## Comparison of 1,4-Dioxane Cometabolism with the Amendment of Different Alkane Gases

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**Background/Objectives.** 1,4-Dioxane (dioxane) groundwater contamination represents a remedial challenge for environmental engineers due to its carcinogenicity, mobility, and recalcitrance for degradation. Gases alkane degrading microorganisms (e.g., methanotrophs, propanotrophs, and butanotrophs) are known for their relaxed substrate range, which enables cometabolism of a large variety of organic pollutants with the presence of their primary substrates. In this study, we compared the biostimulatory performance of methane and propane for decontamination of dioxane using microcosm assays and investigate the putative biotransformation pathways by the enriched consortium.

**Approach/Activities.** Microcosms were prepared in triplicates using 100 mL of groundwater and 50 g of aquifer materials collected at the source zone of a site in west Texas. Initially, 1.5% (v/v) of the headspace of the sealed serum bottles were filled with methane or propane. Dioxane concentrations were monitored weekly or biweekly using a frozen microextraction method followed by GC/MS-SIM. Concentrations of methane and propane in the headspace were measured by GC/FID. Negative controls were prepared with autoclaved samples and dosed with HgCl<sub>2</sub> (200 mg/L), to discern biodegradation from potential abiotic losses.

Results/Lessons Learned. In microcosms stimulated with methane, a lag time of approximately three weeks was observed for methane degradation. Proliferation of methanotrophs was evident by the enhanced methane consumption with no lag time after the second amendment of methane to the microcosms. However, no significant dioxane removal was indicated after 2 amendment cycles of methane. As for propane, it was found to be able to stimulate dioxane cometabolism by the indigenous bacteria, though the lag time was relatively long (i.e., greater than 5 months). No degradation of dioxane was observed until approximately half of the initial propane has been consumed. The consumption rate on propane was approximately three-times slower than methane, suggesting a relatively low abundance of propanotrophs in the field samples. These results showed different treatment performance by adding different gaseous alkane as the stimulants, which underscores the need for a better understanding of the mechanisms of dioxane cometabolisms. It is also notable that using traditional microcosm assays to discover the effectiveness of biostimulation can be quite timeconsuming and unproductive given the long incubation time and high variety of biostimulants. To tackle this problem, molecular tools that allow for quick determination of the prevalence of genes encoding the catabolic enzymes that are proficient in cometablizing dioxane need to be developed.