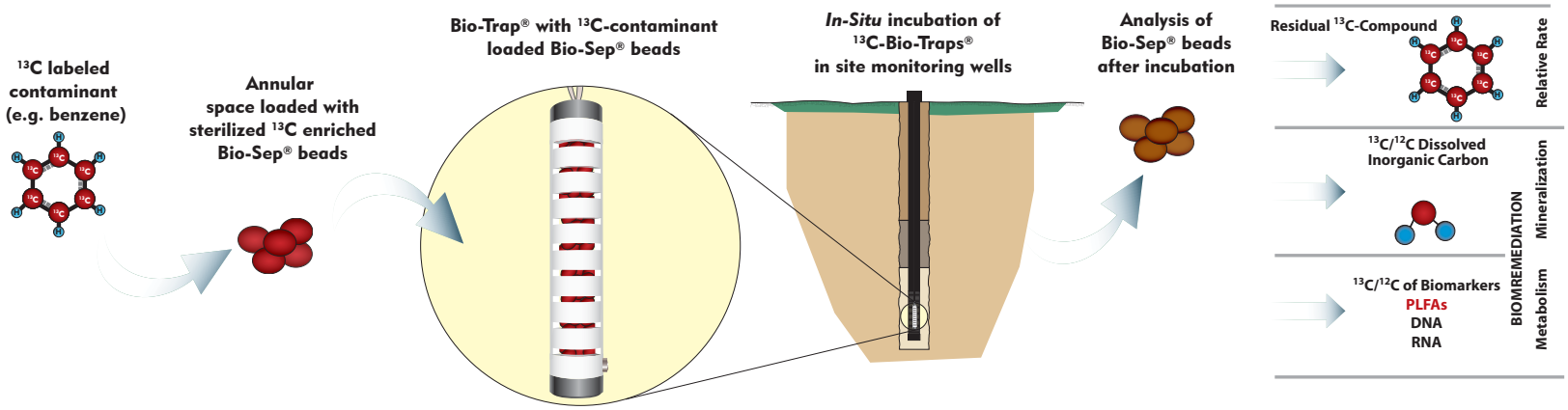
miSIP

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}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 CO_2 or dissolved inorganic carbon).



SIP Applications

- **Assessing monitored natural attenuation (MNA)** — Incorporation of the ^{13}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}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® are "baited" with a specially synthesized form of the contaminant containing "heavy" carbon (^{13}C) as the label.
- Since ^{13}C is rare, carbon originating from the labeled contaminant is readily distinguished from carbon (predominantly ^{12}C) from other sources.
- Bio-Traps® are deployed in a monitoring well and the ^{13}C labeled contaminant is subject to the same physical, chemical, and microbiological processes as the site contaminants.
- Following in-well deployment, the Bio-Traps® 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}C label into PLFA unequivocally shows incorporation of the contaminant into biomass.

  Dissolved Inorganic Carbon (DIC) — Enrichment of ^{13}C labeled DIC (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

and more!

microbialinsights

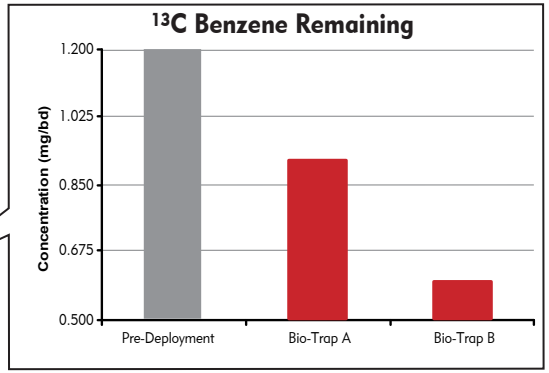
www.microbe.com

Example Stable Isotope Probing (SIP) Results

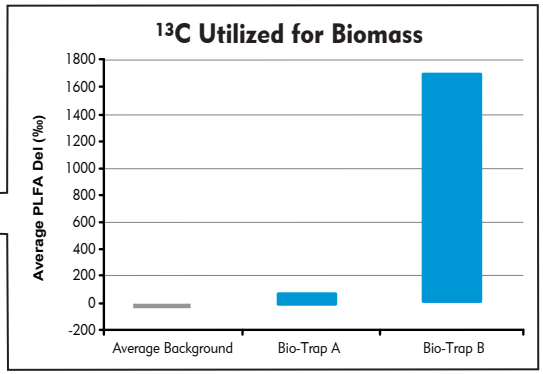
Probably the most common application of the SIP method is demonstrating that biodegradation of a particular contaminant is occurring in situ under monitored natural attenuation (MNA) conditions. In this example, Bio-Trap A and Bio-Trap B were baited with ^{13}C labeled benzene and deployed in existing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

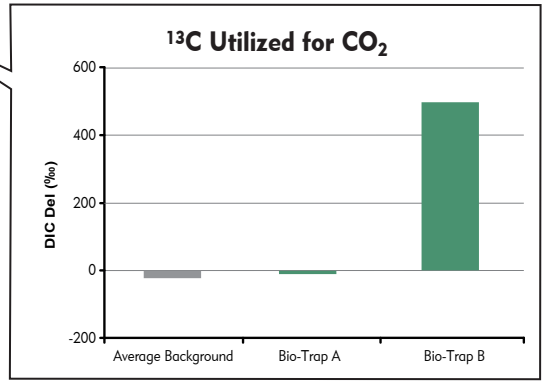
Question: Is benzene biodegradation occuring?

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



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





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

Although ^{13}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.

Answer: Yes, benzene biodegradation is occurring.

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

microbialinsights
www.microbe.com
10515 Research Drive
Knoxville, TN 37932
Phone: 865.573.8188