Novel Organism Deployed for In Situ Bioremediation of 1,4-Dioxane in Groundwater

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Background/Objectives: 1,4-Dioxane is likely a human carcinogen according to U.S. EPA, and is highly prevalent, primarily as a groundwater contaminant. EPA risk assessments have established a Health Reference Level (HRL) for 1,4-dioxane of 0.35 µg/L on the basis of a one-in-one million cancer risk. Treatment of dilute dioxane plumes to this low concentration presents a significant technical challenge and financial burden for many stakeholders. Energy-intensive ex situ technologies (e.g., pump and treat with advanced oxidation processes) with high capital and operating costs are often the only remedy for large dilute plumes. Therefore, ongoing research has aimed to develop low-cost in situ technologies, such as bioaugmentation, for 1,4-dioxane-contaminated groundwater. While microorganisms have been identified and deployed using co-metabolic degradation pathways, few organisms have been commercialized that degrade 1,4-dioxane as a carbon source. Allonnia has identified and grown two microbes that have demonstrated metabolic degradation of 1,4-dioxane. They have been designated as Strain ALL22_0001 & ALL22_0048. Here we describe the laboratory demonstration of 1,4-dioxane degradation in both synthetic media and groundwater. We further describe a pilot study where we introduced ALL22_0001 into a groundwater well to provide a proof of concept for bioaugmentation.

Approach & Activities: Under lab conditions, ALL22_0001 was grown in synthetic media supplemented with down to 1 ppm of 1,4-dioxane. Samples were collected after 96 h incubation and the amount of residual 1,4-dioxane was measured. Similar experiments were conducted using a groundwater matrix as a growth media. For the field study, the Regional Water Quality Control Board for the California site approved inoculation of two separate wells for bioaugmentation with ALL22_0001. After aerating each well, a small amount of the culture was added along with a nutrient mix to facilitate growth. Dilution in the well was minimal. Equipment setup at each well included two five-gallon buckets, one aeration pump and stone, one peristaltic pump, one Horiba multiparameter meter and flow through cell, silicone and HDPE tubing, and a water level meter.

Results: In the laboratory, ALL22_0001 degraded 1 ppm of 1,4-dioxane in synthetic media and groundwater samples to non-detect at 30^0C and 25^0C over 72-96h. To our knowledge, this was the first instance of demonstrating this strain's ability to successfully degrade 1,4-dioxane in groundwater. For the field study, wells are being monitored for a duration of 4-5 months with regular sampling events to track the microbial persistence and 1,4-dioxane concentrations. Results from the first 11 weeks post inoculation show an 88% reduction in 1,4-Dioxane in well 1 and a 54% reduction in well 2. Custom qPCR assays were developed to track the presence of ALL22_0001 in the samples collected and these results validated the presence of Allonnia's bacteria in the system.