

Using Stable Isotope Probing to Confirm Biodegradation of 1,4-Dioxane during In Situ Remediation

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Background/Objectives. 1,4-Dioxane is a common co-contaminant with chlorinated solvents and treatment of 1,4-dioxane via natural or enhanced biodegradation processes provides a viable remedial strategy. While intrinsic metabolic biodegradation of 1,4-dioxane may be limited, co-metabolic biodegradation can occur under a variety of environmental conditions. For example, 1,4-dioxane can be co-metabolically degraded in the presence of alkane gases (e.g., methane or propane) and oxygen by microorganisms that utilize these primary substrates as part of their metabolic processes. At Site 24 at Vandenberg Air Force Base in California, historical use of chlorinated solvents resulted in up to approximately 2,000 micrograms per liter of 1,4-dioxane in groundwater. Previously, in-situ propane biosparging was implemented and resulted in 1,4-dioxane concentration reductions (as published by others). This work builds on the prior field demonstration to confirm biodegradation as the mechanism for 1,4-dioxane concentration decreases via stable isotope probing (SIP). SIP includes addition of a ^{13}C -enriched compound into the test system. The ^{13}C in the 1,4-dioxane acts as a carbon-atom tracer to track the biotransformation of 1,4-dioxane into mineralized carbon dioxide and/or cellular biomass to identify the fate of 1,4-dioxane under biostimulation conditions.

Approach/Activities. This second propane biosparge field demonstration was initiated in December 2015 and included an air compressor, propane tank, a gas mix point, and a lower explosive limit (LEL) meter. Operation of the system included sparging a mixture of air and propane (20 percent of the LEL) at up to 5 standard cubic feet per minute into one sparge point, for 30 minutes every four hours. Bioaugmentation with a propanotrophic culture was conducted, alongside nutrient addition of diammonium phosphate. SIP included use of Bio-Trap[®] samplers “baited” with isotopically enriched 1,4-dioxane. The Bio-Trap[®] samplers were deployed for approximately 30 days during biosparge operation. They were then retrieved and analyzed by Microbial Insights. After completion of the SIP testing, the system was operated under varying conditions to better understand the effect of concentration rebound on the treatment approach.

Results/Lessons Learned. After two months of operation, 1,4-dioxane concentrations decreased approximately 45 to 83 percent at monitoring locations in the test area. The SIP results confirmed the biodegradation mechanism associated with 1,4-dioxane groundwater concentration decreases. The co-metabolic biotransformation of ^{13}C -enriched 1,4-dioxane is expected to result in generation of carbon dioxide which is measured as ^{13}C -enriched dissolved inorganic carbon. The dissolved inorganic carbon delta values were 76 and 20 per mil, values more positive than the average background value which confirms the biotransformation of 1,4-dioxane to carbon dioxide under the propane biosparge conditions. The total biomass values were 1.55×10^6 cells/bead and 5.22×10^5 cells/bead with the relative magnitude of these values correlating to the decreases of 1,4-dioxane in groundwater. A portion of that population incorporated ^{13}C from the 1,4-dioxane into their biomass, approximately 1.5 to 2 percent. Evaluation of this microbial biomass indicated incorporation of ^{13}C into the cellular phospholipids. Because significant carbon uptake into microbial biomass is not commonly associated with co-metabolism, the ^{13}C -enriched biomass values observed here may be attributed to uptake of the mineralization intermediates or the carbon dioxide end product, rather than the 1,4-dioxane directly. These results confirm active biodegradation of 1,4-dioxane under propane biosparge conditions.