

Linking Acetylenotrophs to Microbial Remediation of Chlorinated Solvents

Denise M. Akob (dakob@usgs.gov) and Yesha Shrestha
(U.S. Geological Survey, Reston, VA, USA)

Shaun S. Baesman and Ronald S. Oremland (U.S. Geological Survey, Menlo Park, CA, USA)

Background/Objectives. Groundwater across the United States is contaminated with chlorinated solvents, including trichloroethylene (TCE) and tetrachloroethene (PCE), which were widely used for numerous military, industrial, and commercial applications. Chlorinated solvent remediation strategies include optimizing conditions for abiotic and biotic degradation. Abiotic degradation of PCE and TCE progresses through intermediates resulting in production of acetylene (C_2H_2), whereas biodegradation is catalyzed by dehalogenating microorganisms that vary in their ability to catalyze dechlorination. The success of both strategies is intricately linked to the presence of C_2H_2 : success of abiotic degradation can be masked by consumption of C_2H_2 as an end product, and microbial reductive dehalogenation is inhibited by C_2H_2 . Therefore, research is needed to assess the impacts of acetylene degrading microorganisms (e.g., acetylenotrophs) on chlorinated solvent degradation. In this study, we sought to characterize the activity of and identify anaerobic acetylenotrophs from a TCE-contaminated fractured rock aquifer at the Naval Air Warfare Center (NAWC) Research Site in West Trenton, New Jersey.

Approach/Activities. Laboratory incubations consisted of well water from two sites (73BR and 36BR) that were mixed (1:1) with sterile, anaerobic medium (SeFR1, N_2 headspace) to provide nutrients (N, P, vitamins, and trace elements), and provided with C_2H_2 as the sole carbon and energy source. Enriched microbial populations were characterized using Illumina next-generation 16S rRNA gene sequencing. Genes for acetylene hydratase (AH) were amplified using *Pelobacter*-AH specific primers and sequenced using Sanger sequencing.

Results/Lessons Learned. Acetylene consumption was observed after 20 to 45 days of incubation in 73BR and 36BR cultures, indicating the presence of acetylenotrophs in NAWC groundwater. Over time, the rate of C_2H_2 consumption increased with additional pulses of C_2H_2 . Microbial community characterization showed that the populations enriched from well 73BR differed significantly from those enriched from well 36BR. Communities in 73BR cultures were dominated by members of the Deltaproteobacteria, Betaproteobacteria, and Actinobacteria; whereas, 36BR cultures were dominated by Deltaproteobacteria, Actinobacteria, and Spirochaeta. Despite the anaerobic cultivation conditions, no known anaerobic acetylenotrophs (genus *Pelobacter*) were detected. However, aerobic acetylenotrophic taxa (Nocardioidaceae and Mycobacterium) were present at low abundance. Surprisingly, *Pelobacter*-AH genes were only detected in well 36BR cultures. Together, our results suggest that there is an unknown diversity of acetylenotrophs in contaminated groundwaters. With C_2H_2 impacting the success of chlorinated solvent remediation strategies, our results highlight the need to further understand microorganisms controlling acetylene transformations.