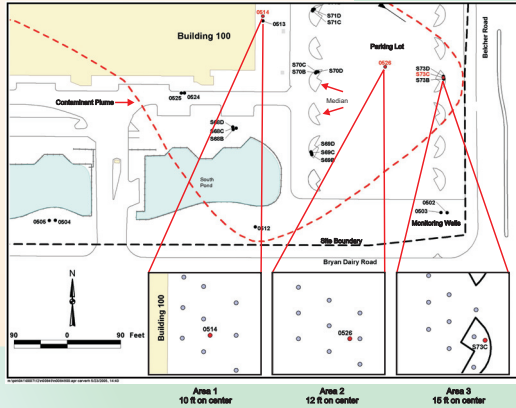


Biostimulation of Dechlorinating Microbial Populations In Groundwater



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Introduction

Safety and Ecology Corporation, under contract to S.M. Stoller Corporation, designed and implemented an in situ enhanced bioremediation technology to control the plume of dissolved contaminants (chloroethenes) at the Building 100 Area of the Young - Rainey Science, Technology, and Research (STAR) Center in Largo, Florida. Hydrogen Release Compound (HRC[®]) was injected in March 2003 in accordance with an approved Remediation Plan as part of a pilot test to assess the efficacy of the technology to treat site contaminants. Seven groundwater sampling events followed the HRC application to track contaminant concentrations and other groundwater parameters.

Objective

Demonstrate the effectiveness of an enhanced bioremediation technology to treat several chloroethenes to the remediation goals (maximum contaminant levels).

Injection Design

This pilot-scale study evaluated the effect of injection-point spacing on the ability of HRC, an injectable, honey-like substance manufactured by Regenesis, to biostimulate indigenous reductive dechlorinating bacteria that degrade *cis*-1,2-dichloroethene (*cis*-DCE) and vinyl chloride (VC). Three grid configurations, each consisting of nine direct-push injection points around a monitoring well, were evaluated at the site. The nine injection points in the first grid area (Area 1) were closely spaced (10-ft centers), the second grid (Area 2) had moderately spaced points (12-ft centers), and the third grid (Area 3) had the widest injection point spacing (15-ft centers).



Direct-Push Injection



Hydrogen Release Compound (HRC)

Acknowledgments

This work was conducted under DOE contract number DE-AC01-02GJ7949 for the U.S. Department of Energy Office of Legacy Management.

Results

Microbial Analysis

- HRC amendment promoted substantial growth of dechlorinating bacteria in groundwater
- Polymerase chain reaction used to detect and monitor *Dehalococcoides* species
- Dehalococcoides* species detected in groundwater samples from Area 1 and in soil samples from Areas 1, 2, and 3 prior to HRC injection
- Dehalococcoides* species present in significant numbers in groundwater samples from all three areas 6 months and 1 year after HRC injection

Groundwater Microbial Analysis

Area 1			Area 2			Area 3		
Day	16S-rRNA gene product	Dehalococcoides 16S-rRNA gene product	Day	16S-rRNA gene product	Dehalococcoides 16S-rRNA gene product	Day	16S-rRNA gene product	Dehalococcoides 16S-rRNA gene product
0	++	++	0	++	-	0	++	-
197	++	++	197	++	++	197	++	++
436	++	++	436	++	++	436	++	++

Soil Microbial Analysis

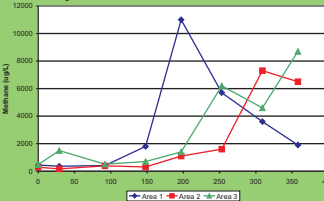
Area 1			Area 2			Area 3					
Sample Location	Day	16S-rRNA gene product	Dehalococcoides 16S-rRNA gene product	Sample Location	Day	16S-rRNA gene product	Dehalococcoides 16S-rRNA gene product	Sample Location	Day	16S-rRNA gene product	Dehalococcoides 16S-rRNA gene product
Area 1 (20)	0	-	ND	Area 2 (8)	0	++	+	Area 3 (8)	0	++	-
Area 1 (24)	0	++	-	Area 2 (16)	0	++	-	Area 3 (16)	0	++	++
Area 1 (30)	0	++	+	Area 2 (23)	0	-	ND	Area 3 (23)	0	++	ND
Area 1 (20)	436	+	+	Area 2 (8)	436	++	-	Area 3 (8)	436	Not tested	Not tested
Area 1 (24)	436	+	+	Area 2 (16)	436	++	-	Area 3 (16)	436	Not tested	Not tested
Area 1 (30)	436	-	-	Area 2 (23)	436	++	++	Area 3 (23)	436	Not tested	Not tested

Day = days since pilot test began.
(20) = sample depth in feet
++ 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" indicates not detected after initial 16S-rRNA analysis.

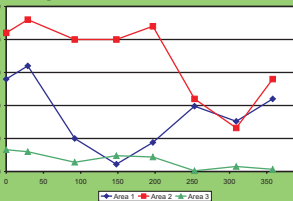
Significant Results in Area 1

- Methane production peaked at day 197 (Graph 1)
- Sulfate concentrations decreased from 140 mg/L to 11 mg/L by day 148 (Graph 2)
- Subsurface conditions were slightly reducing prior to injection; conditions highly reducing following injection
- Reducing conditions persisted in subsurface for entire pilot test period, but appeared to decline when HRC depletion began (Graph 3)
- Injection-point spacing in Area 1 produced the most rapid increase in total organic acids (TOAs) (Graph 4)
- Complete dechlorination observed as declining trend in *cis*-DCE along with increase in VC (Graph 5)

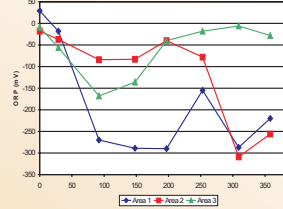
Graph 1 – Methane Concentrations



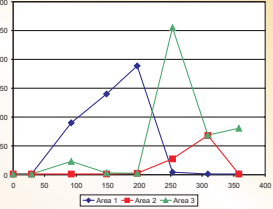
Graph 2 – Sulfate Concentrations



Graph 3 – ORP Comparison



Graph 4 – TOA Comparison



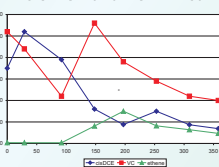
Oxidation-Reduction Potential (ORP) and (TOA) Results

- Most favorable reducing environment initiated by day 90 within Area 1; ORP levels sustained below -250 mV and TOAs spiked to 189 mg/L (Graphs 3 and 4)
- Performance lag in Area 2 and Area 3
- Reducing conditions favorable for dechlorination not produced until approximately day 309 in Area 2
- ORP in Area 3 did not decrease to the desired level (Graph 3)
- TOA concentrations in Area 2 and Area 3 peaked later in pilot test (Graph 4), demonstrating the lower efficiency of wider-spaced injection points

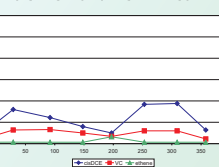
VOC Results

- cis*-DCE concentrations decreased by 75% within 197 days in Area 1, while VC concentrations declined by 62% within 358 days (Graph 5)
- VC concentrations did not decrease to the desired 1 µg/L MCL
- Ethene production observed only in Area 1 with a high of 15 µg/L at post-injection day 197
- Both *cis*-DCE and VC concentrations show minimal decrease in Area 2 and Area 3; concentrations remaining near baseline levels more than 300 days after injection (Graphs 6 and 7)
- Data gathered from Area 2 and Area 3 suggest that electron donor distribution in treatment area was slow to occur; Area 1 results provided strong evidence of efficacy of HRC in subsurface

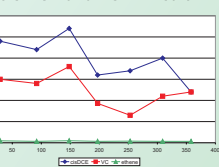
Graph 5 *cis*-DCE and VC in Area 1



Graph 6 *cis*-DCE and VC in Area 2



Graph 7 *cis*-DCE and VC in Area 3



Conclusions

The most favorable results were observed in Area 1. Inefficient and incomplete distribution of HRC in Area 2 and Area 3 apparently caused lags and inconsistencies in many geochemical conditions, including organic acid concentrations, reducing conditions, and downward trends in contaminant concentrations. Geochemical parameters conducive to reductive dechlorination were not sustained in these treatment areas. On the basis of sampling results, closer injection-point spacing provided the most rapid and, with the exception of VC, complete dechlorination and reduction in contaminant concentrations.