

**MICROCOSMS AND FIELD BIOREMEDIATION STUDIES OF
PERCHLOROETHENE (PCE) CONTAMINATED SOIL AND GROUNDWATER**

By

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AN ABSTRACT OF A DISSERTATION

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ABSTRACT

Halogenated organic compounds have had widespread and massive applications in industry, agriculture, and private households, for example, as degreasing solvents, flame retardants and in polymer production. They are released to the environment through both anthropogenic and natural sources. The most common chlorinated solvents present as contaminants include tetrachloroethylene (PCE, perchloroethene), trichloroethene (TCE), trichloroethane (TCA), and carbon tetrachloride (CT). These chlorinated solvents are problematic because of their health hazards and persistence in the environment, threatening human and environmental health. This contribution provides insight on PCE degradation at laboratory and field scale at a former dry cleaning site in Manhattan, KS. Biostimulation experiments included combinations and concentrations of the following nutrients: soy oil methyl esters (SOME), yeast extract (YE), glucose, lactate, methanol and cheese whey. Bioaugmentation studies used KB-1 bacterial consortium (commercially available culture containing *Dehalococcoides*). This culture is known to complete the degradation of PCE to a safe end product, ethene. Concentrations of PCE and its degradation intermediates were monitored in the gas phase of the microcosm vials. Biostimulation of the natural ground water and soil microflora did not completely degrade PCE as cis-DCE (c-DCE) accumulated in the sample. Bioaugmented microcosms containing YE and SOME created reducing conditions for KB-1 culture, resulting in ~ 90% dechlorination of PCE to methane and c-DCE. Cheese whey microcosms containing 0.05% cheese whey inhibited the KB-1 culture. This inhibition was due to a drop of pH that inhibited the culture activity. Lower concentrations of cheese whey (e.g. 0.01% to 0.025%) reduced PCE and generated methane in KB-1 augmented microcosms.

Based on microcosm results, a pilot bioremediation field study was conducted for a dry cleaning site contaminated with PCE. Ground water flow threatened public water wells located 1.5 miles from the source. Concentrations of PCE in the aquifer was 15 mg/L above the maximum contaminant level of 5 µg/L. Tracer studies with potassium bromide (KBr) were conducted before, during and after the bioremediation study. Nutrient solutions prepared with YE, SOME, lactate and glucose were used for biostimulation and preconditioning of ground water prior to KB-1 injection. Nutrients

were provided twice during the pilot study to supplement microbial growth and cheese whey was used. During biostimulation no degradation beyond DCE was evident. The addition of KB-1 reduced PCE and DCE concentrations in the monitoring wells of the pilot study area. Total chlorinated ethene concentrations did not reach background levels 2 years after the last nutrient addition. Tracer results showed that microbial growth decreased ground water velocity during the study, but returned to normal conditions 1 year after the last nutrient addition. In this study we were able to show that native microbial population was not able to degrade PCE to final end products. Therefore, it was necessary to introduce KB-1 culture along with nutrients to support complete reductive dechlorination of PCE.

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TABLE OF CONTENTS

LIST OF FIGURES.....	xi
LIST OF TABLES.....	xxi
ACKNOWLEDGMENTS.....	xxii
DEDICATION.....	xxiv
CHAPTER-1 INTRODUCTION.....	1
Chlorinated Ethenes.....	1
Basic Properties of Chlorinated Ethenes.....	1
Health Hazard	2
Environmental Concerns.....	4
Remediation Approaches	4
Abiotic Pathways.....	4
Abiotic Technologies.....	4
Aerobic Degradation.....	7
Aerobic Oxidation.....	7
Aerobic Cometabolism.....	7
Anaerobic Degradation.....	8
In-Situ Bioremediation.....	11
Biostimulation.....	11
Bioaugmentation.....	12
KB-1 Bacterial Culture.....	14
Factors Affecting Culture Performance.....	15
Advantages and Limitations on In-Situ Bioremediation (ISB).....	17
Field Application of Biodegradation.....	19
Phytoremediation.....	20
Cinderella Dry Cleaning Site.....	21
Site Location and History.....	21
Contaminant Plume Description.....	23
Monitoring Wells Establishment.....	24
Lithology and Ground Water Flow.....	25
Research Objectives and Significance.....	25

CHAPTER-2 EXPERIMENTAL METHODS.....	35
Soil Sample Analysis.....	35
Soil Buffering Capacity.....	35
Water Depth Measurement.....	36
Ground Water Sampling.....	36
Microcosms Preparations.....	37
Reagents and supply.....	37
Soil Microcosms.....	38
Water Microcosms.....	39
Biostimulation Experiment.....	40
Monitoring Well Screening and their Biodegradation Potential...	40
Optimizing Nutrient Concentration for bioremediation.....	41
KB-1 Viability Study.....	41
Cheese Whey Microcosms.....	41
Whey Microcosms/ Set 1.....	42
Whey Microcosms/ Set 2.....	42
Whey Microcosms/ Set 3.....	43
Whey Microcosms/ Set 4.....	43
Whey Microcosms/ Set 5.....	44
Buffered Microcosms.....	44
MW-5 Culture Microcosms.....	45
Analytical Methods.....	45
Pilot Study (Field Scale).....	47
Deep Wells Tracer Study Prior to Biostimulation (Fall 2004).....	47
Shallow Wells Tracer Study Prior to Biostimulation (Fall 2004).....	48
Injection Wells Installation (Fall 2005).....	48
Tracer Study with Biostimulation Experiment (Fall 2005).....	49
KB-1 Injection (Fall 2005).....	50
Nutrient Feeding with Tracer Study (Spring 2006).....	51
Cheese Whey Feeding (Fall 2006).....	51
Final Tracer Study (Fall 2007).....	52

CHAPTER-3 RESULTS AND DISCUSSION.....	62
Soil Core Analysis.....	62
Soil Microcosm Results	62
Water Microcosms Results.....	63
Biostimulation Experiment.....	63
Monitoring Wells Screening and their Biodegradation Potential.....	64
Optimizing Nutrient Concentration Whey for bioremediation.....	65
KB-1 Viability Study.....	66
Cheese Whey Microcosms Studies.....	66
Whey Microcosms/ Set 1.....	66
Whey Microcosms/ Set 2.....	67
Whey Microcosms/ Set 3.....	67
Whey Microcosms/ Set 4.....	68
Whey Microcosms/ Set 5.....	68
Soil Buffering Capacity.....	68
Buffered Microcosms.....	69
MW-5 Culture Microcosms.....	69
Pilot Study (Field Scale).....	70
Groundwater Elevation.....	70
Deep Wells Tracer Prior to Biostimulation.....	71
Shallow Wells Tracer Prior to Biostimulation.....	71
Fall 2005 and Spring 2006 Tracer Studies.....	71
Shallow Zone.....	72
Deep Zone.....	72
Final Tracer Study after Completing Pilot Bioremediation.....	73
Chlorinated Ethenes.....	73
Monitoring Inorganic Ions.....	74
Nitrate Concentrations.....	74
Sulfate and Chloride Concentrations.....	75

CHAPTER-4 CONCLUSIONS AND FUTURE WORK.....	182
Conclusions.....	182
Laboratory Results.....	182
Field Studies.....	182
Future Work and Recommendations.....	183
CHAPTER-5 REFERENCES.....	185

LIST OF FIGURES

Figure 1.1	Sequential reduction of PCE to ethene by anaerobic reductive dechlorination (AFCEE Technology Transfer website, 2008).....	30
Figure 1.2	A. Arial map showing location of former Cinderella and current Stickle drycleaners. B. enlarged image of pilot study area showing location of monitoring wells used in this study. Images were taken from Google earth	31
Figure 1.3	Picture of city parking lot showing location of shallow (S) and deep (D) monitoring wells and spacing between wells. Ground water flow in the subsurface moving eastward.....	32
Figure 1.4	Schematic of screening zones shown in A. Injection wells and B. Monitoring wells and the sampling depths for shallow and deep wells.....	33
Figure 1.5	GIS arial map showing location of PCE source (Oval shape), direction and width of contaminating plume (Dashed lines) in the pilot study area.....	34
Figure 2.1	Picture of A. Soil microcosm vials, B. Water Microcosm vials and C. color change of resazurin indicator from Blue to pink then to highly reduced state when colorless.....	58
Figure 2.2	A. Picture of tools used in sampling; the monitoring wells showing the three stage pump powered by a car battery and collection vials. B. The water level indicator used to take depth to water measurements.....	59
Figure 2.3	KB-1 culture from SiREM A. Picture of the 20 L vessel purchased for the pilot study site injection, B. The 125 ml bottle used for microcosm preparations, provided by SiREM.....	60
Figure 2.4	Pictures of A. First tracer study and B. KB-1 Injections.....	61
Figure 3.1	Chlorinated ethenes and methane concentrations in soil Microcosm study. A. Show control without any substrates, B. SOME treatment, C. SOME + Glucose+ YE treatment.....	84
Figure 3.2 (A-C)	Chlorinated ethenes and methane concentrations in water microcosm (Biostimulation Experiment). Prepared with water from MW-4D, and treated with A. SOME, B. SOME + YE and C. Glucose+ SOME+ YE....	85
Figure 3.2 (D-F)	Chlorinated ethenes and methane concentrations in water microcosm (Biostimulation Experiment). Prepared with water from MW-4D, and treated with D. Glucose+ YE, E. Methanol + YE and F. Methanol.....	86
Figure 3.2 (G)	Chlorinated ethenes and methane concentrations in water microcosm (Biostimulation Experiment). Prepared with water from MW-4D, and treated with G. YE.....	87

Figure 3.3 (A-C)	Chlorinated ethenes and methane concentrations in water microcosm (Biostimulation Experiment). Prepared with water from MW-8S, and treated with A. SOME , B. SOME + YE and C. Glucose+ SOME+ YE	88
Figure 3.3 (D-F)	Chlorinated ethenes and methane concentrations in water microcosm (Biostimulation Experiment). Prepared with water from MW-8S, and treated with D. Glucose+ YE , E. Methanol and F. Methanol+ YE	89
Figure 3.3 (G)	Chlorinated ethenes and methane concentrations in water microcosm (Biostimulation Experiment). Prepared with water from MW-8S, and treated with G. YE	90
Figure 3.4 (A-C)	Chlorinated ethenes and methane concentrations in water microcosm (Biostimulation Experiment). Prepared with water from MW-8D, and treated with A. SOME , B. SOME + YE and C. Glucose+ SOME+ YE	91
Figure 3.4 (D-F)	Chlorinated ethenes and methane concentrations in water microcosm (Biostimulation Experiment). Prepared with water from MW-8D, and treated with D. Glucose+ YE , E. Methanol and F. Methanol+ YE	92
Figure 3.4 (G)	Chlorinated ethenes and methane concentrations in water microcosm (Biostimulation Experiment). Prepared with water from MW-8D, and treated with G. YE	93
Figure 3.5	Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-1D, and treated with A. Glucose + YE and B. Glucose+ YE and KB-1 culture.....	94
Figure 3.6	Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-1D, and treated with A. Lactate+ SOME+ YE and B. Lactate+ SOME+ YE and KB-1 culture.....	95
Figure 3.7	Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-2D, and treated with A. Glucose+ SOME+ YE and B. Glucose+ SOME+ YE and KB-1 culture.....	96
Figure 3.8	Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-2D, and treated with A. Lactate+ YE and B. Lactate+ YE and KB-1 culture.....	97
Figure 3.9	Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-3D, and treated with A. Glucose+ SOME+ YE and B. Glucose+ SOME+ YE and KB-1 culture.....	98

Figure 3.10	Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-3D, and treated with A. Lactate+ YE and B. Lactate+ YE and KB-1 culture.....	99
Figure 3.11	Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-4D, and treated with A. Lactate+ YE and B. Lactate+ YE and KB-1 culture.....	100
Figure 3.12	Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-5D, and treated with A. Glucose + YE and B. Glucose+ YE and KB-1 culture.....	101
Figure 3.13	Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-5D, and treated with A. SOME+ YE and B. SOME+ YE and KB-1 culture.....	102
Figure 3.14	Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-6D, and treated with A. Glucose+ SOME+ YE and B. Glucose+ SOME+ YE and KB-1 culture.....	103
Figure 3.15	Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-6D, and treated with A. Glucose + YE and B. Glucose+ YE with KB-1 culture....	104
Figure 3.16	PCE degradation rates in YE+ Lactate treatments in water microcosm for optimizing concentrations.....	105
Figure 3.17	PCE degradation rates in YE+ SOME treatments in water microcosm for optimizing concentrations.....	106
Figure 3.18	PCE degradation rates in YE+ SOME+ Lactate treatments in water microcosm for optimizing concentrations.....	107
Figure 3.19	CEs and methane concentrations in water microcosms (optimizing substrate concentrations). A. Control and B. Control with KB-1 bacterial Culture..	108
Figure 3.20	CEs and methane concentrations in water microcosms (optimizing substrate concentrations). A. 0.01% YE+ 3mM Lactate, and B. 0.01% YE+ 3mM Lactate with KB-1 bacterial Culture.....	109
Figure 3.21	CEs and methane concentrations in water microcosms (optimizing substrate concentrations). A. 0.01% YE+ 1mM Lactate, and B. 0.01% YE+ 1mM Lactate with KB-1 bacterial Culture.....	110
Figure 3.22	CEs and methane concentrations in water microcosms (optimizing substrate concentrations). A. 0.001% YE+ 3mM Lactate, and B. 0.001% YE+ 3mM Lactate with KB-1 bacterial Culture.....	111

Figure 3.23	CEs and methane concentrations in water microcosms (optimizing substrate concentrations). A. 0.01% YE+ 0.003mM Lactate, and B. 0.01% YE+ 0.003mM Lactate with KB-1 bacterial Culture.....	112
Figure 3.24	CEs and methane concentrations in water microcosms (optimizing substrate concentrations). A. 0.01% YE+ 0.001mM Lactate, and B. 0.01% YE+ 0.001mM Lactate with KB-1 bacterial Culture.....	113
Figure 3.25	CEs and methane concentrations in water microcosms (optimizing substrate concentrations). A. 0.003% YE+ 0.003mM Lactate, and B. 0.003% YE+ 0.003mM Lactate with KB-1 bacterial Culture.....	114
Figure 3.26	CEs and methane concentrations in water microcosms (optimizing substrate concentrations). A. 0.01% YE+0.001% SOME+ 0.3mM Lactate, and B. 0.01% YE+ 0.001% SOME+ 0.3mM Lactate with KB-1 bacterial Culture	115
Figure 3.27	CEs and methane concentrations in water microcosms (KB-1 Viability Study) in control treatment.....	116
Figure 3.28	CEs and methane concentrations in water microcosms (KB-1 Viability Study) treated with YE (0.01%)+ SOME (0.01%)+ Lactate (3 mM). KB-1 is not added to this treatment.....	117
Figure 3.29	CEs and methane concentrations in water microcosms (KB-1 Viability Study) treated with YE (0.01%)+ SOME (0.01%)+ Lactate (3mM). KB-1 is present in this treatment.....	118
Figure 3.30	PCE degradation rate in treatments without KB-1 bacterial culture, (whey microcosm/ Set 1).....	119
Figure 3.31	PCE degradation rate in treatments with KB-1 bacterial culture, (whey microcosm/ Set 1).....	120
Figure 3.32	CEs and methane concentrations in control treatment, (Whey microcosm/ Set 1).....	121
Figure 3.33	CEs and methane concentrations in 0.25% Whey treatment (Whey microcosm/ Set 1) A. without KB-1 and B. With KB-1.....	122
Figure 3.34	CEs and methane concentrations in 0.025% Whey treatment (Whey microcosm/ Set 1). A. without KB-1 and B. With KB-1.....	123
Figure 3.35	CEs and methane concentrations in YE (0.003%) + SOME (0.01%) treatment (Whey microcosm/ Set 1). A. without KB-1 and B. With KB-1	124
Figure 3.36	CEs and methane concentrations in Whey (0.002%) + SOME (0.01%) treatment (Whey microcosm/ Set 2). A. without KB-1 and B. With KB-1.....	125

Figure 3.37	CEs and methane concentrations in Whey (0.025%) + SOME (0.01%) treatment (Whey microcosm/ Set 2). A. without KB-1 and B. With KB-1.....	126
Figure 3.38	PCE degradation rate in filtered whey microcosms treated with cheese whey and KB-1.....	127
Figure 3.39	CEs and methane concentrations in control treatment (Whey microcosm/ Set 3). Standard error is shown based on n= 3.....	128
Figure 3.40	CEs and methane concentrations in Whey (0.25%) treatment (Whey microcosm/ Set 3). A. without KB-1 and B. With KB-1. Standard error is shown based on n=3.....	129
Figure 3.41	CEs and methane concentrations in Whey (0.1%) treatment (Whey microcosm/ Set 3). A. without KB-1 and B. With KB-1. Standard error is shown based on n=3.....	130
Figure 3.42	CEs and methane concentrations in Whey (0.025%) treatment (Whey microcosm/ Set 3). A. without KB-1 and B. With KB-1. Standard error is shown based on n=3.....	131
Figure 3.43	CEs and methane concentrations in Whey (0.025%) treatment (Whey microcosm/ Set 4) prepared with water from MW-8D.....	132
Figure 3.44	CEs and methane concentrations in Whey (0.025%) treatment (Whey microcosm/ Set 4) prepared with water from MW-9D.....	133
Figure 3.45	CEs and methane concentrations in YE (0.01%) treatment (Whey microcosm/ Set 4) prepared with water from MW-8D.....	134
Figure 3.46	CEs and methane concentrations in YE (0.01%) treatment (Whey microcosm/ Set 4) prepared with water from MW-9D.....	135
Figure 3.47	CEs and methane concentrations in control treatment (Whey microcosm/ Set 5) prepared with water from top water of MW-5D. A. with KB-1 and B. without KB-1.....	136
Figure 3.48	CEs and methane concentrations in 0.05% Whey treatment (Whey microcosm/ Set 5) prepared with water from top water of MW-5D. A. with KB-1 and B. without KB-1.....	137
Figure 3.49	CEs and methane concentrations in 0.025% Whey treatment (Whey microcosm/ Set 5) prepared with water from top water of MW-5D. A. with KB-1 and B. without KB-1.....	138
Figure 3.50	CEs and methane concentrations in control treatment (Whey microcosm/ Set 5) prepared with water from bottom water of MW-5D. A. with KB-1 and B. without KB-1.....	139

Figure 3.51	CEs and methane concentrations in 0.05% Whey treatment (Whey microcosm/ Set 5) prepared with water from Bottom water of MW-5D. A. with KB-1 and B. without KB-1.....	140
Figure 3.52	CEs and methane concentrations in 0.025% Whey treatment (Whey microcosm/ Set 5) prepared with water from Bottom water of MW-5D. A. with KB-1 and B. without KB-1.....	141
Figure 3.53	Soil titration curve with 1 M lactic acid. Soil sample used at 42.5 ft bgs during MW-9D installation.....	142
Figure 3.54	Soil titration curve with 0.1 M lactic acid. Soil sample used at 42.5 ft bgs during MW-9D installation.....	143
Figure 3.55	CEs and methane concentrations in soil microcosms prepared with 42.5 ft deep soil MW-9D. All treatments were bioaugmented with KB-1. A. pH not controlled and B. pH controlled with calcium carbonate and occasional addition of tris-base.....	144
Figure 3.56	CEs and methane concentrations in water microcosms prepared with water from MW-5D. All treatments were bioaugmented with KB-1. A. pH not controlled and B. pH controlled with calcium carbonate and occasional addition of tris-base.....	145
Figure 3.57	CEs and methane concentrations in microcosms prepared with water from the top of MW-5D, and bioaugmented with KB-1.....	146
Figure 3.58	CEs and methane concentrations in microcosms prepared with water from the top of MW-5D, and bioaugmented with sediment collected from bottom of MW-5.....	147
Figure 3.59	CEs and methane concentrations in microcosms prepared with water from the top of MW-5D, and bioaugmented with KB-1 and sediment from bottom of MW-5.....	148
Figure 3.60	Groundwater elevation data for the monitoring wells at the pilot study area....	149
Figure 3.61	Bromide concentrations in MW-8D (injection well); injection concentration ~500 mg/L, August 2, 2004, day 0.....	150
Figure 3.62	Bromide concentrations in MW-9D, 10 feet from injection well MW-8D; injection concentration ~500 mg/L, August 2, 2004, day 0.....	151
Figure 3.63	Bromide concentrations in MW-10D, 20 feet from injection well MW-8D; injection concentration ~500 mg/L, August 2, 2004, day 0.....	152
Figure 3.64	Bromide concentrations in MW-9S, (10 ft) east from the injection well MW-8S, injection concentration is ~500 mg/L, 9/16/2004.....	153
Figure 3.65	Bromide concentration in MW-10S, (20 ft) east from injection well MW-8D, injection concentration is ~500 mg/L, 9/15/2004.....	154

Figure 3.66	Bromide concentrations in MW-8S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).....	155
Figure 3.67	Bromide concentration in MW-9S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).....	156
Figure 3.68	Bromide concentration in MW-10S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).....	157
Figure 3.69	Bromide Concentration in MW-12S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).....	158
Figure 3.70	Bromide concentration in MW-8D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).....	159
Figure 3.71	Bromide concentration in MW-9D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).....	160

Figure 3.72	Bromide concentration in MW-10D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).....	161
Figure 3.73	Bromide concentration in MW-12D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).....	162
Figure 3.74	Bromide concentration in MW-8D, (injection well), injection concentration is ~817 mg/L, 7/11/2007.....	163
Figure 3.75	Bromide concentration in MW-9D, (injection well), (10 ft) east from the injection well MW-8D, injection concentration is ~817 mg/L,7/11/2007..	164
Figure 3.76	Bromide concentration in MW-10D, (injection well), (20 ft) east from the injection well MW-8D, injection concentration is ~817 mg/L,7/11/2007..	165
Figure 3.77	Concentrations PCE and DCE in MW-8D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).....	166
Figure 3.78	Chlorinated ethenes concentrations in MW-9D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).....	167
Figure 3.79	Chlorinated ethenes concentrations in MW-10D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).....	168

Figure 3.80	Chlorinated ethenes concentrations in MW-12D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).....	169
Figure 3.81	Chlorinated ethenes concentrations in MW-8S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).....	170
Figure 3.82	Chlorinated ethenes concentration in MW-9S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).....	171
Figure 3.83	Chlorinated ethenes concentrations in MW-10S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).....	172
Figure 3.84	Chlorinated ethenes concentrations in MW-12S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).....	173
Figure 3.85	Nitrate Concentration in MW-8D; Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), day 56 (Oct 13, 2005), day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).....	174
Figure 3.86	Nitrate concentration in MW-9D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), day 56 (Oct 13, 2005), day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).....	175

Figure 3.87	Nitrate concentration in MW-10D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), day 56 (Oct 13, 2005), day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).....	176
Figure 3.88	Nitrate concentration in MW-12D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), day 56 (Oct 13, 2005), day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).....	177
Figure 3.89	Nitrate concentrations in MW-8S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), day 56 (Oct 13, 2005), day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).....	178
Figure 3.90	Nitrate concentration in MW-9S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), day 56 (Oct 13, 2005), day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).....	179
Figure 3.91	Nitrate Concentration in MW-10S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), day 56 (Oct 13, 2005), day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).....	180
Figure 3.92	Nitrate concentration in MW-12S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), day 56 (Oct 13, 2005), day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).....	181

LIST OF TABLES

Table 1.1	Physical-chemical properties of chlorinated ethenes and their degradation products.....	27
Table 1.2	Half-reaction potentials for dechlorination reactions (Vogel <i>et al.</i> , 1987)....	28
Table 1.3	Bioaugmentation cultures used for treatment of chlorinated solvents in groundwater. Adapted from ESTCP, 2005.....	29
Table 2.1	Composition and concentration of amendments and tracer in the nutrient solution used for the preconditioning study on August 18, 2005.....	53
Table 2.2	Anaerobic chase water content used in the preconditioning study on August 18, 2005.....	54
Table 2.3	Content of nutrient and anaerobic chase water barrels (200 L each) used for bioaugmentation study on October 13, 2005.....	55
Table 2.4	Treatments used in water microcosms (optimizing nutrient concentrations for bioremediation).....	56
Table 2.5	Characteristics of cheese whey (Ghaly <i>et. al.</i> , 2003).....	57
Table 3.1	Characteristics of soil samples collected on August 5, 2003 during installation of MW-9D.....	76
Table 3.2	Soil microcosm biostimulation treatments and their ability to produce DCE. Each treatment was done in duplicates and values of 50 % and above is shown in red color.....	77
Table 3.3	Color change of Phenol red indicator in response to the pH of buffer solution.....	78
Table 3.4	Monthly rainfall (inches) in Manhattan for the period May 2003 to April 2007.....	79
Table 3.5	Groundwater elevation (listed value+1000ft above MSL) in the deep zone...80	
Table 3.6	Groundwater elevation (listed value+1000ft above MSL) in shallow zone...81	
Table 3.7	Bromide arrival times compared between Fall 4004 and Fall 2007 tracer studies at the pilot study area.....	82
Table 3.8	Comparison of Groundwater velocities between Fall 2004 and Fall 2007 tracer studies at the pilot study area.....	83

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DEDICATION

TO

my parents Sarab Haris and Hussein Ibbini

my husband Hasan Hatamleh and daughter Nada

And to the loving memory of my grandfathers Abdul-Kareem Haris and Mohammed Ibbini

CHAPTER 1 –INTRODUCTION AND LITRATURE REVEIW

Chlorinated Ethenes

Chlorinated ethenes were first introduced in the 1930's and used in many processes and industries for airplane maintenance and engine manufacturing, paint removal and electronics manufacturing. The two major applications of chlorinated ethenes have been the degreasing machinery and dry cleaning. There are approximately 36,000 active dry cleaning facilities in the United States, and 75% of these facilities have contributed to soil and groundwater contamination (Linn *et al.* 2004). Most releases of chlorinated ethenes occurred in the 1950's through the 1970's before potential effects on health and environment were fully understood (ITRC 2005).

Basic Properties of Chlorinated Ethenes

Physical and chemical properties of chlorinated ethenes are listed in Table 1.1.

The characteristics of chlorinated ethenes help understand the fate and transport of these contaminants in the soil and groundwater, and therefore, provide information for the remediation options. For example, Henry's Law constant (H), determines the tendency of a compound to move from the water phase to the vapor phase at equilibrium (Davis *et al.*, 2001). Vinyl chloride has the highest value of H. Indicating preference for the vapor phase. Water solubility affects the mobility of the compound in groundwater. PCE is the least soluble among other chlorinated ethenes and therefore, it has a high tendency to be retained in the soil matrix. PCE is also heavier than water and tends to form a dense non-aqueous phase liquid (DNAPL) and sink in subsurface formations. The octanol-water partition coefficient (often shown in logarithmic value $\log K_{ow}$) indicates the affinity to organic matter and lipids. High values of $\log K_{ow}$ mean that the chemical is highly

hydrophobic and would prefer to initially associate with organics in the soil. Conversely, chemicals with low log K_{ow} like vinyl chloride would associate with the water phase, and would move more readily with ground water. In general the physical properties of this set of compounds are linked to the number and location of chlorine atoms. With the increase of chlorine content the values of solubility, vapor pressure and H decrease, while log K_{ow} values increase (Lawrence, 2007).

Health Hazard

Humans can be exposed to chlorinated ethenes through inhalation of ambient air, drinking water or by occupational exposure, primarily in dry cleaning operations and in industries that use those chemicals. Acute (short term) exposure to PCE through inhalation may include irritation to upper respiratory tract and eyes, kidney dysfunction, dizziness, headache, sleepiness, and unconsciousness. Low concentrations may have neurological effects such as mood change and coordination impairment. Chronic (long term) inhalation of tetrachloroethene have neurological effects, including sensory symptoms such as headaches, impairments in cognitive and motor neurobehavioral functioning and color vision decrements. Other effects noted in humans include cardiac arrhythmia, liver damage, and possible kidney effects. Reproductive effects such as spontaneous abortions, menstrual disorders, altered sperm structure, and reduced fertility, have been reported with less certainty. Cancer development risk can also be linked to PCE exposure (U.S. EPA, 1994).

Acute and chronic exposure to trichloroethylene (TCE) by inhalation affects the human central nervous system. Case reports of intermediate and chronic occupational

exposures included effects such as dizziness, headache, sleepiness, nausea, confusion, blurred vision, facial numbness, and weakness. Effects to the liver, kidneys, and immune and endocrine systems have also been seen in humans exposed to trichloroethylene occupationally or from contaminated drinking water. TCE exposure can be associated with several types of cancers in humans, especially kidney, liver, cervix, and lymphatic system (U.S. EPA, 2000). Health problems associated with chronic exposure to either cis- or trans-1,2-dichloroethylene include liver, circulatory and nervous system damage from long-term exposure, while acute inhalation can cause central nervous system depression (U.S. EPA, 2006).

Acute exposure to vinyl chloride may cause dizziness, drowsiness, headache, eye and respiratory tract irritation. Short term exposure to high concentrations of vinyl chloride can lead to loss of consciousness, lung and kidney irritation and inhibition of blood clotting. Chronic inhalation or oral exposure leads to liver damage, central nervous system effects as well as peripheral nervous system symptoms (peripheral neuropathy, tingling, numbness and weakness). Developmental effects may occur in pregnant women with high rate of birth defects. Chronic inhalation of vinyl chloride increases the risk of forming angiosarcoma in the liver tissue (U.S. EPA, 2000).

Environmental Concerns

Chlorinated ethenes moving in ground water have the chance to discharge into other ecological systems and surface water. Contaminant discharge into sensitive ecosystems like wetlands has been reported (Lorrah, 2004). Although chlorinated ethenes do not bioaccumulate and they volatilize to the atmosphere, exposing risk to the contaminating receptor ecosystem. Loading of surface water ecosystems need to be evaluated for impacts on public health and the environment (Ward, 1997).

Remediation Approaches

Abiotic Pathways

A number of abiotic processes may degrade chlorinated ethenes under both aerobic and anaerobic conditions. Abiotic pathways include hydrolysis, elimination, dehydrohalogenation, hydrogenolysis, dichloroelimination, and abiotic reductive dechlorination. Many abiotic transformations of chlorinated ethenes occur at rates that are too slow to have significance in contaminant removal (AFCEE, 2004).

Abiotic Technologies

A number of treatment options have been developed for chlorinated ethenes. Those strategies can be adapted alone or in combination with enhanced bioremediation application to achieve on site remediation objectives. Christ *et al.* (2005) showed the advantages of coupling abiotic treatments with microbial reductive dechlorination for DNAPL zone treatment.

- **Natural attenuation:** Natural processes that reduce contaminant levels in groundwater include biodegradation, abiotic transformation, sorption and dilution. This technology is used to address low concentrations of contaminants over large areas, or can be used as a polishing technique, as it can be a feasible and cost effective approach (Lee *et al.*, 1998; Gosset and Zinder 1997). A recent study argues that relying on monitoring of chlorinated ethene products (c-DCE and VC) or non-chlorinated products (ethene and ethane) may not be suitable to determine the efficiency of natural attenuation processes, because reductive dechlorination can be combined with oxidative or mineralization process, to yield CO₂ and methane as final products (Bradley and Chapelle, 2007). Hence one may need to understand the activity of several processes.

- **Thermal Technologies:** These include steam injection or electrical heating of the subsurface water to nearly boiling temperatures at the contaminated site. This leads to volatilization and mobilization of contaminants for subsequent extraction. High temperatures used in this process reduce the population of viable microorganisms during thermal treatment, but dechlorination activity appears to recover (Truex *et al.*, 2007).

- **In Situ Chemical Oxidation:** Chemical oxidation is a widely applied source treatment technology. A strong oxidizing agent such as permanganate (Thomson *et al.*, 2007) or Fenton's reagent is introduced to a contaminated site. Byproducts include carboxylic acids, manganese dioxide and chloride when oxidizing chlorinated compounds (Hood *et al.*, 1998).

In situ oxidation decreases the abundance and activity of microorganisms in the source area. A microbial shift was noted from sulfate- reducing to Fe (III) reducing conditions following Fenton's reagent treatment, which would decrease the efficiency of reductive dechlorination in the injection zone (Chapelle *et al.*, 2005).

- **Zero Valent Iron:** Abiotic degradation by elemental iron involves two simultaneous reactions: oxidation of iron by water and reductive dechlorination with Fe⁰ serving as a source of electrons. Permeable barriers depend either on sorption or destruction of contaminants. Zero valent iron has been shown to be effective for degradation of chlorinated compounds in groundwater. Alterations of the technology can be made with the use of zero valent iron with surfactants (Li *et al.*, 1999; Clark II *et al.*, 2003) or cosolvents (Clayton *et al.*, 2003). Nanoscale zero valent iron was also tested with vegetable oil to create a suitable environment for reductive dechlorination (Geiger *et al.*, 2003).

- **Surfactants and Cosolvents:** These are usually used to increase the dissolution of DNAPL into the aqueous phase, and thereby increase the rate of source mass removal. Cosolvents include alcohols such as ethanol, methanol and isopropanol (ITRC, 2003). Most surfactants and cosolvents can also act as electron donor and can support in situ bioremediation (Strbak, 2000).

- **Pump and treat:** Conventional method depends on pumping contaminated groundwater to the surface and then removing the contaminant by air stripping or

adsorption to charcoal. Over the years this technology failed to perform as expected and was not cost efficient (Ward, 1997). Recirculating the water is an alternative to the conventional pump and treat method and does not affect the configuration of the water table (Major *et al.*, 2002; Ryan *et al.*, 2000); but this technique is also very costly.

Aerobic Degradation

Aerobic Oxidation

Aerobic oxidation is favored for less chlorinated ethenes such as vinyl chloride (VC) (Lee *et al.*, 1998). Anaerobic oxidation of VC can occur under mild reducing conditions like iron and manganese reduction potential. These conditions can be found downgradient of some anaerobic zones. In some cases a combination of an anaerobic reaction zone followed by aerobic oxidation zone would be highly effective for treating chlorinated ethenes (Löffler and Edwards, 2006).

Aerobic Cometabolism

It was discovered that chlorinated ethenes except PCE can be oxidized by aerobic bacteria (Christ *et al.*, 2005). Those organisms grow on a wide range of substrates and produce oxygenases (Hopkins *et al.*, 1993). Cometabolism is the fortuitous transformation of a compound by an enzyme synthesized by the cell for the metabolism of another compound (Zhang and Bennett, 2005; McCarty, 1997). Cometabolism of chlorinated compounds does not yield any energy or growth benefit for the microbe mediating the reaction. Sometimes, cometabolic degradation of chlorinated ethenes has a fast rate of reaction with no intermediate metabolite accumulation of dichloroethene and

vinyl chloride (Bradley *et al.*, 2008). The process also generates a high yield of biomass and it is easy to handle in the field. The abundance of microorganisms harboring mono and di-oxygenase enzymes facilitates the application and establishment of the bacterial culture in situ. Chlorinated ethenes degradation can be enhanced by the addition of methane, aromatic hydrocarbons, ammonia, and propane. The rate of cometabolism increases as the degree of chlorination decreases on the ethene molecule (Johan *et al.*, 2001). Although laboratory results show promising results, field application of aerobic cometabolism was found to be challenging (Azizian *et al.*, 2007). The introduction of oxygen and substrate into the geologic formation was difficult and not cost efficient (McCarty, 1997; Shen and Sewel, 2005). Also, rapid growth of organisms surrounding the injection points limited the success of this application at the field-scale level (Johan *et al.*, 2001; ESTCP, 2005). Another limitation is that TCE concentration in the milligram per liter range is toxic to microbes catalyzing this reaction (Wiedemeier *et al.*, 1998).

Anaerobic Degradation

Microorganisms preferentially reduce the electron acceptor that yields the highest free energy from the redox reaction. Therefore oxygen is the preferred electron acceptor. Presence of substrates stimulates microbial growth and therefore increases oxygen consumption in the subsurface. Bioremediation of organic substrates often depletes the dissolved oxygen (DO) and other terminal electron acceptors (e.g., nitrate and sulfate), and lowers the oxidation reduction potential (ORP) of groundwater, thereby stimulating conditions for anaerobic degradation. After dissolved oxygen is consumed,

microorganisms then use the next available electron acceptor in the following order of preference: nitrate, manganese (IV), iron (III), sulfate and finally carbon dioxide (methanogenesis) (McMahon and Chapelle, 2008). Studies show that chlorinated ethenes can be used as electron acceptors during anaerobic degradation and are reduced to less-chlorinated and or non-chlorinated ethenes (Bradley and Chapelle, 1999). The primary process that drives anaerobic degradation of chlorinated ethenes is reductive dechlorination that is also referred to as halorespiration. Anaerobic reductive dechlorination is a sequential dechlorination reaction catalyzed by microorganisms in which a chlorine atom is replaced by hydrogen (electron donor) on chlorinated ethene (Major *et al.*, 2002). PCE, which contains four chlorine atoms, is sequentially degraded to TCE to DCE to VC and then to ethene as shown in (Figure 1.1). Primarily TCE is degraded to the isomer cis-1,2-dichloroethene (cis-DCE); but infrequently TCE degradation results in the production of trans-1,2-DCE. The process depends on environmental factors that include the presence of strongly anaerobic conditions, availability of fermentable substrates, generation of molecular hydrogen (H₂) and the presence and viability of the appropriate microbial population to facilitate the reaction (Major *et al.*, 2002). More reduced conditions are required for degradation as the oxidation state of the compound is lowered from PCE and TCE to DCE and VC (Bradley *et al.*, 2008). Unlike aerobic conditions, degradation rates under anaerobic conditions decrease with decreasing degree of halogen substitution. As a result it is common for incomplete degradation to occur in contaminated environments where DCE or VC accumulates (Johan *et al.*, 2001; Russell *et al.*, 1992). Vinyl chloride accumulation is not a desirable product since it is more toxic than any of the parent compounds and it is a

known human carcinogen (He *et al.*, 2003; McCarty, 1997). It is found that sulfate and methanogenic groundwater conditions can facilitate reductive dechlorination of DCE and VC (Wiedemeir *et al.*, 1998). Although temporary inhibition of PCE dechlorination was noted in the presence of nitrate and sulfate, the process was resumed after nitrate reduction was followed by sulfate reduction (Shen and Sewel, 2005). Complete reductive dechlorination to ethene without an accumulation of toxic daughter products is most likely to occur under methanogenic conditions. One concern is that methanogens might out-compete dechlorinators for electron donor, but they may fail to grow at high PCE and TCE concentrations near the source zone (Yang and McCarty, 2000).

A number of microorganisms have been isolated in pure and mixed cultures that can reductively dechlorinate PCE and TCE and use them as terminal electron acceptors and obtain energy from the process for cell growth (Löffler and Edwards, 2006). Most cultures known so far to carry out complete biodegradation of PCE and TCE beyond DCE to ethene belong to the genus *Dehalococcoides* (Maymo-Gatell *et al.*, 1997; Yang *et al.*, 2005). The *Dehalococcoides* species occupy a unique ecological niche and are not ubiquitously found in groundwater environments (Major *et al.*, 2002). Several members of the *Dehalococcoides* have been isolated and show different abilities to biodegrade chlorinated compounds e.g. *Dehalococcoides ethenogenes* 195 can degrade PCE all the way to ethene (Maymo-Gatell *et al.*, 1997; Smidt and de Vos, 2004), while *Dehalococcoides* species designated as BAV1 obtains energy from VC dechlorination to ethene (Löffler *et al.*, 2003). Detection of *Dehalococcoides* 16S rRNA gene sequence in a contaminated site suggests the potential for dechlorinating activity (Yang and Zeyer, 2003) but it is not sufficient by itself. Therefore, further evidence needs to be collected

from microcosm experiments to assess the extent of dechlorinating ability of endogenous microflora. PCE-reductive dehalogenases (PCE-RDase) and TCE-reductive dehalogenases (TCE-RDase) were identified as enzymes involved in dehalorespiration (Magnuson *et al.*, 1998; Furukawa, 2006). Moreover, the complete genome sequence of *Dehalococcoides ethenogenes* is available (Seshadri *et al.*, 2005).

In-Situ Bioremediation

In situ bioremediation of chlorinated ethenes is a destruction technology that uses microorganisms to break down contaminants to less toxic end products. Microorganisms can only degrade dissolved contamination (not DNAPL or sorbed contaminant); however, dissolution is enhanced during bioremediation resulting in a reduction of DNAPL mass and sorbed contaminant (ITRC, 2005). Bioremediation can occur naturally to degrade chlorinated ethenes if reductive dechlorinators are present and active in the contaminated site in a process called natural attenuation. However, natural attenuation may not occur at all sites or may not be effective to reduce contaminant level to regulatory standards in a reasonable timeframe. Therefore, enhanced in situ bioremediation is a good alternative approach for remediation. Bioremediation can be enhanced by biostimulation and bioaugmentation through an engineered system (ITRC, 2005).

Biostimulation

In biostimulation, substrates (electron donor /nutrients) are injected into the subsurface to encourage microbial growth and increase the number of microorganisms degrading the contaminant. Fermentation of substrates provides hydrogen that is the

primary electron donor for reductive dechlorination (Yang and McCarty, 1998). Other substrates such as acetate (He *et al.* 2002) lactate (Ellis *et al.* 2000), methanol, ethanol (Cox *et al.* 2002) have been used. Depending on site conditions, slow release or fast release hydrogen compounds can be used as substrates. Slow release substrates e.g. vegetable oil and soybean oil, are commercially available. Hydrogen Releasing Compounds (HRC) have low solubility in water and therefore, provide slow and long lasting hydrogen release. On the other hand more soluble compounds, e.g. lactic acid and molasses, release high concentrations of hydrogen (Moretti, 2005).

Bioaugmentation

Bioaugmentation involves the introduction of microorganisms to a contaminated site lacking dechlorination bacteria or when dechlorination is not complete. For bioaugmentation, a consortium of microorganisms is used to promote complete reductive dechlorination. Dehalorespiration of chlorinated ethenes is currently focusing on *Dehalococcoides* organisms (He *et al.*, 2003; Maymo-Gatell *et al.*, 1999; Löffler *et al.*, 2003), despite the identification of anaerobic organisms like *Desulfitobacterium* sp. Strain Y51 (Suyama *et al.*, 2001), *Desulfuromonas* sp. (Sung *et al.*, 2003), an aerobic denitrifying strain of *Pseudomonas* (Ryoo *et al.*, 2000) and white rot fungus *Trametes versicolor* (Marco-urrea *et al.*, 2006). Several enriched consortia containing *Dehalococcoides* have been used in field demonstrations and are commercially available e.g. KB-1, Bachman Road culture (BAV-1 and BDI), and the Pinellas culture, as summarized in Table 1.3. Other cultures have also been developed with varying degrees of understanding about their performance. More information about pure and mixed

microbial cultures can be found in the ESTCP report (2005). Mixed cultures that contain multiple dechlorinating microorganisms such as *Dehalococcoides* and *Dehalobacter* may show either competitive or complementary dechlorination activities, depending on the available chloro-organic substrates (Grostern and Edwards, 2006).

A decision for use of bioaugmentation can be easy at sites where indigenous *Dehalococcoides* microorganisms are absent and/or where partial dechlorination of PCE and TCE occur following electron donor addition. Bioaugmentation in those cases is necessary to provide the right organisms or to supplement the activity of the existing dechlorinating population. However, bioaugmentation has been applied at sites with competent *Dehalococcoides*. Bioaugmentation at *Dehalococcoides* positive sites can be used to decrease the lag time needed for dechlorination to start after biostimulation, thereby shortening the treatment time (ESTCP, 2005). Bioaugmentation was also used at a site where indigenous *Dehalococcoides* were present but not uniformly distributed in the chlorinated ethene contaminated aquifer (Fennel *et al.*, 2001). Assessment protocols are important to determine the need for bioaugmentation. One method is the direct detection of *Dehalococcoides*; but the unique growth requirements of *Dehalococcoides* and their dependence on other anaerobic microorganisms make conventional plate count techniques not feasible for direct detection of these organisms (Löffler and Edwards 2006). Instead molecular biological techniques have been used. Amplification of the 16S rRNA gene for *Dehalococcoides* using PCR has been used to determine the presence or absence of these organisms in an environmental sample. Advancement in quantitative PCR technology help determine the number of 16S rRNA gene copies in a sample (Smits *et al.*, 2004; Sung *et al.*, 2006). Other studies used DNA sequencing to identify different

strains of *Dehalococcoides*, since different strains have different abilities to halo-respire chlorinated ethenes. For example, *D. ethenogenes* 195 reduces PCE or TCE to ethene, while similar strain FL2 can metabolically dechlorinate TCE but not PCE to ethene. *Dehalococcoides* Strain CBDB1 has the ability to dechlorinate chlorobenzenes and polychlorinated dibenzodioxins (Holmes *et al.*, 2006), Full genomic sequence of this strain is now known (Kube *et al.*, 2005). Furthermore, functional genes like PCE and TCE reductive dehalogenase and vinyl chloride reductase have been recently discovered and sequenced (Seshardi *et al.*, 2005).

Another common assessment protocol is the microcosm testing of soil and groundwater of a particular site for dehalorespiring microorganisms. Microcosms are used to determine degradation rate, acclimation periods (time to initiate reductive dechlorination and achieve complete dechlorination to ethene), to monitor dechlorination products and to determine type and dose of electron donor. Microcosms are often combined with molecular screening to examine the dechlorination activity associated with detected organisms (Fennell *et al.*, 2001).

KB-1 Bacterial Culture

KB-1 is an enriched consortium culture developed by Dr. Elizabeth Edwards at the University of Toronto. It is currently marketed by SiREM laboratories (www.siremLab.com). KB-1 Dechlorinator is a natural non-pathogenic microbial culture grown in an aqueous dilute mineral salt solution medium containing no hazardous ingredients. The microbial composition of KB-1 (as determined by phylogenetic analysis of 16S rRNA gene sequences) identified three genera, *Dehalococcoides* sp., *Geobacter*

sp. and *Methanomethylovorans* sp. (SiREM, 2008). This culture is used to carry out complete dechlorination of chlorinated ethenes to the safe end product ethene. Microorganisms in this culture are strictly anaerobes; they require dissolved oxygen (DO) < 0.2 mg/L, and redox potential of <-50 mV for survival (Duhamel and Edwards, 2006).

Factors Affecting Culture Performance

1- Oxygen tolerance: Microorganisms that can carry out reductive dechlorination vary in their response to oxygen. For example *Dehalococcoides* are strict anaerobes and oxygen toxicity can significantly reduce culture viability and performance (He *et al.*, 2003).

2- Geochemical conditions: Redox potential greatly affects bioaugmentation, and is influenced by the concentration of electron acceptors like nitrate, manganese/iron oxides and sulfate. Generally, anaerobic dechlorination is favored under sulfate reducing and methanogenic conditions. Groundwater pH also affects dechlorination. The optimum pH for KB-1 culture is between 6.0 and 8.3 and the culture loses activity at pH below 5 or above 10 (Woznica *et al.*, 2003). Friis *et al.* (2007) reported temperature dependence of reductive trichloroethene dechlorination by KB-1. The study suggests an ideal temperature for bioaugmentation between 15-30 °C. Microorganisms prefer to use the electron acceptor that provides the most energy. As noted from Table 2 the energy released from the reduction of PCE to TCE is highest. As site conditions become more reducing (lower redox potential values), there would be less energetically favorable acceptors available. Under field conditions, reductive dechlorination occurs at half-reaction potentials more commonly associated with sulfate reduction and methanogenesis (ITRC, 2005).

3- Electron donor selection: Most bioaugmentation cultures have the capability of fermenting a wide range of electron donors. *Dehalococcoides* depend on hydrogen as the sole electron donor for dechlorination (Maymo-Gatell *et al.*, 1995), emphasizing the significant role of other microorganisms that support the process by producing hydrogen. A number of organic substrates were used in the subsurface to generate hydrogen by degradation or fermentation. Other nutrients and cofactors are needed including vitamin B₁₂ (Krasothkina *et al.*, 2001). Soluble substrates such as molasses and lactate are commonly dispersed in the aquifer matrix to generate hydrogen (Lee *et al.*, 1997). This means that continuous or periodic application of substrate is needed to maintain treatment conditions (Moretti, 2005). On the other hand viscous hydrogen releasing compounds (HRC) (Jin *et al.*, 2005) and vegetable oils are used for slow release and long lasting substrates. Single or limited numbers of injections are usually sufficient for site remediation (Jin *et al.*, 2005).

To minimize project cost, substrates are chosen with low number of injection points, low frequency of injection events and low cost of the injected substrate. A combination of different substrates is also used in common practice. For example, lactate and vegetable oil are mixed to produce a fast acting and long lasting product (AFCEE, 2004).

4- VOC concentration: Despite early reports that question the effectiveness of bioremediation of chlorinated ethenes at high DNAPL concentrations, recent data reveal that dechlorinating microorganisms are active over a wide range of chloroethene concentrations (Carr *et al.*, 2000; Cope and Hughes, 2001). In a study, chlorinated ethenes were used near solubility limited concentrations to enrich four KB-1 subcultures.

Each subculture received a periodic dose of one chlorinated ethene (PCE, TCE, c-DCE or VC) to promote reductive dechlorination (Duhamel *et al.*, 2002). Vinyl chloride was utilized by *Dehalococcoides ethenogenes* 195 after PCE and TCE consumption (Maymo-Gatell *et al.*, 2001).

5- Inhibition by selected VOCs: Many sites are contaminated with mixtures of chlorinated solvents. Therefore, studying the effects of co-contamination is important to determine the remediation strategy or the appropriate bioaugmentation culture. Duhamel *et al.* (2002) reported a decrease in the rate of VC dechlorination to ethene when chloroform or 1,1,1-TCA (methylchloroform) are present in the KB-1 culture, and VC degradation was completely inhibited when chloroform concentration reached 450 µg/L (3.8 µM) or a 1,1,1-TCA concentration between 700 - 3000 µg/L (5.2-22 µM). PCE dechlorination was inhibited by chloroform at 4 µM and with carbon tetrachloride at 19 µM (Bagley *et al.*, 2000; Sun *et al.*, 2002; Grostern and Edwards, 2006). Both 1,1,1-TCA and chloroform are known to inhibit methanogenesis, and may interfere with reductive dechlorination (Duhamel *et al.*, 2002; Bagley *et al.*, 2000; Maymo-Gatell *et al.*, 2001). These results suggest that multiple-zone remediation strategies may be required for sites contaminated with mixtures of chlorinated solvents.

Advantages and limitations of In Situ Bioremediation (ISB)

Advantages of ISB can be summarized as follow (Adapted from AFCEE, 2004; ITRC, 2005).

- Can be used to reduce acclimation periods by providing a dechlorinating bacterial population.

- Can reduce contaminant mass without creating process waste, and under carefully controlled reaction zone degradation of contaminants to acceptable environmental end points.
- Can be used for short and long term timeframe, either alone or with more aggressive source treatment technologies.
- Implementation of electron donor addition and bioaugmentation is relatively straightforward.
- Can be a cost effective technology alternative.

Limitations:

- Technology may not be feasible for highly contaminated source zones or DNAPL. Incomplete degradation may also occur, causing the accumulation of toxic intermediates.
- Presence of other contaminants and toxins might affect the microbial population, thereby decreasing the success of bioremediation process.
- Uncontrolled microbial growth and proliferation might reduce permeability.
- Effectiveness varies from site to site and depends upon site hydrogeology and distribution of DNAPL in the subsurface.
- The use of low-cost electron donor amendment is required for cost effective containment. A decision for recirculation approach can be key for economic consideration.

- Some enriched cultures may attach to the aquifer matrix and not migrate with soluble contaminants, making it difficult to distribute the culture in a large volume.

Field Application of Biodegradation

1- A biostimulation case study was carried out at the Idaho National Engineering and Environmental Laboratory (INEEL) Test Area North (TAN). The site was predominantly contaminated with TCE at concentrations up to 20 mg/L in the hot spots. PCE and 1,2-DCE were also detected at lower levels. Biostimulation was implemented at the source zone with sodium lactate periodically injected to enhance degradation of DNAPL material. This gave a temporary increase in the aqueous TCE concentrations. Switching to powdered cheese whey enabled greater mass removal from the DNAPL phase compared to sodium lactate. Reduction of TCE to non-detectable levels was observed in some monitoring wells, including injection wells. There was no accumulation of VC, and ethene detection indicated complete reductive dechlorination at the site (Wymore *et al.*, 2006).

2- At Launch Complex 34 in Cape Canaveral Air Force Station, TCE concentration in soil was detected above the solubility limit for TCE, indicating DNAPL zone presence. Bioaugmentation and biostimulation remediation was implemented in a pilot study area. Recirculation wells were installed to inject ethanol (electron donor) into the source area for 14 weeks before bioaugmentation with KB-1 culture. Results show that TCE in the soil decreased from 8,000 mg TCE/kg soil to 300 mg/kg indicating a large reduction in DNAPL mass. Degradation products c-DCE and VC increased

immediately after biostimulation, but reduced after bioaugmentation. The cis-DCE increased from 32 mg/L to 95 mg/L after biostimulation, but decreased to 20 mg/L after bioaugmentation. Vinyl chloride started at less than 1 mg/L, but increased to 103 mg/L after bioaugmentation. After one month, VC decreased to 8 mg/L. Ethene concentration decreased after biostimulation but then rose from ~ 0.6 mg/L to 22 mg/L post-treatment. Results show the importance of biostimulation and bioaugmentation in chlorinated ethenes treatments (Battelle, 2004).

3- A pilot scale field test was conducted at the Kelly Air Force Base in south central Texas to evaluate biostimulation and bioaugmentation treatment for PCE contamination. Water was recirculated without electron donors for 89 days prior to biostimulation to equilibrate the system. Methanol and acetate were first used as electron donors to establish reducing conditions in the pilot study area before KB-1 injection. During biostimulation (173 days) PCE concentration dropped by 90% and c-DCE dominated the degradation products. After 52 days with KB-1, VC was detected, followed by ethene at later times (Major *et al.*, 2002).

Phytoremediation

Phytoremediation is the use of vegetation and its associated microorganisms, enzymes and water evapotranspiration to contain, extract or degrade contaminants from soil and groundwater. Phytoremediation has been used to contain and/or remediate a number of contaminants including chlorinated hydrocarbons, using the following mechanisms; rhizodegradation (degradation of contaminants by soil microorganisms that surround roots and are influenced by plant roots), phytodegradation (degradation of

contaminants within the plant tissue), phytovolatilization (uptake and transpiration of contaminants from the media through the plant tissue into the atmosphere) and hydraulic control (containment of contaminants within a site by limiting the spread of a contaminant plume through plant evapotranspiration) (McCutcheon and Schnoor, 2003). Hybrid poplar and Phragmites are typical plant species used in treatment (Green and Hoffnagle, 2004). Tree core sampling allows detection of chlorinated ethenes in tree trunk and has been used to assess the presence of shallow groundwater contamination at vegetated sites. This cost effective approach can be used to optimize monitoring well locations (Vroblesky *et al.*, 1999; Vroblesky *et al.*, 2004). It was noted that plants are able to metabolize some portion of PCE and TCE through volatilization (Davis *et al.*, 2002). A case study used a combination of poplar and willow trees as a polishing step in a chlorinated solvent plume while in-situ chemical oxidation with potassium permanganate was used for source control. Chlorinated solvents detected in trees tissues confirmed uptake and phytodegradation (Nzengung, 2005). Moreover, microbial reductive dechlorination seems to be enhanced at phytoremediation sites (Godsy *et al.*, 2003; Nzengung, 2005). Rhizosphere studies on TCE contaminated soil showed that microbial biomass, TCE removal rates and mineralization of [^{14}C] TCE to $^{14}\text{CO}_2$ were greater in rhizosphere soil than non vegetated soils (Walton and Anderson, 1990).

Cinderella Dry Cleaning Site

Site Location and History

The study site is located at and near 1227 Bluemont Avenue, Manhattan, KS. It is in the NW1/4 of section 8, Township 10 South, Range 8 East (Fig. 1.2). Groundwater

contamination in this area is related to Cinderella dry cleaning facility which was in operation from 1967 to 1997. Today Cinderella dry cleaner is no longer in operation and the building is currently occupied by a restaurant. Another dry cleaner, Stickel's, is in the path of the contaminated plume and is still in operation since 1940 (107 m away from Cinderella). Other dry cleaners were documented in the Aggie village area (Davis *et al.*, 2007).

The Drycleaner Environmental Response Act was developed in 1995. Accordingly, the Kansas Department of Health and Environment (KDHE) formed the Kansas Dry-cleaning Program which deals with pollution prevention and proper management of current dry-cleaning facilities. It also utilizes trust fund fees to implement corrective action at contaminated dry cleaning sites (KDHE, 2008).

Volatile organic compounds (VOCs) were discovered in public water supply (PWS) wells PWS-12 and 13 (KDHE a, 2007; KDHE b, 2007). The KDHE and their contractors conducted a Screening Site Inspection (SSI) and found contamination moving towards the public water supply wells which are located about 1.5 miles down gradient of the former Cinderella Cleaners (KDHE a, 2007). Tetrachloroethene (PCE) and its degradation products, trichloroethylene (TCE), cis 1,2 dichloroethylene (DCE) and vinyl chloride (VC), above their maximum contaminant limits (MCL) were noted at depth of 35- 50 ft below ground surface (bgs) along the plume. PCE is assumed to be the source of all chlorinated ethenes along the contaminated plume. Although a high concentration of TCE was recorded close to Stickel's cleaners, it is unlikely to be from current practice but from degradation of PCE from the Cinderella Site. Isoconcentration maps provided by

contractors show TCE concentrations upstream of Stickel's, suggesting that PCE conversion to TCE as the source of the TCE found down gradient from Stickel's.

Contaminant Plume Description

Concentrations of PCE and TCE at the source of Cinderella cleaner site were measured from borehole data obtained in 1997 and 1998. PCE concentration in the soil ranged from 244 - 2837 mg/kg, while TCE concentrations were 1.2 - 7.5 mg/kg. Water samples collected from borehole #5 (18 ft = 5.5 m bgs) contained 18.4 mg/L for PCE, 0.24 mg/L for TCE and 0.17 mg/L for DCE. Monitoring wells # 1-4 were installed in Sep. 2000, and monitoring wells 5 and 6 were installed on April 2002. MW-5, screened at 35-46 bgs, had 26 mg/L PCE, 1.2 mg/L TCE and, 0.5 mg/L c-DCE (BE&K Terranext, 2001). Further investigation on the source done by Terracon in 2004, found the PCE concentration 35 mg/L at MW-5. High PCE concentrations near the source zone suggest the presence of a DNAPL phase at the source area, which provides a long-term source of PCE.

Plume width remained almost constant along a distance of about 600 ft (~200 m) suggesting low transverse dispersion. As it moves east, PCE concentration decreased to near zero ~450 m from the source area. On the other hand, TCE and DCE concentrations rise as the plume moves away from the source. After about 5 blocks DCE concentration generally exceeds that of TCE. The highest concentrations of TCE 4.4 mg/L were reported in MW-6 which is the closest well to Stickel's cleaner (Terracon, 2004). This pattern of contaminant concentration is a sign of reductive dechlorination of PCE along

the plume. With fast conversion of PCE to TCE and slow or no conversion of c-DCE to VC, accumulation of c-DCE is observed in this aquifer.

The pH of the water was 6.5-7 in all wells at ground water temperature of 18-21 °C. Dissolved oxygen values for MW-8S were 0.5-1 mg/L and oxidation-reduction potential was 130 to 225 mV, while MW-8D had 0.35- 0.7 mg/L dissolved oxygen and 0-150 mV for oxidation reduction potential (Terracon, 2004).

Monitoring Wells Establishment

In September 2000 four monitoring wells were established as MW-1 to MW-4. They were 50 ft (15 m) deep and screened in the interval 40-50 ft (12-15 m) of depth. Two years later MW-5 and MW-6 were added and screened at the same depth. MW-1 is considered up-gradient of the contaminated plume, while MW-5 is the closest well to the source area downgradient. Geoprobe studies revealed areal extent of the plume at two depths 35-50 ft (11-15 m) and 45-60 ft (14 - 18 m). Water level data suggest the presence of a deep and a shallow zone in the subsurface. The depths to water in the shallow and deep zone are approximately 12 and 18 feet (4 - 5.5 m) bgs (below ground surface). Cone penetrometer studies were done in July 2003, and additional monitoring wells were constructed along the plume (MW-7 to MW-18). At that time pairs of shallow and deep wells were drilled for monitoring wells 7-18 and the pairs were spaced about 2.5 ft (0.8 m) apart. Figure 1.3 shows a picture of some shallow and deep wells. Shallow wells were drilled 30 ft deep and screened over 10-30 ft (3- 9 m), while deep wells were 55 ft deep and screened from 45-55 ft (14- 17 m) (Figure 1.4); (Terracon, 2004).

Lithology and Ground Water Flow

Cone penetrometer results obtained in August 2003 indicate a clayey subsurface 40 ft (12 m) below ground surface which was interrupted with silty clay layers. A primary clay zone was present 25-30 ft (8- 9 m) bgs. A fine and medium sandy zone exists at 40-45 ft (12- 14 m) bgs (Terracon, Jan 2004). Depths to water in the shallow and deep wells fluctuate between 12-14 ft (4- 4.2 m) and 18-20 ft (5.5- 6 m) below ground surface, respectively. Falling head permeability study was conducted by Alpha-Omega Geotech in 2001 for soils from two locations at 15-17 ft (4.6- 5 m) bgs. One, identified as P-13, was taken from a site close to Stickel's and the other, P-14 was close to MW-5. Tracer study analysis (Santharam *et al.*, 2005) showed groundwater velocities for the shallow zone 18 – 27 ft (5.5- 8 m) bgs and deep zone 40-50 ft (12- 15 m) bgs are 1.1 and 0.7 ft/day (0.34 m/day and 0.21 m/day respectively), which does not match with the soil profile for the site. The clay zone at the top of the profile has a higher groundwater velocity than the sandy layer of the deep zone indicating preferential pathways for water in the shallow zone.

Research Objectives and Significance

The goal of this study was to evaluate the native microbial population at the Cinderella dry cleaner site and to determine if they were capable of carrying out complete reductive dechlorination of PCE, through microcosm experiments stimulated with different amendments. If the native microbial system fails to remove PCE, then bioaugmentation with KB-1 microbial culture would be tested. Results obtained from microcosm experiments were used to carry out a pilot study remediation action at the site,

where nutrients and bacteria were introduced to the subsurface, and the fate of chlorinated ethenes monitored. Hydraulic conductivity of the pilot study area was estimated before and after amendment to check for possible clogging of the wells due to microbial growth.

Understanding site properties and biodegradation potential of indigenous microorganisms would be of great importance for remediation of Cinderella drycleaner site and similar contaminated areas. Lessons learned on microbial behavior and nutrient requirements will affect the success of chlorinated ethene removal from the source area, and mitigate the contaminant migration in the down-gradient plume. Bioremediation will reduce the risk of contaminating city public water wells.

Table 1.1 Physical and chemical properties of chlorinated ethenes and their degradation products.

Property	PCE	TCE	1,1-DCE	trans 1,2-DCE	cis 1,2-DCE	VC	Ethene	Methane
Chemical formula	C ₂ Cl ₄	C ₂ HCl ₃	C ₂ H ₂ Cl ₂	C ₂ H ₂ Cl ₂	C ₂ H ₂ Cl ₂	C ₂ H ₃ Cl	C ₂ H ₄	CH ₄
Molecular weight	165.83	131.40	96.95	96.95	96.95	62.5	28	16
Vapor pressure (mmHg) at 20 °C	18.47	74	500	265	180	2530	80 @ 15 °C	275 @ 15 °C
Density (g/cc) at 20 °C	1.6227	1.465	1.214	1.257	1.2837	0.911	0.97	0.56
Henry's law constant at 25 °C (atm-m³/mol)	0.018	0.011	0.026	0.0094	0.0041	1.2	0.21	0.72
Solubility in water (mg/L) at 25 °C	150	1366	2500	6300	3500	1100	131	22
Log K_{ow} at 25 °C	3.4	2.42	1.32	2.09	1.86	1.36	1.13*	1.09
(MCL)	5	5	7	100	70	2	–	–

* ^a ATSDR, 1997; ATSDR, 1996; ATSDR, 1994; Russell, *et al.*, 1992; U.S. U.S. EPA, 2000; ChemIDplus, 2004; Chemfinder, 2004.

** MCL is defined as Maximum Contaminant Limit, µg/L.

*** All chemical compounds listed in table are colorless. PCE, TCE and DCE are liquid at room temperature, while VC, Ethene and Methane are gases.

Table 1.2 Half-reaction potentials for dechlorination reactions (Vogel *et al.*, 1987).

Half-reaction potential in (mV)	PCE to TCE	TCE to DCE	DCE to VC
	580	550	360

Table 1.3. Bioaugmentation cultures used for treatment of chlorinated solvents in groundwater. Adapted from ESTCP, 2005.

Bioaugmentation culture	Developer	Culture Source	Commercial Vendor	Key References
KB-1	E. Edwards (University of Toronto)	TCE contaminated aquifer, Ontario	SiREM	Duhamel <i>et al.</i> (2002); Major <i>et al.</i> (2002)
Pinellas	Remediation Technologies Development Forum	TCE contaminated aquifer, Pinellas, FL	Terra Systems	Ellis <i>et al.</i> (2000)
Bachman Road culture (BC2, Bio-Dechlor)	F. Löffler (Georgia Institute of Technology)	PCE contaminated aquifer, Oscoda, MI	Regenesis Bioaug- LLC	Löffler <i>et al.</i> (2000); He <i>et al.</i> (2002); He <i>et al.</i> (2003); Lendvay <i>et al.</i> (2003)
WBC-2	USGS	Wetland sediment in West Branch Canal Creek area, Maryland	Geosyntec/ SiREM	Lorah, <i>et al.</i> (2003)

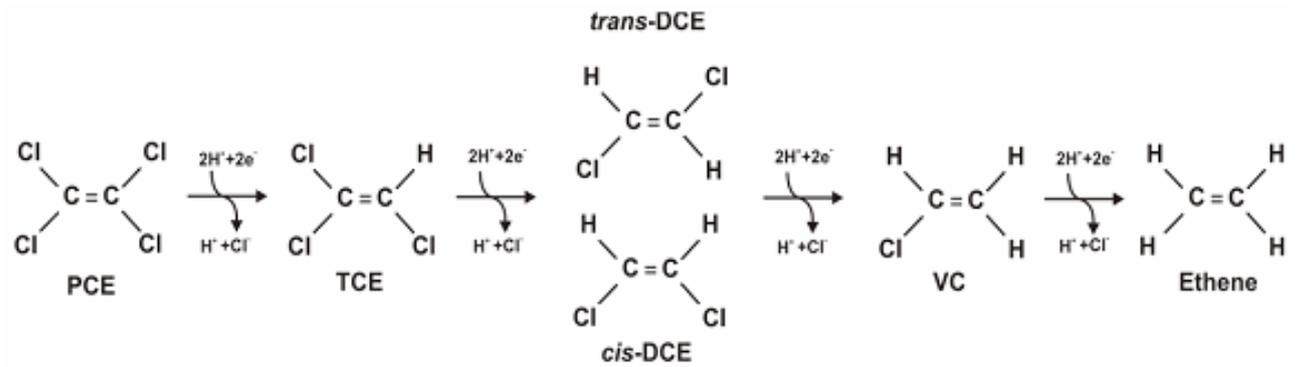


Figure 1.1. Sequential reduction of PCE to ethene by anaerobic reductive dechlorination adapted from (AFCEE, 2004).



Figure 1.2. A. Aerial map showing location of former Cinderella and current Stickle dry cleaners. B. enlarged image of pilot study area showing location of monitoring wells used in this study. Images were taken from Google earth.

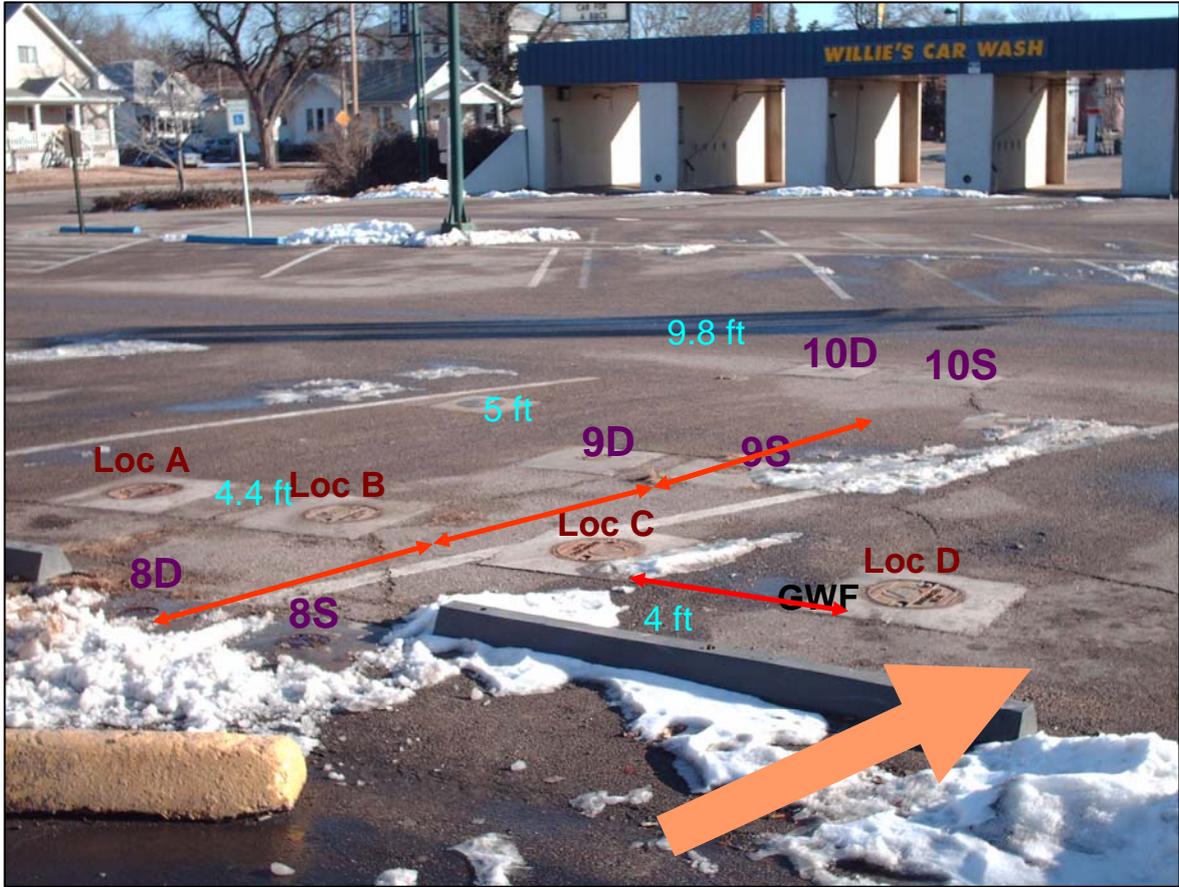


Figure 1.3. Picture of city parking lot showing location of shallow (S) and deep (D) monitoring wells and spacing between wells. Ground water flow in the subsurface moving eastward.

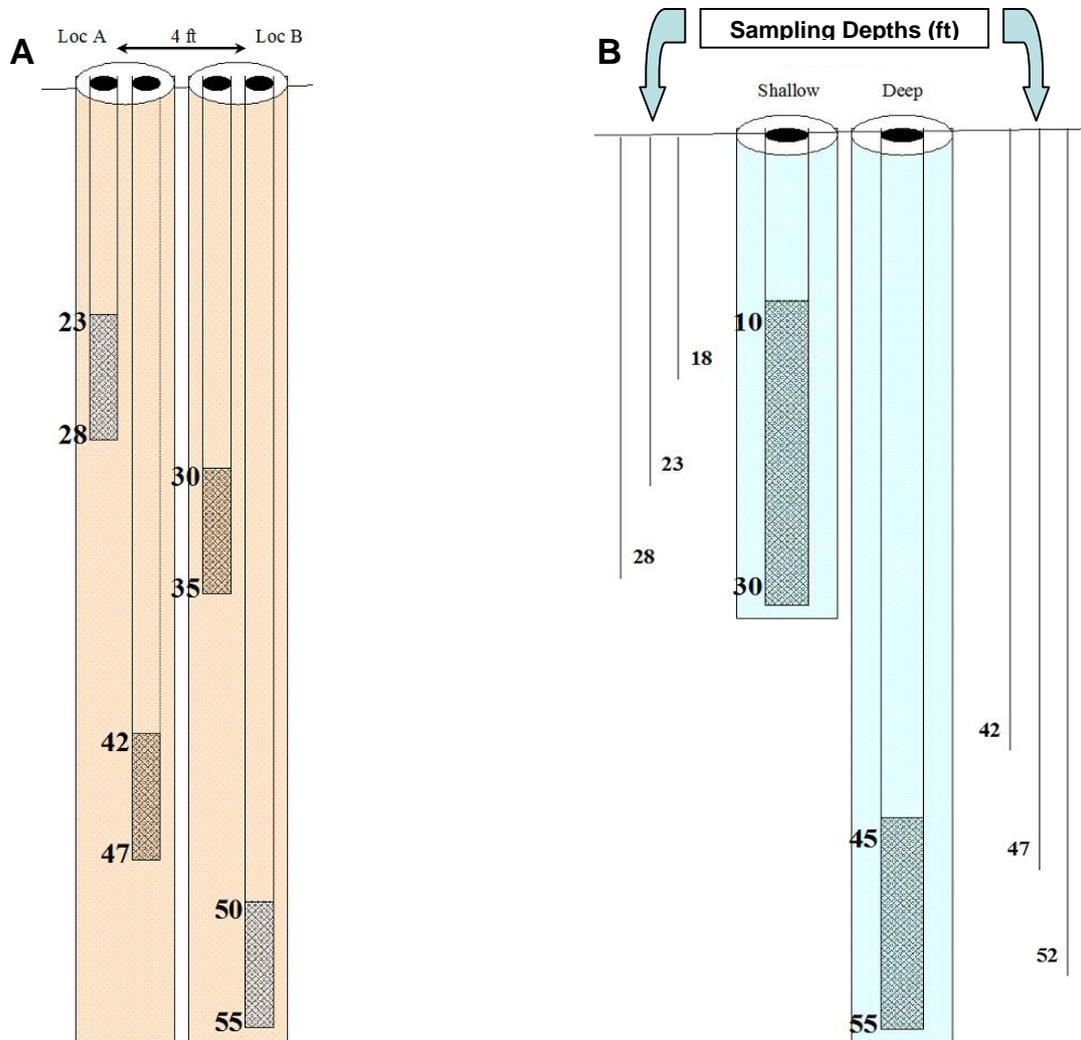


Figure 1.4. Schematic of screening zones shown in **A**. Injection wells and **B**. Monitoring wells and the sampling depths for shallow and deep wells.

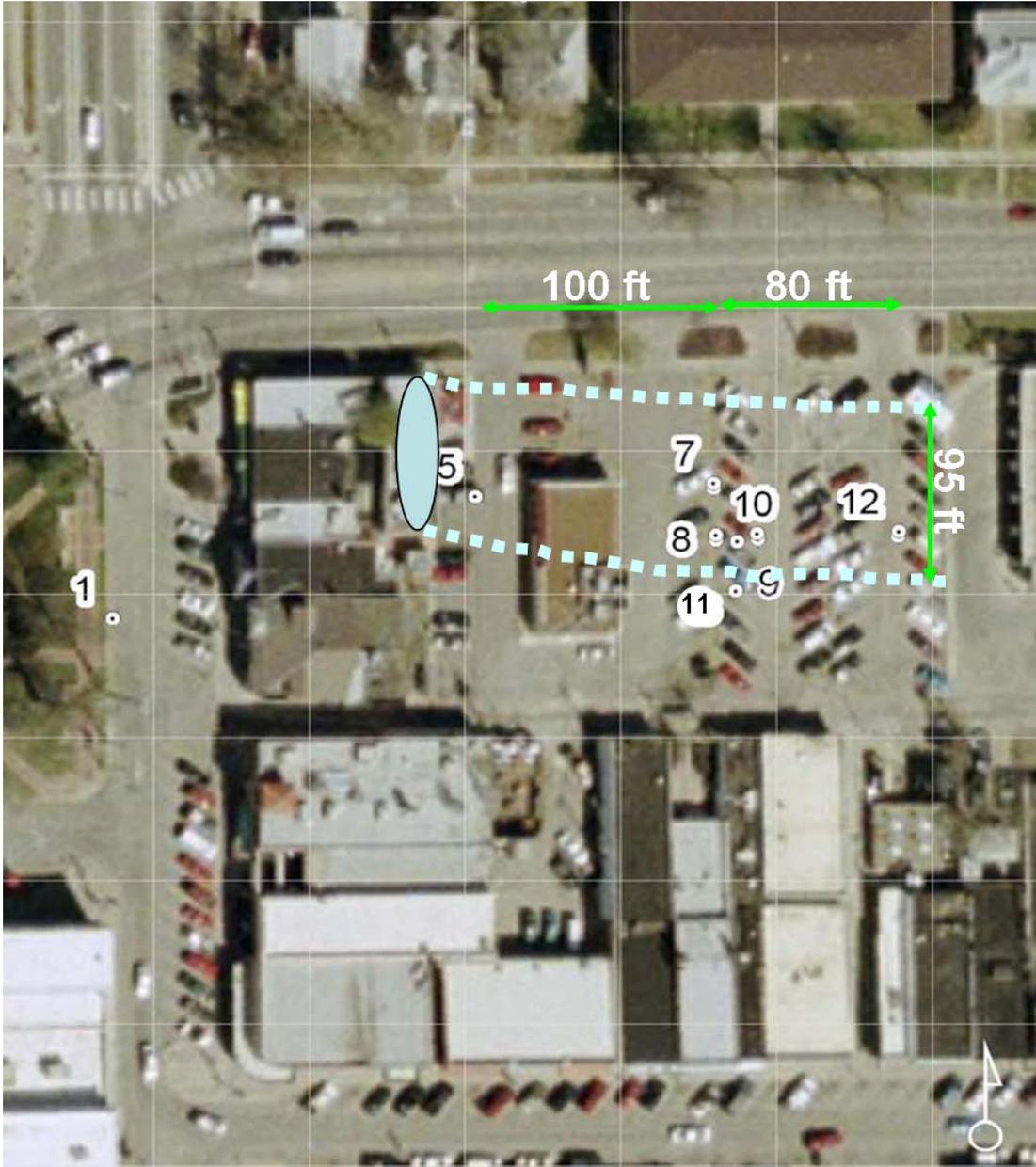


Figure 1.5. GIS aerial map showing location of PCE source (Oval shape), direction and width of contaminating plume (Dashed lines) in the pilot study area.

CHAPTER 2 – MATERIALS AND METHODS

Soil Sample Analysis

Soil core samples were obtained during drilling of the monitoring well MW-9D in the fall of 2003, and stored at 4 °C until processed in January of 2004. Samples were taken at 5 ft (1.5 m) intervals from 2.5 to 47.5 ft (0.8- 14.5 m) below ground surface (bgs), giving a total of 10 samples. Although wells were bored to 55 ft (17 m), the deepest core sample was obtained at 47.5 ft (14.5 m) because the soil below that was sandy and too loose to be collected. A portion of each sample was analyzed by the KSU Soil Testing Laboratory in Throckmorton Hall. Soils were tested for pH, phosphate, potassium, sodium, nitrate, total nitrogen, total carbon and soil texture (% of sand, silt and clay) as shown in Table 3.1. Analysis of soil core samples at 27.5 ft (8.4 m) bgs was repeated to check whether the high total nitrogen and total carbon values were an experimental error or a sample characteristic.

Soil Buffering Capacity

an experiment was conducted to determine the effect of lactic acid on soil pH if lactic acid was formed from cheese whey. Therefore, 100 g of wet soil obtained from MW-9 Deep well drilling at 42.5 ft (13 m) depth. Water from MW-5 D (26 mL) was added to the soil to make slurry. Then 100 μ L of 0.1 M lactic acid was added every 20 min mixed to reach equilibrium; and pH recorded.

Water Depth Measurement

Depth to water measurements in the monitoring wells, were collected frequently to find out the water elevation and direction of water flow. Data were collected with a static water indicator (Sample Pro water level meter model 6000, Q.E.D. Environmental Systems, Inc., Ann Arbor, Michigan). The probe was inserted into the monitoring well from a mark located at the north side of the top of casing. A beeping sound occurred when the probe hit the water, and the depth recorded (Figure 2.2B).

Ground Water Sampling

Ground water samples were collected from monitoring wells using a three stage 12 volt DC submersible pump from Waterra USA Inc. (Bellingham, WA). The pump is fitted to 1/2 " x 3/8 " low density polyethylene tube 55 ft (17 m) long. A portable car battery was used to connect the electrical cable to power the pump. Three water samples were collected from each monitoring well with 5 ft (1.5 m) spacing between them, across the lower portion of the screened zone. Shallow wells were sampled at 18 (5.5 m), 23 (7 m) and 28 (8.5 m) ft below ground surface (bgs), while water from the deep wells was collected at 42 ft (13 m), 47 ft (14.3 m) and 52 ft (16 m) bgs. This sampling allowed detection of a concentration gradient in the monitoring wells and /or it could be considered as triplicate sampling from the same well. Order of sampling was always from the top to the bottom of the well. For example, in shallow wells the pump was first set at 18 ft (5.5m) bgs and then turned on to allow water to flush the tube (~2 – 3 L) after that a sample was taken, then the pump was turned off and lowered to the second level at 23 ft (7 m) bgs, pumped to flush out another liter of water before sample collection, and the

procedure repeated at the third level 28 ft (8.5 m) bgs. After well sampling the tube was flushed with distilled water. This is minimized mixing between wells. The same protocol was followed with the deep wells. Samples were collected in 16.5 mL glass vials, filled to the top, and then immediately capped with mininert caps (Figure 2.1). Vials were then transported to the lab, and 1 mg/L resazurin added as a redox indicator (Figure 2.1C). A 5 mL sample was taken out of the vial with a syringe leaving 5 mL of headspace. While liquid removed, the cap was loosened to allow air replacement. Vials were manually shaken and let stand at least an hour before head space analysis. The water removed was preserved in glass vials, closed with screw caps and stored in a cold room (4°C) for ion analyses the same day.

Microcosms Preparations

Reagents and Supply

Chemicals used in this research included, D-Glucose (Fisher Scientific, Fair Lawn, NJ), Yeast Extract (Sigma-Aldrich, Inc. St. Louis, MO), Soy Oil Methyl Ester (AG Environmental Products, L.L.C.), Methanol (Certified A.C.S. Fisher scientific Co. Fair lawn NJ), Lactic acid (Sigma-Aldrich, Inc. St. Louis, MO), Cheese Whey (Alma Creamery, KS), Resazurin (Baltimore Biological Laboratory Inc. Baltimore, MD). Chlorinated ethenes were obtained from: PCE (Certified A.C.S., Fisher Scientific Co. Fairlawn NJ), TCE (Aldrich Chemical Co. Inc., Milwaukee WI), cis-1,2-DCE (Chem. Service. West Chester PA. Purity 99.4%), VC (Chem. Service. West Chester PA), methane (Matheson Gas Products, A division of Will Ross Inc., E. Rutherford, NJ). The microbial culture KB-1, which was used in microcosm experiments was kindly provided

by SiREM laboratories in Ontario, Canada. A 20 L batch of KB-1 was purchased from SiREM for the pilot study (Figure 2.3). Hamilton gas tight syringes (Hamilton Company, Reno, Nevada) were used to inject gas phase samples into the gas chromatograph with 26 gauge needles, (SUPELCO, Bellefonte, PA). The clear glass 16.5 mL vials fitted with mininert Teflon caps were obtained from SUPELCO (Bellefonte, PA).

Soil Microcosms

Soil microcosms were prepared on February 20, 2004 to study the effect of different nutrient conditions on PCE degradation and to determine the capacity of natural microbial populations to degrade beyond DCE. Soil from (MW-9 D) at 47.5 ft (m) bgs was used to prepare microcosms. The following nutrients were used for biostimulation, glucose (0.02%), yeast extract (YE) (0.025%), soy oil methyl ester (SOME) (0.05%), methanol (0.01%) and lactate (0.04%). Glucose and YE were determined based on (Weight/Volume), while other nutrient concentrations were based on (Volume/Volume). A total of 13 different treatments each with duplicate vials were prepared: Autoclaved (Sterile) control, Live control, YE, Glucose, Glucose + YE, SOME, SOME + YE, Lactic acid, Lactic acid + YE, Methanol, Methanol + YE, Methanol + Lactic Acid + YE, Glucose + SOME +YE. To prepare the microcosms, 5 g dry weight equivalent soil was added to sterile 16.5 mL glass vials and supplemented with nutrients. Sterile distilled water was added to bring the total soil and water volume in the vial to 12 mL. Vials were flushed with argon gas for 30 sec and capped tightly with mininert screw caps. All microcosms were supplied with 0.5 mL of PCE saturated gas. Vials were shaken

manually to allow partitioning and equilibration of PCE into the gas and liquid phases. Microcosms were incubated at ambient temperature ~23-26 °C for 85 days, and were sampled frequently for chlorinated ethenes. In sterile soil treatment, soil was autoclaved for 1 hour over 2 successive days to make sure that the majority of microorganisms were eliminated in the control. KB-1 bacterial culture was not used in this set of microcosms (Figure 2.1A).

Water Microcosms

Water microcosms were prepared from different monitoring wells, at different times, to study the appropriate combinations of nutrients for biostimulation and bioaugmentation studies. Water was collected from wells into 1 L glass bottles, filled to the top, immediately capped, and brought to the lab. Microcosms were prepared by adding treatment nutrients to 16.5 mL glass vials, and then transferring water collected from the site so that final volume was 11 mL. Resazurin was added (1 mg/L) as a redox indicator. Vials were flushed with either argon or nitrogen to maintain anaerobic head space and sealed with mininert Teflon caps. Then 0.5 mL of PCE saturated gas was introduced to the treatment vials for water collected from monitoring wells other than MW-5 (which already contained high PCE). The vials were shaken and allowed to equilibrate for a few hours to overnight before analysis. Vials were maintained under ambient conditions for the duration of the experiment. Headspace analysis was done with a gas chromatograph. The KB-1 bacterial consortium from SiREM was used for bioaugmentation. The culture is sensitive to oxygen, so microcosms were first stimulated with nutrients before adding 10 µL of KB-1, usually after 2-3 days, when resazurin was

reduced from blue to colorless. Redox potential at the colorless stage of resazurin is -100 mV or lower, which is optimum for KB-1 microorganisms (Figure 2.1B).

Biostimulation Experiment

The first set of water microcosms was prepared on March 26, 2004 with water samples from MW-4D, MW-8S and MW-8D. The vials were monitored for 6 weeks, and 7 different treatments were tested for biostimulation activity in each water sample: SOME 0.05%, methanol 0.01%, YE 0.025%, glucose 0.02% + YE 0.025%, methanol 0.01% + YE 0.025%, SOME 0.05% + YE 0.025%, glucose 0.02% + SOME 0.05% + YE 0.025%.

Monitoring Wells Screening and their Biodegradation Potential

On May 26, 2004 a total of 90 microcosm vials were prepared with water from 6 monitoring wells (1-6) with 5 different treatments for each well. Each treatment was repeated 3 times for each monitoring well, the first vial received 0.5 mL of PCE saturated gas. The second received 0.5 mL of PCE gas and 10 μ L of KB-1, the third was a control for that treatment without addition of PCE or KB-1. The treatments included: glucose 0.02% + YE 0.05%, lactate 0.04% + YE 0.05%, SOME 0.05% + YE 0.05%, glucose 0.02% + SOME 0.05% + YE 0.05%, lactate 0.04% + SOME 0.05% + YE 0.05%. KB-1 was added to the vials after 4 days when resazurin color became colorless.

Optimizing Nutrient Concentration for Bioremediation

A third microcosm was set on July 31, 2004 with water taken from MW-5. Microcosms were not spiked with PCE gas because MW-5 was the closest well to the source area and had high concentration of PCE, ~ 20 mg/L. Treatments contained 25 different combinations of YE (0.01%, 0.003%, 0.001%), SOME (0.01%, 0.003%, 0.001%) and lactate (0.04%, 0.013%, 0.004%). Treatment combinations are shown in Table 2.4. Triplicate vials were prepared for each treatment, and 10 µL mineral medium containing KB-1 added to one of three vials in each treatment after 3 days. Chlorinated ethene degradation was monitored in this set for 100 days.

KB-1 Viability Study

This set of microcosms was monitored to determine if KB-1 could maintain activity after nutrient starvation; microcosms were used from an earlier study (July 31, 2004). Selected microcosms that contain appreciable amount of PCE were re-fed with nutrients on March 15, 2005. Nutrients were introduced at the following concentrations, 0.01% yeast extract (YE), 0.04% lactate and 0.1% soy oil methyl esters (SOME).

Cheese Whey Microcosms

Several microcosm experiments were prepared using liquid cheese whey obtained from Dr. Fadi Aramouni's laboratory. They receive the whey from the Alma cheese factory as a waste product. Cheese whey contains 5% lactose and therefore different concentrations were used in microcosms ranging from 0.01 to 0.5 percent of lactose content. Whey was used as sole nutrient and carbon source, or amended with soy oil

methyl esters. One set used cheese whey that was filtered through 0.22 μm filter (CAMEO 25 NS Nylon filter) to eliminate microbial competition with KB-1. Microcosms with and without KB-1 were compared. Water from monitoring wells 5D, 8D and 9D was used for different sets of vials and the same method was used, as described above in water microcosms section. All microcosms were prepared in 16.5 mL clear glass vials, topped with mininert caps. Total volume of liquid phase was 12 mL. Resazurin (1 mg/L) was added as a redox indicator, and vials were flushed with nitrogen gas (30 s) after preparation and sealed immediately.

Cheese Whey / Set 1

A total of 30 microcosm vials were prepared on May 12, 2005 using water from MW-5. Cheese whey was used in some vials to achieve lactose content concentrations of 0.01%, 0.025%, 0.05%, 0.1% and 0.25%. Cheese whey microcosms were compared with the following nutrient combinations: (0.01% YE+0.01% SOME), (0.01% YE+0.003% SOME), (0.003% YE+ 0.01% SOME), (0.003% YE+ 0.003% SOME). Control vials did not receive any nutrients. The KB-1 bacterial consortium was added to one of the three vials for each treatment after 3 days of vials preparation.

Cheese Whey / Set 2

This set was prepared on June 1, 2005 following successful biostimulation with lower concentrations of cheese whey. Therefore, cheese whey concentrations were lowered further (0.05%, 0.025%, 0.01%, 0.005%, and 0.002%). All vials were supplemented with 0.01% SOME. Triplicates of each treatment were prepared including a control resulting in 18 vials. MW-5 water (collected on May 11, 2005) was used in the

experiment. PCE concentrations in this set were lower than what we expected for MW-5. One of the three replicates in each treatment received 10 μ L of KB-1 culture.

Cheese Whey / Set 3

Previous whey microcosms showed reduction in contaminant (PCE) degradation rates when higher concentrations of cheese whey were used (> 0.1%). So this set was prepared with filtered cheese whey (July 21, 2005) to determine if dechlorination inhibition was from microbial competition between KB-1 and cheese whey microbial culture. Five different whey concentrations were used (0.5%, 0.25%, 0.1%, 0.05% and 0.025%), with water from MW-5. Each whey concentration was a triplicate with KB-1 and another triplicate without KB-1 making a total of 33 microcosm vials with control triplicates.

Cheese Whey / Set 4

After 167 days following KB-1 injection in the pilot study area, this set of microcosms was prepared on February 2, 2006 to test the activity of injected KB-1. Hence, water samples were collected from the bottom of MW-8D (upstream of injection wells) and MW-9D (down gradient of injection wells). The microorganisms were stimulated with the following treatments: (0.01% YE+ 0.003% SOME), (0.01% YE), (0.025% Whey), (0.025% Whey+ 0.003% SOME). Each treatment was done in duplicates for each monitoring well and nutrient free controls were also prepared. KB-1 was not added to this set. After 5 days, all PCE was consumed so the vials received 0.5 mL of PCE saturated gas on day 5. Saturated gas was prepared in 125 ml serum vial with

100 L of liquid PCE. Whey treatment vials with samples from MW-8 received an additional PCE dose on days 8 and 20.

Cheese Whey / Set 5

In this set of microcosms fresh KB-1 bacterial cultures were obtained from SiREM. Water was collected from the top and bottom of MW-5 to compare the microbial activity associated with sediment material collected from the bottom of the well (49 ft bgs) to less particulate water from the top of the well 42 ft (13 m) bgs. Duplicate microcosm vials were prepared on May 18, 2006 with 0.05% and 0.025% cheese whey, with and without KB-1; controls were also included. In addition 0.5 mL of saturated PCE gas was added to some vials on day 18.

Buffered Microcosms

Buffered microcosms were set up to counteract the effect of acid generation that occurs when high concentrations of cheese whey were used. Eleven 65 mL bottles amended with 0.25% cheese whey were prepared on January 16 2007, with MW-5 water and soil from the 42.5 ft deep soil core from MW-9D. For the soil microcosms, 10 g of soil was used and the same weight of glass beads was used to prepare the control water microcosms. Each kind of soil and water microcosms were divided into two subsets and only one received 0.1 M calcium carbonate for buffering. Phenol red was added as an acid-base indicator and 1 M tris-base solution was used to bring the color of the vials from yellow (acidic) to orange-red color (neutral) when needed. KB-1 was added to all preparations.

MW-5 Culture Microcosms

On December 3, 2007 water and a large amount of black sediment from the bottom of monitoring well 5 were collected in a 500 mL glass bottle. At the same time 16.5 mL vials were filled with water from the top of the same well. In the lab, 5 mL were taken out of the vials, and 0.025% cheese whey was added to all vials except the control; 1 mg/L resazurin was added to each vial. Vials were flushed with N₂ and allowed to stand for several days; then 10 µL of KB-1 was introduced to selected microcosms, while 10 µL of MW-5 bottom sediment was also used in other vials, and a third set contained 5 µL each of KB-1 and MW-5 culture.

Analytical Methods

Gas chromatograph (Hewlett Packard 5890 Series II, Wilmington, DE) with FID detector and HP-1 column (Dimethyl Polysiloxane matrix, 30 m x 0.53 mm, Agilent Technologies, Wilmington, DE) was used to analyze chlorinated ethenes and methane. Hydrogen was used as the carrier gas. Injection temperature was 200 °C and detector temperature was 300 °C. Parameters were adjusted to obtain detectable peaks that could be distinguished from other compounds by elution time. Different isothermal temperatures were tested (80 °C, 100 °C, and 110 °C). High column temperatures resulted in fast elutions but the peaks did not resolve very well from each other. Lower temperature allows good separation of the compounds, but took more time to finish the run. For example PCE elution time at 80 °C was 4.1 min while at 110 °C the PCE peak was detected after 2.2 min. At the end, a gas phase sample of 100 µL volume was taken with 100 µL Hamilton gas syringe, the column was set on an isothermal temperature of

100 °C, and the run time was 5 min. The previous parameters were able to resolve chlorinated ethene peaks and methane. Gas flow rate was maintained at 1.5 mL/min. Detection limits were in the range of 1-10 µg/L for chlorinated ethenes; differences relate to the compounds and their Henry's constants. This analysis yields different headspace concentrations. Chlorinated ethene standards were prepared to determine elution times of different analytes of interest. A PCE standard was prepared once a month and run prior to each analysis to check the responses of the GC, and determine the relative elution times. The standard was prepared by injecting 10 µL of PCE liquid in a clean amber glass bottle of 4.2 L and allowing it to vaporize completely. The concentration of PCE in the standard bottle was 3.83 mg/L. At detection settings, elution times for chlorinated ethenes were as follow: PCE (2.7 min), TCE (1.6 min), DCE (1.1 min), VC (0.7 min) and Methane (0.6 min). Maintenance was done periodically to make sure that the gas chromatograph operation was uniform across the study. The injection septum (Thermogreen LB-2 / 11 mm diameter) was changed every 200 samples and inner glass column was cleaned every ~1000 samples. Methane and ethene were not resolved on this column at any temperature, and for that another GC was used. It was also difficult to create a calibration curve for vinyl chloride since the standard was prepared in methanol, and a large methanol peak masked the VC peak, even when water or sodium hydroxide was added to the standard.

Methane and ethene were resolved using a thermal conductivity gas chromatograph (Carle 8510) with 1.82 m x 3.2 mm stainless steel column packed with Porapak S. The carrier gas was N₂ and column temperature was between 80-85 °C. Gas

phase standards were prepared in 125 mL serum bottles and used for calibration of elution times and concentration in headspace samples.

Bromide, chloride, nitrate and sulfate were analyzed with an ion chromatograph (Dionex DX500 Series, Sunnyvale, CA) with a conductivity detector and analytical column (Ionpack, AS9-HC, 4 x 250 mm). The elution solvent was 9 mM sodium carbonate at a flow rate of 1 mL/min, and samples were run using an autosampler for 20 minute each. Water samples obtained from the site (portion of the 5 mL taken from the sampling vials) were centrifuged at 10,000 rpm for 2 min in plastic centrifuge tubes to remove debris. The supernatant (1.5 mL) was then carefully transferred to 2 mL glass vials, topped with a septum and sealed with an open top screw cap obtained from the National Scientific Company, (Rockwood, TN). Calibration standards for the ions were obtained from Dionex. Daily standards for Br⁻ were prepared using dried, Infra Red grade potassium bromide. Elution times were 6.3 min for chloride, 9.5 min for bromide, 11 min for nitrate and 18 min for sulfate.

Pilot Study

Deep well Tracer Study Prior to Biostimulation (Fall 2004)

A tracer study was done on August 2, 2004 to determine the direction and velocity of ground water at the pilot study area, before the bioremediation experiment. Potassium bromide was injected in monitoring well-8D. Approximately 200 L of water was withdrawn from the well with a 3-stage pump into a large plastic container and mixed with 1L of concentrated KBr solution (150 g/L) giving ~500 mg/L of Br⁻. Water

was reinjected into the same well at three depths 40 ft (12 m), 45ft (14 m) and 50 ft (15.3 m), each depth received about a third of the total volume (Figure 2.4A).

Ground water samples were collected from three depths at the screening zone 42 ft (13 m), 47 ft (14.3 m) and 52 ft (16 m) bgs to check for preferential flow across the well and to replicate the sampling in each well. After injection, samples were collected daily from MW-8D and MW-9D (~10 ft downgradient from MW-8D), until the bromide peak was passed in MW-9D. Then longer sampling intervals followed until bromide was also detected in MW-10D (~20 ft downgradient of MW-8D).

Shallow Well Tracer Study Prior to Biostimulation (Fall 2004)

After the deep well tracer study was finished in MW-8D and MW-9D, a shallow well tracer study was started on September 16, 2004. Similarly, 200 L of water was taken from MW-8S and KBr stock was added to give ~470 mg/L of analytical Br⁻ concentration. Water-bromide mixture was reinjected into the same well in three equal portions at three depths along the screening zone of the well at 17 ft (5.1 m), 22 ft (7 m), and 27 ft (8.2 m) below top of casing (TOC). Shallow wells were sampled daily at the beginning until bromide was detected in MW-9S.

Injection Wells Installation (Fall 2005)

A pilot study work plan was prepared and approved by KDHE in July, 2005 and the injection well installation started on August 15 and finished on August 17, 2005. The pilot test was done by KDHE contractors. Four injection wells were drilled between MW-8 and MW-9 with 4 ft (1.2 m) in between wells in the north-south direction. Each

injection point had two wells, one shallow and one deep. Loc A was the north injection point and the screening intervals for shallow and deep wells were 23-28 ft (7- 8.5 m) and 42-47 ft (13-14 m). The next injection point, Loc B has screening intervals were 30-35 ft (9-10.7 m) and 50-55 ft (15.2- 16.7 m) for shallow and deep wells, respectively. The screening intervals in Loc A were similar to Loc C and Loc B was similar to Loc D. This allowed wider distribution of nutrients in the four wells intersecting with the contaminated plume, as shown in (Figure 1.4A).

Tracer Study with Biostimulation Experiment -Preconditioning- (Fall 2005)

Ground water pumped from MW-10S and MW- 10D was used to fill 2 barrels from each monitoring well. Each barrel held ~ 200 L of water and was used to prepare both nutrient and anaerobic chase water. Nutrient solution was also supplemented with KBr (670 mg Br⁻/L) as a tracer to monitor nutrient flow in groundwater. The anaerobic chase water was used to push nutrient solution into the newly installed injection points. Nutrient and chase water were prepared a few days before injection to allow the water to become anaerobic. Table 2.1 shows the amount and concentration of SOME, lactate, yeast extract and potassium bromide used to prepare nutrient barrels. Table 2.2 shows composition of chase water solutions.

On August 18, 2005 (Day 0) nutrient solution and chase water were introduced to the aquifer through injection wells. Solutions were injected in the shallow zone first followed by the deep zone. Each shallow well first received about 50 L of nutrient solution followed by 50 L of anaerobic chase water. In shallow wells, siphoning from 5 gal jugs was used to inject the water, while a three stage pump was used for all deep

wells except in Loc C where injection was slow, and therefore, siphoning was used in that well. Ground water samples were collected from monitoring wells in the pilot study area. Shallow wells were sampled at three different depths, top 18 ft (m), middle 23 ft (m) and deep 28 ft (m), while groundwater from deep wells was collected at top 42 ft (m), middle 47 ft (m) and deep 51 ft (m) below top of casing. Ground water sampling was described above in detail. Samples were analyzed for chlorinated ethenes and ions. Redox status in ground water samples were depicted from resazurin treatment in the sampling vials. Color change from blue to pink indicated oxygen reducing potential (ORP) of about -50 mV, while further change from pink to colorless indicates ORP values < -100 mV.

KB-1 Injection (Fall 2005)

After about two months of introducing the first nutrient feeding to the pilot study area on August 18, 2005, the area around the injection wells had become more reducing (based on ORP and dissolved oxygen values recorded by KDHE), and the bromide tracer had moved to the down-gradient wells. On Oct 10, 2005 four barrels were filled (200 L each), two from MW-10S and the other two from MW-10D. A nutrient solution barrel from each well contained 0.2 kg yeast extract, 40 g glucose and 1 L of soy oil methyl ester (SOME). Anaerobic water was prepared with 0.2 kg yeast extract and 40 g of glucose in each chase solution barrel (Table 2.3). The bacterial consortium KB-1 was injected on Oct 13, 2005 in the shallow and deep zones of the four injection points. Deep injection wells received nutrient and chase water from MW-10D, while shallow wells received nutrient and chase water from MW-10S. Each well was first injected with 50 L

of nutrient solution followed by 5 L of KB-1 culture and finally pushed with 50 L of anaerobic chase water, Figure 2.3A shows the 20L KB-1 vessel. To minimize oxygen exposure, the culture was dispensed by pressurizing the vessel with argon gas, pushing the culture through a submerged delivery line into the injection well (Figure 2.4B).

Nutrient Feeding with Tracer Study (Spring 2006)

KDHE reported during February 2006 that the ground water in MW-9 and MW-10 was becoming less reducing as DO and ORP values were rising. Therefore, another nutrient and tracer dose was introduced into the injection wells. As previously described, four 55 gal barrels were filled with groundwater from MW-10 on February 28, 2006, with two barrels filled from MW-10S and 2 from MW-10D. Nutrient barrels received 2 L of soy oil methyl esters and 0.5 kg of yeast extract. KBr was added to nutrient barrels to make a final Br⁻ concentration of 1340 mg/L. Anaerobic chase water received 10 g of yeast extract and 250 mL of glucose stock solution at 80 g/L. Barrels were left in a trailer for 3 days until water became anaerobic as indicated by resazurin. On March 3, 2006 (day 197) injection started in the shallow wells of the injection points and water temperature in the barrels was about 6 °C before injection. Nutrient water (50 L) was injected in the eight wells followed by 50 L of anaerobic chase water.

Cheese Whey Feeding (Fall 2006)

Cheese whey was used as an alternative nutrient source. On August 1, 2006 (day 348) 1/200 (v/v) dilution of cheese whey was used in the injection water that was derived from MW-10 and contained 40 g glucose and 10 g yeast extract per barrel. The four

barrels had the same nutrients. Shallow injection points received 100 L of nutrient solution prepared from MW-10S and deep wells received the same amount of solution from MW-10D. Tracer was not added in this feeding.

Final Tracer Study (Fall 2007)

In July 11, 2007 (day 689) another tracer study was conducted to monitor ground water flow at the end of pilot study. About 50 gal of water was withdrawn from MW-8D and mixed with KBr to give a final analyzed Br⁻ concentration of 817 mg/L. KBr solution was then pumped back into MW-8D.

Table 2.1. Composition and concentration of amendments and tracer in the nutrient solution used for the preconditioning study on August 18, 2005.

	Deep Zone Barrel		Shallow Zone Barrel	
Water (Liters)	188		197	
	Mass (kg)	Concentration (%)	Mass (kg)	Concentration (%)
SOME	8	4	2	1
Lactate	0.8	0.4	0.8	0.4
YE	2	1	0.2	0.1
KBr	0.2	0.1	0.2	0.1

Table 2.2. Anaerobic chase water content used in the preconditioning study on August 18, 2005.

	Deep Zone Barrel		Shallow Zone Barrel	
Water (Liters)	200		200	
	Mass (g)	Concentration (%)	Mass (g)	Concentration (%)
Glucose	40	0.02	40	0.02
YE	10	0.005	10	0.005

Table 2.3. Content of nutrient and anaerobic chase water barrels (200 L each) used for bioaugmentation study on October 13, 2005.

	Nutrient Solution	Chase Water
SOME (L)	1	0
Lactate (L)	0.5	0.5
Yeast extract (kg)	0.2	0.2
Glucose (kg)	0.04	0.04

Table 2.4. Treatments used in water microcosms (optimizing nutrient concentrations for bioremediation).

#	Treatment
1	Control (No substrates)
2	0.01% YE+ 0.01% SOME
3	0.01% YE+ 0.003% SOME
4	0.01% YE+ 0.001% SOME
5	0.003% YE+ 0.01% SOME
6	0.003% YE+ 0.003% SOME
7	0.003% YE+ 0.001% SOME
8	0.001% YE+ 0.01% SOME
9	0.001% YE+ 0.003% SOME
10	0.001% YE+ 0.001% SOME
11	0.01% YE+ 3 mM Lactate
12	0.01% YE+ 1 mM Lactate
13	0.01% YE+ 0.3 mM Lactate
14	0.003% YE+ 3 mM Lactate
15	0.003% YE+ 1 mM Lactate
16	0.003% YE+ 0.3 mM Lactate
17	0.001% YE+ 3 mM Lactate
18	0.001% YE+ 1 mM Lactate
19	0.001% YE+ 0.3 mM Lactate
20	0.01% YE+ 3 mM Lactate+ 0.01% SOME
21	0.003% YE+ 1 mM Lactate+ 0.003% SOME
22	0.001% YE+ 0.3 mM Lactate+ 0.001% SOME
23	0.01% YE+ 1 mM Lactate+ 0.003% SOME
24	0.01% YE+ 0.3 mM Lactate+ 0.001% SOME
25	0.003% YE+ 0.3 mM Lactate+ 0.001% SOME

Table 2.5. Characteristics of cheese whey (Ghaly *et. al.*, 2003).

Characteristic	Percentage %
Total solids	6.8
Suspended solids	2.5
Total Kjeldahl nitrogen	0.16
COD	8.1
Lactose	4.8
Lactic acid	0.22
Vitamin B12	0.025 µg/g
pH	4.9

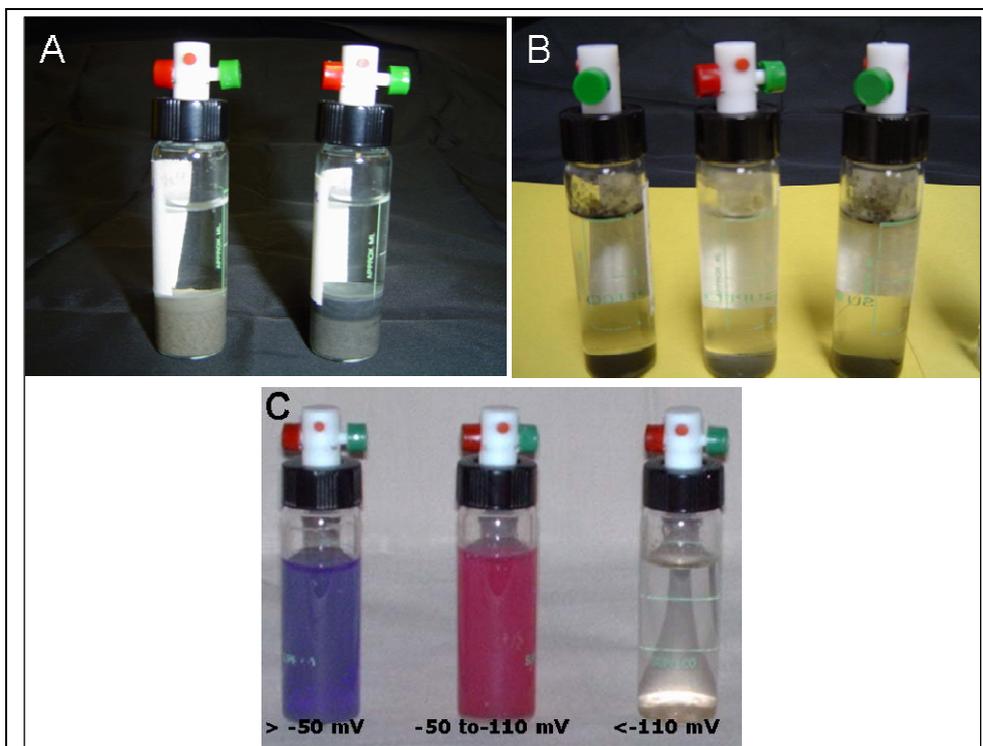


Figure 2.1. A picture of **A.** Soil microcosm vials, **B.** Water Microcosm vials and **C.** color change of resazurin indicator from Blue to pink then to highly reduced state when colorless.

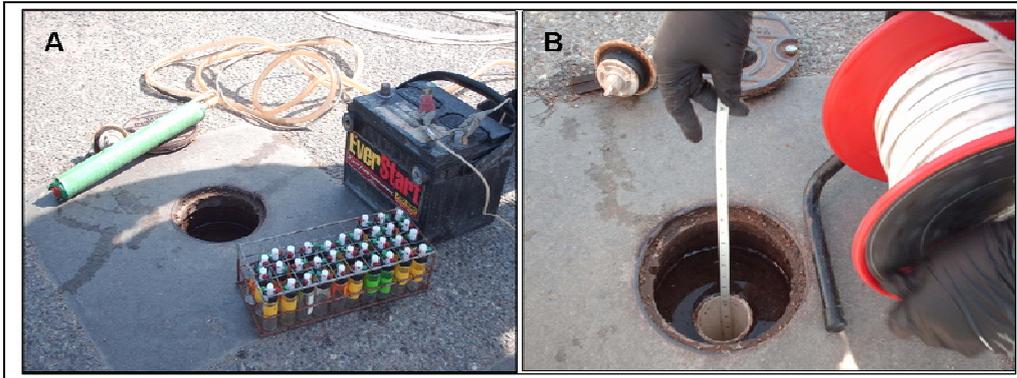


Figure 2.2. **A.** Picture of tools used in sampling; the monitoring wells showing the three stage pump powered by a car battery and collection vials. **B.** The water level indicator used to measure depth to water.

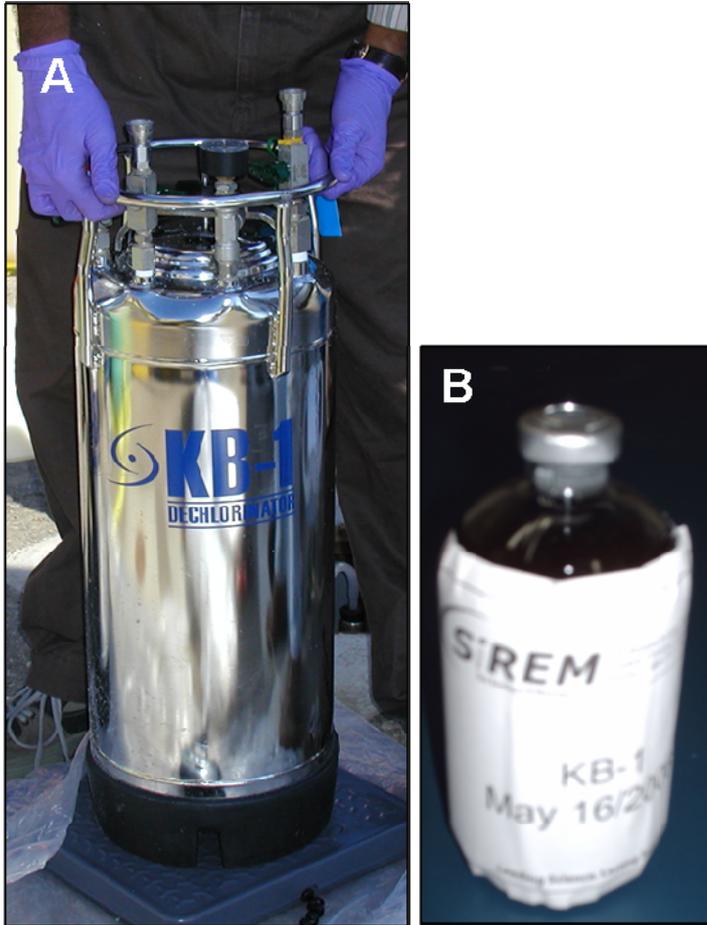


Figure 2.3. KB-1 culture from SiREM **A.** Picture of the 20 L vessel purchased for the pilot study site injection, **B.** The 125 mL bottle used for microcosm preparations, provided by SiREM.



Figure 2.4. Pictures of **A.** First tracer study and **B.** KB-1 Injections.

CHAPTER 3- RESULTS AND DISCUSSION

Soil Core Analysis

Table 3.1 shows results of a soil core sample analysis obtained during MW-9D drilling in 2003. Samples of 5 ft intervals ranging from 2.5 - 47.5 ft (m) below ground surface (bgs) were collected giving a total of 10 samples. Sand content increases and clay decrease with depth. Soil pH was neutral in the shallow soil depth and tended to become slightly alkaline (pH= 8) at depths below 27.5 ft (m). NPK (nitrogen, phosphorus and potassium) values seem to be higher in the upper 22 ft (m) of the soil core. Values of NO₃-N reached 3 ppm at 2.5 ft (m) and 12.5 ft (m) bgs, but were around 1ppm below 27.5 ft. In general the percentage of total nitrogen and total carbon decreased with increasing depth except in the sample at 27.5 ft bgs at which both values were about 10 times higher than the other samples. This sample was then repeated with the first submitted sample and run together for N and C content. Results were in excellent agreement with the first sample. Either the core was anomalous or there was a deposit of relatively high N and C at that depth.

Soil Microcosm Results

The first set of microcosms were to test the ability of the natural microbial community to undergo reductive dechlorination of PCE. Soil microcosms were prepared using soil sample at 47.5 ft (m) bgs that represents the deep aquifer at the site. Anaerobic conditions were obtained by flushing the microcosms with argon, and PCE was introduced to provide a concentration of 20 µg/L. Nutrient screening for biostimulation treatments included yeast extract (YE), soy oil methyl ester (SOME), lactate and glucose. Those treatments were used solely or in different combinations. They were selected because of their common use in literature, and their ability to promote dehalogenation conditions. Table 3.2 lists individual treatments and shows their effect on PCE degradation. Results show that not all nutrient and/ or nutrient combinations were able to promote a significant reduction in PCE under the specified conditions and concentrations. c-DCE was generated in microcosms fed with SOME and YE. SOME fed microcosms,

around 50% of chlorinated ethenes were transformed to DCE. This percentage increased with a combination of glucose, SOME and YE indicating that hydrogen donors provided by glucose and soy oil methyl ester as well as nutrients and vitamins in the yeast extract were needed to support microbial activity. Unfortunately, the microbial community at the site failed to complete the degradation of PCE resulting in the accumulation of c-DCE. Figure (3.1 A-C) illustrates the maximum appearance of daughter products in the actively metabolizing vials after 30 days of incubation and remained steady until the end of this experiment. The rest of the figures can be viewed in the supplementary material of this report (Figure S. 3.1 D-L).

Water Microcosms

Biostimulation Experiment

Water from MW-4D, MW-8S and MW-8D was used to study the extent of chlorinated ethene degradation upon stimulation of native microorganisms. In microcosms prepared with MW-4 (see Figure 3.2. A-G), methane was generated in the following treatments: SOME+YE, Glucose+ YE, Methanol, Methanol +YE, YE. About 50% of PCE decreased in SOME+YE treatment, and two thirds of PCE decreased in methanol and YE treatments. Trichloroethene (TCE) appeared after 6 weeks in SOME+YE, methanol+ YE and YE treatments. Unlike MW-4, addition of nutrients greatly enhanced reductive dechlorination of PCE in all vials except in SOME and methanol treatments. cis-Dichloroethene (c-DCE) was generated in the active microcosms in response to decrease of PCE concentrations during the second week of observation, but no further degradation of DCE was noted after 6 weeks. Figure 3.3. A-G summarizes MW-8D results. MW-8S results -represented in Figure 3.4., A-G show little evidence of reductive dechlorination in this well. The decrease in methanol +YE treatment is more likely due to a leakage in the vial since all other chlorinated ethenes decreased at the same rate.

In general more frequent sampling events were needed to carefully monitor the variation of chlorinated ethene degradation. From this set of microcosms, wells had different responses to nutrient amendments. *Dehalococcoides* sp. that carries out complete degradation of PCE was either absent or not active under these conditions.

Deeper wells may contain microorganisms capable of utilizing chlorinated ethenes relative to shallow depths. This leads to the need to investigate the responses in other wells and to determine their biodegradation potential, and investigate if bioaugmentation is necessary to remediate the contaminated plume at the pilot study area.

Monitoring Wells Screening and their Biodegradation Potential

In this set of microcosms bioaugmentation and biostimulation potential of deep monitoring wells (1-6) were compared. Several nutrient combinations were used to support reductive dechlorination. KB-1 bacterial consortium was added for bioaugmentation studies. Figures 3.5-3.15 represent some of the results. They clearly show that KB-1 was able to drive a reduction of PCE concentration in these wells. However, DCE was generated following PCE reduction. It was noted that biostimulation alone did not decrease the total concentration of chlorinated ethenes (Figure 3.5A-3.15A). On the other hand, total CEs decreased in KB-1 treated vials (Figure 3.8, 3.10, 3.11, 3.12, and 3.14). Methane was generated in most microcosms especially the KB-1 treated cases. This may indicate that PCE was completely converted to methane and not just fermentation from added nutrients. Yeast extract - a complex nutrient mixture- was necessary to support microbial growth in combination with other hydrogen donors like lactate, glucose and SOME. MW-1 is located up-gradient of the contamination source, and detectable concentrations of PCE were found in this well at about 0.2 μM (Burns and McDonnell report May 8, 2007 reported PCE concentration of 52 $\mu\text{g/L}$ in this well). MW-2 was down-gradient but was considered to be out of the contaminated plume. Figure 3.8 shows Lactate+ YE treatment of MW-1, where less than 0.5 μM of DCE was generated within the first week of study. Therefore, within the short monitoring period of this study we were able to see DCE removed from the bioaugmented system. Similar results were obtained with Lactate and YE treatment in MW-3 and MW-4. Higher initial concentrations of CEs in MW-5 and MW-6 may have decreased the dechlorination rate; therefore, longer monitoring periods are needed to evaluate a decrease in DCE concentration.

Optimizing Nutrient Concentration for Bioremediation

For actual site remediation we have to add nutrients which will be added to intercept the contaminated plume. Therefore, finding appropriate nutrient combinations and concentrations was necessary to estimate how much we need for pilot study application. We also, need to determine what will happen to microbial activity if nutrient concentrations are supplied at lower values. From that stand point an array of microcosms was prepared with YE concentrations from 0.01% to 0.001%, Lactate between 3mM and 0.3 mM, and SOME between 0.01% and 0.001%. Combinations with YE+ lactate, YE+ SOME, lactate+ SOME and finally YE+ SOME +lactate were tried. Figure 3.16 shows PCE concentrations in YE+ lactate treatments with KB-1 culture. Over 90% of PCE disappeared in 10-15 days in most treatments except 0.003% YE+3mM lactate and 0.003%YE+ 1mM lactate treatments, as they were similar to the control. Only 75% of PCE disappeared in 10 day in 0.001% YE+ 0.3 mM lactate treatment that had least concentrations of nutrients. A gradient of bioaugmented YE + SOME were tested. The PCE concentration is displayed in Figure 3.17. It is observed that when both concentrations are lowered the rate of PCE removal decreases. For example, PCE removal rate in 0.001% YE+ 0.001% SOME < 0.001% YE + 0.003% SOME < 0.003% YE+ 0.01% SOME < 0.01% YE+ 0.01% SOME. However, when rich nutrient medium is provided by combining YE+ SOME + lactate, all tested concentration gradients show maximum PCE removal in 10 days or less except 0.003% YE+ 0.001% SOME+0.3mM lactate (Figure 3.18). Figures 3.19 – 3.26 represent individual treatments with and without KB-1 bacterial culture, displaying PCE and its degradation products (TCE, c-DCE) as well as methane. Again those results show that -when biostimulated with nutrients- native microorganisms in the water were not able to remove PCE under the specified conditions, whereas, KB-1 bioaugmented microcosms show a decrease in total chlorinated ethenes at appropriate nutrient concentrations. In 0.01% YE+ 3 mM lactate treatment, DCE disappeared and methane was generated as a final end product after 100 days of monitoring (Figure 3.20). Lowering lactate concentration below 3mM leads to c-DCE accumulation (Figure 3.21). The same results were obtained when 0.001% yeast extract was used instead of 0.01% (Figure 3.22). Similarly, when 0.01% YE + 0.003% SOME was used, DCE reached minimum concentrations while methane built up (Figure

3.23), and total chlorinated ethenes remained high when any of the nutrient components decreased (Figures 3.24 and 3.25). Removal of total chlorinated ethenes was also observed in 0.01% YE+ 0.001% SOME+ 0.3mM lactate treatment (Figure 3.26). These results suggest that *Dehalococcoides* were not present at this site and that bioaugmentation was needed for bioremediation of PCE contamination at Cinderella dry cleaning site. Moreover, optimal concentrations of nutrients and vitamins should be provided for bacterial growth and establishment at the introduced environmental system.

KB-1 Viability Study

In this experiment we wanted to investigate the viability and survival of KB-1 bacterial culture after nutrient starvation and whether KB-1 remains dormant after nutrient deprivation and resumes activity after supplement addition or does it lose activity. Therefore, microcosms used in the previous study and bioaugmented with KB-1 were kept for about 8 months then they were re-fed with nutrients. Figure 3.27 represent the control vials without nutrients and so no activity is observed in these vials and PCE concentration remains constant along the study period. Addition of supplements and nutrients to those vials did not enhance native microorganisms (Figure 3.28). KB-1 augmented microcosms were fed with fresh nutrient solutions, but also seem to have little or no activity on chlorinated ethene concentrations. These results suggest that KB-1 bacterial culture could not survive nutrient deprivation for this period of time. So when they are introduced to an environmental system they should be fed with nutrients that provide hydrogen for reductive dechlorination until remediation objectives are achieved.

Cheese Whey Microcosms Studies

Whey Microcosms/ Set 1

We learned in previous studies that nutrients and vitamins were necessary for microbial growth. They should be added frequently during bioremediation action to maintain reducing conditions suitable for reductive dehalogenation. Hence, searching for supplements that would 1) provide essential nutrients 2) easy to implement in actual

remediation plan and 3) to be cost effective is of a great advantage. Cheese whey is a by-product of the dairy industry and can be obtained inexpensively. Powdered whey is more costly, but easier to obtain, ship and store. The AFCEE (2004) report lists cheese whey in experimental substrates. First set of microcosms prepared with cheese whey was prepared to check if the lactate and vitamin B12 content in cheese whey was good for biostimulation and bioaugmentation studies. From here, several concentrations of cheese whey were tested (0.01%, 0.025%, 0.05%, 0.1% and 0.25%) and compared to YE + SOME combinations used in previous studies. As expected, microcosms prepared without KB-1 bacteria were similar to control (Figure 3.32) and PCE remained dominant in the system (see Figure 3.30). On the other hand, PCE concentrations dropped almost 90% in 0.025% whey treatment and had a lag phase of 10 days, and were similar to YE+ SOME treatments. PCE concentrations also dropped in 0.05% and 0.01% whey treatments, but required a longer lag phase of 20 days (Figure 3.31). It was noticed that higher concentrations of cheese whey (above 0.05%) seemed to inhibit KB-1 bacterial culture and PCE remained through the study period as shown in Figure 3.33. Meanwhile, 0.025% whey produced DCE when PCE concentrations dropped (Figure 3.34). Those results were comparable to YE+ SOME combinations used previously (Figure 3.45).

Whey Microcosms/ Set 2

In this study cheese whey and SOME were used in combination to prepare microcosms to see if microbial activity could be enhanced further than cheese whey only. Figures 3.36 and 3.37 show that SOME addition did not affect the rate of PCE degradation and therefore, no gain will occur from this combination.

Whey Microcosms/ Set 3

After noticing inhibition of KB-1 activity at high cheese whey concentrations (> 0.05%), this set was prepared to see if this inhibitory effect is from competition between natural cheese whey culture and the KB-1 culture. Sterile cheese whey was obtained by filtration and used in this set. If the theory is true, then high concentrations of filtered whey should work as well. Figure 3.38 show that whey concentrations of 0.5%, 0.25% and 0.1% were also inhibitory to KB-1 bacterial culture. The 0.05% and 0.025 % whey

treatments were not inhibitory, but here longer lag phase was needed (30 days instead of 20) see Figures 3.39- 3.42. These results support the idea that natural microflora of cheese whey is not competitive with KB-1, and inhibition is caused by other factors in the cheese whey.

Whey Microcosms/ Set 4

At this stage KB-1 had been injected in the pilot study area for 167 days. Therefore, we prepared microcosms from MW-8D and MW-9D located about 5 ft west and east of injection wells to see if the introduced bacteria were able to establish themselves in the injection site. Therefore, KB-1 was not added to this set and organic substrates were introduced. Figure 3.43 shows results in MW-8D obtained with 0.025% cheese whey. PCE decreased within 5 days while DCE concentrations increased and remained till the end of the study. The same results were noticed in MW-9D treated with same concentration of whey (Figure 3.44). A similar trend was followed when 0.01% YE was used in MW-8D (figure 3.45) and MW-9D (Figure 3.46). This indicates that KB-1 injected at the pilot study area on August 18, 2005 was able to grow at the injection site and would be able to convert PCE to DCE in the microcosm setting. Note that this is a measure only of unattached organisms.

Whey Microcosms/ Set 5

Monitoring well 5 top and bottom water was used in this set of microcosms. Figure 3.47 represent the control treatment of MW-5 top water without any substrate addition. No degradation products were observed in these vials, while 0.05% whey (Figure 3.48) and 0.025% whey (Figure 3.49) augmented treatments show decrease in total chlorinated ethenes and appearance of methane after 120 days. Similar results were obtained when MW-5 bottom water was used (figures 3.50-3.52).

Soil Buffering Capacity

Phenol red (also known as phenol sulfonphthalein or PSP) is a pH indicator with $pK_a = 8.0$ at 20 °C. Its color exhibits a graded transition from yellow to red over the pH range of 6.0- 8.0 as indicated in Table 3.3. When phenol red was mixed with cheese whey

solution (0.25%) it was noticed that color changed to yellow overnight and the pH dropped to below pH 5. Therefore, the buffering capacity of soil core sample at 42.5 ft was measured with lactic acid (as this acid is predicted to form during cheese whey fermentation). Figure 3.53 shows soil titration results with 1M lactic acid, while figure 3.54 used 0.1M lactic acid. Results show that the soil is able to reach buffering capacity at pH values between 4-3, which is below the optimum pH of neutrophilic dehalorespirers.

Buffered Microcosms

Based on previous results with cheese whey acidification, soil and water microcosms were prepared with high concentrations of cheese whey. The pH was controlled in one set and compared to non buffered system. Figure 3.55.A shows soil microcosms without buffering power. PCE was detected along the study period and color of microcosm vials turned to yellow, clearly indicating acid formation and inhibition of native and KB-1 microbial cultures. On the other hand buffered soil microcosms shown in Figure 3.55.B responded to pH control converting PCE to DCE and then DCE to methane. Similarly, water microcosms with buffer and pH monitoring were active in reducing total chlorinated ethenes concentrations (Figure 3.56.B). In non buffered water microcosms PCE was the dominant chlorinated ethene compound and decrease in concentration at the end of experiment was due to a leakage in vials as indicated by high error bars at those days (Figure 3.56.A).

MW-5 Culture Microcosms

As we performed the bioremediation study in the field we noticed that the bottom of MW-5 had changed as it becomes more black and mucky, it became harder to sample the bottom of this well as the black sediment blocked the pump, so we had to go little higher to get a representative sample. Also upon analyzing chlorinated ethenes concentrations along the vertical depths of this well, we found that the bottom water had less PCE and more DCE concentrations than the top water sample. We believe that microbial community at this particular well has changed in favor of reductive dechlorination. So a sample of high sediment content was collected for the bottom of

MW-5 and used to prepare microcosms from MW-5 top water. Bioaugmentation potential of KB-1 and MW-5 bottom cultures was compared. Figure 3.57 show that bioaugmentation with KB-1 gave expected results where over 90% of chlorinated ethenes disappeared in 75 days. Surprisingly, the sediment from MW5 bottom show similar results (Figure 3.58), even when the two cultures were mixed together (Figure 3.59). At this point we have no information about the microbial culture composition and whether it is similar to KB-1. Since we use the same pump to sample all wells it is possible that some KB-1 organisms have been transferred from MW-9 (which was located 5 ft east of injection wells where KB-1 was introduced) to other wells and find it suitable to grow at this location.

Pilot Study (Field Scale)

This is a collaborative work between members in the Biochemistry Department (Dr. Lawrence Davis and Jwan Ibbini) and Chemical Engineering Department members (Dr. Larry Erickson and Sathish Kumar). Therefore, detailed information of field work done in the pilot study until the fall of 2007 could be viewed in Dr. Sathish Kumar Santharam PhD thesis (Chemical Engineering Department) (Santharam, 2008). He graduated in August 2008, and his thesis was submitted electronically to graduate school of Kansas State University.

Groundwater Elevation

Precipitation affects the level of groundwater in the subsurface. Table 3.4. lists the amount of rainfall (inches) recorded at Manhattan, KS, during the period from January 2000 to April 2008. Precipitation data was provided by KSU research and extension, <http://av.vet.ksu.edu/webwx/>, 2008. Tables 3.5 and 3.6 present the measured groundwater elevation levels in the pilot study area for the deep and the shallow zone respectively. Variations in the data are due to rainfall events, but generally groundwater table in shallow zone was about 5 ft above that in the deep zone. In the pilot study area, the groundwater elevation is approximately 10 times higher in the shallow zones compared to

deep zones as shown in Figure 3.60. The elevation grades between MW-8 and MW-12, and the general groundwater flow direction is to the east in both zones.

Deep wells tracer prior to biostimulation (Fall 2004)

In this study bromide was injected in MW-8D at a concentration of 500 mg/L. Background concentrations were in the range of 0.3 - 0.6 mg/L. In about 8 days 50 mg/L was detected in MW-8D, and in 28 days, most of the bromide was washed out as shown in Figure 3.61. Bromide concentrations reached peak in MW-9 D after 15 days (Figure 3.62) and MW-10 in about 28 days (Figure 3.63). Based on arrival times ground water velocities in MW-9D and MW-10D were estimated to be (0.67 ft/d) and (0.71 ft/d) respectively (Table 3.7 & 3.8).

Shallow wells tracer study prior to biostimulation (Fall 2004)

At the injected well (MW-8S) initial bromide concentration was 500 mg/L. After about 4 days only 50 mg/L was detected in the well, and in 14 days the injected bromide had been washed out from MW-8S. In down-gradient wells, bromide peak reached MW-9S in 9 days as shown in Figure 3.64; and MW-10S in about 22 days (Figure 3.65). Approximated groundwater velocities in MW9S and MW-10S were 1.02 ft/d and 0.91 ft/day respectively. Groundwater velocity in shallow is higher than deep zone. Although this is supported by the higher elevation gradient (about 10 times) in the shallow zones, soil texture analysis shows that shallow zone composed of higher percentage of clay that would lower permeability. At the same time we don't know if the subsurface is uniformly layered or if there are lenses of sand and silt in the shallow zone causing preferential flow paths.

Fall 2005 and Spring 2006 Tracer Studies

This work has been described extensively in Sathish Kumar PhD dissertation (Santharam, 2008). Here a brief summary is presented:

Shallow zone

On August 18, 2005 first bromide tracer (injection concentration= 670 mg/L) was added with nutrients on day 0 of pilot study. Another tracer study event took place on March 3, 2006 after 197 days of starting pilot study with injection bromide concentration of 1340 mg/L. In both tracer studies bromide was introduced to the subsurface through injection wells located in between MW8 (4.4 ft up-gradient of injection points) and MW-9 (5 ft down-gradient of injection point). Figure 3.66 show bromide concentrations in MW-8S. Bromide reached MW-8S as a result of dispersion and creation of a radius of influence around injection points. Baseline bromide concentration was reached in MW-8S after 7 days in the first tracer and after 22 days in the second.

MW-9S reached peak bromide concentrations (1.5 mg/L) in 5 days in the first tracer, and only reached 1 mg/L in second tracer study after 11 days. Nutrients added to injection location on Aug. 18, 05 and Oct. 13, 2005 appeared to have increased biofilm formation and decreased hydraulic conductivity between injection points and MW-9S (see Figure 3.67).

Higher concentrations of bromide peaks were noticed in MW-10S (Figure 3.68) and water may have bypassed MW-9S due to preferential path formation around the nutrient injection area. MW-12S remained around background levels (Figure 3.69).

Deep Zone

Bromide was injected also in the deep zone on the injection wells on Aug. 18, 2005 and again on March 3, 2006. The Bromide reached MW-8D by the effect on injection in the first tracer, and remained above baseline concentrations after the second nutrient injection in Oct. 13, 2005 (Figure 3.70). This result led to the suggestion that biomass formation in the injection wells may have formed a barrier that decreased permeability at the site. In MW-9D, tracer took 50 days to reach peak concentration in fall 2005 study, and took even longer time (80 days) to reach peak in the second spring 2006 study (Figure 3.71). Bromide in MW-10D peaked after 95 days in first and second tracer, but larger bromide concentrations were observed in the second tracer study (Figure 3.72). the peak of bromide concentration were reached MW-12D (located 75 ft down-gradient from injection wells) after 230 days (Figure 3.73)

Final Tracer Study after Completing Pilot Bioremediation

In July 11, 2007 (day 689 of pilot study) the tracer was introduced to the pilot study area through MW-8D with injected bromide concentration of 817 mg/L. The objective of this tracer was to check ground water velocity in the deep zone at the end of the study, and compare it to first tracer in the Fall 2004 before the bioremediation study. Notice that last nutrient injection took place on Aug. 1, 2006 (day 348 of pilot study). Figure 3.74 shows Fall 2007 tracer results in MW-8D. Bromide was washed out from the well in 10 days but remains constantly above background level of 1 mg/L. Bromide concentrations reached peak after 21 day in MW-9D (vs. 15 days in Fall 2004) and was washed out to a steady level (above background) after 50 days (Figure 3.75). In MW-10D a bromide peak was reached in about 36 days (vs. 28 days in Fall 2004), also concentrations remained above background levels (Figure 3.76). Tables 3.7 and 3.8 compares arrival times and velocities of Fall 2004 and Fall 2007 tracer studies. Estimated velocity of MW-9D was 0.5 ft/d in Fall 2007 vs. 0.67ft/d in 2004. And MW1-10D groundwater velocity was 0.56 ft/d in 2007 vs. 0.71 ft/d in 2004. This shows that biomass barrier may not affect the site in the long run as ground water velocities are becoming closer to values at the beginning of the bioremediation study.

Chlorinated Ethenes

Concentrations of PCE, TCE and DCE were plotted for three depths in deep and shallow wells. Deep well samples were collected at the following depths below top of casing: top (42 ft), mid (47 ft) and bottom (52 ft). Shallow wells sampled at the following depths: top (18 ft), mid (23 ft) and bottom (28 ft).

Figures 3.77 to 3.80 represent chlorinated ethenes concentrations in deep zone of MW-8D, MW-9D, MW-10D and MW12D respectively. In M-8D rapid decrease in PCE and increase in DCE concentrations were noticed after first injection event in day 0. After second nutrient injection with KB-1 PCE remained low and DCE concentrations decreased. PCE concentrations then increased to about 10 μ M before the third nutrient injection in day 197, and decreased rapidly after nutrient addition accompanied by DCE generation which soon decreased to lower levels and remain low for long time (750 days). Similar trend was also followed after last nutrient injection on day 348. Monitoring

the well further we noticed that PCE concentrations are rebounding, but did not reach initial concentrations. This is perhaps due to decrease in microbial biomass and reducing conditions at the site.

MW-9D showed a dramatic response due to nutrient and KB-1 addition. PCE concentrations dropped from about 80 μM before KB-1 to about 10 μM after third nutrient feeding. DCE was generated after PCE decrease then decreased to low levels until about 750 days when concentrations start to rebound to about 7 μM for DCE after 1000 days and around 23 μM for PCE after the same time (Figure 3.78). In MW-10, PCE and DCE concentrations were fluctuating and not reflecting what is happening in MW-9D indicating that this well is receiving water from another path that can create that rebound of PCE concentration to about 40 μM before the nutrient injection in day 197. Total CEs concentrations decreased to 10 μM after 500 days and remained steady until they started to rise again (Figure 3.79). MW-12D showed similar trend (Figure 3.80).

Shallow wells in general had lower PCE concentrations than deep wells ($< 10 \mu\text{M}$) except for MW-10S that had a higher value of 17 μM . MW-8S was not affected much with nutrient addition and concentrations remain similar across the study (Figure 3.81). In MW-9S, PCE concentrations decreased following bacterial injection then rebounded. This was also the case when nutrients were added on day 348. After 1000 days PCE concentrations in this well were close to the starting point at around 6 μM (Figure 3. 82). In MW-10S first response was detected after 230 days when PCE concentrations decreased and DCE increased. Now DCE has gone back to background values while PCE concentrations are reaching initial values (Figure 3.83). In MW-12S, concentrations of PCE and DCE remained the same throughout the study (Figure 3.84).

Monitoring Inorganic Ions

Nitrate Concentrations

In deep wells nitrate concentrations have always been very low except at the last tracer study when concentrations suddenly appeared at high concentrations between day 700 and 800 for MW-8D and MW-9D, and between day 700 and 750 for MW-10. Nitrate concentrations were back to normal levels after this event (see Figures 3. 85 – 3.88).

In shallow wells background concentrations for nitrate before the pilot study were around 15 mg/ L in all wells except MW-12S which was 13 mg/L. Figures 3.89 – 3.92 represent nitrate concentrations in shallow wells. Since higher concentrations of nitrate were observed in shallow wells than in deep wells, concentrations may vary due to precipitation and runoff from surrounding areas during fertilization or nitrification of ammonium. Nutrients added on day 0 significantly reduced nitrate concentrations in shallow wells. A drop in nitrate concentration was coinciding with bromide peak arrival which is associated with nutrient arrival in all shallow wells. Nitrate is an electron acceptor used by microorganisms during nutrient fermentation. When nutrient concentrations decrease due to washing or microbial consumption, nitrate concentrations increase.

Sulfate and Chloride Concentrations

Figures of sulfate and chloride concentrations are given in supplementary material. They show great consistency in levels for individual wells indicating that the analytical system was effective.

Table 3.1. # Characteristics of soil samples collected on August 5, 2003 during installation of MW-9D. Analyzed by soil Testing Laboratory, Throckmorton Hall, Kansas State University; Samples collected by Terracon, Wichita, KS.

Sample Depth (ft)	pH	Bray-1 P ppm	K ppm	Na ppm	Soil Texture			NO ₃ -N ppm	Total N %	Total C %
					Sand %	Silt %	Clay %			
2.5	7	21	520	8	12	40	48	2.9	0.052	0.66
7.5	7.5	28	476	16	14	40	46	2.2	0.036	0.72
12.5	7.6	41	550	31	12	34	54	3	0.036	0.4
17.5	7.7	51	422	15	10	50	40	1.3	0.045	0.36
22.5	7.6	62	389	18	14	46	40	2	0.029	0.17
27.5*	7.1	20	196	17	16	54	30	1.2	0.355 (0.362)	5.29 (5.29)
32.5	8.2	39	145	11	30	48	22	1.1	0.051	0.49
37.5	8.1	45	186	9	30	54	16	1.1	0.022	0.08
42.5	8.2	41	137	7	38	50	12	1.2	0.013	0.06
47.5	8.2	24	73	6	36	42	22	1.1	0.001	0.03

Total N and C are expressed as % (g / 100g dry weight soil)

* Analysis for this sample was repeated and values shown in parenthesis.

Table 3.2. Soil microcosms biostimulation treatments and their ability to produce DCE. Each treatment was done in duplicate and values of 50 % and above are shown in red color.

Treatments	% of CE's converted to DCE in	
	30 Days	85 Days
Control 1	0	38
Control 2	0	7
Autoclaved Control 1	0	19
Autoclaved Control 2	0	0.1
Glucose 1	21	88
Glucose 2	4	8
Lactate 1	6	11
Lactate 2	5	9
Methanol 1	7	3
Methanol 2	8	5
Soy Oil Methyl Ester (SOME) 1	28	52
Soy Oil Methyl Ester (SOME) 2	35	42
Yeast extract (YE) 1	1	8
Yeast extract (YE) 2	1	4
Glucose + YE 1	0	3
Glucose + YE 2	34	75
Lactate+ YE 1	22	23
Lactate+ YE 2	0	21
Methanol +YE 1	0	28
Methanol +YE 2	0	7
SOME + YE 1	27	37
SOME + YE 2	30	34
Glucose + SOME+ YE 1	67	81
Glucose + SOME+ YE 2	30	63
Methanol +Lactate+ YE 1	4	10
Methanol +Lactate+ YE 2	2	7

Table 3.3. Color change of Phenol red indicator in response to the pH of buffer solution.

pH of Potassium Phosphate Buffer	Color of Buffer Solution with Phenol red
5.8	Yellow
6.0	Yellow
6.2	Dark Yellow
6.6	Orange
6.8	Dark Orange
8.0	Red

Table 3.4. *Monthly rainfall (inches) in Manhattan for the period May 2003 to April 2007. The data was recorded at the Kansas State University Campus, Manhattan KS.

Year Month	2000	2001	2002	2003	2004	2005	2006	2007	2008
Jan	0.21	0.71	1.00	0.30	0.63	0.85	0.50	0.68	0.86
Feb	2.03	2.74	0.43	1.10	1.45	2.96	0.01	0.95	1.00
Mar	2.64	1.24	0.60	1.67	5.10	0.84	2.93	4.01	2.59
Apr	1.91	3.40	3.54	4.24	2.06	0.67	3.46	3.08	2.24
May	2.54	5.56	5.67	2.77	3.30	1.45	2.85	11.94	
Jun	5.77	7.03	0.42	7.85	6.60	11.81	1.44	5.93	
Jul	2.17	3.27	3.84	2.60	7.24	2.26	4.10	4.66	
Aug	0.70	6.87	2.93	5.03	6.16	5.61	10.21	2.24	
Sep	0.94	5.67	3.03	3.22	1.35	4.36	1.99	1.96	
Oct	2.42	1.47	5.59	2.57	1.01	3.25	1.95	4.36	
Nov	1.16	0.71	0.39	1.00	1.81	0.68	0.07	0.12	
Dec	0.28	0.23	0.01	1.09	0.46	0.78	0.43	3.71	

* KSU Research and Extension, <http://av.vet.ksu.edu/webwx/>, 2008.

Table 3.5. Groundwater elevation (listed value + 1000 ft above MSL) in the deep zone.

Date	MW-5	MW-7D	MW-8D	MW-9D	MW-10D	MW-11D	MW-12D
3-Apr-02	8.44						
13-Aug-03		7.42	7.39	7.48	7.36	7.39	7.33
3-Sep-03				7.09			7.01
12-Nov-03	7.63						7.45
17-Mar-04			8.7				8.68
27-Apr-04	8.72	7.8	7.76		7.82	7.8	7.73
25-May-04	8.35	9.22	9.22	9.24	9.16	9.18	9.21
24-Jun-04	9.1	8.08	9.09	9.05	9.03		9
29-Jul-04	9.85	9.94	9.84	9.86	9.81		9.76
16-Sep-04	9.29		9.27				9.3
22-Mar-05	8.91		8.89				8.84
27-Jun-05	10.22		10.13				10.08
16-Aug-05			8.42	8.42	8.41		
1-Sep-05			7.97	7.98	7.96		
3-Sep-05	8.12	8.21	8.11	8.12	8.07	8.1	
12-Sep-05	8.12			8.19	8.16	8.17	
15-Sep-05		7.85	7.86	7.85	7.82		
17-Sep-05				8.03			8.95
24-Sep-06		7.97	7.98	7.97	7.96		7.92
10-Oct-06			7.62	7.61			7.76
4-Jan-06			7.42	7.45	7.42		7.41
12-Jan-06			8.07	8.11	8.06		8.07
26-Jan-06			7.5	7.49	7.47		7.45
30-Jan-06	7.93		7.89				7.86
31-Jan-06			7.79	7.8	7.78		7.75
8-Feb-06			7.5	7.51	7.5		7.57
14-Feb-06			7.87	7.89	7.85		7.81
23-Feb-06			7.41	7.43	7.5		7.36
29-Mar-06	8.06		8.02				7.96
25-May-06	8.62	8.6	8.59	8.61	8.56		8.53
10-Jun-06			8.46	8.49	8.43	8.46	8.42
23-Jun-06	7.67	7.65	7.65				7.56
31-Jul-06	7.22	7.19	7.2	7.16	7.16	7.17	7.09
18-Sep-06	7.44		7.4				7.36
22-Sep-06	7.87	7.86	7.86	7.87	7.81		7.79
9-Nov-06		7.52	7.5	7.51	7.49		7.49
26-Nov-06	7.67	7.61	7.62	7.62	7.58		7.56
26-Jan-07			7.46	7.45	7.42		7.39
21-Feb-07			7.23	7.25	7.22		7.18
22-Feb-07	7	5.97					
12-Apr-07	5.16	7.72	7.71	7.72	7.71	7.69	7.65
7-May-07	7.82	8.92	8.91	8.92	9		8.88
12-May-07	9.58	9.56	9.56	9.56	9.49		9.46
22-May-07	10.56		9.32	10.33	9.3		10.28
4-Jun-07	8.52	11.11	11.01	9.17	10.91	10.95	10.99
10-Jul-07	11.15	11.16	11.11	11.17	11.11	11.15	11.09
31-Jul-07	7.22	7.19	7.21	7.16	7.16	7.17	7.09
16-May-08		11.56	11.54		11.52		11.49

Table 3.6. Groundwater elevation (listed value + 1000ft above MSL) in shallow zone.

Date	MW-7S	MW-8S	MW-9S	MW-10S	MW-11S	MW-12S
13-Aug-03	13.35	13.28	13.12	13.04	13.2	12.46
3-Sep-03			13.98			13.11
12-Nov-03						12.48
17-Mar-04	14.59	14.53		14.3		12.58
27-Apr-04	13.6	13.48	13.44	14.27	13.46	12.72
25-May-04	14.93	14.88	14.8	14.69	14.77	14.1
24-Jun-04	14.69	14.84	14.7	14.6		14.01
29-Jul-04	15.32	15.51	15.53	15.32		14.69
15-Sep-04		14.72				13.93
22-Mar-05		14.01				13.24
27-Jun-05		15.29				14.53
18-Aug-05		13.48	13.38	13.29		
1-Sep-05		13.67	13.57	13.51		
5-Sep-05	13.77	13.56	13.42	13.43	13.46	
12-Sep-05			13.21	13.09	13.22	
15-Sep-05	13.27		13.13	13.03		
17-Sep-05		13.21		12.96		12.45
24-Sep-05	13.87	13.84	13.72	13.58		13.38
10-Oct-05		13.45	13.34	13.23		12.65
4-Jan-06		12.63	12.54	12.35		11.98
12-Jan-06		12.55	12.48	12.41		11.88
26-Jan-06		12.34	12.28	12.18		11.68
30-Jan-06		12.75				12.05
31-Jan-06		12.42	12.32	12.22		11.72
9-Feb-06		12.24	12.18	12.08		11.61
14-Feb-06		12.24	12.2	12.08		11.6
23-Feb-06		12.14	12.08	11.93		12.46
29-Mar-06		12.71				11.98
25-May-06	13.58	13.59	13.43	12.98		12.8
10-Jun-06		13.31	13.19	13.07	13.2	12.57
25-Jun-06	12.88	12.91				12.14
31-Jul-06	12.48	12.46	12.32	12.21	12.32	11.69
18-Sep-06		13.23	26.93			12.46
22-Sep-06	13.48	13.46	13.32	13.13		12.61
9-Nov-06	12.93	12.91	12.78	12.68		12.1
28-Nov-06	12.71	12.64	12.53	12.43		11.86
26-Jan-07		12.31	12.17	11.06		11.51
21-Feb-07		11.28	12.18	12.06		11.55
22-Feb-07	12.33					
12-Apr-07	13.56	13.55	13.38	13.18	13.41	12.62
7-May-07	16.12	17.86	16.78	16.67		15.61
12-May-07	17.33	17.27	17.07	13.91		16.05
22-May-07			16.27	15.86		15.26
4-Jun-07	17.43	17.41	17.13	17.03	17.22	16.41
10-Jul-07	16.23	16.23	16.13	15.93	11.02	15.41
31-Jul-07	12.48	12.46	12.32	12.21	12.32	11.69
18-May-08	16.24	16.18		15.96		15.46

Table 3.7. Bromide arrival times compared between Fall 2004 and Fall 2007 tracer studies at the pilot study area.

Well	Distance from MW-8 (ft)	Arrival Time (days)	
		Fall 2004	Fall 2007
MW-9S	10	9	-
MW-9D	10	15	21
MW-10S	20	22	-
MW-10D	20	28	36

Table 3.8. Comparison of Groundwater velocities between Fall 2004 and Fall 2007 tracer studies at the pilot study area.

Well	Distance from MW-8 (ft)	Velocity (ft/day)	
		Fall 2004	Fall 2007
MW-9S	10	1.02	-
MW-9D	10	0.67	0.5
MW-10S	20	0.91	-
MW-10D	20	0.71	0.56

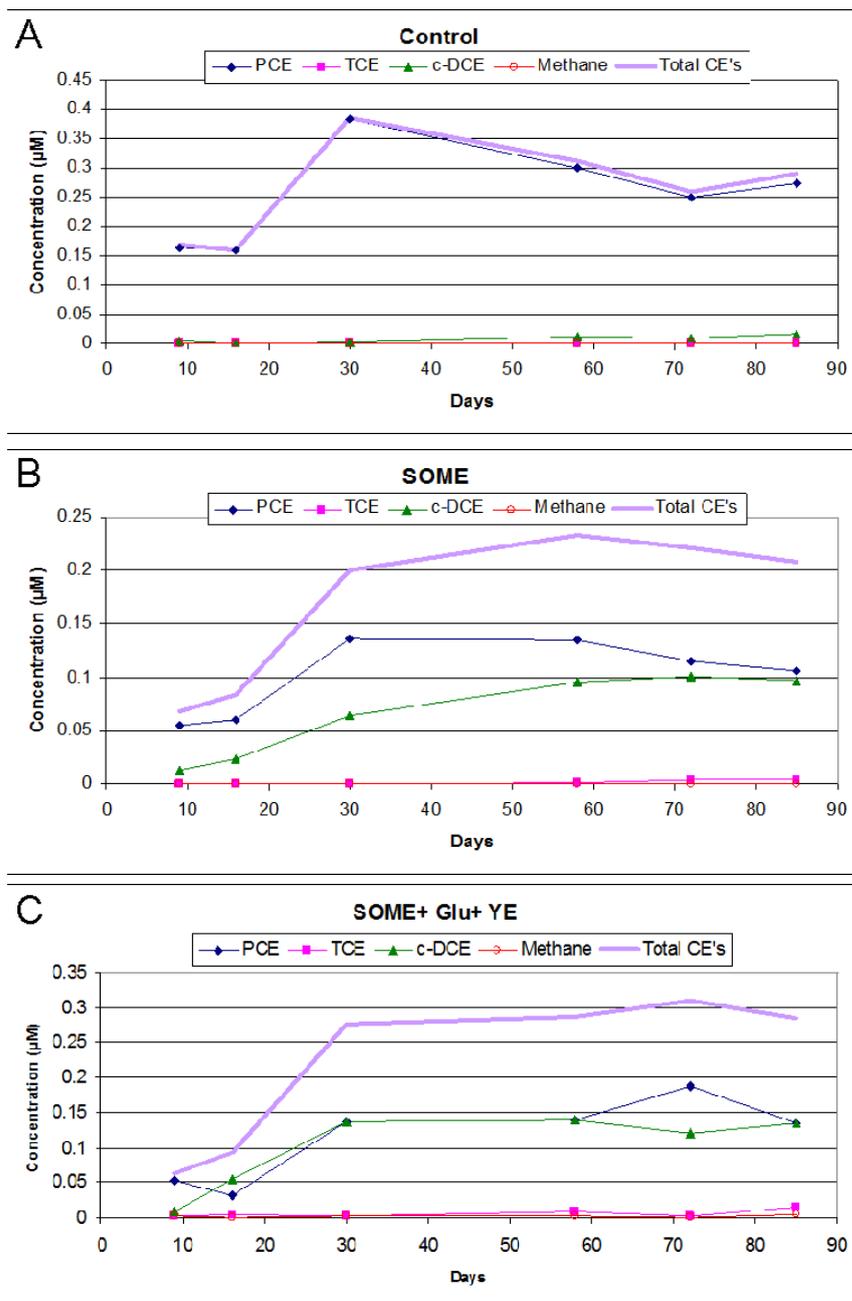


Figure 3.1. Show chlorinated ethenes and methane concentrations in soil Microcosm study. **A.** Control without any substrates, **B.** SOME treatment, **C.** SOME + Glucose+ YE treatment.

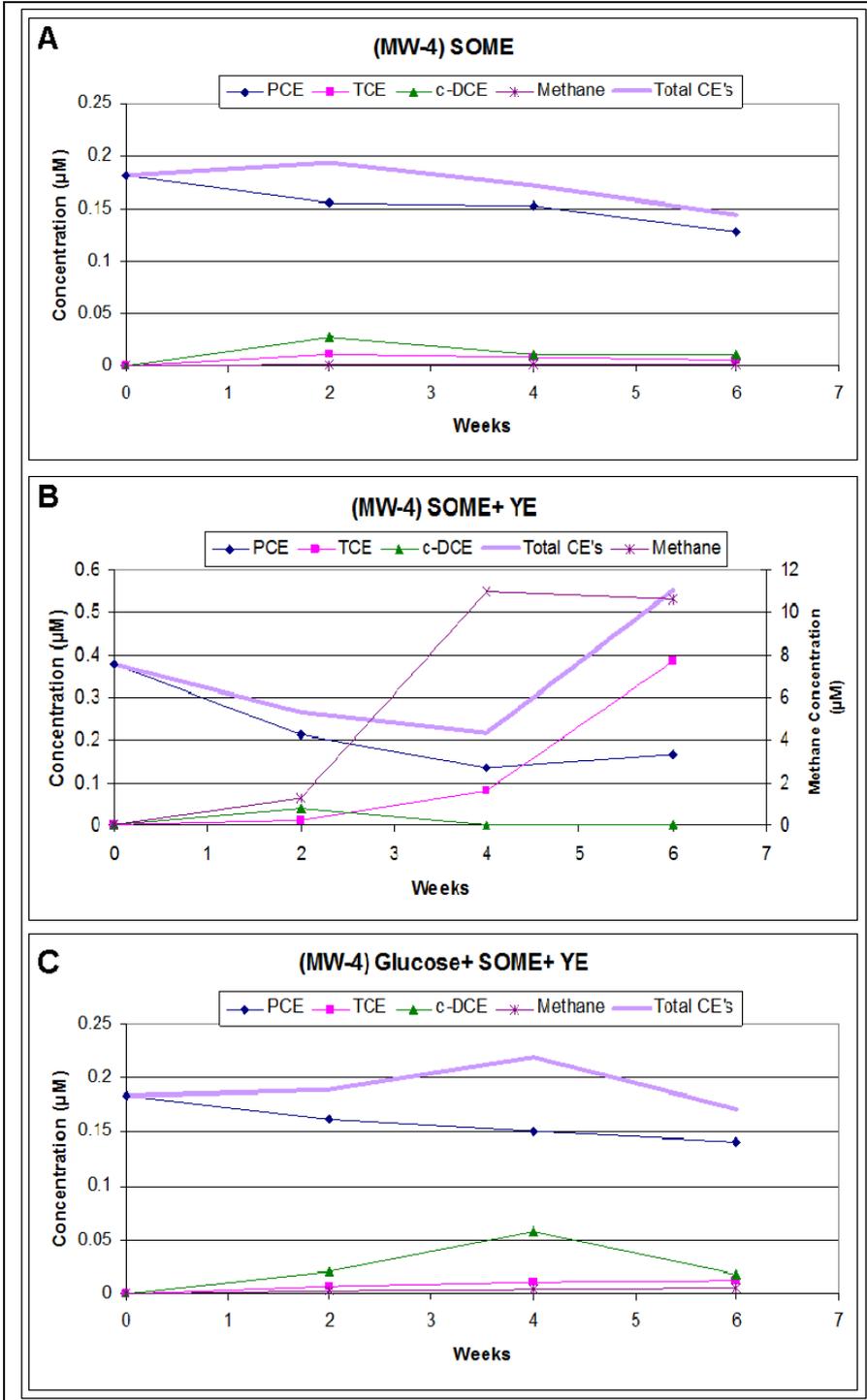


Figure 3.2(A-C). Chlorinated ethenes and methane concentrations in water microcosm (Biostimulation Experiment). Prepared with water from MW-4D, and treated with **A.** SOME, **B.** SOME + YE and **C.** Glucose+ SOME+ YE.

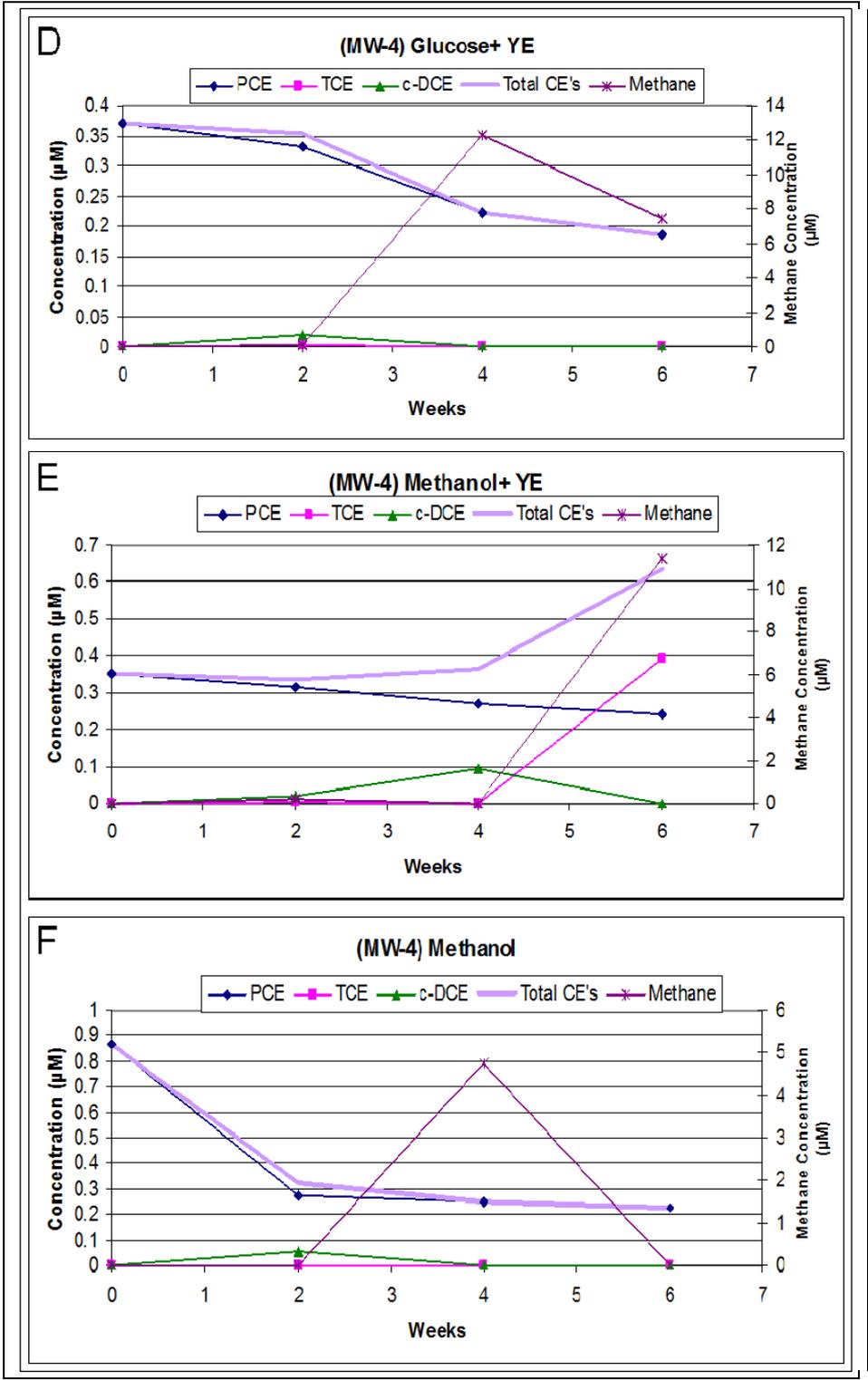


Figure 3.2(D-F). Chlorinated ethenes and methane concentrations in water microcosm (Biostimulation Experiment). Prepared with water from MW-4D, and treated with **D.** Glucose+ YE, **E.** Methanol + YE and **F.** Methanol.

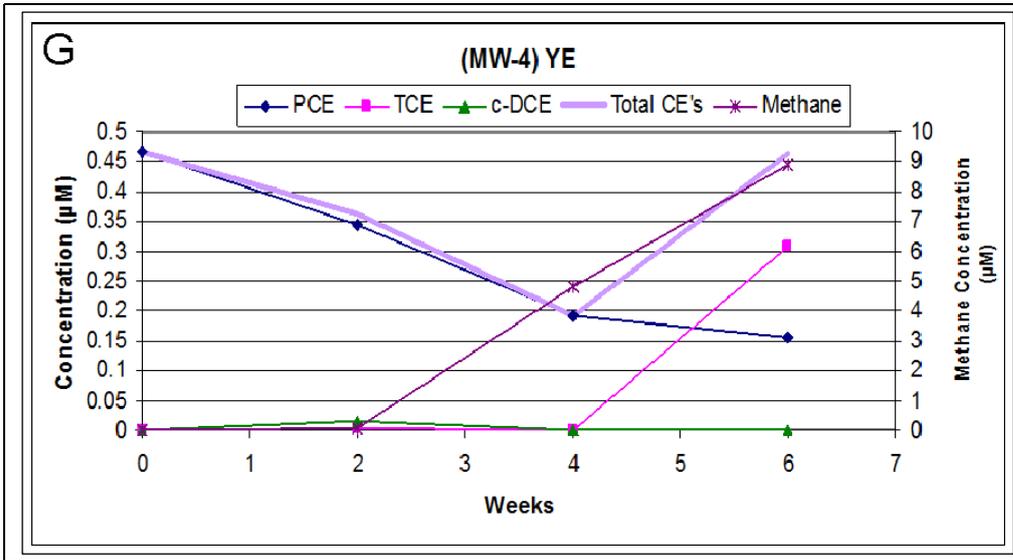


Figure 3.2(G). Chlorinated ethenes and methane concentrations in water microcosm (Biostimulation Experiment). Prepared with water from MW-4D, and treated with **G.** YE.

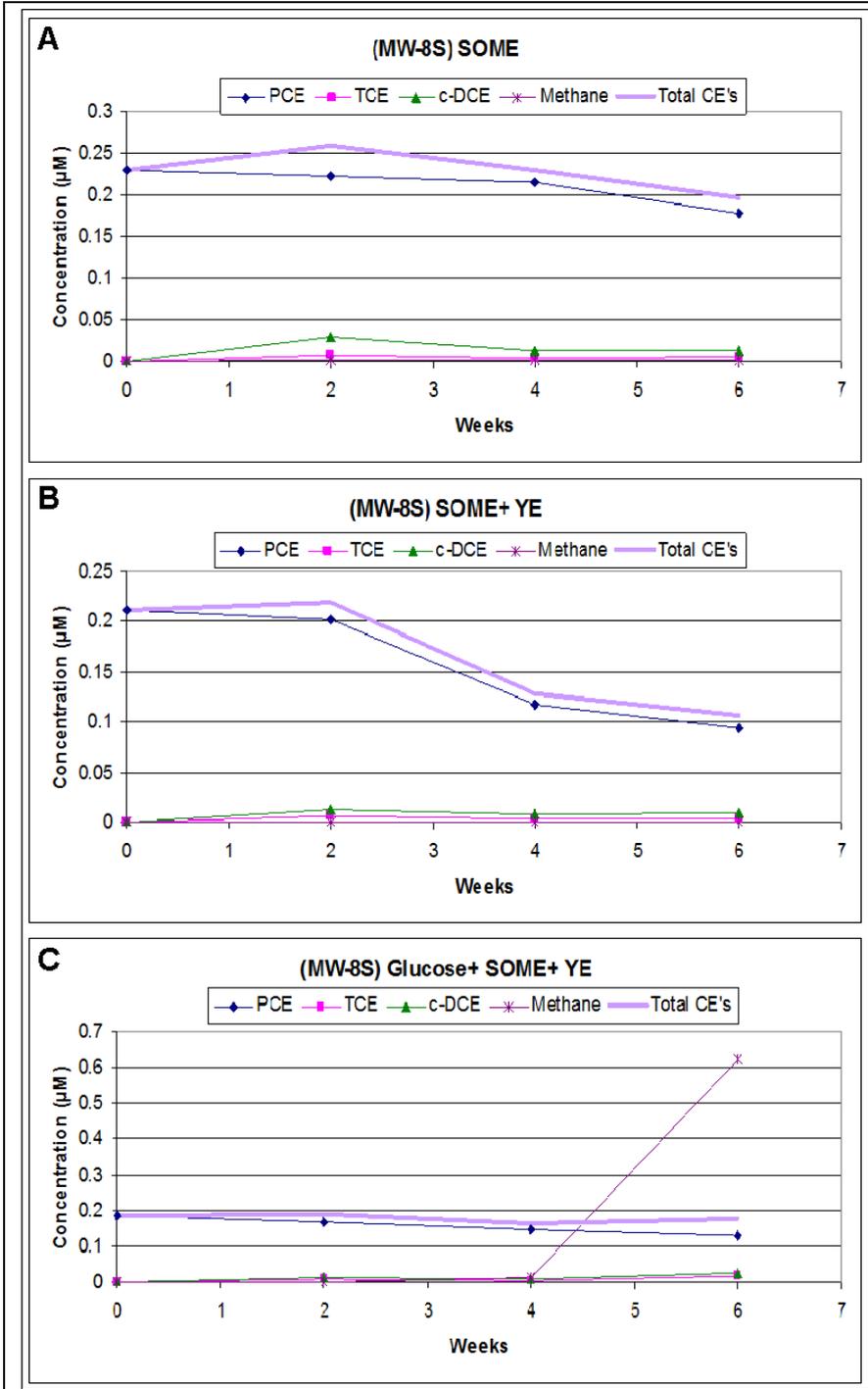


Figure 3.3(A-C). Chlorinated ethenes and methane concentrations in water microcosm (Biostimulation Experiment). Prepared with water from MW-8S, and treated with **A.** SOME, **B.** SOME + YE and **C.** Glucose+ SOME+ YE.

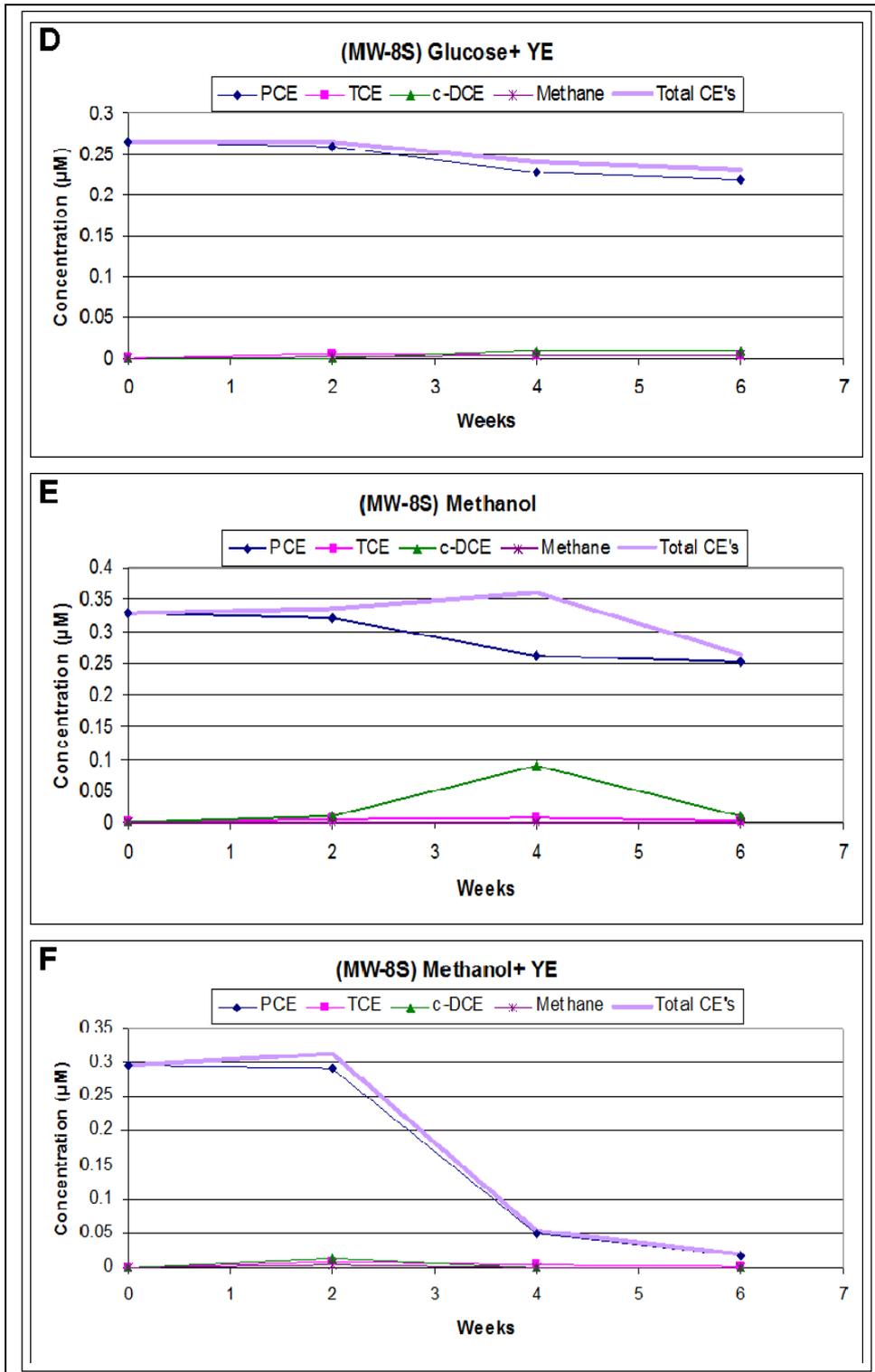


Figure 3.3(D-F). Chlorinated ethenes and methane concentrations in water microcosm (Biostimulation Experiment). Prepared with water from MW-8S, and treated with **D.** Glucose+ YE, **E.** Methanol and **F.** Methanol+ YE.

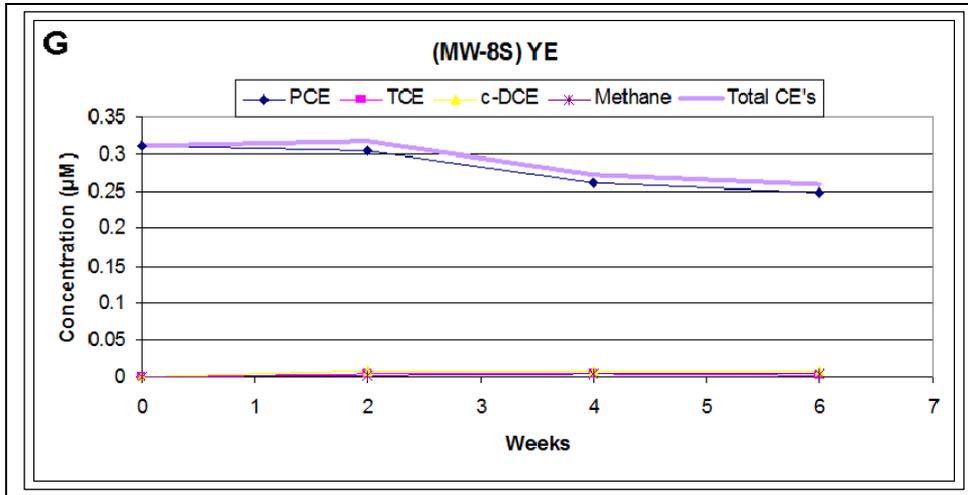


Figure 3.3(G). Chlorinated ethenes and methane concentrations in water microcosm (Biostimulation Experiment). Prepared with water from MW-8S, and treated with **G.** YE

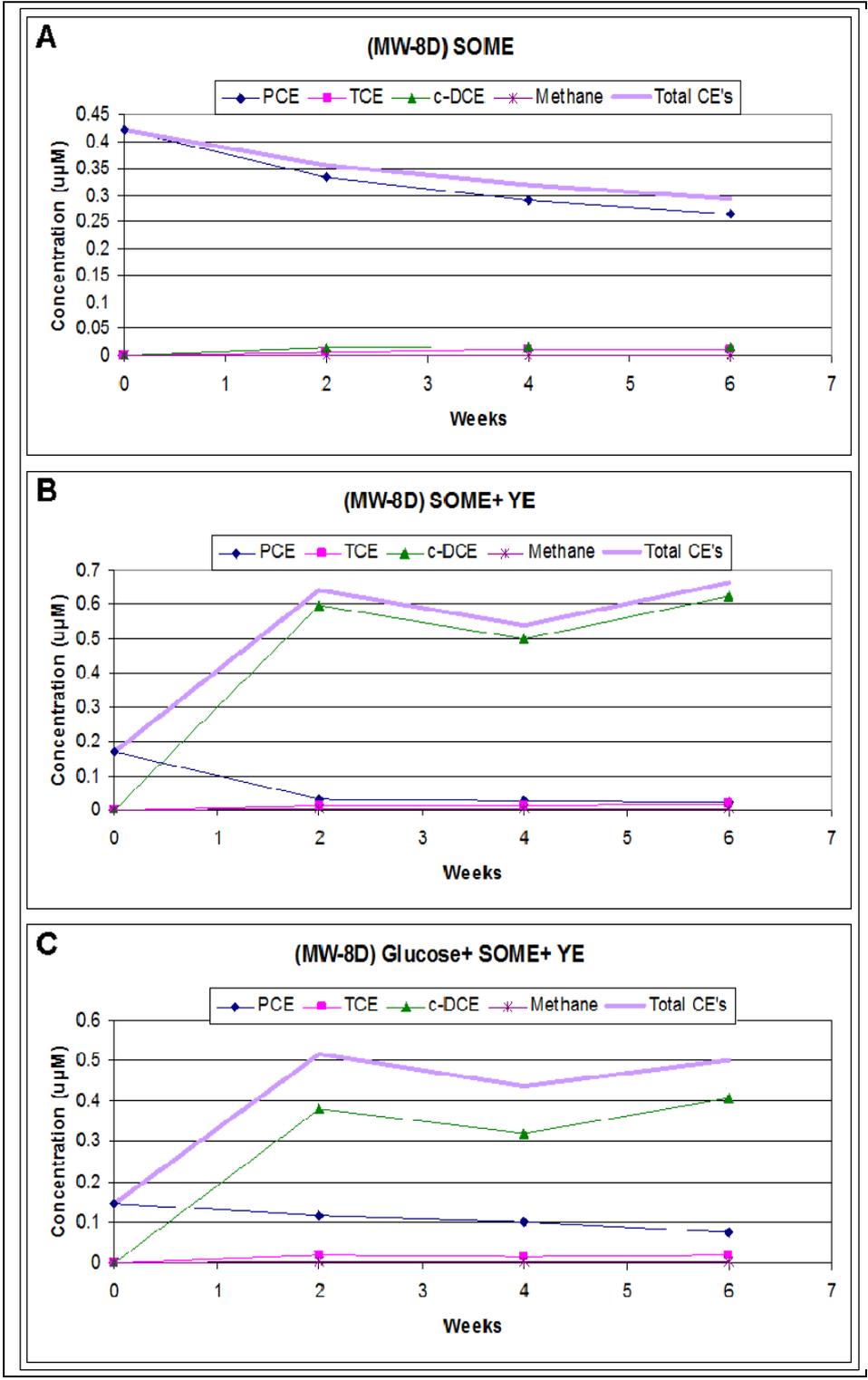


Figure 3.4(A-C). Chlorinated ethenes and methane concentrations in water microcosm (Biostimulation Experiment). Prepared with water from MW-8D, and treated with **A.** SOME, **B.** SOME + YE and **C.** Glucose+ SOME+ YE.

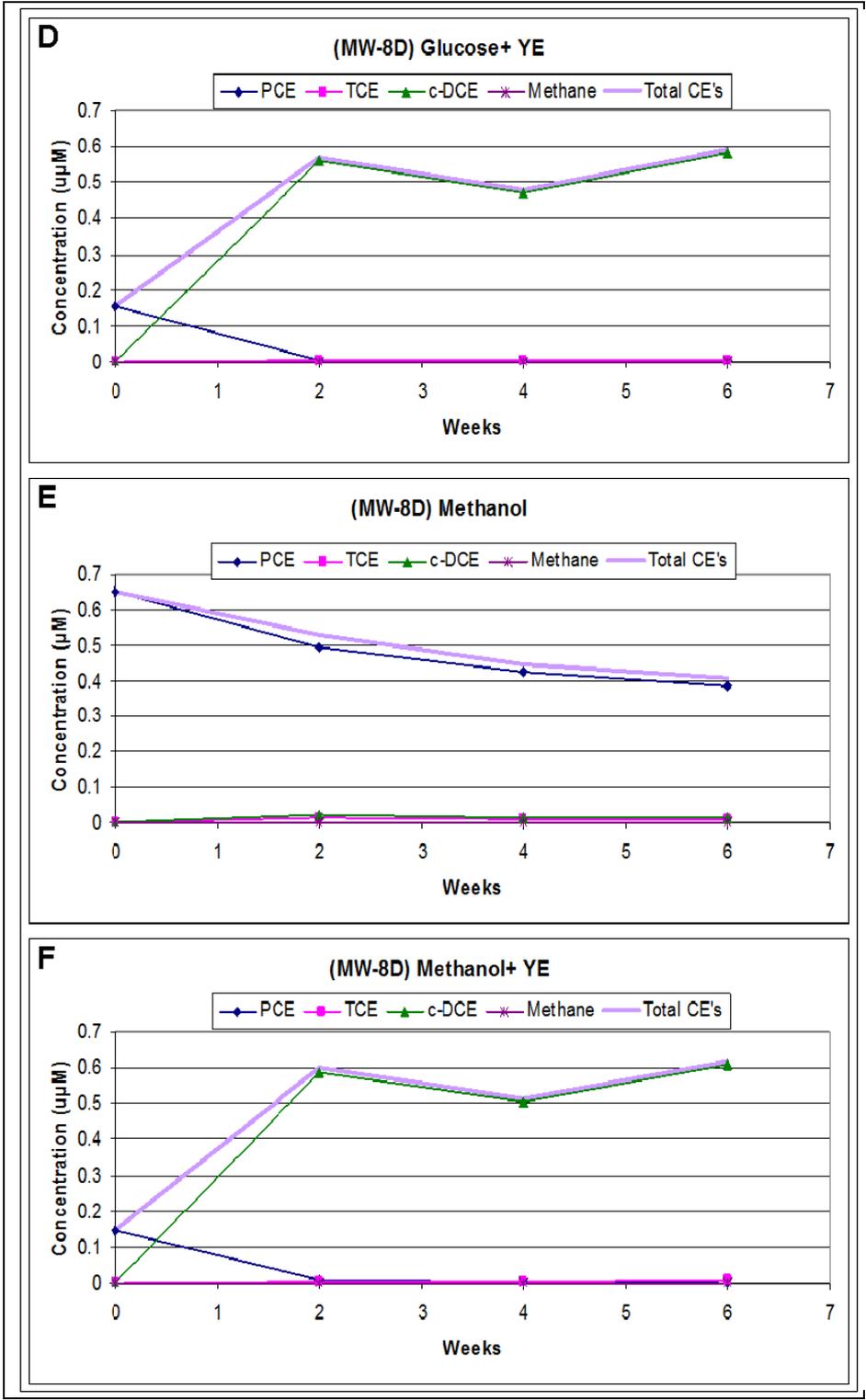


Figure 3.4 (D-F). Chlorinated ethenes and methane concentrations in water microcosm (Biostimulation Experiment). Prepared with water from MW-8D, and treated with **D.** Glucose+ YE, **E.** Methanol and **F.** Methanol+ YE.

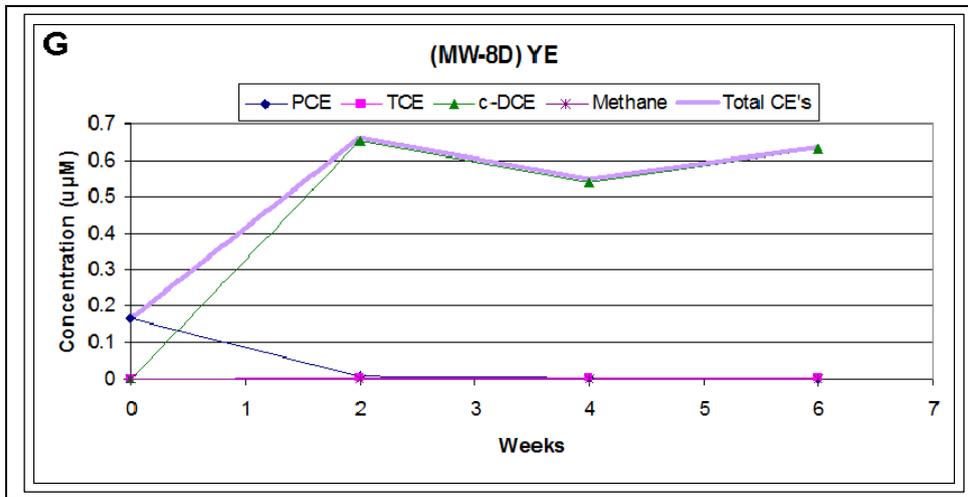


Figure 3.4 (G). Chlorinated ethenes and methane concentrations in water microcosm (Biostimulation Experiment). Prepared with water from MW-8D, and treated with **G.** YE.

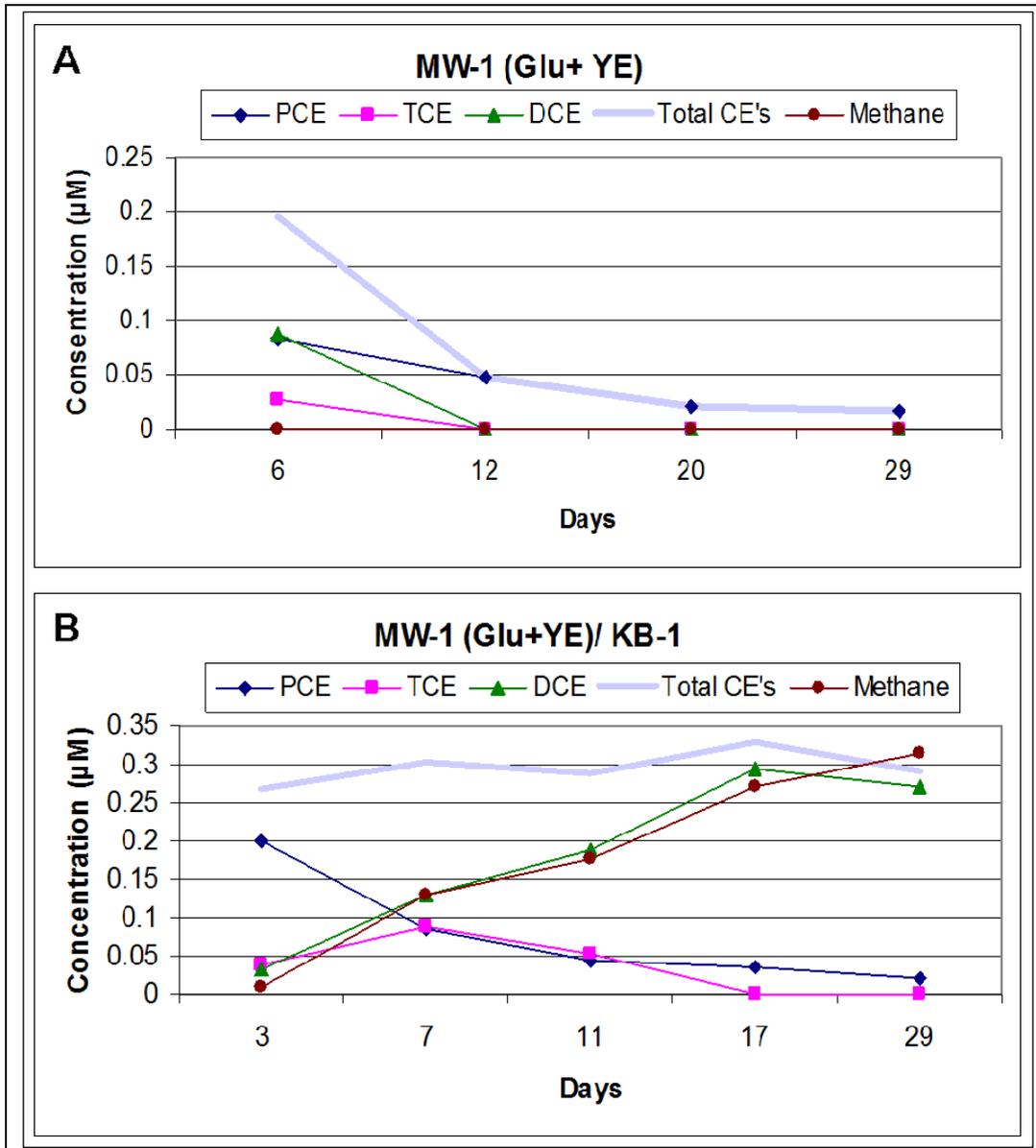


Figure 3.5. Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-1D, and treated with **A.** Glucose + YE and **B.** Glucose+ YE and KB-1 culture.

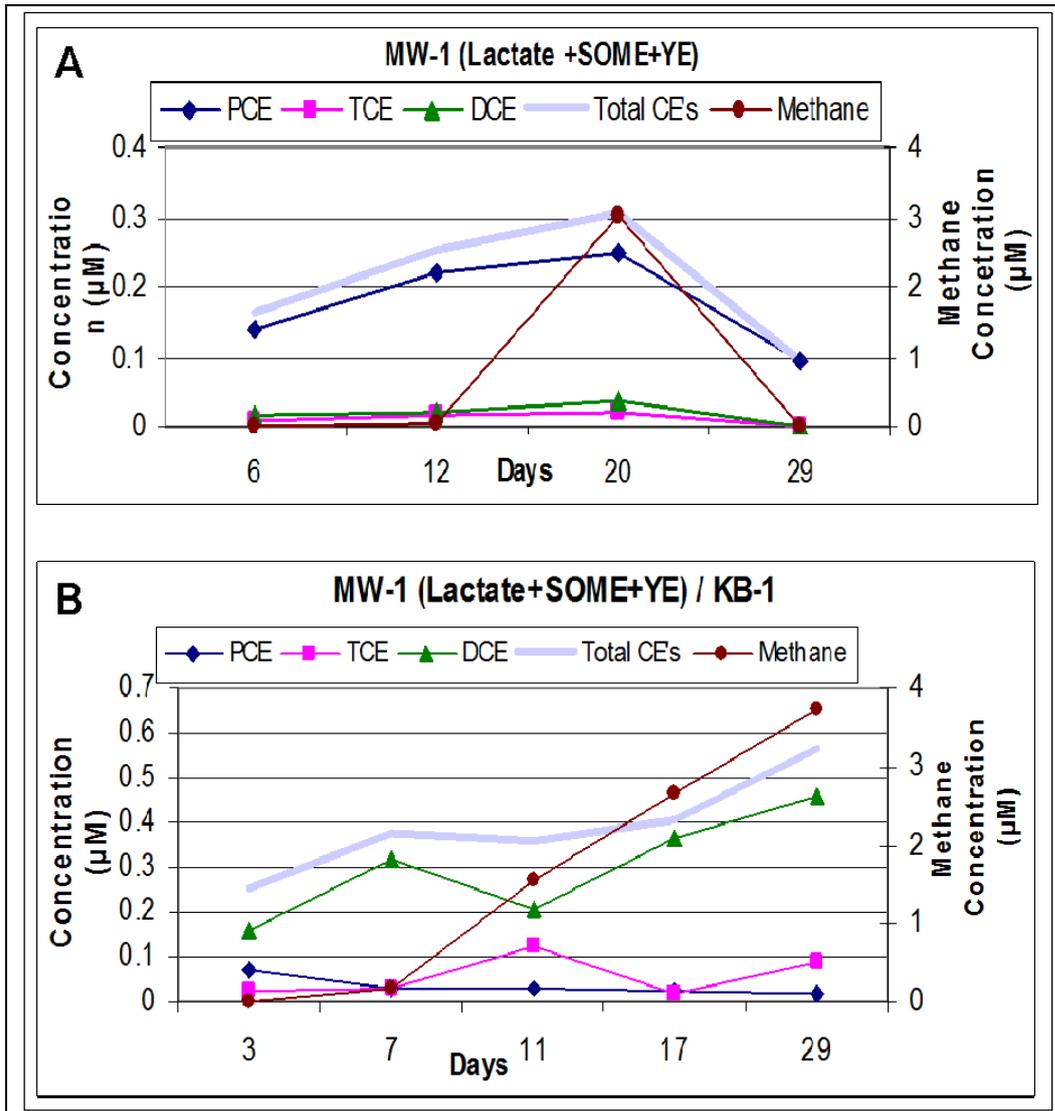


Figure 3.6. Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-1D, and treated with **A.** Lactate+ SOME+ YE and **B.** Lactate+ SOME+ YE and KB-1 culture.

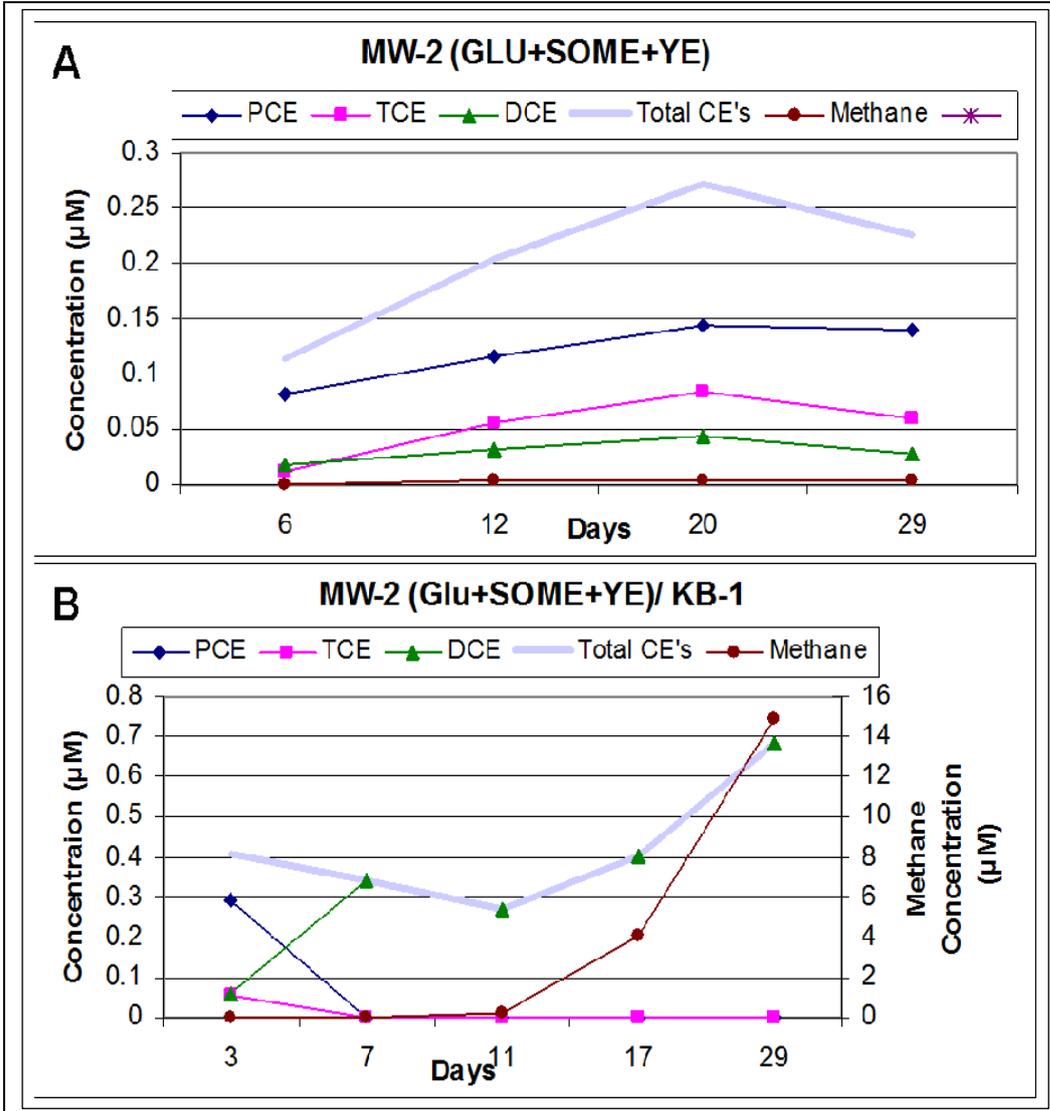


Figure 3.7. Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-2D, and treated with **A.** Glucose+ SOME+ YE and **B.** Glucose+ SOME+ YE and KB-1 culture.

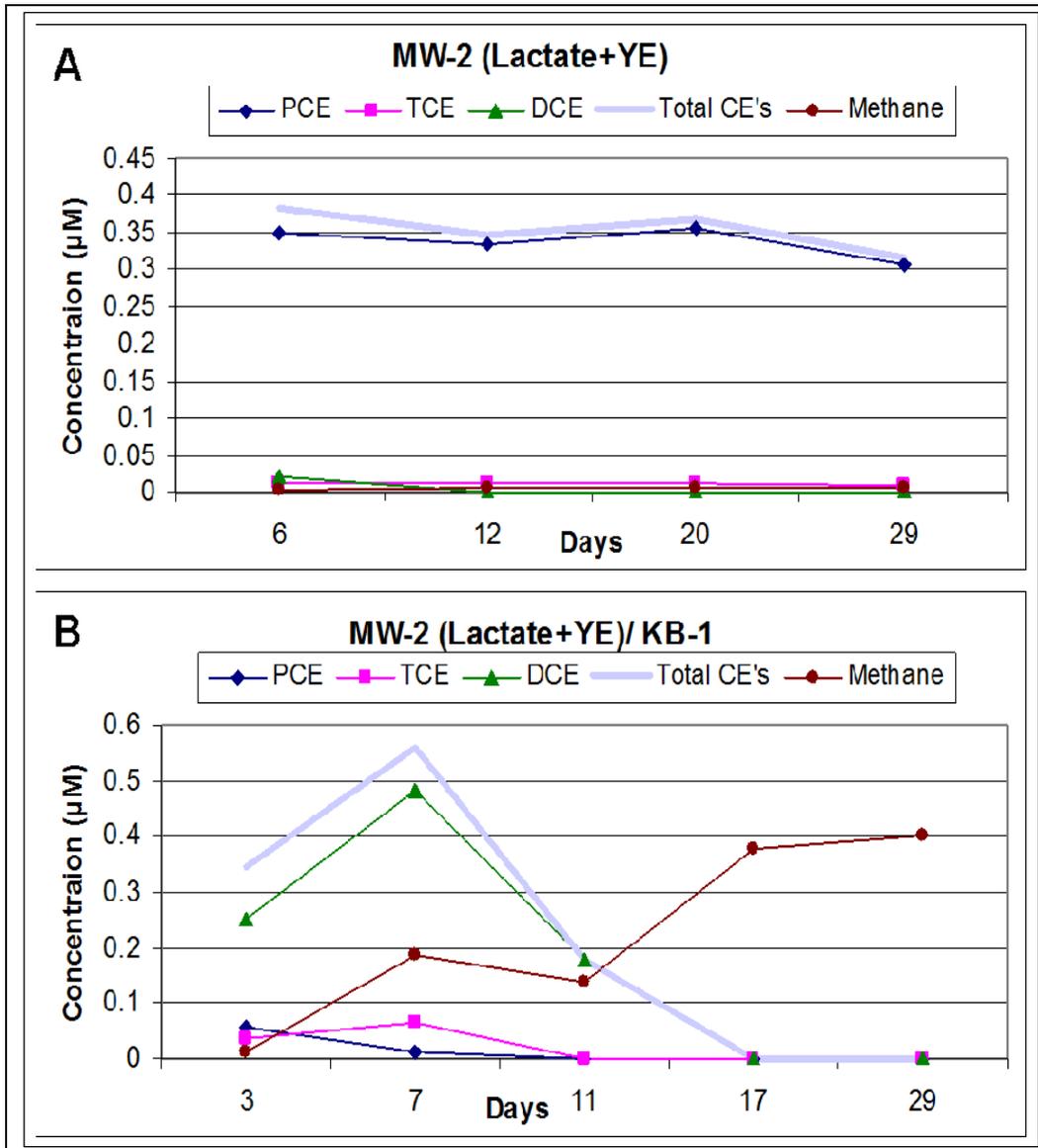


Figure 3.8. Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-2D, and treated with **A.** Lactate+ YE and **B.** Lactate+ YE and KB-1 culture.

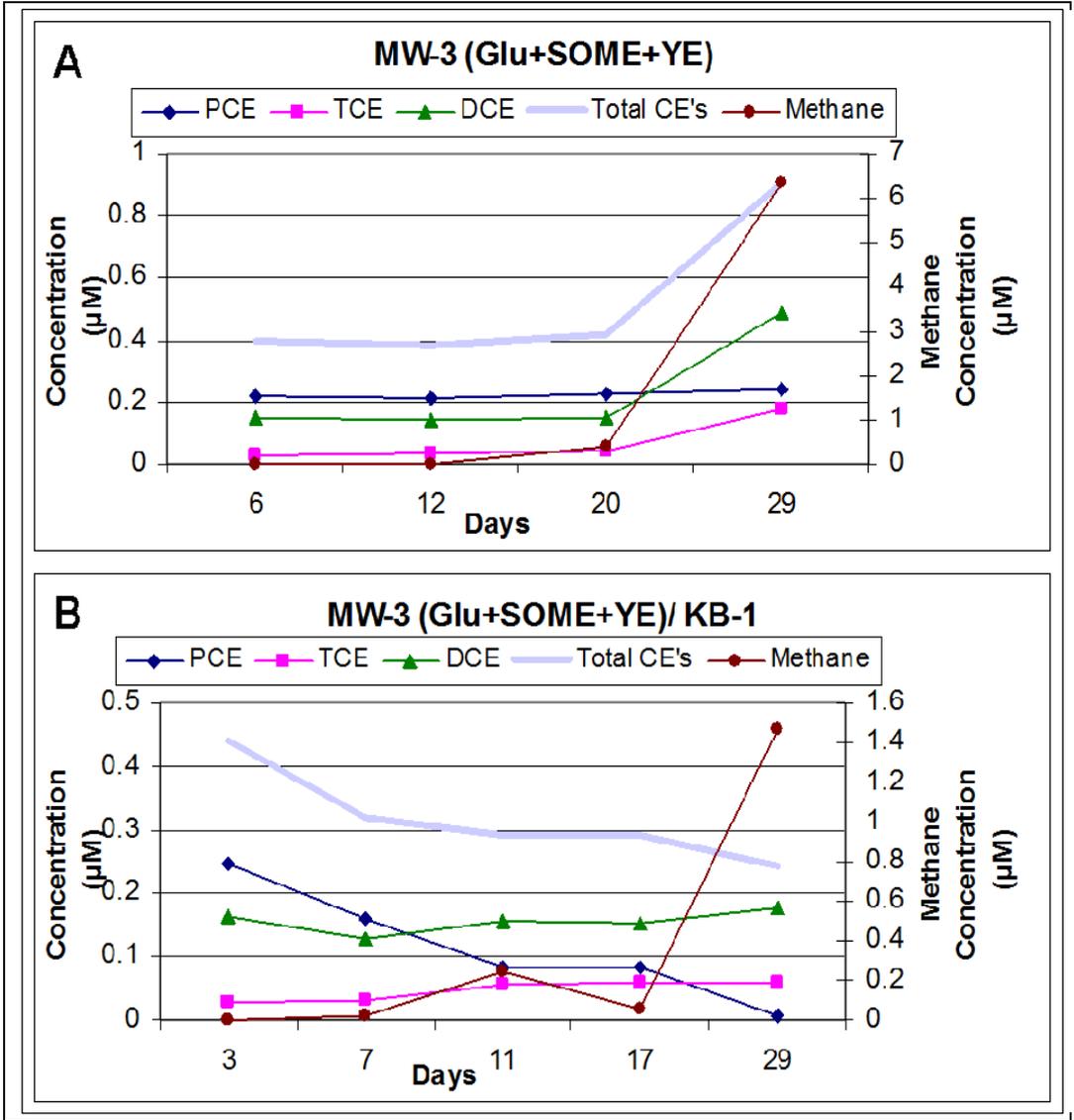


Figure 3.9. Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-3D, and treated with **A.** Glucose+ SOME+ YE and **B.** Glucose+ SOME+ YE and KB-1 culture.

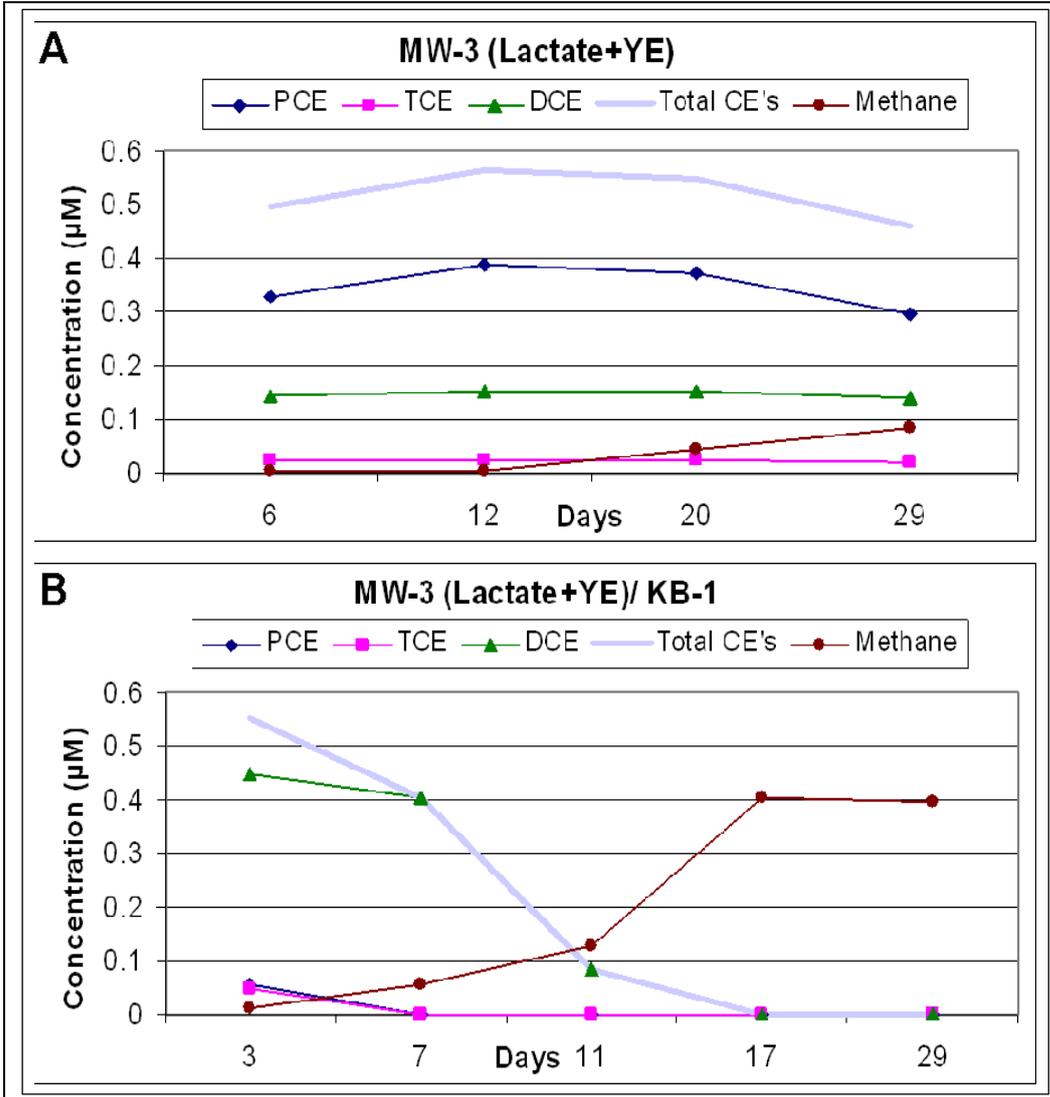


Figure 3.10. Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-3D, and treated with **A.** Lactate+ YE and **B.** Lactate+ YE and KB-1 culture.

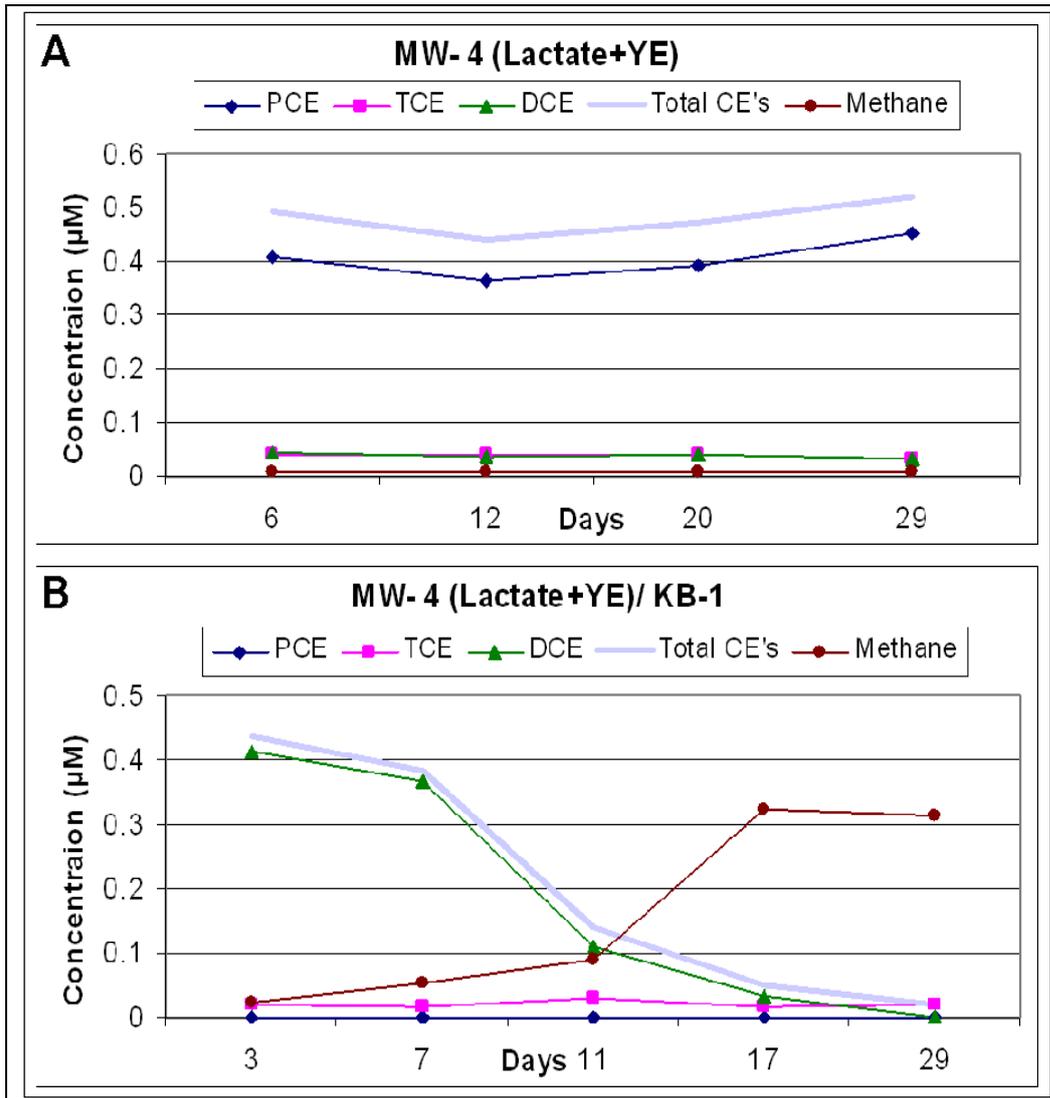


Figure 3.11. Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-4D, and treated with **A.** Lactate+ YE and **B.** Lactate+ YE and KB-1 culture.

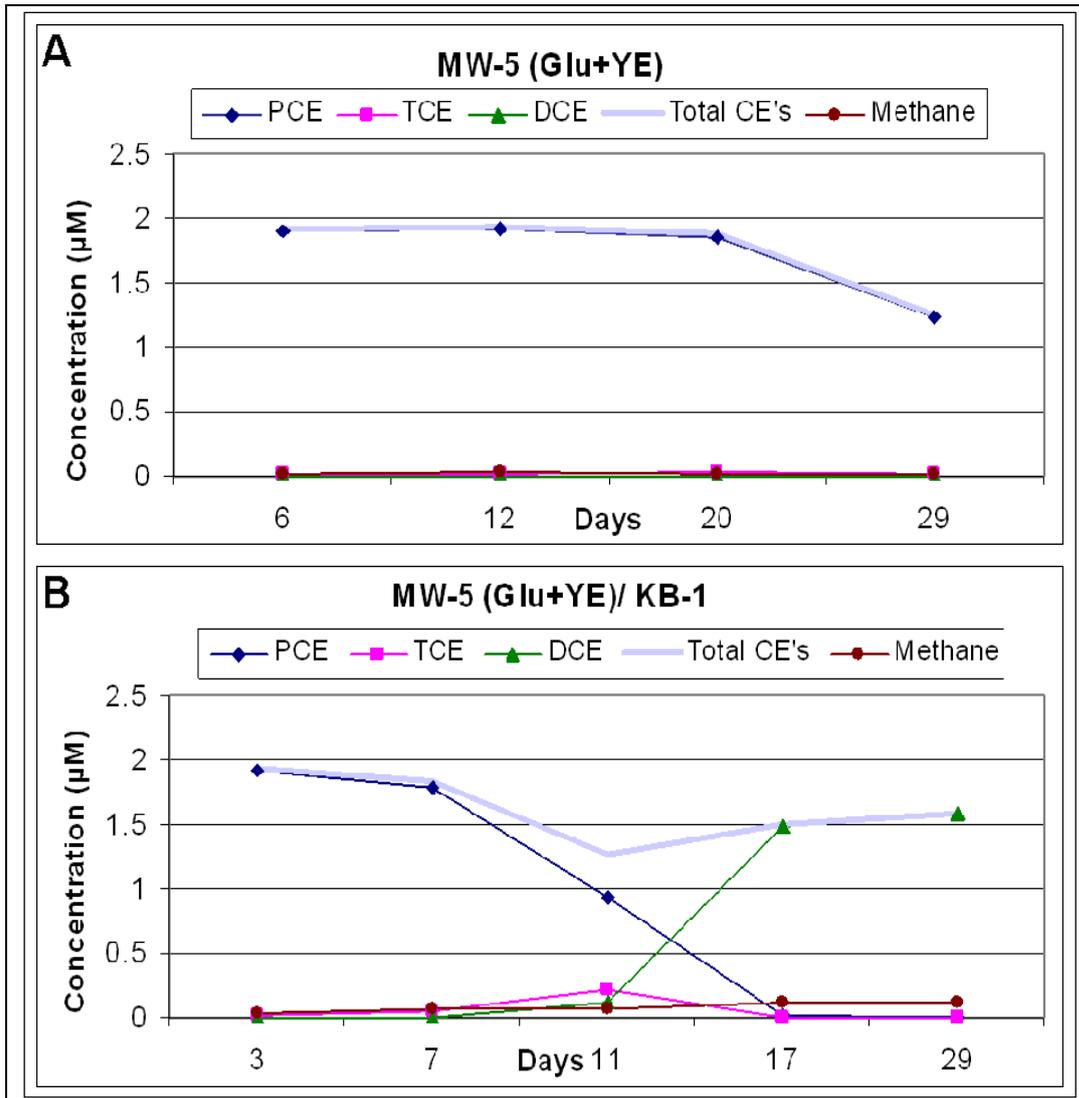


Figure 3.12. Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-5D, and treated with **A.** Glucose + YE and **B.** Glucose+ YE and KB-1 culture.

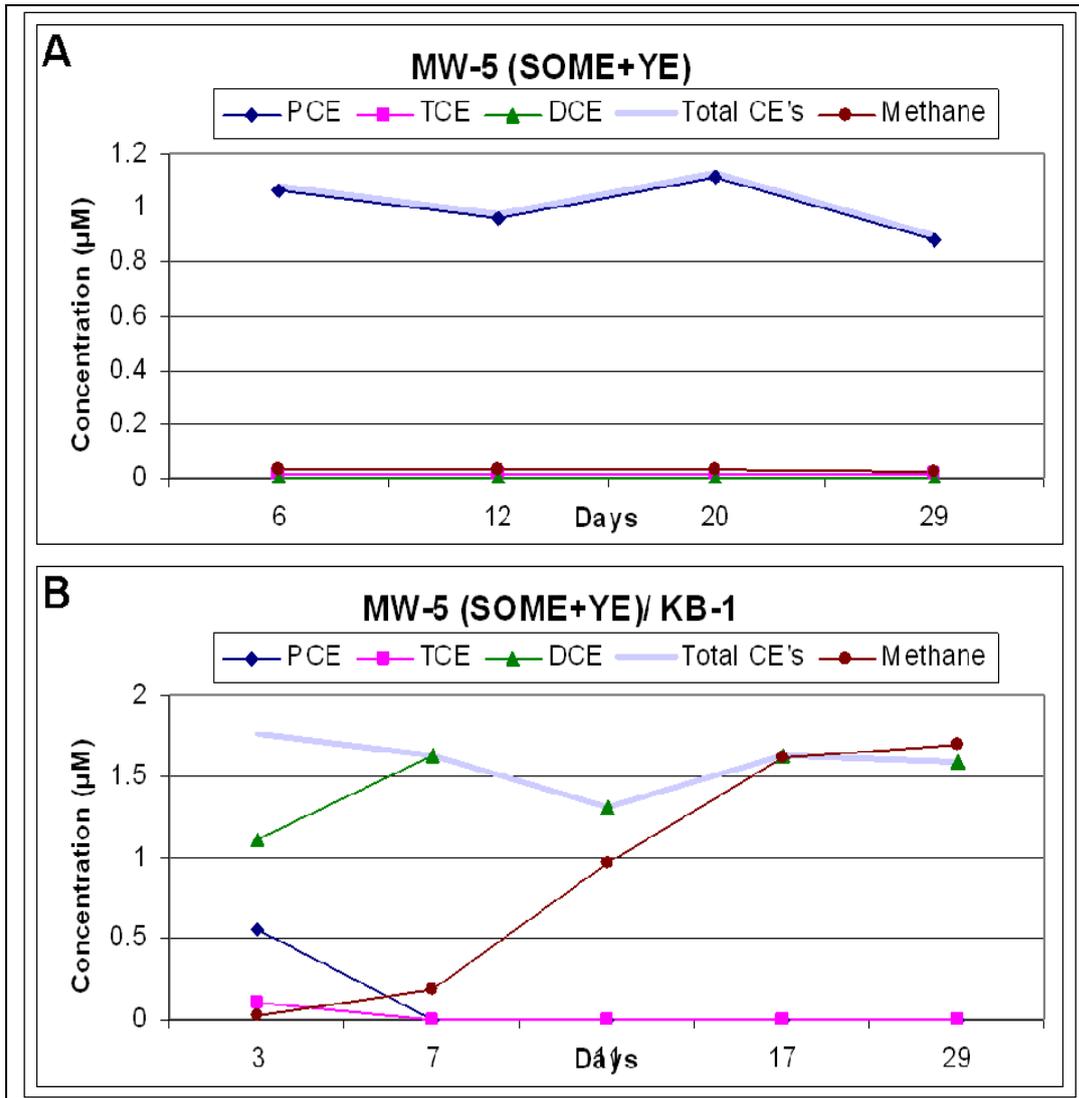


Figure 3.13. Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-5D, and treated with **A.** SOME+ YE and **B.** SOME+ YE and KB-1 culture.

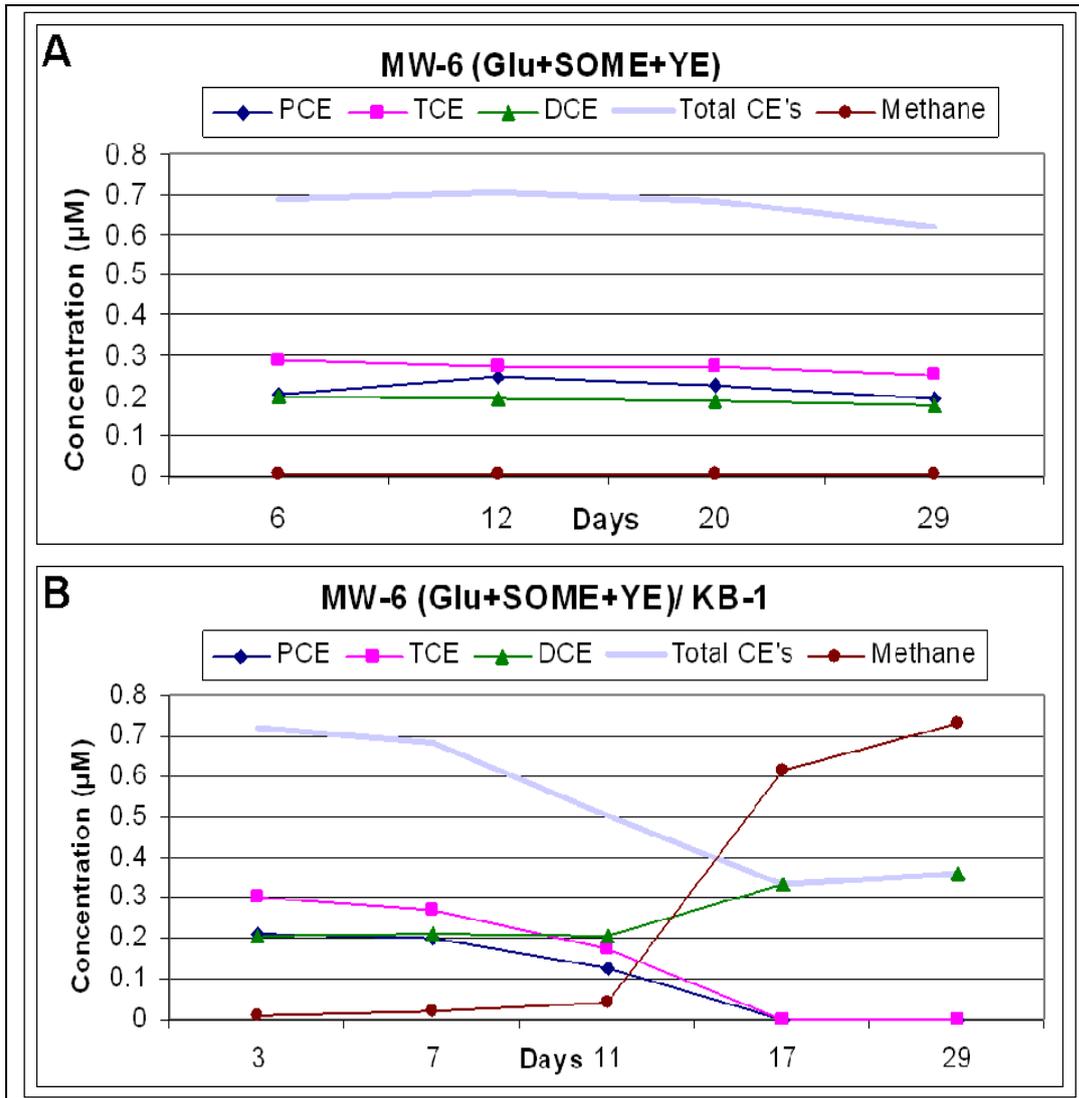


Figure 3.14. Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-6D, and treated with **A.** Glucose+ SOME+ YE and **B.** Glucose+ SOME+ YE and KB-1 culture.

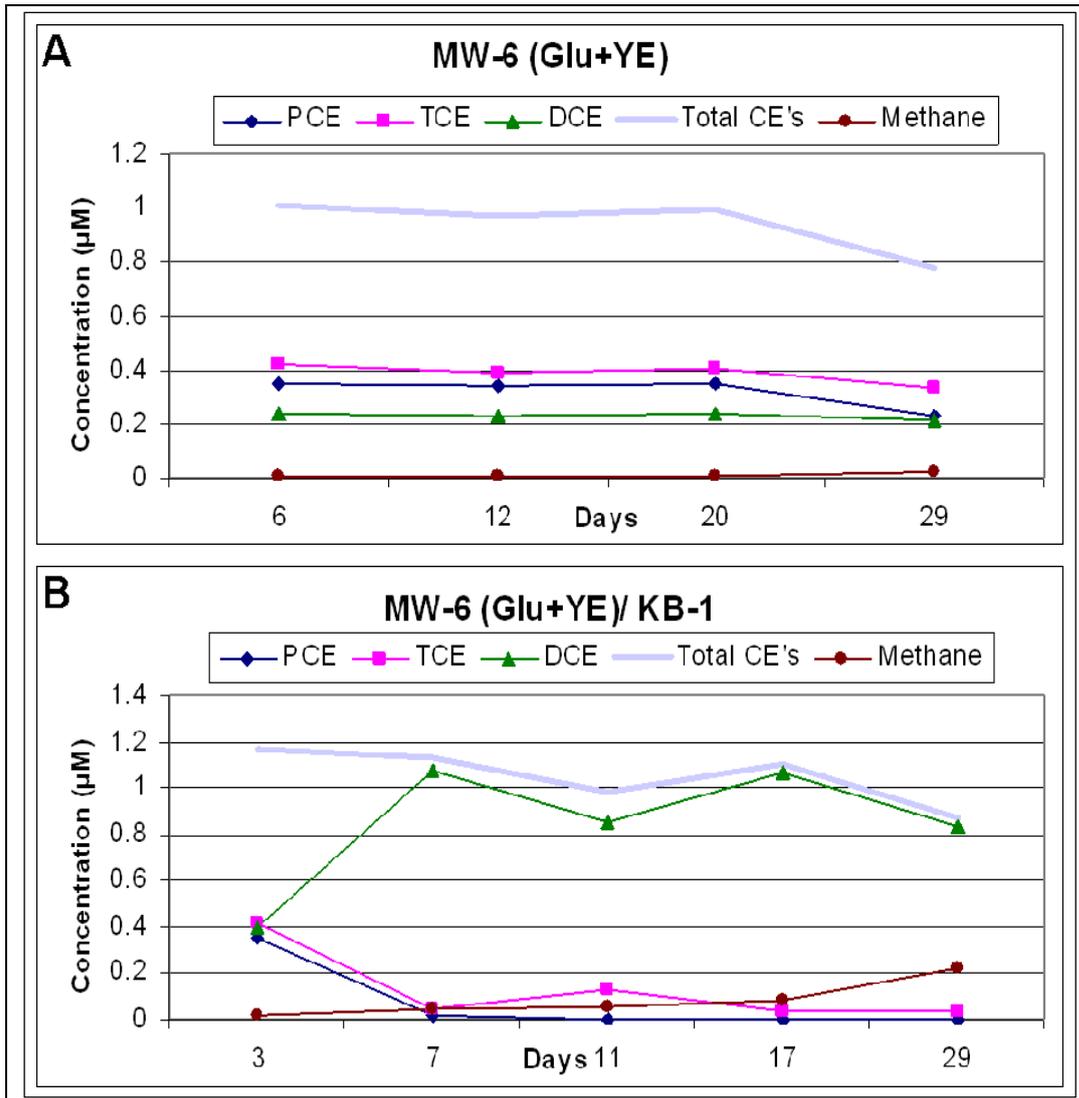


Figure 3.15. Chlorinated ethenes and methane concentrations in water microcosm (Monitoring Well Screening). Prepared with water from MW-6D, and treated with **A.** Glucose + YE and **B.** Glucose+ YE with KB-1 culture.

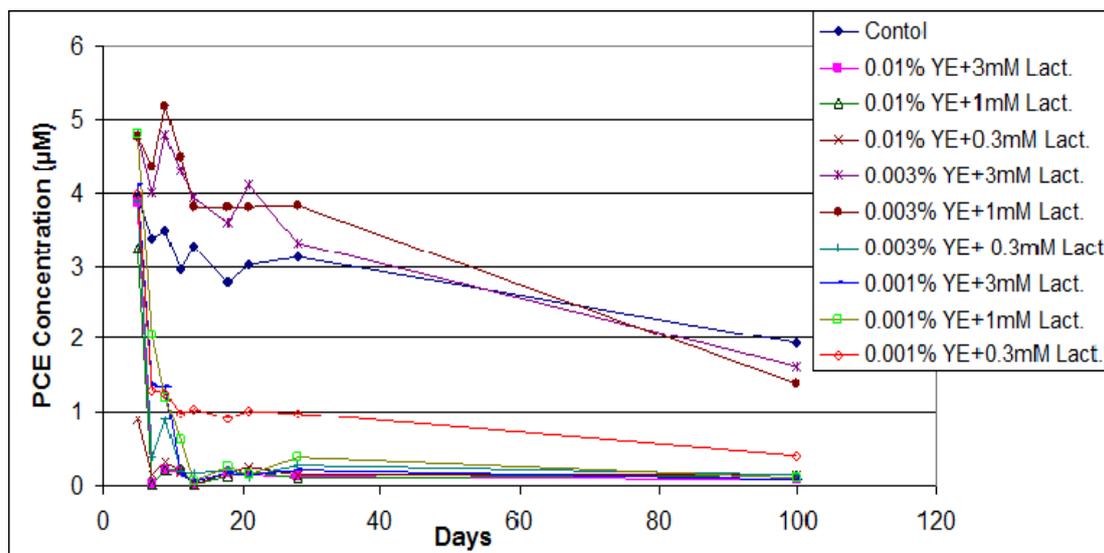


Figure 3.16. PCE degradation rates in YE+ Lactate treatments in water microcosm for optimizing concentrations.

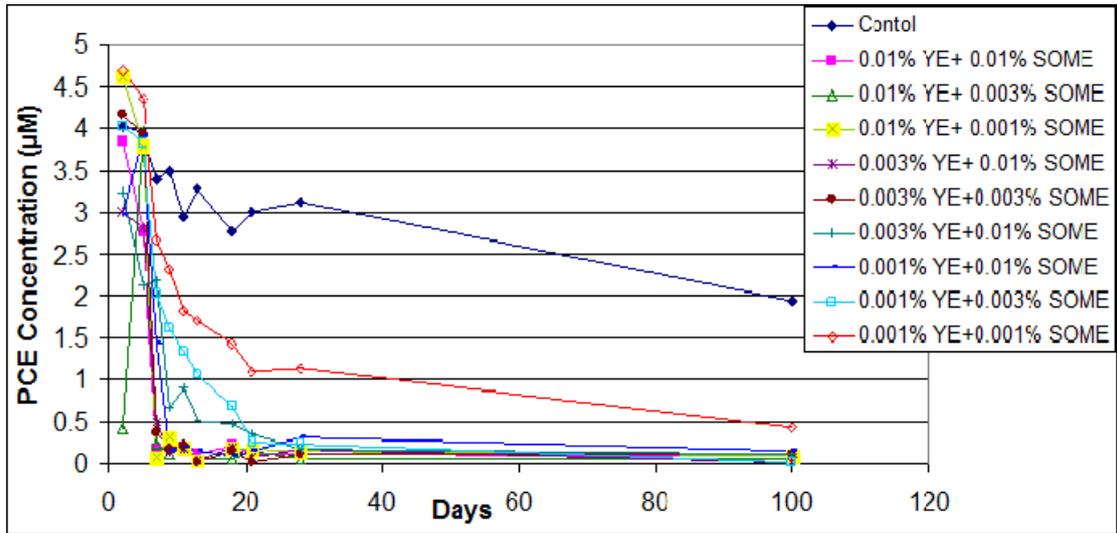


Figure.3.17. PCE degradation rates in YE+ SOME treatments in water microcosm for optimizing concentrations.

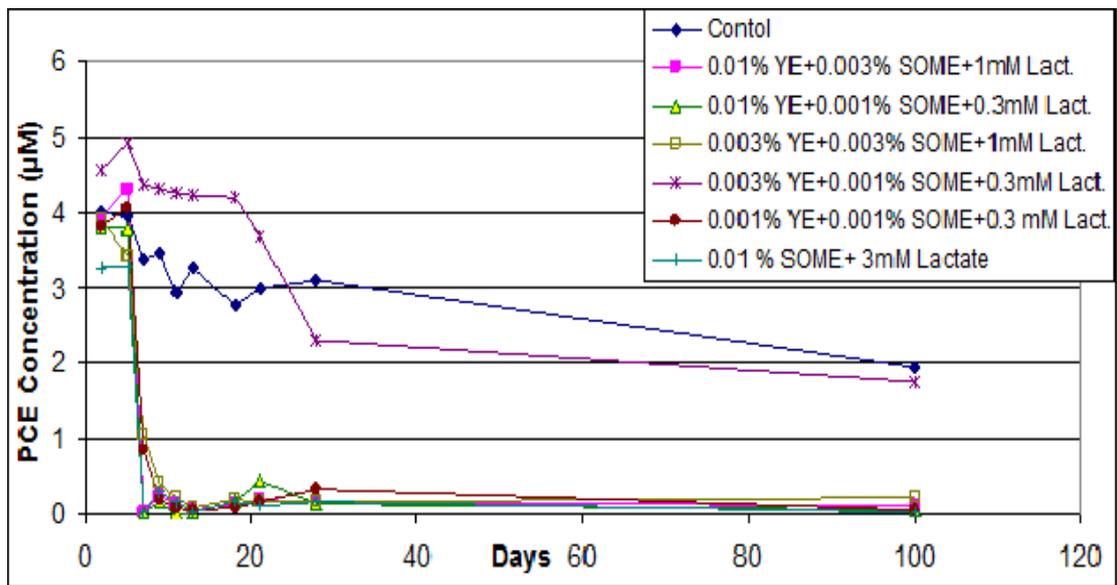


Figure 3.18. PCE degradation rates in YE+ SOME+ Lactate treatments in water microcosm for optimizing concentrations.

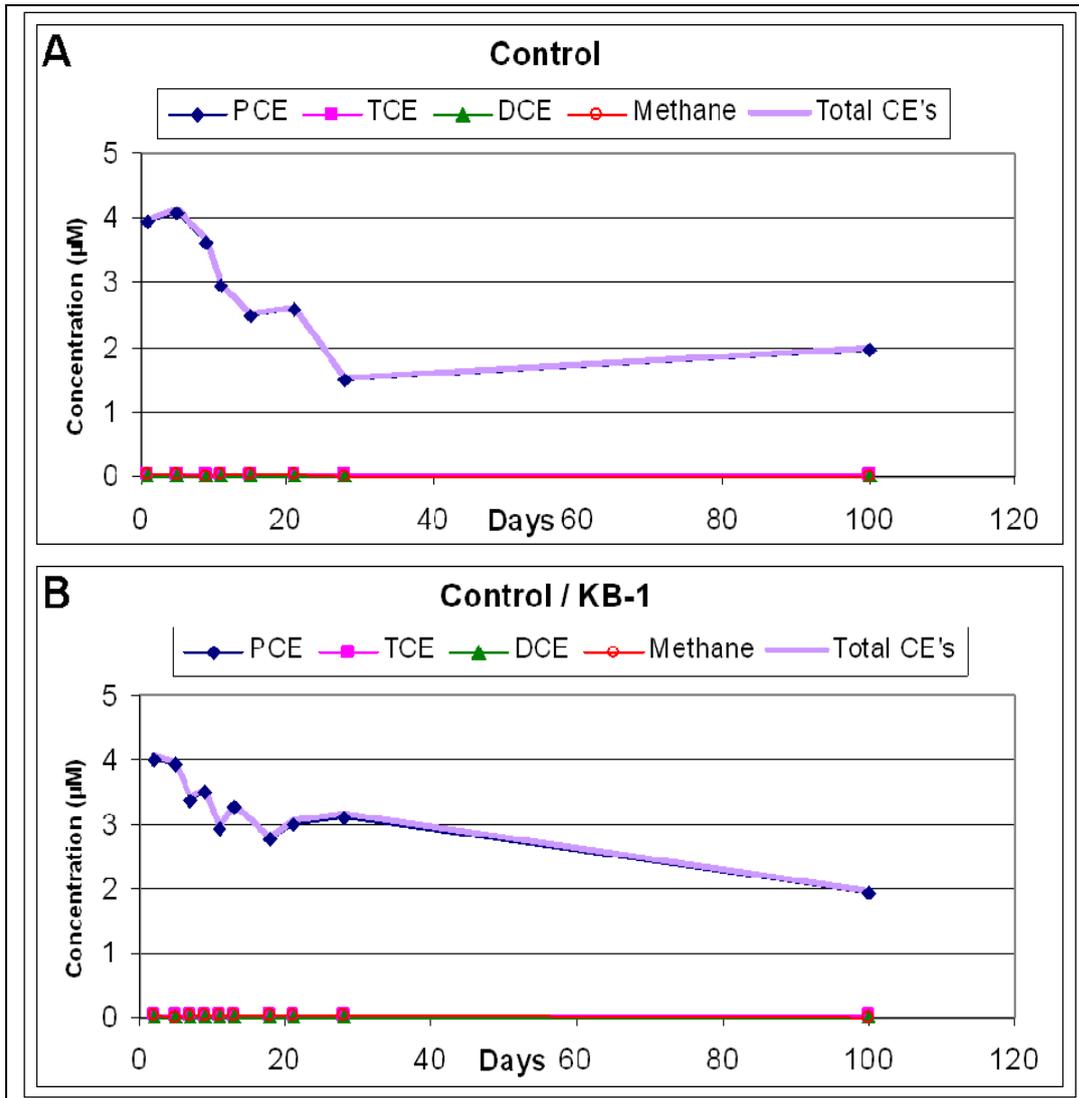


Figure 3.19. CEs and methane concentrations in water microcosms (optimizing substrate concentrations). **A.** Control and **B.** Control with KB-1 bacterial Culture.

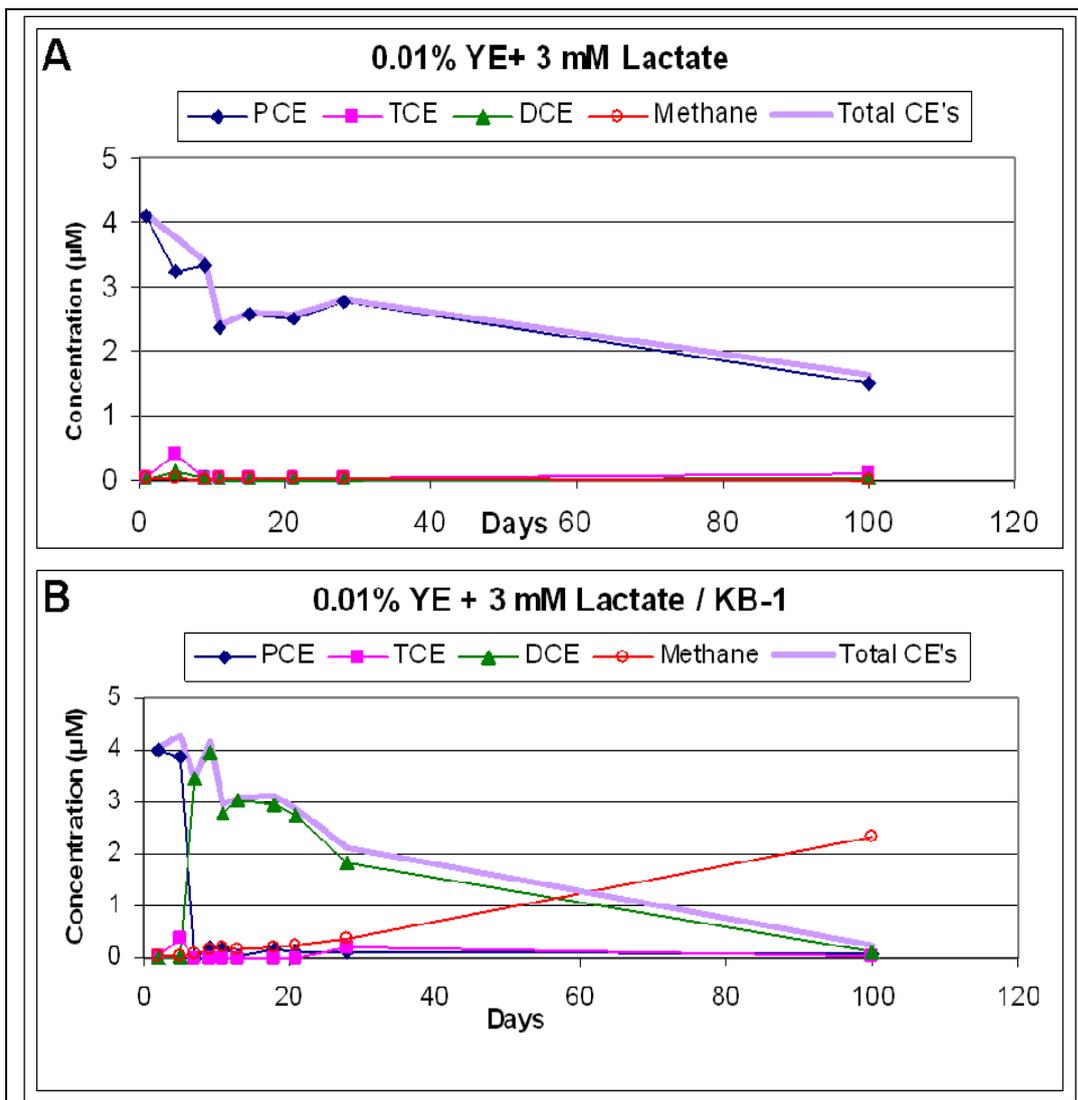


Figure 3.20. CEs and methane concentrations in water microcosms (optimizing substrate concentrations). **A.** 0.01% YE+ 3mM Lactate, and **B.** 0.01% YE+ 3mM Lactate with KB-1 bacterial Culture.

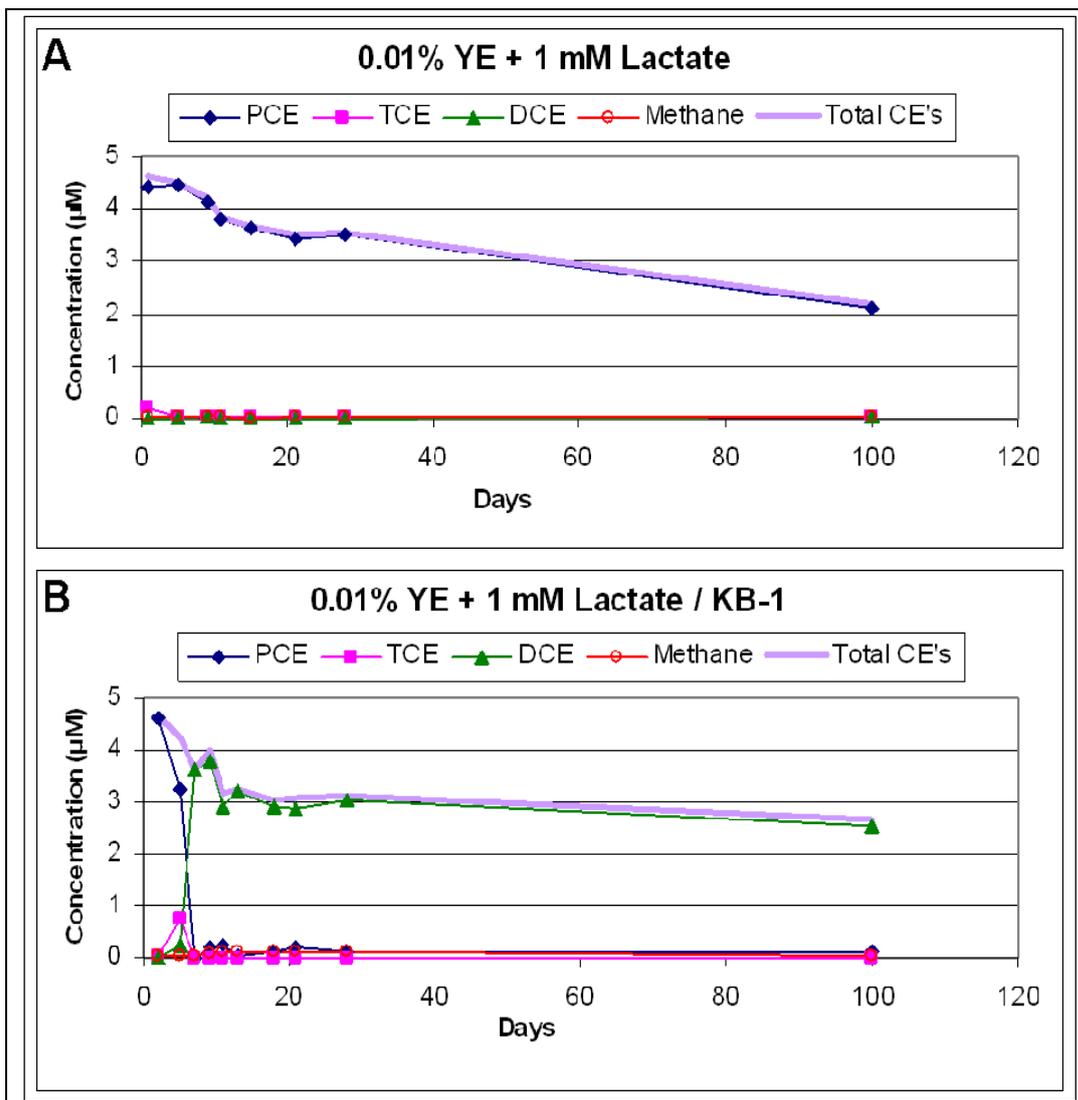


Figure 3.21. CEs and methane concentrations in water microcosms (optimizing substrate concentrations). **A.** 0.01% YE+ 1mM Lactate, and **B.** 0.01% YE+ 1mM Lactate with KB-1 bacterial Culture.

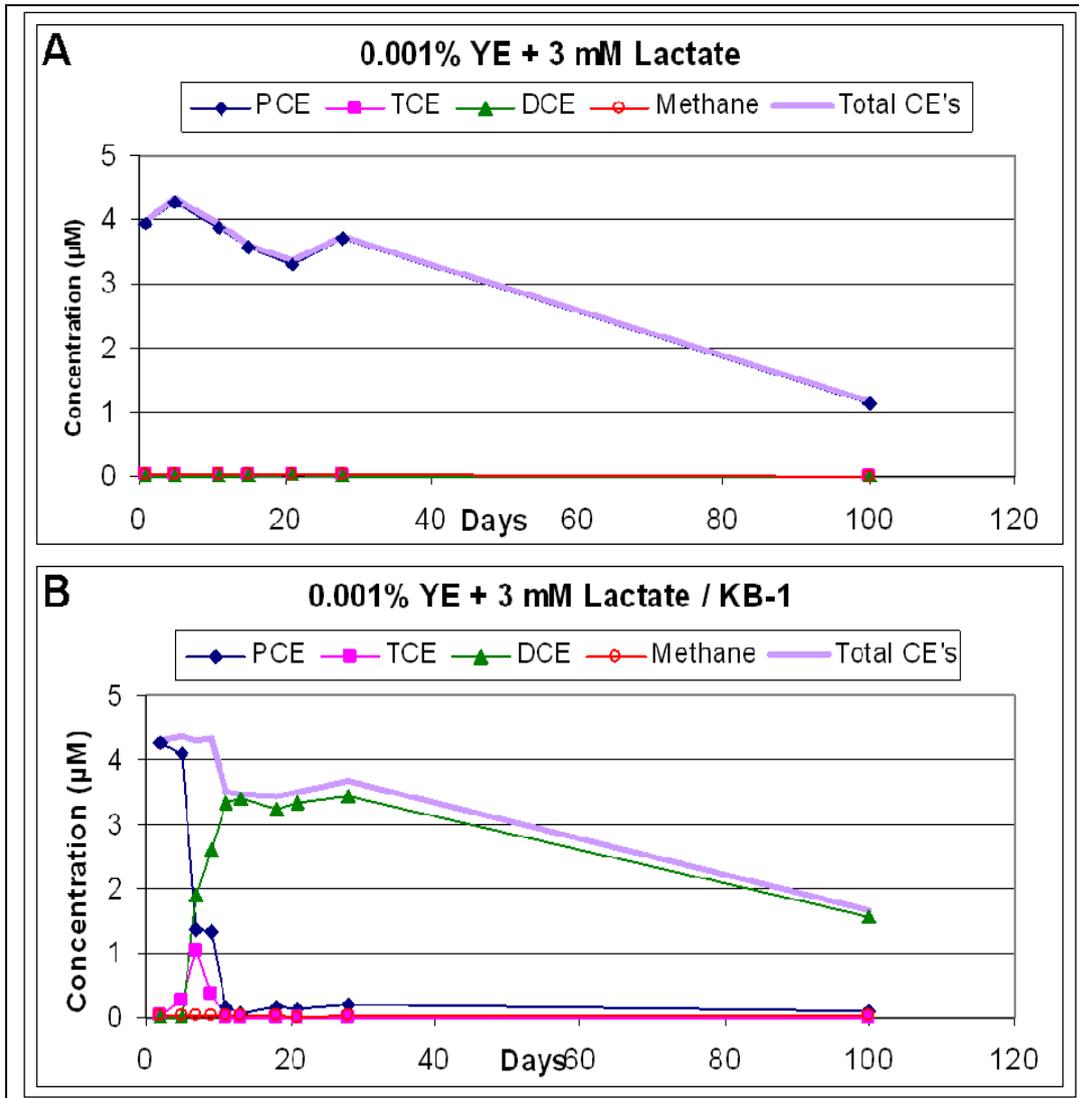


Figure 3.22. CEs and methane concentrations in water microcosms (optimizing substrate concentrations). **A.** 0.001% YE+ 3mM Lactate, and **B.** 0.001% YE+ 3mM Lactate with KB-1 bacterial Culture.

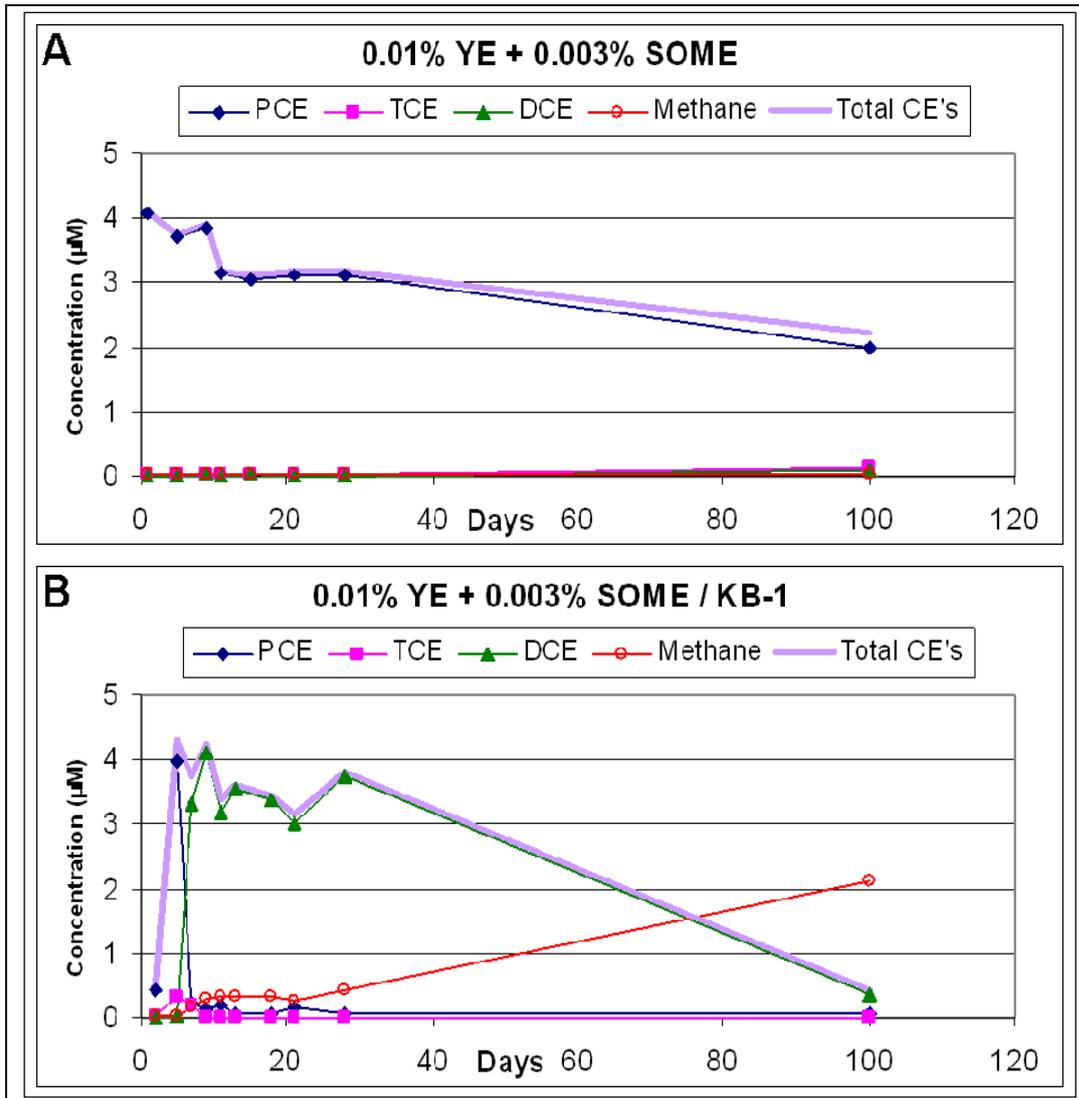


Figure 3.23. CEs and methane concentrations in water microcosms (optimizing substrate concentrations). **A.** 0.01% YE+ 0.003mM Lactate, and **B.** 0.01% YE+ 0.003mM Lactate with KB-1 bacterial Culture.

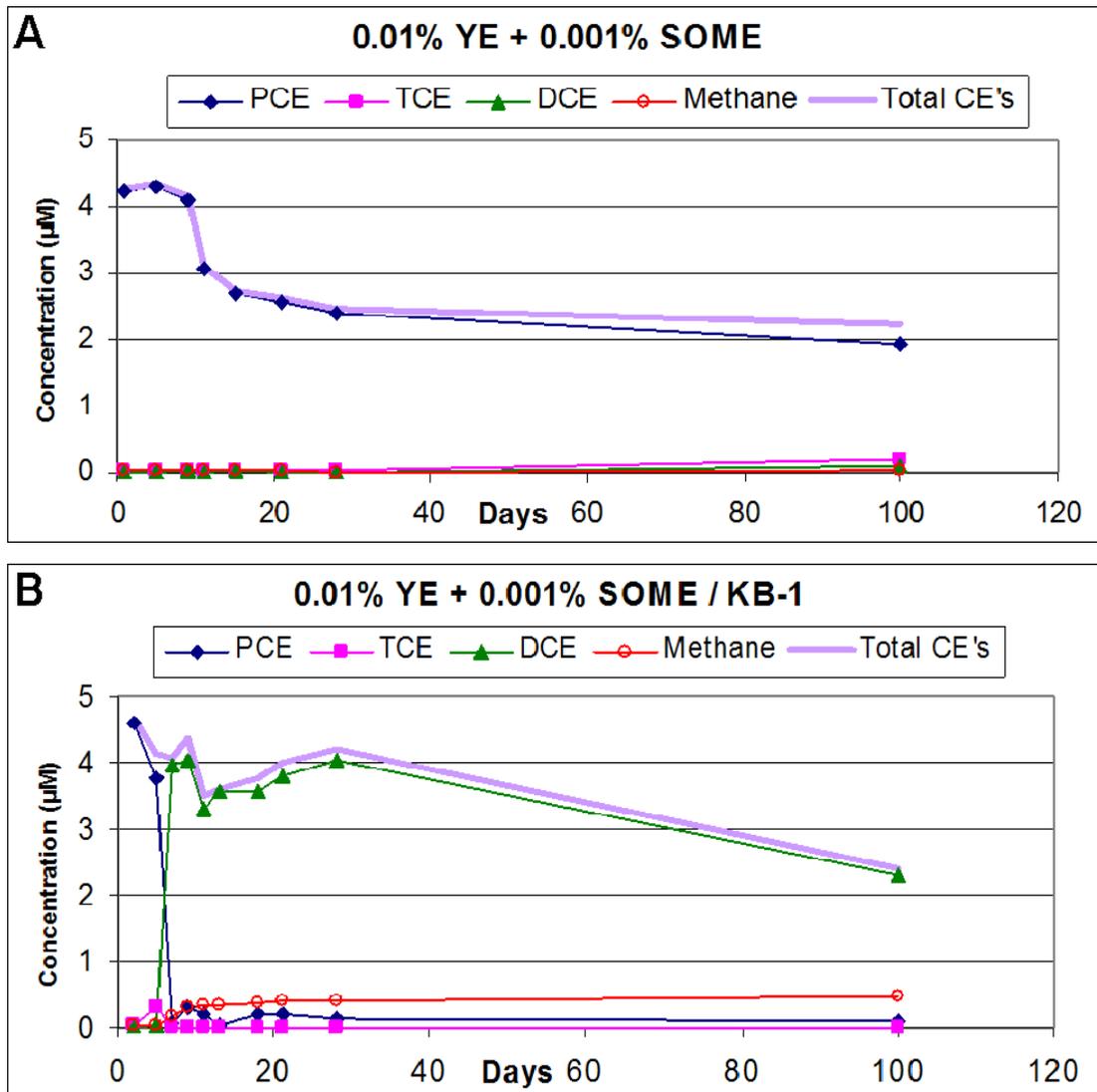


Figure 3.24. CEs and methane concentrations in water microcosms (optimizing substrate concentrations). **A.** 0.01% YE+ 0.001mM Lactate, and **B.** 0.01% YE+ 0.001mM Lactate with KB-1 bacterial Culture.

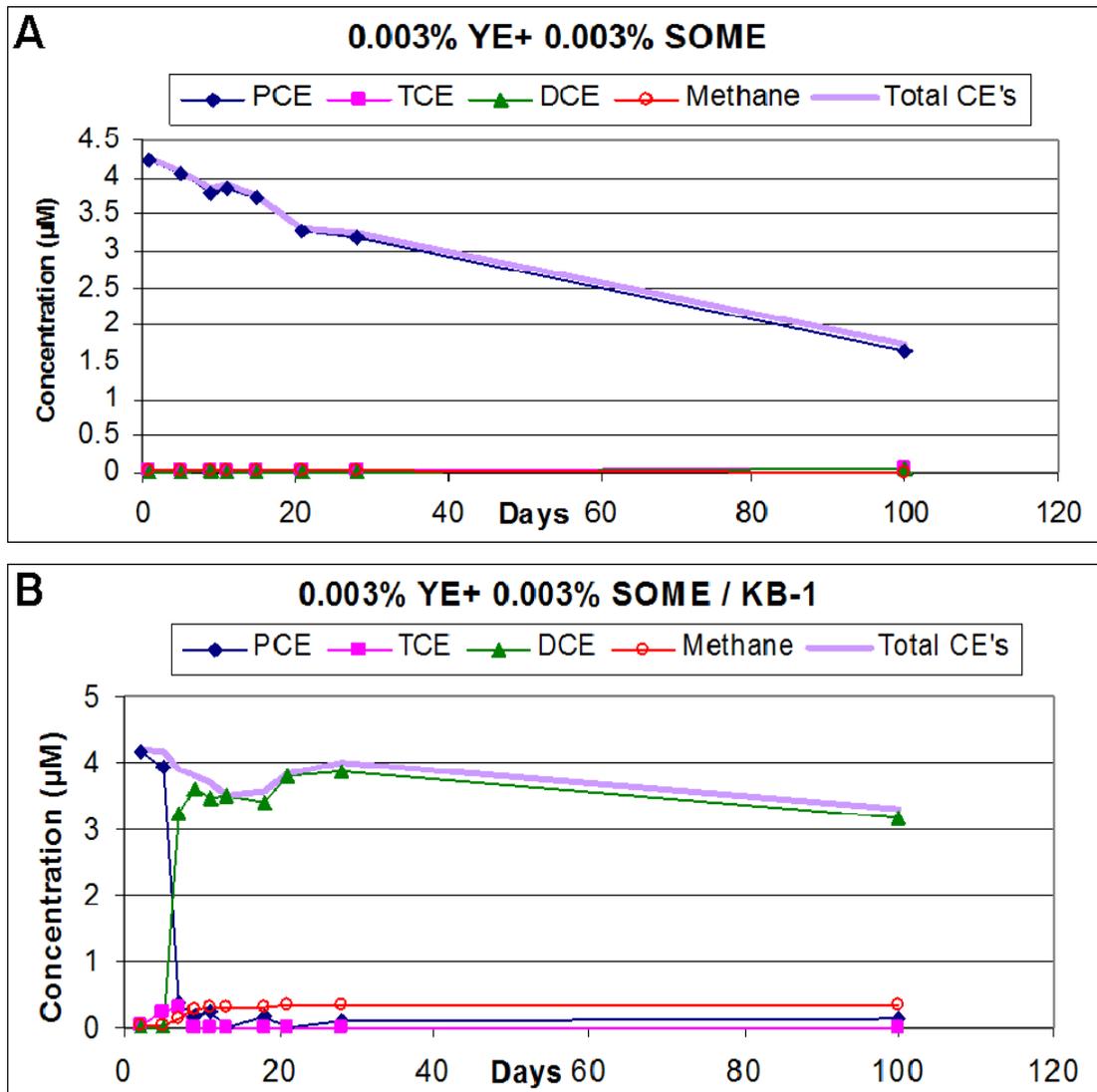


Figure 3.25. CEs and methane concentrations in water microcosms (optimizing substrate concentrations). **A.** 0.003% YE+ 0.003mM Lactate, and **B.** 0.003% YE+ 0.003mM Lactate with KB-1 bacterial Culture.

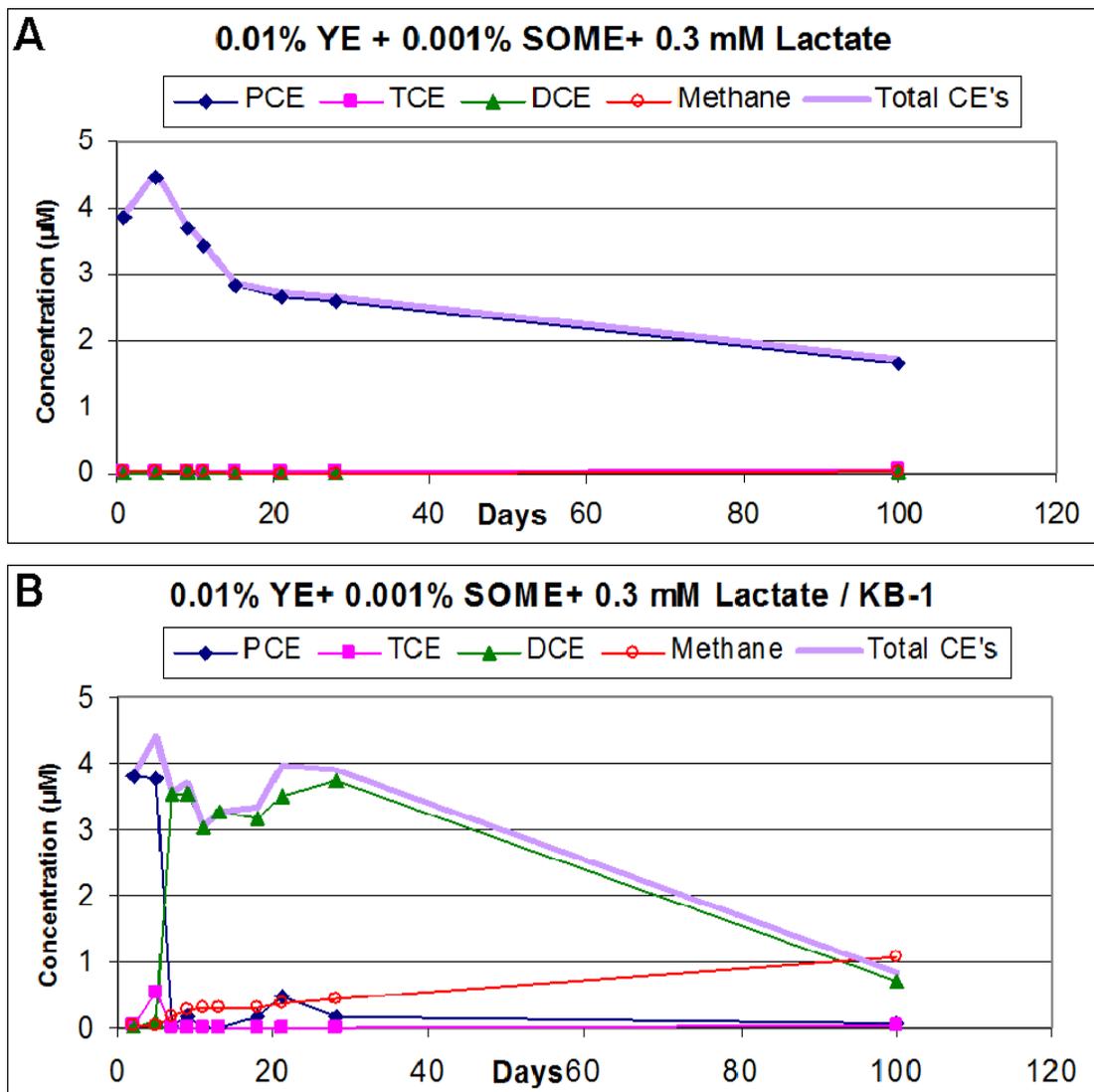


Figure 3.26. CEs and methane concentrations in water microcosms (optimizing substrate concentrations). **A.** 0.01% YE+0.001% SOME+ 0.3mM Lactate, and **B.** 0.01% YE+ 0.001% SOME+ 0.3mM Lactate with KB-1 bacterial Culture.

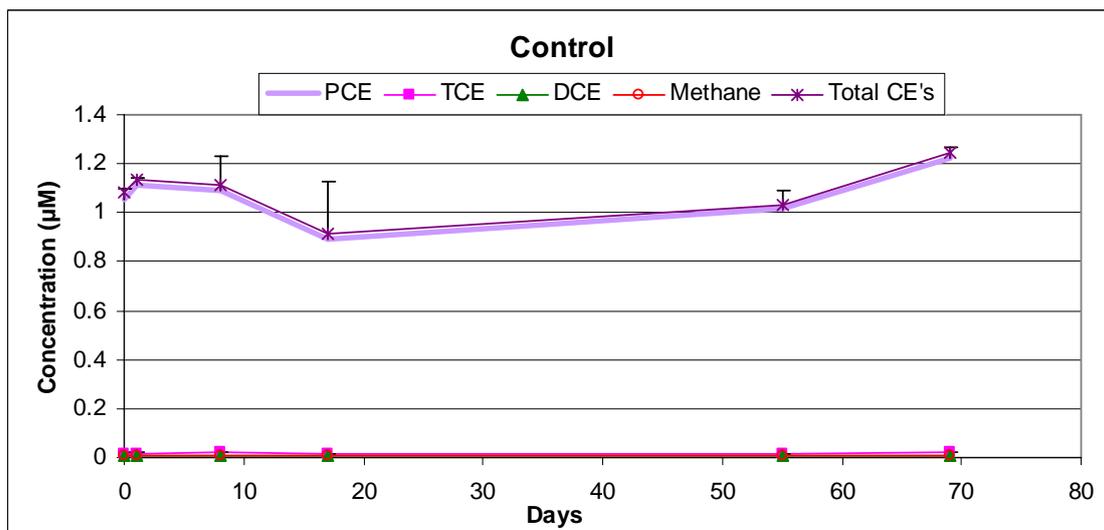


Figure 3.27. CEs and methane concentrations in water microcosms (KB-1 Viability Study) in control treatment.

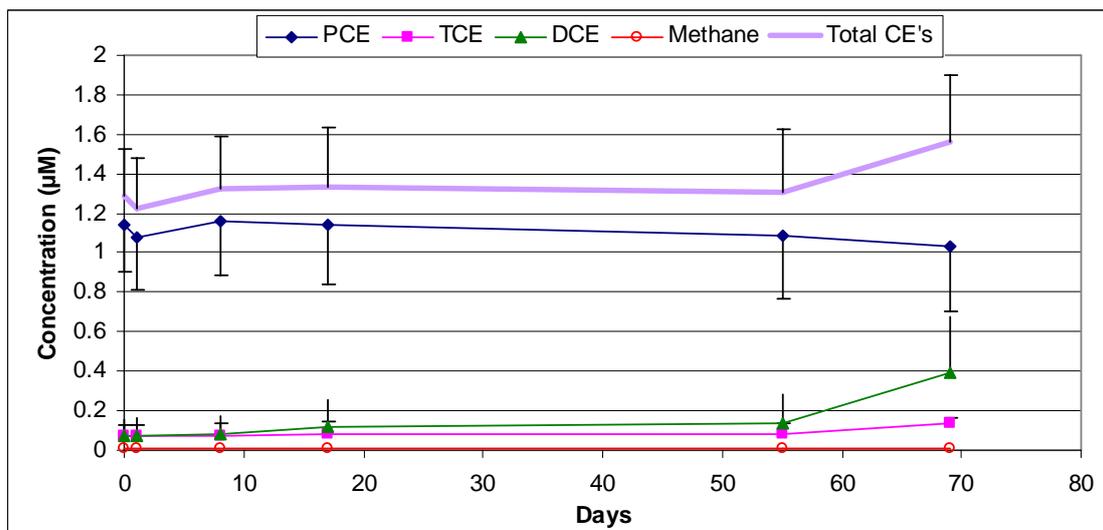


Figure 3.28. CEs and methane concentrations in water microcosms (KB-1 Viability Study) treated with YE (0.01%)+ SOME (0.01%)+ Lactate (3mM). KB-1 is not added to this treatment.

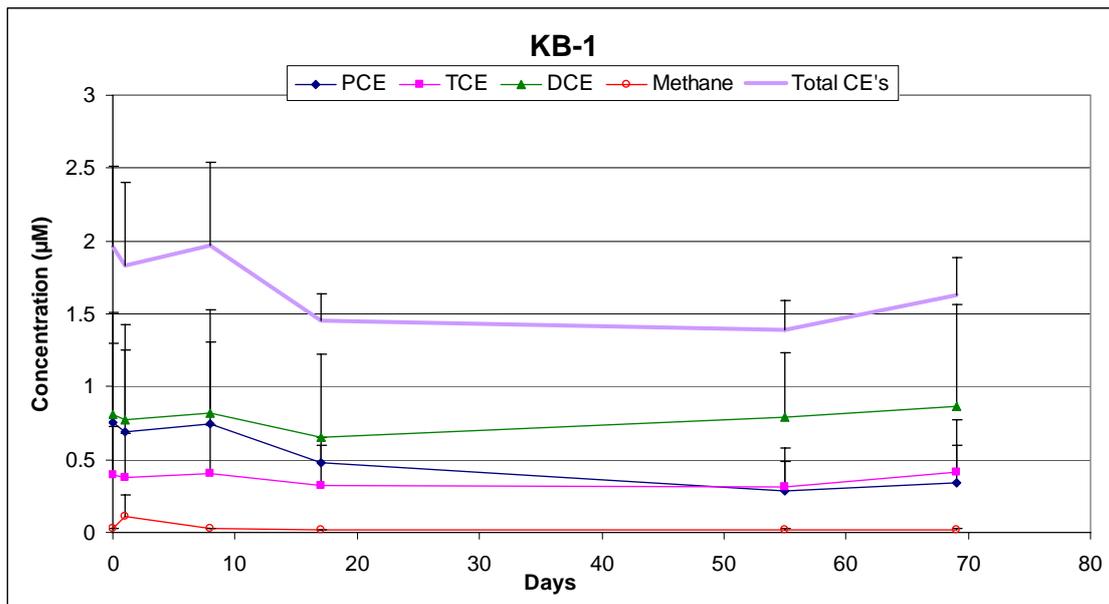


Figure 3.29. CEs and methane concentrations in water microcosms (KB-1 Viability Study) treated with YE (0.01%)+ SOME (0.01%)+ Lactate (3mM). KB-1 is present in this treatment.

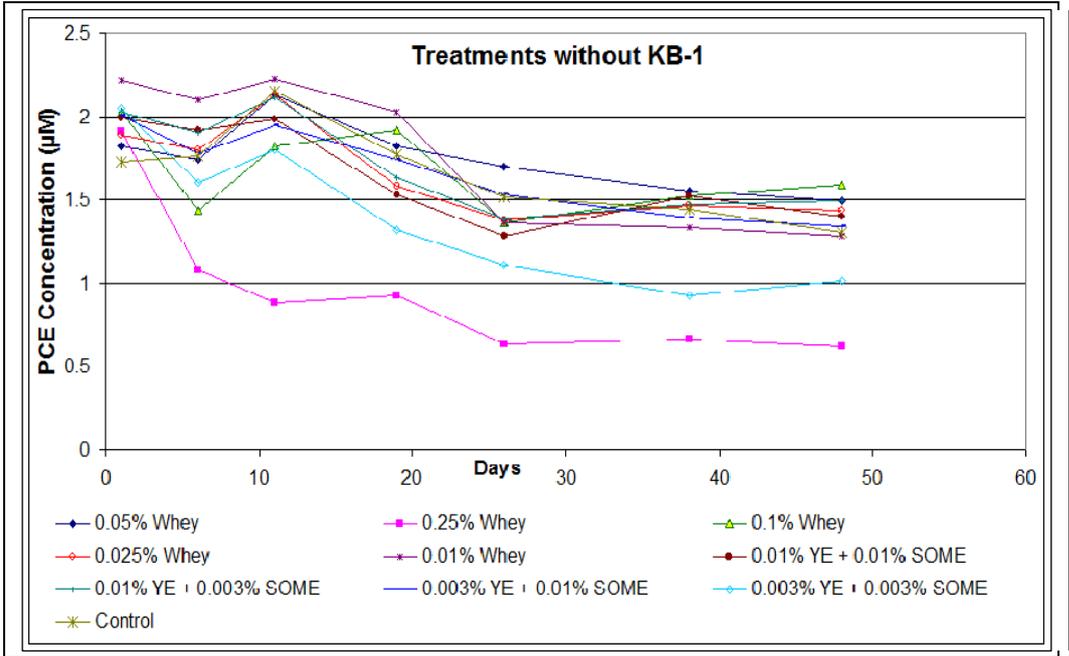


Figure 3.30. PCE degradation rate in treatments without KB-1 bacterial culture, (whey microcosm/ Set 1).

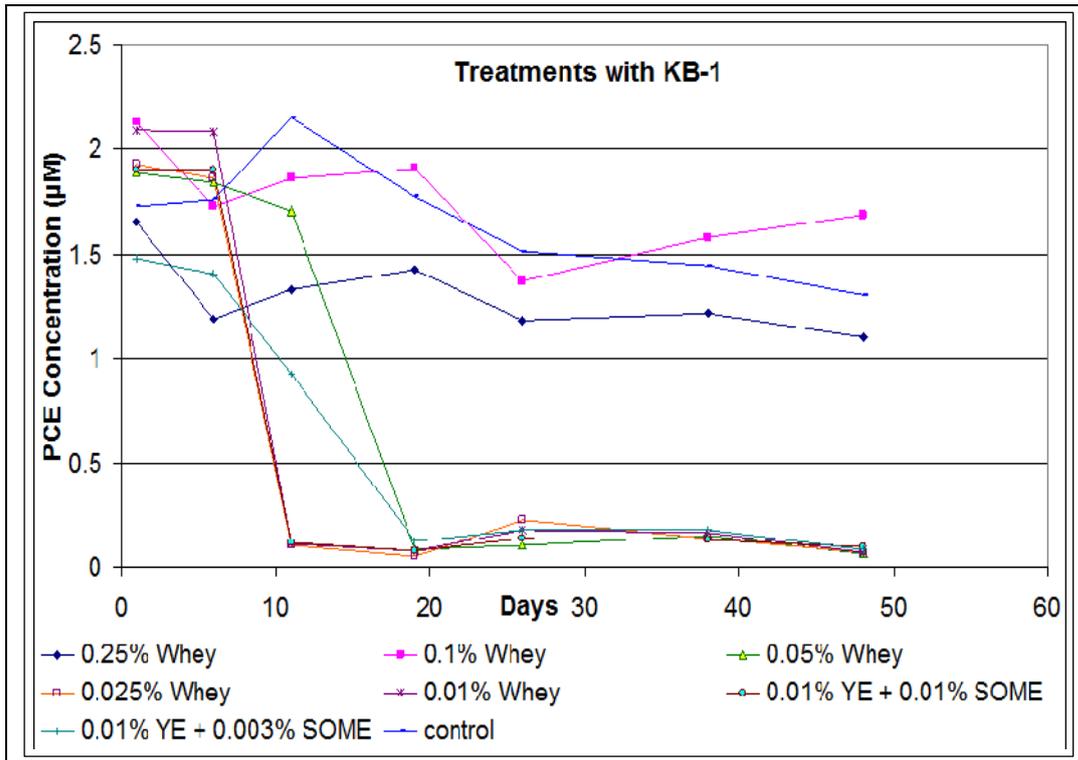


Figure 3.31. PCE degradation rate in treatments with KB-1 bacterial culture, (whey microcosm/ Set 1).

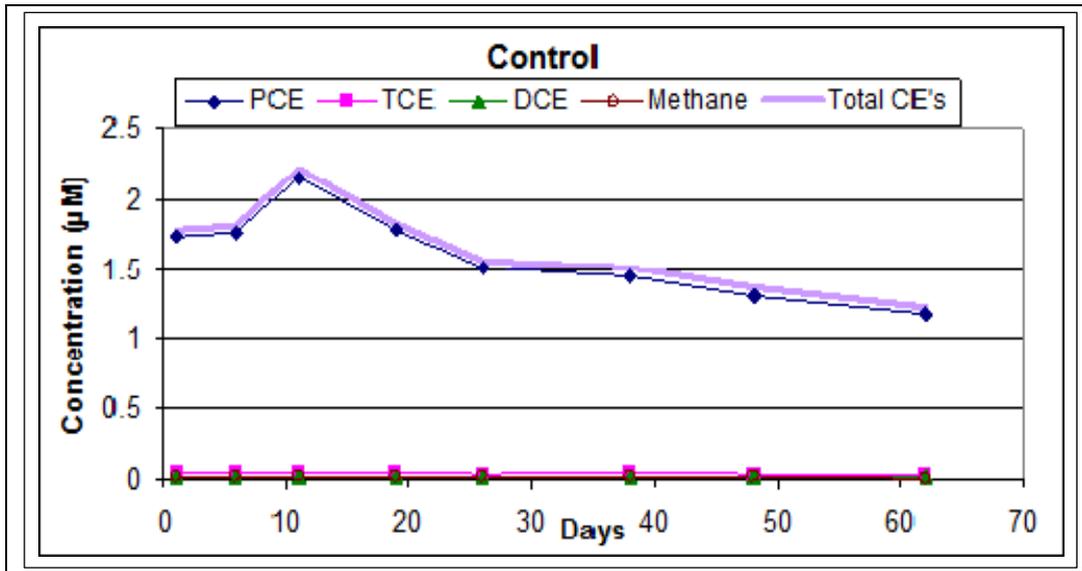


Figure 3.32. CEs and methane concentrations in control treatment, (Whey microcosm/ Set 1).

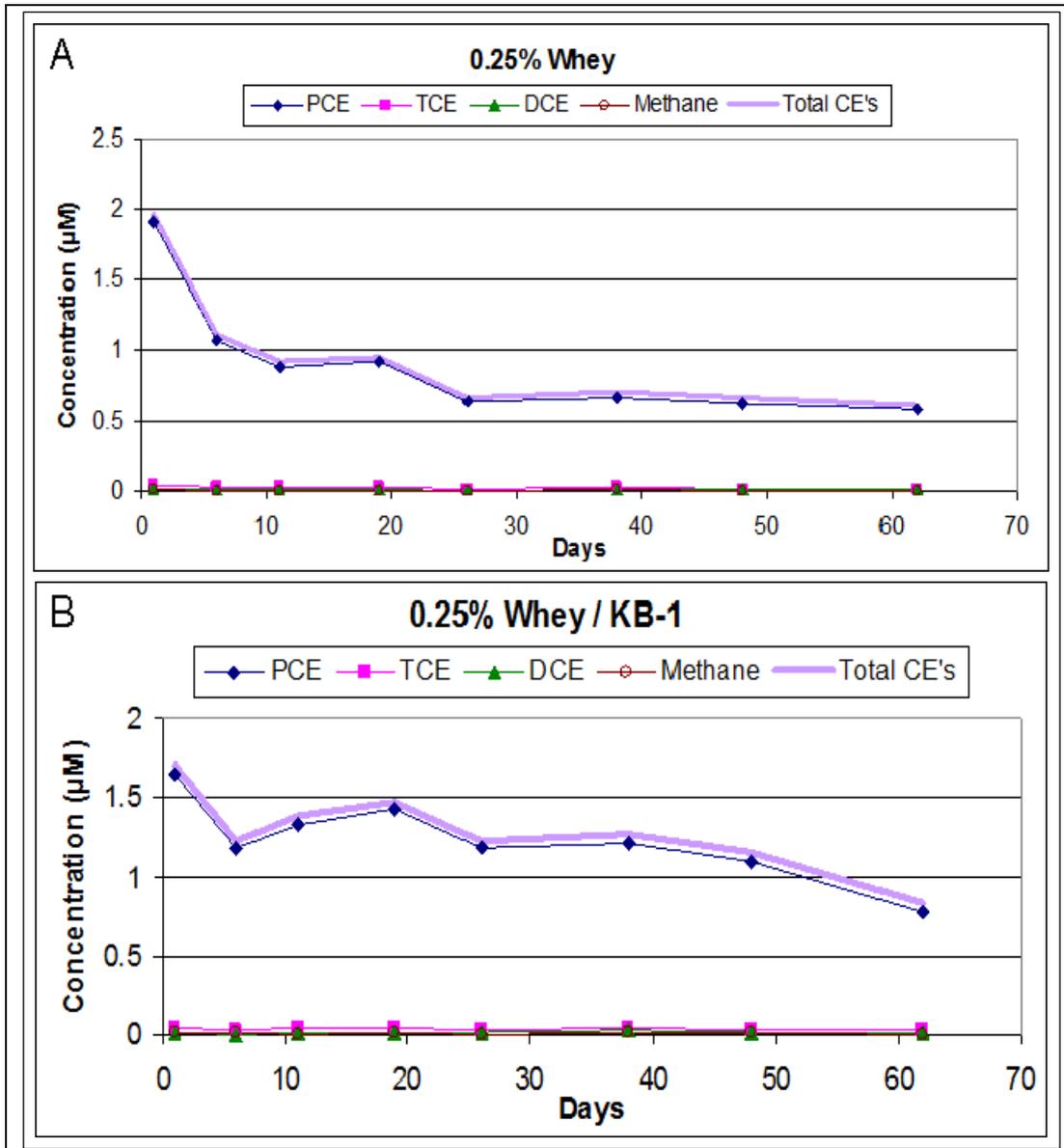


Figure 3.33. CEs and methane concentrations in 0.25% Whey treatment (Whey microcosm/ Set 1) **A.** without KB-1 and **B.** With KB-1.

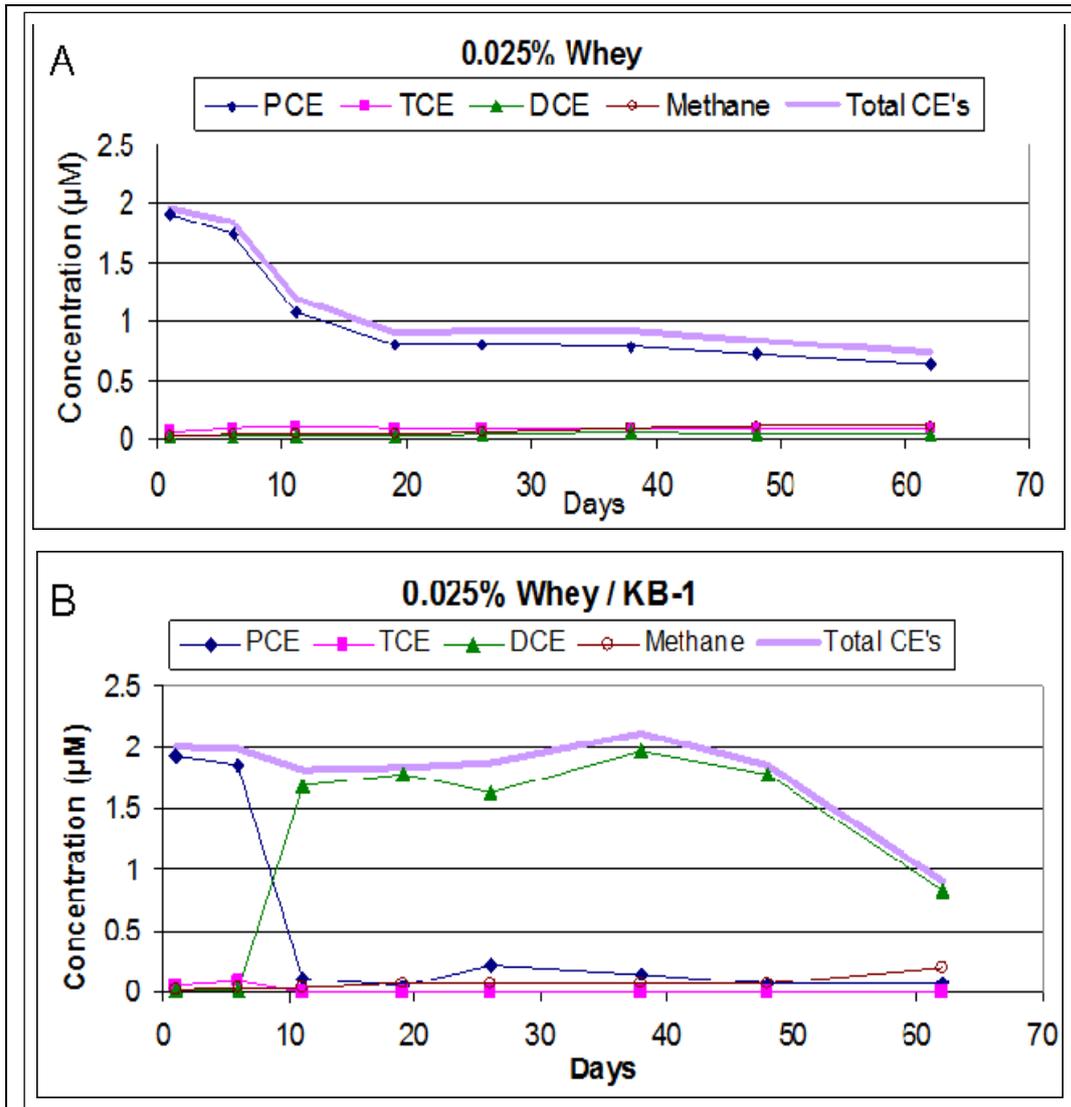


Figure 3.34. CEs and methane concentrations in 0.025% Whey treatment (Whey microcosm/ Set 1). **A.** without KB-1 and **B.** With KB-1.

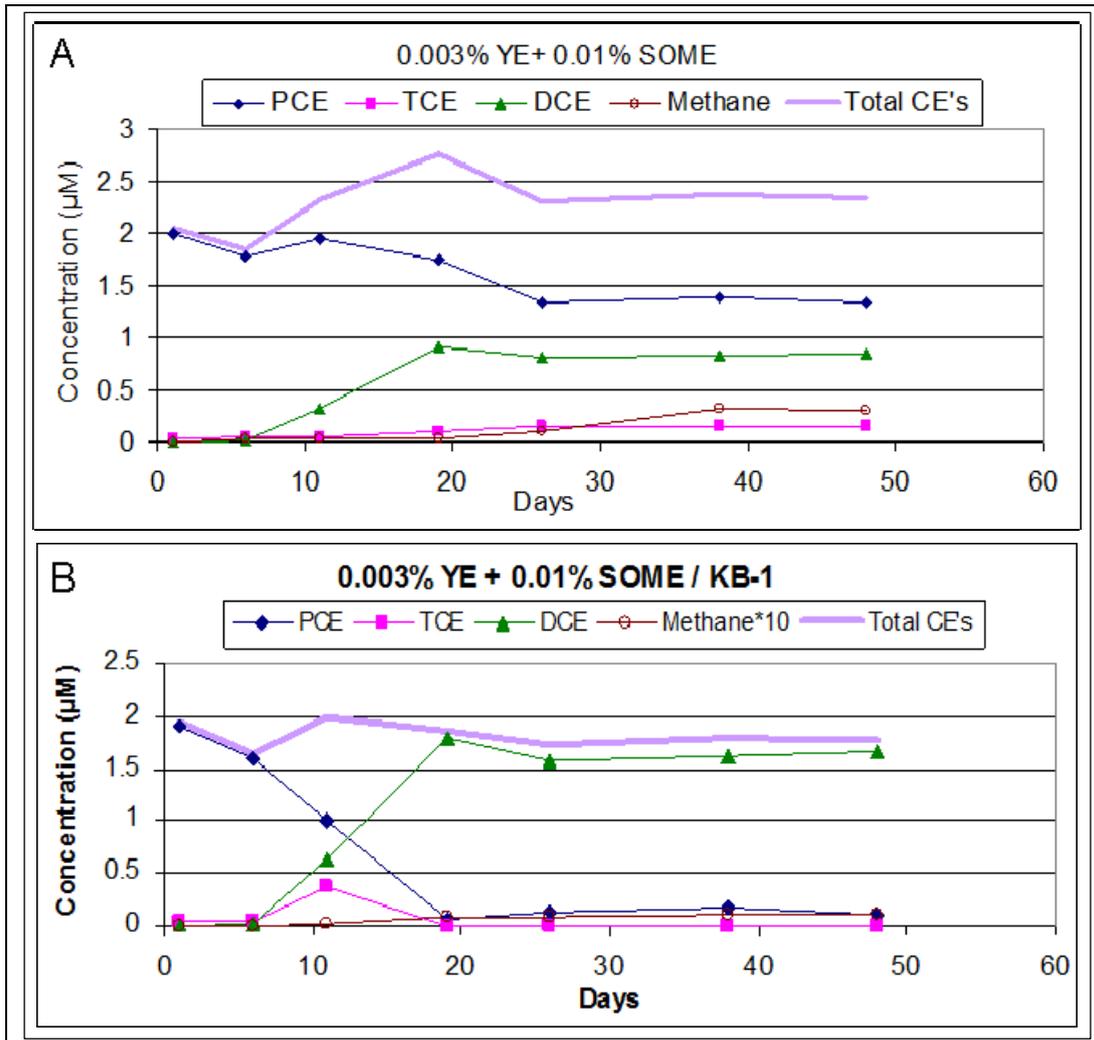


Figure 3.35. CEs and methane concentrations in YE (0.003%) + SOME (0.01%) treatment (Whey microcosm/ Set 1). **A.** without KB-1 and **B.** With KB-1.

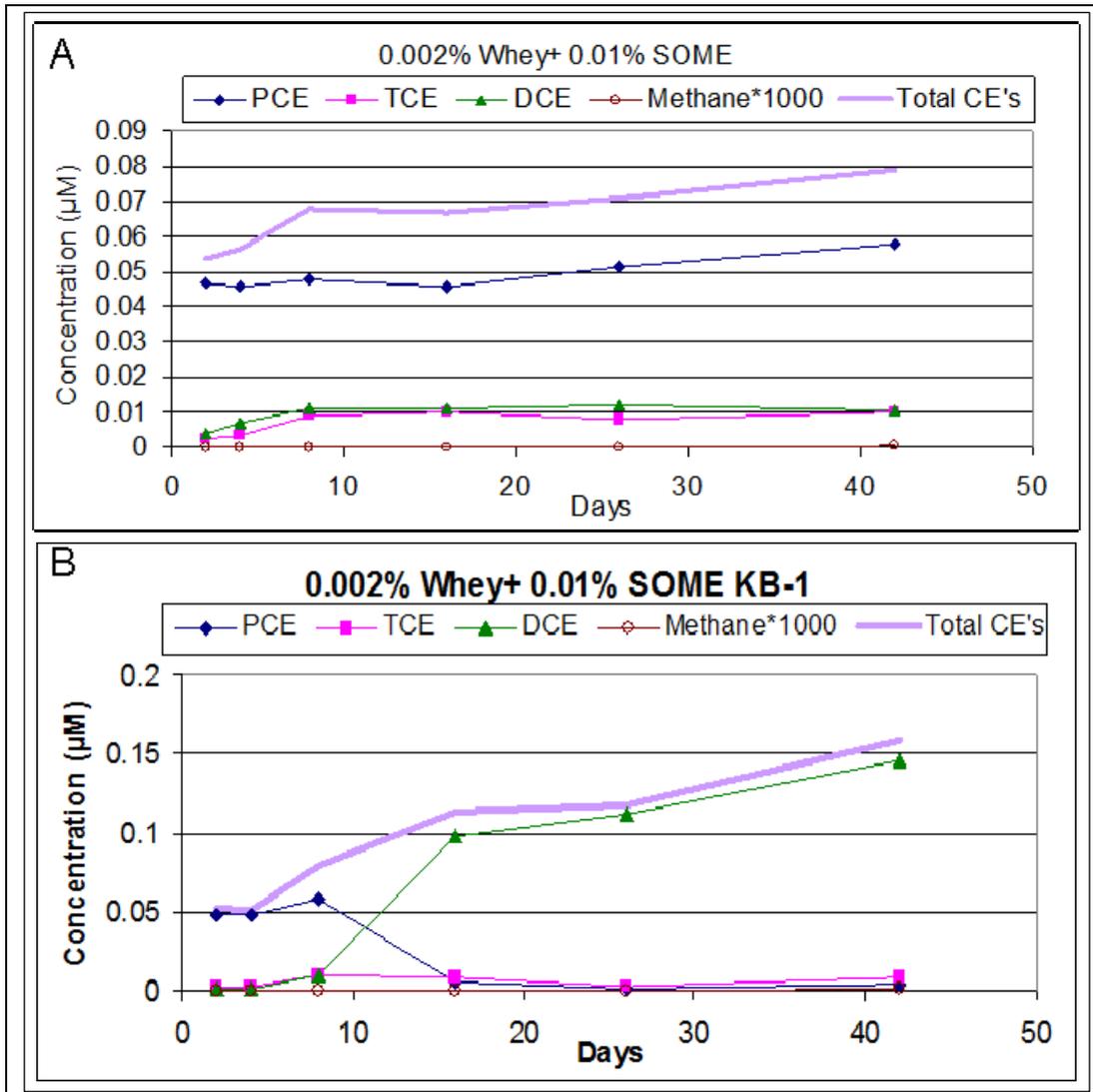


Figure 3.36. CEs and methane concentrations in Whey (0.002%) + SOME (0.01%) treatment (Whey microcosm/ Set 2). **A.** without KB-1 and **B.** With KB-1.

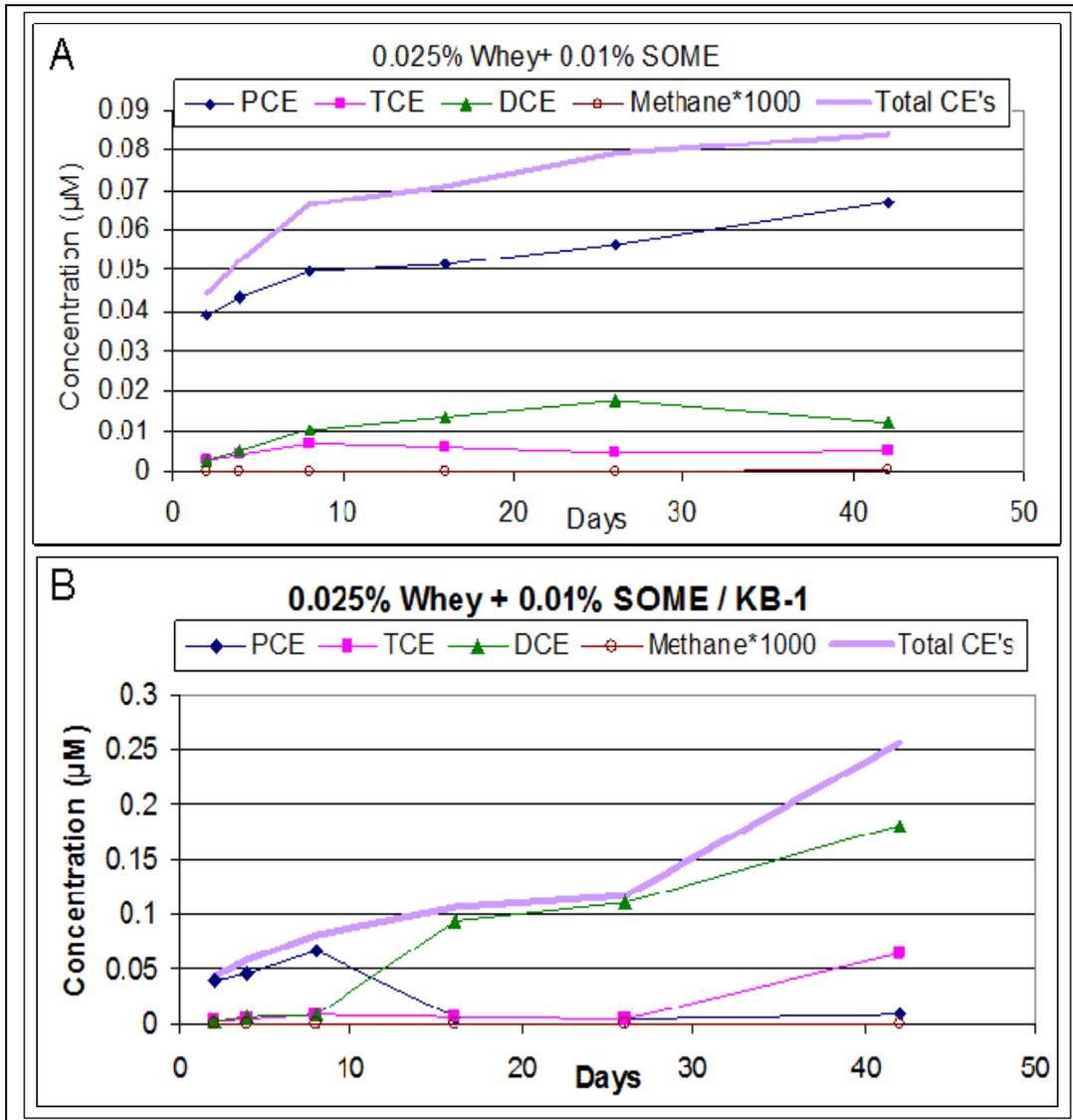


Figure 3.37. CEs and methane concentrations in Whey (0.025%) + SOME (0.01%) treatment (Whey microcosm/ Set 2). **A.** without KB-1 and **B.** With KB-1.

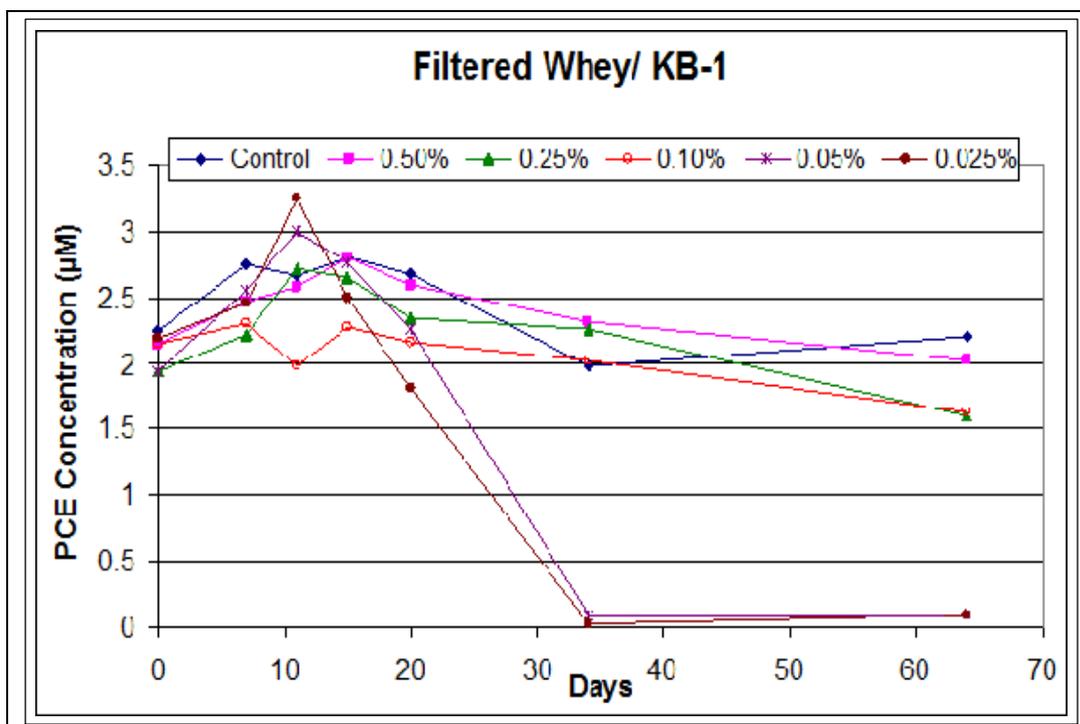


Figure 3.38. PCE degradation rate in filtered whey microcosms treated with cheese whey and KB-1.

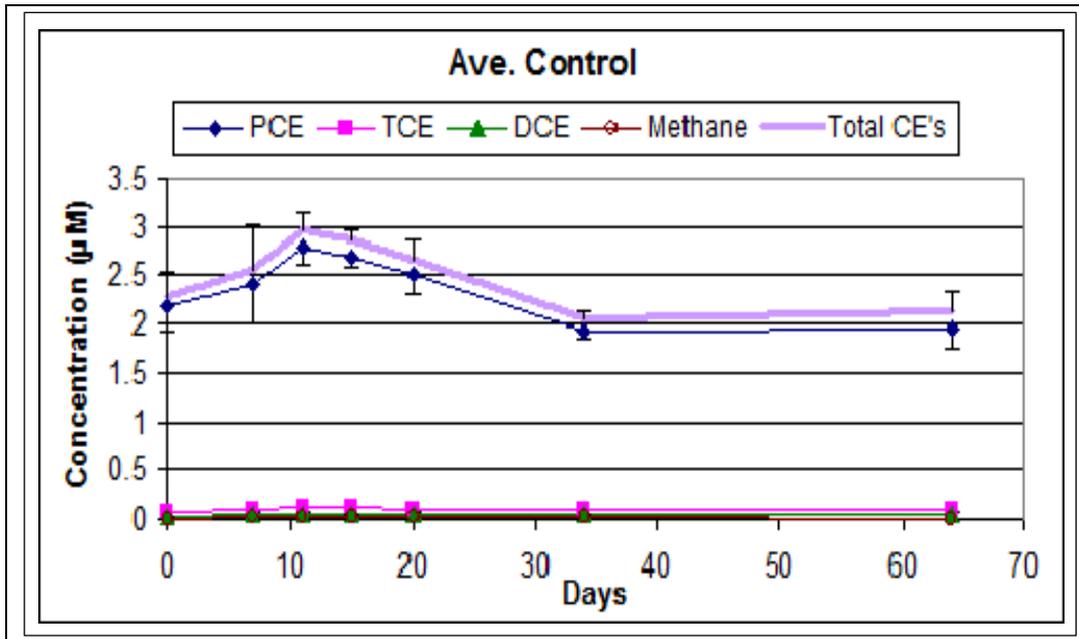


Figure 3.39. CEs and methane concentrations in control treatment (Whey microcosm/ Set 3). Standard error is shown based on n= 3.

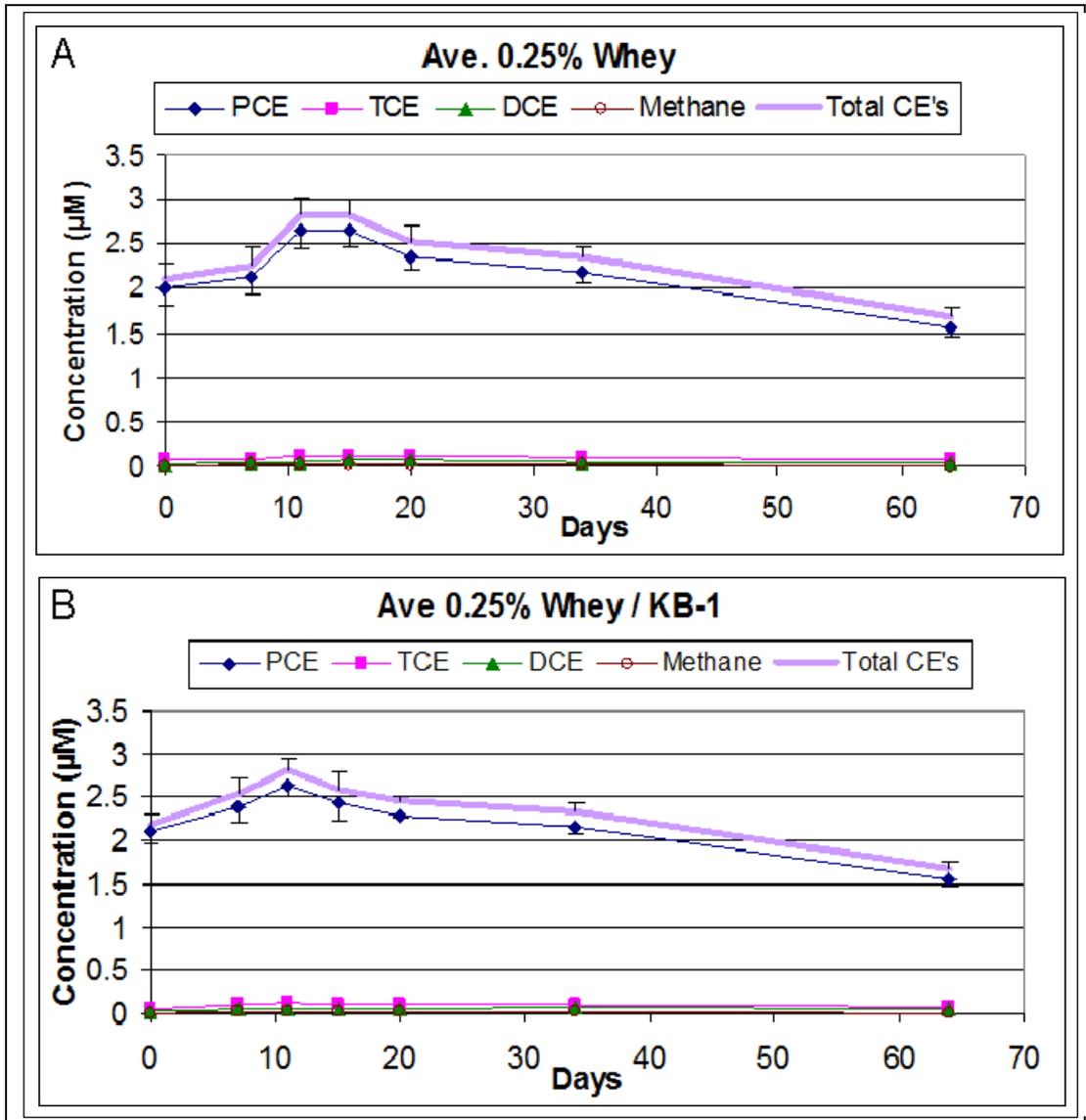


Figure 3.40. CEs and methane concentrations in Whey (0.25%) treatment (Whey microcosm/ Set 3). **A.** without KB-1 and **B.** With KB-1. Standard error is shown based on n=3 .

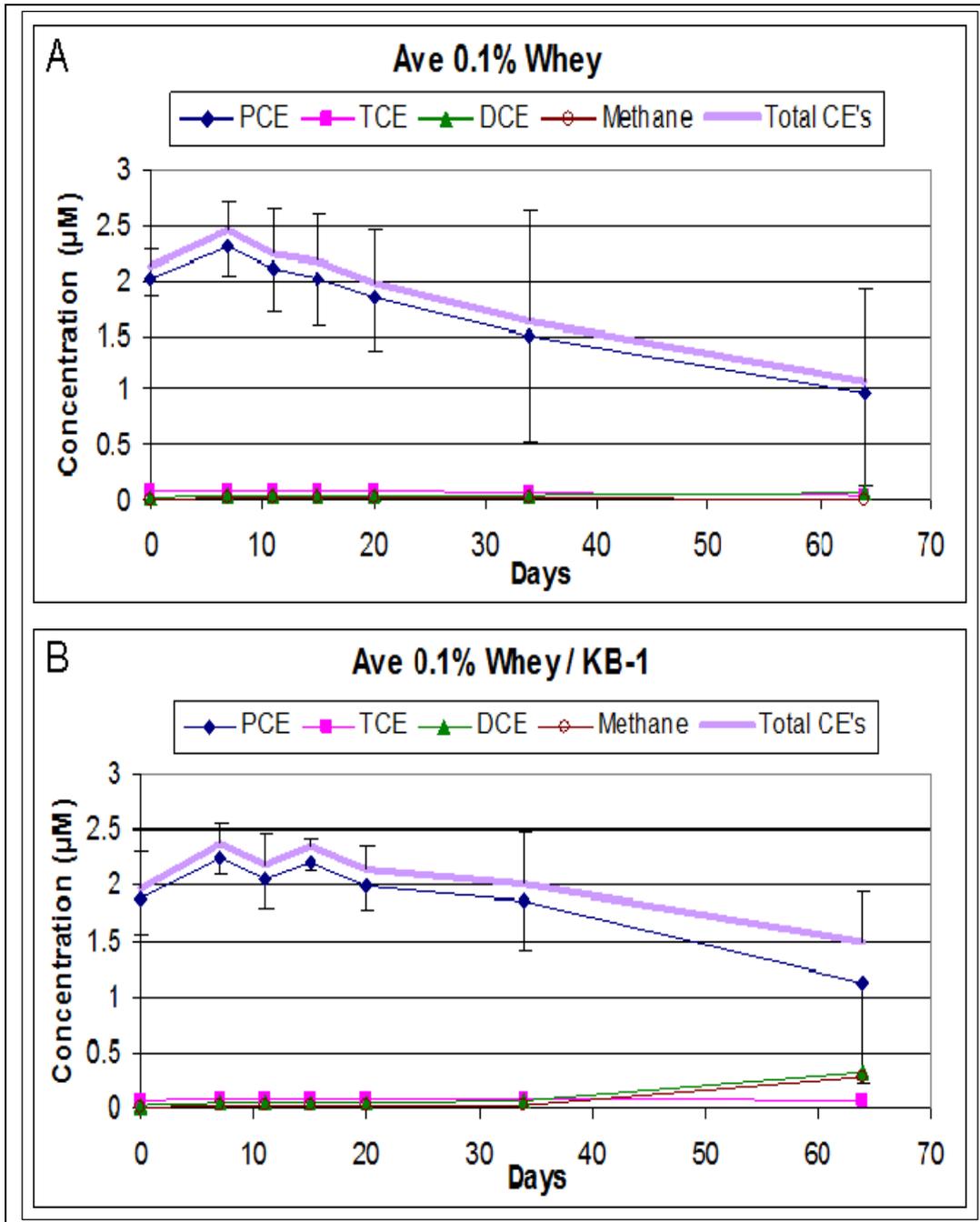


Figure 3.41. CEs and methane concentrations in Whey (0.1%) treatment (Whey microcosm/ Set 3). **A.** without KB-1 and **B.** With KB-1. Standard error is shown based on n=3 .

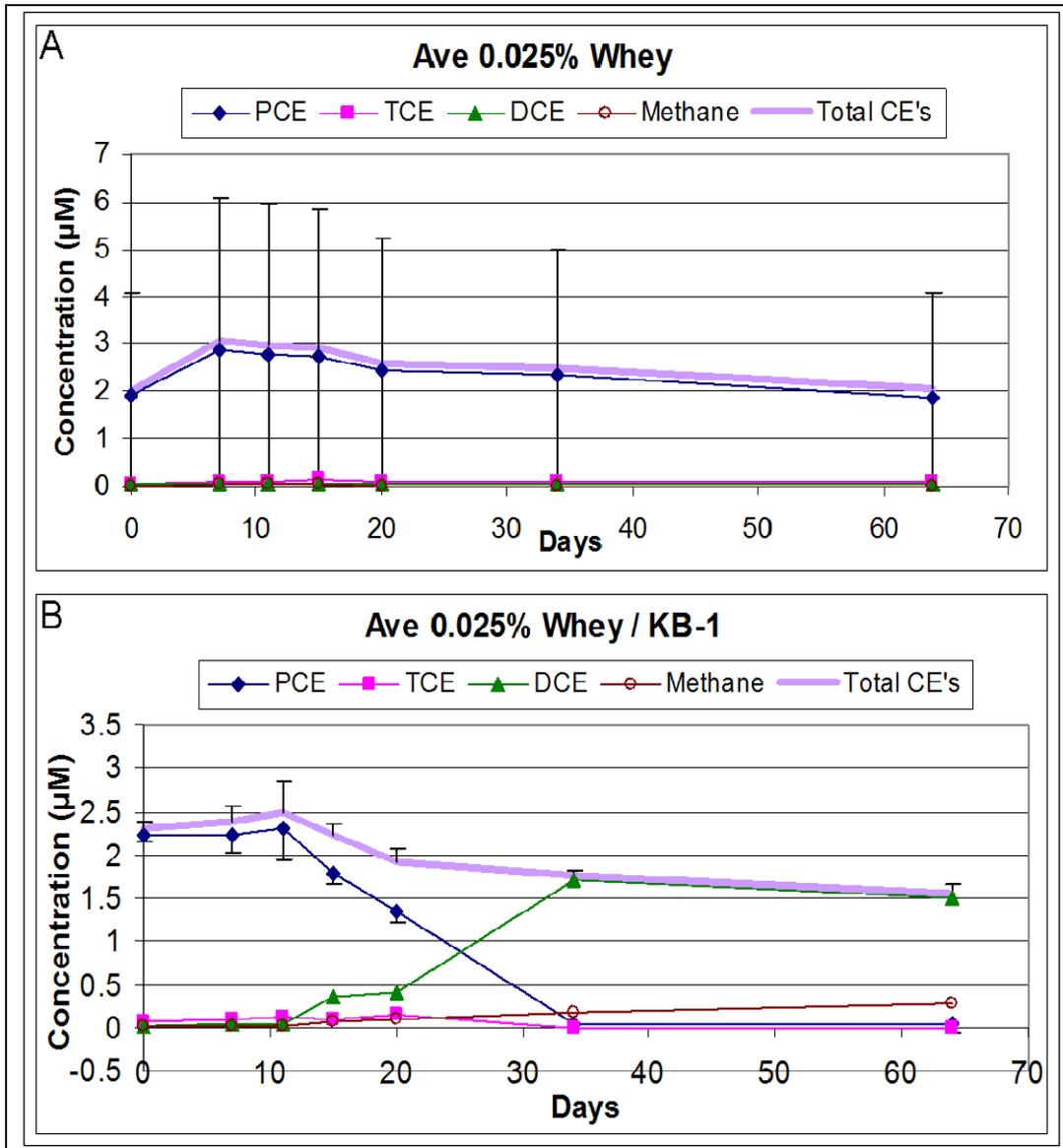


Figure 3.42. CEs and methane concentrations in Whey (0.025%) treatment (Whey microcosm/ Set 3). **A.** without KB-1 and **B.** With KB-1. Standard error is shown based on n=3 .

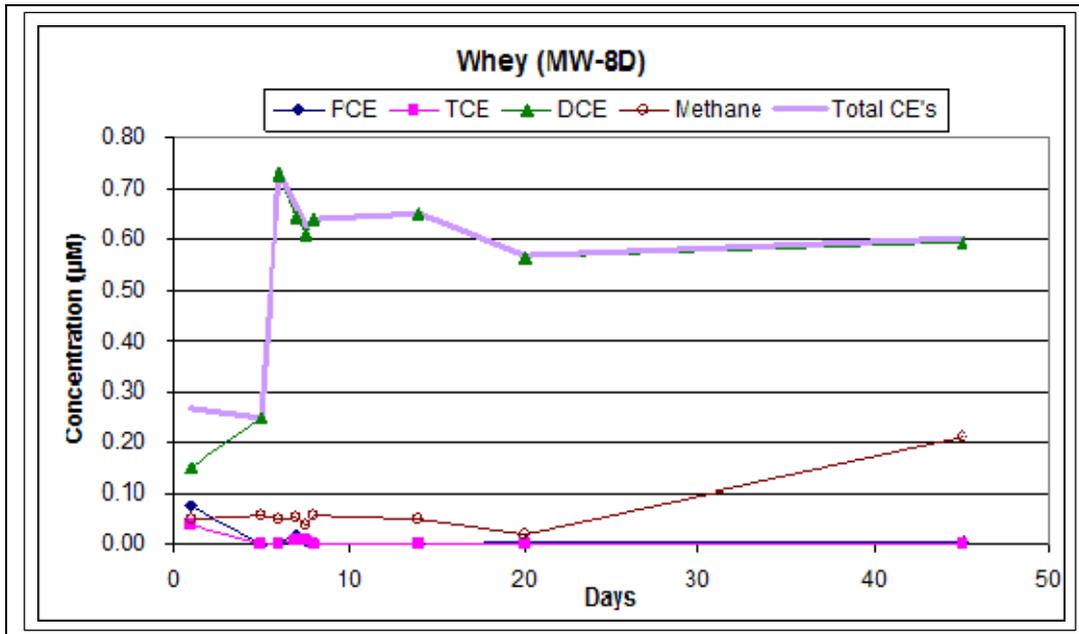


Figure 3.43. CEs and methane concentrations in Whey (0.025%) treatment (Whey microcosm/ Set 4) prepared with water from MW-8D.

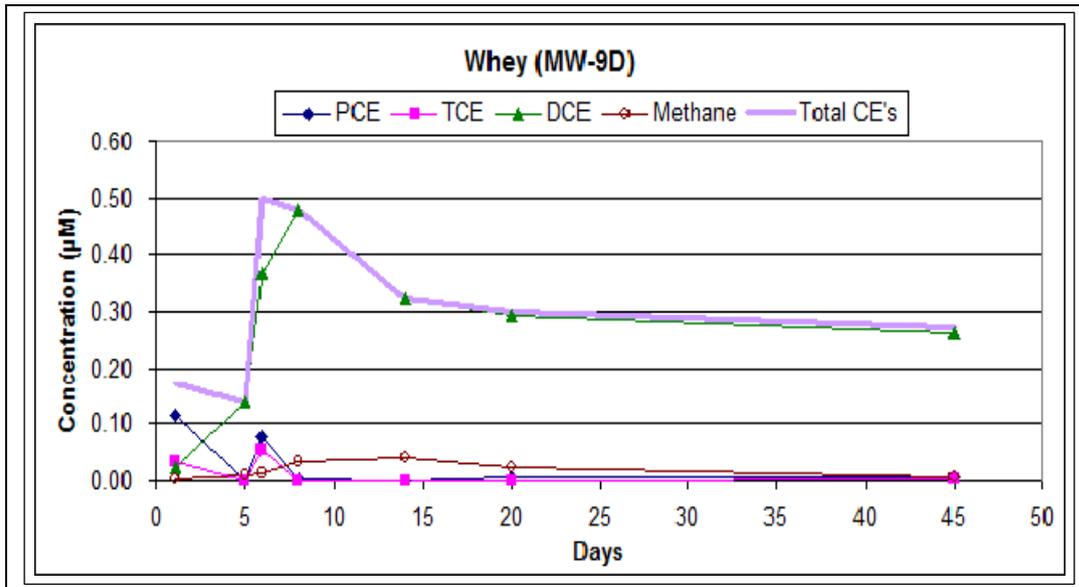


Figure 3.44. CEs and methane concentrations in Whey (0.025%) treatment (Whey microcosm/ Set 4) prepared with water from MW-9D.

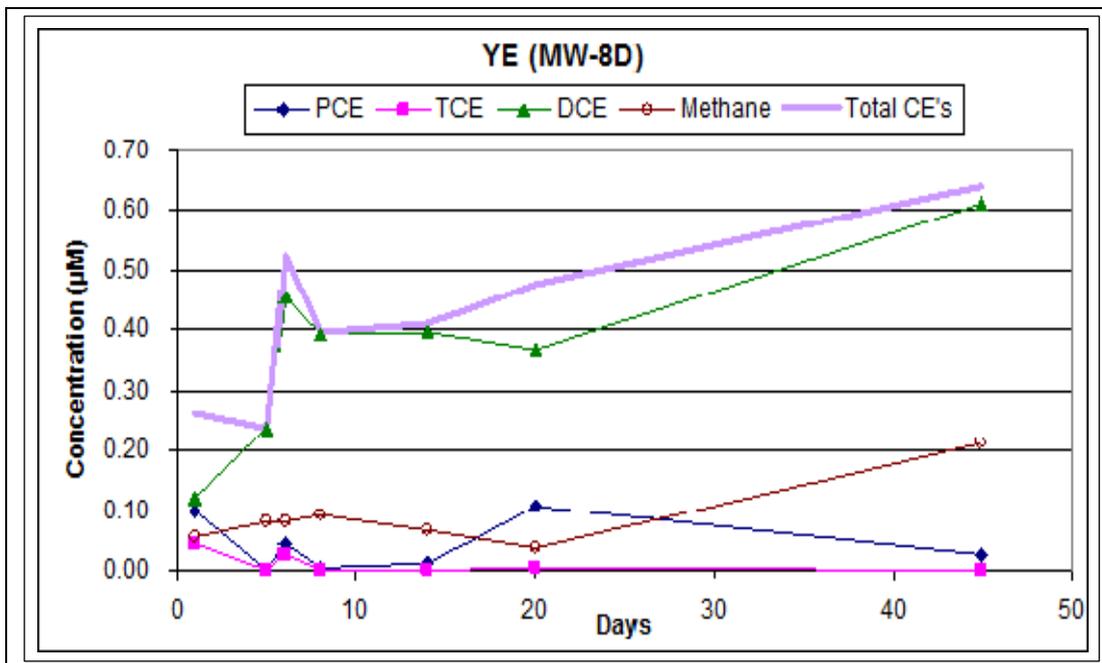


Figure 3.45. CEs and methane concentrations in YE (0.01%) treatment (Whey microcosm/ Set 4) prepared with water from MW-8D.

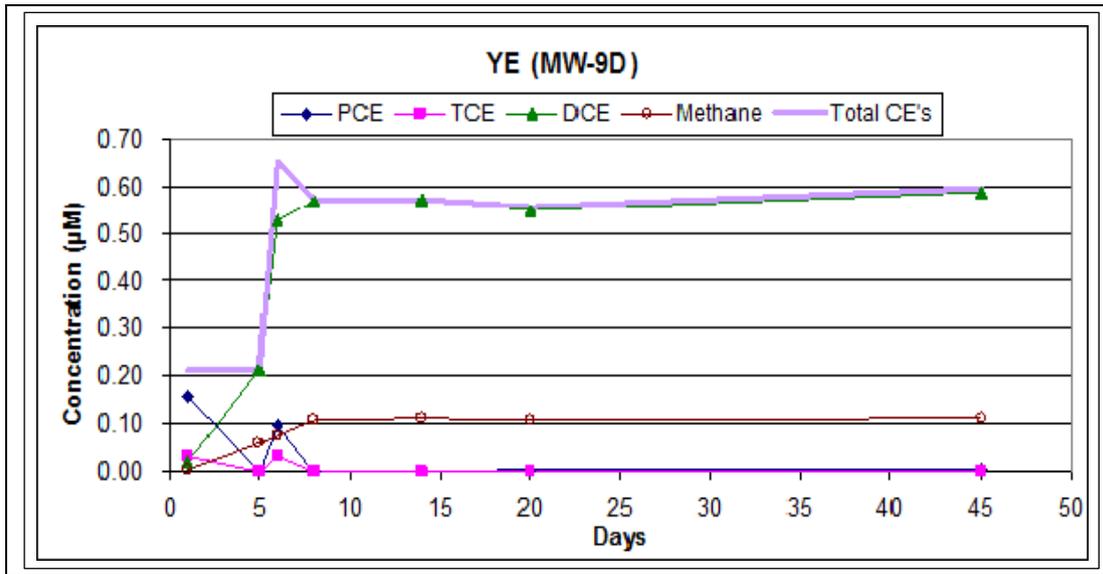


Figure 3.46. CEs and methane concentrations in YE (0.01%) treatment (Whey microcosm/ Set 4) prepared with water from MW-9D.

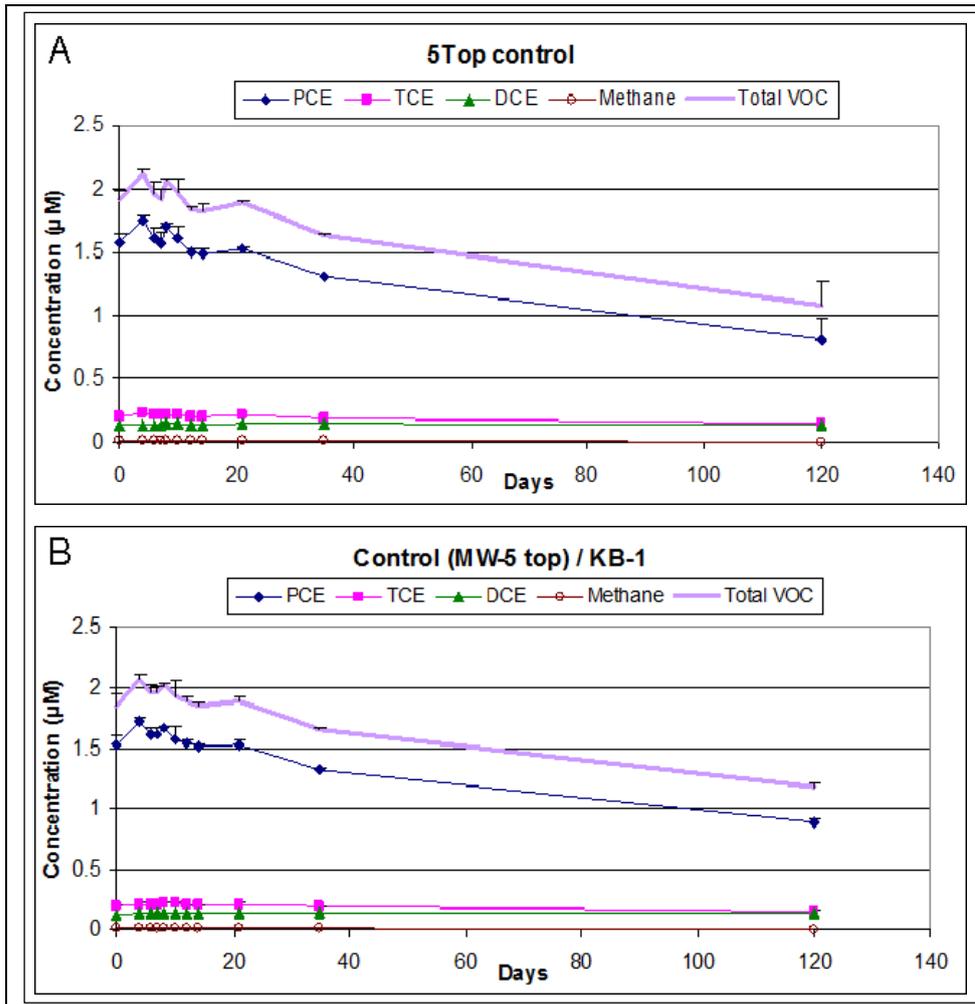


Figure 3.47. CEs and methane concentrations in control treatment (Whey microcosm/ Set 5) prepared with water from top water of MW-5D. **A.** with KB-1 and **B.** without KB-1.

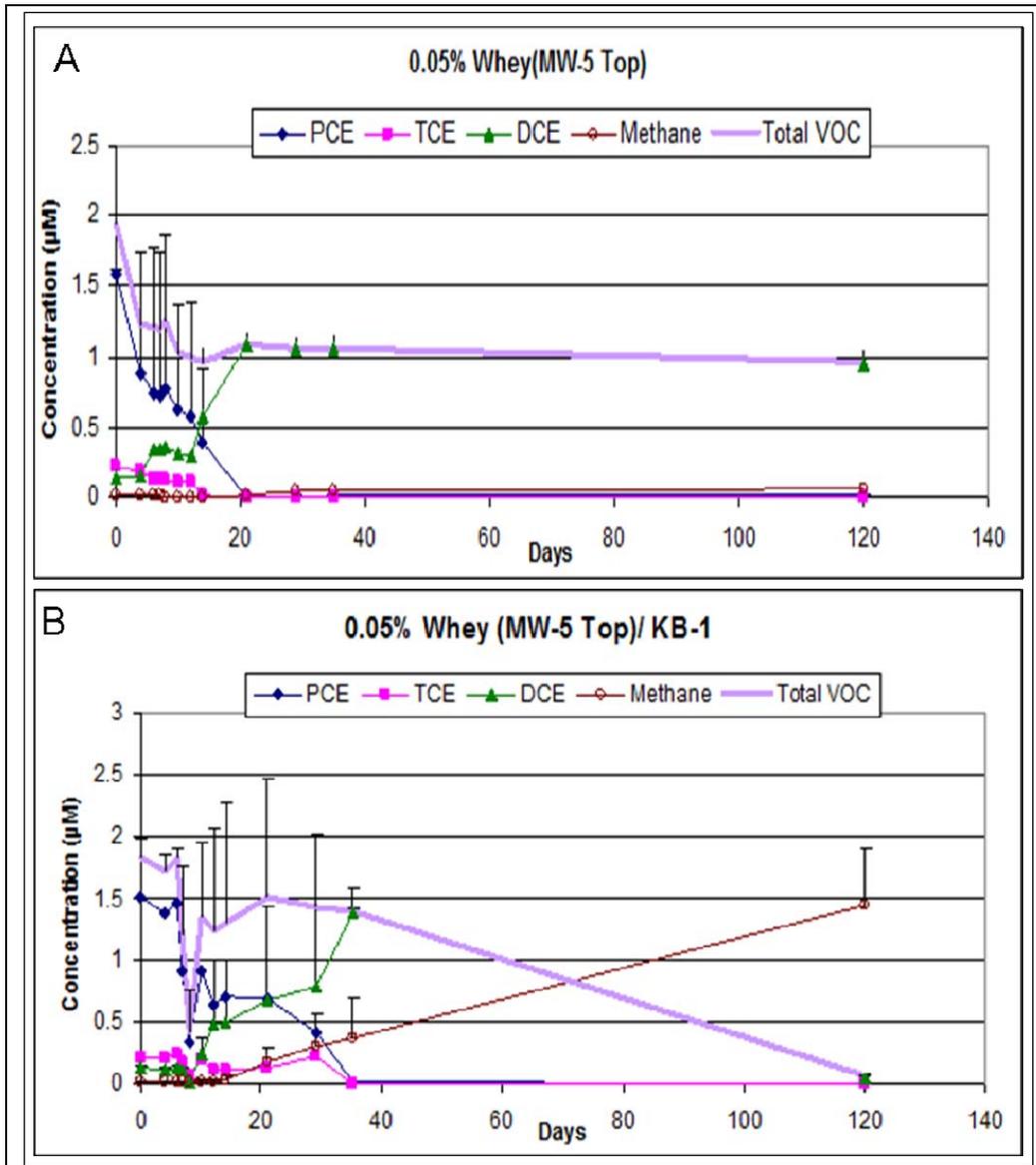


Figure 3.48. CEs and methane concentrations in 0.05% Whey treatment (Whey microcosm/ Set 5) prepared with water from top water of MW-5D. **A.** with KB-1 and **B.** without KB-1.

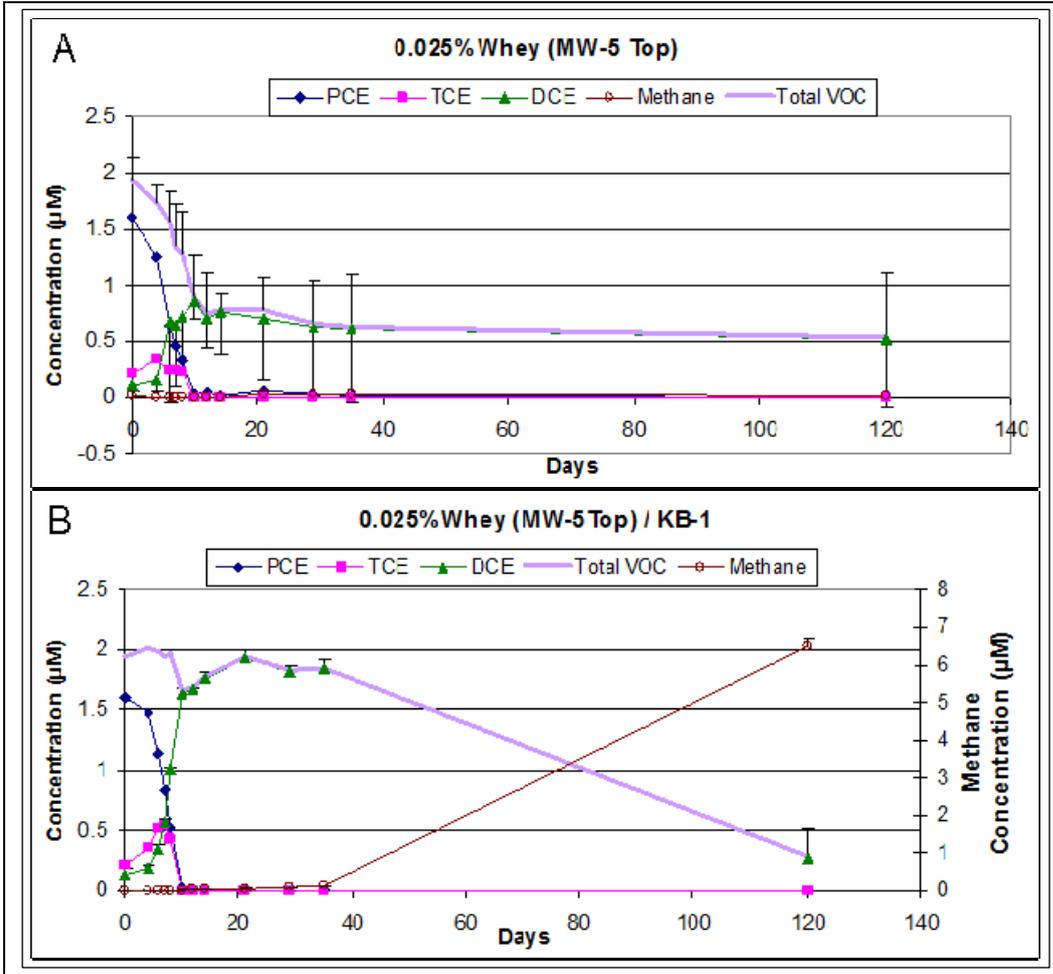


Figure 3.49. CEs and methane concentrations in 0.025% Whey treatment (Whey microcosm/ Set 5) prepared with water from top water of MW-5D. **A.** with KB-1 and **B.** without KB-1.

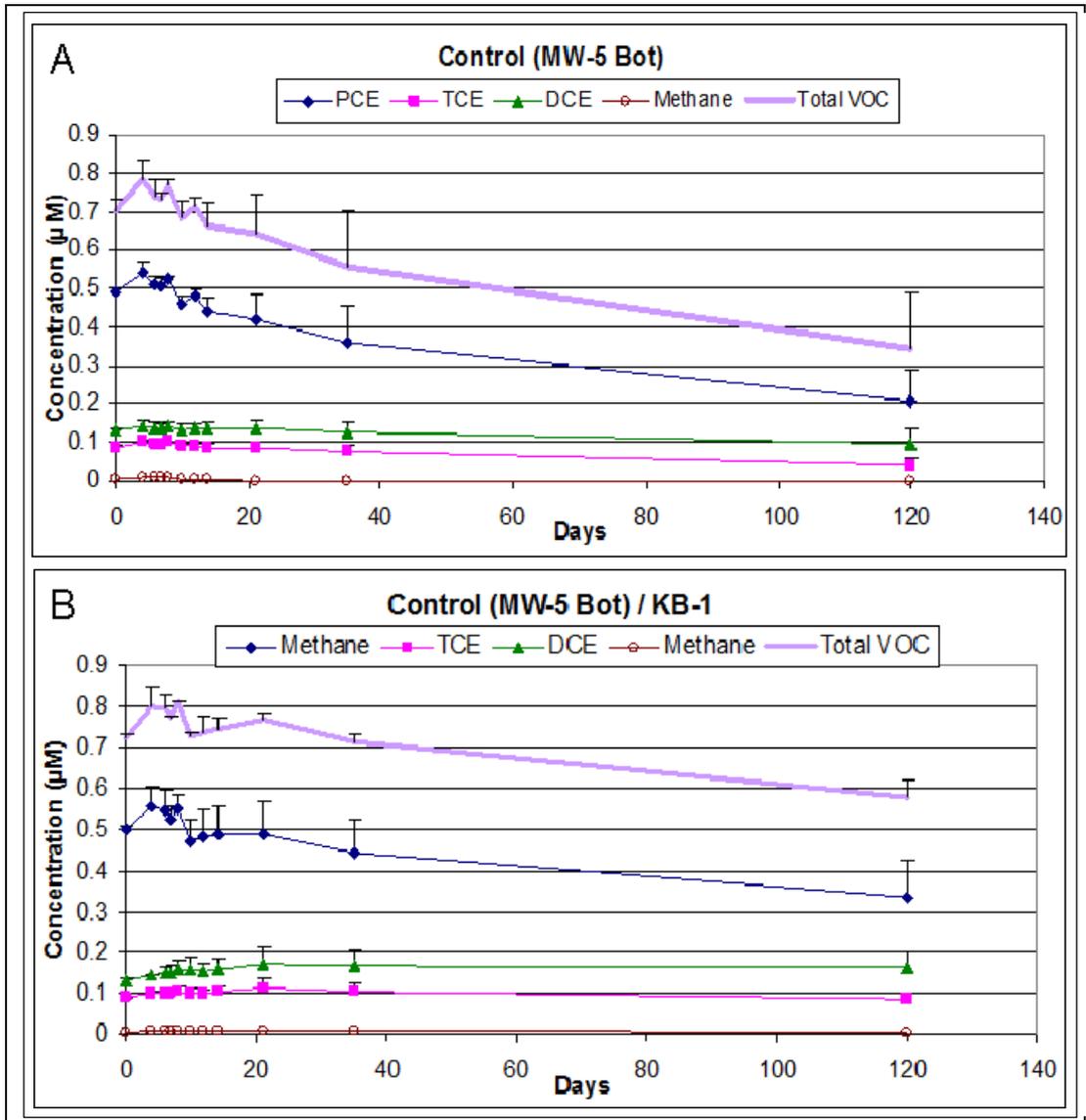


Figure 3.50. CEs and methane concentrations in control treatment (Whey microcosm/ Set 5) prepared with water from bottom water of MW-5D. **A.** with KB-1 and **B.** without KB-1.

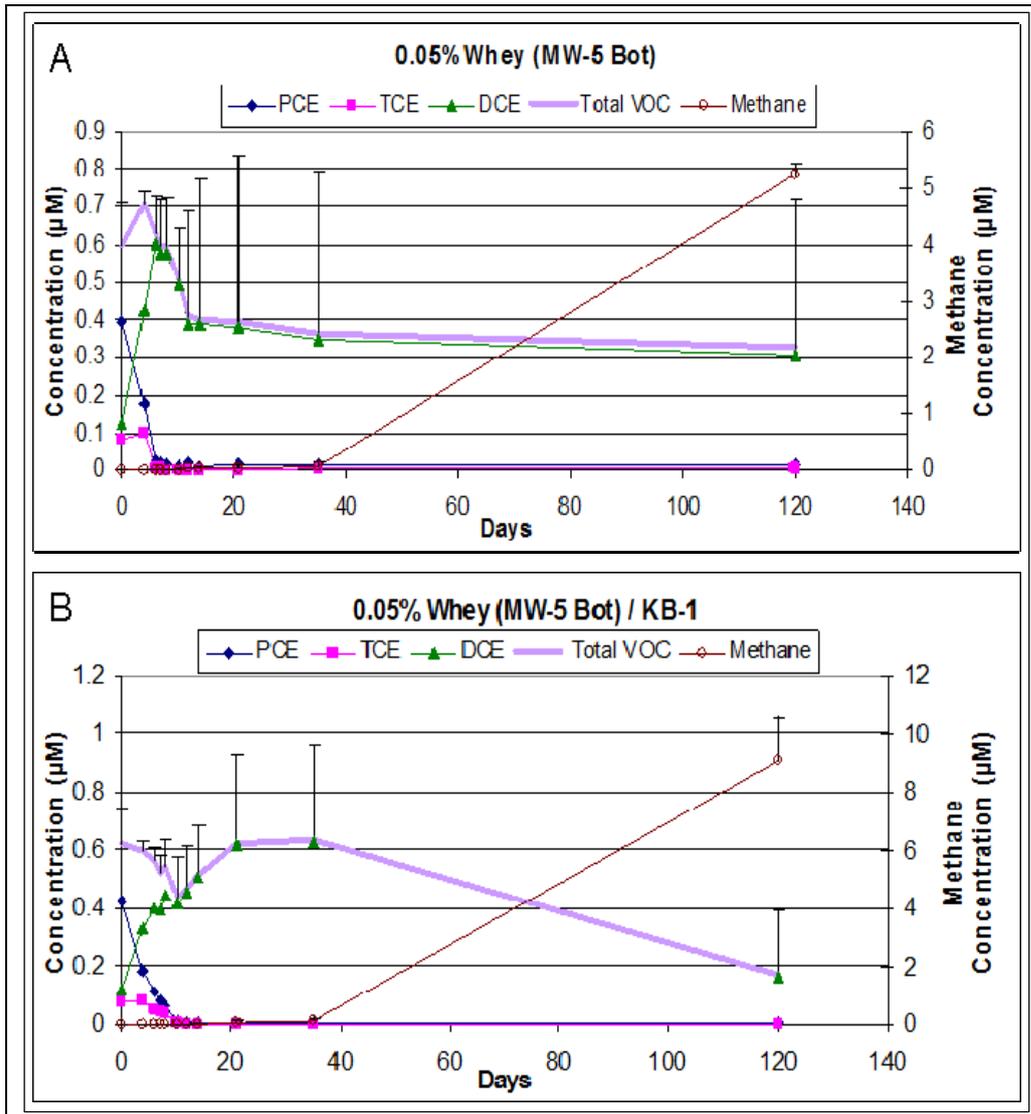


Figure 3.51. CEs and methane concentrations in 0.05% Whey treatment (Whey microcosm/ Set 5) prepared with water from Bottom water of MW-5D. **A.** with KB-1 and **B.** without KB-1.

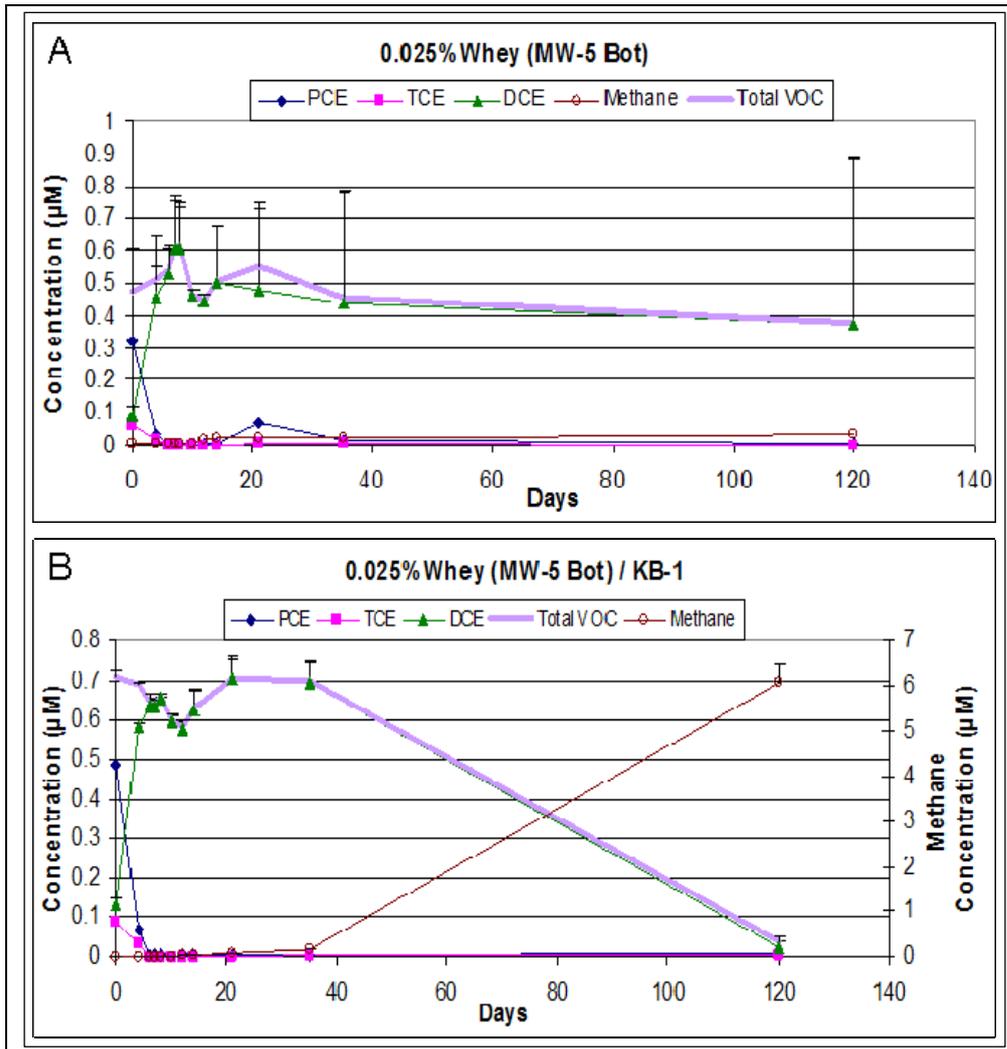


Figure 3.52. CEs and methane concentrations in 0.025% Whey treatment (Whey microcosm/ Set 5) prepared with water from Bottom water of MW-5D. **A.** with KB-1 and **B.** without KB-1.

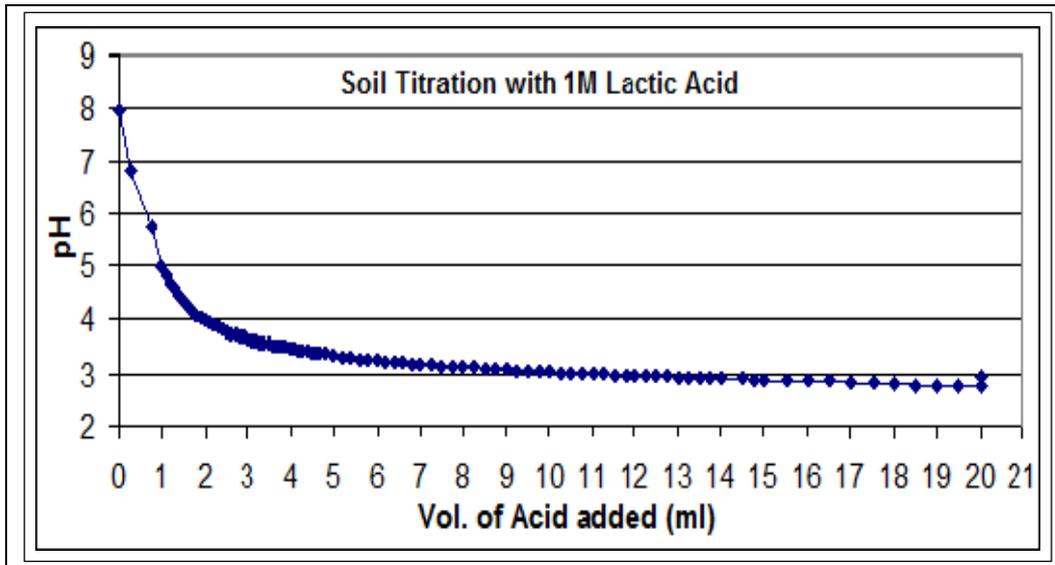


Figure 3.53. Soil titration curve with 1 M lactic acid. Soil sample used at 42.5 ft bgs during MW-9D installation.

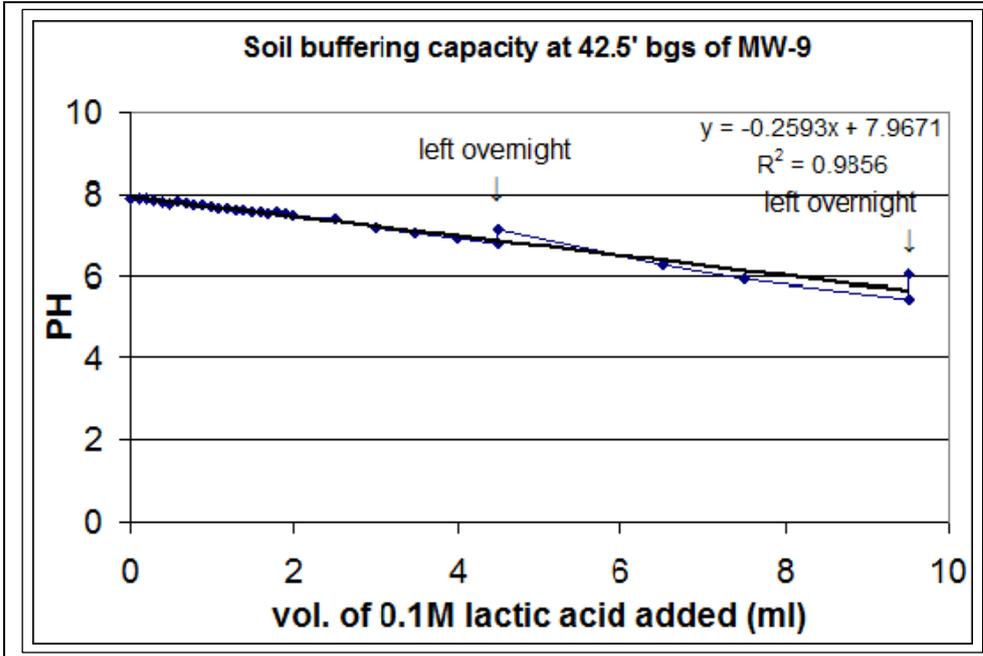


Figure 3.54. Soil titration curve with 0.1 M lactic acid. Soil sample used at 42.5 ft bgs during MW-9D installation.

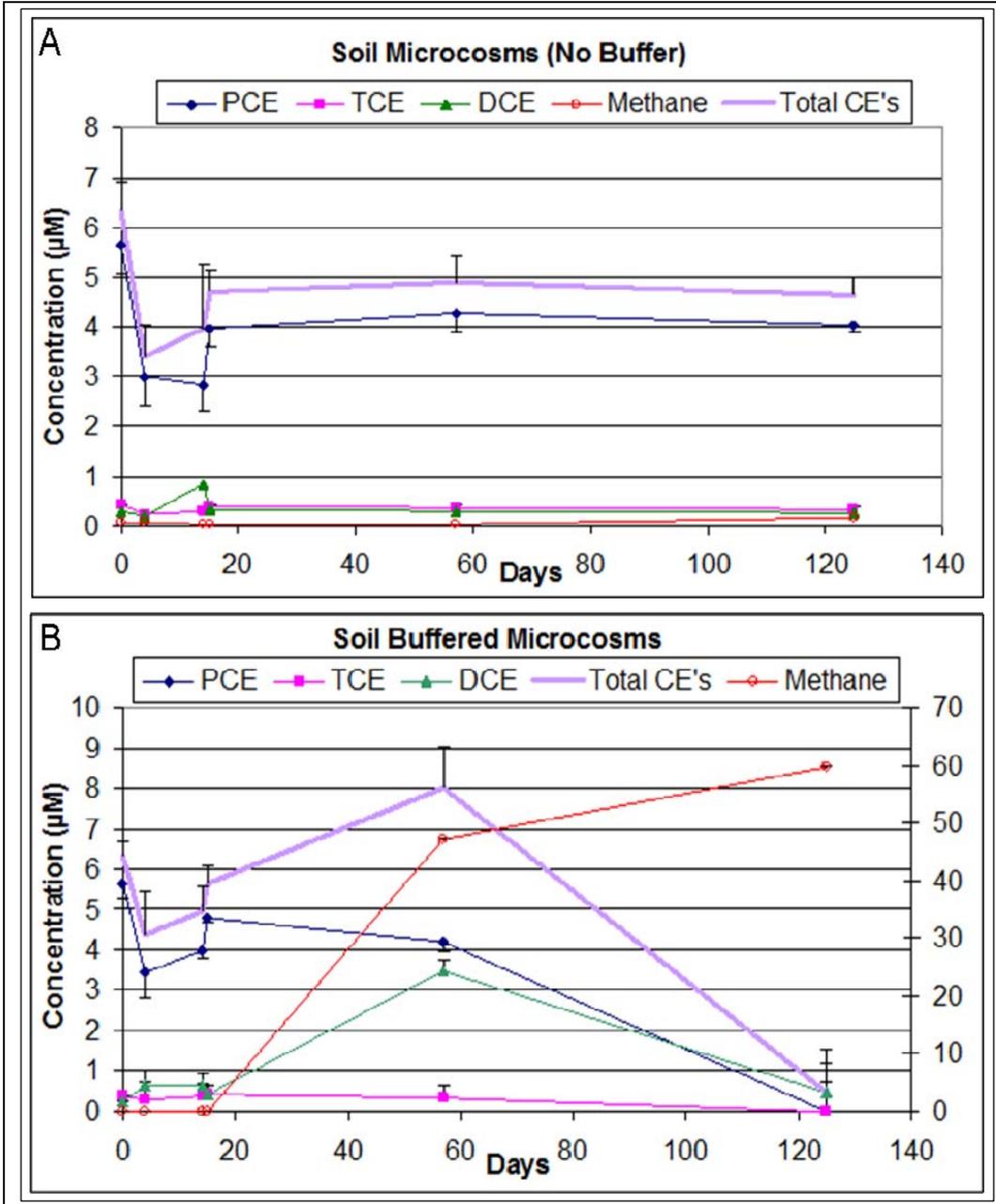


Figure 3.55. CEs and methane concentrations in soil microcosms prepared with 42.5 ft deep soil MW-9D. All treatments were bioaugmented with KB-1. **A.** pH not controlled and **B.** pH controlled with calcium carbonate and occasional addition of tris-base.

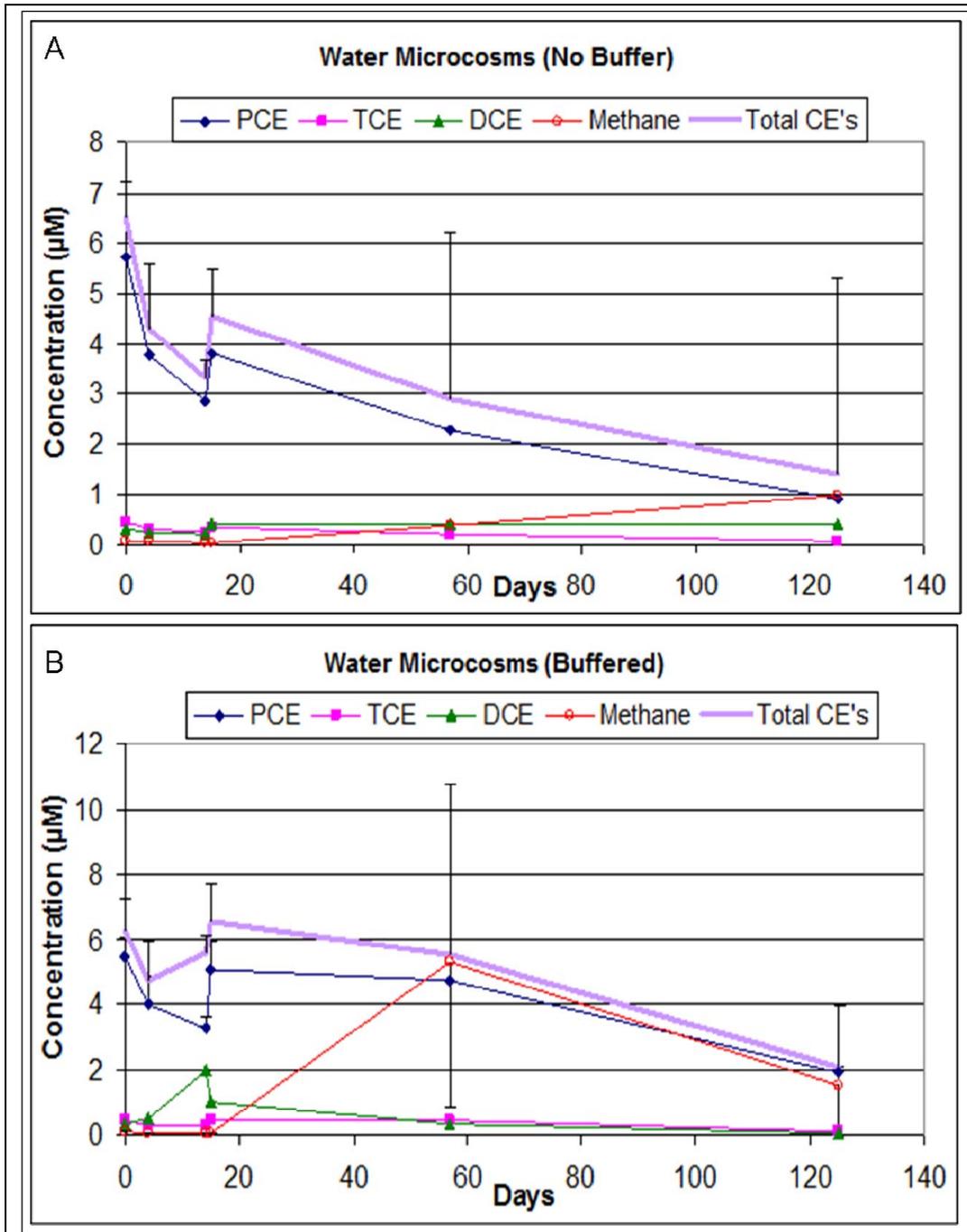


Figure 3.56. CEs and methane concentrations in water microcosms prepared with water from MW-5D. All treatments were bioaugmented with KB-1. **A.** pH not controlled and **B.** pH controlled with calcium carbonate and occasional addition of tris-base.

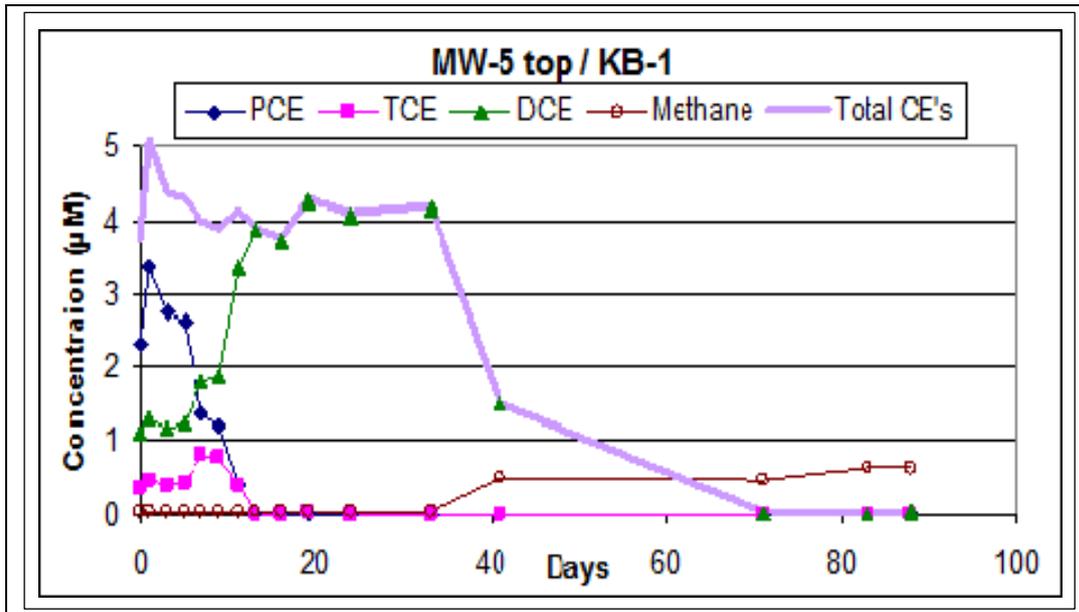


Figure 3.57. CEs and methane concentrations in microcosms prepared with water from the top of MW-5D, and bioaugmented with KB-1.

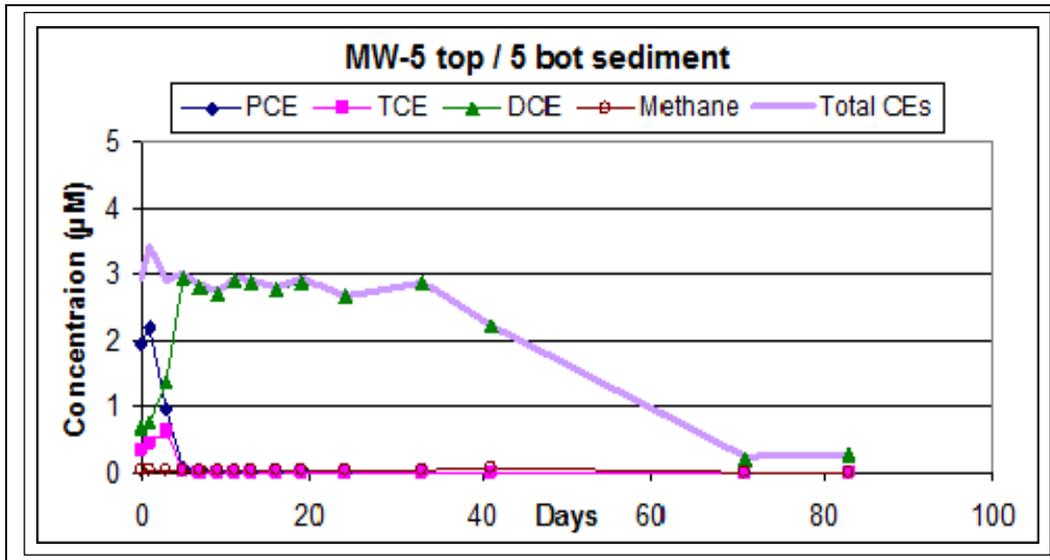


Figure 3.58. CEs and methane concentrations in microcosms prepared with water from the top of MW-5D, and bioaugmented with sediment collected from bottom of MW-5.

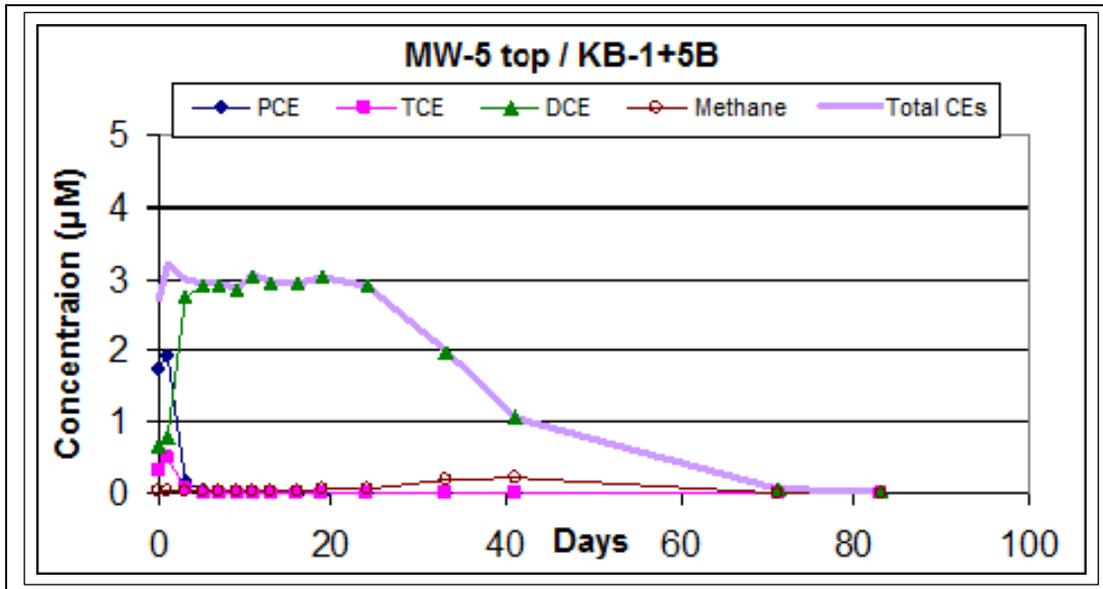
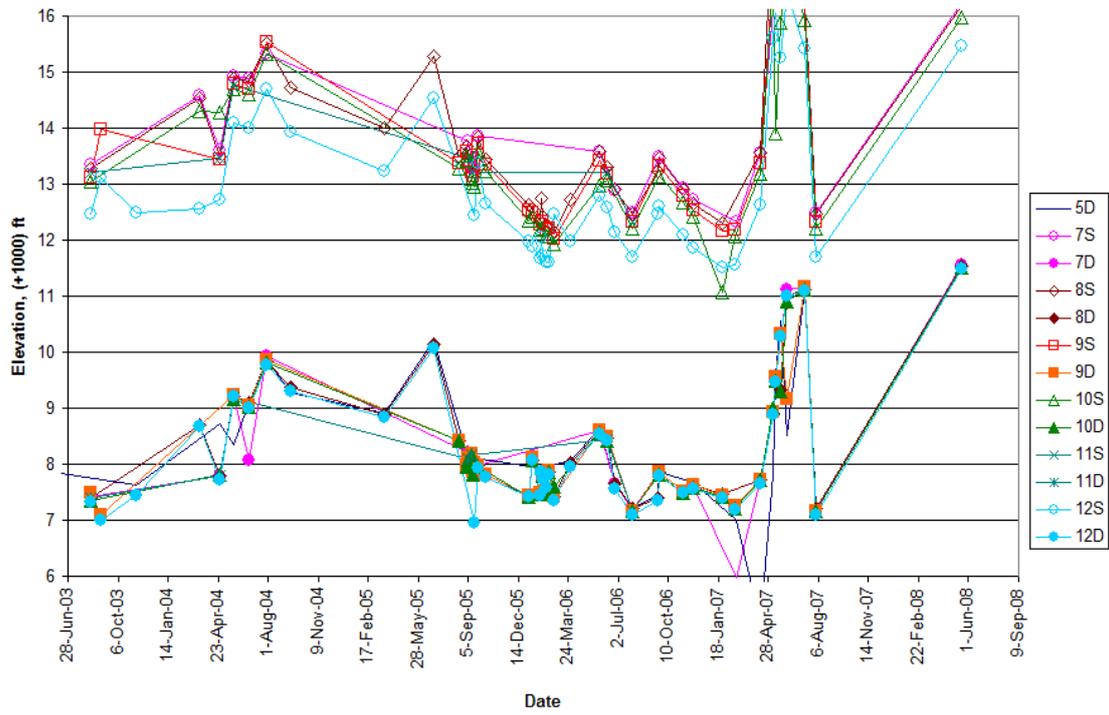


Figure 3.59. CEs and methane concentrations in microcosms prepared with water from the top of MW-5D, and bioaugmented with KB-1 and sediment from bottom of MW-5.



Elevation Data for the monitoring wells at the pilot study area.

Figure 3.60. Groundwater elevation data for the monitoring wells at the pilot study area.

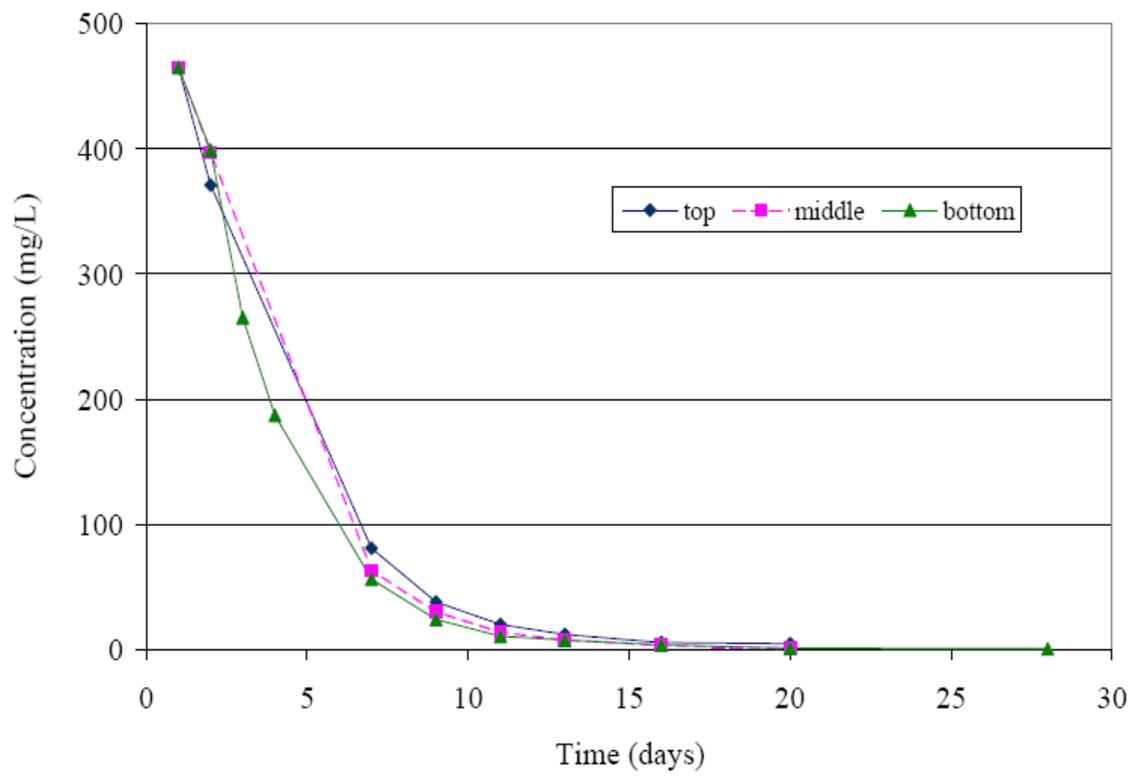


Figure 3.61. Bromide concentrations in MW-8D (injection well); injection concentration ~ 500 mg/L, August 2, 2004, day 0.

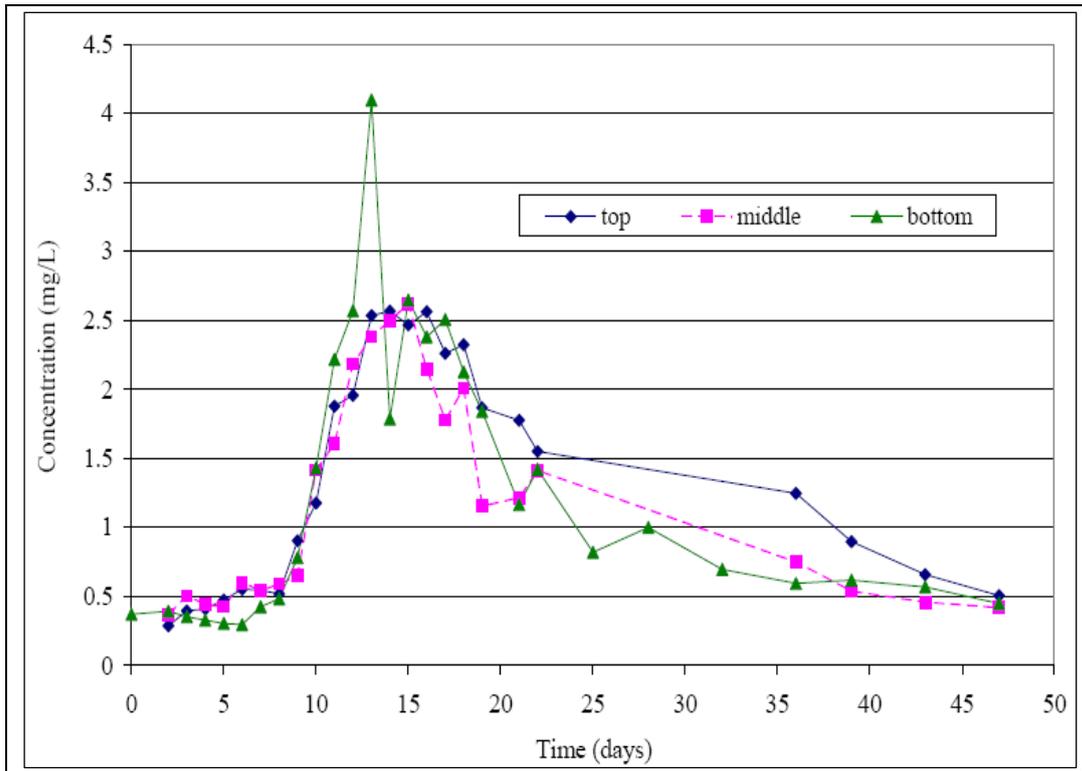


Figure 3.62. Bromide concentrations in MW-9D, 10 feet from injection well MW-8D; injection concentration ~500 mg/L, August 2, 2004, day 0.

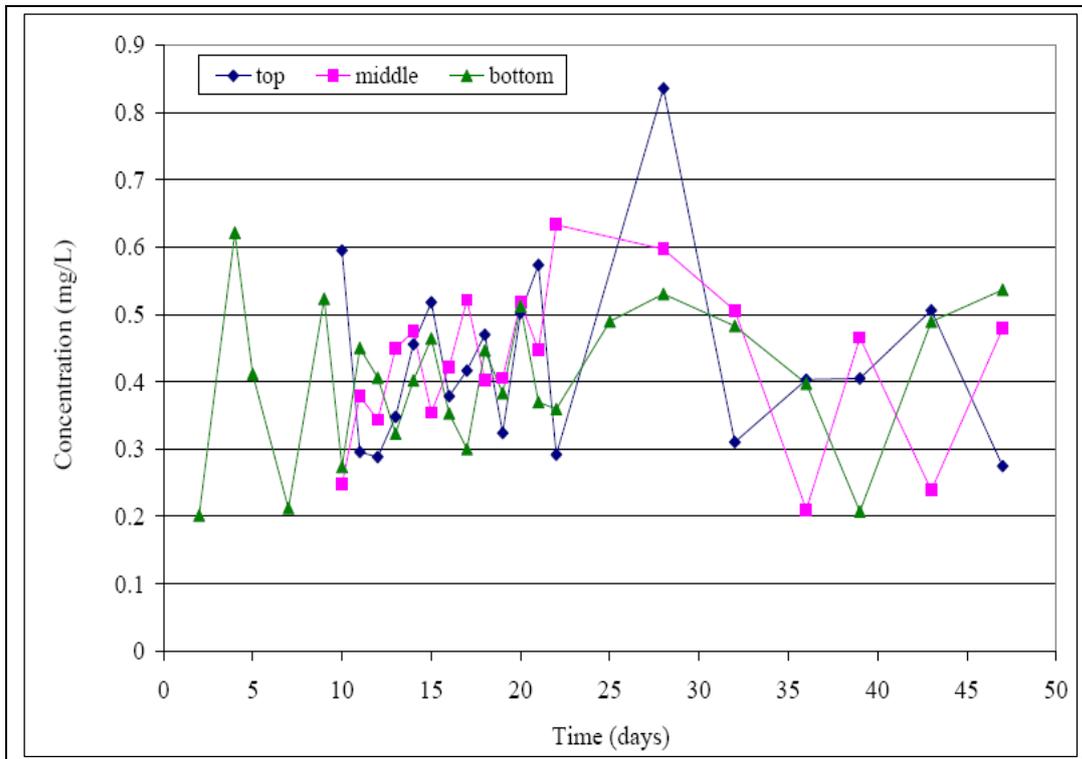


Figure 3.63. Bromide concentrations in MW-10D, 20 feet from injection well MW-8D; injection concentration ~500 mg/L, August 2, 2004, day 0.

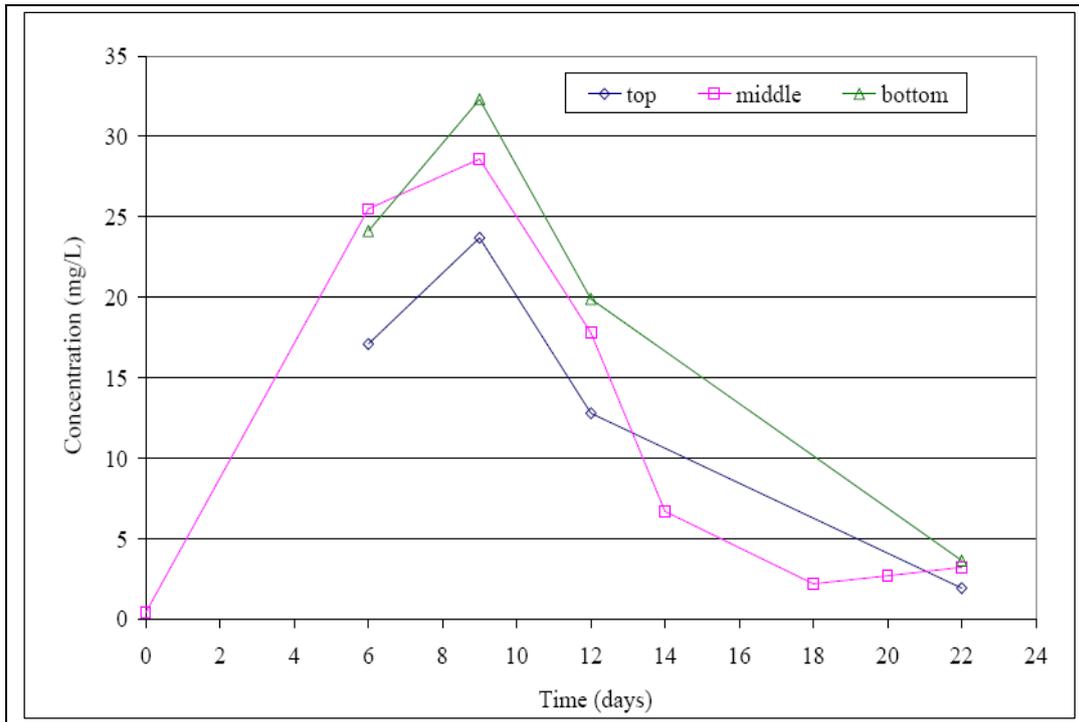


Figure 3.64. Bromide concentrations in MW-9S, (10 ft) east from the injection well MW-8S, injection concentration is ~500 mg/L, 9/16/2004.

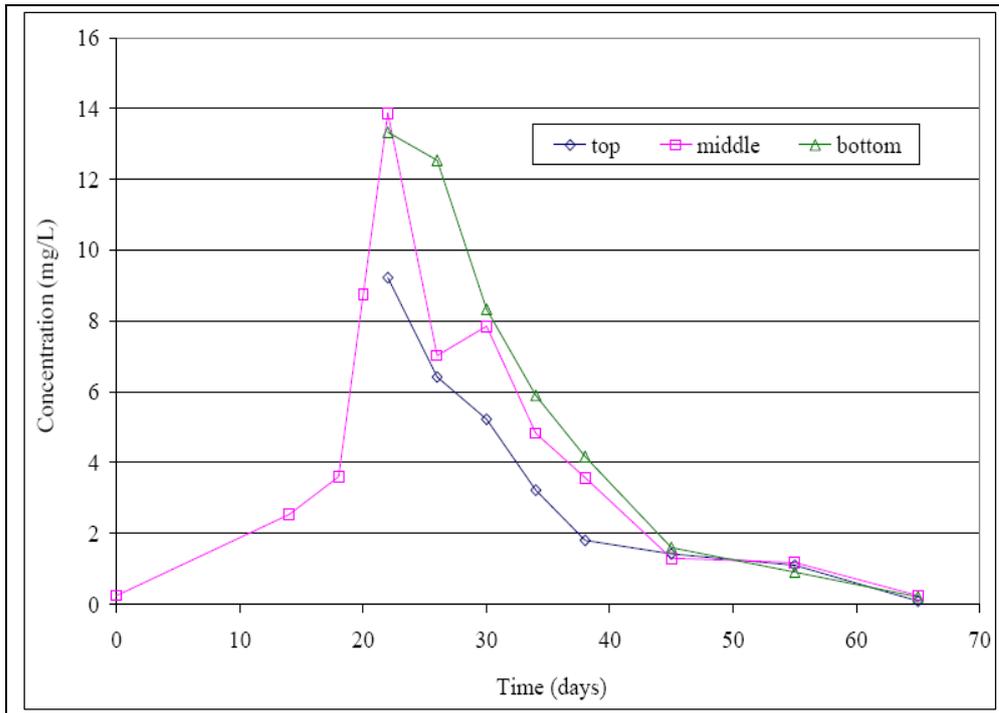


Figure 3.65. Bromide concentration in MW-10S, (20 ft) east from injection well MW-8D, injection concentration is ~500 mg/L, 9/15/2004.

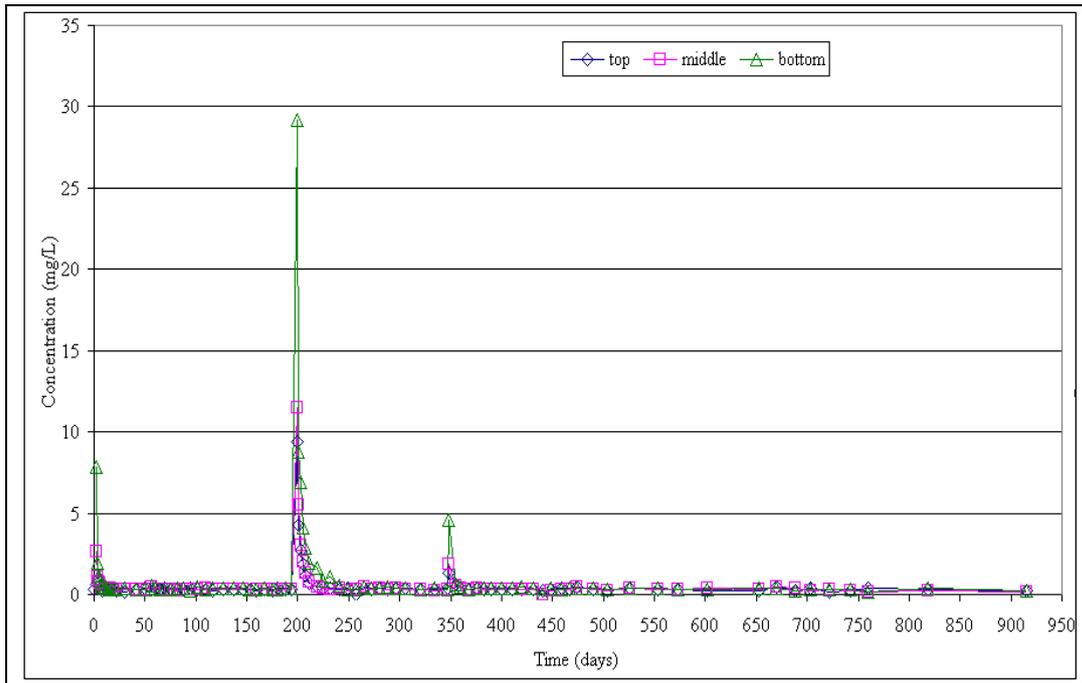


Figure 3.66. Bromide concentrations in MW-8S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).

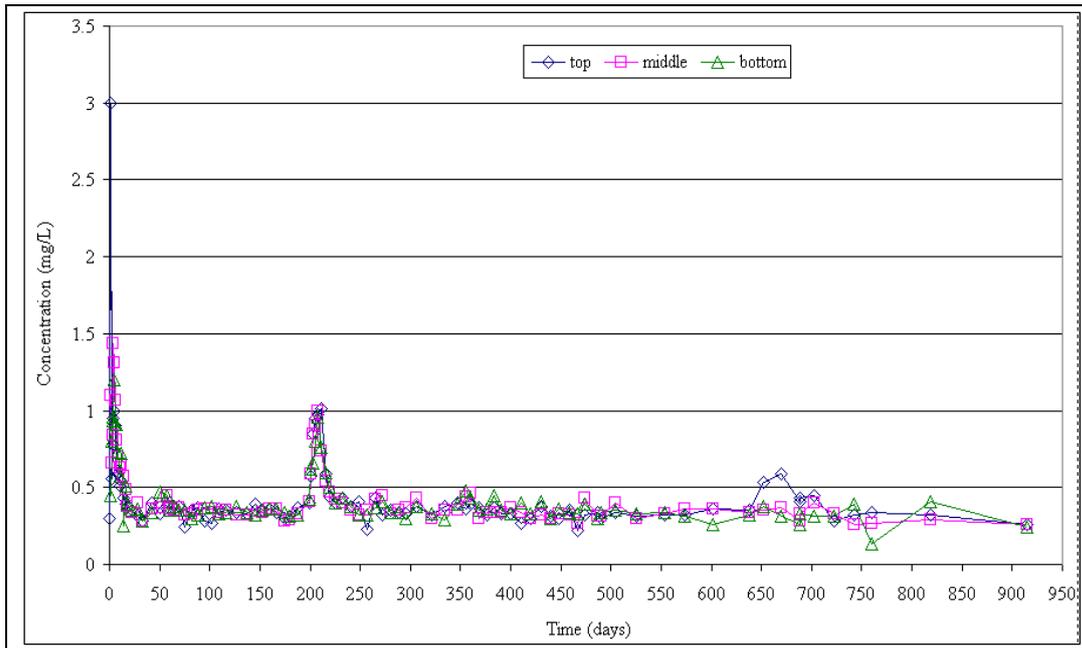


Figure 3.67. Bromide concentration in MW-9S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).

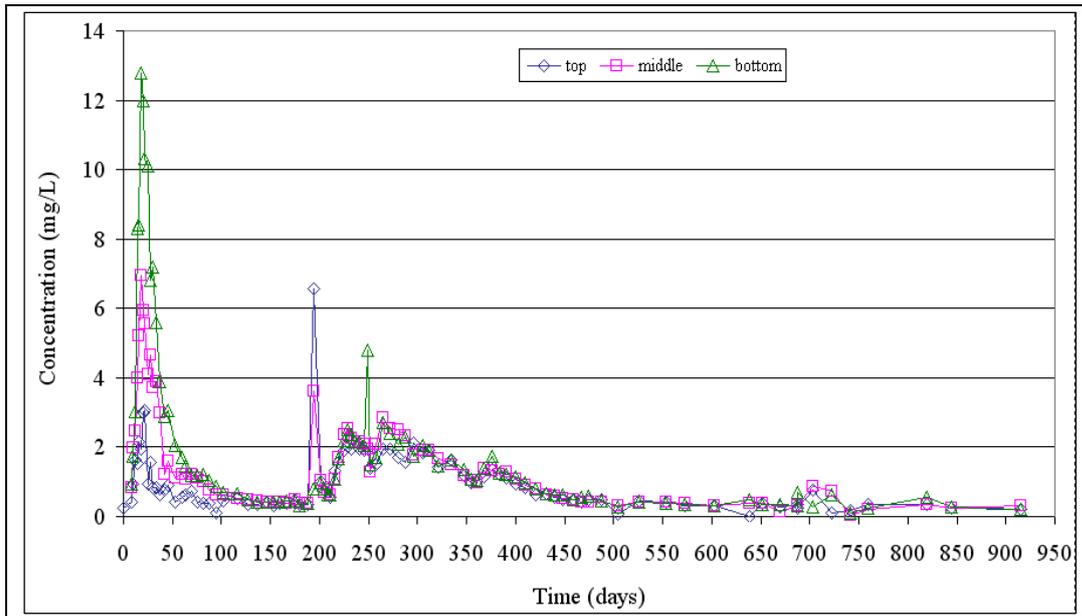


Figure 3.68. Bromide concentration in MW-10S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).

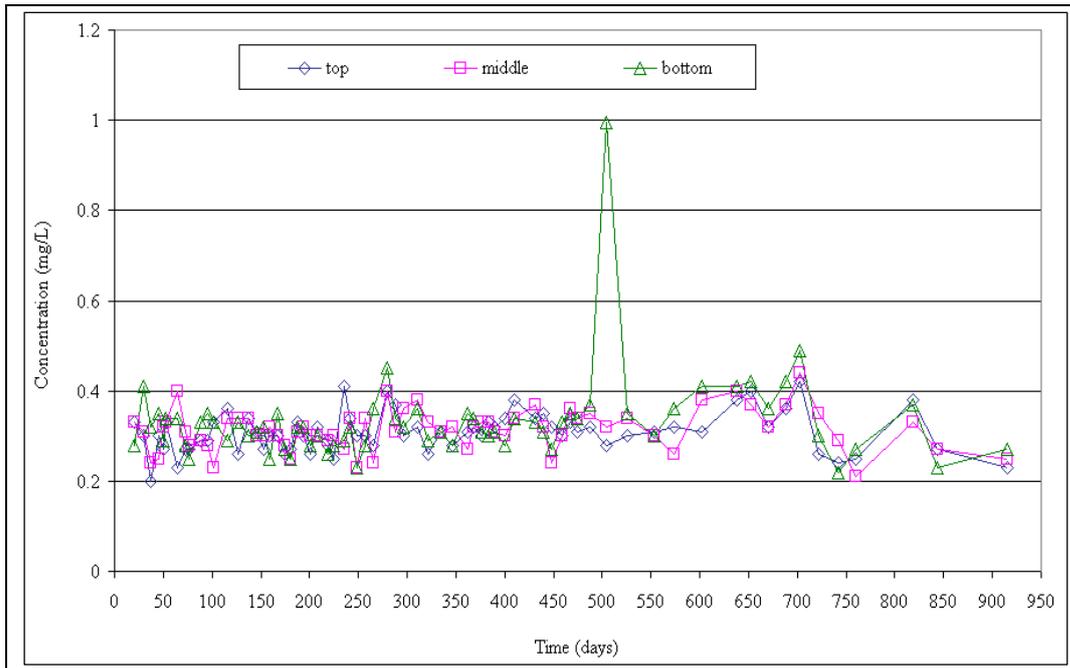


Figure 3.69. Bromide Concentration in MW-12S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).

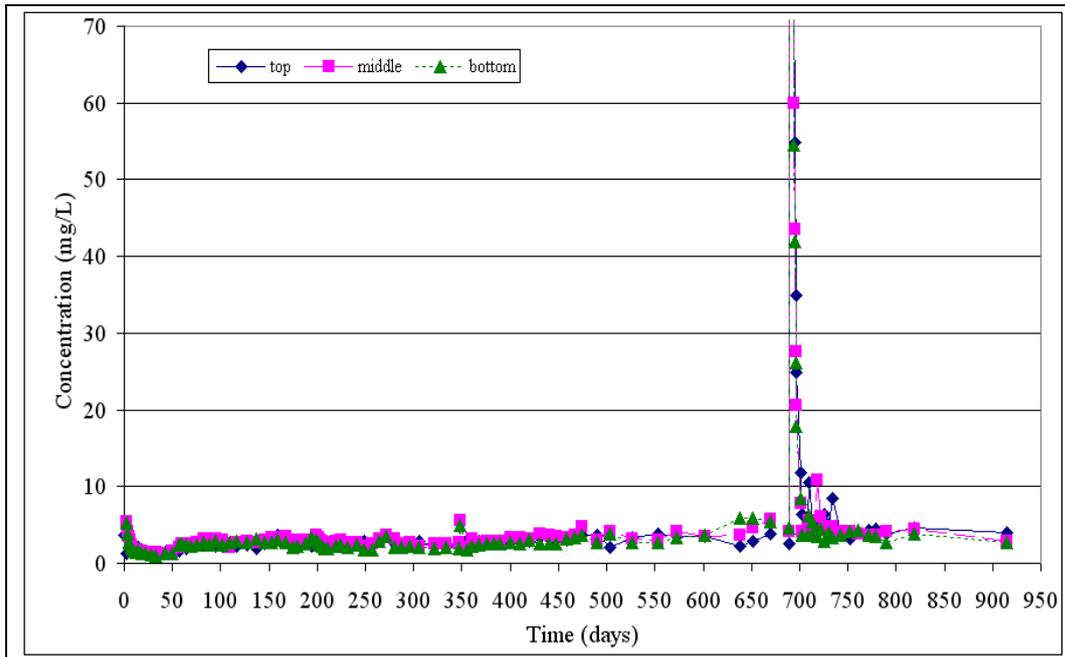


Figure 3.70. Bromide concentration in MW-8D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).

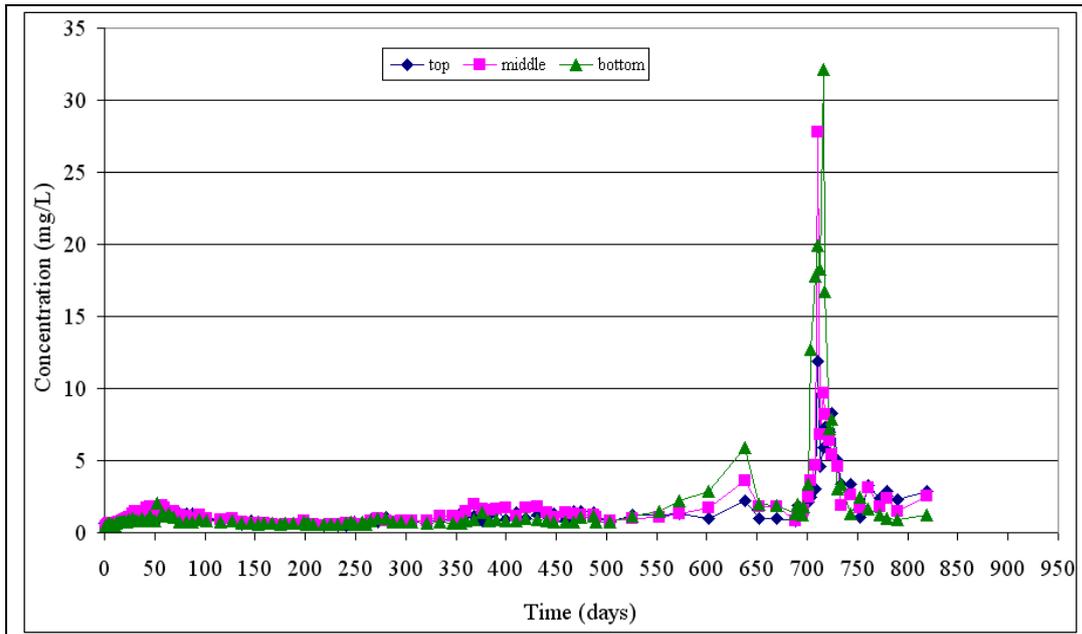


Figure 3.71. Bromide concentration in MW-9D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).

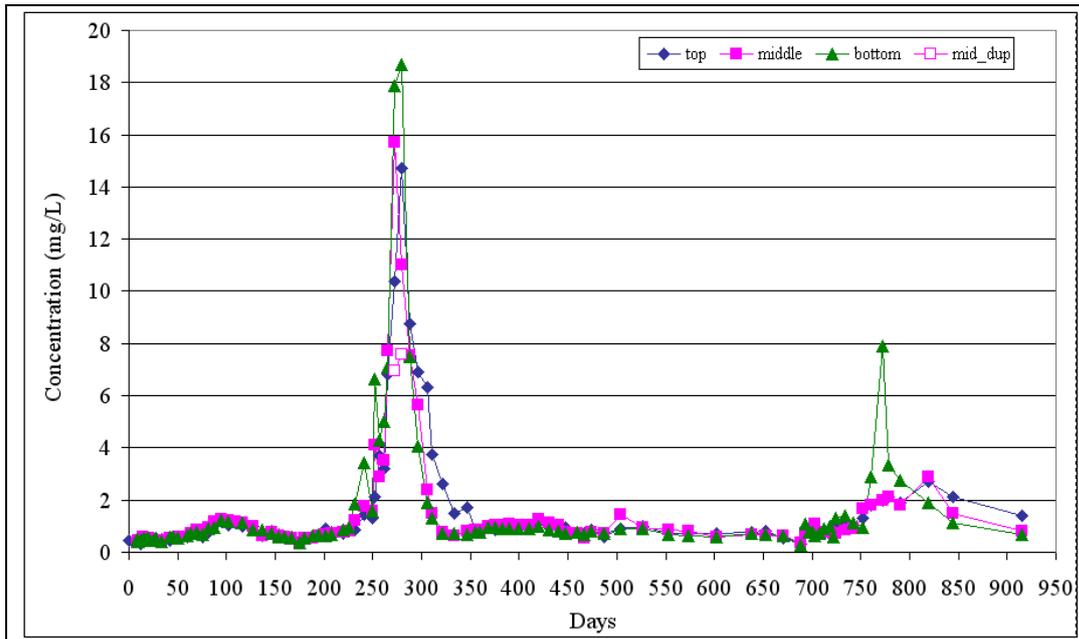


Figure 3.72. Bromide concentration in MW-10D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).

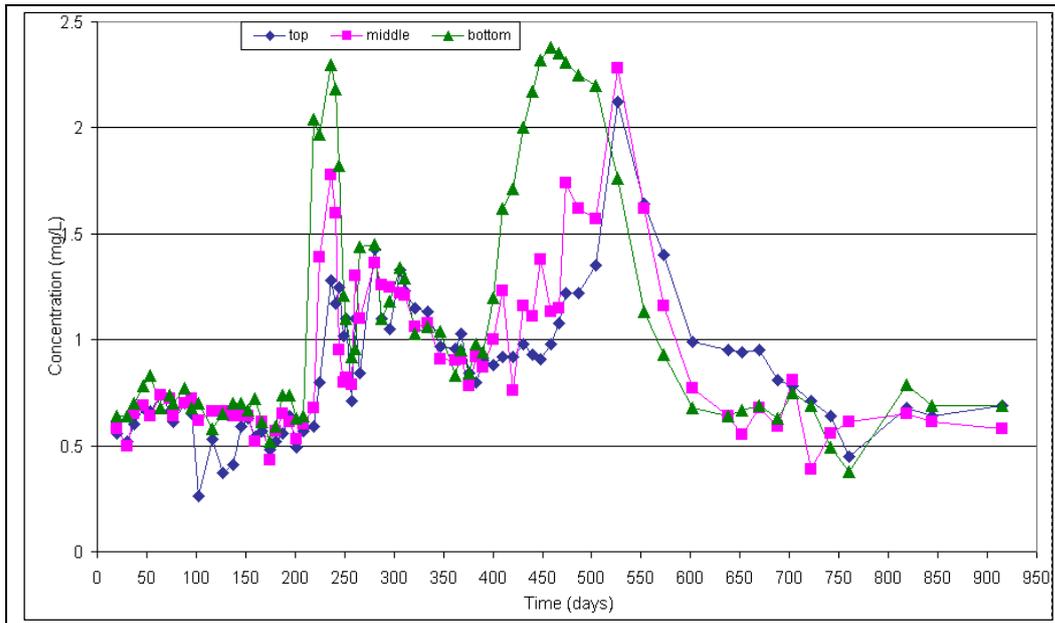


Figure 3.73. Bromide concentration in MW-12D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).

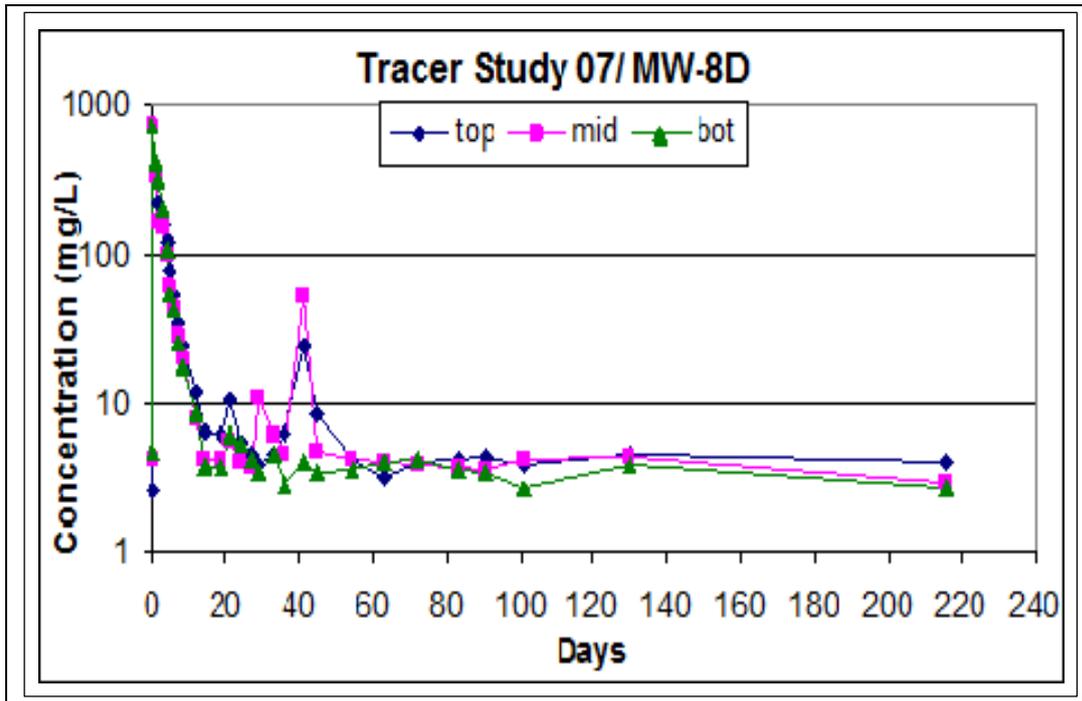


Figure 3.74. Bromide concentration in MW-8D, (injection well), injection concentration is ~817 mg/L, 7/11/2007.

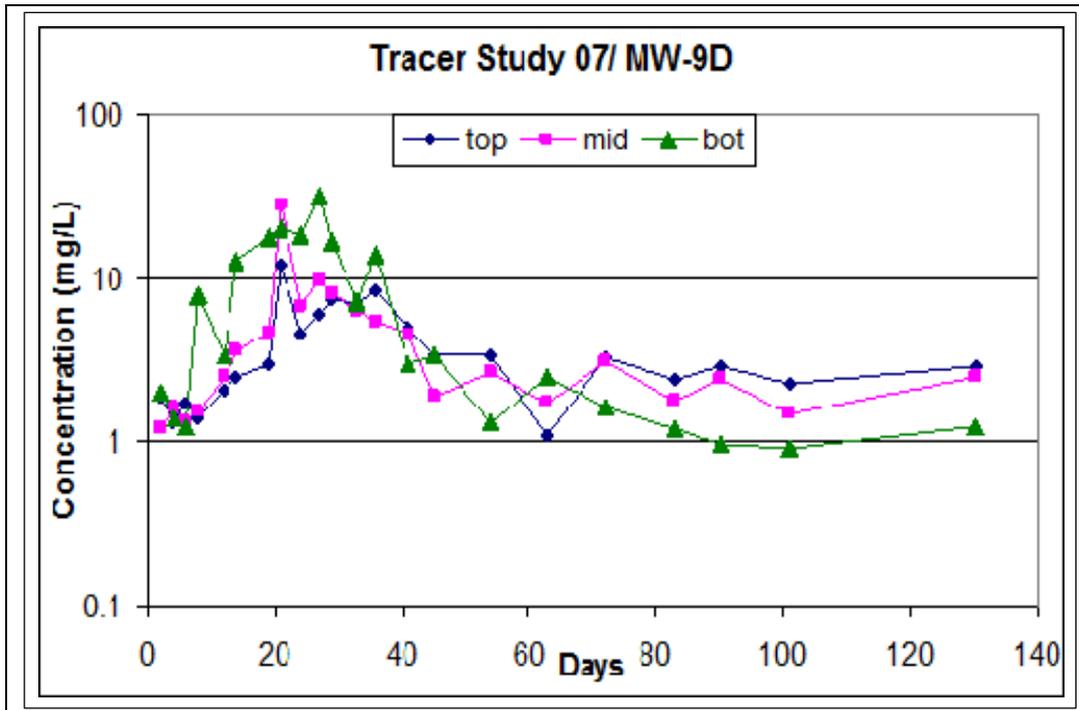


Figure 3.75. Bromide concentration in MW-9D, (injection well), (10 ft) east from the injection well MW-8D, injection concentration is ~817 mg/L, 7/11/2007.

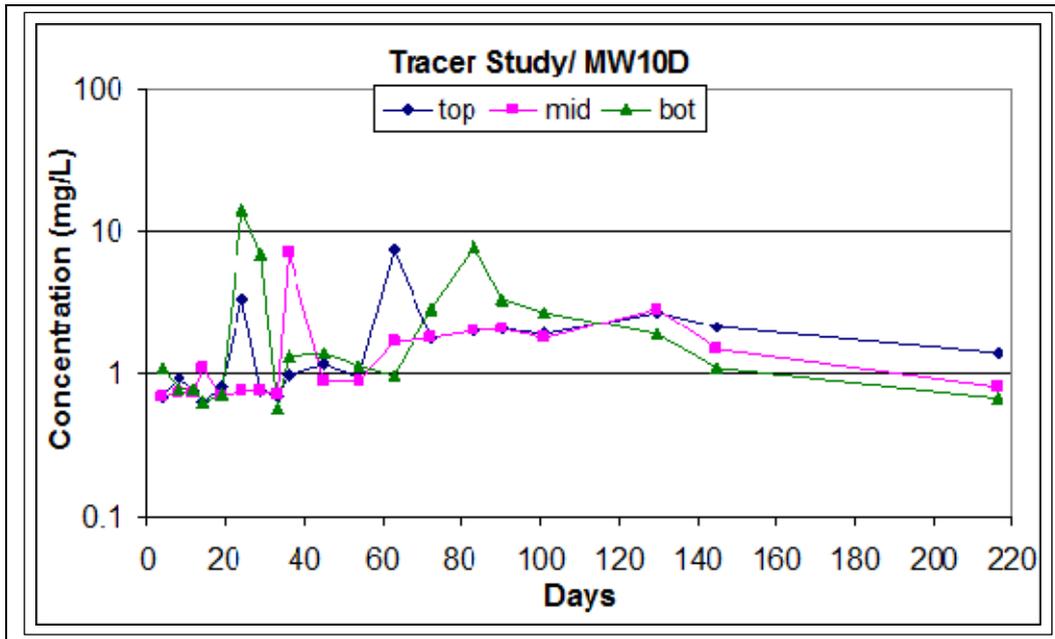


Figure 3.76. Bromide concentration in MW-10D, (injection well), (20 ft) east from the injection well MW-8D, injection concentration is ~817 mg/L, 7/11/2007.

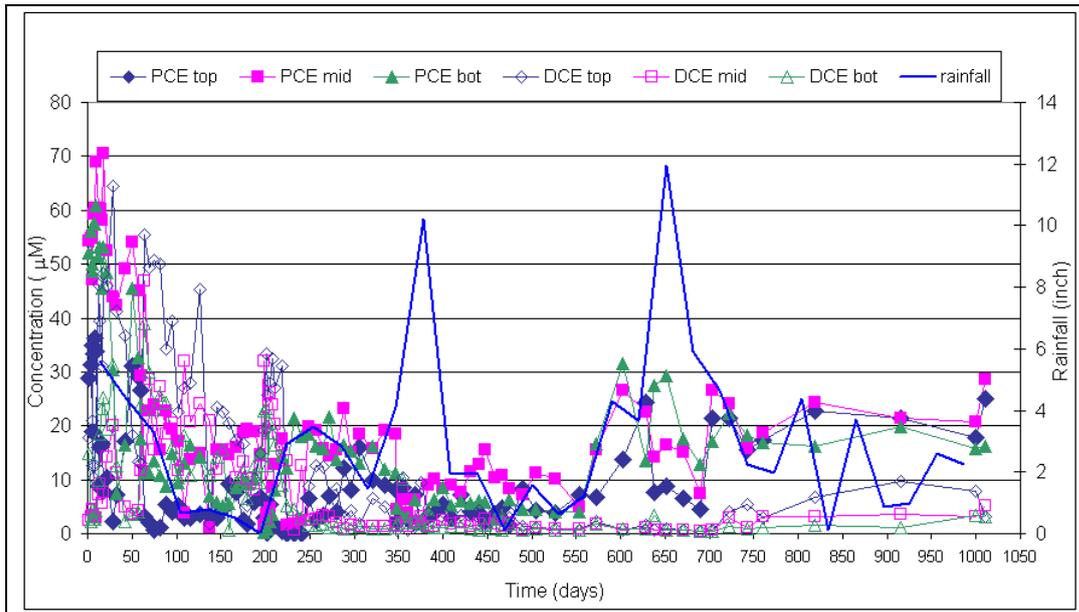


Figure 3.77. Concentrations PCE and DCE in MW-8D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).

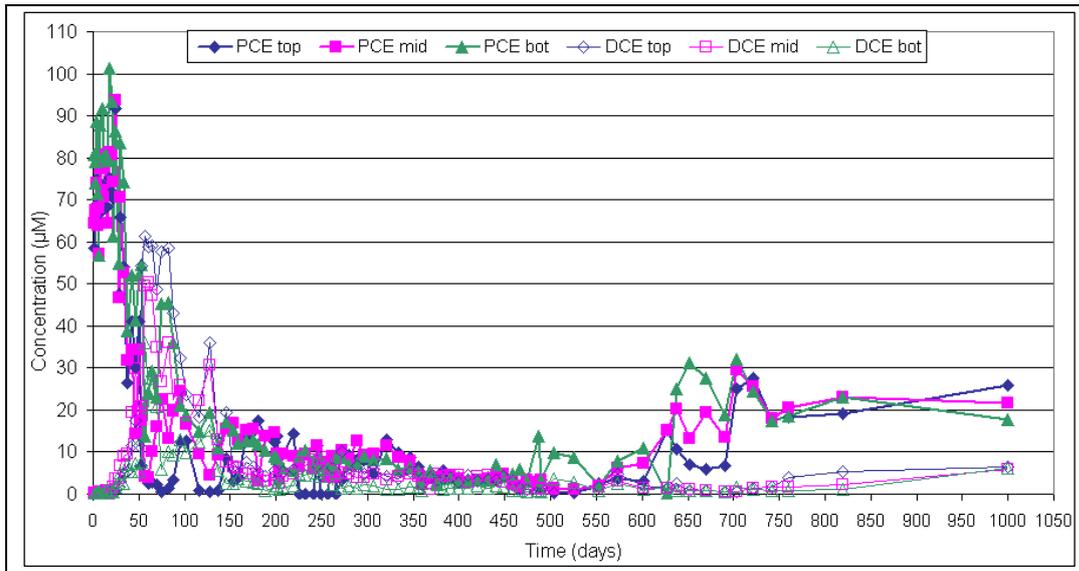


Figure 3.78. Chlorinated ethenes concentrations in MW-9D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).

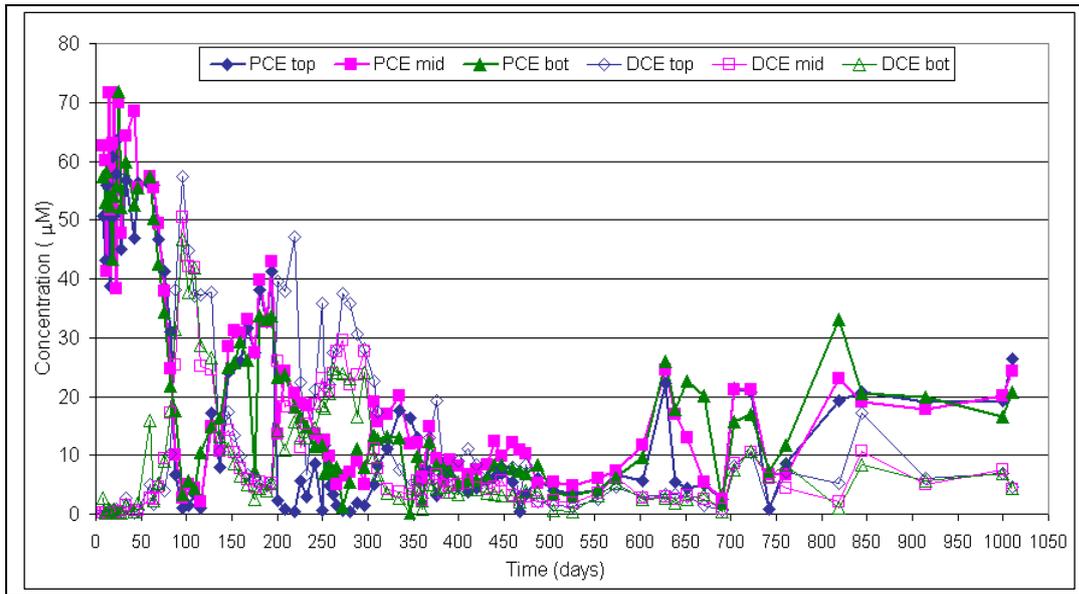


Figure 3.79. Chlorinated ethenes concentrations in MW-10D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).

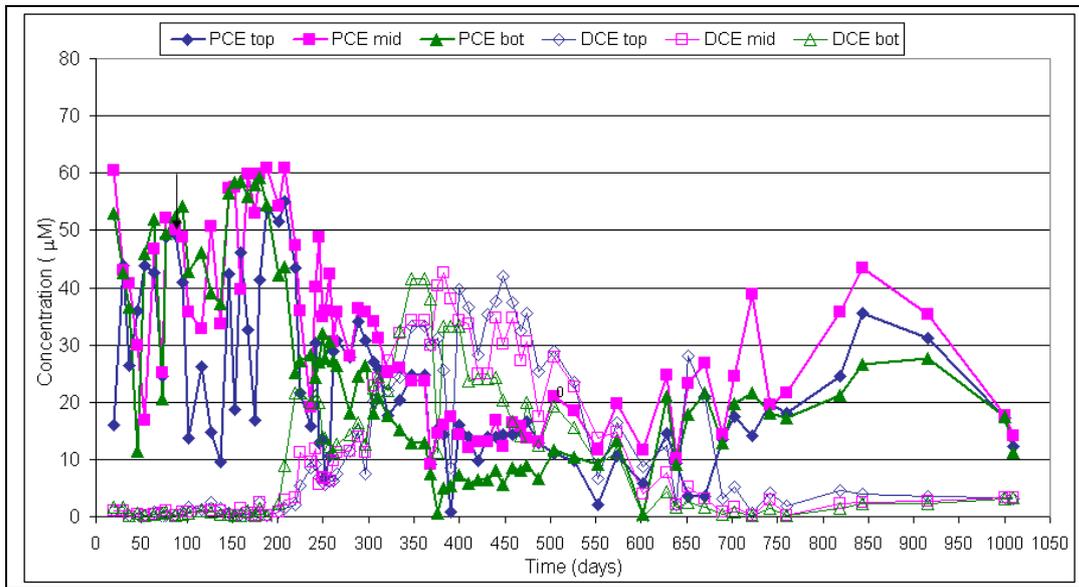


Figure 3.80. Chlorinated ethenes concentrations in MW-12D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).

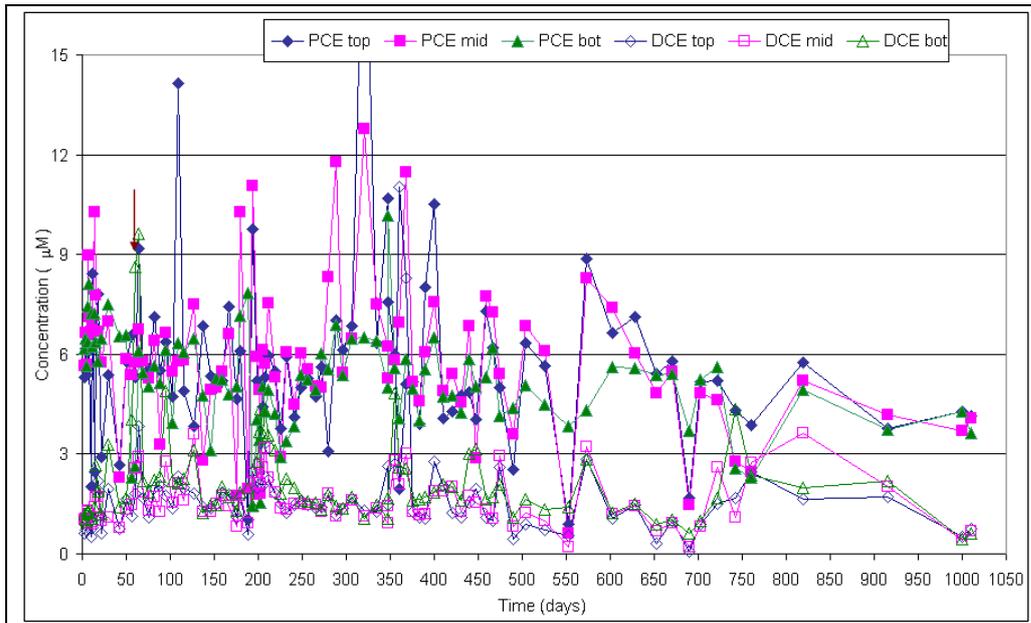


Figure 3.81. Chlorinated ethenes concentrations in MW-8S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).

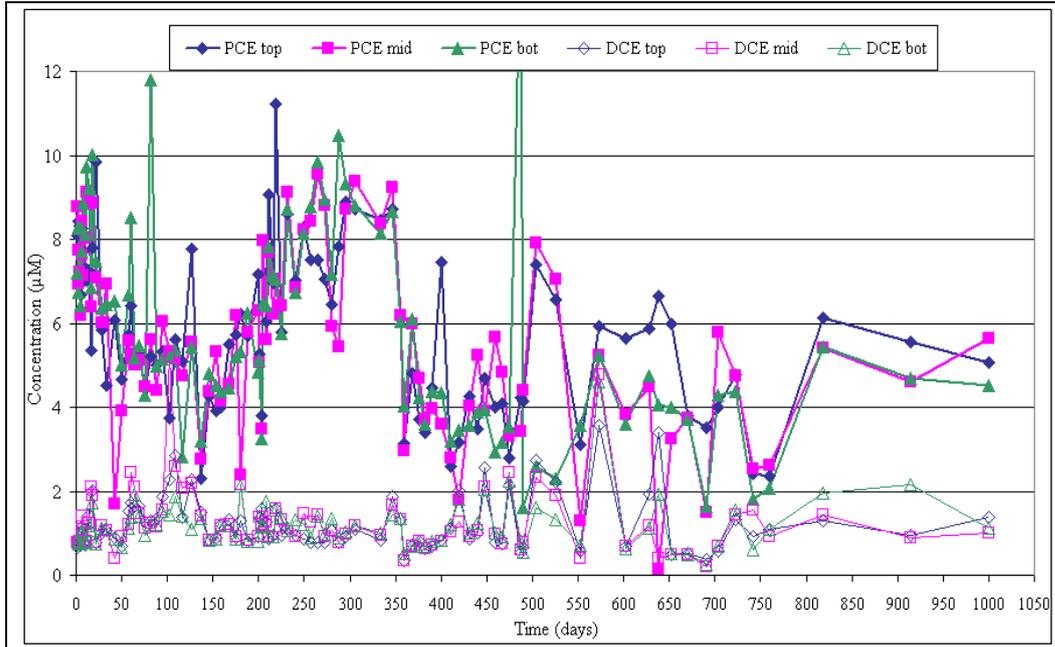


Figure 3.82. Chlorinated ethenes concentration in MW-9S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).

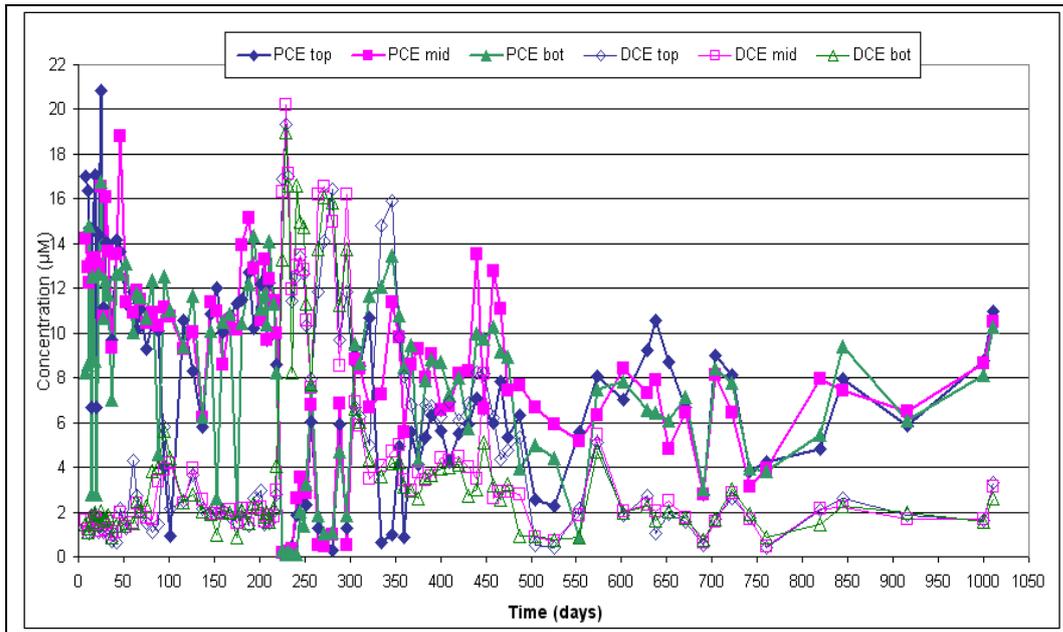


Figure 3.83. Chlorinated ethenes concentrations in MW-10S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).

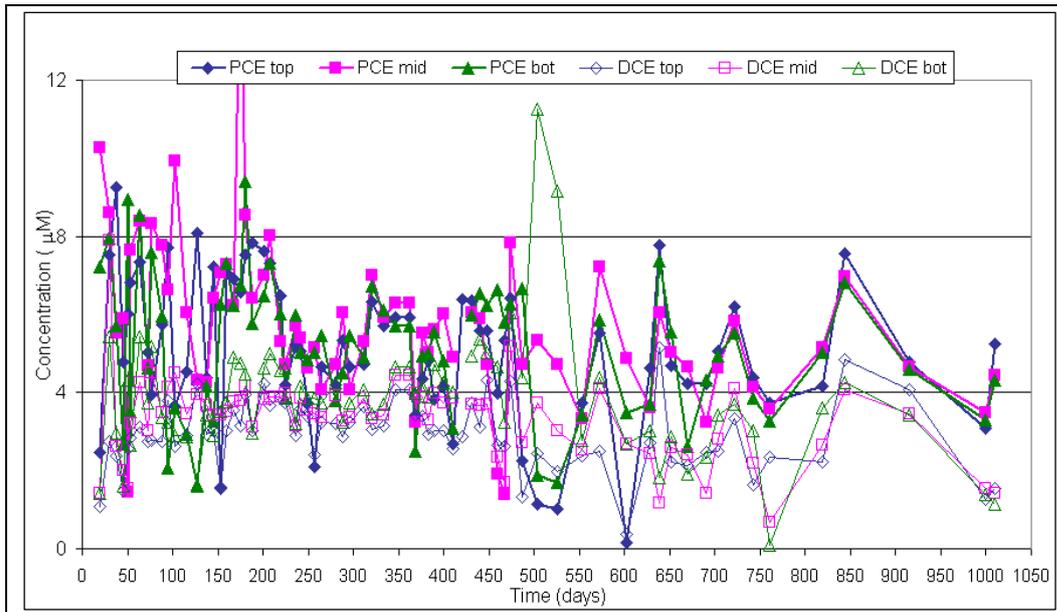


Figure 3.84. Chlorinated ethenes concentrations in MW-12S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), (injected bromide concentration = 670 mg/L); injection of nutrients and KB-1 on day 56 (Oct 13, 2005); injection of nutrients on day 197 (Mar 3, 2006), (Injected bromide concentration = 1340 mg/L); injection of nutrients on day 348 (Aug 1, 2006); and injection of Bromide tracer in MW-8D on day 689 (July 11, 2007), (Injection bromide concentration = 817 mg/L).

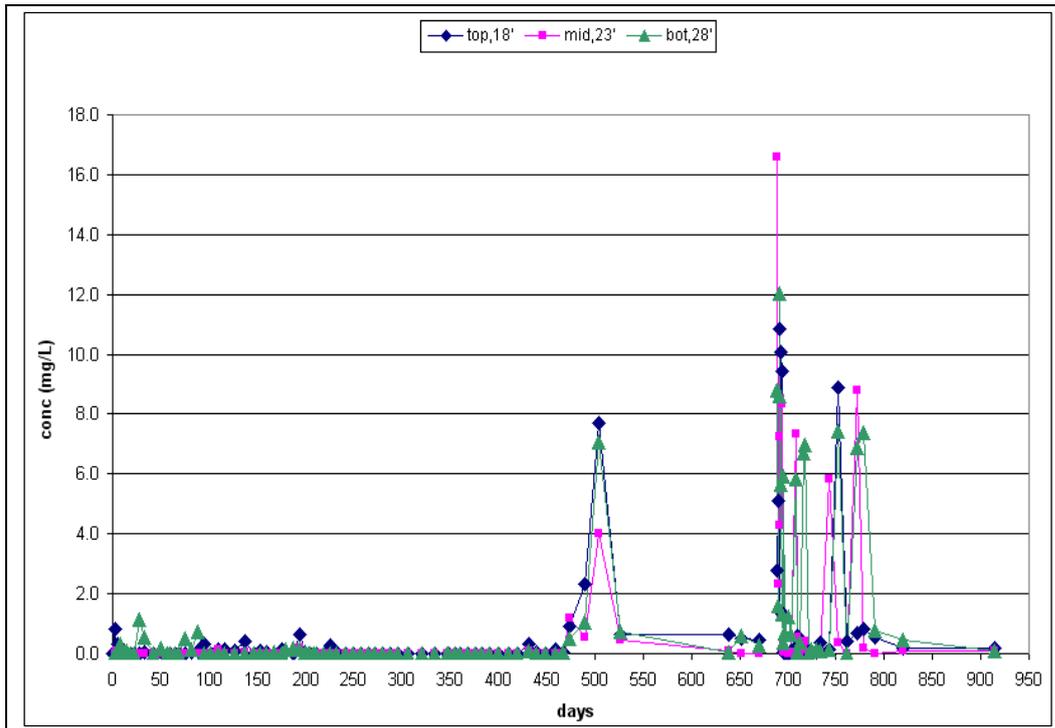


Figure 3.85. Nitrate Concentration in MW-8D; Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), day 56 (Oct 13, 2005), day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).

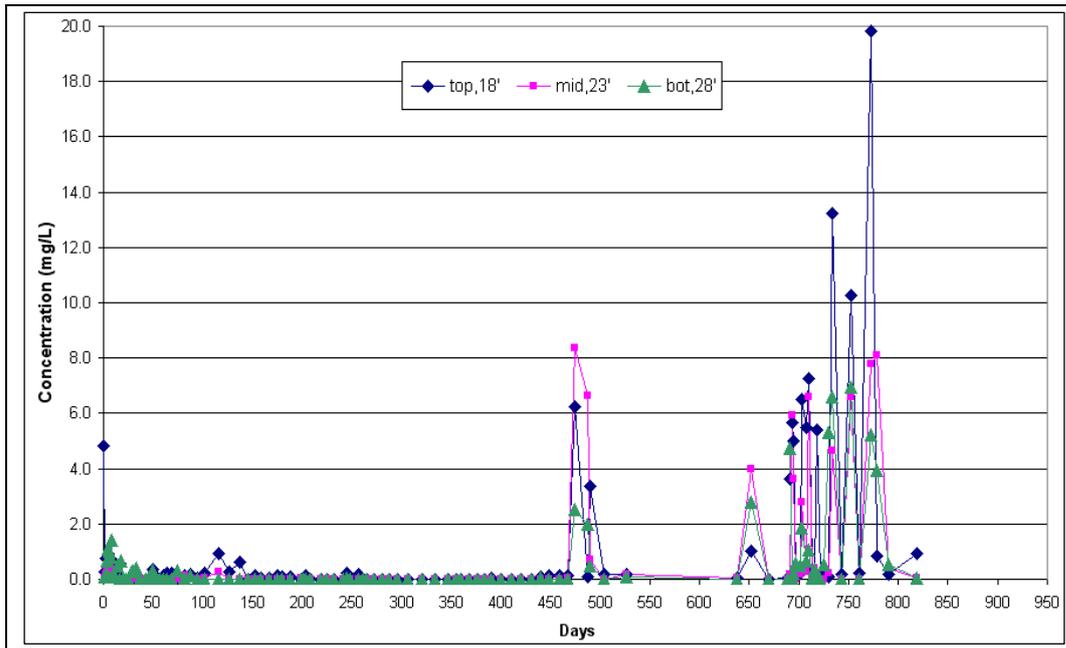


Figure 3.86. Nitrate concentration in MW-9D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), day 56 (Oct 13, 2005), day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).

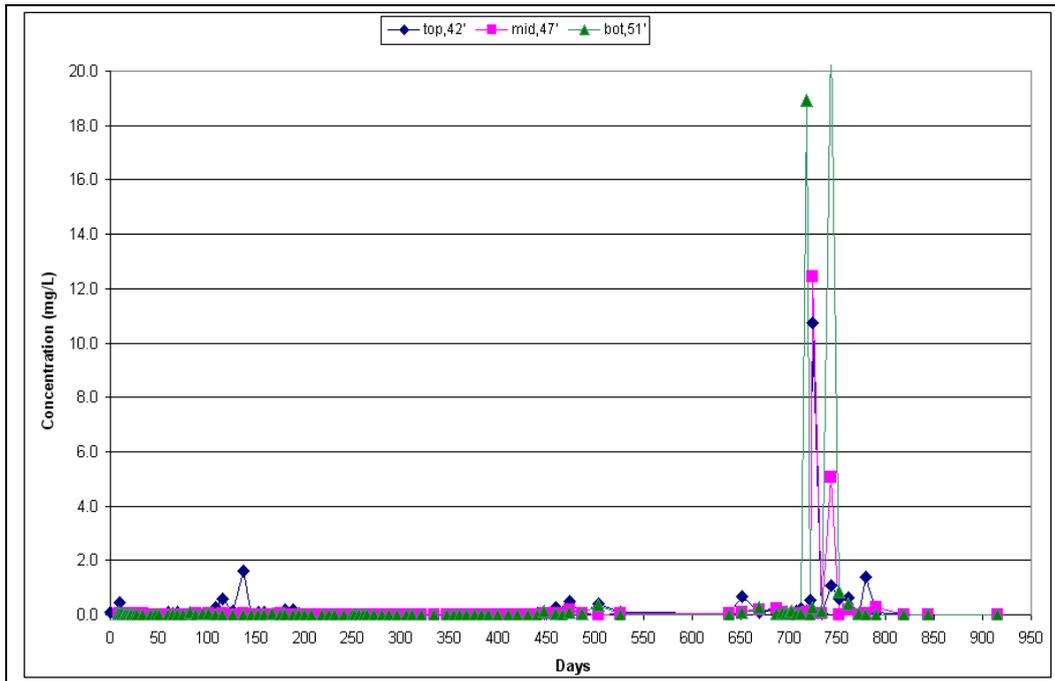


Figure 3.87. Nitrate concentration in MW-10D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), day 56 (Oct 13, 2005), day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).

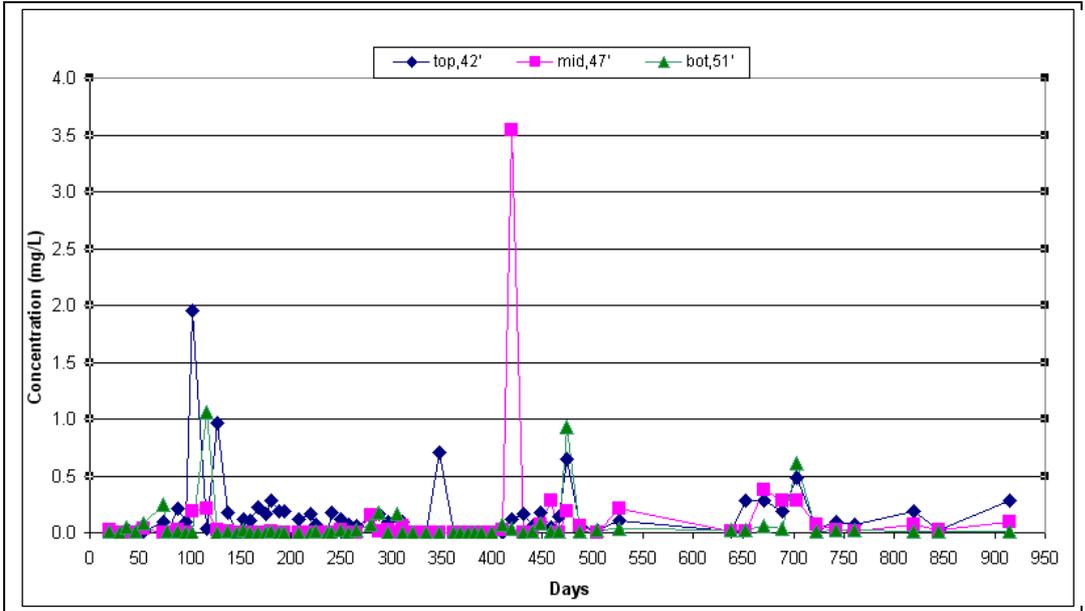


Figure 3.88. Nitrate concentration in MW-12D. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), day 56 (Oct 13, 2005), day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).

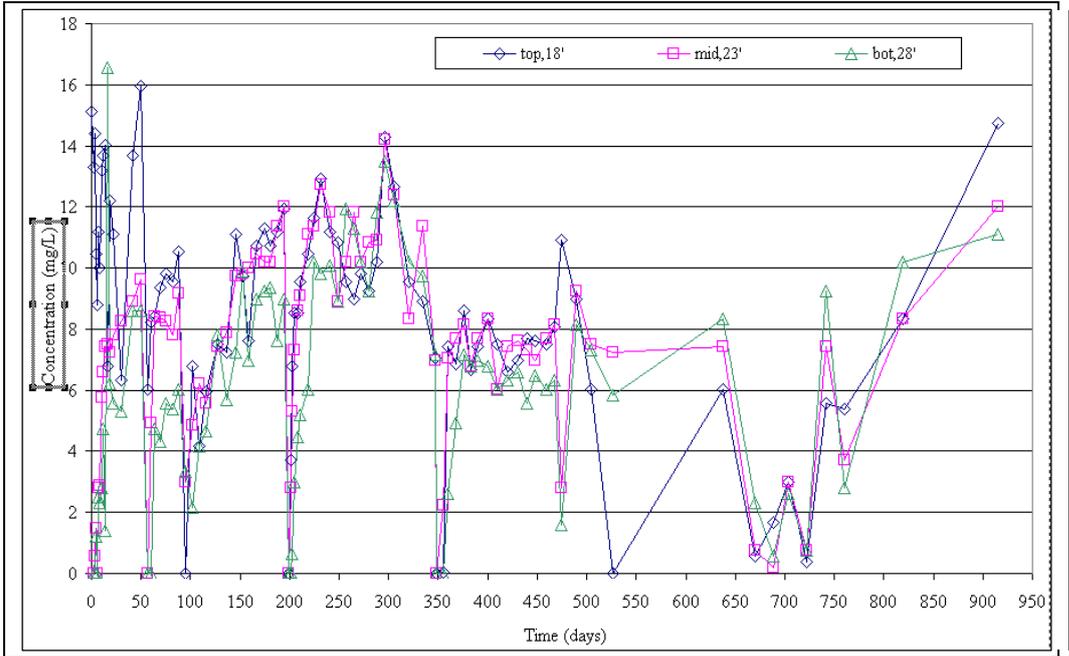


Figure 3.89. Nitrate concentrations in MW-8S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), day 56 (Oct 13, 2005), day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).

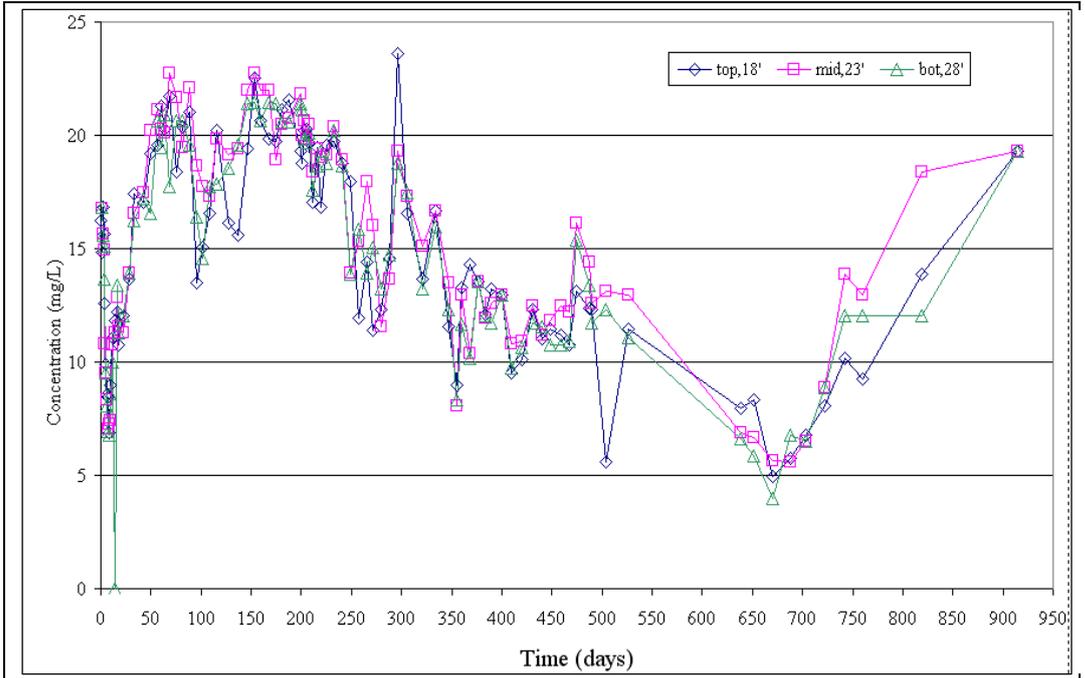


Figure 3.90. Nitrate concentration in MW-9S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), day 56 (Oct 13, 2005), day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).

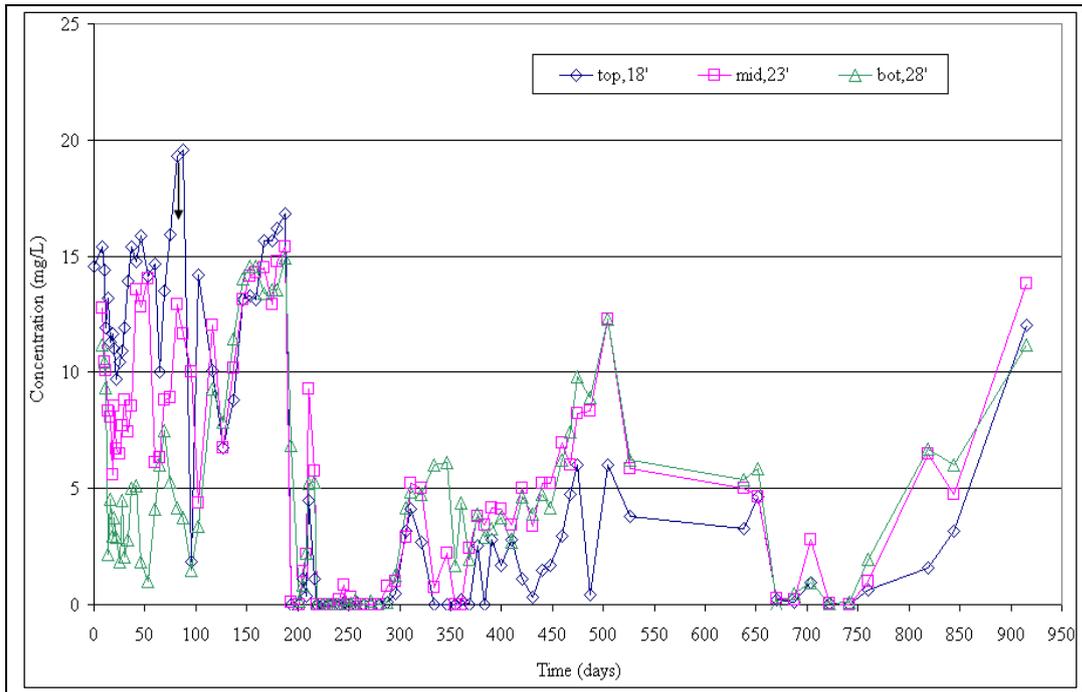


Figure 3.91. Nitrate Concentration in MW-10S. Injection of nutrients between MW-8 and MW-9 on day 0 (Aug 18, 2005), day 56 (Oct 13, 2005), day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).

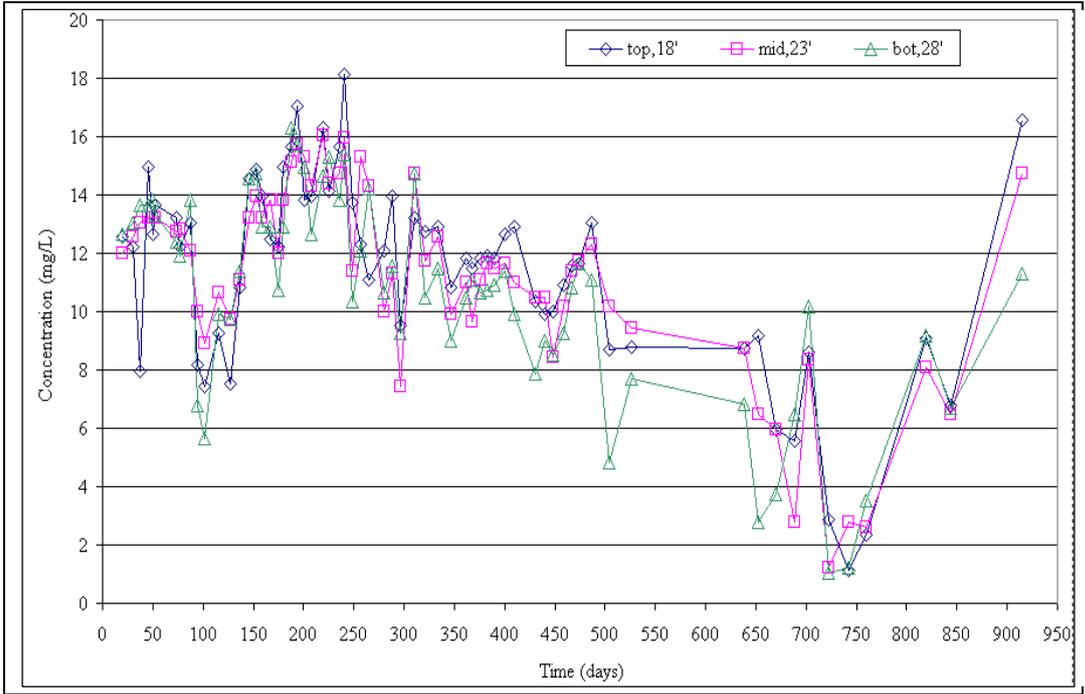


Figure 3.92. Nitrate concentration in MW-12S. Injection of nutrients between MW8 and MW-9 on day 0 (Aug 18, 2005), day 56 (Oct 13, 2005), day 197 (Mar 3, 2006) and day 348 (Aug 1, 2006).

CHAPTER- 4 CONCLUSIONS AND FUTURE WORK

Conclusions

Laboratory Studies

Microcosms experiments indicate that SOME, glucose, lactate, yeast extract, and cheese whey are good electron donors for chlorinated ethene biodegradation. Biostimulation of native microbes results in PCE conversion to DCE, while bioaugmentation with KB-1 bacterial culture was necessary to promote complete degradation of PCE to the end products methane and ethene. Cheese whey provided a good source of carbon and vitamins and can be used in biodegradation studies, though, inhibition of bacterial activity with high cheese whey concentrations was observed. This was due to acid formation and below optimal pH for growth. Therefore, any use of cheese whey should be monitored in terms of concentration and perhaps frequency of addition. Adding small and frequent increments would work better than one bulk application that has a high chance of acidifying the aquifer. It seems that the complex nature of cheese whey would support microbial growth for a long time. It can be added monthly as recommended previously (Moretti, 2005 and AFCEE, 2004). Use of cheese whey will reduce cost of treatment, but this may not apply if pH control is needed. The soil has low buffering capacity at the pilot study area and therefore, careful optimal concentrations of cheese whey should be applied for remediation. Nutrients should be added to maintain KB-1 activity for long period; otherwise the microbial activity may be affected.

Field Studies

Soil tests at the pilot study area revealed that the subsurface is silty clay in the shallow zone grading to more silty sand soil in the deep zone. Tracer studies show that groundwater flow direction is toward the east in shallow and deep zones. Groundwater elevation is influenced by precipitation events. The hydraulic gradient is higher in the shallow zone than the deep zone and this may support the fact that ground water velocity is higher in the shallow zone than the deep zone. Two tracer studies, which were done during the bioremediation study, found that groundwater velocity decreased from 2004 to

2006 due to biomass growth near the injection sites. The results show that KB-1 was successfully established in this location as noted from the decrease in DCE and the total chlorinated ethenes concentrations. The rates of chlorinated ethene degradation in the field is slower than that of microcosms due to difference in temperature of groundwater of about 19 °C and the incubation temperature of microcosms at 23 °C that result in faster microbial growth and enhanced activity. When nutrient feeding was stopped CE concentrations remained low for a long time after the last nutrient feeding, suggesting that substrates used may have provided a long term hydrogen source at the site. The biomass formed earlier would provide a source of organic substrates that can be used to sustain active dehalorespiring organisms and reduce biomass volume. This can also be noted when the final tracer study was conducted, which shows that ground water velocity is approaching the initial state.

Ion analysis showed that nitrate concentrations vary with nutrient availability in the site. As organic substrates arrive they stimulate microbial growth that energetically prefers to use nitrate as an electron acceptor. This can be depicted from bromide data associating with substrate addition. There is a consistent matching of nutrient and bromide arrival with DCE formation and PCE disappearance, indicating low to no sorption of chlorinated ethenes to aquifer material.

The general trend of chlorinated ethenes at the pilot study area show a decrease in PCE, TCE and DCE concentrations but not to the required values below MCLs, perhaps due to mixing with other contaminated water coming from other paths. A rebound of chlorinated ethenes was noticed after nutrient application was terminated, but values did not reach the initial concentrations. In MW-5 the microbial composition seems to be shifting in favor of chlorinated ethenes degradation.

Future Work and Recommendations

Molecular application for the detection and quantification of bacterial species might be effective to determine the need for bioaugmentation along with microcosm settings. For example, fluorescent in-situ hybridization (FISH) can be used to quantify reductive dechlorinating organisms using oligonucleotide probes. Also the use of

degenerate gradient gel electrophoresis (DGGE) would increase the understanding of how community composition can be altered to improve the efficiency of electron donor utilization by dechlorinators. The prediction that community structure was shifted in MW-5 can be investigated and compared with KB-1 culture. This kind of study can be expanded to monitor microbial community composition during each dechlorination step, and determine the dependence of dechlorinating organisms on each other in mixed culture.

Further work is needed with DNAPL phase to study the effect of various nutrients on the dissolution and solubilization of DNAPLs as a start to study feasibility of bioaugmentation for source area bioremediation.

At Cinderella dry cleaner site bioremediation of dissolved plume would require more injection wells to be installed between MW-2 and MW-11 to intercept the total width of the plume. Substrate should be added in small frequent increments rather than one bulk addition to minimize rapid biofilm growth and therefore, prevent clogging of the injection site. Cheese whey would be an effective alternative that can be applied at appropriate concentration.

CHAPTER 5 – REFERENCES

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