Successful field demonstration of bioaugmentation to degrade PCE and TCE to ethene

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Abstract Pilot-scale field demonstrations were conducted in a chlorinated ethene-contaminated aquifer at Kelly Air Force Base, Texas, USA, to evaluate the applicability of accelerated anaerobic bioremediation using bioaugmentation with the microbial consortium KB-1. Results from laboratory microcosm studies and the field demonstrated that anaerobic bioremediation could be enhanced by the supply of organic nutrients but that complete reductive dechlorination of tetrachloroethene and trichloroethene to ethene only occurred after bioaugmentation.

Key words anaerobic bioremediation; bioaugmentation; PCE; reductive dechlorination; TCE

INTRODUCTION

At many contaminated sites, the most significant biodegradation mechanism for tetrachloroethene (PCE) and trichloroethene (TCE) in the subsurface is reductive dechlorination, which occurs under highly reducing anaerobic conditions. This degradative mechanism involves the sequential replacement of chlorine atoms on the solvent molecule with hydrogen atoms and can yield fully dechlorinated end products. Reductive dechlorination requires the presence of organic carbon and energy sources, either natural or anthropogenic.

Although many anaerobic microorganisms can bring about reductive dechlorination by cometabolic reactions (probably due to the transfer of electrons from reduced co-factors involved in anaerobic metabolism), they do not derive energy or any other benefit from the reaction. In contrast, dehalorespiring microorganisms utilise chlorinated solvents as the terminal electron acceptors for respiration and thereby gain energy from the reductive dechlorination process.

Known dehalorespiring bacteria include Dehalospirillum multivorans (Scholz-Muramatsu et al., 1995), Dehalobacter restrictus (Schumacher & Holliger, 1996) and Dehalococcoides ethenogenes (Maymo-Gatell et al., 1997). To date, D. ethenogenes is
the only organism known to completely and rapidly dechlorinate chlorinated ethenes to ethene by dehalorespiration.

Several stable, natural microbial consortia containing *D. ethenogenes* strains have been isolated and have been shown to be capable of achieving the complete dechlorination of TCE to ethene. For example, Ellis *et al.* (2000) reported a field demonstration at Dover Air Force Base, Delaware, USA, where reductive dechlorination of TCE to *cis*-1,2-DCE took place following organic nutrient addition but further dechlorination did not occur until bioaugmentation was performed using a microbial consortium containing *D. ethenogenes*.

In this paper, we report the laboratory and field demonstration of bioaugmentation using the KB-1 microbial consortium 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 the introduced *D. ethenogenes*.

**KB-1 CONSORTIUM**

The KB-1 microbial consortium is a stable, natural mixed population containing *D. ethenogenes*; it is capable of rapid reductive dechlorination of PCE and/or TCE to ethene (Wehr *et al.*, 2001).

Laboratory experiments demonstrated that addition of KB-1 to microcosms in which PCE and/or TCE dechlorination did not previously proceed beyond *cis*-1,2-DCE, resulted in the immediate onset of dechlorination via vinyl chloride to ethene. These bioaugmented systems could achieve complete conversion to ethene in a matter of days (Wehr *et al.*, 2001).

**SITE DESCRIPTION**

Kelly Air Force Base is located near to San Antonio, Texas, approximately seven miles (12 km) southwest of downtown San Antonio. The pilot test area described in this paper was located in the courtyard of Building 360 at the base.

The surficial geology in this area consists of unconsolidated alluvial deposits overlying an undulating erosional surface of Navarro Clay. The alluvial deposits consist of gravel, sand, silt and clay, ranging in thickness from 6 to 12 m. From the surface downward, the geology typically consists of: 0.3–1.3 m of black organic clay (denoted as fill/clay); 2–5 m of tan silt, calcareous clay; and 1–6 m of clayey limestone and chert gravel (denoted as clayey/gravel). The groundwater flow direction is typically to the southeast at a velocity of about 1.0 m day\(^{-1}\) (SAIC, 1999).

Organic contaminants in the groundwater consisted primarily of PCE, with lesser amounts of TCE, and *cis*-1,2-DCE. The site groundwater contained nitrate and sulphate at about 24 and 16 mg l\(^{-1}\), respectively. Dissolved oxygen and redox measurements indicated that the groundwater was aerobic on the macroscale but elevated levels of dissolved manganese suggested the presence of anaerobic microenvironments. Neither methane, ethene or ethane were detected.
LABORATORY MICRO COSM STUDIES

A laboratory microcosm study was performed to evaluate whether intrinsic biodegradation activity in the aquifer at Kelly AFB could be accelerated through organic nutrient supplementation alone, or whether augmentation 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. Microcosms were constructed using 250 ml glass bottles containing 60 g soil and 150 ml groundwater. Incubation was in an anaerobic glovebox at room temperature.

Microcosm headspace and groundwater were sampled through Mininert™ valves for analysis of organic substrates, anions, chlorinated solvents and biogenic gases (ethene, methane). Microcosms amended with mercuric chloride served as sterile controls. Active microcosms were amended with methanol or lactate and TCE at approximately 1 mg l\(^{-1}\). Bioaugmentation treatment microcosms were amended with methanol, KB-1 and TCE at three different concentrations (approximately 1, 8 and 80 mg l\(^{-1}\)). The results (Major et al., 2001) indicated that the indigenous microorganisms in the site groundwater could reductively dechlorinate TCE to cis-1,2-DCE but not beyond. However, bioaugmentation of the microcosms with KB-1 gave complete stoichiometric dechlorination to ethene at all initial TCE concentrations tested.

FIELD DEMONSTRATION

The field pilot test consisted of a closed loop recirculation system comprising three extraction wells, one injection well, and five monitoring wells. Figure 1 presents a schematic representation of the system. Groundwater from the plot was extracted, combined through a common header, amended with substrates (methanol + sodium acetate) and re-injected via the injection well. The electron donor/tracers were metered into injected groundwater to achieve the desired concentration based on the measured groundwater abstraction rate.

Fig. 1 Schematic plan view and cross-section of accelerated anaerobic bioremediation test cell. The surface of the test cell measured approximately 10 x 7 m. Monitoring wells are designated by the prefixes B and T.
Prior to organic supplementation, a bromide tracer test was performed to estimate groundwater pore volume, to verify the hydraulic residence time, and to estimate mass capture efficiency. The time to recirculate one pore volume was approximately 6 days; the travel time between the injection well and the first monitoring well (B1) was between 4 and 8 h. The mass capture efficiency and pore water volume recirculated were estimated to be approximately 90% and 64 m³, respectively. The observed efficiency of the extraction wells indicates that water was brought into the plot from outside the boundaries of the plot, therefore there was a continuous addition of PCE, TCE and cis-1,2-DCE mass into the test area during operation.

The system was fed with organic substrates from day 89 of operation and caused bulk groundwater conditions to change from background conditions (aerobic) to highly reducing. Bioaugmentation with KB-1 took place on day 176 and organic nutrient supply continued. Final sampling was undertaken on day 318; during this entire operating period, a total of 39 pore volumes were re-circulated through the pilot test area. Between bioaugmentation and the final sampling, approximately 24 pore volumes were re-circulated.

Figure 2 presents the chlorinated solvent and ethene concentrations over time at monitoring well B-1 (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 test area declined by more than 90%, with the dominant degradation product being cis-1,2-DCE. However, vinyl chloride and ethene were not produced prior to bioaugmentation. Prior to the addition of KB-1, analysis of soil and groundwater samples using gene probes (16S rRNA analysis) showed that D. ethenogenes was not present at the site (Hendrickson et al., 2001). On day 176, approximately 13 l of KB-1 culture was added through a submerged delivery line placed in the injection well (I-1). Sixteen days after bioaugmentation, trace amounts of VC were detected in monitoring well B-1; ethene was detected after 52 days. By day 318 (142 days after bioaugmentation), ethene was the dominant product throughout the test area.

![Figure 2](image-url)

**Fig. 2** Concentration of chlorinated solvent contaminants and biodegradation products at monitoring Well B-1.
Table 1 compares the concentrations of chlorinated solvents, biodegradation intermediates and ethene between the injection well and monitoring well B-1 over the last three sample periods (i.e. after bioaugmentation), gives the integrated half-life for the conversion of PCE to ethene, and summarizes the mass balance achieved. This demonstrates very close agreement between ethene production and chlorinated solvent destruction. The average integrated half-life of chlorinated solvents over this time period is approximately 3.8 h.

Groundwater samples collected for 16S rRNA analysis demonstrated that the presence of *D. ethenogenes* correlated with the production of vinyl chloride and ethene (data not presented, see Hendrickson et al. (2001)). At the completion of the study, *D. ethenogenes* was detected in all monitoring and extraction wells but was not detected in samples taken from outside the test area.

Table 1 Mass balance of chlorinated solvents, biodegradation intermediates and ethene between the injection well and monitoring well B-1 after bioaugmentation with KB-1. The hydraulic residence time between the injection well and monitoring well B-1 was 4 h.

<table>
<thead>
<tr>
<th>Days after augmentation</th>
<th>Mass detected (μmol)</th>
<th>Ethene recovery (% of theoretical)</th>
<th>Half-life (h)$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Injection well</td>
<td>Monitoring well B-1</td>
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<tr>
<td></td>
<td>Total solvents</td>
<td>Total solvents</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ethene</td>
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<td></td>
<td>1.3</td>
<td>3.0</td>
<td>3.8</td>
</tr>
</tbody>
</table>

$^*$ Recovered ethene expressed as a percentage of the total chlorinated solvents removed from the test area.

$^*$ Integrated half-life for conversion of all chlorinated solvents and intermediates (PCE, TCE, cis-1,2-DCE and vinyl chloride) to ethene.

**CONCLUSIONS**

This project has demonstrated in both laboratory microcosms and a field pilot test that the indigenous microorganisms at Kelly Air Force Base were capable of the reductive dechlorination of PCE and TCE to cis-1,2-DCE, when additional carbon and energy sources were provided. The nutrients were required to ensure that bulk groundwater conditions became highly reducing and their supply was maintained at a controlled rate throughout the test period to ensure that conditions were suitable for reductive dechlorination. However, further dechlorination of cis-1,2-DCE to ethene only occurred when the natural, dehalorespiring, microbial consortium KB-1 was added. Only a small quantity of KB-1 was required to effectively inoculate the test area and resulted in observed half-lives of chlorinated solvents of a few hours. 16S rRNA molecular probing techniques confirmed that the inoculated dehalorespiring organism survived and established itself in the field but was not present beyond the boundary of the test area.
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REFERENCES


