Confirming In Situ Benzene Biodegradation under Anaerobic Conditions Using Stable Isotope Probing

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Background/Objectives. Biodegradation is an important mechanism for contaminant destruction at monitored natural attenuation sites. However, obtaining evidence of in situ biodegradation can be difficult for some contaminants if their metabolites are transient in the environment and/or little is known about the microorganisms and degradation pathways involved. Although many pathways for aerobic benzene biodegradation are well-documented, comparatively less is known about anaerobic benzene biodegradation. Early literature indicated that benzene was recalcitrant in anaerobic environments, but benzene biodegradation has since been demonstrated under iron- reducing, nitrate-reducing, sulfate-reducing, perchlorate-reducing, and methanogenic conditions. Hydroxylation, methylation, and carboxylation have been proposed as potential anaerobic pathways, but many of the genes involved have yet to be identified. Stable isotope probing (SIP) is a versatile molecular biological tool that can be used to provide conclusive proof of in situ biodegradation without requiring prior knowledge of the microorganisms or pathways involved. The current study includes statistical analysis of SIP results from 300 field samples collected from benzene sites around the world, including the United States, Australia, Canada, China, Saudi Arabia, and the United Kingdom.

Approach/Activities. The samples included in the current analysis were Bio-Traps amended with a specially synthesized form of benzene containing carbon-13 (^{13}C). Since ^{13}C is rare, carbon originating from labeled contaminant can be readily distinguished from carbon from other sources (predominantly carbon-12). Following in-well deployment, the Bio-Traps were analyzed for ^{13}C enrichment in dissolved inorganic carbon (DIC) and microbial phospholipid fatty acids (PLFA). ^{13}C incorporation into DIC conclusively demonstrates benzene mineralization during the deployment period. PLFA are a main component of cell membranes, and ^{13}C-enriched PLFA indicate that benzene was metabolized and incorporated into microbial biomass under current field conditions. Furthermore, ^{13}C incorporation into specific fatty acids associated with anaerobic microbial groups indicates that anaerobes were actively involved in the degradation of the ^{13}C-labeled benzene or one of its metabolites.

Results/Lessons Learned. Biodegradation of benzene was documented in 92% (275 of 300) of samples analyzed, and average $\delta^{13}C$ values in the PLFA and DIC will be presented. Over a third of the samples (102 of 300) showed high levels of ^{13}C incorporation ($\delta^{13}C$ > 1,000‰) in either the DIC or PLFA, and 26 samples had $\delta^{13}C$ values exceeding 1,000‰ in both. These results suggest that the capability for benzene biodegradation by indigenous microorganisms is relatively widespread. Additionally, PLFA associated with anaerobic microorganisms were ^{13}C-enriched in 52% (157 of 300) of samples, suggesting that anaerobic microorganisms may play a larger role in benzene degradation than has previously been able to be documented in the field. SIP is a powerful molecular biological tool that can be applied to confirm in situ biodegradation of other persistent contaminants as well, including methyl *tert*-butyl ether, *tert*-butyl alcohol, chlorobenzene, 1,4-dioxane, toluene, and naphthalene among others.