Biodegradation **10:** 287–295, 1999.

Transformation of carbon tetrachloride in an anaerobic packed-bed reactor without addition of another electron donor

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Accepted 13 July 1999

Key words: anaerobic, carbon tetrachloride, electron donor, metabolic transformation, transformation products

Abstract

Carbon tetrachloride (52 μM) was biodegraded for more than 72% in an anaerobic packed-bed reactor without addition of an external electron donor. The chloride mass balance demonstrated that all carbon tetrachloride transformed was completely dechlorinated. Chloroform and dichloromethane were sometimes also found as transformation products, but neither accumulated to significant levels in comparison to the amount of carbon tetrachloride transformed. Transformation of carbon tetrachloride in the absence of an added electron donor suggests that carbon tetrachloride itself is the source of energy for the biological reaction observed, and possibly the source of carbon for cell growth. No such mechanism is yet known. The pathway of carbon tetrachloride transformation is not clear; it may be dehalogenated by hydrolytic reduction to carbon monoxide or formic acid which are electron demanding transformations. Carbon monoxide or formic acid may be further utilized and serve as electron donor. Complete dechlorination of carbon tetrachloride according to this pathway is independent of a second electron donor or electron acceptor, as with a fermentation process. Vancomycin, an inhibitor of gram positive eubacteria, severely inhibited carbon tetrachloride transformation in batch incubations with an enrichment culture from the reactor, indicating that gram positive eubacteria were involved in carbon tetrachloride transformation. Batch experiments with bromoethanesulfonic acid, used to inhibit methanogens, and molybdate, an inhibitor of sulfate reduction in sulfate reducing bacteria, demonstrated that neither methanogens nor sulfate reducers were involved in the complete dechlorination of carbon tetrachloride.

Introduction

Carbon tetrachloride (CT) is a common water pollutant due to widespread use as an industrial solvent. CT resists aerobic biotransformation, but several authors have described transformation of CT under anaerobic conditions in both continuous flow studies (Bae et al. 1990, Bouwer and McCarty 1983a, Bouwer and Wright 1988, Cobb and Bouwer 1991, Petersen et al. 1994, de Best et al. 1998) and batch culture studies (Bouwer and McCarty 1983b, Criddle et al. 1990, Egli et al. 1987 1988, Gälli and McCarty 1989, Picardal et al. 1995). In all of these studies, CT transformation was described as a cometabolic process. During cometabolic transformation, dehalogenation is not coupled to energy conservation and occurs due to a fortuitous reaction with certain enzymes or coenzymes (Holliger and Schumacher 1994). Cometabolic dehalogenation processes require both an external electron donor and electron acceptor to support growth of the active organisms.

Another microbial process for dehalogenation is dehalorespiration. In this process reductive dehalogenation is coupled to energy conservation. The halogenated compound serves as a terminal electron acceptor and only an external electron donor is required (Holli-

ger and Schumacher 1994, Máymo-Gatell et al. 1997, Mohn and Tiedje 1990). Theoretically, CT can also serve as terminal electron acceptor since the redox potential of redox couples for CT transformation are positive and higher than the redox potential for couples of common electron acceptors such as nitrate, sulfate or carbon dioxide (Vogel et al. 1987).

A third anaerobic dehalogenation process, described for dichloromethane (DCM) (Mägli et al. 1996) and chloromethane (Traunecker et al. 1991), is fermentation. Fermentative dehalogenations, are processes that are carried out in the absence of an externally supplied electron acceptor and with an internally balanced oxidation-reduction reaction.

The aim of this work was to investigate the requirement of CT transformation for electron donors and electron acceptors. CT transformation was studied in an anaerobic continuous-flow packed-bed reactor at different electron donor (acetate) concentrations and in the absence of an additional electron donor and electron acceptor. To obtain more information about the pathway and products of CT transformation, ^{13}C-CT transformation was studied. Finally, the role of methanogenic, acetogenic and sulfate reducing microorganisms in CT transformation is discussed.

Material and methods

Packed-bed reactor studies

The experiments were performed in an upflow packed-bed reactor (glass; height 40 cm; inside diameter 4.6 cm; volume 665 ml) (de Best et al. 1997) packed with polyurethane foam (PUR) particles ($5 \times 5 \times 6$ mm, Bayer B.V., Mijdrecht, the Netherlands) mixed with digested sludge from the wastewater treatment plant Kralingseveer (Rotterdam, The Netherlands). The biomass content added to the reactor was about 0.8 g volatile suspended solids (VSS). The packed-bed reactor was wrapped with aluminum foil to prevent growth of phototrophs.

The reactor was continuously fed over a period of 5 years with an anaerobic non-sterile phosphate and bicarbonate buffered mineral medium that contained no organics or yeast extract (de Best et al. 1997). The medium was continuously purged with oxygen free N_2/CO_2 (99.5/0.5%) to remove all oxygen. The medium (pH 7.3 ± 0.2) was pumped into the reactor by means of a peristaltic pump with marprene tubing. All other tubing was either viton or Teflon. CT, acetate (CH_3COOH) and Na_2S (42 μM, to maintain reducing conditions) were added to the medium as a concentrated solution at the influent of the reactor with a syringe pump. Although the medium contained no added sulfate, about 0.15 mM sulfate was present in the influent of the reactor, probably due to oxidation of sulfide by oxygen permeating the tubing. The hydraulic retention time in the packed-bed was 24 h, based on void volume (flow rate 0.411/day). All experiments were carried out at 25 °C.

^{13}O-carbon tetrachioride studies

Experiments with ^{13}C-CT were done in the packed-bed reactor under the same conditions as described above. ^{13}C-CT (56 μM), together with acetate (30 μM) and Na_2S (42 μM), was added to the medium from a concentrated stock solution at the influent of the reactor with a syringe pump. After two weeks of adaptation, daily samples (5 ml) were taken at the influent and effluent (40 cm) of the reactor and at two sample ports at different heights along the reactor (20, 30 cm) for a period of 20 days. Samples were frozen until used.

Immediately after the 20 daily samples taken at the same sample port were pooled, the pH was adjusted to a value below the pKa (4.77) of acetic acid. Each sample was saturated with NaCl and extracted with diethylether (1 : 1). The extract was concentrated by a factor 10 and analyzed for acetic acid and formic acid. CT was analyzed according to the method described at the analytical methods.

Batch culture studies

Batch incubations were done using an anaerobic minimal medium containing (per liter of demineralized water) 80.1 mg $(NH_4)_2HPO_4$, 200 mg $MgSO_4 \cdot 7H_2O$, 1 mg resazurin and 5 ml trace element solution (de Best et al. 1997). The medium was purged with a mixture of CO_2 and N_2 (0.5 : 99.5 v/v%, 700 ml/mm) for 45 min. After purging, $Na_2S \cdot 9H_2O$ (91 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 of medium and was closed with Teflon-lined butyl rubber stoppers and aluminum crimp seals. After sterilization, CT (6.3 μM) and acetate (100 μM) were added as concentrated solutions. All cultures were inoculated with 2 ml of the liquid phase taken from the packed-bed reactor. The cultures were incubated on a shaker (100 rpm) in a canted

position at 25 °C and analyzed regularly for chlorinated compounds (taking into account the partitioning), acetate, sulfate, CO_2, CO, CH_4 and chloride.

To investigate the role of methanogens and gram positive eubacteria in the transformation of CT, inhibitors were added to some batch cultures at $t = 0$. Vancomycin (0.14 mM), 2-bromoethane sulfonic acid (Bres; 6 mM) and molybdate (2 mM) were used as inhibitors.

Analytical methods

CT, CF, DCM and chloromethane were quantified by headspace gas chromatography. 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 sampler connected to a gas chromatograph equipped with an electron capture detector and a CP-Sil 5CB reactor (de Best et al. 1997). Calibration samples were analyzed according to the same method to adjust for air/water partitioning. 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 FID and a methanizer (de Best et al. 1997). Liquid samples (2 ml) from the packed-bed reactor 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. An amount of 50 μl of the headspace was injected into the GC by hand with a 100 μl Hamilton gas and liquid-tight syringe. For batch cultures, 50 μl of the headspace was injected into the GC. A four point calibration curve was used for quantification.

Samples from ^{13}C-CT experiments were analyzed for peaks of molecular weights of 60 (^{12}C-CH_3COOH), 61 and 62 (^{13}C-CH_3COOH) with a gas chromatograph equipped with a mass selective detector (Hewlett Packard, Wilmington, USA) in the selected-ion-monitoring (SIM) mode. The presence of ^{13}C-CH_3COOH besides ^{12}C-CH_3COOH was demonstrated from an increase in the 61/60 amu isotopic intensity ratio (All-ratio). The natural ratio between ^{13}C and ^{12}C is 1.1%. This means that unlabeled CH_3COOH will give a peak in the mass spectrum at 60 amu (^{12}C-CH_3COOH) and a smaller one at 61 amu (^{13}C-CH_3COOH). The intensity of the peak at 61 amu will be about 1.1% of the peak at 60 amu. An increase in this intensity ratio demonstrates the presence of ^{13}C-CH_3COOH in a sample. The detection limit for unlabeled ^{12}C-CH_3COOH was 1.67 μM.

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

Acetate concentrations were determined with an enzymatic test-combination based on the formation of NADH (Boehringer, Mannheim, Germany). NADH formation was measured by the increase in absorbance at 340 nm on a JASCO UV/VIS spectrophotometer (JASCO, The Netherlands). The detection limit was 3 μM.

Chemicals

All chemicals were obtained from commercial suppliers. Carbon tetrachloride was obtained from Baker. Chloroform (CF) and dichloromethane were purchased from Rathburn. ^{13}C-carbon tetrachloride was purchased from C.N. Schmidt B.V. (Amsterdam, The Netherlands) as a 99% pure solution. Vancomycin and sodium molybdate were purchased from Sigma. Chloromethane and 2-bromo-ethanesulfonic acid were obtained from Aldrich. Calibration gases were obtained from AGA (carbon dioxide, methane).

Results

Transformation of CT was studied in a packed-bed reactor under conditions of simultaneous methanogenesis and sulfate reduction to investigate the rate and extent of biological mineralization. Acetate (1 mM) served as a substrate.

First the packed-bed reactor was operated without CT. Acetate was completely utilized by methanogenic and sulfate reducing bacteria. Methane production (0.85 ± 10.05 mM) indicated that 85% of the added acetate was converted by methanogens. Methanogenic bacteria use acetate both as electron acceptor and donor and convert acetate to methane and carbon dioxide. The presence of methanogens was confirmed by fluorescence microscopy (Doddema and Vogels 1978). Sulfate reducing bacteria utilized 15% of the added acetate for the complete reduction of all available sulfate (0.15 ± 0.1 mM).

Four days after CT (1.3 μM) was first added to the influent, complete transformation of CT occurred. Most of the CT transformation (>85%) took place within the first 10 cm of the packed-bed reactor. Chloroform (0.30 μM) was found as a transformation product at the first sample port. Further along the reactor chloroform (CF) was completely degraded.

Since CT is often found in contaminated groundwater's at higher concentrations, CT transformation in the packed-bed reactor was studied at different concentrations. The CT concentration in the reactor was increased stepwise to 60 μM over a period of 15 weeks. Up to a concentration of 40 μM CT was completely transformed. Besides CF (<0.9 μM), dichloromethane (<0.8 μM) was found as a transformation product in the effluent of the reactor. At a CT concentration of 60 μM, low concentrations of CT ($0.9 \pm 0.2 \mu$M) were detected in the effluent of the reactor showing that CT was transformed for more than 98%.

Acetate (1 mM) was completely metabolized up to a CT concentration of 40 μM. Sulfate reducing microorganisms accounted for about 15% of the removal of added acetate as calculated from the amount of sulfate removed, while methanogens converted about 85% to methane according to the amount of methane produced. When the CT concentration was further increased to 60 μM, acetate utilization decreased until only about 65% of the added acetate was metabolized (Table 1). Sulfate reducing bacteria still utilized 0.15 mM of the added acetate for the complete reduction of sulfate. Methane production by methanogens decreased from 0.85 to about 0.55 mM.

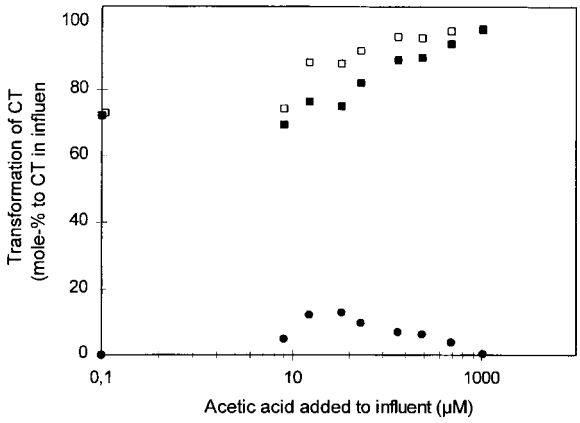

Figure 1. Transformation of carbon tetrachloride in an anaerobic packed-bed reactor at different acetate concentrations. Symbols: CCl_4 transformed (□); CCl_4 mineralized (■); $CHCl_3$ formed (●).

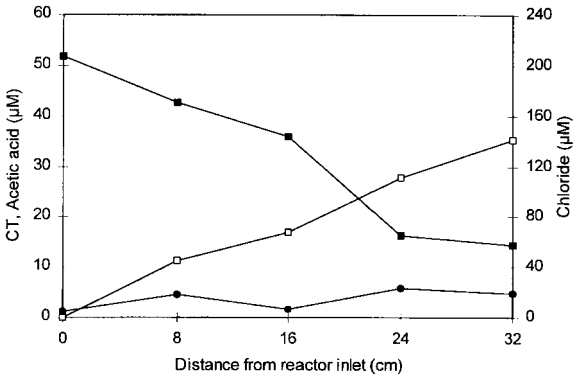

Figure 2. Transformation of carbon tetrachloride in an anaerobic packed-bed reactor without addition of an electron donor. Symbols: CCl_4 (■); Cl (□), CH_3COOH (●).

Effect of the acetate concentration on carbon tetrachloride transformation

To determine the acetate concentration that is sufficient for complete transformation of CT, the acetate concentration was decreased stepwise from 1000 to 0 μM at an influent CT concentration of about 60 μM. At each acetate concentration the reactor was operated for at least 60 days.

Down to an acetate concentration of 8 μM, the amount of CT transformed slowly decreased (Figure 1). This was in great part caused by a decrease in CT mineralization. The amount of CT converted to CF increased. The concentration of dichloromethane (DCM) in the effluent of the reactor was always low (< 0.6 μM) and further decreased during the course of the experiment until DCM was no longer found as a transformation product. At acetate concentrations below 30 μM, the percentage of CT mineralized no longer decreased and remained constant at about 73% of the CT added.

Carbon tetrachloride transformation in the absence of acetate

The continuous absence of acetate in the influent of the reactor had no significant effect on CT transformation. After running the packed-bed reactor in the absence of acetate for 115 days (CT was continuously added at a concentration of about 53 μM) still 37.5 μM (72%) of CT was degraded (Figure 2, Table 1), mainly to non-chlorinated products as indicated by the chloride production (141 μM). CF was no longer found as a transformation product.

Table 1. Mass balance of an anaerobic carbon tetrachloride degrading packed-bed reactor. CH_3COOH (different concentrations), CCl_4 (56.8 ± 4 μM), SO_4^{2-} (0.15 ± 0.1 mM) and CO_2 (1.1 ± 0.05 mM) were present in the influent of the reactor. $CHCl_3$, CH_2Cl_2 or CH_4 were not present in the influent of the reactor. The mass balance was calculated from the difference between influent and effluent concentrations

Influent (μM)		Transformation/formation[e] (μM)							
CH_3COOH	CCl_4	CCl_4 transformed	$CHCl_3$ formed	CH_2Cl_2 formed	Cl^- formed	CH_3CCOOH transformed	SO_4^{2-} transformed	CH_4 formed	CO_2 formed
1030	60.6	59.4	0.3	0.2	–[a]	630	128	570	420
484	56.6	55.3	2.1	0.2	–	319	134	268	224
237	56.3	53.9	3.5	0.1	–	160	105	71	149
132	61.3	58.8	4.3	<[b]	–	101	110	30	115
53	59.1	54.2	5.7	0.5	204	46	46	3	73
33	55.8	49.0	7.2	<	157	15	11	<	–
15	53.5	47.3	6.5	<	161	8	–	<	–
8	59.4	44.1	2.9	<	178	6	<	<	–
0	51.9	37.5	<	<	141	−4[d]	<	<	–
0[c]	53.5	37.3	<	<	143	−5	<	<	–

[a] not determined; [b] <, below detection limit; [c] no Na_2S (42 μM) added to the influent of the reactor; [d] negative sign means production in the reactor; [e] calculated from difference between influent and effluent concentrations.

Table 2. Transformation of ^{13}C-labeled carbon tetrachloride in an anaerobic packed-bed reactor

Sample port	Distance from reactor inlet (cm)	CT (μM)	C (μM)	^{12}C-CH_3COOH (μM)	^{13}C-CH_3COOH (μM)	61/60 All-ratio[a] (%)
1	0 (=influent)	56.2	0	33	<[b]	1.3 ± 0.1
3	20	33.6	ND[c]	28	0.1	1.6 ± 0.1
4	30	18.6	ND	1.7	<	1.6 ± 0.1
5	40 (=effluent)	15.2	157	<	<	–[d]
–	standard[e]	–	33.3	–	1.3 ± 0.1	

[a] All-ratio = amu isotopic intensity ratio; [b] <, below detection limit; [c] ND not determined; [d] cannot be determined; [e] standard = 33.3 μM (2.0 mg/l) unlabeled CH_3COOH.

The complete dechlorination of CT in the reactor was not an abiotic (chemical) conversion for two reasons. First, in a sterile control reactor, filled with PUR particles and autoclaved digested sludge (three times for 20 min at 120 °C) no transformation of CT was detected. This indicated that abiotic hydrolysis of CT to CO_2 (Figure 3, pathway 1), as described by Kriegman-King and Reinhard (1992), was not a significant process in our reactor. Secondly, removal of sulfide, a potential chemical reductant of CT (Figure 3, pathway 5) (Curtis and Reinhard 1994, Kriegman-King and Reinhard 1992), from the influent of the reactor had no significant effect on the transformation of CT (Table 1, bottom line).

Mass balance for carbon tetrachloride transformation

For a better understanding of the mechanism of microbial CT transformation in the reactor, mass balances were determined at different acetate concentrations. Table I shows mass balances of the packed-bed reactor at nine different acetate concentrations. The mass balance at each acetate concentration is the average of at least two steady states. A steady state was based on constant performance for 28 days.

With a decrease in acetate concentration, the production of methane proportionally decreased until no methane production occurred. Sulfate was nearly completely reduced down to an acetate concentration of 132 μM. At acetate concentrations below 132 μM, sulfate reduction gradually decreased until sulfate reduction no longer occurred. Since CT transformation

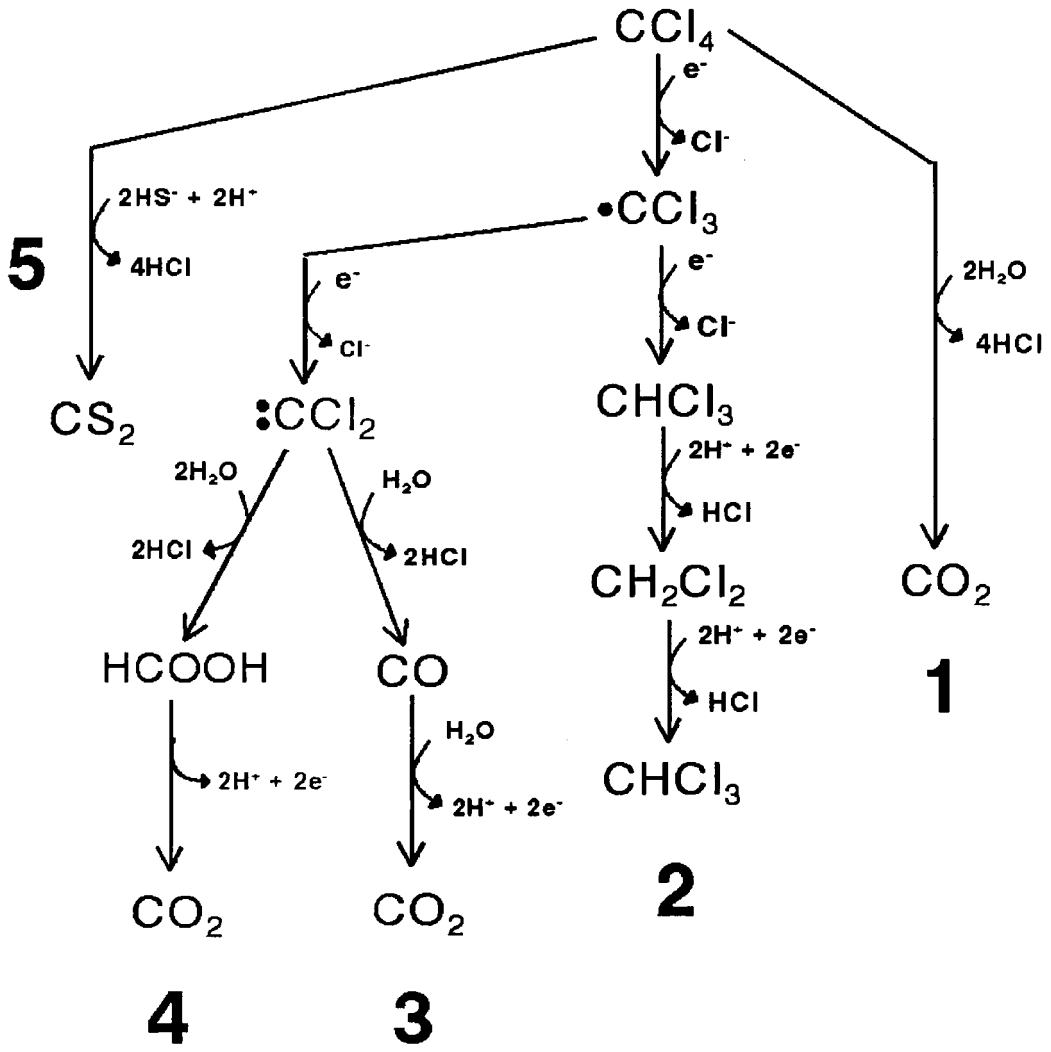

Figure 3. Proposed pathways for the anaerobic transformation of carbon tetrachloride (deduced from Criddle et al. 1991).

still occurred in the absence of methane production and sulfate reduction, these results suggest that neither methanogenic nor sulfate reducing bacteria were involved in CT transformation.

When acetate was no longer added to the influent of the reactor, acetate was still found at different heights in the reactor (Figure 2). A net acetate production of 4 up to 20 μM was also measured at acetate concentrations in the influent of the reactor between 8 and 250 μM (data not shown). Acetate production usually indicates activity by acetogenic bacteria. Besides CT added to the influent, viable and non-viable biological solids present in the reactor are the only possible carbon source which theoretically could serve as a substrate for acetogens. When CT was removed from the influent of the reactor, acetate production no longer occurred. This indicates that viable and non-viable biological solids probably did not serve as a substrate for acetogens. To demonstrate transformation of CT to CH_3COOH in our reactor we carried out experiments with ^{13}C labeled carbon tetrachloride (^{13}C-CT).

Transformation of ^{13}C-CT

The transformation of ^{13}C-CT was studied at a concentration of unlabeled ^{12}C-CH_3COOH in the influent of the reactor of 30 μM. Addition of unlabeled ^{12}C-CH_3COOH to the influent of the reactor was necessary because the concentration of ^{13}C-CH_3COOH was de-

termined from the 61/60 amu isotopic intensity ratio (All-ratio) of unlabeled ^{12}C-CH_3COOH (see material and methods). Unlabeled ^{12}C-CH_3COOH was added to the influent at a low concentration to prevent interference of the peak of unlabeled ^{12}C-CH_3COOH with the peak of ^{13}C-CH_3COOH in the mass spectrum. Besides ^{12}C-CH_3COOH, no other electron donor was added to the influent of the reactor.

When ^{13}C-CT (60 μM) was added, the conversion of CT did not alter. During the 35 days of the experiment, about 80% of the CT added was transformed. CF and DCM were not found as transformation products. The liquid samples taken at the influent (first sample port) and effluent of the reactor and at sample ports at 20 and 30 cm from the inlet of the reactor, were analyzed for $^{13}CH_3COOH$ and $^{12}CH_3COOH$. The results are shown in Table 2.

The 61/60 amu ratio of the standard of unlabeled ^{12}C-CH_3COOH (33.3 μM) was 1.3 ± 0.1%. This ratio differs from the theoretical ratio of 1.1%. This is probably caused by an interference of the solvent used for extraction with the intensity of molecular mass 60 and seemed to have no effect on the determination of ^{13}C-CH_3COOH.

At the first sample port of the reactor, 33 μM of unlabeled acetate was found. The All-ratio was similar to the standard, indicating that no additional ^{13}C-CH_3COOH is present. At sample ports 3 and 4, part of the added acetate was utilized, and an increase in the All-ratio from 1.3 ± 0.1detected. This increase indicated that ^{13}C-CH_3COOH was a product of CT transformation in the reactor. Egli et al. (1988) also found transformation of ^{14}C-CT to $^{14}CH_3COOH$. $^{14}CH_3COOH$ accounted for 38% of the products formed by *Acetobacterium Woodii*.

From the increase in the All-ratio and the unlabeled ^{12}C-CH_3COOH (28 μM) concentration, a concentration of ^{13}C-CH_3COOH of 0.1 μM was calculated for sample port 3, resulting from ^{13}C-CT transformation. The ^{12}C-CH_3COOH concentration of sample port 4 is too low to calculate a reliable ^{13}C-CH_3COOH concentration. These very low concentrations of ^{13}C-CH_3COOH in the reactor were probably caused by an immediate utilization of ^{13}C-CH_3COOH formed. The product of ^{13}C-CH_3COOH transformation, probably ^{13}C-CO_2, could not be detected.

Effect of inhibitors on CT transformation

To determine whether acetogenic bacteria could be involved in the transformation of CT (6.3 μM), batch experiments with an enrichment culture obtained from the reactor were performed in the presence of vancomycin (0.14 mM). Vancomycin, an inhibitor of cell wall synthesis in gram positive eubacteria (Distefano et al. 1992) and used to inhibit acetogenic bacteria, partly inhibited transformation of CT for 14 days (Table 3). In the absence of vancomycin, transformation of CT started immediately. These results indicated that gram positive bacteria, possibly acetogens, were in some way involved in the transformation of CT.

At acetate concentrations in the reactor below 53 μM, no methane production occurred while about 74% of the CT added to the influent of the reactor was removed (Table 1). This suggested that methanogenic bacteria were not involved in the transformation of CT. This was confirmed by batch experiments in the presence of 2-bromoethane sulfonic acid (Bres; 6 mM). Bres, an inhibitor of methanogenesis (Distefano et al. 1992), only reduced the transformation of CT by about 10%, whereas methane production was completely inhibited (Table 3).

At acetate concentrations in the reactor below 8 μM (Table 1) and batch experiments in the absence of inhibitors (Table 3), no sulfate reduction occurred while CT was still transformed. This suggested that sulfate reducing bacteria were also not involved in the transformation of CT. This was confirmed by batch experiments in the presence of molybdate (2 mM). Molybdate, a specific inhibitor of sulfate reducing bacteria (Smith and Klug 1981), only reduced the transformation of CT by about 10% while no sulfate reduction occurred.

In sterile control batches there was no transformation of CT and CH_3COOH (data not shown).

Discussion

Here we report complete biological dechlorination of CT (37.5 μM) in an anaerobic packed-bed reactor without addition of an electron, suggesting that CT degradation in the reactor was independent of an added electron donor. It is also likely that CT served as an electron acceptor since CT is a much stronger electron acceptor than carbon dioxide, the only other potential electron acceptor present in the reactor.

Two possible pathways for the microbial transformation of CT can be distinguished where degradation is independent of a (second) electron donor or electron acceptor, as with a fermentation process. Both pathways start with a 2-electron reduc-

Table 3. Effect of inhibitors on transformation of carbon tetrachloride with 100 μM of acetate and with or without and inhibitor (batch experiments)

Inhibitor	CCl_4 degraded (μM)	$CHCl_3$ formed (μM)	CH_3COOH degraded (μM)	CH_4 formed (μM)	SO_4^{2-} reduced (μM)
None	6.29 (100%)	0.69	37.5	36.5	<
Bres	5.30 (88%)	0.69	33.8	<	<
Vancomycin	2.31 (38%)	<[a]	6.5	4.0	<
Molybdate	5.42 (86%)	0.29	35.5	5.8	<

[a] Below detection limit.

Table 4. Gibbs free energy changes ($\Delta G^{0'}$) of carbon tetrachloride transformation

Reaction		$\Delta G^{\circ'}$ (kJ/reaction)[a]
1. $CCl_4 + H_2O + 2e^-$	$\rightarrow CO + 2H^+ + 4Cl^-$	–528.3
2. $CCl_4 + 2H_2O + 2e^-$	$\rightarrow HCOOH + 2H^+ + 4Cl^-$	–544.8
3. $CO + H_2O$	$\rightarrow CO_2 + 2H^+ + 2e^-$	–91.4
5. HCOOH	$\rightarrow CO_2 + 2H^+ + 2e^-$	–74.9
6. $CO + 1/2H_2O$	$\rightarrow 1/4CH_3COOH + 1/2CO_4$	–39.5
7. HCOOH	$\rightarrow 1/4CH_3COOH + 1/2CO_2 + 1/2H_2O$	–23.0
8. $1/4CH_3COOH + 1/2H_2O$	$\rightarrow 1/2CO_2 + 2H^+ + 2e^-$	–51.9
9. $CCl_4 + 2H_2O$	**$\rightarrow CO_2 + 4H^+ + 4Cl^-$**	**−619.7**

[a] Calculated on the basis of data from Thauer et al. (1977) and Dolfing and Janssen (1994), assuming the following conditions: H^+, 10^{-7} M; Cl^-, 10^{-3} M.

tion of CT, which formally leads to the formation of dichlorocarbene (Figure 3, pathway 3 and 4). Dichlorocarbene is further transformed to either CO or HCOOH through substitutive dehalogenation. CO and HCOOH can be further utilized and serve as electron donor and source of carbon resulting in the formation of carbon dioxide (Figure 3, Table 4). Overall, for the complete dechlorination of CT to CO_2 no net electrons, thus no addition of an electron donor or electron acceptor would be needed. Transformation of CT to CO_2 via HCOOH or CO are also energetically very favorable reactions with an overall $\Delta G^{0'}$ of –619.7 kJ/reaction.

Both biotic transformation of CT to HCOOH and CT transformation to CO have been demonstrated (Hashsham et al. 1995, Dybas et al. 1995, Stromeyer et al. 1992). The mechanism of this conversion is not yet clear, but Egli et al. (1988, 1990) proposed that CT transformation may involve the acetyl-CoA pathway and that cobamides could play a role. There are several reports about the stimulation of CT transformation in the presence of cobamides (Chiu and Reinhard 1996, Hashsham et al. 1995, Stromeyer et al. 1992). It is not clear whether CT was degraded via HCOOH or CO in our reactor. Both the transformation of CT to CO and the transformation of CT to HCOOH are energetically very favorable reactions (Table 4). The samples of the ^{13}C-CT experiment were screened for ^{12}C- and ^{13}C-HCOOH but neither of these compounds were detected in any of the samples, indicating that, at most, less than 2.2 μM was present (detection limit). We were unable to detect for ^{12}CO and ^{13}CO since the mass of ^{12}CO is similar to that of N_2.

The observed formation of ^{13}C-CH_3COOH from ^{13}C-CT indicated that CH_3COOH could be a (minor) product of CT transformation in the reactor. Formation of CH_3COOH would result from the utilization of CO or HCOOH by acetogens (Ljungdahl 1986) (Table 4). CH_3COOH, when formed, again could be oxidized and serve as an electron donor in the reactor, yielding eight electrons per CH_3COOH oxidized (Table 4). Batch experiments with vancomycin strongly suggested that acetogens could be involved in CT transformation in our reactor.

Only isolation of the CT degrading microorganism can give a definitive answer whether acetogenic bacteria or a new physiological type of microorgan-

ism were involved in CT transformation in our open microbial system.

Acknowledgments

This work was financed by a grant No. IOP91225 from the Dutch lOP Environmental Biotechnology program.

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