## Dual-Culture System Enables the Degradation of 1,4-Dioxane and Co-Occurring Chlorinated Aliphatic Hydrocarbons

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**Background/Objectives.** In situ bioaugmentation for 1,4-dioxane (dioxane) remediation is often hindered by the cooccurrence of chlorinated solvents, such as 1,1-dichloroethene (1,1-DCE), 1,1,1-trichloroethane (TCA), and trichloroethene (TCE). TCE and 1,1-DCE's recalcitrance and inhibitory effects are of particular concern. Few dioxane-degrading bacteria are known with a stable ability to degrade TCE or 1,1-DCE. *Azoarcus* sp. DD4 and *Mycobacterium* sp. DT1 are two dioxane-degrading bacteria that are also capable of degrading 1,1-DCE and TCE, respectively. In this study, we exploited the feasibility of different strategies using these two cultures to optimize the removal of commingled contamination of dioxane and chlorinated solvents with propane as the primary substrate.

**Approach/Activities.** Dual-culture batch experiments were carried out with using DT1 and DD4 in the nitrate mineral salts (NMS) medium, spiked with dioxane, propane, TCE, and 1,1-DCE, which were monitored by CG-FID. qPCR was used to monitor the shifting of microbial populations in response to the exposure of different contaminants. The capacity to overcome the inhibitory effects of other chlorinated solvents cooccurring with dioxane, such as 1,1-dichloroethane (1,1-DCA), 1,2-dichloroethane (1,2-DCA), cis-1,2-DCE (c-DCE), trans-1,2-DCE (t-DCE), and vinyl chloride (VC), were also monitored by GC-FID.

**Results/Lessons Learned.** The dual-culture system with DT1 and DD4 was capable of overcoming chlorinated solvent inhibition in the 1,1-DCE, TCE and dioxane contaminated system when amended with propane. Additionally, the system was capable of overcoming other cooccurring inhibitory chlorinated solvents. Overall, this study highlights the use of comingled cultures to tackle complex contamination plumes. The combination of advantageous microbial properties in a mixed culture allows us to tackle complex contamination at sites using in situ bioremediation strategies tailored for the site, as compared to traditional sequential treatments that employ pump-and-treat or other aggressive techniques.