

The Effect of Bioremediation on Microbial Community Dynamics, Transport and Degradation of Chlorinated Solvents in a Fractured-Rock Aquifer

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Background/Objectives. In the United States, trichloroethylene (TCE) is among the most frequently detected volatile organic contaminants in groundwater. A common TCE bioremediation strategy is biostimulation with organic substrates and bioaugmentation with bacterial consortia to select for microbial reductive dechlorination. However, this bioremediation technique can lead to the accumulation of more toxic daughter products such as vinyl chloride (VC) rather than complete degradation to ethene, possibly due to substrate amendments preferentially selecting for organisms incapable of complete TCE degradation. The only known organisms capable of complete reductive dechlorination are some species of *Dehalacoccoides*, whose growth relies on fermentation processes of other members within the microbial community to provide H₂ as the electron donor. In this study, a bioremediation experiment, including bioaugmentation with substrate addition, was performed and monitored from 2008 to 2015 in a contaminated fractured rock aquifer at the Naval Air Warfare Center (NAWC) Research Site in West Trenton, New Jersey. We sought to characterize the microbial community prior to and over the course of the experiment to determine the impact on microbial community dynamics, specifically what organisms (indigenous or bioaugmented) involved in reductive dechlorination were ultimately stimulated.

Approach/Activities. Microbial community analysis was performed on archived DNA samples using Illumina next-generation sequencing of the 16S rRNA gene. Analysis was used to increase our understanding of (1) Pre-bioaugmentation microbial biogeography, (2) Colonization and transport of the added KB-1 enrichment culture (dominated by 36% *Dehalacoccoides* spp., 21% *Acetobacterium* spp., and 10% *Geobacter* spp.) through hydraulically connected fractures within the aquifer, and (3) Effects of the emulsified oil substrate (EOS) and vitamin B₁₂ addition on in-situ microbial community dynamics.

Results/Lessons Learned. Prior to the bioaugmentation, variability in microbial community structures was observed between sample locations, although there was little temporal variation. Many organisms capable of incomplete reductive dechlorination, such as *Geobacter* spp. and *Dehalogenimonas* spp., were already present at the site. Following bioaugmentation, the introduced KB-1 consortium was quickly outcompeted by the native bacteria resulting in 0.1%, 0.0% and 10% abundance for *Dehalacoccoides* spp., *Acetobacterium* spp., and *Geobacter* spp., respectively. At the site of the injection, genera from the family Comamonadaceae were stimulated within the first 3 days followed by the successive stimulation of *Sulfurospirillum* spp., genera from the family Ruminococcaceae and genera from the order Bacteroidales. By 62 days *Geobacter* spp. returned to dominance followed by co-dominance with *Dehalogemimonas* spp. by 302 days. This structure remained relatively stable for the following 5 years. Collectively, these results suggest that targeted *Dehalacoccoides* spp. capable of complete reductive dechlorination were outcompeted, most likely from stimulation of indigenous organisms within the aquifer. Our results highlight the need to fully understand interactions between indigenous and bioaugmented communities in order to develop successful bioremediation strategies.