Single-Well Push-Pull Tests to Assess Aerobic Cometabolism of Isobutene as a Surrogate for 1,4-Dioxane

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Background/Objectives. 1,4-Dioxane is a probable human carcinogen that has emerged as a common groundwater contaminant at military and industrial sites. Aerobic cometabolism is a potentially effective method for remediation due to 1,4-dioxane's miscibility and occurrence at low concentrations in the environment. Pure culture and aquifer microcosm experiments have shown that isobutane, a gaseous hydrocarbon, is an effective primary growth substrate to induce the cometabolism of 1,4-dioxane in laboratory settings. In order to expand the assessment of isobutane as a primary substrate to the field scale, single-well push-pull tests are being performed at an experimental well field at Oregon State University (OSU), the site of a former BTEX plume. 1,4-dioxane is not present at the site, so isobutene is used as an innocuous surrogate for 1,4-dioxane to investigate aerobic cometabolism. The aquifer is shallow (13-foot well used for analysis) with low dissolved oxygen and nitrate concentrations.

Approach/Activities. Aquifer microcosms were used to assess biostimulation with isobutane and bioaugmentation with *Rhodococcus rhodochrous*, a known 1,4-dixoane-degrader when grown on isobutane. Single-well push-pull tests allow for the assessment of aerobic cometabolism on the field scale through the injection and extraction of primary and cometabolic substrates, oxygen, and nitrate in a single well. A bromide tracer is used to assess transport of these compounds in the subsurface. Isobutene is used as a cometabolic substrate surrogate for 1,4-dioxane to investigate aerobic cometabolism at this site because there is no background 1,4-dioxane present. Isobutene and 1,4-dioxane are cometabolized at comparable rates by *R. rhodochrous*.

Results/Lessons Learned. Microcosm studies have shown that microorganisms native to aquifer material from two different sites (including the OSU well field) can be stimulated to utilize isobutane and cometabolically transform 1,4-dioxane. The biostimulation lag in these microcosms was approximately one week. Push-pull tests have shown that biostimulation of isobutane-utilizing microorganisms native to the subsurface also occurred after approximately one week. Subsequent biostimulation tests showed isobutane was consumed at increased rates, indicating growth of an isobutane-utilizing microbial population in the subsurface. A cometabolic activity test with isobutene as a surrogate for 1,4-dioxane showed isobutene was oxidized to isobutene oxide (epoxide). Pure culture and microcosms studies showed inhibition of 1,4-dioxane transformation by the presence of isobutane. Based on these results, push-pull tests are currently being conducted to investigate primary substrate inhibition in the field.