Field-Scale Evaluation of In Situ Biodegradation of 1,4-Dioxane via Bioaugmentation with *Pseudonocardia dioxanivorans* CB1190

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Background/Objectives. 1,4-Dioxane (dioxane) is an emerging contaminant frequently detected at chlorinated solvent sites due to its use as a stabilizer. Although dioxane was once considered a recalcitrant compound, several microorganisms have been isolated that are able to utilize dioxane as a growth supporting substrate. This study involved the use of compound specific isotope analysis (CSIA) and quantitative polymerase chain reaction (qPCR) to evaluate dioxane biodegradation at a trichloroethene (TCE) impacted site under native and enhanced bioremediation in the source zone. At the study site, a shallow unconfined aquifer is impacted by various chlorinated ethenes, including tetrachloroethene (PCE), TCE, cis-1,2-dichloroethene (cis-DCE) and the cocontaminant dioxane.

Approach/Activities. Groundwater samples were periodically obtained for 2D-CSIA to quantify carbon ($d^{13}C$) and hydrogen ($d^{2}H$) isotope fractionation and evaluate dioxane degradation. An aerobic dioxane utilizing bacteria, *Pseudonocardia dioxanivorans* CB1190 (CB1190), uses dioxane monooxygenase (DXMO) to mediate the first step in dioxane metabolism while an aldehyde dehydrogenase enzyme (ALDH) catalyzes continued biodegradation of glycoaldehyde. To investigate the feasibility of bioaugmentation to promote dioxane biodegradation, Bio-Trap samplers were inoculated with the *P. dioxanivorans* CB1190 culture and deployed in source area monitoring wells with and without an oxygen source. In addition, nutrient amended Bio-Trap samplers were also evaluated. qPCR assays targeting the DXMO and ALDH genes were periodically performed to evaluate the potential for metabolic biodegradation of dioxane. The groundwater was monitored for hydrogeochemical properties to establish native conditions, which would impact biodegradation of dioxane.

Results/Lessons Learned. Initial groundwater redox conditions were mildly anaerobic with evidence of reductive dechlorination of PCE and TCE to cis-DCE but limited production of vinyl chloride and ethene. Concentrations of the cocontaminant dioxane were approximately 3 to 5 mg/L in the source area and decreased to approximately 2 mg/L at a downgradient location. Consistent with the generally reducing conditions however, DXMO and ALDH genes were not detected in groundwater samples. Furthermore, dioxane $d^{13}C$ (-31.1 to -30.6‰) and $d^{2}H$ (-51 to -47‰) values did not provide evidence of dioxane degradation within the source area under the initial site conditions. Post-deployment DXMO (10^{4} gene copies/bead) concentrations were comparable to pre-deployment concentrations demonstrating in situ survival of the CB1190 culture despite somewhat unfavorable redox conditions and suggesting bioaugmentation was feasible. The results indicate that the Bio-Trap amended with bioaugmentation in combination with nutrients showed enhanced degradation in comparison to the MNA Bio-Trap. This presentation will discuss the natural attenuation Bio-Trap results in comparison to the Bio-Traps amended with CB1190 plus and minus an oxygen source and nutrients. In addition to the CSIA results, the key target genes associated with dioxane biodegradation and site geochemical data will be presented.