## Co-Metabolic Bioremediation Demonstration Project

FINAL REPORT 08-19
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NEW YORK STATE ENERGY RESEARCH AND DEVELOPMENT AUTHORITY





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STATE OF NEW YORK David A. Paterson, Governor ENERGY RESEARCH AND DEVELOPMENT AUTHORITY Vincent A. DeIorio, Esq., Chairman

# Co-METABOLIC BIOREMEDIATION DEMONSTRATION PROJECT

NYSDEC Inactive Hazardous Waste Site No. 344031 Former Grant Hardware Facility West Nyack, NY

Final Report

Prepared for the

# NEW YORK STATE ENERGY RESEARCH AND DEVELOPMENT AUTHORITY

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and

#### **GUSSACK REALTY**

West Nyack, NY

Prepared by:

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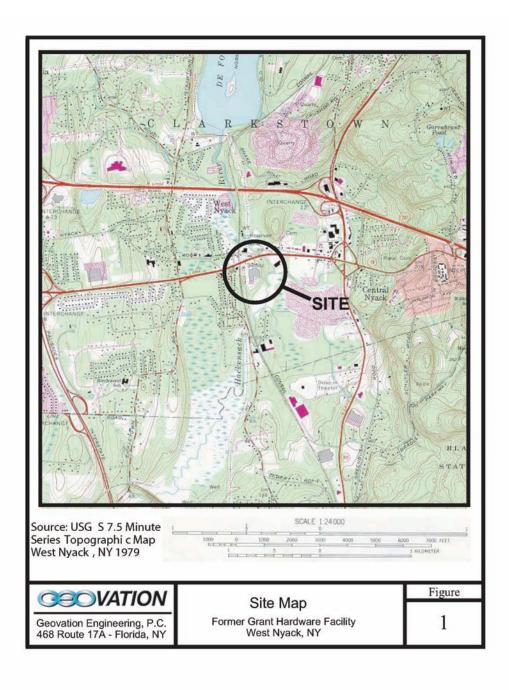
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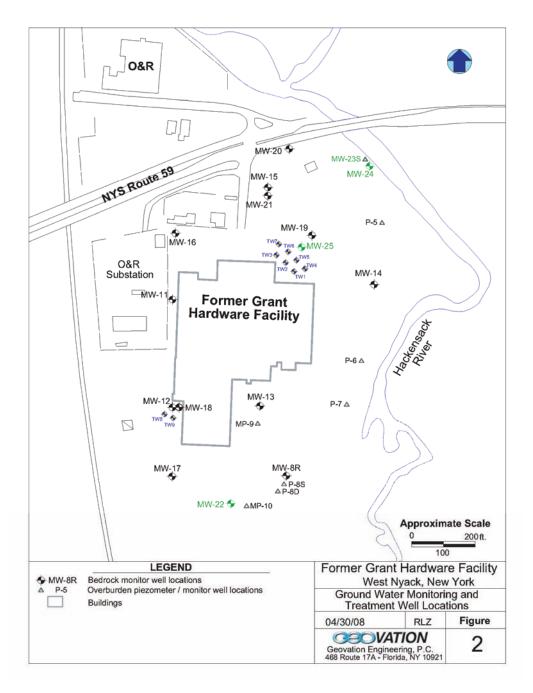
#### 1. INTRODUCTION

From late December 2006 until early January 2008, this bioremediation demonstration project addressed chlorinated solvent contaminated ground water via the addition of Geovation's SRCTM product. Based on evidence gathered during the 12-month duration of this project. Geovation concludes that the application of this product has been successful. During the demonstration project, Geovation's SRCTM product created reducing conditions in all target wells and maintained these conditions, increased biological activity as measured both directly and indirectly, and changed the composition of the biological community. Furthermore, the use of SRC<sup>TM</sup> product resulted in the degradation of ground water contaminants from parent to daughter compounds and the observation of the reduction of total chloroethenes by as much as 99.6% from baseline concentrations. Additional evidence indicated that biological enzymes were available to degrade parent and daughter compounds and that bacteria known to degrade chloroethene contaminants were present in ground water. Analysis of the contaminant concentrations over the duration of the study resulted in the calculation of contaminant degradation rates for parent and daughter compounds. Finally, the use of SRC<sup>TM</sup> through treatment wells was shown to be an effective delivery mechanism in both a bedrock source area and for use in the construction of a line of bedrock treatment wells as a bio-barrier wall.

#### 2. METHODS

This bioremediation demonstration project addressed chlorinated solvent contaminated ground water at the former Grant Hardware facility in West Nyack, NY. Ground water contaminants included total chloroethenes composed of tetrachloroethene (PCE), trichloroethene (TCE), dichloroethenes (cis-1,2 DCE and trans-1,2 DCE), and vinyl chloride (VC). Additional infrastructure required to perform the approved co-metabolic bioremediation demonstration project was installed during the late summer of 2006. Ten bedrock wells, consisting of one monitoring well and nine treatment wells, were installed at the locations shown on Figure 2, and project baseline ground water sampling was performed on August 23, 2006.





Ground water contaminants were treated via the application of Geovation's SRC<sup>TM</sup> to ground water at two locations at the site: (i) in the source area designated as "WN-1, or source area," and (ii) at a down-gradient location designated "WN-2, or barrier area." In both areas, Geovation's SRC<sup>TM</sup> product was introduced into ground water through treatment wells, and the effects of the product were monitored at down gradient monitoring wells (Figure 2).

In the source area, Geovation's SRC<sup>TM</sup> product was added to treatment wells TW-8 and TW-9, which were designed to affect target wells MW-12 and MW-18. In the barrier area, SRC<sup>TM</sup> was added to treatment wells TW-1 through TW-7, and the results were monitored in wells MW-25 and MW-19. SRC<sup>TM</sup> was applied to the subsurface on a monthly schedule. The amounts of Geovation's SRC<sup>TM</sup> product applied during the project are shown in Table 1.

Table 1

Ground-Water Bioremediation Pilot Project SRC<sup>™</sup> Dosing Information
Former Grant Hardware Facility - West Nyack, NY

Treatment Area	Liquid SRC <sup>™</sup> First Quarter (gallons)	Liquid SRC <sup>™</sup> Second Quarter (gallons)	Liquid N- Blend <sup>TM</sup> Second Quarter (gallons)	Liquid SRC <sup>™</sup> Third Quarter (gallons)	Liquid SRC <sup>™</sup> Fourth Quarter (gallons)	Total Liquid SRC <sup>™</sup> (gallons)
WN-1 Source Area Back of Building	407	0	403	472	212	1091
WN-2 Down Gradient Front of Building	211	79	0	48	35	373
Total:	618	79	403	520	247	1464

For each of these areas, a control point was established that was located at a distance far enough away from the treatment wells that they were believed to be beyond the area of influence of the SRC<sup>TM</sup> applications. The control points were monitored to document natural fluctuations in ground water quality during the project. In the source area, MW-17 was selected as the control well, and in the barrier area, MW-11 was selected as the control point (Figure 2). In addition, ground water samples that were collected during quarterly ground water events were split with an independent environmental consultant, Environment Management, Ltd (EML). Four times during the demonstration project, EML observed Geovation collecting the ground water samples, received split samples from Geovation, and independently arranged for and received the results of the groundwater analyses.

Contaminated ground water at this site flows through a bedrock matrix. Typically, primary ground water flow in bedrock is through bedrock faults and fractures. As ground water flows through these faults and fractures, the flow rate can vary considerably. An additional well, MW-24, was added to this project to evaluate potential effects of this variation at great distances from the treatment areas.

Baseline data was collected in August 2006 prior to the addition of any SRC<sup>TM</sup> product. SRC<sup>TM</sup> was first added to site ground water in December 2006, and additional SRC<sup>TM</sup> was amended to site ground water on a monthly schedule (Table 1). Ground water monitoring was performed as follows during the demonstration project:field parameters were monitored monthly; biogeochemical parameters (nutrients, the metals iron and manganese, and alkalinity) and trace gases were monitored on a quarterly basis; and the concentrations of volatile organic compounds (VOCs), including chloroethenes, were monitored quarterly and with supplemental sample collection, which resulted in VOC sampling results for nine months of the 12-month study. The ground water sampling results in the source area are provided in Table 2, and the results obtained in the barrier area are provided in Table 3.

In addition, Biotrap<sup>®</sup> samplers were deployed in target wells to collect microorganisms growing in site ground water to evaluate the composition of the microbial communities and to look for the presence of specific organisms and enzymes to further evaluate if microbial populations were responsible for the degradation of ground water contaminants. Bacterial communities that colonized the Biotrap samplers were analyzed by qPCR and DGGE microbial assay techniques.

Groun	Ground-Water B	r Bior					
0 -1 17-1-0	H						
well Localion Relative to Source	larger	7 70 70					
	Z L-MMM	1.40 O.					
Parameters Measured by EPA Method SW-846 or Off	6/24/2006 od SW-846 o	r Other s					
Nitrate (mod)	0.0151						
Nitrite (ma/l)	0.0216						
Total NO3 + NO2	0.0367						
Ammonium (mg/l)	0.0066						
Orthophosphate (mg/l)	0.0026						
Hydrolyzable Phosphate (mg/l)	0.004						
Field Parameters Measured with Selective Probes or	ective Prob	es or Met					
Dissolved Oxygen (DO) in mg/l	2.17	1.25					
Total Dissolved Solids (mg/l)	717	1,966					
Hd	7.97	5.78					
Temperature (oC)	14.2	14.2					
Conductivity (uS)	1,432	4,030					
Eh / Redox Potential (mv)	-258	-340.					
: Bacteria	Counts via	. Epi-Flu					
Average Population (cells/ml)	1.96E+06						
Metals via SW 846 (mg/l)							
Iron	68'0						
Manganese	0.57						
Bicarbonate Alkalinity (ppm)	290						
Total Organic Carbon (mg/l)	1						
Volatile Organic Compounds via EPA	A Method 8(	021 (ug/l)					
Benzene	ΩN						
Toluene	9						
Ethylbenzene	QN						
M&P Xylenes	QN						
O-Xylenes	QN						
Acetone	20						
Carbon Disulfide	98						
2-Butanone	9						
Chlorotorm	OL S						
Metriylerie Crioride							
1,1,1-Inchloroemane	င္ပင						
1,1,2- Illelioloelialle 1 1-dichoroethane	82						
1.1-dichoroethene	40						
1,2-dichloroethane	QN.						
cis-1,2 -Dichloroethene	38,000						
trans-1,2-Dichoroethene	QΝ						
Tetrachloro ethene	280						
Trichoroethene	67,000						
Vinyl Choride	32,000						
	000 207						
Total Chloroethenes	131,280						
SEMI_Volatile Organic Commonnels vi	is EDA Mot	hod 8270					
Semi-volatile Olyanic Compounds via Er A Metilou							
Ethana	1 600						
Mathana	000,4						
Welliane	) }						

		i					
Grou	Ground-Water Bior	Biore WN-1					
Well Location Relative to Source	Target						
Well Sampled	MW-18	MW-1					
Sampling Date 8/24/2006	8/24/2006	1/19/2(					
Nitrate (mod)	0 0 0 2 4 0 1						
Nitrite (mg/l)	0.0211						
Total NO3 + NO2	0.046						
Ammonium (mg/l)	0.1891						
Hydrolyzable Phosphate (ma/l)	0.0036						
Field Parameters Measured with Selective Probes	lective Probe	s or Me					
Dissolved Oxygen (DO) in mg/l	2.1						
Total Dissolved Solids (mg/l)	411	486					
pH Temperature (oC)	10.33	11.5					
Conductivity (US)	825	971					
Eh / Redox Potential (mv)	-577	-525.					
Microbiological Data: Direct Bacteria Counts via E	ia Counts via	Epi-Flu					
Average Population (cells/ml)	9.13E+05						
Metals via SW 846 (mg/l)							
Iron	0.064						
Manganese Dicarbonato Albalinity (nnm)	2 7						
Total Organic Carbon (mail)	<i>,</i>						
Total Organic Campol (Hg/l)	A Method 80	21 (ua/l					
Benzene							
Toluene	. 9						
Ethylbenzene	9						
M&P Xylenes	P						
O-Xylenes	2						
Acetone	44						
Carbon Disulfide	29						
Z-Dutariorie Chloroform	7						
Methylene Choride	2						
1,1,1-Trichloroethane	29						
1,1,2-Trichloroethane	98						
1,1-dichoroethane	38						
1,2-dichloroethane	9						
cis-1,2 -Dichloroethene	77,000						
trans-1,2-Dichoroethene	290						
Tetrachloroethene Trichoroethene	170						
Vind Charide	240						
A CHICAGO	047						
Total Chloroethenes	131,410						
VOC TICS	84						
SEMI-Volatile Organic Compounds via EPA Method 827	via EPA Meth	lod 827					
Ethene	250						
Methane	6						
	, ,						

Ground-Water Bi												
	/ater Bioren WN-′	en 1-1										
Well Location Relative to Source	Treatment poir	oir										
Well Sampled	TW-8											
Sampling Date	8/24/2006	1										
Parameters Measured by EPA Method SW-846 or	thod SW-846	or										
Nitrate (mg/l)	0.022											
Nitrite (mg/l)	0.0209											
Ammonium (mall)	0.0066			0.3532			6 531			i		1
Orthophosphate (ma/l)	0.0078			10.424			18.895			:		ı
Hydrolyzable Phosphate (mg/l)	0.0146			6.254			3.127			1		1
Field Parameters Measured with Selective Probes or Meters (Geovation Engineering, P.C.)	Selective Prob	es or Meters	; (Geovation	Engineering	g, P.C.)							
Dissolved Oxygen (DO) in mg/l	4.49	1.36	1.74	2.62	1.97	3.07	0.45	3.1	0.45	0.28	0.23	0.19
Total Dissolved Solids (mg/l)	622	28,000	2,780	2,480	11,490	9,680	45,200	3,500	11,220	29,700	5,390	32,800
Hd	7.29	5.72	6.19	5.71	7.03	7.66	6.12	6.83	5.99	6.01	5.22	6.28
Temperature (oC)	13.8	13.5	13.9	14.3	13.7	15.4	14.5	14.1	14.1	14.4	14.5	14.6
Conductivity (uS)	1,242	56,000	5,660	5,040	22,200	19,320	90,300	6,930	21,600	29,600	10,830	65,500
Eh / Redox Potential (mv)	28	-92	-240.9	16.1	-28.1	-48	8.9	-7.1	-55	-134.8	**-48.2	
Microbiological Data: Direct Bacteria Counts via Epi-Fluorescent Microscopy	eria Counts vi	a Epi-Fluore	scent Micro	scopy			100			10,1		Lo
Average Population (cells/ml)	4.68E+U5						4.92E+08			5.16E+U/		1.16E+08
Metals via SW 846 (mg/l)												
Iron	QN			15			9			12		24
Manganese	4.7			16			16			5.6		9.
Bicarbonate Alkalinity (ppm)	350			8800			3700			12000		9600
Volatile Organic Compounds via EPA Method 8021 (ug/l)	EPA Method 8	(021 (ug/l)					ŀ					
Benzene	ND			Q			Q			2		ND
Toluene	Q			2			9			9		R
Ethylbenzene	Q			9			9			9		Q
M&P Xylenes	Q			9			9			9		9
O-Xylenes	2			9			9			9		9
Acetone	9			9			9			9		9
Carbon Disulfide	32			9			9			9		9
2-Butanone	9			9			9			9		9
Chloroform	27			9			9			9		9
Methylene Choride	87.			9!			2 :			9 :		9!
1,1,1-Iricnioroetnane	170			2 9			2 2			2 9		2 2
1, 1, 2- Inchloroemane	/7 8			2 2			2 2			2 2		
1, 1-dichoroemane	90			2 9			2 2		1	2 2	1	2 2
1, I-diction defined and 1.0 dichlorophano	<u></u>		1	2 2			2 2		Ī	2 2	1	2 2
cis-1 2 - Dichloroethene	86,000			28 800			48 800			5 150		2 540
trans-1 2-Dichoroethene	380			S			429			S	Ì	S
Tetrachloroethene	230			9			103			9		40.1
Trichoroethene	190,000			28,600			36,300			6,020		1,590
Vinyl Choride	390			QN			QN			Q		32.3
VOC TICs				QN			QN			340		540
SEMI-Volatile Organic Compounds via EPA		Method 8270 (ug/I)	g/l)									
Ethane	-			-						1		
Ethene				-			-					
Methane	-			:								-

Ground-Water Bio	ter Bioren WN-1	m 1										
Well Location Relative to Source	Control											
Well Sampled	MW-17	THAT I										
Sampling Date	8/24/2006	118200										
Parameters Measured by EPA Method SW-846 or	nod SW-846 c	Jr.										
Nitrate (mg/l)	0.0119											
Nitrite (mg/l)	0.0216											
Ammonium (mg/l)	ON ON			0.2157			0.0362			0.2466		0.042
Orthophosphate (mg/l)	0.0003			3.273			0.0918			0.6838		0.6325
Hydrolyzable Phosphate (mg/l)	0.0005			5.779			0.1117			0.8363		0.8359
Field Parameters Measured with Selective P	elective Prob	es or Meters	Geovation	robes or Meters (Geovation Engineering, P.C.)	g, P.C.)							
Dissolved Oxygen (DO) in mg/l	2.07	1.1	1.8	2.58	1.93	3.58	0.46	0.35	0.47	0.32	0.75	0.65
Total Dissolved Solids (mg/l)	483	409	389	i	333	370	491	900	546	477	397	395
рН	7.13	6.92	7.03	7.03	6.62	13	99'2	6.48	99.9	6.97	6.7	9.9
Temperature (oC)	12.6	13.5	12	13.4	13.1	7.19	13.2	12.8	13.3	13	13.1	13.3
Conductivity (uS)	958	832	871	- 20	673	669	978	1,181	1,087	955	196	787
En / Redox Potential (mV)	-204	5.28.3	-220.4	007-	-789	281-	-138	9 <del>4</del> 4	-180	2/1-	-138.2	:
Microbiological Data: Direct Bacteria Counts via Epi-Fluorescent Microscopy	Ta Counts VI	a Epi-Fluore	scent Micro	scoby								
Average Population (cells/ml)							$\left[ \right]$					
Metals via Sw 846 (mg/l)												
Iron	5.4						27.0			33.0		36.0
Manganese	1.5			1.4			1.6			1.3		1.0
Bicarbonate Alkalinity (ppm)	ŀ			140.0			120.0			160.0		130.0
Volatile Organic Compounds via EPA Metho	ᅙᅵᅵᅙ	8021 (ug/l)										
Benzene	Q			Q			Q			9		9
Toluene	9			Q			9			2		9
Ethylbenzene	2			2			2			2		9
M&P Xylenes	9 9			9 9			9 9			9 9		9 9
Acotono Acotono	2			2 2			2 2			2 2		2 2
Carbon Disulfida	2 2			9 9			2 2			9 9		2 2
2-Butanone	2			2 2			2 2			9 9		2 2
Chloroform	2			2 2			2			2 2		2
Methylene Choride	2			9			9			2		9
1,1,1-Trichloroethane	Q			Q			Q			Q		Q
1,1,2-Trichloroethane	QN			QN			QN			QN		Q
1,1-dichoroethane	ND			QN			ND			QN		QN.
1,1-dichoroethene	QN			QN			QN			Q		Q
1,2-dichloroethane	Q			Q			ND			Q		Q
cis-1,2 -Dichloroethene	51			36			31			33		17
trans-1,2-Dichoroethene	9			Q			Q			9		9
Tetrachloroethene	2			9			9			9		1.2
richoroethene	4/			34 3			33			92,		12
Vinyl Choride	S			41			257			91		»
- CF	,			9			9			í,		Ĺ
VOC IICs		7,0200		2			9			2		2
SEIMI-Volatile Organic Compounds VIa EPA		Method 8270 (ug/l)		·			r			Ţ		c
Ethane	0 L			o (			7 1			4 1		7 (
Ethene	, 200	1		9 000			, 100			, ,		7 7
Wetnane	1,200	1		2,000			1,100			180		440

Grou	Ground-Water Biorem wn-2 B	r Biorem WN-2 B											
Well Location Relative to Source	Target												
Well Sampled	MW-25	MW-25											
Sampling Date	8/23/2006	1/19/2007											
Parameters Measured by EPA Method SW-846 or Other Spe-	od SW-846 o	r Other Spe											
Nitrate (mg/l)	2.3360												
Nitrite (mg/l) Total NO3 + NO2	0.06/1												
Ammonium (ma/l)	0.0538												
Orthophosphate (mg/l)	0.0031			0.0082			0.0033			0.5283			0.0268
Hydrolyzable Phosphate (mg/l)	0.0038			0.0109			0.0037			0.1088			0.0419
Field Parameters Measured with Selective Probes or Meters (Geovati	lective Probe	es or Meters	5	ш	3, P.C.)					1			
Dissolved Oxygen (DO) in mg/l	3.45	1.06		1.48	3.64	2.83	0.32	0.41	0.39	0.034	0.35	1.89	0.48
Total Dissolved Solids (mg/l)	625	998	936	3,090	1,321	1,442	1,621	1,048	1,179	734	775	436	1,558
Hd	7.57	7.06	7.37	7.2	7.52	7.06	7.34	6.35	6.76	7.13	6.99	7.01	6.79
Temperature (oC)	14.7	15.48	14.2	15.1	15.2	15.2	14.8	14.9	14.9	14.8	14.8	15.1	15.1
Conductivity (uS)	1,241	1,741	1,882	6,170	2,690	2,980	3,190	2,030	2,360	1,468	1,549	879	3,220
Eh / Redox Potential (mv)	75.5	-311.5	-329.2	-329.4	- 1	-311	-336.6	-350.7	-344.7	-336.6	-335	**184.8	
Wilcrobiological Data: Direct Bacteria Counts via Epi-riuorescent Mic	la counts via	Epi-riuores	cent Micros	roscopy			!						
Average Population (cells/ml)	3.20E+06		4.38E+06	3.20E+06			5.04E+06		$\Big]$	6.87E+05			2.74E+06
Metals via SW 846 (mg/l)							•						
Iron	QN			2.1			1.7			0.11			2
Manganese	0.3			3.5			3.8		1	8.			2.1
Total Organic Carbon (mg/l)	ij						9.9	11.3	2.7	2.2			2.8
Bicarbonate Alkalinity (ppm)	200			310			330			240			190
Volatile Organic Compounds via EPA Method 8021 (ug/l)	A Method 80	721 (ug/l)											
Benzene	ΩN			Q	QN	ON	QN	QN	QN	QN	ND		ND
Toluene	ΩN			9	Q	ND	QN	Q	QN	QN	Q		QN
Ethylbenzene	QN			Q	Q	ND	Q	Q	QN	QN	Q		Q
M&P Xylenes	QN			Q	Q	QN	QN	Q	QN	QN	Q		QV
O-Xylenes	9			9	2	9	9	2	9	9	9		
Acetone	2 5	<u> </u>	$\uparrow$	2 5	2 5	2 5	2 5	2 5	2 5		9 9		
Carbon Disumae	2 5			2 5	2 5	2 2	2 5	2 5	2 5		2		
Z-butanone	2			2 5	2 5	2 5	2 5	2 5	2 5	2 5	2 5		
Mothydone Cheride	- 2			2 9	2 2	ND 6	2 2	2 2			2 2		2 2
1 1 1-Trichloroethane	2 2			9 9	2 2	120	2 2	2 2	2 2	2 2	2 2		2 9
1.1.2-Trichloroethane	2 8			9 9	2 2	2 2	QN GN	2 2	Q Q	2 2	2 2		2 2
1,1-dichoroethane	Q			2	Q	QN	QN	Q.	QN	QN	2		Q
1,1-dichoroethene	QN			QN	QN	QN	QN	QN	QN	QN	QN		ND
1,2-dichloroethane	QN			QN	ND	ND	ΩN	ND	ON	ON	QN		QN
cis-1,2 -Dichloroethene	3,500			6,050	3,260	2,250	1,990	1,250	1,000	363	333		284
trans-1,2-Dichoroethene	9			9	20	14	32	2	10	Q	9		3.69
l etrachloroetnene	64	<u> </u>		7 ×	N 4								~ 00,
I richoroethene	001.0		1	٥١	2	67	48	28.6	2 2	87	75		60
Vinyl Choride	Q			2	2	115	989	0/8	629	66	202		106
Total Chloroethenes	9.664			6.226	3.375	2.394	2.733	2.213	1.698	588	290		500
VOC TICs	, QN			1,160	742	QN	444	207	Q	44	52		24
Trace Gases (ug/I)													
Ethane	0.34			1.30			0.10			0.11			0.34
Ethene	1.00			4.20			17.00			81.00			71.00
Methane	0.87			14.00			290.00			270.00			200.00

Groun	Ground-Water Bior wn	Bior											
Mell I ocation Relative to Source	Tardet												
Well Sampled	MW-19	MW											
Sampling Date	8/24/2006	1/19/											
Parameters Measured by EPA Method SW-846 or	od SW-846 o	r Othe											
Nitrate (mg/l)	1												
Nitrite (mg/l)													
Total NO3 + NO2	-												
Ammonium (mg/l)				0.1510			0.1244			0.1032			0.1667
Ornophosphate (mg/l)				0.1280			0.0050			0.1237			0.0186
Hydrolyzable Phosphate (mg/l)	 poetivo Broky		or Motors (Consultion Engineering B C)	U.U198	2		0.0083			U.13/4			0.0215
Dissolved Oxygen (DO) in mg/l	2 96		5.39	151	1.52	2 89	0.27	0.55	92.0	1.05	0.36	0.43	4 93
Total Dissolved Solids (mg/l)	532	1,381	3,890	3,420	1.979	1.202	778	957	845	783	708	1.204	4,640
Hd	7.24	6.73	6.42	6.65	6.72	6.58	7.79	6.52	6.62	6.77	6.93	6.7	6.73
Temperature (oC)	15	14.5	14.6	14.6	14.4	14.1	14	13.8	14.4	14.1	14	14.1	14.4
Conductivity (uS)	1,067	2,820	7,860	098'9	3,960	2,410	1,558	1,915	1,69,1	1,568	1,418	2,520	9,280
Eh / Redox Potential (mv)	295		52.8	10.9	-321.5	-349.1	-45.4	8.1	47.3	13.8	4.8	**183.2	:
Microbiological Data: Direct Bacteria Counts via	ia Counts vi	шІ	pi-Fluorescent Microscopy	copy									
Average Population (cells/ml)	5.45E+05		5.45x10^5	1.46E+06			2.96E+06			1.75E+06			2.91E+05
Metals via SVV 846 (mg/l)							ļ						
Iron	QN			0.07			0.53			QN.			QN .
Manganese	QN			3.1			5.4			0.63			99.0
lotal Organic Carbon (mg/l)	1 6			- 60			270	9. 0.	2.5	5.7			_ 0
Volatile Organic Compounds via EDA Method 802	A Mothod 8	024 (110(1)		100			710			200			60
Volatile organic compounds via Er	o nomenio	(1/6n) 170											
Denzene	ON S			2 :	ON C	2	2 5	QN S	ON S	Q :	Q i		Q :
Fibulbonzono				2 2		2					2 2		2
M&P Vylenes				2 2	2 2	2 2	2 2	2 2	2 2	2 2	2 2		2 2
O-Xylenes	2 2			2 2	Q. Q.	2 2	2 2	2 2	Q. Q.	2 2	2 2		2 2
Acetone	ON			QN	ON	QN	QN	ON	QN	QN	ΩN		QN
Carbon Disulfide	QN			QN	QN	QN	QN	QN	QN	QN	Q		QN
2-Butanone	ON			QN	ON	QN	QN	ND	ON	ON	ON		ON
Chloroform	ND			ON	ND	ON	ND	ND	ND	ND	ND		ND
Methylene Choride	ON			ON	ON	QN	QN	ON	QN	QN	ON		QN
1,1,1-Trichloroethane	ON			2	QN	Q	QN	QN	QN	QN	ΩN		Q
1,1,2-Trichloroethane	QN			QN	QN	QN	QN	QN	QN	QN	O.N.		QV
1,1-dichoroetnane	Q :			2 :	Q :	28	2	Q :		2	2		2
1, I-dichloroothana				2	ON C	67		OZ Z	ON C		2 2		2 2
i,z-dicilioloelilaile				UN 600	ON	ON 100	O00 4	ND 4	0 420	ON P	ND.		17 P
trans 1.2 -Dictionoethere	4,200			006,01	066,2	24,6UU 52	006,1	, 100 1	9,130	4,000	0.430		<b>-</b> 9
Tetrachloroethene	170			2 2	Q Q	13	2 2	Q Q	53	2 2	1,		161
Trichoroethene	7,600			551	09	152	1,810	1,750	5,230	2,790	1,110		27
Vinyl Choride	ND			ON	ND	2,230	8,750	3,020	836	123	75		ND
Total Chloroethenes	11,970			11,051	2,610	26,995	18,460	8,950	15,249	7,713	2,652		46
	QN			QN	QN	QN	QN	QN	Q	QN	ON		QN
Trace Gases (ug/l)	0			30 0			0 10			Ç.			2
Ethono	20.00			07.0			8 30			7 10			2 2
Methane	0.19			14.00			100 00			10.00			2 2
	71.5			25:			22.22	1			]		3

Groun	Ground-Water	Bior											
Well Location Relative to Source	Remote Tard	get											
Well Sampled	MW-24												
Sampling Date	8/24/2006	_											
Parameters Measured by EPA Method SW-846 o	10d SW-846	or Oth											
Nitrate (mg/l)	1.988												
Nitrite (mg/l)	0.1056												
Total NO3 + NO2	2.0936			1.1 / 08			2.0850			4.2699			4.1490
Ammonium (mg/l)	0.1837			0.3806			0.0341			0.3521			0.0927
Orthophosphate (mg/l)	0.0019			0.0089			0.0056			0.1088			0.0160
Hydrolyzable Phosphate (mg/l)	0.0028			0.0128			0.0086			0.1221			0.0178
Field Parameters Measured with Selective Probe	elective Prot	es or Meter	s (Geovation	s or Meters (Geovation Engineering, P.C.)	ig, P.C.)								
Dissolved Oxygen (DO) in mg/l	2.84	1.32	1.3	3.14	2.24	3.77	0.38	0.5	0.35	0.28	0.51	0.32	0.29
Total Dissolved Solids (mg/l)	462	495	460		503	328	651	449	459	711	642	807	689
Hd	7.46	7.09	7.19	7.26	7.29	8.1	7.93	7	98.9	7.11	96.9	7.35	7.35
Temperature (oC)	11.5	12	11.9	11.9	11.8	11.7	11.5	11.6	11.7	11.9	11.7	12.4	11.8
Conductivity (uS)	921	1,000	919		1,003	648	1,299	895	905	1,425	1,285	1,624	1,381
En / Redox Potential (mv)	-119		-181.5	-167	-155.5	-53.1	-67.7	-89.8	-75.3	-93.2	-109.3	**-38.2	-
Microbiological Data: Direct Bacteria Counts via	la Counts v		Epi-Fildorescent Microscopy	scopy			1:07 07 0			1011010			10: 11:00
Average Population (cells/ml)	6.52x10 <sup>a</sup> 5			4.62×10 <sup>4</sup> 5			2.42x10 <sup>4</sup> 5			2.73E+05			2.37E+05
Metals via SW 846 (mg/l)													
Iron	<0.05			<0.05			0.1			0.1			0.1
Manganese	0.029			0.042			0.016			<0.01			<b>0.01</b>
Total Organic Carbon (mg/l)	1			1			1 5			1.0			1.2
Sicarbonate Alkalinity (ppm)  Volatile Organic Compounds via EPA Method 80	140.0 P& Method 8	1024 (mail)		110.0			160.0			160.0			160.0
Position of game compounds that I	noinem v	(484)					2						2
Tolliana	2 2			2 2			2 2			2 2	2 2		2 2
Olderie	2 2						2			D 2	2 2		
Mag D Vidence	2						2 2			2	2 2		2
O-Xvlenes	2 2			2 2			2 5			2 2	2 2		2 2
Acetone	2 2			Q Q			2 2			9	2 2		2 2
Carbon Disulfide	S			S S			S			S S	S S		2
2-Butanone	Q			QN			Q			Q	Q.		Q
Chloroform	9			ON			QN			ON	ON		QN
Methylene Choride	QN			ND			QN			ND	ND		QN
1,1,1-Trichloroethane	17			ND			QN			ON	ND		QN
1,1,2-Trichloroethane	6			QN			Q			QN	QN		QN
1,1-dichoroethane	QN 3			Q			Q			QV	Q		Q
1, 1-dichoroethene	74			Q.			9 :			Q !	9		2
1,z-dicfiloroemane	12 CC			ND 2						000 cc	ND 0		ND 77
cis-1,2 -Dichoroethene	12,000			3,140			006,27			73,600	3,640		14,200
Tatrachloroethene	34 <5000			ND 346			718			<sup>0</sup> 828	282		N□ 865
Trichoroethene	48.000			42.800			69.400			86,900	32,000		51.500
Vinyl Choride	11			QN			QN			QN	QZ		QN
VOC TICS	35			ON			QN			ON	ND		ON
Trace Gases (ug/l)													
Ethane	5.90			43.00			3.00			96.0			00'9
Ethene	6.70			90.00			3.10	1		1.00			4.60
Methane	2.00			3.30			2.50	$\rceil$		4.50			3.00

Ground-V	Ground-Water Biore	0 1										
Well Location Relative to Source	Treatment											
Well Sampled	1W-6											
Sampling Date	8/23/2006	1										
Parameters Measured by EPA Method SW-846 or	od SW-846 or											
Nitrate (mg/l)	5.0652											
Nitrite (mg/l)	0.0269						CHAR					
Ammonium (mg/l)	0.1119			0.6173			0.2531			1		1
Orthophosphate (mg/l)	0.0028			11.827			6.525			1		!
Hydrolyzable Phosphate (mg/l)	0.0035			2.774			2.066					-
Field Parameters Measured with Selective P	elective Probes	or Meters	(Geovation	robes or Meters (Geovation Engineering, P.C)	, P.C)							
Dissolved Oxygen (DO) in mg/l	3.41	1.06	1.78	2.5	1.6	2.88	0.5	0.13	1.4	0.15	0.25	0.23
Total Dissolved Solids (mg/l)	518	31,500	4,700	30,300	5,390	20,700	25	14,890	39,900	31,200	17,010	32,100
Hd Tomostatis (20)	7.44	29.6	6.25	6.04	5.92	0.0	6.08	2.5	25.98	2.80	20.08	- u
Conductivity (1.5)	1037	63.200	18.800	60 000	10.680	13.3	6.4 0.4	29 500	70.800	18.2	34 500	13.3
Eh / Redox Potential (mv)	3 8	-129	-240 1	-342 1	-116	-218	-181	-50.2	-46.9	-878	**-72.2	200,45
Microbiological Data: Direct Bacteria Count	U	-pi-Fluores	via Epi-Fluorescent Microscopy	Copy				!!				
Average Population (cells/ml)	ıч			5.07E+07			1.15E+08			5.16E+07		1.81E+08
Metals via SW 846 (mg/l)												
Iron	-			7.5			7.7			21		19
Manganese	:			89.0			3.2			2.3		5.5
Bicarbonate Alkalinity (ppm)	1			1600			4500			12000		0096
Volatile Organic Compounds via EPA Metho	>A Method 802	d 8021 (ug/l)										
Benzene	ON			QN			QN			QN		Q
Toluene	QV			ON			QN			ON		QN
Ethylbenzene	9			Q			Q			9		9
M&P Xylenes	2 :			2			9 !			9 !		2 :
O-Xylenes	2 2						9 <b>7</b>			9 9		NO.
Carbon Disulfide	2 2			CH7			<u>i</u> S			2 2		2 2
2-Butanone	2 2			2 0			2 2			2 2		478
Chloroform	22			2			9			2 2		2
Methylene Choride	ON			ON			ND			ND		ON
1,1,1-Trichloroethane	QN			ON			QN			ON		QN
1,1,2-Trichloroethane	Q			QN			QN			Q		Q
1,1-dichoroethane	2			Q			Q			2		9
1,1-dichoroethene	9			Q			Q			9		9
1,2-dichloroethane	28			9			9 !			2		2
cis-1,2 - Dichloroethene	2,600			D			1/			Q		2
trans-1,2-Dichoroethene	4 6			Q i			2 5			Q (		2
etrachloroethene	84 7						Q <del>Ş</del>			2 5		2 :
I richoroetnene	000,0						=					2
Vinyi Choride				ON			N N					
VOC TICs	S			4 889			3 488			230		298
Trace Gases (IIII/I)	9			200,1			22 (5)					
	9			QN			QN			S		9
Fthene	2 2			S N			<u> </u>			2		2 2
Methane	2 2			2 2			2 2			2 2		2 2
	2			1			2			ì.:		2

Ground-Water B	Vater Biore WN-2	2 2										
Well Location Relative to Source	Control											
Well Sampled	MW-11											
Sampling Date	8/24/2006											
Parameters Measured by EPA Method SW-846 or	od SW-846 or											
Nitrate (mg/l) Nitrite (ಜಾಗ್ರಿ)	2.4994											
Total NO3 + NO2	0.0210			/6/6/			007.5			/0867		/08C/
Ammonium (mg/l)	2			0.2272			0.0404			0.1265		0.1136
Orthophosphate (mg/l)	0.0023			0.02055			0.0237			0.1105		6900'0
Hydrolyzable Phosphate (mg/l) 0.004	0.004			1 0.0408			0.0314			0.124		0.0114
Field Parameters Measured with Sel	lective Probes	s or Meters	Geovation	Engineering	, P.C.)			!				-
Dissolved Oxygen (DO) in mg/l	3.05	6.67	5.61	7.1	7.52	8.33	0.46	4.43	5.27	6.21	5.51	5.25
l otal Dissolved Solids (mg/l)	238	186	206	1 6	424	339	491	308	4/6	465	518	2/4
DH Tomporaturo (OC)	77.0	0.80	75.	0.7 7.4.7	28.0	13 00	13.0	13.7	70.7	0.03	13.0	7.75
Conductivity (1.5)	7.4.7	403	422	į	956	13.30 680	978	7.6	930	932	1 040	546
Eh / Redox Potential (mv)	167	57.8	-76.9	34.3	17.4	18.2	-137.6	-43.5	28.2	2.8	**2.3	2
Microbiological Data: Direct Bacteria Counts	4.	via Epi-Fluorescent Microscopy	cent Micros	copy								
Average Population (cells/ml)												
Metals via SW 846 (mg/l)												
Iron	90.0			QN			0.29			QN		QN
Manganese	QN			<.01			QN			ΩN		QN
Bicarbonate Alkalinity (ppm)	160.00			150.00			160.00			160.00		130.00
Volatile Organic Compounds via EPA Metho	A Method 802	d 8021 (ug/l)										
Benzene	QN			ΟN			QN			ON		QN
Toluene	Q			QN			QN			QN		QN
Ethylbenzene	2			Q :			2			Q :		2
M&P Xylenes	2 2			9 9			9 9			9 9		2 2
Acetone	2 2			2 2			2 2			2 2		2 2
Carbon Disulfide	2 2			2 2			2 2			2 2		2 2
2-Butanone	9			QN			QN			Q		QN
Chloroform	QN			QN			ON			QN		QN
Methylene Choride	Q			QN			ON			QN		ON
1,1,1-Trichloroethane	9			2			2			Q		2
1,1,2-Trichloroethane	2			2			Q.			Q.		2
1, I-dichoroettiane	2 2			2 2						2 2		2 2
1, 1-diction of the second of	2 2			2 2			2 2			2 2		2 2
cis-1.2 - Dichloroethene	290			307			535			217		264
trans-1.2-Dichoroethene	9			2			7			2		9
Tetrachloroethene	14			2			8			13		16
Trichoroethene	940			224			566			884		435
Vinyl Choride	QN			QN			QN			QN		QN
	QN			QN			ND			ΩN		ON
Trace Gases (ug/l)	9			9			2			2		2
Etilalie	2 2	1		2 2			2 2			0 O		200
Ethene Mathana	_ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \			180			⊒ <b>(</b>			0.03		0.03
Melland	20:-	1		25.		$\int$	25.1			000		- 1:5

#### 3. RESULTS

This demonstration project had several objectives, out of necessity, as it was difficult prior to the study to predict the rates of the reaction of the native biological community at the site to SRC<sup>TM</sup> substrate and nutrient enrichment. This was made more difficult by the uncertainties involved with the transport of these products through a bedrock aquifer.

Significant impacts were not observed at monitoring location MW-24, which was located at a considerable distance from the treatment areas; therefore, discussion of the results obtained in this well are not included in this report. However, all data obtained from this well during the project has been included. These results indicate that MW-24 is located beyond the distance that the treatment areas could impact within one year.

#### **Creation of Anaerobic and Reducing Conditions**

The first objective of this project was to use SRC<sup>TM</sup> to create anaerobic and reducing conditions in the subsurface. These geochemical conditions set the stage for the biological reduction of chlorinated compounds. Review of dissolved oxygen values in both the source area and the barrier area indicate that DO concentrations generally remained very low during the project, which indicates anaerobic conditions were achieved (Tables 2 and 3). The exception to this observation is the measurement of some low levels of dissolved oxygen in the barrier area in monitoring well MW-19 near the end of the project (further discussed below). In the source area, the DO concentrations of the control wells also changed in a similar response to that of the targeted monitoring wells. This may indicate that control well MW-17 was not outside the influence of SRC<sup>TM</sup> additions, or it may indicate that DO concentrations naturally declined in portions of the site during the study. In the barrier area, DO concentrations in control well MW-11 changed very little, which supported the view that reductions in DO were achieved via the addition of SRC<sup>TM</sup> product.

Baseline redox potential conditions showed that the source area exhibited preexisting negative eH values. In this area, Geovation's SRC<sup>TM</sup> product was helpful at maintaining these conditions in both target ground water monitoring wells for the duration of the project (Table 2). Comparison of these results to control point MW-17 indicates that ground water in the source area is likely to have remained negative without SRC<sup>TM</sup> additions. In the barrier area, monitoring well MW-25 is located closer to the treatment wells than is MW-19 (Figure 2). In MW-25, eH values became negative after approximately one month of the application of SRCTM product and remained negative for the duration of the project (Table 3). Farther away, at location MW-19, eH values were observed to slowly decrease in response to the application of SRC<sup>TM</sup> product and became negative after four months of SRCTM treatment. The eH values remained negative for approximately three months and then varied between slightly negative and slightly positive for the duration of the project. During this time period, there was some reduction in Geovation's ability to introduce SRC<sup>TM</sup> product in the desired quantities into the treatment wells up gradient of MW-25 and MW-19. It is this reduction in the amounts of SRC<sup>TM</sup> added to the barrier area (Table 1), combined with the greater distance from the treatment wells compared to MW-25, that likely allowed the concentration of dissolved oxygen and redox potential to increase in MW-19 during the final portion of the project. Data obtained from the control point MW-11 indicates that there was considerable natural variation in the redox values of the ground water. Based on this observation of the natural variation in control point MW-11, the consistently negative redox potential measurements obtained from monitoring point MW-25 indicate that addition of SRC<sup>TM</sup> in the barrier area was successful at creating and maintaining anaerobic and reducing conditions.

It also is noted that due to a malfunction of the redox probe during the last two, and a portion of the last third, sampling events, there is no comparable redox data for these events (Tables 2 and 3).

#### **Increased Biological Activity**

The second objective of this pilot project was to measure increased biological activity in response to SRC<sup>™</sup> additions. Biological activity was measured both directly and indirectly. Direct counts of microorganisms in ground water by epi-fluorescent light microscopy (DAPI counts) generally increased in the source area, with cell concentrations measured at one to two orders of magnitude above baseline in MW-12 and MW-18 (Table 2 and Figure 3 – Source Area).

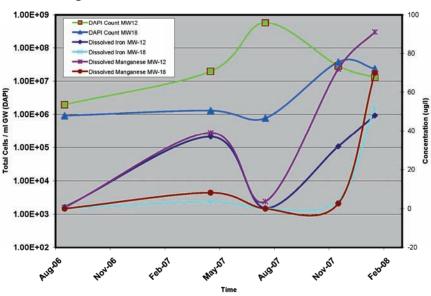


Figure 3 - Source DAPI Counts and Dissolved Metals

In the barrier area, the measured microorganism cell concentrations first increased, then subsequently decreased. As discussed above, in the barrier area, near the end of the study period, Geovation's ability to introduce SRC<sup>TM</sup> product in the desired quantities into the treatment wells was reduced, and this is likely to have resulted in the decreases in cell concentrations observed in MW-19 and MW-25 (Table 3 and Figure 4 – Barrier Area). Cell concentrations in the barrier area also may have decreased in the later portion of the study as contaminant concentrations decreased. Direct cell counts were also collected in one treatment well in the source area and biobarrier area. In the treatment wells, the cell concentrations in ground water consistently increased by two to three orders of magnitude above baseline conditions (Tables 2 and 3).

Indirect measurements of biological activity included measurements of the dissolved metals iron and manganese. The concentrations of dissolved metals in the source area and barrier area were generally elevated above the baseline concentrations throughout the study (Tables 2 and 3, Figures 3 and 4). In the barrier area, the dissolved iron and manganese concentrations were generally observed to decrease near the end of the study, which is consistent with the information collected by direct microscopy counts.

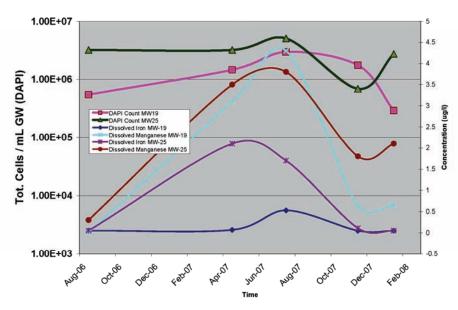
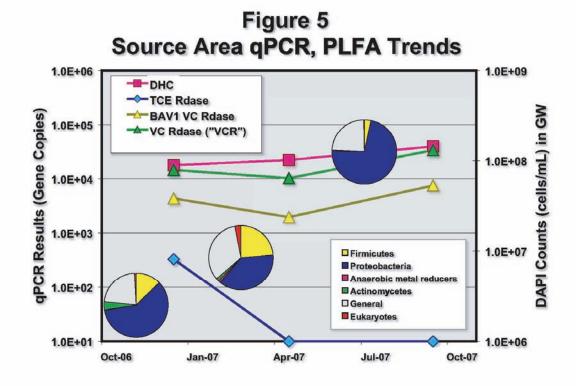


Figure 4 - Barrier Area DAPI Counts and Dissolved Metals

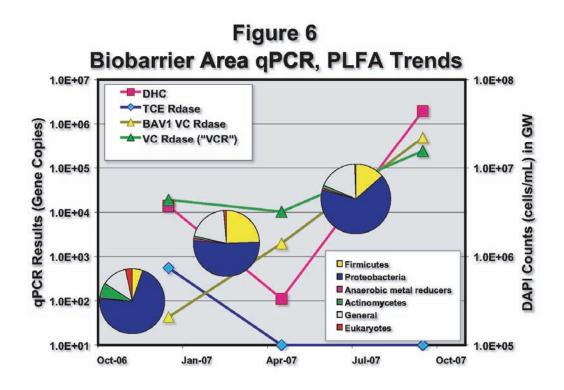
Additional measurements of biological activity included the results obtained from the Biotrap<sup>®</sup> samplers. Biotrap<sup>®</sup> samplers are passive samplers designed for collecting microorganisms by providing a matrix on which to grow. The Biotrap<sup>®</sup> samplers were suspended in monitoring well MW-12 in the source area and, at different time periods, in wells MW-19 and MW-25 in the barrier area. Biotrap<sup>®</sup> samplers were suspended in these wells for minimum 60-day time periods to become colonized.

Biotrap<sup>®</sup> samples were collected at three time points during the study: in December 2006 prior to the first SRC<sup>TM</sup> application (baseline), in April 2007, and in September 2007. The Biotrap<sup>®</sup> samples were analyzed for variants of phospholipids fatty acids (PLFA), which are a main component of the cell membranes of microorganisms. The PLFA profiles obtained from a sample correspond to specific groupings of organisms and reflect the composition of the microbial community in ground water. The changes in the composition of the microbial community during this project are presented on Figure 5.



A copy of the original laboratory data is provided in Appendix A. As shown on Figure 5, both the source and barrier areas were dominated by proteobacteria, which is one of the largest groups of bacteria and represents a wide variety of both aerobes and anaerobes. Firmicutes, indicative of the presence of anaerobic fermenting bacteria (which produce the H<sub>2</sub> necessary for reductive dechlorination, as discussed in more detail below), were also present in both areas monitored during the pilot study. In general, the proteobacteria proportion of the community first decreased, then subsequently increased in response to SRC<sup>TM</sup> treatment. Alternatively, the firmicutes initially increased and then decreased. Eukaryotes consistently represented a small percentage of the community, and actinomycetes, which were present in the baseline sampling, significantly decreased as a percentage of the community, as the study progressed (Figure 5).

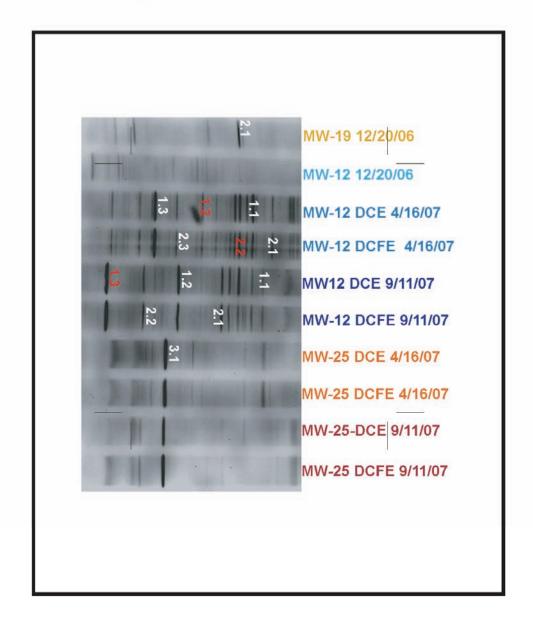
Biotrap® samples were also submitted for qPCR (quantitative real-time polymerase chain reaction) analysis, a molecular biological tool that can detect and quantify genetic code for specific microorganisms and metabolic enzymes. The organisms and enzymes that were targeted were *Dehalococcoides spp* (a group of bacteria known to be capable of dechlorinating PCE and/or TCE to ethene), *TCE R-Dase* "tceA" (an enzyme that is known to catalyze the dechlorination of TCE to VC), and *BAV1 VC R-Dase* and *VC R-Dase* (enzymes that are known to catalyze the direct dechlorination of VC). These target organisms and enzymes were found to be present in both the source and barrier areas, which demonstrated that the biological tools required for the contaminant degradation observed were found to be present in the biological community. A plot of the qPCR data over the three time periods is provided as Figure 6.



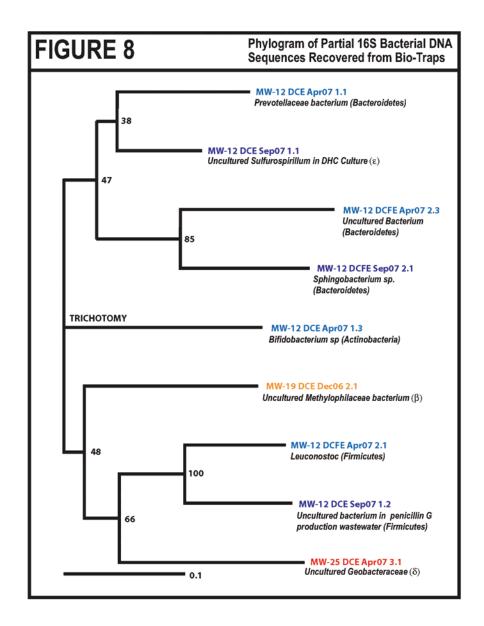
As shown on this figure, the amounts of these targeted enzymes generally increased in response to SRC<sup>TM</sup> treatment. Gene copies of the target organism *Dehalococcoides spp.* increased in both the source and barrier areas. Similarly, gene copies for the enzymes for VC degradation (*VC R-Dase* and *VC R-Dase*) were also observed to increase from the baseline sampling event to the October 2007 sampling event. Gene copies for the TCE degradation enzyme TCE Rdas "tceA" declined to below the analytical method detection limits during the study. However, it is likely that other enzymes were present in the microbial community and involved in TCE dechlorination, as the concentration of TCE was observed to decrease during the study and has been largely eliminated as of the completion of the pilot study in January 2008.

Samples from the Biotraps® were further analyzed by Denatured Gradient Gel Electrophoresis (DGGE), which is a molecular fingerprinting method that separates PCR-generated DNA products and allows further delineation of the microbiological community. The results of the DGGE analysis are shown on Figure 7.

# Figure 7 Diagram of DGGE Gels



On this figure, each "band" theoretically represents a different bacterial population present in the community. Partial 16S bacterial DNA sequences were recovered from the more prominent bands to identify specific bacteria. A phylogram showing relationships of specific identified bacteria is provided as Figure 8.



Of particular interest on this chart is the identification of the Epsilon bacteria, *Sulfurospirillum*, which is a known dechlorinator of PCE and TCE to cDCE, and the identification of the Delta bacteria *Geobacteraceae*, who's family includes many of the most important/known Fe(III) reducers and many sulfate reducers. In general, DGGE analyses support the qPCR analyses, showing the clear dominance of anaerobic fermenters of a microbial community that includes known chloroethene degraders.

Based on the data collected, the application of SRC<sup>TM</sup> product was successful in increasing the biological activity at this site as indicated by: (i) the observation of increased concentrations of iron and manganese dissolved in ground water, (ii) the observation of increased direct counts of microorganisms, (iii) the observation of shifts in the biological community toward anaerobic fermenters, (iv) the observation of increased *Dehalococcoides spp.* amounts and two different microbial enzymes capable of the biodegradation of vinyl chloride, and (v) the identification of specific bacterial populations present at the site that are known to be important in bioremediation of chloroethenes.

#### **Degradation and Reduction of Ground Water Contaminants**

The third objective of this project was to measure decreases in contaminant concentrations in response to SRC<sup>TM</sup> additions. Chlorinated solvents can biodegrade in ground water under strong reducing conditions through a process known as reductive dechlorination. During this process, the chlorinated hydrocarbon is used as an electron acceptor, not as a source of carbon, and a chlorine atom is removed and replaced with a hydrogen atom. Fermentation of another source of carbon would produce H<sub>2</sub>, which serves as the electron donor in this process. Geovation's SRC<sup>TM</sup> product provides the carbon source to accelerate this process.

Biological reductive dechlorination of solvents takes place in a series of discrete steps, with a single chlorine atom removed from the solvent molecule in each step. Tetrachloroethene is progressively converted to trichloroethene (TCE), which is converted to dichloroethene and then to chloroethene (vinyl chloride), which then further degrades to ethene gas and chloride. It should be noted that other degradation pathways do exist for these chlorinated compounds.

Contaminant concentrations are typically measured in ug/l. This measurement of concentration is based on the weight (mass) of the contaminant in a liter of water. The changes in ground water contaminant concentrations for the duration of the study are provided on Tables 2 and 3. This information is presented graphically on Figures 9 through 12.

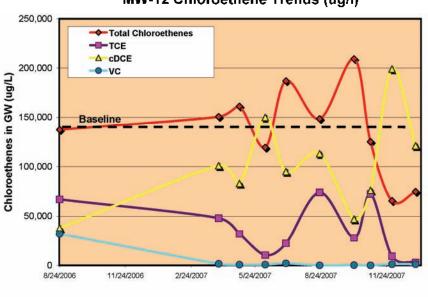


Figure 9
MW-12 Chloroethene Trends (ug/l)

3-8

Figure 10 MW-18 Chloroethene Trends (ug/l)

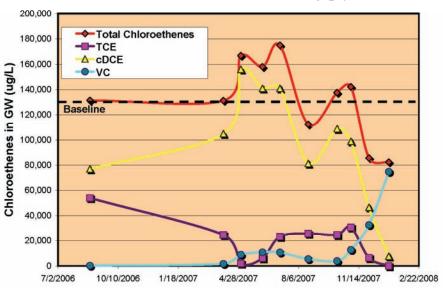
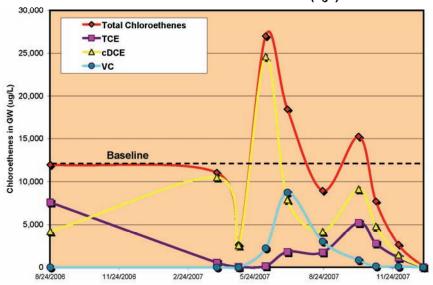
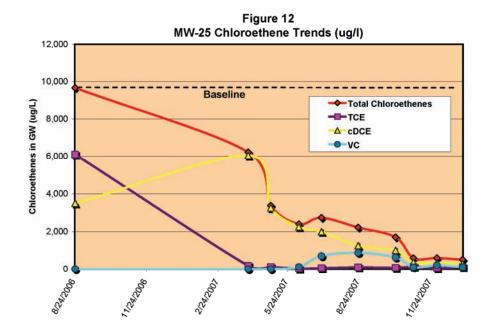


Figure 11 MW-19 Chloroethene Trends (ug/l)



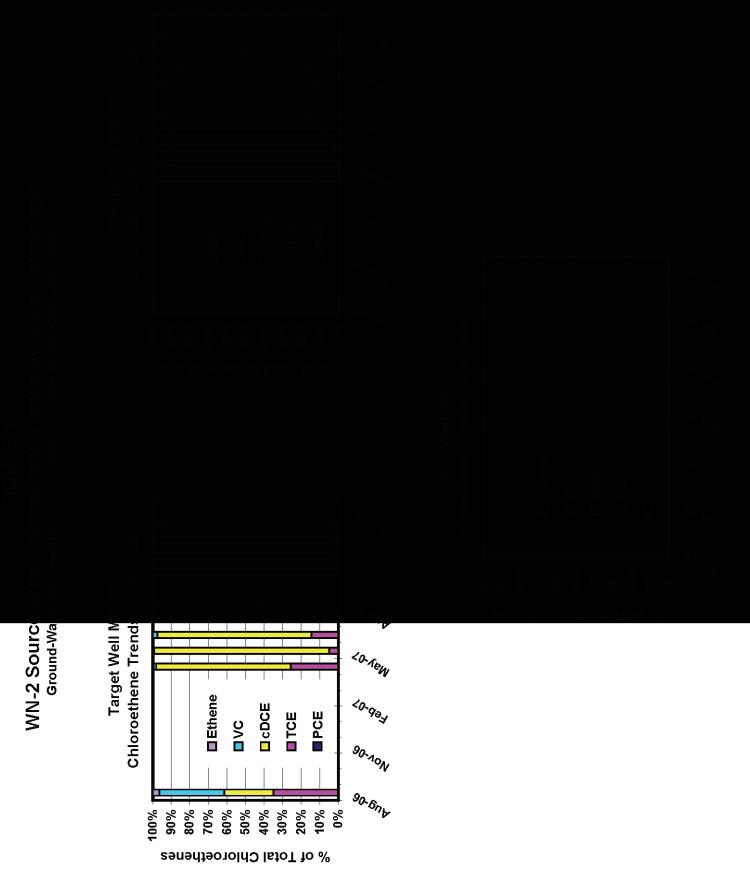


As an analytical technique, the concentrations of the family of related compounds that comprise the chloroethenes -- PCE, TCE, DCE (cis & trans), VC, and ethene -- were converted to molar concentrations to better evaluate the degradation of parent to daughter compounds. This conversion to molar concentration facilitates this understanding by looking at the changes in the amount of molecules from parent to daughter compounds, not their weights.

An example of the conversion of the mass concentration to molar concentration for TCE is provided below.

TCE (moles/liter) = (TCE (ug/l) 
$$\times$$
 (10<sup>-9</sup> g/ug)) / (131.39 g TCE/mole TCE) where 131.39 is the molecular weight of TCE

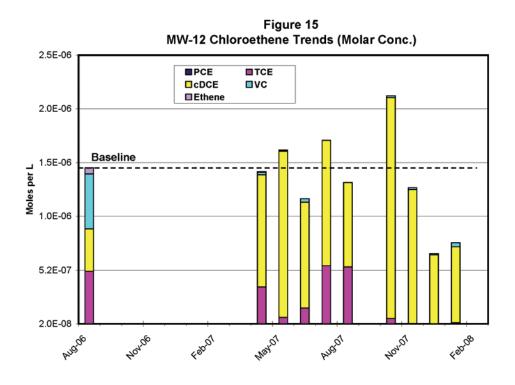
The calculation would be the same for the daughter compounds, with the molecular weight for each of them substituted in the equation. The molar concentrations of the parent and daughter compounds were evaluated in two ways. First, the percentage of each of these constituents as percentage of the total contaminant mass (measured in each well) was calculated for each ground water sampling event, and in this way the composition of the dissolved ground water contamination was evaluated over the duration of the pilot to-date. Charts of the molar percent of contaminants for the target wells and control wells are presented as Figures 13 (source area) and 14 (barrier area).

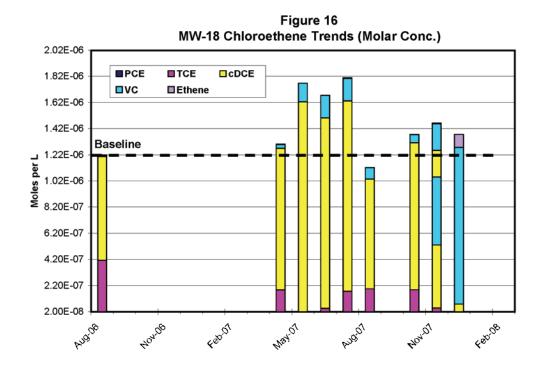


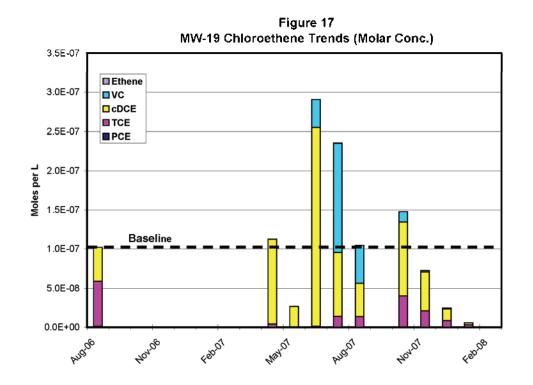
As shown on these figures, the compositions of the contaminants in the control wells (MW-17 and MW-11) have remained relatively stable throughout the pilot project. By comparison, the compositions of the contaminants in the target wells are seen to change in response to SRC<sup>TM</sup> additions as parent compounds are converted to daughters.

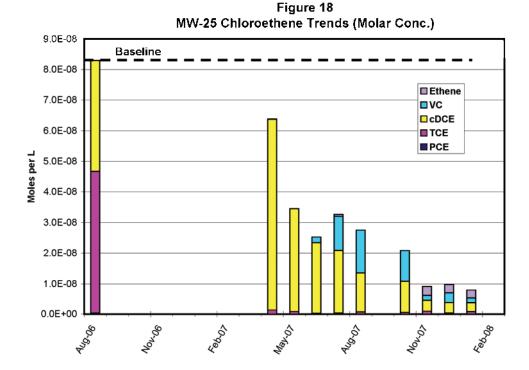
This comparison shows that the changes seen in the reduction of contaminants in the target wells are not the result of simple dilution of aquifer by the additions of "uncontaminated" SRCTM liquid. If simple dilution were the cause for the observed changes in contaminant concentrations, the relative composition of the contaminants should remain largely unchanged and be similar to the background composition changes observed in the control wells. Another use of these percentage composition diagrams is to emphasize the progression of the conversion of parent to daughter compounds. This alteration of contaminant chemistry is direct support for biologically mediated contaminant destruction and the success of this pilot project. The degradation of parent compounds to daughters is seen in all target wells, as the relative proportion of TCE is reduced and the proportion of cDCE increases first, followed by increases in VC and then ethene. This pattern is most clearly observed in monitoring well MW-25 (Figure 14). Baseline conditions show relatively equal proportions of TCE and cDCE in this well. Over time, the enhanced bioremediation caused by the SRC<sup>TM</sup> product is seen to first increase the proportion of cDCE present and then to sequentially initiate the degradation of cDCE and production of VC, and the degradation of VC and production of ethene gas, as predicted by the dechlorination process described above.

The second method used to evaluate the calculated mole concentration data was to directly graph the sum total of the moles of contaminant reported in ground water over time. These data are









In these figures, rather than the bar height representing 100 percent (as in Figures 13 and 14), the bar height represents the amount of contaminants reported during each sampling event, and the amounts are vertically stacked to show the total contaminants measured during each sampling event. The left-most stacked bar on each of these charts represents the baseline conditions prior to the addition of the SRC<sup>TM</sup>. For example, for MW-12 (Figure 15) the total mass of TCE and its daughters was comprised of about one-third each of TCE, cDCE, and vinyl chloride, with a sum total of almost  $1.5 \times 10^{-6}$  moles/liter contaminants. The bars to the right show the changes in the amount of dissolved contaminants over time.

It is Geovation's experience that the increase in the total amount of contaminants above the baseline values indicates some degree of natural variation in the ground water quality as well as desorption of additional source material from the bedrock matrix. It is not unusual in enhanced bioremediation studies to observe temporary increases in contaminant concentrations above baseline values, followed by a subsequent decrease. Temporary increases in the dissolved concentration of a parent or daughter compound are not an indication that the dechlorination process is not occurring. Enhanced desorption of contaminants from the aquifer matrix is a desired outcome of the bioremediation process and, furthermore, will likely be a (if not *the*) limiting factor in the overall success of any type of remedial process implemented at this or other sites.

Figure 15 shows that the responses to SRC<sup>TM</sup> additions in the source area near monitoring well MW-12 were a significant decrease in the amount of TCE in ground water (94% decrease over the project) and an increase in the amount of the daughter product cDCE. It should be noted that, at this location, VC was reported at an elevated concentration (32,000 ug/l) prior to the implementation of the pilot program and is being produced naturally at the site. As a result of the pilot program, VC values at this location have decreased by 92%. It also should be noted that in

May and June 2007, application of SRC™ was suspended in the back area (WN-1), and instead, Geovation's N-Blend® nitrate-based biological nutrients were added to the subsurface in an attempt to further enhance the biodegradation of cDCE. The results of adding N-Blend® during these months were inconclusive, and SRC™ applications were resumed in July 2007. It is likely that this interruption of SRC™ application is responsible for the temporary increase in TCE concentrations observed in July and August 2007.

Data for MW-18, also located in the source area, are depicted in Figure 18 and show that very little vinyl chloride was present prior to the start of the pilot study (240 ug/l – too little to be visible on the chart). In response to SRC<sup>TM</sup> additions, TCE values have decreased to below the detection limit (>99.9%) and concentrations of c-DCE and vinyl chloride both increased, again demonstrating that dechlorination is occurring. Subsequently, c-DCE has declined to below baseline (a 90% removal from the baseline concentration), the VC concentration has increased, and the production of ethene has now been quantified.

In the source area, additional contaminants continue to desorb from the aquifer matrix into ground water, which renews the supply of parent compounds. In the barrier area, renewal of contaminants is limited to up-gradient supply dissolved in ground water. In the barrier area, indicators of the dechlorination of this more limited supply of parent compounds are much more evident. In MW-19, subsequent to SRC<sup>TM</sup> treatment, TCE concentrations were quickly reduced. This was accompanied with the anticipated increase in cDCE concentration and the appearance of VC (Figure 17). Ethene concentrations have not yet been observed to increase significantly; thus, it is likely being removed from the system at the same rate it is being produced (Table 3; the molar concentrations are too low to be visible on Figure 17). In MW-19, TCE concentrations have decreased by 99.6% since the initiation of the pilot study.

Monitoring well MW-25 exhibits the best dataset obtained during this study. TCE has been degraded by 98% since the initiation of the pilot. As discussed above, the complete set of dechlorination daughter products has been observed at this location, which is strong evidence of the great potential for the use of SRC<sup>TM</sup> to remediate ground water at this site. The molar chloroethene trends in MW-25 are depicted in Figure 18. The concentration of cDCE is observed to increase after the initial application of SRC<sup>TM</sup>, with production of VC measured at month five of the pilot. Ethene concentrations increase at approximately month six, and VC concentrations are observed to decrease in month nine. As of January 2008 (month twelve), total VOCs have been reduced by 94.8% on a concentration basis and 90% on a molar basis.

As noted above, the concentrations of parent compound TCE were reduced by at least 94% (by weight) in all target wells. The predicted resultant increases in the first daughter product, cDCE, were observed, and cDCE subsequently was reduced. In the barrier area, the resultant increases and reductions in the second daughter product, VC, were also observed. Based on the observed rates of contaminant reduction and daughter product creation/destruction, Geovation modeled contaminant degradation rates for TCE, cDCE, and VC (when possible). First-order degradation equations were used as follows:

$$\frac{dC_A}{dT} = -k_1 C_A$$

where,

 $dC_A$  = Change in concentration of compound A

dT =Duration of Time period over which  $dC_a$  is measured

k = calculated degradation rate of compound A

 $C_A$  = Initial Concentration of compound A

and

$$\frac{dC_B}{dT} = -k_2C_B + k_AC_A$$

where,

 $dC_B$  = Change in concentration of compound B

dT =Duration of Time period over which  $dC_B$  is measured

 $k_2$  = calculated degradation rate of Compound B

 $C_B$  = Initial Concentration of compound B

Using these equations as the basis of a detailed analytical and numerical evaluation of the data generated, Geovation calculated degradation rates for the ground water contaminants. Curve matching of one or more selected time periods where consistent contaminant mass reductions were observed was the numerical method most often used. More detailed information on the analytical and numerical calculations is provided in Appendix B. The results of this evaluation are summarized in Table 4. As shown on Table 4, Geovation calculated a site-wide average k for TCE of 0.0426, which corresponds to an average half-life of TCE of 16 days. The site-wide average k calculated for cDCE was 0.0269, which corresponds to an average half-life of 26 days. Data was generated in the barrier area to calculate a degradation rate of 0.011 for VC, which corresponds to a half-life of 63 days.

Table 4
Calculated Ground-Water Contaminant Degradation Rates
Former Grant Hardware Facility - West Nyack, NY

Well	k TCE (1/day)	t <sub>1/2</sub> TCE (days)	k cDCE (1/day)	t <sub>1/2</sub> cDCE (days)	k VC (1/day)	t <sub>1/2</sub> VC (days)
Source Area						
MW-12	0.0261	27	0.02	35		
MW-18	0.081	9	0.034	20		
Average Source Area	0.05355	13	0.027	26		
Barrier Area						
MVV-19	0.03135	22	0.042	17		
MW-25	0.032	22	0.0116	60	0.011	63
Average Barrier Area	0.031675	22	0.0268	26	0.011	63
Site-Wide Average	0.042613	16	0.0269	26	0.011	63

#### **Third Party VOC Sampling**

As previously indicated, the four quarterly sampling events were witnessed by an independent third party, Environmental Management, Ltd. (EML), and spilt samples were provided to EML for independent analysis and reporting. A summary of the data obtained by EML is provided in Appendix C. In general, the sampling results obtained by EML from the laboratory correlated very well with the data obtained by Geovation. Comparison of the chloroethene results of the two data sets results in a correlation coefficient of 0.95, which indicates very consistent data sets. Several differences, however, were observed. EML's lab was able to quantify very low concentrations of additional ground water contaminants, which Geovation's lab did not identify. at concentrations above the method detection limit. As the concentrations of the chloroethene compounds of interest in this study were reported in very high concentrations -- with the exception of trans-1,2 dichloroethene, which played a minor role -- this difference in the data sets is not considered to be significant. In addition, the concentrations of the chloroethenes reported by EML's laboratory were consistently lower than those reported by Geovation's laboratory. The fact that EML's laboratory consistently reported lower concentrations of chloroethenes in ground water suggests that Geovation's data may be conservative and site conditions may be slightly better than our data indicates

#### 4. CONCLUSIONS

Data from the barrier area supports the conclusion that the application of SRC<sup>TM</sup> product was successful in creating and maintaining anaerobic and reducing conditions. The source area was anaerobic and reducing prior to the start of the program. Although the source area remained anaerobic and reducing throughout the program, the control well also remained anaerobic and reducing, meaning that the effectiveness of SRC<sup>TM</sup> for this purpose could not be evaluated in the source area.

Reductions of total chloroethenes were observed in all target wells during the demonstration project. The primary contaminant, TCE, was reduced by more than 94% in all target wells, and a reduction of more than 93% of the daughter compound, cDCE, was achieved in the barrier area. Comparison of the contaminant concentrations in the target wells with the control points showed that the contaminant reductions were not the result of simple ground water dilution and were instead a response to SRC<sup>TM</sup> additions. Third- party split sampling of these analyses corroborated the results achieved.

Direct counts of microorganisms in treatment wells showed large increases in the biological community in response to SRC<sup>TM</sup> treatment. Responding in a similar way to ground water treatment as changes observed in microbial populations, concentrations of dissolved metals followed similar patterns and support the observation of increased microorganisms in response to SRC<sup>TM</sup> additions. Biotrap<sup>®</sup> samples provided additional supporting information that the biological community was responsible for the contaminant reductions. One bacteria known to degrade chloroethenes, *Dehalococcoides spp*, was identified in site ground water, and qPCR counts of this organism were seen to increase in response to SRC<sup>TM</sup> additions. In addition, qPCR identified that the DNA code for two enzymes that are capable of VC degradation were present in site ground water and also increased in response to SRC<sup>TM</sup> treatments. DGGE analysis further identified in site ground water the bacteria *Sulfurospirillum*, which is a known dechlorinator of PCE and TCE, and also identified the bacteria *Geobacteraceae*, whose family includes many of the most important known Fe(III) and sulfate reducers for bioremediation technologies.

More detailed analysis of the data obtained on contaminant reductions allowed for the calculation of the rates of contaminant degradation in the target wells. The calculated site-wide average half-life for TCE was 28 days. The average half-life of cDCE was 60 days, and in the barrier area, Geovation was able to calculate a half-life for VC of 63 days.

When combined, the data collected from multiple lines of evidence, including control points and third-party split sampling, indicate that application of SRC<sup>TM</sup> product is capable of effectively degrading site contaminants in both a source area and in a bio-barrier configuration.

#### **Problems Identified and Planned Solutions**

As discussed in the progress reports, the amount of SRC<sup>TM</sup> product that Geovation was able to apply to the subsurface at most of the treatment locations in the front area decreased throughout the duration of the project. Enzyme solutions were applied to the treatment wells on July 11, 2007, to attempt to open up some of the pore space and not negatively impact the biological communities. The results of the use of the enzymes were inconclusive, but the technology warrants a reapplication of the enzymes over a longer duration. Due to the time constraints of this demonstration project, SRC<sup>TM</sup> additions were continued, and the reapplication of enzymes will not be implemented until after the conclusion of the pilot.

#### **Project Schedule**

After a significant delay in starting the nutrient applications, the project was conducted and completed on schedule. As evident during the review of this report, the volume of data collected over the project period is significant, which resulted in a delay in the completion of this report.

#### Financial Report

As discussed in the progress reports, the budget for Geovation's labor and outside laboratory testing was used ahead of schedule. No funds remain in the budget. The co-funding property owner picked up the costs beyond those budgeted, which increased the co-funding obtained on this project. The success of this project most likely will result in the continued application of this bioremediation technology at this property. However, the pilot-phase demonstration project has been completed, and no further expenditures related to this phase of the project remain outstanding.

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### Co-Metabolic Bioremediation Demonstration Project

FINAL REPORT 08-19

STATE OF NEW YORK DAVID A PATERSON, GOVERNOR

NEW YORK STATE ENERGY RESEARCH AND DEVELOPMENT AUTHORITY VINCENT A. DEIORIO, ESQ., CHAIRMAN

