Using Molecular Tools to Monitor a Microbial Consortium Degrading a 12-Chemical Mixture

Rachel E. Hanson
Civil Engineering Department, Colorado State University, Fort Collins

C. Sans
Chemical Engineering and Metallurgy Department, Universistat de Barcelona, Barcelona

M. Hoelscher
Chemical and Biological Engineering Department, Colorado State University, Fort Collins

A. Pruden
Civil Engineering Department, Colorado State University, Fort Collins

K.F. Reardon[1]
Chemical and Biological Engineering Department, Colorado State University, Fort Collins

Abstract. An unexplored issue in studies of the fate and transport of pollutants in groundwater is whether the aquifer microbial community changes along the length of a contaminant plume. Recent developments in molecular biological methods to characterize microbial communities have made it feasible to address this question. To evaluate these methods, we performed a study in which the dynamics of a microbial community in a well mixed continuous-flow bioreactor were tracked after two perturbations: disruption of wall growth and a change in feed composition. The microbial consortium was grown on a 12-chemical mixture consisting of benzene, toluene, phenol, dimethyl phenol, p-cresol, m-xylene, chlorobenzene, dichlorbenzene, trichlorbenzene, acetone, 2-butanone, and hexanone. A suite of complementary molecular techniques, including cloning and sequencing of 16S rDNA genes, denaturing gradient gel electrophoresis (DGGE), and capillary electrophoresis-single strand conformation polymorphism (CE-SSCP), were used to profile the microbial consortium. DGGE and CE-SSCP were used to track changes in the microbial community profile with respect to time. CE-SSCP was also used in conjunction with cloning and sequencing 16S rDNA to identify dominant members of the community. Additionally, pure cultures were isolated on each of the 12 individual chemicals in order to link structure and function of the microbial community.

The results indicate that although chemical removal was stable throughout the 12 month monitoring period, the composition of the consortium was variable. Specifically, changes in the composition of the consortium were observed to correlate with wall-cleaning events and changes in substrate composition. This was confirmed both by DGGE and by cloning. Based on analysis of DGGE gels, it was also found that the composition of the consortium that tended to grow on the walls was different from the composition of the suspended growth consortium. These results indicate that these methods are capable of revealing changes in the microbial population and could be applied to laboratory and field bioremediation studies.

[1] Chemical and Biological Engineering Department
Colorado State University
Fort Collins, CO 80523-1379
Tel: (970) 491-6505
E-mail: reardon@engr.colostate.edu