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Biodegradation of Benzene, Toluene, and *m*-Xylene
by Anaerobic Microorganisms

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Many petroleum products are discharged into the environment in the form of leaks in underground storage tanks, improper disposal technique, and inadvertent spill, and these chemicals could accumulate in the soil and groundwater, leading to serious damages to human. Aromatic hydrocarbons are among the most common soil and groundwater contaminants. The degradation of BTX (benzene, toluene, and *m*-xylene) under anaerobic conditions was studied in batch microcosms developed with aquifer material from the Ulsan and Janglim. Although BTX compounds have a similar chemical structure, the fate of individual BTX compounds differed when the compounds were fed to mixed culture slurries. Interactions between BTX included enhanced degradation of benzene by toluene, toluene by *m*-xylene and *m*-xylene by benzene and toluene. The degradation rate of *m*-xylene was the fastest among the components of BTX mixture. Toluene and *m*-xylene were mineralized to stoichiometric amounts of carbon dioxide and methane under strictly anaerobic conditions. Toluene and *m*-xylene were removed 59% and 68% of initial concentration respectively. Toluene and *m*-xylene were transformed into CO₂ and biomass. Based on the fact that more than 60% toluene- and *m*-xylene-carbon were converted to CO₂ and that significant amounts of methane were formed.

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Development of Molecular Biological Methods to Analyze the Bacterial
Species Diversity in Freshwater and Soil Ecosystem

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Novel techniques were developed for economic and rapid analysis of microbial communities in the natural environment. Our methods are based on terminal restriction length polymorphism (T-RFLP) and PCR-single-strand-conformation polymorphism (PCR-SSCP). The bacterial 16S rRNA gene was amplified by PCR with universal primers, with one of the primers biotinylated at the 5' end. In T-RFLP analysis, the PCR products were digested with restriction enzymes, and then the biotinylated 5' T-RFs were selected by streptavidin paramagnetic particles. The biotinylated strands of the PCR products were selectively isolated for preventing heteroduplex formation in PCR-SSCP analysis. The selected strands of T-RFLP and PCR-SSCP were separated by electrophoresis on polyacrylamide gel, and detected by silver staining. Analysis of PCR products from 10 strains demonstrated those characteristic DNA band patterns. In addition, structural changes of bacterial communities in microcosm treated with phenol could be monitored.