

Bioaugmentation After a Stalled Biostimulation Application

Pawan Sharma
(CDM, Walnut Creek, California)

Al Bourquin
(CDM, Denver, Colorado)

Hypothesis

After a stalled biostimulation test for enhanced reductive dechlorination (ERD) of trichloroethene (TCE), a one-time direct injection event of a bacterial consortium of naturally occurring species of *Dehalococcoides* can successfully increase the rate of complete ERD of cis-1,2-dichloroethene (cDCE).

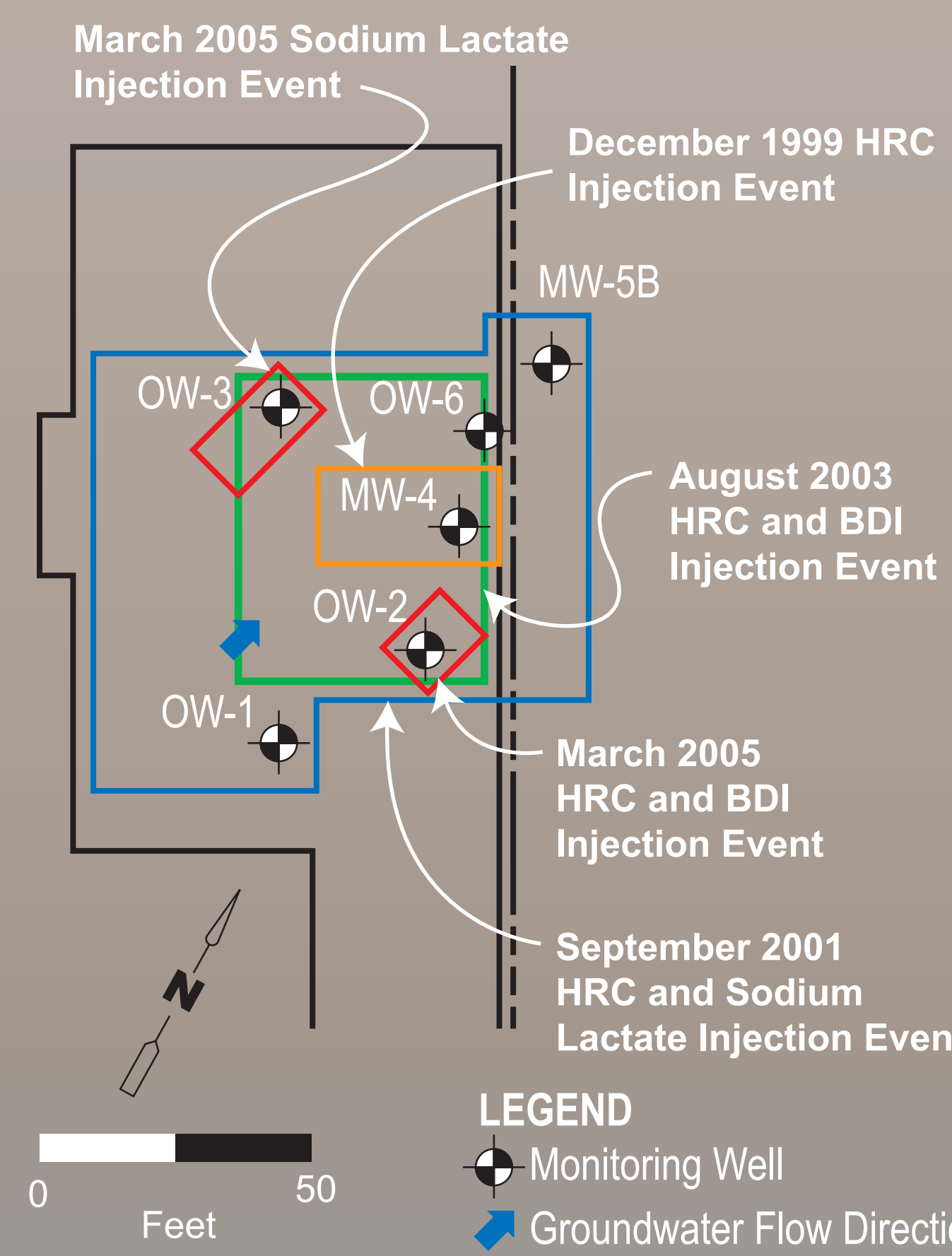
Abstract

Bioaugmentation was tested as an alternative to continued biostimulation of groundwater containing trichloroethene (TCE), cis-1,2-dichloroethene (cDCE), and other chlorinated organic compounds to promote enhanced reductive dechlorination (ERD). Specifically, direct injection of a proprietary bacterial consortium of naturally occurring species of *Dehalococcoides*: Bio-Dechlor INOCULUM™ (BDI), manufactured and provided by Regenesys, was evaluated. Quantitative real-time polymerase chain reaction (PCR) analysis of groundwater and biotrap samples was used to evaluate effectiveness of the injection procedure and *Dehalococcoides* activity in the aquifer system.

Background

Based on results from a natural attenuation study, an in situ biostimulation test for enhanced reductive dechlorination (ERD) of trichloroethene (TCE) was initiated. The biostimulation test included injection of HRC® and sodium lactate and induced strongly reducing methanogenic conditions. Decreases of TCE concentrations in the test area accompanied increases in concentrations of cis-1,2-dichloroethene (cDCE) and to a lesser degree vinyl chloride (VC). Continued HRC and sodium lactate injections maintained methanogenic conditions, but did not lead to complete reductive dechlorination to ethene.

Injection Events



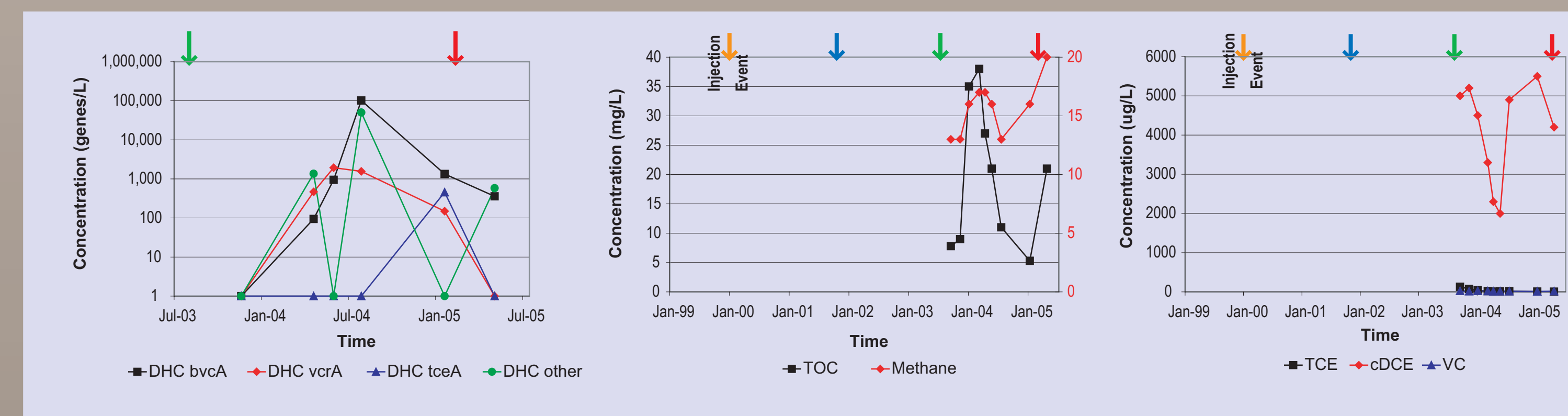
Date	Material	Injection Area (sq. ft.)	Number of Injections	Injection Interval	Amount per Injection Foot
December 1999	HRC	1200	15	8 to 20 feet	6 pounds
September 2001	HRC	7000	20	8 to 20 feet	6 pounds
September 2001	60% NaLac	7000	20	8 to 20 feet	9 pounds
August 2003	HRC	3800	25	8 to 20 feet	4 pounds
August 2003	BDI	3800	25	8 to 20 feet	0.25 liter
March 2005	HRC	200	5	8 to 20 feet	6 pounds
March 2005	BDI	200	3	8 to 20 feet	0.6 liter
March 2005	60% NaLac	300	6	8 to 20 feet	12 pounds

Quantitative PCR Analysis of Biotraps

Location	Date	Sulfate & Iron				
		Methanogens	Reducing	<i>Dehalococcoides</i>	<i>Desulfuromonas</i>	<i>Dehalobacter</i>
Gene Copies Per Bead						
OW-2	Mar-04	434,000	2,880	ND	ND	ND
	Apr-04	3,400,000	27	ND	28	ND
	May-04	608,000	ND	ND	13	ND
OW-3	Mar-04	851,000	ND	ND	31	ND
	Apr-04	2,500,000	160	ND	ND	224
	May-04	427,000	8,450	ND	4	ND
OW-6	Mar-04	211,000	816	ND	ND	ND
	Apr-04	1,420,000	124	ND	19	ND
	May-04	170,000	ND	ND	2	ND

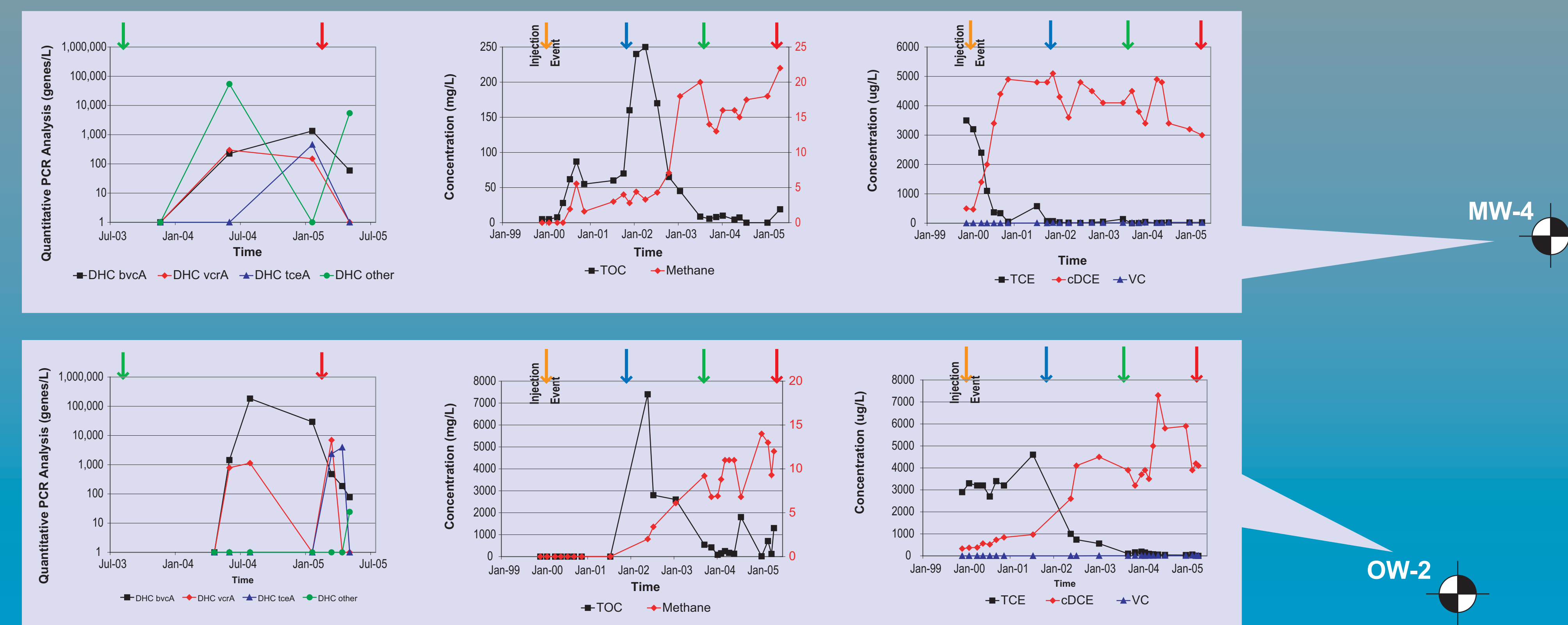
OW-3 Biotraps were suspended in the wells in Feb 2004 and retrieved after 30, 60, and 90 days.

Test Layout and Monitoring Results

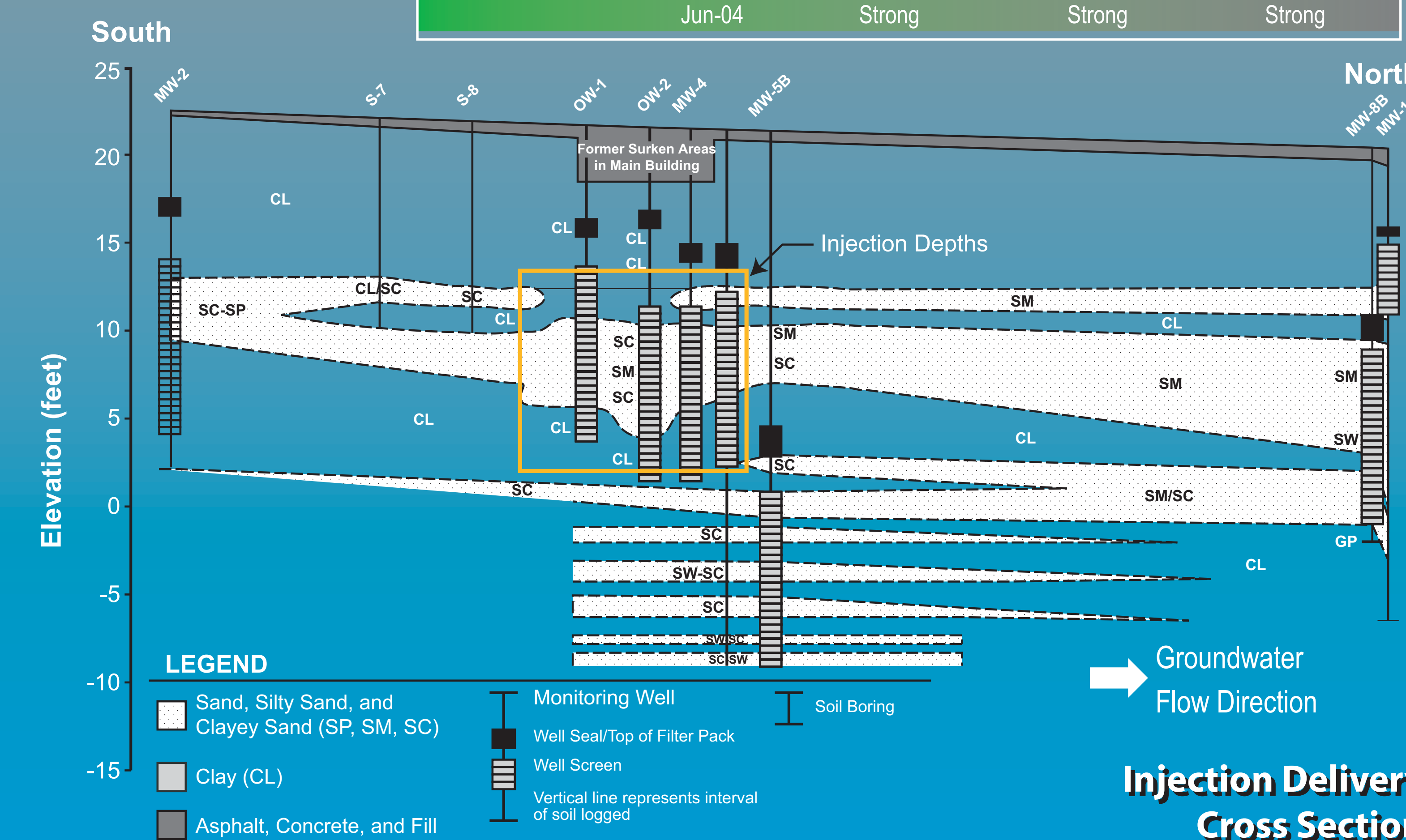


Qualitative PCR Analysis

Location	Matrix	Date	<i>Dehalococcoides</i>	<i>Desulfuromonas</i>	<i>Dehalobacter</i>
Before Bioaugmentation					
MW-4	GW	Jul-03	ND	Strong	ND
OW-1	GW	Jul-03	ND	Strong	ND
BDI-6	Soil	Aug-03	ND	Strong	Strong
After Bioaugmentation					
MW-4	GW	Sep-03	ND	Strong	Strong
		Nov-03	ND	Strong	Strong
		Jan-04	Weak	Strong	Strong
OW-2	GW	Feb-04	Strong	Strong	Strong
		Jun-04	Strong	Strong	Strong
		Jul-04	Strong	Strong	Strong
OW-3	GW	Feb-04	Strong	Strong	Strong
		Jun-04	Strong	Strong	Strong
		Jul-04	Strong	Weak	Weak
OW-6	GW	Sep-03	Strong	Strong	Strong
		Nov-03	ND	Strong	Strong
		Jan-04	ND	Strong	Strong
		Jun-04	Strong	Strong	Strong



OW-6 Converted BDI injection location.



Results and Findings

The introduction of *Dehalococcoides* type microbes (those present in the BDI culture) was successful as evidenced by the results from quantitative and qualitative PCR analyses. However, subsequent growth of these microbes to sufficient populations in the aquifer system appears to have been insufficient to accelerate the complete ERD of TCE and cDCE. While the introduction of low populations of dehalorespiring microbes at low concentrations appears to be successful, the bioaugmentation test has resulted in little change in contaminant concentrations.

The causes for this hindered growth were primarily uneven distribution of electron donor, evidenced by varying total organic carbon (TOC) levels, and potentially competition from non-dehalorespiring microbes. The groundwater concentrations of indigenous microbes, at the time of the initial BDI injections, were 1 to 3 orders of magnitude higher than those of *Dehalococcoides* type microbes injected into the groundwater.

If population competition is a factor in the lack of complete ERD, it is not attributed to microbe characteristics or their suitability to the geochemical conditions in the aquifer, but perhaps simply a function of initial population sizes. The injected *Dehalococcoides* mass was insufficient to gain substantial footing in the aquifer's microbial ecosystem in the time period of the test.

Based on the presence of the *bvcA* gene of *Dehalococcoides*, associated with complete metabolic reductive dechlorination of cDCE and VC to ethene, it is possible that complete dechlorination is currently occurring, but not measurable.

Dehalorespiring microbes can out-compete many anaerobic microorganisms due to a greater enzyme affinity for hydrogen, the electron donor. Therefore, it is expected that complete reductive dechlorination will be evidenced once the *Dehalococcoides* type microbes grow to a suitable population size. This will likely require the injection of additional electron donor.

Two contrasting approaches to DNA sample collection were utilized at the site. One approach was the use of Biotraps that were suspended in monitoring wells in order to allow attachment of indigenous bacteria over a period of 30, 60, and 90 days. The Biotraps were then removed from the wells and DNA was extracted from the attached bacteria. This method is purported to provide a more representative sample of the attached microbial community in the subsurface. The second approach was a DNA extraction directly from groundwater samples using standard methods. Somewhat surprisingly, analysis of samples from the Biotraps never detected *Dehalococcoides*, and frequently did not detect *Desulfuromonas* or *Dehalobacter*. All of these were detected through direct analysis of groundwater. This suggests that while the Biotraps do sample a different portion of the microbial community than groundwater samples, they may be ineffective for detecting the populations of interest.