# Propane Biostimulation for Effective 1,4-Dioxane Removal: Enrichment and Microbial Structure Analysis

***Mengyan Li*** (mengyan.li@njit.edu) and Daiyong Deng (New Jersey Institute of Technology, Newark, NJ, USA)
Yuanyuan Liu (Changsha University of Science and Technology, Changsha, Hunan, China)
Ya He, and Pedro J. J. Alvarez (Rice University, Houston, TX, USA)

**Background/Objectives.** 1,4-Dioxane (dioxane) groundwater contamination represents a remedial challenge for environmental engineers due to its carcinogenicity, mobility, and recalcitrance for degradation. Propane has been reported as an effective auxiliary substrate to stimulate indigenous degraders to remove dioxane via cometabolism at field. However, effectiveness of propane biostimulation is uneven. In addition, scarce information is known regarding the indigenous degraders and enzymes that are associated with such processes. Here, we assessed the biostimulatory performance of propane (in comparison with methane) for decontamination of dioxane using microcosm assays and investigate the putative biotransformation pathways and dominant degraders in 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 propane or methane. 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 $HgCl_2$ (200 mg/L), to discern biodegradation from potential abiotic losses. Enrichment was further conducted by transferring 2 mL of the culture to a bottle with fresh medium. Propane was repeated amended to boost the perforation of propanotrophs that cometabolize dioxane. Genomic DNA of the enriched consortium was obtained using PowerSoil DNA extraction kit following the manufacturer's protocol. Paired-end high-throughput 16S rRNA sequencing was performed using Illumina MiSeq Sequencing System.

**Results/Lessons Learned.** Microcosm assay revealed that amendment of propane can 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. 16S rRNA sequencing analysis demonstrated that the dominant genera in microcosms fed with propane consisted of *Acidobacterium*, *Petrimonas*, and *Aquimonas*, suggesting their potential role in terminal oxidation of propane and dioxane cometabolism. It is notable that using traditional microcosm assays to discover the effectiveness of biostimulation can be quite time-consuming 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.