Dehalobacter-containing microbial consortium (TCA-20^{TM}) for anaerobic bioremediation

1. Dr. Robert J. Steffan, CB&I Federal Services, LLC. (formerly Shaw Environmental, Inc.)
2. Composed of anaerobic bacteria including *Dehalobacter* in an aqueous medium.
3. MSDS and Technical Data Sheet-attached
4. Number of Field-scale Applications to Date: 47+ applications
5. Case Studies – Attached.
6. Technical Summary. The TCA-20^{TM} culture is a pathogen-free, non-genetically altered microbial consortium capable of biologically degrading halogenated aliphatic pollutants including , 1,1,1-TCA, 1,1,2-TCA, 1,1-DCA, 1,2-DCA, 1,2-DBE, TeCA, carbon tetrachloride, chloroform, and also mixtures thereof. It was developed specifically for degrading chlorinated alkanes. Molecular biological analyses of the TCA-20^{TM} culture has demonstrated that the culture has at least one strain of *Dehalobacter* sp. bacteria that degrades chlorinated alkanes. It has now been successfully applied more than 47 times at sites throughout the United States. It is often combined with the SDC-9^{TM} *Dehalococcoides*-containing culture to biodegrade mixtures of halogenated ethenes and halogenated ethanes. The culture has been applied commercially since 2008, and it is sold by licensed distributors.

Material Safety Data Sheet

SECTION 1 – CHEMICAL PRODUCT AND COMPANY IDENTIFICATION

Product Name: TCA-20 microbial consortium.

Manufacturer CB&I 17 Princess Road, Lawrenceville,
NJ 08648. Phone (609) 895-5340

CAS #: N/A (Not Applicable)

Product Use: For remediation of contaminated groundwater (environmental applications).

Material Description: Non-toxic, naturally occurring, non-pathogenic, non-genetically altered anaerobic microbes in a water-based medium. Contains bacteria of the genus *Dehalobacterium* and methanogenic archebacteria.

IN CASE OF EMERGENCY CALL CHEMTREC 24 HOUR EMERGENCY RESPONSE PHONE NUMBER (800) 424-9300

SECTION 2 – COMPOSITIONS AND INFORMATION ON INGREDIENTS

Components	%	OSHA PEL	ACGIH TLV	OTHER LIMITS
Non-Hazardous Ingredients	100	N/A	N/A	N/A

TCA-20 microbial consortium) comprised of microorganism of the genus *Dehalobacter.*

SECTION 3 – HAZARDS IDENTIFICATION

The available data indicates no known hazards associated with exposure to this product. Nevertheless, individuals who are allergic to enzymes or other related proteins should avoid exposure and handling. Health effects associated with exposure to similar organisms are listed below.

Ingestion: Ingestion of large quantities may result in abdominal discomfort including nausea, vomiting, cramps, diarrhea, and fever.

Inhalation: Hypersensitive individuals may experience breathing difficulties after inhalation of aerosols.

Skin Absorption: May cause irritation upon prolonged contact. Hypersensitive individuals may experience allergic reactions..

Eye contact: May cause irritation unless immediately rinsed.

SECTION 4 – FIRST AID MEASURES

Ingestion: Thoroughly rinse mouth with water. Do not induce vomiting unless directed to do so by medical personnel. Get immediate medical attention. Never give anything by mouth to an unconscious or convulsing person.

Inhalation: Get medical attention if allergic symptoms develop.

Skin Absorption: N/A

Skin Contact: Wash affected area with soap and water. Get medical attention if allergic symptoms develop.

Eye Contact: Flush eyes with plenty of water for at least 15 minutes using an eyewash fountain, if available. Get medical attention if irritation occurs.

NOTE TO PHYSICIANS: All treatments should be based on observed signs and symptoms of distress in the patient. Consideration should be given to the possibility that overexposure to materials other than this material may have occurred.

SECTION 5 – FIRE AND EXPLOSION DATA

Flammability of the Product: Non-flammable

Flash Point: N/A

Flammable Limits: N/A

Fire Hazard in Presence of Various Substances: N/A

Explosion Hazard in Presence of Various Substances: N/A

Extinguishing Media: Foam, carbon dioxide, water

Special Fire Fighting Procedures: None

Unusual Fire and Explosion Hazards: None

SECTION 6 – ACCIDENTAL RELEASE MEASURES

Reportable quantities (in lbs of EPA Hazardous Substances): N/A

No emergency results from spillage. However, spills should be cleaned up promptly. Absorb with an inert material and put the spilled material in an appropriate waste disposal container. All personnel involved in the cleanup must wear protective clothing and avoid skin contact. After clean-up, disinfect all cleaning materials and storage containers that come in contact with the spilled liquid.

SECTION 7 – HANDLING AND STORAGE

Avoid breathing breathe aerosol. Avoid contact with skin. Use personal protective equipment recommended in Section 8.
Keep containers tightly closed in a cool, well-ventilated area. The TCA-20 microbial consortium can be supplied in stainless steel kegs designed for maximum working pressure of 130 psi and equipped with pressure relief valves. The kegs are pressurized with Nitrogen up to the pressure of 15 psi. Do not exceed pressure of 15 psi during transfer of TCA-20 microbial consortium from kegs. Don't open keg if content of the keg is under pressure.

TCA-20 microbial consortium may be stored for up to 3 weeks at temperature
2-4°C without aeration. Avoid freezing.

SECTION 8 – EXPOSURE CONTROLS/PERSONAL PROTECTION

Hand Protection: Rubber, nitrile, or vinyl gloves.

Eye Protection: Safety goggles or glasses with side splash shields.

Protective Clothing: Use adequate clothing to prevent skin contact.

Respiratory Protection: N95 respirator if aerosols might be generated.

Ventilation: Provide adequate ventilation to remove odors.

Other Precautions: An eyewash station in the work area is recommended.

SECTION 9 – PHYSICAL/CHEMICAL CHARACTERISTICS

Physical state and appearance: Light greenish murky liquid. Musty odor.

Boiling Point: 100°C (water)

Specific Gravity (H_2O = 1): 0.9 - 1.1

Vapor Pressure @ 25°C: 24 mm Hg (water)

Melting Point: 0°C (water)

Vapor Density: N/A

Evaporation Rate (H_2O = 1): 0.9 - 1.1

Solubility in Water: Soluble

Water Reactive: No

pH: 6.0 - 8.0

SECTION 10 – STABILITY AND REACTIVITY DATA

Stability: Stable

Conditions to Avoid: None

Incompatibility (Materials to Avoid): Water-reactive materials

Hazardous Decomposition Byproducts: None

SECTION 11 – TOXICOLOGICAL INFORMATION

This product contains no toxic ingredients.
TCA-20 consortium has tested negative for pathogenic microorganisms such as Bacillus cereus, Listeria monocytogens, Salmonella sp., Fecal Coliform, Total Coliform, Yeast and Mold and Pseudomonas sp.

SECTION 12 – ECOLOGICAL INFORMATION

Ecotoxicity: this material will degrade in the environment.

SECTION 13 – DISPOSAL CONSIDERATIONS

Waste Disposal Method: No special disposal methods are required. The material is compatible with all known biological treatment methods. To reduce odors and permanently inactivate microorganisms, mix 100 parts (by volume) of TCA-20 consortium with 1 part (by volume) of bleach. Dispose of in accordance with local, state and federal regulations.

SECTION 14 – TRANSPORT INFORMATION

DOT Classification:	N/A
Labeling:	NA
Shipping Name:	Not regulated

SECTION 15 – REGULATORY INFORMATION

Federal and State Regulations: N/A

SECTION 16 – OTHER INFORMATION

MSDS Code: ENV 1080
MSDS Creation Date: 12/04/2006
Last Revised: April 30, 2013.

While the information and recommendations set forth herein are believed to be accurate as of the date hereof, CB&I MAKES NO WARRANTY WITH RESPECT HERETO AND DISCLAIMS ALL LIABILITY FROM RELIANCE THEREON.

Shaw's TCA-20™ Culture

Shaw Environmental & Infrastructure, Inc. (Shaw) provides innovative solutions to environmental and infrastructure challenges for government and private sector clients worldwide. Our skilled scientists and engineers use a wide range of emerging and commercial technologies to solve our clients' problems involving hazardous, toxic and radioactive materials in all types of environmental media. Shaw has developed several high-performance bacterial cultures specifically selected for bioaugmentation treatment of contaminated sites.

Shaw's TCA-20™ culture is a consortium of anaerobic microorganisms specially enriched for their ability to rapidly dehalogenate chlorinated ethanes including 1,1,1-TCA; 1,1,2-TCA; 1,2-DCA and 1,1-DCA (Figure 1). The culture also degrades carbon tetrachloride and chloroform.

The culture is grown in Shaw's Lawrenceville, N.J. fermentation facility by using lactate as an electron donor and 1,1,1-TCA as a sole electron acceptor. During growth in the fermentation process, fermentative microorganisms in the consortium convert lactate to hydrogen which is subsequently used by dehalogenating microbes in the culture to reductively dechlorinate chlorinated alkanes. The culture can be grown to high-cell density in volumes up to 3,000 liters for treatment of large contaminant plumes.

In the field, the culture will thrive on any commonly used electron donor compound (e.g., lactate, vegetable oil, poly-lactate, molasses, etc.), and it can be used either alone or in combination with Shaw's SDC-9™ to treat difficult mixed-contaminant plumes, including those containing carbon tetrachloride and chloroform.

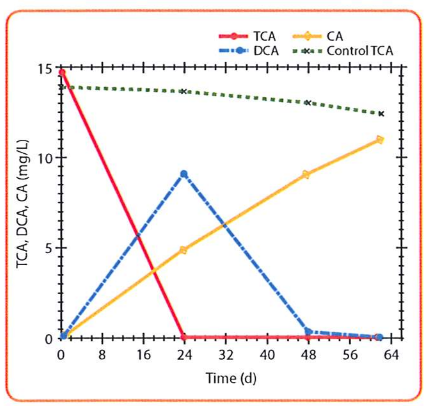

Figure 1: *Biodegradation of 1,1,1-TCA by Shaw's TCA-20™ culture*

Molecular analysis of the TCA-20™ has revealed that it contains at least one *Dehalobacter sp.* strain, and that the *Dehalobacter sp.* strains in the culture are very similar to *Dehalobacter sp.* strain TCA1 (Sun, B., et al. 2002. Science 298:1023-1025) (Figure 2) which is the only pure culture of microorganisms that has been reported in the scientific literature to grow on 1,1,1-TCA as a sole electron acceptor. Like the described TCA1 strain, microbes in the TCA-20™ culture degrade chlorinated alkanes by dehalorespiration, meaning they use these compounds as electron acceptors during their normal respiration and growth.

Figure 2: *PCR Analysis of TCA-20™*

Figure 2 shows agarose gel electrophoresis of polymerase chain reaction (PCR) products from the analysis of the TCA-20™ culture. PCR primers targeted either all bacteria (lane 1), all *Dehalobacter sp.* strains (lane 2) or *Dehalobacter sp.* strain TCA-1-like 16s rRNA gene sequences (lane 3). Positive DNA bands indicating the presence of *Dehalobacter sp.* and TCA-1 homologous DNA sequences are indicated by arrow A and B, respectively.

Contact

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Lawrenceville, NJ 08648
Phone: 609.895.5350
rob.steffan@shawgrp.com
www.shawgrp.com

TCA-20 Bioaugmentation Culture Technical Data

A World of **Solutions**™

About TCA-20

1. Enriched Specifically for Treating Chlorinated Alkanes
   - Used successfully for 1,1,1-TCA, 1,1,2-TCA, 1,1-DCA, 1,2-DCA, CT, CF
   - Successfully used with SDC-9 for mixed cVOCs

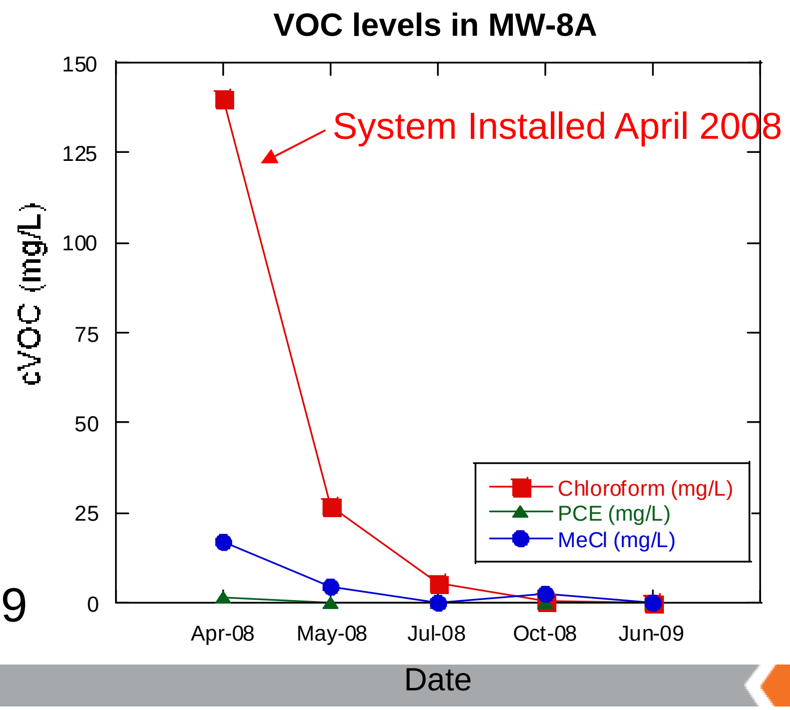
2. Contains *Dehalobacter spp.*

3. Originally Enriched from Groundwater from a New York site, 2006

4. First Commercial Application, Beverly, MA , 3/25/2008

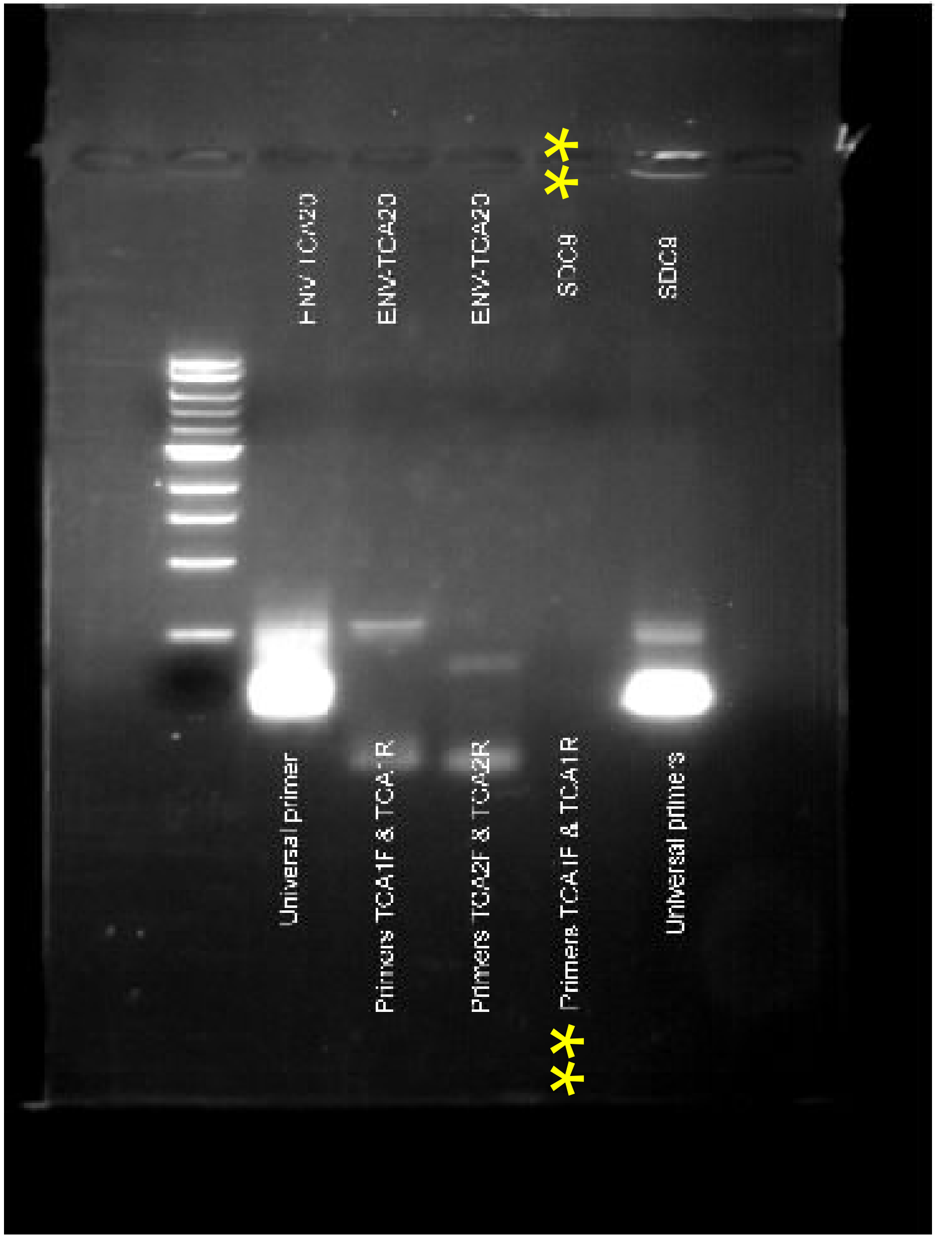
5. Grown Anaerobically with Lactate and 1,1,1-TCA

6. 47 Applications to Date



Data from an Oklahoma site
Bioaugmented with TCA-20 and SDC-9

PCR Screening of TCA-20 and SDC-9



TCA-1-like sequences were identified in another culture, TCA-20

No *Dehalobacter* sp. Strain TCA-1 homologous sequences identified in SDC-9

Microbac®

Microbac Laboratories, Inc.

Baltimore Division

2101 Van Deman Street • Baltimore, MD 21224

Phone: 410-633-1800
Fax: 410-633-6553
www.microbac.com

CERTIFICATE OF ANALYSIS

SHAW ENVIRONMENTAL & INFRA.	Project: CONSORTIUM SAMPLES	Report: 12C0784
17 PRINCESS ROAD	Project Number: CONSORTIUM SAMPLES	Reported: 03/20/2012 13:37
LAWRENCEVILLE, NJ 08648	Project Manager: SIMON VAINBERG	

TCA-20

12C0784-02 (Water) Sampled: 03/13/2012 00:00; Type: Not Specified

Analyte	Result	Reporting Limit	Units	Prepared	Analyzed	Analyst	Method	Notes
Microbac Laboratories, Inc., Baltimore Division								
Microbiology								
Bacillus cereus	ND	3.0	CFU/g	031312 1103	031712 1600	JAT	AOAC 980.31	
Coliform, Total	ND	3.0	MPN/g	031312 1114	031512 0945	DML	FDA-BAM	
E. Coli	ND	3.0	MPN/g	031312 1114	031512 0945	DML	FDA-BAM	
Fecal Coliform	ND	3.0	MPN/g	031312 1130	031512 0945	DML	FDA BAM	
Listeria monocytogens	NEGATIVE		per 25g	031312 1120	031612 0935	JAT	AOAC 2003.12	
Salmonella	NEGATIVE		per 25g	031312 1122	031512 0630	DML	AOAC 2003.09	
Yeast and Mold	ND	10	CFU/g	031312 1123	031812 1140	JAT	FDA-BAM	
Microbac Laboratories, Inc., Central Pennsylvania								
MICROBIOLOGY								
Pseudomonas	ND	10	CFU/g	031412 1845	031612 1600	GLF	ISO 13720	

TCA-20™ Case Study

Treatment of a Fractured Bedrock Aquifer with TCA-20™ and SDC-9™

By

Parsons Corporation

Abstract L-21, in: G.B. Wickramanayake and H.V. Rectanus (Chairs), *In Situ and On-Site Bioremediation—2009.* Tenth International In Situ and On-Site Bioremediation Symposium (Baltimore, MD; May 5–8, 2009). ISBN 978-0-9819730-1-2. Publisher: Battelle Memorial Institute, Columbus, OH. **www.battelle.org/biosymp**

Bioremediation of Chlorinated Ethenes in Fractured Bedrock

James W. Schuetz (james.schuetz@parsons.com)
(Parsons Corporation,Buffalo, New York, USA)
Stephanie Fiorenza (BP, Houston, Texas, USA)
Steve Brauner (Parsons Corporation, Denver, Colorado, USA)
William Barber (Atlantic Richfield Company, Cleveland, Ohio, USA)
Michael D Lee (Terra Systems Inc., Wilmington, Delaware, USA)

A field-scale pilot test of in situ bioremediation of chlorinated volatile organic compounds (CVOCs) in fractured bedrock using emulsified vegetable oil and bioaugmentation. The pilot test injection was completed in 2008.

CVOC concentrations, biogeochemical indicators, and native microbial population composition demonstrate that natural attenuation is occurring at the site. These same data show that that the primary factor that is limiting the rate of complete CVOC degradation by natural attenuation is an insufficient organic carbon source. Furthermore, dense, non-aqueous phase liquid (DNAPL) has been observed at the Site.

During well installations, packer testing, downhole geophysics and downhole cameras were used to characterize the bedrock groundwater hydraulics. Characterization results indicate that groundwater flow is dictated by one or two significant fractures at approximately 10 feet below the top of rock. Hydraulic testing suggests the transmissivity could be 1000 ft^2/day or greater. This high flow potential coupled with high sulfate concentrations are potentially the limiting factors for successful bioremediation.

A bioremediation pilot test was designed and implemented based on site characterization information. Approximately 1000 gallons of vegetable oil-based substrate and a bromide tracer were injected into two injection wells in the pilot test area. Real time substrate breakthrough and hydraulic head were monitored at surrounding observation wells using downhole conductivity probes, a bromide probe, transducers and visual observations. Breakthrough of the substrate occurred significantly sooner than expected and indicated that the effective porosity of the bedrock is less than 1%. Subsequent to the injection, bromide, specific conductivity and oxidation-reduction potential (ORP) were monitored to assess the groundwater flow rate and mobility of the substrate. Approximately 1 month after substrate injection, a bioaugmentation injection was implemented to enhance the degradation of CVOCs. Analysis of CVOCs and geochemistry during performance monitoring were used to evaluate the effectiveness of the substrate injection.

The injection of vegetable oil based substrate resulted in significant decreases in oxidation reduction potential (ORP), sulfate, and chlorinated volatile organics. In the treatment area the ORP decreased to less than -350 mV, sulfate decreased by as much as 1,000 mg/l, and concentrations of total chlorinated ethenes decreased 81 to 97%. TCE concentrations decreased up to 3 orders of magnitude. DCE concentration initially increased with the decrease of TCE, but later decreased to concentrations appreciably lower than observed prior to the injection. Significant increases in VC, ethene and ethane were not observed. PH decreased to slightly below 6.0 in most of the pilot test area; however, it recovered to above 6.0 prior to groundwater buffering.

A Pilot Test for Bioremediation of Chlorinated Ethenes in Fractured Bedrock

J.W. Schuetz (Parsons), S. Fiorenza, (BP America), Steve Brauner (Parsons), M. Lee (Terra Systems Inc.), W. B. Barber (Atlantic Richfield Co.)

OUTLINE

- Site Overview
- Hydrogeology and Geochemistry
- Pilot Test Application
- Results
- Conclusions

BACKGROUND

- Manufacturing facility in Western N.Y.
- Release from a secondary containment tank
- Excavation of source area
- DNAPL in fractured bedrock
- Chlorinated Volatile Organic Compounds (CVOCs)
  - trichloroethene (TCE)
  - cis-1,2-dichloroethene (DCE)
  - vinyl chloride (VC)
    - minor:
      - tetrachlorethene (PCE)
      - trichloroethane (1,1,1-TCA)
      - degradation products
- Silt and clay overlying fractured dolomite

- Site Aerial Photograph

DOLOMITE HYDROGEOLOGY

- Low permeability matrix
- Higher permeability fractures
  - Transmissivity ranging from 200 – 1200 ft^2/day
- Distinct bedding plane joints
- Low porosity


N
lost drillwater

CONCEPTUAL “BLOCK AND CAVITY” MODEL

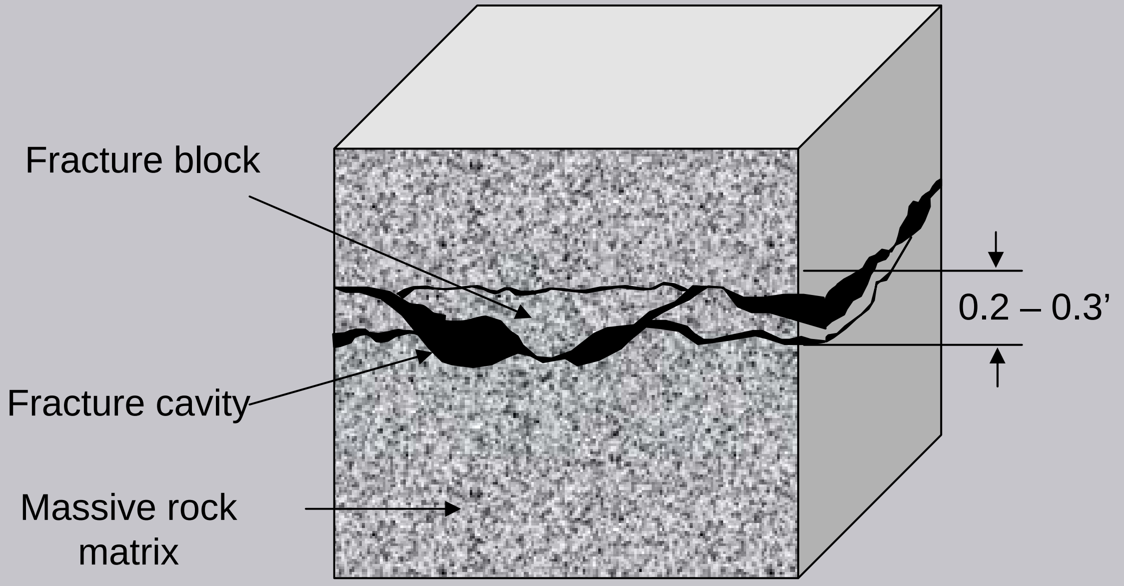
SUMMARY:

•Low Porosity Matrix

•High Transmissivity

•Low Hydraulic Gradient

•Large open fracture accompanied by smaller fractures and cavities



GROUNDWATER GEOCHEMISTRY

- High concentrations of CVOCs in source area
  - TCE > 100 – 200 mg/L
  - DCE > 20 – 50 mg/L
- Strongly Anaerobic
  - Ranges -350 to -180 mv Eh
- Sulfate greater than 1,000 mg/L
- Native *Dehalococcoides sp.* and *Dehalobactor sp.*
- Laboratory treatability testing indicate bioenhanced remediation as promising technology

DESIGN OBJECTIVE:

•Reduce concentrations in the source area

•Distribute substrate throughout the fractures

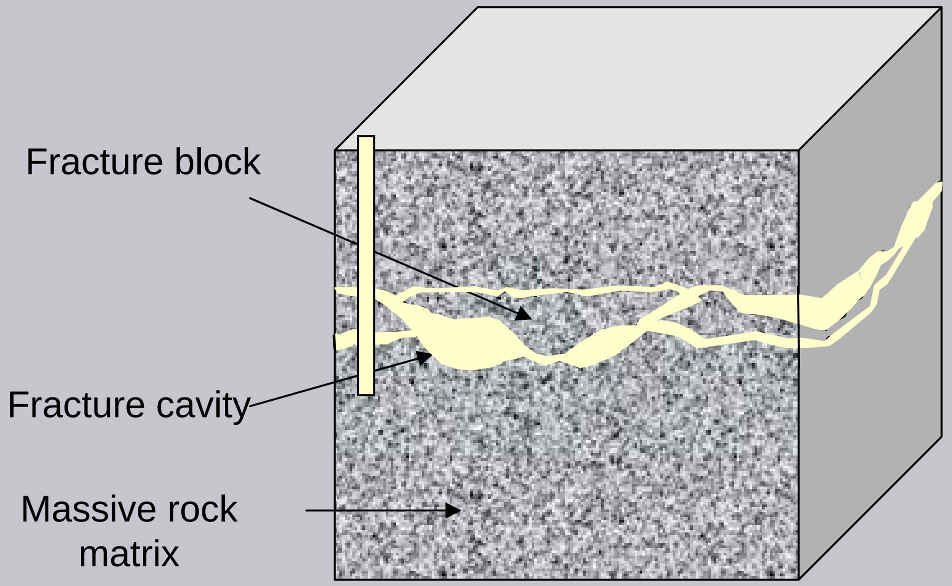
•Limit transport of the substrate out of the pilot test area after injection

•DESIGN

•Specific to the site geology

•Injected substrate with high concentrations of vegetable oil (9% residual)

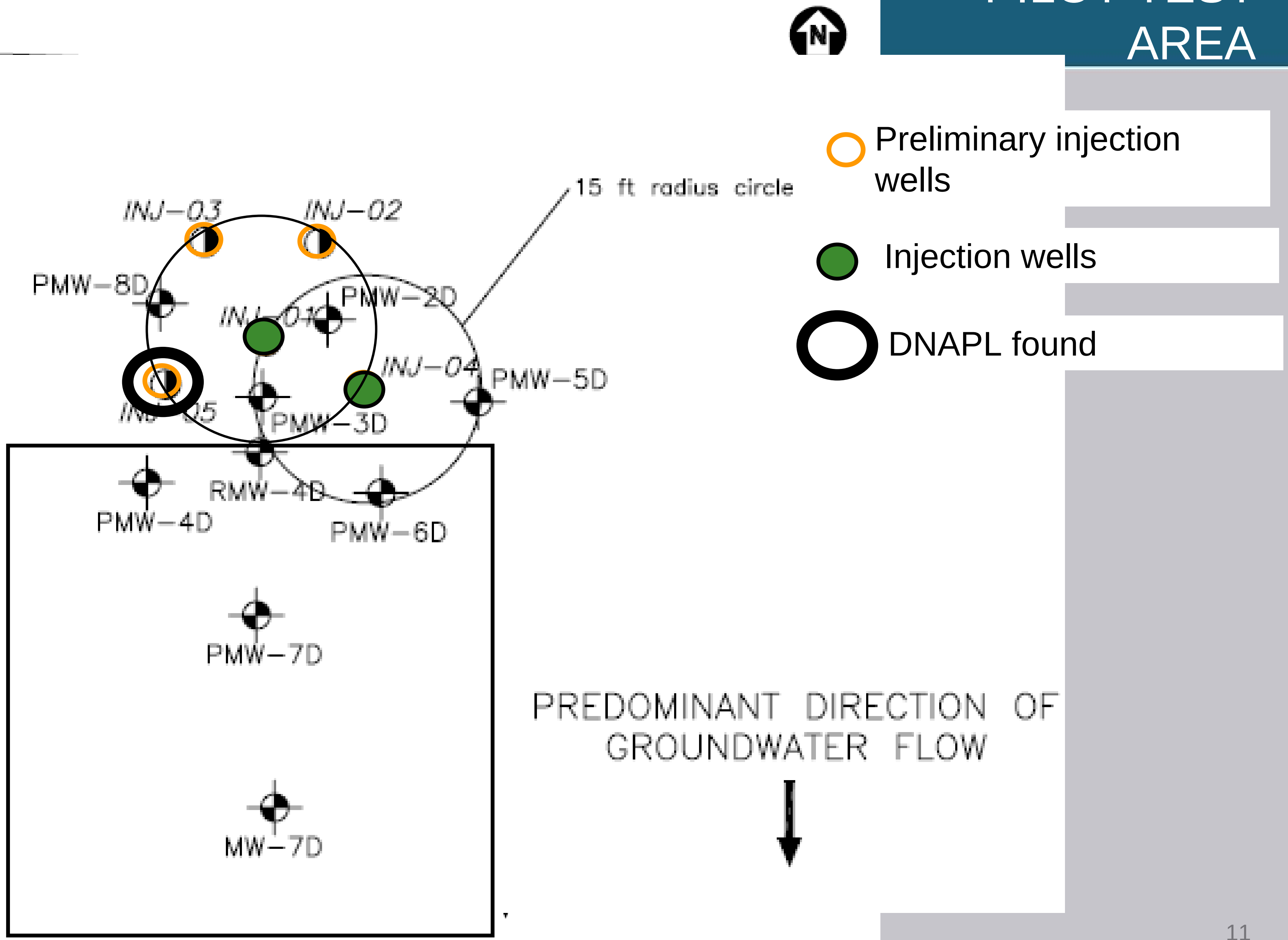
•Coarse emulsion size (30 microns)



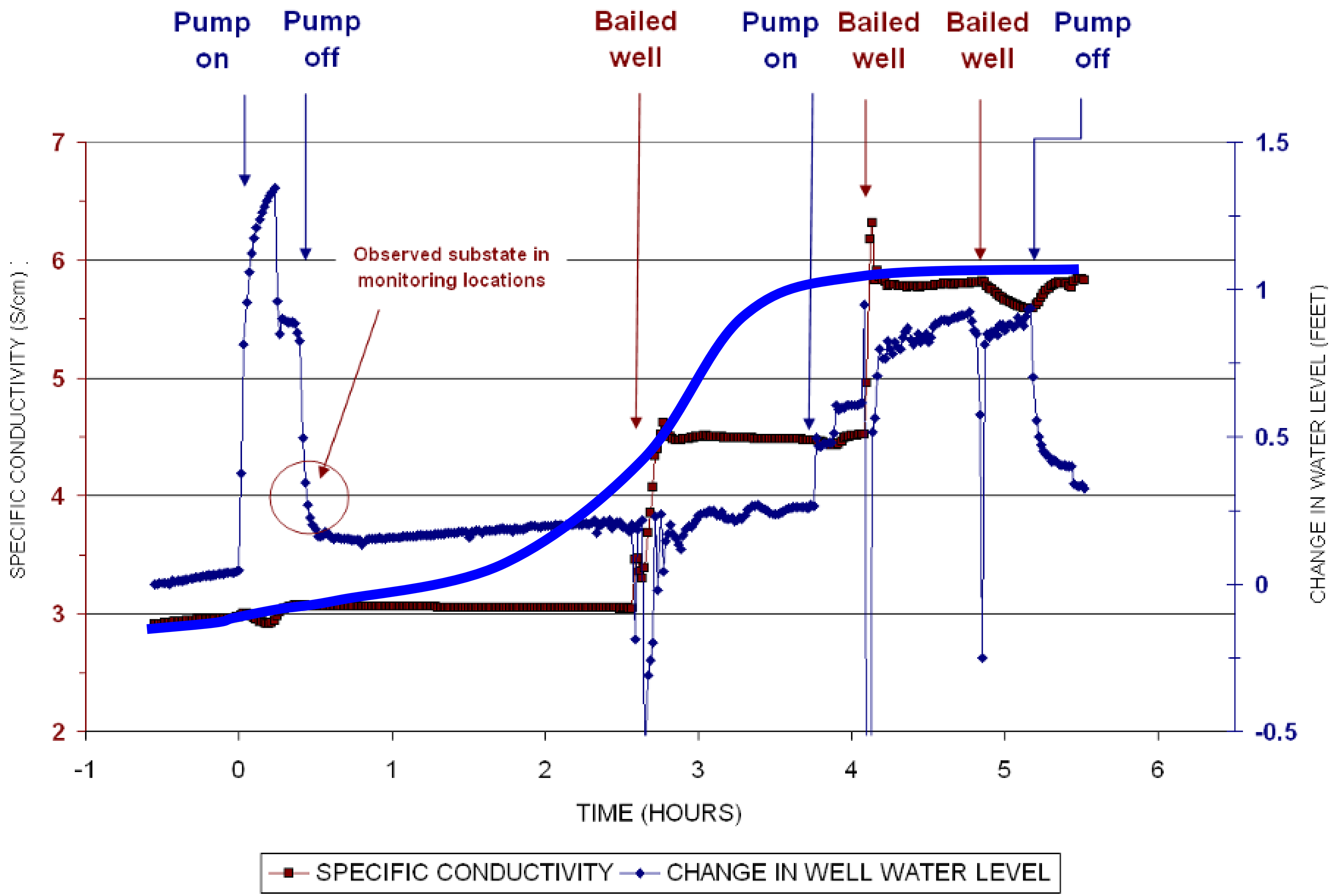
INJECTION

- Transmissive zone was isolated with a well packer
- Anticipated low to zero backpressure
- 450 gallons of site groundwater mixed with 50 gallons of SRS™ vegetable oil substrate
  - Larger droplet size
  - Nutrients added
  - Sodium bromide tracer
- Bioaugmentation 4 weeks after substrate injection.
- Groundwater buffering

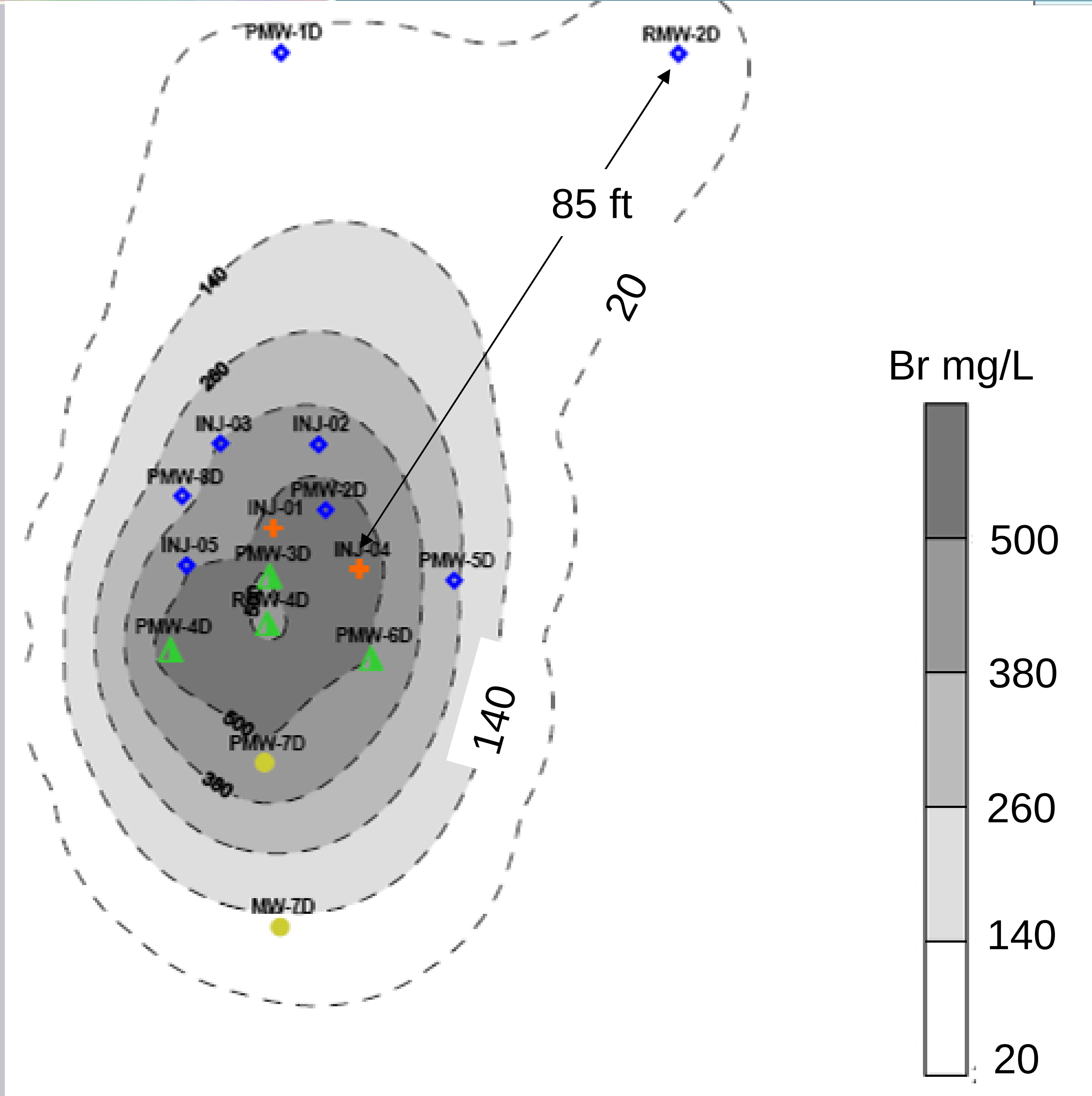
PILOT TEST AREA



BREAKTHROUGH MONITORING



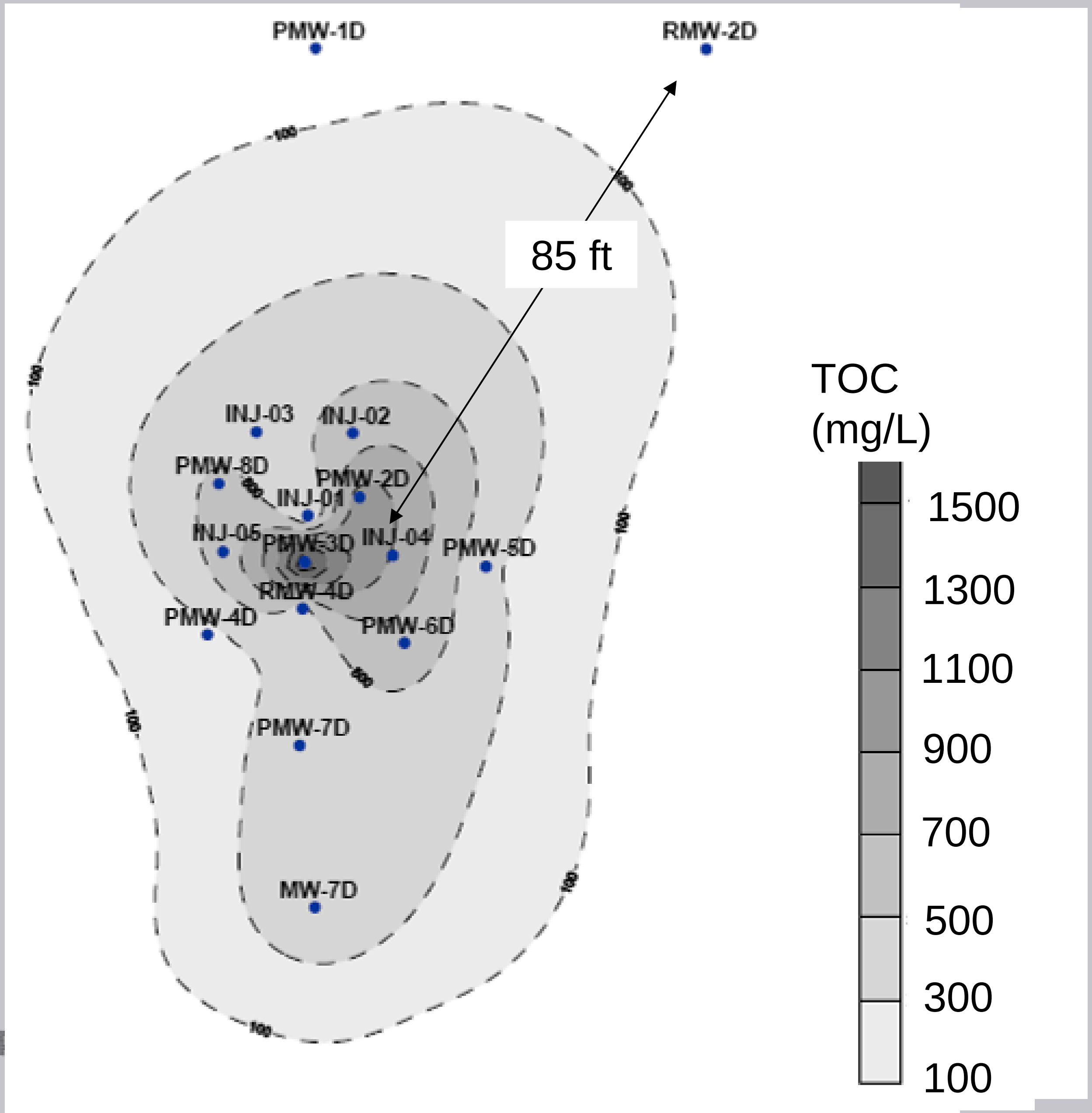
SUBSTRATE DISTRIBUTION



Bromide

- **Field analysis**
- **1 day after injection**
- **Possible interference with sulfate**

SUBSTRATE DISTRIBUTION



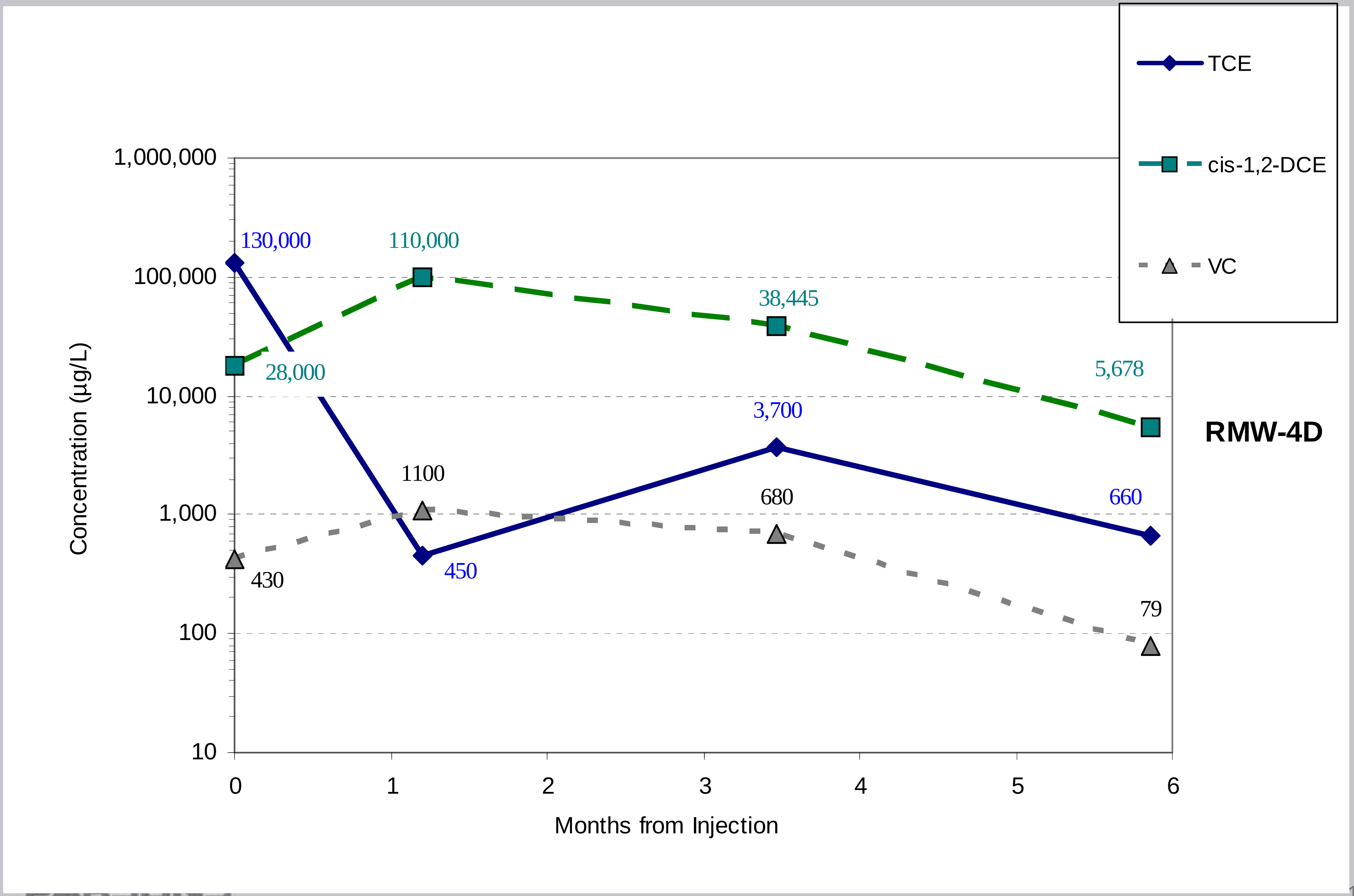
Total Organic Carbon

4-week sampling

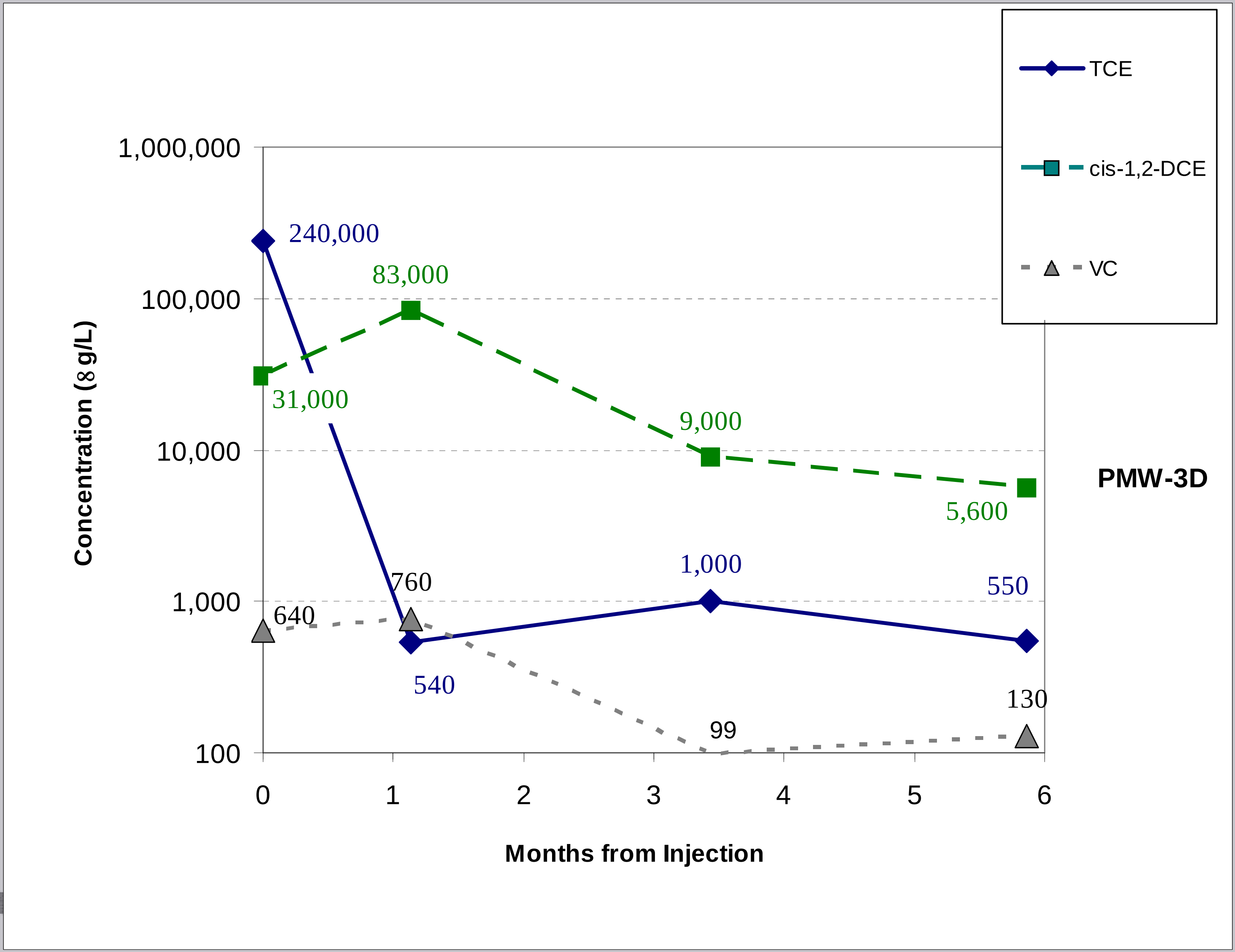
BIOAUGMENTATION

- After 4 week sampling event
- Consortium of *Dehalococcoides sp.* and *Dehalobactor sp.* *($1x10^{10}$ cells/L)*
- *Additional substrate*
- *7 foot radius around the two injection wells*

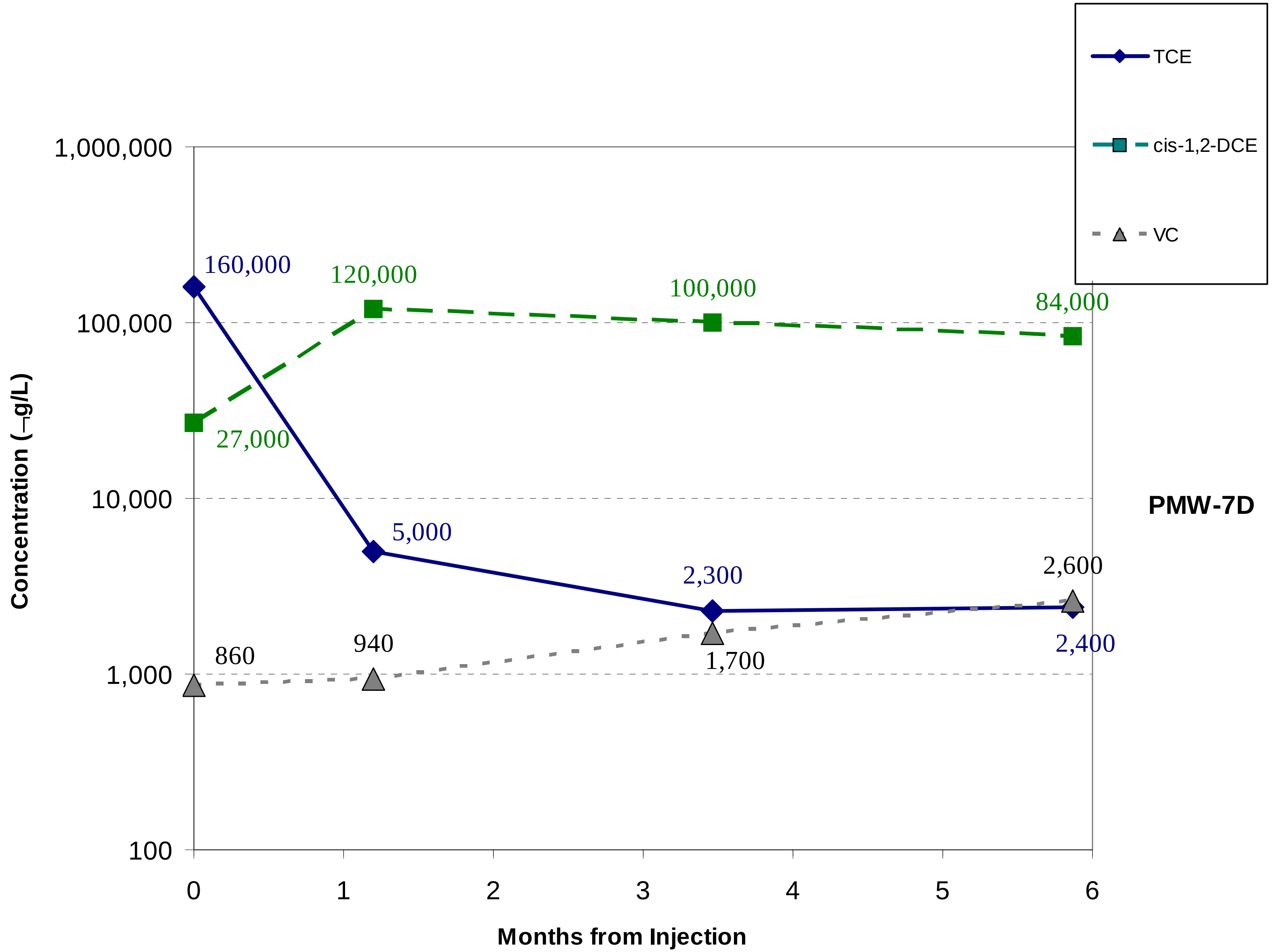
RESULTS
INNER TREATMENT ZONE COCs



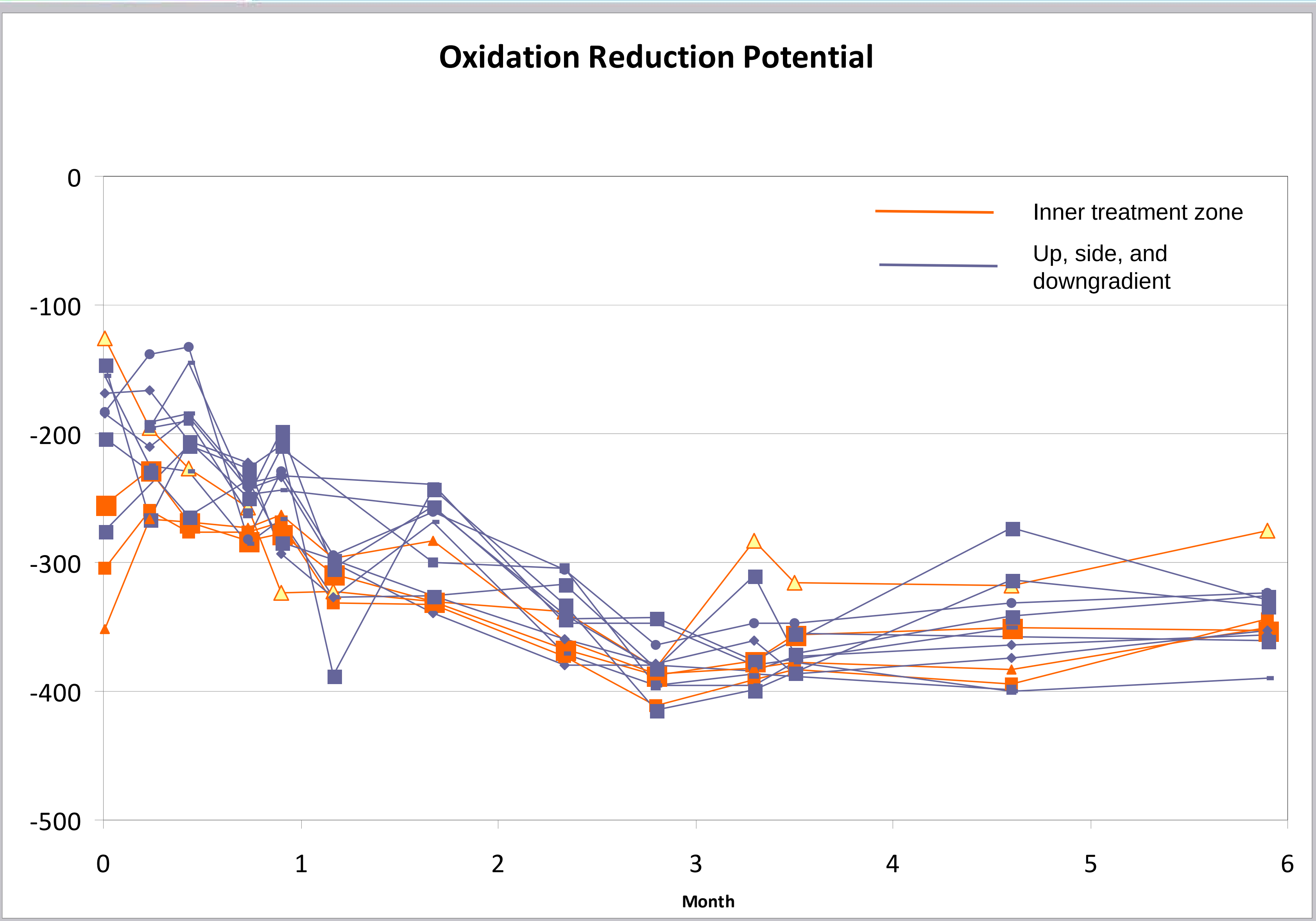
INNER TREATMENT ZONE COCs



DOWNGRADIENT TREATMENT ZONE COCs



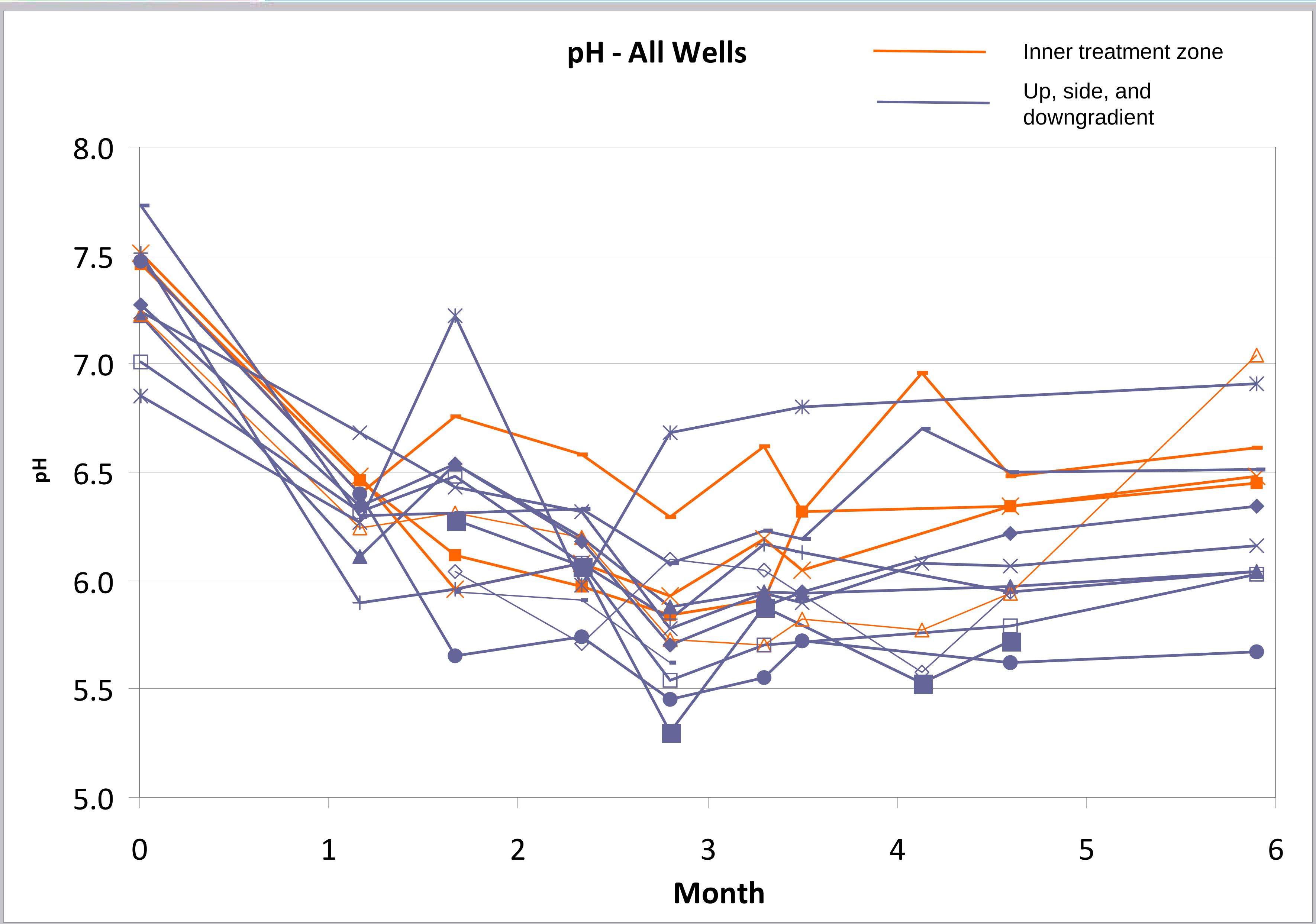
ORP



Oxidation Reduction Potential
0
-100
-200
-300
-400
-500
0
1
2
3
4
5
6
Month
Inner treatment zone
Up, side, and downgradient

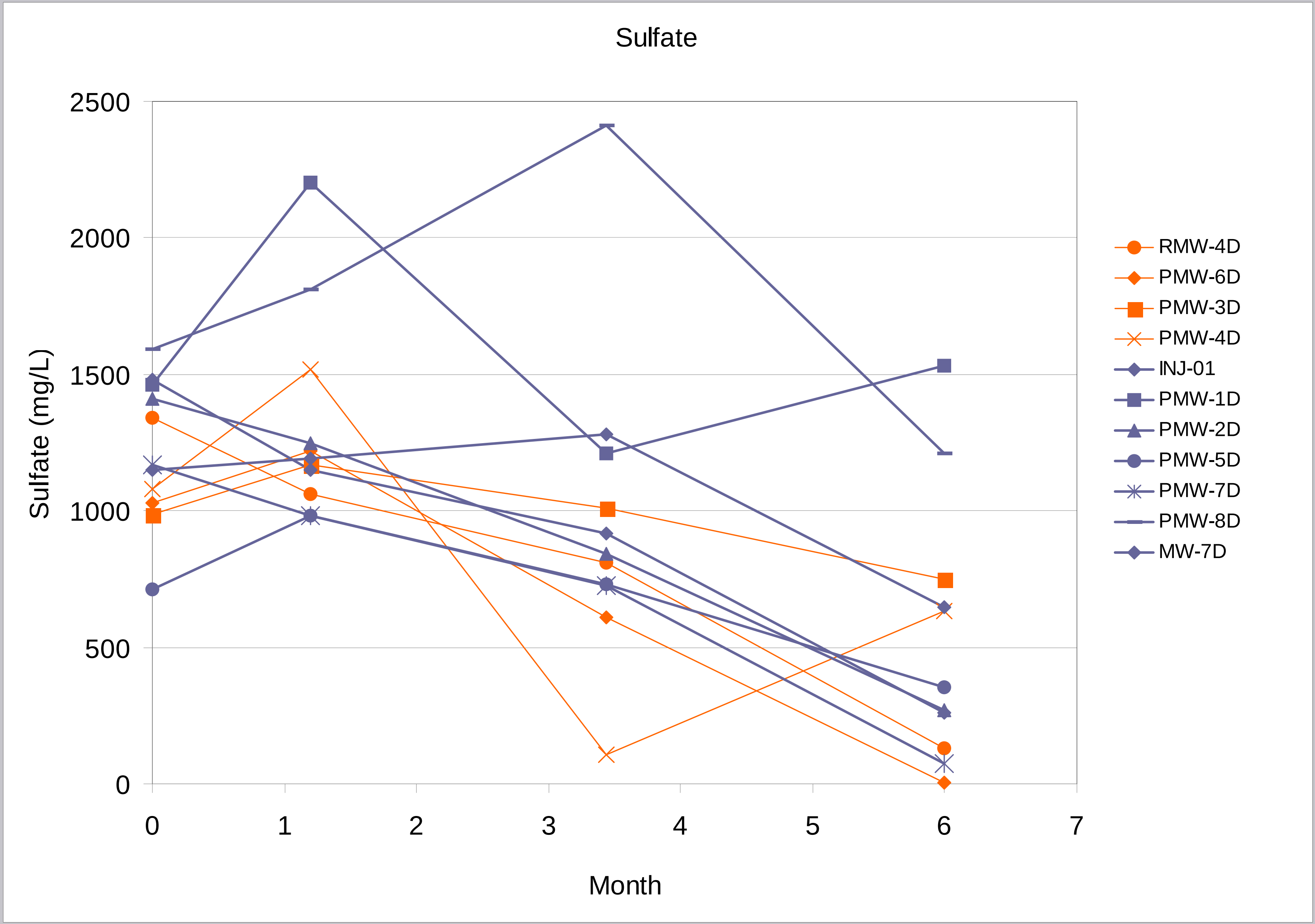
PARSONS

pH

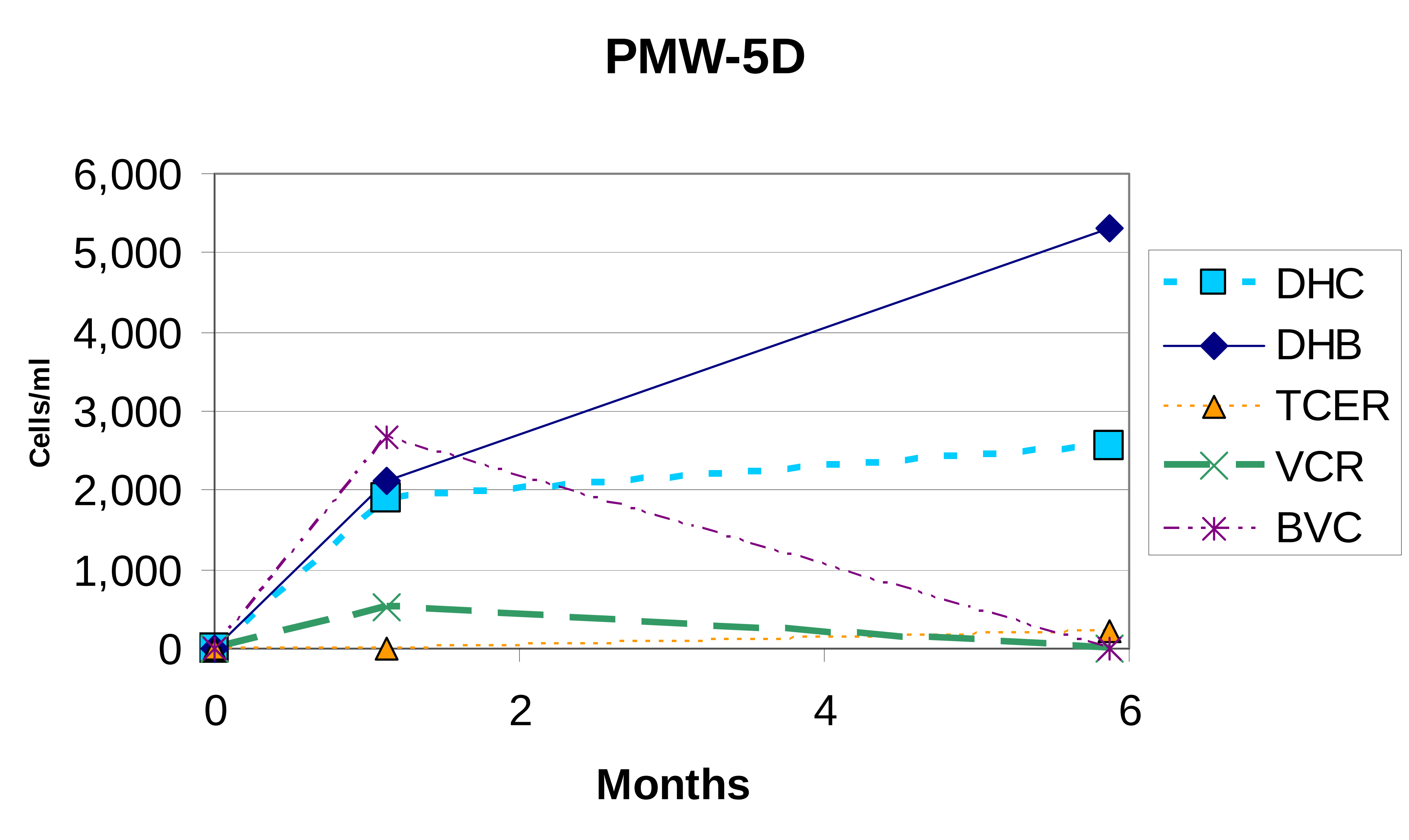


pH - All Wells
Inner treatment zone
Up, side, and downgradient
8.0
7.5
7.0
6.5
6.0
5.5
5.0
pH
0
1
2
3
4
5
6
Month

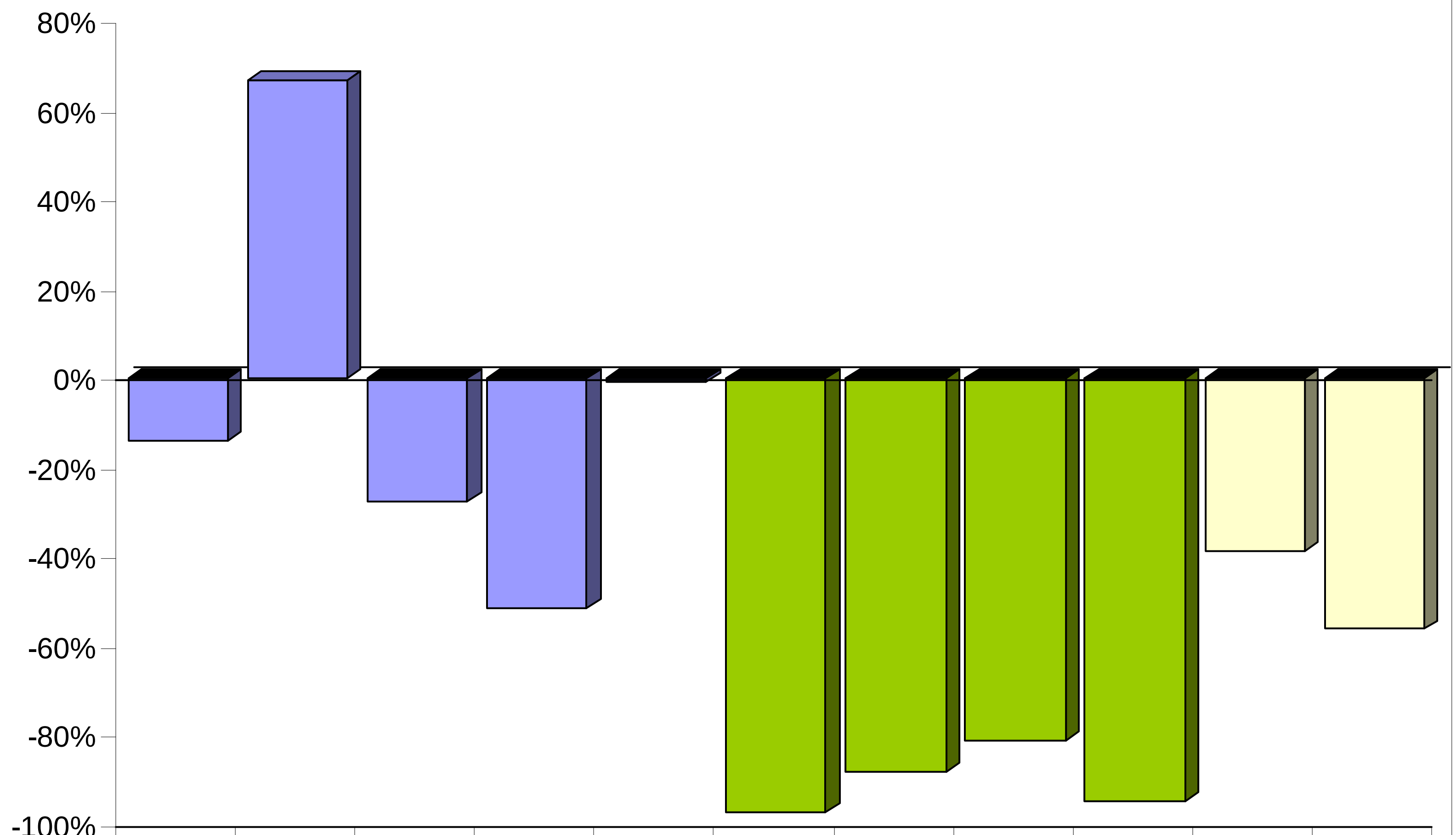
Sulfate



Sulfate
2500
2000
1500
1000
500
0
Sulfate (mg/L)
0
1
2
3
4
5
6
7
Month
RMW-4D
PMW-6D
PMW-3D
PMW-4D
INJ-01
PMW-1D
PMW-2D
PMW-5D
PMW-7D
PMW-8D
MW-7D

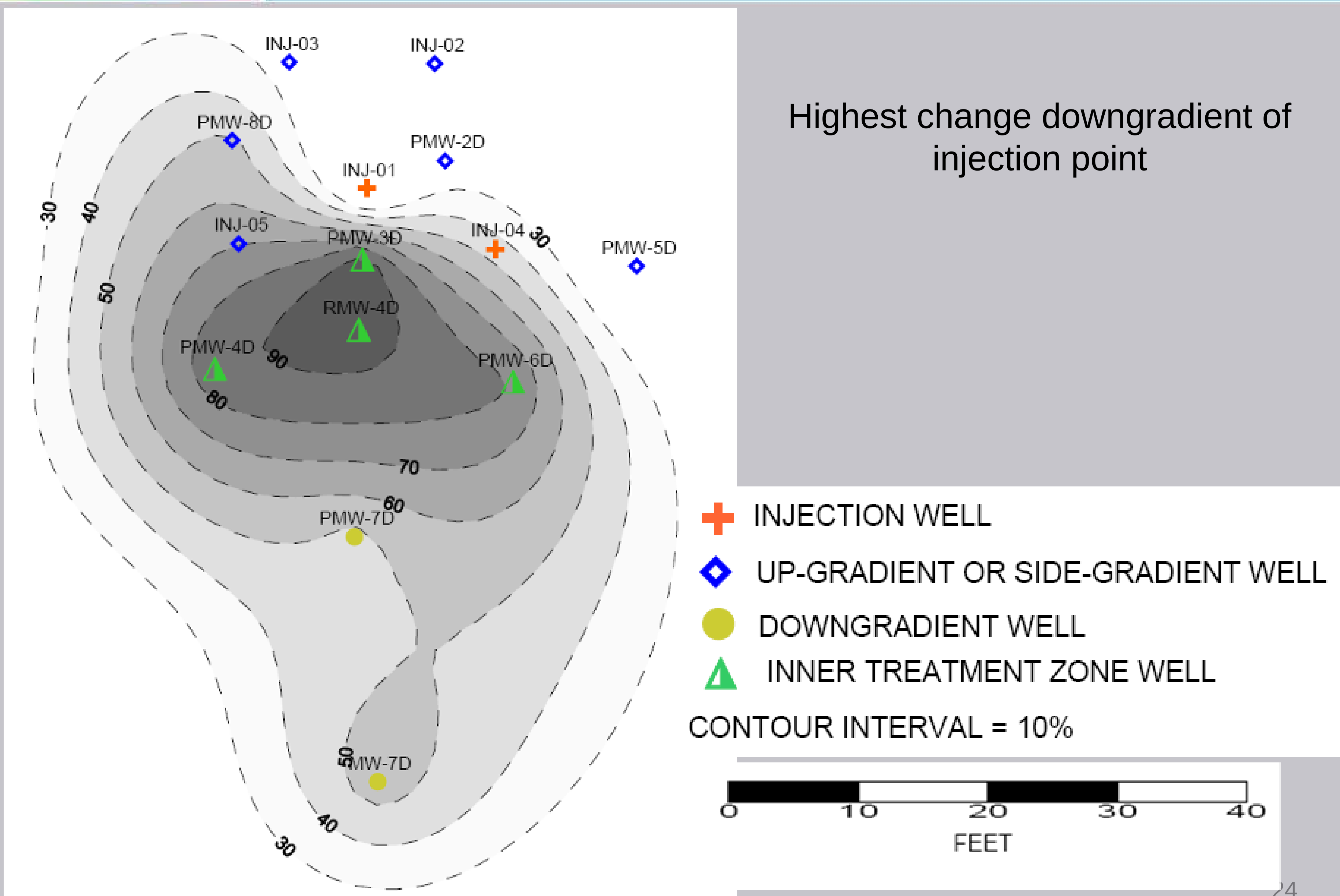

PMW-5D
6,000
5,000
4,000
3,000
2,000
1,000
0
Cells/ml
0
2
4
6
Months
DHC
DHB
TCER
VCR
BVC

RESULTS – % CHANGE TCE+DCE+VC



	PMW-1D	INJ-01	PMW-2D	PMW-8D	PMW-5D	PMW-3D	PMW-4D	PMW-6D	RMW-4D	PMW-7D	MW-7D
Down Gradient										-39%	-56%
Treatement Zone						-97%	-88%	-81%	-95%		
Up and Side Gradient	-14%	67%	-28%	-52%	-1%						

PERCENT CHANGE TCE+DCE+VC



Highest change downgradient of injection point

CONCLUSIONS

- Significant ROI due to transmissive fractures and porosity (1% or less)
- Sustained TOC concentration
  - 100 to 400 mg/l after 6 months
- pH excursions to below 6.0 in some locations
- Microbial results indicate some native growth, and the benefit of bioaugmentation remains unclear
- Significant decrease in CVOCs within inner treatment zone 81 – 97%
- Lack of significant VC and ethene

THE END

Thank you!

Questions?

A Pilot Test for Bioremediation of Chlorinated Ethenes and Ethanes in Fractured Bedrock

Presentation date: May 7,2009

Presented by: James W Schuetz.

Address: 40 La Riviere Dr, Suite 350, Buffalo, NY 14222

Phone: 716-541-0757; Email: James.Schuetz@Parsons.com

TCA-20™ Case Study

Bioaugmentation with TCA-20™ and SDC-9™ After Permanganate Treatment at a Massachusetts Site

Presented at the UMass Soils Conference
October 16, 2007

By

Shaw E&I, Inc.

Bioremediation of TCE and TCA using SDC-9TM after Sodium Permanganate Treatment

Shaw has conducted a highly successful sodium permanganate treatment program at a manufacturing site in New England. The permanganate treatment program involved the injection of over 177,000 gallons of a 20% sodium permanganate solution into the shallow overburden, deep overburden and bedrock aquifers at the site over four years. The sodium permanganate applications have resulted in significant reductions in TCE concentrations across the site.

Subsequent to the sodium permanganate treatment, an enhanced bioaugmentation treatment program was conducted using sodium lactate, Shaw's SDC-9™ culture and a TCA reducing bacteria, TCA-20™ also developed by Shaw. The bioremediation program targeted TCE contamination adjacent to an on-site stream where permanganate injection was not feasible, and residual TCA impacts in the deep overburden that are not amenable to treatment via permanganate. This presentation will discuss the additional steps needed to successfully complete an enhanced bioaugmentation program following permanganate treatment and will provide results of the initial application. In particular, the presentation will focus on the technical aspects of implementing bioaugmentation in an area previously targeted with permanganate, such as the quenching of residual permanganate concentrations with lactate, the potential for solubilizing manganese under reducing conditions, and the ability to achieve complete dechlorination of TCE and TCA via bioaugmentation.

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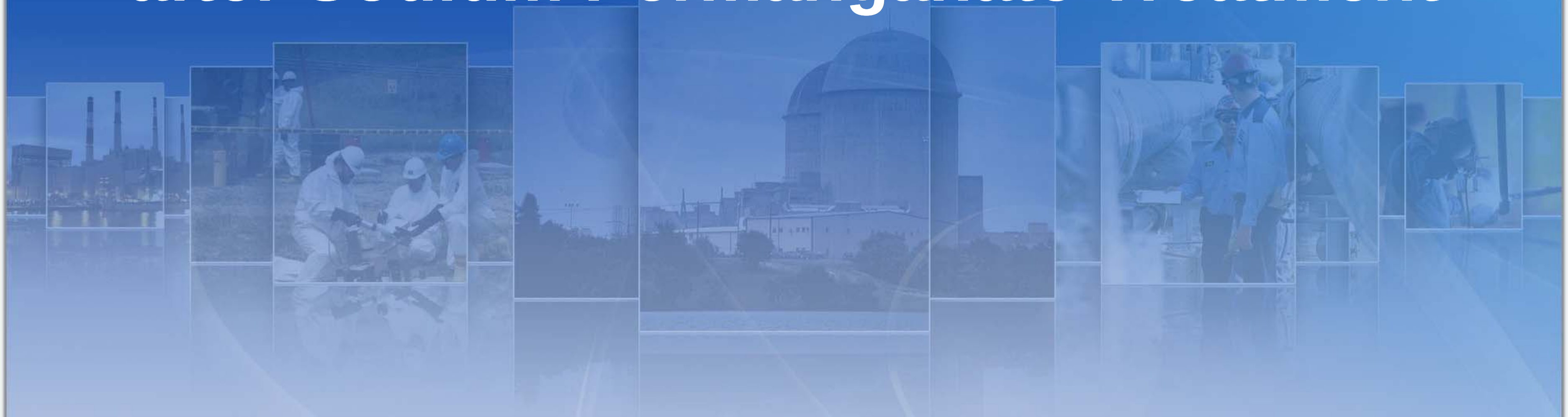
Case Study: Bioremediation of TCE and TCA using SDC-9™ and TCA-20™ after Sodium Permanganate Treatment



Raymond Cadorette

Site Manager, Hopkinton, MA

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Successful Large Scale Permanganate Treatment

- Applied over 180,000 gallons of permanganate (414,000 lbs of oxidant)
- Treated the shallow overburden, deep overburden and bedrock aquifers
- Significant reductions in TCE & PCE concentrations across the site

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Need for Additional Treatment

- Shallow Overburden
  - TCE up to 50 ppm adjacent to a stream
  - Concern about permanganate impacts to stream
- Deep Overburden
  - TCA up to 70 ppm
  - TCA not treated by permanganate

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Solution

- Bioaugmentation
  – Shaw's Dechlorinating Consortium-9 (SDC-9TM) will treat both TCE & TCA
  -- Shaw's TCA-20TM enriched specifically for chlorinated ethanes.
  – Potential impact to stream not as significant as permanganate
  – Application wells available
  – Vapor intrusion not a significant concern

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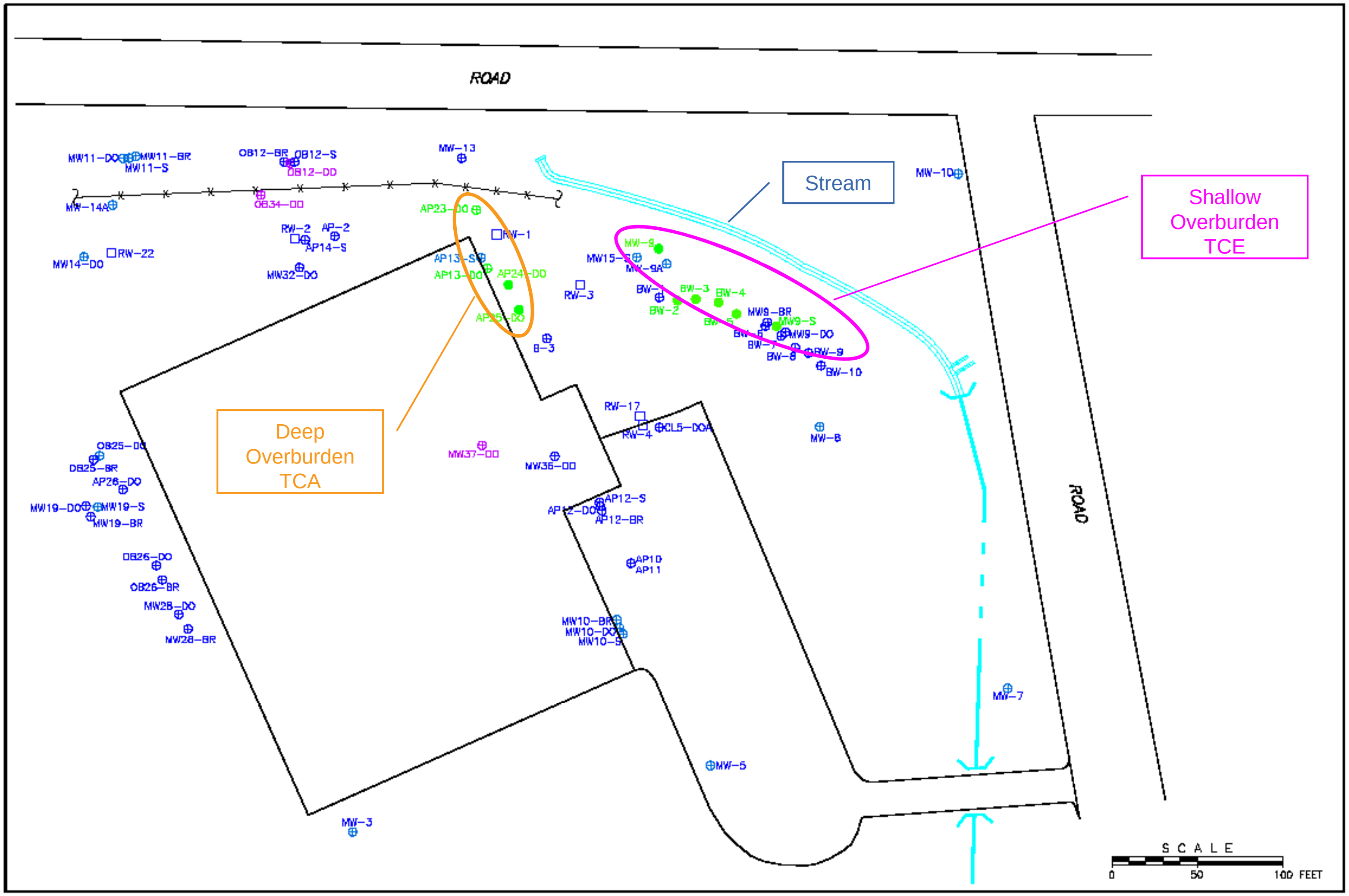
Treatment Areas



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Potential Problems

- Residual permanganate present
  - Baseline permanganate up to 21,000 mg/l
  - Account for permanganate in carbon demand calculation
- Initial high ORP and DO
  - ORP up to 200mV and DO over 6 mg/l
  - Needed to establish anaerobic conditions

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Other Potential Problems

- Permitting
  – Local and state due to proximity to stream
- Solubilizing manganese
  – Would metal reducing bacteria reduce manganese dioxide (forming soluble Mn+2)?

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How did It go?

Residual Permanganate

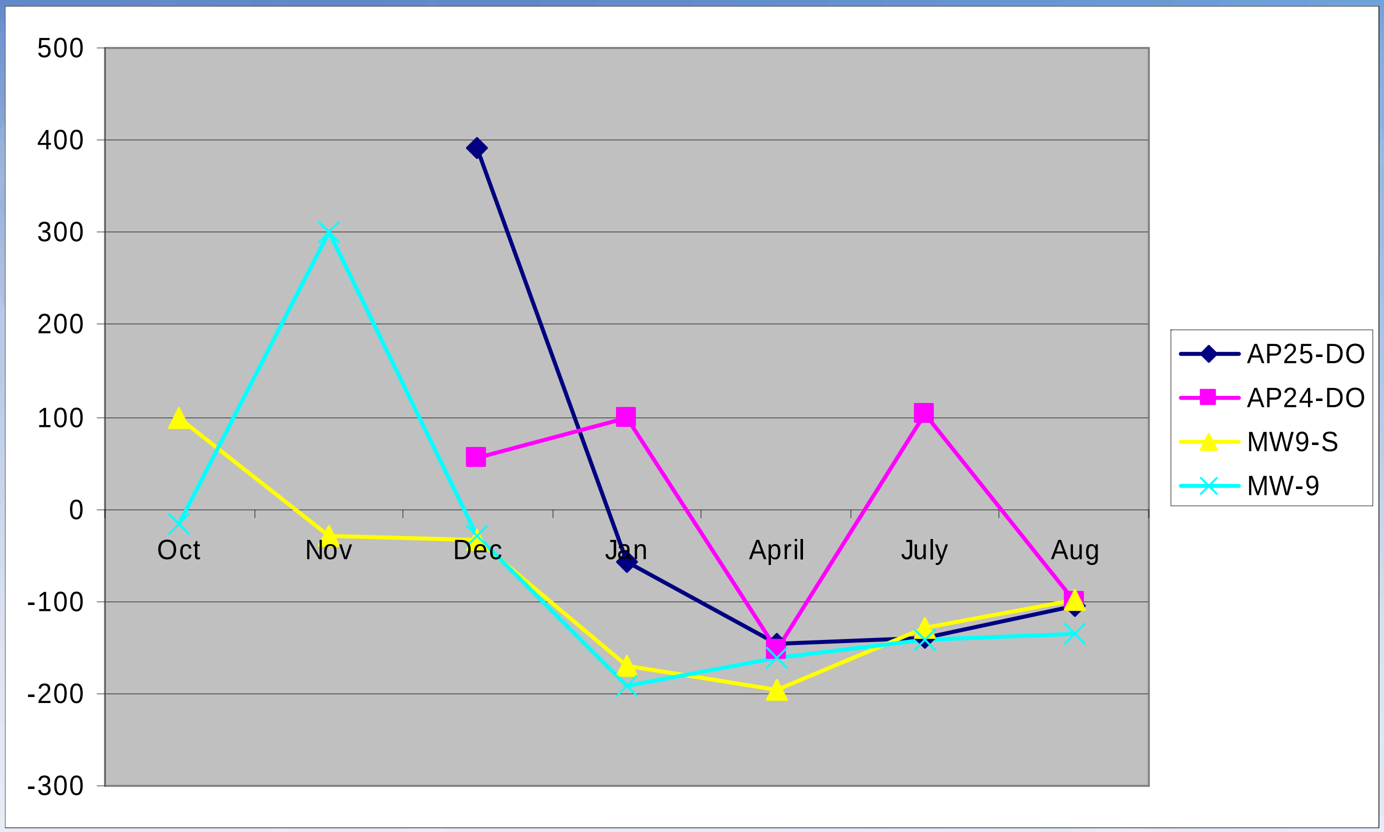
- Calculated lactate demand very high
  - Knew permanganate and VOCs were stratified with good vertical mixing in well
  - Likely permanganate not throughout formation
- How to quench permanganate?
  - Limited chemical options due to stream

Residual Permanganate

- Took an iterative approach
  - Applied lactate until purple color gone
  - Continued application & monitored DO/ORP
  - Used about 6 drums not the calculated 17

Establishing Reducing Conditions

ORP
(mV)



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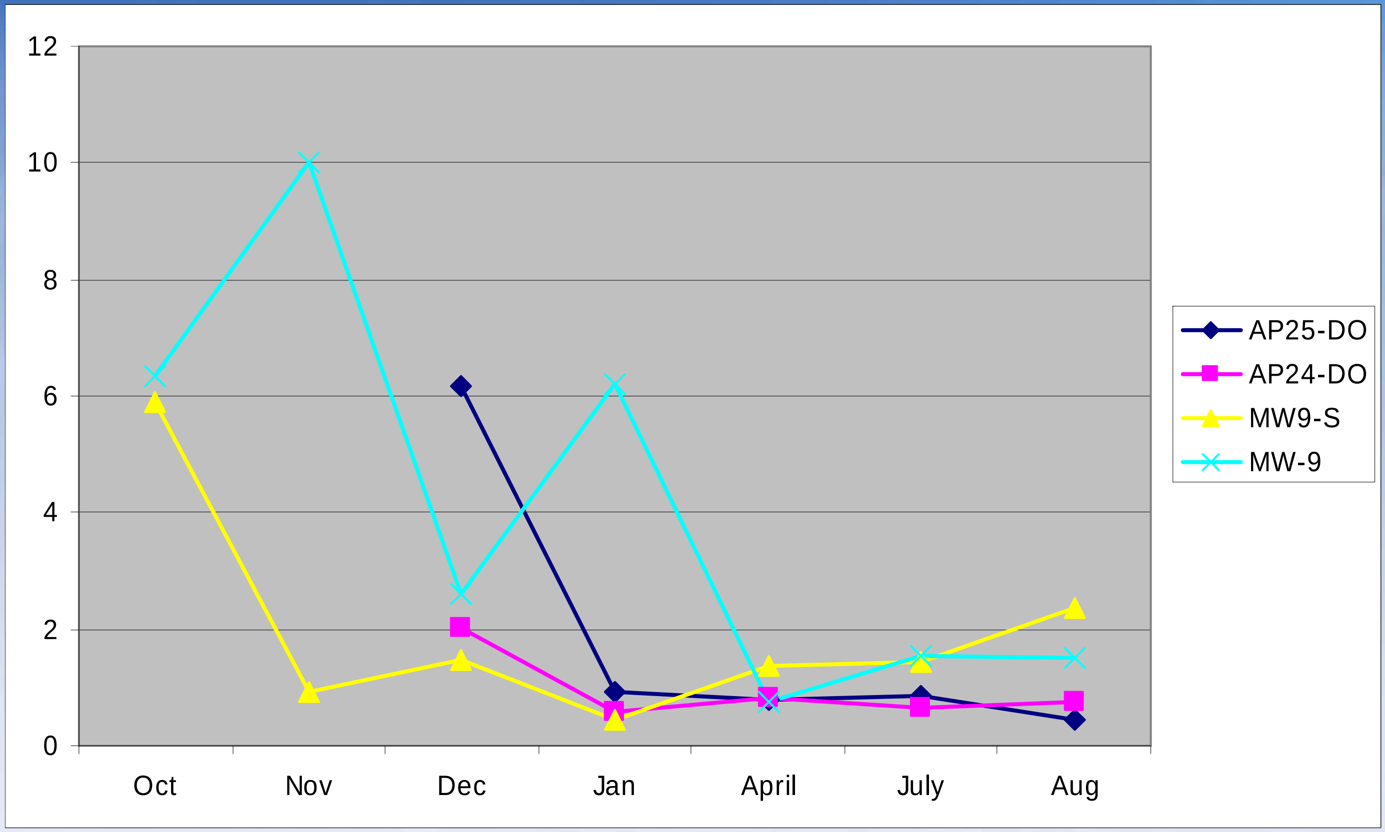
Establishing Anaerobic Conditions

DO
(mg/l)



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Dehalococcoides sp.

Well	Nov 2006	Jan 2007	April 2007	Aug 2007
AP24-DO	ND	$1.9x10^5$	$3.4x10^4$	ND
AP25-DO	ND	$8.0x10^5$	$3.7x10^3$	$6.0x10^4$
MW-9S	ND	$1.3x10^5$	$7.1x10^3$	$8.8x10^6$

Results in cells/ml

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Discussion of VOC Results

- Shallow Overburden
  - Complete breakdown of TCE through DCE to VC
  - Some wells degraded completely to ethene
- Deep Overburden
  - Good reduction in TCE & TCA at AP25-DO
  - Limited success at AP24-DO

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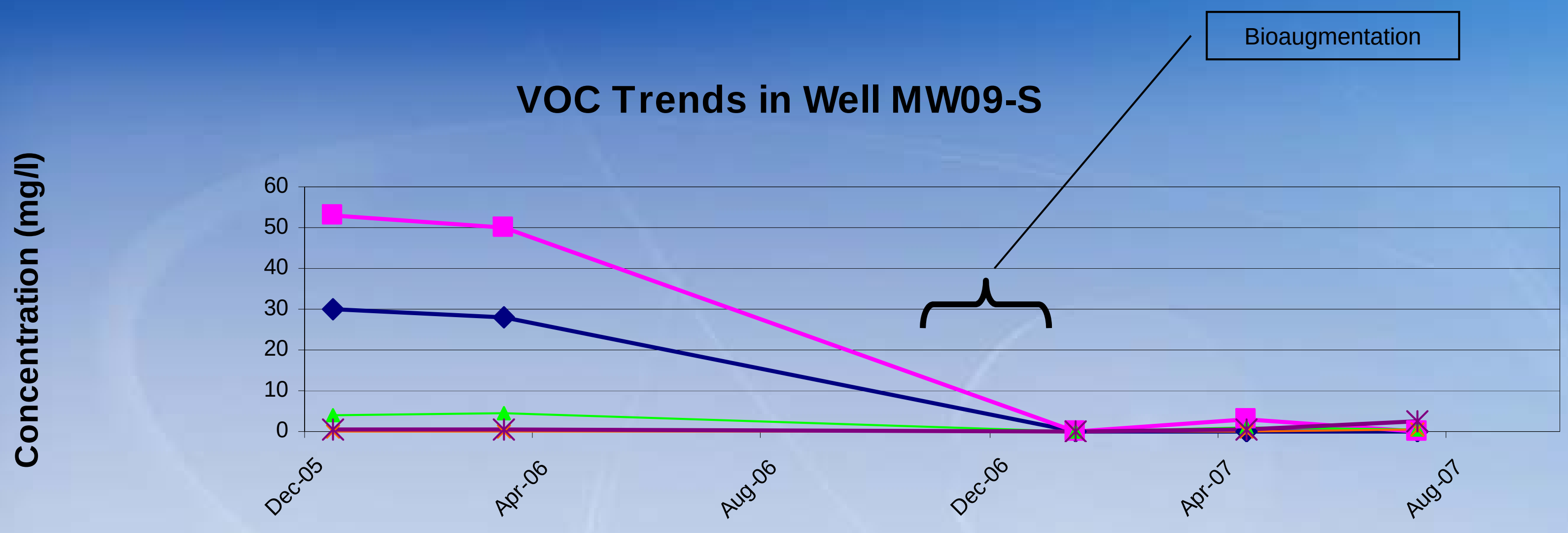
VOC Results



	12/30/2005	3/27/2006	1/30/2007	4/10/2007	7/19/2007
PCE	30	28	0.002	0.18	0.01
TCE	53	50	0.0074	2.8	0.01
cis-1,2-DCE	4.2	4.6	0.17	1	0.64
1,1,1-TCA	0.25	0.25	0.041	0.027	0.48
VC	0.5	0.5	0.071	0.48	2.4

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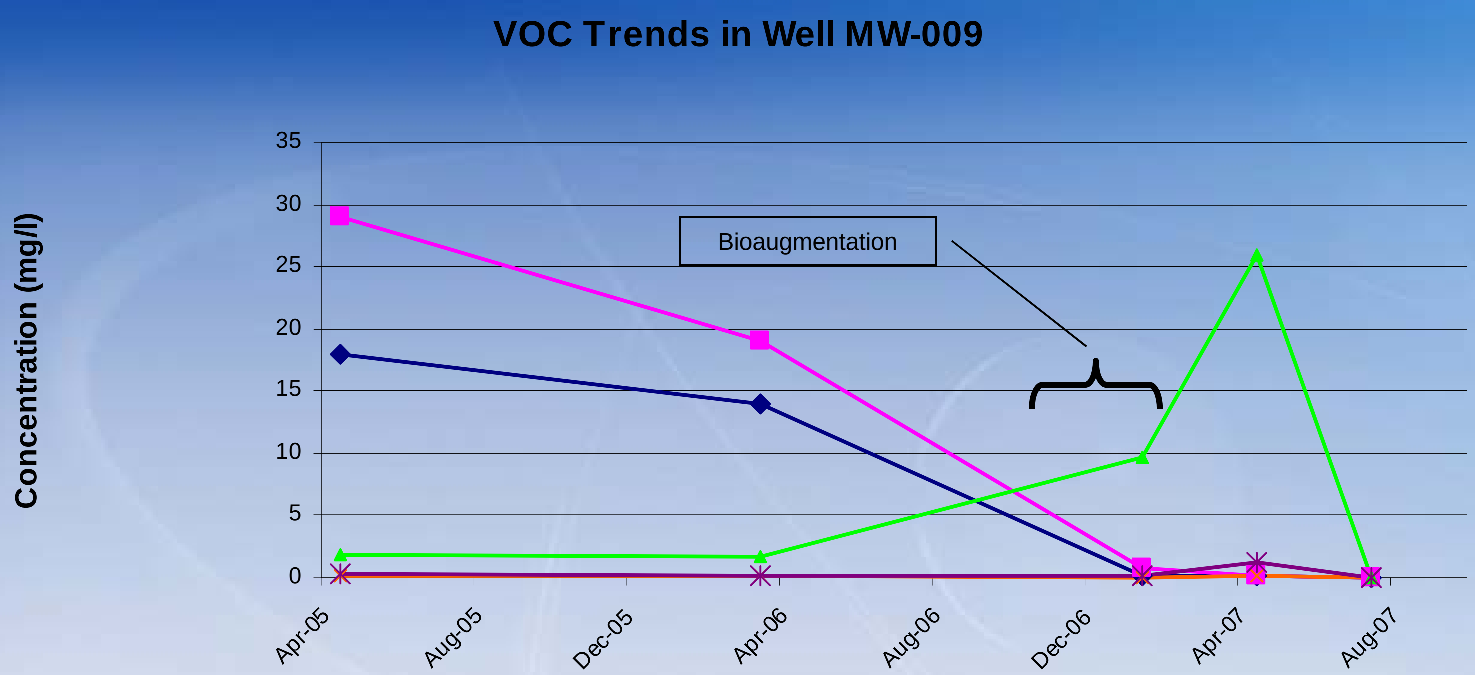
VOC Results



	4/27/2005	3/27/2006	1/30/2007	4/10/2007	7/19/2007
PCE	18	14	0.16	0.125	0.0010
TCE	29	19	0.76	0.125	0.0016
cis-1,2-DCE	1.9	1.7	9.6	26	0.021
1,1,1-TCA	0.125	0.1	0.05	0.125	0.030
VC	0.25	0.2	0.1	1.3	0.016

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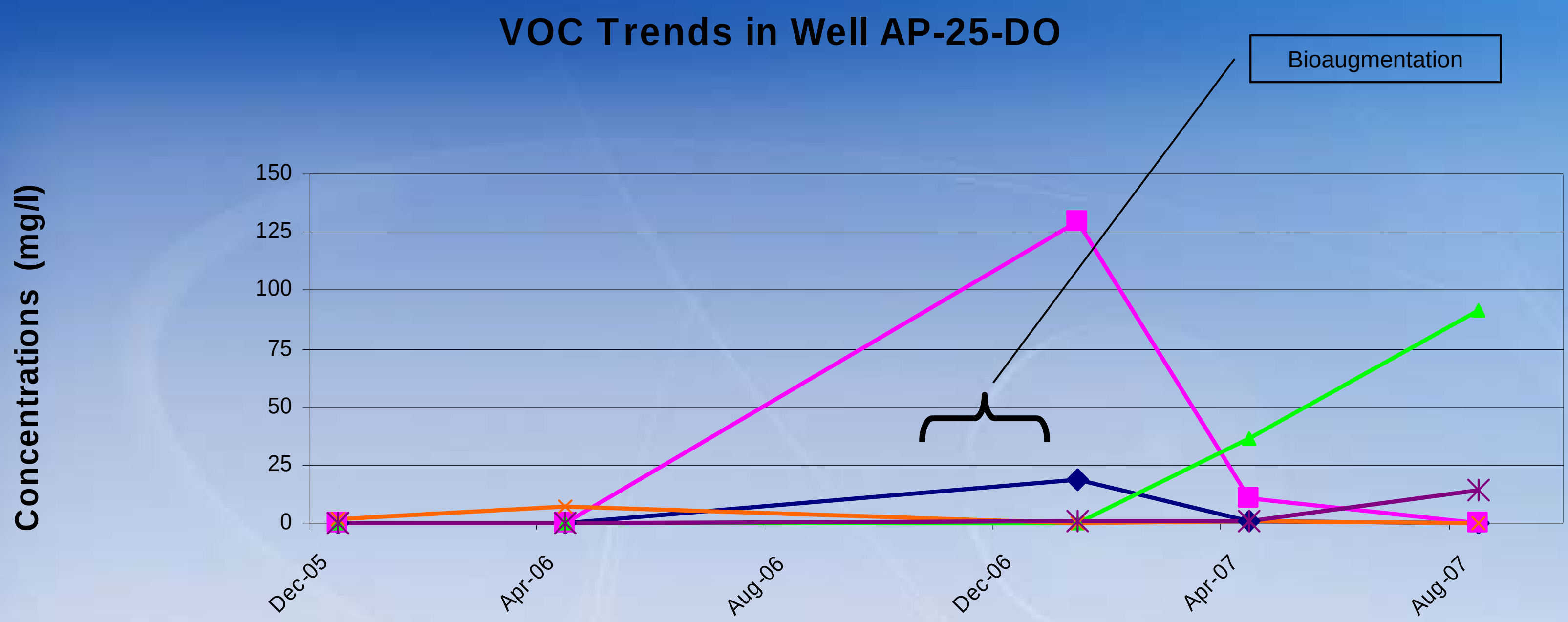
VOC Results



	12/30/2005	4/3/2006	1/30/2007	4/10/2007	8/9/2007
PCE	0.13	0.058	19	0.72	0.05
TCE	0.01	0.025	130	11	0.05
cis-1,2-DCE	0.01	0.025	0.05	36	91
1,1,1-TCA	2.2	7.1	0.05	0.64	0.05
VC	0.02	0.05	1.0	0.5	14

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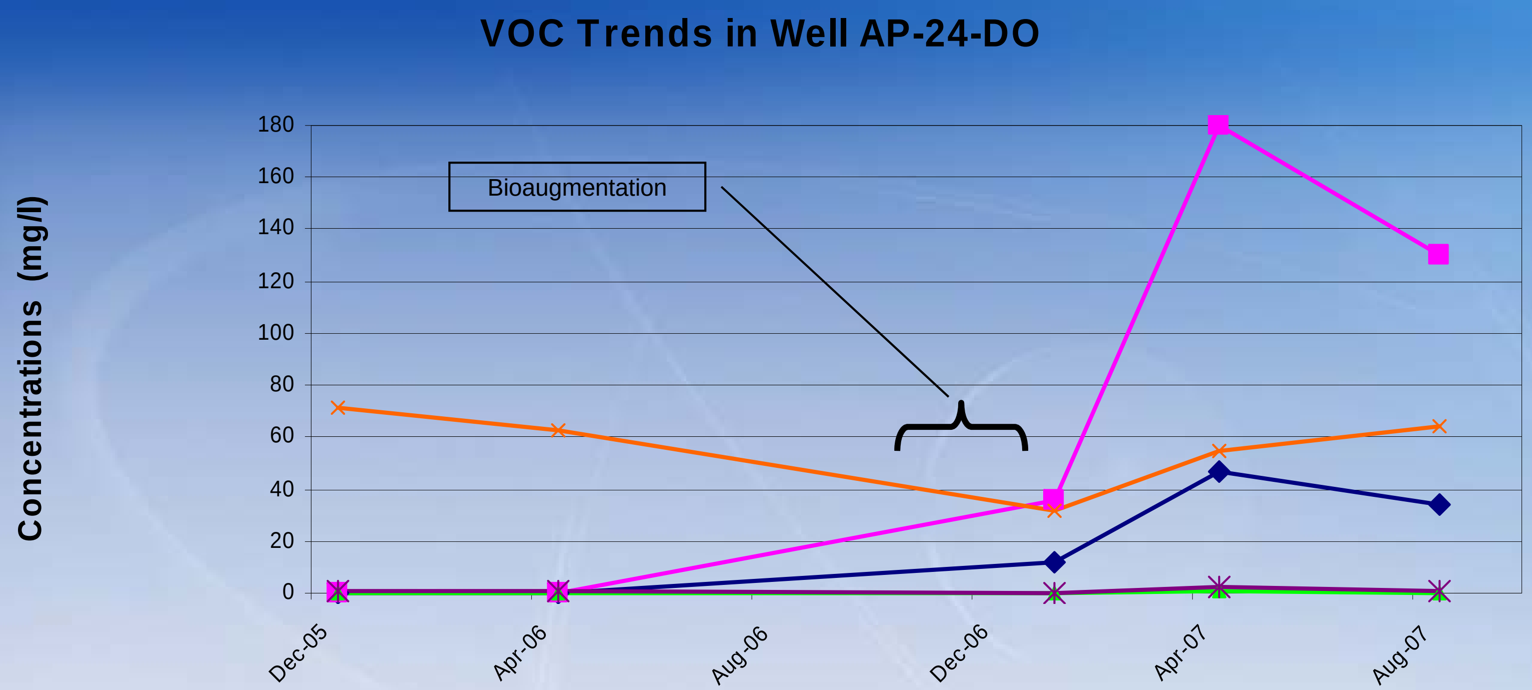
VOC Results



	12/30/2005	4/3/2006	1/30/2007	4/10/2007	8/9/2007
PCE	0.25	0.25	12	47	34
TCE	0.25	0.25	36	180	130
cis-1,2-DCE	0.25	0.25	0.25	1.0	0.05
1,1,1-TCA	71	63	32	55	64
VC	0.5	0.5	0.05	2.0	1.0

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Discussion of VOC Results

- Why limited success at AP24-DO?
  - High initial pH
  - Fewer application wells in deep overburden compared to shallow
  - Adjacent to area with permanganate
  - Mn degraders competing for carbon

Discussion of VOC Results

- Why such a high increase of TCE in deep overburden
  - Up to 400 ppm in deep overburden before permanganate treatment
  - Cosolvent effect of lactate (increases solubility)
  - Mobilized from formation
  - Short in duration, decreased as reductive dechlorination progressed

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Discussion of Mn Results

- Dissolved Mn
  - Previous elevated Mn limited to active permanganate injection wells
  - Mn decreased in some bio wells
  - Mn degraders were present

Dissolved Mn

Well	Nov 2006	Jan 2007	April 2007	Aug 2007
AP24-DO	4430	104	3.17	0.1
AP25-DO	1300	42.8	0.1	0.1
MW-9S	2.52	40	75.2	4.82

Results in mg/l

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Discussion of Mn Results

- Does not appear that soluble Mn+2 is being produced
- Continue to monitor and evaluate

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Going forward

- Additional lactate application in the shallow overburden
  - Complete degradation through VC
- Additional bioaugmentation With TCA-20 in deep overburden
  - Continue degradation of TCE and daughter product
  - Continue to treat TCA

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Conclusions

- Were able to create reductive & anaerobic conditions
- Bioaugmentation with SDC-9 and TCA-20 was successful.
- Observed complete reductive dechlorination in shallow overburden
- Began the reductive dechlorination process in the deep overburden

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TCA-20^{TM} Case Study

Bioaugmentation with TCA-20^{TM} and SDC-9^{TM} Combined with Proton Reduction Technology at an Oklahoma Site

By

Shaw E&I, Inc.

Proton Reduction Technology for Aquifer Remediation

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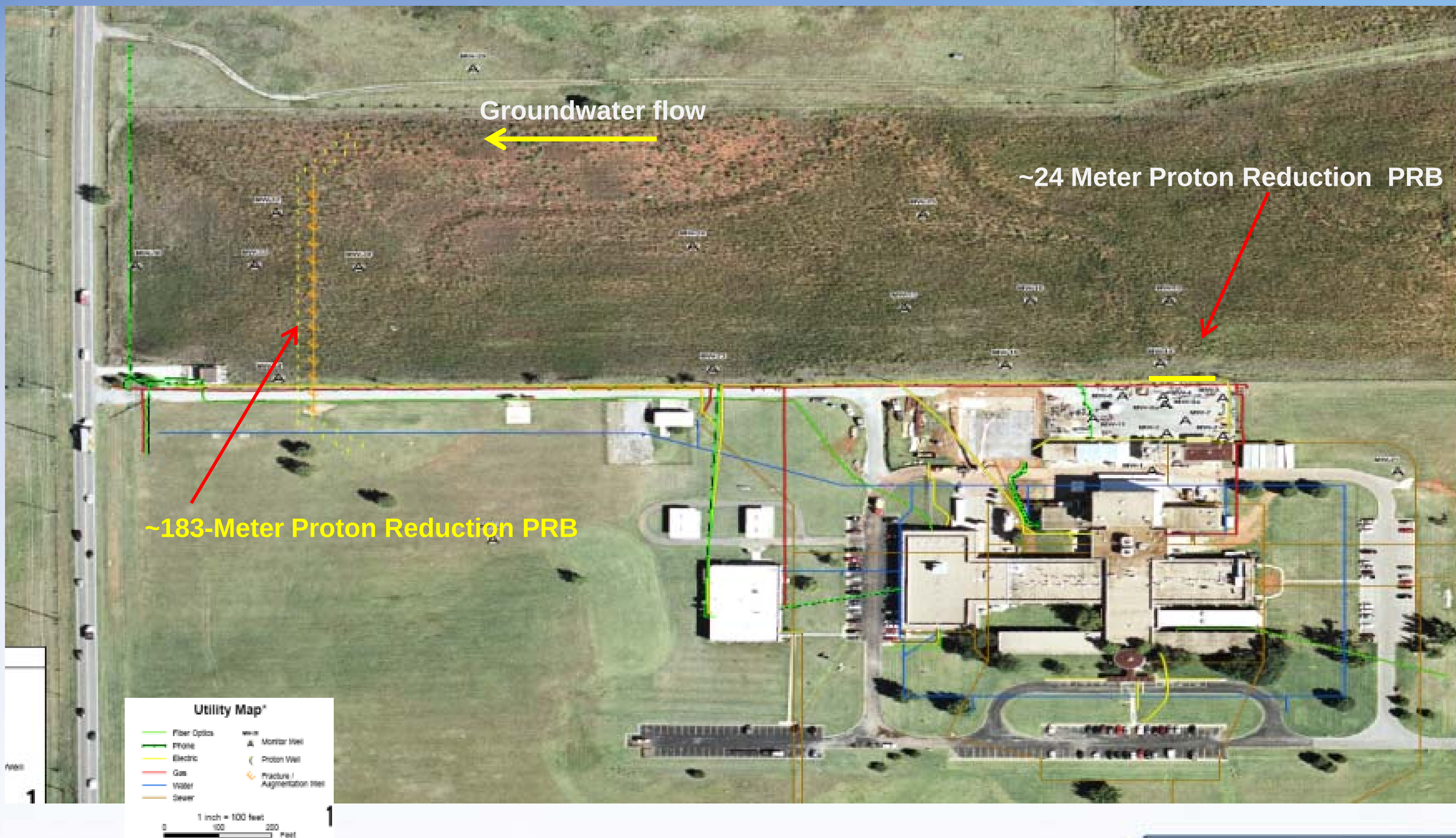
Many of the Nation's most challenging groundwater contaminants (e.g., RDX, TCE, perchlorate) are biodegraded by microbes that use H_2 as an energy source. In most cases the H_2 required by these microbes is produced via fermentation of complex organic substrates referred to as electron donors. An alternative approach is to use electrodes inserted into the aquifer to generate H_2 directly by reduction of protons on the surface of a cathode. This proton reduction occurs at a low voltage (-0.5 V) which can be supplied by using a photo voltaic (i.e., solar) system. Because the process is proton consuming, it also can be used to increase the pH of acidic aquifers. We have applied proton reduction at 5 sites. At an industrial site in the south west, a 600-foot permeable reactive barrier was created by using 42 proton reduction electrodes. A second barrier was created near a source zone by installing nine additional proton reduction wells. Each location also was inoculated with either SDC-9 culture or a combination of SDC-9 and TCA-20 cultures. Near the source zone chloroform concentrations have been reduced from nearly 250 mg/L to <50 µg/L, and PCE concentrations have been reduced from 1.3 mg/L to less than 5 µg /L. At the downgradient barrier chloroform concentrations are being reduced from 700 µg /L to <5 µg /L, effectively preventing offsite migration of the contaminants. During ESTCP project ER-201033, proton reduction is being applied to increase groundwater pH and treat chlorinated solvent contamination at McGuire AFB in New Jersey. During this project, the proton reduction system will be operated to support electrolysis of groundwater at the cathodes to consume protons and produce OH^-, thereby increasing groundwater pH. Bioaugmentation with SDC-9 is being performed to supplement the natural microbial population which lacks an adequate *Dehalococcoides sp*. population. The system used at this site will utilize low cost mixed metal oxide coated titanium electrodes ($2.00/linear foot) inserted in the TCE-contaminated aquifer and powered by a photo voltaic power source.

Case Study: Bioaugmentation with TCA-20 and SDC-9 combined with Proton Reduction technology

Full-Scale Application Near Oklahoma City



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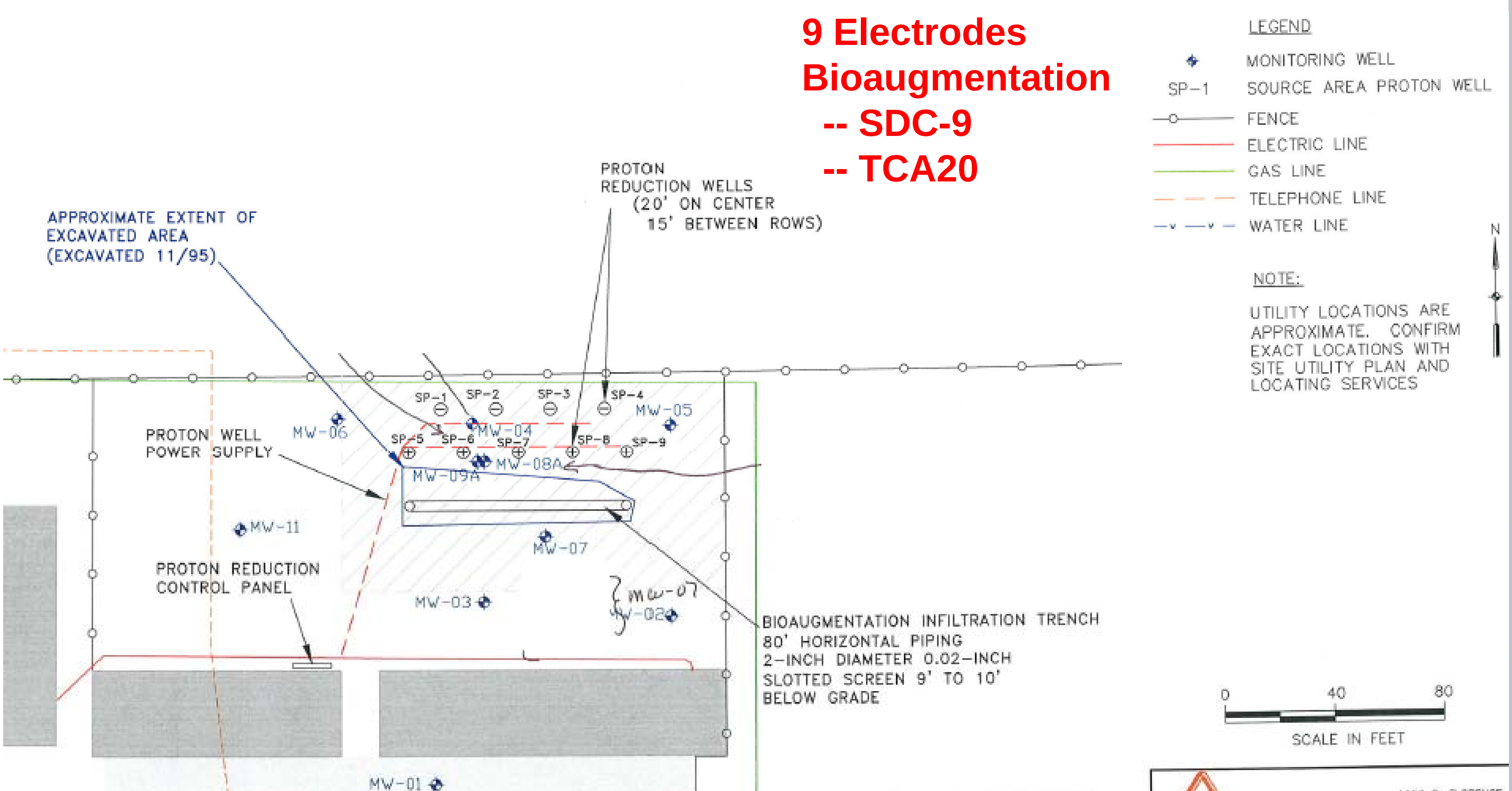
Detail of 80-ft. Barrier Area

9 Electrodes
Bioaugmentation
-- SDC-9
-- TCA20



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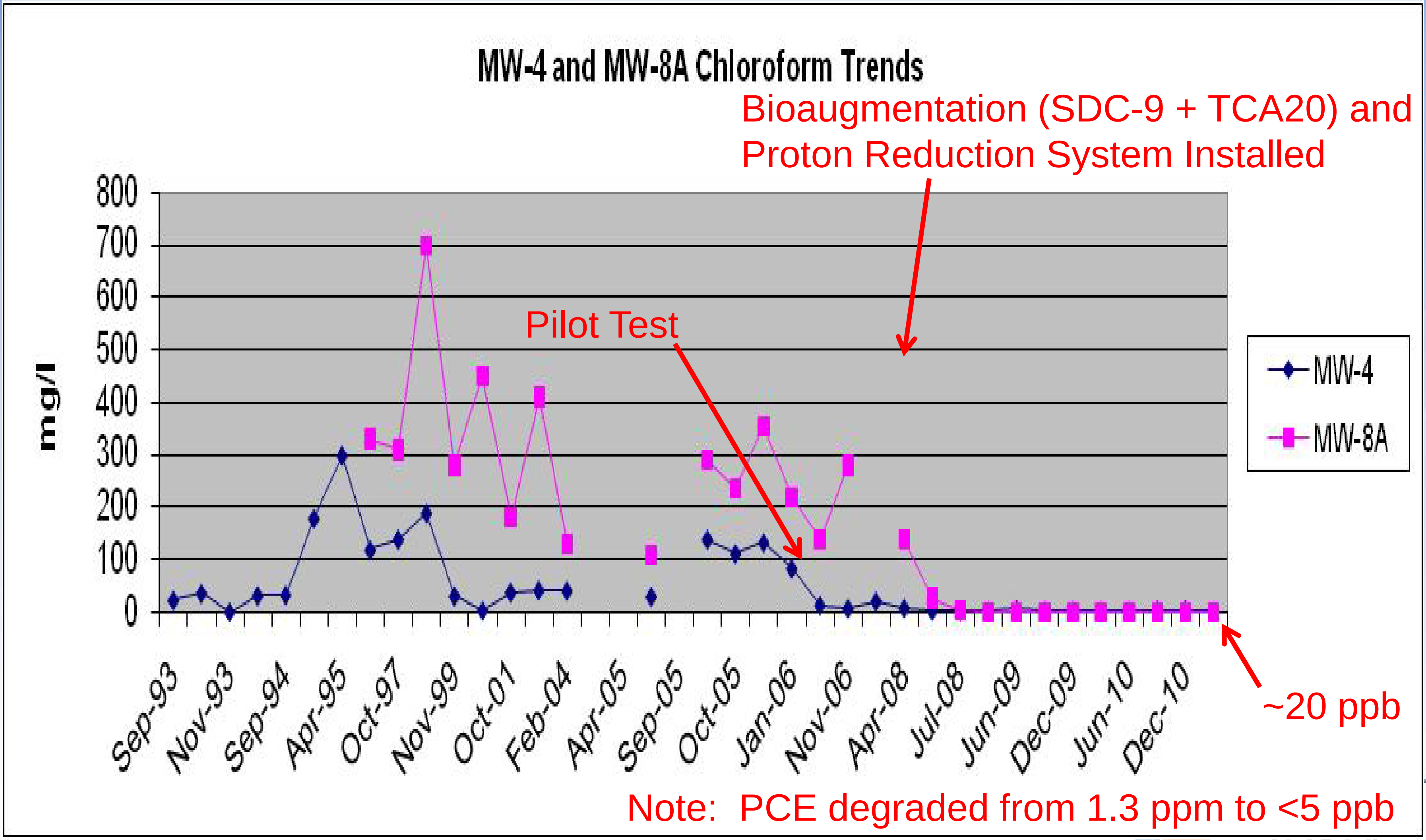
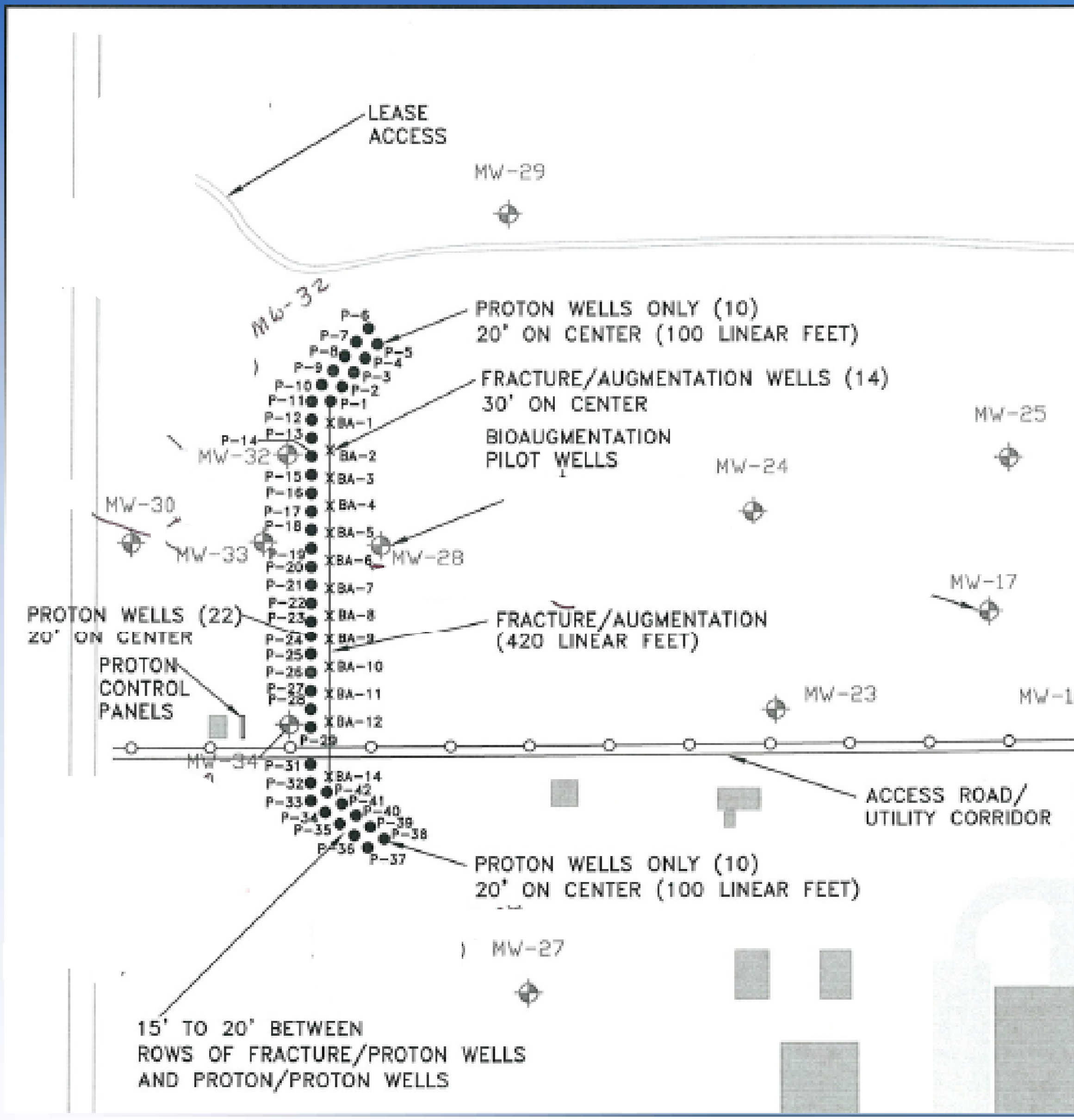
Chloroform in Source Area Monitoring Wells



Detail of 600' Barrier Area

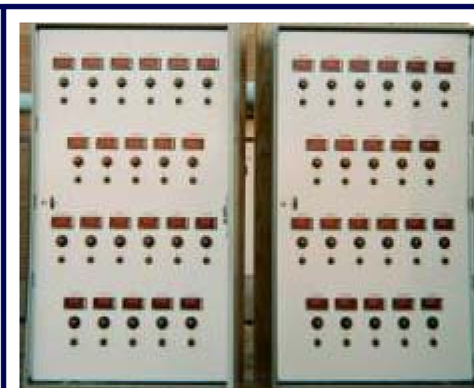


Technologies Employed:

Proton Reduction
-- 44 electrodes

Bioaugmentation
-- SDC-9 + TCA20

Pneumatic Fracturing



Proton Reduction Control Panel

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Completed 600-ft Proton Reduction Barrier at an Oklahoma Site

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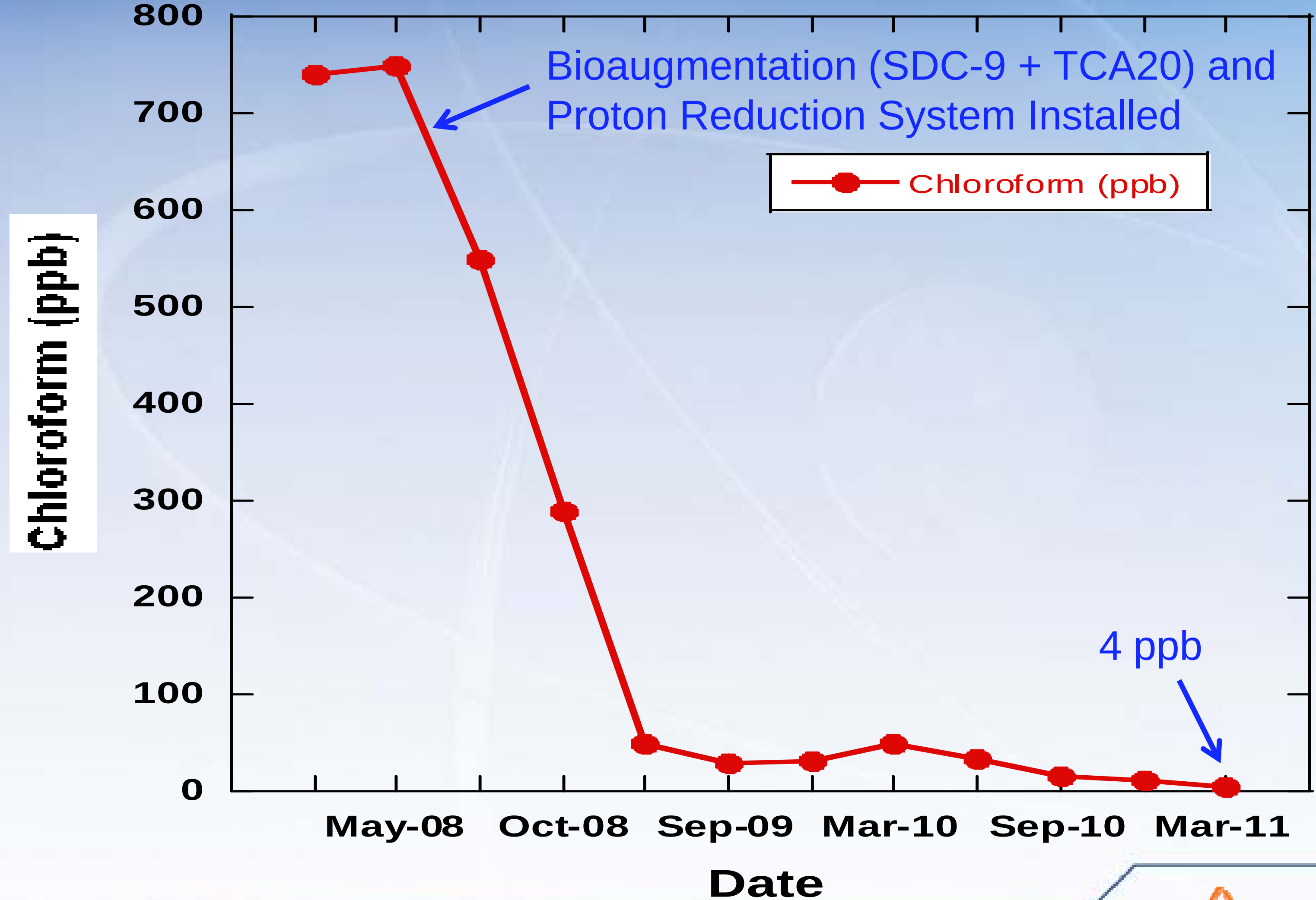
Chloroform in Monitoring Well Down-Gradient of 183-m Barrier



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Summary

- Proton Reduction generates dissolved H_2
- H_2 is used as an electron donor by cVOC-degrading bacteria – SDC-9 and TCA-20
- Bioaugmentation with TCA-20 and SDC-9, combined with proton reduction for electron donor supply, allowed for successful in situ treatment of mixed cVOCs

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