The Microbubble Assisted Bioremediation of Chlorinated Ethenes

by

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(ABSTRACT)

This work focused on using a microbubble dispersion to deliver H_2 and CO_2 to anaerobic consortia to stimulate their ability to reductively dehalogenate tetrachloroethylene (PCE) through a cascade of less chlorinated ethenes all the way to ethene and ethane. A continuous flow system, consisting of six anaerobic soil column bioreactors inoculated with sediments from Virginia Tech's Duck Pond, was designed and built to carry out this study. Two columns received microbubbles containing H_2 and CO_2, two received propionate, which is a biological source of H_2 and CO_2, and two were not fed a substrate. A comprehensive system was designed and built to deliver a 30 _M PCE solution to the consortia. Reliable sampling and analytical procedures were developed to measure and monitor the chlorinated ethenes as well as metabolic substrates and products.

Microbubbles containing a mixture of 90% H_2 and 10% CO_2 were effectively produced in a closed spinning disk generator yielding an average quality of 70% $\pm$ 2% and an average stability of 21% $\pm$ 2%. Microbubbles with these properties were acceptable for delivering the gases to the soil columns. After the biodegradation study was completed, the microbubbles used were found to have a pH of 4.4, caused by the CO_2. Microbubbles amended with NaOH to a final concentration of 0.01 N yielded microbubbles with a pH of 7 and an improved stability of 11% $\pm$ 2%. The quality did not change after adding the NaOH.

Methane was measured as a product in all six columns throughout the duration of the experiment, verifying that methanogens were present. The levels of methane were highest in the propionate columns, showing that the methanogens there were more active and/or more populous. The levels of methane in the microbubble columns were similar to the levels in the

control columns. Propionate and acetate could not be detected in the columns where it was fed, which showed that the proton reducers and acetoclastic methanogens were both active.

Recovery of PCE and the degradation products was almost 90% in the microbubble and control columns where most of the PCE was recovered in the effluent. The predominant product in both systems was TCE, although some ethene was detected in all four columns. The control consortia produced TCE averaging about 5 _M while the microbubble columns averaged about 2 _M TCE. One of the components of the microbubbles was probably the cause of the lower amounts of PCE reduction. The fact that some ethene was seen in the microbubble columns suggests that different conditions can be found that would stimulate the further reduction of PCE with the use of H_2 and CO_2 microbubbles.

The recovery of products in the propionate columns was about 64%. Over half of the injected PCE was completely dechlorinated as measured by ethene and ethane levels. Field studies pumping propionate into an aquifer can be envisioned as a means to completely eliminate chlorinated ethenes from ground water.

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Chapter 1: Introduction

Tetrachloroethylene (PCE) and trichloroethylene (TCE) are two of the most frequent contaminants found in ground water in the United States (Westrick, et al., 1984). They are both regulated under the 1986 amendments to the Clean Water Act. Traditional methods of cleaning aquifers such as pump and treat and vapor extraction are not destructive technologies and additional treatment is always required. The desire for destructive technologies has led to efforts to invent systems in which microorganisms can be utilized to degrade these and many other substances. Microorganisms living under anaerobic conditions have shown that PCE can be biologically degraded (Bouwer and McCarty, 1983; Parsons, et al., 1984). The anaerobic degradation has been shown to be by reductive dechlorination in the laboratory (Vogel and McCarty, 1985; Fathepure and Boyd, 1988A; Freedman and Gossett, 1989). Reductive dechlorination was also the mechanism used in contaminated aquifers undergoing natural attenuation (Fiorenza, et al., 1994; Major, et al., 1994). The products of the dehalogenation consist of less chlorinated ethenes and the nonhalogenated species ethene and ethane. The final two degradation products, ethene and ethane, are not environmental hazards. The need for complete destruction is essential because all the chlorinated ethenes are regulated under drinking water regulations with the values presented in Table 1 (Lexis Law Publishing, 1998).

Other work has shown that aerobic treatment can be applied to partially chlorinated species such as TCE (Wilson and Wilson,

Table 1-1: Maximum Drinking Water Contaminant Levels

Contaminant	MCL (mg/L)
Perchloroethylene	0.005
Trichloroethylene	0.005
Cis-1,2-Dichloroethylene	0.07
Trans-1,2-Dichloroethylene	0.1
Vinyl Chloride	0.002

Other work has shown that aerobic treatment can be applied to partially chlorinated

species such as TCE (Wilson and Wilson, 1985; Nelson, et al., 1987; Fliermans, et al., 1988) and to the less chlorinated ethenes (van Hylckama Vlieg, et al., 1996; Anderson and McCarty, 1997). These species are broken down by epoxidation that decomposes into a variety of products, depending on the initial chloroethene. The degradation process is toxic to the methanotrophic organisms that perform the degradation (Broholm, et al., 1990; Alvarez-Cohen and McCarty, 1991; Oldenhouis, et al., 1991). The initial degradation products are toxic to the organisms performing the degradation, which results in a loss of cell viability and therefore a reduction in their remediation potential.

Treating ground water in situ often requires the addition of chemicals to stimulate the native microorganisms. Many of the essential nutrients can be dissolved in water in sufficient quantities and pumped directly into the ground water, but gaseous species such as O_2, CH_4, CO_2, or H_2 have low water solubilities and therefore require an alternative method of delivery.

Microbubbles can be used deliver these components to an aquifer in a more efficient manner than traditional methods. Microbubbles are a dispersed microfoam with a 60% to 70% gas phase that can efficiently deliver gaseous species to the ground water (Michelsen and Lotfi, 1991). Microbubbles consist of a gas phase encapsulated in a dilute aqueous surfactant solution. The encapsulation of gas into microbubbles, on the order of 50 _m, results from agitation of the gas-liquid interface. The agitated solution forms a microfoam of dispersed bubbles that act as colloidal particles. The dispersion has the bulk properties similar to that of dilute shaving cream and can be easily pumped directly into an aquifer after an initial resistance is overcome. After pumping into the ground water, the microbubbles are stabilized by capillary effects in the soil and most of the injected gas phase is retained in the aquifer. The microbubbles will slowly degrade as the ground water passes by and dissolves away the surfactant, leaving the capillary forces as the means by which the bubbles are stabilized and remain in the ground water. The resulting stability yields a long contact time between the water and the trapped bubbles, which results in almost total dissolution of the gas.

Several methods for delivering gases to an aquifer have been used, but each has its associated problems. Some of the technologies and their respective disadvantages will be outlined below. Sparging, a method involving the direct pumping of a gas through a well into the aquifer, requires large volumes of gas and only a small fraction ends up dissolved in the water. Sparging can cause unwanted vapor stripping by volatilizing the contaminants in question. The volatilized contaminant may also have to be treated. While both the desired and

undesired effects of sparging may clean the aquifer the contaminant still exists and additional regulatory issues may have to be complied with.

A second process involves the addition of O_2 to the water before the water is pumped into the aquifer. The water which is oxygenated by aeration or the bubbling of O_2, requires very large amounts of water to deliver sufficient quantities of oxygen. These processes generally disrupt the natural flow of the ground water that may further disperse the contaminant outside the intended treatment zone.

A chemical method for delivering oxygen also exists. A peroxide solution can be pumped into in aquifer where it decomposes yielding oxygen. The decomposition of hydrogen peroxide may be toxic to the bacteria at the concentrations needed to deliver enough oxygen for complete contaminant degradation. Slow release oxygen compounds, i.e. inorganic peroxides, have also been used to deliver O_2 to contaminated aquifers and has accelerated the degradation of toluene (Wilson, et al., 1997).

The main goal of this work is to demonstrate the use of microbubbles to promote the anaerobic degradation of PCE and it's daughter products. To achieve this aim a number of preliminary goals must be met.

The formation of H_2/CO_2 microbubbles, as opposed to using air or O_2 as the gas phase, of sufficient quality and stability will be demonstrated as the first objective.

The design and construction of a series of continuous flow bioreactors to test the usefulness of microbubbles is the next objective. The bioreactors consist of columns run in a downflow mode and will also have the necessary injection and sampling ports. Feed lines for nutrient salt and PCE streams have to be designed for a controlled delivery to make accurate mass balances around each reactor. A drainage system to safely dispose of any water still contaminated is the last part of the system.

Analytical procedures for the measurement of all of the chloroethenes, ethene, ethane, methane, and certain salts needs to be developed to quantify the biodegradation of chloroethenes, the activity of certain microorganisms, and finally to determine operating parameters of the system like species breakthrough times and residence time distributions. The activities of the organism can be determined by measuring methane, propionate, acetate, and sulfate. Breakthrough and residence times can be determined by measuring the concentration of bromide. The analytical procedures will be developed concurrently with the construction and testing of the bioreactor system.

Once the above objectives are met the main objective can be initiated. Microbubbles with a gas phase mixture consisting of CO_2 and H_2 will be fed to an anaerobic consortium in two columns to accelerate the reduction of chloroethenes as well as to provide growth requirements. The roles of CO_2 and H_2 in the mixture are as the reductant and growth substrate for CO_2 and H_2 will be used as the reducing agent.

Concurrent with the H_2/CO_2 microbubble feeding, treatability comparisons will be made with two columns fed sodium propionate, which provides the consortium both the oxidizing and reducing power needed for growth and dehalogenation. Two control columns receiving only the yeast extract supplements given to the other systems will also be implemented. Propionate was chosen to be the substrate in the comparison studies because it provides a source of H_2. Propionate metabolism produces three moles of H_2 per mole reacted. The gases, propionate, and yeast extract will be injected intermittently. Yeast extract, a solution of complex nutrients, including various fatty acids, will also be injected periodically. These nutrients will assist in the biodegradation of PCE and all of its daughter products. Determination of the ability of each system to degrade PCE to an acceptable product will be examined by the measurement of the degradation products.

Chapter 2: Background

PCE and TCE represent two of the ten most common groundwater pollutants and are considered health hazards. Older technologies to treated contaminated groundwater required recovery of the water then treatment. In situ remediation strategies were desired and a number of technologies have been looked at including immobilization, abiotic destruction and bioremediation.

The implementation of bioremediation necessitated an understanding of the microorganism's nutritional requirements an ability to degrade the contaminants in question. Laboratory studies of the necessary microbiology and biochemistry of organisms from spill sites or treatment plants have been and are currently being done. A number of consortia or pure culture systems have been shown to degrade all the chloroethenes, and the specific conditions that can stimulate these degradations have been elucidated.

Early work concentrated on TCE removal by aerobic bacteria. Aerobic consortia are easier to maintain than anaerobic ones to operate due to difficulties in keeping the later systems oxygen free. These bacteria began the biodegradation by epoxidizing the TCE in a cometabolic process in which the TCE competes with the growth substrate for an enzyme performing the degradation. The chlorinated ethylene oxide epoxide spontaneously breaks down due to its thermodynamic instability. This degradation process was found to be toxic to the organisms capable of performing this degradation. The resultant break-down products are converted to CO_2 and Cl^- by other bacteria in the consortium.

PCE is recalcitrant to aerobic degradation, therefore anaerobic systems that could biodegrade it were studied. Aquifers contaminated with either PCE or TCE often contained dichloroethene isomers and vinyl chloride. The aquifers were found to be anoxic, so the natural attenuation of chloroethenes by reductive dechlorination was determined to be the cause (Parsons, et al. 1984; Fiorenza, et al., 1994; Yager, et al., 1997). Consortia capable of dechlorinating these species were found in contaminated ground waters and anaerobic digesters at treatment plants. Most often, both in the laboratory and at contaminated sites, the degradation stopped at *cis*-dichloroethene (*cis*-DCE) or vinyl chloride. Trans-dichloroethene (*trans*-DCE) was detected in concentrations that were low relative to *cis*-DCE. Ethene and ethane were reported by some investigators, which shows that an anaerobic system can completely remediate waters

contaminated with chloroethenes (Freedman and Gossett, 1989; Rasmussen, et al., 1994).

Both the aerobic and anaerobic systems studied could be stimulated to perform the desired degradations. Many aerobic systems were shown to require CH_4 as the primary substrate in the cometabolic process involving TCE degradation (Wilson and Wilson, 1985; Fliermans, et al., 1988). The early anaerobic processes studied were all conducted with methanogenic (CH_4 producing) consortia and led to the conclusion that methanogenic organisms took part in the dechlorination activity. Virtually all of the methanogens can autotrophically use CO_2 and H_2 for growth (Zehnder, 1988). The biochemical pathway(s) of reductive dechlorination have not been confirmed, although a number of potential pathways have been proposed, with most suggesting that the actual electron donor is H_2, also fulfilling the same role in autotrophic growth. This conclusion about the dechlorinating possibilities with H_2 further implicated methanogens as dechlorinators. The gaseous requirements of either type of biodegradation fostered the expectation that microbubbles could be used as an ideal carrier of these gases to subsoil organisms to stimulate the bioremediation of chloroethene contaminated groundwater.

The replacement of the CO_2 and H_2 with propionate for the stimulation of the original consortium to degrade PCE will also be investigated. The propionate is metabolized yielding acetate, H_2, and CO_2 in the form of bicarbonate. These products can be used by a number of organisms. The acetate can be metabolized to methane by the acetoclastic methanogens. The CO_2 and acetate can be used as a carbon source for many bacteria. The H_2 can be used by methanogens, sulfate reducers, and by any organism dechlorinating PCE. Two controls without either CO_2 and H_2 or propionate will also be operated.

Development of this project requires a description of the technology to be employed and of the microorganisms that will be exploited to perform the degradation. The characteristics of microbubble generating technology and how it can be utilized to produce microbubbles capable of assisting in groundwater remediation is described first. An examination of each of the four groups of anaerobic organisms that have been shown to dechlorinate the chloroethenes is discussed in the following section. All the potential dechlorinators may be present and showing that each is or is not there is beyond the scope of this project. The utilization of certain substrates and the presence or absence of certain products can show that some of the relevant types are definitely present. The pertinent general features of each type and the proposed means by which they dechlorinate will be addressed. The relevant functions of the two groups of bacteria that are

thought to assist the dechlorinators, by providing H_2, will be reviewed after the dechlorinators. The organisms that generate H_2 will be inhibited by the H_2 in the microbubble columns, but they are not needed in those columns. Certain enzymes in one biochemical pathway have been implicated in the reduction of the chloroethenes and will be given separate treatment following the descriptions of the anaerobic microorganisms. A description of the proposed mechanisms shows a rationale for providing certain nutrients and describes one possible reason why the organisms that dehalogenate should actually do it. The important microbiology and biochemical reactions of the aerobic bacteria that can degrade TCE and the less chlorinated organics will be described last. The long term goal is to create a system that accomplishes the complete removal of chlorinated ethenes from ground water. Since the anaerobic systems may only do part of the job, a description of the aerobic degraders, which destroy partially chlorinated ethenes by cometabolism, and their role in a total treatment scenario completes the picture. The aerobes complement the anaerobes in providing a total solution for remediating chloroethene contaminated ground water.

2.1 Microbubble Technology

2.1.1 Microbubble Characteristics

Microbubbles are the name given to a dispersion of bubbles in an aqueous surfactant solution. The bubble dispersion takes on the characteristics of a colloidal suspension, as long as the bubbles are forced to remain dispersed, and has been dubbed either colloidal gas aphrons (CGA) or microbubbles (Sebba, 1987). CGA consists of a 60% to 75% gas phase dispersion of bubbles less than 100 μm in diameter. The diameter of the bulk of the CGA is 55 ± 20 μm. The surfactant in the solution plays two roles in microbubble formation. The first is to lower the surface tension so stable bubbles can form. The second is to stabilize the microbubble by the formation of a bilayer around the water shell surrounding the microbubble. Anionic, non-ionic, and even some cationic surfactants have been utilized while testing the soil retention capabilities of CGA (Longe, 1989).

Knowledge of the properties of the microbubbles in the soil phase is crucial in

determining their applicability for use as a medium of gas delivery to the groundwater for bioremediation. Laboratory research has been completed demonstrating that 70% to 80% of a 65% dispersion of oxygen microbubbles can be injected and retained in a saturated sand matrix (Michelsen, et al., 1984A). The retention was a function of the type of soil studied. In addition, under aseptic condition only 20 to 40% of these microbubbles will be released from sand matrices in 30 days (Michelsen, et al., 1984A; Michelsen, et al., 1985). Subsequently, a series of tests was completed in a 7 ft. by 7 ft. by 5 in. deep vertical slice test cell (VSTC). Oxygen microbubble retention was maintained upon injection into the saturated zone of a VSTC, with oxygen physically transferred to the flowing groundwater as demonstrated by dissolved oxygen measurements. In the VSTC, retention and transfer of the microbubbles are facilitated using a three-foot wide treatment trench with each of two horizontal alluvial sand layers that are covered with a clay layer to enhance performance. After 15 months of intermittent testing in the VSTC, typically 50% to 80% of the oxygen microbubbles were retained upon injection with over 30% to 50% of the oxygen subsequently transferred to the flowing groundwater over 36 to 48 hours (Michelsen, et al., 1993). The oxygen pick-up in the VSTC has typically been in the 5 to 8 mg/liter (ppm) range per pass under aseptic conditions.

Bulk properties of the microbubbles can be measured by two parameters: quality and stability. The quality (Q) is the percentage of the dispersion, at atmospheric pressure, that is occupied by the gas phase. The quality will typically reach a maximum between 70% and 75%. When the gas phase occupies more than 75% of the dispersion the bubbles become too tightly packed. When the bubbles are packed together at these higher qualities, they take on a polyhedral form. The spinning disk generator is not capable of imparting the higher energies required to reach this state. The stability (the lower the better) is alternately defined as the volume of the coalesced liquid (H') in a sample after one minute or more rigorously as the percentage of total liquid that leaves the dispersion (H = f(H',Q)) after one minute. Analytical descriptions and sampling procedure will be described in the section dealing with the production and characterization of microbubbles with a H_2/CO_2 mixture and their comparison to air microbubbles.

2.1.2 Microbubble Generation

Microbubbles have been generated in a number of ways since their early characterization. The feature common to all the generators has been the agitation of the interface between a gas phase and an aqueous surfactant solution. Two of the generation techniques will be described here: the spinning disk generator and the field scale continuous spinning drum generator.

The spinning disk generator is a small scale system for making microbubbles to be studied or injected in a laboratory setting (Sebba, 1985; Sebba, 1987). The set up has been used for almost 15 years. A small disk of about 3 inches is spun just below the interface of the surfactant solution and gas phase. Baffles in the generation chamber aid in the entrainment of the gas during mixing. The microbubbles can be pumped out of the chamber and retain their bulk characteristics if they are used quickly. Details of the spinning disk generator and its modifications will be described in a later section dealing with the production and characterization of microbubbles with a H_2/CO_2 mixture and their comparison to air microbubbles.

A continuous horizontal spinning system generator is important, because it is the system that would be and has been used in field trials at a contaminated site (O'Palko, et al., 1992). The properties of the spinning apparatus are scaled up from the spinning disk system to provide agitation in a similar manner. A concentrated surfactant solution is pumped into a water stream and that mixture is subsequently mixed with a gas stream under pressure. This system can produce up to 30 gallons of microbubbles per minute with greater than 95% of the air stream utilized and consolidated into the microbubble dispersion.

2.1.3 Microbubble Utilization

Microbubbles can be used in a variety of applications of which bioremediation is but one (Sebba, 1987). The high surface area to volume ratio of CGA helps increase the mass transfer to flowing ground water after injection. The oxygen pick-up described in the VSTC translates into a stoichiometric equivalent for biodegradation of 2 to 3 mg/liter xylene dissolved in the flowing ground water as it passes through the 3 foot treatment zone with a contact time of one to three hours (Michelsen and Lotfi, 1991). The unique characteristic of these microbubbles is their ability to be pumped and injected into a saturated soil matrix and be physically held in place,

retained, by the soil matrix, preferably coarse sand. Once injected, oxygen transfer to the adjacent and/or flowing groundwater occurs efficiently for subsequent biodegradation of dissolved or dispersed organics. Oxygen and air microbubbles in the presence of nutrients and microorganisms have demonstrated their ability to help biodegrade dissolved phenol as well as dispersed hexadecane (diesel oil surrogate) (Michelsen, et al., 1984A; Michelsen, et al., 1985; Michelsen, et al., 1984B).

More recently, in situ biodegradation of p-xylene was studied in a 7 cm (2.75 in) diameter layered soil column using oxygen microbubbles to supply the electron acceptor (Jenkins, et al., 1992). A bacterium *Pseudomonas putida,* continuously degraded approximately 7 ppm of p-xylene below detectable limits until the oxygen supply was exhausted. Bulk retention time in the biodegradation zone was approximately 45 minutes (Michelsen, et al., 1993). Vent losses only claimed 5 to 9% of the injected oxygen, with 71 to 82% of the injected oxygen being utilized. Approximately 13% of the oxygen injected must be committed to biodegrade the 150 ppm surfactant used for generating the microbubbles. The pressure drops resulting from increased biomass showed a slight increase over the first few days of the experiment and then a gradual decline indicating that the biomass did not plug the soil matrix. This shows that the stimulation of the microflora in the groundwater does not necessarily affect the groundwater flow (i.e., cause the flow to shift away from the treatment zone at a contaminated site).

2.2 Anaerobic Dehalogenation

Early reports that highly chlorinated organics could be biodegraded under anaerobic conditions led to an extensive search for the mechanisms, organisms, and conditions under which the degradation would take place. The mechanism of chloroethene biodegradation has been determined to be by reductive dehalogenation. Methanogens, homoacetogens, sulfate reducers, and a fourth grouping of species, that can use PCE or TCE as terminal electron acceptors for metabolism, have all been found to be capable of dehalogenating chloroethenes. All four groups require H_2 for either growth or dechlorination, except the acetoclastic methanogens, which use parts of acetate for both the electron donor and electron acceptor. The homoacetogens and some methanogens also require CO_2 for growth. All of these groups will be discussed separately, with descriptions of their relevant microbiology and biochemistry and catalogs of their dechlorination

activities. Two groups of bacteria that do not dechlorinate ethenes, but appear to take part in the overall scheme of some ethene dechlorinations are the fermenters and obligate proton reducers. Their roles in consortia that dehalogenate chloroethenes will also be addressed. The conditions in which degradation can take place can vary depending on the organisms and chemical species being degraded.

The anaerobic consortium that was used to inoculate the treatment columns came from the sandy sediments at the edge of the Duck Pond on campus at Virginia Tech. All four of the dechlorinating groups as well as the two assisting groups were presumably present in the inoculum. The presence of methanogens can be shown by measuring methane. The presence of sulfate reducers will be shown by the loss of sulfate, seen most noticeably in the propionate fed columns. The presence of proton reducers will be verified by measuring only traces of propionate in the effluents. The lack of any acetate in those effluents will demonstrate the presence of acetoclastic methanogens, which metabolize acetate to methane, in the propionate columns. The partial reduction of PCE in all six columns verifies the presence of some type of dechlorinator in all six columns. The yeast extract has fatty acids that the proton reducers metabolize. This provides a bicarbonate and H_2 source for autotrophic methanogens, those capable of using H_2 and CO_2 for growth, homoacetogens, which require the same substrates, and the chloroethene metabolizers that can use the H_2, but may also be utilizing another electron donor found in the yeast extract. If the homoacetogens and the acetoclastic methanogens are living together it would be beyond the scope of this study to show anything but that methanogens are present. Isolation or identification of the bacteria present in each column is not the focus of this study, so each type that may be active and/or dechlorinating PCE must be described.

Reductive dehalogenation is the removal of a halogen atom from a molecule while adding electrons simultaneously. All the dehalogenations that will be described are dechlorinations. There are two types of dehalogenation: hydrogenolysis and vicinal reduction. All biologically mediated dehalogenations of chloroethenes involve hydrogenolysis and are probably catalyzed by enzymatic systems. The first involves replacing a single chlorine or other halogen atom with hydrogen and can be represented in either of two equivalent manners (Mohn and Tiedje, 1992). Examples of both are respectively:

$$PCE + H^+ + 2e^- \text{ ---> } TCE + Cl^- \text{ or} \qquad [2\text{-}1a]$$

$$PCE + H_2 \text{ ---> } TCE + Cl^- + H^+ \text{ and} \qquad [2\text{-}1b]$$

The actual reduction process is not done in one step. H_2 is the initial source of the electrons and protons that eventually are used in the dechlorination process. Many electron and proton carriers are present in cells and it is hypothesized that it is these carriers directly provide the electrons and protons needed for the reduction of chloroethenes through an enzymatic pathway. The possible mechanisms of dehalogenation will be reviewed later.

The methanogens, homoacetogens, and the sulfate reducers all appear to dehalogenate the chloroethenes by a cometabolic process. The chloroethenes are dehalogenated by these organisms although the bacteria derive no energy from the process. This makes dehalogenation by these organisms inefficient and consumes some of the cell's energy. The chloroethene metabolizers actually benefit from the reduction of chlorinated ethenes. The chloroethenes are used as the terminal electron acceptors in the respiration process, which is energy producing. This should make them efficient dehalogenators. One such species, designated strain 195, uses PCE as its only known electron acceptor and can reduce PCE all the way to ethene (Maymo-Gatell, et al., 1997).

The energy required to reductively dehalogenate comes from energy gained by the bacteria during metabolism. The reduction process yields one less chlorinated product after each dehalogenation. For PCE the cascade of products is:

PCE --> TCE --> 1,2-DCE (*cis* or *trans*) --> VC --> Ethene --> Ethane. [2-2]

Four moles of H_2 are required for the complete dechlorination of each mole of PCE. Ethene has been converted to ethane is some of the systems that undergo dehalogenation, but the mechanism for this is unknown. In all the anaerobic studies of PCE dehalogenation, one of the less chlorinated ethenes from the above cascade was found to be the major end product. This cascade excludes 1,1-DCE, which does not seem to be an intermediate in the biologically mediated dechlorination of PCE.

The exact mechanisms of dechlorination by the different types of responsible organisms are not known conclusively, although a number of ideas have been postulated. Highlights of such hypotheses and how they affect this project will be discussed after the descriptions of the microorganisms.

2.2.1 Methanogens

One of the first descriptions of the biodegradation of PCE was in a culture living under methanogenic conditions (Bouwer and McCarty, 1983). The reduction products of different investigations were found to vary depending on the system studied. The final product found by some investigators was TCE (Egli, et al., 1987: Fathepure and Boyd, 1988B). *Cis*-DCE was the final product in one study with bulk aerobic conditions, but anaerobic zones existed in the treatment column as evidenced by the reported production of methane during the degradation (Enzien, et al., 1994). Vinyl chloride and suprisingly some CO_2 were the products in one early study with TCE and DCE measured as intermediates (Vogel and McCarty, 1985). Since vinyl chloride is the most hazardous of the chloroethenes, application of reductive dechlorination to groundwater remediation requires complete reduction to ethene or ethane to be truly effective to decontaminate a spill site. Studies have also shown under methanogenic conditions that ethene could be produced to a significant extent (Freedman and Gossett, 1989; Rasmussen, et al., 1994).

The proof that methanogens were capable of dechlorinating PCE has been shown in different pure culture studies. One culture that also degraded some chloroalkanes is an autotroph, *Methanobacterium Thermoautotrophicum*. Autotrophic methanogens are capable of growing on H_2 as the electron donor and CO_2 as both the terminal electron acceptor for energy production and the source of carbon used for the synthesis of higher organics. Reaction [2-3] represents the overall stoichiometry of the metabolism of autotrophic methanogens in their normal pH range of 6 to 8.

$$4H_2 + HCO_3^- + H^+ \text{ ---> } CH_4 + 3H_2O. \qquad [2\text{-}3]$$

Some methanogenic activity has been found in acidic regimes with a pH of about 4.5, where the predominant carbonate species would be carbonic acid. A number of enzymatically catalyzed reactions are actually occurring during this process. The required ratio of H_2 to CO_2 is 4:1. All but a few of the methanogens can grow autotrophically. The $\Delta G°' = -135.6$ kJ/mol of CH_4 formed (Thauer, et al., 1977). Under typical physiological conditions in an anaerobic digester, which are not at equilibrium or standard concentrations, the actual metabolic energy gain is $\Delta G'$

= -31.6 kJ/mol of CH_4 formed (Zinder, 1984). Standard conditions are taken as one molar for dissolved species and one atmosphere for the gaseous species, although the actual reactant is a dissolved gas. The typical conditions were 37 °C and a pH of 7 with concentrations of fatty acids of 1 mM, bicarbonate of 20 mM, methane of 0.6 atmospheres, and H_2 of 10^{-4} atmospheres, about 80 nanomolar. The reaction can be made to be more energetically favorable by increasing the H_2 and CO_2 partial pressures above the normal physiological levels with the microbubbles to about 6.6 mM for H_2, a 100,000 fold increase, and 0.7 mM for CO_2. This should stimulate the bacteria that require H_2 to become more active. The higher concentrations of dissolved gases that become available make this reaction more energetically favorable than what is present in observed systems and so more energy becomes available to the consortium. This excess energy is used to stimulate cellular activities including the reduction of the chloroethenes. Any bacteria should respond favorably to the process of pushing metabolic reactions to the right by raising the concentrations of primary substrates above normal physiological conditions. More energy reserves are available for use and should stimulate their cellular activities, including those of chloroethene reduction.

Unfortunately, this reaction does not occur in a void and the high concentrations of H_2 may inhibit other reactions within the cell and cause its activity to dramatically decrease. This would remove any apparent advantage in terms of the metabolic reaction gained by increasing the H_2 concentration sharply. The addition of H will be ventured until an inhibition due to H_2 is noticed.

The stimulation of their activity will be done by providing H_2/CO_2 microbubbles that are injected into the column. Three facts that lead to the hypothesis that H_2/CO_2 microbubbles can be used to stimulate the biologically mediated dechlorination of PCE are: that the autotrophs and the process of dechlorination both require H_2, the autotrophs also need CO_2, and that the autotrophs are capable of dechlorination. The stoichiometry of autotrophic growth is four moles of H_2 for every mole of CO_2. H_2 is also needed for the dechlorination of PCE, with four moles required for complete dehalogenation to ethene. To provide enough electrons in the form of H_2 for both processes a gas mixture of 90% H_2 and 10% CO_2 was chosen. To stimulate the use of H_2, headspace phases of 80% H_2 and 20% CO_2 have been used (Egli, et al., 1987; Holliger, et al., 1993; Dong, et al., 1994). One investigation with a bacterium that can grow on H_2 and PCE the gas phase used was 90% H_2 and 10% CO_2 (Scholz-Muramatsu, et al., 1995). The high levels of

both gases relative to background conditions should raise the metabolic activity above normal levels.

The other two methanogenic species shown to be capable of dechlorination are acetoclastic methanogens, which produce methane from the simultaneous reduction and oxidation of parts of acetate. (Egli, et al., 1987; Fathepure and Boyd, 1988A). They are *Methanosarcina mazei* and *Methanosarcina sp*. These two cultures and *Methanobacterium Thermoautotrophicum* stopped their dechlorinations at TCE. The metabolism of the acetoclastic methanogens is represented by reaction [2-4], with standard and physiological free energies respectively of $\Delta G°' = -31.0$ kJ/mol of CH_4 formed (Thauer, et al., 1977).

$$CH_3COO^- + H_2O \text{ ---> } CH_4 + HCO_3^- \qquad [2\text{-}4]$$

Under general physiological conditions the $\Delta G' = -24.5$ kJ/mol of CH_4 formed (Zinder, 1984). The concentrations of propionate fed to the selected consortia that contain bacteria that metabolize propionate to acetate should push the acetoclastic reaction further to the by raising the levels of acetate above what exists under typical conditions in a digester. The higher concentrations of acetate that become available make the forward reaction more thermodynamically favorable than is seen in observed systems and so the acetoclastic methanogens become more active. This greater activity should include the greater levels of chloroethene reduction. Neither the acetoclastic methanogens nor the autotrophs have a strong thermodynamic advantage in energy gained from each mole of methane produced under general physiological conditions. Both groups of organisms should produce more methane after the addition of acetate and H_2/CO_2 microbubbles respectively. The acetoclastic methanogens do not require an additional electron donor, because the needed electrons come from acetate. The choice of propionate as a comparative metabolite selects for the acetoclastic methanogens. The autotrophs should also be present in the comparative consortium, because H_2 is a product of propionate metabolism and bicarbonate is a product of both acetate and propionate metabolism.

The fact the methanogens and methanogenic cultures could degrade chlorinated compounds including the chloroethenes led to the question of how were they accomplishing it. One characteristic is that methanogens have high concentrations of cobalt either in vitamin B_{12} or as an α-ligand modified vitamin B_{12} , such as Factor III found in the enzymes carbon

monoxide dehydrogenase (CODH) and a methyl transferase (MT1). Vitamin B_{12} and its analogs , with different side groups in certain positions along a core structure, all contain a cobalt atom at the center of a corrinoid group. Cobalt atoms are among a group of transition metals that are known to take part in catalyzing dehalogenation reactions as components of biomolecules. The cobalt is bound in a corrinoid structure that is analogous to the more familiar porphyrin structure around the iron atom in hemoglobin. The high concentrations of cobalt in the methanogens along with the known activity of cobalt led investigators to look at cobalt containing enzymes as the possible system that performs the reduction of chloroethenes. Another characteristic of the methanogens is that they contain a unique cofactor, cofactor M, that is utilized in the terminal step of methanogenesis, the hydrogenation of the methyl group. Hypothetically, PCE would be initially dechlorinated then the trichloroethyl group formed would be hydrogenated in the same way as the methyl group in methanogenesis.

Because the methanogens can dechlorinate PCE and have high levels of cobalt, enzymes like CODH were studied as part of the possible source of their dechlorinating activity. This enzyme is responsible for the cleavage of acetyl CoA into methyl and carbonyl fractions and the reverse reaction, acetogenesis. The enzyme also takes part in the transfer of a methyl group to or from the enzyme. The corrinoid containing subunit of the complex is known to be the part that transfers the methyl group to and from the another enzyme system that converts the methyl group to methane. Acetoclastic methanogens, responsible for about two thirds of biological methane production on the planet, use CODH to cleave acetyl CoA, which has been made from acetate, while other methanogens use CODH to build acetyl CoA, which will be used for the incorporation of carbon into the cell. The cobalt has been hypothesized to take part in the dechlorination activity associated with the methanogens. The suspected role of the corrinoid will be dealt with in detail in its own section.

The brominated analog of cofactor M, bromoethanesulfonic acid, BES, is a specific inhibitor of methanogenesis and has been used to determine if the reduction of the chloroethenes in a consortium is performed by methanogens or other organism. BES was added to methanogenic consortia and methane production stopped. The reduction of PCE, however, was not halted, but TCE was the only product formed although these consortia were complete dechlorinaters under non-inhibitory conditions (Freedman and Gossett, 1989; DiStefano, et al., 1992; Rasmussen, et al., 1994). One of the cultures was totally inhibited by BES when methanol was the electron donor, but produced TCE in the presence of BES when H_2 was donating the

electrons. These experiments demonstrated that methanogens take part in dechlorination and, also as importantly, other bacteria can also dechlorinate PCE. These tests with BES imply that a consortium is likely to yield the best scenario for degrading PCE all the way to ethene, as opposed to the utilization of a pure culture.

H_2 is a requirement for all the methanogens except the acetoclastic methanogens. H_2 can serve as the electron donor for reductive dehalogenation, which was shown in one BES test and will be seen again with the organisms that can metabolize PCE. H_2/CO_2 microbubbles should stimulate the autotrophs and any dechlorinators that can otherwise live off the organics in the provided yeast extract. The bacteria that normally produce H_2 will be inhibited by the high concentration of H_2 that exists after the microbubbles are injected. Propionate is the source of H_2 for the comparative column studies, and H_2 production would cease if acetate, another product of propionate metabolism was not consumed by acetoclastic methanogens.

2.2.2 Homoacetogens

The homoacetogenic bacteria, if present in the inoculum will remain active in all of the columns. H_2 and CO_2 are an acceptable substrate for these organisms. The microbubble columns are fed large quantities of H_2 and CO_2 and these species are produced during the metabolism of propionate as well as the metabolism of the fatty acids in the yeast extract. Since their presence is probable, a discussion of their bioreduction of chloroethenes is necessary.

In a mixed anaerobic culture that was capable of dechlorinating PCE to vinyl chloride and ethene, inhibitor studies implicated homoacetogens as taking part in the dechlorination process (DiStefano, et al., 1992). The methanol fed cultures were inhibited with vancomycin, which prevents the cell wall synthesis of eubacteria, the kingdom that has the homoacetogens. Acetogenesis was stopped and the cultures no longer dechlorinated. When the cultures were fed H_2 and given vancomycin, acetogenesis was inhibited, but dechlorination continued, although slower and terminating at vinyl chloride. The implications are that the homoacetogens produce a supply of H_2 for the organisms that were dechlorinating and that H_2 not methanol was the direct electron donor for dechlorination.

The homoacetogens are classified by their ability to get energy by building acetate from smaller molecules. The homoacetogens also employ the acetyl CoA pathway, like the

methanogens, but use it to generate acetate as the metabolic end product as well as for assimilating carbon into the cell. This makes them competitors of the methanogens that can use single carbon species as substrates. If the homoacetogens were also able to degrade PCE this competition for substrate would not really be a problem, since the job would still get done, just at a different rate.

A determination if homoacetogens could dechlorinate was performed with pure cultures of different species. Only one species, *Sporomusa ovata*, could reduce PCE and only to TCE (Terzenbach and Blaut, 1994). Cell extracts of this and three other species could perform the same dechlorination when alternative reducing agents, Ti(III) citrate or CO were provided. They show that something in the cells was able to dechlorinate under in-vitro conditions. Based on other work with CODH and corrinoids, this enzyme was implicated in the extracts. Dechlorination by *S. ovata* was inhibited with propyl iodide, which specifically complexes to reduced cobalt(I). The removal of the propyl iodide restored the dechlorinating ability.

2.2.3 Sulfate Reducers

SO_4^{2-} was fed to the system at very low levels to keep the sulfate reducing population low, but was administered to meet the sulfur requirements of normal growth. The utilization of SO_4^{2-} in the microbubble and control columns was only about 10%, but in the propionate columns it was almost totally consumed. This shows that sulfate reducers were active in these columns, even if they were not given a lot to eat. This result demonstrated the need to understand their chemistry and dehalogenation capabilities, since they may take part in the dechlorination of PCE.

The sulfate reducers, organisms using SO_4^{2-}, sulfite, and/or thiosufate as the terminal electron acceptor during metabolism, have also been found to dechlorinate groundwater contaminants. A number of haloaromatics are susceptible to dechlorination by these bacteria. One culture reduced Chlorophenol isomers and produced stoichiometric amounts of sulfide (Häggblom and Young, 1990). *Desulfomonile tiedjei* degrades meta substituted halobenzoates coupled to sulfate reduction (DeWeerd, et al., 1990). In one case it was demonstrated that different organisms in a consortium have different roles to play in the dechlorination of the

herbicide dicamba (3,6-dichloro-2-methoxybenzoic acid) (Taraban, et al., 1993). One organism, a fermenter, demethylates the dicamba and only then will the sulfate reducer remove a chlorine.

Sulfate reducers are also known to be active in consortia where PCE is being dechlorinated. In a field study, benzoate fed under aerobic conditions could not degrade PCE, but under anaerobic conditions and concurrent with the addition of SO_4^{2-}, the PCE was successfully degraded to ethene and vinyl chloride (Beeman, et al., 1994). When the vinyl chloride began to accumulate more SO_4^{2-}, was added and then only ethene was detected at the monitoring wells. The sulfate reducers definitely aided in the reduction scheme, but the conditions in the aquifer were not described and it can only be implied that they were responsible. A lab study under sulfate reducing conditions and inhibitory to both acetate users and methanogens also dechlorinated PCE to *cis*-DCE with TCE being the major product (Bagley and Gossett, 1990). A more conclusive study was performed with a pure culture sulfate reducer from a sulfate reducing aquifer. TCE was the predominant product and some DCE was also measured (Suflita, et al., 1988).

Another pure culture sulfate reducer has been shown to dechlorinate PCE. This organism, named *Desulfitobacterium* sp. strain PCE1, can use sulfur compounds, PCE, and some chloroaromatics as the terminal electron acceptors (Gerritse, et al., 1996). A number of electron donors sufficed and TCE was the major product, with small amounts of *cis*-DCE produced.

Low concentrations of sulfate were supplied in the nutrient salt solution to minimize the role of the sulfate reducers. The initial concentration of SO_4^{2-} in the feed was 0.013 mM. In one study of sulfate utilization, a sulfate concentration below 0.1 mM was rate limiting (Ingvorsen, et al., 1981). The almost complete consumption of sulfate was seen in some columns and is therefore has a potential role in the effective reduction of PCE to ethene.

2.2.4 Non-associated Dechlorinators

Investigations searching for the actual dechlorinating organisms in different consortia involved in reductive dechlorination has yielded organisms that can not be apparently classified with any of the three previously described groups. These organism all apparently are capable of using chlorinated ethenes as the terminal electron acceptor in metabolism. There is a great

variety among the "orphaned" bacteria and a wide range of dechlorinating and metabolic versatility. It is possible that some organism of this type was present in the inoculum used in this study. It seems that the chloroethenes can be used for energy production, but are not used as a carbon source for incorporation into cellular matter. Whether these organisms use acetylCoA pathway or not is not yet known.

Some of the evidence supporting the existence of these organisms is based on studies where none of the other types of organisms could be performing the dechlorination. An enrichment culture in which methanogenesis was inhibited and the sulfur requirement was provided as sulfide was capable of reducing PCE to ethene (80%) and the remainder to vinyl chloride (DiStefano, et al., 1991). Methanol was the substrate and the electron balance showed they all went to acetate or PCE reduction. Inhibitors of acetogenesis were later given to the same culture and dechlorination went on in the presence of H_2, but not methanol (DiStefano, et al., 1992). The investigators hypothesized that the homoacetogens were only responsible for providing H_2 during acetogenesis, while other bacteria were reducing the PCE. Purification of this culture led to the isolation of a culture, containing only two organisms, that could use PCE as the terminal electron acceptor and H_2 as the electron donor. The products were mostly vinyl chloride and small amounts of ethene (Maymó-Gatell, et al., 1995). Isolation of the dechlorinator in a pure culture was not achieved at this time. Neither methanol nor acetate was acceptable substrates and the culture did not produce CH_4 or acetate when either a H_2/CO_2 mixture or methanol was supplied, demonstrating that methanogens and homoacetogens were not present. This leads into a discussion of pure cultures capable of deriving energy from PCE reduction.

Research in the last six years has led to the discovery of organisms capable generating metabolic energy from the reduction of PCE reduction. The first such culture, PER-K23, was only shown to use formate and H_2 as electron donors and either PCE or TCE as electron acceptors (Holliger, et al., 1993). This does not deny the existence of either other potential electron donors or acceptors. *Cis*-DCE was the major product with no vinyl chloride or ethene detected. PER-K23 could not be assigned with a known group of organisms based on its physiology.

The next discovery was a species also capable of using the H_2 and PCE combination for growth and acetate as a carbon source for cellular incorporation (Scholz-Muramatsu, et al., 1995). This species was capable of utilizing many combinations of electron donors and

acceptors and dechlorinated PCE to *cis*-DCE. The bacterium was found to represent a new genus of proteobacteria.

Another species found to degrade PCE to *cis*-DCE has also been classed as a proteobacterium (Krumholz, et al., 1996). This bacterium uses acetate or pyruvate as electron donors and fumarate and nitriloacetate as well as PCE and TCE as electron donors.

One study has isolated a facultative anaerobe, named MS1, that reduces PCE to *cis*-DCE and has been classified as belonging to the family *Enterobacteriaceae* (Sharma and McCarty, 1996). This organism uses PCE and TCE as two of many possible electron acceptors and oxidizes dozens of substrates. PCE was only reduced when more common natural reductants were not present. MS1 closely resembles a strain of the species *Enterobacter agglomerans*, and when tested it also was capable of reducing PCE and TCE.

Most recently, the first pure culture of an organism capable of reducing PCE all the way to ethene was isolated (Maymó-Gatell, et al., 1997). PCE and H_2 were required for growth, although other compounds would degrade when both were present and PCE reduction would continue after growth stopped. This species is a eubacteria, but was not found to be related to any known group.

At the other end of the chloroethene spectrum a mixed culture has been found that degrades only vinyl chloride under in vivo conditions (Rosner, et al., 1997). Cell extracts of the mixed culture were also able to degrade *cis*-DCE to vinyl chloride at a similar rate. The bulk rate of *cis*-DCE reduction to ethene is about 20 times lower than the vinyl chloride rate. Reduction rates of TCE and PCE were about two and four orders of magnitude slower respectively. Growing the organisms without vinyl chloride present slowed the rate of degradation by two orders of magnitude when vinyl chloride was added. Inhibition studies with propyl chloride stopped dechlorination but the effect could not be reversed by the normal procedure. The mechanism of dechlorination is hypothesized to be different from that or those of PCE reduction. Alternatively, mineralization of vinyl chloride under anaerobic conditions has also been investigated (Bradley and Chapelle, 1997). They found that up to 44% of labeled vinyl chloride was recovered as labeled CO_2. This has implies that mixed culture ethene balances may be low due to mineralization of a supposedly stable root compound. Now that the known or suspected dechlorinators have been described, the probable non-dechlorinating players will be discussed.

2.2.5 Non-Dechlorinators

There are two groups of organisms that are purported to play a role in the overall scheme of deductive dechlorination of chloroethenes, are not dechlorinators. They are fermenters and obligate proton reducers and both groups perform the same role, to produce H_2 that is used for the reduction of chloroethenes. The obligate proton reducers (OPRs) oxidize fatty acids to acetate, H_2, and H^+. The OPRs are synotrophic organisms, in that they must live with organisms that are hydrogenotrophs, consumers of H_2. One example of a reaction performed by an OPR is the utilization of propionate as seen in reaction [2-5]:

$$\text{propionate} + 3H_2O \text{ ---> } CH_3COO^- + HCO_3^- + H^+ + 3H_2 \qquad [2\text{-}5]$$

The standard free energy of propionate oxidation is $\Delta G°'$ = 76.1 kJ/mol, which is highly endergonic, energy consuming (Thauer, et al., 1977). This is primarily due to the use of 1 atm partial pressure for H_2. The reaction becomes thermodynamically favorable at partial pressures less than 10^{-4} atm (Zehnder, 1988). When these organisms are in the presence of a hydrogenotrophs, such as methanogens, sulfate reducers, and the dechlorinators, the partial pressures fall below that value. Below partial pressures of 10^{-6} atm the activity of the H_2 users decreases, so the H_2 concentration generally remains in this range. At a H_2 pressure of 10^{-4} atm, acetate and propionate at 10 mM and bicarbonate at 20 mM, representing general steady state physiological conditions, the free energy of propionate oxidation is $\Delta G'$ = -5.4 kJ/mol that makes the reaction thermodynamically feasible (Zinder, 1984). Reaction 2-5 is obviously inhibited by acetate and H_2, which push the reaction towards the reactants, but it is also inhibited by high concentrations of propionate (Fukuzaki, et al., 1990). The effect of acetate can be mediated by acetoclastic methanogens, but in the presence of acetate they will not metabolize the H_2, that must be done by other organisms (Dong, et al., 1994). Propionate and other fatty acids have been shown to stimulate the reduction of PCE in both methanogenic and nonmethanogenic microcosms (Gibson and Sewell, 1992; Fennell, et al., 1997).

The fermenters that take part in the chloroethene dechlorination scenario perform the same function as the OPRs. They produce H_2 only in the presence of hydrogenotrophs and will be called facultative proton reducers (FPRs). Normally these bacteria ferment substrates to

pyruvate, lactate, ethanol, or a small chain length fatty acid. When the hydrogenotrophs are in the same consortium, the fermenters derive extra energy by oxidizing their regular products to acetate and producing H_2 as an additional product.

Selenomonas ruminantium, a species of fermenter that was not known to produce H_2, will do so in the presence of a hydrogenotrophic methanogen (Scheifinger, et al., 1975). On the other side of the coin, *Syntrophobacter wolinii* is known best for growing along with hydrogenotrophs, usually sulfate reducers or methanogens, but can metabolize propionate with internal sulfate reduction in pure culture (Wallrabenstein, et al., 1994).

The coupled set of interactions between the producers and consumers of H_2 is called interspecies H_2 transfer (Zehnder, 1988). How the transfer occurs exactly and how the OPRs get enough energy to grow from the oxidation of propionate is still unclear, but this symbiosis is a major source of H_2 and acetate for methanogens in treatment plants and helps account for the production of large quantities of acetate, thus allowing the acetoclastic methanogens to be the predominant global methane producers.

The use of H_2 is a competetive process in mixed cultures. The sulfate reducers are better H_2 scavengers than the methanogens (Zehnder, 1988). They are not generally going to contribute to PCE reduction, although some species are capable (Bagley and Gossett, 1990; Gerritse, et al., 1996). This competition led to the choice of a low sulfate medium for the present study.

The outcome of the competition for H_2 between the non-methanogenic dechlorinators and the methanogens is crucial to the complete reduction of PCE. Fortunately, the non-methanogenic dechlorinators have utilization constants approximately an order of magnitude lower than the methanogens (Ballapragada, et al., 1997; Smatlak, et al., 1996). This means that they are capable of using H_2 at one tenth the concentration or partial pressure, thus the dechlorinators will consume the H_2 before methanogens. These observations were made using cultures that dechlorinated PCE without methanogenesis and were compared to methanogenic H_2 consumption.

The OPRs and FPRs are important to this study since propionate is the initial substrate in the comparative columns. The propionate will only be metabolized when there are hydrogenotrophs and less crucially acetate utilizers to consume the reaction products of the metabolism. As will be seen later no propionate or acetate could be measured in the effluents of

either propionate column, which verifies the existence of proton reducers, hydrogenotrophs and acetotrophs.

2.2.6 Mechanisms of Dechlorination

Knowing that certain organisms will reduce PCE is not the same as understanding how it is done or what is required by the bacteria to accomplish the reduction. Determining the mechanism or mechanisms of dechlorination has two purposes. This knowledge will lead to the design of better treatment strategies by being able to properly stimulate the bacteria to degrade chloroethenes more efficiently. This will be done by providing the sufficient quantities of substrate and all the necessary nutrients. The other reason is simply for the gaining of the knowledge. The mechanism in living systems has not been elucidated conclusively. Many implications exist based on inhibitor studies with whole cells, cell extracts, and purified enzymes.

It is possible that some enzyme or cofactor that is released into the surrounding fluid by either excretion or lysis of dead cells is responsible for the dechlorinating ability of a consortium. No research to date has implicated such a mechanism, but many investigators state that this is still a possible means of dechlorination.

The early research into a mechanism of dechlorination focused on the methanogens. The dechlorination was assumed to be related to the process of metabolism. The final step in methanogenesis is the hydrogenation of the cofactor bound methyl group to produce methane and the reduced cofactor The methyl group comes to the cofactor from a cobalimin containing methyl transferase (MT2). The reaction can be shown in either representation as reaction 2-6a or 2-6b:

$$CH_3\text{-cofactor} + 2H^+ + 2e^- \text{ ---> } CH_4 + \text{H-cofactor} \qquad [2\text{-}6a]$$

$$CH_3\text{-cofactor} + H_2 \text{ ---> } CH_4 + \text{H-cofactor} \qquad [2\text{-}6b]$$

The addition of two electrons and an H^+ to the methyl group, resulting in methane, is similar to the hydrogenolysis of PCE and the other chloroethenes. Whether the same enzymes complexes are catalyzing the two reactions or different mechanisms are at work has not been elucidated.

Transition metal containing enzymes are responsible for similar types of reactions in biological systems. The methanogens have relatively large quantities of enzymes with cobalt incorporated in a corrinoid moiety that is part one of its polypeptide subunits.

In vivo studies are the place to begin to describe the mechanism. One investigation studied the effects of different vitamins and only vitamin B_{12} had an effect on dechlorination (Maymó-Gatell, et al., 1995). Concentrations of vitamin B_{12} at 0.05 mg/L or above stimulated the amount of dechlorination by an order of magnitude above what was measured without supplementation.

More often, to determine the potential role of corrinoid containing enzymes, cell extract studies with inhibitor were performed. Propyl iodide selectively binds to reduced cobalt and was used regularly. The most common result was that the addition of propyl iodide reduced the dechlorination ability of the bacteria as seen in a study with the homoacetogen *Sporomusa ovata* and the dechlorinator *Dehalospirillum multivorans* (Terzenbach and Blaut, 1994; Neumann, et al., 1994). The inhibition is reversible by exposing the system to light and the propyl iodide disassociates from the cobalt. The system that preferentially dechlorinated vinyl chloride, relative to the more chlorinated ethenes, was also inhibited by propyl iodide, but the inhibition could not be reversed by the usual method (Rosner, et al., 1997). This suggests a different mechanism of dechlorination by this culture.

The role of transition metal mediated dechlorinations focused on organometallic components common to anaerobes. One study found that the nickel containing coenzyme F_{430}, unique to methanogens, a modified analog of vitamin B_{12}, found in CODH, and hematin, an iron containing species could dechlorinate PCE to ethene as well performing other dechlorinations (Gantzer and Wackett, 1991). Previous to this, corrinoids were shown to dechlorinate all the chloromethanes (Krone and Thauer, 1989).

CODH, an enzyme mentioned earlier, is used as part of the acetyl CoA pathway. This pathway is part of the metabolic paths in the methanogens and is also used by them, the homoacetogens, the sulfate reducers, and many other types of bacteria for the assimilation of carbon from a form of carbonate into cell matter. Some of the pure cultures that can grow on H_2/PCE require a carbon source for cellular incorporation and acetate was acceptable choice for most of the systems mentioned. The means by which the acetate is assimilated into these bacteria has not been described. Investigations into the normal functions of CODH and its component subunits were needed before its role in dechlorination could even be hypothesized.

The CODH enzyme complex has two main components, a nickel iron-sulfur complex, composed of two subunits with some zinc as well, and a cobalt iron-sulfur complex, also with two subunits, as well as a component without a known function at this time (Abbanat and Ferry, 1991). The nickel iron-sulfur component cleaves acetyl CoA into a methyl and carbonyl group, and the cobalt iron-sulfur group transfers the methyl moiety from nickel complex to a methyltransferase enzyme. The oxidation states of the corrinoid center were determined before and after performing anticipated function (Jablonski, et al., 1993). The carbonyl group is oxidized and the electrons are used later in the hydrogenation of the methyl group. Dissolved CO also provides electrons to the nickel complex, although that role in nature is suspect at best. CO binds to the same site as the carbonyl group from acetyl CoA, as shown by labeling studies. Labeled CO mixed with the CODH and acetyl CoA yielded labeled acetyl CoA corresponding to the loss of activity in the CO. A detailed description of the CODH enzyme and its functions can be found in the review literature (Ferry, 1995).

Now the proposed role of CODH and its components in the reduction of the chloroethenes can be discussed. The enzyme was isolated and investigated as the full complex and in parts, the nickel complex, the cobalt complex and the cobalt containing corrinoid (Jablonski and Ferry, 1992). The reduction of TCE was studied. CO and titanium(III) citrate are used as reducing agents. CO only reduced TCE with the complex or when the disassociated parts were in solution together. Neither the nickel component nor the nickel component plus the corrinoid was unable to reduce TCE. The titanium(III) could induce reduction of TCE with the complete enzyme, the cobalt component alone, and with the corrinoid alone. Even the boiled enzyme, usually meaning deactivated, was capable of reducing TCE in the presence of titanium(III). The titanium(III) could not promote reduction with the nickel component. These results imply that the nickel component was transferring electrons from the CO to the cobalt complex and the reduction of TCE was accomplished by the cobalt fraction. The titanium(III) transferred its electrons directly to the cobalt component for TCE reduction. Since in the acetyl CoA pathway the cobalt complex is responsible for the transfer of the methyl group not electrons, these studies can not be conclusive as to the role of CODH or any cobalt containing enzyme in the in vivo dechlorination of TCE or any other chloroethene. The implications of cobalt's role, whatever the mechanism, should be heeded when trying to assure the anaerobic dehalogenation of chloroethenes. The results achieved by these researchers led to the decision to include cobalt, nickel, and zinc salts in the nutrient solution.

2.3 Aerobic Degradation

Anaerobic treatments of chloroethenes have shown that the process does not always go all the way to completion. The partial degradation of PCE, means that the less chlorinated ethenes still have to be destroyed to complete the decontamination of the aquifer. One method of eliminating these less chlorinated species is a follow up treatment by aerobic biodegradation. Any developed treatment scheme must account for only partial dechlorination of PCE under anaerobic conditions and the use of aerobic organisms to complete the decontamination of the ground water is the best possibility. Aerobic bacteria that exist in habitats contiguous with the anaerobic zone offer the optimal in situ dual treatment strategy.

Partially halogenated aliphatic compounds are not degraded by most naturally occurring aerobic bacteria under common environmental conditions. It was discovered that methanotrophs, aerobic bacteria that metabolize methane, will degrade chlorinated species such as TCE (Wilson and Wilson, 1985; Fliermans, et al., 1988). The methanotrophs inhabit the border regions between oxygenated areas and methanogenic consortia that provide them with methane. This makes them the ideal focus of a final in situ treatment following anaerobic dehalogenation. The aerobic biodegradation of the partially chlorinated ethenes is a single stepped process that destroys the chloroethene. No concerns about byproducts like vinyl chloride exist and make this look like a good approach to complete the groundwater remediation.

The degradation occurs because the metabolism of methane uses an enzyme that is nonspecific and catalyzes the degradation reaction, although the organism receives no benefit from the degradation of TCE (Semprini, et al., 1992). This process is called cometabolism. These organisms produce enzymes, called oxygenases, that are expressed to oxidize their specific metabolite in the presence of O_2 and NADH, a common cellular electron donor. For methane the metabolic reaction is (Broholm, et al., 1993):

$$CH_4 + O_2 + NADH + H^+ \xrightarrow{\text{monooxygenase}} CH_3OH + H_20 + NAD^+ \qquad [2\text{-}7].$$

NADH is a reduced coenzyme that provides the necessary electrons to complete the partial

oxidation of methane. NADH and its oxidized counterpart NAD^+ are general cellular sources of electrons used to facilitate controlled partial redox reactions. The catalyzed reaction of TCE degradation by a monooxygenase is the oxidation to an epoxide:

$$TCE + O_2 + NADH + H^+ \xrightarrow{\text{monooxygenase}} \text{TCE-epoxide} + H_20 + NAD^+ \quad [2\text{-}8].$$

The epoxide is very unstable and spontaneously breaks down into compounds that can be metabolized to CO_2 and Cl^- by heterotrophic bacteria in the consortia (Fliermans, et al., 1988; Little, et al., 1988).

Bacteria that metabolize other organics also express oxygenase enzymes similar to the monooxygenase in the methanotrophs, either monooxygenases or dioxygenases. Oxygenase containing bacteria that primarily metabolize substrates such as propane, toluene, or phenol have also been shown to degrade TCE (Wackett and Gibson, 1988; Wackett, et al., 1989; Nelson, et al., 1987). Organisms that employ toluene oxygenases may be more effective degraders of TCE than the methanotrophs. Because these other bacteria do not naturally live in conjunction with methanogenic consortia, their use in a dual treatment system would require the injection of regulated chemicals as substrates. This would limit their practicality for the remediation of ground water.

Due to the cometabolic nature of TCE degradation by methanotrophs the levels of CH_4 must be kept low. If too much CH_4 is present there will be very little if any degradation of TCE because CH_4 will monopolize the oxygenase. Alternately, under CH_4 starvation, normal metabolism does not function and eventually the bacteria will run out of energy. They also will not be able to regenerate the reductants needed to provide the necessary electromotive power for the degradation of TCE (Henry and Grbic-Galic, 1991). The continued metabolism of methanol, the initial product of CH_4 metabolism, provides the necessary energy to both maintain expression of the oxygenase enzymes, and regenerate reductants such as NADH, which would otherwise eventually become depleted.

The methanotrophic bacteria may experience toxic effects from the degradation products of TCE, such as glyoxylic acid or trichloroacetic acid (Broholm, et al., 1990; Alvarez-Cohen and McCarty, 1991; Oldenhouis, et al., 1991). It is the epoxide and/or the primary products from its

degradation that are the candidates for the causing the toxic effects. When methanotrophic organisms are part of a consortium, the heterotrophic bacteria in the consortium metabolize initial degradation products of TCE to CO_2 and Cl^-. It is possible the metabolism of these initial products may take place before toxic effects can occur to the methanotrophs. Also, during in situ or continuous flow ex situ treatment, dilution of the products occurs and they may be washed away from the cellular environment and therefore reduce the effects of toxicity.

The other products of the anaerobic degradation, *cis*-DCE, *trans*-DCE, and vinyl chloride can also be biodegraded aerobically (Hopkins and McCarty, 1995; Chang and Alvarez-Cohen, 1996). These species are epoxidized like TCE (Vogel, et al., 1987). The epoxide degradation products from these compounds may be different from those of TCE, but mineralization is still completed. There is some experimental evidence that vinyl chloride can be used as an energy source during aerobic degradation.

The transformation capacities, the amount of chloroethene degradation that will occur before toxicity reduces a culture's viability, of each of the partially chlorinated ethenes increases with decreasing chlorination (Chang and Alvarez-Cohen, 1996; van Hylckama, et al., 1997). 1,1-DCE is an exception to this trend, but it is not found in ground water when PCE and/or TCE are the only initial contaminants. This trend in transformation capacities means that the aerobes become more efficient at degrading chloroethenes as the dechlorinating efficiencies of the anaerobes decrease.

The opposite trends in chloroethene degradation capacities relative to the number of chlorines and that the aerobes are harmed by the highly chlorinated ethenes suggest two things. The first is that the biodegradation of chloroethenes other than PCE can not easily be accomplished by the aerobes alone. The second and more powerful conclusion is that anaerobic treatment of PCE and TCE spills followed by an aerobic treatment is the surest method of achieving total decontamination of an aquifer. No aerobic study is performed here, because only either minimal or total PCE biodegradation was observed. A full understanding of the aerobic possibilities will help with the interpretation of some conclusions and will also help any researchers that perform follow-up aerobic studies. The aerobic investigations would be performed in sequence with the effluent of an initial anaerobic treatment that used H_2/CO_2 microbubbles and yielded only partial dechlorination.

2.4 Summary

The decontamination of ground water can best be performed in situ. The stimulation of in situ organisms in the anaerobic zones of an aquifer can result in the dechlorination of PCE and the other chloroethenes. Knowledge of the bacteria in an aquifer can be used to determine the best technological method to stimulate their remediative capabilities. Descriptions of the major biological groups that may be present in the study's initial consortium helped determine the conditions of the experiment. These descriptions also showed that the addition of H_2 and CO_2 utilizing microbubble technology should have a stimulatory effect on a consortium that is capable of dehalogenating chloroethenes. The descriptions of the relevant bacteria also help explain why the propionate comparison treatment was planned along side of microbubble experiment.

The microbubble technology and how it can effectively make H_2/CO_2 microbubbles, provides the proper introduction to the technology. The description leads to an understanding of why the microbubble method was chosen as the means to deliver the gases to the bacteria in the columns to stimulate the biodegradation of the chloroethenes.

Chapter 3: H_2/CO_2 Microbubbles

The treatment of contaminated ground water anaerobically can benefit from the ability to deliver H_2 and possibly CO_2 effectively to the aquifer. Microbubbles can be that conduit of effective delivery. Previously, the spinning disk method of generating microbubbles has proved to be effective for laboratory and pilot testing. This method will be shown to be capable of generating microbubbles with H_2 and CO_2, that can be utilized for chloroethene degradation. The microbubbles are characterized by two properties of the bulk dispersion called quality and stability. Adaptations to the spinning disk generator to make it a closed system will be described. Comparisons between H_2/CO_2 microbubbles made in the closed generator and air microbubbles made the traditional way will be made based on the characterizations of each type of microbubble.

3.1 Microbubble Characterization

Delivering gases through the use of CGA is best served if gas phase of the dispersion is maximized, between 60% and 70%, and that the dispersion maintains its integrity until the microbubbles are delivered to and trapped between the soil particles. The upper working maximum for the quality is about 70% to 72%. Dispersions with higher qualities distort the microbubbles into a polyhedral form, which requires the addition of more energy. The volume percentage of the gas phase in the dispersion is defined as the quality. Samples of bubbles are pumped into a 250 ml glass graduated cylinder until full. The volume of liquid that comes out of the dispersion and collects in the bottom after one minute is measured, then the mass of the sample is taken. The parameter defined as stability is a function of the volume of liquid leaving the dispersion.

The formula for calculating the quality in percentage is given in the following equation (Achanta, 1991; Longe, 1989):

$$Q = \frac{(\text{Sample Volume} - \text{Liquid Mass} * \rho\ H_2O\ (@24\ ^\circ C)) * 100\%}{\text{Sample Volume}}$$

[3-1].

Within the error of sampling the density of water can be assumed to be 1 g/ml. The density of water is approximately 1000 times greater than that of air, so the liquid mass is effectively the sample mass. Using the above two simplifications and that the sample volume is always 250 ml the quality calculation can be simplified to:

$$Q = \frac{(250 \text{ ml} - \text{Sample Mass}) * 100\%}{250 \text{ ml}}$$

[3-2].

The stability takes two forms. The rigorous definition is the percentage of the liquid in the dispersion at time zero that has phase separated after the first minute, measured visually as the volume of clear liquid in the bottom of the cylinder. The following expression is used for calculating the stability:

$$H = \frac{(\text{Liquid Phase Volume} - \text{Bottoms Volume}) * 100\%}{\text{Liquid Phase Volume}}$$

[3-3].

Taking the density of the liquid phase to be 1 g/ml as above, the liquid phase mass and volume have the same magnitude so the equation can be calculated more directly from the measured values as:

$$H = 100\% - \frac{(\text{Sample Mass} - \text{Bottoms Volume}) * 100\%}{\text{Sample Mass}}$$

[3-4].

From the definition of stability, the lower the value the more stable the microbubble dispersion. When the quality of a group of samples is nearly the same, the stability becomes directly proportional to the bottoms volume. Under these circumstances the comparisons between samples can be done with the apparent stability (H') defined as:

H’ = Bottoms Volume ml [3-5].

This simplification speeds up the analysis of multiple samples, but is useful only when the qualities are within a few percentage of each other.

Lower stability values are always desired. The quality should be high, but not too high. If the shear generated by the spinning disk is too low then the force of encapsulation is too low to produce small bubbles. The larger the bubbles are initially, the faster they will coalesce and will yield a greater phase separation by the end of the one minute sampling period. When the shear rate is too high the bubbles are too small and forced into an unstable polyhedral shape, so the quality will appear very high in the generator. After the shearing force is removed, these unstable bubbles quickly coalesce. Internal forces on these very small bubbles are high and cause a disruption the surfactant layer, which destabilizes the bubbles and creates the coalescence. Bubbles made under these conditions coalesce faster than bubbles generated that have the desired characteristics when they are generated. Qualities above 75% are characteristic of these kinds of CGA. The generator produces shears in the correct range between 4000 and 7000 rpm (Sebba, 1985).

3.2 Open Microbubble Generator

3.2.1 Apparatus

Describing the microbubble generator as open refers to the solution being in contact with the open air. This is opposed to a closed system that is required to study microbubbles made with other gases. The spinning disk generator that was utilized in previous work to make air microbubbles will be described (Longe, 1989; Jenkins, et al., 1992). The source gas is the open air and is entrained by the disk and baffles during microbubble production. Figure 3-1 shows the configuration of the system before modification and will be the system used to make the air microbubbles for comparison with the H_2/CO_2 microbubbles.

The generator system is comprised of the Plexiglas chamber that has an inner diameter of seven inches and a sufficient height,

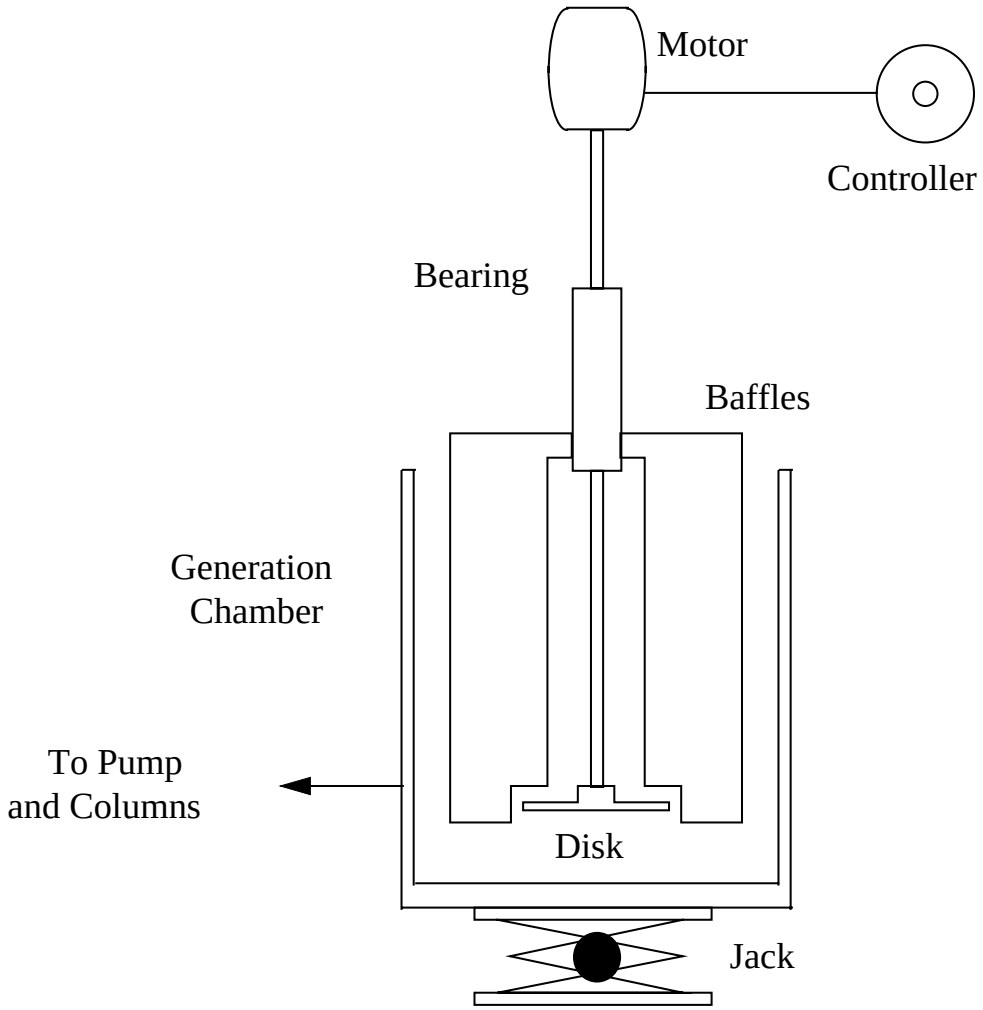

Figure 3-1: Open spinning disk microbubble generator

eight inches, to contain the volume of CGA generated from one liter of surfactant solution. One liter of solution has a depth of just over 1.5 inches and may yield four liters of microbubbles.

The most important of the other features is the disk. It has a diameter of seven centimeters and a thickness of one sixteenth of an inch. The disk is spun by a variable speed motor that permits determination of the optimal level of agitation. The controller can be set to relative values between 0-100. The bearing around the shaft performs two separate functions. The first is to stabilize the shaft during operation by preventing oscillations and keeping the shaft centered with the motor. The second function is to provide an attachment point for the baffles. As the disk spins, it creates a whirlpooling action

and pulls air into the solution where the air becomes entrained. The baffles provide added entrainment capabilities to the generator (Longe, 1989). The role of the baffles is to hinder the bulk flow of the dispersion. The top of the dispersion hits the baffles and folds back over itself and traps bulk air to be entrained into the dispersion. The last part of the generator apparatus is the jack, which controls the depth of the disk within the dispersion by adjusting the cylinder level.

3.2.2 Operating Procedure

Air microbubbles are made using one liter of a dilute surfactant solution, in this work it is a 200 ppm sodium dodecylbenzene sulfonate (NaDBS) solution. This concentration is sufficient to produce high standard microbubbles under the conditions applied. The jack is raised to its highest position, which sets the disk about halfway into the solution. The motor speed is set to 80, which puts the rotational speed of about 6400 rpm. As the microbubbles form, the jack is lowered to keep the disk near the top of the dispersion. This is determined visually by ensuring that a vortex exists and is pulling in both air and the top of the dispersion. Once the dispersion fills most of the generator chamber, the jack is raised and lowered a few times to help ensure that the dispersion is homogenous before sampling. The sampling procedure will be discussed in the microbubble comparison section.

3.3 Closed Microbubble Generator

3.3.1 Apparatus

The closed microbubble generator was designed specifically to produce microbubbles with a H_2/CO_2 gas phase. The adapted CGA generator is shown in Figure 3-2. The modifications center around the need to separate the gas in the chamber from the outside air.

A lid of the same material as the generation chamber was constructed with a central hole slightly larger than the bearing housing and two off-center inlet ports. This extra width of the central hole is necessary because the top moves up and down as the jack is used. If the lid is too

tight around the bearing it will restrict the ability of the chamber height to be adjusted, which is a necessary feature to keep the disk at its optimal depth in the dispersion. The opening is tapered around the bearing and is blocked by wedging a paper towel into it during operation, which slides along the bearing housing without any problems.

A sheet of plastic, the kind used for ground cover, is taped to the edge of the lid and to the top of the chamber wall. This creates opening is tapered around the bearing and is blocked by wedging a paper towel into it during operation, which slides along the bearing

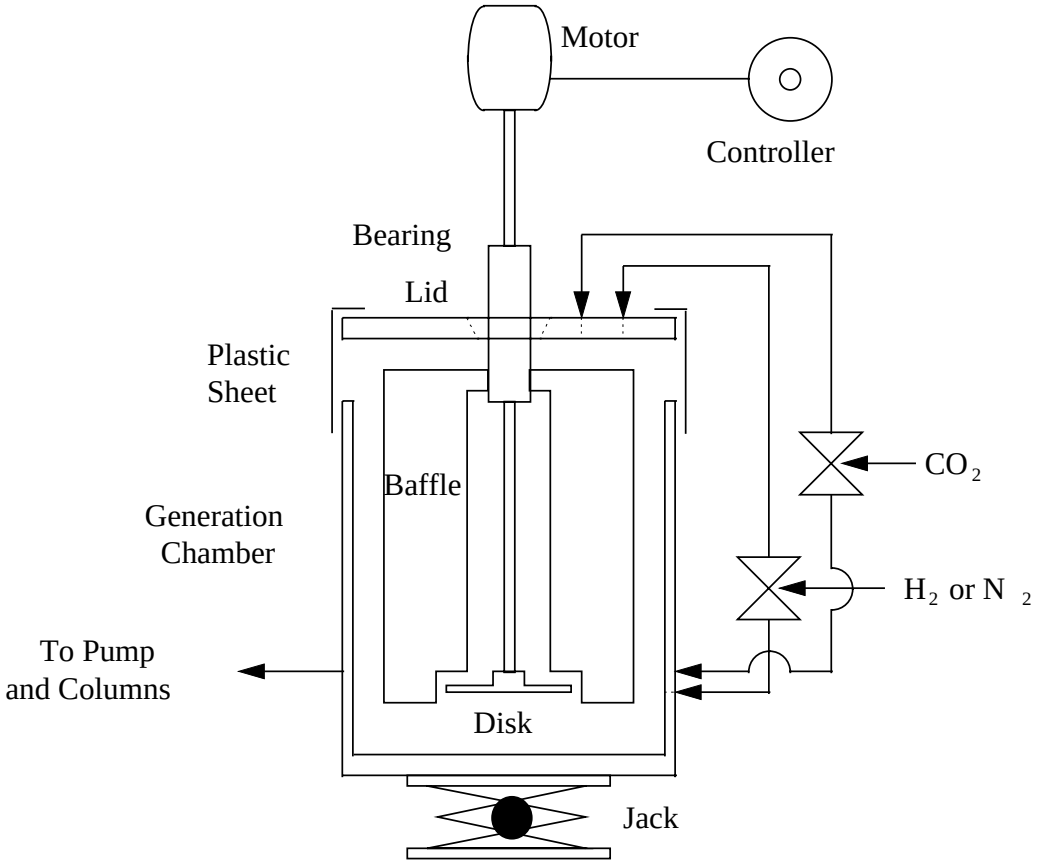

Figure 3-2: Closed spinning disk microbubble generator

housing without any problems.

A sheet of plastic, the kind used for ground cover, is taped to the edge of the lid and to the top of the chamber wall. This creates an enlarged variable volume chamber with a flexible wall and allows the jack to be used to adjust the depth of the spinning disk about 2.5 inches. The disk

level can be changed by an additional one inch due to movement of the lid along the bearing housing. Previous to the plastic, parafilm was used as the flexing material, but it broke under the stress of repeated crinkling. The plastic sheet held up much better. It was originally cut to the same width as the parafilm. Tests using a plastic sheet wide enough to allow the chamber to be raised and lowered the same amount as with the open generator were performed. The resulting CGA were quantitatively the same as the ones with the narrower sheet and the narrow sheet was easier to install.

The gases are injected to the chamber separately through different sets of inlet ports. It is easier to measure and, thereby, control each gas's flow rates before they are mixed. Both gas inlet systems are exactly the same. Each gas flowing from a source tank can be shunted to either a port in the lid or one in the side of the chamber by a three-way valve. The side port is located just below the initial liquid level. The line used for hydrogen is also connected to a nitrogen tank that is used to sparge the system to remove oxygen.

3.3.2 Operating Procedure

The operational protocol associated with the closed microbubble generator has additional steps, but all the steps for the open generator still apply. The introduction of the gases into the chamber is a new element of the procedure.

The solution is prepared the same and is put into the generator. The bottom of the plastic sheet is securely taped on to the chamber wall. Then the chamber is raised to its highest position, following the same procedure as with the open generator. Nitrogen is pumped into the chamber for ten minutes, from one of the upper ports at a high flow rate to flush out the oxygen. The flushing is done for two reasons. The primary reason is to prevent possible explosions when the H_2 is added. The microbubbles are always generated in a hooded environment. The second reason is that many anaerobic organisms are aero-intolerent, so a primary flushing with cheap N_2 will keep the O_2 content as low as possible.

After the primary flushing, the N_2 flow is replaced by H_2 and the CO_2 flow is initiated at the same time. The flows of H_2 and CO_2 are 1.8 and 0.2 L/min respectively. The reasons for these values will be explained in the chapter on chloroethene biodegradation. The procedure for producing the microbubbles changed five months into the degradation experiment. The original

protocol had the two gases always flowing in through the upper ports. The volume of the chamber is five liters. The solution takes up one liter, leaving four liters for the gas. This method flushes the $H_2/CO_2/N_2$ mixture with H_2 and CO_2 that decreases the N_2 content as time for 20 minutes, which is 10 average residence times. The gases are assumed to be well mixed at all times and the addition of H_2 and CO_2 can be treated as a classical continuous flow mixing problem. Mixing is accomplished by the incoming flows and is assumed to be complete, which may not be true. Ideally the H_2/CO_2 mixture should account for over 99% of the gas phase at this point. The chamber is sealed with tape on the plastic sheet and the shaft hole is plugged by a paper towel, so there may be some leaking. Any residual O_2 that ends up in the microbubbles should be consumed by facultative anaerobes near the microbubble inlet ports on the columns.

The newer improved procedure uses the same flow rates, but the gases are injected from the side ports. Three-way valves were installed in the gas lines so the gases can be directed to either the upper or lower injection ports. Figure 3-2 shows the side ports on the right side of the chamber near the base. The new gas delivery method utilizes the same flushing effect as well as the direct displacement of the mixture by surface bubbles filled with either H_2 or CO_2. Large surface bubbles of either pure H_2 or CO_2 fill a significant fraction of the chamber at the time the generator is turned on. Many of them break, and the gases take part in flushing out the $H_2/CO_2/N_2$ mixture just like in the previous method.

Once the H_2 and CO_2 reach their working concentrations the generator is turned on. From this point on the old protocol is the same as for the open system except that the range of motion of the jack is decreased. The jack is only lowered to the point where it is just still supporting the weight of the chamber. The maximum height that the jack can reach is the same, up to the point that the chamber hits the bearing support. The new protocol keeps the gases flowing into the side ports which are across from the disk, where they are entrained quickly. Once the dispersion reaches a volume of about four liters the gases are redirected to the inlet ports in the lid. The disk needs time to reduce the size of the bubbles. The flow of gas into the chamber opposite the disk will keep the bubbles relatively large while the gas gradient exists. Once the gas flow is redirected to the upper inlet ports, the disk can act on the large bubbles and reduce their size to an acceptable one. Then the lowering and raising of the jack takes place to provide a homogeneous dispersion. The sampling procedure, described below, is exactly like that of samples taken from the open generator.

3.4 H_2/CO_2 and Air Microbubbles

After describing the production of microbubbles by both methods, a comparison can be made between the differences in microbubble properties produced by the two methods. This is necessary to validate H_2/CO_2 microbubbles for use in stimulating the bioremediation of chloroethene contaminated ground water. The sampling procedures are independent of the types of microbubbles involved.

3.4.1 Experimental Procedure

Microbubbles were made first with the closed generator and H_2/CO_2 and sampled to determine the dispersion's quality and stability. Several trials were run to be able to define the limits of experimental error and to show reproducibility. The sampling procedure is described in the section below. Then the plastic sheet used to maintain the closed nature of the system was replaced with a longer one that would allow the generator chamber to be raised and lowered over the jack's full range. This gave the same range of motion that was used while making air microbubbles. Only two samples were taken because the qualities and stabilities were statistically exactly the same as with the narrower plastic sheet. Only three samples were taken of air microbubbles, because they all had the same qualities and stabilities. The actual values and errors will be given in the results and discussion section.

During the write-up of the results and discussions, and after the degradation experiment was concluded, it was hypothesized that acidity caused by the CO_2 was responsible for the differences between the H_2/CO_2 and air microbubble stabilities. Additional tests of the quality and stability of H_2/CO_2 microbubbles were run with different amounts of a 1 N NaOH standard solution (J. T. Baker, Inc., Phillipsburg, NJ) added to the NaDBS solution. A solution volume of one liter was maintained. The concentration of NaOH in the surfactant solutions ranged from 10^{-4} M to 10^{-2} M. The purpose of these tests was to try to verify if acidity caused by the. The results of these will be dealt with after the discussion of the previously described experiment comparing

microbubbles made with different gas mixtures. The sampling procedure for all microbubble dispersions was the same regardless of the conditions under which the microbubbles were generated.

The pH of the aqueous phase of microbubble dispersions amended with NaOH was measured after most of the liquid had coalesced. New trials were run without adding any NaOH to the surfactant solution to check the pH of the unamended H_2/CO_2 microbubbles.

3.4.2 Sampling Procedure

Samples are pumped through an exit port in the chamber one inch above the base as seen in Figure 3-3. The exit port is hooked up to a peristaltic pump with one half inch tubing. No valves are needed because no flow can occur when the pump is off. Before the pump is turned on for sampling, the rotational rate of the disk can be measured with a strobe. The pump is set to its maximum speed and turned on when the microbubbles are of sufficient quality. The quality is judged visually at this point. The line is purged with the microbubbles for a few seconds before the sample is collected.

The flow is directed to a 250 ml glass graduated cylinder where a 250 ml sample is collected. The tubing connecting the exit port to the sampling cylinder is six feet long and has an internal diameter of 3/16 inches. When the microbubbles are fed to the columns they have to travel through an additional nine feet of 0.25 inch tubing. The choice to test and compare the microbubbles after only six feet of tubing was made for logistical simplicity. Any effects the extra tubing would have on the bubbles was assumed to be same regardless of gas phase. The sampling arrangement can be seen in Figure 3-3. The cylinder is treated very gingerly because jostling can

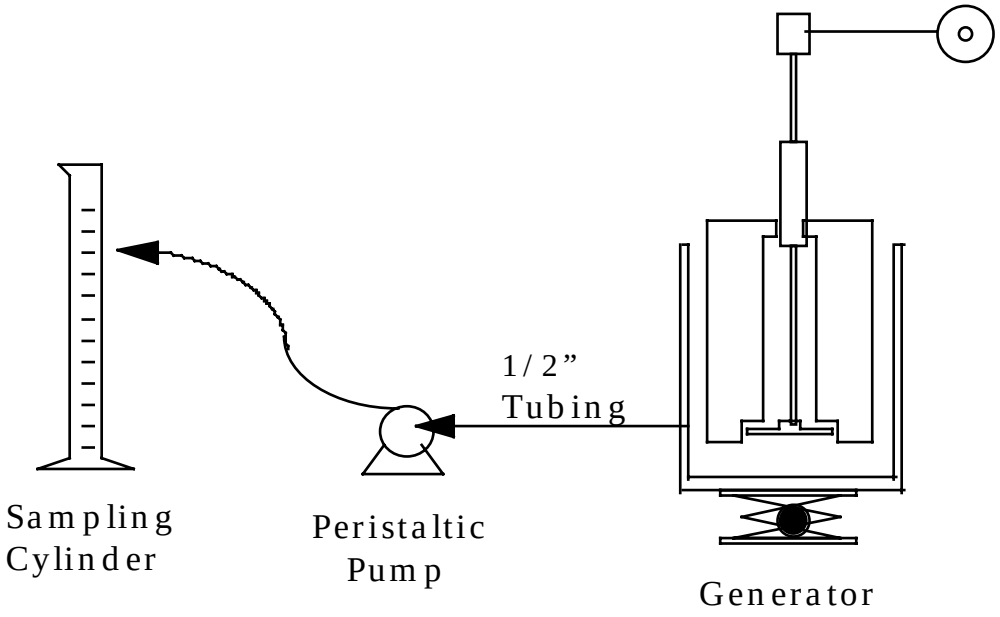

Figure 3-3: Sampling set-up

disrupt the microbubbles and give poorer results than are true for the dispersion. One minute after the sampling is completed, the volume is measured of the clear liquid that has coalesced in the bottom of the cylinder. After the one minute has passed, the mass is taken, then the quality and stability can be determined. After each trial the cylinder is thoroughly dried, because moisture on the walls will destabilize the next sample. There is enough dispersion left in the chamber to take a second sample. The only difference between the two samples from the same solution is the time spent in the generator. The disk is kept away from the top of the dispersion so no further bulk entrainment occurs.

3.4.3 Results and Discussion

Microbubbles were created under three sets of conditions and then compared with respect to quality and stability. Microbubbles were made with a mixture of 90% H_2 and 10% CO_2 in two manners and in the third set of conditions the microbubbles were made with air using the open configuration. The difference in the two sets of H_2/CO_2 microbubbles was the amount of relative movement that the disk had within the dispersion. The first set had a restricted

movement because the plastic sheet used as the flexible wall was only four inches wide, the same width as the parafilm used previously. The second set used an extended plastic sheet six inches wide that permits the same range of relative motion of the disk as the open air system allows. The two anaerobic systems will be referred to as regular sheet and extended sheet during further discussions.

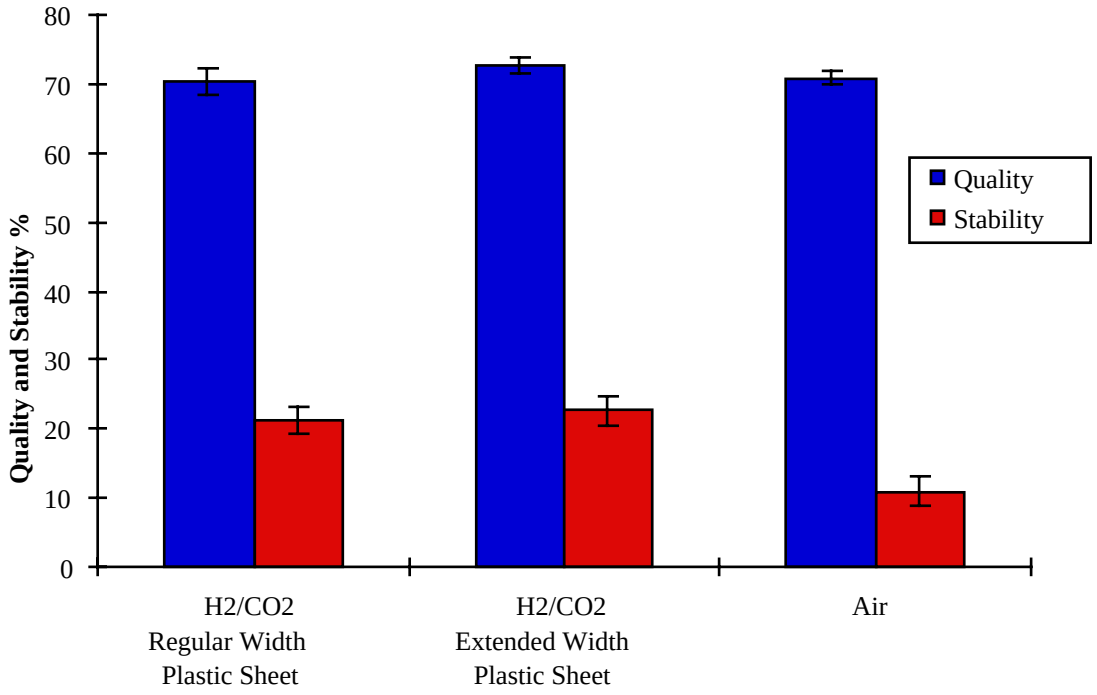

Figure 3-4:Comparison between H_2/CO_2 microbubbles with both regular and extended plastic sheet and air microbubbles

The results can be seen in Figure 3-4. The two sets of anaerobic microbubbles were almost the same. Seven samples were taken when the microbubbles were made with the regular sheet and only two were made with the extended sheet. The extended sheet sampling was minimized because the data resulted in qualities and stabilities that were statistically the same as the ones generated during the regular sheet trials. The qualities of the regular sheet and the extended sheet produced microbubbles are 70% $\pm$ 2% and 73% $\pm$ 1% respectively. These ranges include all of the data. Their respective stabilities were 21% $\pm$ 2%

and 23% $\pm$ 2%. The stabilities ranged from 18% to 23% for regular sheet system and gave 23% for both runs with the extended sheet system. The 2% error listed is derived from measurement errors in the measured variables. The balance reads to $\pm$ 0.05 g and the sample volumes can be read to $\pm$ 1 ml.

One of the seven samples was not included in the results because it was flawed due to sampling before the dispersion reached steady state. This sample was taken as soon as the dispersion's apparent quality reached the visually determined value that was used as a guide of when to sample. The gas entering from the side ports needs some consolidation time to be entrained into microbubbles with the desired properties. It yielded a quality of 68%, but its stability was 43%. There is a gradient of unassimilated gas at the inlet port to well-defined microbubbles somewhere within the dispersion. It takes time to fully entrain the gas entering at the side port, which is why the flow is shifted to the lid ports before the sampling. The disk cannot get anywhere near the top of the dispersion in the closed system, but does not need to, since a gas-liquid interface exists at the side inlets. The time taken to raise and lower the chamber a few times is usually sufficient to allow the disk to generate the proper size bubbles from the initial large bubbles created near the inlet ports. All later samples were taken at least one minute after the quality reached an acceptable level.

The air microbubbles gave an average quality of 71% $\pm$ 1% and the stability was 11% $\pm$ 2%. These ranges include all the data, comprising three points. Limited trials were done since the data were very reproducible.

The qualities of all three sets of data were statistically equal. The generator was able to produce microbubbles from either gas mixture and entrain equal volumes of gas. The surfactant solutions were the same and the rates of agitation used, i.e., the disk speeds were similar. The regular sheet trials, extended sheet trials, and the air trials gave average disk speeds of 6500 $\pm$ 100, 6450 $\pm$ 50, and 6300 $\pm$ 50 respectively. These values are all within the specified range of acceptability. The air microbubble dispersion seemed to offer a greater resistance to the disk as seen in the slower rate of rotation. A dispersion with a thicker consistency would account for the increased drag on the disk. This seems to be correlated to the better stabilities of the air microbubbles.

The stabilities were half as large with the air microbubbles relative to H_2/CO_2 microbubbles under either anaerobic generating condition. The air microbubbles had an average stability of 11% $\pm$ 2% while the regular sheet and extended sheet H_2/CO_2 microbubbles had

stabilities of 21% $\pm$ 2% and 23% $\pm$ 2% respectively. When the samples came out of the tubing the ones made with air seemed more homogeneous and flowed more smoothly. The only differences in the tests were the slight variations in the techniques of microbubble generation and the use of different gas mixtures. If the generation procedure was the cause of the difference, the qualities would not be expected to be the same while the stabilities differed.

Differences in the gases must account for the variation in the stabilities. Equal volumes of gas have the same number of molecules at the same temperature and pressure, so the number of gas-liquid interactions should be the same at the surface of the bubbles, and therefore not the cause. All the gases are non-polar, so charge based interactions between the gases and the bubbles' surfaces seem unlikely either. The likely culprit in reducing the stability of the H_2/CO_2 microbubbles is CO_2. It has a very high solubility in water because it reacts with the water. Water in equilibrium with atmospheric CO_2, 350 ppm, has a pH of approximately 5.7 (Snoeyink and Jenkins, 1980). A 10% CO_2 gas phase in Blacksburg has a pressure of 0.093 atm. The normal air pressure in town is about 93% that at sea level. Water in equilibrium with CO_2 at this pressure could have a pH less than 4.5. The hydrogen ion concentration may be high enough to neutralize the NaDBS to dodecylbenzene sulfonic acid (DBSA). A 10% NaDBS solution in distilled water has a pH of 7.5 to 8.0 indicating it is a weak base and will extract some H^+ from the water (Stepan Co., 1988; Stepan Co., 1989). The 200 mg/L of NaDBS used here will certainly not raise the pH noticeably, but conversely there should be enough CO_2 to have a significant effect on the ionization state of the DBS anion and produce significant amounts of the free sulfonic acid. The loss of the charged end would not make the sulfonic group non-polar, but would significantly lower its polarity and would reduce the hydrogen bonding with water and therefore decrease its activity. The integrity of the bubbles depends on interactions of both the highly polar and non-polar ends of the NaDBS with the water and gas phases. Some of the NaDBS may have already been neutralized during the gas addition phase before the generator has been turned on due to diffusional effects. When the generator is turned on, the contact area between the CO_2 and the aqueous phase goes up tremendously and the deactivation should be even faster.

The hypothesis that the CO_2 was responsible for the worse stabilities of the H_2/CO_2 microbubbles versus the air microbubbles was only developed during the write-up of the overall study. The effects on the bulk parameters can be seen in Figure 3-5. As expected, the qualities

are not affected by the addition of NaOH, since there were no differences in the qualities between the air and original H_2/CO_2 microbubbles. The stabilities are affected by the addition of NaOH. As the concentration of NaOH increases, the microbubbles become more stable and results in lower values for the stability. At a concentration of 10^{-2} N NaOH the stability of the

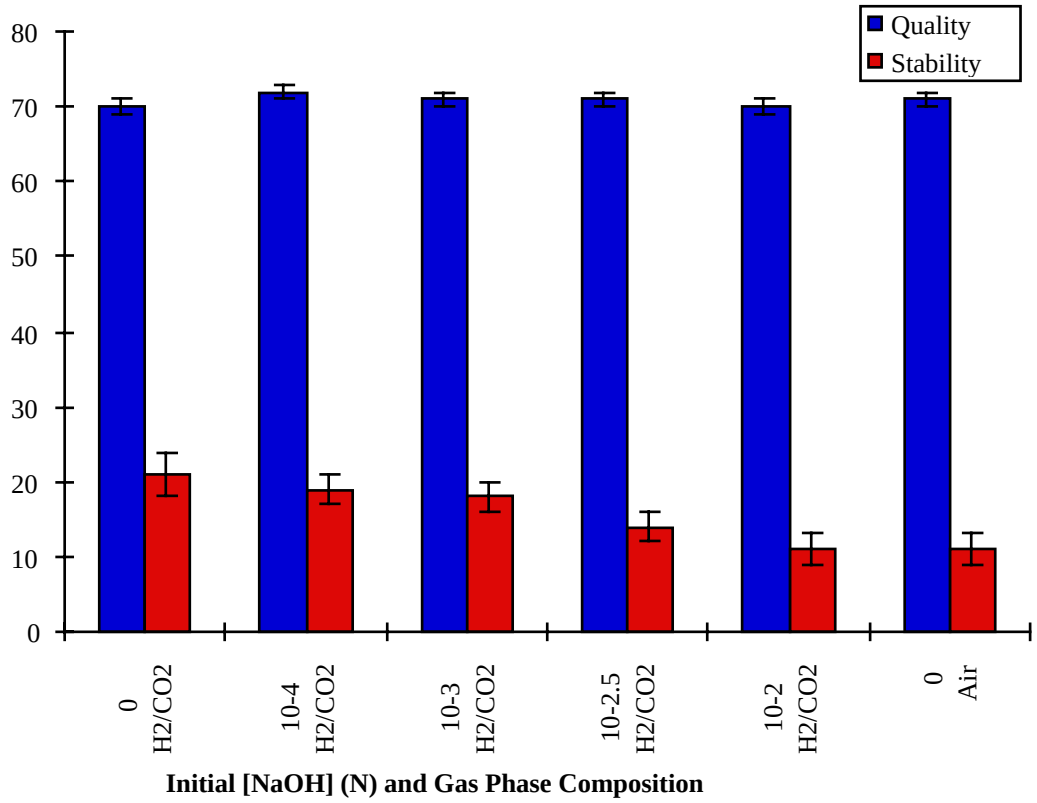

Figure 3-5:Effect of NaOH Levels on the stability of H_2/CO_2 microbubbles and how they compare to air microbubbles

H_2/CO_2 dispersion matches that of the air microbubbles. Since the
NaOH should scavenge hydrogen ions in the solution, the NaDBS should produce microbubbles with improving parameters as the NaOH concentration increases. This is seen best from the comparison between the 10^{-2} N NaOH H_2/CO_2 microbubbles and the air microbubbles. They both have stabilities of 11% $\pm$ 2%. The pH of the H_2/CO_2 microbubbles was measured as a function of the initialconcentration of NaOH. Figure 3-6 shows that the pH of the dispersion, measured after phase separation, increase with

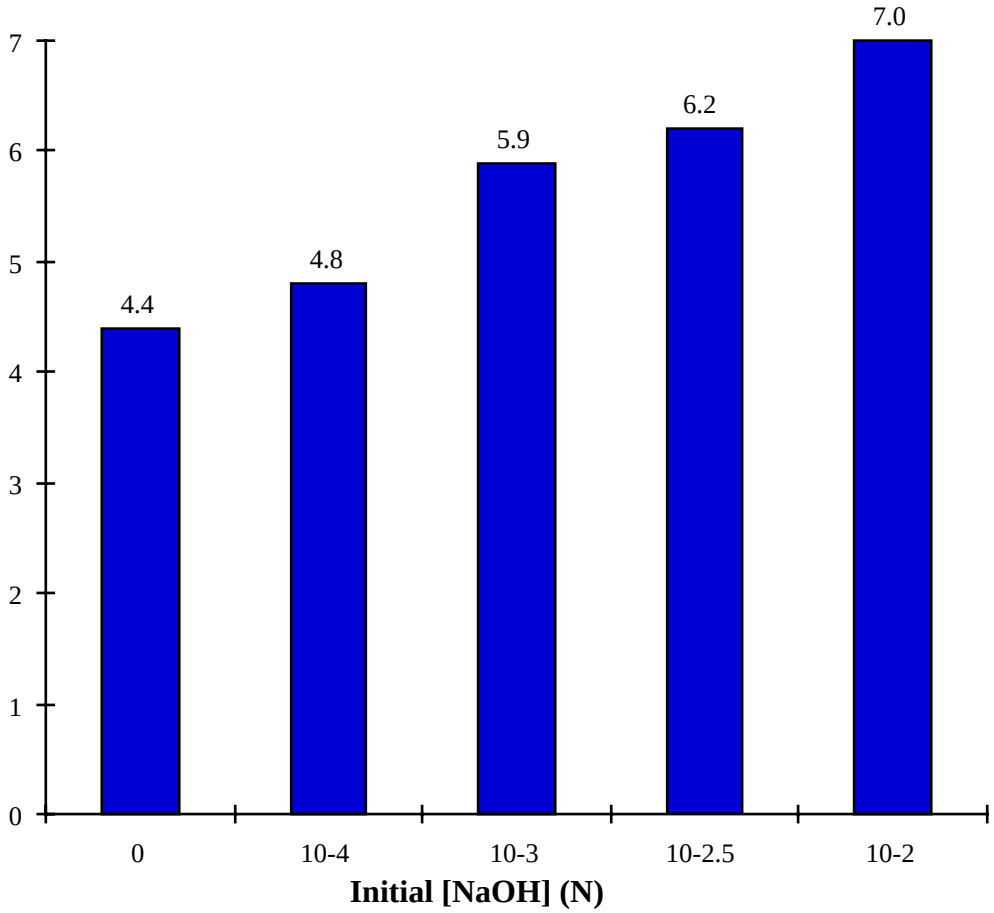

Figure 3-6: Effect of NaOH amendments on the final pH of the microbubble dispersion

increasing initial NaOH concentration. The pH of microbubbles without any NaOH amendment is 4.4. The pH rises steadily as the NaOH concentration increases. At a concentration of $10^{-2.5}$ N NaOH, the final pH of the dispersion enters the range where the various bacteria in anaerobic consortia begin to show normal levels of activity. The range of pHs that are generally preferred by these organisms is about 6 to 8. The 10^{-2} N NaOH amendment yields microbubbles with a pH of 7, which is safely in the middle of the range that the anaerobic bacteria prefer.

The differences in the stabilities are small enough that the H_2/CO_2 microbubbles were still effective in transporting the gases to the soil columns. The microbubbles were injected at 3 ml/sec for 20 seconds twice a week. The time it takes the microbubbles to get from the chamber to the column inlet is less than one minute. The microbubbles travel through 1.85 m (6 feet) of tubing with an inner diameter of 0.79 cm (3/16 inches) then 3 m (9.75 feet) of tubing with an

inner diameter of 0.3175 cm (1/8 inches). The volumes of the two sections are 90.68 ml and 23.75 ml respectively. The total travel time in the tubing is 38 seconds. The stability should be lower, meaning more stable bubbles, due to the 22 second savings between usage and measurement times. The flow process in small diameter tubing may also take part in keeping the dispersion from phase separating. The flow is then pumped to the column inlet port at a high rate to maintain turbulence and the flow is purged from the bypass until the dispersion flows with a visually consistent composition. The flow rate is then reduced to 3 ml/sec and injected for the 20 seconds. The turbulence of the dispersion prior to injection should also help the microbubbles maintain their properties that existed in the generation chamber until actual delivery to the soil.

3.5 Summary

The adapted generator offers an easy way to make microbubbles using any desired gas phase. Using pressurized air from a tank and running the generator in closed mode can provide a more direct comparison with the H_2/CO_2 microbubbles, since the variations in production techniques would no longer exist.

The loss of the complete range of motion of the disk in the dispersion using the four inch wide plastic sheet as the flexible wall instead of the six inch one does not yield poorer H_2/CO_2 microbubbles. The narrower regular plastic sheet is also much easier to install. The regular sheet method and the extended sheet method both produce H_2/CO_2 microbubbles with the same characteristics, qualities of 70% $\pm$ 2% and 73% $\pm$ 1% and stabilities of 21% $\pm$ 2% and 23% $\pm$ 2% respectively. The generator was capable of making microbubbles of consistent quality and independent of the gas phase as seen by the air microbubble values of 71% $\pm$ 1%. Without any amendment to the initial solution, the air microbubbles are twice as stable as the H_2/CO_2 microbubbles. This effect is caused by the acidification of the aqueous phase by CO_2, and the subsequent loss of the ionic character of the NaDBS, which reduces its ability to stabilize the microbubbles. The stabilities attained with the H_2/CO_2 microbubbles are acceptable as gas carriers to the anaerobic consortia in the soil columns.

Only after the degradation experiment was complete, was it learned that the CO_2 acidified the microbubble dispersion to such a high extent. The CO_2 in the dispersion has a negative effect on the stability but not on the quality. The CO_2 made the dispersion acidic, which neutralized

the NaDBS and prevented it from stabilizing the bubbles. Amending the surfactant solution with NaOH led to better microbubbles that showed that the acidifying power of the CO_2 was responsible for the reduction in properties of the dispersion. The pH of the dispersion was dependent on the amount of NaOH that was initially present and that a concentration of 10^{-2} N NaOH yielded a pH neutral dispersion with the best bulk properties.

The microbubbles were used at a 3 ml/sec rate and spent less than 40 seconds outside of the generator before being delivered to and stabilized within the soil matrix. The savings of twenty seconds resulted in the delivery a more stable microbubble dispersion than exists one minute after sampling. The turbulence in the tubing while the microbubbles are flowing to the column port helps the CGA maintain their chamber properties. The dispersion had only been without agitation for up to 25 seconds before injection. The turbulence should have further reduced the degradation of the microbubble dispersion before utilization. Production and delivery methods ensure that microbubbles can be an effective means to transport gases to assist in the biodegradation of contaminated soils.

Chapter 4: Bioremediation of PCE

PCE dehalogenation requires H_2 as the direct electron donor and H_2/CO_2 microbubbles can deliver H_2 to meet that function. Before the bioremediation experiment can be performed, a system that is capable of growing, maintaining, and monitoring the activity of the microbial consortia must be designed and built. The system had to be able to be run continuously for months at a time with well-controlled flow rates. Good control was required to give an accurate measurement of the microbial activity, certain nutrients, and most importantly the amounts of PCE and the chloroethene dehalogenation products. Analytical procedures were developed to measure the chloroethenes, methane, ethene, ethane, propionate and acetate, and bromide and sulfate.

The operating characteristics of the system had to be determined before the PCE addition could begin. Some of the characteristics that had to be verified were that the flow rate stabilities were maintained leaving both the nutrient tank and the PCE solution chamber and entering each column. The inlet PCE concentration to the columns when the remediation experiment began was 30 μM (5 mg/L). Other operating characteristics investigated were that the PCE stream was adequately mixed with the nutrient flow and that, under abiotic conditions, sinks for neither PCE or TCE existed. The breakthrough and the residence time distribution through a column were also determined. A single column abiotic experiment was run to verify that the PCE would be recovered when there was no biological activity. A similar study with TCE was performed to verify it would not interact with the polycarbonate column. The initial breakthrough time of an inert species and the time to it took for that species to reach its maximum concentration were determined. The system was then operated with the consortia present for nine months previous to the addition of PCE.

To meet the operational goals of the bioremediation experiment several improvements were made before the PCE was successfully added to the flow. As the apparent need arose, necessary improvements were made from the time the abiotic recovery experiment began until the PCE addition was initiated.

Once the operational stability of the system was achieved, the PCE flow was initiated. H_2/CO_2 microbubbles were injected twice a week into two columns while the comparison columns received twice daily injections of a sodium propionate solution and the control columns

received no special addition of electron donor or carbon source. The chloroethenes and the two carbon hydrocarbons were measured from the column effluent to monitor the degradation. Methane was measured to monitor the activity of the methanogens. The degradation capabilities of the different consortia were determined under the conditions tested. Recommendations for further improvements to the design of the experimental procedure and monitoring to enhance the biodegradation of PCE with H_2/CO_2 microbubbles will be presented.

4.1 Experimental System

The experiment was designed to control and to monitor the biological remediation of a PCE contaminated water flow. The complete system can be seen in Figure 4-1. The main features of the experimental system are the six columns, where the microbial populations are maintained and perform the dehalogenation of PCE and the other chloroethenes. Flow rates from the PCE chamber and to the six columns are controlled by pumps and measured continuously on-line. The PCE solution chamber and pump can be seen on the left of the figure. One column pump and rotameter can be made out between columns 2 and 3. Two flow streams carrying the PCE and the nutrients are pumped to a meeting point and mixed

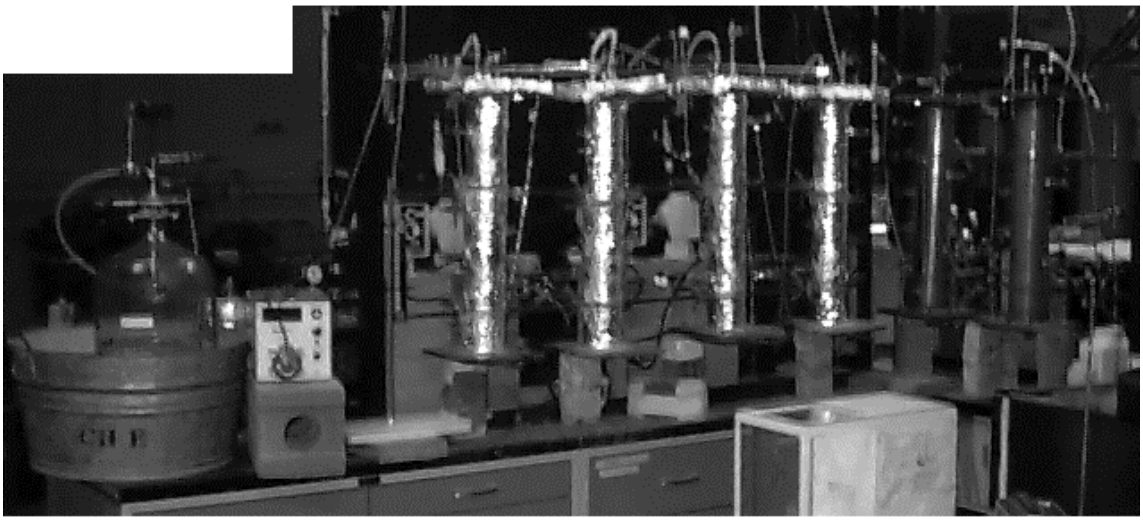

Figure 4-1: Bioremediation system with the six soil column bioreactors.

before reaching the column split-off lines from the manifold. A safe effluent drainage system

completes the system. The flow tubing and mixing chamber are behind the columns and the effluent lines cannot be seen.

A schematic of the system can be seen in Figure 4-2. The system will be divided into sections describing the columns, PCE and nutrient solution flow regions, the manifold, and the drainage lines.

4.1.1 Column Apparatus

The microbial consortia are grown and maintained in soil filled columns. The columns have to have sample ports to monitor the conditions during operation. They also need injection ports to add the required stimulants and nutrients such as the H_2/CO_2 (Industrial Gas, Radford, VA) microbubbles, the sodium propionate (Aldrich

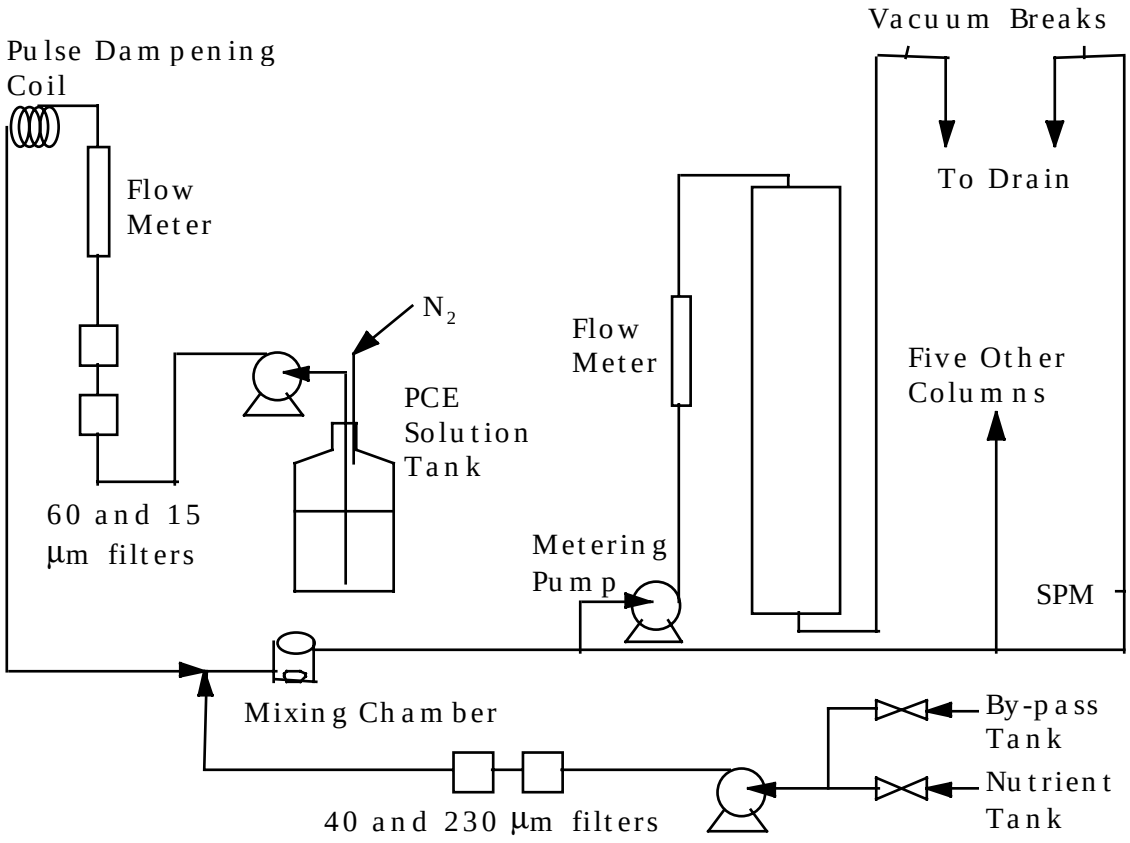

Figure 4-2: Treatment system schematic

Chemical Company, Inc., Milwaukee, WI) and the yeast extract (Sigma Chemical Company, St. Louis, MO). The pump bringing the controlled flow to the column and the rotameter used for flow measurement are considered part of the column apparatus.

The system has six columns. The two microbubble columns and the two propionate columns are made of 0.125 inches thick polycarbonate with an internal diameter of 3.75 inches and 26 inch length. Each column has two 0.5 inches thick polycarbonate plates attached at the top and one or two at the bottom. The overall configuration of a column can be seen in Figure 4-3. The two control columns are made of 0.125 inch stainless steel. They have internal diameters of 4 inches and are 26 inches long. Both have 0.25 inch stainless steel plates on the ends. The plate nearest to the column is sealed around the base of the column either by bonding with methylene chloride or by welding. The outer plate has an o-ring in a groove and the two plates are screwed down firmly to seal the system.

The top plate contains the inlet port for the flowing water as well as the septum lined injection port and a vent. The injection port consists of a Teflon® lined septum placed between a fitting and tubing connector. All of the columns have yeast extract injected through this port and it is also used to feed columns 3 and 4 the propionate solution. The bottom plate connects to the effluent line. Approximately four inches from the bottom plate along the effluent line is the sample port SP5. This is the port used to take virtually all the samples of the study. Along the length of the column are sampling ports (SP1-SP4) that were used to monitor gradient profiles of methane.

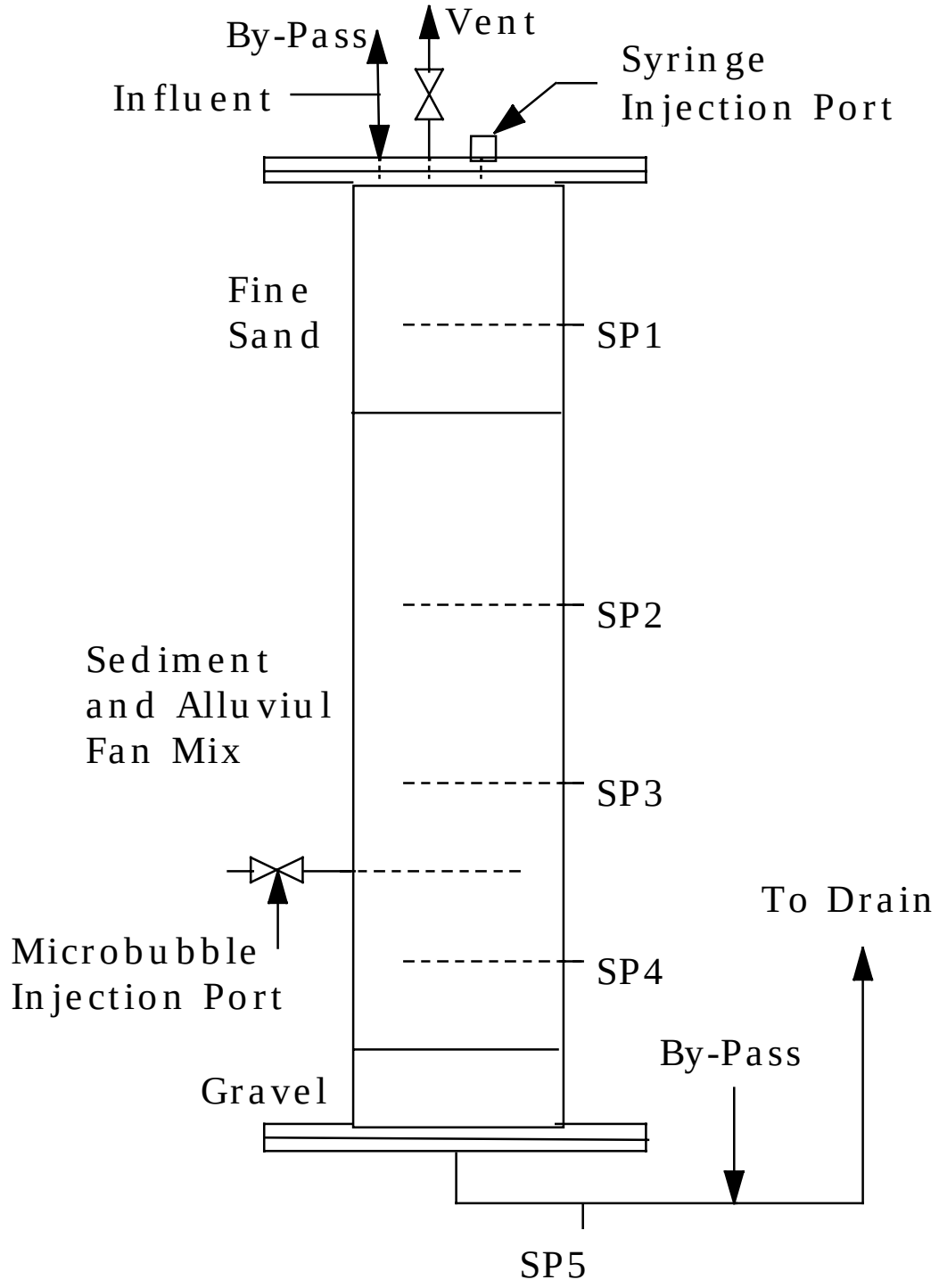

Figure 4-3: Column schematic

With the column structures defined, the column loadings can be addressed. The following discussion will be addressing one column only, but the other five were manipulated in exactly the same manner. The column was filled with a combination of pond sediments, alluvial fan, and sand. The sediments came from the Duck Pond on campus. The different materials are present in different strata. The bottom layer is two inches deep and is made up of a gravel of pebbles sieved from the bulk alluvial fan. The pebbles have diameters between 0.0331 inches and 0.0787 inches. This layer is meant to be a buffer between the effluent line and the small diameter particles in the sediment/fan layer to prevent clogging downstream.

The middle layer was packed with a mixture of anaerobic sediments from the Duck Pond and alluvial fan. This layer was the treatment zone since it has the loading of microorganisms. The layer is made by mixing the sediments with the fan in a ratio of 3:1. The alluvial fan was separated into fractions based on particle size. All particles greater than 0.132 inches were removed. All of the collected material with diameters between 0.0082 inches and 0.132 inches was remixed along with about 20% of the material with diameters less than 0.0082 inches. The sediments were screened to remove twigs, rocks, leaves, litter, and anything else greater than 0.185 inches in diameter. The screening was done in segments while the bulk of the sediments was kept immersed in Duck Pond water to keep them anaerobic as possible. Once the sediments were free of the larger objects, the fan was added and was then mixed by hand. Extra Duck Pond water was added to aid in the mixing. The final mixture was poured into the columns while always maintaining a water layer above the mixture. The water acted as a buffer with the air and helped the mixture settle. This active layer is loaded into the columns until only the last six inches are empty.

The last six inches were filled with fine sand. The sand has a diameter less than 0.0117 inches. The liquid above the treatment layer was removed before the sand was added. The sand was added in stages with deoxygenated water added after each addition. This made easier for the water fill the pores and prevent the accumulation of air pockets. When the column was full, excess sand was removed from the top of the plate so the o-ring was able to make a tight seal between the plates.

Loading the column was hampered by the injection port and sampling ports located along the length of the column. As seen in Figure 4-3, the side ports extend through most of the column, almost to the opposite wall. The ports were extended through most of the soil, so samples would be drawn from a cross section and the injection of microbubbles would be

distributed as widely as possible along that slice of the column. The parts of both the injection and sampling ports that are in the soil layers are made the same way. The section of the ports that are in the soil consists of a 0.25 inch stainless steel slitted tube that is wrapped in a fine stainless steel mesh. The slits are 2.5 inches long for the injection port and one inch long for the sample ports and 0.125 inches wide and are found on both sides of each tube. The ends are crimped shut and the mesh is wrapped around the slitted section. The mesh is sealed to the tube with epoxy. The mesh allowed microbubbles or water to flow through, but did not admit solids. This kept the sample port valves from clogging and helped disperse the microbubbles more evenly upon injection.

The microbubble injection port has a by-pass line so that the microbubbles could be visually inspected before they were pumped into the column. The microbubble injection port was placed in the lower part of the treatment zone since any natural movement would be in the up direction.

The flow into the column could be directed to a by-pass, which meets the effluent line after the sample port SP5. The by-pass line was used during the cleaning of the tubing and rotameters. The cleaning solutions were either dilute NaOH or bleach. Bacteria growing in the feed lines would plug the rotameters, which were essential, and the needle valves used for flow control, which were eventually removed when the metering pumps were put on-line. During normal operation of the system, the by-pass lines were not used, and were therefore left out of Figure 4-2.

The last parts of the column assembly are the metering pump and the rotameter that were used to control and measure the flow rate to the column. The pump is an Electric Metering Pump Model A771-155S (Milton Roy, Acton, MA) which is a diaphragm positive displacement pump. The pump was placed above the manifold since the drawing action of the pump was set vertically. The stroking action of the pump caused the float in the rotameter to pulse regularly over a constant range.

The pump was set and rotameter was calibrated simultaneously by measuring the flow from the effluent stream. The effluent line of the pump was disconnected from the common drain line and was collected for one half of an hour. The samples were massed and converted to volume with the density. As seen in Figure 4-2, the drain lines rise above the columns where the vacuum is broken and the flow is then allowed to drop down to the drain. This maintains a positive pressure at the bottom of the column during operation. While the pump was being set

and the rotameter calibrated the disconnected drain line was taken to the same height before collection of the sample.

Previous to the installation of the metering pumps, the flow into the columns was driven by the peristaltic pump drawing the nutrient solution from the tank. The pressure at the end of the manifold was controlled to maintain a constant driving force for flow into the column. Initially a needle valve was used to maintain a back pressure on the manifold. It was regularly being plugged by contamination in the line. A spring loaded back pressure regulating (BPR) valve was installed in place of the needle valve, but it also became plugged. Another type of BPR valve was tried and also experienced the same plugging phenomenon. Trying to maintain precise control of the flow was attempted for close to two years before the metering pumps were installed. The metering pumps did not require that a back pressure was maintained on the manifold, so the valve was removed and the excess flow in the manifold went to the drain.

Control of each column's flow rate was initially achieved with a needle valve set upstream of the rotameter. As one needle valve plugged the flow rates to the other columns would increase. Efforts to clean and purge the lines were only temporarily successful. A steady flow to each column could not be maintained for over one week periods. This was why the column control valves were replaced by the metering pumps. Besides controlling the flow into each column, the flows were now uncoupled from each. Each metering pump drew up 3 ml/min from the manifold as long as the total flow exceeded the combined flow to the columns.

4.1.2 Nutrient Solution Flow Apparatus

The nutrient solution flow apparatus consists of two holding tanks, a peristaltic pump and two filters. This part of the system can be seen in the bottom part of Figure 4-2. The experiment required high flow rates for months at a time. For this reason a large tank, 1000 liters, was chosen to hold the nutrient solution. The by-pass tank was used while the main tank was being refilled, which took about five hours. The filters were used to prevent fouling due to microbial growth in the stream.

The nutrient solution had a composition of buffering agents and minimal salts dissolved in deionized water. The medium formulation is adapted from one provided by Biosystems (Blacksburg, VA). The deionized water used was made from tap water that flowed through a

deionizing resin (Cole-Parmer, Oak Brook, IL) that yielded a resistivity up to 15 MΩ-cm. One milliliter of the minimal salt solution was added to each liter of medium. The composition of the minimal salt solution is provided in Table 4-1. The amounts of $MnCl_2 \cdot 4H_2O$ and $FeSO_4 \cdot 7H_2O$ (J. T. Baker, Inc., Phillipsburg, NJ) are both calculated for 2 g/L of the anhydrous salt. The $CaCl_2$ and $ZnCl_2$ are from Aldrich Chemical Company, Inc. (Milwaukee, WI). The rest of the minimal salts and the buffers are from Fisher Scientific Co. (Pittsburgh, PA). The amount of NH_4Cl

Table 4-1: Minimal Salt Solution

	(g/L)
$MgBr_2 \cdot 6H_2O$	20
NaCl	2
$MnCl_2 \cdot 4H_2O$	2.62
$FeSO_4 \cdot 7H_2O$	3.66
NH_4Cl	59.33
$CaCl_2$	20
$ZnCl_2$	0.2
$CoCl_2 \cdot 6H_2O$	0.2
$NiCl_2 \cdot 6H_2O$	0.2
$Na_2MoO_4 \cdot 2H_2O$	0.2

used gives an NH_4^+ concentration of 20 g/L. The sulfate concentration in the medium is 0.01 mM. Some sulfur is needed, but the level is kept low to minimize the activity of the sulfate reducers. The buffering agents used were 0.45 g K_2HPO_4 and 0.4 g KH_2PO_4 per liter of medium.

The nutrient solution accounted for approximately 95% of the total flow, the rest coming from the PCE chamber. Maintaining sterile conditions in this tank was not attempted. Efforts were made, however, to keep the microbial growth to a minimum. Nitrogen was periodically sparged through the tank to keep the oxygen level low. The tank was wrapped in opaque plastic to prevent growth of algae. There was no carbon in any of the nutrient salts used. Even with these precautions there was some microbial activity in the flow tubing. The total flow was about 21 ml/min which translates to just over 30 L/day. This allows the system to run off the main

tank for almost one month before it needs to be refilled, since the solution was drawn from the bottom of the tank. To keep the intake free of oxygen the tank was usually refilled when the level got to 200 liters. During refilling, the by-pass tank would be used.

The by-pass tank could hold only 20 liters. Solution from the main tank was placed into the by-pass tank, which was then sparged to remove the oxygen that entered the solution during the transfer. The by-pass tank contained more than enough solution to maintain the flow through the system while the main tank was being refilled.

A peristaltic pump was used to deliver the nutrient flow to the manifold. The tubing used originally was 0.25 inch Tygon® Lab tubing. Every two weeks the tubing was changed and the system was down for about five to ten minutes during the transfer. The new tubing was autoclaved prior to installation. The tubing was wrapped in foil, because it was transparent. When the old tubing was removed growth could be seen on the walls. During the last two months of operation the Tygon® tubing was replaced by norprene tubing. This tubing had many advantages over the Tygon®. First, it has a much longer pumping life and went the two months without being changed. This reduced the chance of contamination at the fitting sites where the tubing was disconnected from the flow stream. Its other advantage was its opacity. The simplified the efforts required to prevent algal growth.

Even with the preventive measures taken, the nutrient tank flow line was where most of the contamination in the main manifold came from. Two filters, of 230 μm followed by one of 60 μm, were placed in-line to trap most of the biological contamination. They appear directly down stream of the nutrient solution pump in Figure 4-2. The filters were cleaned, every two weeks or when the pressure went up in the line, by autoclaving in a dilute bleach solution. The system was off-line for about three hours while this was done. After passing through the filters, the nutrient solution was merged with the PCE solution.

4.1.3 PCE Solution Flow Apparatus

The PCE solution flow apparatus is significantly more complex than the one used for nutrient solution. The apparatus can be seen in Figure 4-2 and includes the equipment from the PCE solution tank to the pulse dampening coil. An aqueous solution saturated with PCE is held in a glass bell jar, referred to from this point on as the PCE solution chamber. Because PCE is

immiscible in water, its solubility is 150 mg/L, the only aqueous solution of PCE in water that will maintain a constant concentration is a saturated solution. About 50 ml of technical grade PCE (Fisher Scientific Co., Pittsburgh, PA) is kept in the bottom of the PCE solution chamber to maintain equilibrium. The original solution was made in the chamber and was mixed for two weeks, to ensure saturation was reached, before it was used. When the chamber needed to be refilled, 17 L of a solution already saturated with PCE was added. The solution was made two weeks before it was added to the PCE solution chamber to ensure that is was saturated. The preparation of this solution and the procedure for getting it into the chamber will be discussed in Section 4.5.1 dealing with the operating procedures of the degradation experiment. The solution was gently stirred through the duration of the experiment. The jar was sealed with a Teflon® cork with two stainless steel lines running through it. The first line barely extends below the cork. This was for the pressurized N_2. The other line extends to about one inch above the bottom of the chamber and was the exit port for the solution.

A peristaltic pump with 4 mm Viton® tubing was the driving force for PCE solution flow. Viton® is a fluoroelastomer that is resistant to PCE. To maintain a constant pressure in the chamber as the solution was used up, the headspace was kept at 1.5 psig with N_2. The flow then passed through 60 μm and 15 μm filters before getting to the rotameter. The flow rate of the PCE solution was about 0.7 ml/min and the clearance between the rotameter float and the tube was small. The filters were there to prevent any plugging.

After the rotameter, there is a coil of Viton® tubing with approximately 20 windings. The metering pumps pulse and decrease the pressure in the manifold. This caused the PCE flow to pulse uncontrollably. The coil effectively dampened the pulse until the rotameter float barely moved. The flow then continued to the point where it met up with the nutrient solution.

Previous to the incorporation of the metering pumps, during the complete growth and maintenance phase of the experiment the driving force for flow was pressurized N_2. Neither the pump nor the coil was present. The pressurized N_2 pneumatically forced the saturated PCE solution to flow. The N_2 gauge pressure was about 12.5 psig. The flow instabilities, due to the needle valves on the columns, affected the manifold pressure, which in turn affected the flow of the PCE solution by changing the driving pressure gradient.

4.1.4 Manifold and Mixing Chamber

The two slow streams meet at a t-junction, which is where the manifold begins, and flow to a mixing chamber, all of which is shown in Figure 4-2. Before the mixing chamber was installed the two solutions did not mix. Experiments showed that the inlet concentration of PCE was highest in column 1 and decreased steadily from each column all the way to the manifold sampling port (SPM). Both flows were very slow and no turbulence was created to integrate the streams.

The combined flow enters through a port near the bottom of the Plexiglas® mixing chamber into a cylindrical region one inch in diameter and 2.5 inches high. A stir bar in the bottom effectively mixes the two solutions. The mixed solution leaves through a port near the top of the mixing chamber. A quick study was performed to verify that the two streams had been mixed into a homogenous solution. Samples were taken from the manifold port (SPM) and the vents at the top of each column. The concentration of PCE measured was the same within experimental error.

After the mixing chamber the manifold has six off-takes for the columns. About one foot after the last column off-take is the SPM. Past the SPM the manifold rises to a vacuum break and the excess flow heads to the drain.

4.1.5 Drainage System

The excess flow from the manifold as well as the effluents from all six columns have to be safely disposed of. The drainage system, found on the right hand side of Figure 4-2, was designed to provide a positive pressure at the exit of each column. All of the effluent lines are taken to a height of five feet above the column exit where the vacuum is broken and the flows can freefall to the drain. The effluent lines from columns 1, 3, and 5 merge before the vacuum is broken. A similar arrangement exists for the effluents from columns 2, 4, and 6. After the vacuums are broken all three lines are brought together. Because there were chloroethenes in the effluents, the drainage line exited into a hood. This is the last part of the system, the whole of which has proven capable of yielding the data required to test the objectives of this study.

4.2 Analytical Procedures

This study required many and varied analyses. The most crucial analyses were for the measurement and monitoring of PCE, TCE, DCE, vinyl chloride, ethene, and ethane. Methane production and sulfate concentrations were measured to monitor the relative activities of the methanogens and sulfate reducers in the six columns. The methane was analyzed alone and with vinyl chloride, ethene, and ethane. All gas analyses were performed to provide the concentrations of the dissolved gases, although the headspaces within the sample vials were the sources of the GC samples. Henry's Law was used to convert these measurements to liquid phase concentrations. The laboratory temperature was between 22 °C and 24 °C and the Henry's Law constants were evaluated from data at 24.9 °C. The sulfate was monitored at the same time as bromide. The bromide was analyzed to determine performance characteristics of the system such as breakthrough time and average residence time. Propionate and its metabolic product acetate were analyzed together to monitor the activity of the proton reducers and the acetoclastic methanogens respectively. The details of sampling procedures will be described along with the conditions of each analytical method.

All of the measured species were determined by some form of chromatography. Peak areas from all GC analyses were converted to species concentrations with linear calibration curves encompassing the range of sample concentrations. Analysis of the multichlorinated ethenes will be discussed first, followed by the technique used for vinyl chloride, ethene, ethane, and methane. The protocol, used for methane when it was the only analyte being measured, will be dealt with next. The bromide and sulfate were analyzed by ion chromatography and the procedure will be described last. Standard preparation protocols and the procedures used to take, store, and prep samples will be described for each type of analysis.

4.2.1 Multichlorinated Ethenes Analysis

The multichlorinated ethenes were analyzed on an HP5890 Gas Chromatograph (GC) (Hewlett Packard, Palo Alto, CA) being operated in split mode. An electron capture detector (ECD), which is a specific analyzer of organic halogens, was used with helium as the carrier gas.

4.2.1.1 Standard Preparation

Mixed standards were made containing the multichlorinated species PCE, TCE (Fisher Scientific Co., Pittsburgh), and *trans-DCE* (Aldrich Chemical Company, Inc., Milwaukee, WI). The standard concentrations ranged from 1 mg/L to 5 mg/L. An inlet concentration of 5 mg PCE/L was used for the degradation experiment. Trans-DCE was the standard used for both isomers of DCE, since the detector does not differentiate these two species anyway. Approximately 25 mg of PCE was diluted with methanol to a final volume of 10 ml. The same was done with the other compounds. One milliliter from each 10 ml volumetric flask was then pipeted into the same 25 ml volumetric flask containing methanol. Different volumes of this mixed methanol standard were pipeted into flasks of deionized water to make standards at five different concentrations. 5 ml samples of each standard were pipeted into vials containing 0.5 ml of HPLC grade n-pentane (Fisher Scientific Co., Pittsburgh, PA). The last drop from a volumetric pipet is usually removed from the pipet by capillary forces. Since pentane and water are immiscible this did not work. The last drop was ejected from the pipet by using the dispenser to blow the last drop out. The vials were then capped. Although this was unorthodox, the samples were prepared in the same manner, so the results can still be interpreted correctly. The pentane was used to extract the chloroethenes from the water. The standards were stored refrigerated until the day of the analysis.

4.2.1.2 Sampling Procedure

Samples were taken from the effluent sample port (SP5). The port was purged of 3 ml to 4 ml before the actual sample was taken. The sample port valve is spring loaded and the collection vial is placed along side and pushed into the valve. This type of sampling valve can not be used to collect samples with accurate volumes. A sample of 6 ml to 7 ml is allowed to run down the side of the vial, then 5 ml is quickly pipeted into another vial containing 0.5 ml pentane. The samples are stored refrigerated until analysis. Manifold samples require a different procedure.

The manifold sample port (SPM) is a ball valve. The valve is opened and all of the fluid

between the valve and the vacuum break in the manifold is purged. The vial is then placed at the valve exit and a 6 ml to 7 ml sample is collected. The pipeting and storage procedures are the same as from SP5.

4.2.1.3 Analytical Procedure

The samples are mixed vigorously immediately prior to injection. After mixing the pentane phase has to coalesce from droplets, and when this is complete sampling for injection can occur. 2 μL samples of pentane are injected. The splitting procedure diluted the samples by a factor of 40 before the sample gets onto the column. The column used is the DB 624, which is a 30 meter capillary column (J&W Scientific, Folsom, California). The support phase is 6% cyanopropylphenyl - 94% dimethyl polysiloxane and the film thickness is 1.8 μm. The detector temperature is 280 °C and the injector temperature is 220 °C. The oven temperature profile begins at 75 °C and is held for one minute. The temperature is then ramped at 15 °C/min to 165 °C, then immediately ramped to 30 °C/min for two minutes.

As a rule the *trans-DCE* gave significantly smaller peaks than PCE. At the same mass concentration, the molarity of *trans-DCE* is almost twice as high as PCE and the molarity of chlorine atoms is almost the same. This means that the detector responses should be similar. The discrepancy is probably due to the fact that *trans-DCE* has a very high solubility in water and did not enter the pentane or that its high volatility fostered large losses during standard preparation.

4.2.2 Vinyl Chloride and Hydrocarbon Analysis

The vinyl chloride should be picked up by the ECD but was not seen. The analyses of the hydrocarbons were also done on an HP5890 Gas Chromatograph. The detector used is a flame ionization detector (FID) which is very sensitive for hydrocarbons.

4.2.2.1 Standard Preparation

Gas standards were prepared for analyzing these compounds. A mixed standard, that contained one percent of ethene, ethane, and methane as well as acetylene, carbon monoxide, and carbon dioxide, was bought (Scott Gases, Plumsteadville, PA). The standard was allowed to flow through a needle into a 70 ml bottle crimp closed over a rubber stopper. Another needle was also put through the stopper to allow gas to be flushed out. After a minute the bottle was full of the standard. Syringes delivered the primary standard to sealed bottles. The bottles had glass beads used to mix the contents. Once the mixing was done the same volume as injected was extracted to keep the pressure atmospheric. This removal of the analytes was taken into account when the calculations were being done. Secondary standards of 0.45% and 0.1% of the analytes were made this way.

A 1000 ppm vinyl chloride standard was bought from the same source and was treated in the same manner to make secondary standards of 450 ppm and 100 ppm. The only difference was that this was done in a hood.

4.2.2.2 Sampling Procedure

The sampling procedure was essentially the same used for samples taken to measure the multichloroethenes. All of the samples were collected from SP5. There were however three minor differences. First, no pentane was used, because no extraction was necessary. Second, open hole caps with teflon lined silicone septa were used and the last difference is that the vials were stored in the refrigerator inverted. This placed the liquid phase as a barrier between the headspace in the vial and the cap, where leaks were most likely to occur. The vial volume was 8,85 ml and the sample was 5 ml. This left 3.85 ml of headspace.

4.2.2.3 Analytical Procedure

It was assumed that the samples had come to equilibrium by the time the analysis was performed, usually after at least a few days. The headspace within the vials was the source of the samples that were actually analyzed with the GC. Vial headspace concentrations were converted to liquid phase concentrations using Henry's Law constants (Gossett, 1987; Liley, et al., 1984).

The hydrocarbons and vinyl chloride were separated in the GC with an HP Alumina PLOT column (Hewlett Packard, Palo Alto, CA). The separation phase was fused aluminum oxide film 15 μm thick. The column was 30 meters long with an inner diameter of 0.53 millimeters. The detector used was an (FID).

Although the samples and standards were not prepared the same the injection procedure was. A 25 μL gas tight syringe was used to take a 15 μL slug from either a standard or sample. Samples were mixed thoroughly before they were injected. The syringe was flushed with the sample before the syringe was withdrawn from the standard bottle or sample vial headspace. The needle was always be checked for plugs before and after each injection to the GC. A completely plugged needle just wastes time since no material is injected to the GC, but a partially plugged needle yields irregularly low values and makes the analysis difficult. If the needle was not checked the data would have to be taken at face value. Most of the plugging occurred during injection to the GC, as opposed to passing through the vial or bottles septa. The way to check for plugging was to depress the plunger while the needle was placed in water. A bubble should immediately form at the tip of the needle. If all was okay the sample volume was reduced to 10 mL and injected into the GC. The needle was then rechecked to make sure the sample was dispensed completely, which would not occur when the needle was partially plugged, or the result may have been lower than was true for the sample.

The detector temperature was 250 °C and the injector temperature was 125 °C. The oven temperature profile started at an initial temperature of 60 °C and was held there for two minutes. After that, the temperature was ramped at 40 °C/min for three minutes.

4.2.2.4 Variations for Methane Analysis

Methane was the only gas analyzed over the compete run of the experiment and began just after the columns were inoculated. Ethene, ethane and vinyl chloride monitoring began when no PCE, TCE, the expected product, DCE, were detected in columns 3 and 4. The analysis of these gases began one month into the degradation experiment. While methane was the only hydrocarbon being followed the analysis was performed on a different system. The analysis was done on an HP5880 packed column GC (Hewlett Packard, Palo Alto, CA) with an FID. The column used was a Molecular Sieve 13X (Supelco, Bellefonte, PA). It was 2.7 m long and had a

0.125 in outer diameter. The molecular sieve separates small gases well, but could not handle the ethene and ethane when these analyses became necessary.

Methane standards were prepared by the same procedure used for the hydrocarbon mixture. The primary standard was different from the one mentioned previously. This standard had a make-up of 1% methane with the balance being N_2 (Mountain State Air, Roanoke, VA). The secondary standards had methane concentrations of 0.45% and 0.1%.

The sampling procedure was the same as described above for the hydrocarbons. The sampling procedure was developed while methane was the only gaseous analyte.

The chromatographic analysis for only methane was a little simpler. The injection protocols, including the needle check, were developed and used for this analysis. The detector and injector temperatures were the same, but the oven was run isothermally. The oven temperature was 150 °C.

4.2.3 Propionate and Acetate Analysis

Propionate and acetate were measured for the columns fed sodium propionate to monitor the activities of the proton reducers and acetoclastic methanogens respectively. These fatty acids were analyzed on two GCs, a Tracor 560 (Tracor Corp.) and the HP5880. The packing used, which will be described later in Section 4.2.3.3, was the same for both GCs. An FID was the detector used with both machines.

4.2.3.1 Standard Preparation

Fatty acid standards were prepared with 99% propionic acid and glacial acetic acid (Fisher Scientific Co., Pittsburgh, PA). Primary standards were made by adding measured masses of each acid to separate volumetric flasks with deionized water. Secondary standards of 50, 20, and 10 mg/L were made by diluting the primary standards. Due to a relatively high volatility when compared to solutions of the sodium salt analogs, the standards were prepared the day of analysis.

4.2.3.2 Sampling Procedure

The sampling procedure follows the general procedure used for the multichlorinated ethenes. Sample port SP5 was purged, then a 6 ml to 7 ml sample was taken. 5 ml of this sample was pipeted into another vial and capped. Samples were stored refrigerated.

4.2.3.3 Analytical Procedure

The procedure used on both GCs was the same. The HP 5880 was used only after the injection heaters on the Tracor machine failed. The glass packed column used had a 0.3% Carbowax 20M/0.1% H_3PO_4/60/80 Carbopak C (Supelco, Bellefonte, PA) as the packing. The detector end of the column had a glass wool plug treated with H_3PO_4. The injector and detector temperatures were both 200 °C. The oven was run isothermally at 120 °C.

The injection volumes were 1 μL. The analysis only works for the free acid so the samples have to be acidified prior to injection. The free acids are more volatile than their salts, so the acidification is done just before injection. 50 μL of formic acid (Mallinckrodt, Paris, KY) is added to a sample which is then mixed by vortexing. At this point the 1 μL can be withdrawn for injection.

4.2.4 Bromide and Sulfate Analysis

Bromide was analyzed to determine minimum column breakthrough times and residence time distributions. Bromide is the ideal species to monitor for two reasons. It does not adsorb on the material in the columns and it is biologically inert. Because species such as the chloroethenes may adsorb onto surfaces within the columns, bromide breakthrough represents a minimum.

Sulfate was included in the media to provide sulfur for incorporation into the bacteria. The measurements of sulfate were made during the bromide time experiment and their analysis showed significant usage in the propionate fed columns.

The anions were measured on a Dionex 2010i ion chromatograph (IC) (Dionex Corp.,

Sunnyvale, CA). The system was run in suppressed ion mode and detected with a conductivity detector.

4.2.4.1 Sampling Procedure

The sampling procedure follows the general procedure used in previous analyses. The only difference is in the amount of sample collected. The sample totally filled the vial, which is about 8.5 ml. The IC required about 2 ml to 2.5 ml for injection and this was the surest method to collect enough sample to adequately rinse the syringe for each sample. Samples of the concentration in the feed tank were taken by dipping a vial into the tank to fill it. There were no analytical standards used in this analysis. The by-pass tank value of bromide was taken as a relative standard for the maximum concentration achievable from the column effluents. The bromide and sulfate concentrations are reported in peak area units, which are proportional to concentration.

4.2.4.2 Analytical Procedure

The procedure used was a set standard for anion analysis. A 2 ml to 2.5 ml sample is injected from a plastic syringe and loaded into the sample loop. Part of the sample purges the sample loop and the last bit fills the loop for injection onto the column. The sample loop volume is significantly smaller than the total sample volume. The sample is then injected onto the column.

4.2.5 Dichloroethene Analysis

Dichloroethene was not detected using the method for the multichlorinated ethenes. The DCE standards used, yielded a linear calibration curve, but the detection was much less than anticipated from the number of chlorines in the standards. The detection problems are probably related to DCE's high vapor pressure or high solubility in water. DCE may not have partitioned into the pentane.

It was hoped that DCE would show up during the headspace analysis of vinyl chloride and the hydrocarbons. The Alumina PLOT column was designed for hydrocarbons, but vinyl chloride eluted quantitatively. A peak that may have been DCE was seen late in many trials, but was not reproducible although the other peaks in the replicate samples were.

Verification of the presence of DCE was done with GC/MS. The Alumina PLOT column was used with the GC. The column pressure was reduced from the usual 10 psig to 5 psig to accommodate the needs of the mass spectrometer. The mass spectrometer used was a 7070 E-HF machine (VG Analytical, Manchester, UK). It is a magnetic sector, double focusing instrument.

The two standards and three samples tested were taken from among those used in the headspace analysis of the hydrocarbons and vinyl chloride. The analysis was performed by the GC/MS technician. Detection required the selected ion mode, which is the most sensitive setting for the machine.

4.3 System Characterization

The operating characteristics of the system had to be determined before the PCE degradation experiment could begin. The integrity of the system had to be verified by showing that PCE would not be lost when the entire process system was run without any microorganisms present. A different apparatus was used to show that TCE would not be lost when the system was free of bacteria. The main concern with TCE was that it may have interacted with the polycarbonate used to fabricate the columns.

After the system integrity was verified, the columns were inoculated with the mixture of alluvium and Duck Pond sediments. Inoculation occurred November 7, 1996. At this point, the activities of the consortia had to be monitored to show that the bacteria were still alive. While the monitoring of the consortia's activities, the flow characteristics through the columns had to be determined. The two characteristics investigated were the breakthrough time of species that did not absorb on to material within the columns and the average residence time for the non-adsorbed species. Bromide, present as the magnesium salt in the nutrient solution, is inert biologically and is the perfect tracer to study the flow properties.

4.3.1 Abiotic PCE Recovery

The first thing that had to be shown to validate the use of the experimental system was that PCE could be completely recovered from when the system was operated under abiotic conditions. Only one column was tested, but the rest of the apparatus was run using normal operating flow rates.

4.3.1.1 Experimental System

The system integrity experiment for PCE was run before the installation of the column metering pumps or the PCE solution peristaltic pump. The regulation of the manifold and column flows with needle valves was imperfect and the flows into the column and through the manifold were not constant. The use of valves to control the flows was the primary hardware variation from the system described in Section 4.1. The flows were monitored regularly and samples were only taken when the system was relatively stable for about 12 hours.

Over time, contamination in the manifold line clogged the needle valve, that was used to control the pressure, at the end of the manifold line. As the manifold needle valve clogged the pressure would go up in the line and the flow rate into the column would increase. The needle valve was replaced with a spring loaded back pressure regulating valve, but the same problems occurred. A different type of BPR valve was tried, and that did not work properly either. Because the PCE flow was pressure driven, it changed in response to the fluctuations in the main manifold. This caused the inlet concentration of PCE to change over time.

The only other difference in this experimental system versus the one used in the degradation experiment was the column loadings. To best simulate the actual soil profile, one liter of 3 mm and 4 mm glass beads was mixed with enough sand to occupy the combined space of the treatment zone and the gravel layer. The top six inches was filled with only sand. As the column was loaded, nitrogen sparged deionized water was added fill the pore space.

4.3.1.2 Operating and Sampling Procedures

The flow rate from the nutrient tank was maintained around 14 ml/min to 15 ml/min over

the duration of the experiment. The PCE flow rate was less stable, but a flow of approximately 0.5 ml/min was the desired rate and was maintained long enough to get reliable samples. This combination of flow rates produced a PCE concentration of about 4.5 mg/L, which was the preselected concentration chosen for the degradation experiment. There was probably some adsorption within the column for the first few days of the experiment, but the flow rates were not steady and no samples were taken.

While the experiment was run the pressures were monitored in the manifold upstream of the filters and immediately downstream of the mixing chamber. The flows into the column and from the PCE tank were determined from rotameter readings. The total flow was measured from the common drain line. When the pressures and flow rates remained steady for about eight hours, samples were taken. Although there were fluctuations in the PCE concentration over time, they were not large. Fluctuations in effluent were probably damped by the adsorption of PCE in the column. If the inlet concentration went up, there would be a delay in seeing the effect in the effluent since the amount adsorbed would increase. The concentration of PCE in the effluent would begin to change only after one breakthrough time and would increase slowly while the adsorption rate was coming to equilibrium with the new inlet concentration. The PCE concentration at the inlet was not steady enough to ever allow the adsorption rate to come to equilibrium. If the inlet concentration went down the opposite effect in the effluent concentration would be seen.

Sampling from both the manifold (SPM) and effluent (MP5) ports was described in the analytical section for multichloroethenes. When sampling from either location, the pressure in the manifold would drop and the PCE flow would instantly increase in a dramatic fashion. After about 15 minutes, the flow rate of the PCE solution would return to its previous value. As long as the manifold port was sampled first, there would be no problem. The change in the column's inlet concentration would not affect the effluent for about five hours. Once the samples were taken in the wrong order and the PCE flow increased 17%. The manifold sample was taken while the flow rate was at this higher level. The analysis of samples taken from each port at every sampling time was used to get the results.

4.3.1.3 Results and Discussion

The system stabilized enough to take samples on four consecutive days after about a

month of monitoring. In that time a small pore filter was installed and then removed when it clogged to quickly and the nitrogen regulator on the PCE line was changed. The concentration of PCE in the results has been normalized to the maximum value found in the samples. The results from these four days of sampling can be seen in Figure 4-4. As can be seen in the results, both the manifold and effluent concentrations of PCE are statistically the same. Random variation should account for the fact that one type of sample did not always give the higher concentration. The samples taken from 10/26 to 10/28 all have the same value. This represented the most stable operation of the system seen to that point. The drop in the PCE concentration on 10/29 is indicative of how hard it was to maintain overall control of the flow rates in the system.

There were no losses of PCE due to interactions with either the sand and glass in the column or with the polycarbonate shell of the column. There was some concern that the polycarbonate would be a perpetual sink for the PCE and possibly other chloroethenes created. It is known that polycarbonate will dissolve in chlorinated solvents, but the low concentration of PCE used should not have been a problem, and it was not.

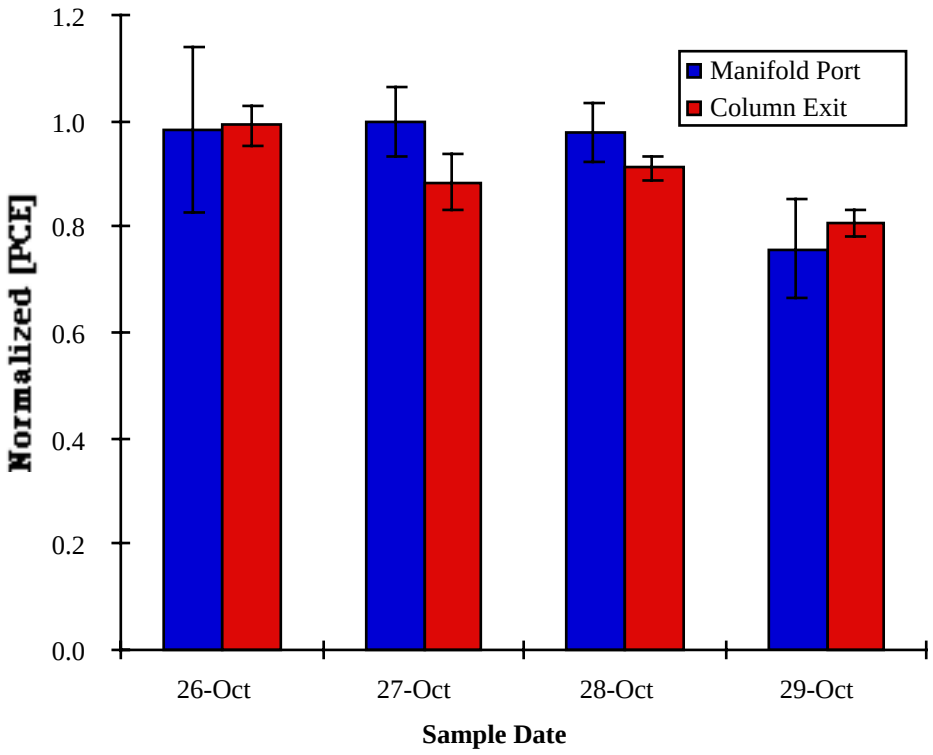

Figure 4-4: PCE concentrations in the abiotic column experiment

4.3.2 Abiotic TCE Recovery

The possible interactions of TCE with the polycarbonate column was also a concern. The successful recovery of TCE was performed using a different apparatus, but the materials used were the same. A solution of TCE dissolved in water was stored in a second glass bell jar, like the one that was used for the PCE solution. The column used in this study was one of the four that was used later in the degradation experiment. The sampling was performed in the same manner as was used in the PCE recovery experiment.

4.3.2.1 Experimental System

TCE was going to be the contaminant in an aerobic experiment that was not performed, but the system was built and used to test TCE recovery. The system used to verify that all of the TCE could be accounted for in an abiotic system was constructed differently. The apparatus used in this experiment can be seen in Figure 4-5. A glass chamber with an aqueous solution having an initial TCE concentration of 1 mg/L was used. Nitrogen was used to pneumatically pump this solution to a polycarbonate column of the same construction as the

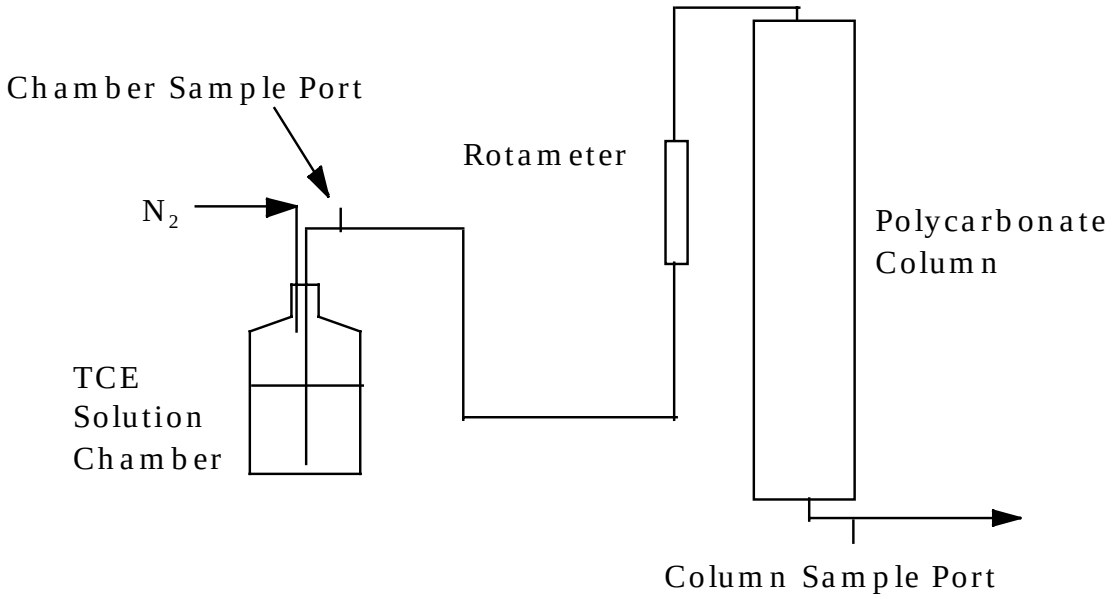

Figure 4-5: System schematic for TCE recovery

ones used in the dehalogenation experiment. Between the chamber and the column was a sample port just after the chamber exit. The column had no fill material, like the sand and glass used in the PCE recovery experiment. The sample port for the column effluent was in the same position as SP5.

4.3.2.2 Operating and Sampling Procedures

The system was operated at 1 ml/min over the duration of the experiment. 18 liters of the TCE solution was made up at 1 mg/L in the glass chamber. The column was filled by quickly pumping some of the solution to the column, through a line by-passing the rotameter. When the column was full the flow was redirected back to main line through the rotameter. This left about 13 liters in the chamber. This gave the chamber and column the same starting concentrations of TCE. Losses in the column due to volatilization during the filling procedure lowered the concentrations a little. The column ended up starting with a slightly lower initial concentration than the chamber because of this. The flow was initiated and the system was operated for eight days. The volume in the TCE solution chamber decreased by 1.44 liters/day. Samples were taken immediately after the system was loaded and the flow was initiated. Samples were taken daily from both ports and stored in the refrigerator until analysis.

4.3.2.3 Results and Discussion

The samples were analyzed using the protocol for multichloroethenes. The results from the system integrity experiment for TCE can be seen in Figure 4-6. The first thing that is noticed is that the concentration of TCE is decreasing. This is not the problem it appears to be at first. While the concentration of TCE is

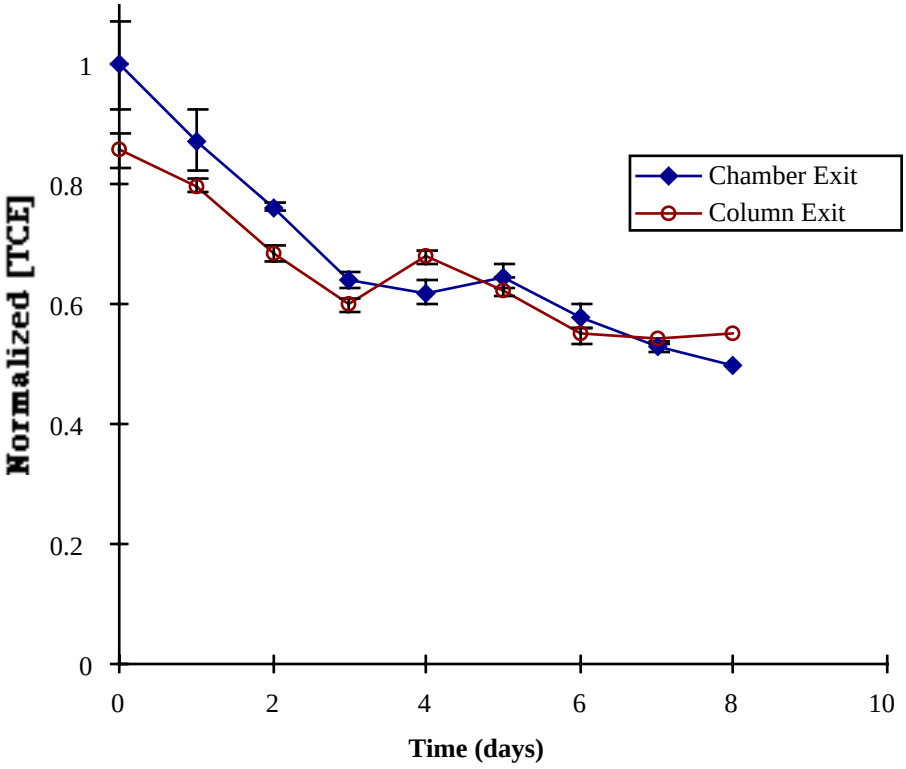

Figure 4-6: TCE concentrations in the abiotic column experiment

decreasing in the effluent line exiting the column, it is also decreasing by the same amount from the chamber concentration. The apparent loss of TCE in the chamber is not due to a leak in the system. The TCE volatilized as fresh nitrogen displaced the parting solution, thereby lowering the concentration in the aqueous phase. The similarities in the concentration profiles demonstrate that there were no abiotic losses due to interactions with the construction materials used in the system. As the concentration of the inlet dropped, the concentration

dropped in the column and at the exit. The effect was delayed due to mixing of the inlet solution with the solution in the column.

4.3.3 Column Flow Characteristics

The breakthrough and average residence times of species flowing through the active columns were investigated to describe the flow characteristics. One reason to measure these operating parameters was to use the results in designing an experiment to follow the degradation of propionate in Columns 3 and 4. Samples for propionate had to be taken from about two hours before breakthrough should have occurred for dissolved species until the time any unmetabolized propionate would have been nearing its maximum concentration at the effluent. Bromide was used as the tracer to measure these times, since it is biologically inert and very water soluble. It should not adsorb onto either the column surface or on the soil particles.

4.3.3.1 Experimental System

The operating system for this system was the complete system described in Section 4.1 as shown in Figure 4-2. The experiment was conducted during the growth and monitoring phase that preceded the degradation experiment. The columns were receiving the nutrient salts from the nutrient tank until the beginning of the experiment, at which time the flow was switched to the by-pass tank containing nutrient solution supplemented with five times as much bromide as usual. This experiment was run before the metering pumps were added and used needle valves to control the flow rates into each column.

4.3.3.2 Operating and Sampling Procedures

The total flow rate into the manifold was about 21 ml/min, which is safely above the 18 ml/min needed to provide the proper flow of about 3 ml/min to each of the columns. The system was cleaned of contamination as well as could be done before the experiment was initiated. This was done to ensure the most stable operating conditions possible. The by-pass tank was filled

with close to 16 liters from the contents of the main nutrient tank and then 1.17 g of $MgBr_2 \cdot 6H_20$ was added to the 16 liters to raise the bromide concentration about 40 mg/L to approximately 50 mg/L. The contents of the by-pass tank were then sparged with nitrogen for 10 minutes. The step change in the bromide was made by shifting the flow from the nutrient tank to the by-pass tank and this is time zero of the experiment.

While the experiment was running four more liters was taken from the nutrient tank and amended with 0.294 g and sparged like the previous solution. The four liters was then added to the by-pass tank. Samples were taken from the SP5 ports on all six columns every hour for 13 hours. One sample was taken from the by-pass tank immediately after the flow was turned back to the main nutrient tank. The method of taking samples was described in Section 4.2.4.

4.3.3.3 Results and Discussion

The bromide concentration profile in each of the columns can be seen in Figure 4-7. At 21 ml/min of total flow the bulk flow velocity was 2.65 m/min. This translates into a time of less than 10 minutes to get from the by-pass tank to the column inlets, which can be ignored.

The initial concentration in all six columns was the same,

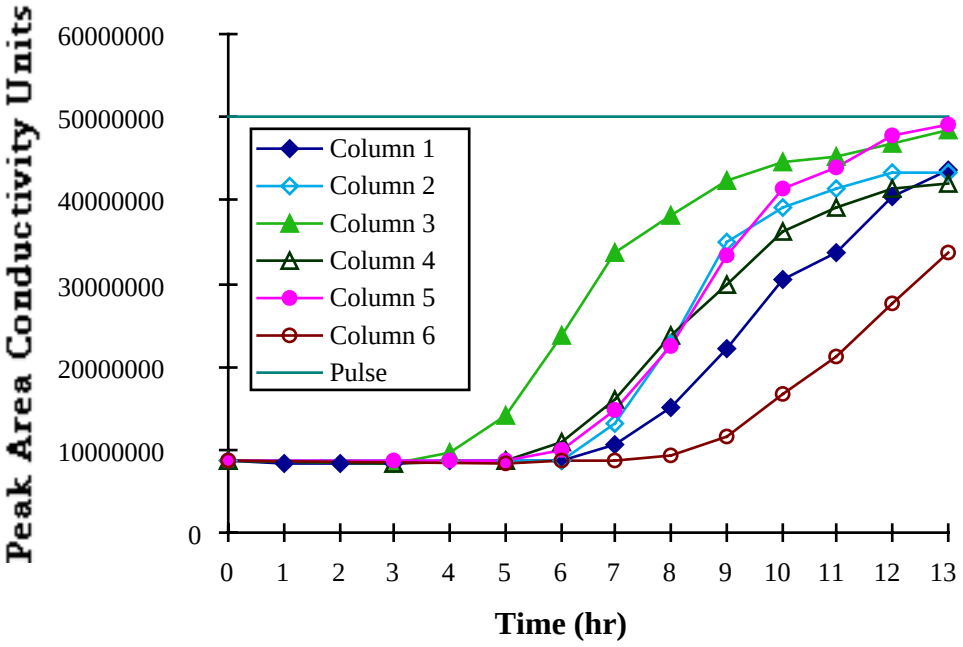

Figure 4-7: Concentration profile of bromide in each of the columns. Time equals zero is when the stepchange in the bromide was made.

which is as it should be for an inert species. Over the length of the experiment each of the columns' flow rates was relatively constant, based on rotameter readings. Column 3 had the highest flow rate of 3.4 ml/min and had the shortest breakthrough time between three and four hours. The flow rates of columns 2, 4, and 5 had flow rates in the range of 2.9 ml/min to 3.1 ml/min and their breakthrough times were all between five and six hours. Column 6 had a flow rate of only 2.8 ml/min and had a breakthrough time between seven and eight hours. The one anomalous result was from Column 1. Its rotameter was steady during the whole experiment and the flow rate was determined to be 2.7 ml/min. This was the lowest of all of the flow rates, yet Column 1's breakthrough time was between five and six hours. The flow rate readings were not totally accurate, since some contaminating material could not be gotten out of the rotameters and the floats may have been stuck in the wrong positions. Three columns had flow rates within 0.1 ml/min of the desired value of 3 ml/min, and they all had breakthroughs between five and six hours. This reproducibility demonstrates that the breakthrough time through a column with a 3 ml/min flow rate is truly between five and six hours. This low flow rate should have been

incapable of forcing channels through the sand and soil. It is possible that gas pockets formed from the addition of microbubbles may have created zones of quick flow in Column 1 and could account for its relatively quick breakthrough time, considering Column 1 had the lowest flow rate.

4.4 Characterization of Microbial Activity

After the columns were inoculated, on November 7, 1996, their activities were monitored until the end of the degradation experiment on December 28, 1997. Any dates given for November or December will have the year denoted, but other dates will not since they are all 1997. This was done to verify that the anaerobic consortia would still be present when the PCE solution was added. Since there were three feeding strategies being used, it was decided to let the cultures grow and acclimate in their new environments, including a higher temperature than they were accustomed to. The temperature in the lab varied between 22 °C and 25 °C over the duration of the experiment. The flow rate of the nutrient salt solution was about 21 ml/min and the column flow rates were kept as close to 3 ml/min as was feasible with the needle valves. Columns 1 and 2 were fed 60 ml of H_2/CO_2 microbubbles at rate of 3 ml/min twice a week. The ratio of the gases was 90:10 respectively. This provided 412 μmol H_2/day. Columns 3 and 4 were fed 10 ml of a 6.916 g/L solution of sodium propionate twice daily. This provided 4320 μmol H_2/day assuming that all of the propionate was metabolized. Each feeding regimen should have selected different organisms to predominate in each consortia. There was also a good probability of having different populations dominate at different depths within any column. The presence of only some of the organisms in any column could be verified by analyzing the effluent for certain substrates or products.

Both the microbubbles and the propionate should have stimulated methanogens, therefore methane was monitored in these columns and in the controls. The presence of methane in any column can only be used to state that methanogens are present and not anything about which types are active. In the propionate fed columns the disappearance of propionate would verify that the obligate proton reducers and/or the fermenters were active. If a H_2 sink was present, then the fermenters would most likely be acting as facultative proton reducers. If no acetate was

detected in these columns, then the acetoclastic methanogens had to be responsible for some of the detected methane production. The yeast extract has fatty acids in it as well as other complex biochemicals, therefore all six columns could produce methane by the scenario presented for propionate metabolism. Fatty acid metabolism also yields H_2 and CO_2 which can be used by the autotrophic methanogens or the homoacetogens, and the acetate produced by the homoacetogens could be used by the acetoclasts. No simple tests can be performed to verify the presence of the bacteria previously referred to as the non-associated dechlorinators. Sulfate, although a minor constituent in the nutrient solution, could be used to foster the sulfate reducers. Sulfate utilization was measured while the bromide tracer study was done. In summary, the measurements of methane, propionate, acetate, and sulfate were only capable of verifying the activity of some types of organisms that were described in the background.

Interruptions to the normal operation of the system occurred when the system needed to be cleaned or leaks had to be stopped. Regular maintenance was done about every two weeks. The peristaltic pump tubing was replaced with cleaned and sterilized tubing. The filters were also removed and sterilized in a bleach solution at the same time. The system was down about four hours while this was being done. The rotameters and valves were cleaned by flushing and flowing a basic bleach solution through them, whenever the need arose. Whenever a column leaked near the inlet point, that column was taken off line so the leak could be fixed. Plugging in an effluent line required the column to be removed from the system so the line could be cleared. The regular maintenance was performed over the complete run of the experiment. The cleaning of the lines was not required after the installation of the metering pumps. Some leaks still had to be fixed after the pumps came on line.

The system had to be turned off while the nutrient solution was being remade. The solution would last about one month. While the tank was being refilled with deionized water the salt and buffer solutions were prepared. The full tank was mixed after the salts had been added. Then N_2 was sparged through the tank for 10 minutes before the flow was restarted. Since this took about six hours the tubing and filter maintenance was usually done during this break.

4.4.1 Methane Monitoring

Methane measurements were used to monitor the activity of the methanogens. Their

activity should have been indicative of the health of the each consortia and how each was responding to feeding and system manipulations. The manipulations of the system included total shutdowns to clean the filters, change the peristaltic pump tubing, and refill the nutrient tank. Individual columns were shutdown to clean rotameters, fix leaks, or remove clogs in the effluent tubing. Methane measurements began when nutrient solution flow was initiated, which was one week after the columns were inoculated. The flow rates were assumed to be 3 ml/min, although there were variations. With constant and equal flow rates in each column, the methane concentration in the effluents would be directly proportional to the headspace concentration in the samples. The methane concentrations are reported as a percentage of the headspace throughout the experiment, and for the first three months was the only way to report methanogenic activity. To take into account the variations in the respective column flows, calculations of the flow rate of methane out of each column were performed. This methane production rate is actually a more appropriate quantity to use and was the method of describing methanogenic activity from the time the metering pumps were installed on August 6, 1997.

4.4.1.1 Experimental and Sampling Procedures

The experimental procedure for system operation is described in the beginning of Section 4.4, and is constant throughout the growth and monitoring phase.

Samples were taken every two or three days while the system was operating, both during the monitoring and degradation phases. The system was down for a couple of days at a time to fix leaks, which happened occasionally, and while the pumps were being installed and tested. This took almost two weeks.

4.4.1.2 Results and Discussion

The history of methanogenic activity over the complete length of the experiment can be seen in Figures 4-8a through 4-8c for microbubble, propionate, and control columns respectively. The percent headspace represents the headspace of the sample vial. The concentration of methane in headspace of the vial after equilibrium

is achieved is directly proportional to the concentration of methane

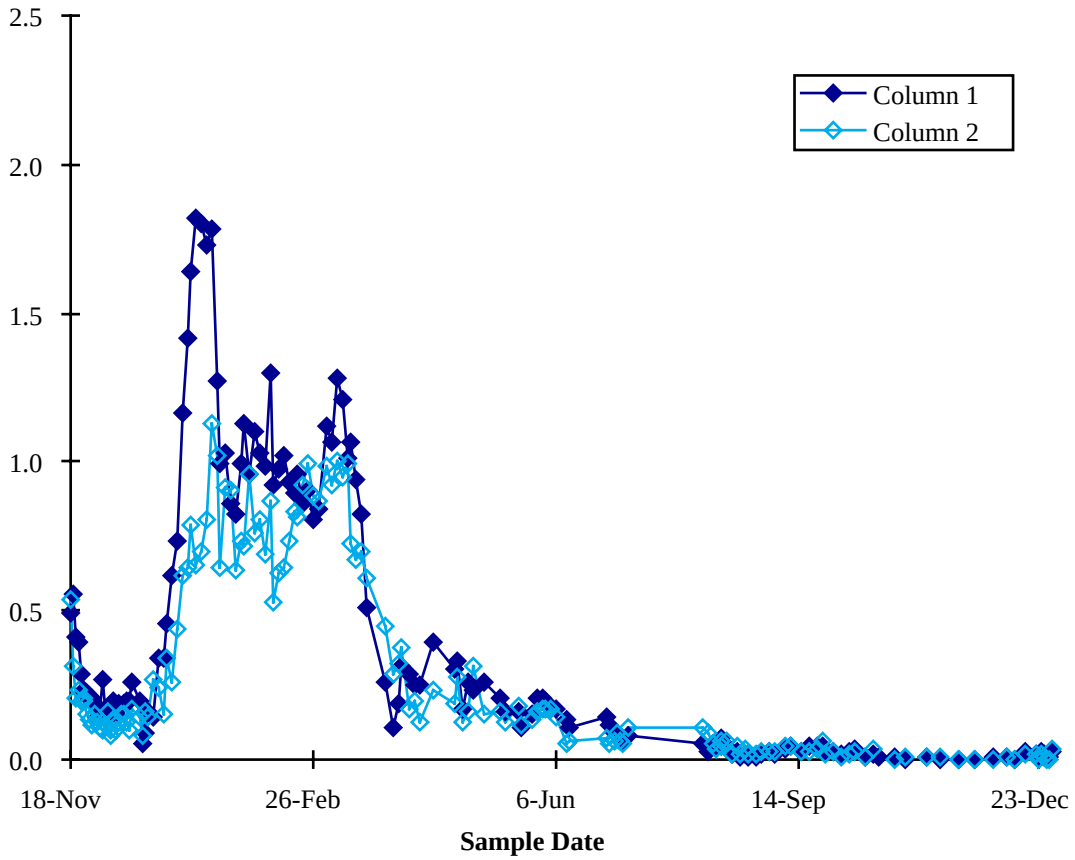

Figure 4-8a:Profile of methane in the headspace of the sample vials as a function of time. Sampling began on November 18, 1996 and went through December 28, 1997. Columns 1 and 2 are fed microbubbles.

dissolved in the effluent. The chief features can be seen even though there is a lot of information in the figure. Initially, the methane production in all of the columns dropped off. This should have been due to the shock of encountering a new environment.

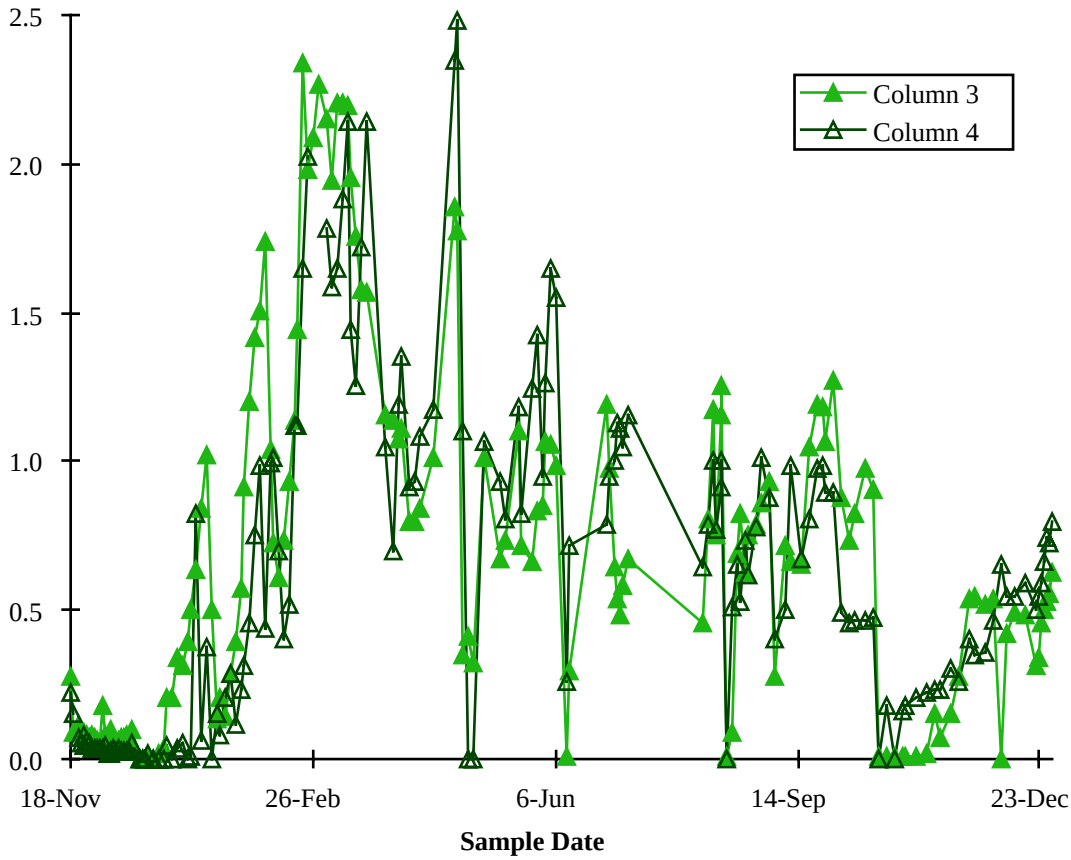

Figure 4-8b: Profile of methane in the headspace of the sample vials as a function of time. Sampling began on November 18, 1996 and went through December 28, 1997. Columns 3 and 4 were fed propionate.

Columns 5 and 6 do the best initially and they are also the ones with the least environmental adaptation to make, since they are not receiving a new feeding substrate.

The microbubble columns, 1 and 2, take-off first and begin to produce significantly more methane. The propionate columns begin to produce as much methane as the microbubble columns a week or two later. In mid-January, oxygen was inadvertently pumped into

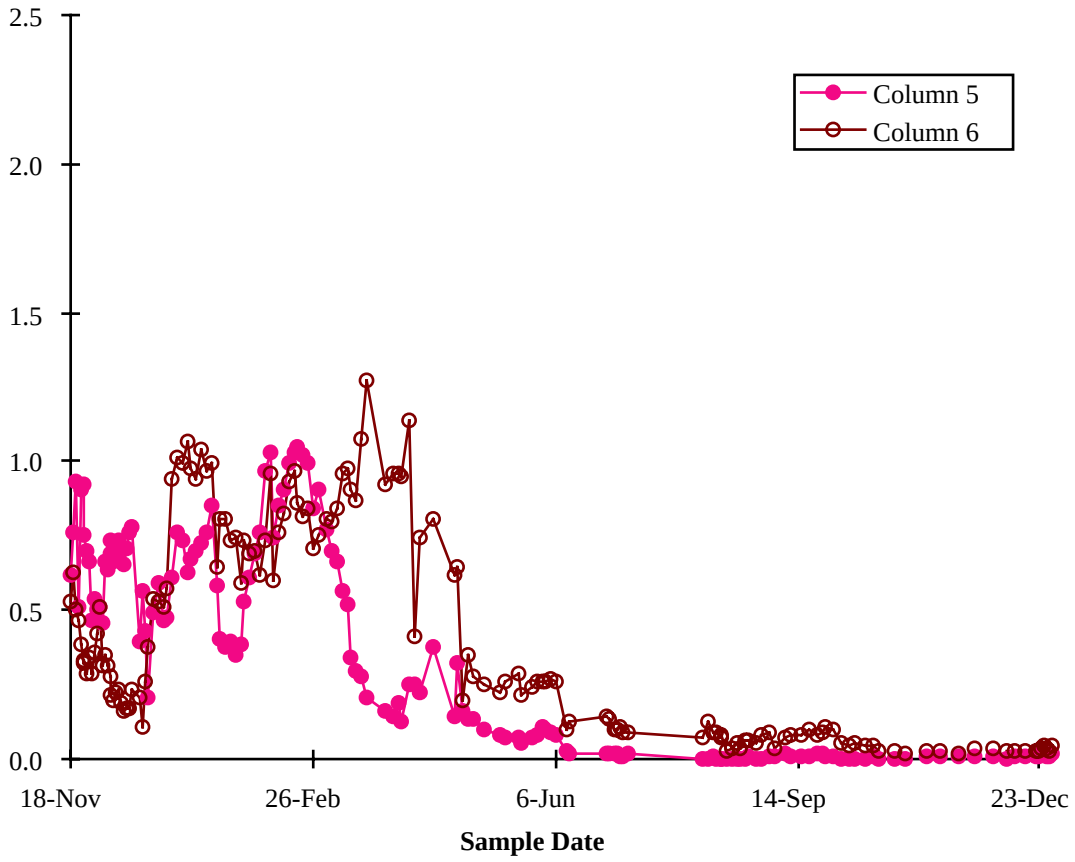

Figure 4-8c: Profile of methane in the headspace of the sample vials as a function of time. Sampling began on November 18, 1996 and went through December 28, 1997. Columns 5 and 6 were fed yeast extract.

the system, and the methane production virtually stopped. This is best seen with column 1. This verifies that the consortia were truly living anaerobically, since the methanogens are strict anaerobes and are impaired in the presence of oxygen.

From February 24 through the end of the degradation experiment the monitoring of methanogenic activity was reported as the rate of methane leaving in the effluent, which was the rate of methane production, assuming that none of the methane reacted within the columns. Figure 4-9 uses this notation and is clearer to interpret because the data is not as compressed.

In early March the methane production in all six columns decreases steadily and stabilizes at the end of the month. PCE flow is initiated on May 23, and this does not seem to have any effect on

methane production. Analysis of the samples for chloroethenes yielded nothing coherent due to the instabilities in the columns' flow rates, so the PCE was shut-down on June 10. The system was shut-down on June 12 for two weeks to clean the lines and fix leaks. The two week period is the small gap in methane levels seen in mid-June.

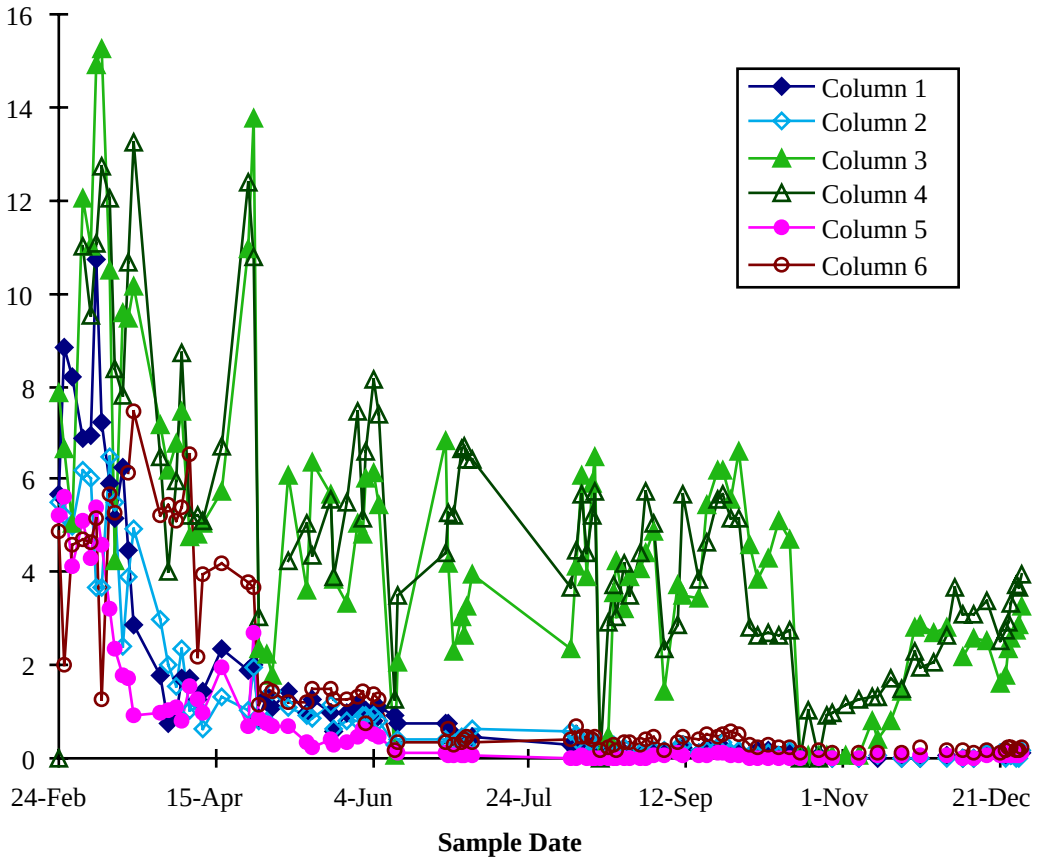

Figure 4-9: Profile of methane production in the effluent

The system is restarted and sampling begins again on June 27. The system flows are felt to be stable, so the PCE is turned on again on July 15. After three days of unstable flows the metering pumps are ordered and the PCE solution flow is stopped. This is the point at which the big gap in methane appears. There was no reason to sample when the results would have very little meaning. It was assumed that the cultures would not die if samples were not taken. The sampling began again on August 6, after the metering pumps were installed. The resumption of methane readings at the same levels as when the sampling was stopped, verified that the assumption was accurate.

Now the final operating system is almost complete. The flow rates are stable from this point on. After two weeks with the pumps on-line, the flow rates were checked and matched their previous values within 0.02 ml/min. The methane profile from the time the pumps went on-line until the end of the degradation experiment can be seen in Figure 4-10. There is a dip in methane production on August 16 for an unknown reason. On August 18 the PCE flow was reinitiated and shut off with in a day. The stroking action of the metering pumps causes the PCE solution flow to pulse also. This prevents an accurate measurement of the flow rate. The peristaltic pump for the PCE solution with Viton® tubing and the Viton® pulse reducing coil were installed on September 18 and PCE flow was truly reinitiated on September 19. The PCE was not turned off again until the experiment was over. The drop in apparent methane production between October 18 and Thanksgiving is probably due to sample

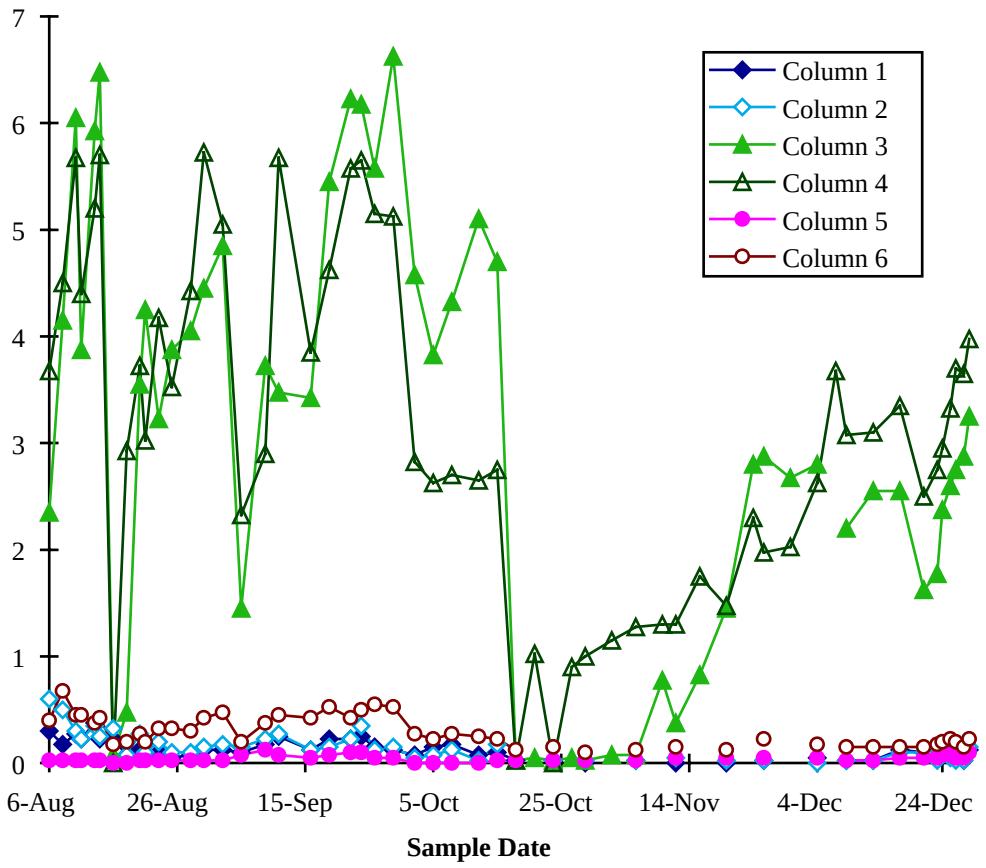

Figure 4-10: Profile of methane production in the effluent from the time the metering pumps were installed

losses due to handling. An alumina PLOT column, that could adequately separate methane as well as ethene, ethane, and vinyl chloride, was ordered and expected in after Thanksgiving. After the holiday, the time for delivery actually went up a week. In early December another alumina PLOT column was ordered and delivered two days later. After installing and conditioning the new column, the HP5880 GC died. All of the samples were removed from the refrigerator and heat back to room temperature before the GC failed. The samples were restored and tested on December 13, 1997. The samples were probably kept too long and the gases were lost progressively over time. The samples that were taken within two weeks of the analysis date, from November 26 on, yielded values that were in the same range as the ones tested before October 18 and also from the samples taken and tested after December 13, 1997. This interpretation will be referred to when analysis of PCE dehalogenation products is discussed. The values for ethene, ethane, and vinyl chloride are probably lower than what truly existed in the vials at the time of sampling.

All six columns showed the presence of methanogens over the duration of the experiment. Simply by measuring methane production, nothing can be said about the types of methanogens that were active. The autotrophs and acetoclastic methanogens may both have been present in all of the columns, since fatty acid metabolism produces H_2 and CO_2 for the autotrophs and acetate for the acetoclastic methanogens. The relative demographics should have been different though with the autotrophs dominating in Columns 1 and 2, at least in the region where the microbubbles were distributed. The acetoclastic methanogens should have dominated in the other columns and were probably the predominant methanogen in the upper regions of Columns 1 and 2.

4.4.2 Propionate and Acetate Monitoring

Propionate and acetate were measured once for only Column 3 and later for both Columns 3 and 4 to detect the activities of the proton reducing organisms and acetoclastic methanogens respectively. The first measurements were made in December 1996, just after the inoculation of the columns. pH readings from Column 3 were also taken at the same time. The addition of a sodium propionate should have stimulated the proton reducers and acetoclasts simultaneously. If the acetoclastic methanogens were stimulated the rate of methane production

would be relatively high, and this was seen over the long term in Figure 4-9. The two conditions that would verify the presence of the acetoclastic methanogens were the production of methane and the lack of fatty acids in the effluent. The lack of propionate in the effluent would also verify the presence of proton reducing organisms.

4.4.2.1 Experimental and Sampling Procedures

The system was operated under the normal conditions for the first fatty acid and pH monitoring experiment. Propionate was injected 15 minutes before the sampling began. The concentration of sodium propionate injected was 1.42 mol/L. pH measurements require a large volume of solution for the probe, so the sample port could not be used as long as the effluent line connected to a common drain line. The effluent lines were disconnected from the drain line and extensions were added that raised the effluent lines up to the same height to where the vacuum is broken in the drain line. This maintained the same back pressure on the columns that existed under normal operation. The complete effluent stream was collected over the length of the experiment. For 30 minutes the effluent was collected in a beaker to be used for pH measurements. For the next three minutes the effluent was collected in a sample vial. 5 ml of this sample were pipeted into another vial and stored. This process was repeated over a 10 hour period.

The second fatty acid monitoring experiment tested the effluents from both Columns 3 and 4. This experiment was performed after adjusting the sodium propionate concentration to 0.071 mol/L, and this was the concentration used until the end of the degradation experiment. This experiment could not be performed until the nutrient tank was refilled with a reformulated buffer that gave a lower pH. The pH of the nutrient solution with the new buffer is about 7, which is in the middle of the range in which the methanogens are active, from 6 to 8. The degradation experiment was performed using the new lower pH buffer. The results from the first pH measurements were about 8, which was considered to be too high. The columns were fed 15 minutes before sampling began and were taken for 9.5 hours. The samples were taken from SP5 and used the regular protocol.

4.4.2.2 Results and Discussion

The samples taken from the first monitoring experiment were so concentrated that they had to be diluted by a factor of 100 to be analyzed. The time profile of propionate can be seen in Figure 4-11. The lowest concentration measured was five times higher than the desired optimal concentration, and the highest was 45 times too high. The concentration of sodium propionate that was fed to the columns was reformulated from 1.42 M to 0.071 M, which was the concentration used immediately after this analysis was completed until the degradation experiment ended. The concentration profile

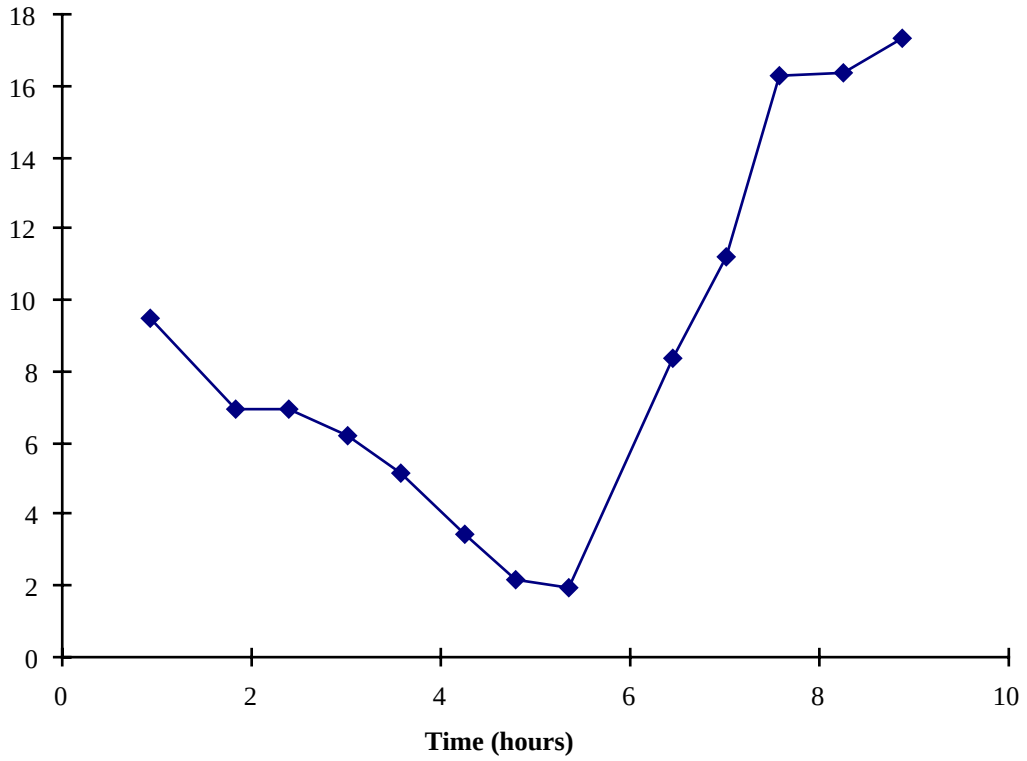

Figure 4-11: Propionate profile in Column 3. The optimal maximum was calculated to be 0.227 mmol/L

shows the breakthrough time for column throughput. The concentration of propionate drops until just after five hours since the previous injection. This agrees with the breakthrough times measured with bromide. Acetate was only detected once. The first data point had acetate measured to be 0.028 mmol/L and the same sample had a propionate concentration of 9.46

mmol/L. The high concentration of propionate probably inhibited the proton reducing bacteria although propionate is their main substrate for growth.

The pH of the samples was always within 7.9 to 8.1, over the 9.5 hours of the experiment. The pH of the buffered nutrient solution was tested and was found to be 7.9. The pHs of all column effluents were found to be between 7.9 and 8.1. These results necessitated a change in the buffering agent. The buffering agent has been previously described as a mixture of the monobasic and dibasic potassium phosphate salts, 0.40 g/L and 0.45 g/L respectively. The mixed buffer is the correction to the buffer used through this analysis. The buffer was 1 g/L of the dibasic salt. The pH of a deionized water solution of the new buffer mixture was 6.95. The mixed buffer was used when the tank needed its next filling. After running with the new buffer for a while, the pH of the nutrient solution was measured to be 6.94 and the effluent pHs ranged between 7.2 and 7.3. Referring back to the section on methane monitoring, it can be seen in Figure 4-8, that the methane concentrations in the effluents began to rise dramatically about the time the buffering agents were changed. The new buffer mixture was first used on December 16, 1996.

The second test to monitor propionate and acetate was conducted about a month after the previous analysis was completed. The test was performed on both Columns 3 and 4. Sampling began 15 minutes after the injection of 10 ml of 6.916 g/L (0.071 mol/L) sodium propionate. Samples were taken every 30 minutes for 9.5 hours. Acetate was not detected in any of the samples. There was no propionate detected in any of the Column 4 samples. Two of the samples from Column 3 had propionate in them. The concentrations detected were 0.0089 mg/L and 0.0062 mg/L. Essentially, all of the propionate was metabolized in both columns. All of the acetate was also metabolized, and this was done by the acetoclastic methanogens. The acetoclastic methanogens do not use the H_2 generated and the proton reducers will become inactive if the H_2 accumulates, therefore there were hydrogenotrophic organisms that were active before the PCE was added.

4.4.3 Sulfate Monitoring

Sulfate was the only other metabolite monitored. The sulfate reducers had to be minimally active, because they produce the sulfide that all the other organisms need to meet their

sulfur requirements. The sulfate reducers are hydrogenotrophic and may have used up a portion of the available H_2, which was also necessary for the reduction of PCE. The initial concentration of sulfate was 10 μM and when the PCE was added later, its initial concentration was about 30 μM. Both sulfate and PCE require four moles of H_2 donated electrons for complete reduction.

4.4.3.1 Experimental and Sampling Procedures

The sulfate concentrations in the effluents were measured along with bromide while the breakthrough and residence time studies were being performed. The operational and sampling procedures were described in Section 4.3.3.2. The sulfate levels were checked before the dechlorination experiment began.

4.4.3.2 Results and Discussion

The levels of sulfate in the effluents of all of the columns can be seen in Figure 4-12. The bromide concentration was stepped up in the nutrient solution, but the sulfate concentration in the by-pass tank was the same as in the nutrient tank. The levels should have remained the same

and that is what they did. The main feature to

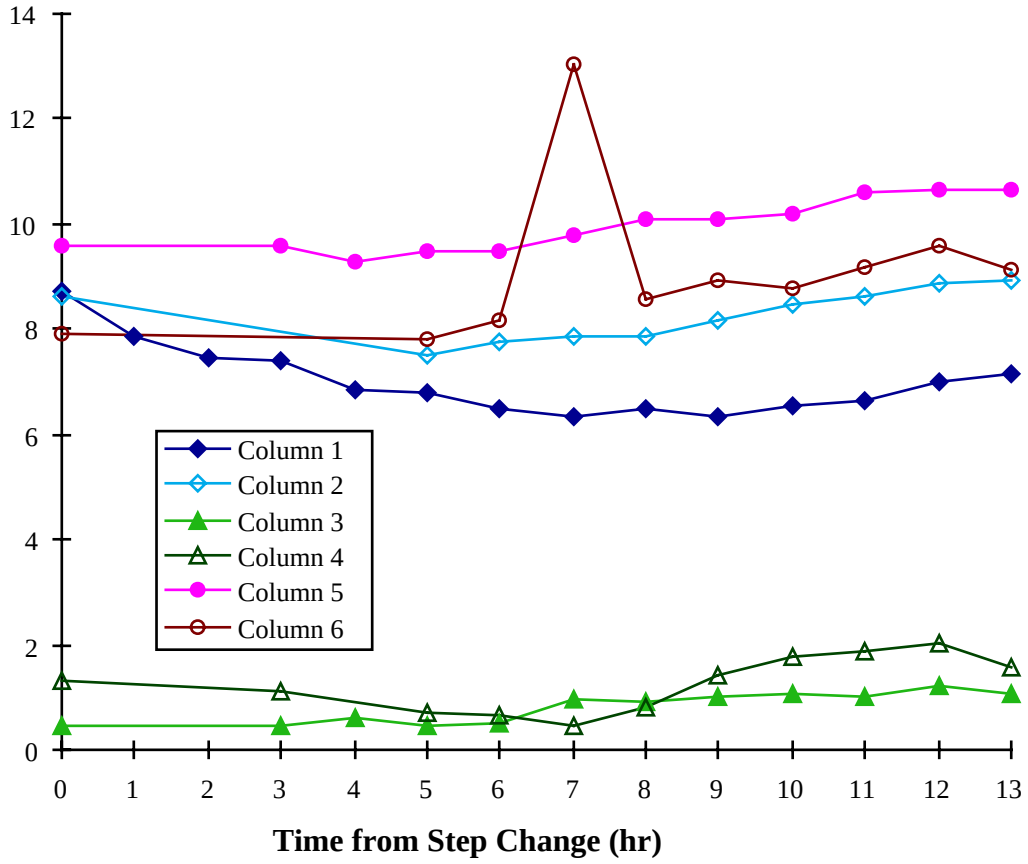

Figure 4-12:Sulfate profile during the breakthrough time experiment

notice is that the sulfate levels in Columns 3 and 4 are only about 15% to 25% of the levels detected in the other columns. The sulfate reducers are significantly more active in the propionate fed columns. The metabolism of propionate provides large amount of H_2 for the sulfate reducers to use. The microbubbles also provide large amounts of H_2, but the sulfate reducers were probably inhibited by the same thing that inhibited methanogenesis and PCE reduction.

It is possible that the differences in the sulfate concentration are due to differences in the total populations or activities of the bacteria in the six columns. The low levels of sulfate in Columns 3 and 4 relative to the other columns correlate inversely with the methane production in the columns. If methane levels in all six columns are indicative of the total populations or activities in each of the columns, then that means Columns 3 and 4 would require more sulfate.

The sulfate levels in Columns 1 and 2 are a little lower than the levels in Columns 3 and 4, but this probably is not statistically significant.

4.5 Biodegradation of PCE

The main objective of this study was demonstrate that H_2/CO_2 microbubbles could stimulate members of an anaerobic consortium and enhance the biological reduction of PCE. To meet this objective, a system was constructed and the operating characteristics were determined. After inoculating the columns the activities of consortia were monitored and characterized. Once all of these preliminary studies were completed, an aqueous solution containing approximately 5 mg/L PCE (30 μM) was added to the main flow of the nutrient solution. PCE, the other multichlorinated ethenes, and methane were measured in samples taken from the effluent sampling ports. PCE was also monitored in the manifold to determine the inlet concentration to the columns.

4.5.1 Experimental System and Procedure

The system that was used was described in Section 4.1. PCE flow was initiated after the last of the system characterization experiments was finished. At that time, the flows into the columns were still being controlled with a BPR valve and needle valves and the PCE flow was pneumatically driven by N_2. The total flow through the manifold was between 22 ml/min and 24 ml/min and the PCE concentration in the manifold was close to 30 μM. The PCE flow was initiated on May 23. Due to the inability to effectively control and measure both the flow rates into the columns and out of the PCE solution chamber, the analysis yielded no interpretable results. The PCE flow was stopped after the analysis was completed, two and a half weeks after flow was initiated. The system was cleaned of any contamination that was plugging the valves or rotameters and some leaking columns were fixed. The PCE flow was restarted on July 15 and shut down two weeks later when the same flow inconsistencies were reoccurred. The PCE flow

was not turned on again until the metering pumps, PCE solution pump, and the pulse reduction coil were installed. The restart occurred on September 19.

The total flow rate through the system ranged between 22 ml/min and 24 ml/min. The flow rates through each column were now constant and all were between 2.84 ml/min and 3.01 ml/min. The PCE flow rate was monitored and found to be constant. The variations in the measured PCE concentration were due to changes in the flow rate of the nutrient solution or systematic errors inherent in the analytical procedure.

The PCE chamber could only hold 17 liters of the saturated solution. When only four liters remained a fresh solution already saturated with PCE was added. The PCE saturated solution was prepared by adding about 10 ml of PCE and four liters of deionized water to bottle and gently stirring the contents for about two weeks at room temperature, before it was added to the PCE solution chamber. As long as a separate PCE phase existed in the bottom of the bottle, the solution could safely be assumed to be saturated. Four such bottles were prepared to provide enough saturated solution to completely refill the PCE solution chamber. The PCE flow was turned off and the new solutions were pumped through a 15 μm filter into chamber through the chamber's exit line. After the tank was refilled and the tubing was reconnected, the solution in the chamber was sparged with N_2 for 15 minutes to deoxygenate it. The PCE flow was only off about 45 minutes while the refilling was done.

4.5.2 Sampling Protocols

The methods involved in taking samples were described in Section 4.2. Sampling for chloroethenes began the day after the PCE solution started to flow. Sampling for methane was already being done and only after October 18 were the gas samples analyzed for ethene, ethane, and vinyl chloride as well.

The effluent lines from the columns entered a common drain manifold approximately one meter after exiting the columns. Samples were drawn from this tubing, therefore the minimum time between samples from any column was 15 minutes. This allowed as effluent line to fill with new flow from its own column. Originally, samples were taken for chloroethene analysis and methane analysis on different days. This procedure was carried over into the first few weeks of samples that were also being analyzed for the other gases. Analysis for ethene, ethane, and

vinyl chloride was done to close the mole balance on PCE degradation. Sampling for the gases shifted from the day after multichloroethene sampling to a period only 15 minutes to 30 minutes afterwards. The analysis was performed by the methods described in section 4.2.

4.5.3 Results and Discussion

The chloroethene profiles for each column were measured over the length of the degradation experiment. The time scale is reported in hours from the time that the PCE flow stabilized, about a day after the flow was initiated. All relevant times will be reported on this scale, with the dates given when they will relate to other facets of the study that have already been discussed. The gaseous degradation products were first measured at 673 hours (October 18) and became reliable at 1611 hours (November 26). The problems associated with the data during this time period were explained during the discussion of methane monitoring.

The results from the H_2/CO_2 microbubble columns will be presented first, followed by the results from the propionate fed comparison columns. The results from the control columns will be presented last. The reproducibility of results from each feeding scheme as well as each column's distribution of the species of the dechlorination cascade will be presented. The overall recoveries from each column and the recoveries of hydrocarbons from Columns 3 and 4 will be presented to bring together the results from all six columns.

Columns 1 and 2 were fed H_2/CO_2 microbubbles to stimulate methanogens that are known to metabolize these species. The major species recovered from these columns is shown in Figure 4-13. The profile shows that both Columns 1 and 2 responding to the experimental conditions is the same manner. They both show that very little dehalogenation occurred in either

column. Some TCE was

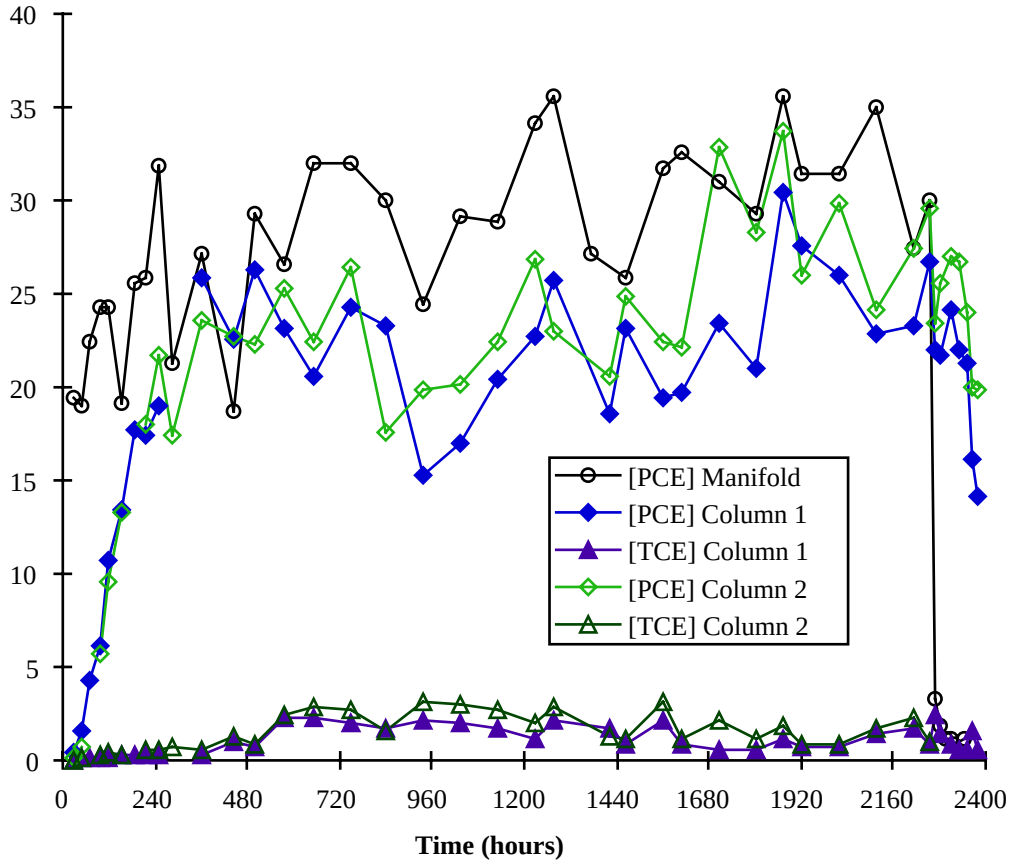

Figure 4-13: Profile of predominant species in the effluents of Columns 1 and 2

produced, but most of the PCE was recovered in the effluent. The PCE concentrations rise over the first 240 hours, until September 29, because most of the entering PCE was being adsorbed on the soil. Only when the adsorption effect reached equilibrium, the PCE concentration plateaued near the inlet values. The PCE flow was
turned off 2266 hours into the experiment on December 23, 1997. The PCE concentration in the manifold dropped immediately to about 3% of the previous inlet concentration. These low concentrations of PCE could still be seen in the manifold for the next few days. The PCE levels in the columns does not begin to decline sharply until two days after the PCE was turned off. There was a delay until desorption of PCE began.

The PCE levels in the effluents were only about 5 μM lower than the amounts that were injected. The TCE concentration in both

columns was about 2 µM and never exceeded 3 µM.

The complete distribution of chloroethenes in Columns 1 and 2 can be seen individually in Figures 4-14 and 4-15 respectively. The new features to notice are that one or more of the gases vinyl chloride, ethene, and ethane are detected at very low concentrations in most of the samples taken after analyses for these species began on October 18, about 670 hours after PCE flow was initiated. Vinyl chloride analysis was imprecise until the last two weeks of the experiment. Until then no values could be reported, because analysis of the standards was not reproducible. From this time, vinyl chloride began to show up at about 1 µM in the samples. Also, the ethene concentration in Column 1 began to rise about 10 days before the PCE flow was stopped. When there was no more PCE entering the column, ethene production stays at about the same level, indicating that residual PCE was desorbing off the soil or that ethene was being given off by the bacteria that were performing the

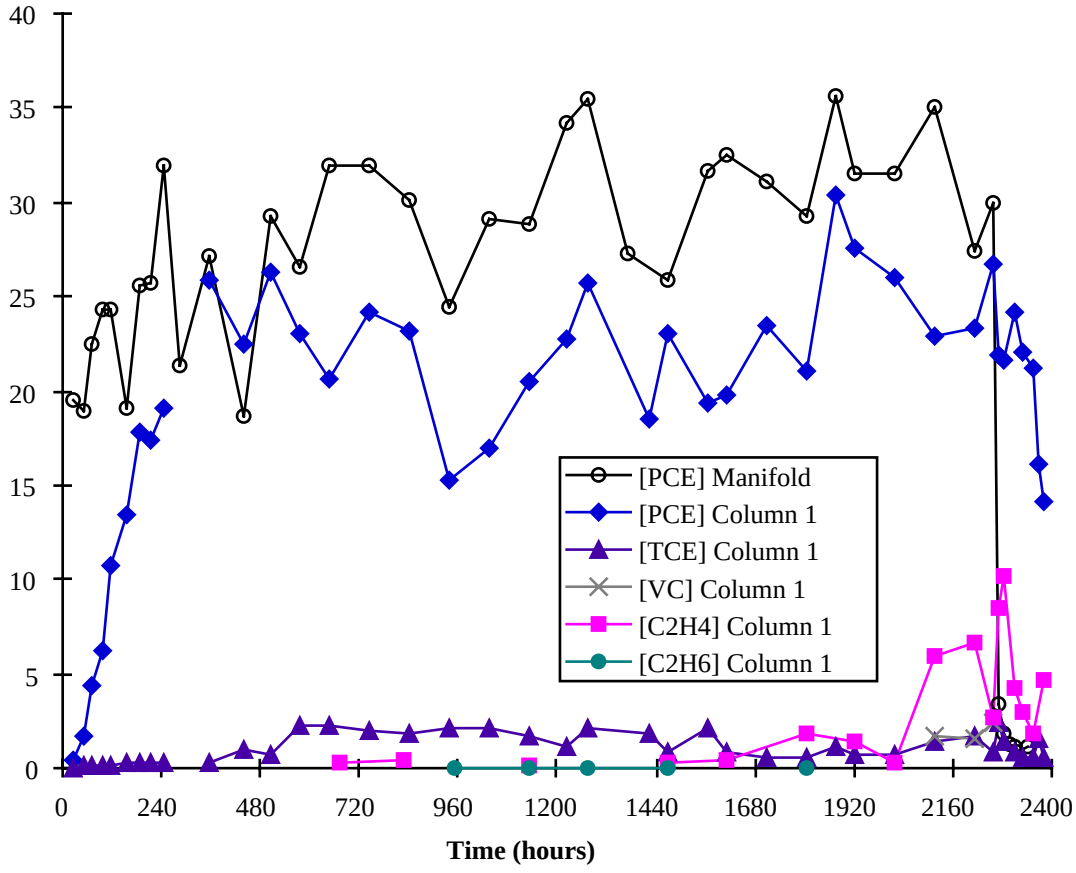

Figure 4-14: Effluent profile of PCE and its reduction products in Column 1

reduction of PCE internally. This effect was also seen to a lesser extent in Column 2. Ethane is only detected occasionally until the PCE is turned off. Figure 4-15 shows the same results as seen in Figure 4-14 with one variation. In Column 2, vinyl chloride and ethene production spiked after the PCE flow was stopped.

The microbubble columns were fed H_2 and CO_2 for 14 months and the consortia that were present in the columns were able to fully reduce PCE to ethene to a small extent. This suggests that the feeding conditions can be modified to make the consortia in Columns 1 and 2 more effective at dehalogenating PCE. If the

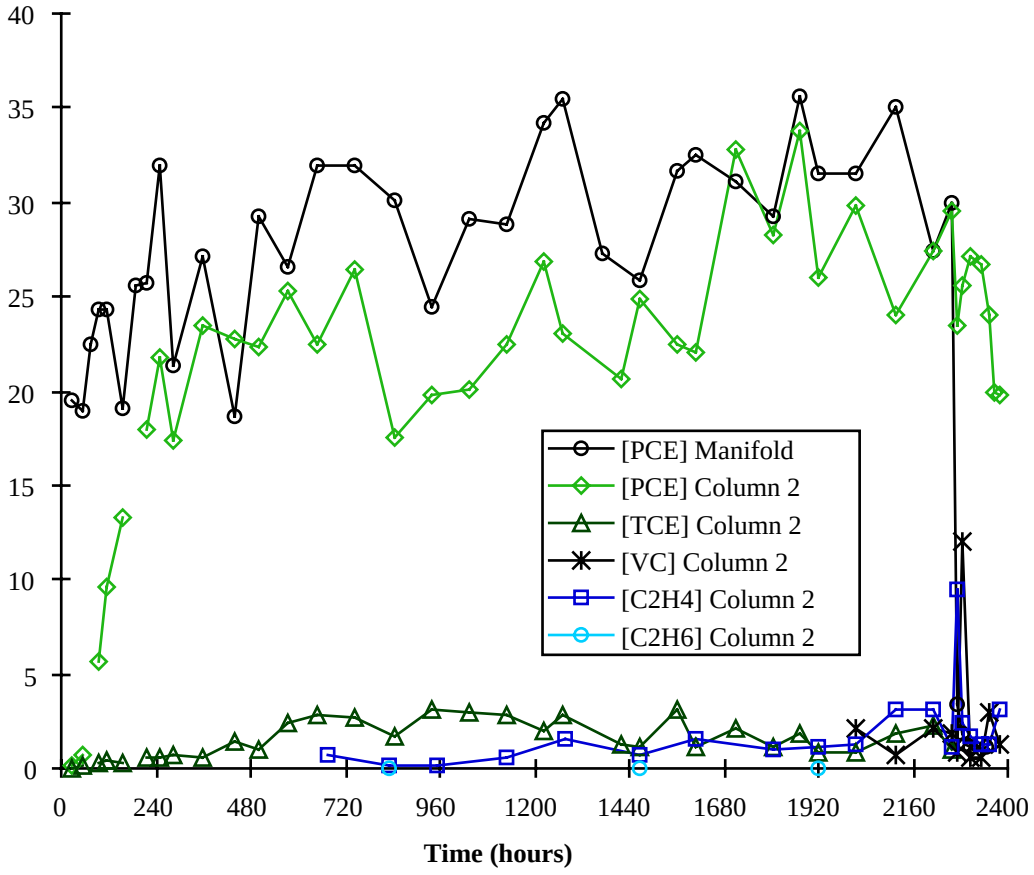

Figure 4-15: Effluent profile of PCE and its reduction products in Column 2

microbubble treatment scheme can be modified to enhance the reduction of PCE, at least completely to TCE or DCE, then anaerobic
treatment of the partially chlorinated ethenes can be considered as an option to complete the decontamination.

Columns 3 and 4 were fed sodium propionate to stimulate the H_2 producing proton reducers and the acetoclastic methanogens. The resulting H_2 should have stimulated the bacteria dechlorinating PCE as well as any hydrogenotrophs. The profiles of the major dehalogenation products in these columns can be seen in Figure 4-16. The profiles show that the consortia in Columns 3 and 4 were able to completely reduce the PCE to ethene. The profiles from both columns are very similar and shows that the results are reproducible. At the very beginning the PCE concentration increases. The consortia possibly needed some time to become efficient at dehalogenating. If they did not begin reducing PCE immediately, then the initial PCE profile would appear more like what was seen in Columns 1 and 2. The TCE profile rises higher than the PCE profile, showing that the reduction of TCE was initially limiting. Within three weeks virtually no PCE or TCE could be detected. DCE was not ever detected in samples analyzed for

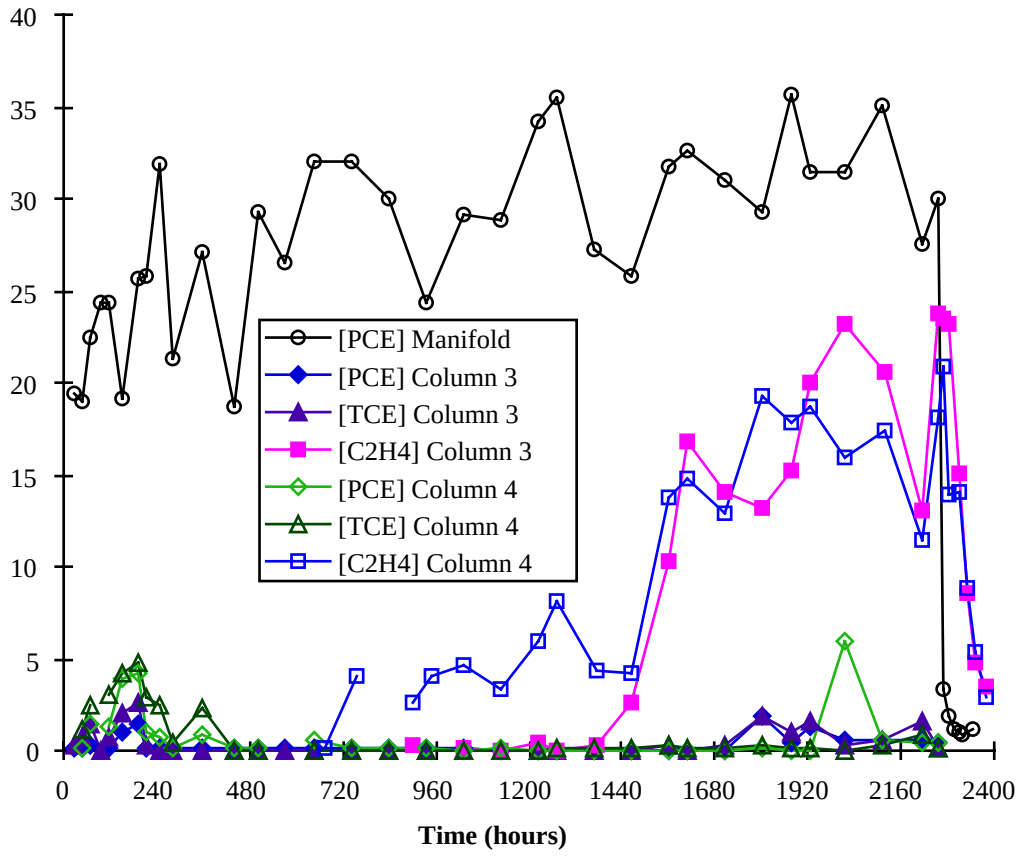

Figure 4-16:Profile of predominant species in the effluents of Columns 3 and 4

multichlorinated ethenes. When the gaseous products were analyzed a peak of an unknown chemical appeared, but was not reproducible and could not be shown to be DCE. Samples analyzed for the gaseous products were analyzed by GC/MS and trace amount of DCE were detected.

Analysis of vinyl chloride, ethene, and ethane began on October 18. The gas analysis of samples taken from that day until November 20,1997 cannot be taken as accurate measurements of the true values. The description of this problem was given in the section on methane analysis, which was analyzed together with the gaseous reduction products of PCE. This explains why there seems to be a delay in the production of ethene and to a lesser extent, vinyl chloride and ethane.

After the 1611 hour mark, November 26, 1997, ethene accounted for about half of the incoming PCE. Before this time the results of the gas analysis were probably low due to a long storage time and the extra heating cycle they experienced. When the PCE was turned off at 2266 hours, the only samples taken from Columns 3 and 4 were analyzed for the gaseous products, not the multichloroethenes. The ethene concentrations in the effluents both columns dropped off immediately, closely mirroring the drop in PCE in the manifold. This immediate loss of ethene production shows two possible things. The first is that PCE was not adsorbed to the soil in these columns at a high concentration. If a lot of PCE was adsorbed, there should have been continued degradation and production while the desorption was taking place. The other reason deals with the bacteria performing the degradation. If the degradation takes place inside the cells, then there could be a delay between the stoppage of PCE and the last of the ethene produced was excreted from the cells.

The complete profile of the dehalogenation products in Columns 3 and 4 can be seen in Figures 4-17 and 4-18 respectively. The only differences in the Column 3 profiles between the figures are the small amounts of ethane and vinyl chloride represented in Figure 4-17. Figure 4-18 shows that small amounts of ethane were occasionally detected in Column 4, just like Figure

4-17 showed for Column 3. Vinyl chloride analysis shows that it was an important

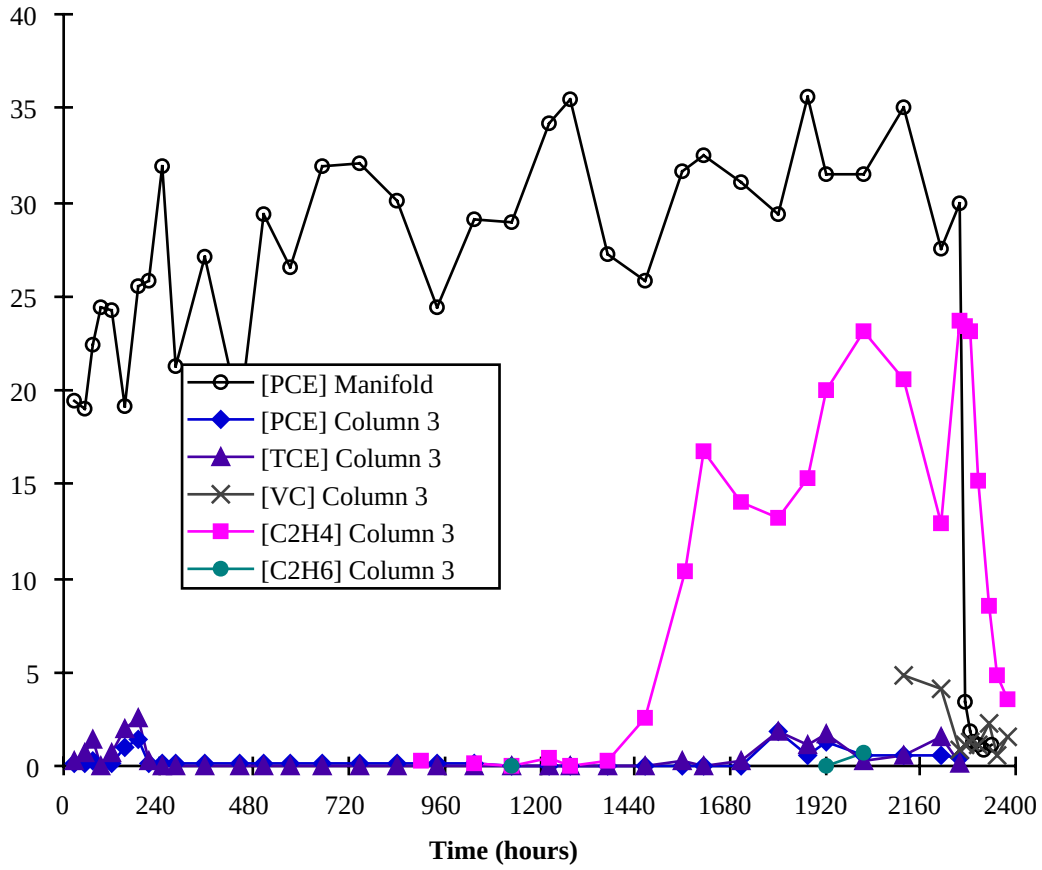

Figure 4-17: Effluent profile of PCE and its reduction products in Column 3

product from the date the analysis became reliable. Vinyl chloride was probably present to a similar extent as seen from the 2000 hour mark until the end of the experiment.

Columns 5 and 6 were controls, and were not fed any special substrate. The were periodically fed yeast extract, which was also being fed to the other four columns. The profile of the predominant species in these columns can be seen in Figure 4-19. Both columns responded the same to the conditions applied. The

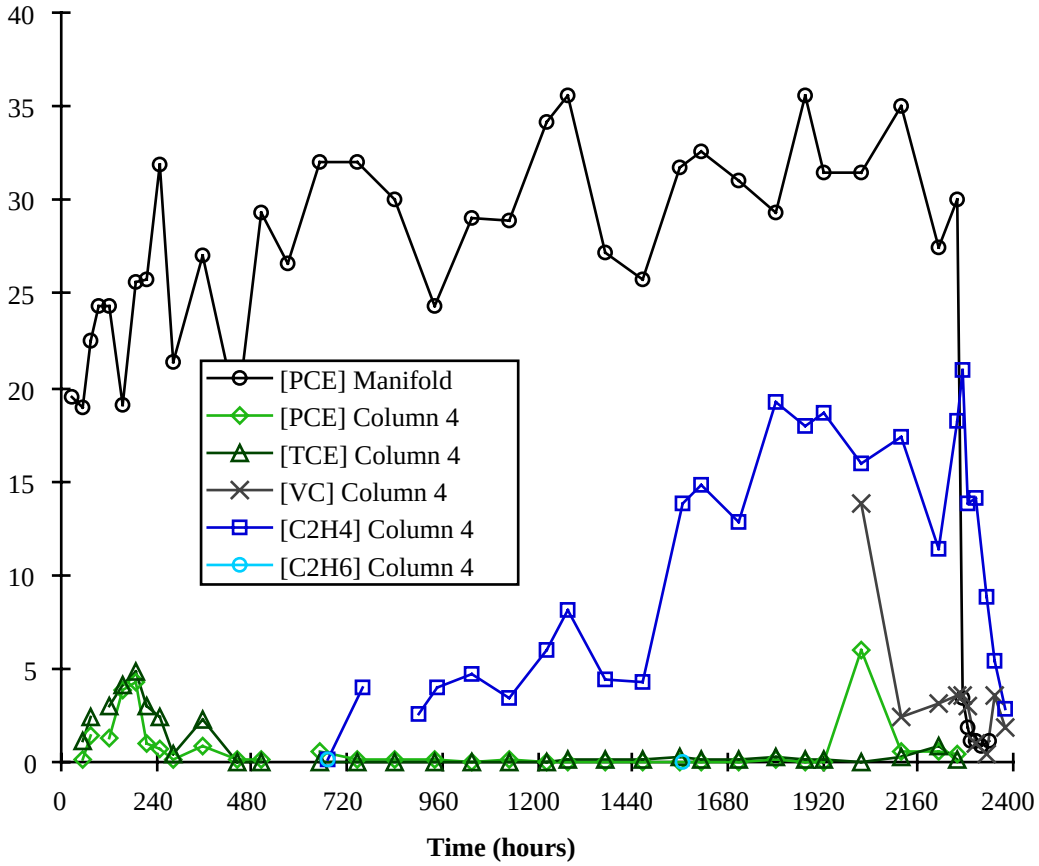

Figure 4-18: Effluent profile of PCE and its reduction products in Column 4

general appearance of the profiles is like that of Columns 1 and 2 in Figure 4-13. The PCE concentration increases dramatically over the

first 10 days then levels off about 10 μM lower than the manifold concentration. The next most predominant species was TCE. This was the same result as seen in Columns 1 and 2. The TCE concentration plateaus in the neighborhood of 5 μM and hits a maximum at about 7 μM. The analyses for vinyl chloride, ethene, and ethane began on October 18, about the 670 hour mark, and ethene was detected in all of the samples taken from this time until the end of the experiment. The level stays very low for almost a

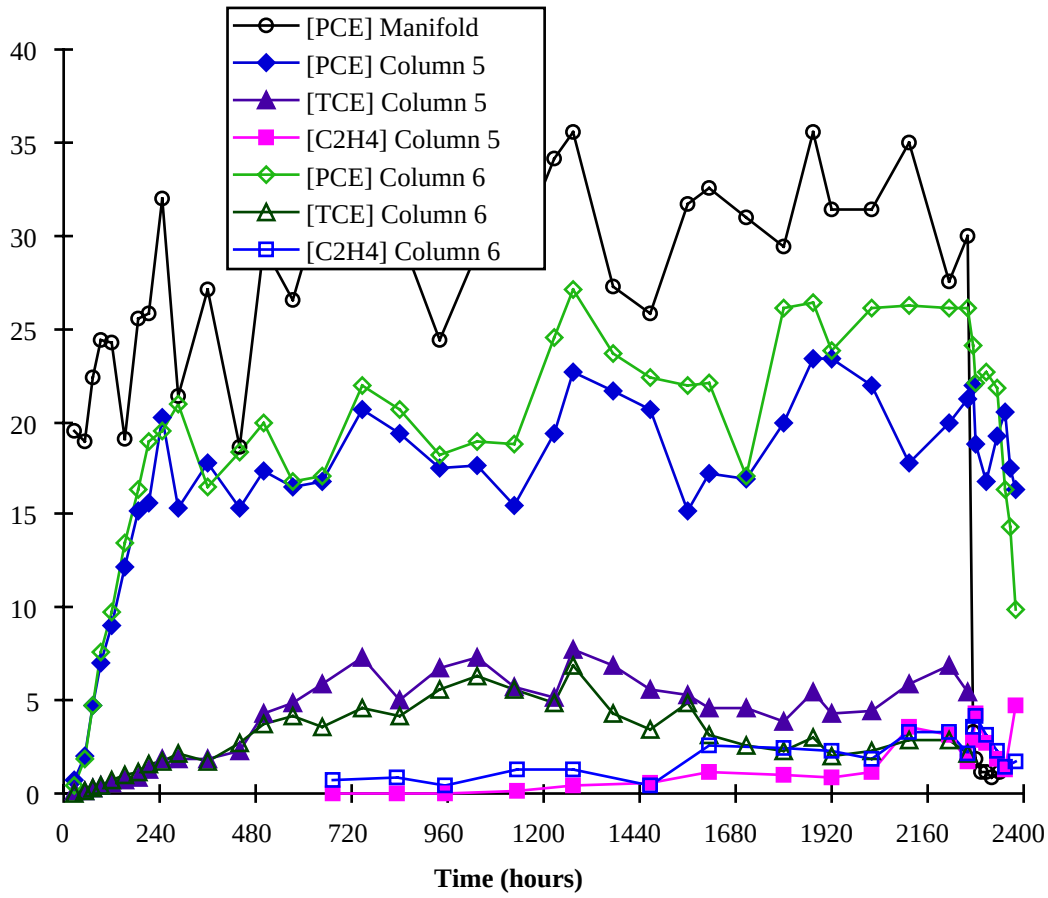

Figure 4-19:Profile of the predominant species in the effluents of Columns 5 and 6

month then climbs to the 1 μM to 2 μM level until the experiment ends. When the PCE flow is shut down, the levels of PCE in the two columns began to drop after a small delay. The profiles for the individual columns can be seen in Figure 4-20 and Figure 4-21 respectively. The figures show, that in both columns, ethane and vinyl chloride were detected as minor products. The vinyl chloride became significant at the end of the experiment in Column 5. This is exactly what was seen in Columns 1 and 2.

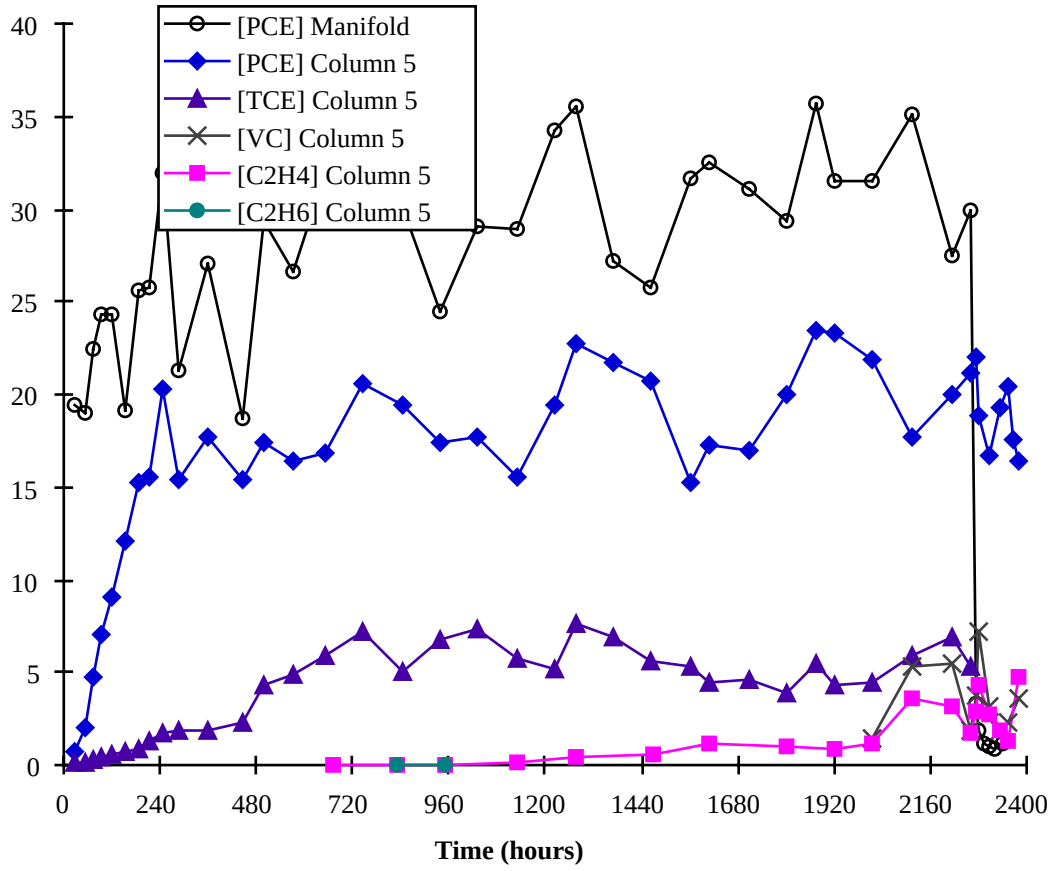

Figure 4-20: Effluent profile of PCE and its reduction products in Column 5

The results from all six columns show that some reductive dehalogenation occurred everywhere. The sampling procedures and analytical techniques were acceptable based on the percent recovery. The recovery of the involved species can be seen in Figure 4-22. The percentage of PCE that entered the columns that was recovered as PCE or one of the degradation products is reported for all of the columns. In Columns 1,2,5, and 6, where the predominant species in the effluent was PCE, the percent recovery is calculated

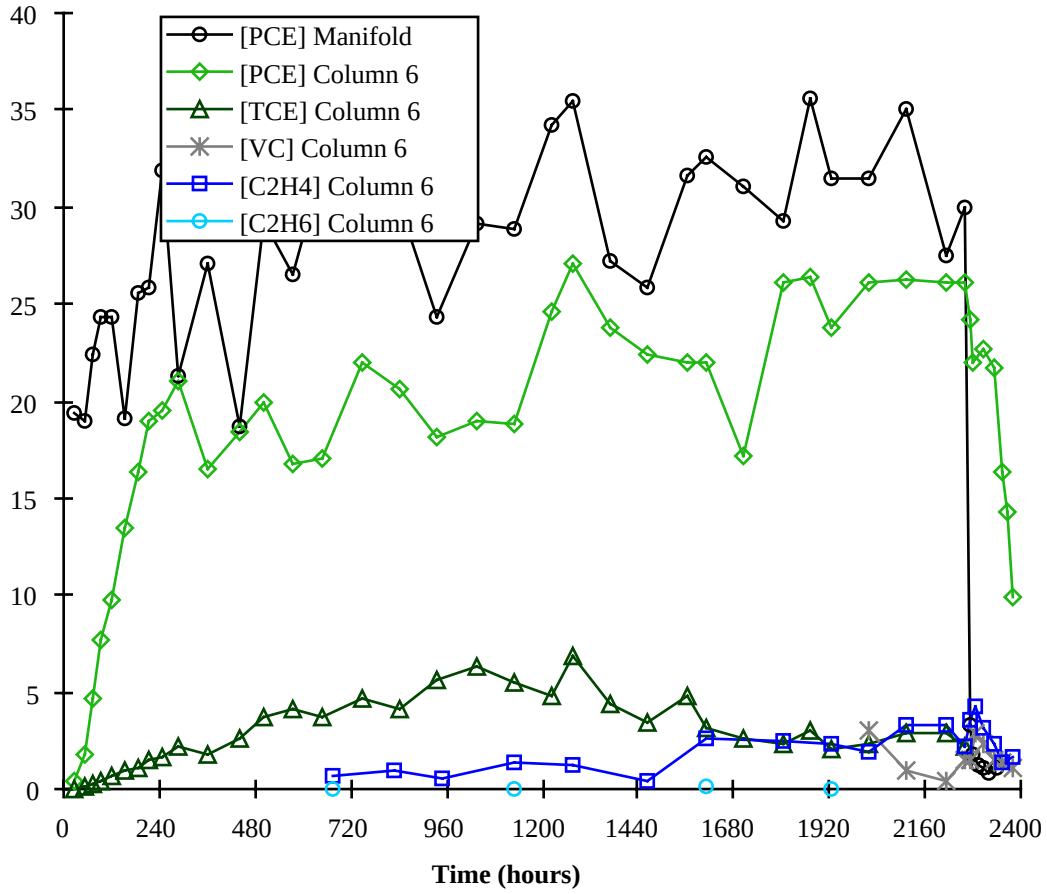

Figure 4-21: Effluent profile of PCE and its reduction products in Column 6

from the all data taken from hour 248 (September 29) until the PCE flow was stopped on December 23, 1997. From each date the effluent concentrations of PCE and all of the reduction products are added together and divided by the PCE concentration in the manifold and multiplied by 100%. That represents the percent recovery for any given day. Recoveries for the four columns were 85%, 93%, 85%, and 90% respectively. The errors in these values ranged from 14% to 17% based on scatter over all of the data. Figure 4-22 shows that the mole balance done around Columns 1, 2, 5, and 6 were

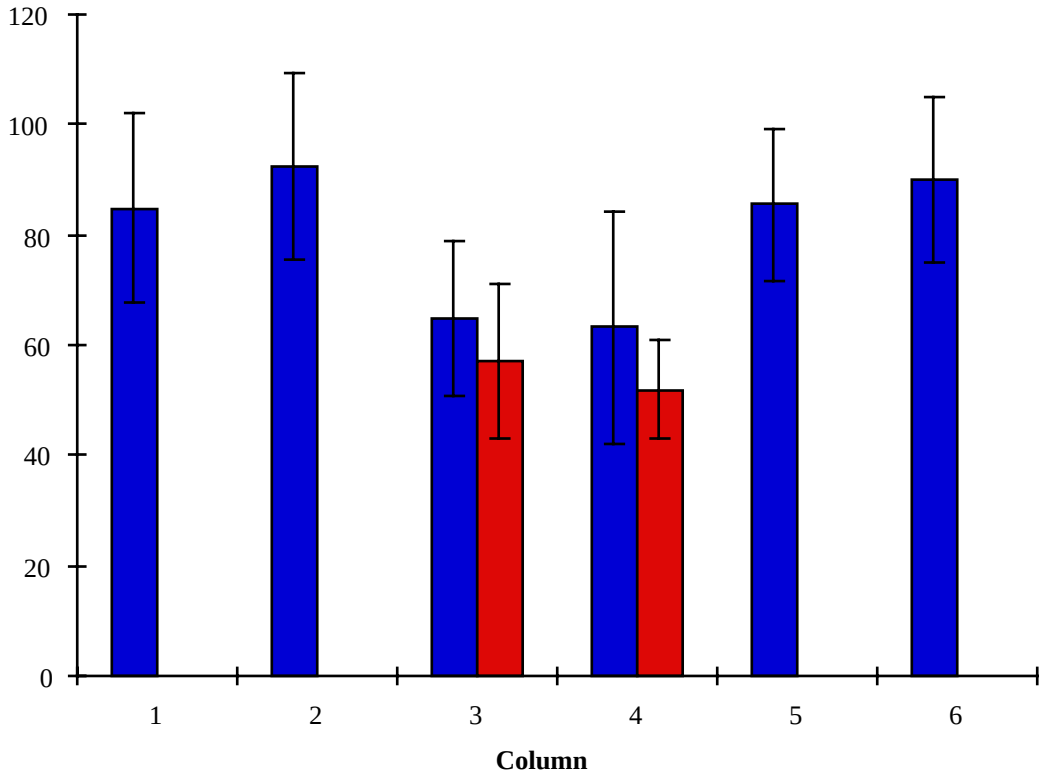

Figure 4-22: Average percent recovery of PCE and the reduction products in all six columns. The average percent recovery as ethene and ethane in Columns 3 and 4 is also presented as the red bars**.**

complete. 248 hours was the time after start-up that PCE reached its steady state value in those columns. The percent recovery for Columns 3 and 4 was calculated from all the data taken after hour 1611 (November 26). This was the first point where the analysis of all of the gases was reliable. The recoveries were only 65% $\pm$ 14% and 63% $\pm$ 21% respectively. The species being recovered were gases, and systematic losses due to their volatility from the samples was high, so closing the mass balance around these columns was more difficult. The values for the gas concentrations were probably higher than what was measured. Columns 3 and 4 also report the percentage of injected PCE that was recovered as ethene. These were 57% $\pm$ 14% and 52% $\pm$ 9% respectively. Ethene accounted for 88% and 83% of the recovered products in Columns 3 and 4 respectively. Ethene accounted for most of the recovered products from both of these columns, with the rest being predominantly vinyl chloride.

Now that the analyses have been demonstrated to account for a majority of the PCE that entered each column, comparisons can be drawn and conclusions can be made about the differences in the treatment schemes. The results seen in Figure 4-16 for Columns 3 and 4 towards the end of the experiment, show that propionate is an effective source of energy and H_2 to stimulate the populations of organisms that are capable of reductively dehalogenating PCE all the way to ethene. The use of bioreactors that have been conditioned to select for consortia that degrade propionate can be effectively utilized to decontaminate ground water with PCE in it. Propionate was known to be an acceptable provider of H_2 for partial PCE reduction and was used as a H_2 source so the results from both feeding schemes could be compared (Gibson and Sewell, 1992; Fennell, et al.,1997). A batch study testing the effects of different acids on the reductive dechlorination of PCE was performed. The propionate fed system yielded vinyl chloride as the major product, accounting for half ot the injected PCE. TCE accounted for an additional 10% of the PCE and only trace amounts of ethene were detected. It seems that DCE was not evaluated at all (Fennell, et al., 1997).

The H_2 and CO_2 fed to Columns 1 and 2 were expected to stimulate the ability of the bacteria to reductively dehalogenate PCE more than would be seen in controls. The controls were not given any electron donors and acceptors other than those that were present in the yeast extract that all six systems received. This did not prove to be the case. The controls reduced more of the PCE to TCE than the columns fed H_2 and CO_2, which can be seen by comparing the concentrations of TCE in the effluents from the microbubble columns versus the controls. The concentration of TCE in Columns 1 and 2 can be seen in Figure 4-13 and generally falls in the range of 1 μM to 2 μM. and for Columns 5 and 6 the TCE concentration range was from 3 μM to 7 μM. Even if the gases were not stimulating the reduction of PCE, they were not expected to have a retarding effect.

Column studies investigating the possible biodegradation of PCE were done by other researchers. One study maintained bulk aerobic conditions in the sediment column, but determined anaerobic sites existed within the column by detecting methane in the effluent. Added PCE and TCE were both reduced to near minimum detection limits using methanol as the electron donor. The average residence time was about 30 hours. *Cis*-DCE was the major product, and no vinyl chloride or ethene were detected. Later additions of methanol resulted in the complete conversion to ethene (Enzien, et al., 1994). Another study ran two sediment

columns from different aquifers. One was previously contaminated with PCE and the other was not. The columns were fed lactate and acetate and had an average residence time of 31 hours. The virgin sediments produced small amounts of TCE and no other products were detected, but most of the PCE was not recovered. The acclimated sediments began to show up to 25% recovery as TCE after 20 pore volumnes with small amounts of *cis*-DCE being detected after 39 pore volumes. When TCE became a significant product the PCE concentration levels fell to near detection limits; together the recoveries of PCE and TCE could only account for half of the influent PCE concentration (Noftsker and Watwood, 997). The propionate columns in this study were superior to the acid fed systems above and comparable to the methanol fed system. The microbubble fed systems were comparable to the sediments in the acid fed study, but the consortia proved to be more versitile since small quantities of ethene and vinyl chloride were detected. The same hold true for the control column consortia.

The cause of the retarding effect the H_2 and CO_2 microbubbles had on Columns 1 and 2 can only be hypothesized without doing more testing or microbiological studies to confirm which bacteria were present in each column and at what density. A few possible causes can be described as well as one that was not so likely. Each of the likely culprits reducing the effectiveness of the consortia in Columns 1 and 2 can be traced to one of the components of the microbubble dispersion, CO_2, H_2, and the surfactant sodium dodecylbenzene sulfonate (NaDBS). How each component may inhibit the dehalogenation of PCE will are discussed below.

The CO_2 may have been the cause of inhibiting the reduction of PCE and the general microbial activity within the columns also. The CO_2 constituted 10% of the gas phase at atmospheric pressure was dissolved in the liquid phase of the microbubbles while they were being made. The pH of the resulting microbubbles was 4.4. This pH will inactivate or dramitically decrease microbial activity of many bacteria, except for acid tolerant populations. When the 60 ml of microbubbles were injected twice a week, 20 ml of acidic water was injected. Since pH is an exponential relation it would take 7960 ml of water to dilute the 20 ml of acid to a pH of 7. At 3 ml/min this would take over 44 hours. The real time should be less, because the acidic water can also flow out of the columns. The shock is possibly to severe to overcome before the next injection of microbubbles, except for the tolerant organisms. After the low pH liquid fraction was diluted, the organisms that survived should have regained at least some activity. The optimal pH range of Methanogenic activity is 6 to 8 (Zehnder, 1988). Many of the background studies controlled or measured the pH of their consortia. In a few studies the pH was

measured to be between 7.0 and 7.4 (Holliger, et al., 1993; Enzien, et al., 1994; Scholz-Muramatsu, et al., 1995). The pH was adjusted to stay between 7.0 and 7.5 in some investigations of chloroethene dehalogenation (Freedman and Gossett, 1989; Maymo-Gatell, et al., 1995). The influent in this study was buffered with potassium phosphate to be right at 7.0. When the effluent pH was measured, it was never following an injection. The effluent pHs were above 7, when measurements were taken. The injected dispersion only delivered H_2 and CO_2 to the bottom of columns. Unfortunately, the part of the column encountering the H_2 and CO_2 food source also had to deal with the acidic environment. This phenomenon may have prevented the gases from being utilized and also may have kept the population activity low in the region from the injection ports to the ends of the columns. These bacteria may have taken part in the reduction of both PCE and the limited amounts of initial degradation products that were created. It is also possible that this is a dead zone and the bacteria never recovered from the acidic shock.

The upper portions of the treatment zone probably never encountered either the acid or the gases and only survived on yeast extract. These upper bacteria lived in an environment that was equivalent to what existed in the control columns. This could account for a similar but reduced degradation pattern in the microbubble columns versus the controls.

Measurements of pH along the length of the columns were not taken to see if the lower ends were acidic. The amount of liquid required for the pH probe to be effective was beyond capabilities of the system. If a sample port along the column were opened long enough to get an adequate sample, the dynamics within the column would have been drastically altered. The pH of the effluents was just above 7 the only time it was taken after the buffer was changed.

pH neutral H_2/CO_2 microbubbles can be made that exhibit the properties required to effectively deliver gases to soils. Further studies involving the transport of CO_2 should use surfactant solutions that have been amended with sodium hydroxide or another base or even a non-ionic surfactant.

The second possible cause for the lower amounts of PCE reduction in Columns 1 and 2 relative to the controls was the H_2 itself. H_2 is a necessary component for both PCE reduction and the growth of the autotrophic methanogens and homoacetogens, and high levels of H_2 should have had a positive effect on both dehalogenation and growth of these organisms. The dissolved concentration of H_2 was in equilibrium with bubbles having a partial pressure of H_2 equal to 0.837 atm, yielding a dissolved concentration of 6.6 mmol/L. This is very high relative to the

levels present in natural environments, which are in equilibrium with gas phase concentrations around 10^{-6} to 10^{-4} atm partial pressures, which translates to 0.79 to 79 nmol/L. The H_2 produced from propionate is used quickly and the concentration of H_2 never builds up. The levels may be higher than natural levels due to the high concentration at the injection point. Some bacteria, like the proton reducers, produce H_2, and its presence thermodynamically inhibits any reaction where H_2 is a product. One kind of syntrophic relationship may exist in the columns, where one bacterium, that cannot produce some essential biochemical nutrient or cofactor, lives in close contact with a bacterium that can. If the bacterium, that produces the chemical, is inhibited by H_2, the other bacterium will not be active. It is possible that the dechlorinating organism is a species that requires such syntrophic assistance to get all of its required nutrients. Then, although the dechlorinator was getting an excess of the H_2 that could be used for the dechlorination of PCE, the rate of dechlorination in such a consortium will be lower than expected. It was possible that some important substance was being produced by an organism that was inhibited by the high concentrations of H_2 present in the columns. Within the dechlorinating organism or organisms, there may be some reaction that was thermodynamically inhibited by the high concentration of H_2 in Columns 1 and 2. Only a detailed study of members of the consortia can determine if an excess of H_2 is the cause of the inhibition of PCE dechlorination. Bacteria would have to be extracted from the growth media, either the soil or from the contents of a beaker, and then be separated by microbiological techniques. Investigating the use of different levels of H_2 in the microbubble dispersion, going as high as the 90% used here, would be one means to see the effect of H_2 on PCE reduction and, simultaneously, on microbial activity.

The last component of the microbubble dispersion is NaDBS. NaDBS is a member of a family of surfactants, linear alkylbenzene sulfonates, that have been shown to inhibit the activity of methanogens (Wagener, et al., 1987). Only 3.6 mg of NaDBS was injected as part of the microbubble dispersion twice a week. The concentration in the liquid phase was 200 mg/L. The surfactant should have been diluted and washed away, allowing the bacteria to regain their activity. Concentrations of NaDBS below 100 mg/L were shown not to have any effect on the methanogens investigated in the earlier study (Wagener, et al., 1987). This would mean that the NaDBS would have had an inhibitory effect in a pulsed manner. More degradation of the PCE should have been detected if the NaDBS had been the cause.

Non-ionic surfactants, such as the family of polyalkylene oxides, do not inhibit methanogens and can be degraded under anaerobic conditions. The compatibility between a surfactant and the consortia chosen for a project can be determined in batch studies. Various amounts of the surfactant can be added to the consortia and the activities can be used to measure any inhibitory effects.

The lowered levels of PCE reduction seen in Columns 1 and 2 relative to the controls may be due to one of the possible causes mentioned or combinations of them. Bacterial consortia are complex systems. Interactions between the effects of low pH, high H_2 concentration, and the surfactant on the consortia may have all played a part in inhibiting both the microbial activity and PCE reduction ability within the columns.

4.6 Summary

A continuous flow system was designed and built that was capable of maintaining anaerobic consortia and delivering controllable amounts of PCE for the consortia to degrade. The system was also designed to be able to easily take samples to monitor the activity of the consortia and the amounts of PCE that was dehalogenated and to what extent.

Analytical techniques were developed that were able to effectively detect and measure PCE and the cascade of products from its reduction. Techniques were established that could effectively measure certain metabolites, propionate and sulfate, and certain metabolic products, methane and acetate. The technique used to measure sulfate also was capable of measuring bromide. DCE was not reliably detected by either of the chromatographic methods designed and used to measure PCE and the rest of compound in the dehalogenation cascade.

The materials of the system did not degrade PCE or TCE under abiotic conditions. None of the materials used provided a continuous sink for PCE, although it was adsorbed onto the soil in equilibrium with the liquid phase concentration during the degradation experiment. Once steady state was achieved in the column, the effluent concentration of PCE under abiotic conditions was the same as at the inlet. TCE was completely recovered from a similar abiotic system.

After the system was inoculated with sediments from the Duck Pond on campus, column performance characteristics were determined. The system operated with a breakthrough time

between five and six hours for the non-adsorbed species, bromide, and it reached its maximum concentration about 7 hours later. The pH in the columns during the degradation experiment was 7.0, based on infrequent measurements. This matched the pH of the buffered nutrient solution. Methane was detected in all of the columns, and showing that methanogens were active throughout the system. Methane was detected throughout the complete duration of the experiment, from inoculation until the PCE flow was terminated. Sulfate reducers were present in all of the columns and it was almost totally consumed in the propionate fed columns, showing that they were very active there. Almost no propionate and no acetate were seen in the effluents Columns 3 and 4, showing that the proton reducers and acetoclastic methanogens were active there.

All of the studied systems remained viable for over a year and were capable of at least partially reducing PCE. Some ethene was produced in the microbubble columns, although PCE was the predominant product. This suggests that the organisms living in the presence of high H_2 concentrations may be stimulated to reduce PCE to a greater extent than was seen here. The control columns accounted for a greater reduction of PCE than the microbubble columns did and was shown by the higher levels of TCE in the controls than in the microbubble columns. Because the only difference between Columns 1 and 2 and the controls was the microbubbles, it is hypothesized that something in the microbubble dispersion was responsible for the lower relative degradation amounts. The consortia that received sodium propionate proved capable of completely reducing at least 50% of the injected PCE to ethene and may lead to a reasonable treatment scheme for PCE contaminated ground water.

Each of components in the microbubble dispersion, CO_2, H_2, or NaDBS, may have inhibited the consortia in Columns 1 and 2. The pH effects of the CO_2, the high dissolved H_2 levels, and the presence of the NaDBS may have inhibited or killed some bacteria in the columns.

The CO_2 may have destabilized the microbubbles by neutralizing the surfactant. If some of the gas escaped from the microbubbles, a bulk gas phase may have opened a channel for the gases to escape with the flow. The effects caused by these species or interactions between them were probably responsible for the lower dehalogenation activity than was seen in the controls.

Chapter 5 Conclusions

The main objective of this study was to demonstrate that microbubbles with a gas mixture of H_2 and CO_2 could effectively stimulate anaerobic consortia to reductively dechlorinate PCE. To meet this objective a system with a series of six anaerobic soil columns was constructed and tested. The consortia were fed either the microbubbles, sodium propionate as a comparison using a conventional feeding strategy, or only the generally administered yeast extract. The results obtained yielded the following conclusions and recommendations.

5.1 Conclusions

- A system was designed, built, and tested that proved effective for maintaining anaerobic organisms, controlling flows, providing reproducible samples, and degrading PCE and other chlorinated ethenes.

- A consortium that had not previously been acclimated to PCE was able to at least partially dechlorinate PCE when fed only yeast extract, yeast extract and H_2 plus CO_2, or yeast extract and sodium propionate.

- After an apparent acclimation time, consortia stimulated by sodium propionate were effective at dechlorinating over half of the PCE that was injected into continuous flow packed soil column bioreactors.

- A microbubble dispersion encapsulating a mixture of H_2 and CO_2 as the gas phase can be made having the properties required to make this an effective means to deliver these gases to a soil column.

- Quantitative and reproducible techniques were developed for the analysis of the multichlorinated ethenes and for the simultaneous analysis of methane and the gaseous

dehalogenated products of PCE, vinyl chloride, ethene, and ethane.

- As presently formulated, H_2/CO_2 microbubbles do not stimulate anaerobic consortia to reductively dehalogenate PCE better than controls.

- The pH of a microbubble dispersion containing CO_2 can be effectively controlled and neutralized with additions of a strong base.

5.2 Recommendations

The use of microbubbles to effectively stimulate the anaerobic reduction of chloroethenes is still feasible in continuous flow column as well as batch studies. To achieve this goal, modifications should be made in the microbubble composition, sampling procedures, and analyses performed. Specific recommendations for modifications to the study that may lead to the demonstration that microbubbles can indeed stimulate anaerobic consortia to reductively dehalogenate PCE are presented below. The first three recommendations deal with the reformulation of the microbubbles and how they might interact with anaerobic consortia. Other recommendations deal with other aspects of involved in an anaerobic treatment of contaminated ground water.

- Additions of base to a microbubble dispersion using NaDBS and other anionic surfactants should be optimized for quality and stability while maintaining a pH neutral dispersion.

- Batch studies with soil microcosms should be performed testing how microbubbles, made with either non-ionic or the amended anionic surfactants, effect the activity and viability of the members in consortia that would be used in a dehalogenation study.
- Following the choice of an acceptable surfactant, continuous flow tests should be done that investigate how microbubbles containing different H_2 contents effect the activity of a consortium, then dehalogenation studies should be done in the same system using microbubbles optimized for H_2 content.

- If further investigations with H_2/CO_2 microbubbles can only enhance the complete reduction of PCE to either TCE or DCE, then a follow-up aerobic treatment scheme can be investigated to complete the decontamination.

- In all investigations, sulfur should be added to the media in the form of sulfide, to eliminate the need to consider the activity of the sulfate reducers in the consortia.

- Field tests should be performed where propionate or optimized microbubbles are either injected into a trench or wells to decontaminate the ground water in situ.

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Appendix A: Characteristics of H_2/CO_2 Microbubbles and Air Microbubbles

Date of Sample	Gases	Sample	Trial	RPM +/- 50	Sample Mass (g) +/- 0.05	Bottoms (ml) +/- 1	Quality (%) +/- 1%	Stability (%) +/- 2%
21-Feb	H_2/CO_2	1	2	3280x2	79.60	14	68	18
21-Feb	H_2/CO_2	3	1	3230x2	77.50	33	69	43
21-Feb	H_2/CO_2	3	2	3180x2	78.75	18	69	23
26-Feb	H_2/CO_2	4	1	6500	69.30	15	72	22
26-Feb	H_2/CO_2	4	2	6500	70.65	15	72	21
26-Feb	H_2/CO_2	5	1	6500	72.90	15	71	21
26-Feb	H_2/CO_2	5	2	6500	72.55	17	71	23
1-Mar	H_2/CO_2	6	1	6450	66.10	15	74	23
1-Mar	H_2/CO_2	6	2	6450	70.75	16	72	23
26-Feb	Air	1	2	6250	72.15	7	71	10
26-Feb	Air	2	1	6300	71.10	8	72	11
26-Feb	Air	2	2	6300	75.30	9	70	12

NOTES:

1) The first trial of all February 21 samples yielded high Stabilities. This was probably due to the short time between the movement of the inlet flow to the top of the generator.

2) The February 21 samples had the disk speed measured at one half of the true rotational rate.

3) The March 1 samples used an extra wide piece of plastic to increase the range of motion of the hand jack.

4) The masses were measured with a triple beam balance.

Appendix B: Characteristics of H_2/CO_2 Microbubbles Amended with NaOH

Gases	Sample	Trial	NaOH (N)	Sample Mass (g) +/- 0.05	Bottoms (ml) +/- 1	Quality (%) +/- 1%	Stability (%) +/- 2%
H_2/CO_2			0			70	21
H_2/CO_2	1	1	10^{-4}	69.25	13	72	19
H_2/CO_2		2	10^{-4}	NM	14		
H_2/CO_2	2	1	10^{-3}	71.45	12	71	17
H_2/CO_2		2	10^{-3}	71.65	13	71	18
H_2/CO_2	3	1	$10^{-2.5}$	73.60	10	71	14
H_2/CO_2		2	$10^{-2.5}$	72.60	10	71	14
H_2/CO_2	4	1	10^{-2}	74.50	8	70	11
H_2/CO_2		2	10^{-2}	75.40	8	70	11
Air			0			71	11

NOTES:

1) NM - not measured

2) The air and the H_2/CO_2 without NaOH are average results from comparative tests between H_2/CO_2 and air microbubbles.

3) All H_2/CO_2 microbubbles were made with the regular width plastic sheet.

4) pH readings were taken from samples, not the generation chamber.

5) The pH was read from the liquid phase after most of the phase separation had occured.

Appendix C: pH of H_2/CO_2 Microbubbles Amended with NaOH

Sample	NaOH (N)	pH
1	0	4.4
2	10^{-4}	4.8
3	10^{-3}	5.9
4	$10^{-2.5}$	6.2
5	10^{-2}	7.0

NOTES:

1) pH readings were taken from samples, not the generation chamber.

2) The pH was read from the liquid phase after most of the phase separation had occured.

Appendix D: PCE Mass Balance Around an Abiotic Column

Date	Sample	Trial	Manifold Port	Column Exit	Manifold Avg. ± St. Dev	Column Avg. ± St. Dev
26-Oct	1	1	9915635	10243680		
	2	1	10965888	10814120	10440762	10528900
	3	1	13176000	8000653	742641	403362
		2		7537984		
27-Oct	1	1	9849632	9380563		
	2	1	10770480	7897370	10611781	9380563
		2		8246115	651138	572214
	3	1	11215232	6624941		
28-Oct	1	1	11014133.3	9838874		
		2	10248663.2		10371695	9676160
	2	1	9852287.18	9513446	590613	230112
		3		7806384		
29-Oct	1	1	7347261	8363021		
		2	8741638	8755123	8044450	8559072
		3			985973	277258

Chamber Exit and Column Exit values are in peak area units from the chromatogram.

Averages and Standard Deviations are normalized to the initial chamber exit value.

Appendix E: TCE Mass Balance Around an Abiotic Column

Date	Sample	Trial	Chamber Exit	Column Exit	Chamber Avg. ± St. Dev	Column Avg. ± St. Dev
12-Apr	1	1	749	642	1.000	0.858
		2	775	649	0.035	0.005
		3		645		
	2	1	717			
		2	738			
		3	780			
13-Apr	1	1	722	635	0.875	0.799
		2	630	622	0.074	0.030
		3	621	591		
	2	1		596		
		2		580		
		3		582		
14-Apr	1	1	577	520	0.763	0.685
		2	612	505	0.053	0.012
		3	533	520		
15-Apr	1	1	477	461	0.641	0.601
		2	480	450	0.008	0.011
		3	488	445		
16-Apr	1	1	470	502	0.621	0.681
		2	476	517	0.014	0.012
		3	455	518		
17-Apr	1	1	501	475	0.647	0.624
		2	487	460	0.020	0.011
		3	471	472		
18-Apr	1	1	444	409	0.581	0.554
		2	446	420	0.019	0.008
		3	420	420		
19-Apr	1	1	413	400	0.531	0.544
		2	383	426	0.020	0.020
		3	403	401		
20-Apr	1	1	377	420	0.499	0.551
		2	381	408	0.010	0.008
		3	367	414		

Chamber Exit and Column Exit values are in peak area units from the chromoatogram.

Averages and Standard Deviaitions are normalized to the initial chamber exit value.

Appendix F: Bromide Concentration from the Breakthrough and Residence Time Experiment

Time (hrs)	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Pulse Solution
0	8578381	8720230	8606285	8510592	8585990	8559565	50165632
1	8453562						50165632
2	8501760						50165632
3	8495110		8449510	8414694	8578266		50165632
4	8513747		9530502		8639104		50165632
5	8533965	8536474	14215144	8568115	8669229	8477357	50165632
6	8675360	8805293	23658560	10982592	9861971	8507110	50165632
7	10564464	13087352	33845280	16084976	14617280	8588064	50165632
8	14946480	22657280	38247104	23881072	22371200	9170694	50165632
9	22012976	34827392	42389376	29795952	33315504	11417320	50165632
10	30575424	39068512	44671040	36216832	41298400	16524088	50165632
11	33738304	41349888	45309600	39123264	43874976	21328832	50165632
12	40519264	43386528	46991232	41240544	47795808	27652352	50165632
13	43674944	43171328	48492992	42094784	49053664	33607648	50165632

Values represent peak areas from the ion chromatograph.

The pulse solution is the concentration of bromide at the inlet.

Appendix G: Methane Peak Areas from the Gas Chromatograph

Date	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
18-Nov	233.40	254.40	132.00	106.32	293.77	252.16
19-Nov	265.91	149.33	41.59	73.62	363.90	298.98
20-Nov	194.52	99.58			445.54	236.84
21-Nov	189.07	111.52	54.07	33.84	244.32	221.65
22-Nov	137.40	93.05	42.75	27.45	430.87	182.34
23-Nov	110.19	96.69	42.35	22.48	439.47	155.57
24-Nov	107.44	92.96	40.06	39.00	358.83	156.55
25-Nov	106.69	71.79	38.27	30.34	333.13	136.79
26-Nov	100.70	65.54	35.76	26.13	317.13	163.81
27-Nov	99.07	56.60	38.38	18.53	222.14	135.54
28-Nov	87.75	71.40	35.87	17.13	256.83	171.45
29-Nov	69.33	58.71	29.84	15.92	239.52	201.41
30-Nov	78.56	51.95	24.37	18.47	244.91	242.33
1-Dec	126.61	48.51	85.79	18.60	218.22	149.42
2-Dec	82.06	57.32	37.18	23.00	315.53	164.47
3-Dec	80.26	78.37	34.35	10.32	304.03	150.44
4-Dec	76.51	47.07	41.76	14.55	328.65	132.46
5-Dec	72.13	38.27	48.05	10.44	351.19	103.20
6-Dec	103.95	68.20	38.09	15.00	349.57	104.51
7-Dec	80.16	49.00	28.33	12.16	357.73	114.76
8-Dec	96.07	66.74	33.27	17.28	385.33	119.52
9-Dec	94.21	77.01	37.39	14.18	370.89	98.05
10-Dec	96.75	77.41	34.21	12.76	343.09	84.17
11-Dec	100.12	64.93	39.00	14.94	370.68	89.94
12-Dec	100.48	51.61	35.91	12.61	398.61	86.80
13-Dec	134.81	86.01	48.94	26.98	408.95	122.92
17-Dec	125.78	99.82	30.48	15.92	227.51	131.49
18-Dec	56.27	70.73	30.49	21.57	312.66	83.01
19-Dec	72.57	107.72	22.76	12.94	245.21	156.70
20-Dec	111.19	99.24	26.44	36.51	132.32	215.24
22-Dec	100.60	161.75	14.44	7.64	275.79	298.53
24-Dec	198.63	148.98	38.30	17.40	323.41	294.96
26-Dec	201.21	102.97	47.94	12.60	262.19	283.94
28-Dec	259.52	200.98	132.90	53.64	268.40	316.72
30-Dec	338.85	161.01	131.80	15.70	333.47	497.27
1-Jan	397.91	246.78	200.59	46.11	411.03	534.99
3-Jan	613.02	336.85	183.97	55.58	397.47	525.27
5-Jan	735.70	350.79	226.92	9.79	342.39	562.49
7-Jan	849.87	423.26	282.03	32.15	362.81	515.20
9-Jan	938.22	356.87	346.77	439.75	377.44	500.41
11-Jan	930.16	379.41	448.60	61.61	389.91	548.54
13-Jan	891.77	432.32	540.99	215.02	408.07	514.69

Appendix G continued

Date	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
15-Jan	922.22	592.38	281.90	7.13	456.81	527.77
17-Jan	665.15	539.40	98.03	106.67	321.24	351.82
19-Jan	525.90	341.44	110.71	42.48	212.73	429.03
21-Jan	543.05	483.66	77.20	108.94	198.77	428.06
23-Jan	454.88	478.12	154.45	155.00	209.20	386.91
25-Jan	433.89	339.11	211.88	64.48	186.43	394.49
27-Jan	526.09	388.18	302.97	126.07	203.19	311.46
29-Jan	595.90	381.04	482.04	165.75	278.36	390.05
31-Jan	505.49	506.48	635.88	242.18	322.08	364.11
2-Feb	584.15	404.10	749.20	396.10	363.16	370.25
4-Feb	518.43	405.14	757.26	495.67	382.05	314.10
6-Feb	494.96	347.31	873.63	221.95	488.71	371.22
8-Feb	655.18	437.64	523.49	499.05	517.56	484.66
10-Feb	484.80	281.93	384.21	535.30	394.51	319.56
12-Feb	516.81	329.67	321.29	368.16	449.95	404.09
14-Feb	540.94	341.96	390.17	213.56	479.01	436.62
16-Feb	492.92	386.60	494.09	273.51	525.80	494.13
18-Feb	474.21	437.66	599.17	590.25	542.74	512.64
20-Feb	503.94	430.41	761.26	591.23	554.79	452.25
22-Feb	454.38	485.84	1233.3	867.77	539.04	428.62
24-Feb	477.26	524.22	1044.4	1066.6	523.95	444.10
26-Feb	446.37	491.81	1160.0		469.42	391.72
28-Feb	468.50	484.02	1261.4		502.56	417.32
4-Mar	621.00	545.42	1193.7	989.97	428.02	449.41
6-Mar	534.58	460.36	971.10	793.16	351.08	397.10
8-Mar	638.62	501.91	1103.2	824.76	330.82	422.12
10-Mar	605.60	473.50	1103.5	938.69	283.49	478.02
12-Mar	504.91	498.99	1097.1	1070.8	259.49	489.48
14-Mar	534.19	362.04	977.24	720.18	168.93	454.11
16-Mar	472.12	334.93	879.40	628.80	149.20	435.34
18-Mar	413.27	349.14	790.39	861.04	139.78	535.59
20-Mar	257.41	304.11	782.64	1069.5	105.23	635.10
28-Mar	129.85	222.61	579.79	524.70	81.57	462.43
31-Mar	53.67	143.19	568.71	351.56	72.97	481.54
2-Apr	92.48	159.57	538.67	597.84	94.52	477.86
4-Apr	156.52	189.05	555.16	674.67	62.60	474.61
7-Apr	143.82	87.03	399.70	456.02	127.43	570.12
9-Apr	127.72	100.23	397.46	466.46	124.42	206.96
11-Apr	124.82	61.11	421.3	544.3	113.87	372.32
17-Apr	198.23	116.46	508.5	586.7	187.08	404.59
25-Apr	130.11	77.02	802.53	1019.34	57.42	266.73
27-Apr	139.67	118.05	767.06	1075.16	138.35	276.34

Appendix G continued

Date	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
29-Apr	75.03	56.46	159.17	501.28	73.23	88.22
1-May	118.34	72.08	186.55	no peak	59.39	157.06
3-May	104.32	140.65	147.09	no peak	58.29	127.16
8-May	119.08	68.38	459.06	485.40	45.50	114.58
14-May	100.06	80.99	336.29	464.26	37.89	110.56
16-May	79.15	63.06	366.56	402.16	32.91	126.66
22-May	88.87	96.35	582.64	626.69	38.69	154.60
23-May	45.68	49.11	349.47	401.57	17.25	98.52
27-May	67.45	58.78	323.18	614.11	25.23	111.57
30-May	96.48	70.85	409.06	701.78	33.69	123.20
1-Jun	96.67	77.34	417.92	465.04	46.59	120.54
2-Jun	91.53	75.70	524.60	621.62	42.90	120.61
4-Jun	75.06	75.95	522.40	814.91	37.89	124.73
6-Jun	78.78	64.92	482.64	766.27	33.35	121.73
11-Jun	71.54	29.45	2.50	135.02	12.88	50.87
12-Jun	57.22	33.23	152.80	375.36	7.74	65.30
27-Jun	75.92	35.80	625.10	415.61	10.28	73.15
28-Jun	61.69	28.25	510.93	500.70	9.52	71.86
30-Jun	43.87	29.94	336.28	527.36	7.09	50.67
2-Jul	40.12	45.79	283.29	594.06	6.23	52.67
3-Jul	35.17	34.30	252.80	583.41	3.77	54.97
4-Jul	30.87	27.63	304.96	553.05	5.43	44.34
6-Jul	39.45	55.49	353.61	605.70	6.66	46.57
6-Aug	28.33	53.75	218.49	309.17	3.22	37.41
8-Aug	16.71	44.37	385.33	378.98	3.85	62.95
10-Aug	26.37	27.12	562.12	478.51	4.50	42.42
11-Aug	22.36	20.29	360.97	369.83	3.52	43.00
13-Aug	35.37	31.74	550.25	437.61	3.74	36.61
14-Aug	21.95	22.62	599.48	480.56	2.93	40.93
16-Aug	21.61	30.38	8.59	3.09	2.29	16.69
18-Aug	9.50	9.65	44.50	236.77	3.29	20.34
20-Aug	13.79	24.98	315.89	301.56	4.52	27.23
21-Aug	7.73	11.74	377.91	244.44	3.76	19.60
23-Aug	11.55	19.73	286.52	336.62	3.66	31.34
25-Aug	6.21	11.65	344.78	284.09	4.68	30.09
28-Aug	8.51	11.60	360.10	356.11	4.62	27.87
30-Aug	12.44	14.34	394.31	461.45	3.78	38.25
2-Sep	14.35	16.68	426.77	402.93	5.59	42.69
5-Sep	7.49	11.14	136.21	199.94	3.83	15.67
9-Sep	14.69	17.66	353.84	248.85	7.69	31.96
11-Sep	22.01	23.25	303.66	450.21	6.25	38.37
16-Sep	12.68	11.75	300.10	305.47	5.77	37.16

Appendix G continued

Date	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
19-Sep	20.87	13.95	477.5	366.45	6.01	46.32
22-Sep	20.95	19.16	543.17	442.97	8.12	38.08
24-Sep	22.58	29.00	539.37	448.58	8.57	42.75
26-Sep	12.93	10.55	486.45	409.57	5.22	48.41
29-Sep	12.63	12.73	578.31	407.31	5.02	46.42
2-Oct	11.11	8.44	465.49	263.43	3.16	30.91
5-Oct	19.65	13.62	389.43	244.27	4.22	26.95
8-Oct	22.12	16.4	439.56	250.47	4.43	29.79
12-Oct	10.46	7.5	517.84	247.43	3.88	27.96
15-Oct	13.84	20.75	479.42	255.53	6.06	25.75
18-Oct	922	ND	1018	514	495	3771
21-Oct			1615	28059		
24-Oct	1034	367	1076	290	608	4237
27-Oct			1535	24825		
29-Oct	339	746	865	27824	576	2654
2-Nov			2004	32280		
6-Nov	692	704	2485	35922	768	3488
10-Nov			23545	36983		
12-Nov	140	1678	11385	36582	1183	4026
16-Nov			24688	50038		
20-Nov	194	483	44842	41991	1033	3316
24-Nov			91939	67562		
26-Nov	424	498	94538	57164	1391	6141
30-Nov			87799	59020		
4-Dec	1471	385	92168	77822	1105	5271
7-Dec				114618		
9-Dec	764	713	70414	93072	631	4337
13-Dec	1286	908	83234	156922	1299	7594
17-Dec	7023	6038	140318	170603	3170	8149
21-Dec	5196	6279	89992	144260	2250	7580
23-Dec	2510	813	97703	158516	2339	8920
24-Dec	7581	6173	130883	169813	4466	11340
25-Dec	5096	2433	143808	191104	3939	12790
26-Dec	3773	1285	151437	213099	2789	10546
27-Dec	2133	1068	159240	210036	2214	7849
28-Dec	7725	9050	180164	228577	4818	12130

Measurements began on 11/18/96 and went through 12/28/97.

From 10/18/97 an Alumina PLOT column was used on the Hp 5890.

Previously a molecular sieve was used on the HP 5880.

Appendix H: Methane Concentration in the Headspace of the Sample Vials (% mole fraction)

Date	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
18-Nov	0.490	0.534	0.277	0.223	0.617	0.530
19-Nov	0.558	0.314	0.087	0.155	0.764	0.628
20-Nov	0.408	0.209			0.936	0.497
21-Nov	0.397	0.234	0.114	0.071	0.513	0.465
22-Nov	0.289	0.195	0.090	0.058	0.905	0.383
23-Nov	0.231	0.203	0.089	0.047	0.923	0.327
24-Nov	0.226	0.195	0.084	0.082	0.754	0.329
25-Nov	0.224	0.151	0.080	0.064	0.700	0.287
26-Nov	0.211	0.138	0.075	0.055	0.666	0.344
27-Nov	0.208	0.119	0.081	0.039	0.466	0.285
28-Nov	0.184	0.150	0.075	0.036	0.539	0.360
29-Nov	0.146	0.123	0.063	0.033	0.503	0.423
30-Nov	0.165	0.109	0.051	0.039	0.514	0.509
1-Dec	0.266	0.102	0.180	0.039	0.458	0.314
2-Dec	0.172	0.120	0.078	0.048	0.663	0.345
3-Dec	0.169	0.165	0.072	0.022	0.638	0.316
4-Dec	0.161	0.099	0.088	0.031	0.690	0.278
5-Dec	0.151	0.080	0.101	0.022	0.737	0.217
6-Dec	0.200	0.132	0.075	0.031	0.667	0.201
7-Dec	0.155	0.096	0.057	0.026	0.683	0.221
8-Dec	0.185	0.130	0.066	0.036	0.735	0.230
9-Dec	0.182	0.149	0.074	0.030	0.708	0.189
10-Dec	0.187	0.150	0.068	0.027	0.655	0.163
11-Dec	0.193	0.126	0.077	0.031	0.707	0.174
12-Dec	0.194	0.101	0.071	0.027	0.760	0.168
13-Dec	0.259	0.166	0.096	0.054	0.780	0.236
17-Dec	0.194	0.142	0.003		0.397	0.205
18-Dec	0.055	0.083	0.003		0.567	0.108
19-Dec	0.087	0.157			0.432	0.255
20-Dec	0.164	0.140		0.015	0.207	0.372
22-Dec	0.143	0.266			0.494	0.539
24-Dec	0.339	0.240	0.019		0.589	0.532
26-Dec	0.344	0.148	0.038		0.466	0.510
28-Dec	0.461	0.344	0.208	0.049	0.479	0.575
30-Dec	0.620	0.264	0.206		0.609	0.937
1-Jan	0.738	0.436	0.343	0.034	0.764	1.012
3-Jan	1.168	0.616	0.310	0.053	0.737	0.993
5-Jan	1.413	0.644	0.396		0.627	1.067
7-Jan	1.642	0.789	0.506	0.006	0.668	0.972
9-Jan	1.818	0.656	0.636	0.822	0.697	0.943
11-Jan	1.802	0.701	0.839	0.065	0.722	1.039
13-Jan	1.726	0.807	1.024	0.372	0.758	0.971

Appendix H continued

Date	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
15-Jan	1.786	1.127	0.506		0.856	0.998
17-Jan	1.272	1.021	0.138	0.155	0.584	0.646
19-Jan	0.995	0.644	0.206	0.076	0.400	0.811
21-Jan	1.027	0.914	0.142	0.202	0.373	0.809
23-Jan	0.860	0.904	0.289	0.290	0.393	0.731
25-Jan	0.820	0.640	0.399	0.119	0.350	0.746
27-Jan	0.996	0.734	0.572	0.236	0.382	0.588
29-Jan	1.128	0.720	0.912	0.311	0.525	0.737
31-Jan	0.956	0.958	1.204	0.456	0.608	0.688
2-Feb	1.106	0.764	1.419	0.749	0.686	0.699
4-Feb	1.030	0.804	1.508	0.985	0.757	0.622
6-Feb	0.983	0.688	1.741	0.437	0.971	0.736
8-Feb	1.304	0.869	1.040	0.991	1.028	0.963
10-Feb	0.919	0.533	0.727	1.014	0.747	0.605
12-Feb	0.979	0.624	0.608	0.697	0.852	0.765
14-Feb	1.025	0.647	0.739	0.403	0.908	0.827
16-Feb	0.934	0.732	0.936	0.517	0.996	0.936
18-Feb	0.898	0.829	1.136	1.119	1.029	0.971
20-Feb	0.955	0.815	1.444	1.121	1.052	0.857
22-Feb	0.861	0.920	2.341	1.646	1.022	0.812
24-Feb	0.904	0.993	1.982	2.024	0.993	0.841
26-Feb	0.804	0.886	2.088		0.845	0.705
28-Feb	0.844	0.872	2.271		0.905	0.752
4-Mar	1.118	0.982	2.149	1.782	0.771	0.809
6-Mar	1.070	0.922	1.943	1.587	0.703	0.795
8-Mar	1.278	1.005	2.207	1.650	0.662	0.845
10-Mar	1.212	0.948	2.208	1.878	0.568	0.957
12-Mar	1.011	0.999	2.195	2.142	0.520	0.980
14-Mar	1.068	0.724	1.954	1.440	0.338	0.908
16-Mar	0.944	0.670	1.759	1.257	0.298	0.870
18-Mar	0.826	0.698	1.581	1.722	0.279	1.071
20-Mar	0.515	0.608	1.565	2.139	0.210	1.270
28-Mar	0.259	0.445	1.159	1.049	0.163	0.925
31-Mar	0.107	0.286	1.137	0.703	0.146	0.963
2-Apr	0.185	0.319	1.077	1.195	0.189	0.955
4-Apr	0.313	0.378	1.110	1.349	0.125	0.949
7-Apr	0.287	0.174	0.799	0.912	0.255	1.140
9-Apr	0.255	0.200	0.795	0.933	0.249	0.414
11-Apr	0.249	0.122	0.842	1.088	0.227	0.744
17-Apr	0.396	0.233	1.017	1.173	0.374	0.809
25-Apr	0.307	0.185	1.853	2.352	0.140	0.621
27-Apr	0.329	0.279	1.772	2.480	0.326	0.643

Appendix H continued

Date	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
29-Apr	0.167	0.126	0.352	1.105	0.163	0.196
1-May	0.262	0.160	0.412		0.133	0.347
3-May	0.231	0.311	0.325		0.130	0.282
8-May	0.264	0.152	1.012	1.070	0.102	0.254
14-May	0.203	0.165	0.676	0.932	0.079	0.224
16-May	0.162	0.129	0.736	0.808	0.069	0.257
22-May	0.164	0.178	1.102	1.186	0.069	0.289
23-May	0.108	0.115	0.716	0.820	0.051	0.214
27-May	0.152	0.134	0.663	1.245	0.067	0.240
30-May	0.210	0.159	0.835	1.420	0.084	0.263
1-Jun	0.210	0.171	0.853	0.947	0.110	0.258
2-Jun	0.200	0.168	1.066	1.260	0.103	0.258
4-Jun	0.167	0.169	1.062	1.647	0.093	0.266
6-Jun	0.174	0.147	0.982	1.549	0.084	0.260
11-Jun	0.138	0.058	0.007	0.258	0.026	0.099
12-Jun	0.111	0.065	0.292	0.715	0.017	0.126
27-Jun	0.146	0.070	1.190	0.792	0.021	0.141
28-Jun	0.119	0.056	0.973	0.953	0.020	0.138
30-Jun	0.085	0.059	0.641	1.004	0.015	0.098
2-Jul	0.078	0.089	0.540	1.131	0.014	0.102
3-Jul	0.069	0.067	0.482	1.110	0.009	0.106
4-Jul	0.061	0.054	0.581	1.053	0.012	0.086
6-Jul	0.077	0.107	0.674	1.153	0.015	0.090
6-Aug	0.056	0.109	0.455	0.645	0.003	0.075
8-Aug	0.031	0.089	0.805	0.792	0.004	0.128
10-Aug	0.051	0.053	1.177	1.001	0.006	0.085
11-Aug	0.043	0.039	0.754	0.773	0.003	0.086
13-Aug	0.070	0.063	1.152	0.915	0.004	0.073
14-Aug	0.042	0.044	1.255	1.005	0.002	0.082
16-Aug	0.042	0.061	0.013	0.001	-0.001	0.031
18-Aug	0.015	0.016	0.092	0.515	0.002	0.039
20-Aug	0.025	0.049	0.689	0.658	0.004	0.054
21-Aug	0.011	0.020	0.826	0.532	0.003	0.037
23-Aug	0.020	0.038	0.625	0.735	0.002	0.063
25-Aug	0.008	0.020	0.753	0.619	0.005	0.060
28-Aug	0.013	0.020	0.787	0.778	0.004	0.056
30-Aug	0.022	0.026	0.862	1.009	0.003	0.078
2-Sep	0.026	0.031	0.933	0.881	0.007	0.088
5-Sep	0.021	0.028	0.278	0.405	0.013	0.037
9-Sep	0.035	0.041	0.713	0.503	0.021	0.070
11-Sep	0.047	0.049	0.666	0.989	0.012	0.083
16-Sep	0.026	0.024	0.658	0.670	0.011	0.080

Appendix H continued

Date	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
19-Sep	0.044	0.029	1.049	0.804	0.011	0.100
22-Sep	0.044	0.040	1.193	0.973	0.016	0.082
24-Sep	0.048	0.062	1.185	0.985	0.017	0.092
26-Sep	0.027	0.021	1.068	0.899	0.010	0.105
29-Sep	0.026	0.026	1.270	0.894	0.009	0.100
2-Oct	0.014	0.009	0.878	0.494	-0.001	0.052
5-Oct	0.030	0.019	0.733	0.457	0.001	0.044
8-Oct	0.035	0.024	0.828	0.469	0.002	0.050
12-Oct	0.013	0.007	0.977	0.463	0.0005	0.046
15-Oct	0.019	0.033	0.904	0.479	0.005	0.042
18-Oct	0.006		0.007	0.003	0.003	0.025
21-Oct			0.011	0.177		
24-Oct	0.007	0.002	0.007	0.002	0.004	0.028
27-Oct			0.010	0.158		
29-Oct	0.002	0.005	0.006	0.176	0.004	0.017
2-Nov			0.013	0.203		
6-Nov	0.005	0.005	0.016	0.225	0.005	0.023
10-Nov			0.150	0.231		
12-Nov	0.001	0.011	0.073	0.229	0.008	0.026
16-Nov			0.157	0.308		
20-Nov	0.001	0.003	0.278	0.261	0.007	0.022
24-Nov			0.537	0.407		
26-Nov	0.003	0.003	0.551	0.349	0.009	0.040
30-Nov			0.516	0.359		
4-Dec	0.010	0.003	0.538	0.463	0.007	0.034
7-Dec			0.000	0.650		
9-Dec	0.005	0.005	0.423	0.543	0.004	0.028
13-Dec	0.004	0.003	0.492	0.545	0.005	0.026
17-Dec	0.024	0.021	0.487	0.592	0.011	0.028
21-Dec	0.018	0.022	0.312	0.501	0.008	0.026
23-Dec	0.009	0.003	0.339	0.550	0.008	0.031
24-Dec	0.026	0.021	0.454	0.590	0.016	0.039
25-Dec	0.018	0.008	0.499	0.664	0.014	0.044
26-Dec	0.013	0.004	0.526	0.740	0.010	0.037
27-Dec	0.007	0.004	0.553	0.729	0.008	0.027
28-Dec	0.027	0.031	0.626	0.794	0.017	0.042

Concentrations are from linear calbration curves of standards over the measured range with correlation coefficients above 0.98.

From 10/18/97 an Alumina PLOT column was used on the Hp 5890.

Previously a molecular sieve was used on the HP 5880

Appendix I: Flow Rate of the Effluents from the Columns (ml/day)

Date	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
24-Feb	4822	4241	3028		4013	4422
26-Feb	8432	4528	2447		5087	2163
28-Feb	7463	4385	1703		3499	4690
4-Mar	4701	4815	4285	4721	5087	4422
6-Mar	5069	5102	4425	4687	4786	4555
8-Mar	6131	2663	4935	4891	5967	4488
10-Mar	4349	2806	5011	4926	5884	957
12-Mar	4582	5102	3751	4414	4860	4555
14-Mar	3798	5963	1703	4550	5477	4555
16-Mar	5196	2806	4285	4857	4713	
18-Mar	4235	4385	4713	4857	4786	4488
20-Mar	4349	6394	5087	4857	3378	4622
28-Mar	5324	5246	4860	4857	4567	4422
31-Mar	5324	5533	4285	4482	5477	4422
2-Apr	4013	3811	4935	3936	4496	4224
4-Apr	4349	4815	5241	5062	4860	4422
7-Apr	4582	4528	4567	4414	4713	4422
9-Apr	3798	4241	4713	4379	4013	4159
11-Apr	4582	4241	4713	3697	3378	4159
17-Apr	4701	4528	4425	4516	4080	4093
25-Apr	4822	4385	4640	4141	3881	4757
27-Apr	4822	5389	6051	3390	6480	4422
29-Apr	4013	5102	5319	2195	4147	4757
1-May	4013	4098	4355	3322	4425	3397
3-May	3693	3667	4285	3765	4355	4029
8-May	4349	5820	4786	3151	5164	3772
14-May	3693	4385	4216	4277	3318	4356
16-May	6271	5389	6835	4311	2754	4628
22-May	4582	5102	4080	3731	4786	4029
23-May	4123	4385	4285	3799	4496	4690
27-May	5069	4672	4013	3492	3815	4093
30-May	4349	4098	4786	4175	4285	3900
1-Jun	3904	4241	4496	4311	4355	4422
2-Jun	4013	4241	4496	4141	4425	2331
4-Jun	3904	4385	4567	3936	4640	4093
6-Jun	4945	4385	4425	3799	4355	3772
11-Jun	5196	4241	3946	3868	5011	1567
12-Jun	5196	5102	5638	3868	4496	2331
27-Jun	4013	4241	4567	4414	4425	1997
28-Jun	4945	4385	3438	4414	3318	3458
30-Jun	4701	4528	2862	4141	3561	2107
2-Jul	4349	4241	4496	4652	4285	2852

Appendix I continued

Date	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
3-Jul	4945	4241	4355	4789	4355	3152
4-Jul	4235	3811	4425	4823	5638	4422
6-Jul	4945	4528	4640	4414	4640	3152
6-Aug	4147	4363	4090	4507	4406	4190
8-Aug	4147	4363	4090	4507	4406	4190
10-Aug	4147	4363	4090	4507	4406	4190
11-Aug	4147	4363	4090	4507	4406	4190
13-Aug	4147	4363	4090	4507	4406	4190
14-Aug	4147	4363	4090	4507	4406	4190
16-Aug	4147	4363	4090	4507	4406	4190
18-Aug	4147	4363	4090	4507	4406	4190
20-Aug	4147	4363	4090	4507	4406	4190
21-Aug	4147	4363	4090	4507	4406	4190
23-Aug	4147	4363	4090	4507	4406	4190
25-Aug	4147	4363	4090	4507	4406	4190
28-Aug	4147	4363	4090	4507	4406	4190
30-Aug	4147	4363	4090	4507	4406	4190
2-Sep	4147	4363	4090	4507	4406	4190
5-Sep	4147	4363	4090	4507	4406	4190
9-Sep	4147	4363	4090	4507	4406	4190
11-Sep	4147	4363	4090	4507	4406	4190
16-Sep	4147	4363	4090	4507	4406	4190
19-Sep	4147	4363	4090	4507	4406	4190
22-Sep	4147	4363	4090	4507	4406	4190
24-Sep	4147	4363	4090	4507	4406	4190
26-Sep	4147	4363	4090	4507	4406	4190
29-Sep	4147	4363	4090	4507	4406	4190
2-Oct	4147	3499	4090	4507	4406	4190
5-Oct	4147	3499	4090	4507	4406	4190
8-Oct	4147	3787	4090	4507	4406	4190
12-Oct	4147	3931	4090	4507	4406	4190
15-Oct	4147	4075	4090	4507	4406	4190
18-Oct	4147	4219	4090	4507	4406	4190
21-Oct			4090	4507		
24-Oct	4147	4363	4090	4507	4406	4190
27-Oct			4090	4507		
29-Oct	4147	4522	4090	4450	4406	4190
2-Nov			4090	4450		
6-Nov	4147	4248	4090	4450	4406	4190
10-Nov			4090	4450		
12-Nov	4147	4248	4090	4450	4406	4190
16-Nov			4090	4450		

Appendix I continued

Date	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
20-Nov	4147	4248	4090	4450	4406	4190
24-Nov			4090	4450		
26-Nov	4147	4248	4090	4450	4406	4190
30-Nov			4090	4450		
4-Dec	4147	3672	4090	4450	4406	4190
7-Dec			4090	4450		
9-Dec	4147	3672	4090	4450	4406	4190
13-Dec	4147	3672	4090	4450	4406	4190
17-Dec	4147	3672	4090	4450	4406	4190
21-Dec	4147	3672	4090	3931	4406	4190
23-Dec	4147	3672	4090	3931	4406	4190
24-Dec	3643	3816	4090	3931	4406	4190
25-Dec	3643	3816	4090	3931	4406	4190
26-Dec	3643	3816	4090	3931	4406	4190
27-Dec	3643	3816	4090	3931	4406	4190
28-Dec	3643	3816	4090	3931	4406	4190

Flow rates from 11/18/96 to 7/6/97 were calculated from rotameter measurements.

Flow rates from 8/6/97 until 12/28/97 were calculated from pump flow rates minus any measurable leaks.

Appendix J: Methane Removal Rate Through the Effluents (μmol/day)

Date	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
24-Feb	5.70	5.51	7.84		5.21	4.86
26-Feb	8.86	5.24	6.68		5.62	1.99
28-Feb	8.23	4.99	5.06		4.14	4.61
4-Mar	6.87	6.18	12.03	11.00	5.12	4.68
6-Mar	6.96	6.03	11.03	9.54	4.32	4.65
8-Mar	10.72	3.66	14.90	11.05	5.41	5.19
10-Mar	7.25	3.66	15.23	12.73	4.60	1.26
12-Mar	5.90	6.49	10.49	12.04	3.22	5.68
14-Mar	5.17	5.50	4.24	8.35	2.36	5.27
16-Mar	6.25	2.39	9.60	7.78	1.79	
18-Mar	4.46	3.90	9.49	10.65	1.70	6.12
20-Mar	2.85	4.95	10.14	13.23	0.90	7.48
28-Mar	1.76	2.98	7.19	6.50	0.95	5.22
31-Mar	0.73	2.02	6.21	4.01	1.02	5.42
2-Apr	0.94	1.54	6.75	5.97	1.08	5.12
4-Apr	1.74	2.33	7.45	8.75	0.78	5.37
7-Apr	1.71	1.02	4.73	5.22	1.56	6.54
9-Apr	1.24	1.09	4.80	5.23	1.28	2.20
11-Apr	1.45	0.66	5.06	5.12	0.98	3.94
17-Apr	2.36	1.34	5.71	6.72	1.94	4.20
25-Apr	1.88	1.03	10.95	12.40	0.69	3.76
27-Apr	2.03	1.93	13.75	10.79	2.71	3.65
29-Apr	0.84	0.81	2.36	3.06	0.85	1.17
1-May	1.33	0.83	2.26		0.74	1.49
3-May	1.08	1.44	1.76		0.71	1.43
8-May	1.45	1.12	6.10	4.25	0.66	1.21
14-May	0.95	0.91	3.59	5.02	0.33	1.23
16-May	1.28	0.88	6.34	4.39	0.24	1.50
22-May	0.95	1.15	5.67	5.57	0.41	1.47
23-May	0.56	0.64	3.86	3.92	0.29	1.26
27-May	0.97	0.79	3.35	5.48	0.32	1.24
30-May	1.15	0.82	5.03	7.47	0.45	1.29
1-Jun	1.03	0.92	4.83	5.14	0.60	1.44
2-Jun	1.01	0.90	6.04	6.57	0.57	0.76
4-Jun	0.82	0.93	6.11	8.16	0.54	1.37
6-Jun	1.09	0.81	5.47	7.42	0.46	1.24
11-Jun	0.90	0.31	0.03	1.26	0.17	0.19
12-Jun	0.72	0.42	2.08	3.48	0.09	0.37
27-Jun	0.74	0.37	6.85	4.40	0.12	0.35
28-Jun	0.74	0.31	4.21	5.30	0.08	0.60
30-Jun	0.50	0.34	2.31	5.24	0.07	0.26
2-Jul	0.43	0.48	3.06	6.63	0.07	0.37

Appendix J continued

Date	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
3-Jul	0.43	0.36	2.65	6.70	0.05	0.42
4-Jul	0.32	0.26	3.24	6.40	0.09	0.48
6-Jul	0.48	0.61	3.94	6.41	0.09	0.36
6-Aug	0.29	0.60	2.34	3.66	0.02	0.39
8-Aug	0.16	0.49	4.15	4.50	0.02	0.68
10-Aug	0.27	0.29	6.06	5.68	0.03	0.45
11-Aug	0.22	0.21	3.89	4.39	0.02	0.46
13-Aug	0.37	0.34	5.93	5.20	0.02	0.39
14-Aug	0.22	0.24	6.47	5.71	0.01	0.43
16-Aug	0.22	0.34	0.07	0.01	0.00	0.16
18-Aug	0.08	0.09	0.48	2.93	0.01	0.21
20-Aug	0.13	0.27	3.55	3.73	0.02	0.29
21-Aug	0.06	0.11	4.25	3.02	0.01	0.20
23-Aug	0.10	0.21	3.22	4.17	0.01	0.33
25-Aug	0.04	0.11	3.88	3.52	0.03	0.32
28-Aug	0.07	0.11	4.05	4.42	0.02	0.29
30-Aug	0.11	0.14	4.44	5.73	0.01	0.41
2-Sep	0.14	0.17	4.86	5.06	0.04	0.47
5-Sep	0.11	0.15	1.45	2.33	0.07	0.20
9-Sep	0.18	0.23	3.72	2.89	0.12	0.37
11-Sep	0.25	0.27	3.47	5.67	0.07	0.44
16-Sep	0.14	0.13	3.43	3.85	0.06	0.43
19-Sep	0.23	0.16	5.46	4.62	0.06	0.53
22-Sep	0.23	0.22	6.21	5.58	0.09	0.44
24-Sep	0.25	0.34	6.17	5.65	0.10	0.49
26-Sep	0.14	0.12	5.56	5.16	0.05	0.56
29-Sep	0.14	0.15	6.62	5.13	0.05	0.53
2-Oct	0.08	0.04	4.57	2.83	-0.01	0.28
5-Oct	0.16	0.08	3.82	2.62	0.01	0.24
8-Oct	0.19	0.12	4.31	2.69	0.01	0.27
12-Oct	0.07	0.04	5.09	2.66	0.00	0.25
15-Oct	0.10	0.17	4.71	2.75	0.03	0.22
18-Oct	0.03		0.03	0.02	0.02	0.13
21-Oct			0.05	1.02		
24-Oct	0.04	0.01	0.04	0.01	0.02	0.15
27-Oct			0.05	0.90		
29-Oct	0.01	0.03	0.03	1.00	0.02	0.09
2-Nov			0.07	1.15		
6-Nov	0.02	0.02	0.08	1.27	0.03	0.12
10-Nov			0.78	1.31		
12-Nov	0.005	0.06	0.38	1.30	0.04	0.14
16-Nov			0.82	1.75		

Appendix J continued

Date	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
20-Nov	0.01	0.02	1.45	1.48	0.04	0.12
24-Nov			2.80	2.31		
26-Nov	0.01	0.02	2.87	1.98	0.05	0.21
30-Nov			2.69	2.04		
4-Dec	0.05	0.01	2.80	2.62	0.04	0.18
7-Dec				3.69		
9-Dec	0.03	0.02	2.20	3.08	0.02	0.15
13-Dec	0.02	0.01	2.56	3.09	0.03	0.14
17-Dec	0.13	0.10	2.54	3.36	0.06	0.15
21-Dec	0.10	0.10	1.63	2.51	0.04	0.14
23-Dec	0.05	0.01	1.77	2.76	0.05	0.17
24-Dec	0.12	0.10	2.37	2.95	0.09	0.21
25-Dec	0.08	0.04	2.60	3.32	0.08	0.24
26-Dec	0.06	0.02	2.74	3.71	0.05	0.20
27-Dec	0.03	0.02	2.88	3.65	0.04	0.15
28-Dec	0.12	0.15	3.26	3.97	0.09	0.22

The effluent removal rates are the flow rates multiplied by liquid phase sample concentrations calculated from the sample headspace concentrations with Henry’s Law.

Appendix K: Propionate Concentration Measured in the Effluent of Column 3

Time hours	[Propionate] mg/L	[Propionate] mmol/L
0.9	700	9.46
1.8	512	6.92
2.4	516	6.98
3.0	459	6.20
3.6	380	5.14
4.3	255	3.45
4.8	159	2.15
5.4	143	1.93
6.5	617	8.34
7.0	828	11.19
7.6	1204	16.27
8.3	1210	16.35
8.9	1281	17.31

The time is measured from the injection of the sodium propionate solution into the column.

Appendix L: Sulfate Concentration Measured in the Effluents of the Columns

Time (hrs)	Column 1	Column 2	Column 3	Column 4	Column 5	Column 6
0	8.73	8.60	0.44	1.30	9.58	7.91
1	7.87					
2	7.44					
3	7.42		0.44	1.12	9.59	
4	6.85		0.62		9.30	
5	6.81	7.52	0.44	0.73	9.46	7.81
6	6.49	7.76	0.52	0.68	9.51	8.16
7	6.33	7.85	0.98	0.44	9.80	13.01
8	6.50	7.86	0.91	0.79	10.09	8.56
9	6.34	8.17	1.00	1.42	10.07	8.93
10	6.54	8.46	1.09	1.75	10.19	8.79
11	6.62	8.60	1.03	1.90	10.61	9.19
12	7.02	8.85	1.20	2.02	10.65	9.58
13	7.15	8.93	1.05	1.58	10.65	9.13

Values represent peak areas from the ion chromatograph in the millions.

Appendix M: PCE Profile in the Manifold as a Function of Time

Time from Stability hours	PCE Sol'n Flowrate mL/min	Total Flowrate mL/min	Peak Area	[PCE] Manifold µmol/L
28.5	0.59		6853827	19.44
51.25	0.61	21.36	6677773	18.94
71.92	0.61	25.67	7908829	22.43
95.75	0.59	23.5	8583069	24.34
118.25	0.66	22.9	8568307	24.30
153.42	0.66	22.66	6737277	19.11
189.25	0.64	23.52	9021786	25.59
213.25	0.61	22.8	9092819	25.79
248.42	0.60	23	11257776	31.93
285.17	0.61	23	6202493	21.34
358.33	0.65	22.8	7871005	27.08
442.33	0.61	22.8	5436490	18.70
501.67	0.60	23.6	8523526	29.32
573.17	0.60	23.9	7726845	26.58
649.67	0.59	22.5	9295168	31.98
745.75	0.62	22.8	9299002	31.99
841.82	0.60	22.9	8740832	30.07
938.57	0.60	22.9	7089744	24.39
1033.57	0.59	22.5	8452691	29.08
1129.92	0.60	22.5	8395366	28.88
1225.75	0.60	22.3	11289328	34.17
1274.17	0.60	22.3	11736744	35.53
1371.33	0.60	23.9	8989734	27.21
1465.58	0.58	22.6	8531654	25.83
1562.75	0.71	22.6	10465504	31.68
1610.92	0.61	22.6	10753856	32.55
1707.58	0.60	22.7	10252984	31.04
1802.17	0.61	21.7	9687315	29.32
1875.08	0.64	21.6	11766280	35.62
1922.67	0.60	22.5	10394960	31.47
2016.92	0.60	22.9	10899408	31.46
2114.33	0.60	22.5	12140816	35.04
2211.50	0.60	22.5	9525203	27.49
2257.25	0.58	22.3	10386664	29.98
2271.08		22	1106891	3.35
2282.83		22.5	609417	1.84
2294.33		22.9	398892	1.21
2307.33		22.9	355935	1.08
2319.08		25.1	293118	0.89
2342.58		22.9	374255	1.13

Appendix N: Chloroethene Profile in Column 1 as a Function of Time

Time (from stability) hours	PCE Peak Area	[PCE] Column 1 µmol/L	TCE Peak Area	[TCE] Column 1 µmol/L
28.5	148910	0.47	3141	0.04
51.25	575892	1.63	5903	0.07
71.92	1531887	4.34	8433	0.11
95.75	2168797	6.15	8868	0.11
118.25	3763722	10.67	16927	0.21
153.42	4723978	13.40	24070	0.30
189.25	6270781	17.78	24019	0.30
213.25	6144125	17.42	25222	0.31
248.42	6718250	19.05	26706	0.33
285.17	Pentane evaporation prior to analysis			
358.33	7512224	25.84	22498	0.34
442.33	6547987	22.53	64739	0.99
501.67	7656189	26.34	46757	0.71
573.17	6716512	23.10	152547	2.33
649.67	5994730	20.62	151306	2.31
745.75	7045290	24.24	129419	1.97
841.82	6758608	23.25	116954	1.78
938.57	4437498	15.26	139370	2.13
1033.57	4927226	16.95	134866	2.06
1129.92	5938173	20.43	115435	1.76
1225.75	7507235	22.72	82765	1.08
1274.17	8477754	25.66	161294	2.11
1420.08	6132474	18.56	136702	1.79
1465.58	7633648	23.11	65516	0.86
1562.75	6405696	19.39	160685	2.10
1610.92	6532221	19.77	65244	0.85
1707.58	7745245	23.44	47024	0.61
1802.17	6951251	21.04	47757	0.62
1875.08	10057368	30.44	89908	1.17
1922.67	9123584	27.62	57572	0.75
2016.92	9008736	26.00	52210	0.66
2114.33	7917341	22.85	114147	1.45
2211.50	8060176	23.26	136914	1.74
2257.25	9278106	26.78	62977	0.80
2271.08	8184979	21.97	209022	2.44
2282.83	8075450	21.67	120133	1.40
2307.33	9011962	24.19	75882	0.89
2330.67	8209984	22.04	53420	0.62
2354.25	7905936	21.22	44420	0.52
2367.33	5994608	16.09	131166	1.53
2379.25	5288483	14.19	49695	0.58

Appendix N continued

Time (from stability) hours	[VC] Peak Area	[VC] µmol/L	[C2H4] Peak Area	[C2H4] µmol/L	[C2H6] Peak Area	[C2H6] µmol/L
673.42			265	0.33		
830.17			359	0.44		
951.25					28	0.03
1130.42			72	0.09	44	0.05
1274.92					21	0.02
1466.17			265	0.33	62	0.07
1611.50			398	0.49		
1802.50			1447	1.79	27	0.03
1922.92			1196	1.48		
2017.33			3832	0.24		
2114.67	1639	1.76	9581	5.97		
2211.92	1514	1.62	10750	6.70		
2257.5	2210	2.40	4273	2.66		
2271.33	2157	2.34	13558	8.45		
2283.25	1546	1.65	16271	10.14		
2307.67	5206	5.74	6850	4.27		
2331.58	1924	2.08	4683	2.92		
2354.58	2235	2.42	2909	1.81		
2379.5	1132	1.19	7569	4.71		

All concentrations are calculated from linear calibration curves of standard concentration versus peak area. The correlation coefficients are all above 0.95. This is method is used for all gas chromatographic analyses in the appendices.

Appendix O: Chloroethene Profile in Column 2 as a Function of Time

Time from Stability hours	PCE Peak Area	[PCE] µmol/L	TCE Peak Area	[TCE] µmol/L
28.5	49093	0.15	3060	0.04
51.25	259396	0.74	8762	0.11
71.92	Pentane evaporation prior to analysis			
95.75	2014417	5.71	20993	0.26
118.25	3382446	9.59	30972	0.39
153.42	4673130	13.25	25227	0.31
189.25	Pentane evaporation prior to analysis			
213.25	6340045	17.98	43123	0.54
248.42	7659795	21.72	50145	0.63
285.17	5070000	17.44	47132	0.72
358.33	6835424	23.51	38196	0.58
442.33	6605024	22.72	88869	1.36
501.67	6498365	22.35	60358	0.92
573.17	7353571	25.30	155734	2.37
649.67	6533683	22.48	185867	2.83
745.75	7676096	26.41	176633	2.69
841.82	5096394	17.53	107414	1.64
938.57	5758115	19.81	206113	3.14
1033.57	5838490	20.08	198032	3.02
1129.92	6528093	22.46	182155	2.78
1225.75	8889312	26.91	151975	1.98
1274.17	7602834	23.01	219291	2.86
1371.33	6797517	20.58	95478	1.25
1465.58	8223683	24.89	88046	1.15
1562.75	7422973	22.47	238720	3.12
1610.92	7295251	22.08	91923	1.20
1707.58	10834544	32.80	162189	2.12
1802.17	9343110	28.28	88183	1.15
1875.08	11156600	33.77	144644	1.89
1922.67	8607789	26.06	62146	0.81
2016.92	10348808	29.87	63958	0.81
2114.33	8340787	24.07	140035	1.78
2211.50	9507123	27.44	180309	2.30
2257.25	10249840	29.58	81243	1.03
2271.08	8733971	23.44	267015	3.12
2282.83	9527680	25.57	189027	2.21
2307.33	10085584	27.07	105151	1.23
2330.67	9933114	26.66	62105	0.73
2354.25	8949594	24.02	36410	0.43
2367.33	7430704	19.94	208914	2.44
2379.25	7386618	19.83	78747	0.92

Appendix O continued

Time from Stability hours	[VC] Peak Area	[VC] µmol/L	[C2H4] Peak Area	[C2H4] µmol/L	[C2H6] Peak Area	[C2H6] µmol/L
673.42			580	0.72		
830.17			165	0.20	27	0.03
951.25			91	0.11		
1130.42			503	0.62		
1274.92			1274	1.57		
1466.17			532	0.66	31	0.04
1611.50			1313	1.62		
1802.50			807	1.00		
1922.92			967	1.20	47	0.05
2017.33	1939	2.09	1949	1.21		
2114.67	663	0.67	5002	3.12		
2211.92	1902	2.05	4944	3.08		
2257.5	1703	1.83	1912	1.19		
2271.33	828	0.85	15284	9.52		
2283.25	10766	11.95	3747	2.33		
2307.67	585	0.58	2650	1.65		
2331.58	532	0.52	2095	1.31		
2354.58	2675	2.91	1984	1.24		
2379.5	1161	1.22	5087	3.17		

Appendix P: Chloroethene Profile in Column 3 as a Function of Time

Time from Stability hours	PCE Peak Area	[PCE] mmol/L	TCE Peak Area	[TCE] mmol/L
28.5	38980	0.12	19813	0.27
51.25	70854	0.20	61107	0.76
71.92	117757	0.33	113484	1.42
95.75	Pentane evaporation prior to analysis			0.00
118.25	26231	0.07	59804	0.75
153.42	358190	1.02	159211	1.99
189.25	501643	1.42	205076	2.56
213.25	56616	0.16	19530	0.24
248.42	40182	0.11	5321	0.07
285.17	26861	0.09	679	0.01
358.33	30174	0.10	1109	0.02
442.33	29637	0.10	986	0.02
501.67	21473	0.07	424	0.01
573.17	22903	0.08	488	0.01
649.67	22621	0.08	446	0.01
745.75	23437	0.08	510	0.01
841.82	23188	0.08	771	0.01
938.57	22264	0.08	391	0.01
1033.57	21082	0.07	391	0.01
1129.92	20467	0.07	1726	0.03
1225.75	960	0.00	5287	0.07
1274.17	2460	0.01	2940	0.04
1371.33	1159	0.00	3074	0.04
1465.58	966	0.00	252	0.00
1562.75	3371	0.01	23685	0.31
1610.92	913	0.00	277	0.00
1707.58	19569	0.06	21973	0.29
1802.17	624216	1.89	139797	1.83
1875.08	164384	0.50	82807	1.08
1922.67	421211	1.28	126377	1.65
2016.92	184653	0.53	24196	0.31
2114.33	181334	0.52	42347	0.54
2211.50	217256	0.63	126584	1.61
2257.25	153491	0.44	14994	0.19

Appendix P continued

Time from Stability hours	[VC] Peak Area	[VC] mmol/L	[C2H4] Peak Area	[C2H4] mmol/L	[C2H6] Peak Area	[C2H6] mmol/L
673.42			trace			
758.42			trace			
830.17			trace			
901.42			236	0.29		
951.25						
1034.92			67	0.08		
1130.42			30	0.04		
1226.25			312	0.39		
1274.92			55	0.07		
1371.92			220	0.27		
1466.17			2110	2.61		
1563.17			8403	10.34		
1611.50			13661	16.75		
1706.83			11474	14.09		
1802.50			10744	13.20		
1875.42			12450	15.27	trace	
1922.92			16350	20.01	7	0.01
2017.33			18967	23.17	604	0.68
2114.67	4329	4.76	33115	20.63		
2211.92	3685	4.04	20830	12.98		
2257.5	814	0.84	38094	23.73		
2271.33	1028	1.08	37599	23.42		
2283.25	1220	1.29	37166	23.15		
2307.67	1117	1.18	24310	15.14		
2331.58	2155	2.33	13661	8.51		
2354.58	534	0.52	7701	4.80		
2379.5	1446	1.54	5637	3.51		

Appendix Q: Chloroethene Profile in Column 4 as a Function of Time

Time from Stability hours	PCE Peak Area	[PCE] mmol/L	TCE Peak Area	[TCE] mmol/L
28.5	Pentane evaporation prior to analysis			
51.25	70854	0.20	87437	1.09
71.92	496962	1.41	192617	2.40
95.75	Pentane evaporation prior to analysis			
118.25	466626	1.32	239689	2.99
153.42	1367958	3.88	331674	4.14
189.25	1485420	4.21	388899	4.85
213.25	369683	1.05	236300	2.95
248.42	257593	0.73	195677	2.44
285.17	25060	0.09	26526	0.40
358.33	235518	0.81	152601	2.33
442.33	30269	0.10	1160	0.02
501.67	21483	0.07	691	0.01
573.17	Pentane evaporation prior to analysis			
649.67	155400	0.53	3612	0.06
745.75	24528	0.08	567	0.01
841.82	23208	0.08	1513	0.02
938.57	21500	0.07	576	0.01
1033.57	20345	0.07	375	0.01
1129.92	22035	0.08	503	0.01
1225.75	751	0.00	4574	0.06
1274.17	19032	0.06	12459	0.16
1371.33	4870	0.01	15527	0.20
1465.58	6623	0.02	11940	0.16
1562.75	11121	0.03	25301	0.33
1610.92	3271	0.01	10643	0.14
1707.58	8824	0.03	6976	0.09
1802.17	28165	0.09	24789	0.32
1875.08	1558	0.00	9043	0.12
1922.67	1872	0.01	8450	0.11
2016.92	2057976	5.94	2074	0.03
2114.33	193011	0.56	22404	0.29
2211.50	173831	0.50	64443	0.82
2257.25	160047	0.46	16052	0.20

Appendix Q continued

Time from Stability hours	[VC] Peak Area	[VC] mmol/L	[C_2H_4] Peak Area	[C_2H_4] mmol/L	[C_2H_6] Peak Area	[C_2H_6] mmol/L
673.42			99	0.12	63	0.07
758.42			3261	4.03		
830.17						
901.42			2103	2.60		
951.25			3235	3.99		
1034.92			3753	4.63		
1130.42			2726	3.37		
1226.25			4874	6.01		
1274.92			6614	8.15		
1371.92			3548	4.38		
1466.17			3439	4.24		
1563.17			11256	13.82	17	0.02
1611.50			12100	14.85		
1706.83			10465	12.86		
1802.50			15727	19.25		
1875.42			14591	17.88		
1922.92			15217	18.64		
2017.33	12400	13.77	25531	15.90		
2114.67	2245	2.43	27868	17.36		
2211.92	2814	3.07	18331	11.42		
2257.5	3271	3.58	29176	18.17		
2271.33	3270	3.58	33484	20.86		
2283.25	2708	2.95	22268	13.87		
2307.67	960	1.00	22578	14.06		
2331.58	456	0.44	14108	8.79		
2354.58	3274	3.58	8600	5.36		
2379.5	1699	1.83	4587	2.86		

Appendix R: Chloroethene Profile in Column 5 as a Function of Time

Time from Stability hours	PCE Peak Area	[PCE] mmol/L	TCE Peak Area	[TCE] mmol/L
28.5	235765	0.74	6855	0.09
51.25	721935	2.05	13753	0.17
71.92	1662750	4.72	22146	0.28
95.75	2498194	7.08	29900	0.37
118.25	3189277	9.04	50896	0.64
153.42	4278557	12.13	62617	0.78
189.25	5371600	15.23	73699	0.92
213.25	5504650	15.61	105375	1.32
248.42	7150304	20.28	143898	1.80
285.17	4466042	15.36	118761	1.81
358.33	5162733	17.76	118305	1.80
442.33	4468083	15.37	149032	2.27
501.67	5056541	17.39	280404	4.28
573.17	4778397	16.44	317823	4.85
649.67	4873568	16.77	388650	5.93
745.75	5986304	20.59	476083	7.26
841.82	5638765	19.40	327191	4.99
938.57	5081050	17.48	442020	6.74
1033.57	5142346	17.69	479534	7.31
1129.92	4503040	15.49	374386	5.71
1225.75	6412346	19.41	393496	5.14
1274.17	7502778	22.71	587376	7.67
1371.33	7171325	21.71	530800	6.93
1465.58	6839693	20.70	429458	5.61
1562.75	5038141	15.25	410088	5.36
1610.92	5682163	17.20	346299	4.52
1707.58	5590608	16.92	351038	4.58
1802.17	6606624	20.00	301597	3.94
1875.08	7738861	23.43	419824	5.48
1922.67	7700909	23.31	327357	4.28
2016.92	7581453	21.88	347236	4.42
2114.33	6147184	17.74	464773	5.92
2211.50	6910253	19.94	540458	6.88
2257.25	7347155	21.20	422679	5.38
2271.08	8196781	22.00	508170	5.94
2282.83	7021370	18.85	579218	6.77
2307.33	6228765	16.72	451810	5.28
2330.67	7174259	19.26	412037	4.81
2354.25	7625261	20.47	404955	4.73
2367.33	6532112	17.53	391082	4.57
2379.25	6096381	16.36	432258	5.05

Appendix R continued

Time from Stability hours	[VC] Peak Area	[VC] mmol/L	[C2H4] Peak Area	[C2H4] mmol/L	[C2H6] Peak Area	[C2H6] mmol/L
673.42			33	0.04		
830.17			31	0.04	54	0.06
951.25			50	0.06	58	0.07
1130.42			156	0.19		
1274.92			299	0.37		
1466.17			505	0.62		
1611.50			968	1.20		
1802.50			772	0.95		
1922.92			712	0.88		
2017.33	1334	1.42	1783	1.11		
2114.67	4885	5.38	5694	3.55		
2211.92	4919	5.42	5124	3.19		
2257.5	1768	1.90	2789	1.74		
2271.33	3405	3.73	4540	2.83		
2283.25	6534	7.22	6893	4.29		
2307.67	2858	3.12	4329	2.70		
2331.58	1756	1.89	2936	1.83		
2354.58	2174	2.36	2046	1.27		
2379.5	3240	3.55	7632	4.75		

Appendix S: Chloroethene Profile in Column 6 as a Function of Time

Time from Stability hours	PCE Peak Area	[PCE] µmol/L	TCE Peak Area	[TCE] µmol/L
28.5	139753	0.44	3420	0.04
51.25	645138	1.83	7299	0.09
71.92	1668375	4.73	17417	0.22
95.75	2692358	7.64	33204	0.41
118.25	3444088	9.77	52265	0.65
153.42	4772995	13.54	77101	0.96
189.25	5780666	16.39	91356	1.14
213.25	6670883	18.92	124637	1.56
248.42	6874525	19.50	133211	1.66
285.17	6097904	20.98	142453	2.17
358.33	4788141	16.47	116897	1.78
442.33	5349536	18.40	175065	2.67
501.67	5808250	19.98	243719	3.72
573.17	4879552	16.79	274483	4.19
649.67	4954701	17.04	239104	3.65
745.75	6373792	21.93	303127	4.62
841.82	6010755	20.68	274428	4.19
938.57	5281456	18.17	367961	5.61
1033.57	5498570	18.92	416636	6.35
1129.92	5461421	18.79	363269	5.54
1225.75	8120192	24.58	369510	4.83
1274.17	8949056	27.09	527365	6.89
1371.33	7835024	23.72	332168	4.34
1465.58	7410538	22.43	265173	3.46
1562.75	7250896	21.95	369022	4.82
1610.92	7274019	22.02	239667	3.13
1707.58	5657395	17.12	201015	2.63
1802.17	8632038	26.13	173897	2.27
1875.08	8719187	26.39	229614	3.00
1922.67	7840477	23.73	154795	2.02
2016.92	9044147	26.10	185317	2.36
2114.33	9079674	26.20	225978	2.88
2211.50	9041984	26.10	221721	2.82
2257.25	9058246	26.14	169669	2.16
2271.08	8990432	24.13	237340	2.77
2282.83	8219680	22.06	193352	2.26
2307.33	8446330	22.67	158774	1.85
2330.67	8114035	21.78	148387	1.73
2354.25	6086605	16.34	137156	1.60
2367.33	5350883	14.36	154752	1.81
2379.25	3705806	9.95	87550	1.02

Appendix S continued

Time from Stability hours	[VC] Peak Area	[VC] µmol/L	[C2H4] Peak Area	[C2H4] µmol/L	[C2H6] Peak Area	[C2H6] µmol/L
673.42			591	0.73	22	0.02
830.17			736	0.91	ND	
951.25			403	0.50	ND	
1130.42			1067	1.32	9	0.01
1274.92			1042	1.29	ND	
1466.17			361	0.45	ND	
1611.50			2095	2.59	79	0.09
1802.50			2027	2.50	ND	
1922.92			1855	2.29	48	0.05
2017.33	2796	3.05	3082	1.92	ND	
2114.67	926	0.96	5207	3.24	ND	
2211.92	382	0.35	5288	3.29	ND	
2257.5	1395	1.49	3469	2.16	ND	
2271.33	1423	1.52	5716	3.56		
2283.25	2589	2.82	6749	4.20		
2307.67	2108	2.28	5174	3.22		
2331.58	1384	1.47	3740	2.33		
2354.58	1332	1.42	2268	1.41		
2379.5	995	1.04	2688	1.67		

Appendix T: Average Percent Recoveries of Chloroethenes in all the Columns and of Ethene and Ethane in Columns 3 and 4

Column	Avg. % Recovery	St. Dev. % Recovery	Avg. % Recovery of HCs	St. Dev. % Recovery of HCs
1	85	17		
2	93	17		
3	65	14	57	14
4	63	21	52	9
5	85	14		
6	90	15		

Values are calculated from the profiles in each of the columns.

The total concentrations of recovered species from a sample date are summed, then the value is divided by the inlet concentration of PCE.

Vita

I was born in the heart of the most important city in the world and grew up in the DC suburb of Rockville, Maryland. Before entering college I spent a year in Israel studying and working the land on a kibbutz. After returning I received by Bachelor of Chemical Engineering Degree from the University of Delaware. I then headed to Columbia University in New York where I got my Master of Science Degree studying in the biochemical engineering field. I left there to pursue my doctorate in the environmental field and came to Virginia Tech. Where I go from here is still an unknown.