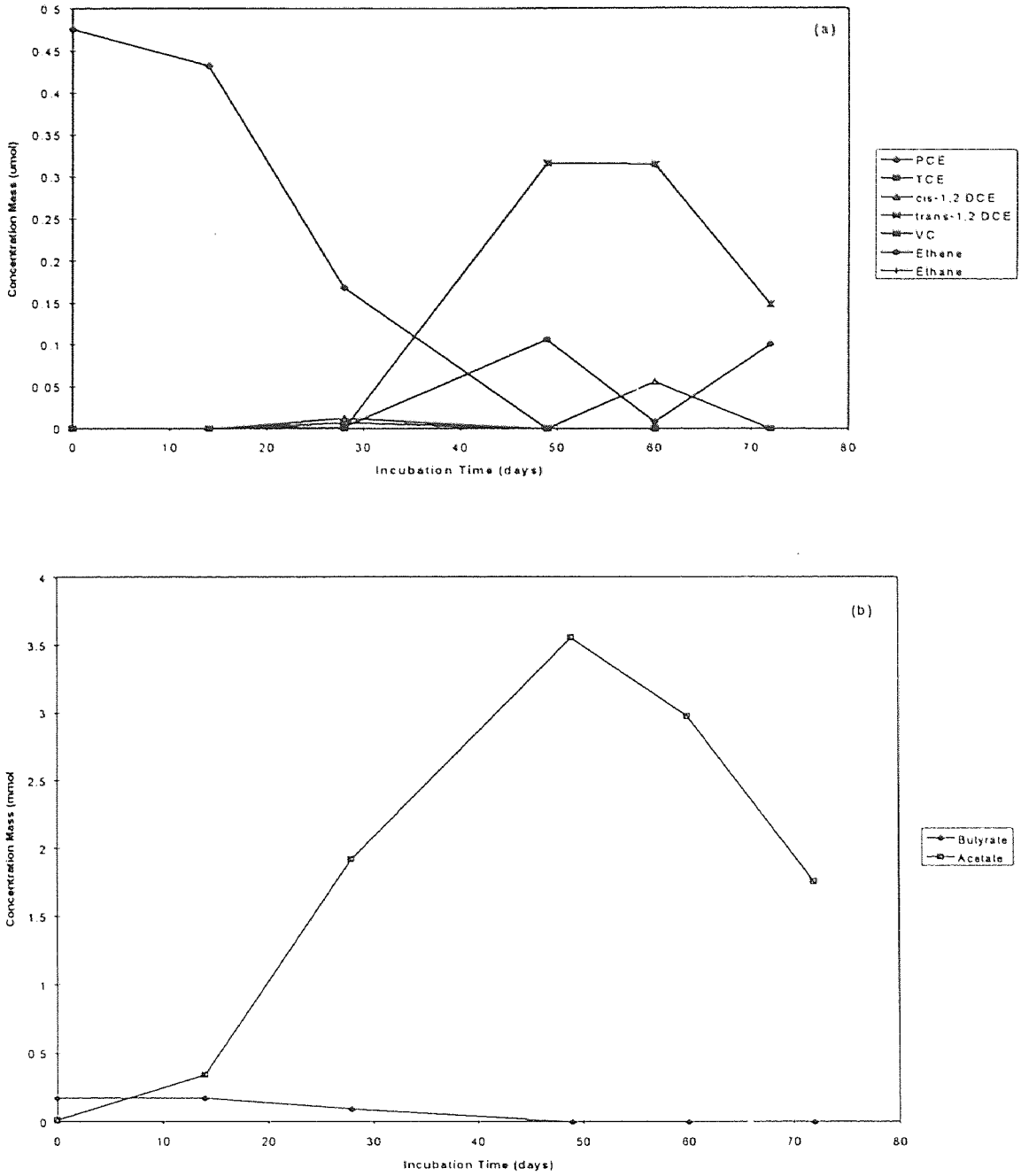


Figure 4.3 Concentration of chloroethenes (a) and electron donors (b) as a function of time in the microcosm supplemented with butyrate

against the initial target level of 0.7125 μmol/bottle and improved to an average of 54.93% ± 22.63% when compared against the the starting concentration measured at t =0. Similar low mass balances were noticed in the negative-control bottles. The mass balance for the chlorinated ethenes for the final sampling period in all the negative-control bottles was between 33.9 and 64% (average of 53.266 ± 16.79%) when compared against the starting concentration measured at t = 0 days. The average loss of almost 50% of the initial PCE added to these negative-control bottles without the presence of dehalogenation products can be explained by the three theories listed in section 4.1.

The variability between the individual bottles showed no pattern between a particular chlorinated ethene and a large standard deviation (Tables E2, E4-E8). The sampling points for which the chlorinated ethenes were detected in all three bottles produced standard deviations which were usually small compared to the average concentration. The largest deviations occurred when a particular chlorinated ethene was only present in one or two of the bottles. No particular PCE dechlorination product was consistently missing in only one or two of the bottles. The individual microcosms seemed to have displayed a true randomness which is common among mixed cultures.

The primary dehalogenation product in all the microcosms, except for the one supplemented with succinate/lactate, was VC at a final value of 0.144 ± 0.066 μm/bottle. The rest of the PCE was either dehalogenated to *cis*-1,2 DCE, ethene, ethane, or unaccounted for. Small amounts of TCE and *trans*-1,2 DCE were also measured but only in a few of the microcosms. The primary dehalogenation product for the microcosm supplemented with succinate/lactate was ethene at final value of 0.185 μm/bottle.

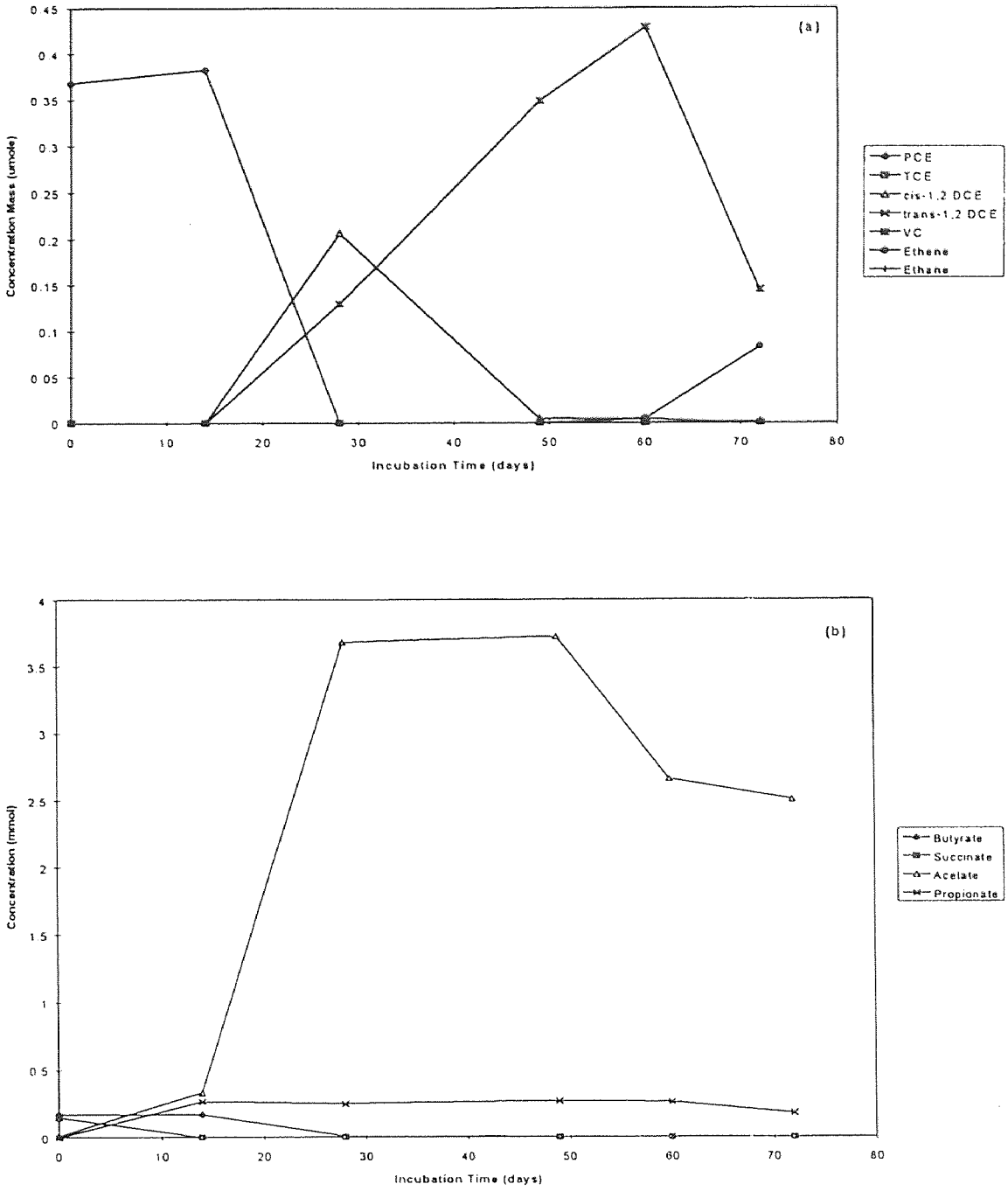


Figure 4.4 Concentration of chloroethenes (a) and electron donors (b) as a function of time for the microcosm supplemented with butyrate/succinate

The microcosm supplemented with a mixture of butyate/succinate produced the greatest concentration of VC (0.429 μM at t = 60 days) and also had the greatest concentration of VC left at the end of the experiment (0.145 μM at t = 72 days). The microcosm supplemented with succinate produced the least VC never exceeding 0.24 μM at any sampling point during the experiment. The lag time before the onset of PCE dehalogenation in the microcosms varied between 15 days, for the microcosm supplemented with lactate, and 49 days for the microcosm supplemented with a butyrate /lactate before *cis*-1,2 DCE was produced.

In all of the microcosms, except for those supplemented with butyate, the added electron donor was completely degraded by t =14 days which was well before any appreciable PCE dehalogenation took place (Tables C3, C6, C7, C9, C11, C12 Figures 4.3, 4.4, B1-B4). In the microcosms supplemented with butyate (Figures 4.3 and 4.4) the PCE and the butyate were both degraded over the same sampling period. In all of the microcosms acetate was produced and in the microcosms supplemented with succinate and lactate, propionate was produced. As previously stated Fennel et al. (23) theorized that since butyrate and propionate are slowly fermentable and produce low levels of H_2 a selective advantage would be afforded to the dechlorinating microorganisms over the methanogens. If this is true then any microcosm supplemented with butyrate or lactate (which was degraded to propionate in these experiments) should have displayed a greater reductive dechlorination than the other microcosms.

The microcosms supplemented with lactate in this experiment were able to reduce the PCE to trace amounts of VC, ethene, and ethane but the final mass balance

was so low (12.35%) that it is hard to draw any conclusions. The microcosms that were supplemented with either butryate or lactate however did produce 56% less VC in the final sampling period than the microcosms supplemented with succinate alone (0.1335 μm/bottle as compared to 0.240 μm/bottle for succinate). It is still hard to draw any definitive conclusions because only one of the microcosms contained an electron donor other than lactate or butyrate.

The microcosms supplemented with lactate had the smallest dehalogenation lag time of all the acid substrates tested. This microcosm dechlorinated 49% of the added PCE by t =15 and by t = 32 all of the PCE had been degraded to *cis*-1,2 DCE, *trans*1,2-DCE, VC, and ethene. Gibson and Sewell (21) also noticed that the microcosms to which lactate were added had a shorter lag time than the microcosms which were amended with butyrate, or propionate. In their experiment TCE was produced by day six while in this experiment TCE was produced by the second sampling period which corresponded to day 15.

The dehalogenation of the PCE could only be accounted for from bacterial action as the autoclaved negative-controls only contained PCE at the final sampling time (Tables C4, C8, C10 Figures D5-D7).

CHAPTER 5

CONCLUSIONS

Of all the different combinations of electron donors tested in this experiment only the combination of butanol/methanol was able to fully reduce the PCE to ethene. In this case however, only 60% of the starting PCE value could be accounted for as ethene. The remainder was either absorbed onto the soil or lost. Methanol appears to be the best electron donor because when butanol was used alone the microcosm was only able to dechlorinate the PCE to VC and a trace amount of ethene. When methanol was used as the sole electron donor the microcosm was able to dechlorinate the PCE to ethene as the major dehalogention product.

A definitive conclusion on which acid served as the best electron acceptor is harder to draw. Each microcosm to which an acid was added as the electron donor was able to dechlorinate the PCE to VC and ethene. VC was the major dehalogenation product in all the microcosms except in the microcosm which was amended with succinate/lactate. However, this microcosm still contained 0.116 um/bottle which accounted for 27% of the added PCE.

The lack of any correlation between the added electron donors and their metabolic products with the reductive dechlorination process suggests that H_2 may indeed be the electron donor in the process. This would mean that the added electron donors mearly serve as substrate which is used by microorganisms in fermentative metabolism. This fermentative metabolism would then generate a hydrogen pool which could be utilized by

the microorganisms involved in the reductive dechlorination process. This theory has been suggested before (23-25) and much more research needs to be under taken to indeed prove if this is true for this experiment.

More research should also be undertaken to determine if the low mass balances in this experiment were a result of the PCE irreversibly binding to the soil or experimental error. In this experiment the sediment slurry was added as a 5-ml volume so it was impossible to determine exactly how much soil was actually in each microcosm. Future experiments should involve adding a carefully measured amount of soil to negative-control bottles to determine the fate of the PCE.

Future studies could also be set up in which inoculum taken from the butanol/methanol microcosm was used to set up a flow through reactor as discussed by Vogel and McCarty (8) to determine the rate at which the organisms degrade PCE. This type of flow through reactor holds the best promise for remediating sites contaminated with PCE because the conditions can be controlled more easily than just pumping a determined amount of electron donor into a PCE contaminated site.

Finally it might be interesting to try to isolate the bacterium or bacteria responsible for the reductive dechlorination process and to determine the role mixed cultures (methanogens, acetogens, sulfate reducers, etc.) have on the reductive dechlorination process.

APPENDIX A

CALIBRATION CURVES

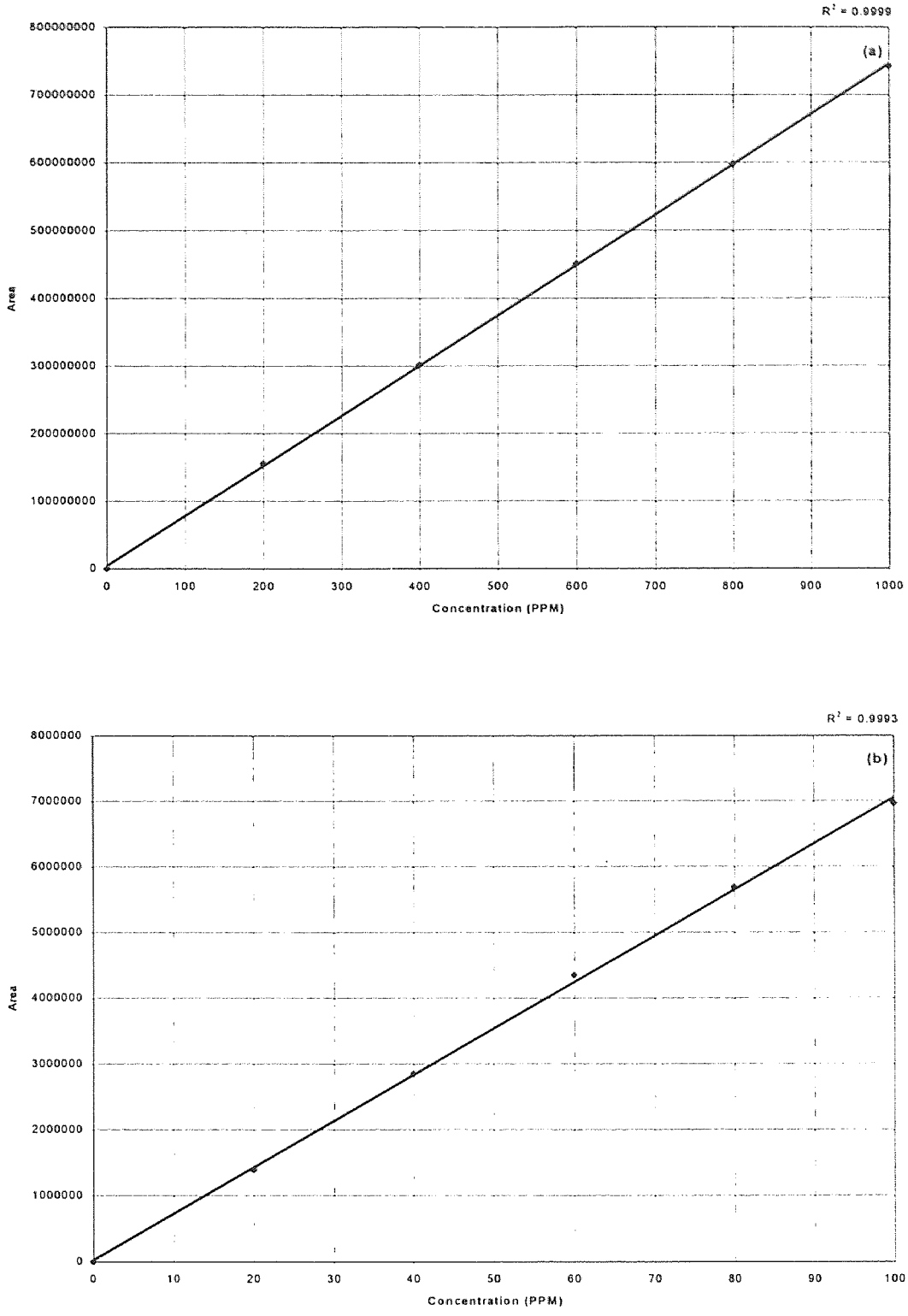


Figure A1 Calibration curves for Ethene (a) and Ethane (b)

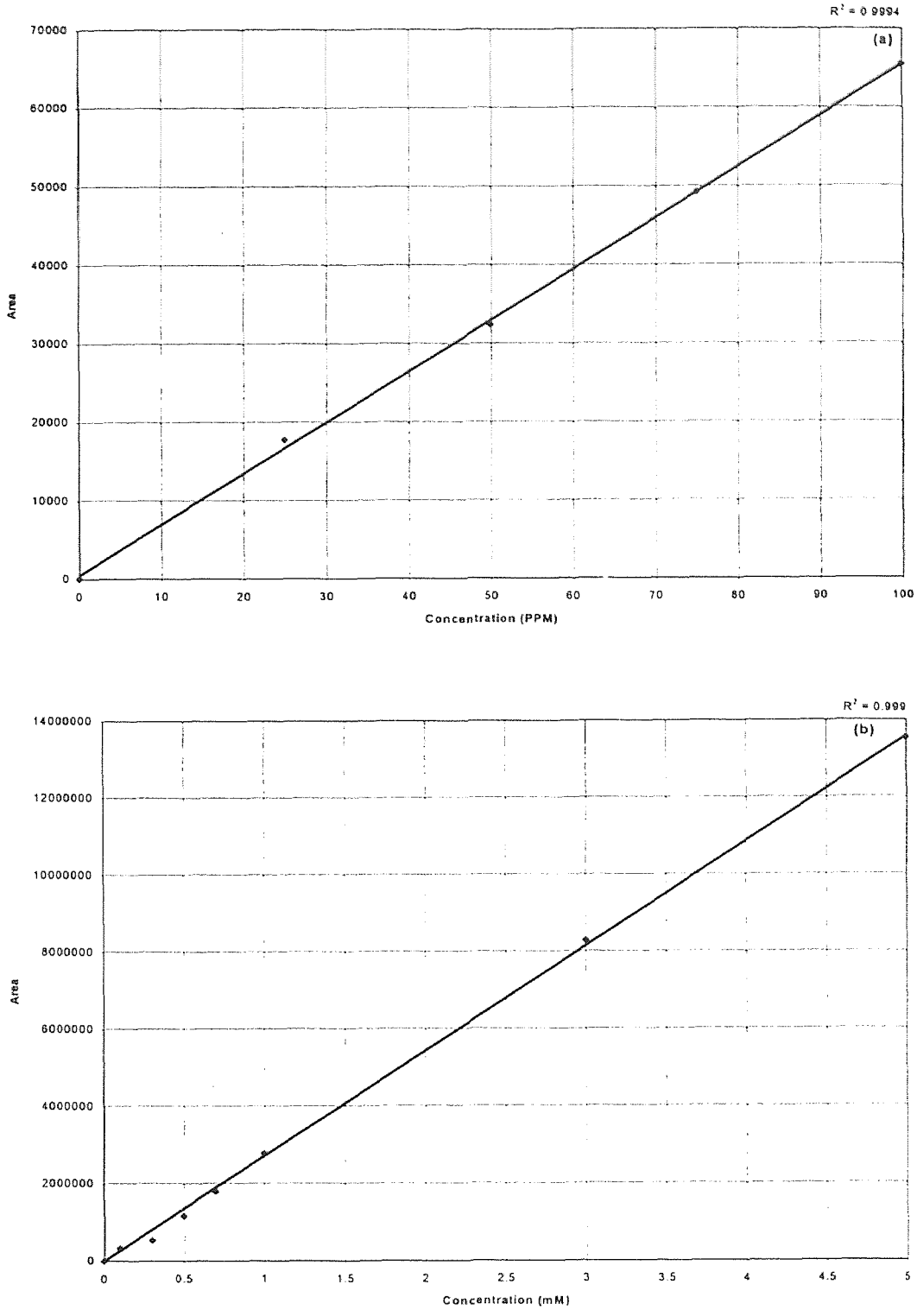


Figure A2 Calibration curves for Methane (a) and Succinate (b)

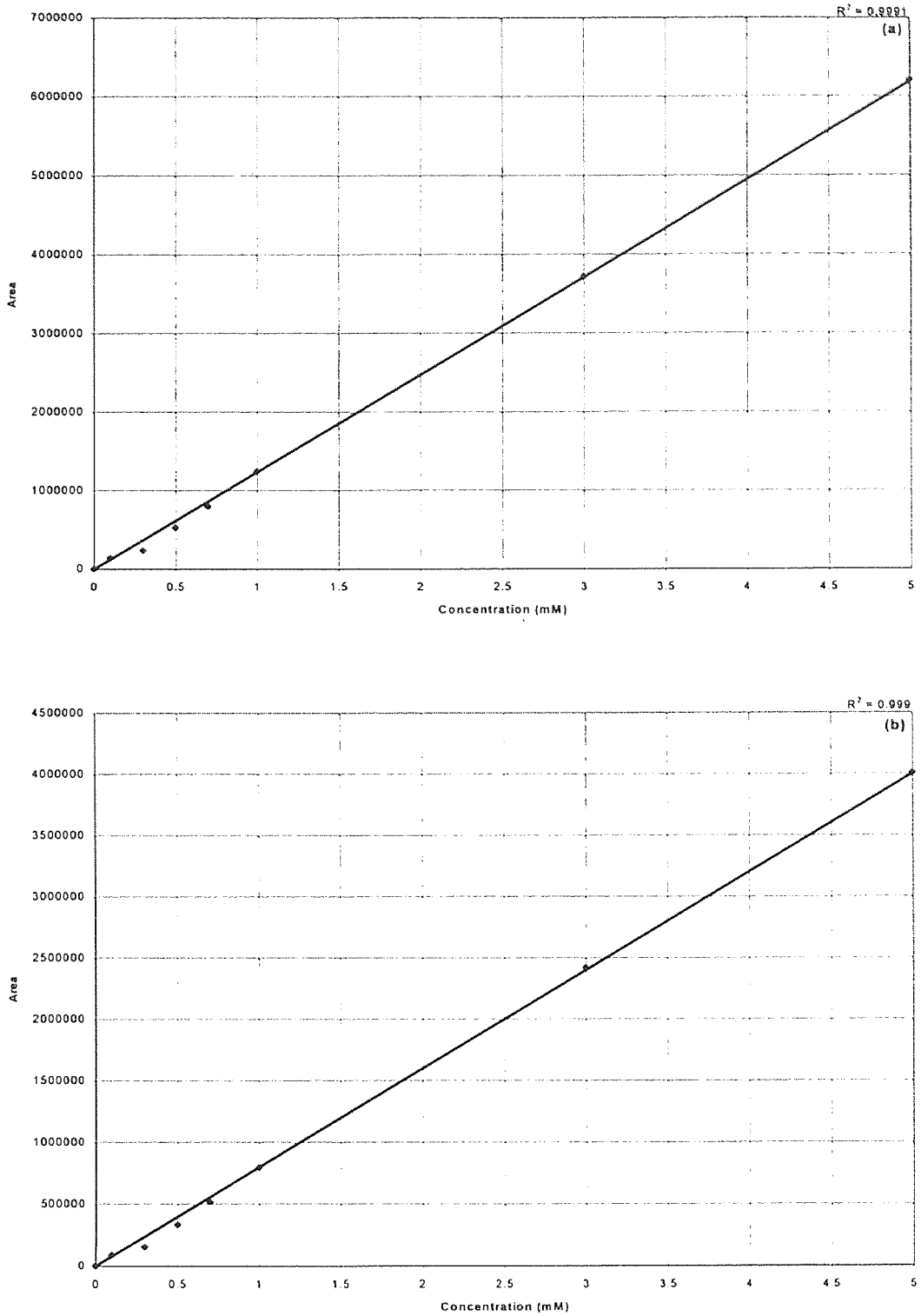


Figure A3 Calibration curves for Lactate (a) and Formate (b)

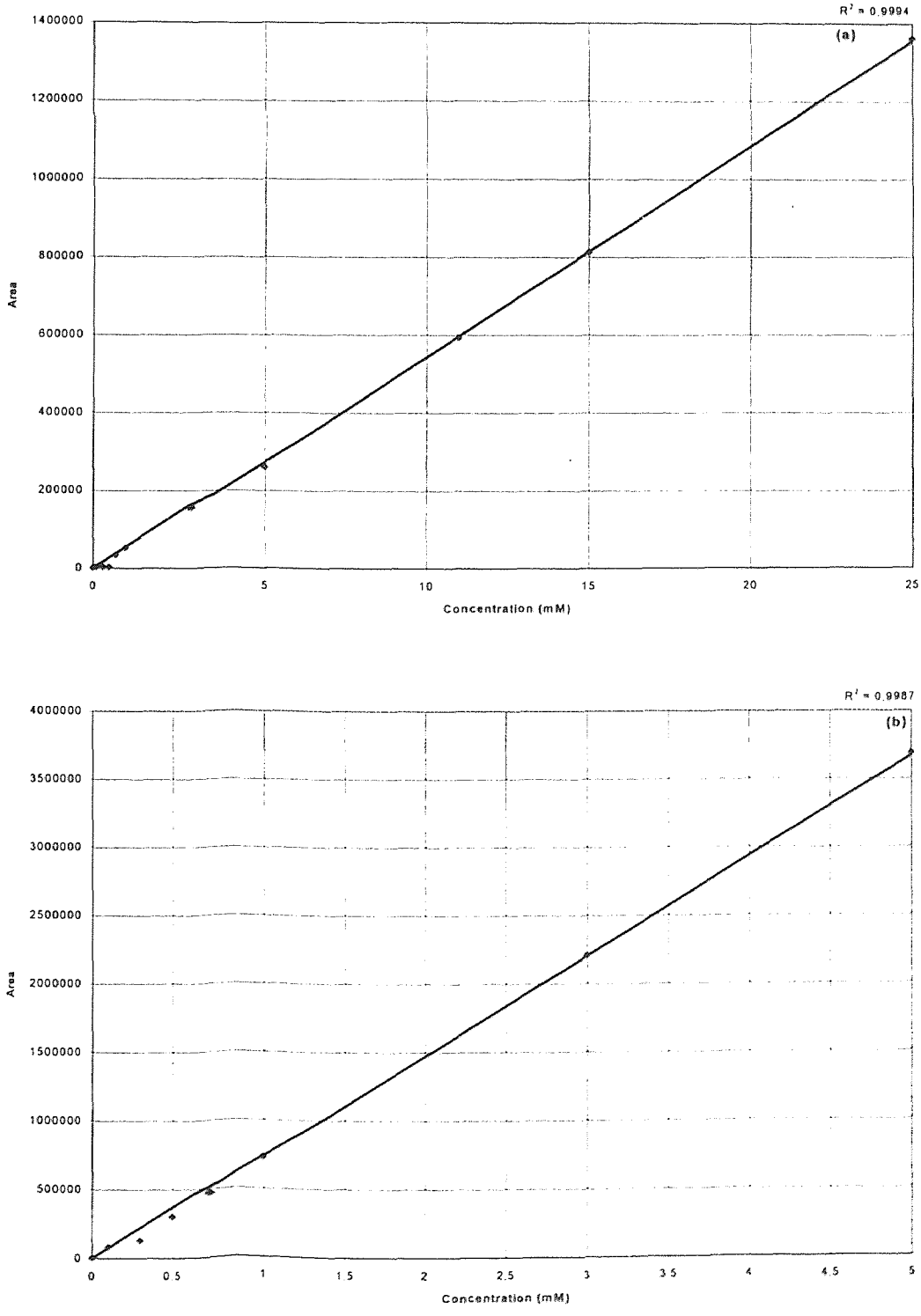


Figure A4 Calibration curves for Acetate (a) and Butyrate (b)

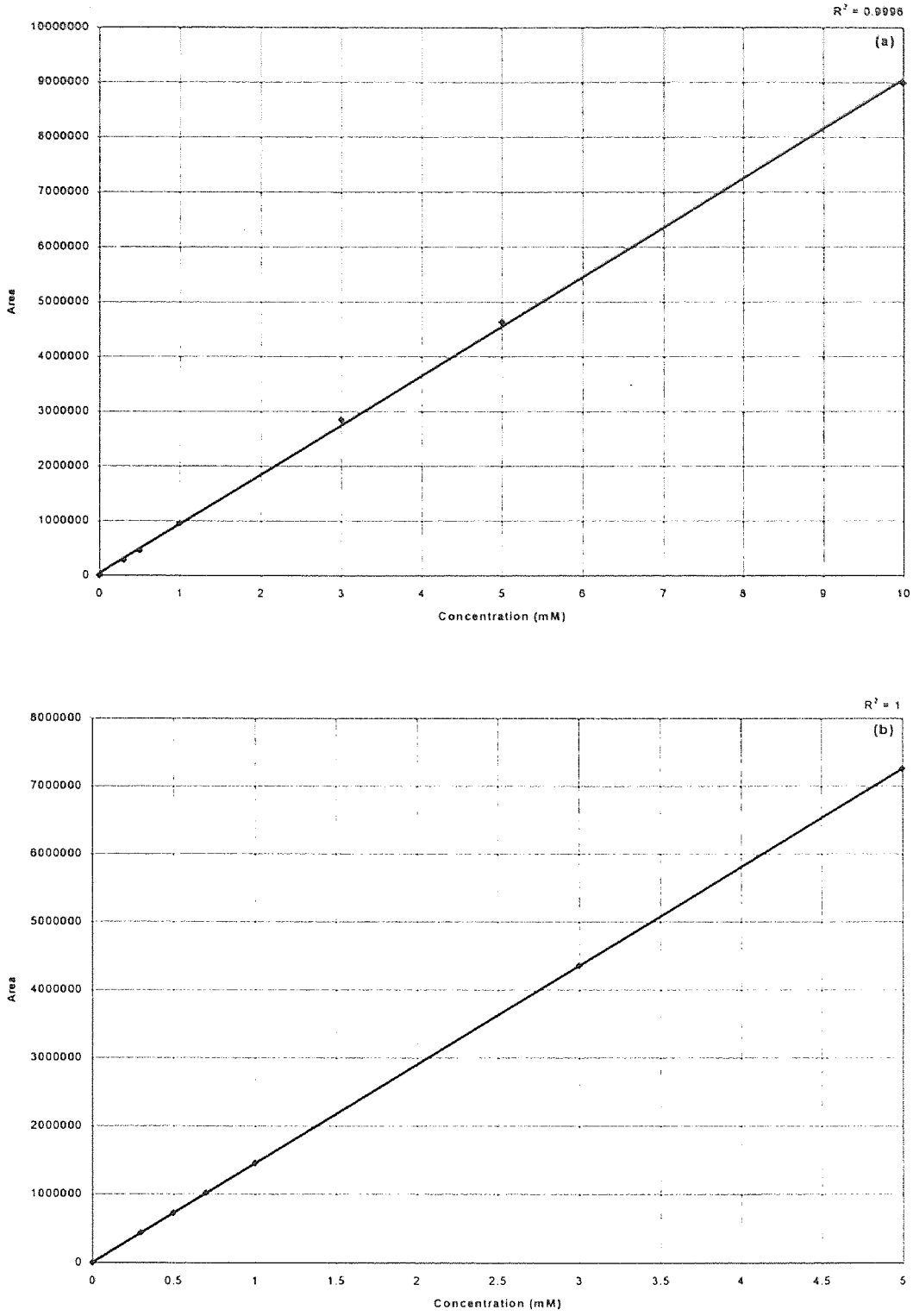


Figure A5 Calibration curves for Propionate (a) and Isobutyrate (b)

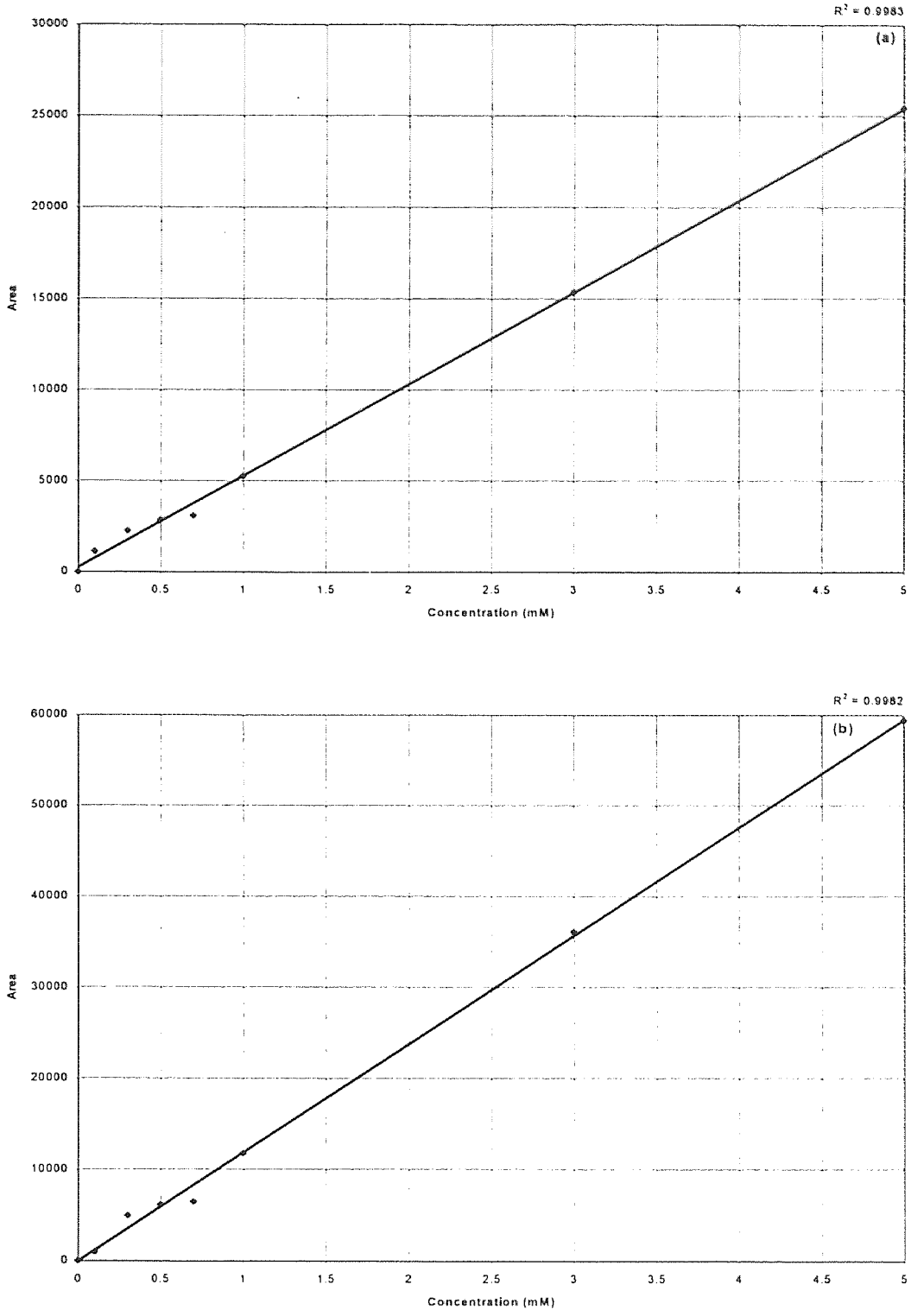


Figure A6 Calibration curves for Methanol (a) and Ethanol (b)

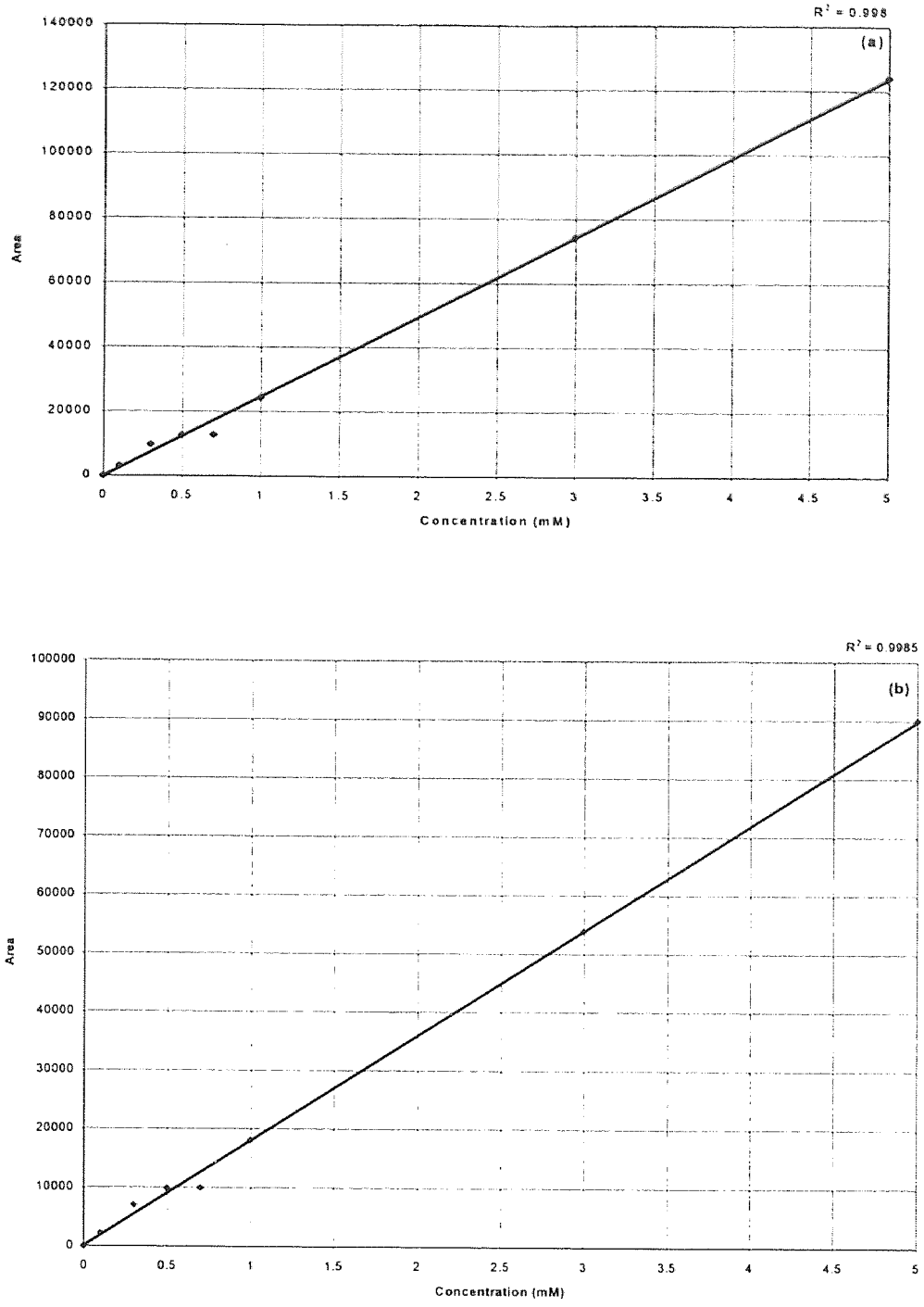
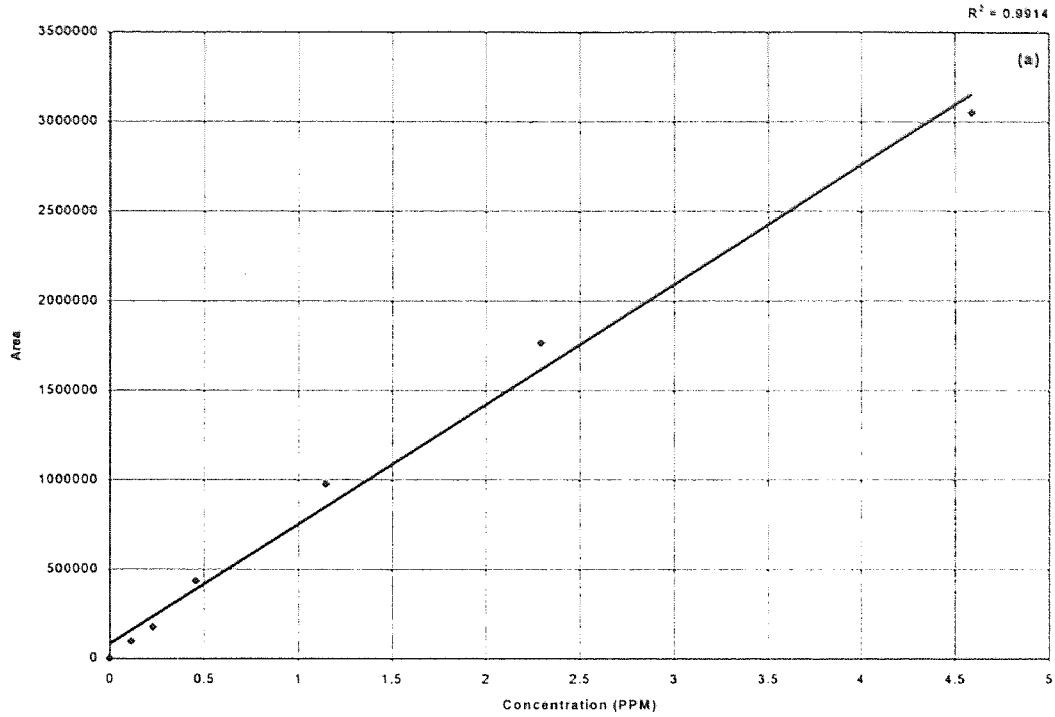


Figure A7 Calibration curves for Butanol (a) and Propanol (b)



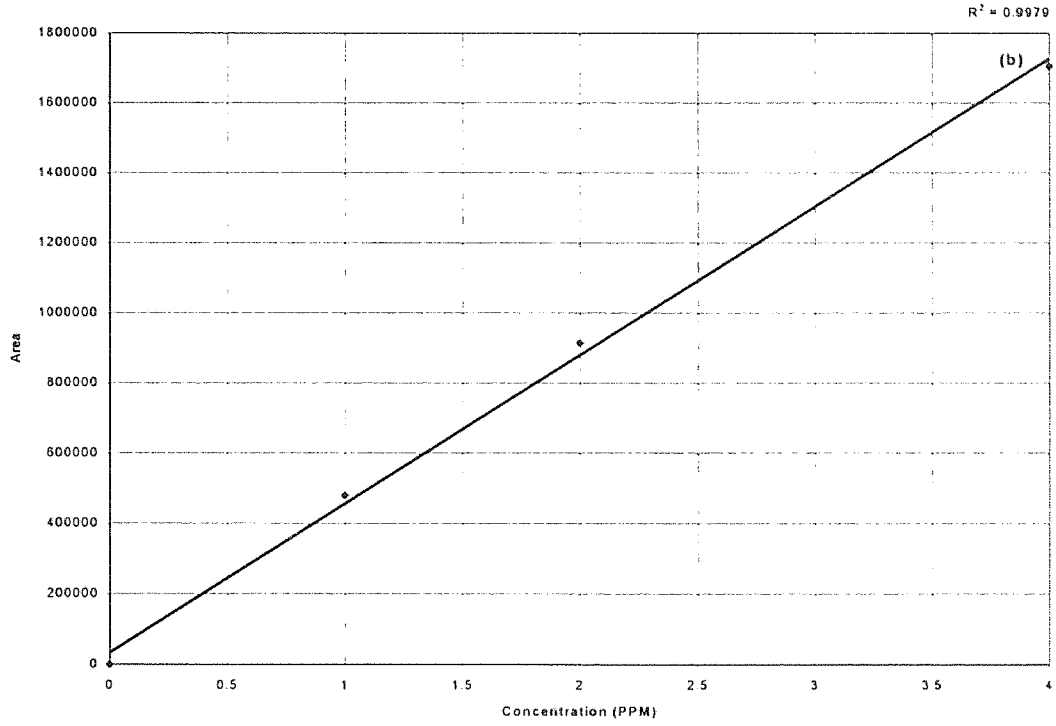


Figure A8 Calibration curves for PCE (a) and VC (b)

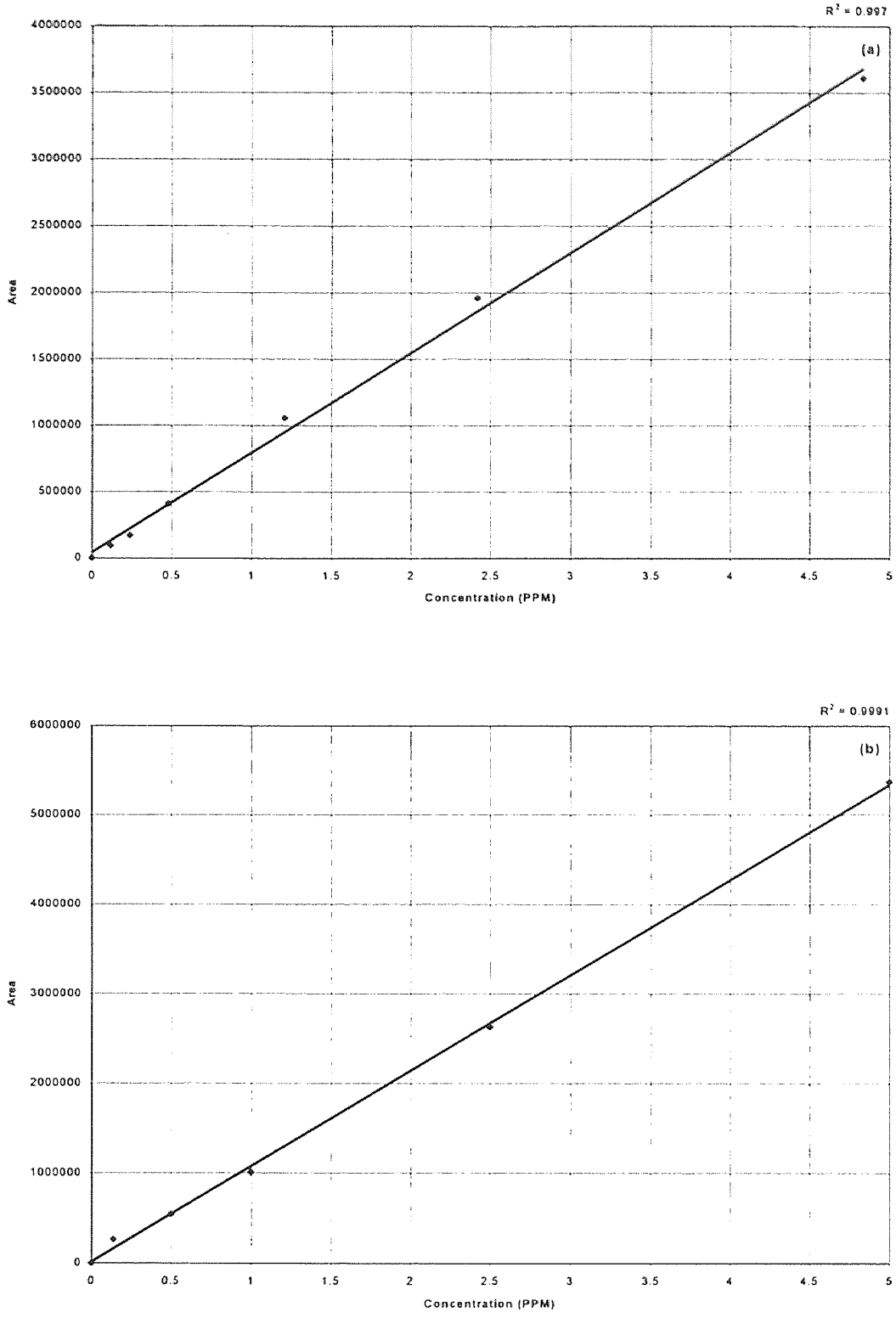


Figure A9 Calibration curves for *trans*-1,2 DCE (a) and *cis*-1,2 DCE (b)

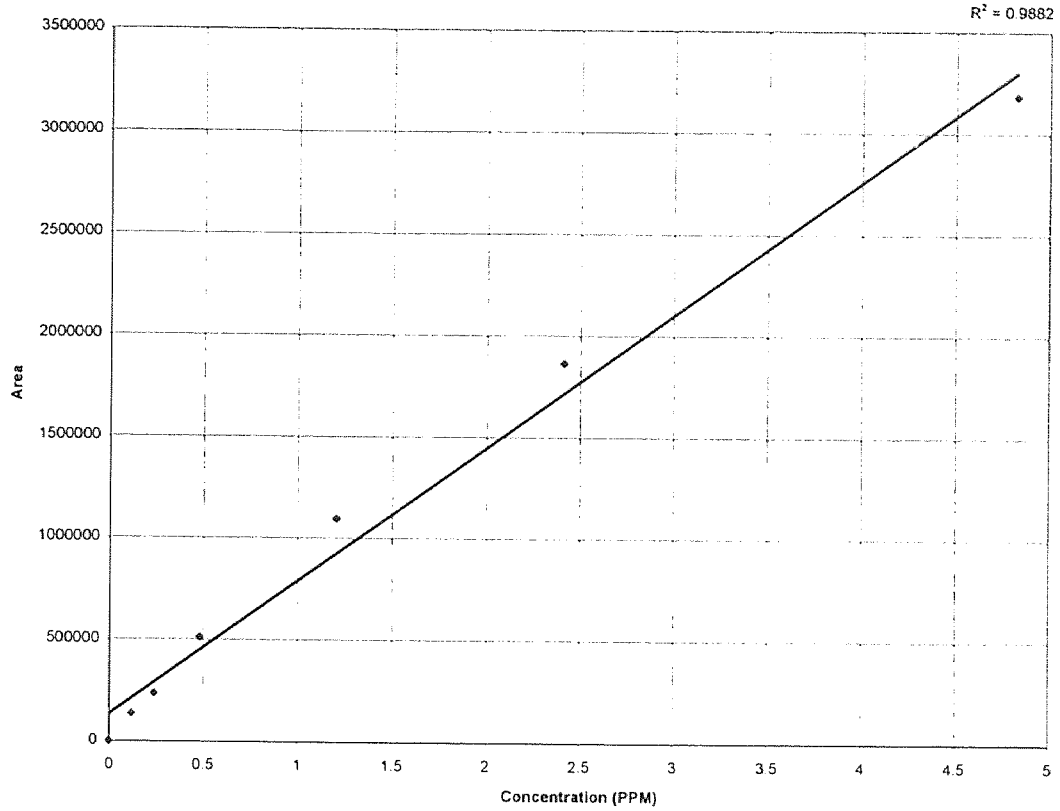


Figure A10 Calibration curve for TCE

APPENDIX B

CHLOROETHENE AND ELECTRON DONOR GRAPHS

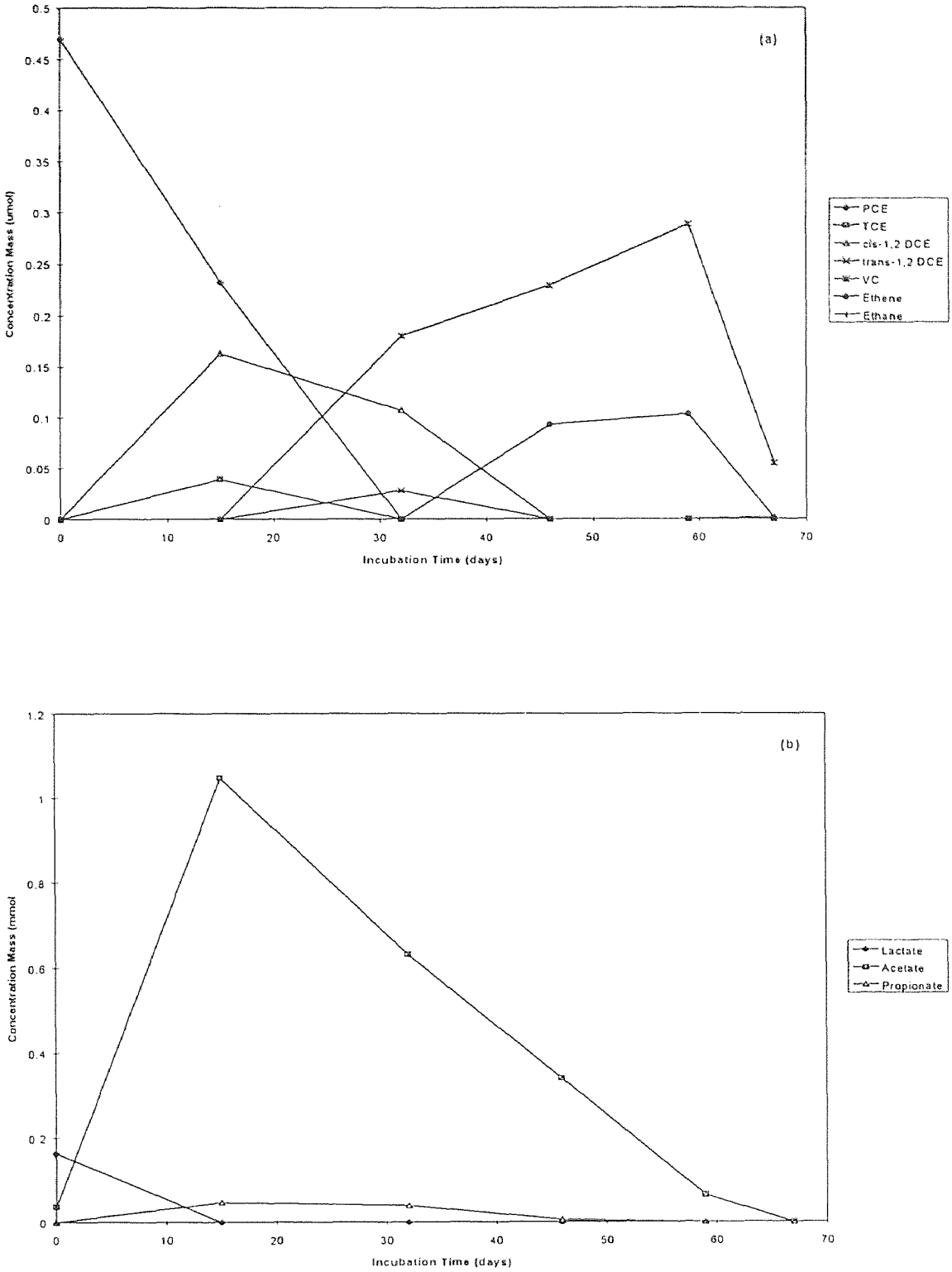


Figure B1 Concentration of chloroethenes (a) and electron donors (b) as a function of time for the microcosm supplemented with lactate

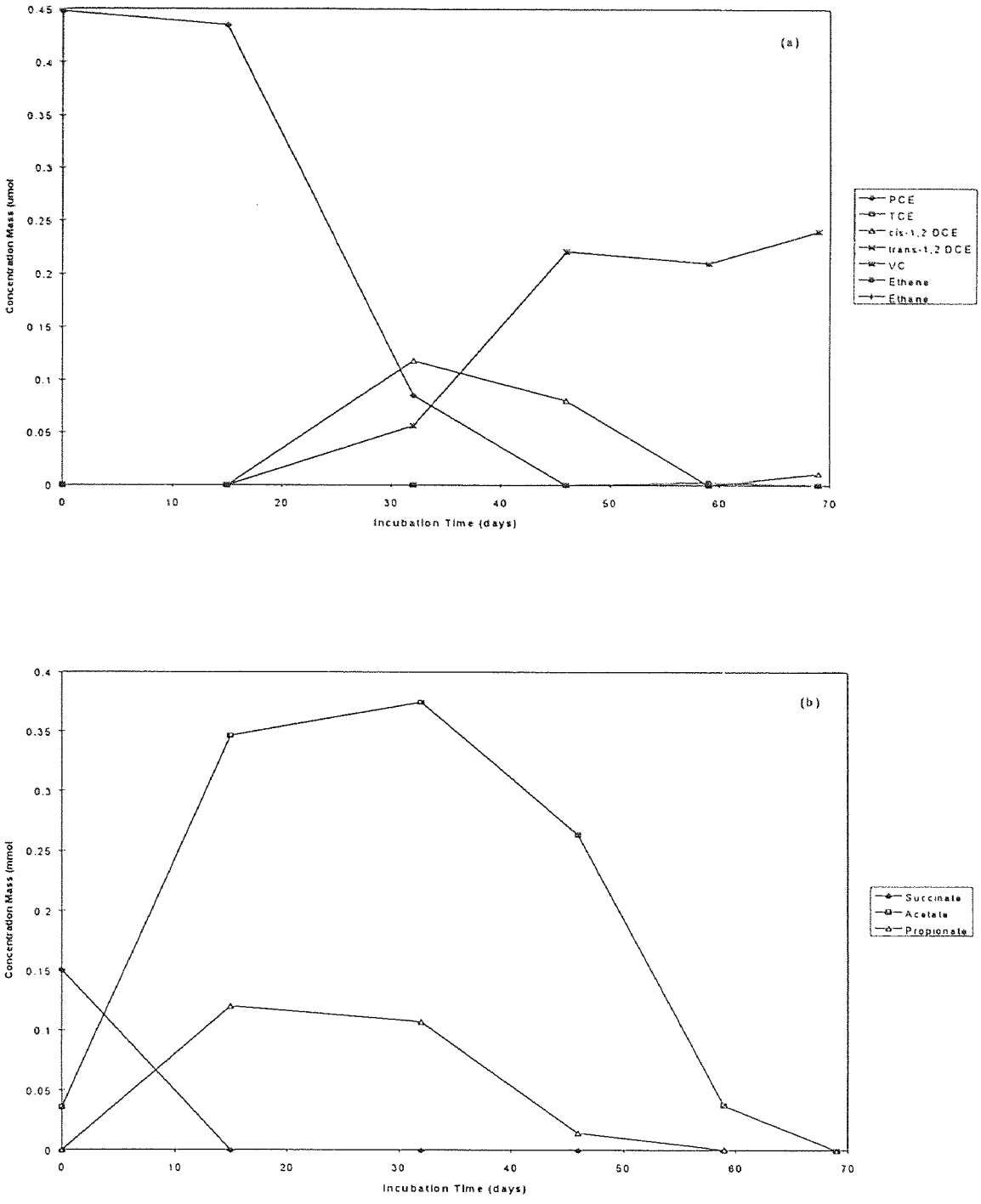


Figure B2 Concentration of chloroethenes (a) and electron donors (b) as a function of time for the microcosm supplemented with succinate

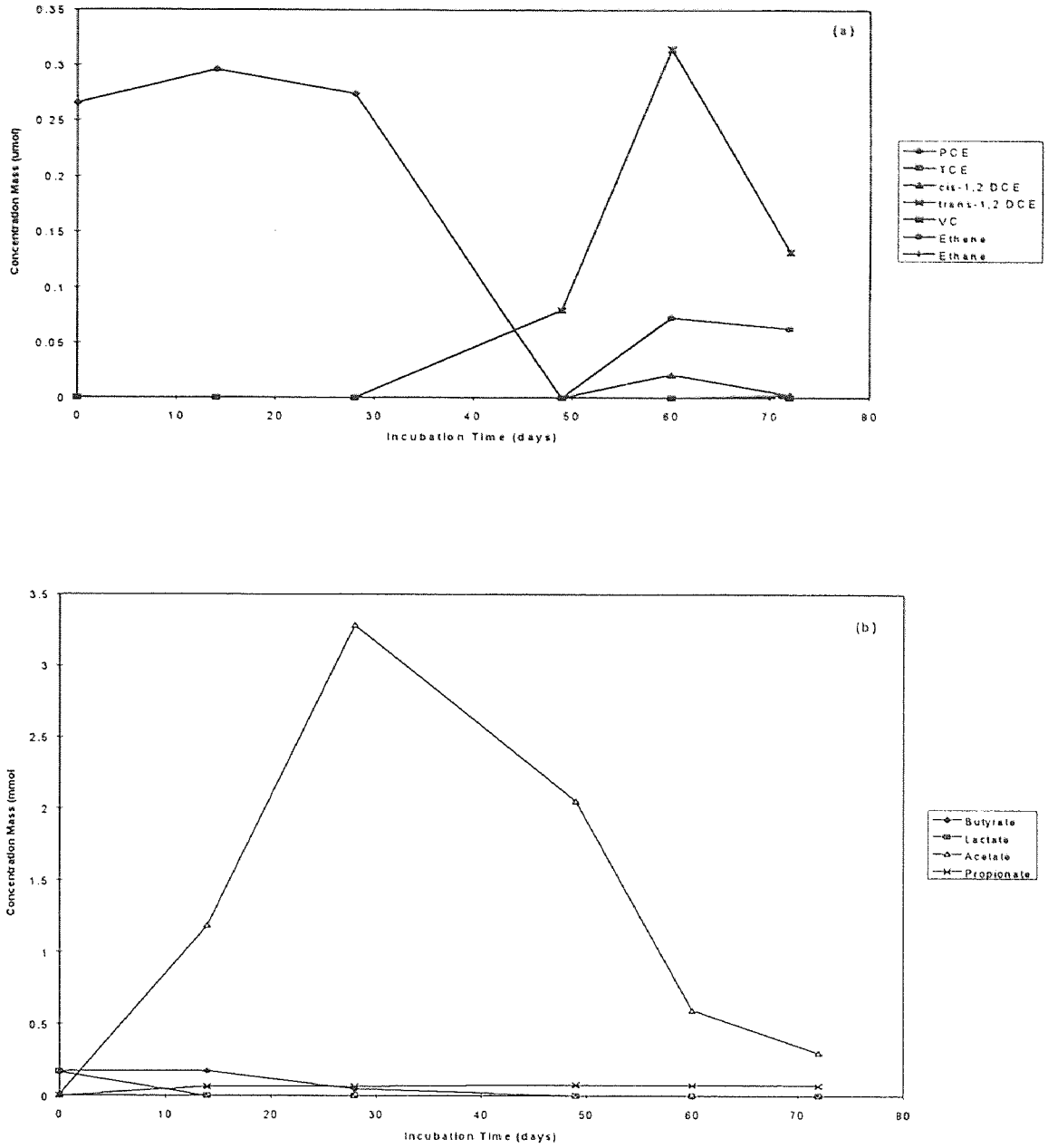


Figure B3 Concentration of chloroethenes (a) and electron donors (b) as a function of time for the microcosm supplemented with butyrate/lactate

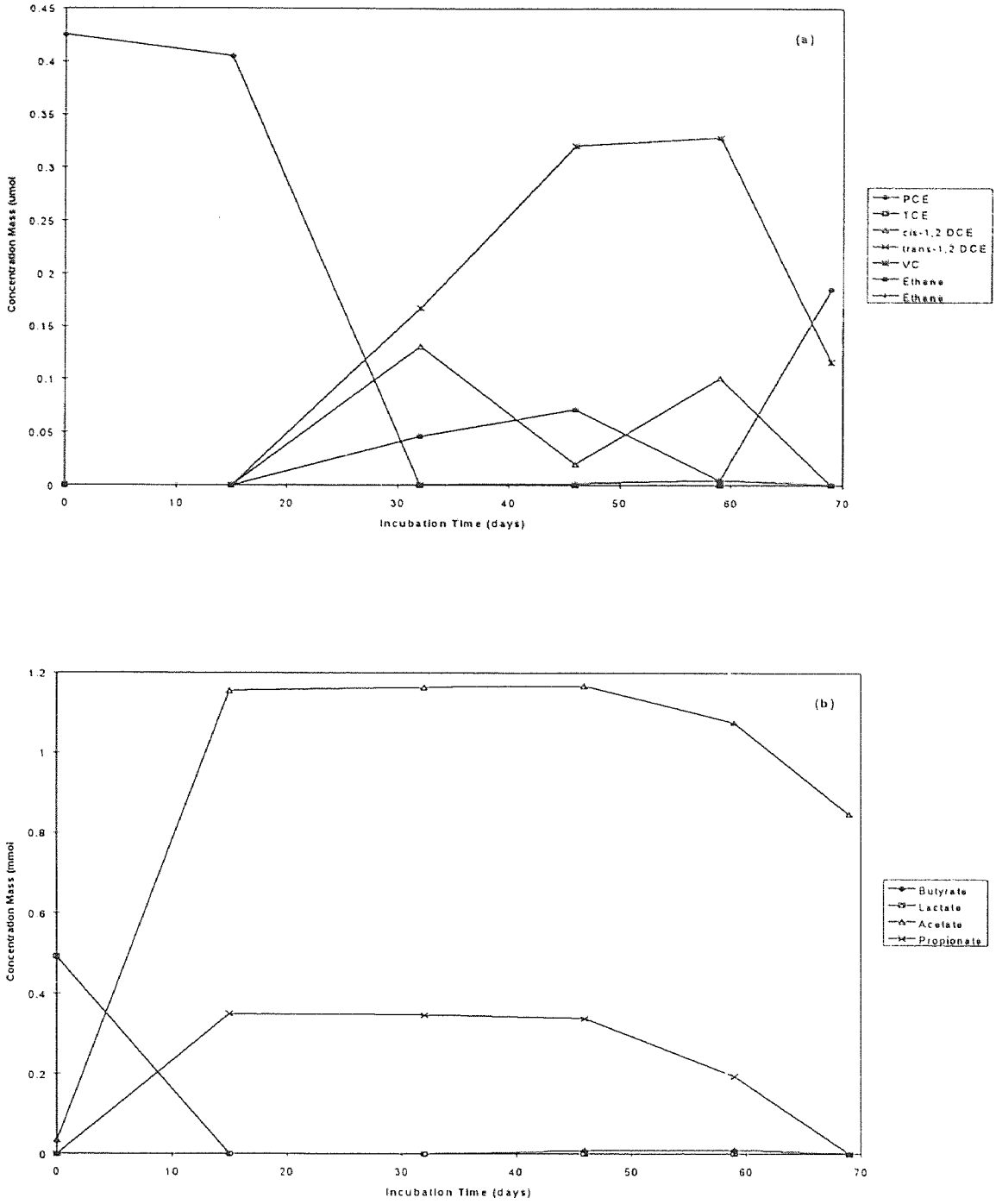


Figure B4 Concentration of chloroethenes (a) and electron donors (b) as a function of time for the microcosm supplemented with succinate/lactate

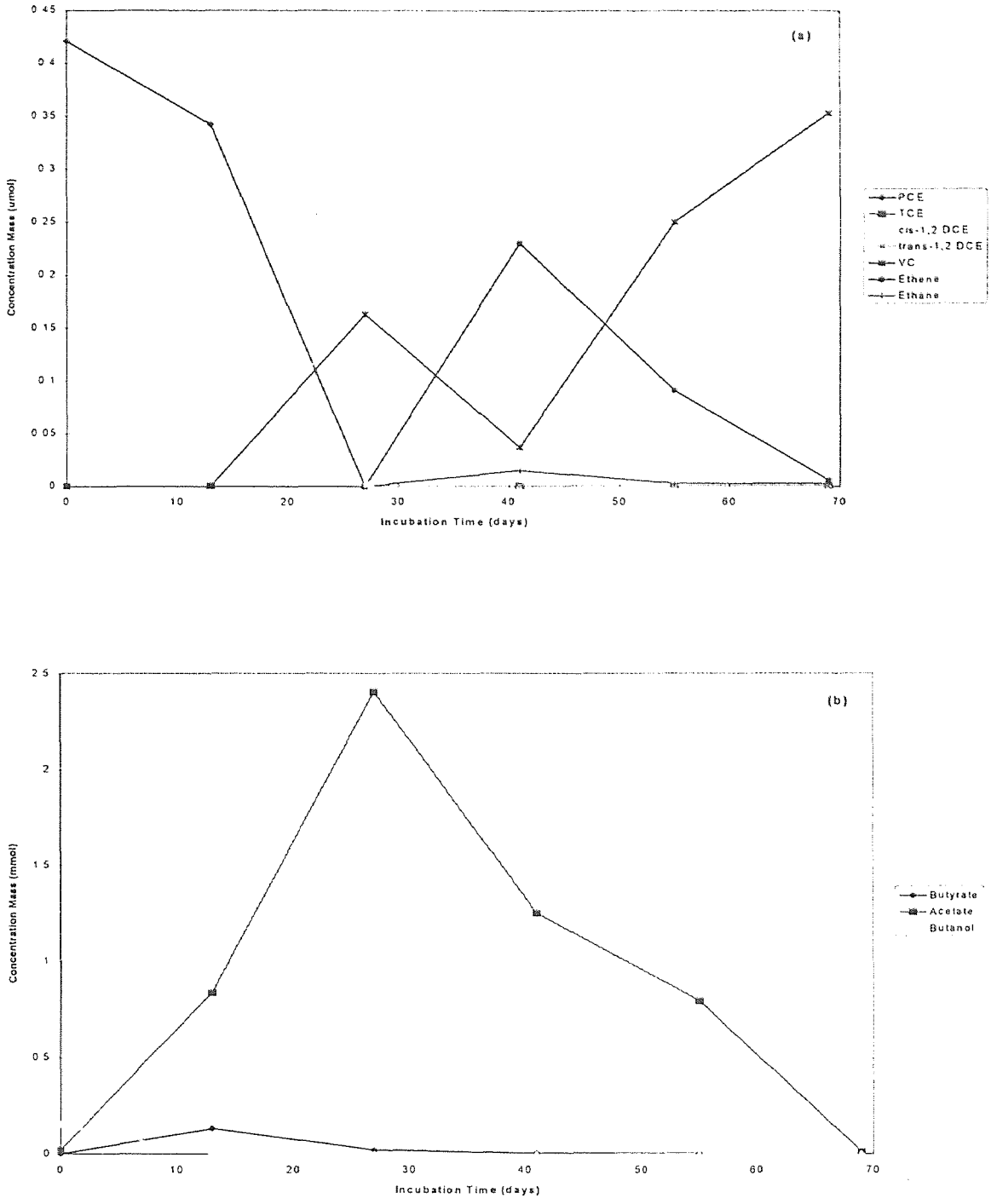


Figure B5 Concentration of chloroethenes (a) and electron donors (b) as a function of time for the microcosm supplemented with butanol

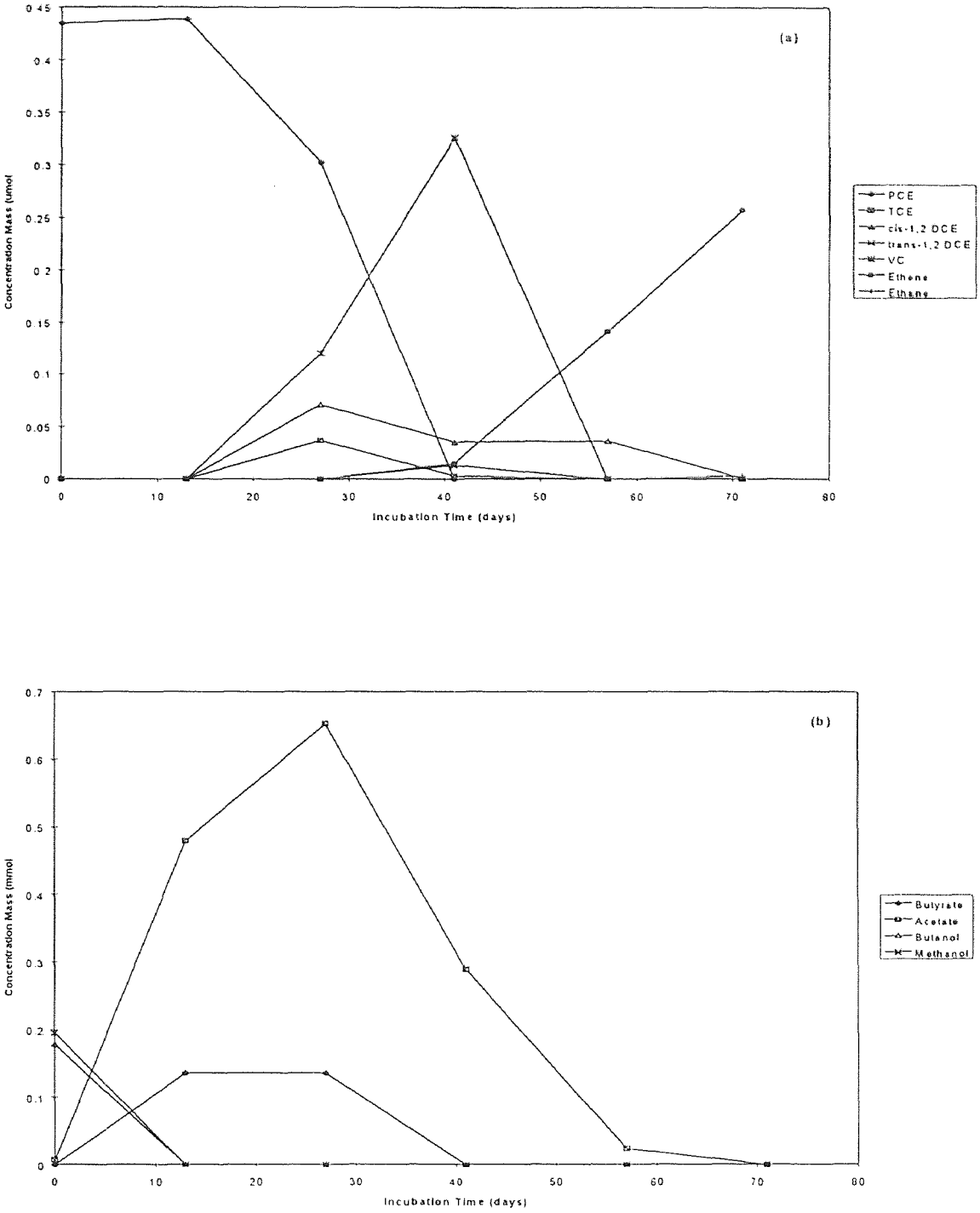


Figure B6 Concentration of chloroethenes (a) and electron donors (b) as a function of time for the microcosm supplemented with butanol/methanol

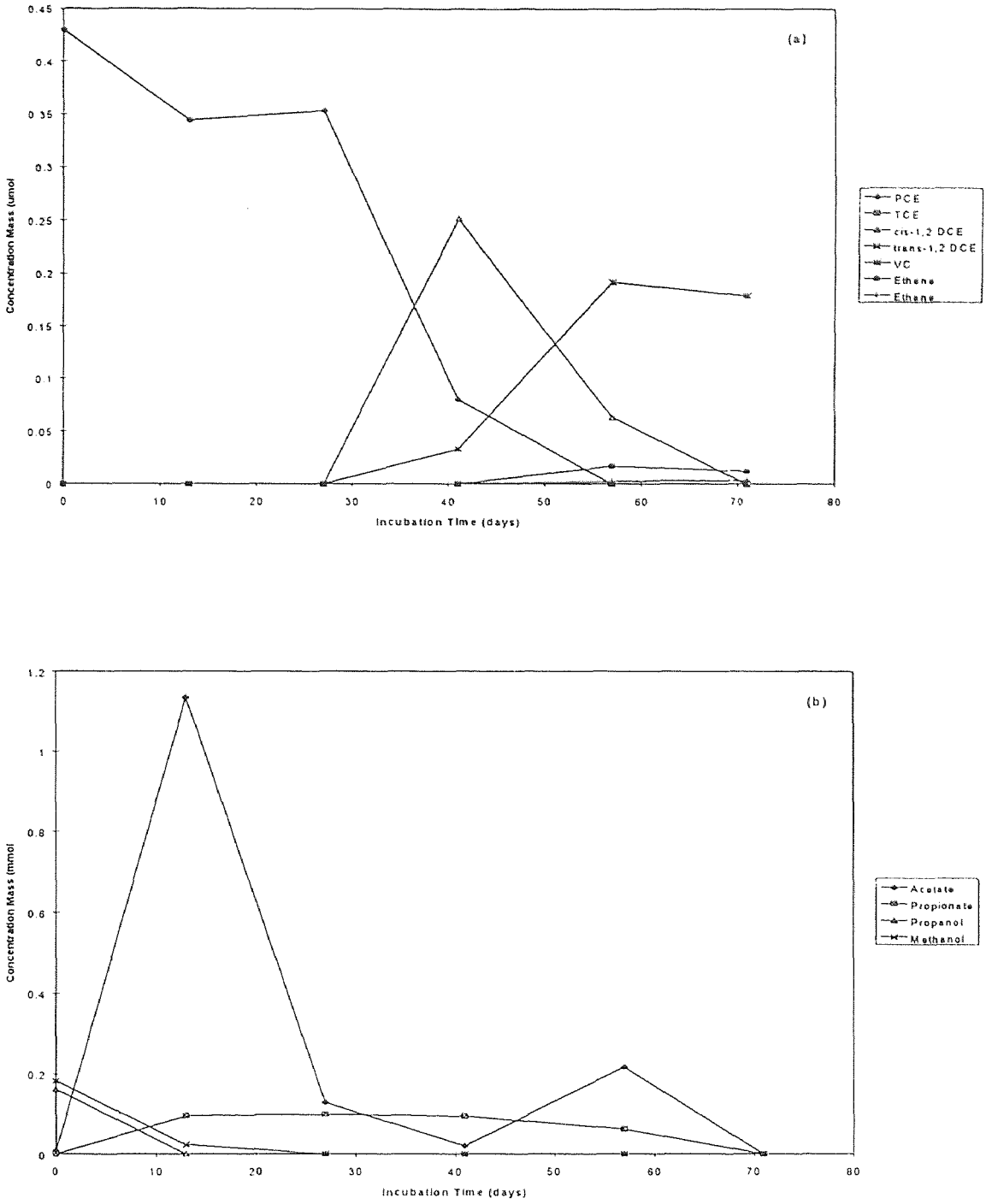


Figure B7 Concentration of chloroethenes (a) and electron donors (b) as a function of time for the microcosm supplemented with propanol/methanol

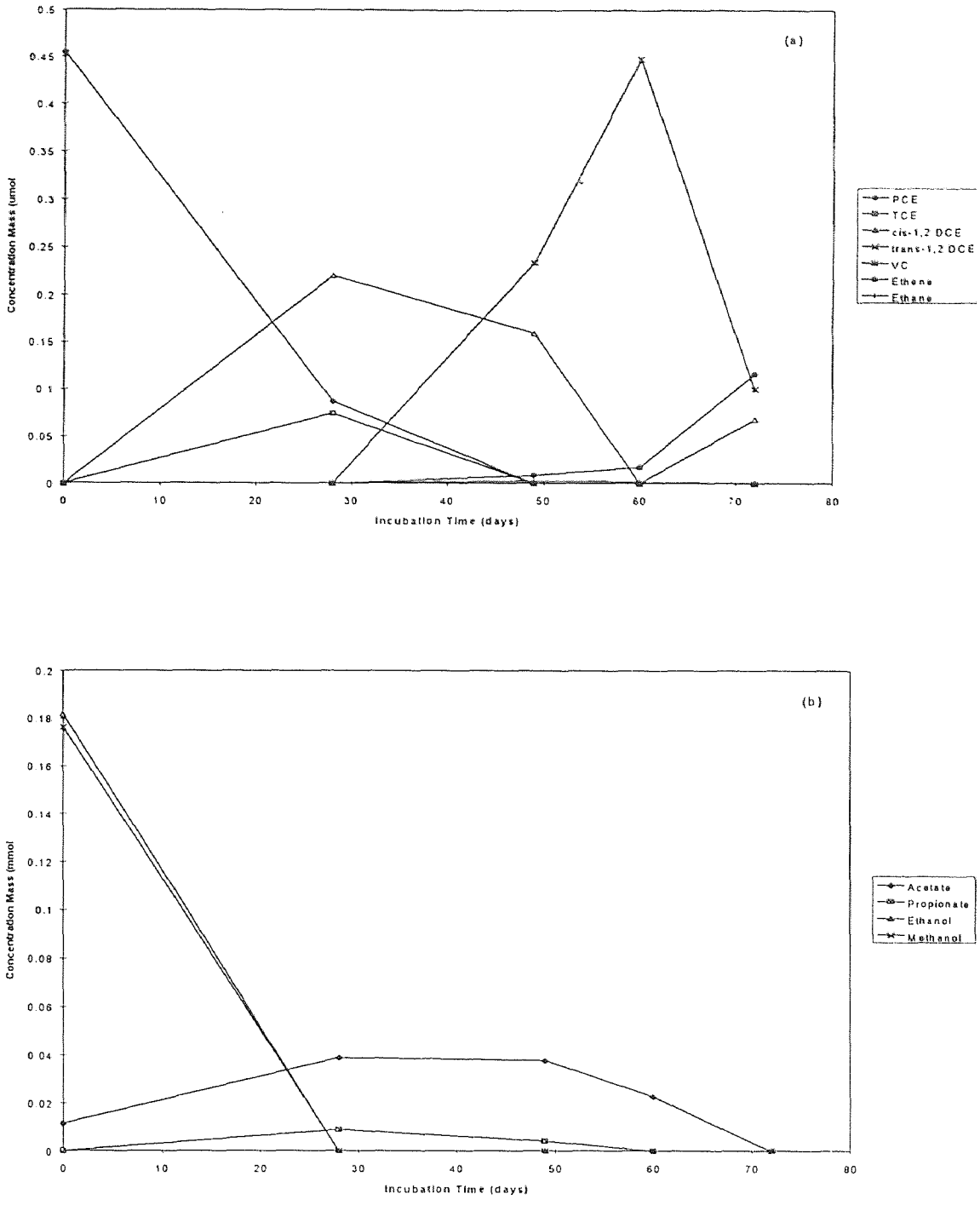


Figure B8 Concentration of chloroethenes (a) and electron donors (b) as a function of time for the microcosm supplemented with methanol/ethanol

APPENDIX C

SAMPLE CALCULATIONS

Sample Calculations to show how the data was calculated for tables C1-C19

1 Calculation of the amount of mass in μmoles for a particular chloroethylene in the *liquid phase*:

(Purge and Trap G.C. reading in uM) * (0.0285 L) = mass in μmoles of the chloroethylene in the bottle

2 Calculation of the amount of mass in μmoles for a particular chloroethylene in the *head space*:

(P&T GC reading in uM) * (Henry's Constant for the specific chloroethylene) * (0.0095 L) = mass in μmoles of the chloroethylene in the headspace

3 Calculation of the total amount of mass in μmoles for a particular chloroethylene in the bottle:

(answer in 1) + (answer in 2) = the total amount of mass in μmoles for that particular chloroethylene

The above procedures were carried out for each individual chloroethylene detected in each individual bottle for each individual sampling period.

4 Calculation of the numbers in tables C1-C19 for the chloroethylenes:

Average of the individual chloroethylenes for the replicate bottles sacrificed at each sampling period

5 Calculation of the amount of mass in μmoles for ethene and ethane in the headspace

(a) Calculation of the amount of moles in 1-ml of a gas at 1-atm:

$$PV=nRT$$
$$(1\ atm)\ (0.001\ L) = (n)\ (0.0821)\ (298)$$
$$n = 4.08733\ E^{-5}\ moles$$

(b) Calculation of the amount of moles in 1-ml of gas at P = 1-atm in a 1000 ppm standard

$$(4.08733\ E^{-5})*(1000 \div 1{,}000{,}000)$$
$$= 4.08733\ E^{-8}\ \text{moles}$$

So we now know that 1-ml of 1000 ppm of the standard at 1-atm contains $4.08733\ E^{-8}$ moles when injected into the G.C.

(c) Calculation to convert the reading from the G.C. to moles

Since 1-ml of the sample was also injected into the G.C. a simple ratio can be set up as follows:

$$\frac{4.08733\ E^{-8}\ \text{moles}}{1000\ \text{ppm}} = \frac{x\ \text{moles in the sample}}{\text{G.C. reading in ppm}}$$

Amount of moles in the 1-ml of sample injected (at its pressure) is equal to:

$$(4.08733\ E^{-11}\ \text{moles} * \text{G.C. reading in PPM})$$

(d) Calculation to determine the total amount of moles of ethane and/or ethene in the headspace

(answer obtained c) * (the headspace volume + the 1-ml volume of the sampling syringe)

The above procedures were carried out for each ethene or ethane measurement detected by the G.C. in each individual bottle for each individual sampling period.

6 Calculation of the numbers in tables C1-C19 for ethene and ethane:

Average of the ethene and ethane data in μmoles obtained from calculation d for the replicate bottles sacrificed at each sampling period

7 Calculation of the amount of moles of PCE in the microcosms

(initial concentration added in moles/L)*(volume of liquid phase in the microcosm in L)

$$\text{or } 25\ E^{-6}\ \text{moles/L}) * (28.5\ \text{ml})$$
$$= 7.125\ E^{-7}\ \text{moles or } .7125\ \mu\text{moles}$$

8 Calculation of the amount of mmoles of alcohol or acid in the *liquid phase*:

(reading from GC or HPLC in mM) (0.0285 L)

Explanation of the data contained in tables C1-C19

1) Time-----Represents the time at which the microcosms were sampled
2) PCE------Represents the average mass in μmoles measured at each sampling time for perchloroethene
3) TCE------Represents the average mass in μmoles measured at each sampling time for trichloroethene
4) c-DCE---Represents the average mass in μmoles measured at each sampling time for *cis*-1,2, dichloroethene
5) t-DCE----Represents the average mass in μmoles measured at each sampling time for *trans*-1,2, dichloroethene
6) VC-------Represents the average mass in μmoles measured at each sampling time for vinyl chloride
7) Ethene---Represents the average mass in μmoles measured at each sampling time of ethene
8) Ethane---Represents the average mass in μmoles measured at each sampling time for ethane
9) Total-----Represents the sum of 2-8 (above) for each time period the microcosms were sacrificed
10) MB%----Represents the mass balance calculated using the average starting (t = 0) measured concentration of PCE ie. (column 9 / PCE conc. t = 0)
11) Mb%---- Represents the mass balance calculated using the initial target level of 0.7125 μmole/bottle ie. (column 9 / 0.7125 μmoles).
12) ND------Represents that the chlorinated ethene was not detected for that sample
13) The alcohols and acids are represented as the average of the three microcosms sampled at each sampling time in millimoles

Table C1 Chloroethylene and electron donor data for the microcosm supplemented with ***methanol*** as an electron donor

Time	*PCE*	*TCE*	*c-DCE*	*t-DCE*	*VC*	*Ethene*	*Ethane*	*Total*	*MB%*	*Mb%*	*Acetate*	*Methanol*
0	0.371	ND	ND	ND	ND	ND	ND	0.371	100	52.07	0.013	0.194
13	0.304	ND	ND	ND	ND	ND	ND	0.304	81.94	42.67	0.185	ND
27	0.056	ND	0.137	ND	0.053	ND	ND	0.246	66.31	34.53	0.181	ND
41	ND	ND	0.061	ND	0.172	0.015	ND	0.248	66.85	34.81	ND	ND
55	ND	ND	ND	ND	0.086	0.166	0.005	0.257	69.27	36.07	ND	ND
69	ND	ND	ND	ND	0.080	0.114	0.005	0.199	53.64	27.93	0.008	ND

Table C2 Chloroethylene and electron donor data for the ***negative control*** microcosm supplemented with ***methanol*** as an electron donor

Time	*PCE*	*TCE*	*c-DCE*	*t-DCE*	*VC*	*Ethene*	*Ethane*	*Total*	*MB%*	*Mb%*	*Formate*	*Acetate*	*Methanol*
0	0.165	ND	ND	ND	ND	ND	ND	0.165	100	23.16	0.0015	0.0115	0.2035
13	0.215	ND	ND	ND	ND	ND	ND	0.215	130	30.17	0.0016	0.0107	0.1896
28	0.210	ND	ND	ND	ND	ND	ND	0.210	127	29.47	0.0020	0.0095	0.1667
41	0.200	ND	ND	ND	ND	ND	ND	0.200	121	28.07	0.0051	0.2583	0.1557
55	0.150	ND	ND	ND	ND	ND	ND	0.150	90.91	21.05	0.0021	0.0178	0.1325
69	0.075	ND	ND	ND	ND	ND	ND	0.075	45.45	10.53	0.0038	ND	0.1343

Table C3 Chloroethylene and electron donor data for the microcosm supplemented with ***butyrate*** as an electron donor

Time	*PCE*	*TCE*	*c-DCE*	*t-DCE*	*VC*	*Ethene*	*Ethane*	*Total*	*MB%*	*Mb%*	*Butyrate*	*Acetate*
0	0.476	ND	ND	ND	ND	ND	ND	0.476	100	66.81	0.1733	0.0112
14	0.432	ND	ND	ND	ND	ND	ND	0.432	90.82	60.67	0.1721	0.3435
28	0.168	0.007	0.0121	ND	ND	0.002	ND	0.189	39.75	26.55	0.0921	1.9206
49	ND	ND	0.0557	ND	0.316	0.106	ND	0.422	88.72	59.27	ND	3.5583
60	ND	ND	ND	ND	0.315	0.008	ND	0.378	79.58	53.17	ND	2.9763
72	ND	ND	ND	ND	0.149	0.1	ND	0.248	52.21	34.88	ND	1.7586

Table C4 Chloroethylene and electron donor data for the ***negative control*** microcosm supplemented with ***butyrate*** as an electron donor

Time	*PCE*	*TCE*	*c-DCE*	*t-DCE*	*VC*	*Ethene*	*Ethane*	*Total*	*MB%*	*Mb%*	*Butyrate*	*Acetate*	*Ethanol*
0	0.423	ND	ND	ND	ND	ND	ND	0.423	100	59.37	0.1724	0.0193	0.0055
14	0.314	ND	ND	ND	ND	ND	ND	0.314	74.20	44.07	0.1804	0.0137	0.0052
27	0.274	ND	ND	ND	ND	ND	ND	0.274	64.80	38.46	0.1899	0.2691	0.0048
49	0.310	ND	ND	ND	ND	ND	ND	0.310	73.30	43.51	0.1820	0.0180	0.0054
72	0.263	ND	ND	ND	ND	ND	ND	0.263	62.20	36.91	0.1542	0.2186	0.0039

Table C5 Chloroethylene and electron donor data for the microcosm supplemented with ***butanol/ethanol*** as an electron donor

Time	*PCE*	*TCE*	*c-DCE*	*t-DCE*	*VC*	*Ethene*	*Ethane*	*Total*	*MB%*	*Mb%*	*Butyrate*	*Acetate*	*Propionate*	*Ethanol*	*Butanol*
0	0.611	ND	ND	ND	ND	ND	ND	0.611	100	85.75	ND	0.0110	ND	0.1608	0.1653
13	0.482	ND	ND	ND	ND	ND	ND	0.482	78.89	67.65	0.1463	2.0804	0.0332	ND	ND
27	0.458	ND	ND	ND	ND	ND	ND	0.458	74.96	64.28	0.1222	1.6220	0.0394	ND	ND
41	0.225	ND	0.117	ND	ND	ND	ND	0.342	55.94	47.97	0.0795	0.9713	0.0259	ND	ND
57	0.183	ND	0.118	ND	ND	ND	ND	0.301	49.33	42.30	0.0010	0.2088	0.0513	ND	ND
71	0.037	ND	0.029	ND	0.075	0.081	0.003	0.225	36.81	31.56	ND	0.0887	ND	ND	ND

Table C6 Chloroethylene and electron donor data for the microcosm supplemented with ***butyrate/succinate*** as an electron donor

Time	*PCE*	*TCE*	*c-DCE*	*t-DCE*	*VC*	*Ethene*	*Ethane*	*Total*	*MB%*	*Mb%*	*Butyrate*	*Succinate*	*Acetate*	*Propionate*
0	0.368	ND	ND	ND	ND	ND	ND	0.368	100	51.65	0.1729	0.1458	0.0126	ND
14	0.383	ND	ND	ND	ND	ND	ND	0.383	104	53.75	0.1706	ND	0.3319	0.2658
28	ND	ND	0.207	ND	0.130	ND	ND	0.337	91.58	47.30	0.0083	ND	3.6831	0.2471
49	ND	ND	0.005	ND	0.349	ND	ND	0.354	96.20	49.68	ND	ND	3.7264	0.2683
60	ND	ND	0.005	ND	0.429	0.005	ND	0.439	119	61.61	ND	ND	2.6606	0.2623
72	ND	ND	ND	ND	0.145	0.083	0.002	0.230	62.50	32.28	ND	ND	2.5057	0.1787

Table C7 Chloroethylene and electron donor data for the microcosm supplemented with ***lactate*** as an electron donor

Time	*PCE*	*TCE*	*c-DCE*	*t-DCE*	*VC*	*Ethene*	*Ethane*	*Total*	*MB%*	*Mb%*	*Lactate*	*Acetate*	*Propionate*
0	0.470	ND	ND	ND	ND	ND	ND	0.470	100	65.94	0.1631	0.0372	ND
15	0.232	0.039	0.163	ND	ND	ND	ND	0.434	92.34	60.88	ND	1.0471	0.0468
32	ND	ND	0.107	0.028	0.18	ND	ND	0.315	67.07	44.22	ND	0.6323	0.0393
46	ND	ND	ND	ND	0.229	0.093	ND	0.322	68.54	45.19	ND	0.3402	0.0067
59	ND	ND	ND	ND	0.288	0.103	ND	0.391	83.23	54.87	ND	0.0643	ND
67	ND	ND	ND	ND	0.055	0.001	0.002	0.058	12.35	8.14	ND	ND	ND

Table C8 Chloroethylene and electron donor data for the ***negative control*** microcosm supplemented with ***lactate*** as an electron donor

Time	*PCE*	*TCE*	*c-DCE*	*t-DCE*	*VC*	*Ethene*	*Ethane*	*Total*	*MB%*	*Mb%*	*Lactate*	*Acetate*	*Isobutyrate*	*Ethanol*
0	0.428	ND	ND	ND	ND	ND	ND	0.428	100	60.07	0.1673	0.0165	0.2539	0.0068
14	0.368	ND	ND	ND	ND	ND	ND	0.368	86.00	51.65	0.1656	0.0112	0.2291	ND
27	0.301	ND	ND	ND	ND	ND	ND	0.301	70.30	42.24	ND	2.8174	0.2539	ND
49	0.286	ND	ND	ND	ND	ND	ND	0.286	66.80	40.14	ND	ND	0.2291	ND
60	0.159	ND	ND	ND	ND	ND	ND	0.159	37.10	22.32	0.1679	0.0172	ND	ND
72	0.145	ND	ND	ND	ND	ND	ND	0.145	33.90	20.35	0.1731	0.0203	ND	ND

Table C9 Chloroethylene and electron donor data for the microcosm supplemented with ***succinate*** as an electron donor

Time	*PCE*	*TCE*	*c-DCE*	*t-DCE*	*VC*	*Ethene*	*Ethane*	*Total*	*MB%*	*Mb%*	*Succinate*	*Acetate*	*Propionate*
0	0.448	ND	ND	ND	ND	ND	ND	0.448	100	62.87	0.1505	0.0356	ND
15	0.435	ND	ND	ND	ND	ND	ND	0.435	97.10	61.05	ND	0.3461	0.1201
32	0.085	ND	0.118	ND	0.056	ND	ND	0.259	57.81	36.35	ND	0.3743	0.1072
46	ND	ND	0.080	ND	0.221	ND	ND	0.301	67.19	42.25	ND	0.2638	0.015
59	ND	ND	ND	ND	0.210	0.003	ND	0.213	47.54	29.89	ND	0.0376	0.0006
69	ND	ND	0.011	ND	0.240	ND	ND	0.251	56.03	35.23	ND	ND	ND

Table C10 Chloroethylene and electron donor data for the ***negative control*** microcosm supplemented with ***succinate*** as an electron donor

Time	*PCE*	*TCE*	*c-DCE*	*t-DCE*	*VC*	*Ethene*	*Ethane*	*Total*	*MB%*	*Mb%*	*Butyrate*	*Succinate*	*Acetate*
0	0.397	ND	ND	ND	ND	ND	ND	0.397	100	55.72	ND	0.1491	0.0144
14	0.379	ND	ND	ND	ND	ND	ND	0.379	95.50	53.19	0.1824	ND	0.0107
27	0.354	ND	ND	ND	ND	ND	ND	0.354	89.20	49.68	ND	0.1509	ND
49	0.350	ND	ND	ND	ND	ND	ND	0.350	88.20	49.12	ND	0.1513	0.0215
60	0.251	ND	ND	ND	ND	ND	ND	0.251	63.20	35.23	ND	0.1530	0.0172
72	0.253	ND	ND	ND	ND	ND	ND	0.253	63.70	35.51	ND	0.1516	0.0196

Table C11 Chloroethylene and electron donor data for the microcosm supplemented with ***butyrate/lactate*** as an electron donor

Time	*PCE*	*TCE*	*c-DCE*	*t-DCE*	*VC*	*Ethene*	*Ethane*	*Total*	*MB%*	*Mb%*	*Butyrate*	*Lactate*	*Acetate*	*Propionate*
0	0.266	ND	ND	ND	ND	ND	ND	0.266	100	37.33	0.1726	0.1662	0.0090	ND
14	0.296	ND	ND	ND	ND	ND	ND	0.296	111	41.54	0.1746	ND	1.1874	0.0669
28	0.274	ND	ND	ND	ND	ND	ND	0.274	103	38.46	0.0511	ND	3.2874	0.0665
49	ND	ND	ND	ND	0.08	ND	ND	0.08	30.08	11.23	0.0014	ND	2.0544	0.0783
60	ND	ND	0.021	ND	0.315	0.073	ND	0.409	154	57.43	ND	ND	0.5975	0.0756
72	ND	ND	0.003	ND	0.132	0.063	0.003	0.201	75.45	28.17	ND	ND	0.3018	0.0732

Table C12 Chloroethylene and electron donor data for the microcosm supplemented with ***succinate/lactate*** as an electron donor

Time	*PCE*	*TCE*	*c-DCE*	*t-DCE*	*VC*	*Ethene*	*Ethane*	*Total*	*MB%*	*Mb%*	*Butyrate*	*Lactate*	*Acetate*	*Propionate*
0	0.425	ND	ND	ND	ND	ND	ND	0.425	100	59.56	ND	0.4915	0.0345	ND
15	0.405	ND	ND	ND	ND	ND	ND	0.405	95.29	56.84	ND	ND	1.1548	0.3498
32	ND	ND	0.131	ND	0.167	0.046	0.001	0.345	81.18	48.42	ND	ND	1.1631	0.3463
46	ND	ND	0.020	ND	0.320	0.071	0.002	0.413	97.18	57.96	0.0096	ND	1.1676	0.3381
59	ND	ND	0.101	ND	0.328	0.005	0.005	0.439	103	61.61	0.0106	ND	1.0757	0.1946
69	ND	ND	ND	ND	0.116	0.185	0.001	0.302	71.06	42.39	0.0014	ND	0.8480	ND

Table C13 Chloroethylene and electron donor data for the microcosm supplemented with ***butanol*** as an electron donor

Time	*PCE*	*TCE*	*c-DCE*	*t-DCE*	*VC*	*Ethene*	*Ethane*	*Total*	*MB%*	*Mb%*	*Butyrate*	*Acetate*	*Butanol*
0	0.421	ND	ND	ND	ND	ND	ND	0.421	100	59.09	ND	0.0183	0.1509
13	0.342	ND	ND	ND	ND	ND	ND	0.342	81.24	48.00	0.1305	0.8366	ND
27	ND	ND	0.175	ND	0.163	ND	ND	0.338	80.29	47.44	0.0198	2.4048	ND
41	ND	ND	ND	ND	0.037	0.230	0.015	0.282	66.98	39.58	0.0017	1.2510	ND
55	ND	ND	ND	ND	0.250	0.091	0.003	0.344	81.71	48.28	ND	0.7932	ND
69	ND	ND	ND	ND	0.353	0.006	0.003	0.362	86.01	50.82	ND	0.0104	ND

Table C14 Chloroethylene and electron donor data for the ***negative control*** microcosm supplemented with ***butanol*** as an electron donor

Time	*PCE*	*TCE*	*c-DCE*	*t-DCE*	*VC*	*Ethene*	*Ethane*	*Total*	*MB%*	*Mb%*	*Acetate*	*Butanol*
0	0.267	ND	ND	ND	ND	ND	ND	0.267	100	37.47	0.0115	0.1674
13	0.237	ND	ND	ND	ND	ND	ND	0.237	88.76	33.26	0.0145	0.1669
28	0.262	ND	ND	ND	ND	ND	ND	0.262	98.13	36.77	0.0197	0.1311
41	0.135	ND	ND	ND	ND	ND	ND	0.135	50.56	18.94	0.0098	0.1314
55	0.132	ND	ND	ND	ND	ND	ND	0.132	49.44	18.53	0.0185	0.1751
69	0.180	ND	ND	ND	ND	ND	ND	0.180	67.42	25.26	0.0193	0.1751

Table C15 Chloroethylene and electron donor data for the microcosm supplemented with ***butanol/methanol*** as an electron donor

Time	*PCE*	*TCE*	*c-DCE*	*t-DCE*	*VC*	*Ethene*	*Ethane*	*Total*	*MB%*	*Mb%*	*Butyrate*	*Acetate*	*Butanol*	*Methanol*
0	0.435	ND	ND	ND	ND	ND	ND	0.435	100	61.05	ND	0.0070	0.1784	0.1951
13	0.439	ND	ND	ND	ND	ND	ND	0.439	101	61.61	0.1364	0.4792	ND	ND
27	0.302	0.040	0.071	ND	0.120	ND	ND	0.533	122	74.33	0.1363	0.6525	ND	ND
41	ND	ND	0.035	0.013	0.326	0.015	ND	0.389	90.12	55.02	ND	0.2890	ND	ND
57	ND	ND	0.036	ND	ND	0.141	ND	0.177	40.69	24.84	ND	0.0239	ND	ND
71	ND	ND	0.001	ND	ND	0.257	0.003	0.261	60.00	36.63	ND	ND	ND	ND

Table C16 Chloroethylene and electron donor data for the microcosm supplemented with ***propanol/methanol*** as an electron donor

Time	*PCE*	*TCE*	*c-DCE*	*t-DCE*	*VC*	*Ethene*	*Ethane*	*Total*	*MB%*	*Mb%*	*Acetate*	*Propionate*	*Propanol*	*Methanol*
0	0.430	ND	ND	ND	ND	ND	ND	0.430	100	60.35	0.0091	ND	0.1613	0.1820
13	0.344	ND	ND	ND	ND	ND	ND	0.344	80.00	48.28	1.1348	0.0958	ND	0.0242
27	0.353	ND	ND	ND	ND	ND	ND	0.353	82.09	49.54	0.1293	0.0992	ND	ND
41	0.080	ND	0.252	ND	0.030	ND	ND	0.365	84.88	51.23	0.0209	0.0939	ND	ND
57	ND	ND	0.063	ND	0.190	0.017	0.003	0.275	63.95	38.60	0.2178	0.0623	ND	ND
71	ND	ND	ND	ND	0.180	0.012	0.004	0.195	45.35	27.38	ND	ND	ND	ND

Table C17 Chloroethylene and electron donor data for the microcosm supplemented with ***methanol/ethanol*** as an electron donor

Time	*PCE*	*TCE*	*c-DCE*	*t-DCE*	*VC*	*Ethene*	*Ethane*	*Total*	*MB%*	*Mb%*	*Acetate*	*Propionate*	*Ethanol*	*Methanol*
0	0.455	ND	ND	ND	ND	ND	ND	0.455	100	63.86	0.0117	ND	0.1817	0.1763
28	0.087	0.074	0.220	ND	ND	ND	ND	0.381	83.74	53.47	0.039	0.0090	ND	ND
49	ND	ND	0.159	ND	0.230	0.009	0.003	0.401	88.92	56.79	0.0378	0.0042	ND	ND
60	ND	ND	ND	ND	0.450	0.017	0.002	0.469	103	65.54	0.0229	ND	ND	ND
72	ND	ND	0.068	ND	0.100	0.116	0.001	0.285	62.64	40.00	ND	ND	ND	ND

Table C18 Chloroethylene and electron donor data for the ***negative control*** microcosm supplemented with ***ethanol*** as an electron donor

Time	*PCE*	*TCE*	*c-DCE*	*t-DCE*	*VC*	*Ethene*	*Ethane*	*Total*	*MB%*	*Mb%*	*Acetate*	*Ethanol*
0	0.258	ND	ND	ND	ND	ND	ND	0.258	100	36.21	0.0145	0.1557
13	0.230	ND	ND	ND	ND	ND	ND	0.230	89.15	32.28	0.2740	0.1595
28	0.187	ND	ND	ND	ND	ND	ND	0.187	72.48	26.25	0.0191	0.1328
41	0.173	ND	ND	ND	ND	ND	ND	0.173	67.05	24.28	0.0177	0.0826
55	0.073	ND	ND	ND	ND	ND	ND	0.073	28.29	10.25	0.3158	0.1650
69	0.175	ND	ND	ND	ND	ND	ND	0.175	67.83	24.56	0.0178	0.1546

Table C19 Chloroethylene and electron donor data for the ***negative control*** microcosm supplemented with ***propanol*** as an electron donor

Time	*PCE*	*TCE*	*c-DCE*	*t-DCE*	*VC*	*Ethene*	*Ethane*	*Total*	*MB%*	*Mb%*	*Acetate*	*Propanol*
0	0.272	ND	ND	ND	ND	ND	ND	0.272	100	38.18	ND	0.1603
13	0.207	ND	ND	ND	ND	ND	ND	0.207	76.10	29.05	ND	0.1649
28	0.212	ND	ND	ND	ND	ND	ND	0.212	77.94	29.75	0.0194	0.1344
41	0.128	ND	ND	ND	ND	ND	ND	0.128	47.06	17.96	ND	0.0862
55	0.130	ND	ND	ND	ND	ND	ND	0.130	47.79	18.25	0.0179	0.1402
69	0.097	ND	ND	ND	ND	ND	ND	0.097	35.66	13.16	0.0192	0.1357

APPENDIX D

NEGATIVE CONTROL CHLOROETHENE GRAPHS

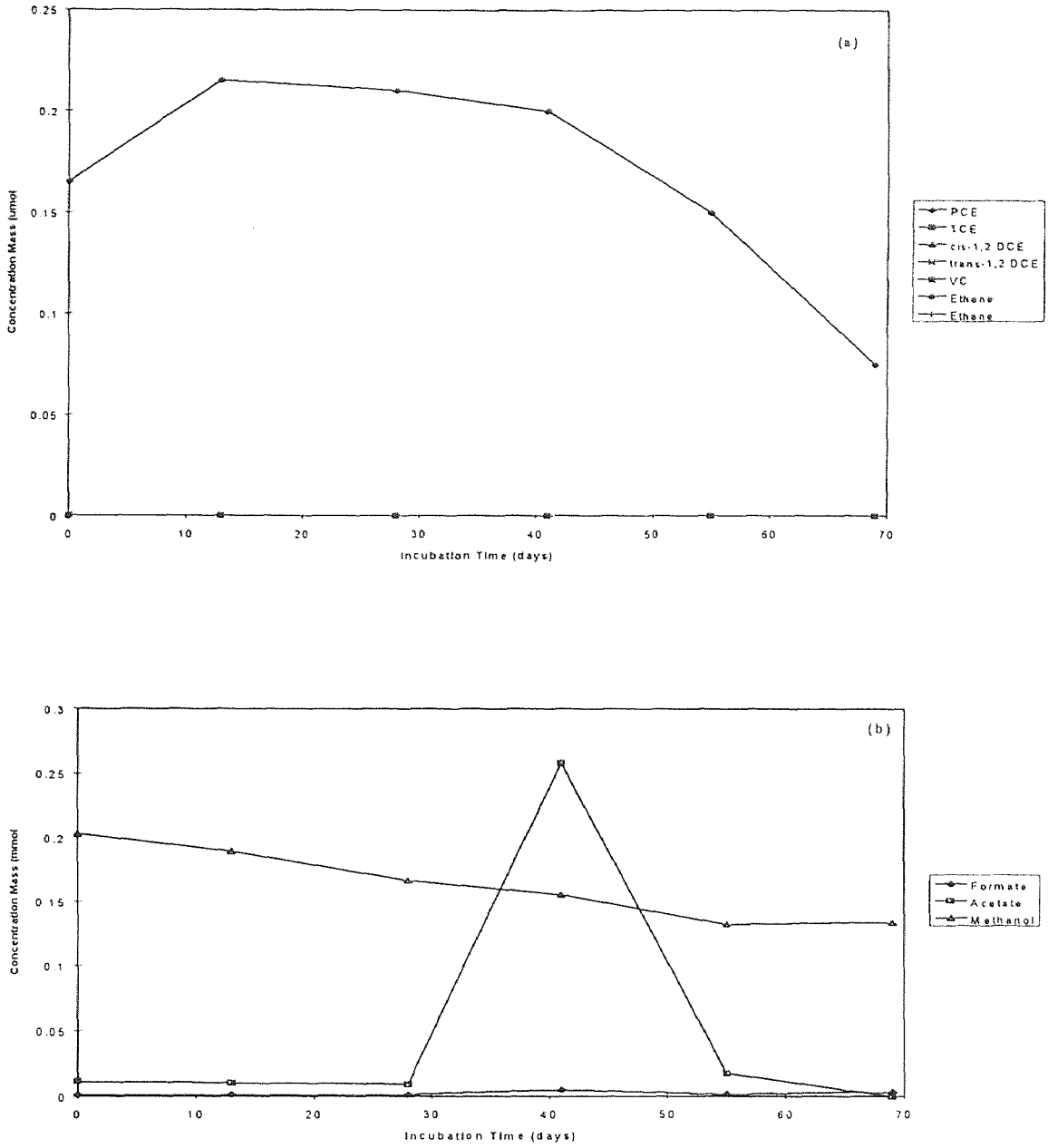


Figure D1 Concentration of chloroethenes (a) and electron donors (b) as a function of time for the ***negative control*** microcosm supplemented with methanol

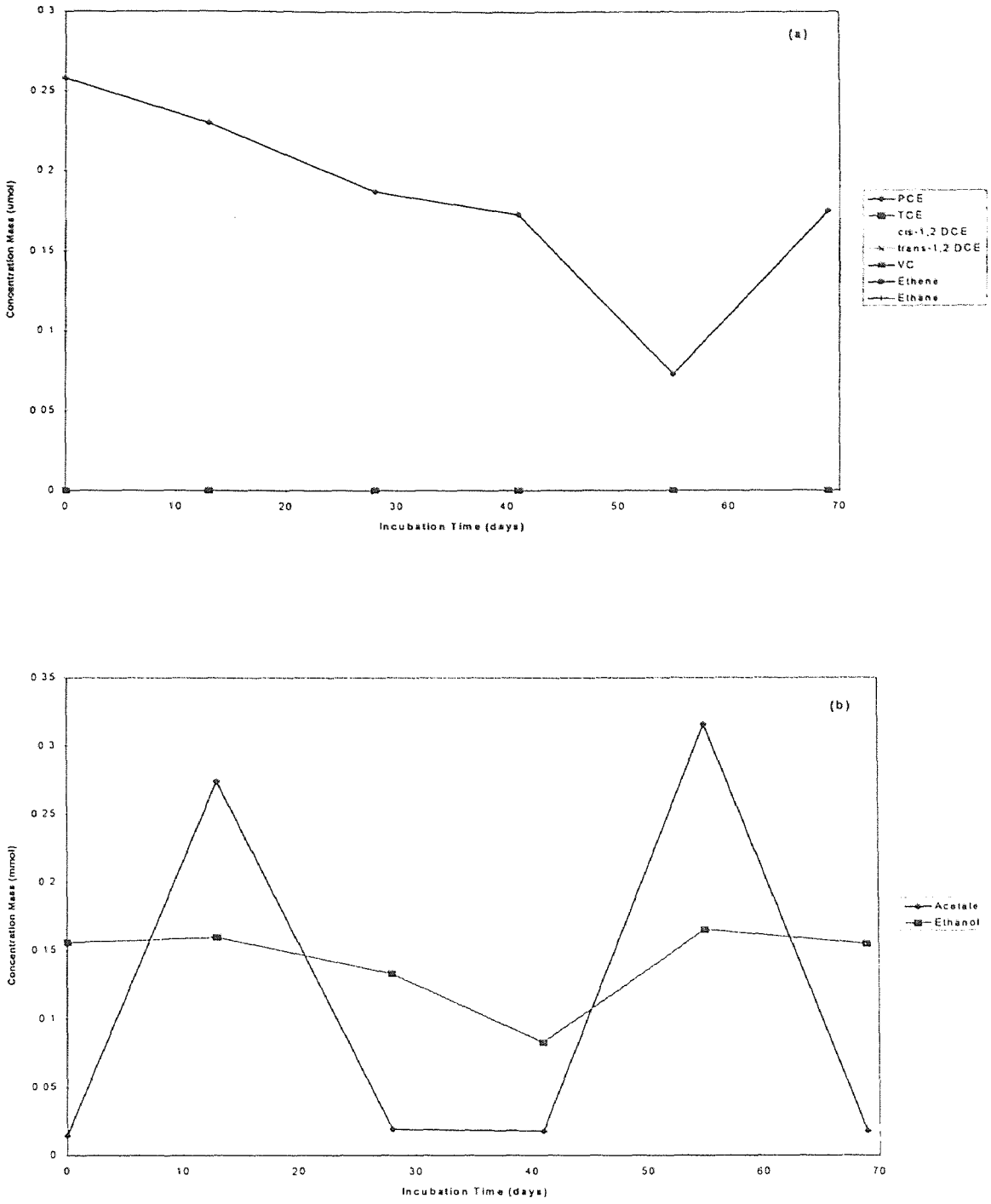


Figure D2 Concentration of chloroethenes (a) and electron donors (b) as a function of time for the ***negative control*** microcosm supplemented with ethanol

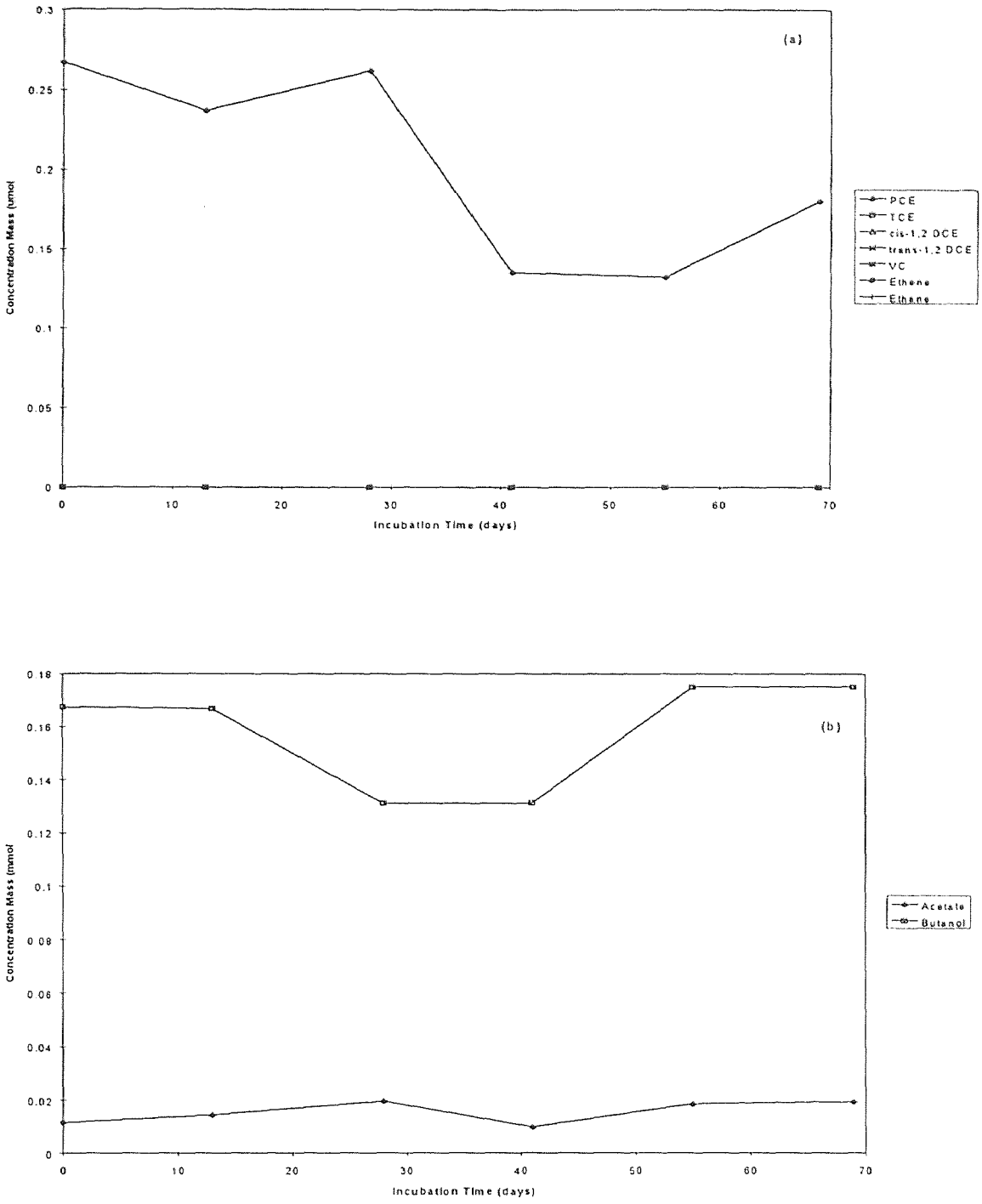


Figure D3 Concentration of chloroethenes (a) and electron donors (b) as a function of time for the *negative control* microcosm supplemented with butanol

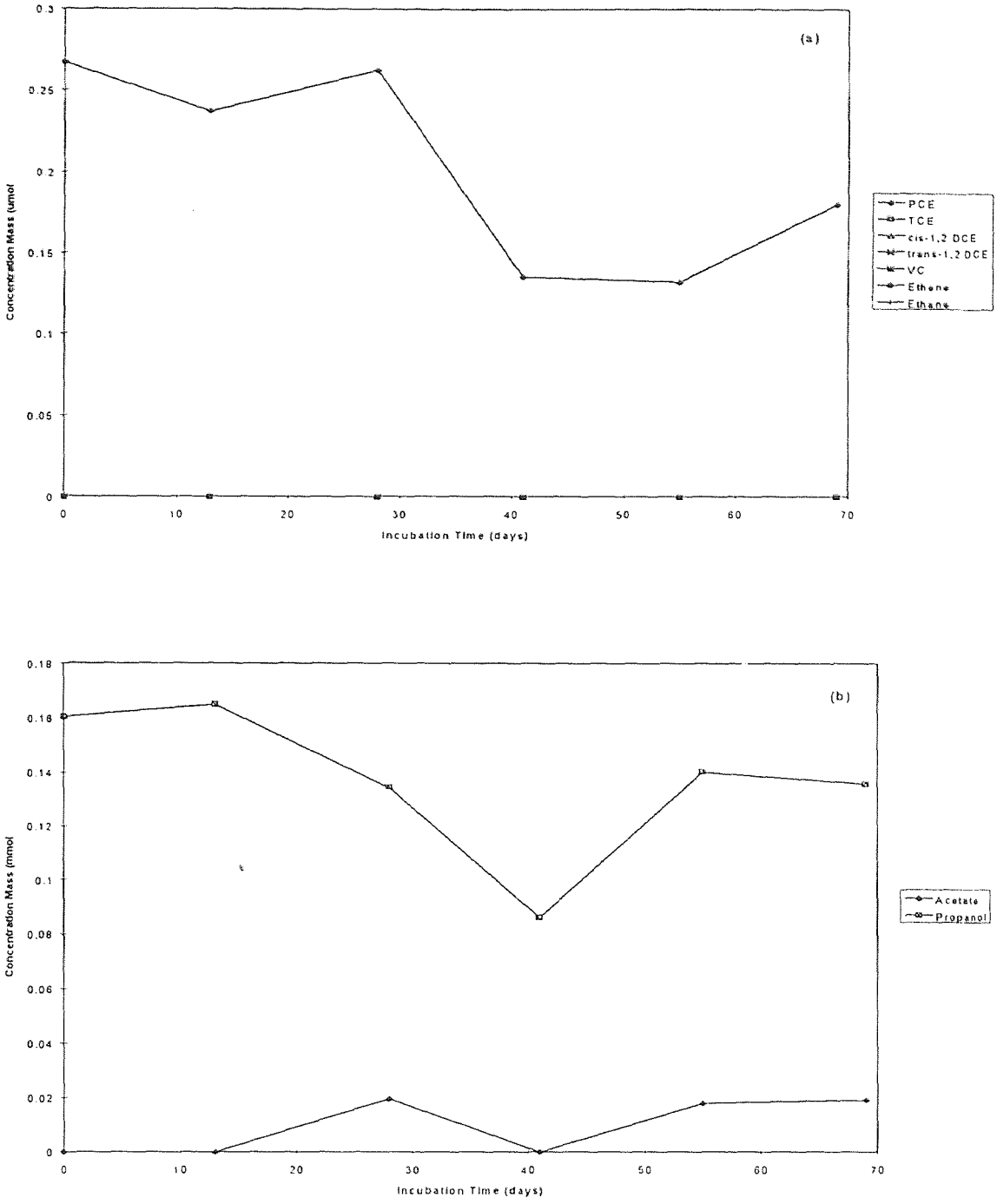


Figure D4 Concentration of chloroethenes (a) and electron donors (b) as a function of time for the ***negative control*** microcosm supplemented with propanol

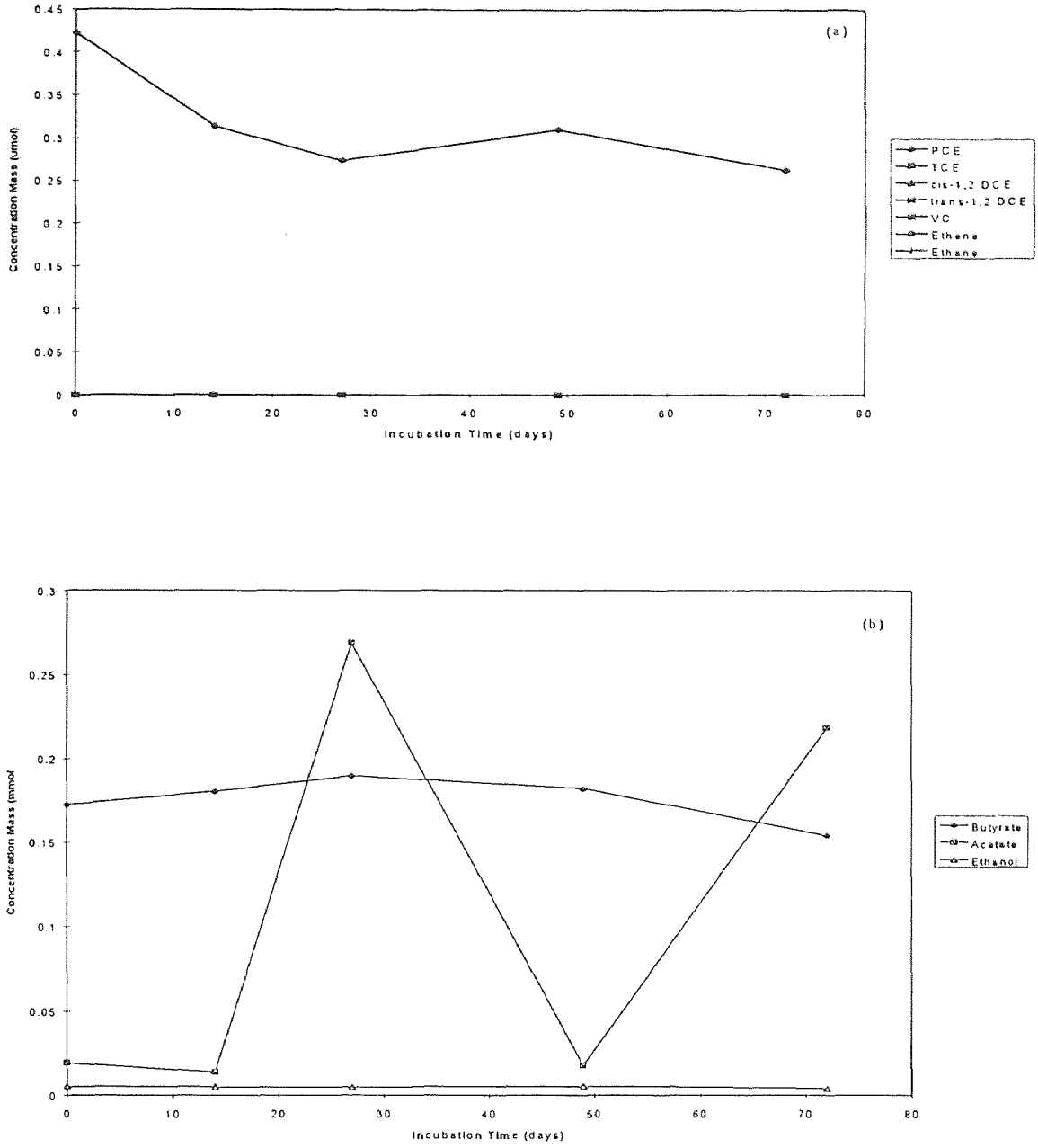


Figure D5 Concentration of chloroethenes (a) and electron donors (b) as a function of time for the ***negative control*** microcosm supplemented with butyrate

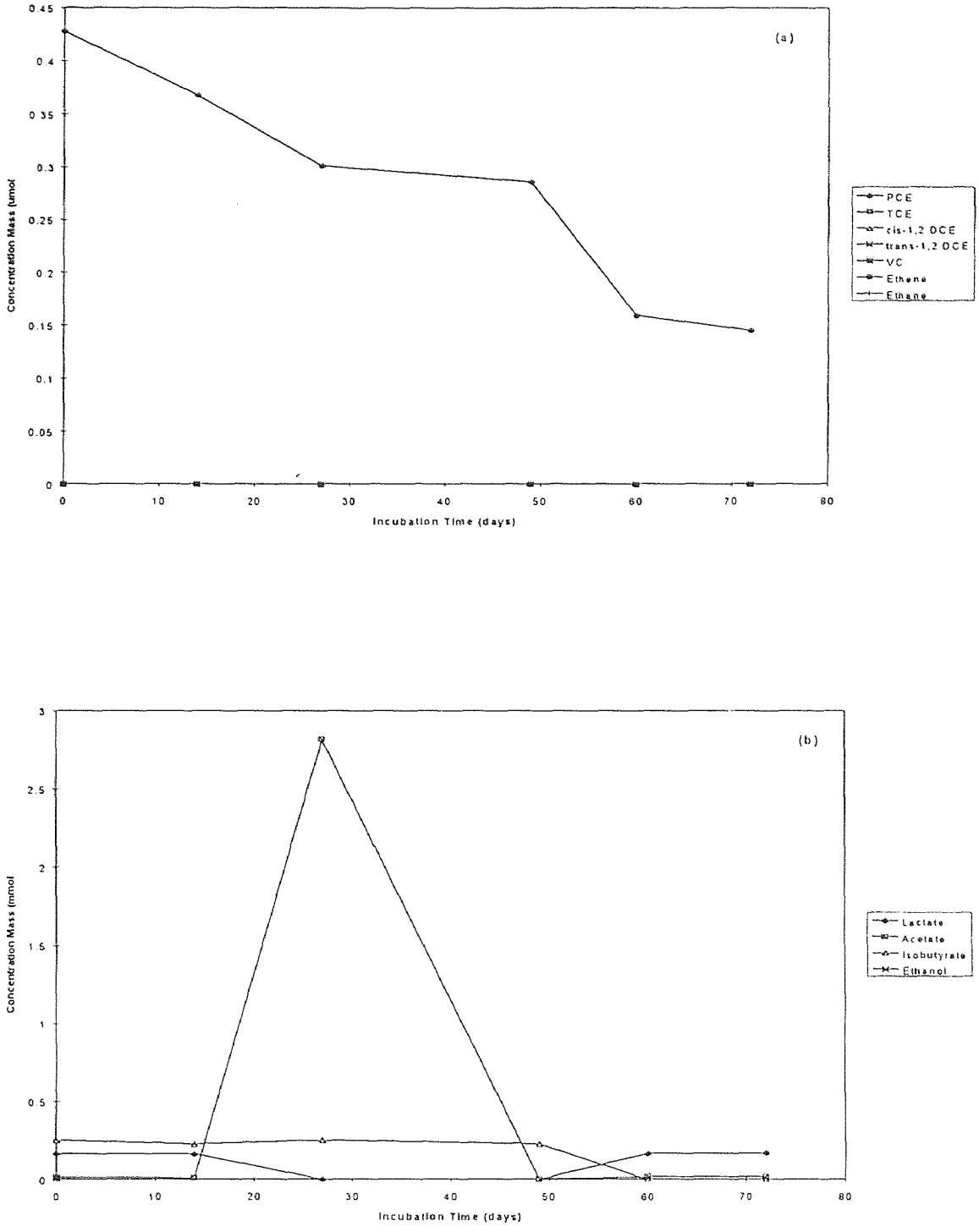


Figure D6 Concentration of chloroethenes (a) and electron donors (b) for the ***negative control*** microcosm supplemented with lactate

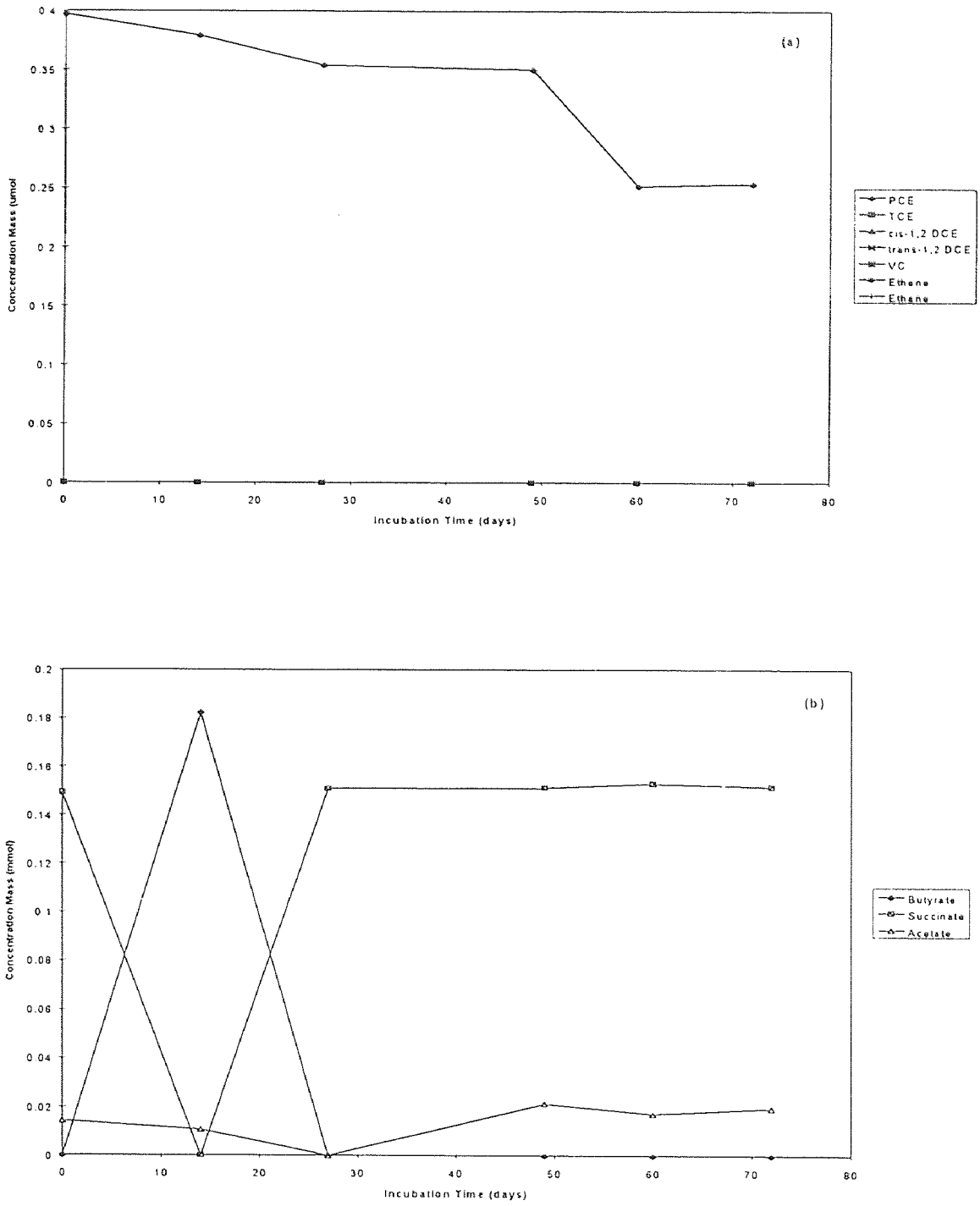


Figure D7 Concentration of chloroethenes (a) and electron donors (b) as a function of time for the ***negative control*** microcosm supplemented with succinate

APPENDIX E

DATA FOR THE INDIVIDUAL MICROCOSMS

Table E1 Chlorinated ethene data for the individual microcosms amended with ***methanol***											
	T=0	T=0	T=0	Average	SD		T=13	T=13	T=13	Average	SD
PCE	0.352	0.39	0.37	0.371	0.019		0.288	0.305	0.3204	0.3044	0.016
TCE	ND	ND	ND	ND			ND	ND	ND	ND	
cis-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
VC	ND	ND	ND	ND			ND	ND	ND	ND	
Ethene	ND	ND	ND	ND			ND	ND	ND	ND	
Ethane	ND	ND	ND	ND			ND	ND	ND	ND	
	T=27	T=27	T=27	Average	SD		T=41	T=41	T=41	Average	SD
PCE	0.097	0	0.07	0.05693	0.051		ND	ND	ND	ND	
TCE	ND	ND	ND	ND			ND	ND	ND	ND	
cis-DCE	0.138	0.174	0.1	0.1373	0.037		0.153	0.018	0.0134	0.0613	0.079
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
VC	0	0.164	0	0.05467	0.095		0.08	0.238	0.2129	0.1771	0.085
Ethene	ND	ND	ND	ND			0	0.031	0.013	0.0147	0.016
Ethane	ND	ND	ND	ND			ND	ND	ND	ND	
	T=55	T=55	T=55	Average	SD		T=69	T=69	T=69	Average	SD
PCE	ND	ND	ND	ND			ND	ND	ND	ND	
TCE	ND	ND	ND	ND			ND	ND	ND	ND	
cis-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
VC	0	0	0.28	0.09233	0.16		0	0	0.246	0.082	0.142
Ethene	0.227	0.233	0.04	0.166	0.111		0.221	0.15	0.005	0.1253	0.11
Ethane	0.002	0.004	0.01	0.00433	0.003		0.004	0.001	0.01	0.005	0.005

Table E2 Chlorinated ethene data for the individual microcosms amended with ***butyrate***

	T=0	T=0	T=0	Average	SD		T=14	T=14	T=14	Average	SD
PCE	0.495	0.501	0.48	0.49267	0.009		0.425	0.456	0.4618	0.4474	0.02
TCE	ND	ND	ND	ND			ND	ND	ND	ND	
cis-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
VC	ND	ND	ND	ND			ND	ND	ND	ND	
Ethene	ND	ND	ND	ND			ND	ND	ND	ND	
Ethane	ND	ND	ND	ND			ND	ND	ND	ND	
	T=28	T=28	T=28	Average	SD		T=49	T=49	T=49	Average	SD
PCE	0.042	0.179	0	0.07367	0.094		ND	ND	ND	ND	
TCE	0.022	0	0	0.00733	0.013		ND	ND	ND	ND	
cis-DCE	0.06	0.269	0.32	0.21683	0.138		ND	ND	ND	ND	
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
VC	ND	ND	ND	ND			0	0.504	0.469	0.3242	0.281
Ethene	0.006	0	0	0.00203	0.004		0.318	0	0	0.106	0.184
Ethane	ND	ND	ND	ND			ND	ND	ND	ND	
	T=60	T=60	T=60	Average	SD		T=72	T=72	T=72	Average	SD
PCE	ND	ND	ND	ND			ND	ND	ND	ND	
TCE	ND	ND	ND	ND			ND	ND	ND	ND	
cis-DCE	0.171	0	0	0.0569	0.099		ND	ND	ND	ND	
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
VC	0	0.525	0.46	0.3283	0.286		0.311	0	0.1322	0.1479	0.156
Ethene	0	0.004	0.02	0.008	0.011		7E-04	0.003	0.284	0.0959	0.163
Ethane	ND	ND	ND	ND			ND	ND	ND	ND	

Table E3 Chlorinated ethene data for the individual microcosms amended with ***butanol/ethanol***

	T=0	T=0	T=0	Average	SD	T=13	T=13	T=13	Average	SD
PCE	0.62	0.64	0.61	0.6237	0.015	0.418	0.513	0.544	0.4916	0.06571
TCE	ND	ND	ND	ND		ND	ND	ND	ND	
cis-DCE	ND	ND	ND	ND		ND	ND	ND	ND	
trans-DCE	ND	ND	ND	ND		ND	ND	ND	ND	
VC	ND	ND	ND	ND		ND	ND	ND	ND	
Ethene	ND	ND	ND	ND		ND	ND	ND	ND	
Ethane	ND	ND	ND	ND		ND	ND	ND	ND	
	T=27	T=27	T=27	Average	SD	T=41	T=41	T=41	Average	SD
PCE	0.53	0.47	0.4	0.467	0.063	0.382	0.308	0	0.2299	0.20253
TCE	ND	ND	ND	ND		ND	ND	ND	ND	
cis-DCE	ND	ND	ND	ND		0	0	0.352	0.1173	0.20323
trans-DCE	ND	ND	ND	ND		ND	ND	ND	ND	
VC	ND	ND	ND	ND		ND	ND	ND	ND	
Ethene	ND	ND	ND	ND		ND	ND	ND	ND	
Ethane	ND	ND	ND	ND		ND	ND	ND	ND	
	T=57	T=57	T=57	Average	SD	T=71	T=71	T=71	Average	SD
PCE	0	0.31	0.25	0.187	0.165	0	0	0.1128	0.0376	0.06513
TCE	ND	ND	ND	ND		ND	ND	ND	ND	
cis-DCE	0.36	0	0	0.119	0.206	0	0	0.0871	0.029	0.05029
trans-DCE	ND	ND	ND	ND		ND	ND	ND	ND	
VC	ND	ND	ND	ND		0	0.233	0	0.0777	0.13452
Ethene	ND	ND	ND	ND		0.27	0	0	0.09	0.15588
Ethane	ND	ND	ND	ND		0.005	0.004	0.002	0.0037	0.00153

Table E4 Chlorinated ethene data for the individual microcosms amended with ***butyrate/succinate***

	T=0	T=0	T=0	Average	SD	T=14	T=14	T=14	Average	SD
PCE	0.37	0.35	0.42	0.3804	0.04	0.369	0.407	0.4144	0.3969	0.02434
TCE	ND	ND	ND	ND		ND	ND	ND	ND	
cis-DCE	ND	ND	ND	ND		ND	ND	ND	ND	
trans-DCE	ND	ND	ND	ND		ND	ND	ND	ND	
VC	ND	ND	ND	ND		ND	ND	ND	ND	
Ethene	ND	ND	ND	ND		ND	ND	ND	ND	
Ethane	ND	ND	ND	ND		ND	ND	ND	ND	

	T=28	T=28	T=28	Average	SD	T=49	T=49	T=49	Average	SD
PCE	ND	ND	ND	ND		ND	ND	ND	ND	
TCE	ND	ND	ND	ND		ND	ND	ND	ND	
cis-DCE	0.26	0.21	0.17	0.2115	0.045	0	0	0.017	0.0057	0.00981
trans-DCE	ND	ND	ND	ND		ND	ND	ND	ND	
VC	0	0.17	0.24	0.1357	0.123	0.391	0.363	0.337	0.3636	0.0269
Ethene	ND	ND	ND	ND		ND	ND	ND	ND	
Ethane	ND	ND	ND	ND		ND	ND	ND	ND	

	T=60	T=60	T=60	Average	SD	T=72	T=72	T=72	Average	SD
PCE	ND	ND	ND	ND		ND	ND	ND	ND	
TCE	ND	ND	ND	ND		ND	ND	ND	ND	
cis-DCE	0	0.02	0	0.005	0.009	ND	ND	ND	ND	
trans-DCE	ND	ND	ND	ND		ND	ND	ND	ND	
VC	0.48	0.42	0.44	0.4468	0.033	0.265	0	0.1876	0.1507	0.13606
Ethene	0	0.02	0	0.005	0.009	0.015	0.008	0	0.0077	0.00751
Ethane	ND	ND	ND	ND		0.004	7E-04	0.001	0.0019	0.00182

Table E5 Chlorinated ethene data for the individual microcosms amended with ***lactate***

	T=0	T=0	T=0	Average	SD		T=15	T=15	T=15	Average	SD
PCE	0.491	0.49	0.48	0.4863	0.0074		0.504	0	0.219	0.241	0.2525
TCE	ND	ND	ND	ND			0	0	0.12	0.04	0.0695
cis-DCE	ND	ND	ND	ND			0	0.368	0.131	0.166	0.1863
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
VC	ND	ND	ND	ND			ND	ND	ND	ND	
Ethene	ND	ND	ND	ND			ND	ND	ND	ND	
Ethane	ND	ND	ND	ND			ND	ND	ND	ND	
	T=32	T=32	T=32	Average	SD		T=46	T=46	T=46	Average	SD
PCE	ND	ND	ND	ND			ND	ND	ND	ND	
TCE	ND	ND	ND	ND			ND	ND	ND	ND	
cis-DCE	0.184	0	0.14	0.1094	0.0968		ND	ND	ND	ND	
trans-DCE	0.086	0	0	0.0286	0.0495		ND	ND	ND	ND	
VC	0	0.39	0.18	0.1881	0.1944		0.324	0	0.392	0.239	0.2094
Ethene	0	0	0	0.0002	0.0003		0	0.278	0	0.093	0.1605
Ethane	ND	ND	ND	ND			ND	ND	ND	ND	
	T=59	T=59	T=59	Average	SD		T=69	T=69	T=69	Average	SD
PCE	ND	ND	ND	ND			ND	ND	ND	ND	
TCE	ND	ND	ND	ND			ND	ND	ND	ND	
cis-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
VC	0.377	0.29	0.23	0.3002	0.0723		0	0.172	0	0.057	0.099
Ethene	0.001	0	0	0.0012	0.0001		0.262	0	0.294	0.185	0.1613
Ethane	ND	ND	ND	ND			0.001	0.001	0.003	0.002	0.0012

Table E6 Chlorinated ethene data for the individual microcosms amended with ***succinate***

	T=0	T=0	T=0	Average	SD		T=15	T=15	T=15	Average	SD
PCE	0.452	0.45	0.44	0.4476	0.0043		0.407	0.431	0.463	0.434	0.0282
TCE	ND	ND	ND	ND			ND	ND	ND	ND	
cis-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
VC	ND	ND	ND	ND			ND	ND	ND	ND	
Ethene	ND	ND	ND	ND			ND	ND	ND	ND	
Ethane	ND	ND	ND	ND			ND	ND	ND	ND	
	T=32	T=32	T=32	Average	SD		T=46	T=46	T=46	Average	SD
PCE	0.255	0	0	0.0851	0.1474		ND	ND	ND	ND	
TCE	ND	ND	ND	ND			ND	ND	ND	ND	
cis-DCE	0	0.16	0.19	0.1183	0.1039		0.055	0.172	0.013	0.08	0.0824
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
VC	0	0.05	0.08	0.0414	0.0393		0.316	0.077	0.271	0.221	0.1271
Ethene	ND	ND	ND	ND			ND	ND	ND	ND	
Ethane	ND	ND	ND	ND			ND	ND	ND	ND	
	T=59	T=59	T=59	Average	SD		T=69	T=69	T=69	Average	SD
PCE	ND	ND	ND	ND			ND	ND	ND	ND	
TCE	ND	ND	ND	ND			ND	ND	ND	ND	
cis-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
VC	0.344	0.29	0	0.2103	0.1843		0.344	0.287	0.318	0.316	0.0282
Ethene	0	0.01	0	0.0033	0.0049		0	5E-04	0	2E-04	0.0003
Ethane	ND	ND	ND	ND			ND	ND	ND	ND	

Table E7 Chlorinated ethene data for the individual microcosms amended with ***butyrate/lactate***											
	T=0	T=0	T=0	Average	SD		T=14	T=14	T=14	Average	SD
PCE	0.2847	0.1944	0.347	0.2754	0.077		0.29	0.3	0.333	0.3062	0.025
TCE	ND	ND	ND	ND			ND	ND	ND	ND	
cis-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
VC	ND	ND	ND	ND			ND	ND	ND	ND	
Ethene	ND	ND	ND	ND			ND	ND	ND	ND	
Ethane	ND	ND	ND	ND			ND	ND	ND	ND	
	T=28	T=28	T=28	Average	SD		T=49	T=49	T=49	Average	SD
PCE	0.3186	0.3219	0.209	0.28313	0.064		ND	ND	ND	ND	
TCE	ND	ND	ND	ND			ND	ND	ND	ND	
cis-DCE	0.5983	0.0413	4.353	1.6643	2.345		6.58	5.01	0.898	4.1641	2.936
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
VC	ND	ND	ND	ND			0	0	0.251	0.0837	0.145
Ethene	ND	ND	ND	ND			ND	ND	ND	ND	
Ethane	ND	ND	ND	ND			ND	ND	ND	ND	
	T=60	T=60	T=60	Average	SD		T=72	T=72	T=72	Average	SD
PCE	ND	ND	ND	ND			ND	ND	ND	ND	
TCE	ND	ND	ND	ND			ND	ND	ND	ND	
cis-DCE	0.006	0	0	0.002	0.003		0	0	0.009	0.003	0.005
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
VC	0.354	0.3702	0	0.2414	0.209		0.21	0	0.222	0.1442	0.125
Ethene	0	0.007	0.212	0.073	0.12		0.16	0.02	0.015	0.063	0.083
Ethane	ND	ND	ND	ND			0	0	0.005	0.003	0.002

Table E8 Chlorinated ethene data for the individual microcosms amended with ***succinate/lactate***

	T=0	T=0	T=0	Average	SD		T=15	T=15	T=15	Average	SD	
PCE	0.4113	0.4651	0.443	0.43983	0.027		0.41	0.42	0.433	0.4188	0.013	
TCE	ND	ND	ND	ND			ND	ND	ND	ND		
cis-DCE	ND	ND	ND	ND			ND	ND	ND	ND		
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND		
VC	ND	ND	ND	ND			ND	ND	ND	ND		
Ethene	ND	ND	ND	ND			ND	ND	ND	ND		
Ethane	ND	ND	ND	ND			ND	ND	ND	ND		
	T=32	T=32	T=32	Average	SD		T=46	T=46	T=46	Average	SD	
PCE	ND	ND	ND	ND			ND	ND	ND	ND		
TCE	ND	ND	ND	ND			ND	ND	ND	ND		
cis-DCE	0.0075	0.0646	0.329	0.1337	0.172		0.02	0.02	0.017	0.0182	0.001	
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND		
VC	0.1618	0.2867	0.021	0.15643	0.133		0.31	0.37	0.318	0.3328	0.036	
Ethene	0.1469	0	0	0.04897	0.085		0.21	0	0	0.071	0.123	
Ethane	0.001	0.001	0.001	0.001	0		0	0	0.003	0.002	0.001	
	T=59	T=59	T=59	Average	SD		T=69	T=69	T=69	Average	SD	
PCE	ND	ND	ND	ND			ND	ND	ND	ND		
TCE	ND	ND	ND	ND			ND	ND	ND	ND		
cis-DCE	0.303	0	0	0.101	0.175		ND	ND	ND	ND		
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND		
VC	0.3533	0.3151	0.36	0.34273	0.024		0	0.16	0.207	0.1212	0.108	
Ethene	0.0013	0.013	6E-04	0.00497	0.007		0.25	0	9E-04	0.0848	0.146	
Ethane	0.015	0	0	0.005	0.009		0	0	0.001	0.001	0	

Table E9 Chlorinated ethene data for the individual microcosms amended with ***butanol***

	T=0	T=0	T=0	Average	SD	T=13	T=13	T=13	Average	SD
PCE	0.416	0.446	0.43	0.42967	0.0152	0.375	0.307	0.364	0.3488	0.0366
TCE	ND	ND	ND	ND		ND	ND	ND	ND	
cis-DCE	ND	ND	ND	ND		ND	ND	ND	ND	
trans-DCE	ND	ND	ND	ND		ND	ND	ND	ND	
VC	ND	ND	ND	ND		ND	ND	ND	ND	
Ethene	ND	ND	ND	ND		ND	ND	ND	ND	
Ethane	ND	ND	ND	ND		ND	ND	ND	ND	
	T=27	T=27	T=27	Average	SD	T=41	T=41	T=41	Average	SD
PCE	ND	ND	ND	ND		ND	ND	ND	ND	
TCE	ND	ND	ND	ND		ND	ND	ND	ND	
cis-DCE	0.196	0.246	0.09	0.17573	0.0823	ND	ND	ND	ND	
trans-DCE	ND	ND	ND	ND		ND	ND	ND	ND	
VC	0.12	0.055	0.33	0.168	0.1432	0.115	0	0	0.0382	0.0661
Ethene	ND	ND	ND	ND		0.154	0.269	0.266	0.2297	0.0655
Ethane	ND	ND	ND	ND		0.025	0.002	0.016	0.0143	0.0116
	T=55	T=55	T=55	Average	SD	T=69	T=69	T=69	Average	SD
PCE	ND	ND	ND	ND		ND	ND	ND	ND	
TCE	ND	ND	ND	ND		ND	ND	ND	ND	
cis-DCE	ND	ND	ND	ND		ND	ND	ND	ND	
trans-DCE	ND	ND	ND	ND		ND	ND	ND	ND	
VC	0.366	0	0.41	0.25723	0.2237	0.374	0.354	0.361	0.363	0.0101
Ethene	0	0.272	0	0.09067	0.157	0.004	0.013	0.003	0.0067	0.0055
Ethane	0.003	0.003	0	0.003	0	0.002	0.001	0.001	0.0013	0.0006

Table E10 Chlorinated ethene data for the individual microcosms amended with ***butanol/methanol***

	T=0	T=0	T=0	Average	SD	T=13	T=13	T=13	Average	SD
PCE	0.478	0.399	0.46	0.44433	0.0408	0.372	0.537	0.435	0.4479	0.0833
TCE	ND	ND	ND	ND		ND	ND	ND	ND	
cis-DCE	ND	ND	ND	ND		ND	ND	ND	ND	
trans-DCE	ND	ND	ND	ND		ND	ND	ND	ND	
VC	ND	ND	ND	ND		ND	ND	ND	ND	
Ethene	ND	ND	ND	ND		ND	ND	ND	ND	
Ethane	ND	ND	ND	ND		ND	ND	ND	ND	
	T=27	T=27	T=27	Average	SD	T=41	T=41	T=41	Average	SD
PCE	0.414	0.38	0.05	0.2808	0.2023	ND	ND	ND	ND	
TCE	0	0	0.12	0.0404	0.07	ND	ND	ND	ND	
cis-DCE	0	0	0.22	0.072	0.1247	0	0.038	0.069	0.0356	0.0344
trans-DCE	ND	ND	ND	ND		0.041	0	0	0.0137	0.0237
VC	ND	ND	ND	ND		0.371	0	0	0.1237	0.2142
Ethene	ND	ND	ND	ND		ND	ND	ND	ND	
Ethane	ND	ND	ND	ND		ND	ND	ND	ND	
	T=57	T=57	T=57	Average	SD	T=71	T=71	T=71	Average	SD
PCE	ND	ND	ND	ND		ND	ND	ND	ND	
TCE	ND	ND	ND	ND		ND	ND	ND	ND	
cis-DCE	0	0	0.11	0.03633	0.0629	ND	ND	ND	ND	
trans-DCE	ND	ND	ND	ND		ND	ND	ND	ND	
VC	0.453	0.34	0.21	0.3353	0.1201	ND	ND	ND	ND	
Ethene	0.011	0.033	0	0.01467	0.0168	0.241	0.317	0.294	0.284	0.039
Ethane	ND	ND	ND	ND		0.002	0.003	0.003	0.0027	0.0006

Table E11 Chlorinated ethene data for the individual microcosms amended with **propa*nol/methanol***

	T=0	T=0	T=0	Average	SD		T=13	T=13	T=13	Average	SD
PCE	0.443	0.4	0.44	0.4298	0.024		0.35	0.3467	0.338	0.3441	0.0053
TCE	ND	ND	ND	ND			ND	ND	ND	ND	
cis-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
VC	ND	ND	ND	ND			ND	ND	ND	ND	
Ethene	ND	ND	ND	ND			ND	ND	ND	ND	
Ethane	ND	ND	ND	ND			ND	ND	ND	ND	
	T=27	T=27	T=27	Average	SD		T=41	T=41	T=41	Average	SD
PCE	0.34	0.35	0.37	0.3536	0.014		0	0	0.24	0.08	0.13856
TCE	ND	ND	ND	ND			ND	ND	ND	ND	
cis-DCE	ND	ND	ND	ND			0.16	0.4001	0.105	0.2232	0.15604
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
VC	ND	ND	ND	ND			0.1	0	0	0.0339	0.05866
Ethene	ND	ND	ND	ND			ND	ND	ND	ND	
Ethane	ND	ND	ND	ND			ND	ND	ND	ND	
	T=57	T=57	T=57	Average	SD		T=71	T=71	T=71	Average	SD
PCE	ND	ND	ND	ND			ND	ND	ND	ND	
TCE	ND	ND	ND	ND			ND	ND	ND	ND	
cis-DCE	0.009	0.18	0	0.0631	0.102		ND	ND	ND	ND	
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND	
VC	0.281	0.08	0.21	0.192	0.1		0	0.2821	0.256	0.1795	0.15596
Ethene	0	0	0.05	0.017	0.029		0.03	0.006	0.006	0.014	0.01386
Ethane	0.003	0	0	0.0037	6E-04		0	0.004	0.005	0.0043	0.00058

Table E12 Chlorinated ethene data for the individual microcosms amended with ***methanol/ethanol***

	T=0	T=0	T=0	Average	SD		T=28	T=28	T=28	Average	SD	
PCE	0.417	0.51	0.44	0.4554	0.046		0.14	0.1383	0	0.0942	0.08163	
TCE	ND	ND	ND	ND			0.18	0.2132	0	0.1321	0.11541	
cis-DCE	ND	ND	ND	ND			0.11	0.1208	0.434	0.2204	0.18518	
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND		
VC	ND	ND	ND	ND			ND	ND	ND	ND		
Ethene	ND	ND	ND	ND			ND	ND	ND	ND		
Ethane	ND	ND	ND	ND			ND	ND	ND	ND		
	T=49	T=49	T=49	Average	SD		T=60	T=60	T=60	Average	SD	
PCE	ND	ND	ND	ND			ND	ND	ND	ND		
TCE	ND	ND	ND	ND			ND	ND	ND	ND		
cis-DCE	0.295	0.05	0.14	0.1591	0.126		ND	ND	ND	ND		
trans-DCE	ND	ND	ND	ND			ND	ND	ND	ND		
VC	0.1	0.34	0.27	0.2338	0.121		0.43	0.4821	0.43	0.4476	0.02991	
Ethene	0	0.03	0	0.0087	0.015		0.02	0.012	0.023	0.0187	0.00586	
Ethane	0.003	0	0	0.0033	6E-04		0	0.003	0.003	0.003	0	
	T=72	T=72	T=72	Average	SD							
PCE	ND	ND	ND	ND								
TCE	ND	ND	ND	ND								
cis-DCE	ND	ND	ND	ND								
trans-DCE	ND	ND	ND	ND								
VC	0	0.29	0	0.0964	0.167							
Ethene	0.312	0.04	0	0.116	0.171							
Ethane	0	0	0	0.002	0.002							

WORKS CITED

1. Holliger, C. "The Anaerobic Microbiology and Biotreatment of Chlorinated Ethenes." *Current Opinion in Biotechnology*. 6 (1995): 347-351.

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

3. Bagley, D., and J. M. Gosset. "Tetrachloroethene Transformation to Trichloroethene and *cis*-1,2-Dichloroethene by Sulfate-Reducing Enrichment Cultures." *Appl. Environ. Microbiol.* 56 (1990): 2511-2516.

4. Gao, J., R. S. Skeen, B. S. Hooker, and R.D. Quesenberry. "Effects of Several Electron Donors on Tetrachloroethylene Dechlorination in Anaerobic Soil Microcosms." *Wat. Res.* 31 (1997): 2479-2486.

5. Haley J. L., B. Hanson, C. Enfield, and J. Glass. "Evaluating the Effectiveness of Groundwater Extraction Systems." *Ground Water Monit. Rev.* 11 (1991): 119-124.

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

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

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

9. Vogel, T. M., and P. L. McCarty. "Abiotic and Biotic Transformations of 1,1,1-Trichloroethane under Methanogenic Conditions." *Environ. Sci. Technol.* 21 (1987): 1208-1213.

10. Kleopfer, R. D., D. M. Easley, B. B. Haas, T. G. Deihl, D. E. Jackson, and C. J. Wurrey. "Anaerobic Degradation of Trichloroethylene in Soil." *Environ. Sci. Technol.* 19 (1985): 277-280.

11. Wilson, B. H., G. B. Smith, and J. F. Rees. "Biotransformations of Selected Alkylbenzenes and Halogenated Aliphatic Hydrocarbons in Methanogenic Aquifer Material: A Microcosm Study." *Environ. Sci. Technol.* 20 (1986): 997-1002.

12. Barrio-Lage, G., F. Z. Parsons, R. S. Nassar, and P. A. Lourenzo. "Sequential Dehalogenation of Chlorinated Ethenes." *Environ. Sci. Technol.* 20 (1986): 96-99.

13. Barrio-Lage, G., F. Z. Parsons, and R. S. Nassar. "Kinetics of the Depletion of Trichloroethene." *Environ. Sci. Technol.* 21 (1987): 366-370.

14. Fatherpure, B. Z., and S. A. Boyd. "Dependence of Tetrachloroethylene Dechlorination on Methanogenic Substrate Consumption by *Methanosarcina* sp. Strain DCM." *Appl. Environ. Microbiol.* 54 (1988): 2976-2980.

15. Fatherpure, B. Z., J. P. Nengu, and S. A. Boyd. "Anaerobic Bacteria that Dechlorinate Perchloroethene." *Appl. Environ. Microbiol.* 53 (1987): 2671-2674.

16. Egli, C., T. Tschan, R. Scholtz, A. M. Cook, and T. Leisinger. "Transformation of Tetrachloromethane to Dichloromethane and Carbon Dioxide by *Acetobacterium woodii*." *Appl. Environ. Microbiol.* 54 (1988): 2819-2824.

17. Stevens, T. O., T. G. Linkfield, and J. M. Tiedje. "Physiological Characterization of Strain DCB-1, a Unique Dehalogenating Sulfidogenic Bacterium." *Appl. Environ. Microbiol.* 54 (1988): 2938-2943.

18. Lessinger, T. "Biodegradation of Chlorinated Aliphatic Compounds." *Current Opinion in Biotechnology.* 7 (1996): 295-300.

19. Hollinger, C., and W. Schumacher. "Reductive Dehalogenation as a Respiratory Process." *Antonie van Leeuwenhoek.* 66 (1994): 239-246.

20. Hollinger, C., and G. Schraa. "Physiological Meaning and Potential for Application of Reductive Dechlorination by Anaerobic Bacteria." *FEMS Microbiol. Rev.* 15 (1994): 297-305.

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

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

23. Fennel, D. E., and J. M. Gosset. "Comparison of Butyric Acid, Ethanol, Lactic Acid, and Propionic Acid as Hydrogen Donors for the Reductive Dechlorination of Tetrachlorethene." *Environ. Sci. Technol.* 31 (1997): 918-926.

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

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

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

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

28. Terzenbach, D. P., and M. Blaut. "Transformation of Tetrachloroethylene to Trichloroethylene by Homoacetogenic Bacteria." *FEMS Microbiol. Lett.* 123 (1994): 213-218.

29. Schink, B. "Inhibition of Methanogenesis by Ethylene and other Unsaturated Hydrocarbons." *FEMS Microbiol. Ecol.* 31 (1985): 63-68.

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

31. Hollinger, C., G. Schraa, A. J. M. Stams, and A. J. B. Zehnder. "A Highly Purified Enrichment Culture Couples the Reductive Dechlorination of Tetrachloroethene to Growth." *Appl. Environ. Microbiol.* 59 (1993): 2991-2997.

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

33. Magnuson, J. K., R. V. Stern, J. M. Gosset, S. H. Zinder, and D. R. Burris. "Reductive Dechlorination of Tetrachloroethene to Ethene by a Two-Component Enzyme Pathway." *Appl. Environ. Microbiol.* 64 (1998): 1270-1275.

34. Smith, R.L., M. L. Ceazan, and M. H. Brooks. "Autotrophic, Hydrogen-Oxidizing, Denitrifying Bacteria in Groundwater, Potential Agents for Bioremediation of Nitrate Contamination." *Appl. Environ. Microbiol.* 60 (1994): 1949-1955.

35. Brock, T. D. "Biology of Microorganisms." ed. Madigan, T. M., J. M. Martinko, and J. Parker. 8th ed. Prentice Hall, New Jersey, 1996, 172-175.

36. Schauder, R., and A. Kroger. "Bacterial Sulfur Respiration." *Arch. Microbiol.* 159 (1993): 491-497.

37. Dolfing, J. "Energetics and Kinetics of Two Complementary Hydrogen Sink Reactions in a Defined 3-Chlorobenzoate Degrading Methanogenic Consortium." In Microbiology and Biochemistry of Strict Anaerobes Involved in Interspecies Transfer. ed. Belaich, J., M. Bruschi, and J. Garcia. Plenum Press, New York, 1990.

38. Ueki, K., and A. Ueki. "Relationships Between Methanogenesis and Sulfate Reduction in Anaerobic Digestion of Municipal Sewage Sludge." In Microbiology and Biochemistry of Strict Anaerobes Involved in Interspecies Transfer. ed. Belaich, J., M. Bruschi, and J. Garcia. Plenum Press, New York, 1990.

39. Guiot, S. R. "Thermodynamical and Microbiological Evidence of Trophic Microniches for Propionate Degradation in a Methanogenic Sludge-Bed Reactor." In Microbiology and Biochemistry of Strict Anaerobes Involved in Interspecies Transfer. ed. Belaich, J., M. Bruschi, and J. Garcia. Plenum Press, New York, 1990.

40. Brock, T. D. "Biology of Microorganisms." ed. Madigan, T. M., J. M. Martinko, and J. Parker. 8th ed. Prentice Hall, New Jersey, 1996, 563-564.

41. Zeikus, J. G., R. Kerby, and J. A. Krzycki. "Single-Carbon Chemistry of Acetogenic and Methanogenic Bacteria." *Science* 227 (1985): 1167-1173.

42. Magli, A., F. A. Rainey, and T. Leisinger. "Acetogenesis from Dichloromethane by a Two-Component Mixed Culture Comprising a Novel Bacterium." *Appl. Environ. Microbiol.* 61 (1995): 2943-2949.

43. Kenealy, W., and J. G. Zeikus. "Influence of Corrinoid Antagonists on Methanogen Metabolism." *J. Bacteriol.* 146 (1981): 133-140.

44. Wolfe, R. S. "1776-1996: Alessandro Volta's Combustible Air." *ASM News* 62 (1996): 529-534.

45. Zeikus, J. G. "The Biology of Methanogenic Bacteria." *Bacteriol. Rev.* 41 (1977): 514-537.

46. Belay, N., and L. Daniels. "Production of Ethane, Ethylene, and Acetylene from Halogenated Hydrocarbons by Methanogenic Bacteria." *Appl. Environ. Microbiol.* 53 (1987): 1604-1610.

47. Johnson, A.C., and M. Wood. "Sulphate-Reducing Bacteria in Deep Aquifer Sediments of the London Basin: Their Role in Anaerobic Mineralization of Organic Matter." *J. Appl. Bacteriol.* 75 (1993): 190-197.

48. Brock, T. D. "Biology of Microorganisms." ed. Madigan, T. M., J. M. Martinko, and J. Parker. 8th ed. Prentice Hall, New Jersey, 1996, 661-691.

49. Shelton, D. R., and J. M. Tiedje. "Isolation and Partial Characterization of Bacteria in an Anaerobic Consortium that Mineralizes 3-Chlorobenzoic Acid." *Appl. Environ. Microbial.* 48 (1984): 840-848.

50. Gosset, G. M. "Measurement of Henry's Law Constants for C1 and C2 Chlorinated Hydrocarbons." *Environ. Sci. Technol.* 21 (1987): 202-208.

51. Zinder, S. H., and M. Koch. "Non-aceticlastic Methanogenesis from Acetate: Acetate Oxidation by a Thermophile Syntrophic Coculture." *Arch. Microbiol.* 138 (1984): 263-272.