Bio-traps and Site Management Strategies for Groundwater Impacted by Petroleum Hydrocarbons

Kerry Sublette
University of Tulsa

Dora Taggart, Anita Biernacki,
Brett Baldwin, Kate Clark
Microbial Insights, Inc.

THE UNIVERSITY of TULSA

microbialinsights

What Are Bio-Trap® Samplers?

Passive sampling tool for microbes

Collects **active** microbes

Integrated sample vs. "snapshot"

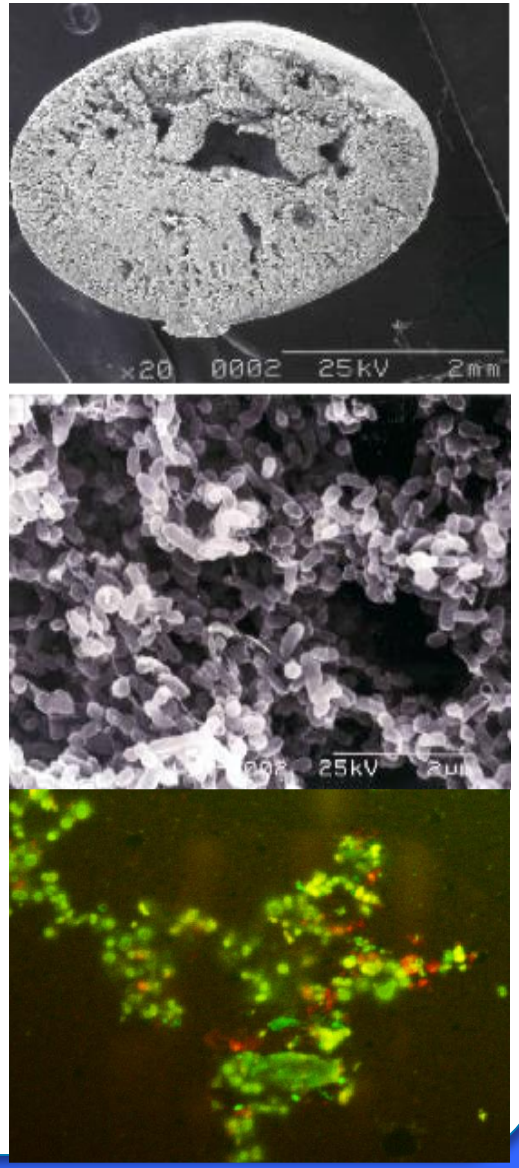
Analyzed using molecular biological tools, analytical chemistry, and stable isotope analysis

microbialinsights

How Do Bio-Traps Work?

Properties of Bio-Sep Beads

- 3-4 mm in diameter
- 25% Nomex and 75% PAC
- 74% porosity
- 600 m^2 of surface area/g
- Heat sterilized 270 ^oC
- Colonized by **active** microbes

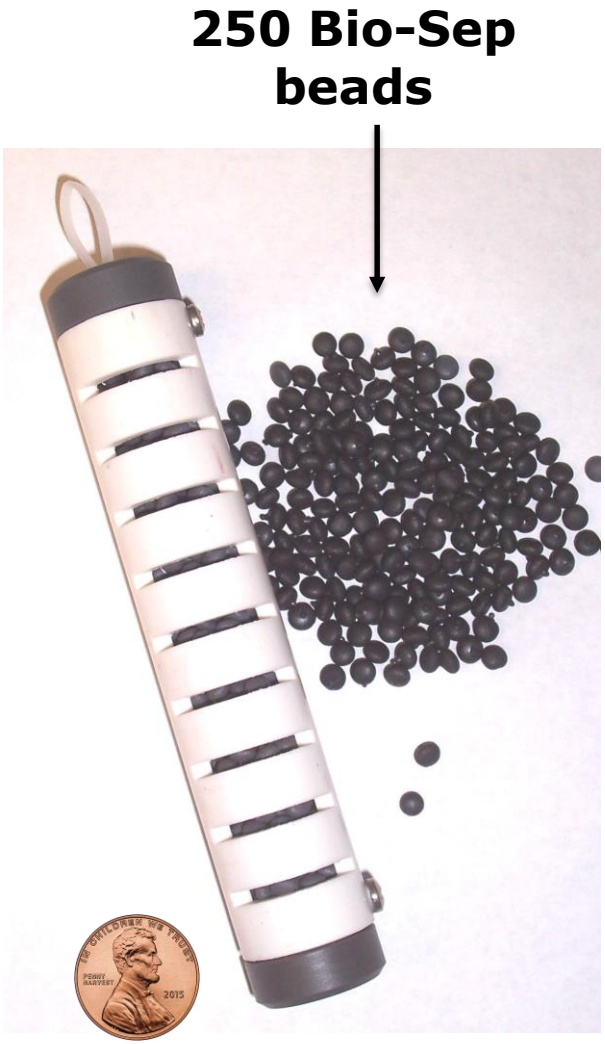


microbialinsights

Types of Bio-Trap Samplers

Standard Bio-Trap

- Basic design
- Sampling groundwater, surface waters, sediments, soils
- Compatible with all MBTs, analytical chemistry, and stable isotope techniques



microbialinsights

Bio-traps may be amended for diagnostic purposes

Electron Donors

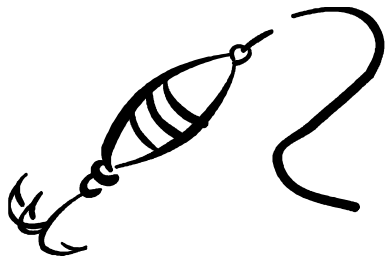
- Vegetable oil
- Molasses
- HRC
- EOS
- Lactate
- And more

Electron Acceptors

- Oxygen (PermeOx, ORC)
- Nitrate
- Iron (III)
- Sulfate
- And more

Stable Isotope Compounds (^{13}C)

- Benzene
- Toluene
- p-Xylene
- MTBE
- TBA
- Naphthalene
- Chlorobenzene
- 1,4-Dioxane
- Sulfolane
- And more



microbialinsights

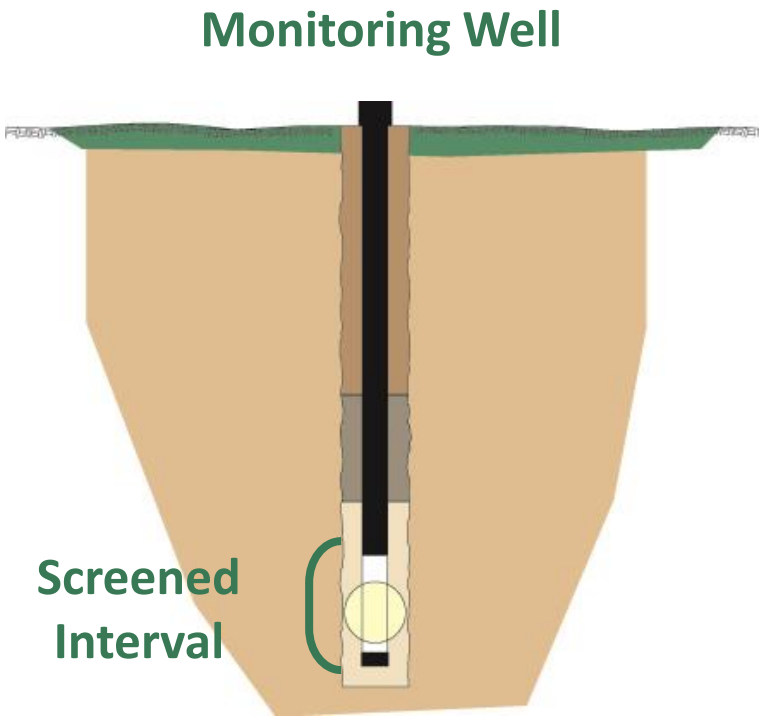
How Are Bio-Trap Samplers Deployed?

Purge monitoring well

Suspend from top of casing

Deploy within the screened interval at depths of interest.

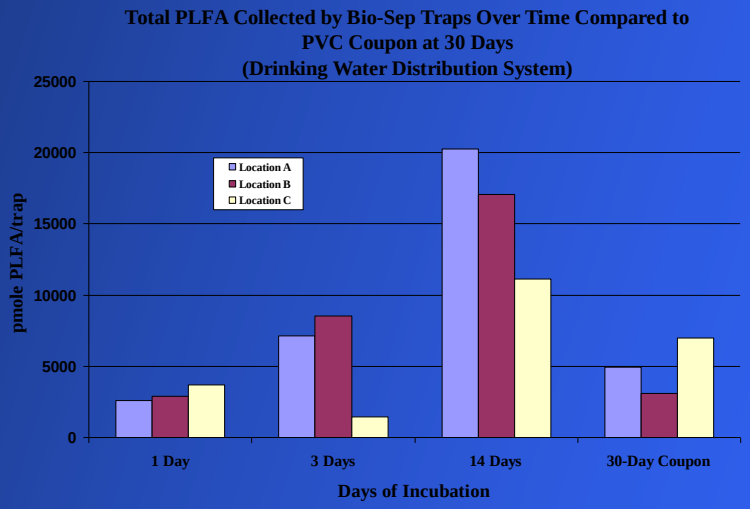
If large fluctuations in the water level are anticipated suspended from a float.



microbialinsights

Specialized Designs

Slip stream from drinking water distribution system

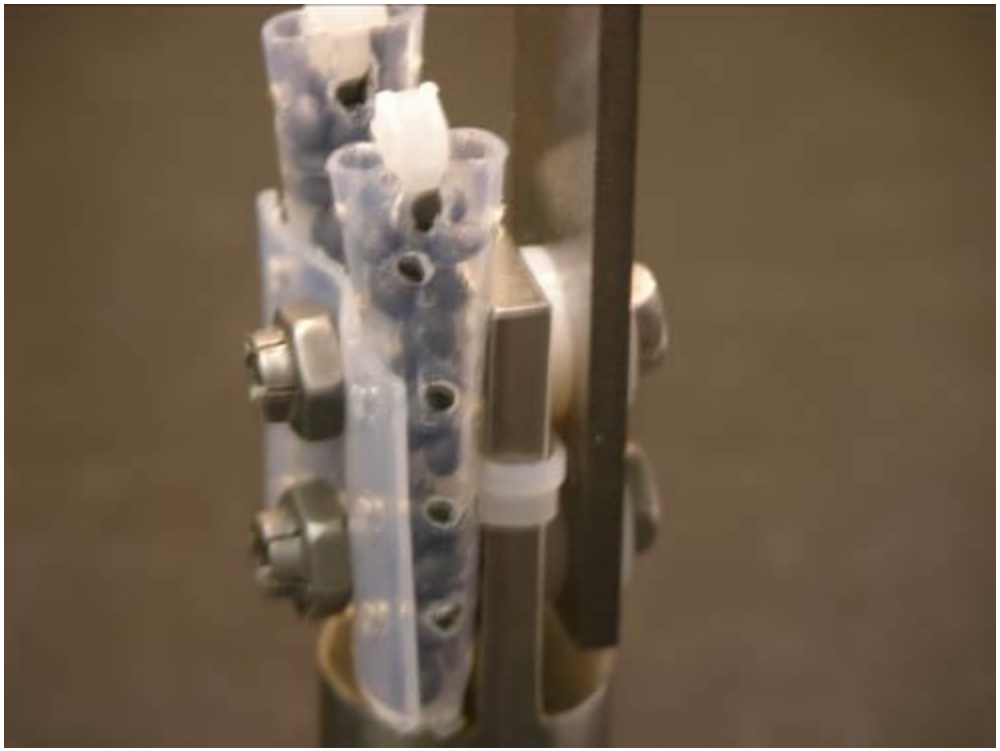


microbialinsights

Specialized Designs

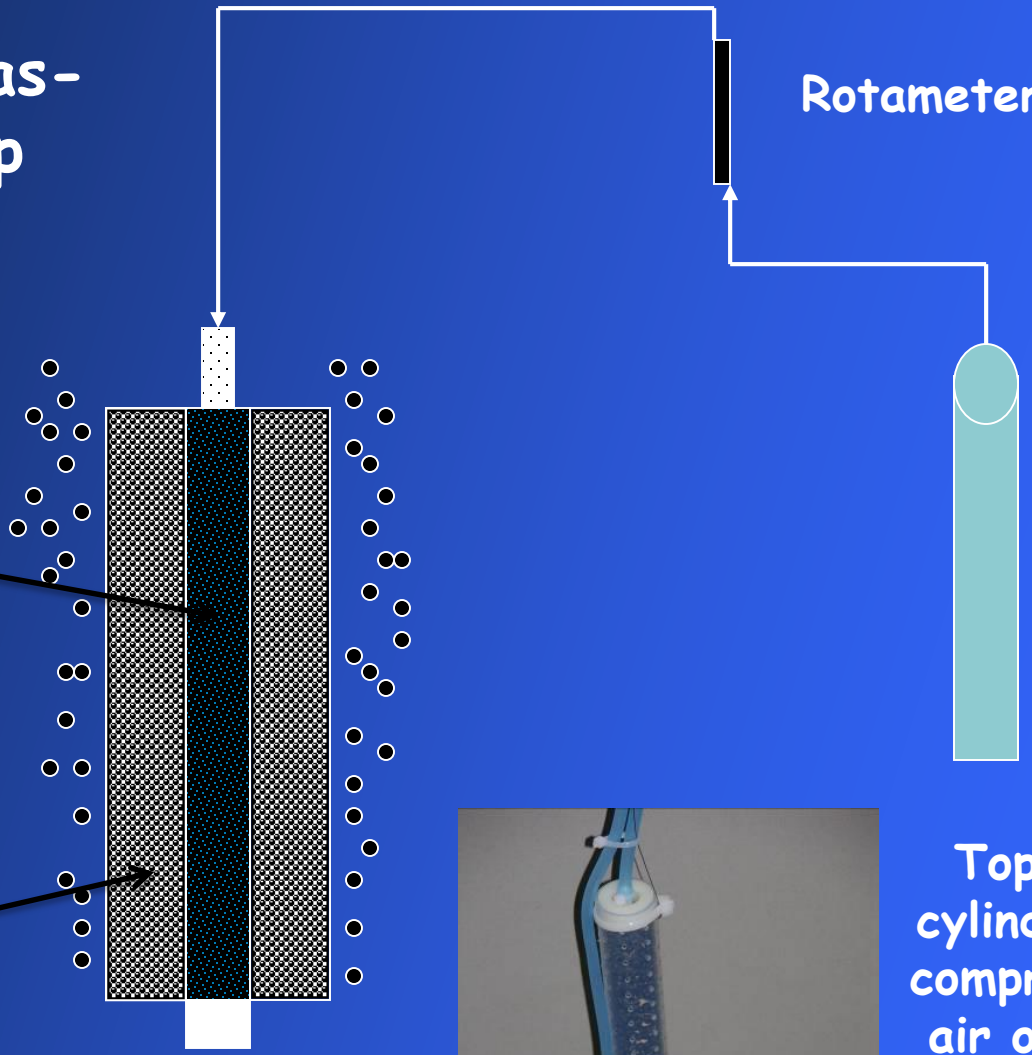
Tank sampling below a hydrocarbon layer in solvent extraction system

Specialized Designs



Sampling
Alaskan pipeline
for MIC

Aerated or Gas-fed Bio-trap



Topside cylinder of compressed air or gas mixture

microbialinsights

Sediment sampling or ocean floor

microbialinsights

Standard and Oil-amended Bio-Traps at 5000 ft in the Gulf of Mexico

microbialinsights

How Are Bio-Trap Samplers Analyzed:

Molecular Biological Tools

- PLFA
- CENSUS (qPCR)
- QuantArrays
- DGGE
- Stable Isotope Probing (SIP)

Chemical Analysis

- Compound specific isotope analysis (CSIA)
- Dissolved Inorganic Carbon (DIC)
- Contaminant Concentrations

microbialinsights

What Can I Do With a Bio-Trap Sampler?

- Determine if known degraders of a COC are present
- Evaluate monitored natural attenuation versus enhanced bioremediation
- Compare effectiveness of amendments designed to stimulate bioremediation
- Prove that bioremediation of a specific compound is occurring

microbialinsights

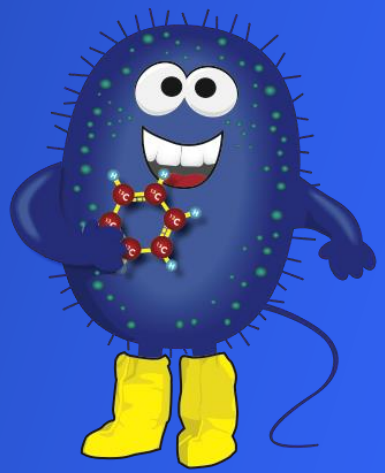
What Can I Do With a Bio-Trap Sampler?

- **Determine if known degraders of a COC are present**
- Evaluate monitored natural attenuation versus enhanced bioremediation
- Compare effectiveness of amendments designed to stimulate bioremediation
- **Prove that bioremediation of a specific compound is occurring**

microbialinsights

SIP

Stable Isotope Probing



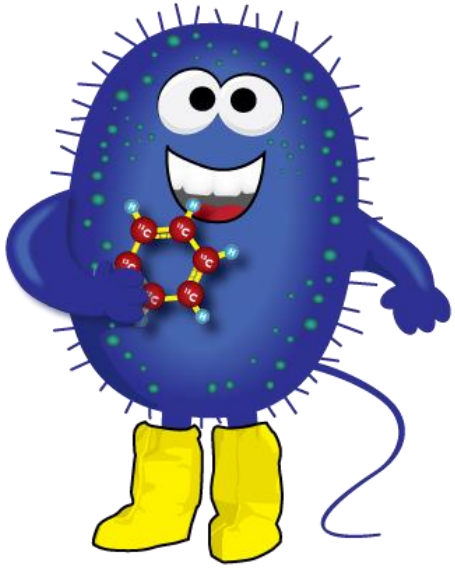
microbialinsights

What is stable isotope probing (SIP)?

Coupling molecular biological tools with stable isotope compounds

a) to prove biodegradation potential under *in situ* conditions and

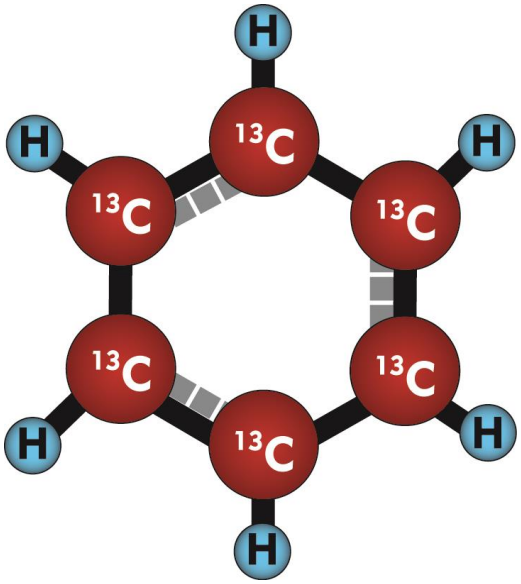
b) to link biodegradation to the responsible microbes



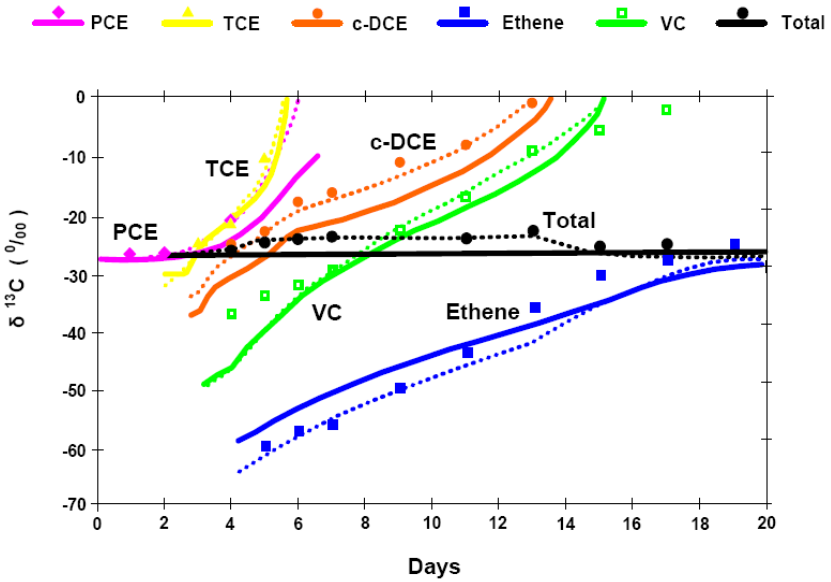
Stable Isotope Compounds

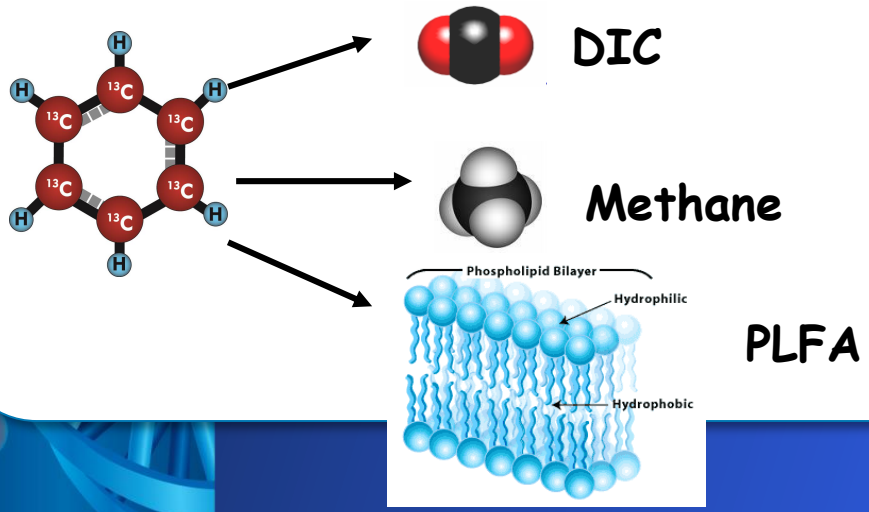
- **Specially produced "heavy" compounds which are composed of 99+% ^{13}C**
  - Natural compounds are 99% ^{12}C
  - Same characteristics as original compound
  - Behave similar to the natural compound

- **Used as "tracers" to increase our understanding of contaminant fate**



CSIA vs. Stable isotope probing

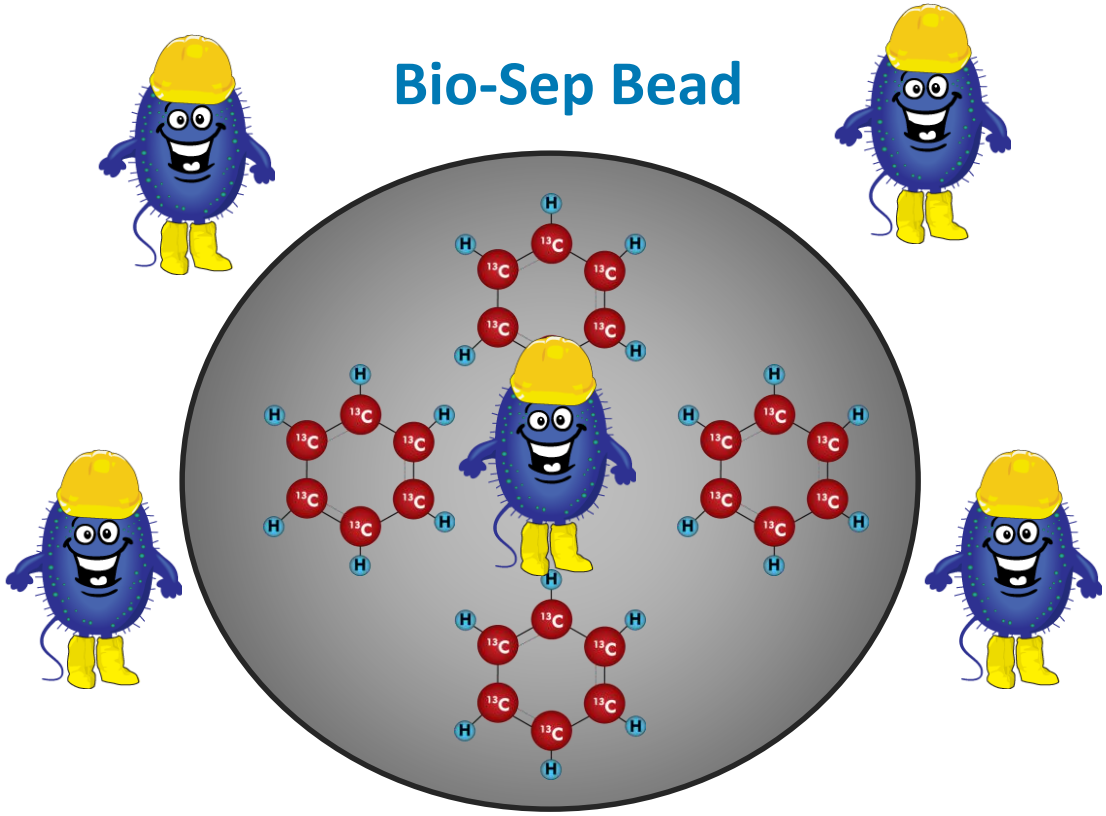




- CSIA
  - Isotopic fractionation results from the differences in the rates of cleavage of carbon-carbon bonds involving ^{12}C and ^{13}C
- Stable isotope probing
  - ^{13}C used as a tracer

microbialinsights

Microbes colonize beads



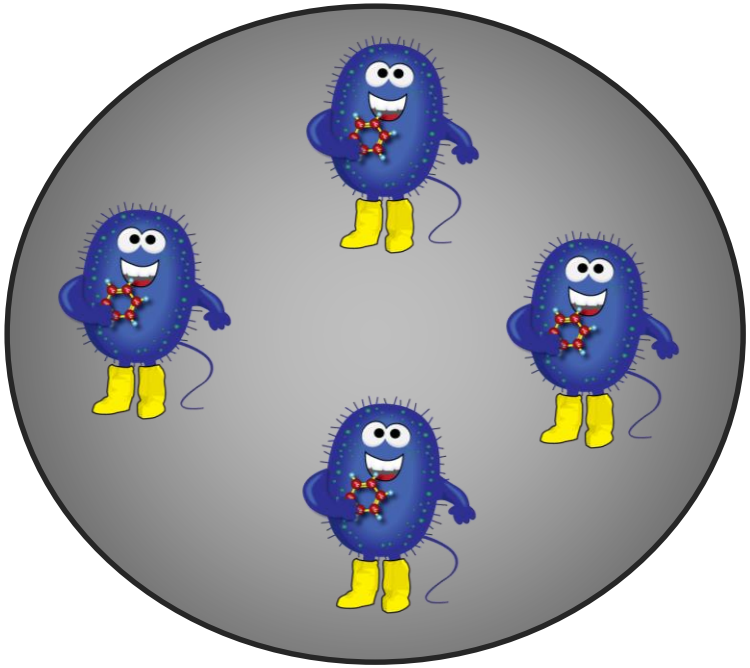
^{13}C-labeled compounds sorbed to Bio-Sep® beads

Bio-Trap colonized by indigenous microorganisms

microbialinsights

Microbes utilize target compound

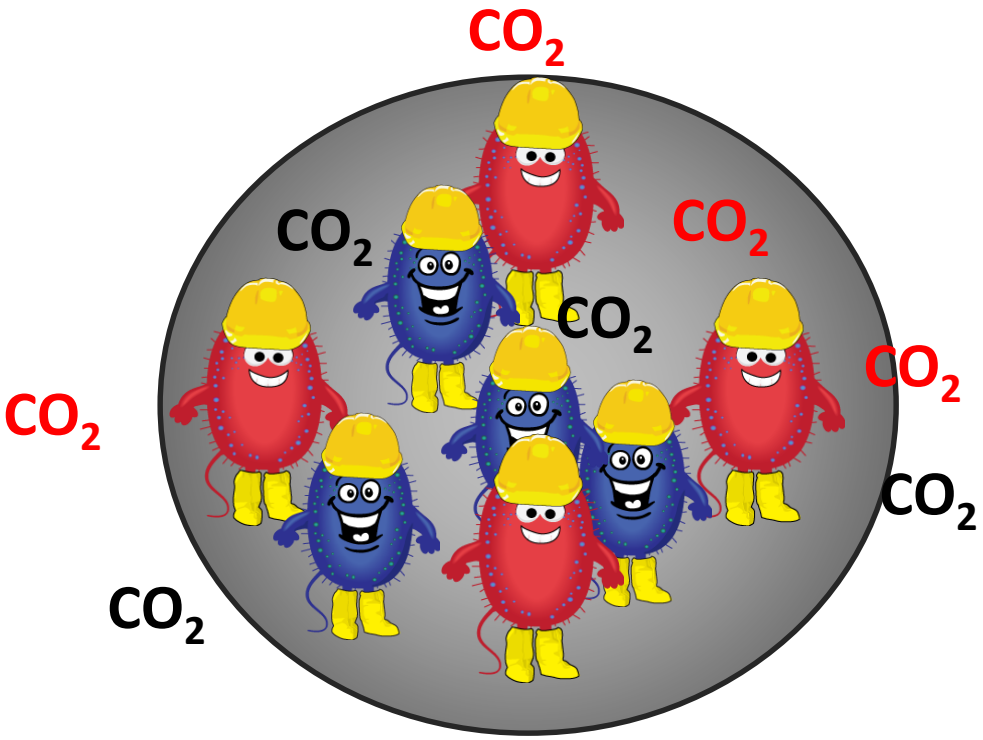
Bio-Sep Bead



Some microbes that colonized the Bio-Sep® bead can utilize ^{13}C labeled target compound.

microbialinsights

^{13}C Incorporation into biomass and CO_2



^{13}C is incorporated into new cells growing in the beads and in CO_2

microbialinsights

Overview of Bio-Trap SIP Approach

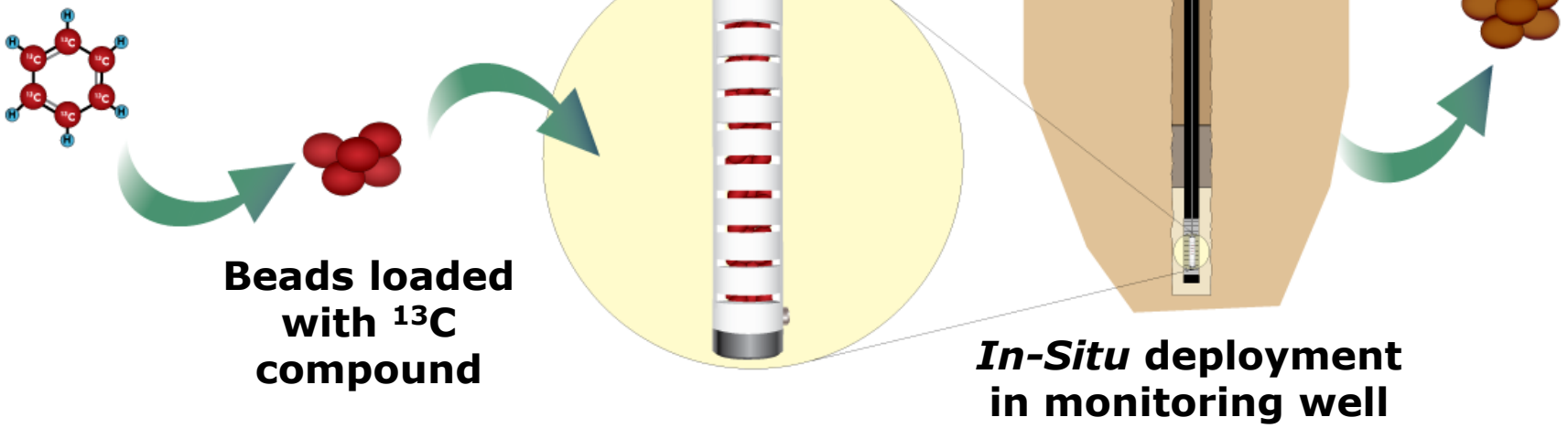
^{13}C labeled Benzene

Beads loaded with ^{13}C compound

Bio-Trap with ^{13}C-benzene loaded beads

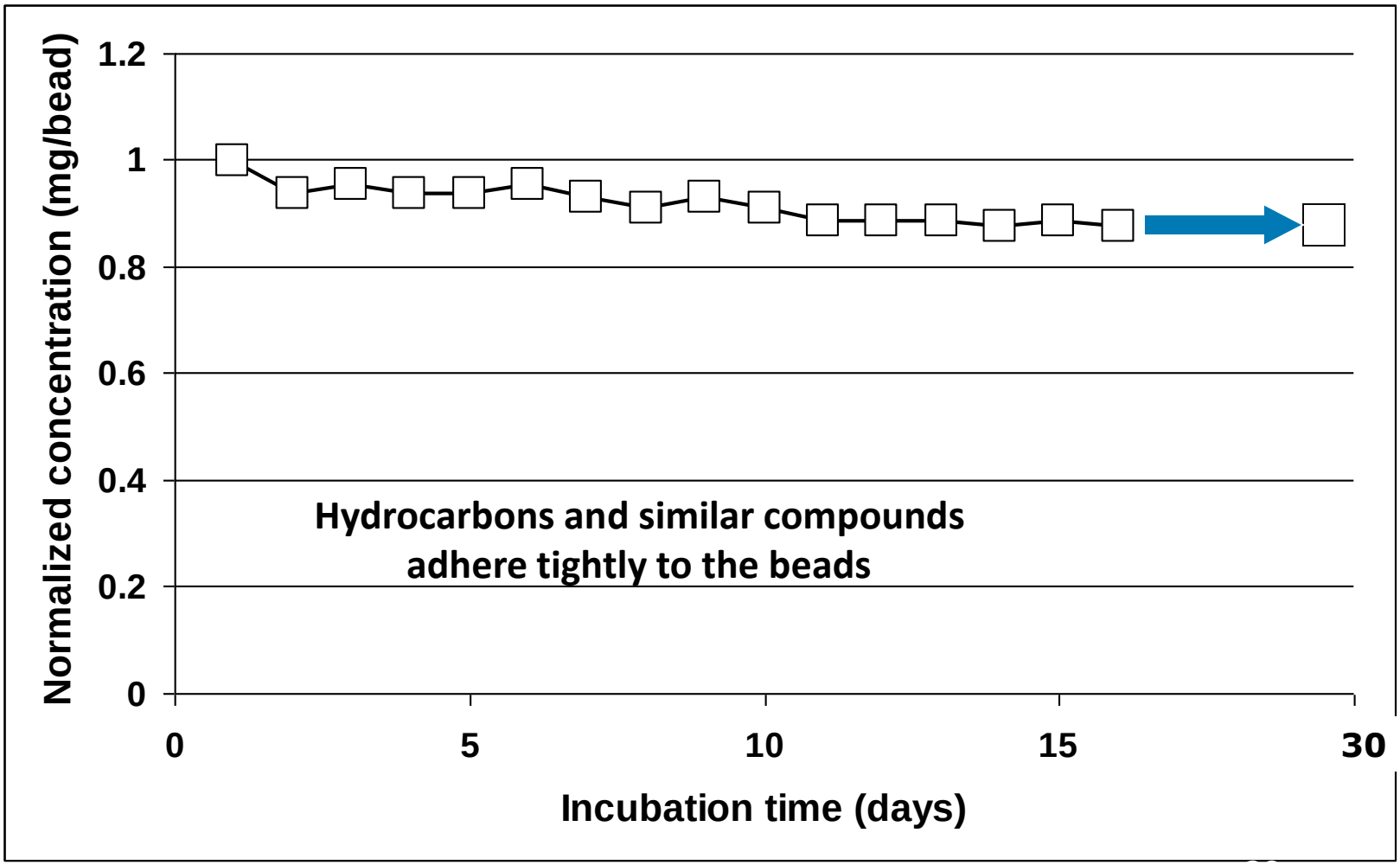
***In-Situ* deployment in monitoring well**

Beads analyzed following deployment



microbialinsights

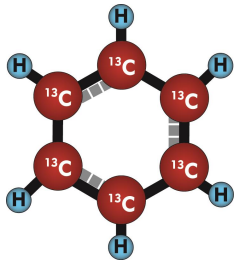
Benzene



microbialinsights

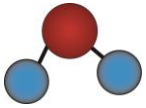
Bio-Trap SIP Analysis

Residual ^{13}C-Compound



Local relative rate

$^{13}C/^{12}C$ Dissolved Inorganic Carbon



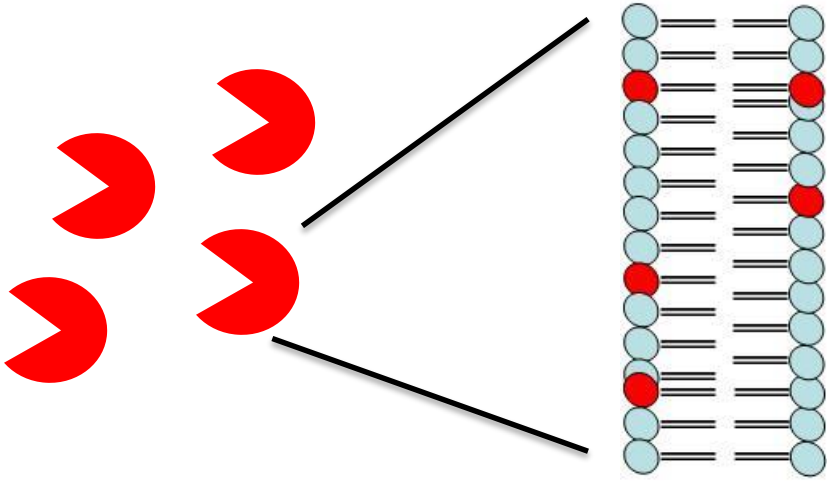
Mineralization
(C for energy)

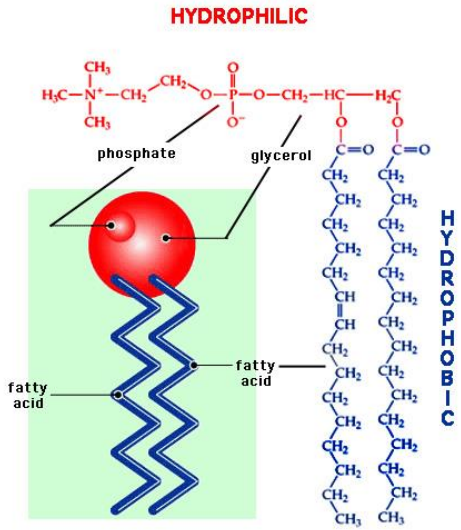
$^{13}C/^{12}C$ of Biomarkers

PLFA
DNA
RNA

Metabolism
(C for growth)

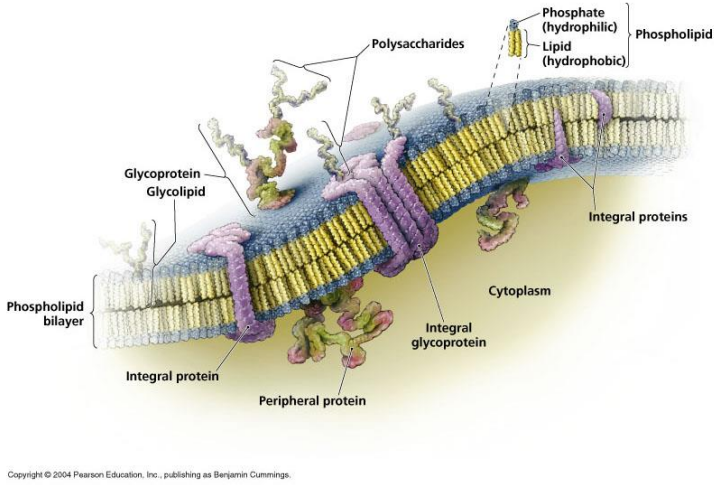
microbialinsights





GC-IRMS measures $^{13}C/^{12}C$ ratios in PLFA fatty acids and CO_2

- High concentrations of phospholipids in microbial cells
- Indicative of viable biomass
- Fatty acid structures give clues to microbial ecology



PLFA Type	Bacterial Group	Potential Relevance to Bioremediation
Monoenoic (Monos)	Abundant in Proteobacteria which includes a wide variety of aerobes and anaerobes	Many hydrocarbon utilizing bacteria are classified within Proteobacteria
Terminally Branched Saturated (TerBrSats)	Characteristic of Firmicutes and *Bacteroides*	Firmicutes include anaerobic fermenting bacteria which produce the H_2 necessary for reductive dechlorination
Branched Monoenoic (BrMonos)	Anaerobes and micro-aerophiles such as sulfate- or iron-reducing bacteria	High proportions are often associated with anaerobic sulfate and iron reducing bacteria
Mid-Chain Branched Saturated (MidBrSats)	Common in sulfate reducing bacteria and also *Actinomycetes*	High proportions are often associated with anaerobic sulfate and iron reducing bacteria
Normal Saturated (Nsats)	Found in all organisms	High proportions often indicate less diverse populations
Polyenoic (Polys)	Found in eukaryotes (fungi, algae, protozoa, plants and animals)	Eukaryotic scavengers often prey on contaminant utilizing bacteria

Unit of measure

Amount of ^{13}C relative to ^{12}C is expressed by the $\delta^{13}C$ notation

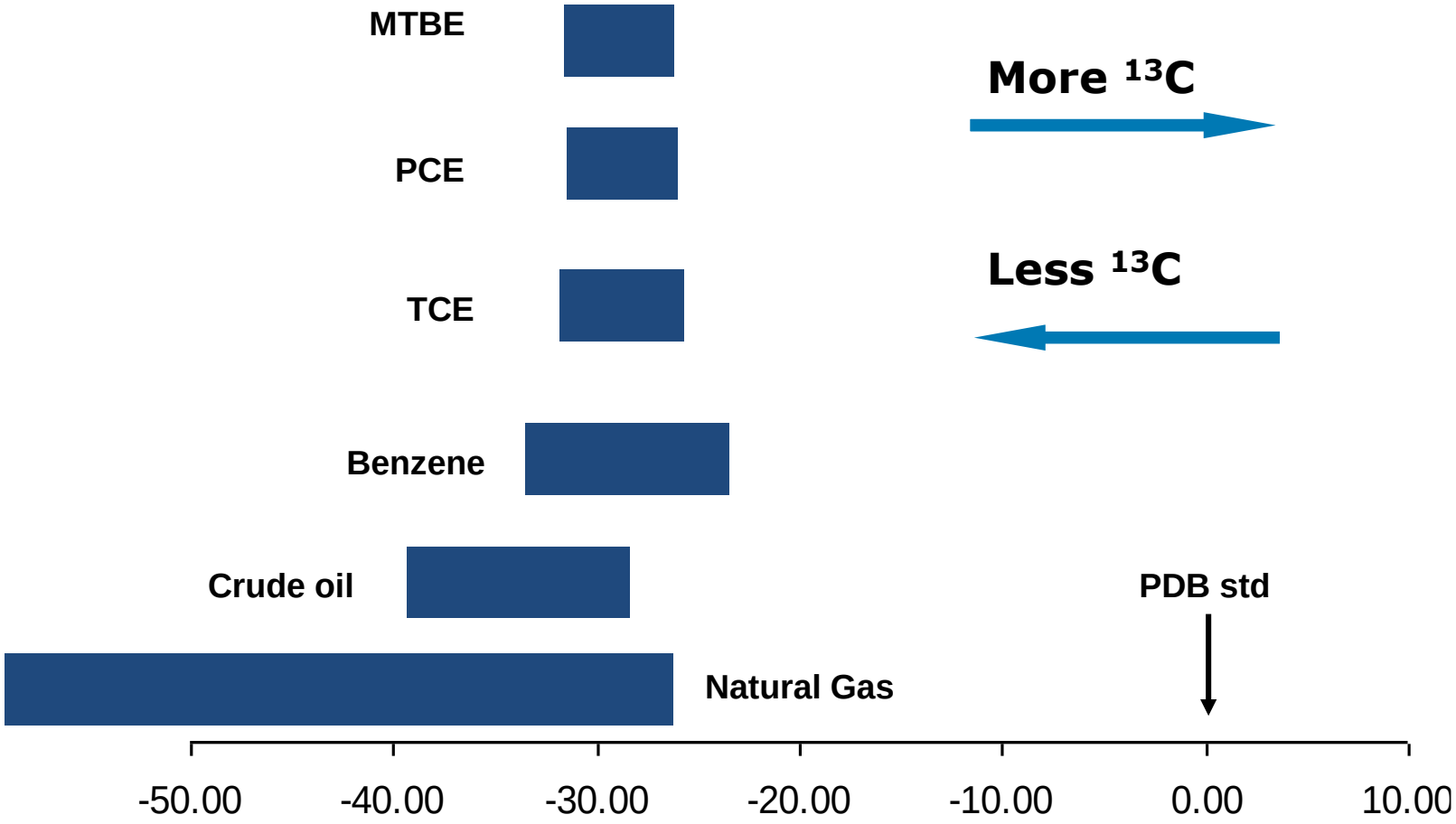
$$\delta^{13}C\,[‰] = \left(\frac{(^{13}C/^{12}C)_{\text{Sample}}}{(^{13}C/^{12}C)_{\text{Standard}}} - 1 \right) \cdot 1000$$

The standard is a specific carbon-containing mineral from a specific location: Pee Dee Belimnite (PDB)

Units of $\delta^{13}C$ are $^{o}/_{oo}$ or "per mill"

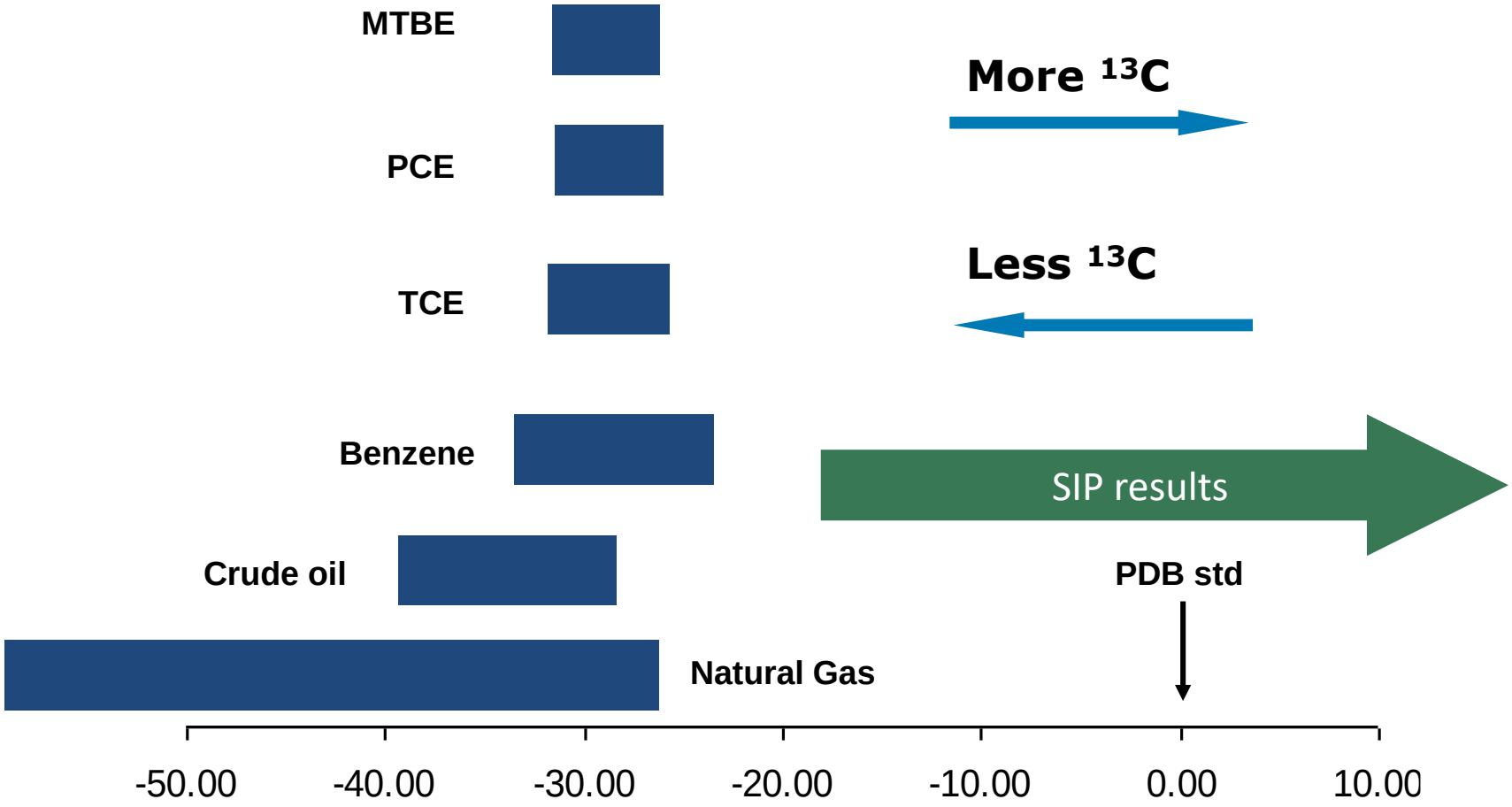
microbialinsights

$\delta^{13}C$ of COCs



microbialinsights

$\delta^{13}C$ of COCs

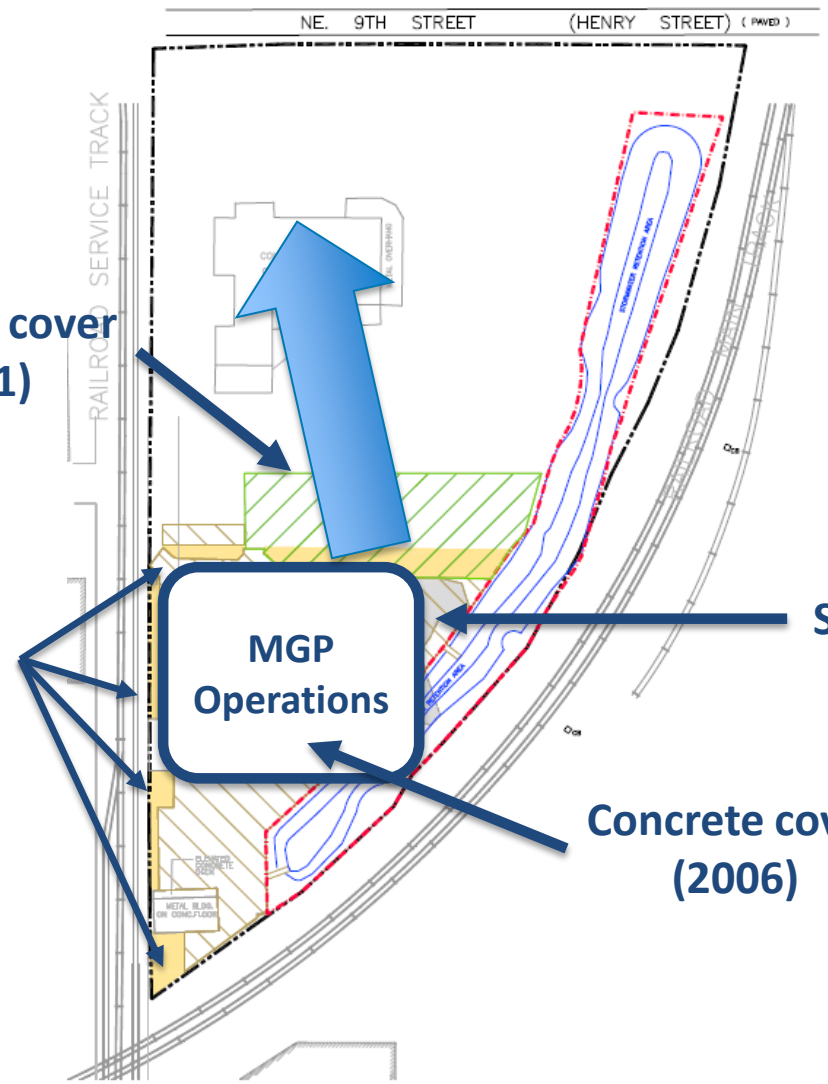


microbialinsights

Investigating the Feasibility of Monitored Natural Attenuation at a Former Manufactured Gas Plant

microbialinsights

Site Map



Concrete cover (2011)

Surface soil removals (2008)

Soil excavation (1990)

Concrete cover (2006)

Benzene, ethylbenzene and PAHs (naphthalenes)

microbialinsights

Groundwater Impacts - PAHs

µg/L

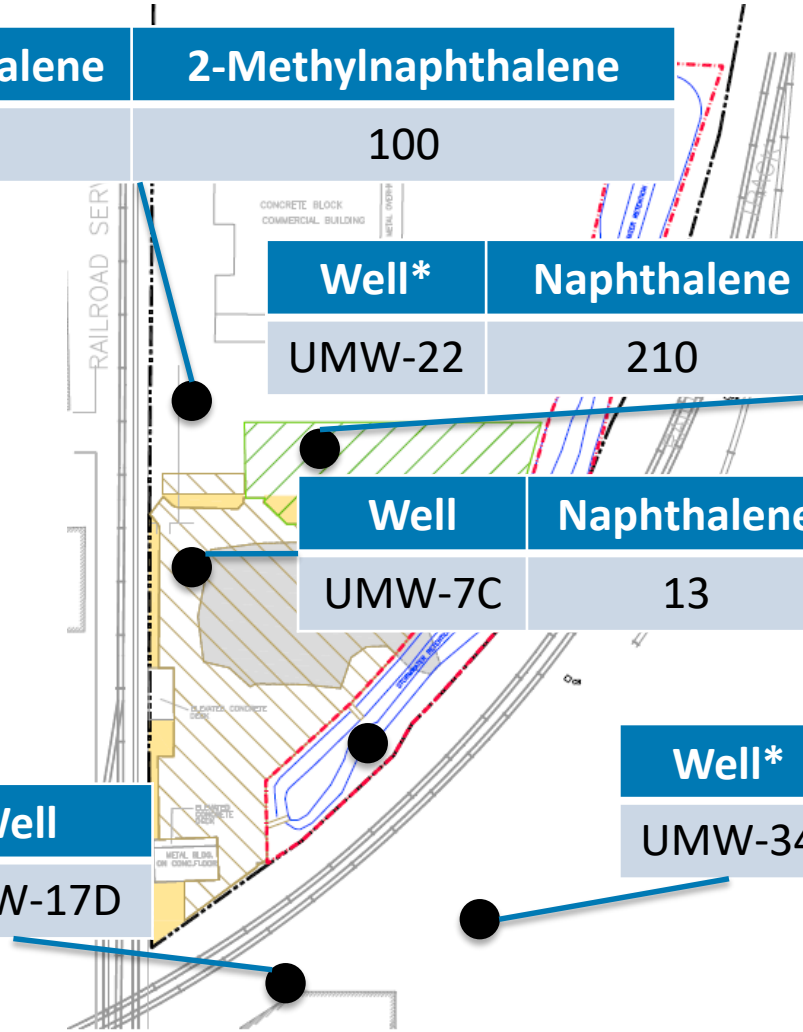
Well	Naphthalene	2-Methylnaphthalene
UMW-44	15	100

Well*	Naphthalene	2-Methylnaphthalene
UMW-22	210	1,700

Well	Naphthalene	2-Methylnaphthalene
UMW-7C	13	1,000

Well
MMW-17D

Well*
UMW-34



* Hard limestone wells

microbialinsights

Contaminant Concentrations

Monitoring Well	Benzene Trend	Naphthalene Trend
UMW-7C	No Trend	Decreasing
UMW-44	No Trend	No Trend
UMW-37	No Trend	Near DL
UMW-6E	Decreasing	Decreasing
UMW-21	Decreasing	No Trend
UMW-22	No Trend	Decreasing

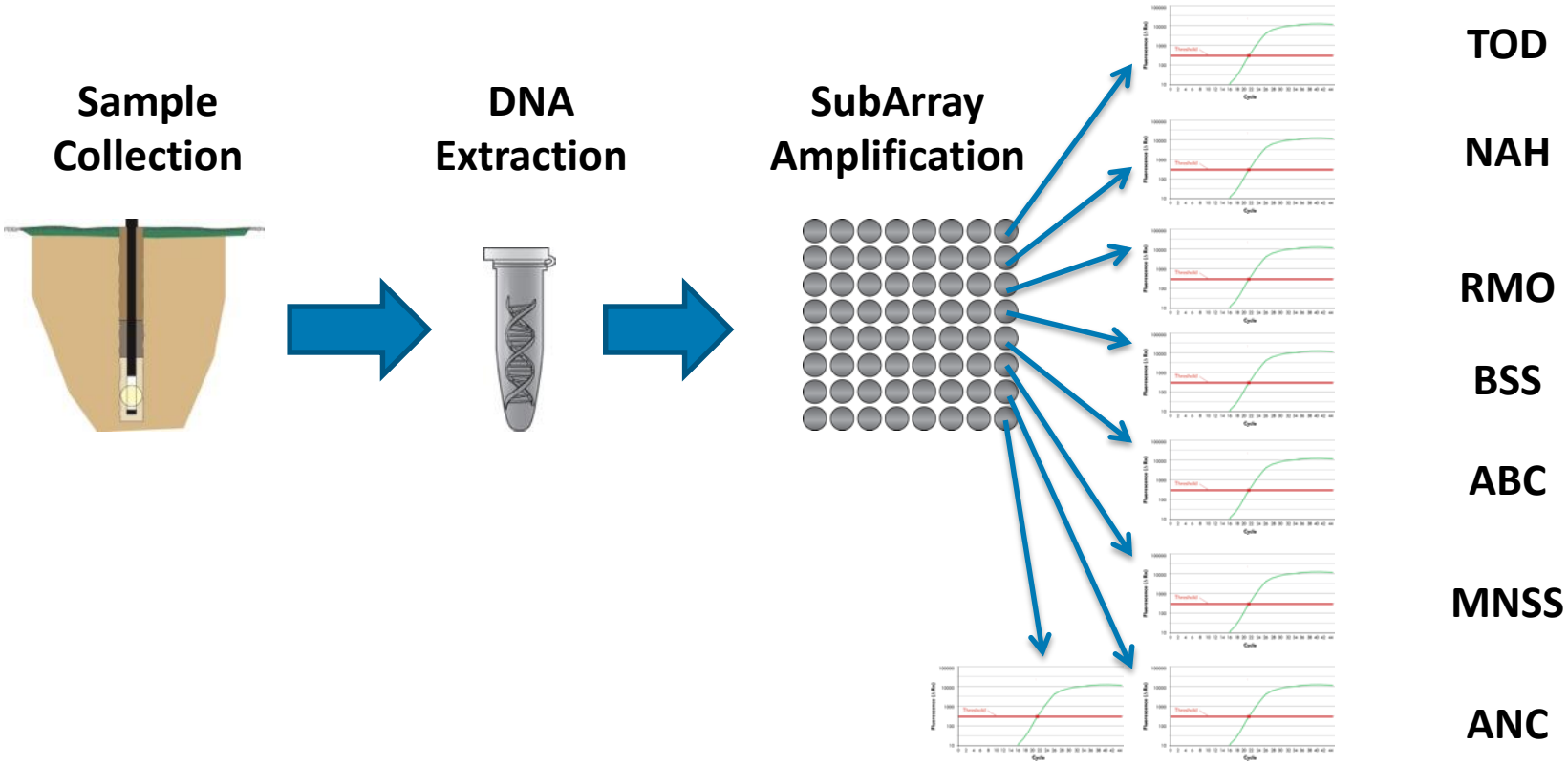
MNA Assessment

- ✓ Contaminant concentrations
- ✓ Geochemistry (utilization of electron acceptors)
- Microbiology

Is biodegradation occurring? → **Stable Isotope Probing (SIP)**

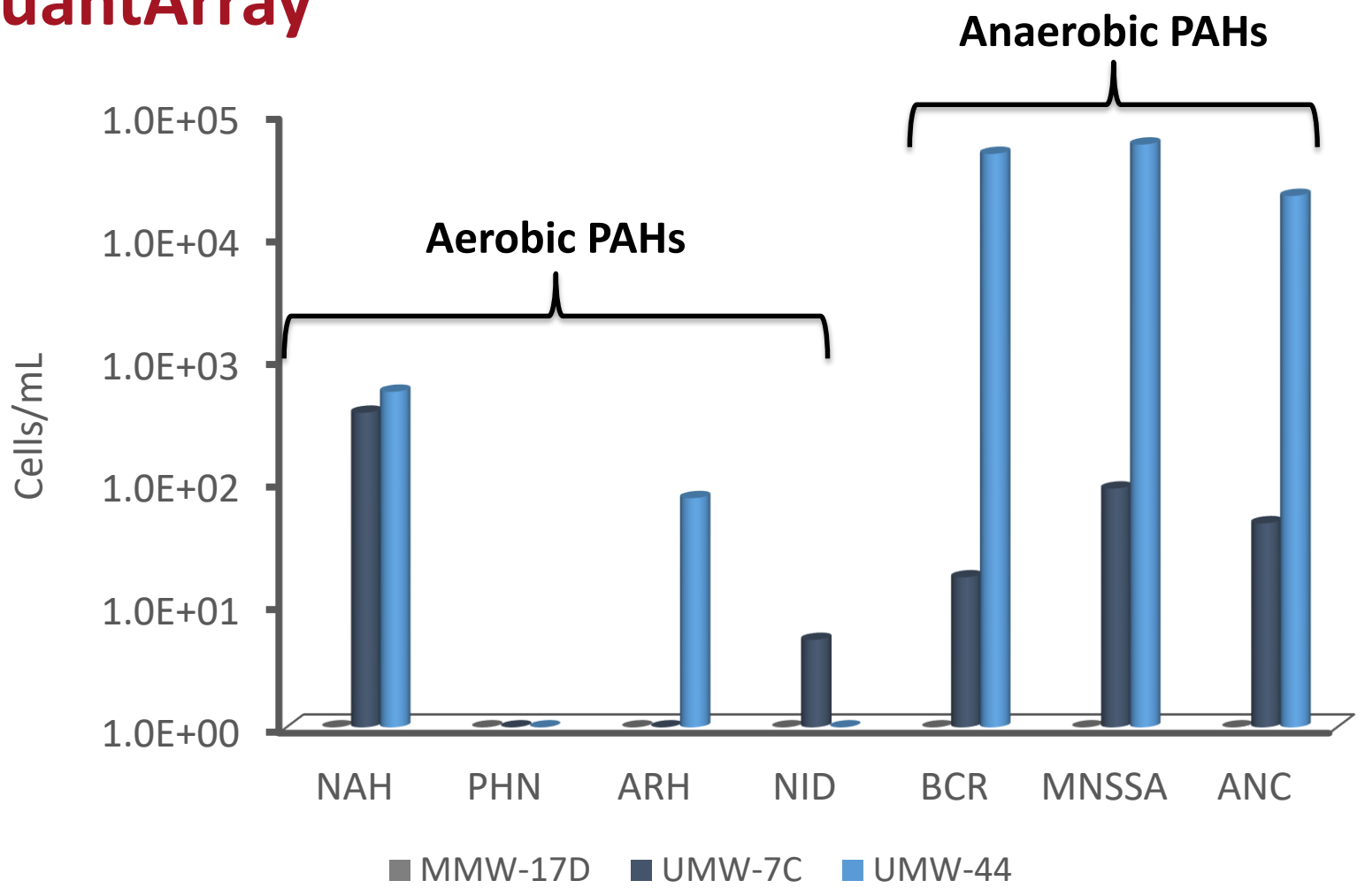
Concentrations of contaminant degrading microorganisms? → **QuantArray qPCR**

QuantArray



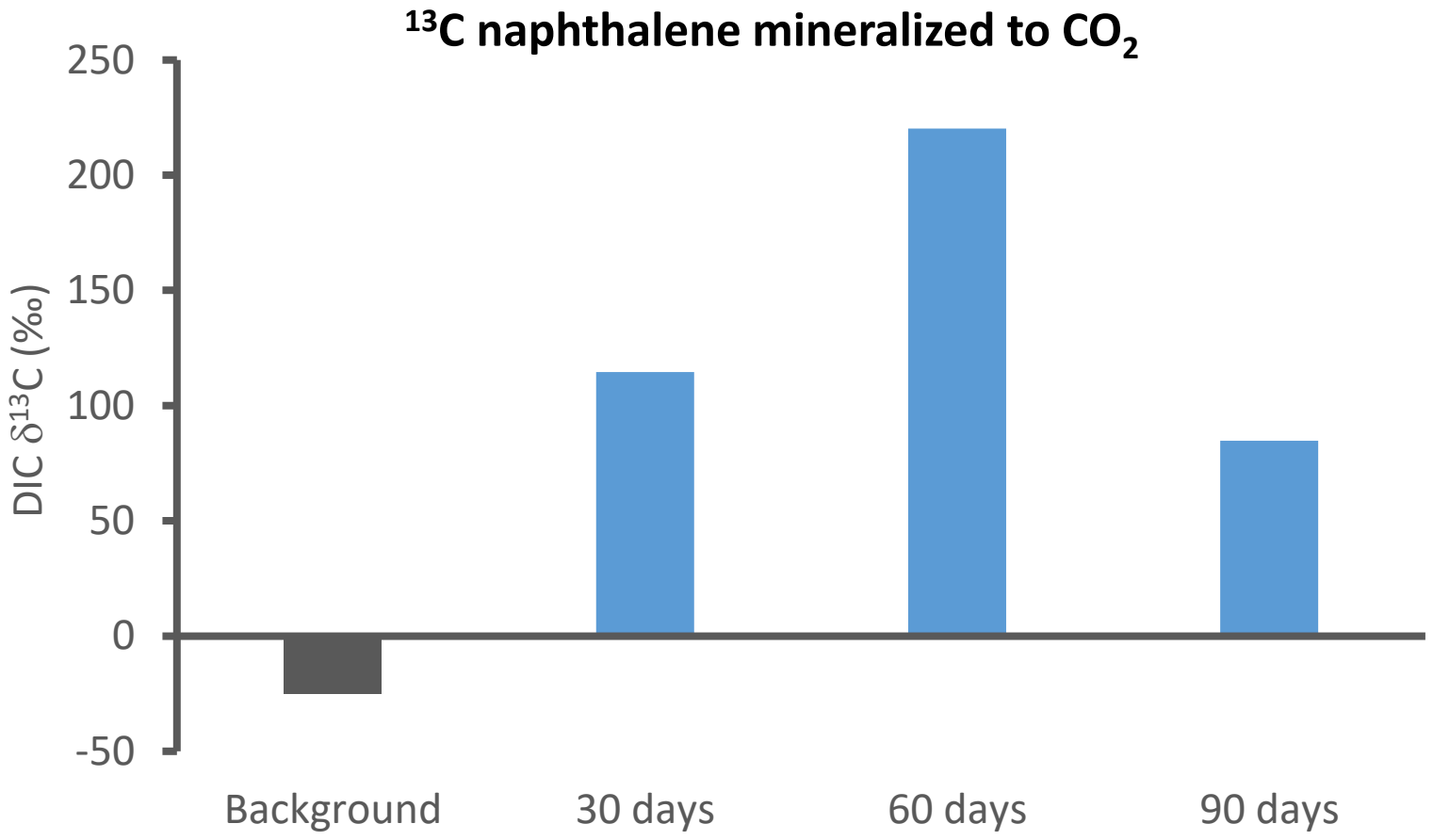
microbialinsights

QuantArray



microbialinsights

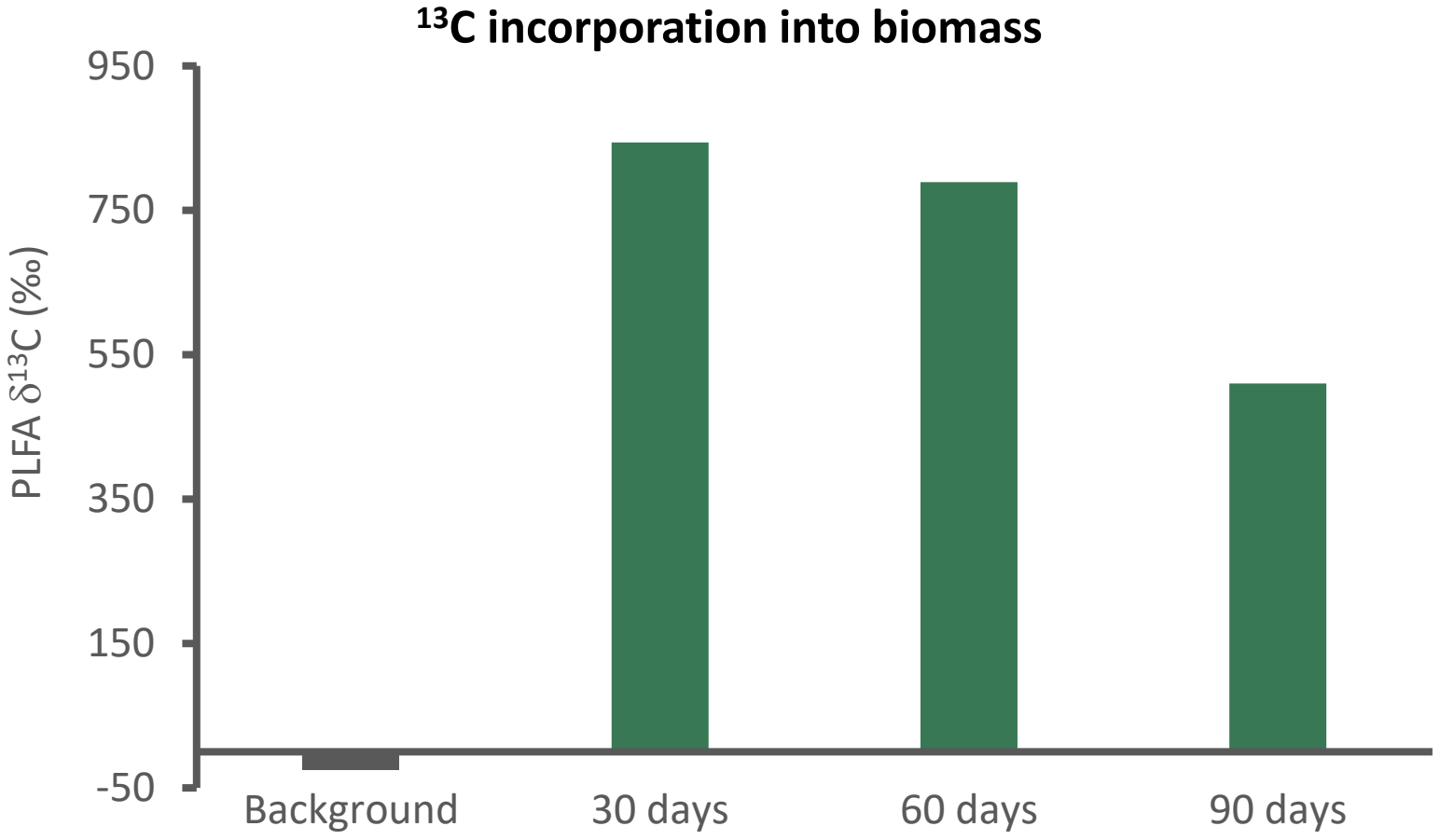
Is naphthalene biodegradation occurring?



UMW-7C

microbialinsights

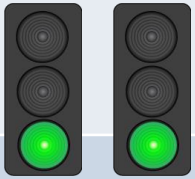
Is naphthalene biodegradation occurring?



UMW-7C

microbialinsights

MNA Assessment

	Chemical	Microbiological	
	Decreasing contaminant concentration?	**Stable Isotope Probing** Did biodegradation occur?	**QuantArray** Concentrations of contaminant degraders?
Naphthalene			

microbialinsights

Stable Isotope Probing (SIP) Bio-Trap Study

Industrial facility in New Jersey evaluating air sparging for a p-xylene contaminated site

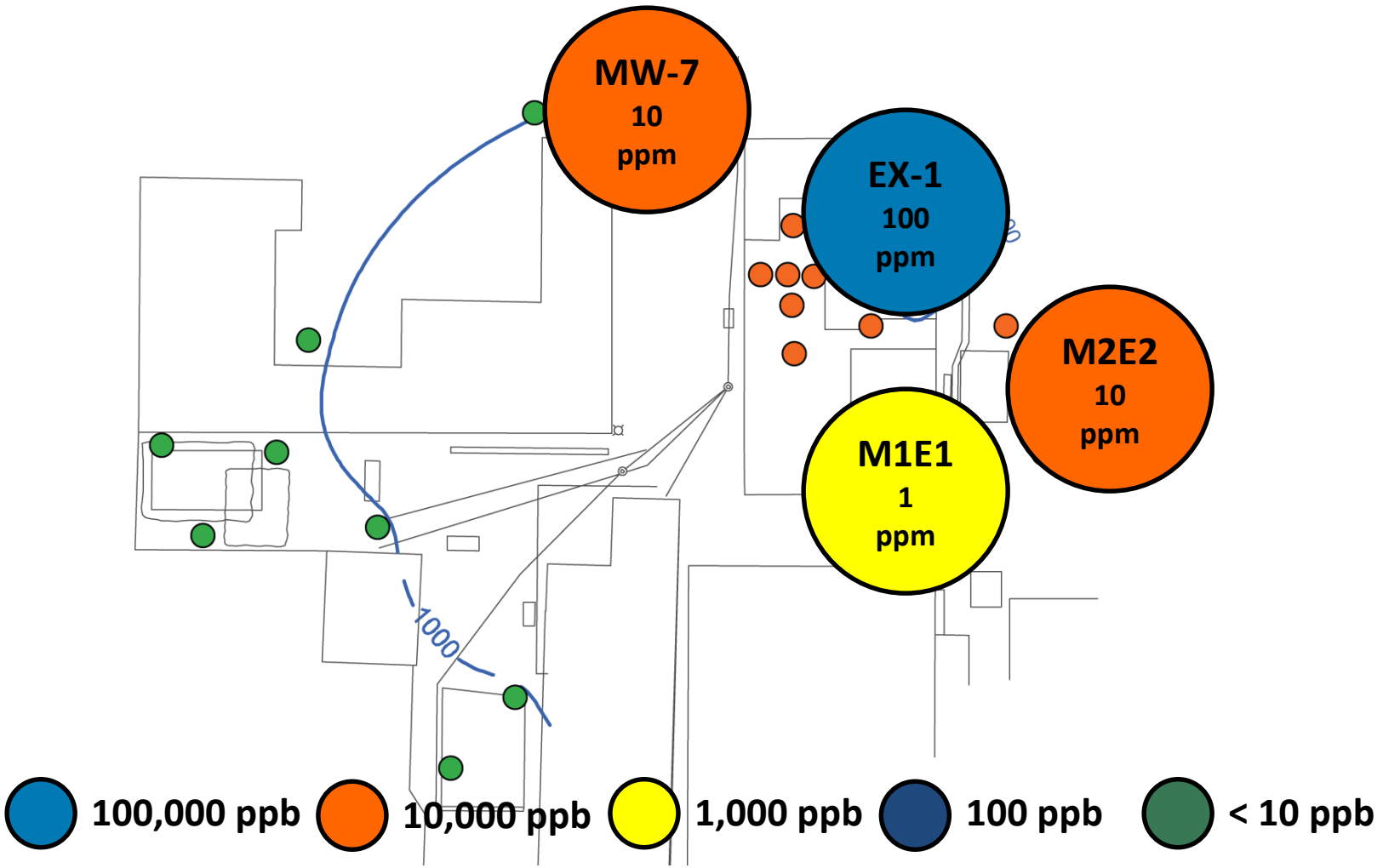
microbialinsights

Bio-Trap Sampler Overview

- Bio-Trap samplers baited with ^{13}C-p-xylene
- Deployed in locations where concentration varied **(100, 10, and 1 ppm)**
- Samplers were analyzed using SIP
- Samplers were deployed for ~30 days.

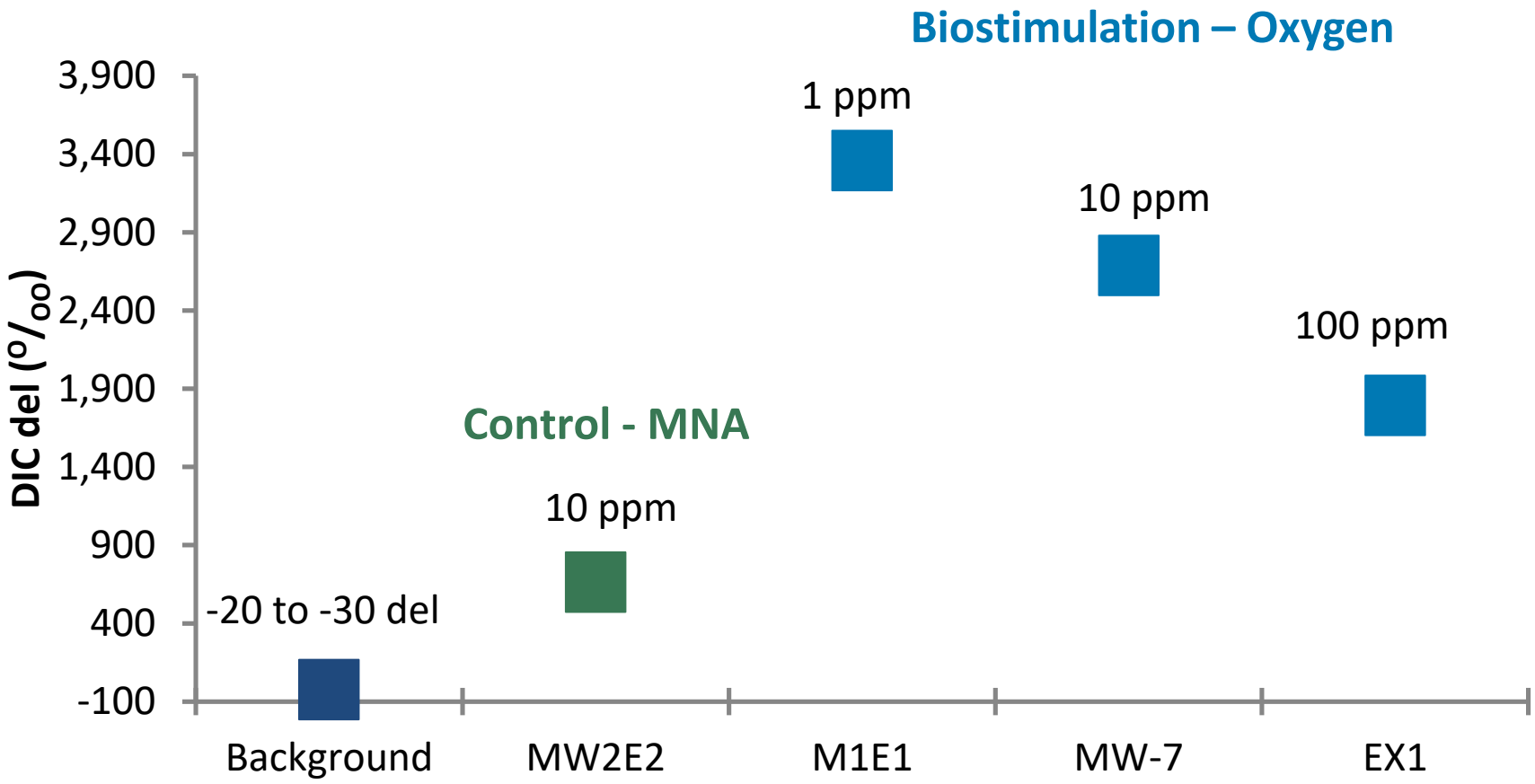
microbialinsights

Xylene Concentration Map



microbialinsights

Bio-Trap Results - Respiration



microbialinsights

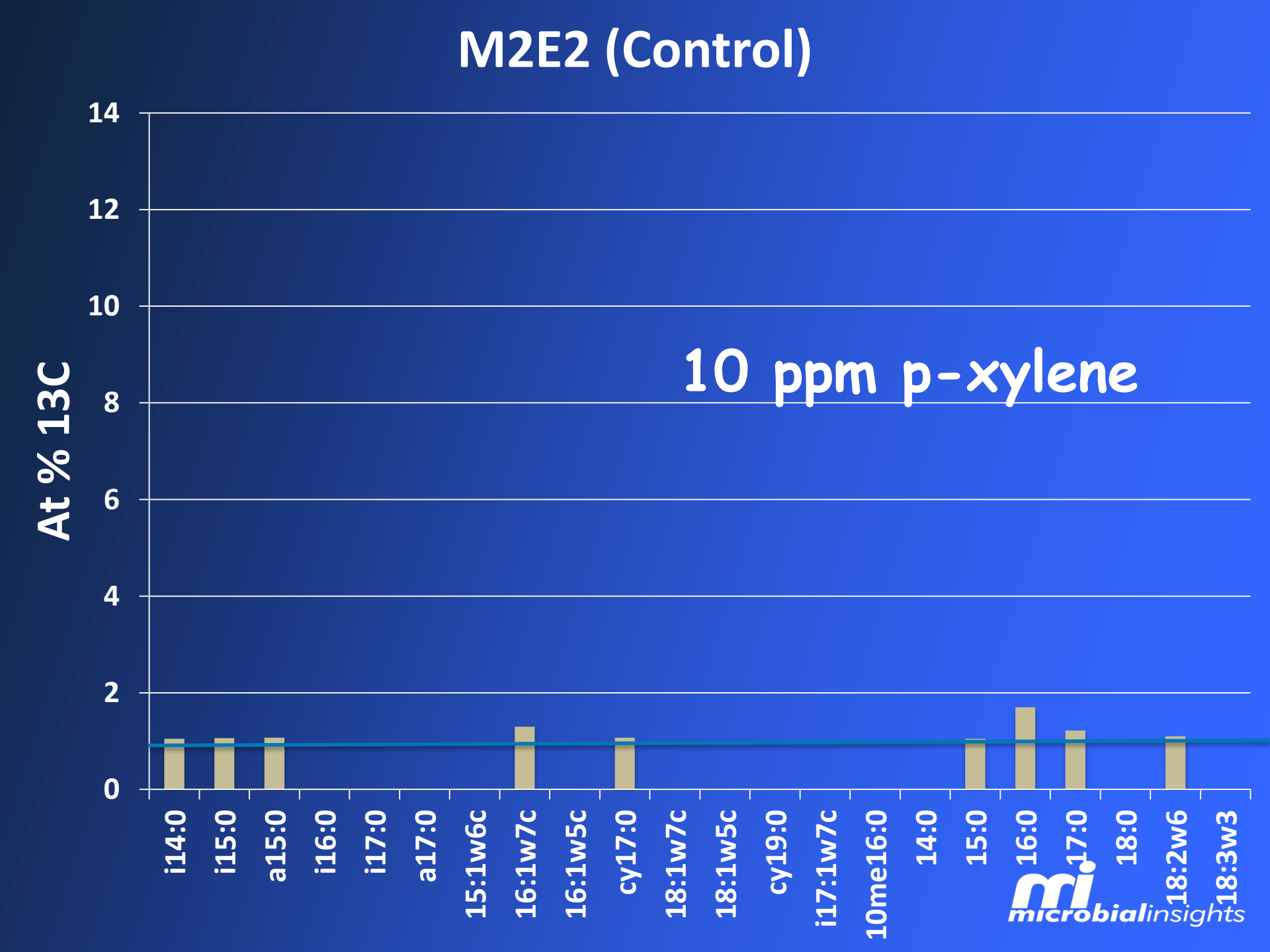
Bio-Trap Results - Metabolism

$^{13}C/^{12}C$ of Biomarkers

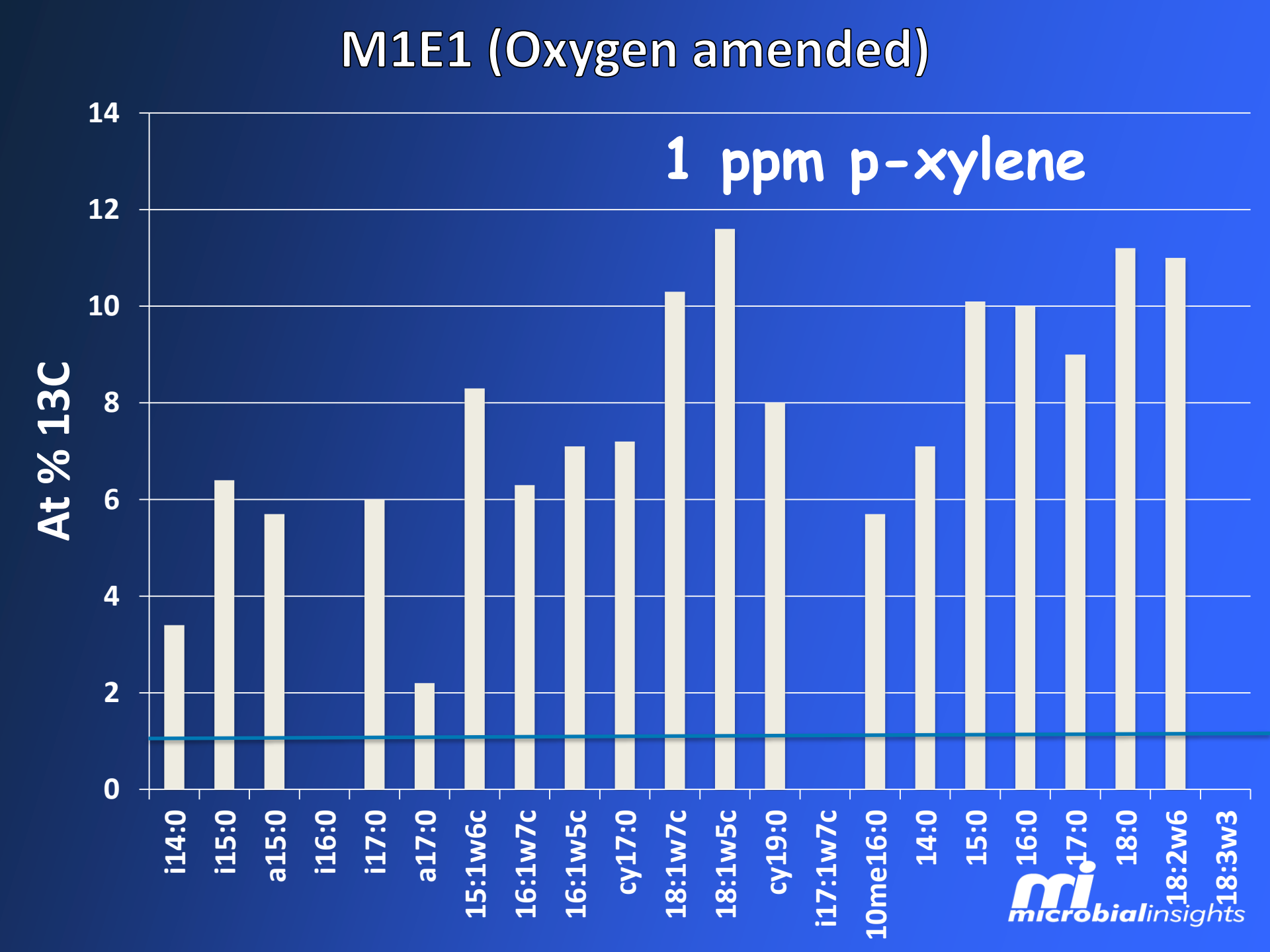
	Biomass (cells/bead)			Del Values		
Sample	Total	13C Enriched	%	**Average**	Minimum	Maximum
Control - MNA						
M2E2	3.27E+05	2.15E+03	1%	**+48**	-50	+547
Biostimulation – Oxygen						
M1E1	2.88E+07	2.14E+06	7%	**+6,288**	+1,009	+10,764
MW-7	2.00E+07	6.24E+05	3 %	**+1,624**	+348	+3,878
EX1	6.77E+07	2.17E+06	3%	**+1,739**	+619	+3,521

microbialinsights

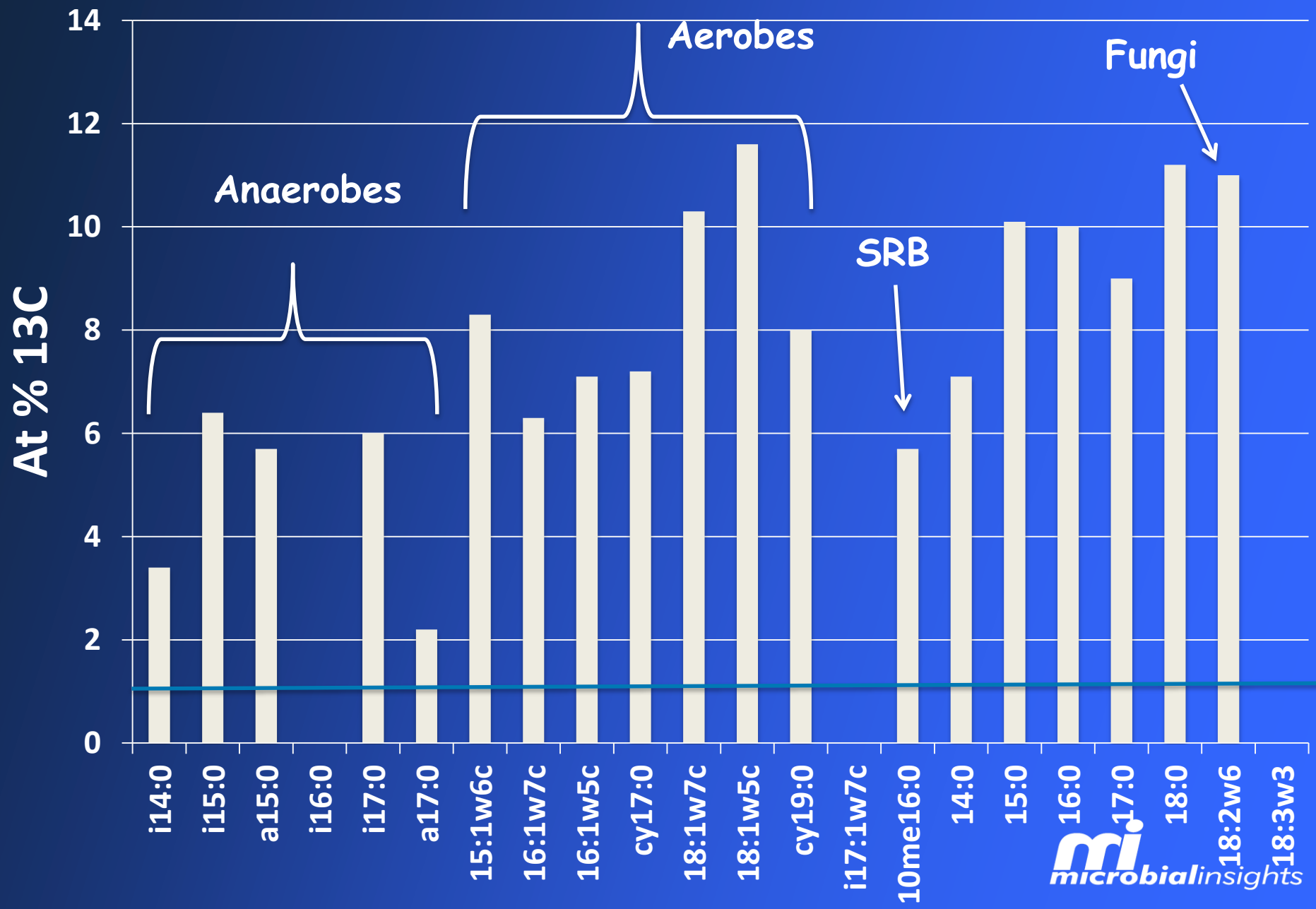
M2E2 (Control)



M1E1 (Oxygen amended)



M1E1 (Oxygen amended)



Bio-traps and Hydrocarbon Impacted Sites

Is biodegradation occurring? → **Stable Isotope Probing (SIP)**

Detect and quantify degraders (Functional genes) → **QuantArray qPCR**

For a copy of this presentation email kerry-sublette@utulsa.edu

For more information go to *microbe.com*

microbialinsights