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The Biological Reductive Dechlorination of Chlorinated Compounds in a High Rate Anaerobic Reactor

by

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A thesis
submitted under the supervision of
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and
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Presented to Ottawa-Carleton Institute for Civil Engineering
in partial fulfillment of the requirements for the degree of
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Department of Civil and Environmental Engineering

The undersigned recommend to the Faculty of Graduate Studies and Research
acceptance of the thesis

**The Biological Reductive Dechlorination of Chlorinated
Compounds in a High Rate Anaerobic Reactor**

submitted by
Jody Wells

in partial fulfillment of the requirements for
the degree of Master of Engineering

Chair, Department of Civil and Environmental Engineering

Co-Supervisor, Dr. Wayne Parker

Carleton University
Ottawa, Ontario, Canada
November, 1998

Abstract

This research investigated the viability of a high rate anaerobic reactor to treat three different target chlorinated compounds fed in a mixture. The target VOC compounds were tetrachloroethylene (PCE), carbon tetrachloride (CT) and dichloromethane (DCM). Batch tests were employed to determine the best cosubstrate for use in a continuous flow process and propylene glycol was selected from ethylene glycol, methanol and ethanolamine as the carbon source to be used in the high rate reactor. Batch tests also indicated that CT was degraded the most rapidly and PCE was the slowest VOC to degrade in the serum bottles.

Continuous flow studies were run under a number of different operating conditions. It was determined that PCE and DCM were not effectively removed from the high rate anaerobic reactor, however the removal of CT was high and consistent regardless of the operating conditions. A rate coefficient of 5.33 $\text{L/gVSS}\cdot\text{d}$ was calculated for CT, from the reactor data, and it was concluded that the process was a viable and effective method for the treatment of CT.

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Glossary

AnSBBR		Anaerobic Sequencing Batch Biofilm Reactor
BESA		Bromoethanesulfonic acid
CF		Chloroform
COD		Chemical Oxygen Demand
2-CP		2-Chloropropene
CT		Carbon tetrachloride
1,1-DCA		1,1-Dichloroethane
1,1-DCE		1,1-Dichloroethene
DCE		Dichloroethylene
DCM		Dichloromethane
1,1-DCPE		1,1-Dichloropropene
ECD		Electron Capture Detector
GC		Gas Chromatograph
HCA		Hexachloroethane
HRT		Hydraulic retention time
OLR		Organic loading rate
PCE		Perchloroethylene
1,1,1-TCA		1,1,1-Trichloroethane
1,2,3-TCP		1,2,3-Trichloropropane
T-1,2-DCE		Trans-1,2-dichloroethene
TCE		Trichloroethylene
TSS		Total Suspended Solids
UASB		Upflow Anaerobic Sludge Blanket
VC		Vinyl chloride
VSS		Volatile Suspended Solids

1. INTRODUCTION

1.1. BACKGROUND

Low level concentrations of chlorinated organic compounds can be found in a number of different air streams such as industrial vents, contaminated soil venting off gases and wastewater treatment off gas streams. These low level concentrations of VOC streams are conventionally treated by adsorption, incineration or biofiltration methods, which have proven to be either costly or ineffective in the past.

A hybrid process that consists of a counter current packed column which transfers the gas phase contaminants into a liquid, which is then directed into a high rate anaerobic treatment process has been proposed for the treatment of these difficult streams. Anaerobic treatment was selected because biological oxidation of the chosen chlorinated organics is not favourable under aerobic conditions, due to the relatively high oxidation state of these compounds. However, many chlorinated organics have been observed to be reductively dechlorinated under anaerobic conditions (Hughes and Parkin, 1996; Vogel and McCarty, 1985). The evaluation of the design and operating variables of the high rate anaerobic treatment unit was the focus of this project.

1.2. OBJECTIVES AND SCOPE OF STUDY

The main objective was to evaluate the biological treatment of a solution that was representative of that which would be obtained from the mass transfer process, in a high rate anaerobic reactor. To meet this goal two sub-objectives were established. The impact of cosubstrate type on anaerobic VOC degradation was determined from serum bottle batch tests, and VOC degradation as a function of hydraulic retention time (HRT) and organic loading rate (OLR) were evaluated during continuous flow studies.

Perchloroethylene (PCE), carbon tetrachloride (CT) and dichloromethane (DCM) were chosen as the target chlorinated organics to be examined due to their wide range in Henry's law coefficients and their molecular make-up with respect to the number of chlorine atoms. Cosubstrates examined in the anaerobic process were methanol, ethylene glycol, propylene glycol and ethanolamine.

2. LITERATURE REVIEW

2.1. BACKGROUND

2.1.1. *Anaerobic Respiration*

Living organisms utilize chemical energy through oxidation-reduction (redox) reactions. Oxidation is defined as the removal of an electron or electrons from a substance, and a reduction is defined as the addition of an electron or electrons to a substance (Brock, 1991). The substance that is oxidized is known as the electron donor and the substance that is reduced as the electron acceptor. The tendency of a substance to give up or accept an electron is measured according to its reduction potential (E_o), which is determined electrically in reference to a standard substance, H_2 (Brock, 1991). A substance with a more negative reduction potential will have more of a tendency to donate electrons, whereas a substance with a more positive reduction potential is more likely to be an electron acceptor. The difference in the reduction potentials of two substances is expressed as ΔE_o , and this value is proportional to the change in free energy (ΔG_o) of the reaction. Therefore, the greater the difference between the reduction potential of two substances, the more energy will be released from their reaction.

An example of a redox reaction is given by the formation of water from molecular hydrogen and oxygen (Brock, 1991). The following equation describes the electron donating half reaction (H_2 is the reductant and becomes oxidized).

The electron accepting half reaction is given by the following (O_2 is the oxidant and becomes reduced):

The net reaction is then equal to the following (the formation of water):

Brock (1991) describes the electron transfer in redox reactions by way of imagining a vertical electron tower. The reduction potentials of oxidation-reduction pairs are arranged with the most negative at the top of the tower and the most positive at the bottom. The most reduced pairs at the top of the tower contain the greatest potential energy, whereas the more oxidized pairs at the bottom of the tower contain the least amount of potential energy. As the electrons from the donors fall from the top of the tower they are 'caught' by acceptors further down, and the greater the difference between the reduction potentials of the acceptor and the donor, the more energy is released.

The metabolic activity of microorganisms induce oxidation -reduction reactions. These reactions transform organic substances into smaller molecules and the energy released is utilized by the microorganisms involved. This process is known as biodegradation (LaGrega. 1994).

One of the most common electron acceptors of living organisms is molecular oxygen. Its position at the bottom of the electron tower makes it the strongest oxidizer, since it is the final electron acceptor. Therefore, when oxygen

is present in a biological system, it will almost always act as the electron acceptor (Brock, 1991). If, however, oxygen is not available, other substances higher up on the electron tower can serve as electron acceptors.

Anaerobic treatment processes are defined as those that occur in the absence of oxygen (LaGrega, 1994). Since oxygen is not available to serve as the terminal electron acceptor, other inorganic compounds will accept electrons. These inorganic compounds which support anaerobic respiration are used in order of preference, according to their reduction potential, and hence the free energy released during the reaction. In general, the reduction of nitrates to nitrogen is favoured over the reduction of sulfates to hydrogen sulfide, which is favored over the reduction of carbon dioxide to methane (LaGrega, 1994).

Most anaerobic systems are heterotrophic, and therefore require a carbon source for the synthesis of biomass (LaGrega, 1994). Some typical carbon sources used as a substrate in anaerobic reactors are methanol, glucose and acetate, and they are usually provided in the feedstock. Normally, the electron donor is the organic pollutant in the feed stock, or the biodegradable COD. However, in the case of chlorinated organics, the contaminant is relatively oxidized due to the presence of the chlorine substitutes. The higher the number of chlorine atoms, the more oxidized the compound is, and the more susceptible it is to reduction (Vogel et al., 1987).

2.1.2. Reductive Dechlorination

Due to the high oxidation state of the chlorinated organic compounds of interest in this research, it is commonly accepted that an aerobic treatment process will not be successful in their degradation. However, there is sufficient evidence to suggest that under anaerobic conditions the transformation of chlorinated compounds will occur by reductive dehalogenation. The general reduction reaction is given by the following (Vogel et al., 1987):

The concept of reductive dehalogenation involves the division of a halogen, in this case a chlorine atom, from an organic compound by reduction (LaGrega, 1994). An example of reductive dechlorination is given in the case of perchloroethylene (PCE). Two electrons that are released by the oxidation of an electron donor are transferred to PCE, thus removing a chlorine atom. It has been found that the highly oxidized PCE is sequentially reduced to trichloroethylene (TCE), followed by dichloroethylene (DCE), vinyl chloride and eventually ethylene (LaGrega, 1994). With each successive reduction, the compound becomes less oxidized and therefore slightly less susceptible to reduction (see figure 2-1).

Figure 2-1: Sequential Anaerobic Reductive Dechlorination of PCE (taken from LaGrega)

2.1.3. *Target Chlorinated Organic Compounds*

The target chlorinated compounds that are of interest in this research are perchloroethylene (PCE), carbon tetrachloride (CT) and dichloromethane (DCM). These compounds are suspected carcinogens and are a concern if introduced into the environment. Sources of low concentrations of chlorinated organic compounds in air streams include industrial vents, contaminated soil venting off gases and wastewater treatment off gas streams. Some industrial applications of these compounds include use as degreasing agents, insect and rodent removal, in the manufacture of plastics, and in dry cleaning (Fathepure et al. 1987).

It has been determined in a number of studies that the biological reductive dechlorination of these toxic compounds in a liquid phase is indeed a feasible method of treatment. Fathepure and Boyd (1988) showed that biological reductive dechlorination of PCE occurred under methanogenic conditions as did Freedman and Gossett (1989). Galli and McCarty (1989), Petrovskis et al. (1994) and Doong and Wu (1995) all found evidence to support the reductive

dechlorination of CT under anaerobic conditions. Freedman and Gossett (1991) were able to utilize DCM as a growth substrate in a methanogenic culture, but did not find evidence to suggest that it underwent reductive dechlorination.

Potential breakdown sequences for the target organics (PCE, CT and DCM) are given in table 2-1.

<p>PERCHLOROETHENE (PCE), C_2Cl_4</p> <p>↓</p> <p>TRICHLOROETHENE (TCE), C_2HCl_3</p> <p>↓</p> <p>DICHLOROETHENE (DCE), $C_2H_2Cl_2$</p> <p>↓</p> <p>VINYL CHLORIDE (VC), C_2H_3Cl</p> <p>↓</p> <p>ETHYLENE (ETH), C_2H_4</p> <p>↓</p> <p>(Freedman and Gossett, 1989)</p>
<p>CARBON TETRACHLORIDE (CT), CCl_4</p> <p>↓</p> <p>CHLOROFORM (CF), $CHCl_3$</p> <p>↓</p> <p>DICHLOROMETHANE (DCM), CH_2Cl_2</p> <p>↓</p> <p>(Galli and McCarty, 1989)</p>
<p>DICHLOROMETHANE</p> <p>↓</p> <p>CO_2, CH_4 and Acetic Acid</p> <p>↓</p> <p>(Freedman and Gossett, 1991)</p>

Table 2-1: Breakdown compounds of PCE, CT and DCM

In the case of perchloroethylene, its conversion to the less chlorinated organics TCE, 1,2-DCEs and vinyl chloride are of little or no benefit (Freedman and Gossett, 1989). These compounds are also considered toxic, and in the case of vinyl chloride, more harmful than the original chlorinated compound. It is therefore desirable for the chlorinated organics to be reduced to non-chlorinated, environmentally acceptable products.

DCM is especially interesting since it is one of the target compounds and also a breakdown component of CT. The fact that DCM may be produced in the system will have to be taken into consideration when performing data analysis.

2.2. PREVIOUS EXPERIMENTAL RESEARCH

2.2.1. Batch Tests

2.2.1.1. General Serum Bottle Method

A general serum bottle test method to assess the degradation of PCE and TCE was described by Freedman and Gossett (1989) and variations on this technique have been described by other researchers. In their technique 160 mL serum bottles were filled with 100 mL of liquid, containing both supernatant and biomass from a laboratory anaerobic reactor. This inoculum contained 50 mg of the auxiliary substrate (glucose, methanol, acetic acid or sodium formate) per liter as well as the necessary nutrient medium required for growth. The nutrient medium consisted of the following: NH_4Cl , K_2HPO_4 , KH_2PO_4 , MgCl_2 , a trace metal, resazurin, Na_2S , FeCl_2 , NaHCO_3 and yeast extract.

The bottles were sealed with Teflon lined rubber septa and aluminum crimp caps. They were then spiked with the contaminant (in this case PCE or TCE) at a concentration of 0.5 to 0.75 mg/L. Incubation was conducted at 35°C under quiescent conditions with the liquid in contact with the septum to minimize losses. In other studies (Galli and McCarty, 1989) serum bottles were placed on a rotary shaker.

The response of the volatile organic compounds with time were routinely determined by injection of headspace samples into a gas chromatograph (GC). Methane production was also determined by GC analysis.

2.2.1.2. *Perchloroethylene*

Several batch test have been performed to determine the fate of perchloroethylene in a biotic anaerobic environment. Freedman and Gossett (1989) reported the complete dechlorination of PCE and TCE to a non-chlorinated end product, ethylene. Their work was conducted using serum bottles, operated on a semi-continuous basis, under methanogenic conditions at a temperature of 35°C. As the initial doses of the contaminant were degraded, the bottles were re-spiked and auxiliary substrate and nutrient medium were also added. Four different auxiliary substrates were examined: methanol, glucose, acetic acid, and sodium formate. The original anaerobic biomass used for the experiments had been obtained from a wastewater treatment plant, and was maintained in a laboratory anaerobic digester. Therefore, the biomass was a mixed culture and no specific organisms were identified.

Results of the experiments indicated that biological reductive dechlorination occurred as shown in figure 2-1. Perchloroethylene (0.5 mg/L) and trichloroethylene (1 mg/L) were completely converted within 2-3 days. Vinyl chloride accumulated and ethylene appeared after repeated additions of PCE. The rate limiting step was observed to be the conversion of vinyl chloride to ethylene, and complete dechlorination of all of the vinyl chloride in the bottle was never achieved. Methanol was found to be the most effective electron donor in terms of maintaining the rate of PCE degradation and the extent to which vinyl chloride was converted to ethylene.

2-Bromoethanesulfonic acid (BESA), a methanogenic inhibitor was used to determine the role of methanogens on the reductive dechlorination of PCE and TCE. Upon the addition of the inhibitor, TCE degradation and methane production were immediately stopped. Repeated additions of the inhibitor were required for the cessation of PCE degradation, and the accumulation of 1,2-DCE and TCE was observed. According to the authors these results suggest that methanogens played a key role in the reductive dechlorination of PCE and TCE and it is important to maintain methanogens in the system. PCE degradation did not stop immediately and it was suspected that the anaerobic culture was degrading the inhibitor.

Fathepure and Boyd (1988) investigated the role of methanogens in the reductive dechlorination of PCE with anaerobic sludges obtained from four different waste water treatment plants in mid-Michigan. The concentration of PCE in the serum bottles was 1 $\mu\text{g/mL}$, and methanol and acetate were

introduced as the primary electron donors. PCE was degraded to TCE at a initial rate of $250 \text{ nmol L}^{-1} \text{ day}^{-1}$ for the first week of incubation, and then at a rate of $445 \text{ nmol L}^{-1} \text{ day}^{-1}$ for the second week. TCE accumulated at a rate of $175 \text{ nmol L}^{-1} \text{ day}^{-1}$ for the first week and then the concentrations leveled off, suggesting its transformation to lower chlorinated compounds, however these products were not identified. The addition of 5 mM of BESA (a methanogenic inhibitor) resulted in a decrease in the dechlorination of PCE, suggesting that methanogenic bacteria had a direct role in the degradation of PCE. Two of the sludges tested showed significantly higher activities as compared to the other two. The reasons suggested by the authors for these differences include the presence or absence of specific groups of microorganisms or the presence or absence of specific electron donors/acceptors. The presence of toxins may have limited activity, and previous exposure of the sludge to chlorinated organics may have aided in the acclimation of the sludge to the toxicant.

Fathepure and Boyd (1988), also obtained direct evidence that methanogens can transform PCE. This was acquired with the use of a pure culture of an acetate cleaving methanogen, *Methanosarcina mazei*, which dechlorinated PCE at an average rate of $56 \text{ nmol L}^{-1} \text{ week}^{-1}$ without a lag time.

Fathepure et al. (1987) identified specific anaerobic bacteria that were successful in the dechlorination of PCE. Two acetate-utilizing methanogenic bacteria showed significant dechlorination of PCE. Both *Methanosarcina sp.* and *Ms. mazei* were able to dechlorinate 1 mg/L of PCE to TCE without a lag period. An obligate anaerobe, known as DCB-1 (a pure-culture dehalogenator), was able

to dechlorinate PCE three to five times faster than that observed for *Methanosarcina* sp. and *M. mazei*. DCB-1 has been more recently been identified as *Desulfomonile tiebjei* (Townsend and Suflita, 1997). Higher PCE transformation rates were achieved when DCB-1 was grown in a methanogenic consortium and maximum TCE yields were obtained when methanol was used instead of acetate as a growth substrate. Although 100% transformation of PCE did not occur the researchers were not alarmed since it had been a general observation that anaerobes are less active in pure culture than when in a mixed consortia under more natural conditions. These findings were significant since *Methanosarcina* spp. are common in anaerobic methanogenic environments.

DiStefano et al. (1991) reported the reductive dechlorination of high concentrations of PCE by an anaerobic culture in the absence of methanogenesis. Methanogenic cultures were initially developed in a fashion similar to that described by Gossett and Freedman (1989). Serum bottle tests were conducted on a semi-continuous basis, at a temperature of 35°C, with methanol as the carbon source and concentrations of PCE reaching 91 mg/L. Each subsequent PCE concentration was increased once dechlorination was verified at a given dosing level. On day 150, when the PCE reached its highest concentration, methane production ceased and was not produced for the remainder of the study. During this period the transformation of PCE was sustained and the conversion of vinyl chloride to ethylene increased. PCE was routinely transformed to 80% ethylene and 20% vinyl chloride within two days, reaching dechlorination rates as high as 275 $\mu\text{mol L}^{-1} \text{ day}^{-1}$. Negligible quantities

of TCE and DCE isomers were observed. An electron balance indicated that approximately two thirds of the methanol reducing equivalents were used to produce acetate and the remaining one third was used in reductive dechlorination. The authors concluded that the high concentrations of PCE inhibited methanogenesis, and suggest that other microorganisms, mainly acetogens, may play a role in the dechlorination of PCE since the accumulation of acetate was observed. However, they did not dismiss the possibility of methanogens being the mediators of reductive dechlorination at the lower PCE concentrations.

In batch experiments conducted by DE Bruin et al. (1992), biomass taken from an anaerobic column in which complete conversion of PCE to ethylene was being observed was used as inoculum. The column's biomass mixture was a 3 to 1 mixture of Rhine River sediment (The Netherlands) and ground granular anaerobic sludge from an upflow anaerobic sludge blanket reactor used to treat sugar beet wastewater. Lactate was supplied as the electron donor and approximately 0.6 mg/L of PCE was added to the serum bottles. After a test period of 16 days, only ethylene was found in the bottles. TCE, cis-DCE and vinyl chloride intermediates were all detected throughout this time period.

In anaerobic serum bottle tests conducted by Long et al. (1993) a mixture of chlorinated aliphatic compounds, including chloroform (CF), carbon tetrachloride (CT), 1,1-dichloroethane (1,1-DCA), 1,1,1-trichloroethane (1,1,1-TCA), hexachloroethane (HCA), 1,1-dichloroethene (1,1-DCE), trans-1,2-dichloroethene (T-1,2-DCE), trichloroethene (TCE), perchloroethene (PCE), 2-

chloropropene (2-CP), 1,1-dichloropropene (1,1-DCPE) and 1,2,3-trichloropropane (1,2,3-TCP) was introduced into bottles. Inoculum consisted of anaerobic biomass taken from a batch fed reactor. Equal COD contributions of glucose, phenol, benzoate and acetate were used as the carbon source (to a concentration of 750 mg/L) and 197 mg/L COD from methanol was contributed since methanol was used as a carrier for the chlorinated compounds. Individual chlorinated organic concentrations were added at a concentration of 120 $\mu\text{g/L}$. The complete disappearance of PCE took 60 days to occur and a maximum specific degradation rate of 30 $\mu\text{g/g VSS-day}$ for the PCE was observed. Methane production measurements, close to those of theoretical values, based on COD feed concentrations, were evidence of an active methanogenic culture.

In a separate serum bottle test with the same operating conditions and PCE as the only chlorinated organic, the specific degradation rate for PCE was determined to be 780 $\mu\text{g/g VSS-day}$. This increase in the specific degradation rate indicates that the dehalogenation rates for individual chlorinated compounds are lower when contained in a mixture with other chlorinated organics, than when fed alone.

Butyric acid, ethanol, lactic acid and propionic acid were evaluated as electron donors in connection with the reductive dechlorination of PCE (Fennell and Gossett, 1997). Both long term and time-intensive studies were conducted. Tests were carried out using serum bottles, with repeated additions of 18 mg/L of PCE in the case of long term testing, and one addition of 18 mg/L in the time-intensive, short term test. Long term observations revealed no significant

difference among the different electron donors with respect to the extent of dechlorination. Vinyl chloride and ethylene were produced in comparable amounts among the different donors. Short term studies indicated that slower degrading butyric acid and propionic acid supported the total dechlorination of PCE to final end products of mostly vinyl chloride and ethylene. Ethanol and lactic acid caused initial rapid dechlorination of PCE, but as the electron donor was depleted, dechlorination drastically slowed and significant quantities of PCE remained, and were slowly degraded. The results make it difficult to conclude which was the best electron donor to support the dechlorination of PCE.

2.2.1.3. Carbon Tetrachloride

Significant research has been conducted regarding specific bacterial cultures that are capable of degrading CT. Egli et al. (1987) observed reductive dechlorination of CT to chloroform (CF) and DCM with a pure culture of the sulfate reducer, *Desulfobacterium autotrophicum*. Complete transformation occurred within 6 days, yielding mostly CF and small amounts of DCM. The concentration of the DCM increased as that of the CF decreased, and there was no significant transformation of the DCM.

Similar results were observed by Egli et al. (1988). *Desulfobacterium autotrophicum*, *A. woodii* and *C. thermoaceticum* were all able to degrade CT to CF and DCM. *Desulfobacterium autotrophicum* reduced CT to mostly CF and a small amount of DCM. Complete disappearance of CT took 18 days. After a prolonged incubation period the concentration of CF decreased with a

subsequent rise in DCM concentration. The acetogen, *A. woodi*, completely degraded the same amount of CT (80 μ M or 12.3 mg/L) in 3 days with only DCM being recovered at the end of the incubation period. CF was detected as a transient intermediate. Only 8 μ M of DCM was recovered at the end of the incubation, resulting in 90% of the original CT being degraded to unknown end products. Radioactive tracer tests indicated that a high percentage of these end products was CO₂. Acetate, pyruvate and cell material also accounted for CT carbon.

Galli and McCarty (1989) isolated a strictly anaerobic bacterium identified as a *Clostridium* sp. from the effluent of an anaerobic suspended growth bioreactor. Serum bottles were dosed with CT at a concentration of 100 μ g/L and incubated at 35°C. Cultures of *Clostridium* sp. in the presence of CT were effective at reductively dechlorinating the CT to CF and DCM. Biotransformation occurred in the first day leading to the formation of CF, corresponding to approximately 55% of the CT. This CF was further degraded to DCM, resulting in about 8% of the theoretical amount of CT and the remainder as unidentified products.

Criddle et al. (1990) determined that the breakdown mechanism for CT is dependent on the electron acceptor conditions of the media in cultures of *Escherichia coli* K-12. Four different media were used to cultivate the cultures. These consisted of media to support aerobic respiration, nitrate respiration, fumarate respiration and fermentation. The major electron donor in the respiring cultures was glycerol and glucose and hydrogen were the only substrates added

to the fermenting cultures. The use of oxygen and nitrate prevented the transformation of CT, however, under fumarate-respiring conditions CT was recovered as CO₂, CF and unidentified nonvolatiles. Fermenting conditions resulted in the production of more CF and cell bound carbon from the CT and little CO₂ production was identified.

Petrovskis et al. (1994) conducted research on the effects of electron acceptors and donors on the transformation of CT by the bacterium *Shewanella putrefaciens* strain MR-1. This particular species is known to utilize manganese(IV) and iron(III) oxides, nitrate, and fumarate as terminal electron acceptors. CT was most effectively dechlorinated under iron(III) respiring conditions with respect to the rate and extent of transformation. Lactate and hydrogen were used as electron donors in all cases. CF was the main transformation product and it was determined that approximately 50% of the CT was transformed to cell-bound material and smaller amounts to CO₂ and unidentified aqueous intermediates. When the lesser chlorinated products, CF and DCM were fermented with the isolated bacterium, no dechlorination products were identified. It was suggested that this lack of activity may have been due to the redox characteristics or the structure of the chlorinated compounds.

The effect of substrate concentration on the biotransformation of CT was investigated by Doong and Wu (1995). An anaerobic mixed culture obtained from an anaerobic digester of the Taiwan Sugar Co. wastewater treatment facility (Hsinchu, Taiwan) was used as an inoculum. Acetic acid was used as the auxiliary substrate and ranged in concentration from 0 to 30 mg/L. This

concentration was increased progressively along with an increase in the concentration of CT, which ranged from 100 to 1000 $\mu\text{g/L}$. In this study 99.9% removal of CT (100 $\mu\text{g/L}$) was observed in 15 days with an auxiliary substrate concentration of 5mg/L. The same removal was observed in only 6 days when 30 mg/L of acetic acid was supplemented. Longer times were required to reach 99.9% removal of CT as the initial concentration of the chlorinated organic increased. Bottles containing no auxiliary substrate transformed 99.9% of CT in 32 days and was attributed to the presence of trace organics in the original medium. CF was produced and was correlated to the decrease in CT. A lower fraction of the total CT transformed could be observed as CF (15-20%), than the 55% reported by Galli and McCarty (1989). Insufficient data was available to determine if this was a result of changing transformation pathways or greater CF transformation.

The effect of substrate type on the biodegradation of CT was also examined by Doong and Wu (1995). Glucose, acetate and humic acid were evaluated as carbon sources and the same inoculum as described above was used. Serum bottles that were fed acetate were more efficient at removing CT (original liquid concentration of 100 $\mu\text{g/L}$) than those fed glucose or humic acid. After 60 days, 48% was removed with acetate addition, whereas in bottles fed with glucose and humic acid 35% and 33% removal efficiencies were observed.

CT was one of the 12 chlorinated aliphatic compounds assessed in serum bottle tests by Long et al. (1993). The experimental set-up was the same as described earlier in section 2.2.1.2. CT, along with its breakdown component,

CF, were both removed from the serum bottles by day six, which was the first sample day. An increase in DCM concentration was observed, followed by its complete disappearance. Relative removal rates for the different compounds in the mixture are shown in table 2-2. The results indicate the CT is removed the fastest, followed by PCE and then DCM.

COMPOUNDS	DEGRADATION RATE ($\mu\text{g/g VSS}\cdot\text{d}$)
CT, CF, HCA	>60
1,1,1-TCA	65
1,1-DCE, TCE	35-40
PCE	30
C-1,2-DCE	70
DCM	20

Table 2-2: Maximum specific degradation rates from anaerobic serum bottle test (Long et. al., 1993)

2.2.1.4. *Dichloromethane*

Freedman and Gossett (1991) showed that dichloromethane (DCM) could be effectively used as a growth substrate under methanogenic conditions. This differs from the utilization of PCE and CT as they were both added as auxiliary compounds along with an additional substrate for growth. Masses of 8 to 12 μmol of DCM, corresponding to aqueous concentrations of 6.3 to 9.5 mg/L, were added to 160 mL serum bottles, inoculated with mixed liquor from a laboratory reactor. DCM was supplied as the sole organic carbon source to the cultures and the bottles were incubated at a temperature of 35°C. The doses of DCM were kept below 12 μmol to avoid inhibition of methanogenesis.

Initial DCM additions were completely degraded after a lag period of 10 days and subsequent additions disappeared with little or no lag time. Average degradation rates of DCM in bottles fed increasing masses of DCM (10 to 200 μmol every other day) were between 6.19 and 7.65 $\mu\text{mol h}^{-1}$ bottle $^{-1}$ (100 ml liquid per bottle). Partial inhibition of methanogenesis was observed at the higher concentration levels. For all conditions examined, CO_2 was the main formation product from the degradation of DCM, followed by CH_4 . Acetic acid was the main formation product when methanogenesis was inhibited.

When DCM is fed as the sole carbon source the theoretical ratio of methane production to DCM consumed is 0.5mol CH_4 /mol of DCM. Experimental results indicated a ratio of 0.492mol CH_4 /mol of DCM. When DCM additions were stopped, methane production levels correspondingly leveled off. This experimental ratio is consistent with the hypothesis that DCM can serve as a growth substrate.

Upon the addition of a methanogenic inhibitor, the degradation of DCM continued. A major product of DCM degradation under non-methanogenic conditions was acetic acid, providing evidence for the presence of an acetogen. Non-methanogens were observed to contribute to the majority of growth when DCM was supplied as the sole carbon source. The combination of these factors suggest that non-methanogens were responsible for the degradation of DCM, oxidizing a portion of it to CO_2 and fermenting the remainder to acetate. Methanogens then converted the degradation products of DCM to CH_4 .

Hughes and Parkin (1992) investigated the effects of mixtures of high concentrations of chlorinated aliphatics on the transformation rates of the individual compounds. The three chlorinated organics used in their study were dichloromethane, chloroform (CF) and trichloroethane (TCA) and acetate was provided as the electron donor. The serum bottles were operated on a fill and draw basis at a temperature of 20°C. The inoculum consisted of biomass from a laboratory anaerobic reactor that had been periodically receiving chlorinated aliphatics and the influent concentration for DCM was 5 mg/L. DCM was biotransformed both alone and in a mixture. An acclimation period of 10 to 15 days was required and the rate of disappearance of DCM increased with the presence of the other chlorinated organics in the feed. The presence of DCM was necessary for the degradation of chloroform to occur.

In the same set of serum bottle tests described earlier, Long et al. (1993) examined the fate of CT and CF. Both compounds disappeared rapidly with a corresponding increase in the concentration of DCM. The DCM was further degraded under anaerobic conditions at a rate of 20 µg/g VSS-d. These results strongly suggest DCM is an intermediate breakdown component of CT and CF.

Hughes and Parkin (1996) determined individual biotransformation rates in mixtures of chlorinated aliphatics. DCM, CF and TCA were selected for the study. Batch tests were conducted similar to those described above with chlorinated organic concentrations for DCM, CF and TCA of 2 and 5 mg/L, 0.125 and 0.250 mg/L, and 0.25 and 1.0 mg/L respectively. DCM transformation was not observed in these systems. One point of interest determined from this study

was that the presence of DCM, CF and TCA decreased the rate of acetate utilization and in all cases CF was found to be the most inhibitory and DCM the least inhibitory.

In other work performed by Hughes and Parkin (1996), in which the effect of chlorinated organics concentration on acetate utilization were considered, DCM transformation was not observed. This result occurred despite previous acclimation of the biomass to DCM.

Table 2-2 presents a summary of the batch tests discussed in this section.

Table 2-3: A summary of the results from previous batch studies using mixed cultures

REFERENCE	COMPOUND	SUBSTRATE	BIOMASS SOURCE	RESULTS
Freedman and Gossett, 1989	PCE (0.5mg/L) TCE (1mg/L)	methanol, glucose, acetic acid, sodium formate	wastewater sludge maintained in an anaerobic digester	-2-3 days for total transformation -accum. Of VC -ethylene detected
Fathepure and Boyd, 1988	PCE (1mg/L)	methanol, acetate	4 different wastewater anaerobic treatment facilities	-PCE deg=250mmol.L ⁻¹ .d ⁻¹ week 1 445mmol.L ⁻¹ .d ⁻¹ week 2, 56mmol.L ⁻¹ week-1 by <i>M. maze</i> -TCE accum.=175mmol.L ⁻¹ .d ⁻¹
DiStefano et al., 1991	PCE ~91mg/L	methanol	wastewater sludge maintained in an anaerobic digester	-80% ethylene -20% VC within 2 days -1%< VC after 4 d -275 µmol L ⁻¹ d ⁻¹
Freedman and Gossett, 1991	DCM (10 to 200 µmol every other day)	DCM	mixed liquor from laboratory anaerobic reactor	-DCM deg= 6.19-7.65µmol h ⁻¹ bottle ⁻¹
DE Bruin et al., 1992	PCE (0.6mg/L)	lactate	river sediment and ground anaerobic sludge	-partial inhibition at high conc. -TCE, <i>cis</i> -DCE and VC intermediates -16 days only ethylene detected
Long et al., 1993	PCE, TCE, DCE CT, CF 120 µg/L	glucose, acetate, benzoate, phenol, methanol	anaerobic biomass taken from batch fed reactor	-complete disappearance of TCE, DCE after day 16, PCE after day 60, CF and CT after 6 days -DCM, VC and <i>cis</i> -1,2-DCE detected, DCM deg=20µg/gVSS-d -PCE deg.= 30µg/gVSS-d in mixture, 780µg/gVSS-d alone
Fennell and Gossett, 1997	PCE (18mg/L)	butyric acid, ethanol, lactic acid, propionic acid	methanol, PCE and yeast enriched culture	-VC and ethylene
Doong and Wu, 1995	CT (100-1000µg/L)	acetic acid	anaerobic mixed culture from a sugar wastewater treatment facility	-99.9% (@ 100 µg/L) removal in 6 days with sufficient electron donor

Table 2-3 continued.

REFERENCE	COMPOUND	SUBSTRATE	BIOMASS SOURCE	RESULTS
Doong and Wu, 1995	CT (100 µg/L)	glucose, humic acid, acetate	anaerobic mixed culture from a sugar wastewater treatment facility	-48% removal with acetate -35% removal with glucose -33% removal with humic acid (all after 60 days)
Hughes and Parkin, 1992	DCM (5mg/L)	acetate	biomass from laboratory reactor periodically fed chlorinated organics	-acclimation period of 10-15 days required -rate of DCM disappearance increased with presence of other compounds
Hughes and Parkin, 1992	DCM (2 and 5mg/L)	acetate	biomass from laboratory anaerobic reactor periodically fed chlorinated organics	no transformation observed

2.2.2. *Continuous Flow Reactor Studies*

Vogel and McCarty (1985) examined the response of PCE in bench scale anaerobic continuous flow reactors. In one study, two reactors with an inside diameter of 2.5 cm, a height of 22 cm and filled with 3 mm glass beads were set up in series. The reactors were operated under anaerobic conditions at a temperature of 22°C and had been in operation for several years prior to the study. The lead reactor was continuously fed a solution containing 100 mg/L of sodium acetate. A PCE and acetate solution was introduced into the second column (110 mg/L PCE and 540 mg/L acetate) via a 10 ml gas tight syringe and mixed with effluent from the first column at a ratio of 1:4.4, resulting in a concentration of 20 mg/L of PCE and 100 mg/L of sodium acetate entering the second column. After 7 months of operation the second column was operated with a liquid detention time of 4 days, and the influent PCE concentration was decreased to 30 µg/L.

A second study employed a larger up-flow plastic reactor with an inside diameter of 20 cm and a height of 200 cm. It was operated at a temperature of 35°C and was filled with smooth quartzite rocks which supported a methanogenic bacterial biofilm. A non-sterile solution containing acetone, isopropanol, o-xylene, 1,1,1-trichloroethane, PCE, TCE, trace nutrients, phosphate and ammonia was stored in a glass bottle at 10°C and was fed into the reactor through a peristaltic pump, resulting in a hydraulic retention time (HRT) of 6 days. Active methanogenic conditions in the reactor had been established over a number of years. Analyses were conducted on samples removed from various

sampling ports over the height of the reactor. Results from the first reactor study indicated a PCE removal of approximately 99%. The formation of trichloroethylene (TCE), dichloroethylene (DCE) and vinyl chloride (VC) were detected, but not quantified. In the second study the larger anaerobic reactor was able to biotransform PCE from 300 $\mu\text{g/L}$ to below 5 $\mu\text{g/L}$ within the first 10 cm of the column. Some DCE was detected, but vinyl chloride continually increased as PCE and TCE disappeared. After 10 days of operation the vinyl chloride concentration at a height of 110 cm was 290 $\mu\text{g/L}$. After 22 days of operation the highest vinyl chloride concentration found in the column was 57 $\mu\text{g/L}$ at a height of 50 cm, indicating some biotransformation had taken place; however, the complete disappearance of vinyl chloride was not observed.

DE Bruin et al. (1992) performed experiments using a fixed bed anaerobic reactor that was 25 cm in length, with an inside diameter of 5.5 cm, and constructed of polyvinyl chloride. Anaerobic sediment from the Rhine River (The Netherlands), along with anaerobic granular sludge used to treat sugar beet wastewater served as the inoculum. The reactor was fed in the upflow mode and an anaerobic mineral medium was delivered through a peristaltic pump, equipped with silicon tubing. The medium was purged with N_2/CO_2 to insure the depletion of oxygen. Stock solutions of PCE were introduced with a syringe pump and a gas exchange chamber was used to remove any traces of oxygen which may have diffused into the silicon tubing. A schematic of the reactor set-up can be seen in figure 2-2.

Figure 2-2:A schematic of the fixed bed column system used by DE Bruin et al. (1992)

The electron donor was lactate which was added along with the PCE via the syringe pump. The initial PCE influent concentration was approximately 0.1mg/L and this was gradually increased to 1.5mg/L. The reactor was operated at temperatures of 20°C and 10°C, and a HRT of 6 hours.

A mixture of the anaerobic granular sludge and clean sand (3:1) was wet packed in a separate reactor and operated for 60 days at a PCE influent concentration of 0.08 mg/L and at an HRT of 24 hours. This was performed to determine the reductive dechlorination potential of the anaerobic sludge alone.

TCE, *cis*-1,2-DCE and vinyl chloride were the only compounds detected in the fixed bed reactor effluent after 2 weeks of operation. Concentration profiles at day 105 indicated that stepwise dechlorination of PCE was occurring via TCE, *cis*-1,2-DCE, VC and ethylene and that ethylene was reduced to ethane.

Between days 250 and 300 the column was run under steady state conditions and only ethane was detected in the effluent. This resulted in 95 to 98% of the PCE being recovered as ethane. The temperature was decreased to 10°C and within one month the concentration profiles were the same as those before the temperature drop. The elimination capacity of the reactor was reported as 3.7 $\mu\text{mol/L/h}$.

In the reactor containing only the ground anaerobic sludge and sand, only *cis*-1,2-DCE was identified and no VC, ethylene or ethane were detected in the effluent. This result would suggest that several microorganisms were required for the complete reductive dechlorination of PCE, and the combination of the two inoculums was necessary in this case to achieve total transformation of the PCE to harmless end products.

An upflow anaerobic sludge blanket (UASB) reactor was used to investigate the dynamic transformation of PCE, by Christiansen et al. (1997). The reactor was a 1 liter glass column inoculated with 115 ml of wet anaerobic granules. All materials used in the set-up were glass, stainless steel or Viton. During startup, the influent PCE concentration was 1 mg/L, and afterwards was varied between 5 and 10 mg/L. Ethanol was fed into the reactor as a primary substrate. Pulse experiments were conducted by stopping the influent flow of the substrate and mineral solutions and closing off the effluent port and then dosing the reactor with PCE, TCE or DCE. The disappearance of the PCE pulse (100 μmol) occurred with the simultaneous appearance of TCE and small amounts of DCE. PCE degradation rates were 134 $\mu\text{M/day}$ until the

concentration reached 10.7 μM , at which time PCE was degraded at a rate of 29 $\mu\text{M}/\text{d}$. Once almost all of the PCE had been transformed, the production rate of DCE increased to 0.6 $\mu\text{M}/\text{h}$ which corresponded to the rate of TCE degradation of 0.8 $\mu\text{M}/\text{h}$, indicating PCE was reductively dechlorinated to TCE and DCE.

The production of DCE was observed during TCE pulse experiments and a decrease in the concentration of TCE lead to a decrease in the production of DCE. A lag phase of 40 hours was observed during DCE pulse experiments at which time DCE was transformed at a rate of 0.02 $\mu\text{M}/\text{h}$. Transformation products for DCE degradation were not identified.

During normal continuous flow operations with PCE as the influent chlorinated organic, vinyl chloride and DCE isomers were detected just above the granular layer; however, no VC was seen in the reactor and only DCE isomers were found in the effluent. PCE transformation rates between 5.5 and 8.6 $\mu\text{mol}/\text{L-d}$ were observed when the respective influent concentrations were between 4.6 and 11.2 μM . The maximum PCE transformation rate of 36.8 $\mu\text{mol}/\text{reactor L-d}$ was observed at an influent concentration of 27 μM . The major transformation product was *trans*-1,2-DCE followed by *cis*-1,2-DCE. Acetate was the main organic component found in the effluent, indicating that the aceticlastic bacteria were inhibited by the chlorinated organics. Small amounts of methane were still detected verifying that some of the acetate was being degraded despite the inhibition of the aceticlastic bacteria.

The use of anaerobic sequencing batch biofilm reactors (AnSBBR) was investigated by Hirl and Irvin (1997) to examine the reductive dechlorination of PCE. Five AnSBBR's were periodically operated in an upflow mode. There were three periods to every cycle: fill, react and draw. The reactors were made from glass and were filled with acid washed pea gravel as a support media. All of the reactor components were made from either Teflon, Viton or glass, to minimize adsorption of the organic contaminant. Anaerobic sludge from a local wastewater treatment plant was used as inoculum. Each AnSBBR had a liquid volume of 1 liter, a headspace of 300 ml and an active bed volume of 400 ml. Operating conditions were 37°C and the process cycled over a 24 hour period. Draw, fill and react times were 5 minutes, 5 minutes and 23 hours 50 minutes respectively. Methanol, acetate, lactate and CO₂/H₂ were the four different electron donors tested for their ability to sustain a microbial consortium able to dechlorinate PCE. The PCE influent concentration was approximately 26 mg/L.

The results from this work showed that the reactors fed acetate and lactate were able to remove all of the PCE and only *cis*-DCE remained in the bulk liquid. The reactor fed methanol was never able to completely remove all of the PCE and both TCE and *cis*-DCE were observed in the bulk liquid.

Skeen et al. (1995) conducted experiments using a batch reactor specifically designed for monitoring process dynamics during biodegradation of volatile organics. The apparatus consisted of a 1 liter stainless steel vessel fitted with gas and liquid sampling ports. The biomass consisted of sediment taken from the Yakima River delta in southeastern Washington state and cultivated

anaerobically with methanol. The initial biomass concentration was 40 mg dry weight/L.

Separate dechlorination tests were conducted, with PCE, TCE, 1,1-DCE and vinyl chloride (VC) respectively. Average maximum specific dehalogenation rates for PCE, TCE, 1,1-DCE and VC were 0.9 ± 0.6 , 0.4 ± 0.1 , 12 ± 0.1 and 2.5 ± 1.7 $\mu\text{mol contaminant/g dry weight/day}$. In general, only the first dechlorination product was observed for each of these tests. A small amount of ethylene was observed by the end of the DCE-fed tests and ethane was observed in both vinyl chloride-fed experiments. Methane production and dechlorination activity were strongly correlated, indicating that biotransformation was mediated by methanogenic organisms. This was also supported by the fact that the addition of a specific methanogenic inhibitor, inhibited both methanogenic activity and the dechlorination of TCE and vinyl chloride.

Hollinger et al. (1993) isolated an anaerobic bacterium referred to as PER-K23 from an anaerobic packed bed column in which PCE was reductively dechlorinated to TCE, *cis*-1,2-DCE, VC and ethylene. Biomass for the anaerobic packed bed column originated from anaerobic sediment from the Rhine River (The Netherlands) and anaerobic granular sludge from a sugar refinery. Growth of PER-K23 was paralleled by the degradation of PCE and the formation of TCE to *cis*-1,2-DCE. H_2 and formate were the only two electron donors that supported growth with PCE or TCE acting as the electron acceptor. The cultures did not grow in the absence of PCE or TCE, even when O_2 , NO_3^- , NO_2^- , SO_4^{2-} ,

SO_3^{2-} , $\text{S}_2\text{O}_3^{2-}$, S or CO_2 were replaced as the electron acceptor. The isolated PER-K23 could not be assigned to a known group of anaerobic bacteria.

The work of Long et al. (1993) involved a sequential anaerobic-aerobic system that was employed to observe the degradation of 12 different chlorinated aliphatic compounds (CACs) including CF, CT, 1,1-DCA, 1,1,1-TCA, HCA, 1,1-DCE, T-1,2-DCE, TCE, PCE, 2-CP, 1,1-DCPE, and 1,2,3-TCP. One aspect of this research consisted of the operation of a continuously mixed, batch fed anaerobic reactor. The reactor consisted of a 20 liter glass bottle with a liquid volume of 16 liters. All connections were Viton, Teflon or stainless steel. Municipal anaerobic digester sludge was used as inoculum and the reactor was initially operated with an SRT of 100 days ($\text{HRT} = \text{SRT}$) and at a temperature of 24°C . The reactors were fed a substrate mixture of glucose, acetate, benzoate and phenol, each contributing 22% of the COD. The remaining 12% of COD was supplied as methanol which was used as a carrier for the chlorinated organics. During testing, the CAC influent concentrations were $120\text{ }\mu\text{g/L}$ and the SRT was decreased to 53 days.

The average influent and effluent CAC concentrations are shown in table 2-3.

COMPOUND	INFLUENT CONC. ($\mu\text{g/L}$)	EFFLUENT CONC. ($\mu\text{g/L}$)
Carbon Tetrachloride	120	nd**
Chloroform	120	21
1,1-Dichloroethane	120	37
1,1,1-Trichloroethane	120	1
Hexachloroethane	120	1
1,1-Dichloroethene	120	2
trans-1,2-Dichloroethene	120	21
2-Chloropropene	120	21
1,1-dichloropropene	120	16
1,2,3-Trichloropropene	120	12
Perchloroethylene	120	3
Trichloroethylene	120	2
cis-1,2-Dichloroethylene	0	10
Vinyl chloride	0	detected*
Dichloromethane	0	2

** not detected, detectable levels were $>1\mu\text{g/L}$. * not quantifiable

Table 2-4: Results from batch fed, anaerobic reactor tests, Long et. al., 1993

These results indicated that carbon tetrachloride and perchloroethylene could be effectively removed, and the presence of vinyl chloride and dichloromethane support the observation that reductive dechlorination of these compounds was occurring.

Hughes and Parkin (1996) described the operation of attached growth systems run in continuous upflow mode, with feed supplied through a syringe pump. The reactors were 40 cm long with an inside diameter of 2 cm, and were filled with 5 mm glass beads. The reactors were seeded with a culture from an anaerobic reactor that had been fed chlorinated organics for over two years. The influent solutions came in contact with only glass or Teflon.

One of the reactors was initially fed only chloroform and eventually chloroform and DCM were fed together. The transformation of DCM was rapid, and 100 % removal was observed. This complete removal might be explained by the acclimation of the biomass to DCM, through the reductive dechlorination of chloroform.

Table 2-5 presents a summary of the reactor studies discussed in this section.

Table 2-5: A summary of the results from previous reactor studies using mixed cultures.

REFERENCE	COMPOUND	SUBSTRATE	BIOMASS SOURCE	RESULTS
Vogel and McCarty	PCE (300µg/L)	acetate	methanogenic conditions had been established over a number of years	-detection of TCE, DCE and VC -complete removal of VC not observed
DE Bruin et al., 1992	PCE (0.1 - 1.5 mg/L)	lactate	river sediment and ground anaerobic sludge	-only TCE and DCE detected after 2 weeks -complete removal of VC and accum. of ethane
Christiansen et al, 1997	PCE (5-10 mg/L)	ethanol	wet granules	- <i>trans</i> -1,2-DCE was main transformation product
Hirl and Irvin	PCE (26mg/L)	methanol, acetate, lactate, CO ₂ /H ₂	anaerobic sludge from local wastewater treatment plant	-acetate and lactate removed 100% PCE, only DCE remained -PCE, TCE and DCE remained when methanol was used
Skeen et al., 1995	PCE, TCE, DCE, VC	methanol	river sediment	-generally, only first dechlorination product observed

Table 2-5 continued.

REFERENCE	COMPOUND	SUBSTRATE	BIOMASS SOURCE	RESULTS
Long et al., 1993	CT, CF, PCE, TCE DCE	glucose, acetate, benzoate, phenol and methanol	municipal anaerobic digester sludge	-complete removal of CT -almost complete removal of PCE, TCE, DCE -detection of VC and DCM
Hughes and Parkin, 1996	DCM, CF	acetate	anaerobic culture that had been fed chlorinated organics for over a year	-100% removal
Hughes and Parkin, 1992	DCM (2 and 5mg/L)	acetate	biomass from laboratory anaerobic reactor periodically fed chlorinated organics	no transformation observed

2.3. DISCUSSION

The results from this review of the literature were encouraging with respect to the reductive dechlorination of PCE. It seems reasonable to expect the transformation of PCE by a mixed anaerobic methanogenic consortia in both batch and continuous flow reactor types. The major concern will be whether or not PCE can be completely degraded to harmless end products, or will more toxic intermediates such as vinyl chloride persist.

It is conclusive from the majority of researchers that PCE can be sequentially reduced to TCE, DCE, vinyl chloride, and in some cases ethylene and ethane, under anaerobic conditions. The extent of PCE transformation and the production of its breakdown products varied among the different researchers.

Freedman and Gossett (1989) reported the dechlorination of PCE to ethylene in batch tests, but the intermediate vinyl chloride was never totally eliminated. Similar results were obtained by DiStefano et al. (1991). In this case, high enough concentrations of PCE were used such that methanogenic activity was terminated. PCE was routinely transformed even under non-methanogenic conditions and, vinyl chloride continued to persist. The only report of complete dechlorination of PCE in batch tests to acceptable end products was presented by DE Bruin et al. (1992). Ethylene was the only end product found in serum bottles after a 16 day test period.

In each of the above cases, a mixed consortium of anaerobic bacteria was used. Freedman and Gossett (1989) and DiStefano et al. (1991) obtained biomass from a laboratory anaerobic digester whereas DE Bruin et al. (1992)

used a mixture of river sediment and granular anaerobic sludge. Methanol was utilized by Freedman and Gossett (1989) and DiStefano et al. (1991) as the electron donor while DE Bruin et al. (1992) utilized lactate. Perhaps the combination of a more varied biomass along with the use of lactate were the factors that contributed to the complete removal of vinyl chloride in the work done by DE Bruin.

In continuous flow studies, PCE degradation results were similar to those found in batch experiments. Vogel and McCarty (1985) were able to attain approximately 99% removal of PCE in a continuous flow reactor, but complete removal of VC was never achieved. The concentration of VC initially increased, but decreased once PCE had disappeared, indicating that some of the VC was being transformed. DE Bruin et al. (1992) were more successful and demonstrated the complete reduction of 1.5 mg/L of PCE in a continuous flow column, to final end products of ethylene and ethane. The origin of biomass in the reactors run by Vogel and McCarty (1985) was not specified, but it was noted that the reactors had been in operation for several years, periodically treating different organic waste streams. The biomass in the reactor study conducted by DE Bruin et al. (1992) consisted of a combination of river sediment and anaerobic granular sludge. One assumption for the difference in end products between these two studies is that the inoculum used by DE Bruin et al. (1992) may have contained a greater, more diverse assortment of bacteria than that used by Vogel and McCarty (1985). This more diverse group of microorganisms was effective at completely degrading PCE to harmless end products, whereas the

older biomass, more specialized from treating specific waste streams, used by Vogel and McCarty (1985) did not contain the necessary microorganisms to carry the degradation of PCE to completion.

Support for this assumption is given by DE Bruin et al. (1992) with results from a column study containing only the ground anaerobic sludge and clean sand without river sediment. Only *cis*-1,2-DCE was found in the effluent (with PCE as the influent organic contaminant) and no vinyl chloride or ethylene were detected, leading one to the assumption that not all of the microorganisms were available for the complete removal of PCE to acceptable end products. Only the combination of the anaerobic sludge and river sediment provided the necessary assortment of microorganisms.

Hollinger et al. (1993) isolated a specific anaerobe from a mixed culture and was able to show its affinity for PCE and TCE as an electron acceptor. This finding indicated that specific bacteria can become highly selective with respect to the conditions under which they will obtain energy to survive. A mixed culture will have a greater probability of becoming acclimated to a chlorinated waste stream, due to a large population of microorganisms, each requiring specific conditions to exist. Microorganisms with an affinity for a chlorinated organic will thrive when that compound is present, and hence the biomass will be effective at degrading that waste.

Another possible factor contributing to the difference in end products in the work conducted by DE Bruin et al. (1992) and Vogel and McCarty (1985), was the length of time over which the experiments were run. DE Bruin et al (1992)

reports results in the reactor after 105 days of operation, whereas Vogel and McCarty (1985) give concentrations after 22 days of reactor operation. It has been observed that the rate limiting step of PCE degradation is that of VC to ethylene (Freedman and Gossett, 1989). Therefore, it may be reasonable to assume that the vinyl chloride detected in Vogel and McCarty's (1985) reactor may have eventually been reduced to ethylene, had a longer acclimation time been permitted.

The effect of the electron donor type on PCE degradation is unclear. DCE isomers were present in an UASB reactor effluent operated by Christiansen et al. (1997), when ethanol was introduced as the auxiliary substrate. *Trans*-1,2-DCE was the major isomer present. AnSBBR's were able to reductively dechlorinate PCE to *cis*-DCE when acetate and lactate were the primary electron donors (Hirl and Irvin, 1997). When methanol was used, not all of the PCE was removed and both TCE and *cis*-DCE were observed. Methanol was used in experiments conducted by Skeen et al. (1995), and in general only the first dechlorination product was observed for individual tests run with PCE, TCE, DCE and VC. Lactate was used by DE Bruin et al. (1992) in experiments that resulted in ethylene and ethane being the only detected compounds in the effluent. A mixture of carbon sources, including glucose, acetate, benzoate, phenol and methanol was used by Long et al. (1993). Almost complete removal of PCE, TCE and CT resulted, but VC and DCM were found in the effluent. H₂ and formate were the only two electron donors that supported growth of the specific bacterium, PER-K23, with TCE and *cis*-1,2-DCE being the only two

transformation products of the electron acceptor, PCE (Hollinger et al., 1993). Methanol, lactate, acetate, glucose, benzoate and phenol were among those used as substrates in batch tests performed by the different researchers. Except in the case of lactate, where only non-chlorinated end products were found in the bottles (DE Bruin et al., 1992), one electron donor did not appear to out perform any others.

Fennell and Gossett (1997) investigated the effects of different electron donors on the dechlorination of PCE. Butyric acid, ethanol, lactic acid and propionic acid all performed approximately the same during long term tests, transforming PCE to vinyl chloride and ethylene. In short term tests, butyric acid and propionic acid faired better in transforming PCE. Due to the wide range of electron donors used in the various studies and the variation among the different results it is difficult to draw any conclusions regarding the best source of an electron donor.

There has been considerable research conducted to determine the role of methanogens in the reductive dechlorination of PCE. Fathepure and Boyd (1988) and Freedman and Gossett (1989) were able to significantly reduce PCE transformation with the use of a specific methanogenic inhibitor. Specific, isolated methanogenic cultures were shown to dechlorinate PCE to TCE. These results suggest that methanogenic bacteria play a key role in the reductive dechlorination of PCE. However, further findings indicate that other microorganisms may be responsible, or aid in the transformation of PCE. DiStefano et al. (1991) described the degradation of PCE under non-

methanogenic conditions. Non-methanogenic conditions were observed when the dosing concentrations of PCE were above 55 μmol , presumably causing an inhibitory effect. Significant amounts of acetate were detected in the bottles, suggesting that acetogens have a role in PCE's degradation.

The literature indicates that sequential reductive dechlorination of carbon tetrachloride to chloroform and dichloromethane commonly occurs in anaerobic environments. Much work investigating specific bacterial cultures has been conducted. Egli et al (1987), Galli and McCarty (1989), Criddle et al (1990), and Petrovskis et al. (1994) were all able to use specific anaerobic microorganisms to achieve transformation of carbon tetrachloride in batch type experiments. Both chloroform and dichloromethane were the final end products in the case of Egli et al.(1987), and Galli and McCarty (1989). Chloroform appeared in the largest quantity and low levels of dichloromethane were detected. Dichloromethane was the only identified chlorinated organic in the case of a specific bacterium isolated to dechlorinate PCE, by Egli et al.(1988); but only 10% of the original carbon tetrachloride was recovered as DCM. It was determined that a high percentage of the end product was CO_2 . Criddle et al. (1990) and Petrovskis et al. (1994) also observed the presence of CO_2 as a breakdown component of carbon tetrachloride. In all of these cases, the complete degradation of carbon tetrachloride took place, but its lower chlorinated transformation products continued to persist. The high percentage of CO_2 produced in some of the degradation tests is encouraging, since it is a non-toxic, environmentally acceptable end product. Since these tests were conducted using only specific

strains of anaerobic bacteria, it seems reasonable to assume that a more diverse population of microorganisms may be more effective at degrading these less chlorinated organics.

Doong and Wu (1995), and Long et al. (1993) both used an anaerobic mixed culture to demonstrate the biodegradation of carbon tetrachloride in batch type experiments. Complete transformation of the carbon tetrachloride took place in both cases, and chloroform was the only breakdown component accounted for by Doong and Wu (1995). The only case where no chlorinated organics were detected as end products was reported by Long et al (1993). DCM was detected as an intermediate, but eventually disappeared. Once again, the mixed culture of anaerobic microorganisms was more effective at transforming the chlorinated organic to desirable end products.

In continuous flow studies conducted by Long et al. (1993) carbon tetrachloride was completely removed from the influent, but chloroform and dichloromethane were both detected in the effluent. This may have been due to the presence of a number of other chlorinated compounds that were present in the feed solution. A competitive type environment may have evolved, whereby the rate and extent of dechlorination would be decreased. This phenomenon was demonstrated by Long et al. (1993) with the use of PCE batch tests. The degradation rate of PCE was significantly increased in bottles when it was the sole chlorinated organic, compared to the rate in bottles where other chlorinated compounds were present.

A variety of electron donor sources were used among the different researchers when testing the biodegradation of CT. A mixture of glucose, phenol, benzoate, acetate and methanol was used by Long et al. (1993), therefore it was not possible to make any conclusions regarding an optimal electron donor. Doong and Wu (1995) experimented with glucose, acetate, and humic acid, and concluded that acetate was the better of the three, with respect to the rate and extent of carbon tetrachloride removal.

The application of DCM as a growth substrate was a significant finding by Freedman and Gossett (1991). Breakdown components of the DCM were determined to be CO_2 and CH_4 when methanogenic conditions existed. Under non-methanogenic conditions acetic acid was the main product formed, leading to the conclusion that acetogens were responsible for the degradation of the DCM. Under methanogenic conditions the acetate was converted to CH_4 by methanogens.

Hughes and Parkin (1992) observed the transformation of DCM both alone and in a mixture with other chlorinated organics, with acetate supplied as the electron donor. However, in a following study the transformation of DCM was not observed (Hughes and Parkin, 1996). The setup and method for batch tests were similar in each case, including inoculum and electron donor. In continuous flow studies, Hughes and Parkin (1992) were able to attain 100% removal of DCM, once the biomass had been acclimated to DCM, through the reductive dechlorination of chloroform in the feed. The biodegradation of DCM

seems to be feasible, although it does not appear to be as readily susceptible to transformation as PCE or carbon tetrachloride.

2.4. CONCLUSIONS

The use of an anaerobic mixed culture to transform PCE, CT and DCM in a continuous flow reactor has been shown to be an effective method for the removal of these potential carcinogenic organics from a liquid stream. Reductive dechlorination of PCE and CT produces lower chlorinated organics, and in some cases non-chlorinated end products have been produced. DCM appears to be less susceptible to degradation, compared to PCE and CT, and degradation was observed in only a few of the studies.

One interesting aspect of the literature was the effect on dechlorination rates when more than one chlorinated organic was present in the solution. In most cases each chlorinated compound was studied individually. The research that will be carried out in this study will employ a mixture of the 3 specified compounds. Slower dechlorination rates may be expected for these compounds as compared to the rates that would be expected if they were treated separately.

The importance of a diverse assortment of microorganisms has been demonstrated. An anaerobic granular mixed culture obtained from a pulp and paper treatment facility will be used for research, and it is expected to be a suitable biomass source.

No conclusions were made with respect to electron donors, although both methanol and acetate were most commonly used.

The rate limiting step of PCE degradation is the reduction of vinyl chloride to ethylene. Enough time must be granted in order to ensure this step is carried to completion, and vinyl chloride does not persist in effluent streams.

The viability of transforming PCE, CT and DCM via reductive dechlorination in an anaerobic environment has been established. This treatment method was the focus of this research. A review of the literature supports the idea that anaerobic biological degradation of these toxic compounds can be anticipated to be a successful treatment process.

3. ANALYTICAL & EXPERIMENTAL METHODS

3.1. ANALYTICAL METHODS

3.1.1. *VOC Measurement / GC Method*

An HP 5890 gas chromatograph (GC) equipped with an electron capture detector (ECD) was used for the measurement of the chlorinated organic compounds. The GC was equipped with a DB624 capillary column that was 30 meters long and had an internal diameter of 0.33 millimeters. The ECD was operated at 300°C and the injector port temperature was 200°C. The GC operating conditions were as follows:

Initial Temperature	30°C for 5 minutes
Rate increase	10°C/minute
Final Temperature	35°C
Final Time	4 minutes
Rate increase	20°C/minute
Final Temperature	120°C
Final Time	0 minutes

Under these conditions the following elution times were determined for the target compounds as well as their hypothesized metabolic breakdown compounds:

VC	1.4 minutes
DCM	3.4 minutes
DCE	2.7 minutes

CF	6.8 minutes
CT	7.7 minutes
TCE	10.5 minutes
PCE	13.5 minutes

Detection limits for the three target compounds were determined to be 0.001, 0.001 and 0.1 mg/L for PCE, CT and DCM respectively.

In this research, liquid and gas phase samples were analyzed for the target compounds. Initially, liquid methanol samples were prepared to generate standards for gas phase analysis. Mass recovery problems were encountered with this method and a headspace equilibrium approach was then selected for the analysis of liquid and gas samples. Prior to analyzing samples, calibration curves and detector linearity tests were determined for each of the target compounds. A known initial liquid concentration was prepared in a 160 ml glass bottle, filled to the 100 mL mark, and sealed with a Teflon coated butyl rubber septa and aluminum crimp cap. Initial liquid concentrations ranged from 0.001 mg/L to 10 mg/L for PCE and CT and 0.01 mg/L to 100 mg/L for DCM. The bottles were placed in a shaker at 35°C for 24 hours. A 50 µl gas headspace sample was drawn, using a Hamilton gas tight glass syringe, and injected into the 5890 GC. Three samples from each bottle were taken and the average gas concentration was plotted against GC counts, producing a standard curve for each compound. Standard curves for each compound can be found in Appendix C.

3.1.2. Methane Measurement / GC Method

A 5710 A Hewlett Packard GC was used to determine methane content in the biogas from the anaerobic process. The GC used a 3.05 m by 0.1 cm O. D. Porapak T 50/80 column. The detector temperature was 150°C and the injection port temperature was 100°C. The elution time for methane was approximately 2.4 minutes. Carbon dioxide and nitrogen were also detected, eluting at 6.3 and 1.9 minutes respectively.

A 0.5 ml gas sample was taken from the anaerobic process with a plastic syringe and was injected into the 5710A GC.

3.1.3. Chemical Oxygen Demand Measurement

Chemical Oxygen Demand (COD) analysis was conducted according to Method 5220 D. Chemical Oxygen Demand (COD) Closed Reflux, Colorimetric Method (APHA, AWWA, WEF, 1992).

3.1.4. Volatile Suspended Solids Determination

A 40 ml sample was drawn from a well mixed sample and poured into a pre-weighed crucible. The sample was dried over night in a Precision Scientific Group mechanical convection oven set at 105°C. Total suspended solids (TSS) was determined and the sample was then combusted in a 62700 Thermolyne muffle furnace at 550°C for 30 minutes. The mass difference between the

sample after being dried and after being combusted was the mass of the volatile suspended solids (VSS).

3.2. EXPERIMENTAL METHODS

3.2.1. *Anaerobic Serum Bottle Batch Tests*

Experiments employing batch anaerobic serum bottles were conducted to determine which cosubstrate was most effective in enhancing the biodegradation of the three target chlorinated organics. All serum bottle tests were conducted using 160 ml glass serum bottles that were sealed with Teflon coated butyl rubber septa and aluminum crimp caps.

A series of preliminary batch tests were performed before a final technique was established and ideal operating conditions were arrived at. In the first test, 8 bottles were employed to allow duplicate evaluation of methanol, ethylene glycol, propylene glycol and ethanolamine as the carbon source. The cosubstrate concentration in each bottle was 0.1% v/v. Each bottle was inoculated with 10 mL of sludge and 50 mL of supernatant taken from an anaerobic acclimation reactor which is subsequently described. The bottles were purged with nitrogen gas for approximately 2 minutes and sealed with a rubber septum and aluminum crimp cap. A mixture of PCE, DCM and CT was then added to each bottle so that an initial liquid concentration of 1 mg/L for each compound was obtained. Bottles were inverted in a G24 Environmental Incubator Shaker at a constant temperature of 35°C.

Methane gas production and VOC headspace concentrations were observed over a period of 8 days. The total biogas volume produced was determined using a manometer (see figure 3-1). A syringe was inserted into the septa on the serum bottle and for each 1 cm of liquid displaced in the glass tube, by the pressure in the serum bottle, 1 mL of gas production in the serum bottle was recorded.

Figure 3-1: A schematic of the manometer set-up for the determination of gas production in the serum bottles.

Fifty μL headspace gas samples were injected into the 5890 GC with a glass syringe and the liquid concentration of target VOC compounds was calculated using the previously described calibration curves. The methane

content of the headspace was determined by injecting a 0.5 mL headspace gas sample with a 1 mL plastic syringe into the 5710A GC.

In this experiment DCM was not detected (no peaks appeared on the GC output), CT disappeared after one day and the PCE concentration in the bottles was inconsistent. The average methane content was approximately 50 to 60 percent. Due to the inconsistency of the PCE concentrations these initial results were not considered reliable and this led to a second set of serum bottle tests which incorporated modifications to the experimental method.

The headspace in the second set of serum bottle tests was decreased to 60 ml by increasing the volume of liquid in the bottle to 90 ml of supernatant plus 10 ml of anaerobic granular sludge. The concentrations of cosubstrates and VOC's and the procedure for determining the methane production and VOC concentrations were consistent with that employed in the first set of tests. The results of the second test again demonstrated non-detectable concentrations of DCM and inconsistent values of PCE.

The DCM concentration was increased to 10 mg/L, the PCE concentration was decreased to 0.5 mg/L and the biomass concentration was decreased to 5 mL, to reduce the impact of any adsorption effects, in a third set of serum bottle tests. The results from this serum bottle test run were preliminary ones and are given in Appendix D.

The preliminary batch tests led to the final methodology for the preparation of the serum bottle batch tests. Serum bottles were spiked with initial concentrations of 0.5, 1 and 10 mg/L of PCE, CT and DCM. The granular

sludge volume was 5 mL and the supernatant volume was 95 mL, resulting in a VSS concentration of 1.95 g/L. A cosubstrate concentration of 0.4% was used in each serum bottle to ensure a sufficient amount of carbon source. Ethylene glycol, propylene glycol and methanol were the 3 cosubstrates considered, since ethanolamine had been eliminated due to poor degradability in the preliminary set of serum bottle tests. Two blanks were also run to observe any abiotic losses from the serum bottles. The final serum bottle set-up can be seen in figure 3-2.

Figure 3-2: Final serum bottle set-up for batch tests

To determine if there was an effect on the biomass activity in the presence of the VOC's an additional 3 serum bottles were prepared as described above, with the exception of the addition of any VOC's. Methane production was

used as an indication of biomass activity. The experimental set-up for the serum bottle tests is given in table 3-1. VOC concentrations and methane volumes were collected over a 68 day period.

Bottle #	Cosubstrate	VOC's	Biomass
60 & 61	methanol	yes	yes
62 & 63	ethylene glycol	yes	yes
64 & 65	propylene glycol	yes	yes
66 & 67	no	yes	no
68	methanol	no	yes
69	ethylene glycol	no	yes
70	propylene glycol	no	yes

Table 3-1: Experimental set-up for serum bottle test.

3.2.2. *Acclimation Anaerobic Reactor*

An anaerobic reactor was employed to support the operation of the batch serum bottles and future continuous flows systems by acclimating anaerobic granular biomass to the 4 cosubstrates and the target VOC's. A glass reactor with an approximate volume of 15 liters was filled with 8 liters of granular anaerobic biomass obtained from the Lake Utopia Paper Mill in New Brunswick. It was believed that the sludge from a paper mill would have had previous exposure to chlorinated aromatics, and therefore be more acclimatized to the compounds observed in this study, than sludge from a waste water treatment facility. A feed solution of 5000 mg COD/L was pumped from a refrigerator into the reactor at a rate of 2 ml/min with a Master Flex pump, resulting in a HRT of 8

days. The 5000 mg COD/L feed solution consisted of 1250 mg COD/L from each of methanol, propylene glycol, ethylene glycol and ethanolamine. The following nutrient mixture was fed at 1.3 g per liter of influent and was mixed with the feed solution to obtain a 1000:7:1 ratio COD to nitrogen to phosphorus.

700 g NaHCO_3
180 g NH_4HCO_3
20 g K_2HPO_4 ,
0.1g yeast extract

Ten milliliters of a 1000mg/L PCE, CT and 10000mg/L DCM methanol solution was injected into the influent line weekly. The acclimation reactor was operated under these conditions throughout the duration of experiments, approximately 14 months.

3.2.3. *Continuous Flow Anaerobic Column*

3.2.3.1. *Column Materials and Schematic*

A 5 L cylindrical glass upflow anaerobic sludge blanket (UASB) reactor, with an inside diameter of 11.7 cm and a height of 40 cm, was started up using anaerobic granular biomass and supernatant from the acclimation reactor. The anaerobic reactor was inoculated with approximately 2.75 L of biomass taken from the acclimation reactor previously described. The VSS content of the biomass was 33.9 g VSS/L, resulting in 93 g VSS in the reactor. All tubing on the reactor was Viton and all connectors were made of either glass or stainless steel.

A Masterflex Easy-load variable speed pump pumped a nutrient mixture into the bottom of the reactor. Supernatant within the reactor was recycled at a rate of 86 ml/minute using a Masterflex pump. A Masterflex pump connected to a ConTrol timer determined the flow rate of the VOC and cosubstrate mixture into the anaerobic reactor.

A 0.5 L Tedlar bag was used to hold a solution of propylene glycol, VOC's and water. The ratio of this solution varied depending on the desired influent concentrations. A nutrient mixture was prepared for each run with a COD:N:P ratio of 1000:7:1 according to the following recipe:

69 g NH_4HCO_3
5 g K_2HPO_4
4 g KH_2PO_4
0.1g yeast extract

Seventy eight grams of the above nutrient mixture was added per liter of propylene glycol in the feed along with 3 g/L feed of sodium bicarbonate to act as a buffer.

Methane gas accumulated and exited at the top of the reactor and was measured by a wet-tip gas meter. Each tip of the meter indicated 80 mL of biogas production. A U-tube was placed on the liquid effluent line to force the gas to exit through the wet-tip meter and not through the unmonitored effluent line.

The temperature of the reactor was maintained at 35°C throughout all experiments. A GCA Precision Scientific hot water bath heated water to the selected temperature and a Cole Palmer centrifugal pump forced the heated

water through tubing that was wrapped around the column. The biomass took on the same temperature as the hot water bath. The following schematic depicts the anaerobic reactor set-up.

Figure 3-3: Anaerobic column set-up

3.2.3.2. *Sampling Procedure*

For each sampling event two 1 mL and one 2 mL samples of the influent and effluent were collected in 2 mL glass vials and immediately sealed with Teflon lined septa and aluminum crimp caps for VOC analysis. The influent samples were collected while the propylene glycol/VOC pump was on. The effluent samples were collected from the bottom of the U-tube and one drop of a 80 mg/L copper sulfate solution was added to inhibit any biological activity in the sample. The four samples with 1 mL of headspace were inverted in a Psycrotherm Controlled Environment Incubator Shaker set at 35°C and 150 RPM. It was determined that equilibrium conditions between the liquid and gas phases, for all of the target compounds, were reached after four hours (Collins, 1998). Therefore, a 50 µl headspace sample was injected into the 5890 GC at least 4 hours after the sample was placed on the shaker. The 2 mL samples were inverted and refrigerated for future analysis if necessary.

Approximately 5 mL of influent and effluent were collected directly from their respective sample ports for the purpose of COD analysis. The effluent sample was centrifuged in a RC5C Sorvall Centrifuge set at 10 000 RPM for 5 minutes to remove any suspended solids. Samples were acidified with sulfuric acid and frozen until COD analysis was performed.

Two 40 mL effluent samples were taken for VSS analysis. The wet-tip meter was monitored daily and a 0.5 mL headspace sample was taken regularly to determine methane content. A 50 µl reactor headspace sample was also

taken to determine VOC concentrations. The pH of the reactor was regularly monitored.

3.2.3.3. *Experimental Plan*

The experimental runs were divided into two categories, a high end and a low end category. The high end runs were described by relatively high organic influent concentrations (3.7-5.5 g COD/L*d) and long HRT's (≥ 2 d). Low end runs were conversely described by low organic influent concentrations (0.4-2.0 g COD/L*d) and short HRT's (≤ 2 d). The experimental plan was designed in this manner in order to examine the two extremes of process operation. The VOC's were added to the reactors in a mixture. The following table presents the operating conditions for all of the runs that were completed.

	<u>RUN 1</u>	<u>RUN 2</u>	<u>RUN 3</u>	<u>RUN 4</u>	<u>RUN 5</u>	<u>RUN 6</u>	<u>RUN 7</u>	<u>RUN 8</u>	<u>RUN 9</u>	<u>RUN 10</u>	<u>RUN 11</u>	<u>RUN 12</u>	<u>RUN 13</u>	<u>RUN 14</u>	<u>RUN 15</u>
Reactor Volume (L)	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Biomass (g/L)	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
Influent (ml/min)	0.87	1.8	1.8	1.8	1.8	3.5	3.5	3.5	7	7.0	10.5	14.0	28.0	14.0	14.0
Prop.Gly./VOC pump on (min/d)	150	200	150	150	200	150	840	840	840	150	150	150	150	150	150
Avg. COD influent conc. (g COD/L)	23.1	14.8	11.1	11.1	7.5	1.0	1.0	0.5	1.0	0.2	0.2	0.2	0.2	0.1	0.5
Avg. Organic loading (g COD/L*d)	6	8	6	6	4	1	1	1	2	0	1	1	2	0	2
HRT (d)	4.0	1.9	1.9	1.9	1.9	1.0	1.0	1.0	0.5	0.5	0.33	0.25	0.12	0.25	0.25
Recycle rate	86	86	86	86	86	86	86	86	86	86	86	86	86	86	86

Table 3-2: Experimental Set-Up

4. RESULTS & DISCUSSION

4.1. DATA QUALITY

Measures were taken in all cases to ensure the highest data quality possible, including duplicate sampling and GC injections. Nevertheless, due to the properties of the compounds, detector sensitivity and the nature of the headspace method that was used to analyze samples, variability in data was observed. The following section describes the data quality prior to the discussion of the experimental results.

4.1.1. VOC Standard Curves

To prepare standard VOC curves, three headspace samples from each serum bottle were analyzed with the GC. Sample counts were averaged and the average count was plotted against initial liquid VOC concentration to produce a standard curve for the particular compound. Confidence in these results was fairly high as indicated by the high coefficient of determination (R^2) value for each curve. PCE, CT and DCM curves all produced a 1.0000 R^2 value. The R^2 value for TCE and CF were slightly less at 0.9997 and 0.9995 respectively, however still high enough to have confidence in the standard curves. These curves are presented in Appendix C.

4.1.2. Serum Bottle Test

In the serum bottle test, two bottles for each condition were prepared in exactly the same manner as described earlier in section 3.2.1. Blanks were also prepared to determine the loss of VOC's from the bottle in the absence of any biological activity. In this test, two 50 μ l headspace samples from each bottle were taken and injected into the GC for each sample period. These two sample injections were then averaged and used to determine the bottle concentration.

Gas phase standard curves were used to calculate the total mass in each bottle. The total mass of the target compound in the serum bottle was calculated by adding the VOC mass in the liquid to the VOC mass in the gas, according to the following equation:

$$M_{\text{TOTAL}} = V_{\text{gas}}C_{\text{gas}} + V_{\text{liquid}} (C_{\text{gas}}/H) \quad (\text{Equation 4-1})$$

Values for Henry's Constant were taken from LaGrega (1994) and converted to the appropriate value for a temperature of 35°C, according to the following equation:

$$H = \exp(A - B/T) \quad (\text{LaGrega, 1994}) \quad (\text{Equation 4-2})$$

where:

A and B are compound specific constants

T = Temperature (K)

The mass of each VOC over time was observed in blank serum bottles to indicate any abiotic activity in the bottles. Blank serum bottle results are presented in figures 4-1 to 4-3.

Figure 4-1: Mass of PCE in blank serum bottle versus time

Figure 4-2: Mass of CT in blank serum bottle versus time

Figure 4-3: Mass of DCM in blank serum bottle versus time

The results from the blank serum bottles show that an insignificant amount of mass was lost over time and therefore any VOC disappearance in the biological serum bottles could be attributed to biological activity.

The averages and standard deviations for each compound's total mass in the serum bottle for each sample day can be seen in Appendix A, tables I to III. The coefficient of variance (COV) was calculated to indicate the magnitude of the error.

The target initial masses of PCE, CT and DCM in the serum bottles were 0.3 μmol (0.5 mg/L), 0.65 μmol (1.0 mg/L) and 11.8 μmol (10 mg/L) respectively. Table 4-1 shows the percent of the measured VOC mass to the target mass.

	PCE	CT	DCM
Methanol Bottle 1	206	72	83
Methanol Bottle 2	86	34	55
Ethylene Glycol Bottle 1	174	107	83
Ethylene Glycol Bottle 2	155	108	38
Propylene Glycol Bottle 1	52	23	55
Propylene Glycol Bottle 2	111	36	53

Table 4-1: % of measured VOC mass in serum bottle to target mass of VOC

The measured initial mass of each VOC in the serum bottles were within the same magnitude as the target masses. In the ethylene glycol serum bottles the initial mass of PCE was as much as 74% higher than target values. In the methanol and propylene glycol bottles the initial mass of PCE was as much as 106% higher and 48% lower than target values. The mass of CT was as low as 23% of the target value in one bottle, but was only 8% higher in another bottle. Differences in target and measured values of DCM were between 38 and 83%. The differences in the target and measured VOC masses were attributed to the difficulties with introducing the compounds into the bottle.

The first sample was taken after only 1 hour and it was possible that the compounds had not yet reached equilibrium between the liquid and gas phase. There were apparent increases in some of the PCE and DCM masses and they may have been attributed to a lack of equilibrium conditions at the first sampling period. If equilibrium conditions did not exist when samples were taken, not all of the mass would have been accounted for, resulting in apparent masses lower

than actual masses. DCM increases may also have been a result of the breakdown of CT.

The low measured initial masses for CT were believed to have been a result of its relatively rapid degradation, which appeared to occur within the first hour.

The largest COV of the total mass for PCE was 32%, which was observed in bottle 64 on day 34. In bottles 61 and 64 on day 22, the COV for DCM mass was larger than 100% and this occurred on the day when DCM was detected for the last time. CT mass standard deviations were generally higher than those of DCM and PCE, but a closer examination of the higher COV's for each of the compounds revealed that the larger errors occurred when the concentrations were relatively small. It was believed that as the compound concentrations approached the analytical detection limits, there was an increased error because of a higher amount of variability present in the analytical method at lower VOC concentrations. In general, the reproducibility of the data among GC injections was acceptable with the average COV for the VOC mass in each bottle below 12% for PCE, 21% for CT and 38% for DCM. The high average COV for DCM mass was a result of two high COV's in bottles 61 and 64 when detection limits were approached. When these high COV's were not considered the average COV for DCM was below 15%, resulting in average COV's below 25% for all compounds.

Results from the serum bottle batch test with respect to data variability were considered acceptable and satisfactory for making conclusions regarding the best cosubstrate to be used in the continuous flow study.

4.1.3. Continuous Flow Study - Runs 2-5

The continuous flow tests were divided into two separate categories based upon HRT and OLR. The first group of runs were operated at an HRT of 2 days and organic loading rates (OLR) between 3.7-5.5 g COD/L*d. A second set of runs were operated at lower HRT's, varying from 24 to 3 hours and lower OLR's, varying from 0.4 to 2 g COD/L*d. The HRT's and OLR's were varied in this fashion to study the relatively large range of conditions that might be employed in the anticipated application. Low end runs (short HRT and low OLR) were used to determine the viability of the process under conditions that would have the lowest capital and operating costs.

Sampling did not begin until the reactor had run for a minimum of three HRT's, at which time it was assumed that steady state conditions had been reached. Figure 4-4 shows typical influent and effluent data obtained over a three day period, after 3 HRT's had passed (data shown for run 8).

Figure 4-4: Typical data showing steady state conditions in the UASB reactor after 3 HRT's.

Figure 4-4 indicates the data obtained from the UASB reactor over the 3 sampling days did not vary significantly suggesting that 3 HRT's was sufficient time for conditions in the reactor to reach steady state.

Tables 4-2 to 4-4 show the results and variability for the initial set of steady state continuous flow runs in the upflow anaerobic sludge blanket reactor. The initial set of runs included those with a long HRT and high cosubstrate loading. Note that there is no data given for run #1 since the reactor failed before sampling could be completed.

Table 4-2: Summary of PCE data for continuous UASB runs 2-5 (HRT 1.9 d, ORL 3.7-5.5 g COD/L*d)

RUN #	DAY	AVG. CONC. IN (mg/L)	STD. DEV.	COV	AVG. CONC. OUT LIQUID (mg/L)	STD. DEV.	COV	AVG. CONC. OUT GAS (mg/L)	STD. DEV.	COV
2	1	0.25	0.13	54	nd*	0	0	nd	0	0
2	2	0.08	0.03	36	nd	0	0	nd	0	0
2	3	0.12	0.03	21	nd	0	0	nd	0	0
3	1	0.31	0.17	55	nd	0	0	nd	0	0
3	2	0.14	0.07	46	nd	0	0	nd	0	0
3	3	0.14	0.04	27	nd	0	0	nd	0	0
4	1	0.08	0.03	36	nd	0	0	nd	0	0
4	2	0.18	0.04	21	nd	0	0	nd	0	0
5	1	0.25	0.03	13	nd	0	0	nd	0	0
5	2	0.20	0.02	8	nd	0	0	nd	0	0
5	3	0.25	0.01	5	nd	0	0	nd	0	0

* not detected

Table 4-3: Summary of CT data for continuous UASB runs 2-5 (HRT 1.9 d, ORL 3.7-5.5 g COD/L*d)

RUN #	DAY	AVG. CONC. IN (mg/L)	STD. DEV.	COV	AVG. CONC. OUT LIQUID (mg/L)	STD. DEV.	COV	AVG. CONC. OUT GAS (mg/L)	STD. DEV.	COV
2	1	0.17	0.13	76	nd	0	0	nd	0	0
2	2	0.07	0.05	70	nd	0	0	nd	0	0
2	3	0.06	0.02	29	nd	0	0	nd	0	0
3	1	0.23	0.16	72	nd	0	0	nd	0	0
3	2	0.10	0.04	42	nd	0	0	nd	0	0
3	3	0.10	0.04	41	nd	0	0	nd	0	0
4	1	0.08	0.03	42	nd	0	0	nd	0	0
4	2	0.10	0.04	37	nd	0	0	nd	0	0
5	1	0.27	0.05	17	nd	0	0	nd	0	0
5	2	0.23	0.05	21	nd	0	0	nd	0	0
5	3	0.25	0.001	0.3	nd	0	0	nd	0	0

Table 4-4: Summary of DCM data for continuous UASB runs 2-5 (HRT 1.9 d, ORL 3.7-5.5 g COD/L*d)

RUN #	DAY	AVG. CONC. IN (mg/L)	STD. DEV.	COV	AVG. CONC. OUT LIQUID (mg/L)	STD. DEV.	COV	AVG. CONC. OUT GAS (mg/L)	STD. DEV.	COV
2	1	2.03	1.2	59	nd	0	0	nd	0	0
2	2	1.49	0.7	47	nd	0	0	nd	0	0
2	3	1.43	1.3	84	nd	0	0	nd	0	0
3	1	1.43	1.8	125	nd	0	0	nd	0	0
3	2	1.12	0.3	22	nd	0	0	nd	0	0
3	3	1.04	0.4	42	nd	0	0	nd	0	0
4	1	1.30	0.4	32	nd	0	0	nd	0	0
4	2	1.30	0.9	7	nd	0	0	nd	0	0
5	1	3.51	0.1	22	nd	0	0	nd	0	0
5	2	4.04	0.4	10	nd	0	0	nd	0	0
5	3	3.51	0.4	12	nd	0	0	nd	0	0

Tables 4-2 to 4-4 gives the averages, standard deviations and the COV for the steady state continuous flow data gathered over runs 2-5. Two influent and effluent samples were collected in runs 2-5 (long HRT, high OLR), and two and sometimes three injections from each sample bottle were performed and the average values were calculated as the average of all of the sample injections. A number of samples were injected in these earlier runs because of a high degree of variability in the data, possibly due to clogged syringes. By run 6 the problem was realized, and corrective measures were taken. Results from GC injections became more consistent hence fewer sample injections were required.

Examining the data for runs 2-5 it was evident that the standard deviations for the influent VOC mass flows were relatively high for all three target

compounds. However, the outlet VOC mass flows, in both the liquid stream and the gas stream, were negligible and essentially 100% treatment was achieved. In these runs there was a high degree of confidence in the fact that all of the influent CT, PCE and DCM were degraded in the reactor for all runs.

4.1.4. Continuous Flow Study - Runs 6-15

Ten low end runs (short HRT, low OLR) were performed, with varying HRT's from 24 to 3 hours and varying OLR's from 0.4 to 2.0 g COD/L*d. A summary of the variability in the data collected from each of these runs is given in tables 4-5 to 4-7.

Table 4-5: Summary of PCE data for runs 6-15

RUN #	DAY	AVG. CONC. IN (mg/L)	STD. DEV.	COV	AVG. CONC. OUT LIQUID (mg/L)	STD. DEV.	COV	AVG. CONC OUT GAS (mg/L)	STD. DEV.	COV
6	1	0.22	0.01	3	0.34	0.02	6	0.44	0.003	1
6	2	0.29	0.004	1	0.30	0.02	8	0.42	0.025	6
6	3	0.25	0.07	26	0.37	0.07	19	0.34	0.029	8
7	1	0.67	0.22	32	0.16	0.05	34	0.18	0.21	121
7	2	0.65	0.05	8	0.24	0.05	19	0.40	0.04	10
7	3	2.12	0.81	38	0.30	0	0	0.41	0.03	7
8	1	0.79	0.06	8	0.42	0.10	23	0.82	0.002	0.4
8	2	0.80	0.02	3	0.37	0.04	12	0.90	0.003	0.3
8	3	0.82	0.03	4	0.55	0.10	19	0.84	0.008	1
9	1	2.92	2.42	83	0.81	0.70	85	1.47	0.44	30
9	2	3.86	0.11	3	1.49	1.27	86	2.05	0.24	12
9	3	3.45	-	-	2.12	0.07	3	0.52	0.03	6
10	1	0.10	0.004	4	0.02	0.001	3	0.03	0.002	6
10	2	0.12	0.03	25	0.03	-	-	0.04	0.004	11
10	3	0.12	0.01	7	0.06	0.004	7	0.05	0.003	6
11	1	0.12	0.05	41	0.11	0.02	16	0.16	0.011	7
11	2	0.17	0.002	1	0.16	0.001	1	0.16	0.002	1
11	3	0.14	-	-	0.12	0.03	24	0.17	0.007	4
12	1	0.12	0.03	26	0.15	0.002	2	0.16	0.023	14
12	2	0.14	0.04	30	0.14	0.004	2	0.16	0.008	5
12	3	0.18	0.01	7	0.15	0.003	2	0.16	0.006	4
13	1	0.07	0.003	5	0.13	0.007	5	0.15	0.008	5
13	2	0.13	0.04	31	0.17	0.03	15	0.16	0.008	5
13	3	0.09	0.01	9	0.18	0.01	6	0.20	0.004	2
14	1	0.14	0.02	14	0.16	0.01	9	0.16	0.002	1
14	2	0.14	0.02	14	0.17	0.02	9	0.20	0.003	2
15	1	0.23	0.003	1	0.19	0.01	7	0.21	0.012	6
15	2	0.26	0.06	22	0.14	0.005	3	0.20	0.011	6

Table 4-6: Summary of CT data for runs 6-15

RUN #	DAY	AVG. CONC. IN (mg/L)	STD. DEV.	COV	AVG. CONC. OUT LIQUID (mg/L)	STD. DEV.	COV	AVG. CONC OUT GAS (mg/L)	STD. DEV.	COV
6	1	0.42	0.12	29	0.001	0.0002	16	0.0019	0.00001	1
6	2	0.44	0.02	4	0.005	0.0017	31	0.0019	0.00001	1
6	3	0.42	0.003	1	0.001	0.00006	6	0.0019	0.00003	3
7	1	0.59	0.19	32	0.01	0.01	96	0.006	0.0002	4
7	2	0.55	0.15	27	0.01	0.005	51	0.003	0.00002	1
7	3	2.17	0.95	44	0.02	0.0004	3	0.0019	0.00009	7
8	1	0.72	0.03	4	0.01	0.008	77	0.0019	-	-
8	2	0.66	0.09	14	0.001	0.002	125	0.0019	0.0003	29
8	3	0.57	0.02	3	0.01	0.0009	9	0.0019	0.0003	32
9	1	3.21	2.66	83	0.05	0.01	26	0.02	0.007	30
9	2	4.86	0.40	8	0.21	0.26	127	0.0019	0	0
9	3	3.57	-	-	0.16	0.12	75	0.0019	0	0
10	1	0.30	0.003	1	0.001*	-	-	0.0019	-	-
10	2	0.31	0.073	24	0.001*	-	-	0.0019	-	-
10	3	0.31	0.02	6	0.004	0.002	49	0.0019	0.00009	9
11	1	0.25	0.08	32	0.001*	-	-	0.0019	-	-
11	2	0.32	0.01	4	0.006	0.001	21	0.003	0.002	60
11	3	0.31	-	-	0.001	0.0012	119	0.003	0.0006	18
12	1	0.26	0.07	25	0.001*	-	-	0.0019	-	-
12	2	0.33	0.04	14	0.001*	-	-	0.0039	0.001	40
12	3	0.38	0.03	9	0.001	0.00005	5	0.0019	0.00004	5
13	1	0.15	0.01	6.5	0.001*	-	-	0.0019	-	-
13	2	0.21	0.07	32	0.001*	-	-	0.0019	-	-
13	3	0.15	0.02	15	0.001*	-	-	0.004	0.002	44
14	1	0.27	0.03	12	0.001*	-	-	0.0019	-	-
14	2	0.27	0.03	12	0.001*	-	-	0.003	0.001	35
15	1	0.41	0.006	2	0.001*	-	-	0.0019	-	-
15	2	0.38	0.11	26	0.003	0.004	114	0.0019	0.00003	6

* Indicates a non-detect and detection limit was used for further calculations.

Table 4-7: Summary of DCM data for runs 6-15

RUN #	DAY	AVG. CONC. IN (mg/L)	STD. DEV.	COV	AVG. CONC. OUT LIQUID (mg/L)	STD. DEV.	COV	AVG. CONC OUT GAS (mg/L)	STD. DEV.	COV
6	1	1.1	0.21	19	2.6	0.1	3	0.60	0.008	1
6	2	1.7	0.06	3	2.7	0.1	4	0.62	0.004	1
6	3	1.2	0.41	33	3.1	0.04	1	0.63	0.012	2
7	1	2.63	0.94	36	0.10	0	0	0.018	0	0
7	2	2.39	0.12	5	0.10	0	0	0.018	0	0
7	3	7.54	2.97	39	0.10	0	0	0.018	0	0
8	1	2.88	0.23	8	1.40	0.08	6	0.41	0.06	14
8	2	2.73	0.27	10	0.85	0.11	13	0.42	0.02	4
8	3	2.89	0.08	3	1.40	0.30	22	0.39	0.05	12
9	1	10.65	9.46	89	0.10	0	0	0.018	0	0
9	2	12.33	0.47	4	0.10	0	0	0.018	0	0
9	3	7.27	5.45	75	0.01	0	0	0.018	0	0
10	1	1.8	0.03	1	1.8	0.01	0.5	0.44	0.016	4
10	2	1.9	0.22	11	2.2	-	-	0.57	0.010	2
10	3	1.8	0.09	5	2.7	0.005	0.2	0.58	0.005	1
11	1	1.5	0.41	27	2.0	0.3	14	0.66	0.029	4
11	2	2.0	0.04	2	2.2	0.2	8	0.49	0.019	4
11	3	1.8	-	-	2.1	0.2	10	0.46	0.016	3
12	1	1.8	0.31	17	1.1	0.01	1	0.30	0.013	4
12	2	2.2	0.35	16	1.0	0.006	0.7	0.24	0.023	10
12	3	2.3	0.01	0.3	0.6	0.01	2	0.20	0.003	1
13	1	1.6	0.10	6	1.0	0.04	4	0.24	0.0004	0.2
13	2	1.6	0.004	0.2	1.0	0.01	1	0.24	0	0
13	3	1.5	0.08	5	0.9	0.01	2	0.28	0.02	7
14	1	2.0	0.18	9	0.10*	-	6	0.09	0.003	3
14	2	2.0	0.18	9	0.10*	-	141	0.05	0.001	2
15	1	2.1	0.02	0.7	0.5	0.1	21	0.17	0.001	1
15	2	2.3	0.29	14	0.2	0.02	8	0.07	0.003	4

* Indicates a non-detect and detection limit was used for further calculations.

The subsequent tables give the averages, standard deviations and the COV for the steady state continuous flow data gathered over runs 6-15. For runs 6-15 (short HRT, low OLR), two influent and two effluent sample vials were taken on each sample day, one GC injection for each bottle was performed, and the average liquid concentration was calculated as the average of the two sample vials. Two reactor headspace samples were taken and the average gas concentration was calculated as the average of the two samples. Average concentrations were then converted to mass flows.

The variability in the influent and outlet data in runs 6-15 had a greater impact on data analysis than that in the first series of runs because the chlorinated compounds were not completely removed.

The standard deviations of the influent mass flows of PCE, CT and DCM were acceptable. Except during run 9, all COV's were below 50% and in most cases were below 20%. The highest COV occurred on sampling day 1 for run 9 (>80%), possibly caused by residual compound in the syringe.

Effluent COV's were at times relatively high (greater than 100% in runs 7 for PCE, runs 8, 9, 11, and 15 for CT and run 14 for DCM) but can be explained as a result of the compounds approaching detection limit concentrations, hence introducing a greater amount of variability due to the lack of sensitivity of the analytical method.

The PCE data was inconsistent, as shown by runs 6, 10, 12 13 and 14 where it was indicated that influent mass flows of PCE were less than effluent mass flows. This was not physically possible and a more detailed mass balance

was examined, which included the production of TCE, to gain additional information regarding data quality. The difference between the total moles of PCE in and the total moles of PCE plus TCE out was calculated for each run. A negative result from the mass balance indicated that the influent data was not a good indication of the actual mass entering the system and therefore could not be considered for further analysis.

The results of the PCE mass balance are given in table 4-8. The differences between the influent PCE mass and the effluent PCE plus TCE mass flows were determined to be negative in runs 6, 10, 12, 13 and 14. Solubility limits for this compound in the dosing solution were inadvertently exceeded during these runs and may have caused the high amount of inconsistency in influent data. This problem was recognized and runs 7, 8 and 9 were prepared so that solubility limits were not exceeded. PCE solubility limits were also exceeded in runs 11 and 15, and therefore there was a lack of confidence in this data. Due to the uncertainty in influent PCE concentrations, it was decided that any further data analysis with runs 6, 10, 11, 12, 13, 14 and 15 would not be advantageous and therefore this data was not further analyzed.

A similar situation as with the PCE occurred with the DCM. However, in this case it was possible for DCM to be produced in the reactor, since it is a breakdown component of CT, and high CT transformation was observed. A DCM mass balance was also examined for more information on data quality.

Table 4-9 describes the mass balance performed on DCM data and its parent compounds. Mass flow is reported in moles/d and it should be noted that

for one mole of CT dechlorinated, one mole of CF is produced and for one mole of CF dechlorinated, one mole of DCM is produced. The DCM mass balance results show that a greater amount of mass (CT plus DCM) was observed entering the system than leaving the system for runs 6, 10 and 11. The total mass out exceeded the total mass in by 50 to 125% in run 6 and therefore this run was not considered useful and the data was discarded from further DCM analysis. Two of three samples produced results with effluent masses exceeding influent masses by less than 10% in both runs 10 and 11 and the highest discrepancy was 38% in run 10. This result was considered to be in an acceptable range and data from runs 10 and 11 were used for further analysis.

Table 4-8: PCE and TCE Mass balance, runs 6-15

RUN #	DAY	MEASURED OLR g COD/L*d	HRT d	MASS FLOW IN PCE ($\mu\text{mol/d}$)	MASS FLOW OUT PCE ($\mu\text{mol/d}$)	MASS FLOW OUT TCE ($\mu\text{mol/d}$)	MASS IN MINUS MASS OUT ($\mu\text{mol/d}$)	MASS $\frac{\text{OUT}}{\text{IN}}$
6	1	0.5	1	6.4	12.7	1.10	-7.4	-116
6	2	0.6	1	8.7	11.3	0.95	-3.55	-41
6	3	0.6	1	7.3	12.9	1.17	-6.77	-93
7	1	0.9	1	20.3	5.1	6.8	8.4	41
7	2	0.5	1	24.2	9.7	9.3	5.2	21
7	3	1.3	1	69.8	10.3	5.1	54.4	78
8	1	0.4	1	24.2	12.9	0.9	10.4	43
8	2	0.7	1	25.8	11.8	0.7	13.3	52
8	3	0.4	1	26.4	17.7	0.6	8.1	31
9	1	2.0	0.5	170.9	47.8	nd	123.1	72
9	2	2.0	0.5	225.3	88.8	nd	136.5	61
9	3	1.8	0.5	195.9	120.1	0.2	75.6	39
10	1	0.4	0.5	5.6	1.3	5.74	-1.44	-26
10	2	0.4	0.5	7.1	1.9	1.51	3.69	52
10	3	0.4	0.5	7.0	3.9	0.62	2.48	35
11	1	0.4	0.33	8.8	8.2	0.36	0.24	3
11	2	0.5	0.33	16.1	14.7	0.12	1.28	8
11	3	0.4	0.33	13.2	11.4	nd	1.799	14
12	1	0.8	0.25	14.2	19.0	nd	-4.801	-34
12	2	0.5	0.25	17.9	18.4	nd	-0.501	-3
12	3	0.8	0.25	16.3	13.8	nd	2.499	15
13	1	1.7	0.12	18.1	32.5	nd	-14.401	-80
13	2	1.5	0.12	30.6	40.9	nd	-10.301	-34
13	3	1.6	0.12	2.7	5.5	nd	-2.801	-104
14	1	0.2	0.25	17.7	20.2	nd	-2.501	-14
14	2	0.3	0.25	18.0	21.2	nd	-3.201	-18
15	1	1.8	0.25	20.0	16.6	nd	3.399	17
15	2	1.7	0.25	32.2	18.3	nd	13.899	43

Table 4-9: DCM, CT & CF mass balance on UASB reactor, runs 6-15

RUN #	DAY	OLR g COD/L*d	HRT d	MASS FLOW IN CT ($\mu\text{mol/d}$)	MASS FLOW IN DCM ($\mu\text{mol/d}$)	MASS FLOW OUT DCM ($\mu\text{mol/d}$)	MASS FLOW OUT CF ($\mu\text{mol/d}$)	MASS IN MINUS MASS OUT ($\mu\text{mol/d}$)	MASS OUT MASS IN
6	1	0.5	1	13.5	64.3	158.1	nd	-80.3	-103
6	2	0.6	1	14.0	95.0	163.3	nd	-54.3	-50
6	3	0.6	1	13.4	68.6	184.6	nd	-102.6	-125
7	1	0.9	1	19.1	154.9	5.7	nd	168.3	97
7	2	0.5	1	22.1	173.9	7.2	nd	188.8	96
7	3	1.3	1	77.0	485.3	6.6	1.4	554.3	99
8	1	0.4	1	23.7	173.0	88.9	2.9	104.9	53
8	2	0.7	1	22.8	171.8	53.1	2.1	139.4	72
8	3	0.4	1	19.6	177.6	88.0	2.4	106.8	54
9	1	2.0	0.5	202.8	1216.7	11.4	61.9	1346.2	95
9	2	2.0	0.5	306.2	1407.2	11.4	50.0	1652	96
9	3	1.8	0.5	109.2	805.2	11.1	69.0	834.3	91
10	1	0.4	0.5	19.5	215.8	217.7	nd	17.6	7
10	2	0.4	0.5	20.2	228.3	262.8	nd	-14.3	-6
10	3	0.4	0.5	20.4	211.5	320.7	nd	-88.8	-38
11	1	0.4	0.33	20.6	226.5	298.5	nd	-51.4	-21
11	2	0.5	0.33	32.0	368.2	415.5	0.8	-16.1	-4
11	3	0.4	0.33	30.5	335.1	384.1	3.1	-21.6	-6
12	1	0.8	0.25	35.3	441.3	266.7	10.3	199.6	42
12	2	0.5	0.25	46.7	553.4	245.9	12.9	341.3	57
12	3	0.8	0.25	37.0	405.0	113.7	10.8	317.5	72
13	1	1.7	0.12	38.9	772.1	453.1	22.5	335.4	41
13	2	1.5	0.12	53.6	748.0	493.1	26.0	282.5	35
13	3	1.6	0.12	39.2	724.0	442.4	24.0	296.8	39
14	1	0.2	0.25	35.6	488.6	11.9	13.2	499.1	95
14	2	0.3	0.25	36.1	495.7	1.5	14.6	515.7	97
15	1	1.8	0.25	35.6	389.3	90.4	16.3	318.2	75
15	2	1.7	0.25	55.9	510.2	53.2	22.3	490.6	86

4.2. SERUM BOTTLE TEST RESULTS

4.2.1. VOC Response in Serum Bottle Test

The main objective of the serum bottle batch test was to examine the impact of the cosubstrates on the biodegradation of the target compounds, in a three compound mixture, and thereby allowing for selection of the cosubstrate which would be employed in continuous flow studies. The choice of the cosubstrate was based on certain criteria. Firstly, the responses of the VOC's in the serum bottles were compared according to the cosubstrate present. The most limiting VOC (i.e., the one with the slowest degradation) was the response chosen to identify the best cosubstrate. Cosubstrates that supported more rapid VOC degradation would be chosen over those that indicated relatively slower VOC degradation. Secondly, the biodegradability of the cosubstrate was considered, as well as the relative toxicity of the cosubstrates to humans. The relative toxicity was considered because it would be a concern in a large scale handling of the material.

The total mass of the three target compounds versus time in the serum bottle test are presented in figures 4-5 to 4-13. Ethanolamine was eliminated in the preliminary set of serum bottle tests due to poor degradability (see Appendix D) and therefore, only methanol, ethylene glycol and propylene glycol were considered in the serum bottle tests. Each plot described the total mass of the respective VOC over time in each serum bottle.

Figure 4-5: PCE and TCE response in serum bottle test - Methanol as cosubstrate

Figure 4-6: PCE and TCE response in serum bottle test - Ethylene glycol as cosubstrate

Figure 4-7: PCE and TCE response in serum bottle test - Propylene glycol as cosubstrate

Figure 4-8: DCM, CT and CF response in serum bottle test - Methanol as cosubstrate

Figure 4-9: DCM, CT and CF response in serum bottle test - Ethylene glycol as cosubstrate

Figure 4-10: DCM, CT and CF response in serum bottle test - Propylene glycol as cosubstrate

Figure 4-11: CT and CF response in serum bottle test - Methanol as cosubstrate

Figure 4-12: CT and CF response in serum bottle test - Ethylene glycol as cosubstrate

Figure 4-13: CT and CF response in serum bottle test - Propylene glycol as cosubstrate

Figures 4-5 to 4-7 present the total mass of PCE and TCE that were measured in the serum bottles over time. These curves were displayed on the same plots to compare the decrease in mass of PCE to the increase in mass of TCE. An acclimation period of approximately 12 days was observed during which time there was a lack of activity as indicated by minimal PCE removal. After this period the mass of PCE decreased and a small amount of TCE was observed in the serum bottles. An accumulation of TCE was observed in propylene glycol bottles, reaching a maximum on day 22 and then decreasing. The presence of TCE in the serum bottles indicated that the TCE degradation

rate was less than the degradation rate of PCE. Vinyl chloride and DCE were not detected.

The response of DCM in the serum bottle batch tests was plotted along with the responses of CT and CF in figures 4-8 to 4-10. This was performed to compare the disappearance of CT with any increases in CF and DCM. In all of the serum bottles, except those containing methanol, the mass of DCM increased by the second sample. Some of the DCM mass increase could have been attributed to a transformation of CT to DCM, however it is apparent from graphs 4-8 to 4-10 that the observed DCM mass increase was much larger than the initial mass of CT. The observed mass increase between days 0 and 5 was an average of 4.0 μmol in the propylene glycol bottles and 4.7 μmol in the ethylene glycol bottles. Only 0.7 μmol of CT was added to the serum bottles and therefore all of the DCM mass increase could not have come from the CT. An increase in DCM mass may have resulted if the DCM had not actually reached equilibrium by the first sample time (1 hour), leaving the impression that an increase in DCM mass occurred. Therefore, it is possible that in the case of the propylene glycol and ethylene glycol serum bottles the observed mass increase in DCM was a combination of both CT degradation and lack of equilibrium conditions. The fact that both DCM and CT disappeared over the course of the tests indicated that both compounds were degradable.

In all cases CT disappeared too rapidly (within a day) to draw any conclusions regarding its rate of degradation (see figures 4-11 to 4-13). The only significant amount of CF was observed in ethylene glycol bottle 2 on day one,

but by day 5 it had disappeared. The fact that some CF was detected would suggest that its rate of degradation was less than the rate of degradation of CT. By day 5, in all of the bottles, the concentration of CT had reached the detection limit and remained at this level for the course of the experiment.

Degradation rates of the target VOC's were calculated from the slope of the steepest part of each curve and an average value was calculated from the duplicate bottles. An example calculation for the degradation of PCE in methanol bottles is given in figure 4-14.

The degradation rates of PCE, CT and DCM, normalized to the amount of biomass, are given in table 4-10 for each cosubstrate. CT and CF were below detection limits in the serum bottles by the time DCM rates were calculated, so it was assumed that the calculated DCM rates were pure degradation rates and did not include any production.

	METHANOL	ETHYLENE GLYCOL	PROPYLENE GLYCOL
Degradation rate $\pm$ s.d ($\mu\text{mol PCE/gVSS}\cdot\text{d}$)	0.107 $\pm$ 0.003	0.097 $\pm$ 0.011	0.110 $\pm$ 0.013
Degradation rate $\pm$ s.d ($\mu\text{mol DCM/gVSS}\cdot\text{d}$)	3.22 $\pm$ 0.93	3.66 $\pm$ 0.32	3.53 $\pm$ 0.91
Degradation rate $\pm$ s.d ($\mu\text{mol CT/gVSS}\cdot\text{d}$) **	0.11 $\pm$ 0.05	0.213 $\pm$ 0.004	0.06 $\pm$ 0.02

Table 4-10: PCE, CT and DCM Degradation rates in a mixture of VOC's in serum bottle test (Rates for CT are minimum and based on detection limits)**

Figure 4-14: Example calculation for the degradation rate of PCE in methanol bottles

An analysis of variance (ANOVA) was performed on the PCE and DCM degradation rates and a significant difference between cosubstrates was not found at a 95 percent confidence level.

The calculated CT rates were minimum rates based on the first sample when the detection limit had been reached.

4.2.2. Methane Production in Serum Bottle Test

Batch serum bottles included bottles with a mixture of VOC's and serum bottles without VOC's, in order to determine the effect of the VOC's on the activity of the biomass. Cumulative methane production results are displayed in figures 4-15 to 4-17 and the line labeled no VOC's indicates the serum bottles prepared without VOC's.

Figure 4-15: Cumulative methane production in serum bottle test - Methanol as cosubstrate

Figure 4-16: Cumulative methane production in serum bottle test - Ethylene glycol as cosubstrate

Figure 4-17: Cumulative methane production in serum bottle test - Propylene glycol as cosubstrate

An observation that can be made from these plots is that the presence of VOC's in the serum bottles inhibited the methanogenic activity of the biomass. An apparent lag period, with respect to bottles containing no VOC's, occurred in all bottles containing VOC's, indicating that the VOC's exhibited some toxicity to the methanogenic organisms. It was not possible to determine from the results which of the three chlorinated organic compounds was the most toxic to the biomass. However, relative inhibition values were calculated for each cosubstrate by determining the percent difference between total methane production for serum bottles with and without VOC's on a particular day. Percent inhibition values were calculated on days 12, 27 and 67 and are reported in Table 4-11. Time to production of 50 mL of CH₄ was also used as an indication of the inhibition of the biomass by the VOC's. 50 mL was chosen as the volume to compare bottles with VOC's to bottles without VOC's instead of a value closer to half of the CH₄ potential, because of the low production of gas in the ethylene glycol bottles. The volume in the ethylene glycol bottles only approached 50 mL of CH₄ by day 68. The results are presented in table 4-12.

DAY	METHANOL	ETHYLENE GLYCOL	PROPYLENE GLYCOL
12	75	98	78
27	59	99	89
67	14	79	52

Table 4-11: Percent inhibition of CH₄ production in serum bottles containing a mixture of VOC's

	METHANOL	ETHYLENE GLYCOL	PROPYLENE GLYCOL
Bottle with VOC's	28	67	38
Bottle without VOC's	19	9	15
Lag time between bottles	9	58	23

Table 4-12: Time to a cumulative volume of 50 mL of CH₄ reported in days - serum bottle test

The inhibition in serum bottles containing a mixture of VOC's was most predominant in the serum bottles containing ethylene glycol as the cosubstrate. Inhibition was as high as 99% and only dropped to 79% by day 67. Serum bottles containing methanol showed the least amount of inhibition, dropping to 14% by day 67.

The time to production of 50 mL of CH₄ offers some information regarding the toxicity of the VOC's to the biomass. The greatest lag time between bottles with and bottles without VOC's was observed in the ethylene glycol bottles at 58 days and the smallest lag time between bottles was observed in methanol bottles at 9 days. This information strongly suggests that the VOC's demonstrated inhibitory effects on the biomass and there maybe implications in continuous flow studies, however the implications may not be as prevalent as the batch tests would indicate. In the batch tests there was an initial high VOC concentration, which was degraded over time. In continuous flow experiments, assuming a continuously well mixed reactor with VOC degradation, the VOC concentration in

the reactor will be much smaller than the inlet concentration, thereby reducing the inhibitory effects observed in the batch tests. It is possible that longer HRT's may be necessary to effectively treat the VOC's in the continuous flow process, although the diluting effects of the continuous flow reactor may be enough to curb the effects of the VOC toxicity to the biomass.

The observed responses of the VOC's and the methane production in the serum bottles offer information regarding the organisms most likely responsible for the VOC biodegradation. In the case of CT it is possible that organisms other than methanogens were responsible for its degradation. The rapid disappearance of the CT, much earlier than any methane production was observed may indicate acetogens were responsible for the degradation of CT.

The PCE response in the serum bottles corresponded more closely to the methane production. Between days 15 and 20 the methane production in the bottles increased considerably (see figures 4-15 to 4-17), and the PCE began to degrade (see figures 4-5 to 4-7). This parallel between PCE degradation and methane production indicates that methanogens were likely responsible for the biodegradation of the PCE.

The DCM response (see figures 4-8 to 4-10) with respect to the methane production was similar to that of CT, even though it took longer for the DCM to disappear. No considerable amount of methane had been produced by day 30 in any of the serum bottles, and by this time almost all of the DCM had disappeared. There was a slight amount of methane production before all of the DCM was removed from the ethylene glycol and propylene glycol bottles and for

this reason it was thought that perhaps a combination of acetogens and methanogens were responsible for the degradation of the DCM.

The findings from the batch tests had some possible implications for UASB reactor studies. CT disappeared rapidly in the batch tests, even before methanogenic activity was observed, suggesting that CT would be easily removed in continuous flow studies. DCM was also removed from the serum bottles before any significant amount of CH_4 was produced, indicating that it may also be easily degraded in continuous flow studies, however slightly longer HRT's may be required than for CT. PCE took the longest of the three target compounds to degrade and not a significant amount of removal was observed until there was a notable production of CH_4 . This would suggest that longer HRT's may be required in the continuous flow process to ensure methanogenesis, which in turn is necessary to treat PCE effectively.

PCE was degraded the slowest of the three target compounds and for this reason was chosen as the indicator VOC for the selection of the cosubstrate to be used in continuous flow studies. Based on the slightly higher PCE degradation rate in the propylene glycol bottle along with the CH_4 production data, indicating that propylene glycol was a highly degradable compound, propylene glycol was selected to be used in continuous flow studies. The fact that propylene glycol was the least toxic of the 3 cosubstrates (personal communication, J. B. Williams, 1998), was also considered when selecting it for future use.

4.3. COMPARISONS BETWEEN EXPERIMENTAL BATCH RESULTS AND LITERATURE

Long et al. (1993) reported a rate of degradation for PCE in a mixture of 30 μ g PCE/g VSS-d, and for DCM in a mixture a value of 20 μ g DCM/g VSS-d. A degradation rate for CT was not reported; however, it was noted that CT did disappear at a much quicker rate than both PCE and DCM.

Degradation rates for PCE and DCM obtained from this study were found to be between 0.097 and 0.110 μ mol PCE/g VSS-d (16.1-18.2 μ g PCE/g VSS-d) and 3.22 and 3.66 μ mol DCM/g VSS-d (312-355 μ g DCM/g VSS-d). The PCE degradation rates were slightly more than half of those reported by Long et al. (1993) and the DCM degradation rates were approximately 20 times higher than those reported by Long et al. (1993). The reason for the difference in the DCM degradation rates may be due to the higher initial DCM concentrations used in this research (10 000 μ g/L) compared to that of Long et al. (120 μ g/L), along with the fact that in Long's work the DCM was produced from the biodegradation of CT, and in this research DCM was added as a target compound.

The PCE degradation rates were slower in this study than those of Long et al. (1993) and may have resulted from differences in biomass and cosubstrates. Methanol was the only common cosubstrate between the two studies.

The accumulation of TCE was observed in batch serum bottle tests with PCE by Fathepure and Boyd (1988), and was consistent with the results found in this study, where small amounts of TCE were observed and in one case

accumulated. This result is also consistent with the theory that as a chlorinated compound becomes less chlorinated, it becomes less susceptible to anaerobic dechlorination and hence its rate of degradation decreases.

Freedman and Gossett (1989) observed complete removal of both PCE and TCE, within 2-3 days. This was much faster than the removal observed in these experiments, perhaps due to acclimation of the biomass to the target compounds. Freedman and Gossett (1989) observed the accumulation of VC, and the presence of ethylene, but it is unknown whether these compounds were formed in the current experiments due to the limited detection capabilities of the equipment.

Fathepure and Boyd (1988) and Freedman and Gossett (1989) both reported the inhibition of the degradation of PCE and TCE when methane production was stopped. This result was consistent with findings from this study and strongly suggests that methanogens were responsible for the degradation of PCE.

The removal of CT by Doong and Wu (1995) was almost 100% when the chlorinated compound was fed alone, and the time for complete degradation depended on the amount of cosubstrate added, as well as the initial CT concentration. Chloroform (CF) production was observed by Doong and Wu (1995) and was correlated to the disappearance of CT. The almost complete degradation of CT agrees with the observations made during this research. In this study a mixture of VOC's containing CT was introduced into serum bottles, and CT disappeared more quickly than in the study conducted by Doong and Wu

(1995), (1 day compared to 6+ days) and CF was observed in small quantities indicating a slower rate of disappearance than CT. The quicker disappearance in this study may have been due to differences in biomass characteristics and cosubstrates used.

Egli et al. (1988) reported the specific acetogens, *Acetobacterium woodii* and *Clostridium thermoaceticum*, completely degraded 80 $\mu\text{mol/L}$ of CT within 3 days. This is consistent with the finding in this study that acetogens, not methanogens, were likely responsible for the dechlorination of the CT.

DCM was observed to degrade in studies conducted by Hughes and Parkin (1992), but no DCM degradation was observed in subsequent studies by the same researchers. The concentration of DCM was relatively higher in this study (10 mg/L compared to 5 mg/L) and yet DCM degradation was observed in both bottle tests. A biomass more acclimated to DCM may have caused this result.

Results from both this work and others suggest that all three of the target compounds, given the right conditions, can potentially be degraded in an anaerobic system. The literature indicates that a long acclimation period of the biomass (i. e., years) may be an important element to the effective degradation of the VOC's, PCE and DCM in particular. However, there are impracticalities of a long acclimation period when dealing with a full scale process. It would be both costly in terms of time and money for an industry to wait years for a biomass to become acclimated to their specific waste. The positive side of a long acclimation period is the degradation rates will be faster in an acclimated

biomass compared to one that is not acclimated and hence the removal of PCE and DCM may occur faster and consistently.

4.4. CONTINUOUS FLOW REACTOR RESULTS

4.4.1. Removal Efficiencies for Runs 2-5

Sampling for the steady state continuous flow experiments began after a minimum equilibrium period of 3 HRT's. Liquid VOC samples were placed on a shaker at 35°C for future analysis, and VOC and methane gas headspace samples were immediately injected into the respective GC. The concentrations measured in these experiments were previously presented and discussed in section 4.1.3.

Tables 4-13 to 4-15 summarize the removal rates of the target VOC's, when fed in a mixture, in the UASB reactor for each experimental run (long HRT, high OLR).

Table 4-13: PCE removal efficiencies when fed in a mixture for runs 2-5 (HRT 2d, OLR 3.7-5.5g COD/L*d)

RUN #	DAY	OLR g COD/L*d	INFLUENT MASS.(μ mol/d)	EFFLUENT MASS (μ mol/d)	REMOVAL EFFICIENCY (%)
2	1	4.8	3.4	nd*	>99
2	2	5.9	1.0	nd	>99
2	3	5.5	1.5	nd	>99
3	1	6.3	4.1	nd	>99
3	2	3.9	2.0	nd	>99
3	3	4.6	3.7	nd	>99
4	1	4.9	1.2	nd	>99
4	2	3.4	2.5	nd	>99
5	1	3.8	2.9	nd	>99
5	2	3.2	2.8	nd	>99
5	3	4.3	3.5	nd	>99

* nd - not detectable

Table 4-14: CT removal efficiencies when fed in a mixture for runs 2-5 (HRT 2d, OLR 3.7-5.5g COD/L*d)

RUN #	DAY	OLR g COD/L*d	INFLUENT MASS.(μ mol/d)	EFFLUENT MASS (μ mol/d)	REMOVAL EFFICIENCY (%)
2	1	4.8	2.5	nd	>99
2	2	5.9	1.1	nd	>99
2	3	5.5	0.8	nd	>99
3	1	6.3	3.2	nd	>99
3	2	3.9	1.4	nd	>99
3	3	4.6	2.9	nd	>99
4	1	4.9	1.2	nd	>99
4	2	3.4	1.6	nd	>99
5	1	3.8	4.0	nd	>99
5	2	3.2	3.3	nd	>99
5	3	4.3	3.8	nd	>99

Table 4-15: DCM removal efficiencies when fed in a mixture for runs 2-5 (HRT 2d, OLR 3.7-5.5g COD/L*d)

RUN #	DAY	OLR g COD/L*d	INFLUENT MASS.(μ mol/d)	EFFLUENT MASS (μ mol/d)	REMOVAL EFFICIENCY (%)
2	1	4.8	55.1	nd	>99
2	2	5.9	25.1	nd	>99
2	3	5.5	35.4	nd	>99
3	1	6.3	37.1	nd	>99
3	2	3.9	32.6	nd	>99
3	3	4.6	55.9	nd	>99
4	1	4.9	35.0	nd	>99
4	2	3.4	35.0	nd	>99
5	1	3.8	139.9	nd	>99
5	2	3.2	107.0	nd	>99
5	3	4.3	97.2	nd	>99

High removal efficiencies were observed in runs 2-5. PCE, DCM and CT were all below detection limits in the effluent in all runs. Therefore, at these reactor conditions, the anaerobic treatment process was an effective treatment method for PCE, CT and DCM. The high removal efficiencies prevented any analysis of the impact of process variables on removal efficiencies.

4.4.2. Removal Efficiencies for Runs 6-15

Tables 4-16 to 4-18 present the removal efficiencies for each VOC for runs 6-15.

Table 4-16: PCE removal efficiencies when fed in a mixture for runs 7, 8, and 9.

RUN #	DAY	OLR g COD/L*d	HRT d	INFLUENT MASS. ($\mu\text{mol/d}$)	EFFLUENT MASS ($\mu\text{mol/d}$)	REMOVAL EFFICIENCY (%)
7	1	0.9	1	20.3	5.1	74
7	2	0.5	1	24.2	9.7	60
7	3	1.3	1	69.8	10.3	85
8	1	0.4	1	24.2	12.9	46
8	2	0.7	1	25.8	11.8	54
8	3	0.4	1	26.4	17.7	33
9	1	2.0	0.5	170.9	47.8	72
9	2	2.0	0.5	225.3	88.8	61
9	3	1.8	0.5	195.9	120.1	39

Table 4-17: CT removal efficiencies when fed in a mixture for runs 6-15

RUN #	DAY	OLR g COD/L*d	HRT d	INFLUENT MASS. (μmol/d)	EFFLUENT MASS (μmol/d)	REMOVAL EFFICIENCY (%)
6	1	0.5	1	13.5	0.004	>99
6	2	0.6	1	14.0	0.18	99
6	3	0.6	1	13.4	0.04	>99
7	1	0.9	1	19.1	0.38	98
7	2	0.5	1	22.1	0.39	98
7	3	1.3	1	77.0	0.53	99
8	1	0.4	1	23.7	0.35	98
8	2	0.7	1	22.8	0.05	>99
8	3	0.4	1	19.6	0.34	98
9	1	2.0	0.5	202.8	3.3	98
9	2	2.0	0.5	306.2	13.1	96
9	3	1.8	0.5	109.2	9.9	91
10	1	0.4	0.5	19.5	0.03	>99
10	2	0.4	0.5	20.2	0.02	>99
10	3	0.4	0.5	20.4	0.28	99
11	1	0.4	0.33	20.6	0.04	>99
11	2	0.5	0.33	32.0	0.58	98
11	3	0.4	0.33	30.5	0.11	>99
12	1	0.8	0.25	35.3	0.05	>99
12	2	0.5	0.25	46.7	0.08	>99
12	3	0.8	0.25	37.0	0.10	>99
13	1	1.7	0.12	38.9	0.09	>99
13	2	1.5	0.12	53.6	0.09	>99
13	3	1.6	0.12	4.9	0.02	>99
14	1	0.2	0.25	35.6	0.03	>99
14	2	0.3	0.25	36.1	0.06	>99
15	1	1.8	0.25	35.6	0.04	>99
15	2	1.7	0.25	55.9	0.50	99

Table 4-18: DCM removal efficiencies when fed in a mixture for runs 7-15

RUN #	DAY	OLR g COD/L*d	HRT d	INFLUENT MASS. (μ mol/d)	EFFLUENT MASS (μ mol/d)	REMOVAL EFFICIENCY (%)
7	1	0.9	1	154.9	5.7	96
7	2	0.5	1	173.9	7.2	96
7	3	1.3	1	485.3	6.6	98
8	1	0.4	1	173.0	88.9	51
8	2	0.7	1	171.8	53.1	69
8	3	0.4	1	177.6	88.0	51
9	1	2.0	0.5	1216.7	11.4	99
9	2	2.0	0.5	1407.2	11.4	99
9	3	1.8	0.5	805.2	11.1	98
10	1	0.4	0.5	215.8	217.7	-1
10	2	0.4	0.5	228.3	262.8	-15
10	3	0.4	0.5	211.5	320.7	-51
11	1	0.4	0.33	226.5	298.5	-32
11	2	0.5	0.33	368.2	415.5	-13
11	3	0.4	0.33	335.1	384.1	-15
12	1	0.8	0.25	441.3	266.7	40
12	2	0.5	0.25	553.4	245.9	56
12	3	0.8	0.25	405.0	113.7	72
13	1	1.7	0.12	772.1	453.1	41
13	2	1.5	0.12	748.0	493.1	34
13	3	1.6	0.12	90.5	55.3	39
14	1	0.2	0.25	488.6	11.9	98
14	2	0.3	0.25	495.7	1.5	99
15	1	1.8	0.25	389.3	90.4	77
15	2	1.7	0.25	510.2	53.2	90

The highest removal efficiencies for PCE were observed during run 7, under operating conditions of a 24 hour HRT and an influent organic loading rate of 0.9 g COD/L*d. The average PCE removal efficiency was 73% over the 3 day sample period. It was not possible to statistically analyze all of the PCE data

because a substantial number of runs were not employed due to a lack of mass balance closure and there was not sufficient data remaining to statistically determine the effects of HRT and OLR on the removal of PCE. However, a t-test was performed on the data from runs 7 and 8 with OLR as the variable (HRT was constant at 24 hours). The results of this t-test indicated that OLR was a significant factor in the removal of PCE at a 95 % confidence level. An average PCE removal of 73% was achieved at an OLR of 0.9 g COD/L*d and an average of 44% was achieved at an OLR of 0.5 g COD/L*d.

An examination of all of the PCE data indicates that HRT may also be a significant factor in the degradation of PCE. Complete removal of PCE was observed in the long HRT, high OLR runs (runs 2-5), and when the HRT was decreased from 48 to 24 hours and less, the removal of PCE was reduced as well. This result was also supported by results observed in the serum bottle batch tests. PCE required a significantly longer time than DCM or CT before significant removal was achieved.

TCE was detected during runs 7, 8 and in one sampling period in run 9 (see table 4-8). The amount of TCE in the reactor significantly dropped off between runs 7 and 8, even though the HRT remained at 1 day. This drop in TCE was probably a result of the OLR effect on the removal of PCE. A higher PCE removal was observed in run 7 and therefore more TCE was produced. In run 8 the removal of PCE decreased resulting in a decrease in the amount of TCE production. DCE and VC were not detected in the reactor for any of the runs.

As in runs 2-5, CT was removed at nearly 100 % efficiency on a continuous basis, regardless of the HRT and the organic loading. The UASB process was a viable treatment method for CT in the presence of PCE and DCM. Degradation rates and the observation of breakdown compounds will be subsequently discussed.

An ANOVA was performed on the data from runs 10, 12 and 13 (HRT 12, 6 and 3 hours) to determine if HRT had an effect on the removal of DCM from the UASB. The influent COD concentration was constant at 0.2 g COD/L for these runs. The results from the ANOVA indicated that at a 95% level of confidence there was a significant difference in the removal of DCM. A Duncan's Multiple range test was performed to determine which HRT's were significantly different and it was found that the removals observed at HRT's of 6 and 3 hours were not significantly different at a 95% confidence level, but HRT's of 6 and 3 hours were significantly different than that with an HRT of 12 hours. The 3 and 6 hour HRT's were the most effective at degrading the DCM and when the HRT was increased to 12 hours DCM actually accumulated in the system.

To clarify what was occurring in the reactor with respect to the production of DCM from CF, table 4-19 was constructed. The column labeled theoretical CF out, was determined by using the total average CT mass flow in during the run. This assumed that all of the CT entering the system was degraded to CF. The column labeled observed CF out was determined by taking the average of the observed CF mass flow out during the run. The observed CF out was also

shown as a percent of the theoretical CF out to indicate the relative amount of CF produced compared to the potential CF production.

RUN #	HRT (d)	AVG. OLR (g COD/L*d)	CF _{OUT} THEORETICAL (μmol/d)	CF _{OUT} OBSERVED (μmol/d)	DCM % REMOVAL	$\frac{\text{OBS. CF}}{\text{THEO CF}}$ (%)
7	1	0.9	39	0.5	97	1
8	1	0.5	22	2.5	57	11
9	0.5	1.9	206	60	99	29
10	0.5	0.4	20	nd	-22	-
11	0.33	0.4	28	1.3	-20	5
12	0.25	0.7	40	11.3	56	28
13	0.12	1.6	31	24	38	77
14	0.25	0.3	36	13.9	98	39
15	0.25	1.7	46	19.3	84	42

Table 4-19: Theoretical CF production and observed CF production in runs 7-15.

In runs 10 and 11 the removal of DCM was negative due to a faster rate of DCM production than degradation. The accumulation of CF was negligible in these runs which suggested that the HRT was long enough to allow the CF to degrade to DCM, but not enough time was allowed for all of the DCM to degrade.

The positive removals of DCM at the short HRT's (3 and 6 hours) probably resulted due to an accumulation of CF. Positive DCM removals were observed in runs 12, 13, 14 and 15 corresponded to an accumulation of CF, as was indicated by a build up of CF as high as 77% of its potential. At the shorter HRT's, the production of CF was faster than the production of DCM, and hence it

accumulated in the reactor. DCM removals were also positive at HRT's of 24 hours and for one of the 12 hour runs. In these cases the accumulation of CF was relatively small, suggesting that there was time enough for the produced DCM to degrade.

An ANOVA ($\alpha=0.5$) was performed to determine the effects of influent COD concentration on the removal efficiency of DCM (runs 7 and 8). The results of the ANOVA indicated that there was a significant difference in the removal of DCM between an influent COD concentration of 0.9 and 0.5 g COD/L. 97% DCM removal was achieved at the higher inlet COD concentration and 57% removal was observed at the lower inlet COD concentration.

4.5. DEGRADATION RATES AND RATE COEFFICIENTS FOR CONTINUOUS FLOW COLUMN

Mass balances were performed on the anaerobic column for each individual VOC and each experimental run. The UASB reactor was assumed to be a well mixed continuous flow reactor under steady state conditions and the following overall mass balance equation was employed:

$$Q_{in} * C_{in} - Q_{out} * C_{out} - Q_{out\ gas} * C_{out\ gas} - r * V = 0 \quad \text{(Equation 4-3)}$$

where: $Q_{in} = Q_{out} = \text{flow rate} \quad (\text{L/d})$

$$Q_{out\ gas} = \text{gas flow rate} \quad (\text{L/d})$$

$$C_{out\ gas} = \text{VOC gas headspace conc.} \quad (\text{mg/L})$$

$$C_{in} = \text{VOC influent conc. and } C_{out} = \text{VOC effluent conc.} \quad (\text{mg/L})$$

V = reactor volume (L)

r = VOC degradation rate (mg VOC/L*d)

The mass balance for DCM was written in a slightly different form to account for the production of DCM from the degradation of CT and CF. The following mass balance equation was used to determine rate coefficients for DCM.

$$Q_{in}C_{in\ DCM} - Q_{out}C_{out\ DCM} - Q_{gas}C_{gas} + Q[f_1C_{in\ CT} - f_1C_{out\ CT} - f_2C_{out\ CF}] + Q_{gas}[-f_1C_{out\ gas\ CT} - f_2C_{out\ gas\ CF}] - rV = 0 \quad \text{(Equation 4-4)}$$

where f_1 = molar mass DCM / molar mass CT

f_2 = molar mass DCM / molar mass CF

Monod kinetics are commonly employed for biological processes. However, at low substrate (VOC) concentrations the Monod equation is simplified and equation 4-5 results. It was assumed that the reactor kinetics followed the first order reaction of equation 4-5, and the equation was employed to determine rate coefficients.

$$r = kXC \quad \text{(Equation 4-5)} \quad \text{(Brock, 1991)}$$

where k = the rate coefficient ($\text{L/gVSS}\cdot\text{d}$)

X = biomass concentration in UASB reactor (20 g VSS/L)

C = effluent VOC concentration (mg/L)

In situations where the outlet mass was less than the detection limit, it was assumed that the effluent concentration was that of the detection limit for the particular compound. This corresponded to a liquid concentration of 0.001 mg/L for PCE and CT and 0.1 mg/L for DCM. Since detection limits were used as effluent concentrations, the resulting rates coefficients were minimum estimates.

4.5.1. Rate Coefficients for runs 2-5

The biodegradation rate coefficients were solved by minimizing the sum of squares between the observed and the model predicted effluent mass flows. Rate coefficients calculated for runs 2-5 are given in table 4-20.

RUN #	HRT d	OLR gCOD/L $\cdot$ d	k PCE (L/g VSS $\cdot$ d)	k CT (L/g VSS $\cdot$ d)	k DCM (L/g VSS $\cdot$ d)
2	1.9	5.5	3.96 ± 0.00	2.45 ± 0.00	0.31 ± 0.51
3	1.9	4.9	4.81 ± 0.00	3.38 ± 0.00	0.22 ± 0.17
4	1.9	5.0	3.03 ± 0.00	1.56 ± 0.00	0.23 ± 0.09
5	1.9	3.7	5.40 ± 0.00	5.43 ± 0.00	0.82 ± 0.30

Table 4-20: Rate Coefficients for PCE, CT and DCM for runs 2-5 ($\pm$ standard error)

The rate coefficients shown in table 4-20 were calculated using the detection limit for each of the target VOC's. Since detection limits were used, the coefficients should be considered as minimum values and it is difficult to assess the impact of HRT or OLR on these coefficients. There are some notable differences between the calculated rate coefficients for the individual compounds. PCE appears to have a slightly higher k value than CT and DCM appears to have the smallest k value. The reason for the smaller DCM k values was because of the higher detection limit employed relative to the detection limit of CT and PCE (0.1 mg/L for DCM versus 0.001 mg/L for CT and PCE).

Also of note is that the k value for all of the compounds increased in run 5. This resulted from the fact that the measured inlet concentration was considerably higher for this run than in the previous runs. Due to effluent concentrations being assumed as the detection limit concentrations, the only variable in the mass balance equation was the influent concentration. Therefore the value of k was more dependent on the influent concentration and this resulted in a higher k value in run 5. However, since the rate coefficients calculated were conservative values, it was reasonable to conclude that the highest k values calculated (run 5) were closest to the actual k values.

4.5.2. Rate Coefficients for Runs 6-15

Rate coefficients were calculated as for runs 2-5, and are presented in table 4-21 for runs 6-15.

Table 4-21: Rate Coefficients for PCE, CT and DCM for runs 6-15 ($\pm$ standard error)

RUN #	HRT d	OLR gCOD/L* d	k PCE (L/g VSS*d)	k CT (L/g VSS*d)	k DCM (L/g VSS*d)
6	1	0.6	-	8.66 ± 1.12	-
7	1	0.9	0.27 ± 1.99	5.32 ± 1.40	$3.46 \pm 12.47^*$
8	1	0.5	0.04 ± 0.46	4.87 ± 1.55	0.08 ± 2.37
9	0.5	1.9	0.12 ± 7.36	2.47 ± 8.55	$11.60 \pm 16.62^*$
10	0.5	0.4	-	$15.15 \pm 1.51^*$	-0.009 ± 2.400
11	0.33	0.4	-	$15.05 \pm 2.28^*$	-0.010 ± 1.366
12	0.25	0.7	-	$36.05 \pm 1.79^*$	0.28 ± 7.40
13	0.125	1.6	-	$40.98 \pm 2.06^*$	0.27 ± 1.12
14	0.25	0.3	-	$32.68 \pm 2.28^*$	$4.15 \pm 0.25^*$
15	0.25	1.7	-	$19.30 \pm 2.34^*$	1.02 ± 22.04

*Indicates detection limit was used for at least one of the sample days during the run.

The rate coefficients calculated for PCE in runs 6-15 were essentially 0. In all cases, the mean value of k was within one standard error of zero and hence it was not distinguishable from zero.

The rate coefficients calculated for CT had less significant standard errors than those found for PCE, however in this case, it was difficult to make conclusions regarding the k values because in a number of the runs detection limit concentrations were used instead of real measured values. It is only valid to discuss runs where CT was actually detected otherwise it is the minimum value that is calculated and it is not valid to make comparisons. The runs where the outlet concentration was measured for CT were runs 6, 7, 8 and 9. In these runs all of the mean values of k were within 2 standard errors of each other and hence it was concluded that they could not be distinguished from each other. An

average rate coefficient of 5.33 L/g VSS*d was calculated using the values found in runs 6, 7, 8 and 9 and this value will be used in further calculations.

Resulting CT rate coefficients from runs 14 and 15 indicated that an increase in OLR may have had a decreasing effect on the k value. The rate coefficient decreased from 32.7 to 19.3 L/g VSS*d when the OLR was increased from 0.3 to 1.7 g COD/L*d. This result was not conclusive since outlet concentrations were not actually detected.

The rate coefficients found for DCM had significant standard errors. In all cases, except in run 14, the mean value of k was within one standard error of zero. In the case of run 14, detection limit concentrations were used which was probably why the standard error was significantly smaller than those calculated for other runs. Because of the large errors associated with the DCM rate coefficients it was not possible to make any conclusions from the data, other than the rates were essentially 0.

To see how well the model outlet concentration values predicted the observed outlet concentrations, figures 4-18 to 4-20 were constructed. Observed outlet concentrations for each target compound are plotted against the mean model predicted concentration.

Figure 4-18: PCE concentration observed values versus model predicted values - runs 7,8 and 9

Figure 4-19: CT concentration observed values versus model predicted values - runs 6-15.

Figure 4-20: DCM concentration observed values versus model predicted values - runs 7-15.

The mean model predicted outlet concentrations represented the observed outlet concentrations well. For all three of the VOC's there appeared to be an even amount of scatter along the ideal trend line. It was concluded that the model was a good representation of the observed CT data and of the kinetics occurring in the anaerobic reactor.

4.6. COMPARISONS BETWEEN EXPERIMENTAL CONTINUOUS FLOW RESULTS AND LITERATURE

Doong and Wu (1995) reported second order rate coefficients of 0.6-4.1 L/g VSS*d for CT in batch fed reactors, based on Monod kinetics. These rate constants had approximately the same range when both low cosubstrate concentrations and no cosubstrate was provided to the inoculum, indicating the

biotransformation of CT can be supported with virtually minimal cosubstrate loading. These results are consistent with those found in this research. An average rate coefficient of 5.33 L/g VSS*d was calculated and is slightly higher than the range reported by Doong and Wu. There was no literature reported that dealt with the degradation of CT in continuous flow reactors, however it is apparent from this study and previous batch studies that a high level of treatment of CT can be expected in anaerobic processes under fairly cost effective conditions (i. e., short HRT's and low OLR's).

Vogel and McCarty (1985) reported high removals of PCE in UASB reactors (approximately 98%). Experiments were conducted at HRT's of 6 days and the active methanogenic conditions in the reactor had been established over a number of years. DE Bruin et al. (1992) also reported high removals of PCE in UASB reactors (98% recovered as ethene) under HRT's of 24 to 6 hours. The HRT's were decreased to 12 and 6 hours after approximately 200 days of operation to ensure the process was stable and steady state conditions had been achieved. High PCE removals were not achieved in the current study when the HRT was decreased below 24 hours. Therefore the high removals of PCE under long HRT's found during this study were consistent with those results found by Vogel and McCarty (1985). The high removals of PCE determined by DE Bruin et al. (1992) at short HRT's were not consistent with results found in this study. It was assumed that acclimation of the biomass to PCE and a relatively long waiting period for steady state conditions may have contributed to the results found by DE Bruin et al. (1992). As mentioned previously, the long

acclimation requirement of the biomass has both positive and negative implications for a full scale process. Degradation rates may tend to increase, but the additional cost and time requirements may not be realistic.

Hughes and Parkin (1996) reported the complete transformation of DCM in an upflow attached growth system. A 283 day period was allowed before the onset of the study, at which time CF was fed alone to develop an acclimated culture. The reactor operated at an HRT of 7 days. The current results showed high removals of DCM at an HRT of 2 days and are consistent with the results reported by Hughes and Parkin (1996).

Results found in this study along with those reported in the literature indicated the PCE and DCM are not treated effectively in a continuous flow system at a high rate (i. e., short HRT). It is evident however, that CT is effectively treated in a system where the HRT is relatively short and minimal cosubstrate is required.

4.7. COD, METHANE, VSS AND pH DATA FOR CONTINUOUS FLOW COLUMN

4.7.1. COD and Methane Data

COD concentrations and methane productions were recorded for each sampling period as an indication of the activity of the biomass. The following tables show the COD data collected over the course of the experiments, the methane fractions, and the volumes of methane produced over each sample period.

Table 4-22: COD concentrations and methane production data for runs 2-15

RUN #	DAY	MEASURED INFLUENT COD (g/L)	MEASURED OLR (g COD/L*d)	EFFLUENT COD (g/L)	COD % REMOVAL	CH ₄ PROD. (L/D)	CH ₄ %
2	1	9.1	4.8	1.1	88	19.3	59
2	2	11.3	5.9	0.8	93	19.3	60
2	3	11.2	5.9	0.9	92	17.7	60
3	1	12.0	6.3	0.4	97	13.1	59
3	2	7.5	3.9	0.4	95	15.8	62
3	3	8.7	4.6	0.4	96	14.2	61
4	1	6.4	3.4	0.2	97	13.0	60
4	2	12.7	6.7	0.3	97	15.1	59
5	1	7.2	3.8	0.3	95	10.6	61
5	2	6.0	3.2	0.2	96	10.2	61
5	3	8.1	4.3	0.02	100	10.5	61
6	1	0.5	0.5	0.9	0	0.6	59
6	2	0.6	0.6	0.8	0	0.6	60
6	3	0.6	0.6	0.8	0	0.7	60
7	1	0.9	0.9	1.0	0	0.3	66
7	2	0.5	0.5	0.3	24	0.2	70
7	3	1.3	1.3	0.9	28	0.1	76
8	1	0.4	0.4	0.3	17	0.0	66
8	2	0.7	0.7	0.4	48	0.0	85
8	3	0.4	0.4	0.4	1	0.2	73
9	1	1.0	2.0	0.8	18	0.0	6
9	2	1.0	2.0	0.9	3	0.0	10
9	3	0.9	1.8	0.7	18	0.0	10
10	1	0.2	0.4	0.3	0	0.18	73
10	2	0.2	0.4	0.16	31	0.12	73
10	3	0.2	0.4	0.18	28	0.34	71
11	1	0.12	0.4	0.09	21	0.06	69
11	2	0.15	0.5	0.08	51	0.16	67
11	3	0.14	0.4	0.10	27	0.20	62
12	1	0.20	0.8	0.11	45	0.50	57
12	2	0.12	0.5	0.10	18	0	41
12	3	0.21	0.8	0.16	27	0.03	40
13	1	0.20	1.7	0.13	34	0	34
13	2	0.18	1.5	0.30	0	0	29
13	3	0.19	1.6	0.30	0	0	29
14	1	0.06	0.2	0.09	0	0	21
14	2	0.07	0.3	0.06	12	0	19
15	1	0.44	1.8	0.33	24	0.02	25
15	2	0.42	1.7	0.30	28	0.04	22

Low CH_4 fractions and gas volumes were observed during run 9, however COD removal was observed. It was suspected that there was a headspace leak in the reactor, which caused the apparent low CH_4 volumes.

In some instances the measured effluent COD was actually higher than the measured influent COD. It was believed that this may have been due to limitations of the COD analysis at these small concentrations. Products of endogenous decay may also have contributed to higher effluent COD values. In such cases where there appeared to be negative COD removal, it was assumed that the removal of COD was 0%.

The following series of plots describe VOC removals with respect to the methane production in the reactor per day normalized to the amount of COD removed.

Figure 4-21: PCE removal in UASB reactor versus normalized methane production for runs 2-15

Figure 4-22: CT removal in UASB reactor versus normalized methane production for runs 2-15

Figure 4-23: DCM removal in UASB reactor versus normalized methane production for runs 2-15

Figures 4-21 to 4-23 describe the normalized methane production per day with respect to the % removal of each VOC from the UASB reactor. The PCE data suggests that there was an increasing trend between methane production and PCE removal. Figure 4-21 indicates that higher PCE removals were observed when the normalized methane production was increased, correlating to an increase in methanogenic activity. This result was consistent with the findings from batch test experiments and suggests that methanogens are responsible for the degradation of PCE.

CT was removed from the UASB reactor regardless of the amount of methane produced, and high removals occurred even when there was no methane production. This result was also consistent with the batch test results and indicates that methanogens are not responsible for the degradation of CT.

There was not an apparent trend in the DCM and normalized methane data. DCM was removed with and without production of methane and no conclusions could be made regarding the relationship between DCM degradation and microbial activity.

A ratio of 0.395 L CH₄/g COD destroyed at 35°C is the theoretical methane equivalence and is a constant, observed in most anaerobic systems. The data collected in this research indicated methane to COD ratios varying from between 0 and 0.9 L CH₄/g COD. It was believed that the relative insensitivity of the gas wet-tip meter (80 mL/tip) may have caused some error with respect to the methane and COD data. If gas production data was collected in between a tip, the sample was not actually representative of the total gas produced. As

well, some of the initial COD influent concentrations were lower than expected, and lower than what the methane production would have suggested. If the COD influent concentrations in the earlier set of runs were assumed to equal the target COD concentration, and the number of tips on the wet-tip meter was increased by one to account for the insensitivity of the meter, the relatively wide range of methane to COD ratios was decreased.

4.7.2. VSS Data

VSS (Volatile Suspended Solids) data was collected during each run and the data is presented in table 4-23. VSS data was collected to assess the amount of biomass leaving the system.

Table 4-23: VSS data collected from runs 2-15

RUN #	DAY	VSS IN EFFLUENT (g/L)
2	1	0.2
2	2	0.4
2	3	0.6
3	1	0.7
3	2	0.3
3	3	0.1
4	1	-
4	2	0.2
5	1	0.1
5	2	0.3
5	3	0.2
6	1	0.4
6	2	0.3
6	3	0.4
7	1	0.2
7	2	0.1
7	3	0.1
8	1	0.2
8	2	0.3
8	3	0.1
9	1	-
9	2	0.5
9	3	-
10	1	0.3
10	2	0.2
10	3	0.3
11	1	0.5
11	2	0.2
11	3	0.3
12	1	0.3
12	2	0.3
12	3	0.3
13	1	0.4
13	2	0.2
13	3	-
14	1	-
14	2	0.3
15	1	0.4
15	2	-

The VSS exiting the system was small during all of the runs, varying from between 0.1 g VSS/L to 0.7 g VSS/L.

4.7.3. *pH Data*

When the pH in an anaerobic process drops below approximately 6.5 the environment becomes too acidic for methanogens to thrive, the reactor becomes unstable, and acetogenic bacteria generally dominate. For this reason the pH of the reactor was regularly monitored and the data was presented in table 4-24.

Table 4-24: pH data collected during runs 2-15

RUN #	DAY	pH
2	1	8.6
2	2	8.5
2	3	8.4
3	1	8.2
3	2	8.3
3	3	8.3
4	1	6.9
4	2	6.8
5	1	6.9
5	2	6.9
5	3	7.0
6	1	7.0
6	2	7.0
6	3	6.9
7	1	7.9
7	2	7.0
7	3	7.1
8	1	7.4
8	2	7.4
8	3	7.4
9	1	6.9
9	2	7.0
9	3	7.1
10	1	7.0
10	2	7.4
10	3	7.6
11	1	7.8
11	2	7.8
11	3	8.0
12	1	7.8
12	2	7.8
12	3	7.5
13	1	7.5
13	2	7.4
13	3	7.4
14	1	7.7
14	2	7.7
15	1	7.4
15	2	7.4

The pH varied from 6.8 to 8.6 in runs 2-15. This was in the viable range for methanogens, and therefore it was assumed that the reactor was stable throughout the course of the experiments.

4.8. SIMULATION OF A TYPICAL FIELD CASE

The UASB reactor studied here is the second part of a hybrid system designed to remove VOC's from a contaminated air stream and treat those VOC's in an anaerobic system. Collins (1998) modeled a full scale packed column where VOC's from a contaminated air stream were removed into an oil stream which was then directed to a proposed anaerobic system. In the proposed anaerobic system VOC-contaminated oil was introduced to the anaerobic reactor above the biomass and recycled reactor water was trickled through the oil layer. Mass transfer of the VOC's from the oil to the water was observed and mass transfer coefficients were determined. It was assumed that the VOC's in the water were treated at 100% efficiency to ensure maximum mass transfer from the oil to the water. This section describes the sizing of the anaerobic reactor to effectively treat the VOC's entering the system from the proposed mass transfer column. For the purpose of this model, CT was the only compound used in determining the reactor volume, since it was the only compound that was removed significantly and consistently during the anaerobic study. CT was also the only compound where a rate coefficient was determined.

Collins (1998) determined that at an inlet CT gas concentration of 0.19 mg/L and gas flow of 1000000 mL/min into the mass transfer packed column, an

oil flow of 50000 mL/min with a concentration of 5.46 mg CT/L resulted to effectively treat the gas stream. The oil stream was then directed into an anaerobic reactor where recycled water was sprinkled over the oil layer and mass transfer of the VOC's from the oil to the water was assumed to occur. Using previously determined mass transfer coefficients it was calculated that the oil layer in the anaerobic column was decreased to a concentration of 1.85 mg CT/L (Collins, 1998).

From the model proposed by Collins (1998) a mass flux of CT was determined from the oil to the liquid phase. This mass flux was incorporated into a rearranged mass balance equation (equation 4-6) to determine the anaerobic reactor volume required to effectively treat the CT-contaminated liquid stream. Assuming an oil flow of 50000 mL/min, an inlet CT concentration in the oil of 5.46 mg/L and an outlet CT concentration in the oil of 1.85 mg/L, a total mass flux of CT of into the water was calculated to be 260000 mg/d. A schematic of the anaerobic system as proposed by Collins (1998) is shown in figure 4-24.

Figure 4-24: Proposed anaerobic treatment system, Collins (1998).

The overall VOC mass balance equation (equation 4-3) was rearranged to solve for the volume of a full scale anaerobic reactor that would satisfactorily remove CT from the liquid phase, according to the mass flux into the system (equation 4-6). The volume that was solved for was the section of the anaerobic reactor labeled “biomass” in figure 4-24.

$$V = (M_{in} - H_{CT}C_{out}Q_{gas})/[(kXC_{out}) + (C_{out}/HRT)] \quad \text{(Equation 4-6)}$$

where M_{in} = total mass flux in (mg/d)

H_{CT} = Henry's Law coefficient for CT

Q_{gas} = biogas flow rate (L/d)

X = biomass concentration (g/L)

The proposed anaerobic system is slightly different from the lab apparatus used in the current experiments. The flow of nutrients and cosubstrate into the reactor is assumed to be relatively small, resulting in a long HRT. A small wasting stream is necessary to prevent the build-up of salts, as well as to minimize the mass of CT exiting the system. Since the volumetric loading and the organic loading to the reactor is small, the production of biogas and the mass flow of CT out of the system may be considered negligible, and the mass balance equation reduces to equation 4-7.

$$V = M_{in} / kXC_{out} \quad \text{(Equation 4-7)}$$

Initially, the anaerobic reactor described in figure 4-30 was modeled using equation 4-6 assuming a number of different HRT's and biogas flows. The required reactor volume was determined for the different conditions. The HRT was varied from 2-40 days and then the flow of biogas was varied between 0-10000 L/d. These results are given in table 4-25.

HRT (Days)	BIOGAS FLOW (L/d)	CT Concentration out (mg/L)	Reactor volume (m ³)
2	100000	0.01	236
4	100000	0.01	237
40	100000	0.01	237
2	1000	0.01	238
2	100	0.01	238
2	0	0.01	238

Table 4-25: Required reactor volumes assuming varying HRT's and biogas flows.

Table 4-25 shows the calculated reactor volumes for the modified anaerobic system assuming a CT rate coefficient of 5.33 L/g VSS*d and varying HRT's and biogas flow rates. It was apparent from the results that a change in either the HRT or the biogas flow rate did not significantly affect the required reactor volume. An increase in the HRT by a multiple of 10 (4 to 40 days) did not cause the reactor volume to change. Decreasing the biogas flow rate from 100000 L/d to 0 L/d only caused the reactor volume to increase by 2 m³. These results indicated that both the HRT and the biogas flow rate were negligible and the reactor was modeled again using the simplified mass balance equation (equation 4-7). The desired outlet CT concentration was varied to observe its impact on the required reactor volume. The results are given in table 4-26.

CONC. CT OUT (mg/L)	REACTOR VOLUME (m ³)
0.1	24
0.01	231
0.001	2306

Table 4-26: Required reactor volumes for varying CT outlet concentrations

The results in table 4-26 emphasizes the impact of the desired outlet CT concentration on the required reactor volume. A decrease in the effluent concentration by a factor of 100 resulted in an increase in the volume by a factor of 100. When deciding on the desired outlet CT concentration it should be taken into consideration that the mass transfer coefficients taken from Collins (1998) were calculated assuming 100% removal of CT from the anaerobic system. Therefore, low CT concentrations in the supernatant are necessary for these mass transfer coefficients to hold true. The results from the model of the anaerobic reactor determined that restrictions regarding the desired effluent CT concentration will be the main factor behind the design of the anaerobic reactor volume.

5. CONCLUSIONS AND RECOMMENDATIONS

5.1. CONCLUSIONS

The removal of PCE, CT and DCM was investigated in a high rate anaerobic process. Batch tests and continuous flow studies were performed to determine the viability of the process.

Batch tests were performed to determine which cosubstrate was to be used in continuous flow studies. VOC removal in serum bottles was observed over time and the cosubstrate with the best removal was to be selected for use in further studies. Propylene glycol was chosen from methanol, ethylene glycol and ethanolamine due to its relatively low toxicity, high degradation potential and the removal of the VOC's in its presence. The degradation rate for PCE and DCM in serum bottles containing propylene glycol was determined to be (0.110 ± 0.013) $\mu\text{mol PCE gVSS}^{-1}\text{d}^{-1}$ and (3.53 ± 0.91) $\mu\text{mol DCM gVSS}^{-1}\text{d}^{-1}$ respectively.

Methane production was also observed in serum bottle tests and it was concluded that CT was most likely degraded by acetogens and PCE by methanogens and DCM by both methanogens and acetogens. CT degradation was very rapid and it was believed that it would degrade relatively easy in continuous flow studies compared to DCM and PCE.

The breakdown compounds of CF and TCE were observed in serum bottle tests indicating their rate of degradation was slower than that of their parent compound. Vinyl chloride and dichloroethene were not detected in serum bottle tests.

Continuous flow studies were divided into two separate categories. The first set of runs was operated under relatively high OLR's (3.7- 5.5 g COD/L*d) and long HRT's (48 hours) and the second set of runs was operated under relatively low OLR's (0.4-2 g COD/L*d) and short HRT's (24-3 hours).

Removals approached 100% for all three target compounds when the UASB was operated under the higher OLR and longer HRT. Rate coefficients were calculated for these runs but comparisons could not be made because detection limit concentrations were used as outlet concentrations caused by non-detects on the GC.

Removals for PCE and DCM dropped when the UASB was run under more strenuous conditions. Data variability was high so direct comparisons between factors were difficult to make. Rate coefficients were calculated and were assumed to be essentially zero since the standard error overlapped zero in all cases.

Removal of CT remained high even when the HRT was shortened and the OLR was decreased. CT removals approached 100% in all cases regardless of the HRT and OLR. Rate coefficients were calculated for CT and an average k value of 5.33 L/g VSS*d was determined.

Methane data corresponded to that of batch tests, suggesting that CT was removed by acetogens and PCE was removed by methanogens. It was believed that DCM was degraded by a combination of methanogens and acetogens.

A full scale UASB was modeled to determine the necessary volume to remove various amounts of CT. The rate coefficient obtained from experimental

data was used in a mass balance equation to back out the volume of a full scale reactor, based on influent CT data obtained from Collins, 1998. Depending on the desired HRT and effluent CT concentration, the reactor volume varied from 2347 to 231 m³.

It was concluded that DCM and PCE were not satisfactorily removed from the high rate anaerobic process. PCE was a particularly difficult compound and more research with respect to its removal in an anaerobic process should be considered.

The most important observations obtained from this research was the fact that CT was degraded in a high rate UASB reactor with relative ease. It was observed that acetogens were the most likely organisms responsible for the degradation of the CT and this was significant, since reactors are much easier to operate under acetogenic conditions than under methanogenic conditions. It is believed that a high rate anaerobic process would be a viable treatment method for CT and it would also be a relatively easy process to operate.

5.2. RECOMMENDATIONS

Further work needs to be conducted regarding the treatment of PCE and DCM in an anaerobic reactor. Longer biomass acclimation periods to PCE and DCM need to be considered before testing begins. This may result in higher degradation rates than those observed in this work.

A more detailed look into the production of the breakdown products of PCE also needs to be examined. The accumulation of VC would cause

problems regarding the viability of the proposed system, due to its relatively higher toxicity.

The anaerobic system needs to be combined with the mass transfer column described by Collins (1998) to assess the viability of the two systems operating simultaneously.

6. REFERENCES

American Public Health Association, American Water Works Association, Water Environment Federation, Standard Methods for the Examination of Water and Wastewater, 18th Edition -1992, pg.5-9 to 5-10.

Brock, T., and Madigan, M., Biology of Microorganisms, 6th ed., 1991, Prentice Hall, NJ.

Christiansen, N., Christensen, S., Arvin, E., and Ahring, B., 1997, 'Transformation of Tetrachloroethene in an Up-Flow Anaerobic Sludgeblanket reactor', Applied Microbiology and Biotechnology, vol. 47, pp. 91-94.

Collins, J., 1998, Enhancing the Scrubbing of Chlorinated Compounds from Air Streams, A thesis submitted to the Dept. of Civil and Environmental Engineering, Carleton University.

Criddle, C., DEWitt, J., and McCarty, P., 1990, 'Reductive Dehalogenation of Carbon Tetrachloride by *Escherichia coli* K-12', Applied and Environmental Microbiology, vol. 56, no. 11, pp. 3247-3254.

DE Bruin, W., Kotterman, M., Posthumus, M., Schraa, G., and Zehnder, A., 1992, 'Complete Biological Reductive Transformation of Tetrachloroethene to Ethane', Applied and Environmental Microbiology, vol. 58, no. 6, pp. 1996-2000.

DiStefano, T., Gossett, J., and Zinder, S., 1991, 'Reductive Dechlorination of High Concentrations of Tetrachloroethene to Ethene by an Anaerobic Enrichment Culture in the Absence of Methanogenesis', Applied and Environmental Microbiology, vol. 57, no. 8, pp. 2287-2291.

Doong, R., and Wu, S., 1995, 'Enhanced Biodegradation of Carbon Tetrachloride by the Supplement of Substrate and Mineral Ions Under Anaerobic Condition', Water Environment Research, vol. 67, no. 3, pp. 276-281.

Doong, R., and Wu, S., 1996, 'Effect of Substrate Concentration on the Biotransformation of Carbon Tetrachloride and 1,1,1-Trichloroethane under Anaerobic Condition', Water Research, vol. 30, no. 3, pp. 577-586.

Doong, R., Wu, S., and Chen, T., 1996, 'Anaerobic Biotransformation of Polychlorinated Methane and Ethene under Various Redox Conditions' Chemosphere, vol. 32, no. 2, pp. 377-390.

Egli, C., Scholtz, R., Cook, A., and Leisinger, T., 1987, 'Anaerobic dechlorination of tetrachloromethane to degradable products by pure cultures of

Desulfobacterium sp. and *Methanobacterium* sp.', FEMS Microbiology Letters, vol. 43, pp. 257-261.

Egli, C., Tschan, T., Scholtz, R., Cook, A., and Leisinger, T., 1988, 'Transformation of Tetrachloromethane to Dichloromethane and Carbon Dioxide by *Acetobacterium woodii*', Applied and Environmental Microbiology, vol. 54, no. 11, pp. 2819-2824.

Fathepure, B., and Boyd, A., 1988, 'Reductive Dechlorination of Perchloroethylene and the Role of Methanogenesis', FEMS Microbiology Letters, vol. 49, pp. 149-156.

Fathepure, B., Nengu, J., and Boyd, A., 1987, 'Anaerobic Bacteria that Dechlorinate Perchloroethene', Applied and Environmental Microbiology, vol. 53, no. 11, pp. 2671-2674.

Fennell, D., and Gossett, J., 1997, 'Comparison of Butyric Acid, Ethanol, Lactic Acid and Propionic Acid as Hydrogen Donors for the Reductive Dechlorination of Tetrachloroethene', Environmental Science and Technology, vol. 31, no. 3, pp. 918-926.

Fogel, M., Taddeo, A., and Fogel, S., 1986, 'Biodegradation of Chlorinated Ethenes by a Methane-Utilizing Mixed Culture', Applied and Environmental Microbiology, vol. 51, no. 4, pp. 720-724.

Freedman, D., and Gossett, J., 1989, 'Biological Reductive Dechlorination of Tetrachloroethylene and Trichloroethylene under Methanogenic Conditions', Applied and Environmental Microbiology, vol. 55, no. 9, pp. 2144-2151.

Freedman, D., and Gossett, J., 1991, 'Biodegradation of Dichloromethane and Its Utilization as a Growth Substrate under Methanogenic Conditions', Applied and Environmental Microbiology, vol. 57, no. 10, pp. 2847-2857.

Galli, R., and McCarty, P., 1989, 'Biotransformation of 1,1,1-Trichloroethane, Trichloromethane, and Tetrachloromethane by a *Clostridium* sp.', Applied and Environmental Microbiology, vol. 55, no. 4, pp. 837-844.

Hirl, P., and Irvine, R., 1997, 'Reductive Dechlorination of Perchloroethylene Using Anaerobic Sequencing Batch Biofilm Reactors (AnSBBR)', Water Science and Technology, vol. 35, no. 1, pp. 49-56.

Hollinger, C., Schraa, G., Stams, A., and Zehnder, A., 1993, 'A Highly Purified Enrichment Culture Couples the Reductive Dechlorination of Tetrachloroethene to Growth', Applied and Environmental Microbiology, vol. 59, no. 9, pp. 2991-2997.

Hughes, J., and Parkin, G., 1992, 'The Effect of Mixtures of Xenobiotics and Primary Electron Donor on the Anaerobic Biotransformation of High Concentrations of Chlorinated Aliphatics', Water Science and Technology, vol. 26, no. 1, pp. 117-126.

Hughes, J., and Parkin, G., 1996, 'Concentration Effects on Chlorinated Aliphatic Transformation Kinetics', Journal of Environmental Engineering, vol. 122, no. 2, pp. 92-98.

Hughes, J., and Parkin, G., 1996, 'Individual Biotransformation Rates in Chlorinated Aliphatic Mixtures', Journal of Environmental Engineering, vol. 122, no. 2, pp. 99-106.

LaGrega, M., Buckingham, P., and Evans, J., 1994, Hazardous Waste Management, McGraw-Hill, Inc.

Long, J., Stensel, H., Ferguson, J., Strand, S., and Ongerth, J., 1993, 'Anaerobic and Aerobic Treatment of Chlorinated Aliphatic Compounds', Journal of Environmental Engineering, vol. 119, no. 2, pp. 300-321.

Petrovskis, E., Vogel, T., and Adriaens, P., 1994, 'Effects of Electron Acceptors and Donors on Transformation of Tetrachloromethane by *Shewanella putrefaciens* MR-1', FEMS Microbiology Letters, vol. 121, pp. 357-364.

Skeen, R., Gao, J., and Hooker, B., 1995, 'Kinetics of Chlorinated Ethylene Dehalogenation Under Methanogenic Conditions', Biotechnology and Bioengineering, vol. 48, pp. 659-655.

Skeen, R., Truex, M., Petersen, J., and Hill, J., 1994, 'A Batch Reactor for Monitoring Process Dynamics During Biodegradation of Volatile Organics', Environmental Progress, vol. 13, no. 3, pp. 174-177.

Townsend, G. T., and Suflita, J. M., 1997, 'Influence of Sulfur Oxyanions on Reductive Dehalogenation Activities in *Desulfomonile tiebjei*', Applied and Environmental Microbiology, vol. 63, pp. 3594-3599.

Vogel, T., Criddle, C., and McCarty, T., 1987, 'Transformation of Halogenated Aliphatic Compounds', Environmental Science and Technology, vol. 21, no. 8, pp. 722-735.

Vogel, T., and McCarty, T., 1985, 'Biotransformation of Tetrachloroethylene to Trichloroethylene, Vinyl Chloride and Carbon Dioxide Under Methanogenic Conditions', Applied and Environmental Microbiology, vol. 49, no. 5, pp. 1080-1083.

APPENDIX A - Final Serum Bottle Test Data

Table I: PCE mass in Serum bottle test

Total PCE Mass (μmol)					
Bottle # 60	Injection #1	Injection #2	AVG	s.d.	COV
Time (d)					
0.04	0.618				
12	0.446	0.435	0.440	0.008	1.71
15	0.413	0.418	0.416	0.003	0.82
22	0.234	0.219	0.226	0.011	4.86
27	0.132	0.189	0.161	0.040	24.91
34	0.093	0.120	0.107	0.019	18.22
67	0.061	0.082	0.071	0.015	20.91
				AVG.COV	11.90
Bottle #61	Injection #1	Injection #2	AVG	s.d.	COV
Time (d)					
0.04	0.259				
12	0.330	0.337	0.334	0.005	1.51
15	0.321	0.316	0.319	0.003	1.08
22	0.080	0.101	0.091	0.014	15.79
27	0.057	0.074	0.065	0.012	17.62
34	0.020	0.019	0.020	0.001	5.20
67	0.096	0.076	0.086	0.015	17.11
				AVG.COV	9.72
Bottle #62	Injection #1	Injection #2	AVG	s.d.	COV
Time (d)					
0.04	0.522				
12	0.617	0.606	0.612	0.007	1.19
15	0.632	0.603	0.618	0.020	3.30
22	0.355	0.365	0.360	0.007	1.98
27	0.374	0.366	0.370	0.005	1.43
34	0.276	0.285	0.280	0.006	2.26
67	0.122	0.131	0.127	0.006	4.92
				AVG.COV	2.51
Bottle #63	Injection #1	Injection #2	AVG	s.d.	COV
Time (d)					
0.04	0.466				
12	0.692	0.664	0.678	0.020	2.98
15	0.659	0.631	0.645	0.020	3.15
22	0.395	0.387	0.391	0.006	1.51
27	0.415	0.428	0.421	0.009	2.18
34	0.282	0.294	0.288	0.008	2.95
67	0.147	0.125	0.136	0.016	11.39
				AVG.COV	4.02

Table I continued....

Bottle #64					
Time (d)	Injection #1	Injection #2	AVG	s.d.	COV
0.04	0.156				
12	0.314	0.311	0.313	0.002	0.57
15	0.270	0.280	0.275	0.007	2.54
22	0.108	0.109	0.108	0.000	0.07
27	0.053	0.055	0.054	0.002	3.49
34	0.035	0.022	0.028	0.009	31.67
67	0.042	0.029	0.036	0.009	25.52
				AVG.COV	10.64
Bottle #65					
Time (d)	Injection #1	Injection #2	AVG	s.d.	COV
0.04	0.332				
12	0.338	0.360	0.349	0.016	4.48
15	0.317	0.328	0.322	0.008	2.34
22	0.125	0.137	0.131	0.008	6.10
27	0.044	0.047	0.045	0.002	4.55
34	0.016	0.015	0.016	0.001	6.94
67	0.037	0.048	0.042	0.008	19.32
				AVG.COV	7.29

Table II: CT Mass in Serum Bottle Test

Total CT Mass (μmol)					
Bottle # 60					
Time (d)	Injection #1	Injection #2	AVG	s.d.	COV
0.04	0.467		0.467		
1	0.021	0.018	0.019	0.0019	9.6
12	0.001	0.001	0.001	0.0002	26.0
15	0.001	0.001	0.001	0.0000	0.6
22	0.001	0.000	0.001	0.0003	45.3
27	0.001	0.001	0.001	0.0001	10.3
34	0.001	0.001	0.001	0.0002	19.7
67	0.001	0.001	0.001	0.0002	36.1
				AVG.COV	21.09
Bottle # 61					
Time (d)	Injection #1	Injection #2	AVG	s.d.	COV
0.04	0.220		0.220		
1	0.023	0.024	0.023	0.0007	3.1
12	0.001	0.001	0.001	0.0000	1.1
15	0.001	0.001	0.001	0.0001	10.6
22	0.001	0.001	0.001	0.0001	9.1
27	0.000	0.000	0.000	0.0000	4.1
34	0.001	0.001	0.001	0.0002	26.5
67	0.001	0.001	0.001	0.0005	45.6
				AVG.COV	14.30

Table II continued....

Bottle #62					
Time (d)	Injection #1	Injection #2	AVG	s.d.	COV
0.04	0.698		0.698		
1	0.016	0.016	0.016	0.0000	0.1
12	0.001	0.001	0.001	0.0000	3.6
15	0.000	0.001	0.001	0.0001	24.0
22	0.001	0.000	0.000	0.0001	32.3
27	0.001	0.001	0.001	0.0000	2.4
34	0.001	0.001	0.001	0.0000	1.8
67	0.000	0.000	0.000	0.0001	27.4
				AVG.COV	13.09
Bottle #63					
Time (d)	Injection #1	Injection #2	AVG	s.d.	COV
0.04	0.703		0.703		
1	0.031	0.035	0.033	0.0027	8.0
12	0.001	0.001	0.001	0.0005	47.1
15	0.001	0.001	0.001	0.0001	7.7
22	0.000	0.000	0.000	0.0000	15.1
27	0.001	0.000	0.001	0.0001	10.7
34	0.000	0.000	0.000	0.0001	11.1
67	0.001	0.001	0.001	0.0001	18.1
				AVG.COV	16.84
Bottle #64					
Time (d)	Injection #1	Injection #2	AVG	s.d.	COV
0.04	0.146		0.146		
1	0.037	0.032	0.034	0.0033	9.5
12	0.001	0.001	0.001	0.0002	25.6
15	0.001	0.001	0.001	0.0003	28.2
22	0.000	0.000	0.000	0.0001	15.9
27	0.000	0.000	0.000	0.0000	4.5
34	0.001	0.000	0.000	0.0001	18.0
67	0.001	0.001	0.001	0.0001	12.9
				AVG.COV	16.37
Bottle #65					
Time (d)	Injection #1	Injection #2	AVG	s.d.	COV
0.04	0.235		0.235		
1	0.036	0.029	0.033	0.0052	15.9
12	0.001	0.001	0.001	0.0002	28.9
15	0.001	0.001	0.001	0.0002	21.7
22	0.000	0.000	0.000	0.0001	19.9
27	0.000	0.000	0.000	0.0000	0.4
34	0.000	0.000	0.000	0.0000	0.9
67	0.001	0.001	0.001	0.0004	40.7
				AVG.COV	18.36

Table III: DCM Mass in Serum Bottle Test

Total DCM Mass (μmol)					
Bottle # 60	Injection #1	Injection #2	AVG	s.d.	COV
Time (d)					
0.04	9.8		9.8	-	-
1	8.3	6.1	7.2	1.5	21.3
5	4.9	5.9	5.4	0.7	12.7
12	6.9	6.9	6.9	0.0	0.1
15	6.8	6.6	6.7	0.1	1.2
22	2.8	2.9	2.9	0.1	2.5
27	0.0	0.0	0.0	0.0	-
34	0.0	0.0	0.0	0.0	-
67	0.0	0.0	0.0	0.0	-
				AVG.COV	7.56
Bottle # 61	Injection #1	Injection #2	AVG	s.d.	COV
Time (d)					
0.04	6.5		6.5	-	-
1	5.9	6.5	6.2	0.4	6.6
5	5.4	5.4	5.4	0.0	0.0
12	4.1	4.2	4.2	0.1	1.6
15	3.8	3.7	3.8	0.1	1.4
22	1.3	0.2	0.8	0.8	106.3
27	0.0	0.0	0.0	0.0	-
34	0.0	0.0	0.0	0.0	-
67	0.0	0.0	0.0	0.0	-
				AVG.COV	2.41
Bottle # 62	Injection #1	Injection #2	AVG	s.d.	COV
Time (d)					
0.04	9.8		9.8	-	-
1	11.1	11.2	11.1	0.1	0.5
12	14.1	13.5	13.8	0.4	2.7
15	13.2	13.2	13.2	0.1	0.4
22	11.2	11.2	11.2	0.0	0.4
27	11.6	11.6	11.6	0.0	0.2
34	0.0	0.0	0.0	0.0	-
67	0.0	0.0	0.0	0.0	-
				AVG.COV	0.84
Bottle # 63	Injection #1	Injection #2	AVG	s.d.	COV
Time (d)					
0.04	4.5		4.5	-	-
1	10.9	14.3	12.6	2.4	19.1
12	14.5	14.6	14.5	0.1	0.5
15	14.3	13.5	13.9	0.6	4.1
22	11.6	11.6	11.6	0.0	0.2
27	11.5	11.8	11.7	0.2	2.0
34	0.0	0.0	0.0	0.0	-
67	0.0	0.0	0.0	0.0	-
				AVG.COV	5.17

Table III continued...

Bottle #64					
Time (d)	Injection #1	Injection #2	AVG	s.d.	COV
0.04	6.5		6.5	-	-
1	6.4	5.8	6.1	0.4	6.8
5	10.5	6.3	8.4	3.0	35.4
12	7.4	7.7	7.5	0.2	2.6
15	4.9	5.0	5.0	0.0	1.0
22	0.2	0.0	0.1	0.1	141.4
27	0.0	0.0	0.0	0.0	-
34	0.0	0.0	0.0	0.0	-
67	0.0	0.0	0.0	0.0	-
				AVG.COV	11.44
Bottle #65					
Time (d)	Injection #1	Injection #2	AVG	s.d.	COV
0.04	6.3		6.3	-	-
1	7.9	4.5	6.2	2.4	38.6
5	11.3	12.8	12.1	1.1	8.8
12	7.9	8.8	8.4	0.7	8.3
15	6.8	6.7	6.8	0.0	0.7
22	0.0	0.0	0.0	0.0	-
27	0.0	0.0	0.0	0.0	-
34	0.0	0.0	0.0	0.0	-
67	0.0	0.0	0.0	0.0	-
				AVG.COV	14.09

APPENDIX B - Continuous Flow UASB Reactor Raw Data

RUN #4

		COUNTS		COUNTS		COUNTS		COUNTS		Avg. mass in		Avg. mass out		Total mass	
		sample 1	sample 2	sample 3	sample 4	μmol		μmol		out μmol		%Removal			
DATE:		Apr. 3	Apr. 3	Apr. 3	Apr. 3										
PCE counts:															
	inlet	44099	53274	79116	85287			1.170							
	outlet	1004	1496	1443	1017					0.00					
	offgas	6648	6133	5348						0.00		0.000		100.0	
CT counts:															
	inlet	33156	36781	62663	70972			1.183							
	outlet	568	840	1291	1300					0.00					
	offgas	0	0	0						0.00		0.000		100.0	
DCM counts:															
	inlet	250	343	377	526			35.032							
	outlet	0	0	0	0					0.00					
	offgas	0	0	0						0.000		0.000		100.0	
COD (mg/L):		MEASURED		AVG(mg/L)		% reduction									
	inlet	55465	6402	96.8											
	outlet	203													
METHANE:					AVG										
	% CH4	59.8		59.8											
	wet-tip	36884													
	total gas (L)	21.68													
	total CH4 (L)	12.96													
VSS, TSS:															
	Crucible(g)														
	+dried solids														
	-Vol. solids														
	TSS (g/L)	0	0.0000	0	0										
	VSS (g/L)	0.0000	0.0000	0	0										
	pH:	6.9													
	Effluent (L):	2.3													
	VSS lost (g)	0.0000	0.0000	0	0										

RUN #5

DATE:		COUNTS		COUNTS		COUNTS		Avg. mass in		Avg. mass out		Total mass			
		sample 1		sample 2		sample 3		μmol		μmol		out μmol		%Removal	
		Mar. 14		Mar. 14		Mar. 14									
PCE counts:															
inlet		135035		126485				3.486							
outlet		2424		2287		3569				0.00					
offgas		5743		4859		6238				0.00		0.000		100.0	
CT counts:															
inlet		111122		111527				3.757							
outlet		334		0		0				0.00					
offgas		0		0		0				0.00		0.000		100.0	
DCM counts:															
inlet		687		812				97.235							
outlet		0		0		0				0.00					
offgas		0		0		0				0.00		0.000		100.0	
COD (mg/L):		MEASURED		AVG(mg/L)		% reduction									
inlet		52639		8068		99.8									
outlet		17													
METHANE:						AVG									
% CH4		61		61		61									
wet-tip		33738													
total gas (L)		17.2													
total CH4 (L)		10.49													
VSS, TSS:															
Crucible(g)		76.6886		64.9565											
+dried solids		76.7854		65.0604											
-Vol. solids		76.7797		65.0529											
TSS (g/L)		2.42		2.5975		2.5087									
VSS (g/L)		0.1425		0.1875		0.1650									
pH:		7													
Effluent (L):		2.35													
VSS lost (g)		0.3349		0.4406		0.38775									

RUN # 6

DATE: 22-Apr											
	COUNTS		COUNTS	Conc. IN 1 mg/L	Conc. IN 2 mg/L	MASS IN μmol	Conc. OUT 1 mg/L	Conc. OUT 2 mg/L	Total mass out (μmol)	Removal %	
	sample1	sample2									
PCE counts:											
inlet	1743101	1775554		0.291	0.297	8.686					
outlet	186383	208853					0.282	0.316		-29.63	
offgas	499993	543853					0.401	0.436	11.260		
CT counts:											
inlet	12552937	13374701		0.426	0.454	14.032					
outlet	20850	13250					0.007	0.004		98.72	
offgas	4328	4282					0.002	0.002	0.179		
DCM counts:											
inlet	42116	44223		1.606	1.687	94.983					
outlet	8071	8495					2.635	2.784		-71.89	
offgas	12024	12125					0.614	0.619	163.268		
TCE counts:											
inlet											
outlet	8936	9661					0.020	0.023			
offgas	17838	18003					0.050	0.051	0.951		
CF counts:											
inlet											
outlet	2056	2176					-0.033	-0.032			
offgas	3754	3727					-0.022	-0.022	-1.375		
COD (mg/L):											
inlet	5277	580	AVG. OVER FLOW		% reduction						
outlet	797				-37.31						
METHANE:											
% CH4	59.5		AVG		59.5						
wet-tip	38268										
total gas (L)	0.96										
total CH4 (L)	0.57										
Crucible(g)	77.7868	66.8582									
+dried solids	77.8877	66.956									
-Vol. solids	77.8739	66.9438									
TSS (g/L)	2.5225	2.445	2.48375								
VSS (g/L)	0.3450	0.3050	0.3250								
pH:	7.0000										
Effluent (L):	4.9										
VSS lost (g)	1.6905	1.4945	1.5925								

RUN # 6

COUNTS		COUNTS	Conc. IN 1	Conc. IN 2	MASS IN	Conc. OUT 1	Conc. OUT 2	Total mass	Removal
sample1	sample2	23-Apr	mg/L	mg/L	μmol	mg/L	mg/L	out (μmol)	%
DATE: 23-Apr									
PCE counts:									
inlet	1227398	1789483	0.205	0.299	7.295				
outlet	273431	209532				0.414	0.317		-76.86
offgas	453348	402095				0.363	0.322	12.902	
CT counts:									
inlet	12363123	12242519	0.420	0.416	13.045				
outlet	3324	3045				0.001	0.001		99.67
offgas	3927	4145				0.002	0.002	0.042	
DCM counts:									
inlet	24536	39404	0.926	1.501	68.581				
outlet	9526	9368				3.146	3.090		-169.14
offgas	12547	12243				0.642	0.626	184.581	
TCE counts:									
inlet									
outlet	11251	10737				0.028	0.026		
offgas	17401	18429				0.049	0.052	1.166	
CF counts:									
inlet									
outlet	2901	2803				-0.028	-0.028		
offgas	4140	4350				-0.020	-0.018	-1.156	
COD (mg/L):			AVG. OVER FLOW		% reduction				
Inlet	5009	551							-52.10
outlet	838								
METHANE:			AVG						
% CH4	60.2								60.2
wet-tip	38282								
total gas (L)	1.12								
total CH4 (L)	0.67								
Crucible(g)	79.5771	77.5093							
+dried solids	79.6655	77.5987							
-Vol. solids	79.6489	77.5804							
TSS (g/L)	2.21	2.235							2.2225
VSS (g/L)	0.4150	0.4575							0.4362
pH:	6.9000								
Effluent (L):	4.8								
VSS lost (g)	1.992	2.196							2.094

RUN # 7

COUNTS		COUNTS	Conc. IN 1	Conc. IN 2	MASS IN	Conc. OUT 1	Conc. OUT 2	Total mass	Removal
sample1	sample2		mg/L	mg/L	µmol	mg/L	mg/L	out (µmol)	%
DATE: 30-Jul-98									
PCE counts:									
inlet	2,947,233	1,694,242	2.687	1.544	69.781				
outlet	200,614	200,830				0.304	0.304		85.24
offgas	487,461	539,119				0.391	0.432	10.303	
CT counts:									
inlet	15,301,436	8,073,352	2.836	1.496	77.032				
outlet	48,558	46,500				0.015	0.015		99.31
offgas	5,027	5,628				0.002	0.002	0.529	
DCM counts:									
inlet	46,272	26,361	9.634	5.437	485.332				
outlet	0	0				0.100	0.100		98.63
offgas	3,464	3,346				0.018	0.018	6.643	
TCE counts:									
inlet	0	0							
outlet	38,106	38,623				0.118	0.120		
offgas	112,750	107,033				0.368	0.348	5.068	
CF counts:									
inlet	0	0							
outlet	12,037	11,540				0.031	0.028		
offgas	30,270	27,876				0.149	0.134	1.392	
COD (mg/L):									
inlet	2179	1307							
outlet	932								
METHANE:									
% CH4	76.2	75.8	AVG	76					
wet-tip	4.36								
total gas (L)	0.11								
total CH4 (L)	0.09								
Crucible(g)	74.2698	66.8652							
+dried solids	74.3607	66.9554							
-Vol. solids	74.3565	66.9561							
TSS (g/L)	2.2725	2.255	2.2637						
VSS (g/L)	0.105	-0.0175	0.04375						
pH:	7.15								
Effluent (L):	5.5								
VSS lost (g)	0.57435	-0.095725	0.2393125						

RUN # 8

DATE: 5-Aug-98									
COUNTS	COUNTS	COUNTS	Conc. IN 1	Conc. IN 2	MASS IN	Conc. OUT 1	Conc. OUT 2	Total mass	Removal
sample1	sample2		mg/L	mg/L	μmol	mg/L	mg/L	out (μmol)	%
PCE counts:									
inlet	897,414	862,642	0.818	0.786	25.817				
outlet	263,539	222,448				0.399	0.337		
offgas	1,115,209	1,120,548				0.895	0.899	11.852	54.09
CT counts:									
inlet	3,181,315	3,902,525	0.590	0.723	22.793				
outlet	0	7,876				0.000	0.003		99.79
offgas	3,927	6,243				0.001	0.001	0.048	
DCM counts:									
inlet	14,436	12,624	2.923	2.541	171.753				
outlet	3,199	2,758				0.923	0.768		69.06
offgas	8,182	8,656				0.408	0.433	53.146	
TCE counts:									
inlet	514	104							
outlet	7,707	7,737				0.016	0.016		
offgas	26,579	26,939				0.079	0.081	0.664	
CF counts:									
inlet	5,787	4,650							
outlet	15,596	13,213				0.054	0.039		
offgas	43,231	44,755				0.233	0.243	2.089	
COD (mg/L):									
inlet	1189	714							
outlet	367								
METHANE:									
% CH4	84.9	85	AVG						
wet-tip	0								
total gas (L)	0.00								
total CH4 (L)	0.00								
Crucible(g)	66.8648	74.2684							
+dried solids	66.9551	74.3617							
-Vol. solids	66.9437	74.3496							
TSS (g/L)	2.26	2.3325	2.295						
VSS (g/L)	0.3	0.3025	0.29375						
pH:	7.4								
Effluent (L):									
VSS lost (g)	1.5219	1.6154	1.568625						

RUN # 9

COUNTS		COUNTS	Conc. IN 1	Conc. IN 2	MASS IN	Conc. OUT 1	Conc. OUT 2	Total mass	Removal
sample1	sample2		mg/L	mg/L	μmol	mg/L	mg/L	out (μmol)	%
DATE: 26-Aug-98									
PCE counts:									
inlet	5,150,688	1,343,053	4.632	1.207	170.875				
outlet	215,037	856,315				0.326	1.300		72.02
offgas	1,443,851	2,228,156				1.159	1.788	47.804	
CT counts:									
inlet	27,858,590	7,311,254	5.093	1.337	202.837				
outlet	140,094	202,478				0.043	0.063		98.35
offgas	93,716	144,307				0.019	0.030	3.352	
DCM counts:									
inlet	84,050	19,495	17.359	3.935	1216.653				
outlet	0	0				0.100	0.100		99.06
offgas	0	0				0.018	0.018	11.418	
TCE counts:									
inlet	141,306	0							
outlet	870	0				-0.007	-0.009		
offgas	0	0				-0.009	-0.009	-0.594	
CF counts:									
inlet	31,232	6,176							
outlet		125,110							
offgas	17,970	31,468				0.070	0.157	61.920	
COD (mg/L):									
inlet		1025							
outlet	840								
METHANE:									
% CH4	4.2	8.4	6.3						
wet-tip	1								
total gas (L)	0.03								
total CH4 (L)	0.00								
Crucible(g)	77.7911	79.5711							
+dried solids	77.90	79.6821							
-Vol. solids	77.8	79.6243							
TSS (g/L)	2.7475	2.775	2.76125						
VSS (g/L)	1.715	1.445	1.58						
pH:	7.0								
Effluent (L):	9.7								
VSS lost (g)	16.644075	14.023725	15.3339						

RUN # 9

COUNTS		COUNTS	Conc. IN 1	Conc. IN 2	MASS IN	Conc. OUT 1	Conc. OUT 2	Total mass	Removal
sample1	sample2		mg/L	mg/L	μmol	mg/L	mg/L	out (μmol)	%
DATE: Aug. 28									
PCE counts:									
inlet	4375681.5	4198823	3.935	3.776	225.270				
outlet	1570084.2	386930.4				2.385	0.587		61.46
offgas	2765474.8	2333730.5				2.220	1.873	86.817	
CT counts:									
inlet	28038482	25138190	5.126	4.595	306.180				
outlet	1275506.5	70232.7				0.394	0.022		95.72
offgas	0	0				0.002	0.002	13.102	
DCM counts:									
inlet	58273.6	61498.2	11.999	12.670	1407.138				
outlet	0	0				0.100	0.100		99.19
offgas	0	0				0.018	0.018	11.408	
TCE counts:									
inlet	0	0							
outlet	4440.7	759.3				0.005	-0.007		
offgas	260101	240891.5				0.860	0.796	-0.059	
CF counts:									
inlet	0	0							
outlet	168816.1	36437.9				1.044	0.189		
offgas	0	0				-0.046	-0.046	50.029	
COD (mg/L):									
inlet		1004							
outlet	975								
METHANE:									
% CH4	9.8	9.8	9.8	9.8	9.8	AVG			
wet-tip	0								
total gas (L)	0								
total CH4 (L)	0.00								
Crucible(g)	73.2178	76.703							
+dried solids	73.3147	76.8029							
-Vol. solids	73.2931	76.7809							
TSS (g/L)	2.4225	2.4975	2.4600						
VSS (g/L)	0.54	0.55	0.545						
pH:	7								
Effluent (L):	9.7								
VSS lost (g)	5.232	5.329	5.281						

RUN # 9

DATE:		COUNTS	COUNTS	Conc. IN 1	Conc. IN 2	MASS IN	Conc. OUT 1	Conc. OUT 2	Total mass	Removal
sample1	sample2	mg/L	mg/L	μmol	mg/L	mg/L	mg/L	mg/L	out (μmol)	%
29-Aug										
PCE counts:										
inlet										
outlet	3840975			195.901						
offgas	1363637.7	1423896.5			2.071	2.163				38.71
	614491.5	669349.3			0.493	0.537	120.066			
CT counts:										
inlet	19544020	0.000		109.236						
outlet	801927.3	247864.6			0.248	0.077				90.92
offgas	0	0			0.019	0.019	9.923			
DCM counts:										
inlet	16998.9	54080.4	3.416	11.127	805.233					
outlet	0	0			0.100	0.100				98.62
offgas	0	0			0.018	0.018	11.074			
TCE counts:										
inlet	0	0								
outlet	3749.3	3271.7			0.003	0.001				
offgas	0	0			-0.009	-0.009	0.161			
CF counts:										
inlet	5431.6	1209.6								
outlet	131672.4	153811.2			0.804	0.947				
offgas	54184.3	28807.5			0.304	0.140	68.972			
COD (mg/L):										
inlet		875								
outlet	722									
METHANE:										
% CH4	9.8	9.9	9.85							
wet-tip	0									
total gas (L)	0									
total CH4 (L)	0.00									
Crucible(g)										
+dried solids										
-Vol. solids										
TSS (g/L)	0	0	0							
VSS (g/L)	0	0	0							
pH:	7.1									
Effluent (L):	9.405									
VSS lost (g)	0	0	0							

RUN # 10

COUNTS		COUNTS	Conc. IN 1	Conc. IN 2	MASS IN	Conc. OUT 1	Conc. OUT 2	Total mass	Removal
sample1	sample2	3-May	mg/L	mg/L	μmol	mg/L	mg/L	out (μmol)	%
DATE:									
PCE counts:									
inlet	594860	846775	0.097	0.138	7.067				
outlet	21254					0.031			72.97
offgas	53256	45739				0.042	0.036	1.910	
CT counts:									
inlet	7820190	10940556	0.259	0.362	20.174				
outlet	1147					0.001			99.89
offgas	4793	3338				0.0019	0.0019	0.023	
DCM counts:									
inlet	47971	56244	1.784	2.095	228.325				
outlet	6900					2.223			-15.11
offgas	11292	11031				0.575	0.561	262.830	
TCE counts:									
inlet									
outlet	8611					0.019	-0.009		
offgas	25484	24443				0.076	0.072	1.505	
CF counts:									
inlet									
outlet	1484								
offgas	2525	2416				-0.037	-0.046		
COD (mg/L):		AVG. OVER FLOW	% reduction			-0.030	-0.031	-3.487	
inlet	2163	232		30.93					
outlet	160								
METHANE:									
% CH4	73.3	73.6	AVG						
wet-tip	38432								
total gas (L)	0.16								
total CH4 (L)	0.12								
Crucible(g)	79.5823	66.8645							
+dried solids	79.6772	66.9563							
-Vol. solids	79.6639	66.9537							
TSS (g/L)	2.3725	2.295							2.33375
VSS (g/L)	0.3325	0.0650							0.1988
pH:	7.4000								
Effluent (L):									
	10								
VSS lost (g)	3.325	0.65		1.9875					

RUN # 10

	COUNTS		COUNTS	Conc. IN 1	Conc. IN 2	MASS IN	Conc. OUT 1	Conc. OUT 2	Total mass	Removal
	sample1	sample2		mg/L	mg/L	μmol	mg/L	mg/L	out (μmol)	%
DATE:	4-May	4-May								
PCE counts:										
inlet	677310	749523		0.110	0.122	6.994				
outlet	39433	43499					0.059	0.065		44.78
offgas	57028	62405					0.045	0.049	3.862	
CT counts:										
inlet	911417	986985		0.301	0.326	20.414				
outlet	17802	8341					0.006	0.003		98.65
offgas	3914	4542					0.0019	0.0019	0.276	
DCM counts:										
inlet	46702	49921		1.736	1.857	211.507				
outlet	8239	8259					2.694	2.701		-51.64
offgas	10910	11046					0.554	0.561	320.723	
TCE counts:										
inlet										
outlet	4969	5107					0.007	0.008		
offgas	14350	14510					0.038	0.039	0.616	
CF counts:										
inlet										
outlet	2428	2596					-0.031	-0.030		
offgas	3405	3556					-0.024	-0.023	-2.540	
COD (mg/L):			AVG. OVER FLOW		% reduction					
inlet	2255	241			27.53					
outlet	175									
METHANE:										
% CH4	71.7	71.2								
wet-tip	38439									
total gas (L)	0.48									
total CH4 (L)	0.34									
Crucible(g)	77.5083	77.7997								
+dried solids	77.6815	77.9687								
-Vol. solids	77.6885	77.958								
TSS (g/L)	4.33	4.225								
VSS (g/L)	0.3250	0.2675								
pH:	8									
Effluent (L):	10									
VSS lost (g)	3.25	2.675								

RUN # 11

COUNTS		COUNTS	Conc. IN 1	Conc. IN 2	MASS IN	Conc. OUT 1	Conc. OUT 2	Total mass	Removal
sample1	sample2	6-May	mg/L	mg/L	μmol	mg/L	mg/L	out (μmol)	%
DATE: 6-May									
PCE counts:									
inlet	924600	512361	0.149	0.082	8.794				
outlet	63790	79354				0.096	0.119		6.20
offgas	214676	195115				0.172	0.156	8.249	
CT counts:									
inlet	9421866	5934273	0.309	0.195	20.618				
outlet	677	1144				0.001	0.001		99.82
offgas	3335	3307				0.0019	0.0019	0.037	
DCM counts:									
inlet	49258	33777	1.815	1.238	226.496				
outlet	6834	5742				2.200	1.816		-31.80
offgas	13168	12409				0.675	0.634	298.532	
TCE counts:									
inlet									
outlet	3944	3953				0.004	0.004		
offgas	6354	5565				0.012	0.009	0.359	
CF counts:									
inlet									
outlet	4578	5742				-0.017	-0.009		
offgas	9828					0.017	-0.046	-1.374	
COD (mg/L):									
inlet	1138	121	AVG. OVER FLOW		% reduction				
outlet	95								
METHANE:									
% CH4	69.3	69.5	AVG						
wet-tip	38457								
total gas (L)	0.08								
total CH4 (L)	0.06								
Crucible(g)	66.8654	79.5833							
+dried solids	67.0564	79.7802							
-Vol. solids	67.042	79.7574							
TSS (g/L)	4.7750	4.9225	4.8488						
VSS (g/L)	0.3600	0.5700	0.4650						
pH:	7.8								
Effluent (L):	12.6								
VSS lost (g)	4.5360	7.1820	5.859						

RUN # 11

DATE:		COUNTS sample1	COUNTS sample2	Conc. IN 1 mg/L	Conc. IN 2 mg/L	MASS IN μmol	Conc. OUT 1 mg/L	Conc. OUT 2 mg/L	Total mass out (μmol)	Removal %
PCE counts:										
	inlet	1075886	1094432	0.173	0.176	16.135				
	outlet	104343	103156				0.157	0.156		9.15
	offgas	197238	193285				0.158	0.155	14.659	
CT counts:										
	inlet	9515006	10054869	0.312	0.330	31.905				
	outlet	20896	15384				0.007	0.005		98.19
	offgas	21822	8443				0.005	0.002	0.578	
DCM counts:										
	inlet	56070	54692	2.069	2.018	368.154				
	outlet	7482	6747				2.428	2.169		-12.85
	offgas	9948	9459				0.502	0.476	415.454	
TCE counts:										
	inlet									
	outlet	3549	2713				0.002	0.000		
	offgas	6354	5565				0.012	0.009	0.122	
CF counts:										
	inlet									
	outlet	8499	7738				0.009	0.004		
	offgas	12637	11977				0.035	0.031	0.795	
COD (mg/L):			AVG. OVER FLOW		% reduction					
	inlet	1435	152		50.75					
	outlet	75								
METHANE:				AVG						
	% CH4	67.1	66.8		66.95					
	wet-tip	38460								
	total gas (L)	0.24								
	total CH4 (L)	0.16								
	Crucible(g)	73.9653	74.2706							
	+dried solids	74.1281	74.4335							
	-Vol. solids	74.1182	74.4262							
	TSS (g/L)	4.07	4.0725	4.07125						
	VSS (g/L)	0.2475	0.1825	0.2150						
	pH:	7.8								
	Effluent (L):	15.3								
	VSS lost (g)	3.78675	2.79225	3.2895						

RUN # 11

COUNTS		COUNTS	Conc. IN 1	Conc. IN 2	MASS IN	Conc. OUT 1	Conc. OUT 2	Total mass	Removal
sample1	sample2	8-May	mg/L	mg/L	μmol	mg/L	mg/L	out (μmol)	%
DATE: 8-May									
PCE counts:									
inlet	883096		0.142		6.607				
outlet	66187	93001				0.099	0.140		-73.27
offgas	220129	207837				0.176	0.166	11.448	
CT counts:									
inlet	9291139		0.305		15.246				
outlet	5433					0.002			99.30
offgas	16958	12934				0.004	0.003	0.107	
DCM counts:									
inlet	50139		1.848		167.559				
outlet	6137	7012				1.955	2.262		-129.23
offgas	9335	8917				0.470	0.447	384.100	
TCE counts:									
inlet									
outlet	1335	1914				-0.005	-0.003		
offgas	4036	3850				0.004	0.003	-0.472	
CF counts:									
inlet									
outlet	9183	12460				0.013	0.034		
offgas	15360	14674				0.053	0.048	3.066	
COD (mg/L):									
inlet	1307	139	AVG. OVER FLOW		% reduction				
outlet	102								
METHANE:									
% CH4	62.4	62.1	AVG						
wet-tip	38464								
total gas (L)	0.32								
total CH4 (L)	0.20								
Crucible(g)	77.5041	77.7959							
+dried solids	77.6615	77.9446							
-Vol. solids	77.6477	77.9356							
TSS (g/L)	3.935	3.7175	3.82625						
VSS (g/L)	0.3450	0.2250	0.2850						
pH:	8								
Effluent (L):	15.4								
VSS lost (g)	5.313	3.465	4.389						

RUN # 12

COUNTS		COUNTS	Conc. IN 1	Conc. IN 2	MASS IN	Conc. OUT 1	Conc. OUT 2	Total mass	Removal
sample1	sample2	12-May	mg/L	mg/L	μmol	mg/L	mg/L	out (μmol)	%
DATE:									
PCE counts:									
inlet	588423	849383	0.094	0.136	14.251				
outlet	96307	98373				0.145	0.148		
offgas	222007	182021				0.178	0.145	18.991	-33.26
CT counts:									
inlet	6682442	9555331	0.218	0.312	35.307				
outlet	550	360				0.001	0.001		99.87
offgas	4540	4603				0.0019	0.0019	0.046	
DCM counts:									
inlet	43848	55812	1.606	2.050	441.284				
outlet	3661	3701				1.085	1.099		39.55
offgas	6417	6063				0.313	0.294	266.745	
TCE counts:									
inlet									
outlet	2671	2161				-0.001	-0.002		
offgas	3848	3746				0.003	0.003	-0.212	
CF counts:									
inlet									
outlet	16370	16375				0.059	0.059		
offgas	25837	25953				0.121	0.121	10.374	
COD (mg/L):									
inlet	1972	208	AVG. OVER FLOW		% reduction				
outlet	114								45.27
METHANE:									
% CH4	57.2	57.2	AVG						
wet-tip	38498								
total gas (L)	0.88								
total CH4 (L)	0.50								
Crucible(g)	66.8628	79.581							
+dried solids	66.9876	79.7152							
-Vol. solids	66.9808	79.6977							
TSS (g/L)	3.1200	3.3550							3.2375
VSS (g/L)	0.1700	0.4375							0.3037
pH:	7.8								
Effluent (L):	20.5								
VSS lost (g)	3.4850	8.9687							6.226875

RUN # 12

COUNTS		COUNTS	Conc. IN 1	Conc. IN 2	MASS IN	Conc. OUT 1	Conc. OUT 2	Total mass	Removal
sample1	sample2		mg/L	mg/L	μmol	mg/L	mg/L	out (μmol)	%
DATE:									
PCE counts:									
inlet	1039089	679223	0.167	0.109	17.865				
outlet	95946	92673				0.145	0.140		-3.11
offgas	186740	200013				0.149	0.160	18.421	
CT counts:									
inlet	11218894	9256401	0.366	0.302	46.693				
outlet	1387	1380				0.001	0.001		99.82
offgas	16049	8689				0.003	0.002	0.082	
DCM counts:									
inlet	52793	66148	1.938	2.434	553.391				
outlet	3324	3350				0.967	0.976		55.57
offgas	5429	4811				0.260	0.227	245.857	
TCE counts:									
inlet									
outlet	1781	2581				-0.004	-0.001		
offgas	2865	2661				0.000	-0.001	-0.360	
CF counts:									
inlet									
outlet	17601	18953				0.067	0.076		
offgas	24060	22864				0.109	0.101	12.914	
COD (mg/L):		AVG. OVER FLOW		% reduction					
inlet	1132	120		18.04					
outlet	98								
METHANE:		AVG							
% CH4	41		41						
wet-tip	38498								
total gas (L)	0								
total CH4 (L)	0.00								
Crucible(g)	73.9752	77.5033							
+dried solids	74.0678	77.5973							
-Vol. solids	74.057	77.5863							
TSS (g/L)	2.315	2.35	2.3325						
VSS (g/L)	0.2700	0.2750	0.2725						
pH:	7.8								
Effluent (L):	21.5								
VSS lost (g)	5.805	5.9125	5.85875						

RUN # 12

COUNTS		COUNTS	Conc. IN 1	Conc. IN 2	MASS IN	Conc. OUT 1	Conc. OUT 2	Total mass	Removal
sample1	sample2	14-May	mg/L	mg/L	μmol	mg/L	mg/L	out (μmol)	%
DATE:									
PCE counts:									
inlet	1065594	1170742	0.171	0.188	16.334				
outlet	98681	101709				0.149	0.153		
offgas	198866	209751				0.159	0.168	13.831	15.32
CT counts:									
inlet	10853802	12243460	0.354	0.399	36.993				
outlet	2963	2718				0.001	0.001		
offgas	3375	3099				0.0019	0.0019	0.102	99.72
DCM counts:									
inlet	61781	62083	2.272	2.283	404.904				
outlet	2368	2413				0.631	0.647		
offgas	4270	4336				0.198	0.202	113.731	71.91
TCE counts:									
inlet									
outlet	1093	1069				-0.006	-0.006		
offgas	2649	2601				-0.001	-0.001	-0.675	
CF counts:									
inlet									
outlet	20194	20478				0.084	0.086		
offgas	31691	32565				0.158	0.164	10.773	
COD (mg/L):									
inlet	2056	217							
outlet	159								
METHANE:									
% CH4	38.9	38.7							
wet-tip	38499								
total gas (L)	0.08								
total CH4 (L)	0.03								
Crucible(g)	77.7974	74.2756							
+dried solids	77.8857	74.365							
-Vol. solids	77.8706	74.3532							
TSS (g/L)	2.2075	2.235							
VSS (g/L)	0.3775	0.2950							
pH:	8								
Effluent (L):	15.1								
VSS lost (g)	5.70025	4.4545	5.077375						

	AVG. OVER FLOW	% reduction
	26.79	

RUN # 13

COUNTS		COUNTS	Conc. IN 1	Conc. IN 2	MASS IN	Conc. OUT 1	Conc. OUT 2	Total mass	Removal
sample1	sample2	16-May	mg/L	mg/L	μmol	mg/L	mg/L	out (μmol)	%
DATE:									
PCE counts:									
inlet	481874	452004	0.077	0.072	18.149				
outlet	85272	91398				0.128	0.138		-78.98
offgas	180990	194599				0.145	0.156	32.483	
CT counts:									
inlet	4769645	4347679	0.155	0.141	38.897				
outlet	531	591				0.001	0.001		99.77
offgas	3911	3132				0.0019	0.0019	0.088	
DCM counts:									
inlet	46329	42674	1.687	1.552	772.112				
outlet	3198	3356				0.922	0.978		
offgas	5050	5039				0.240	0.239	453.073	41.32
TCE counts:									
inlet									
outlet	566	837				-0.008	-0.007		
offgas	1645	1427				-0.004	-0.005	-2.202	
CF counts:									
inlet									
outlet	17063	17805				0.064	0.069		
offgas	25104	25087				0.116	0.116	22.480	
COD (mg/L):			AVG. OVER FLOW		% reduction				
inlet	1881	197						34.12	
outlet	130								
METHANE:			AVG						
% CH ₄	34.2	33.9	34.1						
wet-tip	38502								
total gas (L)	0								
total CH ₄ (L)	0.00								
VSS, TSS:									
Crucible(g)	73.9741	79.5764							
+dried solids	74.077	79.7026							
-Vol. solids	74.0654	79.6823							
TSS (g/L)	2.5725	3.1550	2.8637						
VSS (g/L)	0.2900	0.5075	0.3988						
pH:	7.5								
Effluent (L):	40.5								
VSS lost (g)	11.7450	20.5538	16.149375						

RUN # 13

COUNTS		COUNTS	COUNTS	Conc. IN 1	Conc. IN 2	MASS IN	Conc. OUT 1	Conc. OUT 2	Total mass	Removal
sample1	sample2	17-May	17-May	mg/L	mg/L	μmol	mg/L	mg/L	out (μmol)	%
DATE:										
PCE counts:										
inlet	971808	623915		0.155	0.099	30.651				
outlet	100814	123817					0.152	0.187		-33.35
offgas	195041	209114					0.156	0.167	40.873	
CT counts:										
inlet	7804326	4923775		0.253	0.160	53.629				
outlet	546	615					0.001	0.001		99.84
offgas	3837	3422					0.0019	0.0019	0.088	
DCM counts:										
inlet	43586	43731		1.585	1.591	747.946				
outlet	3579	3526					1.056	1.038		
offgas	5119	5128					0.244	0.244	493.072	34.08
TCE counts:										
inlet										
outlet	553	391					-0.008	-0.008		
offgas	692	636					-0.007	-0.007	-2.409	
CF counts:										
inlet										
outlet	19830	18589					0.082	0.074		
offgas	27771	28144					0.133	0.135	26.044	
COD (mg/L):										
inlet	1750	184		AVG. OVER FLOW			% reduction			
outlet	265								-44.36	
METHANE:										
% CH4	29.1	29.5		AVG			AVG			
wet-tip	38502									
total gas (L)	0									
total CH4 (L)	0.00									
VSS, TSS:										
Crucible(g)	66.8639	77.5026								
+dried solids	66.9443	77.5911								
-Vol. solids	66.9406	77.5789								
TSS (g/L)	2.0100	2.2125							2.1112	
VSS (g/L)	0.0925	0.3050							0.1987	
pH:	7.4									
Effluent (L):	40									
VSS lost (g)	3.7000	12.2000							7.95	

RUN # 13

COUNTS		COUNTS	Conc. IN 1	Conc. IN 2	MASS IN	Conc. OUT 1	Conc. OUT 2	Total mass	Removal
sample1	sample2	17-May	mg/L	mg/L	μmol	mg/L	mg/L	out (μmol)	%
DATE:									
PCE counts:									
inlet	599056	529415	0.095	0.084	2.708				
outlet	124674	115412				0.188	0.174		-101.72
offgas	239764	246785				0.192	0.197	5.463	
CT counts:									
inlet	5095849	4124533	0.165	0.134	4.866				
outlet	1184	1170				0.001	0.001		99.65
offgas	22070	11324				0.005	0.002	0.017	
DCM counts:									
inlet	43731	40797	1.591	1.483	90.467				
outlet	3280	3216				0.951	0.929		38.83
offgas	6012	5482				0.292	0.263	55.335	
TCE counts:									
inlet									
outlet	341					-0.008	-0.009		
offgas	564	595				-0.008	-0.008	-0.339	
CF counts:									
inlet									
outlet	18589	17714				0.074	0.068		
offgas	27156	28788				0.129	0.140	2.969	
COD (mg/L):									
inlet	1845	194							
outlet	257								
METHANE:									
% CH4	28.9	29.1							
wet-tip	38502								
total gas (L)	0								
total CH ₄ (L)	0.00								
VSS, TSS:									
Crucible(g)									
+dried solids									
-Vol. solids									
TSS (g/L)	0.0000	0.0000							
VSS (g/L)	0.0000	0.0000							
pH:	7.4								
Effluent (L):	5								
VSS lost (g)	0.0000	0.0000							
									0

RUN # 14

DATE:									
	COUNTS		Conc. IN 1 mg/L	Conc. IN 2 mg/L	MASS IN μmol	Conc. OUT 1 mg/L	Conc. OUT 2 mg/L	Total mass out (μmol)	Removal %
	sample1	sample2							
PCE counts:									
inlet	807657	979984	0.130	0.157	17.722				
outlet	114852	101677				0.173	0.153		-13.90
offgas	196640	200549				0.157	0.160	20.186	
CT counts:									
inlet	7479292	8887390	0.244	0.290	35.588				
outlet	479	0				0.001	0.001		99.91
offgas	2033	1508				0.0019	0.0019	0.031	
DCM counts:									
inlet	51732	58487	1.899	2.150	488.582				
outlet	719	708				0.1	0.1		
offgas	2181	2262				0.086	0.091	11.916	97.56
TCE counts:									
inlet									
outlet	678	606				-0.007	-0.007		
offgas	967	948				-0.006	-0.006	-0.430	
CF counts:									
inlet									
outlet	19577	18650				0.080	0.074		
offgas	29353	30094				0.143	0.148	4.974	
COD (mg/L):									
inlet	592	63							
outlet	90								
METHANE:									
% CH4	20.9	20.7	AVG						
wet-tip	38505								
total gas (L)	0								
total CH4 (L)	0.00								
VSS, TSS:									
Crucible(g)									
+dried solids									
-Vol. solids									
TSS (g/L)									
VSS (g/L)									
pH:	7.7								
Effluent (L):	20.5								
VSS lost (g)	0.0000	0.0000	0						

RUN # 14

COUNTS		COUNTS		Conc. IN 1	Conc. IN 2	MASS IN	Conc. OUT 1	Conc. OUT 2	Total mass	Removal
sample1	sample2	21-May	21-May	mg/L	mg/L	μmol	mg/L	mg/L	out (μmol)	%
DATE:										
PCE counts:										
inlet		807657	979984	0.130	0.157	17.982				
outlet		119248	105269				0.180	0.159		-18.14
offgas		243858	249524				0.195	0.200	21.243	
CT counts:										
inlet		7479292	8887390	0.244	0.290	36.108				
outlet		498	1117				0.001	0.001		99.85
offgas		18046	10733				0.004	0.002	0.055	
DCM counts:										
inlet		51732	58487	1.899	2.150	495.732				
outlet		514	668				0.1	0.1		
offgas		1505	1483				0.05	0.05	1.549	99.69
TCE counts:										
inlet										
outlet		884	342				-0.007	-0.008		
offgas		867	883				-0.007	-0.007	-0.436	
CF counts:										
inlet										
outlet		23979	16273				0.109	0.059		
offgas		32226	32528				0.162	0.164	5.395	
COD (mg/L):										
inlet		625	66							
outlet		58								
METHANE:										
% CH4		19.2	19.1							
wet-tip		38505								
total gas (L)		0								
total CH4 (L)		0.00								
VSS, TSS:										
Crucible(g)		77.5007	79.5753							
+dried solids		77.5699	79.6467							
-Vol. solids		77.5615	79.6349							
TSS (g/L)		1.7300	1.7850	1.7575						
VSS (g/L)		0.2100	0.2950	0.2525						
pH:		7.7								
Effluent (L):		20.8								
VSS lost (g)		4.3680	6.1360	5.252						

RUN # 15

COUNTS		COUNTS	COUNTS	Conc. IN 1	Conc. IN 2	MASS IN	Conc. OUT 1	Conc. OUT 2	Total mass	Removal
sample1		sample2	mg/L	mg/L	mg/L	μmol	mg/L	mg/L	out (μmol)	%
DATE:		18-May								
PCE counts:										
inlet	1439295	1417150	0.231	0.227	20.037					
outlet	118786	131449					0.179	0.199		17.06
offgas	249832	270380					0.200	0.216	16.618	
CT counts:										
inlet	11728428	11446801	0.383	0.373	35.643					
outlet	1129	718					0.001	0.001		99.88
offgas	3007	3018					0.0019	0.0019	0.042	
DCM counts:										
inlet	61725	62297	2.270	2.291	389.315					
outlet	1854	2300					0.450	0.607		76.78
offgas	3684	3714					0.167	0.168	90.382	
TCE counts:										
inlet										
outlet	747	603					-0.007	-0.007		
offgas	822	847					-0.007	-0.007	-0.800	
CF counts:										
inlet										
outlet	26070	29696					0.122	0.145		
offgas	40682	42054					0.216	0.225	16.273	
COD (mg/L):			AVG. OVER FLOW	% reduction						
inlet	4117	435			24.35					
outlet	329									
METHANE:			AVG							
% CH4	25.3		25.3							
wet-tip	38502									
total gas (L)	0.08									
total CH4 (L)	0.02									
Crucible(g)	74.2779	79.5792								
+dried solids	74.3476	79.6535								
-Vol. solids	74.336	79.6361								
TSS (g/L)	1.7425	1.8575	1.8000							
VSS (g/L)	0.2900	0.4350	0.3625							
pH:	7.4									
Effluent (L):	14.5									
VSS lost (g)	4.2050	6.3075	5.25625							

RUN # 15

COUNTS		COUNTS	Conc. IN 1	Conc. IN 2	MASS IN	Conc. OUT 1	Conc. OUT 2	Total mass	Removal
sample1	sample2	19-May	mg/L	mg/L	μmol	mg/L	mg/L	out (μmol)	%
DATE:									
PCE counts:									
inlet	1344884	1851735	0.216	0.297	32.167				
outlet	97990	93740				0.148	0.141		43.09
offgas	233467	252889				0.187	0.202	18.306	
CT counts:									
inlet	10341856	14980265	0.337	0.489	55.865				
outlet	1619	19039				0.001	0.006		99.19
offgas	2048	2284				0.0019	0.0019	0.454	
DCM counts:									
inlet	51230	62176	1.880	2.286	510.217				
outlet	1225	1154				0.229	0.204		89.58
offgas	1826	1898				0.067	0.071	53.182	
TCE counts:									
inlet									
outlet	440					-0.008	-0.009		
offgas	942	965				-0.006	-0.006	-1.389	
CF counts:									
inlet									
outlet	27025								
offgas	37806	39308				0.128	-0.046		
COD (mg/L):									
inlet	3965	419	AVG. OVER FLOW		% reduction				
outlet	300						0.198	0.208	7.186
METHANE:									
% CH4	22.1	21.9	AVG		22				
wet-tip	38504								
total gas (L)	0.16								
total CH4 (L)	0.04								
Crucible(g)									
+dried solids									
-Vol. solids									
TSS (g/L)									
VSS (g/L)									
pH:	7.4000								
Effluent (L):	20.8								
VSS lost (g)	0	0							0

APPENDIX C - Standard Curves for PCE, CT, DCM, CF and TCE

Gas Concentration of CT vs. GC Counts

Gas Concentration of DCM vs. GC Counts

Gas Concentration of TCE vs. GC counts

Gas Concentration of CF vs GC Counts

Appendix D: Preliminary Serum Bottle Test Results

In the preliminary set of serum bottle tests, duplicate bottles for each condition were prepared in exactly the same manner. For this initial set of bottle tests, standards were prepared using a solution of VOC's dissolved in methanol. Gas samples were collected from the serum bottles and the GC counts were compared to the methanol-prepared standards and Henry's Law was used to convert the gas concentrations to liquid concentrations. Figures II to XIII show the total mass of the target compounds, with time, that were measured in the serum bottles. The two lines on the plots indicate the duplicate bottles.

Figure I describes the response of PCE and CT in blank serum bottles over time.

Figure I: CT and PCE response in preliminary serum bottle test - control serum bottle (no data shown between days 8-37 due to GC complications)

The results of figure I show that the VOC mass in the control serum bottles over time was relatively constant. Therefore there was confidence that VOC responses observed in other bottles was due to biomass, and abiotic mechanisms were negligible.

Figure II: PCE response in preliminary serum bottle test - Methanol as cosubstrate

Figure III: PCE response in preliminary serum bottle test - Ethylene glycol as cosubstrate

Figure IV: PCE response in preliminary serum bottle test - Propylene glycol as cosubstrate

Figure V: PCE response in preliminary serum bottle test - Ethanolamine as cosubstrate

Figure VI: CT response in preliminary serum bottle test - Methanol as cosubstrate

Figure VII: CT response in preliminary serum bottle test - Ethylene glycol as the cosubstrate

Figure VIII: CT response in preliminary serum bottle test - Propylene glycol as cosubstrate

Figure IX: CT response in preliminary serum bottle test - Ethanolamine as cosubstrate

Figure X: DCM response in preliminary serum bottle test - Methanol as cosubstrate

Figure XI: DCM response in preliminary serum bottle test - Ethylene glycol as cosubstrate

Figure XII: DCM response in preliminary serum bottle test - Propylene glycol as cosubstrate

Figure XIII: DCM response in preliminary serum bottle test - Ethanolamine as cosubstrate

The initial VOC masses that were expected in the serum bottles were 0.3 μmol (0.5 mg/L) for PCE, 0.65 μmol (1.0 mg/L) for CT and 6 μmol (5 mg/L) for DCM. It was apparent from figures I to XIII that these initial VOC masses were not attained, even in the blank serum bottles. Hence, additional tests were conducted to assess the fate of the target compounds in the serum bottles.

Initially it was considered that either adsorption onto the serum bottle or adsorption onto the biomass may have been the cause for the low measured VOC concentrations in the serum bottles. To determine if microbial adsorption had an impact a side test was performed. Four, 160 mL serum bottles were filled to the 100 mL mark with a combination of acclimation reactor supernatant and either 5 mL (1.7 mg VSS/L) or 0.1 mL (0.03 mg VSS/L) of sludge granules. Sodium azide was added to each bottle at a 1 % concentration (personal communication, Mr. Ning, Jan. 1998) to inhibit all biological activity. Once methane production had stopped (after approximately 20 hours) VOC's were added to each serum bottle at concentrations of 1 mg/L for PCE and CT and 10 mg/L for DCM. Concentrations were measured over 120 hours and the results are presented in figures XIV and XV.

Figure XIV: Adsorption test - VOC concentration over time with 5 mL biomass

Figure XV: Adsorption test - VOC concentration over time with 0.1 mL biomass

The VOC masses were slightly lower in bottles containing 5 mL of biomass than in those containing 0.1 mL of biomass. This suggested that there was some limited adsorption occurring, however the impacts were small relative to the target concentrations.

The initial target masses were again not detected during the test for adsorption effects, and VOC masses were within the same range as those found in preliminary serum bottle test. These results suggested that adsorption onto the biomass was not the cause of the apparent loss of mass in the serum bottles. A second indication that adsorption did not create a significant loss of mass was that some of the trend lines both increased and decreased over the sampling period and did not change significantly in any of the cases. The observed variations in compound concentrations were attributed to variability in the analytical method as well as the low VOC concentrations detected, and it was concluded that adsorption did not play a significant role in the loss of VOC mass from the serum bottles.

Since it was determined that adsorption was not the cause of the low measured VOC concentrations in the serum bottles, it was thought that there were some problems with the conversion of the gas headspace samples to the liquid standard curves. Therefore, standard curves prepared in methanol were replaced with standard gas curves for the final serum bottle test, in an effort to decrease the error introduced through conversions from liquid to gas phases.

A range of responses were observed in the serum bottles. The disappearance of PCE in the serum bottles was slower than either DCM or CT.

In the cases of methanol and ethanolamine, PCE was above detection limits on day 38 and only approached detection limits around day 30 in the ethylene glycol and propylene glycol bottles. These tests indicated that PCE was a more challenging compound with respect to degradation than either DCM or CT.

The most notable detail about the response of CT was how quickly it disappeared from the bottles. For all cosubstrates, CT disappeared within one day.

The disappearance of DCM from the bottles was less rapid than that of CT and this could have been partly due to its production and accumulation as a breakdown product of CT. The mass of DCM in bottles containing methanol, ethylene glycol and propylene glycol increased around day 5 and then quickly decreased and by day 11 had reached levels below the DCM detection limit.

It should be noted that the VOC target compound breakdown components, CF and TCE, were observed on the GC output, but they were not quantified. Early GC complications combined with the acquisition of a new integrator before standard curves for these compounds could be produced were the reasons contributing to the lack of analysis of CF and TCE.

After an initial examination of all of the serum bottles it was difficult to determine which cosubstrate was the most effective in supporting the degradation of PCE, CT and DCM. CT was not used as an indicator since its degradation was so rapid. Because of problems associated with the determination of the initial DCM and PCE masses, degradation rates were not

calculated and neither DCM or PCE was used as an indicator of the best cosubstrate.

Methane Production in Preliminary Serum Bottle Test

Methane production was recorded over time for each serum bottle.

Figures XVI to XVX show the cumulative methane production for the duplicate serum bottles, over time.

Figure XVI: Cumulative methane production with methanol as the cosubstrate in preliminary serum bottle test

Figure XVII: Cumulative methane production with ethylene glycol as cosubstrate in preliminary serum bottle test

Figure XVIII: Cumulative methane production with propylene glycol as cosubstrate in preliminary serum bottle test

Figure XIX: Cumulative methane production with ethanolamine as the cosubstrate in preliminary serum bottle test

Two methods were used to determine the degradability of the four cosubstrates. The first method calculated the maximum methane production rate for each cosubstrate from the steepest part of the curve and was reported in Table IV. Methane production rates were reported as ml CH₄/d. The second method used lag time as an indication of the compounds degradability. A target value of 40 mL of CH₄ was chosen (a little over half of the potential CH₄ production) and the number of days to reach this value was read from the plot and recorded in table IV.

	METHANOL	ETHYLENE GLYCOL	PROPYLENE GLYCOL	ETHANOLAMINE
Rate of CH₄ Prod.	5.3±0.4	5.3±0.14	6.2±0.16	2.7±0.43
Days to 40 mL CH₄	10	5	6	33

Table IV: Maximum CH₄ production rates ± s. d., reported as ml CH₄/d and Days to reach 40 mL of CH₄ production

Maximum methane production rates indicated that propylene glycol (6.2 ± 0.16 mL/d) had a higher rate of degradability than the other compounds, and ethanolamine was the least degradable (2.7 ± 0.43 mL/d).

The lag period test indicated that the biomass fed ethanolamine took 5 to 6 times longer to acclimate than those fed ethylene glycol or propylene glycol. The low CH₄ production rates along with the relatively long acclimation period required for ethanolamine resulted in it not being considered for future use in bottles tests and continuous flow experiments.

In view of the fact that the criteria pertaining to the selection of an indicator VOC for the selection of a cosubstrate was not attained, a second, final set of serum bottle tests was performed.

IMAGE EVALUATION TEST TARGET (QA-3)

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