Environ. Sci. Technol. 1994, 28, 1124–1128

Comparison of Reductive Dechlorination of Hexachloro-1,3-butadiene in Rhine Sediment and Model Systems with Hydroxocobalamin

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Transformations of hexachloro-1,3-butadiene were studied in columns packed with Rhine River sediment and in batch incubations containing titanium(III) citrate and hydroxocobalamin. Columns were operated under various redox conditions. Transformation was observed in a methanogenic column at influent concentrations of 4 and 400 nmol/L but not in columns where oxygen or nitrate were fed as terminal electron acceptors. Hexachloro-1,3-butadiene was reductively dechlorinated to (*E,E*)-1,2,3,4-tetrachlorobutadiene (>90%) and traces of a trichloro-1,3-butadiene isomer (<5%). (*E*)-1,1,2,3,4-Pentachloro-1,3-butadiene was detected as intermediary product. Reductive dechlorination in the column was ascribed to the activity of anaerobic microorganisms. In the batch experiments with titanium(III) citrate and hydroxocobalamin, hexachloro-1,3-butadiene (5 and 500 μmol/L) was transformed to an isomer of pentachloro-1,3-butadiene and two compounds with molar masses of 154 and 52, tentatively identified as trichloro-1-buten-3-yn, and 1-buten-3-yn respectively.

Introduction

Several reports exist in which hexachloro-1,3-butadiene, which has been shown to be toxic to rats and humans (*1*), is described as a pollutant present in sediment samples in Western Europe and North America (*2–4*). Hexachloro-1,3-butadiene has been used as a heat-transfer fluid in transformators, as an intermediate to produce lubricants, and as an intermediate in the manufacture of rubber compounds (*1, 5*), while it is also formed as a byproduct during the production of vinyl chloride, trichloroethene, and tetrachloroethene. The latter may be the cause for the detection of high concentrations in soil near tetrachloroethene-producing facilities (*3, 6*). Hexachloro-1,3-butadiene has been applied as a fungicide in the former Soviet Union (*7, 8*).

No information has been reported thus far about transformations of hexachloro-1,3-butadiene in soil or groundwater. The saturation of the molecule with chlorines may limit aerobic transformations but may be suitable for anaerobic reductive dechlorination reactions. Results of the transformation of hexachloro-1,3-butadiene in columns packed with Rhine River sediment and operated under various redox conditions are presented in this study. The possible reduction of hexachloro-1,3-butadiene in model systems with hydroxocobalamin (vitamin B_{12a}) and titanium(III) citrate was also investigated because transition metal coenzymes are known to catalyze such reactions and may provide a reference for comparison of dechlorination in environmental samples (*9*).

Materials and Methods

Chemicals. Hexachloro-1,3-butadiene (98% pure) was purchased from E. Merck (Amsterdam, The Netherlands). 1,1,4,4-Tetrachloro-1,3-butadiene (96% pure) was obtained from V. I. Potkin and R. V. Kaberdin of the Institute of Physical Organic Chemistry of the Byelorussian Academy of Sciences; deuterated chloroform (99.8% D) was obtained from Janssen Chimica (Belgium); and hydroxocobalamin was obtained from Fluka (Oud-Beijerland, The Netherlands). Titanium(III) citrate (pH 9, 100 mM) was prepared from $TiCl_3$ and sodium citrate as described previously (*10, 11*).

Column Experiments. Columns were constructed of hard PVC (25-cm length, 5.5-cm i.d.) and were equipped with stainless steel capillaries (2.0 mm in diameter) extending into the center of the column at various heights (*12*). They served as sampling ports for concentration profile measurements. Columns were wet packed with sediment from the Rhine River near Wageningen, The Netherlands, and were continuously percolated in an upflow mode with autoclaved mineral medium prepared with highly purified Milli-Q water (Millipore, USA) closely resembling the mineral composition of Rhine water (*12*). The flow rate was 1 cm/h, which resulted in a residence time of 1 day. The medium contained NH_4Cl (27 mg/L), $MgCl_2 \cdot 6H_2O$ (102 mg/L), K_2HPO_4 (12 mg/L), $CaCl_2$ (222 mg/L), $NaHCO_3$ (215 mg/L), Na_2SO_4 (7 mg/L), and 0.15 mL/L of a trace element solution (*13*). The synthetic medium was amended with an excess of granulated marble ($CaCO_3$) and continuously aerated. The equilibrium between $CaCO_3$, HCO_3^-, and CO_2 resulted in a pH of 8.3 ± 0.1. In the anaerobic experiments, the originally aerated medium was depleted from oxygen by its continuous replacement with nitrogen gas amended with 0.5% CO_2 in a gas-exchange chamber (*14*). Reducing conditions were maintained by the addition of Na_2S (10 mg/L final concentration) via the solution of chlorinated hydrocarbons (*15*). One column was operated without sulfide as a reducing agent, but with nitrate (final concentration 35 mg/L) added as an electron acceptor. The columns were operated aseptically up to the influent port where a bacterial filter (cellulose nitrate, 0.2 μm, Sartorius GmbH, Germany) prevented back growth of microorganisms from the sediment into the feeding lines. The medium was pumped into the columns by a peristaltic pump (Watson Marlow Ltd., U. K.) at a flow rate of 12.0 mL/h. A solution of hexachloro-1,3-butadiene was added continuously with a syringe pump (Perfusor VI; B. Braun Medical B. V., Germany) at a flow rate of 0.6 mL/h. This solution was prepared by adding 100 μL of a stock solution in methanol

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to 70 mL of Milli-Q water. Final concentrations in the influent were either 4 or 400 nmol/L, depending on the experiment. The solutions were autoclaved before use. Mixing of the hexachloro-1,3-butadiene with the mineral medium took place in a mixing chamber just before entering the sediment columns. The columns were operated in a climate room at a temperature of 20 °C. The occurrence of methane production in the anaerobic column was incidentally verified by measuring methane with a Packard GC 417 gas chromatograph equipped with a flame ionization detector and a packed 1.5-m column (Porapack R; Chrompack).

Column Sampling and Analyses. Columns were sampled with glass syringes as previously described (*12,15*). Sample volumes varied from 1 to 50 mL. They were analyzed routinely by hexane extraction followed by on-column injection into a gas chromatograph (United Technologies, The Netherlands) equipped with an electron capture detector (ECD) and a 25-m capillary column (Sil 5CB, 1.2 μm; Chrompack, The Netherlands).

To identify the various transformation products, samples of 50 mL were purged with nitrogen gas at a flow rate of 10 mL/min during 30 min at a temperature of 90 °C by means of a purge and trap system (Chrompack, The Netherlands). Components present in the outflowing gas were trapped in a glass tube packed with 90 mg of Tenax TA. The components were released from the Tenax in a thermodesorption cold trap (TCT) unit (Chrompack, The Netherlands) at 250 °C for 10 min with a helium flow of 10 mL/min. The desorbed compounds were cryofocused in a cold trap at −100 °C. Fast heating of this cold trap gave a sharp injection of the compounds onto the analytical column (Supelcowax-10, 60-m length, 0.25-μm film thickness). After an initial oven temperature of 60 °C during 4 min, the temperature was raised to 270 °C at a rate of 4 °C/min. The GC was connected to a VG MM7070F mass spectrometer operating in the 70-eV EI ionization mode.

Aqueous samples (50 mL) for proton NMR were taken from the methanogenic column as described above. The samples were injected directly into 2-mL aliquots of deuterated chloroform in 60-mL extraction tubes and shaken for 5 min. After settling, the aqueous phase was removed by syringe. Four additional samples were treated in a similar manner with the same aliquot of deuterated chloroform. Thus, five samples of 50 mL were extracted resulting in an increase in concentration by a factor of 125 at maximum. After extraction, 1 mL of deuterated chloroform could be separated from the aqueous sample and was measured directly in the NMR apparatus (Bruker AC-E 200).

Reduction of Hexachloro-1,3-butadiene by Titanium(III) Citrate. The assay was carried out in a series of 13-mL serum bottles (*11*). The bottles were filled inside the anaerobic glovebox and sealed with viton stoppers (Maag Technic, Switzerland) and aluminium crimp caps. The reaction mixture contained 8.58 mL of 100 mM Tris/HCl (pH 9), 2.2 mL of titanium(III) citrate (100 mM), and 220 μL of hydroxocobalamin (5 mM). Three control series were prepared: one without titanium(III) citrate, one without hydroxocobalamin, and one without both. Bottles were stored at 0 °C before use. After the gas phase was changed with 100% N_2, 11 μL of hexachloro-1,3-butadiene in ethanol (5 mM) was added by syringe. It was assured that ethanol was not transformed to ethene

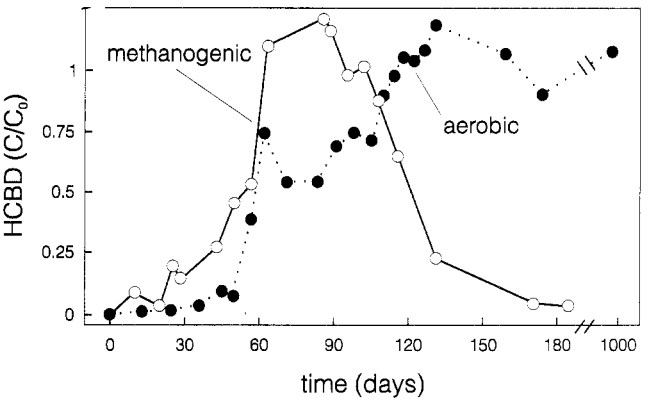

Figure 1. Breakthrough of hexachloro-1,3-butadiene (HCBD) in aerobic and methanogenic columns. Removal took place under methanogenic conditions only. Influent concentrations were 4 nmol/L.

by GC/MSD analysis (see below). The reaction was carried out at room temperature (22 ± 1 °C). After different time intervals, the reaction was stopped by the injection of 2 mL of hexane into one bottle of each series. The bottles were stored at 4 °C after extraction and analyzed within 48 h by means of a Hewlett-Packard mass spectrometric detector 5970B connected to an HP 5890 gas chromatograph (GC/MSD). The GC/MSD was equipped with a 25-m capillary column (Sil 5CB, 1.2 μm; Chrompack, The Netherlands). After an initial oven temperature of 40 °C (5 min), the oven temperature was raised to its final value of 200 °C at a rate of 10 °C/min.

A second assay was performed to determine transformation products. The reaction mixture contained 7.0 mL of 100 mM Tris/HCl (pH 9), 1.8 mL of titanium(III) citrate (100 mM), 180 μL of hydroxocobalamin (5 mM), and 350 μL of 500 mM hexachloro-1,3-butadiene in ethanol. After 1 day of incubation, three bottles were analyzed via hexane extraction to determine nongaseous transformation products. Head spaces of three other bottles were analyzed for possible gaseous dechlorination products by injection into the GC/MSD equipped with a 25-m capillary column (Sil 5CB, 1.2 μm; Chrompack, The Netherlands).

Results

Column Experiments. Initially, hexachloro-1,3-butadiene (4 nmol/L) was added to an aerobic column, a column fed with nitrate as the terminal electron acceptor, and a methanogenic column in a mixture of other chlorinated compounds. Removal of hexachloro-1,3-butadiene was only observed under methanogenic conditions (*15*) after an acclimation time of approximately 4 months (Figure 1). No disappearance of hexachloro-1,3-butadiene was detected within the experimental period of 3 years in the presence of nitrate and under aerobic conditions. The aerobic breakthrough curve of hexachloro-1,3-butadiene is more retarded than the methanogenic curve. Apparently, hexachloro-1,3-butadiene adsorbs better to the sediment under aerobic than under anaerobic conditions.

The methanogenic column was first used to assess the transformation pathway of trichloro- and dichlorobenzenes (*15*). No hexachloro-1,3-butadiene was fed to the column during this study. After its completion, the chlorinated benzenes in the influent were replaced by hexachloro-1,3-butadiene as the sole organic contaminant at 400 nmol/L (≈100 μg/L). Hexachloro-1,3-butadiene was never detected in the effluent but disappeared within the first

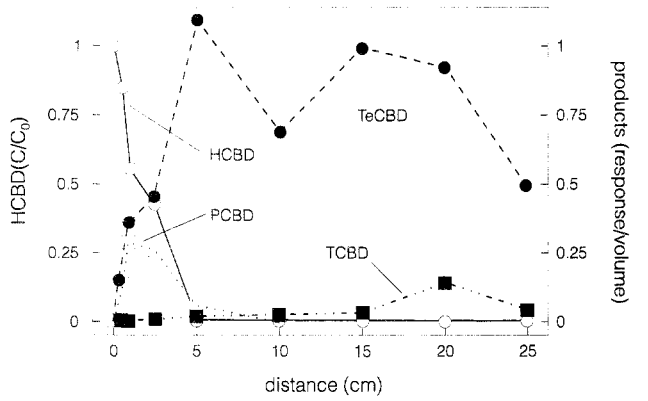

Figure 2. Disappearance of hexachloro-1,3-butadiene (HCBD) in a methanogenic column and the appearance of penta- (PCBD), tetra- (TeCBD), and trichloro-1,3-butadiene (TCBD). The influent concentration of hexachloro-1,3-butadiene was 400 nmol/L.

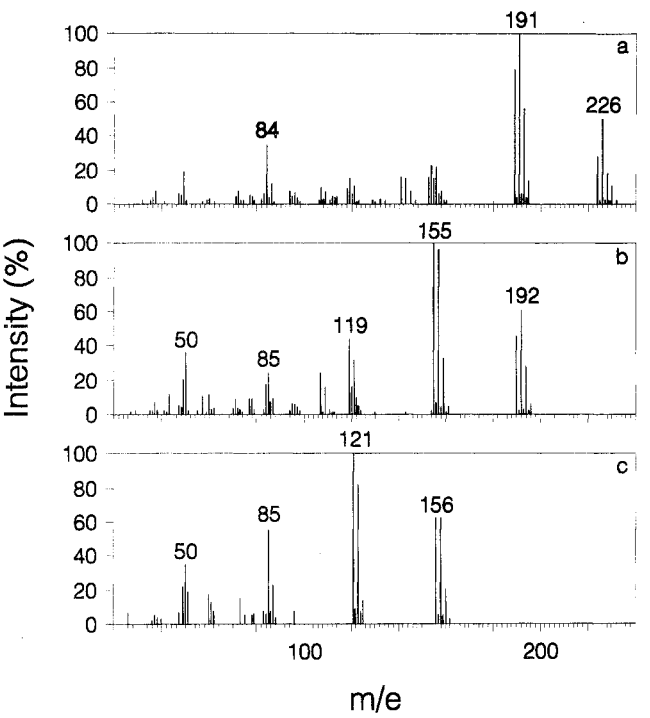

Figure 3. Mass spectra of penta- (a), tetra- (b), and trichloro-1,3-butadiene (c) obtained with column samples taken at different distances from the influent port.

5 cm of the column (Figure 2), despite its absence in the influent over a period of 1.5 year in which the experiments with chlorinated benzenes were done. Some unknown compounds appeared in the column simultaneously (Figure 2). A pseudo-first-order rate constant of 0.36 h^{-1} was calculated from the measurements at 0, 0.5, 1.0, and 2.5 cm from the inlet. The mass spectra of the products show the characteristic pattern caused by the natural abundance of the chlorine isotopes (Figure 3). The molecular ions in the spectra a–c (Figure 3) indicate the presence of five, four, and three chlorine atoms, respectively, in the corresponding compounds. They were identified as penta-, tetra-, and trichloro-1,3-butadiene. Judging from peak responses in the GC/MS analyses and taking into account the mass loss resulting from dechlorination, hexachloro-1,3-butadiene was completely converted to tetra- (>90%) and trichloro-1,3-butadiene (<5%).

There are nine possible isomers of tetrachloro-1,3-butadiene (*16*). Since mass spectrometry only yields information on the gross formulas of compounds, further identification was done by means of proton NMR. A sample taken from the methanogenic column at 20-cm height where tetrachloro-1,3-butadiene was the dominant transformation product (at least 90%, confirmed by GC/MS) gave a spectrum with a singlet at $\delta = 6.47$ ppm (Table 1). Hence, the tetrachloro-1,3-butadiene is symmetrical since a singlet can only result from the presence of two identical protons in one molecule. This restricted the number of possible structures to the three symmetrical isomers (Table 1). The product was identified as (*E,E*)-1,2,3,4-tetrachloro-1,3-butadiene by comparison of the values in Table 1. The concentration of trichloro-1,3-butadiene in the extract was below the detection limit of the NMR apparatus.

Table 1. NMR Data of Symmetrical Tetrachlorobutadienes

isomer	chemical shift (ppm)	reference
1,1,4,4-tetrachlorobutadiene	6.60	32
	6.59	this study
(*Z,Z*)-1,2,3,4-tetrachlorobutadiene	6.93	33
	7.12	34
	7.05	35
(*E,E*)-1,2,3,4-tetrachlorobutadiene	6.47	36
	6.47	37
	6.47	this study

Reduction of Hexachloro-1,3-butadiene by Hydroxocobalamin and Titanium(III) Citrate. In the incubations with titanium(III) citrate and hydroxocobalamin, 5 μM hexachloro-1,3-butadiene was completely reduced within 2 h with a first-order rate constant of 2.5 ± 0.5 h^{-1}. The low concentration prevented detection of degradation products in this experiment. No reduction was observed in controls lacking hydroxocobalamin, titanium(III) citrate, or both. To detect and identify transformation products, a second experiment was done with an initial hexachloro-1,3-butadiene concentration of 0.5 mM. Too little of the initial amount of hexachloro-1,3-butadiene was removed in this experiment to be able to determine the transformation rate. The GC/MSD analysis revealed the appearance of two dechlorination products in the hexane extracts and a third in the head space. One product was identified as pentachloro-1,3-butadiene from its mass spectrum, which was similar to the spectrum in Figure 3a. The second product in the hexane extract showed the characteristic pattern caused by the natural abundance of the chlorine isotopes (Figure 4a). The molecular ions in spectrum a of Figure 4 indicate the presence of three chlorine atoms in the compound. The molecular ion with a mass of 49 results from the presence of four carbons and one hydrogen in the molecule and not from one carbon, two hydrogens, and one chloride, because a molecular ion with a mass of 51 is lacking. Thus, the compound was tentatively identified as trichloro-1-buten-3-yn. The product detected in the head space had a molar mass of 52 and showed the typical mass spectrum of 1-buten-3-yn (Figure 4b), which was confirmed by comparison with a published reference spectrum (*17*).

Discussion

Hexachloro-1,3-butadiene was only removed under methanogenic conditions and not when oxygen or nitrate were present in the column experiments. The observed removal of hexachloro-1,3-butadiene under methanogenic

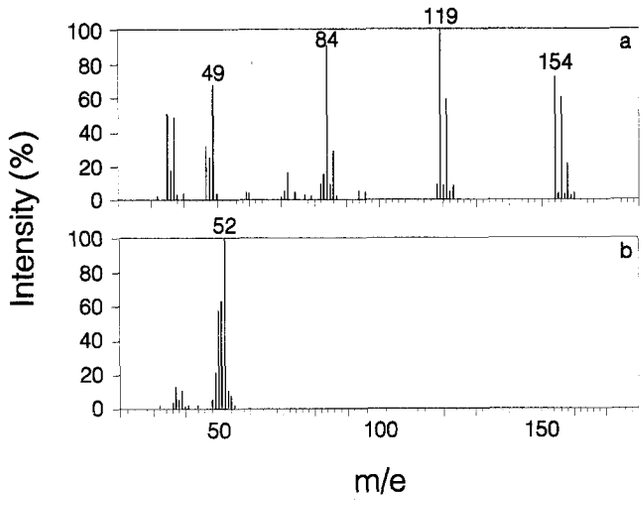

Figure 4. Mass spectra of trichloro-1-buten-3-yn (a) and 1-buten-3-yn (b) obtained with samples from batch incubations of hexachloro-1,3-butadiene with titanium(III) citrate and hydroxocobalamin.

conditions was attributed to microbial activity because (i) a long acclimation time preceded its disappearance and (ii) elimination was also observed in anaerobic batch experiments inoculated with column material (after a lag time of about 2 weeks) but not in controls with autoclaved column material. Reducing equivalents for dechlorination had to come from the organic matter in the sediment or from the methanol that was added together with hexachloro-1,3-butadiene. Methanogenesis developed because of the presence of either methanol or of low molecular weight organic molecules produced by other anaerobic organisms. Attempts to enrich for dechlorination activity on hexachloro-1,3-butadiene failed.

The end products of hexachloro-1,3-butadiene transformation in the methanogenic column were (*E,E*)-1,2,3,4-tetrachloro-1,3-butadiene (>90%) and an isomer of trichloro-1,3-butadiene (Figure 5). A small amount of pentachloro-1,3-butadiene was detected as an intermediary product. Assuming that no chlorine atoms are translocated in the molecule during the dechlorination reaction, it follows that the observed pentachloro-1,3-butadiene was (*E*)-1,1,2,3,4-pentachloro-1,3-butadiene (Figure 5). So, hexachloro-1,3-butadiene is reduced by consecutive steps in which one chlorine is substituted by one hydrogen (hydrogenolysis). A similar mechanism was responsible for reductive dechlorination of other chlorinated hydrocarbons in sediment samples, such as tetrachloroethene (*18–21*), chlorinated benzenes (*15, 22, 23*) and chlorophenols (*24*).

Electrochemical and biological reductive dechlorinations of aryl and vinylic compounds probably occur via a mechanism that involves two consecutive single electron transfers (*25*). The inductive effect of the substituents is therefore expected to determine where electron transfer takes place. Hence, one would expect that electron transfer occurs at the 2- (or 3-) position (*25, 26*). However, dechlorinations in the methanogenic column actually occurred at the 1- and 4-positions, resulting in the formation of (*E,E*)-1,2,3,4-tetrachloro-1,3-butadiene. This may be due to steric interferences between the 1- and 3- and 2- and 4-substituents, respectively (Figure 5), resulting in a gauche configuration of the hexachloro-1,3-butadiene. Indeed, the angle in the single C–C bond (φ) of gaseous hexachloro-1,3-butadiene is 78.1 ± 1.1° (*27*). Furthermore, each chlorine substituent is forced out of the planar

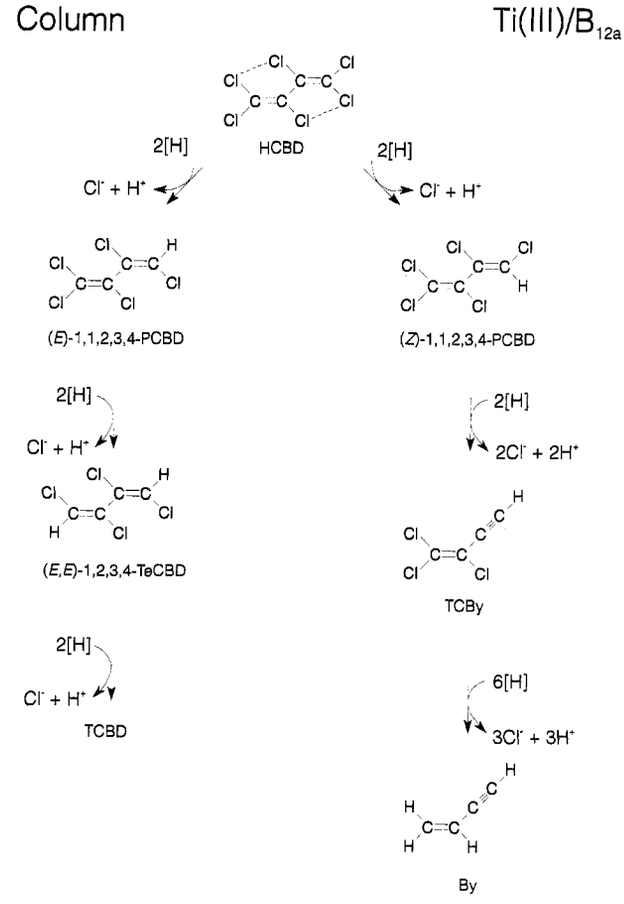

Figure 5. Tentative pathways of the reductive dechlorination of hexachloro-1,3-butadiene (HCBD) in a methanogenic sediment column and in batch systems with titanium(III) citrate. (PCBD = pentachlorobutadiene, TeCBD = tetrachlorobutadiene, TCBD = trichlorobutadiene, TCBy = trichloro-1-buten-3-yn, By = 1-buten-3-yn). The steric interactions between 1- and 3- (2- and 4-) chlorines in hexachloro-1,3-butadiene are indicated by dashed lines.

configuration of each double carbon bond, because their van der Waals radii overlap. As a consequence, the positively charged carbons in the middle of the molecule are completely surrounded by electron-dense chlorines which prevents electron transfer.

Reductive dechlorination of hexachloro-1,3-butadiene in the batch incubations with titanium(III) citrate and hydroxocobalamin yielded pentachloro-1,3-butadiene, trichloro-1-buten-3-yn, and 1-buten-3-yn as products (Figure 5). The system had a pH of 9, which was chosen for optimal reaction rates. The pH did not have an effect on the transformation pathway of 1,2-dichloroethane (*11*), and such an effect is not expected for hexachloro-1,3-butadiene either. The presence of pentachloro-1,3-butadiene implies that the first step in the reductive dechlorination of hexachloro-1,3-butadiene was a hydrogenolysis, similar to observations with tetrachloroethene and chlorinated benzenes (*28*). The retention time of the pentachloro-1,3-butadiene in the GC analysis differed from that of the intermediate (*E*)-1,2,3,4,4-pentachloro-1,3-butadiene obtained from the methanogenic column. Therefore, assuming that dechlorination in this experiment also took place at the 1-position because of steric protection of the 2-position, (*Z*)-1,1,2,3,4-pentachloro-1,3-butadiene is proposed to be the first intermediate of reductive dechlorination of hexachloro-1,3-butadiene by titanium(III) citrate and hydroxocobalamin. The presence of

trichloro-1-buten-3-yn suggests that pentachloro-1,3-butadiene was dechlorinated via a dihalo elimination (Figure 5). During reductive dihalo elimination of an alkane, two vicinal halogens are released from the molecule, giving the respective alkene (*9*). Similarly, reductive dihalo elimination of an alkene would result in the corresponding alkyn. Dihalo elimination by vitamin B_{12} has also been demonstrated with chlorinated alkanes (*9, 11, 28*). Finally, trichloro-1-buten-3-yn was dechlorinated by the replacement of two chlorines by two hydrogens (Figure 5).

Methanogenic and acetogenic bacteria contain considerable amounts of cofactors like hydroxocobalamin [50–800 nmol/g of dry weight (*29*)]. Hence, by analogy to observations with chlorinated ethanes (*11, 30*) and tetrachloroethene (*28, 31*), one may conclude that they should have the potential to reduce hexachloro-1,3-butadiene. However, the column results show that dechlorination under environmental conditions may involve other catalysts, which was also found for the reductive dechlorination of 1,2-dichloroethane by a pure culture of *Methanobacterium thermoautotrophicum* (*11*). While hexachloro-1,3-butadiene can potentially be dechlorinated completely by hydroxocobalamin, dechlorination in the column followed a different pathway and lead to the formation of (*E,E*)-1,2,3,4-tetrachloro-1,3-butadiene and an isomer of trichloro-1,3-butadiene as dead-end products. These products are known as antifungal agents (*7*), which means that the dechlorination in the column does not lead to a complete detoxification. However, the partially dechlorinated products may be susceptible to aerobic degradation, an option that has to be tested in future experimentation.

Acknowledgments

We thank Dr. D. de Bie and G. Lelyveld of the Department of Organic Chemistry and J. W. Simons and W. Roelofsen of the Department of Microbiology for technical assistance. We are grateful to Mrs. I. Davidova for translating Russian literature and for establishing the contact with Dr. Potkin and Dr. Kaberdin. T.N.P.B. was partially supported by The Netherlands Integrated Soil Research Programme.

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Received for review September 1, 1993. Revised manuscript received February 7, 1994. Accepted February 8, 1994.[⊗]

[⊗] Abstract published in *Advance ACS Abstracts,* March 15, 1994.