## Application of Biostimulation and Bioaugmentation to Promote In Situ Biodegradation of Chlorinated Ethenes in Complex Hydrogeology

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**Background/Objectives.** The purpose of the in situ bioremediation system was to promote biodegradation of chlorinated ethenes in groundwater via biostimulation in combination with bioaugmentation. The groundwater contains elevated concentrations of the following chlorinated volatile organic compounds (cVOCs): tetrachloroethene (PCE), trichloroethene (TCE), cis-1,2-dichloroethene (cis-1,2-DCE), and 1,1-dichloroethene. Elevated levels of 1,4-dioxane have also been observed in some areas of the site. A preliminary natural attenuation evaluation indicated that reductive dechlorination was occurring in several of the on-site monitoring wells. An in situ microcosm evaluation using Bio-Traps demonstrated that the combination of biostimulation and bioaugmentation showed the highest level of conversion of chlorinated ethenes to the final end product, ethene. The site is located in a groundwater discharge zone where groundwater moves not only laterally along fractures and bedding planes but also upward from depth.

Approach/Activities. A pair of recirculation wells was installed to inject the carbon sources and microbial consortium into the groundwater. The injection program was conducted in two steps: 1. biostimulation with EOS QR, a quick release carbon substrate (e.g. 99% glycerin) to promote rapid microbial growth and an emulsified oil substrate (EOS 100) to sustain the microbial population for several years and support reductive dechlorination, and 2. Bioaugmentation with Dehalococcoides microbial consortium (BAC-9) to promote complete reductive dechlorination of the chlorinated ethenes. For the first step, approximately 570 pounds of EOS QR (99% soluble substrate) and 2100 pounds of EOS 100 (85% soybean oil, 15% surfactant and 4% soluble substrate) were injected to promote reduced conditions in a rapid time frame and maintain low ORP. The EOS 100 was also amended with CoBupH-Mg to maintain optimum pH conditions in the groundwater. Once the oxidation reduction potential (ORP) was maintained at less than -150 mV for several weeks, 20 liters of the Dehalococcoides consortium (BAC-9) was injected into the groundwater and recirculated using the paired wells. The groundwater was monitored over time for the following parameters: dissolved oxygen (DO), ORP, pH, conductivity, temperature, chlorinated ethenes and ethanes, total organic carbon, electron acceptors (nitrate, iron, manganese and sulfate) and dissolved gasses (methane, ethane and ethene).

**Results/Lessons Learned.** Within one week of injecting the carbon substrates, there was in increase in conductivity in downgradient wells. The carbon substrate impacted wells further downgradient than was anticipated based on current knowledge of the hydrogeologic conditions. Within two months after the EOS QR and EOS 100 injection, there was a significant decline in the DO levels to less than 0.5 mg/L and likewise, there was a corresponding decrease in the ORP levels to less than -150 mV in the recirculation and downgradient impacted wells. The *Dehalococcoides* population and corresponding key enzymes (e.g. tceA reductase, vcrA reductase) markedly increased in downgradient wells after the bioaugmentation event. The geochemistry indicated that competing electron acceptors, oxygen, nitrate and iron were considerably reduced in the groundwater and conditions were appropriate for reductive dechlorination, which was demonstrated by the major decreases in PCE, TCE and cis-1,2-DCE concentrations in downgradient wells. Likewise, ethene levels are increasing in downgradient wells. This presentation will discuss how the injection approach impacted distribution of the various amendments in the complex hydrogeology and impacted biodegradation of cVOCs.