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MEMBRANE DELIVERED ETHENE TO STIMULATE MICROBIAL DEGRADATION OF DCE

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ABSTRACT

A significant obstacle to the application of microbial reductive dechlorination of PCE and TCE is the undesirable accumulation of cis-DCE and vinyl chloride. In this study, lab-scale aquifer sediment column experiments were conducted to evaluate the feasibility of using ethene to stimulate cometabolic and/or auxiliary aerobic degradation of DCE in aquifer sediments from Edwards Air Force Base (EAFB). A mixture of ethene in air was supplied to gas-permeable membranes installed in a test column to stimulate aerobic degradation of DCE by ethenotrophic populations. Membranes in a parallel column were supplied with air or nitrogen as a negative control. The experimental results indicated that simply supplying ethene and air to the EAFB aquifer sediments alone did not produce conditions favorable for growth of DCE-degrading ethenotrophs. Moreover, amending the aquifer sediments with nutrients and bioaugmenting with enriched and pure (Nocardioides strain JS614) ethenotrophic cultures failed to stimulate growth of DCE-degrading ethenotrophs. This may have been due to the presence of inhibitory substrates or the absence of requisite growth factors. Parallel microcosm studies demonstrated that both the enriched ethenotrophic culture and the Nocardioides strain JS614 culture rapidly cometabolized DCE in mineral salts media, but did not readily acclimate to the EAFB aquifer sediments. The results of the column study and microcosm study together indicated that achieving ethenotrophic degradation of cis-DCE may be more difficult under in situ conditions than under ideal microcosm conditions.

KEYWORDS: bioaugmentation bioremediation, dichloroethene, cometabolism, ethene, membrane

1. INTRODUCTION

1.1 Background

Cis-dichloroethene (cis-DCE) and vinyl chloride (VC) are formed during anaerobic reductive dechlorination of perchloroethene (PCE) and trichloroethene (TCE). Although complete dechlorination to ethene in situ has been demonstrated, persistence of cis-DCE and VC under anaerobic conditions has impeded success of both natural attenuation and enhanced reductive dechlorination at a large number of sites. One explanation is that cis-DCE/VC dehalorespirers are often absent from contaminated sites (Fennell et al., 2001). Another potential

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explanation is that *cis*-DCE/VC dehalorespirers are subject to substrate-limited growth; for example, Cupples *et al.* (2004) concluded that dehalorespirers theoretically should be unable to grow on VC at concentrations below ~44 µg/L.

As an alternative to reductive dechlorination, many studies have demonstrated that *cis*-DCE and VC can be readily degraded under aerobic conditions (Coleman 2002a and references therein). Several studies have verified the existence of bacteria that can grow by oxidizing VC as the sole carbon source (Hartmans and de Bont, 1985; Verce *et al.*, 2000, 2001, 2002). Coleman *et al.* (2002a) conducted a study to assess the diversity and distribution of aerobic bacteria that grow on VC and detected VC degradation in 23 of 37 samples collected from 22 sites, and furthermore isolated 11 *Mycobacterium* strains and 1 *Nocardioides* strain. Thus, bacteria capable of growing on VC appear to be common in the environment, but not ubiquitous.

At many sites bioaugmentation may be required to promote growth of aerobic DCE/VC-oxidizing bacteria in the aquifer. Coleman *et al.* (2002b) also recently conducted a study to assess the existence of aerobic bacteria that grow on *cis*-DCE and obtained 2 active cultures from 18 enrichments, and furthermore isolated one aerobic β-proteobacterium strain that can grow on *cis*-DCE as a sole carbon and energy source. They conducted a study to assess the existence of aerobic bacteria that grow on *cis*-DCE as a sole carbon and energy source and concluded that these organisms are rare in the environment and may only exist in highly selective environments.

Successful bioaugmentation may be impeded by inefficient transport and dispersal of injected bacteria in the subsurface. Microbial transport in the subsurface depends on the characteristics of both the cells and the surrounding porous media (Ginn *et al.*, 2002). Several field studies with forced gradient groundwater flow found that injected bacteria do not travel far from the injection point (ESTCP, 2005). In general, observed advective transport of less than 3 m appears to be common for bacteria in silt or fine sand deposits (Harvey, 1997).

*VC and cis-DCE may only support net growth of aerobic bacteria when present at concentrations well above MCL values.* Microbial growth rate is a function of substrate concentration. At sufficiently low concentration (S<sub>min</sub>) the decay rate exceeds the growth rate. Bradley and Chapelle (2000) found that bacteria indigenous to stream sediments could utilize *cis*-DCE as a sole carbon substrate for aerobic metabolism, but could not grow at concentrations below 4850 µg/L, and Rittmann and Brunner (1984) have reported that aerobic bacteria cannot sustain net growth on simple compounds at concentrations below 100 to 1000 µg/L.

Ethene can serve as an auxiliary substrate to support secondary aerobic metabolism of *cis*-DCE and VC. Aerobic ethene-utilizing bacteria can readily cometabolize *cis*-DCE (Koziollek *et al.*, 1999), and known VC-assimilating bacteria readily grow on ethene (Coleman *et al.*, 2002a). In aerobic VC assimilation, the first step involves monooxygenase-catalyzed reactions that produce VC epoxide (Verce *et al.*, 2000). Chloroethene epoxides are chemically unstable and rapidly degrade to a variety of products that are toxic to cells (Fox, 1990), which accounts for the low VC transformation yields attained through cometabolic degradation by methanotrophs and other oxygenase-expressing bacteria (Dolan and McCarty, 1995). Unlike all other substrates that have been used to support aerobic degradation of VC and *cis*-DCE via cometabolism, ethene can serve as a co-substrate for growth-coupled secondary metabolism of VC (Fogel *et al.*, 2005). Coleman *et al.* (2003) recently found that *Mycobacterium* strains isolated on either ethene or VC possessed epoxyalkane:coenzyme M transferase (EaCoMT) enzymes that channel the generated epoxides into metabolic pathways and thus guard against accumulation of the toxic epoxides in
the cytoplasm. This probably explains why cis-DCE transformation yields (i.e., moles of cis-DCE mineralized per mole of substrate utilized) attained with ethene as an auxiliary growth substrate are an order of magnitude greater than when methane is used (Koziollek et al., 1999).

Ethenotrophic degradation of VC and cis-DCE is inhibited by high ethene concentrations. As described above, ethene possesses advantages over other substrates (e.g., methane). However, as is the case for the other substrates, ethene will compete for monoxygenase active sites and thus competitively inhibit VC and cis-DCE degradation.

Gas-permeable membranes can be used to supply dissolved gases to the subsurface with very high transfer efficiency. The iSOC® membrane technology has recently been used to supply ethene and oxygen to a VC-contaminated aquifer (Fogel et al., 2005; LeBlanc et al., 2005). Gas-permeable hollow-fiber membranes have also been used for in situ hydrogen transfer to stimulate reductive dechlorination (Edstrom et al., 2005; Clapp et al., 2004a; Ma et al., 2003; Fang et al., 2002). The use of gas-permeable membranes has the advantage of preventing gas bubble formation, and thus avoiding potential accumulation of explosive gas mixtures in the vadose zone or well head.

It may be possible to concentrate VC/DCE-degrading ethenotrophs on gas-permeable membranes installed within groundwater circulation wells. Previous studies (Clapp et al., 1999; Sumani et al., 2005) have demonstrated that robust methanotrophic biofilms will rapidly grow on gas-permeable membranes supplied with CH$_4$ and air. These studies also indicated that high CH$_4$ concentrations and low TCE concentrations at the biofilm interior resulted in optimal methanotrophic growth conditions, while low CH$_4$ and high TCE concentrations near the biofilm exterior minimized competitive inhibition. These results suggest that bioaugmented cis-DCE and VC degrading ethenotrophs could similarly be concentrated on gas-permeable hollow-fiber membranes.

By combining the in-situ membrane approach with groundwater circulation well (GCW) technology, the number of wells required to effectively treat the width of a plume can be minimized. In GCWs, groundwater is extracted from one screened interval and discharged through a second screened interval at a different level, creating a groundwater circulation pattern around the wells. If the GCWs are equipped with membrane-attached ethenotrophic biofilms, they will effectively become in-situ bioreactors (Veerasekaran, 2004).

1.2 Research Objectives

As described above, degradation of cis-DCE and VC under aerobic conditions may be impeded by absence of the requisite microbial populations and/or substrate-limited growth conditions. In addition, cis-DCE and VC typically coexist with PCE and TCE in groundwater plumes (Suarez et al., 2004), and thus supplying oxygen to promote aerobic conditions would inhibit reductive dechlorination of PCE and TCE. These obstacles could be circumvented by developing a bioremediation technology involving (i) bioaugmentation with an aerobic microbial population capable of growing on cis-DCE and VC, (ii) supply of an auxiliary growth substrate capable of sustaining growth of the bioaugmented population without inhibiting secondary metabolism of cis-DCE and VC, and (iii) supply of oxygen without inhibiting anaerobic PCE/TCE dechlorination (i.e., by simultaneously supplying oxygen and an excess of an electron donor such that aerobic conditions are localized within a small aerobic zone).
Towards developing such a technology, the specific research objectives of this study were:

1. Using experiments with aquifer sediment columns, assess the ability of membrane-delivered ethene and air to stimulate aerobic degradation of cis-DCE by ethenotrophic bacteria that might be indigenous to EAFB aquifer sediments;

2. Using the same aquifer sediment column experiments, determine if nutrient amendment and bioaugmentation with an enriched ethenotrophic culture would stimulate cis-DCE degradation in the EAFB aquifer sediments;

3. Using parallel microcosm experiments, determine if cis-DCE degrading ethenotrophs were indigenous to the EAFB aquifer sediments;

4. Using the same parallel microcosm experiments, determine if nutrient amendment and bioaugmentation with an enriched ethenotrophic culture would improve cis-DCE degradation in the EAFB aquifer sediments;

5. Using enriched and pure cultures, verify that ethenotrophs are capable of rapidly degrading cis-DCE.

2. MATERIALS AND METHODS

2.1 Aquifer Sediment Collection and Characterization

Edwards Air Force Base (EAFB) is located in the Mojave Desert of Southern California. The aquifer sediments were obtained from bore MW03, Site 284, Operable Unit 5. Aquifer sediment cuttings were collected at a depth of between 127 to 192 feet using a hollow-stem auger using water as the drilling fluid (Carter, 2006). The cuttings were placed in a 55-gallon drum and transferred to the Base Environmental Analysis Laboratory (BEAL) facility for use in the soil column experiments. Approximately four liters of the collected sediments were also shipped to Texas A&M University-Kingsville (TAMUK) for use in the microcosm experiments.

The collected aquifer sediments were uncontaminated alluvial deposits and primarily consisted of unconsolidated fine to medium sand, with minor amounts of gravel and lacustrine silts and clays. The hydraulic conductivity in the vicinity of Site 284 ranges between \(1.66 \times 10^{-3}\) and \(3.74 \times 10^{-3}\) cm/sec, and the porosity is approximately 0.30 (EAFB, 2006). The fraction of organic carbon (\(f_{oc}\)) in the EAFB sediments is 0.01 - 0.04% and the bulk density is approximately 1.75 g/mL (McCarty et al., 1998). Since the sediments were from an uncontaminated background location, no microbial characterization analyses were performed.

2.2 Groundwater Collection and Characterization

Approximately 80 L of groundwater was initially collected from the 284-MW03 monitoring well and transferred to the BEAL facility in two plastic carboys. Approximately 5 L of the groundwater sample was shipped to TAMUK for the microcosm experiments. Thereafter, groundwater was collected from the same monitoring well about once per month. Since the pH of the groundwater samples increased when CO\(_2\) escaped, the pH of the feed groundwater was adjusted to approximately 8.0 using HCl. Table 1 summarizes the chemistry of the groundwater
used for the column and microcosm studies (EAFB, 2006). Concentrations of trace constituents are also available in the EAFB groundwater monitoring report.

Table 1 – Chemistry of the groundwater used in the column and microcosm studies.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Conc. (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bromide</td>
<td>1.6 – 1.8</td>
</tr>
<tr>
<td>Chloride</td>
<td>324 – 354</td>
</tr>
<tr>
<td>Fluoride</td>
<td>0.7 – 1.1</td>
</tr>
<tr>
<td>Nitrate (as nitrogen)</td>
<td>0.39 – 0.86 *</td>
</tr>
<tr>
<td>Sulfate</td>
<td>161– 190</td>
</tr>
<tr>
<td>Total alkalinity (as CaCO₃)</td>
<td>95 – 150 **</td>
</tr>
<tr>
<td>Hardness (as CaCO₃)</td>
<td>169 – 189</td>
</tr>
<tr>
<td>TDS</td>
<td>1,100 – 1,190</td>
</tr>
</tbody>
</table>

* Groundwater feed was amended with 10 mg/L of NO₃-N at start of study F (see below).

** From experimental measurements performed during the study.

2.3 Aquifer Sediment Column Studies

Aquifer sediment columns were used to assess the ability of membrane-delivered ethene and air to stimulate degradation of cis-DCE by ethenotrophic bacteria indigenous to EAFB aquifer sediments. The same aquifer sediment columns were used to determine if nutrient amendment and bioaugmentation with an enriched ethenotrophic culture would improve cis-DCE degradation in the EAFB aquifer sediments. Two aquifer sediment columns were fabricated (Figure 1a). Each column consisted of three 9.2-cm i.d. × 31 cm glass sections, with two 3.5-cm thick polycarbonate membrane modules (Figure 1b) placed in between the sections. To allow collection of aqueous samples, one sampling port in the bottom glass sections, three in the middle and top sections, and one in each of the membrane modules were installed. Each membrane module contained two lengths of gas-permeable Varglas ES-4400 (Varflex Corp., Rome, NY) silicone-coated fiberglass membrane tubing (1.54-mm o.d. × 100 cm) threaded in parallel through aluminum cross supports in a spiral configuration as shown in Figure 1b to provide a uniform ethene and oxygen supply across the column cross sections. The total membrane gas-transfer surface area in each module was 97 cm², or 194 cm² in each column. The bottom column sections were filled with 1-mm glass beads to provide uniform hydraulic flow. Two aquifer sediment columns were wet-packed with the homogenized aquifer sediments collected from the EAFB site. Tubing was connected to the inlet and outlet of columns. The outlet lines delivered the effluent to collection flasks. The inlet lines were connected to a 50-L carboy that served as a groundwater feed reservoir. Two piston pumps were used to deliver the groundwater to the columns. The soil columns operated in a saturated up-flow mode.

To simulate typical groundwater conditions, the groundwater was pumped from the feed carboy to the two columns at 0.5 mL/min, resulting in a linear water velocity of approximately 31 cm/day through the aquifer media. Although this was about four times greater than the typical groundwater velocity of 8 cm/day at EAFB (McCarty et al., 1998), it allowed quasi-steady-state conditions to be attained in a reasonable time. The water velocity through the membrane modules was approximately 11 cm/day (due to porosity approximately three times greater than for the aquifer media). A tee was installed in each inlet line and connected to a
A multi-channel syringe pump that was used to spike the groundwater with a concentrated cis-DCE solution.

An overview of the column operating parameters for the cis-DCE degradation studies is presented in Table 2. The only difference between the test column (C1) and control column (C2) was the gas supplied to the membrane modules. A mixture of 6% ethene in air (for the test column) and either air or nitrogen (for the control column) were supplied to the membrane modules from gas cylinders (the value of 6% ethene was chosen to provide ethene and oxygen at a 1:3 stoichiometric ratio as per the reaction \( \text{C}_2\text{H}_4 + 3\text{O}_2 \rightarrow 2\text{CO}_2 + 2\text{H}_2\text{O} \)). An infrared ethene sensor was connected to the outlet gas line of the test column so that continuous ethene delivery could be monitored. The gas flow through each hollow-fiber membrane was adjusted to either 3 or 1 mL/min using low-flow needle valves. The total gas pressure within the membranes decreased from \( \sim 1.05 \) atm at the inlets to \( \sim 1.00 \) atm (ambient) at the outlets. The aquifer sediment column cis-DCE degradation studies were comprised of seven phases (A-G), as described in the results and discussion section.

![Figure 1](http://scholarworks.umass.edu/intljssw/vol2/iss3/2)

**Figure 1.** (a) Aquifer sediment column reactors with piston pumps and syringe pump on the right; (b) 15-cm o.d. membrane module with two 100-cm hollow-fiber silicone membranes.

<table>
<thead>
<tr>
<th>Study</th>
<th>Start</th>
<th>C1</th>
<th>C2</th>
<th>C1</th>
<th>C2</th>
<th>C1</th>
<th>C2</th>
<th>C1</th>
<th>C2</th>
<th>C1</th>
<th>C2</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) Br tracer</td>
<td>01/26/07</td>
<td>0</td>
<td>0</td>
<td>air</td>
<td>air</td>
<td>3</td>
<td>3</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>B) DCE retardation</td>
<td>03/12/07</td>
<td>50</td>
<td>50</td>
<td>air</td>
<td>air</td>
<td>3</td>
<td>3</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>C) Ethene turned on for C1</td>
<td>04/03/07</td>
<td>500</td>
<td>500</td>
<td>eth/air</td>
<td>air</td>
<td>3</td>
<td>3</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>D) Lowered gas flows</td>
<td>05/24/07</td>
<td>500</td>
<td>500</td>
<td>eth/air</td>
<td>air</td>
<td>1</td>
<td>1</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>E) Switched to N(_2) in C2</td>
<td>07/28/07</td>
<td>500</td>
<td>500</td>
<td>eth/air</td>
<td>N(_2)</td>
<td>1</td>
<td>1</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>F) Added culture &amp; nutrients</td>
<td>10/19/07</td>
<td>500</td>
<td>500</td>
<td>eth/air</td>
<td>N(_2)</td>
<td>1</td>
<td>1</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>G) Turned off gas to C2</td>
<td>11/15/07</td>
<td>500</td>
<td>500</td>
<td>eth/air</td>
<td>none</td>
<td>1</td>
<td>0</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

1 The groundwater flow rate through both aquifer sediment columns was maintained at 0.5 mL/min (or 0.72 L/day) throughout all the studies, corresponding to a linear velocity of \( \sim 31 \) cm/day.

2 A mixture of 6% ethene in air was supplied to column C1 during studies C-G.
2.4 Aquifer Sediment Microcosm Studies

To complement the aquifer sediment column studies, EAFB aquifer sediment microcosm studies (Figure 2a) were conducted to determine (1) if growth of indigenous ethenotrophic bacteria present in the EAFB aquifer sediments could be stimulated in the presence of ethene and oxygen, (2) if nutrient (N and P) availability in the EAFB aquifer sediments limited growth of ethenotrophic bacteria, and (3) if bioaugmentation with an enriched ethenotrophic culture would stimulate growth of ethenotrophs in the EAFB aquifer sediments (Bandyopadhyay, 2008). To meet these objectives, samples of the homogenized aquifer sediments (6 L) and groundwater (2 L) collected from the EAFB site were shipped to TAMUK. Approximately 50 g (dry weight) of sediment was mixed with EAFB groundwater to a volume of 100 mL and then added to each serum bottle. Three additional autoclaved serum bottles were filled with 100 mL of DI water to serve as negative controls (see Table 3). The pH of all the microcosm bottles was initially adjusted to between 7 and 8 using HCl. The 15 serum bottles were then capped with Teflon-lined rubber septa and a gas-tight syringe was used to add ethene to the 60-mL headspaces to yield a ~5% ethene in air mixture. All the serum bottles were then placed on a platform shaker at 150 rpm (Figure 2a).

Figure 2. (a) sediment microcosm bottles being analyzed for ethene and CO₂. (b) Subcultures of pure and enriched ethenotrophic cultures on a platform shaker.

To assess indigenous ethenotroph activity in the EAFB sediments, ethene, carbon dioxide, and methane concentrations in the headspaces of the 12 sediment microcosm bottles and 3 control bottles were measured once per week for 16 weeks. During this period, the microcosm bottles were not amended with nutrients or an enriched ethenotrophic culture. After 16 weeks of continuous monitoring with little ethene utilization in all of the microcosms, all of the sediment microcosm bottles (see Table 3) were amended with 5 mL of nitrate mineral salts medium (Table 4). In addition, microcosm bottles 1-6 were each bioaugmented with 250 µL of a DCE-degrading mixed ethenotrophic culture and 250 µL of a pure culture of DCE-degrading Nocardioides strain JS614. Both cultures were grown at TAMUK in 160-mL serum bottles containing 40 mL of a nitrate mineral salts medium and supplied with a 10% ethene in air headspace (see Section 2.5 for the ethenotroph culture growth methods). Both bacterial cultures were inoculated just after stationary growth phase was reached. Ethene, carbon dioxide, and methane concentrations in the headspaces of the 12 sediment microcosm bottles and 3 control bottles were subsequently analyzed once per week for another 5 weeks.
Table 3. Microcosm test conditions.

<table>
<thead>
<tr>
<th>Bottle Numbers</th>
<th>1-6</th>
<th>7-12</th>
<th>13-15</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EAFB aquifer sediments</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Nutrient addition</strong></td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Bioaugmentation</strong></td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

1 Bottles 13-15 were negative controls filled with 50 mL of DI water only.
2 Nutrient amendment and bioaugmentation occurred at the beginning of week 16.

Table 4. Nitrate mineral salts medium.

<table>
<thead>
<tr>
<th>Salt</th>
<th>Conc. (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K₂HPO₄</td>
<td>3880</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>3590</td>
</tr>
<tr>
<td>(NH₄)₂SO₄</td>
<td>700</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>50</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>300</td>
</tr>
<tr>
<td>EDTA</td>
<td>10</td>
</tr>
<tr>
<td>ZnSO₄·7H₂O</td>
<td>2</td>
</tr>
<tr>
<td>CaCl₂·2H₂O</td>
<td>1</td>
</tr>
<tr>
<td>FeSO₄·7H₂O</td>
<td>5</td>
</tr>
<tr>
<td>NaMoO₄·2H₂O</td>
<td>0.20</td>
</tr>
<tr>
<td>CuSO₄</td>
<td>0.08</td>
</tr>
<tr>
<td>CoCl₂·6H₂O</td>
<td>0.4</td>
</tr>
<tr>
<td>MnCl₂·4H₂O</td>
<td>1.0</td>
</tr>
</tbody>
</table>

2.5 Enriched and Pure Ethenotrophic Culture Studies

Enriched ethenotrophic culture studies were performed to verify that ethenotrophic cultures could rapidly degrade cis-DCE with high transformation yields. A small serum bottle containing a suspended-growth enriched cis-DCE degrading ethenotrophic culture was obtained from Dr. Sam Fogel at Bioremediation Consulting Inc. (BCI, Watertown, MA). In addition, an agar plate with colonies of a pure-culture of *Nocardioides* strain JS614 was obtained from Dr. Tim Mattes at the University of Iowa. To grow subcultures, 160-mL serum bottles were filled with 40 mL of nitrate mineral salts medium (Table 4) and autoclaved along with Teflon-line septa and aluminum crimps. After the bottles cooled, the suspended-growth BCI culture was sub-cultured by injecting 250 µL into three of the sterile 160-mL serum bottles and then crimping the caps onto the bottles. A syringe was then used to remove 6 mL of the headspace and replace it with 6 mL of ethene gas, resulting in a 5% ethene headspace. The pure culture *Nocardioides* strain JS614 was similarly subcultured, except that a sterile inoculation loop was used to pick colonies from the surface of the agar plate and transfer them to the 160-mL serum bottles. After inoculation, the serum bottles were agitated on a shaker set at 150 rpm (Figure 2b). The subculturing was conducted under sterile conditions to prevent contaminating the cultures; however, no analyses were conducted to assess the purity of the subcultures.

To assess the ethene utilization in the subcultures, the ethene concentrations in the headspaces of the serum bottles were measured just before inoculation, and then again after the cultures had reached the stationary growth phase after approximately 7 days. Observed growth yields were determined by determining the mass of dry cells (as volatile suspended solids) and dividing by the mass of ethene consumed. To assess cis-DCE degradation, 160-mL serum bottles containing 40 mL of ethenotrophic culture in the stationary growth phase were opened and briefly (< 1 minute) sparged with air to remove residual ethene. The bottles were then recapped with Teflon-coated septa and then spiked with 2 ppm cis-DCE (based on the initial liquid phase volume). The headspace cis-DCE concentrations were then measured, taking samples approximately once every hour. After the cis-DCE concentrations had decreased by at 50-90%, the bottles were respiked to obtain the original 2 ppm concentration. The observed cis-DCE transformation yields were then calculated by divided the cumulative mass of cis-DCE degraded by the mass of ethene utilized to grow the cells.
Table 5. Analytical methods and sampling schedule used during the sediment column studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Analyte</th>
<th>Period</th>
<th>Method</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Br tracer study</td>
<td>bromide</td>
<td>Feb 07</td>
<td>IC</td>
<td>~20 total (2 samples per day over 2 weeks)</td>
</tr>
<tr>
<td>DCE retardation study</td>
<td>headspace DCE</td>
<td>Mar 07</td>
<td>GC-ECD</td>
<td>~30 total (2 samples per day over 3 weeks)</td>
</tr>
<tr>
<td>DCE degradation studies</td>
<td>headspace DCE</td>
<td>Apr 07-Mar 08</td>
<td>GC-ECD</td>
<td>~18 samples once per week for 12 months</td>
</tr>
<tr>
<td></td>
<td>headspace C₂H₄, CH₄</td>
<td>Feb 08</td>
<td>GC-TCD</td>
<td>5 samples (one-time analysis)</td>
</tr>
<tr>
<td></td>
<td>DO and pH</td>
<td>Feb 07-Mar 08</td>
<td>DO/pH meters</td>
<td>~2 samples once per month for 12 months</td>
</tr>
<tr>
<td></td>
<td>Alkalinity</td>
<td>Feb 07-Mar 08</td>
<td>Acid titration</td>
<td>2 samples once per week for 12 months</td>
</tr>
<tr>
<td></td>
<td>gas-phase C₂H₄</td>
<td>June 07-Mar 08</td>
<td>ethene sensor</td>
<td>1 sample once per week for 10 months</td>
</tr>
<tr>
<td></td>
<td>gas flows</td>
<td>Feb 07-Mar 08</td>
<td>bubble meter</td>
<td>2 samples once per week for 12 months</td>
</tr>
<tr>
<td></td>
<td>water flows</td>
<td>Feb 07-Mar 08</td>
<td>effluent collection</td>
<td>2 samples once per week for 12 months</td>
</tr>
</tbody>
</table>

Table 6. Analytical methods and sampling schedule used during the microcosm and pure/enriched ethenotrophic culture studies.

<table>
<thead>
<tr>
<th>Study</th>
<th>Analyte</th>
<th>Period</th>
<th>Method</th>
<th>Number of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcosm study</td>
<td>gas-phase C₂H₄, CH₄</td>
<td>June 07-Mar 08</td>
<td>GC/TCD</td>
<td>~15 once per week for 12 months</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>June 07-Mar 08</td>
<td>pH meter</td>
<td>~15 once every 2 months for 12 months</td>
</tr>
<tr>
<td>Pure and mixed culture</td>
<td>gas-phase C₂H₄</td>
<td>Sept 07-Mar 08</td>
<td>GC/TCD</td>
<td>~5 once per week for 7 months</td>
</tr>
<tr>
<td>DCE degradation study</td>
<td>headspace DCE</td>
<td>Jan 07-Mar 08</td>
<td>GC-ECD</td>
<td>~5 twice per week for 3 months</td>
</tr>
</tbody>
</table>
3. RESULTS AND DISCUSSION

3.1 Results of Aquifer Sediment Column Studies

3.1.1 Conservative tracer (study A)

The hydraulic characteristics of the columns were evaluated using a tracer study, with the columns operated in an up-flow mode. To simulate groundwater conditions, groundwater was pumped through the two columns at 0.5 mL/min. The groundwater feed to one of the columns was spiked with 60 mg/L of bromide, and 5-mL water samples were collected from the last port once a day for 16 days and analyzed for Br\(^-\) breakthrough using ion chromatography (Figure 3). The column cross-sectional area was \(A_c = 67 \text{ cm}^2\), and thus the specific discharge was \(v = Q/A_c = 10.7 \text{ cm/day}\). Since the porosity of the membrane modules was 1.0, this was also the groundwater linear velocity through the membrane modules. Bromide at the top sampling port reached 30 mg/L (50% of the inlet concentration) at approximately \(\theta = 4\) days after initiating the bromide spiking. Since total distance from the bottom of the column to the top sampling port was \(L = 123 \text{ cm}\), the average linear groundwater velocity through the column was \(v_{\text{lin}} = L/\theta = 31 \text{ cm/day}\). The effective porosity of the porous media was calculated as \(\eta = v/v_{\text{lin}} = 0.34\).

3.1.2 cis-DCE breakthrough curve (study B)

After the tracer study, a cis-DCE breakthrough study was performed. The influent line to each column was spiked with a concentrated cis-DCE solution at 5 \(\mu\)L/hr using a syringe pump. To characterize the cis-DCE breakthrough curves, 1-mL water samples were withdrawn from the last port of each column every day over two weeks and analyzed for cis-DCE using an equilibrium headspace method with a gas chromatograph equipped with an electron capture detector (GC-ECD). Figure 3 compares the breakthrough curves for bromide and cis-DCE. The cis-DCE concentration at the top sampling port reached 25 \(\mu\)g/L (50% of the inlet concentration) at approximately \(\theta = 15\) days after initiating the cis-DCE spiking. Thus, the cis-DCE took roughly 3.7 times as long to break through the columns, implying a low retardation factor of 3.7, which was consistent with the low organic carbon content of the sediments.

![Figure 3. Bromide and cis-DCE breakthrough data for aquifer sediment column.](image-url)
3.1.3 Baseline cis-DCE removal (study C)

Following the bromide tracer and cis-DCE breakthrough studies, a baseline cis-DCE degradation study was conducted to assess whether supply of ethene and air through the gas-permeable membranes would stimulate growth of indigenous ethenotrophic bacteria in the EAFB aquifer sediments. Ethene supply (6% in air by volume) to column C1 was initiated, while column C2 continued to be supplied with air alone as a negative control. The gas flow rate through each hollow-fiber membrane was maintained at ~3 mL/min. The DO concentrations in the effluent from both columns were consistently above 4 mg/L, indicating that aerobic conditions existed in both columns. The cis-DCE concentrations at the bottom and top sampling ports for the ethene-supplied test column (C1) and air-supplied control column (C2) are shown in Figure 4. The average cis-DCE removals in the ethene-supplied test column (C1) and air-supplied control column (C2) during the study C were 82 ± 6% and 78 ± 6%, respectively (with the ± values indicating 90% confidence intervals for the mean).

Study C showed that supply of 6% ethene and 94% air to the test column did not result in significantly greater cis-DCE removal than in the air-supplied control column after 49 days of continuous operation. The immediate attainment (i.e., with no lag period) of >72% removal in both columns after adding cis-DCE to the feed groundwater indicated that the removal was probably not due to biodegradation [e.g., Coleman et al. (2002b) found that the lag time before aerobic VC degradation occurred in sediment samples ranged from 20 to 110 days]. A similar column study by Ma et al. (2006) found that stripping of PCE through the gas-permeable hollow-fiber membranes accounted for between 19% and 32% of the contaminant removal. It was thus hypothesized that back-diffusion of cis-DCE through the hollow-fiber membranes was primarily responsible for the high removals observed in both columns.

3.1.4 cis-DCE removal after lowering gas flows (study D)

To test the preceding hypothesis, the gas flows were decreased from ~3 mL/min per hollow-fiber membrane to ~1 mL/min per fiber (or, since there were four fibers per column, from a total of ~12 mL/min per column to ~4 mL/min per column). The DO concentrations in the effluent from both columns were consistently above 4 mg/L, indicating that aerobic conditions still existed in both columns. The decrease in gas flow rates coincided with average cis-DCE removals in columns C1 and C2 decreasing from 82 ± 6% and 78 ± 6% to 49 ± 8% and 50 ± 10%, respectively (Figure 4), which supported the cis-DCE back-diffusion hypothesis.

Study D showed that supply of 6% ethene and 94% air to the test column did not result in significantly greater cis-DCE removal than in the air-supplied control column after 115 days of continuous operation. Although the decrease in removal with decreased gas flow was consistent with cis-DCE back-diffusion through the membranes being a dominant removal mechanism, the possibility that supply of oxygen was stimulating aerobic biodegradation of cis-DCE in both columns could not be ruled out.
Figure 4. cis-DCE removals in the ethene-supplied test column (C1) and control column (C2) over time. The operating conditions for each period are summarized in Table 2. The open symbols on day 318 were for split sample analyses conducted at a second laboratory.

Figure 5. Average cis-DCE removal efficiencies in the test column (C1) and control column (C2) during the three different gas flow periods. Error bars represent 90% confidence intervals. The first letters on the x-axis indicate the study period (C, D-F, or G).
3.1.5 *cis*-DCE removal after switching from air to \( \text{N}_2 \) in control column (study E)

To test the possibility that aerobic *cis*-DCE degradation was occurring in the control column (C2), air was replaced with \( \text{N}_2 \) for the gas supply in study E. Counter to the \( \text{O}_2 \) supply hypothesis, *cis*-DCE removal in column C1 remained comparable to that in column C2 (66 ± 11% and 69 ± 15%, respectively; see Figure 4). However, the possibility that DO in the feed groundwater was supporting aerobic degradation still could not be ruled out because the inlet and outlet DO concentrations remained greater than 4 mg/L.

Study E showed that supply of 6% ethene and 94% air to the test column did not result in significantly greater *cis*-DCE removal than in the control column after 189 days of continuous operation. This provided strong evidence that the supply of ethene and air to the test column had not stimulated significant growth of DCE-degrading ethenotrophs. It was thus hypothesized that either lack of nutrients and/or absence of indigenous ethenotrophs in the EAFB sediments was preventing growth of DCE-degrading ethenotrophs in the test column.

3.1.6 *cis*-DCE removal after bioaugmentation and nutrient amendment (study F)

To test the preceding hypothesis, the feed groundwater for both columns was amended with 10 mg/L of \( \text{NO}_3^-\text{N} \) and 2 mg/L of \( \text{PO}_4^-\text{P} \) (as \( \text{NaNO}_3 \) and \( \text{KH}_2\text{PO}_4 \), respectively), and the test column was bioaugmented with two enriched DCE-degrading cultures. A DCE-degrading mixed ethenotrophic culture was obtained from Bioremediation Consultants Inc. (Watertown, MA) and a pure culture of *Nocardioides* strain JS614 was obtained from University of Iowa. Both cultures were grown to concentrations of approximately 500 mg VSS/L in serum bottles containing 40 mL of a nitrate mineral salts medium (Table 4) and supplied with a 6% ethene in air headspace (Figure 2a). To bioaugment the test column (C1), 10 mL of each culture was injected into both the bottom and top membrane module sampling ports. The DO concentrations in the effluent from both columns remained above 2 mg/L, indicating that aerobic conditions still existed in both columns. Counter to the bioaugmentation hypothesis, *cis*-DCE removal in column C1 remained comparable to that in column C2 (38 ± 7% and 44 ± 10%, respectively; see Figure 4).

Study F showed that bioaugmenting the test column and continuing to supply it with 6% ethene and 94% air did not result in significantly greater *cis*-DCE removal than in the non-bioaugmented and \( \text{N}_2 \)-supplied control column over 27 days (note that, because the inoculated DCE-degrading strains may have needed more time to acclimate to the new conditions, *cis*-DCE removal continued to be monitored for an additional 121 days during study G). However, significant (>31%) *cis*-DCE removal continued to be observed in both columns. It was thus hypothesized that, even at the decreased gas-flow rate of 1 mL/min per hollow-fiber membrane, back-diffusion of *cis*-DCE continued to be the primary removal mechanism in both column.

3.1.7 *cis*-DCE removal after turning off gas supply to control column (study G)

To test the preceding hypothesis, all gas flow to the control column (C2) was stopped. The DO concentrations in the effluent from both columns remained above 2 mg/L, indicating that aerobic conditions still existed in both columns. There was no significant change in the *cis*-DCE removal in the ethene-supplied test column (C1), indicating that *cis*-DCE removal had not improved significantly over the 151 days after nutrient addition and bioaugmentation. In contrast, the average *cis*-DCE removal in the control column decreased to 11 ± 7% during study
G (Figure 4), which coincided with the cessation of gas flow through the column’s four hollow-fiber membranes.

Studies F and G together showed that after 151 days the bioaugmented test column supplied with 6% ethene and 94% air did not attain significantly greater cis-DCE removal than in the non-bioaugmented and N$_2$-supplied control column. Study G also showed that back-diffusion through the membranes – and not biodegradation – had probably been the primary cis-DCE removal mechanism in both columns throughout the entire study.

3.1.8 Comparison of cis-DCE removal under different gas flow conditions (studies C-G)

To characterize the effect of gas flow rate, Figure 5 compares the cis-DCE removals in both columns under the three different gas flow conditions used. It is evident that the time-averaged cis-DCE removal rates decreased significantly when the gas flow rate was decreased from ~3 mL/min/fiber to ~1 mL/min/fiber (for both columns), and again when the gas flow rate was decreased from ~1 mL/min/fiber to zero (for column C2 only). In contrast, there was no statistically significant difference between the cis-DCE removals in the ethene-supplied test column (C1) and the air- or N$_2$-supplied control column (C2) when both were supplied with gas at the same flow rates. These results were all consistent with the cis-DCE removal in both columns resulting primarily from back-diffusion through the hollow-fiber membranes, and not from biodegradation. This interpretation of the data was supported by average cis-DCE concentration profiles for both columns that consistently showed decreases in cis-DCE concentrations between the sampling ports immediately upstream and downstream of the two membrane modules, but not between other sampling ports (Clapp et al., 2008). As will be discussed in section 3.2, this interpretation of the data was also consistent with the results of the EAFB aquifer sediment microcosm study that showed little stimulation of ethenotrophic activity, even after bioaugmentation.

3.1.9 Sources of variability in determined cis-DCE removals

Figure 4 shows substantial variability in the cis-DCE removal with respect to time throughout studies C-G. This variability can be partly attributed to removal being calculated based on measured concentrations at the bottom and top sampling ports at a given time $t$; that is:

$$\% R_t = \frac{C_{bot,t} - C_{top,t}}{C_{bot,t}} \times 100$$

where $\% R_t$ is the percent removal and $C_{bot,t}$ and $C_{top,t}$ are the measured concentrations at the bottom and top sampling ports, respectively, at time $t$. This calculation method did not account for the approximate four-day hydraulic residence time (HRT) difference between the bottom and top sampling ports. Consequently, the calculated removals tended to be high when the feed cis-DCE concentrations were above normal and low when the feed concentrations were below normal. Fluctuations in the feed cis-DCE concentrations resulted from a number of factors, including: (1) occasional brief power interruptions that caused the syringe pump – but not the groundwater feed pumps – to stop for up to 48 hours; (2) small decreases in groundwater feed

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1 This HRT difference could not be accounted for by using $C_{top,t-4}$ instead of $C_{top,t}$ in Eq. 1 because: (1) top-port samples were not always collected four days after bottom-port samples; and (2) dispersion and adsorption/desorption dampened transient concentration fluctuations near the top sampling port.
flows that occurred due to clogging of the filters installed on the inlet lines (followed by groundwater flow increases when the filters were replaced); and (3) small changes in the concentration of the cis-DCE spiking solution that occurred due to human error when preparing the spiking solution. Finally, there were also changes in temperature over time (approximately ±8°C) that may have contributed to variability in cis-DCE stripping and adsorption. It should be noted that all the aforementioned sources of experimental variability affected both columns almost equally. This is consistent with Figure 4, which shows that despite significant removal variability with time, the fluctuations followed the same general trends in both columns.

Analytical error, characterized following standard quality control protocols (Eaton et al., 2005), also contributed to the cis-DCE removal variability evident in Figure 4. At least four laboratory-fortified blank (LFB) samples were analyzed with each set of samples (for a total of 122 calibration verification analyses). The mean and standard deviation for the percent recovery for the LFB samples was 99 ± 15%. In addition, at least two duplicate (DUP) samples were analyzed with each set of samples (for a total of 58 duplicate sample analyses). The mean and standard deviation for the DUP sample relative percent difference was 5 ± 5% (absolute value). It should be noted that, unlike the experimental variability, the analytical variability did not affect the removal data for both columns at any given time equally. Thus, the variability in the difference between the removal efficiency between the two columns during any given study period (C-G) can be largely attributed to analytical error.

### 3.1.10 Dissolved ethene concentrations in test column

During study G, liquid samples were collected (using 10-mL vials with no headspace) from sampling ports in the bottom and top membrane modules of the test column (C1). In addition, the gas outlet line from the test column was placed in a 20-mL glass vial filled with DI water such that the gas bubbled into the water. The collected samples were analyzed for ethene using a GC equipped with a thermal conductivity detector. The DI water that had been bubbled with the outlet gas had an ethene concentration of 6.40 ± 0.28 mg/L. In comparison, using a Henry’s law constant for ethene at 25°C of 213 atm/M (Sander, 1999), the theoretical ethene concentration for water at equilibrium with ethene gas at a partial pressure of 0.06 atm was calculated to be 7.89 mg/L. Thus, the ethene concentration in the water that had been bubbled with the outlet gas from the membrane modules was only slightly lower (19%) than the theoretical saturation concentration assuming an ethene partial pressure of 0.06 atm, indicating that the ethene composition of the gas flowing through the membranes was close to the target of 6%.

The dissolved ethene concentrations in the water samples collected from the bottom and top membrane module sampling ports were similar at 0.83 ± 0.28 and 0.87 ± 0.09 mg/L, respectively. In comparison, a dimensionless membrane mass transfer correlation developed by Fang et al. (2002) for creeping groundwater flow in a very similar column system was used to predict that the ethene concentrations would have been 2.52 mg/L under abiotic conditions. Thus, the actual measured ethene concentrations, 0.83 and 0.87 mg/L, were only 33% and 35%, respectively, of the concentration predicted by the mass transfer correlation. This experimental finding was consistent with the possibility that ethenotrophic bacteria were utilizing ethene within the test column. It should be noted that lower experimental ethene concentrations than predicted by the dimensionless mass transfer correlation implied that the ethene transfer rates were higher, not lower, than would be predicted for abiotic conditions (Essila et al., 2000).
3.1.11 Presence of biofilms on membranes in the test column

At the conclusion of the experiment, the columns were disassembled and the membrane modules visually inspected. Figure 6 shows that significantly more robust biofilms developed on the membranes in the test column than on the membranes in the control column, indicating more microbial activity in the test column. This could be partly attributable to the test column having been supplied with oxygen for 237 days longer than the control column (i.e., during studies E-G). However, the observations that (i) both columns remained aerobic over the entire study, and (ii) microbial growth in the test column appeared to occur primarily on the membranes indicated that the biofilm development in the test column was largely due to the supply of ethene. Finally, evidence of minor mineral scaling (e.g., CaCO$_3$) was also observed on the membranes from both columns.

![Figure 6. Biofilms on the bottom membranes in the control column (CB) and test column (TB).](image)

3.1.12 Alkalinity and pH data

The effluent alkalinity and pH for the two columns were measured weekly. During studies C-E, the effluent alkalinitities ranged from 90 to 120 mg/L (as CaCO$_3$) in both columns. However, the effluent alkalinitities in both columns increased to between 120 and 160 mg/L as CaCO$_3$ during studies F-G. Although the feed groundwater was spiked with 10 mg/L of NO$_3$-N (as NaNO$_3$) and 2 mg/L of PO$_4$-P (as KH$_2$PO$_4$) during studies F and G, the theoretical change in alkalinitity associated with these amendments was negligible (< 1 mg/L as CaCO$_3$). Furthermore, although denitrification of 10 mg/L of NO$_3$-N would theoretically increase alkalinitity by 35 mg/L as CaCO$_3$ (Metcalf & Eddy Inc. et al., 2002), the measured effluent DO concentrations were never below 2.0 mg/L, suggesting that denitrification did not occur. Thus, the increase in alkalinitity from both columns was probably due to an increase in the alkalinitity of the feed groundwater.

Despite periodic additions of HCl to the groundwater feed to lower the influent pH, the effluent pH values for the control column (C2) were consistently between 8.7 and 9.1. The effluent pH values for the ethene-supplied test column (C1) also fell within this range during studies C and D. However, during studies E-G, the test column effluent pH generally decreased to between 8.2 and 8.8, or 0.5 units lower, on average, than for the control column. This result was consistent with the possibility that ethenotrophic bacteria were active in the ethene-supplied test column, since ethene oxidation to CO$_2$ would decrease the effluent pH. For example,
equilibrium mass balance calculations showed that oxidation of 5 mg/L of ethene to CO$_2$ would theoretically decrease the pH by 1.9 units in a closed system. That the pH in the test column was only 0.5 pH units lower than in the control column could be attributed to its being an open system (i.e., some microbial-produced CO$_2$ was removed via back-diffusion through the hollow-fiber membranes) and to the possibility that the delivered ethene was not completely oxidized.

3.2 Results of Aquifer Sediment Microcosm Studies

3.2.1 Evaluation of ethene utilization in non-bioaugmented EAFB aquifer sediments

Figure 7 compares the average headspace ethene concentrations in the 12 microcosm bottles and the 3 control bottles over time. During days 1-49, the average headspace ethene concentrations in the microcosm and control bottles were consistently between 4.4 and 4.8, indicating negligible activity of ethenotrophic bacteria. However, between days 49 and 105, the average microcosm bottle headspace ethene concentrations slowly decreased from $4.4 \pm 0.3\%$ to $2.9 \pm 0.5\%$, whereas the average control bottle headspace ethene concentrations only decreased from $4.3 \pm 0.4\%$ to $3.8 \pm 0.4\%$. Correspondingly, the average headspace CO$_2$ concentrations in the microcosm bottles slowly increased from $0.23 \pm 0.02\%$ to $0.55 \pm 0.05\%$, whereas the average headspace CO$_2$ concentrations in the controls did not increase significantly (from $0.05 \pm 0.01\%$ to $0.11 \pm 0.07\%$). These results were consistent with the possibility that ethenotrophic bacteria were active in the microcosm bottles. Finally, no methane was detected, indicating that methanogens were not active.

![Figure 7](image-url)

*Figure 7.* Average headspace ethene concentrations in the 12 microcosm bottles and 3 control bottles over time. On day 112 (vertical dashed line), bottles 1-12 were amended with nutrients, bottles 1-6 were bioaugmented, and control bottles 13-15 were inadvertently opened.

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5 mg/L was the minimum total dissolved ethene concentration estimated to have been supplied to the groundwater flowing through the test column.
3.2.2 Assessment of nutrient amendment and bioaugmentation on ethenotroph activity

Although the headspace ethene and CO$_2$ analyses did suggest that ethenotrophic bacteria may have been active in the microcosms, the disappearance of ethene was very slow. Therefore, on day 112, all the microcosm bottles were amended with 5 mL of mineral salts media (MSM) to supply nutrients (N and P), and bottles 1-6 were bioaugmented with 250 µL of the DCE-degrading mixed ethenotrophic culture and 250 µL of the pure culture of *Nocardioides* strain JS614. Surprisingly, as shown in Figure 7, the average headspace ethene concentrations in the bioaugmented bottles (1-6) did not decrease at all between day 105 (before bioaugmentation) and day 147 (35 days after bioaugmentation), whereas the average headspace ethene concentrations in the non-bioaugmented bottles did decrease slightly from 2.8 ± 0.5% on day 105 to 2.6 ± 0.5% on day 147 (which was not statistically significant). These results suggested that, although the mixed ethenotrophic culture and pure culture of *Nocardioides* strain JS614 both grew readily in MSM (as described below), the cultures did not acclimate readily to the EAFB aquifer sediments.

3.3 Results of Pure and Enriched Culture cis-DCE Degradation Study

As described above, both a mixed ethenotrophic culture and a *Nocardioides* strain JS614 pure culture were grown in serum bottles (Figure 2b). Observed growth yields, $Y_{obs}$, were 1.62 ± 0.05 and 1.89 ± 0.35 mg of VSS per mg of ethene, respectively. The $Y_{obs}$ values were determined by measuring initial and final ethene concentrations in the headspace, as well as initial and final VSS concentrations. To assess cis-DCE degradation, 160-mL serum bottles containing 40 mL of both ethenotrophic cultures in early stationary growth phase were opened and briefly (< 1 minute) sparged with air to remove residual ethene. The bottles were then recapped with Teflon-coated septa and spiked with 10 ppm cis-DCE (based on initial liquid phase volume). Negative controls were similarly prepared by spiking 40 mL of DI water. A dimensionless Henry’s constant of 0.167 for cis-DCE at 25°C (Sander 1999) was used to compute the aqueous and headspace cis-DCE concentrations. The bottles were then placed on a platform shaker at 150 rpm, and the headspace cis-DCE concentrations over time were measured by GC-ECD. The first time the cultures were spiked with cis-DCE, it took about 6 hrs to remove more than 90% of cis-DCE (Figure 8). However, with each respiking the rate of cis-DCE transformation decreased, and after the sixth respiking it almost ceased completely, indicating that in the absence of ethene the resting cells had a finite cis-DCE transformation capacity, $T_c$. The $T_c$ values for the mixed ethenotrophic culture and the *Nocardioides* strain JS614 culture were determined to be 0.079 ± 0.020 and 0.083 ± 0.022 mg cis-DCE/mg VSS, respectively. These results indicated that neither culture was able to utilize cis-DCE as a growth substrate, and that the degradation occurred via cometabolism. The cis-DCE transformation yields, $T_y$, defined as the maximum mass of cis-DCE transformed by the resting ethenotrophic cells per unit mass of ethene used for cell growth (Alvarez-Cohen and McCarty, 1991), were determined to be 0.128 ± 0.042 and 0.156 ± 0.015 mg cis-DCE/mg ethene (calculated as $T_y = Y_{obs} \times T_c$), for the mixed ethenotrophic culture and the *Nocardioides* strain JS614 culture, respectively. Finally, a peak for a significant transient cis-DCE transformation product appeared in the GC-ECD chromatograms that may have been dichloroacetaldehyde, although this was not confirmed.
4. CONCLUSIONS

The studies with the enriched mixed ethenotrophic culture and the pure *Nocardiooides* strain JS614 culture demonstrated that both were able to rapidly degrade cis-DCE when grown in a nitrate mineral salts medium. The cis-DCE degradation rates decreased significantly over time, indicating that neither culture could utilize cis-DCE as a growth substrate, and that the degradation occurred via cometabolism. The cis-DCE transformation yields were determined to be $0.128 \pm 0.042$ and $0.156 \pm 0.015$ mg cis-DCE/mg ethene, respectively. However, these cultures did not appear to acclimate to the EAFB aquifer sediments readily.

The experimental results of the aquifer sediment column studies indicated that simply supplying ethene and air to the EAFB aquifer sediments alone did not produce conditions favorable for growth of ethenotrophs capable of degrading cis-DCE over 230 days. Moreover, amending the EAFB aquifer sediments with nutrients and bioaugmenting with the enriched and pure ethenotrophic cultures failed to produce conditions favorable for growth of DCE-degrading ethenotrophs over an additional 151 days. This may have been due to the presence of inhibitory substrates [e.g., copper is known to inhibit expression of the soluble methane monooxygenase enzyme responsible for rapid chloroethene cometabolism by methanotrophs (Alvarez-Cohen and Speitel, 2000)], or the absence of requisite growth factors (e.g., micronutrients).

Biodegradation and gas stripping through the hollow-fiber membranes were competing removal mechanisms in the column reactors, such that lower biodegradation removals corresponded to higher stripping removals. Thus, it was not surprising that cis-DCE stripping...
was a more significant removal mechanism in this study than for TCE in a similar previous study (Ma et al., 2006). One possible explanation for the ethene-supplied test column not achieving higher cis-DCE removal rates than the control column (when both columns had the same gas flow per membrane fiber) was that high pH conditions (8.2 to 9.1) inhibited growth of cis-DCE degrading ethenotrophic bacteria. However, a 5% ethene in air headspace did not stimulate significant growth of ethenotrophs in the same EAFB aquifer sediments during pH-controlled microcosm studies (over a 21-week period), even after nutrient addition and bioaugmentation with DCE-degrading ethenotrophic cultures.

Future research should focus on the causes for inhibited growth of ethenotrophs in the aquifer sediments, including the possibility of the presence of inhibitory substrates (e.g., metals) or the absence of requisite growth factors (e.g., micronutrients).

5. ACKNOWLEDGMENTS

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6. REFERENCES


