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Pinellas Environmental Restoration Project

Young - Rainey STAR Center Building 100 Area Enhanced Bioremediation Pilot Test Final Report

January 2005

U.S. Department of Energy

Office of Legacy Management

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Enhanced Bioremediation Pilot Test Final Report

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Work Performed by S.M. Stoller Corporation under DOE Contract No. DE–AC01–02GJ79491
for the U.S. Department of Energy Office of Legacy Management, Grand Junction, Colorado

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1.0 Introduction

This document is the final report for the enhanced bioremediation pilot test conducted at the Building 100 Area in 2003 and 2004. The subcontractor who conducted the pilot test has submitted their final report for the project, but another sampling event beyond the subcontractor's scope was conducted, and this report presents the results of that event. Additionally, this report includes supplemental data collected during the pilot test that were not reported in the subcontractor's final report.

The subcontractor's final report is included as Appendix A. Much of the information included in that report, such as the pilot test design details and the subcontractor's interpretation of the data, will not be reiterated in this report.

2.0 Results from the May 2004 (P8) Sampling Event

The subcontractor's scope for the pilot test ended with the P7 sampling event in March 2004. However, based upon the continued presence of the Hydrogen Release Compound (HRC) metabolic acids in one of the monitoring wells and the associated potential for contaminant degradation, it was determined that additional pilot test sampling should occur, so the P8 event was conducted by Stoller in May 2004. The results from this sampling event are shown in Tables 1, 2, and 3. Also shown in these tables are the results from the previous pilot test sampling events conducted by the subcontractor (Baseline and P1 through P7 events). Trichloroethene (TCE) was not detected during the pilot test, so the data for cis- and trans-1,2-dichloroethene (DCE) and vinyl chloride (VC) from the Baseline through P8 sampling events for the three pilot test wells are shown graphically in Figures 1 through 3. In these figures, the contaminant concentration units have been converted from micrograms per liter (μg/L) to micromoles per liter (μmol/L) to allow direct comparison of contaminant amounts.

As discussed in the subcontractor's final report (Appendix A), decreasing concentration trends were noted for cis-1,2-DCE and VC in well 0514, and ethene (the major end product of TCE/DCE/VC biodegradation) was also measured in this well. While these data demonstrate that bioremediation was enhanced and that some portion of the contamination was degraded completely (to ethene), one of the major goals of the pilot test was to show that the contaminants could be degraded to below their maximum contaminant levels (MCLs). VC has the lowest MCL (1 μg/L) and the other contaminants degrade to form VC, so this contaminant is the most significant in terms of meeting the MCL. As can be seen in Table 1 and Figure 1, VC concentrations in well 0514 never decreased to the 1 μg/L MCL. Additionally, VC concentrations in wells 0526 and S73C never decreased to the MCL, although the baseline contaminant concentrations were much lower in these wells relative to well 0514.

At the conclusion of the pilot test, the reason(s) for the lack of contaminant degradation to MCLs was unknown, although several hypotheses were discussed. Subsequently, as part of a separate project, a laboratory microcosm test was conducted using site media from the 4.5 Acre Site. The results of this test suggested that the contaminants could be degraded to below detection limits in site soils when nutrients such as yeast or nitrogen and phosphorous compounds were added. These results suggest that a potential nutrient limitation existed in the area of the Building 100 Area Pilot Test. Further testing in the field will be necessary to conclusively show that adding

nutrients along with an electron donor (such as HRC, lactate, etc.) can degrade the contaminants to below MCLs.

The P8 event results also provided other useful information. These data showed that most of the metabolic acids (lactic, butyric, pyruvic, propionic, and acetic acids; all are breakdown products from HRC) that act as electron donors (to promote biodegradation) were still present in well S73C at 14 months after HRC injection. This confirmed that the effects of HRC injection could last at least this long in the subsurface at the site.

3.0 Microorganism Data

The three pilot test wells were sampled for microorganism analysis in January 2003, approximately two months before the pilot test began. The purpose of this was to provide qualitative baseline information concerning the presence of *Dehalococcoides ethenogenes* (DHC), which is the microorganism that is responsible for biodegradation of TCE, DCE, and VC. The samples were submitted to Microbial Insights, a microbiology laboratory in Tennessee. The results showed a moderate abundance of DHC in wells PIN12−0514 and −0526; no DHC was detected in well PIN12−S73C. The laboratory report is included as Appendix B.

Table 4 shows data from a separate set of analyses conducted by the pilot test subcontractor concerning DHC and other microorganism concentrations in ground water and soil just prior to, during, and at the end of the pilot test. The entire report from the subcontractor is included as Appendix C. These data show that the number of specific microorganisms increased during the pilot test at some locations. For example, in ground water, DHC was not detected in wells 0526 and S73C during the baseline sampling in March 2003, but were strongly detected in both wells in the two subsequent sampling events in September 2003 and May 2004. Thus, it would appear that the injection of HRC into the subsurface spurred growth of the microorganisms that cause contaminant degradation. The soil data are not as conclusive, and in fact it appears that the microorganism concentrations may have decreased in samples collected from near wells 0514 and S73C. This apparent decrease may be due to the fact that the soil samples from March 2003 and May 2004 were collected from similar locations but not the exact same locations, or could simply be due to the inherent high variability associated with soil sampling.

During the pilot test baseline sampling event in March 2003, the soil samples for microorganism analysis were collected by the subcontractor from one of the injection points near each well and were labeled with the well ID instead of a separate soil boring ID. Additionally, the soil samples collected in May 2004 were also labeled with the nearby well ID. However, when Stoller loaded these data into the Pinellas database, it was determined that the soil sample IDs should be changed from a ground water sample ID to a soil sample ID. Therefore, the soil samples were given the label from the injection point (IP) at which they were collected in March 2003. The soil sample collected near well 0514 was labeled as IP14, the soil sample from near well 0526 was labeled as IP25, and the soil sample from near well S73C was labeled as IP35. The injection point locations and the nearby monitoring wells are shown on Figure 4. Although the May 2004 soil samples were not collected from the exact same location as the March 2003 borings, the borings for these samples were placed as close as possible to the March 2003 boring locations, and therefore were given the same boring ID for consistency and so the data can be directly compared in the future. The soil samples in the lab report in Appendix C remain labeled with the well IDs to keep that report in its original condition, but the IDs were changed for Table 4 (and

in the database), and these new soil IDs will be used in future work. Additionally, in the laboratory report in Appendix C, well S73C is mislabeled as 573C.

4.0 Additional Data

This section includes additional data that were associated with the pilot test but were not included in the subcontractor's final report.

Ground water samples were collected in February 2002 from the six Building 100 Area wells that were under consideration for inclusion in the pilot test. These samples were analyzed for a typical suite of parameters that help define the subsurface geochemistry as it relates to enhanced bioremediation activities. These data were collected to provide information for the Statement of Work that was sent to the various prospective enhanced bioremediation vendors. A similar set of data was collected during each pilot test sampling event, so the data in Table 5 simply provide a perspective on the baseline conditions a year before the pilot test began.

Table 6 contains additional data concerning bioavailable iron that were voluntarily measured by the pilot test subcontractor. Bioavailable iron is a terminal electron acceptor that, in high enough concentrations, may interfere with the biodegradation of the contaminants. The laboratory report is included as Appendix D. According to the pilot test subcontractor, these data show bioavailable iron present at relatively low concentrations, indicating that bioavailable iron should not present an obstacle to implementing enhanced bioremediation at the site.

5.0 Conclusions

The conclusions here are based on the subcontractor's final report, the additional data discussed in this report, and subsequent data evaluation and research by Stoller and other subcontractors. The subsequent research and data evaluation certainly were not within the scope of the pilot test, but the knowledge gained during these activities directly impacts the conclusions drawn from the pilot test.

The injection of HRC into the subsurface at the Building 100 Area resulted in enhancement of contaminant biodegradation around pilot test well 0514. This conclusion is based on several lines of evidence including decreasing contaminant concentrations and the production of significant concentrations of ethene. The production of ethene also demonstrates that complete dechlorination of contaminants was occurring. Significant enhancement of biodegradation at the other two pilot test wells was not observed.

Injection point spacing was closest around well 0514, the only well showing significant contaminant degradation, indicating that injection point spacing for potential future work should be no larger than approximately 10 feet on center. The lack of observable biodegradation in the other two wells with wider injection point spacing also indicates that subsurface heterogeneity may have a significant effect on distribution and effectiveness of injected amendment chemicals. As learned from a different subcontractor subsequent to the pilot test, a ground water recirculation approach could potentially overcome the limitations of subsurface heterogeneity on amendment distribution under the "inject and leave" scenario used during the pilot test.

Laboratory microcosm testing conducted more than a year after the conclusion of the pilot test suggested that adding nutrients to site media could degrade contaminants to below soil detection limits. No nutrients were used during the pilot test, so it is possible that addition of nutrients during the pilot test could have resulted in significant additional contaminant biodegradation, possibly to below the MCLs.

Voluntary work conducted by the pilot test subcontractor showed that the number of DHC, the main microorganism responsible for contaminant biodegradation, increased significantly in ground water during the pilot test. The natural presence of DHC in the subsurface and ability to increase their population strongly indicates that enhanced bioremediation should be a viable remedial option not only for the Building 100 Area but also for the remainder of the STAR Center and the 4.5 Acre Site.

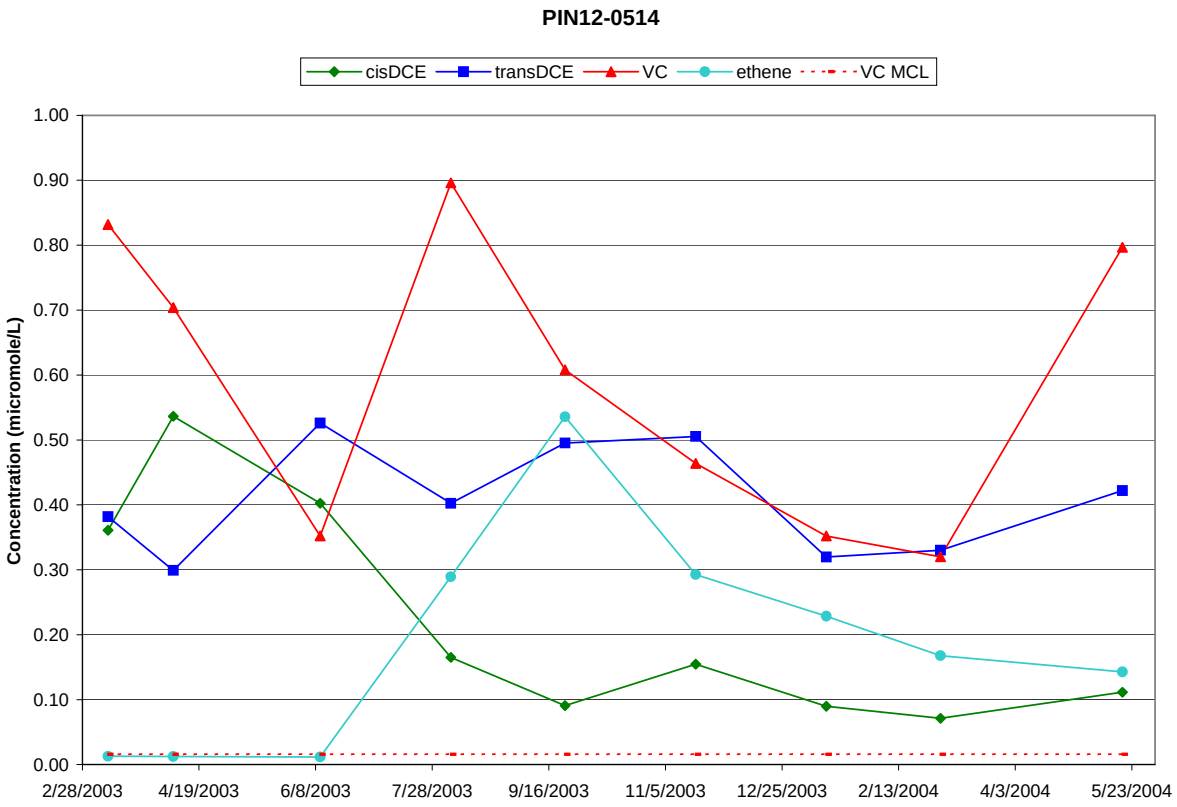

Figure 1. Contaminant Concentration Trends in Well PIN12-0514 During the Pilot Test

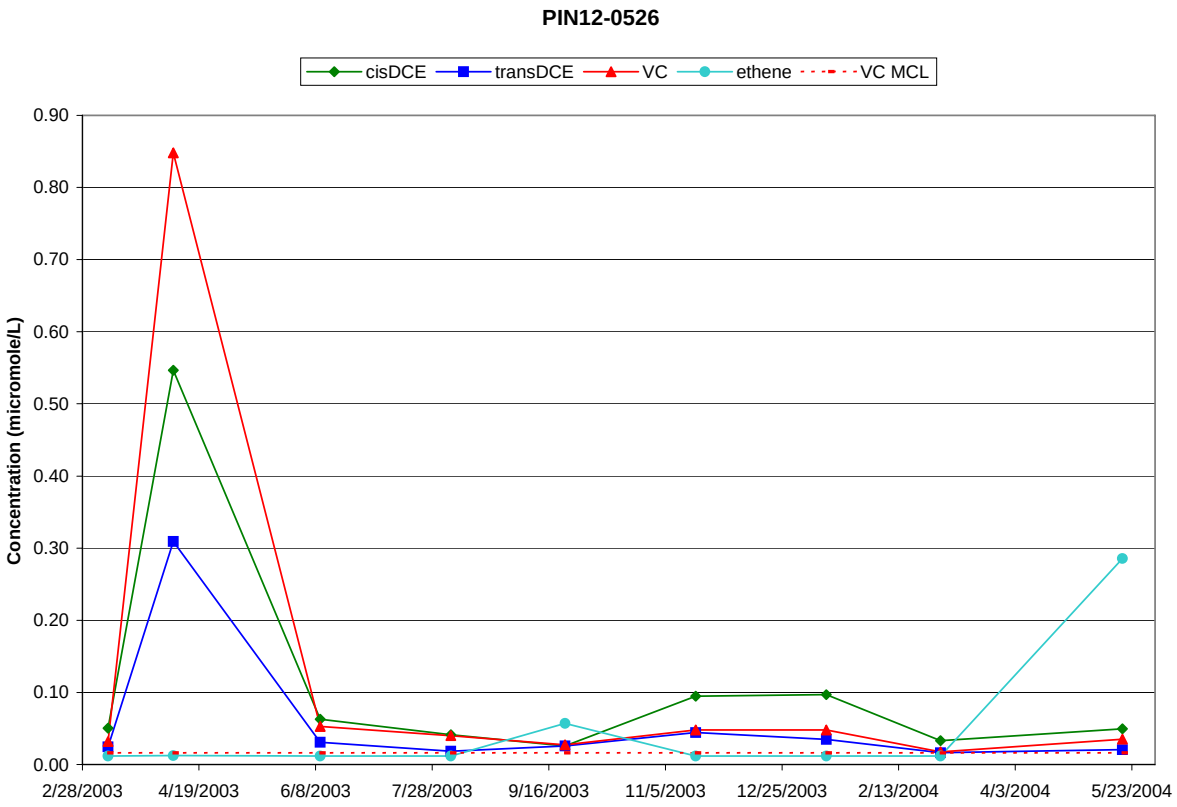

Figure 2. Contaminant Concentration Trends in Well PIN12-0526 During the Pilot Test. The final ethene data point in May 2004 is a non-detect value at 0.29 micromole/L; this elevated detection limit is the result of different lab conducting the P8 event analysis.

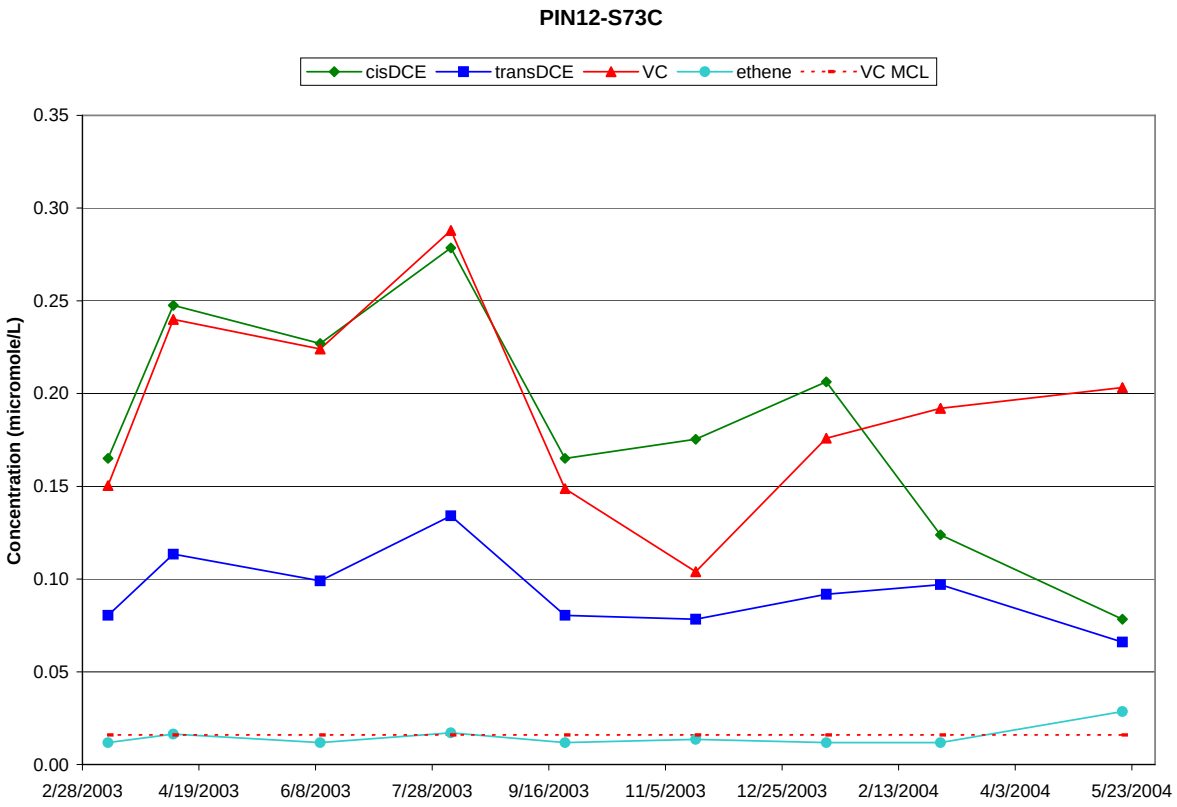

Figure 3. Contaminant Concentration Trends in Well PIN12-S73C During the Pilot Test

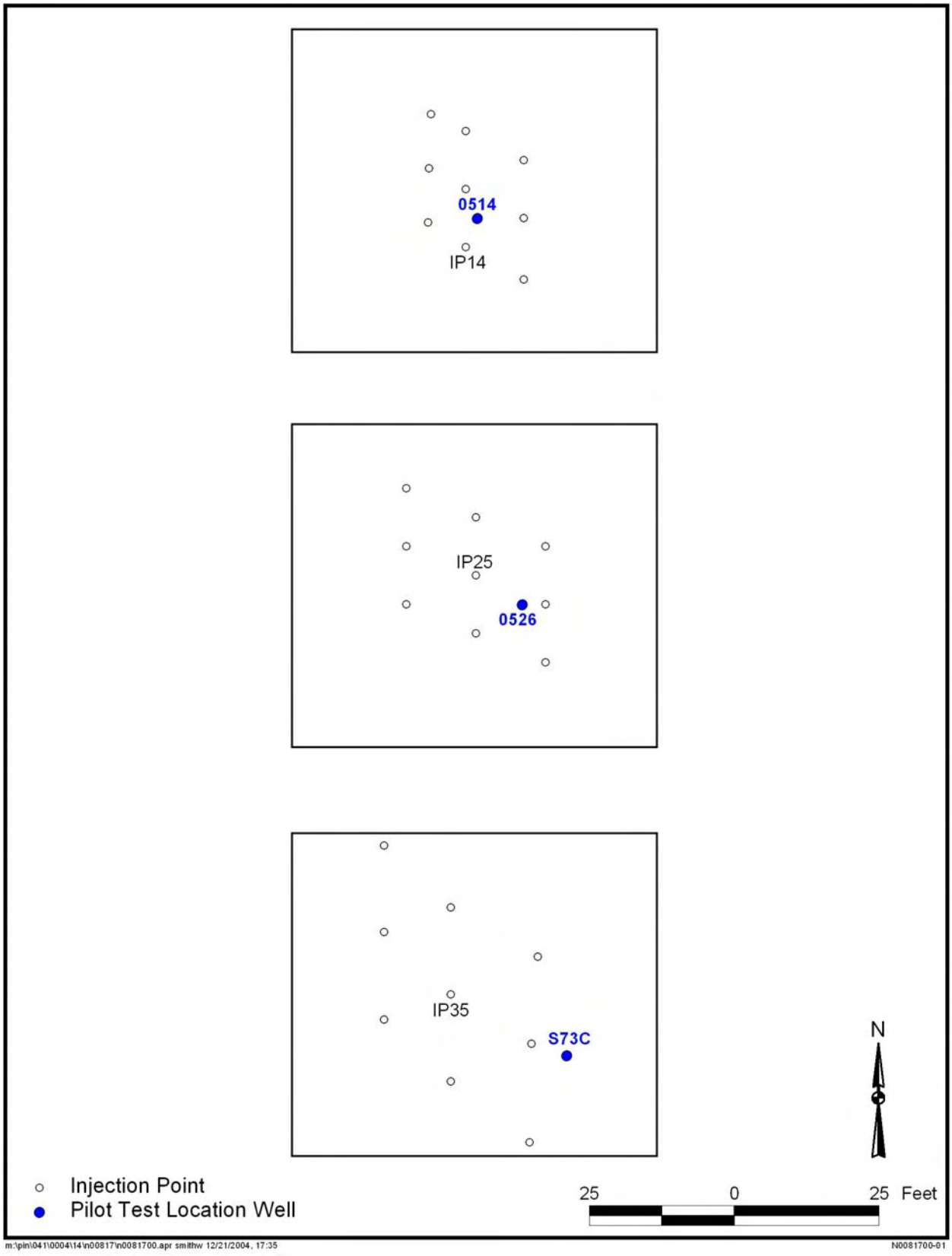

Figure 4. Location of Soil Samples Collected from Injection Points

Table 1. Pilot Test Data from Well PIN12-0514

Analyte	Units	Baseline	P1	P2	P3	P4	P5	P6	P7	Stoller P8
		3/11/2003	4/8/2003	6/10/2003	8/5/2003	9/23/2003	11/18/2003	1/13/2004	3/2/2004	5/19/2004
Temperature	C	23.5	23.78	24.61	24.56	24.83	24.72	24.11	24.11	24.3
Specific Conductance	umhos/cm	1,650	1,640	1,640	1,620	1,570	1,630	1,570	1,550	618
pH	s.u.	6.5	6.53	6.17	6.02	6.77	6.79	7.06	7.05	6.53
Oxidation Reduction Potential	mV	29	-18	-270	-289	-290	-155	-287	-220	-21.3
Dissolved Oxygen	mg/L	0.76	0.14	0.09	0.08	0.16	0.16	0.49	0.4	0.96
Turbidity	NTU	132	124	118	132	188	163	150	156	188
PCE	µg/L	1 U	1 U	1 U	1 U	1 U	1 U	1 U	1 U	0.5 U
TCE	ug/L	1 U	1 U	1 U	1 U	1 U	1 U	1 U	1 U	0.5 U
cis-1,2-DCE	ug/L	35	52	39	16	8.8	15	8.7	6.9	10.8
trans-1,2-DCE	µg/L	37	29	51	39	48	49	31	32	40.9
1,1-DCE	µg/L	1 U	1 U	1 U	1 U	0.38 J	1 U	1 U	1 U	0.5 U
Vinyl chloride	µg/L	52	44	22	56	38	29	22	20	49.8
1,1,1-TCA	µg/L	1 U	1 U	1 U	1 U	1 U	1 U	1 U	1 U	0.5 U
1,1-DCA	µg/L	11	12	17	13	9.1	9.5	8.2	8.2	12.4
Chloroethane	µg/L	1 U	1 U	1 U	1 U	1 U	2.4	0.42 J	1 U	1 U
Benzene	µg/L	1 U	1 U	1 U	1 U	1 U	1 U	1 U	1 U	0.5 U
Total Organic Carbon	mg/L	64	58	NA	130	NA	59	NA	54	78.6
Methane	µg/L	450	360	410	1,800	11,000	5,700	3,600	1,900	4400
Ethene	µg/L	0.36	0.35 U	0.33 U	8.1	15	8.2	6.4	4.7	4 U
Ethane	µg/L	0.35 U	0.44	0.35 U	1	1.8 U	1.2	1.2	0.35 U	3 U
Carbon Dioxide	mg/L	98	79	170 D	270 E	230	66	80	110	560
Acetic Acid	mg/L	1 U	1 U	69	79	110	2.3	1 U	0.5 U	0.34 U
Propionic Acid	mg/L	1 U	1 U	17	49	59	1.4	1 U	0.5 U	0.31 U
Pyruvic Acid	mg/L	10 U	10 U	50 U	10 U	100 U	10 U	10 U	2 U	2.6 U
Butyric Acid	mg/L	1 U	1 U	3.7 J	12	20	1 U	1 U	0.5 U	0.16 U
Lactic Acid	mg/L	25 U	25 U	120 U	25 U	250 U	25 U	25 U	10 U	2.1 U
Nitrate as NO_3	mg/L	0.1 U	0.1 U	NA	0.1 UH	NA	0.1 UH	NA	0.1 U	NA
Sulfate	mg/L	140	160	50	11	44	99 D	76 D	110	108
Chloride	mg/L	220	210	NA	180	NA	230 D	NA	*	NA
Bromide	mg/L	0.5 B	0.6 B	0.92 J	0.99	1.6	0.49 J	0.56 J	0.3 J	NA
Iron (total)	mg/L	5.1	5.4	2	0.77	1.8	5.2	4	1.8	1.75
Manganese (total)	mg/L	0.016	0.016	0.016	0.013	0.014	0.014	0.012	0.012	NA
Iron (dissolved)	mg/L	2.6	4.1	1.3	0.55	0.43	1.2	1.2	0.62	0.134 B
Manganese (dissolved)	mg/L	0.017	0.016	0.015	0.011	0.012	0.0083 J	0.0077 J	0.011	NA
Color	PCU	180	200	NA	120	NA	250	NA	180 H	NA
TDS	mg/L	1,100	1,000	NA	1,000	NA	1,000	NA	1,100	NA

* = Replicate analysis not within control limits.
B = Detected in laboratory method blank.
D = Analyte determined in diluted sample.
H = Holding time expired, value suspect.
J = estimated quantity below reporting limit but above the instrument detection limit.
NA = Not Analyzed.
U = Undetected.
E = Inorganic: Estimate value because of interference, see case narrative.
Organic: Analyte exceeded calibration range of the GC-MS.

Table 2. Pilot Test Data from Well PIN12−0526

Analyte	Units	Baseline	P1	P2	P3	P4	P5	P6	P7	Stoller P8
		3/11/2003	4/8/2003	6/10/2003	8/5/2003	9/23/2003	11/18/2003	1/13/2004	3/2/2004	5/19/2004
Temperature	C	29.5	28.9	30.89	30.22	31.22	30.22	29.61	29.61	29.5
Specific Conductance	µmhos/cm	2,090	2,300	2,100	2,120	2,130	1,980	1,970	1,950	853
pH	s.u.	6.54	6.55	6.3	6.25	6.8	6.97	7.03	7.03	6.43
Oxidation Reduction Potential	mV	-18	-37	-84	-83	-40	-78	-309	-256	-14.6
Dissolved Oxygen	mg/L	4.31	0.11	0.06	0.01	0.2	0.45	0.34	0.18	1.17
Turbidity	NTU	45.9	38.9	42.5	48	48	48	66	72	12.5
PCE	µg/L	1 U	1 U	1 U	1 U	1 U	1 U	1 U	1 U	0.5 U
TCE	µg/L	1 U	1 U	1 U	1 U	1 U	1 U	1 U	1 U	0.5 U
cis-1,2-DCE	µg/L	4.9	53	6.1	4	2.5	9.2	9.4	3.2	4.8
trans-1,2-DCE	µg/L	2.4	30	3	1.8	2.5	4.3	3.4	1.6	2
1,1-DCE	µg/L	1 U	1 U	1 U	1 U	0.36 J	1 U	1 U	1 U	0.5 U
Vinyl chloride	µg/L	2	53	3.3	2.5	1.7	3	3	1.1	2.2
1,1,1-TCA	µg/L	1 U	1 U	1 U	1 U	1 U	1 U	1 U	1 U	0.5 U
1,1-DCA	µg/L	1 U	12	1 U	1 U	0.73 J	0.17 J	1 U	1 U	0.5 U
Chloroethane	µg/L	1 U	1 U	1 U	1 U	1 U	1 U	1 U	1 U	1 U
Benzene	µg/L	1 U	1 U	1 U	1 U	1 U	1 U	1 U	1 U	0.5 U
Total Organic Carbon	mg/L	66	54	NA	72	NA	74	NA	64	77.5
Methane	µg/L	290	170	390	310	1,100	1,600	7,300	6,800	6,830
Ethene	µg/L	0.33 U	0.35 U	0.33 U	0.33 U	1.6 U	0.33 U	0.33 U	0.33 U	8 U
Ethane	µg/L	0.35 U	0.33 U	0.35 U	0.35 U	1.8 U	0.35 U	0.35 U	0.35 U	6 U
Carbon Dioxide	mg/L	110	97	110	160 E	200	66	100	420	850
Acetic Acid	mg/L	1 U	1 U	1 U	1 U	1 U	16 D	51 D	0.5 U	0.34 U
Propionic Acid	mg/L	1 U	1 U	1 U	1 U	1 U	11	16 D	0.5 U	0.31 U
Pyruvic Acid	mg/L	10 U	10 U	10 U	10 U	10 U	10 U	10 U	2 U	2.6 U
Butyric Acid	mg/L	1 U	1 U	1 U	1 U	1 U	1 U	1	0.5 U	0.16 U
Lactic Acid	mg/L	25 U	25 U	25 U	25 U	25 U	25 U	25 U	10 U	2.1 U
Nitrate as NO_3	mg/L	0.1 U	0.21	NA	0.1 UH	NA	0.1 UH	NA	0.1 U	NA
Sulfate	mg/L	210	230	200 D	200	220 D	110 D	66 D	140	85.6
Chloride	mg/L	300	300	NA	330	NA	280 D	NA	*	NA
Bromide	mg/L	1.4	1.8	2.1	2.1	1.5	1.5	1.1	1.3	NA
Iron (total)	mg/L	8.7	9.7	8.6	7.5	5.8	1.8	2.3	1.1	1.27
Manganese (total)	mg/L	0.017	0.017	0.016	0.017	0.016	0.019	0.013	0.014	NA
Iron (dissolved)	mg/L	3.9	5.3	3.9	2.5	3.5	1.1	1.3	0.54	1.33
Manganese (dissolved)	mg/L	0.016	0.018	0.016	0.016	0.016	0.02	0.012	0.013	NA
Color	PCU	180	200	NA	140	NA	120	NA	120 H	NA
TDS	mg/L	1,300	1,400	NA	1,300	NA	1,100	NA	1,400	NA

* = Replicate analysis not within control limits.
D = Analyte determined in diluted sample.
H = Holding time expired, value suspect.
J = Estimated quantity below reporting limit but above the instrument detection limit.
NA = Not Analyzed.
U = Undetected.

Table 3. Pilot Test Data from Well PIN12−S73C

Analyte	Units	Baseline	P1	P2	P3	P4	P5	P6	P7	Stoller P8
		3/10/2003	4/8/2003	6/10/2003	8/5/2003	9/23/2003	11/18/2003	1/13/2004	3/2/2004	5/19/2004
Temperature	C	27.67	28.83	29.22	30.06	27.67	29.50	26.94	26.72	29.1
Specific Conductance	µmhos/cm	1,610	1,610	1,600	1,610	1,610	1,860	1,670	1,770	771
pH	s.u.	6.39	6.51	6.15	6.05	6.85	6.71	6.88	6.98	6.34
Oxidation Reduction Potential	mV	-9	-56	-168	-136	-40	-18	-6	-28	-9.6
Dissolved Oxygen	mg/L	0.19	0.14	0.07	0.07	0.25	0.3	0.31	0.38	1.38
Turbidity	NTU	179	157	142	155	166	87	60	66	136
PCE	µg/L	1 U	1 U	1 U	1 U	1 U	1 U	1 U	1 U	0.5 U
TCE	µg/L	1 U	1 U	1 U	1 U	1 U	1 U	1 U	1 U	0.5 U
cis-1,2-DCE	µg/L	16	24	22	27	16	17	20	12	7.6
trans-1,2-DCE	µg/L	7.8	11	9.6	13	7.8	7.6	8.9	9.4	6.4
1,1-DCE	µg/L	1 U	1 U	1 U	0.96 J	0.51 J	1 U	0.39 J	0.23 J	0.5 U
Vinyl chloride	µg/L	9.4	15	14	18	9.3	6.5	11	12	12.7
1,1,1-TCA	µg/L	1 U	1 U	1.5	1 U	1 U	1 U	1 U	1 U	0.5 U
1,1-DCA	µg/L	1.2	1.7	1.1	1.9	0.99 J	0.45 J	0.5 J	0.36 J	0.5 U
Chloroethane	µg/L	1 U	1 U	1 U	1 U	1 U	1 U	1 U	1 U	1 U
Benzene	µg/L	1 U	1 U	1 U	1 U	1 U	1 U	1 U	1 U	0.5 U
Total Organic Carbon	mg/L	75	50	NA	50	NA	210	NA	110	177
Methane	µg/L	480	1,500	510	700	1,400	6,200	4,600	8,700	10,300
Ethene	µg/L	0.33 U	0.46	0.33 U	0.48	0.33 U	0.38	0.33 U	0.33 U	0.8 U
Ethane	µg/L	0.35 U	0.36	0.35 U	0.43	0.35 U	0.35 U	0.35 U	0.35 U	0.6 U
Carbon Dioxide	mg/L	100	100	120	150 E	160 D	93	130	120	747
Acetic Acid	mg/L	1 U	1 U	8.2	1 U	1 U	120	26 D	46	100
Propionic Acid	mg/L	1 U	1 U	14	1.4	1.4	110 D	33 D	31	76
Pyruvic Acid	mg/L	10 U	10 U	10 U	10 U	10 U	10 U	10 U	2 U	80
Butyric Acid	mg/L	1 U	1 U	0.67 J	1 U	1 U	25 D	9.8	3.5	10
Lactic Acid	mg/L	25 U	25 U	25 U	25 U	25 U	25 U	25 U	10 U	2.1 U
Nitrate as NO_3	mg/L	0.1 U	0.18	NA	0.14 H	NA	0.1 UH	NA	0.1 U	NA
Sulfate	mg/L	33	30	14	24	22	1.1	7.6	3.3	4.4
Chloride	mg/L	270	260	NA	260	NA	300 D	NA	*	NA
Bromide	mg/L	0.68 B	0.87 B	1.6	1.4	0.81 J	1.4	1.5	0.74 J	NA
Iron (total)	mg/L	14	14	12	15	12	16	13	13	15.4
Manganese (total)	mg/L	0.0084 B	0.0081 J	0.01	0.0093 J	0.0074 J	0.0091 J	0.0081 J	0.0072 J	NA
Iron (dissolved)	mg/L	2.4	2.1	0.97	1.7	1.3	2.2	3.3	1.4	13.7
Manganese (dissolved)	mg/L	0.0068 B	0.0069 I	0.0085 J	0.0071 J	0.0079 J	0.01	0.0072 J	0.0066 J	NA
Color	PCU	180	200	NA	180	NA	120	NA	180 H	NA
TDS	mg/L	1,100	970	NA	1100	NA	1,200	NA	1,100	NA

* = Replicate analysis not within control limits.
B = Detected in laboratory method blank.
D = Analyte determined in diluted sample.
E = Inorganic: Estimate value because of interference, see case narrative.
Organic: Analyte exceeded calibration range of the GC-MS.
H = Holding time expired, value suspect.
I = Increased detection limit due to required dilution.
J = Estimated quantity below reporting limit but above the instrument detection limit.
NA = Not Analyzed.
U = Undetected.

Table 4. Microorganism Results From the Building 100 Pilot Test

The soil samples labeled IP14 were collected from a location near monitoring well 0514. The soil samples labeled IP25 were collected from a location near monitoring well 0526. The soil samples labeled IP35 were collected from a location near monitoring well S73C.

Sample Location	Date	16S rRNA gene product [a]	*Dehalococcoides* 16S rRNA gene product [a]	*Desulfuromonas* 16S rRNA gene product [a]	*Dehalobacter* 16S rRNA gene product [a]
Ground Water					
0514	Mar-03	++	++	++	-
	Sep-03	++	++	-	-
	May-04	++	++	++	-
0526	Mar-03	++	-	-	-
	Sep-03	++	++	+	-
	May-04	++	++	+	-
S73C	Mar-03	++	-	-	-
	Sep-03	++	++	-	-
	May-04	++	++	-	-
Soil					
IP14 (20-22')	Mar-03	-	nd	nd	nd
IP14 (24-26')	Mar-03	++	-	-	-
IP14 (30-32')	Mar-03	++	+	-	-
IP14 (20')	May-04	-	-	-	-
IP14 (24')	May-04	+	+	-	-
IP14 (30')	May-04	-	-	-	-
IP25 (8-10')	Mar-03	++	+	-	-
IP25 (14-16')	Mar-03	++	-	+	-
IP25 (23-24')	Mar-03	-	nd	nd	nd
IP25 (8')	May-04	++	-	-	-
IP25 (16')	May-04	++	-	-	-
IP25 (23')	May-04	++	++	-	-
IP35 (8-10')	Mar-03	++	-	+	-
IP35 (14-16')	Mar-03	++	++	-	-
IP35 (23-24')	Mar-03	-	nd	nd	nd
IP35 (8')	May-04	-	+	-	-
IP35 (16')	May-04	-	-	-	-
IP35 (23')	May-04	-	-	-	-

[a](++) indicates amplification product in two replicate DNA extractions, (+) indicates amplification product obtained in one of two replicates DNA extractions, (-) indicates no visible product in either replicate, and (nd) = not detected after initial 16S rRNA analysis.

Table 5. Field and Laboratory Geochemistry Data From Selected Wells Downgradient From Building 100, February 2002. Samples collected for analysis of alkalinity, hardness, ammonia, copper, manganese, nitrate, and orthophosphate were filtered in the field through a 0.45 micrometer filter. Samples for the remaining analytes were not filtered.

Analyte	Units[a]	PIN12–0513	PIN12–0514	PIN12–0524	PIN12–0525	PIN12–0526	PIN21–0512	PIN21–0512 Dup
Methane	µg/L	170	220	680	44	190	120	220
Ethane	µg/L	0.73	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35	< 0.35
Ethene	µg/L	3.3	< 0.33	< 0.33	2	< 0.33	< 0.33	< 0.33
Carbon Dioxide	mg/L	20	32	40	23	43	22	37
Alkalinity as $CaCO_3$	mg/L	340	320	360	300	320	310	310
Hardness as $CaCO_3$	mg/L	340	530	460	370	600	350	360
Ammonia as Nitrogen	mg/L	1.6	0.52	0.37	1	0.48	0.41	0.49
Nitrate as Nitrogen	mg/L	< 0.05	< 0.05	< 0.05	< 0.05	0.01 J	< 0.05	< 0.05
Othophosphate as phosphorus	mg/L	0.11	0.073	0.094	0.079	0.07	0.07	0.074
Sulfate	mg/L	47	80	3.8 J	61	50	26	28
Sulfide	mg/L	0.04	< 0.01	0.01	< 0.01	< 0.1	< 0.01	< 0.01
Biochemical Oxygen Demand	mg/L	2.8	4.8	< 2	< 2	2.8	< 2	< 2
Chemical Oxygen Demand	mg/L	40	160	220	63	160	98	140
Total Organic Carbon	mg/L	21	62	62	20	68	42	42
Dissolved Total Iron	mg/L	3.6	3.4	3.6	3.2	5.2	2.3	2.2
Dissolved Ferrous Iron	mg/L	2.7	3.5	3.1	2.3	4.4	1.9	1.9
Copper	mg/L	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02
Manganese	mg/L	0.019	0.017	0.0088 J	0.012	0.016	0.011	0.011
Dissolved Oxygen	mg/L	0.49	0.7	1	0.79	1.05	0.78	NM
Oxidation Reduction Potential	mV	-124	-78	-88	-90	-78	-84	NM
pH		6.85	6.7	6.69	6.92	6.6	6.81	NM
Turbidity	NTU	10.9	63.8	8.4	21.1	29.7	59.8	NM
Specific Conductance	µmhos/cm	396	729	820	479	882	570	NM
Temperature	°C	23.1	23.7	25.5	25.2	29.1	23.3	NM

[a]µg/L = micrograms per liter.
mg/L = milligrams per liter.
mV = millivolt.
NTU = Nephelometric Turbidity Units.
µmhos/cm = micromhos per centimeter.
NM = not measured.

Table 6. Bioavailable Iron Data From Soil Samples Collected Near the Pilot Test Wells During the Baseline Sampling Event in March 2003

Soil Sample Location	Sample Depth (feet below land surface)	Ambient Fe^{+2} (g/kg)	Bioavailable Fe^{+3} (g/kg)
Near 0514	20	0.16	< 0.07
Near 0514	30	0.11	< 0.07
Near 0526	12	0.17	0.15
Near 0526	22	0.18	< 0.07
Near S73C	12	0.37	< 0.07
Near S73C	22	0.14	0.076

Appendix A

Pilot Test Subcontractor's Final Report

FINAL REPORT

S.M. Stoller Corporation
Grand Junction, CO
Contract Number 03-733-SF

In-Situ Enhanced Bioremediation Technology to Control the Plume of Dissolved Contaminants at the Building 100 Area of the Young-Rainey STAR Center Pilot Test

Revision 0

April 2004

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1.0 Introduction

Under S.M. Stoller Corporation (Stoller) Subcontract No. 03-733-SF, Safety and Ecology Corporation (SEC) was contracted to design and implement an in-situ enhanced bioremediation technology to control the plume of dissolved contaminants at the Building 100 Area of the Young-Rainey STAR Center. SEC designed and performed the injection of Hydrogen Release Compound (HRC) from March 11 to March 14, 2003 in accordance with an approved Remediation Plan as part of a pilot test to assess the efficacy of the technology at treating site contaminants. SEC performed sampling of groundwater on the following dates:

- March 10, 2003 (Baseline event),
- April 8, 2003 (P1 event),
- June 10, 2003 (P2 event),
- August 5, 2003 (P3 event),
- September 23, 2003 (P4 event),
- November 18, 2003 (P5 event),
- January 13, 2004 (P6 event), and
- March 2, 2004 (P7 event).

The purpose of these sampling events was to track contaminant concentrations and other groundwater parameters. A sampling report summarizing the results was prepared for each event. A previous report, entitled "Injection Completion Report" was developed and submitted to summarize injection activities. This document (the Final Report) summarizes project sampling and analytical activities from initial baseline sampling through completion of the P7 sampling event and discusses the results achieved over the one-year life-cycle of the project and how these results can be used to design an effective barrier for full-scale plume control.

1.1 Scope and Objectives

The primary objective of the pilot scale bioremediation project is to design, implement, monitor and summarize the results of the enhanced bioremediation technology and assess its applicability to full-scale plume control. The pilot test is being performed to determine the efficacy of the technology for reducing contaminants to concentrations below the remediation goals and is being critically evaluated to determine if the bioremediation technology will control the dissolved phase contaminant plume near the eastern and southern boundaries of the Building 100 Area, so that contaminant concentrations above remediation goals do not move off property. Remediation goals are listed below in Table 1. Two overall objectives were developed for the pilot test remediation:

- Demonstrate the effectiveness of the technology at treating the contaminants to the remediation goals as listed below
- Minimize the disruption of business activities at the Center and minimize the visual impact of remediation to the public and Center employees.

Table 1. Remediation goals and maximum observed concentrations in the area of interest for the contaminants of concern at the STAR Center.

COC	*Remediation Goals (ug/L)*	*Maximum Observed Concentration (ug/L)*
PCE	3	Not detected
TCE	3	0.68
cis-1,2-DCE	70	99
trans-1,2-DCE	100	130
1,1-DCE	7	1.2
VC	1	120
1,1,1-TCA	200	Not detected
1,1-DCA	70	38
Chloroethane	12	Not detected
Benzene	1	Not detected

Sampling and analysis objectives for the pilot test include:

- Track intermediate progress and bioremediation success during the period of performance by assessing contaminant levels at specified monitoring periods
    - Determine the most effective and efficient design for optimal reductive dechlorination
- Track aquifer conditions during the period of performance to assess the efficacy of the HRC at producing conditions conducive to bioremediation
- Confirm that bioremediation goals have been met (contamination levels are at or below remediation goals listed in Table 1) at the conclusion of the pilot test.

A total of three monitoring wells were sampled during each assessment. Each monitoring well corresponds directly with an HRC treatment area. The monitoring wells that were sampled during each sampling event include:

- Well PIN 12-0514
- Well PIN 12-0526
- Well PIN 12-S73C

The locations of these wells are shown below in Figure 1.

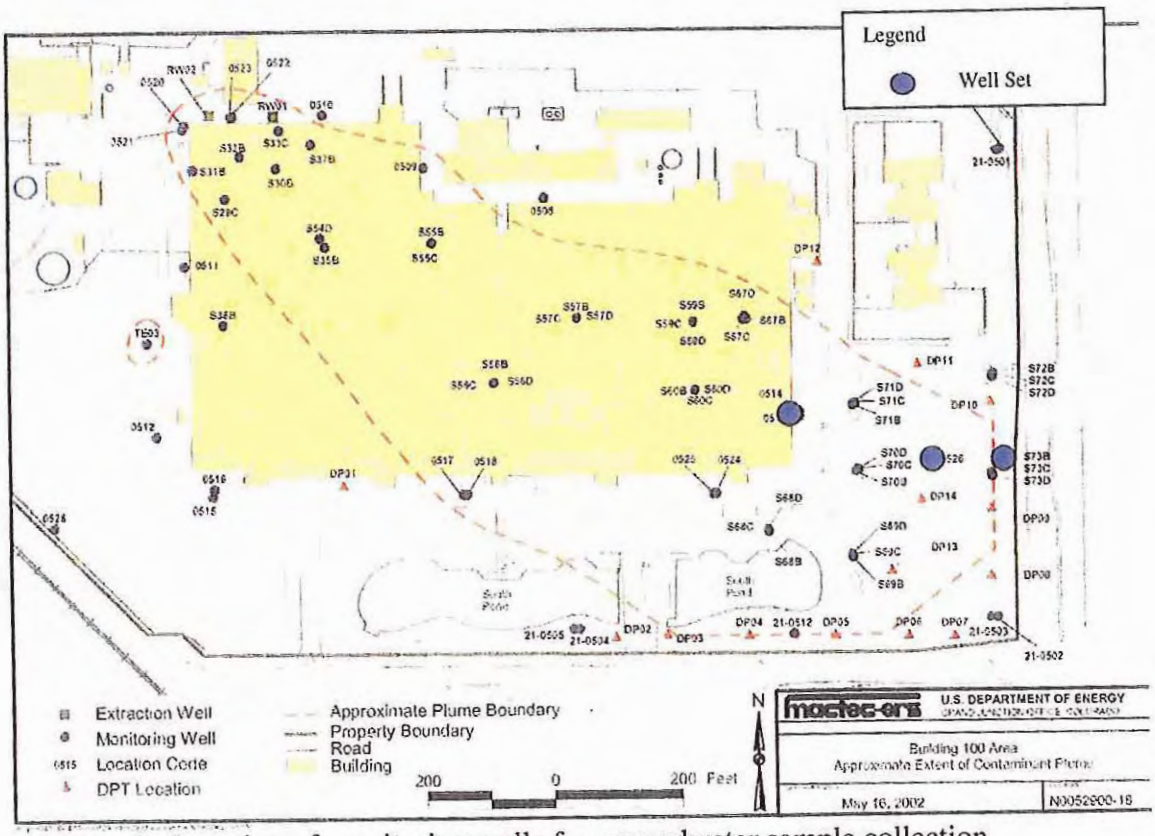


Figure 1. Location of monitoring wells for groundwater sample collection.

2.0 Injection Design

One of the stated goals of the pilot test was to determine the most effective and efficient injection point spacing and dose rate of HRC (per foot of injection interval) for achievement of remediation goals. To meet this goal, SEC and Regenesis designed the pilot test with different injection point spacing and dose rates in each injection area so the most effective and efficient design could be determined.

Table 2. Injection design parameters for each treatment area.

Area	Injection Point Spacing	Injection Point	Survey Data		Injection Interval (ft. bgs)	HRC dose (lbs. per foot)	Total HRC injected (lbs)
			Northing	Easting			
S73C	15 feet on center	5	1287213.0047	258923.6345	35 to 10	8	200
		6	1287198.0296	258923.6052	35 to 10	8	200
		7	1287183.0056	258923.6537	35 to 10	8	200
		34	1287172.3200	258935.1200	35 to 10	8	200
		35	1287187.3200	258935.1200	35 to 10	8	200
		36	1287202.3200	258935.1200	35 to 10	8	200
		37	1287193.8200	258950.1200	35 to 10	8	200
		38	1287178.8200	258949.0200	35 to 10	8	200
		39	1287161.8200	258948.7200	35 to 10	8	200
0526	12 feet on center	21	1287215.0900	258836.9800	35 to 10	6	150
		22	1287205.0900	258836.9800	35 to 10	6	150
		23	1287195.0900	258836.9800	35 to 10	6	150
		24	1287190.0900	258848.9800	35 to 10	6	150
		25	1287200.0900	258848.9800	35 to 10	6	150
		26	1287210.0900	258848.9800	35 to 10	6	150
		27	1287205.0900	258860.9800	35 to 10	6	150
		28	1287195.0900	258860.9800	35 to 10	6	150
		29	1287185.0900	258860.9800	35 to 10	6	150
0514	10 feet on center	2	1287279.5183	258642.4370	45 to 20	4.5	112.5
		3	1287288.8588	258642.6454	45 to 20	4.5	112.5
		4	1287298.2066	258642.9817	45 to 20	4.5	112.5
		14	1287275.3300	258648.9800	45 to 20	4.5	112.5
		15	1287285.3300	258648.9800	45 to 20	4.5	112.5
		16	1287295.3300	258648.9800	45 to 20	4.5	112.5
		17	1287290.3300	258658.9800	45 to 20	4.5	112.5
		18	1287280.3300	258658.9800	45 to 20	4.5	112.5
		19	1287269.6800	258658.9800	45 to 20	4.5	112.5

Actual injection point layouts for each injection area are depicted below in Figures 2 through 4.

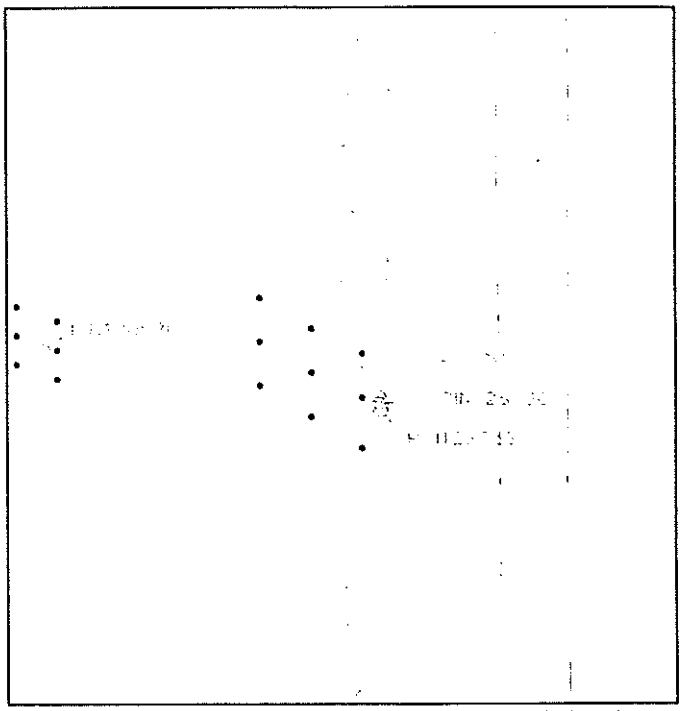


Figure 2. Injection point layout in PIN12-S73C injection area.

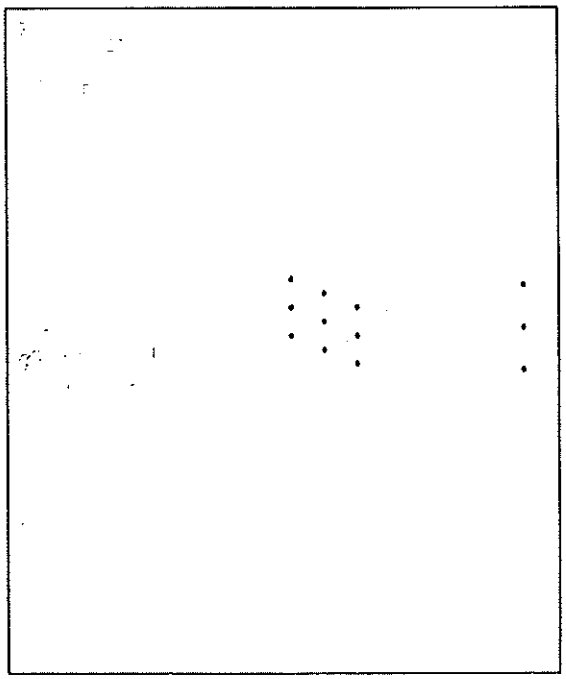


Figure 3. Injection point layout in PIN12-0526 injection area.

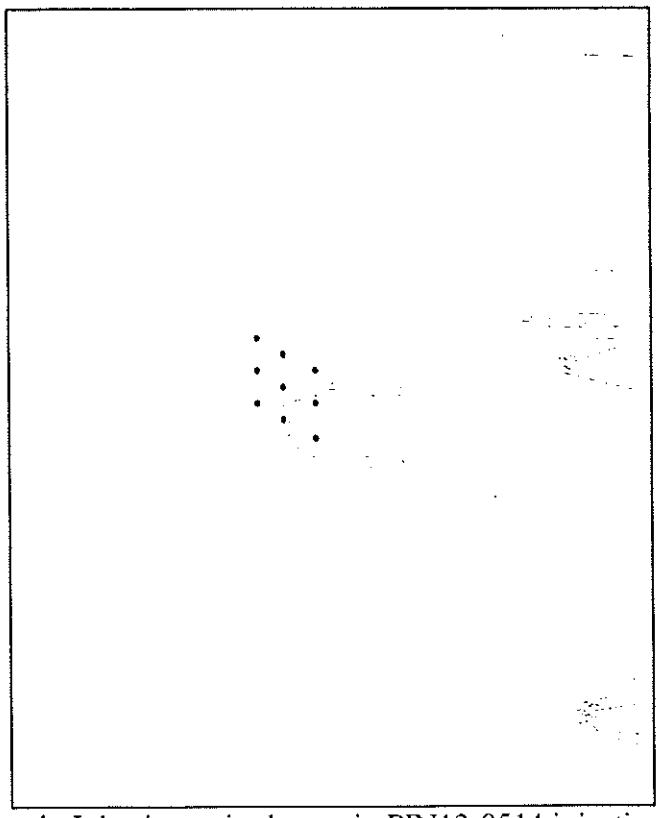


Figure 4. Injection point layout in PIN12-0514 injection area.

3.0 Sample Collection and Analysis

At each sampling event, groundwater sampling was performed through the use of dedicated bladder pumps and in accordance with the following *Pinellas Environmental Restoration Project Sampling Procedures:*

- FD 1000 Documentation Procedures
- FQ 1000 Field Quality Control Procedures
- FS 1000 General Sampling Procedures
- FS 2000 General Aqueous Sampling
- FS 2200 Groundwater Sampling
- FT 1000 General Field Testing and Measurement
- FT 1100 Field Measurement of Hydrogen Ion Activity
- FT 1200 Field Measurement of Specific Conductance
- FT 1400 Field Measurement of Temperature
- FT 1500 Field Measurement of Dissolved Oxygen
- FT 1600 Field Measurement of Turbidity
- FT 2100 Oxidation –Reduction Potential

Prior to sample collection, wells were purged using dedicated bladder pumps in accordance with sampling procedures, until selected parameters stabilized. Once parameters stabilized, groundwater samples were collected from each well. Samples were collected in containers (preservative already added as needed) provided by the laboratory. Samples were immediately cooled using ice and coolers. Samples were then delivered to Severn-Trent Laboratories (STL) in Tampa where they were then shipped to the Tallahassee lab location, prepped and analyzed.

Field parameters, including pH, dissolved oxygen (DO), oxygen-reduction potential (ORP), Temperature, Turbidity, and Conductivity, were analyzed onsite according to the referenced FDEP procedure. These field parameters were measured using a QED Micropurge Basics MP20 Flow Cell Meter (Serial Number MP20-1213). The unit was calibrated in accordance with FDEP procedures and manufacturer's guidelines prior to each sampling event.

Table 3, below, describes the groundwater samples collected and analyses performed during the each of the sampling events.

Table 3. Samples collected during performance of the pilot test.

Parameter	Methodology	Data Use	Number of Samples per Sampling Event							
			Baseline	P1	P2	P3	P4	P5	P6	P7
Chlorinated VOCs and remediation goal compounds	EPA 8021	Track Bioremediation progress by observing contaminant levels versus time	4	3	4	3	4	3	3	4
Trip Blank	EPA 8021	QA/QC Check	1	1	1	1	1	1	1	1
pH, DO, ORP, Temperature	SEC-EM-307	Track aquifer properties to determine if anaerobic conditions are being produced and reductive dechlorination conditions are favorable	4	3	4	3	4	3	3	4
TOC	EPA 415.1 or EPA 9060	TOC in the aquifer matrix influences contaminant migration, sorption and desorption and biodegradation rates.	4	3	0	3	0	3	0	4
Metabolic gases (carbon dioxide, ethane, ethane, methane)	EPA 003C or ASTM D1945	Track Bioremediation progress	4	3	4	3	4	3	3	4
Metabolic Acids (lactic, pyruvic, acetic, propionic, and butyric)	SW846 8000 and 8015	Track the distribution of HRC in the aquifer to assess if the design ensures sufficient coverage	4	3	4	3	4	3	3	4
Nitrate	EPA 353.1 or 9056	Substrate for microbial respiration in depleted oxygen environments	4	3	0	3	0	3	0	4
Bromide	SW846 9056 or SW846 9211	Track tracer movement in aquifer	4	3	4	3	4	3	3	4
Sulfate	EPA 375.3 or 9056	Substrate for microbial respiration in depleted oxygen environments.	4	3	4	3	4	3	3	4
Total Dissolved Solids, color, Cl	EPA 0160.1, EPA 0110.1, and SW846 9056	Track aquifer conditions in zone of discharge for permit variance	4	3	0	3	0	3	0	4
Total and dissolved Fe and Mn	EPA 6000 (with filtered [0.1 to 0.4 um] and unfiltered samples)	Indicative of reductive conditions in aquifer that facilitate reductive dechlorination	4	3	4	3	4	3	3	4

4.0 Results

Sampling results for each well are presented below in Tables 4 through 6.

Table 4. Analytical results for Well PIN12-0514.

Parameter	Units	Baseline	P1	P2	P3	P4	P5	P6	P7
Date	NA	3/10/2003	4/8/2003	6/10/2003	8/5/2003	9/23/2003	11/18/2003	1/13/2004	3/2/2004
Temperature	Degrees F	74.3	74.8	76.3	76.2	76.7	76.5	75.4	75.4
Conductivity	mS/cm	1.65	1.64	1.64	1.62	1.57	1.63	1.57	1.55
pH	NA	6.5	6.53	6.17	6.02	6.77	6.79	7.06	7.05
ORP	mV	29	-18	-270	-289	-290	-155	-287	-220
DO	mg/L	0.76	0.14	0.09	0.08	0.16	0.16	0.49	0.4
Turbidity	NTU	132	124	118	132	188	163	150	156
PCE	ug/L	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U
TCE	ug/L	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U
cis-1,2-DCE	ug/L	35	52	39	16	8.8	15	8.7	6.9
trans-1,2-DCE	ug/L	37	29	51	39	48	49	31	32
1,1-DCE	ug/L	1.0U	1.0U	1.0U	1.0U	0.38J	1.0U	1.0U	1.0U
Vinyl Chloride	ug/L	52	44	22	56	38	29	22	20
1,1,1-TCA	ug/L	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U
1,1-DCA	ug/L	11	12	17	13	9.1	9.5	8.2	8.2
Chloroethane	ug/L	1.0U	1.0U	1.0U	1.0U	1.0U	2.4	0.42J	1.0U
Benzene	ug/L	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U
Total Organic Carbon	mg/L	64	58	NA	130	NA	59	NA	54
Methane	ug/L	450	360	410	1800	11000	5700	3600	1900
Ethene	ug/L	0.36	0.44	0.33	8.1	15	8.2	6.4	4.7
Ethane	ug/L	0.35U	0.35U	0.35	1	1.8U	1.2	1.2	0.35U
Carbon Dioxide	ug/L	98000	79000	170000	270000	230000	66000	80000	110000
Acetic Acid	mg/L	1.0U	1.0U	69	79	110	2.3	1.0U	0.5U
Propionic Acid	mg/L	1.0U	1.0U	17	49	59	1.4	1.0U	0.5U
Pyruvic Acid	mg/L	10U	10U	50U	10U	<100	10U	10U	2.0U
Butyric Acid	mg/L	1.0U	1.0U	3.7	12	20	1.0U	1.0U	0.5U
Lactic Acid	mg/L	25U	25U	25U	25U	<250	25U	25U	10U
Nitrate	mg/L	0.10UQ	0.10UQ	NA	0.1UQ	NA	0.1UQ	NA	0.1U
Sulfate	mg/L	140 F42	160	50	11	44	99D	76D	110
Chloride	mg/L	220 F42	210	NA	180	NA	230D	NA	210
Bromide	mg/L	0.50B	0.60B	0.92	0.99	1.6	0.49J	0.56J	0.30J
Iron (total)	ug/L	5100	5400	2000	770	1800	5200	4000	1800
Manganese (total)	ug/L	16	16	16	13	14	14	12	12
Iron (dissolved)	ug/L	2600	4100	1300	550	430	1200	1200	620
Manganese (dissolved)	ug/L	17	16	15	11	12	8.3J	7.7J	11
Color	PCU	180	200	NA	120	NA	250	NA	180Q
Total Dissolved Solids	mg/L	1100	1000	NA	1000	NA	1000	NA	1100

Table 5. Analytical results for Well PIN12-0526

Parameter	Units	Baseline	P1	P2	P3	P4	P5	P6	P7
Date	**NA**	**3/10/2003**	**4/8/2003**	**6/10/2003**	**8/5/2003**	**9/23/2003**	**11/18/2003**	**1/13/2004**	**3/2/2004**
Temperature	Degrees F	85.1	84	87.6	86.4	88.2	86.4	85.3	85.3
Conductivity	mS/cm	2.09	2.3	2.1	2.12	2.13	1.98	1.97	1.95
pH	NA	6.54	6.55	6.3	6.25	6.8	6.97	7.03	7.03
ORP	mV	-18	-37	-84	-83	-40	-78	-309	-256
DO	mg/L	4.31	0.11	0.06	0.01	0.2	0.45	0.34	0.18
Turbidity	NTU	45.9	38.9	42.5	48	48	48	66	72
PCE	ug/L	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U
TCE	ug/L	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U
cis-1,2-DCE	ug/L	4.9	8	6.1	4	2.5	9.2	9.4	3.2
trans-1,2-DCE	ug/L	2.4	3.6	3.0	1.8	2.5	4.3	3.4	1.6
1,1-DCE	ug/L	1.0U	1.0U	1.0U	1.0U	0.36J	1.0U	1.0U	1.0U
Vinyl Chloride	ug/L	2	3.2	3.3	2.5	1.7	3	3	1.1
1,1,1-TCA	ug/L	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U
1,1-DCA	ug/L	1.0U	1.0U	1.0U	1.0U	0.73J	0.17J	1.0U	1.0U
Chloroethane	ug/L	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U
Benzene	ug/L	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U
Total Organic Carbon	mg/L	66	54	NA	72	NA	74	NA	64
Methane	ug/L	290	170	390	310	1100	1600	7300	6500
Ethene	ug/L	0.33U	0.33U	0.33U	0.33U	1.6U	0.33U	0.33U	0.33U
Ethane	ug/L	0.35U	0.35U	0.35U	0.35U	1.8U	0.35U	0.35U	0.35U
Carbon Dioxide	ug/L	110000	97000	110000	160000	200000	66000	100000	150000
Acetic Acid	mg/L	1.0U	1.0U	1.0U	1.0U	1	16D	51D	0.5U
Propionic Acid	mg/L	1.0U	1.0U	1.0U	1.0U	1	11	16D	0.5U
Pyruvic Acid	mg/L	10U	10U	10U	10U	<10	10U	10U	2.0U
Butyric Acid	mg/L	1.0U	1.0U	1.0U	1.0U	<1	1.0U	1	0.5U
Lactic Acid	mg/L	25U	25U	25U	25U	<25	25U	25U	10U
Nitrate	mg/L	0.10UQ	0.21Q	NA	0.1UQ	NA	0.10UQ	NA	0.1U
Sulfate	mg/L	210 F42	230	200D	200	220D	110D	66D	140
Chloride	mg/L	300 F42	300	NA	330	NA	280D	NA	330
Bromide	mg/L	1.4	1.8	2.1	2.1	1.5	1.5	1.1	1.3
Iron (total)	ug/L	8700	9700	8600	7500	5800	1800	2300	1100
Manganese (total)	ug/L	17	17	16	17	16	19	13	14
Iron (dissolved)	ug/L	3900	5300	3900	2500	3500	1100	1300	540
Manganese (dissolved)	ug/L	16	18	16	16	16	20	12	13
Color	PCU	180	200	NA	140	NA	120	NA	120Q
Total Dissolved Solids	mg/L	1300	1400	NA	1300	NA	1100	NA	1400

Table 6. Analytical results for Well PIN12-S73C

Parameter	Units	Baseline	P1	P2	P3	P4	P5	P6	P7
Date	NA	3/10/2003	4/8/2003	6/10/2003	8/5/2003	9/23/2003	11/18/2003	1/13/2004	3/2/2004
Temperature	Degrees F	81.8	83.9	84.6	86.1	86.5	85.1	80.5	80.1
Conductivity	mS/cm	1.61	1.61	1.6	1.61	1.61	1.86	1.67	1.77
pH	NA	6.39	6.51	6.15	6.05	6.85	6.71	6.88	6.98
ORP	mV	-9	-56	-168	-136	-40	-18	-6	-28
DO	mg/L	0.19	0.14	0.07	0.07	0.25	0.3	0.31	0.38
Turbidity	NTU	179	157	142	155	166	87	60	66
PCE	ug/L	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U
TCE	ug/L	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U
cis-1,2-DCE	ug/L	16	24	22	27	16	17	20	12
trans-1,2-DCE	ug/L	7.8	11	9.6	13	7.8	7.6	8.9	9.4
1,1-DCE	ug/L	1.0U	1.0U	1.0U	1.0U	0.51J	1.0U	1.0U	0.23J
Vinyl Chloride	ug/L	9.4	15	14	18	9.3	6.5	11	12
1,1,1-TCA	ug/L	1.0U	1.0U	1.5	1.0U	1.0U	1.0U	1.0U	1.0U
1,1-DCA	ug/L	1.2	1.7	1.1	1.9	0.99J	0.45J	0.39J	0.36J
Chloroethane	ug/L	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U
Benzene	ug/L	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U	1.0U
Total Organic Carbon	mg/L	75	58	NA	50	NA	210	NA	110
Methane	ug/L	480	1500	510	700	1400	6200	4600	8700
Ethene	ug/L	0.33U	0.36	0.33	0.48	0.33U	0.35U	0.33U	0.33U
Ethane	ug/L	0.35U	0.46	0.35	0.43	0.35U	0.38	0.35U	0.35U
Carbon Dioxide	ug/L	100000	100000	120000	150000	160000D	93000	130000	120000
Acetic Acid	mg/L	1.0U	1.0U	8.2	1	<1	120D	26D	46
Propionic Acid	mg/L	1.0U	1.0U	14	1.4	1.4	110D	33D	31
Pyruvic Acid	mg/L	10U	10U	10U	10U	<10	10U	10U	2.0U
Butyric Acid	mg/L	1.0U	1.0U	0.67J	1.0U	<1	25D	9.8	3.5
Lactic Acid	mg/L	25U	25U	25U	25U	<25	25U	25U	10U
Nitrate	mg/L	0.10UQ	0.18Q	NA	0.14Q	NA	0.10UQ	NA	0.1U
Sulfate	mg/L	33	30	14	24	22	1.1	7.6	3.3
Chloride	mg/L	270 F42	missing datum	NA	260	NA	300D	NA	300
Bromide	mg/L	0.68B	0.87B	1.6	1.4	0.81J	1.4	1.5	0.74J
Iron (total)	ug/L	14000	14000	12000	15000	12000	16000	13000	13000
Manganese (total)	ug/L	8.4B	8.1J	10	9.3	7.4J	9.1J	8.1J	7.2J
Iron (dissolved)	ug/L	2400	2100	970	1700	1300	2200	3300	1400
Manganese (dissolved)	ug/L	6.8B	6.9J	8.5	7.9J	7.9J	10	7.2J	6.6J
Color	PCU	180	200	NA	180	NA	120	NA	180Q
Total Dissolved Solids	mg/L	1100 Q1	970	NA	1100	NA	1200	NA	1100

Where: B – Compound was detected in the associated method blank.
U- Compound was analyzed for but not detected.
J- The reported value is between the laboratory method detection limit and the laboratory practical quantitation limit.
Q – Samples were analyzed outside holding time.

5.0 Interpretation and Discussion

In order for enhanced reductive dechlorination to be successful, both the electron donor (HRC) and electron acceptor (contaminant) must be in sufficient contact in the presence of the correct bacteria (in sufficient biomass). These three factors essentially control the process of reductive dechlorination. In order to affect these three factors, several engineering aspects can be manipulated, including:

- Delivery
- Distribution
- Degradation, and
- Duration

When designing the pilot test, SEC and Regenesis experimented with these design factors to determine the most effective and efficient HRC injection design for achievement of remediation goals and for application to full scale plume control.

During the design phase, indicator parameters were used to support the assumption that the correct bacteria were present to stimulate the dechlorination process. The prevalence of vinyl chloride indicated that some degree of dechlorination had occurred in the past, and, if properly stimulated, would likely occur again. Microbial sampling performed at the baseline event indicated that bacteria were either not present or (the more likely scenario) were present but inactive in groundwater taken from wells PIN12-0526 and PIN12-S73C. Bacteria were detected in groundwater samples taken from well PIN12-0514. However, microbial samples were taken again during the P4 sampling event (in September 2003) and bacteria were found to be present and active in groundwater samples collected from each well area, indicating that HRC injection had stimulated microbial growth and activity in the treatment areas.

During the design phase, the presence of electron acceptor was known – concentrations of contaminants had been well documented. In addition to the target contaminants, additional compounds can compete as electron acceptors. These compounds, such as iron, sulfate, and nitrate, also act as electron acceptors and will compete with the target contaminants to accept the electrons released from HRC. Sufficient HRC must be injected to satisfy the needs of these competing electron acceptors as well as the target contaminants. Historic concentrations and assumptions were used to determine competing electron acceptor concentrations. These concentrations and assumptions were very close to the actual situation, as revealed during subsequent sampling events. Inadequately calculating these concentrations could result in the early consumption of

electrons released from HRC, effectively prematurely halting the reductive dechlorination process.

Remembering that the presence of an electron acceptor is only part of the equation to successful dechlorination, Regenesis calculated the mass of electron donor needed to stoichiometically satisfy the demand (of both target contaminants and competing electron acceptors), with appropriate contingency factors included. Not only would the injection of HRC serve as an input of electron donor material, it would also serve as a food source to stimulate the growth and activation of the microbial biomass needed to mediate the dechlorination process.

The challenge was to determine the appropriate delivery mechanism to ensure that this mass of donor could effectively be in contact with the mass of acceptor for the maximum period of time to produce the needed reduction in contaminant mass. In each well area, SEC and Regenesis designed slightly different injection schemes to determine the most effective design for achieving reductive dechlorination at the site. Injection point spacing and injection dose rates were varied in each area. The following sections will discuss the results achieved in each injection area.

5.1 PIN 12-0514 Area

Results from the pilot test indicate that the injection design implemented at the well PIN 12-0514 area proved to be the most effective for stimulating reductive dechlorination at the site. Selected results are graphically displayed below, in Figures 5 through 9, and discussed in detail.

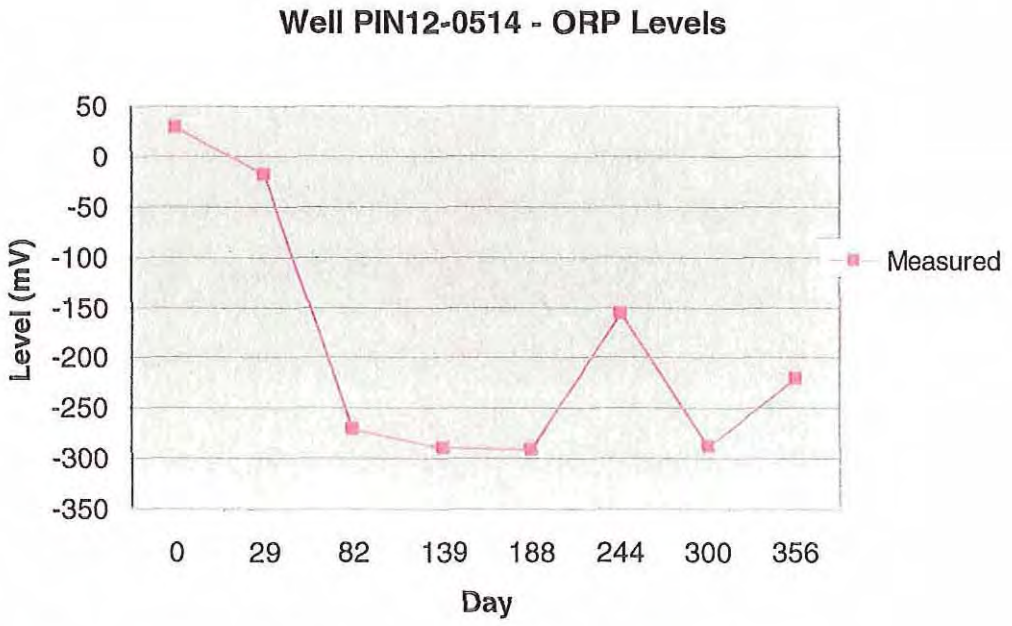


Figure 5. ORP levels in Well PIN12-0514 during pilot test.

Figure 5, above, depicts oxidation–reduction potential in the Well PIN12-0514 area during the pilot test. Reductive dechlorination is favored when ORP is below -200 mV. Prior to injection, conditions in the subsurface were slightly reducing. However, following injection, conditions became highly reducing, favoring the dechlorination process. Reducing conditions persisted in the subsurface for the entire period of the pilot test, but appear to be becoming less reducing as HRC reaches the end of its useful life in the subsurface.

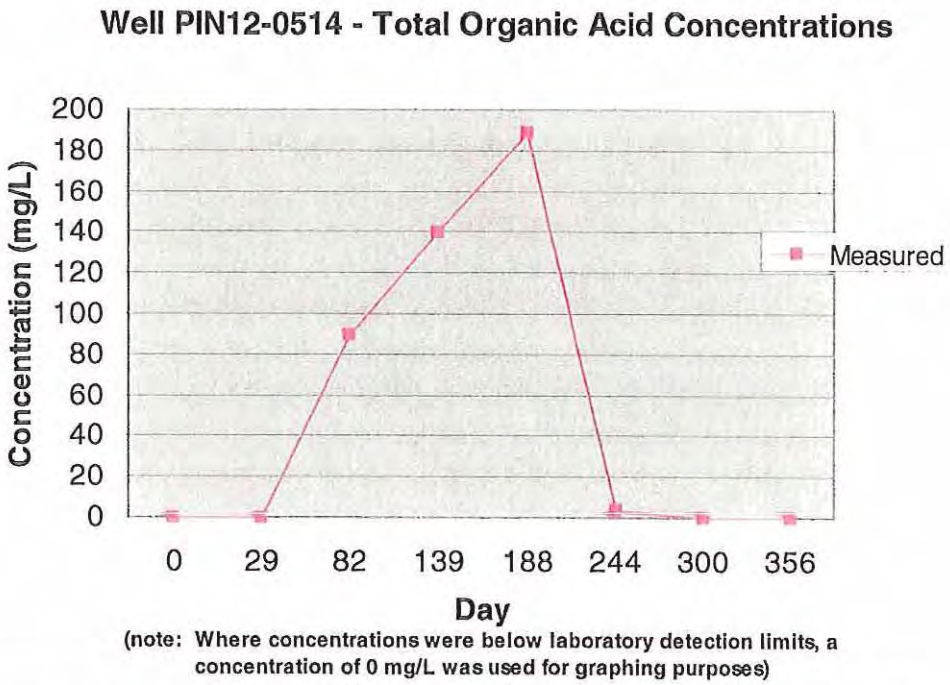


Figure 6. Total Organic Acid levels in Well PIN12-0514 during pilot test.

Figure 6, above, depicts total organic acid concentrations in the Well PIN12-0514 area during the pilot test. For graphing purposes, organic acid concentrations that were below the laboratory detection limits, were assumed to be zero. Total organic acid concentration includes the summation of the concentration of acetic, propionic, pyruvic, butyric, and lactic acids – all of which are produced during the step-wise metabolism of HRC. As the HRC is metabolized into the acids, the microbial biomass uses the released carbon as an energy source and the released hydrogen as an electron donor to replace chlorine on the contaminant (acceptor). Typically, organic acid concentrations in the 100 to 150 mg/L range indicate that HRC metabolism is occurring in the subsurface and conditions are becoming favorable for reductive dechlorination. Within one month of injection, apparent HRC metabolism was occurring in the well area. The injection point spacing in this area produced the most rapid increase in organic acid concentration. The longevity of the acids in the subsurface at the site appears to be approximately 200 days. Following this period, organic acid concentrations declined sharply. Reductive dechlorination can occur for several months following acid concentration decline because hydrogen released during metabolism of the acids remains for a longer time.

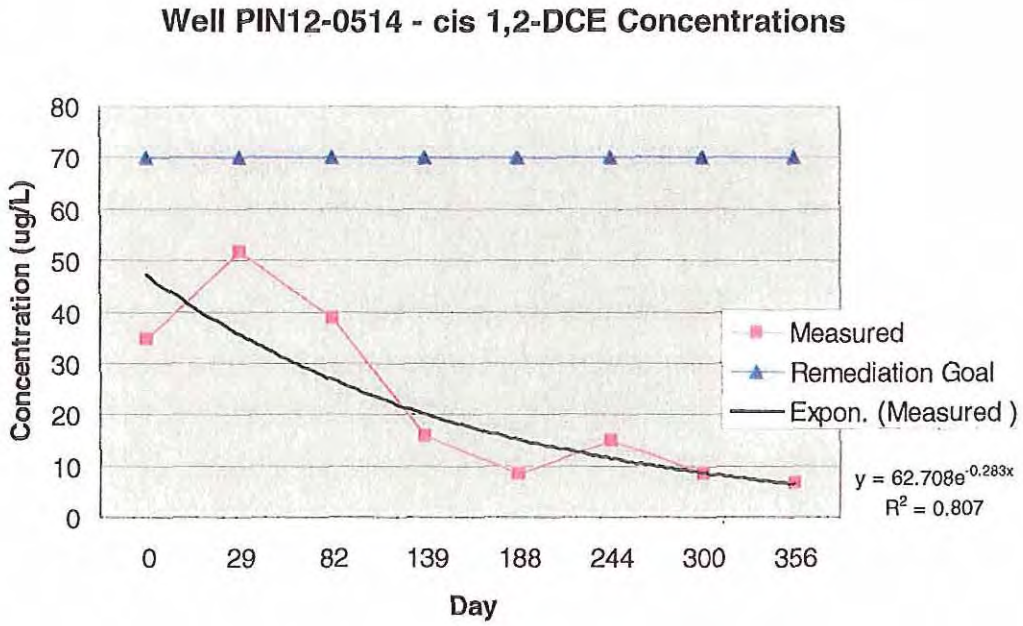


Figure 7. cis-1,2-DCE concentrations in Well PIN12-0514 during pilot test.

Figure 7, above, depicts cis-1,2-DCE concentrations in the Well PIN12-0514 area during the pilot test. Also shown in the graph is the remediation goal for cis-1,2-DCE and an exponential trend line fitted to the data. Along with the exponential trend line is the equation of the line (represented by the $y=62.708e^{-0.283x}$) and the relative fit of the trend line to the actual data (represented by the $R^2 = 0.807$). In general a fit the where R^2 is greater than or equal to 0.7 is considered a good fit. (An $R^2 = 1$ means that the trend line exactly fits the data points). The trend line above represents a fairly good fit to the data.

The decline in cis-1,2-DCE concentrations corresponds with the increase in reducing conditions and the increase in organic acid concentrations, shown earlier. Furthermore, an increase in ethene (shown below in Figure 9) that stoichiometically corresponds to the decrease in cis-1,2-DCE molar concentrations indicates the complete reductive dechlorination of the cis-1,2-DCE occurred in the treatment area. The complete dechlorination of cis-1,2-DCE provides strong evidence of the efficacy of HRC in the subsurface. The rapid distribution of HRC in the treatment area due to the close injection point spacing produced a slow reduction in cis-1,2-DCE concentrations.

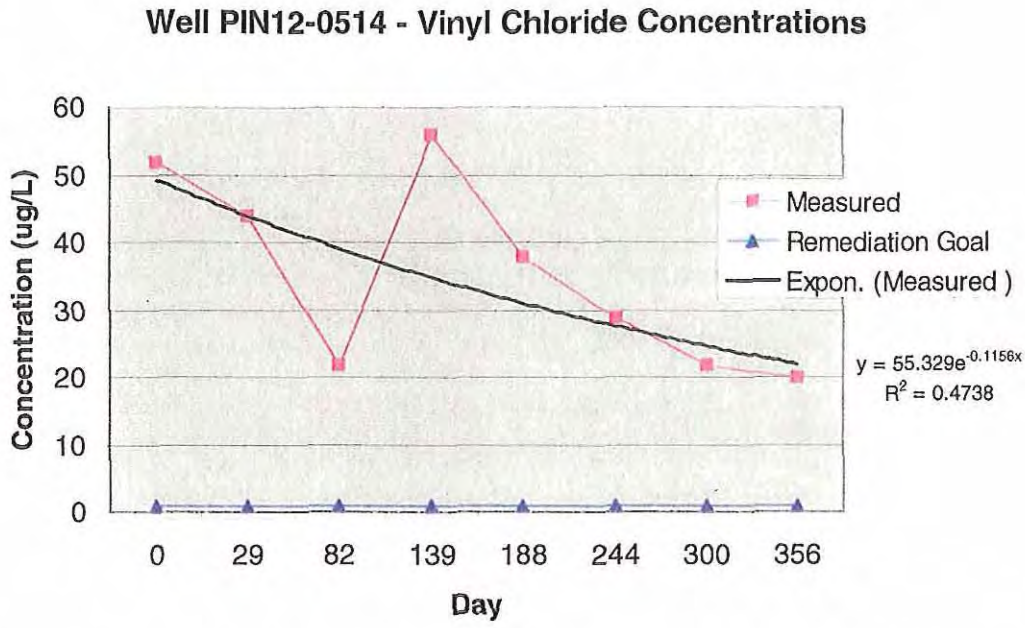


Figure 8. Vinyl Chloride concentrations in Well PIN12-0514 during pilot test.

Figure 8, above, depicts vinyl chloride concentrations in the Well PIN12-0514 area during the pilot test. Also shown in the graph is the remediation goal for vinyl chloride and an exponential trend line fitted to the data. The fit of the trend line to the data is marginal (as evidenced b the R^2 value). The poor fit is likely due to the sharp increase in concentration on day 139. Periodic spikes in vinyl chloride concentration, as seen on day 139, are expected as vinyl chloride is both produced (from the dechlorination of parent products) and degraded during the step-wise dechlorination process. However, the downward exponential trend line provides a fairly good fit to the data. The trend line indicates that continued decline in vinyl chloride concentrations can be expected, provided microbial stimulation continues to occur and electron donor continues to be readily available. The data indicate that although the remediation goal for vinyl chloride was not reached during the pilot test, the goal is certainly achievable. Additional HRC injection (or injection of extended release HRC (known as HRC-x) should provide sufficient energy source to continue to stimulate microbial activity and sufficient donor to continue the reduction in vinyl chloride concentration to meet remediation goals.

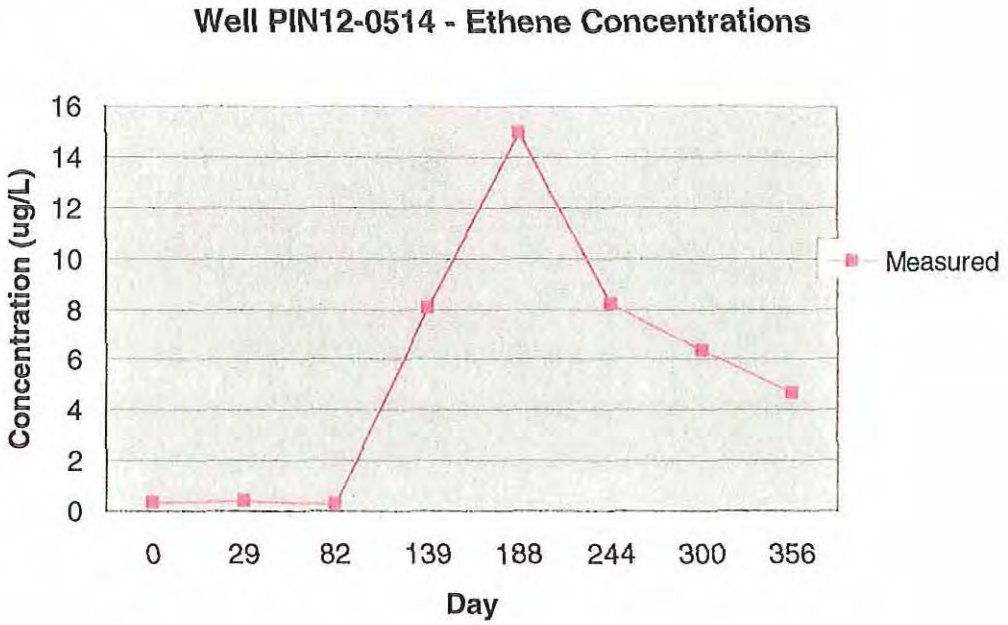


Figure 9. Ethene concentrations in Well PIN12-0514 during pilot test.

Figure 9, above, depicts ethene concentrations in the Well PIN12-0514 area during the pilot test. Ethene is the final end product produced during the complete reductive dechlorination of the chlorinated organic compounds such as cis-1,2-DCE and vinyl chloride. The sharp increase in ethene concentration corresponds to the decrease in vinyl chloride and cis-1,2-DCE concentrations, indicating that complete reductive dechlorination is occurring in the treatment area.

Data gathered from the Well PIN12-0514 treatment area throughout the pilot test indicate rapid and complete distribution of HRC in the subsurface. The rapid increase in organic acid concentrations, coupled with rapid decline in ORP and dissolved oxygen concentrations indicate that HRC was well distributed in the subsurface and that injection point spacing was sufficient. The decrease in contaminant concentrations and a corresponding increase in ethene production indicate that reductive dechlorination occurred in this treatment area. Furthermore, the data suggest that remediation goals for vinyl chloride can be met. Experimental and field data from other sites indicate that the useful life of HRC in the subsurface ranges from 12 to 18 months. After 12 months, continued reductions in contaminant concentrations are still being observed in this pilot test. However, ORP levels are becoming less reducing and organic acid concentrations have decreased indicating that HRC may be reaching the end of its lifecycle in the subsurface. The data indicate that the injection point spacing and dosing used in the Well 0514 treatment area are the most effective for the site and that a reinjection of HRC or injection of extended release HRC (HRC-x) would provide the donor and energy source needed to continue to stimulate dechlorination to meet remediation goals.

5.2 PIN 12-0526 Area

Results from the pilot test indicate that the injection design implemented at the well PIN 12-0526 did not prove to be an effective scheme for stimulating rapid reductive dechlorination at the site. HRC was not adequately and quickly distributed in the treatment area. Selected results are graphically displayed below, in Figures 10 through 14 and discussed in detail below.

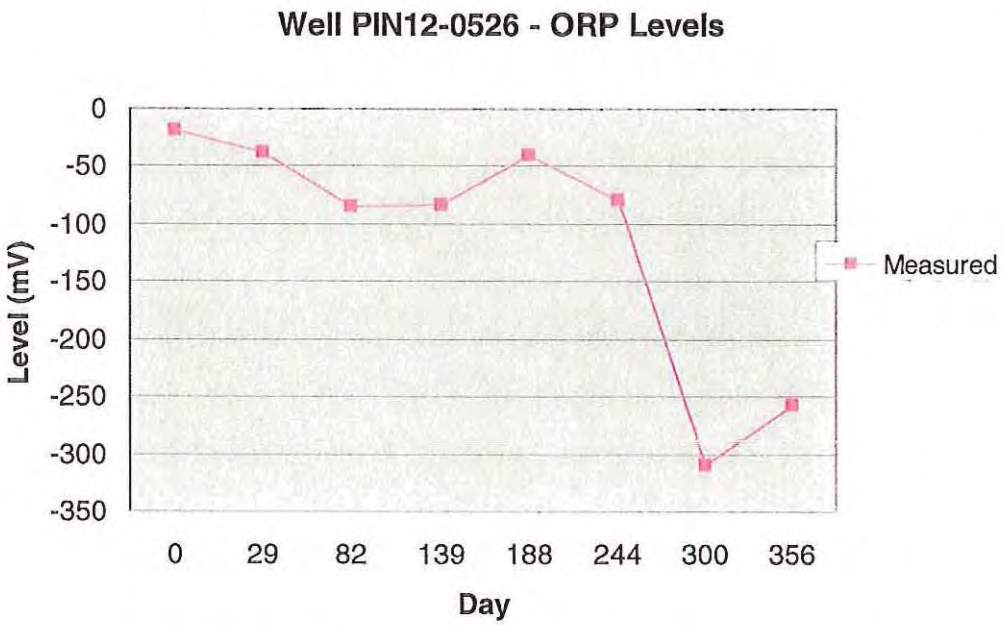


Figure 10. ORP levels in Well PIN12-0526 during pilot test.

Figure 10, above, depicts oxidation –reduction potential in the Well PIN12-0526 area during the pilot test. Reductive dechlorination is favored when ORP is below -200 mV. Prior to injection, conditions in the subsurface were slightly reducing. Unlike in the Well PIN12-0514 treatment area, reducing conditions favorable for dechlorination were not produced until approximately day 260. This significant lag in the production of reducing conditions may help to explain the absence of significant decreasing trends in contaminant concentrations until recently.

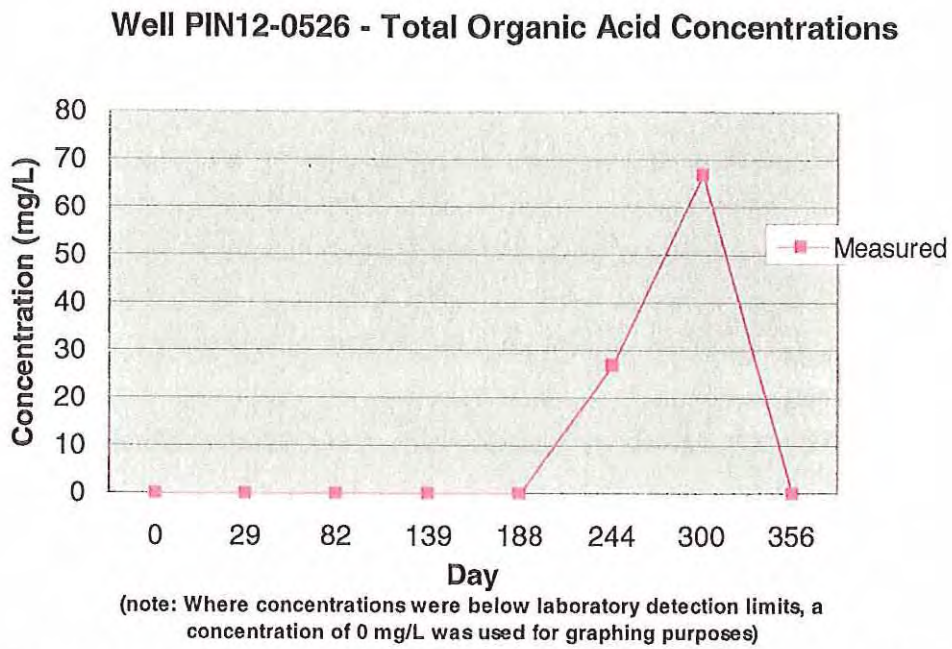


Figure 11. Total Organic Acids levels in Well PIN12-0526 during pilot test.

Figure 11, above, depicts total organic acid concentrations in the Well PIN12-0526 area during the pilot test. For graphing purposes, organic acid concentrations that were below the laboratory detection limits, were assumed to be zero. Again, a significant lag in organic acid concentration increase after injection is observed in this treatment area, which helps to explain the lag in development of reducing conditions as shown above and the lag in significant decreasing trends in the contaminant data, as shown below. The lack of organic acids in the subsurface at concentrations sufficient to stimulate significant reductive dechlorination may be due to inefficient injection point spacing.

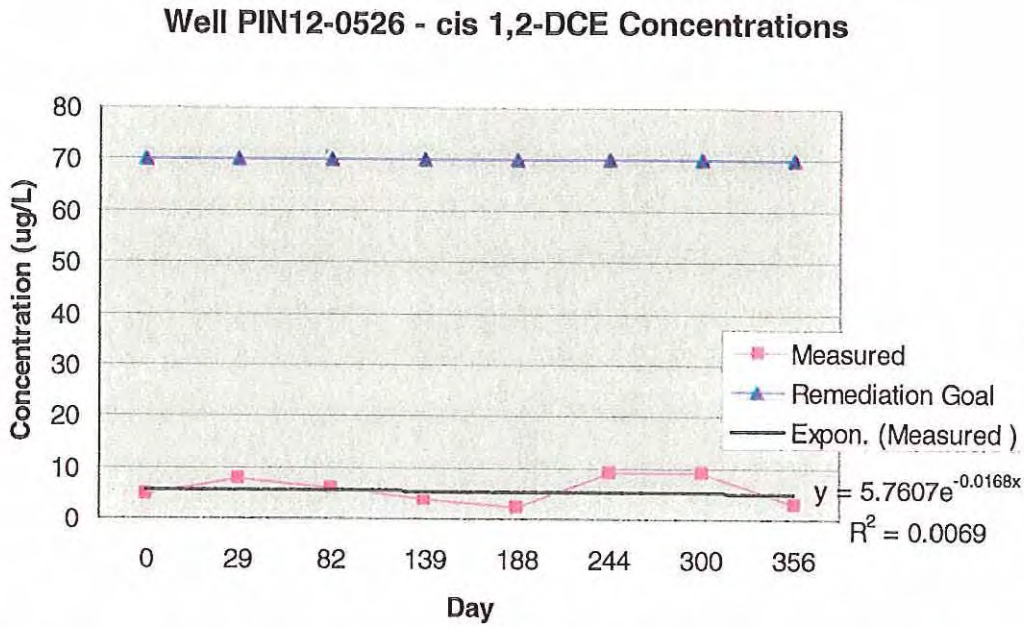


Figure 12. cis-1,2-DCE concentrations in Well PIN12-0526 during pilot test.

Figure 12, above, depicts cis-1,2-DCE concentrations in the Well PIN12-0526 area during the pilot test. Also shown in the graph is the remediation goal for cis-1,2-DCE and an exponential trend line fitted to the data. Although well below the remediation goal, the concentrations of cis-1,2-DCE never showed a significant downward trend. The fitted trend lines indicates a very slight downward trend in cis-1,2-DCE concentrations, however the fit of the line to the data is very poor, as evidenced by the very low R^2 value. Again, this poor trend may be due to ineffective distribution of HRC in the subsurface as indicated by the lag in development of reducing conditions and the lag in the increase in organic acid concentrations.

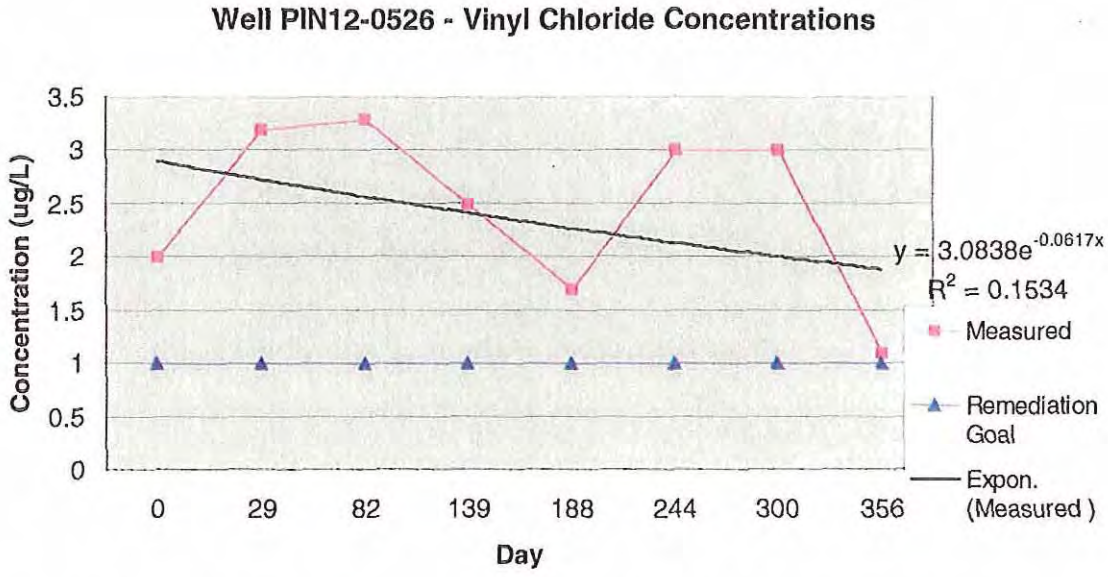


Figure 13. Vinyl Chloride concentrations in Well PIN12-0526 during pilot test.

Figure 13, above, depicts vinyl chloride concentrations in the Well PIN12-0526 area during the pilot test. Also shown in the graph is the remediation goal for vinyl chloride and an exponential trend line fitted to the data. Periodic spikes in vinyl chloride concentration, as seen on day 29 through 82 and day 244 through 300, are expected as vinyl chloride is both produced (from the dechlorination of parent products) and degraded during the step-wise dechlorination process. It does appear that concentrations of vinyl chloride are approaching remediation goals during the last sampling event. In fact, a strong decrease in contaminant concentrations toward then end of the test period corresponds to the presence of organic acids in the subsurface and the development of reducing conditions.

Overall, the data indicate a potential slight downward trend. However the fit of the line to the data is poor as evidenced by the very low R^2 value. Again, this poor trend may be due to ineffective distribution of HRC in the subsurface as indicated by the lag in development of reducing conditions and the lag in the increase in organic acid concentrations.

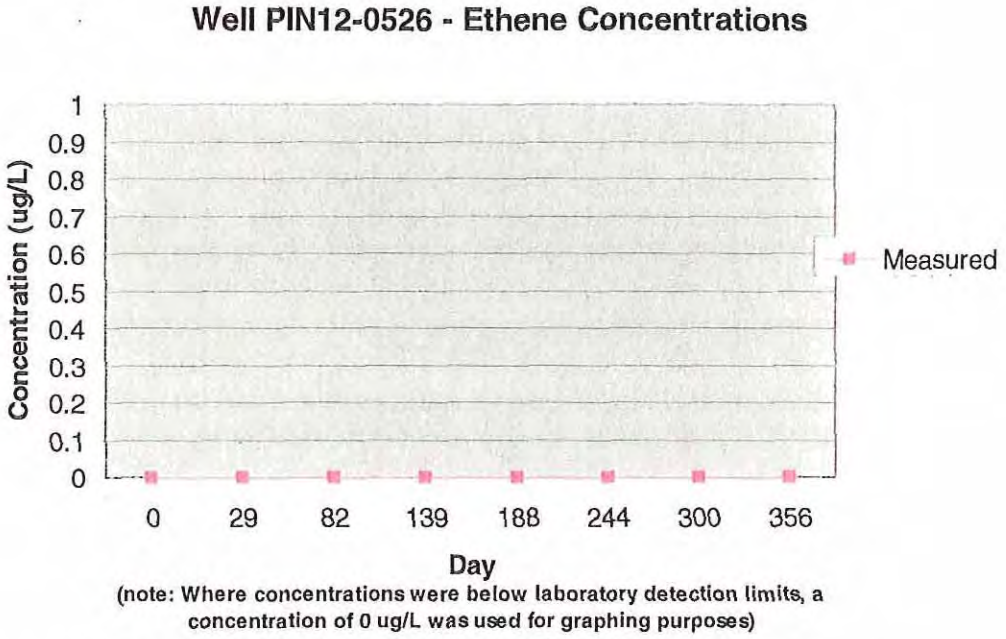


Figure 14. Ethene concentrations in Well PIN12-0526 during pilot test.

Figure 14, above, depicts ethene concentrations in the Well PIN12-0526 area during the pilot test. Ethene is the final end product produced during the complete reductive dechlorination of the chlorinated organic compounds such as cis-1,2-DCE and vinyl chloride. Ethene concentrations have never been detected in above laboratory detection limit (which is 0.33 ug/L) in this treatment area.

Data gathered from the Well PIN12-0526 treatment area throughout the pilot test indicate that HRC distribution in the treatment area has been slow to occur. Consequently, a lag in the production of reducing conditions and in the development of organic acids was also observed. The incomplete and inefficient distribution of HRC and development of conditions conducive to reductive dechlorination have resulted in weak decreasing trends in contaminant concentrations. The data indicate that the injection point spacing and dosing used in this treatment area are not the most effective for full scale plume control.

5.3 PIN 12-S73C Area

Results from the pilot test indicate that the injection design implemented at the well PIN 12-S73C did not prove to be an effective scheme for stimulating rapid reductive dechlorination at the site. HRC was not adequately and quickly distributed in the treatment area and trends in this area are very difficult to establish. Selected results are graphically displayed below, in Figures 15 through 19 and discussed in detail.

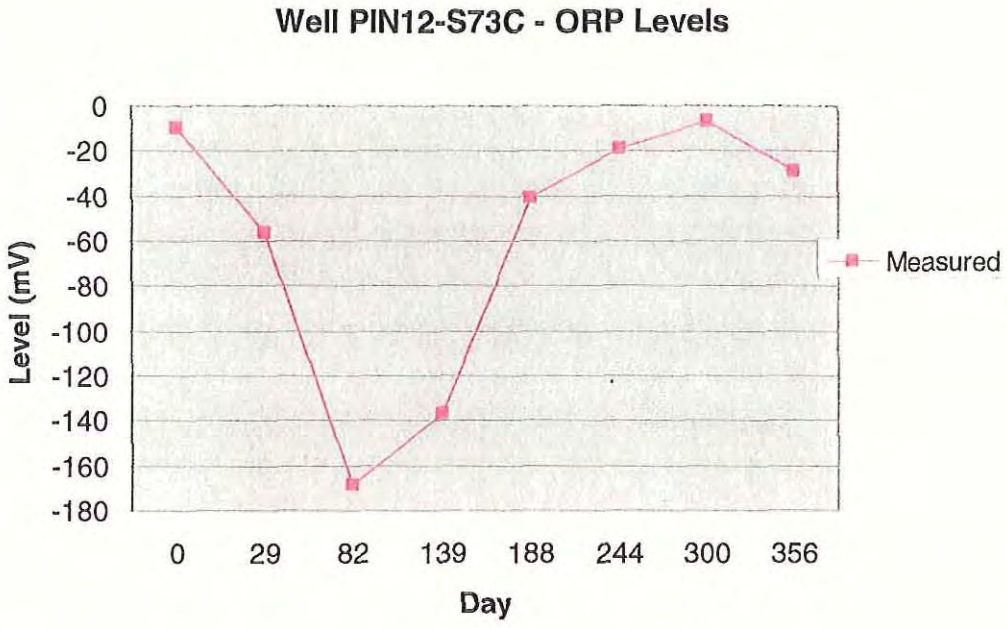


Figure 15. ORP levels in Well PIN12-S73C during pilot test.

Figure 15, above, depicts oxidation –reduction potential in the Well PIN12-S73C area during the pilot test. Reductive dechlorination is favored when ORP is below -200 mV. Prior to injection, conditions in the subsurface were slightly reducing. Conditions quickly became more reducing, but this trend did not last. Additionally, conditions never became reducing enough to significantly favor reductive dechlorination.

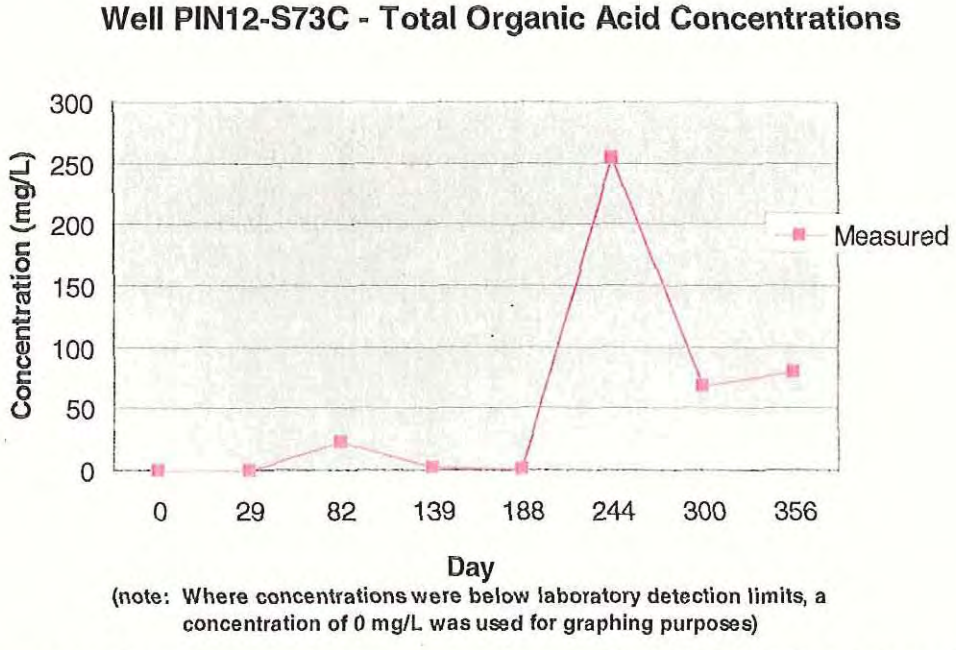


Figure 16. Total Organic Acid concentrations in Well PIN12-S73C during pilot test.

Figure 16, above, depicts total organic acid concentrations in the Well PIN12-S73C area during the pilot test. A significant lag in organic acid concentration increase after injection is observed in this treatment area, however, this lag does not correspond with the decrease in ORP noted above. The increase in organic acid concentrations does correspond with a decrease in vinyl chloride concentration, shown below, around day 200. The poor distribution of organic acids in the subsurface may be due to inefficient injection point spacing.

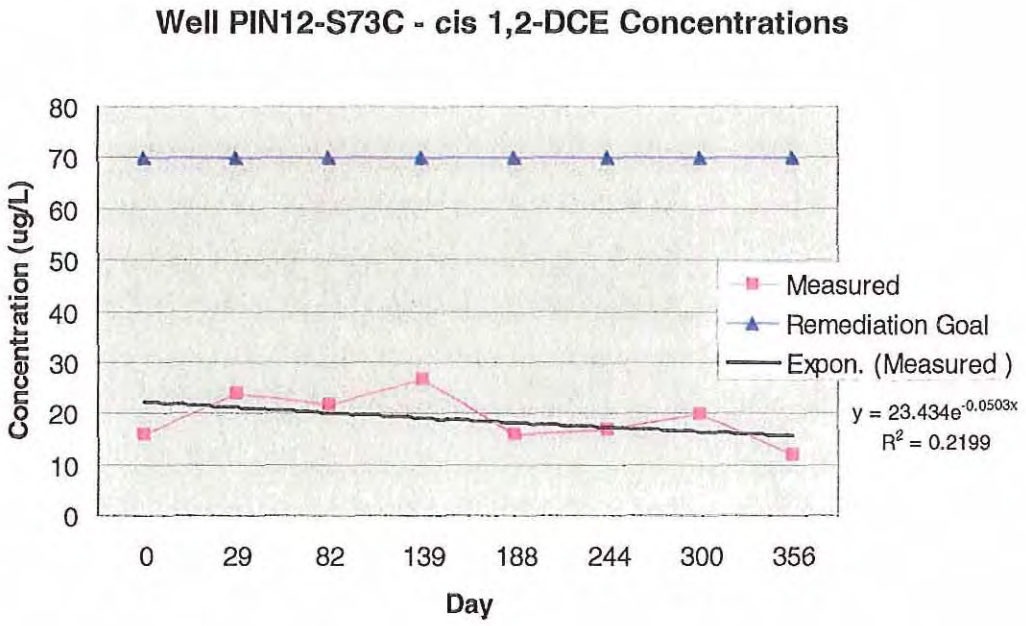


Figure 17. cis-1,2-DCE concentrations in Well PIN12-S73C during pilot test.

Figure 17, above, depicts cis-1,2-DCE concentrations in the Well PIN12-S73C area during the pilot test. Also shown in the graph is the remediation goal for cis-1,2-DCE and an exponential trend line fitted to the data. Although well below the remediation goal, the concentrations of cis-1,2-DCE shows a very slight downward trend and the fit of the trend line is not very good. Again, this poor trend may be due to ineffective distribution of HRC in the subsurface as indicated by the lag in development of reducing conditions and the transient increase in organic acid concentrations.

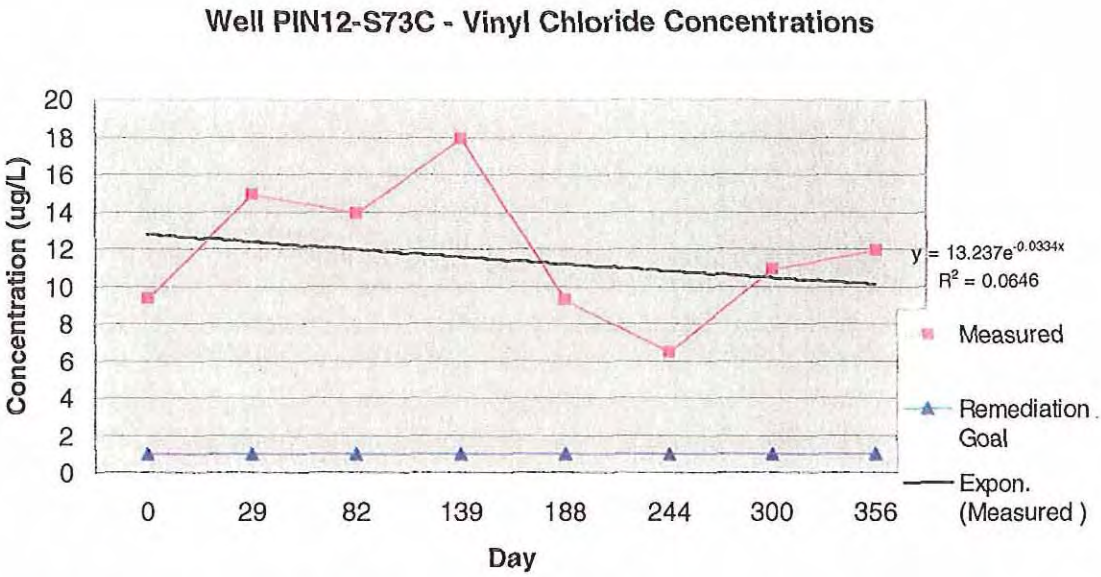


Figure 18. Vinyl Chloride concentrations in Well PIN12-S73C during pilot test.

Figure 18, above, depicts vinyl chloride concentrations in the Well PIN12-S73C area during the pilot test. Also shown in the graph is the remediation goal for vinyl chloride and an exponential trend line fitted to the data. Periodic spikes in vinyl chloride concentration are expected as vinyl chloride is both produced (from the dechlorination of parent products) and degraded during the step-wise dechlorination process. The data do not indicate any real trends in the concentrations. A transient decline in concentration, which corresponds to the increase in organic acid concentration, is observed between day 139 and day 244. However, concentrations then begin a steady increase and the fit of the trend line to the data is very poor. Again, this poor trend may be due to ineffective distribution of HRC in the subsurface as indicated by the lag in development of reducing conditions and the transient increase in organic acid concentrations.

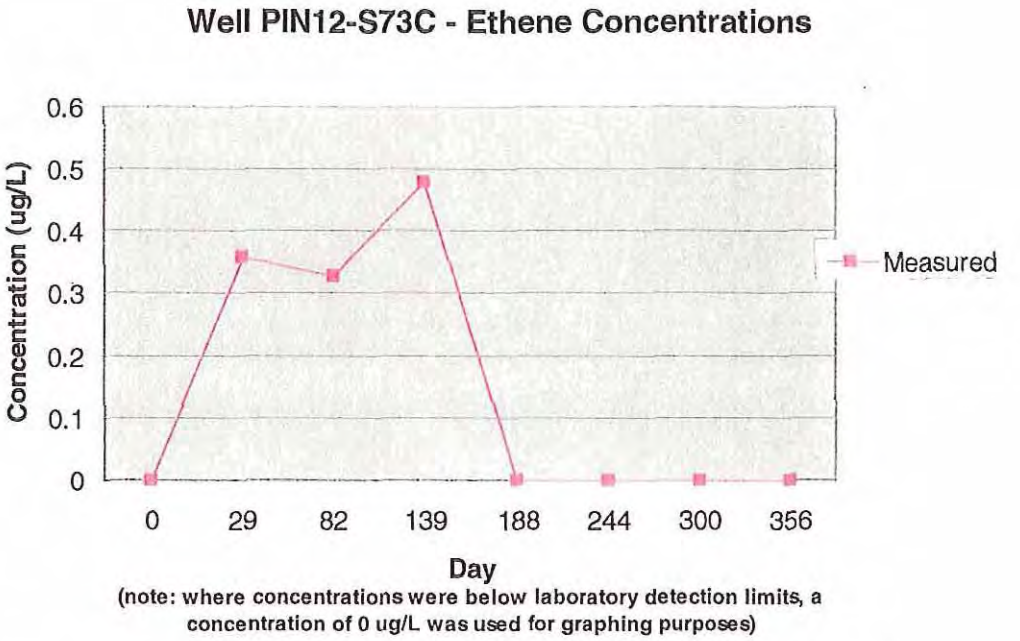


Figure 19. Ethene concentrations in Well PIN12-S73C during pilot test.

Figure 19, above, depicts ethene concentrations in the Well PIN12-S73C area during the pilot test. Ethene is the final end product produced during the complete reductive dechlorination of the chlorinated organic compounds such as cis-1,2-DCE and vinyl chloride. The increases in ethene concentration may be indicating that complete reductive dechlorination is occurring in the treatment area. However, significant sustained production of ethene has not been observed in the treatment area nor have sustained decreases in contaminant concentrations.

Data gathered from the Well PIN12-S73C treatment area throughout the pilot test indicate that HRC distribution in the treatment area has been slow to occur and trends are difficult to establish. The incomplete and inefficient distribution of HRC and development of conditions conducive to reductive dechlorination have resulted in weak decreasing trends in contaminant concentrations. The data indicate that the injection point spacing and dosing used in this treatment area are not the most effective for full scale plume control.

It appears from the pilot test data that the injection layout in the PIN 12-0514 area is the most effective design to achieve quick results.

5.4 Conclusions

When assessing success of HRC at enhancing reductive dechlorination in the subsurface, it is important to look at multiple lines of evidence. The inherent heterogeneity of the subsurface coupled with common difficulties in sampling and analyses (particularly with low concentrations) and other unknowns make it difficult to establish clear trends. Rather than looking solely at contaminant concentrations, other factors, including

production of metabolic gases and end products (such as ethene), increases in organic acids, and development of geochemical conditions conducive to dechlorination should also be considered.

Establishing clear trends in the Well PIN12-0526 and Well PIN12-S73C treatment areas is difficult. It appears that inefficient and incomplete distribution of HRC in the subsurface caused lags and inconsistencies in organic acid concentrations, development of reducing conditions, and development of downward trends in contaminant concentrations and upward trends in end-product concentrations. Geochemical parameters conducive to reductive dechlorination were not sustained in these treatment areas. The injection point spacing in these areas was greater than in the PIN12-0514 area. It appears that the closer injection point spacing resulted in the most rapid and complete dechlorination and reduction in contaminant concentrations.

Within the 0514 treatment area, the following lines of evidence were observed, which support the conclusion that reductive dechlorination was stimulated by injection of HRC.

- Rapid and significant increases in the concentration of organic acids, by-products of the metabolism of HRC in the subsurface, were observed. These increases corresponded with decreases in ORP and dissolved oxygen concentrations and the development of reducing conditions conducive to dechlorination. The increases lasted approximately 200 days, although reductive dechlorination appears to have continued throughout the remainder of the pilot test.
- Rapid and significant decreases in ORP were observed, which favors reductive dechlorination. These decreases were maintained throughout the 360 day period of performance, although conditions appear to be becoming less reducing towards the end of the pilot test.
- Significant downward trends were observed in both cis-1,2-DCE and vinyl chloride concentrations during the pilot test. Although remediation goals for vinyl chloride were not reached, data indicate that they are achievable if continued dechlorination occurs.
- Significant upward trends in ethene, an end-product of the reductive dechlorination process, were observed which stoichiometrically correspond with the decreases in contaminant concentrations, indicating that complete dechlorination is occurring.

One concern that is raised from the results of the test in the PIN 12-0514 test area is that the longevity of HRC in the subsurface is not sufficient to stimulate reductive dechlorination for a period of time long enough to reach remediation goals. Several potential solutions to this concern should be considered:

- Reinjection of HRC over a similar grid pattern after a period of 300 to 365 days.
- Injection of HRC-x (in some conjunction with injection of HRC), a slow-release formulation of HRC that will persist in the subsurface for up to 3 years to continue the stimulation of reductive dechlorination for a longer period of time

SEC and Regenesis have incorporated these lessons learned from the pilot test and developed a revised preliminary design for full scale plume design that is presented in Appendix A along with a cost estimate for implementation of this design which is included in Appendix B.

Appendix A
Preliminary Design for Full Scale Plume Control

PRELIMINARY DESIGN OF FULL SCALE PLUME CONTROL

S.M. Stoller Corporation
Grand Junction, CO
RFP-1639

In-Situ Enhanced Bioremediation Technology to Control the Plume of Dissolved Contaminants at the Building 100 Area of the Young-Rainey STAR Center

Full Scale Plume Control
April 2004

Prepared and Submitted by:
Safety and Ecology Corporation
SEC Business Center
2800 Solway Rd.
Knoxville, TN 37931
865-690-0501
865-539-9868 (fax)

SCOPE AND OBJECTIVES

The primary objectives of Phase II of the project are listed below:

- Use an enhanced bioremediation technology to control the plume of dissolved contaminants such that contaminants at concentrations above the remediation goals listed in *Table 1* do not move off the property.
- Implement the technology such that visual impacts to site employees, visitors, and the general public are minimized.
- Control the plume from moving offsite during the period of performance.
- Ensure the plume does not extend past its current boundaries.
- Comply with Stoller's Operational Plan.
- Comply with applicable regulatory requirements.

Table 1. Remediation goals and maximum observed concentrations in the area of interest for the contaminants of concern at the STAR Center.

COC	Remediation Goals (ug/L)	Maximum Observed Concentration (ug/L)
PCE	3	0.63
TCE	3	2
Cis-1,2-DCE	70	1800
Trans-1,2-DCE	100	130
1,1-DCE	7	430
VC	1	490
1,1,1-TCA	200	4.7
1,1-DCA	70	47
Chloroethane	12	14
Benzene	1	2.6

REMEDIATION ACTIVITIES

Overview of Remediation Activities

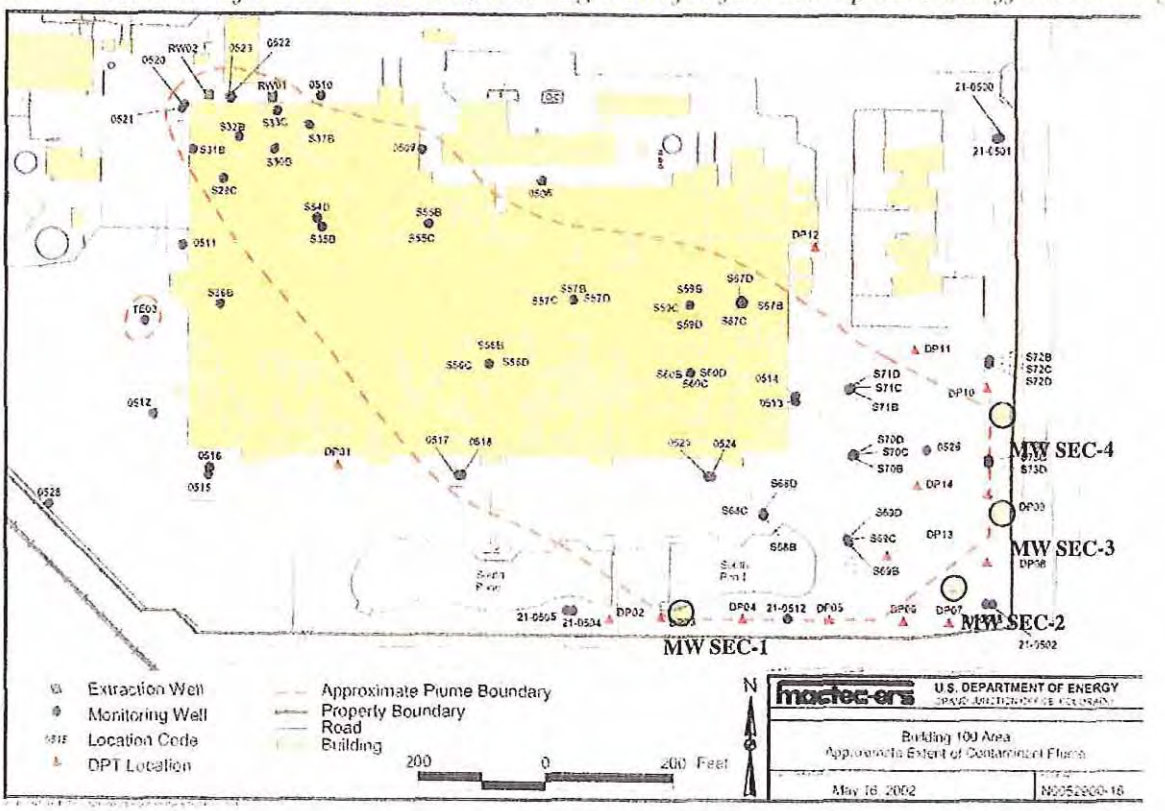
Based on information gathered during the pilot-scale testing conducted over the past year, Regenesis and SEC have developed a barrier-wall approach to remediation to ensure that contaminants (above regulatory levels) do not migrate off-site. The approach involves the installation of a barrier wall of HRC and Extended Release-HRC (HRC-X) along the southern and eastern boundaries of the current plume. The barrier wall will be created by injecting HRC and HRC-X along two rows of injection points. A total of 18,000 pounds of HRC and HRC-X will be injected to create the barrier-wall. The use of HRC-X will ensure that the wall remains effective for the entire length of the project period of performance (and for up to one year following project completion). Data gathered during the pilot test indicate that after a period of 200 days, organic acids were no longer detected in the remediation zone. The use of the

extended release formulation will ensure that organic acids continue to be detected in the treatment area during the entire period of performance.

Prior to injection, SEC will contract George F. Young, a local engineering company, to perform utility location in and around the injection areas. Exact locations of injection points may be moved slightly to offset known or discovered utility lines. Prior to advancement of a Geoprobe rod into the subsurface, SEC will either hand-excavate or utilize a drum-vac to remove the first four feet of soil from each injection point. Following injection, SEC will perform the necessary point restoration (i.e. ready-mix concrete or cold-patch asphalt, depending on the existing pavement).

Additionally, to facilitate the monitoring of contaminant levels, SEC will install four monitoring wells between the plume and site boundary. The monitoring wells will be installed at the locations indicated below in *Figure 1*. Monitoring wells will be of similar size and construction as the wells currently utilized on site.

Figure 1. Locations of SEC-installed monitoring wells for full-scale plume cutoff monitoring.



Once the injections have been performed, SEC will demobilize the injection equipment and on-site personnel. Support personnel will return to the site for periodic sampling and monitoring. Prior to final demobilization from the site, SEC will perform a walkdown of the site with Stoller personnel to ensure that all field activities are complete. Interim groundwater sampling and final verification groundwater sampling results will be reported to Stoller by SEC's project manager. After mobilization and analysis of the final samples, SEC will prepare a final report detailing project activities and results.

Injection Design Parameters

The overall design of SEC's remedial approach is based on the conceptualization of a three-dimensional rectangle of contaminated groundwater. The rectangle is created using the length of the barrier wall (approximately 900 feet) and the depth of the contaminated zone (approximately 25 feet). The barrier wall is approximately 20 feet in width. However the conceptual rectangle is wider, as the conceptual rectangle takes into account not only the width of the barrier wall, but also the width of the column of water that will pass through the wall over the period of performance of the cut-off project. Conservatively estimating the groundwater flow over the area is approximately 15 feet per year (1.25 feet per month) and the period of performance is 16 months, the conceptual rectangle is an additional 20 feet wide (1.25 feet per month x 16 months). The overall dimensions of the conceptual rectangle are then 900 feet x 25 feet x 40 feet. The volume of contaminated groundwater requiring treatment is then the volume of the rectangle, which is approximately 25,488,000 liters.

The overall design of the HRC injection is based on a mass balance between the mass of contaminants in the dissolved phase and the sorbed phase and the mass of HRC required to treat the contaminants. A number of additional demand factors and safety factors are built into the mass balance. The mass of contaminants was conservatively calculated using recent sampling data in the well PIN12-0526 well area, as this well is generally upgradient of the plume boundary and should be representative of concentrations at the plume boundary.

SEC's approach estimates the total mass of TCE and associated chlorinated hydrocarbons (in grams) present in pilot test area(s), and uses this mass to stoichiometrically calculate the mass of HRC required to reduce the contaminants into ethane and ethene. The total mass of TCE and other contaminants is equal to the dissolved phase contaminant concentrations and the sorbed phase contaminant concentrations. Sorbed phase contaminant concentrations were estimated using dissolved phase concentrations and site geochemical conditions.

These concentrations are input into the bioremediation model and used to perform a mass balance and stoichiometrically calculate the mass of HRC needed to dechlorinate the chlorinated hydrocarbons, with the understanding that dechlorination is a step-wise process. Both the dissolved phase and the sorbed phase masses contribute to the overall design of the remediation approach.

In addition to the overall mass of contamination, additional properties and factors must be considered. These additional parameters must be estimated or measured and factored into the design to accurately determine the mass of electron donor needed to both satisfy the demand factors and to enhance the reductive dechlorination of the target contaminants. These additional factors include:

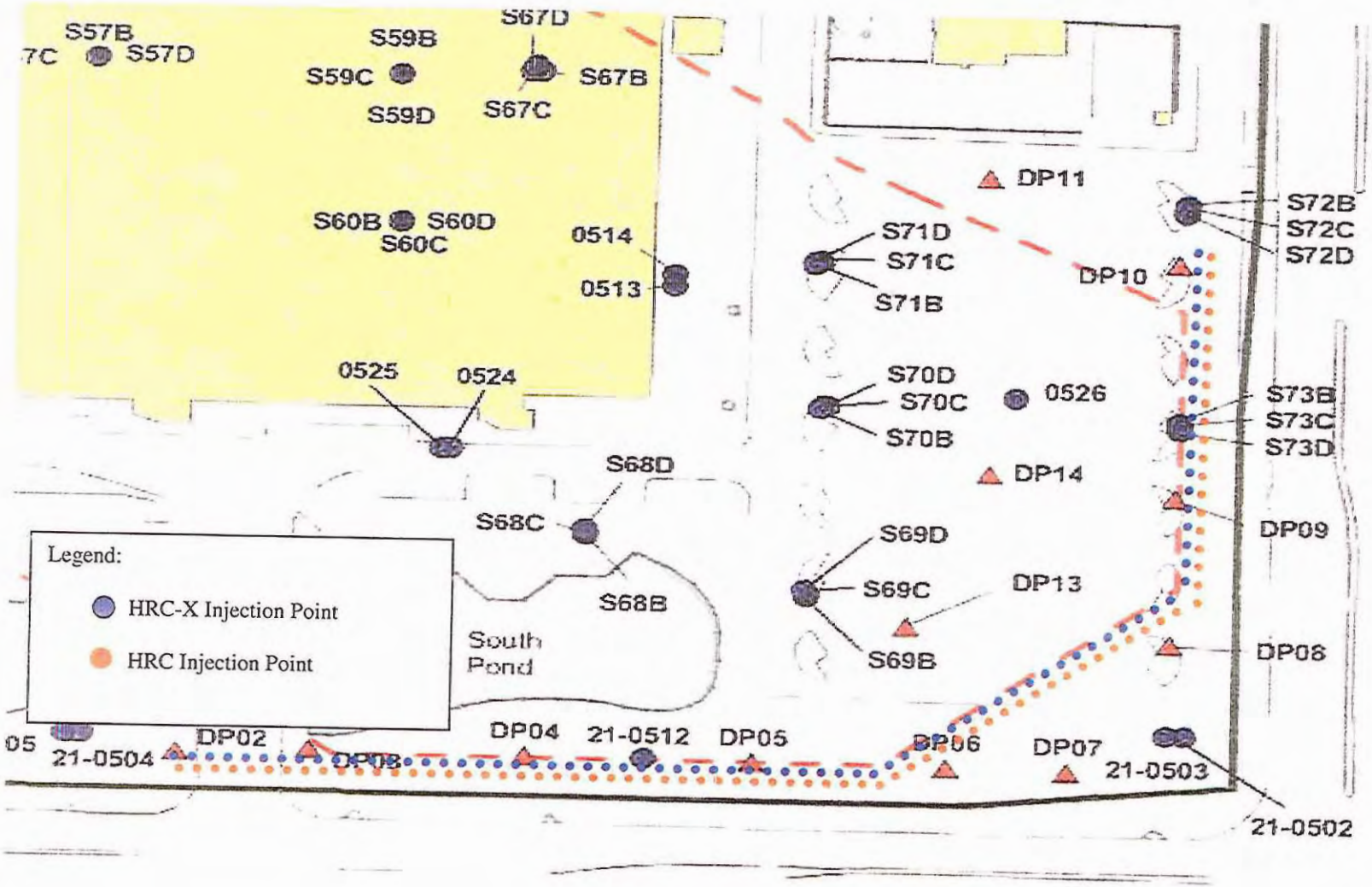
- Basic Site Characteristics – To effectively design the system, SEC and Regenesis input the basic site characteristics such as width and depth of the plume, thickness of the contaminated zone, porosity, hydraulic gradient and conductivity into the design model. These characteristics determine the depth of injection and the injection point spacing, and evaluate flow dynamics for the grid.
- Dissolved-Phase Groundwater Concentrations – The Dissolved phase concentrations were determined from previous sampling data and are representative of an entire hydrogeologic cycle, rather than a one-time monitoring event.

- Sorbed Phase Contaminant Mass – Generally the mass of contaminant bound to the saturated soil matrix is a multiple of the total dissolved contaminant mass. The mass of contaminant sorbed to the aquifer matrix is a function of the bulk density of the aquifer matrix, the fraction of organic carbon (foc) in the matrix, and the contaminant partitioning coefficient (Koc).
- Competing Electron Acceptor (CEA) Concentrations – The concentrations of dissolved competing electron acceptors such as oxygen, nitrate, ferric iron, and sulfate have an effect on the amount of HRC required to enhance bioremediation. Hydrogen from HRC is used to reduce these CEAs and create redox conditions that are conducive to reductive dechlorination. CEA concentrations were calculated from the previous sampling data and are representative of an entire hydrogeologic cycle, rather than a one-time monitoring event. These concentrations were input into the model to determine a conservative HRC dose amount that would satisfy the CEAs and dechlorinate the contaminants.
- Microbial Demand Factor – In addition to the contaminant and CEA demand for HRC, subsurface microbes will use some of the lactic acid as a source of energy or structural carbon. Therefore, when designing an initial or re-injection of HRC, these competing microbial processes must be taken into account. Sampling for the additional demand factor can be very difficult. SEC chose to rely on Regenesis' overly conservative demand factor of 3.
- Additional Demand Factor – Additional demand factor is used for uncertainty about the potential sinks for electron donor, and can be though of as a contingency. SEC chose to use a factor of 2 for additional demand for preliminary design purposes. It will become better defined after the initial injection and monitoring.

Injection Design

SEC and Regenesis have developed a barrier-wall approach to remediation to ensure that contaminants (above regulatory levels) do not migrate off-site. The approach involves the installation of a barrier wall of HRC and Extended Release-HRC (HRC-X) along the southern and eastern boundaries of the current plume. The barrier wall will be created by injecting HRC and HRC-X along two rows of injection points. Each injection point will be spaced 10 feet apart, and the two rows will be spaced 10 feet apart. Each row will consist of 90 injection points. The southernmost row will receive injection of HRC at 4 pounds per linear foot and will be located along the southern boundary of the existing plume. The next row (to the north) will receive injection of HRC-X at 4 pounds per linear foot and will be located 10 feet to the north of the initial injection row. The rows will follow the general contours of the existing plume and will extend roughly from MW-21-0504 to DP-10 (a total distance of approximately 900 feet). A conceptual design layout is depicted below in *Figure 2*.

Figure 2. Full-scale preliminary design for HRC barrier wall.



Injection details of the HRC barrier wall are presented below in *Table 2.*

Table 2. HRC barrier wall details.

Injection Detail	*Row 1 (southernmost)*	*Row 2 (15 feet north of Row 1)*
Injection Point Spacing within rows (ft.)	10 ft.	10 ft.
Injection Point Spacing (between rows)	10 ft.	10 ft.
Total injection points	90	90
Vertical extent of injection (ft.)	25 ft.	25 ft.
Amount of stimulant per linear foot per injection (lbs.)	4 lbs per ft.	4 lbs per ft.
Amount of stimulant injected per point (lbs.)	100	100
Total HRC injected (lbs.)	9,000 lbs.	0
Total ERHRC injected (lbs.)	0	9,000 lbs.
Total Stimulant Injection (HRC + ERHRC)	**18,000 lbs.**	

HRC-X ensures that the barrier wall will remain effective for the entire period of performance of the cut-off project. Created of the same formulation as HRC, the HRC-X has much more viscous consistency (approximately 1,000,000 centipoise) that will allow it to react with the groundwater much more slowly, extending its effective life in the aquifer. Studies have shown that HRC remains effective for as long as 14-18 months in the aquifer. HRC-X will remain active for a period of over 2.5 years. While the project is only expected to last for 16 months, SEC's approach will ensure that contaminants will not move off-site for over a year after the project is complete.

A number of buried utility lines have been identified within the remediation area. Injection activities will maintain at least a three-foot distance from these identified lines. SEC will contract with a local utility locator to identify buried utilities prior to injection. Injection points will be adjusted as needed depending on the results of the utility location.

Additionally, SEC will hand-excavate or drum-vac the first four feet of each injection point.

SAMPLING AND ANALYSIS

Objectives

Sampling and analysis objectives for the full scale plume control project include:

- Track intermediate progress and bioremediation success during the period of performance by assessing contaminant levels at specified monitoring periods.
- Track aquifer conditions during the period of performance to assess the efficacy of the HRC at producing conditions conducive to bioremediation.
- Confirm that bioremediation goals have been met (contamination levels are at or below regulatory limits) at the conclusion of the project.

- Confirm that contaminants are not moving off-site and contaminants are not spreading from existing plume boundaries.

Groundwater Sampling

SEC has chosen to perform full scale monitoring at quarterly intervals (rather than twice per year as specified as the minimum). The more frequent monitoring will allow for better tracking and trouble shooting (if necessary). The wells that have been chosen for the quarterly groundwater sampling as well as the preliminary sampling dates are shown in *Table 3*.

Table 3. Preliminary wells to be sampled by quarter

Quarter	*Wells Sampled*
Baseline	MW21-0504, MW21-0512, MW21-0502, MWS-73C, MWS-72C, MWSEC-1, MWSEC-2, MWSEC-3, MWSEC-4, MW 0526
F1	MW21-0504, MW21-0512, MW21-0502, MWS-73C, MWS-72C, MWSEC-1, MWSEC-2, MWSEC-3, MWSEC-4, MW 0526
F2	MW21-0504, MW21-0512, MW21-0502, MWS-73C, MWS-72C, MWSEC-1, MWSEC-2, MWSEC-3, MWSEC-4, MW 0526
F3	MW21-0504, MW21-0512, MW21-0502, MWS-73C, MWS-72C, MWSEC-1, MWSEC-2, MWSEC-3, MWSEC-4, MW 0526
F4	MW21-0504, MW21-0512, MW21-0502, MWS-73C, MWS-72C, MWSEC-1, MWSEC-2, MWSEC-3, MWSEC-4, MW 0526
F5	MW21-0504, MW21-0512, MW21-0502, MWS-73C, MWS-72C, MWSEC-1, MWSEC-2, MWSEC-3, MWSEC-4, MW 0526
F6	MW21-0504, MW21-0512, MW21-0502, MWS-73C, MWS-72C, MWSEC-1, MWSEC-2, MWSEC-3, MWSEC-4, MW 0526

Analyses

In addition to chlorinated hydrocarbon concentrations, a number of other important parameters will be analyzed to track bioremediation progress. *Table 4*, below, describes the number of samples and associated analyses that will be performed at each groundwater monitoring event.

Table 4. Quarterly groundwater sample analyses.

Sampling Event	*Number of Samples*	*Analysis Parameter*	*Methodology*	*Notes*
	10	Nitrate	EPA 353.1 or 9056	None
	10	Sulfate	EPA 375.3 or 9056	None
F2	20	Chlorinated VOCs and GCN-listed compounds	EPA 8021	1
	10	pH, Dissolved Oxygen (DO); Oxidation-Reduction Potential (ORP); Temperature	SEC-EM-307	None
	10	Metabolic Acids (including lactic, pyruvic, acetic, propionic and butyric) – generated from HRC release.	SW846 8000 and 8015	None
	10	Metabolic Gases (including hydrogen, methane, ethane, ethene, and carbon dioxide)	EPA 003C or ASTM D1945	None
	10	Total and dissolved Fe and Mn	EPA 6000 with filtered and unfiltered samples	None
F3	20	Chlorinated VOCs and GCN-listed compounds	EPA 8021	None
	10	pH, Dissolved Oxygen (DO); Oxidation-Reduction Potential (ORP); Temperature	SEC-EM-307	None
	10	Total Organic Carbon (TOC)	EPA 415.1 or EPA 9060	None
	10	Metabolic Acids (including lactic, pyruvic, acetic, propionic and butyric) – generated from HRC release.	SW846 8000 and 8015	None
	10	Metabolic Gases (including hydrogen, methane, ethane, ethene, and carbon dioxide)	EPA 003C or ASTM D1945	None
	10	Total and dissolved Fe and Mn	EPA 6000 with filtered and unfiltered samples	None

Table 4. Quarterly groundwater sample analyses.

Sampling Event	*Number of Samples*	*Analysis Parameter*	*Methodology*	*Notes*
	10	Nitrate	EPA 353.1 or 9056	None
	10	Sulfate	EPA 375.3 or 9056	None
	10	Total Dissolved Solids, color, Cl	Various	None
F4	20	Chlorinated VOCs and GCN-listed compounds	EPA 8021	1
	10	pH, Dissolved Oxygen (DO); Oxidation-Reduction Potential (ORP); Temperature	SEC-EM-307	None
	10	Metabolic Acids (including lactic, pyruvic, acetic, propionic and butyric) – generated from HRC release.	SW846 8000 and 8015	None
	10	Metabolic Gases (including hydrogen, methane, ethane, ethene, and carbon dioxide)	EPA 003C or ASTM D1945	None
	10	Total and dissolved Fe and Mn	EPA 6000 with filtered and unfiltered samples	None
F5	20	Chlorinated VOCs and GCN-listed compounds	EPA 8021	None
	10	pH, Dissolved Oxygen (DO); Oxidation-Reduction Potential (ORP); Temperature	SEC-EM-307	None
	10	Total Organic Carbon (TOC)	EPA 415.1 or EPA 9060	None
	10	Metabolic Acids (including lactic, pyruvic, acetic, propionic and butyric) – generated from HRC release.	SW846 8000 and 8015	None
	10	Metabolic Gases (including hydrogen, methane, ethane, ethene, and carbon dioxide)	EPA 003C or ASTM D1945	None

Table 4. Quarterly groundwater sample analyses.

Sampling Event	*Number of Samples*	*Analysis Parameter*	*Methodology*	*Notes*
	10	Total and dissolved Fe and Mn	EPA 6000 with filtered and unfiltered samples	None
	10	Nitrate	EPA 353.1 or 9056	None
	10	Sulfate	EPA 375.3 or 9056	None
	20	Chlorinated VOCs and GCN-listed compounds	EPA 8021	None
	10	pH, Dissolved Oxygen (DO); Oxidation-Reduction Potential (ORP); Temperature	SEC-EM-307	None
F6	10	Metabolic Acids (including lactic, pyruvic, acetic, propionic and butyric) – generated from HRC release.	SW846 8000 and 8015	None
	10	Metabolic Gases (including hydrogen, methane, ethane, ethene, and carbon dioxide)	EPA 003C or ASTM D1945	None
	10	Total and dissolved Fe and Mn	EPA 6000 with filtered and unfiltered samples	None
	10	Total Dissolved Solids, color, Cl	Various	None

Notes: 1) Duplicate samples will be taken from each of the wells for VOC analysis

Appendix B
Preliminary Budgetary Estimate for Implementation of Full Scale Plume Control

PROJECT: Building 100 Plume Control LOCATION: Largo, Florida

ITEM NO.	TASK DESCRIPTION	INITIAL ESTIMATED QUANTITY	UNIT OF MEASURE	UNIT PRICE	LINE ITEM EXTENDED PRICE
XXXXXXX	Year 1 Full Scale Remediation	XXXXXXXX	XXXXXXXXX	XXXXXXXX	XXXXXXXXXX
01	Submittal and Approval of Final Full Scale Design	1	LS	$ 15,189.40	$ 15,189.40
02	Obtain UIV Permit Exemption	1	LS	$ 6,346.60	$ 6,346.60
03	Submittal of Baseline Monitoring Report	1	LS	$ 18,498.00	$ 18,498.00
04	Completion of Amendment Injection and Submittal of Installation Completion Report	1	LS	$ 366,091.00	$ 366,091.00
05	Submital of First Performance Monitoring Report	1	LS	$ 19,849.75	$ 19,849.75
06	Submital of Second Performance Monitoring Report	1	LS	$ 19,849.75	$ 19,849.75
07	Submital of Third Performance Monitoring Report	1	LS	$ 19,849.75	$ 19,849.75
08	Submital of Fourth Performance Monitoring Report	1	LS	$ 19,849.75	$ 19,849.75
09	Submital of Fifth Performance Monitoring Report	1	LS	NA	NA
10	Submital of Sixth Performance Monitoring Report	1	LS	NA	NA
11	Submittal of Final Project Report for Year 1	1	LS	$ 7,920.00	$ 7,920.00

Year 1 Full Scale Total $ 493,444.00

SIGNATURE DATE

Safety and Ecology Corporation
COMPANY NAME

BFyear1.xls:1639

PROJECT: Building 100 Plume Control LOCATION: Largo, Florida

ITEM NO.	TASK DESCRIPTION	INITIAL ESTIMATED QUANTITY	UNIT OF MEASURE	UNIT PRICE	LINE ITEM EXTENDED PRICE
XXXXXXX	Year 2 Full Scale Remediation	XXXXXXXX	XXXXXXXXX	XXXXXXXX	XXXXXXXXXX
01	Submital of First Performance Monitoring Report	1	LS	$ 22,643.50	$ 22,643.50
02	Submital of Second Performance Monitoring Report	1	LS	$ 22,643.50	$ 22,643.50
03	Submittal of Final Project Report for Year 2	1	LS	$ 8,158.00	$ 8,158.00
	Year 2 Full Scale Total				$ 53,445.00

SIGNATURE DATE

Safety and Ecology Corporation
COMPANY NAME

BFyear2.xls:1639

Appendix B

Microbial Insights Microorganism Analysis Laboratory Report

microbial insights

2340 Stock Creek Blvd.
Rockford TN 37853-3044
Phone (865) 573-8188
Fax: (865) 573-8133
Email: microbe@microbe.com

Microbial Analysis Report

Client: Charles Tabor
SM Stoller
2597 B ¾ Rd.
Grand Junction, CO 81503

Phone: 970-248-6515

Fax:

MI Identifier: 1 SMS **Date Rec.:** 01/10/03 **Report Date:** 01/14/03

Analysis Requested: TGD for DHc

Project: Pinellas Environmental Restoration Program-Young Rainey Star Center

Comments:

All samples within this data package were analyzed under U.S. EPA Good Laboratory Practice Standards: Toxic Substances Control Act (40 CFR part 790). All samples were processed according to standard operating procedures. Test results submitted in this data package meet the quality assurance requirements established by Microbial Insights, Inc.

Reported by:

Reviewed by:

NOTICE: This report is intended only for the addressee shown above and may contain confidential or privileged information. If the recipient of this material is not the intended recipient or if you have received this in error, please notify Microbial Insights, Inc. immediately. The data and other information in this report represent only the sample(s) analyzed and are rendered upon condition that it is not to be reproduced without approval from Microbial Insights, Inc. Thank you for your cooperation.

microbial insights

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Email: microbe@microbe.com

Microbial Analysis Report

Results:

The microbial communities from three samples were screened for the presence of *Dehalococcoides* (DHc) by a targeted gene detection approach. Results from this analysis confirmed the presence of DHc in samples PIN12-0514 and PIN12-0526.

Table 1. Results from DNA amplification using primers specific for DHc. Presence is noted with a plus sign, and the relative abundance is presented by the number of plus signs.

Sample	Dehalococcoides
PIN12-0514	++
PIN12-0526	++
PIN12-S73C	-
Dehalococcoides etheneogenes positive control	+++
E.coli negative control	-

Overview of Approach:

Targeted Gene Detection for DHc

The recovery of DNA and its subsequent analysis provides a powerful tool for characterizing bacterial community structure. All cells (animals, plants, fungi, and bacteria) contain DNA that allows for their identification. These cells also contain ribosomes, which are required for normal cell functions. The favored target in DNA identification for bacteria is the small sub-unit ribosomal RNA gene, generally referred to as "16S rDNA" in prokaryotes). This target is favored because during the course of evolution, different regions of the ribosome have mutated (or changed) at different rates, with the overall result that some regions of this gene are virtually the same between all organisms (conserved), while other regions differ among even closely related species.

Specific primers directed to a conserved region of the 16S rRNA gene of Dehalococcoides (*DHc*) *was* used to determine its presence. Based upon Loffler *et al.* (1) the sensitivity of these primers is ~10^3 cells/mL or g of sample. Cloned *Dehalococcoides ethenogenes* 16S rDNA was used as a positive control to verify test results.

References

1. Löffler, F. E., Q. Sun, et al. (2000). "16S rRNA gene-based detection of tetrachloroethene-dechlorinating Desulfuromonas and Dehalococcoides species." Appl Environ Microbiol 66(4): 1369-1374.

Appendix C

Microorganism Analysis Laboratory Report

Pinellas Site Summary

June 2004

Objective:

Determine the composition of the dechlorinating bacterial populations in groundwater samples from the Pinellas Site Project using 16S rRNA gene-based tools.

Table 1. Samples obtained in May, 2004.

Sample ID	Date	Preservative	Matrix
PIN120526-P4007	05/19/04	blue ice	groundwater
PIN120514-P4007	05/19/04	blue ice	groundwater
PIN12753c-P4007	05/19/04	blue ice	groundwater
PIN12-0514-20'	05/18/04	blue ice	sediment core
PIN12-0514-24'	05/18/04	blue ice	sediment core
PIN12-0514-30'	05/18/04	blue ice	sediment core
PIN12-0526-8'	05/18/04	blue ice	sediment core
PIN12-0526-16'	05/18/04	blue ice	sediment core
PIN12-0526-23'	05/18/04	blue ice	sediment core
PIN12-573C-8'	05/18/04	blue ice	sediment core
PIN12-573C-16'	05/18/04	blue ice	sediment core
PIN12-573C-23'	05/18/04	blue ice	sediment core

Methods:

DNA isolation. All groundwater samples were analyzed in duplicate. Groundwater samples were concentrated onto polyether sulfone membrane filters (0.2 μm pore) and DNA extractions were performed using the MoBio Water DNA kit. (MoBio Laboratories Inc, Carlsbad, CA). The DNA was concentrated and its presence was confirmed by visualization on a 1% agarose gel. Sediment samples (1 g each) were also analyzed in duplicate, following DNA extraction with the MoBio Soil DNA kit. (MoBio Laboratories Inc, Carlsbad, CA).

Detecting dechlorinating bacteria. For increased sensitivity in detecting dechlorinating bacteria, a nested PCR approach was used. First, an initial amplification of the communities' 16S rRNA genes was performed (in duplicate for each sample material) using universal bacterial primers 8F and 1541R and conditions as described [1]. After gel electrophoresis verified that 16S rRNA genes could be amplified from the community DNA, 1:50 dilutions of the 16S rRNA gene amplicons were used as templates for primer pairs that specifically target the 16S rRNA genes of three dechlorinating bacterial groups (*Dehalococcoides* spp., *Desulfuromonas* spp., and *Dehalobacter* spp.), as published previously [2, 3, 4, 5]. Even if no visible 16S rRNA gene amplicons were seen in the sediment extractions, the "product" was analyzed by nested PCR, without dilution. Positive controls included genomic DNA of *Dehalococcoides* sp. strain FL2, *Desulfuromonas michiganensis* strain BB1, and *Dehalobacter restrictus* (DSM 9455) for the *Dehalococcoides*-, *Desulfuromonas*-, and *Dehalobacter*-targeted primers, respectively. Gel electrophoresis was used to visualize amplicons corresponding to the known groups of dechlorinating bacteria: 620, 815 and 828 bp respectively.

Quantifying Dehalococcoides spp.

Real-Time (RTm) PCR was used to estimate the number of *Dehalococcoides* cells present in the groundwater samples, since these yielded high-quality amplifiable DNA [3]. RTm PCR uses a probe that contains a reporter dye at its 5' end and a quencher dye at its 3' end. The reporter signal is blocked by the quencher dye as long as the probe remains intact. When the probe is cleaved by the 5' exonuclease activity of Taq polymerase as it extends the sequence from an upstream primer, the dye and the quencher dissociate and the reporter dye fluoresces when stimulated with the appropriate wavelength of light. A probe is cleaved each time a primer is extended and the fluorescent signal increases in proportion to the amount of PCR product generated. The spectrofluorimetric thermal cycler (ABI Prism 7700 Sequence Detection System, Applied Biosystems) records the emitted fluorescence intensities of each reaction at regular intervals, then compares these values to a standard curve to determine the initial target copy number. In addition to the *Dehalococcoides*- targeted primer and probe set, the newly developed *bvcA* and *tceA* specific primer pairs were utilized to determine the gene copy numbers of the *bvcA* gene (of the VC-to-ethene dechlorinating *Dehalococcoides* sp. strain BAV1) and the *tceA* gene (the TCE and cis-DCE dechlorinating gene identified in strains FL2 and 195).

Results:

Table 1 summarizes the results of the dechlorinator-targeted analysis of the Pinellas site samplings from March 2003, September 2003 and May 2004. Table 2 shows the results of the Quantitative PCR for May 2004.

- Sediment core DNA extractions do not reproducibly yield high quality DNA for community analysis. 14 of 26 DNA extractions did not yield a visible 16S rRNA gene product with Bacteria-targeted PCR primers. Groundwater consistently yielded amplifiable DNA.
- In March 03, *Dehaloccoccoides* populations were below the detection limit of RTm PCR in all samples.
- Site 0514 indicated the presence of *Dehalococcoides* populations in groundwater obtained in all three sampling events. *Dehalococcoides* amplicons were detected in 30' sediment cores in the March 03 sampling and at 24' in May 04 (No 16S rRNA gene amplicons were detected at 30'). In May 04 groundwater, *Dehalococcoides* were present at below the detection limit of RTm PCR.
- *Dehalococcoides* populations were not detected at Site 573c in the March 03 groundwater (but in the 14-16' core they were present) and were detected in both replicate extractions in September 03 and May 04. In May 04, RTm PCR quantified between 10-100 copies of the *Dehalococcoides* 16S rRNA gene copies per ml groundwater, and both the *tceA* and *bvcA* genes were present at a high proportion of the *Dehalococcoides* populations.
- Site 0526 did not have detectable *Dehalococcoides* populations in the March 03 groundwater (but in the 8-10' core they were present in one replicate). In September 03 and May 04 *Dehalococcoides* populations were detected in both replicate DNA extractions from groundwater. Well 0526 had very few (0.1-10) *Dehalococcoides* cells per ml, and no *bvcA* or *tceA* genes could be detected.

Table 1. Detecting dechlorinating populations in Pinellas samples: September, 2003 in bold, groundwater samplings in red.

Sample[b]	Date	16S rRNA gene product[a]	*Dehalococcoides* 16S rRNA gene product[a]	*Desulfuromonas* 16S rRNA gene product[a]	*Dehalobacter* 16S rRNA gene product[a]
0514 (GW)	Mar 03	++	++	++	-
0514 (S:20-22')	Mar 03	-	nd	nd	nd
0514 (S:24-26')	Mar 03	++	-	-	-
0514 (S:30-32')	Mar 03	++	+	-	-
0514 (GW)	Sep 03	++	++	-	-
0514 (GW)	**May 04**	++	++	++	-
0514 (S 20')	**May 04**	-	-	-	-
0514 (S 24')	**May 04**	+	+	-	-
0514 (S 30')	**May 04**	-	-	-	-
573C (GW)	Mar 03	++	-	-	-
573C (S:8-10')	Mar 03	++	-	+	-
573C (S:14-16')	Mar 03	++	++	-	-
573C (S:23-24')	Mar 03	-	nd	nd	nd
573C (GW)	Sep 03	++	++	-	-
573C (GW)	**May 04**	++	++	-	-
573C (S 8')	**May 04**	-	+	-	-
573C (S 16')	**May 04**	-	-	-	-
573C (S 23')	**May 04**	-	-	-	-
0526 (GW)	Mar 03	++	-	-	-
0526 (S:8-10')	Mar 03	++	+	-	-
0526 (S:14-16')	Mar 03	++	-	+	-
0526 (S:23-24')	Mar 03	-	nd	nd	nd
0526 (GW)	Sep 03	++	++	+	-
0526 (GW)	**May 04**	++	++	+	-
0526 (S 8')	**May 04**	++	-	-	-
0526 (S 16')	**May 04**	++	-	-	-
0526 (S 23')	**May 04**	++	++	-	-

[a] (++) indicates amplification product in two replicate DNA extractions, (+)indicates amplification product obtained in one of two replicates DNA extractions, (-) indicates no visible product in either replicate.

[b] (GW) indicates a groundwater sample, (S) indicates a sediment core sample, and depth.

Table 2. Quantifying *Dehalococcoides* genes in the May 2004 groundwater samples. Duplicate DNA extractions (a and b) were each quantified in triplicate.

	gene copies per ml of groundwater		
	Dhc	BvcA	TceA
PIN12-0514 a	not detected	not detected	not detected
PIN12-0514 b	not detected	not detected	not detected
PIN12-573c a	14 (±7)*	2.0 (±2)	141 (±214)
PIN12-573c b	34 (±41)	1.1 (±0.7)	1355 (±423)
PIN12-0526 a	0.03 (±0.05)	not detected	not detected
PIN12-0526 b	23 (±39)	not detected	not detected

* Indicates one standard deviation of triplicate assays.

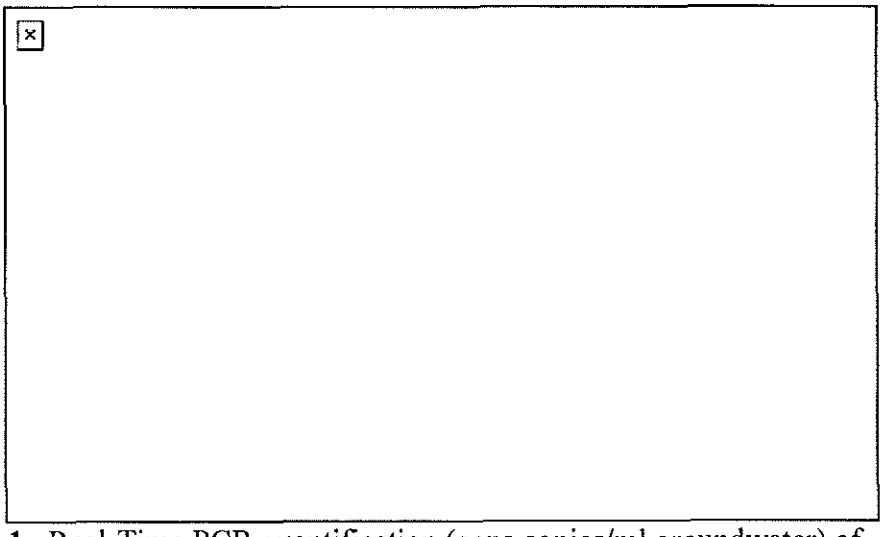

Figure 1. Real-Time PCR quantification (gene copies/ml groundwater) of *Dehalococcoides* genes in May 04 DNA samples.

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(2) **Löffler, F. E., Sun, Q., Li, J. & Tiedje, J. M.** 16S rRNA gene-based detection of tetrachloroethene (PCE)-dechlorinating *Desulfuromonas* and *Dehalococcoides* species. *Appl. Environ. Microbiol.* **66**, 1369-1374 (2000)

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Appendix D

Bioavailable Iron Data

Bioavailable Ferric Iron Assay Report

CDM

Client:	Regenesis
Site:	Pinellas
Sample Receipt Date:	3/13/2003
Analyst:	M.T.
Analysis Date:	3/18/2003
CDM Project Number:	20944-36061-PINELLAS

Sample	Solids (%)	Ambient Fe II (g/kg)	Bioavailable Fe III (g/kg)
S7 3C-12'	76%	0.37	<.07
S7 3C-22'	72%	0.14	0.076
526-12'	72%	0.17	0.15
526-22'	71%	0.18	<.07
514-20'	69%	0.16	<.07
514-30'	76%	0.11	<.07

Bioavailable Ferric Iron Assay QAQC

CDM

QAQC Sample: S7 3C-12'

	Ambient Fe II (g/kg)	Final Fe II (g/kg)	Bioavailable Fe III (g/kg)
Result	0.367	0.237	<.07
Duplicate	0.169	0.224	<.07
RPD	54%	6%	NA

Reagent Lot Number: 91802

Reagent Analyte	Units	Average Value	Coefficient Variation (%)
pH	-	6.49	0.14
Calcium	mg/l	226	6.6
Magnesium	mg/l	6.30	7.3

Bacteria Lot Number: 101100

Concentration: 1.2×10^6 Cells/ml

Date: 6/13/2002

QAQC Reviewer: Patrick J. Evans, Ph.D.

CDM

Bioavailable Ferric Iron Assay

Background

Ferric iron (Fe III) is a widespread terminal electron acceptor used by iron-reducing bacteria under anaerobic conditions. These bacteria can oxidize various organic compounds and in turn reduce ferric iron (Fe III) to ferrous iron (Fe II). Some of the organic compounds that can be oxidized by certain iron-reducing bacteria include benzene, toluene, vinyl chloride (VC), *cis*-dichloroethene (cDCE), and methyl tertiary butyl ether (MTBE). Additionally, iron oxides play an important role in the immobilization of metals in aquifers and bacterial iron reduction is one factor affecting the transport of metals in aquifers.

Not all ferric iron can be biologically reduced. A definition of bioavailable ferric iron is:

> *Ferric iron (Fe III) that is capable of being reduced by microorganisms that oxidize another chemical species and derive energy from the electron transfer.*

Prediction of the amount of bioavailable ferric iron is difficult because it is affected by many factors. Factors that can determine whether ferric iron is bioavailable include iron oxide crystallinity and surface area, groundwater pH and specific conductivity, concentrations of divalent cations, concentrations of electron shuttles such as humic acids, and adsorbed ferrous iron.

Assay Description

The assay is a bioassay that uses the iron-reducing bacterium *Shewanella alga* BrY to give an estimate of the maximum concentration of bioavailable ferric iron in soil or other solid materials. The assay medium contains a phosphate buffer to control the pH around 6.5, calcium chloride as a source of divalent cations, 1,5-anthraquinonedisulfonic acid (AQDS) and humic acids as sources of electron shuttles, and sodium lactate as the electron donor. A five-gram soil sample is incubated in the assay medium along with the bacteria for a period of one month. During this time bioavailable ferric iron is reduced to ferrous iron. The newly formed ferrous iron plus the originally present ambient ferrous iron is extracted with weak acid (0.5 N HCl) at the end of the incubation period and measured using a Hach kit following dilution. The ambient ferrous iron concentration is measured by similarly extracting a soil sample that has not been incubated or exposed to the assay reagents. The ambient ferrous iron concentration is subtracted from the concentration in the incubated sample to obtain the bioavailable ferric iron concentration.

CDM

Assay Method

Soil samples are typically collected from the saturated zone. A four-ounce jar of soil is sufficient for the bioavailable ferric iron assay. Jars should be filled with water-saturated soil and kept refrigerated until analysis. Recommended holding times for soil samples have not been determined. Preferably, analyses should be initiated within one week of sample collection.

The sample is wet-sieved through a 3/16-inch sieve if necessary and two five-gram sub-samples of the sieved material are placed in each of two assay tubes labeled T_0 and T_{30}. The T_0 tube is filled with distilled water and one milliliter of concentrated HCl. The tube is capped and then placed on a tube rotator for 48 hours during which time the acid extracts weakly associated ferrous iron (Fe II) from the soil. Following the incubation period, the extract liquid is filtered if necessary and diluted prior to measurement of the ferrous iron concentration using the Hach phenanthroline method. The T_{30} tube, which also contains the assay reagent and lyophilized bacteria, is filled with distilled water, capped, mixed by hand, and then incubated in the dark at room temperature for 30 days. Following the incubation one milliliter of liquid is withdrawn, discarded, and replaced with one milliliter of concentrated hydrochloric acid. The tube is then rotated for 48 hours and analyzed for ferrous iron. This concentration is the final ferrous iron concentration and is the sum of the ambient ferrous iron and the bioavailable ferric iron. The ambient ferrous iron and the bioavailable ferric iron concentrations on the soil are calculated as follows:

Ambient Fe II = (T_0 Fe II)/{(217)(solids fraction)}

Bioavailable Fe III = (T_{30} Fe II - T_0 Fe II)/{(217)(solids fraction)}

The terms in these equations are defined as follows:

Ambient Fe II – The concentration of Fe II in the soil sample (units of grams Fe per kilogram dry soil) prior to conducting the assay.

Bioavailable Fe III – The concentration of biologically reducible Fe III in the soil sample (units of grams Fe per kilogram dry soil) determined using the assay.

T_0 Fe II – The Fe II concentration measured in the T_0 tube (units of milligrams Fe II per liter) following acid extraction. Measured using a Hach phenanthroline kit.

T_{30} Fe II – The Fe II concentration measured in the T_{30} tube (units of milligrams Fe II per liter) following acid extraction. Measured using a Hach phenanthroline kit.

217 – A conversion factor to convert the liquid Fe II concentration to the soil concentration. It incorporates tube volume (25 milliliters), soil mass (5 grams), soil particle density (2.6 grams per milliliter), and unit conversions.

Solids fraction – Solids fraction in the soil sample (units of grams dry soil per gram wet soil). Measured separately and used to convert the ambient and bioavailable iron results from a wet-soil basis to a dry-soil basis. This term is optional if results expressed per kilogram of wet soil are acceptable.

Applications

The concentration of bioavailable ferric iron in soil is one parameter that may be used to determine the potential for oxidative degradation of organic chemicals and the transport of metals. It also may be used to determine the potential for inhibition of reductive dechlorination of chlorinated ethenes by maintenance of low dissolved hydrogen concentrations.

The assimilative capacity for oxidation of organic chemicals can be calculated by calculation of the electron equivalents that can potentially be accepted by the bioavailable ferric iron. This is calculated as follows:

Electron accepting equivalents = (Bioavailable Fe III)(eq./56 g) equiv./kg

As an example, vinyl chloride oxidation to carbon dioxide requires 10 electron equivalents/mole or 6 g/equiv. If the measured bioavailable ferric iron concentration is 1 g/kg, the assimilative capacity for vinyl chloride oxidation is:

0.11 g VC/kg soil = (1 g bioavailable Fe III/kg soil)(1 equiv. /56 g Fe III)(6 g VC/equiv.)

Another application is calculation of how much historical contaminant oxidation is attributable to iron reduction. This calculation requires comparison of background samples to samples in the contaminant plume. The difference in bioavailable ferric iron between these two samples can be used to calculate the amount contaminant that has been oxidized as shown in the following example. If the background sample contains 1 g/kg and the sample in the plume contains 0.5 g/kg bioavailable ferric iron, then the amount of vinyl chloride oxidation theoretically attributable to iron reduction is:

0.054 g VC/kg soil = {(1-0.5) g bioavailable Fe III/kg soil}(1 equiv. /56 g Fe III)(6 g VC/equiv.)

Note that oxidation of organic chemicals other than VC is also possible. The above calculations do not take into account oxidation of other chemicals and are provided for example only. They should be modified to meet the requirements of specific sites.

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Disclaimer

The bioavailable ferric iron assay is a developmental analytical method that was developed for the U.S. Air Force under the Small Business Innovative Research program. It is currently being evaluated by the Department of Defense under the Environmental Security Technology Certification Program (ESTCP) and by the EPA. It is not an EPA-approved test method. The results of the assay are only one of several types of analytical data that should be considered in assessing soil conditions and are intended to be used in combination with these other data. Users of the bioavailable ferric iron assay should not rely solely on the assay results and should exercise best professional judgment in determining the extent to which reliance on the assay results is appropriate in a particular instance. The user shall be solely responsible for inconsistent or erroneous assay results or injuries that occur due to improper or negligent use or handling of the test kit or of any results of assays performed with the test kit.

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