

## Laboratory Evaluation of Alternative Substrates for Enhancing the Cometabolic Biodegradation of 1,4-Dioxane and Tetrahydrofuran

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**Background/Objectives.** Few treatment methods have proven successful and economically feasible for removing 1,4-dioxane (1,4-D) from groundwater. Alkane gases, including methane, ethane, and propane, have been used successfully in the past to stimulate cometabolic degradation of a number of different contaminants, including chlorinated solvents, nitrosamines, and fuel oxygenates, among others. Laboratory treatability testing has shown that several alkane gases and tetrahydrofuran (THF) have the potential to act as cometabolic substrates for the treatment of 1,4-D in groundwater, but that the most effective substrates may be site specific. Cometabolic treatment of pollutants is particularly useful when the target chemical: 1) is not a growth substrate for bacteria; 2) produces poor growth yield of degradative microbes; or 3) must be degraded to very low levels, including levels insufficient to support bacterial growth, to meet regulatory goals. During cometabolism, robust growth of the degradative microorganism is supported by the primary substrate (e.g., propane) while the enzymes produced to grow on the primary substrate (e.g., propane monooxygenase) also catalyze degradation of the target contaminant, even though that contaminant does not provide carbon or energy that can be utilized by the organism for growth.

**Approach/Activities.** Laboratory treatability studies were performed to evaluate the effectiveness of five different cometabolic substrates (propane, ethane, methane, isobutane, and THF) for the aerobic treatment of 1,4-D. The effect of inorganic nutrients on the degradation kinetics of these target contaminants was also evaluated. Microcosms were constructed using sand-rich aquifer media and groundwater collected from two distinct locations within an aquifer containing both 1,4-D and THF. The first set of microcosms was prepared using site materials from a location within the core of the plume that exhibits extremely anaerobic conditions, while the second set of microcosms were set up using site materials collected from a location at the downgradient edge of the plume that exhibits slightly aerobic conditions (2-3 mg/L DO). A total of 17 treatments, including live and killed controls, were prepared to evaluate the ability of THF and the four alkane substrates to stimulate cometabolic degradation of 1,4-D. Inorganic nutrients were added to a subset of these treatments to evaluate whether N or P was limiting cell growth on any of the primary substrates. Regular headspace sampling for oxygen and alkane gases was conducted with select gases and air added as necessary. Monthly sampling of 1,4-D and THF was performed to evaluate treatment effectiveness.

**Results/Lessons Learned.** Data collected during the study indicated that propane, methane, ethane, and isobutane were all being consumed in both sets of treatments, but only when inorganic nutrients were added. In the presence of nutrients and oxygen, THF (present in the groundwater used in all of the microcosms) was biodegraded in both sets of treatments, irrespective of the presence of alkane gases. Propane was the most effective substrate to stimulate the biodegradation of 1,4-D, with concentrations reduced below regulatory levels in both studies. Isobutane and ethane were also effective substrates in stimulating 1,4-D biodegradation, although kinetics were slower (especially with ethane) than those realized in the propane treatments. Microcosm data, in conjunction with enrichment testing data, suggest that methane is, at best, a very poor cometabolic substrate for degradation of 1,4-D at this site.

Significant 1,4-D biodegradation was not observed in any microcosms that were not amended with inorganic nutrients, suggesting nutrients are limited at the study Site. The role of THF as a potential cometabolic substrate continues to be evaluated through enrichment testing. Overall, the data indicate that organisms capable of degrading each of the added primary substrates are present in the aquifer (even in the highly anaerobic source area), and that the addition of some of these substrates, in conjunction with oxygen and nutrients, is a viable approach for in situ degradation of 1,4-D and THF in the aquifer.