Propane and 1-Propanol as Auxiliary Substrate Alternatives for Effective Cometabolic Bioremediation of 1,4-Dioxane

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Background/Objectives. Cometabolic bioremediation is trending for the treatment of 1,4-dioxane (dioxane) and other emerging contaminants to meet stringent regulatory goals (e.g., <10 μg/L) since the biodegradation activities can be fueled by the supplementation of auxiliary substrates. Previous studies on dioxane cometabolic degradation were centered on utilizing propane, iso-butane, or other short-chain alkane gases as the auxiliary substrates. Compared to gaseous alkanes, liquid alcohols (e.g., 1-propanol and 1-butanol) can be advantageous considering their ease of dosing at the injection wells and monitoring through routine water sampling. Liquid alcohols are highly soluble or even miscible, allowing direct injection of high concentrations without the need of specialized distribution systems to optimize the radius of influence. Liquid alcohols are also safer for storage and use, while gaseous alkanes such as propane are a concern due to their flammable and explosive nature, requiring appropriate onsite management. However, liquid alcohols are not specific to dioxane degraders. Many native bacteria can grow with liquid alcohols, which thus may pose the potential to outcompete the dioxane degraders. Therefore, it is of great application value to discern the effectiveness of liquid alcohols for bioremediation in a range of environmental matrices consisting of different microbiomes.

Approach/Activities. In this study, we compared and investigated the effectiveness of two types of common auxiliary substrates, short-chain alkane gases (e.g., propane and butane) and primary alcohols (e.g., 1-propanol, 1-butanol, and ethanol), for dioxane removal in diverse environmental matrices with *Azoarcus* sp. DD4 as the inoculum. Parallel microcosm assays were conducted to assess the compatibility of DD4 bioaugmentation in diverse microbiomes recovered from five different environmental samples, including shallow and deep aquifer groundwater, contaminated river sediment, and municipal activated sludge. Dioxane disappearance and substrate consumption were monitored by GC-FID. The shifting of microbial communities was analyzed by 16S rRNA amplicon-based sequencing.

Results/Lessons Learned. Physiochemical characterization at the pure culture level revealed that propane and 1-propanol are advantageous for stimulating cell growth and dioxane biodegradation by DD4. In addition, both substrates can effectively upregulate the transcription of the *tmoA* gene, which encodes the toluene monooxygenase responsible for the alpha hydroxylation of dioxane, as compared to the housekeeping gene. Furthermore, microcosm studies revealed that propane was effective in sustaining efficient dioxane removal and the dominance of DD4 across all environmental matrices. Notably, amendment of 1-propanol promoted superior dioxane degradation in the deep aquifer groundwater, in which low pre-treatment biomass and post-treatment diversity were observed, suggesting its potential for intrinsic field applications. The combination of microbial community analysis and differential ranking identified *Ochrobactrum* and several other indigenous bacteria were boosted by the inoculation of DD4, implying their commensal or mutualistic relationship. Collectively, propane and 1-propanol can be effective auxiliary substrate alternatives tailored for in situ bioaugmentation and their effectiveness is affected by the density and structure of environmental microbiomes.