Controlling Trichloroethene Aerobic Cometabolism Rate and Microbial Biomass Using Acetylene

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Background/Objectives. Bioremediation of large diffuse contaminant plumes often experiences challenges, such as limited substrate transport and excessive microbial growth near the substrate injection points, which leads to bioclogging. Bioclogging decreases permeability and effectiveness of in situ bioremediation. To minimize bioclogging, microbial inhibitors may be used to deter microbial growth allowing for better operational control. In this project, we are investigating the effect of acetylene, a microbial inhibitor, on cometabolic biodegradation of trichloroethene (TCE) in soil-free microcosms and soil columns containing sediment and groundwater from a TCE-contaminated site. To achieve cometabolism, growth substrate (e.g., propane) and oxygen must be injected into the subsurface therefore increasing the likelihood of bioclogging. Acetylene binds to the monooxygenase enzymes which both metabolically degrade hydrocarbons (in this case propane) and cometabollically degrade TCE.

Approach/Activities. In the first phase of the project, batch studies were conducted using two microbial cultures - a pure culture of the hydrocarbon degrading bacteria *Mycobacterium austroafricanum* JOB5 and a soil-derived propane-oxidizing and TCE-cometabolizing mixed culture enriched at Arizona State University, named COMET1. These cultures were fed with propane and oxygen and were exposed to TCE to verify aerobic cometabolic capacity. They were then exposed to the monooxygenase-inhibiting acetylene gas at 5% v/v for differing lengths of time (no exposure, 1 day, 2 days, 4 days, and 8 days) to verify if microbial growth and TCE cometabolic capacities were altered. In the second phase of the project soil columns filled with sediment from a TCE-contaminated site were bioaugmented with a mixture of the two cultures. Flow through the columns consisted of groundwater from the contaminated site with O_2, with O_2 and propane, and with O_2, propane, and acetylene, respectively. TCE was added at 50 µM. TCE, propane, O_2, and CO_2 concentrations were measured during operation to verify effects on acetylene's effects on TCE cometabolism.

Results/Lessons Learned. In the soil-free microcosms, both cultures displayed the capacity for aerobically degrading TCE through cometabolism and exhibited time-dependent relationships between acetylene exposure and microbial growth. TCE degradation and microbial growth, rates decreased as a function of increasing exposure to acetylene. Microbial community analysis of COMET1 microcosms showed differences between treatments in relative abundance of families known to contain TCE cometabolizers. In the soil columns, we documented delayed and minimal TCE removal in columns without propane. Columns fed with propane showed a significantly higher TCE degradation that columns without propane. Acetylene slowed TCE degradation in propane-fed columns. Consumption of propane in acetylene-inhibited columns also occurred to a lesser extent. Additionally, acetylene exposed columns had lower total organic carbon (TOC), a proxy measurement for biomass production, proximal to the columns' inlet. Microbial ecology analysis of both soils and groundwater from the soil column showed assemblage shifts due to acetylene exposure. From these two studies we conclude that acetylene is a viable microbial inhibitor meriting of field testing for enhancing in situ aerobic cometabolism based bioremediation schemes.