

Coupled Biodegradation of Chlorinated Benzenes at Anaerobic-Aerobic Interfaces

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Background/Objectives. We investigated the biodegradation of chlorinated benzenes (CBs) across anaerobic-aerobic interfaces commonly found at shallow wetland sites. Contaminated wetlands contain complex redox gradients, which facilitate a variety of biogeochemical processes including desirable contaminant biodegradation pathways. Under anaerobic conditions highly chlorinated CBs can be reductively dechlorinated to monochlorobenzene and benzene, with increased environmental mobility compared to their parent compounds. These dechlorinated products may be readily mineralized by aerobic bacteria given sufficient oxygen availability. We aim to study the dynamics between anaerobic and aerobic CB biodegradation pathways in order to understand the factors affecting their coupling for bioremediation in the field. Ultimately, these results will be used to develop an effective in situ sorptive bio-barrier capable of interception, sequestration, and degradation of CBs discharged from the subsurface.

Approach/Activities. We developed a novel laboratory column system designed to simulate an anaerobic-aerobic interface. Columns were packed with either sand or a mixture of wetland sediment and sand inoculated with aerobic and anaerobic CB-degrading cultures. Steady inputs of 1,2,4-trichlorobenzene, electron donor sodium lactate (NaLac), and oxygen were maintained in simulated groundwater media. Two separate year-long experiments were conducted to study this model system. The first evaluated the effects of varying electron donor concentrations on biodegradation pathways. The second examined the dynamics of varying degrees of nitrate- and sulfate-reducing conditions on contaminant biodegradation and microbial community structure. In both experiments, porewater was sampled at various times across the length of the columns for CBs, organic acids, and inorganic anions. At the end of the experiments, columns were sacrificially sampled and analyzed using 16S rRNA amplicon sequencing.

Results/Lessons Learned. Both anaerobic and aerobic biodegradation activity were maintained on simple sand as well as sand and sediment matrices. Sand columns were able to achieve the highest degree of aerobic mineralization (0.58 mg CB / mg O₂). Anaerobic transformations in sand columns were highly affected by electron donor availability, with no apparent reductive dechlorination at low doses (15 mg/L NaLac). In contrast, sediment mixtures had moderately less aerobic mineralization (0.41 mg CB / mg O₂) and near complete reductive dechlorination to monochlorobenzene under all tested NaLac doses. An analysis of putative anaerobic dechlorinator *Dehalobacter* showed low enrichment in sand biofilms (maximum 3% relative abundance) compared to high enrichment in mixed sand and sediment biofilms (up to 31%), demonstrating that site sediment may provide a favorable environment for reductive dechlorinators and facilitate more parsimonious utilization of electron donor. Competitive inhibition between electron donor and O₂ occurred as electron donor inputs increased. Under moderate sulfate (250 mg/L) and nitrate (30 mg/L) reducing conditions, we observed both CB transformation pathways to persist, despite apparent increased electron donor demand from these alternative electron acceptors. Biofilm microbial communities showed bifurcation across the anaerobic-aerobic interface and stratification within their respective redox zones. Analysis of porewater microbial communities under varying electron acceptor conditions is ongoing.