## Biodegradation of 1,4-Dioxane in a Fixed-Film Bioreactor

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Background/Objectives. 1,4-Dioxane is a common cocontaminant with chlorinated solvents. Treatment of 1,4-dioxane via enhanced biodegradation processes provides a viable remedial strategy. Both metabolic (i.e., energy producing) and co-metabolic (i.e., fortuitous) biodegradation of 1,4-dioxane can occur. Metabolic biodegradation occurs under aerobic conditions, and co-metabolic biodegradation must occur in the presence of both oxygen and a primary substrate (e.g., methane or propane) by microorganisms that utilize these primary substrates for growth. At the RACER Trust facility in Lansing, Michigan, historical use of chlorinated solvents resulted in up to approximately 3,300 micrograms per liter of 1,4-dioxane in groundwater. The initial remedial design called for ex situ treatment via advanced oxidation processes (AOPs), which is relatively expensive for long-term removal of 1,4-dioxane considering the power and chemical costs for operation. Instead, the RACER Trust is exploring an ex situ biological treatment approach, using pilot fixed-film bioreactors.

Approach/Activities. Three bioreactors were piloted to treat extracted groundwater with approximately 300 µg/L 1,4-dioxane prior to reinjection: one designed for metabolic biodegradation of 1,4-dioxane and two designed for co-metabolic biodegradation of 1,4-dioxane (for concurrent testing of different conditions). The bioreactor pilot system consisted of an aerated iron oxidation tank and primary clarifier, oxygen and/or propane cylinders, gas injection points, a nutrient feed tank, the three bioreactors filled with approximately 40 percent media in parallel, a biological solids settling tank, an effluent cartridge filter, a lower explosive limit meter, and associated controls. The metabolic bioreactor was seeded with a known 1,4-dioxane degrading organism, *Pseudonocardia dioxanivorans* CB1190 (provided by Dr. Shaily Mahendra, University of California, Los Angeles). The co-metabolic bioreactors were seeded with a propanotrophic culture, *Rhodococcus ruber* ENV425 (provided by EOS Remediation, LLC). ENV425 utilizes propane as its primary substrate and has been used elsewhere to facilitate co-metabolic biodegradation of 1,4-dioxane. The bioreactors also received micro- and macronutrients to promote bacterial colonization.

Results/Lessons Learned. Several challenges were experienced during initial operation of the bioreactors, primarily establishment of a working microbial population. Efforts undertaken to facilitate growth included: 1) a period of recirculation and extra nutrient addition after the initial seeding with the microbial cultures, 2) a second seeding of the ENV425 microbial culture, 3) reconfiguration of one of the co-metabolic bioreactors to allow for cycling on and off of the propane, and 4) increasing the hydraulic residence times to mitigate microbial washout. After these efforts, 83% removal of 1,4-dioxane was observed in the co-metabolic bioreactor with cycled propane. However, unforeseen system upsets limited continued performance. A post-mortem evaluation of the microbial population in the bioreactors concluded that the seeded microbial cultures were outcompeted by native microorganisms (iron oxidizers).