SUCCESSFUL FIELD DEMONSTRATION OF BIOAUGMENTATION TO DEGRADE PCE AND TCE TO ETHENE

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ABSTRACT: Field demonstrations were conducted at Kelly Air Force Base in Texas to evaluate the applicability of accelerated anaerobic bioremediation via bioaugmentation to treat chlorinated ethenes in groundwater. KB-1, a natural, non-pathogenic microbial consortium isolated by University of Toronto and GeoSyntec is being used for bioaugmentation. Results of laboratory studies and the field pilot tests show that complete dechlorination to ethene occurred only after bioaugmentation with KB-1.

INTRODUCTION

In most subsurface environments, the main biodegradation mechanism for chlorinated ethenes is reductive dechlorination, which involves the sequential replacement of chlorine atoms on the alkene molecule by hydrogen atoms. The chlorinated ethenes serve as electron acceptors and hydrogen produced from the anaerobic metabolism of sugars, alcohols, fatty acids or other complex carbohydrates or carbon sources, serves as the electron donor during dechlorination reactions.

Although a number of anaerobic microorganisms can reductively dechlorinate through co-metabolic reactions (likely due to the transfer of electrons from reduced co-factors involved in anaerobic metabolism), they do not necessarily derive energy from the reaction. In contrast, specific dehalorespiring microorganisms use chlorinated solvents as their terminal electron acceptors and gain energy from reductive dechlorination. Dehalorespiring bacteria that have been identified, include *Dehalospirillium multivorans* (Scholz-Muramatsu et al., 1995), *Dehalobacter restrictus* (Schumacher and Holliger, 1996) and *Dehalococcoides ethenogenes* (Maymo-Gatell et al., 1997). Of these microorganisms, *D. ethenogenes* is able to completely dechlorinate the chlorinated ethenes and possibly ethanes. *D. ethenogenes* does not appear to be ubiquitous at all sites, or alternatively, are present but are not active. As a result, dechlorination of teratchloroethene (PCE) and trichloroethene (TCE) stalls at *cis*-1,2-dichloroethene (cis-1,2-DCE) at many sites, resulting in a build up of this dechlorination product.

Several stable, natural microbial consortia containing *D. ethenogenes* strains have been isolated that are capable of mediating complete dechlorination of TCE to ethene. A field demonstration by the Remediation Technologies Development Forum (RTDF) at Dover Air Force Base in Delware showed that TCE dechlorination had stalled at cis-1,2-DCE despite continued electron donor

addition. Following bioaugmentation, complete dechlorination of cis-1,2-DCE via vinyl chloride (VC) to ethene was observed (Ellis et al., 2000).

GeoSyntec, working with the University of Toronto (UT), has isolated a stable, natural microbial consortia (referred to as KB-1) capable of stimulating rapid dechlorination of PCE and/or TCE to ethene at sites where this activity is otherwise deficient. Experiments have shown that addition of KB-1 to microcosms in which PCE and/or TCE dechlorination had stalled at cis-1,2-DCE, despite continued electron donor addition, immediately stimulated dechlorination of cis-1,2-DCE via VC to ethene; cis-1,2-DCE was completely transformed to ethene in a matter of days (Cox et al., 1998, Wehr et al, 2001). Development of these and similar microbial cultures now provides the ability to accelerate bioremediation of TCE and related chlorinated solvents at sites where complete dechlorination reactions do not otherwise occur.

A laboratory and field test was conducted at Kelly AFB to assess if enhancement by electron donor addition or bioaugmentation with KB-1 was required to achieve complete reductive dechlorination of PCE to ethene. A key component of this demonstration was the ability to assess before the field demonstration the absence of *D. ethenogenes* at the site and track the spread of KB-1 strains of *D. ethenogenes*.

Site Description: Kelly AFB is located in south central Texas, approximately seven miles southwest of downtown San Antonio, Texas. The main base covers an area of 3,929 acres and is located in an area generally consisting of residential, commercial, and light industrial land usage. The pilot test area (PTA) is located in the courtyard of Building 360 of Kelly AFB. The geology in the vicinity of Building 360 consists of unconsolidated alluvial deposits overlying an undulatory erosional surface of Navarro Clay. The alluvial deposits consist of gravel, sand, silt and clay, ranging in thickness from 20 to 40 feet. From the surface downward, the geology typically consists of: 1 to 4 feet of black organic clay (denoted as fill/clay); 6 to 16 feet of tan silty, calcareous clay; and 4 to 20 feet of clayey limestone and chert gravel (denoted as clayey/gravel). The groundwater flow direction is typically to the southeast and the flow velocity is about 3.0 ft/day (SAIC, 1999). Volatile organic compounds (VOCs) in site groundwater consisted primarily of PCE, with lesser amounts of TCE, and cis-1,2-DCE. The site groundwater contained nitrate and sulfate at about 24 and 16 mg/L, respectively. Dissolved oxygen and redox measurements indicated that the groundwater was aerobic and oxidizing on a macro-scale; but elevated levels of dissolved manganese suggest anaerobic microenvironments. Methane, ethene and ethane were not detected in the sampled groundwater.

RESULTS FROM MICROCOSM STUDIES

A laboratory microcosm study was used to evaluate whether the intrinsic biodegradation activity could be accelerated through electron donor addition, or if bioaugmentation with KB-1 was required to completely degrade PCE and TCE to ethene. A series of treatment and control microcosms were constructed in

triplicate using site soil and groundwater, and incubated at room temperature in an anaerobic glovebox. Microcosms were constructed using 250 mL nominal volume glass bottles filled with 60 g soil and 150 mL groundwater. Headspace and groundwater were sampled through Miniert™ valves for analysis of electron donors, anions, VOCs, and biogenic gases (ethene, methane). Microcosms amended with TCE and mercuric chloride or just TCE served as sterile and intrinsic (biotic) controls. Electron Donor treatment microcosms were amended with only electron donors (methanol or lactate) and TCE at approximately 1 mg/L. Bioaugmentation treatment microcosms were amended with methanol, KB-1 and TCE at three different concentrations (approximately 1, 8 and 80 mg/L). Table 1 shows distribution of TCE and its dechlorination products under each control and treatment condition at the end of the experiment. The results indicate that the indigenous microorganisms in the site groundwater can reductively dechlorinate TCE to cis-1,2-DCE but cannot mediate further dechlorination of cis-1,2-DCE to VC and ethene. Bioaugmentation of the microcosms with KB-1 promoted complete dechlorination to ethene at all initial TCE concentrations tested.

TABLE 1. Initial Mass of TCE and Final Distribution of VOC Mass in Microcosm Treatments and Controls

Treatment	Mass Distribution at Final Sampling Point (umoles/bottle)					Conversion to Ethene (%)
	TCE Initial	TCE	DCE	VC	Ethene	
Sterile Control	10.3	11.4	0.03	<0.05	<0.01	0%
Intrinsic Control	11.2	10.7	0.06	<0.05	<0.01	0%
Methanol	1.04	1.02	0.87	<0.05	<0.01	0%
Lactate	1.16	<0.01	1.51	<0.05	<0.01	0%
Low TCE & KB-1	1.16	<0.01	<0.01	<0.05	1.91	164%
Mid TCE & KB-1	10.4	<0.01	<0.01	<0.05	10.8	105%
High TCE & KB-1	117	0.02	0.24	44.7	104	89%

Notes: Values represent average of triplicate microcosms.
Final data are after 70 or 100 days of incubation
KB-1 ammended treatment received methanol as electron donor

FIELD DEMONSTRATION

The field pilot test consisted of a closed loop recirculation system, including 3 extraction wells, one injection well, and 5 biomonitoring wells. Figure 1 presents a schematic of the system in plan view and cross section. Groundwater was extracted, combined through a common header, amended with electron donors (methanol/acetate) and tracer (bromide) as required, and injected via the injection well. The electron donor/tracers were metered into injected groundwater

to achieve the desired concentration based on the extracted groundwater measured flow rate.

FIGURE 1A . Plan View of Pilot Test Area

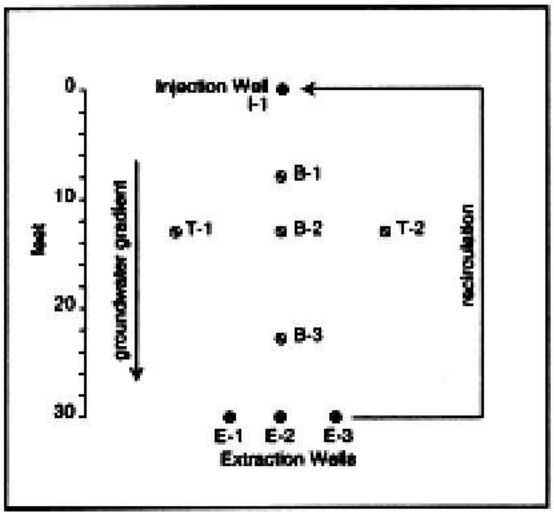
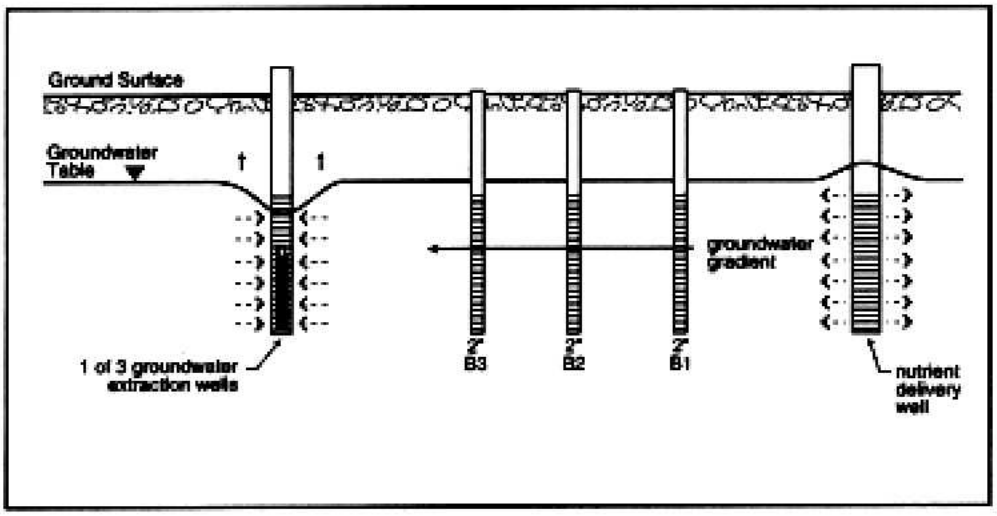


FIGURE 1B. Cross-Section Schematic of Pilot Test Area

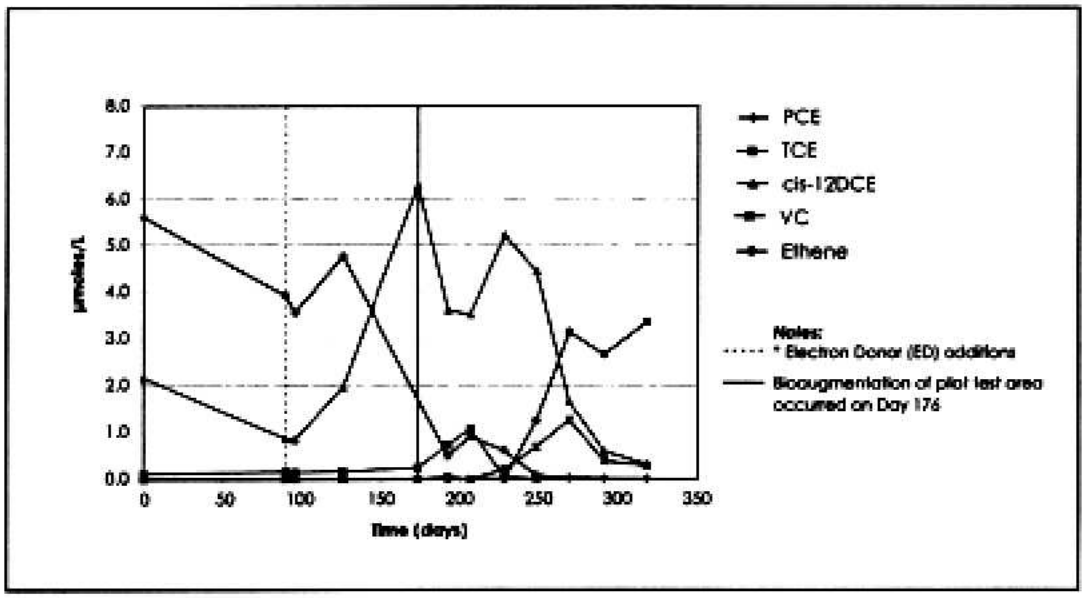


A bromide tracer test was used to verify residence times of the entire system and between monitoring wells, and estimate mass capture efficiency and the groundwater pore volume (PV). The time to capture and recirculate one pore volume was approximately 6 days, and the time between the injection well and the first monitoring well (B1) was between 4 to 8 hours. The mass capture efficiency and number of groundwater PVs recirculated were estimated to be approximately 90% and 64 m^3, respectively. The extraction wells efficiency indicates that either background water or recirculated water that was taking longer flow paths, was being re-injected into the pilot test area. This resulted in the continuous addition of PCE and cis-1,2-DCE into the pilot test area over the test period. From Day 89 (electron donor started) to Day 318 (last sample event) a

total of 39 PV were re-circulated through the pilot test area (PTA). Since bioaugmentation (Day 176), approximately 24 PVs were re-circulated.

Figure 2 presents the VOC and ethene concentrations over time at B1 (similar results were observed at all monitoring wells at the end of the test). When both acetate and methanol additions were made PCE concentrations in the PTA declined by more than 90%, with the dominant degradation products being cis-1,2-DCE. However, VC and ethene were not produced prior to bioaugmentation.

FIGURE 2. Micromoles of CVOC and Ethene Over Time at Monitoring Well B1



Prior to the addition of KB-1, analysis of soil and groundwater samples through gene probes (16S rRNA analysis) showed that *D. ethenogenes* was not detected at the site (Hendrickson et al. 2001). On 6 May 2000 (Day 176) approximately 13 L of KB-1 was added to the PTA through a submerged delivery line in the injection well (IW). Sixteen days after bioaugmentation, trace amounts of VC were reported in well B1, and ethene was detected after 52 days. By day 318 (142 days after bioaugmentation), ethene was the dominant product in the PTA.

Table 2 compares the concentration of cVOCs and ethene between the injection and B1 wells over the last three sample periods, the associated integrated half-life (PCE to ethene), and the mass balance between cVOCs removed and ethene produced. Recall, the residence time between the injection well and B1 is 4 hours. Table 2 shows that we obtained very good agreement between ethene production and cVOC loss, and that the average integrated half-life of cVOCs to ethene over this time period is approximately 3.8 hours.

Groundwater samples collected for molecular analysis showed that detection of *D. ethenogenes* correlated with the production of VC and ethene in the PTA (Data not shown, see Hendrickson et al., 2001). At the completion of the study, *D. ethenogenes* was detected in all monitoring and extraction wells. *D. ethenogenes* was not detected in soil and groundwater samples taken from outside the PTA. In addition, 12/13C stable isotope analysis was also conducted on groundwater samples, and verified the loss of cVOCs was attributed to biodegradation due to 12/13 C stable isotope ratios in the cVOC parent and daughter products (data not shown, see Morrill et al., 2001).

TABLE 2. Mass Conversion of Chlorinated VOCs to Ethene in the Pilot Test Area

	μmoles							
	Total VOCs	Ethene	Total VOCs	Ethene	Total VOCs	Ethene		
Well ID	IW		B1		Δ IW-B1		% of Expected*	Half-life (Hours)#
Days After KB-1 addition								
93	4.5	0.9	2.9	3.1	1.6	2.3	144%	6.5
115	2.9	1.1	1.0	2.7	1.9	1.5	79%	2.6
142	2.0	1.9	0.6	3.4	1.4	1.4	102%	2.3
Average	3.1	1.3	1.5	3.0	1.6	1.7	108%	3.8

Notes:

IW = Injection well

B1 = Monitoring well B1

* percentage of ethene expected given mass of total cVOCs loss

Integrated half-life (i.e., conversion of PCE, TCE, cis-1,2-DCE and VC to ethene)

CONCLUSIONS

We demonstrated through a laboratory and a field pilot test that the indigenous microorganisms at the Kelly AFB were capable of reductively dechlorinating PCE to cis-1,2-DCE when electron donors were present. However, complete dechlorination to ethene was only observed when KB-1, a natural, non-pathogenic, dehalo-respiring, microbial consortium was added to microcosms or to the aquifer. KB-1 was shown to completely dechlorinate TCE up to 80 mg/L. Only a small volume of KB-1 was required to effectively inoculate the PTA and resulted in observed half-lives of cVOCs to ethene in the order of hours. The study also verified that 16S rRNA molecular probing techniques can be used to assist in the monitoring, tracking and verification of KB-1's establishment at field scale. Isotope analysis confirmed that the degradation was biologically mediated.

ACKNOWLEDGMENTS

The authors gratefully acknowledge funding provided by the Army Corp of Engineers (Tulsa District) and the Remediation Technologies Development Forum, and the Kelly AFB for providing access to the site. We also appreciate data review and input by RTDF members during the execution of this project.

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