

The SABRE (Source Area BioREmediation) project was conducted to evaluate accelerated anaerobic bioremediation of chlorinated solvents in areas of high concentration, such as DNAPL source areas. To study performance of this technology, a test cell was constructed with a longitudinal multilevel sampler (MLS) transect and two transverse MLS transects, one in the source zone and one in the plume zone. Four fully screened wells were also distributed along the length of the cell. Using this sampling network, the cell was monitored for VOCs, inorganic species, pH and other parameters during the more than 600 days of operation. In addition, samples were collected to characterize the microbial community present throughout the field operations, including during the baseline, biostimulation with SRS, and post-bioaugmentation periods. This characterization relied primarily on groundwater samples collected along the length of the test cell from four fully screened wells. Groundwater samples were collected from the cross sectional transects on a less frequent basis. During site investigation, a few soil samples were collected. Extensive soil sampling was conducted at the conclusion of the study. The microbial community was characterized using two primary techniques: quantitative polymerase chain reaction (qPCR) for enumeration of *Dehalococcoides* organisms (DHC) and for the vinyl chloride reductase (*vcrA*) gene; and phospholipid fatty acid analysis (PFLA). DHC were tracked because these microbes are capable of complete reductive dechlorination of TCE to ethene and are the most common qPCR targets at chlorinated solvent sites. The *vcrA* test was also included as this analyte provides quantitative information regarding the relative abundance of the gene that codes for the enzyme that dechlorinates vinyl chloride to ethene. This test determines if the detected DHC will degrade VC to ethene efficiently and may be used to evaluate the activity of indigenous and bioaugmented DHC. Additional PCR testing was undertaken in order to identify primers specific to the bioaugmented DHC found in the bioaugmentation culture (KB-1<sup>®</sup>). PFLA was used to describe the size and structure of the microbial community based on the phospholipids present in cell membranes. In combination, these three molecular data sets provide a robust understanding of the microbial community throughout test cell operations, rather than a snapshot in time and space.

Prior to amendment additions, *vcrA* levels ranged from  $10^3$  (the method detection limit) to  $10^5$  gene copies per liter. After electron donor addition and bioaugmentation, gene copies per liter increased to a range of  $10^4$  to  $10^8$  with a median level of  $8 \times 10^5$  over the 605 days of test cell operation. The *vcrA* and DHC concentrations tended to agree throughout the bulk of test cell operations. Operational factors such as pH and TOC appear to affect *vcrA* concentrations. Soil concentrations at the conclusion of test cell operations ranged from  $10^4$  to  $10^7$  gene copies per g dry soil with a median of  $10^6$ . Spatial variation was observed as a function of length and depth in the test cell for both ground water and soil densities.