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Transformation of 1,1,1-trichloroethane in an anaerobic packed-bed reactor at various concentrations of 1,1,1-trichloroethane, acetate and sulfate

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Abstract Biotransformation of 1,1,1-trichloroethane (CH_3CCl_3) was observed in an anaerobic packed-bed reactor under conditions of both sulfate reduction and methanogenesis. Acetate (1 mM) served as an electron donor. CH_3CCl_3 was completely converted up to the highest investigated concentration of 10 μM. 1,1-Dichloroethane and chloroethane were found to be the main transformation products. A fraction of the CH_3CCl_3 was completely dechlorinated via an unknown pathway. The rate of transformation and the transformation products formed depended on the concentrations of CH_3CCl_3, acetate and sulfate. With an increase in sulfate and CH_3CCl_3 concentrations and a decrease in acetate concentration, the degree of CH_3CCl_3 dechlorination decreased. Both packed-bed reactor studies and batch experiments with bromoethanesulfonic acid, an inhibitor of methanogenesis, demonstrated the involvement of methanogens in CH_3CCl_3 transformation. Batch experiments with molybdate showed that sulfate-reducing bacteria in the packed-bed reactor were also able to transform CH_3CCl_3. However, packed-bed reactor experiments indicated that sulfate reducers only had a minor contribution to the overall transformation in the packed-bed reactor.

Introduction

1,1,1-Trichloroethane CH_3CCl_3 is a ubiquitous contaminant in groundwater, mainly because of accidental spills in industrial processes. Because the compound is toxic for both man and animals, remediation of contaminated sites is necessary. Microbial remediation could be an attractive clean-up technique if rapid degradation can be achieved. However, CH_3CCl_3 is one of the chlorinated aliphatic hydrocarbons that are difficult to degrade biologically. So far, its dechlorination has not been described under aerobic or denitrifying conditions. Only Oldenhuis et al. (1989) have reported the partial conversion of CH_3CCl_3 to trichloroethanol by *Methylosinus trichosporium* OB3b under aerobic conditions. Transformation of CH_3CCl_3 in anaerobic continuous-flow systems has been reported under both sulfate-reducing and methanogenic conditions (Bouwer and McCarty 1983; Bouwer and Wright 1988; Cobb and Bouwer 1991; Vogel and McCarty 1987a; Wrenn and Rittmann 1996). Transformation only occurred after a long acclimatization period (10–12 weeks) and was only investigated at CH_3CCl_3 concentrations below 1 μM. Also the involvement of methanogenic and/or sulfate-reducing bacteria in the transformation was not established.

1,1-Dichloroethane (CH_3CHCl_2) has been found to be the main product of CH_3CCl_3 biotransformation (Egli et al. 1987, 1988; Gälli and McCarty 1989a, b; Parsons et al. 1985; Vogel and McCarty 1987a) but conversion to chloroethane (CH_3CH_2Cl) (Parsons and Lage 1985; Vogel and McCarty 1987a) and complete dechlorination to CO_2 (Vogel and McCarty 1987a), acetic acid (Gälli and McCarty 1989a) and unknown products (Gälli and McCarty 1989a) was also detected. Transformation of CH_3CCl_3 to CH_3CHCl_2 and CH_3CH_2Cl occurs through reductive dechlorination. The pathway of complete dechlorination is not yet clear.

The aim of this study was to explore the potential of complete CH_3CCl_3 dechlorination, in an anaerobic packed-bed reactor under methanogenic conditions, of concentrations as high as 1.3 mg/l (10 μM). For the application of CH_3CCl_3 biotransformation in the treatment of contaminated groundwaters it is important to obtain more information about the effect of important

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process parameters on the transformation. Therefore, the effects of varying sulfate and acetate (primary substrate) concentrations on the transformation of CH_3CCl_3 were investigated. For a better understanding of these effects, the role of methanogenic and sulfate-reducing bacteria in the transformation of CH_3CCl_3 was studied.

Materials and methods

Packed-bed reactor studies

The experiments were performed in an upflow packed-bed reactor (glass, height 32 cm, inside diameter 4.42 cm, volume 492 ml) (Fig. 1) packed with polyurethane foam particles (5 × 5 × 6 mm; Bayer B. V., Mijdrecht, The Netherlands) mixed with digested sludge (20 v/v%) from the wastewater treatment plant Kralingseveer (Rotterdam, The Netherlands). The packed-bed reactor was wrapped with aluminum foil to prevent growth of phototrophs.

The reactor was continuously fed with an anaerobic non-sterile mineral medium containing (mg/l) K_2HPO_4 (8), KH_2PO_4 (3.6), $NaHCO_3$ (40), NH_4Cl (26.6), $MgCl_2 \cdot 6H_2O$ (101.6), $CaCl_2 \cdot 2H_2O$ (62.6), resazurine (1). From a trace element solution, 0.125 ml/l was added. The trace element solution contained (mg/l) $FeSO_4 \cdot 7H_2O$ (2800), H_3BO_3 (50), $Al_2(SO_4)_3 \cdot 16H_2O$ (118.3), $MnCl_2 \cdot 4H_2O$ (50), $CuSO_4 \cdot 5H_2O$ (92.8), EDTA (500), $ZnCl_2$ (50), $(NH_4)_6Mo_7O_{27} \cdot H_2O$ (50), $CoCl_2$ (27.3), $NiCl_2 \cdot 6H_2O$ (91.6), 1 ml HCl (37%). The medium was continuously purged with a mixture of N_2 and CO_2 (99.5%/0.5%) (Hoek Loos B. V., Dieren, The Netherlands) to remove all oxygen.

The medium (pH 7.3 ± 0.2) was pumped into the packed-bed reactor by means of a peristaltic pump with Marprene tubing (Watson Marlow, England). All other tubing was either Viton or Teflon. CH_3CCl_3, acetate and Na_2S (42 μM, to maintain reducing conditions) were added to the medium as a concentrated solution at the influent of the packed-bed reactor with a syringe pump (Fig. 1). The hydraulic retention time in the packed-bed was 24 h. All experiments were carried out at 25 °C.

Batch culture studies

Experiments were done with the following medium (g/l): KH_2PO_4 (0.43), $Na_2HPO_4 \cdot 2H_2O$ (0.53), NH_4Cl (0.3), $CaCl_2 \cdot 2H_2O$ (0.12), $MgSO_4 \cdot 7H_2O$ (0.13) resazurine (0.0005). The medium also contained 1 ml trace element solution (see above) and 1 ml vitamin solution. The vitamin solution contained (mg/l): biotin (2), folic acid (2), riboflavin (5), thiamine (5), cyanocobalamin (5), nicotinamide (5), *p*-aminobenzoic acid (5).

The medium was purged with a mixture of CO_2 and N_2 (0.5:99.5 v/v%, 700 ml/min) for 30 min. After 15 min, $Na_2S \cdot 9H_2O$ (67 mg/l) and $NaHCO_3$ (100 mg/l) were added. The medium was transferred to 120-ml bottles (brown glass) in an anaerobic glove-box. Each bottle contained 60 ml medium and was closed with Teflon-lined butyl rubber stoppers and aluminum crimp seals. CH_3CCl_3 (5.8 μM) and acetate (1000 μM) were added as concentrated solutions.

All batch cultures were inoculated with 2 ml liquid phase taken from the packed-bed reactor (1 ml liquid phase from sample port 2 and 1 ml from sample port 3; Fig. 1). The cultures were incubated on a shaker (100 rpm) in a canted position (90°) at 25 °C. In sterile control batch cultures, there was no transformation of CH_3CCl_3 and acetate (data not shown).

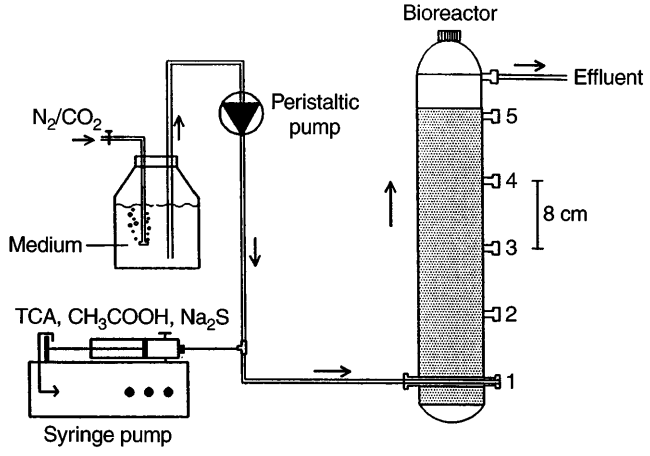

Fig. 1 Schematic presentation of packed-bed reactor

Inhibitor studies

To investigate the role of methanogenic, acetogenic and sulfate-reducing microorganisms in the transformation of CH_3CCl_3, three inhibitors were used. After complete transformation of the first amount of added CH_3CCl_3, vancomycin (0.14 mM), 2-bromoethanesulfonic acid (6 mM) or molybdate (2 mM) was added to the batch cultures together with more CH_3CCl_3 (5.8 μM) and acetate (500 μM).

Analytical methods

CH_3CCl_3, CH_3CHCl_2 and CH_3CH_2Cl were quantified by headspace gas chromatography using a headspace sampler. Liquid samples (100–1000 μl) were injected in 10-ml headspace autosampler vials with Teflon-lined butyl rubber stoppers and aluminum crimp seals. The final volume was adjusted to 2 ml with demineralized water. The vials were analyzed using a Hewlett-Packard 19395A headspace autosampler connected to an HRGC 5300 Carlo Erba gas chromatograph equipped with an electron-capture detector and a CP-Sil 5CB column (length 25 m, inner diameter 0.53 mm, film thickness 2 μm; Chrompack, The Netherlands). Helium (16 ml/min) served as a carrier gas for the headspace sampler. The gas chromatograph had the following settings: injection temperature, 200 °C; oven temperature, 35 °C; detector temperature, 300 °C. The flow rate of the carrier gas (helium) in the column was 20 ml/min. The detector make-up gas was nitrogen. The detector signal was processed with the Mosaic chromatography data system (Chrompack, Bergen op Zoom, The Netherlands). A four-point curve was used for calibration.

Carbon dioxide, carbon monoxide and methane concentrations were routinely analyzed on a Varian 3700 gas chromatograph and flame ionization detector after separation on a Carboplot P7 column (length 12.5 m, inner diameter 0.53 mm, film thickness 25 μm; Chrompack, Bergen op Zoom, The Netherlands) and reduction of CO_2 and CO by a methanizer at 400 °C (Varian, Houten, The Netherlands). The carrier gas was helium (40 ml/min). Injector, oven and detector temperatures were set at 280 °C, 50 °C and 280 °C respectively. Liquid samples (2 ml) were injected in 10-ml headspace autosampler vials with Teflon-lined butyl rubber stoppers and aluminum crimp seals and equilibrated at 80 °C for 45 min. A 50-μl sample of the headspace gas was injected into the gas chromatograph by hand with a 100-μl Hamilton gas- and liquid-tight syringe. A four-point calibration curve was used for quantification.

Sulfate was determined by ion chromatography. Liquid samples were centrifuged (14 000 *g* for 10 min) and injected in 2-ml screw-cap vials with Teflon-lined silicone liners. The vials were sampled (50 μl) with a Marathon-XT autosampler (Spark Holland, Emmen, The Netherlands) and the samples injected into a Dionex DX-100 ion chromatograph (Dionex, Breda, The Netherlands) equipped with a conductivity detector, thermal stabilizer and ASRS

suppressor. Sulfate was separated on an IONPAC AG9-SC guard column and IONPAC AG9-SC anion column (Dionex, Breda, The Netherlands). $NaHCO_3$ (1.7 mM)/Na_2CO_3 (1.8 mM) was used as effluent at a flow rate of 2 ml/min. The detector signal was processed with the Maestro chromatography data system (Chrompack, Bergen op Zoom, The Netherlands). A ten-point calibration curve was used.

Acetate concentrations were determined with an enzymatic test combination (Boehringer, Mannheim, Germany) based on the formation of NADH (Boehringer Mannheim GmbH Biochemica 1989). NADH formation was measured by the increase in absorbance at 340 nm on a JASCO 7800 UV/VIS spectrophotometer.

Chemicals

All chemicals were obtained from commercial companies. CH_3CCl_3 and CH_3CH_2Cl were obtained from Fluka. CH_3CHCl_2 and sodium acetate were obtained from Janssen Chimica. Vancomycin and sodium molybdate were purchased from Sigma. 2-Bromoethanesulfonic acid was obtained from Aldrich. Calibration gases were obtained from AGA (carbon dioxide, methane).

Results

Transformation of CH_3CCl_3 in an anaerobic packed-bed reactor inoculated with digested sludge was studied to establish whether its complete dechlorination under methanogenic conditions was possible. Before the addition of CH_3CCl_3, the packed-bed reactor was operated for 2 weeks with acetate (1 mM) as the sole substrate. After 2 weeks, 75% of the added acetate was utilized. Methane production (0.62 mM) indicated that 62% of the acetate added was converted by methanogens. The presence of methanogens was confirmed by fluorescence microscopy according to the method described by Doddema and Vogels (1978). Sulfate-reducing bacteria utilized 13% of the added acetate for the complete reduction of all available sulfate (0.13 mM). Although no sulfate was added, about 0.13 mM was present in the influent of the packed-bed reactor, probably because of (microbial) oxidation of sulfide by traces of oxygen in the influent.

When CH_3CCl_3 (0.75 μM) was added to the packed-bed reactor it took 7 days until its complete breakthrough. This period of time is a result of initial sorption of CH_3CCl_3 to polyurethane foam, the carrier material of the packed-bed reactor. This sorption was taken into account when steady-state samples were collected. A steady state was characterized by a constant degree of removal of CH_3CCl_3 for a period of 14 days after at least seven hydraulic retention times.

Seven days after complete breakthrough of CH_3CCl_3, its transformation started and CH_3CHCl_2 was found to be the only transformation product. Two days later, CH_3CH_2Cl was also detected. During the next 20 days, the concentration of CH_3CHCl_2 in the effluent decreased until CH_3CCl_3 was completely recovered as the monochloro derivative. Subsequently, the concentration of CH_3CH_2Cl in the effluent of the bioreactor also decreased. In a steady state, 62.5% of the added CH_3CCl_3 was recovered as CH_3CH_2Cl (Fig. 2A) while 38.5% was converted to unknown products. These results indicate that part of the CH_3CCl_3 was probably mineralized, or completely converted to ethane by reductive dechlorination.

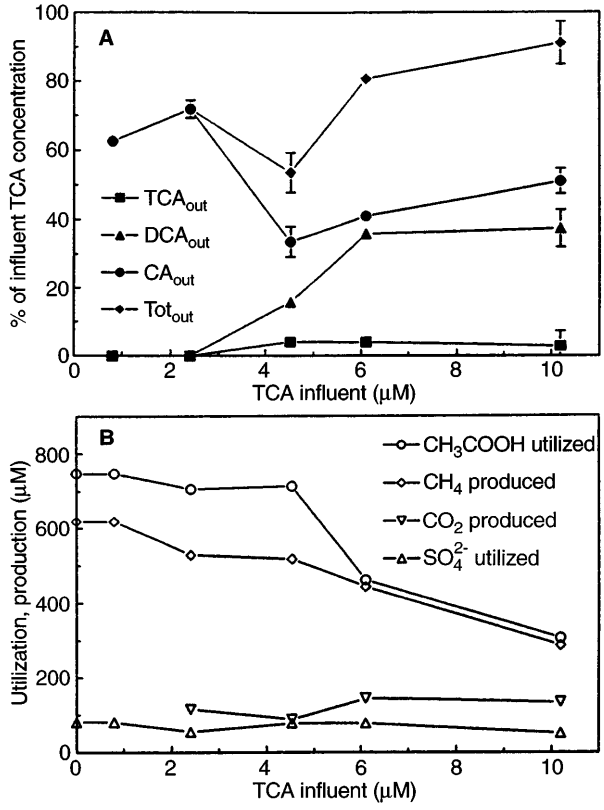

Fig. 2 Effect of 1,1,1-trichloroethane (*TCA*) concentration on its transformation (**A**) and the substrate conversion (**B**) in an anaerobic packed-bed reactor. Chlorinated ethanes in the effluent of the reactor are expressed as the percentage of trichloroethane in the influent. *Error bars* standard deviations on two to five measurements taken within 7 days. $Tot_{out} = TCA_{out} + DCA_{out} + CA_{out}$ (*DCA* 1,1-dichloroethane, *CA* chloroethane)

Transformation at different 1,1,1-trichloroethane concentrations

The transformation of CH_3CCl_3 in the packed-bed reactor was studied in a concentration range from 1.3 μM to 10 μM under starting conditions. At all concentrations tested, CH_3CCl_3 was completely transformed to CH_3CHCl_2, CH_3CH_2Cl and unknown products (Fig. 2A). Up to a CH_3CCl_3 concentration of 2.5 μM, CH_3CH_2Cl was found to be the only chlorinated transformation product. At higher initial concentrations, CH_3CHCl_2 was also detected. With an increase in CH_3CCl_3 concentration, the percentage recovered as chlorinated intermediates also increased, while the percentage of CH_3CCl_3 transformed to unknown products decreased. At the highest concentration tested, CH_3CCl_3 (10 μM) was nearly completely converted to the dichloro and monochloro derivatives.

Up to a CH_3CCl_3 concentration of 4.5 μM, acetate (1 mM) was utilized to an extent of about 75% by both methanogenic and sulfate-reducing bacteria (Fig. 2B). Methane was produced (0.529 mM) and sulfate (0.055 mM) completely reduced. At higher concentrations, sulfate was still completely removed, but less acetate was metabolized. This was caused by a decrease in methanogenic activity, as evident from a decrease in methane production. These results indicate that methanogenic activity is probably inhibited by CH_3CCl_3 at concentrations higher than 4.5 μM. This is close to the inhibition level between 6 μM and 15 μM that was reported by Vargas and Ahlert (1987) for semi-batch culture studies with a mixed anaerobic culture.

Effect of sulfate concentration on the transformation of 1,1,1-trichloroethane

The effect of the sulfate concentration on CH_3CCl_3 removal was tested by increasing the sulfate content of the influent of the packed-bed reactor in four steps from 0.06 mM to 0.95 mM. Under the starting conditions, CH_3CCl_3 (2.5 μM) was completely degraded to CH_3CH_2Cl and unknown products (Fig. 3A); 70% of the added acetate (1 mM) was utilized. Sulfate-reducing bacteria utilized 0.06 mM and methanogenic bacteria converted 0.61 mM to methane (Fig. 3B).

Up to a concentration of 0.33 mM, sulfate had no significant effect on the transformation of CH_3CCl_3 (Fig. 3A). At higher concentrations, sulfate clearly influenced its transformation. CH_3CHCl_2 again was found to be a transformation product and the concentration of CH_3CH_2Cl in the effluent of the packed-bed reactor decreased, indicating that the transformation of CH_3CCl_3 became less complete. At a sulfate concentration of 0.95 mM, the degree of CH_3CCl_3 removal decreased rapidly. After 7 days of operation at a sulfate concentration of 0.95 mM, only 56.5% of the added CH_3CCl_3 was transformed. CH_3CHCl_2 (0.72 μM) was found to be the only transformation product. No formation of CH_3CH_2Cl occurred. To prevent complete loss of CH_3CCl_3-transforming capacity, the sulfate concentration was decreased to the original concentration of 0.06 mM before a steady state was reached.

During the increase in sulfate concentration in the influent of the packed-bed reactor, the amount of sulfate reduced increased from 0.055 mM at an influent sulfate concentration of 0.060 mM to 0.85 mM at an influent sulfate concentration of 0.95 mM (Fig. 3B). At the same time, methane production by methanogenic bacteria decreased from 0.61 mM to 0.09 mM. These results indicate that sulfate-reducing bacteria were not involved in the transformation of CH_3CCl_3. Methanogenic bacteria probably play a role in the transformation of CH_3CCl_3 since the decrease of its transformation coincided with the decrease of methane production by methanogenic bacteria.

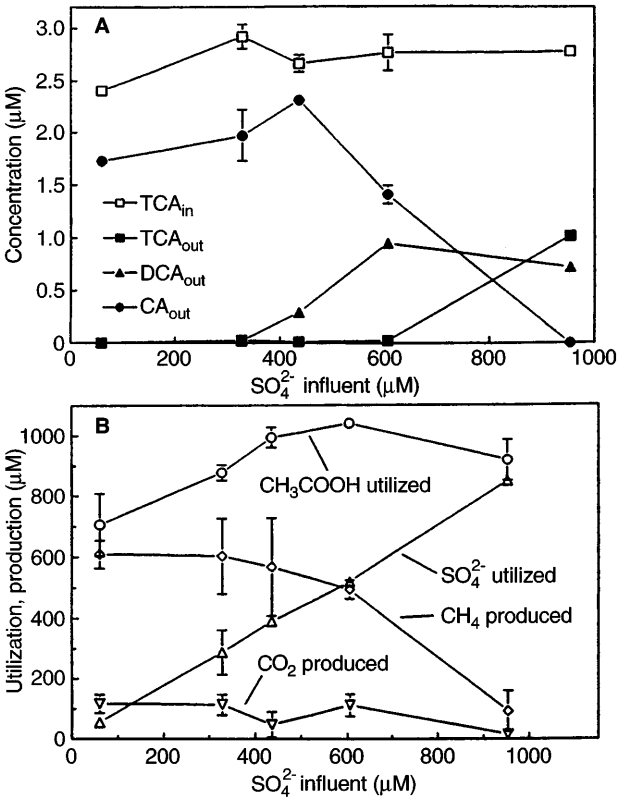

Fig. 3 Effect of sulfate concentration on the transformation of 1,1,1-trichloroethane (*TCA*) (**A**) and acetate conversion (**B**) in an anaerobic packed-bed reactor. *Error bars* standard deviations on two to five measurements taken within 7 days

Effect of acetate concentration on 1,1,1-trichloroethane transformation

The effect of the electron donor concentration on CH_3CCl_3 transformation was tested in a range from 0.25 mM to 1 mM. The packed-bed reactor was first operated under starting conditions at a CH_3CCl_3 concentration of 10 μM, and it was mainly converted to CH_3CHCl_2 (3.8 μM) and CH_3CH_2Cl (5.2 μM) (Fig. 4A). About 10% was converted to unknown products. Acetate (1 mM) was utilized to 23% by methanogens and sulfate-reducing bacteria (Fig. 4B).

In the range studied, the acetate concentration did not have a significant effect on the transformation (Fig. 4A). CH_3CCl_3 transformation remained complete at all times. However, the acetate concentration had a profound effect on the transformation products formed. At lower acetate concentrations, less CH_3CCl_3 was converted to CH_3CH_2Cl while the concentration of CH_3CHCl_2 in the effluent of the packed-bed reactor increased. The changes in the transformation products formed coincided with a decrease in methanogenic activity (Fig. 4B). Again, this indicates that methanogenic bacteria were involved in the transformation of CH_3CCl_3. Sulfate reduction (0.06 mM) did not change and remained complete at all times.

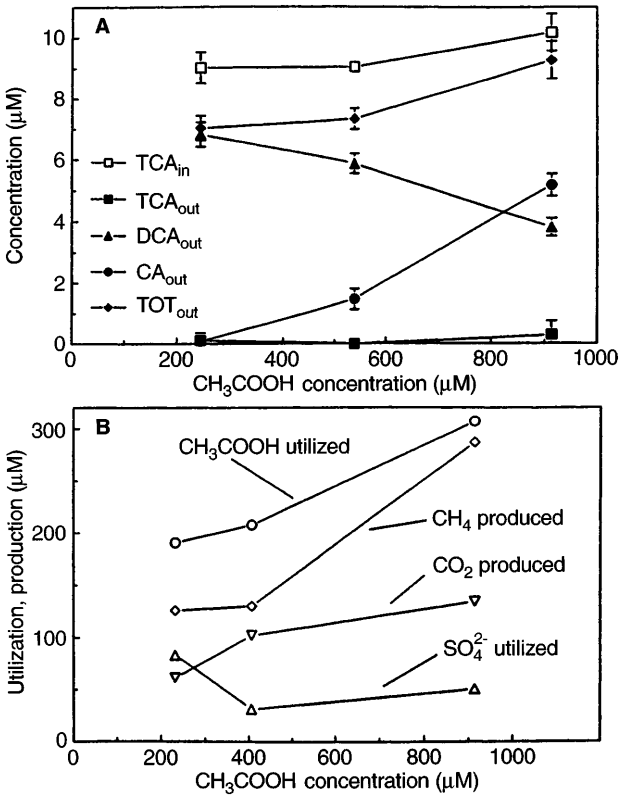

Fig. 4 Effect of acetate concentration on 1,1,1-trichloroethane (*TCA*) transformation (**A**) and acetate conversion (**B**) in an anaerobic packed-bed reactor

Transformation of 1,1,1-trichloroethane in batch cultures

The results presented above indicate that methanogenic bacteria and sulfate-reducing bacteria were both present in the packed-bed reactor. The involvement in CH_3CCl_3 transformation of these two bacterial groups and of acetogenic bacteria was investigated by adding specific inhibitors to batch cultures. First, a CH_3CCl_3-degrading microbial population was cultivated in the absence of inhibitors. The cultures were inoculated with liquid from the packed-bed reactor. In all batch cultures, transformation started within 1 week. After 23 days, CH_3CCl_3 (5.83 μM) was completely converted. Acetate (1000 μM) was utilized to an extent of 55%–60% and converted to about 580 μM methane. When CH_3CCl_3 was completely transformed, it was added again together with acetate (500 μM). At the same time specific inhibitors were added (Table 1).

2-Bromoethanesulfonic acid, an inhibitor of methanogenesis (Distefano et al. 1992), completely inhibited CH_3CCl_3 transformation and methane production. This confirms the findings in the packed-bed reactor that methanogenic bacteria were involved in the transformation of CH_3CCl_3.

In the presence of molybdate, an inhibitor of sulfate reduction (Smith and Klug 1981), CH_3CCl_3 transformation was partly inhibited. Molybdate also had an effect on the ratio of transformation products that were formed. More CH_3CCl_3 was converted to CH_3CHCl_2 (87% compared to 71% in the absence of inhibitors). This means that sulfate-reducing bacteria could be involved in the transformation, although no significant reduction of sulfate was detected in any of the batch cultures.

Vancomycin is an inhibitor of cell wall synthesis in gram-positive eubacteria and was used to inhibit acetogenic bacteria (Distefano et al. 1992). Vancomycin did not affect the transformation of CH_3CCl_3. This means that acetogenic bacteria, as described by Egli et al. (1988), and *Clostridia*, as described by Gälli and McCarty (1989a, b), were not responsible for the CH_3CCl_3 transformation observed in our packed-bed reactor.

Discussion

In this paper, CH_3CCl_3 transformation in an anaerobic packed-bed reactor, in which both sulfate reduction and methanogenesis occurred, is described. In the range (0.75–10 μM) studied, over 95% degradation was obtained under standard conditions (0.1 mM sulfate and 1 mM acetate). CH_3CHCl_2 and CH_3CH_2Cl were found to be the main transformation products. Part of the CH_3CCl_3 was converted to unknown non-chlorinated products. This is the first report of CH_3CCl_3 transformation in a continuous-flow reactor with CH_3CH_2Cl as the main transformation product (above 90%). Vogel and McCarty (1987a) also described CH_3CH_2Cl as a transformation product in a continuous-flow reactor, but this only accounted for 5% of the transformation products formed, whereas over 90% was transformed to

Table 1 Effect of inhibitors on the transformation of 1,1,1-trichloroethane in batch cultures. – Disappearance, + formation (CH_3CCl_3 1,1,1-trichloroethane; CH_3CHCl_2 1,1-dichloroethane; *UP* unknown products; *BES* 2-bromoethanesulfonic acid)

Inhibitors	Chlorinated hydrocarbons			Substrate conversion		
	CH_3CCl_3 (μM)	CH_3CHCl_2 (μM)	UP (μM)	CH_3COOH (μM)	CH_4 (μM)	SO_4^{2-} (μM)
None	−5.8	+4.0	+1.8	−350	+135	−15.0
Molybdate	−2.5	+2.2	+0.3	−167	+147	−16.7
Vancomycin	−5.0	+3.7	+1.3	−183	+157	−6.7
BES	0	0	0	0	+ 20	+33.3

CH_3CHCl_2. We observed that up to 90% of added CH_3CCl_3 was transformed to CH_3CH_2Cl.

The results of the batch experiments with inhibitors and of the packed-bed reactor studies suggest that methanogenic bacteria are involved in CH_3CCl_3 transformation. In the packed-bed reactor, both higher sulfate concentrations and lower acetate concentrations inhibited the transformation. This inhibition coincided with a decrease in methanogenic activity. Inhibition of CH_3CCl_3 transformation by sulfate has not been reported before, but there are several reports of the inhibition of dechlorination of other chlorinated compounds by sulfate (Suflita et al. 1988; Kuhn et al. 1990; Sharak Genther et al. 1989; Kohring et al. 1989; Gibson and Suflita 1986). In all cases, the inhibition of dechlorination activity was caused by the inhibition of methanogenic activity. Cobb and Bouwer (1991) reported no effect of sulfate on the transformation of CH_3CCl_3 in a biofilm reactor. However, sulfate was only added at very low concentrations (0.1 mM) at an acetate concentration (electron donor) of 1 mM. Under these conditions, no inhibition of CH_3CCl_3 transformation was observed in our packed-bed reactor, since methanogenesis was not suppressed. Wrenn and Rittmann (1996) also reported no effect of sulfate on CH_3CCl_3 transformation in a methanogenic biofilm reactor even at a sulfate/formate ratio of 1. However, in these experiments no time for adaptive changes in the microbial population was allowed.

Batch experiments with molybdate indicated that sulfate-reducing bacteria in the packed-bed reactor could also transform CH_3CCl_3, as observed before by Egli et al. (1987) for *Desulfobacterium autotrophicum*. However, the effect of both acetate and sulfate concentrations on CH_3CCl_3 transformation showed that sulfate-reducing bacteria only accounted for a minor percentage of the transformation in the packed-bed reactor.

Transformation of CH_3CCl_3 in the packed-bed reactor mainly occurred through reductive dechlorination to the di- and monochloro derivatives (Fig. 5). The percentage of CH_3CCl_3 transformed to CH_3CH_2Cl depended on the methanogenic activity. With an increase in methanogenic activity, the extent of CH_3CCl_3 dechlorination also increased. This suggests that its transformation by methanogens in the packed-bed reactor is a cometabolic process with no benefit for the organisms.

Part of the CH_3CCl_3 was converted to unknown products. For its transformation to products other than CH_3CHCl_2 and CH_3CH_2Cl there are two possibilities: either CH_3CH_2Cl, when formed, was further converted or CH_3CCl_3 was transformed via other initial reactions (Fig. 5). CH_3CH_2Cl can undergo both biotic transformation to ethene or ethane (pathways 3 and 4; Belay and Daniels 1987; Vogel and McCarty 1987a) and abiotic transformation to ethanol (pathway 5; Vogel and McCarty 1987b). Three pathways have been described for the transformation of CH_3CCl_3 via other initial reactions. First, it can be converted to non-volatile halocarbons according to pathways 6, 7 and 8 (Gälli and McCarty 1989a). We did occasionally test for halogenated acetic acids but never detected any of these compounds. The second pathway has been described by Gälli and McCarty (1989a). They found transformation of CH_3CCl_3 to acetic acid by a *Clostridium* sp. (pathway 1). Finally it can undergo abiotic transformation to 1,1-dichloroethene (pathway 2), which can be further degraded (Gälli and McCarty 1989b; Vogel and McCarty 1987a, b). We never detected any 1,1-dichloroethene in the packed-bed reactor as expected, since the first-order rate coefficient for abiotic 1,1-dichloroethene formation is only 0.0024 day^{-1} (Gälli and McCarty 1989b). Our results suggest that part of the CH_3CCl_3 in the packed-bed reactor was converted to non-chlorinated products. It is not yet clear via which pathway complete dechlorination of CH_3CCl_3 occurred and to which products it was converted.

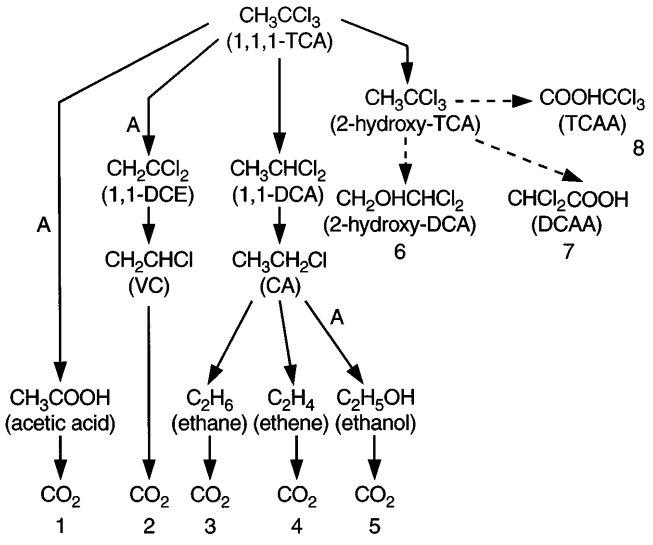

Fig. 5 Pathways for the transformation of 1,1,1-trichloroethane (deduced from Vogel and McCarty 1987). Transformations that have been reported are shown with solid lines. Proposed pathways are shown with dotted lines. *A* Abiotic transformations. *TCA* 1,1,1-trichloroethane; *DCA* 1,1-dichloroethane; *CA* chloroethane; *VC* vinyl chloride; *DCE* 1,1-dichloroethene

Our results suggest that CH_3CCl_3 removal by methanogens is a feasible option, provided that sulfate can be removed and a sufficient amount of suitable electron donor is added. Complete dechlorination occurred, but usually the di- and monochloro derivatives accumulated as undesirable transformation products. Sequential anaerobic/aerobic transformation of CH_3CCl_3 now seems a feasible option for its complete mineralization, since both CH_3CHCl_2 and CH_3CH_2Cl can be degraded under aerobic conditions (Oldenhuis et al. 1989; Scholtz et al. 1987). CH_3CHCl_2 transformation under oxic conditions is much slower than CH_3CH_2Cl transformation and appears to be a cometabolic process (Vogel et al. 1987; McCarty and Semprini 1994). Therefore, a packed-bed reactor that would completely transform CH_3CHCl_3 to

CH_3CH_2Cl and not form any CH_3CHCl_2 is of great interest. Further research will focus on the possibilities of complete transformation of CH_3CCl_3 to CH_3CH_2Cl under methanogenic conditions and the mechanism of this transformation in methanogens.

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