Dechlorination of PCE Contamination by *Desulfovromonas michiganensis*, Strain BB1 and *Dehalococcoides mccartyi*, Strain 195 in a Model Aquifer

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**Overall Objective:** Evaluate the ecological interactions between two dehalorespiring bacteria species in an intermediate-scale flow cell (ISFC), or model aquifer, and examine how their spatial distribution affects the extent of tetrahydrothiophene (PCE) dissolution and dechlorination under different hydrodynamic conditions.

**Specific Objectives:**
- Design and construct an ISFC that remains anaerobic during use.
- Develop sample analysis protocols and a project outline.

**Background:**
- Chlorinated ethenes are common groundwater contaminants found in industrial wastewaters at dry-cleaning and metal degreasing facilities, and pose acute and long-term health risks to the public.
- Such dense non-aqueous phase liquids (DNAPLs) move downward through the saturated zone, and form pools on low-permeability surfaces. Complete dissolution of a PCE pool may take several hundred years due to its low solubility (Yang and McCarty, 2000).
- Dehalorespiring microbes use a process called reductive dechlorination to detoxify PCE contamination while conserving energy in anaerobic groundwater.
- Complete reductive dechlorination involves the transformation of PCE into tetrahydrothiophene (TCE) → cis-1,2-dichloroethene (cDCE) → vinyl chloride (VC) → ethane daughter products by sequentially removing chlorine atoms on each molecule:

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\[ \text{C-C} \rightarrow \text{C-Chl-C} \rightarrow \text{Cl-Cl} \rightarrow \text{H-Cl} \rightarrow \text{H-H} \]
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- *Desulfovromonas michiganensis*, strain BB1 and *Dehalococcoides mccartyi*, strain 195 are two dehalorespiring bacteria that utilize PCE and TCE as electron acceptors. Although *D. michiganensis* populations exhibit faster substrate utilization rates, *D. mccartyi* 195 also dechlorinates cDCE and VC into non-toxic ethene (Becker and Seagren, 2009).
- When dehalorespiring bacteria dechlorinate aqueous-phase PCE, the dissolution of DNAPL-phase PCE is enhanced because of the greater concentration gradient at the NAPL/water interface (Seagren et al., 1994).

**Methodology:**

The following modifications were made to a previously-assembled ISFC (Song and Seagren, 2008):
- **Anaerobic conditions in the ISFC.** To ensure strict anaerobic conditions in the ISFC, a gas-tight lid and an influent delivery system were developed and/or constructed to maintain positive pressure using O₂-free gas. To test the effectiveness of these measures, anaerobic media was pumped through the sand tank for one week. Dissolved O₂ levels in the influent sand tank and effluent sample ports were measured using an O₂ microelectrode.
- **DNAPL source zone in the ISFC.** A rectangular DNAPL source zone was filled into a 4 cm thick aluminum plate at the bottom of the ISFC sand tank (Figure 1). The source zone will be filled with ½ inch glass marbles and Nile Red dyed-PCE, and covered with a wire screen at the DNAPL/media interface. Subsequently, ASTM 20/30 Ottawa coarse sand was back-packaged into the sand tank to a 26 cm depth in ~5 cm increments. To select an appropriate mesh size and practice filling the source zone, Sudan IV-dyed PCE was injected into a malleable-filled depression in a saturated, sand-filled glass pan.

A flow chart outlining the overall experimental plan was created, and the following sample analysis protocols were tested and/or developed:

**Results and Discussion**

- **ISFC dissolved oxygen levels.** During anaerobic testing, sand in the ISFC gradually turned black due to the build-up of Fe(II) (Figure 2). Dissolved O₂ concentrations in pore water samples generally remained below 2%. A oxidation-reduction indicator in the media began to change color at a dissolved O₂ level of 8%.
- **Preliminary DNAPL source zone formation.** As shown in Figure 3, dyed PCE was injected into a malleable-filled depression with a wire screen cover. Both 40 x 40 mesh and 60 x 60 mesh screens contained the DNAPL within the source zone. The 40 x 40 mesh screen was selected for ISFC experiments because the opening size is closer to the pore space in the coarse sand.
- **Experimental plan and sample analysis protocols.** Standard curves were generated for assays measuring chlorinated ethanes (i.e., PCE, TCE, cDCE, VC), chloride, bromide, acetate, nuclic acid and protein concentrations (data not shown). Upcoming work will include calibrating the H₂ microsensor, obtaining an ethane standard curve, and continued optimization of the chloride electrode protocol. Figure 4 provides a flow chart of planned ISFC experiments.

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**Figure 1.** An image of the ISFC with the empty DNAPL source zone installed.

**Figure 2.** Images of the ISFC sand tank (a) before and (b) after anaerobic testing.

**Figure 3.** Images of the preliminary DNAPL source zone (a) before injection, (b) immediately after injection, (c) after sprinkling sand on the 40 x 40 mesh wire screen, and (d) dislodging the screen.

**Figure 4.** Flow plan for upcoming ISFC experiments.

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**References**


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**Figures and Tables**

- **Figure 1:** An image of the ISFC with the empty DNAPL source zone installed.
- **Figure 2:** Images of the ISFC sand tank (a) before and (b) after anaerobic testing.
- **Figure 3:** Images of the preliminary DNAPL source zone (a) before injection, (b) immediately after injection, (c) after sprinkling sand on the 40 x 40 mesh wire screen, and (d) dislodging the screen.
- **Figure 4:** Flow plan for upcoming ISFC experiments.

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**Conclusions**

- **ISFC lid and influent assembly modifications results in O₂-free sand tank conditions.**
- A 40 x 40 mesh screen will separate the DNAPL source zone from the bulk-phase coarse sand, without creating an artificial bottleneck boundary layer.
- **Sample analysis protocols will be developed by the time ISFC sampling begins**.