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Bioaugmentation of *TreeWells*® to Enhance the Aerobic Degradation of 1,4-Dioxane at High Concentrations

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Background/Objectives. A phytoremediation system was installed at a former 1,4-dioxane (dioxane) manufacturing facility where dioxane concentrations in groundwater range from parts per billion to percent levels. The phytoremediation system consists of 240 hybrid poplar trees planted inside *TreeWell*® units down gradient of the source area. The objectives of the *TreeWell*® phytoremediation system are hydraulic control of the dioxane plume and contaminant uptake. A bench scale study was designed to evaluate additional mechanisms (e.g., microbial biodegradation) that can accelerate the degradation of high concentrations of dioxane. This bench study assessed the influence of root exudates on the microbial communities within the phytoremediation system. More specifically the study evaluated the efficacy of bioaugmentation with *Pseudonocardia dioxanivorans* (CB1190), which can directly metabolize dioxane under aerobic conditions. Growth of CB1190 on dioxane is relatively slow and results in low cell yields, however the root exudates present in the rhizosphere may provide a carbon source for increased growth and cell yield of the actinomycete.

Approach/Activities. Soil and groundwater collected from *TreeWell*® units were used to construct microcosms simulating natural attenuation (with root extract), biostimulation (with root extract and diammonium phosphate (DAP)), and bioaugmentation (with root extract, DAP and CB1190). Abiotic control microcosms were also established. All microcosms were conducted in triplicate and incubated on a shaker at 225 rpm at 25 ^{o}C, under aerobic conditions. Samples were collected and analyzed for total bacteria (16sDNA), dioxane monoxygenase (DXMO), and aldehyde dehydrogenase (ALDH) enzymes using qPCR, and for dioxane using GC/MS-SIM with an SPME fiber extraction with isotope dilution.

Results/Lessons Learned. Baseline groundwater qPCR analysis indicated presence of total bacteria at 6X10^6 copies/mL, of which 5X10^5 copies/mL are *Pseudonarcardia* like. Both biomarkers DXMO and ALDH were observed at concentrations above the detection limit for DNA, however gene transcripts were not detected, indicating that the indigenous microbes had the potential for degrading dioxane, but they were not active. Preliminary results from bioaugmented microcosms suggest enhanced degradation with over ninety percent of the dioxane biodegraded within 11 days. These biodegradation rates are similar to others published in literature using the CB1190 culture and high dioxane concentrations.