Aerobic Biodegradation of Aromatic, Chlorinated Aliphatic, and Ether Contaminants by *Pseudonocardia* sp. Strain ENV478 and Native Populations

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Background/Objectives. Decades of remediation efforts at a Superfund site located in the northeastern United States have resulted in removal of the vast majority of contaminant mass. However, small pockets containing ppb to low ppm concentrations of a number of different compounds including aromatics, chlorinated aliphatics, and various ethers including diethyl ether, tetrahydrofuran (THF), and 1,4-dioxane (DX) remain, preventing site closure. A laboratory study was conducted to evaluate aerobic biodegradation as a remedial option for the site. Biostimulation of native bacteria and bioaugmentation with *Pseudonocardia sp.* strain ENV478 (ENV478), a co-metabolic degrader of ethers including DX, were evaluated. ENV478 had not previously been tested to determine if it could also degrade one or more of the aromatic and chlorinated aliphatic co-contaminants present at this site.

Approach/Activities. Groundwater microcosms were constructed to evaluate the potential for biodegradation of site constituents by native microorganisms and by ENV478 under aerobic conditions. Biostimulation treatments with diammonium phosphate (DAP) were tested to determine N and P limitations, and treatment with propane as a carbon substrate was conducted to stimulate native propanotrophs to co-metabolize DX and other compounds. Additionally, since the ambient pH at the site is approximately 5.8, select microcosms were adjusted to pH 7.0 to potentially enhance degradation rates of the COCs. To differentiate between degradation performed by native bacteria from that by bioaugmented ENV478, biodegradation was also monitored in sterile COC-spiked basal salt medium (BSM) augmented with ENV478 only (cell density approximately 10^7 cells/mL).

Results/Lessons Learned. Biostimulation with N and P significantly accelerated biodegradation of many of the COCs by native degraders, and further treatment with potassium carbonate to increase the pH from 5.8 to 7.0 resulted in >95 % removal of THF, diethyl ether, ethyl chloride, and 1,2-dichloroethane (1,2-DCA), while DX showed about 75 % removal in less than 50 days. Similarly, non-detectable levels of benzene, toluene, o-xylene, and 1,2-dichlorobenzene (1,2-DCB), were observed while 1,1-dichloroethane (1,1-DCA) and 1,1,1-trichloroethane (1,1,1-TCA) were not degraded by native populations. Treatments with propane to induce co-metabolic degradation of 1,1-DCA and 1,1,1-TCA by native propanotrophs were unsuccessful. In COC-spiked growth medium, ENV478 rapidly degraded all ethers, benzene, and toluene to non-detectable levels within two weeks. 1,2-Dichloroethane was degraded by about 85 % over the course of two weeks, but degradation stalled at that point. Other compounds, including 1,1-DCA, 1,1,1-TCA, 1,2-DCB, and o-xylene were not degraded by ENV478. The results demonstrate that, in addition to DX, ENV478 also can degrade various other ethers, benzene, toluene and 1,2-DCA. Chlorinated ethanes where multiple chlorine atoms were located on the same carbon (1,1-DCA and 1,1,1-TCA) remained recalcitrant to both native and bioaugmented organisms. The study indicates that inorganic nutrients and low pH are limiting the rates and extents of aerobic biodegradation of many of the residual COCs at the site, with the exception of 1,1-DCA and 1,1,1-TCA, which were persistent under all conditions tested.