Biodegradation and Bioaugmentation of Aniline and *p*-chloroaniline at a Contaminated Chemical Manufacturing Site in Southern Jersey

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Background/Objectives. Aniline and parachloroaniline are used in manufacturing industries including those producing: plastics, dyes, pharmaceuticals, and fossil fuels. These compounds have entered the environment and are problematic groundwater pollutants. Aniline is toxic to aquatic, terrestrial and human life. These compounds affect the central nervous and endocrine systems, and according to EPA, aniline is a probable human carcinogen.

Approach/Activities. We are evaluating the potential for aniline and *p*-chloroaniline biodegradation by native aquifer microorganisms from a large, chemically and geologically complex industrial site historically contaminated with many different chemicals including aniline and chlorinated aniline. A 55-ft sediment core was recovered from the site along with groundwater from a multi-level sampler. The sediment core was divided into 2-inch slices for detailed interrogation. The ~300 sediment core slices were shared among multiple teams and characterized for in situ geochemistry and microbial community via next-generation sequencing of 16S rRNA genes. We used this multifaceted data to develop hypotheses for microbial activity and tested the hypotheses through laboratory scale microcosms simulating in situ conditions. Microcosms were established to determine if aniline and paprchloroaniline biotransformation occurred under these conditions. Sediment from two to three 2-in. core slices from different specific elevations (~ -10, -13, -17, -34 ft) were used to establish four sets of targeted microcosms. In addition, sediments from -9.2 to -10 ft were mixed and used for composited microcosms. Aerobic, sulfate-, nitrate-, and iron-reducing microcosms were established and incubated in dark at room temperature. Microcosms were ~15% sediment slurry in groundwater from a matching depth or in minimal medium. Autoclaved controls were also established. Experiments were performed under sterile conditions using triplicates. Microcosms were amended with aniline and parachloroaniline and monitored by HPLC. Active microcosms were plated (0.1 mL) on minimal media agar plates without carbon and nitrogen sources for isolation. Non-active microcosms were bioaugmented using pure isolates.

Results/Lessons Learned. The targeted microcosms showed no significant loss of aniline or *p*-aniline. The aerobic and sulfate-reducing condition of the composited microcosms were active, and aniline was depleted several times after being re-amended. *Janibacter* HA1 and *Rhodococcus* HA2 were isolated from the aerobic microcosms. HA1 uses aniline as a carbon source, and HA2 uses aniline as a carbon and nitrogen source. Analysis of 16S rRNA genes showed a shift in the microbial community at the genus level in comparison to the original community. Aniline was depleted in non-active targeted microcosms two weeks after bioaugmentation with the pure isolates. Understanding the microbial community behavior, detecting the activity of microorganisms, and isolation of active pure cultures from the contaminated site will be useful in developing future bioremediation strategies for the site.