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Evaluating Enhanced Bioremediation of 1,4-Dioxane following Bioaugmentation with 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 often described as recalcitrant, an increasing number of microorganisms capable of utilizing dioxane as a growth supporting substrate have been isolated. Furthermore, pathways for aerobic metabolism and co-oxidation of dioxane have been elucidated suggesting that under appropriate environmental conditions, biodegradation is a potential attenuation mechanism. In the current study, compound specific isotope analysis (CSIA) and quantitative polymerase chain reaction (qPCR) were performed to assess dioxane biodegradation at a TCE-impacted site under existing conditions and to evaluate the performance of enhanced bioremediation in the source zone.

Approach/Activities. At the study site, a shallow unconfined aquifer is impacted by PCE, TCE, 1,1,1-TCA and the co-contaminant dioxane. 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, most notably *Pseudonocarida dioxanivorans* CB1190, uses dioxane monooxygenase (DXMO) to mediate the first step in dioxane metabolism while an aldehyde dehydrogenase enzyme (ALDH) catalyzes continued biodegradation of a key intermediate. qPCR assays targeting the DXMO and ALDH genes were periodically performed to evaluate the potential for metabolic biodegradation of dioxane. Based in part on qPCR results under initial site conditions, an in situ microcosm study was conducted to evaluate the feasibility of bioaugmentation with *P. dioxanivorans* CB1190. Ultimately, full scale CB1190 bioaugmentation may be implemented in the source area.

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 co-contaminant dioxane were on the order of 4 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 $d^{2}H$ values did not provide evidence of dioxane degradation within the source area under the initial site conditions. 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. 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. A pilot system employing bioaugmentation with CB1190 is being considered in the source area.