

## ISM & SIP

Combining *In Situ* Microcosms and Stable Isotope Probing



### PROJECT SUMMARY



- An *In Situ* Microcosm (ISM) study was conducted to evaluate MNA and enhanced aerobic bioremediation at a chlorobenzene impacted site.
- For both the MNA and Oxygen Amended ISM units, stable isotope probing (SIP) was performed to conclusively evaluate chlorobenzene biodegradation.
- The SIP results demonstrated that chlorobenzene biodegradation was occurring during MNA but also indicated that oxygen addition could enhance biodegradation.

### PROJECT CHALLENGE



A former industrial facility impacted by chlorobenzene was undergoing monitored natural attenuation (MNA). Site managers wanted to determine whether observed decreases in chlorobenzene concentrations were due to biodegradation or dilution. Injection of an oxygen releasing product (e.g. ORC<sup>®</sup>, PermeOx<sup>®</sup>) was also being considered. Site managers needed to conclusively determine whether chlorobenzene biodegradation was occurring under existing conditions and to cost effectively evaluate MNA and enhanced aerobic biodegradation as remediation strategies.

### IN SITU MICROCOSMS AND ANALYSIS



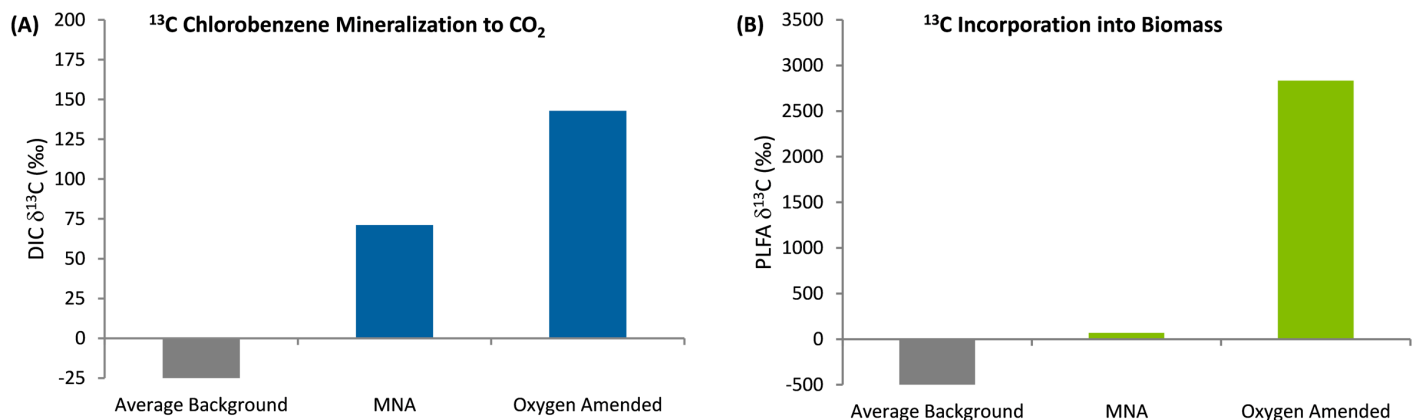
*In Situ* Microcosms (ISMs) are field deployed microcosm units, each representing a different treatment strategy. In this study, ISMs composed of an unamended MNA unit and a BioStim unit amended with an oxygen releasing product were deployed in existing monitoring wells for approximately 40 days, then retrieved and shipped to Microbial Insights for analysis.

ISM units contain passive samplers for post-deployment analyses to determine the effectiveness of each remediation strategy. In this case, stable isotope probing (SIP) was used to conclusively evaluate chlorobenzene biodegradation. Both ISM units contained Bio-Traps<sup>®</sup> amended with <sup>13</sup>C chlorobenzene. If chlorobenzene biodegradation occurred, the <sup>13</sup>C “label” would be detected in the end products of biodegradation – microbial biomass and/or CO<sub>2</sub>.

SIP RESULTS FOR *IN SITU* MICROCOSM UNITS

In a SIP study, the detection of  $^{13}\text{C}$  enriched dissolved inorganic carbon (DIC) demonstrates mineralization of the contaminant to  $\text{CO}_2$ . Similarly, the detection of  $^{13}\text{C}$  enriched phospholipid fatty acids (PLFA), a major component of bacterial cell membranes, demonstrates incorporation of contaminant carbon into microbial biomass.

Thus, the detection of  $^{13}\text{C}$  enriched DIC and/or  $^{13}\text{C}$  enriched PLFA during a SIP study conclusively demonstrates *in situ* biodegradation of the contaminant of concern.



**MNA Unit:** As shown in Figures 1A and 1B, the DIC  $\delta^{13}\text{C}$  (71‰) and PLFA  $\delta^{13}\text{C}$  (70‰) values were much greater in the MNA unit than typical background levels of -25‰ demonstrating  $^{13}\text{C}$  chlorobenzene mineralization and incorporation into biomass, respectively.

- Although relatively low,  $^{13}\text{C}$  incorporation into DIC and biomass in the MNA unit conclusively demonstrated that chlorobenzene biodegradation occurred under existing site conditions.

**Oxygen Amended Unit:** Levels of  $^{13}\text{C}$  chlorobenzene mineralization (143‰) and  $^{13}\text{C}$  incorporation into biomass PLFA (2,835‰) were considerably greater in the Oxygen Amended unit than in the MNA unit.

- While results for the MNA unit confirmed chlorobenzene biodegradation under existing conditions, greater levels of  $^{13}\text{C}$  incorporation in the Oxygen Amended Unit indicated that oxygen addition could enhance chlorobenzene biodegradation.

**Decision:** Chlorobenzene biodegradation was occurring under existing conditions and MNA was feasible. However, oxygen addition will enhance chlorobenzene biodegradation and may decrease time to closure.

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## KEY BENEFITS



- **Conclusive:** Demonstrated *in situ* biodegradation of chlorobenzene under existing site conditions.
- **Actionable:** While MNA was feasible, oxygen addition would enhance biodegradation.
- **Saved Money:** Screened MNA and enhanced bioremediation as treatment strategies for a fraction of the cost of a pilot or lab study.

## LAB LOCATIONS



### **Microbial Insights, Inc. USA**

10515 Research Drive, Knoxville, TN 37932 USA

### **Microbial Insights Canada, c/o EBPI**

735 Griffith Court, Burlington Ontario, L7L 5R9

### **Microbial Insights (Australia) Pty Ltd, c/o AGRF Ltd**

Plant Genomics Centre, Hartley Grove, Urrbrae SA 5064, Australia

### **Microbial Insights Europe, c/o Avecom**

Industrieweg 122P, B-9032 Wondelgem, Belgium

### **Microbial Insights Europe (Germany), c/o Sensatec**

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### **Microbial Insights China, Xiuying Li (cell# 13204027102)**

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