## Evolution of a Chlorinated Solvent-Degrading Microbial Community in a Geochemically Diverse Aquifer Undergoing Heating

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**Background/Objectives.** This presentation will describe observed effects of a combined biological and thermal remedial action on microbial populations and the usefulness of such observations in evaluating the remedy. Multi-technology remedies are often required to treat aquifers contaminated with dense nonaqueous phase liquids (DNAPLs). The remedy at the Well 12A Superfund site in Tacoma, Washington, includes enhanced anaerobic bioremediation (EAB) in an aerobic, transmissive aquifer to reduce contaminant mass discharge from a high-concentration, dissolved-phase, chlorinated solvent plume. During installation of injection wells, two DNAPL hotspots were identified within the larger EAB treatment zone. To accelerate cleanup of the hotspots, a low-energy electrical resistance heating (ERH) system was installed to increase temperatures to 35-45C, to speed DNAPL dissolution and biodegradation kinetics, and to enhance the overall treatment rates. The chlorinated solvent-degrading microbial community was evaluated using a suite of molecular diagnostic tools, including quantitative polymerase chain reaction (qPCR) and metagenomics.

**Approach/Activities.** EAB was initiated by injection of emulsified vegetable oil amendment. Reducing conditions were quickly established within a discrete vertical zone of the contaminated aquifer. Reductive dechlorination of site contaminants was evident at many monitoring wells within six months of injection. Yet input of oxygen from vertical and horizontal groundwater flow created a geochemically diverse system. Approximately one year after EAB was initiated, a three-phase ERH system was installed around the DNAPL hotspots and heating operations commenced. Groundwater samples were collected before and approximately 6 and 12 months after heating and analyzed for known dehalogenating bacteria using qPCR. In addition, metagenomics was conducted to evaluate the microbial community more comprehensively.

**Results/Lessons Learned.** Preliminary bioinformatics results of the 16S rRNA DNA sequences obtained across the three sample sets identified approximately 1503 sequences similar enough to known microorganisms that the physiology could be assessed with reasonable certainty. The vast majority of results were associated with anaerobic primary and secondary fermentation of complex organic carbon. In addition, nine populations associated with Dehalorespiration were identified, including Dehalogenomonas and Dehalococcoides. However, the testing also identified significant populations of microaerophiles and aerobic methanotrophs associated with oxidation of chlorinated chemicals, which was consistent with a loss of mass balance of lower chlorinated cis-DCE, trans-DCE, VC and ethene and ethane. Overall, these data support the general observation of anaerobic conditions throughout the EAB treatment zone, but with geochemical transition zones and support of some aerobic populations. Tracking the relative abundance of biodegrading populations along with the other relevant populations will aid in understanding the conditions and community dynamics that facilitate efficient biodegradation of contaminants, and impacts of heating on reductive and oxidative degradation pathways.