University of Groningen

Complete transformation of 1,1,1-trichloroethane to chloroethane by a methanogenic mixed population

de Best, JH; Hage, A; Doddema, HJ; Janssen, DB; Harder, W

Published in:
Applied Microbiology and Biotechnology

DOI:
[10.1007/s002530051393](https://doi.org/10.1007/s002530051393)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1999

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

de Best, JH., Hage, A., Doddema, HJ., Janssen, DB., & Harder, W. (1999). Complete transformation of 1,1,1-trichloroethane to chloroethane by a methanogenic mixed population. *Applied Microbiology and Biotechnology*, 51(2), 277-283. <https://doi.org/10.1007/s002530051393>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

ORIGINAL PAPER

J. H. de Best · A. Hage · H. J. Doddema
D. B. Janssen · W. Harder

Complete transformation of 1,1,1-trichloroethane to chloroethane by a methanogenic mixed population

Received: 19 June 1998 / Received revision: 14 September 1998 / Accepted: 17 September 1998

Abstract A methanogenic mixed population in a packed-bed reactor completely transformed 1,1,1-trichloroethane (10 μ M) to chloroethane by a cometabolic process. Chloroethane was not further transformed. Acetate and methanol served as electron donors. Complete transformation of 1,1,1-trichloroethane to chloroethane only occurred when sufficient electron donor was fed into the reactor. Otherwise, besides chloroethane, 1,1-dichloroethane was also found as a product. The products of 1,1,1-trichloroethane transformation also depended on the type of electron donor present. With acetate, the degree of dechlorination was higher, i.e. more 1,1,1-trichloroethane was transformed to chloroethane than with methanol. In an enrichment culture obtained from the reactor contents, 1,1,1-trichloroethane was only transformed to 1,1-dichloroethane and was not further metabolized. Methanol, acetate, formate, ethanol, 2-propanol, trimethylamine and H_2 , but not dimethylamine and methylamine, served as electron donors for 1,1,1-trichloroethane transformation by this enrichment culture. Both nitrate and nitrite inhibited 1,1,1-trichloroethane transformation; while nitrate completely inhibited 1,1,1-trichloroethane dechlorination, some conversion did occur in the presence of nitrite. The product(s) of this conversion remain unknown, since no chlorinated hydrocarbons were detected.

Introduction

The toxic solvent 1,1,1-trichloroethane TCA ($Cl_3C_2H_3$) is often encountered as a contaminant in soil and groundwater. The evidence available to date indicates that it can only be biodegraded at a significant rate under anaerobic conditions. Its complete mineralization has been described (Vogel and McCarty 1987; Gälli and McCarty 1989; de Best et al. 1997) but usually 1,1-dichloroethane DCA ($Cl_2C_2H_4$) and chloroethane CA (ClC_2H_5) are found as the main transformation products (Parsons et al. 1985; Gälli and McCarty 1989; Vogel and McCarty 1987; de Best et al. 1997).

Bioremediation can only be considered as a useful remediation technique for TCA contaminated sites if complete dechlorination can be achieved. Sequential anaerobic/aerobic transformation of TCA seems a feasible option since both DCA and CA, products of anaerobic TCA transformation, can be degraded under aerobic conditions (Oldenhuis et al. 1989; Scholtz et al. 1987). DCA transformation under oxic conditions is much slower than CA transformation and appears to be a cometabolic process (Vogel et al. 1987; McCarty and Semprini 1994). Therefore, complete transformation of TCA to CA under anaerobic conditions without formation of DCA is of interest.

Previous studies showed that the ratio of DCA to CA, as products of TCA transformation by a methanogenic population in a packed-bed reactor, depended on the electron donor concentration in the reactor feed and was inhibited by sulfate (de Best et al. 1997). The aim of this study was to obtain the complete transformation of TCA to CA in this reactor and establish how it is influenced by process conditions. Therefore, the transformation of TCA and its products was studied at different electron donor concentrations in the reactor feed. The effect of the type of electron donor on the products of TCA transformation was also investigated. Furthermore, the effects of the electron acceptors nitrate and nitrite on TCA

J. H. de Best¹ (✉) · A. Hage · H. J. Doddema · W. Harder
TNO Environmental Technology and Process Engineering
Division, Department of Environmental Biotechnology,
Apeldoorn, The Netherlands

D. B. Janssen
Department of Biochemistry,
University of Groningen, Groningen, The Netherlands

Present address:

¹ Grontmij A&T, PO Box 119, 3990 DC,
Houten, The Netherlands
Tel.: +31-30-63-44-818
Fax: +31-30-63-79-415

transformation is described. Finally the effect of the pH and the temperature, both important process parameters, is discussed.

Materials and methods

Packed-bed column studies

The experiments were performed in an upflow packed-bed column (glass: height 32 cm; inside diameter 4.42 cm; volume 492 ml) (de Best et al. 1997) packed with polyurethane foam particles ($5 \times 5 \times 6$ mm; Bayer B.V., Mijdrecht, the Netherlands), which were 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 column was continuously fed with an anaerobic non-sterile phosphate- and bicarbonate-buffered mineral medium (de Best et al. 1997). The medium (pH 7.3 ± 0.2) was continuously purged with oxygen-free N_2/CO_2 (99.5%/0.5%) to remove oxygen, and was pumped into the column by means of a peristaltic pump with Marprene tubing. All other tubing was of either Viton or Teflon.

TCA, Na_2S (41.8 μM , to maintain reducing conditions), acetate and/or methanol were added to the medium as a concentrated solution at the influent of the column with a syringe pump. The medium contained no sulfate, but about 20 μM sulfate tended to be present in the influent of the reactor, probably as a result of oxidation of sulfide by oxygen permeating the tubing. The hydraulic retention time in the reactor was 24 h. All experiments were carried out at 25 °C.

Batch-culture studies

Batch-culture studies were done with enrichment cultures, which were obtained from the TCA-transforming packed-bed reactor according to the method described previously (de Best et al. 1997).

Effect of nitrate and nitrite

TCA (5 μM), acetate (1 mM) and methanol (1 mM) were added to the batch cultures as concentrated solutions. After inoculation, nitrate (0, 240, 490, 950 and 2010 μM) or nitrite (0, 80, 187, 397 and 1092 μM) was added as a concentrated solution. The batch cultures were analyzed daily for chlorinated ethanes, methane, carbon dioxide, sulfate, nitrate and nitrite.

Effect of temperature and pH

After addition of TCA (5 μM), acetate (1 mM) and methanol (1 mM), the batch cultures were inoculated and incubated at different temperatures (11.1; 20.5; 25.0; 30.0; 37.3 and 44.0 °C). The cultures were analyzed daily for chlorinated ethanes.

The effect of the pH (6.71; 6.82; 7.26; 7.45; 7.54; 7.87 and 8.17) on TCA transformation (5 μM) was tested in batch cultures with 50 ml medium and 10 ml different buffer solutions to obtain the different pH values. After addition of acetate (1 mM) and methanol (1 mM) as electron donors, the batch cultures were inoculated and analyzed daily for chlorinated ethanes.

Different electron donors

To test the effect of different electron donors on TCA transformation, sulfate was omitted from the medium and replaced by $MgCl_2$. TCA (4.0 μM) and all electron donors tested were added as concentrated solutions. H_2 was added to the headspace with a gas-tight syringe. The final concentration of all electron donors was

1.0 mM. The cultures were analyzed regularly for chlorinated ethanes, methane, carbon dioxide and electron donor.

Analytical methods

TCA, DCA and CA were quantified by headspace gas chromatography. Liquid samples (100–1000 μl) were injected into 10-ml headspace autosampler vials closed 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 in a Hewlett Packard 19395A headspace sampler connected to a gas chromatograph equipped with an electron capture detector and a CP-Sil 5CB column (de Best et al. 1997). Calibration samples were analyzed by the same method to adjust for air/water partition. A four-point curve was used for calibration.

Carbon dioxide and methane concentrations were determined after separation on a CarboPlot P7 column, using a gas chromatograph equipped with a flame ionization detector and a methanizer (de Best et al. 1997). For the reactor, liquid samples (2 ml) were injected into 10-ml headspace gas autosampler vials closed with Teflon-lined butyl rubber stoppers and aluminum crimp seals and equilibrated at 80 °C for 45 min. A sample of 50 μl headspace gas was injected into the GC by hand with a 100- μl Hamilton gas- and liquid-tight syringe. For batch cultures, 50 μl headspace gas was injected into the GC. A four-point calibration curve was used for quantification.

Sulfate, nitrate and nitrite were determined after separation on an Ionpac AG9-SC guard column and Ionpac AG9-SC anion column (Dionex, Breda, the Netherlands) on an ion chromatograph equipped with a conductivity detector, thermal stabilizer, and anion self regenerating suppressor (de Best et al. 1997).

Methanol was quantified by gas chromatography. Liquid samples were centrifuged (10 000 g for 10 min) and injected into 2-ml screw-cap vials with Teflon-lined silicone liners. The vials were sampled (10 μl) with a Chrompack CP 9010 liquid sampler and analyzed on a Chrompack 9001 gas chromatograph (Chrompack, Bergen op Zoom, The Netherlands) equipped with a split injector (split ratio 1:10), a Chrompack CP-Poraplot Q column (length 25 m, inner diameter 0.32 mm, film thickness 10 μm) and a flame ionization detector. Helium served as a carrier gas (0.8 ml/min). The GC had the following settings: injection temperature, 250 °C; oven temperature, 100 °C; detection temperature, 275 °C. The detector signal was processed with the Maestro chromatography data system (Chrompack, Bergen op Zoom, The Netherlands). A five-point calibration curve was used for quantification.

Acetate concentrations were determined by an enzymatic test combination (Boehringer, Mannheim, Germany).

Results

Electron donor concentration

Previous studies showed that cometabolic transformation of TCA (10 μM) by a methanogenic population was possible in an anaerobic packed-bed reactor (de Best et al. 1997). DCA and CA were found as transformation products in the reactor effluent. To determine whether TCA could be completely transformed to CA in the reactor, TCA transformation was studied at different electron donor concentrations.

In the starting conditions, TCA (10.4 μM) was completely transformed to DCA (2.4 μM) and CA (8.0 μM). Acetate (0.97 mM) and methanol (0.69 mM), serving as electron donors, were completely converted. Methane production (0.9 mM) indicated that part of the acetate and methanol added was converted by met-

hanogens. Sulfate-reducing bacteria utilized part of the available electron donor for the reduction of all available sulfate (20 μM).

With an increase of the acetate concentration from 0.97 mM to 1.5 mM, leaving the concentration of methanol (0.69 mM) unchanged, the proportion of TCA that was transformed to CA increased, and less DCA was found as a transformation product (Fig. 1). At an acetate concentration of 2.1 mM, TCA was completely transformed to CA. DCA was no longer detected as a transformation product. CA was not further transformed. Even when the acetate concentration was increased from 2.1 mM to 5.0 mM, all TCA transformed was recovered as CA in the effluent of the reactor. The maximum dechlorination rate of $9.6 \text{ g m}^{-3} \text{ day}^{-1}$ that was calculated for TCA transformation in the reactor is similar to the transformation rates for TCA described previously (Bouwer and McCarty 1983; Bouwer and Wright 1988). More than 97% of both the acetate and methanol was utilized by the methanogenic population in the reactor at all concentrations tested.

Different electron donors

The difference between methanol and acetate as electron donors for TCA transformation in the reactor was examined. In the presence of acetate (1.56 mM), TCA (10.4 μM) was completely transformed to DCA (2.5 μM) and CA (8.1 μM) (Table 1). Acetate was completely utilized, mainly by methanogens, as indicated by methane production (0.99 mM). Sulfate reduction did not occur. Replacement of acetate by methanol (1.53 mM) as electron donor in the reactor had a profound effect on TCA transformation. When a steady state was reached, TCA was still completely transformed but no longer primarily transformed to CA (1.7 μM) but

Table 1 Transformation of different electron donors in a TCA (10 μM) transforming packed-bed reactor. – Not determined. The ratio of the measured CH_4 production ($\text{CH}_4 \text{ m}$) and the theoretically expected CH_4 production ($\text{CH}_4 \text{ th}$)

Electron donor	CH_3COOH (μM)	CH_3OH (μM)
Influent	1562	0
CH_3COOH	0	1529
CH_3OH	10.4	9.8
TCA		
Effluent		
TCA transformed	10.4	9.8
DCA formed	2.4	7.8
CA formed	8.0	1.7
CH_3COOH utilized	1562	–
CH_3OH utilized	– ^a	1529
CH_4 formed	990	759
CO_2 formed	909	203
SO_4^{2-} reduced	< 2	< 2
Ratio $[\text{CH}_4 \text{ m}]/[\text{CH}_4 \text{ th}]^b$	0.63	0.66

to DCA (7.8 μM). The production of methane decreased (Table 1), as predicted from the higher [methane]/[electron donor] ratio with acetate as an electron donor (Eq. 1), compared to that obtained with methanol (Eq. 2).

A theoretical $[\text{CH}_4 \text{ acetate}]/[\text{CH}_4 \text{ methanol}]$ of $1/0.75 = 1.33$ can be calculated from the stoichiometry of these reactions. This ratio is close to the observed ratio of $990/759 = 1.3$ in the reactor.

The results indicated that acetate was a more suitable electron donor than methanol for transformation of TCA by methanogens as the degree of dechlorination in the reactor was much better, i.e. more TCA was transformed to CA with acetate as an electron donor than with methanol as an electron donor.

To determine whether other electron donors supported cometabolic transformation of TCA by methanogens, an enrichment culture from the reactor was used (Table 2). This enrichment culture converted TCA to DCA in a batch culture using acetate as substrate. DCA was not further transformed. With all electron donors tested, except methylamine and dimethylamine, significant transformation of TCA was observed (Table 2). DCA was detected as the only product of TCA transformation with a recovery higher than 91%. Both trimethylamine and methanol were poor electron donors for TCA transformation by this enrichment culture since it was only partially transformed.

Fig. 1 Effect of the electron donor concentration on the transformation of 1,1,1-trichloroethane (TCA) by methanogens in a packed-bed reactor. Chlorinated ethanes in the effluent are expressed as the percentage of 1,1,1-trichloroethane in the influent. The concentration of CH_3OH in the influent of the reactor was 0.69 mM. The concentration of CH_3COOH varied between 1 mM and 5 mM. ■ 1,1-Dichloroethane; ● chloroethane

Effect of nitrate and nitrite on 1,1,1-trichloroethane transformation

Previous studies on TCA transformation (de Best et al. 1997) showed that sulfate – an electron acceptor often found in groundwater – has an effect on (the products of) the transformation. Therefore we studied the effect

Table 2 Effect of different electron donors (1 mM) on the transformation of trichloroethane (TCA) by an enrichment culture from a trichloroethane-transforming packed-bed reactor. The percentage of trichloroethane transformed after 48 days and the percentage of trichloroethane transformed to dichloroethane after 48 days is shown in parentheses

Electron donor	TCA transformed (μM)	DCA formed (μM)
Formic acid	3.87 (100%)	3.74 (97%)
Acetic acid	3.94 (100%)	3.63 (92%)
Methanol	0.90 (23%)	1.09 (121%)
Ethanol	4.01 (100%)	4.15 (103%)
2-Propanol	4.04 (100%)	3.66 (91%)
Methylamine	0.24 (6%)	< 0.01
Dimethylamine	0.21 (5%)	< 0.01
Trimethylamine	1.16 (30%)	1.07 (92%)
H ₂ /CO ₂	3.76 (100%)	3.74 (99%)
None	< 0.01	< 0.01

of two other naturally occurring electron acceptors – nitrate and nitrite – using an enrichment culture from the reactor at nitrate concentrations between 0 mM and 2.01 mM and nitrite concentrations between 0 mM and 1.09 mM. Acetate (1 mM) and methanol (1 mM) served as electron donors.

In the absence of nitrate or nitrite, 1.63 μM TCA was completely transformed to DCA (Table 3). No other transformation products were found. Acetate and methanol were utilized by methanogens and sulfate-reducing bacteria, as indicated by the production of methane (0.47 mM) and the reduction of all available sulfate (0.48 mM) (Table 3).

When nitrate or nitrite was present in the batch cultures, besides methane production and sulfate reduction, nitrate reduction (Table 3) and nitrite reduction (Table 4) to N₂ occurred respectively. Both nitrate and nitrite inhibited the reduction of TCA to DCA in enrichment cultures (Tables 3, 4). The inhibition by nitrite was much stronger than inhibition by nitrate. At nitrate concentrations over 490 μM , conversion of TCA to DCA was only partially inhibited, while at a nitrite concentration of 119 μM , TCA transformation still occurred but neither DCA nor CA was found as a product. The inhibition of the conversion of TCA to DCA coincided with a decrease or complete inhibition of methane production and sulfate reduction in the batch cultures. Since previous studies showed that methanogens are probably involved in this conversion (de

Best et al. 1997), inhibition of TCA conversion probably resulted from a decrease in methanogenic activity.

Effect of pH and temperature on TCA transformation

The effect of two other important environmental conditions, namely pH and temperature, on the transformation of TCA was determined using the enrichment cultures isolated from the reactor. The rate of DCA production, the only product of TCA transformation, was used as a measure for the dechlorination activity.

Transformation of TCA to DCA by the methanogenic population was observed at temperatures between 11 °C and 44 °C (Fig. 2A), with an optimum between 26 °C and 33 °C. The temperatures in groundwater usually are between 10 °C and 15 °C. This means that, for this mixed population, the rate of in situ or on-site biotransformation of TCA could be significantly reduced. However, it has been reported that dechlorinating microorganisms can adapt to temperatures below 15 °C without a significant effect on the kinetics of dechlorination (de Bruin et al. 1992).

TCA transformation by the enrichment culture occurred between pH 6.7 and 8.5 with an optimum between pH 7.4 and 7.6 (Fig. 2B). Usually, contaminated groundwaters have a pH within this range, so that no problems are expected for in situ and on-site biotransformation. However, there may be exceptions, like systems with high concentrations of humic acid, sulfide or carbonate creating an extremely high or low pH (Wilson et al. 1996), where the transformation rates may be considerably lower.

Discussion

Previously we found CA to be the main product of TCA transformation in a packed-bed reactor (de Best et al. 1997), while other studies reported DCA as the main transformation product of biotic TCA transformation (Egli et al. 1987; Gälli and McCarty 1989) and sometimes also traces of CA (Vogel and McCarty 1987). This paper describes the complete biological cometabolic transformation of TCA to CA and the conditions necessary for this complete transformation.

Table 3 Effect of nitrate on the transformation of 1,1,1-trichloroethane after 39 days by an enrichment culture from a 1,1,1-trichloroethane-transforming packed-bed reactor

NO ₃ ⁻ (mM)	TCA transformed (μM)	DCA formed (μM)	CH ₃ COOH utilized (mM)	CH ₃ OH utilized (mM)	NO ₃ ⁻ reduced (mM)	SO ₄ ²⁻ reduced (mM)	CH ₄ formed (mM)
0	1.63	1.62	0.75	1.13	< 0.01	0.48	0.47
0.24	1.52	1.42	0.86	1.07	0.23	0.44	0.44
0.49	1.52	1.63	0.89	1.24	0.48	0.42	0.44
0.95	0.71	0.71	0.84	0.96	0.95	0.01	0.16
2.01	0.17	0.10	0.92	1.06	2.01	< 0.01	0.11

Table 4 Effect of nitrite on the transformation of 1,1,1-trichloroethane after 37 days by an enrichment culture from a 1,1,1-trichloroethane-transforming packed-bed reactor

NO ₂ ⁻ (mM)	TCA transformed (μM)	DCA formed (μM)	CH ₃ COOH utilized (mM)	CH ₃ OH utilized (mM)	NO ₂ ⁻ reduced (mM)	SO ₄ ²⁻ reduced (mM)	CH ₄ formed (mM)
0	1.63	1.62	0.75	1.05	<0.01	0.48	0.47
0.08	1.74	0.55	0.95	1.15	0.08	0.45	0.43
0.19	1.24	<0.01	0.72	<0.05	0.19	<0.01	0.06
0.40	1.19	<0.01	0.57	<0.05	0.40	<0.01	<0.01
1.09	1.17	<0.01	0.96	<0.05	1.09	<0.01	0.03

In general, the rate of cometabolic dechlorination processes increases with an increase in electron donor concentration (Doong and Wu 1996; Wrenn and Rittmann 1996). Here we report that, not only the rate of dechlorination, but also the nature of the products depend on the electron donor concentration.

By extrapolation of the data presented in Fig. 1 it can be calculated that an electron donor concentration (acetate + methanol) of 2.75 mM is necessary for complete transformation of TCA to CA under these conditions. The corresponding molar ratio of [acetate + methanol] to [TCA] of about 275 (2750/10) confirmed that TCA transformation was a cometabolic process. This ratio is within the range of 100–1000 found for other cometabolic transformations of chlorinated compounds (Bouwer and McCarty 1983; Vogel and McCarty 1987; Bouwer and Wright 1988).

Besides the electron donor concentration, the microbial transformation of TCA also depended on the type of electron donor present. Methylamine and dimethylamine did not support TCA transformation by an enrichment culture from the reactor, while methanol and trimethylamine were very poor electron donors compared to others such as acetate and H₂. The final transformation products of TCA also depended on the type of electron donor present. With acetate as an electron donor, the degree of dechlorination in the re-

actor was much better, i.e. more TCA was transformed to CA than with methanol as an electron donor. The effect of different electron donors on the products of biological dechlorination has never been described, but there are several reports of the effect of different electron donors on (the rate of) microbial dechlorination (Bagley and Gossett 1990; Holliger 1992; Lewis and Crawford 1993; Petrovskis et al. 1994; Doong et al. 1996; Wrenn and Rittmann 1996).

Under appropriate conditions, CA was the end-product of TCA transformation in the reactor. Reduction of CA to ethane was not found, although it has been stated that it can be reduced to ethane by cell suspensions of *Methanosarcina barkeri* with a dechlorination rate of 0.58 mmol mol CH₄⁻¹ in the presence of acetate as an electron donor (Holliger 1992). The pseudo-first-order rate constant for abiotic hydrolysis of CA to ethanol of 0.0010 day⁻¹ (Vogel and McCarty 1987) indicated that no significant amount of CA could be transformed to ethanol in the reactor as the hydraulic retention time in the reactor was only 24 h.

Both nitrate and nitrite inhibited reductive dechlorination of TCA to DCA in enrichment cultures from the reactor. The decrease in methanogenic activity in the presence of nitrate was a result of the competition for the available electron donor between the TCA-transforming methanogens and nitrate reducers or sulfate

Fig. 2A,B Effect of temperature (A) and pH (B) on the production of dichloroethane (DCA) from 1,1,1-trichloroethane by an enrichment culture from a trichloroethane-transforming packed-bed reactor. Acetate (1 mM) and methanol (1 mM) served as electron donors. **A** pH = 7.5; **B** T = 25 °C

reducers (competitive inhibition), since both acetate and methanol were nearly completely utilized at all nitrate concentrations. Competitive inhibition of the TCA-transforming methanogens could be prevented by adding excess electron donor. The decrease in methanogenic activity in the presence of nitrite was not a result of competition for the available substrate, but was caused by the toxicity of nitrite. At a nitrite concentration of 0.19 mM or higher, sufficient substrate was available for nitrite reduction as well as sulfate reduction and methane production but only nitrite reduction occurred (Table 4). Nitrite is often found to be toxic for microorganisms because of its inhibitory effects on electron carriers (Stouthamer 1988). Electron carriers, such as cobalamins (Krone et al. 1989a, 1991) or factor F₄₃₀ (Krone et al. 1989b; Gantzer and Wackett 1991), are often involved in the cometabolic transformation of chlorinated hydrocarbons under anaerobic conditions.

Although TCA transformation to DCA was completely inhibited at nitrite concentrations above 80 µM, about 1.2 µM of TCA was still transformed (Table 4). The products of this transformation are unknown but no chlorinated hydrocarbons were found as transformation products. Others also described complete dechlorination of TCA to CO₂ (Vogel and McCarty 1987; Gälli and McCarty 1989), acetic acid and other unknown products (Gälli and McCarty 1989), but these products only accounted for a minor percentage of the TCA transformed. Methanogens and sulfate-reducing bacteria were probably not involved in this transformation of TCA in the presence of nitrite. While methane production and sulfate reduction no longer occurred, TCA was still transformed. It is not clear whether nitrite-reducing bacteria play a role in this transformation of TCA to non-chlorinated products.

Fast and complete biological dechlorination of TCA is only likely to occur in a sequential anaerobic/aerobic process. Here we have described the conditions necessary for the anaerobic process: complete transformation of TCA to CA by a methanogenic mixed population without the formation of DCA. CA, when formed, can be further dechlorinated under aerobic conditions (Keuning et al. 1985; Scholtz et al. 1987). Whether TCA was completely transformed to CA depended both on the electron donor concentration and on the type of electron donor present in the reactor. Further research should focus on the development and optimization of a sequential anaerobic/aerobic reactor system for TCA mineralization.

Acknowledgements This work was financed by a grant IOP91225 from the Dutch IOP Environmental Biotechnology program.

References

- Bagley DM, Gossett JM (1990) Tetrachloroethene transformation to trichloroethene and *cis*-1,2-dichloroethene by sulfate-reducing enrichment cultures. *Appl Environ Microbiol* 56: 2511–2516
- Best JH de, Jongema H, Weijling A, Doddema HJ, Janssen DB, Harder W (1997) Transformation of 1,1,1-trichloroethane in an anaerobic packed-bed reactor at various concentrations of 1,1,1-trichloroethane, acetate and sulfate. *Appl Microbiol Biotechnol* 48: 417–423
- Bruin WP de, Kotterman MJJ, Posthumus MA, Schraa G, Zehnder AJB (1992) Complete biological reductive transformation of tetrachloroethene to ethane. *Appl Environ Microbiol* 58: 1996–2000
- Bouwer EJ, McCarty PL (1983) Transformations of 1- and 2-carbon halogenated aliphatic organic compounds under methanogenic conditions. *Appl Environ Microbiol* 45: 1286–1294
- Bouwer EJ, Wright JP (1988) Transformations of trace halogenated aliphatics in anoxic biofilm columns. *J Cont Hydrol* 2: 155–169
- Doong RA, WU S-C (1996) Effect of substrate concentration on the biotransformation of carbon tetrachloride and 1,1,1-trichloroethane under anaerobic conditions. *Water Res* 30: 577–586
- Doong RA, Chen TF, Chang WH (1996) Effect of electron donor and microbial concentration on the enhanced dechlorination of carbon tetrachloride by anaerobic consortia. *Appl Microbiol Biotechnol* 46: 183–186
- Egli C, Scholtz R, Cook AM, Leisinger T (1987) Anaerobic dechlorination of tetrachloromethane and 1,2-dichloroethane to degradable products by pure cultures of *Desulfobacterium* sp. and *Methanobacterium* sp. *FEMS Microbiol Lett* 43: 257–261
- Gälli R, McCarty PL (1989) Biotransformation of 1,1,1-trichloroethane, trichloromethane, and tetrachloromethane by a *Clostridium* sp. *Appl Environ Microbiol* 55: 837–844
- Gantzer CJ, Wackett LP (1991) Reductive dechlorination catalyzed by bacterial transition-metal coenzymes. *Environ Sci Technol* 25: 715–722
- Holliger C (1992) Reductive dehalogenation by anaerobic bacteria. PhD-thesis Wageningen Agricultural University, Wageningen, The Netherlands, pp 19–28
- Keuning S, Janssen DB, Witholt B (1985) Purification and characterization of hydrolytic haloalkane dehalogenase from *Xanthobacter autotrophicus* GJ10. *J Bacteriol* 163: 635–639
- Krone UE, Thauer RK, Hogenkamp HPC (1989a) Reductive dehalogenation of chlorinated C₁-hydrocarbons mediated by corrinoids. *Biochemistry* 28: 4908–4914
- Krone UE, Laufer K, Thauer RK, Hogenkamp HPC (1989b) Coenzyme F₄₃₀ as a possible catalyst for the reductive dehalogenation of chlorinated C₁-hydrocarbons in methanogenic bacteria. *Biochemistry* 28: 10061–10065
- Krone UE, Thauer RK, Hogenkamp HPC, Steinbach K (1991) Reductive formation of carbon monoxide from CCl₄ and FREONs 11, 12 and 13 catalyzed by corrinoids. *Biochemistry* 30: 2713–2719
- Lewis TA, Crawford RL (1993) Physiological factors affecting carbon tetrachloride dehalogenation by the denitrifying bacterium *Pseudomonas* sp. strain KC. *Appl Environ Microbiol* 59: 1635–1641
- McCarty PL, Semprini L (1994) Ground-water treatment for chlorinated solvents. In: Norris RD, Hincsee RE, Brown R, McCarty PL, Semprini L, Wilson JT, Kampbell DH, Reinhard M, Bouwer EJ, Borden RC, Vogel TM, Thomas JM, Ward CH, Matthews JE (eds) *Handbook of bioremediation*. Lewis, Boca Raton, Fla, pp 87–116
- Oldenhuis R, Vink RLJM, Janssen DB, Witholt B (1989) Degradation of chlorinated aliphatic hydrocarbons by *Methylosinus trichosporium* OB3b expressing soluble methane monooxygenases. *Appl Environ Microbiol* 55: 2819–2826
- Parsons F, Barrio-Lage G, Rice R (1985) Biotransformation of chlorinated organic solvents in static environments. *Environ Toxicol Chem* 4: 739–742
- Petrovskis EA, Vogel TM, Adriaens P (1994) Effects of electron acceptors and donors on transformation of tetrachloromethane

- by *Shewanella putrefaciens* MR-1. FEMS Microbiol Lett 121: 357–364
- Scholtz R, Schmucke A, Cook AM, Leisinger T (1987) Degradation of eighteen 1-monohaloalkanes by *Arthrobacter* sp. strain HA1. J Gen Microbiol 133: 267–274
- Stouthamer AH (1988) Dissimilatory reduction of oxidized nitrogen compounds. In: Zehnder AJB (ed) Biology of anaerobic microorganisms. Wiley-Liss, New York, pp 245–304
- Vogel TM, McCarty PL (1987) Abiotic and biotic transformations of 1,1,1-trichloroethane under methanogenic conditions. Environ Sci Technol 21: 1208–1213
- Vogel TM, Criddle CS, McCarty PL (1987) Transformation of halogenated aliphatic compounds. Environ Sci Technol 21: 722–736
- Wilson BH, Wilson JT, Luce D (1996) Design and interpretation of microcosm studies for chlorinated compounds. In: Symposium on natural attenuation of chlorinated organics in ground water. Office of research and development U.S. environmental protection agency, Washington, DC, pp 21–28
- Wrenn BA, Rittmann BE (1996) Evaluation of a model for the effects of substrate interactions on the kinetics of reductive dehalogenation. Biodegradation 7: 49–64