

1-OSU-03: Development of the Push-Pull Test to Monitor Bioaugmentation with Dehalogenating Cultures

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Goal: The overall goal is to modify the single-well push-pull groundwater test as a means for obtaining quantitative information on in situ dechlorinating activity before and after bioaugmentation. The specific objectives include: 1) modifying TCFE and fumarate assays to determine TCE-transformation potential for use in monitoring bioaugmentation, 2) developing methods for monitoring the transport of dehalogenating cultures during push-pull tests, and 3) evaluating the ability of push-pull tests to monitor changes in TCE-transformation potential resulting from the injection of dehalogenating cultures.

Rationale: Technologies are needed to enhance the in situ remediation of groundwater contaminated by chlorinated aliphatic hydrocarbons (e.g., trichloroethene or TCE). Bioaugmentation may be a viable alternative for remediating TCE source zones. Currently it is difficult to assess if bioaugmentation is increasing in situ dechlorination activity. The single-well 'push-pull' tests with the TCE surrogate trichlorofluoroethene or TCFE, can provide quantitative information on in situ biological activity and can be modified for use in determining the effectiveness of bioaugmentation.

Approach: Two cultures (Evanite and Pt. Mugu) that transform TCE to ethene will be characterized in collaboration with Dr. Semprini (Developing and Optimizing Biotransformation Kinetics for the Bio-remediation of Trichloroethylene at NAPL Source Zone Concentrations). The transport of the culture(s) will be determined during injection into anaerobic physical aquifer models (PAMs). Spatial distributions of dechlorinating activity and redox will be determined from a suite of assays conducted at sampling ports and at the injection/extraction well. Push-pull tests will be conducted at the injection/extraction well to assess changes in reductive dechlorination activity resulting from bioaugmentation.

Status: The background activity of sediment collected from a site with known indigenous reductive dechlorination activity has been characterized with respect to the kinetics of TCE, TCFE, fumarate, and succinate utilization and product formation. These four substrates were proposed for this project as substrates that could be used to assay for reductive dechlorination potential in situ. The microcosm study was used to determine the relationship between TCE and TCFE transformation rates and product speciation when fed fumarate and succinate prior to initiating the assays in PAMs. The microcosms were operated over a period of approximately 250 days. Succinate- and fumarate-fed microcosms produced very similar results for lag times, transformation rates and product speciation, with very similar results from triplicate microcosms at each condition. Lag time to the onset of TCE transformation in both fumarate- and succinate-fed microcosms was about two weeks. The corresponding lag times for TCFE transformation under the same conditions was about six weeks. TCE transformation rates, based on a first order

model fit after the lag time, were from 3.3 times (fumarate-fed) to five times (succinate-fed) faster than microcosms without exogenous electron donor addition. TCFE transformation rates were about 2.4 times faster than control microcosms and about four to five times slower than TCE transformation rates. TCE transformation products were cis-DCE and trans-DCE in approximately a 2:1 ratio and TCFE transformation products were cis-DCFE and trans-DCFE in approximately 2:1 ratio as well. TCE was ultimately reduced to VC, but very little ethene was observed. TCFE was transformed into a mixture of DCFEs and CFEs, with no FE formation. CAH transformation rates were not affected by sulfate addition. From these tests it was determined that succinate was a potential electron donor for further experiments and that TCFE transformation rates would have to be assessed in the sediments used in the PAM tests to determine the relationship to TCE rates.

A seed culture was obtained from Dr. Semprini's group from their Evanite culture reactor, was serially fed butanol and PCE for about two months, and has shown complete dehalogenation of PCE to ethene. This culture will be used in future tests related to this project. A series of microcosms have been prepared with the same sediments that were used to pack the PAM, and will be used to test the survivability of the bioaugmented culture under different geochemical conditions. The water phase in the microcosms consists of tap water or tap water amended with 5% media solution used in the culture reactor. Both lactate and butanol will be tested as fermentable substrates and bioaugmentation doses of 0.1, 1, and 10 mL of reactor culture will be tested. The microcosms have been recently inoculated and survivability should be assessed within about 30 days. The results will be used to determine the necessary water amendments and bioaugmentation dose for the PAM experiments.

A glass column of 5 cm diameter and 34 cm length has been packed with the same sediments used to pack the PAMs, and will be used to evaluate the transport characteristics of the bioaugmentation culture. A feed rate approximating the same linear average velocity to be used in the PAM will be used with an influent culture concentration approaching that found in the mother reactor (~25-40 mg/L protein). Effluent samples will be acquired and analyzed for *Dehalococcoides* sp. using group-specific PCR primers and compared to influent concentrations. The Evanite culture was tested using *Dehalococcoides* group-specific primers in PCR reactions and universal bacterial primers for T-RFLP analyses and was found to be highly enriched in *Dehalococcoides* sp. Serial dilutions of the Evanite culture were extracted and analyzed using *Dehalococcoides* group-specific PCR and dilutions down to 10^{-4} were detectable by this process. Future work includes expanding this testing to limited real-time quantifiable PCR analyses to attain better enumeration of *Dehalococcoides* sp. within the effluent samples and for use in the PAM tests. A bromide tracer test is currently underway on the column to determine the flow characteristics of the system.

The PAMs have been packed with sediment from the Hanford, Washington, site and have been saturated with oxygen-free water to produce anoxic conditions for the start of the test. Lactate solution will be added to the PAM just prior to bioaugmentation in an effort to assure anaerobic conditions prior to bioaugmentation. Information in the literature on

Dehalococcoides sp. involved in the critical step of VC transformation to ethane indicate an extreme sensitivity to oxygen, and every effort will be made to assure anaerobic conditions in the PAM before onset of bioaugmentation.