Microbial and Isotopic Evidence of Concurrent Aerobic and Anaerobic Degradation of Chlorinated Benzenes in Wetland Sediments and a Bioaugmented-Activated Carbon Reactive Barrier

Michelle M. Lorah (mmorah@usgs.gov) (U.S. Geological Survey, Baltimore, MD, USA)
Denise Akob (U.S. Geological Survey, Reston, VA, USA)
Steven Chow and Edward J. Bouwer (Johns Hopkins University, Baltimore, MD, USA)
Amar R. Wadhawan (Arcadis, Columbia, MD, USA)
Neal D. Durant (Geosyntec Consultants, Columbia, MD, USA)

Background/Objectives. Aerobic and anaerobic bacteria capable of degrading chlorinated benzenes can naturally coexist in wetland sediments. However, natural biodegradation rates may be insufficient to achieve complete degradation without accumulation of chlorobenzene or benzene before exposure at land surface or surface water. We conducted small-scale pilot tests to evaluate the use of bioaugmented, granular activated carbon (GAC) in a reactive barrier to enhance biodegradation of chlorinated benzenes at the Standard Chlorine of Delaware Superfund wetland site. To evaluate biodegradation, microbial community data and stable isotope probing in in situ microcosms were used, along with chlorinated benzene analyses.

Approach/Activities. GAC was bioaugmented with a commercially available anaerobic, dechlorinating culture (WBC-2) and an aerobic consortium (15B) that was enriched from the site for chlorinated benzene degradation. Reactive barriers were constructed by mixing bioaugmented GAC, sand, and chitin into the upper 25 centimeters (cm) of wetland sediment in two plots. At 0.5, 5, 9, and 18-19 months post-installation, sediment cores (45-cm-long) were collected from the test plots and adjacent control areas and sectioned at 5-10 cm intervals for microbial community analysis by Illumina next generation sequencing and for analysis of volatile organic compounds after methanol extraction. At 19 months, Bio-Trap in situ microcosms with ^{13}C-labeled chlorobenzene were conducted in the test plots and control areas to evaluate chlorobenzene degradation mechanisms and key microbes and functional genes.

Results/Lessons Learned. Microorganisms associated with anaerobic reductive dechlorination and aerobic oxidation of chlorobenzenes and benzene, occurring together, were prevalent in reactive barrier sediment and in situ microcosms. Anaerobic members of the Desulfuromonadales order and aerobic members of the Burkholderiales order showed enhanced population abundances in Illumina analyses of bioaugmented GAC and reactive barrier sediment samples compared to the control sediment samples. Desulfuromonadales included *Desulfuromonas* and *Geobacter* genera, while Burkholderiales included *Alcaligenes* and three genera of Comamonadaceae bacteria (*Aquabacterium, Ideonella,* and *Polaromonas)* that are associated with oxidation of chlorinated benzenes and benzene. From the in situ microcosm analyses, *Desulfuromonas* was the only indicator microbe of reductive dechlorination that was elevated by more than an order of magnitude in the reactive barriers compared to the controls. Multiple functional genes associated with aerobic degradation of aromatics and chlorinated benzenes were elevated in the reactive barriers compared to control areas— toluene dioxygenase (TOD), trichlorobenzene dioxygenase (TCBO), phenol hydroxylase genes/benzene monooxygenase (PHE), toluene monooxygenase-2/phenol hydroxylase (RDEG), and toluene monooxygenase-3 and -4 (RMO). Incorporation of 13C into the microbial biomass and dissolved inorganic carbon was 2 to 4 orders of magnitude higher in the reactive barriers than those in the control areas, conclusively demonstrating enhanced biodegradation. The microbial and isotopic data confirmed that biodegradation was responsible

for the observed mass removal of chlorinated benzenes in the reactive barriers and could regenerate the GAC sorption capacity to provide long-term effectiveness of the barriers.