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Transformation of carbon tetrachloride under sulfate reducing conditions

Jappe H. de Best*, E. Salminen, Hans J. Doddema, Dick B. Janssen[1] & Wim Harder
TNO Environmental Technology and Process Engineering Division, Department of Environmental Biotechnology, P.O. Box 342, 7300 AH Apeldoorn, The Netherlands; [1]Department of Biochemistry, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands (author for correspondence, present address: Grondmij A & T, P.O. Box 119, 3990 DC Houten, The Netherlands)*

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Abstract

The removal of carbon tetrachloride under sulfate reducing conditions was studied in an anaerobic packed-bed reactor. Carbon tetrachloride, up to a concentration of 30 μM, was completely converted. Chloroform and dichloromethane were the main transformation products, but part of the carbon tetrachloride was also completely dechlorinated to unknown products. Gram-positive sulfate-reducing bacteria were involved in the reductive dechlorination of carbon tetrachloride to chloroform and dichloromethane since both molybdate, an inhibitor of sulfate reduction, and vancomycin, an inhibitor of gram-positive bacteria completely inhibited carbon tetrachloride transformation. Carbon tetrachloride transformation by these bacteria was a cometabolic process and depended on the input of an electron donor and electron acceptor (sulfate). The rate of carbon tetrachloride transformation by sulfate reducing bacteria depended on the type of electron donor present. A transformation rate of 5.1 nmol·ml^{-1}·h^{-1} was found with ethanol as electron donor. At carbon tetrachloride concentrations higher than 18 μM, sulfate reduction and reductive dechlorination of carbon tetrachloride decreased and complete inhibition was observed at a carbon tetrachloride concentration of 56.6 μM. It is not clear what type of microorganisms were involved in the observed partial complete dechlorination of carbon tetrachloride. Sulfate reducing bacteria probably did not play a role since inhibition of these bacteria with molybdate had no effect on the complete dechlorination of carbon tetrachloride.

Abbreviations: CT – carbon tetrachloride; CF – chloroform; DCM – dichloromethane

Introduction

Carbon tetrachloride is a toxic and carcinogenic compound of great environmental concern, as it is often found as a contaminant in groundwater and soil. Bioremediation is an attractive clean-up technique for sites contaminated with CT, provided complete dechlorination occurs and no toxic metabolites accumulate. Whether complete dechlorination takes place depends on the environmental conditions, with the redox potential as the most important factor. CT resists aerobic biodegradation, but under anaerobic conditions it has been shown that in the presence of several electron acceptors, except sulfate, CT can be completely dechlorinated (Bouwer & McCarty 1983; Gälli & McCarty 1989; Criddle et al. 1990; Picardal et al. 1995). CT transformation under sulfate reducing conditions in continuous flow reactors has been described (Bouwer & Wright 1988; Cobb & Bouwer 1991) but the products of CT transformation are unknown. In pure cultures of *Desulfobacterium autotrophicum*, CT was for more than 70% transformed to CF and DCM (Egli et al. 1987; 1988), but unidentified water-soluble products were also detected (Egli et al. 1990). Accumulation of the metabolites CF and DCM as metabolites is undesirable because, like CT, both compounds are toxic and carcinogenic.

Since sulfate is often present in groundwater, the aim of this study was to determine the products of CT transformation under sulfate reducing conditions. CT transformation in the presence of sulfate was studied in an anaerobic packed-bed reactor. The role of sulfate reducing bacteria in the transformation of CT, the toxicity of CT and the effect of different electron donors on CT transformation have been investigated. Furthermore, we established how complete dechlorination of CT can be achieved in groundwater containing high sulfate concentrations.

Material and methods

Packed-bed reactor studies

These 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 (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 the anaerobic non-sterile mineral medium described previously (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 and Na_2S (41.8 μM, to maintain reducing conditions) were added to the medium as a concentrated solution at the entrance of the reactor with a syringe pump. 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 a minimal anaerobic medium that contained (per liter of demineralized water) 80.1 mg $(NH_4)_2HPO_4$, 200 mg $mgSO_4 \cdot 7H_2O$, 1 mg resazurine and 5 ml trace element solution (de Best et al. 1997). The medium was purged with oxygen free N_2/CO_2 (99.5%/0.5%; 700 ml/min) 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 (11 μM) and an electron donor (1 mM) were added form concentrated solutions. All cultures were inoculated with 2 ml of an enrichment culture from the packed-bed reactor. The cultures were incubated on a shaker (100 rpm) in a canted position (90 °) at 25 ° C and analyzed regularly for chlorinated compounds, electron donor, sulfate and chloride. Sterile batches were used to test for abiotic losses.

To investigate the role of sulfate reducing microorganisms in the degradation of CT, inhibitors were added to some of the batch cultures at t = 0. Vancomycin (0.14 mM), 2-bromoethane sulfonic acid (Bres; 6 mM), molybdate (2 mM) and H_2O_2 (5.8 mM) were used as inhibitors. The effect of sulfate on CT transformation was also tested in batch cultures. $MgSO_4 \cdot 7H_2O$ was replaced by $Mg(CH_3COO)_2 \cdot 4H_2O$ to remove all sulfate.

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, carbon monoxide 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.

Table 1. Carbon tetrachloride (2.5 μM) transformation in a sulfate reducing packed-bed reactor at different sulfate concentrations

Substrate utilization				CT transformation		
SO_4^{2-} influent (μM)	CH_3COOH utilization (μM)	SO_4^{2-} reduction (μM)	CH_4 production (μM)	CT conversion (%)	CF formation (%)	DCM formation (%)
510	622	511	0	100	51.8	15 9
510	999	350	672	100	0	0
5000	974	963	0	100	46.0	13.1

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 (Boehringer, Mannheim, Germany).

Chemicals

All chemicals were obtained from commercial suppliers. CT was obtained from Baker. CF and DCM were purchased from Rathburn. Vancomycin and sodium molybdate were purchased from Sigma. Chloromethane and 2-Bromo- ethanesulfonic acid were obtained from Aldrich. Calibration gases (carbon dioxide, carbon monoxide, methane) were obtained from AGA.

Results

Although the biotransformation of CT has been studied extensively, little information is available on the products of CT transformation and the physiological factors affecting CT transformation under sulfate reducing conditions, especially in continuous flow systems. Here, CT transformation in the presence of sulfate (0.51 mM) was studied in an anaerobic packed-bed reactor, inoculated with digested sludge. Acetate (1 mM) served as an electron donor.

After 3 weeks of operation, sulfate reducing bacteria utilized 55% of the added acetate for complete reduction of sulfate. Acetate was not further utilized by other bacteria. CT (2.5 μM), which was then added to the influent of the reactor, was transformed without delay. CF (1.3 μM) and DCM (0.44 μM) were found as main transformation products (Table 1). No further transformation to chloromethane was detected. Part of CT (0.77 μM) was converted to unknown products. In a sterile control packed-bed reactor, which was run under the same conditions except for the inoculation with digested sludge, no transformation or loss of CT occurred. This suggests that CT transformation is a biological process rather than an abiotic chemical transformation caused by reaction with sulfide or other reducing compounds present in the reactor.

After complete reduction of sulfate (0.51 mM) in the reactor, about 0.4 mM of acetate was still available for conversion by non sulfate reducing microorganisms. After 23 weeks of operation methane was detected in the packed-bed reactor, indicating that methanogens had started to grow. When acetate was completely utilized, 0.67 mM of methane was produced in the reactor. Sulfate reduction had decreased to 0.35 mM (Table 1). The development of a methanogenic population had a profound effect on CT transformation. Instead of reductive dechlorination to CF and DCM, CT was completely dechlorinated. Under methanogenic conditions more than 99% of the added CT was mineralized.

In a subsequent experiment, the sulfate concentration in the influent of the reactor was increased from 1 mM to 5 mM (Table 1). When a new steady state was reached, sulfate reducing bacteria utilized all available acetate for the reduction of 0.96 mM of sulfate. Methane production no longer occurred. CT was still completely transformed but CF and DCM again were found as main transformation products.

These results demonstrate that the products of CT transformation depend on the microbial population present in the reactor. The sulfate reducing population mainly transformed CT to CF and DCM. When

Table 2. Transformation of carbon tetrachloride in anaerobic packed-bed reactor under sulfate reducing conditions at different carbon tetrachloride concentrations

CT influent (μM)	CT effluent (μM)	CF effluent (μM)	DCM effluent (μM)	Cl^- formation (μM)	SO_4^{2-} reduced (μM)	CH_3COOH utilized (μM)
2.5	0 (0%)[1]	1.5 (46%)	0.3 (13%)	–[2]	960	970
11.8	0 (0%)	4.6 (39%)	2.5 (21%)	–	900	1000
20.9	0 (0%)	7.2 (34%)	1.6 (8%)	–	980	1010
29.6	0 (0%)	12.3 (42%)	1.0 (3%)	–	970	910
56.6	23.5 (42%)	<0.1 (0%)	<0.1 (0%)	114	40	310

[1] % of CT in influent; [2] not determined.

methanogens were present in the reactor, CT was completely dechlorinated. CF and DCM were not found as intermediates in the effluent nor at several sample ports at different heights of the reactor (data not shown). Complete dechlorination of CT under strictly sulfate reducing conditions by the microbial population in the reactor apparently is not possible. CT transformation under these conditions was studied further to obtain a better understanding of the factors affecting CT transformation and the microorganisms involved in this process.

CT transformation at different concentrations

The performance of the reactor was studied in a CT concentration range from 2.5 μM to 56.6 μM. Under the starting conditions used (CT 2.5 μM, acetate 1 mM, SO_4^{2-} 5 mM), CT was completely transformed, primarily to CF and DCM via reductive dechlorination (Table 2). Part of CT (41%) was converted to unknown products. Nearly all available acetate was utilized by sulfate reducing bacteria for the reduction of 0.96 mM sulfate.

Up to a concentration of 29.6 μM, CT was mainly transformed to CF and DCM. Part of the CT was converted to unknown products. At CT concentrations higher than 11.8 μM, reductive dechlorination of CT to DCM was partially inhibited and the percentage of CT transformed to unknown products increased. At the highest CT concentration tested (56.6 μM), reductive dechlorination of CT to CF and DCM was completely inhibited, and about 23.5 μM of CT remained in the effluent of the reactor. According to the amount of chloride formed (Table 2) up to 28.5 μM of the added CT was completely dechlorinated.

Sulfate reduction was nearly completely inhibited at a CT concentration of 56.6 μM. This inhibition coincided with the inhibition of reductive dechlorination to CF and DCM. This is a further indication that sulfate reducing bacteria are probably involved in the reductive dechlorination of CT to CF and DCM. Other bacteria probably play a role in the complete dechlorination of CT since significant dechlorination of it still occurred when sulfate reduction was nearly completely inhibited.

The reactor was run for 30 weeks at a CT concentration of about 57 μM. The removal of CT, measured by the production of chloride, did not change and remained at about 50%. Sulfate reduction and methane production were not detected in the reactor.

Role of sulfate reducing bacteria in CT transformation

To determine the role of sulfate reducing bacteria in CT transformation, specific inhibitors were added to an enrichment culture from the packed-bed reactor. This CT transforming enrichment culture was obtained at a CT concentration in the reactor of 18.2 μM (Figure 3), using the liquid phase of the packed-bed reactor as an inoculum. Ethanol (1 mM) served as an electron donor.

In the absence of inhibitors, CT (11.3 μM) was completely transformed within 115 hours at a maximum CT transformation rate of 5.1 $nmol \cdot ml^{-1}$ þh^{-1} (Figure 1). CF (9.4 μM) was found as the only chlorinated transformation product. Part of CT (1.9 μM) was transformed to unknown products. Ethanol was completely utilized for the reduction of 0.49 mM sulfate according to the following reactions.

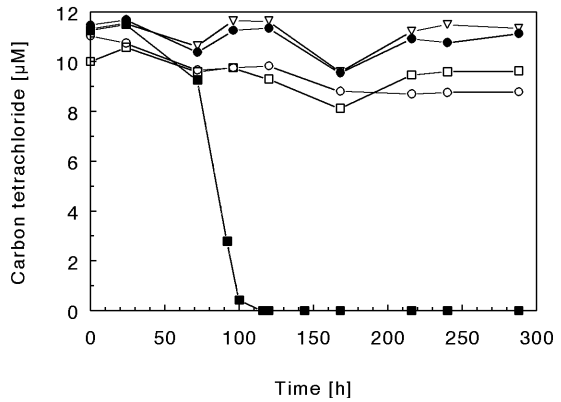

Figure 1. Effect of inhibitors on the transformation of carbon tetrachloride (11 μM) by a sulfate reducing mixed culture in a minimal medium. Ethanol (1 mM) served as an electron donor. Symbols: no inhibitor (■); molybdate (●); vancomycin (▽); H_2O_2 (○); medium without sulfate (□).

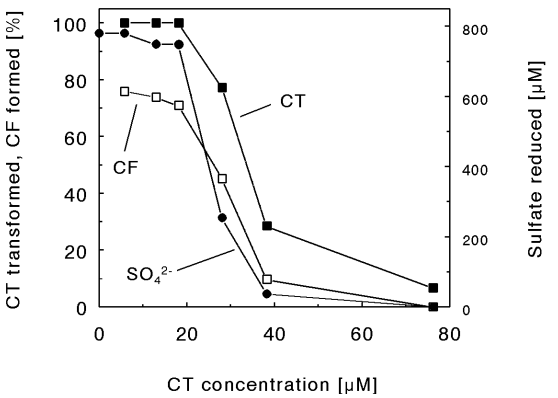

Figure 2. Effect of the carbon tetrachloride concentration on carbon tetrachloride transformation by a sulfate reducing mixed culture in a minimal medium. Ethanol (1 mM) served as an electron donor. The batch cultures were analyzed after 27 days. Symbols:% of CT transformed (■);% of CT transformed to CF (□); concentration of sulfate reduced (●).

$$CH_3CH_2OH + \tfrac{1}{2}SO_4^{2-} \rightarrow CH_3COO^- + \tfrac{1}{2}HS^- + \tfrac{1}{2}H^+ + H_2O$$
$$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$$
$$CH_3CH_2OH + \tfrac{1}{2}SO_4^{2-} \rightarrow 2HCO_3^- + 1\tfrac{1}{2}HS^- + \tfrac{1}{2}H^+ + H_2O$$

DCM was not found as a transformation product during this experiment, probably because of the short duration of the incubations (13 days). In other long-term batch culture experiments up to 27% of the CF formed was further transformed to DCM but only after 4 weeks of incubation. DCM was not transformed by the sulfate reducing mixed culture (data not shown).

Molybdate, an inhibitor of sulfate reduction in sulfate reducing bacteria (Smith & Klug 1981) and vancomycin, an inhibitor of cell wall synthesis in gram positive eubacteria (Distefano et al. 1992), completely inhibited CT transformation (Figure 1) and sulfate reduction. This demonstrated that gram-positive sulfate reducing bacteria were involved in the reductive dechlorination of CT to CF. Reductive dechlorination by these bacteria is a cometabolic process since no CT transformation occurred in the absence of an electron donor (ethanol) or a suitable electron acceptor (sulfate). Inhibition of CT transformation by hydrogen peroxide suggested that CT transformation also depended on the input of reducing equivalents.

Toxicity of CT for sulfate reducing bacteria

At a CT concentration in the packed-bed reactor of 56.6 μM, sulfate reduction was nearly completely inhibited. This suggests that CT is toxic to sulfate reducing bacteria. The toxicity level of CT for the sulfate reducing population in the packed-bed reactor was determined in batch cultures. Ethanol (2 mM) served as an electron donor.

In the absence of CT, ethanol was utilized by the sulfate reducing population to nearly complete reduction of all available sulfate (811 μM) (Figure 2). Up to a CT concentration of 18.2 μM, sulfate reduction did not significantly change. However, at higher CT concentrations the amount of sulfate reduced rapidly decreased and at a CT concentration of 38.2 μM, sulfate reduction was nearly completely inhibited. This results in an toxicity level of CT for the sulfate reducing population of about 38 μM. Egli et al. (1988) described inhibition of the autotrophic growth of *Desulfobacterium autotrophicum* at a CT concentration of 80 μM.

Since sulfate reduction became inhibited at CT concentrations above 20 μM, the amount of CT transformed to CF also decreased. At the highest CT concentration tested (76.3 μM), no sulfate reduction and thus no reductive dechlorination of CT to CF occurred.

Effect of different electron donors on CT transformation by sulfate reducing bacteria

The rate and the products of cometabolic transformation of chlorinated hydrocarbons often depend on the type of electron donor that is available (Mikesell & Boyd 1990; Lewis & Crawford 1993). To determine whether this also applies for CT transformation by the sulfate reducing enrichment culture from the packed-bed reactor, different electron donors were tested (Table 3; Figure 3).

Table 3. Transformation of carbon tetrachloride by a sulfate reducing mixed batch culture in the presence of different electron donors. The initial concentration of the different electron donors was 1 mM

electron donor	$\Delta G^{0\prime}$[1] (kJ/mol)	t=0 CT (μM)	t=13 days CT (μM)	CF (μM)	R_{max}[2] (nmol · ml^{-1} · h^{-1})	SO_4^{2-} reduced (mM)
H_2/CO_2	− 9.5	10.1	2.2 (22%)[3]	5.2 (51%)[4]	1.83	0.049
formate	−36.7	10.9	2.4 (22%)	6.5 (60%)	1.72	0.246
methanol	−73.0	10.3	10.3 (100%)	<0.1 (0%)	0	0.003
acetate	−47.6	9.7	<0.1 (0%)	6.6 (68%)	2.03	0.369
ethanol	−66.4	10.4	<0.1 (0%)	9.4 (90%)	5.09	0.486
lactate	−80.0	10.3	7.9 (77%)	2.1 (20%)	0.21	0.333
propionate	−37.7	10.1	7.2 (71%)	3.4 (34%)	0.15	0.494

[1] $\Delta G^{0\prime}$ values are taken from Thauer et al. (1977); [2] R_{max} = maximum observed rate of CT transformation; [3] percentage of CT remaining after 13 days; [4] percentage of CT transformed to CF after 13 days.

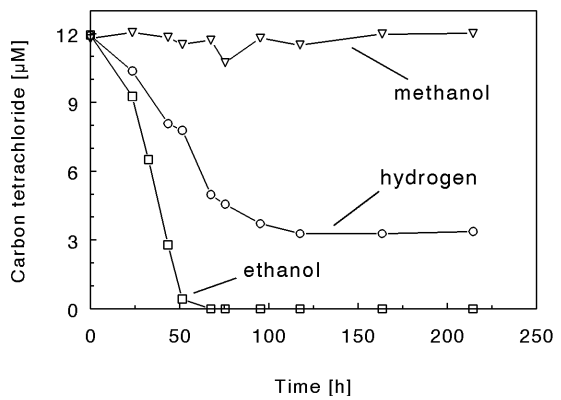

Figure 3. Transformation of carbon tetrachloride (12 μM) by a sulfate reducing mixed culture with different electron donors (1 mM) in a minimal medium. Symbols: ethanol (□); hydrogen (○); methanol (▽).

With all electron donors tested, except methanol, both sulfate reduction and CT transformation to chloroform occurred. Methane production was not found. The concentration of CT transformed seems unrelated to either the amount of sulfate reduced or the energy yield per mole of electron donor (Table 3).

Only with acetate or ethanol as electron donors complete CT transformation occurred. The maximum observed rate of CT transformation (R_{max}), which was calculated from the rate of CT disappearance in the batch cultures (Figure 3), was 3 times higher with ethanol then with acetate (Table 3). However, with ethanol CT was nearly completely (>90%) transformed to CF, while with acetate only 68% was transformed to CF and 32% was transformed to unidentified products. This corresponds with our findings in the packed-bed reactor. With all other electron donors tested, the percentage of CT transformed to unidentified products was below 27%.

For application of microbial CT transformation under sulfate reducing conditions, complete dechlorination of CT is to be preferred over the formation and accumulation of CF or DCM. Acetate therefore appeared a more promising electron donor than ethanol, although complete dechlorination of CT under sulfate reducing conditions without the formation of CF is not likely to occur.

Discussion

Bioremediation processes can only be applied for clean-up purposes at CT contaminated sites if complete mineralization can be established. Complete dechlorination of CT without the formation of CF or DCM does not appear to be possible by the sulfate reducing population in our reactor. The main pathway of CT transformation by sulfate reducing bacteria was found to be reductive dechlorination to CF and DCM (Figure 4). Both CF and DCM accumulated and were not further dechlorinated to chloromethane or methane. The accumulation of CF and DCM probably resulted from a decrease in the redox potential for reductive dechlorination with each reductive step making the transformation less favorable. Reductive dechlorination of DCM to chloromethane and methane has been described but only under methanogenic and acetogenic conditions and at very low transformation rates (Egli et al. 1988; Mikesell & Boyd 1990). Besides reductive dechlorination, DCM can also be fermented by anaerobic bacteria, a process which supports growth (Mägli et al. 1996). We did not find

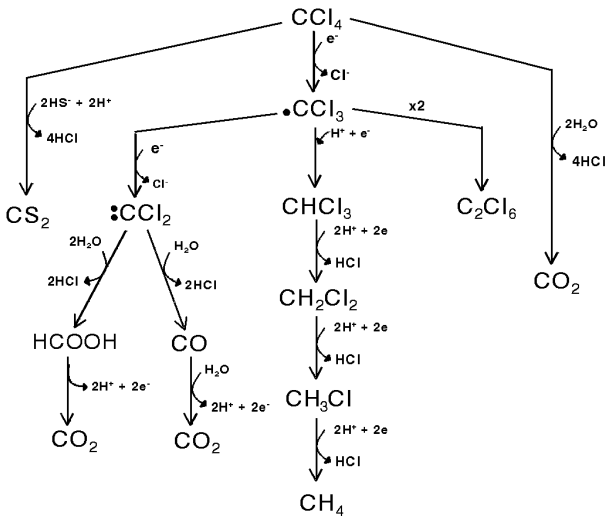

Figure 4. Proposed pathways for the anaerobic transformation of carbon tetrachloride (deduced from Criddle et al. 1991).

evidence for fermentation of DCM in our experiments. This could be a result of the toxicity of CF, the other product of CT transformation, to DCM utilizing bacteria (Mägli et al. 1996). Inhibition of DCM transformation is probably not caused by sulfate. When all sulfate present in the reactor was completely reduced, DCM transformation still did not occur (Table 1).

Part of CT transformed in the reactor was converted to unknown products. Hexachloroethane, besides CF and DCM the only other reported chlorinated product of CT transformation under anerobic conditions (Criddle et al. 1991; Figure 4), was not found as a product of CT transformation. The formation of chloride at a CT concentration of 56.6 μM (Table 2) suggested that CT was completely dechlorinated. Two main products for complete CT dechlorination under anaerobic conditions have been described, carbon disulfide and carbon dioxide.

In the presence of sulfide, CT can be transformed to CS_2 (Figure 4; Kriegman-King & Reinhard 1992; Curtis & Reinhard 1994). The reaction of CT with sulfide is an abiotic transformation which can be catalyzed by corrinoids, present in anaerobic bacteria (Hashham et al. 1995). CT transformation to CS_2 probably did not occur in the reactor. At a CT concentration of 56.6 μM, sulfate reduction, and thus the formation of sulfide, were completely inhibited in the reactor (Table 2) while CT transformation to unknown products still occurred. Removal of sulfide (42 μM) from the influent of the reactor, added to maintain reducing conditions, also had no significant effect on the transformation of CT (data not shown).

Carbon dioxide can be formed as a result of both biotic (Bouwer & McCarty 1983 1983a; Egli et al. 1988 1990; Criddle et al. 1990) and abiotic (Kriegman-King & Reinhard 1992) transformation of CT. The pathway of biological CT transformation to CO_2 is not yet clear. It is generally agreed that the first step is a one electron reduction of CT to give a trichloromethyl radical and a chlorine ion (Criddle and McCarty 1991). A second electron transfer step would lead to the formation of dichlorocarbene, which hydrolyzes either to formic acid or carbon monoxide (Figure 4). Transformation of CT to formic acid has never been reported but carbon monoxide formation from CT has been demonstrated (Krone et al. 1991; Stromeyer et al. 1992; Hashham et al. 1995; Chiu and Reinhard, 1996). Both formic acid and carbon monoxide, when formed, can be biologically oxidized to CO_2. Biological transformation of CT to CO_2 in the reactor is possible. Abiotic hydrolysis of CT to CO_2 probably did not occur since in a sterile control reactor no transformation of CT was detected.

Although CT was mainly transformed to CF and DCM in the presence of sulfate in the reactor, under two different conditions CT transformation without the formation of CT and DCM occurred. First, at CT concentrations higher than 40 μM, when sulfate reduction (and methane production) was completely inhibited. Secondly, when besides sulfate reducing bacteria also methanogens were present in the packed-bed reactor. Under methanogenic conditions, CT is readily mineralized (Bouwer & McCarty 1983; Egli et al. 1987; de Best et al. unpubl.).

These results suggested that inhibition of sulfate reduction and/or stimulation of methanogenic activity can lead to complete CT mineralization. This could be used for the bioremediation of CT contaminated groundwaters containing high sulfate concentrations. Stimulation of methanogenic activity at sites with high sulfate concentrations could be achieved by the addition of an excess of electron donor resulting in simultaneous sulfate reduction and methanogenesis.

Besides stimulation of methanogenic activity, the development of acetogenic activity or denitrification could possibly also lead to CT mineralization. Mineralization of CT to CO_2 is also the predominant transformation pathway under these electron accepting conditions (Bouwer & McCarty, 1983a; Egli et al. 1988; Criddle et al. 1990). CT transformation under iron reducing conditions has only been stud-

ied by Picardal et al., (1993). They found CF as the only transformation product of CT transformation in *Shewanella putrefaciens* 200.

Although complete dechlorination of CT by the sulfate reducing bacteria in our reactor did not occur without simultaneous formation of CF and DCM, there are possibilities to achieve complete anaerobic biological dechlorination of CT present in groundwater that also contains high sulfate concentrations. More information is needed on the costs and the possibilities for application of these techniques to assess whether bioremediation of contaminated groundwater is an attractive option that can compete with other techniques such as activated carbon absorption, extraction or stripping.

Acknowledgements

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References

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 & McCarty PL (1983a) Transformations of halogenated organic compounds under denitrification conditions. Appl. Environ. Microbiol. 45: 1295–1299

Bouwer EJ & Wright JP (1988) Transformations of trace halogenated aliphatics in anoxic biofilm column. J. Cont. Hydrol. 2: 155–169

Chiu P-C & Reinhard M (1996) Transformation of carbon tetrachloride by reduced vitamin B_{12} in aqueous cysteine solution. Environ. Sci. Technol. 30: 1882–1889

Cobb GD & Bouwer EJ (1991) Effects of electron acceptors on halogenated organic compound biotransformations in a biofilm column. Environ. Sci. Technol. 25: 1068–1074

Criddle CS, DeWitt JT, Grbic-Galic D & McCarty PL (1990) Transformation of carbon tetrachloride by *Pseudomonas* sp. strain KC under denitrification conditions. Appl. Environ. Microbiol. 56: 3240–3246

Criddle CS & McCarty PL (1991) Electrolytic model system for reductive dehalogenation in aqueous environments. Environ Sci Technol 25: 973–978

Curtis GP & Reinhard M (1994) Reductive dehalogenation of hexachloroethane, carbon tetrachloride, and bromoform by anthrahydroquinone disulfonate and humic acid. Environ. Sci. Technol. 28: 2393–2401

de Best JH, 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

Distefano TD, Gossett JM & Zinder SH (1992) Hydrogen as an electron donor for dechlorination of tetrachloroethene by an anaerobic mixed culture. Appl. Environ. Microbiol. 58: 3622–3629

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

Egli C, Tschan T, Scholtz R, Cook AM & Leisinger T (1988) Transformation of tetrachloromethane to dichloromethane and carbon-dioxide by *Acetobacterium woodii*. Appl. Environ. Microbiol. 54: 2819–2824

Egli C, Stromeyer SA, Cook AM & Leisinger T (1990) Transformation of tetrachloromethane and chloroform to CO_2 by anaerobic bacteria is a non-enzymic process. FEMS Microbiol. Lett. 68: 207–212

Gälli R & McCarty PL (1989) Biotransformation of 1,1,1-trichloroethane, trichloromethane, and tetrachloromethane by a *Clostridium* sp.. Appl. Environ. Microbiol. 55: 837–844

Hashham S, Scholze R & Freedman DL (1995) Cobalamin-enhanced anaerobic transformation of carbon tetrachloride. Environ. Sci. Technol. 29: 2856–2863

Kriegman-King MR & Reinhard M (1992) Transformation of carbon tetrachloride in the presence of sulfide, biotite and vermiculite. Environ. Sci. Technol. 26: 2198–2206

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

Mägli A, Wendt M & Leisinger T (1996) Isolation and characterization of *Dehalobacterium formicoaceticum* gen. nov. sp. nov., a strictly anaerobic bacterium utilizing dichloromethane as source of carbon and energy. Arch. Microbiol. 166: 101–108

Mikesell MD & Boyd SA (1990) Dechlorination of chloroform by *Methanosarcina* strains. Appl. Environ. Microbiol. 56: 1198–1201

Picardal FW, Arnold RG, Couch H, Little AM & Smith ME (1993) Involvement of cytochromes in the anaerobic biotransformation of tetrachloromethane by *Shewanella putrefaciens* 200. Appl. Environ. Microbiol. 59: 3763–3770

Picardal FW, Arnold RG & Huey BB (1995) Effects of electron donor and acceptor conditions on reductive dehalogenation of tetrachloromethane by *Shewanella putrefaciens* 200. Appl. Environ. Microbiol. 61: 8–12

Smith RL & Klug MJ (1981) Electron donors utilized by sulfate-reducing bacteria in eutrophic lake sediments. Appl. Environ. Microbiol. 42: 116–121

Stromeyer SA, Stumpf K, Cook AM & Leisinger T (1992) Anaerobic degradation of tetrachloromethane by *Acetobacterium woodii*: separation of dechlorinative activities in cell extracts and roles for vitamin B_{12} and other factors. Biodegradation 3: 113–123

Thauer RK, Jungermann K & Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. 41: 100–180