Concurrent Biodegradation of 1,4-Dioxane and 1,1-Dichloroethylene by a Gram-Negative Propanotroph *Azoarcus* sp. DD4

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Background/Objectives. Bioremediation of 1,4-dioxane is strongly challenged by the concurrent contamination of chlorinated solvents, particularly 1,1-dichloroethylene (1,1-DCE), a potent inhibitor of archetypic 1,4-dioxane degraders (e.g., *Pseudonocardia* and *Mycobacterium*). Vegetative growth and aggregation behavior of these Gram-positive Actinomycetes also hinder their field application. Cometabolic bioremediation is an advantageous technique for treating commingled contamination as the fortuitous degradation can destroy multiple pollutants of disparate properties with little reliance on their concentrations. Theoretically, cometabolism can diminish contaminants to sub-parts per billion levels, which are sufficient for stringent cleanup goals (e.g., <1 μg/L for 1,4-dioxane). In this study, we isolate and characterize a nonfilamentous Gram-negative bacterium that can effectively remove both 1,4-dioxane and 1,1-DCE in lab media and contaminated groundwater samples using propane as the primary substrate.

Approach/Activities. Enrichment was initiated with 2 g of activated sludge and 20 mL of nitrate mineral salt (NMS) medium amended with an appropriate amount of 1,4-dioxane and propane of instrument purity (>99.5%). After enrichment for 2 months, a pure isolate (designated as DD4) was obtained and verified for its capability of degrading 1,4-dioxane and 1,1-DCE. To assess the feasibility of DD4 for in situ bioaugmentation, groundwater samples were collected in January 2017 from three monitoring wells in the source zone of a site in southern California. Three samples were pooled in equal volumetric portions (1:1:1) to make one mixed sample detected with 10.1 ± 0.5 mg/L 1,4-dioxane. Microcosms were prepared with 20 mL of mixed groundwater, DD4 inoculum (0.024 mg of protein), and propane (8 mg/L in headspace equivalent to a percent volume of 0.15%) in 150 mL serum bottles, leaving sufficient headspace to maintain aerobic conditions during the treatment period. To further mimic the source zone condition, microcosms were prepared with groundwater spiked with 3.28 ± 0.19 mg/L (as the aqueous concentration) 1,1-DCE. Aliquots and headspace samples were periodically collected to monitor the depletion of propane, 1,4-dioxane, and 1,1-DCE by GC-FID. When lower than 1 mg/L, 1,4-dioxane concentrations were analyzed by GC/MS with the microextraction method (method detection limit [MDL] of 0.38 μg/L). All treatments were performed in triplicate, and abiotic controls were conducted using killed DD4 cells.

Results/Lessons Learned. The primary superiority of DD4 lies in its capability of co-oxidizing both 1,4-dioxane and 1,1-DCE. DD4 can sustain the concurrent degradation of 1,4-dioxane and 1,1-DCE using propane as the primary substrate without significant formation of clumps. Microcosm assays prepared with source zone groundwater samples from a contaminated site indicated DD4 can efficiently remove 1,4-dioxane, with the concentration decreasing from an initial value of 10.4 mg/L to <0.4 μg/L within 14 days of incubation. Removal of 1,4-dioxane was partially inhibited when an excessive amount of 1,1-DCE (3.28 ± 0.19 mg/L) was artificially spiked into the microcosms but significantly accelerated immediately after the complete depletion of 1,1-DCE. Furthermore, a gene encoding a putative propane monooxygenase was discovered, which may contribute to the oxidation of propane, 1,4-dioxane, and/or 1,1-DCE. Detection of 2-S-glutathionyl acetate and synchronic dechlorination suggest that DD4 detoxifies its primary metabolite, 1,1-DCE epoxide, via conjugation with glutathione. All these findings indicate the suitability of DD4 as a robust inoculum candidate for in situ bioaugmentation to remediate co-contamination by 1,4-dioxane and 1,1-DCE.