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Exploring anaerobic reductive dechlorination at low pH environments

Yi Yang
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To the Graduate Council:

I am submitting herewith a thesis written by Yi Yang entitled "Exploring anaerobic reductive dechlorination at low pH environments." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Environmental Engineering.

Frank E. Loeffler, Major Professor

We have read this thesis and recommend its acceptance:

Chris Cox, Qiang He

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

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Exploring Anaerobic Reductive Dechlorination at Low pH Environments

A Thesis Presented for the
Master of Science
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Yi Yang
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ABSTRACT

Chlorinated aliphatic hydrocarbons (CAHs), such as tetrachloroethene (PCE) and trichloroethene (TCE), are ubiquitously pollutants in aquifer sediments and groundwater due to their heavy usage in industry and inappropriate disposal in the last century. Among about 1300 NPL (National Priorities List) sites, PCE and TCE are the two most frequently detected hazardous contaminants. Engineered bioremediation, including biostimulation and bioaugmentation, is a promising technology to clean those PCE and/or TCE contaminated sites. However, in many contaminated groundwater systems and hazardous waste sites, pH can be lower than 5 to 6. And release of HCl (strong acid) from anaerobic reductive dechlorination may lower the pH of groundwater. Besides, another main source of acidity comes from the fermentation of additive electron donors such as alcohols, organic acids and etc.

Decreasing pH has been proved to be detrimental to the microbes that dechlorinated PCE or TCE. We intended to enrich and isolate microorganisms, which can perform anaerobic reductive dechlorination at low pH environments, by establishing microcosms, which will be beneficial to *in situ* bioremediation. We also screened some existing cultures for dechlorinating activity at low pH and determined the pH tolerance of consortium BDI, which had been successfully, applied for *in situ* bioremediation. Besides, this study investigated and explored the effects of solids on BDI consortium under low pH conditions.

Generally, various dechlorinating pure cultures and consortium BDI show highest dechlorination rates and extent at circumneutral pH. Only *Sulfurospirillum* multivorans among tested cultures dechlorinated PCE to *c*DCE at pH 5.5. The screening efforts suggest that microbes capable of dechlorination below pH 5.5 are not common. It was observed that solids play an important role for enhancing microbial activities under low pH conditions. And BDI consortium can recover

from up to 8 weeks exposure to low pH conditions, although the VC-to-ethene dechlorination step was affected.

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SYMBOLS AND ABBREVIATIONS

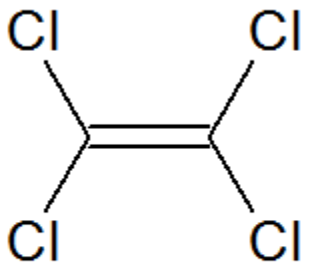
PCE	Tetrachloroethene
CAHs	Chlorinated Aliphatic Hydrocarbons
TCE	Trichloroethene
*c*DCE	*cis*-1,2-Dichloroethene
VC	Vinyl Chloride
ATSDR	Agency for Toxic Substances and Disease Registry
NPL	National Priorities List
DNAPL	Dense Non-aqueous Phase Liquid
DoD	Department of Defense
DoE	Department of Energy
ITRC	Interstate Technology Regulatory Council
Dhc	*Dehalococcoides mccartyi*
FID	Flame Ionization Detected
PCR	Polymerase Chain Reaction
qPCR	Real-time Polymerase Chain Reaction
T-RFLP	Terminal Restriction Fragment Length Polymorphism
TGGE	Temperature Gradient Gel Electrophoresis
DGGE	Denaturing Gradient Gel Electrophoresis

CHAPTER I
Introduction and General Information

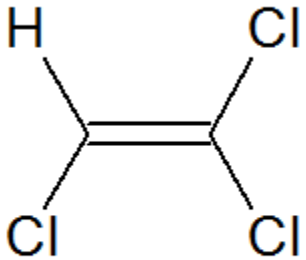
1.1 Chlorinated Aliphatic Hydrocarbons

Chlorinated aliphatic hydrocarbons (CAHs), such as tetrachloroethene (PCE) and trichloroethene (TCE), are ubiquitously pollutants in aquifer sediments and groundwater due to its heavy usage in industry and inappropriate disposal in the last century. [1-9] (Figure 1.1) The extent of soil and groundwater contamination was reflected by the National Priorities List (NPL), which is a primary guidance for EPA in determining which sites warrant further investigation. Up to February 21, 2012, there are more than 1200 sites listed. Among these sites, PCE and TCE are the two most frequently detected hazardous contaminants. Furthermore, in the ATSDR (Agency for toxic substances and disease registry) 2011 Priority List of Hazardous Substance, PCE and TCE are ranked #16 and #33 of toxicological profiles, respectively.

Tetrachloroethene, also known as perchloroethylene, PCE, pert, tetrachloroethylene, perclene and perchlor, is a synthetic chemical that is widely used in dry cleaning of fabric and as a mixture with other chlorocarbons to degrease metal parts in the automotive and other industries. Historically, PCE has also been used as the intermediates to produce the refrigerants in the refrigerators and automobile air conditioners. Adverse effects, such as immunological, neurological, reproductive, developmental and carcinogenic effects have been reported and discussed in the toxicological profile for tetrachloroethene. Furthermore, PCE is also a common groundwater and soil contaminant and can form a dense non-aqueous phase liquid(DNAPL) in these contaminated sites due to its specific gravity greater than 1, which results in the difficulty of effective cleanup. The main properties of PCE are listed in Table 1 for reference.



PCE



TCE

Figure 1.1 Structure Formulas of PCE and TCE

Trichloroethene, also known as trichloroethylene, TCE, trichlor, triclene, trimar, has been extensively used as industrial solvent for different purposes, such as dry cleaning and rocket engine flushing. It is reported that about 1400 military properties of Department of Defense (DoD) and 23 sites of Department of Energy (DoE) have been contaminated with TCE. Because of its irreversible health effects on human and other lives, regulation on TCE has been proposed. The main chemical and physical properties of TCE are listed in Table1.1.

Table 1.1 Chemical and Physical Properties of PCE and TCE

Name	Molecular Formula	Molecular Weight (g/mol)	Density (g/cm^3)	Melting Point (K)	Boiling Point (K)	Solubility in Water (g/ml)	Henry's Constant @293K
PCE	C_2Cl_4	165.83	1.622	254	394	0.015	0.546*
TCE	C_2Cl_3H	131.39	1.46	200	360	1.280	0.294*

* Value calculated using thermodynamic data reported in Washington, J.W. 1996. Ground Water. Vol. 34. pp. 709-718. Via EPA On-line Tools for Site Assessment Calculation

1.2 Bioremediation of Chlorinated Solvents

Bioremediation, by definition, is the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. [10] Generally, bioremediation technologies can be classified into *in situ* and *ex situ*. *In situ* bioremediation is a process to treat the contaminated soils and groundwater at the site, for example, biosparging, bioventing and bioaugmentation; while *ex situ* bioremediation is a process involving excavation or removal of contaminated materials to be treated elsewhere, such as landfarming, composting, biopiles and bioreactors. [11-14] Among these technologies, natural attenuation, biostimulation, and bioaugmentation are of more importance. [15]

Natural attenuation is defined in ‘Natural Attenuation for Environmental Restoration Interim Army Policy’ by US Army as follows:

"The reduction of contaminant concentrations in the environment through biological processes (aerobic and anaerobic biodegradation, plant and animal uptake), physical phenomena (advection, dispersion, dilution, diffusion, volatilization, sorption/desorption), and chemical reactions (ion exchange, complexation, abiotic transformation). Terms such as intrinsic remediation or bio-transformation are included within the more general natural attenuation definition."

ITRC (Interstate Technology Regulatory Council) has issued a guideline report on the natural attenuation, in which detailed principles, methods and case studies can be referred. However, wide application of this strategy is constrained by long time frame for remediation and monitoring, the absence of contaminants-degrading microorganisms *in situ*, the inhibition of high solvent concentrations , competitiveness from other microbes, etc. [16-19]

Biostimulation involves injecting substrates as limiting nutrients and/or electron donors into the contaminated sites in order to stimulate the growth of indigenous microbes utilizing the contaminants. Requirements for the successful application of biostimulation include: presence of contaminants-degrading microorganisms, efficiency of nutrients delivery, ability to stimulate targeted microbes and balanced nutrients ratio (C: N: P). [20]

Bioaugmentation involves introducing enriched or isolated microorganisms into the contaminated sites to degrade the toxic or hazardous chlorinated compounds. Bioaugmentation technology started in the later 1980s and early 1990, accompanied with the increasing acceptance of bioremediation to treat petroleum hydrocarbon pollution. Various environmental companies and government agencies realized the great potential of bioaugmentation and developed different microbial inoculants to bioremediate groundwater and soil contamination.

Chlorinated solvents bioremediation has always been exerted great research efforts for its emergency and harmful effects on human health. Laboratory and field research, producing quantities of scientific paper, put light into the mechanisms and involving microorganisms of chlorinated solvents bioremediation. Microbial metabolisms of chlorinated solvents (chlorinated ethene, ethane and methane) can be categorized broadly into four areas: energy-yielding solvent oxidations, co-metabolic oxidations, energy-yielding reductions-dehalorespiration, and co-metabolic reductive dehalogenation processes. [21, 22] And different microorganisms responsible for degradation pathways have been identified and isolated.

Due to limited available oxygen in the sub-surface area and recalcitrance under aerobic degradation, anaerobic reductive dechlorination is a preferred approach for biodegradation of these chlorinated aliphatic hydrocarbons (CAHs), compared to other treatment methods. Dechlorination, also known as chlororespiration, is a process in which chlorinated compounds serve as metabolic electron acceptors

for energy generation under the strict anaerobic condition. [22] Especially, in 1989 when Freedman and Gossett demonstrated that vinyl chloride can be further biodegraded into environmental-friendly ethene, great interests in this pathway and mechanisms were intrigued.

1.3 Anaerobic Reductive Dechlorination

Highly chlorinated hydrocarbons such as PCE and TCE can be readily degraded into less chlorinated or non-chlorinated compounds through anaerobic reductive dechlorination. Anaerobic reductive dechlorination involves the replacement of chlorine with hydrogen, with a net input of one proton and two electrons. [23] PCE can be sequentially dechlorinated into ethene; TCE, *cis*-1, 2-dichloroethene (*c*DCE) and vinyl chloride (VC) are typical intermediates in this process. (Figure 1.2)

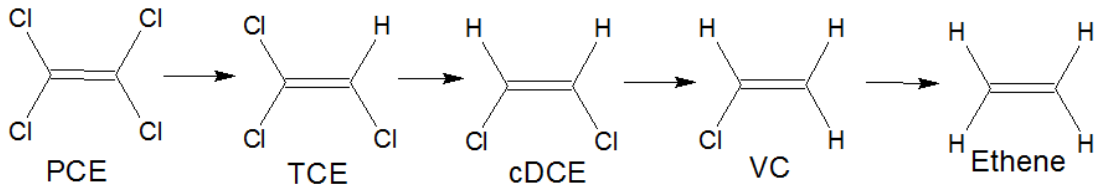


Figure 1.2 Sequential Reduction of PCE to Ethene by Anaerobic Reductive Dechlorination

Several bacteria have been isolated that can couple the reductive dechlorination of chlorinated aliphatic and aromatic hydrocarbons to energy conservation. [23] By constructing phylogenetic tree of these dechlorinating bacteria based on bacterial 16S rRNA gene sequences (See Figure 1.3), there are four distinct groups of microorganisms: the genera *Dehalobacter* [24-27] and *Desulfitobacterium* [28-35] in the *Firmicutes*, *Dehalococcoides mccartyi* [36-47] in the *Chloroflexi*, *Anaeromyxobacter*, [48-51], *Desulfuromonas* [52-55],

Desulfomonile [56-58], and *Desulfovibrio* [59-62] in the Delta-Proteobacteria, and *Sulfurospirillum sp.* [63, 64] in the Epsilon-Proteobacteria. [65] And these bacteria show differences in physiology, biochemistry, ecology and genetics. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. [66] The tree with the highest log likelihood (-5461.0340) is shown. Initial tree(s) for the heuristic search were obtained automatically as follows. When the number of common sites was < 100 or less than one fourth of the total number of sites, the maximum parsimony method was used; otherwise BIONJ method with MCL distance matrix was used. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 27 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 1250 positions in the final dataset. Evolutionary analyses were conducted in MEGA5. [67] (See in Figure 1.3)

One of the most important and interesting microbes is *Dehalococcoides mccartyi*, because it is, up to date, the only known genera that can degrade PCE and TCE farther than *c*DCE to the harmless ethene [47, 68, 69] compared with other pure cultures which can only dechlorinate PCE or TCE to *c*DCE or VC (VC is known as a Human Carcinogen). Attempt to compare the rates of dechlorination by different cultures in terms of kinetics seems not useful [70-72] because the various growth conditions including temperature, electron donor source, pH value, volume of bio-reaction and etc., differ extensively. Properties of these dechlorinators are listed in Table 1.2.

Field –scale bioaugmentation projects for chlorinated solvent remediation, for most of the times, utilized commercial consortia containing *Dehalococcoides mccartyi* rather than pure cultures. KB-1^{TM}, Bio-Dechlor Inoculum™ (BDI) and SDC-9^{TM} are of great interests for laboratory research and *in situ* field test. Useful information about these consortia has been listed in Table 1.3.

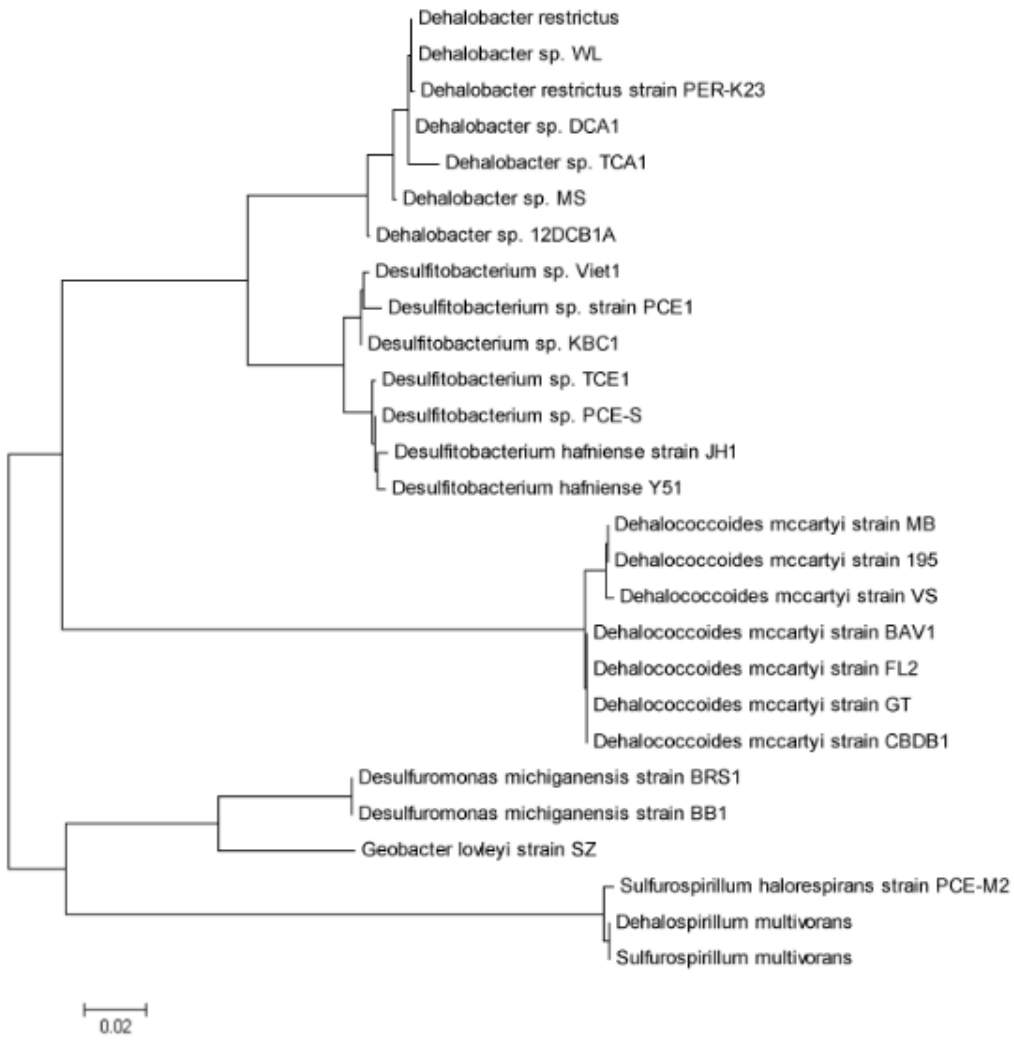


Figure 1.3 Molecular Phylogenetic Analysis of Dechlorinators by Maximum Likelihood method

Table 1.2 Properties of Selective Isolates/Strains

Strains/Isolates	Utilized chlorinated compounds	End-products	Electron donor	pH range	Temperature range	Reference and remarks
***Desulfitobacterium hafniense* Y51**	PCE, chlorinated ethane (hexa, penta, tetra)	DCEs	formate, lactate, pyruvate	6.5-7.5	23-40	[35, 73-75]
***Desulfitobacterium sp.* PCE-1**	PCE	TCE	formate, pyruvate, lactate	7.2 (Optimal)	19-42	[32]
***Desulfitobacterium hafniense* JH1**	PCE, TCE	*cis*-DCE	Acetate, pyruvate	-	-	[76]
***Dehalobacter restrictus* PER-K23**	PCE, TCE	*Cis*-DCE	H_2	6.5-8.0	10-37	[77]
Desulfuromonas michiganensis BB1	PCE,TCE	*cis*-DCE	Acetate, pyruvate,lactate, succinate,fumarate,malate	6.8-8.0	10-35	[55]
***Geobacter lovleyi* SZ**	PCE, TCE	*Cis*-DCE	H_2, acetate	-	-	[78, 79]
Sulfurospirillum multivorans	PCE,TCE	*cis*-DCE	H_2, formate, lactate, pyruvate	7.0-7.5	15-33	[64, 80]
***Dehalococcoides mccartyi* 195**	PCE,TCE,DCEs,DCA etc.	VE, ETH	H_2	-	-	[36, 38, 81, 82]
***Dehalococcoides mccartyi* BAV1**	*cis*-DCE, trans-DCE, 1,1-DCE, VC	ethene	H_2	-	-	[47]
***Dehalococcoides mccartyi* FL2**	TCE, *cis*-DCE, transDCE	VC, ethene	H_2	-	-	[45]
***Dehalococcoides mccartyi* GT**	TCE, *cis*-DCE, 1,1-DCE, VC	Ethene	H_2	-	-	[83]

Table 1.3 Consortia Used for Treatment of Chlorinated Solvents in Groundwater

Bioaugmentation consortia	Developer	Culture Source	Target Contaminants	Commercial Vendors	Primary Degradation Pathway	Key Microbial Species	Growth Conditions	Field test
KB-1™	E.Edwards (University of Toronto) and GeoSyntec Consultants	TCE contaminate aquifer, Ontario	Chloroethenes	SiREM	Reductive dechlorination (dehalorespiration)	*Dehalococcoides mccartyi, Acetobacterium, Geobacter sp., Methanospirillum, Methanosaeta*	Anaerobic defined mineral media with TCE/methanol at 23C PH=7.0	23 sites in 12 states
Bio-Dechlor Inoculum™ (BDI)	F. Loeffler (Georgia Institute of Technology)	PCE-contaminated aquifer, Oscoda, MI	Chloroethenes	Regenesis, Bioaug-LLC	Reductive dechlorination (Dehalorespiration)	*Dehalococcoides mccartyi., Desulfuromonas michiganensis BRS1, Desulforomonas chloroethenica*	Inoculum was grown in anoxic bicarbonate-buffered mineral salts medium with lactate as electron donor and PCE as electron acceptor	27 sites in 14 states
SDC-9™	Technology Application Group laboratory of Shaw Environmental Inc.	Not reported	chlorinated solvent contaminated aquifers	Shaw Environmental Inc.	Reductive dechlorination (Dehalorespiration)	*Dehalococcoides mccartyi* (DHC) bacteria	Produced in quantities up to 4,000 L per batch	12 sites

*Table was adapted from *Bioaugmentation for Remediation of Chlorinated Solvents: Technology Development, Status, and Research Needs*

1.4 Effects of pH on *in situ* Bioremediation

The success of natural attenuation, biostimulation and bioaugmentation also depends on the appropriate physical and geochemical factors [84], such as temperature, dissolved oxygen/redox level, salinity, pH and etc. Increased acidification can affect bacteria either directly, by interfering with pH homeostasis or indirectly, by increasing the concentration of toxic metal ions. [85] pH is one of the main concerns for site mangers, since low pH has a great impact on the microorganisms dechlorinating chlorinated solvents. Most of these important dechlorinators responsible for dechlorination belong to neutrophile, of which microbes the optimum pH is between 6 and 8. For example, KB-1^{TM} did not show any dechlorination activity below pH 5 either above pH 10, and the optimum pH for dechlorination is between 6.0 and 8.0.[86] So maintaining the optimum pH in the subsurface or contaminated groundwater systems can be very conducive to the growth of dechlorinators and anaerobic reductive dechlorination.

However, in some contaminated groundwater systems and hazardous waste sites pH values can be lower than 5 to 6, respectively. Achieving effective and successful application of bioremediation will be in doubt. Furthermore, the release of HCl (strong acid) from anaerobic reductive dechlorination will reduce the pH of groundwater that lacks strong buffer capacity. Generally, low pH increases the solubility of many metals and metalloids, of which the dissolved concentrations may exceed regulatory limits and impact the activities of microorganisms such as nitrate reducers, sulfate reducers, iron reducers, and methanogens. Another main source of acidity comes from the fermentation of additive electron donors such as alcohols, organic acids, emulsified vegetable oil (EVO), and complex organic materials (e.g. molasses, corn cobs, wood chips, microbial biomass, chitin and etc.) *in situ*, which generates quantities of organic acids and carbon dioxide.

There are various methods and approaches to deal with low pH problems *in situ* bioremediation. One of them is to adjust pH to the neutral condition by adding calcium carbonate ($CaCO_3$), baking soda ($NaHCO_3$), soda ash (Na_2CO_3) or caustic soda (NaOH). But the effectiveness of these buffering agents may be limited. For example, in some sites the low hydraulic permeability prevents the distribution of these agents. Furthermore, calcium carbonate has low solubility; soda ash and baking soda in acid condition may form carbon dioxide, which may clog the pore spaces, leading to reduced permeability; and caustic soda is a very strong chemical base and may cause the pH to overshoot and inhibit dechlorination. [87-91] Although other synthetic commercial buffering agents are available, the effectiveness of these agents is uncertain *in situ*. Another approach is to investigate the pH tolerance of pure cultures or enrichment. If we accept the "Doctrine of Infallibility" and "Survival of the fittest", [20] then bioremediation under low pH was feasible and such microbes and enrichment do exist to be discovered.

1.5 Hypothesis and Objectives

Based on the fact that pH values of some contaminated groundwater systems are below 6, and/or less chlorinated solvents such as *c*DCE and VC exist, which are generally the sub-products of dechlorination of PCE and TCE, it is hypothesized that microorganisms or enrichment can survive at low pH by harvesting the energy from dechlorination activity. Then consequences of decreasing pH on reductive dechlorination activity and microbial community structure will be evaluated by performing a series of experiments of increasing complexity.

Laboratory studies are of importance and focus, leading to a better understanding of these dechlorination processes and their potential for *in situ* bioremediation. [92] And two distinct strategies have been used to identify and isolate several anaerobic pure strains: some researchers have selected

previously isolated pure anaerobic stains to test their dehalogenation capacities, and other researches have focused on the isolation of new anaerobic strains from dechlorinating consortia. [93] Both ways are used to explore the dechlorination activity at low pH.

The specific tasks and objectives of exploring pH effects on dechlorinators and dechlorination activities are listed below:

Task 1: Dechlorinators screening. Batch culture experiments with a variety of dechlorinating pure and mixed cultures available in the lab (*Desulfitobacterium Michiganensis* strain BB1; *Desulfitobacterium* sp*.* strain Viet1; *Desulfitobacterium* sp. strain JH1; *Geobacter lovleyi* strain SZ; *Sulfurospirillum Multivorans*; and BDI consortium) will be tested for the pH range of reductive dechlorination activity.

Task 2: Enriching or isolating chlorinated solvents dechlorinators at low pH. Sediments and groundwater samples from pristine or contaminated sites were collected from geographically diverse locations across the US. These samples will be sent to the lab for microcosm setup. Especially, samples from sites with low pH conditions and dechlorination activity detected are preferred for microcosm setup.

Task 3: BDI consortium tolerance at low pH. Bacteria have additional strategies for surviving without growth during periods of exposure to pH values outside their growth range. Survival without growth is assessed by the resumption of growth on the return of bacteria to a permissive pH (a near neutral pH for neutrophils). [94] BDI consortium can be used for *in situ* bioremediation for chlorinated solvents cleanup. Under low pH condition, dechlorination activity of BDI will be inhibited or stopped. And recovery of BDI after transferred back to the neutral defined basal salts medium will be evaluated.

Task 4: Improved dechlorinating performance of BDI at low pH by incorporation of sterile soils. Ahring et al. [95] demonstrated that *Desulfomonile tiedjei* formed microcolonies inside the granules; Christiansen and Ahring [96] proved that granules could be constructed which possessed specific abilities such as a dechlorinating activity and at the same time be high performing; and Horber et al. [97] investigated the performance and distribution of the PCE-dechlorinating *Sulfurospirillum multivorans* added to sterile and non-sterile granular sludge. Similar approach was taken for investigating the performance of BDI at low pH by incorporating sands and sediments.

CHAPTER II
Literature Review

Several topics have been of interest and importance in the research area of anaerobic reductive dechlorination since 1980s: 1) identifying and isolating new pure cultures (dechlorinators) from enrichment; 2) identifying genes and proteins responsible for dechlorination; 3) kinetics and rates of dechlorination activity; last but not least 4) field experiments and *in situ* bioremediation. With the development of molecular biology, genomics, proteomics and applied bioinformatics, new tools and methods have been used extensively to enhance the research. Especially, with advancement of sequencing technologies and decreasing expenses, high-throughput sequencing has been used to investigate the microbial community. Lots of publications and reviews have been published on anaerobic reductive dechlorination. This literature review mainly focuses on the effect of low pH on reductive dechlorination.

2.1 The Effect of Low pH on Reductive Dechlorination

Vainberg *et al.* [98] from Shaw Environmental Company intended to produce large scale of bacterial consortia such as SDC-9^{TM} for remediating sites contaminated with chlorinated solvent compounds. They reported that no dechlorination of chloroethenes by SDC-9^{TM} was detected when pH dropped to below 5.0, and the pH of medium in the 4000L fermenter decreased from an initial pH 7.4 to approximately 6.1 during the first days of cell growth.

Schaefer *et al.* [99] conducted an *in situ* bioremediation experiments to investigate the effects of bioaugmentation dosage on dechlorination. Bulk injections containing sodium carbonate powder, as buffer system, mixed with groundwater were performed to further elevate groundwater pH values, which still largely remained below 5.5 standard units after several weeks of system operation. Besides, at pH levels ranging from 4.9 to 5.8 during 64 days period,

dechlorination activities by consortium with *Dehalococcoides mccartyi* (*Dhc*) was severely inhibited.

In a ESTCP Project report [86], low pH condition impacted the proliferation and growth of *Dehalococcoides mccartyi* microorganisms, resulting in the accumulation of VC in groundwater. pH can be reduced in the source area by dechlorination and buffering may be required for maintaining the desired pH value.

Eaddy [100] has tested SRS culture's susceptibility to extreme pH levels. Originally, SRS culture was maintained in buffered minimal media with pH range of 6.5-7.5. Then the SRS culture was exposed to different pH levels, including 5.5, 6.0, 6.5, 7.0 and 8.5. It was reported that at pH 6.0 accumulated concentrations of *c*DCE and VC were higher than at pH 7.0; at pH 5.5, *c*DCE was the end product, and no VC and ethene were produced; and at pH 8.5, the dechlorination activity was severely inhibited.

Zhuang P. and Pavlostathis S.G. [101] investigated the effect of pH on the microbial reductive dechlorination. At pH 7.0, the culture achieved both the highest PCE dechlorination rate and extent as well as methane production. The extent of dechlorination of PCE at pH 4, 6, 7, 8 and 9.5 were 25.4%, 72.9%, 97.2%, 90.9% and 27.3%, respectively. Besides, the end products for chlorinating PCE were different under the discrepancy of pH values.

Aulenta *et al*. [102] discussed that during DNAPL dechlorination hydrochloric acid release can be a significant problem, making a high buffer necessary to prevent adverse pH condition. Besides, acetic acid from fermentation of substrates as electron donor may further deteriorate the pH condition. Then additional buffer was required.

Holmes *et al*. [103] demonstrated that isolated *Dehalococcoides mccartyi* strains which have little variance of 16S rRNA gene sequences, yet, bear notably different metabolic abilities. It was suggested that using *Dhc* 16S rRNA gene sequences to characterize the unknown community would not be adequate enough for predicting the activities of the community or for identifying the number of functionally distinct *Dhc* strains in that community. Furthermore, microcosm setup would be beneficial for characterizing the physiology of the microorganisms or community.

Middeldorp *et al*. [104] indicated that in order to stimulate the microorganisms capable of completely dechlorinating PCE to ethene, hydrogen as the main electron donor must be provided. The amendment of hydrogen can only be achieved by anaerobic degrading substrate such as short chain organic acids. This process often leads to acidification of the soil.

EI Fantroussi *et al*. [105] reported that the first pure dehalogenase enzyme was active over temperatures ranging from 20 to 55ºC with an optimum at 37ºC and pH from 5.5 to 8.7 with an optimum at 7.2. The maximum initial rate of 2, 3-DCP dechlorination by *D. chlororespirans* strain Co23 was observed between pH 6.2 and 7.0 with an optimum pH at 6.5. Another enzyme purified from *Sulfurospirillum multivorans* exhibited an optimum pH at about 8.

In a column study by Nathan Cope and Joseph Hughes, [106] they found that effluent pH for all columns (which were still in the viable range for dechlorinators pH 6.4-7.2), were lower than that observed in the inoculum cultures. And one of the columns had pH values near 6.3 for about 2 weeks. Pyruvate was serving as electron donor and carbon source; and it was degraded to acetate and propionate by fermenting bacteria.

Damborsky J. compiled a list of major tetrachloroethene-degrading microorganisms. [7] In this review, the pH range and optimum pH for the dechlorinators were listed in Table 2.1:

Table 2.1 pH Range and Optimum pH for Some Isolates/Strains

Strain/isolate	pH range	pH optimum
***Desulfitobacterium dehalogenans* JW/IU-DC1**	6.0-9.0	7.5
***D. tiedjei* DCB1**	6.5-7.8	6.8-7.0
***D. restrictus* PER-K23**	6.5-8.0	6.8-7.6
S. multivorans	6.0-8.0	7.0-7.5
***D. chloroethenica* TT4B**	6.5-7.4	7.4

Scheutz *et al.* reviewed [107] a field case. In that field experiment, it was observed that low pH at the site 5-5.5 was inhibitory to the dechlorination activities. They speculated that the presence of TCA DNAPL resulted in the low pH condition *in situ*, which was caused by generation of HCl and acetic acid from TCA degradation.

McCarty P.L. *et al.* discussed the pH problem on biological reductive dechlorination to biologically enhanced dense non-aqueous phase (DNAPL) chlorinated solvent dissolution. Large amounts of hydrochloric acid and acetic acid can be produced during the dechlorination process. They compared the effects of different electron donors (glucose, lactate, hydrogen, formate and ethanol) on pH. Formate has a potential advantage over other electron donors because formate can be disproportionated to sodium bicarbonate, which can be used to neutralize hydrochloric acid. [108]

Robinson C. *et al.* discussed the acid-forming process of dechlorination. Although the soil has some buffer capacity, it cannot sustain because of the extensive dechlorination. Bicarbonate addition is a method to control the pH value. Besides they developed a model to predict the amount of bicarbonate required to maintain the pH at a suitable level for dehalogenation bacteria, which may be a useful tool for *in situ* bioremediation. [109]

Armenante *et al.* were investigating into the effect of pH on the anaerobic dechlorination of 2, 4, 6-trichlorophenols by growing an inoculum in a defined medium. It was discussed that any attempt to lower the pH to values at or below 6.8 using phosphate buffer additions severely inhibited or completely repressed dehalogenation. Besides, they reported that beyond the range between 8.0 and 8.8, any dehalogenation of 2, 4, 6-trichlorophenol was prevented from occurring. [110]

Adamson D.T. *et al*. reported a pH drop as low as 4.9 in active systems involved biological dechlorination of tetrachloroethene dense non-aqueous phase liquid, indicating the large amount of HCl release overwhelmed the buffering capacity. And it was suggested that rapid dechlorination of PCE DNAPL can alter chemical characteristics in source zone regions, and further affect the activities of microorganisms *in situ*. [111]

Fam S.A. *et al*. reported a full-scale field enhanced anaerobic dechlorination at a NAPL strength 1, 1, 1-TCA source area. In the microcosm test, TCA dechlorination was inhibited by the site's relatively low pH (5-5.5) as well as high concentration of TCA. The dechlorination resumed after adjusting the pH and TCA concentration. Besides, it was indicated by the site data that the lack of groundwater in the vicinity of MW-303 was likely due to low pH (5.0) and/or inhibitory concentration of TCA. [112]

2.2 Enhanced Reductive Dechlorination by Incorporation of Granules

Aktas *et al*. investigated the effect of granular activated carbon on reductive dechlorination rates and growth of *Dehalococcoides mccartyi.* They found that addition of granular activated carbon could enable biological dechlorination down to ethene by adsorbing PCE onto granule activated carbon. They suggested that the combination of microbial reductive dechlorination and GAC adsorption could be a promising method applied to *in situ* bioremediation. [113]

Horber C. [97], Christianse N. [96], Ahring B.K. [95], Prakash S.M. [114] and Schmidt J.E. [115] had investigated the inoculation of granular sludge with dechlorinators, such as *Sulfurospirillum multivorans* and *Desulfomonile tiedjei* in upflow anaerobic granular-sludge blanket reactors to enhance the dechlorination of PCE. Microorganisms had colonized the sludge granules and immobilized in the living confirmed by using fluorescein-labeled antibody probes.

Collins R. and Picardal F. found that anaerobic reductive dehalogenation of carbon tetrachloride (CT) by *Shewanella putrefaciens 200* was enhanced by the presence of a high organic carbon soil. It was suggested that abiotic electron transfer mediators in the soil were catalyzing the reaction, such as humic acid. Furthermore, they investigated the effect of different pH on the CT transformation. At pH 3.6, little CT transformation was observed; at circumneutral pH, CT transformation required the presence of reductant dithiothreitol; and at pH 8.7, CT transformation occurred regardless the presence of a reductant. [116]

CHAPTER III
Materials and Methods

3.1 Samples

3.1.1 Pure Cultures and Enrichment

All isolates and BDI^{TM} consortium for pH range tests were transferred from pure culture stock maintained in Dr. Loeffler's lab. Cultures (*ca.* 2ml stock) were transferred to 160mL bottles containing 100 mL DCB-1 defined medium (see appendix A for details) for growth at pH_n 7.2[1] as the stock bottle for following experiments. PCE was spiked into the bottles as electron acceptor, and 5mM lactate plus 5mL hydrogen served as electron donors.

3.1.3 Sediments and Groundwater

Sediments and groundwater samples (listed in Table 3.1) from geographically diverse locations were sent to our lab for microcosm setup and further analysis. Samples were put in the glovebox for anaerobic aeration overnight, and microcosms were setup the next day. Then samples were stored at 4℃. Before microcosm setup in anaerobic glovebox, some other samples, which had been stored at 4℃, were mixed in the glovebox first.

3.2 Main Chemicals

3.2.1 Chlorinated Ethenes and Ethene

PCE was 99+% spectrophotometric grade, purchased from ACROS ORGANICS. (FW 165.83, n_D^{20}=1.5056, Density=1.62).TCE was obtained from Fisher Scientific with assay 99.9%. *c*DCE was bought from SUPELCOTM analytical and

[1] n in pH_n stands for nominal. m in pH_m stands for measured.

stored at 4℃. Vinyl chloride (99.5+%, FW62.5, Density=0.911) and ethene (≥99.5%) gases were purchased from Sigma-Aldrich Chemicals.

3.2.2 Buffers

MES (MW=195.24，$C_6H_{13}NO_4S$, 99 %, < 1% Water) was purchased from ACROS ORGANICS. Sodium bicarbonate was purchase from Fisher Scientific with assay 100.1%. Tris-base (white crystals or crystalline powder, for molecular biology) and Tris-hydrochloride (white flakes, for molecular biology) were purchased from Fisher Scientific.

3.3 Experimental Design

DCB-1 defined basal salts medium was used to set up microcosms for different experiments. Lactate and hydrogen were constantly used as electron donors; and neat PCE and TCE, as electron acceptors, were added to serum bottles by Hamilton syringes.The constituents of DCB-1 defined medium and the procedures of making DCB-1 medium were listed in appendix A for reference.

3.3.1 Experiment 1: pH Range Screening of Pure Cultures and Enrichment

Isolates, such as *Desulfitobacterium Michiganensis* strain BB1, *Desulfitobacterium* sp. strain Viet1, *Desulfitobacterium* sp. strain JH1, *Geobacter lovleyi* strain SZ, *Sulfurospirillum Multivorans* and BDI consortium were selected for determining the pH range suitable for respiratory reductive dechlorination. The aim of the screening was to test whether these pure cultures and consortium can grow at low pH. If so, they would be promising candidates for bioaugmentation under low pH conditions. Although some of these isolates had been tested for optimum pH range, low pH condition was of interest in this experiment.

Before growth experiments with different pH values, isolates and consortium were grown in 160mL stock bottles containing 100mL DCB-1 medium spiked with 2.5μL PCE. When PCE were totally degraded into less chlorinated compounds (Gas Chromatograph measurement), 3% v/v medium from stock bottles were transferred to new 160mL serum bottles (160 mL nominal capacity, Wheaton Co., Millville, New Jersey) containing 100mL DCB-1 medium with different pH values (5.5, 6.0, 7.2 and 8.0, ±0.2 standard units). The medium had been adjusted with 25mM Good buffers [117] (MES [5.5-6.5] for maintaining targeted pH 5.5 and 6.0; TRIS [7.5-9] for maintaining targeted pH 8.0) and 30mM sodium bicarbonate for maintaining pH 7.2. Before any inoculum, neat PCE (*ca.* 2.5μL, 24.45 μmol/bottle) and 5mL hydrogen (*ca.* 0.207mmol/bottle) were spiked into the 160mL serum bottle with 5μL Hamilton syringe and 5mL sterile plastic syringe respectively. Chlorinated compounds were measured by gas chromatography to monitor the dechlorination activities under different pH conditions.

3.3.2 Experiment 2: Microcosms Setup to Enrich or Isolate Microbes at Low pH

Soil/sediments and groundwater samples from all over the world had been collected and transported to the lab for microcosms study. Sample information details are displayed in Table 3.1.

As it is described in the previous section, DCB-1 medium with different pH values (5.5, 6.0, 7.2 and 8.0) were prepared in advance. Medium was distributed into each vial or serum bottle (20mL vials or 160mL serum bottles), which was then capped with a black stopper (Geo-Microbial Technologies) and crimped with an aluminum ring (Wheaton Aluminum Seals). The autoclaved medium and soil/sediments samples were brought into glove box together. Inside the glove box with a mixture of 97% N_2 and 3% H_2 [vol/vol], soil/sediment samples were added to the 20mL vials containing 10mL DCB-1 medium or 160 mL serum bottles with 100 mL DCB-1 medium by sterilized spatulas. The vials and bottles were re-sealed with black stoppers and aluminum crimp seals. Then pure hydrogen (0.12mmol for 20 mL vial and 0.41mmol for 160 mL serum bottle) and neat PCE or TCE (1µL for 20 mL vial and 5µL for 160mL serum bottle) were added to the microcosms after bottles were removed from glove box. Sterile hydrogen was added via syringe; while PCE and TCE were added by Hamilton micro-syringe. The serum bottles or vials were shaken and then put into the dark area. After 24 hours, initial PCE or TCE concentrations was measured by gas chromatography manually. Replicate or triplicate microcosms had been set up under different pH conditions and monitored regularly by GC manual measurement. Autoclaved microcosms and DCB-1 medium without inoculum were established as the abiotic and negative control groups.

Whenever all PCE and TCE were degraded into less chlorinated compounds (GC measurement), 3% (vol/vol) of aqueous cultures with sediments were transferred to new 20mL vials or 160mL serum bottles. Consecutive transfers were made in order to get sediment-free enrichment for further investigation.

3.3.3 Experiment 3: BDI Tolerance at Low pH

BDI consortium was maintained in 160mL serum bottle with 100mL DCB-1 medium (pH_n=7.2) amended with 5µL PCE and 10mL H_2. The dechlorination

activity of BDI was monitored by measuring chlorinated compounds with GC. When all PCE was degraded into *c*DCE, VC and ethene, biomass was collected from the serum bottle in the anaerobic glove box. Biomass was dissolved into pH 5.5 DCB-1 medium and then transferred into another 160mL serum bottle containing 100mL DCB-1 medium (pH_n=5.5). Neat PCE (*ca.* 5μL) and 10 mL H_2 were spiked into the serum bottles. BDI consortium was exposed to pH_n 5.5 medium for 1, 2 and 4 weeks in batch culture incubations before the inoculum was transferred back to DCB-1 medium with neutral pH. Dechlorination activity had been monitored to determine whether BDI can recover from the low pH exposure.

3.3.4 Experiment 4: BDI Performance by Incorporation of Sterile Soils

Based on previous experimental results (Experiment 2: Microcosms Setup to Enrich or Isolate Microbes at Low pH), dechlorination activity can be detected at low pH (pH_n=5.5) with the presence of soils or sediments. However, when the liquid medium was free of soils or sediments, the dechlorination activity stopped. To explore the role of soils or sediments conducive to the microbial dechlorination under low pH conditions, a bioaugmentation experiment was conducted.

US silica sands and sediment samples from Third Creek, Tennessee were chosen for experiment. These samples were autoclaved twice. Soil or sediment samples (*ca.* 20g) were added to 160mL serum bottles with 100mL DCB-1 medium (pH_n 5.5); then 160mL serum bottles with DCB-1medium were autoclaved again. BDI cultures (*ca.* 3mL) were transferred to the 160mL serum bottles. PCE (*ca.* 2.5μL) and H_2 (*ca.* 5mL) were added. Chlorinated compounds were monitored by GC. The microcosms without BDI inoculum were established as the control group to exclude the possibility of abiotic dechlorination.

Table 3.1Sample Information for Microcosms Study

(Adapted from Lab Stock Table)

Sample Name	Information	Place	Received Date	Stored in	Labeling			Type of Sample
					Sample ID	Sample Depth	Labeled as	Sediment/GW
Ft. Pierce Samples	Florida 1,2 D Project	Ft. Pierce Florida	August, 2010	4°C Frige (705)	MW-46-BOS-0175	25.5'-26.5'	Sample 1	Sediment
					MW-49-BOS-0180	26'-27'	Sample 2	Sediment
					MW-49-BOS-196	46'-47'	Sample 3	Sediment
					MW-47-BOS-0173	47'-48'	Sample 4	Sediment
					MW-49-BOS-200	95'-96'	Sample 5	Sediment
PNNL Samples	Contaminated by chlorinated compounds.	PNNL CT	November, 2010	4°C Frige (705)	Sample ID	Sample Depth	Labeled as	Sediment/GW
					300 Area, well C7656,int 22	63.5'-66.0'	-	Sediment
					301 Area, well C7656,int 23	66.0'-68.5'	-	Sediment
					301 Area, well C7656,int 25	71.0'-73.5'	-	Sediment
					302 Area, well C7656,int 26	73.22'-75.7'	-	Sediment
					302 Area, well C7656,int 27	76.0'-78.5'	-	Sediment
					303 Area, well C7656,int 28	78.5'-81.0'	-	Sediment
DuPont's Oakley Samples	High concentration of chlorinated methanes	Denver, Colorado	December, 2010	4°C Frige (705)	Sample ID	Sample Depth	Labeled as	Sediment/GW
					MW-171-95-2W	26.5'-27.0'	Sample 1	Sediment
					MW-171-95-2W	28.0'-28.5'	Sample 2	Sediment
					MW-171-95-2W	29.0'-29.5'	Sample 3	Sediment
					MW-171-95-2W	30.5'-31.0'	Sample 4	Sediment
					MW-171-95-2W	31.5'-32.0'	Sample 5	Sediment
					MW-171-95-2W	32.5'-33.0'	Sample 6	Sediment
					MW-171-95-2W	33.0'-34.0'	Sample 7	Sediment
					MW-171	-	Sample 8	GW (5 containers)
Brasil Samples	High in chloroform	Brasil	December, 2010	4°C Frige (705)	Sample ID	Sample Depth	Labeled as	Sediment/GW
					001-ST-SO	2.70-2.94	Sample 1	Sediment
					002-ST-SO	5.70-5.85	Sample 2	Sediment
					003-PI-AS	-	Sample 3	GW (1 container)
Third Creek, Tennessee	(1,1,1 TCA), (PCE), (TCE) reductive dechlorination	Tennessee		4°C Frige (705)	Sample ID	Sample Depth	Labeled as	Sediment/GW
					Location 1	Location 1	Sample 1	Sediment w/ surface water
					Location 2	Location 2	Sample 2	
					Location 3	Location 3	Sample 3	
Neckar River		Germany	May, 2011	4°C Frige (705)	Sample ID	Sample Depth	Labeled as	Sediment/GW
								Sediment
Rotenberg Creek&Trest		Germany	May, 2011	4°C Frige (705)	Sample ID	Sample Depth	Labeled as	Sediment/GW
								Sediment
McGuire AFB(Delivered by Shaw Environmental)			Feb, 2012	4°C Frige (705)	Sample ID	Sample Depth	Labeled as	Sediment/GW
					Above coarse			Sediment
					Below Clay			Sediment
					PMW-65 Water			GW (3 containers)

3.4 Chlorinated Ethenes and Ethene GC Analysis

Total chlorinated solvent mass or concentrations of chlorinated compounds were measured by analyzing 100μL headspace gas samples (for 20mL vials and 160mL serum bottles) or 1mL liquid sample (extracted from 160mL serum bottles)

on a gas chromatography with a flame ionization detector, and normalizing the values to a standard curve generated using bottles with the same gas to liquid ratio and maintained at the same temperature as the pure cultures and microcosms. Gas sample (*ca.* 100μL) was extracted from the headspace of 20mL or 160mL serum bottle by a 250μL Hamilton syringe and then injected into the GC manually. Liquid sample (*ca.* 1mL) was extracted from 160mL serum bottle by a 1mL sterile plastic syringe with $25\frac{7}{8}$ gauge needle and injected into a 20mL vial previously sealed with a Teflon-lined gray butyl septum secured with an aluminum crimp cap for autosampler injection and measurement.

Samples were measured by Agilent 7890A gas chromatography equipped with an Agilent DB624 column (30 m x 0.53 mm I.D., 3 μm.) with a flame ionization detected (FID). The parameters of manual injections and autosampler injection are listed in Appendix C Table C.1 and C.2.

The retention times were determined by injecting pure compounds into the GC for analysis. The retention time is used as the identity for the specific chlorinated compound. The retention times for different chlorinated compounds are listed in Appendix C Table C.3.

3.5 Buffer Systems and pH Measurement

When microorganisms grow or reproduce, substrates from the environment are utilized and end-products are released into the environment by microbes. In these processes, the pH of the environment may be changed, resulting in the inhibition of microorganisms' metabolisms, growth or reproduction. This problem exists not only in the closed environment of a batch culture in a lab study, but also in the open environment such as a contaminated site.

Buffer solutions were used to maintain a stable optimum pH for microbial growth since all microbes have evolved to grow within a particular range of external pH values. Of various buffer systems, Good's buffers are of significance and used extensively in biological studies, including MES, PIPES, TES, MOPS, PIPES and etc. The criteria to select the Good's buffers are listed as follows: pKa value between 6.0 and 8.0, high water solubility, limited permeability of biological membranes., minimum salt effects, minimal changes due to temperature and concentration, limited interaction with mineral cations, enzymatic and hydrolytic stability, no light absorbance in the visible or ultraviolet regions, and ease of preparation. [117]

Based on the above discussion, buffer systems for different pH ranges have been selected for the experiments (Table 3.2). The concentration of buffer typically needs to be as high as 100mM, and the pH of the medium must be measured both before and after microbial growth. [118] Buffer chemicals were added into the medium at 25mM or 50mM concentrations, then pH was adjusted to the targeted value using a strong base (Sodium hydroxide) or acid (Hydrochloric acid).

Table 3.2 Buffers for DCB-1 Medium[2]

Buffer abbreviation	Chemical Name	pKa at 20°C	ΔpK/ΔT	Useful pH range
HOMOPIPES	Homopiperazine-N,N'-bis-2-ethanesulfonic acid	4.84	-0.017	3.9-5.1
MES	2-(N-morpholino)-ethanesulfonic acid	6.15	-0.011	5.5-6.7
PIPES	piperazine-N,N'-bis-2-ethanesulfonicacid	6.81	-0.009	6.1-7.5
MOPS	3-(N-morpholino)propanesulfonic acid	7.11	−0.015	6.5-7.9
HEPES	2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid	7.55	-0.014	6.0-8.0

[2] Most of the data compiled from: http://www.sigmaaldrich.com/life-science/core-bioreagents/biological-buffers/learning-center/buffer-reference-center.html

TRIS/TRIZMA	tris(hydroxymethyl)aminomethane	8.30	-0.031	7.0–9.0
CHES	2-(Cyclohexylamino)ethanesulfonic acid	9.33	-0.015	8.6–10.0

The pH of the microcosms, pure cultures and enrichment were measured by extracting 1mL liquid samples from 20mL vials or 160mL serum bottles into 2mL Eppendorf tubes. The Eppendorf tubes were centrifuged for 30 seconds at maximum speed. Supernatants were measured by a pH meter (Fisher Scientific). The pH meter was calibrated at 4.0 and 7.0 before every measurement.

CHAPTER IV
Results and Discussion

4.1 pH Range Screening of Pure Cultures and Consortium

4.1.1 Desulfuromonas michiganensis strain *BB1*

BB1 strain was cultivated in replicate 160mL bottles. Within one week, PCE was degraded to *c*DCE. Then BB1 isolate was readily for pH screening.

BB1 strain was cultivated under various pH_n (n stands for nominal) values (5.5, 6.0, 7.2 and 8.0) in triplicate. There was no dechlorination activities detected under pH_n 5.5 and 8.0 after 50 days; while all PCE could be degraded to *c*DCE under pH_n 6.0 and 7.2 within 50 days. (See in Table 4.1) And final pH values of the medium were verified within the range of 0.2 standard units.

Table 4.1 Reductive Dechlorination by BB1 under Different pH Values

Targeted pH_n	Day 1 (Initial Products)	Day 50 (Final Products)	Comments
5.5	PCE	PCE	No dechlorination
6.0	PCE	*cis*-DCE	Dechlorination. Final pH values were 6.2.
7.2	PCE	*cis*-DCE	Dechlorination.
8.0	PCE	PCE	No dechlorination

Then a second triplicate screening on strain BB1 was conducted. The initial pH_m (m stands for measured) values in DCB-1 medium with different buffer systems were about 5.70±0.01, 5.96±0.01, 7.32±0.04 and 8.34±0.01, respectively. When pH_n values were 5.50, 6.0 and 8.0, PCE cannot be degraded to *c*DCE. (See Figure 4.1) The decreasing total mass of PCE was probably due to the absorption to the black stopper. By comparison, BB1 strain could transform PCE to *c*DCE at neutral pH_n 7.2 within two weeks at room temperature. (See Figure 4.2) After the experiment, pH_m values were measured again, which were

5.79±0.00, 6.04±0.01, 7.38±0.02 and 8.34±0.02, indicating the pH values remained in the acceptable ranges.

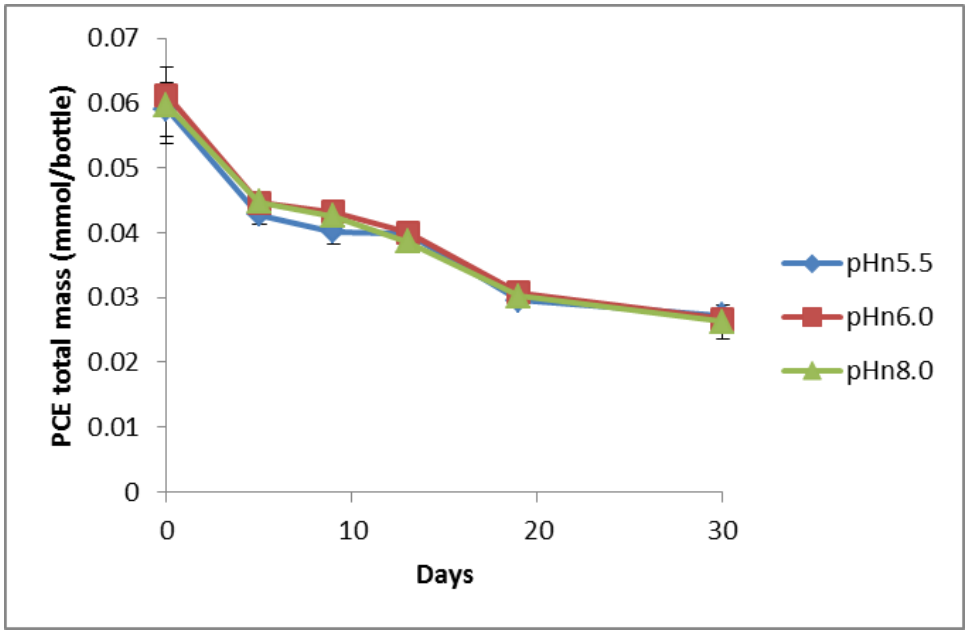


Figure 4.1 Reductive Dechlorination of PCE by Strain BB1 at different pH values[3]

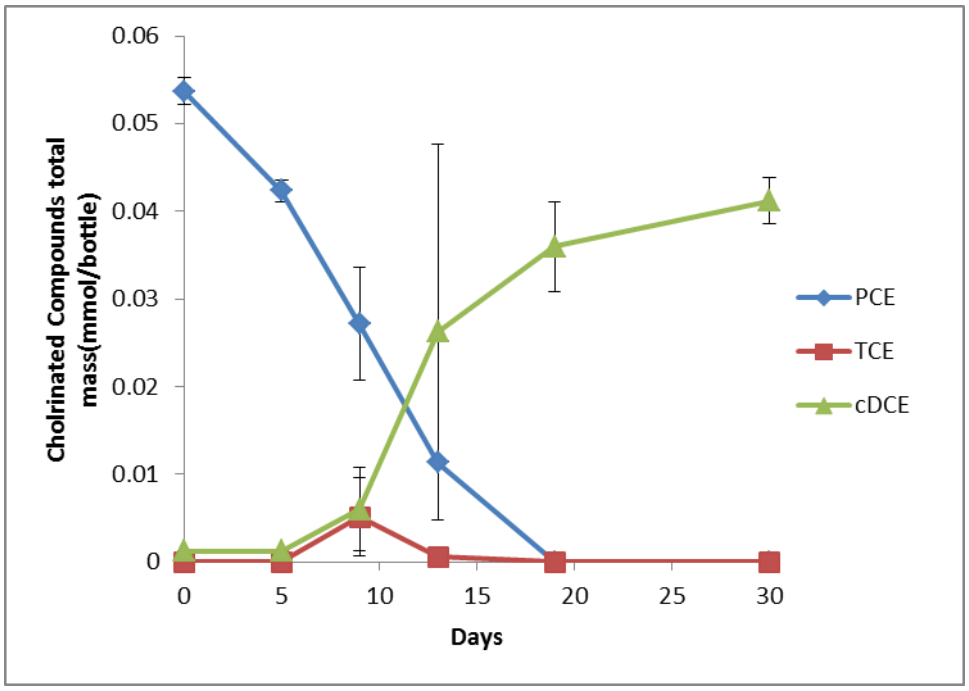


Figure 4.2 Reductive Dechlorination of PCE by Strain BB1 under pH_n 7.2

[3] Error bars indicating standard deviation in all figures.

4.1.2 BDI Consortium

The BDI consortium was maintained in a 160mL serum bottle before screening experiment. When PCE was degraded to *c*DCE, VC and ethene, aqueous cultures (3% v/v) were transferred to the new medium with targeted pH_n values 5.5, 6.0, 7.2 and 8.0. When pH_n values were around 6.00 and 7.20, PCE could be degraded. (See in Table 4.2) But after 50 days, there was no ethene production when pH_n was about 6.00 compared to the production of ethene when pH_n was around 7.20. No dechlorination activities were detected below pH_n 6.0 or above pH_n 8.0.

Table 4.2 Reductive Dechlorination by BDI at Different pH Values

Targeted pH_n	Day 1 (Initial Products)	Day 50(Final Products)	Comments
5.5	PCE	PCE	No dechlorination
6.0	PCE	cDCE,VC	Dechlorination. Final pH_m values were about 6.2.
7.2	PCE	cisDCE, VC, Ethene	Dechlorination.
8.0	PCE	PCE	No dechlorination

A second triplicate screening experiment was conducted. The initial pH_m values were 5.76±0.01, 6.02±0.00, 7.47±0.05 and 8.33±0.05. In Figure 4.3, it can be inferred that there were no dechlorination detected when pH_n values were about 5.50, 6.00 and 8.00; while BDI consortium could perform dechlorination around neutral pH (Figure 4.3 and 4.4). The pH_m values were measured again after the experiment, which were 5.81, 6.03, 7.37 and 8.20 respectively.

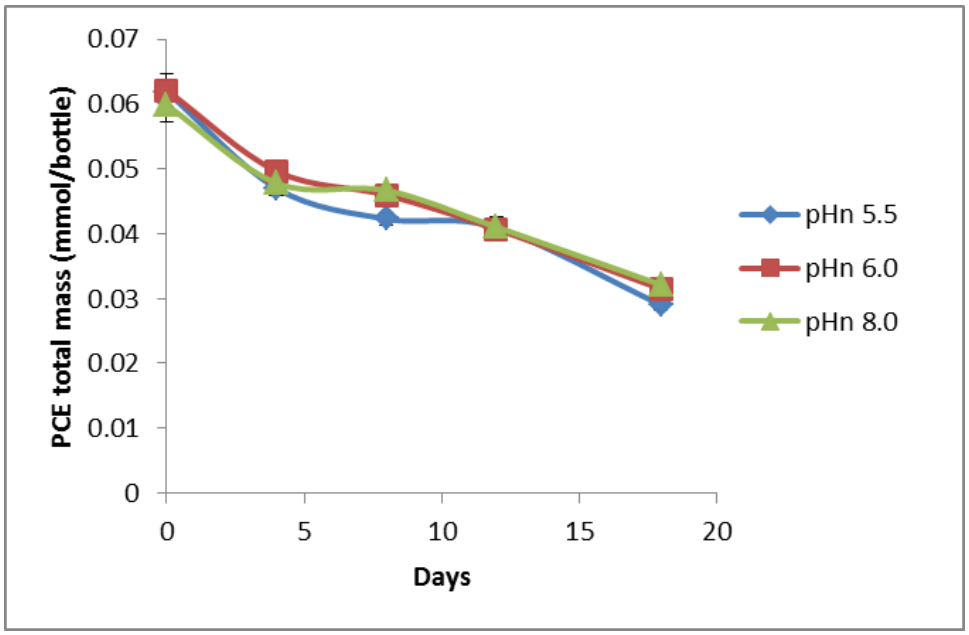


Figure 4.3 Reductive Dechlorination of PCE by BDI at different pH values

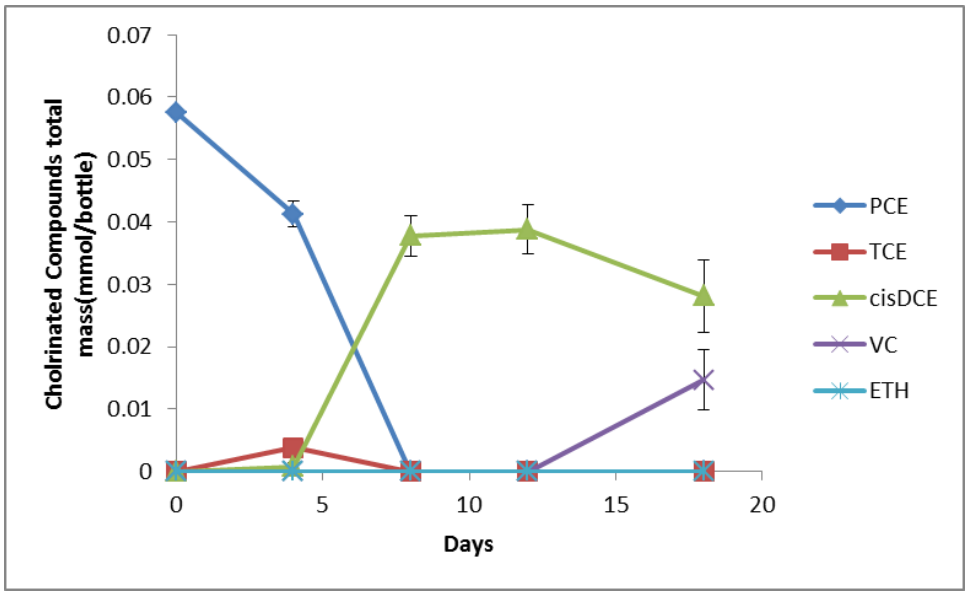


Figure 4.4 Reductive Dechlorination of PCE by BDI at pH_n 7.2

4.1.3 Sulfurospirillum multivorans

S. multivorans was screened for growth under different pH conditions. *S. multivorans* was fed with 2.5µL PCE (electron acceptor) and 5mM lactate plus 5mL H_2 (electron donors) in 160mL serum bottles. Without any lag phase, *S. multivorans* readily degraded PCE to *c*DCE within 24hours. Then *S. multivorans* was 3% (v/v) transferred to the new medium with different pH_n values (5.5, 6.0, 7.2, and 8.0, targeted). The initial pH_m values were around 5.77±0.01, 6.01±0.01, 7.31±0.11 and 8.38±0.02.

S. multivorans could degrade PCE to *c*DCE under pH_n 5.50, 6.00 and 7.20 within four days. (Figure 4.5, 4.6, and 4.7) It also degraded PCE at pH_n 8.00, but this process was much slower. (Figure 4.8) Within one week, only partial PCE can be degraded to TCE and *c*DCE. At the end of the experiment, pH_m values were verified to be 5.80, 6.05, 7.28 and 8.30 respectively.

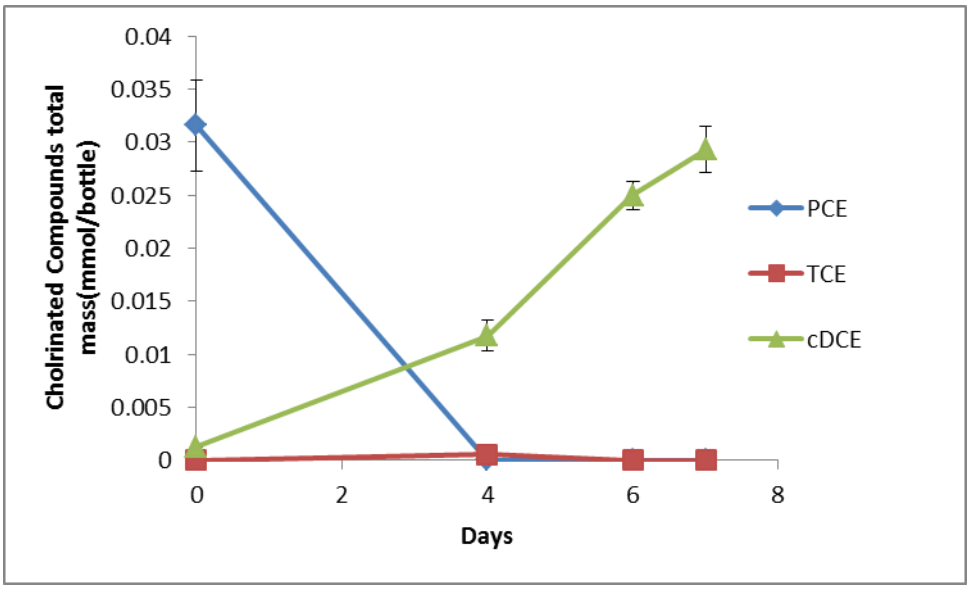


Figure 4.5 Reductive Dechlorination of PCE by *S. multivorans* at pH_n 5.5

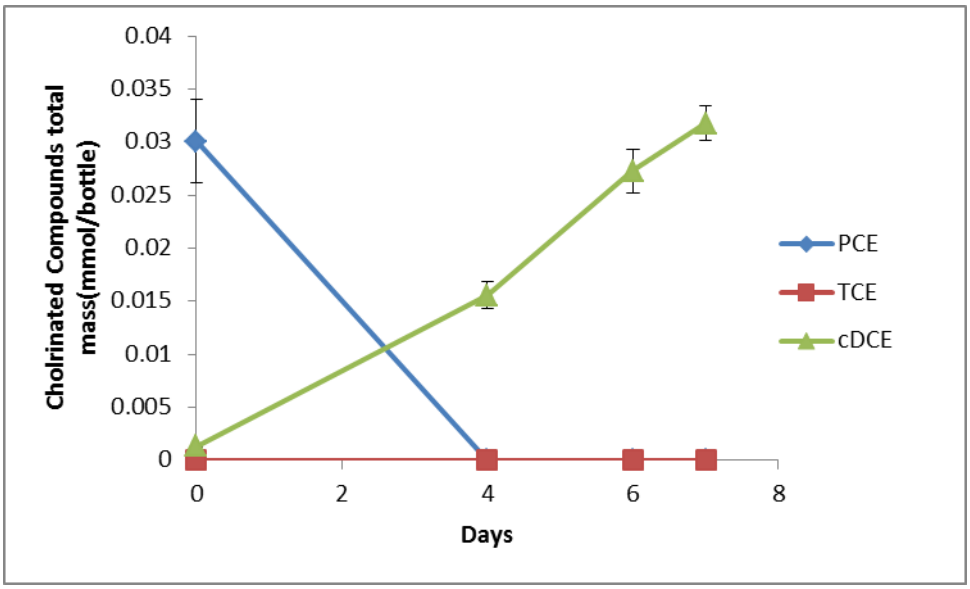


Figure 4.6 Reductive Dechlorination of PCE by *S. multivorans* at pH_n 6.0

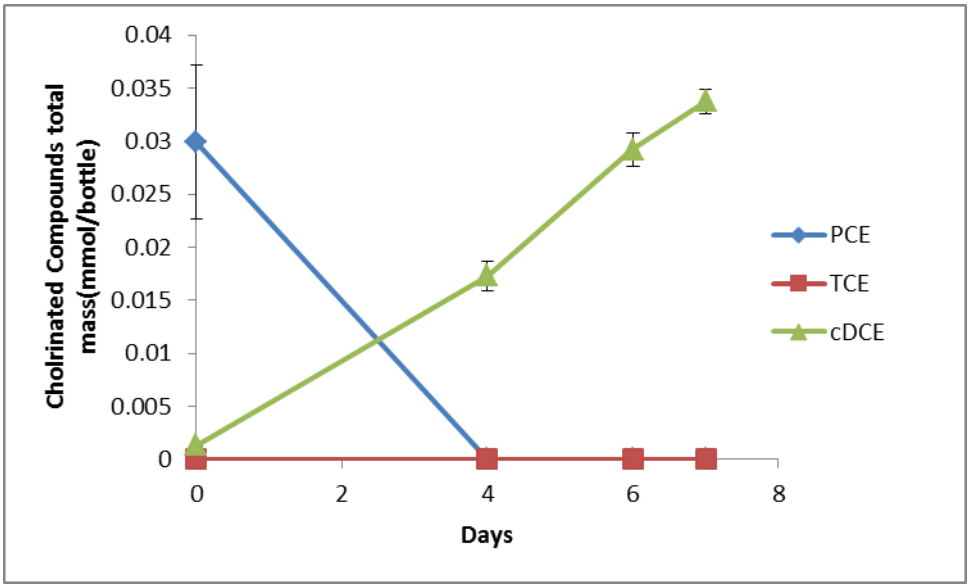


Figure 4.7 Reductive Dechlorination of PCE by *S. multivorans* at pH_n 7.2

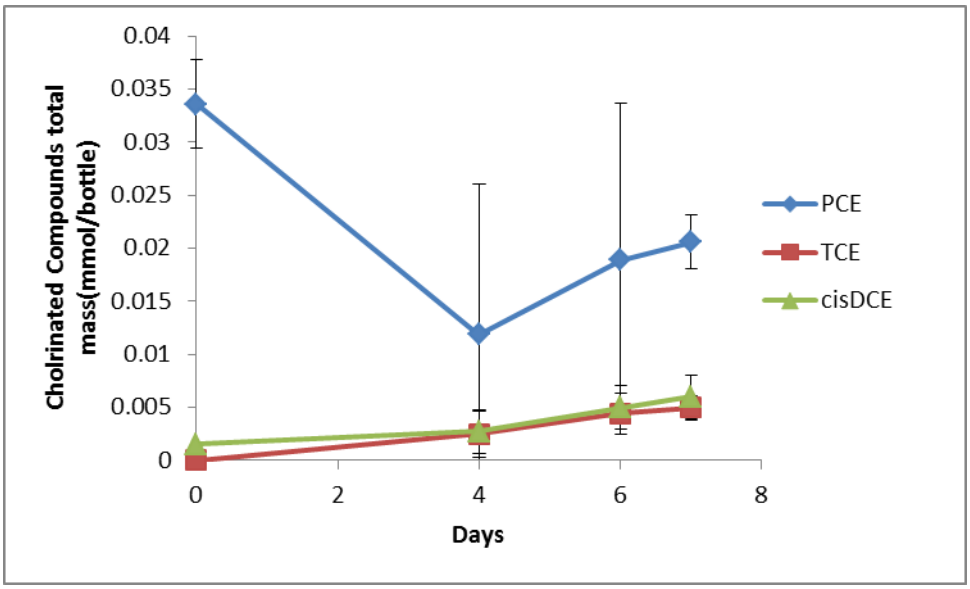


Figure 4.8 Reductive Dechlorination of PCE by *S. multivorans* at pH_n 8.0

For the interest of studying low pH dechlorination and verifying dechlorination activity below 6.0 by *S. Multivorans*, two continuous transfers were conducted. *S. multivorans* sustained to dechlorinate PCE to *c*DCE in these two transfers.

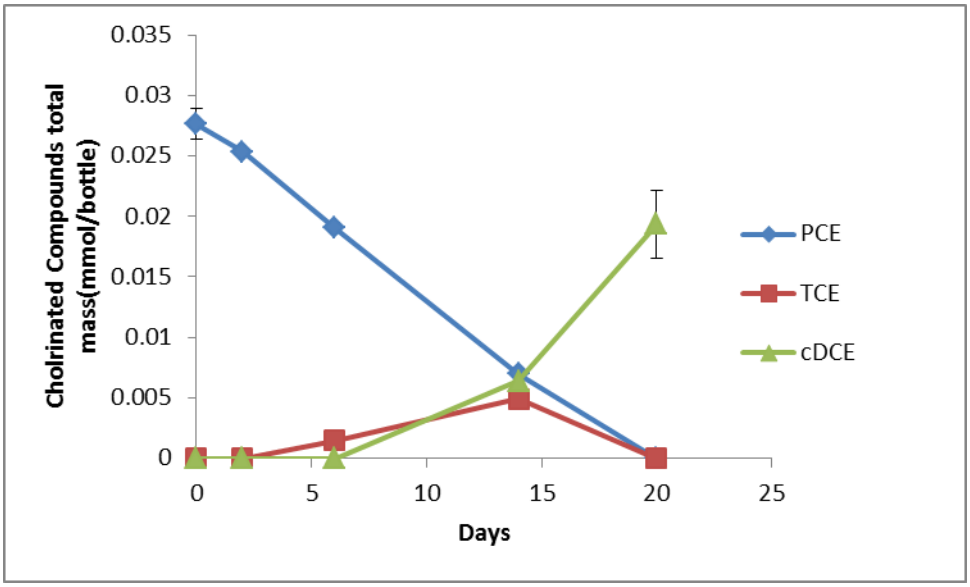


Figure 4.9 Reductive Dechlorination of PCE by *S. multivorans* at pH_m 5.5

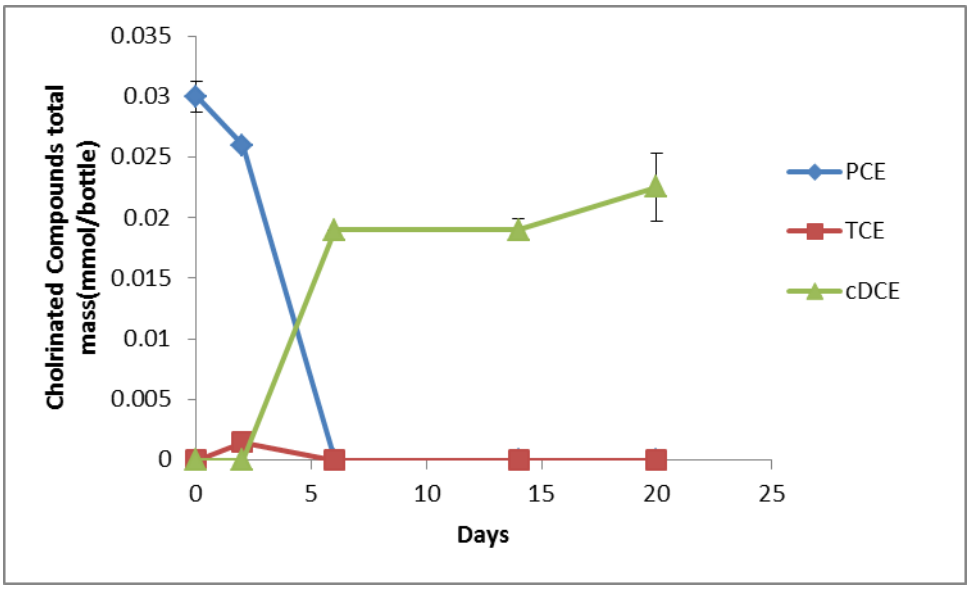


Figure 4.10 Reductive Dechlorination of PCE by *S. multivorans* at pH_m 5.8

In Figure 4.9, the pH was about 5.5; Dechlorination by *S. multivorans* was completed within three weeks. However, when the pH increased to about 5.8, the dechlorination rate was much faster. (See Figure 4.10) When pH was adjusted to 5, the *S. multivorans* can't dechlorinate PCE to *c*DCE. (See Figure4.11)

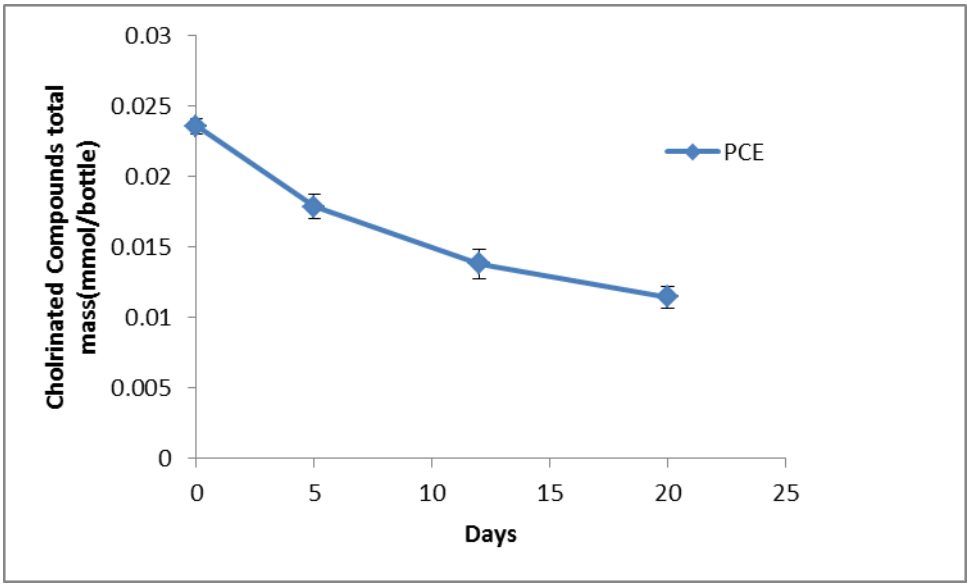


Figure 4.11 Reductive Dechlorination of PCE by *S. multivorans* at pH 5.0

4.1.4 Other Isolates

Strain Viet1 and JH1 showed the same pH range as strain BB1. Both of them can only dechlorinate PCE to TCE or *c*DCE at neutral pH value. No dechlorination activities were detected under other pH_n values (5.5, 6.0 and 8.0). (Appendix D)

Geobacter lovleyi strain SZ can dechlorinate PCE to *c*DCE within two days at room temperate and neutral pH with acetate provided as electron donor. When pH_n was around 6.0, PCE was degraded to *c*DCE within two weeks. No dechlorination activity was detected when pH_n values were 5.5 and 8.0 during two weeks monitoring. (Appendix D)

Generally, all of the dechlorinators tested can perform dechlorination around neutral pH range. However, no dechlorination can be detected below pH_n 6.0 or above 8.0 except *S. multivorans*. The optimum growth conditions for *S. multivorans* were pH (7.3-7.6) and temperature (30ºC). [80] Since *S. multivorans* can degrade PCE to *c*DCE on the edge of neutral pH range (5.5), it guaranteed the potential existence of other microbes which can survive at low pH and perform dechlorination activity.

Moreover, it is worth mentioning the effects of different buffer system on anaerobic microbial dechlorination activity. It was discussed above that, no dechlorination activities were detected above pH_n 8.0 when Tris buffer was used. Then HEPES buffer system, which has a pKa of 7.48 (at 25ºC) and widely used in cell culture, was used to maintain the pH_n of medium at 8.0. Strain Viet1, strain SZ, strain BB1 and BDI consortium could dechlorinate PCE to less chlorinated compounds slowly. And the pH_m values were stable at 8.25±0.02. The reasons for the differences are to be explored and explained by further experiments.

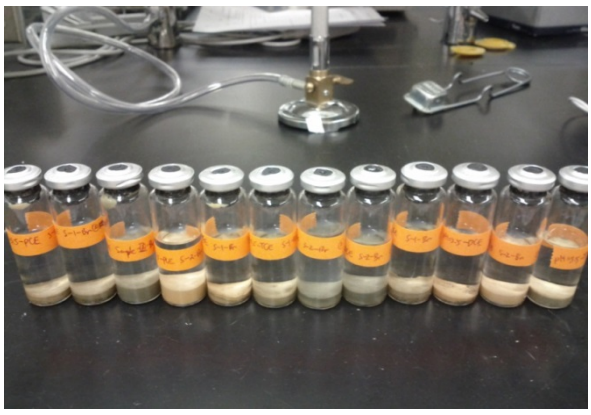
4.2 Microcosms Setup to Enrich or Isolate Microbes at Low pH

4.2.1 Microcosms with 20mL Vials

Microcosms were established in 20mL vials by using soil samples from Ft. Pierce site, PNNL site, DuPont's Oakley site, and Brazil. Different buffers were used to main the targeted pH values (5.5±0.2 and 7.2±0.2). (See Figure 4.12) Pure PCE, TCE or *c*DCE (*ca.* 1μL) were spiked into the microcosms to stimulate the growth of dechlorinators. Abiotic control groups, which were autoclaved twice, were established to exclude the possibility of abiotic dechlorination.

DuPont Samples

Ft. Pierce Samples



Brazil Samples

PNNL Samples

Figure 4.12 Microcosms Setup for Reductive Dechlorination Detection

After more than one year's monitoring, no dechlorination activities were detected in these microcosms. PCE, TCE or *c*DCE stayed in the vials without being degraded to less chlorinated compounds. Although there were some mass losses of these chlorinated compounds due to the absorption to the black stoppers or adsorption to the sediments in the microcosms and abiotic control groups, what interested and concerned us was chlorinated ethenes biodegradation. No further investigation was conducted yet.

4.2.3 Microcosms with 160mL Serum Bottles

For the convenience of experiment measurement, serum bottles (160mL) were used to establish microcosms for soil and groundwater samples (McGuire AFB soils and groundwater; and AS-MW-21 soil and groundwater samples) McGuire AFB and AS-MW-21 samples were used to establish microcosms. (See Figure 4.13) PCE and 5mM lactate+ 5mL hydrogen were served as the electron acceptor and electron donors respectively. The initial pH values were listed in Table 4.3. The pH values of groundwater were as low as about 3.9 for McGuire AFB samples and about 5.5 for AS-MW-21 sample. Although the groundwater pH values were low, indicating the potential of dechlorination activity at low pH, no dechlorination activity was detected in these microcosms after up to two months monitoring. PCE was not degraded into less chlorinated compounds.

Then another set of microcosms was established with TCE (electron acceptor) and 5mM lactate + 5mL hydrogen (electron donors). (See Figure 4.14) These microcosms have been monitored for up to two months. Less chlorinated compounds such as *c*DCE, VC and ethene were not detected in the microcosms yet. Although TCE concentrations decreased, it was speculated that the losses were due to the absorption and adsorption by black stoppers and sediments.

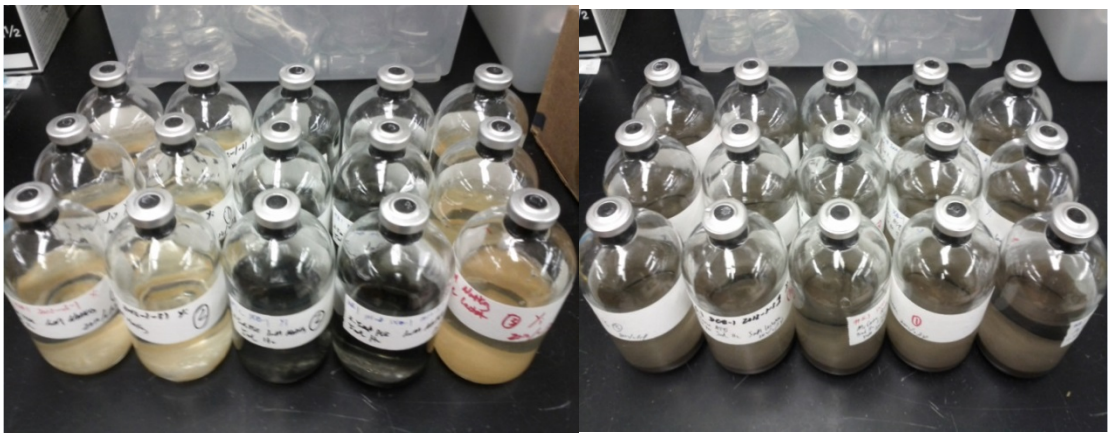


Figure 4.13 McGuire AFB (Below) and AS-MW-21(Above) Microcosms Spiked with PCE

Table 4.3 Initial pH Values of the Microcosms

Target	AS-MW-21 Samples pH_m (Triplicate)			McGuire AFB samples pH_m(Triplicate)		
MES Buffer (pH 5.5)	**5.73**	**5.73**	**5.73**	**4.25**	**4.47**	**4.16**
MES Buffer (pH 6.0)	**5.94**	**5.90**	**5.94**	**4.85**	**5.31**	**5.03**
Bicarbonate (pH 7.2)	**7.02**	**6.91**	**6.82**	**6.66**	**6.48**	**6.5**
Tris buffer (pH 8.0)	**7.64**	**7.63**	**7.68**	**6.27**	**5.92**	**6.07**
No buffer (Ground Water)	**5.42**	**5.47**	**5.52**	**3.92**	**3.87**	**3.96**

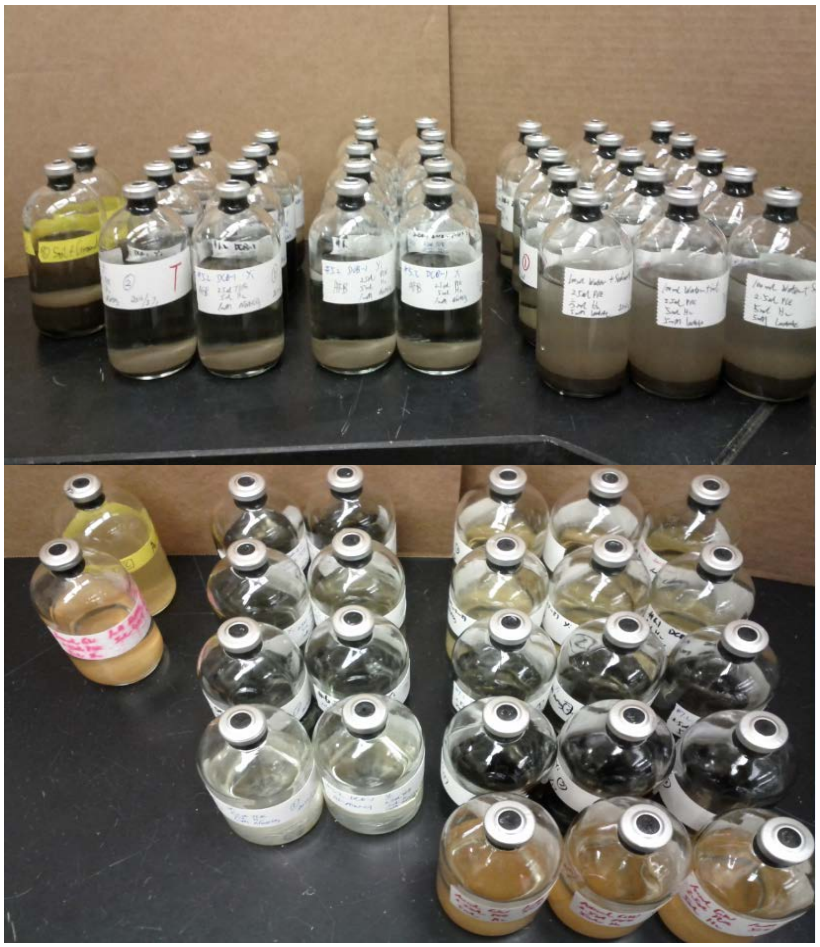


Figure 4.14 McGuire AFB (Above) and AS-MW-21(Below) Microcosms Spiked with TCE

4.2.3 Other Active Microcosms

4.2.3.1 Neckar River Samples

Neckar river samples were from Germany. When the soil samples arrived at the lab, replicate microcosms with different pH values (5.5±0.2 and 7.2±0.2) were set up in the glove box immediately in case of oxygen exposure. PCE and TCE were spiked into the microcosms respectively as electron acceptors. PCE and TCE could be degraded to ethene in all of the microcosms. And Neckar samples could degrade TCE to less chlorinated compounds more rapidly than PCE, which is

also seen in the later transfers when pH values were around 7.2. (Figure 4.15 and 4.16)

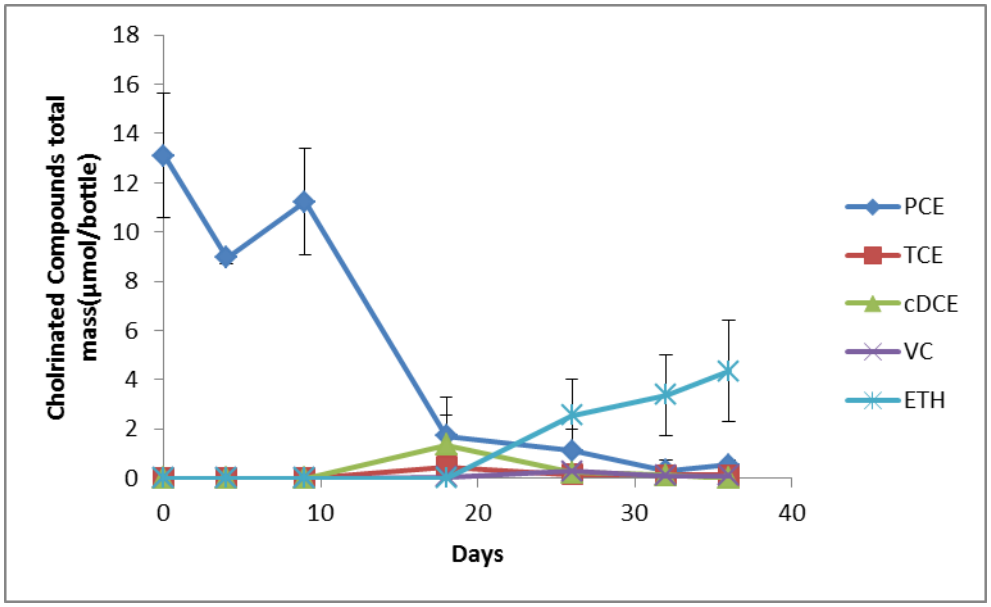


Figure 4.15 Neckar Sample Reductive Dechlorination of PCE at pH_n 7.2

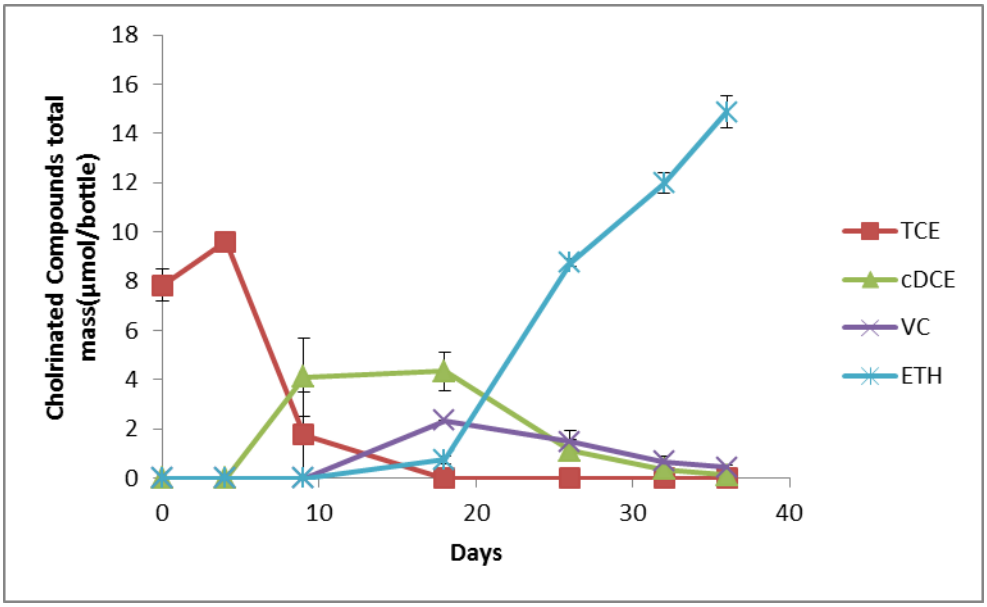


Figure 4.16 Neckar Sample Reductive Dechlorination of TCE at pH_n 7.2

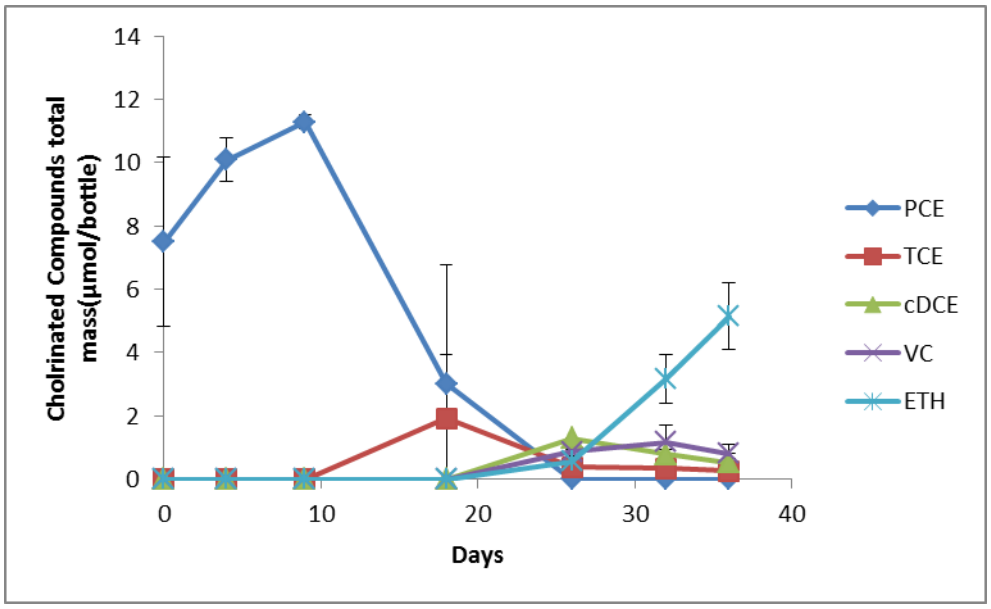


Figure 4.17 Neckar Sample Reductive Dechlorination of PCE at pH_n 5.5

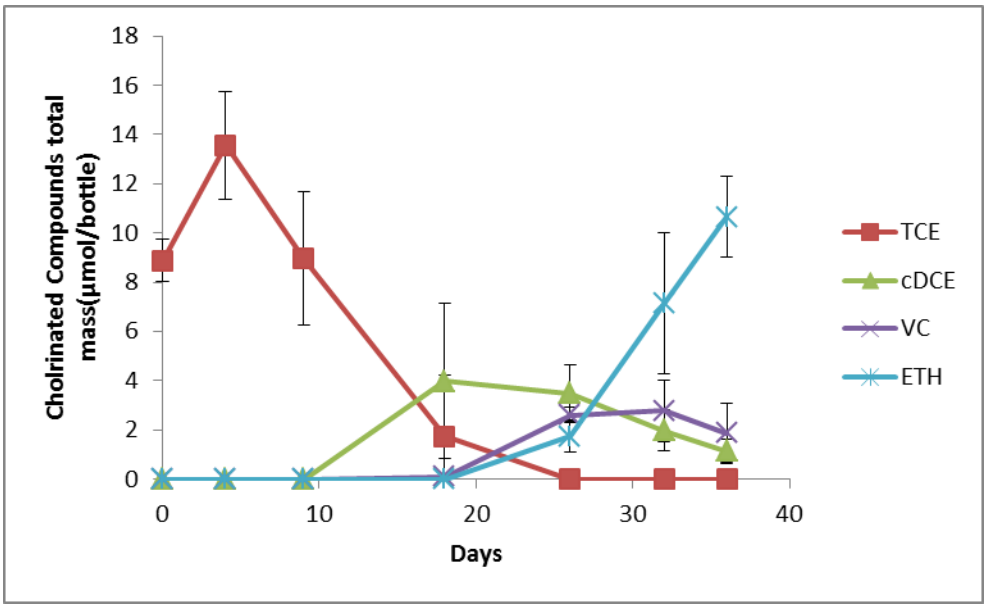


Figure 4.18 Neckar Sample Reductive Dechlorination of TCE at pH_n 5.5

After PCE and TCE were degraded, about 1% v/v liquids were transferred to the new medium spiked with PCE or TCE again in order to enrich consortium or isolate pure cultures which could perform dechlorination activity at low pH. When pH was 7.2±0.2, the dechlorination activity could maintain continuously. After 12th

transfer, PCE and TCE could still be dechlorinated to ethene. However, when pH was 5.5±0.2, the dechlorination activity can't sustain when sediments were removed from DCB-1 medium gradually. (Figure 4.17 and 4.18) After second transfer to the new medium with pH 5.5±0.2, only partial PCE and TCE could be degraded to *c*DCE, VC and ethene. When ethene appears in the vials, the third transfer was conducted. Third transfer had been monitored for about two months; no PCE and TCE dechlorination were detected in the vials. There were still some mass loss due to the absorption to the blacker stoppers. It was then speculated that sediments have an effect on the survival or dechlorination activities of these dechlorinators when the pH value was low. The dechlorinators could not perform dechlorination when the medium was sediment free. After another unsuccessful attempt to cultivate dechlorinators under pH5.5±0.2, pH6.0±0.2 media were proposed to cultivate Neckar samples. About 1% v/v liquids from original pH5.5±0.2 microcosms were transferred to the media with pH6.0±0.2. After three transfers, the Neckar river enrichment can only dechlorinate PCE or TCE to *c*DCE. VC and ethene could not be detected in the enrichment.

4.2.3.2 Rotenberg Trester and Creek samples

Rotenberg Trester and Creek samples, also from Germany, were pristine samples without exposure to known chlorinated contaminants before. pH_n 5.5 and pH_n 7.2 microcosms using Rotenberg Creek sediments were established. PCE and TCE were spiked into the vials. After up to two months' monitoring, no dechlorination activities were found in all of replicate microcosms. (Appendix E) No less chlorinated compounds or ethene had been detected in the microcosms. It was thought that no dechlorination can happen in these microcosms. However, after about eight months, TCE, *c*DCE and VC were detected in the microcosms spiked with PCE; and *c*DCE and VC were detected in the microcosms spiked with TCE.

Microcosms with different pH values (5.5 and 7.2) using Rotenberg Trester sediments were set up and monitored weekly by measuring headspace with GC. Dechlorination activities were detected after one month in all replicate microcosms. *c*DCE, *t*DCE and VC were the degradation products. (Appendix E) In some of the microcosms, ethene was detected. Further experiments were in progress for enrichment and identifying the dechlorinators in the microbial community.

4.2.3.3 Third Creek Samples

Third Creek was exposed to chlorinated contaminants in history. And aqueous sediments were taken to the lab to established microcosms with different pH values (5.5±0.2 and 7.2±0.2). (Appendix E) As same as Neckar River samples, Third Creek samples have been maintained for 12 transfers when the pH of the medium was 7.2. Although dechlorination activities were detected in the initial microcosms with pH5.5, the dechlorination activity also could not sustain after three transfers (sediment free).

Up to now, more than 10 different geographical soil or groundwater samples have been used to establish microcosms to enrich or isolate dechlorinators which can perform dechlorination at low pH. No enrichments have been obtained to dechlorinate PCE or TCE at low pH. And no isolates or enrichment have been reported to be capable of dechlorinating PCE or TCE to ethene when the medium is sediment free. More efforts are needed to enrich or isolate these microbes.

4.3 BDI Tolerance at Low pH

BDI consortium has been successfully applied to field experiments, and it is promising to be used in other *in situ* bioaugmentation or bioremediation. However, the decreasing pH *in situ* may affect the dechlorination performance of BDI. This

experiment was to investigate the tolerance of BDI consortium at low pH. As is shown in Figure 4.19, there was no dechlorination by BDI when the pH of medium was about 5.5. No less chlorinated compounds were detected in the bottles.

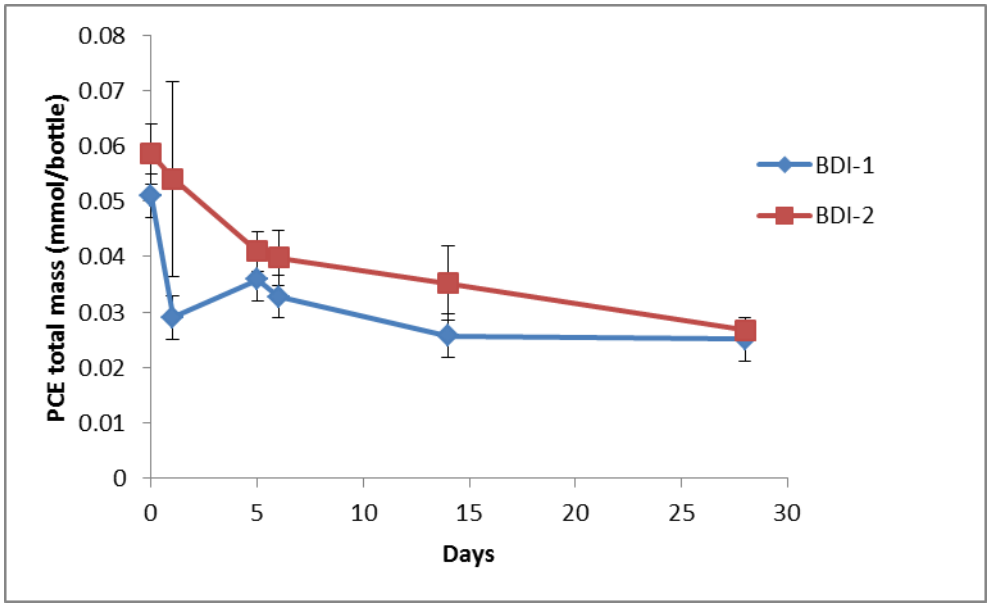


Figure 4.19 BDI Exposed to pH 5.5 DCB-1 Medium

After 1 week, 2 weeks, 4 weeks and 8 weeks, biomass were collected and transferred back to neutral pH to investigate whether BDI can recover dechlorinating PCE. As Figure 4.20-4.26 show, BDI could recover from up to 8 weeks' exposure to low pH condition. However, VC was the end product, compared with ethene supposedly. The reason why ethene was not produced was unknown. The possible explanation was that low pH condition has an effect on the microbes which were responsible for biotransforming VC to ethene.

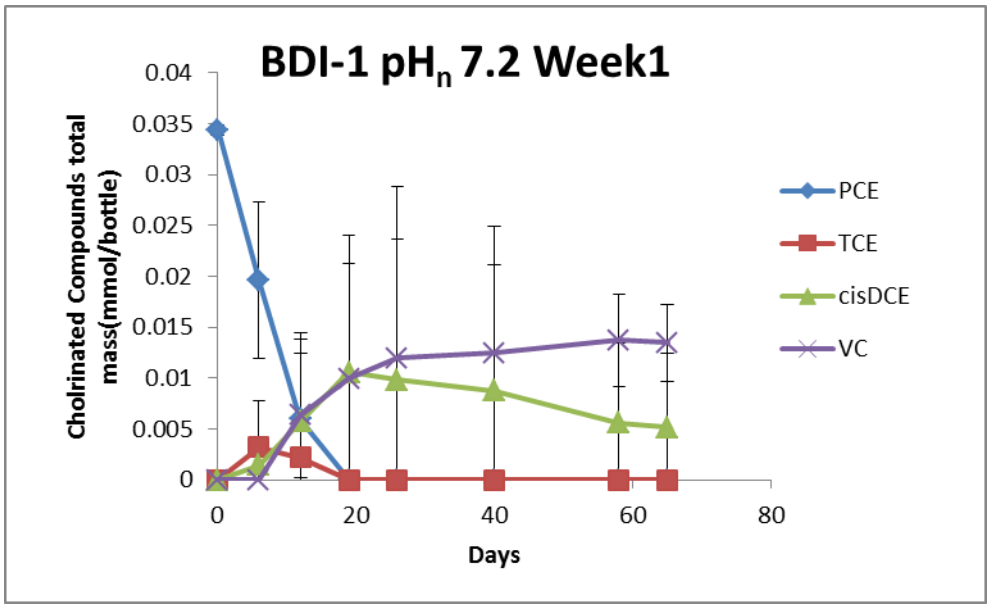


Figure 4.20 Reductive Dechlorination of PCE by BDI after 1 week's Low pH Exposure

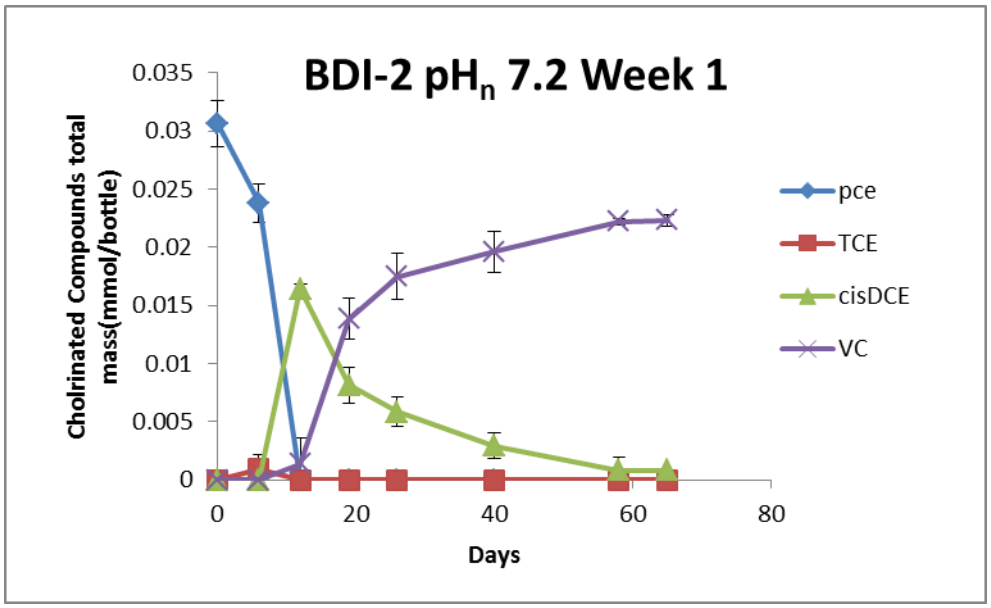


Figure 4.21 Reductive Dechlorination of PCE by BDI after 1 week's Low pH Exposure

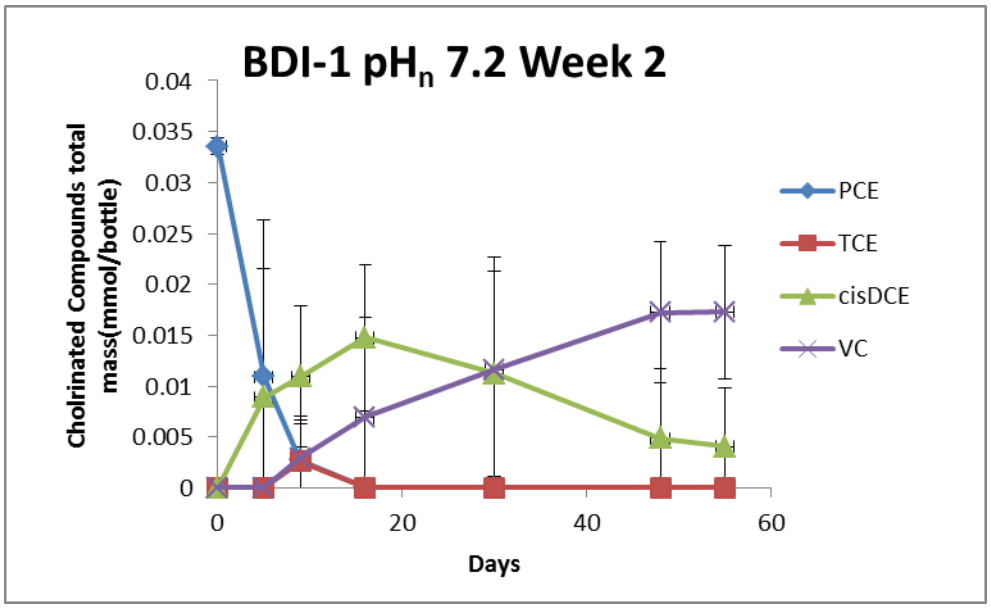


Figure 4.22 Reductive Dechlorination of PCE by BDI after 2 weeks' Low pH Exposure

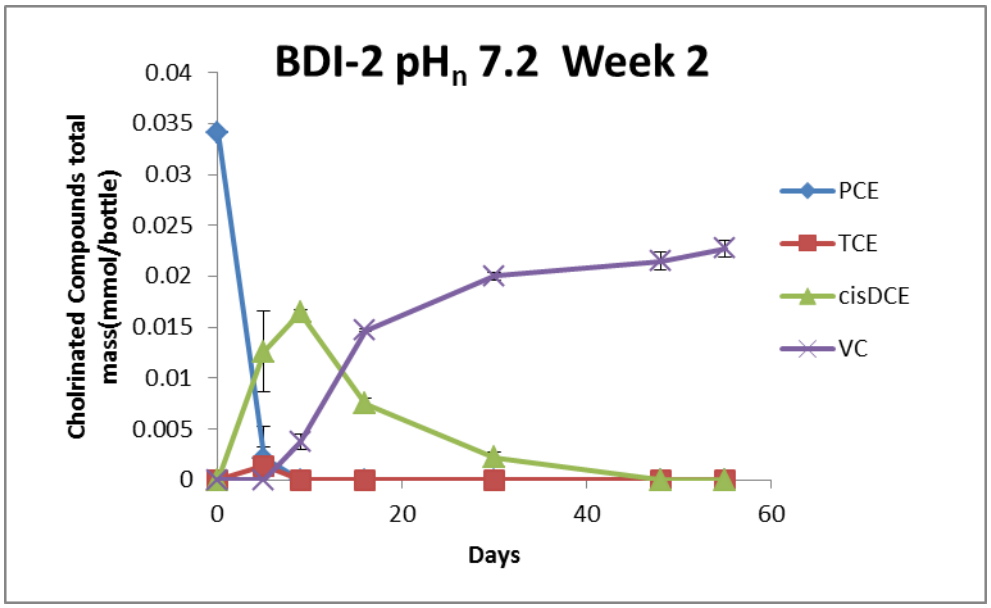


Figure 4.23 Reductive Dechlorination of PCE by BDI after 2 weeks' Low pH Exposure

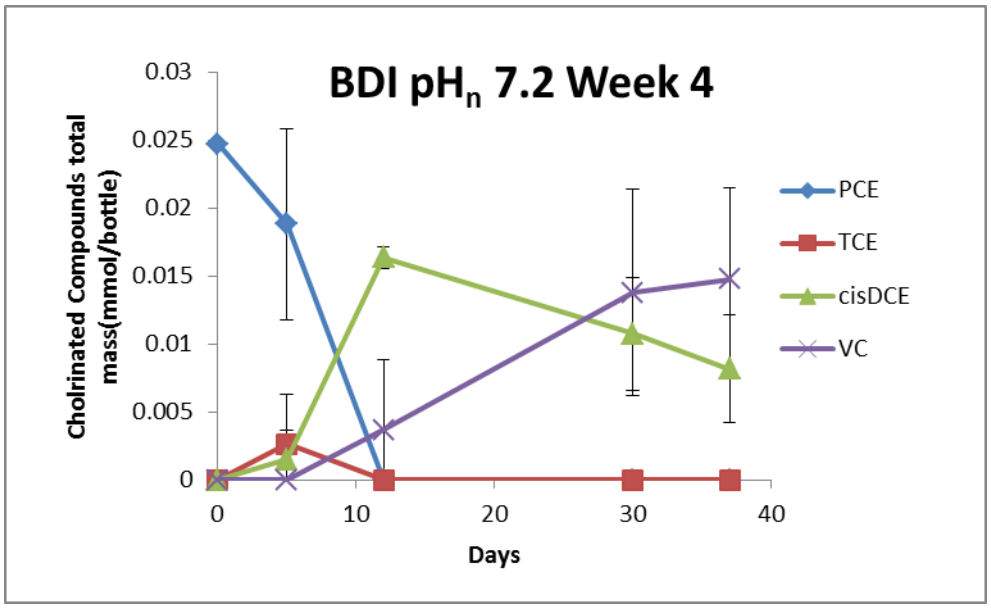


Figure 4.24 Reductive Dechlorination of PCE by BDI after 4 weeks' Low pH Exposure

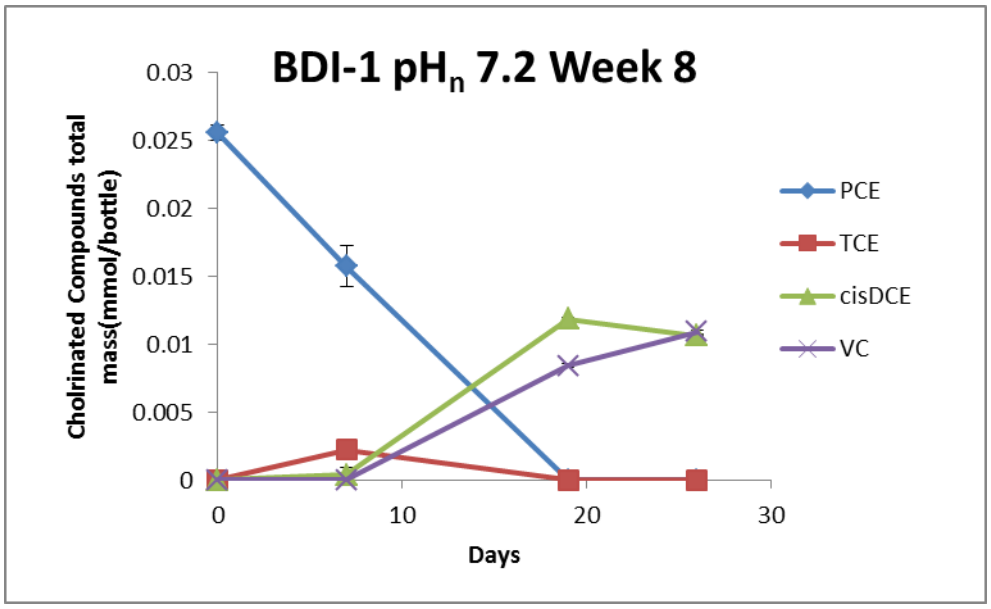


Figure 4.25 Reductive Dechlorination of PCE by BDI after 8 weeks' Low pH Exposure

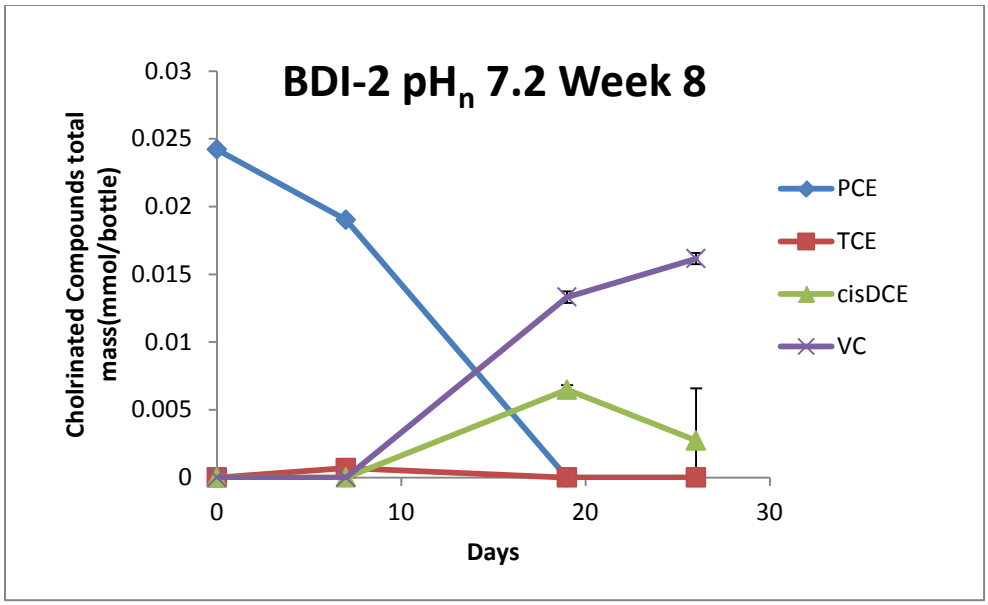


Figure 4.26 Reductive Dechlorination of PCE by BDI after 8 weeks' Low pH Exposure

pH stress can be defined as 'survival outside the pH range of growth with recovery of colony-forming capacity after the pH is brought within the growth range'.[85, 118] And resistance to low pH condition of a strain also depends on the degree of acidity designated 'extreme', the time of exposure, and the death rate.[85, 118] In this experiment, BDI has been exposed to pH 5.5 for up to 8 weeks. BDI could not perform dechlorination activity at low pH. But after brought back to neutral pH, ability of BDI to dechlorinate PCE to VC was recovered. However, VC-to-ethene step was affected by exposure to low pH.

4.4 Enhanced Performance of BDI by Incorporation of Sterile Soils

From previous microcosm experiments, it was shown that soils or sediments could help microbes survive low pH condition to perform dechlorination. In this experiment, the aim was to investigate BDI performance by incorporation of sterile soils. As Figure 4.27 shows, no dechlorination activities were detected in

the medium only and medium with sand only with up to 150 days monitoring (pH_m=5.5±0.2). By comparison, PCE could be degraded to ethene in the bottles amended with autoclaved Third Creek samples after about 150 days incubation. (See Figure 4.28)

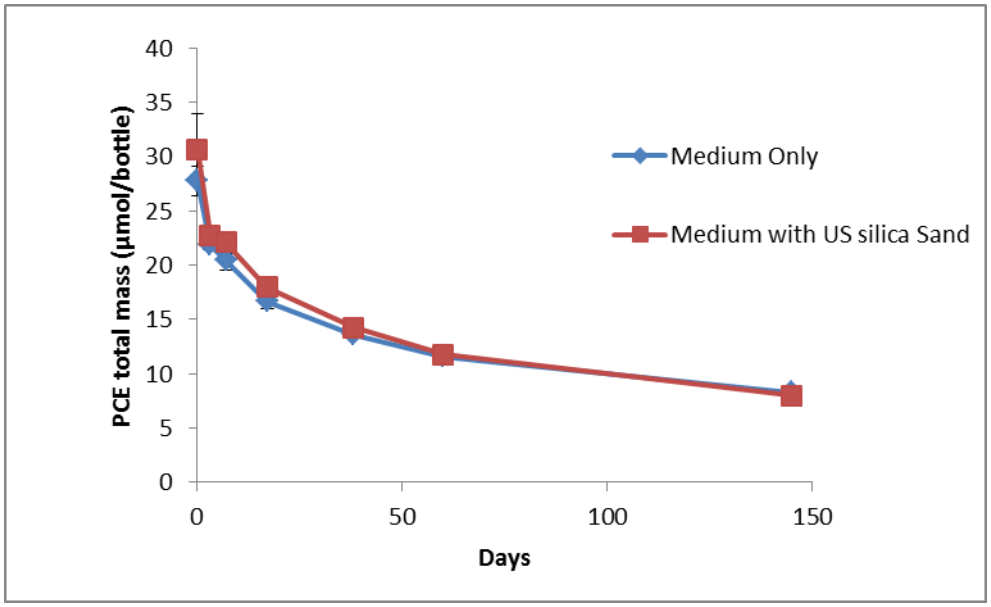


Figure 4.27 Reductive Dechlorination of PCE by BDI without Solids or with Sands

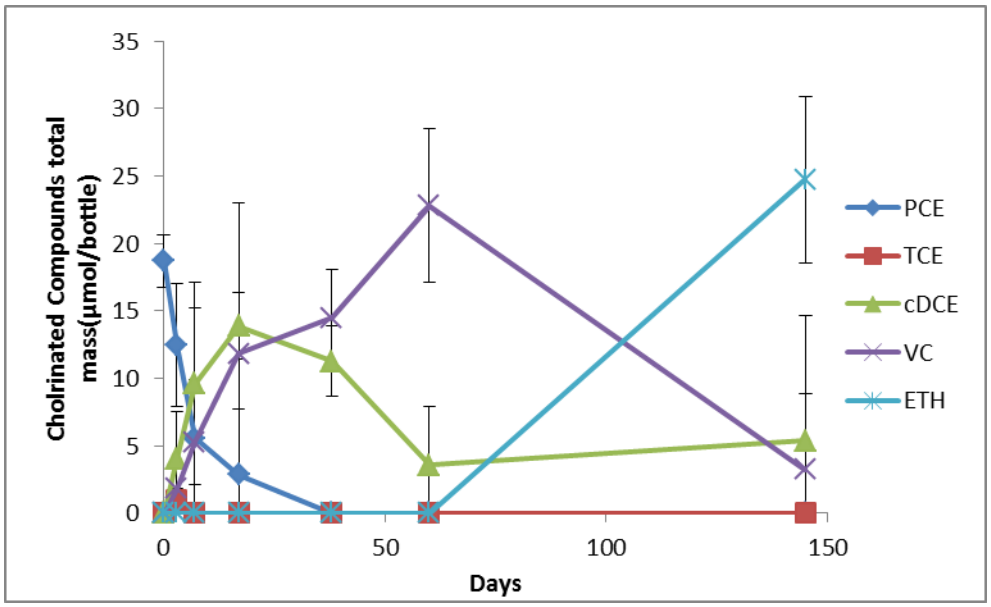


Figure 4.28 Reductive Dechlorination of PCE by BDI with Third Creek Sediments

For the verification of Third Creek sediments effect on the dechlorination activity, another triplicate 160mL bottles were set up. BDI can perform dechlorination activity with the addition of sediments, even though the pH was as low as 5.5±0.2. However, ethene was not produced within 40 days monitoring. (See Figure 4.29) It has been reported that dechlorination activities had been improved in UASB by incorporation of granules in the reactors. It was analyzed that granules could help microbes form biofilms attached to the granules, which enhanced the microbial dechlorination activities. However, why BDI could not perform dechlorination at the presence of sand was interesting and unresolved.

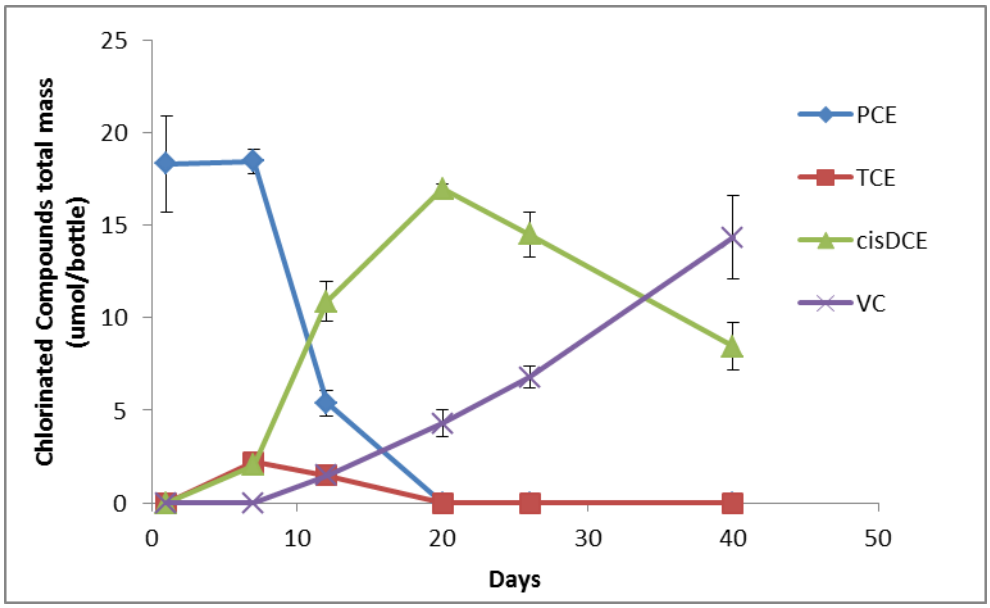


Figure 4.29 Reductive Dechlorination of PCE by BDI with Third Creek Sediments

It was suggested by some researchers that 'soil-microorganism complex should be considered an integrated, self-organizing system that can remodel its state in response to change' due to the importance of interactions in soil system dynamics. [119] Interaction between soils or sediments and microorganism has been of interests to soil microbiology. However, due to limited sampling and

sample processing methods, these studies are of difficulties. More methods are needed to explore the mechanisms and interactions between microbes and sediments as regard to microbial anaerobic reductive dechlorination at low pH.

CHAPTER V
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

From above experiments to explore anaerobic reductive dechlorination at low pH, preliminary conclusions can be generalized:

- Various pure strains and consortium, generally, have neutral pH ranges for dechlorination activity. Most of them can only perform reductive anaerobic dechlorination at neutral pH. Only *S. multivorans* can dechlorinate PCE to *c*DCE at low pH (pH 5.5). But this dechlorinator cannot perform dechlorination at pH 5.0. *S. multivorans* will be a promising candidate to be applied *in situ* bioremediation at low pH. BDI cannot perform dechlorination activities at low pH, which indicates the inhibition of *Dehalococcoides mccartyi*, *Geobacter spp.* and other bacteria.

- Although there were differences between experiments in the lab and *in situ*, microcosm experiment is still a good method to infer the possibility of *in situ* dechlorination activity by using field soil samples. It was difficult to enrich and isolate microbes which can dechlorinate PCE or TCE below pH 6.0. It is possible that some microbes can dechlorinate PCE to *c*DCE on the lower edge of neutral pH range. What is more interesting and important is to find microbes that can dechlorinate chlorinated ethenes to non-toxic ethene at low pH.

- From enrichment experiments, Neckar samples and Third Creek samples dechlorinated PCE to ethene at low pH in the presence of soils or sediments. But the dechlorination ability was lost at low pH when soils and

sediments were removed from medium after several transfers. So it should be cautious to generate conclusion of dechlorination activity at low pH condition. More efforts are needed to enrich or isolate microbes which can perform anaerobic reductive dechlorination at low pH.

- BDI can recover from long time exposure to low pH environment. (Up to 8 weeks) It can be indicated that the dechlorinators in BDI were only inhibited rather than killed. Although low pH has an inhibition effect on dechlorination, it did not kill these dechlorinators.

- BDI can dechlorinate PCE to VC by incorporation of abiotic sediments when pH was as low as 5.5. However, sand alone cannot provide effective shelter for BDI enrichment to biodegrade PCE to less chlorinated compounds, which is to be explained by further experiments.

5.2 Recommendations for Future Research

- More samples from *in situ* previously contaminated with chlorinated compounds, especially low pH sites will be used to establish more microcosms to investigate the potential low pH dechlorination.

- Due to the diversity of dechlorinators, it is of interests to test acidophiles to see whether they can perform anaerobic dechlorination at low pH.

- Why sediments can help dechlorinators to perform dechlorination? What is the mechanism and interaction between soils and dechlorinators?

- Molecular tools, such as polymerase chain reaction (PCR), real-time polymerase chain reaction (qPCR), terminal restriction fragment length polymorphism (T-RFLP), temperature Gradient Gel Electrophoresis (TGGE), denaturing Gradient Gel Electrophoresis (DGGE) and etc., high throughput sequence and applied bioinformatics tools can provide promising tools to investigate the change of community structure by pH stress.

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APPENDIX

Appendix A DCB-1 Medium

A-1: Constituents of DCB-1 medium (from Lab protocols written by Dr. Frank Loeffler)

- **Salts solution:**

Table A.1 Constituents of Salts Solution

Salts	1 x [g/L]	100 x stock [g/L]
NaCl	1.0	100.0
MgCl2 x 6 H2O	0.5	50.0
KH2PO4	0.2	20.0
NH4Cl	0.3	30.0
KCl	0.3	30.0
CaCl2 x 2 H2O	0.015	1.5

- **Trace element solution**

Per liter: HCl (25% solution, w/w), 10 ml; FeCl2 x 4 H2O, 1.5 g; CoCl2 x 6 H2O, 0.19 g; MnCl2 x 4 H2O, 0.1 g; ZnCl2, 70 mg; H3BO3, 6 mg; Na2MoO4 x 2 H2O, 36 mg; NiCl2 x 6 H2O, 24 mg; CuCl2 x 2 H2O, 2 mg

- **Se/Wo solution**

Per liter: 6 mg Na2SeO3 x 5 H2O, 8 mg Na2WO4 x 2 H2O and 0.5 g NaOH

- **Wolin Vitamins**

Wolin, F. A., M. J. Wolin, and R. S. Wolfe. 1963. Formation of methane by bacterial extracts. J. Biol. Chem. **238:**2882-2886.

Table A.2 Constituents of Vitamin Solutions

Vitamins	1000 x [mg/L]	Final conc.
biotin	20 mg/L	0.02 mg/L
folic acid	20 mg/L	0.02 mg/L
pyridoxine hydrochloride	100 mg/L	0.1 mg/L
riboflavin	50 mg/L	0.05 mg/L
thiamine	50 mg/L	0.05 mg/L
nicotinic acid	50 mg/L	0.05 mg/L
pantothenic acid	50 mg/L	0.05 mg/L
vitamin B12	1 mg/L	0.001 mg/L
p-aminobenzoic acid	50 mg/L	0.05 mg/L
thioctic acid	50 mg/L	0.05 mg/L

A-2: Preparation of DCB-1 medium

1) In a 3L three-neck, round bottom flask, add the chemicals listed in step 1 of Table A.2.
2) Boil and then cool down the medium to room temperature under flushing with N2 (for PH 5.5, 6 and 8.0 medium) or with N2/CO2=80/20 (for PH 7.2 medium).
3) Add the chemicals listed in step 3 of Table A.3.
4) Then adjust the PH to targeted PH with HCL/NaOH (for PH 5.5, 6 and 8.0 medium) or with CO2 (for PH 7.2 medium).
5) Flush 100ml serum bottles with N2 (for PH 5.5, 6 and 8.0 medium) or with N2/CO2=80/20 (for PH 7.2 medium).
6) Dispense medium and close the bottles with black rubber stoppers.
7) Add 0.1ml NaHCO3 to the PH 5.5, 6 and 8.0 medium.
8) Autoclave the medium.
9) After medium turning to clear and cool down, add 0.1ml vitamin stock solution to the serum bottles.

Table A.3 Preparation of DCB-1 Medium

DCB-1 media									
Steps		1L Media				2L Media			
Step 1	100xSalts (ml)	10				20			
	Trace Elements (ml)	1				2			
	Se/Wo solutions (ml)	1				2			
	Resazurin<0.1%> (ml)	0.25				0.5			
	Electon donor (0.5 mM 60% w/w lactate)(g)	0.934				1.868			
	H_2O	Up to 1L				Up to 2L			
Step 2	Ph	5.5	6	7.2	8	5.5	6	7.2	8
	Boil&Cool	N_2	N_2	N_2&CO_2	N_2	N_2	N_2	N_2&CO_2	N_2
Step 3	0.2mM L-cystein (175.63g/mol)	0.035				0.07			
	0.2mM Na_2S	0.048				0.096			
	0.5mM DL-dithiothreito	0.077				0.154			
	Buffer	25mM MES		30mM $NaHCO_3$	25mM Tris-Base	25mM MES		30mM $NaHCO_3$	25mM Tris-Base
	(g/L)	4.88		0.084	3.03	9.76		0.168	6.06
	1M $NaHCO_3$ (ml)	0.1		N/A	0.1	0.1		N/A	0.1
Step 4	Vitamin (1000x) (ml)	0.1 in 100ml Serum bottles				0.1 in 100ml Serum bottles			

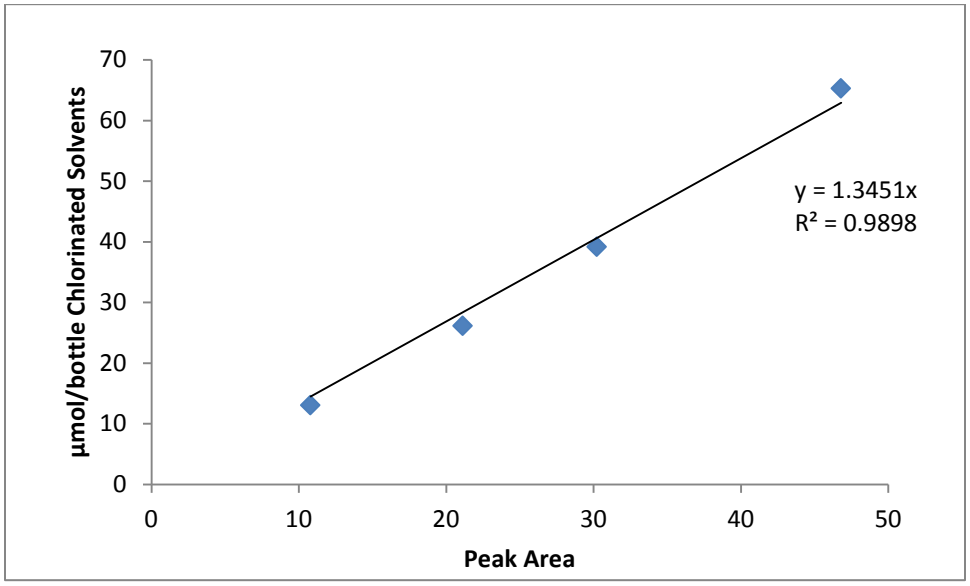
Appendix B GC Conversion Factors and Calibration Curves

Table B.1 GC Peak Area versus Chlorinated Solvents Total Mass/Bottle

Conversion Factor						
GC1 (umol/bottle/PA)	160ml Headspace	R^2	160ml Liquid	R^2	20ml Headspace	R^2
PCE	0.8207	0.9976	1.3451	0.9898	0.2516	0.9946
TCE	0.7968	0.9994	0.9048	0.999	0.139	0.9942
cis-DCE	1.5127	0.9991	0.7907	0.9993	0.1642	0.9932
VC	0.3203	0.9944	1.1841	0.996	0.0466	0.9968
Ethene	0.1449	0.9907	4.4732	0.9985	0.0272	0.9971
GC2 (umol/bottle/PA)	160ml Headspace	R^2	160ml Liquid	R^2	20ml Headspace	R^2
PCE	0.6505	0.9743	N/A	N/A	0.2425	0.9901
TCE	0.7173	0.9975	N/A	N/A	0.1408	0.994
cis-DCE	1.4399	0.999	N/A	N/A	0.169	0.9905
VC	0.3192	0.9979	N/A	N/A	0.0458	0.9951
Ethene	0.1441	0.9955	N/A	N/A	0.0274	0.9966

Table B.2 GC Peak Area versus Chlorinated Solvents Concentration in Liquid

Conversion Factors						
GC1(Mm/PA)	160 Headspace	R^2	160 Liquid	R^2	20 Headspace	R^2
PCE	0.0061	0.9976	0.0099	0.9898	0.0158	0.9946
TCE	0.0067	0.9994	0.0076	0.999	0.0105	0.9942
cis-DCE	0.014	0.9991	0.0073	0.9993	0.0144	0.9932
VC	0.002	0.9944	0.0075	0.996	0.0024	0.9968
Ethene	0.0002	0.9907	0.0068	0.9985	0.0003	0.9971
GC2(mM)	160 Headspace	R^2	160 Liquid	R^2	20 Headspace	R^2
PCE	0.0048	0.9743	N/A	N/A	0.0152	0.9901
TCE	0.006	0.9975	N/A	N/A	0.0106	0.994
cis-DCE	0.0133	0.999	N/A	N/A	0.0149	0.9905
VC	0.002	0.9979	N/A	N/A	0.0023	0.9951
Ethene	0.0002	0.9955	N/A	N/A	0.0003	0.9966



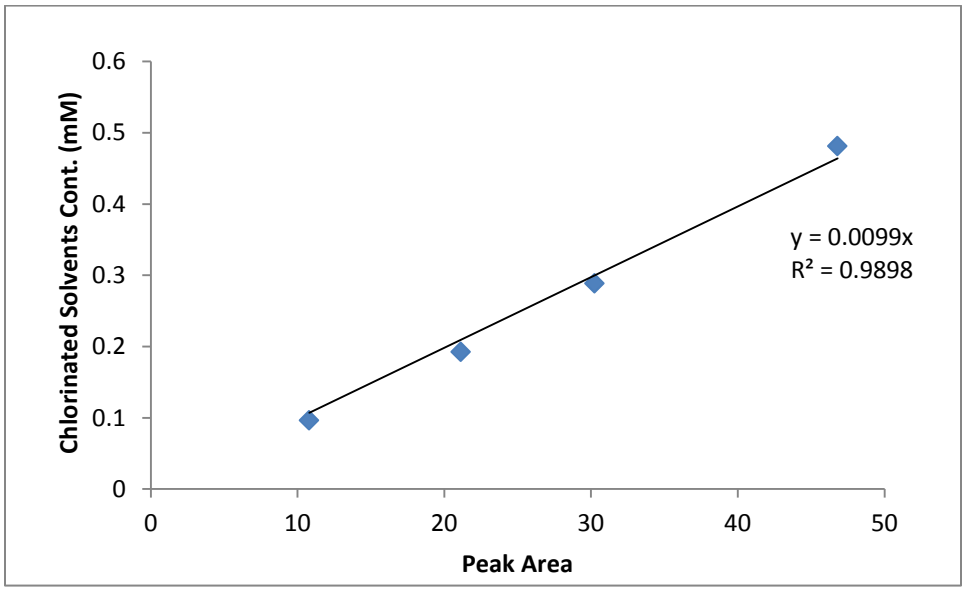
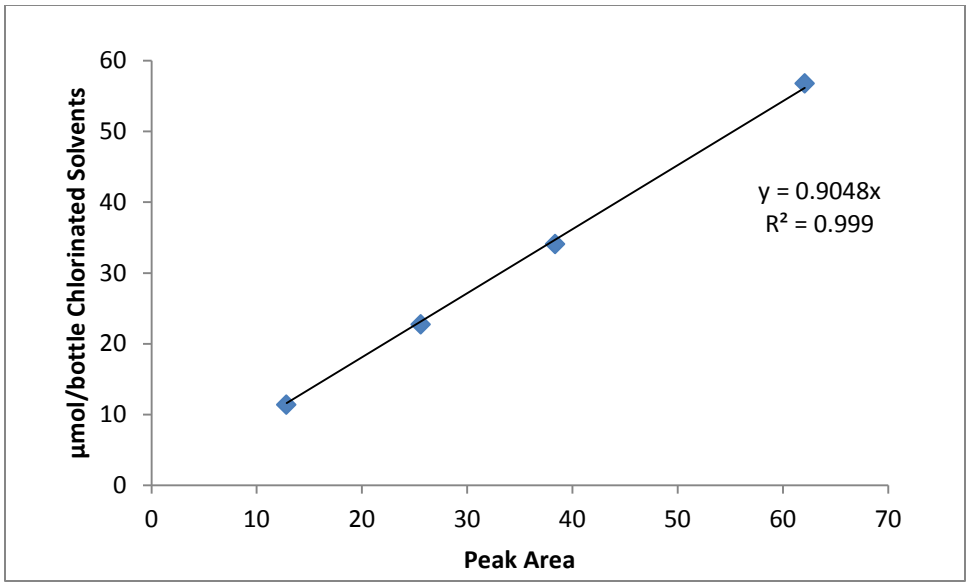


Figure B.1 Calibration Curve for PCE (GC1-1mL Autosample from 160mL Serum Bottle)



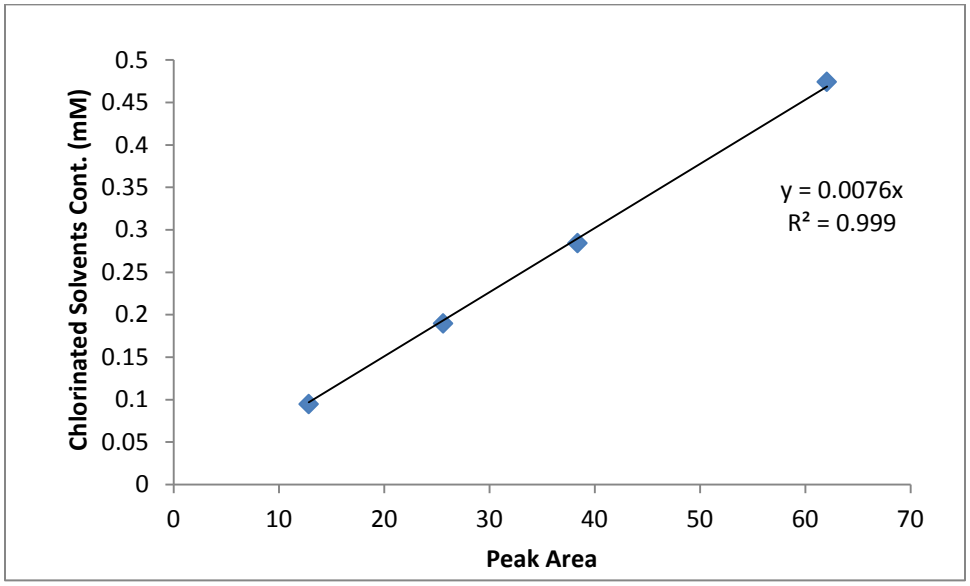
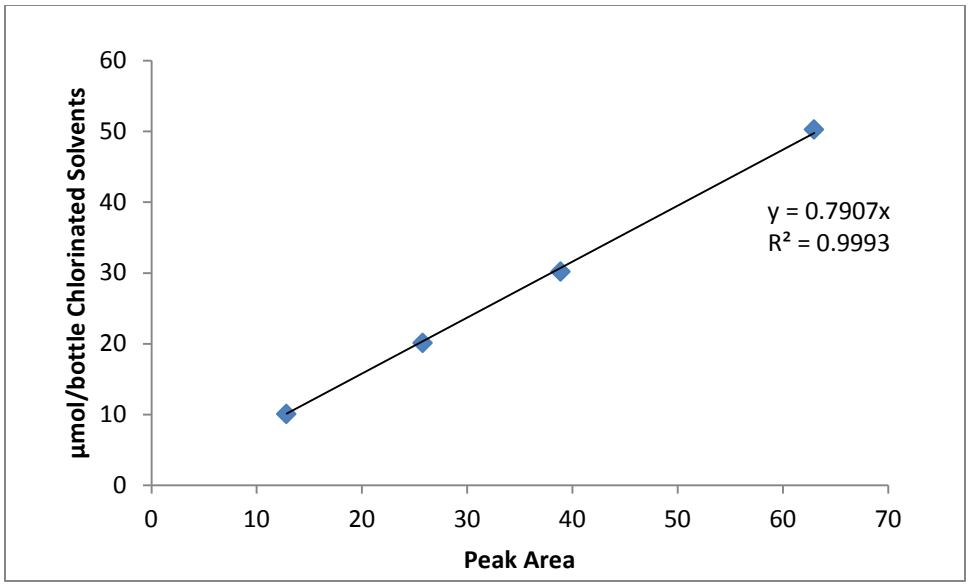


Figure B.2 Calibration Curve for TCE (GC1-1mL Autosample from 160mL Serum Bottle)



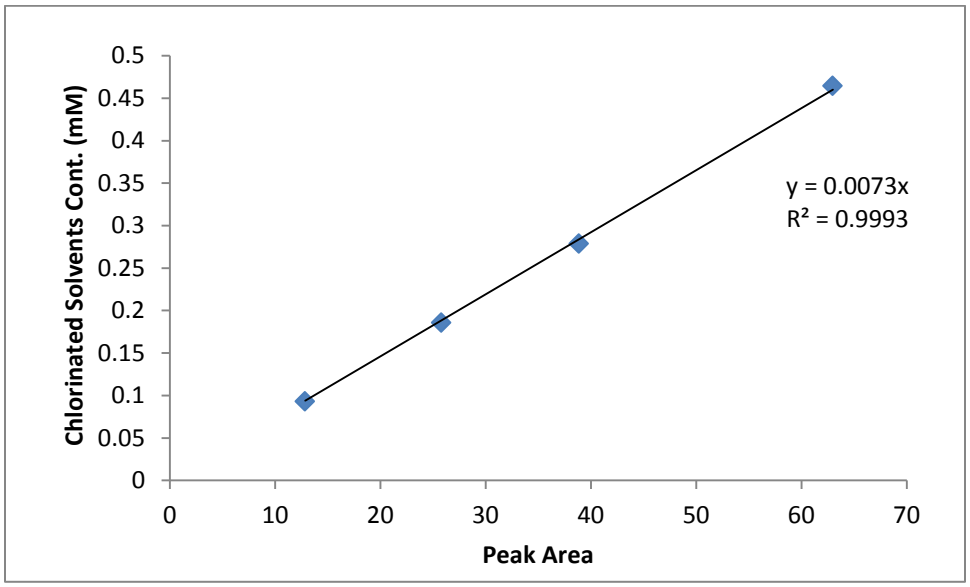
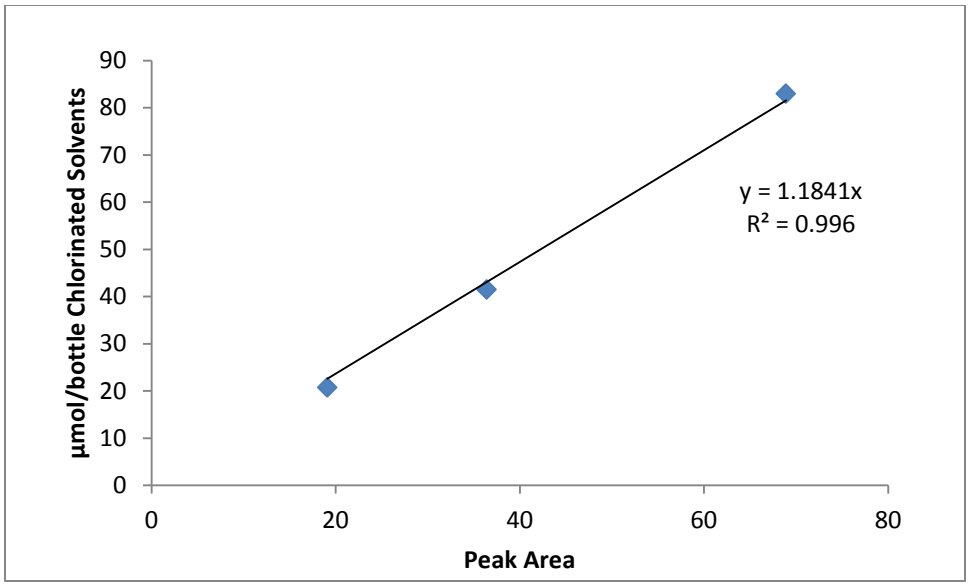


Figure B.3 Calibration Curve for *c*DCE (GC1-1mL Autosample from 160mL Serum Bottle)



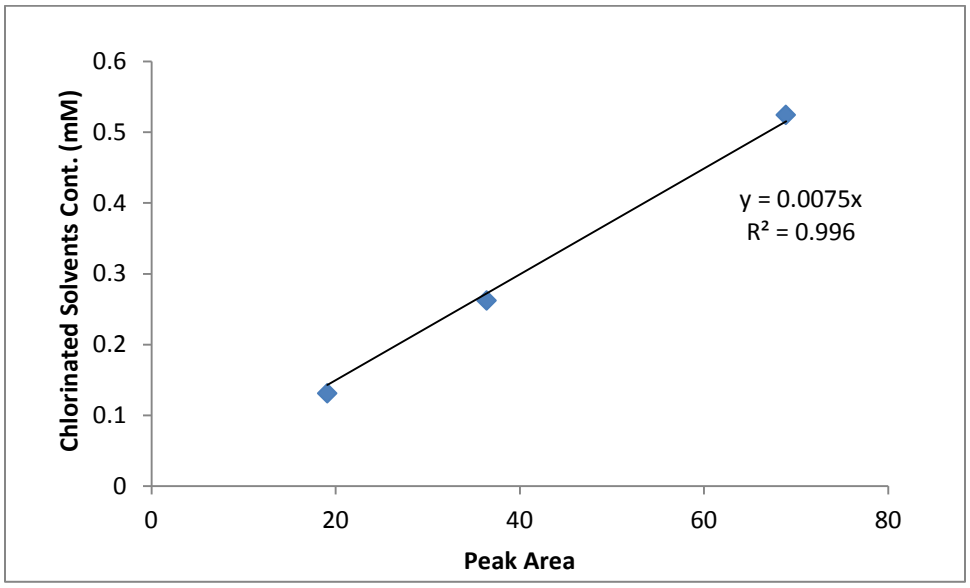
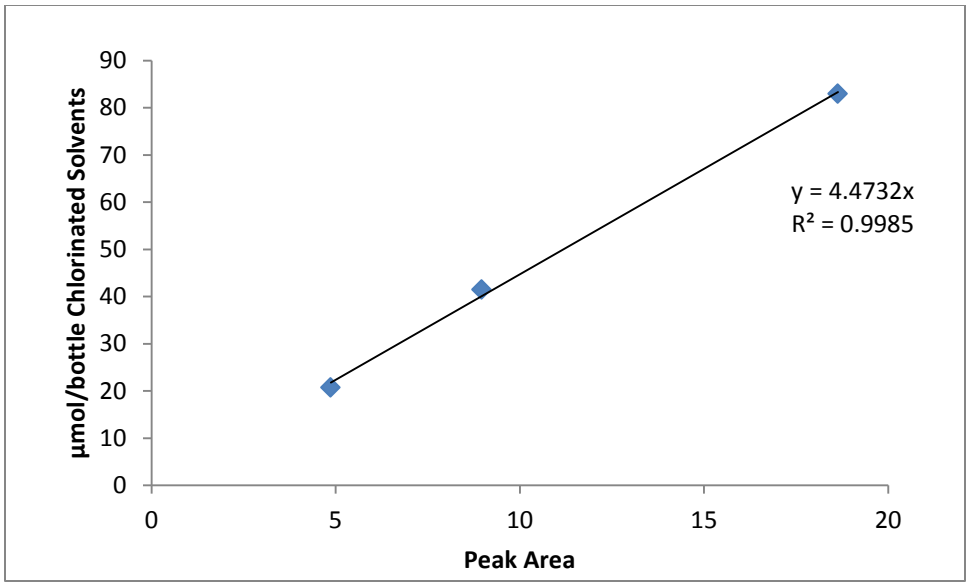


Figure B.4 Calibration Curve for VC (GC1-1mL Autosample from 160mL Serum Bottle)



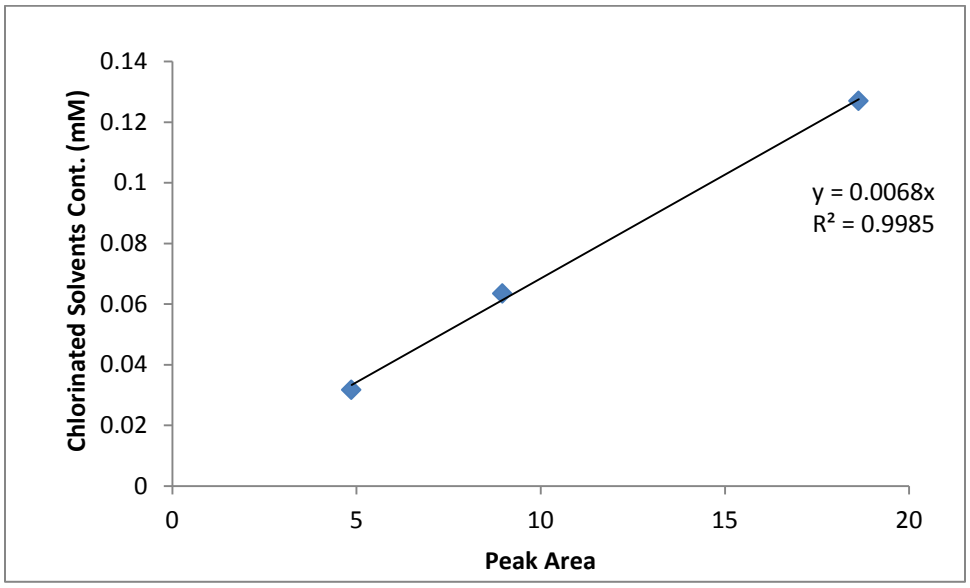
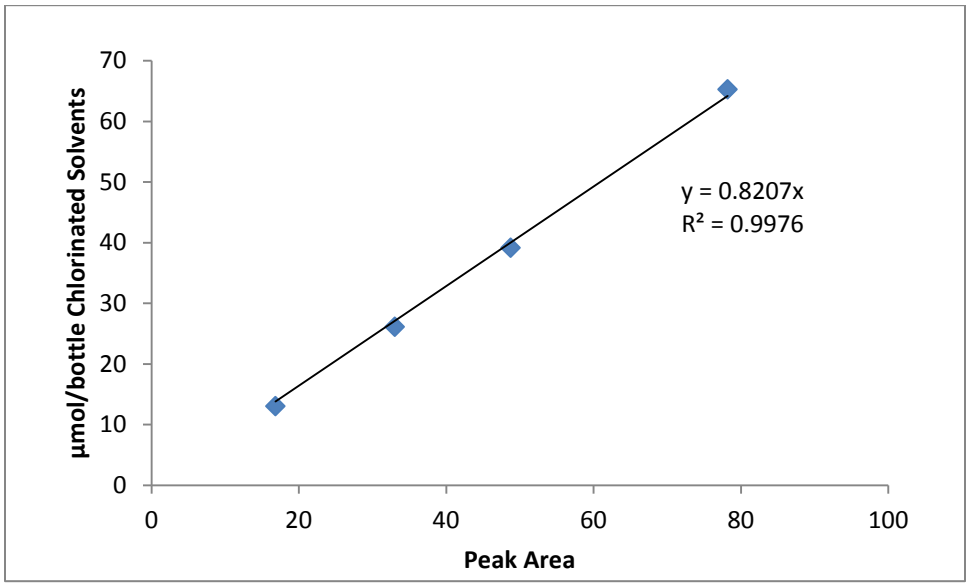


Figure B.5 Calibration Curve for ETH (GC1-1mL Autosample from 160mL Serum Bottle)



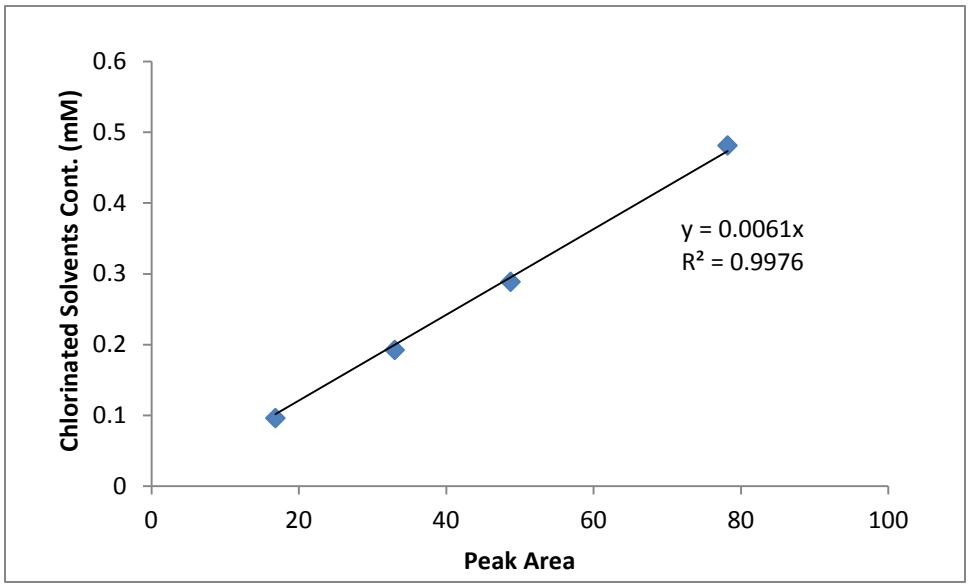
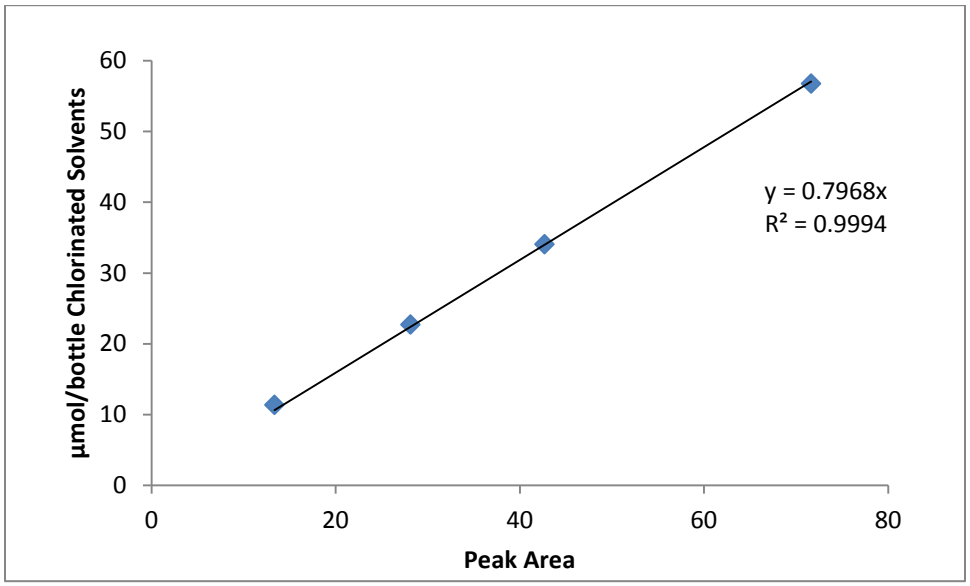


Figure B.6 Calibration Curve for PCE (GC1-100µL Headspace from 160mL Serum Bottle)



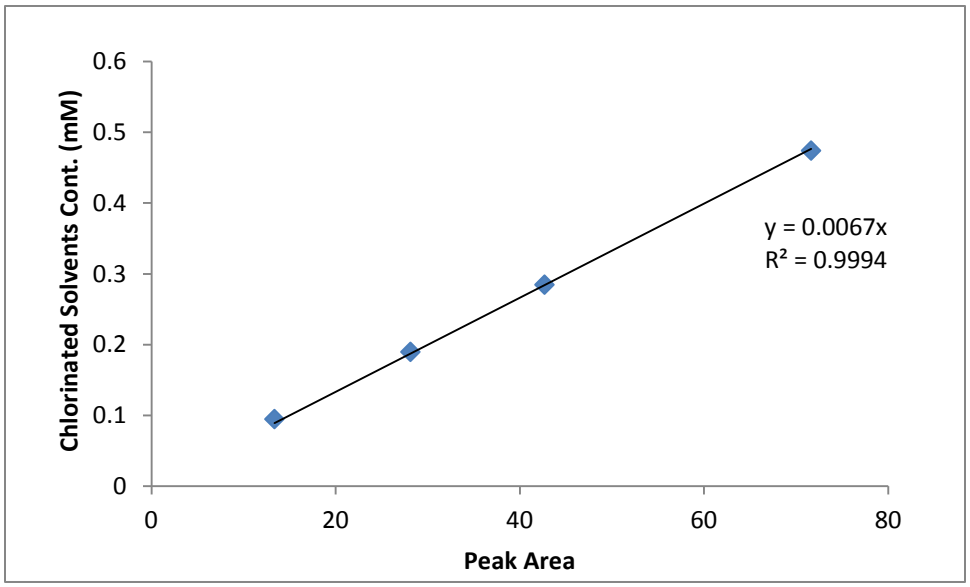
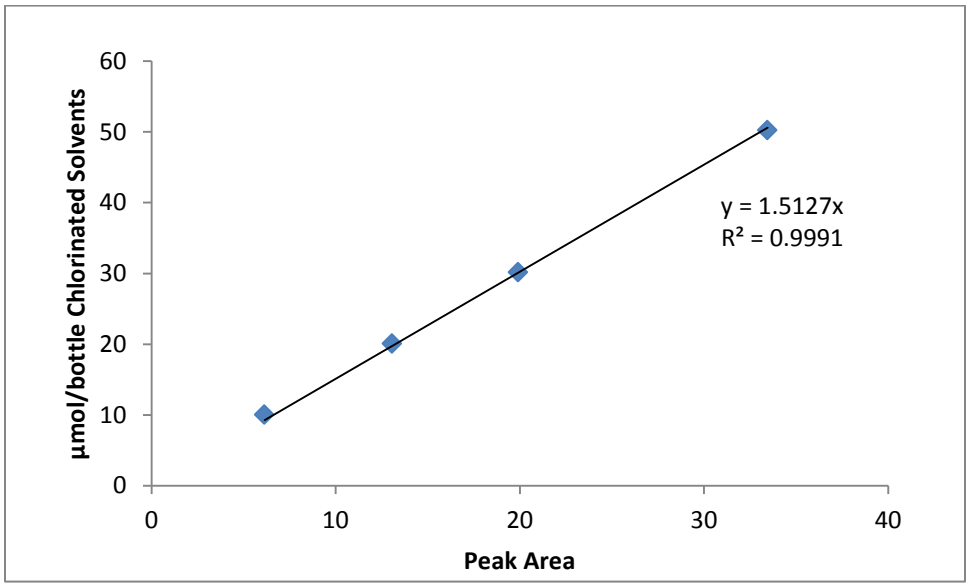


Figure B.7 Calibration Curve for TCE (GC1-100µL Headspace from 160mL Serum Bottle)



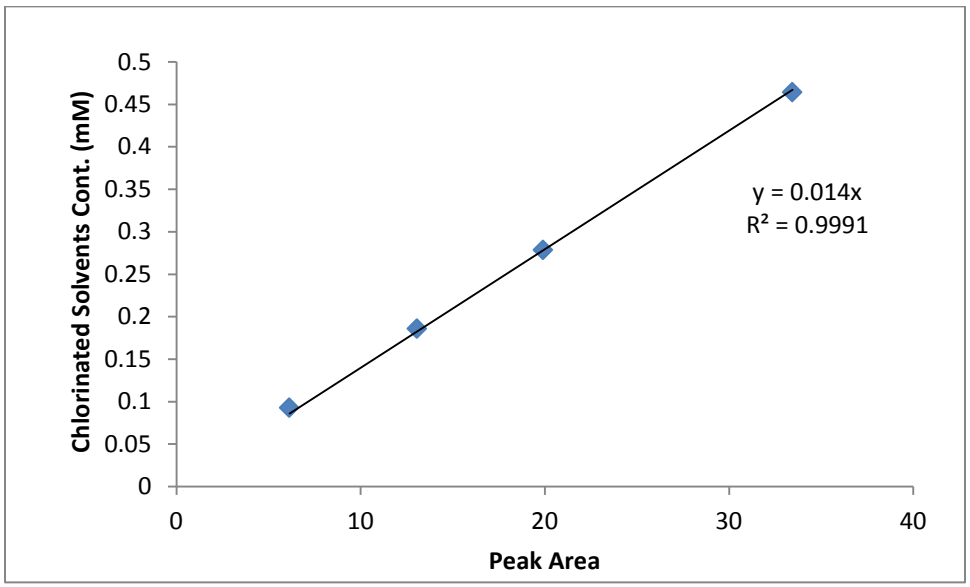
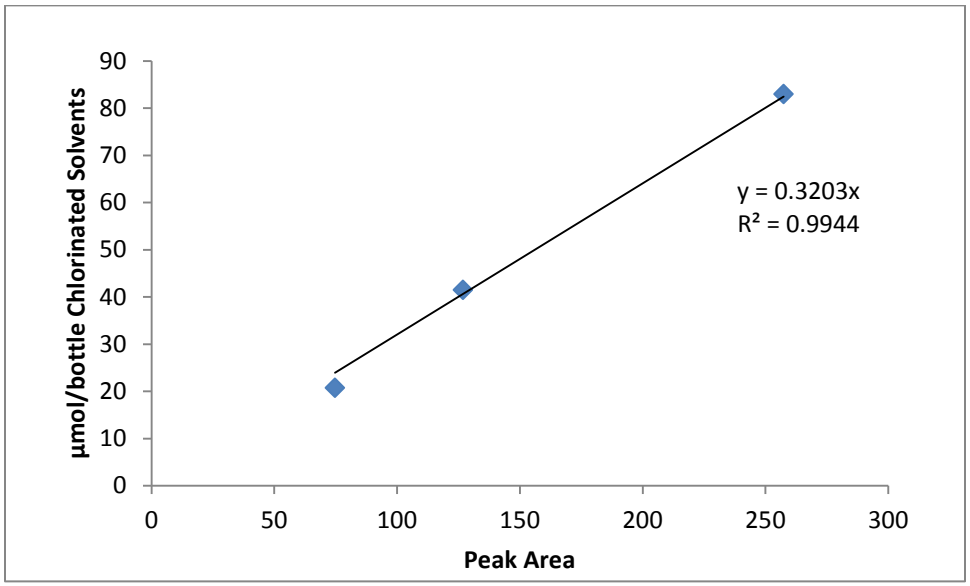


Figure B.8 Calibration Curve for *c*DCE (GC1-100µL Headspace from 160mL Serum Bottle)



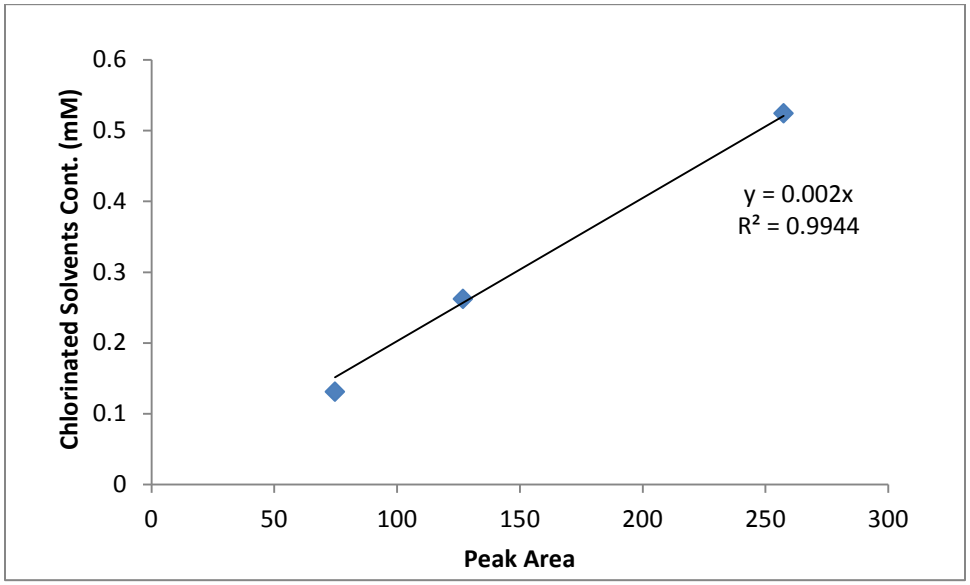
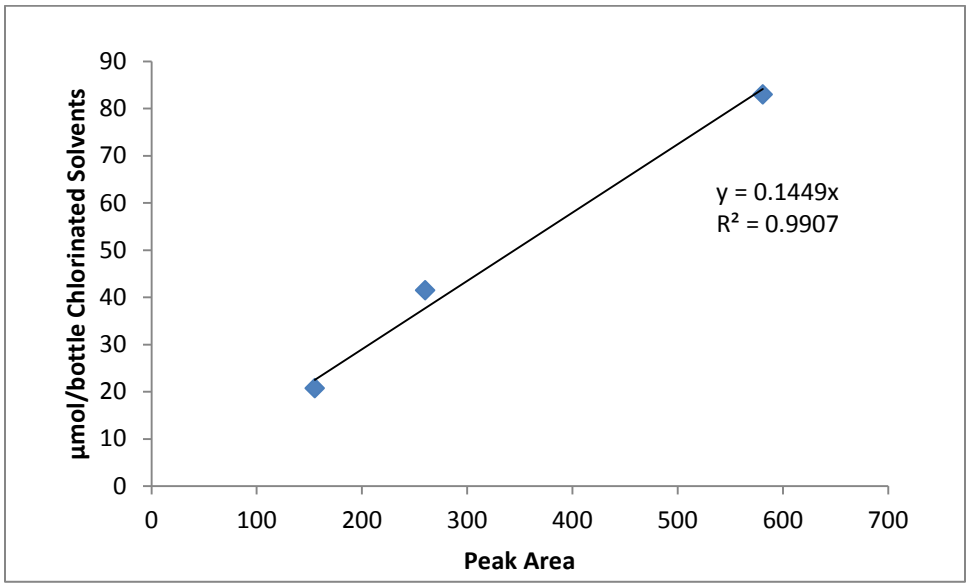


Figure B.9 Calibration Curve for VC (GC1-100µL Headspace from 160mL Serum Bottle)



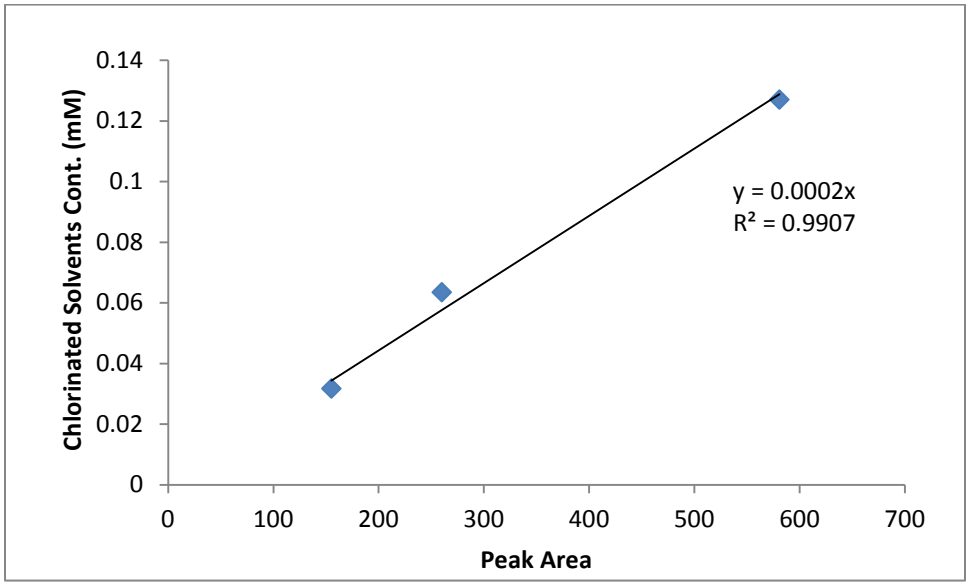
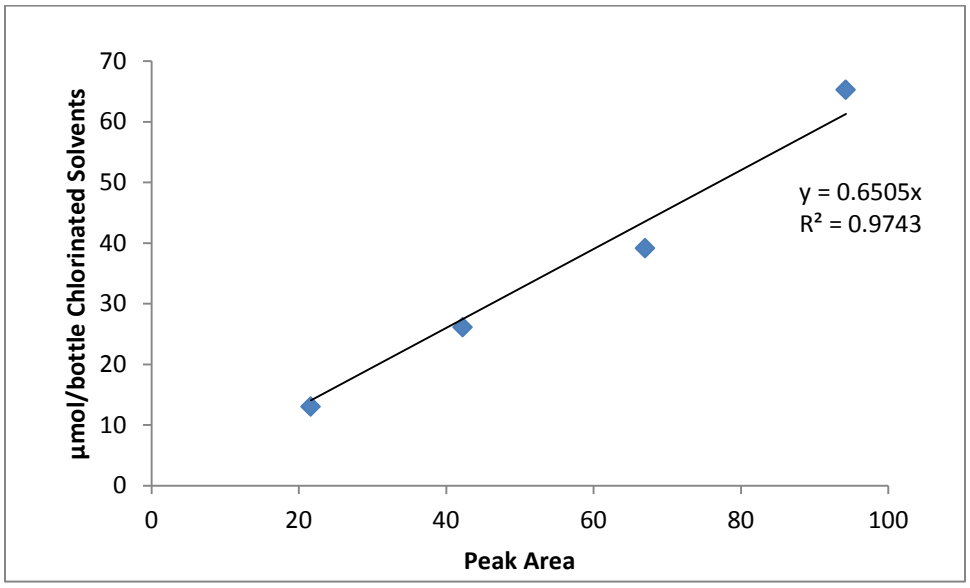


Figure B.10 Calibration Curve for ETH (GC1-100µL Headspace from 160mL Serum Bottle)



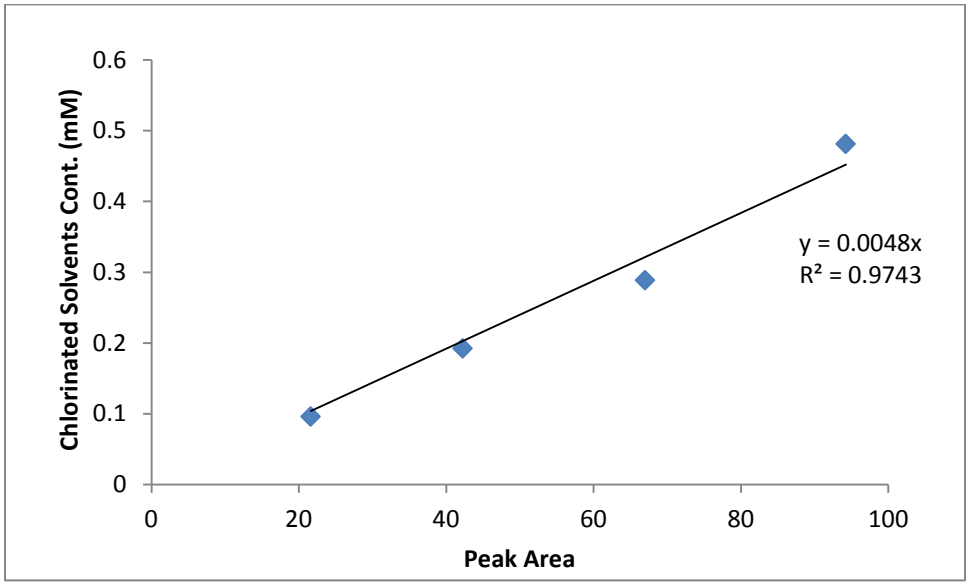
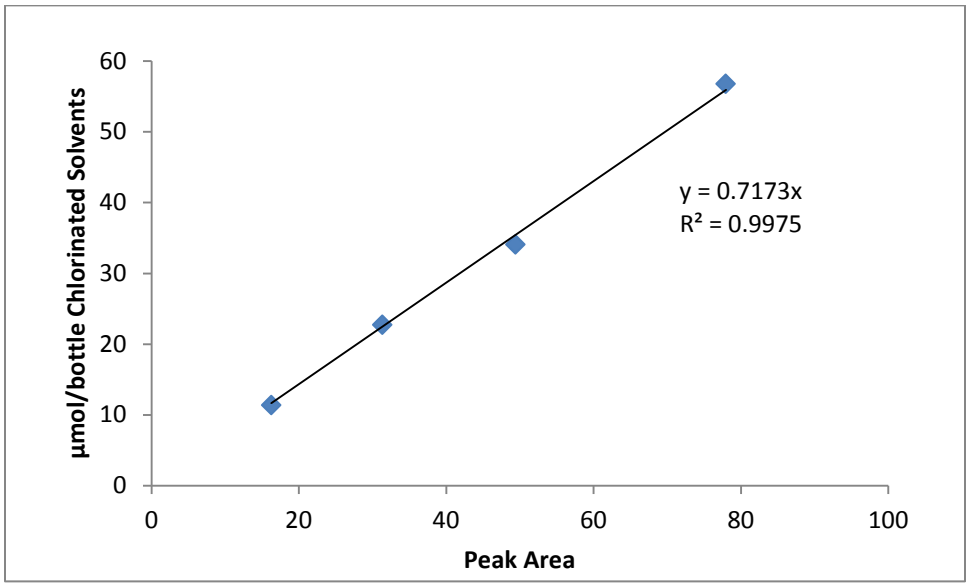


Figure B.11 Calibration Curve for PCE (GC2-100µL Headspace from 160mL Serum Bottle)



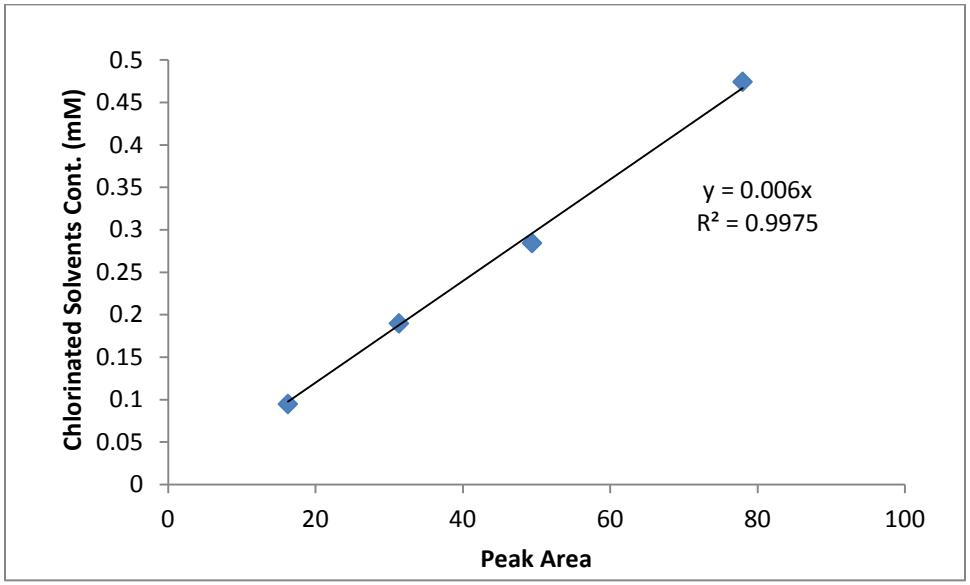
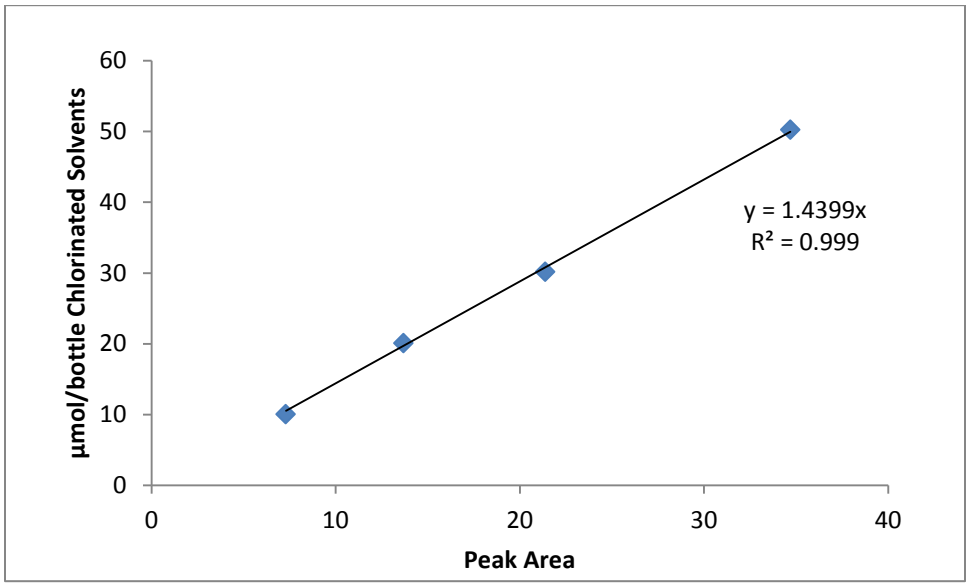


Figure B.12 Calibration Curve for TCE (GC2-100µL Headspace from 160mL Serum Bottle)



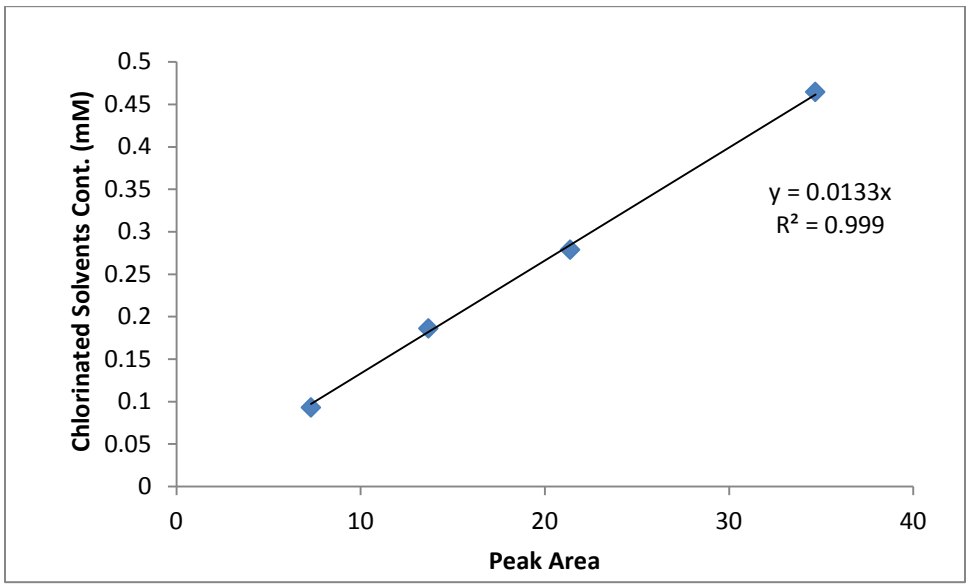
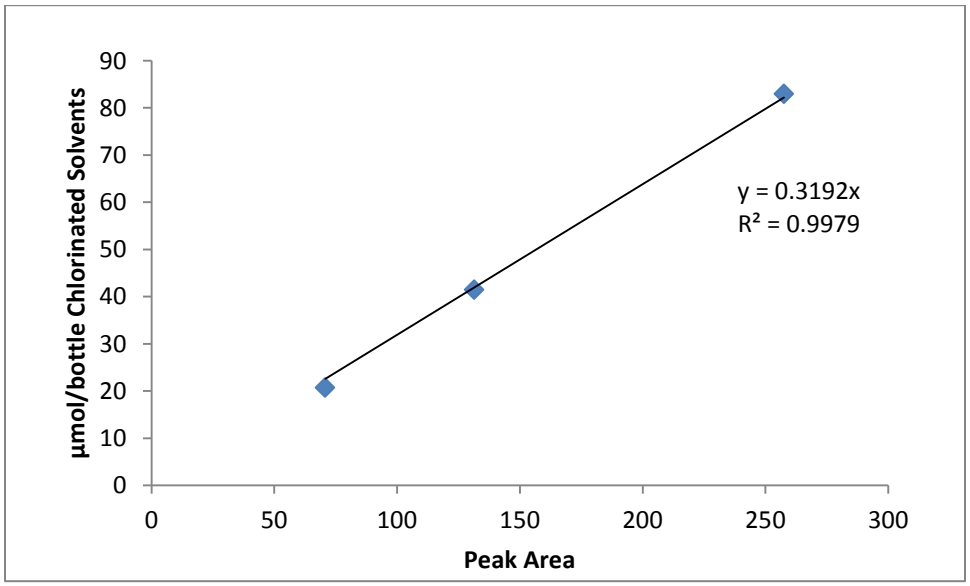


Figure B.13 Calibration Curve for *c*DCE (GC2-100µL Headspace from 160mL Serum Bottle)



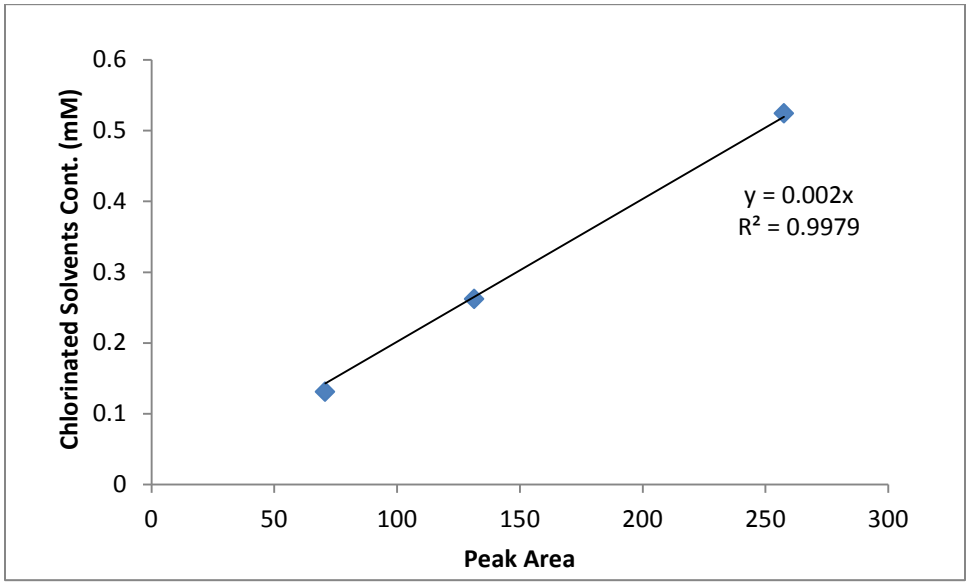
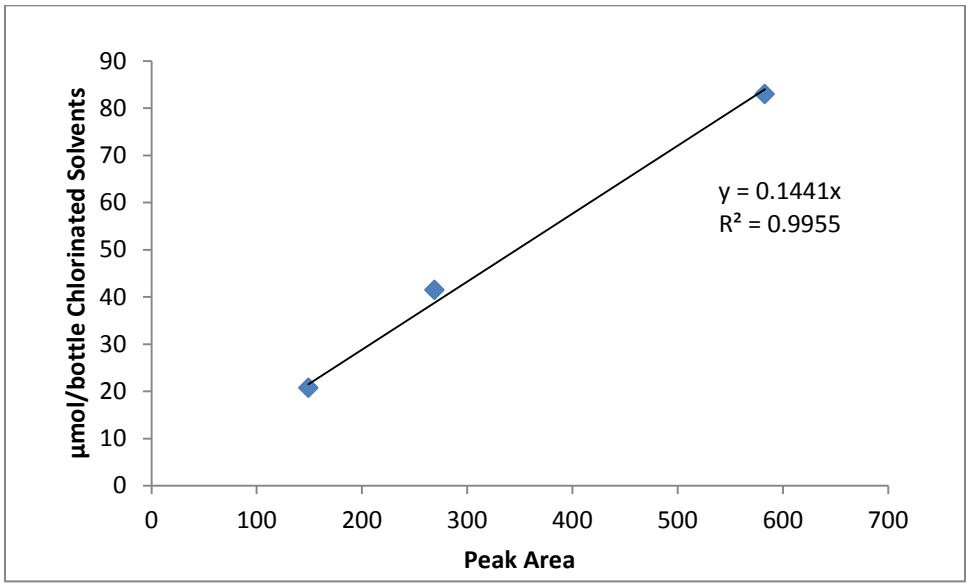


Figure B.14 Calibration Curve for VC (GC2-100µL Headspace from 160mL Serum Bottle)



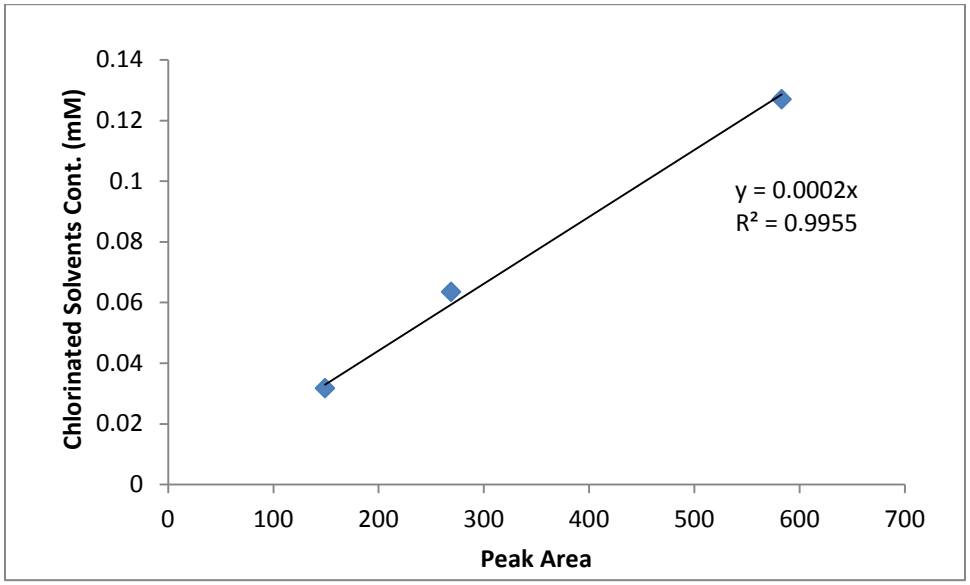
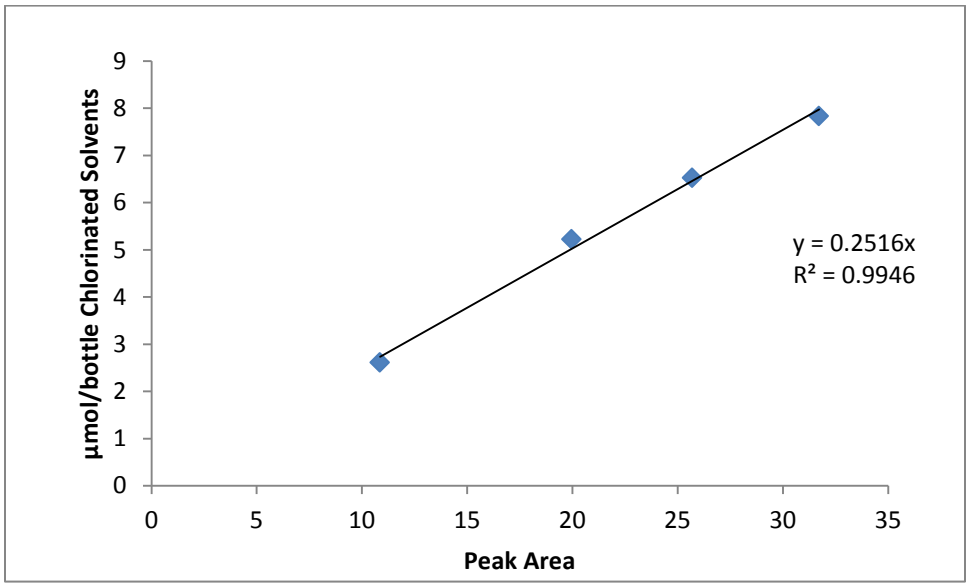


Figure B.15 Calibration Curve for ETH (GC2-100µL Headspace from 160mL Serum Bottle)



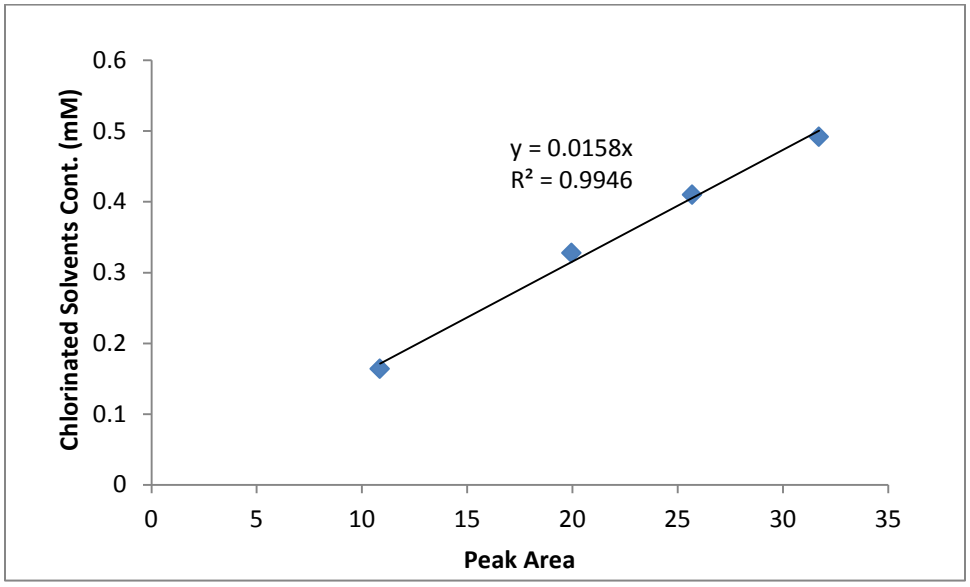
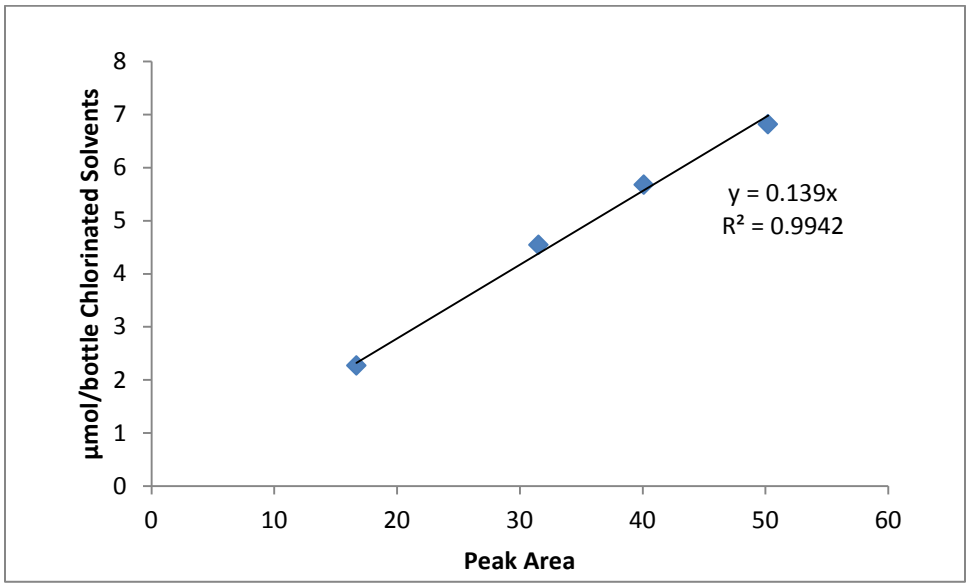


Figure B.16 Calibration Curve for PCE (GC1-100µL Headspace from 20mL Serum Bottle)



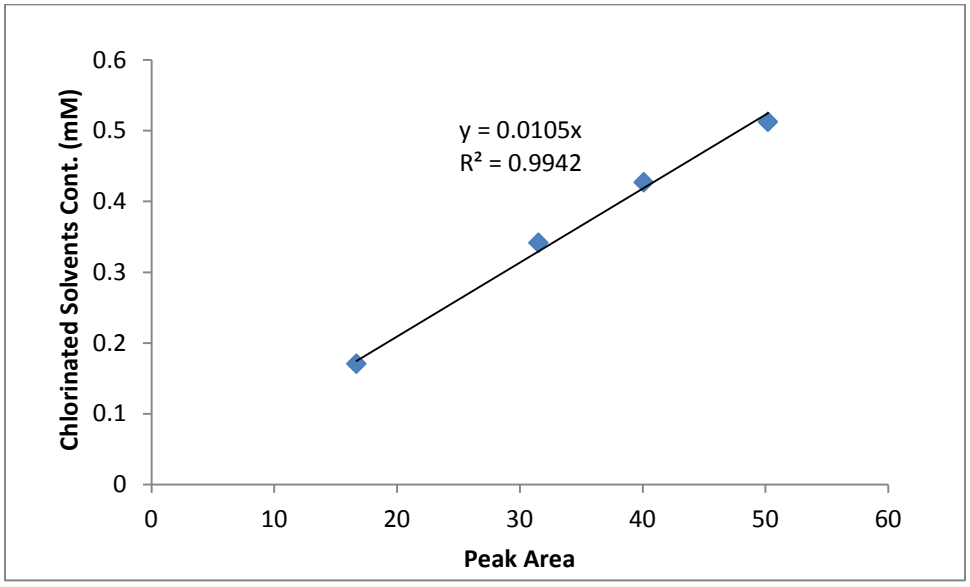
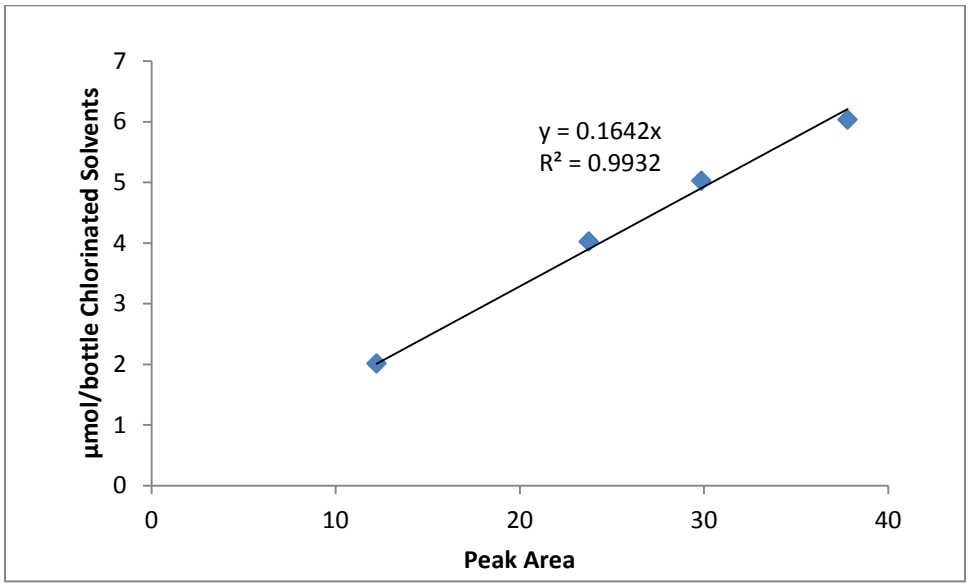


Figure B.17 Calibration Curve for TCE (GC1-100µL Headspace from 20mL Serum Bottle)



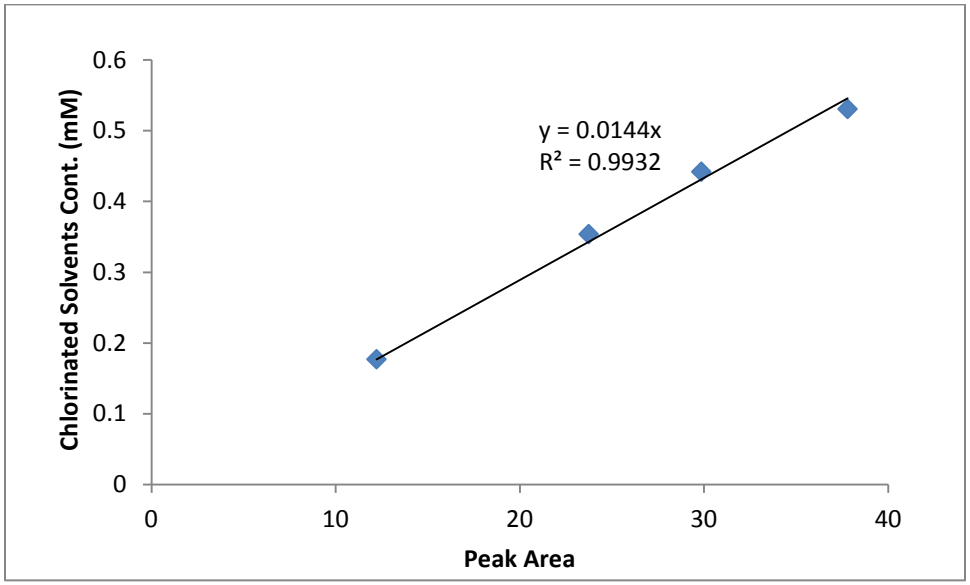
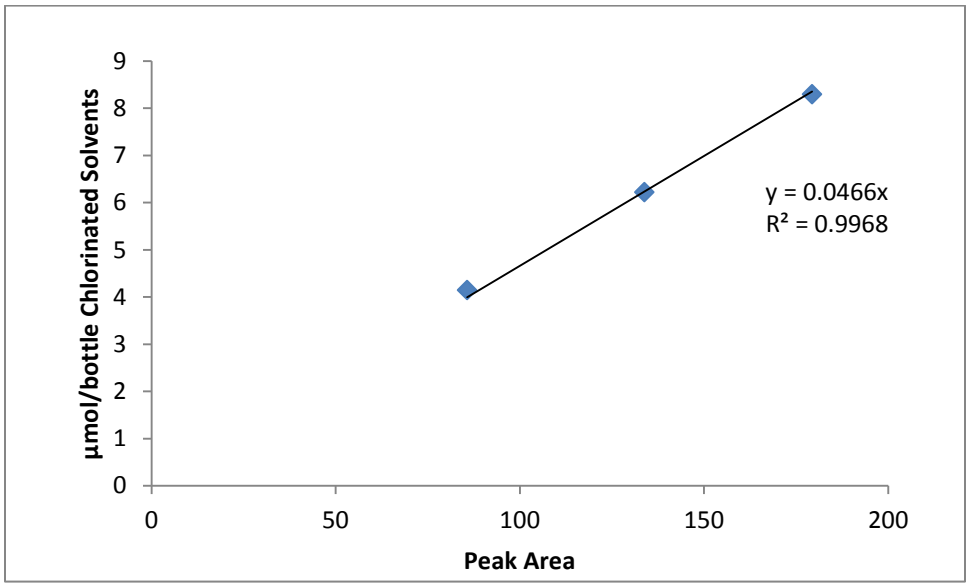


Figure B.18 Calibration Curve for *c*DCE (GC1-100µL Headspace from 20mL Serum Bottle)



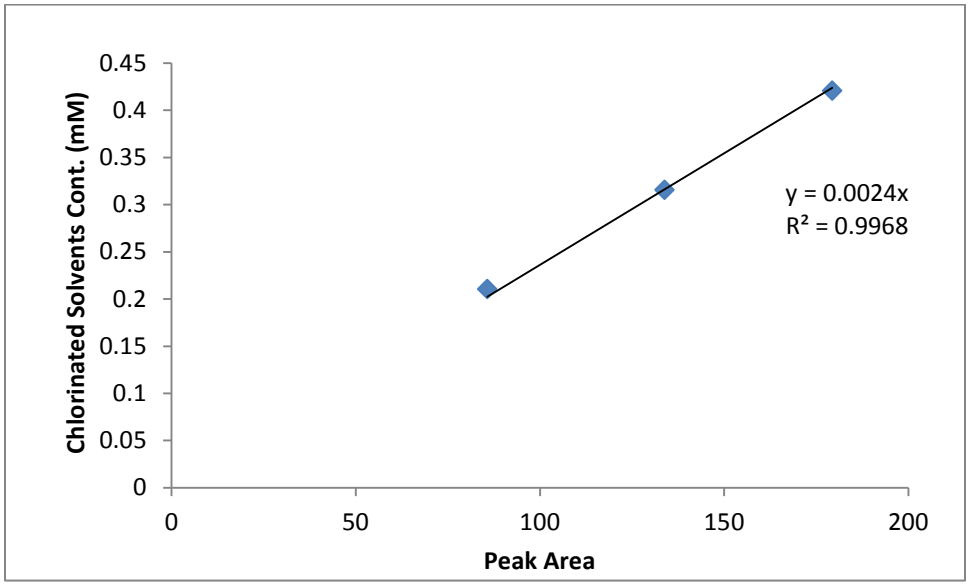
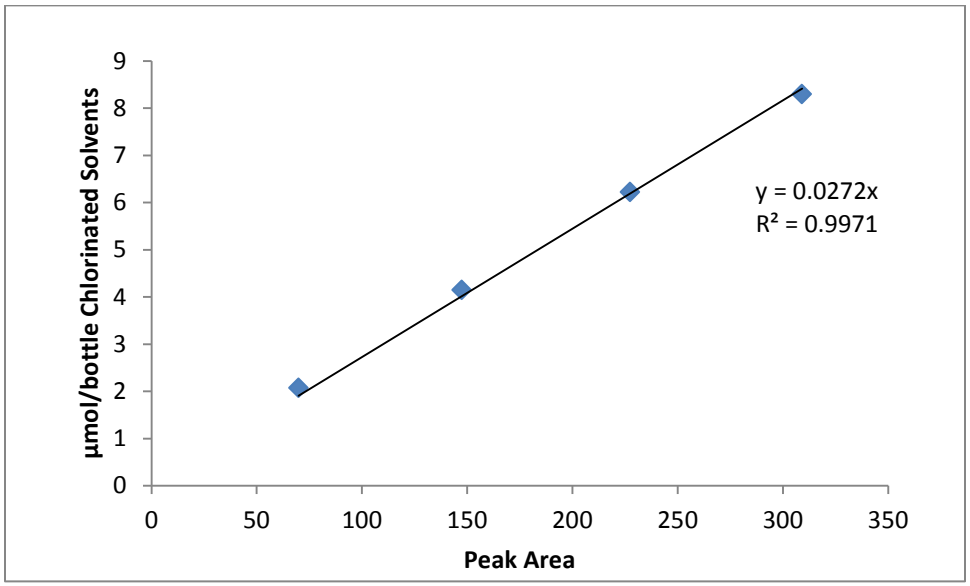


Figure B.19 Calibration Curve for VC (GC1-100µL Headspace from 20mL Serum Bottle)



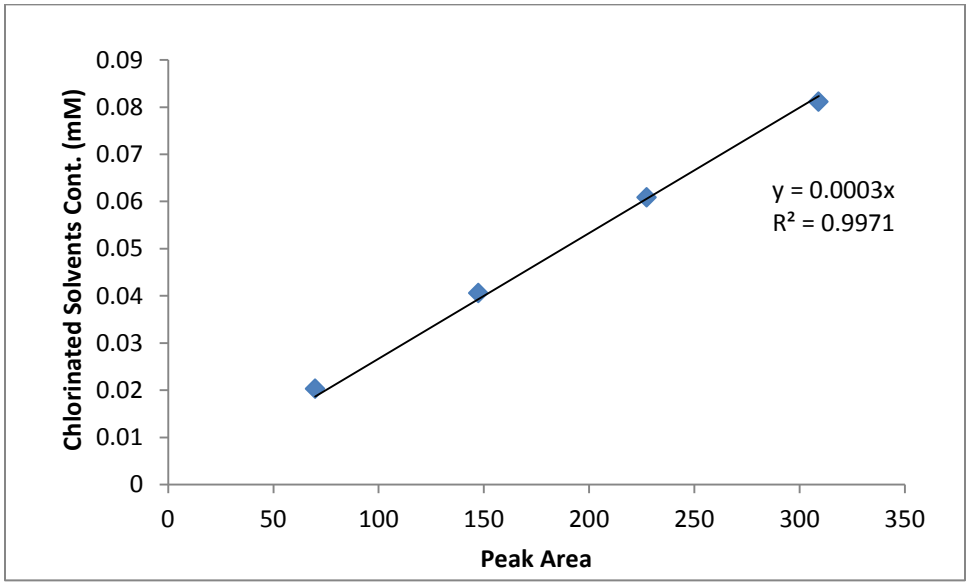
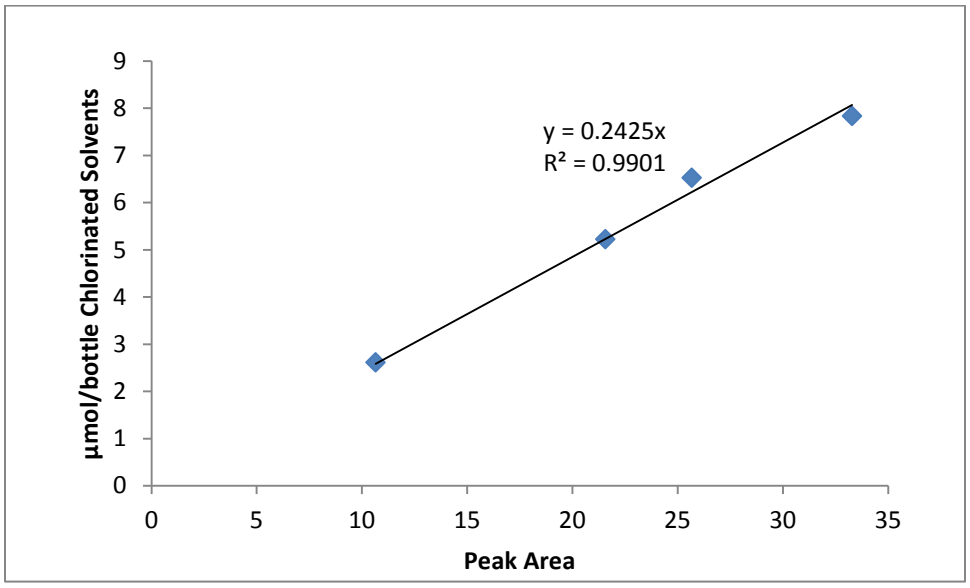


Figure B.20 Calibration Curve for ETH (GC1-100µL Headspace from 20mL Serum Bottle)



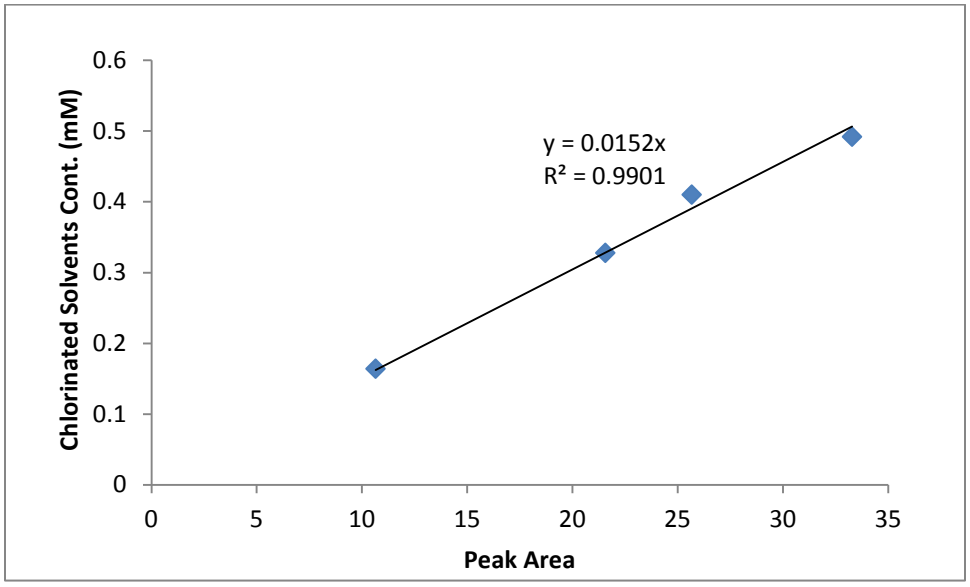
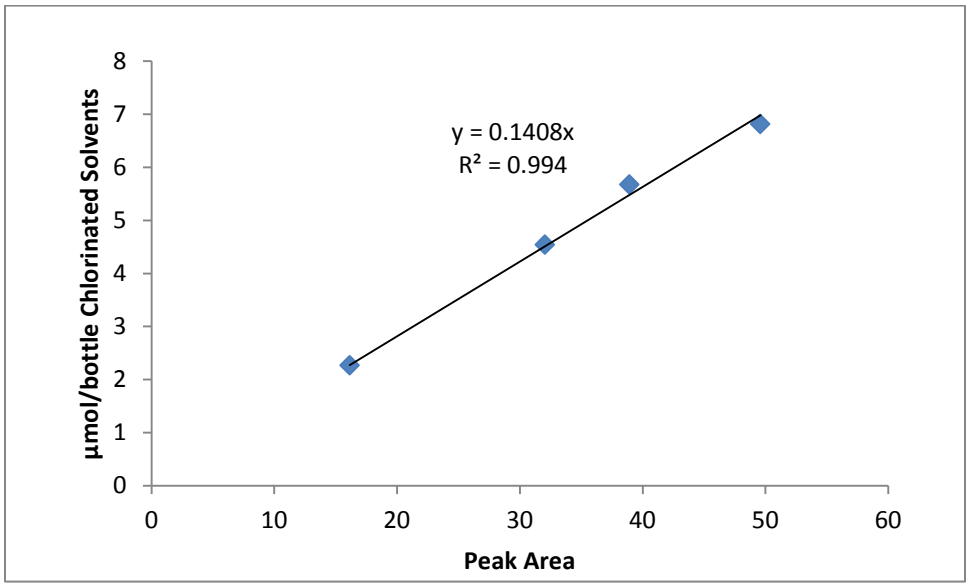


Figure B.21 Calibration Curve for PCE (GC2-100µL Headspace from 20mL Serum Bottle)



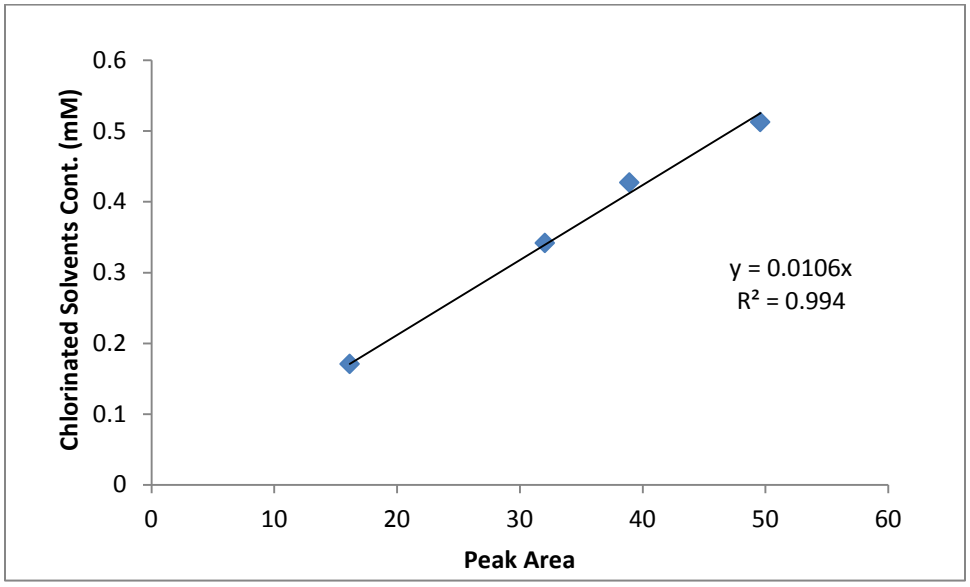
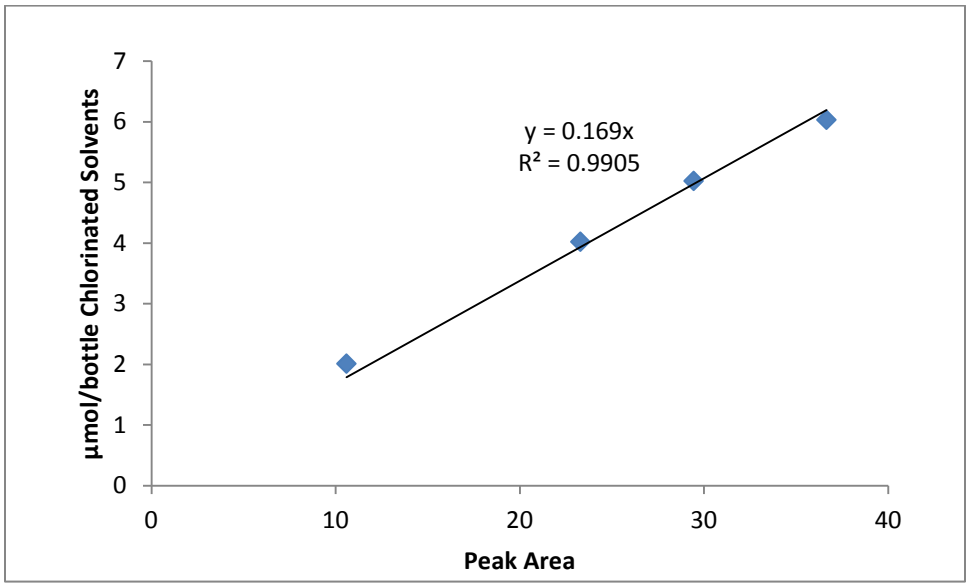


Figure B.22 Calibration Curve for TCE (GC2-100µL Headspace from 20mL Serum Bottle)



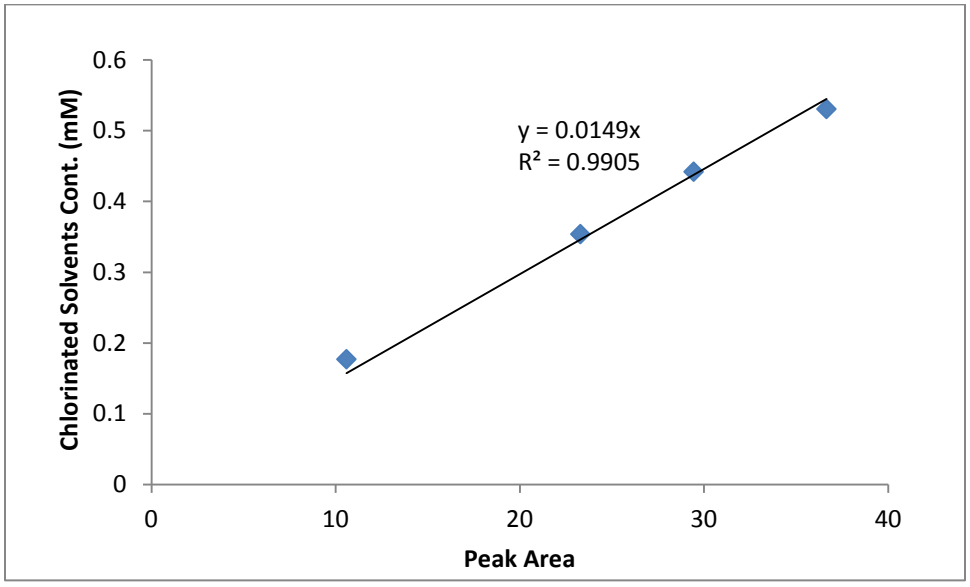
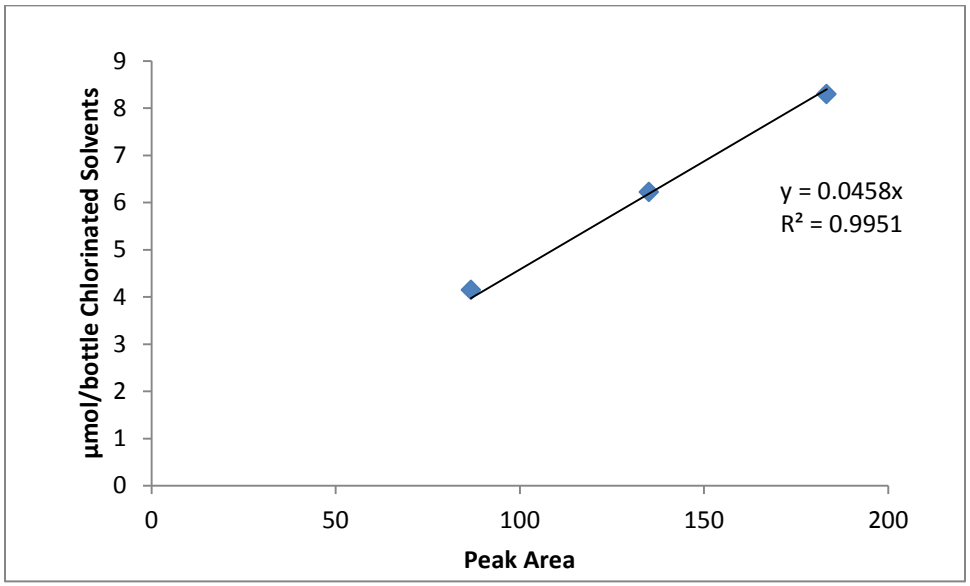


Figure B.23 Calibration Curve for *c*DCE (GC2-100µL Headspace from 20mL Serum Bottle)



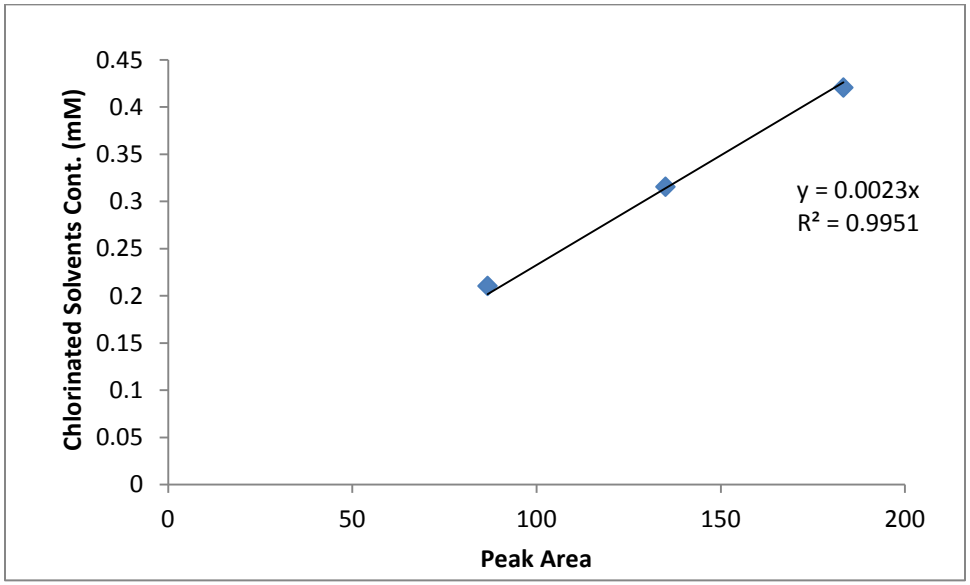
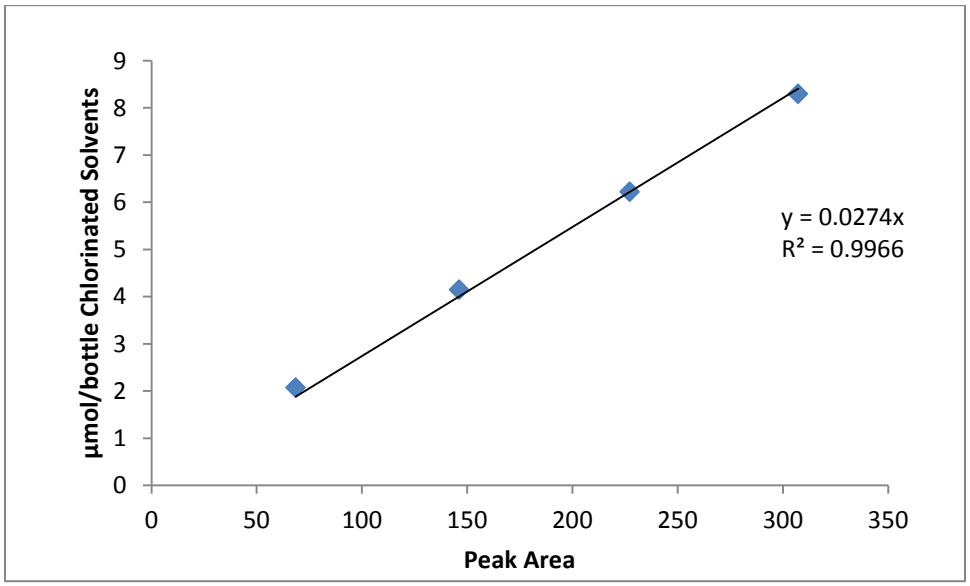


Figure B.24 Calibration Curve for VC (GC2-100µL Headspace from 20mL Serum Bottle)



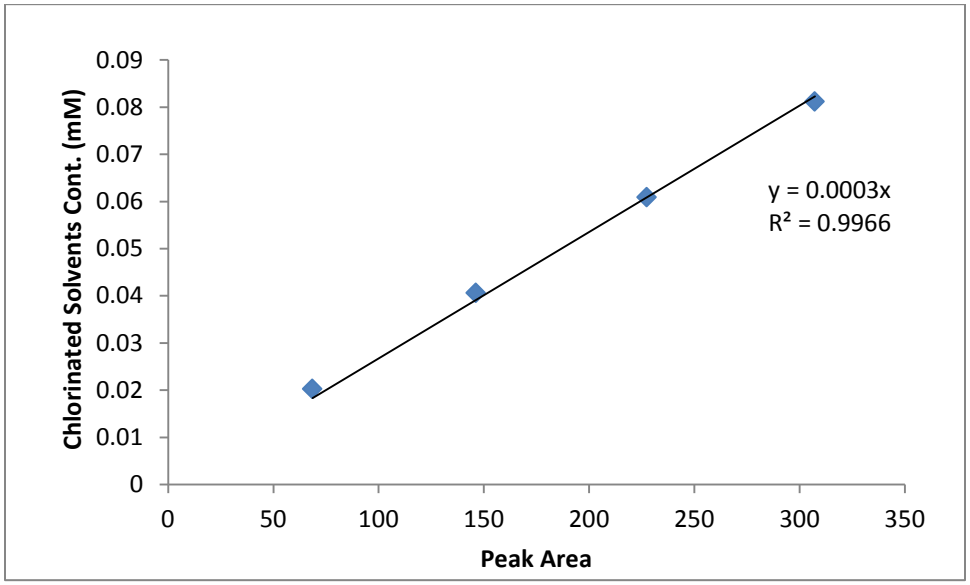


Figure B.25 Calibration Curve for ETH (GC2-100µL Headspace from 20mL Serum Bottle)

Appendix C Gas Chromatography Parameters and Retention Time

Table C.1 Parameters for gas chromatograph methods

		Manual injection	Autosampler injection
Oven	Equilibration Time	0.5 min	0.5 min
	Max Temperature	260 ^{o}C	260 ^{o}C
	Oven Program	60 ^{o}C for 2min then 25 ^{o}C/min to 200 ^{o}C for 0 min	60 ^{o}C for 2min then 25 ^{o}C/min to 200 ^{o}C for 1 min
Front SS Inlet He	Mode	Split	Split
	Heater	200 ^{o}C	200 ^{o}C
	Pressure	23.193 psi	23.193 psi
	Total Flow	153 ml/min	154.5 ml/min
	Septum Purge Flow	No	1.5 ml/min
	Split Ratio	50:1	50:1
	Split Flow	150 ml/min	150 ml/min
Column	Column	Agilent DB624 column	Agilent DB624 column
	Initial	60 ^{o}C	60 ^{o}C
	Pressure	23.193 psi	23.193 psi
	Flow	3ml/min	3ml/min
	Average Velocity	37.299 cm/sec	37.299 cm/sec
	Holdup Time	2.681 min	2.681 min
	Run time	7.6 min	8.6 min
Front Detector FID	Heater	300^{o}C	300^{o}C
	H2 Flow	30 ml/min	30 ml/min
	Air Flow	400 ml/min	400 ml/min
	Makeup Flow	25 ml/min	25 ml/min

Table C.2 Autosampler parameters

Parameters	
Extractions Per Vial	2
GC Cycle Time (min)	14.0
Inject Time (min)	0.50
Loop Equilibrium Time (min)	0.05
Loop Fill Time (min)	0.03
Loop Temperature	125
Oven Temperature	70
Shake	High
Transfer Line Temperature	125
Vial Equilibration Time (min)	15.0
Vial Pressurization Time (min)	0.50

Table C.3 Retention Times of Hydrocarbons and CAHs[3]

Retention Time (min)		Compound	Abbreviation	Henry's constant @ 20 °C (dimensionless) [1,2]	Special notes
Manual	**Autosampler**				
2.827		Methane	CH_4	28.4[1]	
2.884	**2.921**	Ethene	C_2H_4	7.7[1](9.22)[4]	
3.053		Propene	C_3H_6	3.5 @ 10 °C[1]	
		Chloromethane	CM	0.278[2]	Not tested
3.417	**3.455**	Vinyl Chloride	VC	0.901-0.981[2](0.9715)[4]	
3.59	**3.585**	Methanol	MeOH		
	4.080	Ethanol	EtOH		
4.323		2-Chloropropane	2-CP		
4.398	**4.427**	1,1-Dichloroethene	1,1-DCE	0.886-0.904[2]	
4.408		Dichloromethane	DCM	0.0745-0.0816[2]	
4.408		Isopropanol	Isop		
4.755		1-Chloropropane	1-CP		
5.482	**5.502**	1,2-*cis*--DCE	cDCE	0.127-0.135[2](0.137)[4]	
		1,2-*trans*-DCE	tDCE	0.308-0.318[2]	Not tested
5.646	**5.663**	Chloroform	CF	0.117-0.122[2]	
5.810		1,1,1-Trichloroethane	1,1,1-TCA	0.539-0.570[2]	
5.916	**5.931**	Carbon Tetrachloride	CT	0.997-1.01[2]	
		1,2-dichloroethane	1,2-DCA	0.0318-0.0388[2]	Not tested here
6.032		Benzene	BEN	0.181-0.183[2]	
6.385	**6.399**	Trichloroethylene	TCE	0.294-0.335[2](0.3295)[4]	
6.535		1,2-Dichloropropane	1,2-DCP	0.0883-0.0907[2]	
7.143		Toluene	TOL	0.193-0.211[2]	
7.30		1,1,2-trichloroethane	1,1,2-TCA	0.0286-0.0287[2]	
7.486	**7.495**	Perchloroethylene	PCE	0.546-0.579[2](0.594)[4]	
8.910		1,2,3-Trichloropropane	1,2,3-TCP		
		1,2,4-Trichlorobenzene	1,2,4-TCB	0.0402[2]	Not tested here
		1,2,3-Trichlorobenzene	1,2,3-TCB		Not tested here

1. Virginia *et al.* 1995. Retardation of dissolved oxygen due to a trapped gas phase in porous media, Groundwater, 33(3), pp 391-398
2. U.S. EPA On-line Tools for Site Assessment Calculation, http://www.epa.gov/athens/learn2model/part-two/onsite/esthenry.html
3. Table prepared by Jun Yan
4. Average Values from OSWER method and Washington(1996) method at 21ºC (Room Temperature)

Appendix D Data for pH Range Screening

Table D.1 Geobacter lovleyi strain SZ Screening (1mL liquid GC autosampler measurement)

Geobacter lovleyi SZ		pHn 5.5			pHn 6.0			pHn 7.2			pHn 8.0		
		Triplicate 1	Triplicate 2	Triplicate 3	Triplicate 1	Triplicate 2	Triplicate 3	Triplicate 1	Triplicate 2	Triplicate 3	Triplicate 1	Triplicate 2	Triplicate 3
Mar-22-2012	PCE	27.5	27.1	26.6	27	25.9	27.2	24.6	22.6	25.1	24.5	22.2	23.2
	TCE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	cDCE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Mar-24-2012	PCE	17.2	17.9	16.9	17.1	17.8	18.4	ND	ND	ND	15.5	14.4	16.6
	TCE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	cDCE	ND	ND	ND	ND	ND	ND	23.3	24.9	24.4	ND	ND	ND
Mar-26-2012	PCE	15.4	15.2	14.5	15.9	14.3	16.1	ND	ND	ND	14.2	14.1	15
	TCE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	cDCE	ND	ND	ND	ND	ND	ND	28.6	28	29.9	ND	ND	ND
Apr-02-2012	PCE	12.3	12.4	12.1	10.3	ND	12.4	ND	ND	ND	11.5	10.3	10.8
	TCE	ND	ND	ND	1.2	ND	ND	ND	ND	ND	ND	ND	ND
	cDCE	ND	ND	ND	ND	21.8	1.6	29.2	30.2	29.7	ND	ND	ND

Table D.2 *Desulfitobacterium hafniense* strain JH1 Screening (1mL liquid GC autosampler measurement)

Desulfitobacterium spp. Strain JH1		pHn 5.5			pHn 6.0			pHn 7.2			pHn 8.0		
		Triplicate 1	Triplicate 2	Triplicate 3	Triplicate 1	Triplicate 2	Triplicate 3	Triplicate 1	Triplicate 2	Triplicate 3	Triplicate 1	Triplicate 2	Triplicate 3
Feb-23-2012	PCE	35.9	33.5	38.3	30.9	23.3	31.8	34.9	37.2	32.2	312	34	37.8
	TCE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	cDCE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Feb-28-2012	PCE	17.6	18.6	19.8	17.6	17.3	17.4	7.8	7.7	8.4	16.4	15.5	17
	TCE	ND	ND	ND	ND	ND	ND	4.3	4.1	4.8	ND	ND	ND
	cDCE	ND	ND	ND	ND	ND	ND	10.8	10.2	11.3	ND	ND	ND
Mar-04-2012	PCE	14.3	14.7	15.2	15.2	15.5	15.2	3.1	3	1.7	14.2	14.1	16.1
	TCE	ND	ND	ND	ND	ND	ND	2.1	2	ND	ND	ND	ND
	cDCE	ND	ND	ND	ND	ND	ND	22.6	22.6	24.5	ND	ND	ND
Mar-09-2012	PCE	12.6	13.6	14.2	14.1	14.3	13.7	ND	ND	ND	12.8	12.3	12.3
	TCE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	cDCE	ND	ND	ND	ND	ND	ND	29	26.4	26.9	ND	ND	ND
Mar-19-2012	PCE	11.7	11.9	12.4	12.8	11.7	11	ND	ND	ND	10.2	8.4	11.9
	TCE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	cDCE	ND	ND	ND	ND	ND	ND	29.6	31.1	32.2	ND	ND	ND

Table D.3 *Desulfitobacterium* sp. strain Viet1 Screening (1mL liquid GC autosampler measurement)

Desulfitobacterium sp. Strain Viet1		pHn 5.5			pHn 6.0			pHn 7.2			pHn 8.0		
		Triplicate 1	Triplicate 2	Triplicate 3	Triplicate 1	Triplicate 2	Triplicate 3	Triplicate 1	Triplicate 2	Triplicate 3	Triplicate 1	Triplicate 2	Triplicate 3
Feb-23-2012	PCE	18.4	21.7	21.5	21.3	19.9	20.4	13.8	19.6	21	13.9	13.3	20.1
	TCE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Feb-28-2012	PCE	14.1	14.7	14.5	14	13.6	14.3	3.7	6.5	ND	14.1	13.9	13.8
	TCE	ND	ND	ND	ND	ND	ND	14.4	9.4	18	ND	ND	ND
Mar-04-2012	PCE	14.1	13.4	13.3	13.8	13.1	13.2	1.3	4.4	ND	14	13.6	13.8
	TCE	ND	ND	ND	ND	ND	ND	19	13.3	22.3	ND	ND	ND
Mar-09-2012	PCE	12.5	12.3	11.3	12.6	11.5	10.9	ND	2.4	ND	13.3	13.2	12.6
	TCE	ND	ND	ND	ND	ND	ND	22	13.6	21.6	ND	ND	ND
Mar-19-2012	PCE	10.7	10.4	9.9	10.6	9.2	9.4	ND	ND	ND	11.2	10.5	10.4
	TCE	ND	ND	ND	ND	ND	ND	20.4	16.7	22.4	ND	ND	ND

Appendix E Data for Microcosms

Table E.1: Rotenberg Trester Sample pH_n=5.5 (PCE as electron acceptor, 100µL headspace GC measurement)

	May-19-2011		May-23-2011		May-28-2011		June-6-2011		June-15-2011		June-20-2011		July-5-2011		March-20-2012	
Peak Aera	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2
PCE	27.803	30.256	32.283	37.561	28.754	23.417	19.003	17.122	15.084	14.651	14.082	13.533	2.858	4.492	ND	ND
TCE	ND	ND	ND	ND	ND	ND	ND	ND	2.044	1.807	3.892	2.995	3.24	8.578	2.505	2.663
DCE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	7.924	3.214	13.524	13.145
VC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	17.975	9.474
ETHENE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table E.2: Rotenberg Trester Sample pH_n=5.5 (TCE as electron acceptor, 100µL headspace GC measurement)

	May-19-2011		May-23-2011		May-28-2011		June-6-2011		June-15-2011		June-20-2011		July-5-2011		March-20-2012	
Peak Aera	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2
TCE	81.294	79.214	43.207	62.98	80.611	51.328	41.614	46.457	36.284	36.516	32.243	34.233	11.183	17.097	6.393	4.005
DCE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	4.763	1.353	21.481	15.469	16.661	22.058
VC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	25.885	23.251
ETHENE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table E.3: Rotenberg Trester Sample pH_n=7.2 (PCE as electron acceptor, 100µL headspace GC measurement)

	May-19-2011		May-23-2011		May-28-2011		June-6-2011		June-15-2011		June-20-2011		July-5-2011		March-20-2012	
Peak Aera	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2
PCE	21.682	20.562	21.323	21.976	7.11	30.923	1.849	ND	ND	ND	ND	ND	ND	ND	ND	ND
TCE	ND	ND	ND	ND	ND	3.727	2.016	6.216	ND	2.021	ND	1.522	ND	ND	ND	ND
DCE	ND	ND	ND	ND	ND	ND	16.982	12.265	17.77	18.542	23.759	20.55	12.699	14.866	10.38	13.982
VC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	62.941	51.943
ETHENE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	20.609	24.61

Table E.4: Rotenberg Trester Sample pH_n=7.2 (TCE as electron acceptor, 100µL headspace GC measurement)

	May-19-2011		May-23-2011		May-28-2011		June-6-2011		June-15-2011		June-20-2011		July-5-2011		March-20-2012	
Peak Aera	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2
TCE	53.739	45.579	52.646	44.849	56.621	48.162	9.388	4.794	3.536	3.365	2.27	2.385	ND	ND	ND	ND
DCE	ND	ND	ND	ND	ND	ND	45.004	16.727	32.183	25.77	35.768	31.501	19.902	22.496	32.969	19.241
VC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	55.682
ETHENE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	15.17

Table E.5: Rotenberg Creek Sample pH_n=5.5 (PCE as electron acceptor, 100µL headspace GC measurement)

	May-19-2011		May-23-2011		May-28-2011		June-6-2011		June-15-2011		June-20-2011		July-5-2011		March-20-2012	
Peak Aera	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2
PCE	56.313	41.798	58.539	45.742	30.32	31.531	15.664	14.991	10.025	10.463	11.327	10.189	10.35	10.309	1.591	3.504
TCE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	3.137	ND
DCE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	10.851	ND
VC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.588	1.396
ETHENE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table E.6: Rotenberg Creek Sample pH_n=5.5 (TCE as electron acceptor, 100µL headspace GC measurement)

	May-19-2011		May-23-2011		May-28-2011		June-6-2011		June-15-2011		June-20-2011		July-5-2011		March-20-2012	
Peak Aera	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2
TCE	50.214	76.177	65.992	83.185	63.896	81.224	39.807	49.703	32.381	39.743	33.863	36.996	29.577	39.79	9.16	14.276
DCE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	6.71	7.295
VC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.22	1.34
ETHENE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table E.7: Rotenberg Creek Sample pH_n=7.2 (PCE as electron acceptor, 100µL headspace GC measurement)

	May-19-2011		May-23-2011		May-28-2011		June-6-2011		June-15-2011		June-20-2011		July-5-2011		March-20-2012	
Peak Aera	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2
PCE	49.829	50.462	51.966	58.346	32.437	37.9	15.015	14.566	10.316	11.505	10.384	13.172	9.997	10.132	ND	3.7
TCE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.819	ND
DCE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	7.152	1.5
VC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.472	51.943
ETHENE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table E.8: Rotenberg Creek Sample pH_n=7.2 (TCE as electron acceptor, 100µL headspace GC measurement)

	May-19-2011		May-23-2011		May-28-2011		June-6-2011		June-15-2011		June-20-2011		July-5-2011		March-20-2012	
Peak Aera	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2
TCE	72.763	103.709	77.325	65.38	76.238	82.294	49.606	41.426	45.429	33.89	45.149	34.227	35.136	29.886	ND	2.009
DCE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	2.46	4.796	29.838	17.364
VC	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.984	3.297
ETHENE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table E.9: Third Creek Sample pH_n=5.5 (PCE as electron acceptor, 100µL headspace GC measurement)

	May-27-2011		May-30-2011		June-07-2011		June-17-2011		June-29-2011		July-11-2011	
Peak Aera	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2
PCE	144.681	132.279	100.656	93.4	8.799	9.834	ND	1.682	ND	ND	ND	ND
TCE	ND	ND	ND	ND	1.837	1.81	ND	ND	ND	ND	ND	ND
DCE	ND	ND	ND	ND	23.082	11.806	16.321	22.993	25.429	25.354	20.909	31.148
VC	ND	ND	ND	ND	3.502	1.785	4.704	11.308	29.93	31.314	39.889	63.989
ETHENE	ND	ND	ND	ND	11.557	ND	5.296	3.032	15.343	11.814	24.618	38.374

Table E.10: Third Creek Sample pH_n=5.5 (TCE as electron acceptor, 100µL headspace GC measurement)

	May-27-2011		May-30-2011		June-07-2011		June-17-2011		June-29-2011		July-11-2011	
Peak Aera	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2	Replicate-1	Replicate-2
TCE	138.788	161.745	92.615	121.979	19.869	18.383	18.326	ND	ND	ND	ND	ND
DCE	ND	ND	ND	ND	21.58	27.944	22.113	19.615	32.075	45.204	27.315	37.893
VC	ND	ND	ND	ND	12.625	10.241	21.113	12.335	44.093	62.871	55.042	65.294
ETHENE	ND	ND	ND	ND	1.707	1.988	6.168	2.246	15.343	19.398	23.822	23.462

VITA

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