

SUCCESSFUL FIELD DEMONSTRATIONS OF BIOAUGMENTATION TO REMEDIATE TRICHLOROETHENE IN GROUNDWATER

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Recent research has shown that tetrachloroethene (PCE) and trichloroethene (TCE) can be reductively dechlorinated to ethene by specific dehalorespiring bacteria from the group *Dehalococcoides* (DHE). While these dehalorespiring bacteria have been determined to be present at a significant number of sites, the relatively common occurrence of PCE or TCE dechlorination stalling at cis-1,2-dichloroethene (cis-1,2-DCE) and/or vinyl chloride (VC) suggests that these microorganisms are not ubiquitous in groundwater environments. Fortunately, field demonstrations at Dover Air Force Base in Delaware (RTDF; Ellis et al. 2001) and Kelly Air Force Base in Texas (GeoSyntec; Major et al., 2001) have shown that natural (i.e., not genetically modified or engineered), non-pathogenic, dehalorespiring microbial consortia such as KB-1 can be added to aquifers at sites where dechlorination stalls at cis-1,2-DCE, to promote complete dechlorination of the cis-1,2-DCE via VC to ethene.

In September 2000, a field demonstration was initiated to assess TCE dechlorination in a deep aquifer at the Aerojet Superfund site in California. Previous laboratory microcosm studies for the Aerojet site had shown that TCE dechlorination consistently stalled at cis-1,2-DCE, unless bioaugmented with dehalorespiring bacteria (Figure 1).

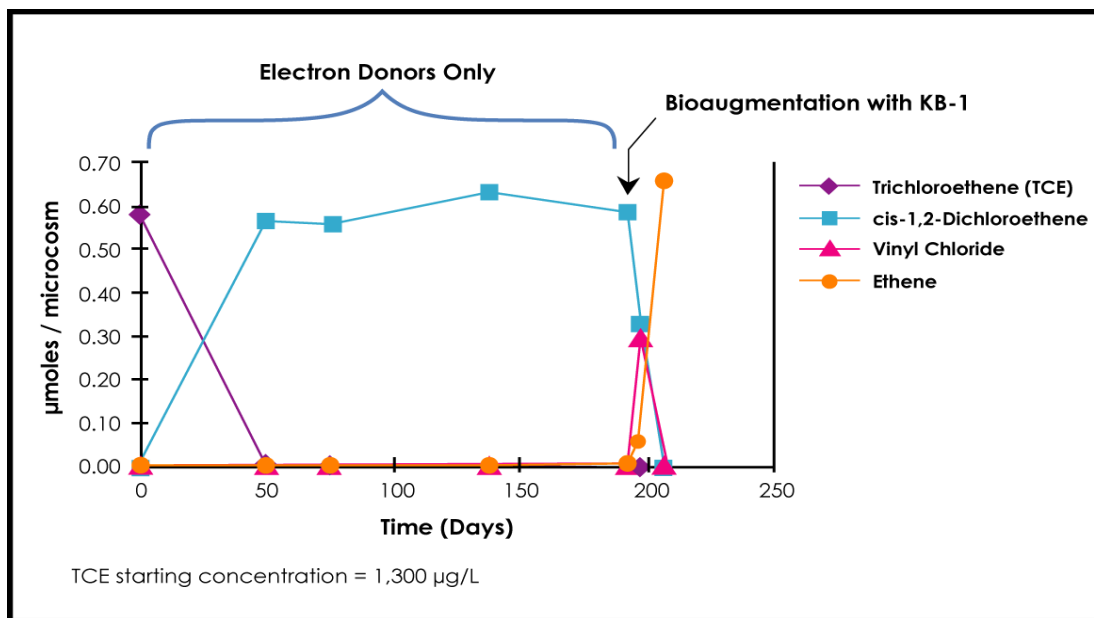


Figure 1. Bioaugmentation of Laboratory Microcosms with KB-1 Dehalorespiring Microbial Culture to Improve the Rate and Extent of TCE Dechlorination

The addition of lactate alone to the pilot test area (PTA) groundwater in a closed-loop recirculation system failed to promote significant TCE dechlorination past cis-1,2-DCE (VC and ethene were not produced). Bioaugmentation of the PTA with KB-1 at 157 days into the study immediately accelerated the rate of TCE and cis-1,2-DCE dechlorination, and VC and ethene production from cis-1,2-DCE were observed within 8 days following bioaugmentation (Figure 2). Within 125 days, the concentrations of TCE (starting from 2 mg/L), cis-1,2-DCE, 1,1-DCE and VC were below respective maximum contaminant limits (MCLs) in the PTA (see Figure 2). Molecular characterization techniques (16S rRNA screening using a sequence primer for DHE) developed by the Dupont Corporate Center for Engineering Research were used to evaluate the presence of DHE: i) prior to bioaugmentation, to assess the effects of electron donor addition alone; and ii) following bioaugmentation to track the success of KB-1 addition, and to assess its transport and survival in the PTA groundwater. Initial sample analyses were negative (Table 1), suggesting that DHE was not present in the PTA groundwater. A few days after bioaugmentation, a strong signal representative of the DHE strain in KB-1 was detected in the PTA well where KB-1 was introduced to the aquifer. A final sample round for DHE was collected 75 days after bioaugmentation, and all wells in the PTA, to a distance of 50 feet from the point of introduction, indicated moderate to strong DHE signal, suggesting KB-1 transport through the aquifer (Table 1).

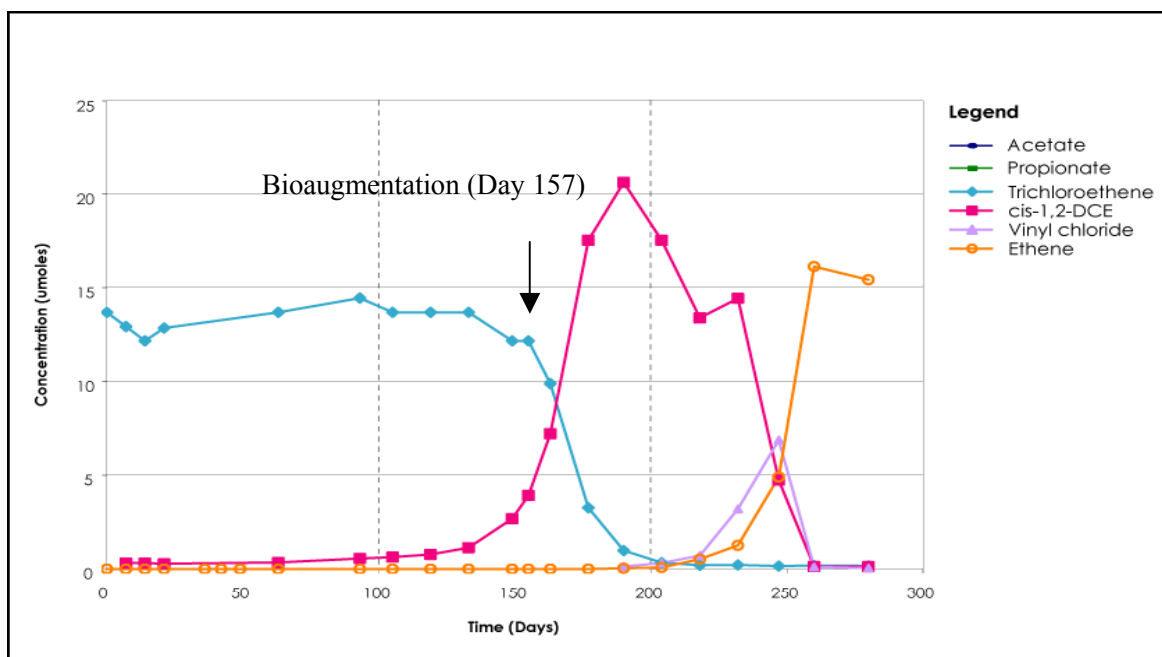


Figure 2. Results of KB-1 Bioaugmentation Field Demonstration #1

Following successful demonstration of TCE biodegradation in the first study, a follow-on demonstration was conducted to scale up the recirculation system into an active biobarrier capable of providing treatment across a 600-foot wide, 100-foot deep portion of the plume. The second demonstration, initiated in the summer of 2001, involved extraction of impacted

groundwater via 2 extraction wells at 10 gallons per minute (gpm) each, amendment of the blended groundwater (from both wells) with soluble electron donors (ethanol), and recharge of the amended groundwater to the aquifer to promote TCE biodegradation.

Table 1. Molecular Analysis (16sRNA) of DHE in Groundwater Following Bioaugmentation

| Pilot Test | Sample Date | Day | Monitoring Wells (distance from donor delivery well) | | | | |
|------------|--------------|-----|--|--------------|---------------|-----------------|----------------|
| | | | 3601 (15 ft) | 3600 (35 ft) | 100 (65 feet) | 3618 (100 feet) | 3617 (50 feet) |
| 1 | 11 July 2000 | 0 | -- | -- | -- | nt | nt |
| | 15 Dec 2000 | 157 | Bioaugmentation with KB-1 (via well 3601) | | | | |
| | 21 Dec 2000 | 163 | +++ | -- | -- | nt | nt |
| | 28 Feb 2001 | 232 | +++ | +++ | ++ | nt | nt |
| 2 | 20 Nov 2001 | 0 | System Re-Start (Pilot Test 2) | | | | |
| | 31 Jan 2002 | 72 | nt | +++ | +++ | +++ | + |

Notes:

- DHE - Dehalococcoides ethenogenes
- DHE not detected
- ++ DHE detected
- nt - Not Tested

The results of the second field demonstration confirmed earlier studies. Specifically, the addition of ethanol at a time-weighted average (TWA) concentration of 55 mg/L (about 3 times the stoichiometric demand, based on electron acceptor concentrations) promoted rapid and complete dechlorination of TCE (2 mg/L) to ethene within 35 to 65 feet from the electron donor delivery well. Figure 3 presents the concentrations (in $\mu\text{moles/L}$) of TCE, cis-1,2-DCE, VC and ethene at the start of demonstration #2 and at 72 days following initiation of ethanol addition. At the start of the demonstration (Day 1), TCE was the dominant VOC in the biobarrier influent and all downgradient and transgradient performance monitoring wells (the ethene in well 3618 was a relic from the first demonstration). By Day 72, ethene was the dominant product at wells located 35 and 65 feet downgradient. Steady state TCE, cis-1,2-DCE, and VC concentrations were typically at or below MCLs at wells 3600 and 100; and concentrations were continuing to decline at downgradient well 3618. Based on these data, the half-life for TCE dechlorination (to cis-1,2-DCE) under steady state conditions ranged between 1.3 to 3.7 days, while the half-life (first-order approximation) for TCE dechlorination (to ethene) ranged between 4.1 to 11 days, which is faster than observed in the first demonstration.

Of particular note, the system did not require a second bioaugmentation (with KB-1) of the aquifer following a seven month shutdown interval (April to November 2001) between demonstrations #1 and #2, when no electron donor was added, and aerobic groundwater was allowed to flux through the PTA. Following system re-start and resumption of electron donor

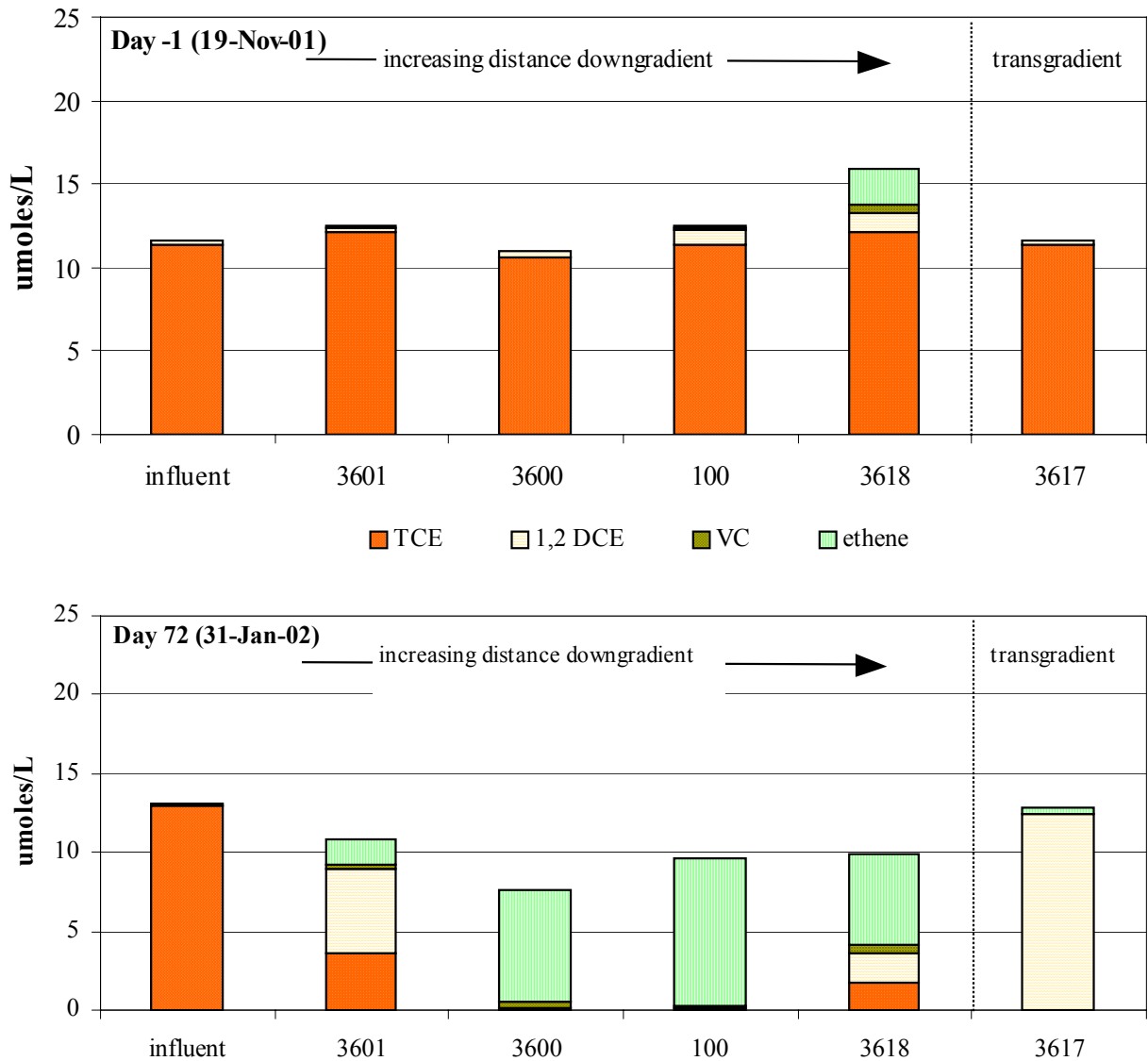


Figure 3. Results of KB-1 Bioaugmentation Field Demonstration #2

addition, the existing KB-1 culture from demonstration #1 resumed its highly effective TCE dechlorination activity in the downgradient leg of the PTA, confirming the robust nature of this natural microbial consortia. Interestingly, transgradient well 3617 was located outside of the original area of influence of the bioaugmentation conducted during demonstration #1. As a result, TCE was only dechlorinated to cis-1,2-DCE along the transgradient flowpaths to well 3617 over the 72-day test period, confirming that bioaugmentation is required at the Aerojet site to achieve complete TCE reduction to ethene.

These data, in conjunction with similar success stories at the Dover and Kelly Air Force Base sites, indicate that bioaugmentation is a promising remediation technique for PCE and TCE-impacted sites. DHE analyses and KB-1 are now commercially available from SiREM Laboratories (www.SiREMLab.com).