Biodegradation **6**: 309–318, 1995.

Anaerobic dechlorination of trichloroethene, tetrachloroethene and 1,2-dichloroethane by an acetogenic mixed culture in a fixed-bed reactor

Alex P. Wild[1], Wolfgang Winkelbauer[2] & Thomas Leisinger[1,*]
[1]*Mikrobiologisches Institut ETH, ETH-Zentrum, CH-8092 Zürich, Switzerland;* [2]*Mercedes-Benz AG, D-70327 Stuttgart, Germany (* Corresponding author)*

Accepted 9 January 1995

Key words: Acetogenesis, anaerobic dehalogenation, 1,2-dichloroethane, perchloroethene, reductive dechlorination, trichloroethene

Abstract

An anaerobic enrichment culture with glucose as the sole source of carbon and energy plus trichloroethene (TCE) as a potential electron acceptor was inoculated with material from a full size anaerobic charcoal reactor that biologically eliminated dichloromethane from contaminated groundwater (Stromeyer et al. 1991). In subcultures of this enrichment complete sequential transformation of 10 μM TCE via *cis*-dichloroethene and chloroethene to ethene was reproducibly observed. Maintenance of this activity on subcultivation required the presence of TCE in the medium. The enrichment culture was used to inoculate an anaerobic fixed-bed reactor containing sintered glass Raschig elements as support material. The reactor had a total volume of 1780 ml and was operated at 20 °C in an up-flow mode with a flow rate of 50 ml/h. It was fed continuously with 2 mM glucose and 55 μM TCE. Glucose was converted to acetate as the major product and to a minor amount of methane; TCE was quantitatively dehalogenated to ethene. When, in addition to TCE, tetrachloroethene or 1,2-dichloroethane were added to the system, these compounds were also dehalogenated to ethene. In contrast, 1,1,1-trichloroethane was not dehalogenated, but at 40 μM severely inhibited acetogenesis and methanogenesis. When the concentration of TCE in the feed was raised to 220 μM, chloroethene transiently accumulated, but after an adaptation period ethene was again the only volatile product detected in the effluent. The volumetric degradation rate at this stage amounted to 6.2 μmol/l/h. Since complete transformation of TCE occurred in the first sixth of the reactor volume, the degradation capacity of the system is estimated to exceed this value by factor of about ten.

Abbreviations: CA – chloroethane, 1,1-DCA – 1,1-dichloroethane, 1,2-DCA – 1,2-dichloroethane, 1,1-DCE – 1,1-dichloroethene, c-DCE – *cis*-1,2-dichloroethene, t-DCE – *trans*-1,2-dichloroethene, PCE – tetrachloroethene, perchloroethene, 1,1,1-TCA – 1,1,1-trichloroethane, TCE – trichloroethene, VC – chloroethene, vinyl chloride

Introduction

Trichloroethene (TCE) is a frequent groundwater contaminant (Howard 1991) whose persistence, toxicity (Van Duuren & Banerjee 1976) and carcinogenicity (Shahin & Von Borstel 1977) give rise to concern. Microbial oxidation of TCE thus has become a field of intensive research in recent years. Various bacterial mixed and pure cultures cometabolically transforming this compound to innocuous products have been described (Ensley 1991), and some of them have been tested for in situ bioremediation (Hopkins et al. 1993) and for use in a bioreactor (Ensley & Kurisko 1994).

Biotransformation of TCE has also been observed under anaerobic conditions. Information on the anaerobic transformation of the compound stems mostly from work on the reductive dechlorination of tetrachloroethene (PCE), a pollutant which is microbially attacked exclusively under anoxic conditions. In strictly anaerobic mixed cultures PCE has been shown to be subject to sequential reductive dehalogenation, yielding TCE, *cis*-1,2-dichlorethene (c-DCE),

and chloroethene (VC) as intermediates and either ethene (Freedman & Gossett 1989; Di Stefano et al. 1991) or ethane (De Bruin et al. 1992) as the end-product. The electrons for reductive dechlorination in these systems were derived from organic compounds such as methanol, formate, lactate, acetate or glucose. The recent description of a pure culture (Neumann et al. 1994) and of a microscopically pure enrichment culture (Holliger et al. 1993) utilizing H_2 as an electron donor and PCE as the electron acceptor represents a significant advancement in the understanding of reductive dechlorination. The properties of these organisms indicate that reductive dechlorination of PCE and TCE is the result of a novel type of anaerobic respiration and that the occurrence of these compounds in anaerobic environments may offer selective advantages to highly specialized bacteria.

For the application of reductive dehalogenation in the treatment of groundwater contaminated with PCE or TCE it is important to attain high degradation rates while avoiding the accumulation of VC, a product more toxic than the parent compounds (Easter & Von Burg 1994). In the present contribution we describe an anaerobic acetogenic enrichment culture which reductively dehalogenates TCE to ethene. In a continuously operated fixed-bed column this culture transformed not only TCE without the accumulation of VC, but also PCE and 1,2-dichloroethane (1,2-DCA). The performance and the versatility of this system thus appear promising for the treatment of contaminated groundwater.

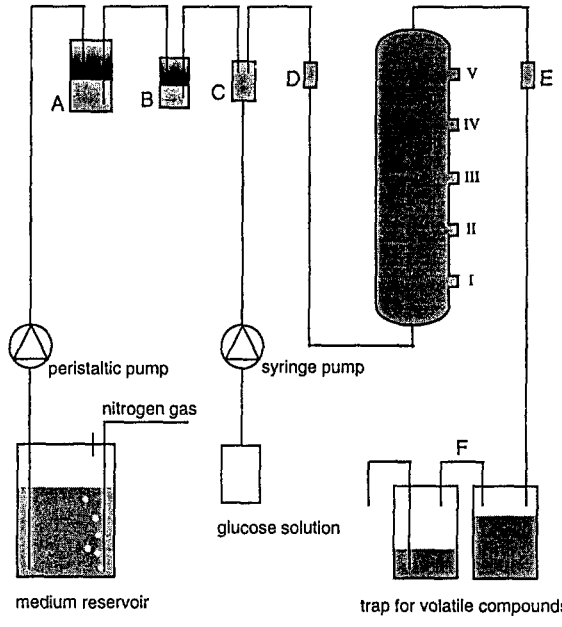

Fig. 1. Schematic diagram of the fixed-bed reactor system. The mineral medium was purged with nitrogen gas (10 ml/min). All tubing was of stainless steel or Viton. A) Continuous addition of TCE dissolved in hexadecane. B) continuous addition of 1,2-DCA and PCE dissolved in hexadecane. This bottle was only installed in certain experiments. C) Mixing chamber for the addition of glucose. D and E) Sampling ports for completed medium and reactor effluent. F) Collection bottles for spent medium and gaseous products.

Materials & methods

Chemicals

All chemicals used were of reagent grade or better and were obtained from Fluka (Buchs, Switzerland). Ethene was from Matheson (Oevel, Belgium), and other gases were purchased from Carbagas (Liebefeld-Bern, Switzerland). Bi-distilled water was used throughout.

Growth medium and enrichment conditions

The composition of the defined, low-chloride salts medium containing trace elements (Eichler & Pfennig 1986) and vitamins (Widdel & Pfennig 1981) is shown in Table 1. For batch cultures a 100-fold concentrated stock solution of buffer (Table 1) was diluted in oxygen-free water, dispensed in 200 ml portions into 330 ml serum bottles which were sealed with Viton stoppers (Egli et al. 1987), gassed with an 80% N_2/20% CO_2 gas mixture to a final pressure of 2.8 bar and autoclaved. Five hundred- to 2000-fold concentrated sterile stock solutions of mixtures A through E (Table 1) were then added aseptically by syringe. Glucose or methanol were added from sterile 1 M stock solutions to final concentrations of 1 mM, yeast extract from a 100-fold concentrated stock solution to a final concentration of 0.5 g/l, and TCE from a saturated aqueous stock solution to final concentrations between 5 and 10 μM.

Non-sterile medium for feeding the fixed-bed reactor was prepared by adding concentrated buffer and the sterile stock solutions A through E to bi-distilled water that had been sparged for two hours with nitrogen gas. The final composition of the medium is given in Table 1. Glucose and chlorinated aliphatics were added separately to the feed of the reactor (Fig. 1).

The inoculum (1% w/v) for the enrichment of the TCE-dehalogenating mixed culture was biofilm-coated charcoal from the previously described fixed-bed reac-

Table 1. Synthetic low-chloride medium used for batch and bioreactor experiments.

Solution	Substance	Concentration of stock solution	Final concentration [g/l]	[mM]
Buffer	di-Sodium hydrogen phosphate	× 100	1.417	10.0
	Potassium dihydrogen phosphate		0.648	4.8
	Resazurine		0.001	0.004
A	Ammonium bromide	× 500	0.098	1.0
	Calcium bromide		0.080	0.4
	Magnesium bromide Hexahydrate		0.292	1.0
E	Sodium sulfide Hydrate	× 500	0.5	2.1
			[mg/l]	[μM]
B	EDTA Disodium salt Dihydrate	× 1000	5.200	14.0
	Ferrous(II)chloride Tetrahydrate		2.000	10.1
	Zinc(II)chloride		0.070	0.5
	Manganese(II) chloride Dihydrate		0.100	0.6
	Cobaltous(II) chloride Hexahydrate		0.190	0.8
	Cupric(II) chloride Dihydrate		0.002	0.01
	Nickel(II) chloride Hexahydrate		0.024	0.1
	Sodium molybdate Dihydrate		0.036	0.15
	Boric acid		0.006	0.1
C	Sodium selenite	× 1000	0.003	0.02
	Sodium tungstate Dihydrate		0.004	0.01
D	Cyanocobalamin	× 2000	0.050	0.03
	4-Aminobenzoic acid		0.040	0.29
	(+)-Biotin		0.010	0.04
	Nicotinic acid		0.100	0.81
	D-Panthothenic acid Calcium salt		0.050	0.10
	Pyridoxine hydrochloride		0.150	0.73
	Thiamine hydrochloride		0.100	0.30

tor which biologically eliminated dichloromethane (100 μM) from contaminated groundwater (Stromeyer et al. 1991). This groundwater also contained up to 0.2 μM TCE, 0.03 μM of PCE and up to 5.2 μM of 1,2-DCA (Winkelbauer & Kohler 1991). Inocula for subcultures were free of charcoal.

Dehalogenation of TCE in batch cultures

Enrichment cultures were incubated without shaking at 20 °C in the dark and transferred (10% v/v) in two-week intervals into fresh medium. The aqueous and gas phases were sampled through the septum. Headspace-liquid partitioning of volatile compounds within bottles was not considered, and all concentrations are given as nominal concentrations in the liquid phase.

Dehalogenation in the fixed-bed reactor

A diagram of the fixed-bed reactor (63 cm in length, 6 cm inside diameter) is shown in Fig. 1. The reactor had a total volume of 1780 ml, and it was filled with sintered glass Raschig elements (9 × 9 mm, Schott, Muttenz, Switzerland). It was equipped with five sampling ports for following concentration profiles in the column. The tubing was either of stainless steel (1 mm inner diameter) or of Viton (0.5 mm inner diameter). The column was operated at 20 °C in an upflow mode with a standard flow rate of 50 ml/h. To ensure a constant flow of TCE to the column feed, the medium was pumped from the reservoir (Fig. 1) through a magnetically stirred serum flask (330 ml, Fig. 1A) that was thermostated at 20 °C and contained 1 to 15 ml of

TCE dissolved in 200 ml hexadecane (Holliger et al. 1993). For certain experiments chlorinated aliphatic compounds other than TCE were added by passing the medium through a second 156 ml stirred flask (Fig. 1B). This flask contained either 1 ml of PCE or 0.1 ml of 1,2-DCA dissolved in 80 ml of hexadecane plus 0.4 ml of TCE. For the addition of 1,1,1-trichloroethane (1,1,1-TCA) in certain experiments, the glucose stock solution was saturated with this compound. Glucose was added to the feed in a separate mixing chamber (Fig. 1C) from a 250 mM stock solution by a syringe pump (Ismatec, Basel, Switzerland). The blue color of rezazurine always disappeared before the feed entered the reactor. Samples of the completed medium were drawn from a sampling vial (Fig. 1D) before the feed entered the column. Another sampling vial (Fig. 1E) enabled sampling of the column effluent. The spent medium and the gaseous products formed in the column were collected over periods between 12 and 100 h in a two-flask system (Fig. 1F). At the start of the sampling period the first of these flasks was empty and the second filled with water. For quantitative analysis the concentrations of ethene, ethane and methane were determined in the liquid phase and gas phase of each bottle. Reductive dehalogenation and growth in the collection system were prevented by the presence of air and 2 ml of 20% sodium azide in the first bottle.

For start-up the fixed-bed reactor was percolated (50 ml/h) for two weeks with anaerobic medium not containing glucose or TCE. Twenty ml aliquots of a fully glucose-grown TCE-dehalogenating enrichment culture were then injected at the bottom and at each sideport, and the concentration of glucose was brought to 2 mM. The system was allowed to equilibrate without flow of medium for ten days. The concentrations of glucose and TCE in the column feed were then set to 2 mM and 10 μM, respectively, and the flow rate was adjusted to 10 ml/h.

Analytical methods

Chlorinated aliphatic hydrocarbons, ethene, ethane and methane were quantified by gaschromatographic analysis of headspace gas. For the analysis of the fixed-bed reactor, headspace gas was drawn from 2 ml samples of feed or effluent that had been incubated for 60 min at 60 °C in 5 ml glass vials with Teflon seals. Headspace gas (20 °C) from batch cultures was obtained directly from serum bottles.

200 μl of headspace gas were analyzed with a GC 8700 gas chromatograph (Perkin Elmer, Beaconsfield, UK) equipped with a flame ionization detector and a Teflon coated stainless steel column (0.3 mm I. D. × 1.8 m) packed with Porapak P, mesh 80/100 (Bellafonte, PA). Operating conditions were as follows. Injector: 220 °C; detector: 250 °C; oven: 0.8 min at 50 °C, 50 °C to 190 °C at 25 °C/min, 3.5 min at 190 °C; nitrogen carrier gas flow: 25 ml/min. Water spiked with 13 compounds (methane, ethane, ethene, chloroethane (CA), 1,1-dichloroethane (1,1-DCA), 1,2-DCA, 1,1-dichloroethene (1,1-DCE), c-DCE, *trans*-1,2-dichloroethene (t-DCE), PCE, 1,1,1-TCA, TCE, VC) served as external standard. The detection limit was 0.07 μM for ethane, ethene and VC, 0.1 μM for methane and TCE and 0.15 μM for all other compounds. Chloride, acetate and formate were quantified by ion chromatography (Dionex, Sunnyvale, CA). The system consisted of an AS 10, 4 mm column, suppressor and conductivity detector. Samples were loaded for overnight runs on a LKB 2157 autosampler. 10 to 100 μl of filtered sample were injected. Operating conditions were as follows: 30 mM NaOH for 3.4 min, 60 mM NaOH for 5.7 min and 180 mM NaOH for 6.5 min, flow rate: 1.0 ml/min. The detection limit was 1 μM for all three compounds. For chloride, the background concentration of 14 μM present in the medium was subtracted.

Results

Enrichment cultures to utilize TCE as an electron acceptor under anaerobic conditions

Batch enrichment cultures were set up to obtain material for the inoculation of a laboratory fixed-bed reactor reductively dehalogenating TCE. The inoculum for these enrichments originated from a 43 m^3 anaerobic fixed-bed reactor which was packed with activated charcoal and operated continuously for over four years to eliminate dichloromethane from anaerobic groundwater (Stromeyer et al. 1991). Since the groundwater treated in this system contained traces of TCE, the biofilm on the charcoal of the reactor was suspected to contain organisms reductively dehalogenating this compound. This was shown to be the case when a series of batch cultures containing either yeast extract, methanol or glucose as an electron donor and inoculated with biofilm-coated charcoal (Materials & methods) degraded 6 μM TCE within 30 days (not shown).

Three primary enrichment cultures, one each on medium with 0.5 g/l of yeast extract, 1 mM methanol

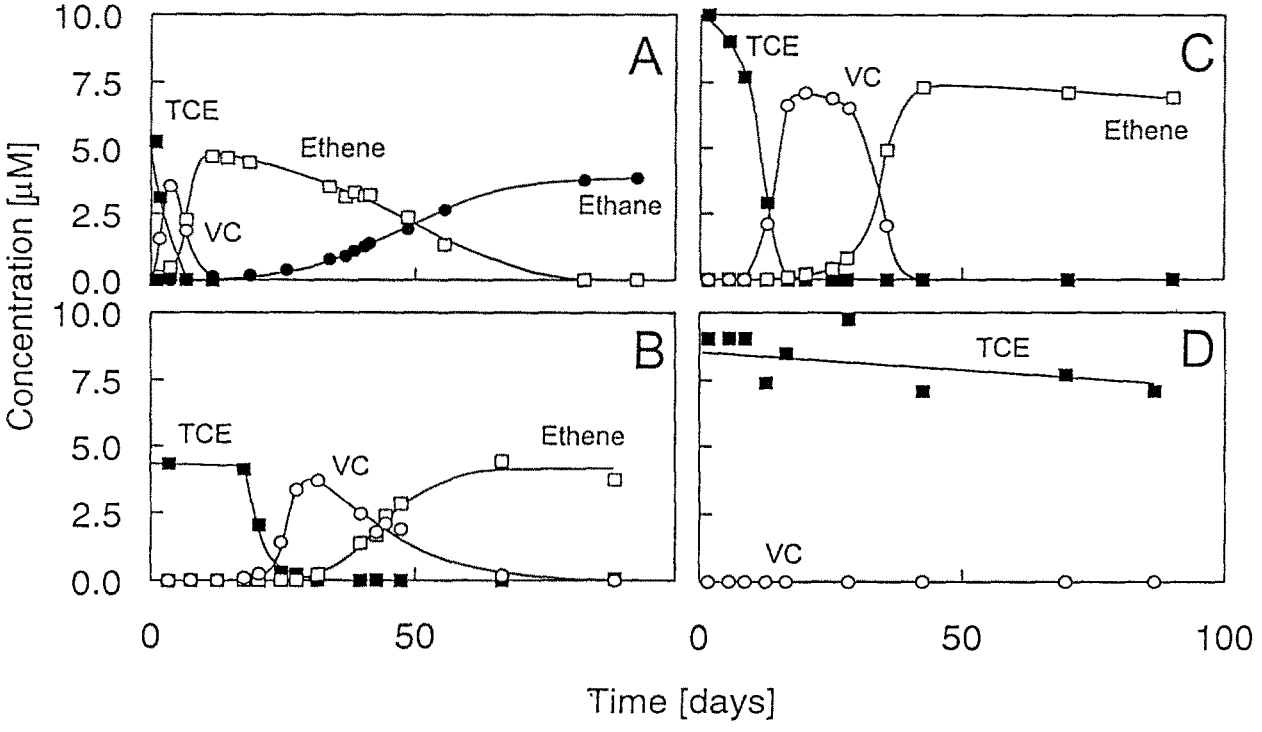

Fig. 2. TCE transformation in batch enrichment cultures subcultured three times with (A–C) or without TCE (D). Carbon sources were: 0.5 g/l yeast extract in A), 1 mM methanol in B) and 1 mM glucose in C) and D).

or 1 mM glucose, were subcultured without charcoal in the presence of 5 to 10 μM TCE. As shown in Fig. 2 for the fourth generation of these cultures, TCE was sequentially dehalogenated to c-DCE, VC and ethene irrespective of the electron donor supplied. In the enrichment with yeast extract (Fig. 2A) ethene was further reduced to ethane, whereas in the enrichments with methanol (Fig. 2B) and glucose (Fig. 2C) the transformation process stopped at the stage of ethene. The enrichment with methanol as an electron donor was least efficient with respect to TCE dehalogenation, but it was not investigated whether this was due to the low amount of carbon (12 mg C/l) present in the methanol medium as compared to the yeast extract (250 mg C/l) and the glucose (72 mg C/l) medium.

The primary enrichment on the medium with 1 mM glucose and TCE was also transferred for three generations on 1 mM glucose without TCE. When the fourth generation of this line was re-exposed to TCE, the dehalogenative capacity had been lost (Fig. 2D). This indicates that maintenance of the reductively dehalogenating organisms present in the primary enrichment requires selective pressure. The observation is in accordance with the finding of Holliger et al. (1993) that a quasi-pure culture of a PCE- and TCE-dehalogenating bacterium derives a selective advantage from utilizing these compounds as physiological electron acceptors. With a view to the large scale application of TCE reductive dehalogenation, glucose was chosen as the electron donor for all further experiments. Advantages of this compound include its low price, its non-toxicity and its non-flammability.

Performance of a TCE-dehalogenating enrichment culture in an anaerobic fixed-bed reactor

After inoculation with a fifth generation enrichment culture on 1 mM glucose and 10 μM TCE (compare Materials & methods) the bioreactor was operated for 93 days at a flow rate of 10 ml/h. During this period 2 mM glucose and 10 μM TCE were continuously added to the mineral medium. On day 94 the concentration of TCE was increased to 50 μM, and on day 174 the flow rate was set at 50 ml/h. On day 195 the reactor consistently transformed 55 μM TCE to ethene and chloride (Fig. 3). Based on the stoichiometry of the reaction, the recoveries of ethene and chloride amounted to 82% and 94% of the theoretical values. VC was

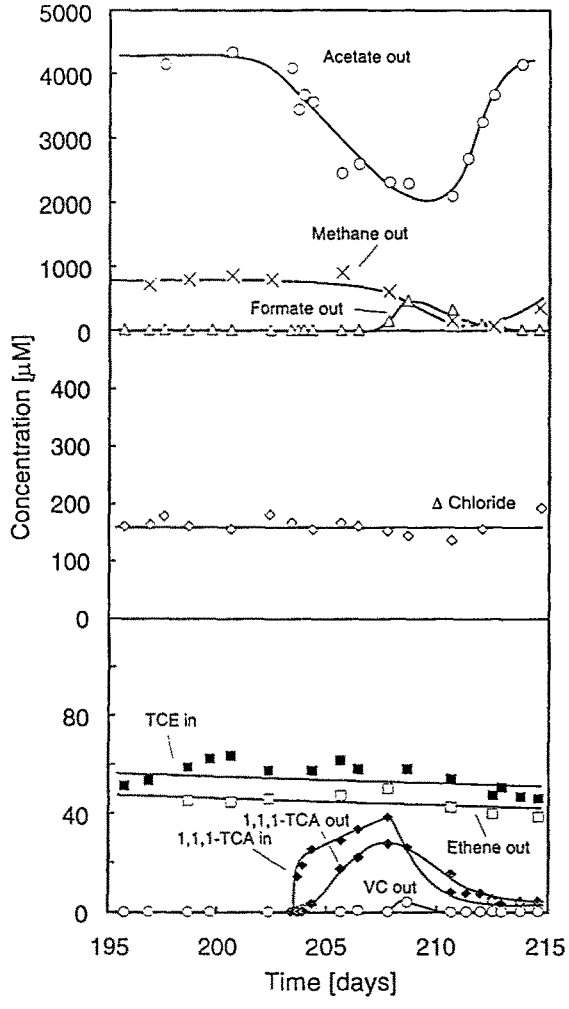

Fig. 3. Addition of 1,1,1-TCA to the fixed-bed reactor continuously degrading 55 μM TCE to ethene. 1,1,1-TCA was added from day 203 to day 209 to the system. No chlorinated compounds other than VC and 1,1,1-TCA were detected in the effluent (detection limit: 0.15 μM).

not detectable (< 0.07 μM) in the effluent. Since 95% of the chlorinated ethenes had disappeared at sideport I (not shown), the dehalogenative reactions occurred largely in the first 307 cm^3 of the reactor. 4.3 mM acetate and 0.8 mM methane were recovered in the effluent, and these products amounted to 78 % of the carbon from 2 mM glucose. Assuming the formation of equimolar amounts of methane and carbon dioxide (which was not measured) from glucose (Buswell 1933) and considering that about 10% of the carbon are fixed in biomass (Heijnen & van Dijken 1992), total estimated carbon recovery ranges around 95%.

Fate of chlorinated aliphatic hydrocarbons other than TCE in the anaerobic fixed-bed reactor

To test the stability and the versatility of the TCE dehalogenation system, the reactor was challenged with chlorinated aliphatic hydrocarbons to which the microbial population on the column had not been exposed in its previous history. Pulses of 1,1,1-TCA, 1,2-DCA and PCE were applied to the reactor feed.

1,1,1-TCA, added for a six-day period to a final concentration of 40 μM in the inflowing medium, was largely recovered in the effluent (Fig. 3). Potential transformation products such as 1,1-DCA and 1,1-dichlorethene were not detected. It thus appears that 1,1,1-TCA was not metabolized by the microorganisms in the reactor. The compound, however, exerted a marked toxic effect in that it inhibited both methanogenesis and acetogenesis and led to the transient accumulation of formate and VC in the effluent. Transformation of TCE to ethene was not affected by the presence of 1,1,1-TCA, and acetogenesis as well as methanogenesis recovered when 1,1,1-TCA was omitted from the reactor feed (Fig. 3).

1,2-DCA was added during a four-day period, initially to a concentration of 480 μM which gradually decreased to 90 μM (Fig. 4). From the start 1,2-DCA was transformed at a rate of 1.4 μmol/l/h to ethene in addition to the basal rate of TCE dehalogenation which proceeded at a rate of 1.4 μmol/l/h. The formation of methane and acetate were unaffected by the spike of 1,2-DCA in the system which, however, caused a transient accumulation of VC. The transformation rate of 1,2-DCA to ethene was constant and independent of the initial concentration of 1,2-DCA. This suggests that the preformed catalysts involved in this reaction operated at their maximum capacity. Since the dehalogenation of TCE was not affected by 1,2-DCA, these catalysts appear to be different from the activities responsible for the reduction of TCE to ethene.

PCE is known to be reductively dehalogenated via TCE as the first intermediate (Freedman & Gossett 1989). We wondered whether the enrichment culture in the fixed-bed reactor, which had never been exposed to significant levels of PCE, was able to dehalogenate this compound. As shown in Fig. 5 PCE added to concentration levels of 25 μM and 60 μM was readily transformed to ethene. The increase of the concentrations of ethene (65 μM) and chloride (240 μM) in the effluent upon installing 60 μM PCE in the feed was in accordance with the stoichiometry of PCE transformation to ethene. The production of methane and acetate

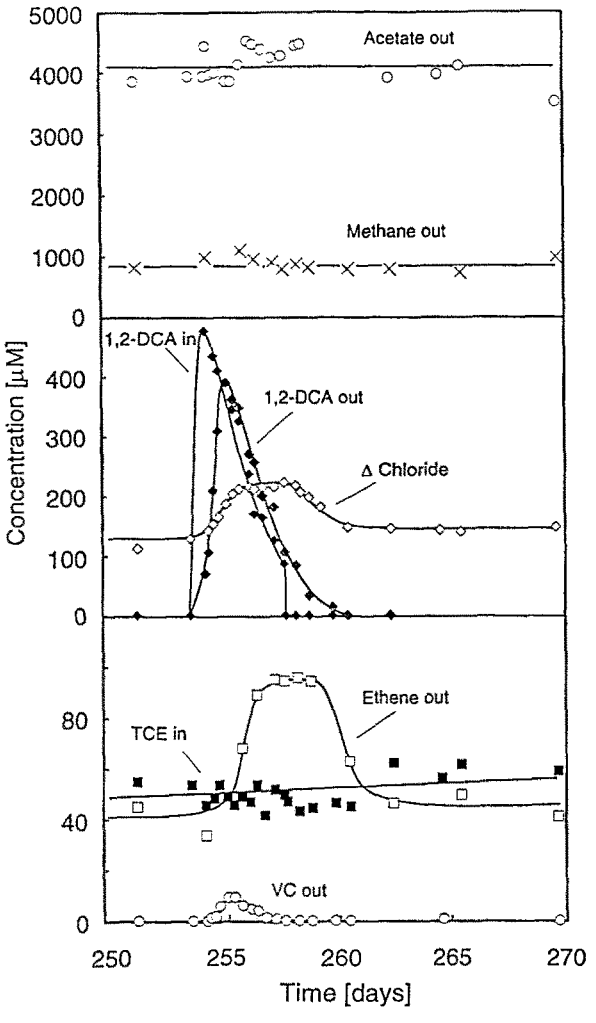

Fig. 4. Addition of 1,2-DCA to the fixed-bed reactor degrading 50 μM TCE to ethene. 1,2-DCA was added from day 254 to day 258 to the system. No chlorinated compounds other than VC and 1,2-DCA were detected in the effluent (detection limit: 0.15 μM).

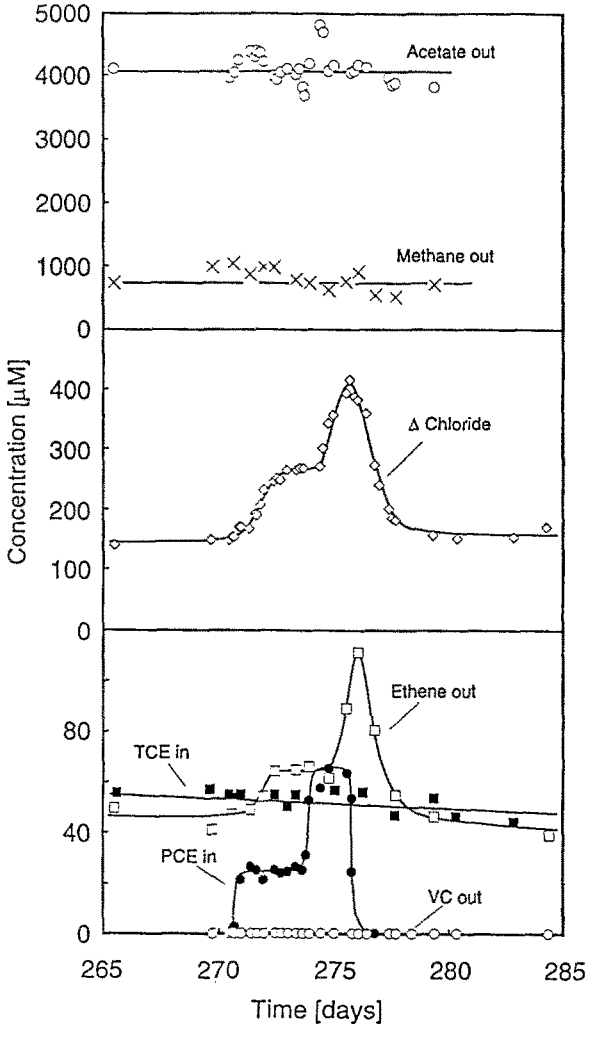

Fig. 5. Complete, simultaneous transformation of 60 μM PCE and 50 μM TCE to ethene in the fixed-bed reactor. No chlorinated compounds were detected in the effluent (detection limit: 0.15 μM).

was not affected by PCE, and there was no accumulation of VC. The instantaneous transformation of PCE indicates that the microbial activity responsible for the dehalogenation of this compound was present in the system and most likely is identical with the biocatalyst transforming TCE to c-DCE.

Maximum degradation capacity of the fixed-bed reactor

The previous experiment (Fig. 5) indicated that the capacity of the reactor for TCE degradation was larger than the 3.1 μmol/l/h attained with a total of 110 μM TCE plus PCE in the feed. We thus explored the limit of reactor performance by increasing the concentration of TCE in the medium at a constant flow rate of 50 ml/h. In a first step (data not shown) the concentration of TCE was increased to 220 μM. c-DCE and VC transiently accumulated, but after an adaptation period of 14 days ethene was again the only volatile product detected in the effluent.

In a second step the concentration of TCE was increased from 220 μM to 825 μM. As shown in Fig. 6, the sudden change in the TCE load led to massive accumulation of c-DCE and VC and to a corresponding decrease in the concentration of ethene in the effluent. The system slowly recovered, but when on day 318 (Fig. 6) the concentration of TCE in the feed was readjusted to 900 μM, adaptation to complete

dehalogenation of TCE was transiently interrupted. At high concentrations of TCE the reactor had apparently become sensitive to changes in the TCE load. A second adjustment of the TCE concentration on day 328 was paralleled by an increase in the concentration of glucose in the feed from 2 mM to 4 mM. Adaptation of the system to complete dehalogenation of TCE continued, and on day 335 TCE (820 μM) was transformed to 780 μM ethene and 4 μM VC. At this stage the volumetric degradation rate for TCE of the reactor amounted to 23 μmol/l/h. However, at this high rate the system had become unstable, as indicated by a renewed increase in the concentration of VC (Fig. 6). We conclude that reliable operation of the reactor for complete dehalogenation of TCE is possible with 220 μM but not with 800 to 900 μM TCE in the feed. The concentration profiles of TCE, VC, ethene and chloride in the fixed bed column on day 340 are shown in Fig. 7. They demonstrate that also at the high freight of 710 μM TCE dehalogenation occurred exclusively in the first sixth of the reactor volume.

Discussion

The fixed-bed reactor described in this study quantitatively dehalogenated TCE via c-DCE and VC to ethene. Evidence for this reaction sequence is based on the transient appearance of these intermediates in the batch culture used to inoculate the reactor and on their accumulation in the reactor when the system was disturbed (Fig. 6). The acetogenic mixed culture in the reactor thus catalyzed the same dehalogenation reactions as the anaerobic batch culture described by DiStefano et al. (1991) and the anaerobic fixed-bed reactor studied by de Bruin et al. (1992). While these authors have enriched and operated their systems with PCE, we have exposed our enrichments to TCE and have used this compound as the target for dehalogenation in the fixed-bed reactor. It was thus interesting to note that PCE was readily dehalogenated to ethene by our fixed-bed system, which had never been in contact with concentrations of PCE above 0.15 μM. This suggests that both PCE and TCE served as electron acceptors for the same fraction of the mixed culture and is in line with the properties of the organism characterized by Holliger et al. (1993) that dehalogenates these compounds to c-DCE. It has been suggested that several microorganisms are involved in the sequential reductive dehalogenation of PCE or TCE to ethene (de Bruin et al. 1992). Our data on the performance of

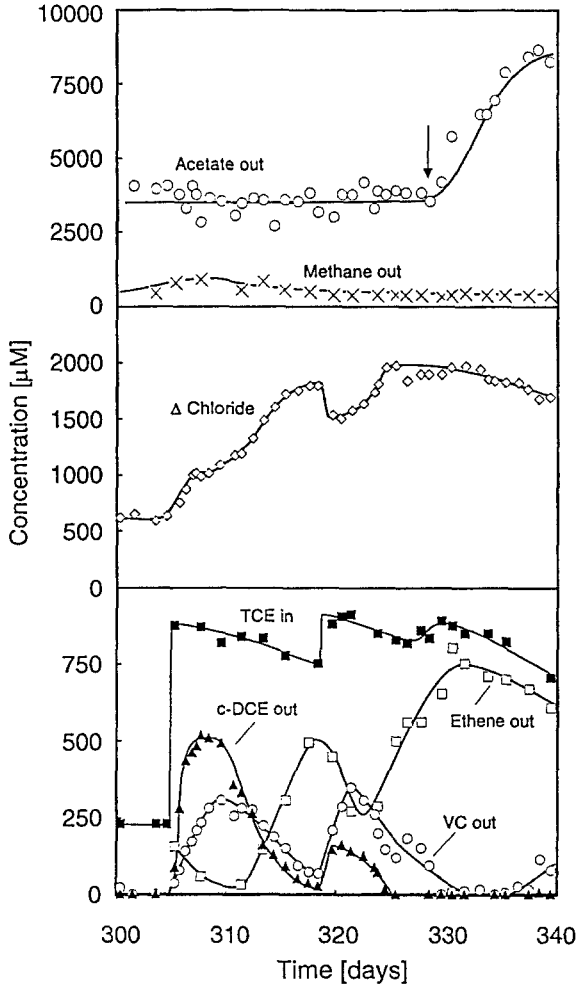

Fig. 6. Adaptation of the fixed-bed reactor to 825 μM TCE. On day 328 the glucose concentration in the feed was increased from 2 to 4 mM (arrow).

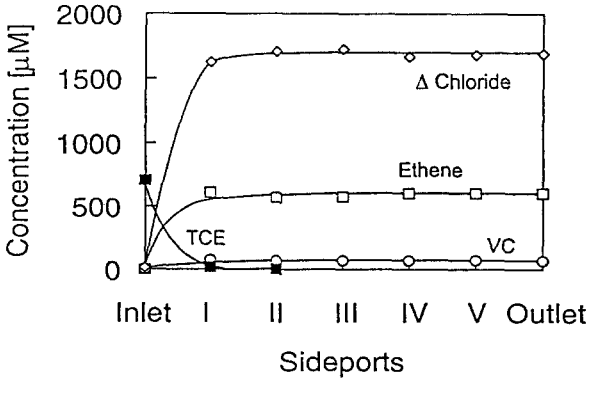

Fig. 7. Concentration profile of the fixed-bed reactor on day 340 of operation. 15 μM c-DCE detected at sideport I are not shown in the figure.

the fixed-bed reactor neither confirm nor exclude this view. They are, however, in accordance with previous observations that the conversion of VC to ethene is the critical step in the reaction sequence. Whenever the fixed-bed reactor was disturbed by the addition of a toxic compound or by a sudden increase in the TCE load, VC accumulated (Figs 3, 4, 6).

In view of the potential of TCE reductive dehalogenation for bioremediation applications we explored the maximum volumetric degradation rate of the fixed-bed reactor. At a TCE concentration of 220 μM in the feed the system performed complete dehalogenation, with VC remaining below the detection limit of 0.07 μM (5 μg/l). This corresponded to a volumetric degradation rate of 6.2 μmol/l/h. The value is comparable to the degradation rate of 3.7 μmol PCE/l/h observed in a fixed-bed reactor supplied with 1 mM lactate as electron donor (de Bruin et al. 1992) and to the PCE degradation rate of 5.7 μmol/l/h achieved in batch cultures with 1.5 mM methanol as electron donor (DiStefano et al. 1991).

Our experiments indicate that the degradation capacity of the fixed-bed reactor is about ten times larger than the actual degradation rate measured. Firstly, complete transformation of TCE occurred within the first sixth of the reactor volume (Fig. 7). Expanding the biologically active population throughout the reactor column thus would lead to a corresponding increase of the volumetric degradation rate. Secondly, the system transiently exhibited an actual degradation rate of 23 μmol/l/h, though it had become unstable at the high TCE load installed to achieve this rate (Fig. 6). We anticipate that optimization of the engineering parameters of the fixed-bed system should lead to actual degradation rates in the range of 50 to 100 μmol TCE/l/h. Such rates are of the same order of magnitude as those achieved in the aerobic degradation of TCE using a methanotrophic attached-film bioreactor (Fennell et al. 1993) or bioreactors with phenol-metabolizing pseudomonads (Folsom & Chapman 1991; Ensley & Kurisko 1994).

The ratio of the TCE concentration in the feed to the flow rate is an important parameter with respect to the application of TCE reductive dehalogenation in groundwater bioremediation. In the experiments presented here this ratio was high. Since in practice large volumes of low-level contaminated water have to be treated, the performance of the fixed-bed reactor needs to be explored when TCE freights similar to the ones we have used here are applied in larger volumes.

In addition to dehalogenating TCE and PCE the microbial population in the fixed-bed reactor also transformed 1,2-DCA to ethene and this process occurred without adaptation (Fig. 4). 1,2-DCA is known to be reductively dehalogenated by hydrogenotrophic (Egli et al. 1987; Belay & Daniels 1987) and aceticlastic (Holliger et al. 1990) methanogenic bacteria. In pure cultures of these organisms the compound undergoes dihaloelimination to ethene as well as hydrogenolysis to ethane via chloroethane as an intermediate, and both processes have been shown to be catalyzed simultaneously by purified methyl-CoM reductase, a key enzyme of methanogenesis (Holliger et al. 1992). Judged by the constant but low rate of formation of methane in the fixed-bed reactor, the system contained metabolically active methanogens. However, upon addition of 1,2-DCA we observed formation of ethene only, but not of choroethane and ethane, the products of the simultaneously occurring hydrogenolysis pathway of 1,2-DCA. This may mean that reductive dehalogenation of 1,2-DCA to chloroethane by methanogens was preferentially inhibited under the conditions in the reactor. Alternatively it is possible that organisms or catalysts other than methanogens were responsible for the dihaloelimination of 1,2-DCA to ethene.

When 40 μM 1,1,1-TCA were applied to the fixed-bed reactor, methanogenesis and acetogenesis were severely inhibited while 1,1,1-TCA was not metabolized (Fig. 3). The inhibitory effect we observed is in accordance with a report by Vargas & Ahlert (1987) who have found strong inhibition of methanogenesis and acetogenesis by 30 μM 1,1,1-TCA. The absence of product formation from 1,1,1-TCA, however, is striking in view of the presence of methanogens in the reactor. Methanogens (Egli et al. 1987) and methanogenic mixed cultures (Vogel & McCarty 1987) have been shown to transform 1,1,1-TCA to 1,1-DCA as the major product, as has a *Clostridium* sp. (Gälli & McCarty 1989). It is possible that the absence of 1,1,1-TCA dehalogenation results from the simultaneous presence of TCE, which itself and whose dehalogenation products may compete for electrons.

Acknowledgement

This study was supported by a grant from Mercedes-Benz AG.

References

Belay N & Daniels L (1987) Production of ethane, ethylene and acetylene from halogenated hydrocarbons by methanogenic bacteria. Appl. Environ. Microbiol. 53: 1604–1610

Buswell AM & Symons GE (1933) The methane fermentation of carbohydrates. J. Am. Chem. Soc. 55: 2028–2036

De Bruin WP, Kotterman MJJ, Posthumus MA, Schraa G & Zehnder AJB (1992) Complete biological transformation of tetrachloroethene to ethane. Appl. Environ. Microbiol. 58: 1996–2000

DiStefano TD, Gossett JM & Zinder SH (1991) Reductive dechlorination of high concentrations of tetrachloroethene to ethene by an anaerobic enrichment culture in the absence of methanogenesis. Appl. Environ. Microbiol. 57: 2287–2292

Easter MD & Von Burg R (1994) Toxicology update, vinyl chloride. J. Appl. Toxicol. 14: 301–307

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

Eichler B & Pfennig N (1986) Characterization of a new platelet-forming purple sulfur bacterium, *Amoebobacter pedioformis* sp. nov. Arch. Microbiol. 146: 295–300

Ensley BD & Kurisko PR (1994) A gas lift bioreactor for removal of contaminants from the vapor phase. Appl. Environ. Microbiol. 60: 285–290

Ensley BD (1991) Biochemical diversity of trichloroethylene metabolism. Annu. Rev. Microbiol. 45: 283–299

Fennell DE, Nelson YM, Underhill SE, White TE & Jewell JW (1993) TCE degradation in a methanotrophic attached-film bioreactor. Biotechnol. Bioeng. 42: 859–872

Folsom BR & Chapman PJ (1991) Performance characterization of a model bioreactor for the biodegradation of trichloroethylene by *pseudomonas cepacia* G4. Appl. Environ. Microbiol. 57: 1602–1608

Freedman DL & Gossett JM (1989) Biological reductive dechlorination of tetrachloroethylene and trichloroethylene to ethylene under methanogenic conditions. Appl. Environ. Microbiol. 55: 2144–2151

Gälli R & McCarty PL (1989) Kinetics of biotransformation of 1,1,1-trichloroethane by *Clostridium* sp. strain TCAIIB. Appl. Environ. Microbiol. 55: 845–851

Heijnen JJ & van Dijken JP (1992) In search of a thermodynamic description of biomass yields for the chemotrophic growth of microorganisms. Biotechnol. Bioeng. 39: 833–858

Holliger C, Kengen SWM, Schraa G, Stams AJM & Zehnder AJB (1992) Methyl-coenzyme M reductase of *Methanobacterium thermoautotrophicum* ΔH catalyzes the reductive dechlorination of 1,2-dichloroethane to ethylene and chloroethane. J. Bacteriol. 174: 4435–4443

Holliger C, Schraa G, Stams AJM & Zehnder AJB (1990) Reductive dechlorination of 1,2-dichloroethane and chloroethane by cell suspensions of methanogenic bacteria. Biodegradation 1: 253–261

— (1993) A highly purified enrichment culture couples the reductive dechlorination of tetrachloroethene to growth. Appl. Environ. Microbiol. 59: 2991–2997

Hopkins GD, Semprini L & McCarty PL (1993) Microcosm and in situ field studies of enhanced biotransformation of trichloroethylene by phenol-utilizing microorganisms. Appl. Environ. Microbiol. 59: 2277–2285

Howard PH (1990) Handbook of environmental fate and exposure data for organic chemicals. Vol 3 (pp 467–473) Lewis Publishers Inc. Michigan

Neumann A, Scholz-Muramatsu H & Dieckert G (1994) Tetrachloroethene dechlorination to cis-1,2-dichloroethene in cell suspensions and cell extracts of *Dehalospirillum multivorans* Spec. nov. Poster, VAAM Frühjahrstagung in Hannover

Shahin MM & Von Borstel RC (1977) Mutagenic and lethal effects of α-benzene hexachloride, dibutyl phthalate and trichloroethylene in *Saccharomyces cerevisiae*. Mutat. Res. 48: 173–180

Stromeyer SA, Winkelbauer W, Kohler H, Cook AM & Leisinger T (1991) Dichloromethane utilized by an anaerobic mixed culture: acetogenesis and methanogenesis. Biodegradation 2: 129–137

Van Duuren BL & Banerjee S (1976) Covalent interaction of metabolites of the carcinogen trichloroethylene in rat hepatic microsomes. Cancer Res. 36: 2419–2422

Vargas C & Ahlert RC (1987) Anaerobic degradation of chlorinated solvents. Res. J. Water Pollut. Control Fed. 59: 964–968

Vogel TM & McCarty PL (1987) Abiotic and biotic transformations of 1,1,1-trichloroethane under methanogenic conditions. Environ. Sci. Technol. 21: 1208–1213

Widdel F & Pfennig N (1981) Studies on dissimilatory sulfate-reducing bacteria that decompose fatty acids. Arch. Microbiol. 129: 395–400

Winkelbauer W & Kohler H (1991) Biologischer Abbau von Dichlormethan unter anaeroben Bedingungen in einer Aktivkohle-Anlage. GWF, Gas Wasserfach: Wasser/Abwasser 132: 425–432