



NOVEL USES OF ADVANCED DIAGNOSTIC TOOLS FOR CHEMICAL OXIDATION AND BIOREMEDIATION APPLICATIONS

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Introduction

- Recent and affordable commercial availability of molecular biological tools (MBT) and compound specific isotope analysis (CSIA) is changing our approach to site assessment, remedy selection and performance monitoring
 - Screening tool to assess viability of MNA and bioremediation
 - Replace microcosm studies with *in-situ* diagnostic tools
 - Track remedial progress




Introduction Continued

- Use of the advanced diagnostic tools allows remediation professionals to make better decisions to expedite site closure and minimize costs
- WSP has implemented these tools at approximately 30 sites world wide, most of which are in the United States
 - Monitored Natural Attenuation (MNA)
 - Bioremediation
 - Chemical Oxidation



Monitored Natural Attenuation

- Challenges related to traditional approach
 - Time consuming (many rounds of data)
 - Cost
 - Data interpretation
 - Conflicting data
 - Kinetics
- Advanced diagnostic tools resolve these challenges



MNA Example Georgia Site

- Historical release of coolant containing chlorinated solvent (PCE/TCE) and lesser concentrations of petroleum constituents
- Continuing vadose zone source area
- Dissolved VOC concentrations exceeding 50 mg/l in source area
- 1,200-foot long dissolved plume

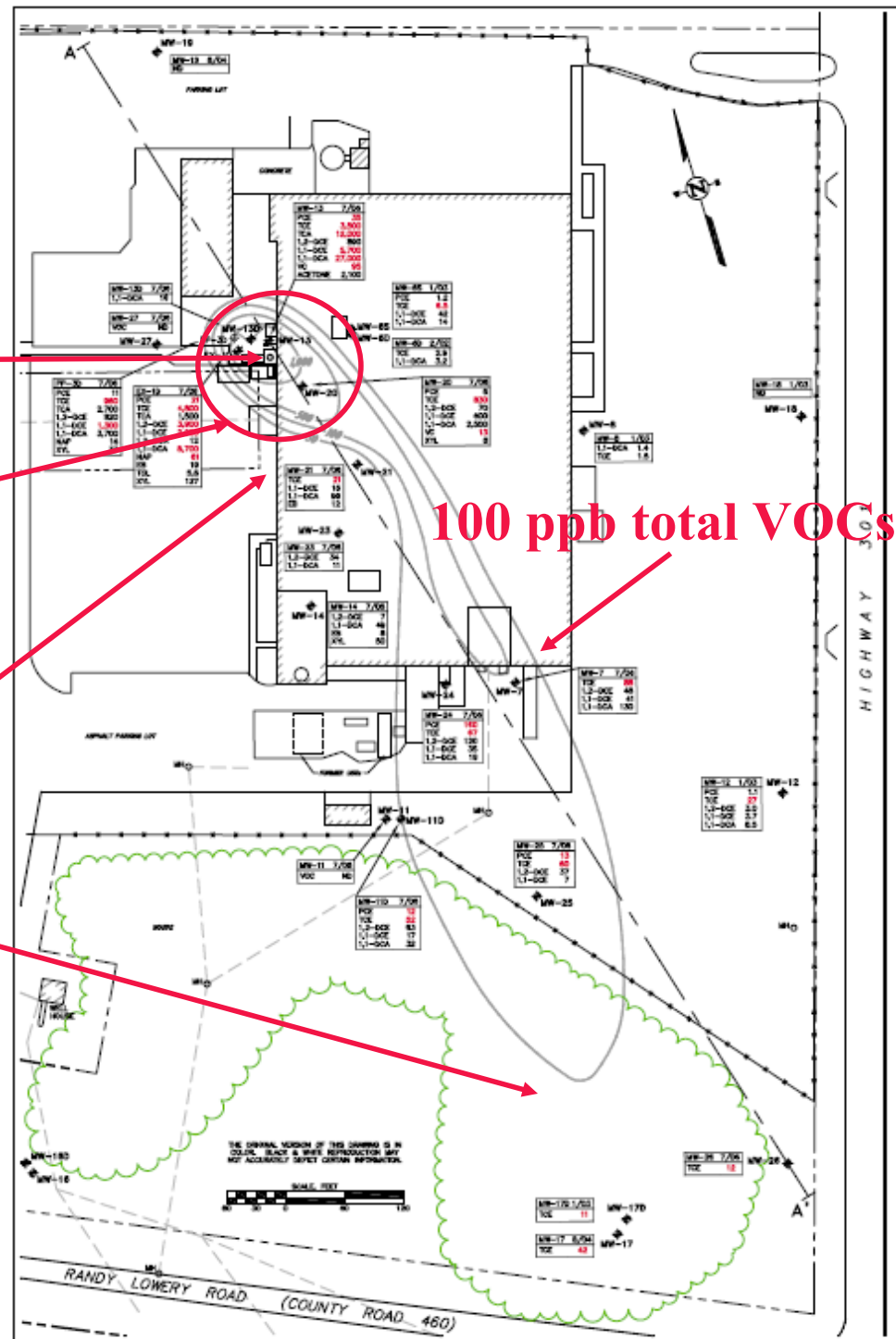
MNA Example Georgia Site

Corrective Actions:

Source area soils were excavated

Source area groundwater was treated with Perozone™


MNA proposed for downgradient area of plume



MNA Example Georgia Site

Traditional MNA Assessment:

- Plume Stability
 - Increasing source area dissolved VOC concentrations
 - Decreasing downgradient area VOC concentrations indicating attenuation
- Daughter Products
 - Reductive daughter products, including end products present
- Competing Electron Acceptors
 - Elevated dissolved oxygen
 - Redox couple data consistent with aerobic conditions
- Electron Donors
 - Elevated concentrations of naturally occurring and some anthropogenic electron donor compounds present
- Conclusion
 - Aerobic environment not consistent with MNA



MNA Example Georgia Site


- Challenge
 - Definitively identify plume stability mechanism for the observed decreasing VOC trend
 - Show that the mechanism is active
 - Gain regulatory approval
- Microbial Insights Bio-trap[®] deployed
 - Bio-trap[®] samples collected from 4 wells
 - 7 week incubation period
- Phospholipid Fatty Acids (PLFA)
 - PLFA data show the presence of a microbial population that is consistent with MNA
- Quantitative Polymerase Chain Reaction (qPCR)
 - Identified the presence of a significant population of *Dehalococcoides spp* (DHC)

MNA Example

Bio-trap® Deployment




WSP Sampler Tim Huff




MNA Example Georgia Site

- Conclusion with MBTs
 - MBTs conclusively demonstrated the mechanism (i.e., the DHC) of observed plume stability and daughter product production
 - DHC is an obligate anaerobe therefore anaerobic micro-environments that support microbial attenuation of the chlorinated volatile organic compounds (VOCs) must be present
 - State approved MNA
 - Cost savings



MNA Example Tennessee Site


- In 1983, a control valve for a diesel AST was inadvertently left open, and released approximately 5,000 gallons of diesel fuel.
- In 1987, 7,000 gallons of waste coolant was released
- Elevated concentrations of BTEX and TPH present in groundwater
- No NAPL
- Decreasing dissolved BTEX concentration trends observed



MNA Example Tennessee Site

Evaluation Process

- Microbial Insights Biotrap[®] deployed for 55 days
- Three tests were performed by MI:
 - PLFA to gather data on the broader community structure of the microbial ecology.
 - Density gradient gel electrophoresis (DGGE) that provides specific information about individual species of microorganisms.
 - A stable isotope probing (SIP), which proves that the chemicals of concern, in this case represented by benzene, are metabolized by the cells.




MNA Example Tennessee Site

■ Results

- The PFLA test showed an adaptable population of organisms capable of utilizing the chemicals of concern at this site
- The DGGE test provided more specificity about organisms that are known to degrade benzene and related compounds
- The most important evidence is provided by the SIP test which demonstrated that labeled benzene is being incorporated into the biomass of the organisms at the site


■ Remedy Selection

- The results of these tests provide conclusive evidence supporting natural attenuation
- Based on an evaluation of the sampling results, WSP proposed MNA as a long-term groundwater remedy for the site
- The MNA was approved as a remedy by the State



MNA Example New York Site

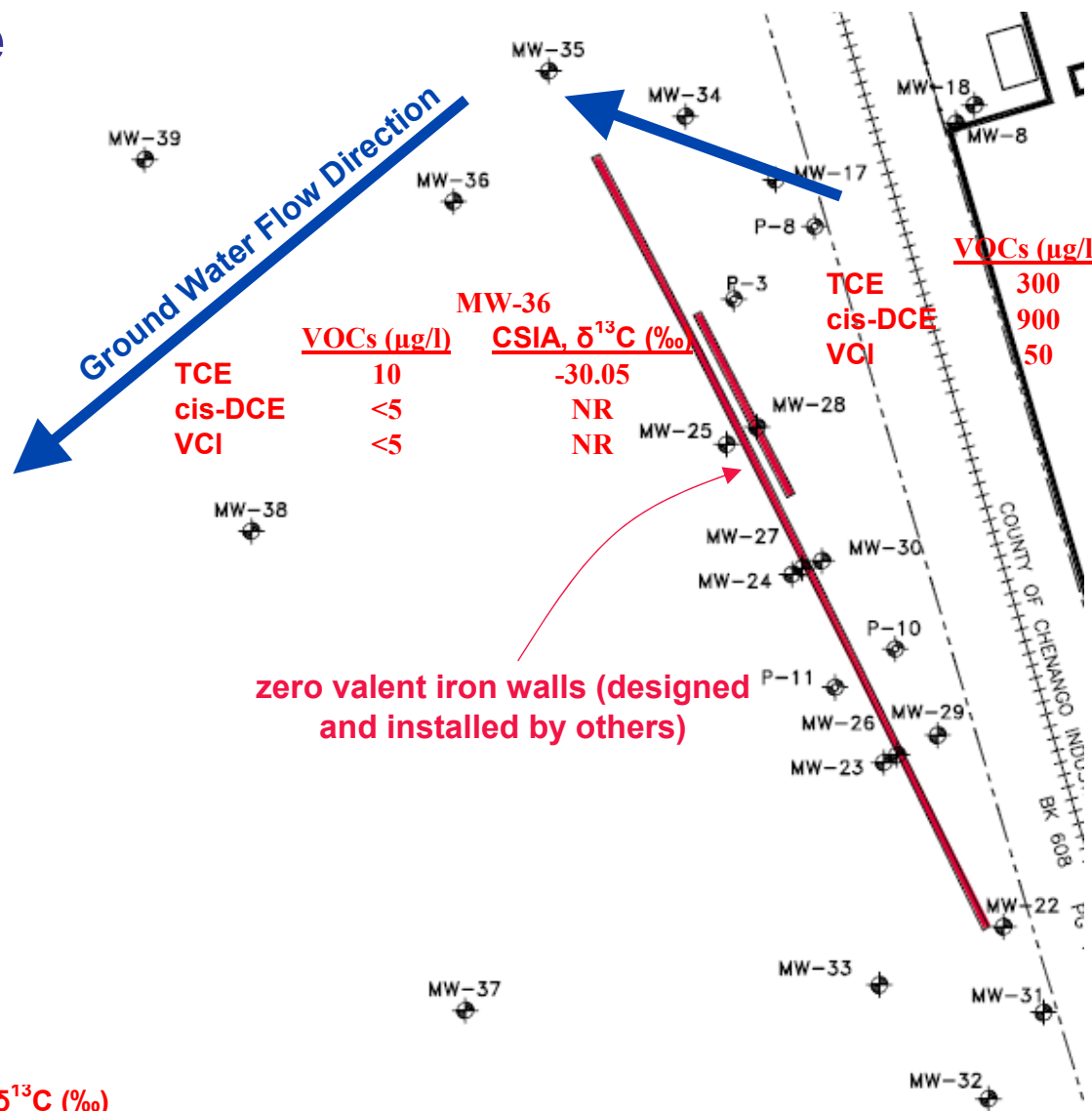
- Permeability of zero valent iron wall (designed and installed by others) has decreased causing VOCs (TCE, cis-DCE, vinyl chloride) to bypass the wall
- Dissolved VOCs have migrated approximately 500 feet offsite to an actively farmed field
- Historically VOC concentrations have decreased throughout the plume including upgradient of the iron wall
- Propose MNA as remedy



MNA Example New York Site

- Advanced diagnostics implemented from select wells along plume flow path
 - Microbial Insights Bio-trap[®] deployed
 - CSIA samples collected and analyzed by Microseeps
- PLFA and PCR results
 - PLFA showed a diverse healthy population of microbes consistent with conditions that support reductive dechlorination
 - PCR data showed a significant population of known chloro-respiring microbes but no DHC

MNA Example New York Site



TCE
cis-DCE
VCI

<u>VOCs (µg/l)</u>	<u>CSIA, δ¹³C (‰)</u>
10	-30.05
<5	NR
<5	NR

MW-36
TCE
cis-DCE
VCI

<u>VOCs (µg/l)</u>	<u>CSIA, δ¹³C (‰)</u>
10	-30.05
<5	NR
<5	NR

TCE
cis-DCE
VCI

<u>VOCs (µg/l)</u>
300
900
50

P-8


<u>CSIA, δ¹³C (‰)</u>
-32.3
-32.87
-24.05

zero valent iron walls (designed and installed by others)

TCE
cis-DCE
VCI

MW-21

<u>VOCs (µg/l)</u>	<u>CSIA, δ¹³C (‰)</u>
50	-30.71
20	-25.71
<5	NR



MNA Example New York Site

CSIA results (one round of data)

- Source area daughter product fractionation (VCL $\delta^{13}\text{C} = -24.05\text{‰}$) greater than parent (TCE $\delta^{13}\text{C} = -32.3\text{‰}$) indicating that the degradation pathway is complete
- TCE fractionation increased from the source area ($\delta^{13}\text{C} = -32.3\text{‰}$) to the furthest downgradient sampling point ($\delta^{13}\text{C} = -30.71\text{‰}$)
- Significant DCE fractionation in downgradient sample observed without the accumulation of vinyl chloride
 - Probable oxidative degradation pathway
 - *Nitrosomonas* species are known to fortuitously oxidize TCE and its daughter products
 - Plan additional Biotrap[®] deployment to assess nitrogen cycle and effects on contaminant concentrations



Bioremediation

Challenges related to traditional approach

- Scalability of and costs of microcosm tests
- Cost of pilot test
- Data interpretation

Advanced diagnostic tools resolve these challenges

- Amended Bio-Trap® Samplers
- CSIA



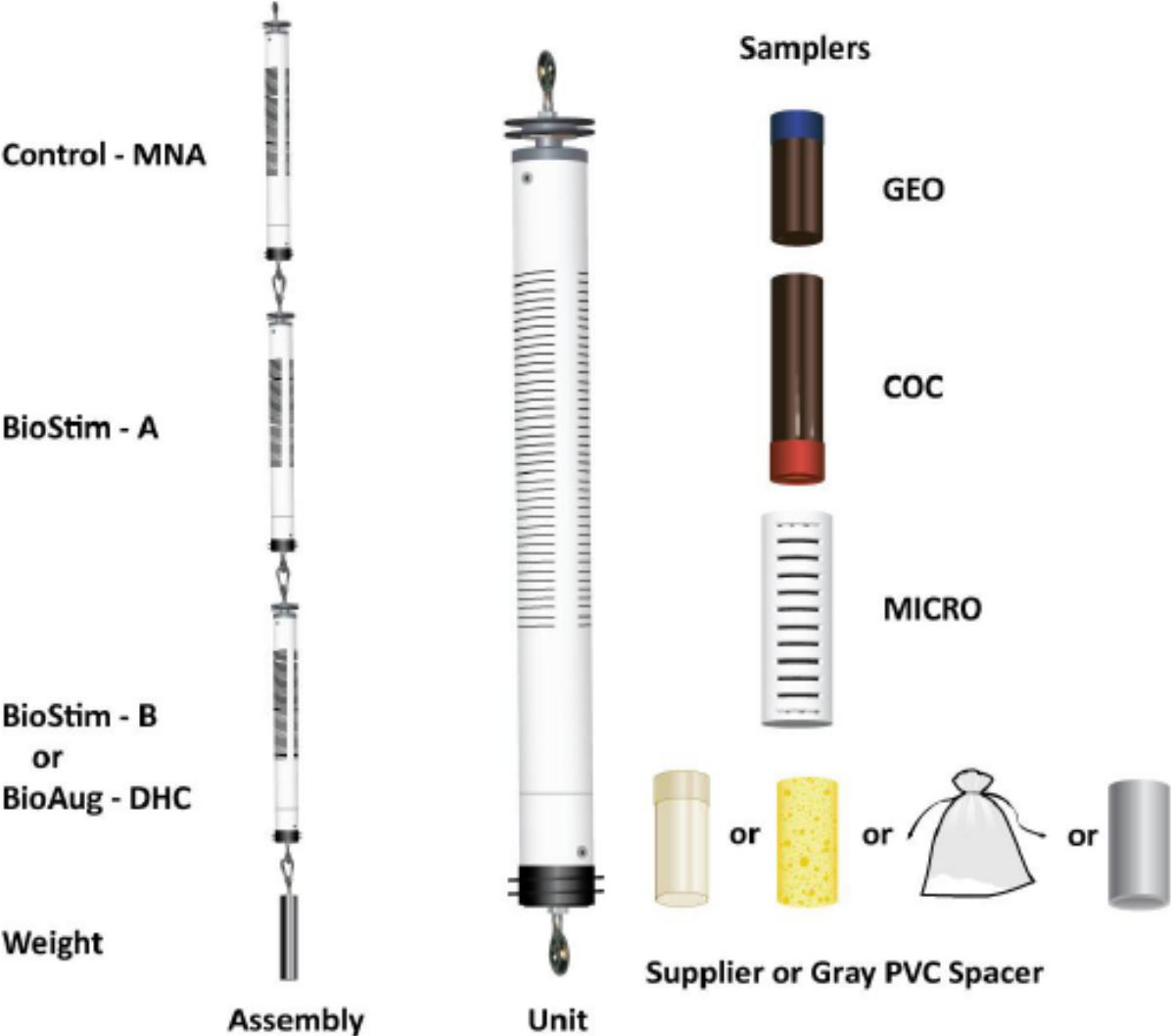
Bioremediation (Continued)

Bio-Trap[®] Samplers

- Bio-Trap[®] Samplers can be “baited” with various amendments or compounds to answer site-specific questions and screen remedial alternatives:
 - Contaminants of concern (¹³C labeled or unlabeled)
 - Contaminant analogs
 - Various electron donors such as HRC[®], EOS[®] or lactate
 - Various electron acceptors such as oxygen releasing materials, nitrate or sulfate
 - Microbial augments
- Sorbed VOCs can be extracted from Bio-Sep[®] beads to assess effect of biostimulants
 - Contaminant analog concentration trends
 - CSIA fractionation

Bioremediation (Continued)

Microbial Insights Assembly

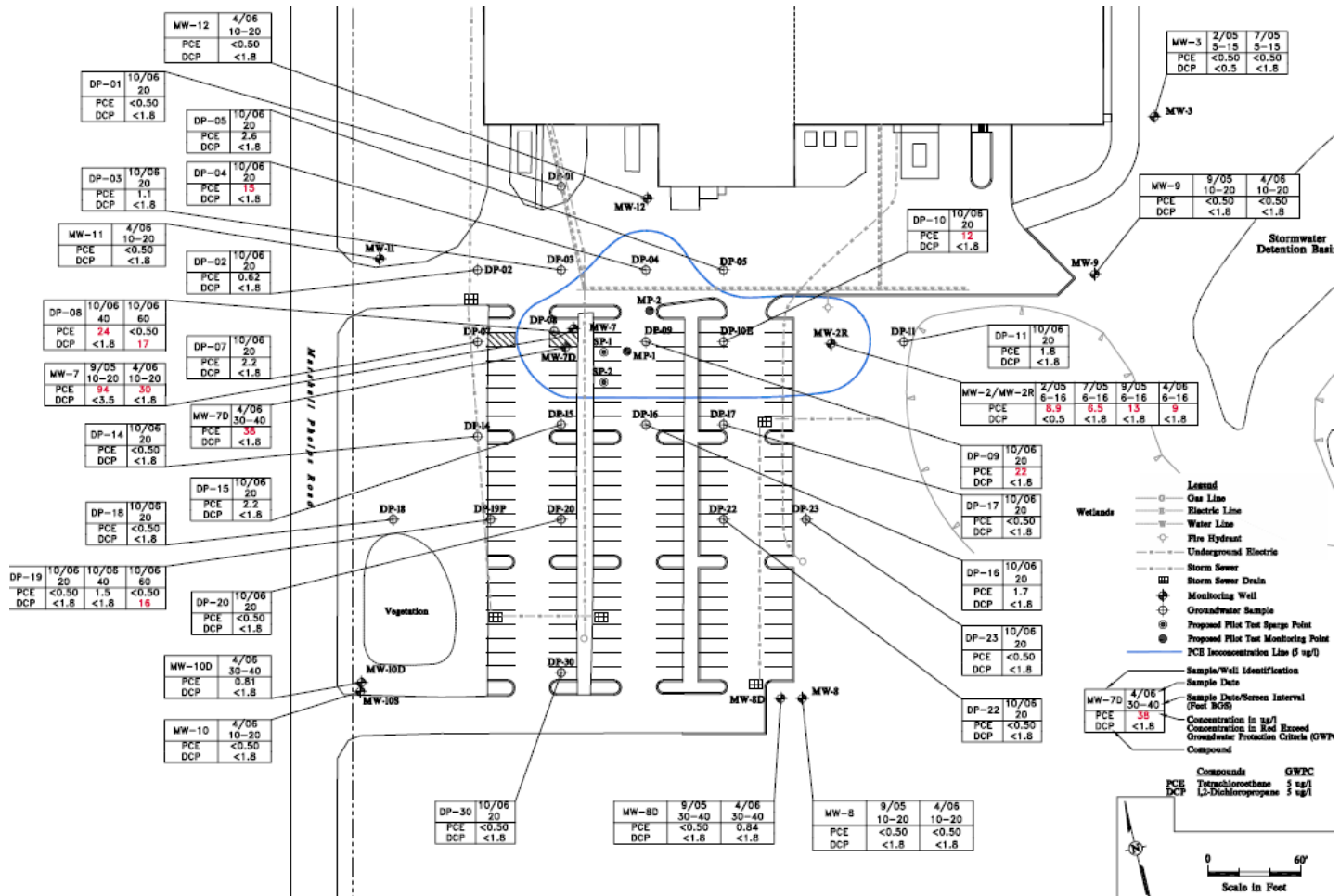




Bioremediation Example Connecticut Site

- Low concentrations of PCE (less than 50 $\mu\text{g/l}$) across a 1,000 SF area (20-foot saturated thickness)
- Aerobic aquifer (DO = 5 mg/l)
- No reductive daughter products present
- MCL cleanup goal
- Air sparge pilot test failed
 - Presence of thin clay/silt lenses within sandy aquifer

Bioremediation Example Connecticut Site





Bioremediation Example Connecticut Site

Challenge

- Assess feasibility of reductive dechlorination in aerobic aquifer
- Microbial Insights Bio-trap[®] Assembly deployed
 - MNA (non-amended Bio-trap[®]), biostimulant (amended Bio-trap[®] with HRC) and bioaugment (amended Bio-trap[®] with HRC and augmented with KB1)
 - 7-week incubation period

Bioremediation Example Connecticut Site

Well Name Treatment	MP-2 Control	MW-7D Bio-Stim	MW-7 Bio-Aug
Microbial Populations (cells/bd)			
Total Eubacteria	2.93E+08	3.40E+08	3.51E+07
Dehalococcoides spp.	31.1j	46.9j	5.21E+03
tceA Reductase	ND	ND	4.93E+02
bvcA Reductase	ND	8.10j	ND
vcrA Reductase	ND	ND	1.18E+03
Contaminant of Concern (µg/L)			
PCE	ND	ND	10.6
TCE	ND	ND	ND
cis-1,2 DCE	ND	ND	ND
trans-1,2 DCE	ND	ND	ND
Vinyl Chloride	ND	ND	ND
Dissolved Gases (µg/L)			
Ethene	ND	ND	ND
Ethane	ND	ND	ND
Methane	ND	2	4
Anions (mg/L)			
Bromide	0	0	0
Chloride	1.15	1.49	430.3
Fluoride	0	0.3	0
Nitrate	0.31	0	14.67
Sulfate	0	33.82	0
Phosphate	0.44	0	27.27
Volatile Fatty Acids (mg/L)			
Acetic	0	7.5	0
Propionic	0	7.3	0
Formic	0	0	0
Lactic	0	3.7	0
Pyruvic	0	0	0
Butyric	0	0	0
Other Parameters			
pH	6.55	6.6	6.6
Total Organic Carbon (mg/L)	ND	12600	3900
PLFA			
Cells/bead	1.06E+05	1.82E+05	1.12E+06
Firmicutes (TerBrSats)	0.47	5.13	9.04
Proteobacteria (Monos)	77.85	71.80	60.71
Anaerobic metal reducers (BrMonos)	0.00	0.79	2.35
Actinomycetes (MidBrSats)	0.00	0.00	0.38
General (Nsats)	20.55	21.82	26.18
Eukaryotes (polyenoics)	1.14	0.47	1.35
Slowed Growth	0.53	0.18	0.61
Decreased Permeability	0.19	0.27	0.11

PCR data showed that DHC survived the 7 week deployment period in high DO aquifer

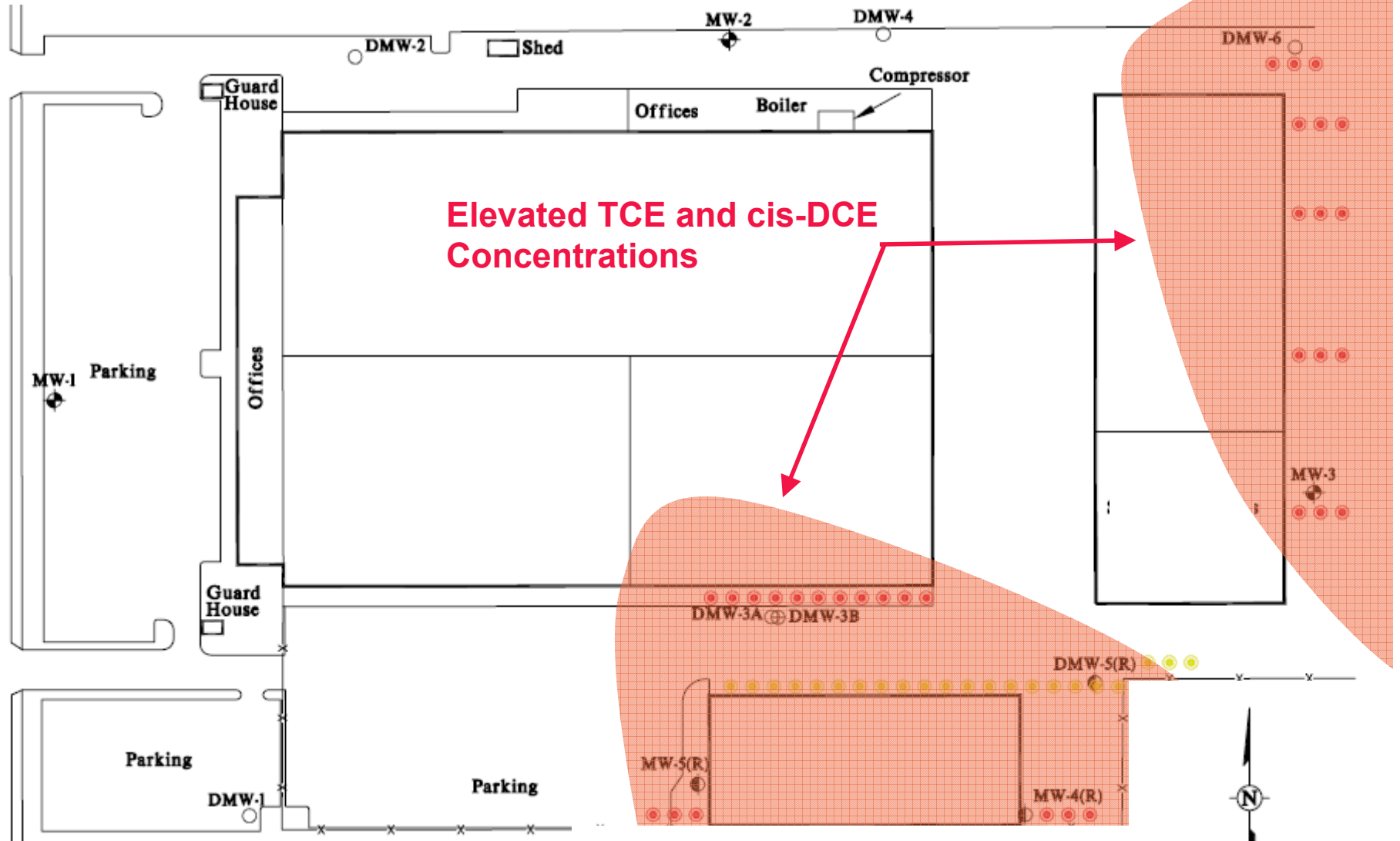
PLFA data show that the microbial population shifted in response to the biostimulant to an ecology consistent with reductive dechlorination

Note: the increase in percent composition of firmicutes within the microbial population

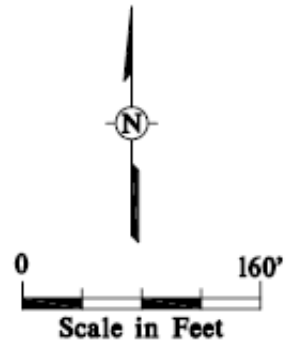
Conclusion:

Increased confidence in recommending pilot testing sequential biostimulation and bioaugmentation (if necessary)

Bioremediation Example California Site



- ⊙ 3DMe Application Point 20-foot spacing
- ⊙ 3DMe Application Point 15-foot spacing





Bioremediation Example California Site

Post 3DMe application

- Higher energy yielding electron acceptors depleted in concentration, the redox potential decreased, and conditions favorable for microbially mediated dechlorination were established
- DCE Stall

Challenge: assess the likelihood of successful bioaugmentation

Microbial Insights Bio-trap[®] Assembly deployed

- MNA (non-amended Bio-trap[®]) and bioaugment (amended Bio-trap[®] augmented with SDC-9) were deployed in separate wells within the 3DMe-applied area
- 62-day incubation period

Bioremediation Example California Site

Sample Name Treatment	DMW-3A Control	MW-4R BioAug		
Microbial Populations (cells/bd)				
Total Eubacteria	3.78E+07	3.97E+07		
Dehalococcoides spp.	5.07E+01	1.25E+07		
tceA Reductase	ND	5.68E+05		
bvcA Reductase	9.00E+00 (J)	7.44E+04		
vcrA Reductase	3.86E+01	1.05E+07		
Dehalobacter spp.	9.60E+03	2.43E+05		
Desulfuromonas spp.	2.86E+02	1.85E+03		
Methanogen	1.00E+03	2.38E+03		
Contaminant of Concern (µg/L)				
PCE	6.0	--	ND	--
TCE	9.0	--	ND	-22.16
cis-1,2 DCE	89	-22.08	530	-6.61
trans-1,2 DCE	ND	-22.60	87	-34.96
Vinyl Chloride	ND	--	400	-6.08
PLFA				
Total Biomass (cells/bd)	8.26E+03	7.27E+05		
Firmicutes (TerBrSats)	0.00	13.34		
Proteobacteria (Monos)	62.39	62.02		
Anaerobic metal reducers (BrMonos)	6.92	0.99		
SRB/Actinomycetes (MidBrSats)	1.23	0.85		
General (Nsats)	29.46	21.95		
Eukaryotes (polyenoics)	0.00	0.83		
Slowed Growth	2.07	0.08		
Decreased Permeability	0.00	0.28		

PCR data showed that very little DHC in the MNA biotrap and low concentrations of other dechlorinating microbes

Augmented biotrap contained high concentrations of DHC, other dechlorinating microbes, and genes that code for key reductase enzymes

CSIA data showed ¹³C in cis-DCE and vinyl chloride to be much more enriched (less negative δ¹³C values) than the ¹³C in TCE proving that the degradation pathway is complete

PLFA data show that the augmented biotrap microbial population is diverse and includes a high fraction hydrogen producing firmicutes which DHC uses as an energy source

Conclusion: Bioaugmentation is viable

In Situ Chemical Oxidation Example New Jersey Site

- CSIA used in conjunction with ISCO
 - Confirm contaminant destruction
 - Track “rebound” attributable to desorption or delivery inefficiencies

	MW-23S			MW-A		
	Pre-ISCO		Post-ISCO	Pre-ISCO		Post-ISCO
	<u>11/27/2007</u>	<u>12/9/2008</u>	<u>3/5/2009</u>	<u>11/29/2007</u>	<u>7/21/2008</u>	<u>3/5/2009</u>
TCE: CSIA, $\delta^{13}\text{C}$ (‰)	-34.44	-25.66	-30.10	-29.55	-3.74	-24.04
TCE Concentration ($\mu\text{g/l}$)	300	500	600 J	3,600	80	1000 J

- Data Interpretation
 - MW-23 fractionation data show that despite increasing concentration trends contaminant was oxidized in this area
 - MW-A shows significant initial fractionation with later addition of additional contaminant consistent with incomplete delivery (mass from untreated area entered treatment zone) and desorption



Conclusions

- Advanced diagnostic tools can provide
 - Definitive data
 - Current data, not a footprint of the past
 - Near real-time actionable performance monitoring data
 - Environmental forensics and potential liability relief
- Affordability of advanced diagnostic tools
 - No need for endless layers of data
 - Quick and easy to collect samples
 - Saves the client money

Questions?

