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Modeling Aerobic Cometabolism of 1,4-Dioxane and Chlorinated Solvents by Isobutane-Utilizing Bacteria

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Background/Objectives. 1,4-Dioxane, a probable human carcinogen, is a common groundwater cocontaminant in chlorinated solvent plumes. As a non-volatile compound with low K_{ow}, 1,4-dioxane is resistant to traditional pump-and-treat remediation techniques. Several bacteria have been isolated that directly metabolize 1,4-dioxane, however transformation rates are slow and the low concentrations typical of 1,4-dioxane plumes would likely not sustain an active population of these bacteria. In contrast, aerobic cometabolism has the potential to rapidly transform low levels of 1,4-dioxane to meet the U.S. Environmental Protection Agency 1×10^{-6} cancer risk level for drinking water of 0.35 µg/L. The objectives of this study were: 1. to determine the effectiveness of isobutane as a primary substrate to promote aerobic cometabolism of 1,4-dioxane and chlorinated solvent mixtures by both a pure culture *Rhodococcus* species and as a biostimulant in aquifer microcosms; 2. to develop a competitive inhibition model to predict transformation rates of 1,4-dioxane and chlorinated solvent mixtures for applications as an in situ bioremediation strategy.

Approach/Activities. The effectiveness of isobutane (2-methylpropane) as a primary substrate for aerobic cometabolism was assessed in pure culture and microcosm experiments. Pure culture experiments with *Rhodococcus rhodochrous* (ATCC 21198) were used to determine Monod kinetic parameters for the development of the model. Microcosms were created using aquifer solids from Fort Carson, CO, a site with 1,4-dioxane and trichloroethene (TCE) cocontamination, and artificial groundwater. Microcosms containing TCE and/or 1,4-dioxane were used to compare the effect of biostimulation with isobutane and bioaugmentation with *R. rhodochrous*.

Results/Lessons. Isobutane is an effective primary substrate to promote rapid transformation of 1,4-dioxane by *R. rhodochrous*. When bioaugmented in microcosms, *R. rhodochrous* allows for immediate, rapid transformation of 1,4-dioxane. Isobutane is also an effective biostimulant for microorganisms native to Fort Carson aquifer solids. After an initial lag period, 1,4-dioxane transformation rates approach those in bioaugmented microcosms. TCE is transformed much more slowly than 1,4-Dioxane, but does not inhibit 1,4-dioxane transformation at the concentration tested. 1,4-dioxane-cometabolizing microbial populations remain active in bioaugmented and biostimulated microcosms with repeated additions of isobutane over several months, though transformation rates slow without nutrient amendment. Microcosm experiments show that 1,4-dioxane transformation rates correlate to isobutane utilization rates. Monod kinetic parameters determined in high biomass pure culture experiments simulate resting cell transformation of 1,4-dioxane and chlorinated solvent mixtures, but when incorporated into a competitive inhibition model they do not accurately predict isobutane utilization and 1,4-dioxane transformation by active cells at lower concentrations of biomass and primary or cometabolic substrates. The cause of the difference between Monod kinetic parameters determined from pure culture and microcosm experiments is currently under investigation.