Molecular Methods and Bio-traps for the Assessment of Biodegradation in Contaminated Groundwater

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What can I do with a bio-trap?

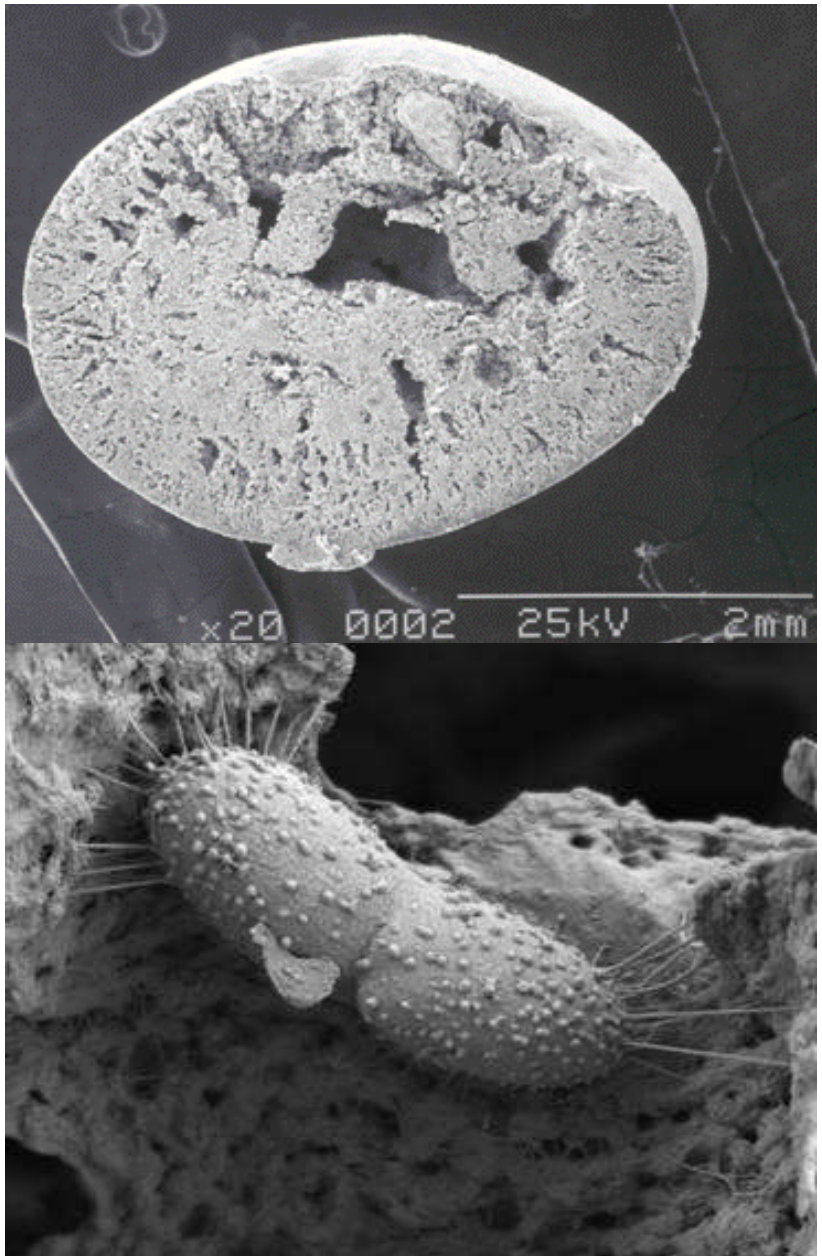
- Characterize and quantify active components of a microbial community in terms of phylogenetic groups or functional genes
  - e.g. Is an aquifer microbial community responding to contaminants of concern?
- Predict the response of a microbial community to a potential remediation amendment
  - e.g. How will dechlorinating bacteria in an aquifer respond to an injection of HRC?
- Evaluate actual *in situ* response to remediation amendments
  - e.g How did the microbes respond to a field-scale injection of HRC?
- Provide irrefutable proof of the biodegradation potential of target compounds
  - e.g. Is benzene or other hydrocarbon being degraded under aquifer conditions by indigenous microbes?

How do bio-traps work?
Bio-Sep® Beads

- 3-4 mm in diameter
- 25 % Nomex, 75% PAC
- 74% porosity
- 600 m^2 of surface area/g
- Autoclavable
- Cleaned of fossil biomarkers by heating to 300 ^oC

What do Bio-Sep® beads collect in the aquifer?

- Bacteria have to enter the bead and grow there to be detected
- How do bacteria enter the beads?
  - Motility by means of flagella
  - Adsorption to the bead surface followed by
    - twitching motility (bacterial crawling)
    - Gliding motility
  - Brownian motion
- Organisms collected in the beads are more likely to be the environmentally competent, active fraction of the subsurface community



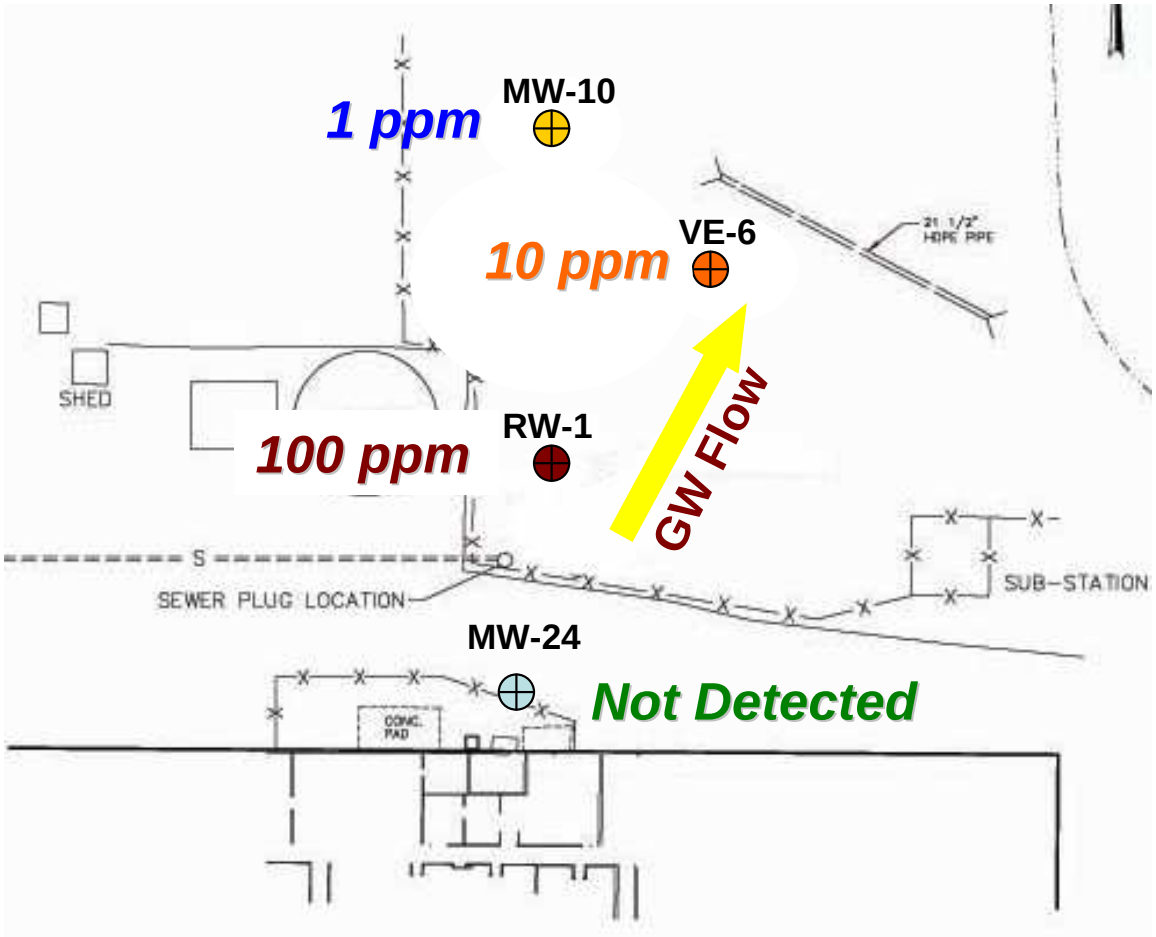
Case Study: Documenting the *in situ* biodegradation of chlorinated hydrocarbons

- Remediation of chlorinated hydrocarbons is typically stimulated by injection of an easily degraded organic substrates which drives down the redox potential and favors the growth of dehalogenating species
  - *Dehalococcoides* sp. (DHC)
    - PCE $\longrightarrow$ ethene + Cl^-
- Bio-traps can be used to document relative activity of DHC in an aquifer
- Baited bio-traps can be used to evaluate potential remediation amendments

Site investigation: PCE/TCE Plume

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

Sampling Locations



Analysis:

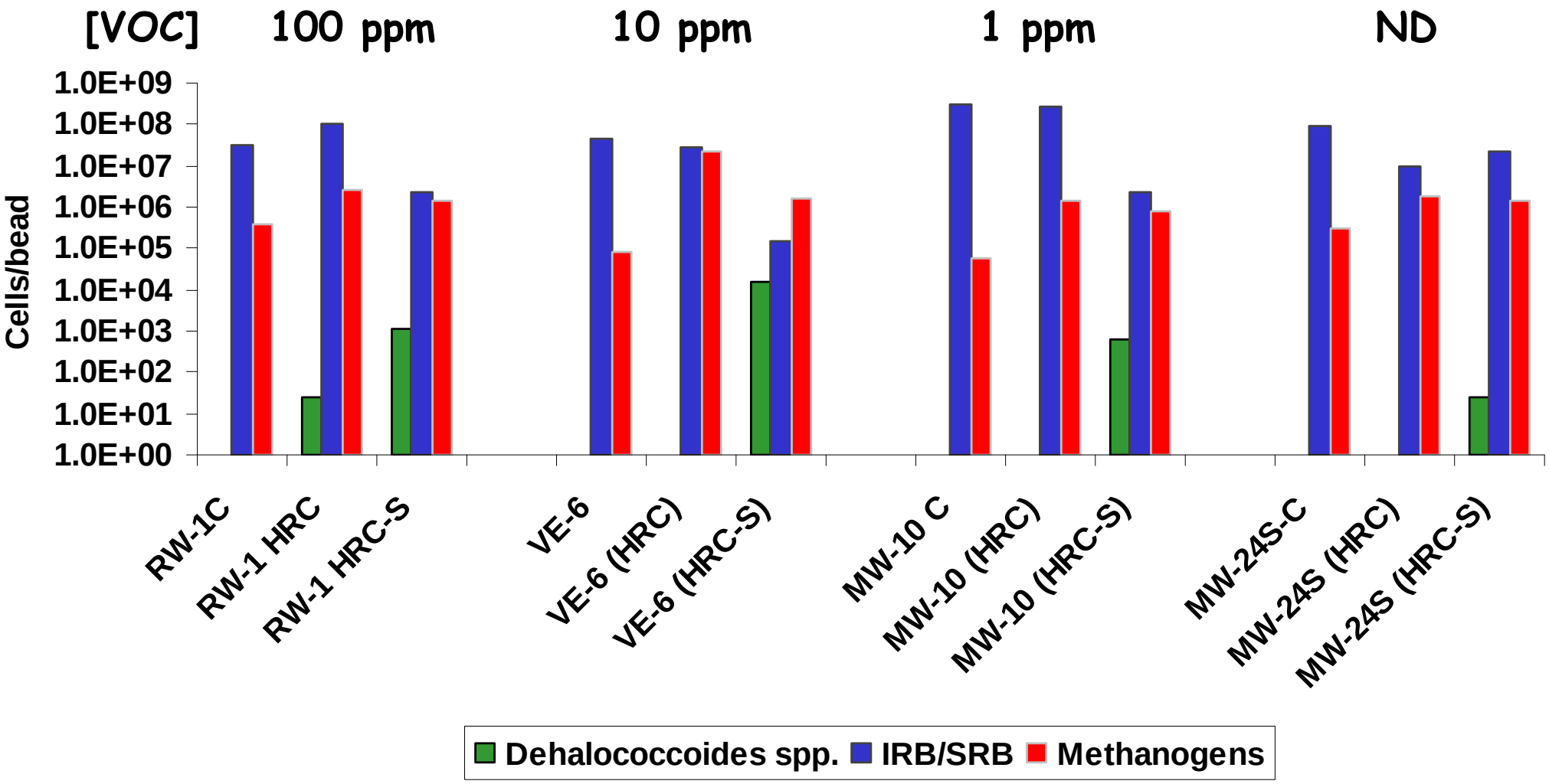
- qPCR
  - SRB/IRB
  - Methanogens
  - DHC
  - Functional genes

Initial groundwater conditions

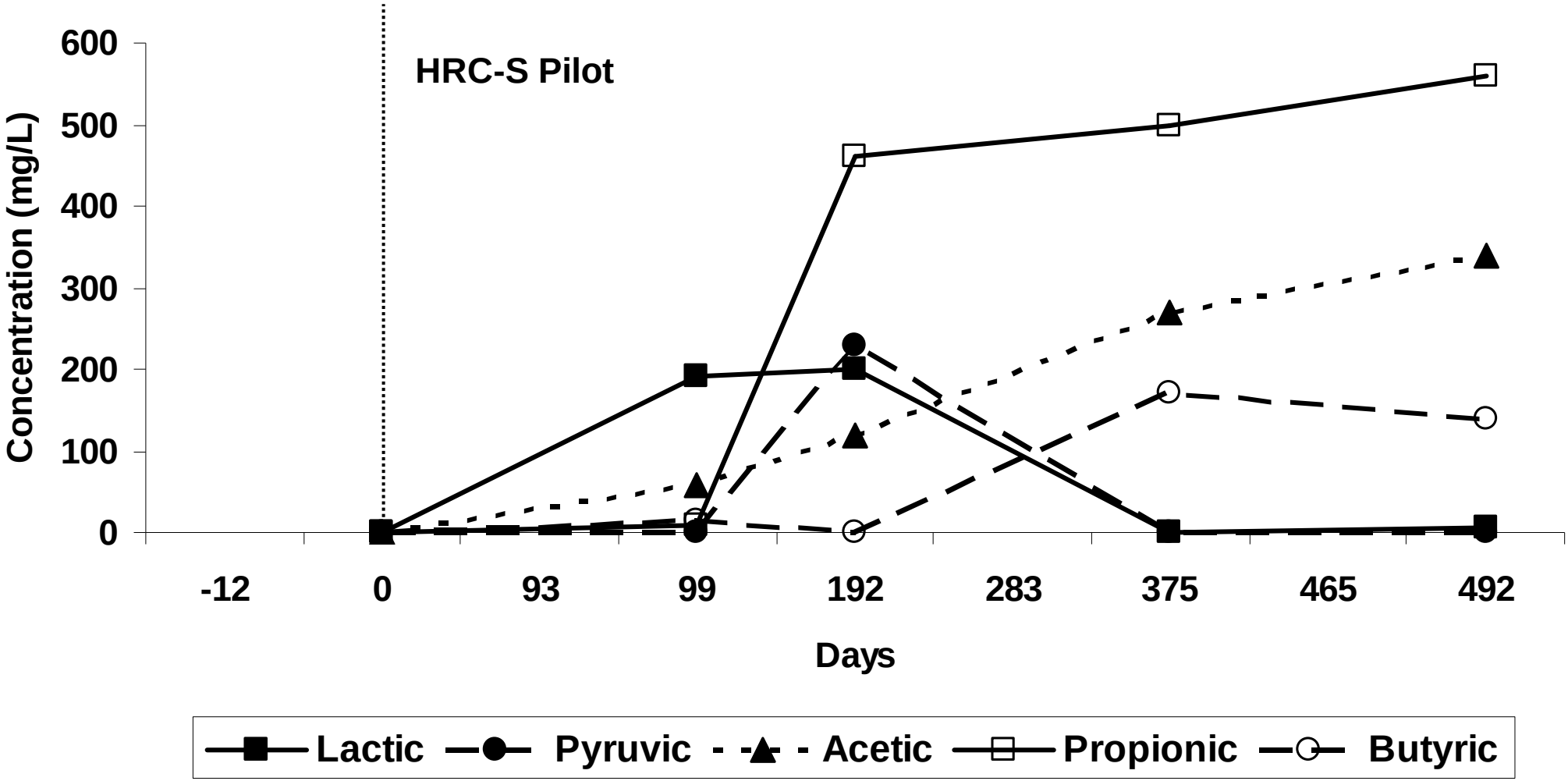
Parameter	Source (RW-1)	Mid-Plume (VE-6)
ORP	79	194
DO	0.48	0.34
Sulfate	842	233
Methane*	140	29
PCE	20	10
TCE	ND	6
cis-DCE	130	13
TCA	59	8
VC	ND	ND
Total CVOCs	215 ppm	38 ppm

*All parameters reported as ppm (mg/L) except methane which was reported as ug/L

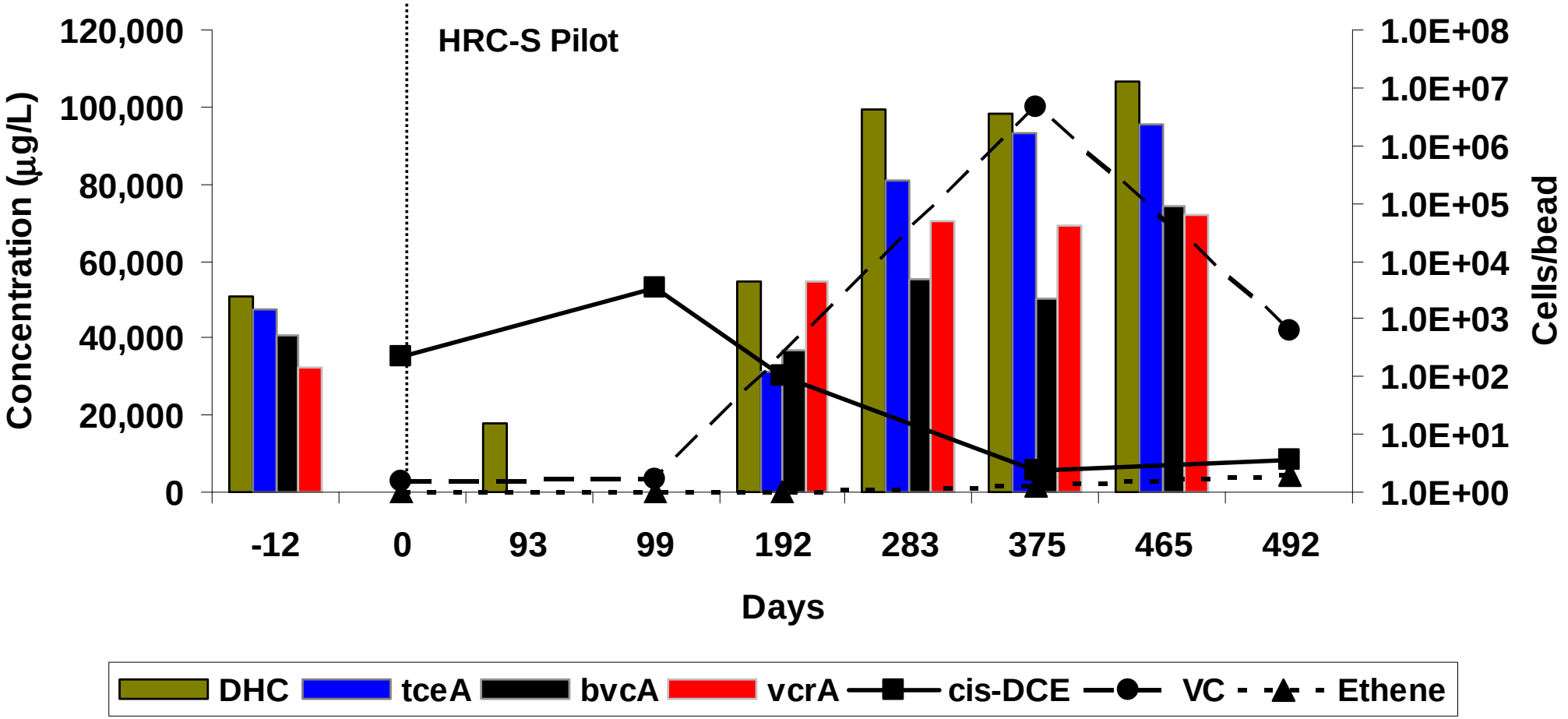
Baited bio-traps evaluated remediation amendments



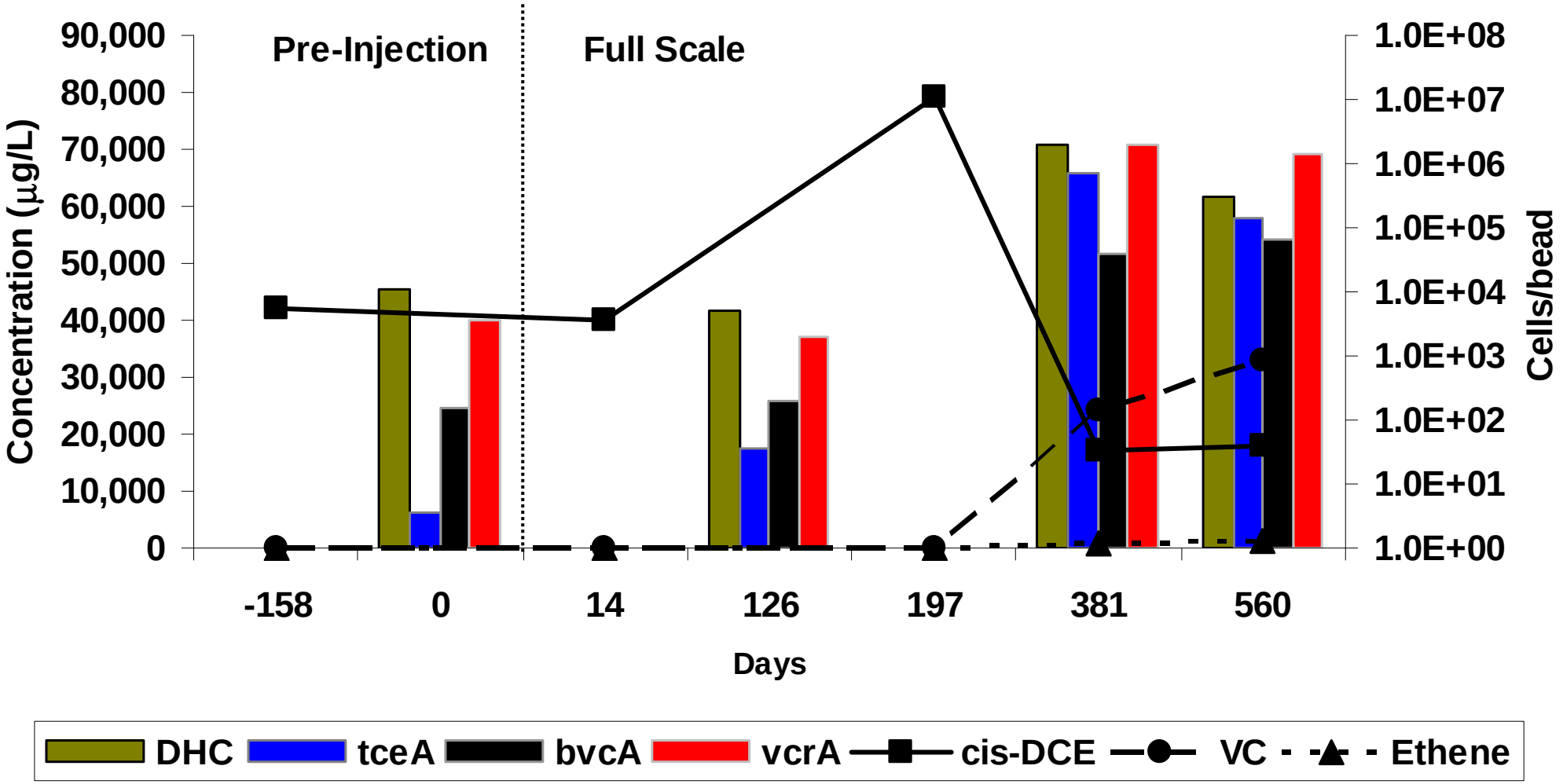
HRC-S injection pilot test



HRC-S injection pilot test

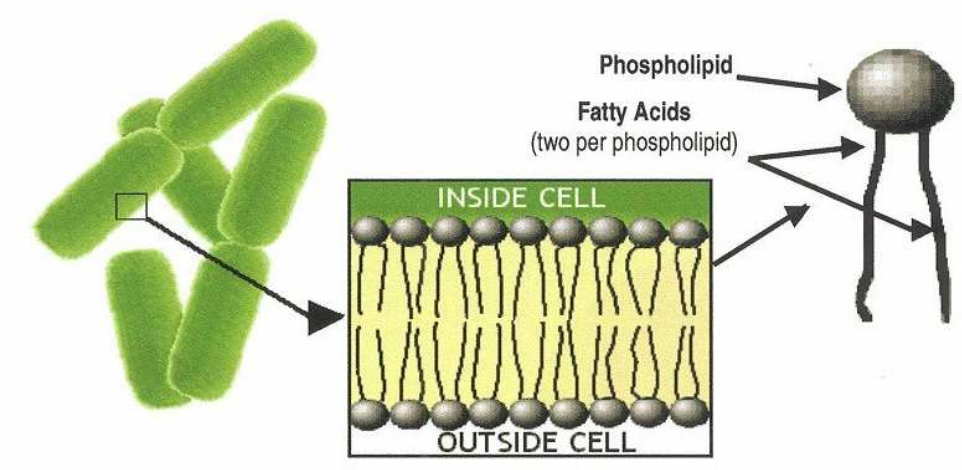


Full-scale treatment with HRC-S



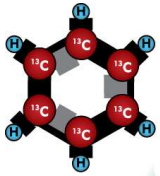
Case study: Demonstrating the *in situ* biodegradation potential of p-xylene during an air sparging pilot test

- Bio-traps baited with ^{13}C-labeled p-xylene (11% ^{13}C)
- Incorporation of ^{13}C into membrane phospholipids demonstrates metabolism of labeled compound
- Incorporation of ^{13}C in dissolved inorganic carbon demonstrates mineralization of labeled compounds

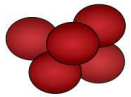


Overview of Bio-Trap SIP Approach

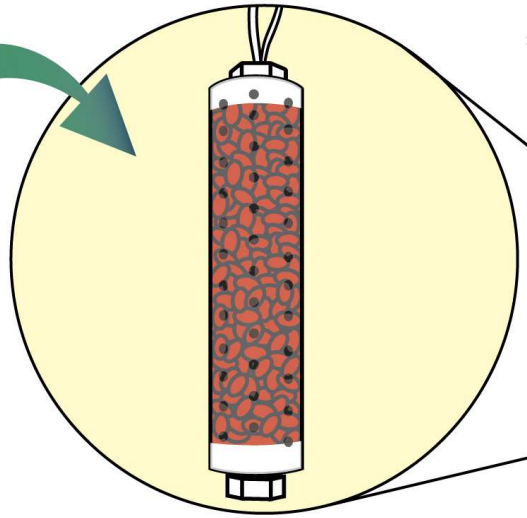
^{13}C-labeled p-xylene



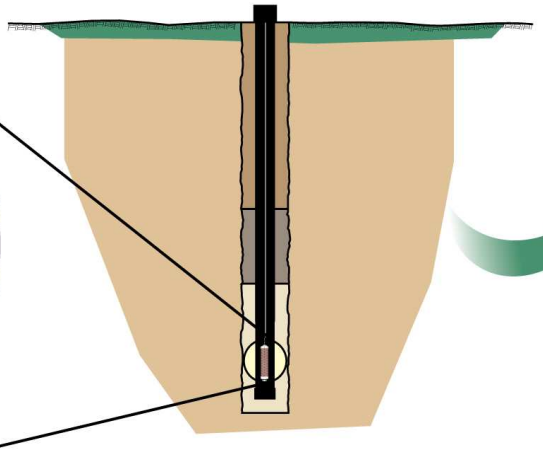
^{13}C-p-xylene loaded into Bio-Sep beads



Bio-trap with ^{13}C-p-xylene loaded Bio-Sep beads



In situ incubation of ^{13}C-p-xylene bio-trap in site monitoring well



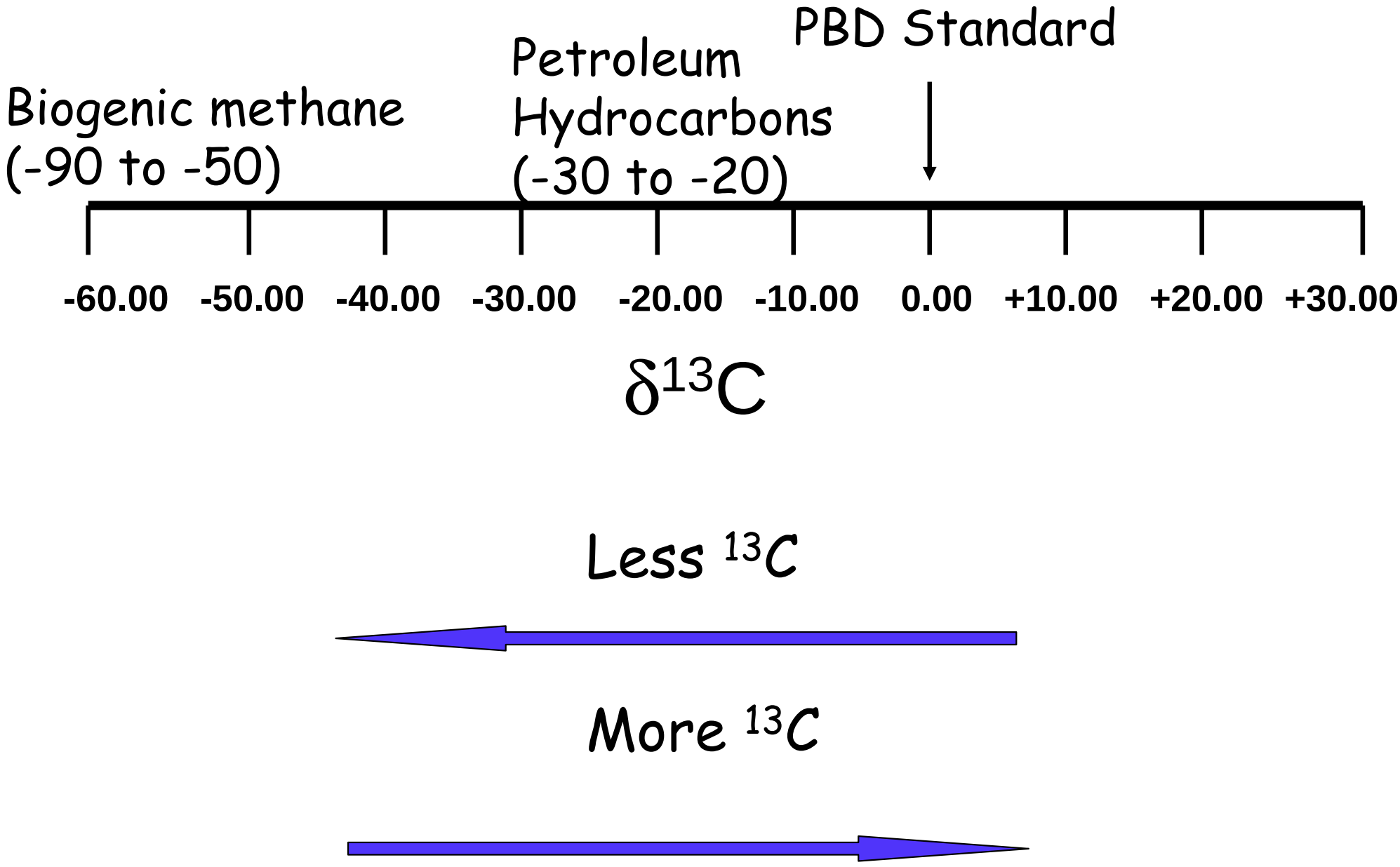
Analysis of post-incubated Bio-Sep beads

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

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

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

- The standard is a specific carbon-containing mineral from a specific location: Pee Dee Belimnite (PDB)
- Units of $\delta^{13}C$ are °/₀₀ or "per mill"


PBD Standard
Petroleum Hydrocarbons (-30 to -20)
Biogenic methane (-90 to -50)
-60.00
-50.00
-40.00
-30.00
-20.00
-10.00
0.00
+10.00
+20.00
+30.00
δ13C
Less 13C
More 13C

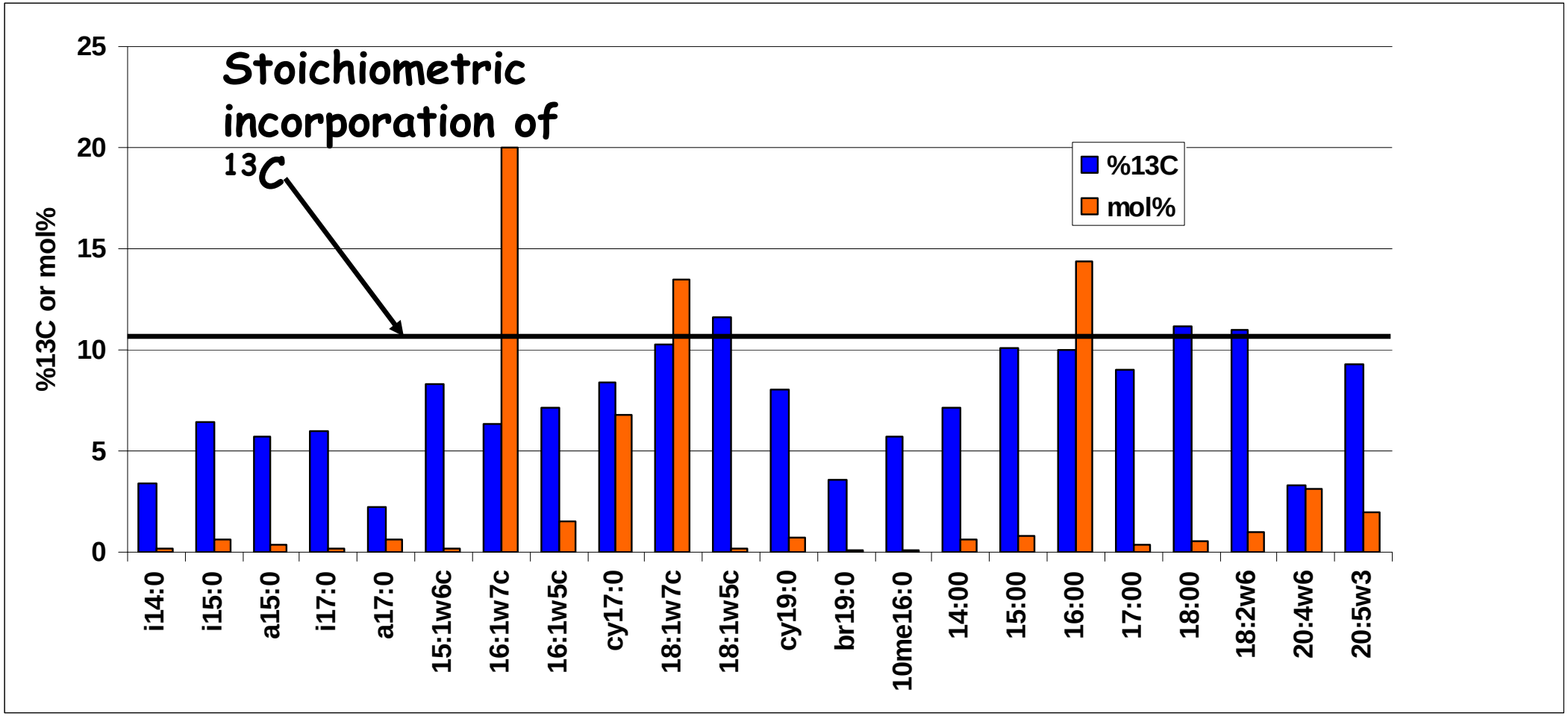
Observed $\delta^{13}C$ values for selected phospholipid fatty acids

Well/FA	Plume 1 (‰)	Plume 2 (‰)	Plume 3 (‰)	Control (‰)
15:1w6c	+1758	+1300	+7105	
16:1w7c	+1652	+1795	+4991	+159
cy17:0	+3229	+2379	+7245	
18:1w7c	+2735	+2592	+9219	
16:0	+2239	+2364	+8986	+547

^{13}C incorporation in phospholipids and dissolved inorganic carbon

Well	^{13}C in phospholipids (pmoles/bead)	^{13}C in DIC (%)
Plume 1	1095	3.0
Plume 2	356	4.0
Plume 3	1530	4.6
Control (Outside plume)	2.5	1.8

^{13}C content of phospholipid fatty acids from Plume Well 3



Conclusions

- Bio-Sep® bio-traps can reveal details of the microbial ecology of contaminant impacted aquifers including:
  - Concentrations of active degraders
  - Concentrations of relevant functional genes
  - Predicting response to potential remediation amendments (baited bio-traps)
- Bio-Sep® bio-traps coupled with stable isotope probing can provide proof of *in situ* biodegradation potential
  - The ^{13}C enrichment of fatty acids and CO_2 derived from the bio-trap biofilms conclusively link the biodegradation of ^{13}C-labeled contaminants of concern with indigenous microorganisms under actual aquifer conditions
  - Labeling patterns of phospholipid fatty acids enriched with ^{13}C can provide clues to the identity of degraders

EnviroGene


Bio-trap
sampler
EnviroGene