

## **Bench-Scale Evaluation of 1,4-Dioxane Biodegradation Via Alkane Gas-Mediated Cometabolism in the Presence and Absence of 1,1-DCE and 1,1-DCA**

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**Background/Objectives.** Groundwater and soil from an industrial site have been impacted with 1,4-dioxane, 1,1-dichloroethylene (1,1-DCE) and 1,1-dichloroethane (1,1-DCA) which constitutes a challenge for bioremediation since 1,1-DCE has been shown to be inhibitory to 1,4-dioxane biodegradation. A bench-scale laboratory study was performed to evaluate alternatives for in situ bioremediation of site groundwater; specifically, 1,4-dioxane bioremediation via aerobic cometabolism and 1,1-DCE and 1,1-DCA via reductive dechlorination.

**Approach/Activities.** Two sets of microcosm experiments were performed using soil and groundwater from the site. The first set was prepared under aerobic conditions with the following treatments: 1) sterile controls; 2) intrinsic controls; 3) propane and bacteria culture *Rhodococcus ruber* ENV425 (ENV425) amended; 4) propane and ENV425 amended after removal of 1,1-DCE and 1,1-DCA; and 5) butane amended. The second set of microcosms, prepared under anaerobic conditions to promote reductive dechlorination, had the following treatments: 1) sterile anaerobic controls; 2) intrinsic anaerobic controls; and 3) vegetable oil and bacteria culture KB-1 Plus® amended. All biostimulated and bioaugmented microcosms received nutrients. An additional experiment on methane-mediated cometabolism of 1,4-dioxane was performed by adding oxygen to microcosms that underwent complete reductive dechlorination of 1,1-DCE and 1,1-DCA. Data collected included aqueous concentrations of 1,4-dioxane, 1,1-DCE, 1,1-DCA, hydrocarbon gases, sulfate, chloride, key biomarkers, pH and oxidation-reduction potential.

**Results/Lessons Learned.** Aerobic microcosms amended with propane and ENV425 showed propane consumption and 1,4-dioxane biodegradation when 1,1-DCE and 1,1-DCA were previously removed from the groundwater via sparging. Biodegradation in these microcosms was further supported by the increase in propane monooxygenase biomarker concentrations. No decreases of COC concentrations was observed in the remaining aerobic microcosms (propane or butane amended), which may be explained in part by the inhibitory effects of the chlorinated COCs. Results from the set of anaerobic microcosms indicated that reductive dechlorination with vegetable oil and KB-1 Plus® was effective at treating 1,1-DCE and 1,1-DCA. Methane produced in these anaerobic microcosms was consumed after oxygen amendment. However, only about 25% of 1,4-dioxane was biodegraded in these microcosms. The results of this study indicate that bioremediation of groundwater impacted with 1,4-dioxane and chlorinated solvents, especially 1,1-DCE, requires that solvents are treated first to decrease inhibition. Reductive dechlorination could be an effective method of removing 1,1-DCE prior to treatment of 1,4-dioxane. However, creating the appropriate redox conditions to promote methane oxidation may be challenging. In addition, even if methane consumption is established, the appropriate community of bacteria should be present to effectively degrade 1,4-dioxane via cometabolism. In contrast, aerobic cometabolism of 1,4-dioxane by oxygen and propane amendment and ENV425 bioaugmentation may be a more suitable alternative following reductive dechlorination, if adequate oxygen levels are maintained.