Microcosm Study of Isobutane and Isobutene Utilizing Microorganisms for the In Situ Bioremediation of 1,4-Dioxane, 1,1-Dichloroethene and Trichloroethene

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1,4-Dioxane (1,4-D) and chlorinated solvents such as trichloroethylene (TCE) and 1,1-dichloroethene (1,1-DCE) are hazardous chemicals commonly found in groundwater. Bioremediation through aerobic cometabolism is a potential option for the remediation of aquifers containing mixtures of these compounds. The purpose of this study was to examine the use of multiple primary substrates to stimulate bacteria that can perform concurrent aerobic cometabolism of 1,4-D, 1,1-DCE, and TCE. Isobutane and isobutene were tested as primary growth substrates, and the bioaugmentation with two different pure bacterial cultures was evaluated.

Batch microcosms containing groundwater and sediment from the Naval Air Station North Island, San Diego, California were used to determine if native aerobic bacteria could be stimulated to biodegrade TCE, 1,1-DCE, and 1,4-D. Isobutane and isobutene were selected as primary growth substrates to promote cometabolism of these compounds. Select microcosms were also bioaugmented with the isobutane-utilizing culture *Rhodococcus rhodochrous* ATCC 21198 or the isobutene-utilizing bacterium *Mycobacterium* ELW1.

After 52 days of incubation, isobutene uptake was observed in non-bioaugmented microcosms. Once isobutene was completely utilized, rapid 1,1-DCE transformation and slow TCE degradation were observed. 1,4-D was not effectively transformed in the native or bioaugmented microcosms fed isobutene. Isobutene was found to strongly inhibit the cometabolism of both 1,1-DCE and TCE. Microcosms bioaugmented with ELW1 showed a similar transformation response as observed in the microcosms with native bacteria only. Isobutene was effectively utilized in repeated additions in the microcosms, and successive additions of 1,1-DCE and TCE were transformed. In microcosms with isobutane as the primary substrate, no substrate uptake or loss of contaminants was observed after 130 days. It is hypothesized that the transformation of 1,1-DCE to a toxic epoxide was inhibitory to the native microbes, not allowing significant population growth. Bioaugmentation with a high concentration of ATCC 21198 was an effective strategy to stimulate transformation of 1,4-D and 1,1-DCE. Although 1,1,-DCE was likely toxic to the culture, the population density was high enough to biodegrade all of the contaminant, allowing 1,4-D to also be transformed. TCE was minimally biodegraded in the microcosms. After multiple additions of primary substrate and contaminants, 1,1-DCE and 1,4-D were transformed repeatedly. 1,1-DCE was transformed prior to the utilization of isobutane, indicating potential inhibition of isobutane uptake by 1,1-DCE. The data also show potential inhibition of 1,4-D transformation by isobutane. Microcosm and column studies are currently being performed to evaluate how the combination of isobutane and isobutene addition, along with bioaugmentation of 21198 and ELW1, can be optimized to achieve effective in situ treatment of 1,4-D, 1,1-DCE, and TCE at the site.