Biodegradation of 1,1,1,2-tetrachloroethane under methanogenic conditions

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Abstract Chlorinated aliphatic hydrocarbons are widely used as solvents and as intermediates in chemical synthesis, so they can be found in industrial wastewaters and released to the environment where they became a serious health risk due to their toxic properties and high chemical stability. Most of these compounds are xenobiotic and recalcitrant to biodegradation. In this article we report the effect of different co-substrates in the 1,1,1,2-tetrachloroethane (1,1,1,2-TeCA) degradation by anaerobic granular sludge, and its degradative pathway. Our results show that this compound is easy and rapidly biodegradable under methanogenic conditions, even in the absence of external electron donors. 1,1,1,2-TeCA is equimolecularly degraded to 1,1-dichloroethene (1,1-DCE) by reductive dichloroelimination. 1,1-DCE is only completely biodegraded in the presence of lactic acid as co-substrate. Although 1,1,1,2-TeCA can be apparently removed by autoclaved granular sludge, the compound is not transformed but retained inside the granules. The primary biodegradation of 1,1,1,2-TeCE to 1,1-DCE is a biotic process mediated by anaerobic bacteria.
Keywords Chlorinated aliphatic hydrocarbons; tetrachloroethane; biodegradation; co-metabolism

Introduction

Chlorinated aliphatic hydrocarbons (CAH) are used for a wide variety of applications (Holliger, 1995) and, due to their toxicity and carcinogenic properties, most of them are included on List I of The Council Directive 76/464 (EC) and EPA List (USA) of priority environmental pollutants. This group of compounds, frequently found in industrial wastewaters, are cited as significant contributors to the inhibition of anaerobic digestion (Mohn and Tiedje, 1992), being considered more toxic to anaerobic than to aerobic microorganisms.

CAH have been considered xenobiotic compounds, although some of them – especially chloromethanes – are also released by algae, fungi or volcanoes (Eekert, 1999). In any case, most CAH have an anthropogenic origin, and they are considered recalcitrant to degradation. The majority of the available information on the biodegradation of chlorinated compounds was related to oxidative degradation, since aerobic processes were considered the most efficient and easily applicable. However, increasing information is now available on anaerobic biotransformation of CAH. In fact, highly chlorinated xenobiotic compounds have proven to be biodegradable only under anaerobic conditions (Mohn and Tiedje, 1992; Speece, 1996; Eekert, 1999). Anaerobic dechlorination can be broadly subdivided into two groups: biologically and non-biologically mediated reactions. Reductive dehalogenation (mainly hydrogenolysis and dichloroelimination) is the most important route for anaerobic biodegradation, and it requires an electron donor. Abiotic dehalogenations exhibit, in general, extremely low reaction rates.

In this work, we have focused our attention on the biodegradation of 1,1,1,2-tetrachloroethane ($C_2H_2Cl_4$, 1,1,1,2-TeCA) which, as it is not known to occur as a natural product, is included in the US-EPA pollutant list, and there is limited evidence for its carcinogenicity. 1,1,1,2-TeCA has been found in water supply systems (Golfinopoulos *et al.*, 1998). We report its biodegradative pathway, the effect of primary substrates (co-substrates) and its maximum degradation rate, both in batch and UASB reactors.

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Materials and methods

Chemicals

Chloroaliphatic hydrocarbons: 1,1,2-trichloroethane (1,1,2-TCA), 1,1,1,2-tetrachloroethane (1,1,1,2-TeCA), 1,1-dichloroethylene (1,1-DCE), trichloroethylene (TCE), and tetrachloroethylene (TeCE) were obtained from Aldrich Chemical Co. (Milwaukee, USA). The other chemicals used were purchased from Merck (Darmstadt, Germany), with the exception of yeast extract, which was obtained from Difco (Detroit, USA).

Batch degradation assays

The biomass used as inoculum was a mixture of anaerobic granular sludge, obtained from a UASB (Upflow Anaerobic Sludge Blanket) reactor located at the "El Águila" brewery (Valencia), and activated sludge digested from the Universidad Autónoma sewage treatment plant (Madrid). The biomass was stored before use at 4ºC. VSS (volatile suspended solids) content was 9.1% and its initial specific activity was 1.6 gDQO/gVSS·d. Assays were carried out with 1.7 gVSS/l of elutriated granular sludge. In control experiments with sterile biomass, the sludge was autoclaved (inactivating all microbial activity) for 30 minutes at 120ºC. Biomass was washed to remove any possible substrates and co-substrates.

Assays were carried out in Pyrex bottles (250 ml) adapted to use screw tops Mininert (Supelco) to avoid gas leakage. Nutrients were prepared as described in Sanz *et al*. (1997). Incubation was conducted at 30±1ºC under static conditions and all experiments were performed in duplicate. Standards were prepared using milli-Q water.

Analytical methods

The chloroaliphatic hydrocarbons were measured using a gas chromatograph (Varian Star 3400 CX) equipped with a split injector (ratio 1:50) and an electron capture detector. A capillary column SPB 624 (30 m × 0.25 mm × 0.25 µm) manufactured by Supelco was used. Temperatures of the column, injector and detector were 210, 200 and 275ºC respectively. Nitrogen was used as carrier gas. Measurements were made taking 50 µl from the gaseous phase with a gas-tight syringe (Dynatech). Samples were directly injected into the gas chromatograph. Nominal concentrations were determined from the gaseous phase concentration using the previously calculated Henry's constant (Sanz *et al*., 1997). VSS were determined according to the 2540 E method of the Standard Methods (1995). pH was measured with an Orion 420A pH-meter (Cambridge, USA).

Results and discussion

1,1,1,2-TeCA degradation in the presence and in the absence of external electron donors

Different degradation experiments showed that 1,1,1,2-TeCA was completely removed only in presence of anaerobic microbial consortia. Measured degradation velocities (V) were independent of the co-substrates added (lactic acid, methanol, saccharose, acetic acid or formic acid) and they remained constant in their absence. Control assays indicated that there was no degradation of 1,1,1,2-TeCA in the absence of microorganisms (biomass), and that the degradation was not mediated by the different components of the incubation media. The presence or absence of macronutrients and trace elements, with or without yeast extract and Na_2S, both as hypothetical electron donors, did not modify the degradation rate.

The experiments shown in Table 1 demonstrate that the sludge, by itself and without co-substrate, was able to degrade 1,1,1,2-TeCA fast and efficiently. As far as we know, this possibility has not been previously reported for other CAH. In order to determinate the kinetic parameters, 1,1,1,2-TeCA degradation was studied at eight different concentrations ranging from 0,1 and 15 ppm. The results obtained suggest a maximum specific degradation rate of approximately 47 ppm/gVSS·d and a Ks of 5 ppm.

Table 1 1,1,1,2-TeCA degradation velocities using different electron donors

		Na_2S (0.1 g/l)	Nutrients	Velocities (µmol/gVSS·d)
Co-substrate (10 mM)	Lactic acid	+	+	219
	Methanol	+	+	224
	Saccharose	+	+	275
	Acetic acid	+	+	130
	Formic acid	+	+	186
Without co-substrate		+	+	234
		–	+	238
		–	+*	193
		–	–	238

Biomass: 1.7 gVSS/l
1,1,1,2-TeCA: 7 ppm
* Nutrients without yeast extract

Degradative pathway of 1,1,1,2-TeCA

To identify and quantify the possible 1,1,1,2-TeCA degradation products, three different concentrations (1,7,15 ppm), with and without co-substrate were employed. The compounds identified were 1,1-DCE, 1,1,2-TCA, TCE and TeCE, but only 1,1-DCE and 1,1,2-TCA corresponded to biotransformation products. TCE and TeCE were present at low and constant concentrations – 0,2 ppm and 0,01 ppm respectively – as impurities, even in the 1,1,1,2-TeCA standards.

Experiments carried out with 1,1-DCE demonstrated that 1,1-DCE concentration remained constant either in the absence of co-substrate or in the presence of sodium lactate (both at neutral pH). However, DCE was rapidly eliminated with lactic acid at pH 5.5, but it did not occur using phosphoric acid at pH 3 or 5.5. These results allowed an abiotic effect due to chemical acid catalysis to be discarded.

Using several 1,1,1,2-TeCA concentrations, 0.62±0.05 ppm of 1,1-DCE were formed per ppm of 1,1,1,2-TeCA degraded (1.07±0.08 µmol 1,1-DCE/µmol 1,1,1,2-TeCA). It means that 100% of 1,1,1,2-TeCA was converted to 1,1-DCE.

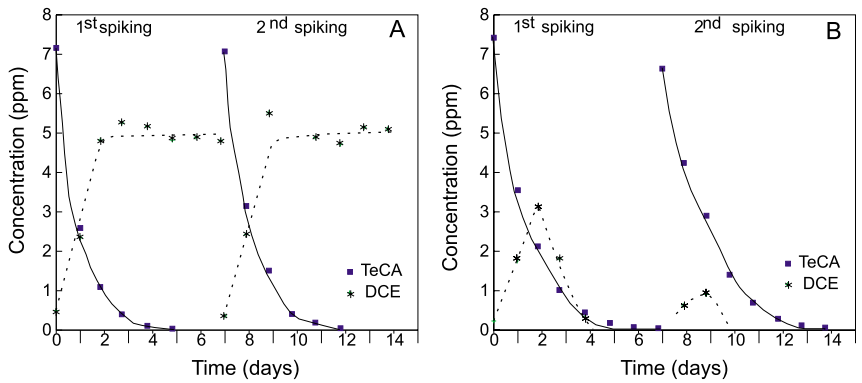


Figure 1 Biotransformation of 1,1,1,2-TeCA (7 ppm) by anaerobic granular sludge DCE production: A) in the absence of co-substrate, B) in the presence of 10 mM lactic acid. Similar results were obtained with 15 ppm of 1,1,1,2-TeCA

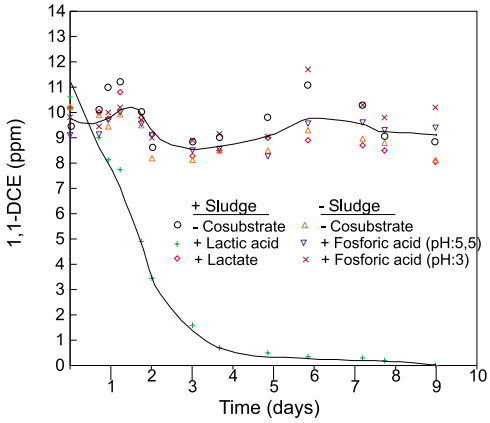


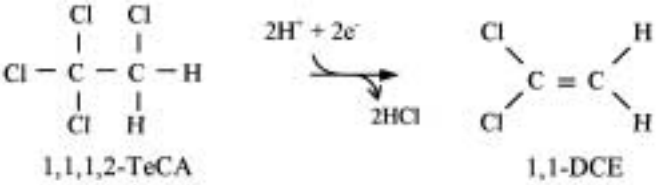
Figure 2 1,1-DCE degradation in different conditions

Table 2 Relation of 1,1,1,2-TeCA concentration degraded

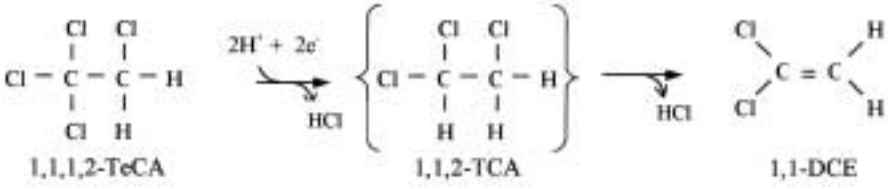
TeCA (ppm) degraded	2.8	2.92	4.46	5.5	5.7	6.5	8.6	9.5 1	0.36	13.4
DCE (ppm) formed	1.6	1.7	2.82	3.15	4.16	4.3	5.45	6.1	6.95	7.9
ppm DCE formed/ ppm TeCA degraded	0.57	0.58	0.63	0.57	0.73	0.66	0.63	0.64	0.67	0.59
µmol DCE formed/ µmol TeCA degraded	0.99	1.01	1.10	0.99	1.27	1.15	1.10	1.11	1.17	1.03

In accord with our results, there are two possible biodegradation pathways:

1) Dichloroelimination:



2) Reductive dechlorination and dehydrochlorination:



Due to the fact that 1,1,2-TCA was rarely observed – in low and non-quantify amounts – the alternative isomer 1,1,1-TCA was never detected, and that dehydrochlorination proceeds slowly (Van Eekert, 1999; Chen *et al.*, 1993), it can be assumed that dichloroelimination is the principal pathway through which it takes place. Taking into consideration that the elimination of two chlorides and the correspondent double bond formation requires electron donor intervention, our results suggest that electron donors derive from the endogenous sludge digestion.

In order to confirm the former results, experiments using two UASB reactors (with and without co-substrate) were carried out. 1,1,1,2-TeCA biotransformation was complete in both system up to 180 mg TeCA/l. Noteworthy, for this 1,1,1,2-TeCA concentration the methanogenic activity were strongly affected dropping to 32% (reactor with co-substrate) and 15% (reactor without co-substrate) from the initial one.

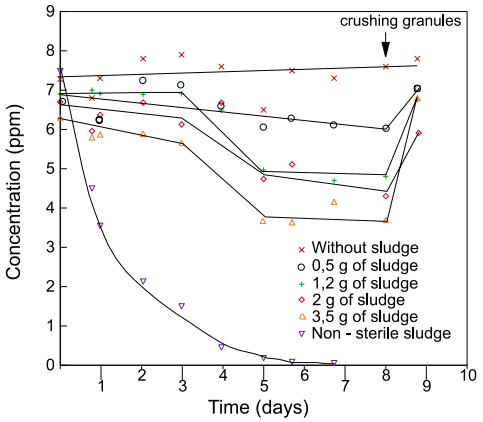


Figure 3 Removal of 1,1,1,2-TeCA by sterile granular sludge

Effect of biomass in the disappearance of 1,1,1,2-TeCA

TeCA was degraded and converted to DCE only in the presence of alive sludge. In assays carried out with autoclaved sludge, in absence or presence of co-substrate, it was observed that 1,1,1,2-TeCA concentrations decreased gradually. Using different sludge concentrations (Figure 3), it was shown that there was a relation between the biomass added and the 1,1,1,2-TeCA which disappeared, although without any formation of any detectable product. However, when the granules were disrupted by mechanical procedures, 1,1,1,2-TeCA was released and approximately its initial concentration recovered. This experiment proved that 1,1,1,2-TeCA was indeed not degraded by the sterile sludge, but had been retained (by adsortion or absortion) inside the granules. These results are in agreement with the hydrophobic nature of 1,1,1,2-TeCA and the granular sludge (Grotenhuis *et al*., 1992). It is important to underline that the disappearance of hydrophobic compounds, like CAH, in the presence of inactive granular sludge could be confused with an apparent abiotic degradation, especially at high biomass/CAH ratios.

Conclusions

Our experiments confirm that anaerobic sludge is able to biotransform 1,1,1,2-TeCA rapidly in presence or absence of external electron donors. The highest degradation rate observed was 280 µmol/gVSS·d.

Dichloroelimination is the major degradative pathway for the 1,1,1,2-TeCA under methanogenic conditions. 1,1-DCE is the final product obtained in this process. Complete dehalogenation only takes place with lactic acid as co-substrate.

All the results presented led to the conclusion that 1,1,1,2-TeCA transformation is a biotic process mediated by the anaerobic microbial consortia. Removal of this compound can be observed in the presence of sterile biomass due to adsorption or absorption mechanisms.

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