

Bioaugmentation for Accelerated In Situ Anaerobic Bioremediation

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A successful anaerobic bioaugmentation was carried out on a trichloroethene (TCE)-contaminated aquifer at Dover Air Force Base, DE, using a microbial enrichment culture capable of dechlorinating TCE to ethene. A hydraulically controlled pilot system 12 × 18 m was constructed 15 m below ground surface in an alluvial aquifer to introduce nutrients and substrate into the groundwater. Ambient TCE and *cis*-1,2-dichloroethene (cDCE) concentrations in groundwater averaged 4800 and 1200 μg/L. The pilot operated for 568 days. Results by day 269 confirmed previous laboratory work showing that dechlorination did not proceed past cDCE. By this time, most of the TCE was dechlorinated to cDCE, and cDCE was the predominant contaminant. An ethene-forming microbial enrichment culture from the Department of Energy's Pinellas site in Largo, FL, was injected into the pilot area. After a lag period of about 90 days, vinyl chloride and ethene began to appear in wells. The injected culture survived and was transported through the pilot area. By day 509, TCE and cDCE were fully converted to ethene.

Introduction

Chlorinated solvents are widely used as solvents, cleaners, and degreasing agents. As a result of spills and past disposal practices, these compounds are contaminants in groundwater, soil, and sediments. Standard remedial approaches have proven to be ineffectual and costly at removing these substances from the environment. Within the last 15 years, basic research on natural microbial dechlorination mechanisms has suggested that the destruction of chlorinated compounds can be practically achieved by stimulating bacterial reductive dechlorination in the field (1).

The Remediation Technologies Development Forum (RTDF) is a consortium composed of industrial corporations

and government agencies. This paper describes the RTDF's application of anaerobic microbial dechlorination of chlorinated solvents in a pilot study at Dover Air Force Base (AFB), DE. In this process, electron donors and/or nutrients are added to the subsurface to create anaerobic conditions and to stimulate organisms that can completely reductively dechlorinate chlorinated solvents.

Initially, substrates and nutrients were injected into the groundwater to stimulate indigenous microbiology, and an ethene-forming enrichment culture was injected into the groundwater later. The observed biodegradation confirmed experiments by Harkness et al. (2, 3) which showed that indigenous Dover AFB bacteria were unable to dechlorinate trichloroethene (TCE) beyond *cis*-1,2-dichloroethene (cDCE); that the nonindigenous bacterial consortium added to the subsurface was able to reductively dechlorinate TCE and cDCE to ethene; and that the injected culture survived in the new environment and was physically transported throughout the pilot study area. Stapleton et al. (4) characterized Dover AFB microbial populations in contaminated and uncontaminated sediments based on catabolic genotypes.

Methods

Physical Site Characterization. Dover AFB is underlain by sands and silts of the Pleistocene Columbia Formation that overlie the Miocene Calvert Clay aquitard (5). The pilot area was characterized as part of the Area 6 sitewide study conducted by the RTDF in March 1995 (6). Figure 1 shows the location of the pilot in relation to the contaminant plume. Groundwater velocity under natural nonpumping conditions is approximately 0.05 m/day. Total organic carbon (TOC) levels in the native soils were less than 1%.

Chemical Site Characterization. Historic maintenance and repair activities over approximately 50 years of operation at Dover AFB have resulted in solvent spills and subsequent groundwater contamination. Remedial investigations at the AFB have concluded that, except for source areas, solvent contamination is much more widespread in the deep zones of the shallow aquifer. Therefore, pilot activities focused on the deep zone. Sampling was conducted on monitor wells in and near the pilot area (Figure 2). A certified commercial laboratory determined the concentrations of chlorinated volatile organic compounds (CVOs), TOC, major cation and anions (Table 1), and important geochemical parameters. Contaminant and geochemical data were used for designing the pilot feeding strategy and equipment.

Groundwater Modeling and System Design. Groundwater flow and transport modeling was used as a tool to design the pilot system. The Columbia Formation was modeled as one unconfined aquifer system with three layers to determine the three-dimensional flow regime caused by pumping and to evaluate pilot system scenarios. The combined pumping rate determined by groundwater modeling was established at 11.6 L/min, 3.9 L min⁻¹ well⁻¹. Figure 2 presents the predicted groundwater flow lines for the final design. These were verified under operating conditions by using a bromide tracer. Details of modeling, design, and operation will be published separately.

Figure 2 also presents the plan view of extraction, injection, and monitor well locations. One row of three extraction wells and one row of three injection wells were installed aligned perpendicular to groundwater flow to create a hydraulically semi-isolated recirculation cell in the lower aquifer unit. The wells in each row were spaced 6 m apart. Extraction wells were installed 18.3 m downgradient from the injection wells. Wells were screened from 12 to 15 m below ground surface.

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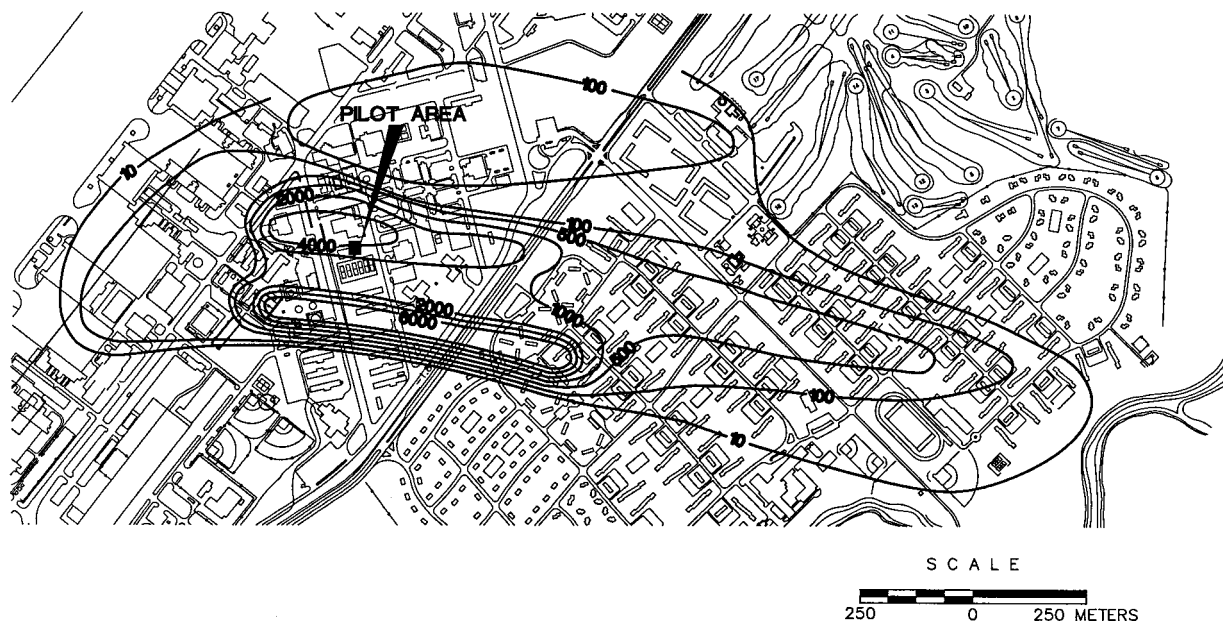


FIGURE 1. Total chlorinated solvent plume and pilot study location at Dover AFB.

Monitor wells were spaced along the central downgradient flow path from the injection wells at distances of 1.2, 2.4, 4.0, 9.1, 12.2, and 16.8 m. Monitor wells in the downgradient side flow paths were installed at greater intervals.

System Operation. Groundwater was extracted from the three downgradient wells, combined, amended, and re-injected into the three upgradient wells. The combined groundwater and pumping rate of 11.6 L/min created a subsurface residence time of approximately 60 days. Electron donors and nutrients were delivered on a 7-day pulsed feeding schedule to minimize injection well biofouling. The initial substrate feed delivered 100 mg/L as lactate. The substrate was prepared and stored as a mixture containing 94.5 L of 60% sodium lactate, 198.5 L of tap water, and 100 g of yeast extract. Substrate was fed into the injected groundwater for 3.75 days, followed by unamended groundwater injection for 0.25 day. A nutrient mix consisting of 5 mg/L ammonia and 5.5 mg/L phosphate was then fed into the flow stream for 2.75 days, after which unamended groundwater was injected for 0.25 day.

A routine groundwater sampling and analysis schedule was followed to assess microbial growth and chlorinated solvent biodegradation. Samples from monitor wells inside the pilot area were tested at a commercial laboratory for CVOCs, methane, ethane, ethene, propane, TOC, and ammonia. Analyses for lactate, sulfate, phosphate, and bromide (tracer) were performed in a nearby RTDF facility using ion chromatography. pH, temperature, conductivity, dissolved oxygen, and redox potential were measured in the field using a flow-through cell. Declining dissolved oxygen concentration and redox potentials were indicative of the development of anaerobic conditions. However, methane production and declining sulfate concentrations were more indicative of strongly reducing conditions conducive to reductive dechlorination. Additional wells outside the pilot area were sampled monthly to assess recirculation system impact outside of the pilot area.

Microbiology. Static Microcosms for Evaluation of Reductive Dechlorination. Sealed microcosms were used to assess the site microbes' potential to reductively dechlorinate TCE. Triplicate microcosms were set up in 26-mL glass bottles with Teflon-faced butyl rubber septa and filled with a 25% vol/vol sediment/groundwater slurry. The bottles were amended with either sodium butyrate, sodium benzoate,

sodium acetate, ethanol, or molasses normalized to 200 mg of carbon/L (wt/vol). Two sets of these microcosms were prepared, with and without 10 mM sodium sulfate. Only the indigenous chlorinated ethenes were monitored for degradation; no exogenous solvents were added. Sacrificial sampling of triplicate microcosms was performed over a 6-month period. Microbial methods are described in detail in Davis et al. (7).

Semicontinuously Amended Microcosms for Evaluation of Reductive Dechlorination. A 45% (vol/vol) sediment/groundwater slurry was placed in 44-mL Teflon-faced butyl rubber-sealed microcosms to fill 90% of the microcosm total volume. Sodium lactate-amended microcosms were constructed generated with the following lactate concentrations: 0, 67, 180, 220, 1400, 2400, 14 000, and 23 000 mg/L. Nitrogen and phosphorus were added as ammonium chloride and sodium phosphate to a final concentration of 20 and 2 mg/L, respectively. Yeast extract was added to all microcosms at a ratio of 0.0035 mg of yeast extract/mg of sodium lactate. All bottles were spiked with 5 mg/L TCE. Aqueous supernatant from the bottles was replaced with fresh groundwater amended with lactate, yeast extract, TCE, and nutrients every 2 weeks. TCE, cDCE, and vinyl chloride (VC) were analyzed by gas chromatography/mass spectrography (GC/MS) as previously described (8). Ethene, ethane, and methane were determined by GC.

Pinellas Culture: Growth and Scale-up. A crude enrichment suspended culture isolated from the Department of Energy's (DOE's) Pinellas site in Largo, FL, was used as the source material to scale-up the inoculum to be injected at Dover AFB (2, 3). This culture was grown on a chloride-free, minimal salts medium composed of the following ingredients: ammonium acetate, 5.4 g; potassium dihydrogen phosphate, 1 g; sodium sulfate, 0.5 g; magnesium acetate, 3.8 g; calcium acetate 2.1 g; mineral solution 1, 120 mL (9); sodium bicarbonate, 21.6 g; *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid (HEPES) buffer at pH 7.0, 900 mL; sodium lactate, 45 mL of 2 M syrup; yeast extract (10% solution), 18 mL; and distilled water, 18 L.

Scale-up was conducted through serial 10% (vol/vol) transfers until 180 L of culture was obtained. Cultures were incubated at room temperature (approximately 24 °C) for up to 14 days. All cultures were spiked with an aqueous TCE-saturated solution to a final concentration of 5 mg/L TCE.

DM316D

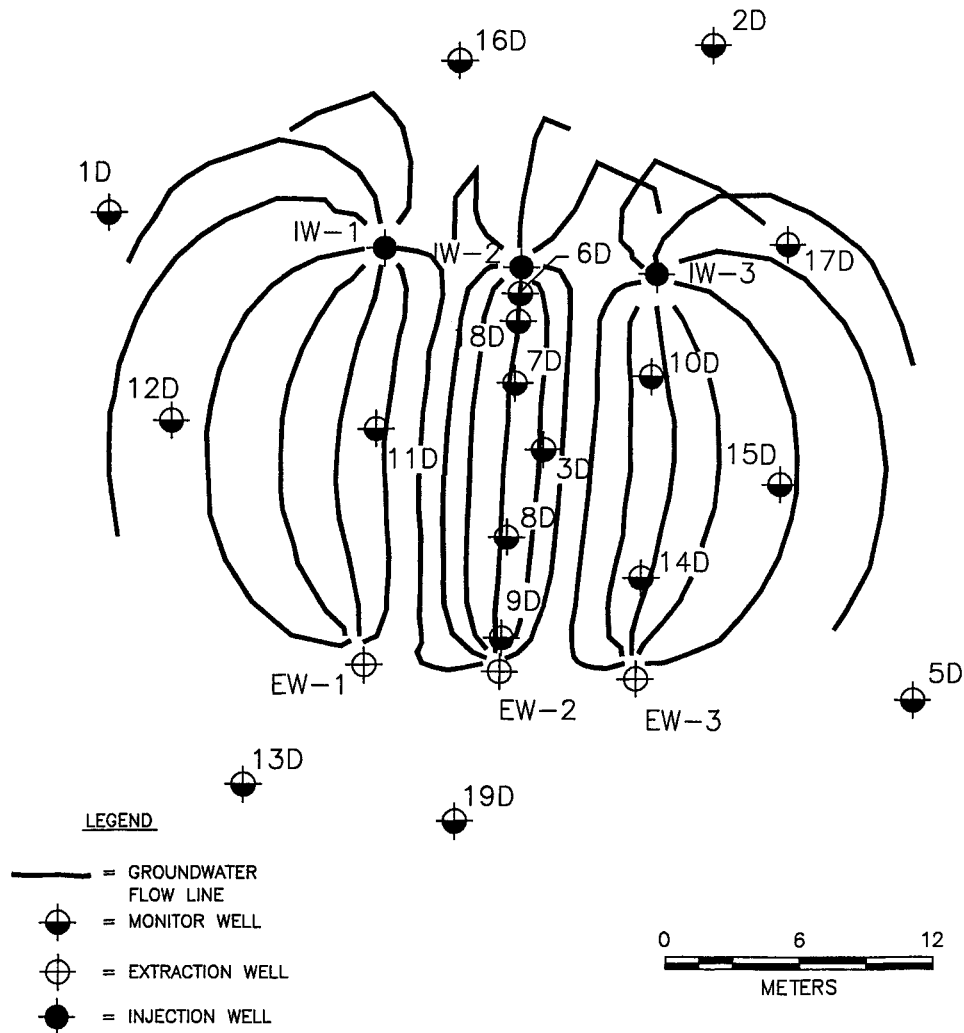


FIGURE 2. Monitor well locations and groundwater flow lines for pilot system.

TABLE 1. Geochemistry of Dover AFB Test Aquifer before and after Pilot Operation

parameter	value before operation	value after operation
dissolved oxygen	2.5 mg/L	0.3 mg/L
nitrate	2.0 mg/L	nondetect
Fe ²⁺	0.01 mg/L	60 mg/L
sulfate	2 mg/L	nondetect
methane	0.02 mg/L	1.4 mg/L
E _h	100 mV	-200 mV
pH	6.0	6.5

TCE and its dechlorination products were measured by duplicate sample analysis during growth to certify that the culture continued to completely dechlorinate. Before ship-

ment of the 20-L cultures to the field, certified laboratory analyses were conducted to verify that all of the chlorinated solvent in the cultures had been fully reduced to ethene. The carboys containing the Pinellas culture were sparged with nitrogen to remove any residual chlorocarbons or daughter products before injection into Dover AFB groundwater.

Pinellas Culture: Field Bioaugmentation. Written approval of the RTDF bioaugmentation work plan (10) was obtained from the U.S. Environmental Protection Agency (EPA) and the Delaware Department of Natural Resources and Environmental Control (DNREC) before bioaugmentation was conducted. Injection well (IW)-2 was targeted for initial injection because of its central position in the pilot area. IW-1 and IW-3, which flank IW-2, were used as control wells for recirculation in the absence of bioaugmentation.

TABLE 2. Effect of Lactate Concentration on Reductive Dechlorination of Added TCE (5 mg/L) in Semicontinuously Fed Microcosms

lactate (mg/L)	% TCE converted to cDCE after 75 days	
	replicate 1	replicate 2
0	0	0
67	92	77
180	79	35
220	69	69
1 400	80	62
2 400	72	114
14 000	0	0
23 000	0	0

After 269 days of substrate/nutrient injection, 180 L of Pinellas culture was injected under a nitrogen blanket into IW-2. On day 284, another 171 L of aqueous culture was injected into IW-2. During injection, unamended groundwater recirculation to the well was maintained at 1.5 L/min. The discharge tube of the pump was positioned at the well midscreen. A surge block/packer was positioned at the top of the screen to ensure that the injected culture would immediately enter the aquifer outside of the injection well. Substrate feed concentrations were returned to 200 mg/L as lactate 1 day after bioaugmentation.

Results

Indigenous Dechlorination Activity. The effect of lactate additions on dechlorination in microcosms is shown in Table 2. Two replicates were analyzed at day 75 for each lactate loading. Sodium lactate concentrations from 67 to 2400 mg/L supported reductive dechlorination of TCE to cDCE at comparable rates. After 75 days of semicontinuous feeding, reductive dechlorination of TCE to cDCE was observed in most microcosms with the exception of the unamended, control, and 14 000–23 000 mg/L lactate microcosms. The data suggest an optimum concentration range above which lactate is inhibitory. No VC or ethene was detected in any lactate-amended microcosm, suggesting that the indigenous microbial population was incapable of dechlorination beyond cDCE.

Other carbon sources were investigated using sealed, static microcosms that were not repeatedly fed. Molasses, butyrate, benzoate, and acetate at 200 mg/L as carbon were assessed under methanogenic and sulfate-reducing conditions in the presence of 10 mg/L yeast extract. In these 6-month experiments, no evidence of cDCE dechlorination to VC or ethene was observed.

Column studies with Dover AFB soil and groundwater show showed no dechlorination beyond cDCE after 371 days (2, 3). These three independent sets of experiments show that the microbes in Dover AFB sediments were incapable of dechlorination beyond cDCE irrespective of the carbon source, the presence or absence of sulfate, or the length of the experiments. Ethene-forming microbes were either absent or could not be stimulated under a wide range of laboratory conditions.

Pinellas Culture: Characterization. Previous work by Harkness et al. clearly showed the ability of the Pinellas culture to carry out complete dechlorination of TCE to ethene in column studies (2, 3). RTDF experiments confirmed this observation. The culture was robust, yielded reproducible growth, and reliably dechlorinated TCE to ethene. In chloride-free medium, the culture reached maximal cell densities within 4 days, and complete dechlorination of 5 mg/L TCE was achieved typically within 10 days of inoculation. Maximum cell densities obtained were typically 2×10^8 bacteria/mL. Doubling times at room temperature in chloride-free

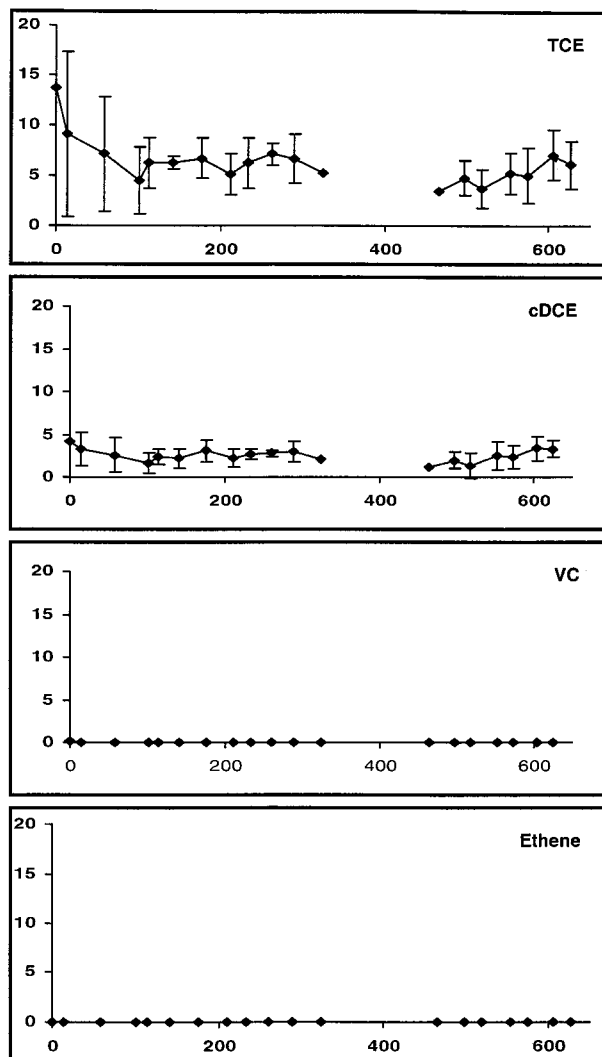


FIGURE 3. CVOC concentrations (μM) versus days in background well 316D, 35 m upgradient from pilot area. (Error levels are shown at the 95% confidence level, estimated using analyses from three consecutive events. Nondetect results were assumed to be 0.5 of the detection level.)

media were estimated at 30–40 h with fairly low yield. However, dechlorination was relatively rapid and extended well into the stationary phase of growth.

Molecular characterization of the culture by terminal restriction enzyme fragment length polymorphism (T-RFLP) analysis showed the presence of *Dehalococcoides ethenogenes*. However, the abundance of this organism was low relative to other phylogenetic types found by 16SrRNA analysis of the culture (11, 12).

Reductive Dechlorination: Prebioaugmentation. Before the pilot study was initiated, the following compounds were present in groundwater at this average concentration: tetrachloroethene at 46 $\mu\text{g/L}$, TCE at 7500 $\mu\text{g/L}$, cDCE at 1200 $\mu\text{g/L}$, 1,2-dichloroethane at 96 $\mu\text{g/L}$, VC at 34 $\mu\text{g/L}$, methane at 80 $\mu\text{g/L}$, and ethene in trace amounts. 1,1-Dichloroethene and *trans*-1,2 dichloroethene were present at trace levels of approximately 10 $\mu\text{g/L}$. Although CVOC concentrations were elevated on one side of the pilot area as compared to the other, concentrations became more uniform after recirculation. Figure 3 shows that the concentration of solvents outside the pilot area declined during the test period and that the upgradient well, MW-316, still contains TCE. The well data also show no significant change in the ratio of TCE to cDCE and no production of VC or

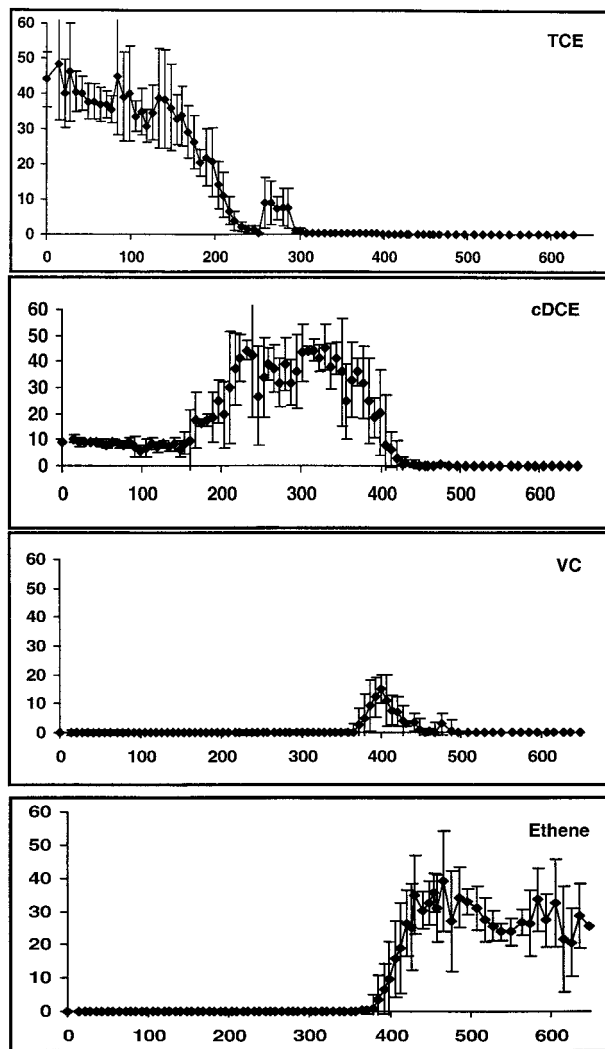


FIGURE 4. CVOC concentrations (μM) versus days in well 7D. (Errors were estimated by the same method as Figure 3.)

ethene. The data demonstrate that the capability for reductive dechlorination of TCE or cDCE to ethene did not spontaneously develop in nearby, untreated portions of the pilot area aquifer during this test.

Construction and hydraulic testing of the pilot system was completed before injection of substrate and nutrients began on day 1. The initial carbon concentration was approximately 40 mg/L. On day 144, analytical data suggested that insufficient carbon was being transported away from the injection wells and that dechlorination of TCE to cDCE was insufficient to remove all TCE. The substrate feed concentration was therefore increased to approximately 130 mg/L as lactate. After increasing the lactate concentration, an increase in the proportion of cDCE concentrations was noted in the monitor wells nearest to the injection point, and TCE concentrations decreased in these wells. Nearly stoichiometric conversion of TCE to cDCE was observed by day 291; however, no evidence for dechlorination beyond cDCE was observed, confirming previous detailed column studies (2, 3). Typical biodegradation results are shown in Figure 4 for well 7D. The redox potential in well 7D dropped from 251 mV on day 10 to between 0 and 121 mV from day 24 to day 150. The potential fell below 0 mV after the substrate loading was increased to 130 mg/L as lactate. At the time of bioaugmentation, redox potential had been below -100 mV for 4 months.

Reductive Dechlorination: Postbioaugmentation. Bioaugmentation was conducted on days 269 and 284. The first

TABLE 3. First Appearance of VC and Ethene Occurring at Greater than $2\times$ the Detection Limit

well no.	first appearance (days)		total travel distance from IW-2 (m)	total travel time (days)
	VC	ethene		
Center Bioaugmented Flow Path (Bioaugmented on Days 269 and 284)				
6D	381	374	1.2	1
18D	360	367	2.4	5
7D	381	388	5.2	15
3D	388	395	8.2	27
8D	402	409	12.2	42
9D	402	409	16.8	58
Right Flow Path				
11D	479	486	32.3	106
Left Flow Path				
10D	430	430	22.9	74
14D	458	465	26.5	87

indication of reduction beyond cDCE was the occurrence of VC 91 days after the first bioaugmentation in well 18D on day 360 (Table 3). Ethene was detected shortly afterward in this well. The appearance of VC and ethene first in well 18D rather than well 6D may be due to the short travel time between IW-2 and well 6D not providing a long enough residence time for the dechlorination reaction to occur before the Pinellas culture had completed its growth. The nearly simultaneous appearance of VC or ethene in adjacent wells (e.g., wells 8D and 9D) reflects the weekly sampling interval rather than a true difference in travel time.

VC and ethene concentrations then continuously increased relative to cDCE throughout the pilot area. VC concentrations declined later throughout the pilot area as the VC was further and completely dechlorinated to ethene. By day 479, reductive dechlorination was stoichiometrically degrading influent TCE and cDCE to levels below EPA maximum contaminant levels (MCLs) without creating toxic byproducts.

Reductive dechlorination of cDCE to VC was first observed in MW-18D. As a result, the Pinellas organisms are believed to have been transported by groundwater at least 2.4 m downgradient from IW-2. Ethene concentrations initially increased over the first 2.4 m of flow, indicating that the ethene-creating bacteria was transported at least 2.4 m. Beyond a distance of 2.4 m, the relative delays in the first appearance of cDCE dechlorination to VC and ethene are very close to groundwater travel times (Table 3), indicating advective transportation of VC and ethene away from the bioreactive zone that was created downgradient from IW-2.

Figure 4 shows that 75–80% of the original molar concentrations of TCE and cDCE have been recovered as ethene. This recovery ratio is similar in wells across the pilot area, indicating that little or no mass was lost by volatilization during recirculation. The loss of mass mirrored the decline in overall plume concentrations during the pilot operation (Figure 3) and was probably due to groundwater containing lower CVOC concentrations becoming entrained in the pilot circulation system.

The pattern of lactate degradation changed with time. Early in the test, lactate was fully removed in the first 3 m of downgradient flow. However, downgradient lactate concentrations increased over time and stabilized at approximately 50 mg/L. The reason for incomplete use of lactate is unknown.

Table 1 shows that, prior to pilot operation, the Dover AFB aquifer was aerobic. By day 151, redox and dissolved oxygen measurements indicated that pilot area conditions had become anaerobic. After 568 days of operation, dissolved oxygen, nitrate, and sulfate were completely depleted,

indicating that the aquifer was anaerobic and conducive to reductive dechlorination. Also consistent with anaerobic conditions is that ferrous iron concentrations increased to approximately 60 mg/L and methane concentrations increased to 1.4 mg/L. Microbial characterization of the aquifer by culture-based methods and molecular (16SrRNA) methods have shown that iron-reducing bacteria are a major component of the microbial community at Dover AFB (13).

Discussion

There are many sites with perchloroethene (PCE) or TCE contamination where reductive dechlorination stops at cDCE irrespective of the amount or type of electron donor available (14). These sites appear to lack organisms that have the anaerobic capability to further dechlorinate cDCE to ethene. Anaerobic bioaugmentation with microorganisms that carry out complete dechlorination has the potential to overcome that metabolic hurdle and allow much broader application of anaerobic bioremediation to chlorinated solvents. In addition, bioaugmentation may increase the use and value of natural attenuation as a remedy at sites where sufficient electron donors are available in groundwater but where indigenous bacteria do not have the metabolic capability to carry out reductive dechlorination to ethene.

Results indicate that an ecological niche existed at Dover AFB for organisms that could derive energy from the complete dechlorination of chlorinated solvents to ethene. Chlorinated solvents such as PCE and TCE are known to be energy-yielding respiratory electron acceptors for certain anaerobic dechlorinating bacteria (15). This bioenergetic advantage of anaerobic dechlorinators over organisms that co-metabolize chlorinated solvents without bioenergetic benefit suggests that the anaerobic processes will be more sustainable in the long term.

A number of mixed cultures have been shown to carry out the reduction of TCE to ethene (15); therefore, there may be no single culture that is uniquely useful for anaerobic bioaugmentation. The primary differences that can be observed between these cultures are their rate of dechlorination and their survival at a particular site. There is little useful data on the relative efficiency in use of electrons for dechlorination between different cultures (16).

In Situ Bacterial Growth. The timing of bioaugmentation was based on the column studies of Harkness et al., which showed that indigenous Dover AFB bacteria were not capable of dechlorinating cDCE to VC or ethene even after a 371-day exposure to electron donors (2, 3). The absence of VC and ethene in groundwater samples through day 269 was interpreted as confirming the absence of in situ cDCE-dechlorinating bacteria, allowing bioaugmentation to proceed.

The quantity of bacteria added to soil at Dover AFB was quite small, estimated to be less than 35 g on a dry weight basis, based on optical density measurements. The Pinellas culture is a relatively slow-growing culture with a doubling time of 30–40 h under optimal laboratory conditions. It can be anticipated that the doubling time will be significantly longer under field conditions where the temperatures are on the order of 10 °C lower than the laboratory and overall nutritional conditions are unknown. The slower growth rate under field conditions is believed to be the cause of the 90-day lag period observed before the first appearance of VC and an additional 60 days before complete conversion of all chlorinated solvents to ethene. This significant lag time is likely due to slow growth and adaptation of one or more members of the Pinellas culture to suboptimal Dover AFB aquifer conditions. Over time and as the population of cDCE dechlorinators increased, the observed activity moved closer to the point of substrate injection. The decrease in lag times and more rapid treatment were probably caused by an

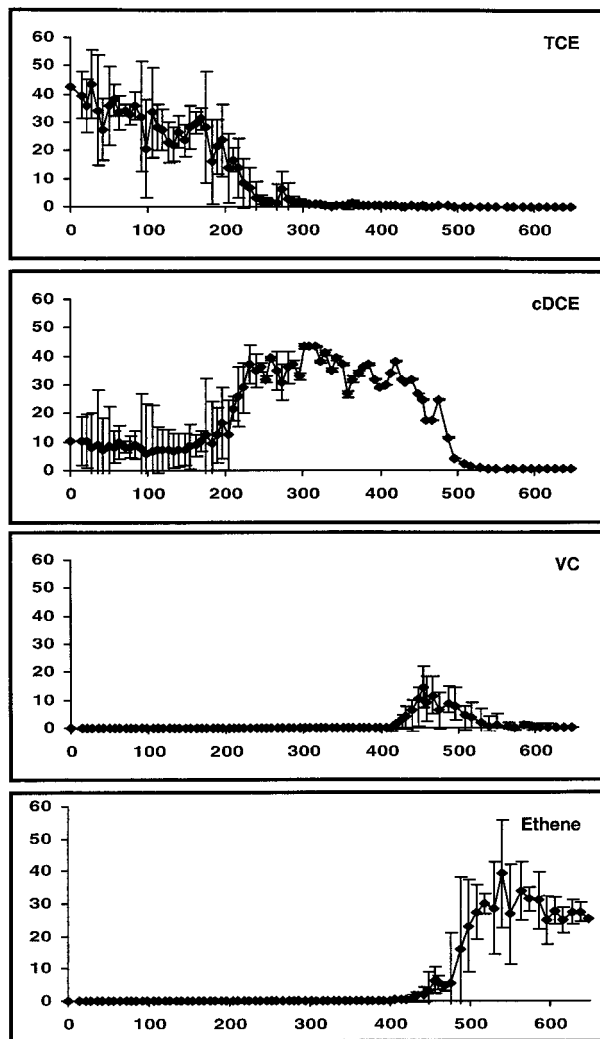


FIGURE 5. CVOC concentrations (μM) versus days in well 10D. (Errors were estimated by the same method as Figure 3.)

increase in the population of cDCE-dechlorinating bacteria in soil close to the point of injection where CVOC concentrations were highest. At the end of this study, complete dechlorination of TCE to ethene occurred between IW-2 and MW-6, a well less than 1.4 m (approximately 1 day travel time) downgradient from IW-2.

Transport. Groundwater flow from the three recovery wells was mixed before re-injection until day 424, when flows were separated. The separation of flow paths was probably not performed early enough because chemical data indicated that reductive dechlorination of cDCE to ethene began to occur in both of the side flow paths approximately 70 days later than in the bioaugmented central flow path (Table 3 and Figure 5). It is important to note that it takes approximately 70 days for water injected at IW-2 to circulate through the center flow path and re-injection system and arrive at MW-10D. The most likely explanation for the delayed appearance of cDCE reduction to ethene in the unaugmented side flow paths is that the dechlorinating bacteria were carried in groundwater completely down the length of the center flow path, through the surface equipment, and re-injected into the side flow paths before separation. The 160-day delay in VC appearance in MW-10D seems to confirm the need for a 90-day growth period before the number of cDCE-reducing bacteria is sufficient to observe impact on groundwater composition. Therefore, the loss of the control baseline is concluded to be due to transport from the center flow path.

Preparation for Bioaugmentation. Bioaugmentation was performed on an aquifer that was aerobic in its natural state. The pilot area was highly reduced by lactate addition before addition of the Pinellas culture. It is clear that the use of anaerobic cultures such as Pinellas requires either a naturally anaerobic aquifer or prereduction of the aquifer via substrate addition. Prereduction of the aquifer through substrate addition will by itself induce the development of an adapted microbial population. Field data showed that the adapted population at Dover AFB was incapable of dechlorination beyond cDCE, consistent with the microcosm and column data. Careful testing is necessary to design successful bioaugmentation remedies. Laboratory studies are highly recommended to ensure that the bacterial culture in question will colonize the site soil, acclimatize to the subsurface, and carry the desired reaction to completion. In order for bioaugmentation to succeed, a niche must be created that is specifically available to the added microorganism. In the case at Dover AFB, a strongly reducing anaerobic environment was created via lactate addition and the environment contained an energy-yielding electron acceptor in the form of chlorinated solvents. This combination of conditions allowed the dechlorinating bacteria in the Pinellas culture to gain energetic advantage over other anaerobes in the sediments. Site soils that already contain organisms capable of complete dechlorination will neither need nor benefit from bioaugmentation.

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Literature Cited

- (1) Beeman, R. E.; Howell, J. E.; Shoemaker, S. H.; Salazar, E. A.; Buttram, J. R. A Field Evaluation of In Situ Microbial Reductive Dehalogenation by the Biotransformation of Chlorinated Ethenes. In *Bioremediation of Chlorinated and Polycyclic*

Aromatic Hydrocarbon Compounds; Hinchee, R., Leeson, A., Semprini, L., Ong, S., Eds.; Lewis Publishers: Boca Raton, FL, 1994.

- (2) Harkness, M. R.; Bracco, A. A.; Brennan, M. J., Jr.; DeWeerd, K. A.; Spivack, J. L. *Environ. Sci. Technol.* **1999**, *33*, 1100–1109.
- (3) DeWeerd, K. A.; Flanagan, W. P.; Brennan, M. J.; Principe, J. M.; Spivack, J. L. *Bioremed. J.* **1998**, *2* (1), 29–43.
- (4) Stapleton, R. D.; Ripp, S.; Jimenez, L.; Cheol-Koh, S.; Fleming, J. T.; Gregory, I. R.; Sayler, G. S. *J. Microbiol. Methods* **1998**, *32*, 165–178.
- (5) Dames & Moore. *Area 6 Remedial Investigation, Dover Air Force Base, Dover: Delaware, Volume I*; Bethesda, MD, July 1994.
- (6) DuPont Environmental Remediation Services. *Site Characterization of Area 6, Dover Air Force Base, in Support of Natural Attenuation and Enhanced Anaerobic Bioremediation Projects*; Project 7124; Wilmington, DE, March 1997.
- (7) Davis, J. W.; Odom, J. M.; DeWeerd, K. A.; De Carolis, J.; Stahl, D. A.; Fishbain, S. S.; West, R. J.; Klecka, G. M. Natural Attenuation of Chlorinated Solvents at Area 6, Dover Air Force Base. 2. Characterization of Microbial Community Structure. Submitted to *J. Contam. Hydrol.*
- (8) Odom, J. M.; Tabinowski, J.; Lee, M. D.; Fathepure, B. Z. In *Bioremediation of Chlorinated Solvents*; Hinchee, R., Leeson, A., Semprini, L., Eds.; Battelle Press: Columbus, OH, 1995; pp 17–24.
- (9) Odom, J. M.; Jessie, K.; Knodel, E.; Emptage, M. H. *Appl. Environ. Microbiol.* **1991**, *57*, 727–733.
- (10) RTDF Bioremediation Consortium. *Bioaugmentation Work Plan: Dover Air Force Base*; DuPont Corporate Remediation Group: Wilmington, DE, 1996.
- (11) Elbersen, M. A.; Ebersole, R. C.; Young, R. M.; Tabinowski, J. A.; Perry, M. P.; Fahnestock, S.; Ellis, D. E.; Hendrickson, E. R. *American Society for Microbiology, 99th General Meeting*, Chicago, IL; 1999; Abstract Q32, p 539.
- (12) Hendrickson, E. R. *RTDF Bioremediation Consortium 1999 Annual Meeting Summary*; SLG Inc., Gaithersburg, MD, 1999.
- (13) Stahl, D. A. Personal communication, 1999.
- (14) Ellis, D. E. Intrinsic Remediation in the Industrial Marketplace. Presented at EPA Symposium on Natural Attenuation of Chlorinated Organics in Groundwater, Dallas, TX, September 11–13, 1996.
- (15) McCarty, P. L. *Science* **1997**, *276*, 1521–1522.
- (16) Lee, M. D.; Odom, J. M.; Buchanan, R. J., Jr. *Annu. Rev. Microbiol.* **1998**, *52*, 423–452.

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