# STABLE ISOTOPE PROBING TO DOCUMENT BIODEGRADATION UNDER MONITORED NATURAL ATTENUATION CONDITIONS

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## INTRODUCTION

Depending on site conditions including contaminant concentrations, availability of electron donors or acceptors, groundwater velocity, and proximity to potential receptors, monitored natural attenuation (MNA) can be an effective remediation strategy. However, MNA is sometimes viewed as a "do nothing" solution in which decreases in contaminant concentrations result from physical processes (e.g. dilution) rather than biodegradation. Ultimately, the feasibility and regulatory acceptance of MNA as a remediation strategy rests upon demonstrating contaminant biodegradation under existing site conditions.

Stable isotope probing (SIP) is an innovative method to track the environmental fate of a "labelled" contaminant of concern to unambiguously determine whether biodegradation is occurring and evaluate the feasibility of MNA. With the SIP method, a Bio-Trap® sampler is baited with a specially synthesized form of the contaminant containing "heavy" carbon ($^{13}C$). When the baited Bio-Trap is deployed in a monitoring well, the $^{13}C$ labelled contaminant is subject to the same physical, chemical, and microbiological processes as the unlabelled contaminant present at the site. If biodegradation is occurring, the $^{13}C$ label from the synthesized contaminant in the Bio-Trap will be incorporated into the end products of biodegradation: microbial biomass and dissolved inorganic carbon ($CO_2$)

This presentation focuses on case studies in which SIP was performed to evaluate MNA at sites impacted by gasoline (benzene), fuel oxygenates (MTBE), diesel components (naphthalene), and other industrial contaminants (aniline, nitrobenzene, chlorobenzene).

## METHODS

Bio-Trap samplers were amended with $^{13}C$ labelled contaminants of concern and deployed in existing monitoring wells in the dissolved plume for 30 to 90 days. Briefly, Bio-Traps are passive sampling tools that collect microbes over time for the purpose of better understanding biodegradation potential. Bio-Traps contain Bio-Sep® beads, an engineered composite of Nomex® and powdered activated carbon (PAC) that provides a large surface area for microbial colonization and biofilm formation. Due to the PAC component of the Bio-Sep beads, Bio-Traps can be "baited" with $^{13}C$ labelled compounds by vapour phase adsorption. During field deployment, biofilms characteristic of in situ aquifer conditions are formed on and within the Bio-Sep beads (Busch-Harris et al., 2006, Sublette et al., 2006 and 2008). Following field deployment, the Bio-Trap® is recovered and two methods are used to conclusively demonstrate biodegradation of the contaminant of concern:

(a) Quantification of $^{13}C$ enriched phospholipid fatty acids (PLFA) indicates contaminant incorporation into microbial biomass.
(b) Quantification of $^{13}C$ enriched dissolved inorganic carbon (DIC) indicates contaminant mineralization.

## RESULTS AND DISCUSSION

At gasoline impacted sites, benzene is often the contaminant of principal concern due to its known toxicity, mobility, and slower rates of biodegradation under anaerobic conditions typical of MNA. Bio-Traps amended with $^{13}C$ labelled benzene were deployed in select monitoring wells at gasoline contaminated sites where MNA was being considered as a remediation alternative. Based on traditional groundwater monitoring data, subsurface conditions at the sites were similar in terms of contaminant concentrations and redox state. SIP results and thus the feasibility of MNA at the sites, however, were markedly different (Figure 1).

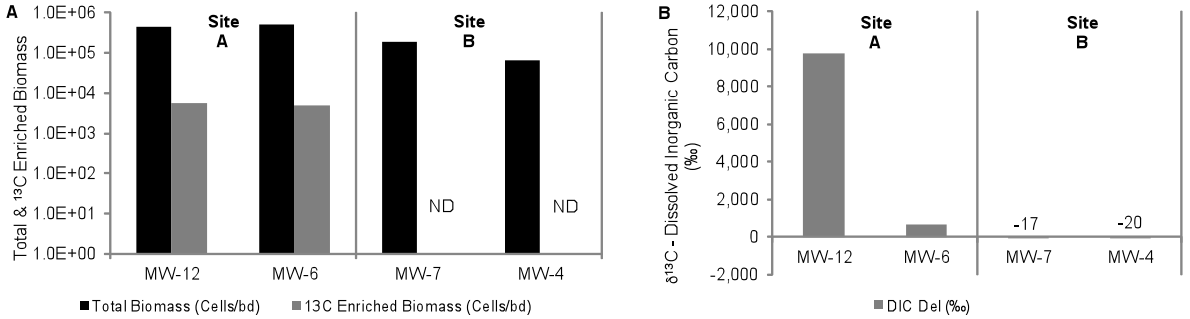


**Fig. 1. (A) Quantification of total and $^{13}C$ enriched biomass. (B) Quantification of $^{13}C$ enriched dissolved inorganic carbon (DIC). Bio-Traps were amended with $^{13}C$ labelled benzene and suspended in existing monitoring wells for approximately 30 days prior to recovery and analysis.**

At Site A, substantial incorporation of $^{13}C$ into biomass (~$10^3$ enriched cells/bd) at both monitoring wells demonstrated benzene biodegradation under existing site conditions. Although lower at MW-6 than at MW-12, detection of $^{13}C$ enriched DIC in both wells at Site A documented mineralization of benzene. Conversely, neither $^{13}C$ enriched biomass nor DIC was detected in study wells at Site B. In terms of site management decisions, the SIP results indicated that MNA may be a feasible remediation strategy at Site A but enhanced remediation alternatives should be considered at Site B.

## CONCLUSIONS

For a broad spectrum of common contaminants of concern, SIP can provide the unequivocal evidence of in situ biodegradation required to evaluate the feasibility and performance of MNA as a site remediation strategy.

## REFERENCES


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