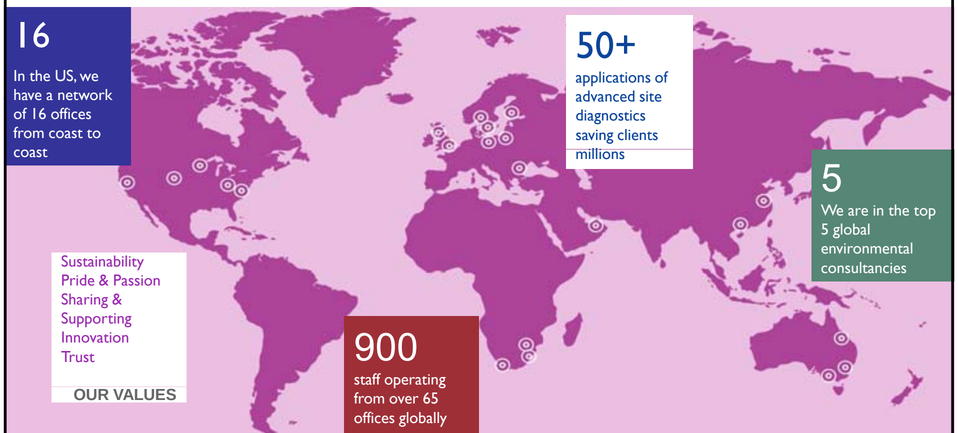
Combining Advanced Site Diagnostics with In Situ Microcosm Studies to Optimize Bioremediation Remedy Selection



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Introduction

- Most applications of *in situ* remediation are inherently green

Proposed Program Policy DER-31, March 17, 2010,
New York State Department of Environmental Conservation

> *"Encourage development and evaluation of low energy alternatives such as enhanced bioremediation, phytoremediation, permeable reactive barriers (PRBs), source removal with monitored natural attenuation (MNA), enhanced attenuation of chlorinated organics (EACO), engineered wetlands, and remedies which can be driven to MNA or monitoring only (e.g., remedies which will not need external power indefinitely)"*

Green Remediation: Incorporating Sustainable Environmental Practices into Remediation of Contaminated Sites
U.S. Environmental Protection Agency
Office of Solid Waste and Emergency Response
April 2008, EPA 542-R-08-002

> *"Passive energy remediation systems use little or no external energy to power mechanical equipment or otherwise treat contaminated environmental media. These systems commonly involve technologies such as bioremediation, phytoremediation, soil amendments, evapotranspiration covers, engineered wetlands, and biological permeable reactive barriers. Cleanup strategies can combine elements of these technologies to achieve novel hybrid systems, paving the way for yet more innovative applications."*

Introduction

- *In situ* technologies have a perception problem
- Mechanisms of destruction are unseen
  - Mechanisms are not well understood
  - Physical effects of amendment application (e.g., dilution and displacement) effect concentration data in an identical manner as the desired reactive mechanism

Introduction Continued

- Uncertainties have lead to the weight of evidence approach when proving viability:
  - Multiple rounds of data collection
  - Bench studies
  - Pilot studies

Kinetics?
Conflicting data?
Scale up?

- Skipping these steps has resulted in many failed *in situ* applications
  - even if the steps are completed the success rate of in situ technologies was not that high

Introduction Continued

- Traditional assessment data is often tangential to the desired information:
  - Is contaminant being destroyed or just being pushed around and diluted?
  - What is the mechanism of the destruction and can it be monitored directly?

Introduction Continued

- Advanced site diagnostics (ASD) change the approach to assessing viability of in situ technologies
  - Compound specific isotope analysis (CSIA) provides definitive data on contaminant destruction that is not concentration related
  - Molecular biological tools (MBT) can identify microbial-mediated mechanisms of destruction.

Definitive
Cost effective
Easy to implement

Introduction continued

- Use of the advanced diagnostic tools allows remediation professionals to make better decisions to expedite site closure and minimize costs
- Through a series of case studies ASDs will be introduced and their benefits highlighted

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

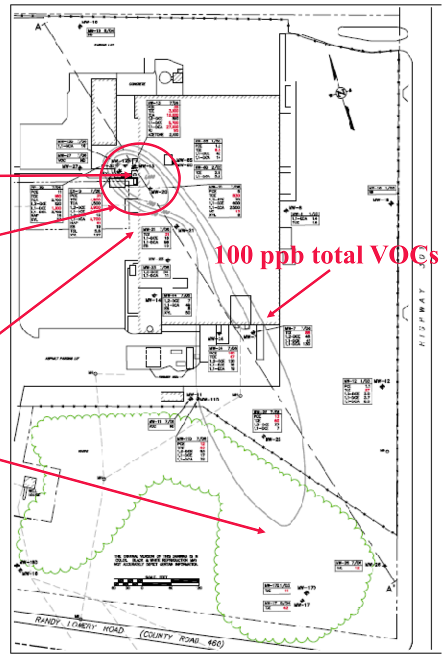
MNA Example
Georgia Site

Multiple Corrective Actions:

Source area soils were excavated

Source area groundwater was treated with Perozone™

MNA proposed for downgradient area of plume

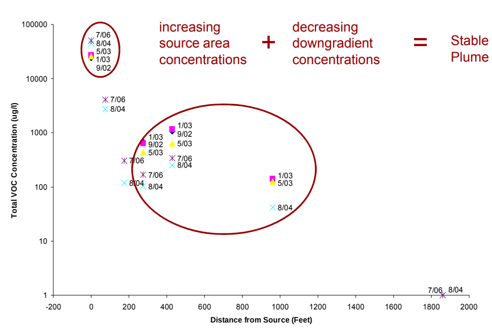


MNA Example
Georgia Site

Traditional MNA Assessment:

- Plume Stability
  - Increasing dissolved VOC concentrations in source area
  - Decreasing VOC concentrations downgradient indicating attenuation

Total VOC Trend with Time and Distance



MNA Example
Georgia Site

Traditional MNA Assessment Continued:

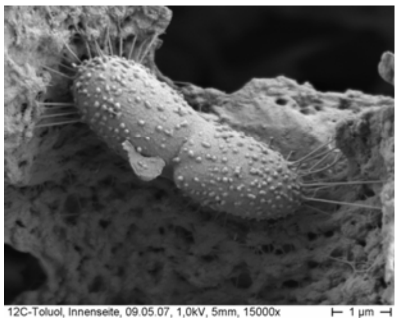
- Daughter Products
  - Reductive daughter products, including end products present
- Electron Donors
  - Elevated concentrations of naturally occurring and some anthropogenic electron donor compounds present
- Competing Electron Acceptors
  - Slightly elevated dissolved oxygen (2 to 3 mg/l)
  - Redox couple data consistent with aerobic conditions
- Conclusion
  - Aerobic environment not consistent with traditional MNA assessment criteria

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

Microbial Insights Bio-trap® Samplers

- Bio-Trap® samplers are passive sampling tools that collect actively colonizing microbes



- Composed of 2-4 mm diameter Bio-Sep® beads (25% Nomex® and 75% powdered activated carbon).
  - Large surface area (~600 m2/g)
  - 74% porosity
  - Readily colonized by subsurface microorganisms.

MNA Example
Georgia Site

- Phospholipid Fatty Acids (PLFA)
  - PLFAs are components of cell walls and can be used to identify groups of microbes
  - Georgia Site: PLFA data show the presence of a microbial population that is consistent with MNA
- Quantitative Polymerase Chain Reaction (qPCR)
  - qPCR identifies microbes based on nucleotide sequences (i.e., DNA & RNA)
  - Georgia Site: qPCR data identified the presence of a significant population of *Dehalococcoides spp* (DHC)

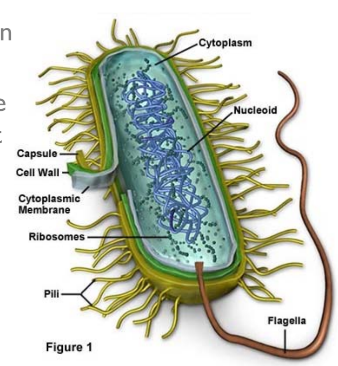


Figure 1

MNA Example
Georgia Site

- Conclusion with MBTs
  - MBTs conclusively demonstrated the mechanism (i.e., the DHC) of observed plume stability and showed that the mechanism was active
  - DHC is an obligate anaerobe therefore, at a minimum, anaerobic micro-environments that support microbial attenuation of the chlorinated volatile organic compounds (VOCs) must be present
  - State approved MNA
  - Cost savings
  - MNA CO_2 emissions over the lifespan of the project are calculated to be 16-percent of the emissions calculated for pump and treat.

(calculated using AFCEE SRT Rev.2 – tier 1 calculations)

MNA Example - Tennessee Site

- Elevated concentrations of BTEX and TPH present in groundwater over a 6.5 acre area
- No NAPL
- Decreasing dissolved BTEX concentration trends observed

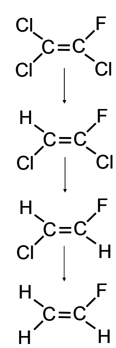
MNA Example - Tennessee Site

Evaluation Process Included:

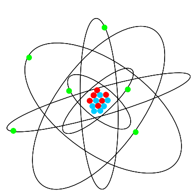
- Traditional assessment (electron acceptors, concentration data etc.)
- Deployed amended (^{13}C labeled benzene) Microbial Insights Bio-Traps® for 55 days

Bio-trap® Samplers continued

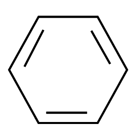
- Bio-Trap® Samplers can be amended to answer site specific questions
  - Electron acceptors (ORC, PermeOx Plus, sulfate, etc.)
  - Electron donors (HRC®, EOS®, lactate, etc.)
  - Microbes (SDC-9, KB-1, PM-1, etc.)
  - Contaminant analogs (trichlorofluroethene)
  - ^{13}C labeled contaminants



The TCFE degradation Pathway parallels that of PCE/TCE



^{13}C Stable Isotope Natural Abundance ~ 1%



^{13}C enriched (~ 10 – 20 %) manufactured contaminant

^{13}C enriched contaminant loaded on a bio-trap

Stable Isotope Probing

CSIA used to track ^{13}C enrichment in biomass and dissolved inorganic carbon

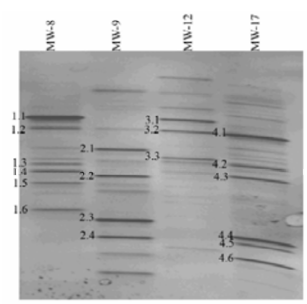
MNA Example
Tennessee Site

Evaluation Process

- 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 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

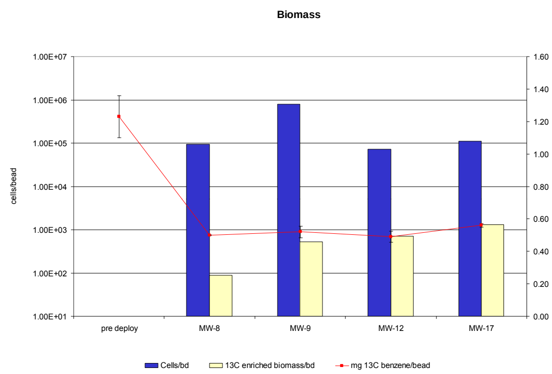
- Results
  - The PFLA test showed an adaptable population of organisms capable of utilizing the chemicals of concern at this site
  - The DGGE test provided more specificity about organisms that are known to degrade benzene and related compounds



Genera of Bacteria Identified Include:
- *Azozrcus spp.*
- *Pseudomonas spp.*
- *Geobacter spp.*

MNA Example
Tennessee Site

- Results Continued
  - 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



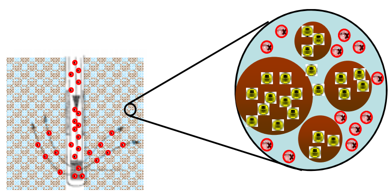
MNA Example
Tennessee Site

- 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 CO_2 emissions over the lifespan of the project are calculated to be 17-percent of the emissions calculated for pump and treat MNA CO_2

    (calculated using AFCEE SRT Rev.2 – tier 1 calculations)

MNA Example
New Jersey Site

- Use CSIA to track cleanup at a chlorinated solvent release site
  - The majority of all carbon is present as the ^{12}C, but a small percentage of carbon is naturally present as the stable (i.e., not radioactive) ^{13}C isotope.
  - Chemical bonds involving the ^{13}C isotope are slightly stronger than those of ^{12}C and as a result react slower in bond-breaking reactions.
  - The slower reaction rate leads to an accumulation of the ^{13}C isotope in the residual contaminant (e.g., TCE). The accumulation of the ^{13}C is referred to as fractionation.



Oxidant Applied
Sodium Permanganate

MNA Example
New Jersey Site

Initial Post-Treatment Results

	MW-1		MW-2	
	Pre-ISCO	Post-ISCO	Pre-ISCO	Post-ISCO
TCE: CSIA, $\delta^{13}C$ (‰)	-29.6	-3.7	-34.4	-25.7
TCE Concentration (μg/l)	3,000	80	400	500

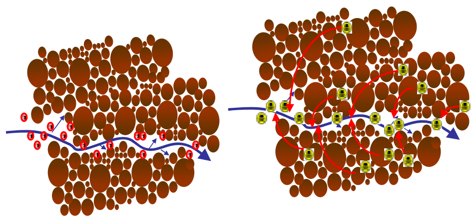
- MW-1: expected concentration decreases and ^{13}C fractionation
- MW-2: concentration increase with fractionation – TCE was oxidized

MNA Example
New Jersey Site

Rebound

	MW-1		
	Pre-ISCO	Post-ISCO T-1	Post-ISCO T-2
PCE: CSIA, $\delta^{13}C$ (‰)	-27.3	-16.8	-33.1
PCE Concentration (µg/l)	6,000	80	600

- Rebound beyond initial isotopic signature observed
  - Cause: non-degraded PCE diffusing into preferential flow paths after depletion of oxidant
  - Note that the Pre-ISCO isotopic signature is fractionated likely due to biodegradation.



Preferential flow paths affect oxidant distribution and a large rebound is observed

MNA Example
New Jersey Site

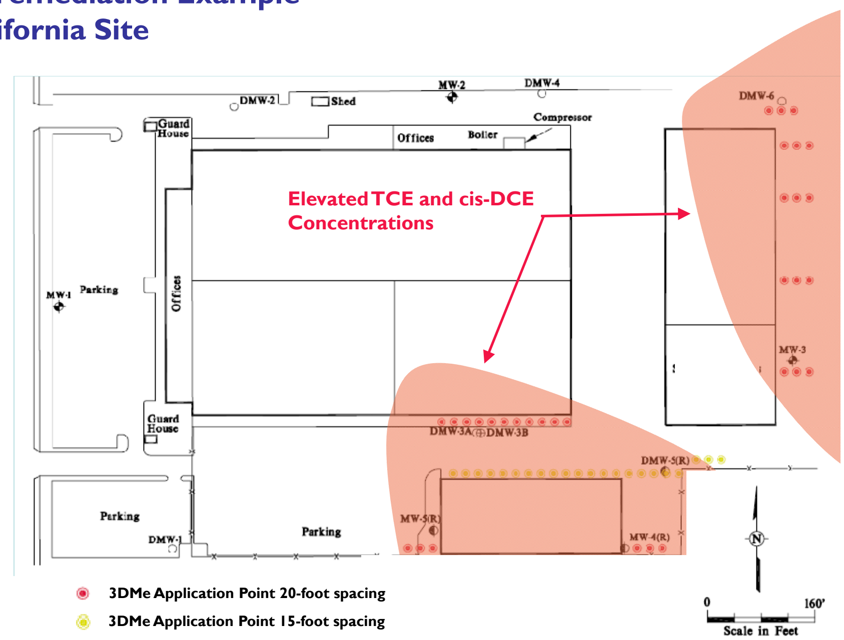
Pneumatic fracturing completed to aid delivery

	MW-1			
	Pre-ISCO	Post-ISCO T-1	Post-ISCO T-2	Post-Fracturing
PCE: CSIA, $\delta^{13}C$ (‰)	-27.3	-16.8	-33.1	-23.6
PCE Concentration (µg/l)	6,000	80	600	3,000

	MW-3			
	Pre-ISCO	Post-ISCO T-1	Post-ISCO T-2	Post-Fracturing
PCE: CSIA, $\delta^{13}C$ (‰)	-28.5	-27.8	-28.7	-25.8
PCE Concentration (µg/l)	30,000	20,000	9,000	60,000

- Fractionation of a larger concentration observed
- Improved remedy performance

Bioremediation Example
California Site

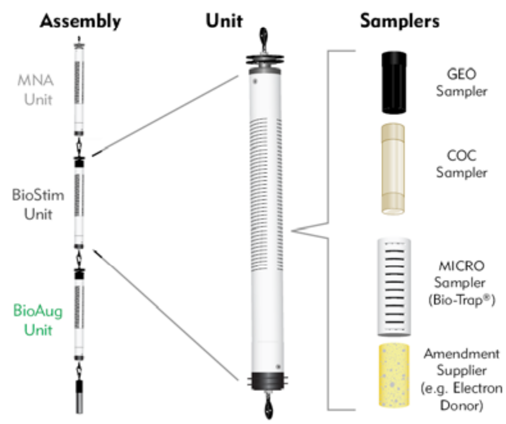


Bioremediation Example
California Site

- 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 as an assembly
  - 62-day incubation period

Bioremediation Example
California Site

Bio-Trap Assembly



Bioremediation Example
California Site

	Control	Bioaugment
Microbial Population (cells/bead) by qPCR		
Total Eubacteria	3.87E+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
Isotopic Signature (δ13C (‰)) by CSIA		
Trichloroethene	ND	-22.16
cis-1,2-Dichloroethene	-22.08	-6.16
trans-1,2-Dichloroethene	-22.06	-34.96
Vinyl Chloride	ND	-6.08

ND = analytical target not detected
J = result between the method detection limit and the reporting limit

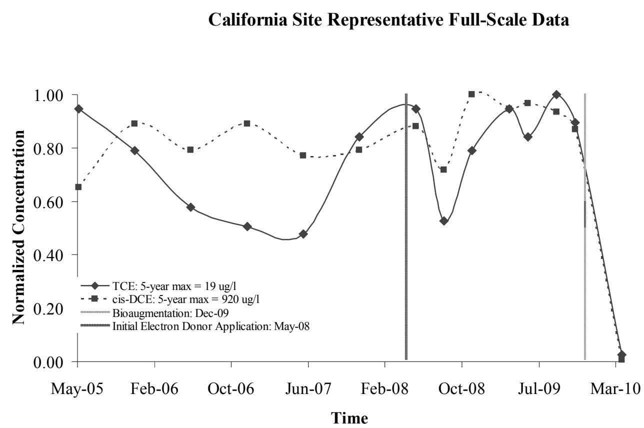
PCR data showed that very little DHC in the MNA biotrap and low concentrations of other dechlorinating microbes

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

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

- In situ microcosm data demonstrates that bioaugmentation would likely be successful

Bioremediation Example
California Site



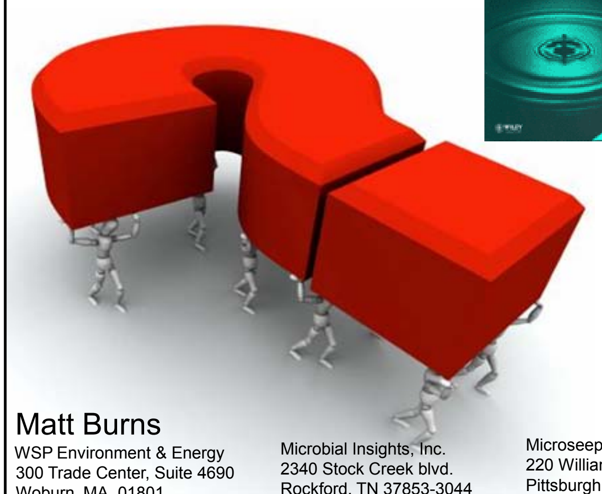
Implement Full-Scale Bioaugmentation

Concentrations approach maximum contaminant levels (MCLs) within three months

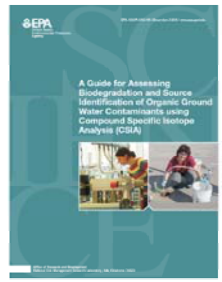
Conclusions

- Advanced diagnostic tools can:
  - Identify contaminant destruction
  - Identify the microbial-mediated mechanism of the destruction
  - Provide definitive data
  - When used in combination with Bio-Traps can provide data on viability of a contemplated remedy (i.e., microcosm study)
  - Provide near real-time actionable performance monitoring data
  - Improves/supports inherently green *in situ* remediation
- Affordability of advanced diagnostic tools
  - No need for endless layers of data
  - Quick and easy to collect samples
  - Costs are comparable to some traditional analytical methods

Questions?



Burns, M. and Myers, C. (2010), Evolution of predictive tools for in situ bioremediation and natural attenuation evaluations. Remediation Journal, 20: 5–16. doi: 10.1002/rem.20259



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