Bioremediation and Sustainable Environmental Technologies—2017. Fourth International Symposium on Bioremediation and Sustainable Environmental Technologies (Miami, FL; May 2017). A. Barton and S. Rosansky (Chairs)

Potential for Natural or Enhanced Biodegradation of 1,4-Dioxane with Methane and Ethane as Cosubstrates

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Background/Objectives. The objective of this research was to evaluate the potential for two alkane substrates, methane and ethane, to stimulate the biological degradation of 1,4-dioxane (1,4-D) in groundwater aquifers via cometabolism. During cometabolism, microorganisms grow on a primary substrate (e.g., methane) but the enzyme(s) produced during growth on that substrate (e.g., soluble methane monooxygenase; sMMO) also degrade the target contaminant, sometimes to very low concentrations (e.g., parts-per-trillion). Methane is generated in anaerobic aquifers via methanogenesis whereas ethane may be derived from multiple sources, including generation through reductive dechlorination processes. Studies were conducted herein to determine whether either of these alkane substrates may support degradation of 1,4-D in aquifers or be applied to enhance its degradation rate.

Approach/Activities. Experiments with aquifer microcosms, enrichment cultures, pure cultures, and pure enzyme (methane only) were performed to evaluate the potential for methane and ethane to serve as substrates for enhancing the aerobic biodegradation of 1,4-D. Aquifer microcosms from several sites were amended with methane or ethane. Killed and unamended controls were also prepared. Various other treatments, including the addition of copper chelators (to support production of sMMO) and various inorganic nutrients were also evaluated. In microcosms where the primary substrate was degraded, enrichment cultures and/or pure cultures were isolated and evaluated for their potential to biodegrade 1,4-D as well as chlorinated solvents that commonly co-occur with this compound in groundwater. Studies with several different known pure cultures of ethane and methane oxidizers were also conducted to assess the kinetics and extent of 1,4-dioxane degradation by these different strains, as well as inhibition of 1,4-D degradation by the parent substrate (ethane only).

Results/Lessons Learned. The data from microcosm, enrichment culture and pure culture studies indicate that methane does not typically stimulate the biodegradation of 1,4-D at environmentally relevant concentrations, and that 1,4-D is not a substrate for sMMO in the methanotrophic bacteria that were tested. This result was supported by subsequent testing with the pure sMMO enzyme. However, the experimental data from multiple sites as well as pure and enrichment cultures suggest that ethane does support 1,4-D biodegradation. Experiments are underway with a pure culture to evaluate which enzyme is responsible for ethane and 1,4-D degradation. Much like methane, ethane can occur in anoxic environments, particularly from the biotic or abiotic reductive dechlorination of chlorinated ethanes and ethenes. Thus, degradation of 1,4-D with ethane rather than methane as a co-substrate is feasible based on the current lab studies.