

RECLAMATION

Managing Water in the West

Final Report for the Enhanced Anaerobic Bioremediation Pilot Test

Bountiful/Woods Cross Superfund Site, Bountiful, Utah



U.S. Department of the Interior
Bureau of Reclamation
Technical Service Center
Denver, Colorado

December 2006

MISSION STATEMENTS

The mission of the Department of the Interior is to protect and provide access to our Nation's natural and cultural heritage and honor our trust responsibilities to Indian Tribes and our commitments to island communities.

The mission of the Bureau of Reclamation is to manage, develop, and protect water and related resources in an environmentally and economically sound manner in the interest of the American public.

Final Report for the Enhanced Anaerobic Bioremediation Pilot Test

Bountiful/Woods Cross Superfund Site, Bountiful, Utah



**U.S. Department of the Interior
Bureau of Reclamation
Technical Service Center
Denver, Colorado**

December 2006

Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test:
Table of Contents

<i>section</i>	<i>page</i>
Executive Summary	1-1
1. Introduction	1-1
1.1 Project and Regulatory Background	1-1
1.2 Objectives	1-1
1.3 Technical Approach	1-2
1.3.1 Electron Donor	1-2
1.3.1.1 Aqueous Electron Donors	1-3
1.3.1.2 Slow Release Donors	1-3
1.3.2 Dechlorinating Bacteria	1-4
1.4 Report Organization	1-4
2. Summary of Activities	2-1
3. Electron Donor Delivery and Distribution	3-1
3.1 Delivery	3-1
3.1.1 Sodium Lactate	3-1
3.1.2 Emulsified Oil Substrate	3-2
3.1.3 Chitin	3-2
3.2 Distribution	3-2
3.2.1 Sodium Lactate	3-3
3.2.2 Emulsified Oil Substrate	3-6
3.2.3 Chitin	3-9
3.2.4 Summary of Distribution	3-12
4. EAB Results	4-1
4.1 Redox Conditions	4-1
4.1.1 Oxidation-Reduction Potential (ORP)	4-1
4.1.2 Inorganic Electron Acceptors	4-3
4.1.2.1 Ferrous Iron	4-3
4.1.2.2 Sulfate	4-5
4.1.2.3 Methane	4-7
4.1.2.4 Methane Splits	4-9
4.1.3 Redox Summary	4-10

**Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test**

4.2	Dechlorination	4-10
4.2.1	Sodium Lactate Cell.....	4-11
4.2.2	Emulsified Oil Substrate Cell	4-13
4.2.3	Chitin Cell	4-16
4.2.4	Ethene Splits	4-19
4.2.5	HMW2S and HMW10S Wells.....	4-20
4.2.6	Dechlorination Summary	4-22
4.3	Bioaugmentation	4-22
5.	Pilot Scale Summary and Conclusions	5-1
6.	Full-Scale Implementation of EAB	6-1
6.1	Selected Electron Donor	6-1
6.2	Overview of Remedy	6-1
6.3	Injection Strategy	6-3
6.4	Bioaugmentation	6-3
6.5	Groundwater Monitoring	6-4
References	7-1

Final Report for the Enhanced Anaerobic Bioremediation Pilot Test: **Figures and Tables**

<i>figure</i>		<i>page</i>
2-1	Pilot Study Layout	2-3
3-1A	SLMU Organic Acids	3-4
3-1B	SLM1 Organic Acids	3-5
3-1C	SLM2 Organic Acids	3-5
3-2A	OSMU Organic Acids	3-7
3-2B	OSM1 Organic Acids	3-7
3-2C	OSM2 Organic Acids	3-8
3-2D	OSM3 (HMW3S) Organic Acids	3-8
3-3A	CMU Organic Acids	3-10
3-3B	CM1 Organic Acids	3-10
3-3C	CM2 Organic Acids	3-11
3-3D	CM3 Organic Acids	3-11
4-1A	SLM Cell ORP	4-2
4-1B	OSM Cell ORP	4-2
4-1C	CM Cell ORP	4-3
4-2A	SLM Cell Ferrous Iron	4-4
4-2B	OSM Cell Ferrous Iron	4-4
4-2C	CM Cell Ferrous Iron	4-5
4-3A	SLM Cell Sulfate	4-6
4-3B	OSM Cell Sulfate	4-6
4-3C	CM Cell Sulfate	4-7
4-4A	SLM Cell Methane	4-8
4-4B	OSM Methane	4-8
4-4C	CM Methane	4-9
4-5A	SLMU VOC	4-12
4-5B	SLM1 VOC	4-12
4-5C	SLM2 VOC	4-13
4-6A	OSMU VOC	4-14
4-6B	OSM1 VOC	4-15
4-6C	OSM2 VOC	4-15
4-6D	OSM3 VOC	4-16
4-7A	CMU VOC	4-17
4-7B	CM1 VOC	4-18
4-7C	CM2 VOC	4-18
4-7D	CM3 VOC	4-19

**Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test**

4-8A	HMW2S VOC.....	4-21
4-8B	HMW10S VOC.....	4-21
4-9	Q-PCR Results for Dehalococcoides 16s rRNA gene at Bountiful OU1.....	4-23
6-1	Conceptual Full Scale EAB Layout.....	6-5
<i>table</i>		<i>page</i>
4-1	Comparison of Methane data from Utah State and Microseeps	4-10
4-2	Comparison of Ethene data from Utah State and Microseeps	4-20

Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test:
Abbreviations and Acronyms

ACI	American Concrete Institute
BOR	Bureau of Reclamation
CDM	Camp, Dresser, and McKee, Inc.
CM	chitin monitoring
COCs	chloroethenes
DCE	dichloroethene
DNA	dense non-aqueous
OSM	emulsified oil substrate monitoring
EAB	enhanced anaerobic bioremediation
EPA	Environmental Protection Agency
gpm	gallons per minute
HMW#S	HatchCo monitoring well # shallow
HMW#D	HatchCo monitoring well # deep
L	liter
MDL	method detection limit
mg/L	milligrams per liter
mL	milliliter
mV	millivolts
Q-PCR	quantitative polymerase chain reaction
RPD	relative percent difference
TCE	trichloroethene
ORP	oxidation reduction potential
OU	operable unit
PCE	tetrachloroethene/perchloroethene
RI	remedial investigation
RNA	ribonucleic acid

**Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test**

Site	Bountiful/Woods Cross Superfund Site
SLI	sodium lactate injection
SLM	sodium lactate monitoring
VC	vinyl chloride
VOCs	volatile organic compounds

Final Report for the Enhanced Anaerobic Bioremediation Pilot Test: **Executive Summary**

Pursuant to the Proposed Plan for Operable Unit 1 (OU-1) of the Bountiful/Woods Cross/5th South Superfund Site (Site) in southern Davis County Utah, a pilot test of enhanced anaerobic bioremediation (EAB) was performed from July 2005 through August 2006. Based on the results of the pilot test, this technology has been selected for the Record of Decision (ROD).

The pilot test consisted of three side-by-side treatment cells designed to compare the performance of three different electron donors at the Site. Sodium lactate, the aqueous electron donor was compared to two slow-release electron donors, emulsified oil and chitin. Based on preliminary DNA analysis, which showed that the indigenous biological community might be limited in its ability to perform complete dechlorination, each of the treatment cells was bioaugmented with a commercially available dechlorinating culture. The sodium lactate was injected monthly into a permanent 4-inch well; the emulsified oil was injected one time in three 1-inch temporary wells with pre-packed screens; and the chitin was injected one time via six temporary, direct-push injection points. The injection strategy for the lactate was effective initially, as donor was observed 10 feet from the injection point, but was reduced considerably (to less than 5 feet) by the end of the pilot test. This might have been due to the growth of the bacterial community utilizing lactate as electron donor. This suggests that the injection strategy for sodium lactate needs to be optimized to increase the extent of distribution. The chitin injection was problematic because the fittings on the direct-push rig were not designed to facilitate injection of the large particle size of chitin. As a result, a field decision was made to screen out these large particles, which resulted in only the smallest size fraction of the chitin being injected. This effectively reduced the distribution and longevity of chitin in the subsurface. The emulsified oil was easily injected and effectively distributed showing the highest concentrations of organic acids and was also the longest lived electron donor (projected to last approximately 2 years). The organic acids acetate, propionate, and butyrate were generated by all three electron donors.

All three treatment cells exhibited reducing conditions within 1 month of injection, and all eventually became methanogenic. *Dehalococcoides spp.* numbers increased dramatically in all treatment cells, and ethene production was achieved first with chitin, but was short-lived. Complete dechlorination was also achieved with lactate and emulsified oil, except in the monitoring well for emulsified oil that had the highest organic acid concentrations (OSM2, 5 feet

**Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test**

downgradient from the injection point). This well appeared to be directly impacted by the oil emulsion, which might have inhibited complete dechlorination.

Based on the results of the pilot test, emulsified oil (e.g., EOS®) is recommended as the electron donor to be used for the full-scale remediation at the Site.

Final Report for the Enhanced Anaerobic Bioremediation Pilot Test:

I. Introduction

A pilot test of enhanced anaerobic bioremediation was performed at Operable Unit 1 of the Bountiful/Woods Cross/5th South PCE Plume Superfund Site (Site) in southern Davis County Utah, approximately 10 miles north of Salt Lake City. This report presents the data collected and the data analysis for the pilot test. This introductory section provides an overview of the project background, pilot test objectives, technical approach, and document organization. Details are provided in subsequent sections.

1.1 Project and Regulatory Background

The Site includes two Operable Units: Operable Unit 1 (OU1), a trichloroethene (TCE) plume; and Operable Unit 2 (OU2), a perchloroethene (PCE) plume. The pilot test discussed in this report was performed near the source area of the OU1 TCE plume. A Remedial Investigation (RI) that characterized the Site and the contaminant plume was completed in 2003, and a Focused Feasibility Study to evaluate remedial alternatives was completed in 2004. Based on these documents EPA published a Proposed Plan for remediation of OU1 in 2004. The Proposed Plan identified a combination of Monitored Natural Attenuation and Enhanced In situ Biological/Chemical Remediation as the preferred alternatives. The potential need for a pilot test was also identified in the Proposed Plan. A work plan providing the pilot test design and preliminary full-scale design for the Enhanced In situ Remediation portion of the preferred alternative was prepared in May 2005. The pilot test described herein was performed in accordance with this work plan from July 2005 to August 2006.

1.2 Objectives

As presented in the work plan, the primary objective of the pilot test was to determine the site-specific requirements for full-scale implementation of the preferred alternative. Three specific objectives contribute to the overall objective for the pilot test:

Final Report for the Enhanced Anaerobic Bioremediation Pilot Test

- ◆ Determine substrate requirements
- ◆ Determine the injection strategy
- ◆ Determine biodegradation capability of the indigenous microbial community

The third objective was modified slightly since the work plan, and was rephrased as “Determine effectiveness of biodegradation following bioaugmentation with a commercially available dechlorinating culture.” The purpose of the pilot test was ultimately to verify the efficacy of bioremediation at the site, and to finalize a full-scale design if appropriate.

The work plan called for an interim report, which was submitted to EPA on April 3, 2006. The interim report summarized the initial results with respect to electron donor delivery and distribution, and illustrated that these issues do not represent any fatal flaws for the full-scale implementation of the technology at the Site. This report also provided a preliminary comparison of the efficacy of the three electron donor compounds being tested with respect to redox conditions and biodegradation of chloroethenes (COCs), as well as the efficacy of bioaugmentation.

1.3 Technical Approach

The *in situ* biological/chemical remediation approach selected for pilot testing was enhanced anaerobic bioremediation (EAB), whereby TCE will be completely degraded following the reductive dechlorination pathway: TCE → dichloroethene (DCE) → vinyl chloride (VC) → ethene (Freedman and Gossett, 1989). This process is generally facilitated through the addition of fermentable carbon compounds that serve as “electron donors” for subsurface bacteria that use the chloroethenes as “electron acceptors”. Bacteria utilize the electron donor first to remove naturally occurring electron acceptors such as oxygen, nitrate, ferric iron, and sulfate, and then transform the chlorinated hydrocarbons to ethene. Details regarding this process were provided in an appendix to the work plan. The two primary requirements for successful implementation of EAB are: 1) adequate spatial distribution of the electron donor to achieve strongly reducing conditions, and 2) the presence of a microbial community capable of complete reductive dechlorination of the contaminants.

1.3.1 Electron Donor

In order to satisfy the first requirement, an appropriate electron donor and injection strategy had to be selected. Electron donors can generally be categorized as aqueous or “slow-release” donors.

1.3.1.1 Aqueous Electron Donors

Aqueous electron donors are generally of a viscosity similar to water, and are therefore relatively easy to distribute in the subsurface. This implies that fewer injection locations can be used to deliver donor to a given area. As aqueous electron donors can facilitate the rapid onset of strongly reducing conditions to quickly poise the subsurface for efficient dechlorination, they can be considered “fast release”. In addition, they generally have low unit costs (i.e. per pound or per gallon) compared to slow release electron donors. Also, they can cause significant enhanced dissolution from residual sources when injected directly into a source area (i.e. contaminants are released from the non-aqueous phase source faster as compared to ambient conditions). This can result in more rapid degradation of a source term as contaminant mass is driven into the aqueous phase and then degraded. However, as the term implies, fast release electron donors are utilized rapidly in the subsurface and therefore have a short to medium longevity in the field (few weeks to months), implying that they have to be reinjected periodically, in order to ensure that adequate electron donor is available in the subsurface.

1.3.1.2 Slow Release Donors

Slow release donors have much higher longevities (on the order of months to years) than aqueous donors, but are typically high-viscosity liquids, or solids that have relatively low solubilities, which can limit the ability to distribute them over large areas. This implies that numerous closely spaced injection points or trenches might be required in order to achieve adequate distribution. In addition, the relatively slow utilization can result in longer timeframes for establishment of appropriate redox conditions. Also, slow release donors generally do not enhance mass transfer from residual source areas to the extent that fast release donors do. In fact, some slow release donors can actually sequester contaminants because the hydrophobic chlorinated solvents can actually partition into the donor itself. Another consideration is that in general, slow release donors are more expensive than fast release donors on a unit basis. However, their longevity can result in a single application being sufficient to provide treatment at a site for several years. In addition, at sites with variable saturation, slow release donors can be especially long lived. At this site, slow-release donors are a candidate because of the shallow depth of the treatment zone, and the ability to emplace a large amount of electron donor using a grid of direct-push injection points.

The pilot test consisted of evaluating three different electron donor addition strategies: two “slow-release” electron donors and one aqueous electron donor for comparison. All three were implemented at a small scale in the high concentration portion of the chloroethene plume on the HatchCo property.

1.3.2 Dechlorinating Bacteria

To provide an initial assessment of the second requirement for successful EAB described above, samples were collected in April 2005 in order to analyze the DNA of indigenous bacteria at the site. These data suggested that native bacteria at the site might be limited in their capability to perform complete detoxification of the TCE to ethene without a significant lag time. Therefore, “bioaugmentation” with a microbial culture that has this capability was performed during the pilot test.

1.4 Report Organization

The report is organized in keeping with the objectives of assessing electron donor delivery and distribution, providing results regarding the overall effectiveness of EAB in this pilot test, and describes the design of the full scale remediation of the site using EAB. Section 2 provides an overview of all field activities performed at the site. Section 3 summarizes the results pertaining to electron donor delivery and subsequent distribution. Section 4 presents the results for EAB, including redox conditions, dechlorination, and bioaugmentation. A summary of the conclusions of the pilot scale study is provided in Section 5, and Section 6 describes the design of the full scale implementation of EAB at the Site.

Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test:
2. Summary of Activities

This section provides a timeline of activities performed, followed by a brief description of those activities:

Date	Objective
07/01/05	Kickoff meeting - EPA, BOR, and CDM
07/11/05	Site visit to mark drilling locations for monitoring well and sodium lactate injection installation
07/11/05 - 7/29/05	Well installation, completion, and development
07/27/05 - 7/29/05	DNA Sampling & Baseline
08/31/05 - 9/06/05	Installation of emulsified oil and chitin injections wells, and initial injection of all electron donors
10/04/05 - 10/07/05	Lactate injection and sampling event (results not included due to data quality concerns)
10/26/05 - 10/28/05	1 st Sampling Round
11/21/05 - 11/22/05	DNA Sampling and Inoculation
11/29/06 - 12/01/05	2 nd Sampling Round
01/03/06 - 01/05/06	DNA Sampling + 3 rd Sampling Round
01/31/06 - 02/02/06	4 th Sampling Round
02/28/06 - 03/02/06	5 th Sampling Round
03/28/06 - 03/30/06	DNA Sampling + 6 th Sampling Round

**Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test**

04/25/06 - 04/27/06	7 th Sampling Round
05/30/06 - 06/01/06	DNA Sampling + 8 th Sampling Round
06/27/06 - 06/29/06	Ethane/ethene/methane split sampling + 9 th Sampling Round
07/25/06 - 07/27/06	10 th Sampling Round
08/28/06 - 08/31/06	11 th Sampling Round

By July 27, 2005, all of the wells had been installed, and their development was being completed. The three treatment cells were installed as shown in Figure 2-1. The lactate cell (furthest north) was comprised of the injection well, two downgradient monitoring wells (5 and 10 feet downgradient), and one upgradient monitoring well (20 feet upgradient). The emulsified oil cell was comprised of three downgradient monitoring wells (two at 5 feet and one at 10 feet downgradient); and one upgradient monitoring well (20 feet upgradient). The chitin cell was analogous to the emulsified oil cell, with three downgradient monitoring locations and one upgradient. The injection locations for the emulsified oil and the chitin were not installed until the end of August. Baseline samples were collected during the July 27, 2005 sampling event.

The results of the baseline sampling supported moving ahead with electron donor injections at the end of August. Initial injections occurred from August 31 through September 6, 2005. Approximately 14 gallons of 60% sodium lactate solution was injected at a 20:1 dilution rate with potable water on Thursday, September 1, 2005 using a proportional mixer. The injection well was not readily accepting the injection solution, which limited the volume that could be injected. On Tuesday, September 6, 2005 another 23 gallons of 60% sodium lactate solution was injected at a 20:1 ratio with potable water. At this point the well readily accepted the solution, and no further difficulties with injection were encountered throughout the pilot test.

Due to the inability to direct-push the emulsified oil injection wells on September 3, 2005, the drilling contractor was forced to return on September 6, 2005 with different equipment. The planned quantity of emulsified oil was successfully injected into three temporary 1-inch injection points. The injection was performed with a proportional mixer; analogous to sodium lactate injections, except that the resultant concentration was approximately 1%. The total volume of emulsified oil injected was 275 gallons, or approximately 91 gallons per injection point.

Final Report for the Enhanced Anaerobic Bioremediation Pilot Test

The chitin was to be injected in six direct push injection points. Initial attempts to pump the chitin slurry resulted in pump failure due to clogging of the lines by the coarse chitin flakes. A backup pump provided by the drilling contractor also clogged immediately. A field decision was made to screen the large chitin flakes out of the suspension before pumping, resulting in the injection of a dark brown solution that contained the finer chitin particles. This approach was used for the first two and a portion of the third northern-most injection points on September 1 and 2. Upon Dr. Kent Sorenson's arrival on September 2, 2005, injection was ceased due to the concern that the vast majority of the coarse chitin flakes were being screened out, in particular the flakes that contribute to chitin's longevity in the subsurface. By September 3, an approach for grinding the remaining dry chitin into smaller particles was implemented, and the finer ground material was readily injected into the third of the northern-most injection points and the three remaining southern-most injection points. Approximately, a total of 108 to 120 gallons (mass unknown) of the ground chitin were injected as a slurry from 25 to 37 feet below ground surface into each of the six wells.

On October 3 and 4, 2005, another sodium lactate injection (37 gallons at 20:1) was performed. Sampling was also conducted, but data from this sampling event were discarded due to data quality concerns. These issues were addressed prior to the next sampling event, which occurred during October 24-26, 2005. This was considered the first official sampling event following electron donor injection. Because of the proximity to the October 3-4 event, 2005, sodium lactate was not injected during this sampling event. All the following monthly sampling and lactate injection events went as planned throughout the pilot test.

Field data from both October events indicated that conditions were appropriate for addition of the dechlorinating culture by November. Inoculation of all three cells with a commercially available dechlorinating culture (*Dehalococcoides ethenogenes*) occurred on November 22, 2005 following a standard sodium lactate injection on November 21, 2005. Approximately 25 gallons of culture was procured, out of which about 15 gallons was injected in one cell and 5 gallons in each of the others. Due to this inconsistency, an additional 25 gallons of culture was procured and injected on November 29, 2005 to provide equal amounts of culture to all treatment cells.

DNA testing of the site was performed during the January 3, March 28, and May 30, 2006 sampling events to monitor the growth and proliferation of the bioaugmented dechlorinating culture at the site. Methane and ethene were produced in all cells during the pilot test but the concentrations were lower than expected. In order to determine whether the lower than expected concentrations were the result of analytical problems, an additional set of samples for analysis of ethene/ethane/methane was sent to the Microseeps, Inc. laboratory during the June 27, 2006 sampling event along with the usual set of samples sent to the Utah State University laboratory.

Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test:

3. Electron Donor Delivery and Distribution

This section provides a description of the delivery methods used to inject each electron donor, along with a discussion of the apparent effectiveness of delivery and distribution based upon electron donor concentrations in the monitoring wells. The results in this section address the first decision question in the data quality objectives section of the work plan, namely determining substrate requirements.

3.1 Delivery

Three different electron donors, one aqueous (sodium lactate) and two slow release (emulsified oil and chitin) were injected at the site, each using a different delivery method. The sodium lactate was injected monthly during the sampling events. The emulsified oil substrate and chitin were injected one time at the beginning of the pilot test.

3.1.1 Sodium Lactate

Sodium lactate was injected monthly into a standard 4-inch well (SLMI, Figure 2.1) having a 15-foot screened interval installed to the same depth as the monitoring wells. Unamended water for the injection was obtained from a nearby fire hydrant. The water was routed through a proportional mixer (Dosatron) that served to meter the 60% stock sodium lactate solution into the flow of water at a dilution rate of about 20:1, the resultant concentration was approximately 3-5% wt/wt. The injection well was able to accept a flow rate of approximately 2-3 gpm, which required an average of about 6 to 8 hours to complete each injection. After the first day of injection, the sodium lactate delivery became routine, and few problems were encountered.

The volume of water injected was calculated based on the readings on the fire hydrant valve totalizer. This volume was then used to calculate the % wt/wt sodium lactate solution injected. Although the target sodium lactate concentration range injected during each injection event was 3-5 % wt/wt, it appeared to vary from 5-9.5 % wt/wt during the April to August 2006 sampling events. It should be noted that the readings on the totalizer might not be accurate at the low flow rates involved for these injections. This was supported by the fact that the Dosatron setting was not changed throughout the course of the pilot study and the pump

Final Report for the Enhanced Anaerobic Bioremediation Pilot Test

was operated approximately at the same rate (2-3 gpm) and for the same duration of time (6-8 hours) excluding during the March 28, 2006 sampling event. During this event the sodium lactate solution was injected at a flow rate of 2-3 gpm in only 3.75 hours, but the totalizer was clearly not functioning properly as the readings showed that only 10 gallons of water were injected during that time period.

3.1.2 Emulsified Oil Substrate

The emulsified oil was injected using the same proportional mixing strategy as used for lactate injection; however, the injection points consisted of three temporary 1-inch wells with 10-foot screens (Figure 2.1). Although the original intent was to install these wells with direct-push technology, this was unsuccessful and the holes had to be pre-drilled using an auger rig. The 1-inch wells were then installed inside the auger string. The oil emulsion was successfully injected along with the hydrant water using a proportional mixer at an approximate concentration of 1% (wt/wt). The total volume of emulsified oil injected was 275 gallons, or approximately 91 gallons per injection point.

3.1.3 Chitin

The chitin was injected into six direct-push points (Figure 2.1) using a bottom-up approach from 25 to 37 feet below ground surface. The total volume of ground chitin slurry injected was approximately 108 to 120 gallons (mass unknown), or 9 to 10 gallons were injected per foot. As noted in Section 2, the equipment brought to the site was inadequate for injecting the coarse chitin flakes. The first two and half of the third injection point (northern) did not receive the quantity or the consistency of chitin intended because the coarse flakes were screened out. After grinding the chitin to a smaller particle size, the available equipment was able to inject the material easily into the half of the third and the three remaining (southern) injection points. It was concluded that successful delivery would require either chitin of a smaller particle size, more robust injection equipment (including pumps, fittings, joints, etc.), or both.

3.2 Distribution

The distribution of each electron donor was monitored by measuring the concentrations of organic acids (initial breakdown products of biodegradation of the electron donors) in the monitoring wells. These data are used to answer the decision question posed in the work plan: "Can electron donor(s) be effectively distributed at the Site to affect the reducing conditions necessary to support complete dechlorination of contaminants?" This section presents the organic acid data for each of the three treatment cells. It should be noted that well HMW2S (230 feet upgradient of the injection wells) and HMW10S (270 feet downgradient

of the injection wells) wells were sampled during the baseline (July 27, 2005), first (October 26, 2005) and last (August 28, 2006) sampling events, and no organic acids were measured in these wells (data not shown).

3.2.1 Sodium Lactate

Organic acids were measured at three locations in the sodium lactate treatment cell, SLMU (20 feet upgradient), SLM1 (5 feet downgradient), and SLM2 (10 feet downgradient) (Figure 2.1). Typically, the organic acids propionate, butyrate, and acetate are produced from lactate fermentation under anaerobic conditions. Figure 3-1 shows the organic acids data for the sodium lactate cell. SLMU (Figure 3-1A) shows two peaks of organic acids, one during the January 3, 2006 sampling event and the other during the June 27, 2006 sampling event. The concentrations of organic acids in these peaks are near-detection limit and consist of isobutyrate, isovalerate, and valerate, which are products not typically produced upon lactate biodegradation. Given the spotty detections of these organic acids at concentrations near-detection limits in a well 20 feet upgradient, the data suggest analytical issues are the likely cause, and not the actual lactate distribution.

Surprisingly, organic acids were observed at SLM1 just 5 feet downgradient (Figure 3-1B) only after a 3-month period following the initial lactate injection. A significant concentration of valerate (178 mg/L) was observed in the February 28, 2006 sampling event at SLM1. As mentioned earlier, valerate is not typically produced upon lactate biodegradation, hence this peak is suspected to be an analytical issue. March 28 and April 25, 2006 sampling events showed significant organic acids concentrations on the order of a few hundred mg/L. Donor concentrations at SLM1 decreased starting with the May 30, 2006 sampling event, and did not exceed 50 mg/L during the remainder of the pilot test.

The SLM1 data alone might cast doubt on the efficacy of lactate distribution; however, SLM2 about 10 feet downgradient (Figure 3-1C) had significant organic acids concentrations on the order of a few hundred mg/L immediately following the initial lactate injection. Organic acid concentrations continued to be significant for the first eight months of the pilot test and declined thereafter. Sampling events from April 25, 2006 through August 28, 2006 revealed organic acid concentrations near-zero at this well.

As expected for lactate, the organic acids observed in the SLM treatment cell consisted primarily of acetate and propionate. The relatively high concentrations of propionate are considered very positive for facilitating reductive dechlorination. Taken together, SLM1 and SLM2 results indicate that while some preferential flow (likely due to heterogeneity of the aquifer) was initially observed at SLM1, sodium lactate and associated organic acids were effectively distributed initially to distances of at least 10 feet downgradient from the injection point at concentrations that would be expected to induce reducing conditions in the

Final Report for the Enhanced Anaerobic Bioremediation Pilot Test

groundwater. Organic acids concentrations remained at significant levels only during the initial months which gradually decreased and were reduced to near-zero concentrations as observed after the May 30, 2006 sampling event at SLM1 and April 25, 2006 sampling event at SLM2.

The lower concentrations observed during the later part of the pilot test may have been due to a number of factors, most likely because the bacterial community increased both in numbers and in their lactate utilization rates during the course of the pilot test, resulting in decreased distribution of lactate as the pilot test progressed. Over the time frame of 12 months, the extent of distribution had considerably decreased, suggesting that the injection strategy would need to be changed to increase distribution.

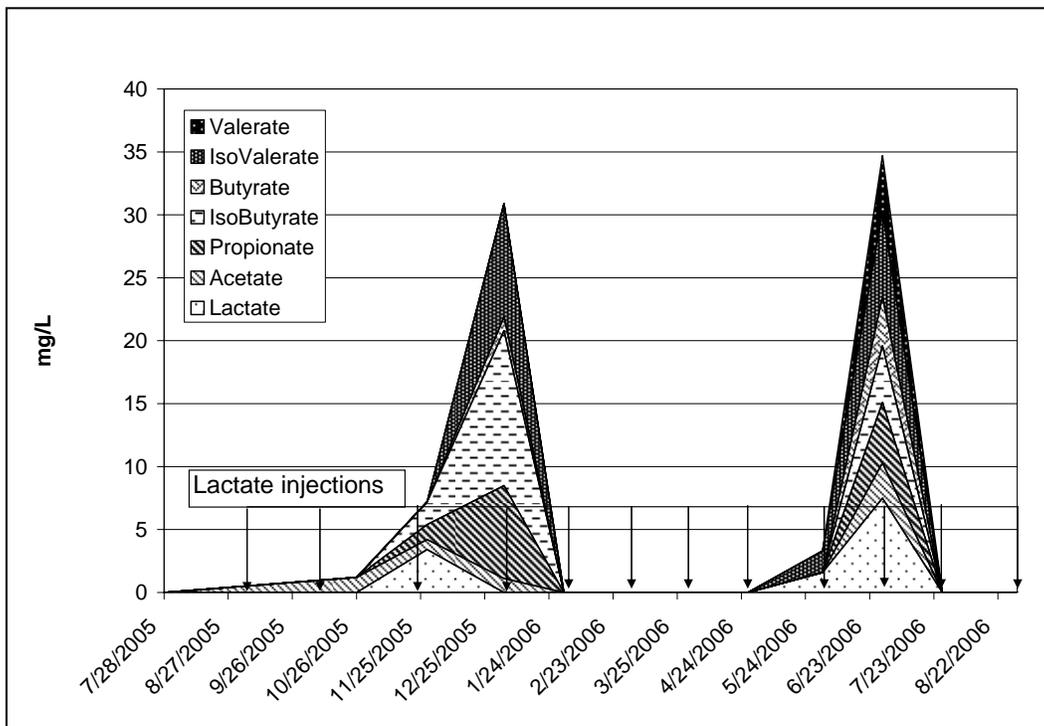


Figure 3-1A.—SLMU Organic Acids

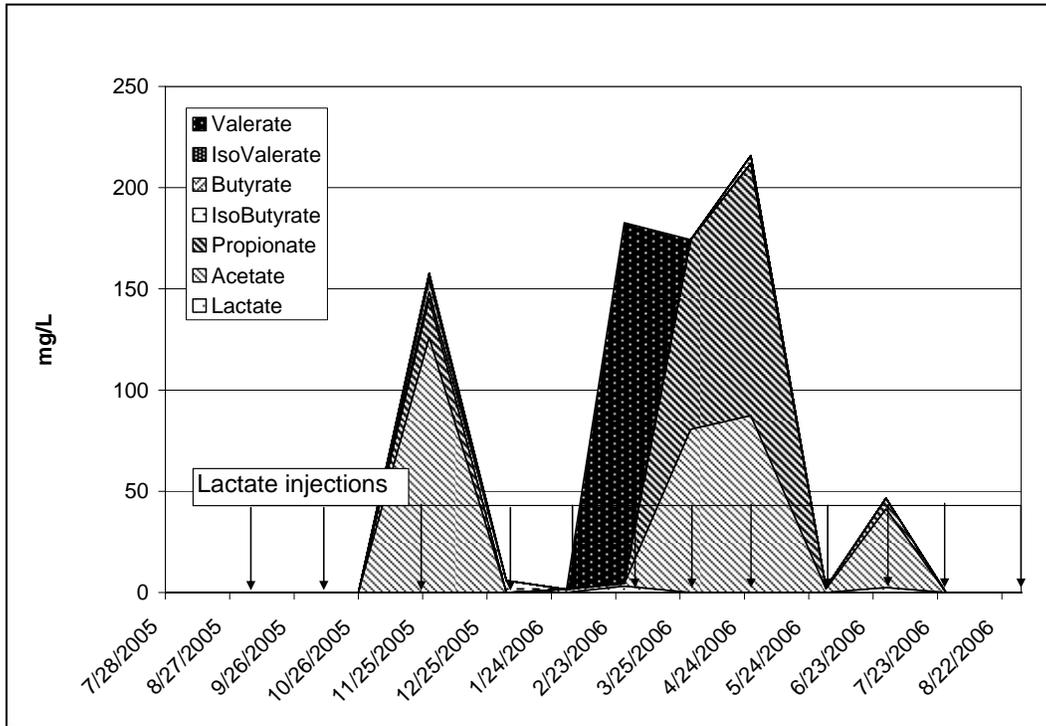


Figure 3-1B.—SLM1 Organic Acids

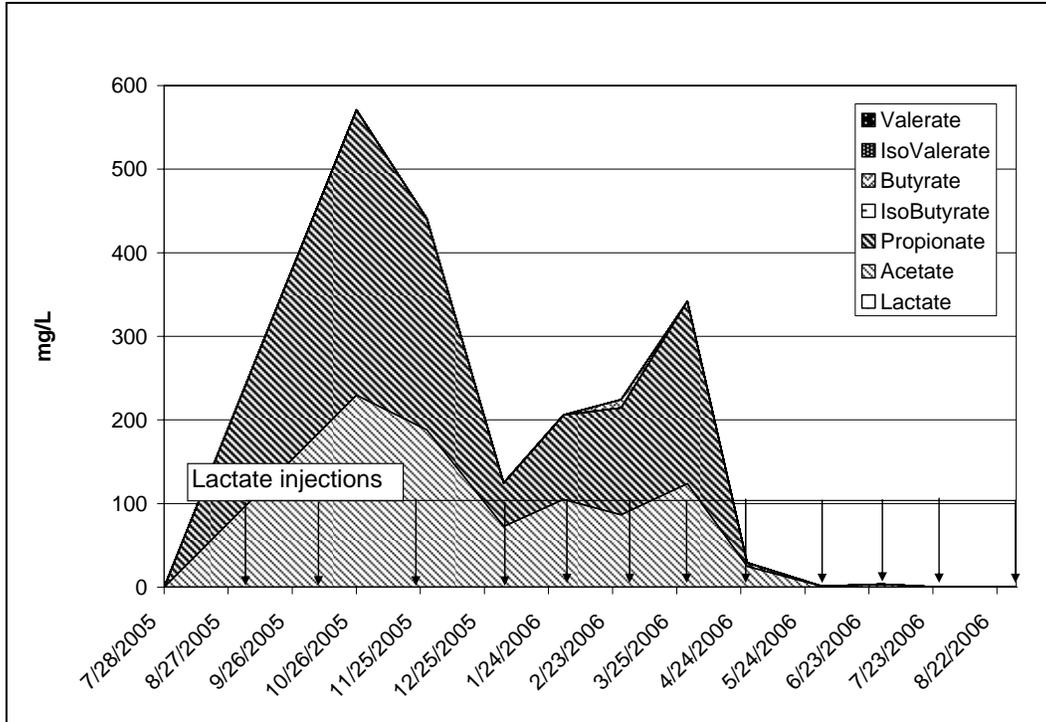


Figure 3-1C.—SLM2 Organic Acids

3.2.2 Emulsified Oil Substrate

Figure 3-2 illustrates the concentrations of organic acids measured in the monitoring wells of the emulsified oil substrate cell. As with lactate, the organic acids propionate, butyrate, and acetate are produced from degradation of emulsified oil substrate under anaerobic conditions. The organic acids were measured at four locations in the emulsified oil substrate treatment cell, OSMU (20 feet upgradient), OSM1 and OSM2 (5 feet downgradient), and OSM3 (10 feet downgradient) (Figure 2.1). A peak of organic acids was observed in the upgradient well, OSMU (Figure 3.2A) during the February 28, 2006 sampling event. The peak was reported to be mostly composed of lactate at a concentration of 113 mg/L. Lactate was not injected at this cell, is not a typical product of emulsified oil biodegradation, and the approximate 40 feet distance separating the sodium lactate and emulsified oil substrate cells and the groundwater flow direction makes it unlikely for migration of lactate to this well. Thus, the single report of lactate at this well is suggestive of an analytical issue.

Figures 3-2B and 3-2C show the organic acids measured 5 feet downgradient from the injection points at OSM1 and OSM2, respectively. It should be noted that OSM1 is located approximately directly downgradient from an injection point, while OSM2 is located approximately between two injection points (Figure 2-1). Both monitoring wells showed several hundred mg/L of organic acids, primarily as acetate, but with appreciable concentrations of other compounds, most notably propionate for both wells, and butyrate for well OSM2. OSM1 had organic acids at concentrations as high as approximately 550 mg/L in March 28, 2006 sampling event, while OSM2 had surprisingly high concentrations, as high as approximately 3400 mg/L during the May 30, 2006 sampling event. It is suspected that the injected oil emulsion that reached OSM2 is acting as a localized source of the organic acids. The concentrations at OSM1 have been gradually decreasing since they peaked and were measured to be at approximately 65 mg/L during the last (August 28, 2006) sampling event. At OSM2 the concentrations of organic acids have slightly reduced from the peak level but are still considerably high (2250 mg/L observed during the August 28, 2006).

OSM3 (Figure 3-2D) had significant concentrations of organic acids, especially considering it is located 10 feet downgradient of the injection points. The concentration peaked at approximately 690 mg/L during the January 3, 2006 sampling event before starting its decline. The concentrations observed were still significant for the last sampling round, and appeared to be stabilizing at approximately 200 mg/L. It should be noted that even though OSM3 is located approximately directly downgradient from OSM1, the concentrations observed at OSM3 over the last couple of months were higher than that observed at OSM1. Overall, these data indicate that not only was the emulsified oil delivered successfully, the subsequent distribution of organic acids was better than expected, both in terms of the concentrations observed and the longevity of those concentrations.

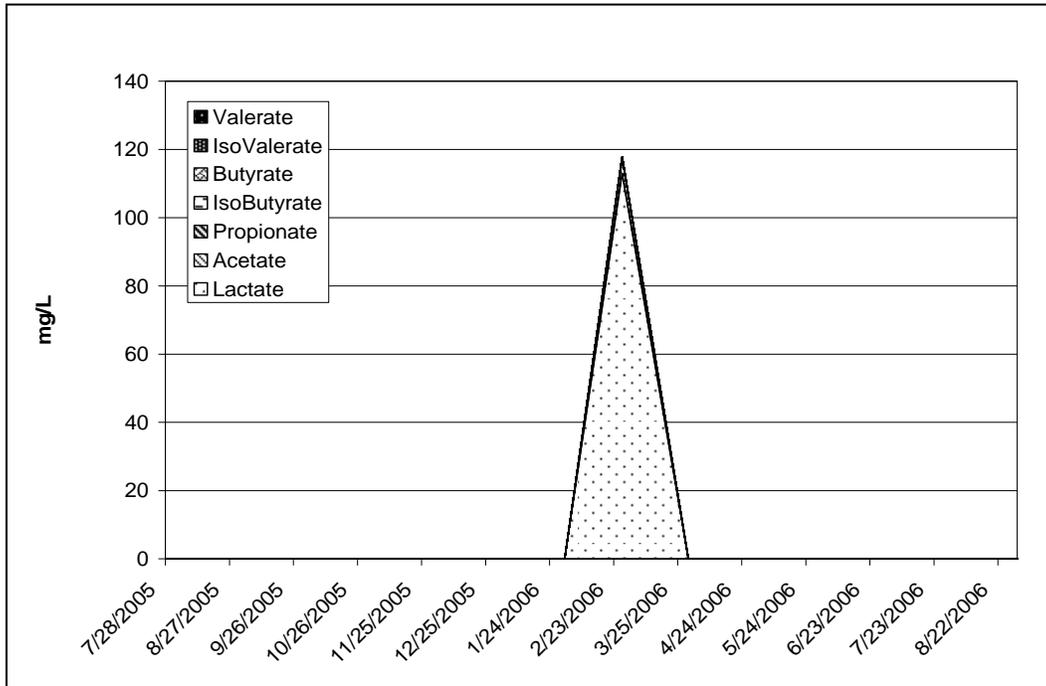


Figure 3-2A.—OSMU Organic Acids

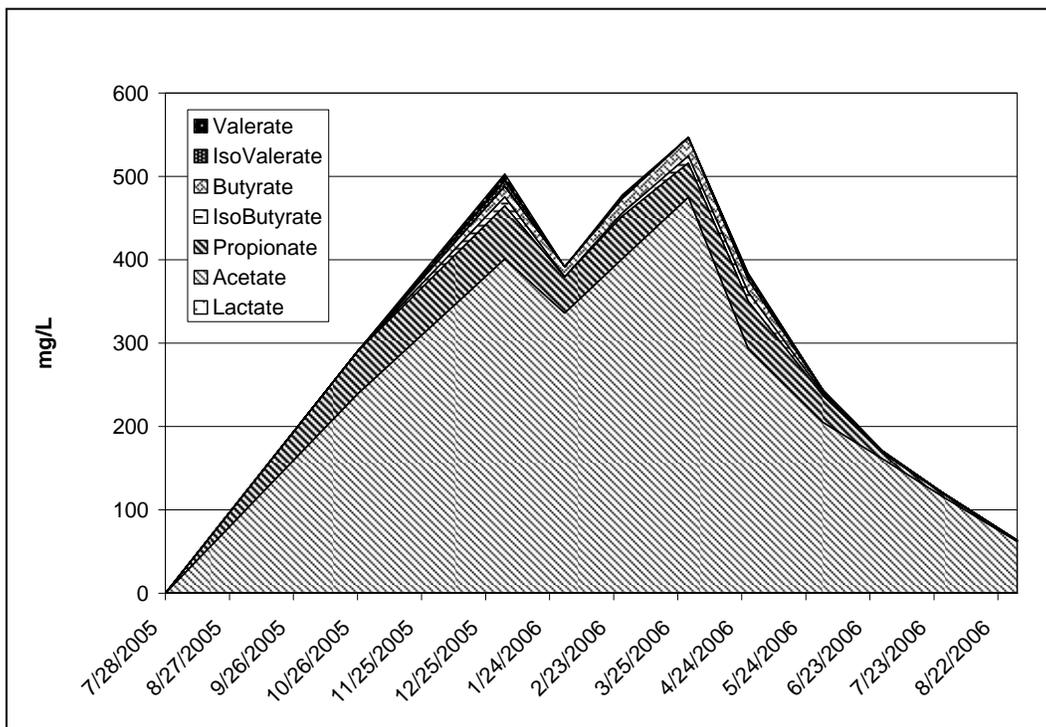


Figure 3-2B.—OSM1 Organic Acids

**Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test**

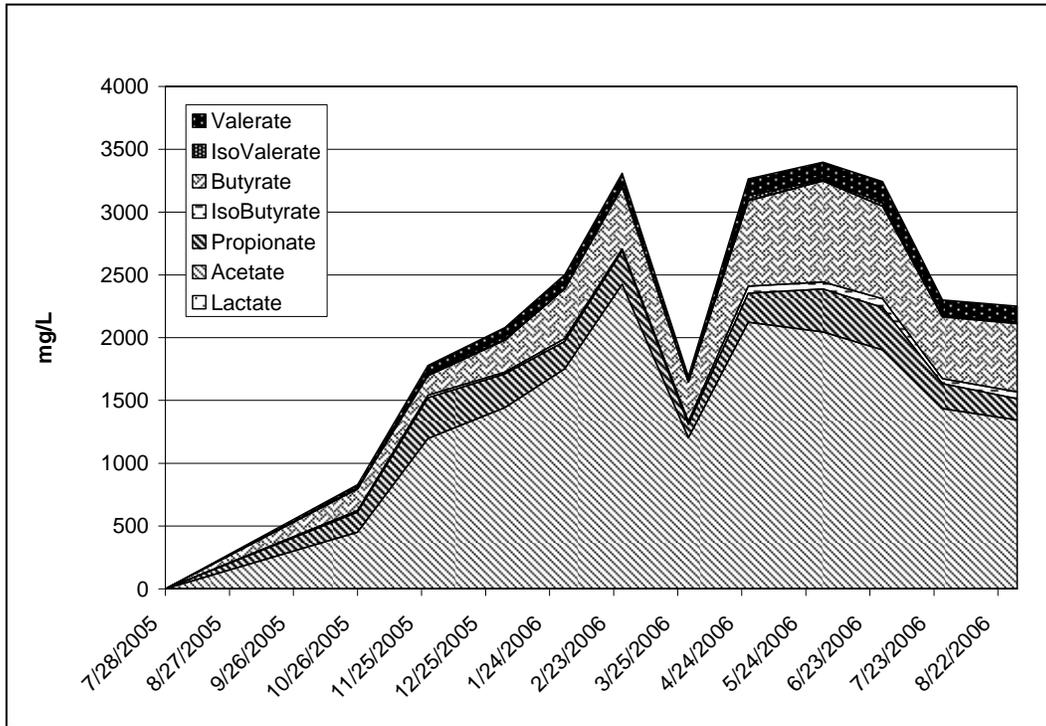


Figure 3-2C.—OSM2 Organic Acids

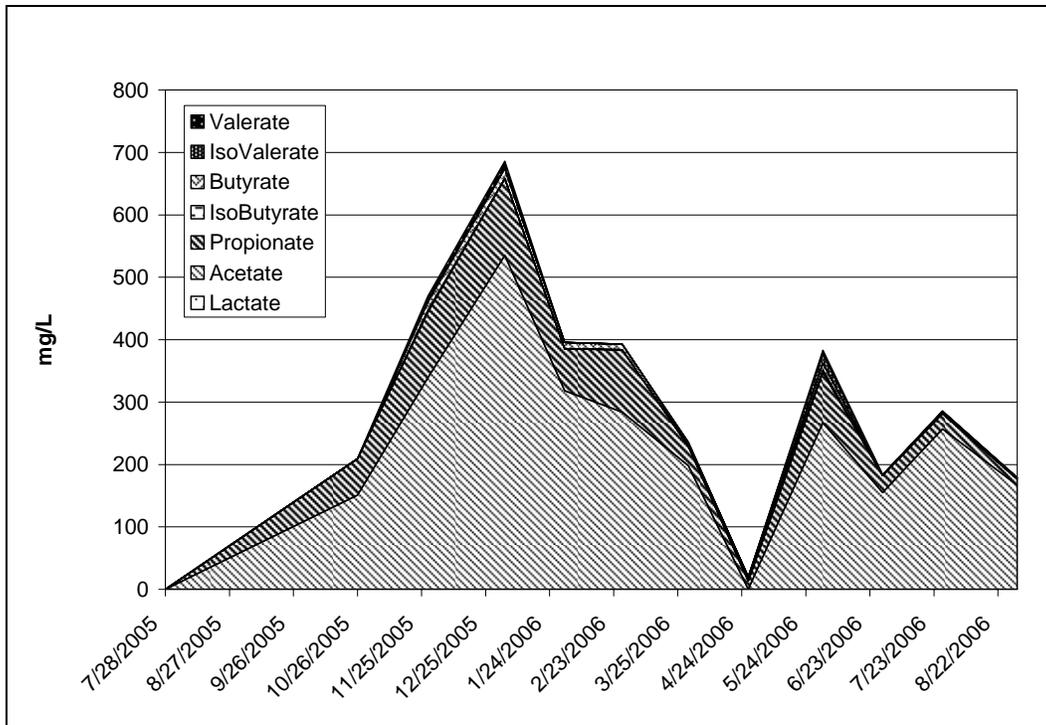


Figure 3-2D.—OSM3 (HMW3S) Organic Acids

3.2.3 Chitin

The organic acids produced from the chitin injection are shown in Figure 3-3. As with the other donors tested, chitin produces propionate, butyrate, and acetate, but can also produce isobutyrate, valerate, and isovalerate. The organic acids were measured at four locations in the chitin treatment cell, CMU (20 feet upgradient), CM1 and CM2 (5 feet downgradient), and CM3 (10 feet downgradient) (Figure 2.1). It should be noted here that the northern-most injection points received the filtered chitin solution containing mainly the smallest chitin particles while the southern-most injection points received the ground chitin solution containing larger and longer lived chitin flakes.

A peak of organic acids was observed in the upgradient well, CMU (Figure 3.3A) during the June 27, 2006 sampling event. The peak was mostly composed of lactate at near-detection limit concentrations. As mentioned earlier for the oil cell, lactate was not injected at this cell, is not a typical product of chitin biodegradation, and the approximate 80 feet distance separating the sodium lactate cell and chitin cell and the groundwater flow direction makes it unlikely for migration of lactate to this cell. Thus, the one time near-detection limit lactate peak at this upgradient well is suggestive of an analytical issue.

Organic acid concentrations were initially in the range of a few hundred mg/L in both CM1 and CM2 (Figure 3.3B and 3.3C). These concentrations represent good donor distribution from the chitin considering the issues faced during injection, but they were sustained only for a few months. For CM3, the well 10 feet downgradient (Figure 3.3D), only one significant peak of organic acids (approximately 150 mg/L) was observed, during the November 28, 2005 sampling event. The composition of the acids was dominated by acetate, but with a secondary presence of others. The concentrations in the wells 5 feet downgradient (CM1 and CM2) were sufficient to induce strongly reducing conditions required for reductive dechlorination during the first few months. Thus, the fairly good distribution of the chitin observed only during the initial few months and the limited longevity of the organic acids, can be attributed to the inability to inject the larger chitin flakes at this cell.

**Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test**

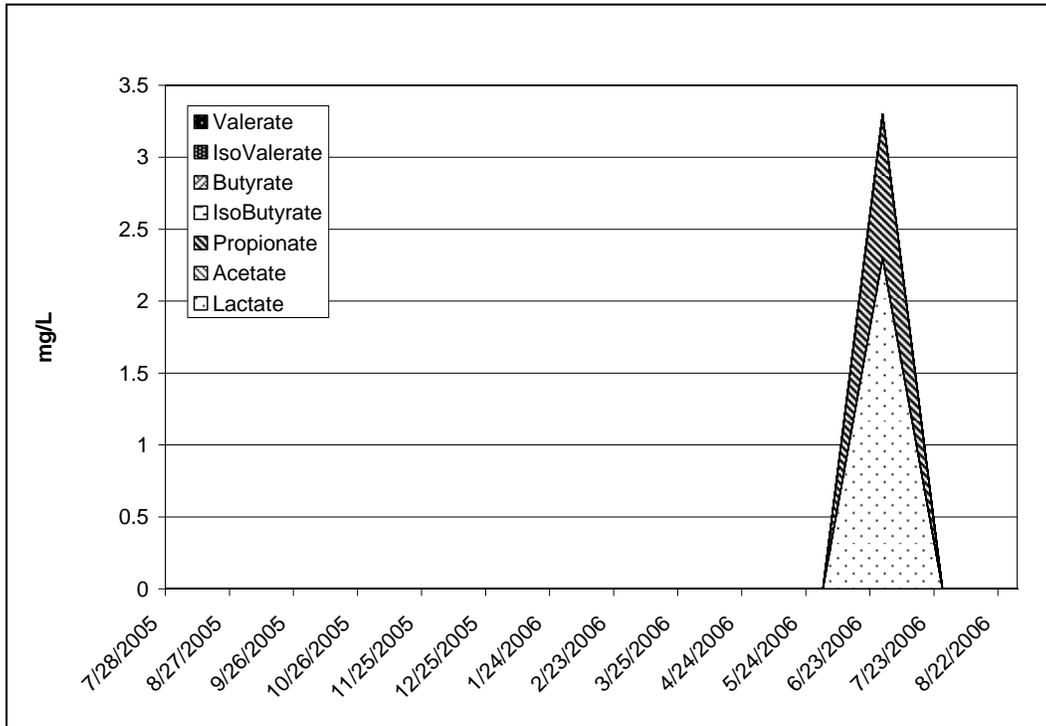


Figure 3-3A.—CMU Organic Acids

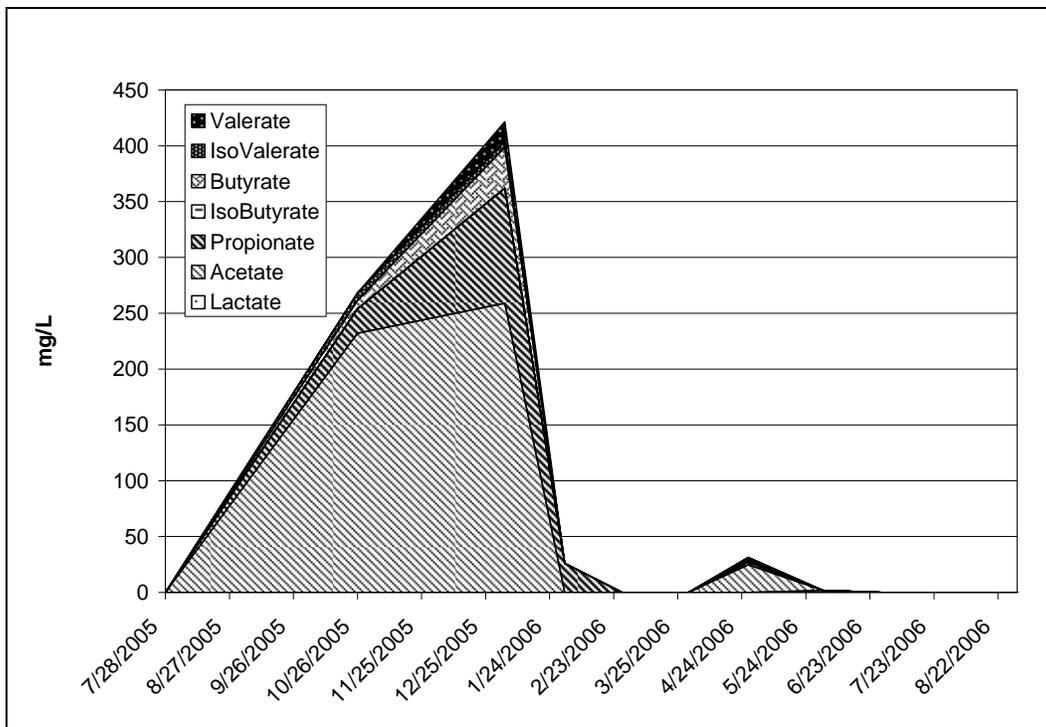


Figure 3-3B.—CM1 Organic Acids

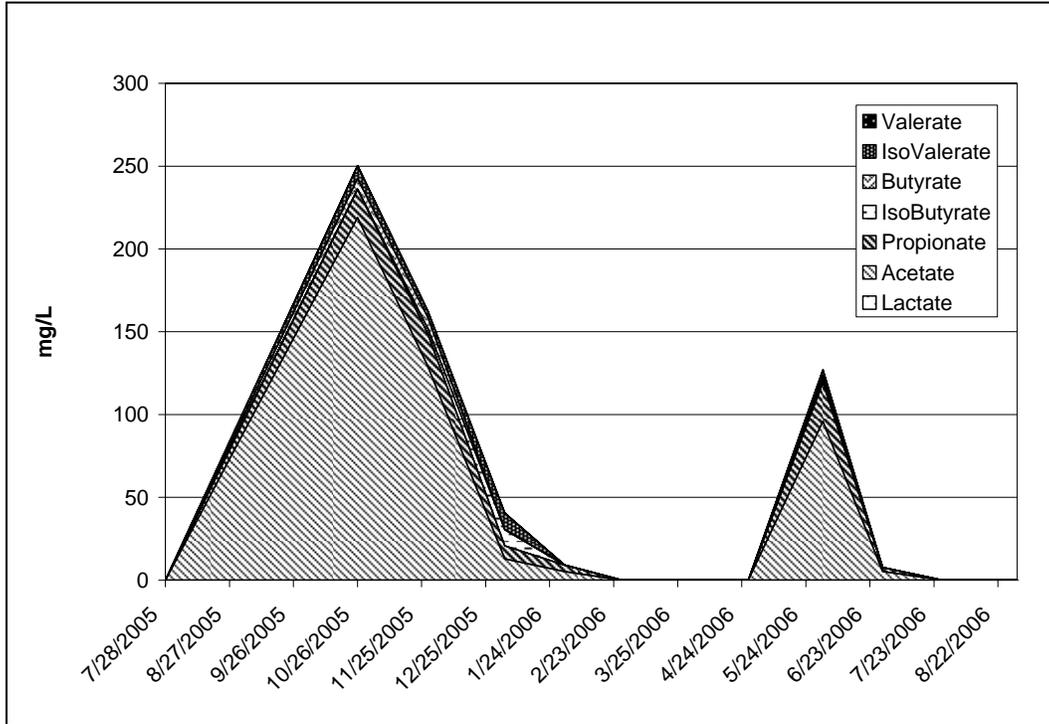


Figure 3-3C.—CM2 Organic Acids

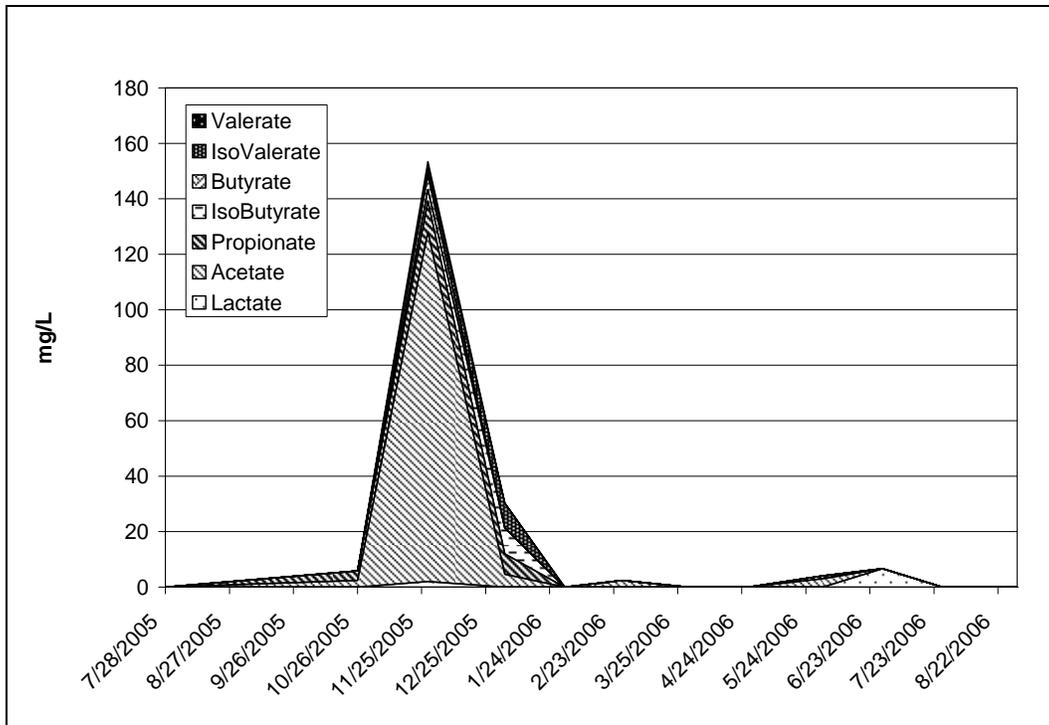


Figure 3-3D.—CM3 Organic Acids

3.2.4 Summary of Distribution

Overall, the results indicate that electron donor distribution can be achieved at the Site using all three electron donors, but that the injection strategy for sodium lactate and chitin needs to be optimized. The emulsified oil was easily injected and effectively distributed showing the highest concentrations of organic acids and was also the longest lived. For lactate, the injection strategy could be optimized by either increasing the volume of lactate injection or installing more injection wells. For chitin, the injection strategy can be optimized by applying a strategy that allows for injection of the coarse long-lived chitin flakes. The extent to which these donors create the appropriate redox conditions and stimulate reductive dechlorination is discussed in the next section.

Final Report for the Enhanced Anaerobic Bioremediation Pilot Test:

4. EAB Results

In order for complete reductive dechlorination of TCE to ethene to occur, electron donor must be adequately distributed, redox conditions must be sufficiently reducing and appropriate microbial populations must be present and active. The electron donor distribution was discussed in the previous section. The redox conditions and microbial populations are discussed in this section.

4.1 Redox Conditions

Redox conditions are frequently monitored by measuring the oxidation-reduction potential (ORP). It is a simple indicator of redox conditions and can be easily measured on site during the field activities. However, it is not the most accurate parameter in assessing the actual redox conditions, and if considered alone can sometimes be misleading. Thus, it is required to monitor concentrations of certain inorganic electron acceptors in addition to ORP in order to assess the accurate redox conditions at a site.

4.1.1 Oxidation-Reduction Potential (ORP)

ORP is measured in a flow-through cell during sampling. Figure 4-1 illustrates ORP over time in all of the treatment cells. Figures 4.1A, 4.1B, and 4.1C illustrate the ORP for the sodium lactate, emulsified oil substrate and chitin cells respectively. Initial values generally ranged from about -100 mV to 200 mV for all cells, except for the downgradient SLM wells where ORP was below -300 mV. Following electron donor injections, ORP decreased in all of the cells and ranged from about -100 mV to -300 mV as measured by the water quality instrument used for all except the last (August 28, 2006) sampling event when a different instrument was used. The August 28, 2006 sampling event revealed an ORP of +218 mV for SLMU, +124 mV for OSMU, and -65 mV for CMU. ORP ranged from -50 mV to -400 mV for all the downgradient monitoring wells during this sampling event. Even though the ORP was lower for CMU (-65 mV) compared to the other two upgradient wells it was higher as compared to the three CM downgradient wells (ranged from -150 mV to -200 mV). An ORP of -100 mV to -300 mV is within the appropriate range to facilitate reductive dechlorination. The ORP should be viewed as a screening parameter, however, because the electrodes often require long periods of exposure to water that is significantly different in ORP than a previous measurement before they will register an accurate reading.

**Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test**

This effect could have been responsible for the fact that ORP readings observed at the upgradient wells were similar to those measured in the pilot study wells during several sampling events.

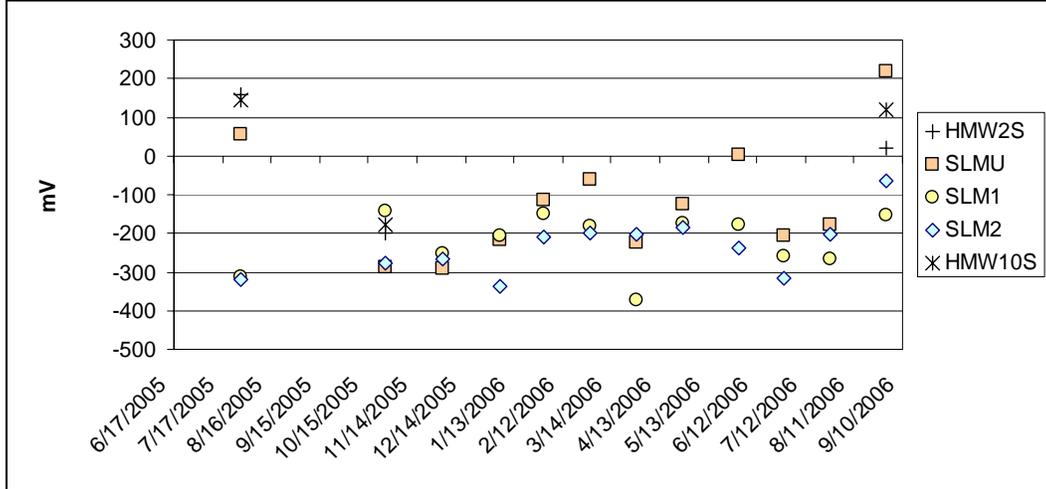


Figure 4-1A.—SLM Cell ORP

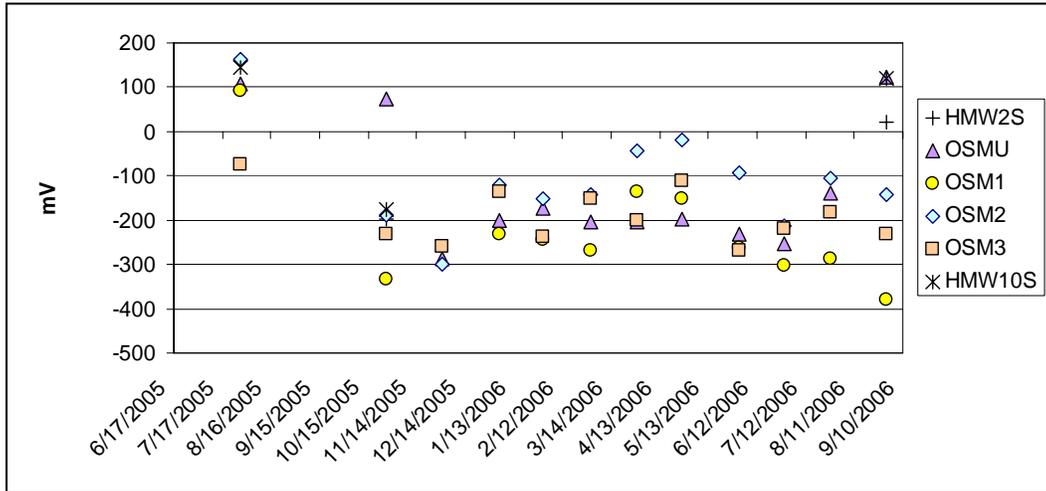


Figure 4-1B.—OSM Cell ORP

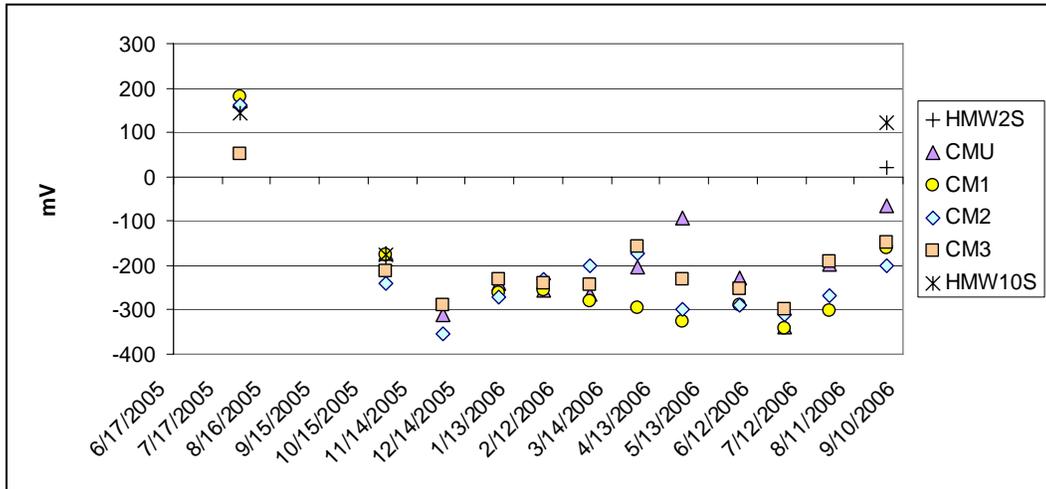


Figure 4-1C.—CM Cell ORP

4.1.2 Inorganic Electron Acceptors

As discussed earlier the more reliable indicator of redox conditions is the aqueous concentrations of inorganic electron acceptors and their reduced products. As discussed in the work plan, redox conditions typically progress from aerobic → nitrate reducing → iron reducing → sulfate reducing → methanogenic following addition of a sufficient supply of electron donor. The data indicate that this progression has occurred in all of the treatment cells.

4.1.2.1 Ferrous Iron

Figure 4-2 illustrates the dissolved (ferrous) iron concentrations in all of the treatment cells. Ferrous iron is the product of ferric iron reduction. Figures 4.2A, 4.2B, and 4.2C illustrate the ferrous iron for the sodium lactate, emulsified oil substrate and chitin cells respectively. In general, the ferrous iron concentrations correlated with the organic acids concentration. Ferrous iron was not produced in any of the upgradient wells, except for low concentrations in CMU at the end of the pilot test.

In the lactate treatment cell, ferrous iron concentrations correlated with the organic acids concentrations in wells SLM1 and SLM2. At both of these wells, the concentrations of ferrous iron was observed to increase with the increase in the concentrations of organic acids during the initial months, but started declining once the concentrations of organic acids were depleted.

The emulsified oil generated the most ferrous iron production compared to the other two cells with OSM2, which also had the highest organic acid concentrations, exhibiting the highest concentration (approximately 65 mg/L). At both wells OSM1 and OSM3 the concentrations of ferrous iron were observed to increase initially with increase in the concentrations of organic acids and

**Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test**

decreased with organic acids depletion. OSM1 showed a faster decline in ferrous iron concentration because the concentrations of organic acids were lower compared to OSM3.

Surprisingly, chitin showed iron reduction throughout the pilot test, even though organic acids were observed only during the initial few months at these wells. The concentrations were low compared to the other two cells but were still measurable. The production of low levels of ferrous iron in absence of significant electron donor at these wells can be attributed to the presence of some long-lived chitin flakes still producing organic acids and thereby inducing reduced conditions near the injection points in this cell.

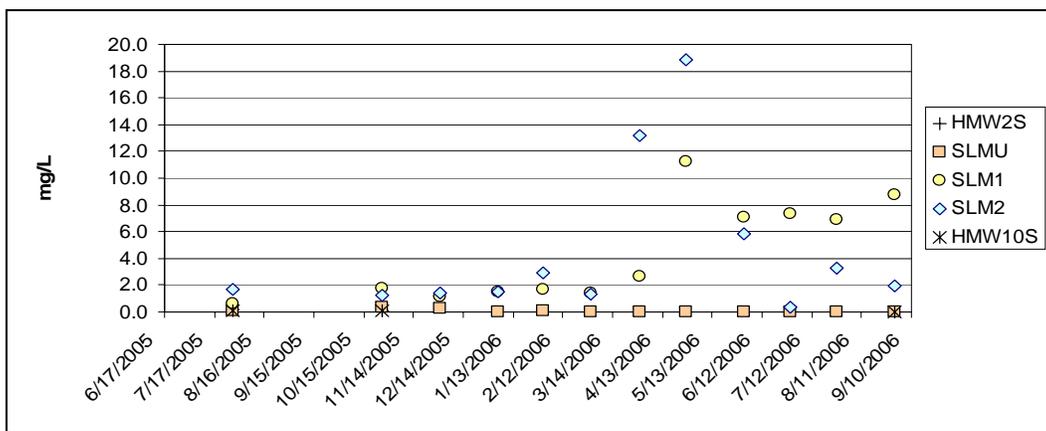


Figure 4-2A.—SLM Cell Ferrous Iron

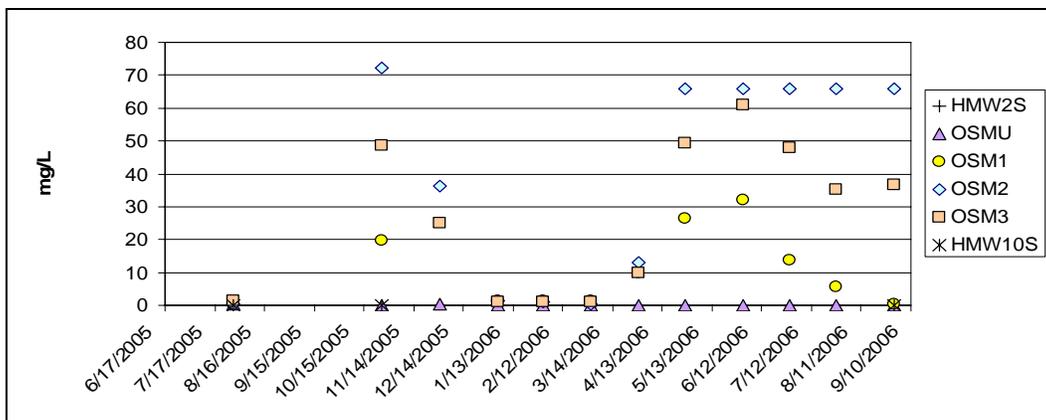


Figure 4-2B.—OSM Cell Ferrous Iron

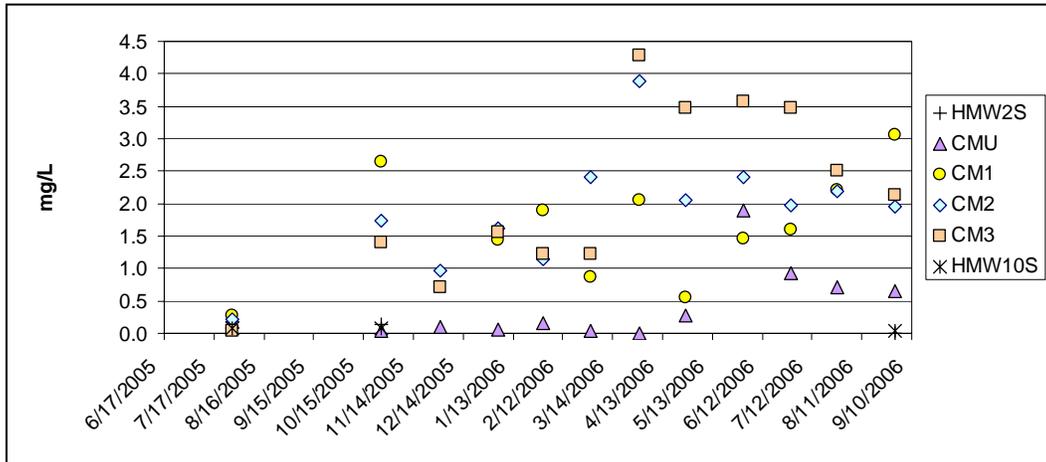


Figure 4-2C.—CM Cell Ferrous Iron

4.1.2.2 Sulfate

Figure 4-3 shows sulfate concentrations over time in all of the cells. Figures 4.3A, 4.3B, and 4.3C illustrate the sulfate concentrations for the sodium lactate, emulsified oil substrate and chitin cells, respectively. The extent of sulfate reduction is quite variable for the different electron donors but is consistent with the organic acids distribution for sodium lactate and emulsified oil substrate.

The sulfate concentrations are near background concentrations in the upgradient wells for all three cells throughout the pilot test. For sodium lactate, sulfate was completely removed in SLM2 when significant concentrations of organic acids were observed. Sulfate reduction was minimal initially in SLM1 when organic acids were not observed, but with increase in organic acids at this well some sulfate reduction was observed. With the depletion of organic acids the concentrations of sulfate were observed to rebound to the background levels at both SLM1 and SLM2.

In the emulsified oil cell, sulfate has been almost completely removed from initial concentrations of 400 to 500 mg/L. The reduction has been sustained throughout the pilot test in all three downgradient monitoring wells.

The chitin injections have not induced much sulfate reduction as this cell did not have a good organic acids distribution. The little sulfate reduction observed can be attributed to the presence of some long-lived chitin particles still producing organic acids near the injection points in this cell. CM1 and CM2, the wells 5 feet downgradient showed more sulfate removal than CM3, the well 10 feet downgradient.

**Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test**

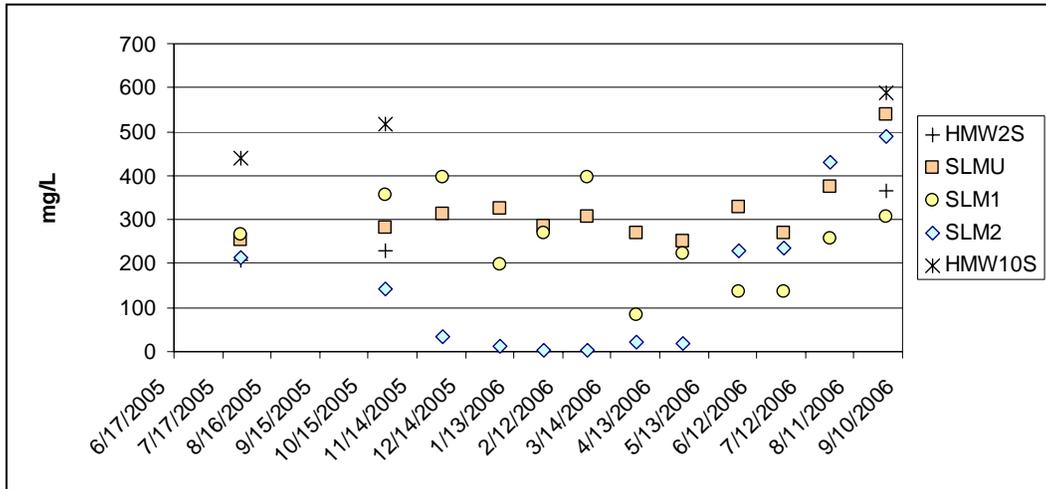


Figure 4-3A.—SLM Cell Sulfate

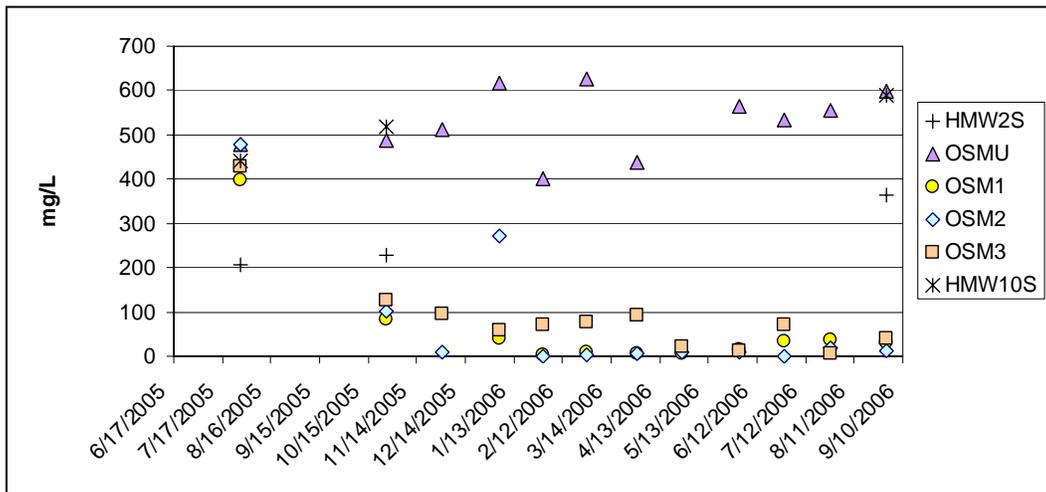


Figure 4-3B.—OSM Cell Sulfate

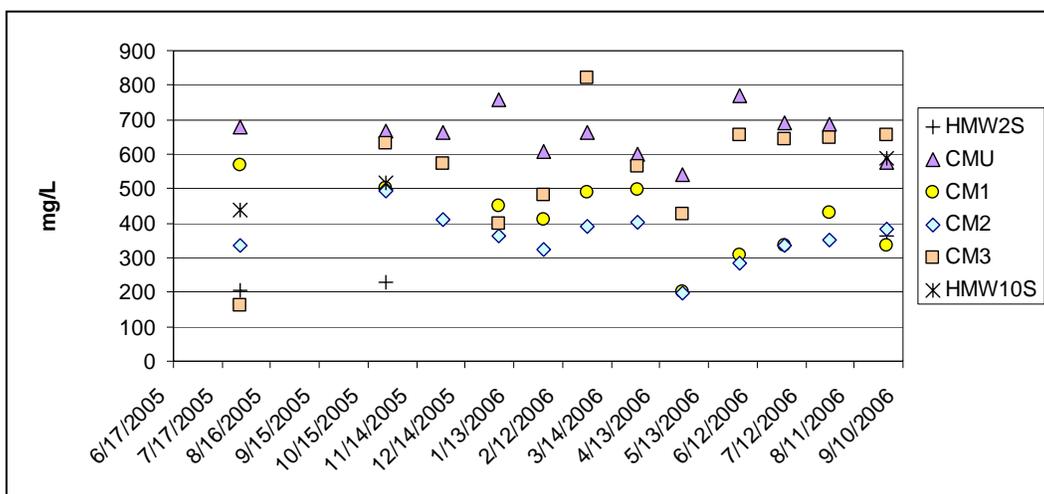


Figure 4-3C.—CM Cell Sulfate

4.1.2.3 Methane

Methane provides an indication of conditions most conducive to complete reductive dechlorination of TCE to ethene. Figure 4-4 shows methane concentrations over time in all of the cells. Figures 4.4A, 4.4B, and 4.4C illustrate the methane concentrations for the sodium lactate, emulsified oil substrate and chitin cells, respectively. Methane was observed at well SLM1, but the concentrations did not exceed 0.5 mg/L throughout the pilot test. For well SLM2, the concentration reached its peak (2.5 mg/L) in the June 27, 2006 sampling event before decreasing again with decreases in organic acids.

The wells OSM1 and OSM3 showed significant methane production peaking in the June 27, 2006 sampling event at about 12 mg/L and 10 mg/L, respectively, and declining thereafter. OSM2 showed the least methane production compared to other emulsified oil downgradient wells even though it showed the highest amounts of organic acids. It is suspected that the oil emulsion present near the well might be inhibiting methanogens.

The levels of methane observed at CM1 and CM2, the wells 5 feet downgradient, throughout the pilot test were surprisingly high given notable electron donor concentrations were observed only for the initial few months. Near-zero concentrations of methane were observed in the CM3, the well 10 feet downgradient. The presence of methane further strengthens the concept of coarse, long-lived chitin particles still being present near the injection points at this cell. Methane concentrations often reach as high as 15 mg/L in a strongly reducing environment with an excess of electron donor. The concentrations of methane observed at these wells indicate that methanogenesis is occurring very near the injection points, and the methane is being transported to the CM wells downgradient.

**Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test**

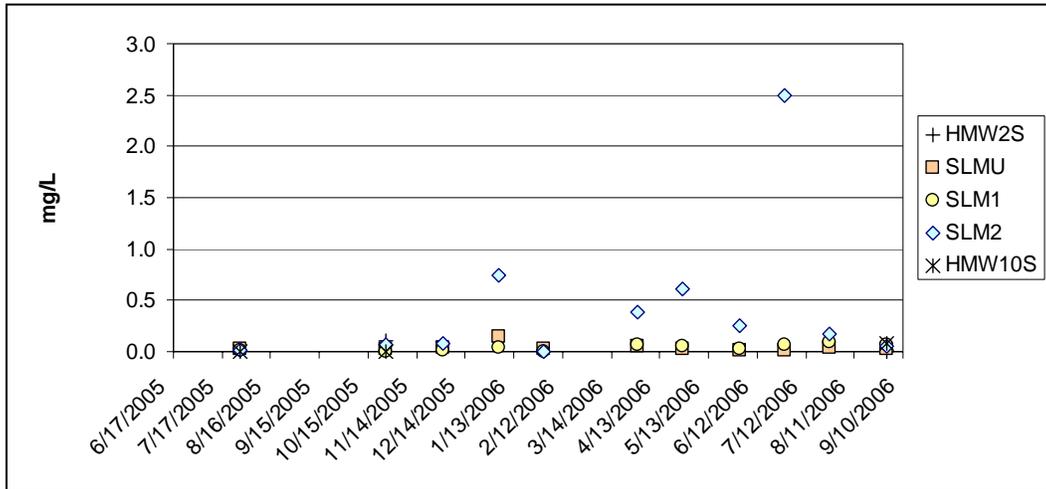


Figure 4-4A.—SLM Cell Methane

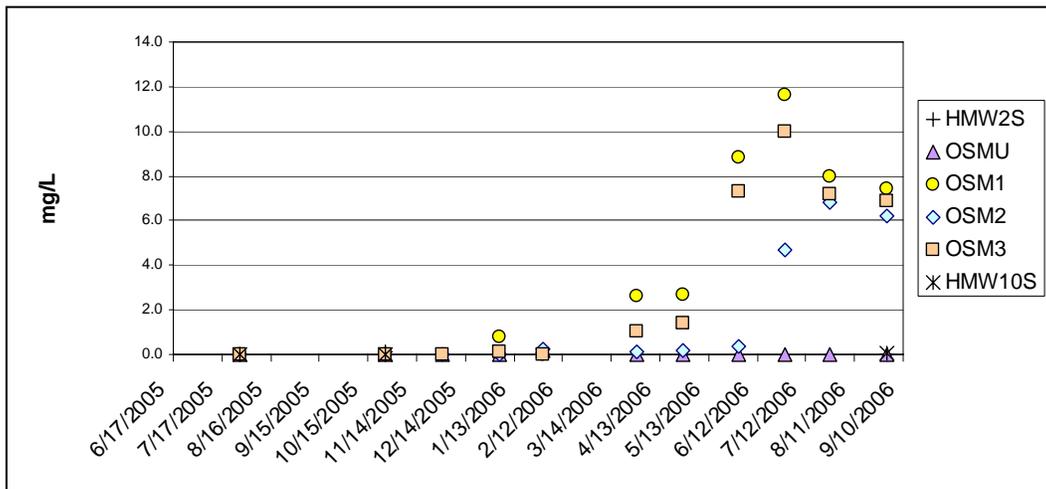


Figure 4-4B.—OSM Methane

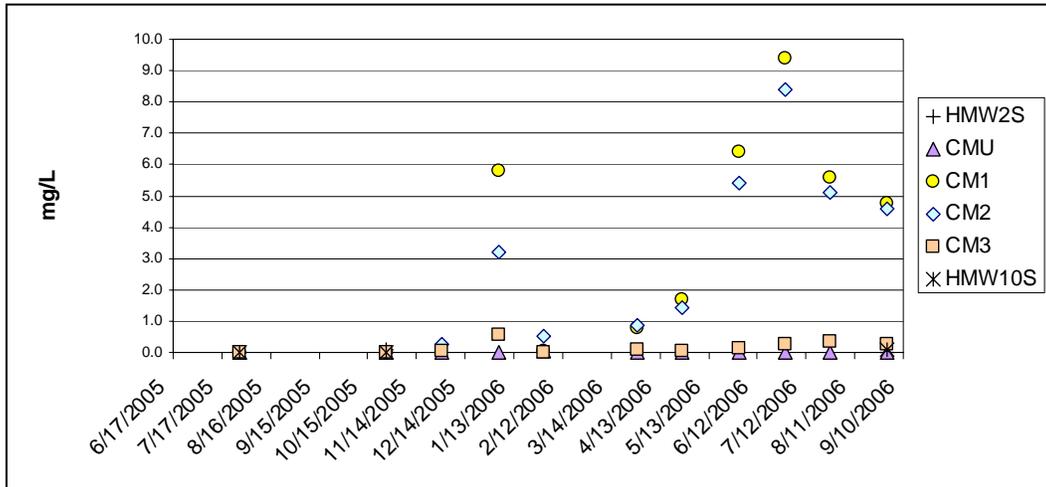


Figure 4-4C.—CM Methane

4.1.2.4 Methane Splits

As mentioned earlier, methane was produced at all the cells but the concentrations were lower than expected. Hence, an additional set of samples for analysis of ethene/ethane/methane was sent to the Microseeps, Inc. laboratory during the June 27, 2006 sampling event along with the usual set of samples sent to the Utah State University laboratory. Table 4-1 presents the comparison between the methane concentrations reported by each lab, using relative percent difference (RPD) as the metric. This comparison indicates that Microseeps results showed significantly higher concentrations of methane than the Utah State results for most samples. The comparison shows that more methane is likely being produced than previously reported, which only further strengthens the argument that electron donor addition is effectively creating appropriate redox conditions for complete reductive dechlorination at this site. Based on these results, it is recommended that the analytical laboratories be re-evaluated during the final remedial action planning for the Site.

**Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test**

Table 4.1.—Comparison of Methane data from Utah State and Microseeps.

Methane (µg/L)			
Well	Utah State Result	Microseeps Result	RPD
SLMU	11	130	168.8
SLM1	72	390	137.7
SLM2	2,500	4,300	52.9
OSMU	2	11	138.5
OSM1	11,800	14,000	17.1
OSM2	4,700	7,800	49.6
OSM3	10,000	10,000	0.0
CMU	2	19	161.9
CM1	9,400	10,000	6.2
CM2	8,400	9,200	9.1
CM3	260	1,300	133.3

4.1.3 Redox Summary

Based on the results discussed in this section it can be concluded that redox conditions have shifted in accordance with the electron donor distribution, and that conditions are generally favorable for reductive dechlorination where the electron donor is present. While little to no change in redox indicators is apparent in the upgradient wells for all three treatment cells, ferric iron reduction, sulfate reduction, and methanogenesis (in that order) are apparent in the downgradient wells for both the sodium lactate and emulsified oil cells, although the extent depended on the concentrations of electron donor.

The ORP of all three downgradient wells in the chitin cell was in the appropriate range (-100 mV to -300 mV) to facilitate dechlorination. In addition, some production of ferrous iron was observed, and high concentrations of methane were observed in the wells 5 feet downgradient even after the depletion of organic acids. However, sulfate concentrations remained near background levels throughout the pilot study for all three chitin cell monitoring wells, indicating that active sulfate reduction was not occurring in the chitin cell. These redox data, combined with the electron donor data, suggest that the larger and long-lived chitin flakes that were successfully injected into 3+ injection points are still present near their respective injection locations, but that the more soluble chitin fractions were initially transported to the chitin monitoring wells and are now depleted.

4.2 Dechlorination

The concentrations of organic acids and redox conditions only indicate whether conditions are favorable for reductive dechlorination to progress at the site. Thus, the concentrations of chloroethenes and ethene need to be monitored as direct evidence of the removal of chloroethenes, which are the contaminants of concern

at the Site. Molar concentrations are used in the figures in this section so that an evaluation of mass balance can be made (1 mole of DCE is produced from reductive dechlorination of 1 mole of TCE, 1 mole of VC is produced from 1 mole of DCE, and so on).

4.2.1 Sodium Lactate Cell

Figure 4-5 shows the molar concentrations of chlorinated ethenes upgradient and downgradient of the sodium lactate injection (SLI) well (Figure 2.1). Total chloroethene concentrations in SLMU, the upgradient well, primarily consisted of TCE and DCE with some amounts of VC and very little ethene. At SLM1 concentrations of chloroethenes decreased significantly initially unlike the increase in concentrations observed at SLMU. SLM1 was not receiving enough electron donor during the initial few months and was thus dominated by DCE following bioaugmentation until the end of February. As previously discussed, these results can be attributed primarily to the apparent relative hydraulic isolation of this monitoring well during that time period. The well received significant concentrations of electron donors in the months of March and April, which led to gradual conversion of DCE and VC to ethene. After the depletion of electron donor as measured during the end of May sampling event, the concentration of TCE, DCE and VC started rebounding with very little ethene production.

At SLM2, as seen in the figure, chloroethene concentrations increased initially. Following bioaugmentation, however, TCE was rapidly converted to DCE. DCE and VC concentrations then decreased gradually through the end of January before apparently plummeting at the end of February. Although the concentrations of organic acids were decreasing and reached near-zero concentration as measured at the end of March, the quantities were sufficient to support complete reductive dechlorination until the end of April, after which the concentrations of TCE, DCE, and VC rebounded with very little ethene production.

Once complete reductive dechlorination was achieved, loss of mass balance was observed at both SLM1 and SLM2. Although, it was unexpected, this phenomenon has been observed at other sites with similar conditions, namely shallow, relatively “thin” contaminated aquifers (e.g. French et al, 2003). This result can be attributed to the volatilization of VC and ethene to the vadose zone. Interestingly, once electron donor was depleted, and TCE and c-DCE rebound occurred at SLM2, the mass balance was restored. Overall, it can be concluded that complete reductive dechlorination of TCE was successfully stimulated where lactate was distributed following bioaugmentation.

**Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test**

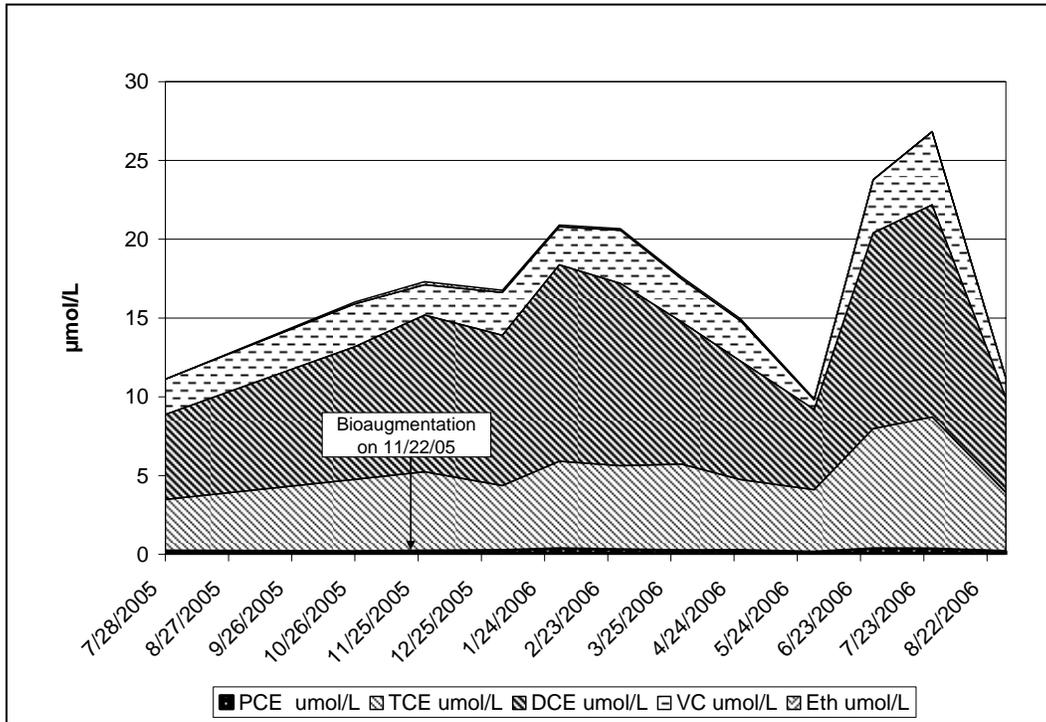


Figure 4-5A.—SLMU VOC

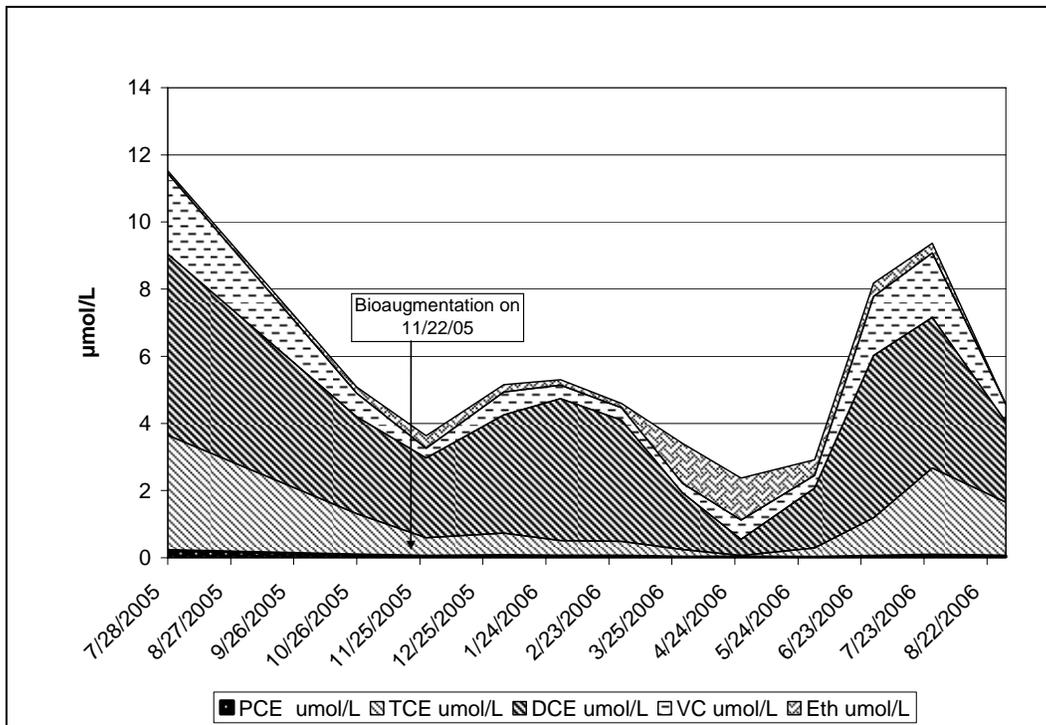


Figure 4-5B.—SLM1 VOC

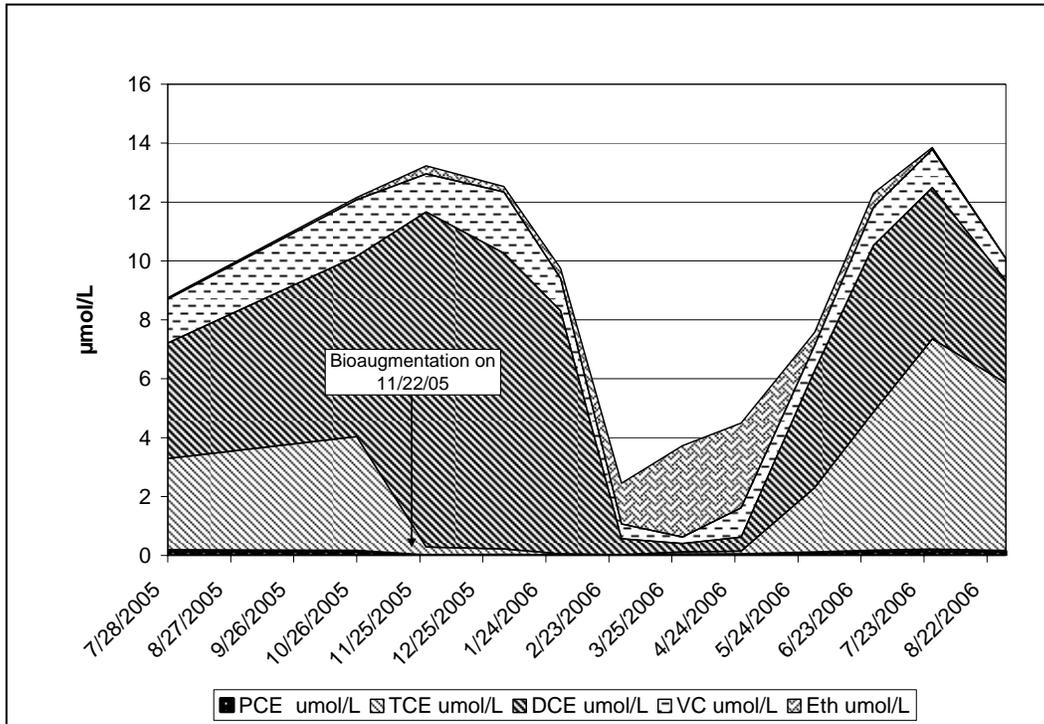


Figure 4-5C.—SLM2 VOC

4.2.2 Emulsified Oil Substrate Cell

Figure 4-6 provides the chloroethene concentrations over time upgradient and downgradient of the emulsified oil injection wells. As observed in SLMU, total chloroethene concentrations in the OSMU well primarily consisted of TCE and DCE with some amounts of VC and little to no ethene. Downgradient concentrations showed somewhat surprising behavior. Because TCE is hydrophobic, one of the concerns with the use of emulsified oil was that the TCE would partition out of the aqueous phase into the oil phase, resulting in a drop in TCE concentration without any decrease in overall contaminant mass. This was not observed during the pilot test, and in fact the opposite effect was seen. Chloroethene concentrations remained fairly constant in all three downgradient wells prior to bioaugmentation, but then began to increase significantly through January and even into February. The factor of increase ranged from about 3-6 to 11-20 μmol/L and significantly exceeded the change in upgradient concentrations. In addition, the composition of the chloroethenes shifted, with TCE being largely removed, DCE increasing dramatically at all wells, and VC increasing significantly at OSM1 and OSM3.

It is not clear whether the enhanced bioavailability of the chloroethenes is related to the simultaneous increase in organic acids at these locations, transport of the surfactant in the particular emulsified oil formulation used, the bioaugmentation event, or some other factor. Following February, DCE concentration rapidly

**Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test**

started decreasing as it was converted to VC and finally to ethene at OSM1 and OSM3. As in the lactate treatment cell, once dechlorination proceeded past c-DCE, a loss of mass balance was observed. Even though the concentration of organic acids have decreased at these wells the chloroethene concentrations are observed still to be near-zero, suggesting that the concentrations of organic acids are still enough to support complete dechlorination.

The same trends were not observed at OSM2 well where the highest concentrations of organic acids were observed. Dechlorination was found to stall at DCE with very minute quantities of VC and ethene. It is suspected that as with the methanogens, the oil phase might also be inhibiting the dechlorinators. It can be concluded from the data set that the emulsified oil clearly enhanced the bioavailability of the contaminants and stimulated complete reductive dechlorination where concentrations of organic acids were observed in the appropriate range.

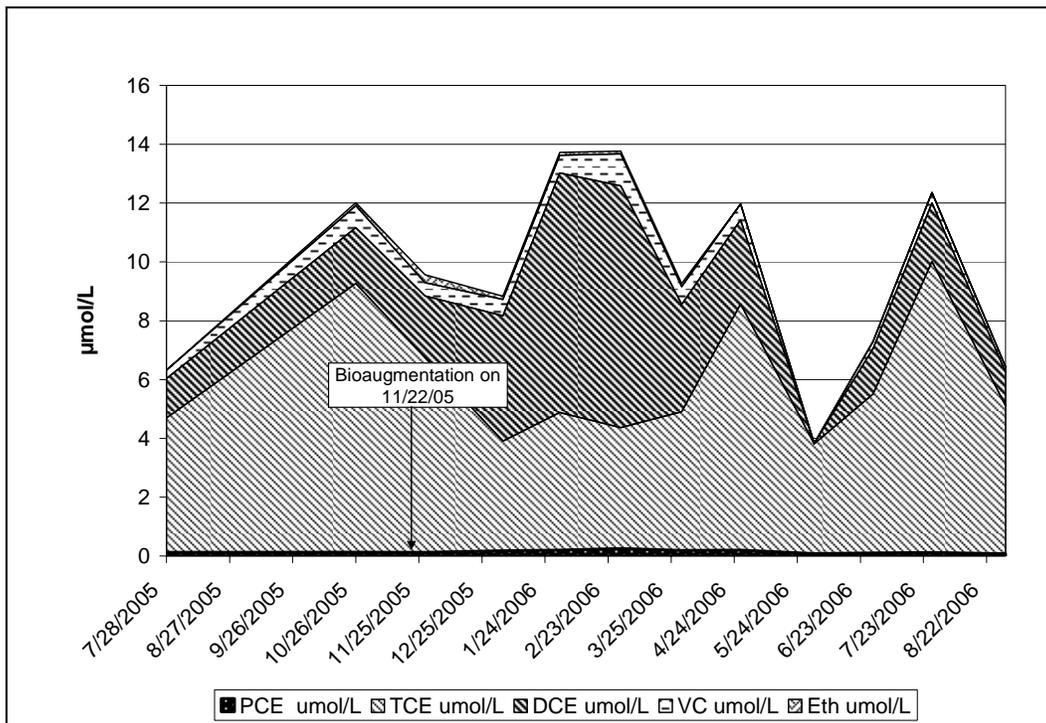


Figure 4-6A.—OSMU VOC

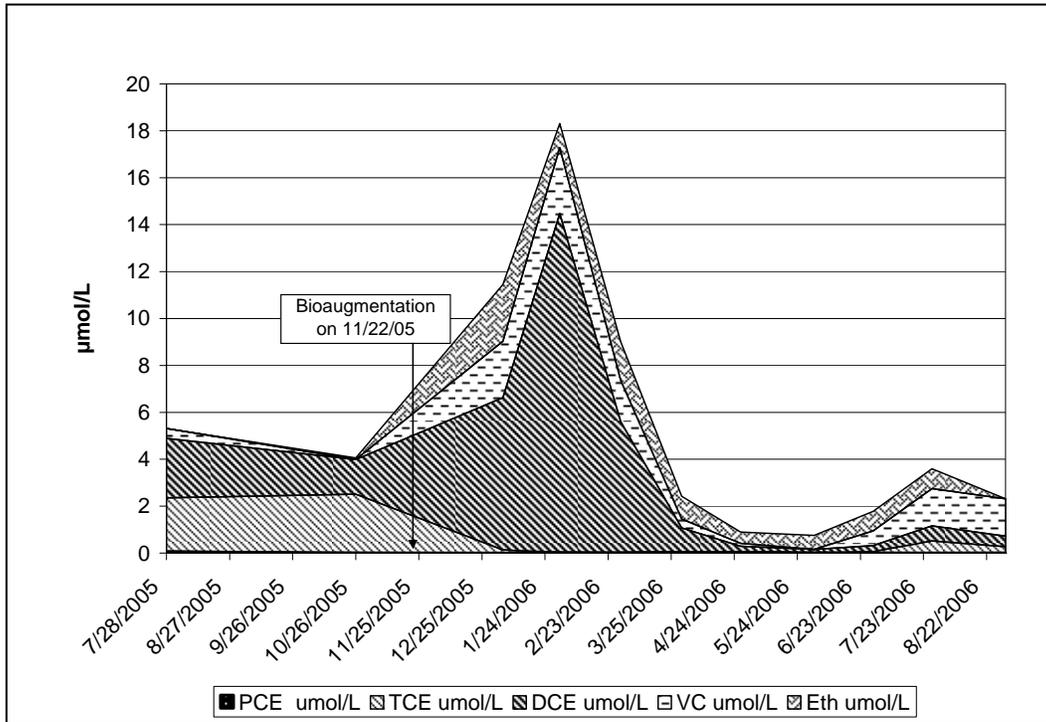


Figure 4-6B.—OSM1 VOC

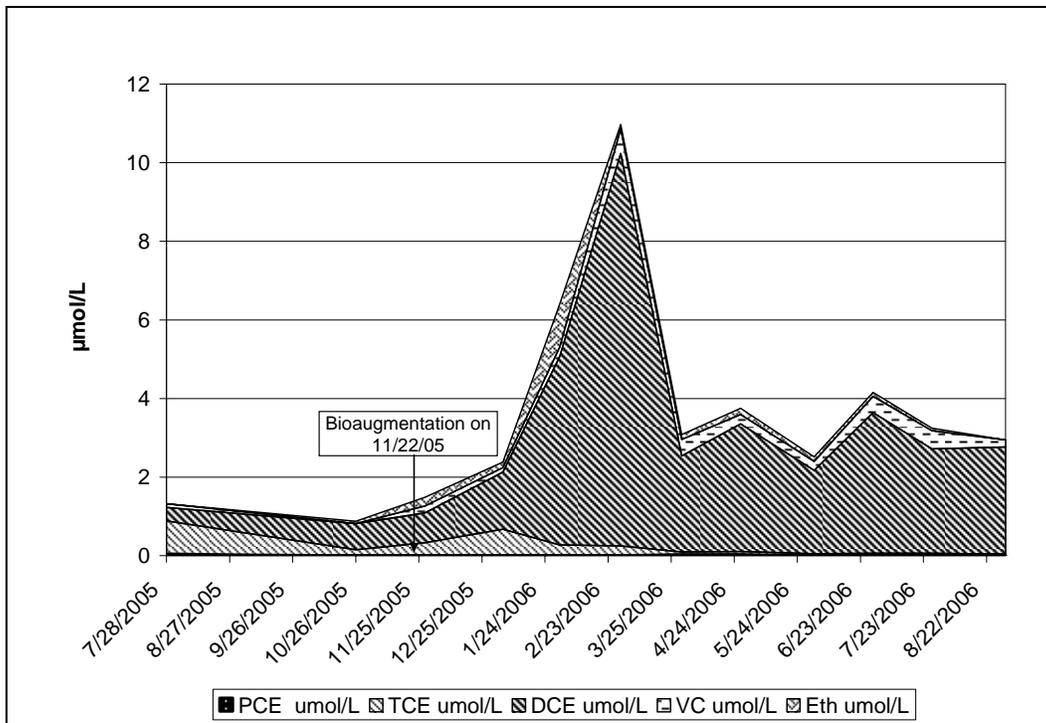


Figure 4-6C.—OSM2 VOC

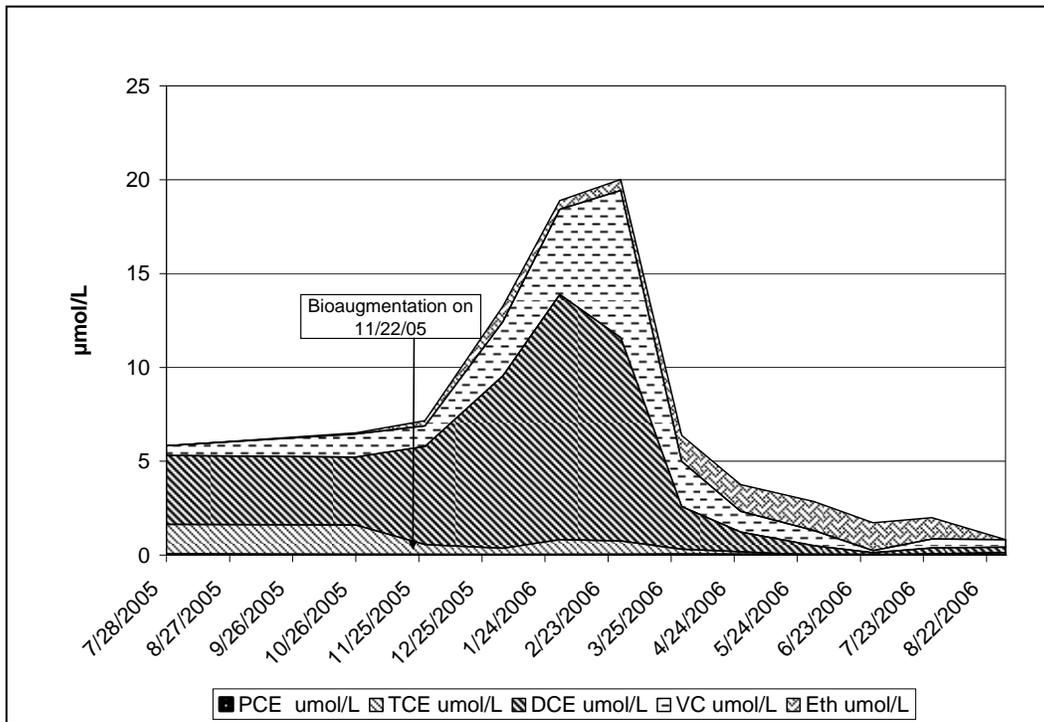


Figure 4-6D.—OSM3 VOC

4.2.3 Chitin Cell

Chloroethene concentrations at the upgradient well of the chitin cell were the lowest of the three cells to start, and then dropped precipitously even before bioaugmentation (Figure 4-7). TCE and DCE were the primary chloroethenes present at this well. The downgradient monitoring wells did not show a drop similar to that seen at the upgradient well, but chloroethene concentrations were much lower in these wells at the beginning of the pilot test. VC and ethene concentrations increased in both the wells 5 feet downgradient (CM1 and CM2) following bioaugmentation. Ethene production was first observed in the chitin cell, but was short-lived.

CM3, well the 10 feet downgradient, showed less production of VC and ethene compared to the wells 5 feet downgradient, with no production during the last three sampling events. The presence of all chloroethenes at the chitin wells following bioaugmentation suggests that concentrations of organic acids produced by chitin initially were sufficient to produce conditions favorable for dechlorination but not enough to remove all TCE present. Production of ethene after the initial few months with no organic acids present at these wells suggest that the long-lived chitin particles are present near the injection points. Thus, chitin injection and bioaugmentation clearly started the reductive dechlorination process, but removal of all TCE was not achieved due to the inability to inject

enough coarse long-lived chitin flakes. The production of ethene until the end of the pilot test in the wells 5 feet downgradient clearly showed that chitin could be a very efficient electron donor if injected successfully.

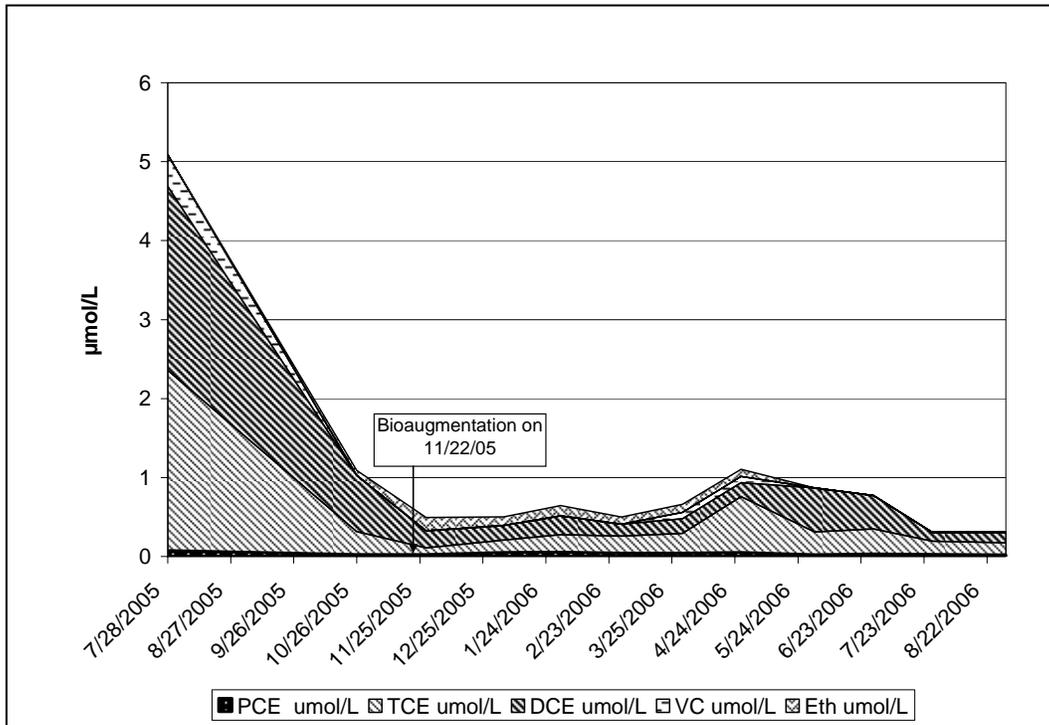


Figure 4-7A.—CMU VOC

**Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test**

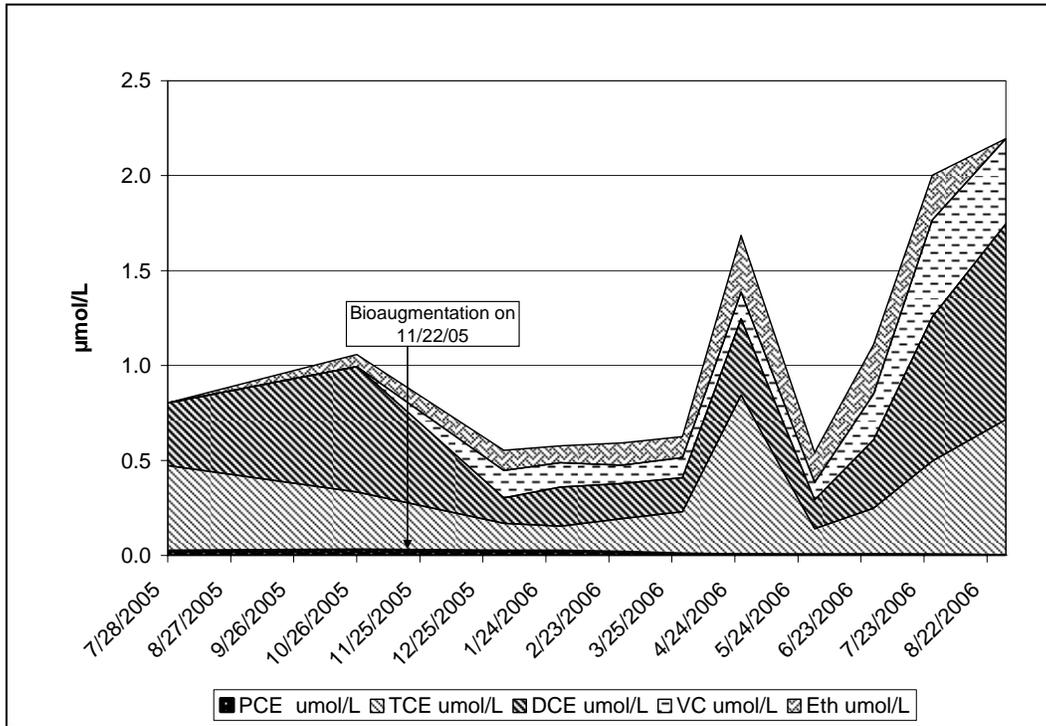


Figure 4-7B.—CM1 VOC

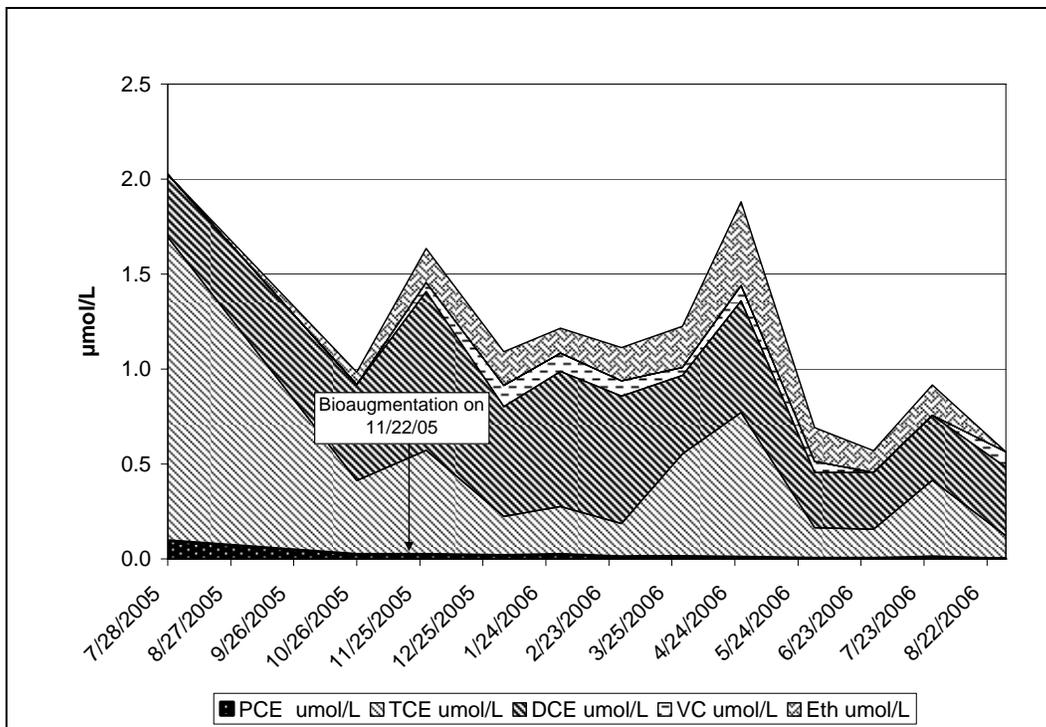


Figure 4-7C.—CM2 VOC

**Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test**

Table 4.2.—Comparison of Ethene data from Utah State and Microseeps.

Ethene (µg/L)			
Well	Utah State Result	Microseeps Result	RPD
SLMU	<MDL	3.4	NA
SLM1	11.2	21.0	60.9
SLM2	13.3	27.0	68.0
OSMU	<MDL	0.4	NA
OSM1	23.8	46.0	63.6
OSM2	2.8	4.4	44.4
OSM3	41.6	84.0	67.5
CMU	<MDL	0.1	NA
CM1	7.3	16.0	74.7
CM2	3.2	8.8	93.3
CM3	1.8	2.9	46.8

4.2.5 HMW2S and HMW10S Wells

As mentioned earlier, HMW2S (230 feet upgradient of the injection wells) and HMW10S (270 feet downgradient of the injection wells) wells were sampled during the baseline (July 27, 2005), first (October 26, 2005) and last (August 28, 2006) sampling events. The concentration of chloroethenes at both the wells remained fairly consistent during all three sampling events. At well HMW2S, which is located in the source area, chloroethene concentrations were in the range of 25-35 µmol/L. The concentrations of chloroethenes at the HMW10S were in the range of 0.2-1.2 µmol/L. The total chloroethene concentrations in both wells primarily consisted of TCE and DCE with some amounts of VC and no ethene.

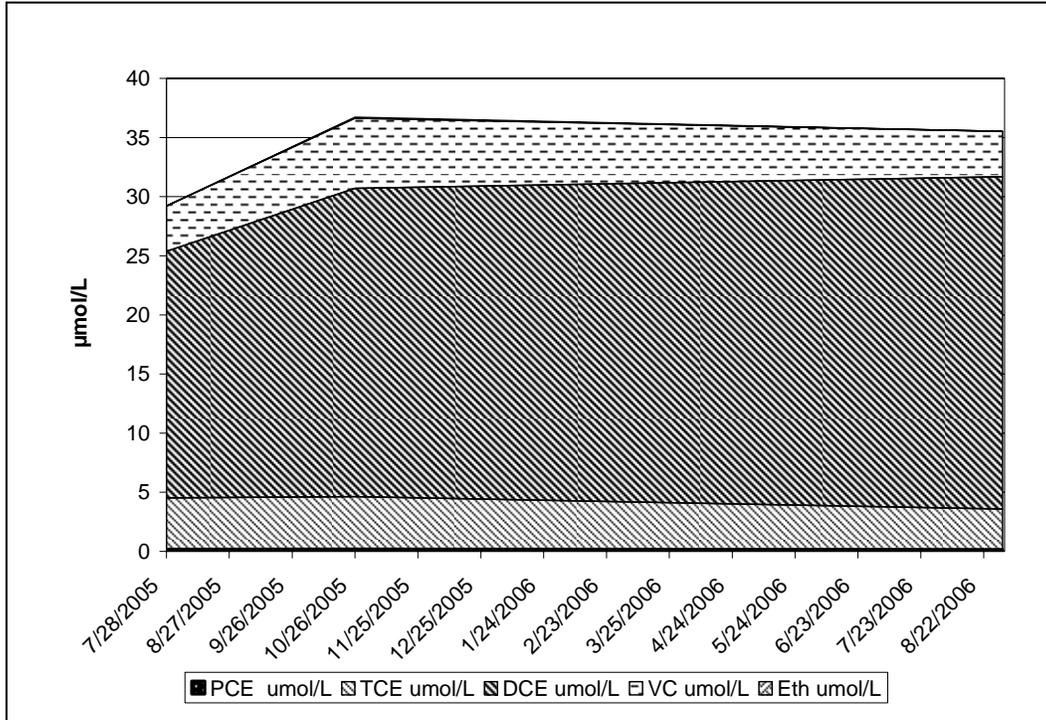


Figure 4-8A.—HMW2S VOC

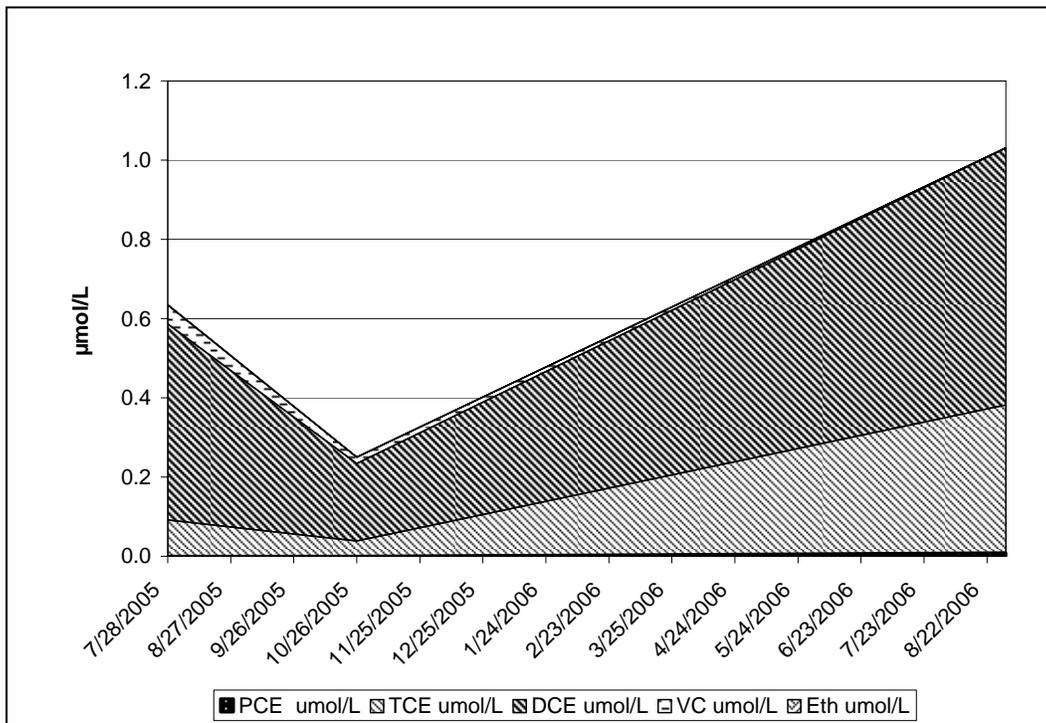


Figure 4-8B.—HMW10S VOC

4.2.6 Dechlorination Summary

Overall, each of the electron donors was successful in inducing complete dechlorination following bioaugmentation. The emulsified oil substrate was the only cell that showed complete removal of TCE throughout the pilot test, as it was the only one that had consistent electron donor distribution. The sodium lactate cell showed complete removal of TCE only during the initial months when good distribution of organic acids was observed.

The chitin cell was the first to show ethene production and showed partial removal of TCE at both wells 5 feet downgradient throughout the pilot test even though organic acids were observed only during the initial few months. These wells were being influenced by the larger and long-lived chitin particles present at the injection points throughout the pilot test. These results suggest that chitin could be a very effective electron donor if the larger chitin flakes could be injected successfully.

4.3 Bioaugmentation

Groundwater samples were analyzed for DNA of *Dehalococcoides spp.* bacteria at selected wells during baseline sampling on July 27, 2005 and none was detected. This suggested that stimulating complete dechlorination of TCE to ethene might be problematic, or at least slower than desired. In order to expedite complete dechlorination in the pilot test, it was decided to inoculate each of the three treatment cells with a microbial culture containing active *Dehalococcoides spp.* Groundwater samples were analyzed again for DNA immediately before bioaugmentation was performed on November 20, 2005 and this time a very low level detection was found in OSM1 (Figure 4-8). DNA was analyzed in the January 3, March 28, and May 30, 2006 sampling events and it was found that *Dehalococcoides spp.* numbers have been maintained above 10^8 cells/mL in the oil and chitin cells during all three sampling events, and increased above 10^8 cells/mL in the lactate cell as measured during the March and May sampling events. The fact that DNA numbers are lower in the lactate cell is actually consistent with the fact that SLM1 was used to sample for DNA, and this well had the least electron donor initially. *Dehalococcoides* numbers increased eventually once the electron donor distribution improved. Thus, the DNA results suggest that the added dechlorinating bacteria are thriving in their new surroundings.

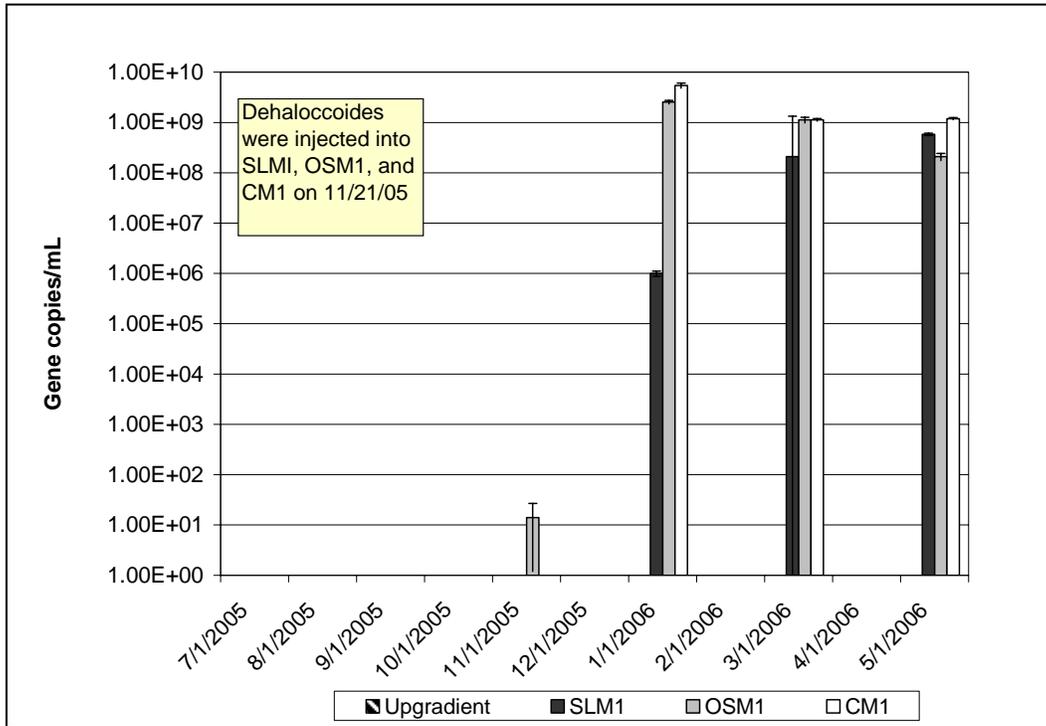


Figure 4-9.—Q-PCR Results for Dehalococcoides 16s rRNA gene at Bountiful OU1

Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test:

5. Pilot Scale Summary and Conclusions

The primary objective of the pilot test, determining the site-specific requirements for full-scale implementation of the EAB at the Site, was successfully accomplished. Three electron donors, one aqueous (sodium lactate) and two “slow-release” (emulsified oil and chitin) were tested at the Site.

In terms of electron donor distribution, sodium lactate was successfully delivered to the aquifer, but the injection strategy needs to be optimized to increase the extent of distribution if lactate is to be considered for use in the full scale remedy. The emulsified oil substrate was successfully delivered and effective donor distribution was maintained throughout the pilot test. For the chitin treatment cell, the delivery of the larger and long-lived chitin flakes was problematic. Because of this, only the smaller chitin particles were successfully injected, which significantly reduced the longevity of chitin in the subsurface.

All three treatment cells showed strongly reducing conditions within 1 month of electron donor injection, and in general these favorable reducing conditions were maintained as long as organic acids were available. In addition, complete reductive dechlorination of TCE was achieved at all three treatments cells as a function of electron donor distribution. Also, bioaugmentation appeared to have been successful at the site as the dechlorinating bacteria (*Dehalococcoides spp.*) were found to be proliferating. Not only the counts of these bacteria have increased to a high number ($>10^8$ cells/L), but also these bacteria have been successful in facilitating complete dechlorination at the Site.

Overall, the emulsified oil treatment cell showed the most consistent performance over the course of the pilot test. Based on the pilot test results, emulsified oil with bioaugmentation is recommended as the electron donor to be used for full-scale implementation of EAB at the Site. An overview of the full-scale remedy is provided in the next section.

Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test:
6. Full Scale Implementation of EAB

This section provides guidance for design, and implementation of EAB for full-scale remediation of chlorinated solvent-contaminated groundwater at the Site. A technical memorandum for the cost estimate for the application of this remedy was prepared and submitted to EPA, September 19, 2006.

6.1 Selected Electron Donor

While all three electron donors showed the potential to achieve complete dechlorination at the site, emulsified oil is recommended for full-scale application based on the following characteristics identified in the pilot study:

- ◆ Was easily distributed in site soils
- ◆ Achieved reducing conditions fairly rapidly and maintained them
- ◆ Supported growth and proliferation of the *Dehalococcoides spp.* bioaugmentation culture
- ◆ Facilitated complete dechlorination of contaminants to ethene without significant accumulation of vinyl chloride
- ◆ Was the longest lived electron donor, with data supporting an *in situ* longevity well in excess of 1 year (projected to last approximately 2 years)
- ◆ Displayed apparent enhanced mass transfer of contaminants from the sorbed phase to the aqueous phase, thereby expediting mass removal.

6.2 Overview of Remedy

For the source area, it is recommended that emulsified oil be used to inundate the area with contaminant concentrations greater than 200 µg/L, as described in Section 6.3 below. For the downgradient plume, the anticipated application of EAB based on the pilot test is a relatively passive system that relies on the ambient flow of contaminated groundwater through the treatment zone to effect treatment. The design of the bio-barriers, however, must account for the current

Final Report for the Enhanced Anaerobic Bioremediation Pilot Test

understanding of groundwater flow conditions at the site. In addition to source area treatment, it is assumed that up to three bio-barriers will be installed perpendicular to groundwater flow in the downgradient plume. The first will be installed upgradient of HMW-10S, just west of the HatchCo property (Figure 2.1). The location of the second and third bio-barriers will be finalized based on the need to treat downgradient “hot spots” as determined from ongoing groundwater monitoring. Depending on electron donor longevity, it is anticipated that it will be necessary to “recharge” the bio-barriers with electron donor every 2 years until contaminants have decreased to concentrations that can be treated by MNA. Two injection events in the source area and three in each of the bio-barriers are anticipated to be sufficient to reduce concentrations appropriately. Depending on the effectiveness of bioremediation amendments, the natural attenuation efficiency and the degradation capability of the microbial community, it is anticipated this approach will take about 4 years to reduce groundwater concentrations to levels that will be conducive for MNA in the source area. In the plume, the bio-barriers are anticipated to reduce groundwater contaminant concentrations to levels conducive to MNA in about 8 years. Groundwater monitoring is expected to be required for at least 15 years.

In terms of specific concentrations that are expected to remain following treatment (i.e. the concentrations conducive for MNA discussed above), pilot test data from well OSM3 can be used as an indicator. The reason is that this well is located 10 feet downgradient of the emulsified oil injection points, and represents conditions at the downgradient edge of the active biological treatment zone.

VOC data from OSM3 during the last months of the pilot study showed that essentially all of the PCE and TCE were degraded, and concentrations of c-DCE were below the MCL of 70 µg/L. The only VOC constituent that was detected above MCLs at OSM3 was VC, which was measured at approximately 25 µg/L (0.4 µmol/L). In order to estimate what concentrations of VC might remain following source area treatment, the chloroethene concentrations in OSM3 need to be “scaled” to those observed historically in the source area. The total concentrations of VOCs observed at the OSM3 peak were approximately 2,630 µg/L (20 µmol/L) as TCE, which are similar to the concentrations historically observed near the source area. Thus, based on the OSM3 data, it is reasonable to expect that concentrations of VC leaving the treatment area should be below 25 µg/L, and that all other chloroethenes will be below MCLs.

Even though VC might remain at concentrations greater than MCLs, it is expected that attenuation would occur rapidly. One reason is that VC can be biodegraded by a number of different pathways (e.g. oxidized or reduced anaerobically and cometabolically oxidized), as opposed to just reductive dechlorination. In addition, any VC leaving the source area will be intercepted by the downgradient bio-barriers before it reaches potential receptors. Thus the concentrations of VC are unlikely to pose health hazard to the residents of the area.

Given that VC may still be present above MCLs following treatment, and given that some volatilization of VC is thought to have occurred during the pilot study, it is important to consider the impacts of VC vapor migration. At other sites with similar conditions, low concentrations of VC have been measured in the vadose zone (French et al, 2003). This could occur during full scale remediation at the Site, but would be limited to the actual treatment areas. Given that the areas currently planned for injection are not directly adjacent to residential areas, the hazards to residents is expected to be minimal. Still, vapor monitoring will be included in the final remedy in order to confirm that VC vapor is not problematic.

6.3 Injection Strategy

Based on the pilot study results, emulsified oil (e.g., EOS®) is recommended as the electron donor. As in the pilot study, the electron donor will be injected using 1-in. temporary wells with pre-packed screens. In the source area, an area about 200 ft wide by 250 ft long is assumed to require treatment (Figure 6.1). This design is based on the estimated extent of contaminant concentrations in the source area above 200 µg/L, not including the area treated during the pilot test. An injection grid will be created that injects electron donor on 20-ft centers across the width of the source area, and 40-ft centers along the length of the source area, for a total of 77 injection points. For the bio-barriers, injection points will be on 20-ft centers in two parallel, but staggered rows 10 to 20 ft apart. This gives 22 injection points per barrier, or 66 total. It is assumed that approximately 1000 gallon of electron donor solution will be injected at each location. The solution will be composed of 10% emulsified oil as delivered from the manufacturer, and 90% water. In other words, 100 gallon of emulsified oil product will be injected at each location.

6.4 Bioaugmentation

Given the low numbers of *Dehalococcoides spp.* bacteria in the source area, it will likely be necessary to inoculate new treatment areas with a dechlorinating culture. This will be accomplished simply by pumping groundwater from the pilot test area into wells located in new treatment zones. It should be noted that redox conditions should not be allowed to become oxidizing in an area to be used as a source for the dechlorinating culture before scale-up is accomplished. If groundwater cannot be pumped directly from one well to another, it can be pumped into a container that is under an inert gas headspace and then transported to the inoculation location and injected. Once a new area is inoculated and is achieving complete dechlorination, groundwater from that area can be used to inoculate other areas.

6.5 Groundwater Monitoring

The groundwater monitoring frequency and parameter list will be reduced in the full scale remedy as compared to the pilot test. This reduction is justified because the remediation process has already been demonstrated, and the objective of monitoring will simply be to measure the change in conditions expected from the electron donor injection, and progress toward completion. Monitoring parameters will include contaminants and degradation products, key redox parameters, electron donor concentrations (based on a surrogate such as total organic carbon or chemical oxygen demand), and standard purging parameters. In addition, soil vapor monitoring will be included to ensure that gaseous degradation products such as vinyl chloride and methane are not posing a hazard. The sampling frequency will be reduced to semiannual for the first 2 years and then annual thereafter. A total of 15 years of monitoring is assumed for cost estimating purposes. It is anticipated that 15 wells and 4 soil vapor monitoring wells are sampled during each event.



Figure 6-1.—Conceptual Full Scale EAB Layout

Final Report for the
Enhanced Anaerobic Bioremediation Pilot Test:
References

- CDM. 2006a. Final Pilot Study Implementation Plan for Operable Unit 1 – Enhanced Anaerobic Bioremediation at the Bountiful/Woods Cross 5th South PCE Plume Superfund Site Davis County, Utah, May 2005.
- _____. 2006b. Interim Report for Operable Unit 1 – Enhanced Anaerobic Bioremediation at the Bountiful/Woods Cross 5th South PCE Plume Superfund Site Davis County, Utah, April 2006.
- Freedman, D. L.; Gossett, J. M. 1989. Applied and Environmental Microbiology 55, 2144-2151.
- French, J. H., A. Rossi, K. T. Kirk, D. B. Blackwelder, K. S. Sorenson, B. Rahm, L. Alvarez-Cohen, S. Le, M. Pound, and P. Tamashiro. 2003. “Phased In Situ Biostimulation/Bioaugmentation Pilot Testing in a Coastal Aquifer.” Proceedings of the 8th International In Situ and On-Site Bioremediation Symposium, Battelle Press, Columbus, Ohio, June.

