Transport of *Pseudonocardia* through Soil for Bioremediation of 1,4-Dioxane

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Background/Objectives. Bioaugmentation is feasible only when microbes are able to move through an aquifer in the direction of contaminated groundwater. For 1,4-dioxane, bioaugmentation with a culture that grows on the contaminant offers several advantages over cometabolic biodegradation, including reduced chances for aquifer clogging, no need to deliver a primary substrate, and reduced demand to supply oxygen. *Pseudonocardia dioxanivorans* CB1190 is the best studied of microbes that use 1,4-dioxane as a sole source of carbon and energy under aerobic conditions. A potential concern with using CB1190 is its propensity to clump during growth, presumably hindering its ability to move through soil. The objective of this study was to evaluate the movement of CB1190 and another strain of *P. dioxanivorans* through soil columns.

Approach/Activities. *P. dioxanivorans* BERK-1 was isolated from a contaminated site in the southeastern U.S. based on its ability to use 1,4-dioxane as a sole carbon and energy source. Identification of the isolate as a new strain was evident from whole genome sequencing. Kinetic parameters for BERK-1 were measured, including its yield, maximum specific growth rate, decay rate, half saturation constant, and affinity for dissolved oxygen, all of which differ somewhat from CB1190. The cell surface properties of BERK-1 are distinctively different from those of CB1190. BERK-1 does not form clumps during growth at high rates to the same extent that CB1190 does. The movement of both strains through soil was evaluated. Bench-scale columns were constructed in vertical glass tubes with 12.7 cm of saturated sand (porosity ~0.4) and mineral medium containing 100 ppm of 1,4-dioxane. The end of each column was immersed in medium contained within an Erlenmeyer flask. Inoculum was applied to the top of the columns and liquid was allowed to flow through the soil by gravity. Recirculation was accomplished by removing liquid from the reservoir and adding it back to the column. The medium was constantly stirred to provide oxygen. Samples were taken from the reservoir to analyze 1,4-dioxane. Treatments included columns inoculated with CB1190, BERK-1, and no inoculum, with and without recirculation. A second set of columns was prepared in the same manner using silty sand (porosity ~0.2).

Results/Lessons Learned. BERK-1 moved through the sand columns at a higher rate than CB1190, although this difference was reduced with recirculation. Because CB1190 grows at a higher rate than BERK-1, it consumed the 1,4-dioxane at a higher rate even though it took longer to travel through the sand. There was no removal of 1,4-dioxane in the uninoculated controls. Similar results were obtained for the columns filled with lower porosity silty sand. Thus, in spite of its propensity to form clumps during growth at high cell densities, CB1190 was able to move through soil. This suggests that a different cell surface prevails when the cells are in contact with soil and are growing at less than the maximum rate. The reduced propensity for cell clumping by BERK-1 during suspended growth corresponded to faster rates of movement through soil. However, CB1190 grows at a faster rate. This will likely be less of a disadvantage for BERK-1 in situ since the majority of aquifers contaminated with 1,4-dioxane have concentrations well below what is needed for high rates of growth. The results suggest that at lower growth rates, CB1190 does not clump excessively and therefore is able to move reasonably well through soil, although not as fast as BERK-1. Additional cultures that grow on

1,4-dioxane as a sole source of carbon and energy should be evaluated for their rate of movement through soil during bioaugmentation.