

Applications of Bio-traps

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Advanced Tools for *In Situ* Remediation Workshop, Albany, NY

What is a Bio-trap?

- Passive sampling tool for investigating the degradative capabilities microorganisms
- Collects only **environmentally competent, active microbes**
- **Integrative** sample (vs. snapshot)
- Analyzed using molecular biological tools (MBTs) and stable isotope analysis



What can I do with a bio-trap?

Site characterization and performance verification

- Determine if known degraders of a COC are present and active
 - Standard bio-traps + qPCR (16SrDNA, functional genes)
- Predict the response of a microbial community to a potential remediation amendment
 - Baited bio-traps + qPCR
- Evaluate actual *in situ* response to remediation amendments
 - Standard bio-traps + qPCR
- Provide irrefutable proof of the biodegradation potential of target compounds
 - Baited bio-traps + stable isotope analysis of phospholipid fatty acid analysis (PLFA) or DNA

How do bio-traps work?

Bio-Sep Beads

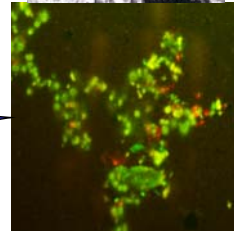
- 3-4 mm in diameter
- 25 % Nomex, 75% PAC
- 74% porosity
- 600 m² of surface area/g
- Surrounded by ultrafiltration-like membrane with 1-10 micron holes
- Autoclavable
- Cleaned of fossil biomarkers by heating to 300 °C

Biofilms Form Rapidly in Bio-Sep® Beads

- High surface area
- Low shear
- Concentration of nutrients by PAC
- Rapid formation of pre-conditioning films



*Live-dead stain of
biofilm in Bio-Sep
bead*

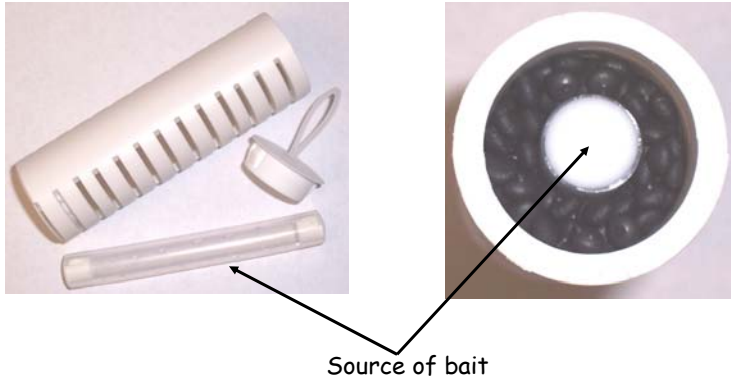


Bio-trap Configurations

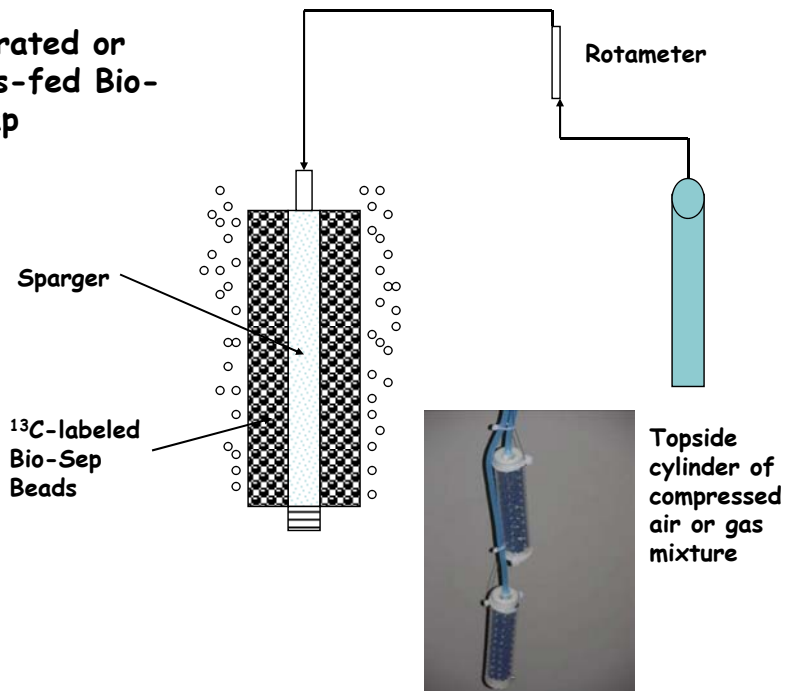
- Standard monitoring well design
- "Baited" designs
- Specialized designs
 - Flow-through
 - Micro traps
 - Gas-fed



Tube-in-tube Baited Bio-trap

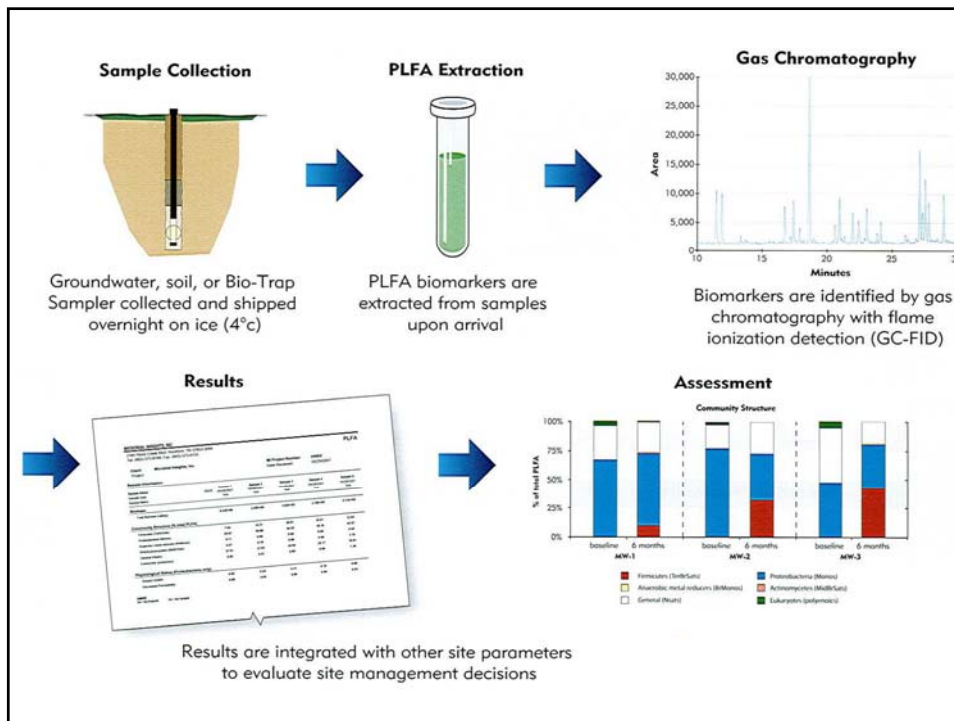
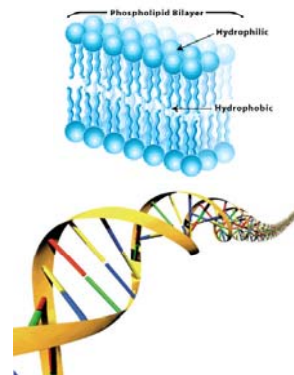


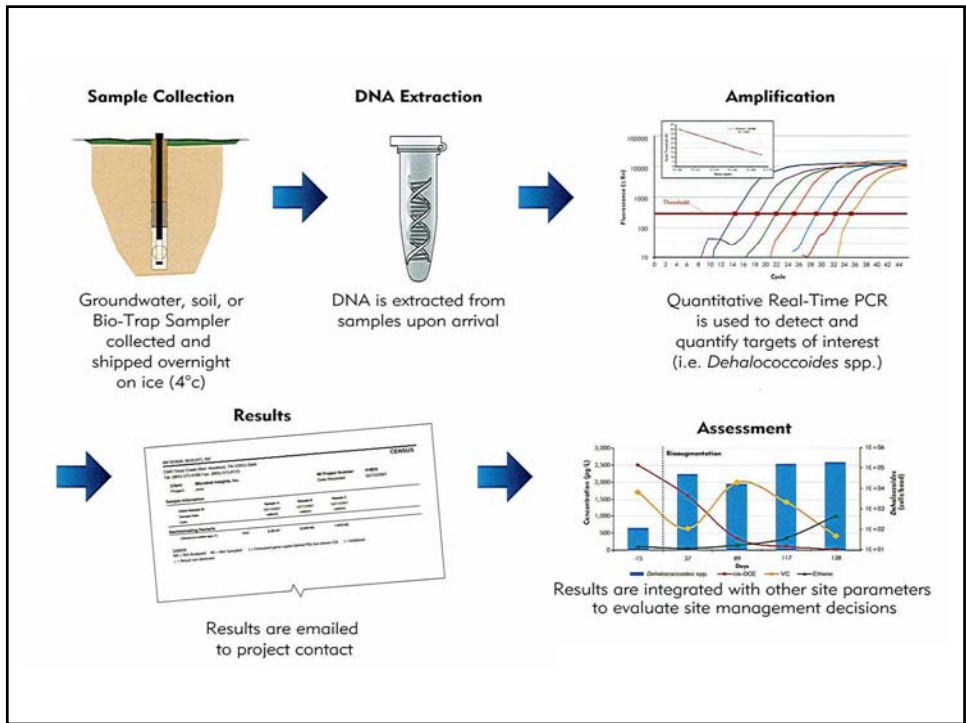
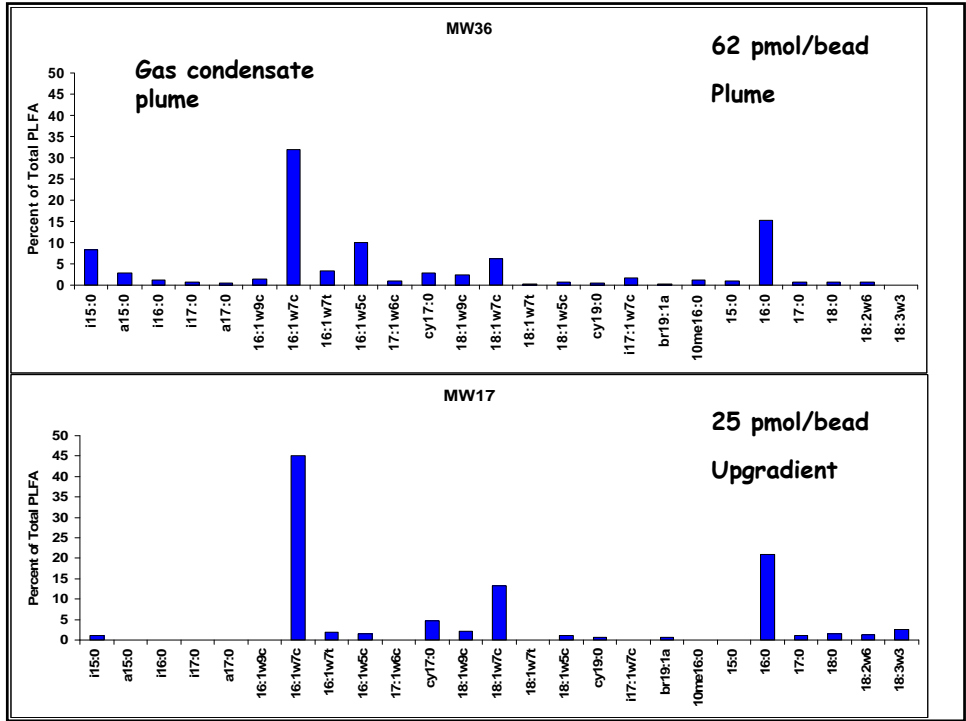
Aerated or Gas-fed Bio-trap



Analysis of Bio-traps: Molecular Biological Tools

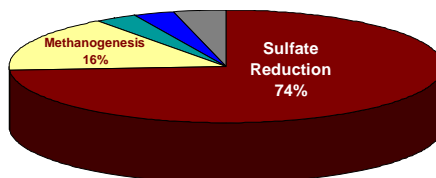
- Phospholipid fatty acid analysis (PLFA)
- DNA methods
 - Denaturing gradient gel electrophoresis (DGGE)
 - Quantitative PCR (qPCR)
- Stable isotope probing



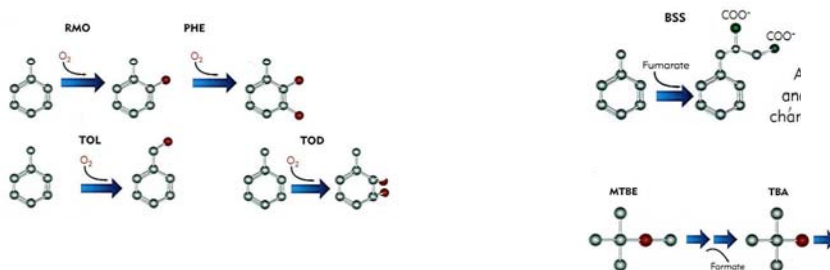


Taxonomic Groups

- Denitrifying Bacteria (DNF)
- SRB/IRBs (SRB/IRB)
- Methanogens (MGN)

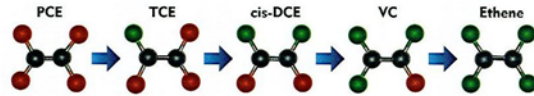


Target	Code	Contaminants	Environmental Relevance / Data Interpretation
Ring-hydroxylating Toluene Monooxygenases	qRMO	BTEX	Catalyzes the initial (and sometimes second) hydroxylation of BTEX compounds Presence indicates the potential for aerobic BTEX biodegradation
Phenol Hydroxylase	qPHE	BTEX	Catalyzes further oxidation of BTEX compounds Presence indicates the potential for aerobic BTEX biodegradation
Toluene Monooxygenase	qTOL	Toluene, Xylene	Attacks toluene and xylenes at the methyl group
Toluene Dioxigenase	qTOD	Benzene, Toluene Ethylbenzene	Catalyzes biodegradation of benzene and toluene by incorporation of oxygen into the aromatic ring
Naphthalene Dioxigenase	qNAH	Naphthalene	Catalyzes aerobic biodegradation of naphthalene and other PAHs by incorporation of oxygen into the aromatic ring
MTBE utilizing PM1	qPM1	MTBE	Targets <i>Methylbium petroleiphilum</i> PM1, one of the few bacteria isolated that is capable of growth on MTBE Indicative of the potential for aerobic MTBE biodegradation
Benzylsuccinate Synthase	qBSS	Toluene, Xylene	Targets gene encoding enzyme in anaerobic biodegradation of toluene Indicative of the potential for anaerobic BTEX biodegradation

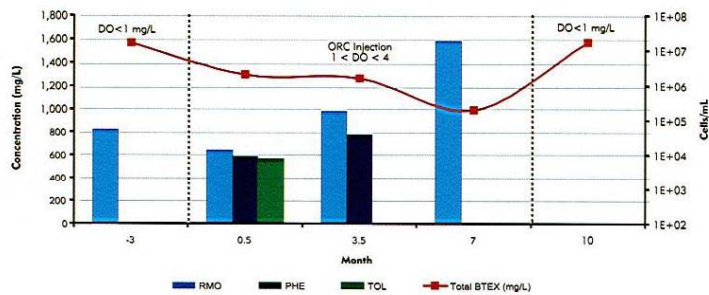


qPCR for Reductive Dechlorination

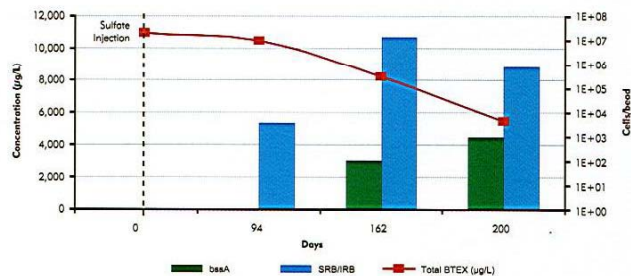
Target	Code	Contaminants	Environmental Relevance / Data Interpretation
Dehalococoides	qDHC	PCE, TCE, DCE, VC	Only known group of bacteria capable of complete dechlorination of PCE and/or TCE to ethene Absence of <i>Dehalococoides</i> suggests dechlorination of DCE and VC is improbable and accumulation of daughter products is likely The presence of <i>Dehalococoides</i> even in low copy numbers indicates the potential for complete reductive dechlorination Higher copy numbers and the presence of daughter products suggest that dechlorination may be occurring
Dehalococoides Functional Genes	qTCE qVC	TCE, VC	Functional genes encoding reductive dehalogenases for TCE and VC Presence of TCE reductase indicates the ability to reduce TCE to DCE and VC Presence of VC reductase indicates the potential for reductive dechlorination of VC to ethene Absence of VC reductase suggests that VC may accumulate
Dehalobacter	qDHB	PCA, TCA, PCE, TCE	Capable of dechlorination of PCE and TCA to cis-DCE Converts TCA, a common co-contaminant at PCE/TCA-impacted sites to chloroethane
<i>Desulfuromonas</i>	qDSM	PCE, TCE	Capable of dechlorination of PCE and TCE to cis-DCE using acetate as an electron donor
<i>Desulfitobacterium</i>	qDSB	PCE, TCE	Capable of dechlorination of PCE and TCE to cis-DCE
Total bacteria	qEBAC		Index of total bacterial biomass Domain level
Methanogens	qMGN		Methanogens utilize hydrogen and carbon dioxide to produce methane Compete with dechlorinating bacteria for available hydrogen
Iron and Sulfate Reducing Bacteria	qSRB/IRB		Targets delta-Proteobacteria Index of iron and sulfate reducing bacteria including <i>Geobacter</i> , <i>Pelobacter</i> , <i>Desulfovibrio</i> , and <i>Desulfuromonas</i>



Aerobic BTEX Bioremediation



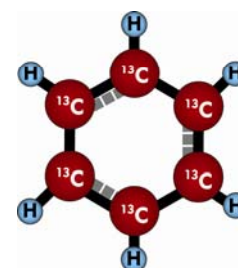
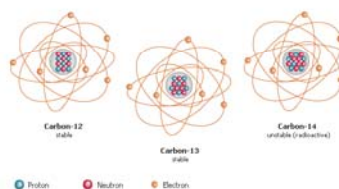
Anaerobic BTEX Bioremediation



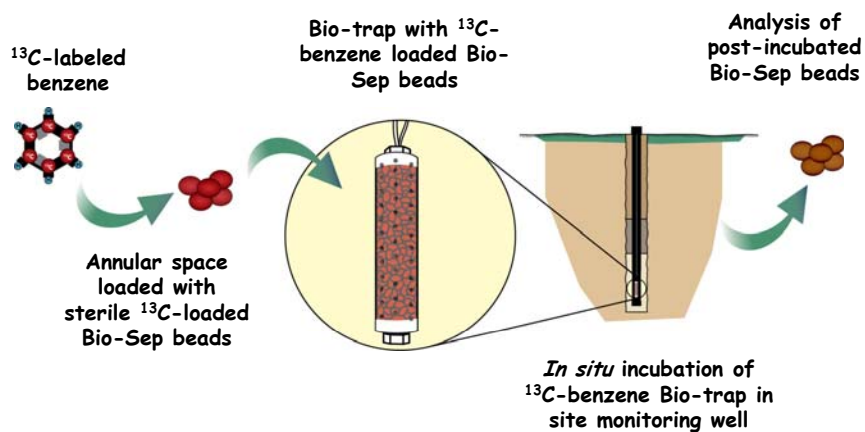
Stable Isotope Probing (SIP)

What is stable isotope probing?

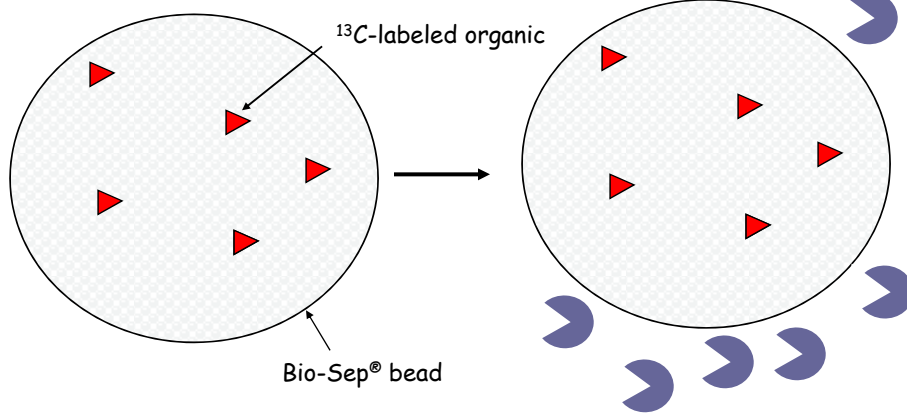
- Specially produced "heavy" compounds which are composed of 99% ^{13}C
 - Natural compounds are 99% ^{12}C
 - Same characteristic as original compound
 - Behave similar to the natural compound
- Used as tracers to increase our understanding of contaminant fate



Stable Isotope Probing with Bio-traps

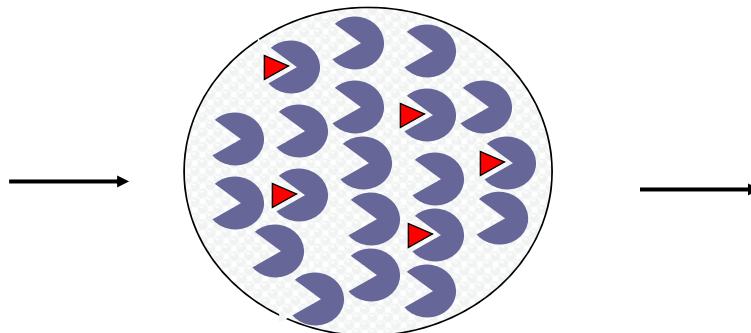


Stable isotope probing with Bio-Sep beads



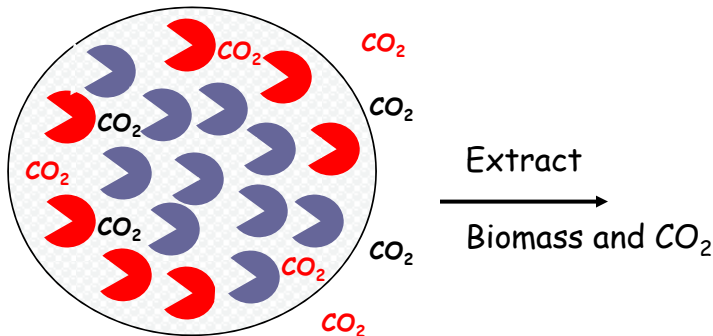
1. ^{13}C -labeled compounds adsorbed to PAC within the beads. Approximately 15% of the loaded organic contains ^{13}C stable isotope (red triangle = ^{13}C)
2. Substrate-baited Bio-Sep beads attract microorganisms; microenvironment provides optimal conditions for colonization

Microbes utilize the target compound



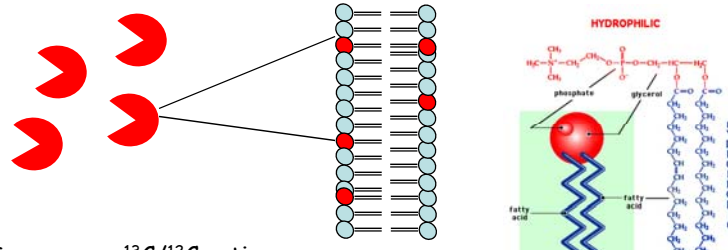
3. Indigenous microbes cultivated inside the Bio-Sep[®] bead utilize target compound.

¹³C Incorporation into biomass and CO₂

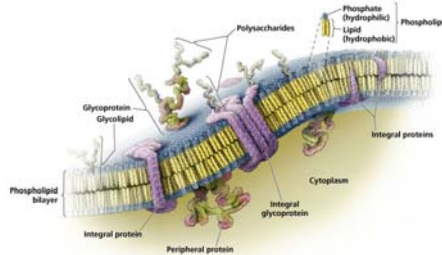


4. ¹³C is incorporated into new cells growing in the beads and in CO₂, the desired end product of mineralization of the ¹³C-organic.
5. Extract biomass and CO₂ from beads and look for ¹³C in cell biopolymers (e.g., membrane lipids) and the CO₂.

Biomarkers of choice: Phospholipids (PLFA)

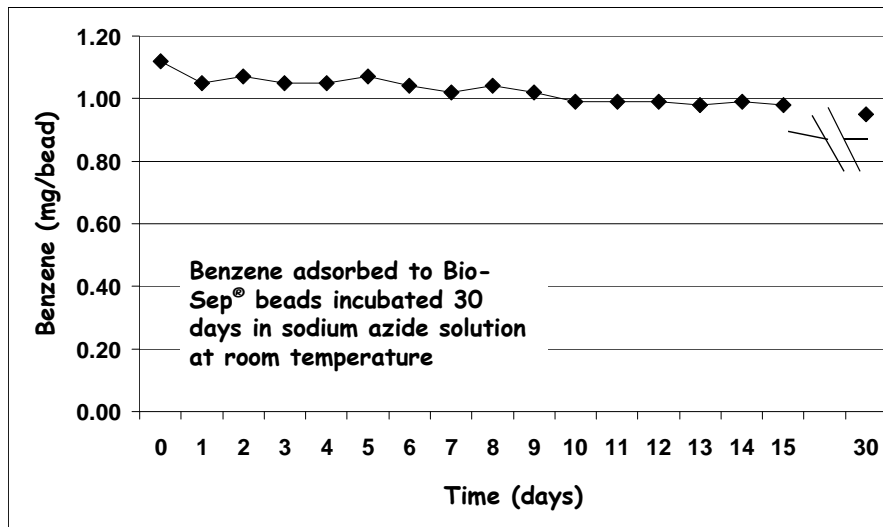


6. GC-IRMS measures ¹³C/¹²C ratios in PLFA fatty acids and CO₂
 - High concentrations of phospholipids in microbial cells
 - Indicative of viable biomass
 - Fatty acid structures give clues to microbial ecology

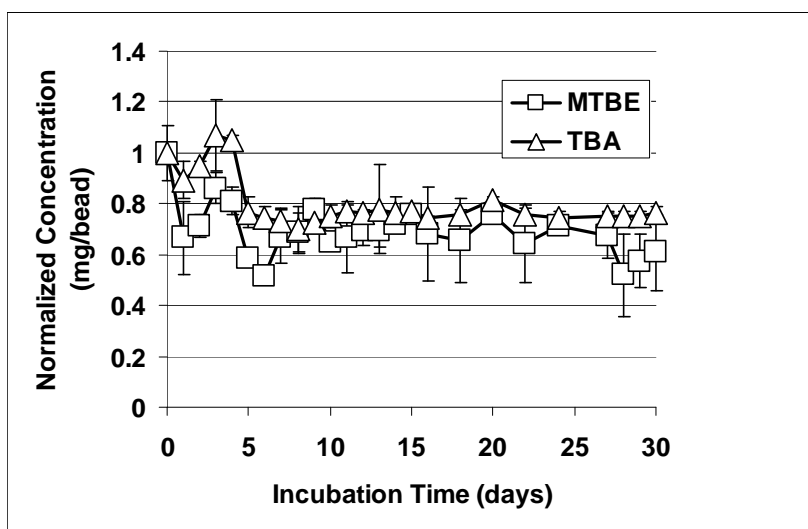


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Adsorbed benzene is retained in beads during extended aqueous incubation under abiotic conditions (25 mM Na azide)



Adsorbed MTBE and TBA are retained in beads during extended aqueous incubation under abiotic conditions (25 mM Na azide)

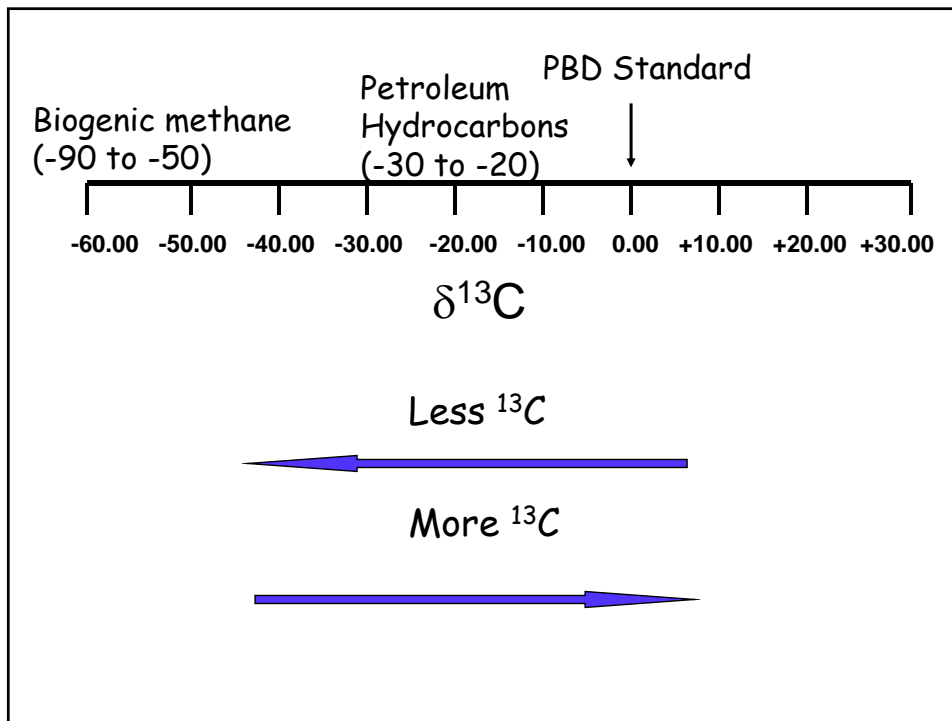


The $^{13}\text{C}/^{12}\text{C}$ Isotope Ratio

- The amount of ^{13}C relative to ^{12}C in a sample is expressed by the $\delta^{13}\text{C}$ notation where

$$\delta^{13}\text{C} [\text{‰}] = \left(\frac{(^{13}\text{C}/^{12}\text{C})_{\text{Sample}}}{(^{13}\text{C}/^{12}\text{C})_{\text{Standard}}} - 1 \right) \cdot 1000$$

- The standard is a specific carbon-containing mineral from a specific location: Pee Dee Belimnite (PDB)
- Units of $\delta^{13}\text{C}$ are ‰ or "per mill"

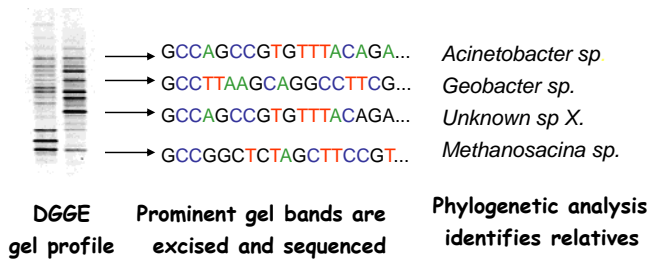


Bio-trap Case Studies

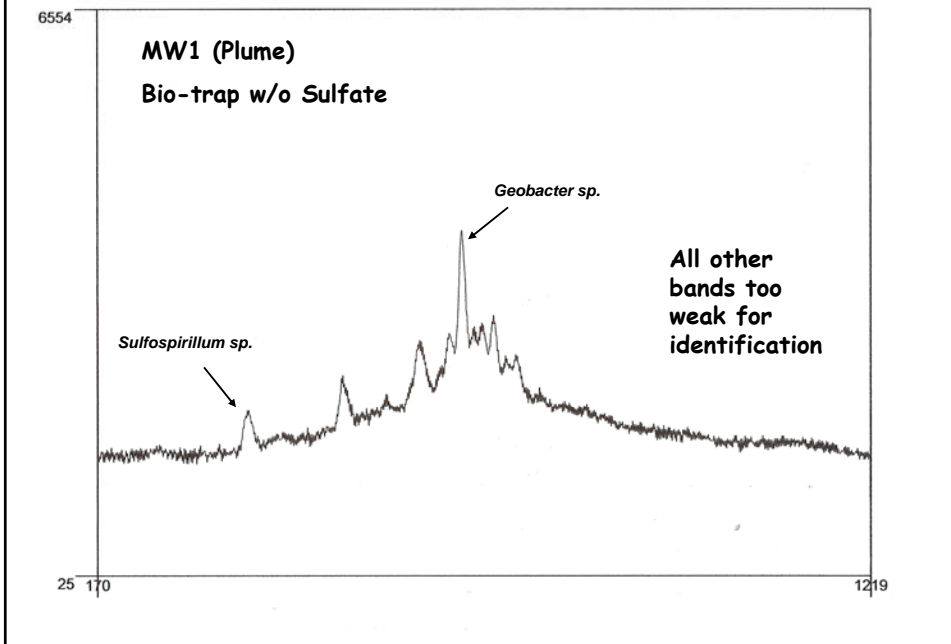
Hydrocarbons and Oxygenates

Standard Bio-traps and DGGE Predict the Effect of Sulfate Injection in a BTEX-impacted Aquifer

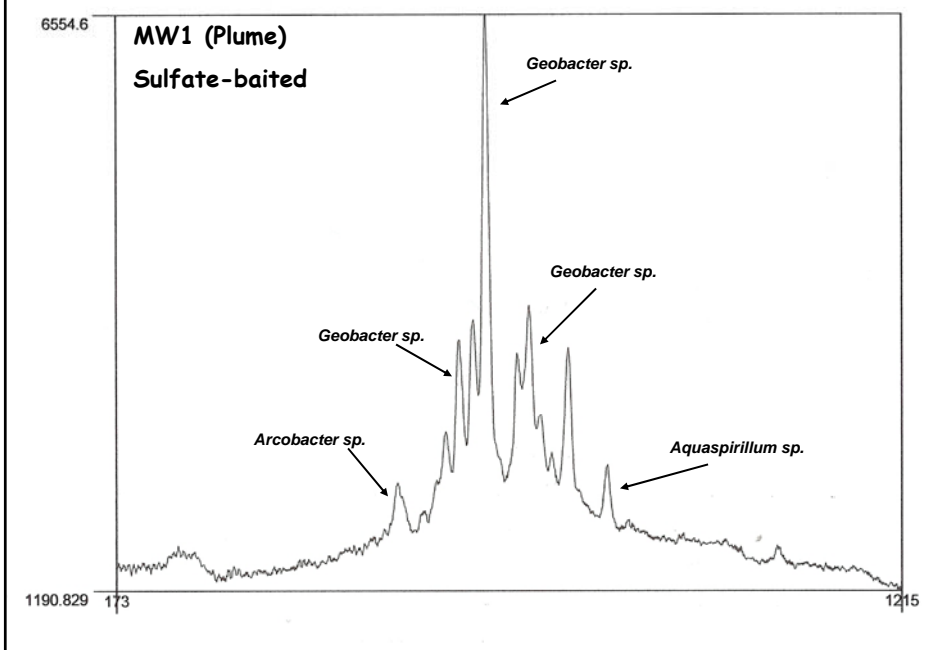
Results obtained:



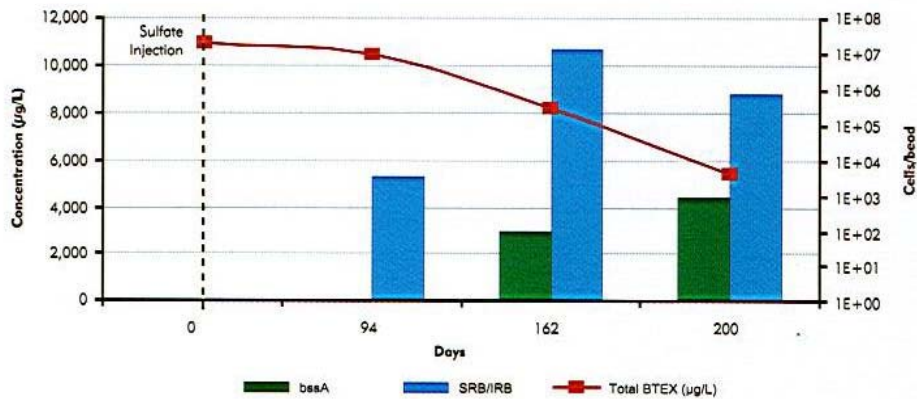
x-y plot of a scanned DGGE gel. Each peak is a different band from the gel



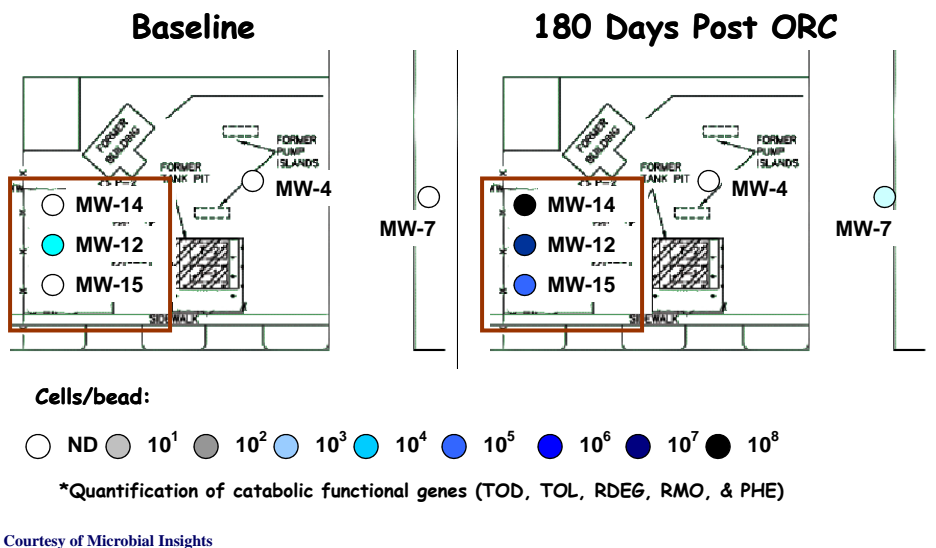
x-y plot of a scanned DGGE gel. Each peak is a different band from the gel



Effect of Sulfate Injection *In Situ*



Standard Bio-traps and qPCR Document Microbial Response to ORC Injection at a LUST Site

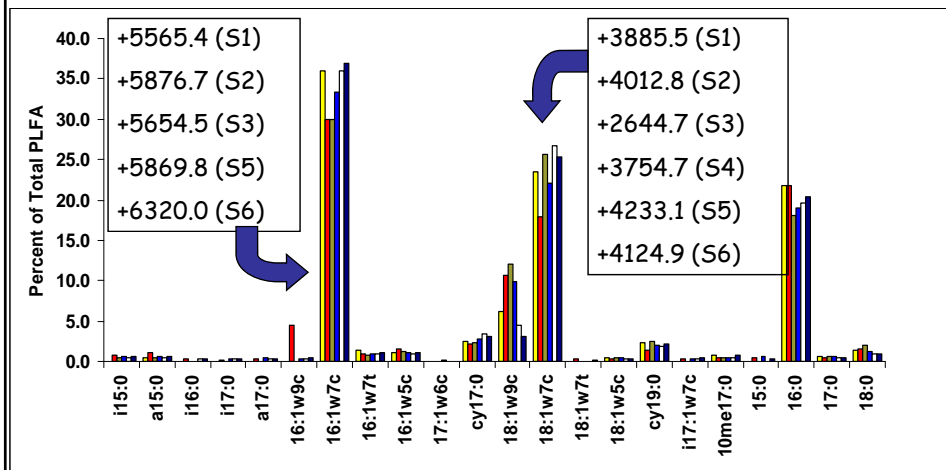


Applications of Bio-trap SIP Yucaipa, CA

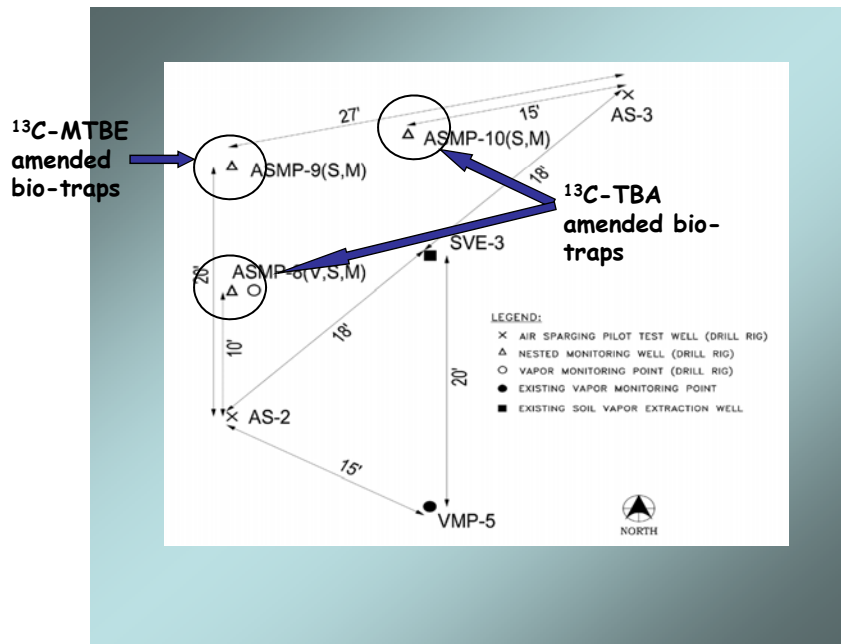
- Site of gasoline LUST
- Is there intrinsic bioremediation of benzene at this site?
- Bio-Sep bio-traps with ^{13}C -benzene installed in MW20 suspended 30 cm below water table with float; incubated for 45 days

Parameter	Background well	MW20
Benzene	ND	6.6 $\mu\text{g/L}$
TEX	ND	ND
MTBE	ND	1.2 $\mu\text{g/L}$
pH	7.4	7.5
Nitrate-N	9.4 mg/L	8.6 mg/L
Sulfate	23 mg/L	19 mg/L
Alkalinity	140 mg/L	130 mg/L
TDS	280 mg/L	250 mg/L

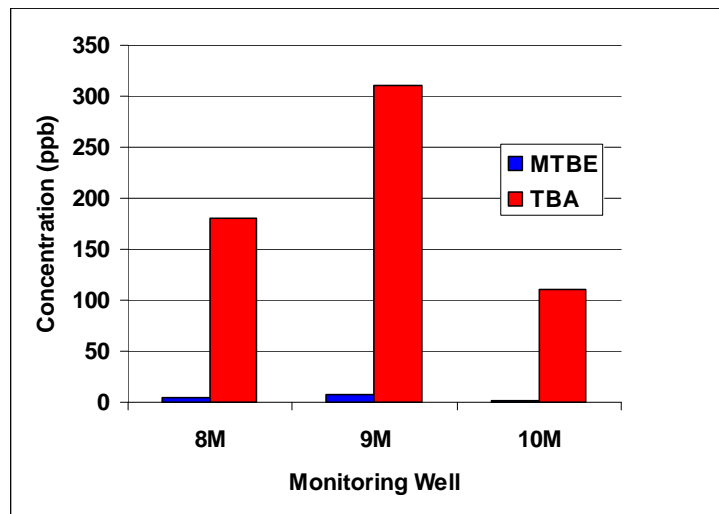
$\delta^{13}\text{C}$ Values of Individual Fatty Acids Derived from Phospholipids Extracted from Bio-traps



Deployment of ¹³C-MTBE and ¹³C-TBA Amended Bio-traps in an Air-sparged Aquifer



Pre-deployment levels of MTBE and TBA



Post-deployment 8M-TBA
(10 ft from a sparging well)

Fatty acid	$\delta^{13}\text{C}$ (‰)	Fatty acid	$\delta^{13}\text{C}$ (‰)
i15:0	+997	cy19:0	+421
a15:0	+2550	10me16:0	+381
i16:0	+2002	14:0	+799
i17:0	+1504	15:0	+834
a17:0	+2799	16:0	+564
16:1w7c	+297	17:0	+1293
cy17:0	+226	18:0	+464
18:1w9c	+1464		
18:1w7c	+326		
18:1w5c	+295	DIC $\delta^{13}\text{C}$ (‰)	+32.2, +23.1

Post-deployment 10M-TBA
(15 ft from a sparging well)

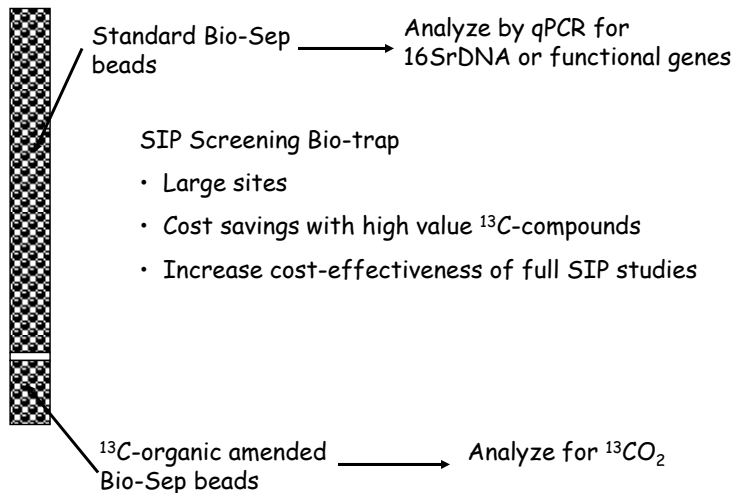
Fatty acid	$\delta^{13}\text{C}$ (‰)	Fatty acid	$\delta^{13}\text{C}$ (‰)
i15:0	+87	10me16:0	+134
a15:0	+74	14:0	+73
i16:0	+175	15:0	+189
i17:0	+328	16:0	+98
16:1w7c	+60	17:0	+343
cy17:0	+59	18:0	+93
18:1w9c	-21.9		
18:1w7c	+32		
cy19:0	+73	DIC $\delta^{13}\text{C}$ (‰)	+238, +181

Post-deployment 9M-MTBE (20 ft from a sparging well)

Fatty acid	$\delta^{13}\text{C}$ (‰)	Fatty acid	$\delta^{13}\text{C}$ (‰)
i15:0	-19.4	18:1 ω 5c	-21.7
a15:0	-20.4	cy19:0	-21.7
i16:0	-29.8	10me16:0	-32.6
i17:0	-21.0	15:0	-18.8
a17:0	-19.0	16:0	-34.2
16:1 ω 7c	-31.9	17:0	-20.6
cy17:0	-38.7	18:0	-20.5
18:1 ω 9c	-32.0		
18:1 ω 7c	-33.0	DIC $\delta^{13}\text{C}$ (‰)	+415, +708

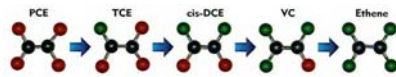
Only very low levels of incorporation of ^{13}C into biomass indicated

What's new in SIP? SIP Screening Bio-trap

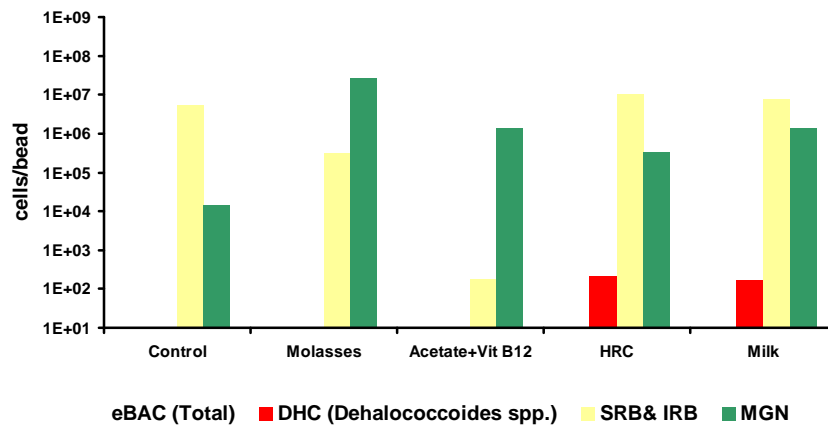


Bio-trap Case Studies

Chlorinated Hydrocarbons



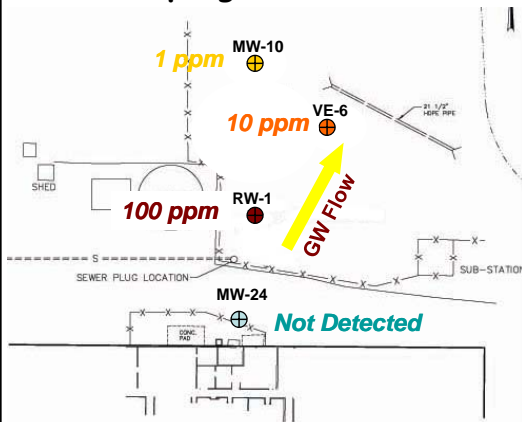
What Amendments Stimulate DHC? Baited Bio-traps at a Dry Cleaners Site



Site Investigation: PCE/TCE Plume

Control & HRC Bio-traps placed in MWs that represented a gradient across the site

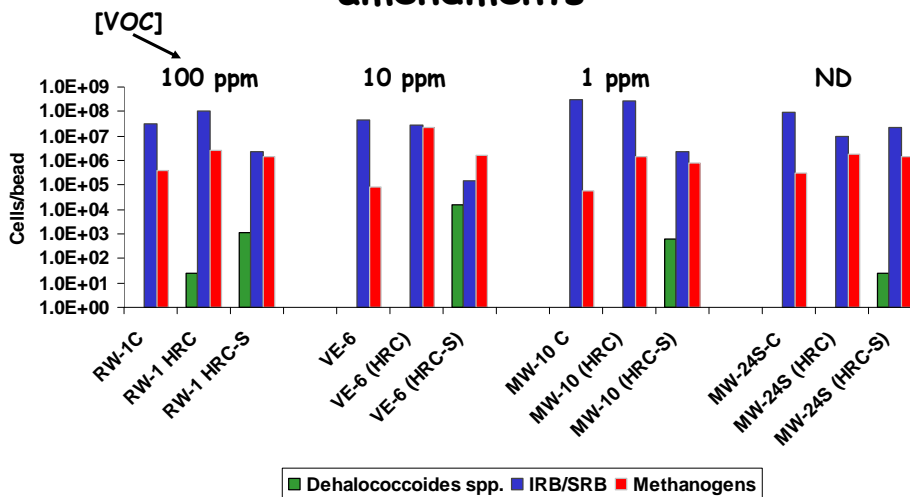
Sampling Locations



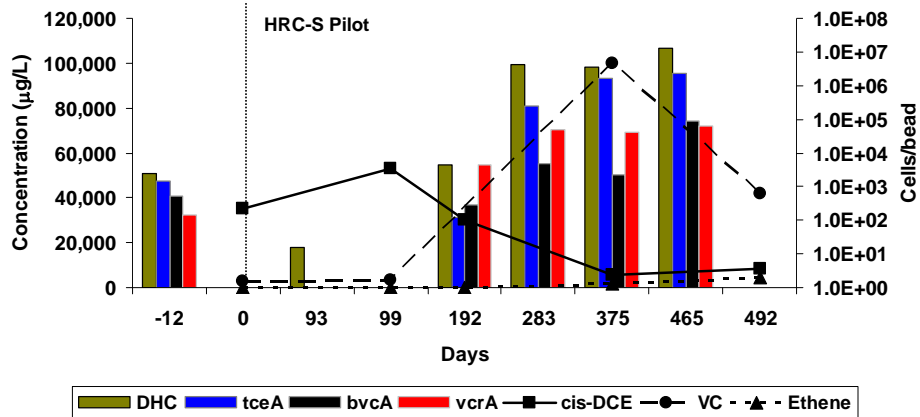
Analyzed for:

- PLFA
- DGGE
- qPCR
 - Bacterial
 - SRB/IRB
 - Methanogens
 - DHC

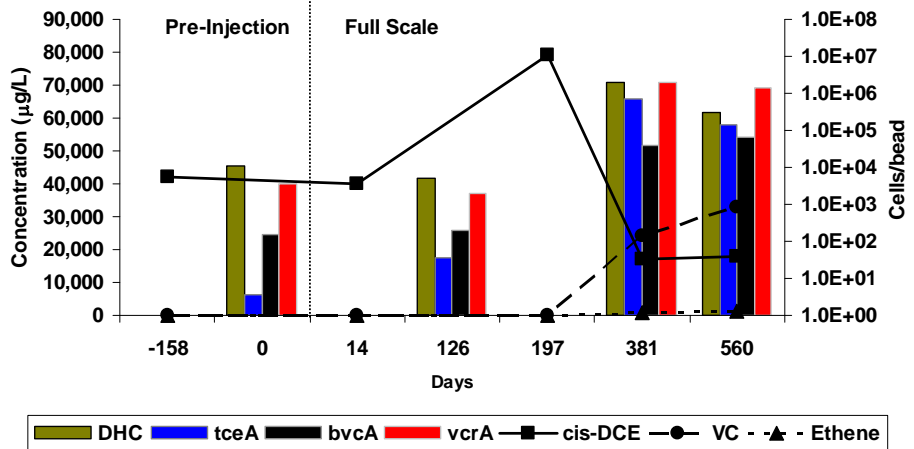
Baited bio-traps evaluated remediation amendments



HRC-S injection pilot test



Full-scale treatment with HRC-S



What's new in bio-traps for chlorinated hydrocarbons?

- Advanced bio-trap assembly

