Optimizing a Mixed Microbial Community to Biodegrade Chlorinated Ethenes and 1,4-Dioxane

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Background/Objectives. Improper storage, discharges, and accidental spills of chlorinated ethenes and 1,4-dioxane (dioxane) have led to widespread groundwater contamination. Anaerobic biological reduction is a common remediation strategy for chlorinated ethenes. However, under some conditions, intermediate daughter products, such as cis-1,2-dichloroethene (cDCE) and vinyl chloride (a known human carcinogen) accumulate. Aerobic cometabolic biodegradation of chlorinated ethenes can be burdensome due to the additional amendments required by microbial cometabolic populations. Currently, anaerobic microorganisms have not been isolated that are capable of biodegrading dioxane. However, aerobic microbes have been shown to effectively biodegrade dioxane. Opposing redox conditions favored by chlorinated ethene-and dioxane-degrading bacteria pose a challenging problem for concurrent bioremediation of both contaminants. We formulated a microbial community to simultaneously or sequentially degrade chlorinated ethenes and dioxane.

Approaches/Activities. A mixed microbial community composed of the anaerobic microbial consortium (KB-1®) containing *Dehalococcoides* and aerobic *Pseudonocardia dioxanivorans* CB1190 (CB1190) bacteria was used to evaluate the biodegradation of chlorinated ethenes and dioxane under changing redox conditions. Triplicate batch reactors containing CB1190 alone or with KB-1® were used for the experimental set-up as follows: 1) CB1190 only that transitioned from anaerobic to aerobic conditions, 2) CB1190 only that remained anaerobic, and 3) CB1190 and KB-1® that transitioned from anaerobic to aerobic. Once the TCE was degraded to cDCE by KB-1®, oxygen was amended to allow CB1190 to reactivate and degrade dioxane. Gas chromatography (GC) mass spectrometry and GC flame ionize detection was used to measure low (<1ppm) and higher (>1ppm) concentrations of dioxane and chlorinated solvents, respectively. Liquid biomass samples were extracted using the phenol/chloroform method. qPCR assays targeting the 16S, *dxmB*, and *aldH* genes were tested over the course of the experiment to evaluate growth and gene expression of CB1190. Dissolved oxygen, pH, and ATP were also monitored.

Results/Lessons Learned. Results showed that the aerobic, dioxane degrader, CB1190, was able to survive anaerobic incubation and still grow in optimized medium when conditions turn aerobic. The monooxygenase enzyme was reactivated after little to no lag, and able to catalyze dioxane degradation. The mixed microbial community was able to mitigate CVOC inhibition of 1,4-dioxane aerobic biodegradation. Also, the mixed microbial community was able to biodegrade cis-1,2-dichloroethene under aerobic conditions. As a plume disperses downgradient, the redox conditions frequently change from anaerobic (source zone) to aerobic (downgradient). The results of this study demonstrate that the mixed microbial community can withstand these changing redox conditions downgradient and over time, and biodegrade chlorinated ethenes as well as dioxane. Quantitative data describing the biodegradation kinetics and microbial growth will be presented. This approach could expand the sites where bioremediation could be a viable remedy for these compounds.