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# Characterizing the Microbial Community in SABRE Microcosm Studies

Sandra Dworatzek and Jeff Roberts (SiREM, Guelph, ON, Canada)
Ronald F. Herrmann, Tracy Dahling, and ***Carolyn M. Acheson*** (U.S. EPA, Cincinnati, OH, USA)
David W. Major (Geosyntec Consultants, Guelph, ON, Canada)
Mark Harkness, Angela Fisher, Antonio Possolo, and Richard Royer (GE Global Research, Schenectady, NY, USA)
Michael Lee (Terra Systems, Inc, Wilmington, DE, USA)
E. Erin Mack and Jo Ann Payne (Dupont, Newark, DE, USA)

The SABRE (Source Area BioREmediation) project will evaluate accelerated anaerobic bioremediation of chlorinated solvents in areas of high concentration, such as DNAPL source areas. In preparation for a field scale pilot test, laboratory microcosm studies were conducted to design the system and obtain modeling information. System design questions evaluated in a microcosm study included: selecting an electron donor; evaluating the necessity or advantages of bioaugmentation; evaluating the necessity or advantages of nitrogen, phosphorous, and micronutrient amendment; and evaluating the effect of trichloroethylene (TCE) concentration.

In the microcosm study, performance was assessed based on the chemical concentrations of TCE and dechlorination products. In addition, the microbial community was characterized using three techniques: quantitative polymerase chain reaction (qPCR) for enumeration of *Dehalococcoides* organisms (DHC); qPCR for the vinyl chloride reductase (*vcr*) gene; and phospholipid fatty acid analysis (PLFA). DHC was tracked as these microbes are capable of complete reductive dechlorination of TCE to ethene. The *vcr* test provides quantitative information regarding the relative abundance of the gene that codes for the enzyme that dechlorinates vinyl chloride to ethene. This test provides further characterization of any *Dehalococcoides* detected (i.e., will they degrade VC to ethene efficiently?) and can be used to evaluate the activity of indigenous and bioaugmented DHC. PLFA was used to describe the size and structure of the microbial community based on the phospholipids present in cell membranes. Microbial diversity was also assessed by PLFA. In combination, these three molecular techniques provide a robust understanding of the microbial community.

PLFA was used to evaluate all microcosms and the bioaugmentation culture (KB-1). Two measures of biomass were calculated from PLFA data: total biomass and KB-1 equivalent biomass. The microcosm study evaluated the effects of six electron donors, bioaugmentation, nutrient amendment, and TCE concentration on total biomass and KB-1 equivalent biomass at the end of the experiment. Carried out under the assumption that biomass amounts were comparable across jars at the beginning of the experiment, the statistical analysis was based on linear models fitted to suitably transformed total biomass and KB1 equivalent biomass, with significance assessed via univariate (ANOVA), and bivariate (MANOVA) analyses of variance. MANOVA, in particular, suggests that all of the experimental factors have statistically significant effects upon final biomass. PLFA

community structure was also evaluated using hierarchical cluster analysis. In addition, DHC and *vcr* were analyzed for microcosms with statistically superior treatments and to evaluate bioaugmentation. Initial samples from the field site were positive for DHC.