

Stable Isotope Probing to Demonstrate Contaminant Biodegradation

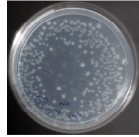
Kerry Sublette, Dora Taggart, Brett Baldwin, Anita Biernacki, Kate Clark, Kerry Sublette





Why do we need MBTs?

Contaminant concentrations and geochemistry don't always provide the complete picture.

Plate counts do not accurately reflect *in situ* microbial community




< 1 % of bacteria can be cultured in the laboratory




Questions that MBTs can answer

Is biodegradation occurring?	What is the concentration of contaminant degraders?	What microorganisms are present?
Stable Isotope Probing	qPCR	Next Generation Sequencing (metagenomics)
Compound Specific Isotope Analysis	QuantArray	



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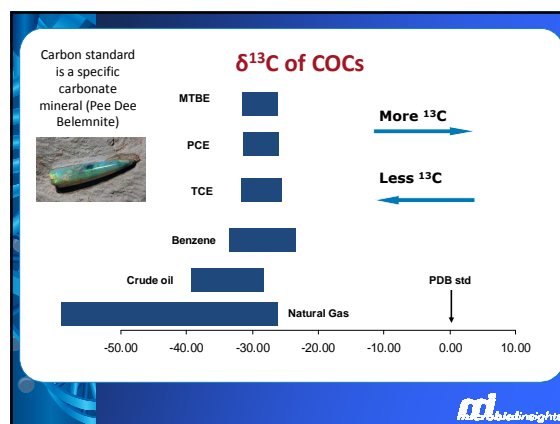


SIP vs. CSIA

- Both used to demonstrate biodegradation
- Both based on isotopic analysis (mostly ^{13}C)
- Same notation and units for expressing ^{13}C content

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

Units of $\delta^{13}\text{C}$ (delta thirteen C) are ‰ or “per mill”

How are they different?

SIP

Source of ^{13}C added as tracer
We ask where does it go? and What does that mean?

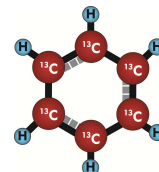
CSIA

Naturally occurring ^{13}C only
We ask has the ^{13}C content of a compound changed over time? and What does that mean?



SIP: The Tracers

- 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 a “probe” or “tracer” to determine if biodegradation is occurring
 - If biodegradation occurs, the ^{13}C will be incorporated biomass or mineralized to $^{13}\text{CO}_2$.

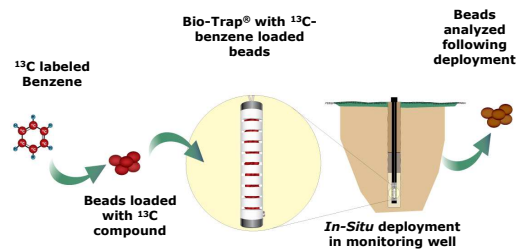


How is it Done? Bio-Trap® Samplers

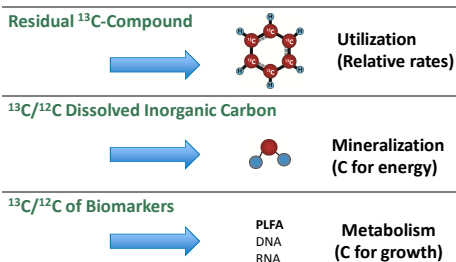
- Passive microbial sampling tool
- Colonized by active microbes
- 25% Nomex and 75% PAC
- Used in conjunction with
 - Stable isotope probing
 - qPCR and QuantArray
 - Other MBTs



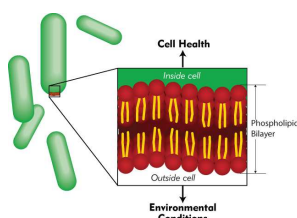
Overview of Bio-Trap SIP Approach



Bio-Trap SIP Analysis

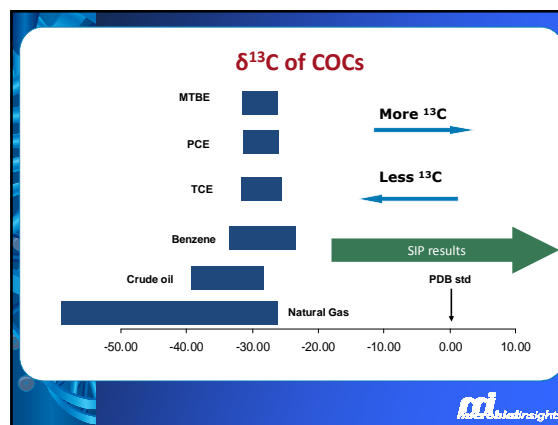
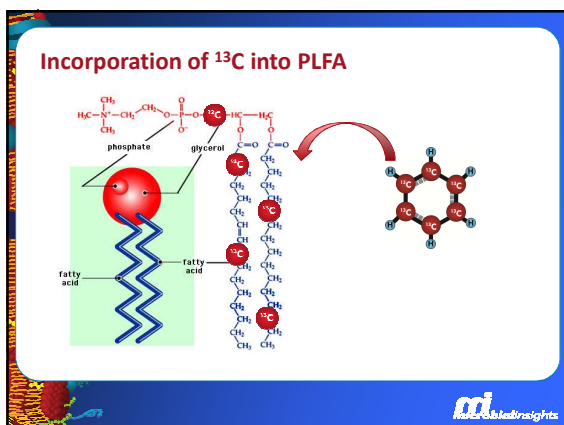


What are phospholipids?



- Phospholipid fatty acids (PLFA) are a main component of the cell membrane of all bacteria.
- The “skin” of a bacterial cell



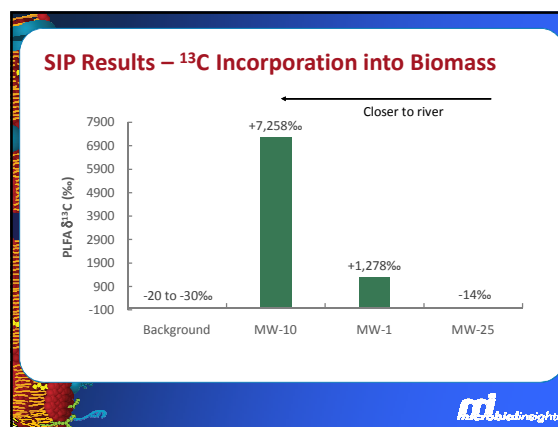
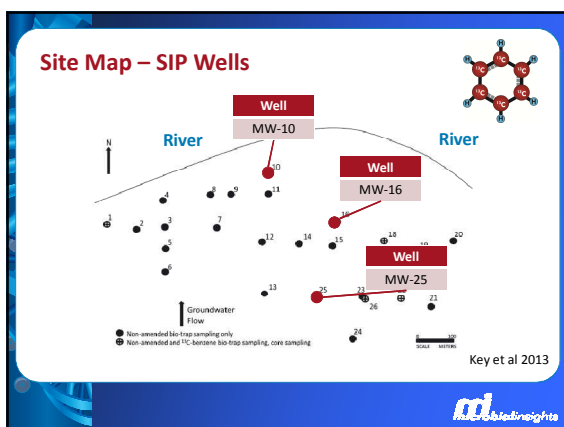


Stable Isotope Probing Evaluating MNA at a Refinery

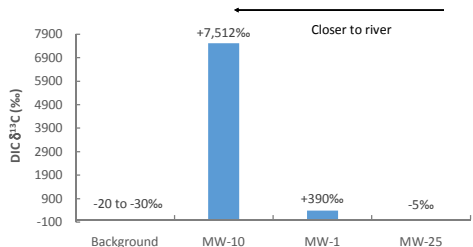
Site Background

- Operating refinery
- Historical releases of gasoline, diesel and naphtha
- NAPL present
- Groundwater influenced by river

Key et al 2013. (Kate Clark)
Groundwater Monitoring and Remediation 33(4): 57-68



SIP Results – ^{13}C Mineralization



Conclusions

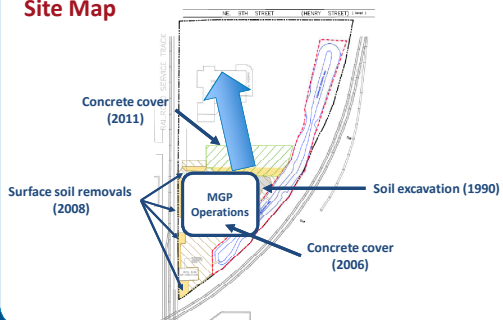
- SIP provided direct evidence of benzene biodegradation in 6 out of 8 wells studied.
- Highest levels of ^{13}C incorporation into biomass and DIC observed at wells closer to river
- Periodic influx of oxygen and nutrients

Stable Isotope Probing Evaluating MNA at a MGP Site

Site Background

- Former manufactured gas plant (MGP)
- Operated from 1890s until 1953
- NAPL present
- Benzene, ethylbenzene
- **Naphthalene**, methylnaphthalenes, and other PAHs

Site Map



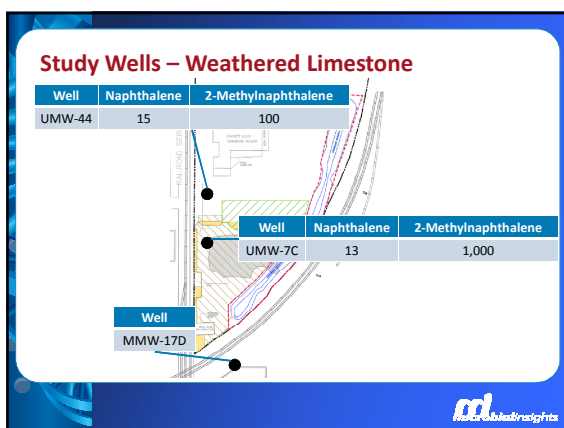
- Tar staining at discrete intervals in a peratures
- Vertical migration



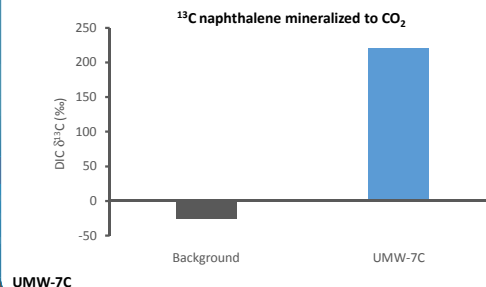


Contaminant Concentrations

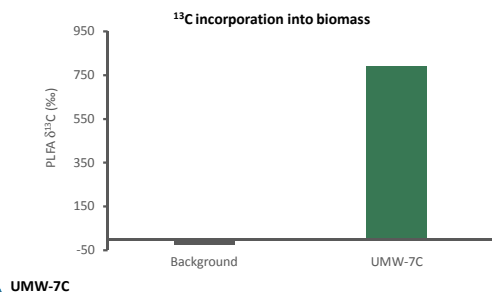
Monitoring Well	Benzene Trend	Naphthalene Trend
UMW-7C	No Trend	Decreasing
UMW-44	No Trend	No Trend
UMW-37	No Trend	Near DL
UMW-6E	Decreasing	Decreasing
UMW-21	Decreasing	No Trend
UMW-22	No Trend	Decreasing



Is naphthalene biodegradation occurring?



Is naphthalene biodegradation occurring?



SIP Strengths

- Conclusive evidence of current *in situ* biodegradation activity **under actual aquifer conditions**
- Little prior knowledge needed
 - Don't need to know what organisms are involved
 - Don't need to know biodegradation pathway
 - Just need to know that the contaminant is likely to be used as a carbon and energy source
- Can be used in source areas with NAPL

SIP Strengths

- Inexpensive for many common contaminants
- Broad applicability
 - BTEX
 - MTBE, TBA
 - Naphthalene
 - Chlorobenzene
 - Emerging contaminants (dioxane, sulfolane)



SIP Limitations

- Analysis of ^{13}C -DNA required for identification of degraders
- Large or complex ^{13}C compounds may be more expensive or not available
- Not applicable to contaminants used as electron acceptors (like chlorinated ethenes under anaerobic conditions)



The End

