Metrics of Performance for the SABRE Microcosm Study

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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, a laboratory microcosm study was conducted to provide information on four design issues: selection of an electron donor (six considered); evaluation of the necessity or advantages of bioaugmentation; evaluation of the necessity or advantages of nitrogen, phosphorous, and micronutrient amendment; and evaluation of the effect of trichloroethylene (TCE) concentration (two levels considered). A fractional factorial experimental design was used and each treatment combination was tested in triplicate (168 microcosms including controls). The microcosms were distributed among four labs. The performance was monitored based on the chemical concentrations of TCE and dechlorination products measured at 2-3 week intervals. In addition, the following measurements were included on a less frequent basis: pH, temperature, total organic carbon, and anions such as sulfate. The microbial community was assessed using: quantitative polymerase chain reaction (PCR) enumeration of *Dehalococcoides* organisms (DHC); semi-quantitative PCR of vinyl chloride reductase; microbial diversity by denaturing gel electrophoresis, and total biomass and microbial community structure by phospholipid fatty acid analysis.

The objective of the microcosm study was to identify the treatment combination yielding the quickest, most robust, and complete dechlorination of TCE to ethene. As a result, the analysis of the microcosm study must incorporate data for TCE, cis-dichloroethylene (DCE), vinyl chloride, and ethene. Other measurements such as DHC and sulfate concentration are also useful. Due to the complexity of the experimental design, statistical analysis is required to interpret the information. Many metrics and statistical methods are available to draw conclusions, but it was not clear which ones would be most useful and appropriate for this data.   To fill this gap, several metrics and statistical methods were evaluated. Some metrics, such as molar chlorinated volatile organic compound ratio and chlorine number, incorporated chlorinated solvent data explicitly. These quantities were tracked as a function of treatment time. Other metrics incorporated time into calculations, including time to reach ethene endpoint, rate of TCE dechlorination, and rate of ethene formation. Different metrics yielded insight into different steps in the dechlorination process. Statistical techniques used in analyzing the SABRE microcosm data included: analysis of variance (ANOVA), multivariate ANOVA, correlations, and regressions. The advantages and disadvantages of these metrics and

statistical analysis techniques are discussed in the context of the SABRE microcosm study. Based on this analysis, electron donors were selected for further consideration in a column study, and conclusions about the necessity or benefits of bioaugmentation and nutrient amendment and the effects of TCE concentration were reached.