Is a Mineral Surface Critical to Rapid and Successful Anaerobic Benzene Biodegradation?

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Background/Objectives. Microbial communities modus operandi in contaminated soils remains relatively elusive. Numerous factors affect the success of in situ petroleum hydrocarbon (PHC) biodegradation such as nutrient availability, contaminant concentrations, climate, soil composition, and microbial community structure. As part of the Sustainable In Situ Remediation Cooperative Alliance (SIRCA), PHC contaminated sites are amended with solutions composed of critical respiration nutrients and alternative electron acceptors for anaerobic PHC degradation in the cold, calcareous, and clayey soils of Western Canada. Benzene is suspected to be degraded by a consortia of bacteria under anaerobic conditions rather than a single species. However, benzene degradation is still an unusually slow process under the kinetically favoured pathway of nitrate reduction; leading researchers to investigate why. Most laboratory experiments investigate planktonic cells, however, there is the potential that degrative communities form biofilms on mineral surfaces in situ. We hypothesized that mineral surfaces impact degradation rates. Initially, we investigated if the addition of electronically active minerals such as hematite, or inert minerals such as corundum, stimulated benzene degradation under anaerobic conditions. Following this, we explored if microbial adhesion to the minerals was required for stimulation or alternatively, minerals were altering bulk solution behavior.

Approach/Activities. We explored benzene degradation stimulation using an enrichment culture (gifted to us from Dr. Ania Ulrich, University of Alberta) under nitrate-reducing conditions with benzene as carbon source. In the first experiment, cultures incubated with a 10 g L^{-1} slurry of hematite (α-Fe_2O_3) and media. The second ongoing experiment is assessing the importance of a mineral surface to benzene degradation. Anaerobic cultures were set up in air tight serum bottles with the following treatments: 1) media only sterile controls and live cultures, 2) media only sterile controls and live cultures with inoculant inside and outside dialysis tubing (DT), 3) hematite sterile controls and live cultures with inoculant inside and outside DT, and 4) corundum (α-Al_2O_3) sterile controls and live cultures with inoculant inside and outside DT. The samples are being analyzed for benzene concentrations weekly, and for nutrients, DNA (concentrations and sequencing), and aerobic and anaerobic hydrocarbon degradation metabolites at t_i and t_f.

Results/Lessons Learned. The first experiment showed that hematite accelerated benzene degradation rates under nitrate reducing conditions compared to a media control culture (degradation slope: - 1.5 versus - 0.37). However, we cannot distinguish whether these rates increased due to the mineral surface or if the mineral changed solution chemistry. To investigate this further, our second experiment is using dialysis tubing in which microbes and mineral cannot pass through, but molecules can. This will allow us to discern if the mineral surface or solution chemistry is critical. We hypothesize that inoculating into media only is less detrimental to the microbial community and cultures may begin degrading sooner in comparison to inoculating into a mineral slurry. However, we also suspect that once the degradative community has established with the mineral presence, that benzene degradation rates will increase rapidly due to a biofilm with an unique microbial community.