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ORIGINAL PAPER

# Effect of pore velocity on biodegradation of *cis*-dichloroethene (DCE) in column experiments

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**Abstract** Column experiments were conducted to evaluate the effect of pore velocity on the extent of biodegradation of *cis*-dichloroethene (*cis*-DCE) during transport in porous media. Columns were filled with homogeneous glass beads and inoculated with a culture capable of complete dechlorination of tetrachloroethene to ethene. A constant concentration of *cis*-DCE was maintained in the columns' influent. Three different pore velocities were tested in duplicate, subjecting each column to a constant velocity. At high flow velocity, degradation of *cis*-DCE to ethene was nearly complete within the residence time of the columns. However, at medium and low flow velocities, incomplete dechlorination was observed. After 7 weeks, DNA was harvested from the columns to determine differences in the microbial populations. Results suggest that *Dehalococcoides* sp. were present in higher quantities in the high-velocity columns, consistent with the observed dechlorination. These results suggest that, at contaminated groundwater sites, heterogeneity of groundwater velocity may be one factor that contributes to heterogeneous distribution of biological activity.

**Keywords** Bioremediation · Chlorinated solvents · PCE · TCE · Vinyl chloride · KB-1

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## Introduction

In the United States, chlorinated ethenes are common groundwater contaminants. The prevalence and the potential adverse health effects of these chemicals place them among the most important groundwater contaminants. Therefore, biodegradation of chlorinated ethenes under anaerobic conditions has been studied extensively (e.g., McCarty 1997; Maymó-Gatell et al. 1997; Löffler and Edwards 2006). Briefly, tetrachloroethene (also called perchloroethene, or PCE) is reduced sequentially to trichloroethene (TCE), dichloroethene (DCE), vinyl chloride (VC), and finally ethene, a benign end-product. The goal of bioremediation in a chloroethene-contaminated aquifer is to attain complete dechlorination of PCE to ethene, because VC is the most toxic and carcinogenic of all the chlorinated ethenes.

Unfortunately, under "natural attenuation" clean-up conditions (e.g., MacDonald 2000), complete dechlorination of PCE to ethene has been observed only at some sites, with accumulation of DCE and VC often observed (Hendrickson et al. 2002).

Several factors, related mainly to biological processes, have been identified that may affect the degree of dechlorination observed at contaminated groundwater sites. These factors include the presence or absence of dechlorinating species (e.g., Fennell et al. 2001; Hendrickson et al. 2002), the presence and activity of fermentative bacteria to produce hydrogen for the dechlorinating species (e.g., Cabirol et al. 1998; Duhamel and Edwards 2006), a sufficient supply of electron-donating compounds (e.g., He et al. 2002; Cupples et al. 2004), and competition among dechlorinating, sulfate-reducing, and methanogenic bacteria for nutrients and electron donors (e.g., Carr and Hughes 1998; Fennell and Gossett 1998; Yang and McCarty 1998).

Recent field studies have found that dechlorination rates vary spatially in contaminated aquifers (Fennell et al. 2001; Sandrin et al. 2004; Allen-King et al. 2006; Hoelen et al. 2006). This spatial variability has potentially important consequences for site bioremediation. For instance, caution must be used when selecting remediation by natural attenuation; even if evidence of successful reductive dechlorination is observed in some parts of a contaminated aquifer, natural attenuation might nonetheless fail if the dechlorination rates are too slow in other parts of the aquifer. Therefore, if in situ bioremediation is desired, it may be necessary to employ an engineered approach that is able to overcome sparse or heterogeneous biological activity (e.g., Major et al. 2002; Hoelen et al. 2006). Hence, in order to select and design an appropriate remediation technology, it may be necessary to know how biodegradation rates vary spatially. Unfortunately, the causes of spatial variability of biological reactivity are not yet understood. For instance, what factors might lead to the natural growth of necessary microorganisms (such as *Dehalococcoides* sp.) in some parts of a contaminated aquifer, but not in others?

It is well known that the groundwater pore velocity in an aquifer varies spatially, so it is reasonable to ask if the heterogeneity of pore velocity has important consequences for heterogeneity of biological activity. There is already a body of literature that suggests this could be the case. For instance, when biofilms are used to treat wastewater in trickling filters, the performance is enhanced when the hydraulic loading is increased to an optimum range, in part because the increased hydraulic loading increases the rate of electron-acceptor mass transfer to the biofilms (Rittmann and McCarty 2001). Also, it is known that aquifer bacteria tend to proliferate in coarser-grained deposits (Fredrickson et al. 1989; Sinclair et al. 1990), perhaps due to better availability of nutrients in layers or facies with faster groundwater flow. Furthermore, Major et al. (2002) observed dechlorination rates in a forced-gradient field system up to 10 times greater than the rates observed in static laboratory microcosms, suggesting that faster flow and greater mass flux of electron donor to the relevant bacteria promotes faster growth and faster dechlorination. Taken together, all of these suggest that a faster flow velocity could lead to increased biological activity in contaminated aquifers.

Therefore, the objective of the present study is to determine the effect of pore velocity on the extent of dechlorination of *cis*-DCE to ethene. The main contribution of this paper is the demonstration that pore velocity can have an important effect on the extent of dechlorination and may also affect the microbial community composition. This influence of pore velocity may help to explain the spatial heterogeneity of biological activity and the incomplete dechlorination observed in some contaminated groundwater sites.

## Materials and methods

### Chemicals and culture

Liquid *cis*-1,2-dichloroethene (99%; Pfaltz and Bauer, Inc., Waterbury, Connecticut) was used to prepare stock solutions and standards, and vinyl chloride standard in methanol (2,000 μg/ml; Restek Corp., Bellefonte, Pennsylvania) was used to prepare analytical standards. We chose *cis*-DCE (as opposed to TCE or PCE) as our primary target because *cis*-DCE and vinyl chloride have often been observed to accumulate at contaminated sites, so it is important to improve our understanding of their biodegradation.

Commercially available KB-1® culture (SiREM, Guelph, Ontario, Canada) was used to inoculate

columns filled with glass beads (described subsequently). KB-1® was originally derived from a TCE-contaminated aquifer in southern Ontario, Canada (Duhamel and Edwards 2006; Duhamel et al. 2002, 2004). According to SiREM, the concentration of *Dehalococcoides* sp. in the KB-1® culture is approximately $1.6 \times 10^9$ cells/l. The KB-1® culture contains two different strains of *Dehalococcoides* (Duhamel et al. 2004; Waller et al. 2005) as well as acetogens including *Sporomusa*, methanogens, other dechlorinators including *Geobacter*, and putative fermenters including Bacteroidetes and *Syntrophus* (Duhamel and Edwards 2006, 2007; Sleep et al. 2006; Amos et al. 2007).

### Anaerobic medium

Anaerobic medium, spiked with *cis*-DCE, was prepared as a feed solution to support the KB-1® culture in the columns. The medium was prepared in a gas-tight system. A glass container was filled with basal medium, prepared as described by Yang and McCarty (1998), and contained 50 mg/l of yeast extract. Yeast extract (YE) is a complex mixture of organic substrates simulating what might be present in a contaminated aquifer (Aulenta et al. 2005), and thereby acts as both a nutritional supplement and an electron donor to support hydrogen production (Fennell et al. 1997). Therefore, YE should be an appropriate electron donor to simulate natural-attenuation conditions in the column experiments. Since YE is the soluble portion of autolysed yeast (containing a variety of cofactors, vitamins, and organic substrates), it is difficult to calculate *a priori* if 50 mg/l of YE is stoichiometrically sufficient to support complete dechlorination of 30 μM of *cis*-DCE to ethene. Furthermore, to the best of our knowledge, it has not been demonstrated in the peer-reviewed literature that *Dehalococcoides* strains in KB-1® can grow on yeast extract. Therefore, batch microcosm tests with KB-1® were conducted. Results from batch experiments (not shown) proved that 50 mg/l YE with no additional electron donor could support complete dechlorination of *cis*-DCE to ethene, including sufficient donor supply to account for losses to methanogenesis and/or other processes that might be occurring under the relevant experimental conditions.

The medium in the container was purged with a gas mixture of 80% $N_2$ and 20% $CO_2$ (Air Liquide, Houston, Texas) to attain anaerobic conditions and a stable pH of 6.9–7.2. The container was closed with a three-valve cap (Ace Glass, Inc., Vineland, New Jersey) to permit delivery of the anaerobic medium from the container while maintaining anaerobic conditions. Two valves of the cap were closed and the other valve was fitted with a septum to permit injection of *cis*-DCE to the anaerobic medium. Pure *cis*-DCE was injected with a gas-tight syringe through the septum to attain the *cis*-DCE concentration desired (30 μM) in the medium. Then, the three valves of the cap were fitted with Viton® tubing. One valve of the cap was connected to a foil gas bag (SKC, Inc., Eighty-Four, Pennsylvania). The gas bag was filled with $N_2/CO_2$ gas mixture to keep anaerobic conditions in the medium as the level in the glass container drained. The gas-tight system was allowed to equilibrate for at least 12 h prior to connection to the columns.

### Column construction and set-up

Eight glass columns of 5 cm internal diameter and 60 cm length were custom made (Ace Glass, Inc., Vineland, New Jersey). Each column was equipped with five sampling ports, equally spaced 10 cm apart (Fig. 1). To attain a good seal (and thereby maintain anaerobic conditions), each column was assembled with a Teflon adapter at the top and bottom. The adapter includes a stainless steel mesh (mesh #100, 0.0045-inch wire diameter, Purolator EFP, Shelby, North Carolina) to retain the filling material, and brass connectors to fit Viton® tubing at the inlet and outlet of the column. Sampling ports were fitted with Mininert® valves (Valco Instruments Company, Inc., Houston, Texas) to allow for sampling while preserving anaerobic conditions in the columns. All tubing and connectors were made of Viton®, Teflon®, brass, or stainless steel to minimize sorption of chlorinated ethenes to the material. The columns were filled with homogeneous soda lime glass beads of 0.8–1.0 mm diameter (Norstone, Inc., Wyncote, Pennsylvania) with a measured porosity of 0.33 in the columns. Glass beads were used as the porous medium in order to prevent any complicating factors that would be introduced by a more complex medium such as a natural soil or sediment; this enabled us to control the experiment to test only the effect of pore velocity.

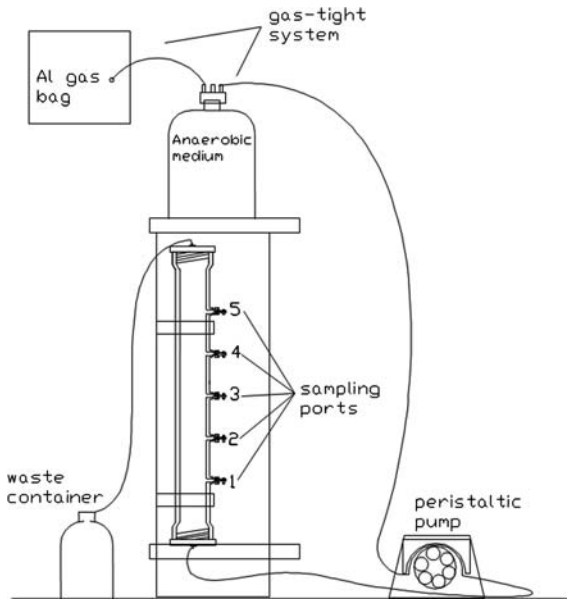


**Fig. 1** Schematic representation of the set-up of one column. An arrangement of eight similar columns was used during the experiments

Columns were equipped to be fed with the anaerobic medium in an upflow mode (Fig. 1). The sampling ports of each column were numbered following the direction of the flow, i.e., port number 1 refers to the port at the bottom of the column and port number 5 refers to port at the top of the column. The entire assembly was constructed and operated in a fume hood for safety. All experiments were conducted at room temperature (22.5 ± 1.5°C).

## Column preparation and inoculation

After being filled with the glass beads, the columns were conditioned by purging with $N_2$ gas for at least five pore volumes, but were not sterilized. Then, the columns were connected to the gas-tight system with anaerobic medium (Fig. 1) in pairs, i.e., two columns were connected to the same gas-tight system through the valves in the three-hole cap of the anaerobic medium. This was done in order to maintain the same *cis*-DCE influent concentration and anaerobic conditions in duplicate columns, i.e., duplicate columns were fed solution from the same bottle for consistency. The columns were then purged with five pore volumes of anaerobic medium that was prepared as described above, but without *cis*-DCE. All columns were wrapped in aluminum foil to avoid the growth of photosynthetic bacteria.

After conditioning, the columns (except for non-bioreactive control columns, described subsequently) were inoculated with KB-1® culture. The inoculation was performed within 24 h of receiving the culture from SiREM to minimize changes in the microbial community composition. One ml of the culture was injected through each sampling port. Then, static conditions were maintained for 24 h with no flow through the columns; this static period guaranteed the same inoculation conditions in all columns and also allowed the culture time to establish without being washed out. Experiments were then initiated by pumping the anaerobic medium and *cis*-DCE through the column at the desired flow rate.

## Flow velocities in columns

To obtain constant velocities in the range of realistic groundwater flows, the columns were connected to a peristaltic pump (Masterflex L/S® Brushless variable-speed digital drive equipped with L/S® 12-channel, 6-roller cartridge pump head; Cole-Parmer, Vernon Hills, Illinois) that allowed nine tubes to be connected at a time. The pump was run at a defined speed with three different sizes of tubing employed to obtain three different constant velocities. The pump was calibrated for the speed of 1.6 RPM, and tubing inner diameters were 0.89, 1.6, and 3.1 mm, with respective flow rates of $0.016 \pm 8 \times 10^{-6}$, $0.036 \pm 2 \times 10^{-4}$, and $0.23 \pm 2 \times 10^{-4}$ ml/min. Based on the column diameter (5 cm) and the bulk porosity of the beads (0.33), this corresponds to initial pore velocities of 0.036, 0.080, and 0.51 m/day, respectively. Velocities of 0.036–0.51 m/day are realistic for groundwater flow. A velocity of 0.51 m/day is probably close to the upper limit of what might be encountered under natural-gradient conditions, but would be relatively common under forced-gradient conditions such as might be encountered during engineered bioremediation (e.g., Scheutz et al. 2008). We note that pore velocities may have changed over the course of the experiment if a growth of biomass led to a decrease in porosity, but a post-experimental inspection of the columns did not suggest that a significant reduction in porosity occurred. Therefore we believe that the pore velocities remained close to constant throughout the duration of the experiment.

### Experimental conditions tested

Over the course of the experiment, the eight columns were used to test a total of 11 different sets of conditions, as summarized in Table 1. Six tests were used to measure the transport and biodegradation of *cis*-DCE under the three different pore velocities (each conducted in duplicate); these tests are called "bioreactive column" tests. Three column control tests were conducted (one at each pore velocity) with no KB-1® culture; these columns are called "non-bioreactive control columns" and they test for abiotic losses of *cis*-DCE. Finally, two control tests were conducted (at two different velocities) in which columns were inoculated with KB-1® but the feed solution did not contain any *cis*-DCE; these are called "bioreactive control columns" and they test for production of vinyl chloride or ethene from any mechanism other than degradation of *cis*-DCE. The three different pore velocities tested are referred to as low, medium, and high (0.036, 0.080, and 0.51 m/day, respectively).

### Sampling and chemical analysis

During the experiments, 1.0 ml liquid samples were taken for analysis of *cis*-DCE, VC, and ethene. All the columns were sampled by means of 1-ml gas-tight syringes introduced into the sampling ports via the Mininert® valves. Samples were taken at different time intervals depending on the flow rate tested. Samples for *cis*-DCE, VC, and ethene analysis were then prepared following a procedure modified from Major et al. (2002) by adding the 1-ml sample to a 10-ml vial containing 1.0 ml of acidified water (0.195 M boric acid, 0.048 M citric acid, and 0.0025 M tertiary sodium phosphate). After sample addition, the pH was approximately 2.6. The sample was shaken for 1 h and equilibrated statically for at least 12 h. Then, 1.0 ml of headspace was collected from the vial with a gas-tight syringe and injected manually into a gas chromatograph (GC). The headspace samples were analyzed for *cis*-DCE, VC, and ethene using GC with flame ionization detection (FID). For analysis of *cis*-DCE and VC, the GC/FID was calibrated using standards of known concentration. For analysis of ethene, the calibration curve was calculated from the vinyl chloride and *cis*-DCE calibration curves by correcting for differences in Henry's constants. Further details of the GC method and the calibration procedure are provided by Mendoza-Sanchez (2007).

### Column disassembly

At the end of the column experiments, the pore water from each column (389 ml per column) was collected by gravity. A container was placed at the inlet of the column (see Fig. 1) and the water was allowed to drain through the stainless steel mesh (see Column construction and set-up, above). During the drainage, the biofilms attached to the glass beads were essentially undisturbed. After the water was drained, visual inspection of the glass beads was conducted by opening the columns from the top and collecting the glass beads in 10-cm sections, from top to bottom. DNA was extracted from the collected pore water with the Fast-DNA kit (BIO 101 Systems, Qbiogene, Inc., Irvine, California) according to the manufacturer's instructions.

**Table 1** Experimental conditions tested (11 tests total)

| Columns | Pore velocity (m/day) | Inoculated with KB-1®? | Fed with *cis*-DCE? |
|---|---|---|---|
| 2 Bioreactive columns | Low (0.036) | Yes | Yes |
| 2 Bioreactive columns | Medium (0.08) | Yes | Yes |
| 2 Bioreactive columns | High (0.51) | Yes | Yes |
| 1 Bioreactive control | Medium (0.08) | Yes | No |
| 1 Bioreactive control | High (0.51) | Yes | No |
| 1 Non-bioreactive control | Low (0.036) | No | Yes |
| 1 Non-bioreactive control | Medium (0.08) | No | Yes |
| 1 Non-bioreactive control | High (0.51) | No | Yes |

### Microbiological analysis

The extracted DNA samples were frozen and sent to SiREM laboratories to be analyzed via denaturing gradient gel electrophoresis (DGGE) and quantitative polymerase chain reaction (qPCR). The DGGE analysis included cloned reference standards to allow rapid identification of each band that appeared. The samples and the reference standards were run in parallel and the identification of each species was based on co-migration in the gel.

The qPCR analysis was used to estimate the percentage of *Dehalococcoides* sp. in the microbial population at the end of the experiments; this percentage was calculated by dividing the number of *Dehalococcoides* 16S rRNA gene copies identified in qPCR by the total number of bacteria estimated from the mass of DNA extracted. According to SiREM, the primers used for DNA replication during PCR were 5′-ATTACCGCGGCTGCTGG-3′ and 5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3′, following Muyzer et al. (1993). It should be noted that different strains of *Dehalococcoides* with the same 16S rRNA gene sequence can have different dehalogenating abilities, and therefore targeting strain-specific reductive dehalogenase functional genes (e.g., *vcrA* and/or *bvcA*) is necessary to provide information about actual dechlorination activity (Ritalahti et al. 2006; Lookman et al. 2007; Behrens et al. 2008; Cupples 2008; Tas et al. 2009). However, use of 16S rRNA for quantifying *Dehalococcoides* is an established technique (e.g., Cupples et al. 2003; Lendvay et al. 2003; Smits et al. 2004; Buergmann et al. 2008; Cupples 2008) and was deemed appropriate for the analysis presented here.

## Results

### Control experiments

Breakthrough of *cis*-DCE was monitored in the non-bioreactive control columns (those lacking KB-1® culture). In all sample ports, *cis*-DCE broke through and persisted within ±11% of its injected concentration of 30 μM. In other words, *cis*-DCE did not experience any loss mechanism when KB-1® culture was not present. This demonstrates that any observed disappearance of *cis*-DCE in the bioreactive column tests must be due to biodegradation, not due to another mechanism such as sorption. Also, as expected, breakthrough of *cis*-DCE was observed faster in the high-velocity columns than in the low-velocity columns.

In the bioreactive control columns (those which were inoculated but did not receive *cis*-DCE in the feed solution), measured vinyl chloride concentrations were always below 0.6 μM and measured ethene concentrations were always below 0.9 μM. In other words, essentially no vinyl chloride or ethene was produced if no *cis*-DCE was fed to the column. This demonstrates that, as expected, any observed production of vinyl chloride and ethene in the bioreactive columns is due only to biodegradation of *cis*-DCE by the KB-1® culture, and cannot be reasonably explained by any other mechanism.

### Dechlorination results

Concentrations of *cis*-DCE, VC, and ethene from the six bioreactive columns were quantified over time and are shown in Fig. 2. Data points represent averages taken from two duplicate columns, e.g., data points in a low-flow-rate graph represent averages of the concentrations found in the two individual low-flow-rate columns. Error bars indicate the actual concentrations measured in the individual columns; the top of the error bar represents the concentration in one column, and the bottom of the error bar represents the concentration in the other column. For clarity, Figs. 3, 4, and 5 are larger versions of some of the graphs. Figure 3 shows data from sample port 4 of the high-flow-rate columns (which are not displayed in Fig. 2); Fig. 4 shows data from sample port 3 of the medium-flow-rate columns (same as Fig. 2e); Fig. 5 shows data from sample port 4 of the low-flow-rate columns (not displayed in Fig. 2).

DCE was efficiently converted to ethene in high-flow-rate columns (Figs. 2, 3). DCE initially broke through at close to its influent concentration of 30 μM, but over time the effluent DCE concentration decreased significantly. By about 20 days, *cis*-DCE was almost completely degraded in the first half of the column, as evidenced by data from sample ports 3, 4, and 5. This shows that dechlorination rates were faster at the end of the experiment than at the beginning, which probably indicates that key species

in the KB-1® culture adapted and/or grew over the course of the 6-week experiment. The vinyl chloride data indicate that vinyl chloride was produced near the influent of the column (see data from port 1) but that vinyl chloride was removed almost completely by the time the water exited the column (see data from ports 4 and 5). This is precisely what one would expect if *cis*-DCE is sequentially reduced to VC and then to ethene as the water travels through the column. Ethene concentrations in all sample ports rose over the first 20 days, again indicating that the biodegradation process became more effective during the first 20 days of the experiment. In sample port 4 (Fig. 3), the ethene concentration after day 20 remained stable at around 25 μM, indicating approximately 85% conversion of *cis*-DCE to ethene. Taken together, the concentration data clearly demonstrate that sequential reductive dechlorination occurred in the high-velocity columns, converting *cis*-DCE almost completely to ethene within the residence time of the columns.

In contrast, dechlorination failed over time in the low-velocity columns. These columns did show an initial degradation of *cis*-DCE (Figs. 2, 5). However, after about 10–20 days, the degradation rate slowed. In all sample ports, the *cis*-DCE concentration

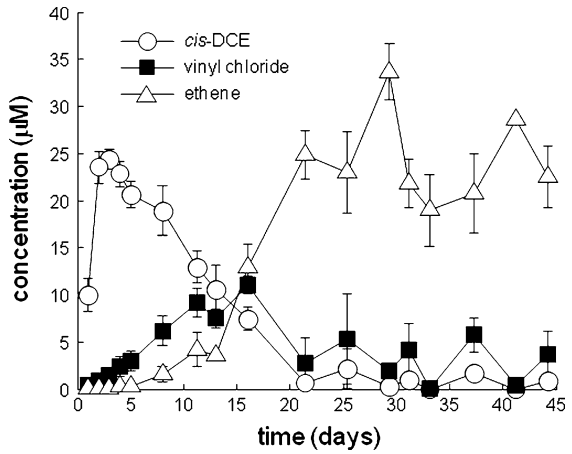


**Fig. 3** Concentration of *cis*-DCE, VC, and ethene in the high-flow-rate column as a function of time at sample port 4. *Error bars* represent results of duplicate column experiments

increased over time, and the ethene concentration decreased over time. Near the inlet of the columns (Fig. 2g), the concentration of *cis*-DCE was equal to the injected concentration (30 μM), and no VC or ethene production was observed after about 20 days. Near the effluent of the columns (Figs. 2a, 5), there was a little conversion of *cis*-DCE to VC and ethene even after 44 days, but the *cis*-DCE concentration

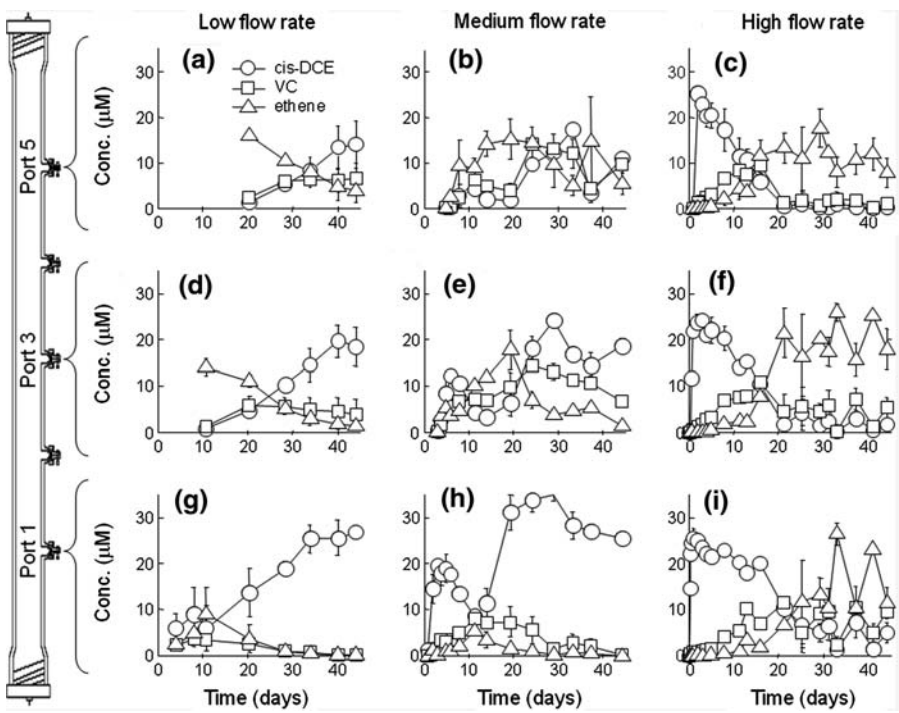


**Fig. 2** Concentrations of *cis*-DCE, VC, and ethene in the low-, medium-, and high-velocity columns as a function of both time and position. *Circles* indicate *cis*-DCE concentrations, *squares* indicate VC concentrations, and *triangles* indicate ethene concentrations. *Error bars* represent results of duplicate column experiments. The injected concentration of *cis*-DCE was 30 μM

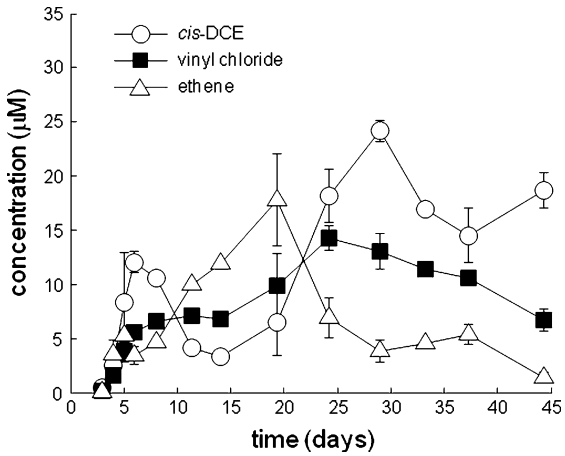


**Fig. 4** Concentration of *cis*-DCE, VC, and ethene in the medium-flow-rate column as a function of time at sample port 3. *Error bars* represent results of duplicate column experiments

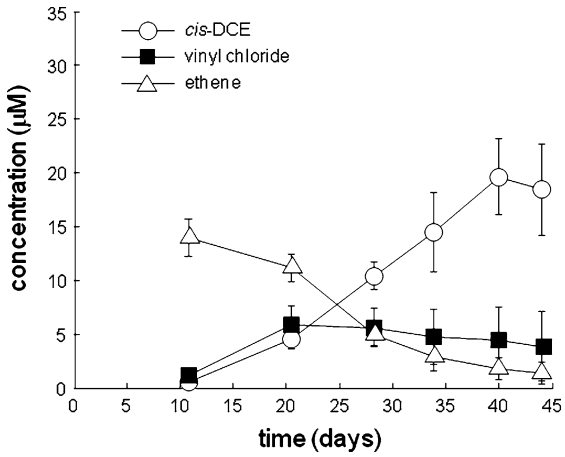


**Fig. 5** Concentration of *cis*-DCE, VC, and ethene in the low-flow-rate column as a function of time at sample port 4. *Error bars* represent results of duplicate column experiments

was increasing with time, suggesting that dechlorination might have ceased altogether had the columns run for longer than 6 weeks.

Similar results were observed for the medium-velocity columns (Figs. 2, 4). The *cis*-DCE was degraded initially, and the data even seem to indicate that the biodegradation process became more efficient over the first two weeks, with increasing ethene concentrations observed in most sampling ports. However, after 14–20 days, the degradation rate slowed; an increase in *cis*-DCE concentration was observed accompanied by a decrease in VC and ethene concentrations. The effect was observed in all sample ports, but was especially pronounced in sample port 1 (Fig. 2h). The dechlorination rate appears to have decreased over time, similar to what was observed in the low-velocity columns. Overall, it can be concluded that dechlorination failed over time in the low- and medium-velocity columns.

Another way to analyze the data is by examining concentration profiles rather than concentration histories. Figure 6 graphs concentrations of *cis*-DCE, VC, and ethene versus longitudinal position along the columns. The column influent is denoted 0 cm, the column effluent is denoted 60 cm, and sample ports are located every 10 cm, as shown in Fig. 1. The data shown in Fig. 6 were all collected on day 44 of the experiment, which was the last day of sample collection. Therefore, Fig. 6 shows the concentration profiles in the columns at the end of the experiment, after 6 weeks of operation. The results visible from Fig. 6 are very consistent with those discussed in the preceding paragraphs. In the high-flow-rate columns (top panel of Fig. 6), degradation of *cis*-DCE was apparent within the first 10 cm of the columns. Within 40–50 cm, *cis*-DCE was converted almost completely to ethene. Vinyl chloride was produced within the first 10 cm of the column, but was converted to ethene downgradient of the first sample port. In contrast, in both the medium- and low-flow-rate columns, there was little or no biodegradation of *cis*-DCE within the first 10–20 cm. By the last sample port (50 cm from column inlet), about half of the *cis*-DCE had been degraded, but it was converted mostly to vinyl chloride, with only low concentrations of ethene. These results again show that, in high-flow-rate columns, *cis*-DCE was converted almost completely to ethene within the residence time of the columns, but that biodegradation was not significant in the medium- and low-velocity columns at the end of the experiment.

These results were consistent and reproducible in the duplicate columns, i.e., the two high-velocity columns behaved similarly to each other, the two medium-velocity columns behaved similarly to each other, and the two low-velocity columns behaved similarly to each other. This is evidenced by the tight error-bar ranges on most data points. Furthermore, the dashed lines in Fig. 6 show that we were generally able to achieve good mass balances with our measured concentrations. The dashed lines in

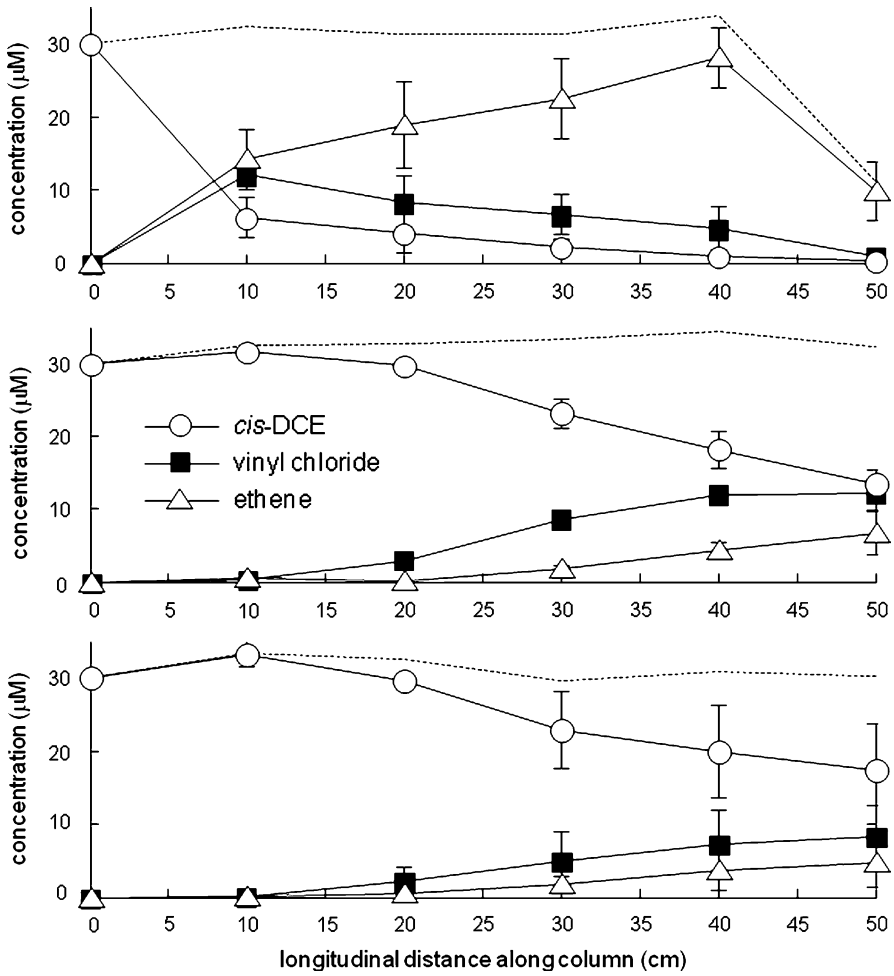


**Fig. 6** Concentration profiles in the columns on day 44 of the experiment. *Top panel* is for high-velocity columns, *middle panel* is for medium-velocity columns, and *bottom panel* is for low-velocity columns. *Dashed lines* indicate the sum of measured *cis*-DCE, VC, and ethene concentrations

Fig. 6 show the sums of measured *cis*-DCE, vinyl chloride, and ethene concentrations. In theory, the sum of these concentrations at any sample port should equal 30 μM, i.e., the injected concentration of *cis*-DCE. As seen in Fig. 6, the observed mass balances were very close to 30 μM (within 15%) in all sample ports except for sample port #5 of the high-velocity columns. Overall, the consistency between columns and the good mass balances at nearly all sample ports increase our confidence in the experimental data. Hence we are confident in the conclusion that it was velocity, not some other unidentified factor, that led to the different dechlorination behavior at the three velocities tested. The logical conclusion is that at the high velocity tested, dechlorination was supported by the specific conditions in the columns. However, at the low and medium velocities tested, the conditions were not favorable for dechlorination.

## Microbiological results

Columns were operated for about 6–7 weeks, after which they were disassembled. When the columns were disassembled, the glass beads at the bottom of the low-flow-rate columns presented a yellowish color. Dark spots, apparently from the culture, were found at the sampling ports where the culture was injected initially, but were not found elsewhere in the column. Similar observations were made in the medium-velocity columns, e.g., the glass beads at the bottom of the column had a similar yellowish color. The observed color pattern suggests that the culture grew near the column influent but did not spread through the column. However, the two high-velocity columns presented a dark color spread all around the column, suggesting that the culture grew and distributed throughout the column.

DNA was harvested from the columns and analyzed by DGGE and qPCR (both performed by SiREM). The purpose of these analyses was to test the hypothesis that the greater dechlorination activity in the high-velocity columns could be linked to greater concentrations of *Dehalococcoides* bacteria; this would imply that higher flow velocity was more favorable than lower flow velocity for the growth of *Dehalococcoides* sp. Overall, the results of the DGGE and qPCR analyses are consistent with this hypothesis, but are somewhat inconclusive, as described subsequently.

In the DGGE analysis, the intensity of the *Dehalococcoides* band was greater in DNA extracted from the high-velocity columns than in DNA extracted from the medium- or low-velocity columns. However, the difference in intensity between *Dehalococcoides* bands was relatively small, and therefore does not correlate well with the very different performance observed in terms of *cis*-DCE dechlorination. Also, using band intensity to infer differences in relative abundance is somewhat tenuous, because intensity is also related to the amount of DNA loaded onto the gel, which is difficult to control precisely. Thus, we believe the DGGE results are consistent with our hypothesis that higher flow velocity was more favorable than lower flow velocity for *Dehalococcoides*, but the results are not conclusive.

Similar conclusions can be drawn from the qPCR results. Estimates of the *Dehalococcoides* concentrations from the qPCR analysis were as follows: $9 \times 10^7$ cells/l in pore water from the high-velocity columns; $1 \times 10^7$ cells/l in pore water from the medium-velocity columns; and $2 \times 10^7$ cells/l in pore water from the low-velocity columns. The relative abundance of *Dehalococcoides* as a percentage of the entire microbial community was 2–5, 0.3–0.8, and 0.5–2% for high, medium, and low velocity, respectively. However, we do not have a reliable estimate of the uncertainty in the cell concentrations determined by qPCR; therefore we cannot say that the reported cell concentrations ($9 \times 10^7$, $1 \times 10^7$, and $2 \times 10^7$ cells/l) are significantly different at a 95% confidence level. Hence, overall, the qPCR results are consistent with the hypothesis that *Dehalococcoides* was probably present in higher quantities in the high-velocity columns than in the medium- or low-velocity columns, but the results are again inconclusive.

Finally, it must be noted that we analyzed only DNA harvested from pore water, not from attached biofilms. This is a relatively common practice when analyzing for *Dehalococcoides* in groundwater at field sites (e.g., Lendvay et al. 2003; Lu et al. 2006; Lookman et al. 2007). However, it is not yet clear which is more relevant in terms of dechlorination activity, attached bacteria or pelagic ("free") bacteria. Although it is generally believed that most active biomass in contaminated aquifers resides in fixed biofilms (e.g., Rittmann 1993), Schaefer et al. (2009) found that aqueous concentrations of *Dehalococcoides* sp. are better predictors of dechlorination activity than are attached soil-phase concentrations. Even if dechlorinating activity occurs primarily in biofilms, we speculate that analysis of DNA from the pore water is indicative of the biofilm community composition, and hence relevant to dechlorination activity.

## Discussion

The dechlorination results show clearly that dechlorination was sustained in the high-velocity columns but not at lower velocities. This observed dechlorination behavior is consistent with three preliminary findings from the microbiological analysis: (1) the inoculated culture spread throughout the high-velocity columns, but was found only near the inoculation points and the column inlet in medium- and low-velocity columns; (2) the high-velocity columns seemed to exhibit greater microbial diversity than the medium- and low-velocity columns, as determined by the DGGE analysis; and (3) the high-velocity columns may have had *Dehalococcoides* organisms present in greater abundance than the medium- and low-velocity columns. A reasonable hypothesis is that dechlorination failed in the medium- and low-velocity columns because some organism(s) necessary for sustained dechlorination could not be supported except at high velocity. The key organism could be *Dehalococcoides*, or some other organism that works in conjunction with *Dehalococcoides*, such as fermenters that provide acetate or hydrogen to the dechlorinators, or some other species that provide trace nutrients (Duhamel and Edwards 2006).

This hypothesis naturally leads to the question of why high-velocity conditions might be preferable for

the key organism(s). Probably the simplest explanation is that flow rate controls microbial community composition by controlling the flux of electron donor through the column. Because the columns were operated at fixed influent concentrations of 50 mg/l yeast extract (the electron donor), the flux of electron donor into the columns is directly proportional to the velocity. Hence, the failure of low- and medium-velocity columns to sustain dechlorination could be because the flux of electron donor was inadequate for that process. This hypothesis is supported by the finding that in low- and medium-velocity columns, the bacterial culture grew close to the column inlet, but not throughout the entire column. However, based on this hypothesis, we might expect to see dechlorination activity close to the column inlet (where donor is present) but a lack of activity downgradient (after the donor has been completely consumed). This is not what is observed in Fig. 6; instead, in both low- and medium-velocity columns, we see little dechlorination in the first 10 cm, but partial conversion of *cis*-DCE to VC and ethene further downgradient. Hence, it is still somewhat unclear if donor limitation is the sole explanation for the observed effect of velocity on dechlorination. Other possible explanations could be that velocity influences the microbial community composition by affecting the rate of mass transfer of chemicals from the bulk groundwater to attached biofilms, and/or by selecting for bacteria with certain adherence characteristics under different shear conditions.

These findings have interesting implications for contaminated field sites. Under natural-gradient conditions, we would expect to see dechlorination activity distributed heterogeneously; this is, not surprisingly, observed in practice (Fennell et al. 2001; Sandrin et al. 2004; Allen-King et al. 2006; Hoelen et al. 2006). However, to the best of our knowledge, it is not yet apparent if spatially-variable dechlorination activity is correlated with pore velocity under natural attenuation conditions. This lack of information is perhaps because of the difficulty associated with trying to measure local degradation rates in the field, although the use of strain-specific genes or other biomarkers could perhaps be used to yield indirect evidence of whether such a correlation exists. In cases where natural attenuation is inadequate, chemical augmentation and/or bioaugmentation (the introduction of bacterial species not present naturally) are often used to stimulate biodegradation (e.g., Ellis et al. 2000; Major et al. 2002; Lendvay et al. 2003; Hoelen et al. 2006; Scheutz et al. 2008). This is typically accomplished by injection into wells, which also serve to pump the groundwater under a forced gradient, thereby increasing pore velocity. Based on the results of the study presented here, it is interesting to speculate whether an increased pore velocity (e.g., induced by pumping via wells) would, in some cases, be able to stimulate dechlorination activity without an accompanying chemical or biological augmentation. To answer that question, we will need to more fully investigate the underlying cause of why, in these experiments, a higher pore velocity proved so essential to sustained dechlorination.

Finally, this study underscores the importance of further understanding the interaction of physical and biological factors at the microscopic scale (pore scale or biofilm scale). Physical factors such as pore velocity can, as demonstrated here, have a pronounced effect on biological processes. Biodegradation as a technology for site remediation is not likely to be optimized until our collective understanding of such effects is more complete.

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