Field Demonstration of Bioaugmentation for Remediation of Trichloroethene-Contaminated Groundwater in Japan

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Background/Objectives. Chlorinated ethenes such as tetrachloroethene and trichloroethene (TCE) are widely used as solvents, cleaners, and degreasing agents. As a result of spills and past disposal practices, these compounds are some of the most pervasive contaminants in groundwater, soil, and sediments. Biodegradation of these contaminated pollutants is a promising remedial alternative in many cases. In situ biodegradation of chlorinated ethenes can be performed by nutrients that are purposefully added to support activity of indigenous microorganisms at contaminated sites (biostimulation). The lack of an adequate microbial population capable of completely dechlorinating PCE and TCE to ethene at some sites, however, may lead to the accumulation of *cis*-1,2-dichloroethene (*cis*-DCE) and vinyl chloride (VC). Thus, we enriched an anaerobic consortium in which the dominant microorganisms were *Dehalococcoides* species (DHC) possessing VC reductive dehalogenase gene (*vcrA*) that code for dechlorinating *cis*-DCE and VC to ethene, and have considered using the consortium for bioaugmentation applications. In this study, a pilot-scale field test was conducted to evaluate the effectiveness of bioaugmentation by injecting the dechlorinating consortium into a TCE-contaminated aquifer.

Approach/Activities. The pilot test area was located at the operating factory in the western part of Japan. The alluvial aquifer consisted of gravel and sand, and the aquifer extended from the water table (depth to water was 6 m below ground surface [m bgs]) to a depth of 19 m bgs. The groundwater flow velocity was approximately 6 cm/day. The pilot test consisted of direct-push system, including two injection wells. One injection well (AIW) was for bioaugmentation and the other (SIW) was about 20 m away for biostimulation. Groundwater samples were collected from the injection wells to be analyzed for the concentration of chlorinated ethenes and ethene by GC-MS and the numbers of DHC-16S rRNA gene and *vcrA* gene by quantitative PCR assays. Before injection, the site groundwater contained about 0.2 mg/L of TCE and lower amounts of *cis*-DCE and VC, and DHC-16S rRNA gene copies were lower detection limit (10 copies/mL). The dechlorinating consortium used for bioaugmentation was grown on VC as electric acceptor in 10 L fermentation reactor, and then transported to the site. Approximately 25L of the dechlorinating consortium that had a DHC-16S rRNA gene of 10^8 copies/mL was injected into AIW with nutrients.

Results/Lessons Learned. TCE was rapidly dechlorinated to *cis*-DCE in both wells, however, first indication of reduction beyond *cis*-DCE and VC was the occurrence of ethene 63 days after bioaugmentation in AIW. Along with ethene production, DHC-16S rRNA gene and *vcrA* gene mounted to around 10^6 copies/mL. By day 119, all chloroethenes declined to levels below Environmental Quality Standards for Groundwater Pollution in Japan (TCE: 0.01 mg/L, cis-DCE: 0.04 mg/L, and VC:0.002 mg/L). Though indigenous DHC also grew to around 10^6 copies/mL 182 days after injection of nutrients in SIW, *cis*-DCE and VC remained at high concentration until 371 days. These results confirmed that bioaugmentation contributed to shortening of clean-up time rather than biostimulation by increasing initial DHC population in groundwater.