THE EFFECT OF CFCs ON PCE BIODEGRADATION

by

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ABSTRACT

Biodegradation of PCE has been well studied. In studies where PCE was combined with other chlorinated solvents, the biologically mediated degradation of PCE and its sequentially produced byproducts was inhibited.

The objectives of this study were to examine the effect of selected CFCs on the degradation of PCE and the sequential degradation of PCE byproducts including TCE, cDCE, VC. The degradation of ethanol, acetate and propionate and the biodegradability of the CFCs were also examined. Two CFCs were initially selected for testing in combination with the chlorinated ethenes. These were 1,2,2-trichlorotrifluoroethane (CFC113) and 1,2-dichlorotetrafluoroethane (CFC114). A third CFC, 1,2-dichlorotrifluoroethane (HCFC123a), was produced as a breakdown product of CFC113 and was tested for its effect on the rate of PCE degradation.

All CFCs tested were found to inhibit PCE degradation and products of PCE degradation, with CFC113 being the most inhibitory. Degradation of CFC113 was also observed. Further research could examine methods to enhance CFC113 degradation in order to reduce inhibition of other chlorinated compounds.

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LIST OF ABBREVIATIONS[1]

Chemical Name (and alternate name)	Abbreviation
1,1-Dichloroethene	1,1DCE
1,2,2-Trichlorotrifluoroethane (1,2,2-Trichloro-1,1,2-trifluoroethane)	CFC113
1,2-Dichlorotetrafluoroethane	CFC114
1,2-Dichlorotrifluoroethane	HCFC123a
1-Chloro-1,2,2trifluoroethane	HCFC133
2-Chloro-1,2,2trifluoroethane	HCFC133b
Carbon Tetrachloride (Tetrachloromethane)	CT
Chlorofluorocarbene	CFCl
Chloroform (Trichloromethane)	CF
Chlorotrifluoroethene	CTFE
Chlorotrifluoromethane	CFC13
cis-Dichloroethene	cDCE
cis-Dichlorofluoroethene	cDCFE
Dichlorodifluoromethane	CFC12
Dichlorofluoromethane	CFC21
Difluorocarbene	CF_2
Distilled water	dH_2O
Ethanol	EtOH
Ethene	ETH
Perchloroethene (Tetrachloroethene)	PCE

[1] Occasionally the work of others may refer to a compound by its general name, without specifying the actual isomer. Where such reporting has been referenced in this work, the general name of the compound is provided in the text.

Tetrafluoromethane	CFC14
trans-Dichloroethene	tDCE
Trichloroethene	TCE
Trichlorofluoroethene	TCFE
Trichlorofluoromethane	CFC11
Vinyl Chloride (Chloroethene)	VC

1 INTRODUCTION

Chlorinated solvents such as perchloroethene (PCE) and trichloroethene (TCE) have for many years been widely used as cleaners, degreasers, dry-cleaning fluids and fumigants. These substances are suspected carcinogens and frequent groundwater contaminants, and they represent a serious threat to human health (Fathepure and Boyd, 1988). Due to their water solubility, 150 mg/L for PCE and 1100 mg/L for TCE (MacKay et al., 1993), these compounds are highly mobile in groundwater. A contaminant plume can extend for a great distance beyond the point at which contaminants are introduced to the groundwater (Fathepure and Boyd, 1988).

The need exists for the development of cost-effective methods for reducing and eliminating groundwater contamination. In many cases, natural attenuation of chlorinated compounds by bacterial processes may be the best solution. Cost generally favors biological methods over physical/chemical methods (Adamson and Parkin, 2000). Physical removal often results simply in the relocation of the contaminant. Through an understanding of the natural processes by which PCE can be eliminated from the environment, steps can be taken to improve the conditions under which these processes can be initiated or their removal rate increased.

The need for this study was brought about by conditions at a former industrial site where PCE-contaminated ground water was found in combination with polyhalogenated methanes and polyhalogenated ethanes. This condition has persisted for many years and it has been thought that over this period of time, contaminant levels would have naturally abated and that products of PCE breakdown would be found. Groundwater test results have shown the following concentrations: PCE 5.8mg/L, 1,2-dichlorotetrafluoroethane

(CFC114) 3.2mg/L, dichlorofluoromethane (CFC21) 3.7mg/L, dichlorotrifluoroethane (HCFC123) 3.6mg/L, 1,2,2-trichloro-1,1,2-trifluoroethane (CFC113) 12.0mg/L, carbon tetrachloride (CT) 1.0mg/L, chloroform (CF) 2.9mg/L and trichlorofluoromethane (CFC11) 12.0mg/L (Lauridsen, 1999). The analysis provided did not specify which HCFC123 isomer was detected. No other CFCs or chlorinated ethenes that could result from the degradation of PCE were detected. These data were obtained using purge and trap gas chromatography and mass spectra analysis (GC/MS) (Lauridsen, 1999).

Krone et al. (1991) studied the toxicity of CFCs to anaerobic bacteria. It is thought that CFCs inhibit PCE removal and/or the further degradation of PCE degradation products. The inhibitory effects of CT and CF on PCE degradation has been well studied (e.g. Bagley et al., 2000, Kaseros et al., 2000). The objectives of this work were to examine and describe the effects of selected CFCs on biodegradation of PCE and how the presence of these could account for the absence of PCE degradation seen at this industrial site. In addition, the biodegradability of selected CFCs was examined. Further studies beyond the scope of this work could lead to methods to overcome inhibitory effects of CFCs on PCE degradation. With an understanding of which chlorinated compounds are degradable under which circumstances, it may be possible to develop a remediation program for this industrial site that targets individual chlorinated compounds.

In order to examine the effect of various CFCs on anaerobic PCE-degrading cultures a number of experiments were carried out using live anaerobic bacterial cultures. The CFCs initially chosen for testing with PCE degradation were the haloethanes CFC113 and CFC114. Later, when the CFC113 breakdown product 1,2-

dichlorotrifluoroethane (HCFC123a) was formed and detected in several of the cultures being studied, additional experiments testing HCFC123a were conducted. The anaerobic degradation of CFCs has been described in other research and was expected to occur in this work, however the focus of this study was the degradation of PCE and the ways in which PCE degradation was influenced by other chlorinated compounds.

2 BACKGROUND

2.1 Degradation of Chlorinated Ethenes

Chlorinated alkenes were once thought to be resistant to biodegradation. In the 1980's, it was shown that perchloroethene (PCE) and other lesser-chlorinated species could be microbially degraded under anaerobic conditions in-vitro and in the environment (Vogel and McCarty, 1985; Freedman and Gossett, 1989). It was found that degradation proceeds via sequential reductive dechlorination resulting in the formation of lower chlorinated ethenes (Fathepure and Boyd, 1988). The discovery of anaerobic dechlorination of PCE by methanogens was important because these organisms are widespread in nature and have the potential to enable environmental technologies aimed at remediation of PCE contaminated soil and groundwater (Fathepure and Boyd, 1988).

Vogel and McCarty (1985) discussed the 'biotransformation' of PCE through trichloroethene (TCE), dichloroethene, vinyl chloride (VC) and carbon dioxide. It was reported that about 25% of PCE at a concentration less than 60μg/L was converted to carbon dioxide (Vogel and McCarty, 1985). Other subsequent researchers have not reported the formation of carbon dioxide.

Freedman and Gossett (1989) described complete reductive dechlorination of PCE to ethene, an environmentally acceptable product. The final products of the dechlorination of PCE were ethene, H^+ and Cl^- (Freedman and Gossett, 1989). The formation of ethane from the reductive dechlorination of PCE was described by DeBruin, et al. (1992). Anaerobic Rhine river sediment with 1μM lactate converted 9μM PCE to ethene and finally to ethane (95-98%). Other researchers did not observe ethane formation in similar studies.

Reductive dechlorination is the step-wise process of removing chlorine and replacing it with hydrogen on the PCE molecule. Other halogenated alkenes and alkanes may also degrade by a similar process. Under aerobic conditions, PCE and many chlorinated ethenes are resistant to biodegradation. Under anaerobic conditions, mixed methanogenic bacterial cultures have been found to cometabolise the degradation of PCE and lesser-chlorinated species via a process of sequential dechlorination. Each step of this process results in the formation of a lesser-chlorinated product plus the release of hydrogen (H^+) and chlorine (Cl^-) ions as shown in Fig 2.1 below.

The halogenated compound in this process is the electron acceptor. The electron donor shown in Fig 2.1 is hydrogen but another compound could also serve as the electron donor (DiStefano et al., 1991; Bagley et al., 2000).

Fig 2.1 Anaerobic dechlorination sequence for PCE (Freedman and Gossett, 1989)

Cometabolic reductive dechlorination tends to be more rapid for those compounds having a higher number of halogens per molecule (Fathepure and Boyd, 1988). Lesser-chlorinated species such as VC are often seen to persist in the environment after their precursors have been degraded. Unfortunately, the more persistent and lesser-chlorinated products such as vinyl chloride are often more of an environmental health risk to humans than their precursors. VC produced by the dechlorination of PCE tends to accumulate in the anaerobic environment (Fathepure and Boyd, 1988). Since both aerobic and anaerobic

pathways for the degradation of VC have been found, it has been suggested that the ideal treatment method for decontamination of VC-containing ground water is a sequential anaerobic/aerobic system (Fathepure and Boyd, 1988).

Fathepure and Boyd (1988) examined pure cultures of *Methanosarcina sp.* strain DCM in the dechlorination of PCE and the formation of TCE. It was hypothesized that methanogenesis was necessary to cause reductive dechlorination and that dechlorination would not occur once methane production ceased. Freedman and Gossett (1989) investigated the possible link between degradation of the chlorinated compound and methanogenesis and were inconclusive in this regard but noted that indirect evidence suggests that methanogens play a key role. Bagley and Gossett (1990) reductively dechlorinated PCE to produce TCE and cDCE using sulphate-reducing enrichment cultures. That study was initially based on the hypotheses that dechlorination took place only when the organisms are actively producing methane (Bagley and Gossett, 1990), however degradation of PCE to TCE and cDCE in the absence of methanogenesis was demonstrated. This was regarded as an indication that organisms other than methanogens may be responsible for dechlorination. Cultures that were inhibited with bromoethane sulphonic acid (BES) or with fluoroacetate (an acetate inhibitor) produced very little methane but readily degraded PCE (Bagley and Gossett, 1990).

By 1991, it was clear that PCE was being reduced to various lesser-chlorinated products in anaerobic systems and that reductive dechlorination could take place in the absence of methanogenesis. This degradation was being observed under different conditions, with different organisms and electron donors. But what was not clear at that time was whether microbial growth was taking place, using PCE as the electron acceptor.

DiStefano et al. (1991) showed that microbial growth was resulting from the degradation of PCE as the electron acceptor. A study was conducted using a methanogenic culture to which increasing amounts of PCE were being added, as high as 55 mg/L in aqueous solution. At 55mg/L of PCE, methane production ceased entirely and the number of methanol-utilizing methanogens had decreased dramatically, and non-methanogenic PCE dechlorinating organisms began to dominate. Table 2.1 provides a few examples of mixed cultures and microbes and their varying abilities to degrade PCE and their various end products.

A PCE grown strain of *Dehalococcoides ethenogenes 195* has been identified that will dechlorinate PCE completely to ethene (Maymó-Gatell et al., 1997; Maymó-Gatell et al., 1999). The substrate utilization pattern was similar to that seen in mixed culture except that VC utilization was slower in the pure culture (Maymó-Gatell et al., 1999). *Dehalospirillum multivorans* catalyses the dehalogenation of TCE to cDCE. Other organisms thought to participate in the reductive dechlorination of PCE include *Sporomusa ovata* and *Dehalobacter restrictus* (Ellis, 2000). *Dehalococcoides ethanogenes 195* is reported to produce the trans isomer of dichloroethene, tDCE from TCE (Ellis, 2000). Many organisms making use of various electron donors in the reductive dechlorination of PCE have now been identified.

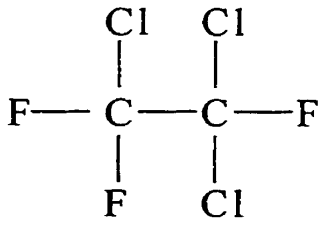
Table 2.1 PCE Dechlorinating Organisms

Organism or Culture	Action	End Product	Electron Donors	Reference
Methanosarcina sp. Strain DCM	dechlorinated PCE, only during formation of methane	TCE	acetate, methanol, methylamine, trimethylamine	Fathepure and Boyd, 1988
Sulphate reducing enrichment culture (unidentified organism)	dechlorinated to TCE and cDCE	cDCE	(various)	Bagley and Gossett, 1990
Thermophilic enrichment culture (85% *Dehalobacter Restrictus*)	dechlorinated PCE at 60°C	cDCE	various VFAs	Kengen et al.,1999
Desulfitobacterium sp. PCE1	bacterial growth by dechlorination of PCE and ortho-chlorinated phenols	TCE, cDCE, tDCE, phenols dechlorinated at the ortho-position	various VFAs	Gerritse, et al., 1996
Desulfitobacterium frappieri TCE1	dechlorinated PCE	cDCE	L-lactose	Gerritse, et al., 1999
Dehalococcoides ethenogenes 195	dechlorinated PCE	Ethene	hydrogen, methanol, butyrate	Maymó-Gatell et al., 1997, Maymó-Gatell et al., 1999

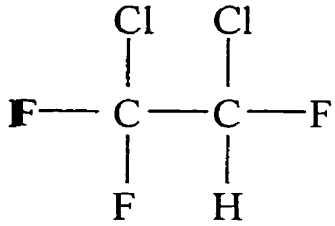
2.2 Degradation of Selected CFCs

A study was conducted by Lesage et al. (1992) to test the degradation of CFC113 by anaerobic mixed microbial cultures. That study showed that the degradation products of CFC113 included 1,2-dichlorotrifluoroethane (HCFC123a) (Fig 2.2) and chlorotrifluoroethene (CTFE) (Fig 2.3). CTFE is the most toxic byproduct of CFC113 degradation and is of concern in the environment (Lesage et al. 1992). These two byproducts of CFC113 degradation are not produced sequentially as was once

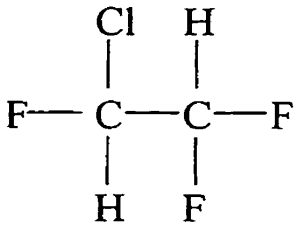
hypothesized by Lesage et al. (1992) and are now believed to follow different degradation pathways. Lesage et al. (1992) also provided evidence for the formation of breakdown products of HCFC123a that included 1-chloro-1,2,2-trifluoroethane (HCFC133) and 2-chloro-1,2,2-trifluoroethane (HCFC133b) (Fig 2.2).



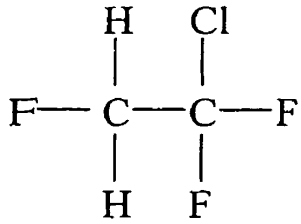
1,2,2-Trichlorotrifluoroethane
CFC113



1,2-Dichlorotrifluoroethane
HCFC123a



1-Chloro-1,2,2-trifluoroethane
HCFC133



2- Chloro-1,2,2-trifluoroethane
HCFC133b

Fig 2.2 Halogenated ethanes

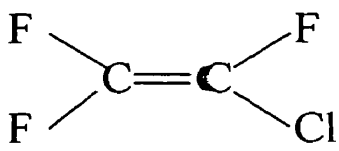


Fig 2.3 Chlorotrifluoroethene (CTFE)

The amount of published research on the degradation of CFCs is small compared with the work published on PCE degradation. Those studies relevant to this work are summarized in Table 2.2.

Table 2.2 Summary of CFC Degradation Studies

CFC	Degradation Product	System	Reference
CFC11 CFC12 CFC13	CO formate (HCOOH)	Corrinoid catalyst, titanium citrate III electron donor	Krone et al., 1991
CFC113	HCFC123a CTFE	Anaerobic landfill leachate, hematin	Lesage et al., 1992
HCFC123a	HCFC133 HCFC133b	Anaerobic landfill leachate, hematin	Lesage et al., 1992
CFC11, CFC12, CFC113	Products not identified	Enrichment culture, digester sludge, freshwater sed.	Denovan and Strand, 1992
CFC113, several halogenated compounds	Products not identified	*P. putida* G786 (pHG-2)	Hur et al., 1994
CFC11	Products not identified	Anaerobic groundwater with TCE	Sonier et al., 1994
Trichlorofluoroethene (TCFE)	cis-dichlorofluoroethene (cDCFE)	Anaerobic groundwater, mixed VFA electron donors	Sanjay, et al., 1999

There has been very little published work done on biodegradation of CFC113 and CFC114, other than that of Lesage (1992) and Hur et al., (1994) listed above. It is apparent however, that CFCs are degraded by similar microbial systems to those seen in PCE degradation and for this reason, it is reasonable to expect CFC113 and CFC114 to be degraded by PCE degrading cultures.

2.3 Relevant Biochemistry of Dehalogenating Systems

Methanogens, acetogens and sulfate-reducing bacteria have been found to contain reduced metal cofactors including corrinoids, hemes and cofactor F_{430} which can act as catalysts and reductively dechlorinate PCE in what is regarded as a cometabolic process (Lesage et al., 1992; Maymó-Gatell et al., 1999).

> "Large cyclical metal containing molecules known as metallomacrocycles or corrinoids, found in bacteria have been found to catalyze reductive dehalogenation reactions *in vitro*. Nickel containing coenzyme F_{430}, cobalt containing vitamin B-12 and iron-porphyrins (hematin) are redox active cofactors produced by diverse bacterial species. *In vitro* the reduced cofactors can catalyze an overall two-electron transfer to carbon-halogen bonds, yielding a free halide anion. *In vivo*, bacterial proteins containing metal cofactors may figure prominently in environmental reductive dehalogenase reactions."
>
> (Li and Wackett, 1993).

Corrinoids are non-polymeric complex structures, found in mammals and are produced by microbes in the gut. The corrinoids are a group of compounds containing four reduced pyrrole rings joined into a macrocyclic ring by links between their α positions. Three of these links are formed by a one-carbon unit (methylidyne radicals) (the (a) positions in Fig 2.4); and the other by a direct Cα-Cα bond (position (b) in Fig 2.4). They include various B-12 vitamins, Cytochrome P-450_{CAM} monoxygenase and derivatives based upon the skeleton of *corrin*, $C_{19}H_{22}N_4$ pictured in Fig 2.4.

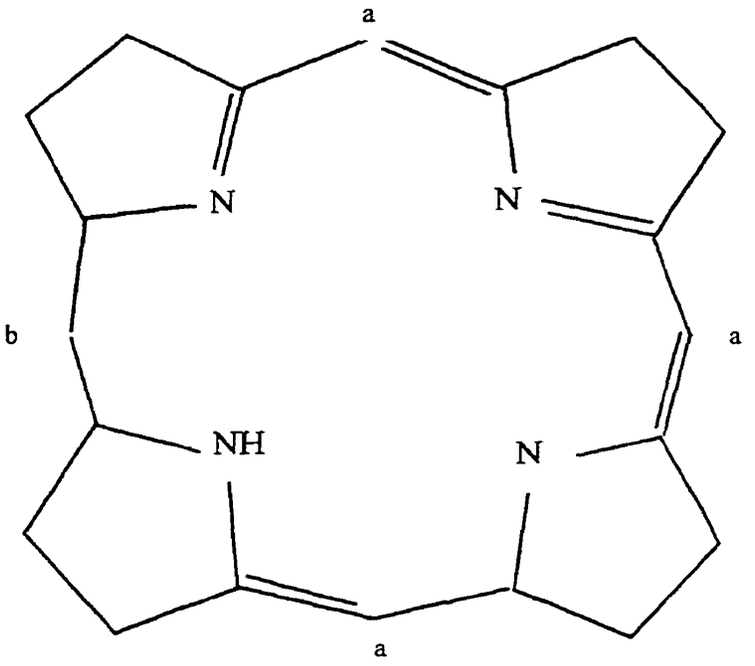


Fig 2.4 Structure of Corrin (Modified from Moss, 2000)

The structure in Fig 2.4 is the basic corrin macrocyclic ring without the central metal atom found in microbial metal-corrinoids. This is the typical base upon which those metallomacrocycles are formed. The name "corrin" was proposed by those who established its structure because it is the core of the vitamin B-12 molecule; the letters "co" of corrin are *not* derived from the fact that vitamin B-12 contains cobalt (Moss, 2000).

In order to catalyze reactions, enzymes join with the substrate at the enzyme's active site to form enzyme-substrate complexes. Only certain substrates can bind to the enzyme's active site (Krone et al., 1989). The rate of reaction generally decreases with the number of halogens per molecule (Lesage et al. 1992).

Cytochrome P-450_{CAM} monoxygenase, is a heme protein that is known to catalyze non-physiological reductive reactions including the reduction of polyhalogenated

compounds such as PCE. The products, including TCE, acted as substrates for toluene dioxygenase and were further degraded (Li and Wackett, 1993).

The cytochrome P-450 enzyme is known to be capable of catalyzing reductive dechlorination in microbial and mammalian systems. In methanogenic bacteria, corrinoids and coenzyme F_{430} may also be involved as catalysts (Krone et al., 1989). Corrinoids have been shown to catalyze the sequential reduction of tetrachloromethane, trichloromethane, dichloromethane and chloromethane with titanium citrate as the electron donor (Krone et al., 1989). The recovery of the byproducts of tetrachloromethane degradation of was less than 50% suggesting that other products were being formed.

Many anaerobic bacteria catalyze the first two steps of sequential reductive dehalogenation using the cofactors heme, cofactor F_{430} and corrinoids such as aquocobalamin and methylcobalamin (Tripp et al., 2000). A recent study investigated the effect of increased concentrations of hydroxocobalamin on the biodegradation of 72mg/L aqueous CT in anaerobic pure cultures of *Acetobacterium woodii.* It was shown that the CT degradation rate was increased thirty times over that of control cultures (Hashsham and Freedman, 1999). Table 2.3 lists a summary of dehalogenating corrinoids.

Table 2.3 Corrinoids in Organisms

Name	Metal	Location/Purpose	Reference
cobalamins	Co	gut microbes	Krone et al., 1989
Cytochrome P-450	Fe	diverse bacterial species	Li and Wackett, 1993
Hematin	Fe	diverse bacterial species	Lesage et al., 1992
Coenzyme F430	Ni	diverse bacterial species	Li and Wackett, 1993
Cytochrome P-450$_{CAM}$	Fe(III)	liver micrososmes	Li and Wackett, 1993
Cytochrome P-450$_{CAM}$	Fe(III)	genetically engineered *P. pitudaG786*	Wackett et al., 1994
hydroxocobalamin (Vitamin B12)	Co	uses the Acetyl-CoA pathway	Hashsham and Freedman, 1999

A methylated corrinoid protein may be the mediator of the dehalogenation of chlorinated C_1 hydrocarbons in anaerobic bacteria (Krone et al., 1991). A similar process may be involved in the dehalogenation reactions of C_2 based CFCs. All bacteria capable of reductive reactions contain this corrinoid protein.

Reductive dehalogenation can be facilitated by the use of a cyclical transfer of electrons through the acetyl-CoA pathway (Krone et al., 1991). The anaerobes known to mediate dechlorination reactions have in common that they use an acetyl-CoA/carbon monoxide dehydrogenase pathway for the degradation and synthesis of acetyl-CoA. This process includes the key step of the reversible transfer of the methyl group of acetyl-CoA (Krone et al., 1991). Organisms that were found to rapidly transform CT at high rates made use of the acetyl CoA pathway and have correspondingly high levels of corrinoids (Hashsham and Freedman, 1999).

Krone et al. (1991) examined the reduction of halomethanes and found that reduced corrinoids were found to reductively dechlorinate chlorotrifluoromethane (CFC11), dichlorodifluoromethane (CFC12), and trichlorofluoromethane (CFC13). Krone et al. (1991) also found that tetrafluoromethane (CFC14) was not reduced,

suggesting that the higher strength fluorine-carbon bond is resistant to reductive dehalogenation catalyzed by corrinoids.

2.4 Inhibition of PCE Degradation

Various factors are known to affect the rate and completeness of PCE dechlorination, including competition from other anaerobic microorganisms for added electron donor and hydrogen, byproduct concentrations and microbial ecology of the system including concentration of PCE degrading biomass (Bagley, 1998). In addition to these factors, the presence of other halogenated aliphatic compounds can inhibit to degradation rate. It has also been shown that in some systems, PCE is inhibitory to VC degradation and that rapid VC degradation begins only when PCE is depleted (Maymó-Gatell et al, 1999; DiStefano, 1999).

Mixtures of chlorinated methanes and chlorinated ethenes have been found to degrade more slowly than anaerobic cultures containing only chlorinated ethenes. It has been found in studies with carbon tetrachloride (CT) that the addition of 19μM CT completely inhibited PCE degradation as did 4μM chloroform (CF) although 0.7μM CF was not inhibitory (Bagley et al., 2000). In addition to being more recalcitrant, CF was a more potent inhibitor of anaerobic systems than CT (Kaseros, et al., 2000). An anaerobic PCE degrading culture in a column study that had been receiving PCE for 161 days had not developed PCE degradation capability while CT was being degraded to CF and dichloromethane (DCM) (Bagley et al., 2000). *In situ* the degradation of PCE could be adversely affected by the presence of CT and CF (Bagley et al., 2000).

Maymó-Gatell et al. (1999) showed that that the presence of PCE inhibited the reduction of VC to ethene. A dose of PCE added to a butyrate-grown enrichment culture that had previously received several doses of PCE was converted stoichiometrically to VC following zeroth order kinetics. VC conversion to ethene began once PCE had been depleted and followed first order kinetics (Maymó-Gatell et al., 1999). Another study (DiStefano, 1999) using mixed anaerobic digester sludge inoculum concluded that the presence of PCE and/or TCE, DCE is (are) required to sustain VC dechlorination and that complete dechlorination may be a multi step process. Differences in the microbial consortium may account for the opposing conclusions of the two similar studies.

No studies have been found that explore the possible inhibition of biodegradation of PCE by CFCs in an anaerobic system. The reductive dehalogenation of CFCs is catalyzed by corrinoids and may involve the intermediate formation of dihalocarbenes. The intermediate formation of chlorofluorocarbene (CFCl) in the reduction of $CFCl_3$ (CFC11) was noted by Krone et al. (1991), as was the intermediate formation of difluorocarbene (CF_2) from reduction of CF_2Cl_2 (CFC12). The reductive dehalogenation of CCl_4 to CO may also involve a dichlorocarbene that is expected to be more reactive than chlorofluoro- or difluorocarbene. In the cases of CCl_4, $CFCl_3$ and CF_2Cl_2, the formation of very reactive carbenes under reducing conditions may explain their toxicity to anaerobic bacteria. These compounds may be considered mechanism-based inhibitors of the enzyme system that catalyses the reversible formation of acetyl-CoA in the acetyl-CoA/CO pathway (Krone et al. 1991).

3 METHODS AND MATERIALS

3.1 Experimental Approach

The primary objective of these experiments was to examine the effect of a particular concentration of a CFC on the rate of PCE degradation as compared with a control not containing CFC. Other objectives were to examine the effect of the presence of CFC on:

a) The formation and degradation of PCE byproducts

b) PCE degradation over time

c) The ability of CFC inhibited cultures to recover their PCE degrading ability after the CFC was removed

d) The possible degradation of CFC

e) The effect of CFC breakdown products on PCE degradation.

All cultures were prepared from a robust anaerobic parent culture that had been actively degrading PCE and producing lesser-chlorinated products including ethene. For each experiment, groups of between 2 and 5 replicate PCE degrading daughter culture bottles were typically prepared. One or more of these was selected for use as a control to be fed only a solution of PCE and ethanol. The others were fed an initial dose of a solution of CFC, PCE and ethanol. This initial feeding was to provide a dose of CFC that remained for the duration of the experiment, sometimes degrading slowly. Subsequent feedings were done using the same PCE/ethanol stock solution being fed to the PCE degrading control. These sets of culture bottles were then subjected to PCE degradation rate tests.

The analytical method for most compounds of interest was headspace analysis using various gas chromatography methods, depending on the analyte. To facilitate the use of uniform calibration for all bottles, all cultures, water controls and standards were prepared in the same size bottles having the same liquid/headspace ratio. This enabled the use of the same calibration data and the same ratios for determining liquid phase concentration of a particular analyte given total mass. Note that all bottles were prepared in the same manner unless otherwise stated. The average bottle volume was 121.7mL with a liquid volume of 70mL and the balance headspace volume. The experimental temperature was 22°C and the internal pressure of the bottles was approximately 1 atmosphere.

PCE degradation rate testing was often finished within the same 24-hour period as the PCE dose was added. Taking several measurements over a period of time (usually four to twenty-four hours) following the addition of PCE, the PCE concentration and time were recorded and plotted separately for each period in which the PCE concentrations were greater than zero. The slope of the best-fit straight line through the data set following each addition of PCE and the instantaneous PCE degradation rate for that period was calculated.

3.2 Materials Used

Perchloroethene (PCE) (neat liquid), 99%, 1,2-dichlorotrifluoroethane (HCFC123a) (neat liquid), 99.5%, were purchased from Caledon Labs, Georgetown, Ontario. Trichloroethene (TCE) (neat liquid), 99.5%, cis-dichloroethene (cDCE) (neat liquid), 97%, trans-dichloroethene (tDCE) (neat liquid), 98%, 1,1-dichloroethene (1,1-

DCE) (neat liquid), 99%, 1,2,2-trichloro-1,1,2-trifluroethane (CFC113) (neat liquid), 99.8% and 1,2-dichlorotetrafluoroethane (CFC114) (pure gas), 99%, were purchased from Sigma-Aldrich Canada, Mississauga, Ontario. Vinyl chloride (VC) (gas), 1000ppm by volume, was purchased from Scott Specialty Gases, Plumsteadville, PA, USA. Acetic acid, 99.8% and propionic acid, 99% were purchased from BDH, Poole, England. Ethanol, 200 Proof, 99.5%, was purchased from Commercial Alcohols Inc., Brampton, Ontario. Compressed gases: nitrogen UHP 5.0 grade, compressed air Ultra Zero grade, helium UHP 5.0 grade, hydrogen UHP 5.0 grade used in purging and gas chromatography were obtained from Praxair, Brampton, Ontario.

Several of the materials analysed in this study are gases at ambient lab temperature. Samples of these, to be used in preparing calibration standards were obtained in gaseous form, including CFC114, HCFC123a and VC. In these cases, gases were measured by volume at atmospheric pressure rather than weight, and using the ideal gas law, molar quantities of these materials were determined.

3.3 Culture Source and Maintenance

3.3.1 Culture Source

The culture used in this research was originally obtained from a sludge sample taken from the sludge digester at the Main Sewage Treatment Plant in Toronto in 1996. An anaerobic bench scale reactor was started using 1.5L of anaerobic sludge that was diluted with 1.5L of basal medium (See Fig 3.1). The mixture was purged with N_2 to maintain anaerobic conditions and fed with ethanol. The culture produced methane. The culture was used in continuous flow column experiments during 1997 (Lalonde, 1997). A

2m x 100mmID stainless steel column having injection valves spaced at 100mm intervals was used for column experiments with a mixture of PCE, TCE and tetrachloromethane (CT). Ethanol was the electron donor. The column was inoculated by injecting 5mL of the anaerobic culture to each injection port on the column. The column was initially fed a mixture containing 5mg/L each of PCE, TCE and CT and 100mg/L ethanol, but later was re-inoculated and received only PCE, ethanol and yeast extract. PCE degradation was then observed but cDCE was recalcitrant in both column and microcosm studies undertaken using the culture (Lalonde, 1997).

V. Kaseros assumed operation of the column and its contents. By late 1997, PCE was being completely converted to VC and ethene. In 1998, experiments involving PCE, CT and chloroform (CF) were conducted. These experiments showed the inhibitory effects of CT and CF on PCE degradation (Kaseros, 1998).

A. Brown (2000) assumed operation of the column in 1999. The column was run for five months with 5 mg/L PCE, 2.8 mg/L ethanol and 2 mg/L yeast extract following which, PCE was increased to 10 mg/L and ethanol and yeast extract were gradually eliminated entirely. Complete PCE degradation to ethene was observed. The column was operated as such for 368 days with slowly decreasing PCE degradation capability (Brown, 2000).

In August 1999, while the column was in continuous operation by Brown, G. Zimmer withdrew a 150mL sample from the column. This sample was stored under a 90% N_2/ 10% CO_2 headspace in a 250mL bottle fitted with a Mininert® valve and fed with methanol. In February 2000, two 122mL-culture bottles were started using 10mL samples from this reactor plus 60mL of basal medium. These were labeled A1a0202 and

A1b0202 respectively (See Fig 3.2). All the PCE degrading cultures used in this work were descended from A1b0202.

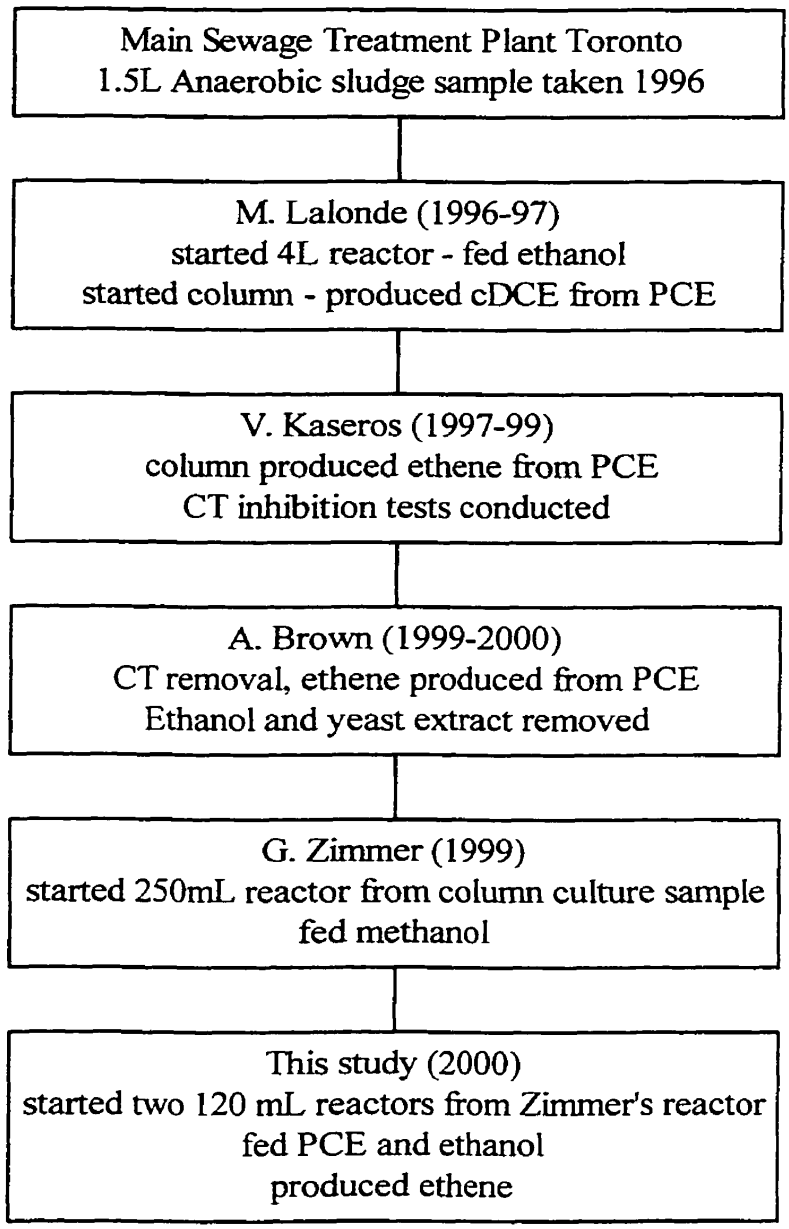


Fig 3.1 Culture Development

Culture bottles were prepared as groups of two to five bottles as required for the experiments contemplated. The chart shown in Fig 3.2 describes the genealogy of the groups of cultures used in each experiment in this work. All culture bottles prepared in the lab were identified as per the identification code described in Figure 3.3.

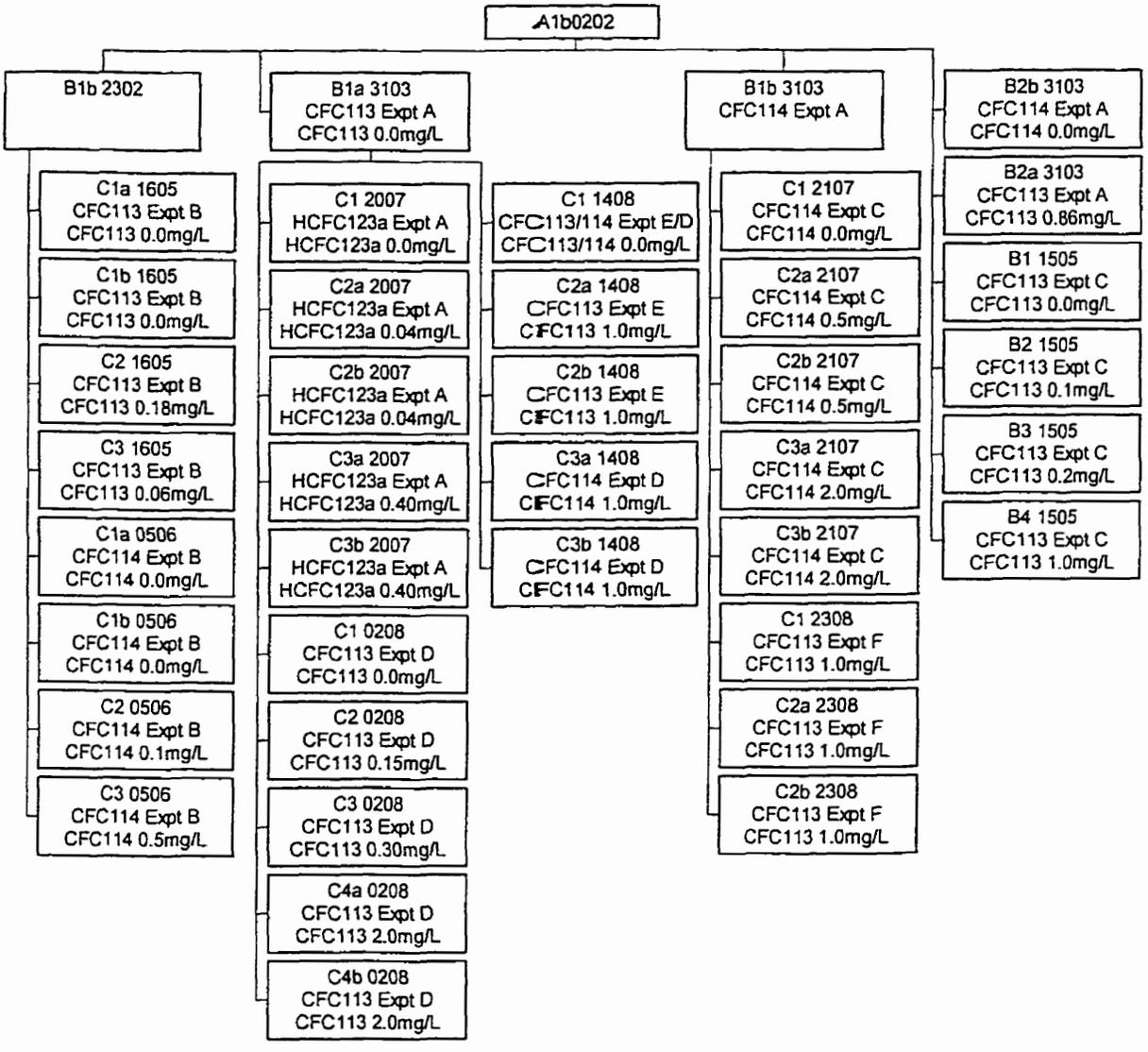


Fig 3.2 Culture Genealogy and initial CFC concentrations

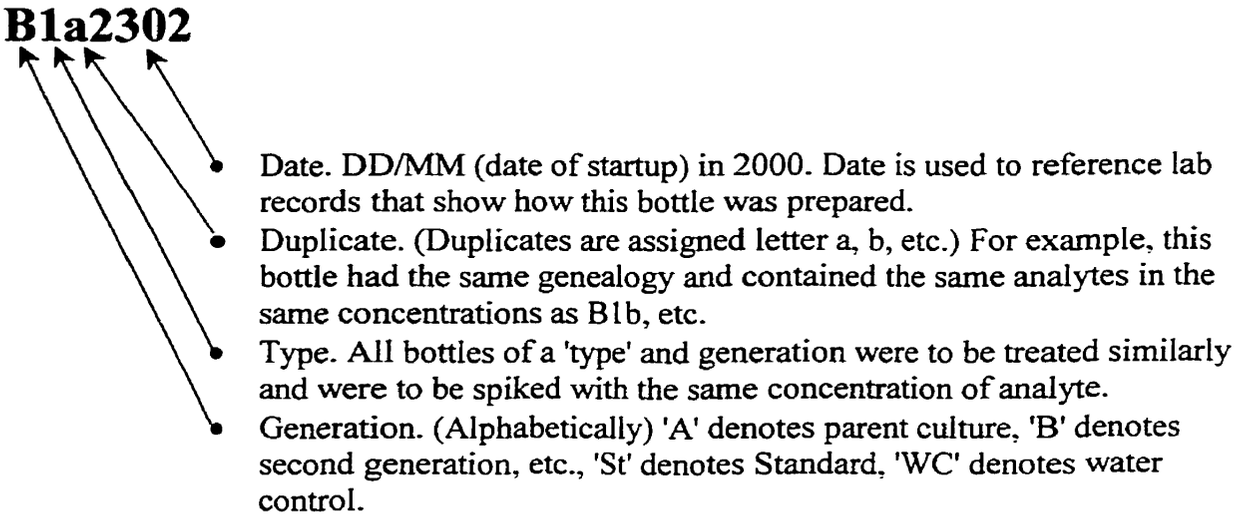


Fig. 3.3 Description of Bottle Nomenclature

3.3.2 Culture Maintenance and Transfer

The basal medium used to prepare and dilute cultures was formulated based on the recipes used by other researchers examining anaerobic dechlorination. In previous work done using the same source culture degrading PCE, Lalonde (1997) and Kaseros (1998) used yeast extract at 100mg/L (See Appendix A Table A1). This resulted in robust degradation of PCE. The basal medium described in Table 3.1 is very similar to that used by previous researchers except for an increase in $NaHCO_3$ in order to buffer the anticipated pH depressing effects of reductive dechlorination and the formation of acetic and propionic acids.

Table 3.1 Basal Medium

Constituent	mg/L
NH_4Cl	60
K_2HPO_4	20
$CaCl_2 2H_2O$	50
$MgCl_2$	100
$FeSO_4$	15
$NaHCO_3$	2000
$Na_2S 9H_2O$	100
$FeCl_2 4H_2O$	20
Resazurin	1
Yeast Extract	100
Trace Metals	
$MnSO4H_2O$	0.86
$CoCl_2 6H_2O$	1.7
$ZnSO_4$ $7H_2O$	2.1
H_3BO_3	0.19
$NiCl_2 6H_2O$	0.5
$NaMoO_4$ $2H_2O$	0.2

All cultures were prepared in 121.7mL clear glass serum vials, fitted with Teflon® lined rubber septum seals and aluminum crimp caps. Prior to the transfer of inoculum, parent cultures were purged with nitrogen to remove chlorinated ethenes and other volatile substances using the purging apparatus as described later (Section 3.4.1). The transfer took place inside an anaerobic glove box containing a 10% CO_2, 90% N_2 mix. In all cases, 10mL of inoculum from the parent culture plus 60 mL of basal medium were added to each bottle to provide a total of 70mL of liquid in each bottle. A PCE/ethanol stock solution consisting of 35% by mass PCE, balance ethanol was prepared. This stock solution was designed to provide 10mg/L aqueous PCE and 27mg/L ethanol per 70mL culture from a 3μL injection (See Appendix A, Table A2, p.115). The sets of bottles were fed the PCE/ethanol stock solution at two to five day intervals until rapid PCE

degradation was apparent, typically in the range of 1-2 μmols $bottle^{-1}$ hr^{-1}. Following the start-up of a new generation, two or three feedings were usually required to ensure that the culture was sufficiently active to begin an experiment.

3.4 Experimental Techniques

3.4.1 Preparation for Experimentation

The performance of the control culture was often seen to vary in different experiments, increasing significantly with frequent feedings and decreasing following periods without feeding. For these reasons, at least one PCE degrading control was maintained in all experiments for use in comparison with bottles that had been spiked with CFCs.

Except in some early experiments, the bottles were purged before PCE rate testing began. This was done to remove accumulated methane, products of PCE degradation and other volatile substances. Purging was also used as a means of CFC removal in some experiments.

The purging apparatus used in purging is described in Fig 3.4. Nitrogen was passed through a stripping solution containing titanium chloride and sodium citrate, to remove any traces oxygen from the nitrogen gas. The stripping solution was prepared in distilled water with sodium citrate (1600mg/L), titanium chloride 15% solution (3.6mL/L), and sodium bicarbonate (4500mg/L). The nitrogen was then passed through the bottle's septum using an 80mm needle. This needle was long enough to sparge gas through the liquid portion of the bottle, enabling volatile substances to be stripped out of solution.

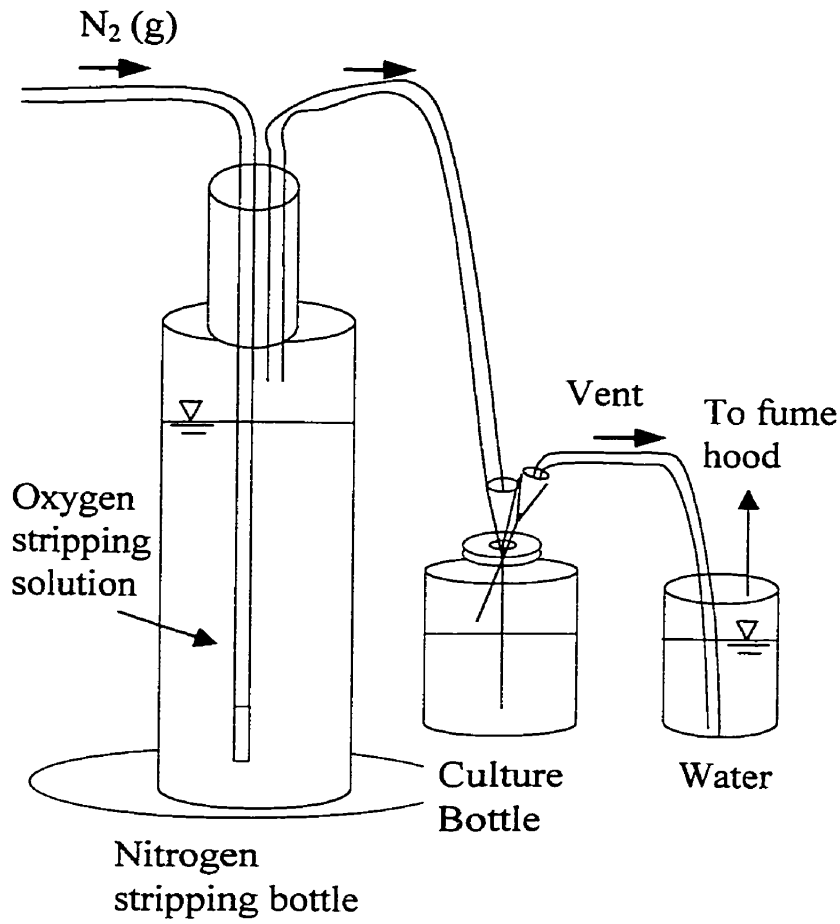


Fig 3.4 Purging Apparatus

To achieve a liquid phase concentration of 10mg/L PCE in a bottle having 70mL liquid and 51.7mL headspace, the Bottle Concentration Conversion Table (Appendix A, Table A3) indicates that 6.2μmol PCE/bottle is required (See Section 3.6 for discussion). This is equivalent to 1.03mg of PCE or 0.63μL of pure PCE. Volumes of CFC113 added were smaller, thus the need for stock solutions to provide precise delivery (See Appendix A, Table A2). The default setting in Table A2 was for the delivery of 3μL of stock solution and this is the volume of stock solution usually added to each bottle. Therefore, the amount of ethanol delivered with each 3μL injection of stock solution ranged between 15 and 25mg/L in solution depending on the amount of CFC113 and PCE present. The

stock solution was designed to have a total volume of 1950μL, within a 2mL-glass vial with screw cap and Teflon® septum. This resulted in a minimal 50μL headspace and minimum volatilization of the analyte.

3.4.2 Measurements Conducted

These tests involved the addition of a quantity of the PCE/ethanol stock solution to provide about 6μmol/bottle PCE. This quantity of PCE was often transformed into lesser-chlorinated products in less than four hours by active PCE degrading cultures.

Because one of the objectives in many of the experiments was to determine the threshold concentration at which an effect was seen, very small amounts of CFC were sometimes added and the effect was often subtle or 'no-effect'. For this reason, it was often necessary to determine the PCE degradation rate, expressed in $\mu mol\ bottle^{-1}hr^{-1}$. This was accomplished by first adding a 3μL dose of the PCE/ethanol stock solution to provide 10mg/L PCE and 25mg/L ethanol. The bottles were inverted, placed on an orbital shaker (Labline Instrument Inc., Melrose Pk, IL) and shaken for one hour at 200 rpm. A headspace sample of 20μL was then injected on the Vocol column using the CRESTECH method (described in Section 3.5.1) at hourly intervals thereafter until the PCE was consumed unless no evidence of PCE degradation was apparent.

3.5 Analytical Methods

3.5.1 Gas Chromatography as a Measurement Tool

The gas chromatography methods used to determine constituents of interest are summarized in Table 3.2:

Table 3.2 Gas Chromatography Methods

Method Name[1]	Sample Type/Volume	Analytes Quantified[2]	Detector
CRESTECH	Headspace - 20μL	PCE** TCE** cDCE tDCE 1,1-DCE VC CFC113** CFC114 HCFC123a**	Flame ionization detector (FID)
IAN01	Liquid - 1μL	Ethanol Acetate Propionate	Flame ionization detector (FID)
ETHENE	Headspace - 20μL	Methane** Ethene	Flame ionization detector (FID)
CFC01	Headspace - 20μL	CFC113 CFC114** HCFC123a PCE TCE	Electron capture detector (ECD)
HYDROGEN	Headspace - 20μL	Hydrogen Methane Carbon dioxide	Thermal conductivity detector (TCD)

[1]Detailed method description can be found in Appendix B.
[2]Where an analyte is present in more than one Gas Chromatography Method, the preferred method for measurement is marked above with a double asterisk (**).

For headspace samples, the Sample Volume noted in Table 3.2 was withdrawn directly from experimental bottles using a 25μL gas tight syringe (Hamilton 1702, Reno, NV) and analysed by direct injection. For liquid samples, 1mL samples were prepared and placed in 2mL vials using a 2.5mL gas-tight syringe, (Hamilton 1002LT, Reno, NV). Samples were then acidified prior to injection as noted in Appendix C.

CFC113 and HCFC123a were quantified using the CRESTECH method and had elution times of 2.1min and 1.8min respectively. Any constituents that were not retained on the column eluted prior to this time, including methane and ethene. Because all of the cultures used in this study formed methane, and many produced ethene, a large 'not

retained' peak was usually seen at 0.93min. This presented a problem in attempting to quantify constituents such as VC and CFC114, which had similar elution times of 1.12 and 1.26 minutes respectively. Therefore to measure CFC114, a gas chromatograph equipped with an electron capture detector (ECD) was used. The ECD is insensitive to methane. This method used with the ECD is referred to as the CFC01 method.

The ECD demonstrated poor sensitivity to VC however and a series of tests were conducted to estimate the influence of methane and CFC114 peaks on VC peak area using the CRESTECH Method. These tests were done using six 9mL-glass vials containing laboratory air only. The vials were capped with Teflon® seals and aluminum crimp caps and each was spiked with an amount of VC equivalent to 1.5μmol/bottle in the 122mL culture bottles. These results are presented in Appendix A, Table A6 (See p. 119). The effect of methane and CFC114 on VC peak area was apparent even at the lowest concentrations tested (actual gas phase concentrations of 0.06mg/L methane and 0.78mg/L CFC114). At the highest concentrations tested, the effect resulted in a 50% overestimation of the VC concentration (actual gas phase concentrations 14mg/L methane and 197mg/L CFC114). This overestimation would be less severe when the VC concentration was higher. Because the culture bottles tested during the PCE/ CFC experiments had VC concentrations typically in the 20-40μmol/bottle range, the accuracy of VC quantification afforded by the CRESTECH method was deemed sufficient for the experiments contemplated.

The CFC01 method was used to quantify CFCs and was very sensitive to those CFCs tested. The advantage of this method for CFCs was good separation and lack of interference by methane or other substances having early elution time.

The ETHENE Method was used to quantify ethene and methane. During PCE degradation rate testing, headspace samples were injected into two chromatographs, one running the CRESTECH Method and the other running the ETHENE Method to obtain quantification of all the chlorinated analytes required to complete a mass balance on PCE.

3.5.2 Mass Spectra Gas Chromatography as an Identification Tool

To confirm the formation of CFC dehalogenation products, some CFC degrading cultures were sent to an independent lab equipped with Gas Chromatography/ Mass Spectroscopy (York-Durham Regional Environmental Laboratory, Pickering, Ontario). The results of that analysis confirmed the existence of CFC113 breakdown products including HCFC123a and Chlorotrifluoroethene (CTFE). VC, TCE and cDCE were also detected.

3.6 Determining Liquid Concentration Using Henry's Law Constants

All culture bottles and standards bottles were prepared in the same manner using nominally 120mL clear glass bottles with rubber/Teflon seals and aluminum crimp caps. The bottles, seals and caps were obtained from Sigma-Aldrich of Mississauga, Ontario. The actual volume of each individual bottle was measured before using and recorded directly on each bottle. A sampling of 14 empty bottles taken randomly from a case of 144 bottles on Sept. 14th, 1999 showed that the bottles had a mean volume of 121.7mL, +/-0.45mL(+/- standard deviation).

All bottles including cultures and standards were filled with 70mL total liquid leaving the balance (about 52mL) as headspace. The chlorinated ethenes and breakdown products partition between gas and liquid phases according to Henry's Law.

It was intended that the microcosms used in these experiments would mimic or reflect field conditions under which PCE is present. It was therefore important in experimental design and monitoring, to know the actual concentrations in the liquid and gas phases that would result from the addition of a certain mass of chlorinated ethene to a bottle. An Excel® spreadsheet was prepared to convert the total mass or molarity into liquid or gas concentrations and visa-versa (See Appendix A Table A3). This spreadsheet allowed for the input of bottle volume (default 121.7mL) and liquid volume (default 70mL). The conversion values contained in the spreadsheet are based on Henry's Law constants (Hc) for each analyte at the ambient lab temperature of 22°C however the Hc value can be edited if required for use at different temperatures. The values shown in the spreadsheet are conversion values. The values are grouped in rows with each group having one of its columns of value set at unity. This is the only difference between groups; the same essential information is replicated. If the ratios of values across the rows are compared between groups, they are the same. Because the spreadsheet was prepared in this way, conversions can be made from any phase or total concentration to another phase or total concentration of different units was straightforward. This spreadsheet proved to be very convenient in lab calculations. A description of the Table A3 terms and short-forms used is found in Appendix A Table A4.

3.6.1 Determining Henry's Constant Values

Henry's Constants (Hc) can be determined by considering the definition of Hc and the mass balance in a closed system with aqueous and gas phases. The definition of Hc is given in Equation 3.1 (Gossett, 1987) where C_g = moles constituent/L gas and C_w = moles constituent/L water:

$$Hc = C_g / C_w \tag{3.1}$$

Total Moles is given by:

$$M = C_w V_w + C_g V_g \tag{3.2}$$

Where M = total moles of constituent in the closed system

V_w = Volume of water phase

V_g = Volume of gas phase

Substituting for C_w this becomes:

$$M = \frac{C_g}{Hc} V_w + C_g V_g \tag{3.3}$$

If two closed systems of equal total volume but different liquid and gas phase volumes receive the same mass, then:

$$M = \frac{C_{g1}}{Hc} V_{w1} + C_{g1} V_{g1} \tag{3.4}$$

$$M = \frac{C_{g2}}{Hc} V_{w2} + C_{g2} V_{g2} \tag{3.5}$$

where the subscripts 1 and 2 refer to the different liquid and gas phase volumes. Equations 3.4 and 3.5 can then be set equal to one another:

$$\frac{C_{g1}}{Hc}V_{w1} + C_{g1}V_{g1} = \frac{C_{g2}}{Hc}V_{w2} + C_{g2}V_{g2} \tag{3.6}$$

Solving for Hc gives:

$$Hc = \frac{\frac{C_{g2}}{C_{g1}}V_{w2} - V_{w1}}{V_{g1} - \frac{C_{g2}}{C_{g1}}V_{g2}} \tag{3.7}$$

Because C_g is directly proportional to the chromatographic peak area of a headspace sample, if the same headspace sample volumes are used, C_g can be replaced with peak area. Then:

$$Hc = \frac{\frac{PA_2}{PA_1}V_{w2} - V_{w1}}{V_{g1} - \frac{PA_2}{PA_1}V_{g2}} \tag{3.8}$$

where PA represents peak area from chromatograms. Because Henry's Law constants (Hc) are critical to quantification of the analytes being used in these experiments, early preparations for this work included the determination of Hc values for many analytes. Laboratory tests using the method described by Gossett (1987) were undertaken to determine the Hc values for many analytes of interest.

The Hc values for PCE, TCE, cDCE and CFC113 were determined using a pair of 121.7mL bottles, one bottle containing 20mL of distilled water, the other containing 100mL of distilled water. Since the amount of each analyte being added to each bottle was small, more accurate measurements could be made by first preparing a dilute stock

solution, then adding equal amounts of the stock solution to each bottle. The stock solution was prepared in ethanol plus an amount of each analyte equal to 1% of the total mass.

Each 121.7mL bottle capped with Teflon® seals and aluminum crimp caps received a quantity of a previously prepared stock solution. Duplicates each containing the same mass of each analyte were also prepared. Four bottles in all were used, each containing identical masses of PCE, TCE, cDCE and CFC113. The bottles were inverted and shaken for one hour at 200 RPM. A 20μL headspace sample was manually injected on gas chromatograph (GC) using the CRESTECH method. The GC peak area results from these two sets were averaged and used in Eq. 3.8 to obtain a value for Hc (Appendix A, Table A5, p. 118).

To determine the Hc values of CFC114, VC and CH_4, separate tests for each analyte were conducted using 9mL bottles with Teflon® lined septa and aluminum crimp caps. Six of these bottles contained 1mL of distilled H_2O (dH_2O) and another six bottles contained 7mL of dH_2O. To all of these bottles, the same mass of each analyte was added. The average peak area of those six bottles containing 1mL of liquid and those six containing 7mL of liquid were calculated separately. The average values were then used to calculate the Hc values. To determine the Hc of CFC114, 5μL of 99% CFC114 as a gas at atmospheric pressure was added to each bottle. To determine the Hc of VC, 400μL of 1000.5ppm (by mol) as a gas at atmospheric pressure was added to each bottle. To determine the Hc of CH_4, 10μL of 23.7% CH_4/ balance CO_2 as a gas at atmospheric pressure was added to each bottle. In all cases, the bottles were inverted, placed on an orbital shaker and shaken for one hour at 200 rpm (Labline Instrument Inc., Melrose Pk,

IL). Headspace samples of 20μL were injected on the Vocol column at 35°C isothermal for 1.5 minutes or as required for elution. Other GC parameters were the same as the CRESTECH method. All measurements were taken at 22°C. Using the peak area data thus obtained, Hc values were determined and are listed in Table 3.3.

The measured values were similar to those reported by Gossett thus providing confidence in both sets of data. Where literature values existed, they were used in the calculations. Where literature values were not found, the measured values were used. HCFC123a was a late subject of interest in this study and the Hc value was not found in the available literature.

Table 3.3 Measured and Literature Hc Values

Compound	Temperature (°C)	Measured value	Literature value[1]	Value used
PCE	22	0.82	0.634(1)	0.634
TCE	22	0.46	0.343(1)	0.343
cDCE	22	0.21	0.145(1)	0.145
tDCE	22	ND[2]	0.340(1)	0.340
1,1DCE	22	ND	0.202(1)	0.202
VC	22	0.93	1.01(1)	1.01
CH4	22	15.22	-	15.22
Ethene	22	ND	8.69(2)	8.69
CFC113	22	12.07	-	12.07
HCFC123a	22	0.93	-	0.93
CFC114	22	12.85	-	12.85

[1]Source: (1) Gossett(1987), (2) CRC Handbook, 1999.
[2] ND= not determined

4 THE EFFECT OF CFC113 ON PCE BIODEGRADATION

4.1 Experimental Design

In each PCE degrading experiment undertaken, the first step was to establish a set of replicate culture bottles actively degrading PCE and producing ethene. Ethene was the final product of reductive dechlorination of PCE in the mixed microbial culture used except in cases where degradation was inhibited. The rates of degradation were rapid in cultures that were not inhibited. PCE was removed at a rate of 1-2 μmol $bottle^{-1}$ hr^{-1}. At this rate, a 6μmol spike of PCE was often consumed in less than four hours.

Several sets of experiments were conducted to test the hypothesis that CFC113 would cause a rate reduction or cessation of PCE biodegradation. Additional objectives were to describe the overall effect of CFC113 on the PCE degradation rate, the effect on degradation of PCE breakdown products and to determine the threshold concentration at which an effect is seen. Details of the combined CFC113/PCE tests conducted are shown in Table 4.1. The data are presented in ascending order of CFC113 concentration and show the range of CFC113 concentrations studied. The genealogy of these various cultures is described in Chapter 3 (Fig 3.2).

PCE degrading controls (those not containing CFC113) used in each experiment are discussed as a group in Section 4.2.1. Each experimental group of cultures contained at least one control. Cultures within an experimental group are compared directly with one another in order to draw conclusions as to the effect of CFC113 on a particular culture.

Table 4.1 Experimental Conditions for CFC113 Studies[1]

Study Code (Bottle)[3] (Expt)	Initial CFC113 Aqueous Conc. (mg/L)	Study Time (days)	Number Tested	Gas Purging (y/n)[2]	H_2 Addition (y/n)[2]
C2b 1605 (Ex B)	0.06	15	1	y	n
B2 1505 (Ex C)	0.1	22	1	y	n
C2b 0208 (Ex D)	0.15	76	1	y	y
C2a 1605 (Ex B)	0.18	15	1	y	n
B3 1505 (Ex C)	0.2	22	1	y	n
C2a 0208 (Ex D)	0.3	76	1	y	y
B2a 3103 (Ex A)	0.86	8	1	n	n
B4 1505 (Ex C)	1.0	22	1	y	n
C2a/C2b 1408 (Ex E)	1.0	50	2	y	n
C3a/C3b 0208 (Ex D)	2.0	76	2	y	y

[1]PCE added at 6μmol/bottle each feeding.
[2]y = yes, n = no
[3] for genealogy see Fig 3.2.

The details of each experiment conducted are provided in the following sections of this report. The Study Time in Table 4.1 is the number of days that each culture was under observation. Study Time was not determined in advance but was based on the results, most often an identifiable trend or in some cases, a lack of activity. The first experiments conducted generally had the shorter Study Times. The later experiments, especially those in which complete inhibition was seen, tended to have the longest Study Times.

The column 'Number Tested' (Table 4.1) refers to the number of replicates used. In most cases, cultures were purged of chlorinated compounds prior to beginning an experiment as indicated by the column 'Gas Purging' (Table 4.1). Some cultures were tested for the consumption of hydrogen as an electron donor. This is indicated in the column 'H_2 Addition' in Table 4.1.

In figures throughout this chapter, cultures that did not contain CFC113 are depicted with open markers (eg. □) and those that contained CFC113 are depicted with closed markers (eg. ■).

4.2 Effects of CFC113 on PCE Degradation

4.2.1 Cultures Receiving No CFC113 (PCE Degrading Controls)

Significant PCE degradation rate differences existed between the control cultures used in this work, although all shared the same parent or grandparent culture. The cause of these differences is unclear as all cultures were prepared and handled in the same manner but differences could be related to the length of time and the number of feedings prior to purging and beginning the rate tests. In general, PCE removal rates in the controls tended to increase with the number of feeding and presumably, with the growth of the culture. The control culture PCE removal rates described in Table 4.2 are individually used in the following sections of this report. In all cases, the PCE removal rate could be described as 'Rapid' (0.5 to 1.0μmol bottle^{-1} hr^{-1}) or 'Very Rapid' (>1.0μmol bottle^{-1} hr^{-1}).

Table 4.2 PCE degradation rates in cultures receiving no CFC113[1] (μmol bottle^{-1} hr^{-1})

Study Code Bottle; Expt	*Day*									
	1	*3*	*6*	*8*	*15*	*18*	*22*	*29*	*41*	*62*
B1a3103, Ex. A	-1.55	-1.06	-1.08	-1.38						
C1a1605, Ex. B	-1.24	-1.44	-1.16	-2.44	-2.44					
C1b1605, Ex. B	-1.44	-1.07	-1.24	-1.95	-2.91					
B11505, Ex. C	-1.85	-2.30				-1.32	-2.40			
C10208, Ex. D	-0.74				-0.47	-1.14		-2.55	-1.77	
C11408, Ex. E	-1.62		-2.14		-2.96[2]		-0.25			-0.76

[1] blank space indicate no test conducted

[2] purged to remove chlorinated compounds including CFC113.

The data from the 'Day 8' PCE degradation rate test are displayed graphically in Fig 4.1. Each curve is labeled with the PCE degradation rate. Although the quantitative PCE degradation rates differed as indicated in Table 4.1 and Fig 4.1, culture bottles containing no CFC113 showed rapid degradation of PCE overall (Fig 4.2). TCE formed during PCE degradation was also rapidly consumed in all PCE degrading controls (See Fig 4.3).

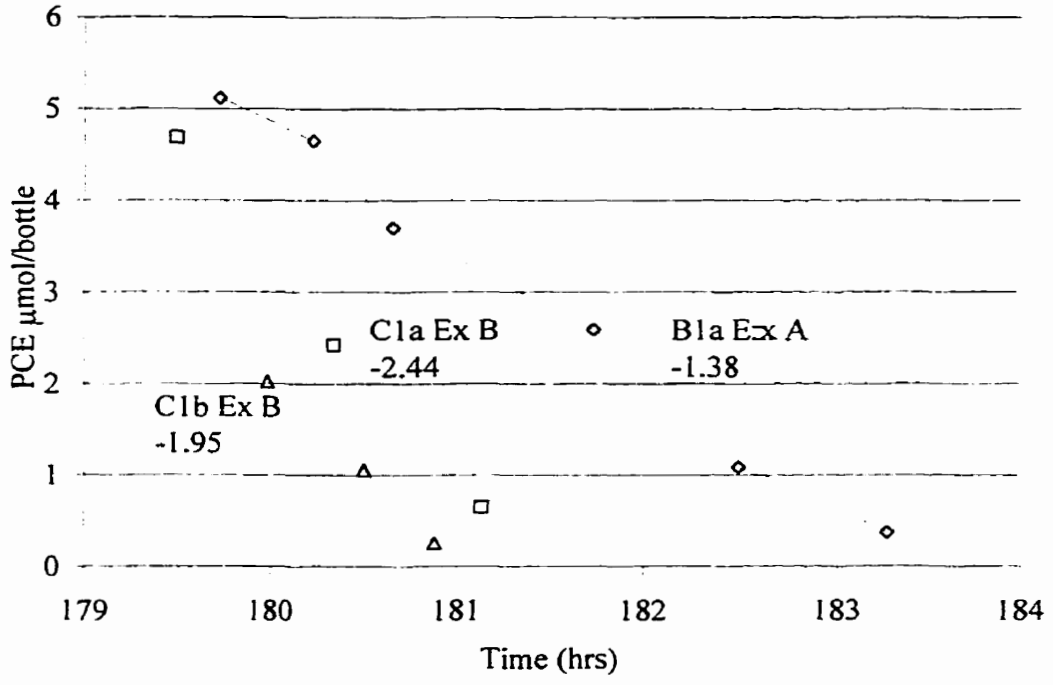


Fig 4.1 PCE degradation on the 8th day in cultures receiving no CFC113

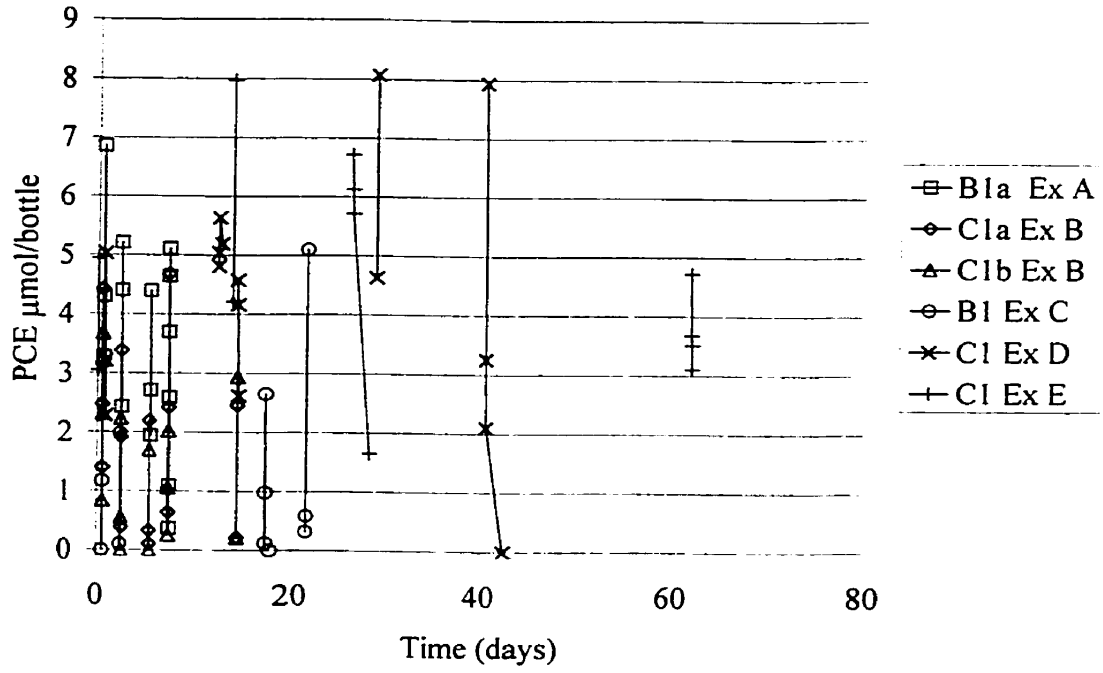


Fig 4.2 Degradation of PCE in cultures receiving no CFC113

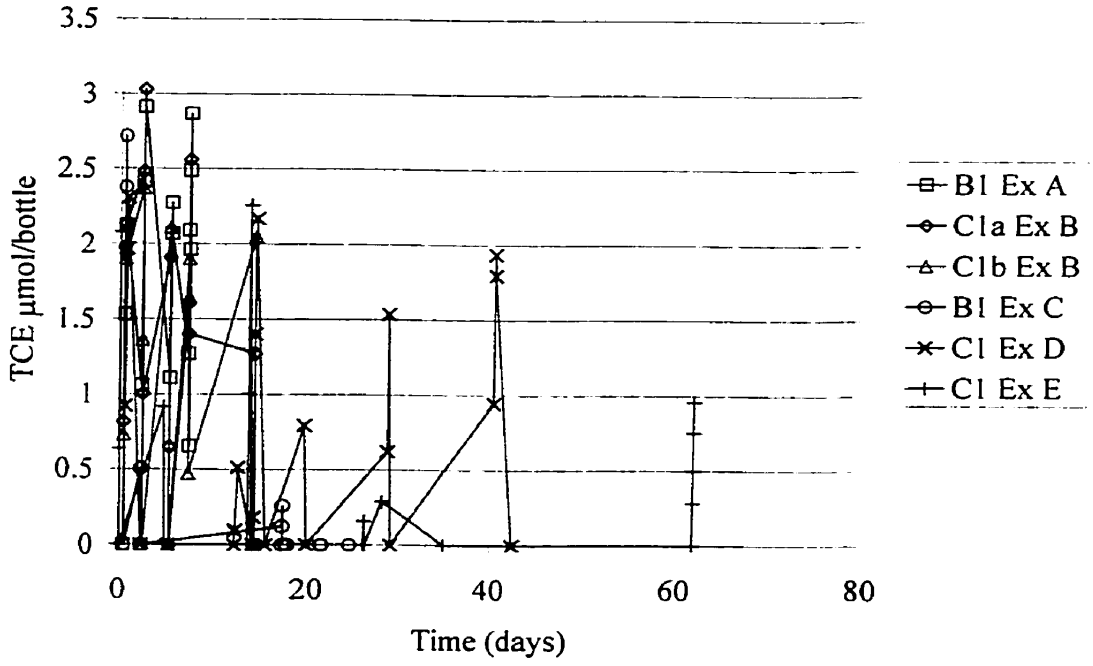


Fig 4.3 Production and degradation of TCE in cultures receiving no CFC113

The degradation of cDCE formed as a byproduct of PCE degradation occurred in all cultures receiving no CFC113 but the actual degradation rate varied greatly (See Fig 4.4)

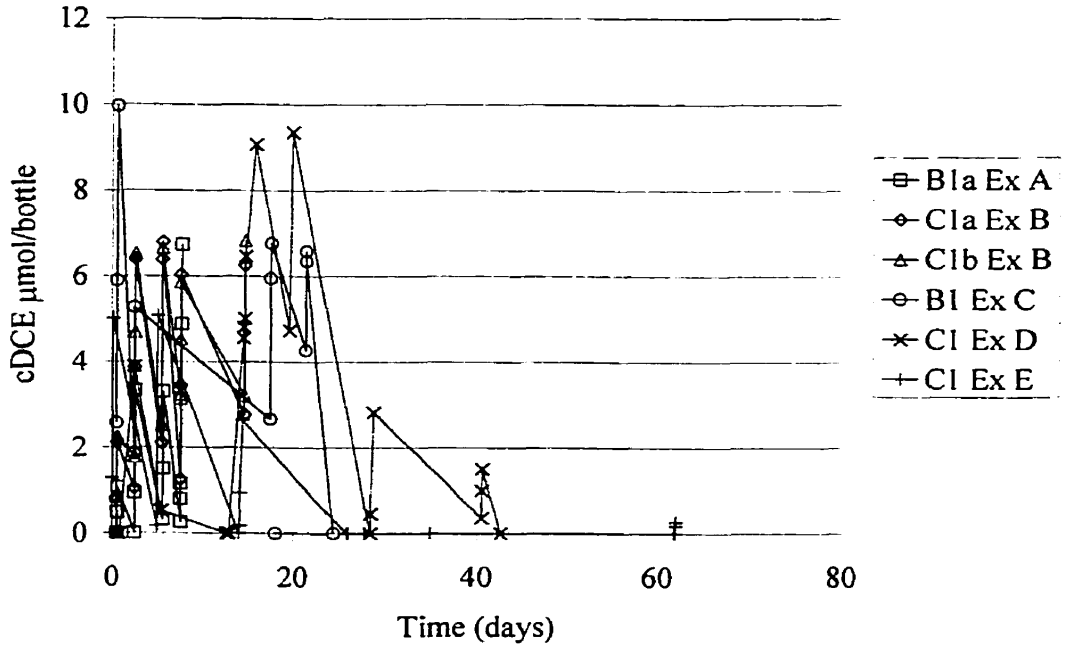


Fig 4.4 Production and degradation of cDCE in cultures receiving no CFC113

Both production and degradation of VC varied greatly between cultures not receiving CFC113 (Fig 4.5). The quantity of VC present varied in relation to the cDCE degradation rate (Fig 4.5). VC degradation appeared to begin after PCE had been consumed and after VC had accumulated to relatively high concentrations. All cultures receiving no CFC113 produced ethene (Fig 4.6). The amount of ethene produced was directly proportional to the amount of PCE added to each. Bottle C1 Ex D did not begin to produce a significant amount of ethene until Day 20 (Fig 4.6).

Ethene that formed in bottle C1 Ex E was purged at Day 15 as were all cultures in Ex E. Ethene was being formed both before and after purging.

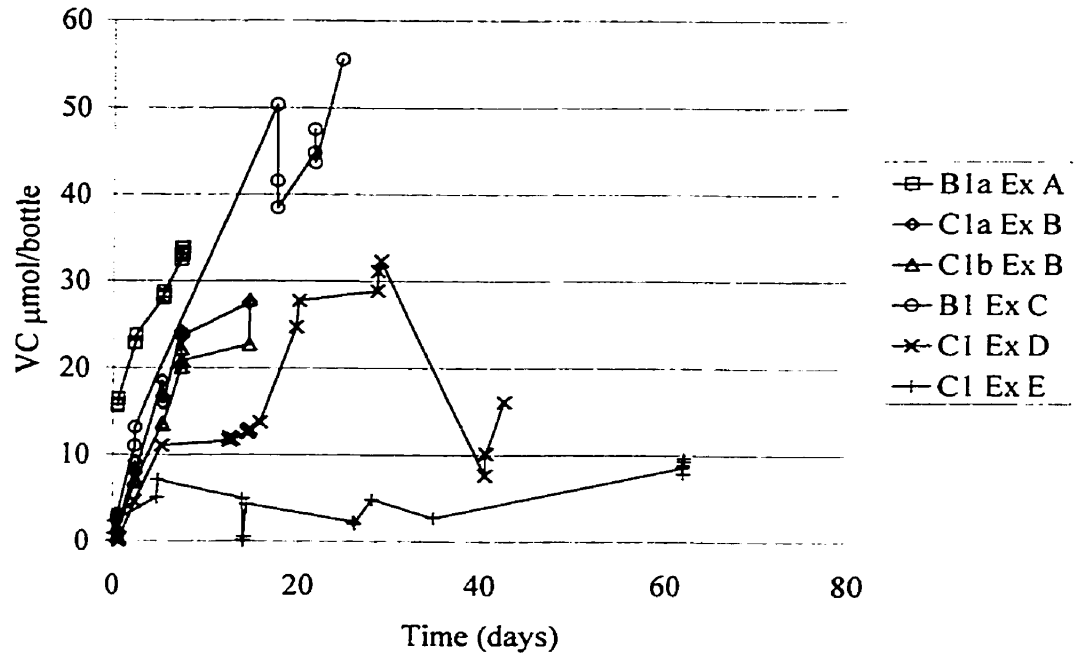


Fig 4.5 Production and degradation of VC in cultures receiving no CFC113

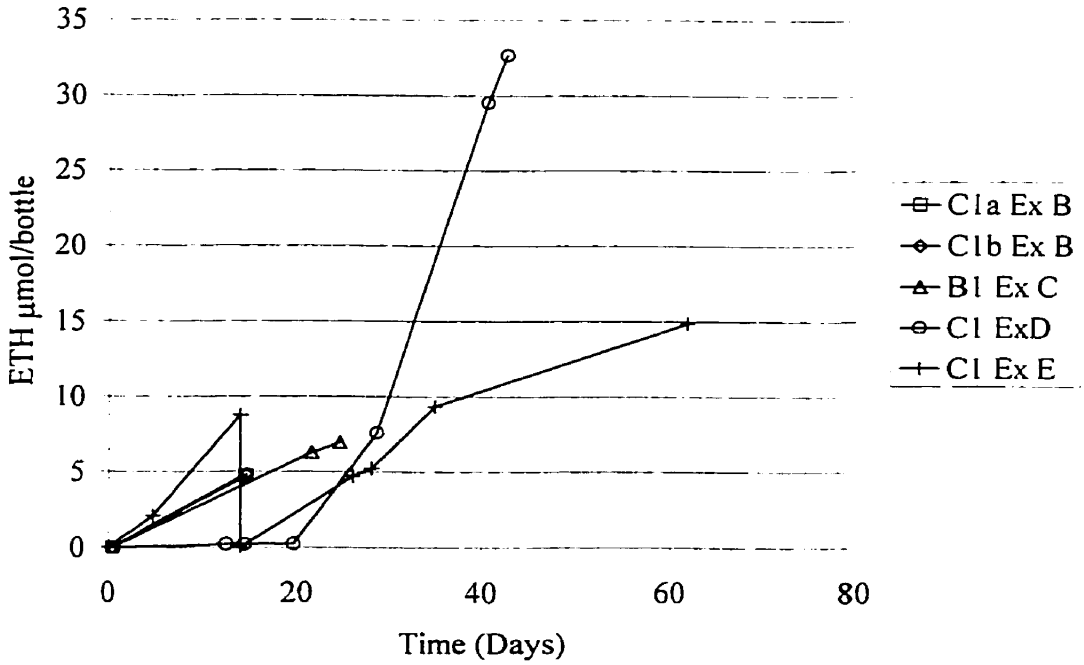


Fig 4.6 Production of ethene in cultures receiving no CFC113

4.2.2 Cultures Receiving CFC113

The cultures that received CFC113 are listed in Table 4.3 in order of increasing CFC113 concentration. Note that although there was variation in the Day 1 PCE degradation rates, the Day 1 rates for all bottles could be described as 'Very Rapid' (>1.00μmol/b/hr) or 'Rapid' (0.50-1.00μmol/b/hr). Inhibition was evident in all cultures rate tested after Day 1 except C3 Ex B (CFC113: 0.06mg/L) and B2 Ex C (CFC113: 0.1mg/L) which were the lowest concentrations tested and performed at the rate 'Very Rapid' throughout.

Table 4.3 PCE degradation rates in cultures receiving CFC113[3] (μmol bottle^{-1} hr^{-1})

Study Code (Bottle) (Expt)	Initial CFC113 Aqueous Conc. (mg/L)	Day							
		1	3	6	8	15	18	22	41-50
C3 1605 Ex B	0.06	-1.37	-0.93	-0.95	-1.51	-1.02			
B2 1505 Ex C	0.1	-1.32	-2.17				-1.32	-2.33	
C2 0208 Ex D	0.15	-0.77	DND[1]			DND[1]		DND[1]	DND[1]
C2 1605 Ex B	0.18	-1.47	-1.08	-0.71	-0.93	-0.24			
B3 1505 Ex C	0.2	-1.37	-1.81					-0.88	
C3 0208 Ex D	0.3	-0.80	DND[1]			DND[1]		DND[1]	DND[1]
B2a 3103 Ex A	0.86	-1.40	-0.67	-0.46	-0.08				
C2a 1408 Ex E	1.0	-1.28		DND[1]		P[2]	DND[1]		DND[1]
C2b 1408 Ex E	1.0	-0.94		DND[1]		P[2]	DND[1]		DND[1]
B4 1505 Ex C	1.0	-1.67	-0.46					-0.15	
C4a 0208 Ex D	2.0	-0.51	DND[1]			DND[1]		DND[1]	DND[1]
C4b 0208 ExD	2.0	-0.68	DND[1]			DND[1]		DND[1]	DND[1]

[1] DND = PCE degradation not detected
[2] P = purged to remove chlorinated compounds including CFC113.
[3] blank spaces indicate no test done

4.2.2.1 CFC113 Expt A.

This experiment was started May 3rd, 2000. Two replicate PCE degrading culture bottles were selected. One received no PCE and was named B1, the other bottle received CFC113 at an initial aqueous concentration of 0.86mg/L and was B2. Both bottles were fed a PCE/ EtOH stock solution and were rate tested every second or third day for eight days.

A comparison of the performance of B1 and B2 is made in Table 4.4.

Table 4.4 PCE degradation rates during Expt A (μmol bottle^{-1} hr^{-1})

Bottle Study Code	Initial CFC113 (mg/L)	Day			
		1	3	6	8
B1 a3103	0.0	-1.55	-1.06	-1.08	-1.38
B2 a3103	0.86	-1.40	-0.67	-0.46	-0.08

Bottle B2 received a stock solution containing PCE / EtOH/CFC113 on Day 1 and another stock solution containing only PCE/EtOH on Days 3, 6 and 8. The first injection provided 0.86mg/L aqueous CFC113 that began to slowly degrade during the experiment. (See Section 4.4, Degradation of CFC113.) Degradation of the PCE was initially rapid but slowed on Day 3 and nearly ceased on Day 8 (Table 4.4). However, the inhibition of PCE degradation acquired over the seven-day experiment did not result in complete cessation.

TCE was produced in both cultures as PCE degraded (Fig 4.7). TCE degradation rates were not determined but over the course of the experiment the removal of TCE appeared similar in the presence and absence of CFC113. cDCE was readily formed by both cultures (Fig 4.8). In culture B2, cDCE accumulated. Degradation of cDCE was apparently inhibited by CFC113 at 0.86mg/L (Fig 4.8). Less VC is formed in B2 than in B1 (Fig 4.9). This was expected since cDCE was not readily degraded in B2 (Fig 4.8).

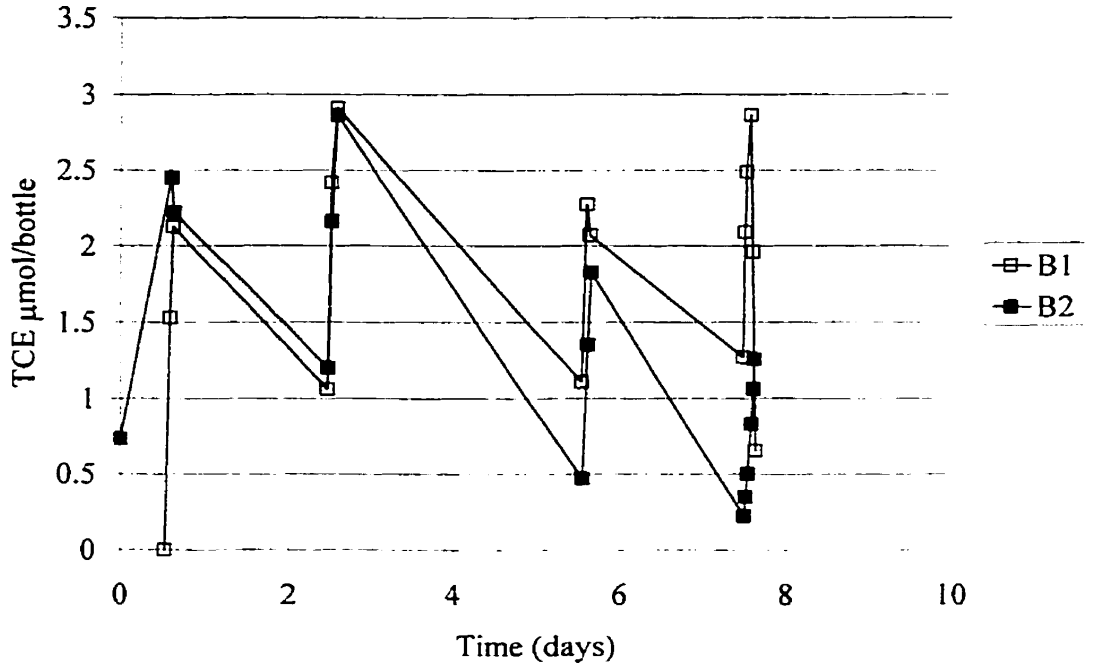


Fig 4.7 Production and degradation of TCE in a culture containing no CFC113 (B1) and a culture containing CFC113 at 0.86mg/L (B2)

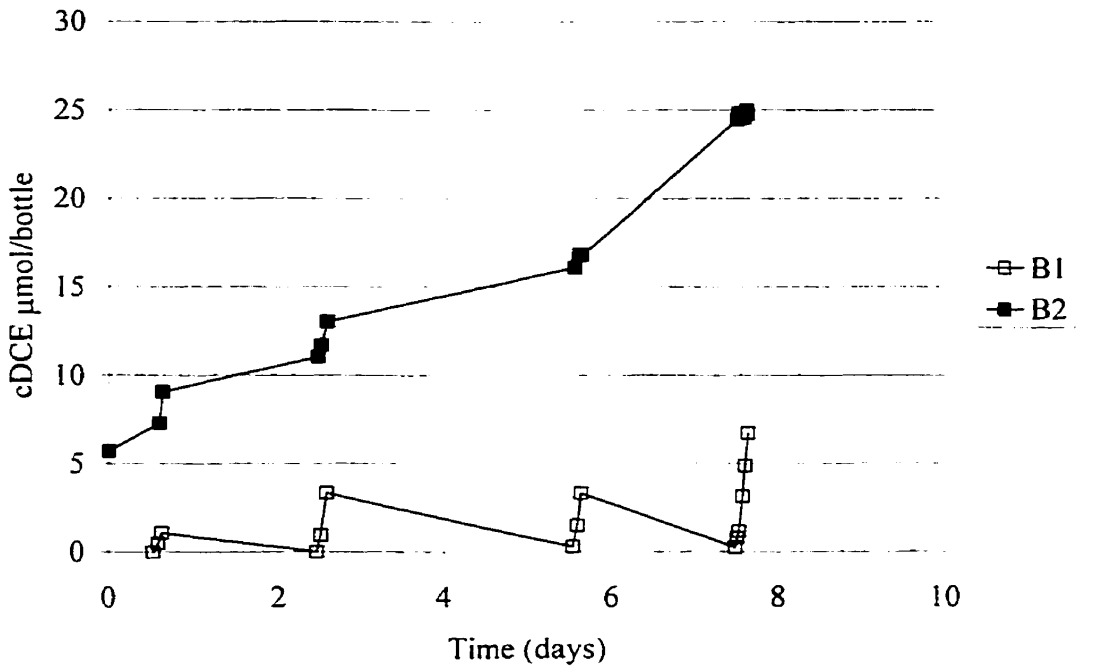


Fig 4.8 Production and degradation of cDCE in a culture containing no CFC113 (B1) and a culture containing CFC113 at 0.86mg/L (B2)

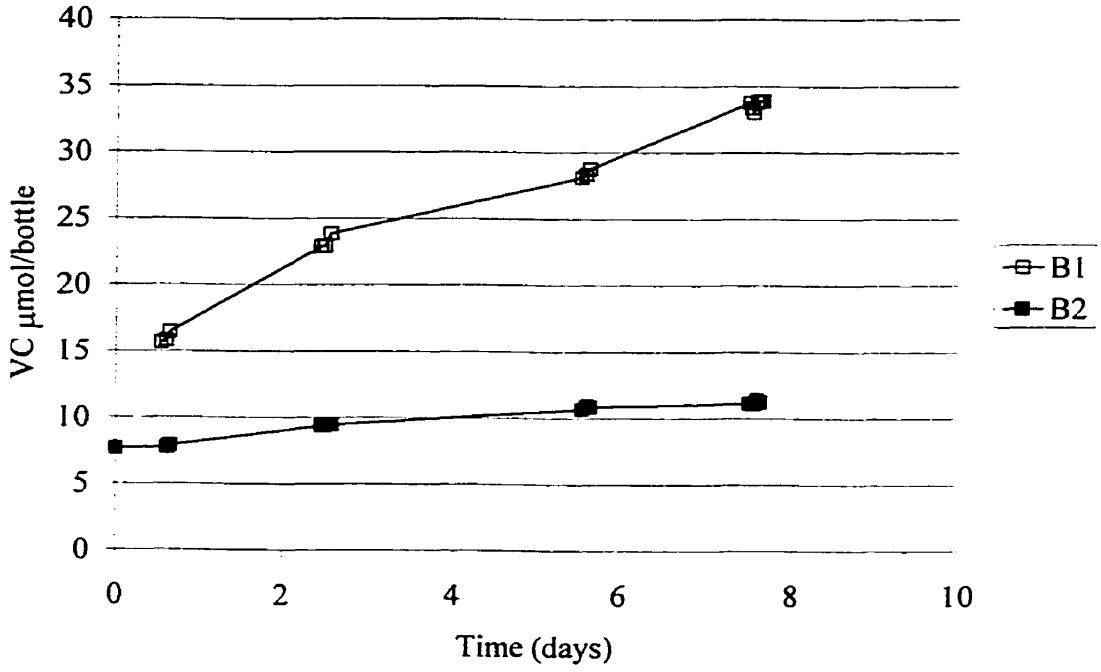


Fig 4.9 Production and degradation of VC in a culture containing no CFC113 (B1) and a culture containing CFC113 at 0.86mg/L (B2)

4.2.2.2 CFC113 Expt B

This experiment consisted of four bottles and was started May 31st, 2000. The experiment included two PCE degrading cultures containing no CFC113 (C1a and C1b), one culture spiked with CFC113 at 0.18mg/L (C2) and another PCE/CFC113 degrading culture spiked with CFC113 at 0.06mg/L AL (C3). Five PCE rate tests were conducted over fifteen days. The PCE data are summarized in Table 4.5.

Table 4.5 PCE degradation rates during Expt B (µmol bottle^{-1} hr^{-1})

Bottle Study Code	Initial CFC113 (mg/L)	Day				
		1	3	6	8	15
C1a 1605	0.0	-1.24	-1.44	-1.16	-2.44	-2.44
C1b 1605	0.0	-1.44	-1.07	-1.24	-1.95	-2.91
C2 1605	0.18	-1.47	-1.08	-0.71	-0.93	-0.24
C3 1605	0.06	-1.37	-0.93	-0.95	-1.51	-1.02

The cultures containing no CFC113 (C1a and C1b) performed similarly throughout. Although the Day 1 PCE degradation rates were similar in all four cultures, C2 and C3 had reduced PCE degradation rates in subsequent days. The most profound PCE degradation rate reduction effect was seen in C2, which had the highest CFC113 concentration. TCE was formed and degraded in all the bottles (Fig 4.10). Fig 4.11 below shows the cDCE concentrations over time for all four cultures. Note a similar pattern in all bottles except C2 (CFC113 0.18mg/L). C2 also demonstrated the lowest PCE degradation rate especially at Day 15 (Table 4.5). cDCE accumulated after five days in culture C2. VC was produced in all four cultures (Fig 4.12). C2 had the lowest total formation of VC as expected due to the lowest cDCE degradation rate.

Ethene formation was measured on Day 1, Day 15 and again on Day 20 following the completion of the PCE rate testing (Fig 4.13). Both cultures containing CFC113 showed very little formation of ethene while those cultures without CFC113 had started to produce ethene. Ethene formation was apparently inhibited by the presence of CFC113.

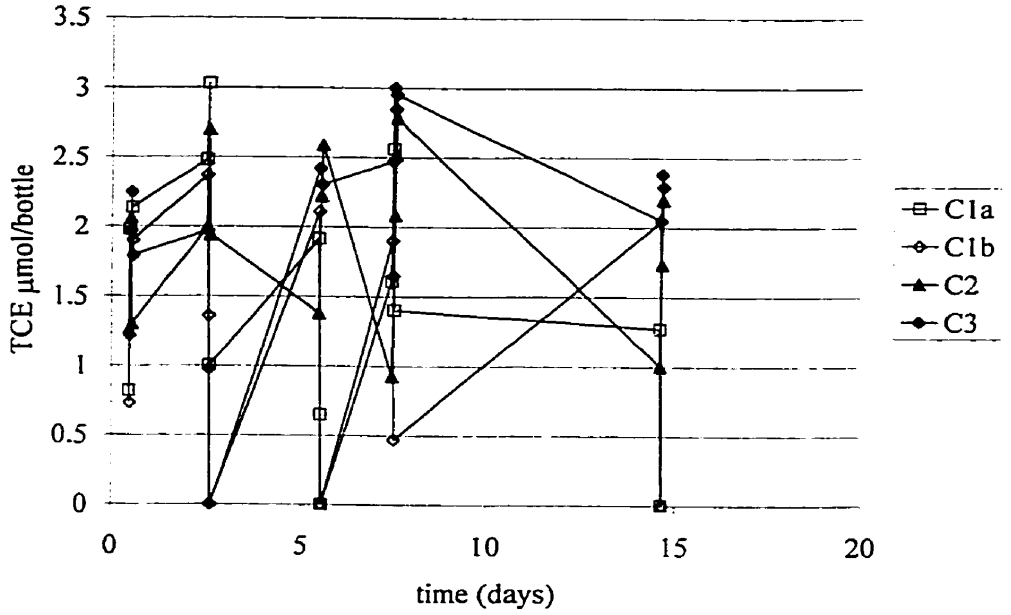


Fig 4.10 Production and degradation of TCE in two cultures containing no CFC113 (C1a and C1b), one culture containing CFC113 at 0.18mg/L (C2) and one culture containing CFC113 at 0.06mg/L (C3)

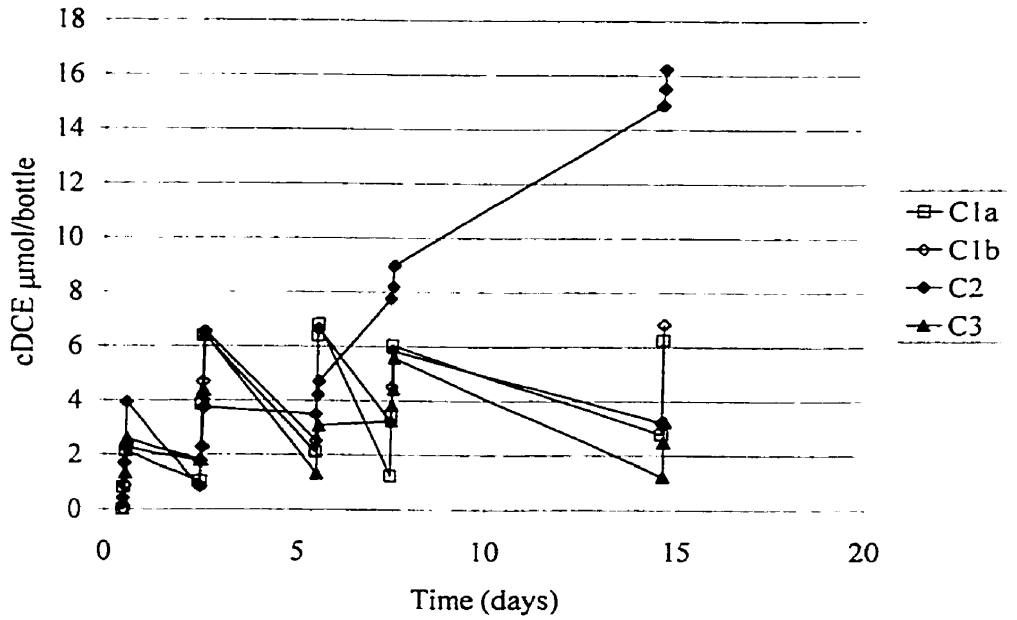


Fig 4.11 Production and degradation of cDCE in two cultures containing no CFC113 (C1a and C1b), one culture containing CFC113 at 0.18mg/L (C2) and one culture containing CFC113 at 0.06mg/L (C3)

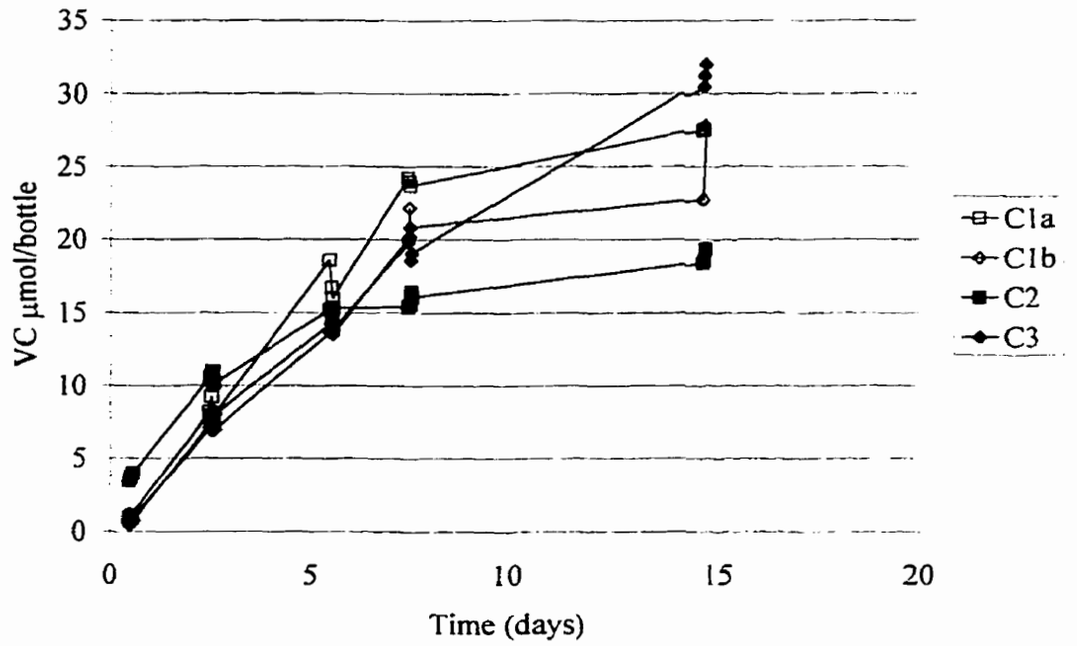


Fig 4.12 Production and degradation of VC in two cultures containing no CFC113 (C1a and C1b), one culture containing CFC113 at 0.18mg/L (C2) and one culture containing CFC113 at 0.06mg/L (C3)

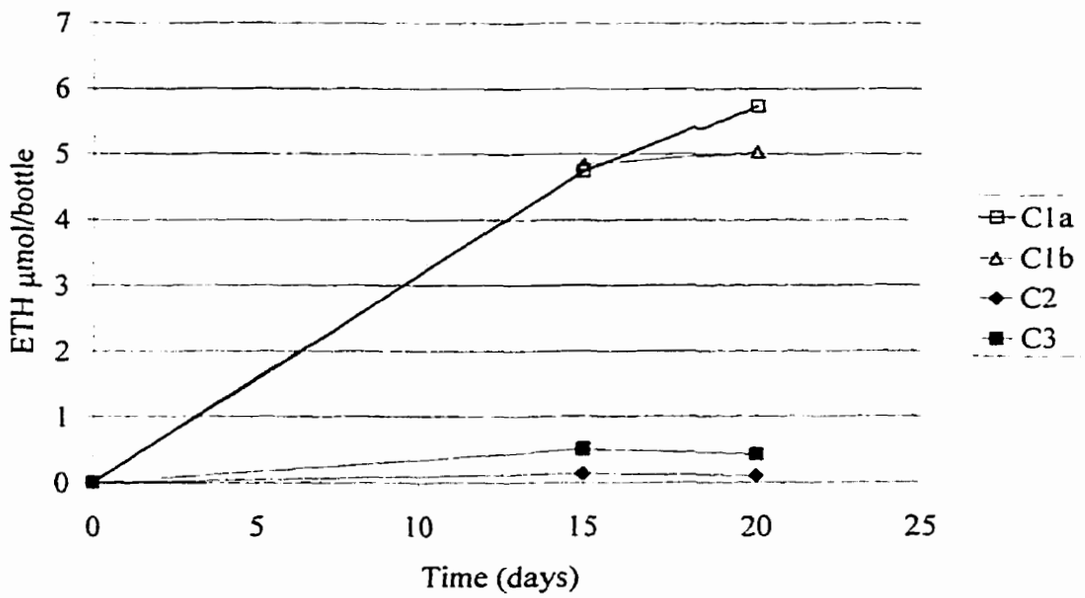


Fig 4.13 Production of ethene in two cultures containing no CFC113 (C1a and C1b), one culture containing CFC113 at 0.18mg/L (C2) and one culture containing CFC113 at 0.06mg/L (C3)

4.2.2.3 CFC113 Expt C

This experiment consisted of four bottles and was started June 28th, 2000. The experiment included one PCE degrading culture containing no CFC113 (B1), one culture spiked with CFC113 at 0.10mg/L (B2), one culture spiked with CFC113 at 0.20mg/L (B3) and one culture spiked with CFC113 at 1.0mg/L (B4). Four PCE rate tests were conducted over 22 days. (Table 4.6).

Table 4.6 PCE degradation rates during Expt C (μmol bottle^{-1} hr^{-1})

Bottle Study Code	Initial CFC113 (mg/L)	Day			
		1	3	18	22
B1 1505	0.0	-1.85	-2.30	-1.32	-2.40
B2 1505	0.1	-1.32	-2.17	-1.32	-2.33
B3 1505	0.2	-1.37	-1.81	no data	-0.88
B4 1505	1.0	-1.67	-0.46	-1.19	-0.15

The objectives of this experiment were to confirm the findings of CFC113 Expts A and B, and to determine the threshold concentration of CFC113 for which inhibition occurred. Several problems developed during the course of this experiment. The first was a GC breakdown in the first two weeks of July 2000 that prevented sampling in the period between Day 3 and Day 18. Another problem was the unusual accumulation of cDCE in the culture containing no CFC113. Finally, partially plugged GC headspace injection needles caused erratic data. Nevertheless, sufficient data were collected for analysis. The results of this experiment were included in this report in order to show all the available information on the effect of CFC113 on PCE degradation but due to the difficulties associated with this experiment, another similar subsequent experiment was undertaken with the intention of obtaining more descriptive data.

At 0.1mg/L CFC113 (B2), there was little effect on the PCE degradation rate (Table 4.6). In CFC113 Expt B, an effect was seen at 0.06mg/L (See Table 4.5). It is

thought that this difference may be attributed to the characteristics of the culture itself, but both seem to be in the range of concentrations at which inhibition begins to occur. At 0.2mg/L CFC113 (B2), inhibition is seen at Day 22 although PCE degradation was still detectable. At 1.0mg/L CFC113 (B3), PCE degradation has slowed significantly by Day 22.

Fig 4.14 shows the TCE concentrations in the four cultures. There is no clear trend or a pattern in TCE concentration except that the lowest TCE level overall is seen in the culture containing no CFC113. Fig 4.15 shows cDCE concentrations in the four cultures. At the start of this experiment, cDCE accumulated in all four cultures then steadily decreased. Toward the end of the experiment, cDCE degradation in those cultures containing CFC113 was inhibited and cDCE accumulated. The greatest accumulation was in those bottles having the highest CFC113 concentration. VC data are shown in Fig 4.16. As the CFC113 concentration increased, the bottles accumulated less VC. This is thought to be the result of CFC113 inhibition of cDCE to degrade and form VC.

Ethene formation is shown in Fig 4.17. The culture having the lowest CFC113 concentration 0.1mg/L (B2) also showed significant ethene formation inhibition although partially inhibited, indicating that VC degradation was also inhibited by CFC113. Ethene formation was completely inhibited in the two highest CFC113 concentrations.

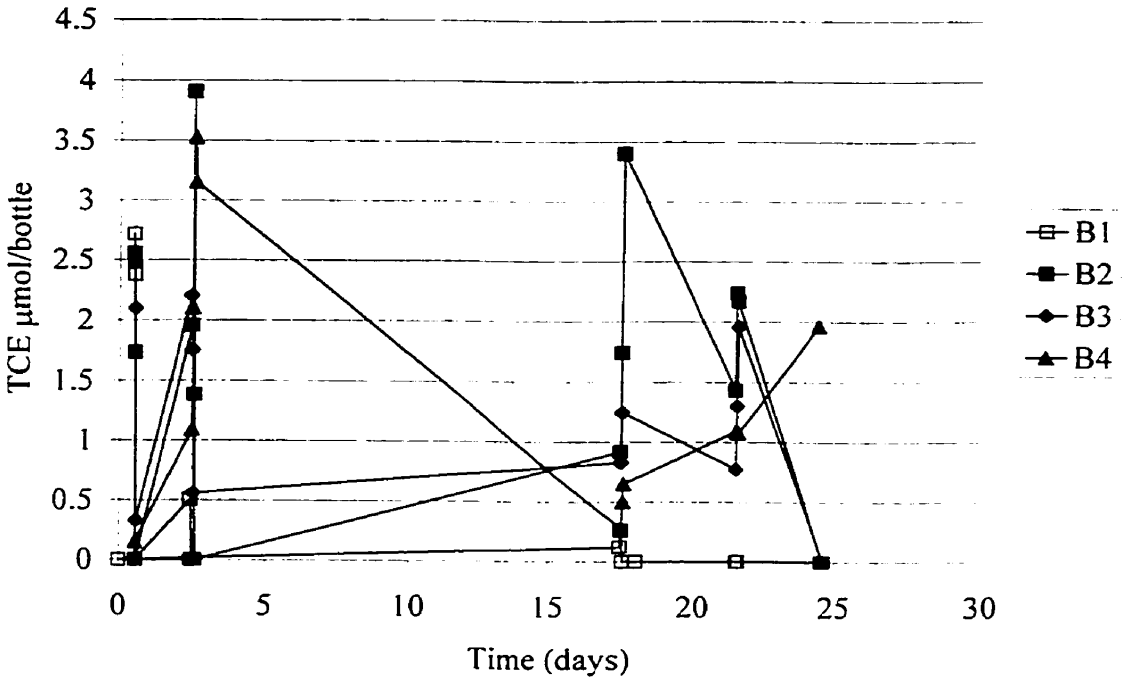


Fig 4.14 Production and degradation of TCE in one culture containing no CFC113 (B1) and cultures containing CFC113 at 0.10mg/L (B2), 0.20mg/L (B3) and 1.0mg/L (B4)

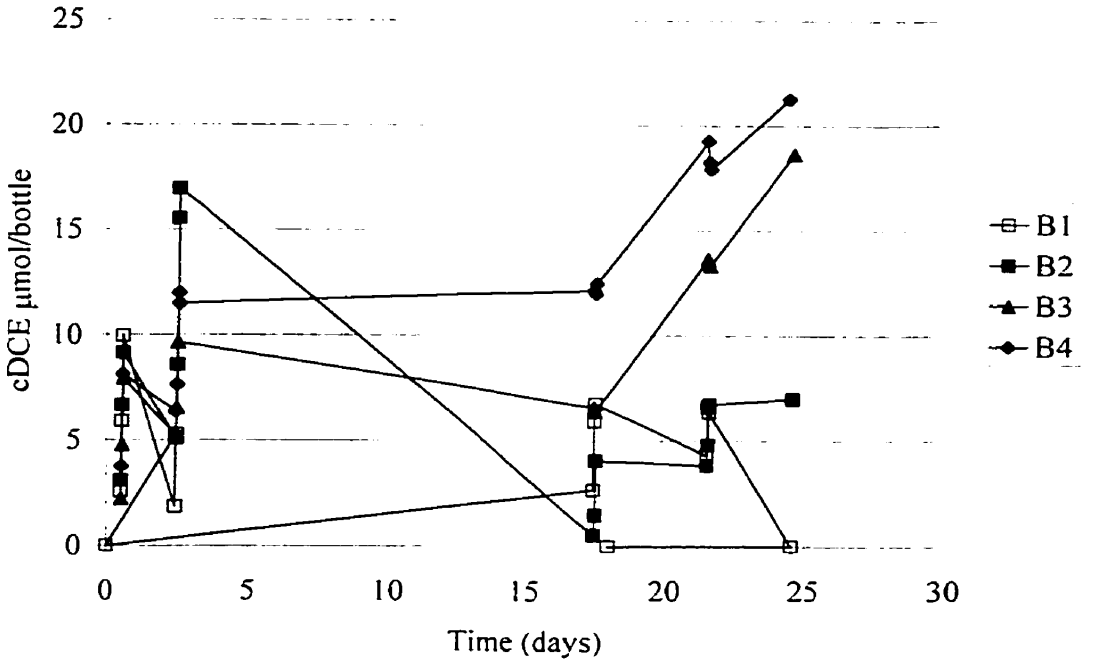


Fig 4.15 Production and degradation of cDCE in one culture containing no CFC113 (B1) and cultures containing CFC113 at 0.10mg/L (B2), 0.20mg/L (B3) and 1.0mg/L (B4)

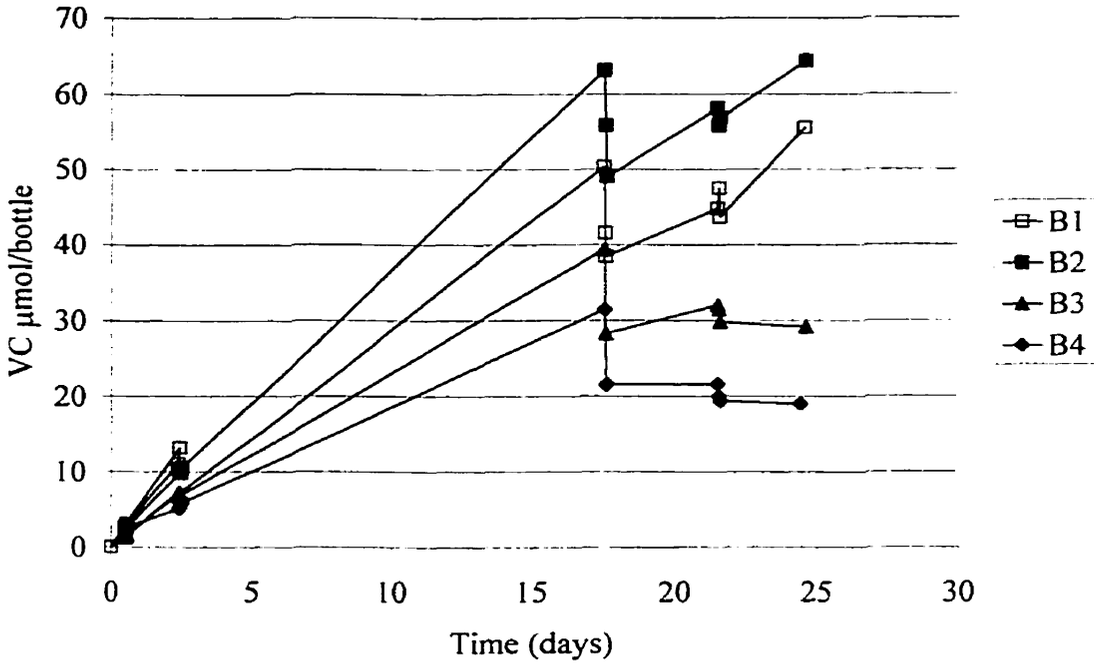


Fig 4.16 Production and degradation of VC in one culture containing no CFC113 (B1) and cultures containing CFC113 at 0.10mg/L (B2), 0.20mg/L (B3) and 1.0mg/L (B4)

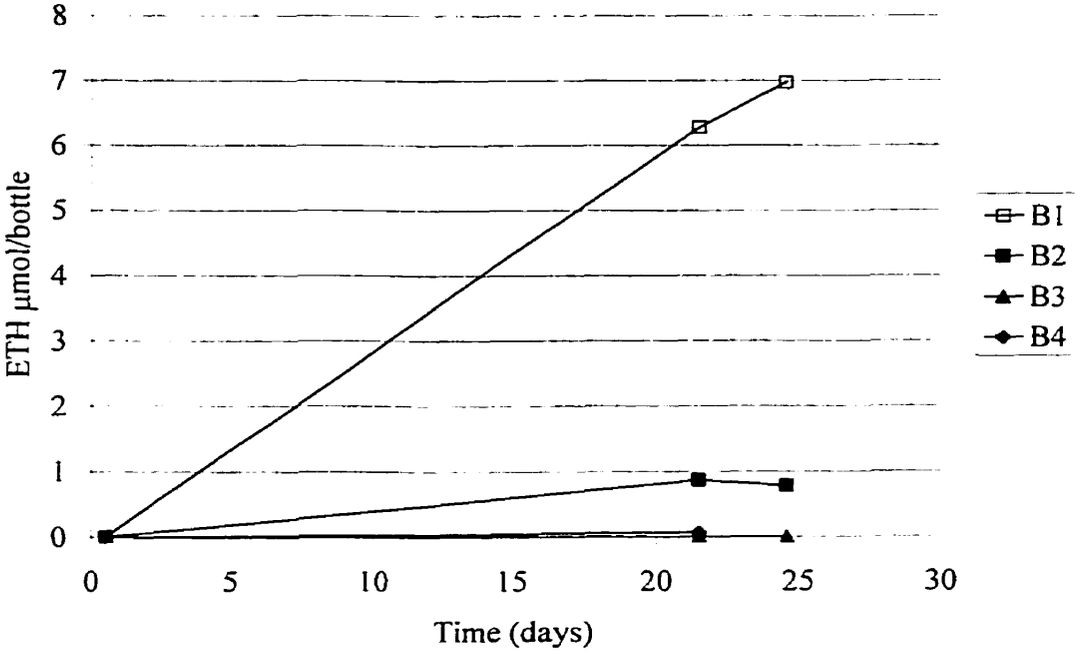


Fig 4.17 Production of ethene in one culture containing no CFC113 (B1) and cultures containing CFC113 at 0.10mg/L (B2), 0.20mg/L (B3) and 1.0mg/L (B4)

4.2.2.4 CFC113 Expt D

Consisting of five culture bottles in total, this experiment was started on August 9th, 2000. The experiment including one culture containing no CFC113 (C1), one culture spiked with CFC113 at 0.15mg/L (C2), one culture spiked with CFC113 at 0.3mg/L (C3) and two cultures spiked at with CFC113 at 2.0mg/L (C4a and C4b).

All cultures degraded PCE at a similar rate on Day 1 (Table 4.7). By Day 13 the initial dose of PCE added on Day 1 had been entirely consumed in C1 and C2 (Fig 4.18) but remained in C3, C4a and C4b. Cultures C1 and C2 were re-spiked with PCE on Day 13. C1 continued to degrade PCE while the re-spike in C2 was not degraded.

Table 4.7 PCE degradation rates during Expt D (μmol bottle^{-1} hr^{-1})

Bottle Study Code	Initial CFC113 (mg/L)	Day				
		1	13	20	29	41
C1 0208	0.0	-0.74	-0.47	-1.14	-2.55	-1.77
C2 0208	0.15	-0.79	DND[1]	DND[1]	DND[1]	DND[1]
C3 0208	0.3	-0.80	"	"	"	"
C4a 0208	2.0	-0.51	"	"	"	"
C4b 0208	2.0	-0.68	"	"	"	"

[1]DND = PCE degradation not detected or very slow

All culture bottles, to which CFC113 was added, also degraded CFC113, resulting in lowered CFC113 concentrations as the experiment progressed (See Section 4.4 for CFC113 degradation results). Culture C2, having the lowest initial CFC113 concentration, entirely consumed its initial CFC113 content by Day 50. After Day 50, PCE degradation began to recover slowly in C2 and by Day 75, all PCE had been consumed (Fig 4.18). During the same time period, PCE was recalcitrant in the other cultures that still contained decreased concentrations of CFC113.

TCE produced by the initial stage degradation of PCE also persisted in C3, C4a and C4b (Fig 4.19). In C1 and C2, TCE did not persist and rapidly disappeared following

the breakdown of PCE. In these cultures, the threshold for TCE degradation lay between 0.15 and 0.30mg/L CFC113.

cDCE produced by the initial stage degradation of TCE persisted in C3, C4a and C4b throughout the experiment (Fig 4.20). In the culture containing no CFC113 (C1) cDCE disappeared quickly following the initial degradation of PCE and TCE. Culture C2 contained slowly degrading CFC113 initially at 0.15mg/L. cDCE persisted in C2 until Day 30 at which time cDCE began to degrade. The CFC113 concentration at the time when the cDCE began to degrade (about 30 Days) is estimated to have been 0.1mg/L (Fig 4.37). Therefore, the threshold for cDCE degradation in these cultures is estimated to be 0.1mg/L CFC113. VC is produced and consumed in C1 following the several additions of PCE at various times during the experiment (See Fig 4.21). In cultures C2 and C3, a considerable amount of VC was formed following the initial PCE feeding but persisted throughout the experiment. In C4a and C4b, little VC was formed and that which was formed persisted.

The culture used in Expt D appears to have been less robust than the cultures used in previous CFC113 experiments. This may be due in part to the culture generation used, as later generations may have been more dilute than earlier ones. Also, the incubation time allowed for the culture to develop before adding CFC113 may affect the concentration of active biomass. Cultures used in Expt D were incubated 7 days prior to adding CFC113 whereas in the incubation times in other experiments were, Expt A: 36 days, Expt B: 15 days, Expt C: 44 days.

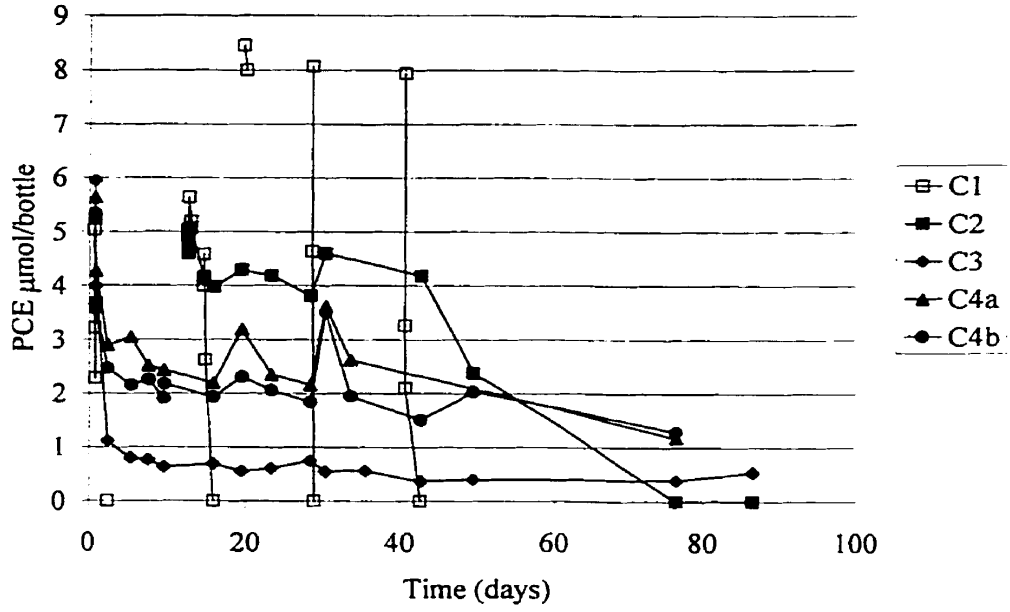


Fig 4.18 Degradation of PCE in one culture containing no CFC113 (C1) and cultures containing CFC113 at 0.15mg/L (C2), 0.30mg/L (C3) and 2.0mg/L (C4a and C4b)

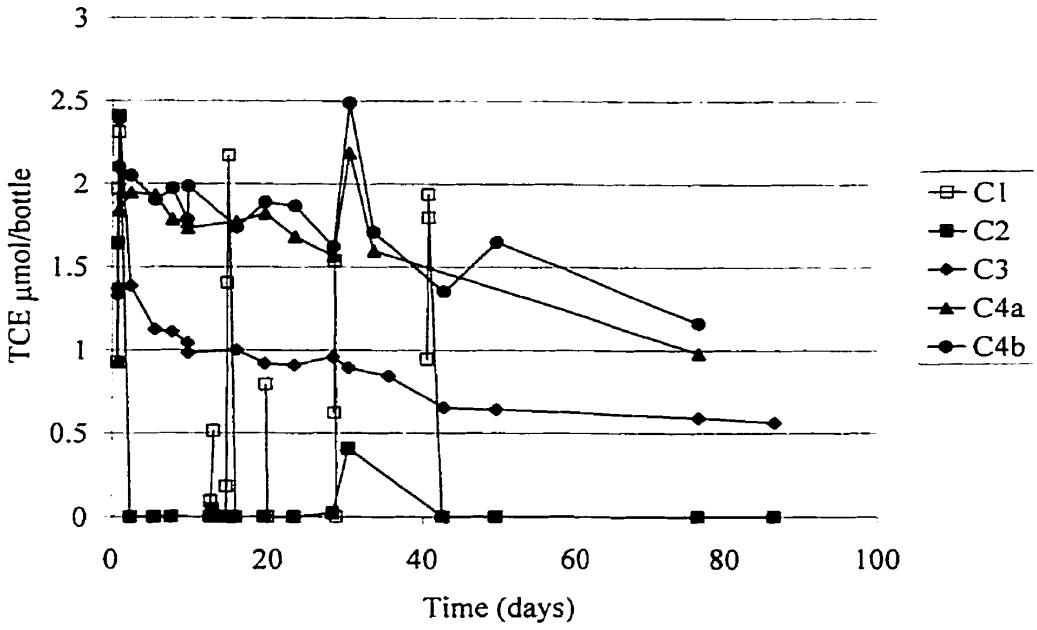


Fig 4.19 Formation and degradation of TCE in one culture containing no CFC113 (C1) and cultures containing CFC113 at 0.15mg/L (C2), 0.30mg/L (C3) and 2.0mg/L (C4a and C4b)

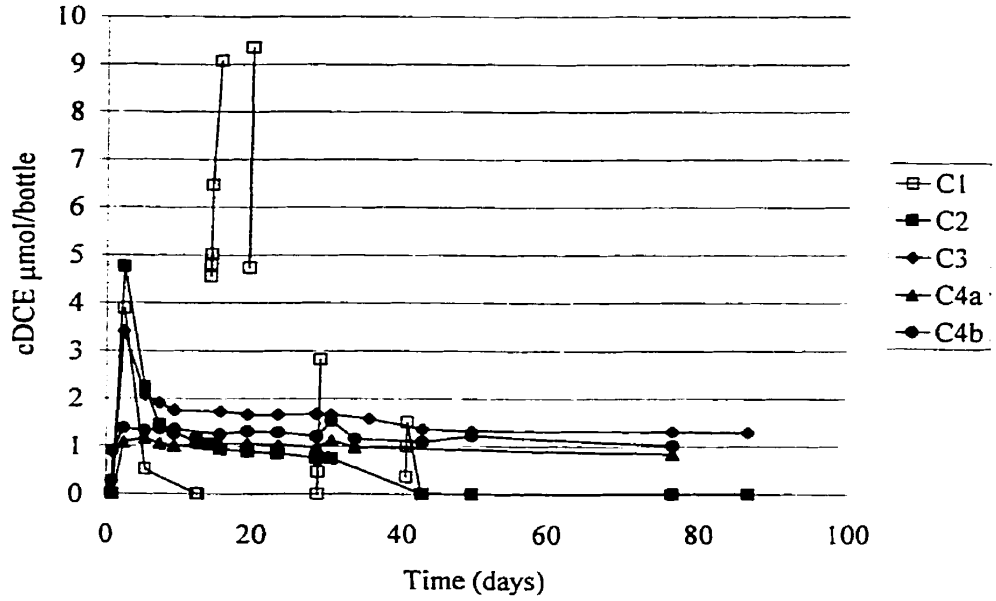


Fig 4.20 Formation and degradation of cDCE in one culture containing no CFC113 (C1) and cultures containing CFC113 at 0.15mg/L (C2), 0.30mg/L (C3) and 2.0mg/L (C4a and C4b)

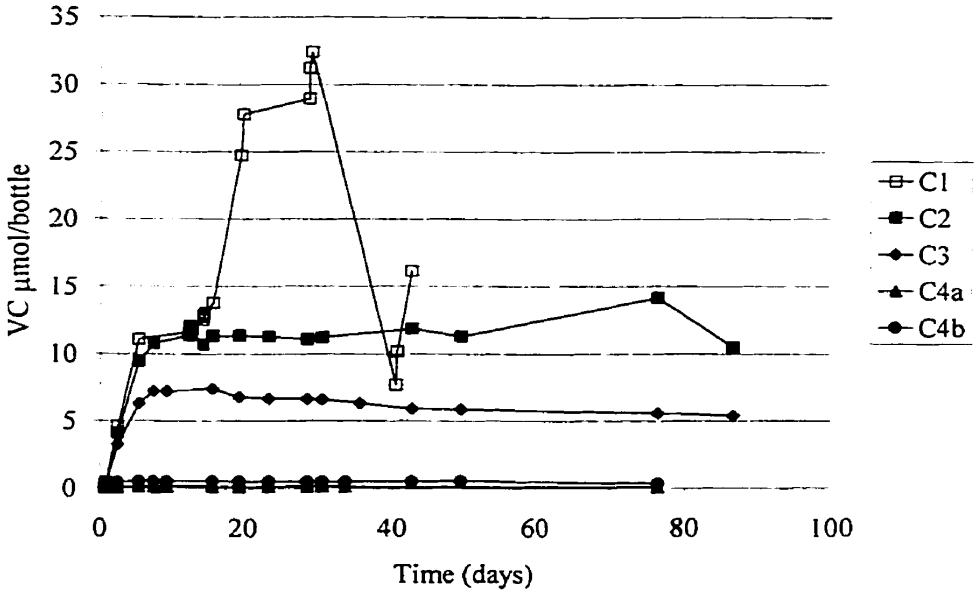


Fig 4.21 Formation and degradation of VC in one culture containing no CFC113 (C1) and cultures containing CFC113 at 0.15mg/L (C2), 0.30mg/L (C3) and 2.0mg/L (C4a and C4b)

C1 began to rapidly form ethene after 20 days (Fig 4.22). C2 began to slowly form ethene after 50 days. No ethene was formed in C3, C4a or C4b. Note in culture C2, cDCE consumption and ethene production coincided between 30 and 50 days, while VC concentration remained constant.

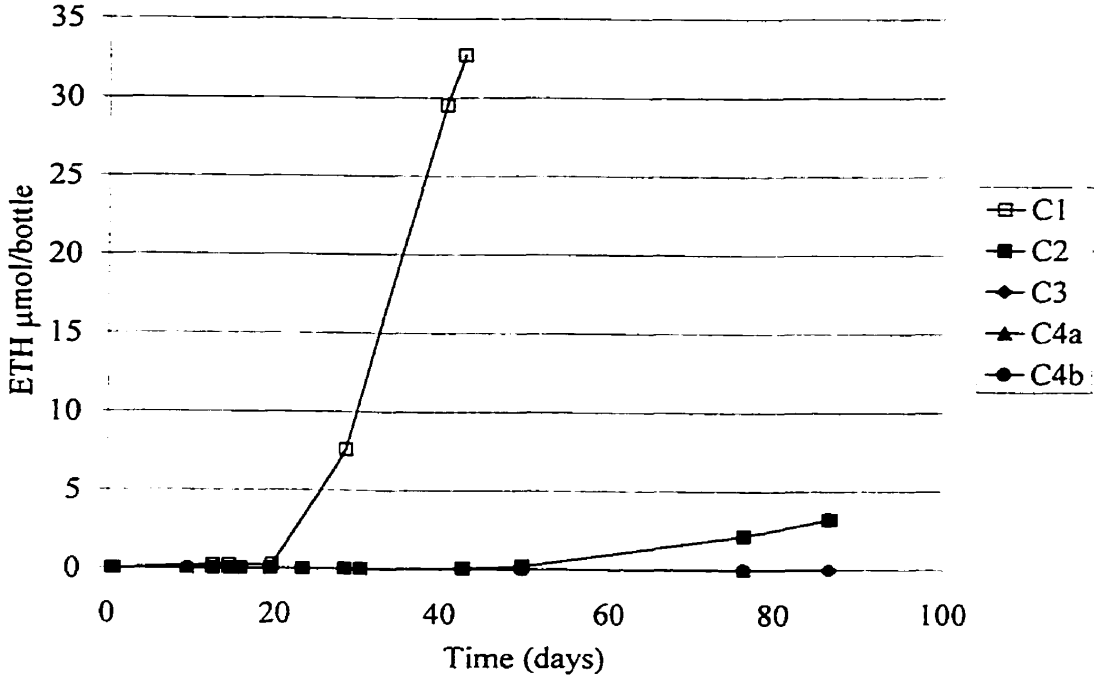


Fig 4.22 Formation of ethene in one culture containing no CFC113 (C1) and cultures containing CFC113 at 0.15mg/L (C2), 0.30mg/L (C3) and 2.0mg/L (C4a and C4b)

4.2.3 Effect of Adding and Removing CFC113

4.2.3.1 CFC113 Expt E

CFC113 Expt E began on August 23, 2000. The purpose of this experiment was to determine the ability of a CFC113 inhibited culture, to recover PCE degrading ability after removal of CFC113. Three new culture bottles were used including one PCE degrading culture containing no CFC113 (C1) and two PCE degrading culture bottles containing CFC113 at 1.0mg/L (C2a and C2b). The three bottles received PCE on Day 1. The rate test results on Day 1 were similar in all three bottles (Table 4.8). A second rate test done on Day 6 showed that in the two CFC113 spiked bottles (C2a and C2b), PCE degradation was severely inhibited. By Day 15 most of the PCE that had been added to C2a and C2b on Day 6 remained (See Fig 4.23).

Table 4.8 PCE degradation rates during Expt E (μmol bottle^{-1} hr^{-1})

Bottle Study Code	Initial CFC113 (mg/L)	Day				
		1	6	15[1]	27	62
C1 1408	0.0	-1.62	-2.14	-2.96	-0.25	-0.76
C2a 1408	1.0	-1.28	DND[2]	DND[2]	DND[2]	DND[2]
C2b 1408	1.0	-0.94	"	"	"	"

[1] all bottles purged on Day 15
[2] DND = PCE degradation not detected

On Day 15, all three bottles were purged with nitrogen gas sparged through the liquid portion of the culture bottle. This procedure removed nearly all ethene, methane and measurable chlorinated substances. Following purging on Day 15, additional PCE/Ethanol stock solution was added to all bottles. C1 resumed degrading PCE following the purging while PCE continued to be recalcitrant in C2a and C2b (Fig 4.23). At Day 27, twelve days after purging, C2a and C2b have not begun to degrade PCE. When testing on Day 50, PCE was recalcitrant in both C2a and C2b.

TCE was formed and degraded in C1 with each addition of PCE. In C2a and C2b, TCE was seen following the initial feeding of PCE after which only traces are seen (See Fig 4.24). Note that C2a shows a small amount of TCE on Day 50, indicating that PCE degradation may have begun at that time. cDCE was formed in C1 on Day 1 but was degraded rapidly. cDCE was formed in C2a which had been consumed by Day 6. cDCE was formed in C2b on Day 1 but persisted until Day 15 (Fig 4.25). Prior to purging, VC had accumulated in all bottles due to the initial PCE degradation (Fig 4.26). Following purging, only C1 produced a significant amount of VC. A small amount of VC remained in C2a and C2b although this may be residual not removed by purging. A considerable amount of ethene was produced in the control (C1) before and after purging (Fig 4.27). Ethene was being formed in C2a before purging but not in C2b. No ethene was formed in either of C2a or C2b after purging (Fig 4.27).

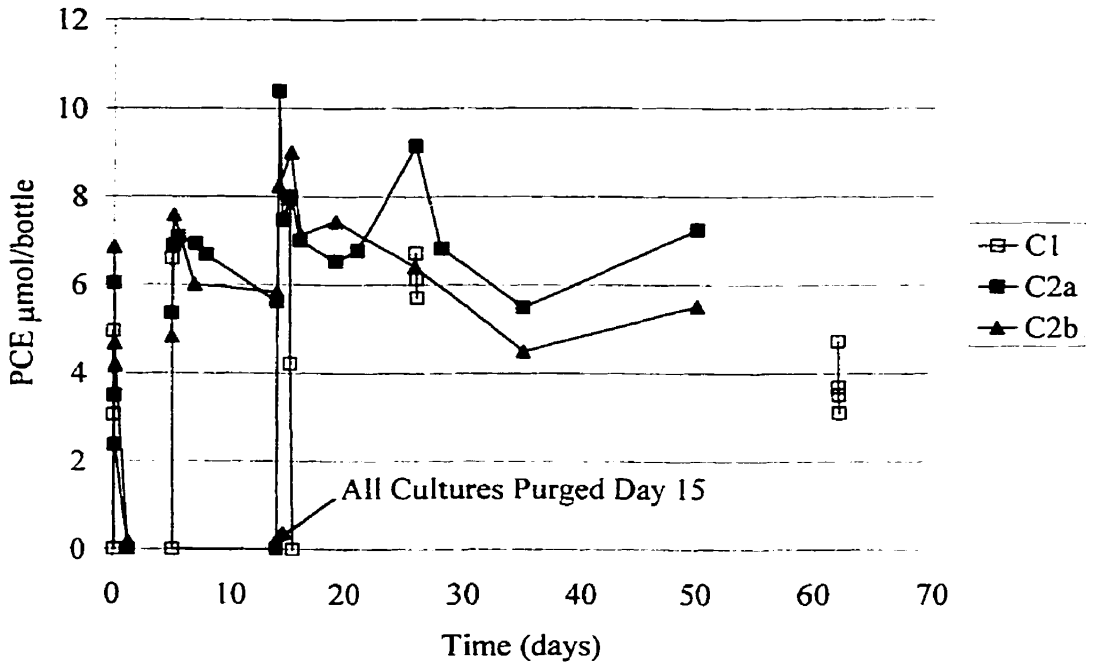


Fig 4.23 Degradation of PCE, one culture containing no CFC113 (C1) and two cultures containing CFC113 at 2.0mg/L (C2a and C2b) all purged on Day 15

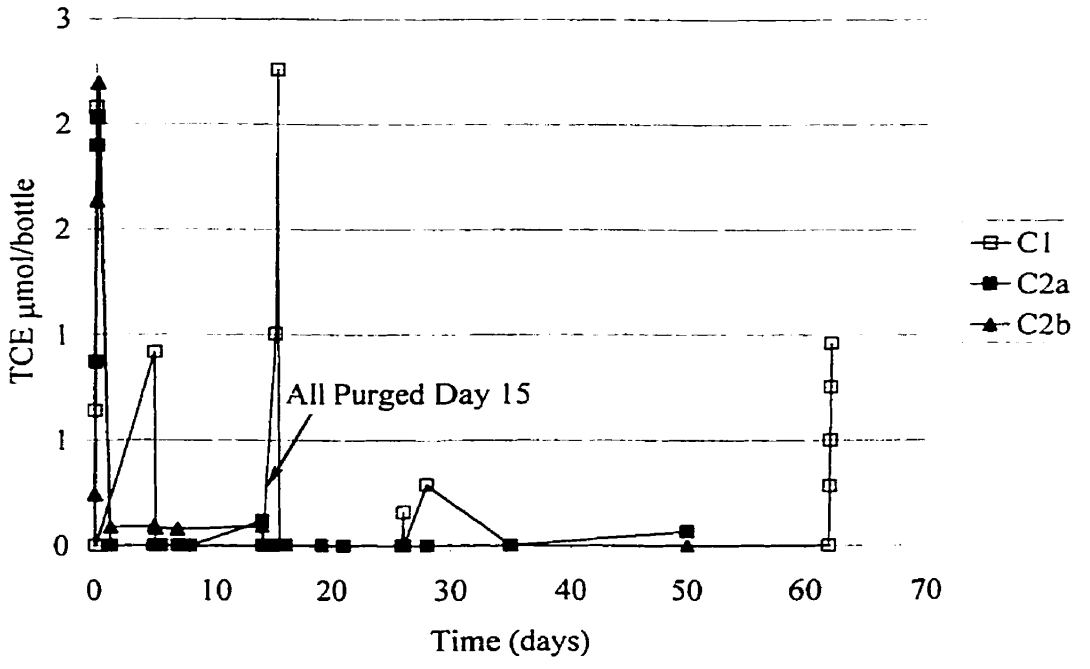


Fig 4.24 Formation and degradation of TCE, one culture containing no CFC113 (C1) and two cultures containing CFC113 at 2.0mg/L (C2a and C2b), all purged on Day 15

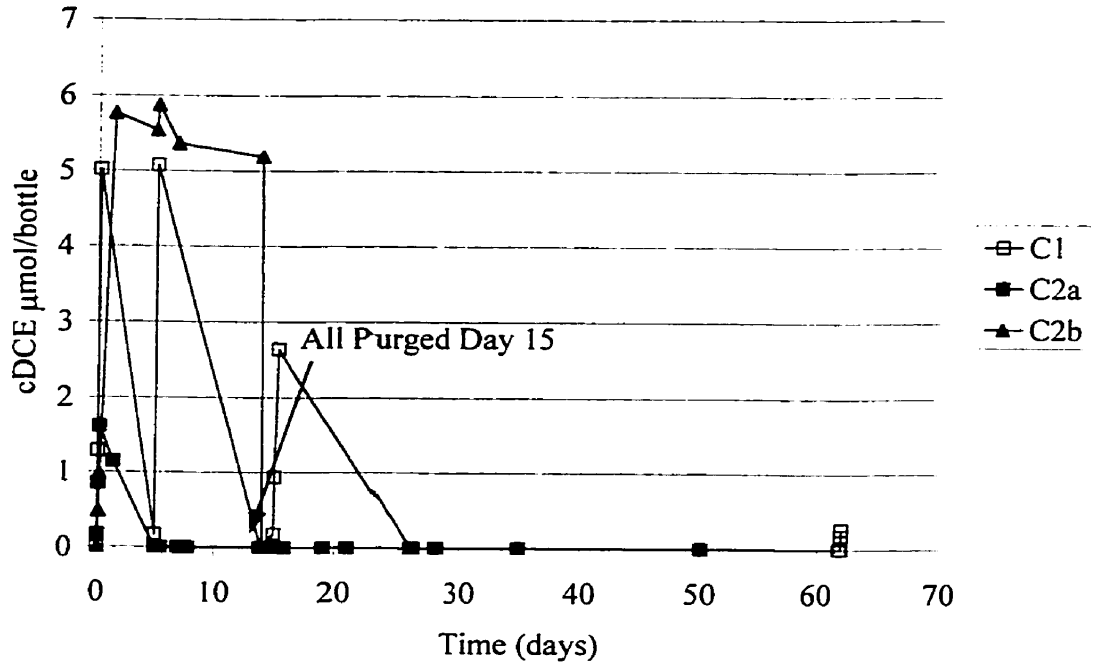


Fig 4.25 Formation and degradation of cDCE, one culture containing no CFC113 (C1) and two cultures containing CFC113 at 2.0mg/L (C2a and C2b), all purged on Day 15

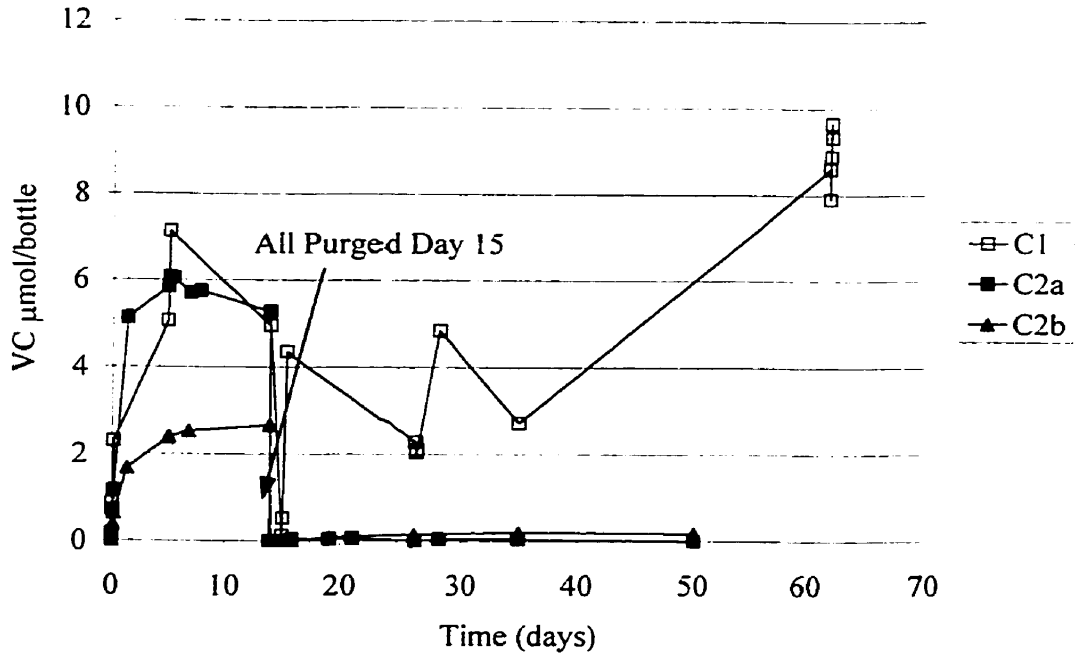


Fig 4.26 Formation and degradation of VC, one culture containing no CFC113 (C1) and two cultures containing CFC1 13 at 2.0mg/L (C2a and C2b), all purged on Day 15

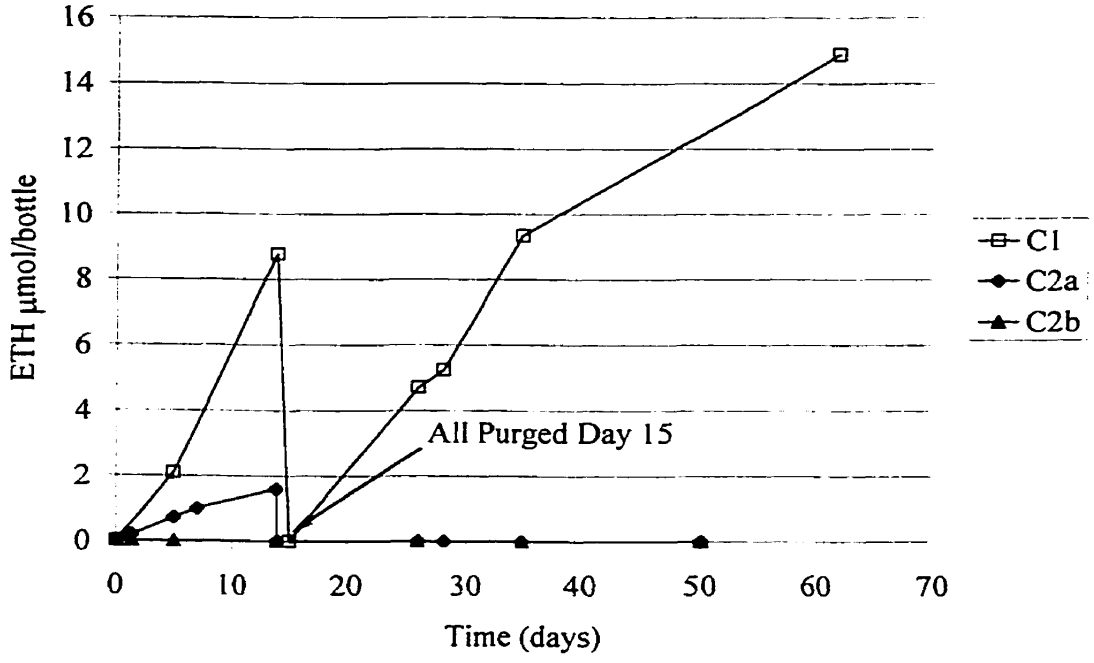


Fig 4.27 Formation of ethene, one culture containing no CFC113 (C1) and two cultures containing CFC113 at 2.0mg/L (C2a and C2b), all purged on Day 15

4.2.4 Effect of Adding Hydrogen

Hydrogen is known to serve as an electron donor for PCE degradation (See Section 2). The rates of hydrogen consumption in cultures that were PCE inhibited with CFC113 were tested. The purposes of this test were to ensure that PCE inhibition was not due to insufficient electron donor and to examine any differences in hydrogen consumption by cultures having different CFC113 concentrations. Three culture bottles from CFC113 Expt. D were used for this purpose. These were C2 (CFC113: 0.15mg/L), C3 (CFC113: 0.30mg/L) and C4b (CFC113: 1.0mg/L). All of these cultures contained CFC113 and recalcitrant concentrations of PCE .

A 1mL syringe of pure hydrogen gas, equivalent to 42µmol of H_2, was injected in to each of the PCE-degrading culture bottles. The bottles were inverted, placed on an

orbital shaker (Labline Instrument Inc., Melrose Pk, IL) and shaken for one hour at 200 rpm. Note that the initial measurement of hydrogen on Day 1 was less than 42μmol/bottle, probably due to transference losses (Fig 4.28). Headspace samples from each bottle were analysed over a period of several days to determine the rate at which hydrogen was being consumed (Fig 4.28). The slopes of the 'best fit' lines through the resulting data were deemed to be the Hydrogen Removal Rates and are summarized in Table 4.9.

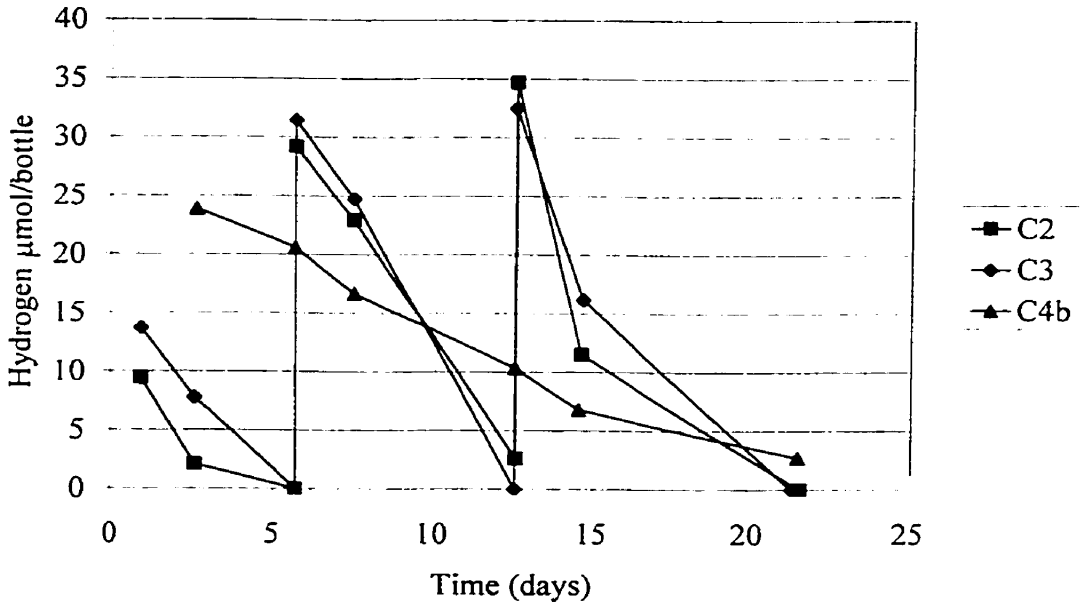


Fig 4.28 Consumption of hydrogen in cultures containing CFC113 at 0.15mg/L (C2), 0.30mg/L (C3) and 2.0mg/L (C4b)

Table 4.9 Hydrogen removal rates in PCE degrading cultures inhibited with CFC113

Bottle Study Code	CFC113 (mg/L) (initial)	Hydrogen Removal Rate (μmol bottle^{-1} day^{-1})
C2 0208 (Ex D)	0.15	-0.16
C3 0208 (Ex D)	0.3	-0.15
C4b 0208 (Ex D)	1.0	-0.05

The hydrogen consumption rate was lowest in the culture having the highest CFC113 concentration. Additional hydrogen was present in all three bottles during the period of Day 28 to Day 49 of CFC113 Expt D. The chlorinated ethene degradation data in CFC113 Expt. D indicate that the degradation rates for the chlorinated ethenes did not increase as a result of the added hydrogen in any of the cultures tested. (Figs 4.29-4.31).

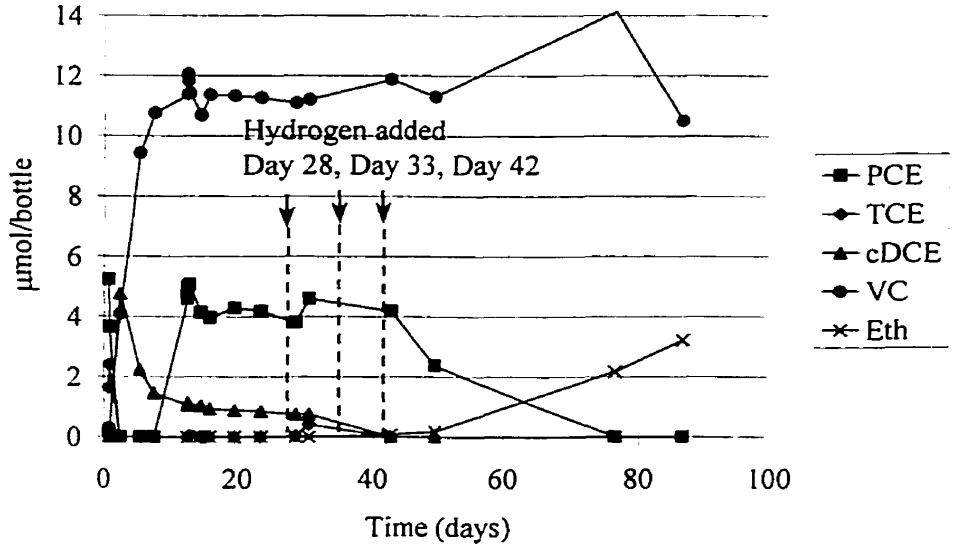


Fig 4.29 Chlorinated ethenes with the addition of hydrogen, CFC113 Expt D, culture C2 (0.15mg/L CFC113)

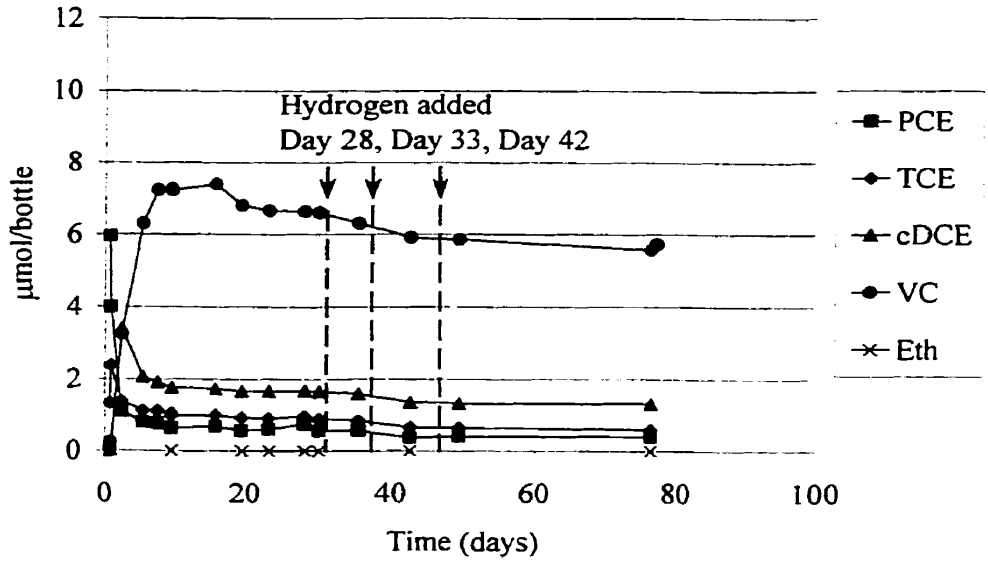


Fig 4.30 Chlorinated ethenes with the addition of hydrogen, CFC113 Expt D, culture C3 (0.30mg/L CFC113)

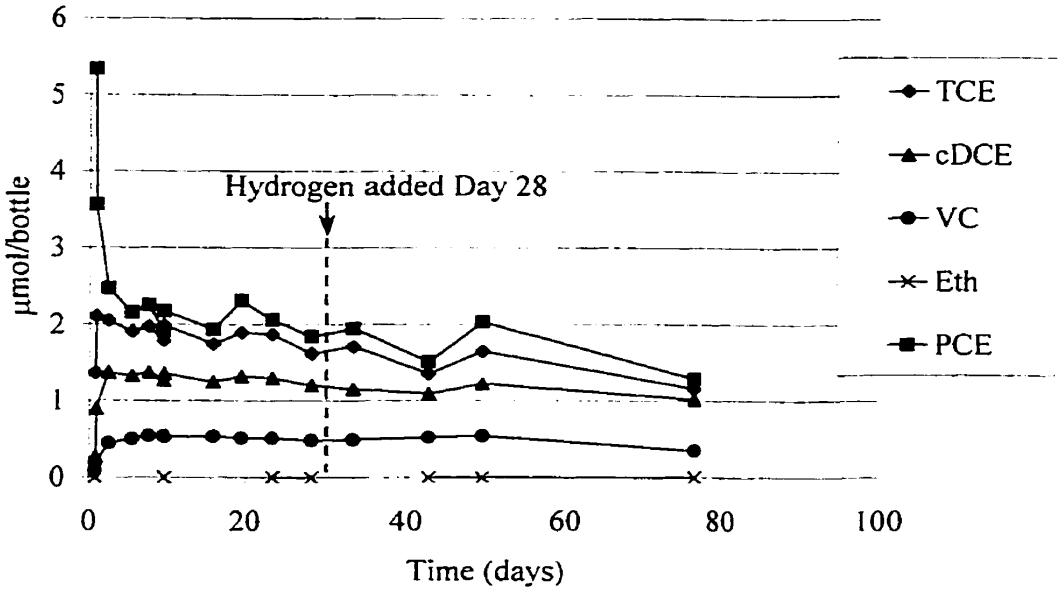


Fig 4.31 Chlorinated ethenes with the addition of hydrogen, CFC113 Expt D, Culture C4b (1.0mg/L CFC113)

4.3 Effect of CFC113 on Electron Donor Degradation and Methane Formation

The concentration of available electron donor was monitored during CFC113 Expt D. Ethanol (EtOH) was the added electron donor and was added as PCE/EtOH stock solution. The 'design' of this stock provided about 25mg/L ethanol in the culture and 6μmol PCE per 3μL injection. Removal of ethanol usually occurred in less than 24 hours, with acetate and propionate formed. These cultures were fed on a regular basis and little change was seen in the concentrations of acetate (350-400mg/L) and propionate (50-100mg/L) throughout the monitoring period (Fig 4.32 and 4.33).

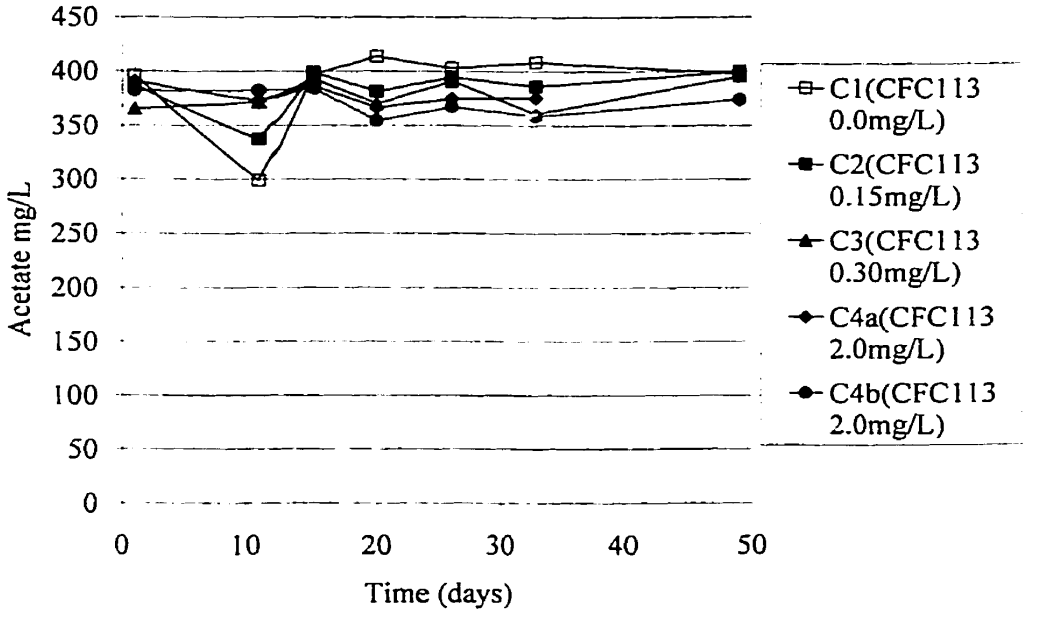


Fig 4.32 Acetate concentration in cultures containing a range of CFC113 concentrations

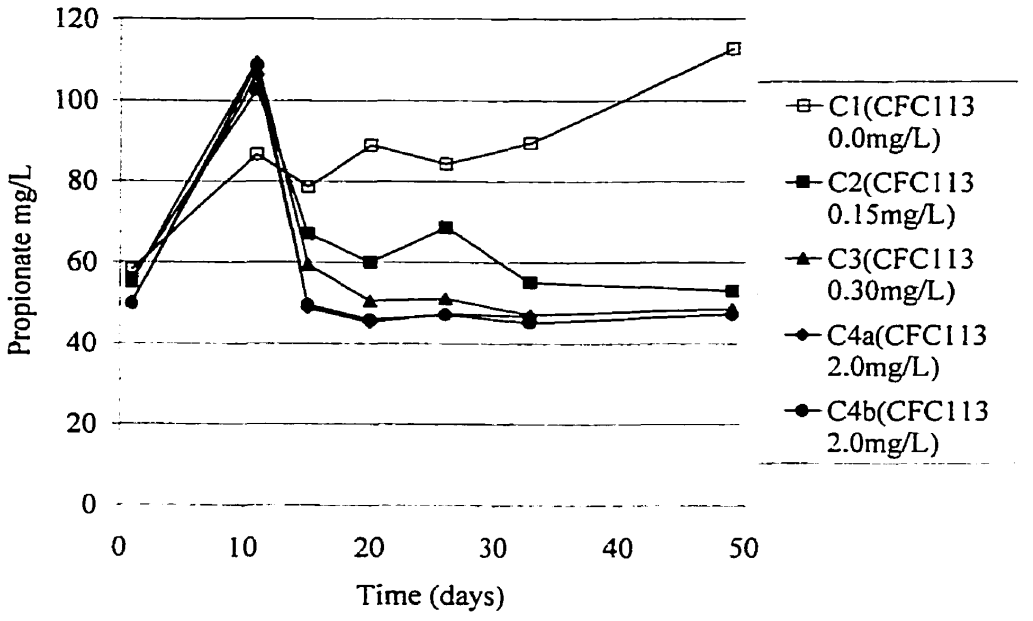


Fig 4.33 Propionate concentration in cultures containing a range of CFC113 concentrations

Methane production in CFC113 Expt D is shown in Fig 4.34. Formation of methane is directly related to the quantity of available electron donor. Bottles C3, C4a and C4b were fed ethanol only once, while bottle C2 was fed twice, about 25mg/L ethanol per feeding. Culture (C1) was fed ethanol six times as indicated by the arrows in Fig 4.34. Prior to Day 12, all cultures had received only one feeding of ethanol and no hydrogen. At Day 12, C1 had produced about 25μmol/bottle methane, C2 about 20μmol/bottle, C3 about 18μmol/bottle and C4a and C4b less than 10μmol/bottle each. Prior to Day 28 C3, C4a and C4b had received only one feeding of ethanol and no feedings of hydrogen. At Day 28, the methane produced by the 2.0mg/L CFC113 cultures (C4a and C4b) was about half that of the 0.3mg/L CFC113 culture (C3) (Fig 4.34). Methane production in cultures C2, C3 and C4b increased starting at Day 28 once

hydrogen addition commenced, suggesting that aceticlastic methanogens were more severely inhibited by CFC113 than hydrogenotrophic methanogens.

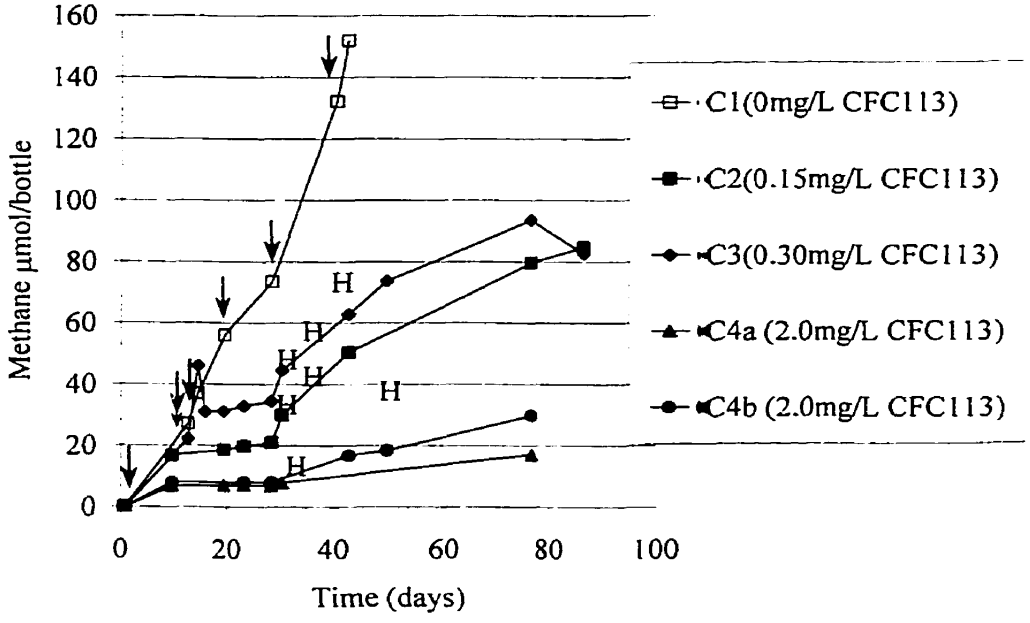


Fig 4.34 Methane concentration in cultures containing a range of CFC113 concentrations. (Ethanol additions are indicated by arrows, hydrogen additions are indicated by (H) symbol.)

4.4 Degradation of CFC113

Other researchers have shown that mixed anaerobic microbial systems can degrade CFC113 and form 1,2-dichlorotriflouroethane (HCFC123a) (See Section 2). During the course of all the CFC113 experiments undertaken, CFC113 was slowly removed and an unknown product was formed. The unknown product was positively identified as HCFC123a by York-Durham Regional Environmental Lab, Oct 10th, 2000, using GC/MS. For example, a steady decline in CFC113 and an increase in HCFC123a occurred in culture B2 from CFC113 Expt A over an eight-day period (Fig 4.35). HCFC123a was first detected on Day 6 of CFC113 Expt A. This result is consistent with the CFC113 degradation pathway proposed by Lesage et al. (1992).

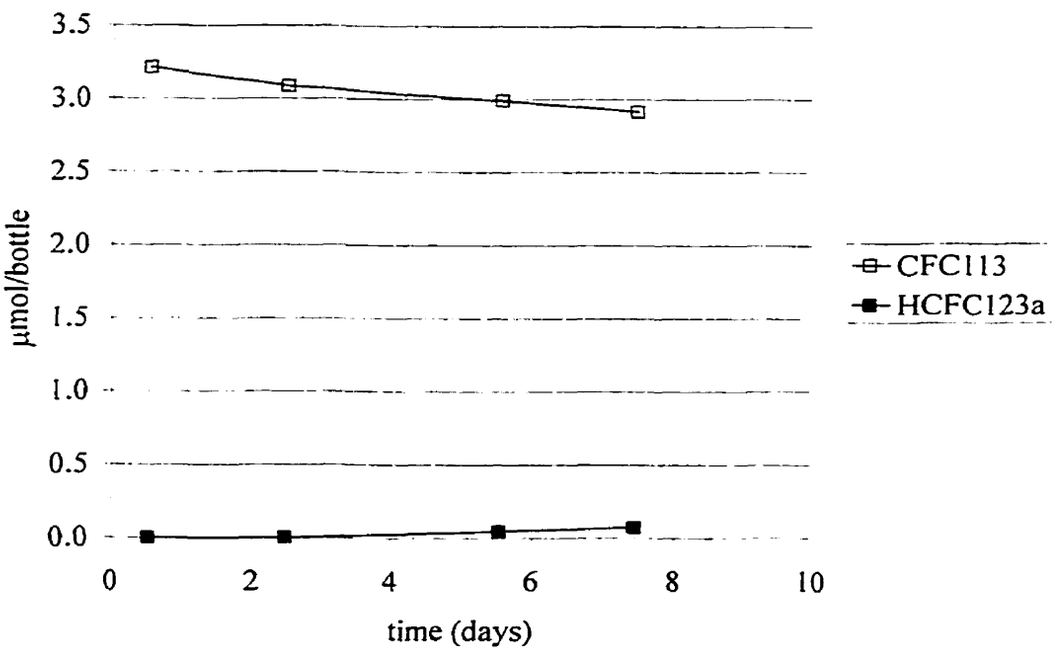


Fig 4.35 CFC113 Degradation and HCFC123a Formation, culture B2, CFC113 Expt A, CFC113 starting at 0.86mg/L (3.2µmol/b)

In CFC113 Expt D, four culture bottles containing three different concentrations of CFC113 at 0.15, 0.30 and 2.0mg/L were examined. The slopes of the CFC113 degradation curves appeared to decrease with time suggesting that the rate of CFC113 degradation was concentration dependent (Figs 4.36-4.37). Note in Fig 4.37 that the HCFC123a curve for culture C2 became flat after 45 days once the CFC113 concentration approached zero, suggesting that HCFC123a was not being removed once formed.

The average CFC113 degradation rates in Table 4.10 are calculated based on the slope of the best fit straight line through the data collected over a 76-day period. These data are displayed graphically in Figs 4.36 and 4.37. The CFC113 degradation rates appeared to be directly related to the initial concentration of CFC113. Additionally, the HCFC123a production rates in all cases were less than the CFC113 removal rates, indicating that other undetected degradation products were formed (Figs 4.36 and 4.37; Table 4.10).

Table 4.10 Comparison of CFC113 degradation rates in CFC113 Expt D

Bottle Study Code	Initial CFC113 (mg/L) (μmol bottle^{-1})	CFC113 degradation rate (μmol bottle^{-1} day^{-1})	HCFC123a Formation Rate (μmol bottle^{-1} day^{-1})
C2 0208	0.15mg/L (0.55μmol/bottle)	-0.008	0.003
C3 0208	0.30mg/L (1.1μmol/bottle)	-0.015	0.006
C4a 0208	2.0mg/L (7.4μmol/bottle)	-0.070	0.020
C4b 0208	2.0mg/L (7.4μmol/bottle)	-0.062	0.021

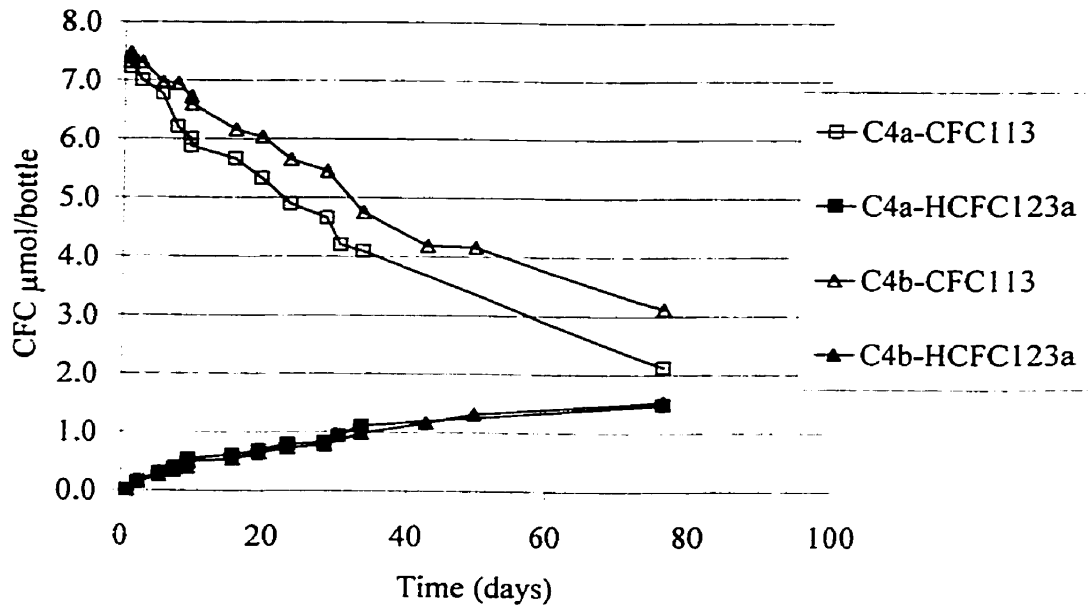


Fig 4.36 CFC113 Degradation and HCFC123a Formation in C4a and C4b (2.0mg/L CFC113), CFC113 Expt D.

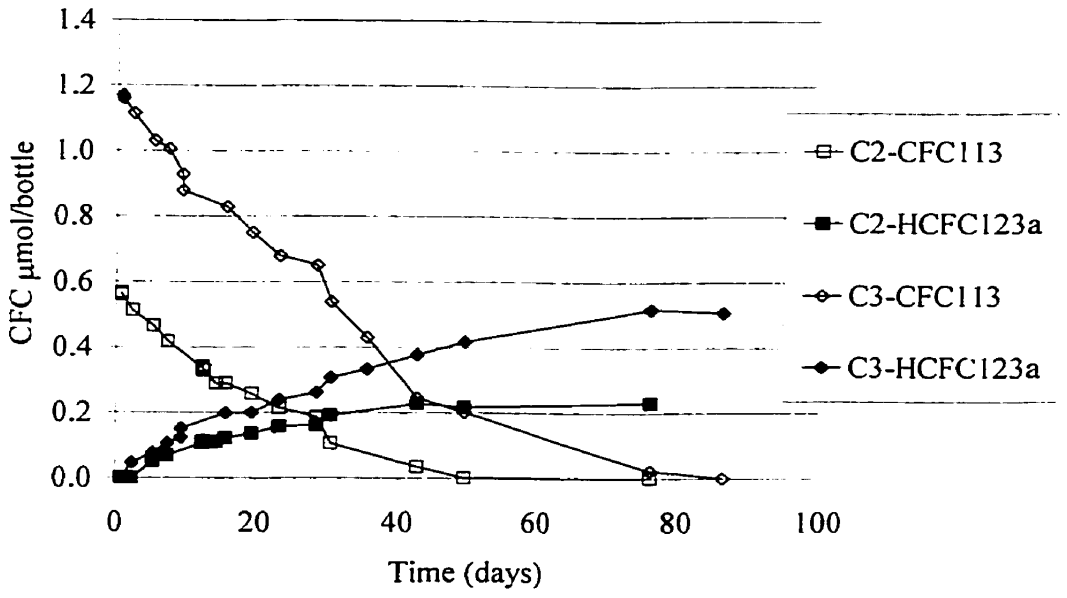


Fig 4.37 CFC113 Degradation and HCFC123a Formation in C2 (0.15mg/L CFC113) and C3 (0.30mg/L CFC113), CFC113 Expt D.

4.4.1.1 CFC113 Expt. F

To test whether CFC113 conversion to HCFC123a was the result of a biological process, a separate experiment CFC113 Expt F was conducted using three PCE degrading cultures that had not previously been exposed to CFC113 and one water control. Two of the cultures were autoclaved for 20 minutes at 115°C then allowed to cool. All three cultures were then spiked with CFC113 at about 1.0mg/L (4.0μmol/bottle). These cultures are referenced as C11408 (C1), C2a1408 (C2a) and C2b1408 (C2b) in Fig 3.2, Section 3. Culture C1 was the active CFC113 degrading culture, C2a and C2b were the autoclaved replicates. The CFC113/HCFC123a concentration data for the live and autoclaved cultures are pictured in Fig 4.38 and for the water control in Fig 4.39. CFC113 was readily removed in the live culture and formed HCF123a. Very little CFC113 was removed in the two autoclaved controls except for a small amount similar to the loss seen in a water control. In the water control, CFC113 was slowly removed but no HCFC123a was formed. Furthermore, after 91 days, only 15% of the CFC113 was removed from the water control compared to 32% removed from the live culture, C1.

Regressing the best fit straight line through the water control CFC113 data over 83 days gives an average removal rate of -0.0024μmol/bottle/d (Fig 4.39). This rate is well below -0.0140μmol/bottle/d calculated from the slope of CFC113 curve for the live culture in Fig 4.38 and less than any of the rates in Table 4.10. That biological processes were responsible for CFC113 removal and conversion to HCFC1213a is probable.

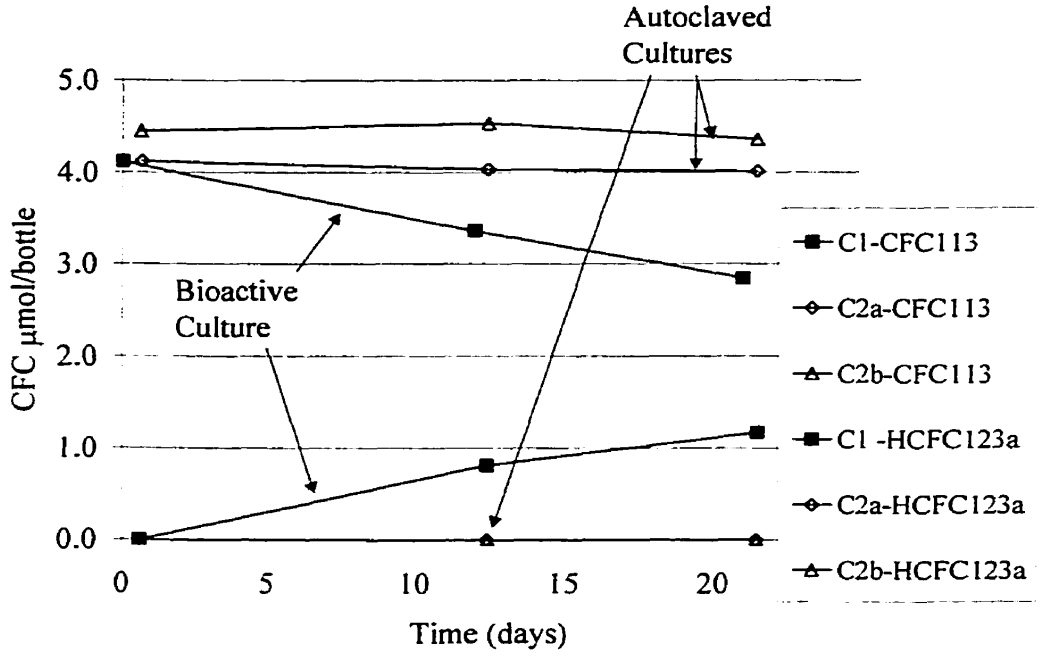


Fig 4.38 One bioactive CFC113 degrading culture compared with two autoclaved CFC113 degrading cultures.

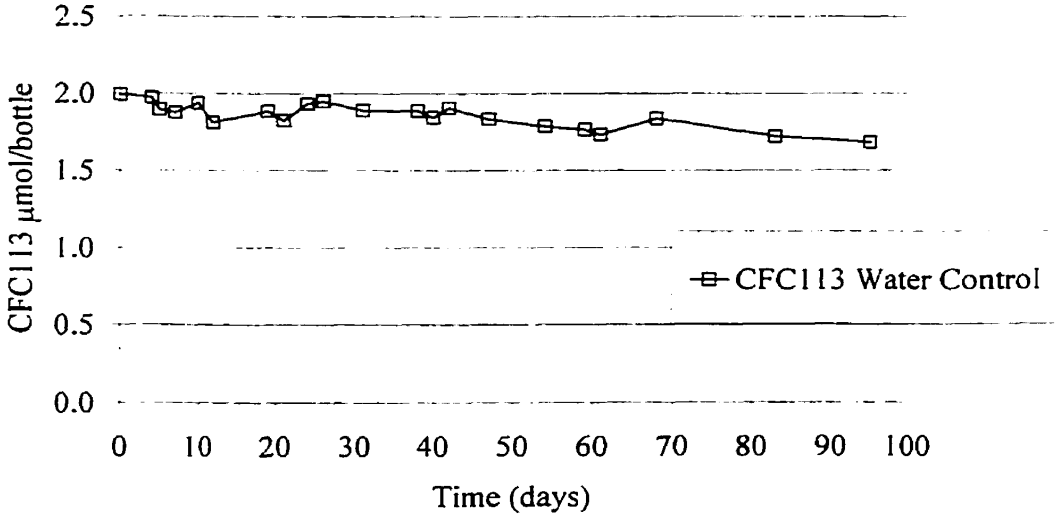


Fig 4.39 CFC113 water control over 95 day period

4.4.1 CFC113 Degradation Products

Several cultures selected from CFC113 experiments were tested by York-Durham Regional Environmental Lab, using GC/MS to determine the presence of CFC113 degradation products (Table 4.11). The results provided were qualitative and indicate only 'yes or no' as to the finding of a substantial amount of the analytes listed. As previously mentioned, HCFC123a was confirmed in several cultures.

Table 4.11 CFC113 degradation products

Bottle Study Code	CFC113 Expt	Initial CFC113 (mg/L)	Culture Type	Days incubation	Analytes Confirmed
C3 0208	D	0.30	PCE degrading	136	HCFC123a
B2a 3103	A	0.86	PCE degrading	157	CFC113, HCFC123a
B4 1505	C	1.0	"	104	CFC123a, HCFC123a
C1 2308	F	1.0	CFC113 degrading	72	CFC113, HCFC123a, CTFE
C2a 2308	F	1.0	autoclaved	72	CFC113
C2b 2308	F	1.0	autoclaved	72	CFC113
C4a 0208	D	2.0	"	62	CFC113, HCFC123a, CTFE

5 THE EFFECT OF HCFC123a ON PCE BIODEGRADATION

5.1 Experimental Design

The purpose of these experiments was to determine the effect of HCFC123a on biodegradation of PCE and lesser-chlorinated PCE byproducts and to determine the concentration of HCFC123a required for an effect to be seen. The same method for evaluating the effect of HCFC123a on the rate of PCE degradation was used for testing the effect of CFC113 on PCE degradation as described in Section 4. Table 5.1, provides details of the combined HCFC123a/PCE tests conducted. The genealogies of the various cultures of HCFC123a Expt A are described in Fig 3.2.

PCE degradation inhibition was observed in the CFC113/PCE experiments when the HCFC123a concentration was in the 0.1-0.2mg/L range. The choice of the HCFC123a concentrations used in HCFC123a Expt A was intended to bracket the 0.1-0.2mg/L HCFC123a range of concentrations.

Table 5.1 Experimental conditions for HCFC123a studies[1]

Study Code (Bottle)	HCFC123a Aqueous Conc. (mg/L)	Study Time (days)	Number Tested	Gas Purging[2] (y/n)
C2a/ C2b2007	0.04	31	2	y
C3a/ C3b2007	0.40	31	2	y

[1]PCE added at 6μmol/bottle each feeding.
[2]y = yes; n = no

5.2 Effects of HCFC123a

5.2.1 Effect on PCE Degradation

5.2.1.1 HCFC123a Expt A

This experiment was begun on July 31st, 2000 and was the only group of cultures, used in HCFC123a testing. Five culture bottles were used including one PCE degrading

control (C1). Two PCE degrading cultures contained HCFC123a at 0.04mg/L (0.03μmol/b) (C2a and C2b) and two PCE degrading cultures contained HCFC123a at 0.40mg/L (0.33μmol/b) (C3a and C3b) (Table 5.2).

Table 5.2 PCE degradation results for HCFC123a Expt A (μmol bottle^{-1} hr^{-1})

Bottle Study Code	HCFC123a (mg/L)	Days					
		1	10	12	26	29	31
C1 2007	0.0	-1.72	-1.13	-1.16	-1.13	no test	no test
C2a 2007	0.04	-1.37	-1.11	-1.41	-0.85	no test	-1.80
C2b 2007	0.04	-2.06	-1.10	-1.61	-1.31	no test	-2.10
C3a 2007	0.40	-1.66	-0.37	-1.72	no test	-0.13	no test
C3b 2007	0.40	-1.76	-0.85	-1.24	no test	-0.19	no test

A sample of the PCE degradation curve is displayed in Fig 5.1, and is typical of the data used to compile Table 5.2. This figure shows the Day 26 data for C1, C2a and C2b and includes the actual degradation rate calculated from the slope of the curve for each culture. Fig 5.2 provides the Day 29 PCE degradation results for C3a and C3b. For comparison, the same scale abscissa and ordinate have been used in both Figs 5.1 and 5.2.

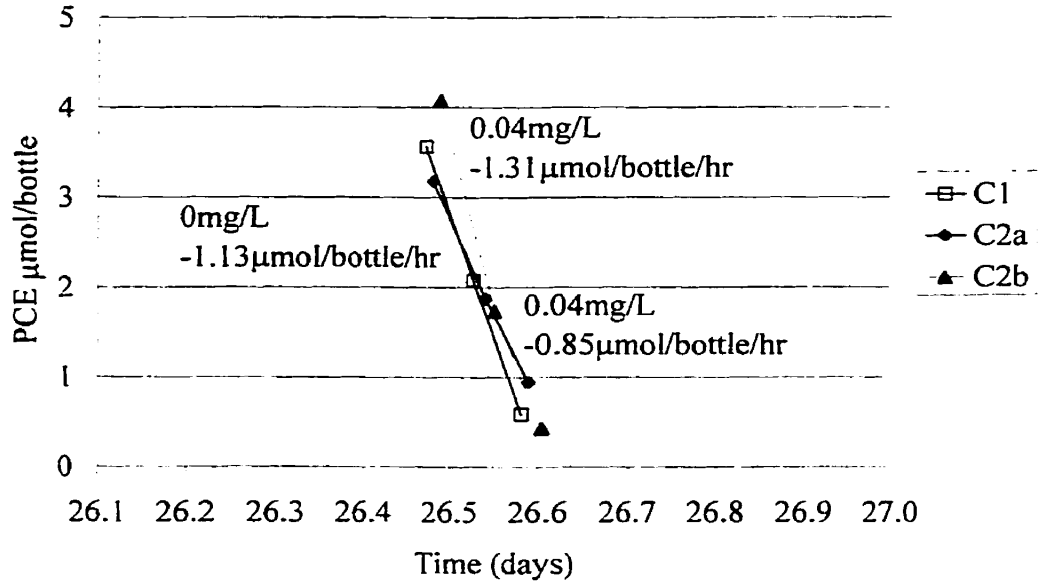


Fig 5.1 PCE degradation on Day 26 for HCFC123a Expt A (C1, C2a, C2b)

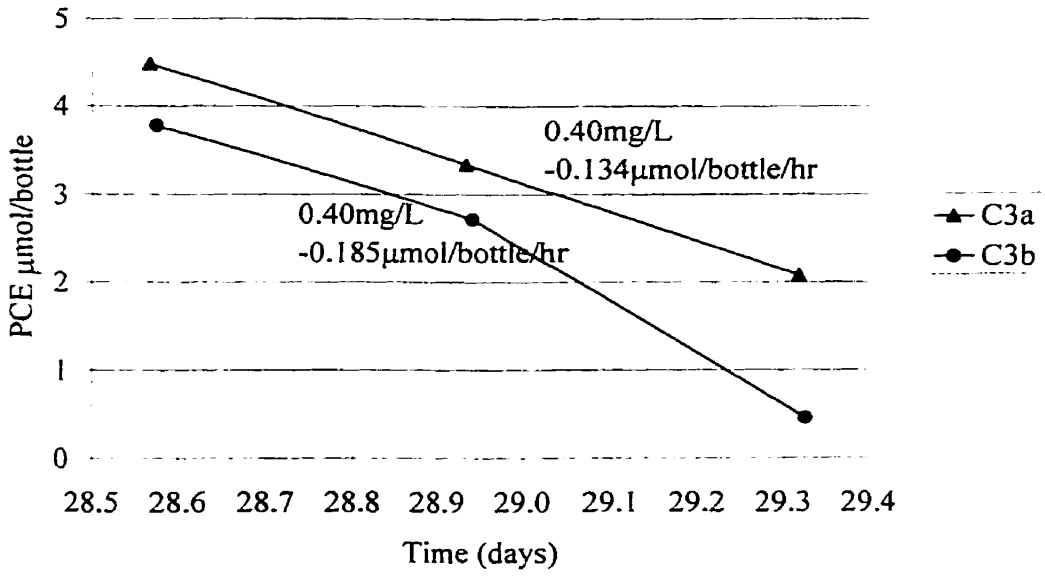


Fig 5.2 PCE degradation on Day 29 for HCFC123a Expt A (C3a, C3b)

HCFC123a at 0.04mg/L (C2a and C2b) did not significantly affect the rate of PCE degradation when compared with the culture containing no HCFC123a (C1). At 0.40mg/L HCFC123a (C2a and C2b) however, PCE removal was inhibited. The inhibition seen at 0.40mg/L HCFC123a was not severe enough to result in cessation of PCE degradation. Substantial PCE inhibition did not begin to occur until sometime between the twelfth and the twenty-sixth day although there was some evidence of a rate reduction on Day 10. In all bottles, PCE was ultimately removed within a 'suitable time period' and did not remain.

The PCE dechlorination products are presented in Figs 5.3-5.6. Degradation rates should not be inferred from the slopes of these curves because the data that is presented includes measurements of zero indicating the analyte had been entirely consumed. This measurement may have been obtained some time after the complete consumption of a particular analyte. TCE was formed in all cultures with and without HCFC123a. The TCE results are presented in Fig 5.3. The rates of consumption varied but in no case did TCE accumulate from one PCE addition to the next. The cDCE results are presented in Fig 5.4. cDCE was rapidly formed and consumed. No impact on cDCE degradation due to HCFC123a was observed. VC was formed quickly following the addition of PCE and the sequential breakdown of PCE products (Fig 5.5). VC accumulated shortly after PCE addition but then slowly declined during the periods between PCE additions.

Ethene data pictured in Fig 5.6 below provided a clear indication of the similar behavior in all cultures tested with and without HCFC123a. In all cases, the total amount of ethene being formed was very similar indicating that HCFC123a had no effect on total ethene formation in the time period analysed.

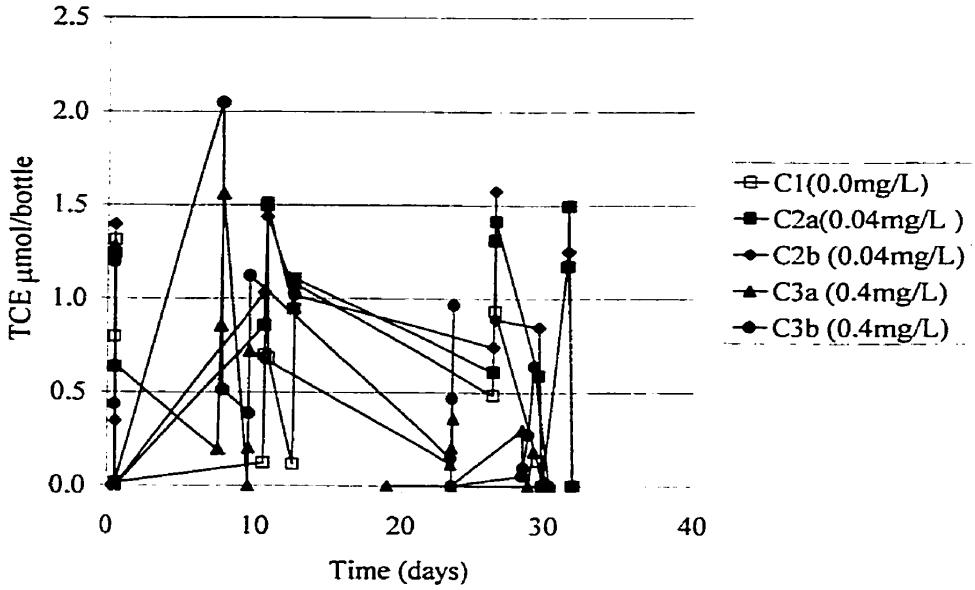


Fig 5.3 Production and degradation of TCE in one culture containing no HCFC123a (C1), two cultures containing HCFC123a at 0.04mg/L (C2a/b) and two cultures containing HCFC123a at 0.4mg/L (C3a/b) (Expt A)

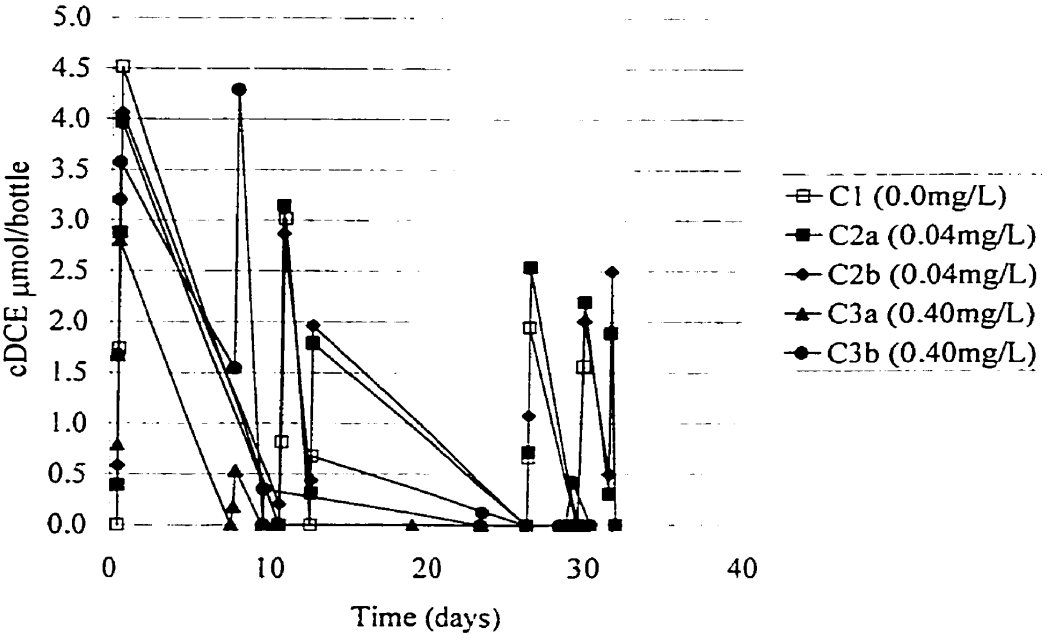


Fig 5.4 Production and degradation of cDCE in one culture containing no HCFC123a (C1), two cultures containing HCFC123a at 0.04mg/L (C2a/b) and two cultures containing HCFC123a at 0.4mg/L (C3a/b) (Expt A)

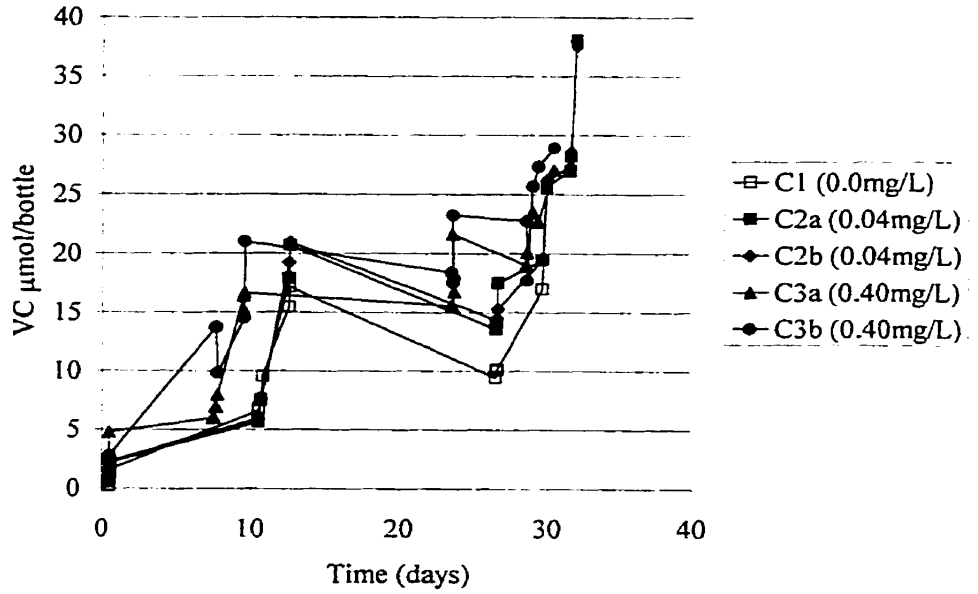


Fig 5.5 Production and degradation of VCin one culture containing no HCFC123a (C1), two cultures containing HCFC123a at 0.04mg/L (C2a/b) and two cultures containing HCFC123a at 0.4mg/L (C3a/b) (Expt A)

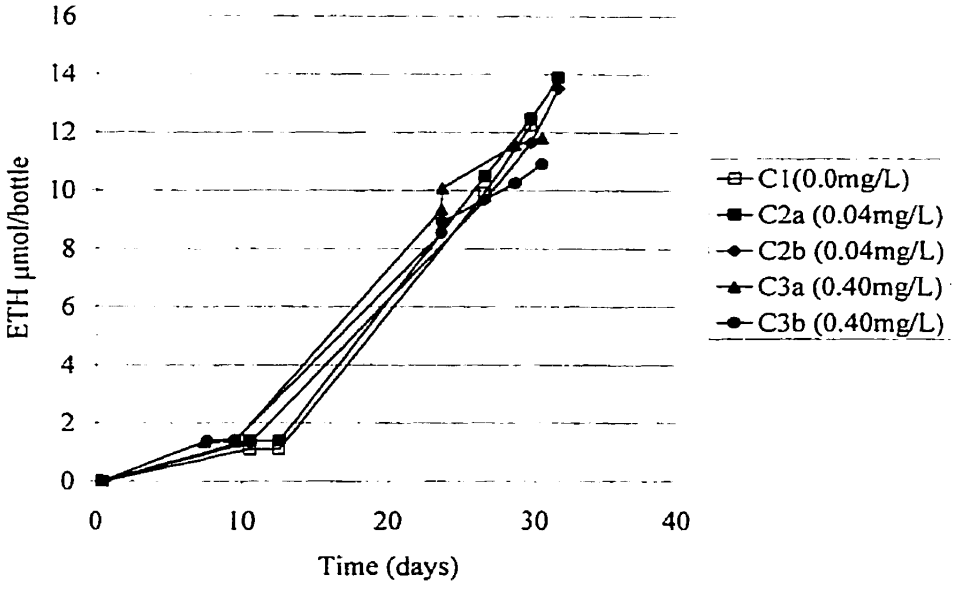


Fig 5.6 Production and of ethene in one culture containing no HCFC123a (C1), two cultures containing HCFC123a at 0.04mg/L (C2a/b) and two cultures containing HCFC123a at 0.4mg/L (C3a/b) (Expt A)

HCFC123a addition slowed the rate of PCE degradation but did not cause cessation at the concentrations tested. PCE degradation products continued to be degraded without excess accumulation in those cultures containing HCFC123a and total ethene formation was similar in all bottles.

5.2.2 Effect of HCFC123a on Electron Donor Degradation and Methane Formation

A greater amount of acetate was found in cultures having the higher HCFC123a concentration (See Fig 5.7). The amount of acetate detected in cultures C3a and C3b is too high to have been formed solely by the ethanol added. Only four additions of ethanol amounting to 100mg/L total were conducted. If all the ethanol was converted to acetate and hydrogen, not degraded, the increase in acetate concentration would have been 130mg/L. The observed increase of almost 400mg/L to over 550mg/L indicates that either acetate production from non-ethanol sources was stimulated by the presence of HCFC123a or acetate was inadvertently added, or extra ethanol was inadvertently added or the measurements in bottles C3a and C3b on Days 30 and 38 were incorrect.

Propionate formed readily in all cultures but may have started to be consumed in those having the higher HCFC123a concentration (Fig 5.8).

Methane was produced in all bottles following the addition of HCFC123a (Fig 5.9). Methane formation was greatest in those cultures containing the higher concentration of acetate.

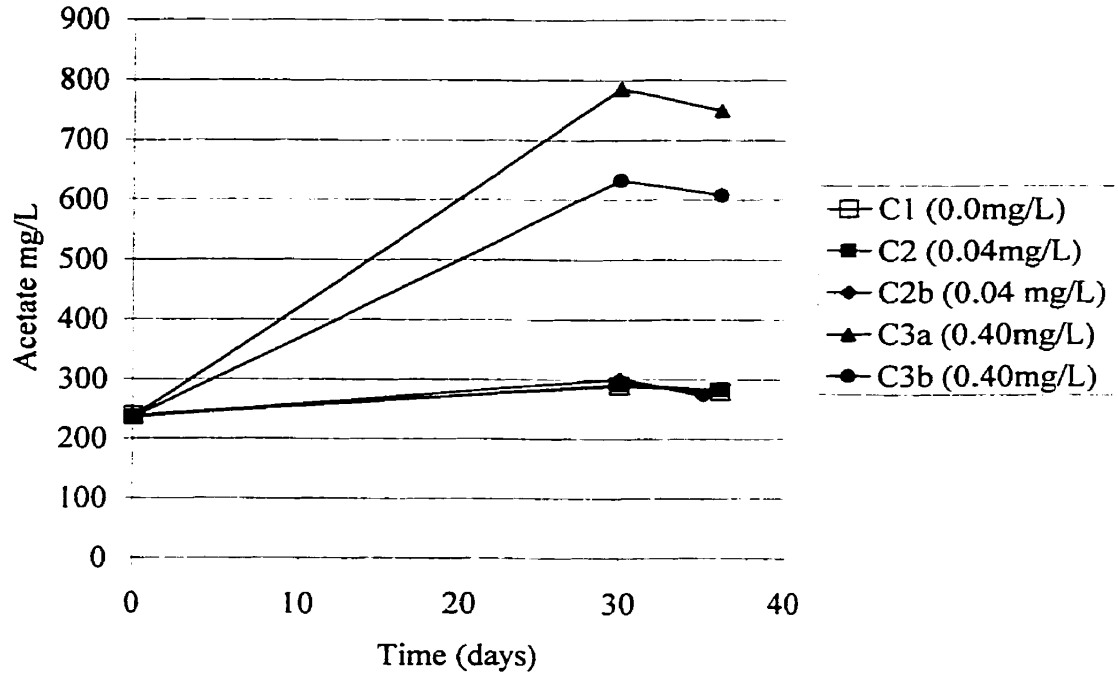


Fig 5.7 Concentration of acetate in HCFC123a Expt A

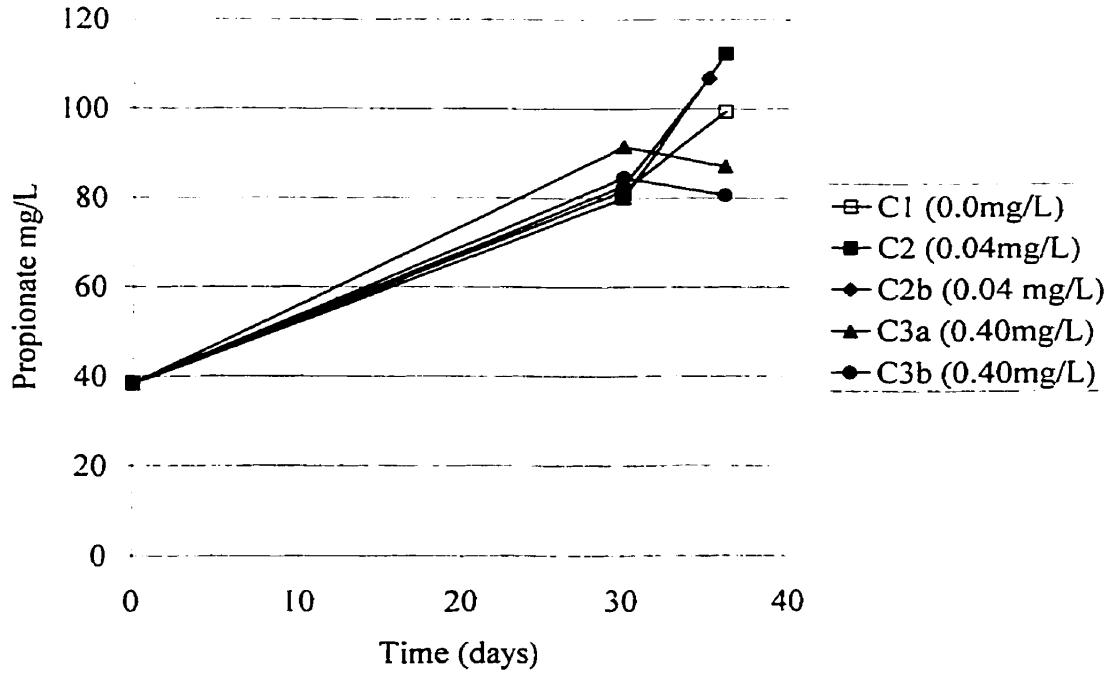


Fig 5.8 Concentration of propionate in HCFC123a Expt A

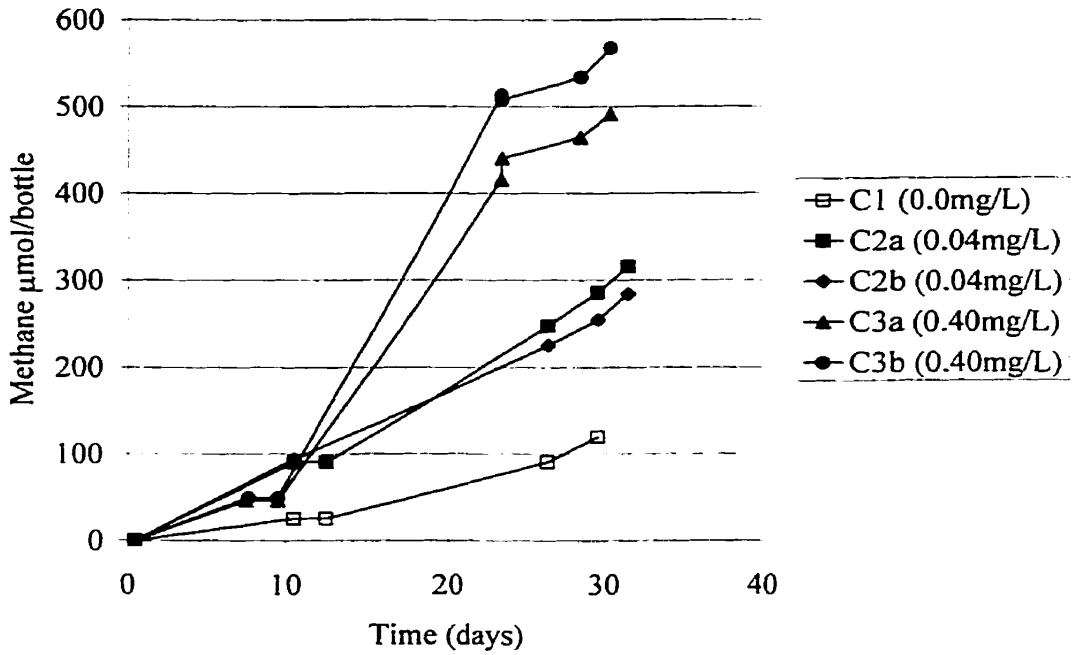


Fig 5.9 Formation of methane in HCFC123a Expt A

5.3 Degradation of HCFC123a

HCFC123a degradation data are presented in Fig 5.10 below. One HCFC123a water control data was examined. Note that the sampling time between points in the water control data exceeded the scale of the HCFC123a degradation data thus the right side data point of the water control does not appear in Fig 5.10.

A visual comparison of curves shows that the HCFC123a removal rate is similar in both bottles C2a and C2b as bottles C3a and C3b also demonstrated similar removal rates. The HCFC123a removal rates in both C3a and C3b appeared to be greater than that in the water control, suggesting that HCFC123a may have been degraded in those cultures having the higher concentration of HCFC123a and that the degradation rate of HCFC123a was concentration dependent.

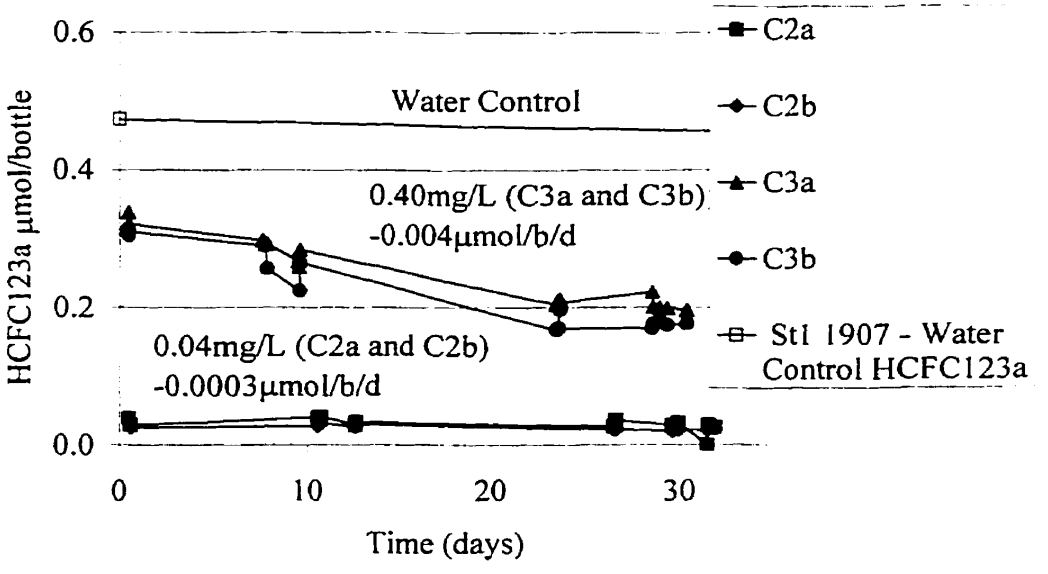


Fig 5.10 Concentration of HCFC123a in HCFC123a Expt A, in cultures and water control

5.3.1 HCFC123a Degradation Products

While removal rates of HCFC123a in C3a and C3b appeared to be greater than the removal rate in the water control, no degradation products were detected with the usual experimental methods described in Section 3. To further test for the formation of HCFC123a degradation products, culture C3a2007 was submitted to an independent lab equipped with Gas Chromatography/ Mass Spectroscopy (York-Durham Regional Environmental Laboratory, Pickering, Ontario). The results provided were qualitative and indicate only 'yes or no' as to the finding of a substantial amount of the analytes listed. The results of that analysis, done 145 days after adding HCFC123a, confirmed only the existence of HCFC123a and no degradation products. Chlorotrifluoroethene (CTFE) was not detected.

6 THE EFFECT OF CFC114 ON PCE BIODEGRADATION

The purpose of this set of experiments was to test the hypothesis that CFC114 inhibits biodegradation of PCE. Objectives include determining the threshold concentration of CFC114 at which inhibition occurs and the threshold concentration of CFC114 at which PCE biodegradation ceases. The effect of CFC114 on biodegradation of PCE breakdown products and the tendency of CFC114 to be degraded were also investigated. The effect of CFC114 on electron donors and methane formation was also examined. This series of experiments was conducted using the same methods as those used to test the effects of CFC113 and HCFC123a on PCE degradation.

6.1 Experimental Design

In each test conducted, a set of replicate culture bottles was prepared that were actively degrading PCE and producing ethene. Ethene was the final product of reductive PCE dechlorination in the mixed microbial cultures.

Table 6.1 provides combined details of the CFC114/PCE tests conducted. The genealogy of these various cultures is described in Chapter 3 (Fig 3.2). A total of five PCE degrading cultures not containing CFC114 were used in these experiments and are discussed separately in Section 6.2.1. All of the culture bottles in each experiment group were prepared at the same time, from the same source (same genealogy) and are compared directly with one another within the group. This comparison is used to draw conclusions as to the effect of CFC114 on a particular culture, recognizing that various cultures may produce various responses.

Table 6.1, Experimental Conditions for CFC114 Studies[1]

Study Code (CFC114 Expt)	CFC114 Aqueous Conc. (mg/L)	Study Time (days)	Number Tested	Gas Purging[2] (y/n)
C2 0506 (Ex B)	0.1	11	1	y
C3 0506 (Ex B)	0.5	11	1	y
C2a/ C2b 2107 (Ex C)	0.5	36	2	y
B2b 3103 (Ex A)	1.0	4	1	n
C3a/C3b 1408 (Ex D)	1.0	27	2	y
C3a/ C3b 2107 (Ex C)	2.0	36	2	y

[1]PCE added at 6μmol/bottle each feeding.
[2]y= yes; n = no

6.2 Effects of CFC114

6.2.1 Cultures Receiving No CFC114

Table 6.2 summarizes the rates obtained from one-day PCE degradation rate tests of the cultures containing no CFC114, used in CFC114 experimentation. The rates for cultures not receiving CFC114 are described as Very Rapid (>1.00μmol bottle^{-1} hr^{-1}) or Rapid (0.50-1.00μmol bottle^{-1} hr^{-1}). One exception is bottle C1 Expt D. In this case, the rate fell to -0.25μmol bottle^{-1} hr^{-1} on day 26 following purging but later recovered to the Rapid range.

Table 6.2 PCE degradation Rates in Cultures Receiving No CFC114[1] (μmol bottle^{-1} hr^{-1})

Study Code (Expt)	Day									
Day	*1*	*3*	*5*	*6*	*11*	*15*	*20*	*26*	*31*	*62*
B1 3103 (Ex A)	-1.29		-0.76							
C1a 0506 (Ex B)	-0.98			-1.32						
C1b 0506 (Ex B)	-1.48			-1.58	-1.93					
C1 2107 (Ex C)	-1.47	-1.58	-1.66			-1.17	-1.46		-1.44	
C1 1408 (Ex D)	-1.62	-2.14		-2.14		-2.94[2]		-0.25		-0.76

[1] blank space indicates no test conducted
[2]purged to remove chlorinated compounds.

6.2.2 Effects of CFC114 on PCE Degradation

The cultures that received CFC114 are listed in Table 6.3 below in order of increasing CFC114 concentration. Although there was significant variation in the Day 1 degradation rates, the Day 1 rates for all bottles could be described as Very Rapid (>1.00μmol bottle^{-1} hr^{-1}) or Rapid (0.50-1.00μmol bottle^{-1} hr^{-1}). Inhibition was evident at all concentrations of CFC114 listed below except C3 Ex. B (CFC 114 0.1mg/L) which performed in the 'Rapid' or 'Very Rapid' range throughout, suggesting that this concentration of CFC114 was not inhibitory to PCE degradation.

Table 6.3 PCE degradation Rates in cultures receiving CFC114[1] (μmol bottle^{-1} hr^{-1})

CFC114 Aqueous Conc. (mg/L)	Study Code (Bottle) (Expt)	*Day*								
		1	*3*	*6*	*11*	*15*	*20*	*26*	*31*	*36*
0.1	C3 0506 (Ex B)	-1.03		-0.64	-0.99					
0.5	C2 0506 (Ex B)	-1.43			-0.49					
0.5	C2a2107 (Ex C)	-1.04	-0.26	-0.20		-0.08	-0.04		-0.22	-0.31
0.5	C2b2107 (Ex C)	-1.11	-0.34	-0.17		-0.18	-0.09		-0.40	-0.24
1.0	B2 3103 (Ex A)	-1.82		-0.01						
1.0	C3a1408 (Ex D)	-0.95		-0.15		-0.11[2]	-0.13	-0.90		
1.0	C3b1408 (Ex D)	-1.03		-0.11		-0.21[2]	-0.10	-1.39		
2.0	C3a2107 (Ex C)	-.95	-0.26	-0.14		-0.01	-0.06		-0.11	-0.09
2.0	C3b2107 (Ex C)	-1.03	-0.22	-0.13		-0.02	-0.05		-0.16	-0.05

[1] blank space indicates no test conducted

[2] purged to remove chlorinated compounds including CFC114

6.2.2.1 CFC114 Expt A

The purpose of CFC114 Expt A was to conduct a preliminary examination of the effect of CFC114 on PCE biodegradation. Begun on May 31st, 2000, two culture bottles were used including one PCE degrading control (B1) and one PCE degrading culture (B2) containing CFC114 at 1.0mg/initial aqueous concentration (Table 6.4).

Table 6.4 PCE degradation rates during CFC114 Expt A (μmol bottle^{-1} hr^{-1})

Bottle	Initial CFC114 (mg/L)	Day	
		1	4
B1 a3103	0.0	-1.29	-0.76
B2 a3103	1.0	-1.82	-0.012

On Day 4, PCE degradation in bottle B2 had nearly stopped while B1 continued to degrade PCE rapidly. No unknown GC peaks that may have been CFC114 degradation products were detected.

The TCE and cDCE results are shown in Figs 6.1 and 6.2, respectively. No accumulations of TCE or cDCE were apparent. TCE was readily formed and degraded in both cultures although on Day 4, TCE appeared to accumulate in B2. TCE did not persist following the PCE rate test. On Day 4, cDCE does not form in bottle B2 during the PCE rate test because TCE had temporarily accumulated.

These cultures were not purged prior to PCE rate testing and therefore contained a substantial amount of VC. Therefore, VC concentrations are not reported for this experiment. Ethene was not tested in this most preliminary work.

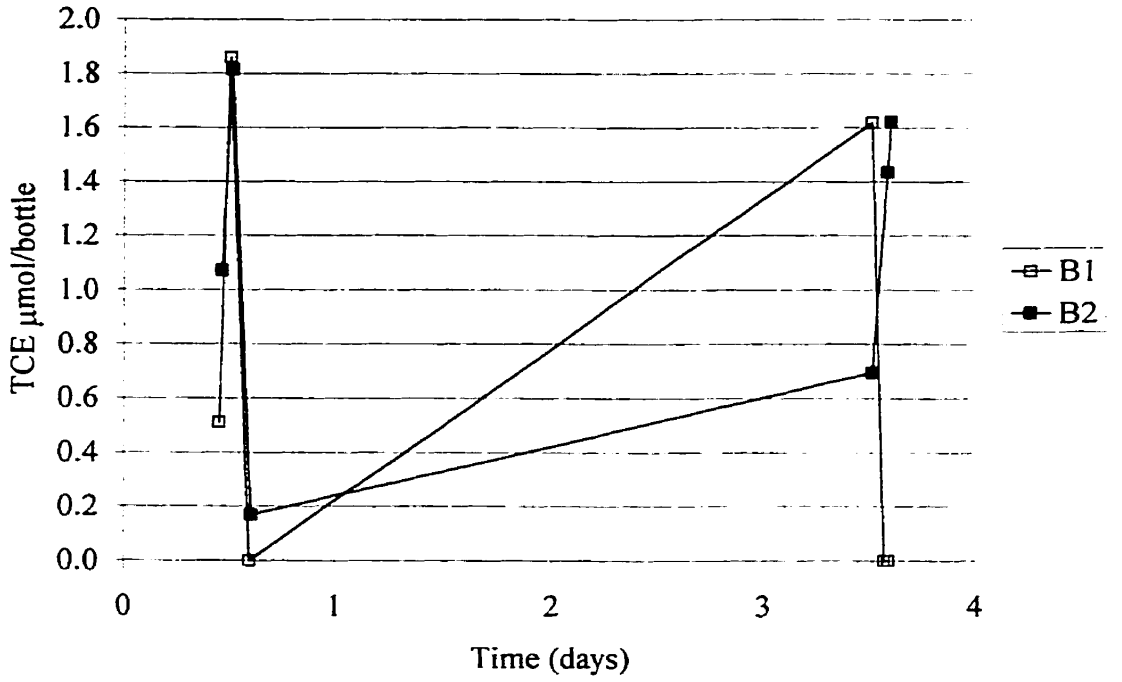


Fig 6.1 TCE formation in the absence and presence of CFC114 at 1.0mg/L, Expt A

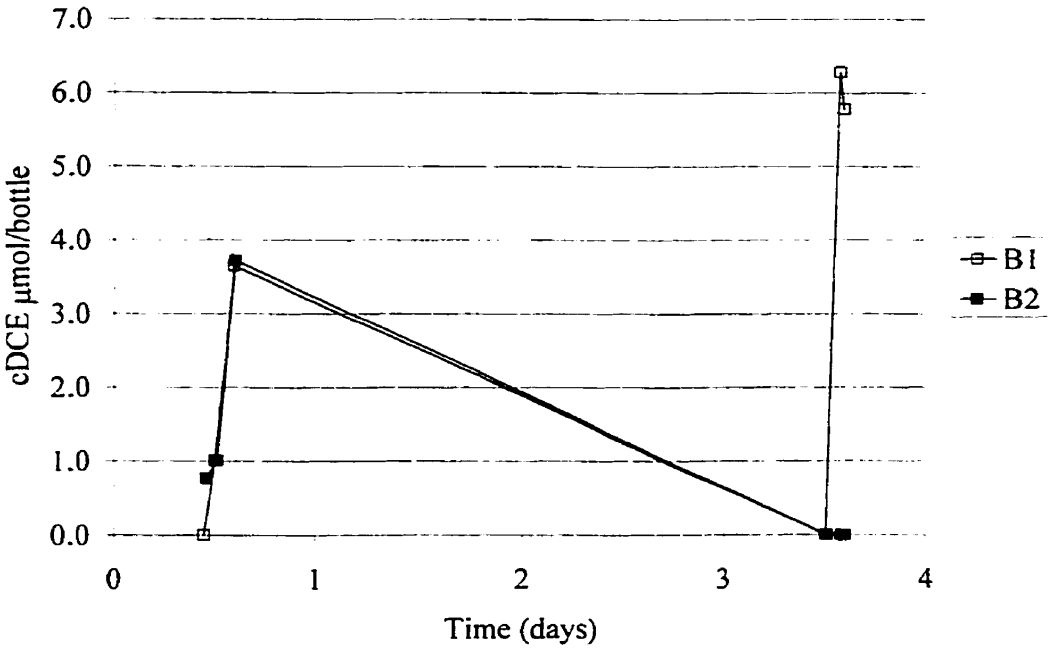


Fig 6.2 cDCE formation in the absence and presence of CFC114 at 1.0mg/L, Expt A

6.2.2.2 CFC114 Expt B

This experiment was started on June 9th, 2000. The purpose of this experiment was to confirm that CFC114 had an inhibiting effect on biodegradation of PCE, and to determine the threshold concentration at which an inhibiting effect was seen. The bottles were purged with nitrogen before starting therefore the methane and ethene concentrations were zero and the VC concentration was near zero at the outset.

Four culture bottles were used including two PCE degrading cultures not containing CFC114 (C1a and C1b), one culture receiving CFC114 at 0.1mg/L (C2) and another receiving CFC114 at 0.5mg/L (C3) (Table 6.5). The cultures containing CFC114 showed slowing PCE degradation by Day 6 but may have recovered somewhat by Day 11.

Table 6.5 PCE degradation rates during CFC114 Expt B (μmol bottle^{-1} hr^{-1})

Bottle Study Code	Initial CFC114 (mg/L)	Day		
		1	6	11
C1a 0506	0.0	-0.98	-1.32	≥1.0[1]
C1b 0506	0.0	-1.48	-1.58	-1.93
C2 0506	0.1	-1.03	-0.64	-0.99
C3 0506	0.5	-1.43	<1.0[1]	-0.49

[1] not enough data to calculate rate.

TCE formed readily following each addition of PCE and was quickly degraded (Fig 6.3). cDCE also formed readily and was degraded quickly (Fig 6.4). VC formed following each addition of PCE but was not rapidly degraded between feedings (Fig 6.5). Ethene was produced slowly in all cultures tested with and without CFC114 at about the same rate (Fig 6.6).

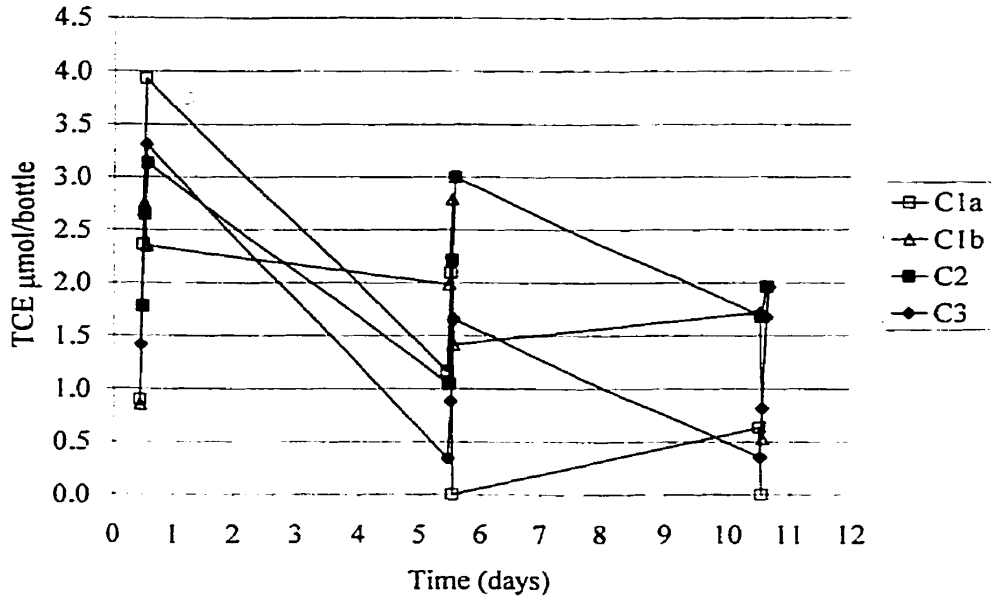


Fig 6.3 Production and degradation of TCE in two cultures containing no CFC114 (C1a and C1b), one culture containing CFC114 at 0.1mg/L (C2) and one culture containing CFC114 at 0.5mg/L (C3)

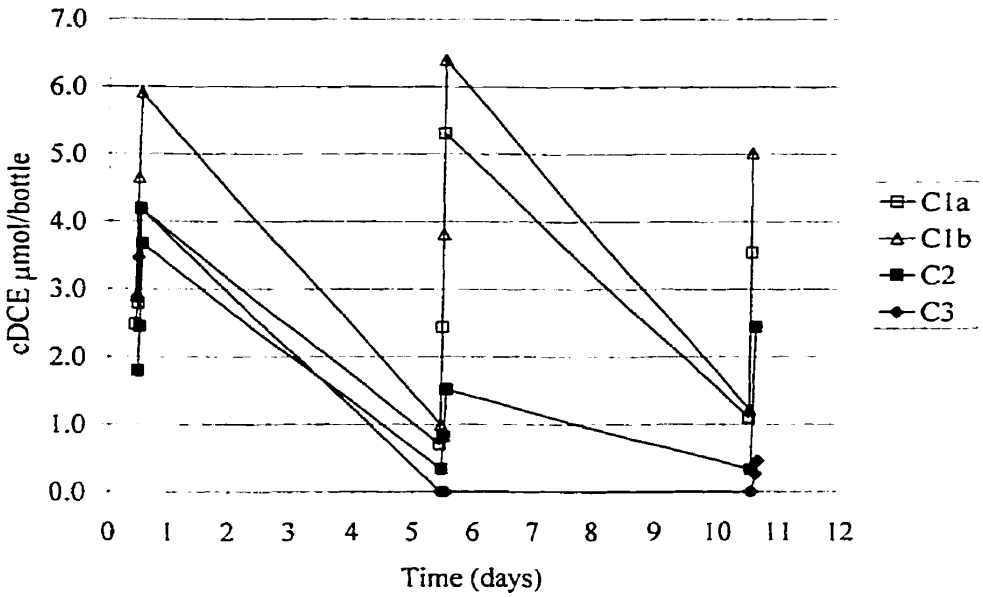


Fig 6.4 Production and degradation of cDCE in two cultures containing no CFC114 (C1a and C1b), one culture containing CFC114 at 0.1mg/L (C2) and one culture containing CFC114 at 0.5mg/L (C3)

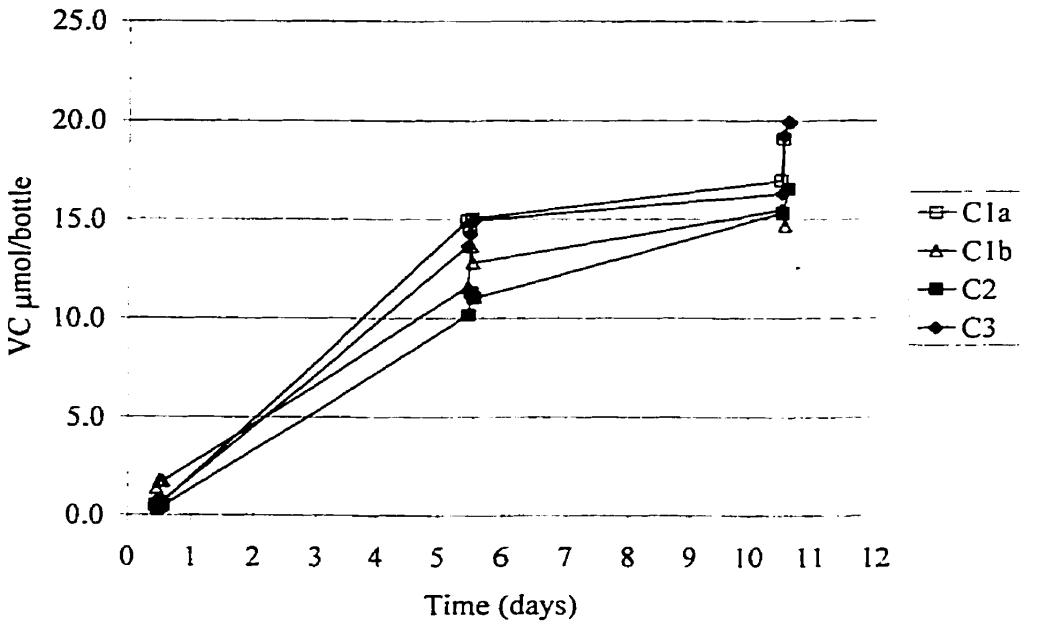


Fig 6.5 Production and degradation of VC in two cultures containing no CFC114 (C1a and C1b), one culture containing CFC114 at 0.1mg/L (C2) and one culture containing CFC114 at 0.5mg/L (C3)

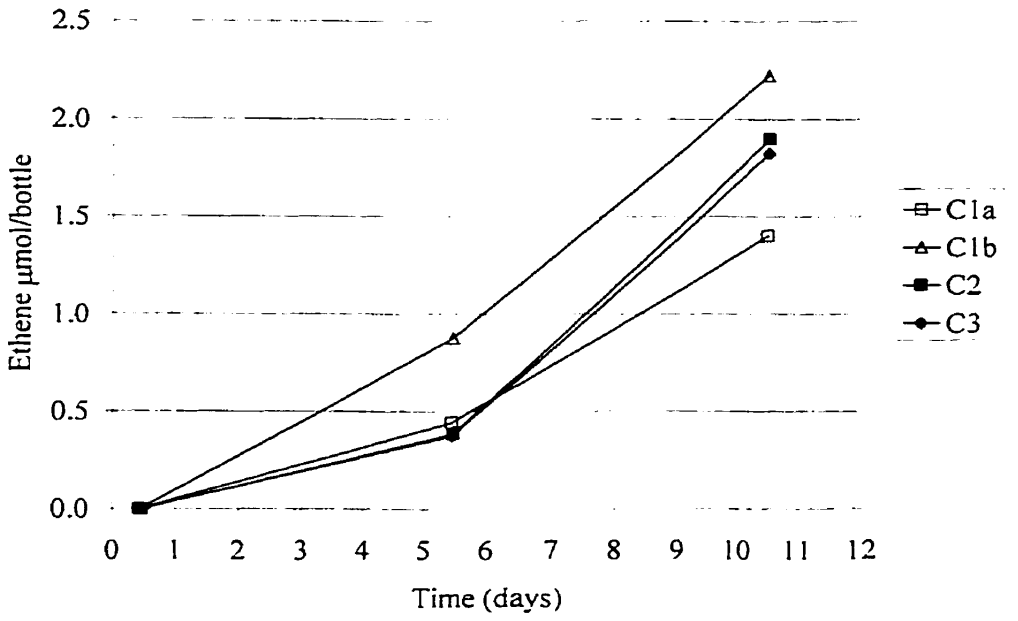


Fig 6.6 Production of ethene in two cultures containing no CFC114 (C1a and C1b), one culture containing CFC114 at 0.1mg/L (C2) and one culture containing CFC114 at 0.5mg/L (C3)

6.2.2.3 CFC114 Expt. C

This experiment was begun on July 26, 2000. The objectives of CFC114 Expt C were to determine or confirm the threshold concentration at which an inhibiting effect was detected and to determine the threshold concentration at which cessation of PCE degradation was seen. Five culture bottles were used including one PCE degrading culture not containing CFC114 (C1), two PCE degrading cultures containing CFC114 at 0.5mg/L (C2a and C2b) and two PCE degrading cultures containing CFC114 at 2.0mg/L (C3a and C3b).

PCE degradation rates are summarized in Table 6.6:

Table 6.6 PCE degradation rates during CFC114 Expt C (μmol bottle^{-1} hr^{-1})

Bottle Study Code	Initial CFC114 (mg/L)	Day 1	Day 3	Day 5	Day 15	Day 20	Day 31	Day 36
C1 2107	0.0	-1.47	-1.58	-1.66	-1.17	-1.46	-1.44	≥1.0
C2a 2107	0.5	-1.04	-0.26	-0.20	-0.08	-0.04	-0.22	-0.31
C2b 2107	0.5	-1.11	-0.34	-0.17	-0.18	-0.09	-0.40	-0.24
C3a 2107	2.0	-0.95	-0.26	-0.14	-0.01	-0.06	-0.11	-0.09
C3b 2107	2.0	-1.03	-0.22	-0.13	-0.02	-0.05	-0.16	-0.05

In all cultures spiked with CFC114, the PCE degradation rates were significantly below that of the control at all times. Although the onset of inhibition was delayed, inhibition had begun to occur even on Day 1. By Day 3, the PCE degradation rates cultures C2a and C2b (0.5mg/L CFC114) had slowed to between -0.25 to -0.34μmol bottle^{-1} hr^{-1}. By Day 15 the rate had declined to between -0.08 to -0.18μmol bottle^{-1} hr^{-1}. The PCE degradation rate increased slightly by Day 36 to the range of -0.24 to -0.31μmol bottle^{-1} hr^{-1}. In cultures C3a and C3b (2.0mg/L CFC114) the PCE degradation rate declined in the Day 3 rate test to between -0.22 to -0.26μmol bottle^{-1} hr^{-1} and continued to decline until Day 15 when the rate was between -0.01 to -0.02μmol bottle^{-1} hr^{-1}. By

Day 36, the PCE degradation rates in C3a and C3b had increased slightly to the range of -0.05 to -0.09μmol b^{-1} hr^{-1}. The PCE rate decline was more severe due to the higher concentration of CFC114 and recovered more slowly than at the lower concentration.

TCE formed most rapidly in the controls, as shown by the large peaks following PCE additions (Fig 6.7). In cultures containing CFC114, the TCE peaks were smaller, probably due to the slower degradation of PCE. TCE did not accumulate in any of the cultures tested.

cDCE pictured in Fig 6.8 did not persist in any of the cultures tested (Fig 6.8). VC accumulated between PCE additions (Fig 6.9) but was also degraded to ethene (Fig 6.10). Ethene formation was very consistent in the control and all CFC114 cultures at 17-21μmol $bottle^{-1}$ after 7 PCE feedings. Ethene formation was not affected by CFC114.

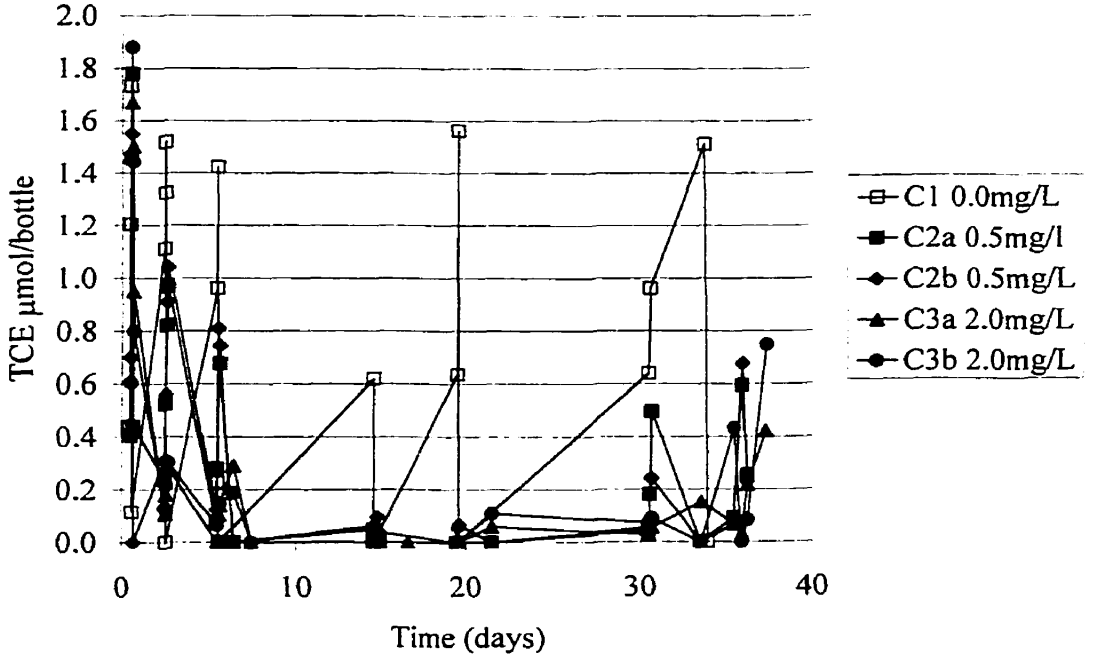


Fig 6.7 Production and degradation of TCE in one culture containing no CFC114 (C1), two cultures containing CFC114 at 0.5mg/L (C2b and C2b) and two cultures containing CFC114 at 2.0mg/L (C3a and C3b)

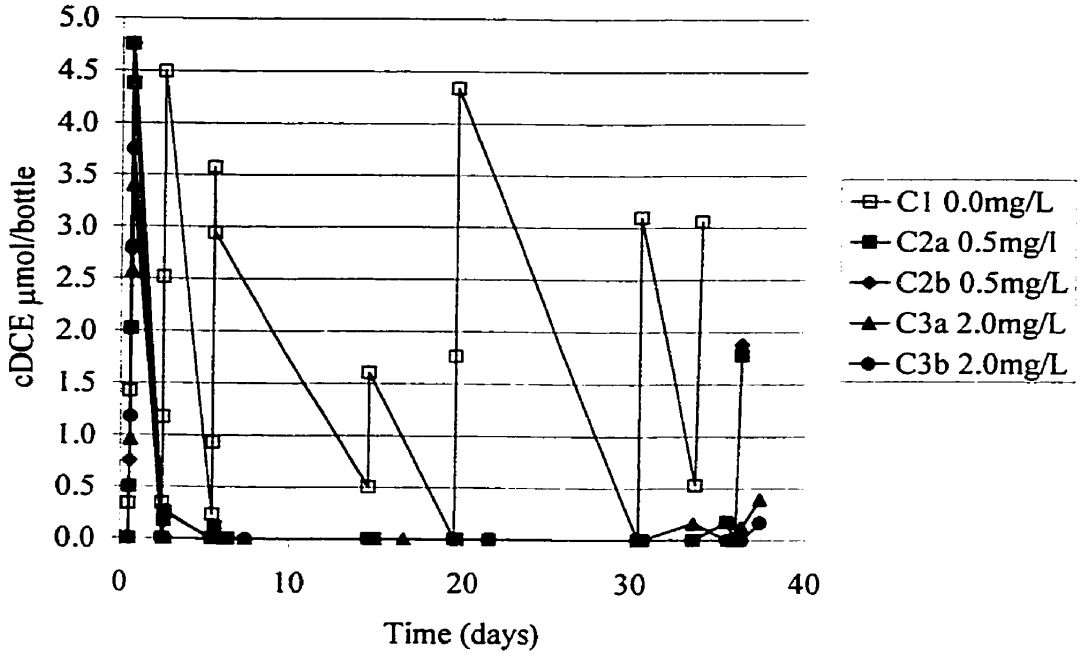


Fig 6.8 Production and degradation of cDCE in one culture containing no CFC114 (C1), two cultures containing CFC114 at 0.5mg/L (C2b and C2b) and two cultures containing CFC114 at 2.0mg/L (C3a and C3b)

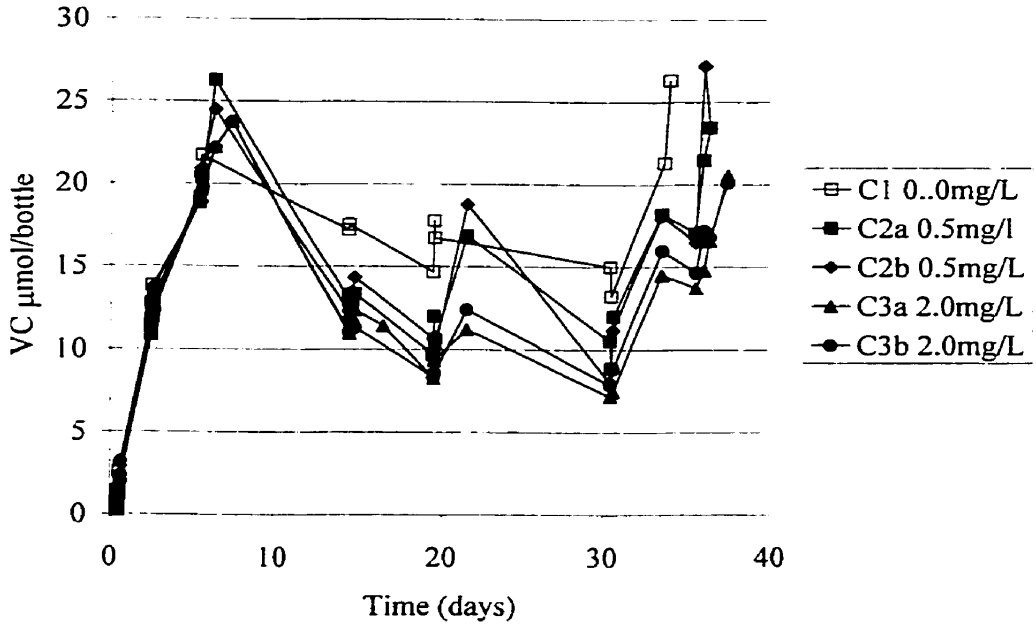


Fig 6.9 Production and degradation of VC in one culture containing no CFC114 (C1), two cultures containing CFC114 at 0.5mg/L (C2b and C2b) and two cultures containing CFC114 at 2.0mg/L (C3a and C3b)

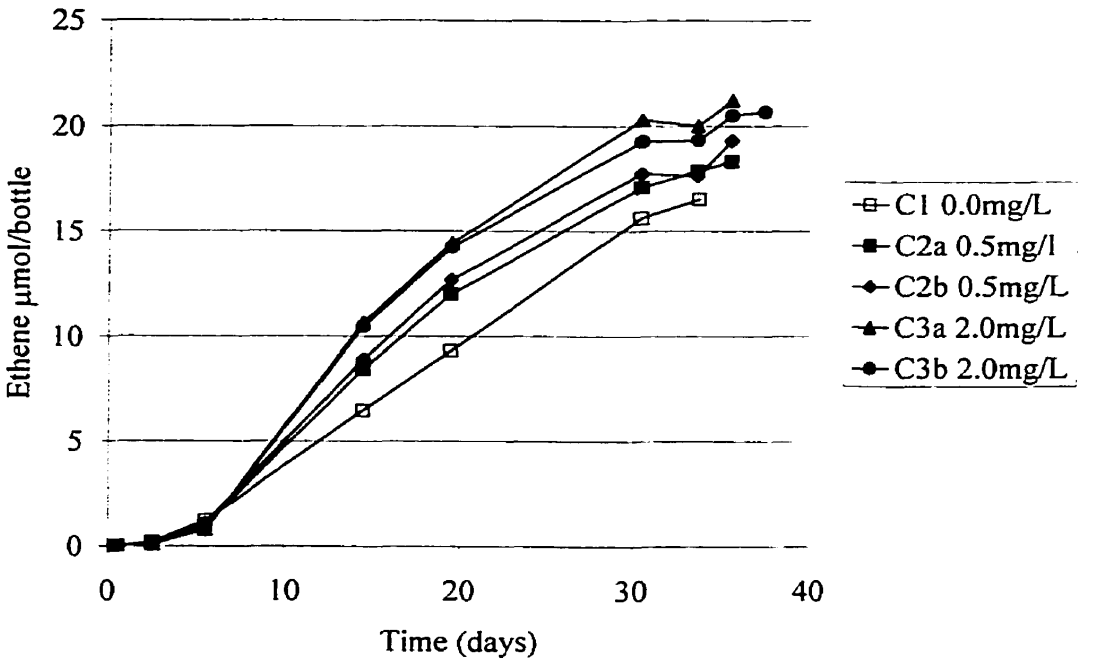


Fig 6.10 Production of ethene in one culture containing no CFC114 (C1), two cultures containing CFC114 at 0.5mg/L (C2b and C2b) and two cultures containing CFC114 at 2.0mg/L (C3a and C3b)

6.2.2.4 CFC114 Expt D - CFC114 Removal

This experiment was started August 23rd, 2000. The purpose of this experiment was to determine the ability of a PCE degrading culture to recover following exposure to and inhibition by CFC114. Three culture bottles were used including one PCE degrading control (C1) and two bottles receiving CFC114 at 1.0mg/L (3a and C3b).

The three bottles were purged and fed PCE in an ethanol stock solution on Day 1. The PCE rate test results shown in Table 6.7, show that the PCE degradation rate observed in C1 on Day 1 (-1.62µmol bottle^{-1} hr^{-1}) was similar to the rates observed in C3a and C3b (-0.95 and -1.03µmol bottle^{-1} hr^{-1}). A second rate test done on Day 6 showed that PCE degradation in C3a and C3b had slowed considerably to the range of -0.11 to -0.15µmol bottle^{-1} hr^{-1}. These results are consistent with the results CFC114 Expt C over

the same time period. On Day 15, following the rate test, the three bottles were purged with nitrogen gas sparged through the liquid portion of the culture bottle. This procedure removed nearly all ethene, methane and measurable chlorinated substances. The maximum residual ethene was 0.05μmol bottle^{-1} and the maximum residual VC was 0.20μmol bottle^{-1} following purging.

Table 6.7 PCE degradation results CFC114 Expt D (μmol bottle^{-1} hr^{-1})

Bottle Study Code	Initial CFC114 (mg/L)	Day 1	Day 6	Day 15 (purged)	Day 22	Day 26	Day 62
C1 1408	0.0	-1.62	-2.14	-0.80	no test	no test	-0.76
C3a 1408	1.0	-0.95	-0.15	-0.05	-0.13	-0.90	no test
C3b 1408	1.0	-1.03	-0.11	-0.20	-0.10	-1.39	no test

The control (C1) continued degrading PCE following the purging. Bottles C3a and C3b did not quickly resume PCE degradation and on Day 22, seven days after being purged were degrading PCE sluggishly. A test done on Day 26 (Fig 6.12) shows evidence of a strong recovery when compared with the rate test done on Days 14-15 (Fig 6.11). By Day 26, PCE degradation rates in C3a and C3b were in a range similar to that of C1. These cultures recovered their ability to degrade PCE at a rate similar to the control after removal of the CFC114.

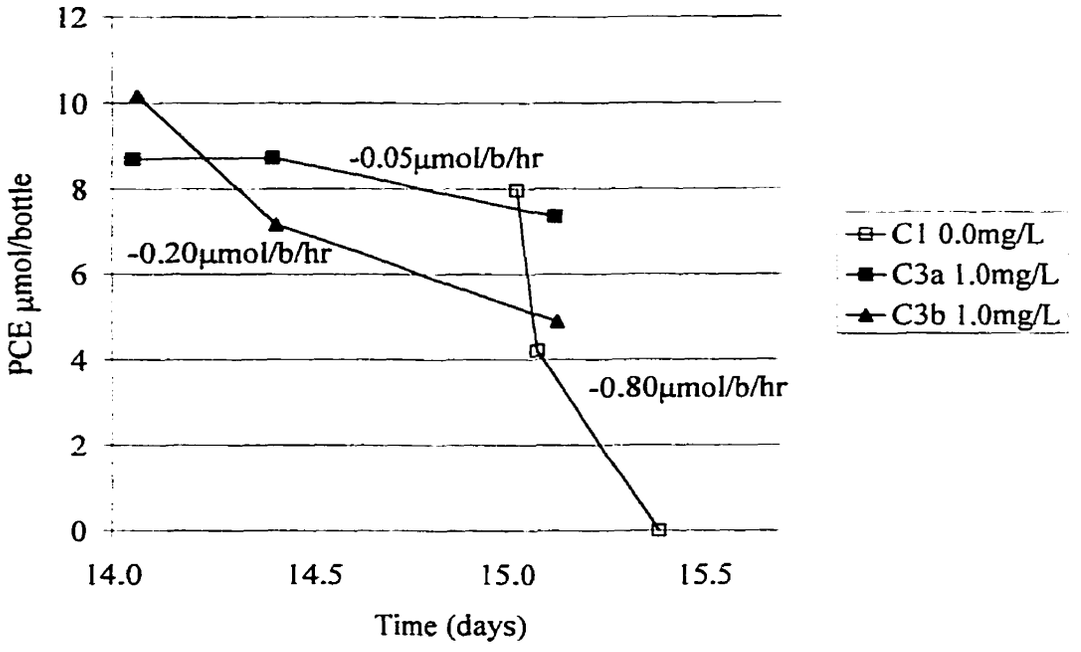


Fig 6.11 PCE degradation rate test Days 14-15 CFC114 Expt D

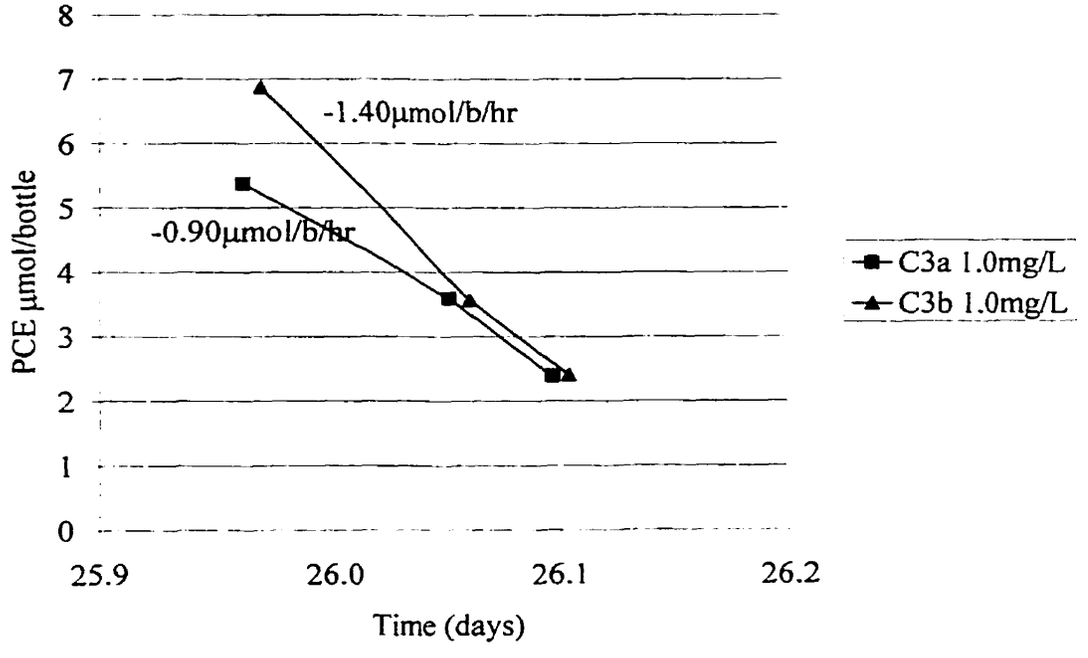


Fig 6.12 PCE degradation rate test Days 25-26 CFC114 Expt D (C1 not tested)

Cultures C3a and C3b demonstrated similar characteristics to those seen in previous CFC114 experiments. The onset of inhibition was delayed following the addition of CFC114. Inhibition of PCE degradation was observed but there was no accumulation of PCE degradation products. Ethene formed readily before and after purging (Fig 6.13).

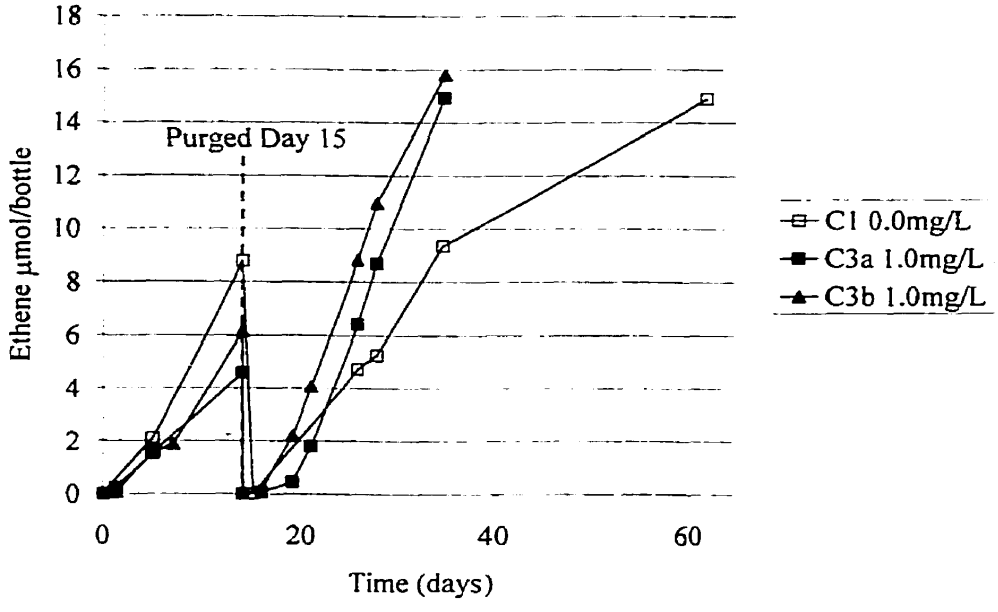


Fig 6.13 Ethene Formation before and after removal of CFC114, CFC114 Expt. D

6.2.3 Effects Of CFC114 on Electron Donor Degradation and Methane Formation

Acetate and propionate were produced as a result of ethanol consumption (Figs 6.14-6.15). CFC114 appeared to have no effect on acetate or propionate concentration over the range of CFC114 concentrations tested. Methane was formed both in the absence and the presence of CFC114 (Fig 6.16). After 36 days, the cultures containing the greater CFC114 concentrations produced less methane, suggesting that methanogenesis may have been slightly inhibited (Fig 6.16).

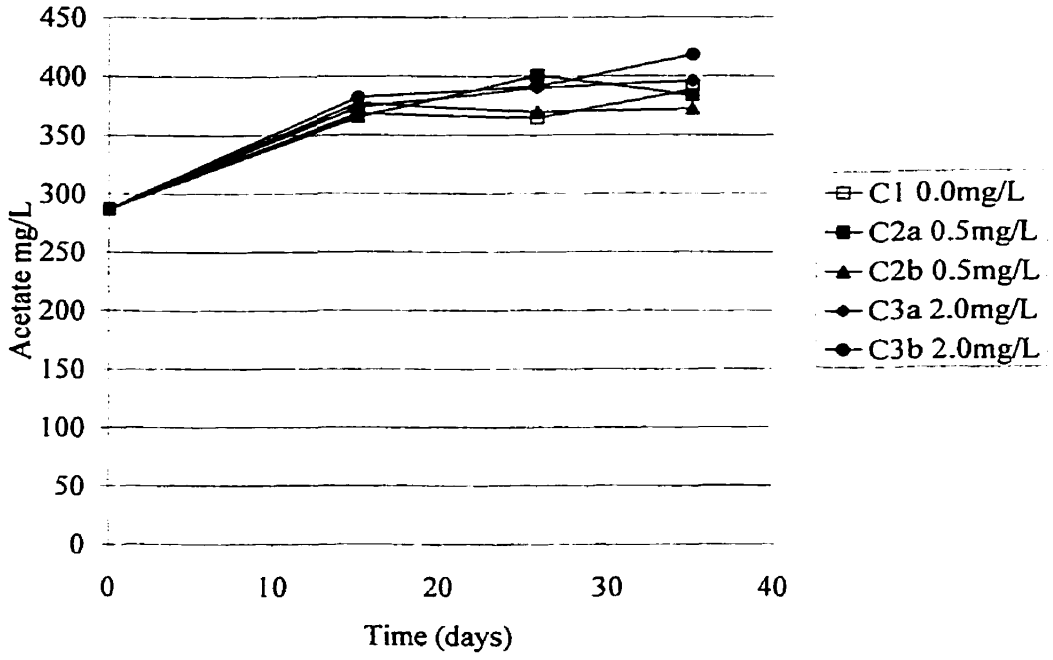


Fig 6.14 Acetate concentration in cultures having a range of CFC114 concentrations CFC114 Expt C

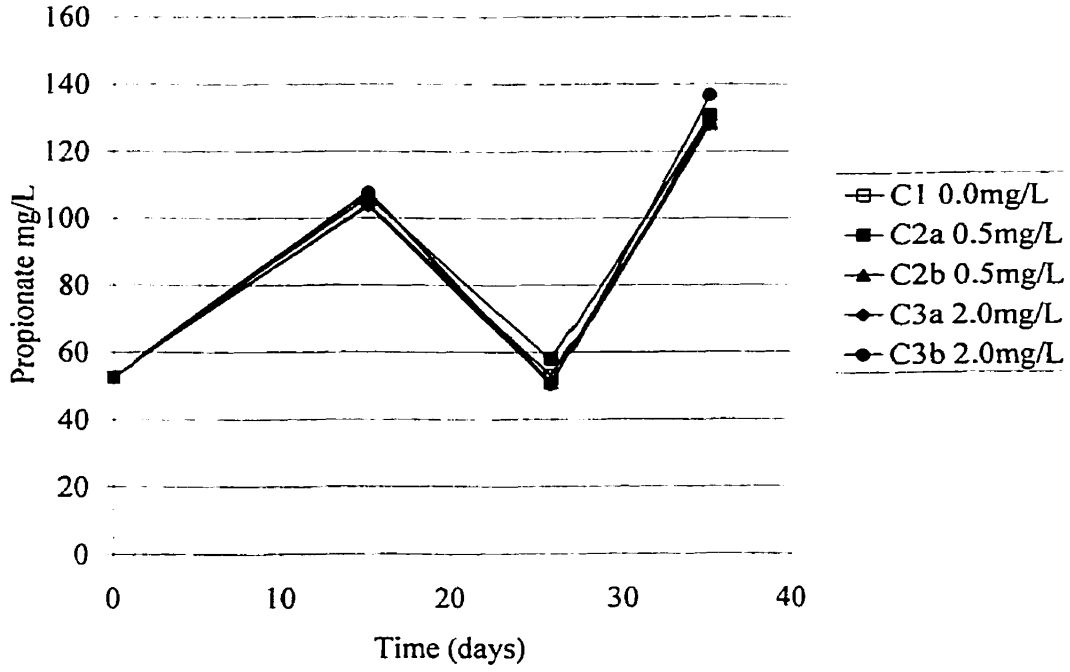


Fig 6.15 Propionate concentration in cultures having a range of CFC114 concentrations CFC114 Expt C

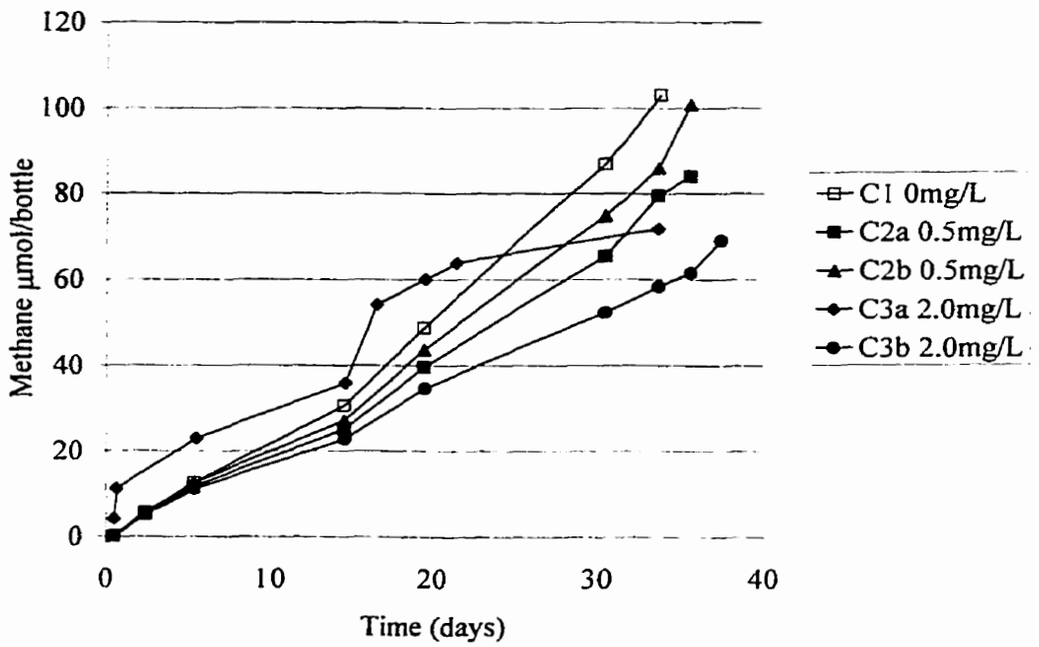


Fig 6.16 Methane formation in cultures having a range of CFC114 concentrations CFC114 Expt C

In CFC114 Expt. D, the bottles were purged of methane, CFC114 and other analytes at 15 days (Fig 6.17). Prior to purging, significantly less methane had been formed in the cultures containing 1.0mg/L CFC114 than in the control. It appears that methane formation prior to purging was inhibited, however the inhibition was not severe. Following purging, it appears that the rates in all cultures were similar (Fig 6.17).

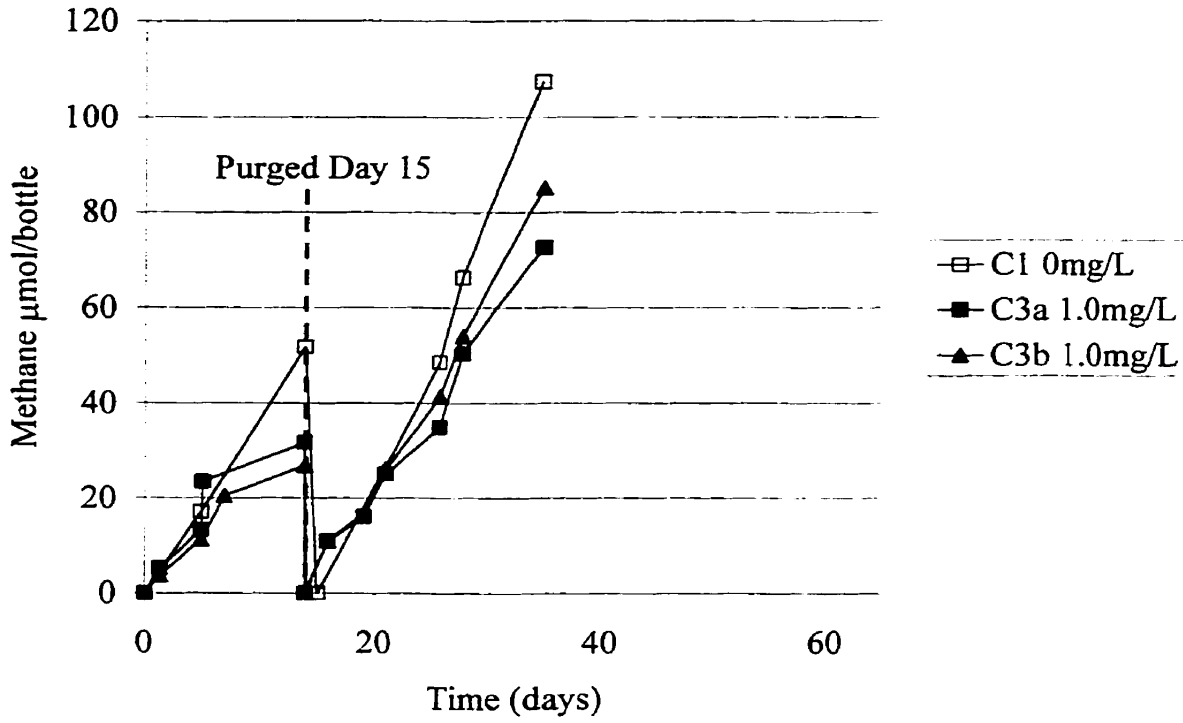


Fig 6.17 Methane Formation before and after removal of CFC114, CFC114 Expt. D

6.3 Degradation of CFC114

CFC114 concentrations were monitored over time in CFC114 Expt A (B2b3103) and Expt C (C3a2107 and C3b2107) (Fig 6.18). The initial CFC114 concentrations are those listed in the chart legend. There was no clear indication of CFC114 degradation as CFC114 removal in the live bottle was similar to that in the water control. Additionally, no degradation products were detected using the usual experimental methods described in Section 3.

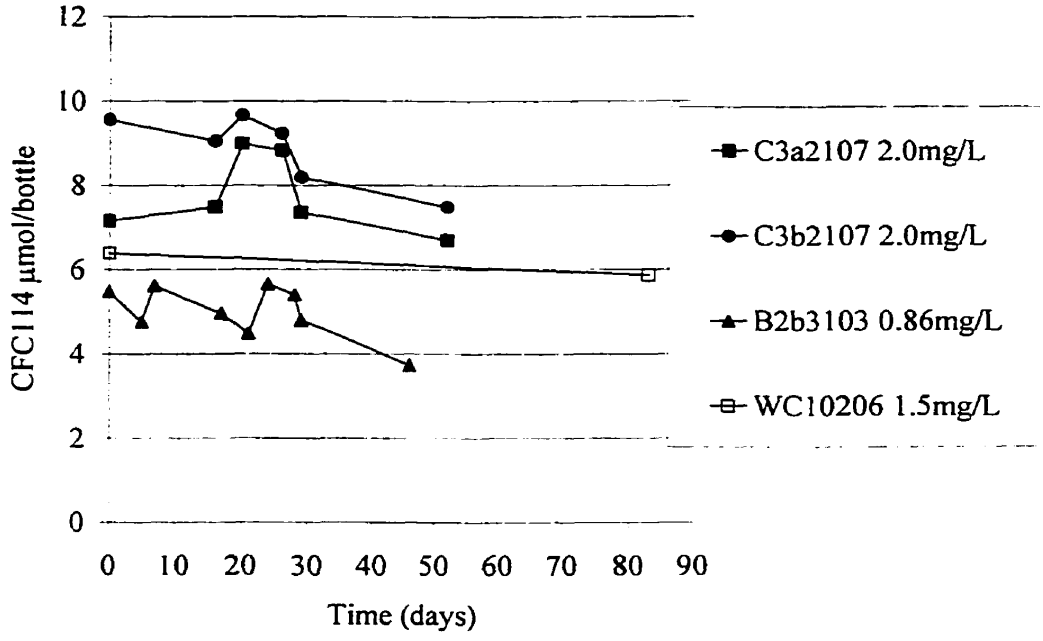


Fig 6.18 CFC114 concentrations in three PCE degrading cultures and one water control

To further test for the formation of CFC114 degradation products, culture C3a2107 Expt C, (initial CFC114=2.0mg/L) was submitted to an independent lab equipped with Gas Chromatography/ Mass Spectroscopy (York-Durham Regional Environmental Laboratory, Pickering, Ontario). The results of that analysis, done 160days after adding CFC114, confirmed only the existence of CFC114 and no degradation products.

7 DISCUSSION AND CONCLUSIONS

7.1 Discussion

All of the CFCs tested in this study demonstrated inhibition of PCE degradation, but with different effects. Chemically, the three CFCs tested are closely related single bonded C2 aliphatics having either two or three chlorine atoms and three or four fluorine atoms. PCE and PCE byproduct degradation were severely inhibited by CFC113 ($C_2Cl_3F_3$) and were somewhat inhibited by CFC114 ($C_2Cl_2F_4$) and HCFC123a ($C_2HCl_2F_3$). In this study, CFC113 was degraded while the others were not. These findings suggest that the inhibitory effect of CFC113 on PCE is related to its propensity to degrade and/or the presence of a third chlorine atom.

The inhibition of PCE degradation by CFC113 showed a characteristic delayed onset in all cases (Section 4). Gradual degradation of CFC113 and the appearance of HCFC123a also accompanied inhibition. The delayed inhibition characteristic of CFC113 raised the possibility that inhibition was caused by some substance formed after CFC113 was added. To examine the possibility that inhibition was caused by the slowly formed HCFC123a rather than CFC113 itself, PCE degrading cultures received HCFC123a without CFC113. A comparison of the results of CFC113 Expt D (Section 4) to those of the HCFC123a experiment presented in Section 5 provides an indication of the causes of PCE inhibition. In the CFC113 experiments, cultures receiving 0.15 to 0.30 mg/L of CFC113 produced HCFC123a concentrations of about 0.2 mg/L after 48 hours (HCFC123a is much more soluble than CFC113 and will have a higher aqueous concentration due to the same mass in the experimental systems examined). Cultures receiving 0.65 mg/L (aqueous) of CFC113 had produced about 0.65 mg/L (aqueous) of

HCFCl23a, although the total molarity of the HCFC123a produced in the system was less than the molarity of CFC113 consumed. In all cultures except the one receiving 0.15 mg/L CFCl13, PCE degradation was incomplete, as were the degradations of PCE by-products. Upon receiving additional PCE, the culture with 0.15 mg/L CFC113 also showed cessation of PCE and product degradation.

In contrast, the HCFC123a cultures with initial HCFC123a concentrations similar to those seen after 48 hours in the CFC113 cultures, showed only very moderate inhibition of PCE degradation. In such cases, the PCE degradation rate was slowed but repeated PCE additions were completely removed and PCE byproducts were formed and removed with no apparent inhibition. This pattern of inhibition was completely different from that observed with CFC113. The presence of HCFC123a did not cause the inhibition of PCE degradation observed in cultures receiving CFC113.

This comparison does not prove that CFC113 is inhibitory but it does indicate that HCFC123a did not cause the inhibition due to CFC113 degradation. The delayed inhibition onset due to CFC113 suggests that the process of degrading CFC113, not its mere presence, may be inhibitory. This inhibition may result from the intermediate chlorinated products (carbenes) formed during CFC degradation that are toxic to bacteria (Krone et al., 1991). Furthermore, the mass balance on CFC113 indicates that HCFC123a was not the only product of CFC113 degradation (Figs 4.36, 4.37, p.73). Other undetected products may have caused the inhibition of PCE degradation. Further experimentation is required to answer these questions.

There is some preliminary evidence in this study for the degradation of HCFC123a. The HCFC123a concentration can be seen to decrease over time in the

cultures at a rate that was greater than in the water control, although no degradation products were detected (Fig 5.10). If HCFC123a degradation was occurring, the process was very slow. Longer-term observation of HCFC123a and CFC114 containing cultures is recommended in order to continue monitoring the formation of degradation products. The formation of CTFE observed in some of the CFC113 containing cultures in this study is consistent with the findings of previous researchers (Lesage et al., 1992). Biotic pathways for the formation of HCFC133 and HCFC133b from the degradation of HCFC123a have also been proposed in previous research (Lesage et al., 1992) but those products were not seen in this study

In CFC113 Expt D culture C2 (Fig 4.37, p.73), the added CFC113 was completely consumed within 50 days. Once the CFC113 was consumed, PCE degradation (Fig 4.18, p.57) and production of ethene (Fig 4.20, p.58) began. This is a possible indication that if CFC113 can be degraded *in situ,* subsequent PCE degradation may be possible. Removal of both PCE and CFC113 in the laboratory was accomplished in mixed anaerobic cultures with added electron donor. Based on the results of this study, any efforts to remove both PCE and CFC113 either in the laboratory or *in situ* should begin with the removal of CFC113. *In situ* CFC113 degradation could be stimulated in several ways. Adequate electron donor should be available and various VFAs, alcohols or yeast extract have been used for this purpose. Furthermore, bioaugmentation of groundwater with cultures such as those used in this study could be conducted. In the future, genetically engineered microorganisms may be produced which could more rapidly produce cofactors that would target the desired substrate. The cofactors or the cultures themselves could be added to the groundwater *in situ*. Hydroxocobalamin

(vitamin B12) has been suggested for this purpose (Hashsham and Freedman, 1999).

In order to investigate methods for increasing the CFC degradation rate(s) it is recommended that the CFCs used in this study be tested in combination with various electron donors, and the possibility be explored, that available nutrients are limiting the degradation rate(s). The microbial community composition could also be examined to determine the types of microbial species and/or specific microbial strains that are effective in CFC degradation. The change in total biomass as it is related to the change in CFC concentration could also be measured. Finally, further studies should examine degradation rates using higher initial concentrations of CFCs that more closely reflect site conditions.

7.2 Conclusions

This study has lead to the following conclusions:

1. All CFCs tested in this study produced partial or complete inhibition of PCE degradation. Degradation of lesser-chlorinated byproducts was also inhibited. This effect was seen in all CFCs tested at concentrations below 2mg/L.
2. In all cases, inhibition was not seen until 3 to 10 days following the addition of CFC to the PCE degrading culture.
3. The most highly chlorinated CFC tested, CFC113, was also the most inhibitory to PCE degradation. In addition, CFC113 was the only CFC for which degradation was confirmed.

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APPENDIX A - LAB REFERENCE TABLES

Table A1 Basal Media

Constituent (mg/L)	DiStefano	Bagley	LaLonde	Kaseros	This work
NH_4Cl	200	285	60	60	60
K_2HPO_4	100	100	20	20	20
$CaCl_2 2H_2O$	0	0	50	50	50
$MgCl_2$	200	200	100	100	100
$FeSO_4$	0	0	15	15	15
$NaHCO_3$	5000	6000	1000	1000	2000
$Na_2S 9H_2O$	500	500	30	30	100
$FeCl_2 4H_2O$	100	100	100	0	20
$FeCl_2 4H_2O$	55	55	0	0	0
Resazurin	1	1	1	1	1
Yeast Extract	0	0	2	100	100
Trace Metals					
$MnSO_4 H_2O$	0	0	0.86	0.86	0.0
$CoCl_2 6H_2O$	1.7	1.7	1.7	1.7	1.7
$ZnSO_4\ 7H_2O$	0	0	2.1	2.1	2.1
H_3BO_3	0.19	0.19	0.19	0.19	0.19
$NiCl_2 6H_2O$	0.5	0.5	0.5	0.5	0.5
$NaMoO_4\ 2H_2O$	0.2	0.2	0.2	0.2	0.2
Nitrilotriacetic acid	0	0	5	5	0
NH_4VO_3	0	0.2	0	0	0
Kl	0	4	0	0	0
$ZnCl_2$	1	1	0	0	0
$CaCl_2$	2	2	0	0	0
$MnCl_2 4H_2O$	1	1	0	0	0
cysteine HCl	0	4	0	0	0

Table A2 Stock Solution Spreadsheet

Stock solutions					
PCE/CFC113/EtOH					
	in 70mL culture	PCE	CFC113	EtOH	
SS0	final mg/L	**10.00**	**0.00**	26.84	
	inj(µL)			**3.00**	
	density	1.62	1.57	0.79	
	µL/bottle	0.63	0.00	2.37	3.00
	vol fraction	0.21	0.00	0.79	
	mass/b(mg)	1.03	0.00	1.88	2.91
	mass frac	0.35	0.00	0.65	
	avg density				0.97
	sample mass				2.91
	ss vol(µL)				**1950.00**
	vol reqd(mL)	412.51	0.00	1537.49	1950.00
	mass reqd(mg)	669.50	0.00	1221.38	1890.88
	resulting conc (mg/L)	*10.00*	*0.00*	*26.84*	
	in 70mL culture				
		PCE	CFC113	EtOH	
SS1	final mg/L	**10.00**	**0.20**	25.83	
	inj(µL)			**3.00**	
	density	1.62	1.57	0.79	
	uL/bottle	0.63	0.09	2.28	3.00
	vol fraction	0.21	0.03	0.76	
	mass/b(mg)	1.03	0.14	1.81	2.98
	mas frac	0.35	0.05	0.61	
	avg density				0.99
	sample mass				2.98
	ss vol(µL)				**1950.00**
	vol reqd(µL)	412.51	57.80	1479.70	1950.00
	mass reqd(mg)	669.50	90.74	1175.47	1935.71
	resulting conc (mg/L)	*10.00*	*0.20*	*25.83*	
	in 70mL culture				

Table A3 Bottle Concentration Conversions[1]

Bottle concentration conversion chart			Updated 13Oct00					
Bottle Volume:	122		*(some values interpolated from Gossett's)					
Liquid Volume:	70		**CRC value at 25C					
Headspace Volume:	52							
This chart may be used to determine bottle concentration in terms of Nominal liquid,								
Actual gas phase concentration, Actual liquid phase concentration or mols per bottle.								
			(NL)	(AG)	(AL)	(AL)	Per Bottle	Per Bottle
	Hc	FW(g/mol)	NomLq(mg/L	ActGas(mg/L)	ActLq(mg/L)	AL(umol/L	umol/b	mg/b
ETH**	8.69	28	1	1.166	0.134	4.79	2.500	0.070
CH4	15.22	16	1	1.237	0.081	5.08	4.375	0.070
CFC114	12.85	170.92	1	1.219	0.095	0.55	0.410	0.070
VC*	*1.01*	62.5	1	0.577	0.571	9.14	1.120	0.070
CFC113	12.07	187.39	1	1.211	0.100	0.54	0.374	0.070
HCFC123a	0.93	152.93	1	0.550	0.591	3.87	0.458	0.070
cDCE*	*0.145*	97	1	0.131	0.903	9.31	0.722	0.070
tDCE*	*0.34*	97	1	0.271	0.798	8.23	0.722	0.070
1,1DCE*	*0.202*	97	1	0.176	0.870	8.96	0.722	0.070
TCE*	*0.343*	131.5	1	0.273	0.797	6.06	0.532	0.070
PCE*	*0.634*	165.83	1	0.431	0.680	4.10	0.422	0.070
ETH	8.69	28	0.858	1	0.115	4.11	2.145	0.060
CH4	15.22	16	0.809	1	0.066	4.11	3.537	0.057
CFC114	12.85	170.92	0.821	1	0.078	0.46	0.336	0.057
VC*	*1.01*	62.5	1.733	1	0.990	15.84	1.941	0.121
CFC113	12.07	187.39	0.826	1	0.083	0.44	0.308	0.058
HCFC123a	0.93	152.93	1.818	1	1.075	7.03	0.832	0.127
cDCE*	*0.145*	97	7.639	1	6.897	71.10	5.513	0.535
tDCE*	*0.34*	97	3.684	1	2.941	30.32	2.659	0.258
1,1DCE*	*0.202*	97	5.693	1	4.950	51.04	4.109	0.399
TCE*	*0.343*	131.5	3.658	1	2.915	22.17	1.947	0.256
PCE*	*0.634*	165.83	2.320	1	1.577	9.51	0.979	0.162
ETH	8.69	28	7.455	8.690	1	35.71	18.639	0.522
CH4	15.22	16	12.306	15.220	1	62.50	53.840	0.861
CFC114	12.85	170.92	10.546	12.850	1	5.85	4.319	0.738
VC*	*1.01*	62.5	1.750	1.010	1	16.00	1.960	0.123
CFC113	12.07	187.39	9.966	12.070	1	5.34	3.723	0.698
HCFC123a	0.93	152.93	1.691	0.930	1	6.54	0.774	0.118
cDCE*	*0.145*	97	1.108	0.145	1	10.31	0.799	0.078
tDCE*	*0.34*	97	1.253	0.340	1	10.31	0.904	0.088
1,1DCE*	*0.202*	97	1.150	0.202	1	10.31	0.830	0.081
TCE*	*0.343*	131.5	1.255	0.343	1	7.60	0.668	0.088
PCE*	*0.634*	165.83	1.471	0.634	1	6.03	0.621	0.103
ETH	8.69	28	0.209	0.243	0.028	1	0.522	0.015
CH4	15.22	16	0.197	0.244	0.016	1	0.861	0.014
CFC114	12.85	170.92	1.802	2.196	0.171	1	0.738	0.126
VC*	*1.01*	62.5	0.109	0.063	0.063	1	0.123	0.008
CFC113	12.07	187.39	1.868	2.262	0.187	1	0.698	0.131
HCFC123a	0.93	152.93	0.259	0.142	0.153	1	0.118	0.018
cDCE*	*0.145*	97	0.107	0.014	0.097	1	0.078	0.008
tDCE*	*0.34*	97	0.121	0.033	0.097	1	0.088	0.009
1,1DCE*	*0.202*	97	0.112	0.020	0.097	1	0.081	0.008
TCE*	*0.343*	131.5	0.165	0.045	0.132	1	0.088	0.012
PCE*	*0.634*	165.83	0.244	0.105	0.166	1	0.103	0.017

Table A3 Bottle Concentration (cont.)

ETH	8.69	28	0.400	0.466	0.054	1.92	1	0.028
CH4	15.22	16	0.229	0.283	0.019	1.16	1	0.016
CFC114	12.85	170.92	2.442	2.975	0.232	1.35	1	0.171
VC*	*1.01*	62.5	0.893	0.515	0.510	8.16	1	0.063
CFC113	12.07	187.39	2.677	3.242	0.269	1.43	1	0.187
HCFC123a	0.93	152.93	2.185	1.202	1.292	8.45	1	0.153
cDCE*	*0.145*	97	1.386	0.181	1.251	12.90	1	0.097
tDCE*	*0.34*	97	1.386	0.376	1.106	11.41	1	0.097
1,1DCE*	*0.202*	97	1.386	0.243	1.205	12.42	1	0.097
TCE*	*0.343*	131.5	1.879	0.514	1.497	11.38	1	0.132
PCE*	*0.634*	165.83	2.369	1.021	1.611	9.71	1	0.166
ETH	8.69	28	14.286	16.651	1.916	68.43	35.714	1
CH4	15.22	16	14.286	17.668	1.161	72.55	62.500	1
CFC114	12.85	170.92	14.286	17.407	1.355	7.93	5.851	1
VC*	*1.01*	62.5	14.286	8.244	8.162	130.59	16.000	1
CFC113	12.07	187.39	14.286	17.301	1.433	7.65	5.336	1
HCFC123a	0.93	152.93	14.286	7.857	8.449	55.25	6.539	1
cDCE*	*0.145*	97	14.286	1.870	12.897	132.95	10.309	1
tDCE*	*0.34*	97	14.286	3.878	11.405	117.58	10.309	1
1,1DCE*	*0.202*	97	14.286	2.509	12.422	128.06	10.309	1
TCE*	*0.343*	131.5	14.286	3.905	11.385	86.58	7.605	1
PCE*	*0.634*	165.83	14.286	6.157	9.712	58.56	6.030	1

[1]Valid at 22°C.

Table A4 Bottle Concentration Conversion Table Legend

Short-form	Term(units)	Explanation
Hc	Henry's Law Constant (unitless)	The ratio of the partition of analyte between liquid and gas at ambient lab temperature 22°C and at equilibrium, stated in mass per volume (gas)/ mass per volume (liquid).
FW	Formula Weight (g/mol)	Based on analysis provided by supplier.
NL	Nominal Liquid Conc. (mg/L)	A theoretical value determined by the total mass of the analyte divided by the volume of liquid present. Analogous to mass per bottle.
AG	Actual Gas Concentration (mg/L)	The actual concentration in the gas phase at 22°C and at equilibrium.
AL	Actual Liquid Conc. (mg/L)	The actual concentration in the liquid phase at 22°C and at equilibrium.
AL(µmol/L)	Actual Liquid Conc. (µmol/L)	Same as AL above, converted to µmol/bottle

Table A5 Testing Henry's Law Constants (Hc) - Results[1]

Determination of Hc for various solutes							
Vol water	Vol gas	trial	PCE	TCE	cDCE	CFC113
		1	27807	24770	13692	27574
		2	27488	20719	11059	23476
St1	121.13	3	37978	26099	13383	25908
20	101.13		31091	23862.7	12711.3	25652.7
		1	30287	23855	12823	26280
St2	121.92	2	34000	23458	12071	23412
20	101.92		32143.5	23656.5	12447	24846
		1	28946	14249	4817	84378
St3	122.03	2	25368	13823	4861	89011
100	22.03		27157	14036	4839	86694.5
		1	22433	13881	4956	88063
		2	27124	13760	4582	82195
St4	121.68	3	34159	15871	5248	84404
100	21.68		27905.3	14504	4928.67	84887.3
Hc						
	1		0.84616	0.45363	0.20192	12.5946
	2		0.80404	0.46612	0.20997	11.5498
	avg		0.8251	0.45987	0.20594	12.0722

[1]Values are integrated peak areas obtained from gas chromatograms.

Table A6 VC Attenuation Experiment[1]

VC Attenuation Experiment						
Analyte added per 9mL bottle(uL)				*CG Peak area*		
Bottle	CH4	CFC114	VC	*CH4*	*CFC114*	*VC*
A1,11/11	1	1	1000	*1850*	*2245*	*4224*
A2,11/11	5	5	1000	*9225*	*10271*	*4573*
A3,11/11	10	10	1000	*17000*	*21000*	*4637*
A4,11/11	50	50	1000	*82000*	*103000*	*5147*
A5,11/11	100	100	1000	*168000*	*205000*	*5661*
A6,11/11	250	250	1000	*394000*	*490000*	*6535*
These were 9mL bottles, spiked with gas volumes of analyte at the concentrations shown below.						
CH4: 76.1%						
CFC114: 99.5%						
VC: .001mol/mol						
The peak areas given represent the concentrations shown in 122mL bottles with 70mL liquid, 52mL headspace						

[1]This experiment was done to determine the effect of increasing CFC114 and CH_4 on the measured value of VC while the concentration of VC was held constant.

APPENDIX B- GAS CHROMATOGRAPH METHODOLOGY

CRESTECH Method

Hardware: HP5890 II Manual Injection, Auto pressure control
Column: Supelco 25320-U, 30m x 0.53mm x 0.3μm film Vocol
Detector: Flame ionization detector (FID)
Oven Temperature: 35°C/2min, +20°/min, 110°C/2min
Injector/det: 240/200°C
Carrier Gas: N_2 @8mL/min
Injection volume: 20μL headspace

Compound	Peak time(min)	Detection Limit (mg/L AL)	Calibration p.a. =	Comments
CH4	0.938		--	Preferred method for compounds listed. All compounds well separated and detected. Use Hamilton 1702 25uL syringe with new #2 tip. Pump 2x in bottle and inject ASAP. Hold needle in injection port 10 seconds.
VC	1.259	0.05	12578x[μmol/b]	
CFC114	1.123		--	
EtOH	1.633		--	
HCFC123a	1.80	0.04	39262x[μmol/b]	
CFC113	2.11	0.005	49786x[μmol/b]	
1,1DCE	2.20		--	
tDCE	2.87	0.15	6866x[μmol/b]	
cDCE	3.69	0.20	5686x[μmol/b]	
TCE	4.66	0.12	13156x[μmol/b]	
PCE	6.015	0.08	20165x[μmol/b]	

Peak time (above) refers to the elution time for each compound using the method described above. Calibration refers to the peak area resulting from 1μmol/bottle of analyte.

CFC01 Method

Hardware: HP5890 II Manual Injection, manual pressure control
Column: Hewlett-Packard HP-624 - 30m x 0.053mm x 0.03μm film.
Detector: Electron Capture Detector (ECD)
Injector/det: 240/240°C
Carrier Gas: He @8mL/min
Make-up gas: Argon/methane mix @1mL/min
Oven Temperature: 40°C/ 5 min, +10°/min to 120°, 120°/1 min
Injection volume: 20μL headspace

Compound	peak time(min)	Detection Limit (mg/L AL)	Calibration P.A. =	Comments
CFC-114	1.56	0.005	46590[μmol/b]	Method required for CFC114. Allows separation in the presence of methane. Useful for monitoring changes in CFC113 and HCFC123a concentrations. Detection limit varies widely but sensitive to highly chlorinated compounds. PCE and TCE should be used for check on other methods only. Calibration curves are forced through the origin (0,0). Use Hamilton 1702 25uL syringe with new #2 tip. Pump 2x in bottle and inject ASAP. Hold needle in injection port 10 seconds.
HCFC-123a	3.38	0.010	117172 [μmol/b]	
CFC-113	3.665	0.0001	$2.32x10^6$[μmol/b]	
TCE	*8.44*	*0.0004*	*$3.72x10^6$[μmol/b]*	
PCE	*10.84*	*0.005*	*$0.304x10^6$[μmol/b]*	

Peak time (above) refers to the elution time for each compound using the method described above. Calibration refers to the peak area resulting from 1μmol/bottle of analyte. Not used for quantification of PCE or TCE but large peaks seen when present.

ETHENE Method

Hardware: HP5890 II Manual Injection, Auto pressure control
Column: Supelco 2-5461, Carboxen 1006 Plot, 30m x 0.53
Detector: Flame ionization detector (FID)
Injector/det: 240/200°C
Carrier Gas: He @8.0/min
Temperature: isothermal 120°C, 5min.
Injection volume: 20μL headspace

Compound	peak time(min)	Detection Limit (μmol/b)	Calibration curve p.a. =	Comments
CH4	1.515	0.08	12176 [μmol/b]	Use Hamilton 1702 25uL syringe with new #2 tip. Pump 2x in bottle and inject ASAP. Hold needle in injection port 10 seconds.
ETH	4.90	0.05	21581[μmol/b]	

IAN01 Method

Hardware: HP5890 II Autosampler Injection, Auto pressure control
Column: Supelco 4% Carbowax 20M, 80/120 Carbopack B-DA
Detector: Flame ionization detector (FID)
Injector/det: 200/180°C
Carrier Gas: He @35mL/min
Temperature: 125°C/1min., +30°C to 180°C, 180°C/4min.
Injection volume: 1μL liquid

Compound	peak time(min)	Detection Limit (mg/L)	Calibration curve p.a. =	Comments
EtOH	1.338	0.60	1778x[mg/L liq]	Use Hamilton 2μL syringe with flat tip. Autosampler injection of 1μL.
Acetate	3.969	1.20	830x[mg/L liq]	
Propionate	5.943	0.70	1388x[mg/L liq]	

Each 1.0mL sample is acidified with the addition of 100μL of 0.3M oxalic acid.

An oxalic acid GC peak is seen at about 1 minute.

HYDROGEN Method

Hardware: HP5890 II Manual Injection, Auto pressure control
Column: Supelco 2-5461 Carboxen 1006 Plot 30m x 0.53 mm
Detector: TCD
Injector: 240°C
Carrier Gas: N_2 @8mL/min
Temperature: isothermal
Injection volume: 20µL headspace

Compound	peak time(min)	Detection Limit (µmol/b)	Calibration curve p.a. =	Comments
H_2	1.08	1.20	826[µmol/b]	Use Hamilton 1702 25uL syringe with new #2 tip. Pump 2x in bottle and inject ASAP. Hold needle in injection port 10 seconds.
CH_4	1.80		--	
CO_2	3.30		--	

Used only for hydrogen quantification. CH_4 is detected when present but results obtained from ETHENE method are used. CO_2 has been suggested as the final breakdown product of ethane. CO_2 is measured but no significant amount was produced in the cultures under analysis.

APPENDIX C - LIQUID SAMPLING PROTOCOL

Liquid samples were removed from culture bottles for testing acetate, propionate and ethanol concentrations.

1. Using a 2.5mL gas-tight Hamilton Luer-lock syringe #1002, a 2mL sample is withdrawn from each bottle.
2. The syringe, containing the 2mL sample is then fitted with a Acro Disk® 0.45μm, 13mm Teflon® disposable filter. 1mL of the sample is passed through the filter in order to purge air. The remaining 1mL is then placed in a 2mL-glass vial fitted with screw cap and Teflon® lined rubber septa.
3. The 1.0mL sample is then acidified with Oxalic acid by the addition of 100μL of 0.3M Oxalic acid to provide 0.03M Oxalic acid upon dilution as required for chemical stability of the Gas Chromatograph column packing material.
4. 2mL of basal media (See Table 3.1) is added back to culture bottle.

Bottles are shaken by hand and placed in vial holder for automatic injection.

APPENDIX D - CALIBRATION CURVES

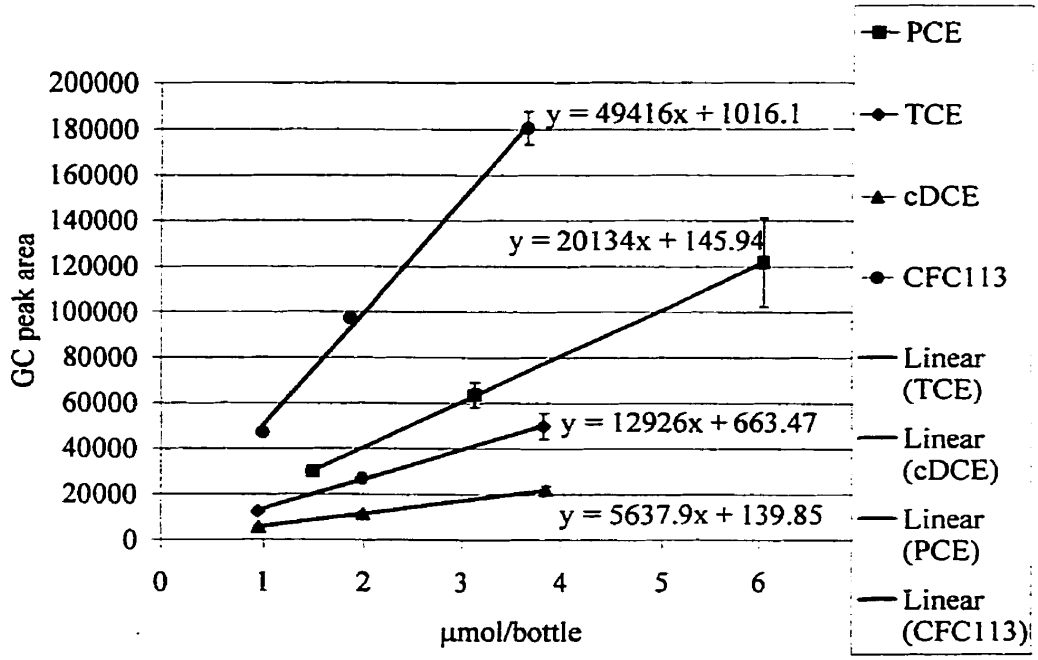

Fig D1 CRESTECH Method Calibration

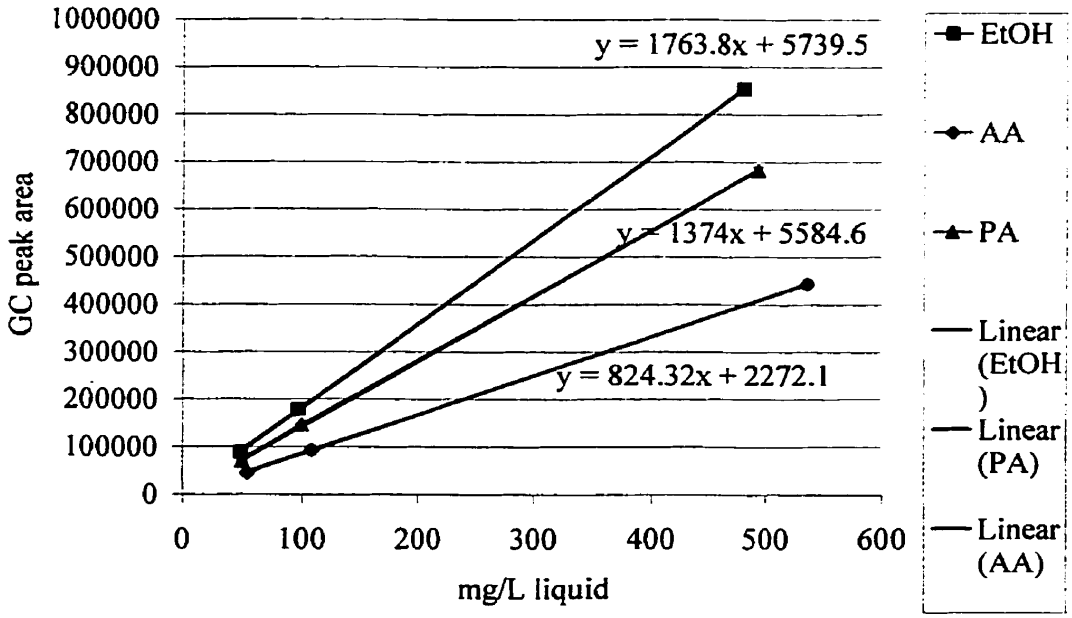

Fig D2 IAN01 Method Calibration

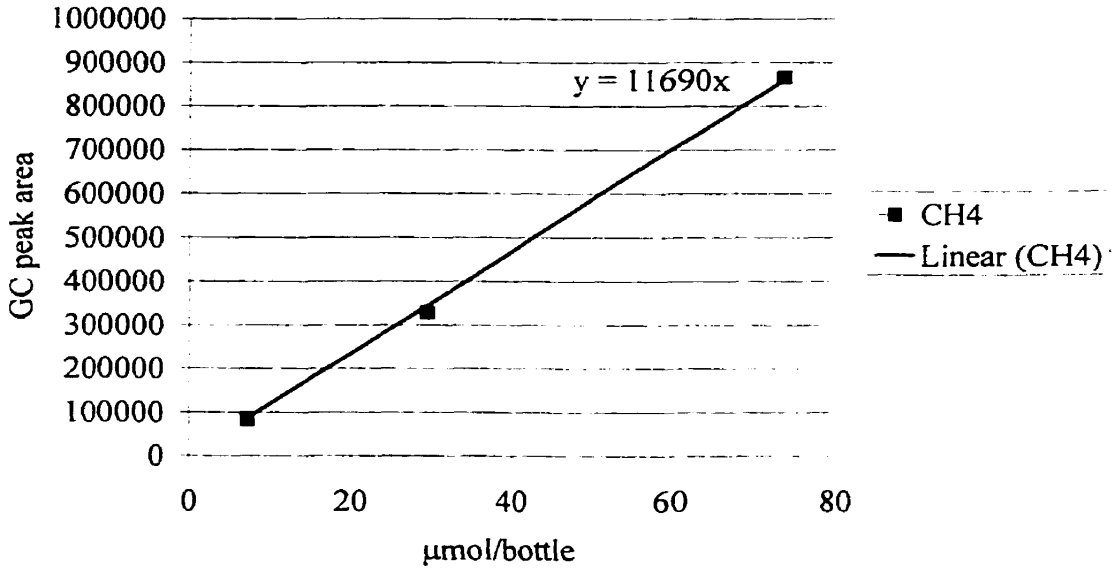

Fig D3 ETHENE Method - Methane Calibration

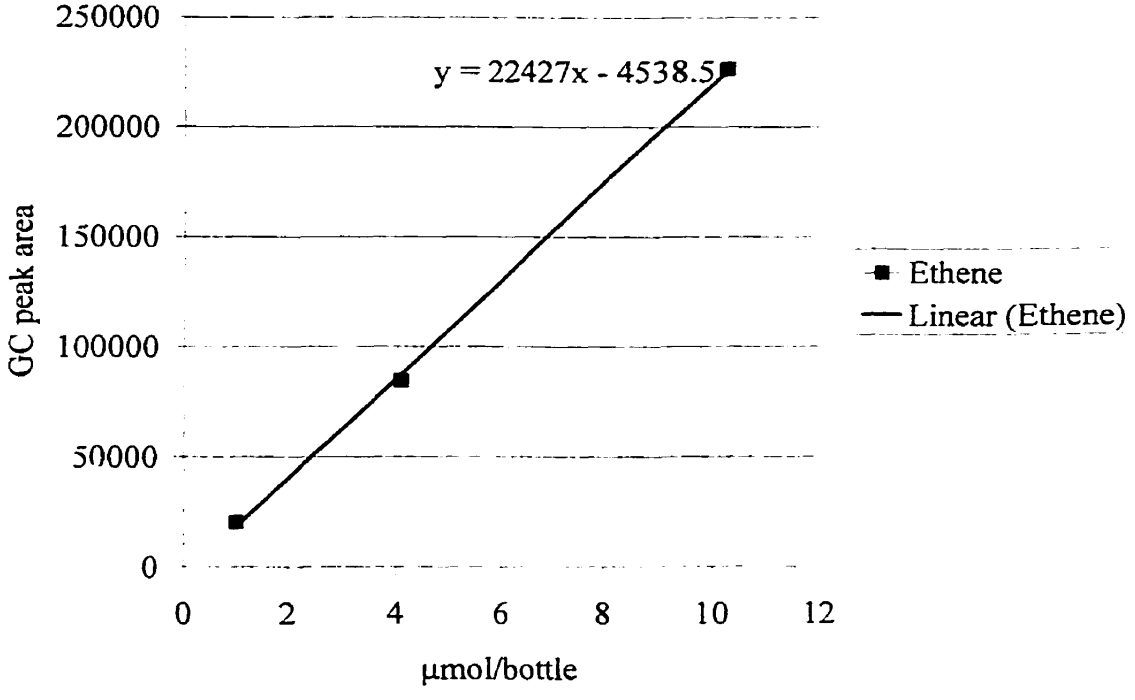

Fig D4 ETHENE Method - Ethene Calibration

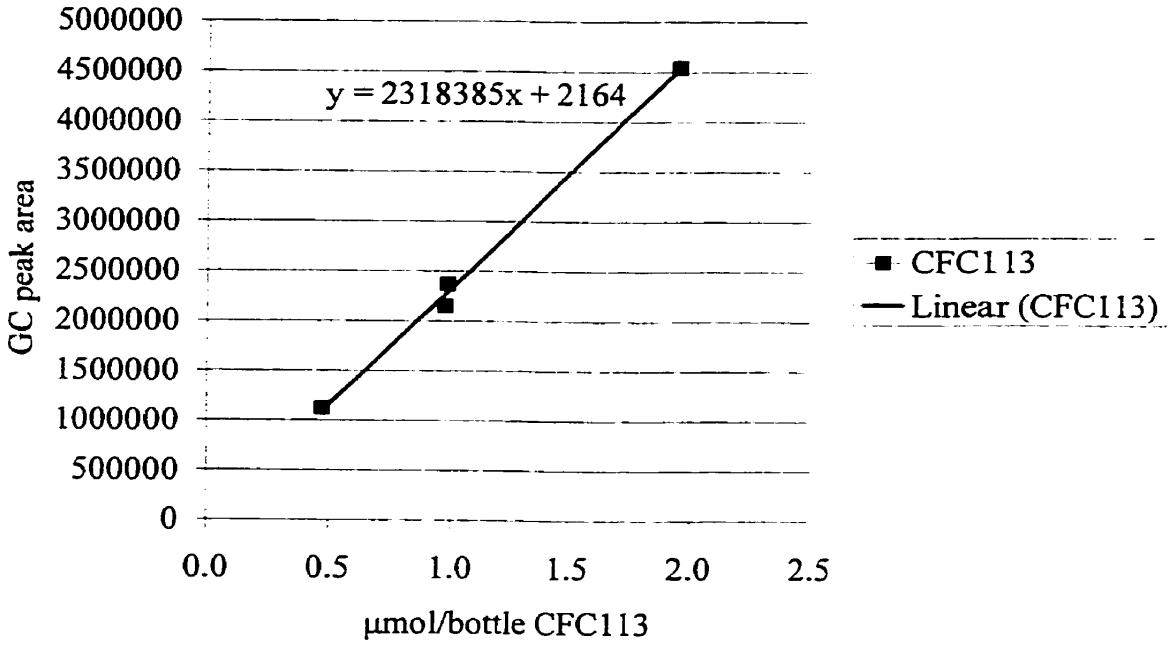

Fig D5 CFC01 Calibration - CFC113

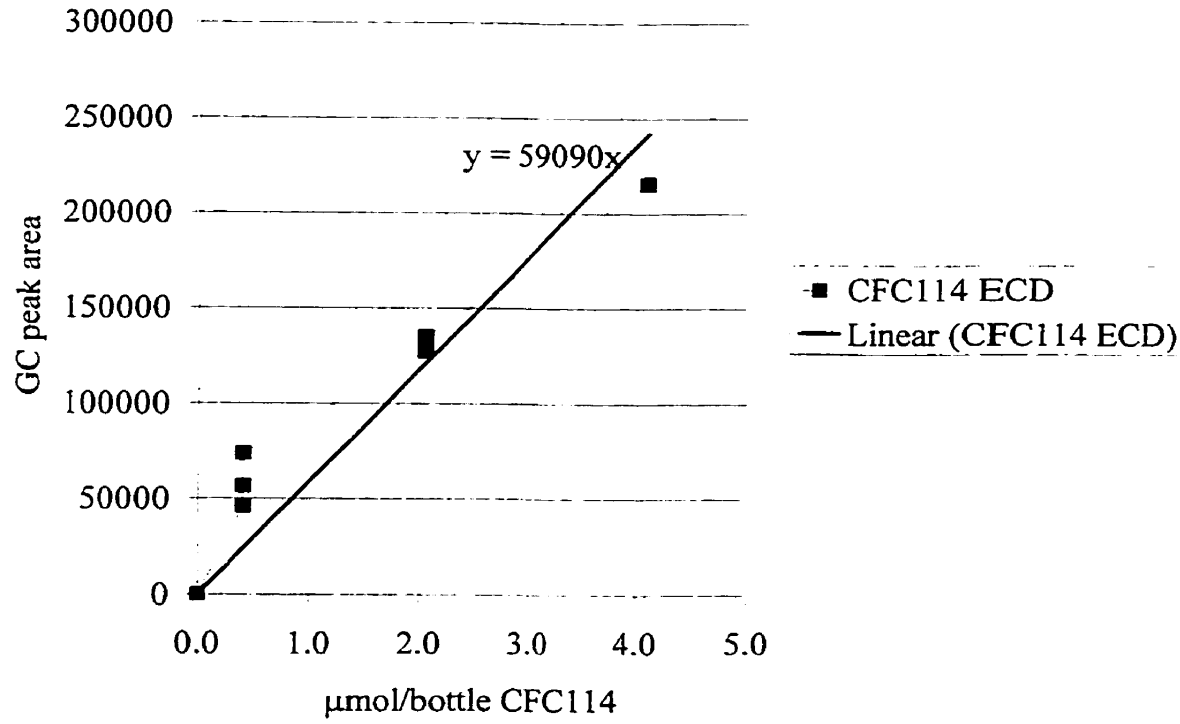

Fig D6 CFC01 Method - CFC114