

Field demonstration of bioaugmentation for remediation of trichloroethene-contaminated groundwater in Japan



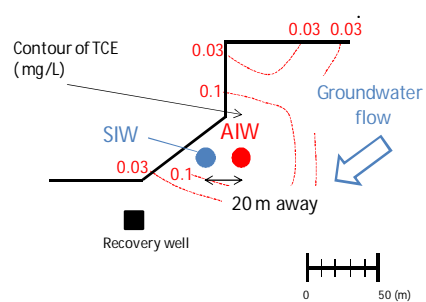
Background/Objectives

Chlorinated ethenes such as tetrachloroethene and trichloroethene (TCE) are widely used as solvents, cleaners, and degreasing agents. 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.

Site characterization



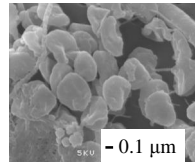
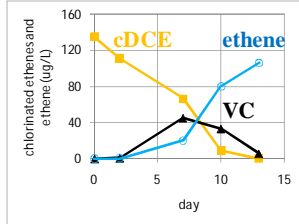
Geology of the aquifer	
Soil type	sand
Groundwater level	GL-6 m
Thickness of aquifer	13 m
Groundwater velocity	6 cm/d
porosity	0.25

Into AIW, 25 L of culture (DHC 16S rDNA : 10^9 copies/mL) with 10 m^3 of substrates were directly injected
 Into SIW, only 10 m^3 of substrates were directly injected

Our culture

The characterization of our DHC containing culture is detailed as below.

- ◆ Enriched from TCE-contaminated groundwater in Japan
- ◆ Maintained with organic acid and VC anaerobically
- ◆ Consortium mainly contain the DHC possessing *vcrA*
- ◆ No pathogens detected by DNA analysis
- ◆ Approved to comply with the Japanese guidelines since 2008



Approach/Activities

The pilot test consisted of direct-push system, including 2 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).



Whole view of culture and nutrient injection



Culture injection unit by using N₂ cylinder

Results/Lessons learned

First indication of reduction beyond *cis*-DCE and VC was the occurrence of ethene 63 days after bioaugmentation in AIW (day0). Along with ethene production, DHC-16S rRNA 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 (day0), *cis*-DCE and VC remained at high concentration until 360 days. These results confirmed that bioaugmentation contributed to shortening of clean-up time rather than biostimulation by increasing initial DHC population in groundwater.

Figure 1. Bioaugmentation

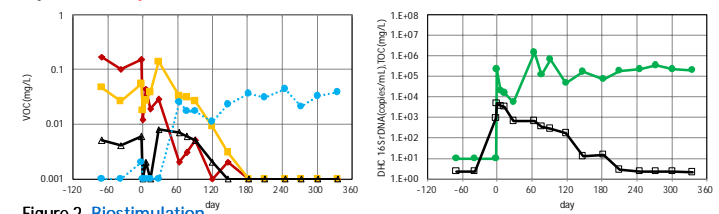
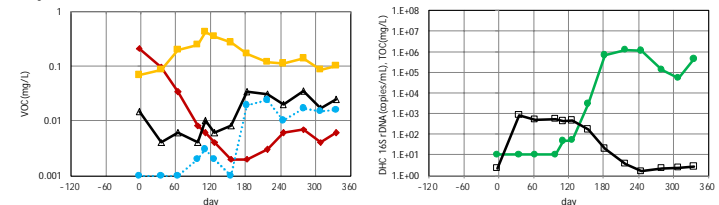


Figure 2. Biostimulation



Application expansion

We have introduced more than 15 cases of bioaugmentation technology, and we are continuing to expand its application in the future.

