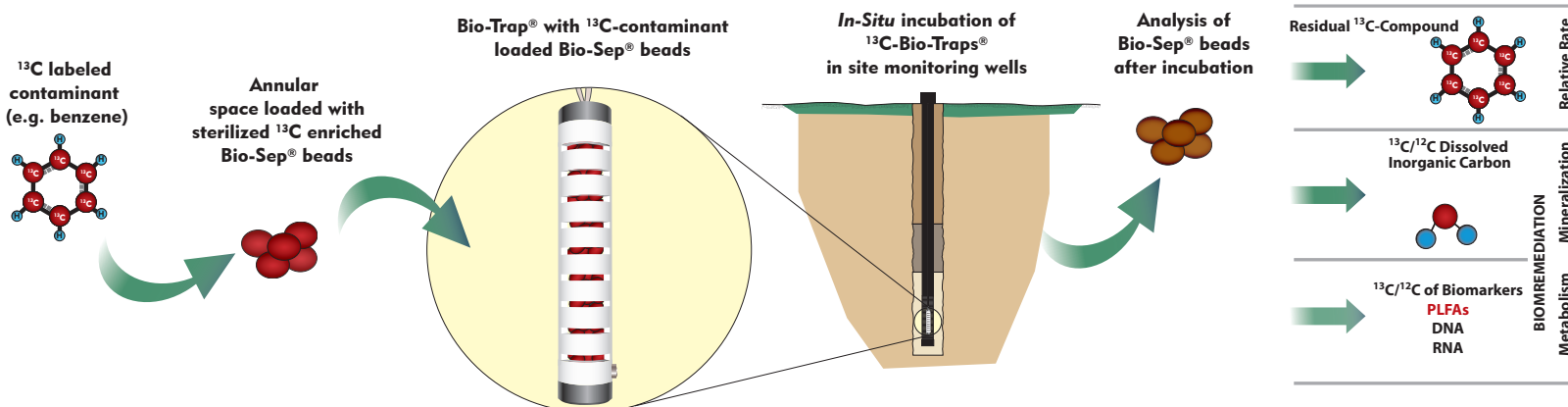




MOLECULAR BIOLOGICAL TOOL

The big question—Is biodegradation occurring at the site?

**Stable Isotope Probing (SIP)** is an innovative method to track the environmental fate of a “ $^{13}\text{C}$ -labeled” contaminant of concern to unambiguously demonstrate biodegradation. The label serves as a tracer which can be detected in the end products of biodegradation (new biomass and  $\text{CO}_2$  or dissolved inorganic carbon).



## SIP Applications

- **Assessing monitored natural attenuation (MNA)**—Incorporation of the  $^{13}\text{C}$  label into biomass and dissolved inorganic carbon (DIC) conclusively demonstrates that biodegradation of the contaminant is occurring in situ.
- **Evaluating enhanced bioremediation**—Greater levels of  $^{13}\text{C}$  incorporation into biomass and DIC relative to a control demonstrate that the addition of the amendment (electron acceptor, nutrients, etc.) promoted biodegradation.

## How does SIP work?

- Bio-Traps<sup>®</sup> are “baited” with a specially synthesized form of the contaminant containing “heavy” carbon ( $^{13}\text{C}$ ) as the label.

- Since  $^{13}\text{C}$  is rare, carbon originating from the labeled contaminant is readily distinguished from carbon (predominantly  $^{12}\text{C}$ ) from other sources.
- Bio-Traps<sup>®</sup> are deployed in a monitoring well and the  $^{13}\text{C}$  labeled contaminant is subject to the same physical, chemical, and microbiological processes as the site contaminants.
- Following in-well deployment, the Bio-Traps<sup>®</sup> are recovered and two methods are used to document in situ biodegradation of the contaminant.

**Phospholipid Fatty Acids (PLFA)**—PLFA are a major component in the membranes of all microbes, thus, incorporation of the  $^{13}\text{C}$  label into PLFA unequivocally shows incorporation of the contaminant into biomass.

**Dissolved Inorganic Carbon (DIC)**—Enrichment of  $^{13}\text{C}$  labeled DIC ( $\text{CO}_2$  and carbonates) demonstrates contaminant mineralization.

SIP studies can be performed for any compound that microbes use as a carbon source. Some of the more common include:

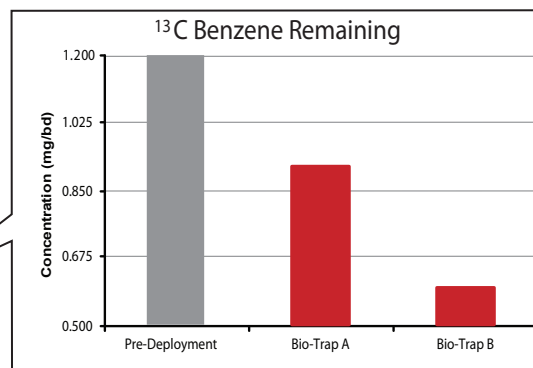
- Benzene
- MTBE (methyl tert-butyl ether)
- TBA (tert-butyl alcohol)
- Chlorobenzene
- Toluene
- Xylenes
- Naphthalene

## Example Stable Isotope Probing (SIP) Results

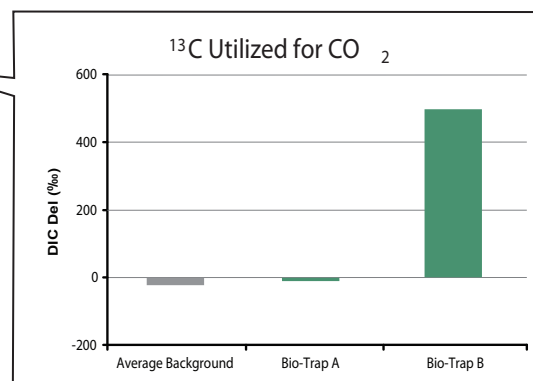
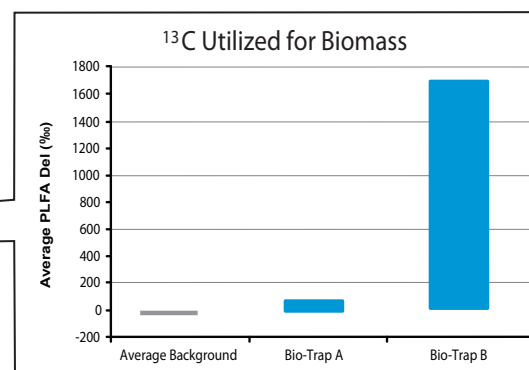
Probably the most common application of the SIP method is demonstrating that biodegradation of a particular contaminant is occurring in situ under monitored natural attenuation (MNA) conditions. In this example, Bio-Trap A and Bio-Trap B were baited with  $^{13}\text{C}$  labeled benzene and deployed in existing monitoring wells representing different zones of the dissolved plume

Question: Is benzene biodegradation occurring?

Sample Name	Bio-Trap A	Bio-Trap B
<b><math>^{13}\text{C}</math> Contaminant Loss</b>		
Benzene Pre-deployment (mg/bd)	1.2	1.2
Benzene Post-deployment (mg/bd)	0.9	0.6
% Loss	24%	50%
<b>Biomass &amp; <math>^{13}\text{C}</math> Incorporation</b>		
Total Biomass (Cells/bd)	3.53E+04	1.15E+05
$^{13}\text{C}$ Enriched Biomass (Cells/bd)	6.58E+01	3.30E+03
% $^{13}\text{C}$ Incorporation	0.19%	2.87%
Average PLFA Del ( ‰)	76	1,710
Maximum PLFA Del ( ‰)	122	3,018
<b><math>^{13}\text{C}</math> Mineralization</b>		
DIC Del ( ‰)	-12	506
% $^{13}\text{C}$	1.09	1.66



Comparison of Pre- and Post- Deployment  $^{13}\text{C}$  benzene concentrations are used to document loss of the contaminant.



Although  $^{13}\text{C}$  incorporation into biomass demonstrated that benzene biodegradation was occurring at both locations, contaminant incorporation into biomass was substantially greater in Bio-Trap B which was consistent with a greater decrease in benzene concentration.

### Conclusions

- Results of the SIP study conclusively demonstrates that benzene biodegradation is occurring.

What is a Del (‰) Value? The del value represents the isotopic ratio ( $^{13}\text{C}/^{12}\text{C}$ ) of the sample compared to a standard. When biodegradation of the  $^{13}\text{C}$  labeled contaminant is occurring, the  $^{13}\text{C}/^{12}\text{C}$  ratio and thus the del value of the PLFA biomass and DIC will increase above background values.

Similarly, incorporation of  $^{13}\text{C}$  into DIC was moderate in Bio-Trap B while only minor mineralization was observed in Bio-Trap A.