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February 23, 2015

Mr. Stephen A. Cobb, Chief  
c/o Mrs. Brandi Little  
Governmental Hazardous Waste Branch Land Division  
Alabama Department of Environmental Management  
P.O. Box 301463  
Montgomery, Alabama 36130-1463

*Via Email*

**SUBJECT:** *Remedy Selection Update / Addendum to Final Corrective Measures Implementation Plan Training Area T-6 (Naylor Field), Parcel 183(6) and Cane Creek Training Area, Parcel 501(7), McClellan, Anniston, Alabama dated August 2008*

Dear Mr. Cobb:

On behalf of the McClellan Development Authority (MDA), Matrix Environmental Services, LLC (MES) is pleased to submit this *Remedy Selection Update / Addendum to Final Corrective Measures Implementation Plan Training Area T-6 (Naylor Field), Parcel 183(6) and Cane Creek Training Area, Parcel 501(7), McClellan, Anniston, Alabama dated August 2008 (Final CMIP)* to document the transition to in situ bioremediation (ISB) as requested in the Alabama Department of Environmental Management (ADEM) comments dated December 23, 2014 on the *Corrective Measures Effectiveness Report, June 2013 to March 2014 Monitoring Events* dated May 20, 2014 and as agreed to during the technology transition meeting on November 14, 2013.

Groundwater sample results from monitoring wells and air monitoring data collected from the soil vapor extraction / air sparging (SVE/AS) system summarized in the Corrective Measures Effectiveness Reports for the Site indicate the corrective action constituents of concern (COC) concentrations have generally decreased as a result of SVE/AS operation, but operational data indicate the SVE/AS system achieved near maximum benefit. Given the heterogeneities in the Site geology and concomitant difficulty in removing volatile organic compounds (VOCs) from groundwater, a treatment train involving a combination of remediation technologies was described in the *Final CMIP* as shown below. The SVE/AS system was shut down in November 2013 with ADEM's concurrence to begin transitioning to ISB as described in the *Final CMIP*.



A groundwater sample was collected from well CWM-183-MW23 in December 2013 for biotreatability (laboratory) testing to: (i) evaluate optimum conditions to facilitate COC biodegradation; and (ii) serve as proof of performance testing to support field implementation of the ISB. The results of the biotreatability testing indicated the SRS<sup>®</sup>-SD, an emulsified vegetable oil (EVO) amendment, in combination with KB-1<sup>®</sup> Plus bioaugmentation culture, and near neutral pH conditions are effective in achieving reductive dechlorination of COCs. The biotreatability study report is included as Appendix A.

Implementation of ISB at T-6 was performed by MES in collaboration with Geosyntec Consultants and drilling and injection subcontractors. The work included (i) installation of four additional monitoring wells; (ii) using eight existing SVE wells (Figure 1) as ISB injection wells (IWs); (iii) injection of ISB materials into the eight IWs to establish a biologically active zone; and (iv) monitoring and reporting as required by the underground injection control (UIC) Permit Number ALSI9908664 and CMER. The eight SVE wells are installed to the top of bedrock or into the bedrock (up to 12 to 15 ft) and therefore the target injection interval not only targets shallow groundwater in the residuum but also the top of rock/transition between residuum and bedrock which is a preferential pathway for contamination. Four borings were advanced into bedrock using rotasonic drilling to further evaluate geologic conditions and converted into monitoring wells (CWM-183-MW32, CWM-183-MW33, CWM-183-MW34, and CWM-183-MW35) to monitor the injection fluids in bedrock downgradient of SVE-5 and SVE-8. Boring logs/well completion forms and survey data are included in Appendix B.

In December 2014, prior to the injection activities, a baseline groundwater sampling was performed. In addition to the routine VOCs, ferrous iron, dissolved hydrocarbon gases, anions, total organic carbon, and alkalinity were also measured in select wells as summarized in Table 1. The analytical results will be included in the next CMER.

The baseline sampling included ammonium, nitrate, and sulfate from two locations (CWM-183-MW03 and CWM-183-MW15) in accordance with the UIC permit. The data will be reported under separate cover via the ADEM electronic environmental (E2) DMR reporting system and also included in the CMER. Sampling was also attempted at CWM-183-MW05, as specified in the UIC permit, on December 11, 2014; there was, however, insufficient water in the well to allow for sample collection.

ISB implementation was completed between January 6 and 13, 2015 by Vironex, Inc. (Millersville, MD). Injection was performed in eight existing SVE wells, and included the following permitted amendments: (i) EVO; (ii) sodium bicarbonate; (iii) sodium bromide (injection wells SVE-5 and SVE-8 only); (iv) KB-1<sup>®</sup> Plus; (v) sodium sulfite; and (vi) potable water.

The overall injection quantities are summarized in Table 2, and quantities per each well are presented in Table 3. These quantities included a total volume of approximately 20,480 gallons (gal) of solution containing 2,000 gal of EVO, 6,680 pounds (lb) of sodium bicarbonate, 50 lb of sodium bromide, 16 liters of KB-1<sup>®</sup> Plus; and approximately 0.2 lb of sodium sulfite. The total amendment quantities are in general agreement with those outlined in the UIC Permit Application.

During the EVO injection, monitoring was performed at select locations to assess radius of influence (ROI). ROI monitoring consisted of collecting grab samples and visual inspection for EVO, which has a milky white color, and field measuring conductivity and turbidity, which

increase when EVO is present (Table 4). Because of the subsurface heterogeneities and time variant nature of the ROI, it is difficult to assess the ROI of the injection fluids. However, EVO, as well as increases in conductivity and/or turbidity, was observed in wells CWM-183-MW-06, CWM-183- MW08, CWM-183-MW-21 and AS-5 and indicates that the magnitude of the ROI may be on the order of up to approximately 40 feet - 50 feet. These observations are generally supported by depth to water observations during the injection event (Figure 2), as a slight rise in water elevation during injection was noted in wells CWM-183-MW06 and CWM-183-MW08. Water level rise was also observed in CWM-183-MW23, suggesting a broader influence resulting from the injection event than identified by assessing conductivity, turbidity, and/or presence of EVO. Over time the best estimate of the effective ROI will be determined based on the reduction of COC concentrations.

An electronic copy of this document has been provided to Mrs. Brandi Little via e-mail and two hard copies will follow by mail. Please contact me at (256) 847-0780 (Anniston) or (770) 594-0331 (Atlanta) should you have any questions or comments.

Sincerely,  
**MATRIX ENVIRONMENTAL SERVICES, LLC**



Richard Satkin, P.G  
McClellan Program Manager

#### Enclosures

Attachments: Table 1: Baseline Sampling  
Table 2: ISB Injection Overview  
Table 3: ISB Injection Details  
Table 4: ROI Monitoring During ISB Injection  
Figure 1: Injection and Monitoring Well Locations  
Figure 2: Water Levels During ISB Injection  
Appendix A: Laboratory Biotreatability Study Report  
Appendix B: Well Logs and Survey Data

cc: Mrs. Brandi Little, ADEM (two paper copies)  
Mr. Robin Scott, MDA (one paper copy)  
Ms. Lisa Holstein, U.S. Army (one paper copy)  
MES Files (one paper copy)

**TABLE 1: PERFORMANCE MONITORING PROGRAM - BASELINE SAMPLING FOR QUARTERLY COMPLIANCE MONITORING WELLS**  
**Training Area T-6 (Naylor Field)**  
**McClellan, Anniston, Alabama**

Well ID	Baseline (Pre-Injection) Sampling for Compliance Monitoring Wells							
	Field Parameters <sup>(1)</sup>	Ferrous Iron <sup>(2)</sup>	VOCs <sup>(3)</sup>	DHGs <sup>(4)</sup>	Anions for Performance Monitoring <sup>(5)</sup>	Anions for UIC Permit Monitoring <sup>(6)</sup>	TOC <sup>(7)</sup>	Alkalinity <sup>(8)</sup>
CWM-183-MW04	✓	✓	✓	✓	✓		✓	✓
CWM-183-MW06	✓	✓	✓	✓	✓		✓	
CWM-183-MW11	✓	✓	✓	✓	✓		✓	✓
CWM-183-MW13	✓	✓	✓	✓	✓		✓	
CWM-183-MW07	✓		✓	✓			✓	
CWM-183-MW08	✓		✓	✓			✓	
CWM-183-MW09	✓		✓	✓			✓	
CWM-183-MW20	✓		✓	✓			✓	
CWM-183-MW21	✓		✓	✓			✓	
CWM-183-MW22	✓		✓	✓			✓	
CWM-183-MW23	✓		✓	✓			✓	
CWM-183-MW28	✓		✓	✓			✓	
CWM-183-MW15	✓		✓			(✓)	✓	
CWM-183-MW16	✓		✓				✓	
CWM-183-MW17	✓		✓				✓	
CWM-183-MW25	✓		✓				✓	

Notes:

- Field parameters include depth to water (DTW), temperature, turbidity, conductivity, pH, oxidation-reduction potential (ORP) and dissolved oxygen (DO).
- A field kit can be used for ferrous iron. Geosyntec recommends CHEMets Kit K-6210.
- Suite of volatile organic compounds (VOCs) is the same as long-term monitoring.
- Dissolved hydrocarbon gases (DHGs; i.e., methane, ethane, and ethene) via RSK Method 175 or equivalent.
- Anions for performance monitoring include chloride, bromide, sulfate, and nitrate. Chloride and bromide via USEPA Method 300.1; sulfate and nitrate via USEPA Method 300.
- Anions for monitoring per UIC Permit include sulfate, nitrate, and ammonium. Ammonium via USEPA Method 300.7 or 350.2 or 350.3; sulfate and nitrate via USEPA Method 300.
- Total organic carbon (TOC), preserved with H<sub>3</sub>PO<sub>4</sub> and analyzed via USEPA Method 415.1.
- Alkalinity via USEPA Method 310.1 or 310.2.
- Gray shading indicates analytes overlapping with quarterly compliance monitoring, per the Performance, Compliance, and Monitoring Plan (PCMP).

**TABLE 2: ISB INJECTION OVERVIEW**  
**Training Area T-6 (Naylor Field)**  
**McClellan, Anniston, Alabama**

Anmendment Injection Parameters	Target Value	Injected Value
<b>Donor/Buffer</b>		
Donor/Buffer Injection Volume [gal]	20,000	20,000
Volume of SRS <sup>®</sup> -SD [gal]	2,000	2,000
Sodium Bicarbonate Mass [lb]	6,680	6,680
Mass of NaBr (application to 2 wells) [lb]	50	50
<b>Bioaugmentation Culture/Anaerobic Chase Water</b>		
Bioaugmentation Culture Volume [L]	16	16
Sodium Sulfitte Mass [lb]	0.21	0.21
Anaerobic Water [gal]	400	480
<b>Total Injection Volume (Donor/Buffer + Anaerobic Water)</b>	<b>20,400</b>	<b>20,480</b>

Notes:

ISB - in situ bioremediation

SRS<sup>®</sup>-SD - emulsified vegetable oil (EVO) product

gal - gallons

L - liters

lb - pounds

NaBr - sodium bromide

**TABLE 3: ISB INJECTION DETAILS**  
**Training Area T-6 (Naylor Field)**  
**McClellan, Anniston, Alabama**

Well ID	Injection Dates		SRS®-SD Volume (gal)	Sodium Bicarbonate (lb)	Sodium Bromide (lb)	Donor/Buffer Solution Volume (gal)	KB-1® Plus Volume (L)	Sodium Sulfite (lb)	Anaerobic Water Volume (gal)	Total Volume of Injectate (gal)
	Started	Completed								
SVE-1	1/7/2015	1/10/2015	250	835	0	2,500	2	0.026	60	2,560
SVE-2	1/10/2015	1/12/2015	250	835	0	2,500	2	0.026	60	2,560
SVE-3	1/7/2015	1/10/2015	250	835	0	2,500	2	0.026	60	2,560
SVE-4	1/7/2015	1/10/2015	250	835	0	2,500	2	0.026	60	2,560
SVE-5	1/10/2015	1/13/2015	250	835	25	2,500	2	0.026	60	2,560
SVE-6	1/7/2015	1/10/2015	250	835	0	2,500	2	0.026	60	2,560
SVE-7	1/10/2015	1/12/2015	250	835	0	2,500	2	0.026	60	2,560
SVE-8	1/10/2015	1/13/2015	250	835	25	2,500	2	0.026	60	2,560
<b>Total</b>			<b>2,000</b>	<b>6,680</b>	<b>50</b>	<b>20,000</b>	<b>16</b>	<b>0.21</b>	<b>480</b>	<b>20,480</b>

Notes:

- ISB - in situ bioremediation
- SRS®-SD - emulsified vegetable oil (EVO) product
- gal - gallons
- L - liters
- lb - pounds
- NaBr - sodium bromide

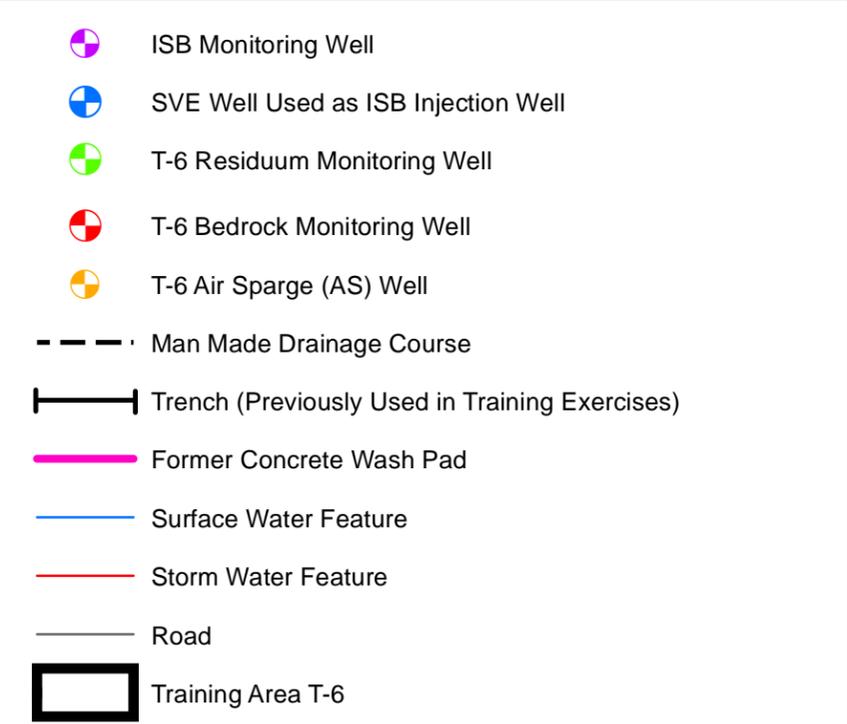
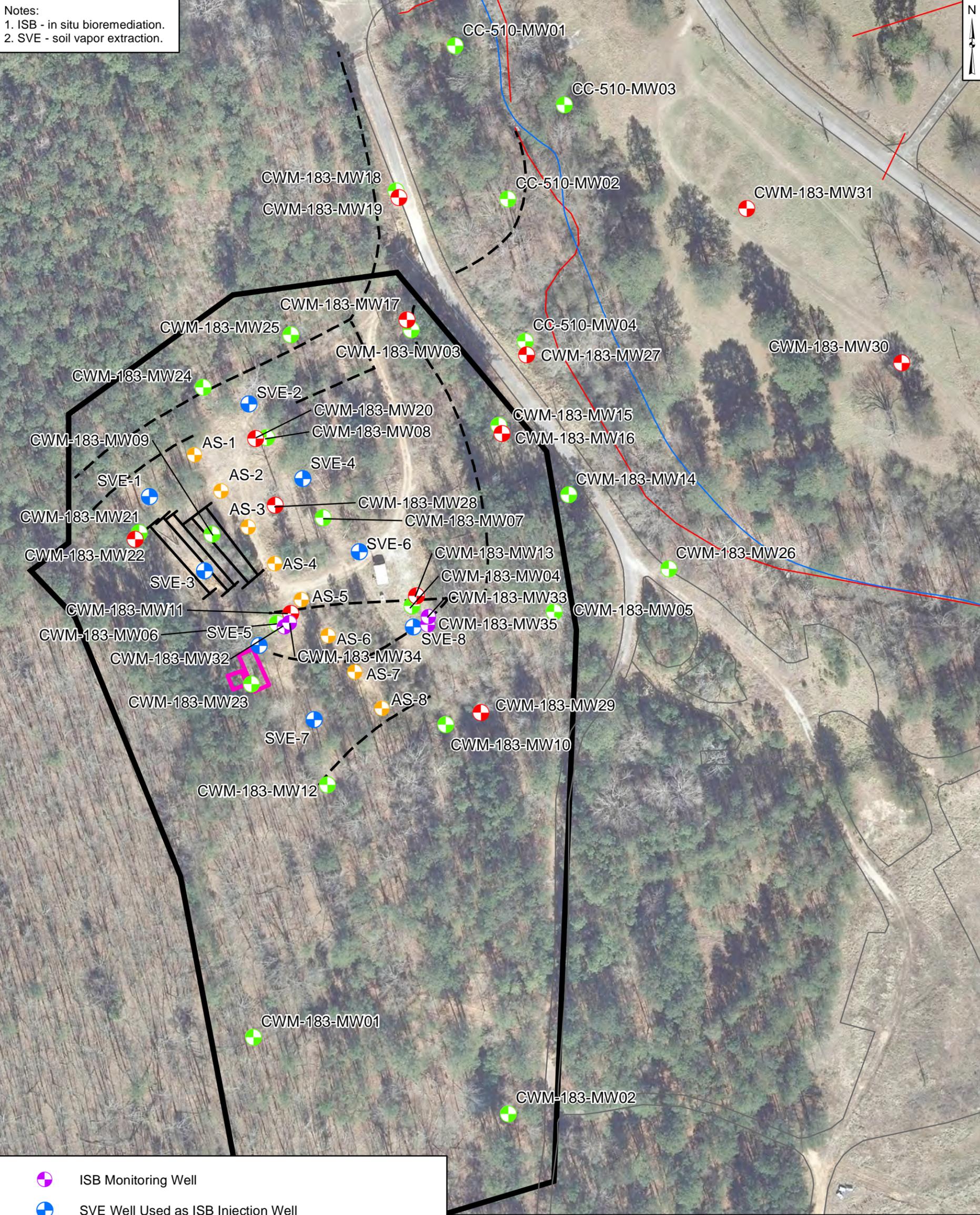
**TABLE 4: ROI MONITORING DURING ISB INJECTION**

**Training Area T-6 (Naylor Field)  
McClellan, Anniston, Alabama**

Location	Date	Time	pH	DO (mg/L)	ORP (mV)	Conductivity (µS/cm)	Temperature (°C)	Turbidity (NTU)	Comments
CWM-183-MW04	12/12/2014	845	6.13	2.31	15.6	349	12.00	18.12	clear, colorless
CWM-183-MW04	1/11/2015	1237	5.65	2.1	119.4	502	14.25	60.55	cloudy
CWM-183-MW04	1/12/2015	1141	6.34	1.61	108.9	491	15.18	55.66	cloudy
CWM-183-MW04	1/13/2015	0844	6.61	1.32	154.5	499	14.48	48.65	cloudy
CWM-183-MW04	1/14/2015	0743	6.73	2.13	112.4	162	14.18	42.08	cloudy
CWM-183-MW06	12/15/2014	915	6.45	4.57	38.7	462	16.22	84.56	slightly cloudy, colorless
CWM-183-MW06	1/11/2015	1407	5.98	4.23	122.1	322	14.42	4.37	clear
CWM-183-MW06	1/12/2015	1248	6.3	4.17	122.6	463	15.49	550	milky, EVO white
CWM-183-MW06	1/13/2015	1008	6.84	3.00	146.8	1945	13.49	183.13	milky, EVO white
CWM-183-MW06	1/14/2015	0913	7.00	0.91	84.17	2463	13.53	58.42	milky, EVO white
CWM-183-MW07	12/11/2014	1336	--	--	--	--	--	--	well too dry to sample
CWM-183-MW07	1/14/2015	0958	6.84	5.17	115.9	301	14.44	16.68	clear
CWM-183-MW08	12/11/2014	1250	5.62	2.59	171.9	404	17.68	5.86	clear, colorless
CWM-183-MW08	1/14/2015	1053	7.06	1.65	68.7	657	14.62	998.7	milky, EVO white
CWM-183-MW09	12/11/2014	1325	--	--	--	--	--	--	dry
CWM-183-MW09	1/14/2015	0959	6.98	2.26	28.9	336	13.86	689.5	cloudy
CWM-183-MW11	12/15/2014	910	7.37	4.98	20.5	241	16.93	4.09	clear, colorless
CWM-183-MW11	1/11/2015	1327	6.51	4.39	60.3	277	14.03	3.47	cloudy
CWM-183-MW11	1/12/2015	1225	7.3	4.3	61.9	268	14.86	1.9	clear
CWM-183-MW11	1/13/2015	0943	7.61	4.24	-24.1	268	13.16	1.01	clear
CWM-183-MW11	1/14/2015	0834	7.57	3.14	87.1	247	13.32	0.16	clear
CWM-183-MW13	12/11/2014	1436	6.08	4.94	167.7	324	14.69	5.86	clear, colorless
CWM-183-MW13	1/11/2015	1225	5.85	3.99	186.9	259	14.53	6.81	clear
CWM-183-MW13	1/12/2015	1130	6.54	3.43	188.9	260	15.24	1.74	clear
CWM-183-MW13	1/13/2015	0832	6.76	3.32	188.5	264	14.32	2.18	clear
CWM-183-MW13	1/14/2015	0731	6.57	3.14	139.6	235	11.7	3.20	clear
CWM-183-MW20	12/11/2014	1050	6.94	1.97	64.8	357	16.66	52.59	slightly cloudy, colorless
CWM-183-MW20	1/14/2015	1040	7.74	2.37	69.8	306	14.53	70.82	cloudy
CWM-183-MW21	12/9/2014	1050	7.16	1.58	-4.3	272	15.79	40.2	slightly cloudy, colorless
CWM-183-MW21	1/14/2015	1027	7.12	2.17	55.8	323	13.58	758.1	milky, EVO white
CWM-183-MW22	12/9/2014	1152	7.91	1.2	25.2	154	15.47	0.33	slightly cloudy, colorless
CWM-183-MW22	1/14/2015	1012	8.75	2.97	47.3	138	14.21	0.72	clear
CWM-183-MW23	12/16/2014	1150	6.4	4.01	23.7	133	15.35	70.47	slightly cloudy, colorless
CWM-183-MW23	1/14/2015	0925	7.35	1.97	84.8	250	13.15	32.02	cloudy
CWM-183-MW28	12/10/2014	915	8.61	3.9	-73.9	299	13.76	6.97	clear, colorless, sulfur-like odor
CWM-183-MW28	1/14/2015	0948	8.39	1.73	-24.2	328	13.97	13.77	clear
CWM-183-MW32	12/15/2014	913	8.01	0.35	-39.3	253	16.68	40.52	--
CWM-183-MW32	1/11/2015	1355	7.09	2.66	32.1	293	14.28	8.23	clear
CWM-183-MW32	1/12/2015	1304	7.19	2.79	-12.9	285	15.45	12.4	clear
CWM-183-MW32	1/14/2015	0859	7.84	2.46	21.0	264	13.76	23.76	clear
CWM-183-MW33	12/12/2014	1015	8.42	0.33	23.5	397	16.18	48.68	slightly cloudy, colorless
CWM-183-MW33	1/11/2015	1349	8.89	2.01	128.5	393	14.75	79.61	cloudy
CWM-183-MW33	1/12/2015	1152	8.87	1.75	118.3	364	15.27	57.46	cloudy
CWM-183-MW33	1/13/2015	0856	8.40	1.14	94.9	392	14.68	22.13	clear
CWM-183-MW33	1/13/2015	0952	7.47	2.02	-52.12	284	13.91	24.53	clear
CWM-183-MW33	1/14/2015	0756	7.99	2.36	-17.4	373	13.42	24.77	clear
CWM-183-MW34	12/15/2014	911	7.83	2.51	46.3	305	17.51	37.96	clear, colorless
CWM-183-MW34	1/11/2015	1341	7.41	2.74	91.5	378	14.31	68.83	clear
CWM-183-MW34	1/12/2015	1239	7.97	3.46	87.4	342	15.61	13.26	clear
CWM-183-MW34	1/13/2015	0929	8.12	2.70	-29.3	355	14.06	26.50	clear
CWM-183-MW34	1/14/2015	0845	8.21	3.03	98.8	334	13.43	34.47	cloudy
CWM-183-MW35	12/12/2014	1150	7.01	0.86	15.7	511	17.47	29.97	clear, colorless
CWM-183-MW35	1/11/2015	1300	6.30	3.08	-18.9	585	14.81	86.47	cloudy
CWM-183-MW35	1/12/2015	1159	7.03	1.36	-31.6	558	15.1	11.74	clear
CWM-183-MW35	1/13/2015	0908	7.30	1.42	-72.3	553	13.09	13.80	clear
CWM-183-MW35	1/14/2015	0808	7.40	1.58	-67.5	526	14.26	17.95	clear
AS-5	12/15/2014	903	7.33	1.11	20.1	297	14.5	30.06	clear, colorless
AS-5	1/11/2015	1316	6.91	1.92	43.7	2250	13.23	322.8	white, milky
AS-5	1/12/2015	1218	7.28	1.91	8.9	528	15	789.3	milky, EVO white
AS-5	1/13/2015	0918	7.56	1.68	-90.5	344	13.62	135.5	cloudy
AS-5	1/14/2015	0821	7.58	2.11	118.3	341	13.28	139.1	cloudy

Notes:  
 ROI - radius of influence  
 ISB - in situ bioremediation  
 DO - dissolved oxygen  
 mg/L - milligrams per liter  
 ORP - oxidation-reduction potential  
 mV - millivolts  
 °C - degrees Celsius  
 µS/cm - micro-Siemen per centimeter  
 NTU - nephelometric turbidity unit  
 EVO - emulsified vegetable oil

Notes:  
 1. ISB - in situ bioremediation.  
 2. SVE - soil vapor extraction.



**Injection and Monitoring Well Locations  
 Training Area T-6 (Naylor Field)  
 Parcel 183(6)**

McClellan, Anniston, AL



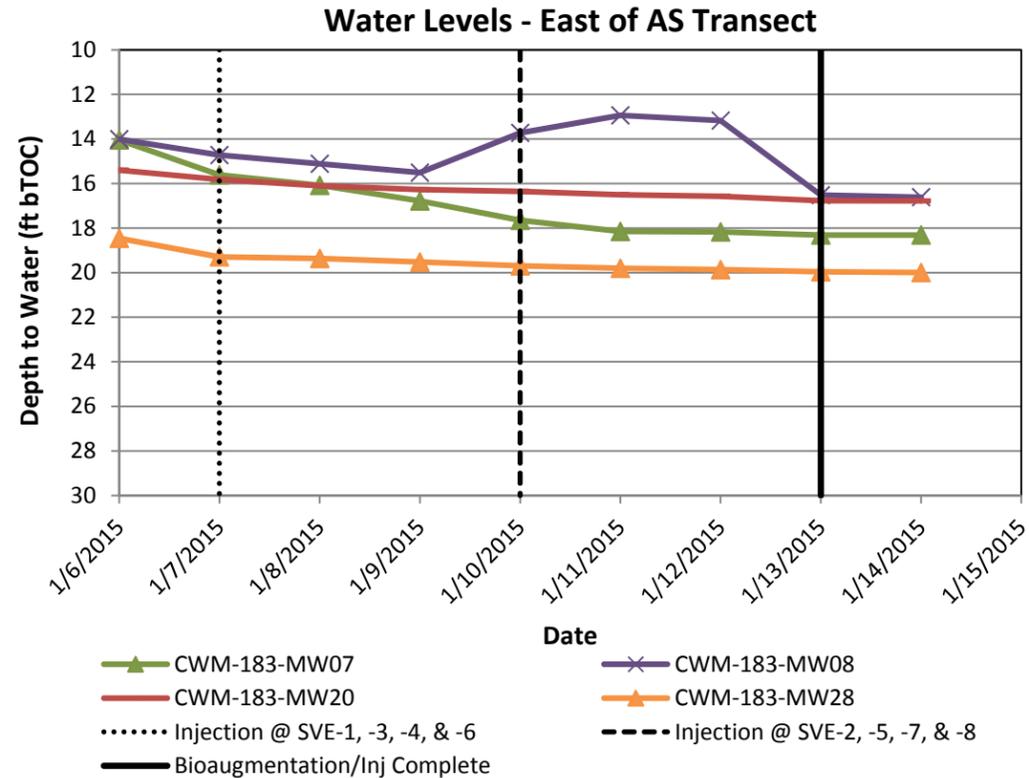
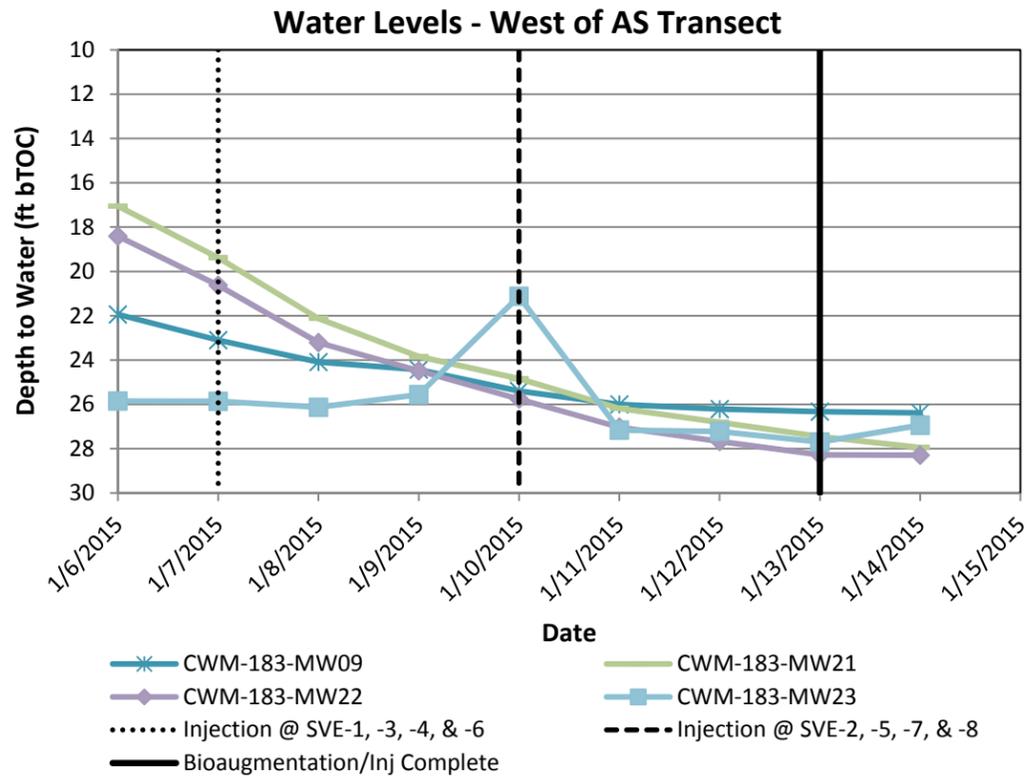
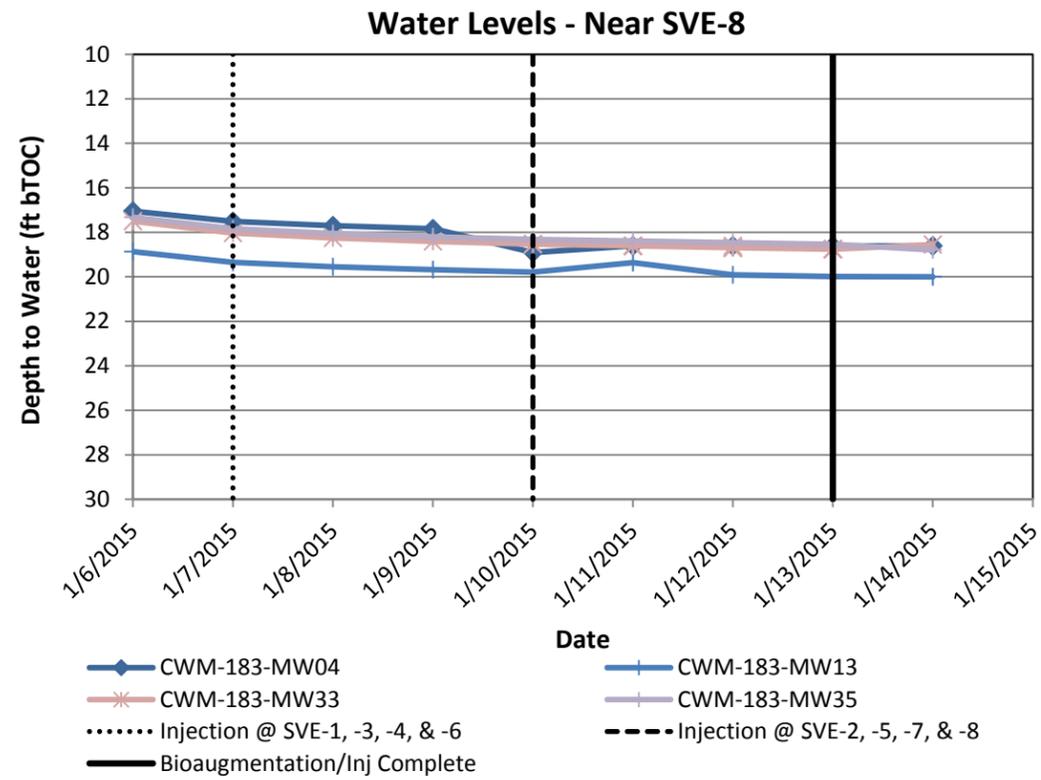
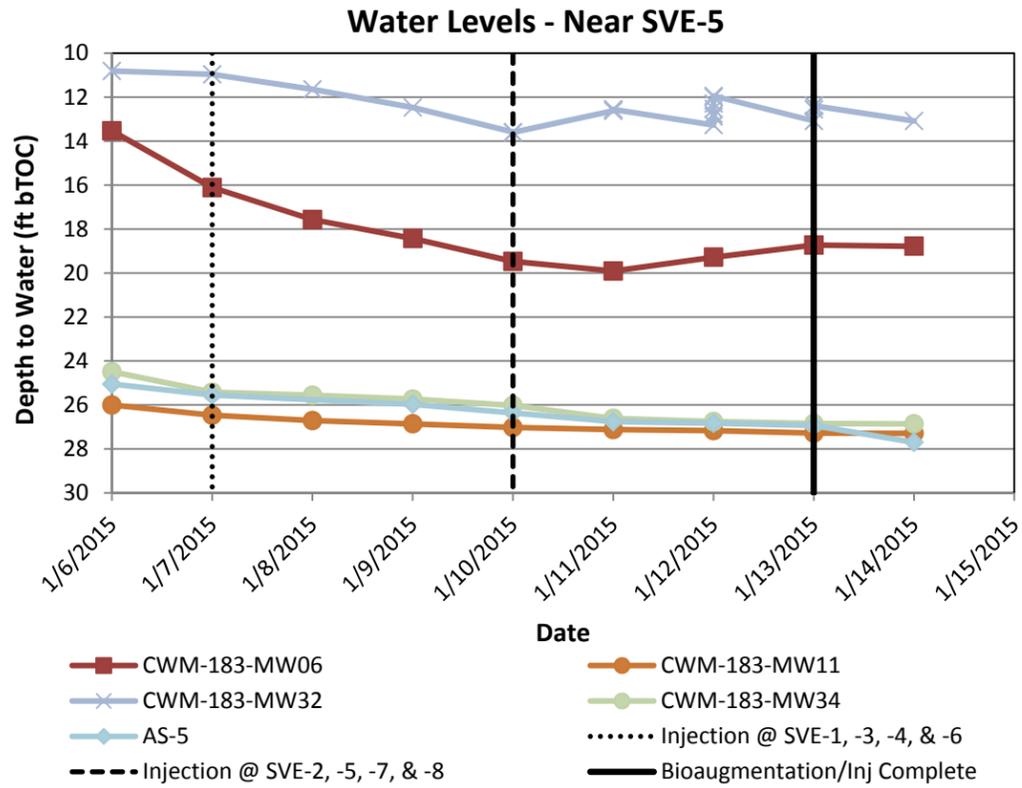
Figure

1

Kennesaw, GA

February 2015

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- Notes:**
1. ISB - in situ bioremediation
  2. ft bTOC - feet below top of casing
  3. Inj - injection
  4. SVE - soil vapor extraction
  5. AS - air sparge

**Water Level Data During ISB Injection**  
Training Area T-6  
McClellan, Anniston, AL



# **Appendix A**

## Laboratory Biotreatability Study Report

**Prepared for:**

**Geosyntec Consultants**  
2240 Sutherland Avenue, Suite 107  
Knoxville, Tennessee 37919

**Final Report**

**Laboratory Biotreatability Study to  
Evaluate Remediation of Chlorinated  
VOCs in Groundwater**

Training Area T-6, McClellan, Anniston, Alabama

**Prepared by:**



130 Research Lane, Suite 2  
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SiREM Ref: GR5429.02

19 August 2014

[siremlab.com](http://siremlab.com)

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### LIST OF APPENDICES

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## LIST OF ABBREVIATIONS

CA	chloroethane
cDCE	<i>cis</i> -1,2-dichloroethene
cells/L	cells per liter
CO <sub>2</sub>	carbon dioxide
cVOC	chlorinated volatile organic compound
1,1-DCA	1,1-dichloroethane
1,2-DCA	1,2-dichloroethane
1,2-DCP	1,2-dichloropropane
1,1-DCE	1,1-dichloroethene
<i>Dhb</i>	<i>Dehalobacter</i>
<i>Dhc</i>	<i>Dehalococcoides</i>
<i>Dhg</i>	<i>Dehalogenimonas</i>
°C	degrees Celsius
°C/min	degrees Celsius per minute
DHG	dissolved hydrocarbon gases
ERD	enhanced reductive dechlorination
Fe <sup>2+</sup>	ferrous iron
Fe <sup>3+</sup>	ferric iron
GC	gas chromatograph
gene copies/L	gene copies per liter
IC	ion chromatograph
µg/L	micrograms per liter
min	minutes
mg/L	milligrams per liter
mL	milliliters
mL/min	milliliters per minute
mM	millimolar
mmol/bottle	millimoles per bottle
Mn	manganese
NaHCO <sub>3</sub>	sodium bicarbonate
PCE	tetrachloroethene
%	percent
QL	quantitation limit
qPCR	quantitative polymerase chain reaction
RPM	revolutions per minute
rRNA	16 S ribosomal ribonucleic acid
SiREM	SiREM Laboratory
1,1,1-TCA	1,1,1-trichloroethane
1,1,2-TCA	1,1,2-trichloroethane
1,2,3-TCP	1,2,3-trichloropropane
TCE	trichloroethene
tDCE	<i>trans</i> -1,2-dichloroethene
TECA	1,1,2,2-tetrachloroethane

VC	vinyl chloride
VFA	volatile fatty acid
VOC	volatile organic compound

## 1. INTRODUCTION

Geosyntec Consultants (Geosyntec) retained SiREM Laboratory (SiREM) to perform a laboratory biotreatability study to assess the potential for in situ bioremediation of chlorinated volatile organic compounds (cVOCs) in groundwater at the Training Area T-6 site of the former Fort McClellan (McClellan) in Anniston, Alabama (the Site). The purpose of the study was to assess anaerobic biodegradation of the Site contaminants namely 1,1,2,2-tetrachloroethane (TECA) and chlorinated ethenes (tetrachloroethene [PCE] and trichloroethene [TCE]). Degradation products (cis-1,2-dichloroethene [cDCE], trans-1,2-dichloroethene [tDCE] and vinyl chloride [VC]) as well as chlorinated ethanes (1,1,2-trichloroethane [1,1,2-TCA], 1,2-dichloroethane [1,2-DCA] and chloroethane [CA]) were also monitored in this study.

The groundwater samples labelled CWM-183-MW23 used in this study was collected by Matrix Environmental Services, LLC personnel on 17 December 2013 and received by SiREM on 19 December 2013. Refer to Appendix A for the chain of custody documentation received with the groundwater.

The remainder of this report contains a summary of key biodegradation processes (Section 1.1), the experimental materials and methods (Section 2), the results and discussion of the microcosm study (Section 3), conclusions (Section 4) and report references (Section 5).

### 1.1 Summary of Biodegradation Processes

Biological degradation products of PCE and TCE include cDCE, VC and the fully dechlorinated end product ethene. Biological reductive dechlorination breakdown products of TECA include 1,1,2-TCA, 1,2-DCA and CA although the more common pathway for TECA degradation is by dehaloelimination to tDCE and by the elimination reaction of TECA to TCE. Both TCE and tDCE follow the reductive dechlorination pathway to ethene. Figure 1 contains degradation pathways for the chlorinated ethenes and Figure 2 contains degradation pathways for TECA.

Natural attenuation processes can occur in situ and are often mediated by indigenous microbial populations present at contaminated sites. Enhanced reductive dechlorination (ERD), can in certain cases, be achieved by stimulating the indigenous microbial populations through the addition of electron donors. Bioaugmentation is the process in which a microbial population known to promote ERD or other biodegradation processes is introduced to groundwater to enhance the rate or extent of biodegradation. KB-1<sup>®</sup> Plus is a custom formulated natural microbial consortium containing microorganisms (*Dehalococcoides* [Dhc], *Dehalobacter* [Dhb] and *Dehalogenimonas* [Dhg]). Dhc are known to be responsible for mediating the complete dechlorination of PCE, TCE, cDCE, 1,1-dichloroethene (1,1-DCE), tDCE and VC to ethene (Major et al., 2002; Duhamel et al., 2002). Dhb are known to dechlorinate chlorinated ethanes including 1,1,1-trichloroethane (1,1,1-TCA) and 1,1-dichloroethane (1,1-DCA) to CA (Grostern and Edwards 2006) and TECA, 1,1,2-TCA and 1,2-DCA to ethene (Lorah et al., 2007). Dhg are also known to dechlorinate 1,2,3-trichloropropane (1,2,3-TCP), 1,2-dichloropropane (1,2-DCP), TECA, 1,1,2-TCA, 1,2-DCA (Moe et al. 2009) and tDCE (Manchester et al. 2012). KB-1<sup>®</sup> Plus is used to introduce Dhc, Dhb and Dhg and to complete dechlorination activity at sites

exhibiting slow or incomplete dechlorination of chlorinated ethene and chlorinated ethane compounds.

## 2. MATERIALS AND METHODS

The following sections describe the materials and methods used for microcosm construction and incubation (Section 2.1), and microcosm sampling and analysis (Section 2.2).

### 2.1 Microcosm Construction and Incubation

Biotreatability microcosms were constructed in an anaerobic glove bag containing the Site groundwater and all of the materials required to construct the treatment and control microcosms. The anaerobic glove chamber (Coy Laboratory Products, Grass Lake, MI) was filled with an atmosphere of approximately 80 percent (%) nitrogen, 10% carbon dioxide (CO<sub>2</sub>) and 10% hydrogen (Linde Gases, Guelph, ON). Hydrogen in the anaerobic chamber functions to scavenge trace oxygen via a palladium catalyst to protect any microorganisms present in the site materials from oxygen exposure. During microcosm construction, the Site water was mixed thoroughly to ensure reproducibility between replicates.

Microcosms were constructed by filling sterile 250 milliliter (mL) (nominal volume) screw cap Boston round clear glass bottles (Systems Plus, New Hamburg, ON) with 200 mL of Site groundwater. The bottles were capped with Mininert™ closures to allow repetitive sampling with minimal chlorinated volatile organic compound (cVOC) loss and to allow nutrient amendment, as needed, throughout the incubation period. All control and treatment microcosms were constructed in triplicate. Table 1 summarizes the details of microcosm construction and the amendments used for the treatment and control microcosms.

Anaerobic sterile control microcosms were constructed to quantify potential abiotic and experimental cVOC losses from the microcosms. The sterile controls were constructed by amending respective microcosms with mercuric chloride and sodium azide as described in Table 1.

All microcosms were sampled and incubated in the anaerobic chamber. Anaerobic conditions in the anaerobic chamber were verified using an indicator containing resazurin (Sigma, St. Louis, MO) in a mineral medium, which turns pink in the presence of oxygen. During quiescent incubation, all microcosms were covered to minimize photodegradation, and stored horizontally to minimize cVOC losses via the (submerged) Mininert™ closure. Microcosms were incubated for a period of up to 199 days at approximately 22 degrees Celsius (°C) (room temperature).

Geosyntec specified that the initial PCE, TCE and TECA concentrations in the microcosms should all be 1.5 milligrams per liter (mg/L) to represent concentrations measured at the Site. The initial concentrations measured in the prepared microcosms were not at these target concentrations; therefore on 23 December 2013 (Day 0), the microcosms were amended with PCE, TCE and TECA to reach the target concentration in the microcosms.

Treatment microcosms were amended with electron donor on 23 December 2013 (Day 0). SRS<sup>®</sup>-SD (Terra Systems, Claymont, DE) was the selected electron donor evaluated in this study. The first microcosm of each treatment and control was amended with resazurin (Sigma, St. Louis, MO) to monitor redox conditions. Resazurin turns from pink to clear in the absence of oxygen and can be used to indicate the on-set of reducing conditions. Details of PCE, TCE and TECA spiking, electron donor addition and resazurin amendment are provided in Table 1 and Table 2.

Bioaugmentation may improve the extent and rate of PCE, TCE and TECA dechlorination. Microcosms are typically bioaugmented after reducing conditions required by the KB-1<sup>®</sup> Plus culture are achieved. Suitable reducing conditions are typically achieved after electron donor addition and are assessed qualitatively by both changes in the resazurin indicator color (from pink to clear) and the on-set of sulfate reduction. The onset of sulfate reduction was observed on 6 January 2014 (Day 14) in the SRS<sup>®</sup>-SD amended microcosms and the resazurin color had changed from pink to clear. The respective microcosms were bioaugmented with KB-1<sup>®</sup> Plus on 20 January 2014 (Day 28).

The optimum pH for reductive dechlorination is between 6.8 and 7.5 (Middledorp et al., 1999) and complete dechlorination can occur between a pH range of 6.0 and 8.0 (SiREM, unpublished data). On 17 February 2014 (Day 56) the pH in the treatment microcosms had decreased to below a pH of 6.0. To maintain a pH in the optimal range for reductive dechlorination saturated sodium bicarbonate (NaHCO<sub>3</sub>) was amended to treatment microcosms on 19 February 2014 (Day 58). The treatment microcosms were continually monitored for pH and NaHCO<sub>3</sub> was amended to the treatment microcosms on Days 120 and 165 to maintain a pH above 6.0.

## 2.2 Microcosm Sampling and Analysis

### 2.2.1 Microcosm Sampling

Aqueous samples were collected from the control and treatment microcosms approximately biweekly (i.e. every two weeks) for analysis of cVOCs, dissolved hydrocarbon gases (DHGs – ethene, ethane, and methane) and pH. Aqueous samples were also collected less frequently for analysis of volatile fatty acids (VFAs – lactate, acetate, propionate, formate, butyrate and pyruvate) and anions (sulfate, nitrate, nitrite, chloride, phosphate, bromide). The microcosms were sampled using gas-tight 1 mL Hamilton glass syringes. Separate sets of syringes were used for the bioaugmented and non-bioaugmented treatments to minimize the potential for transfer of KB-1<sup>®</sup> Plus microorganisms from bioaugmented to non-bioaugmented treatments. Syringes were cleaned with acidified water (pH ~2) and rinsed 10 times with deionized water between samples to ensure that volatile organic compounds (VOCs) and microorganisms were not transferred between different samples or treatments. The analytical methods employed by SiREM are described below.

### 2.2.2 Analysis of cVOCs and Dissolved Hydrocarbon Gases

This section describes the methods used to quantify the cVOCs and DHGs. The quantitation limits (QL) for the chlorinated ethenes and DHGs were typically 10 micrograms per liter (µg/L) in

the microcosms based on the lowest concentration standards that were included in the linear calibration trend.

Aqueous cVOC and DHG concentrations in the microcosms were measured using a Hewlett-Packard (Hewlett Packard 7890) gas chromatograph (GC) equipped with an auto sampler (Hewlett Packard G1888) programmed to heat each sample vial to 75°C for 45 min. prior to headspace injection into a GSQ Plot column (0.53 millimeters x 30 meters, J&W) and a flame ionization detector. Sample vials were heated to ensure that all VOCs in the aqueous sample would partition into the headspace. The injector temperature was 200°C, and the detector temperature was 250°C. The oven temperature was programmed as follows: 35°C for 2 min, increased to 100°C at 50 degrees Celsius per minute (°C/min), then increased to 185°C at 25°C/min and held at 185°C for 6.80 min. The carrier gas was helium at a flow rate of 11 milliliters per minute (mL/min).

Aqueous TECA concentrations in the microcosms were also measured using a Hewlett-Packard GC equipped with an auto sampler. The analysis of TECA differed from cVOC and DHG analysis as the headspace was injected into a DB-624 column (0.53 millimeters x 30 meters, J&W) and a flame ionization detector. The oven temperature was programmed at 40 °C for 5 min and increased to 200 °C by 10°C/min and held at 200°C for 5 minutes (min).

After withdrawing a 1.0 mL sample (as described in section 2.2.1), the sample was injected into a 10 mL auto sampler vial containing 5.0 mL of acidified deionized water (pH ~2). The water was acidified to inhibit microbial activity between microcosm sampling and GC analysis. The vial was sealed with an inert Teflon<sup>®</sup>-coated septum and aluminium crimp cap for automated injection of 3 mL of headspace onto the GC. One cVOC standard was analysed with each set of samples to verify the instrument five-point calibration curve. Calibration was performed using external standard solutions (Sigma, St Louis, MO), where known volumes of standard solutions were added to acidified water in auto sampler vials and analysed as described above for microcosm samples. Data were integrated using Chemstation Software (Agilent Technologies, Santa Clara, CA).

### 2.2.3 Analysis of Anions and Total Volatile Fatty Acids

Anions and total VFA analysis was performed on a Dionex DX-600 ion chromatograph (IC) equipped with a Dionex AS-40 auto sampler and an AS18 column, the sample loop volume was 25 µL. An isocratic separation was performed using 33 millimolar (mM) reagent grade sodium hydroxide (Fisher Scientific, Ottawa, ON) eluent for 13 min. One standard was analysed with each set of samples tested in order to verify the seven-point calibration using external standards of known concentrations. External standards were prepared gravimetrically using chemicals of the highest purity available (Sigma St Louis, MO or Bioshop, Burlington, ON). Data were integrated using Peaknet Chromatography software (Dionex, Oakville, ON). The QLs were as follows: 0.07 mg/L total VFA, 0.07 mg/L chloride, 0.09 mg/L nitrite, 0.09 mg/L nitrate, 0.07 mg/L sulfate, 0.07 mg/L phosphate and 0.08 mg/L bromide. The total VFA value includes lactate, formate, acetate, propionate, pyruvate and butyrate (valerate has not been confirmed). The VFA method described below (Section 2.2.4) is used to quantify individual VFAs.

A 0.5 mL sample was withdrawn (as described in section 2.2.1), after which the sample was placed in a 1.5 mL micro-centrifuge tube. Samples were centrifuged for five minutes at 13,000 revolutions per minute (RPM) to remove solids. The supernatant was removed, diluted 50-fold in deionized water and placed in a Dionex auto sampler vial with a cap that filters the sample during automated injection onto the IC.

#### 2.2.4 Analysis of Volatile Fatty Acids

Individual VFAs (lactate, acetate, propionate, formate, butyrate and pyruvate) analysis was performed on a Dionex DX-600 IC equipped with a Dionex AS-40 auto sampler and an AS11-HC column, the sample loop volume was 25  $\mu$ L. A gradient separation was performed using the following eluent profile; 1.0 mM sodium hydroxide for 8.0 min to 15 mM at 18.0 min and proceeding to 30 mM at 28.0 min. with a flow rate of 1.5 mL/min. Calibration was performed using external standards of known concentrations. One standard was analysed with each set of samples to verify the instrument's seven-point calibration curve produced using external standards of known concentrations. External standards were prepared gravimetrically using chemicals of the highest purity available (Sigma St Louis, MO or Bioshop, Burlington, ON). Data were integrated using Peaknet chromatography software (Dionex, Oakville, ON). The QLs were as follows: 0.40 mg/L lactate, 0.54 mg/L acetate, 0.31 mg/L propionate, 0.23 mg/L formate, 0.41 mg/L butyrate and 0.69 mg/L pyruvate.

A 0.5 mL sample was withdrawn (as described in section 2.2.1), after which the sample was placed in a 1.5 mL micro-centrifuge tube. Samples were centrifuged for five minutes at 13,000 RPM in a microcentrifuge to remove solids. The supernatant was removed, diluted 50-fold in deionized water and placed in a Dionex auto sampler vial with a cap that filters the sample during automated injection onto the IC.

#### 2.2.5 Analysis of pH

The pH measurements were performed using an Oakton pH spear with a combination pH electrode (Oakton, Vernon Hills, IL). A 0.5 mL sample was taken (as described in section 2.2.1), the vial was removed from the glove box and the pH was measured on the lab bench. The pH spear was calibrated at each sampling event according to the manufacturer's instructions using pH 4.0, 7.0 and 10 standards.

#### 2.2.6 Gene-Trac<sup>®</sup> Dehalococcoides, Dehalobacter and Dehalogenimonas Testing

Gene-Trac<sup>®</sup> quantitative polymerase chain reaction (qPCR) testing was performed in this study to quantify and characterize *Dhc*, *Dhb* and *Dhg* microorganisms. *Dhc* facilitate the dechlorination of PCE to ethene, whereas *Dhb* facilitate the dechlorination of 1,1-DCA to CA and TECA and 1,1,2-TCA to ethene. *Dhg* also facilitates the dechlorination of 1,2,3-TCP, 1,2-DCP, TECA, 1,1,2-TCA and 1,2-DCA. The Gene-Trac<sup>®</sup> *Dhc*, *Dhb* and *Dhg* tests quantify the total *Dhc*, *Dhb* and *Dhg* populations by targeting the 16S ribosomal ribonucleic acid (rRNA) gene. The method for the analysis is provided in Appendix B.

As per Geosyntec's request, on 10 July 2014 (Day 199) a 10 mL sample from the first and third replicates of the bioaugmented treatment microcosms were collected for end-point sampling. Samples were submitted for Gene-Trac<sup>®</sup> *Dhc*, *Dhb* and *Dhg* testing. Refer to Appendix B for the Gene-Trac test certificates.

### 3. RESULTS AND DISCUSSION

The following sections present and discuss the results of the biotreatability study:

- Redox processes (Section 3.1),
- Chlorinated ethenes and chlorinated ethanes biodegradation results (Section 3.2),
- Volatile Fatty Acids and pH (Section 3.3),
- Gene-Trac<sup>®</sup> *Dehalococcoides*, *Dehalobacter* and *Dehalogenimonas* Testing (Section 3.4).

Tables 2, 3, 4 and 5 provide cVOC, ethene, ethane, methane, anion, VFA and pH data from the control and treatment microcosms over the incubation period for the study. All cVOC, ethene, ethane, and methane concentrations are presented in units of mg/L and millimoles per microcosm bottle (mmol/bottle) to demonstrate mass balances on a molar basis. Concentrations were converted from mg/L to mmol/bottle using Henry's Law as demonstrated in Appendix C. Table 6 summarizes the Gene-Trac<sup>®</sup> results and Figures 3 through 6 present trends in the concentrations of chlorinated ethenes and chlorinated ethanes in the control and treatment microcosms over the incubation period for the study.

#### 3.1 Redox Processes

The addition of electron donor typically results in microbial activity that promotes changes in the redox conditions in groundwater. Aerobic or mildly reducing redox conditions will be reduced, resulting in more strongly reducing conditions required to support anaerobic degradation of cVOCs.

The sequence of redox reactions in groundwater is well known (Appelo and Postma, 1994). Oxygen is first consumed, followed by nitrate (denitrification), iron, manganese (Mn) and sulfate reduction. Ferric iron ( $\text{Fe}^{3+}$ ) is reduced to ferrous iron ( $\text{Fe}^{2+}$ ), manganese ( $\text{Mn}^{4+}$ ) is reduced to manganese ( $\text{Mn}^{2+}$ ) and sulfate is reduced producing sulfides. The final step is  $\text{CO}_2$  reduction producing methane (methanogenesis). The consumption of each species in sequence indicates that conditions are becoming increasingly reducing. Dechlorination of chlorinated solvents typically occurs in the range of sulfate reducing to methanogenic conditions.

In the sterile and active control microcosms, nitrate and sulfate concentrations remained relatively stable (Table 3). Methane concentrations did not increase in the sterile controls (Table 2) and increased only slightly in the active controls. This suggests that reducing

conditions were not achieved in the sterile or active control microcosms. These observations are consistent with low levels of microbial activity expected in control microcosms.

In the SRS<sup>®</sup>-SD amended and the SRS<sup>®</sup>-SD amended/KB-1<sup>®</sup> Plus bioaugmented treatment microcosms the on-set of sulfate reduction was observed by Day 14. A change in resazurin color from pink to clear was also observed in both treatment microcosms indicating reducing conditions were achieved.

Methane concentrations were observed to increase after additional SRS<sup>®</sup>-SD was amended to the electron donor only microcosms on Day 107. In the SRS<sup>®</sup>-SD/KB-1<sup>®</sup> bioaugmented treatment microcosms, methane concentrations were observed to increase after bioaugmentation by Day 35 (Table 2). These results suggest that methanogenic organisms known to be present in the KB-1<sup>®</sup> Plus culture as well as some indigenous organisms were active and consumed a portion of the available electron donor.

## 3.2 Chlorinated Ethenes and Chlorinated Ethanes Biodegradation Results

### 3.2.1 Sterile and Active Controls

PCE concentrations in the sterile and active controls remained relatively stable over the incubation period with no increases in degradation products. TECA concentrations decreased slightly in both the sterile and active control microcosms with a corresponding increase in TCE (Table 2 and Figures 3 and 4). These results indicate that there was no mass loss of PCE in the control microcosms resulting from abiotic degradation or experimental losses (eg., sorption or loss through microcosm closures) during the incubation period. TECA was likely degraded by abiotic elimination or dehydrochlorination (Figure 2) to TCE over the incubation period.

### 3.2.2 SRS<sup>®</sup>-SD amended Microcosms

In the SRS<sup>®</sup>-SD amended microcosms, some dechlorination of PCE and TCE was observed with a corresponding increase in cDCE (Figure 5). TECA concentrations also decreased in the SRS<sup>®</sup>-SD amended microcosms likely by elimination to TCE similarly to the control microcosms. TCE produced by TECA elimination was further dechlorinated to cDCE. Some increases in tDCE concentrations were also observed likely from the dihaloelimination of TECA. VC concentrations increased only slightly indicating some dechlorination of cDCE and tDCE likely occurred, but ethene was not detected. These data suggest that indigenous microbial activity may be capable of promoting partial degradation of TECA, PCE and TCE to VC.

### 3.2.3 SRS<sup>®</sup>-SD amended/KB-1<sup>®</sup> Plus Bioaugmented Microcosms

In the SRS<sup>®</sup>-SD amended/KB-1<sup>®</sup> Plus bioaugmented microcosms PCE, TCE and TECA, remained relatively stable prior to bioaugmentation. After bioaugmentation with KB-1<sup>®</sup> Plus on Day 28, PCE and TCE dechlorinated rapidly to cDCE with some increases in VC and the complete dechlorination end product ethene were detected in all microcosm replicates by Day 35 (Figure 6). TECA remained relatively stable to Day 35, after which degradation varied

between the three treatment replicates. Data for Replicates 1, 2 and 3 are provided in Figures 6a, 6b and 6c respectively.

In summary, Replicates 1 and 2 had complete transformation of the Site contaminants (i.e., PCE, TCE, and TECA). In Replicate 3, concentrations of TECA have continued to slowly decrease with a corresponding increase in tDCE. Transformation of intermediate daughter products in Replicate 3 has also proceeded at a slower rate throughout the study. To better understand the observed lag, bacterial characterization of Replicate 1 and 3 was included in the endpoint sampling (reported in Section 3.4 below). The bacterial characterization indicated that the microbial counts of key dechlorinating bacteria in Replicate 3 were two to three orders of magnitude lower than the counts in Replicate 1, which correlates to the slower dechlorination rates. Since Replicate 3 is performing similarly to the other replicates, just at a slower rate, this is unlikely to have significant long-term bearing on performance of the remedy, even if the aquifer behaves more like Replicate 3 than Replicates 1 and 2.

### 3.3 Volatile Fatty Acids and pH

In all the SRS<sup>®</sup>-SD amended treatment microcosms, lactate was detected at an average concentration of 47 mg/L, with low concentrations of acetate and formate observed at time zero (Table 4). Lactate decreased to non-detect by Day 56, indicating that the lactate portion of the SRS<sup>®</sup>-SD was consumed. By Day 56 increases in acetate, propionate and butyrate were also observed. On day 107 SRS<sup>®</sup>-SD was re-amended to the treatment microcosms and by Day 199 lactate had once again decreased to levels of non-detect. By Day 199 in the SRS<sup>®</sup>-SD amended microcosms acetate, propionate and butyrate concentrations increased to averages of 286 mg/L, 158 mg/L and 17 mg/L respectively. In the SRS<sup>®</sup>-SD amended/KB-1<sup>®</sup> Plus bioaugmented microcosms acetate, propionate and butyrate concentrations increased to an average of 367 mg/L, 67 mg/L and 30 mg/L respectively. The increase in acetate, propionate and butyrate indicate that fermentation of soybean oil portion of the SRS<sup>®</sup>-SD electron donor was occurring. SRS<sup>®</sup>-SD contains 4% sodium lactate, providing a soluble and easily fermentable electron donor source to increase microbial activity when initially added. The fermentation of both lactate and soybean oil results in the production of hydrogen, which is the ultimate electron donor used by dechlorinators.

The pH remained relatively stable around 6.5 in both the sterile and active control microcosms over the incubation period (Table 5). In all of the SRS<sup>®</sup>-SD amended microcosms pH decreased below a pH of 6.0 by Day 56 and microcosms were buffered with saturated sodium bicarbonate (NaHCO<sub>3</sub>). The bioaugmented microcosms decreased to a pH of 6.0 by Day 120 and NaHCO<sub>3</sub> was amended to increase the pH up to pH 6.5. The pH in the electron donor only amended microcosms continued to decrease and by Day 163 had decreased to below 6.0 and were also buffered again with NaHCO<sub>3</sub>. These results indicate that the acid buffering properties of the Site material were not sufficient to maintain a relatively neutral pH during reductive dechlorination and electron donor fermentation (both acid producing processes). The optimum pH for reductive dechlorination is 6.8 to 7.5 (Middledorp et al., 1999) and complete dechlorination can occur between a pH range of 6.0 and 8.0 (SiREM, unpublished data). These results suggest that application of buffering agents may be required to support ERD at the Site.

### 3.4 Gene-Trac<sup>®</sup> Dehalococcoides, Dehalobacter and Dehalogenimonas Results

Table 6 summarizes the Gene-Trac<sup>®</sup> test results for aqueous samples collected from the treatment microcosms at the end-point of the study. Samples were collected from Replicates 1 and 3 from the bioaugmented treatment microcosms. In consultation with Geosyntec, these replicates were chosen for Gene-Trac<sup>®</sup> analysis as Replicate 1 showed the highest amount of dechlorination and Replicate 3 the least. Gene-Trac<sup>®</sup> analysis was performed to determine if the bacterial counts may indicate reasons for the slower dechlorination in Replicate 3. Typically *Dhc* concentrations above  $1 \times 10^7$  cells/L are required for high rates of in situ reductive dechlorination and ethene production (Lu et al., 2006). Although similar studies have not been performed for *Dhb* and *Dhg* the  $1 \times 10^7$  cells/L value is often used to indicate robust populations for these other dechlorinators.

In the first SRS<sup>®</sup>-SD amended/KB-1<sup>®</sup> Plus bioaugmented replicate *Dhc* was detected at  $3 \times 10^8$  cells per liter (cells/L) indicating a robust population of *Dhc* following bioaugmentation. *Dhb* concentrations at Day 199 were at  $8 \times 10^7$  gene copies per liter (gene copies/L) and *Dhg* populations were at a concentration of  $9 \times 10^6$  gene copies/L indicating high populations of these dechlorinators were also present. The microbial populations in the third bioaugmented replicate were lower than the first at concentrations of  $3 \times 10^6$  cells/L,  $3 \times 10^4$  gene copies/L and  $4 \times 10^4$  gene copies/L of *Dhc*, *Dhb* and *Dhg* respectively. These lower microbial populations correlate to the slower dechlorination rate of TECA, cDCE, tDCE and VC in the third replicate and suggest that the bioaugmented bacteria were not able to thrive in the third replicate as they did in the first.

## 4. CONCLUSIONS

The laboratory biotreatability study results suggest the following conclusions:

1. The extent of intrinsic degradation of cVOCs in groundwater appears to be limited by the lack of available nutrients (e.g. electron donors) and appropriate microorganisms to promote complete dechlorination. However, TECA degradation to TCE was observed by an abiotic dehydrochlorination pathway in the control microcosms.
2. SRS<sup>®</sup>-SD amendment promoted the appropriate geochemical conditions (i.e., sulfate reducing conditions) for bioremediation of TECA, PCE and TCE.
3. Complete dechlorination of PCE and TCE to ethene was achieved with the addition of SRS<sup>®</sup>-SD as the electron donor in combination with KB-1<sup>®</sup> Plus bioaugmentation.
4. Complete TECA degradation to tDCE was observed in two of three replicates with the addition of SRS<sup>®</sup>-SD as the electron donor in combination with KB-1<sup>®</sup> Plus bioaugmentation. tDCE degradation was the slowest of all the cVOCs evaluated.
5. pH adjustment was required to maintain the pH in the dechlorinating range (6.0 to 8.0).

The results of this study indicate that ERD using SRS<sup>®</sup>-SD amendment combined with KB-1<sup>®</sup> Plus bioaugmentation and pH adjustment has the potential to be an effective remedial approach for the chlorinated ethenes and TECA at the Site. tDCE had a slower degradation rate than the other cVOCs evaluated and may persist longer at the Site.

## 5. REFERENCES

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**TABLES**

**Table 1: SUMMARY OF MICROCOSM CONTROLS, TREATMENTS AND AMENDMENTS**  
 Training Area T-6, McClellan, Anniston, Alabama

Treatment/Control	Number of Microcosms	Geologic Material (mL)	Groundwater (mL)	Headspace (mL)	Sodium Azide	Mercuric Chloride	Resazurin	Chlorinated Compounds	SRS®-SD	KB-1®
Anaerobic Sterile Control	3	NA	200	50	Amended with 0.5 mL of a 5% solution on Day -1.	Amended with 2.8 mL of a 2.7% solution on Day -1.	Amend first replicate with 100 µL resazurin.	Spiked with PCE, TCE and 1,1,2,2-TeCA to a target concentration of 1.5 mg/L each on Day 0.	NA	NA
Anaerobic Active Control	3	NA	200	50	NA	NA	Amend first replicate with 100 µL resazurin.	Spiked with PCE, TCE and 1,1,2,2-TeCA to a target concentration of 1.5 mg/L each on Day 0.	NA	NA
SRS®-SD	3	NA	200	50	NA	NA	Amend first replicate with 100 µL resazurin.	Spiked with PCE, TCE and 1,1,2,2-TeCA to a target concentration of 1.5 mg/L each on Day 0.	Amended with 333µL of SRS®-SD to a target concentration of 0.1 % as oil on Day 0 and on Day 107.	NA
SRS®-SD and KB-1® Plus	3	NA	200	50	NA	NA	Amend first replicate with 100 µL resazurin.	Spiked with PCE, TCE and 1,1,2,2-TeCA to a target concentration of 1.5 mg/L each on Day 0.	Amended with 333µL of SRS®-SD to a target concentration of 0.1 % as oil on Day 0 and on Day 107.	Bioaugmented with KB-1® Plus on Day 28.

**Notes:**

- g - grams
- mg/L - milligrams per liter
- mL - milliliters
- NA - not applicable
- PCE - tetrachloroethene
- 1,1,2,2-TeCA - 1,1,2,2-tetrachloroethane
- TCE - trichloroethene
- % - percent
- µL - microliters

**TABLE 2: SUMMARY OF MICROCOSM cVOCs, ETHENE, ETHANE AND METHANE RESULTS**  
 Training Area T-6, McClellan, Anniston, Alabama

Treatment	Date	Day	Replicate	Chlorinated Ethenes						Chlorinated Ethanes					Methane	Comment	
				PCE	TCE	cDCE	tDCE	VC	Ethene	Total Ethenes	1,1,2,2-TECA	1,1,2-TCA	1,2-DCA	CA	Ethane		Methane
				mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mmol/bottle	mg/L	mg/L	mg/L	mg/L	mg/L		mg/L
Anaerobic Sterile Control	19-Dec-13	-4														Poisoned with mercuric chloride and sodium azide.	
	23-Dec-13	0														Amended the first replicate with 100 µL of resazurin.	
				ANSC-1	1.3	1.8	<0.010	<0.010	<0.010	<0.010	--	1.4	<0.010	<0.010	<0.010	<0.010	0.024
				ANSC-2	1.6	1.8	<0.010	<0.010	<0.010	<0.010	--	1.2	<0.010	0.088	<0.010	<0.010	0.024
				ANSC-3	1.5	1.8	<0.010	<0.010	<0.010	<0.010	--	1.4	<0.010	<0.010	<0.010	<0.010	0.024
				Average Concentration (mg/L)	1.5	1.8	ND	ND	ND	ND	--	1.3	ND	0.029	ND	ND	0.024
				Standard Deviation (mmoles)	1.9E-04	3.4E-05	0.0E+00	0.0E+00	0.0E+00	0.0E+00	--	1.4E-04	0.0E+00	1.0E-04	0.0E+00	0.0E+00	1.4E-05
				Average Total mmoles	<b>0.0022</b>	<b>0.0031</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>5.3E-03</b>	<b>0.002</b>	<b>ND</b>	<b>0.00006</b>	<b>ND</b>	<b>ND</b>	<b>0.0023</b>
	06-Jan-14	14		ANSC-1	1.3	1.9	<0.010	<0.010	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.027
				ANSC-2	1.5	1.8	<0.010	<0.010	<0.010	<0.010	--	--	<0.010	0.019	<0.010	<0.010	0.027
				ANSC-3	1.5	1.8	<0.010	<0.010	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.023
				Average Concentration (mg/L)	1.4	1.8	ND	ND	ND	ND	--	--	ND	0.0063	ND	ND	0.025
				Standard Deviation (mmoles)	1.5E-04	6.0E-05	0.0E+00	0.0E+00	0.0E+00	0.0E+00	--	--	0.0E+00	2.2E-05	0.0E+00	0.0E+00	2.3E-04
				Average Total mmoles	<b>0.0021</b>	<b>0.0031</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>5.2E-03</b>	<b>--</b>	<b>ND</b>	<b>0.000013</b>	<b>ND</b>	<b>ND</b>	<b>0.0025</b>
	10-Mar-14	77		ANSC-1	1.3	2.1	<0.010	<0.010	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.022
				ANSC-2	1.5	2.0	<0.010	<0.010	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.021
				ANSC-3	1.4	2.0	<0.010	<0.010	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.021
				Average Concentration (mg/L)	1.4	2.0	ND	ND	ND	ND	--	--	ND	ND	ND	ND	0.021
			Standard Deviation (mmoles)	1.6E-04	6.4E-05	0.0E+00	0.0E+00	0.0E+00	0.0E+00	--	--	0.0E+00	0.0E+00	0.0E+00	0.0E+00	2.9E-05	
			Average Total mmoles	<b>0.0021</b>	<b>0.0034</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>5.5E-03</b>	<b>--</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>0.0021</b>	
10-Jul-14	199		ANSC-1	1.4	2.6	<0.010	<0.010	<0.010	<0.010	--	0.80	<0.010	<0.010	<0.010	<0.010	0.021	
			ANSC-2	1.6	2.5	<0.010	<0.010	<0.010	<0.010	--	0.71	<0.010	<0.010	<0.010	<0.010	0.02	
			ANSC-3	1.6	2.6	<0.010	<0.010	<0.010	<0.010	--	0.81	<0.010	<0.010	<0.010	<0.010	0.02	
			Average Concentration (mg/L)	1.5	2.6	ND	ND	ND	ND	--	0.77	ND	ND	ND	ND	0.02	
			Standard Deviation (mmoles)	1.7E-04	1.3E-04	0.0E+00	0.0E+00	0.0E+00	0.0E+00	--	7.8E-05	0.0E+00	0.0E+00	0.0E+00	0.0E+00	4.4E-05	
			Average Total mmoles	<b>0.0023</b>	<b>0.0044</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>6.7E-03</b>	<b>0.0011</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>0.002</b>	
Anaerobic Active Control	19-Dec-13	-4														Amended the first replicate with 100 µL of resazurin.	
	23-Dec-13	0														Spiked with PCE, TCE and 1,1,2,2-TeCA to a target concentration of 1.5 mg/L.	
				ANAC-1	1.6	1.8	<0.010	<0.010	<0.010	<0.010	--	1.4	<0.010	<0.010	<0.010	<0.010	0.023
				ANAC-2	1.7	1.9	<0.010	<0.010	<0.010	<0.010	--	1.4	<0.010	<0.010	<0.010	<0.010	0.024
				ANAC-3	1.6	1.8	<0.010	<0.010	<0.010	<0.010	--	1.2	<0.010	<0.010	<0.010	<0.010	0.025
				Average Concentration (mg/L)	1.6	1.9	ND	ND	ND	ND	--	1.3	ND	ND	ND	ND	0.024
				Standard Deviation (mmoles)	4.7E-05	7.1E-05	0.0E+00	0.0E+00	0.0E+00	0.0E+00	--	1.6E-04	0.0E+00	0.0E+00	0.0E+00	0.0E+00	7.3E-05
				Average Total mmoles	<b>0.0024</b>	<b>0.0032</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>5.6E-03</b>	<b>0.002</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>0.0023</b>
	06-Jan-14	14		ANAC-1	1.6	1.9	<0.010	<0.010	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.023
				ANAC-2	1.6	1.9	<0.010	<0.010	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.023
				ANAC-3	1.6	1.9	<0.010	<0.010	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.023
				Average Concentration (mg/L)	1.6	1.9	ND	ND	ND	ND	--	--	ND	ND	ND	ND	0.023
				Standard Deviation (mmoles)	9.5E-06	6.8E-06	0.0E+00	0.0E+00	0.0E+00	0.0E+00	--	--	0.0E+00	0.0E+00	0.0E+00	0.0E+00	9.5E-06
				Average Total mmoles	<b>0.0023</b>	<b>0.0032</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>5.5E-03</b>	<b>--</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>0.0022</b>
	10-Mar-14	77		ANAC-1	1.6	2.2	<0.010	<0.010	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.021
				ANAC-2	1.5	2.2	<0.010	<0.010	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.021
				ANAC-3	1.5	2.1	<0.010	<0.010	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.021
				Average Concentration (mg/L)	1.5	2.1	ND	ND	ND	ND	--	--	ND	ND	ND	ND	0.021
			Standard Deviation (mmoles)	8.4E-05	1.1E-04	0.0E+00	0.0E+00	0.0E+00	0.0E+00	--	--	0.0E+00	0.0E+00	0.0E+00	0.0E+00	1.1E-05	
			Average Total mmoles	<b>0.0022</b>	<b>0.0037</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>5.9E-03</b>	<b>--</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>0.0021</b>	
10-Jul-14	199		ANAC-1	1.6	2.8	0.031	<0.010	<0.010	<0.010	--	0.61	<0.010	<0.010	<0.010	<0.010	0.31	
			ANAC-2	1.7	2.9	<0.010	<0.010	<0.010	<0.010	--	0.56	<0.010	<0.010	<0.010	<0.010	0.056	
			ANAC-3	1.7	2.9	<0.010	<0.010	<0.010	<0.010	--	0.41	<0.010	<0.010	<0.010	<0.010	0.057	
			Average Concentration (mg/L)	1.7	2.9	0.01	ND	ND	ND	--	0.52	ND	ND	ND	ND	0.14	
			Standard Deviation (mmoles)	6.3E-05	9.2E-05	3.9E-05	0.0E+00	0.0E+00	0.0E+00	--	1.5E-04	0.0E+00	0.0E+00	0.0E+00	0.0E+00	1.4E-02	
			Average Total mmoles	<b>0.0025</b>	<b>0.005</b>	<b>2.3E-05</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>7.5E-03</b>	<b>0.00077</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>0.014</b>	
SRS®-SD Amended	19-Dec-13	-4														Amended the first replicate with 100 µL of resazurin.	
	23-Dec-13	0														Amended with SRS®-SD to a target concentration of 0.1% as oil.	
																Spiked with PCE, TCE and 1,1,2,2-TeCA to a target concentration of 1.5 mg/L.	
				SRS-SD-1	1.6	1.8	<0.010	<0.010	<0.010	<0.010	--	1.4	<0.010	<0.010	<0.010	<0.010	0.024
				SRS-SD-2	1.6	1.8	<0.010	0.011	<0.010	<0.010	--	1.3	<0.010	<0.010	<0.010	<0.010	0.024
				SRS-SD-3	2.5	1.9	<0.010	<0.010	<0.010	<0.010	--	1.3	<0.010	<0.010	<0.010	<0.010	0.024
			Average Concentration (mg/L)	1.9	1.8	ND	0.0035	ND	ND	--	1.4	ND	ND	ND	ND	0.024	
			Standard Deviation (mmoles)	7.2E-04	1.1E-04	0.0E+00	1.3E-05	0.0E+00	0.0E+00	--	1.6E-05	0.0E+00	0.0E+00	0.0E+00	0.0E+00	2.2E-05	
			Average Total mmoles	<b>0.0028</b>	<b>0.0031</b>	<b>ND</b>	<b>7.7E-06</b>	<b>ND</b>	<b>ND</b>	<b>5.9E-03</b>	<b>0.002</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>0.0023</b>	

**TABLE 2: SUMMARY OF MICROCOSM cVOCs, ETHENE, ETHANE AND METHANE RESULTS**  
Training Area T-6, McClellan, Anniston, Alabama

Treatment	Date	Day	Replicate	Chlorinated Ethenes							Chlorinated Ethanes					Methane	Comment
				PCE	TCE	cDCE	tDCE	VC	Ethene	Total Ethenes	1,1,2,2-TECA	1,1,2-TCA	1,2-DCA	CA	Ethane	Methane	
				mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mmol/bottle	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	
SRS®-SD Amended (Cont'd)	06-Jan-14	14	SRS-SD-1	1.4	1.3	0.54	<0.010	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.022	
			SRS-SD-2	1.6	1.7	<0.010	<0.010	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.026	
			SRS-SD-3	2.3	1.8	<0.010	0.017	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.023	
			<b>Average Concentration (mg/L)</b>	1.8	1.6	0.18	0.0056	ND	ND	--	--	ND	ND	ND	ND	0.024	
	Standard Deviation (mmoles)	7.1E-04	4.9E-04	6.9E-04	2.1E-05	0.0E+00	0.0E+00	--	--	0.0E+00	0.0E+00	0.0E+00	0.0E+00	1.9E-04			
	<b>Average Total mmoles</b>	<b>0.0026</b>	<b>0.0027</b>	<b>0.0004</b>	<b>0.000012</b>	<b>ND</b>	<b>ND</b>	<b>5.7E-03</b>	--	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>0.0023</b>			
	20-Jan-14	28	SRS-SD-1	1.3	0.99	0.82	<0.010	<0.010	<0.010	--	1.3	<0.010	<0.010	<0.010	<0.010	0.022	
			SRS-SD-2	1.5	1.6	0.015	<0.010	<0.010	<0.010	--	1.2	<0.010	<0.010	<0.010	<0.010	0.021	
			SRS-SD-3	2.5	1.9	0.016	0.018	<0.010	<0.010	--	1.2	<0.010	<0.010	<0.010	<0.010	0.022	
			<b>Average Concentration (mg/L)</b>	1.8	1.5	0.28	0.006	ND	ND	--	1.2	ND	ND	ND	ND	0.022	
	Standard Deviation (mmoles)	9.9E-04	8.1E-04	1.0E-03	2.3E-05	0.0E+00	0.0E+00	--	8.2E-05	0.0E+00	0.0E+00	0.0E+00	0.0E+00	4.5E-05			
	<b>Average Total mmoles</b>	<b>0.0026</b>	<b>0.0026</b>	<b>0.00063</b>	<b>0.000013</b>	<b>ND</b>	<b>ND</b>	<b>5.8E-03</b>	<b>0.0018</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>0.0021</b>			
	17-Feb-14	56	SRS-SD-1	1.3	0.99	0.83	<0.010	<0.010	<0.010	--	1.3	<0.010	<0.010	<0.010	<0.010	0.022	
			SRS-SD-2	1.5	1.7	0.02	<0.010	<0.010	<0.010	--	1.3	<0.010	<0.010	<0.010	<0.010	0.022	
			SRS-SD-3	2.5	1.9	0.022	0.012	<0.010	<0.010	--	1.2	<0.010	<0.010	<0.010	<0.010	0.088	
			<b>Average Concentration (mg/L)</b>	1.8	1.5	0.29	0.0039	ND	ND	--	1.2	ND	ND	ND	ND	0.044	
	Standard Deviation (mmoles)	9.8E-04	8.1E-04	1.0E-03	1.5E-05	0.0E+00	0.0E+00	--	1.1E-04	0.0E+00	0.0E+00	0.0E+00	0.0E+00	3.7E-03			
	<b>Average Total mmoles</b>	<b>0.0026</b>	<b>0.0026</b>	<b>0.00065</b>	<b>8.6E-06</b>	<b>ND</b>	<b>ND</b>	<b>5.9E-03</b>	<b>0.0018</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>0.0043</b>			
	10-Mar-14	77	SRS-SD-1	1.3	1.0	0.85	<0.010	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.021	
			SRS-SD-2	1.6	1.7	<0.010	0.011	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.031	
			SRS-SD-3	1.6	1.7	0.03	0.01	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.023	
			<b>Average Concentration (mg/L)</b>	1.5	1.5	0.29	0.0071	ND	ND	--	--	ND	ND	ND	ND	0.025	
	Standard Deviation (mmoles)	2.5E-04	6.9E-04	1.1E-03	1.3E-05	0.0E+00	0.0E+00	--	--	0.0E+00	0.0E+00	0.0E+00	0.0E+00	4.8E-04			
	<b>Average Total mmoles</b>	<b>0.0022</b>	<b>0.0026</b>	<b>0.00065</b>	<b>0.000016</b>	<b>ND</b>	<b>ND</b>	<b>5.5E-03</b>	--	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>0.0024</b>			
09-Apr-14	107															Amended with SRS®-SD to a target concentration of 0.1% as oil.	
07-May-14	135	SRS-SD-1	1.3	1.0	0.83	<0.010	0.01	<0.010	--	--	<0.010	0.013	<0.010	<0.010	0.14		
		SRS-SD-2	1.6	1.7	0.036	<0.010	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.35		
		SRS-SD-3	0.018	0.027	3.3	0.026	0.02	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	7.4		
		<b>Average Concentration (mg/L)</b>	0.97	0.93	1.4	0.0087	0.01	ND	--	--	ND	0.0045	ND	ND	2.6		
Standard Deviation (mmoles)	1.3E-03	1.5E-03	3.8E-03	3.3E-05	4.0E-05	0.0E+00	--	--	0.0E+00	1.6E-05	0.0E+00	0.0E+00	4.0E-01				
<b>Average Total mmoles</b>	<b>0.0014</b>	<b>0.0016</b>	<b>0.0031</b>	<b>0.000019</b>	<b>0.00004</b>	<b>ND</b>	<b>6.2E-03</b>	--	<b>ND</b>	<b>9.1E-06</b>	<b>ND</b>	<b>ND</b>	<b>0.26</b>				
04-Jun-14	163	SRS-SD-1	1.2	1.0	0.82	0.01	0.012	<0.010	--	1.1	<0.010	<0.010	<0.010	<0.010	0.21		
		SRS-SD-2	1.4	1.6	0.037	0.011	<0.010	<0.010	--	0.96	<0.010	<0.010	<0.010	<0.010	0.37		
		SRS-SD-3	0.032	0.33	3.2	0.031	0.025	<0.010	--	0.75	<0.010	<0.010	<0.010	<0.010	2.9		
		<b>Average Concentration (mg/L)</b>	0.90	1.0	1.3	0.017	0.012	ND	--	0.92	ND	ND	ND	ND	1.2		
Standard Deviation (mmoles)	1.1E-03	1.1E-03	3.6E-03	2.6E-05	4.9E-05	0.0E+00	--	2.3E-04	0.0E+00	0.0E+00	0.0E+00	0.0E+00	1.5E-01				
<b>Average Total mmoles</b>	<b>0.0013</b>	<b>0.0017</b>	<b>0.003</b>	<b>0.000038</b>	<b>4.8E-05</b>	<b>ND</b>	<b>6.1E-03</b>	<b>0.0014</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>0.11</b>				
10-Jul-14	199	SRS-SD-1	1.5	1.3	1.0	0.014	0.020	<0.010	--	1.0	<0.010	<0.010	<0.010	<0.010	0.46		
		SRS-SD-2	1.8	2.0	0.054	0.012	0.011	<0.010	--	1.0	<0.010	<0.010	<0.010	<0.010	0.54		
		SRS-SD-3	0.034	0.079	4.0	0.05	0.057	<0.010	--	0.71	<0.010	<0.010	<0.010	<0.010	2		
		<b>Average Concentration (mg/L)</b>	1.1	1.1	1.7	0.025	0.029	ND	--	0.92	ND	ND	ND	ND	0.99		
Standard Deviation (mmoles)	1.4E-03	1.7E-03	4.6E-03	4.7E-05	9.6E-05	0.0E+00	--	2.7E-04	0.0E+00	0.0E+00	0.0E+00	0.0E+00	8.3E-02				
<b>Average Total mmoles</b>	<b>0.0016</b>	<b>0.0019</b>	<b>0.0038</b>	<b>0.000056</b>	<b>0.00012</b>	<b>ND</b>	<b>7.4E-03</b>	<b>0.0013</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>0.096</b>				
SRS®-SD Amended and KB-1® Plus Bioaugmented	19-Dec-13	-4															Amended the first replicate with 100 µL of resazurin.
SRS®-SD Amended and KB-1® Plus Bioaugmented	23-Dec-13	0															Amended with SRS®-SD to a target concentration of 0.1% as oil.
																	Spiked with PCE, TCE and 1,1,2,2-TeCa to a target concentration of 1.5 mg/L.
	SRS-SD&KB-1+1	2.2	1.6	<0.010	<0.010	<0.010	<0.010	--	1.3	<0.010	<0.010	<0.010	<0.010	0.026			
	SRS-SD&KB-1+2	2.5	1.9	<0.010	<0.010	<0.010	<0.010	--	1.4	<0.010	<0.010	<0.010	<0.010	0.025			
	SRS-SD&KB-1+3	2.4	1.8	<0.010	<0.010	<0.010	<0.010	--	1.3	<0.010	<0.010	<0.010	<0.010	0.026			
	<b>Average Concentration (mg/L)</b>	2.3	1.8	ND	ND	ND	ND	--	1.3	ND	ND	ND	ND	0.026			
	Standard Deviation (mmoles)	2.5E-04	2.1E-04	0.0E+00	0.0E+00	0.0E+00	0.0E+00	--	5.3E-05	0.0E+00	0.0E+00	0.0E+00	0.0E+00	6.4E-05			
	<b>Average Total mmoles</b>	<b>0.0035</b>	<b>0.003</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>6.5E-03</b>	<b>0.0019</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>0.0025</b>			
	06-Jan-14	14	SRS-SD&KB-1+1	2.3	1.7	<0.010	0.024	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.022	
			SRS-SD&KB-1+2	2.4	1.8	<0.010	<0.010	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.022	
SRS-SD&KB-1+3			2.4	1.8	<0.010	0.012	<0.010	<0.010	--	--	<0.010	<0.010	<0.010	<0.010	0.022		
<b>Average Concentration (mg/L)</b>			2.3	1.8	ND	0.012	ND	ND	--	--	ND	ND	ND	ND	0.022		
Standard Deviation (mmoles)	8.2E-05	5.8E-05	0.0E+00	2.6E-05	0.0E+00	0.0E+00	--	--	0.0E+00	0.0E+00	0.0E+00	0.0E+00	1.3E-05				
<b>Average Total mmoles</b>	<b>0.0035</b>	<b>0.003</b>	<b>ND</b>	<b>0.000026</b>	<b>ND</b>	<b>ND</b>	<b>6.5E-03</b>	--	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>0.0022</b>				
20-Jan-14	28	SRS-SD&KB-1+1	2.4	1.9	0.011	0.024	<0.010	<0.010	--	1.1	<0.010	<0.010	<0.010	<0.010	0.023		
		SRS-SD&KB-1+2	2.4	1.8	0.019	<0.010	<0.010	<0.010	--	1.2	<0.010	<0.010	<0.010	<0.010	0.024		
		SRS-SD&KB-1+3	2.5	1.8	0.012	<0.010	<0.010	<0.010	--	1.2	<0.010	<0.010	<0.010	<0.010	0.023		
		<b>Average Concentration (mg/L)</b>	2.4	1.8	0.014	0.0082	ND	ND	--	1.2	ND	ND	ND	ND	0.023		
Standard Deviation (mmoles)	5.3E-05	5.1E-05	1.0E-05	3.1E-05	0.0E+00	0.0E+00	--	1.0E-04	0.0E+00	0.0E+00	0.0E+00	0.0E+00	7.7E-05				
<b>Average Total mmoles</b>	<b>0.0036</b>	<b>0.0031</b>	<b>3.1E-05</b>	<b>0.000018</b>	<b>ND</b>	<b>ND</b>	<b>6.7E-03</b>	<b>0.0017</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>ND</b>	<b>0.0022</b>				

TABLE 2: SUMMARY OF MICROCOSM cVOCs, ETHENE, ETHANE AND METHANE RESULTS

Training Area T-6, McClellan, Anniston, Alabama

SIREM

Treatment	Date	Day	Replicate	Chlorinated Ethenes							Chlorinated Ethanes					Methane	Comment
				PCE	TCE	cDCE	tDCE	VC	Ethene	Total Ethenes	1,1,2,2-TECA	1,1,2-TCA	1,2-DCA	CA	Ethane	Methane	
				mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	mmol/bottle	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L	
SRS®-SD Amended and KB-1® Plus Bioaugmented (Cont'd)	27-Jan-14	35	SRS-SD&KB-1+-1	<0.010	<0.010	1.9	0.093	0.67	0.033	--	1.1	<0.010	<0.010	<0.010	<0.010	0.26	
			SRS-SD&KB-1+-2	<0.010	<0.010	2.1	0.076	0.59	0.022	--	1.1	<0.010	<0.010	<0.010	<0.010	0.42	
			SRS-SD&KB-1+-3	<0.010	<0.010	2.3	0.079	0.42	0.011	--	1.2	<0.010	<0.010	<0.010	<0.010	0.32	
			Average Concentration (mg/L)	ND	ND	2.1	0.083	0.56	0.022	--	1.1	ND	ND	ND	ND	0.33	
	Standard Deviation (mmoles)	0.0E+00	0.0E+00	4.4E-04	1.9E-05	5.1E-04	2.5E-04	--	6.1E-05	0.0E+00	0.0E+00	0.0E+00	0.0E+00	8.0E-03			
	Average Total mmoles	ND	ND	0.0046	0.00018	0.0022	0.00049	7.5E-03	0.0016	ND	ND	ND	ND	0.032			
	17-Feb-14	56	SRS-SD&KB-1+-1	<0.010	<0.010	<0.010	0.65	0.034	0.26	--	<0.010	<0.010	<0.010	<0.010	<0.010	1.1	
			SRS-SD&KB-1+-2	--	--	--	--	--	--	--	0.90	--	--	--	--	--	
			SRS-SD&KB-1+-3	<0.010	0.013	1.6	0.093	0.67	0.021	--	1.0	<0.010	<0.010	<0.010	<0.010	0.39	
			Average Concentration (mg/L)	ND	0.0063	0.82	0.37	0.35	0.14	--	0.65	ND	ND	ND	ND	0.76	
	Standard Deviation (mmoles)	0.0E+00	1.5E-05	2.6E-03	8.6E-04	1.8E-03	3.8E-03	--	8.3E-04	0.0E+00	0.0E+00	0.0E+00	0.0E+00	5.1E-02			
	Average Total mmoles	ND	0.000011	0.0018	0.00082	0.0014	0.0031	7.1E-03	0.00095	ND	ND	ND	ND	0.074			
	10-Mar-14	77	SRS-SD&KB-1+-1	<0.010	<0.010	<0.010	0.61	<0.010	0.23	--	--	<0.010	<0.010	<0.010	<0.010	2.2	
			SRS-SD&KB-1+-2	<0.010	0.055	0.55	0.18	0.85	0.099	--	--	<0.010	<0.010	<0.010	<0.010	1.3	
			SRS-SD&KB-1+-3	0.012	0.053	0.58	0.17	0.85	0.11	--	--	<0.010	<0.010	<0.010	<0.010	1.5	
			Average Concentration (mg/L)	0.0039	0.036	0.38	0.32	0.56	0.14	--	--	ND	ND	ND	ND	1.7	
	Standard Deviation (mmoles)	1.0E-05	5.3E-05	7.3E-04	5.6E-04	1.9E-03	1.7E-03	--	--	0.0E+00	0.0E+00	0.0E+00	0.0E+00	4.7E-02			
	Average Total mmoles	5.8E-06	0.000061	0.00084	0.0007	0.0022	0.0032	7.0E-03	--	ND	ND	ND	ND	0.16			
	31-Mar-14	98	SRS-SD&KB-1+-1	<0.010	<0.010	<0.010	0.57	0.013	0.22	--	<0.010	<0.010	<0.010	<0.010	<0.010	2.5	
			SRS-SD&KB-1+-2	0.01	0.049	0.16	0.25	0.49	0.17	--	0.52	<0.010	<0.010	<0.010	<0.010	1.6	
			SRS-SD&KB-1+-3	0.017	0.064	1.6	0.11	0.63	0.026	--	0.89	<0.010	<0.010	<0.010	<0.010	1.4	
			Average Concentration (mg/L)	0.009	0.038	0.58	0.31	0.38	0.14	--	0.47	ND	ND	ND	ND	1.8	
	Standard Deviation (mmoles)	1.3E-05	5.7E-05	1.9E-03	5.2E-04	1.3E-03	2.2E-03	--	6.6E-04	0.0E+00	0.0E+00	0.0E+00	0.0E+00	5.7E-02			
	Average Total mmoles	0.000013	0.000064	0.0013	0.00069	0.0015	0.0031	6.7E-03	0.00069	ND	ND	ND	ND	0.18			
09-Apr-14	107															Amended with SRS®-SD to a target concentration of 0.1% as oil.	
07-May-14	135	SRS-SD&KB-1+-1	<0.010	<0.010	<0.010	0.51	0.032	0.21	--	--	<0.010	0.015	<0.010	<0.010	6.2		
		SRS-SD&KB-1+-2	0.013	0.021	<0.010	0.49	<0.010	0.24	--	--	<0.010	0.029	<0.010	<0.010	4.3		
		SRS-SD&KB-1+-3	0.035	0.10	1.6	<0.010	0.56	0.021	--	--	<0.010	<0.010	<0.010	<0.010	1.9		
		Average Concentration (mg/L)	0.016	0.04	0.52	0.33	0.20	0.15	--	--	ND	0.015	ND	ND	4.2		
Standard Deviation (mmoles)	2.6E-05	9.1E-05	2.0E-03	6.3E-04	1.3E-03	2.6E-03	--	--	0.0E+00	2.9E-05	0.0E+00	0.0E+00	2.1E-01				
Average Total mmoles	0.000024	0.000069	0.0012	0.00073	0.00079	0.0034	6.2E-03	--	ND	0.00003	ND	ND	0.4				
04-Jun-14	163	SRS-SD&KB-1+-1	<0.010	<0.010	<0.010	0.44	0.033	0.19	--	<0.010	<0.010	<0.010	<0.010	<0.010	8.7		
		SRS-SD&KB-1+-2	<0.010	0.012	<0.010	0.44	<0.010	0.21	--	<0.010	<0.010	0.013	<0.010	<0.010	4.1		
		SRS-SD&KB-1+-3	0.019	0.10	1.5	0.13	0.51	0.018	--	0.67	<0.010	<0.010	<0.010	<0.010	1.7		
		Average Concentration (mg/L)	0.0065	0.038	0.49	0.34	0.18	0.14	--	0.22	ND	0.0045	ND	ND	4.8		
Standard Deviation (mmoles)	1.7E-05	9.6E-05	1.9E-03	4.0E-04	1.1E-03	2.3E-03	--	5.7E-04	0.0E+00	1.6E-05	0.0E+00	0.0E+00	3.5E-01				
Average Total mmoles	9.6E-06	0.000066	0.0011	0.00074	0.00071	0.0031	5.7E-03	0.00033	ND	9.1E-06	ND	ND	0.47				
10-Jul-14	199	SRS-SD&KB-1+-1	<0.010	<0.010	<0.010	0.44	0.092	0.21	--	<0.010	<0.010	<0.010	<0.010	<0.010	14		
		SRS-SD&KB-1+-2	<0.010	0.015	<0.010	0.52	0.011	0.25	--	<0.010	<0.010	0.014	<0.010	<0.010	5.4		
		SRS-SD&KB-1+-3	0.024	0.15	1.9	0.15	0.77	0.025	--	0.60	<0.010	<0.010	<0.010	<0.010	2.1		
		Average Concentration (mg/L)	0.0081	0.056	0.64	0.37	0.29	0.16	--	0.20	ND	0.0047	ND	ND	7.1		
Standard Deviation (mmoles)	2.1E-05	1.4E-04	2.4E-03	4.3E-04	1.6E-03	2.7E-03	--	5.1E-04	0.0E+00	1.7E-05	0.0E+00	0.0E+00	5.9E-01				
Average Total mmoles	0.000012	0.000095	0.0014	0.00082	0.0011	0.0036	5.9E-03	0.00029	ND	9.7E-06	ND	ND	0.69				

Notes:

- not analyzed
- % - percent
- < - compound not detected, the associated value is the detection limit
- 1,1,2,2-TECA - 1,1,2,2-tetrachloroethane
- 1,1,2-TCA - 1,1,2-trichloroethane
- 1,2-DCA - 1,2-dichloroethane
- ANSC - anaerobic sterile control
- ANAC - anaerobic active control
- cDCE - cis-1,2-dichloroethene
- CA - chloroethane
- µL - microliters
- mg/L - milligrams per liter
- mmoles - millimoles
- mmoles/bottle - millimoles per bottle
- ND - not detected
- TCE - trichloroethene
- tDCE - trans-1,2-dichloroethene
- PCE - tetrachloroethene
- VC - vinyl chloride

**TABLE 3: SUMMARY OF MICROCOSM ANION RESULTS**  
Training Area T-6, McClellan, Anniston, Alabama

Treatment	Date	Day	Treatment Replicate	Total VFAs	Chloride	Nitrite-N	Nitrate-N	Sulfate	Phosphate
				mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Anaerobic Sterile Control	23-Dec-13	0	ANSC-1	0.87	96	<0.09	16	4.6	0.36
			ANSC-2	0.27	119	<0.09	21	5.4	0.13
			ANSC-3	0.66	103	<0.09	19	4.7	0.14
			<b>Average Concentration</b>	<b>0.60</b>	<b>106</b>	<b>ND</b>	<b>19</b>	<b>4.9</b>	<b>0.21</b>
	6-Jan-14	14	ANSC-1	2.8	95	<0.09	16	4.8	0.10
			ANSC-2	3.1	105	<0.09	17	5.5	0.18
			ANSC-3	2.2	99	<0.09	18	5.3	0.08
			<b>Average Concentration</b>	<b>2.7</b>	<b>100</b>	<b>ND</b>	<b>17</b>	<b>5.2</b>	<b>0.12</b>
	10-Mar-14	77	ANSC-1	2.4	84	<0.09	14	3.6	0.12
			ANSC-2	3.0	83	<0.09	15	3.3	0.14
			ANSC-3	3.2	84	<0.09	16	3.9	0.17
			<b>Average Concentration</b>	<b>2.9</b>	<b>84</b>	<b>ND</b>	<b>15</b>	<b>3.6</b>	<b>0.15</b>
10-Jul-14	199	ANSC-1	12	98	<0.09	<0.09	8.1	<0.07	
		ANSC-2	11	97	<0.09	<0.09	8.0	<0.07	
		ANSC-3	11	43	<0.09	<0.09	4.8	<0.07	
		<b>Average Concentration</b>	<b>12</b>	<b>80</b>	<b>ND</b>	<b>ND</b>	<b>7.0</b>	<b>ND</b>	
Anaerobic Active Control	23-Dec-13	0	ANAC-1	1.5	3.9	<0.09	0.72	4.8	0.35
			ANAC-2	0.54	3.5	<0.09	0.14	4.4	<0.07
			ANAC-3	0.53	3.4	<0.09	<0.09	4.9	<0.07
			<b>Average Concentration</b>	<b>0.86</b>	<b>3.6</b>	<b>ND</b>	<b>0.29</b>	<b>4.7</b>	<b>0.12</b>
	6-Jan-14	14	ANAC-1	2.6	4.0	<0.09	0.32	4.8	<0.07
			ANAC-2	2.1	3.8	<0.09	0.27	4.4	<0.07
			ANAC-3	2.9	5.2	<0.09	1.1	5.6	<0.07
			<b>Average Concentration</b>	<b>2.5</b>	<b>4.3</b>	<b>ND</b>	<b>0.55</b>	<b>4.9</b>	<b>ND</b>
	10-Mar-14	77	ANAC-1	2.8	2.6	<0.09	<0.09	1.8	0.07
			ANAC-2	3.8	4.3	<0.09	0.16	2.2	<0.07
			ANAC-3	2.4	3.8	<0.09	0.13	1.8	<0.07
			<b>Average Concentration</b>	<b>3.0</b>	<b>3.6</b>	<b>ND</b>	<b>0.10</b>	<b>1.9</b>	<b>ND</b>
10-Jul-14	199	ANAC-1	14	4.0	<0.09	0.12	2.8	<0.07	
		ANAC-2	13	3.6	<0.09	<0.09	2.2	<0.07	
		ANAC-3	13	3.4	<0.09	0.2	2.3	<0.07	
		<b>Average Concentration</b>	<b>13</b>	<b>3.7</b>	<b>ND</b>	<b>0.10</b>	<b>2.4</b>	<b>ND</b>	
SRS®-SD Amended	23-Dec-13	0	SRS-SD-1	52	3.3	<0.09	0.10	6.3	5.8
			SRS-SD-2	50	3.8	<0.09	<0.09	4.9	0.62
			SRS-SD-3	58	3.3	<0.09	0.11	4.8	0.34
			<b>Average Concentration</b>	<b>53</b>	<b>3.5</b>	<b>ND</b>	<b>0.07</b>	<b>5.3</b>	<b>2.3</b>
	6-Jan-14	14	SRS-SD-1	93	4.2	<0.09	0.29	0.83	0.09
			SRS-SD-2	81	3.0	<0.09	0.21	0.72	<0.07
			SRS-SD-3	65	3.5	<0.09	0.30	0.78	<0.07
			<b>Average Concentration</b>	<b>80</b>	<b>3.5</b>	<b>ND</b>	<b>0.27</b>	<b>0.78</b>	<b>0.03</b>
	10-Mar-14	77	SRS-SD-1	173	6.8	<0.09	0.93	4.6	0.52
			SRS-SD-2	168	6.6	<0.09	0.44	0.34	0.38
			SRS-SD-3	147	2.9	<0.09	0.14	0.19	0.08
			<b>Average Concentration</b>	<b>163</b>	<b>5.4</b>	<b>ND</b>	<b>0.50</b>	<b>1.7</b>	<b>0.33</b>
10-Jul-14	199	SRS-SD-1	383	3.8	<0.09	0.12	2.1	<0.07	
		SRS-SD-2	367	4.0	<0.09	0.09	2.4	<0.07	
		SRS-SD-3	426	5.2	<0.09	0.12	2.2	<0.07	
		<b>Average Concentration</b>	<b>392</b>	<b>4.4</b>	<b>ND</b>	<b>0.11</b>	<b>2.2</b>	<b>ND</b>	
SRS®-SD amended/KB-1® Plus Bioaugmented	23-Dec-13	0	SRS-SD/KB-1+1	55	3.3	<0.09	0.21	0.29	0.17
			SRS-SD/KB-1+2	48	4.5	<0.09	0.13	4.8	0.17
			SRS-SD/KB-1+3	68	5.2	<0.09	0.20	0.63	<0.07
			<b>Average Concentration</b>	<b>57</b>	<b>4.3</b>	<b>ND</b>	<b>0.18</b>	<b>1.9</b>	<b>0.11</b>
	6-Jan-14	14	SRS-SD/KB-1+1	63	3.6	<0.09	0.25	0.81	<0.07
			SRS-SD/KB-1+2	69	4.1	<0.09	0.28	1.0	0.12
			SRS-SD/KB-1+3	74	4.7	<0.09	0.28	1.0	<0.07
			<b>Average Concentration</b>	<b>68</b>	<b>4.1</b>	<b>ND</b>	<b>0.27</b>	<b>0.94</b>	<b>0.04</b>
	10-Mar-14	77	SRS-SD/KB-1+1	166	8.1	<0.09	0.12	0.28	0.33
			SRS-SD/KB-1+2	109	2.2	<0.09	<0.09	0.21	<0.07
			SRS-SD/KB-1+3	139	7.1	<0.09	<0.09	0.27	0.32
			<b>Average Concentration</b>	<b>138</b>	<b>5.8</b>	<b>ND</b>	<b>0.04</b>	<b>0.25</b>	<b>0.22</b>
10-Jul-14	199	SRS-SD/KB-1+1	311	10	<0.09	0.14	2.3	<0.07	
		SRS-SD/KB-1+2	462	11	<0.09	0.10	2.2	<0.07	
		SRS-SD/KB-1+3	379	6.6	<0.09	0.09	2.2	<0.07	
		<b>Average Concentration</b>	<b>384</b>	<b>9.4</b>	<b>ND</b>	<b>0.11</b>	<b>2.2</b>	<b>ND</b>	

**Notes:**

ANAC - anaerobic active control  
 ANSC - anaerobic sterile control  
 ND - not detected  
 mg/L - milligrams per liter  
 VFAs - total volatile fatty acids, calibrated as lactate but may include other VFAs such as formate, acetate, propionate, pyruvate and butyrate  
 < - compound not detected, the associated value is the detection limit

**TABLE 4: SUMMARY OF MICROCOSM VFA RESULTS**  
 Training Area T-6, McClellan, Anniston, Alabama

Treatment	Date	Day	Treatment Replicate	Lactate	Acetate	Propionate	Formate	Butyrate	Pyruvate
				mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
SRS®-SD Amended	23-Dec-13	0	SRS-SD-1	48	0.60	<0.31	0.84	<0.41	<0.69
			SRS-SD-2	45	0.54	<0.31	0.50	<0.41	<0.69
			SRS-SD-3	46	0.57	<0.31	0.51	<0.41	<0.69
			<b>Average Concentration</b>	<b>47</b>	<b>0.57</b>	<b>ND</b>	<b>0.62</b>	<b>ND</b>	<b>ND</b>
	17-Feb-14	56	SRS-SD-1	<0.39	102	82	<0.22	3.3	<0.69
			SRS-SD-2	<0.39	98	78	0.41	5.5	<0.69
			SRS-SD-3	<0.39	56	40	0.48	5.0	<0.69
			<b>Average Concentration</b>	<b>ND</b>	<b>85</b>	<b>67</b>	<b>0.30</b>	<b>4.6</b>	<b>ND</b>
	10-Jul-14	199	SRS-SD-1	<0.39	269	172	0.37	13	1.4
			SRS-SD-2	<0.39	245	163	0.48	4.7	2.2
			SRS-SD-3	<0.39	344	140	0.33	35	<0.69
			<b>Average Concentration</b>	<b>ND</b>	<b>286</b>	<b>158</b>	<b>0.39</b>	<b>17</b>	<b>1.2</b>
SRS®-SD amended/KB-1® Plus Bioaugmented	23-Dec-13	0	SRS-SD/KB-1+-1	44	0.81	<0.31	0.37	<0.41	<0.69
			SRS-SD/KB-1+-2	48	0.58	<0.31	0.67	<0.41	<0.69
			SRS-SD/KB-1+-3	49	0.21	<0.31	0.29	<0.41	<0.69
			<b>Average Concentration</b>	<b>47</b>	<b>0.53</b>	<b>ND</b>	<b>0.44</b>	<b>ND</b>	<b>ND</b>
	17-Feb-14	56	SRS-SD/KB-1+-1	<0.39	112	36	0.25	8.9	<0.69
			SRS-SD/KB-1+-2	<0.39	107	40	0.31	2.7	<0.69
			SRS-SD/KB-1+-3	<0.39	107	55	0.29	4.4	<0.69
			<b>Average Concentration</b>	<b>ND</b>	<b>109</b>	<b>44</b>	<b>0.28</b>	<b>5.3</b>	<b>ND</b>
	31-Mar-14	98	SRS-SD/KB-1+-1	<0.39	206	41	0.29	22	<0.69
			SRS-SD/KB-1+-2	<0.39	176	39	<0.22	16	<0.69
			SRS-SD/KB-1+-3	<0.39	168	71	0.67	4.0	<0.69
			<b>Average Concentration</b>	<b>ND</b>	<b>184</b>	<b>50</b>	<b>0.32</b>	<b>14</b>	<b>ND</b>
	10-Jul-14	199	SRS-SD/KB-1+-1	<0.39	316	13	0.34	43	<0.69
			SRS-SD/KB-1+-2	<0.39	444	34	0.26	33	<0.69
			SRS-SD/KB-1+-3	<0.39	343	153	0.62	15	<0.69
			<b>Average Concentration</b>	<b>ND</b>	<b>367</b>	<b>67</b>	<b>0.41</b>	<b>30</b>	<b>ND</b>

**Notes:**

ANAC - anaerobic active control  
 ANSC - anaerobic sterile control  
 mg/L - milligrams per liter  
 ND - not detected  
 < - compound not detected, the associated value is the detection limit

**TABLE 5: SUMMARY OF MICROCOSM pH RESULTS**  
 Training Area T-6, McClellan, Anniston, Alabama

Treatment	Date	Day	Treatment Replicate	pH	
Anaerobic Sterile Control	23-Dec-13	0	ANSC-1	6.34	
			ANSC-2	6.38	
			ANSC-3	6.34	
			<b>Average Concentration</b>	<b>6.35</b>	
	6-Jan-14	14	ANSC-1	6.36	
			ANSC-2	6.39	
			ANSC-3	6.43	
			<b>Average Concentration</b>	<b>6.39</b>	
	10-Mar-14	77	ANSC-1	6.13	
			ANSC-2	6.17	
			ANSC-3	6.22	
			<b>Average Concentration</b>	<b>6.17</b>	
10-Jul-14	199	ANSC-1	6.21		
		ANSC-2	6.25		
		ANSC-3	6.28		
		<b>Average Concentration</b>	<b>6.25</b>		
Anaerobic Active Control	23-Dec-13	0	ANAC-1	6.54	
			ANAC-2	6.51	
			ANAC-3	6.53	
			<b>Average Concentration</b>	<b>6.53</b>	
	6-Jan-14	14	ANAC-1	6.57	
			ANAC-2	6.60	
			ANAC-3	6.58	
			<b>Average Concentration</b>	<b>6.58</b>	
	10-Mar-14	77	ANAC-1	6.33	
			ANAC-2	6.30	
			ANAC-3	6.39	
			<b>Average Concentration</b>	<b>6.34</b>	
10-Jul-14	199	ANAC-1	6.36		
		ANAC-2	6.42		
		ANAC-3	6.44		
		<b>Average Concentration</b>	<b>6.41</b>		
SRS®-SD Amended	23-Dec-13	0	SRS-SD-1	6.47	
			SRS-SD-2	6.44	
			SRS-SD-3	6.47	
			<b>Average Concentration</b>	<b>6.46</b>	
	6-Jan-14	14	SRS-SD-1	6.27	
			SRS-SD-2	6.34	
			SRS-SD-3	6.46	
			<b>Average Concentration</b>	<b>6.36</b>	
	20-Jan-14	28	SRS-SD-1	5.65	
			SRS-SD-2	6.00	
			SRS-SD-3	6.33	
			<b>Average Concentration</b>	<b>5.99</b>	
	17-Feb-14	56	SRS-SD-1	5.22	
			SRS-SD-2	5.35	
			SRS-SD-3	5.99	
			<b>Average Concentration</b>	<b>5.52</b>	
	Buffered with 0.5 mL of Saturated Sodium Bicarbonate				
	19-Feb-14	58	SRS-SD-1	6.31	
			SRS-SD-2	6.44	
			SRS-SD-3	6.78	
			<b>Average Concentration</b>	<b>6.51</b>	
	10-Mar-14	77	SRS-SD-1	6.15	
			SRS-SD-2	6.25	
			SRS-SD-3	6.23	
<b>Average Concentration</b>			<b>6.21</b>		
7-May-14	135	SRS-SD-1	6.14		
		SRS-SD-2	6.28		
		SRS-SD-3	6.26		
		<b>Average Concentration</b>	<b>6.23</b>		
22-May-14	150	SRS-SD-1	6.08		
		SRS-SD-2	6.00		
		SRS-SD-3	6.00		
		<b>Average Concentration</b>	<b>6.03</b>		
4-Jun-14	163	SRS-SD-1	5.85		
		SRS-SD-2	5.91		
		SRS-SD-3	5.92		
		<b>Average Concentration</b>	<b>5.89</b>		
Buffered with 0.5 mL of Saturated Sodium Bicarbonate					
17-Jun-14	176	SRS-SD-1	6.28		
		SRS-SD-2	6.28		
		SRS-SD-3	6.30		
		<b>Average Concentration</b>	<b>6.29</b>		
10-Jul-14	199	SRS-SD-1	6.40		
		SRS-SD-2	6.45		
		SRS-SD-3	6.36		
		<b>Average Concentration</b>	<b>6.40</b>		

**TABLE 5: SUMMARY OF MICROCOSM pH RESULTS**  
 Training Area T-6, McClellan, Anniston, Alabama

Treatment	Date	Day	Treatment Replicate	pH	
<b>SRS®-SD amended/KB-1® Plus Bioaugmented</b>	23-Dec-13	0	SRS-SD/KB-1+-1	6.45	
			SRS-SD/KB-1+-2	6.51	
			SRS-SD/KB-1+-3	6.52	
			<b>Average Concentration</b>	<b>6.49</b>	
	6-Jan-14	14	SRS-SD/KB-1+-1	6.55	
			SRS-SD/KB-1+-2	6.46	
			SRS-SD/KB-1+-3	6.40	
			<b>Average Concentration</b>	<b>6.47</b>	
	20-Jan-14	28	SRS-SD/KB-1+-1	6.31	
			SRS-SD/KB-1+-2	6.27	
			SRS-SD/KB-1+-3	6.04	
			<b>Average Concentration</b>	<b>6.21</b>	
	27-Jan-14	35	SRS-SD/KB-1+-1	6.33	
			SRS-SD/KB-1+-2	6.08	
			SRS-SD/KB-1+-3	5.80	
			<b>Average Concentration</b>	<b>6.07</b>	
	17-Feb-14	56	SRS-SD/KB-1+-1	5.64	
			SRS-SD/KB-1+-2	5.65	
			SRS-SD/KB-1+-3	5.49	
			<b>Average Concentration</b>	<b>5.59</b>	
	Buffered with 0.5 mL of Saturated Sodium Bicarbonate				
	19-Feb-14	58	SRS-SD/KB-1+-1	--	
			SRS-SD/KB-1+-2	6.76	
			SRS-SD/KB-1+-3	6.63	
			<b>Average Concentration</b>	<b>6.70</b>	
	10-Mar-14	77	SRS-SD/KB-1+-1	6.41	
			SRS-SD/KB-1+-2	6.57	
			SRS-SD/KB-1+-3	6.51	
<b>Average Concentration</b>			<b>6.50</b>		
31-Mar-14	98	SRS-SD/KB-1+-1	6.42		
		SRS-SD/KB-1+-2	6.51		
		SRS-SD/KB-1+-3	6.24		
		<b>Average Concentration</b>	<b>6.39</b>		
9-Apr-14	107	SRS-SD/KB-1+-1	6.14		
		SRS-SD/KB-1+-2	6.26		
		SRS-SD/KB-1+-3	6.06		
		<b>Average Concentration</b>	<b>6.15</b>		
22-Apr-14 start	120	SRS-SD/KB-1+-1	6.02		
		SRS-SD/KB-1+-2	6.15		
		SRS-SD/KB-1+-3	5.91		
		<b>Average Concentration</b>	<b>6.03</b>		
Buffered with 0.5 mL of Saturated Sodium Bicarbonate					
22-Apr-14 end	120	SRS-SD/KB-1+-1	6.46		
		SRS-SD/KB-1+-2	6.65		
		SRS-SD/KB-1+-3	6.47		
		<b>Average Concentration</b>	<b>6.53</b>		
7-May-14	135	SRS-SD/KB-1+-1	6.74		
		SRS-SD/KB-1+-2	6.85		
		SRS-SD/KB-1+-3	6.66		
		<b>Average Concentration</b>	<b>6.75</b>		
22-May-14	150	SRS-SD/KB-1+-1	6.36		
		SRS-SD/KB-1+-2	6.44		
		SRS-SD/KB-1+-3	6.32		
		<b>Average Concentration</b>	<b>6.37</b>		
4-Jun-14	163	SRS-SD/KB-1+-1	6.42		
		SRS-SD/KB-1+-2	6.43		
		SRS-SD/KB-1+-3	6.28		
		<b>Average Concentration</b>	<b>6.38</b>		
17-Jun-14	176	SRS-SD/KB-1+-1	6.29		
		SRS-SD/KB-1+-2	6.27		
		SRS-SD/KB-1+-3	6.12		
		<b>Average Concentration</b>	<b>6.23</b>		
10-Jul-14	199	SRS-SD/KB-1+-1	6.46		
		SRS-SD/KB-1+-2	6.40		
		SRS-SD/KB-1+-3	6.26		
		<b>Average Concentration</b>	<b>6.37</b>		

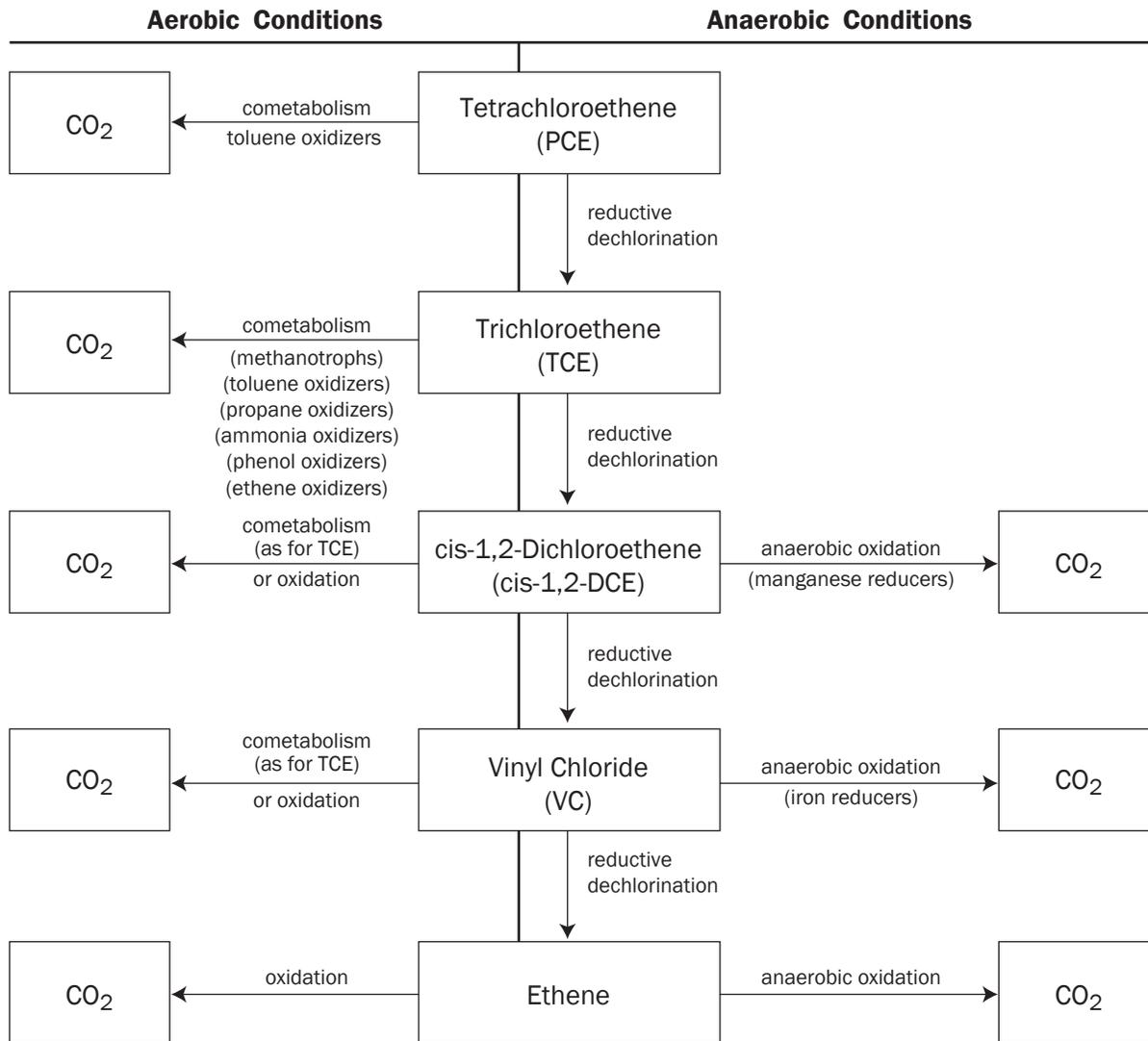
**Notes:**

ANAC - anaerobic active control  
 ANSC - anaerobic sterile control  
 mL - milliliter

**TABLE 6: SUMMARY OF GENE-TRAC® RESULTS**  
 Training Area T-6, McClellan, Anniston, Alabama

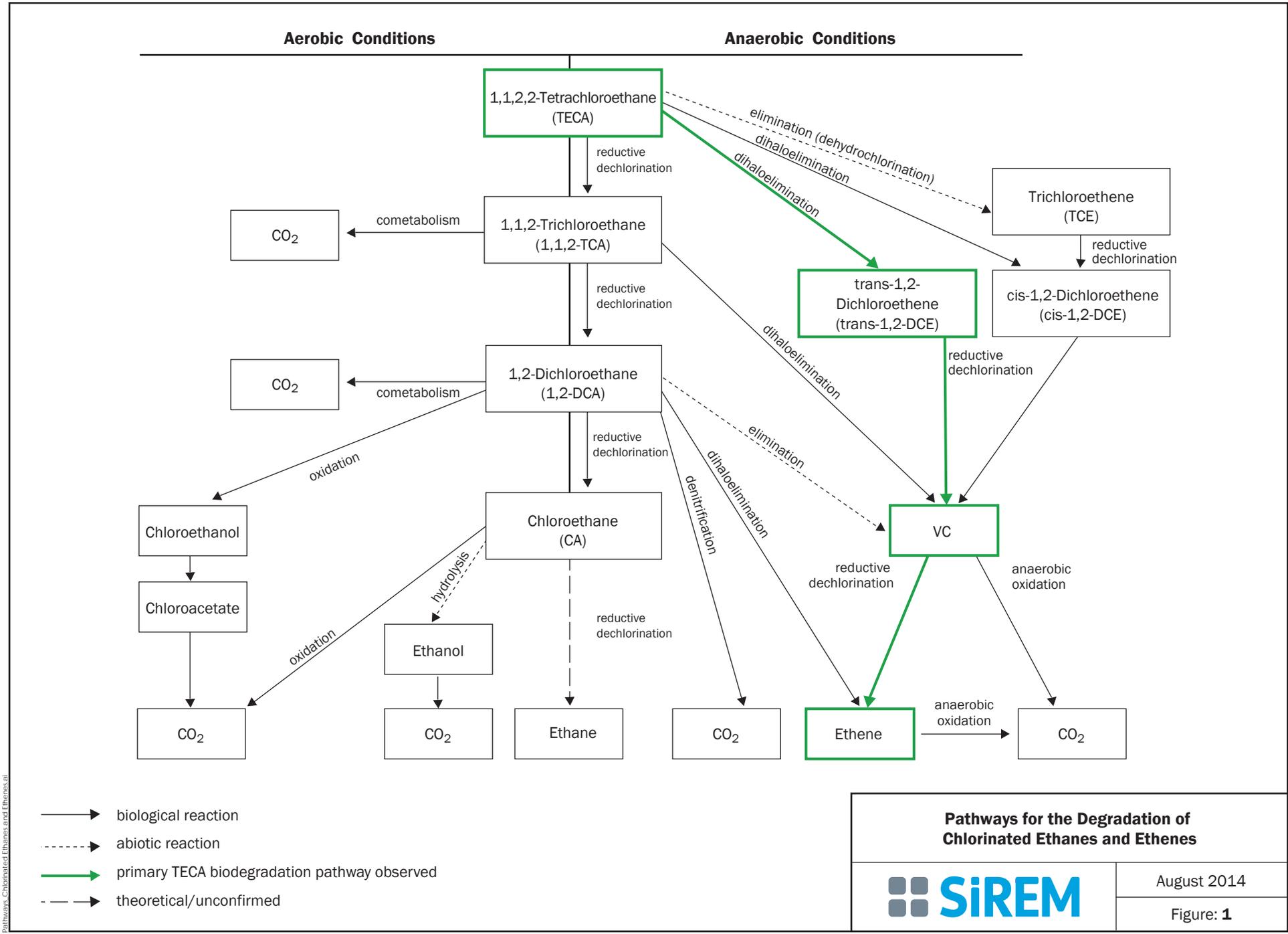
<b>Sample ID</b>	<b>Replicate Sample ID</b>	<b>Sample Date</b>	<b>Day</b>	<b><i>Dehalococcoides</i> Enumeration/Liter</b>	<b><i>Dehalobacter</i> Gene Copies/Liter</b>	<b><i>Dehalogenimonas</i> Gene Copies/Liter</b>
SRS®-SD Amended and KB®-1 Plus Bioaugmented (Replicate 1)	Ft Mc-Bio-10	10-Jul-14	199	3 x 10 <sup>8</sup>	8 x 10 <sup>7</sup>	9 x 10 <sup>6</sup>
SRS®-SD Amended and KB®-1 Plus Bioaugmented (Replicate 3)	Ft Mc-Bio-12	10-Jul-14	199	3 x 10 <sup>6</sup>	3 x 10 <sup>4</sup>	4 x 10 <sup>4</sup>

**FIGURES**



**Pathways for the Degradation of Chlorinated Ethenes**

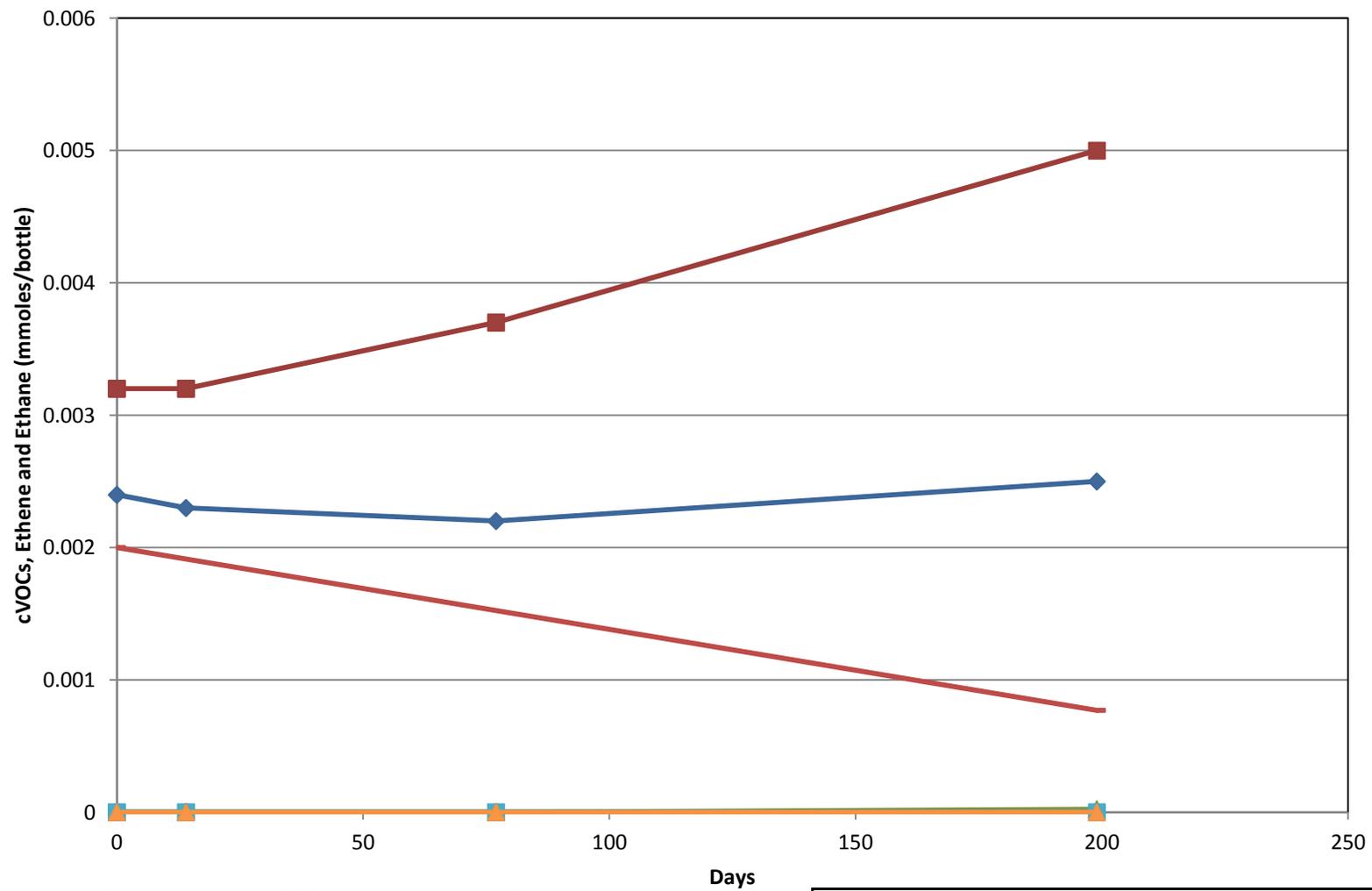
	June 2012
	Figure: 1



**Pathways for the Degradation of Chlorinated Ethanes and Ethenes**

**SiREM** | August 2014

Figure: 1

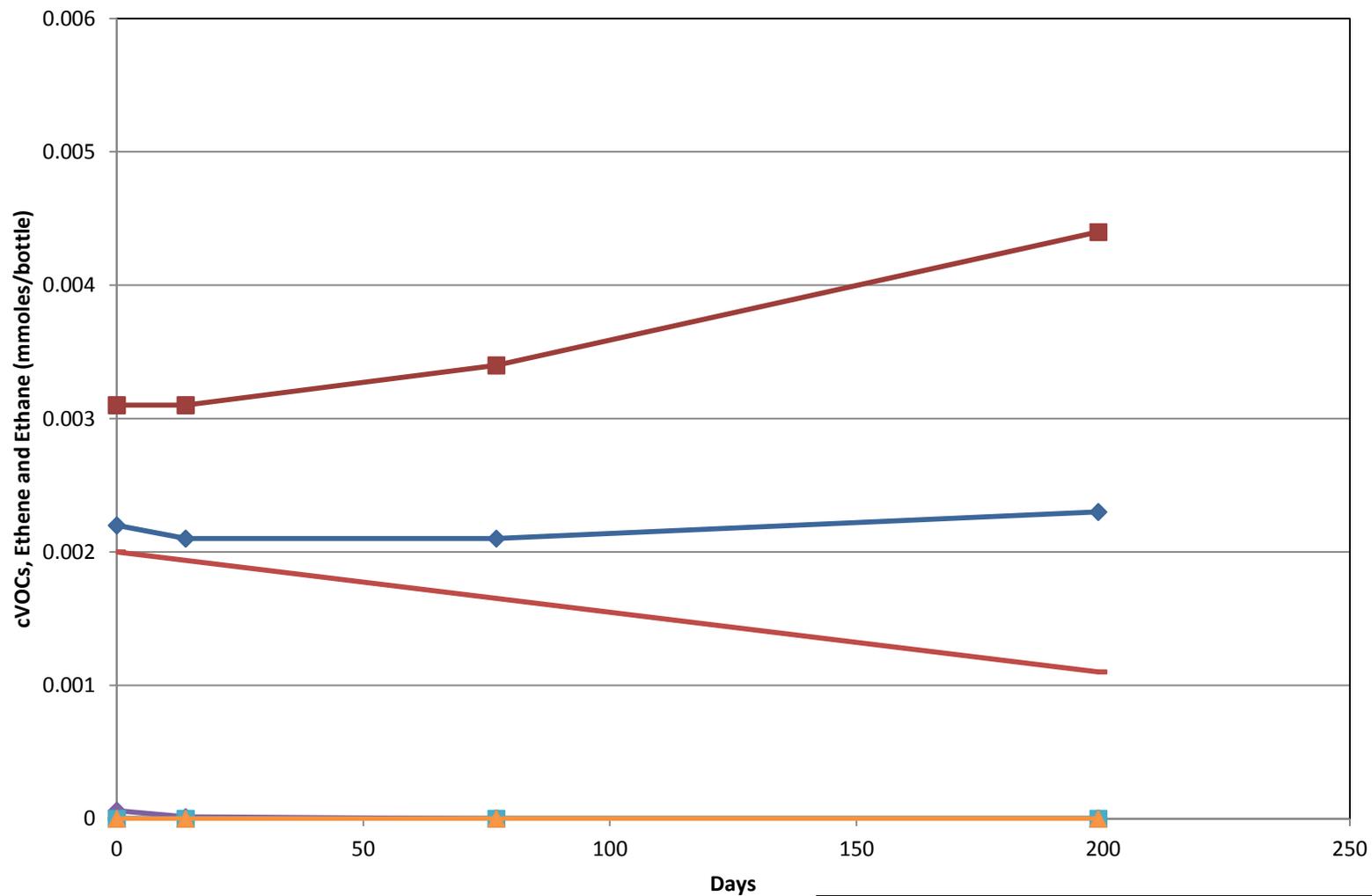


cVOC, Ethene and Ethane Concentration Trends  
 in Anaerobic Sterile Control Microcosms  
 Training Area T-6, McClellan, Anniston, Alabama



August-14

Figure: 3

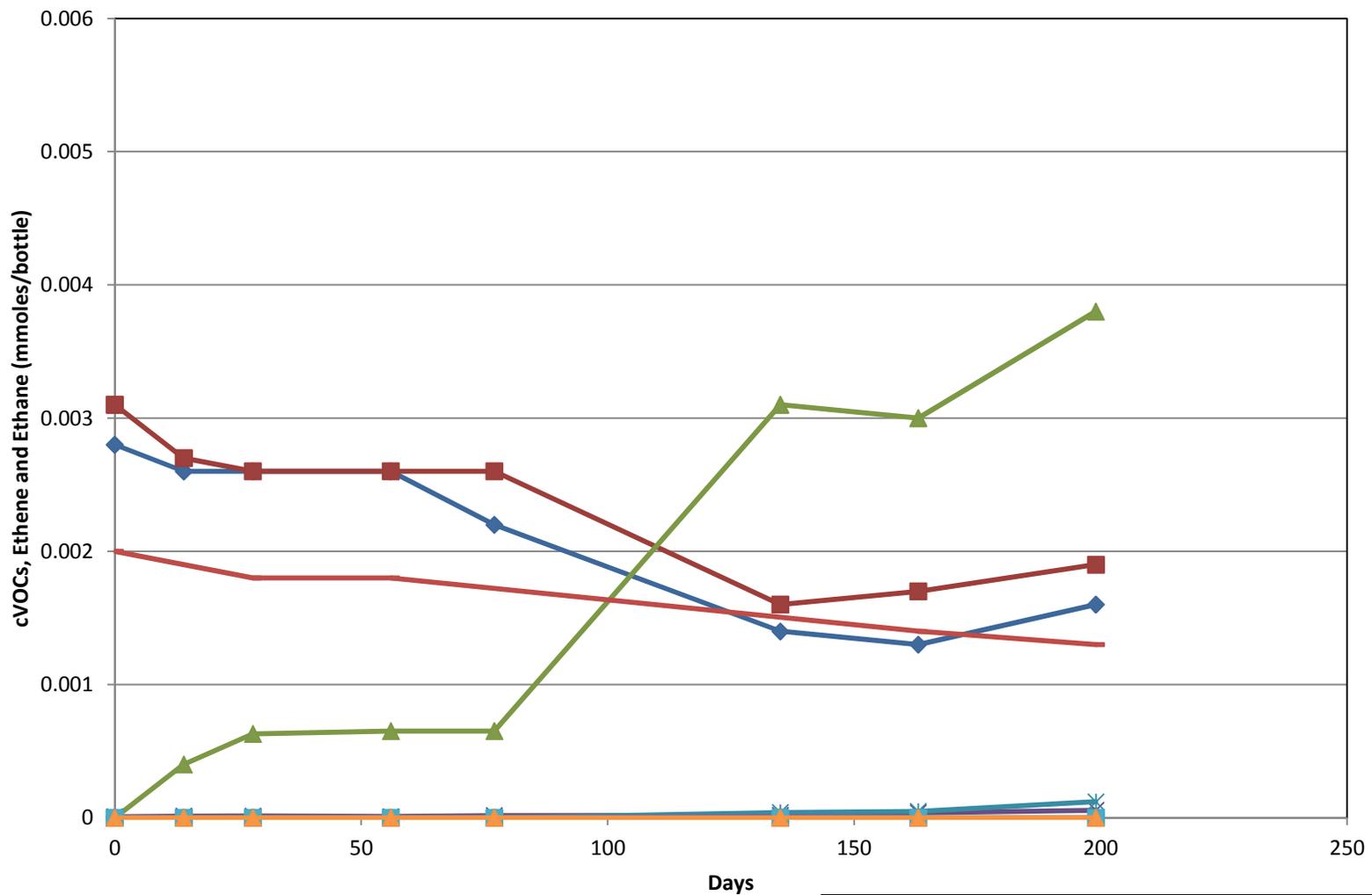


cVOC, Ethene and Ethane Concentration Trends  
 in Anaerobic Active Control Microcosms  
 Training Area T-6, McClellan, Anniston, Alabama

August-14

Figure: 4



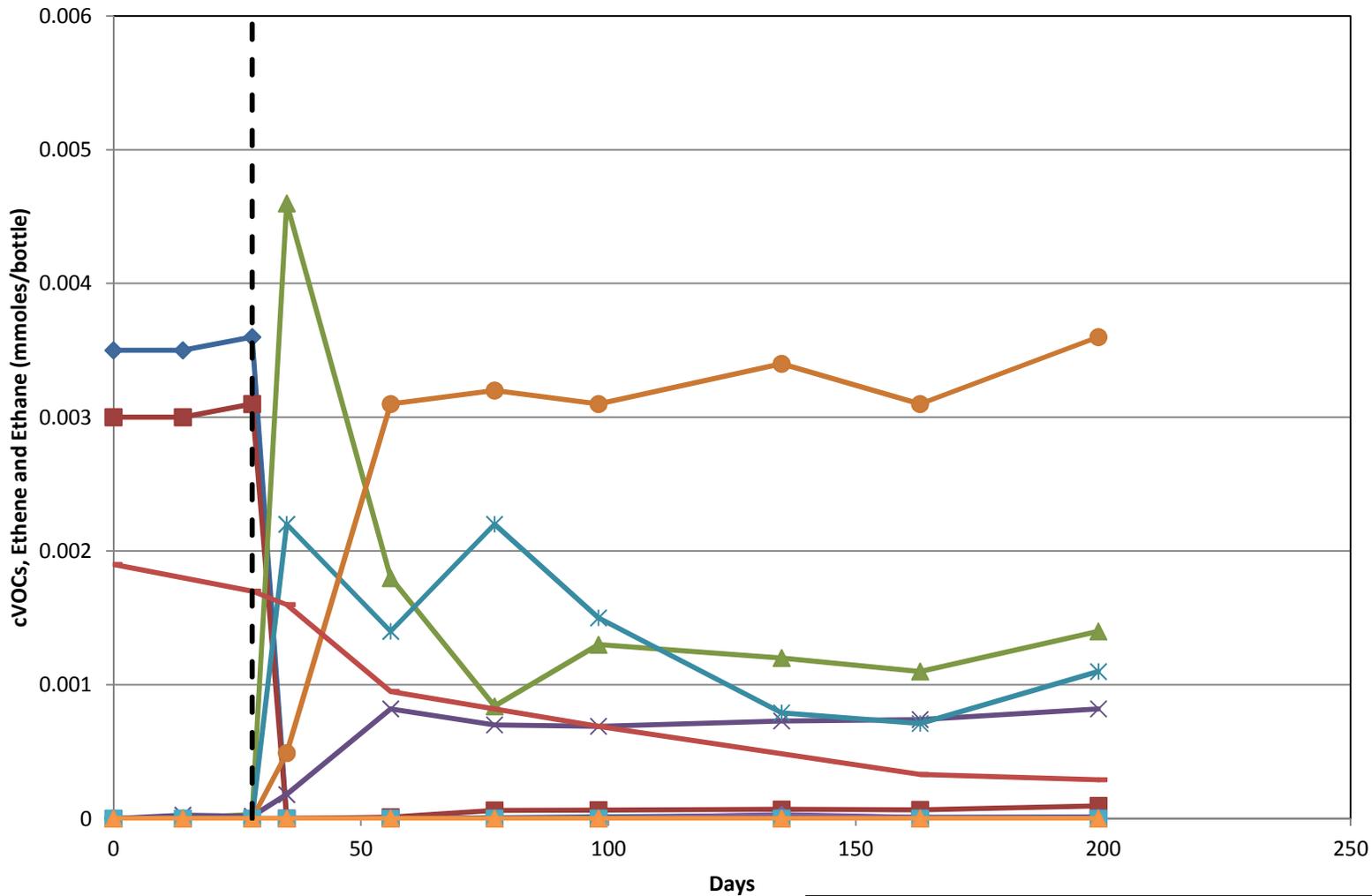


cVOC, Ethene and Ethane Concentration Trends  
in SRS®-SD Amended Microcosms  
Training Area T-6, McClellan, Anniston, Alabama

August-14

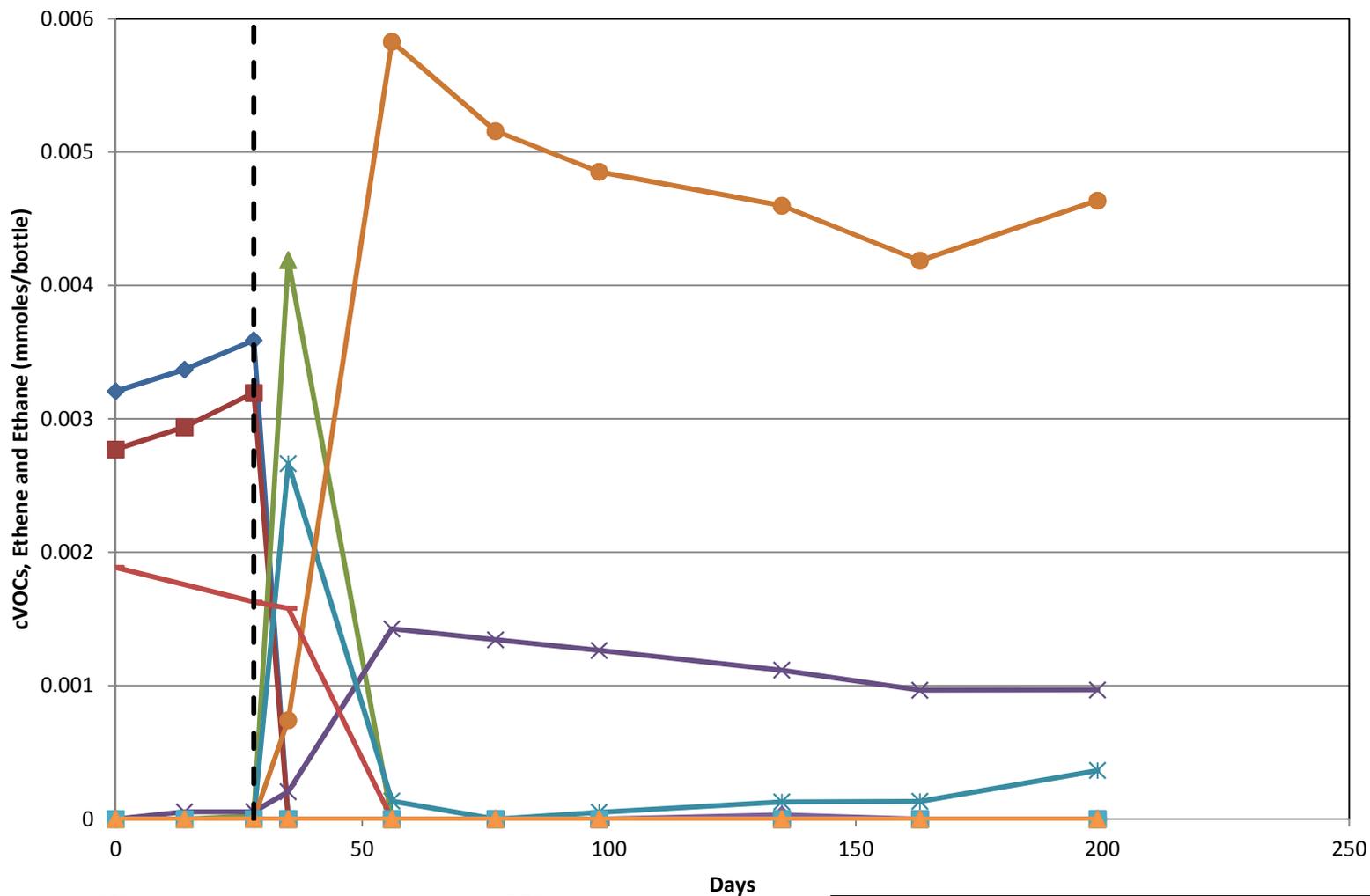
Figure: 5





- ◆ PCE
- ▲ cDCE
- \* VC
- 1,1,2,2-TECA
- ◆ 1,2-DCA
- ▲ Ethene
- TCE
- \* tDCE
- Ethene
- ▲ 1,1,2-TCA
- \* CA
- Bioaugmtented with KB-1 Plus

<p>cVOC, Ethene and Ethane Concentration Trends in  SRS®-SD Amended and KB-1® Plus Bioaugmtented Microcosms  Training Area T-6, McClellan, Anniston, Alabama</p>	
	<p>August-14</p>
<p>Figure: 6</p>	

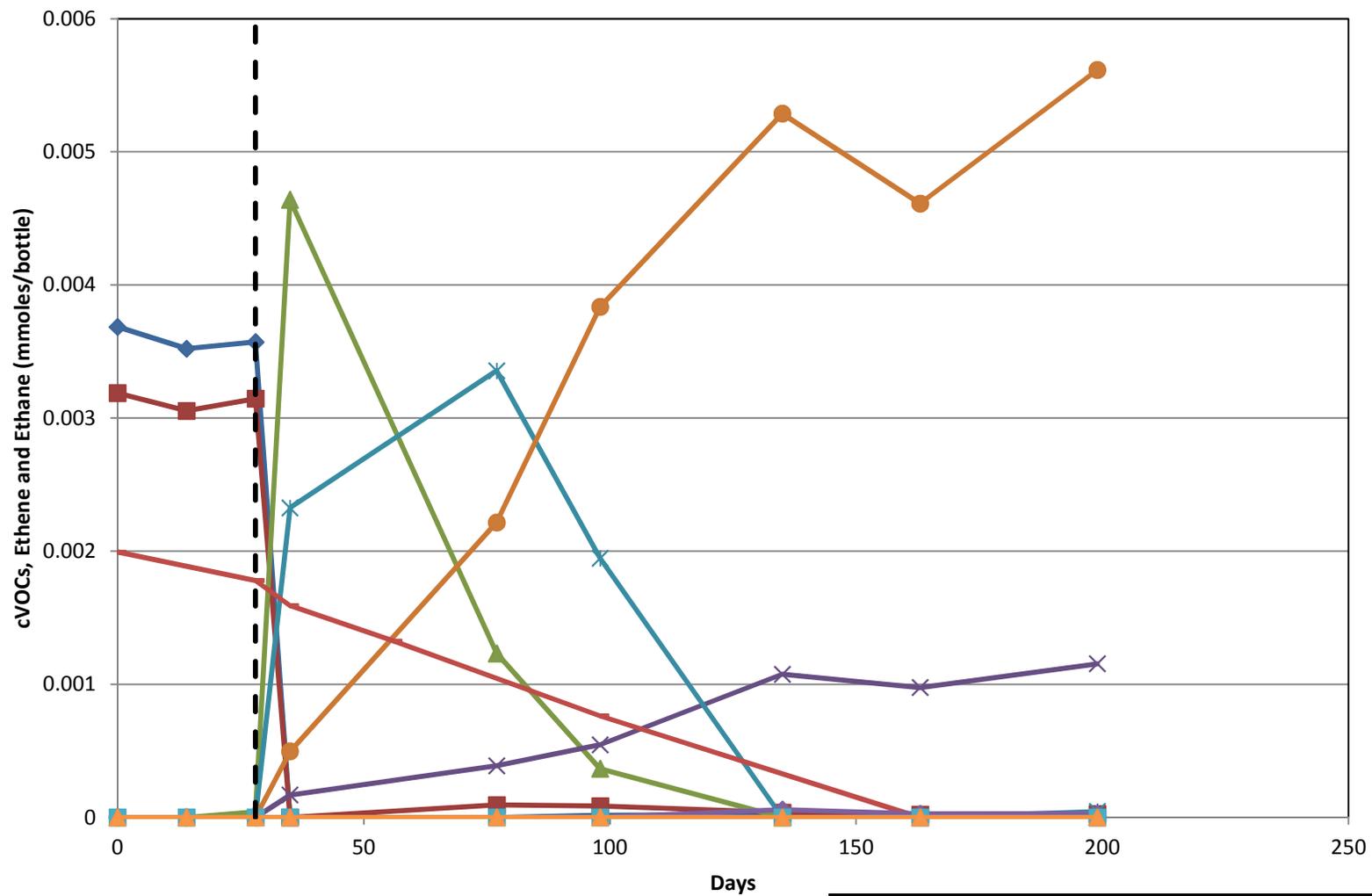


cVOC, Ethene and Ethane Concentration Trends in SRS®-SD Amended and KB-1® Plus Bioaugmented Microcosms (Replicate 1)  
 Training Area T-6, McClellan, Anniston, Alabama

August-14

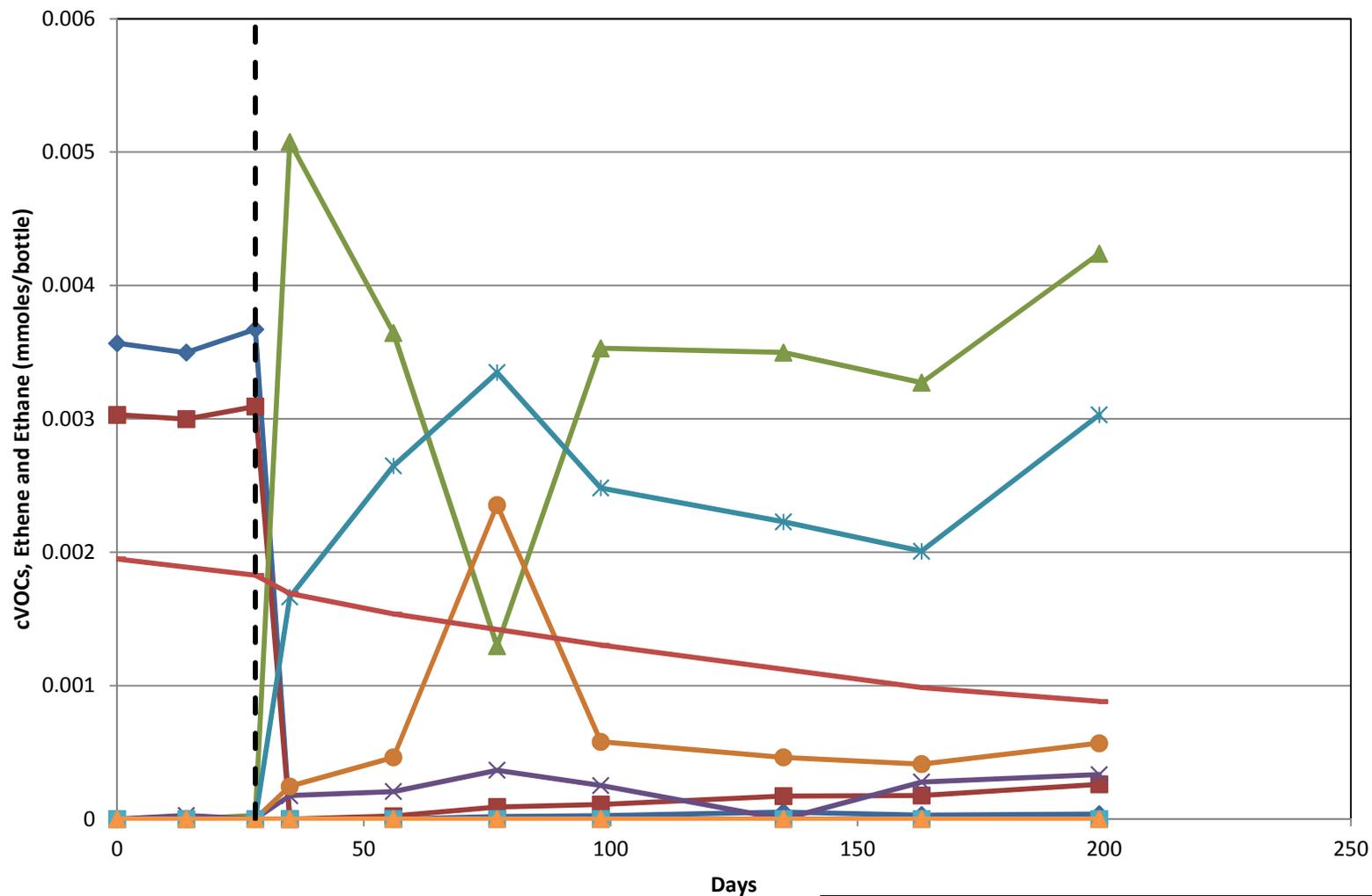
Figure: 6a





cVOC, Ethene and Ethane Concentration Trends in SRS®-SD Amended and KB-1® Plus Bioaugmented Microcosms (Replicate 2)  
 Training Area T-6, McClellan, Anniston, Alabama  
 August-14  
 Figure: 6b





cVOC, Ethene and Ethane Concentration Trends in SRS®-SD Amended and KB-1® Plus Bioaugmented Microcosms (Replicate 3)  
 Training Area T-6, McClellan, Anniston, Alabama  
 August-14  
 Figure: 6c



**APPENDIX A: Chain of Custody Documentation**

# Chain-of-Custody Form

130 Research Lane, Suite 200 Guelph, Ontario, Canada N1G 5G3 Phone (519) 822-2265 or toll free 1-866-251-1747 Fax (519) 822-3151

www.siremlab.com

Lab #  
S-3058

Project Name <b>McClellan Tb</b>		Project # <b>13.094.14-11.1</b>		Analysis															
Project Manager <b>Joseph Owens</b>				Preservative															
Email Address <b>joseph-owens@matrixdesigngroup.com</b>																			
Company <b>Matrix Environmental Services</b>																			
Address <b>283 Rucker Street Anniston, AL 36205 USA</b>																			
Phone # <b>256-613-4256</b>		Fax # <b>256-847-0180</b>																	
Sampler's Signature <b>Joseph Owens</b>		Sampler's Printed Name <b>Joseph Owens</b>																	
Customer Sample ID		Sampling		Matrix		# of Containers		Other Information											
		Date Time																	
<b>CWM-183-MW23 A</b>		<b>12/11/13 1516</b>				<b>1</b>		<b>Microcosm study per Geosyntec - Duane Graves</b>											
<b>CWM-183-MW23 B</b>		<b>12/17/13 1516</b>				<b>2</b>													

Cooler Condition: <b>Sample Receipt</b> <b>good</b>		P.O. #		Billing Information		Turnaround Time Requested		For Lab Use Only			
Cooler Temperature: <b>70C</b>		Bill To:				Normal <input type="checkbox"/>		Proposal #: _____			
Custody Seals: Yes <input checked="" type="checkbox"/> No <input type="checkbox"/>						Rush <input type="checkbox"/>					

Relinquished By: Signature <b>Joseph Owens</b>		Received By: Signature <b>Fed EX</b>		Relinquished By: Signature		Received By: Signature <b>Kela Ashworth</b>		Relinquished By: Signature		Received By: Signature	
Printed Name <b>Joseph Owens</b>		Printed Name <b>Fed Ex</b>		Printed Name		Printed Name <b>Kela Ashworth</b>		Printed Name		Printed Name	
Firm <b>Matrix Environmental</b>		Firm		Firm		Firm <b>SiREM</b>		Firm		Firm	
Date/Time <b>12/17/13</b>		Date/Time <b>12/17/13 1800</b>		Date/Time		Date/Time <b>19 Dec 13 15:30</b>		Date/Time		Date/Time	

## Appendix B: Gene-Trac<sup>®</sup> Reports







**Table 2: Detailed Test Parameters, Gene-Trac Test Reference S-3273**

<b>Customer Sample ID</b>	Ft Mc-Bio-10	Ft Mc-Bio-12
<b>SiREM Dhc Sample ID</b>	DHC-10637	DHC-10638
<b>SiREM Dhb Sample ID</b>	DHB-1167	DHB-1168
<b>SiREM Dhg Sample ID</b>	DHG-0030	DHG-0031
<b>Date Received</b>	10-Jul-14	10-Jul-14
<b>Sample Temperature</b>	N/A	N/A
<b>Volume Used for DNA Extraction</b>	10 ml	10 ml
<b>Filtration Date</b>	10-Jul-14	10-Jul-14
<b>DNA Extraction Date</b>	16-Jul-14	16-Jul-14
<b>DNA Concentration in Sample (extractable)</b>	1332600 ng/L	216975 ng/L
<b>PCR Amplifiable DNA</b>	Detected	Detected
<b>Dhc qPCR Date Analyzed</b>	22-Jul-14	22-Jul-14
<b>Dhb qPCR Date Analyzed</b>	21-Jul-14	21-Jul-14
<b>Dhg qPCR Date Analyzed</b>	24-Jul-14	24-Jul-14
<b>Laboratory Controls (see Tables 3, 4, &amp; 5)</b>	Passed	Passed
<b>Comments</b>	--	--

**Notes:**

Refer to Tables 3, 4, & 5 for detailed results of controls.

°C = degrees Celsius

Dhb = *Dehalobacter*

Dhc = *Dehalococcoides*

Dhg = *Dehalogenimonas*

DNA = Deoxyribonucleic acid

mL = milliliters

ng/L = nanograms per liter

PCR = polymerase chain reaction

qPCR = quantitative PCR

vcrA = vinyl chloride reductase

**Table 3: Gene-Trac Dhc Control Results, Test Reference S-3273**

Laboratory Control	Analysis Date	Control Description	Spiked Dhc 16S rRNA Gene Copies per Liter	Recovered Dhc 16S rRNA Gene Copies per Liter	Comments
<b>Positive Control Low Concentration</b>	22-Jul-14	qPCR with KB1 genomic DNA (CSLD-0770)	$1.3 \times 10^5$	$9.9 \times 10^4$	--
<b>Positive Control High Concentration</b>	22-Jul-14	qPCR with KB1 genomic DNA (CSHD-0770)	$1.5 \times 10^7$	$6.7 \times 10^6$	See Note 1
<b>DNA Extraction Blank</b>	22-Jul-14	DNA extraction sterile water (EB-2224)	0	$2.6 \times 10^3$ U	--
<b>Negative Control</b>	22-Jul-14	Tris Reagent Blank (TBD-0729)	0	$2.6 \times 10^3$ U	--

**Notes:**

Dhc = *Dehalococcoides*

DNA = Deoxyribonucleic acid

qPCR = quantitative PCR

16S rRNA = 16S ribosomal ribonucleic acid

U Not detected, associated value is the quantification limit.

<sup>1</sup>Outside recovery limit guideline of +/- 50%.

**Table 4: Gene-Trac Dhb Control Results, Test Reference S-3273**

Laboratory Control	Analysis Date	Control Description	Spiked Dhb 16S rRNA Gene Copies per Liter	Recovered Dhb 16S rRNA Gene Copies per Liter	Comments
<b>Positive Control Low Concentration</b>	21-Jul-14	qPCR with SC05 genomic DNA (CSLDB-0266)	$1.8 \times 10^6$	$1.6 \times 10^6$	--
<b>Positive Control High Concentration</b>	21-Jul-14	qPCR with SC05 genomic DNA (CSHDB-0266)	$2.8 \times 10^8$	$2.0 \times 10^8$	--
<b>DNA Extraction Blank</b>	21-Jul-14	DNA extraction sterile water (EB-2224)	0	$2.6 \times 10^3$ U	--
<b>Negative Control</b>	21-Jul-14	Tris Reagent Blank (TBDB-0266)	0	$2.6 \times 10^3$ U	--

**Notes:**

qPCR = quantitative PCR

Dhb = *Dehalobacter*

DNA = Deoxyribonucleic acid

16S rRNA = 16S ribosomal ribonucleic acid

U Not detected, associated value is the quantitation limit.

**Table 5: Gene-Trac Dhg Control Results, Test Reference S-3273**

Laboratory Control	Analysis Date	Control Description	Spiked Dhb 16S rRNA Gene Copies per Liter	Recovered Dhb 16S rRNA Gene Copies per Liter	Comments
<b>Positive Control Low Concentration</b>	24-Jul-14	qPCR with <i>Dehalogenimonas</i> plasmid DNA (CSLDG-0016)	$8.8 \times 10^4$	$5.7 \times 10^4$	--
<b>Positive Control High Concentration</b>	24-Jul-14	qPCR with <i>Dehalogenimonas</i> plasmid DNA (CSHDG-0016)	$9.9 \times 10^5$	$8.0 \times 10^5$	--
<b>DNA Extraction Blank</b>	24-Jul-14	DNA extraction sterile water (EB-2224)	0	$2.6 \times 10^3$ U	--
<b>Negative Control</b>	24-Jul-14	Tris Reagent Blank (TBDG-0016)	0	$2.6 \times 10^3$ U	--

**Notes:**

qPCR = quantitative PCR

Dhb = *Dehalogenimonas*

DNA = Deoxyribonucleic acid

16S rRNA = 16S ribosomal ribonucleic acid

U Not detected, associated value is the quantitation limit.

## SiREM Technical Note 1.5:

### Guidelines for Interpretation of Gene-Trac<sup>®</sup> Test Results

This document provides technical background information and guidelines for interpreting the results for the following Gene-Trac<sup>®</sup> assays:

- (1) Gene-Trac<sup>®</sup> Dhc
- (2) Gene-Trac<sup>®</sup> VC
- (3) Gene-Trac<sup>®</sup> Dhb

SiREM Technical Note 1.4 - *Quantitative Gene-Trac<sup>®</sup> Assay Test Procedure and Reporting Overview* provides detailed information on Gene-Trac<sup>®</sup> test procedures and reporting. Explanation of data qualifiers and commonly used notes is provided as Appendix A. Table 1 provides a brief interpretation for some common scenarios, more detailed interpretation information is provided in the following sections.

**Table 1: Common Gene-Trac<sup>®</sup> Test Result Scenarios and Interpretation**

Gene-Trac <sup>®</sup> Dhc ( <i>Dehalococcoides</i> )	Gene-Trac <sup>®</sup> VC ( <i>vcrA</i> )	Gene-Trac <sup>®</sup> Dhb ( <i>Dehalobacter</i> )	Interpretation
>1 x10 <sup>7</sup> /L	>1 x10 <sup>7</sup> /L	Not Analyzed	Complete dechlorination to ethene likely as Dhc high and <i>vcrA</i> high
1 x10 <sup>7</sup> /L	Not Detected	Not Analyzed	VC accumulation possible as <i>vcrA</i> negative
Not Detected	Not Detected	Not Analyzed	Dhc negative/ lack of dechlorination or <i>cis</i> -DCE accumulation likely
Not Analyzed	Not Analyzed	1 x10 <sup>6</sup> /L	Dhb positive, potential for biodegradation of 1,1,1-TCA, 1,2-DCA, carbon tetrachloride and chloroform, PCE and TCE to <i>cis</i> -DCE
Not Analyzed	Not Analyzed	Not Detected	Biodegradation of 1,1,1-TCA, carbon tetrachloride and chloroform not expected as Dhb negative

## **Gene-Trac<sup>®</sup> Dhc -Total *Dehalococcoides* Test**

### **Background:**

Gene-Trac<sup>®</sup> Dhc is a quantitative PCR (qPCR) test for total *Dehalococcoides* (Dhc) microbes that targets Dhc specific sequences of the 16S ribosomal ribonucleic acid (rRNA) gene, a gene commonly used to identify microbes. Dhc are the only known microorganisms capable of complete dechlorination of chloroethenes (i.e., tetrachloroethene, trichloroethene, cis-1,2-dichloroethene [cis-DCE] and vinyl chloride) to non-toxic ethene. Gene-Trac<sup>®</sup> Dhc may also be used to assess the in situ growth of Dhc containing bioaugmentation cultures such as KB-1<sup>®</sup>.

### ***Negative Gene-Trac<sup>®</sup> Dhc Test Results (U qualified)***

A non-detect in the Gene-Trac<sup>®</sup> Dhc assay (e.g., 4,000U) indicates that Dhc were not detected in the sample. The absence of Dhc is frequently associated with a lack of complete dechlorination or incomplete dechlorination of chlorinated ethenes. Where Dhc are absent the accumulation of cis-DCE is commonly observed, particularly after addition of electron donors. Bioaugmentation with Dhc containing cultures, such as KB-1<sup>®</sup>, is commonly used to improve bioremediation performance at sites that lack an indigenous Dhc population.

### ***Positive Gene-Trac<sup>®</sup> Dhc Test Results***

The detection of Dhc has been correlated with the complete biological dechlorination of chlorinated ethenes to ethene at contaminated sites (Hendrickson et al., 2002). A positive Gene-Trac<sup>®</sup> Dhc test indicates that Dhc DNA was detected in the sample and is encouraging for dechlorination of chlorinated ethenes to ethene. Note not all Dhc are capable of conversion of vinyl chloride to ethene; this capability can be determined by the Gene-Trac<sup>®</sup> VC test (see Section 2) which is commonly performed as a follow-on analysis after positive Gene-Trac<sup>®</sup> Dhc tests. In most cases Dhc must be present at sufficient concentrations in order for significant dechlorination to be observed, guidelines for expected impacts at various Dhc concentrations are indicated below.

**Values of 10<sup>4</sup> Dhc gene copies per liter (or lower):** indicates that the sample contains low concentrations of Dhc which may indicate that site conditions are suboptimal for high rates of dechlorination. Increases in Dhc concentrations at the site may be possible if conditions are optimized (e.g., electron donor addition).

**Values of 10<sup>5</sup>-10<sup>6</sup> Dhc gene copies per liter:** indicates the sample contains moderate concentrations of Dhc which may, or may not, be associated with observable dechlorination activity (i.e., detectable ethene).

**Values at or above 10<sup>7</sup> Dhc gene copies per liter:** indicates that the sample contains high concentrations of Dhc that are often associated with high rates of dechlorination (Lu et al., 2006) and the production of ethene.

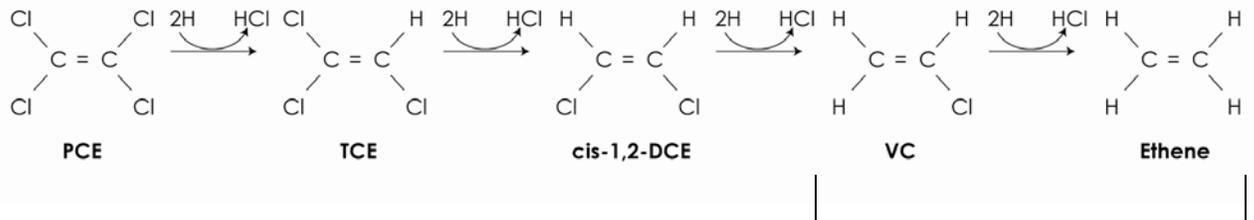
**Values of 10<sup>9</sup> Dhc gene copies per liter** are generally the highest observed for groundwater samples with rare exceptions.

# Gene-Trac<sup>®</sup> VC- Vinyl Chloride Reductase (*vcrA*) Test

## Background

Gene-Trac<sup>®</sup> VC is a qPCR test for the vinyl chloride reductase (*vcrA*) gene that codes for a Dhc enzyme that converts (VC) to ethene, a critical step in reductive dechlorination of chlorinated ethenes. Gene-Trac<sup>®</sup> VC is commonly used where Gene-Trac<sup>®</sup> Dhc test results are positive to confirm that the Dhc detected are capable of complete dechlorination to ethene. #

The vinyl chloride reductase gene (*vcrA*) (Müller et al., 2004) produces an enzyme that is found in many (but not all) Dhc and is reported to be the most common identified VC reductase in the environment (van der Zaan et al., 2010).



**Key activity of vinyl chloride reductase *vcrA* gene/enzyme**

## Interpretation of Gene-Trac<sup>®</sup> VC Results

### Detect in Gene-Trac<sup>®</sup> VC Test

A detect in the Gene-Trac<sup>®</sup> VC test indicates that a Dhc population has the *vcrA* gene and the prospects for complete dechlorination to ethene are good. As a minimal requirement, *vcrA* copies exceeding  $10^5$ /L combined with observed increases over time (i.e., cell growth) are required for robust VC dechlorination (van der Zaan et al., 2010). Also the guidelines for detection of ethene provided under Gene-Trac<sup>®</sup> Dhc are conservative for interpretation of Gene-Trac<sup>®</sup> VC (i.e.,  $> 1 \times 10^7$  gene copies/L indicate a high likelihood of detection of ethene). In one study, more than 90% of samples where *vcrA* enumeration exceeded  $1 \times 10^7$  gene copies/L had detectable ethene (Dennis, 2009). In cases where *vcrA* gene copies are lower the likelihood of detectable ethene decreases.

### Non-Detect in Gene-Trac<sup>®</sup> VC Test (*U* qualified)

A non-detect in the Gene-Trac<sup>®</sup> VC test indicates that *vcrA* gene sequences in the sample are below the detection limit of the assay (typically  $4 \times 10^3$  *vcrA* gene copies/L). This indicates VC accumulation (VC stall) is possible. Note negative Gene-Trac<sup>®</sup> VC test results do not indicate with 100% certainty that a VC-stall will occur as there are other vinyl chloride reductase genes, such as *bvcA* (van der Zaan et al., 2010) that also convert VC to ethene.

## Comparing Gene-Trac<sup>®</sup> VC and Gene-Trac<sup>®</sup> Dhc Test Results

Sites may contain different types of Dhc populations. At some sites the Dhc population is homogenous while other sites have Dhc populations that are mixtures of different types of Dhc. This can lead to differing results for Gene-Trac<sup>®</sup> Dhc and Gene-Trac<sup>®</sup> VC.

In many cases, the numerical results of Gene-Trac<sup>®</sup> VC test are identical to those obtained in the Gene-Trac<sup>®</sup> Dhc test, indicating that the entire Dhc population contains the *vcrA* gene. In other cases, Gene-Trac<sup>®</sup> VC results may differ significantly (i.e., more than an order or magnitude) from the total Dhc for a number of reasons.

Table 3 provides some common scenarios for Gene-Trac<sup>®</sup> VC and Gene-Trac<sup>®</sup> Dhc test results. In general, where Gene-Trac<sup>®</sup> VC results are non-detect, or significantly lower than Gene-Trac<sup>®</sup> Dhc, accumulation of VC is more likely.

**Table 2: Interpretation of Gene-Trac<sup>®</sup> VC in Relation to Gene-Trac<sup>®</sup> Dhc**

Gene-Trac <sup>®</sup> Dhc (16S rRNA gene copies/ L)	Gene-Trac <sup>®</sup> VC ( <i>vcrA</i> gene copies/L)	Results Summary	Interpretation	Potential Site Implications
$2 \times 10^8$ /L	$3 \times 10^8$ /L	Total Dhc and <i>vcrA</i> are ~the same (within 3-fold)	Entire Dhc population has <i>vcrA</i> gene	Potential for complete dechlorination high. VC stall unlikely-sites with <i>vcrA</i> above $1 \times 10^7$ /L typically have detectable ethene
$1 \times 10^8$ /L	Non-detect	Total Dhc high; <i>vcrA</i> non-detect	High concentration of Dhc and entire population lacks the <i>vcrA</i> gene	Likelihood for VC accumulation high as <i>vcrA</i> non-detect
$1 \times 10^8$ /L	$1 \times 10^6$ /L	Total Dhc is significantly higher (100 fold) than <i>vcrA</i>	<i>Dhc</i> population consists of different types, some with the <i>vcrA</i> gene (~1%) and some without (~99%)	VC-accumulation possible; Dhc/ <i>vcrA</i> proportions may change over course of remediation
$1 \times 10^6$ /L	$1 \times 10^8$ /L	<i>vcrA</i> orders of magnitude higher than Dhc	Significantly higher <i>vcrA</i> may indicate the presence of populations of non-Dhc microorganisms with <i>vcrA</i> like genes	Potential for VC-stall likely low

## **Gene-Trac<sup>®</sup> Dhb-Total *Dehalobacter* Test**

Gene-Trac<sup>®</sup> Dhb is a qPCR test targeting the 16S rRNA gene sequences unique to *Dehalobacter* (Dhb). Dhb are implicated in the biodegradation of 1,1,1-trichloroethane (to chloroethane), 1,1,2-trichloroethane and 1,2-dichloroethane to ethene (Grostern and Edwards, 2006) and chloroform (to dichloromethane) (Grostern et al., 2010) as well as incomplete dechlorination of PCE and TCE to cis-DCE (Holliger et al., 1998). Gene-Trac<sup>®</sup> Dhb may also be used as a tool to assess the impact of bioaugmentation with the KB-1<sup>®</sup> Plus cultures which contain high concentrations of Dhb.

### ***Positive Gene-Trac<sup>®</sup> Dhb Test Results (Detects)***

A positive Gene-Trac<sup>®</sup> Dhb indicates that a member of the *Dehalobacter* (Dhb) genus was detected in the sample. The detection of Dhb indicates that some or all of the dechlorination activities attributed to Dhb may be present at the subject site. Increasing concentrations of Dhb are indicative of increased potential to degrade some or all of these compounds.

Note: the Gene-Trac<sup>®</sup> Dhb test will not differentiate the type of Dhb; therefore, observations of the specific biodegradation pathways and end products based on chemical analytical methods in conjunction with Gene-Trac<sup>®</sup> Dhb will increase the interpretability of Gene-Trac<sup>®</sup> Dhb results.

Note: Dhb have been reported to contain multiple copies (up to 4 per cell) of the 16S rRNA gene (Grostern and Edwards, 2008). This means that, unlike Dhc, there is not a 1:1 ratio between the 16S rRNA gene copy and the number of Dhb cells in a sample. Calculating the number of Dhb cells requires dividing the Gene-Trac<sup>®</sup> Dhb test result by the 16S rRNA gene copy number (often 3-4 copies/cell).

### ***Non-detect Gene-Trac<sup>®</sup> Dhb Results (U qualified)***

In cases where Gene-Trac<sup>®</sup> Dhb is not detected (e.g., 4,000U) this indicates that *Dehalobacter* species were not identified in the sample and that anaerobic reductive dechlorination of 1,1,1-TCA, 1,1,2-TCA, 1,2-DCA or chloroform, which are dechlorinated by *Dehalobacter*, may not be observed. This activity can be introduced at sites through the addition of bioaugmentation cultures containing *Dehalobacter* such as KB-1<sup>®</sup> Plus.

## **Key Elements of Gene-Trac® Data**

Gene-Trac® test results include two key values (a) Target Gene Enumeration, an enumeration of target gene sequence by quantitative PCR (e.g. “Dhc Enumeration” “Dhb 16S Gene Copies” or “*vcrA* gene copies”) and (b) Target gene percent (e.g. “Percent Dhc”), an estimated percentage of the microbial population comprised by microbes harboring the target gene and other microbes present in sample. Further explanation of these values is provided below.

### **a) Target Gene Enumeration**

This value is the concentration of Dhc or Dhb 16S rRNA or *vcrA* gene copies detected in the sample. Results may be reported as either gene copies per liter (for groundwater) or per gram (for soil). In general, the greater the number of gene copies in a sample the greater the likelihood of related dechlorination activity. Dhc 16S gene copies are typically equivalent to the number of Dhc as they have 1 gene copy per cell this is not necessarily true for Dhb or *vcrA* which have the potential be present in multiple gene copies per cell. Guidelines for relating target gene presence and concentration to observable dechlorination activity for groundwater samples are provided below in previous sections.

### **b) Target Gene Percent (%Dhc, %Dhb, %*vcrA*)**

This value estimates the percentage of the target gene (e.g., %Dhc) relative to other microorganisms in the sample based on the formulas/assumptions presented below. For example, %Dhc is a measure of the predominance of Dhc and, in general, the higher this percentage the better.

$$\%Dhc = \frac{\text{Number Dhc}}{\text{Number Dhc} + \text{Number other Bacteria}}$$

Where:

$$\text{Number other Bacteria} = \frac{\text{Total DNA in sample (ng)} - \text{DNA attributed to Dhc (ng)}}{4.0 \times 10^{-6} \text{ ng DNA per bacterial cell}}$$

\*Paul and Clark, (1996).

Percent Dhc (and % *vcrA*) values can range from very low fractions of percentages, in samples with low numbers of Dhc and a high number of other bacteria (incompletely colonized by Dhc), to greater than 50% in Dhc enriched locations (highly colonized by Dhc).

In addition to determining the predominance of the target gene target gene percent is also useful for interpretation of Dhc counts from different sampling locations, or the same location over time. For example, the %Dhc value can be used to correct Dhc counts where samples are biased due to non-representative sampling. Example 1 illustrates a hypothetical scenario where the %Dhc value improved data interpretation.

**Example 1, use of %Dhc to interpret enumeration data**

Table 2 presents results from MW-1 sampled in April, May and June. Based on the Dhc enumeration alone one would conclude that the concentration of Dhc held steady between April and May; however, the %Dhc indicates the proportion of Dhc actually increased from April to May and the unchanged count in May could be a case of low biomass recovery during sampling or other losses such as sample degradation in transit. The higher raw count and the higher percentage of Dhc in June confirm the trend of increasing Dhc concentrations over time.

**Table 3: Use of % Dhc\* Value to Diagnose Sampling Bias**

Sample	Dhc Enumeration	%Dhc	Interpretation Based on %Dhc
MW-1, April	$1.0 \times 10^5$ /Liter	0.1%	Dhc is a low proportion of total microbial population
MW-1, May	$1.0 \times 10^5$ /Liter	1%	Dhc proportion increased 10-fold from April. Dhc enumeration was unchanged possibly due to low biomass recovery from monitoring well, non-biased sample would be $[(1.0/0.1) \times 1.0 \times 10^5] = 1.0 \times 10^6$ /Liter
MW-1, June	$1.0 \times 10^7$ /Liter	10%	Dhc has increased 100-fold from April and confirms May sample was likely low biased

*\*Note: the above approach is also applicable to the “%vcrA” and “%Dhb” values provided on their respective test certificates*

## References

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## Appendix A: Data Qualifiers

## **Data Qualification**

Data qualifiers and notes are used to clarify Gene-Trac® test results. Additional explanation beyond that provided on the test certificate is provided below.

**“U” Not detected, associated value is the quantitation limit.** Indicates that the target gene (microbe) was not detected in the sample above the quantitation limit of the assay. Note the quantitation limit value can change between samples as the volume filtered can vary; thus, a sample in which 100 ml was tested would have a 5-fold higher quantification limit compared with a sample in which 500 ml was tested.

**“J” The associated value is an estimated quantity between the method detection limit and quantitation limit.** Indicates that the target gene was conclusively detected but the concentration is below the quantitation limit where it cannot be accurately quantified.

**“I” Sample inhibited the test reaction.** This means universal primers were incapable of amplifying DNA from this sample. The inability to amplify with universal primers suggests that the sample may be imparting matrix interference. Matrix interference is commonly attributed to humic compounds, polyphenols and metals. Non-detects with an “I” qualifier are more likely to be false negative.

**“B” Analyte was also detected in the method blank.** Indicates that DNA was detected in a method blank or negative control; detectable contamination of the blanks with microbes or DNA containing the gene of interest is not uncommon as the test reaction is extremely sensitive. In most cases, blank contamination is at a very low level relative to test results (often orders of magnitude lower). In these cases, blank contamination is not relevant to interpretation of test results. The potential of test samples being contaminated (i.e. false positives) should be considered in cases where blank results are within 1 order of magnitude of test results.

## APPENDIX C: Henry's Law Calculation

The following Henry's Law calculation was used to convert aqueous concentrations (Table 2) to total mmoles of each analyte per microcosm bottle (Figures 2 to 5):

$$\text{Total mmoles} = \frac{C_{\text{liq}} \times (V_{\text{liq}} + H \times V_{\text{gas}})}{\text{Molecular Weight (mg/mmol)}}$$

Where

$C_{\text{liq}}$  = liquid concentration (mg/L)

$V_{\text{liq}}$  = liquid volume (0.225 L) per bottle

$V_{\text{gas}}$  = headspace volume (0.025 L) per bottle

H = Henry's Law constant (dimensionless)

The Henry's Law constants used are summarized in the table below.

Analyte	Henry's Law Constant <sup>a</sup> (dimensionless)
Tetrachloroethene	0.60
Trichloroethene	0.42
cis-1,2-dichloroethene	0.18
Trans-1,2-dichloroethene	0.39
Vinyl chloride	1.08
Ethene	8.76
1,1,2-trichloroethane	0.04
1,1,2,2-tetrachloroethane	0.019
1,2-dichloroethane	0.05
Chloroethane	0.49
Ethane	20.4
Methane	27.2

<sup>a</sup> Source: Montgomery, J.H. 2000. *Groundwater Chemicals Desk Reference, Third Edition*. CRC Press LLC, Boca Raton, FL.

## **Appendix B**

Boring Logs / Well Completion Forms

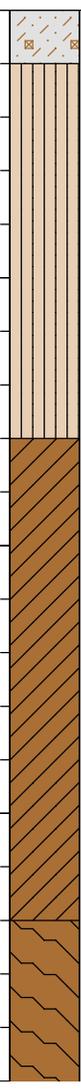
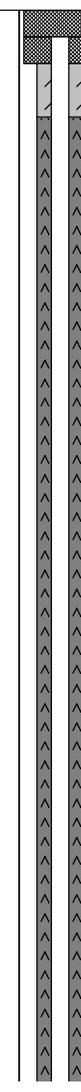
Survey Data

## BORING AND WELL LOG LEGEND

LITHOLOGY	WATER LEVEL	WELL/BORING COMPLETION	COLLECT				DESCRIPTION	MEASURE	
			Sample Type	Date & Time	Blow Counts	Recovery (%)		PID (ppm)	Lab Sample
						ASPHALT CONCRETE FILL Well graded GRAVEL (GW) Poorly graded GRAVEL (GP) Silty GRAVEL (GM) Clayey GRAVEL (GC) Well graded SAND (SW) Poorly graded SAND (SP) Silty SAND (SM) Clayey SAND (SC) SILT (ML) Lean CLAY (CL) Organic SOIL (OL) Organic SOIL (OH) Elastic SILT (MH) Fat CLAY (CH) PEAT (PT) BEDROCK Volume Descriptors: Trace = <5% Few = 5-10% Little = 15-25% Some = >=30%  Water Level During Drilling Water Level In Completed Well  Cap Riser Screen Backfill Filter Pack Bentonite Grout Bentonite Seal Cement  GR EN SS SH CO DP  Grab Encore Split Spoon Shelby Tube Core Barrel Direct Push Sample Analysis			

NOTES:

Hole Clear. Date: <b>12/01/2014</b>	Boring Depth (ft): <b>42</b>	Well Depth (ft): <b>42</b>
Hole Clear. Company: <b>Underground Det.</b>	Boring Diameter (in): <b>8</b>	Well Diameter (in): <b>2</b>
Hole Clear. Method: <b>GPR/ER</b>	Sampling Method(s): <b>Core Barrel</b>	Screen Slot in: <b>0.010</b>
Drilling Start Date: <b>12/07/2014</b>	Logged By: <b>Joseph Ivanowski</b>	Riser Material: <b>Sch 40 PVC</b>
Drilling End Date: <b>12/07/2014</b>	Boring Location (X): <b>670242.55</b>	Screen Material: <b>Sch 40 PVC Slotted</b>
Drilling Company: <b>Cascade Drilling</b>	Boring Location (Y): <b>1166390.92</b>	Seal Material(s): <b>Bentonite Grout/Chips</b>
Drilling Method: <b>Sonic</b>	Casing Elevation (Z): <b>810.53</b>	Filter Pack: <b>20/40 Mesh Silica</b>

DEPTH (ft.)	LITHOLOGY	WATER LEVEL	WELL/BORING COMPLETION	COLLECT				SOIL/ROCK VISUAL DESCRIPTION	MEASURE		DEPTH (ft.)
				Sample Type	Date & Time	Blow Counts	Recovery (%)		PID (ppm)	Lab Sample	
0								(0') Clayey GRAVEL with sand (GC); coarse grained gravel, little fine-medium sand, few silt, little clay, dense, dry, light reddish-brown, gravel surface; fill material.	0.0		0
								(1') SILT (ML); trace fine-coarse gravel, little fine sand, little clay, stiff, dry, light reddish-brown.	0.0		
5								Pre-cleared using hand auger to 5' bgs.	0.0		5
10								(8') Lean CLAY (CL); little fine sand, little silt, very stiff, dry, light reddish-brown.	0.0		10
15								(17') Fat CLAY (CH); trace fine sand, little silt, very stiff, dry, light yellowish-brown, iron-oxide streaks throughout.	0.0		15
20									0.0		20

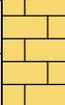
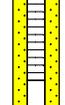
NOTES:

Hole Clear. Date: <b>12/01/2014</b>	Boring Depth (ft): <b>42</b>	Well Depth (ft): <b>42</b>
Hole Clear. Company: <b>Underground Det.</b>	Boring Diameter (in): <b>8</b>	Well Diameter (in): <b>2</b>
Hole Clear. Method: <b>GPR/ER</b>	Sampling Method(s): <b>Core Barrel</b>	Screen Slot in: <b>0.010</b>
Drilling Start Date: <b>12/07/2014</b>	Logged By: <b>Joseph Ivanowski</b>	Riser Material: <b>Sch 40 PVC</b>
Drilling End Date: <b>12/07/2014</b>	Boring Location (X): <b>670242.55</b>	Screen Material: <b>Sch 40 PVC Slotted</b>
Drilling Company: <b>Cascade Drilling</b>	Boring Location (Y): <b>1166390.92</b>	Seal Material(s): <b>Bentonite Grout/Chips</b>
Drilling Method: <b>Sonic</b>	Casing Elevation (Z): <b>810.53</b>	Filter Pack: <b>20/40 Mesh Silica</b>

DEPTH (ft.)	LITHOLOGY	WATER LEVEL	WELL/BORING COMPLETION	COLLECT				SOIL/ROCK VISUAL DESCRIPTION	MEASURE		DEPTH (ft.)
				Sample Type	Date & Time	Blow Counts	Recovery (%)		PID (ppm)	Lab Sample	
20									0.0		20
25								(24') Lean CLAY (CL); few fine-coarse gravel, few fine sand, few silt, soft, wet, dark yellowish-brown, some iron-oxide inclusions; soft white clay inclusions.	0.0		25
30									0.0		30
35								(31') BEDROCK: Dark bluish-gray limestone; very dense; white calcite-healed fractures.	0.0		35
40								(as above, some greenish brown discoloration at suspected fracture surfaces)	0.0		40

NOTES:

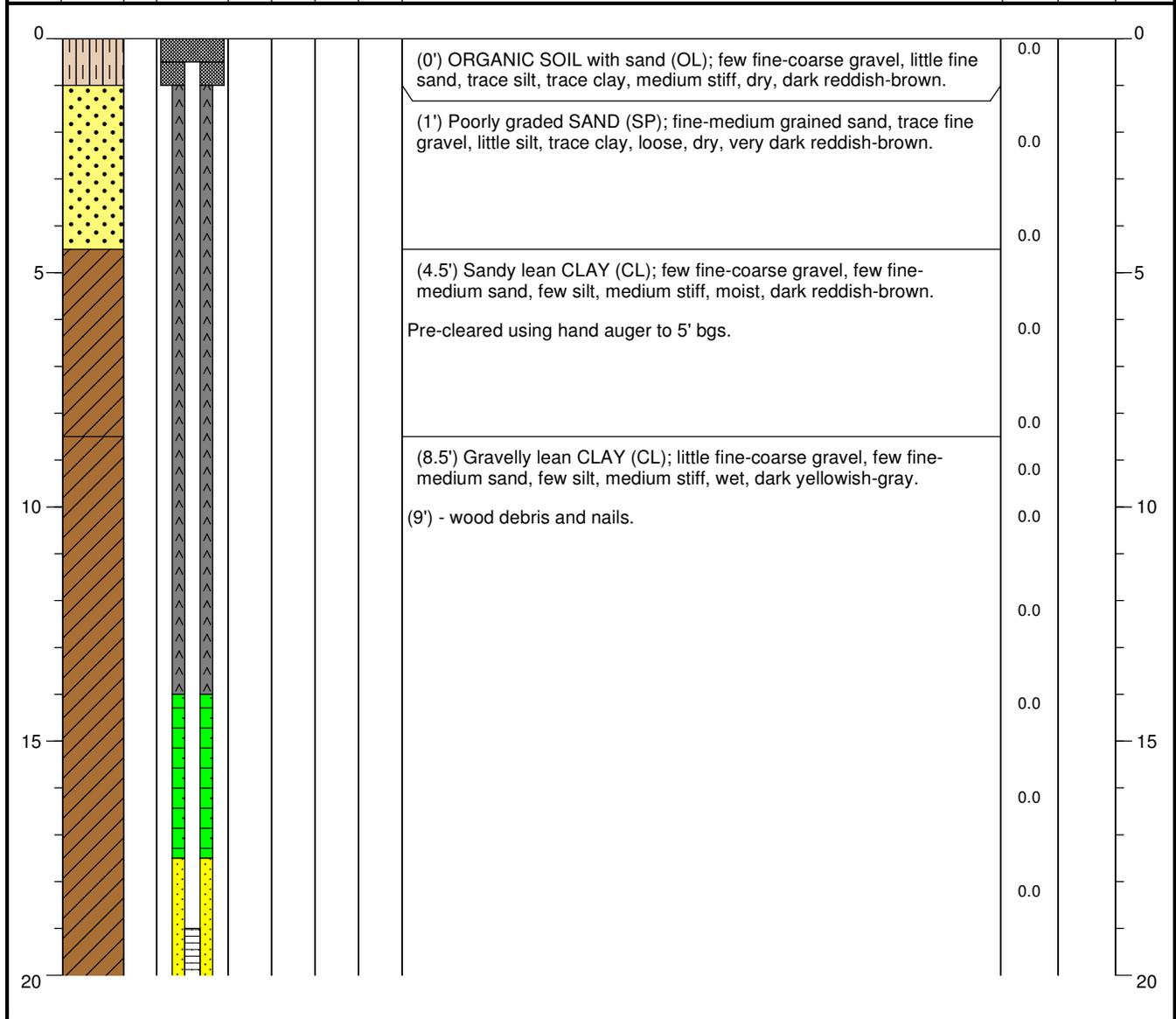
Hole Clear. Date: <b>12/01/2014</b>	Boring Depth (ft): <b>42</b>	Well Depth (ft): <b>42</b>
Hole Clear. Company: <b>Underground Det.</b>	Boring Diameter (in): <b>8</b>	Well Diameter (in): <b>2</b>
Hole Clear. Method: <b>GPR/ER</b>	Sampling Method(s): <b>Core Barrel</b>	Screen Slot in: <b>0.010</b>
Drilling Start Date: <b>12/07/2014</b>	Logged By: <b>Joseph Ivanowski</b>	Riser Material: <b>Sch 40 PVC</b>
Drilling End Date: <b>12/07/2014</b>	Boring Location (X): <b>670242.55</b>	Screen Material: <b>Sch 40 PVC Slotted</b>
Drilling Company: <b>Cascade Drilling</b>	Boring Location (Y): <b>1166390.92</b>	Seal Material(s): <b>Bentonite Grout/Chips</b>
Drilling Method: <b>Sonic</b>	Casing Elevation (Z): <b>810.53</b>	Filter Pack: <b>20/40 Mesh Silica</b>

DEPTH (ft.)	LITHOLOGY	WATER LEVEL	WELL/BORING COMPLETION	COLLECT				SOIL/ROCK VISUAL DESCRIPTION	MEASURE		DEPTH (ft.)
				Sample Type	Date & Time	Blow Counts	Recovery (%)		PID (ppm)	Lab Sample	
40								End of Boring	0.0		40
45											45

NOTES:

Hole Clear. Date: <b>12/01/2014</b>	Boring Depth (ft): <b>29.5</b>	Well Depth (ft): <b>29.5</b>
Hole Clear. Company: <b>Underground Det.</b>	Boring Diameter (in): <b>8</b>	Well Diameter (in): <b>2</b>
Hole Clear. Method: <b>GPR/ER</b>	Sampling Method(s): <b>Core Barrel</b>	Screen Slot (in): <b>0.010</b>
Drilling Start Date: <b>12/05/2014</b>	Logged By: <b>Joseph Ivanowski</b>	Riser Material: <b>Sch 40 PVC</b>
Drilling End Date: <b>12/06/2014</b>	Boring Location (X): <b>670397.94</b>	Screen Material: <b>Sch 40 PVC Slotted</b>
Drilling Company: <b>Cascade Drilling</b>	Boring Location (Y): <b>1166401.82</b>	Seal Material(s): <b>Bentonite Grout/Chips</b>
Drilling Method: <b>Sonic</b>	Casing Elevation (Z): <b>800.58</b>	Filter Pack: <b>20/40 Mesh Silica</b>

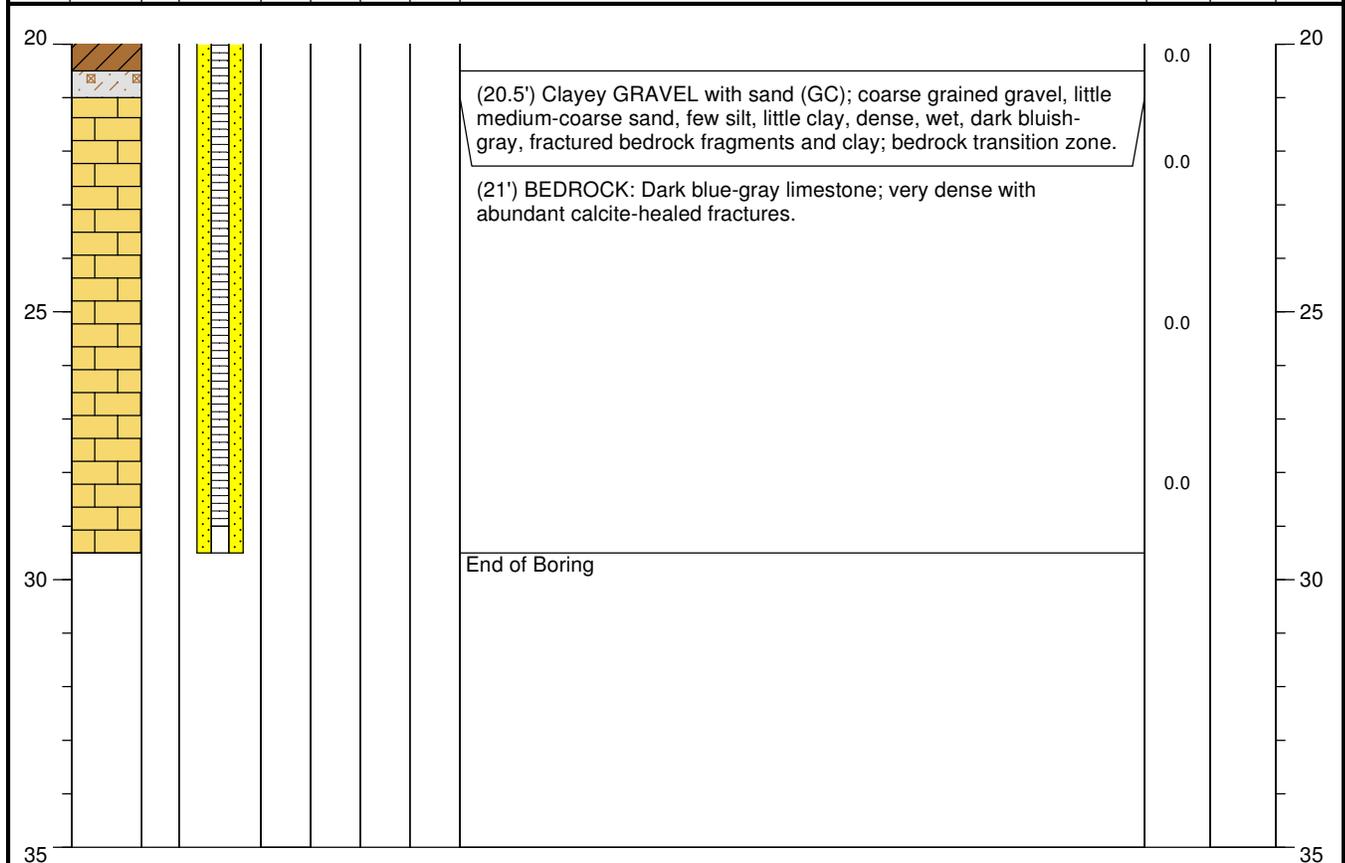
DEPTH (ft.)	LITHOLOGY	WATER LEVEL	WELL/BORING COMPLETION	COLLECT				SOIL/ROCK VISUAL DESCRIPTION	MEASURE		DEPTH (ft.)
				Sample Type	Date & Time	Blow Counts	Recovery (%)		PID (ppm)	Lab Sample	



NOTES:

Hole Clear. Date: <b>12/01/2014</b>	Boring Depth (ft): <b>29.5</b>	Well Depth (ft): <b>29.5</b>
Hole Clear. Company: <b>Underground Det.</b>	Boring Diameter (in): <b>8</b>	Well Diameter (in): <b>2</b>
Hole Clear. Method: <b>GPR/ER</b>	Sampling Method(s): <b>Core Barrel</b>	Screen Slot (in): <b>0.010</b>
Drilling Start Date: <b>12/05/2014</b>	Logged By: <b>Joseph Ivanowski</b>	Riser Material: <b>Sch 40 PVC</b>
Drilling End Date: <b>12/06/2014</b>	Boring Location (X): <b>670397.94</b>	Screen Material: <b>Sch 40 PVC Slotted</b>
Drilling Company: <b>Cascade Drilling</b>	Boring Location (Y): <b>1166401.82</b>	Seal Material(s): <b>Bentonite Grout/Chips</b>
Drilling Method: <b>Sonic</b>	Casing Elevation (Z): <b>800.58</b>	Filter Pack: <b>20/40 Mesh Silica</b>

DEPTH (ft.)	LITHOLOGY	WATER LEVEL	WELL/BORING COMPLETION	COLLECT				SOIL/ROCK VISUAL DESCRIPTION	MEASURE		DEPTH (ft.)
				Sample Type	Date & Time	Blow Counts	Recovery (%)		PID (ppm)	Lab Sample	

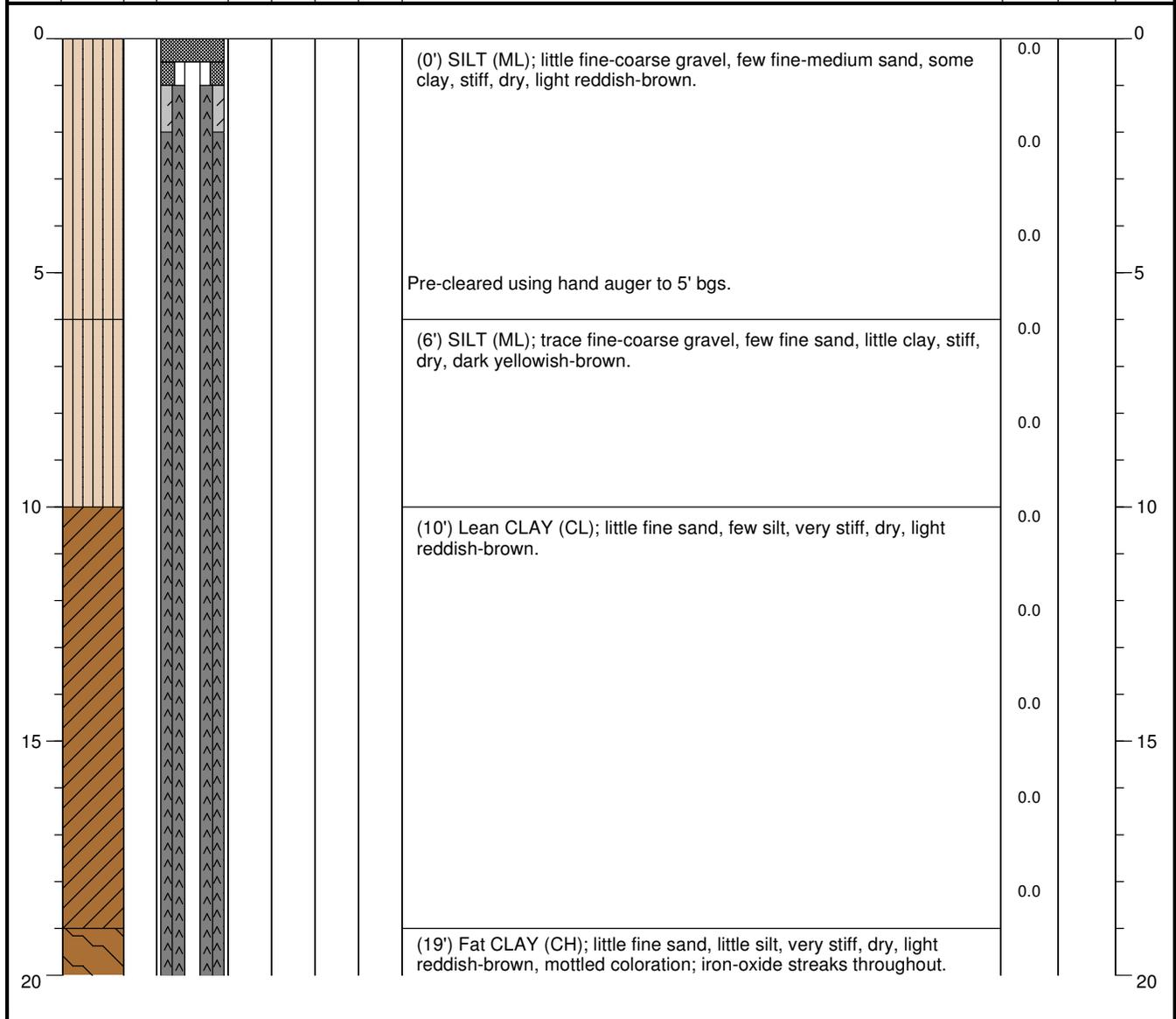


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NOTES:

Hole Clear. Date: <b>12/01/2014</b>	Boring Depth (ft): <b>57</b>	Well Depth (ft): <b>57</b>
Hole Clear. Company: <b>Underground Det.</b>	Boring Diameter (in): <b>12(boring), 8(casing)</b>	Well Diameter (in): <b>2</b>
Hole Clear. Method: <b>GPR/ER</b>	Sampling Method(s): <b>Core Barrel</b>	Screen Slot (in): <b>0.010</b>
Drilling Start Date: <b>12/04/2014</b>	Logged By: <b>Joseph Ivanowski</b>	Riser Material: <b>Sch 40 PVC</b>
Drilling End Date: <b>12/08/2014</b>	Boring Location (X): <b>670247.76</b>	Screen Material: <b>Sch 40 PVC Slotted</b>
Drilling Company: <b>Cascade Drilling</b>	Boring Location (Y): <b>1166395.49</b>	Seal Material(s): <b>Bentonite Grout/Chips</b>
Drilling Method: <b>Sonic</b>	Casing Elevation (Z): <b>809.78</b>	Filter Pack: <b>20/40 Mesh Silica</b>

DEPTH (ft.)	LITHOLOGY	WATER LEVEL	WELL/BORING COMPLETION	COLLECT				SOIL/ROCK VISUAL DESCRIPTION	MEASURE		DEPTH (ft.)
				Sample Type	Date & Time	Blow Counts	Recovery (%)		PID (ppm)	Lab Sample	



NOTES:

Hole Clear. Date: <b>12/01/2014</b>	Boring Depth (ft): <b>57</b>	Well Depth (ft): <b>57</b>
Hole Clear. Company: <b>Underground Det.</b>	Boring Diameter (in): <b>12(boring), 8(casing)</b>	Well Diameter (in): <b>2</b>
Hole Clear. Method: <b>GPR/ER</b>	Sampling Method(s): <b>Core Barrel</b>	Screen Slot (in): <b>0.010</b>
Drilling Start Date: <b>12/04/2014</b>	Logged By: <b>Joseph Ivanowski</b>	Riser Material: <b>Sch 40 PVC</b>
Drilling End Date: <b>12/08/2014</b>	Boring Location (X): <b>670247.76</b>	Screen Material: <b>Sch 40 PVC Slotted</b>
Drilling Company: <b>Cascade Drilling</b>	Boring Location (Y): <b>1166395.49</b>	Seal Material(s): <b>Bentonite Grout/Chips</b>
Drilling Method: <b>Sonic</b>	Casing Elevation (Z): <b>809.78</b>	Filter Pack: <b>20/40 Mesh Silica</b>

DEPTH (ft.)	LITHOLOGY	WATER LEVEL	WELL/BORING COMPLETION	COLLECT				SOIL/ROCK VISUAL DESCRIPTION	MEASURE		DEPTH (ft.)
				Sample Type	Date & Time	Blow Counts	Recovery (%)		PID (ppm)	Lab Sample	
20									0.0		20
25									0.0		25
30								(26') Lean CLAY (CL); few fine-coarse gravel, little fine-medium sand, few silt, soft, wet, light yellowish-brown, gravel-sized limestone inclusions, iron-oxide streaking.	0.0		30
35								(32') BEDROCK: Very dark bluish-gray limestone; very dense with abundant calcite-healed fractures.	0.0		35
40								Note: 8-inch steel surface casing installed in 12-inch borehole to 37' bgs. Note: ~8 inches of grout cored through from permanent casing installation. Note: Driller reports soft zone at 39-40; no staining, but more fractured in core.	0.0		40

NOTES:

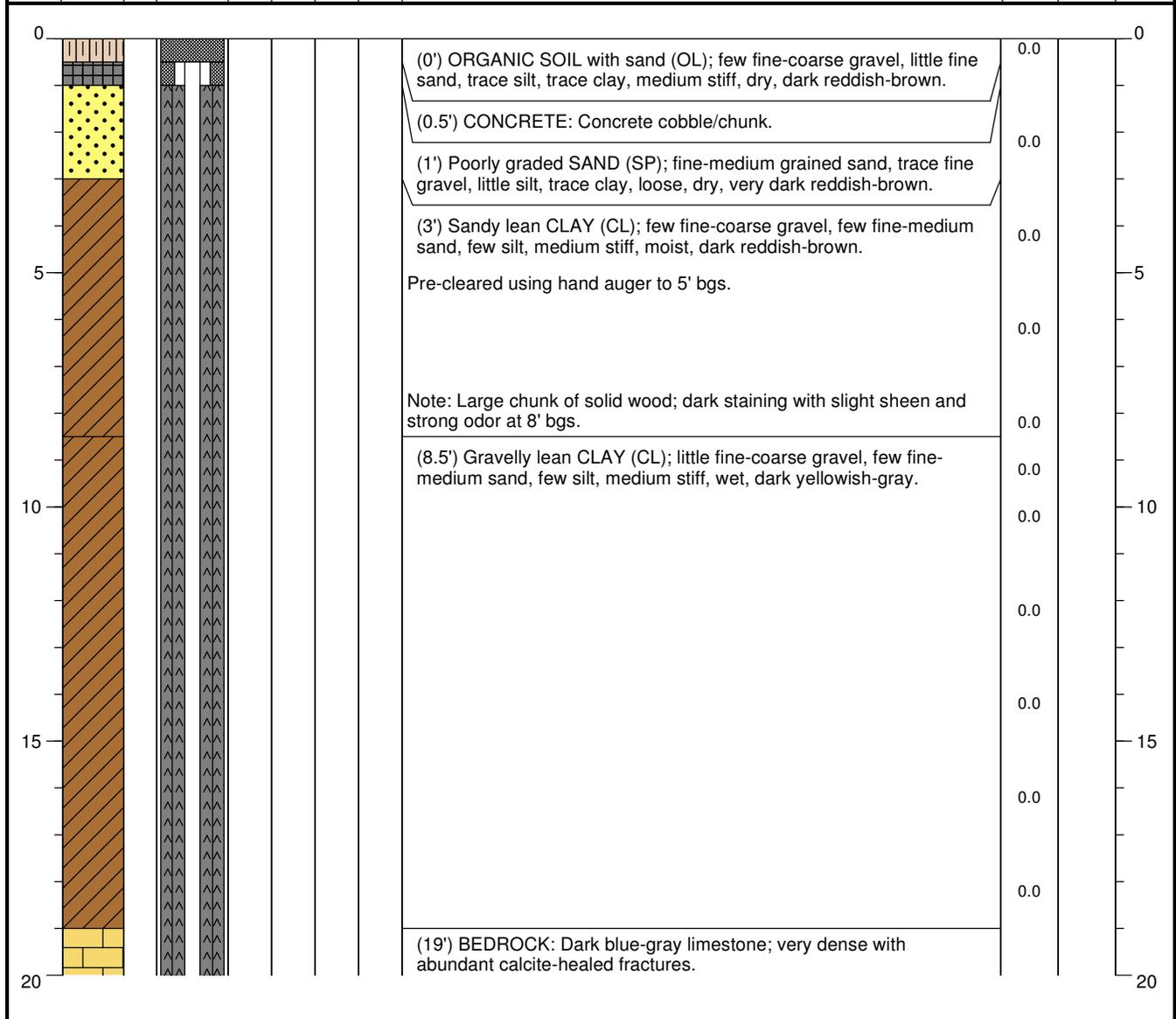
Hole Clear. Date:	12/01/2014	Boring Depth (ft):	57	Well Depth (ft):	57
Hole Clear. Company:	Underground Det.	Boring Diameter (in):	12(boring), 8(casing)	Well Diameter (in):	2
Hole Clear. Method:	GPR/ER	Sampling Method(s):	Core Barrel	Screen Slot (in):	0.010
Drilling Start Date:	12/04/2014	Logged By:	Joseph Ivanowski	Riser Material:	Sch 40 PVC
Drilling End Date:	12/08/2014	Boring Location (X):	670247.76	Screen Material:	Sch 40 PVC Slotted
Drilling Company:	Cascade Drilling	Boring Location (Y):	1166395.49	Seal Material(s):	Bentonite Grout/Chips
Drilling Method:	Sonic	Casing Elevation (Z):	809.78	Filter Pack:	20/40 Mesh Silica

DEPTH (ft.)	LITHOLOGY	WATER LEVEL	WELL/BORING COMPLETION	COLLECT				SOIL/ROCK VISUAL DESCRIPTION	MEASURE		DEPTH (ft.)
				Sample Type	Date & Time	Blow Counts	Recovery (%)		PID (ppm)	Lab Sample	
40								<p>Note: Steeply dipping (~75 deg) fracture with some weathering along surface.</p> <p>Note: Minor iron-oxide staining along fracture surface at 50 ft bgs.</p> <p>Note: Driller reports soft zone from 52-53 ft bgs.</p> <p>Note: Densely fractured zone at 55.5-56 ft bgs; no staining.</p>	0.0		40
45							0.0			45	
50							0.0			50	
55							0.0			55	
60							End of Boring	0.0		60	

NOTES:

Hole Clear. Date: <b>12/01/2014</b>	Boring Depth (ft): <b>65</b>	Well Depth (ft): <b>44</b>
Hole Clear. Company: <b>Underground Det.</b>	Boring Diameter (in): <b>12(boring), 8(casing)</b>	Well Diameter (in): <b>2</b>
Hole Clear. Method: <b>GPR/ER</b>	Sampling Method(s): <b>Core Barrel</b>	Screen Slot (in): <b>0.010</b>
Drilling Start Date: <b>12/02/2014</b>	Logged By: <b>Joseph Ivanowski</b>	Riser Material: <b>Sch 40 PVC</b>
Drilling End Date: <b>12/06/2014</b>	Boring Location (X): <b>670397.26</b>	Screen Material: <b>Sch 40 PVC Slotted</b>
Drilling Company: <b>Cascade Drilling</b>	Boring Location (Y): <b>1166393.11</b>	Seal Material(s): <b>Bentonite Grout/Chips</b>
Drilling Method: <b>Sonic</b>	Casing Elevation (Z): <b>800.40</b>	Filter Pack: <b>20/40 Mesh Silica</b>

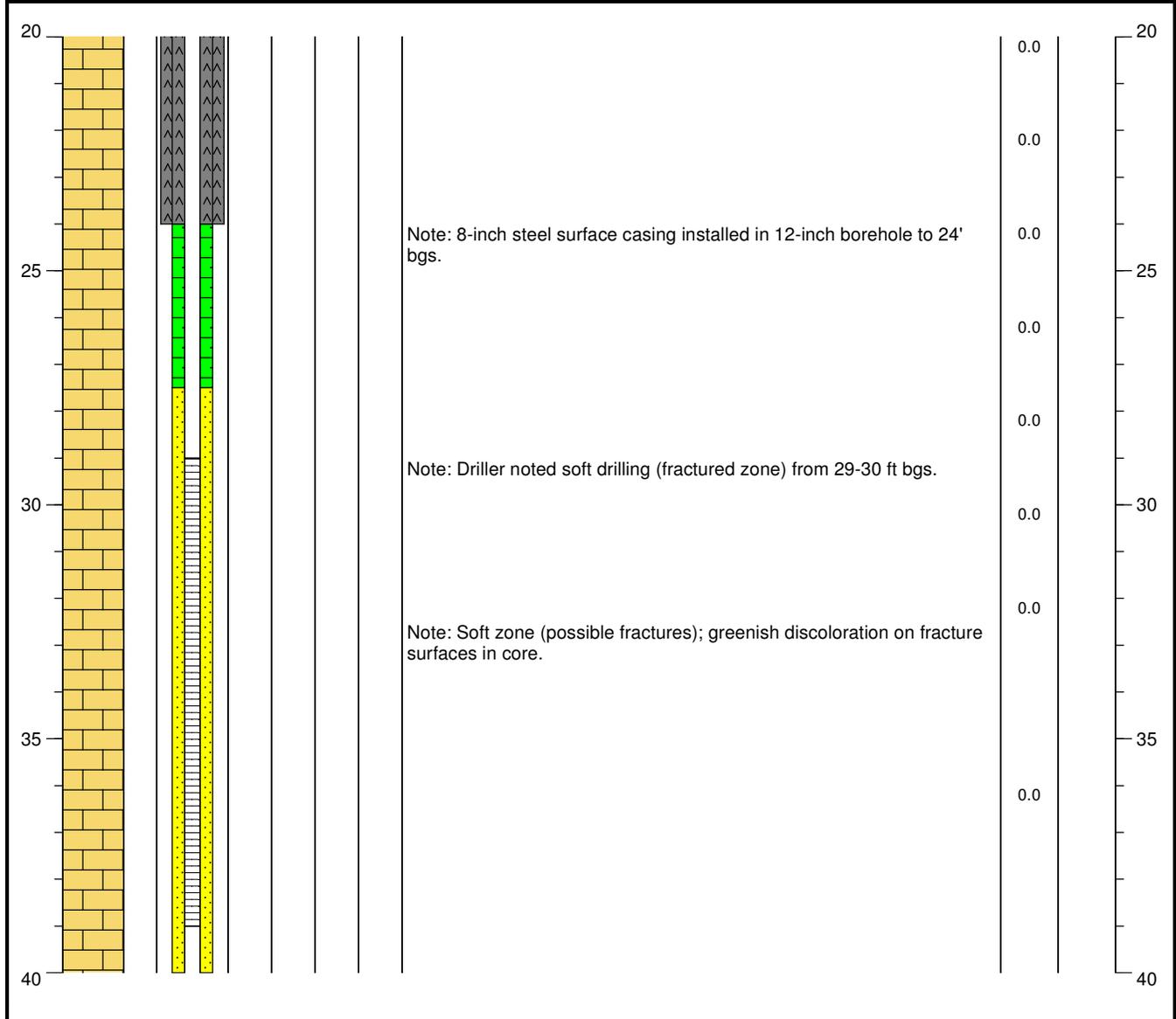
DEPTH (ft.)	LITHOLOGY	WATER LEVEL	WELL/BORING COMPLETION	COLLECT				SOIL/ROCK VISUAL DESCRIPTION	MEASURE		DEPTH (ft.)
				Sample Type	Date & Time	Blow Counts	Recovery (%)		PID (ppm)	Lab Sample	



NOTES:

Hole Clear. Date: <b>12/01/2014</b>	Boring Depth (ft): <b>65</b>	Well Depth (ft): <b>44</b>
Hole Clear. Company: <b>Underground Det.</b>	Boring Diameter (in): <b>12(boring), 8(casing)</b>	Well Diameter (in): <b>2</b>
Hole Clear. Method: <b>GPR/ER</b>	Sampling Method(s): <b>Core Barrel</b>	Screen Slot (in): <b>0.010</b>
Drilling Start Date: <b>12/02/2014</b>	Logged By: <b>Joseph Ivanowski</b>	Riser Material: <b>Sch 40 PVC</b>
Drilling End Date: <b>12/06/2014</b>	Boring Location (X): <b>670397.26</b>	Screen Material: <b>Sch 40 PVC Slotted</b>
Drilling Company: <b>Cascade Drilling</b>	Boring Location (Y): <b>1166393.11</b>	Seal Material(s): <b>Bentonite Grout/Chips</b>
Drilling Method: <b>Sonic</b>	Casing Elevation (Z): <b>800.40</b>	Filter Pack: <b>20/40 Mesh Silica</b>

DEPTH (ft.)	LITHOLOGY	WATER LEVEL	WELL/BORING COMPLETION	COLLECT				SOIL/ROCK VISUAL DESCRIPTION	MEASURE		DEPTH (ft.)
				Sample Type	Date & Time	Blow Counts	Recovery (%)		PID (ppm)	Lab Sample	



NOTES:

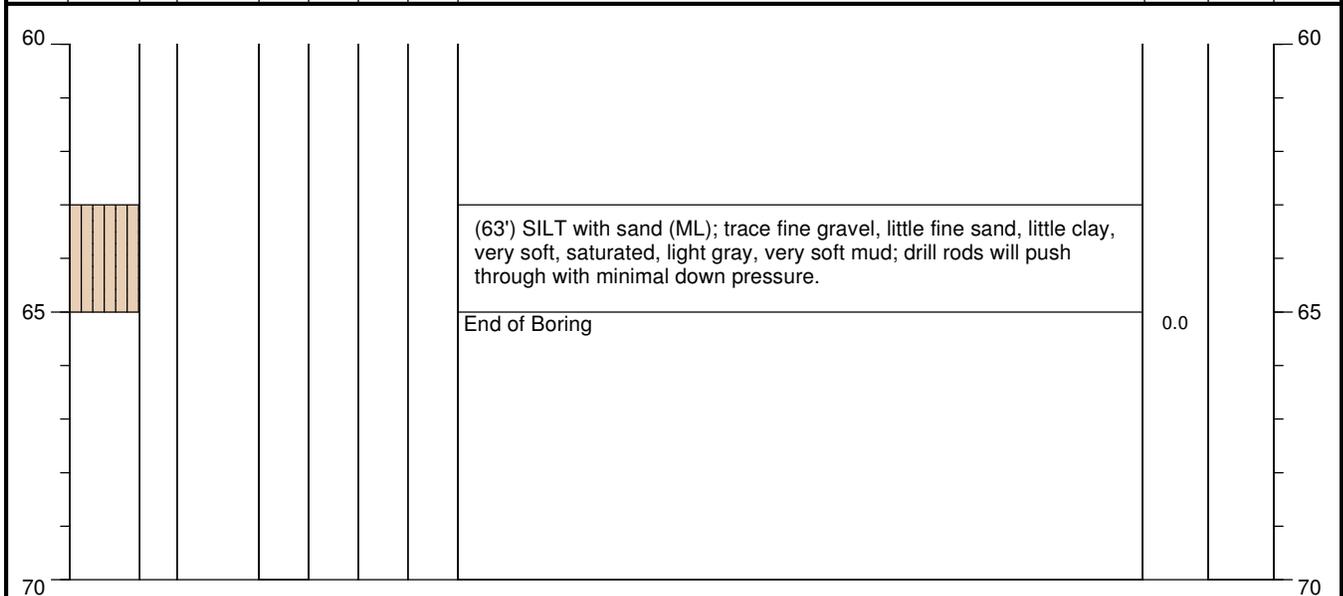
Hole Clear. Date: <b>12/01/2014</b>	Boring Depth (ft): <b>65</b>	Well Depth (ft): <b>44</b>
Hole Clear. Company: <b>Underground Det.</b>	Boring Diameter (in): <b>12(boring), 8(casing)</b>	Well Diameter (in): <b>2</b>
Hole Clear. Method: <b>GPR/ER</b>	Sampling Method(s): <b>Core Barrel</b>	Screen Slot (in): <b>0.010</b>
Drilling Start Date: <b>12/02/2014</b>	Logged By: <b>Joseph Ivanowski</b>	Riser Material: <b>Sch 40 PVC</b>
Drilling End Date: <b>12/06/2014</b>	Boring Location (X): <b>670397.26</b>	Screen Material: <b>Sch 40 PVC Slotted</b>
Drilling Company: <b>Cascade Drilling</b>	Boring Location (Y): <b>1166393.11</b>	Seal Material(s): <b>Bentonite Grout/Chips</b>
Drilling Method: <b>Sonic</b>	Casing Elevation (Z): <b>800.40</b>	Filter Pack: <b>20/40 Mesh Silica</b>

DEPTH (ft.)	LITHOLOGY	WATER LEVEL	WELL/BORING COMPLETION	COLLECT				SOIL/ROCK VISUAL DESCRIPTION	MEASURE		DEPTH (ft.)
				Sample Type	Date & Time	Blow Counts	Recovery (%)		PID (ppm)	Lab Sample	
40								<p>Note: Very soft zone, drill rods dropped about 4 inches from 42.5-43 ft bgs.</p> <p>Formation packer (inverted Fernco fitting) installed at bottom of 5 ft sump (44 ft bgs) using bentonite chips from 39.5 to 44 ft bgs to separate well from void below.</p> <p>(as above)</p>	0.0		40
45										0.0	
50									0.0		50
55											55
60								(45.5') No Recovery: Open void; drill rods drop with no resistance.			60

NOTES:

Hole Clear. Date: <b>12/01/2014</b>	Boring Depth (ft): <b>65</b>	Well Depth (ft): <b>44</b>
Hole Clear. Company: <b>Underground Det.</b>	Boring Diameter (in): <b>12(boring), 8(casing)</b>	Well Diameter (in): <b>2</b>
Hole Clear. Method: <b>GPR/ER</b>	Sampling Method(s): <b>Core Barrel</b>	Screen Slot (in): <b>0.010</b>
Drilling Start Date: <b>12/02/2014</b>	Logged By: <b>Joseph Ivanowski</b>	Riser Material: <b>Sch 40 PVC</b>
Drilling End Date: <b>12/06/2014</b>	Boring Location (X): <b>670397.26</b>	Screen Material: <b>Sch 40 PVC Slotted</b>
Drilling Company: <b>Cascade Drilling</b>	Boring Location (Y): <b>1166393.11</b>	Seal Material(s): <b>Bentonite Grout/Chips</b>
Drilling Method: <b>Sonic</b>	Casing Elevation (Z): <b>800.40</b>	Filter Pack: <b>20/40 Mesh Silica</b>

DEPTH (ft.)	LITHOLOGY	WATER LEVEL	WELL/BORING COMPLETION	COLLECT				SOIL/ROCK VISUAL DESCRIPTION	MEASURE		DEPTH (ft.)
				Sample Type	Date & Time	Blow Counts	Recovery (%)		PID (ppm)	Lab Sample	



NOTES:

### T-6 Survey of Well Location and Elevation

Well ID	Northing	Easting	Top of Casing	Ground Surface
			Elevation	Elevation
CWM-183-MW-35	1166393.11	670397.26	800.40	798.04
CWM-183-MW-33	1166401.82	670397.94	800.58	798.14
CWM-183-MW-34	1166395.49	670247.76	809.78	807.71
CWM-183-MW-32	1166390.92	670242.55	810.53	808.37

Elevation in feet

Surveyed by L.I. Smith & Associates, Inc.