Isolation, Adaptation, and Preparation of Bacterial Strains for 1,4-Dioxane Bioaugmentation

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Background/Objectives. Currently, 12% of the US population is served by drinking water with 1,4-dioxane concentrations above the EPA health advisory level. However, remediation of dioxane-contaminated sites is a challenging task because of its resistance to biodegradation, low tendency to volatilize from water, and high mobility in groundwater. As such, dioxane can be extremely difficult and costly to remediate. Thus, there is a pressing need for innovative approaches that leads to faster, more economical, and more sustainable remediation of dioxane-contaminated groundwater, particularly when located near large population centers. Bioaugmentation offers great potential to enhance dioxane removal in many circumstances. However, strain selection has been shown to be one of the most critical determinants for bioaugmentation success, and there are few choices available for dioxane bioremediation. Moreover, the bacterial strains known to degrade dioxane tend to grow slowly, are fastidious, and form aggregates. These characteristics make large-scale culturing difficult, decrease the probability of survival in situ, and hinder subsurface dispersion. Thus, the creation of a diverse collection of dioxane-degrading bacteria along with data to inform their implementation in the field is needed.

Approach/Activities. We will conduct a comparative study of several well-known dioxane-degrading strains, including *Pseudonocardia dioxanivorans* CB1190, *Mycobacterium dioxanotrophicus* PH-06, and *Rhodococcus aetherivorans* 10BC-312. Each strain will be assessed for its ability to degrade dioxane in not only microcosms, but in column studies, for which data is generally lacking. We will also report on efforts to scale up the growth of each strain, the isolation of new dioxane degrading strains and/or consortia, practices to enhance the success of bioaugmentation studies, and challenges that remain.

Results/Lessons Learned. Contrary to prior reports detailing the widespread occurrence of dioxane-degrading bacteria, our recent results suggest many dioxane-impacted sites may lack bacteria with degradative capacity, which precludes the use of MNA or biostimulation as remediation strategies. Additionally, preliminary data from column studies with CB1190 suggests very limited distribution after injection even when the cultures have been subjected to deaggregation protocols. However, recent efforts to identify bacterial strains with enhanced subsurface distribution potential have yielded a variety of strains that appear more promising than CB1190. Other data and analyses expected at the time of presentation include methods for culture scale-up that maintain dioxane-degradation activity, a comparative analysis of different methods for deaggregating cell clumps (and enhancing subsurface distribution), and the results of efforts to adapt tetrahydrofuran-degrading cultures to growth on dioxane.