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Complete Biological Reductive Transformation of Tetrachloroethene to Ethane

WIL P. DE BRUIN,[1]* MICHIEL J. J. KOTTERMAN,[1] MAARTEN A. POSTHUMUS,[2] GOSSE SCHRAA,[1] AND ALEXANDER J. B. ZEHNDER[1]

Department of Microbiology[1] and Department of Organic Chemistry,[2] Wageningen Agricultural University, Hesselink van Suchtelenweg 4, 6703 CT Wageningen, The Netherlands

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Reductive dechlorination of tetrachloroethene (perchloroethylene; PCE) was observed at 20°C in a fixed-bed column, filled with a mixture (3:1) of anaerobic sediment from the Rhine river and anaerobic granular sludge. In the presence of lactate (1 mM) as an electron donor, 9 μM PCE was dechlorinated to ethene. Ethene was further reduced to ethane. Mass balances demonstrated an almost complete conversion (95 to 98%), with no chlorinated compounds remaining (<0.5 μg/liter). When the temperature was lowered to 10°C, an adaptation of 2 weeks was necessary to obtain the same performance as at 20°C. Dechlorination by column material to ethene, followed by a slow ethane production, could also be achieved in batch cultures. Ethane was not formed in the presence of bromoethanesulfonic acid, an inhibitor of methanogenesis. The high dechlorination rate (3.7 $\mu mol \cdot l^{-1} \cdot h^{-1}$), even at low temperatures and considerable PCE concentrations, together with the absence of chlorinated end products, makes reductive dechlorination an attractive method for removal of PCE in bioremediation processes.

The potential of microorganisms to transform halogenated compounds into innocuous products is a major advantage of biological remediation techniques in comparison with physicochemical techniques for the cleanup of contaminated soil and groundwater. Tetrachloroethene (perchloroethylene; PCE) is widely employed as a dry-cleaning and degreasing solvent and is a pollutant of major concern. It is commonly found as a groundwater contaminant. PCE is not degraded by microorganisms under aerobic conditions, but several investigators have observed biotransformation of PCE under strict anaerobic conditions (2–9, 12, 14, 15). The transformation proceeds via sequential reductive dechlorination steps to replace the chlorine atoms with hydrogen atoms. The electrons for the reductive dechlorination are derived from the oxidation of organic compounds. Trichloroethene (TCE), dichloroethene (DCE) isomers, vinylchloride (VC), and ethene are often formed as intermediates and/or end products. For example, a partial mineralization of PCE (24% mineralization) was observed in a continuous-flow, fixed-film column (15), and it was hypothesized that some of the VC produced was transformed via an alcohol and an aldehyde to CO_2. Simultaneous conversion of PCE to VC and ethene was demonstrated in a mixed bacterial culture (9). Initially, the dechlorination of VC to ethene was slow and only partial. In a follow-up study (5), this mixed bacterial culture was developed into enrichment cultures capable of dechlorinating high concentrations (330 μM) of PCE to ethene and small amounts of VC.

In this study, we provide evidence for the complete sequential reductive dechlorination of PCE to ethene and ethane in a continuous-flow, fixed-bed column at rates which are promising for bioremediation applications.

MATERIALS AND METHODS

Chemicals. PCE (99% pure) and TCE (99% pure) were purchased from E. Merck, Darmstadt, Germany. 1,1-DCE (99% pure), *cis*-1,2-DCE (97% pure), and *trans*-1,2-DCE (98% pure) were obtained from Janssen Chimica, Beerse, Belgium. Ethene (99.9% pure), ethane (99.5% pure), and VC (99.95% pure) were obtained as gases from Hoek Loos, Amsterdam, The Netherlands.

Column experiments. The experiments were performed with a fixed-bed column (Fig. 1), which was constructed of hard polyvinyl chloride (25-cm length; 5.5-cm inside diameter). It was equipped with stainless steel capillaries (2.0 mm in diameter), extending into the center of the column at various heights (0, 0.5, 1, 2.5, 5, 10, 15, 20, and 25 cm above the influent port). They served as sampling ports for concentration profiles in the column. The column was wet packed with a mixture (3:1) of anaerobic sediment from the Rhine river (near Wageningen, The Netherlands) and ground anaerobic granular sludge from an upflow anaerobic-sludge blanket reactor that was used for the treatment of sugar beet wastewater (CSM Breda, The Netherlands). The column was continuously percolated in an upflow mode under saturated conditions with an anaerobic mineral medium containing (per liter of demineralized water) 27 mg of NH_4Cl, 102 mg of $MgCl_2 \cdot 6H_2O$, 12 mg of K_2HPO_4, 222 mg of $CaCl_2$, 215 mg of $NaHCO_3$, 7 mg of Na_2SO_4, and 0.15 ml of a trace element solution (17). The medium was depleted of oxygen by boiling followed by cooling under an N_2/CO_2 purge (99.5/0.5%). An excess of granulated marble served as carbonate buffer in combination with CO_2 in the purge gas. The medium was pumped into the column by a peristaltic pump that was equipped with silicone tubing. Traces of oxygen which may have diffused into the silicone pump tubing were removed by replacement with nitrogen in a gas exchange chamber (18). Reducing conditions were maintained by the presence of Na_2S (final concentration, 10 mg/liter). Lactate was used as an electron donor (final concentration, 1 mM). A stock solution of PCE was added with a syringe pump, together with the Na_2S and lactate (as sodium salt). Before entering the column, the stock solution was mixed with mineral medium in a 114-ml mixing cham-

* Corresponding author.

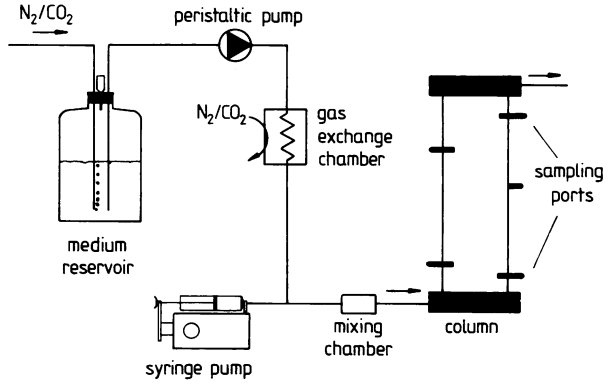

FIG. 1. Schematic diagram of fixed-bed column system.

ber. Stock solutions were prepared by making an appropriate dilution of PCE-saturated water.

The column was routinely percolated at a flow rate of 15 ml/h (column detention time of 24 h), in a 20°C environmental chamber in the dark. The initial PCE influent concentration was 0.6 μM.

Effect of PCE concentration, flow rate, and temperature. Several factors which can influence the dechlorination process were tested. The PCE concentration was gradually increased from 0.6 to 9 μM. Upon process stabilization, the flow rate was increased fourfold to 60 ml/h (detention time, 6 h). The effect of temperature on the process was tested by operating the column both at 20 and at 10°C.

Reductive dechlorination potential of anaerobic sludge. The effect of the addition of ground anaerobic granular sludge to Rhine river sediment as inoculum was tested by wet packing a column, as described before, with a mixture (1:3) of the sludge and bare sand (particle size, 0.2 to 0.6 mm). Bare sand served as a matrix in the column to prevent clogging. The column was operated for 60 days at a PCE influent concentration of 0.5 μM, a lactate concentration of 1 mM, and a detention time of 24 h.

Batch experiments. The degradation of PCE and intermediates was also studied in two batch experiments. Experiments were conducted with 114-ml serum bottles that were sealed with viton stoppers and filled with 20 ml of mineral medium no. 3 (10). The gas phase was N_2/CO_2 (80/20%). Suspensions (1 ml each) of a mixed sample from the first 5 cm of the column in which complete conversion of PCE to ethane took place served as inoculum. In the first experiment, lactate (1 mM) served as electron donor. A total of 260 nmol of PCE per bottle was added, which corresponds to an initial aqueous concentration of 3.4 μM. The headspace was analyzed for PCE and its conversion products. In the second experiment, several inocula (1-ml suspensions) were tested for their ability to reduce ethene to ethane. Besides Rhine river sediment plus granular sludge from the column, the following materials were tested: fresh Rhine river sediment, fresh ground anaerobic granular sludge (similar to that in the column), fresh Rhine river sediment plus fresh ground anaerobic granular sludge, and anaerobic sediments from two Dutch harbors (Rotterdam and Zierikzee). Two concentrations of ethene (0.15 and 8.32 μmol per bottle) were tested. The experiments were done with hydrogen (H_2/CO_2, 80/20%) or lactate (1 mM) as electron donor and in the presence and absence of 5 mM 2-bromoethanesulfonic acid (BrES), an inhibitor of methanogenesis.

Analytical methods. Amounts of PCE and its conversion products were routinely determined in the column by taking water samples with a gas-tight glass syringe connected to a sample port. The syringe was allowed to be filled by the flow pressure of the pumps in the system. PCE and TCE were analyzed by hexane extraction of the water samples, followed by split-flow injection into a GC 436 gas chromatograph (United Technologies, Delft, The Netherlands) equipped with an electron capture detector and a capillary column (25 m by 0.32 mm [inside diameter]; Sil 5CB [1.19 μm]; Chrompack, Middelburg, The Netherlands). Amounts of PCE, TCE, and *cis*-1,2-DCE in batch experiments were determined by direct injection of headspace samples into this gas chromatograph. *cis*-1,2-DCE in the aqueous column samples was analyzed by purge-and-trap injection into a GC 438 gas chromatograph (Chrompack), which was equipped with a flame ionization detector and a capillary column (25 m by 0.32 mm [inside diameter]; Sil 5CB [1.22 μm]; Chrompack). VC, ethene, and ethane in the column were analyzed by injection of an aqueous column sample into a rubber-capped test tube. The headspace was analyzed with a GC 417 gas chromatograph (Packard) equipped with a flame ionization detector and a packed 1.5-m (inside diameter, 0.3 cm) column (Porapack R; Chrompack). Amounts of VC, ethene, and ethane in batch experiments were determined by direct injection of headspace samples into this gas chromatograph. Ethene and ethane were identified and confirmed with a gas chromatograph-mass spectrometer (VG MM7070F), which was equipped with a capillary column (25m by 0.32 mm; Poraplot Q [10 μm]; Chrompack). The gas chromatograph oven was kept isothermal at 60°C, and the spectra were obtained with 70 eV of electron impact ionization. Ethane was positively identified as the peak corresponding to the molecular ion peak with the mass 30.0469 atomic mass units [amu] (calculated C_2H_6 mass, 30.0469 amu).

Mass balance. Fluctuations in the PCE influent concentration, which were caused by stepwise pumping of the syringe pump, hindered an accurate comparison of influent and effluent concentrations. Mass balances were therefore determined over 24-h periods. PCE in the influent was measured four times in 24 h, while the effluent was sampled during 24 h in a system which limited the loss of volatile products. It was collected in an empty 3-liter glass bottle, which was connected to a second 2-liter glass bottle completely filled with ethane-free water. Both bottles had rubber-capped gas-sampling points. A constant headspace volume and a nearly constant headspace pressure (atmospheric pressure minus height of the water column in the second bottle) were created by an outlet at the bottom of the second bottle. Headspace pressure varied from 0.980 atm at the beginning to 0.995 atm at the end of the 24-h sampling periods. The amount of ethane was determined as the sum of the amounts present in the headspace of the two bottles and the amounts dissolved in the effluent in the first bottle and in the water in the second bottle. The loss of ethane via the displaced water from the second bottle was less than 1%.

RESULTS

After 2 weeks of operation of the column, PCE was no longer detected in the effluent and instead the less-chlorinated compounds TCE, *cis*-1,2-DCE, and VC appeared. In time, the PCE influent concentration was increased to around 9 μM. At day 105, concentration profiles in the column were determined (Fig. 2). These profiles indicate that PCE was dechlorinated stepwise via TCE, *cis*-1,2-DCE, and VC to ethene, and that ethene was reduced to ethane. At

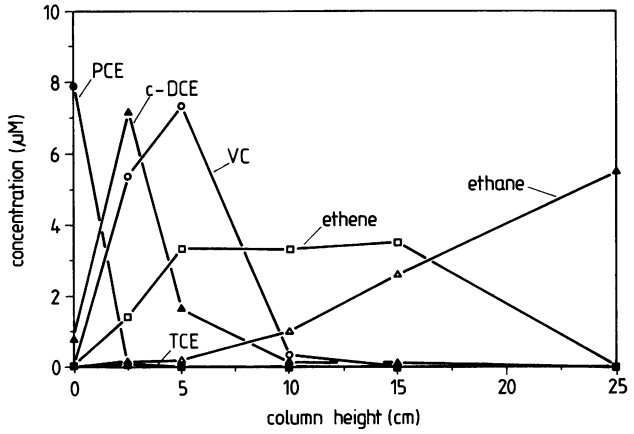

FIG. 2. Concentration profiles of PCE, TCE, *cis*-1,2-DCE (c-DCE), VC, ethene, and ethane in a fixed-bed column after 105 days of operation at 20°C. Liquid detention time was 24 h.

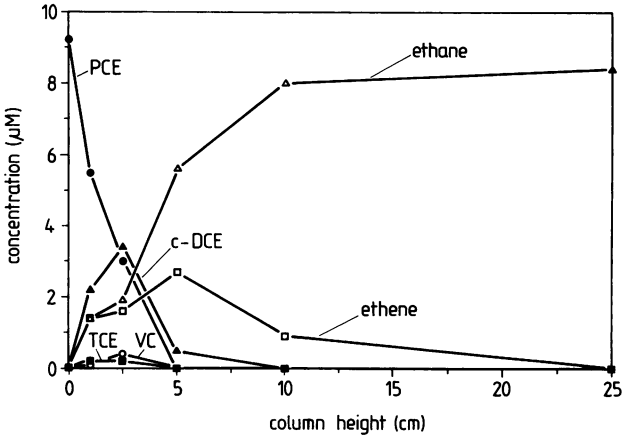

FIG. 3. Concentration profiles of PCE, TCE, *cis*-1,2-DCE (c-DCE), VC, ethene, and ethane in a fixed-bed column 14 days after a decrease in temperature from 20 to 10°C. Liquid detention time was 6 h.

days 185 and 210, the flow rate was increased. This resulted in detention times in the column of 12 and 6 h, respectively. Despite these increases in organic load in the system, the flow distance needed for the dechlorination of PCE decreased from the entire column at the start to the first 5 cm at day 240. Between days 250 and 300, during which the column was operated under a steady-state condition and only ethane was detected in the effluent, two mass balance experiments were performed. Measurements were taken during a period of 24 h. The total amount of PCE fed to the column was calculated by multiplying the average PCE influent concentration with the influent volume. Ethane was measured as the total amount produced during this 24-h period. A total of 95 to 98% of the PCE could be recovered as ethane (Table 1).

The effect of temperature was examined by transferring the column from 20 to 10°C. The decrease in temperature had only a temporary effect on the kinetics of dechlorination. Upon transfer, traces of PCE (up to 2% of the influent concentration) could be measured in the effluent, but within 2 weeks only ethene and ethane could be detected past the first 10 cm of the column (Fig. 3). Within 1 month of operation at 10°C, the concentration profiles were the same as those before the temperature drop. Process performance at 10°C remained constant for more than 600 days.

In the column with only ground anaerobic granular sludge as inoculum, PCE was initially converted to both TCE and *cis*-1,2-DCE. After 2 months of operation, *cis*-1,2-DCE was the only end product. VC, ethene, and ethane were never detected. In an earlier column study with only Rhine river sediment, mainly *cis*-1,2-DCE and small quantities of VC were observed as intermediates (2).

Complete dechlorination of PCE by material from the column could also be achieved in batch cultures. Within an extensive test period of 16 days, only ethene was formed (Fig. 4). Measurements of these cultures afterwards (at day 44) revealed that a large part of the ethene was eventually transformed to ethane (Fig. 4).

A small production of 0.05 μmol of ethene from 0.15 μmol of ethane and 0.6 μmol of ethene from 8.3 μmol of ethane in 25 days was observed in batches with material from the column. Both hydrogen and lactate could serve as electron donor, while BrES prevented the transformation. The ability to reduce ethene to ethane was restricted to material from the column which was inoculated with the Rhine river sediment and granular sludge from the column. No ethane production in fresh Rhine river sediment, fresh granular sludge, or a combination of both or in harbor sediments was witnessed.

TABLE 1. Mass balance determined over 24 h for steady-state conversion of PCE to ethane in a fixed-bed column

Total PCE added (μmol) (±SD)[a]	Total ethane produced (μmol) (±SD)[b]	Conversion (%)
9.70 ± 0.83	9.31 ± 0.46	98
10.32 ± 0.93	9.83 ± 0.49	95

[a] Calculated as the product of the total influent volume and four independent concentration measurements in 24 h. Deviations are given as total analytical error (5%) of each batch.

[b] Calculated as the product of concentration and total volume in headspace and effluent after 24 h. Deviations are given as total analytical error (5%) of each batch.

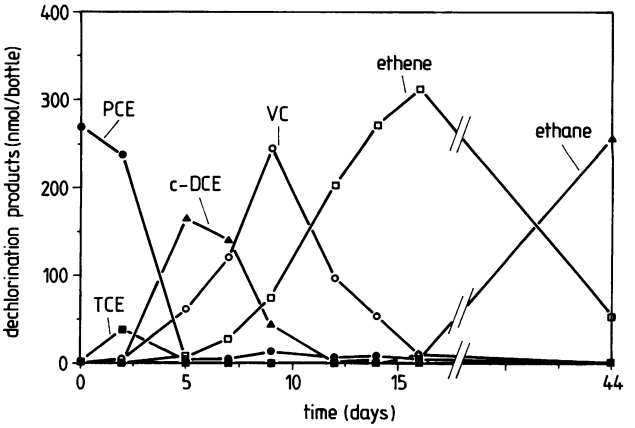

FIG. 4. Dechlorination of PCE and formation of intermediates in a batch culture inoculated with column material. A total of 260 nmol of PCE per bottle was added, which corresponds to an initial aqueous concentration of 3.4 μM. c-DCE, *cis*-1,2-DCE.

DISCUSSION

A complete reductive dechlorination of PCE, via TCE, *cis*-1,2-DCE, and VC, to ethene, followed by a reduction of ethene to ethane, has been demonstrated to occur readily in a continuous-flow, fixed-bed column that is filled with Rhine river sediment and ground granular sludge. This is the first report of such a complete reaction sequence. Most of the researchers who have reported on the anaerobic reductive dechlorination of PCE have found only a partial dechlorination. Fathepure and coworkers (6–8) demonstrated that pure cultures of two methanogenic bacteria (*Methanosarcina* strains) and a chlorobenzoate-dechlorinating organism (strain DCB-1) were able to convert PCE to TCE slowly. A further degradation of TCE to an unknown compound(s) was possible when strain DCB-1 was present in the methanogenic consortium that was able to degrade 3-chlorobenzoate. TCE and *cis*-1,2-DCE were reported to be the products of the reductive dechlorination of PCE by sulfate-reducing enrichment cultures (1). *cis*-1,2-DCE was also the product in an anaerobic enrichment culture in which benzoate acted as electron donor and PCE as electron acceptor (14). A dechlorination beyond *cis*-1,2-DCE was reported by Vogel and McCarty (15), who observed the formation of VC in a continuous-flow, fixed-film methanogenic column. Freedman and Gossett (9) observed not only the dechlorination of PCE to VC in methanogenic enrichment cultures but also a partial dechlorination of VC to ethene. DiStefano et al. (5) were able to develop these cultures into enrichment cultures which were able to convert PCE to ethene and small amounts of VC at high rates, with methanol as electron donor.

The finding in our study that PCE was stoichiometrically converted to ethene and eventually to ethane is of great importance. The presence of a suitable electron donor is necessary to prevent the accumulation of chlorinated intermediates. In several studies, dechlorination of PCE was stimulated by electron donors like lactate under sulfate-reducing conditions (1), and by acetate (9, 15), benzoate (14), glucose, formate, and methanol (9) under methanogenic conditions, but a complete dechlorination was not achieved. Because all studies were carried out in mixed cultures, not all reducing equivalents may have been available for reductive dechlorination. In our study, 1 mM lactate together with about 8 μM PCE was used. This is 150 times the minimum reducing equivalents necessary for a complete reduction of PCE to ethane and at least 10 times more than the amount supplied in other studies (1, 9, 14, 15). At this moment, it is not clear whether a smaller amount of lactate or another electron donor will also lead to a complete dechlorination.

It is not known which organisms are responsible for the complete dechlorination of PCE. In an earlier column study with only Rhine river sediment, mainly *cis*-1,2-DCE and small quantities of VC were observed as intermediates, but the dechlorination was far from complete (2). A column experiment with just anaerobic granular sludge with bare sand as a matrix resulted in a conversion of PCE to *cis*-1,2-DCE. Complete dechlorination could be achieved only by a combination of Rhine river sediment and anaerobic granular sludge. Apparently, organisms from both inoculum sources are necessary for the full dechlorination process. On the basis of the data presented here and a study by Fathepure et al. (8), who observed a dechlorination of PCE to TCE by strain DCB-1 in pure culture and a further dechlorination in a defined consortium, it may be hypothesized that several different microorganisms are needed to achieve complete dechlorination of PCE. This idea is supported by two preliminary studies in our laboratory. Two different enrichment cultures were obtained from the column in which PCE was completely converted to ethane. The first enrichment culture dechlorinates PCE and TCE stoichiometrically to *cis*-1,2-DCE as end product, while the second one dechlorinates *cis*-1,2-DCE and VC to ethene.

The reduction of ethene to ethane is a new phenomenon. Although it has been suggested elsewhere that ethene can be reduced in anaerobic sediments via biological reactions (16), evidence was lacking, and several authors report ethene to be persistent under anaerobic conditions (11, 13). Our observation of ethane production was restricted to the column and to batch experiments with material from this column, while several other sediments did not produce any ethane. Interestingly, BrES, an analog of methyl coenzyme M and a specific inhibitor of the last step in the formation of methane by methanogenic bacteria, also inhibited ethane formation. The role of methanogenic bacteria in the reduction of ethene remains unclear, because ethene is considered to be an inhibitor of methanogenesis (13).

A high dechlorination rate at groundwater temperature is a prerequisite for the exploitation of reductive dechlorination as a bioremediation process. The effects of a temperature reduction from 20 to 10°C on the process performance were small. Although it was expected that the dechlorination rate would drop at this lower temperature, only very small amounts of PCE were found in the effluent. Within a few weeks, the microbial population was capable of a 100% dechlorination of PCE in the first 10 cm of the fixed-bed column, at an influent concentration of 9 μM and a liquid detention time of 2.4 h in this 10 cm. Both at 20 and 10°C, considerable quantities of PCE can be completely dechlorinated at high rates (elimination capacity, 3.7 μmol/liter/h).

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