Aerobic Cometabolism of 1,4-Dioxane and Chlorinated Solvent Mixtures: Experimental and Modeling Analysis of Multiple Primary Substrates

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Background/Objectives. 1,4-dioxane (1,4-D), a probable human carcinogen, has emerged as a common groundwater contaminant at military and industrial sites due to its use as a stabilizer for the chlorinated solvent 1,1,1-trichloroethane (TCA). Aerobic cometabolism is an attractive remediation approach due to low concentrations of 1,4-D in the environment. Previous work has shown 1,4-D is cometabolized by isobutane-grown microorganisms, including *Rhodococcus rhodochrous* ATCC 21198. However, 1,4-D contamination also often occurs in mixtures of chlorinated solvents such as TCA, trichloroethene (TCE), and their transformation products. Some of the chlorinated compounds are also readily transformed by isobutane-grown bacteria, however others are only slowly transformed or exert a toxic effect. Therefore, a second gaseous growth substrate, isobutene, and model microorganism, *Mycobacterium* ELW1, were investigated for additional degradation capabilities. The objectives of the study were to: 1. investigate the combined use of isobutane and isobutene as primary substrates to cometabolically degrade mixtures of 1,4-D and chlorinated solvents, and 2. incorporate transformation capacity and competitive inhibition into a kinetic model to predict degradation of contaminant mixtures.

Approach/Activities. The aerobic cometabolic transformation abilities of isobutane-grown 21198 and isobutene-grown ELW1 were investigated in batch studies, both in pure culture experiments and when augmented in aquifer microcosms. Rates of 1,4-D and chlorinated solvent transformation were used to develop a Michaelis-Menten/Monod kinetic model to predict cometabolic transformation of contaminant mixtures. The numerical model is evaluated in MATLAB and includes the influence of transformation capacities and competitive inhibition for primary substrate and among co-contaminants. A column study will be used to assess the combined use of isobutene and isobutane to induce cometabolic transformation of contaminant mixtures in a continuous flow and transport environment.

Results/Lessons Learned. Batch tests have shown isobutane-grown 21198 is able to cometabolize 1,4-D below the EPA lifetime cancer risk level of 0.35 ppb. 21198 also rapidly cometabolizes several chlorinated compounds, including 1,1-dichloroethene (1,1-DCE), cis-dichloroethene, and vinyl chloride. However, the preferential transformation of 1,1-DCE results in the formation of a toxic epoxide which inhibits transformation of other compounds, including isobutane (its growth substrate). Modeling analysis confirmed, as shown in microcosm studies, that stimulation of native isobutane-utilizing microbes will not occur in the presence of high 1,1-DCE concentrations. By contrast, the presence of isobutene inhibits 1,1-DCE transformation, which allows for microbial growth prior to cometabolic transformation of chlorinated solvents. In addition, isobutene-grown microorganisms transform TCE at greater rates, but do not readily transform 1,4-D. Therefore, a combination of primary substrates is required to achieve transformation of complex contaminant mixtures. It is anticipated the column study will show that spatial or temporal pulsing of isobutene followed by isobutane will result in complete transformation of complex mixtures.