

Development and Testing of a 1,4-Dioxane Bioaugmentation Culture

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Background/Objectives. Results from enhanced in situ bioremediation (EISB) bench-scale and field trials for 1,4-dioxane treatment have often varied widely and consistent and effective application approaches for in situ treatment have yet to be developed. Academic research has predominantly focused on the development of pure cultures that have been demonstrated to effectively degrade 1,4-dioxane in laboratory studies. A mixed microbial consortium that effectively degrades 1,4-dioxane at concentrations ranging up to 1,000 mg/L to low concentrations (e.g., less than 10 µg/L) has been enriched and is a potential bioaugmentation culture for field application. Mixed consortia may include multiple 1,4-dioxane degraders imparting beneficial redundancy compared to pure cultures.

Approach/Activities. Specific challenges associated with evaluating EISB for 1,4-dioxane remediation include: analysis (low site concentrations and specialty analytical methods); inhibitory effects by co-contaminants; and the fact that bioaugmentation using metabolic 1,4-dioxane degrading organisms has yet to be successfully demonstrated in the field. Bench-scale batch microcosm and column tests were completed to i) demonstrate the effectiveness of the developed bioaugmentation culture and ii) to identify the conditions and treatments associated with successful outcomes, as well those that were ineffective or negatively affected outcomes. Next generation and metagenomic sequencing was used to characterize the microbial community and to identify possible biomarkers for this novel culture.

Results/Lessons Learned. Results from bench-scale studies, including studies testing bioaugmentation with aerobic metabolic 1,4-dioxane degrading populations, or biostimulation of co-metabolic populations, is increasingly demonstrating that bioremediation as a feasible approach for 1,4-dioxane contamination. Specifically, this study demonstrates that bioaugmentation with a 1,4-dioxane culture can be used to successfully optimize bioremediation at concentrations often encountered in the field.

Results from molecular characterization suggest that this 1,4-dioxane bioaugmentation culture lacks the *Pseudonocardia* dioxane monooxygenase biomarkers commonly associated with 1,4-dioxane degradation, although several organisms in the culture are putative 1,4-dioxane degraders and their potential roles in biodegradation of 1,4-dioxane will be discussed.