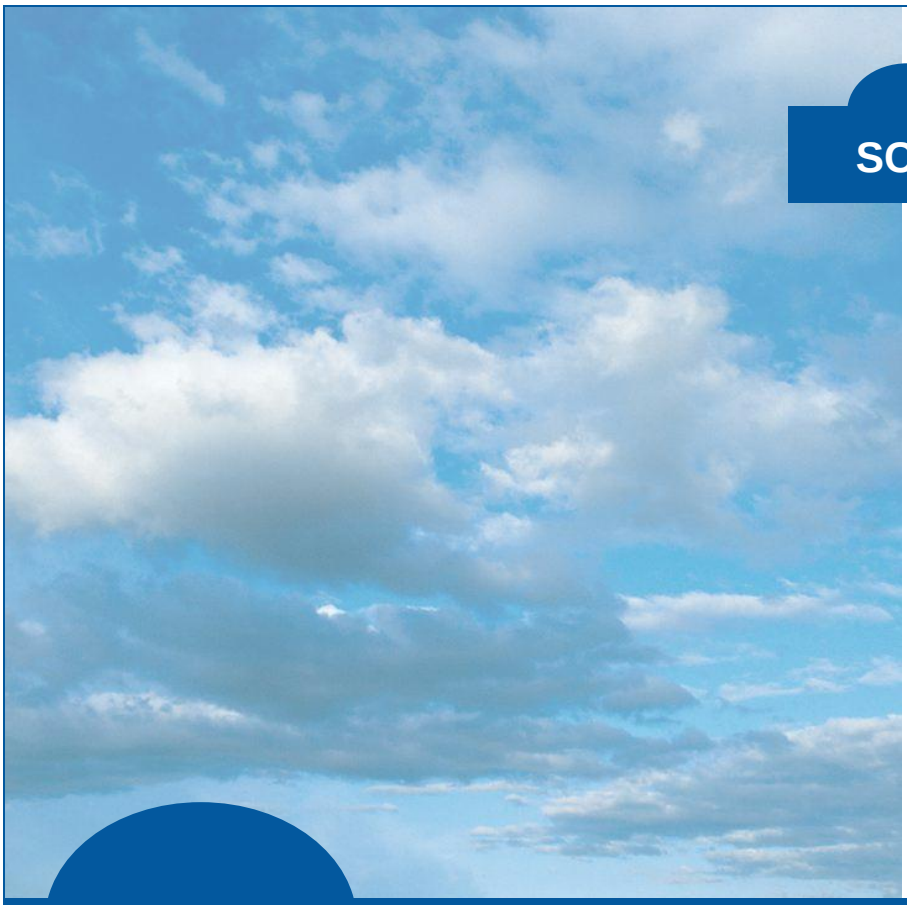

SCIENCE DOSSIER

Biodegradability of chlorinated solvents and related chlorinated aliphatic compounds

J. A. Field & R. Sierra-Alvarez
University of Arizona

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Foreword

The Monitoring & Environmental Chemistry Working group (MECW) is a science group of Euro Chlor, which represents the European chlor-alkali industry. The main objectives of the group are to identify both natural and anthropogenic sources of chlorinated substances, study their fate, gather information on the mechanisms of formation and degradation in the environment, and achieve a better knowledge of the persistence of such substances. The MECW often uses external specialists to assist in developing reports that review the state of existing knowledge of the different aspects mentioned.

Since 2001 Prof. Jim Field has been Associate Professor at the Department of Chemical & Environmental Engineering, University of Arizona. One of his main research topics is the natural production of organohalogens by fungi. He has been working closely with Euro Chlor for five years, researching biodehalogenation and biodegradation of chlorinated chemicals in the environment. Dr Reyes Sierra-Alverez has also been at the University of Arizona since 2001 and has 16 years research experience in environmental biotechnology. Her current research includes work on the biotransformation of chemicals in the environment. Dr Sierra-Alverez has a particular interest in the microbially catalysed transformation of metals and hazardous organic pollutants, bioremediation, biological treatment of industrial wastewaters and biotechnology for environmental benign manufacturing.

In *Biodegradability of chlorinated solvents and related chlorinated aliphatic compounds*, Field and Sierra-Alvarez demonstrate the capacity of micro-organisms to degrade many chlorinated organics such as chloromethanes, chloroethanes, chloroacetic acids and chloroethenes. This science dossier summarises and discusses the results of the most relevant studies that provide conclusive evidence of microbial biotransformation or mineralisation of chlorinated compounds. It is often the case that certain chlorinated chemicals are completely broken down into harmless matter due to the fact that micro-organisms grow by feeding off the compounds. Also illustrated are the various redox conditions that can be favourable for biodegradation, even of the most difficult compounds. Highly chlorinated structures tend to resist the degradation process under aerobic conditions, but under anaerobic conditions they are dehalogenated to lower chlorinated moieties. For the different compound categories, this dossier also summarises data on biodegradation rates in the environment.

Summary

The biodegradability of chlorinated methanes, chlorinated ethanes, chlorinated ethenes, chlorofluorocarbons (CFCs), chlorinated acetic acids, chlorinated propanoids and chlorinated butadienes was evaluated based on literature data. Evidence for the biodegradation of compounds in all of the compound categories evaluated has been reported.

A broad range of chlorinated aliphatic structures are susceptible to biodegradation under a variety of physiological and redox conditions. Microbial biodegradation of a wide variety of chlorinated aliphatic compounds was shown to occur under five physiological conditions. However, any given physiological condition could only act upon a subset of the chlorinated compounds. Firstly, chlorinated compounds are used as an electron donor and carbon source under aerobic conditions. Secondly, chlorinated compounds are cometabolised under aerobic conditions while the micro-organisms are growing (or otherwise already have grown) on another primary substrate. Thirdly, chlorinated compounds are also degraded under anaerobic conditions in which they are utilised as an electron donor and carbon source. Fourthly, chlorinated compounds can serve as an electron acceptor to support respiration of anaerobic micro-organisms utilising simple electron donating substrates. Lastly chlorinated compounds are subject to anaerobic co-metabolism becoming biotransformed while the micro-organisms grow on other primary substrate or electron acceptor.

The literature survey demonstrates that in many cases, chlorinated compounds are completely mineralised to benign end products. Additionally, biodegradation can occur rapidly. Growth rates exceeding 1 d^{-1} were observed for many compounds. Most compound categories include chlorinated structures that are used to support microbial growth. Growth can be due to the use of the chlorinated compound as an electron donor or alternatively to the use of the chlorinated compound as an electron acceptor (halorespiration). Biodegradation linked to growth is important, since under such conditions, rates of degradation will increase as the microbial population (biocatalyst) increases. Combinations of redox conditions are favourable for the biodegradation of highly chlorinated structures that are recalcitrant to degradation under aerobic conditions. However, under anaerobic conditions, highly chlorinated structures are partially dehalogenated to lower chlorinated counterparts. The lower chlorinated compounds are subsequently more readily mineralised under aerobic conditions.

1. Introduction

The objective of this manuscript is to review the literature on the biodegradability of chlorinated solvents and related chlorinated aliphatic compounds. Seven compound categories are examined as follows:

1. chlorinated methanes
2. chlorinated ethanes
3. chlorinated ethenes
4. chlorofluorocarbons
5. chlorinated acetic acids
6. chlorinated propanoid compounds
7. chloro-1,3-butadienes.

Each category is examined closely in separate sections with respect to the biodegradation of the compounds in the environment, in engineered systems and the microbiological basis for the observed biodegradation. A brief introduction is provided here on the sources of chlorinated solvents, basic principles of biodegradation and mechanisms of microbial dehalogenation.

1.1 Sources

Chlorinated solvents and related chlorinated aliphatic structure enter into the biosphere from both industrial and natural sources. Literature data on industrial production and emissions of selected chlorinated solvents are summarised in Tables 1 and 2, respectively. Global industrial emissions of halogenated methanes range from 100 to 550 kt y^{-1} (Harper 2000; Laturnus *et al*. 2002). Production of chlorinated ethanes and chlorinated ethene solvents in the U.S. ranges from 78 to 8000 kt y^{-1} (Fetzner 1998; Swoboda-Colberg 1995). The scale of production is similar in Western Europe (Fetzner 1998). Emission of chlorinated solvents at 80 production plants in Europe is on the decline. Releases of chlorinated solvents ranged from 0.1 to 3.5 kt y^{-1} in 1997, which are approximately 20% of the levels discharged in 1985 (Lecloux 2003). The chlorinated solvents are used principally for degreasing and dry cleaning and have entered the environment through leakage in storage tanks and irresponsible disposal in the past. Additionally related compounds such as vinyl chloride (VC) and 1,2-dichloroethane (1,2-DCA) are important bulk chemicals used as intermediates in the synthesis of other chemicals. The world production of VC is estimated at $14x10^6$ to $20x10^6$ t y^{-1} (Fetzner 1998; WHO 1990). The combined production of 1,2-DCA in the USA, Western Europe and Japan is estimated at $17.5x10^6$ t y^{-1}.

Chlorinated compounds are also introduced into the environment through natural sources. Over 3800 natural organohalogen compounds have been identified (Gribble 2003; Winterton 2000). Chlorinated methanes, alkanes and alkenes are produced either biologically or through chemical reactions in soils at ambient temperatures. Chlorinated solvents such as perchloroethylene (PCE) and trichloroethylene (TCE) are produced by marine algae (Abrahamsson *et al*. 1995). VC, chloromethane (CM), trichloroacetic acid (TCAA) and chloroform (CF) are generated during the oxidation of soil humus (Hoekstra 2003; Hoekstra *et al.* 1998a; Keppler *et al.* 2000 & 2002; Laturnus *et al*. 2002). CM and CF are also produced by plants and fungi (Harper 2000; Hoekstra *et al.* 1998b). The natural production of chloromethane is estimated at $4x10^6$ t y^{-1} (Gribble 2003), which far exceeds the industrial production. Additionally, the natural production of CF is estimated at $0.7x10^6$ t y^{-1} and thus exceeds the industrial emission (Laturnus *et al.* 2002).

1.2 Principles of Biodegradation

The microbial degradation of a contaminant typically occurs because a micro-organism can benefit from the use of the contaminant as an electron donor and carbon source to support growth. The redox reactions result in the flow of electrons from the substrate to a terminal electron acceptor (e.g. an oxidant such as O_2) and the release of energy that is used to support cell synthesis (Tiedje 1993). Growth of the microbial population results in an

increase in the biocatalyst and, thus, the rates of biodegradation can increase. Aside from O_2, a large number of alternative terminal electron acceptors can be utilised in the absence of O_2 to support anoxic respiration. Ecological significant electron acceptors include: the use of NO_3^-, known as denitrification; the use of Fe^{3+} bearing minerals such as ferrihydrite ($Fe(OH)_3$), known as dissimilatory iron reduction; the use of SO_4^{2-}, known as sulfate reduction; and the use of CO_2, known as methanogenesis. The standard reduction potential at pH 7 ($E°'$) of the common terminal electron acceptor redox pairs is 0.82, 0.75, 0.00, -0.22 and –0.25 V for O_2/H_2O, NO_3^-/N_2, $Fe(OH)_3/Fe^{2+}$ SO_4^{2-}/H_2S and CO_2/CH_4, respectively. The standard Gibb's free energy change is more negative for reactions linked to terminal electron acceptors with higher values of $E°'$ and as such are more energetically favourable.

On the other hand, the chlorinated compound can serve as the electron acceptor in a process known as halorespiration. During this type of metabolism anoxic decomposition of simple organic substrates (e.g. lactic acid) is linked to the use of chlorinated compounds as electron acceptors resulting in their reductive dehalogenation (McCarty 1997). Halorespiration is also linked to growth contributing to increases in biocatalyst concentration as degradation proceeds. The $E°'$ of chlorinated solvents used as terminal electron acceptors are relatively high, for example the $E°'$ for the couple PCE/TCE is 0.58V. Thus, halorespiration is an energetically favourable microbiological process.

Micro-organisms can also fortuitously transform chlorinated compounds while they are degrading non-halogenated primary substrates. In this case, enzymes expressed to degrade the primary substrate accidentally display activity with the chlorinated compound. This type of metabolism is referred to as co-metabolism (Wackett 1995). A special type of co-metabolism involves the oxidation of chlorinated solvents by oxygenases, which is known as cooxidation (Alvarez-Cohen & Speitel Jr 2001). The classic example is the oxidation of chlorinated solvents by the methane monooxygenase expressed by methylotrophic organisms to oxidise methane. Under anaerobic conditions, a common form of co-metabolism is the reaction of reduced enzyme cofactors with chlorinated solvents, resulting in their reductive dehalogenation (Gantzer & Wackett 1991; Krone *et al.* 1989). The degradation of chlorinated solvents during co-metabolism is not linked to growth. Thus the concentration of the biocatalyst will depend on the addition of the primary electron donating substrates and electron acceptors to the system.

1.3 Mechanisms of Dehalogenation

As chlorinated compounds are being metabolised by micro-organisms, chlorine substituents are enzymatically removed to form non-halogenated intermediates that can enter into normal biochemical routes of metabolisms, such as the tricarboxylic acid cycle. Several review articles are available which discuss in detail the mechanisms of different enzymes involved in dehalogenation (Fetzner 1998; Janssen *et al.* 1994; van Pee & Unversucht 2003). An overview of the enzymatic strategies towards dehalogenation is given in Figure 1. An oxygenolytic attack of chlorinated solvents typically involves a monooxygenase that uses elemental oxygen to insert one oxygen atom into the molecule. If the oxygen is inserted onto the same carbon as a chloro group, the resulting molecule is chemically unstable and will spontaneously convert to an aldehyde, releasing the chloro group as HCl. A monooxygenase attack of chlorinated ethenes results in the formation of unstable epoxides that spontaneously decompose to organic acids, including chloroacetic acids (Fetzner 1998; Wackett 1995).

Three common mechanisms of reductive dechlorination are shown in Figure 1. Reductive hydrogenolysis is the most common and involves the sequential replacement of chloro-substituents with H from protons, releasing HCl (Holliger *et al.* 2003). Reductive dichloroelimination is also common for chlorinated alkanes, in which vicinal chloro-groups on neighboring carbons are eliminated and replaced by a double bond (De Wildeman & Verstraete 2003). Hydrolytic reduction is common for halomethanes, in which two halogen substituents are removed leaving a dicarbene radical to react with water or sulfhydryl groups (van Eekert *et al.* 1998).

Many dehalogenases involve hydrolytic mechanisms in which the chlorine-carbon bond is hydrolysed, resulting in the replacement of the chloro group with a hydroxyl substitution (Janssen *et al.* 1994). Haloalkane dehalogenases utilise this mechanism and are commonly employed by aerobic micro-organisms degrading chlorinated alkanes and chloroacetic

acids. Similar substitutive strategy is used by thiolytic enzymes, in which a chloro group is replaced by a sulfhydryl group such as from the common biochemical glutathione (Fetzner 1998; Leisinger *et al.* 1994; van Pee & Unversucht 2003). The metabolism of chloromethane is characterised by a dehalogenation reactions carried out by methyl transferase enzymes (Fetzner 1998; McDonald *et al.* 2002).

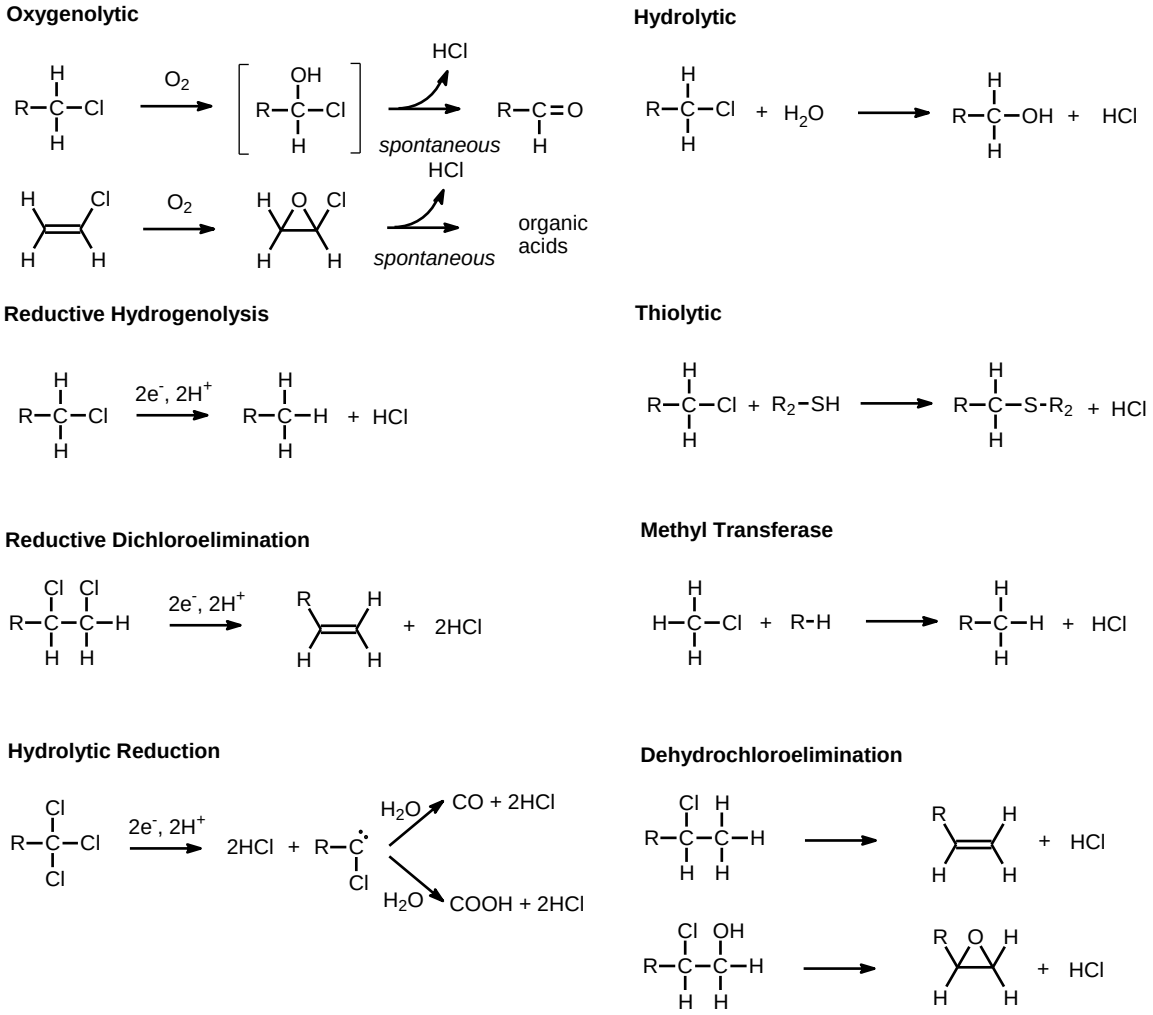

Figure 1: Mechanisms of enzymatic bioconversion of chlorinated compounds. Adapted in part from Fetzner (1998); van Eekert (1999); van Pee & Unversucht (2003); Wackett (1995). R = rest of molecule.

Lastly, the elimination of HCl from vicinal hydrogen and chlorine atoms on neighboring carbons is referred to as dehydrochloroelimination. This type of mechanism is a common abiotic dehalogenation reaction occurring in anaerobic sludge with higher chlorinated alkanes, resulting in the formation of chloroethenes (De Wildeman & Verstraete 2003; van Eekert *et al.* 1999). A similar mechanism is used during catalysis of chloropropanes by haloalcohol dehalogenase, which eliminates an HCl from neighboring hydroxyl and chloro-substituents to yield an epoxide. The enzyme can also work in the reverse direction, causing the halogenation of an epoxide (Fetzner 1998; van Pee & Unversucht 2003).

1.4 Scope of Review

The main purpose of the review is to demonstrate the vast capacity of micro-organisms in the environment to degrade chlorinated solvents and related chloroaliphatic contaminants. The main strategies and pathways involved in the degradation for each compound category are elucidated. Possible intermediates and end products of degradation are inventoried, so as to provide information on changes in risk associated with compound biotransformation and biodegradation. An attempt was also made to provide kinetic information on the microbial bioconversion processes to provide insights on the rates of compound dissipation in the environment as well as in engineered bioremediation and biotreatment systems.

2. Chloromethanes

2.1 Introduction

Chloromethanes can be divided into two categories; either the lower- or higher chlorinated methanes, based on the distinct behaviour patterns in their biodegradability. Lower chlorinated methanes, which include chloromethane (CM) and dichloromethane (DCM), can be utilised as primary growth substrates by both aerobic and anaerobic micro-organisms. In addition, lower chlorinated methanes are also known to be cometabolised. Higher chlorinated methanes, which include chloroform (CF) and carbon tetrachloride (CT), are only known to be cometabolised. The biodegradation of CT is almost exclusively under anaerobic conditions.

The only review articles available on the biodegradation of chloromethanes are concerned with the simpler compounds, CM and DCM. Harper (2000) reviewed both anaerobic and aerobic biodegradation of CM in relation to possible sinks of CM in the global atmospheric CM budget. McDonald *et al.* (2002) reviewed the latest developments in the biochemistry and genetics of aerobic biodegradation of chloromethane. Leisinger *et al.* (1994) reviewed the microbiology, biochemistry and genetics of DCM degradation.

2.2 Biodegradation of lower chlorinated methanes

2.2.1 Degradation of lower chlorinated methanes in the environment

Little information is available on the biodegradation of lower chlorinated methanes in the environment. Khalil & Rasmussen (2000) have monitored the uptake of chloromethane by soils in Brazil and Greenland at rates averaging 10 $\mu g\ m^{-2}\ d^{-1}$. The average rate extrapolated over the land area of the earth accounts for an estimated terrestrial sink of CM of 0.5 $Tg\ y^{-1}$. Strains of aerobic bacteria capable of utilising CM as a growth substrate have been isolated from a variety of pristine terrestrial, freshwater, estuarine and marine environments (Coulter *et al.* 1999; McAnulla *et al.* 2001). Also anaerobic sludge that was not previously exposed to chloromethanes was capable of degrading CM and DCM (van Eekert *et al.* 1998).

2.2.2 Degradation of lower chlorinated methanes in engineered systems

No information could be found on the biodegradation of CM in engineered systems. On the other hand, DCM degradation in engineered systems has been described in bioreactors treating DCM-containing liquid and gaseous effluents. In an anaerobic packed bed bioreactor, a DCM biodegradation rate of 1.25 $kg\ m^{-3}$ reactor d^{-1} from a liquid effluent was achieved with acetate and formate as electron donors (De Best *et al.* 2000). DCM degradation in an anaerobic chemostat operated under methanogenic conditions was 0.054 $kg\ m^{-3}$ reactor d^{-1} with propionate as primary substrate (Rhee & Speece 2000). Biodegradation rates of 34 to 40 $kg\ m^{-3}$ reactor d^{-1} were achieved in aerobic fluidised bed reactors treating DCM in liquid effluents (Flanagan 1998; Galli 1987; Herbst & Wiesmann 1996). The treatment of DCM in waste gases has also been reported at maximum rates ranging from 3.8 to 4.8 $kg\ m^{-3}$ reactor d^{-1} (Diks & Ottengraf 1991; Hartmans & Tramper 1991).

2.2.3 Microbiology and biochemistry of lower chlorinated methane biodegradation

Under both anaerobic and aerobic conditions, micro-organisms have been found that can degrade CM and DCM as sole sources of carbon and energy. Additionally, aerobic cooxidation of these compounds has been observed.

Anaerobic growth on lower chlorinated methanes as electron donor. An anaerobic acetogenic bacterium isolated from sewage sludge, *Acetobacterium dehalogenans* strain MC, is capable of utilising CM as a sole source of energy (Traunecker *et al.* 1991). The organism requires 2 moles of CO_2 to form 3 moles of acetate per 4 moles of CM consumed. The dechlorinating enzyme involved is a methyltransferase, which transfers the methyl group to a corrinoid protein (vitamin B12 containing protein). Subsequently, a second

methyltransferase transfers the methyl group to tetrahydrofolate (an important coenzyme of C_1-metabolising organisms) (Harper 2000; Messmer *et al.* 1996).

Anaerobic degradation of DCM as a sole source of carbon and energy has been reported for methanogenic and acetogenic mixed cultures (Brausstromeyer *et al.* 1993; Freedman & Gossett 1991; Magli *et al.* 1995). Magli *et al.* (1996) isolated *Dehalobacterium formicoaceticum* as the responsible DCM degrading bacterium from the acetogenic enrichment culture. *Dehalobacterium formicoaceticum* converted 3 moles of DCM and one mole of CO_2 to formate and acetate in a 2:1 molar ratio. Similar to CM metabolism, the reaction involves corrinoid proteins and the transfer of the methylene group onto tetrahydrofolate (Magli *et al.* 1998). DCM is also degraded under denitrifying conditions (De Best *et al.* 2000; Freedman *et al.* 1997). *Acinetobacter* sp. isolated from activated sludge rapidly metabolised DCM with nitrate as the terminal electron acceptor with a growth rate (0.89 d^{-1}) and specific activity (3.8 g DCM (g biomass volatile suspended solids (VSS))$^{-1}$ d^{-1}) comparable to that under aerobic conditions (Freedman *et al.* 1997). *Hyphomicrobium* sp. DM2 is another isolate capable of degrading DCM under denitrifying conditions with a growth rate of 0.36 d^{-1}, which is 4-fold slower than under aerobic conditions (Kohler-Straub & Leisinger 1995).

Aerobic growth on lower chlorinated methanes. Many aerobic bacteria have also been isolated which are capable of growth with CM as the primary substrate. Hartmans *et al.* (1986) isolated the first bacterium (strain MC1) capable of growing on CM from industrial sewage. The isolate, identified as a *Hyphomicrobium*, used CM but not methane as a growth substrate. One research group (Doronina *et al.* 1996; McDonald *et al.* 2001) isolated eight CM-utilising bacteria belonging to the genera *Hyphomicrobium* and *Methylobacterium* from polluted soil at a petrochemical factory. Additionally CM-utilising bacteria belonging to the genera *Rhizobium*, *Aminobacter* and *Nocardioides* have been isolated from unpolluted environmental samples (Coulter *et al.* 1999; McAnulla *et al.* 2001). Similar to anaerobic growth on CM, the biochemistry of aerobic CM-utilisation also involves corrinoid-dependent methyltransferases (Coulter *et al.* 1999; McDonald *et al.* 2002). The biochemistry of the aerobic and anaerobic CM-utilisers is similar. The aerobes however utilise only one methyltransferase (CmuA) for the transfer of the methyl group to tetrahydrofolate; whereas two methyltransferases are required for the anaerobe.

Many aerobic bacterial isolates originating from activated sludge have been identified which can utilise DCM as a growth substrate (Leisinger *et al.* 1994). The isolates belong to the genera *Hyphomicrobium*, *Methylobacterium*, *Methylophilus* and *Pseudomonas*. Recently aerobic DCM utilising strains have also been described from the genera *Paracoccus*, *Albibacter*, and *Methylopila* (Doronina *et al.* 1998; 2000 & 2001), attesting to a large biodiversity. The dehalogenation of DCM by these aerobic facultative methylotrophic bacteria is catalysed by glutathione S transferases yielding S-chloromethylglutathione, an unstable intermediate subject to abiotic hydrolysis to formaldehyde (Bader & Leisinger 1994; Gisi *et al.* 1998; Leisinger *et al.* 1994). The pathway of aerobic DCM degradation is illustrated in Figure 2 (Leisinger *et al.* 1994).

Aerobic cooxidation of lower chlorinated methanes. Both CM and DCM are cometabolised by various aerobic micro-organisms oxidising other compounds as primary substrates. The reactions involve cooxidation of the lower chlorinated methanes with the oxygenases produced to oxidise the primary substrates. CM is cooxidised by *Methylosinus trichosporium* (Bartnicki & Castro 1994) and *Methylococcus capsulatus* (Stirling & Dalton 1979) with methane as the primary substrate and by a butane-oxidising enrichment culture (Kim *et al.* 2000). With *Methylosinus trichosporium*, a half-life of 9.5 min for CM was reported with a cell density of 100 g wet weight l^{-1}. Involvement of methane monooxygenase was confirmed by inhibition due to acetylene (Bartnicki & Castro 1994). Additionally a methyl bromide utilising isolate (strain IMB-1) was shown to cometabolise CM (Schaefer & Oremland 1999). CM was cooxidised by the nitrifying bacterium *Nitrosomonas europaea* with ammonia as the primary substrate and formaldehyde was recovered as sole end product of the reaction (Rasche *et al.* 1990). DCM was also cooxidised by *Nitrosomonas europaea* with ammonia (Vannelli *et al.* 1990) and by *Methylosinus trichosporium* with methane as the primary substrates (Bartnicki & Castro 1994; Oldenhuis *et al.* 1989) as well as by the butane-oxidising enrichment culture (Kim *et al.* 2000).

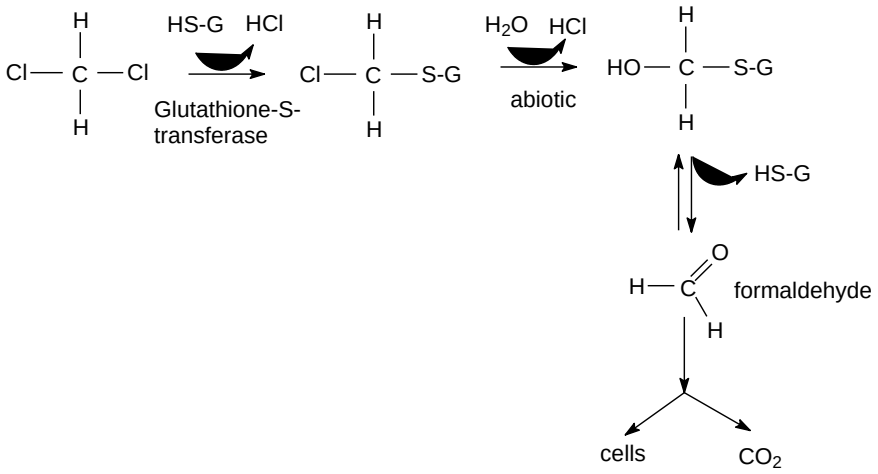

Figure 2: Proposed pathway for the aerobic degradation of DCM (Leisinger *et al.* 1994). HS-G = glutathione (5-L-glutamyl-L-cysteinylglycine, $C_{10}H_{17}N_3O_6S$).

Kinetic data on lower chlorinated methane biodegradation. Kinetic parameters of lower chlorinated methane degradation are shown in Table 3. Growth rates during the anaerobic utilisation of CM and DCM range from 0.36 to 0.89 d^{-1}; whereas those of aerobic bacteria range from 0.89 to 3.65 d^{-1}. Cell yields of both anaerobic and aerobic utilising bacteria are similar and range from 0.09 to 0.20 g cell dry weight (dwt) g^{-1} chlorinated methane consumed. Specific activities of the anaerobes range from 1700 to 9100 chlorinated methane mg (g dwt)$^{-1}$ d^{-1} while that of the aerobes range from 8500 to 26000mg(g dwt)$^{-1}d^{-1}$. There are only three data points for the specific activity of aerobic cooxidation of lower chlorinated methanes and these values were many magnitudes lower than the specific activities of aerobic bacteria directly utilising lower chlorinated methanes.

2.3 Biodegradation of higher chlorinated methanes

2.3.1 Degradation of higher chlorinated methanes in the environment

A decade of field data collected at a CT contaminated industrial site constructed over an estuary in France corroborated natural attenuation of a CT due primarily to anaerobic biodegradation (Davis *et al.* 2003). CT degradation was observed in anoxic marine sediments (Lee *et al.* 1999a). Also CT degradation was demonstrated with *in situ* microcosms placed in several boreholes at landfill leachate contaminated aquifers in Denmark (Bjerg *et al.* 1999; Nielsen *et al.* 1992). CT injected into a landfill leachate plume dominated by iron-reducing conditions was degraded with a first-order rate constant greater than 0.7 d^{-1} (Rugge *et al.* 1999). Also methanogenic landfill leachate samples were shown to degrade CT (Kromann *et al.* 1998). Both CT and CF are readily biotransformed by unadapted methanogenic sludge from various sources (Olivas *et al.* 2002; van Eekert *et al.* 1998; Yu & Smith 1997). Removal of atmospheric CT was demonstrated in the unsaturated zone of soil profiles, with only 25% of the initial atmospheric CT present in the gas phase at 30 cm depth (Happell & Wallace 1998). A CT flux of 8300 pmol m^{-2} soil surface d^{-1} was estimated in the latter study.

2.3.2 Degradation of higher chlorinated methanes in engineered systems

Numerous reports indicate the degradation of CT and CF in anaerobic bioreactors provided with simple organic compounds electron donating substrates (Boucquey *et al.* 1995; Bouwer & McCarty 1983; Fathepure & Vogel 1991; Long *et al.* 1993). CT was removed at a rate of 11 to 31 and 19 g m^{-3} reactor d^{-1} in methanogenic (Boucquey *et al.* 1995; Jin & Englande 1998; Sponza 2002) and sulfate-reducing (de Best *et al.* 1997a) biofilm reactors, respectively. CF was removed at a rate of 47 g m^{-3} reactor d^{-1} by a CF-degrading enrichment culture in a chemostat operated under sulfate-reducing conditions (Gupta *et al.* 1996). In an anaerobic chemostat operated under methanogenic conditions, CF degradation was 78 g m^{-3} reactor d^{-1} with propionate as primary substrate (Rhee & Speece 2000). In anaerobic bioreactors, CT is converted to CF, DCM and other unidentified products (de Best *et al.* 1997a). Under similar conditions CF is converted to DCM (Gupta *et al.* 1996). Bouwer & McCarty (1983) observed that a large fraction of radiolabeled CT and

CF was converted to CO_2 in anaerobic bioreactors indicating the occurrence of alternative processes other than reductive hydrogenolysis.

Also CT degradation was demonstrated during a field bioremediation study with acetate injection in a sulfate-reducing aquifer (Devlin & Muller 1999). CT was completely transformed producing CF and CS_2 at a ratio of approximately 2:1. Lee *et al.* (1998). reviewed five anaerobic bioremediation pilot studies in which CT was one of the major contaminants. In all of the pilot studies, CT degradation was associated with CF formation. In one study, DCM and CS_2 were additionally detected.

2.3.3 Microbiology and biochemistry of higher chlorinated methane biodegradation

In stark contrast to lower chlorinated methanes, CF and CT are not utilised as sole sources of carbon and energy. Rather biodegradation is limited to fortuitous co-metabolism (Alvarez-Cohen & Speitel Jr 2001; Fetzner 1998). Biodegradation of CT occurs almost exclusively under anaerobic conditions, while CF can be cometabolised under both anaerobic and aerobic conditions (Alvarez-Cohen & Speitel Jr 2001; Ferguson & Pietari 2000).

Anaerobic co-metabolism of higher chlorinated methanes. Under anaerobic conditions CT and CF degradation has been observed to take place by pure cultures of methanogens (Bagley & Gossett 1995; Egli *et al.* 1987; Mikesell & Boyd 1990), acetogenic bacteria (Egli *et al.* 1988), fermentative bacteria (Galli & McCarty 1989), sulfate-reducing bacteria (Egli *et al.* 1987) and iron-reducing bacteria (Picardal *et al.* 1993) without any apparent benefit to the microbe responsible for the degradation, suggesting co-metabolism (Holliger & Schraa 1994). In these experiments, CT and CF are sequentially reduced forming CF, DCM and even traces of CM as products. In some studies, radiolabeled CO_2 as well as CS_2 were also observed from the anaerobic conversion of radiolabeled CT or CF (Bagley & Gossett 1995; Egli *et al.* 1988; Hashsham *et al.* 1995; Mikesell & Boyd 1990). Heat-killed cells of methanogens, acetogens and methanogenic mixed cultures are also able to catalyze the dechlorination of CT and CF (Egli *et al.* 1990; van Eekert *et al.* 1998; Yu & Smith 1997) forming similar products as the living cells including CO_2 and CS_2. Reduced enzyme cofactors of the anaerobic microbes have been implicated as the catalyst of these biotransformations. The heat stable cobalt-containing cofactor, vitamin B12, was shown to directly catalyze the dechlorination of CT and CF when supplied with an appropriate reducing agent such as Ti(III)-citrate or hydrogen sulfide (Assaf-Anid & Lin 2002; Gantzer & Wackett 1991; Krone *et al.* 1991; Tanaka 1997). Vitamin B12 is a common cofactor of strict anaerobes, especially those involved in C_1 metabolism. Addition of vitamin B12 greatly accelerated CT and CF dechlorination by whole cells of anaerobic micro-organisms (Becker & Freedman 1994; Hashsham *et al.* 1995; Zou *et al.* 2000). The unique nickel containing coenzyme F430 of methanogens was also shown to catalyze the reductive dechlorination of CT to CF, DCM, CM and methane (Krone *et al.* 1989). Recently extracellular cell exudates of a methanogen (*Methanosarcina thermophila*), characterised as zinc-containing porphorinogen-type molecules, were shown to catalyze the reductive dechlorination of CT (Koons *et al.* 2001). The pathway of CT degradation by strict anaerobes is shown in Figure 3. CT is either degraded by sequential reductive hydrogenolysis in which the organochlorine groups are replaced by hydrogen. Alternatively, CT can be reduced to a dichlorocarbene radical, which is unstable and can undergo substitutive dechlorination reactions with water yielding CO and formate that are degraded further to CO_2. In the presence of hydrogen sulfide, substitutive reactions could lead to the formation of CS_2.

CT degradation under denitrifying conditions by *Pseudomonas stutzeri* KC has also been reported with CO_2 as the major product and with little or no formation of CF as an intermediate (Criddle *et al.* 1990b). The responsible catalyst is a siderophore excreted by the organism, which has been identified as pyridine-2,6-bis(thiocarboxylic acid) (PDTC) complexed with copper (Lewis *et al.* 2001). Biodegradation proceeds through the formation of phosgene and thiophosgene as intermediates (Lewis & Crawford 1995). Under iron-reducing conditions, CT degradation is catalysed by biogenic Fe(II) adsorbed onto reactive metal oxide surfaces (Kim & Picardal 1999; McCormick *et al.* 2002).

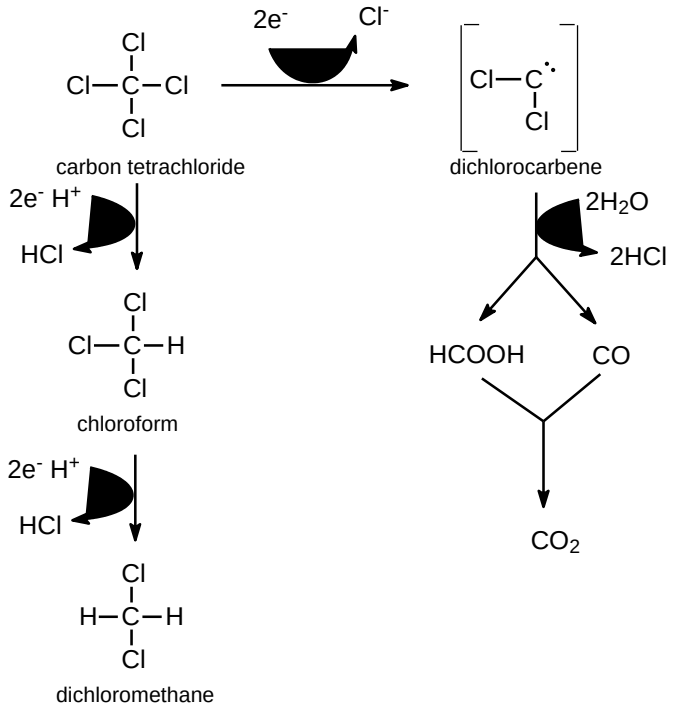

Figure 3: Pathways of anaerobic cometabolism of carbon tetrachloride (Egli *et al*. 1990; van Eekert *et al*. 1998).

Aerobic cooxidation of higher chlorinated methanes. Under aerobic conditions CF biodegradation has been observed during the oxidation of other primary substrates. CF cooxidation by the methane-oxidising bacterium *Methylosinus trichosporium* OB3b has been reported with formate or methane as the additional substrate (Aziz *et al.* 1999; Chang & Alvarez-Cohen 1996; Oldenhuis *et al.* 1989). At cell densities of 100 g wet weight l^{-1}, the half-life of CF was 38 min (Bartnicki & Castro 1994). Several studies demonstrate CF cooxidation by the nitrifying bacterium *Nitrosomonas europaea* with ammonia as the additional substrate (Ely *et al.* 1997; Rasche *et al.* 1991; Vannelli *et al.* 1990). High rates of CF cooxidation were also noted with butane-grown cells of *Pseudomonas butanovora* and *Mycobacterium vaccae* (Hamamura *et al.* 1997). Methane monooxygenase (Alvarez-Cohen & McCarty 1991; Bartnicki & Castro 1994; Jahng & Wood 1994), ammonia monooxygenase (Ely *et al.* 1997; Rasche *et al.* 1991) and butane monooxygenases (Hamamura *et al.* 1997) were implicated as the responsible enzymes for CF cooxidation. The pathway of CF degradation by methane oxidisers starts by insertion of oxygen into the molecule resulting in the formation of phosgene (dichloroformaldehyde) that subsequently decomposes abiotically to CO_2 (Bartnicki & Castro 1994). Alternatively reductive reactions caused by reduced cytochromes could result in the formation of a trichloromethane radical that could undergo chemical reactions with O_2 (Castro 1993; Criddle *et al.* 1990a; Picardal *et al.* 1993). The latter mechanism was also implicated in the oxidation of CT by lignin peroxidase of the white-rot fungus *Phanerochaete chrysosporium* (Khindaria *et al.* 1995).

Kinetic data on higher chlorinated methane biodegradation. Kinetic parameters on the biodegradation of higher chlorinated methanes are shown in Table 4. The specific activities observed for the aerobic cooxidation of CF were approximately two orders of magnitude higher than the corresponding activities of anaerobic co-metabolism of CF. The specific activities during the anaerobic co-metabolism of CT by pure cultures of methanogens and acetogens were approximately one to two orders of magnitude faster than those of CF.

3. Chloroethanes

3.1 Introduction

As was the case with chloromethanes, chloroethanes will be divided into two categories because of differences in their biodegradability, the lower chlorinated ethanes or the higher chlorinated ethanes. Lower chlorinated methanes, which include chloroethane (CA), 1,1-dichloroethane (1,1-DCA) and 1,2-dichloroethane (1,2-DCA), are utilised as primary growth substrates by aerobic micro-organisms but only cometabolised by anaerobic micro-organisms. Higher chlorinated ethanes are only known to be cometabolised. The higher chlorinated ethanes include 1,1,1-trihloroethane (1,1,1-TCA), 1,1,2-trichloroethane (1,1,2-TCA), various isomers of tetrachloroethane (TeCA), pentachloroethane (PCA), and hexachloroethane (HCA). The biodegradation of PCA and HCA occurs for the most part only under anaerobic conditions.

There is no comprehensive review article on the biodegradation of chlorinated ethanes, however several reviews of chlorinated aliphatics or chlorinated solvents cover chlorinated ethanes. De Wildeman & Verstraete (2003) reviewed the anaerobic degradation of chlorinated ethanes as part of a review on chlorinated alkanes. Ferguson & Pietari (2000) reviewed selected studies on the anaerobic biodegradation of chlorinated solvents, including chloroethanes. Semprini (1997) and Alvarez-Cohen & Speitel Jr (2001) reviewed the aerobic cooxidation of chlorinated solvents including chloroethanes. Janssen *et al.* (1994) reviewed the use of chlorinated aliphatic compounds as growth substrates, including lower chlorinated ethanes.

3.2 Biodegradation of lower chlorinated ethanes

3.2.1 Degradation of lower chlorinated ethanes in the environment

Several reports indicate degradation of dichloroethanes in the environment. First-order rate half-lives of 1,2-DCA in the environment are reported as 3-6 months and one year under aerobic and anaerobic conditions, respectively (Howard *et al.* 1991). The natural attenuation of 1,2-DCA in an anaerobic contaminated shallow subsurface site on the Gulf Coast in the USA was demonstrated based on degradation in microcosms, depletion of 1,2-DCA in the field and recovery of products, 2-chloroethanol, ethanol, ethene and ethane (Lee *et al.* 1999b). Both aerobic and anaerobic micro-organisms isolated from pristine and historically contaminated sites were shown to degrade 1,2-DCA (Klecka *et al.* 1998). Anaerobic and aerobic biotransformation resulted in ethene and CO_2 formation, respectively. The micro-organisms isolated from historically contaminated sites initiated degradation without a lag phase; whereas those from pristine sites had lag phases of 7 to 8 weeks under anaerobic conditions and 13 weeks under aerobic conditions. Van Eekert *et al.* (1999) demonstrated that unadapted methanogenic sludge converted 1,1-DCA and 1,2-DCA to CA, ethene and ethane without any lag phase. Also municipal digestor sludge converted to 1,1-DCA and CA to ethane without any lag phase (Chen *et al.* 1999).

3.2.2 Degradation of lower chlorinated ethanes in engineered systems

Biodegradation of dichloroethanes has been reported in bioreactor studies. In high-rate anaerobic biofilm reactors operated under methanogenic conditions with ethanol as primary substrate, 1,2-DCA was removed at a rate of 71.8 and 38.8 g m^{-3} reactor d^{-1} in a reactor with and without granular activated carbon support matrix, respectively (De Wildeman *et al.* 2001). In an anaerobic CSTR reactor, 1,1-DCA was partially removed. The removal was complete in a sequential anaerobic-aerobic reactor (Long *et al.* 1993). The anaerobe, *Desulfitobacterium dichloroeliminans* strain DCA1, was successfully used to bioaugment a 1,2-DCA-contaminated groundwater in a laboratory-scale experiment (De Wildeman *et al.* 2004). During exponential growth the bacterium dechlorinated 1,2-DCA at a rate of 350 nmol Cl^- (mg protein)$^{-1}$ min^{-1}.

Aerobic packed-bed reactors have been utilised to treat 1,2-DCA from contaminated groundwater. Several bench-scale bioreactors inoculated with the aerobic 1,2-DCA utilising

bacterium *Xanthobacter autotrophicus* GJ10 degraded 1,2-DCA at influent concentrations ranging from 0.6 to 25 mg l^{-1} at rates varying from 0.6 to 120 g m^{-3} reactor d^{-1} (Friday & Portier 1991; Stucki & Thuer 1994; Stucki *et al.* 1992). Based on the bench-scale studies, a full-scale plant was constructed to treat 1,2-DCA-contaminated groundwater, which has operated successfully for many years (Stucki & Thuer 1995). An aerobic fluidised-bed reactor was utilised to treat 1,2-DCA in a synthetic liquid feed (5 g L^{-1}) achieved a degradation rate of 14.4 kg $m^{-}3$ d^{-1} (Herbst & Wiesmann 1996). A biofilter reactor with activated carbon to adsorb phenol as a primary substrate was used to support the cooxidation of 1,2-DCA in a waste gas effluent (Veenstra *et al.* 1999). Hartmans *et al.* (1992) used an aerobic chemostat to characterise the utilisation of 1,2-DCA in waste gas by several 1,2-DCA-utilising bacterial strains. A gas-lift reactor with a membrane to facilitate O_2-transfer was used to treat a wastewater containing 1600 mg L^{-1} of 1,2-DCA, which was almost completely removed at an exceptionally high loading rate of 51.2 kg m^{-3} reactor d^{-1} (Frietos dos Santos & Livingstone 1995). *In situ* bioremediation of CA, 1,1-DCA, and 1,2-DCA with either anaerobic or combined anaerobic-aerobic approaches was reviewed by Lee *et al.* (1998). At two sites, relatively high concentrations of the lower chlorinated ethanes (20 to 230 mg L^{-1}) were successfully remediated under combined anaerobic-aerobic conditions.

3.2.3 Microbiology and biochemistry of lower chlorinated ethane biodegradation

Micro-organisms that can utilise lower chlorinated ethanes as a sole source of carbon and energy have only been observed under aerobic conditions and exclusively with 1,2-DCA. Co-metabolism of lower chlorinated ethanes is well documented under both anaerobic and anaerobic conditions. Under anaerobic conditions, there are a few reports confirming the co-metabolism lower chlorinated ethanes and three reports indicating the use of 1,2-DCA as a terminal electron acceptor.

Aerobic growth on lower chlorinated ethanes. The growth of bacteria linked to the use of 1,2-DCA as carbon and energy source was first reported by Stucki *et al.* (1983) for an isolate, *Pseudomonas* strain DE2, with a growth rate of 1.92 d^{-1}. Several other bacterial strains have been isolated that can also grow with 1,2-DCA as a substrate, such as *Xanthobacter autotrophicus* GJ10 (Janssen *et al.* 1985), *Ancylobacter aquaticus* AD20 (van den Wijngaard *et al.* 1992), and *Pseudomonas* sp. strain DCA1 (Hage & Hartmans 1999), with growth rates of 2.64, 1.92 and 3.36 d^{-1}, respectively. The initial dehalogenation in *X. autotrophicus* GJ10 and *A. aquaticus* AD20 is catalysed by a hydrolytic dehalogenase referred to as haloalkane dehalogenase (DhlA), an enzyme that removes a chlorine group and replaces it with a hydroxy group from water, yielding 2-chloroethanol as the first product (Janssen *et al.* 1994; Keuning *et al.* 1985; van den Wijngaard *et al.* 1992). Chloroethanol is oxidised by a dehydrogenase to chloroacetaldehyde. The dehalogenation by *Pseudomonas* sp. strain DCA1 is carried out by a monooxygenase that forms an unstable 1,2-dichloroethanol intermediate that chemically decomposes to chloroacetaldehyde (Hage & Hartmans 1999). From chloroacetaldehyde onwards all the 1,2-DCA-utilising bacteria have a shared pathway as shown in Figure 4.

Aerobic cooxidation of lower chlorinated ethanes. Rapid cooxidation of 1,2-DCA by the methane-oxidising bacterium *Methylosinus trichosporium* OB3b (Chang & Alvarez-Cohen 1996; Oldenhuis *et al.* 1989 & 1991) and the ammonia-oxidising bacterium *Nitrosomonas europaea* (Ely *et al.* 1997) has been reported. *M. trichosporium* OB3b could also cooxidise 1,1-DCA (Oldenhuis *et al.* 1989). Both 1,1-DCA and 1,2-DCA were completely degraded with stoichiometric release of inorganic chloride (Oldenhuis *et al.* 1989). *Nitrosomonas europaea* also cooxidised CA and 1,1-DCA (Rasche *et al.* 1990 & 1991). CA was converted primarily to acetaldehyde and to a lesser extent to 2-chloroethanol (Rasche *et al.* 1990). A butane-grown mixed culture was shown to cooxidise CA, 1,2-DCA and 1,1-DCA (Kim *et al.* 2000; Kim *et al.* 2002). However, the recovery of inorganic chloride from the reaction was variable corresponding to 90, 60 and 37%, respectively (Kim *et al.* 2000), suggesting an accumulation chlorinated products for the dichloroethanes.

Anaerobic co-metabolism lower chlorinated ethanes. The literature concerning the conversion of lower chlorinated ethanes by anaerobic mixed cultures is summarised in Table 5. 1,1-DCA, 1,2-DCA and CA were shown to be degraded under anaerobic conditions by unadapted methanogenic sludge and enrichment cultures. Some products of the conversion have been identified but they are recovered in low yields. 1,1-DCA is converted to CA and ethane. CA was converted to ethane. On the other hand, 1,2-DCA is

converted to CA and ethene. Heat-killed sludge controls showed small losses of added lower chlorinated ethane parent compounds but no products were detected (Chen *et al.* 1999; van Eekert *et al.* 1999), suggesting that the conversions were catalysed biologically. Radiolabeled 1,1-DCA and CA fed to anaerobic columns or anaerobic batch fermentors, respectively, were converted to a limited extent to $^{14}CO_2$ (8 to 16%) demonstrating that a small portion of the lower chlorinated ethanes is mineralised (Vogel & McCarty 1987).

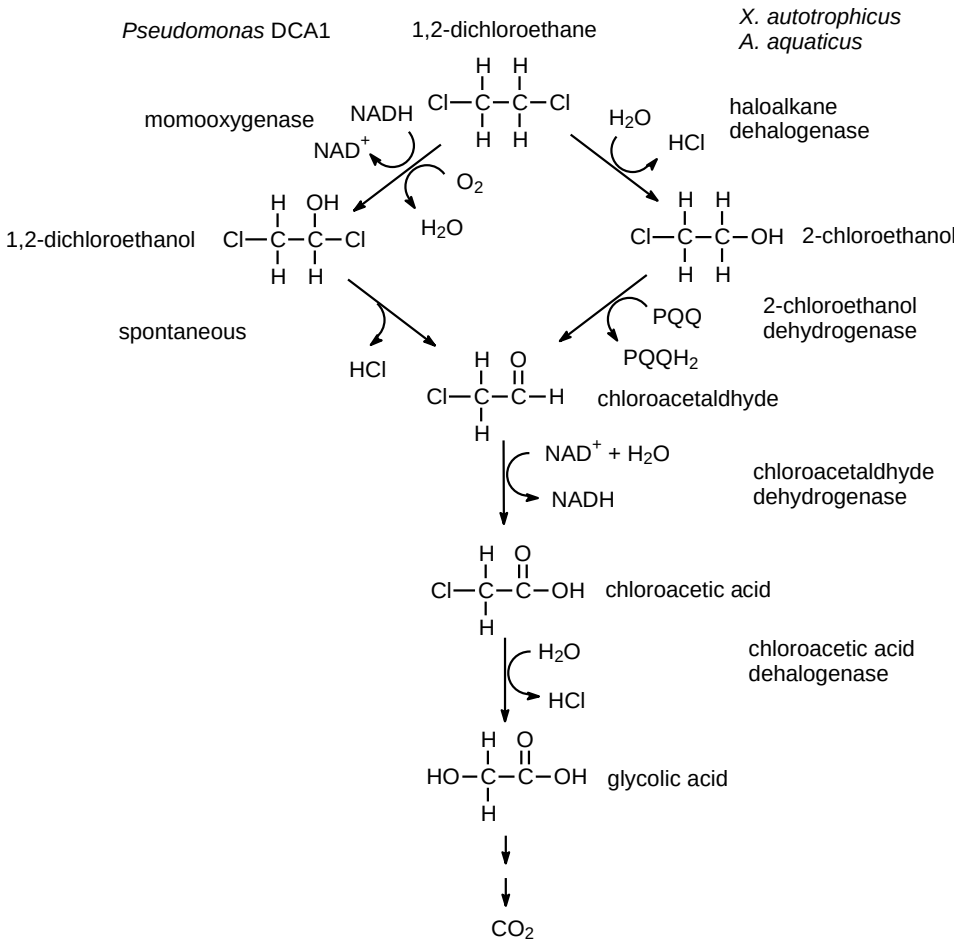

Figure 4: Biodegradation pathways proposed for the aerobic utilisation of 1,2-DCA as a sole source of carbon and energy (Hage & Hartmans 1999).

Anaerobic enrichment cultures adapted to the dehalogenation of chlorinated solvents also readily convert 1,2-DCA to ethene suggesting a preference for dichloroelimination (Table 5). An enrichment culture acclimated to methanol and perchloroethylene rapidly dechlorinated 1,2-DCA to ethene in high yields (Tandol *et al.* 1994). Similarly, an enrichment culture acclimated to 1,2-dichloropropane dechlorination converted 1,2-DCA to ethene (Loffler *et al.* 1997). An ethanol fed UASB reactor acclimated to 1,2-DCA, converted the compound in high yields to ethene (65-80%) (De Wildeman *et al.* 2001). Killed sludge had no activity, indicating that conversion was truly a biological reaction.

Pure cultures of methanogens were shown to reductively dechlorinate 1,2-DCA to CA or cause dichloroelimination to ethene (Egli *et al.* 1987; Holliger *et al.* 1990). A key methanogenic enzyme of *Methanobacterium thermoautotrophicum*, coenzyme M reductase, was shown to be directly responsible for the reductive dechlorination of 1,2-DCA to CA and the dichloroelimination to ethene (Holliger *et al.* 1992a). The enzyme had a K*m* of 119 μM for 1,2-DCA and a V_{max} of 0.02 nmol min^{-1} (mg protein)$^{-1}$ estimated from CA formation. Also enzyme cofactors in methanogens, such as vitamin B12 and the nickel-containing cofactor F430, catalysed the reductive dechlorination of 1,2-DCA with Ti(III)-citrate as reductant (Holliger *et al.* 1992b). A strain of the acetogenic bacterium, *Acetobacterium*, cometabolised 1,2-DCA with an exceptionally high activity of 2 nmol Cl^- (mg protein)$^{-1}$ min^{-1} (De Wildeman *et al.* 2003b). The presence of 1,2-DCA had no effect of the growth of the *Acetobacterium* strain.

Halorespiration of lower chlorinated ethanes. Finally, growth of the halorespiring bacteria *Dehalococcoides ethenogens* strain 195 and *Dehalococcoides* strain BAV1 was demonstrated with 1,2-DCA as a terminal electron acceptor and hydrogen as electron donor (He *et al.* 2003b; Maymo-Gatell *et al.* 1999). Additionally, *Desulfitobacterium dichloroeliminans* strain DCA1 was isolated which also grows by use of 1,2-DCA as a terminal electron acceptor with formate or hydrogen as electron donor (De Wildeman *et al.* 2003a). These observation demonstrate that at least one lower chlorinated ethene can support microbial growth in an energy conserving reaction. Ethene was the primary product of the conversion (De Wildeman *et al.* 2003a; Maymo-Gatell *et al.* 1999) and traces of vinyl chloride were formed as well (Maymo-Gatell *et al.* 1999).

Kinetic data on lower chlorinated ethanes. Table 6 summarises the rates of lower dichloroethane degradation. The kinetics of 1,2-DCA degradation have been thoroughly studied and the specific rates of degradation are high. Specific activities of aerobic bacteria utilising the compound ranged from 2100 to 27,000 mg (g cell dwt)$^{-1}$ d^{-1}, with growth rates of 1.9 to 4.6 d^{-1}. The affinity constants for 1,2-DCA degradation are generally high (2 to 57 mg L^{-1}), with the exception of *Pseudomonas* strain DCA (<0.05 mg L^{-1}). The specific activity of a halorespiring anaerobe that utilises 1,2-DCA as an electron acceptor was 12,500 mg (g cell dwt)$^{-1}$ d^{-1}. Aerobic cooxidation of 1,2-DCA was also comparable with these rates.

3.3 Biodegradation of higher chlorinated ethanes

3.3.1 Degradation of higher chlorinated ethanes in the environment

Higher chlorinated ethanes are degraded in the environment. 1,1,1-TCA was readily degraded in samples from an alluvial aquifer under both methanogenic and sulfate-reducing conditions (Klecka *et al.* 1990). First-order rate constants for 1,1,1-TCA ranging from 0.0034 to 0.015 d^{-1} were estimated in the aquifer material. No degradation of 1,1,1-TCA was observed in aerobic or denitrifying zones of the aquifer. 1,1,1-TCA was degraded with a first-order rate constant in the range of 0.0044-0.0054 d^{-1} when it was injected into a landfill leachate plume dominated by iron-reducing conditions (Rugge *et al.* 1999). Also *in situ* microcosm studies conducted in landfill leachate plumes demonstrated the conversion of 1,1,1-TCA (Bjerg *et al.* 1999; Nielsen *et al.* 1992). Natural attenuation of 1,1,1-TCA and 1,1,2-TCA was observed in contaminated groundwater as evidenced by the formation of 1,1-DCA and 1,2-DCA (Cline & Viste 1985; Lesage *et al.* 1990). Natural attenuation of 1,1,2,2-TeCA was observed in tidal wetland sediments (Lorah & Olsen 1999). Disappearance of 1,1,2,2-TeCA coincided with the formation of reductive dechlorination products, 1,1,2-TCA and 1,2-DCA. Both 1,1,2,2-TeCA and 1,1,2-TCA were removed by natural attenuation in an industrially polluted site at Necco Park, New York (Lee *et al.* 1998). HCA was transformed by anaerobic sediments (Reinhard *et al.* 1990; Vogel *et al.* 1987). HCA, PCA, 1,1,2,2-TeCA, 1,1,1-TCA and 1,1,2-TCA were all readily dechlorinated by unadapted methanogenic sludge from either a bioreactor or municipal digestor sludge (Chen *et al.* 1996 & 1999; van Eekert *et al.* 1999).

3.3.2 Degradation of higher chlorinated ethanes in engineered systems

Engineered systems have been utilised to degrade higher chlorinated ethanes under both anaerobic and aerobic conditions. An anaerobic packed-bed reactor operated under methanogenic or sulfate-reducing conditions was used to degrade 1,1,1-TCA (de Best *et al.* 1997b & 1999). The parent compound was converted to CA at a rate of 9.6 g m^{-3} reactor d^{-1} with transient formation of 1,1-DCA as an intermediate (de Best *et al.* 1999). A methanogenic packed-bed reactor was also shown to degrade 1,1,2,2-TeCA and 1,1,1-TCA (Bouwer & McCarty 1983). 1,1,2-TCA was detected as an intermediate in the degradation of 1,1,2,2-TeCA. An anaerobic filter treating a mixture of chlorinated aliphatic compounds removed HCA, 1,1,2,2-TeCA and 1,1,1,2-TeCA (Boucquey *et al.* 1995). HCA, which was one of the main components of the mixture, was degraded at a rate of 30 g m^{-3} reactor d^{-1}. An anaerobic fed-batch reactor completely removed 1,1,1-TCA and HCA (Long *et al.* 1993). Several examples of 1,1,1-TCA and 1,1,2-TCA removal during bioremediation under anaerobic or combined anaerobic-aerobic conditions were reviewed by Lee *et al.* (1998). Also 1,1,1-TCA bioremediation was demonstrated by injection of acetate, nitrate and sulfate into groundwater (Semprini *et al.* 1992). Bioaugmentation of an anaerobic 1,1,1-TCA dechlorinating culture, *Dehalobacter* sp. strainTCA1, into groundwater resulted in improved degradation of TCA at a field site in Michigan (Sun *et al.* 2002). Engineered

cometabolic degradation of higher chlorinated ethanes under aerobic conditions has also been demonstrated for 1,1,1-TCA. 1,1,1-TCA was degraded in a methanotrophic biofilm reactor with methane as the primary substrate with a pseudo first-order rate constant of 0.0064 l mg^{-1} viable VSS d^{-1} (Arvin 1991). In a closed suspended-growth reactor, a mixed methane-oxidising culture also degraded 1,1,1-TCA with a pseudo first-order rate constant of 0.00212 l mg^{-1} VSS d^{-1} (Strand *et al.* 1990). 1,1,1-TCA degradation in groundwater microcosms from Moffet Field site in California was stimulated by bioaugmentation with butane oxidisers that promoted cooxidation of the chlorinated solvent (Jitnuyanont *et al.* 2001).

3.3.3 Microbiology and biochemistry of higher chlorinated ethane biodegradation

Higher chlorinated ethanes are cometabolised under anaerobic and aerobic conditions. One example is known in which a higher chlorinated ethane, 1,1,1-TCA, is used as terminal electron acceptor supporting microbial growth.

Anaerobic co-metabolism higher chlorinated ethenes. Several studies have examined the conversion of higher chlorinated ethanes under anaerobic conditions by mixed methanogenic cultures (Table 5). HCA, PCA, 1,1,1,2-TeCA and 1,1,2,2-TeCA are primarily converted by dichloroelimination or dehydrochloroelimination resulting in the formation of chlorinated ethenes. The reactions of hexa-, penta- and tetrachlorinated ethanes are readily catalysed by both living- and heat-killed cells. Trichlorinated ethanes were subject to reductive hydrogenolysis and dichloroelimination, resulting in the formation of both lower chlorinated ethenes and ethanes as the main products. 1,1,2-TCA is predominantly converted to vinyl chloride and 1,2-DCA. On the other hand 1,1,1-TCA is primarily transformed to 1,1-DCA and CA. While in some cases limited catalysis by heat-killed sludge was observed leading to the formation of chlorinated ethenes, the biological catalysis of trichloroethanes provided by living sludge was clearly far superior.

Cell free extracts prepared from the methanogenic sludge converted 1,1,1-TCA to 1,1-DCA to acetic acid (Chen *et al.* 1999). Conversion of radiolabeled 1,1,1-TCA by biofilms from an acclimated anaerobic bioreactor was evaluated by Vogel & McCarty (1987). The study indicates that aside from 1,1-DCA and CA formation, $^{14}CO_2$ was identified as a product. In long-term batch incubations of up to 80 days, 17% of the label was recovered as $^{14}CO_2$. Vogel & McCarty (1987) also reviewed the existing data on the chemical hydrolysis of TCA to acetic acid and estimated a first-order rate constant for the chemical hydrolysis of 0.2 y^{-1}.

Several studies demonstrate co-metabolism of 1,1,1-TCA by pure cultures of anaerobes. The methanogen, *Methanobacterium thermoautotrophicum*, converted 1,1,1-TCA to 1,1-DCA (11%) in 8 days (Egli *et al.* 1987). Cell exudates of the methanogen *Methanosarcina thermophila* converted 1,1,1-TCA (Baeseman & Novak 2001). The sulfate-reducing bacterium, *Desulfobacterium autotrophicum*, removed 1,1,1-TCA by 50% with stoichiometric recovery of 1,1-DCA (Egli *et al.* 1987). The proteolytic *Clostridium* sp. completely removed 1,1,1-TCA yielding 1,1-DCA (30 to 40%) and acetic acid (7%) as identified products (Galli & McCarty 1989).

The results taken as a whole indicate three major types of reactions occurring during anaerobic co-metabolism of chlorinated ethanes: dehydrochloroelimination, dichloroelimination, and reductive hydrogenolysis (Chen *et al.* 1996; van Eekert *et al.* 1999). Dehydrochloroelimination is the main abiotic mechanism. Dichloroelimination and reductive dehydrogenolysis are the preferred biologically catalysed reactions. Dichloroelimination predominates for chlorinated ethanes with higher numbers of chloro groups; whereas, reductive dehydrogenolysis progressively becomes more important as the chloro number decreases. An example of the three reactions operating during the degradation of 1,1,2,2-TeCA is shown in Figure 5. In addition, the partial formation of acetate and CO_2 from either 1,1-DCA or 1,1,1-TCA is indicative of hydrolytic reactions, which in some cases clearly appear to be biologically catalysed (Chen *et al.* 1999; Galli & McCarty 1989; Vogel & McCarty 1987).

Halorespiration of higher chlorinated ethenes. An anaerobic bacterium, *Dehalobacter* sp. strainTCA1, has recently been isolated that utilises 1,1,1-TCA as a terminal electron acceptor supporting growth with hydrogen as electron donor (Sun *et al.* 2002). *Dehalobacter* sp. strainTCA1 rapidly converts 1,1,1-TCA to CA with transient accumulation of 1,2-DCA as an intermediate. A cell yield of 5.6 g dwt per mol of Cl dechlorinated was

observed. A perchloroethylene (PCE) halorespiring bacterium, *Desulfitobacterium* sp. strain Y51, was isolated which also converts HCA, PCA and TeCA at fast rates, forming dichloroethenes as products of the dehalogenation reaction (Suyama *et al.* 2001). The purified PCE-dehalogenase from *Desulfitobacterium* sp. strain Y51 displayed high activities with HCA, PCA, 1,1,1,2-TeCA and 1,1,1,2-TeCA of 148, 876, 42 and 773 nmol (mg protein)$^{-1}$ min^{-1}, respectively; yielding dichloroethenes (Suyama *et al.* 2002).

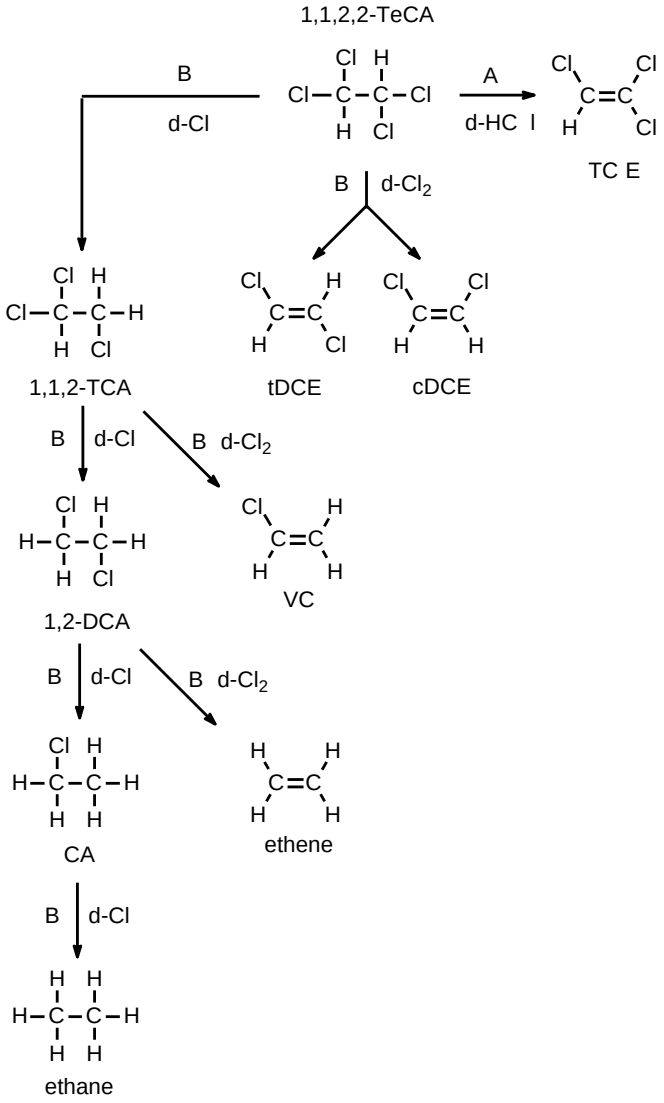

Figure 5: Pathways of cometabolism of 1,1,2,2-TeCA in anaerobic sludge (Chen *et al*. 1996; van Eekert *et al*. 1999). A = abiotic; B = biotic; d-Cl = reductive hydrogenolysis; d-HCl = dehydrochloroelimination; d-Cl_2 = dichloroelimination.

Aerobic cooxidation of higher chlorinated ethanes. Several higher chlorinated ethanes are also degraded aerobically by cooxidation with monooxygenases expressed for the conversion of one of several different primary substrates. Evidence for cooxidative degradation of 1,1,1-TCA has been observed with either pure cultures or enrichment cultures with methane (Arvin 1991; Oldenhuis *et al.* 1989; Strand *et al.* 1990), ethane (Hashimoto *et al.* 2002; Yagi *et al.* 1999), propane (Malachowsky *et al.* 1994), butane (Jitnuyanont *et al.* 2001; Kim *et al.* 2000) and ammonia (Vannelli *et al.* 1990) as the primary substrates. Degradation proceeds initially via 2,2,2-trichloroethanol, which has been recovered in high yields (40-86%) (Hashimoto *et al.* 2002; Oldenhuis *et al.* 1989). 2,2,2-trichloroethanol is readily converted to chloral (trichloroacetaldehyde). Chloral is abiotically converted to chloroform and formic acid at high pH or can be oxidised biologically to trichloroacetic acid. Both trichloroacetic and dichloroacetic acids have been observed as intermediates of 1,1,1-TCA cooxidation (Hashimoto *et al.* 2002). Cooxidation of 1,1,2-TCA occurred under methane-oxidising (Henson *et al.* 1989) and ammonia-oxidising conditions (Rasche *et al.* 1991; Vannelli *et al.* 1990). 1,1,2-TCA was decomposed to

dichloroacetaldehyde during aerobic cooxidation by a *Pseudomonas* strain (Castro 1993). 1,1,1,2-TeCA and 1,1,2,2-TeCA were also cooxidised under ammonia-oxidising conditions with the nitrifying bacterium *Nitrosomonas europaea* (Rasche *et al.* 1991). 1,1,1,2-TeCA was converted to chloral (Rasche *et al.* 1991). An overview of the pathways of higher chlorinated ethane cooxidation is provided in Figure 6.

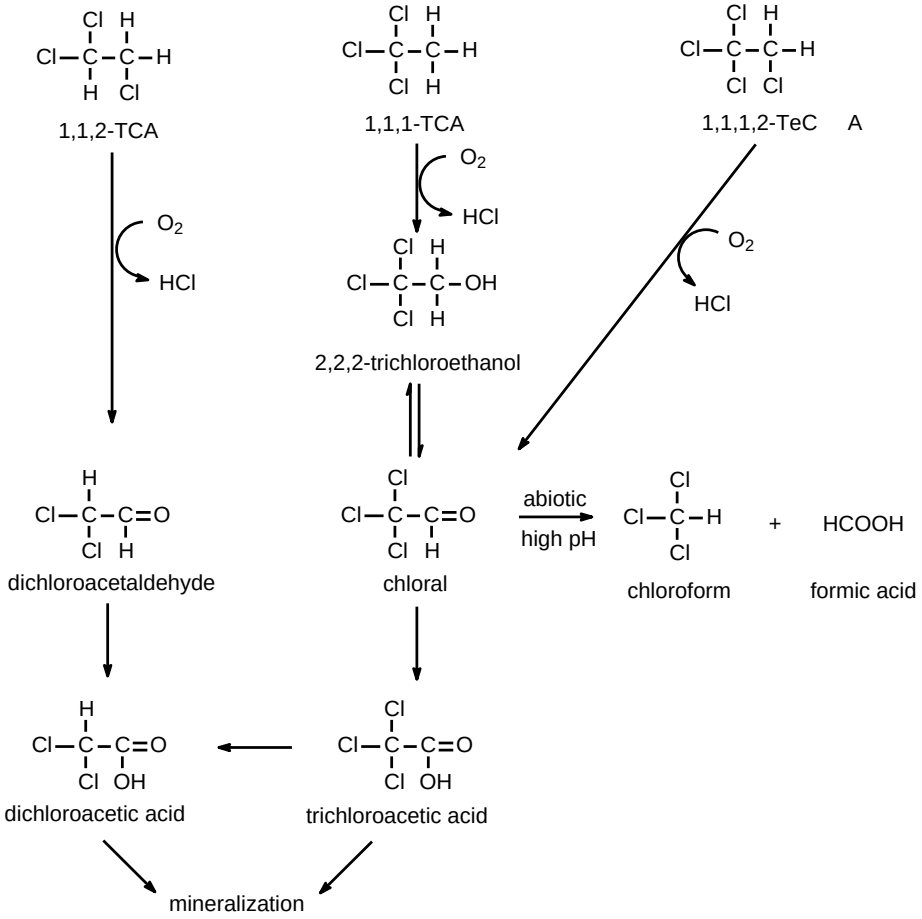

Figure 6: Pathway of aerobic cooxidation of 1,1,1-TCA, 1,1,2-TCA and 1,1,1,2-TeCA (Castro 1993; Hashimoto *et al*. 2002; Oldenhuis *et al*. 1989; Rasche *et al*. 1991).

Kinetic data higher chlorinated ethanes. Table 7 summarises the rates of higher dichloroethane degradation. There is limited data for TCA isomers. Under anaerobic conditions, kinetic data has not yet been reported for halorespiring cultures of TCA. The only data available is that referring to the slow anaerobic co-metabolism of TCA in mixed methanogenic cultures with specific activities 0.42 mg (g cell dwt)$^{-1}$ d^{-1} or less. Aerobic cooxidation of 1,1,1-TCA has been shown to be relatively fast with specific activities ranging from 21 to 4600 mg (g cell dwt)$^{-1}$ d^{-1}. Resting cells of the PCE-halorespiring culture, *Desulfitobacterium* strain Y5, were shown to metabolise TeCA, PCA and HCA at high rates ranging from 1700 to 12,100 mg (g cell dwt)$^{-1}$ d^{-1}. On the other hand, slow anaerobic co-metabolism of TeCA to HCA in mixed methanogenic cultures was observed at rates ranging from 1 to 3 42 mg (g cell dwt)$^{-1}$ d^{-1}.

4. Chloroethenes

4.1 Introduction

As was the case with chloromethanes and chloroethanes, chloroethenes will also be divided into categories of lower and higher chlorinated compounds due to distinct behavior in their biodegradability. Lower chlorinated ethenes include monochlorinated ethene, vinyl chloride (VC), and the dichlorinated ethenes, 1,1-dichloroethene (1,1-DCE), *trans*-dichloroethene (tDCE) and *cis*-dichloroethene (cDCE). Higher chlorinated ethenes include trichloroethene (TCE) and tetrachloroethene (also known as perchloroethylene or PCE). As a general rule lower chlorinated ethenes are more prone to aerobic degradation and less prone to anaerobic degradation compared to the higher chlorinated ethenes. Nonetheless, there is good evidence for aerobic degradation of TCE, DCE isomers and VC as well as good evidence for the anaerobic biotransformation of all chlorinated ethenes.

Several review articles have been published on the degradation chlorinated ethenes. Bradley (2003) wrote an extensive review on the microbial degradation of chlorinated ethenes covering their reductive dechlorination and oxidation under anaerobic conditions as well as their cooxidation and use as growth substrate under aerobic conditions. Middeldorp *et al.* (1999) reviewed the anaerobic reductive dechlorination of chlorinated ethenes, covering aspects of anaerobic co-metabolism and halorespiration. Chen (2004) also wrote a short review on anaerobic reductive dechlorination of chlorinate ethenes, with a focus on the competition between halorespiring and other micro-organisms for hydrogen as electron donor. Alvarez-Cohen & Speitel Jr (2001) wrote an extensive review on the kinetics of chlorinated solvent cooxidation, with an emphasis on TCE. Lee *et al.* (1998) reviewed the bioremediation of chlorinated ethene contaminated sites in the field.

4.2 Biodegradation of lower chlorinated ethenes

4.2.1 Degradation of lower chlorinated ethenes in the environment

Due to the large scope of the chlorinated ethene contamination problem, there are now several accounts providing evidence for the degradation of lower chlorinated ethenes in the environment. Laboratory studies confirmed aerobic biodegradation of the three dichloroethene (DCE) isomers in authentic surface and subsurface soils with no previous exposure to DCE (Klier *et al.* 1999). DCE degradation rates were faster in surface soils compared to subsurface sediments. Radiolabeled cDCE and tDCE were supplied as the only exogenous carbon source and they were partially oxidised to $^{14}CO_2$. In a similar fashion, aerobic VC degradation was demonstrated in aquifer material taken from a shallow aquifer with no previous exposure to VC (Davis & Carpenter 1990). Radiolabeled VC as the sole exogenous carbon source was highly converted to $^{14}CO_2$ (65%) without any lag phase. Hartmans & de Bont (1992) isolated several strains of the bacterium *Mycobacterium aurum* from pristine environments that could utilise VC as a growth substrate. VC degradation was also detected in 23 out of 27 microcosms or enrichment cultures obtained from PCE- or TCE-contaminated sites worldwide, suggesting that capacity for the aerobic mineralisation of VC is ubiquitous (Coleman *et al.* 2002a). Cometabolic degradation of cDCE and tDCE was observed in aerobic groundwater microcosms supplemented with phenol and methane, respectively (Hopkins *et al.* 1993). Natural attenuation of cDCE and VC at the anaerobic/aerobic interface at a natural attenuation site contaminated with PCE and TCE is probably attributable to a combination of aerobic cometabolic and direct use of the daughter products (Witt *et al.* 2002). Anaerobic natural attenuation of higher chlorinated ethenes and ethanes results in the accumulation of cDCE, VC and ethene among others as daughter products as well as methane. Ethene and methane was shown to help stimulate aerobic degradation by facilitating cooxidation of VC and cDCE down gradient in aerobic zones (Freedman *et al.* 2001). VC was degraded as a sole substrate after a 75-day lag phase. Lee *et al.* (1998) reviewed several studies on the natural attenuation of lower chlorinated ethenes. Half-lives based on first-order rate constants estimated from field data vary from 0.8 to 22 years for DCE and 0.2 to 6.3 years for VC. VC degradation was associated with the use of elemental oxygen as a terminal electron acceptor at several of the sites.

Unadapted methanogenic sludges have been shown to slowly convert 1-DCE, tDCE and cDCE to VC, ethene and ethane (Komatsu *et al.* 1994; van Eekert *et al.* 2001), providing evidence for their reductive dehalogenation in the natural anoxic environments.

4.2.2 Degradation of lower chlorinated ethenes in engineered systems

Aerobic cooxidation of DCE and VC has been reported in aerobic bioreactors and bioremediation systems. In an aerobic batch-fed aerobic bioreactor, 1-DCE and tDCE were removed by cooxidation with a mixture of glucose, acetate, benzoate and phenol as primary substrates (Long *et al.* 1993). Likewise cDCE and VC that were detected as intermediates in an anaerobic bioreactor of other higher chlorinated solvents (e.g. TCE), were subsequently removed in the aerobic bioreactor of a sequential anaerobic-aerobic treatment system. In a similar fashion, aerobic methane-oxidising bacteria grown in an oxic down-flow fixed bed reactor were shown to be effective in the cooxidation of cDCE formed as intermediates in an anaerobic bioreactor treating PCE and TCE (Gerritse *et al.* 1995). The volumetric conversion rate was 2.6 g cDCE m^{-3} reactor d^{-1}. A mixture of cDCE and VC produced in the anaerobic stage of a sequential anaerobic-aerobic chemostat were subsequently rapidly co-oxidised in the aerobic chemostat utilising phenol as the primary substrate (Gerritse *et al.* 1997). The aerobic chemostat converted 1.1 mol of chlorinated ethenes (or approx. 104 g cDCE equivalents) m^{-3} reactor d^{-1}, which is an extremely high volumetric rate. Based on inorganic chloride measurements, the chlorinated ethenes were highly mineralised. VC was cooxidised in a methane-oxidising attached-film expanded-bed bioreactor at a rate of 30 g VC m^{-3} reactor d^{-1} (Nelson & Jewell 1993). Cooxidation of VC, 1-DCE, cDCE and tDCE with phenol and/or toluene as the primary substrates was demonstrated in the field as an *in situ* bioremediation technology (Hopkins & McCarty 1995; Hopkins *et al.* 1993).

There are only a few examples of engineered bioreactor system in which lower chlorinated ethenes are used as an aerobic growth substrate. One such example is a study evaluating an aerobic chemostat for the degradation of VC in waste gases (Hartmans *et al.* 1992). VC effectively removed from the gas phase (95%) with stoichiometric recovery of inorganic chloride.

The anaerobic degradation of lower chlorinated ethenes is probably feasible in engineered systems since such compounds occur as intermediates of anaerobic TCE and PCE degradation and sometimes are subsequently further biotransformed to VC and ethene and ethane in engineered bioreactor or bioremediation systems (Bradley 2000; Lee *et al.* 1998; Middeldorp *et al.* 1999). Except for the complete removal of 1-DCE in an anaerobic reactor filled with municipal solid wastes (Ejlertsson *et al.* 1996), there are no other specific examples of the anaerobic degradation of lower chlorinated ethenes in engineered systems where the lower chlorinated ethenes have been introduced as the parent compounds.

4.2.3 Microbiology and biochemistry of lower chlorinated ethene biodegradation

Aerobic growth on lower chlorinated ethenes. Pure cultures of micro-organisms have been isolated that utilise lower chlorinated ethenes as a sole source of carbon and energy supporting growth under aerobic conditions. These include several strains of VC-utilising bacteria and one cDCE utilising strain. The VC-utilising strains belong to the genera of *Mycobacterium* (Coleman *et al.* 2002a; Hartmans & de Bont 1992; Hartmans *et al.* 1985), *Nocardioides* (Coleman *et al.* 2002a), and *Pseudomonas* (Verce *et al.* 2000 & 2001), as well as an unidentified strain of the from order *Actinomycetales* (Phelps *et al.* 1991) and others isolated from activated sludge (Aulenta *et al.* 2003). Studies with the VC-utilising strains indicate that VC degradation is initiated with a VC induced monooxygenase (Coleman *et al.* 2002a; Hartmans & de Bont 1992; Verce *et al.* 2000). VC-epoxide has been identified as the initial degradation intermediate (Hartmans & de Bont 1992; Verce *et al.* 2000). Recently, the second step in the degradation pathway has been shown to be catalysed by epoxyalkane:coenzyme M transferase, which degrades the epoxide by forming an 2-hydroxyethyl adduct with coenzyme M (Coleman & Spain 2003a & 2003b). Coenzyme M (thioethanesulfonate) was previously only known as a coenzyme utilised during methanogenesis.

VC degradation as a sole carbon and energy source is associated with stoichiometric release of chloride (Hartmans *et al.* 1985; Verce *et al.* 2000) and radiolabeled VC is converted largely to $^{14}CO_2$ (Davis & Carpenter 1990; Phelps *et al.* 1991), indicating the

extensive mineralisation of the compound. Also one isolate, referred to as strain JS666 (closely related to *Polaromonas vacuolata*), has been obtained that utilises cDCE as a sole source of carbon and energy (Coleman *et al.* 2002b). As with VC, bacterial growth on cDCE involves a monooxygenase. An epoxide was postulated to be the first intermediate since cDCE grown JS666 cells converted ethene to epoxyethane (Coleman *et al.* 2002b). Conversion of cDCE was also paralleled by stoichiometric recovery of inorganic chloride.

Aerobic cooxidation of lower chlorinated ethenes. The cometabolic oxidation of VC and DCE has been observed with various primary substrates eliciting monooxygenase activity such as methane (Aziz *et al.* 1999; Janssen *et al.* 1988; Oldenhuis *et al.* 1989 & 1991), ethane (Freedman & Herz 1996; Verce & Freedman 2000), ethene (Freedman & Herz 1996; Koziollek *et al.* 1999; Verce *et al.* 2001), propene (Ensign *et al.* 1992), toluene (Chauhan *et al.* 1998; Schafer & Bouwer 2000), isopropanol (Kuntz *et al.* 2003) and ammonia (Ely *et al.* 1997; Rasche *et al.* 1991; Vannelli *et al.* 1990). Also strain JS666 cells grown on cDCE have the capacity to cometabolically oxidise VC and tDCE (Coleman *et al.* 2002b) and *Mycobacterium aurum* L1 cells grown on VC have the capacity to cometabolise cDCE, tDCE and 1-DCE (Hartmans & de Bont 1992). The degradation VC and DCE via cooxidation also appears to proceed via epoxide intermediates that are formed by the activity of monooxygenases (Arvin 1991; Castro *et al.* 1992a; Janssen *et al.* 1988; van Hylckama Vlieg *et al.* 1996). The use of specific inhibitors of monooxygenase has consistently resulted in a loss in VC or DCE cooxidation capacity (Ensign *et al.* 1992; Kim *et al.* 2000; Vannelli *et al.* 1990). In support of the involvement of monooxygenases, a purified soluble monooxygenase from the methylotrophic bacterium, *Methylosinus trichosporium* OB3b, was shown to directly oxidise VC and the three isomers of DCE (Fox *et al.* 1990). Likewise heterologous expression of toluene/o-xylene monooxygenase (from *Pseudomonas stutzeri* OX1) in *Escherichia coli* enabled the host to degrade 1DCE (Chauhan *et al.* 1998). Three fates of VC epoxide (chlorooxirane) have been observed (Castro *et al.* 1992a; van Agteren *et al.* 1998). Firstly, the reduction of chlorooxirane to ethylene oxide and its subsequent transformation to ethylene glycol, glycoaldehyde and glycolic acid as intermediates prior to mineralisation. Secondly, chlorooxirane may be subject to biotic or abiotic hydrolysis yielding glycoaldehyde. Epoxide dehydrogenase activity was detected in the VC-utilising *Mycobacterium aurum* L1 (Hartmans & de Bont 1992). Finally the chlorooxirane can abiotically rearrange to form chloroacetaldehyde prior to becoming oxidised to chloroacetic acid. An alternative route via direct hydrolysis of VC to acetaldehyde was suggested to be catalysed by resting cells of a *Pseudomonas* strain isolated from soil (Castro *et al.* 1992b). The loss of tDCE epoxide formed from tDCE in methane-oxidising cultures was shown to be abiotic following a first-order rate decay with a half-life of 31 h (Janssen *et al.* 1988). During cooxidation, there is a high percentage organochlorine released as inorganic chloride (Chauhan *et al.* 1998; Janssen *et al.* 1988; Oldenhuis *et al.* 1989; Verce & Freedman 2000), conversion of labeled substrates to CO_2 (Castro *et al.* 1992b; Malachowsky *et al.* 1994), and loss of soluble chemical oxygen demand (Verce & Freedman 2000), all suggesting a high degree of mineralisation.

Anaerobic oxidation of lower chlorinated ethenes. In a series of publications, Bradley and Chapelle have demonstrated the anoxic oxidation of radiolabeled cDCE and VC to $^{14}CO_2$ in creek bed and aquifer sediments with different terminal electron acceptors, ranging from methanogenic, iron-reducing, humus-reducing and manganese-reducing conditions (Bradley & Chapelle 1996, 1997 & 2000; Bradley *et al.* 1998a & 1998b). Under methanogenic conditions, a specific methanogenic inhibitor did not halt anaerobic VC oxidation, instead VC was converted to acetate, indicating the involvement of acetogenic bacteria (Bradley & Chapelle 2000). To date, no isolates or enrichment cultures capable of oxidising VC or cDCE under anaerobic conditions have yet been obtained.

Anaerobic halorespiration of lower chlorinated ethenes. Both VC and cDCE are known to serve as terminal electron acceptors to support the growth of halorespiring bacteria. Three recent reports describe strains of *Dehaloccocoides* (strain VS and BAV1) or an enrichment culture containing *Dehaloccocoides* capable of utilising VC as the sole electron acceptor with hydrogen as electron donor (Cupples *et al.* 2003; He *et al.* 2003a; He *et al.* 2003b). In all cases, VC was reduced to ethene and growth of the bacteria linked to VC dechlorination was demonstrated. The enrichment culture also utilised lactate and pyruvate as electron donors (He *et al.* 2003a). An enrichment culture developed by subculturing an active TCE halorespiring mixed culture with VC as the only electron acceptor, was capable of

dechlorinating VC to ethene, but growth measurements were not made (Duhamel *et al.* 2002). The subculture was also composed of *Dehaloccocoides* species.

The use of cDCE as a growth supporting electron acceptor has also been reported for isolates and enrichment cultures identified as (or containing) *Dehaloccocoides* (Cupples *et al.* 2003; He *et al.* 2003a; Maymo-Gatell *et al.* 1997 & 2001). Also all three DCE isomers were utilised as sole electron acceptor and were converted to ethene with VC as an intermediate by an enrichment culture (He *et al.* 2003a) and by *Dehaloccocoides* sp. strain BAV1 isolated from the enrichment culture (He *et al.* 2003b). 1-DCE was also used as a growth supporting electron acceptor by *Dehaloccocoides ethenogens* strain 195 (Maymo-Gatell *et al.* 1999). Both cDCE and VC dechlorinating subcultures developed from a TCE dechlorinating enrichment culture utilised cDCE as the sole electron acceptor (Duhamel *et al.* 2002).

There is only one reductive dehalogenase isolated so far that has demonstrated activity with lower chlorinated ethenes. A TCE-dechlorinating dehalogenase isolated from a *Dehaloccoides* containing enrichment culture had an activity with 1-DCE and cDCE of 8-12 nmol (mg protein)$^{-1}$ min^{-1} whereas activities with VC and tDCE were 2 orders of magnitude lower (Magnuson *et al.* 1998). All four compounds were converted to ethenes. Inhibition of the enzyme by iodopropane indicates the involvement of cobalt-containing corrinoids as a cofactors of the enzyme.

Anaerobic co-metabolism of lower chlorinated ethenes. Anaerobic co-metabolism of lower chlorinated ethenes can be divided into two categories: either co-metabolism during halorespiration or fortuitous reduction by common coenzymes of anaerobes. Halorespiring bacteria can sometimes cometabolise lower chlorinated ethenes while utilising higher chlorinated ethenes as the primary electron acceptor. The best documented example is VC metabolism in *Dehaloccocoides ethenogens* strain 195 that could slowly reduce VC to ethene but the reduction was not linked to growth. The presence of PCE was required to sustain the reaction (Maymo-Gatell *et al.* 2001). A similar dependence on PCE for VC dechlorination was also observed with a VC enrichment culture (Distefano 1999). Likewise, tDCE was converted by cells of *Dehaloccocoides ethenogens* strain 195 previously grown with 1-DCE or 1,2-DCA as electron acceptor (Maymo-Gatell *et al.* 1999). An enrichment culture whose cells were cultivated while using PCE as an electron acceptor could also dechlorinate VC and cDCE (Haston & McCarty 1999).

Lower chlorinated ethenes can also be slowly converted fortuitously by pure cultures or consortia of anaerobes. Pure cultures of the methanogens, *Methanobacterium thermoautotrophicum, Methanococcus deltae* and *Methanobacterium thermolitho-autotrophicus,* converted DCE to acetylene (Belay & Daniels 1987). VC was slowly converted to ethene in minor amounts after 49 days in methanogenic sludge (van Eekert *et al.* 2001). A methanogenic consortium converted 38 μg l^{-1} radiolabeled 1-DCE to VC (54%) and CO_2 (6%) after 107 d incubation period (Vogel & McCarty 1987). 1-DCE was converted approximately to VC (30%) after 63 days in methanogenic sludge (van Eekert *et al.* 2001). Conversion of cDCE and tDCE to VC and ethene also occurred but in minor amounts (van Eekert *et al.* 2001). The conversion cDCE to ethene and ethane was observed in anaerobic sewage sludge (Komatsu *et al.* 1994).

The fortuitous co-metabolism of lower chlorinated ethenes in methanogenic consortia may be due to the activity of reduced coenzymes common in methanogens, such as the cobalt-containing cobalamin or nickel-containing factor 430 (F430). Both cobalamin and F430 reduced with Ti(III)-citrate displayed the capacity to catalyze the slow conversion of cDCE, tDCE and VC (Gantzer & Wackett 1991; Glod *et al.* 1997). The reactions followed first-order kinetics. The conversion of cDCE proceeded through the intermediate formation of VC to produce ethene as the final product after several months of reaction time. The study of Glod *et al*. (1997) was consistent with observations in methanogenic sludge (van Eekert *et al.* 2001) in that the rates of 1-DCE dechlorination by cobalamin proceeded significantly faster than VC, cDCE and tDCE.

Kinetic data on lower chlorinated ethene biodegradation. Kinetic parameters of VC biodegradation are shown in Tables 8 and 9, respectively. Growth rates of aerobic micro-organisms on VC ranged from the moderately fast growing *Mycobacterium aurum* (0.96 d^{-1}) to the very slow growing *Pseudomonas aeruginosa* (0.0048 d^{-1}). Half-saturation constants

for VC, ranging from 0.016 mg L^{-1} to 0.2 mg L^{-1}, are very low compared to those for the degradation of other chlorinated compounds. Cell yields were between 0.17 and 0.33 g cell dwt (g VC consumed)$^{-1}$. Specific activities of the aerobic VC degradation ranged from 26 to 4950 mg (g cell wt)$^{-1}$ d^{-1}. There is only one data point in the literature concerning the kinetics of the growth of aerobic bacteria on DCE. This corresponds to an unidentified strain, JS666, which had a growth rate of 0.23 d^{-1} on cDCE. Specific activities of VC and DCE degradation during aerobic cooxidation were generally comparable or faster than direct utilisation.

Growth rates and specific activities of the VC-respiring *Dehaloccocoides* strains were rapid and comparable in magnitude to aerobic use of VC as electron-donating growth substrate as shown in Table 8. The rates of fortuitous VC co-metabolism in methanogenic sludge are four- or more orders of magnitude slower than VC halorespiration. Likewise, the fortuitous co-metabolism of DCE in methanogenic sludge was slow.

4.3 Biodegradation of higher chlorinated ethenes

4.3.1 Degradation of higher chlorinated ethenes in the environment

The chlorinated ethenes, PCE and TCE are among the most commonly occurring groundwater contaminants. Therefore the body evidence for their biodegradation in the environment is growing. A large number of reports indicate the natural attenuation of PCE and TCE contaminated sites under anaerobic conditions. One of the first observations of higher chlorinated ethene degradation in the natural environment was from Parsons *et al*. (1984), who observed the transformation of PCE incubated in anaerobic muck from an aquifer recharge basin with methanol. TCE, cDCE, tDCE and VC were observed as products after 3 weeks. Thereafter, Kleopfer *et al.* (1985) observed conversion of TCE to cDCE in anaerobically incubated soil. Since then several microcosms studies have been conducted in which microcosms were prepared from sediments or groundwater collected at PCE- or TCE-contaminated sites and the degradation of PCE or TCE was observed to proceed either to cDCE (Loffler *et al.* 2000; Pavlostathis & Zhuang 1993; Vancheeswaran *et al.* 1999) or to ethene and/or VC (Fennell *et al.* 2001; Loffler *et al.* 2000).

Field evidence from natural attenuation of PCE- or TCE-contaminated sites also provides compelling evidence for degradation of higher chlorinated ethenes in the environment. The Interstate Technology and Regulatory Council (ITRC 1999) reviewed 24 PCE/TCE natural attenuation sites and Lee *et al.* (1998) reviewed nine PCE/TCE natural attenuation sites. Some sites converted PCE or TCE mainly to DCE; whereas other sites converted the higher chlorinated ethenes to VC or ethene. Natural attenuation requires a source of organic electron-donating substrates, which is either provided as co-contamination or natural occurring soil organic matter. Common types of co-contamination that supports PCE or TCE natural attenuation include petroleum hydrocarbons (e.g. BTEX), landfill leachate and co-disposed solvents (e.g. methanol, acetone, DCM). Successful natural attenuation is generally associated with methanogenic and sulfate-reducing zones of the aquifer. TCE and PCE half-lives estimated from field studies ranged from 0.6 to 4.3 years and 0.1 to 1.6 years, respectively (Bourg *et al.* 1992; Lee *et al.* 1998; Rugge *et al.* 1999). TCE degradation to DCE and VC in wetland sediments was also reported (Lorah & Olsen 1999). The naturally high levels of organic carbon in the wetland environment were suggested to support the reductive dechlorination. Reductive dechlorination of PCE and TCE was observed during *in situ* microcosms set up in an aquifer affected by landfill leachate (Bjerg *et al.* 1999).

PCE and TCE dechlorinating micro-organisms have also been detected at various field sites utilising 16S rRNA gene detection techniques. Loffler *et al.* (2000) examined several contaminated and pristine sediment samples from the environment for the detection of the H_2 utilising *Dehalococcoides* (known to dechlorinate PCE to ethene) or the acetate-utilising *Desulfuromonas* (known to dechlorinate PCE to cDCE). *Desulfuromonas* was detected at three wells at a PCE contaminated site from which the microcosms established with the groundwater samples converted PCE to cDCE with acetate as electron donor. *Dehalococcoides* was detected at one contaminated site where the corresponding microcosm converted PCE to VC and ethene with H_2 as electron donor. An interesting finding was that both *Desulfuromonas* and *Dehalococcoides* were detected in river sediments from three pristine locations. Microcosms established from the three locations

also converted PCE to cDCE with acetate and PCE to ethene with H_2. Hendrickson *et al.* (2002) examined 24 sites where PCE degradation was occurring, located in the USA, Canada, Holland end England, for the presence of *Dehalococcoides*. *Dehalococcoides* 16S RNA genes were absent at sites where cDCE was the major end product of PCE conversion but the genes were consistently present at sites where ethene was the major end product. Fennel *et al*. (2001) examined several wells at a TCE-contaminated site for the presence of *Dehalococcoides ethenogens*. Wells with activity for the conversion of TCE to VC and ethene were positive for the presence of *D. ethenogens*. The results taken as a whole suggest a good correspondence between PCE dechlorinating activity in the environment and the presence of the responsible micro-organisms.

Slow dechlorination of PCE and TCE has also been observed in unadapted methanogenic sludge (Bouwer & McCarty 1983; Chen *et al.* 1996; van Eekert *et al.* 2001). Therefore, the conversion of higher chlorinated ethenes should be expected in methanogenic niches in the environment.

4.3.2 Degradation of higher chlorinated ethenes in engineered systems

Anaerobic reductive dechlorination of both PCE and TCE has been reported in bioreactor systems as well as *in situ* bioremediation. Of the higher chlorinated ethenes, aerobic cooxidation in engineered systems has only been applied to TCE. The first report of higher chlorinated ethene degradation was that of Bouwer & McCarty (1983) who observed the conversion of PCE in a continuous-flow anaerobic columns supplied with acetate as electron donor. TCE was the only product identified from the conversion. A large number of other bioreactor studies have been conducted since then, and the results have been summarised in Table 10. The volumetric conversion rates in biofilm reactors ranged from 4.6 to 79 g PCE m^{-3} reactor d^{-1}. In some studies, PCE was completely converted to ethene and ethane (Debruin *et al.* 1992; Wild *et al.* 1995) or mainly to VC (Carter & Jewell 1993; Chu & Jewell 1994; Vogel & McCarty 1985). Otherwise, the main dechlorination product was typically cDCE (Fathepure & Tiedje 1994; Guiot *et al.* 1995; Horber *et al.* 1998). The most rapid volumetric conversion rates of 558 g PCE m^{-3} reactor d^{-1} were observed in an anoxic chemostat with a PCE dechlorinating enrichment culture yielding primarily cDCE as the end product (Gerritse *et al.* 1997).

In situ anaerobic bioremediation is commonly applied towards the clean up of PCE or TCE contaminated sites. Lee *et al.* (1998) reviewed five the bioremediation results at five field sites contaminated with higher chlorinated ethenes. PCE and TCE were highly converted to ethene at the Victoria, Texas site utilising benzoate as electron donor. PCE was highly converted to cDCE at the Breda site (The Netherlands) utilising methanol as electron donor and cDCE was subsequently removed down-gradient by aerobic cooxidation with phenol. Low maintenance permeable reactive barriers (PRB) that intersect the plume and prevent their further spreading from the contaminated site are being proposed as an alternative to *in situ* bioremediation. Vital to the success of biological PRB's applied to higher chlorinated ethenes is the use of slow-release electron-donating substrates. The application of peat as a slow-release substrate was shown to be effective in supporting PCE/TCE dechlorination (Kao & Lei 2000). Bioremediation can also be stimulated by adding *Dehaloccocoides* strains capable of complete conversion of PCE to ethene to the aquifer. Two examples of successful application of *Dehaloccocoides* bioaugmentation in the field have recently been published (Ellis *et al.* 2000; Lendvay *et al.* 2003). In both cases, the bioaugmentation enabled the complete conversion of PCE/TCE to ethene instead of their partial reduction to cDCE. A mixed PCE-dechlorinating culture containing *Dehaloccocoides* was used to bioaugment a dense non-aqueous phase liquid (DNAPL) source zone (Adamson *et al.* 2003).

There are also several examples of engineered systems for the aerobic cooxidation of TCE. A biofilm bioreactor and a completely mixed bioreactor were utilised to cooxidise TCE with methane as the primary substrate (Alvarez-Cohen *et al.* 1992; Arvin 1991). An aerobic semi-continuous completely stirred reactor was utilised to degrade TCE with toluene and phenol supplied as primary substrates (Long *et al.* 1993). Several other bioreactor configurations for the *ex situ* treatment of TCE were reviewed by Semprini (1997). Semprini (1997) also reviewed four *in situ* field studies where a strategy of aerobic cooxidation was used to bioremediate contaminated aquifers. In these studies, either phenol, toluene or methane was utilised as a primary substrate to sustain the cooxidation of TCE. One of the most successful field demonstrations of TCE cooxidative bioremediation was performed at

Edwards Air Force Base in California (McCarty *et al.* 1998). Toluene was utilised as the primary substrate and oxygen was supplied with hydrogen peroxide. The average removal of TCE in groundwater that passed through the treatment zone was 87%.

Several examples of sequential anaerobic-aerobic bioreactors utilised for the complete biodegradation of either PCE or TCE are reported in the literature (Fathepure & Vogel 1991; Gerritse *et al.* 1995 & 1997; Guiot *et al.* 1995; Long *et al.* 1993). Radiolabeled [^{14}C]TCE was converted by 96% to $^{14}CO_2$, illustrating a high degree of mineralisation in the sequential anaerobic-aerobic bioreactor systems (Fathepure & Vogel 1991). A sequential series of anoxic and oxic chemostats mineralised PCE completely at a volumetric conversion rate of 173 g $m^{-3}.d^{-1}$ (1041 μM d^{-1}) with stoichiometric recovery of organochlorine in PCE as inorganic chloride (Gerritse *et al.* 1997).

4.3.3 Microbiology and biochemistry of higher chlorinated ethene biodegradation

The biodegradation of higher chlorinated ethenes occurs readily under anaerobic conditions, either cometabolically or linked to growth-supporting halorespiration. Additionally, TCE (but generally not PCE) is susceptible to aerobic cooxidation.

The anaerobic metabolism of higher chlorinated ethene proceeds through sequential reductive dechlorination in which hydrogen replaces the chloro-group, leading to progressively more dechlorinated intermediates (Middeldorp *et al.* 1999). These include TCE, DCE, VC and eventually ethene as shown in Figure 7. Of the three isomers of DCE, cDCE is the most commonly encountered intermediate from biological reductive dechlorination. Occasionally, tDCE and 1-DCE are also found (Christiansen *et al.* 1997; Gerritse *et al.* 1996; Kastner 1991; van Eekert *et al.* 2001). Formation of 1-DCE is mainly due to abiotic reduction of PCE with sulfide (Kastner 1991).

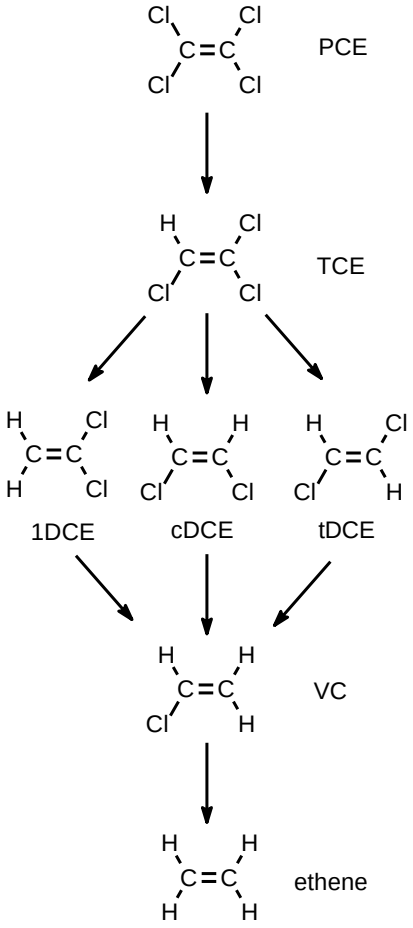

Figure 7: The anaerobic biotransformation of perchloroethylene (PCE) to trichloroethene (TCE), *cis*-1,2-dichloroethene (cDCE), *trans*-1,2-dichloroethene (tDCE), 1,1-dichloroethene (1-DCE), vinyl chloride (VC) and ethene (Middeldorp *et al*. 1999).

Anaerobic co-metabolism of higher chlorinated ethenes. Methanogens as well as acetogenic bacteria have been shown to slowly cometabolise PCE and TCE. Several pure strains of methanogens from the genus *Methanosarcina* converted to PCE to TCE with methanol as the electron-donating substrate (Fathepure & Boyd 1988a & 1988b; Fathepure *et al.* 1987). Recently a *Methanosarcina* strain FR was isolated that completely dechlorinates PCE with methanol as primary substrate (Cabirol *et al.* 1998). Another methanogen, *Methanobacterium thermoautotrophicum,* converted PCE to TCE with H_2 as electron donor. Several acetogenic bacteria have also been shown to dechlorinate PCE to TCE such as *Acetobacterium woodii* (Egli *et al.* 1988; Terzenbach & Blaut 1994) and *Sporomusa ovata* (Terzenbach & Blaut 1994).

Boiled cell extracts of *Methanosarcina* strain FR were capable of dechlorinating PCE to TCE (Cabirol *et al.* 1998). The dechlorinating active fractions of the cell free extract contained cobalamin and factor F430 suggesting the involvement of these coenzymes in the initial dechlorination reaction. A specific inhibitor was used to demonstrate the involvement of cobalamin in the dechlorination reaction of *Sporomusa ovata* (Terzenbach & Blaut 1994). In agreement with these observations cobalamin and F430 incubated with strong reducing agents were shown to catalyze the dechlorination of PCE (Egli *et al.* 1988; Habeck & Sublette 1995; Lesage *et al.* 1996). With cobalamin, PCE was mainly dechlorinated to TCE with some accumulation of cDCE as well (Gantzer & Wackett 1991). TCE was also dechlorinated by both cobalamin and F430 but the rates were slower compared to PCE (Gantzer & Wackett 1991). A purified carbon monooxygenase dehydrogenase from the methanogen, *Methanoscarcina thermophila*, was shown to reduce TCE to cDCE, tDCE, 1-DCE, VC and ethene (Jablonski & Ferry 1992). The enzyme contained a cobalamin cofactor that was also revealed to catalyze the same reaction with Ti(III) as reductant. Extracellular cell exudates of *Methanoscarcina thermophila* were also shown to slowly reduce PCE and TCE (Baeseman & Novak 2001).

Anaerobic enrichment cultures with higher chlorinated ethenes. A large number of studies have been conducted with anaerobic enrichment cultures in which micro-organisms specialised in the dechlorination higher chlorinated ethenes have developed. Enrichment cultures either dechlorinate PCE or TCE to cDCE (Bagley & Gossett 1990; Garant & Lynd 1996; Gerritse *et al.* 1995 & 1997; Holliger *et al.* 1993; Kastner 1991; Kengen *et al.* 1999; Pavlostathis & Zhuang 1991) or to ethene (Distefano *et al.* 1991; Gerritse *et al.* 1997; Nielsen & Keasling 1999; Tandol *et al.* 1994). The more rapid rates observed in enrichment suggest micro-organisms in the enrichment cultures are benefiting from the reductive dechlorination. The difference in product spectrum from the various enrichment cultures also indicates that there are different micro-organisms involved depending on the environment from which the enrichment culture originated.

Anaerobic halorespiration of higher chlorinated ethenes. Presently different bacteria have been isolated and described which are capable of utilising PCE or TCE as an electron acceptor to support growth. The PCE/TCE halorespiring bacteria come from four distinct phylogenetic groups. The bacteria involved in PCE halorespiration are shown in Table 11 together electron donors utilised as well as the products of the PCE dechlorination. Kinetic data of these bacteria are given in Table 14. PCE halorespirers from the low G+C Gram positive bacteria, δ-Proteobacteria, ε-Proteobacteria converted PCE to cDCE with the sole exception of *Desulfitobacterium* sp. strain PCE1 that only converted PCE to TCE. On the other hand, the only halorespiring isolates capable of converting PCE to ethene are from the genus *Dehaloccocoides* of the Green Non-Sulfur Bacteria. The first strain described from this genus, *Dehaloccocoides ethenogens* 195, could only completely convert PCE to ethene if PCE or TCE was present. VC, an accumulating intermediate, could only be degraded further to ethene if it was cometabolised in the presence of the higher chlorinated ethenes (Maymo-Gatell *et al.* 2001). Strains vary on their requirements for electron donor, several strains are quite restrictive such as *Dehaloccocoides* and *Dehalobacter* that can only utilise H_2. On the other hand, several strains (e.g. *Dehalospirillum* and *Desulfitobacterium*) are quite versatile utilising a broad spectrum of electron donors. *Desulfuromonas chloroethenica* and *Desulfuromonas michiganensis* are unique since they are the only known strains that can utilise acetate as electron donor to support the dechlorination of PCE.

The biochemical basis for the halorespiration of higher chlorinated ethene is also becoming better understood with the purification of dehalogenases and identification of dehalogenase

genes (Holliger *et al.* 1998 & 2003; Smidt *et al.* 2000). PCE specific dehalogenases have been purified from *Dehaloccocoides ethenogens* (Magnuson *et al.* 1998) and *Desulfitobacterium* sp. strain PCE1 (van de Pas *et al.* 2001). Dehalogenases utilising either PCE or TCE have been purified from *Dehalospirillum multivorans* (Neumann *et al.* 1995, 1996), *Dehalobacter restrictus* (Schumacher *et al.* 1997), *Desulfitobacterium* sp. strain PCE-S (Miller *et al.* 1998), *Desulfitobacterium* sp. strain Y51 (Suyama *et al.* 2002), and *Desulfitobacterium frappieri* (Holliger *et al.* 2003). Also a TCE-specific dehalogenases has been purified from *Dehaloccocoides ethenogens* (Magnuson *et al.* 1998). All of these dehalogenases contain corrinoid as an enzyme cofactor, many also contain iron-sulfur (Fe/S) clusters as cofactor as well. All of these dehalogenases are membrane bound with the exception of the dehalogenase from *Dehalospirillum multivorans*, which is located in the cytoplasm. The evidence indicates the involvement of the PCE and TCE dehalogenases in respiration. Firstly the membrane localisation of most dehalogenases is consistent. Secondly, important components of the respiratory chain are consistently found in the halorespiring bacteria (Holliger *et al.* 2003). Thirdly, specific inhibitors of the respiratory chain inhibit halorespiration as well as the translocation of protons (Schumacher & Holliger 1996).

The specific activity of the dehalogenases from *Desulfitobacterium* PCE-S, *Dehalobacter restrictus*, *Dehalospirillum multivorans* and *Dehalococcoides ethenogens* were 57,600, 44,000, 108,000 and 33,120 nmol PCE (mg protein)$^{-1}$ min^{-1} (Magnuson *et al.* 1998; Miller *et al.* 1998; Neumann *et al.* 1996; Schumacher *et al.* 1997). These activities are from 500 to 2000-fold faster than those of corresponding bacterial cell suspensions. *Desulfitobacterium* sp. strain PCE-S PCE/TCE-dehalogenase has an apparent *K*m of 10 and 4 μM for PCE and TCE, respectively (Miller *et al.* 1998). *Dehalobacter restrictus* dehalogenase had a similar affinity with a *K*m for PCE of 20 μM (Schumacher *et al.* 1997). The *K*m values were somewhat higher for *Dehalospirillum multivorans* dehalogenase corresponding to 200 and 240 μM for PCE and TCE, respectively (Neumann *et al.* 1996).

The different dehalogenases of higher chlorinated ethenes have little homology with each other (only 27-32% similarity based on amino acid sequence) (Holliger *et al.* 2003), indicating a high level of biodiversity. Based on known gene sequences, the different dehalogenases do share conserved regions encoding for Fe/S cluster binding motifs but there is absolutely no consensus for the corrinoid-binding motif (Holliger *et al.* 2003).

Aerobic Cooxidation. Aerobic cooxidation is another mechanism resulting in the degradation of higher chlorinated ethenes. TCE is readily degraded by this mechanism (Alvarez-Cohen & Speitel Jr 2001; Wackett 1995). PCE on the other hand is almost always reported to not be subject to cooxidation under aerobic conditions (Chang & Alvarez-Cohen 1995 & 1996; Fox *et al.* 1990; Hage *et al.* 2001; Kim *et al.* 2000; Oldenhuis *et al.* 1989; Vannelli *et al.* 1990). Only one research group has noticed slow PCE cooxidation with the bacterium *Pseudomonas stutzeri* OX1 expressing toluene-o-xylene monooxygenase (Ryoo *et al.* 2000).

The cooxidation of TCE has been described for a large number of micro-organisms utilising a variety of primary substrates such as methane, ammonia, phenol, toluene, and propane (Alvarez-Cohen & Speitel Jr 2001; Arp *et al.* 2001; Semprini 1997; van Agteren *et al.* 1998; Wackett 1995). In most cases, the enzymes responsible for the oxidation of TCE are monooxygenases involved in the oxidation of the primary substrate. However, TCE oxidation is also feasible with micro-organisms expressing certain dioxygenases, such as toluene dioxygenase of *Pseudomonas putida* F1 (Wacket & Gibson 1988). The most studied organism capable of TCE cooxidation is the methane-oxidising bacterium *Methylosinus trichosporium* OB3b. Its methane monooxygenases (MMO) are responsible for the oxidation of methane to methanol and have been implicated in the cooxidation of TCE. There are two distinct forms of MMO, a soluble methane monooxygenase (sMMO) expressed under copper-limiting conditions and a particulate (membrane bound) methane monooxygenase (pMMO) expressed under copper sufficiency (Wackett 1995). Early studies established that sMMO was responsible for high rates of TCE cooxidation (Oldenhuis *et al.* 1989; Tsien *et al.* 1989). The fact that purified sMMO can directly cooxidise TCE *in vitro* confirmed the enzyme role as the main catalyst (Fox *et al.* 1990). The enzyme has a relatively low *K*m for TCE of 35 μM and a high specific activity of 682 nmol mg^{-1} min^{-1}.

A large list of other micro-organisms have been identified which can cooxidise TCE but at slower rates compared to *Methylosinus trichosporium* OB3b. TCE oxidation is known from several other methane-oxidising bacteria, such as *Methylocystis sp. strain* M (Saeki *et al.* 1999), *Methylomonas* sp. strain MM2 (Henry & Grbic Galic 1990, 1991), *Methylomonas methanica* 68-1 (Koh *et al.* 1993), and *Methylomicrobium album* BG8 (Lontoh *et al.* 2000). Other bacteria are known which utilise other primary substrates to cometabolise TCE. These include: *Burkholderia cepacia* G4 expressing toluene-2-monooxygenase (Landa *et al.* 1994; Mars *et al.* 1996), *Pseudomonas stutzeri* OX1 expressing toluene/o-xylene monooxygenase (Chauhan *et al.* 1998), *Nitrosomonas europaea* expressing ammonia monooxygenase (Arciero *et al.* 1989; Ely *et al.* 1997; Rasche *et al.* 1991; Vannelli *et al.* 1990), *Mycobacterium vaccae* JOB5 or *Rhodococcus rhodochrous* expressing propane monooxygenase (Malachowsky *et al.* 1994; Wackett *et al.* 1989; Wilcox *et al.* 1995), *Alcaligenes eutrophus* or *Burkholderia cepacia* BR1 expressing phenol hydroxylase (Harker & Kim 1990; Inguva & Shreve 1999), *Mycobacterium* sp. TA27 expressing ethane monooxygenase (Hashimoto *et al.* 2002), and *Pseudomonas* sp. strain DCA1 expressing 1,2-dichloroethane monooxygenase (Hage *et al.* 2001). The toluene-2-monooxygenase from *Burkholderia cepacia* G4 has been purified and shown to directly cause the oxidation of TCE *in vitro* (Newman & Wackett 1997). The specific activity of 37 nmol mg^{-1} min^{-1} is approximately 20-fold lower than sMMO. The *K*m of the toluene-2-monooxygenase for TCE was found to be 12 μM.

The cooxidation of TCE leads to short-lived toxic intermediates that react with proteins, leading to the inactivation of cells and enzymes. An important parameter during the cooxidation of solvents is the transformation capacity (Tc), which indicates how much chlorinated solvent is converted per unit mass of cells before the cells are completely inactivated. An extensive review of Tc values for TCE from the literature revealed that the average Tc for TCE cooxidation by methane-oxidising bacteria and aromatic (toluene or phenol) oxidising bacteria was 150 and 86 μg TCE (mg dwt cells)$^{-1}$, respectively. When monooxygenase inhibitors were applied to methane- or ammonia-oxidising cultures, TCE no longer caused toxicity (Alvarez-Cohen & McCarty 1991; Rasche *et al.* 1991). These results suggest that the oxidised intermediates of TCE rather than TCE itself are responsible for cell inactivation. Furthermore, numerous studies have demonstrated the incorporation of radiolabeled TCE into cell biomass (Malachowsky *et al.* 1994; Shurtliff *et al.* 1996) as well as covalent bonding of radiolabeled intermediates onto proteins including the monooxygenases involved in the transformation (Fox *et al.* 1990; Newman & Wackett 1997; Oldenhuis *et al.* 1991; Rasche *et al.* 1991).

To understand the pathway of TCE cooxidation, the products and intermediates identified from the biological oxidation are listed in Table 12. The cooxidation of TCE by monooxygenases is initiated by the formation of a short-lived TCE-epoxide. Direct experimental evidence for the formation of the epoxide has been obtained in at least two studies (Fox *et al.* 1990; van Hylckama Vlieg *et al.* 1996). Based on measurements of TCE-epoxide together with kinetic considerations, van Hylckama Vlieg *et al.* (1996) estimated that 94% of the TCE cooxidised by *Methylosinus trichosporium* OB3b passes through the TCE-epoxide intermediate. TCE-epoxide is very short lived with an abiotic half-life of 21 s (van Hylckama Vlieg *et al.* 1996). TCE-epoxide is expected to readily undergo hydration in aqueous media to form 1,2-dihydroxy-TCE. The latter compound is unstable and it can either undergo elimination of two HCl equivalents to form glyoxylic acid or rearrangement via C-C bond scission to yield formic acid and carbon monoxide, which are the products observed from the reaction of TCE with sMMO (Fox *et al.* 1990; Wackett 1995). The same products are formed by chemical decomposition of a synthetically prepared TCE-epoxide (Fox *et al.* 1990), emphasising the central role of the TCE-epoxide in the degradation pathway. In addition to the products listed above, trichloroacetaldehyde (chloral) was observed as a product from catalysis with whole cells or with purified sMMO but not form the decomposition of synthetic TCE-epoxide, suggesting that chloral is formed by rearrangement of an enzyme intermediate prior to formation of TCE-epoxide (Fox *et al.* 1990). Chloral is usually observed as a minor product (3-6%) from the cooxidation of TCE by methane-oxidising bacteria (Newman & Wackett 1991). The chloral formed can be metabolised by the bacteria to 2,2,2-trichloroethanol, trichloroacetic acid and dichloroacetic acid (Hashimoto *et al.* 2002; Newman & Wackett 1991; Saeki *et al.* 1999), accounting for the occurrence of such compounds during TCE cooxidation as shown in Table 12. A distinct pathway of TCE degradation has been noted for *Pseudomonas putida* expressing toluene dioxygenase (Li & Wacket 1992; Wacket & Gibson 1988). The dioxygenase causes

the initial formation of a putative iron-bridged dioxygenated intermediate. The intermediate partitions to form formic acid and glyoxylic acid, without formation of carbon monooxide. The different pathways of TCE cooxidation are shown in Figure 8.

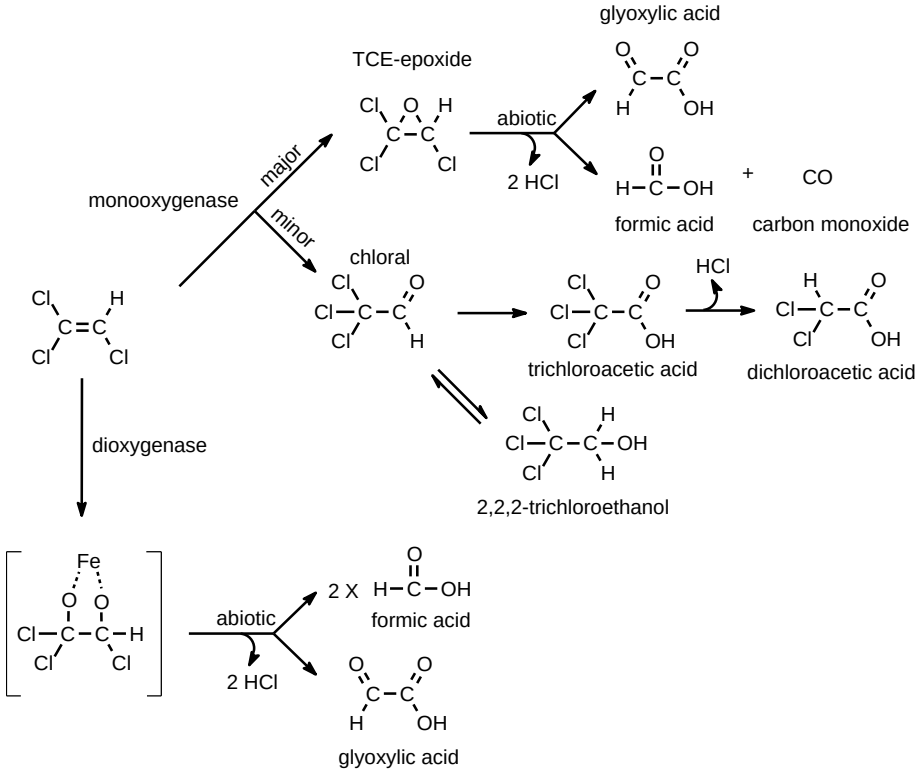

Figure 8: Pathways of TCE cooxidation by bacteria expressing either monooxygenase or dioxygenase (Wackett 1995).

Cooxidation of TCE is usually associated with a high degree of dechlorination, ranging from 55-90% in different experiments (Chauhan *et al.* 1998; Hashimoto *et al.* 2002; Jahng & Wood 1994; Oldenhuis *et al.* 1989). The mineralisation of organic carbon in TCE has been indicated by the conversion of [^{14}C]-TCE to $^{14}CO_2$, which has been recovered in yields of 19 to 28% (Lontoh *et al.* 2000; Malachowsky *et al.* 1994; Shurtliff *et al.* 1996).

Finally evidence for the cometabolic degradation of TCE has also been observed with white rot fungus, which mineralised [^{14}C]-TCE by 20.3% (Khindaria *et al.* 1995). Experiments with purified lignin peroxidase from *Phanerochaete chrysosporium* demonstrated the partial dechlorination of both TCE and PCE with the fungal metabolites, oxalic acid and veratryl alcohols as cofactors in the reaction.

Kinetic data on higher chlorinated ethene biodegradation. Kinetic parameters of TCE and PCE biodegradation are summarised in Tables 13 and 14, respectively. Specific activities of anaerobic dehalogenation of TCE and PCE are two- to six-fold higher in enrichment cultures compared to mixed methanogenic cultures or pure cultures of methanogens or acetogens. The results clearly suggest enrichment of halorespiring micro-organisms that benefit from the use of TCE and PCE as terminal electron acceptors. Pure cultures of halorespiring bacteria utilise these compounds at rates ranging from 850-37,500 mg (g cell dwt)$^{-1}$ d^{-1}. Growth rates of micro-organisms that halorespire PCE range from 0.23 to 5.76 d^{-1}, indicating that they are among the fastest metabolisers of chlorinated compounds.

Kinetic rates for the aerobic cooxidation of TCE have been extensively reported. The highest activities are observed in pure cultures of methanotrophic micro-organisms expressing soluble methane monooxygenase with specific activities ranging from 3700 to 55,000 mg (g cell dwt)$^{-1}$ d^{-1}. The rates with toluene as cosubstrate range from 60 to 1300 mg (g cell dwt)$^{-1}$ d^{-1}. One data point show that rates with ammonia as cosubstrate by a nitrifying bacterium is comparable at 1600 mg (g cell dwt)$^{-1}$ d^{-1}.

5. Chlorofluorocarbons

5.1 Introduction

Chlorofluorocarbons (CFC) and hydrochlorofluorocarbons (HCFC) represent a group of persistent pollutants. Evidence for the cometabolic biotransformation of several CFC compounds is now known. The CFC compounds and their abbreviations are indicated below in Table 15. CFC compounds are encoded by four numbers the first number refers to the number of double bonds, the second number refers to the number of carbons minus one, the third number refers to the number of hydrogen plus one, the fourth number refers to the numbers of fluorines. If one of the numbers equates to zero it is not shown. Letter codes *a*, *b* and *c* refer to isomers with progressively less evenly distributed molecular weights across the two carbon atoms. The most even isomer has no letter code.

Recently several review articles have been published. One review article concerns the fate and biodegradation of CFC in the environment (Hohener *et al.* 2003). Another review article focused on the ecotoxicity testing of various HCFC compounds (Berends *et al.* 1999). The latter summarised studies on the aerobic biodegradability testing of eight HCFC compounds. None of the HCFC compounds were found to be readily biodegradable in the aerobic biodegradability tests performed.

5.2 Biodegradation of CFC and HCFC

5.2.1 Degradation of CFC and HCFC in the environment

There are numerous accounts attesting to the biodegradation of certain CFC compounds in the environment. Termite mounds and rice paddies were shown to be sinks for atmospheric CFC-11 and CFC-12 (Khalil & Rasmussen 1989; Khalil *et al.* 1990). CFC-11 and to a lesser extent CFC-12 were shown to be biodegraded in peat samples from swamp and bog sites (Bauer & Yavitt 1996). CFC-11 and CFC-12 showed a first-order rate constant of 0.122 d^{-1} and 0.055 d^{-1}, respectively in the peat cores from the swamp site. Direct measurements of CFC-11 and CFC-12 in strongly reducing subsurface anoxic zones of the Black Sea and Saanich Inlet on the coast of British Columbia (Canada) provided evidence for the degradation of CFC-11 (Bullister & Lee 1995). The removal of CFC-11 in the anaerobically incubated Saanich Inlet bottom water samples corresponded to first-order decay rates of 0.5 and 1.2 y^{-1} at 8ºC and 25ºC, respectively (Lee *et al.* 1999a). No degradation occurred in aerobically incubated oxygenated water samples. CFC-11 and CFC-113 (but not CFC-12) were degraded by bay sediments (Denovan & Strand 1992). Both CFC-11 and CFC-12 were consumed in microcosms established with various anaerobic freshwater (river, pond, marsh, swamp) sediments (Lovley & Woodward 1992). Biodegradation was indicated by lowered or no uptake in heat-killed controls. No degradation occurred in an aerobic soil microcosm. CFC-11 degradation was also observed in microcosms established with an anaerobic groundwater sample (Sonier *et al.* 1994). CFC-11 and CFC-113 were significantly depleted in an anoxic groundwater leaching from a hazardous waste site (Semprini *et al.* 1990). Evidence for biotransformation of CFC-113 was also observed in an aquifer contaminated by a hazardous waste dump (Lesage *et al.* 1990) as well as in landfill leachate (Lesage *et al.* 1992 & 1993). The first-order rate constant for CFC-133 degradation in landfill leachate was 0.14 d^{-1} (Lesage *et al.* 1992). Groundwater from a TCE contaminated aquifer was shown to readily dechlorinate CFC-1111 under anaerobic conditions (Vancheeswaran *et al.* 1999).

Unadapted methanogenic municipal digestor sludge significantly degraded CFC-11, CFC-12 and CFC-113 within 14 days (Denovan & Strand 1992). CFC-11 and to a lesser extent CF-12 was degraded in anaerobically incubated municipal solid waste, suggesting their conversion in landfills (Ejlertsson *et al.* 1996).

There is also evidence for degradation of some HCFC in the environment. Anaerobic degradation of HCFC-21 and HCFC-123 occurred in freshwater and salt marsh sediments

(Oremland *et al.* 1996). Aerobic degradation of HCFC-21 (but not of HCFC-123) occurred in aerobic soils supplied with methane (Oremland *et al.* 1996).

5.2.2 Degradation of CFC in engineered systems

Little has been published on the biodegradation of CFC compounds in engineered systems. *In situ* bioremediation of CF-11 and CF-113 was obtained by stimulating indigenous micro-organisms with the addition acetate as electron donor and sulfate as electron acceptor (Semprini *et al.* 1992). *In situ* bioremediation of CFC-1111 injected into an aquifer (as a surrogate to TCE) was obtained by introducing formate into the aquifer as electron-donating substrate (Hageman *et al.* 2001). Rates of CFC-1111 biotransformation ranged from 0.05 to 0.3 μM d^{-1}. Biodegradation of CFC-11, CFC-12 and CFC-113 was confirmed in laboratory-scale anaerobic digesters operated under simulated landfill conditions (Deipser & Stegmann 1997). Biodegradation of CFC-11, CFC-12 and CFC-113 was also observed in an anaerobic batch reactor filled with compost and operated with gas recirculation (Deipser 1998). The volumetric degradation rates of CFC-11, CFC-12 and CFC-113 were 0.17, 0.02 and 0.03 g m^{-3} reactor d^{-1}, respectively. HCFC-22 (but not HCFC-21) was degraded in a similar reactor filled with marl but operated under aerobic cooxidation conditions with methane in the gas phase (Deipser 1998). HCFC-22 was degraded at a volumetric rate of 0.24 g m^{-3} reactor d^{-1}.

5.2.3 Microbiology and biochemistry of CFC biodegradation

The biodegradation of CFC and HCFC compounds is only known to occur under cometabolic conditions, either under anaerobic conditions with electron-donating compounds or under aerobic cooxidation conditions with primary substrates that induce monooxygenase activity.

Anaerobic co-metabolism of CFC and HCFC. The literature citations listed in the previous sections provide strong evidence that several CFC and HCFC compounds are subject to anaerobic biotransformation by natural mixed cultures in the environment and engineered systems. There are several reports of CFC degradation by pure or highly enriched anaerobe cultures. Anaerobic sludge which was previously acclimatised to the dechlorination of a mixture of chlorinated organics, displayed significantly faster rates of CFC-11, CFC-12 and CFC-113 degradation compared to unadapted anaerobic sludge (Denovan & Strand 1992). The methanol grown cells of *Methanosarcina barkeri* was found to catalyze the reductive dehalogenation of CFC-11 (Krone & Thauer 1992). HCFC-21, carbon monoxide (CO) and fluoride were observed as products of the reaction along with traces of HCFC-31. Another anaerobe, *Clostridium pasteurianum,* was shown to deplete CFC-12 faster than corresponding heat-killed cells, suggesting its capability to biologically transform the compound (Lovley & Woodward 1992). Cells of the aerobic bacterium, *Pseudomonas putida* G786, were shown to reductively biotransform CFC-112a to HCFC-122 and CFC-1112 (Hur *et al.* 1994). *Pseudomonas putida* G786 harbors a cytochrome P-450 monooxygenase which is known to carry out reductive dechlorinations at low oxygen tensions, such as the conversion of PCA to TCE (Wackett *et al.* 1994).

The biochemical basis for the degradation of CFC is still poorly understood. None the less, the involvement of reduced coenzymes in the process has been demonstrated. Corrinoid coenzymes, which are present in methanogens including *Methanosarcina,* could catalyze the dehalogenation of CFC compounds *in vitro* if a bulk reductant was supplied such as Ti(III)citrate (Krone *et al.* 1991). Like the whole cells of *Methanosarcina barkeri*, corrinoids also converted CFC-11 to HCFC-21, HCFC-31, CO and formic acid as well as a number of other products, *e.g.* CFC-41 and CFC-1112. Corrinoids also catalysed the transformation of CFC-12 and CFC-13, yielding CO as one of the products detected in both cases. The occurrence of CO, formic acid as well as 2 carbon products suggests the involvement of dihalocarbene intermediates. Like corrinoids, another reduced porphyrinogen coenzyme, hematin, has been shown to catalyze the reduction of CFC-11 (Lovley & Woodward 1992) and CFC-113 (Lesage *et al.* 1992). Heat-killed cells of anaerobic sludge supplied with the bulk reductant Ti(III)citrate also catalysed the abiotic reduction of CFC-11 as was observed with the purified coenzymes (Olivas *et al.* 2002). Cytochrome P450 monooxygenase is well known for carry out oxidative reactions; however, in the absence of elemental oxygen the reduced heme group can transfer its electrons to halogenated organics (Li & Wackett 1993). Purified cytochrome P450 incubated anaerobically and supplied with an appropriate electron donor was able to catalyze the dehalogenation of CFC-11 and CFC-113a (Li &

Wackett 1993). CFC-11 was converted to CO as the major product and traces of HCFC-21. CFC-113a was converted to an equimolar mixture of CFC-1112a and HCFC-123.

In order to clarify the pathways of anaerobic CFC degradation, Table 16 summarises the products observed in several studies with pure cultures and mixed cultures. The biotransformation pathway proposed for CFC-11 is shown in Figure 9. The main pathway involves reductive hydrogenolysis, accounting for the commonly observed intermediates HCFC-12 and HCFC-13. Chlorine atoms are successively removed and replaced with hydrogen. The formation of a dihalocarbene radical from CFC-11 must also occur, as is the case during anaerobic CT biotransformation. A fluorochlorocarbene radical would account for the formation of CO and small release of fluoride via hydrolysis with water (Krone & Thauer 1992; Li & Wackett 1993). The formation of formate is only known from in vitro experiments with corrinoids (Krone *et al.* 1991). CFC-12 biotransformation occurs similar to the reductive hydrogenolysis of CFC-11, accounting for HCFC-22, the only known anaerobic conversion product.

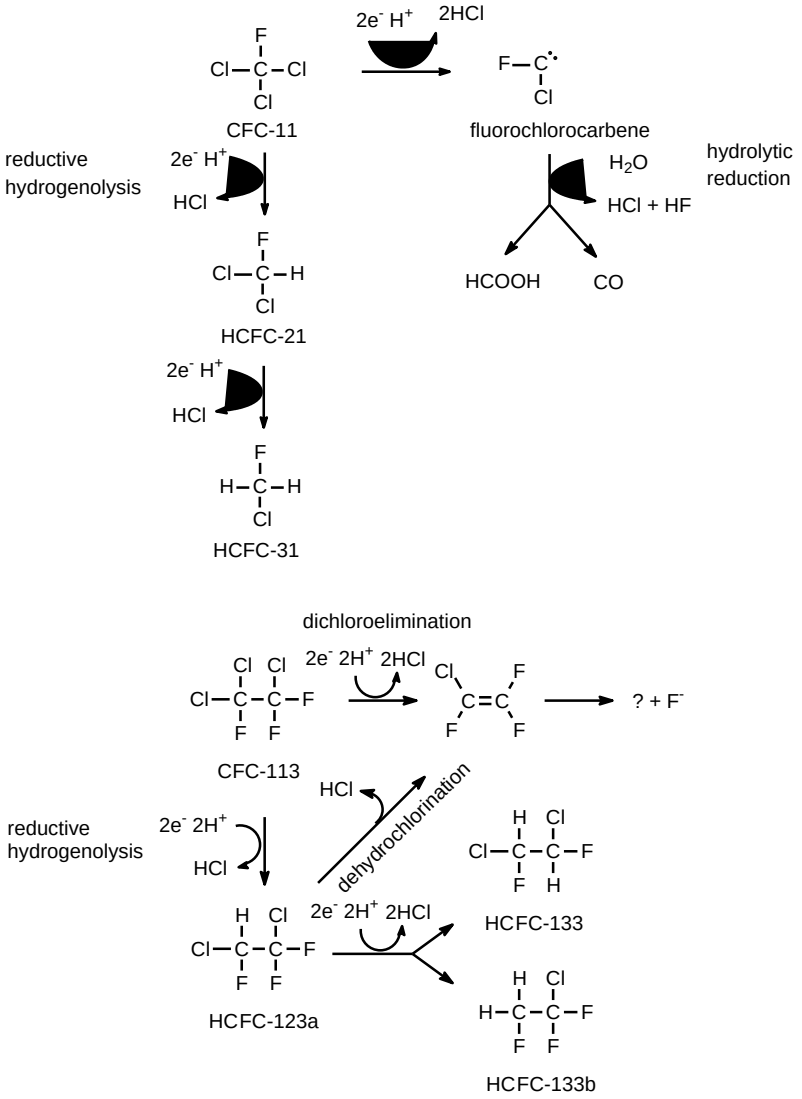

Figure 9: Proposed pathways of the anaerobic biotransformation of CFC-11 and CFC-113 (Deipser 1998; Krone & Thauer 1992; Krone *et al*. 1991; Lesage *et al*. 1990 & 1992).

The pathway proposed for the anaerobic biotransformation of CFC-113 is shown in Figure 9. The main pathway involves reductive hydrogenolysis leading to two successive dechlorinations. The first yielding HCFC-123a as a product and subsequently HCFC-123a is converted to HCFC-133 and HCFC133b as products (Lesage *et al.* 1992). Another isomer, HCFC-123 was reductively dechlorinated to HCFC-133a (Oremland *et al.* 1996). Both CFC-113 and HCFC-123a are abiotically converted to CFC-1113 in anaerobic medium by dichloroelimination and dehydrochlorination, respectively (Lesage *et al.* 1992).

CFC-1113 is not stable since, hydrogen sulfide (produced as a biogenic product by sulfate-reducing bacteria) could chemically reduce CFC-1113 to unidentified products and fluoride ions (Lesage *et al.* 1992).

Several investigators have evaluated the anaerobic biotransformation CFC-1111 (trichloroflouroethylene). Anaerobic CFC-1111 conversion is also caused by reductive hydrogenolysis leading to the formation of *cis*-HCFC-1121 and HCFC-1131 in a manner paralleling the formation of cDCE and VC during TCE conversion (Hageman *et al.* 2001; Vancheeswaran *et al.* 1999).

Aerobic cooxidation of CFC and HCFC. While there is no report (yet) in which CFC or HCFC are utilised as aerobic growth-supporting substrates, there is ample evidence suggesting that some CFC and HCFC compounds are readily cooxidised when alternative primary substrates are supplied. Several examples of HCFC-21 and HCFC-22 cooxidation in the environment and bioreactors were discussed in the previous sections. Three HCFC compounds, HCFC-21, HCFC-141b and HCFC-131, were shown to be cooxidised by *Methylosinus trichosporium* OB3b expressing sMMO (Deflaun *et al.* 1992). In the same study, HCFC-123, HCFC-142b and CFC-11 were also tested but were not cooxidised. HCFC-21 was completely dehalogenated by the cooxidation reaction as evidenced by the high recovery of chloride and fluoride ions (Deflaun *et al.* 1992). A mixed culture of methanotrophic aerobic bacteria was observed to cooxidise HCFC-22, HCFC-142b and HCFC-123 (Chang & Criddle 1995). CFC-1113 was tested as a substrate of purified sMMO from *Methylosinus trichosporium* OB3b and was found to be cooxidised at a rate which was only 2% of that observed for ethene (Fox *et al.* 1990). One product from the reaction was identified, oxalic acid (15%). In addition to methane monooxygenase, several HCFC compounds were reported in a conference abstract to be susceptible to cooxidation with ammonia monooxygenase from *Nitrosomonas europaea* (Hyman *et al.* 1992). Lastly, the genetically engineered bacterium *Pseudomonas putida* G786(pHG-2) expressing recombinant toluene dioxygenase was shown to be able to oxidise CFC-1112a and HCFC-1121 (Hur *et al.* 1994). CFC-1112a was oxidised stoichiometrically to oxalic acid (Wackett *et al.* 1994).

Kinetics of CFC and HCFC biodegradation. Table 17 summarises the limited information available in the literature on the kinetics of CFC and HCFC biodegradation. The anaerobic co-metabolism of CFC-11 by a pure culture of methanogens was comparable in rate to the analogous chlorinated compound, CT, incubated under similar conditions (see Table 4). The rate of anaerobic co-metabolism of CFC-12 by a fermentative bacterium, *Clostridium*, was several orders of magnitude slower. Aerobic cooxidation of the HCFC compound, HCFC-21, by a methanotrophic culture was comparable in rate to the anaerobic CFC-11 co-metabolism by methanogens. The fastest bioconversion reported so far was observed for the aerobic cooxidation of CFC-1113 by a purified soluble methane monooxygenase of *Methylosinus trichosporium*.

6. Chloroacetic Acids

6.1 Introduction

Chloroacetic acids include chloroacetic acid (CAA), dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA). This family of compounds includes important intermediates in the degradation of other chlorinated solvents. DCAA and TCAA are important byproducts from drinking water disinfection with chlorine (Hu *et al.* 1999; Peters *et al.* 1991). They are also significant secondary atmospheric pollutants formed from the oxidation of atmospheric PCE and 1,1,1-TCA or natural compounds formed during oxidation of humic substances (McCulloch 2002). Slater *et al.* (1997) wrote a review article on the biodegradation of halogenated alkanoic acids, including chloroacetic acids. More recently, McCulloch (2002) wrote a review on the sources and environmental fate of TCAA.

6.2 Biodegradation of Chloroacetic acids

6.2.1 Degradation of chloroacetic acid in the environment

Several lines of evidence indicate the rapid degradation of chloroacetic acids in the environment. In an extensive study of chloroacetic acids in rain- and surface waters in Switzerland, the ratios of CAA, DCAA and TCAA to trifluoroacetic acid (TFAA) dramatically increased when moving from precipitation to surface waters (Berg *et al.* 2000). Biodegradation was suggested to be responsible for the increase in the ratios, with TFAA serving as a conservative solute. Biodegradation of TCAA, DCAA and CAA in pond water was demonstrated in field studies as in as with laboratory microcosms (Ellis *et al.* 2001). After an initial lag-phase, the compounds were degraded. The half-lives during the degradation phase were 14.4, 1.2 and 4.7 days for TCAA, DCAA and CAA, respectively in the field, and 15.7, 0.8 and 6.5 days, respectively in the laboratory. Early studies demonstrated the biodegradation of radiolabeled TCAA in agricultural soils (Lignell *et al.* 1984). Biodegradation of radiolabeled TCAA was also studied in a plant-soil system with 4 year-old spruce trees (Forczek *et al.* 2001; Matucha *et al.* 2003). TCAA was rapidly degraded in the soil and some TCAA was incorporated into spruce tree needles. The only volatile product from TCAA degradation was CO_2. DCAA degradation was also observed in the soil from the spruce tree nursery (Matucha *et al.* 2003). CAA degradation under semi-natural field conditions was tested in submerged plots with aquatic plants. The half-life of CAA degradation in the water column of the plots ranged from 3.6 to 21.8 d (Hanson *et al.* 2002). Deep aquifer sediments in an aerobic aquifer storage system were tested for their ability to degrade drinking water disinfection byproducts and CAA was shown to be partially mineralised (Landmeyer *et al.* 2000).

6.2.2 Degradation of CFC in engineered systems

Degradation of chlorinated acetic acids has been accomplished in aerobic as well as anaerobic bioreactor systems. DCAA was rapidly biodegraded aerobically by free and immobilised cells of *Xanthobacter autotrophicus* GJ10 applied in a packed-bed fermentor (Meusel & Rehm 1993). DCAA applied at volumetric loading rates of 25.1 kg m^{-3} reactor d^{-1} could be completely degraded. DCAA grown cells had specific activities of 20 nmol chloride formed (mg protein)$^{-1}$ min^{-1}. In a similar study, DCAA degradation as also described in air bubble and packed-bed reactors with free, calcium-alginate entrapped and adsorptive immobilised cells of *Xanthobacter autotrophicus* GJ10 (Heinze & Rehm 1993). A purified haloacid dehalogenase from *Azotobacter* sp. was immobilised into a column by ion exchange (Diez *et al.* 1996b). Activity of the immobilised enzyme showed a 4-fold increase compared to the native enzyme. Anaerobic degradation of CAA in a fluidised-bed reactor was evaluated in which cells from an anaerobic CAA-degrading enrichment culture were immobilised on charcoal (Egli *et al.* 1989). CAA, applied at volumetric loads up to 1.7 kg m^{-3} reactor d^{-1}, could be completely degraded. A mass balance was performed when a volumetric load of 0.5 kg m^{-3} reactor d^{-1} was applied and 100% of the chlorine was recovered as chloride in the effluent. The carbon was recovered for 97% as methane, carbonic acid and biomass. The results clearly indicate that CAA was completely mineralised in the anaerobic fluidised bed.

6.2.3 Microbiology and biochemistry of chloroacetic acid biodegradation

Chloroacetic acids are cometabolised and degraded as electron donating growth substrates both under aerobic as anaerobic conditions. Under anaerobic conditions there is also evidence for both co-metabolism and halorespiration of chlorinated acetic acids.

Aerobic degradation of chloroacetic acids. Jensen (1957) first reported the isolation of several bacterial strains capable of cometabolically degrading on mono-, di- and trichloroacetic acid with other primary substrates. An unidentified strain labeled 3-Cl was shown to cause TCAA degradation only when supplied with vitamin B12, an *Agrobacterium* strain was responsible for DCAA degradation and a *Pseudomonas* strain was implicated in CAA degradation. Since then numerous studies have confirmed aerobic degradation of chlorinated acetic acids with a wide variety of isolates.

Pseudomonas sp. strain CE1 (Stucki & Leisinger 1983), *Pseudomonas* sp. strain E4 (Hardman & Slater 1981), *Pseudomonas* sp. strain DCA1 (Hage & Hartmans 1999; Hage *et al.* 2001) as well as *Ancylobacter aquaticus* strains AD20 and AD25, (van den Wijngaard *et al.* 1992) were all shown to grow on CAA as the sole carbon and energy source. In addition, Diez *et al.* (1995) isolated seven strains of soil bacteria that could grow on CAA. Three of them were identified as *Pseudomonas* spp., and the remaining four strains were identified as *Alcaligenes* sp., *Agrobacterium* sp., *Arthrobacter* sp., and *Azotobacter* sp. Co-metabolism of CAA has also been reported for *Nocardia* sp. strain 398 (Hirsch & Alexander 1960) and *Pseudomonas dehalogens* (Little & Williams 1971). Extensive mineralisation of CAA has been demonstrated by release of the substrate organochlorine as inorganic chloride (Ellis *et al.* 2001; Hirsch & Alexander 1960; Jensen 1957). Mineralisation of CAA was also demonstrated by CO_2 production (Hage *et al.* 2001) and conversion [^{14}C]-TCA to $^{14}CO_2$ (Landmeyer *et al.* 2000). The degradation of chloroacetic acid is catalysed by 2-haloacid dehalogenases that hydrolytically cleave the chlorine group of CAA to yield glycolic acid (Diez *et al.* 1996a; Little & Williams 1971; Ridder *et al.* 1995; Schneider *et al.* 1993; van den Wijngaard *et al.* 1992). Glycolic acid was observed as an intermediate during the degradation of CAA by pond water (Ellis *et al.* 2001). Four groups of 2-haloacid dehalogenases can be recognised based on the stereo selectivity of the reaction (Fetzner 1998; Janssen *et al.* 1994; Slater *et al.* 1997).

Xanthobacter autotrophicus GJ10 (Heinze & Rehm 1993; Janssen *et al.* 1985; Meusel & Rehm 1993), *Pseudomonas* sp. DCA1 (Hage *et al.* 2001), and *Pseudomonas* sp. strain CE1 (Stucki & Leisinger 1983) have been shown to grow on DCAA as a sole source of carbon and energy. In addition, *Pseudomonas* sp. strain 409 (Hirsch & Alexander 1960) cometabolised DCAA. Mineralisation of organochlorine bound in DCAA to inorganic chloride has been demonstrated on many occasions (Ellis *et al.* 2001; Hirsch & Alexander 1960; Janssen *et al.* 1985; Jensen 1957; Meusel & Rehm 1993). 2-haloacid dehalogenases convert their 2,2-dihalogenated substrates to the corresponding 2-aldehyde (Slater *et al.* 1997), presumably via chemical rearrangement of the initial 2-chloro-2-hydroxy- product. Supporting this mechanism of degradation is the observation that glyoxylate was detected as an intermediate during the degradation of DCAA by micro-organisms in pond water (Ellis *et al.* 2001). Glyoxylate is also known as in intermediate in the conversion of DCAA by mammalian hepatic cells (James *et al.* 1997).

Aerobic biodegradation TCAA as a sole source of carbon and energy has been reported for several isolated strains that were all shown to be closely related to *Acinetobacter calcoaceticus* (Yu & Welander 1995). Stoichiometric dechlorination and growth of biomass linked to the metabolism of TCAA was demonstrated. Previously, TCAA-degrading *Pseudomonas* and *Arthrobacter* strains were found that grew very poorly on TCAA when offered as a sole carbon and energy source (Gemmell & Jensen 1964; Kearney *et al.* 1969). Cell synthesis linked to TCAA degradation was demonstrated for the *Arthrobacter* strains (Gemmell & Jensen 1964). Co-metabolism of TCAA was reported for a defined coculture composed of *Pseudomonas carboxydohydrogens* and an unidentified facultative methanotroph (Weightman *et al.* 1992), and *Pseudomonas* sp. strain 409 (Hirsch & Alexander 1960). Extensive mineralisation of organochlorine to chloride is associated with the aerobic bacterial decomposition of TCAA (Gemmell & Jensen 1964; Hirsch & Alexander 1960; Jensen 1957; Kearney *et al.* 1969; Weightman *et al.* 1992; Yu & Welander 1995). Conversion of [^{14}C]-TCAA to $^{14}CO_2$ has also been demonstrated (Forczek *et al.* 2001; Kearney *et al.* 1969). Several studies have attempted to identify degradation products

during the aerobic decomposition of TCAA. A *Pseudomonas* strain converted TCAA to serine and two additional products that were not identified (Kearney *et al.* 1969). Oxalic acid was identified in pond water incubated with TCAA (Ellis *et al.* 2001). The putative product of TCAA conversion by haloacid dehalogenase activity is oxalate, yet the product has not been detected in defined bacterial cultures degrading TCAA including those expressing 2-haloacid dehalogenase (Weightman *et al.* 1992). TCAA conversion to CO_2 in cell free extracts required two cofactors, NADPH and Coenzyme A (Kearney *et al.* 1969). The fact that [1-^{14}C]-TCAA and [2-^{14}C]-TCAA did not produce equal amounts of $^{14}CO_2$ in either whole cultures or cell free extracts provides evidence against the formation of the symmetrical metabolite, oxalate, as a major intermediate (Kearney *et al.* 1969).

Anaerobic degradation of chloroacetic acids. There are several reports confirming the anaerobic degradation of chlorinated chloroacetic acids. A mixed anaerobic culture derived from the mouse and rat gastrointestinal microflora was shown to dechlorinate TCAA to DCAA (Moghaddam *et al.* 1996). A phototrophic anaerobe, *Rhodospirillum photometricum* utilised CAA a sole substrate (Moghaddam *et al.* 1996) for growth. CAA was dechlorinated to acetate and subsequently acetate was degraded. A methanogenic consortium enriched to degrade CAA as sole source of carbon and energy in a biofilm reactor with charcoal as a supporting matrix was assayed with labeled substrates or with inhibitors to unravel the pathway of biodegradation (Egli *et al.* 1989). [2-^{14}C]-CAA was recovered as $^{14}CH_4$ (11%), $^{14}CO_2$ (60%) and biomass (15%). [2-^{13}C]-CAA was incubated to monitor intermediates by ^{13}C-NMR. Glycolate was observed as an intermediate and bicarbonate accumulated as a product in the assay. When a selective methanogenic inhibitor was applied to the assays with CAA, both glycolate and acetate were recovered. Also glycolate was shown to be converted to acetate in the methanogenic-inhibited culture. The results taken as a whole suggest a pathway of conversion (Figure 10) involving a haloacid dehalogenase converting CAA to glycolate. Glycolate is subsequently converted to bicarbonate and interspecies reducing equivalents (e.g. H_2). Bicarbonate and H_2 are further transformed to methane or acetate via either methanogenic or acetogenic metabolism, respectively, depending on the absence or presence of the methanogenic inhibitor. Both CAA and higher chlorinated acetic acids, DCAA and TCAA, were converted stoichiometrically to inorganic chloride by the CAA grown biofilm. CAA was also abiotically converted to thioglycolate in the presence of sulfide (Egli *et al.* 1989).

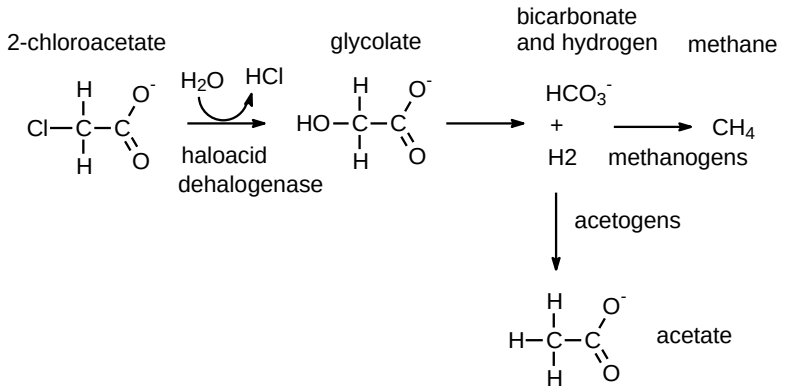

Figure 10: Proposed pathway of anaerobic chloroacetic acid biodegradation based on evidenced published by Egli *et al*. (1989).

TCAA is also used as an electron acceptor by the anaerobic halorespiring bacterium, *Trichlorobacter thiogens* (De Wever *et al.* 2000). TCAA is reductively dechlorinated to DCAA. While acetate is the substrate of the bacterium, it is not the direct electron donor supporting dechlorination. Instead acetate supports the reduction of elemental sulfur to sulfide, and sulfide is the direct electron donor of the dechlorination reaction. The dechlorination causes sulfide to become oxidised to elemental sulfur, occurring as intracellular granules in the bacterium. *Trichlorobacter thiogens* is closely related to *Geobacter*, a genus well known for bacteria capable of utilising elemental sulfur as an electron acceptor. Snoeyenbos-West *et al.* (2001) have argued that *Trichlorobacter* should more appropriately be classified as *Geobacter*.

Kinetic data on the biodegradation of chloroacetic acids. Table 18 summarises the limited data in the literature on the rates of chlorinated acetic acid biodegradation. Aerobic bacteria that grow on chlorinated acetic acids at moderate rates ranging from 0.65 to 1.68 d^{-1}. Growth during anaerobic halorespiration on TCAA was faster (2.77 d^{-1}). The only directly measured specific activity data available from the literature is for cell free extracts with values ranging from 123,000 to 389,000 mg (g protein)$^{-1}$ d^{-1}, observed for aerobic bacterial cells incubated with CAA and DCAA, respectively. A specific activity of 24,000 mg (g cell dwt)$^{-1}$ d^{-1} could be calculated from the growth rate and cell yield data for an aerobic bacterium growing on TCAA.

7. Chloropropanes, Chloropropenes & Epichlorohydrin

7.1 Introduction

Various chloropropanoids, such as chloropropanes (CPrpA) and chloropropenes (CPrpE) will be evaluated in this chapter. These compounds were introduced into the environment primarily through use as fumigants in agriculture, such as 1,2-dichloropropane (1,2-DCPrpA) and 1,3-dichloropropene (1,3-DCPrpE). Additionally the related structure 3-chloro-1,2-epoxypropane, also known as epichlorohydrin (EPC), was used in large quantities for the industrial synthesis of chemicals (WHO 1984).

7.2 Biodegradation of chloropropanoids

7.2.1 Degradation of chloropropanoids in the environment

Only a few studies have evaluated the degradation of chloropropanoid compounds in the environment. In soil, average half-lives for the degradation of (*Z*)-1,3-DCPrpE, (*E*)-1,3-DCPrpE, 1,2-DCPrpA and 2,3-DCPrpE were 13, 8, 52 and 26 days, respectively (van Dijk 1980). In another study, a half-life of 1.5 days was reported for (*Z*)- and (*E*)-1,3-DCPrpE when measured in top soil (Vink & Groen 1992). Radiolabeled (*Z*)-1,3-DCPrpE and (*E*)-1,3-DCPrpE applied to soil was converted to corresponding 3-chloroallyl alcohols, which became strongly bound to soil. Additionally, (*Z*)- and (*E*)-3-chloroacrylic acids were found as minor products (Roberts & Stoydin 1976). In contrast, there was only evidence for slight degradation of radiolabeled 1,2-DCPrpA applied to soil (Roberts & Stoydin 1976). In open field or microcosms experiments, volatilisation is an important component of chloropropanoid dissipation, even though under such conditions some evidence for biological decomposition of 1,3-DCPrpE is still apparent (Roberts & Stoydin 1976; van Dijk 1980). Biodegradation rates of 1,3-DCPrpE in soil are consistently observed to be faster at sites with a history of field applications compared to pristine sites (Chung *et al.* 1999; Ou *et al.* 1995; Verhagen *et al.* 1995). The observation is consistent with enrichment of 1,3DCPrpE-degrading micro-organisms. Amendments of organic matter to soil were also shown to increase the rate of 1,3-DCPrpE degradation (Dungan *et al.* 2001). The half-life of 1,3-DCPrpE in soil treated with manure was only 0.5 days (Dungan *et al.* 2001).

In sediments, field evidence for 1,2-DCPrpA degradation was observed in an iron-reducing zone of an aquifer contaminated by agricultural activity (Tesoriero *et al.* 2001). The field evidence indicated that no degradation was occurring in aerobic or denitrifying zones. Microcosms established with the iron-reducing sediments confirmed degradation 1,2-DCPrpA to 1- and 2-CPrPA under anaerobic conditions, and the absence of any degradation under aerobic conditions (Tesoriero *et al.* 2001). In other studies, anaerobic stream or river sediments converted 1,2-DCPrpA to 1- and 2-CprPA and subsequently to propene (Hauck & Hegemann 2000; Loffler *et al.* 1997).

7.2.2 Degradation of chloropropanoids in engineered systems

A bacterial biofilm in an extractive membrane biofilm reactor operated under aerobic conditions, was shown to mineralise a technical mixture 1,3-DCPrpE after its diffusion through the membrane (Katsivela *et al.* 1999). The biofilm showed a specific activity of 33 g 1,3-DCPrpE (g protein)$^{-1}$ d^{-1} and the reactor could handle volumetric loadings up to 399 g m^{-3} reactor d^{-1}.

Microbial degradation of 1,2-DCPrpA was studied in a laboratory-scale continuous-flow fluidised-bed reactor using polyurethane foam cubes as a carrier for the mixed culture. Continuous 1,2-DCPrpA removal efficiencies over 90% were achieved at substrate loading rates of up to 79 g m^{-3} reactor d^{-1}with methanol and sodium acetate as cosubstrates (Hauck & Hegemann 2000). Propene was formed as the end product of anaerobic 1,2-DCPrpA conversion.

7.2.3 Microbiology and biochemistry of chloropropanoid biodegradation

Under aerobic conditions several chloropropanoid compounds can support growth as a carbon and energy source for bacteria. Several reports also indicate aerobic cooxidation of chloropropanes. Anaerobic bacteria can also cometabolise and halorespire chloropropanes.

Aerobic growth on chloropropanoid compounds. Several bacterial strains have been described which can grow on 1,3-DCPrpE. These include *Pseudomonas cichorii*, *Pseudomonas corrugata*, *Pseudomonas putida*, *Alcaligenes paradoxus,* and *Rhodococcus erythropolis* (Katsivela *et al.* 1999; Poelarends *et al.* 1998; Verhagen *et al.* 1995). However, data on their growth rates, is missing. A pathway has been proposed for the mineralisation of 1,3-DCPrpE (Poelarends *et al.* 1998), as shown in Figure 11. First a haloalkane dehalogenase is responsible for the initial hydrolytic dehalogenation of 1,3-DCPrpE to 3-chloroallyl alcohol, which is later oxidised to 3-chloroacrylic acid. Subsequently a 3-chloroacrylic acid dehalogenase catalyzes the hydrolytic dehalogenation of 3-chloroacrylic acid to malonate semialdehyde which is further mineralised to CO_2 via acetaldehyde. The initial hydrolysis of 1,3-DCPrpE to 3-chloroallyl alcohol also occurs chemically (Castro & Belser 1966).

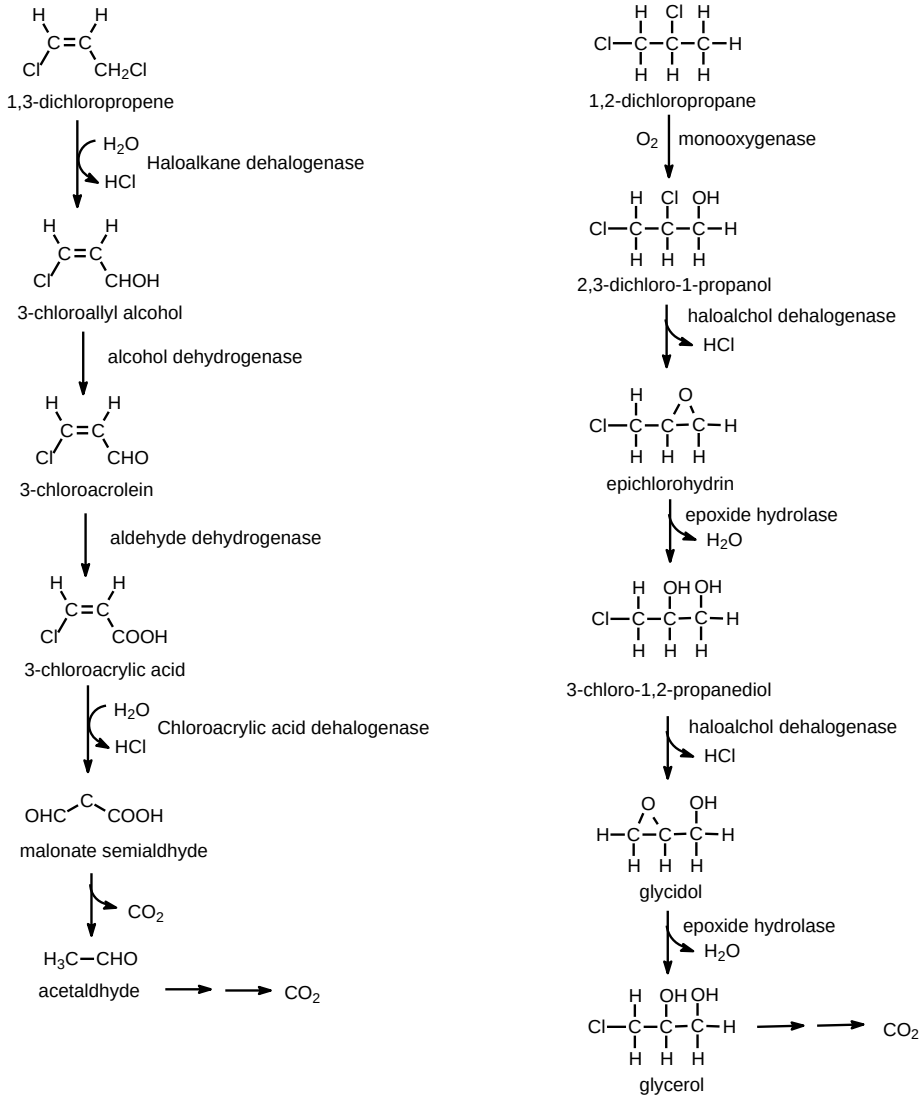

Figure 11: Pathways of aerobic degradation of 1,3-DCPrpE (Poelarends *et al*. 1998), epichlorohydrin (van den Wijngaard *et al*. 1989) and 1,2-DCPrpA (van Agteren *et al*. 1998).

Both 1-CPrpA and 1,3-DCPrpA were used as growth substrates by *Xanthobacter autotrophicus* GJ10 (Janssen *et al.* 1985). No study has yet reported on an aerobic bacterium utilising 1,2-DCPrpA as a growth substrate with the exception of one

unsubstantiated report in which only 1,2-DCPrpA disappearance was demonstrated (Vandenbergh & Kunka 1988). Likewise, repeated attempts to select and enrich bacterial cultures that can use 1,2,3-trichloropropane (1,2,3-TCPrpA) as a sole source of carbon and energy have been unsuccessful (Bosma *et al.* 1999). Using a combined strategy of random mutagenesis and genetic engineering, an organism (*Agrobacterium radiobacter* AD1(pTB3)) was recently constructed that was capable of growth on 1,2,3-TCPrpA (Bosma *et al.* 2002). The problematic step in the aerobic degradation of TCP is the initial dehalogenation to 2,3-dichloro-1-propanol. The latter compound is good growth substrate for several bacterial strains.

Pseudomonas sp. strain AD1 isolated from freshwater sediments utilised epichlorohydrin as a growth substrate (van den Wijngaard *et al.* 1989). *Pseudomonas* sp. strain OS-K-29, isolated from soil with 2,3-dichloro-1-propanol as growth substrate was also shown to utilise epichlorohydrin (Kasai *et al.* 1990). Both isolates were shown to express epoxide hydrolase (Jacobs *et al.* 1991; Kasai *et al.* 1990). Epichlorohydrin is converted to 3-chloro-1,2-propanediol by the epoxide hydrolase. The chlorinated propanediol is dehalogenated yielding glycidol, which is further degraded to glycerol before being mineralised Figure 11.

Aerobic cooxidation of chloropropanoids. Many bacteria are also implicated in the aerobic cooxidation of chloropropanoids. Numerous reports are available concerning the co-metabolism of chloropropanes as outlined in Table 19. For the most part the cometabolic reactions are initiated by monooxygenases, resulting in the intermediate formation of chlorinated propanols. A pathway for 1,2-dichloroprpane has been proposed based on the composite information available in the literature (van Agteren *et al.* 1998) as shown in Figure 11. The initial reaction involves the corporation of oxygen, catalysed by monooxygenase, resulting in the formation of 2,3-dichloro-1-propanol as a major product. The dichlorinated alcohol is than further metabolised to 3-chloro-1,2-propanediol via the intermediate formation of epichlorohydrin. Thereafter, the chlorinated diol is mineralised via glycidol and glycerol.

In contrast with chloropropanes, reports on the aerobic co-metabolism of chloropropenes and epichlorohydrin are rare. Only one study reports on the co-metabolism of 1,3-DCPrpE. *Rhodococcus* sp. strain AS2C cometabolised 1,3-DCPrpE with either tryptone, tryptophan, or alanine as the primary organic substrate (Ou *et al.* 2001). Both 3-chloroallyl alcohol and 3-chloroacrylic acid were observed as intermediates. Likewise, only one report is available on the co-metabolism of epichlorohydrin. *Xanthobacter* strain PY2 converted epichlorohydrin to chloroacetone and acetone while actively metabolising propylene (Small *et al.* 1995).

Anaerobic metabolism of chloropropanes. Anaerobic biotransformation of chloropropanoids is limited to studies regarding the chloropropanes. A sediment free enrichment culture developed from creek sediments, dechlorinated 1,2-DCPrpA to propene with a specific activity of 5 nmol min^{-1} (mg protein)$^{-1}$ while utilising either acetate/H_2 mixture or glycerol/pyruvate mixture as electron donors (Loffler *et al.* 1997). The enrichment culture was also able to convert 1,1-DCPrpA to 1-CPrpA and 1,2,3-TCPrpA to 1,2-DCPrpA and propene. A halorespiring bacterium, *Desulfitobacterium dichloroeliminans,* was recently isolated that converted 1,2-DCPrpA to propene, utilising either hydrogen or formate as electron donor (De Wildeman *et al.* 2003a). The results suggest a dichloroelimination mechanism of reductive dechlorination. A microbial consortium from an anaerobic biofilm reactor, actively dechlorinating 1,2-DCPrpA, was shown to contain bacteria closely related to *Dehalobacter restrictus*, a known halorespiring bacterium (Schlotelburg *et al.* 2002; von Wintzingerode *et al.* 2001). A PCE dehalogenase, isolated from *Clostridium bifermentans*, was also shown to dechlorinate 1,2-DCPrpA (Okeke *et al.* 2001). A PCE respiring bacterium, *Desulfitobacterium* sp. strain Y51, displayed activity with heptachloropropane, yielding pentachloropropene as the biotransformation product (Suyama *et al.* 2001).

Kinetic data on the biodegradation of chlorinated propanoids. The limited data available in the literature on the kinetics of chloropropanoid degradation are summarised in Table 20. Aerobic bacteria grow relatively fast on lower chlorinated propanes with growth rates between 2 to 3 d^{-1}. Only one data point is available concerning the growth rate on epichlorohydrin, which is comparable to growth rates on lower chlorinated ethanes. The kinetic data on the aerobic degradation of 1,3-DCPrpE are limited to one study in which specific activities of 15,000 to 55,000 mg (g cell dwt)$^{-1}$ d^{-1} were observed in the biofilm and

isolates from the biofilm in a 1,3-DCPrpE-degrading bioreactor. Rates of anaerobic chloropropane conversion are limited to two studies, involving either a halorespiring enrichment or pure culture and were in the 400 to 4000 mg (g cell dwt)$^{-1}$ d^{-1} range.

8. Chlorobutadienes

8.1 Introduction

Two compounds fall under the category of chlorobutadienes. The first one, 2-chloro-1,3-butadiene (2-CBD) also known as chloroprene, was mainly used for the synthesis of neoprene. The second one, hexachlorochloro-1,3-butadiene (HCBD), is a byproduct of the manufacture of other chlorinated solvent . HCBD has also been used as a heat transfer fluid, a solvent in rubber production and as a fungicide (Booker & Pavlostathis 2000; Bosma *et al.* 1994).

8.2 Biodegradation of chlorobutadienes

8.2.1 Degradation of chlorobutadienes in the environment

Biodegradation of chlorobutadienes in the environment has not been adequately studied. Several report indicated lack of HCBD degradation in aerobic environments (Bosma *et al.* 1994; Ghisalba 1983; Richards & Shieh 1986). Anaerobic biotransformation was observed in anaerobic columns composed of Rhine river sediment (Bosma *et al.* 1994). A first-order rate constant of 8.64 d^{-1} was estimated for HCBD after active metabolism was established.

8.2.2 Degradation of chlorobutadienes in engineered systems

There are no reports on the direct biotreatment of chlorobutadienes in aerobic treatment systems. Two studies evaluated the fate of HCBD during the anaerobic digestion of waste activated sludge and both studies concluded that partial HCBD losses were due to adsorption and volatilisation (Govind *et al.* 1991; Schroder 1987). Two studies report on the degradation of a mixture of chlorinated aliphatic pollutants containing a large fraction of HCBD (51% by weight) in a laboratory-scale anaerobic fixed-film reactor (Boucquey *et al.* 1995; Modesto *et al.* 1992). The support material for used for biofilm development was polyurethane foam blocks doped with activated carbon. After 476 days, a mass balance of HCBD in the gas, effluent and biofilm/support material indicated from 52 to 58% of the HCBD applied had been biodegraded (Modesto *et al.* 1992). In a second experiment, the HCBD degradation was complete and the elimination of HCBD was associated with an increase in inorganic chloride (Boucquey *et al.* 1995). The degradation rates of HCBD of up to 12 g m^{-3} reactor d^{-1} were achieved.

8.2.3 Microbiology and biochemistry of chlorobutadiene biodegradation

No studies are available on the aerobic degradation of chlorobutadienes by micro-organisms. However, the aerobic metabolism of chlorobutadienes in rat liver microsomes has been studied extensively and thus provides a clue of possible biotransformation pathways expected in eukaryotic micro-organisms such as fungi. Chloroprene is metabolised by an epoxidation reaction yielding intermediates such as (1-chloro-ethenyl)oxirane and 2- chloro-2-ethenyloxirane (Cottrell *et al.* 2001). 2-Chloro-2-ethenyloxirane was very unstable in the presence of the microsomal mixture and was rapidly converted to 1- hydroxybut-3-en-2-one and 1-chlorobut-3-en-2-one. These metabolites were further metabolised reductively to 1-hydroxybutan-2-one and 1-chlorobutan-2-one. Epoxide hydrolase activity in the microsomes converted (1-chloroethenyl)oxirane to 3- chlorobut-3-ene-1,2-diol (Cottrell *et al.* 2001). The initial steps of HCBD metabolism in rat microsomes involve the conjugation with glutathione forming pentachlorobutadienyl-L-glutathione metabolites (Birner *et al.* 1998; Koob & Dekant 1992). The glutathione conjugate is further metabolised to the corresponding cysteine conjugate metabolite.

Better evidence for microbial degradation of chlorobutadienes is available under anaerobic conditions. HCBD supplied at 0.1 mg L^{-1} was metabolised in continuously fed Rhine river sediment columns (Bosma *et al.* 1994). Biologically mediated disappearance of HCBD occurred after 4 months of column operation. HCBD disappeared completely in the first 5 cm of the sediment column. Isomers of pentachlorobutadiene (PCBD), tetrachlorobutadiene (TeCBD) and to a lesser extent trichlorobutadiene (TCBD) accumulated as a result of

HCBD dechlorination in the sediment column. At the outlet of the column, approximately 90% and 5% of the HCBD was recovered as TeCBD and TCBD, respectively. The predominant isomer of TeCBD was determined to be (*E,E*)-1,2,3,4-tetrachloro-1,3-butadiene. Sequential reductive dechlorination was also achieved with a methanogenic enrichment culture originally derived from estuarine sediments using methanol or lactate as electron donors (Booker & Pavlostathis 2000). HCBD was converted primarily to isomers of tri- and dichlorochlorobutadiene; however PCBD and TeCBD were detected as intermediates. Also traces of 2-CBD were detected. The maximum rates of HCBD degradation were 1.44 mg HCBD g^{-1} VSS d^{-1} (Booker & Pavlostathis 2000). Both methanol and lactate served equally as well as electron donor for the process. A selective methanogenic inhibitor had no effect on HCBD dechlorination, suggesting that methanogens were not responsible for the observed reductive dechlorination (Booker & Pavlostathis 2000). The results taken as a whole suggest that under methanogenic conditions, HCBD degradation occurs via successive reductive dechlorinations, in which reductive hydrogenolysis is the main mechanism as shown in Figure 12.

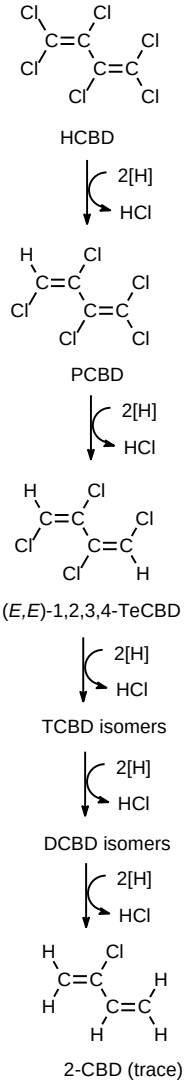

Figure 12: Sequential reductive dechlorination of hexachlorobutadiene HCBD in methanogenic sediments and enrichment cultures (Booker & Pavlostathis 2000; Bosma *et al*. 1994). Isomer identification has only been established for tetrachlorobutadiene (TeCBD).

Reductive HCBD transformation was also catalysed by the enzyme cofactor, hydroxycobalamin (Bosma *et al.* 1994). Using titanium(III)-citrate as a bulk reductant, HCBD was converted to three products identified by GC-MS as PCBD, trichloro-1-buten-3-yne, and 1-buten-3-yne. The results suggest that cobalamin mediated reduction of HCBD includes dichloroelimination or dehydrochloroelimination reactions resulting in the formation of triple bonds.

9. Summary & Conclusions

In this manuscript the biodegradability of chlorinated methanes, chlorinated ethanes, chlorinated ethenes, chlorofluorocarbons (CFCs), chlorinated acetic acids, chlorinated propanoids and chlorinated butadienes was evaluated based on literature data. Evidence for the biodegradation of compounds in all of the categories evaluated has been reported. An overview of the findings from this review article for individual compounds is given in Table 21.

9.1 Scope of Different Physiological Approaches to Biodegradation

In Table 21, biodegradability is indicated for five physiological conditions. The first physiological condition is growth under aerobic conditions in which the chlorinated compound is being used as an electron and carbon source. This type of metabolism results in the mineralisation of the chlorinated compound to CO_2 and Cl^-. The use of chlorinated as aerobic growth substrates was observed in almost all compound categories with the exception of CFCs and chlorobutadienes. Typically, the lower chlorinated compounds in each compound category were readily used as aerobic growth substrates.

The second physiological condition is aerobic co-metabolism (CoM) in which the chlorinated compounds are degraded while the micro-organism is growing (or otherwise already has grown) on another primary substrate. In most cases, aerobic co-metabolism involves cooxidation with an oxygenase expressed to degrade the primary substrate. Aerobic co-metabolism was observed in all compound categories. However, the most chlorinated members in each compound category were normally not susceptible to aerobic cooxidation.

The third physiological condition is growth under anaerobic conditions in which the chlorinated compound is being used as an electron donor and carbon source. Under anaerobic conditions, this may result in the anoxic oxidation of the chlorinated compound to CO_2 and Cl^- or fermentation products such as acetate and formate. Anaerobic growth on chlorinated compounds as electron donors was clearly the least common form of biodegradability. In the entire review article, evidence for this type of metabolism was limited to five compounds, CM, DCM, VC, cDCE and CAA.

The fourth physiological condition is the use of the chlorinated compound as an electron acceptor (EA) to support growth of a micro-organism utilising a simple electron-donating substrate, such as H_2 or lactate. This physiological process is referred to as halorespiration and results in either the partial or complete reductive dechlorination of the compound. Evidence for halorespiration was observed for chlorinated ethanes, chlorinated ethenes, chlorinated acetates and chlorinated propanes.

The fifth condition is the anaerobic co-metabolism in which the chlorinated compounds are biotransformed while the micro-organism is growing on other primary substrate and electron acceptors. Anaerobic co-metabolism also results in the partial or complete reductive dechlorination of chlorinated compounds. This type of metabolism is the most widespread, occurring in all compound categories and for both lower and higher chlorinated members in each category. Rapid anaerobic co-metabolism is observed in a few cases (e.g. PCA) due to an enzymatic reduction by a halorespiring dehalogenase expressed for another chlorinated compound. The more common, slow anaerobic co-metabolism results from the direct reaction of the chlorinated compound with common occurring reduced enzyme cofactors (e.g. vitamin B12).

9.2 Biodegradation per Chlorinated Compound Category

A summary and some conclusions are provided in the following sections with respect to the biodegradability of each compound category. To support the discussion, Table 21 also provides information on highest values of certain kinetic data observed for each compound under aerobic and anaerobic conditions. The kinetic data summarised are growth rates,

volumetric loading capacities of bioreactors and first-order rate constants observed in environmental samples.

9.2.1 Chlorinated Methanes

Lower chlorinated methanes (CM and DCM) were observed to be excellent growth substrates for both aerobic and anaerobic bacteria. Under aerobic conditions, relatively high growth rates have been observed. Of all the compounds considered in this review article, the second highest bioreactor-loading rate was reported with DCM (40 kg m^{-3} d^{-1}). Under aerobic conditions, CM and DCM are mineralised to CO_2 and Cl^-. Lower chlorinated methanes are also readily cooxidised under aerobic conditions. Under anaerobic conditions, growth rates were about four-fold lower than under aerobic conditions. Both aerobes and anaerobes initiate CM degradation by a dehalogenation catalysed by methyl transferase enzymes. Aerobic DCM degradation proceeds via thiolytic mechanism involving glutathione-S-transferase. Rates of anaerobic DCM conversion in bioreactors (1.25 kg m^{-3} d^{-1}) were the second highest under anaerobic conditions reported in this review. CM and DCM are completely dehalogenated anaerobically but the carbon is converted to fermentation products (acetate and formate). Higher chlorinated methanes are not growth substrates. Both CT and CF and are cometabolised under anaerobic conditions resulting in lower chlorinated methanes from reductive hydrogenolysis as well as CO_2 or CS_2 originating from substitution reactions between H_2O or H_2S and reduced chlorinated methane radicals. The highest loading capacities of CT and CF in anaerobic bioreactors were at least 16-fold lower than that observed with DCM. The aerobic cooxidation of higher chlorinated methanes is limited to CF, resulting in toxic intermediates (e.g. phosgene) that are eventually mineralised abiotically. CT is not degraded under aerobic conditions. There is also no evidence yet of the use of chlorinated methanes as terminal electron acceptors for halorespiration.

9.2.2 Chlorinated Ethanes

The only chlorinated ethane known to be used as an electron-donating growth substrate is 1,2-DCA under aerobic conditions. 1,2-DCA is the best growth substrate of all the chlorinated compounds considered in this review article, supporting the highest growth rates as electron donor (4.56 d^{-1}) and the highest volumetric loads applied to bioreactors (51 kg m^{-3} d^{-1}). Two strategies of degradation have been observed one involving a monooxygenase and the other involving a hydrolytic mechanism catalysed by haloalkane dehalogenase. Both mechanisms lead to the intermediate chloroacetaldehyde, which is mineralised to CO_2 and Cl^- via CAA. The possible use of CA and 1,1-DCA as growth substrates has not yet been studied. Aerobic cooxidation has been reported for mono-, di-, tri and tetrachloroethane. Chloral and chlorinated acetic acids are intermediates in the cooxidative pathway. All chlorinated ethanes are reported to be biotransformed by anaerobic co-metabolism. Both reductive hydrogenolysis, forming lower chlorinated ethanes, and reductive dichloroelimination, forming lower chlorinated ethenes, are important mechanisms of anaerobic co-metabolism. Volumetric loading rates of chlorinated ethane co-metabolism in anaerobic bioreactors (10-72 g m^{-3} d^{-1}) are more than 700-fold lower than that observed for the aerobic degradation of 1,2-DCA. Halorespiration has been reported for 1,2-DCA and 1,1,1-TCA. 1,2-DCA is converted to ethene; whereas 1,1,1-TCA is converted to CA. Halorespiration of tetra-, penta- and hexachloroethane has not yet been reported; however, a purified dehalogenase from PCE-halorespiring micro-organisms readily converted such compounds.

9.2.3 Chlorinated Ethenes

VC and cDCE are utilised as aerobic growth substrates. Degradation is initiated by a monooxygenase, resulting in the formation of an epoxide which is further degraded by a newly discovered epoxyalkane:coenzyme M transferase. Aerobic degradation of VC as a sole electron donor and carbon source is associated with stoichiometric release of Cl^-. Maximum growth rates on VC and cDCE are clearly lower than growth rates observed with other lower chlorinated aliphatics. However, VC-degrading aerobes also display an unusually high affinity for VC with half-saturation constant (k_s) values as low as 16 μg l^{-1}. Higher chlorinated ethenes are not utilised as aerobic growth substrates. There is extensive evidence indicating that VC, DCE isomers, and TCE are readily cooxidised by a wide variety of micro-organisms. DCE isomers were cooxidatively degraded up to volumetric loading rates of 104 g m^{-3} d^{-1} in a bioreactor. The fastest rates of chloroethene cooxidation are observed with methane oxidisers, expressing soluble methane monooxygenase. TCE cooxidation proceeds through TCE epoxide as a major intermediate, which abiotically

decomposes to organic acids and Cl^-. PCE is generally not metabolised under aerobic conditions. One research group has reported the anoxic oxidation of VC and cDCE under different redox conditions; however anoxic oxidation of these compounds is often not observed. All chlorinated ethenes are slowly cometabolised under anaerobic conditions to lower chlorinated ethenes and ethene. The reactions are catalysed by methanogens and acetogenic bacteria, which are micro-organisms containing reduced enzyme cofactors. Rates of anaerobic chlorinated ethene degradation are considerably faster by halorespiring bacteria, which utilise all chlorinated ethenes, including all isomers of DCE, as an electron acceptor for growth. Many genera of halorespiring micro-organisms partially dechlorinate chlorinated ethenes (e.g. the conversion of PCE to cDCE). However complete dechlorination to ethene is only observed with strains of bacteria from one genus, *Dehaloccocoides*. Corrinoid-containing dehalogenase enzymes have been isolated from PCE-halorespiring bacteria that are responsible for the reductive dechlorination reactions. The highest growth rate (6.65 d^{-1}) reported in this review article corresponds to a PCE-halorespiring micro-organism. The third highest volumetric loading rate applied to an anaerobic bioreactor (0.56 kg m^{-3} d^{-1}) of all the compounds considered in this review article, corresponds to a chemostat with PCE-halorespiring bacteria.

9.2.4 Chlorofluorocarbons

Too date, there are no micro-organisms known that can grow on CFC or HCFC as an electron donor or electron acceptor. The microbial conversion of CFC and HCFC compounds is limited to aerobic cooxidation or anaerobic co-metabolism. Aerobic cooxidation has mostly been observed with HCFC compounds and not CFC compounds with the sole exception of CFC-112a cooxidation observed with a genetically modified micro-organism. HCFC cooxidation is slow as evidenced by the very low volumetric loading rates of HCFC-22 applied to an aerobic bioreactor (Table 21). On the other hand, slow anaerobic co-metabolism has mostly been observed with CFC and not HCFC compounds with the exception of HCFC-123 and HCFC-123a, which are analogues of PCA. The mechanisms of anaerobic CFC-11 bioconversion during anaerobic co-metabolism parallel those observed with the analogue, CT. Reductive hydrogenolysis reactions occur that successively dechlorinate CFC-11 to HCFC-21 and HCFC-31. Also substitution reactions with fluorochlorocarbene radical formed from CFC-11 reduction, results in the formation of formate, carbon monoxide, F^- and Cl^-. Rates of anaerobic CFC conversion in bioreactors are the lowest observed for any compound in this review article. CFC-11 and CFC-12 were converted in batch reactors at volumetric rates that were at least 1800-fold lower than those observed for the analogue, CT.

9.2.5 Chlorinated Acetic Acids

CAA, DCAA and TCAA have all been observed to be excellent growth substrates for aerobic bacteria as sole sources of electron donor and carbon with growth rates ranging from 0.65 to 1.68 d^{-1}. In all cases, mineralisation of the chlorinated acetic acids results in stoichiometric release of Cl^- and formation of CO_2. The aerobic degradation of CAA is catalysed by a hydrolytic enzyme, 2-haloacid dehalogenase, that produces glycolic acid as an intermediate. The volumetric rate of aerobic DCAA degradation in a bioreactor (25.1 kg m^{-3} d^{-1}) was the third highest rate observed in this review article. Additionally, CAA, DCAA and TCAA are all known to be cometabolised under aerobic conditions. Two studies have demonstrated the anaerobic degradation of CAA as the sole source of carbon. The first study describes a phototrophic bacterium that initially converts CAA to acetate. The second study describes an anaerobic biofilm that converts radiolabeled CAA to CH_4 and CO_2. Glycolic acid was observed as an intermediate of the metabolism in the biofilm. The volumetric loading of CAA in the biofilm reactor (1.7 kg m^{-3} d^{-1}) was the highest observed for an anaerobic reactor for compounds considered in this review article. Lastly, halorespiration has been observed with TCAA resulting in the formation of DCAA. The growth rate during halorespiration exceeds the aerobic growth rate of TCAA by 4-fold.

9.2.6 Chloropropanoids

Several chloropropanoid compounds (1,3-DCPrpE, 1-DCPrpA, 1,3-DCPrpA and epichlorohydrin) can be utilised as electron donors to support growth of aerobic bacteria. Epichlorohydrin and the chlorinated propanes are degraded with relatively high growth rates ranging from 2 to 3 d^{-1}. Although no growth rates were reported for 1,3-DCPrpE, a high rate can be inferred from the high specific cell activities of isolated micro-organisms and moderate volumetric loading rates (0.4 kg m^{-3} d^{-1}) observed in an aerobic bioreactor treating the compound. The aerobic biodegradation of 1,3-DCPrpE is initiated with a

hydrolytic enzyme, haloalkane dehalogenase, yielding 3-chloroallyl alcohol as an intermediate. This intermediate is oxidised by dehydrogenases to 3-chloroacrylic acid, which is also attacked by a hydrolytic dehalogenase. Epichlorohydrin degradation is initiated by epoxide hydrolase, forming 3-chloro-1,2-propanediol that is subsequently dehalogenated to glycidol before being mineralised via glycerol to CO_2. There are presently no reports of 1,2-DCPrpA being used a an electron-donating substrate supporting growth. However, 1,2-DCPrpA along with several other chlorinated propanes (1-DCPrpA, 2-DCPrpA, 1,3-DCPrpA and 1,2,3-TCPrpA) are readily cooxidised under aerobic conditions. Cooxidation is catalysed by monooxygenases, forming chlorinated propanols. 2,3-dichloro-1-propanol, formed from the cooxidation of 1,2-DCPrpA, is dehalogenated by a haloalcohol dehalogenase to epichlorohydrin. Under anaerobic conditions, 1,2-DCPrpA is dechlorinated to propene via reductive dichloroelimination by cometabolic enrichment cultures as well as by a pure culture of a halorespiring bacterium. The anaerobic enrichment culture also reduced 1,1-DCPrpA to 1-CPrpA and 1,2,3-TCprpA to propene. Rates of 1,2-DCPrpA conversion in anaerobic bioreactors were comparable to those observed for chlorinated ethanes.

9.2.7 Chlorobutadienes

Chlorinated butadienes are not yet known to be used as growth substrates by micro-organisms. Additionally, there is no information available on their cometabolic degradation under aerobic conditions by micro-organisms. Cooxidation is suspected based on findings that 2-CBD is oxidised by liver microsomes. Under anaerobic conditions, solid evidence for microbial biodegradation of chlorinated 1,3-butadienes has been found in several studies. HCBD is sequentially dehalogenated by reductive hydrogenolysis via PCBD and TeCBD to TCBD and DCBD isomers. The enzyme cofactor, vitamin B12 was also shown to carry out the reduction of HCBD. Conversion of HCBD in anaerobic bioreactors has also been demonstrated.

9.3 Concluding Remarks

The results taken as a whole suggest a tremendous capacity of micro-organisms in the environment to degrade chlorinated solvents and related chlorinated aliphatic structures. This literature survey has revealed that a broad range of chlorinated structures are susceptible to biodegradation under a variety of physiological and redox conditions. In many cases, lower chlorinated compounds are completely mineralised to benign end products. Additionally, biodegradation can occur rapidly with growth rates exceeding 1 d^{-1} for many of the lower chlorinated compounds. Most compound categories include chlorinated structures that are used to support growth of micro-organisms. Growth can be due to the use of the chlorinated compound as an electron donor or, alternatively, to the use of the chlorinated compound as an electron acceptor (halorespiration). Lower chlorinated structures supporting growth as electron donors were subject to mineralisation. Lower and higher chlorinated structures supporting halorespiration were either partially or fully reductively dechlorinated if electron donating substrates were provided. Biodegradation linked to growth is important, since under such conditions rates of degradation will increase as the micro-organism population (biocatalyst) increases. When growth was not feasible, chlorinated compounds are potentially subject to co-metabolism. Under aerobic conditions compounds such as CF, TCA and TCE could be rapidly cooxidised if primary substrates were added that support the expression of oxygenases. However cooxidation of perchlorinated compounds, such as CT and PCE, was not feasible. Slow anaerobic co-metabolism involving non-specific reactions with reduced cofactors was evident for many of the compound categories, especially halomethanes and chlorinated ethanes.

The literature survey also reveals that combinations of redox conditions can be favorable for the biodegradation of the most difficult compounds. The most highly chlorinated structures are generally recalcitrant to degradation under aerobic conditions. However, under anaerobic conditions, highly chlorinated structures are partially dehalogenated to lower chlorinated counterparts. The lower chlorinated compounds would, subsequently, be subject to mineralisation under aerobic conditions.

When chlorinated structures are released to the environment, micro-organisms can be expected to play a major role in their natural attenuation. Ecological and health risk assessments should therefore take into account the microbial mediated dissipation of

chlorinated compounds. However, chlorinated compounds will persist in the environment if the conditions for biodegradation are inadequate such as a poor supply of electron acceptors, electron donors or cometabolites.

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Appendix: Tables

Table 1: Industrial production of chlorinated solvents in the U.S., Europe and the World.

Compound[†]	Global Production		US Production		European Production	
	t y^{-1}	Year of estimate	t y^{-1}	Year of estimate	t y^{-1}	Year of estimate
Chloromethanes						
CM	530,000[*]	1983[a]	390,000	1992[a]	164,000	1998[m]
DCM	515,000[*]	1983[a]	183,200	1994[b]	291,000	1998[m]
CF	325,000[*]	1983[a]	254,000	1994[c]	300,000	1998[m]
CT	575,000[*]	1983[a]	143,000	1991[a]	48,000	1998[m]
Chloroethanes						
1,2-DCA	15,868,000[*]	2002[d]	9,328,000	2002[d]		
1,1,1-TCA	600,000[+]	1984[e]	364,800	1990[f]	40,000	1998[m]
1,1,2-TCA	200,000-220,000[+]	1984[e]	170,000	1984[e]		
Chloroethenes						
1,1-DCE	150,000-200,000[+]	1986[e]	104,500	1989[f]	12,000	1998[m]
VC	13,600,000	1985[e]	6,000,000	1992[a]	5,533,000	1998[m]
TCE	390,000[*]	1984[e]	110,000	1984[e]	123,000	1998[m]
PCE	650,000	1993[e]	123,000	1993[g]	122,000	1998[m]
Chloroacetic acid	290,000[&]	1983[h]				
Epichlorohydrin						
					211,000	1998[m]
3-Chloropropene	800,000	1997[i]				
Chloro-1,3-butadiene	648,000[#]	1983[e]				
Chlorofluorocarbons						
CFC-12	425,000	1987[j]				
CFC-11	382,000	1987[j]				
HCFC-22	243,000	1987[j]				
CFC-113	251,000	1987[j]	78,000	1995[k]		
HCFC 141b	113,000	1995[j]				
HCFC 142b	38,000	1995[j]				

[†] CM= chloromethane; DCM = dichloromethane; CF = chloroform; CT = carbon tetrachloride; 1,2-DCA = dichloroethane; TCA = trichloroethane; 1,1-DCE= 1,1-dichloroethene; VC = vinyl chloride; TCE = trichloroethylene; PCE = perchloroethylene; CFC-12 = dichlorodifluromethane; CFC-11 = trichlorofluromethane; HCFC-22 = chlorodifluoromethane; CFC-113 = 1,2,2-trichlorotrifluoroethane; HCFC 141b = 1,1-dichloro-1-fluoroethane; HCFC 142b= 1,1-dichloro-1- fluoroethane.

[*] Global production estimated as sum of production in Japan, Western Europe and USA.

[+] Production of the "Western world" (Western Europe and USA).

[&] Worldwide consumption.

[#] World capacity

References: a- Anonymous 1993; b- Anonymous 1996; c- Anonymous 1995; d- Anonymous 2003b; e- Rossberg *et al.* 2002; f- Anonymous 2003; g- Anonymous 1994; h- Koenig *et al.* 2002; i- Keene *et al.* 1999; j- Siegemund and Schwertfeger 2002; k- Swoboda-Colber *et al.* 1995; m- Anonymous 2000.

Table 2: Industrial emission of chlorinated solvents in the U.S., Europe and the World.

Compound[†]	Global Industrial Emissions		US Emissions			European Emissions at 80 Production Plants in 1997[a]	
	t y^{-1}	Year of estimate	Air t y^{-1}	Water t y^{-1}	Year of estimate	Air t y^{-1}	Water t y^{-1}
Chloromethanes							
CM	10,000	1990[b]	2,030	4.8	1996[c]		
	163,000[*]	1990[b]					
DCM	584,400	1990[d]	18,360	177.6	1998[c]	810	3.4
	73,000	1999[e]	6,070	151	1993[c]	430	16.2
CF	160,000[*]	1999[f]					
	90,000	1978-1985[c]	130	51.8	2001[c]	97	3.8
CT	100,000[*]	1990[g]					
Chloroethanes							
1,2-DCA			250	0.4	1999[c]	3,460	23.8
1,1,1-TCA	679,000	1989[h]	52,270	10.6	1992[c]	0.2	0.6
1,1,2-TCA			10,000-20,000		1979[i]	164	2.2
Chloroethenes							
1,1-DCE			130	0.4	1991[c]		
VC	181,800	1982[j]	460	0.1	1993[c]	2,470	4.7
TCE	241,400	1990[d]	13,730	2.5	1993[c]		
PCE	366,200	1990[d]	4,870	4.6	1993[c]	230	2.9
Chlorofluoro-carbons							
CFC-12	550,000	1990[g]					
CFC-11	440,000	1990[g]					
HCFC-22	170,000	1990[g]					
CFC-113	200,000	1990[g]					

† CM= chloromethane; DCM = dichloromethane; CF = chloroform; CT = carbon tetrachloride; 1,2-DCA = dichloroethane; TCA = trichloroethane; 1,1-DCE= 1,1-dichloroethene; VC = vinyl chloride; TCE = trichloroethylene; PCE = perchloroethylene; CFC-12 = dichlorodifluromethane; CFC-11 = trichlorofluromethane; HCFC-22 = chlorodifluoromethane; CFC-113 = 1,2,2-trichlorotrifluoroethane.

* "Anthropogenic emissions" to the atmosphere (various sources e.g., industrial activity, coal combustion, incineration, etc). References: a- Lecloux 2003; b- McCulloch *et al.* 1999; c- Anonymous 2003; d- McCulloch *et al.* 1999b; e- Laturnus *et al.* 2002; f- Keene *et al.* 1999; g- Prather & Watson 1990; h- Thomas *et al.* 1982; i- Midgley 1989; j- Hartmans *et al.* 1985.

Table 3: Summary of kinetic data on lower chlorinated methane biodegradation available in the literature.

Cmpd	Role†	Redox‡	Cosubstr	Culture	Growth Rate	Pseudo 1^0 k*	ks or km	Activity*	Cell Yield*	Refs$
					d^{-1}	L mg^{-1} dwt d^{-1}	mg L^{-1}	mg g^{-1} dwt d^{-1}	g dwt g^{-1}	
CM	ED	AN F		*Acetobacterium dehalogenans*	0.55		<5.05	*3554*	0.156	a
CM	ED	A		*Hyphomicrobium* MC1	2.16			*10909*	0.198	b
CM	ED	A		*Methylobacterium* CM4	2.88			*25969*	0.111	c
CM	CoO	A	Ammonia	*Nitrosomonas europaea*				1854		d
CM	CoO	A	Butane	enrichment culture				1151		e
DCM	CoM	AN-M		*Methanosarcina barkeri*				5		f
DCM	ED	AN F		Mixed culture				1726		g
DCM	ED	AN F		Methanogenic consortium					0.145	h
DCM	ED	AN D		*Acinetobacter*	0.89			9084	0.096	i
DCM	ED	AN F		Strain DMC in coculture	0.83			6609	0.125	j
DCM	ED	AN F		*Dehalobacterium formicoaceticum* DMC	0.32			6010	0.081	k
DCM	ED	AN D		*Hyphomicrobium* DM2	0.36					l
DCM	ED	A		Mixed culture	0.89		11.60	*8457*	0.105	m
DCM	ED	A		*Hyphomicrobium* DM1	2.64			*14293*	0.185	n
DCM	ED	A		*Hyphomicrobium*	2.64			*15529*	0.170	o
DCM	ED	A		*Methylobacterium* sp. DM4	3.58		0.05			p
DCM	ED	A		*Methylophilus* sp. DM11	3.65		2.07			p
DCM	ED	A		*Acinetobacter*	1.10			9084	0.130	i
DCM	CoO	A	Butane	Enrichment culture				571		q

† ED = growth linked to use of chlorinated compound as electron donor; EA = growth linked to use of chlorinated compound as electron acceptor; CoM = cometabolism; CoO = cooxidation

‡ A = aerobic; AN = anaerobic; M = methanogenic; F = fermentative or acetogenic; S = sulfate-reducing; D = denitrifying; I = Iron-reducing; H =halorespiring

* pseudo first-order rate constants are normalised per mg cell dwt l^{-1}; activity is expressed in mg compound converted per g cell dwt (values in italic were calculated from growth rate and cell yield data) ; yield expressed as g cell dwt produced per g compound consume. Assumptions were: 0.5 g cell protein per g cell dwt; 0.9 g volatile suspended solids per g cell dwt

$ References: a - Traunecker *et al.* 1991; b - Hartmans *et al.* 1986; c - Vannelli *et al.* 1998; d - Rasche *et al.* 1990; e - Kim *et al.* 2000; f - Krone *et al.* 1989; g - Brausstromeyer *et al.* 1993; h - Freedman & Gossett 1991; i - Freedman *et al.* 1997; j - Magli *et al.* 1995; j - Magli *et al.* 1996; k - Kohler-Straub & Leisinger 1995; l - Herbst & Wiesmann 1996; m -Brunner *et al.* 1980; n - Diks & Ottengraf 1991; o - Gisi *et al.* 1998; p – Kim *et al.* 2000.

Table 4: Summary of kinetic data on higher chlorinated methane biodegradation available in the literature. Definition footnotes are given in Table 3.

Cmpd	Role†	Redox‡	Cosubstr	Culture	Growth Rate d^{-1}	Pseudo 1^0 k* L mg^{-1} dwt d^{-1}	ks or km mg L^{-1}	Activity* mg g^{-1} dwt d^{-1}	Cell Yield* g dwt g^{-1}	Refs.*
CF	CoM	AN F		*Acetobacterium woodii*				36		a
CF	CoM	AN-M		*Methanosarcina barkeri*				29		b
CF	CoM	AN M		Anaerobic digester sludge				1		c
CF	CoM	AN S		Enrichment culture				162		d
CF	CoM	AN-M		Enrichment culture				0.26		e
CF	CoO	A	Methane	*Methylosinus trichosporium* OB3b		0.400				f
CF	CoO	A	Formate	*Methylosinus trichosporium* OB3b		1.000	3.10	3,100		g
CF	CoO	A	Ammonia	*Nitrosomonas europaea*		0.320	31.91	10,153		h
CF	CoO	A	Methane	Aerobic methanotrophic biofilm		0.560	1.50	840		
CT	CoM	AN M		Anaerobic digester sludge				0.1		j
CT	CoM	AN F		Anaerobic digester sludge				0.1		j
CT	CoM	AN I		Anaerobic digester sludge				0.1		j
CT	CoM	AN S		Anaerobic digester sludge				0.1		j
CT	CoM	AN D		Anaerobic digester sludge				0.1		j
CT	CoM	AN M		Methanogenic biofilm				0.3		k
CT	CoM	AN M		Anaerobic biofilm				8		l
CT	CoM	AN M		Anaerobic biofilm				10.8		m
CT	CoM	AN M		Anaerobic biofilm			1.37	2.4		n
CT	CoM	AN M		Anaerobic biofilm		0.004				o
CT	CoM	AN Mx		Enrichment culture				3		j
CT	CoM	AN F		*Acetobacterium woodii*				732		p
CT	CoM	AN M		*Methanobacterium thermoautotrophicum*				60		p
CT	CoM	AN S		*Desulfobacterium autotrophicum*				11,98		p

* References: a- Egli *et al.* 1990; b- Krone *et al.* 1989; c- Yu & Smith 1997; d- Gupta *et al.* 1996; e- Yu *et al.* 1997; f- Speitel *et al.* 1993; g- Aziz *et al.* 1999; h- Ely *et al.* 1997; i- Alvarez-Cohen & McCarty 1991a; j - Boopathy 2002; k- van Eekert *et al.* 1998; l- Rhee & Speece 2000; m- Rhee & Speece 2000; n- Sponza 2002; o- Doong & Wu 1996; p- Egli *et al.* 1990.

Table 5: Biological and abiotic conversions of chlorinated ethanes under anaerobic conditions by methanogenic mixed or enrichment cultures.

Compound	Culture[†]	Elimination (%)	Products[1] (% recovered mol)	Refs.[&]
HCA	GS-live	92	PCE (42); TCE (6)	a
HCA	GS-killed	98	PCE (55); TCE (10)	a
PCA	GS-live	100	PCE (35); TCE (6); c/tDCE (8), 1DCE (4)	a
PCA	GS-killed	100	PCE (25); TCE (9); c/tDCE (6)	a
1,1,1,2-TeCA	GS-live	100	1,1-DCE (35); VC (8)	a
1,1,1,2-TeCA	GS-killed	100	1,1-DCE (15)	a
1,1,2,2-TeCA	GS-live	96	cDCE (73); tDCE (32); VC (11) TCE (7); E (tr); A (tr)	a
1,1,2,2-TeCA	GS-killed	100	cDCE (68); tDCE (33); TCE (18); VC (tr[**])	a
1,1,2,2-TeCA	MS-live	100	cDCE (52); tDCE (21); TCE (17); 1,1,2-TCA (3); 1,2-DCA (1); E (0.6) ; A (0.3)	b
1,1,2,2-TeCA	MS-killed	40	TCE (40)	b
1,1,2,2-TeCA	EC-live	NQ[*]	cDCE; tDCE; TCE	c
1,1,2,2-TeCA	EC-killed	NQ	TCE	c
1,1,1-TCA	GS-live	100	1,1-DCA (36); 1DCE(12); CA (5); E (tr); A (tr)	a
1,1,1-TCA	GS-killed	45	None detected	a
1,1,1-TCA	MS-live	79	1,1-DCA (25); CA (20), A (tr), E (tr); Cl^- (45%)[‡]	b
1,1,1-TCA	MS-killed	-	1DCE (tr)	b
1,1,2-TCA	GS-live	86	VC (40); 1,2-DCA (11); E (4)	a
1,1,2-TCA	GS-killed	51	VC (18)	a
1,1,2-TCA	MS-live	100	VC (80); 1,2-DCA (20); E (3)	b
1,1,2-TCA	MS-killed	-	None detected	b
1,1,2-TCA	EC-live	37	1,2-DCA	d
1,1-DCA	GS-live	31	CA (15); A (tr)	a
1,1-DCA	GS-killed	10	None detected	a
1,1-DCA	MS-live	70	CA (5); A (tr)	b
1,1-DCA	MS-killed	30	None detected	b
1,2-DCA	GS-live	37	CA (10); E (10); A (tr)	a
1,2-DCA	GS-killed	13	None detected	a
1,2-DCA	MS-live	40	CA (25); E (10)	b
1,2-DCA	MS-killed	15	None detected	b
1,2-DCA	EC-live	100	E (83)	e
1,2-DCA	EC-live	NQ	E	c
CA	MS-live	80	A (0.4)	b
CA	MS-killed	30	None detected	b

[†] GS = methanogenic granular sludge from a methanol fed upward-flow anaerobic sludge-blanket reactor; MS = anaerobically-digested municipal sludge; EC = enrichment culture (1,2-dichloropropane, 3-chlorobenzoate or perchloroethylene-dechlorinating);

[‡] recovery of inorganic chloride as a percent of organic chlorine

[*] NQ = not quantified

[**] tr = trace

[1] PCE = perchloroethylene; TCE = trichloroethylene; 1DCE = 1,1-dichloroethene; tDCE = *trans*-dichloroethene; cDCE = *cis*-dichloroethene; c/tDCE = unspecified mixture of *cis*- and *trans*- dichloroethene; VC = vinyl chloride; E = ethene; A = ethane; Cl^- = inorganic chloride

[&] References: a- van Eekert et al. 1999; b- Chen et al. 1996; c- Loffler et al. 1997; d- Fathepure & Tiedje 1994; e- Tandol et al. 1994.

Table 6: Summary of kinetic data on lower chlorinated ethane biodegradation available in the literature (definition abbreviations see Table 3)

Cmpd	Role	Redox	Cosubstr	Culture	Growth Rate d^{-1}	Pseudo 1^0 k $l\ mg^{-1}\ dwt\ d^{-1}$	ks or km $mg\ L^{-1}$	Activity $mg\ g^{-1}\ dwt\ d^{-1}$	Cell Yield $g\ dwt\ g^{-1}$	Refs.*
CA	CoO	A	Butane	Butane-grown mixed culture				2601		a
CA	CoO	A	Ammonia	*Nitrosomonas europaea*				5574		b
1,1-DCA	CoM	AN M						0.42		c
1,1-DCA	CoO	A	Butane	Butane-grown mixed culture			1.98	1188		b
1,1-DCA	CoO	A	Butane	Butane-grown mixed culture				451		b
1,2-DCA	CoM	AN M		Methanogenic biofilm				0.04		c
1,2-DCA	CoM	AN M		Acclimatised anaerobic biofilm				3		d
1,2-DCA	CoM	AN M		Acclimatised anaerobic biofilm + GAC				6		d
1,2-DCA	CoM	AN F		*Acetobacterium*				71		e
1,2-DCA	EA	AN H		*Desulfitobacterium dichloroeliminans*				12469		f
1,2-DCA	ED	A		mixed culture	4.56		57.00	26824	0.170	g
1,2-DCA	ED	A		*Xanthobacter autotrophicus*	2.64		25.73			h
1,2-DCA	ED	A		*Pseudomonas* strain DE2	1.92			2138		i
1,2-DCA	ED	A		*Ancylobacter aquaticus*	1.92		2.38			j
1,2-DCA	ED	A		*Pseudomonas* strain DCA1	3.36		<0.05	8835		k
1,2-DCA	ED	A		*Xanthobacter autotrophicus*	2.23		52.45	6056		l
1,2-DCA	CoO	A	Formate	*Methylosinus trichosporium* OB3b		1.200	7.60	9300		m, n
1,2-DCA	CoO	A	Ammonia	*Nitrosomonas europaea*		0.370	99.00	36000		o
1,2-DCA	CoO	A	Butane	Butane-grown mixed culture				261		b

* References: a- Kim *et al.* 2000; b- Rasche *et al.* 1990; c- van Eekert *et al.* 1999; d- De Wildeman *et al.* 2001; e- De Wildeman *et al.* 2003; f- De Wildeman *et al.* 2004; g- Herbst & Wiesmann 1996; h- Janssen *et al.* 1985; i- Stucki *et al.* 1983; j- van den Wijngaard *et al.* 1992; k- Hage & Hartmans 1999; l- Hartmans *et al.* 1992; m- Chang & Alvarez-Cohen 1996; n- Oldenhuis *et al.* 1991; o- Ely *et al.* 1997.

Table 7: Summary of kinetic data on higher chlorinated ethane biodegradation available in the literature (definition abbreviations see Table 3)

Compound	Role	Redox	Cosubstr	Culture	Growth Rate d^{-1}	Pseudo 1^0 k L mg^{-1} dwt d^{-1}	ks or km mg L^{-1}	Activity mg g^{-1} dwt d^{-1}	Cell Yield (g dwt) g^{-1}	Refs.*
1,1,1-TCA	CoM	AN M		Methanogenic biofilm				0.42		a
1,1,1-TCA	CoM	AN M		Anaerobic biofilm		0.0003				b
1,1,1-TCA	CoM	AN M		Anaerobic bioreactor mixed culture				0.02		c
1,1,1-TCA	CoM	AN M		Mixed culture bioreactor				0.05		d
1,1,1-TCA	CoO	A	Methane	Mixed methanotrophic biofilm		0.0064				e
1,1,1-TCA	CoO	A	Butane	Mixed butane-oxidising biofilm			16.01	608		f
1,1,1-TCA	CoO	A	Methane	Mixed methanotrophic biofilm		0.0001				g
1,1,1-TCA	CoO	A	Formate	*Methylosinus trichosporium* OB3b		0.1600	28.5	4600		h
1,1,1-TCA	CoO	A	Ethane	*Mycobacterium* sp. TA27			0.41	21		i
1,1,2-TCA	CoM	AN M		Methanogenic biofilm				0.42		a
1,1,1,2-TeCA	CoM	AN M		Methanogenic biofilm				3		a
1,1,1,2-TeCA	CoM	AN H		*Desulfitobacterium* sp, strain Y51				11626		j
1,1,2,2-TeCA	CoM	AN M		Methanogenic biofilm				1		a
1,1,2,2-TeCA	CoM	AN M		Municipal digester sludge				10		k
1,1,2,2-TeCA	CoM	AN H		*Desulfitobacterium* sp, strain Y51				3879		j
PCA	CoM	AN M		Methanogenic biofilm				3		a
PCA	CoM	AN H		*Desulfitobacterium* sp, strain Y51				12133		j
HCA	CoM	AN M		Methanogenic biofilm				3		a
HCA	CoM	AN H		*Desulfitobacterium* sp, strain Y51				1705		j

* References: a- van Eekert *et al.* 1999; b- Doong & Wu 1996; c- Rhee & Speece 2000; d- Long *et al.* 1993; e- Arvin 1991; f- Kim *et al.* 2002; g- Strand *et al.* 1990; h- Chang & Alvarez-Cohen 1996; i- Hashimoto *et al.* 2002; j- Suyama *et al.* 2001; k- Chen *et al.* 1996.

Table 8: Summary of kinetic data on vinyl chloride (VC) biodegradation available in the literature (definition abbreviations see Table 3)

Cmpd	Role	Redox	Cosubstr	Culture	Growth Rate	Pseudo 1^0 k	ks or km	Activity	Cell Yield	Refs.*
					d^{-1}	L mg^{-1} dwt d^{-1}	mg L^{-1}	mg g^{-1} dwt d^{-1}	g dwt g^{-1}	
VC	CoM	AN M		Methanogenic biofilm				0.001		a
VC	CoM	AN M		Methanol-degrading enrichment				0.141		b
VC	EA	AN H?		PCE-dechlorinating enrichment culture			0.163	19		c
VC	EA	AN H		VC-dechlorinating enrichment culture			0.313			d
VC	EA	AN H		*Dehaloccoides* strain BAV1	0.320			6030	0.008	e
VC	EA	AN H		*Dehaloccoides* strain VS	0.400			3047	0.133	f
VC	ED	AN M		Creek bed sediments			0.081			g
VC	ED	AN I		Creek bed sediments			0.475			g
VC	ED	A		Unidentified strains (active sludge)	0.190		0.700			h
VC	ED	A		*Mycobacterium* sp. Strain JS617	0.230	*14.400*	0.050	720	0.192	i
VC	ED	A		*Mycobacterium* sp. Strain JS616	0.170	*3.465*	0.200	693	0.173	i
VC	ED	A		*Mycobacterium* sp. Strain JS60	0.220	*13.968*	0.031	437	0.211	i
VC	ED	A		*Mycobacterium aurum* L1	0.960	*24.750*	0.200	4950	0.220	j
VC	ED	A		*Nocardioides* JS614	0.710	*25.800*	0.075	1935	0.330	i
VC	ED	A		*Pseudomonas* DL1	0.046	*3.094*	0.073	226	0.210	k
VC	ED	A		*Pseudomonas aeruginosa* MF1	0.005	*1.577*	0.016	26	0.200	l
VC	CoO	A	butane	Butane-oxidising enrichment culture				435		m
VC	CoO	A	formate	Methane oxidising mixed culture		1.600	3.6	5700		n
VC	CoO	A		cDCE grown cells strain JS666				297		o
VC	CoO	A	methane	Methane-oxidising biofilm				2.3		p
VC	CoO	A		Ethene grown cells *Pseudomonas* DL1			0.301	274		k
VC	CoO	A	ethane	*Pseudomonas* sp. strain EA1		*0.508*	0.039	20		q
VC	CoO	A	Cl-prpnol	*Pseudomonas* sp.		0.0001				r

* References: a- van Eekert *et al.* 2001; b- Skeen *et al.* 1995; c- Haston & McCarty 1999; d- He *et al.* 2003a; e- He *et al.* 2003b; f- Cupples *et al.* 2003; g- Bradley & Chapelle 1997; h- Aulenta *et al.* 2003; i- Coleman *et al.* 2002a; j- Hartmans & De Bont 1992; k- Verce *et al.* 2001; l- Verce *et al.* 2000; m- Kim *et al.* 2000; n- Chang & Alvarez-Cohen 1996; o- Coleman *et al.* 2002b; p- Nelson & Jewell 1993; q- Verce & Freedman 2000; r - Castro *et al.* 1992.

Table 9: Summary of kinetic data on dichloroethenes (DCE) biodegradation available in the literature (definition abbreviations see Table 3)

Cmpnd	Role	Redox	Cosubstr	Culture	Growth Rate d^{-1}	Pseudo 1^0 k L mg^{-1} dwt d^{-1}	ks or km mg L^{-1}	Activity mg g^{-1} dwt d^{-1}	Cell Yield g dwt g^{-1}	Refs.*
1-DCE	CoM	AN M		Methanogenic mixed culture				0.034		a
1-DCE	CoM	AN M		Methanogenic biofilm				0.018		b
1-DCE	CoM	AN M		Methanol-degrading enrichment				1.05		c
1-DCE	CoO	A	Butane	Butane-oxidising enrichment culture				791		d
1-DCE	CoO	A		VC-grown cells Mycobacterium aurum L3				1396		e
1-DCE	CoO	A	Formate	Methylosinus trichosporium OB3b smmo		1.700	0.490	840		f
1-DCE	CoO	A	Ammonia	Nitrosomonas europaea		1.130	0.890	1000		g
cDCE	CoM	AN M		Methanogenic mixed culture				0.063		a
cDCE	CoM	AN M		Anaerobic sewage sludge				0.366		h
cDCE	CoM	AN M		Methanogenic biofilm				0.002		b
cDCE	EA	AN H?		PCE-dechlorinating enrichment culture		0.101	0.320	32		i
cDCE	EA	AN H		VC-dechlorinating enrichment culture			0.863			j
cDCE	CoO	A	Methane	Methane-oxidising biofilm		0.009				k
cDCE	CoO	A	Butane	Butane-oxidising enrichment culture				465		d
cDCE	ED	A		Strain JS666	0.228		0.155	1173	0.213	l
cDCE	CoO	A		VC-grown cells Mycobacterium aurum L2				4188		e
cDCE	CoO	A	Formate	Methylosinus trichosporium OB3b sMMO		8.800	2.9	25400		f
cDCE	CoO	A	Formate	Methylosinus trichosporium OB3b PP359		8.600	1.1	9500		m
tDCE	CoM	AN M		Methanogenic biofilm				0.001		b
tDCE	EA	AN H		VC-dechlorinating enrichment culture			0.824			j
tDCE	CoO	A	Methane	Methane-oxidising biofilm		1.100				k
tDCE	CoO	A	Methane	Methane-oxidising mixed culture				465		n
tDCE	CoO	A		cDCE-grown cells strain JS667				279		l
tDCE	CoO	A		VC-grown cells Mycobacterium aurum L1				3490		e
tDCE	CoO	A	Formate	Methylosinus trichosporium OB3b sMMO		3.200	14.4	46200		f
tDCE	CoO	A	Formate	Methylosinus trichosporium OB3b PP358		3.900	6.4	24800		m

* References: a- Long *et al.* 1993; b- van Eekert *et al.* 2001; c- Skeen *et al.* 1995; d- Kim *et al.* 2000; e- Hartmans & De Bont 1992; f- Oldenhuis *et al.* 1991; g- Ely *et al.* 1997; h- Komatsu *et al.* 1994; i- Haston & McCarty 1999; j- He *et al.* 2003a; k- Arvin 1991; l- Coleman *et al.* 2002b; m- Aziz *et al.* 1999; n- Janssen *et al.* 1988.

Table 10: Anaerobic bioreactors utilised for the treatment of higher chlorinated ethenes (adapted in part from Middeldorp *et al.* 1999)

Bioreactor	Substrate	Products	Electron Donor	Volumetric Conversion Rate g m^{-3} reactor d^{-1}	Ref.*
Attached-film expanded bed	PCE	VC, E	Sucrose	79.0	a
Attached-film expanded bed	PCE	VC	Sucrose		b
Upflow anaerobic sludge bed	PCE	DCE	Ethanol	6.1	c
Upflow anaerobic sludge bed (*Dehalospirillum multivorans*)	PCE	DCE	Formate, acetate	31.5	d
Anaerobic reactor treating DCM	TCE	E	Glucose	19.5	e
Fixed film reactor	PCE	VC	Acetate	10.2	f
Sediment-granular sludge column	PCE	E, A	Lactate	14.7	g
Upflow anaerobic sludge bed	PCE	DCE	Mixture	4.7	h
Fixed-film reactor (*Desulfomonile*)	PCE	DCE	3-chlorobenzoate	41.0	i
Fixed-film stationary-bed	PCE		Vinasse	17.1	j
Anoxic chemostat	PCE	DCE	Glucose, formate	557.8	k

* References: a- Carter & Jewell 1993; b- Chu & Jewell 1994; c-Christiansen *et al.* 1997; d- Horber *et al.* 1998; e- Wild *et al.* 1995; f- Vogel & McCarty 1985; g- Debruin *et al.* 1992; h- Guiot *et al.* 1995; i- Fathepure & Tiedje 1994; j- Boucquey *et al.* 1995; k- Gerritse *et al.* 1997.

Table 11: Halorespiring bacteria that utilise PCE as an electron acceptor to support growth

Species	Electron Donors	Dechlorination Products	Ref[&].
Low G+C Gram Positive Bacteria			
Desulfitobacterium frappieri TCE1	H_2, lactate, formate, pyruvate, ethanol, butyrate	cDCE	a
Desulfitobacterium sp. strain PCE1	H_2, lactate, formate, pyruvate, ethanol, butyrate, succinate	TCE	b
Desulfitobacterium sp. strain Y51	Pyruvate, formate, lactate	cDCE	c
Clostridium bifermentans	Yeast extract, glucose	cDCE	d
Dehalobacter restrictus TEA	H_2	TCE, cDCE	e
Dehalobacter restrictus PER-K23	H_2	cDCE	f
δ-Proteobacteria			
Desulfuromonas chloroethenica TT4B	Acetate, pyruvate	cDCE	g, h
Desulfuromonas michiganensis BB1 and BRS1	Acetate, lactate, pyruvate, succinate, malate, fumarate	cDCE	i
ε- Proteobacteria			
Dehalospirillum multivorans[†]	Pyruvate, lactate, ethanol, formate, glycerol, H_2	cDCE	j
Sulfurospirillum halorespirans	Lactate	cDCE	k
Green Non-Sulfur Bacteria			
Dehalococcoides ethenogens 195[*]	H_2	VC, ethene	l, m
Dehaloccocoides sp.	H_2	Ethene	n

[*] PCE is only cometabolised if either cDCE or VC is utilised as electron acceptor

[†] Dehalospirillum multivorans has recently been reclassified as Sulfurospirillum multivorans (Luijten *et al.* 2003)

[&] References: a- Gerritse *et al.* 1999; b- Gerritse *et al.* 1996; c- Suyama *et al.* 2001; d- Chang *et al.* 2000; e- Wild *et al.* 1997; f- Holliger *et al.* 1998; g- Krumholz 1997; h- Krumholz *et al.* 1996; i- Sung *et al.* 2003; j- Scholzmuramatsu *et al.* 1995; k- Luijten *et al.* 2003; l- Maymo-Gatell *et al.* 1999; m- Maymo-Gatell *et al.* 1997; n- He*et al.* 2003a.

Table 12: Products formed from the cooxidation of TCE by whole cells (*in vivo*) and purified monooxygenases (*in vitro*).

Microorganism / Enzyme [†]	Products/Intermediates	Refs[*]
In Vivo		
Mycobacterium sp. strain TA27 ethane MO[†]	2,2,2-trichloroethanol (4.4%), trichloroacetic acid (3.1%), chloral (0.6%), dichloroacetic acid (0.6%), inorganic chloride (90%)	a
Rhodococcus rhodochrous propane MO[†]	CO_2 (28%), unidentified aqueous soluble products (40%), cell bound material (10%)	b
Phenol Oxidising Mixed Culture	CO_2 (22%), unidentified aqueous soluble products (42%), cell bound material (8.8%)	c
Pseudomonas stutzeri OX1 ToMO[†] expressed by recombinant *Escherichia c*oli	Inorganic chloride (87%)	d
Methylosinus trichosporium OB3b sMMO[†]	TCE-epoxide (12%)	e
Methylosinus-Trichosporium Ob3b sMMO[†]	Inorganic chloride (90%), chloral (0.7%), 2,2,2-trichloroethanol (0.8%)	f
Methylocystis sp. strain M methane MO	Trichloroacetic acid (7,8%), 2,2,2-trichloroethanol (0.04%), dichloroacetic acid (1.8%)	g
Various methane oxidising bacterial strains	Chloral	h
Methylosinus trichosporium OB3b sMMO expressed by recombinant *Pseudomonas putida*	Inorganic chloride (55%)	i
Methylomicrobium album BG8 expressing pMMO[†]	CO_2 (19%), formic acid (7%), glyoxylic acid (28%)	j
In Vitro		
Methylosinus trichosporium OB3b purified sMMO[†]	Carbon monoxide (53%), formic acid (35%), glyoxylic acid (5%), dichloroacetic acid (5%), chloral (6%), TCE-epoxide (qualitative evidence)	k
Methylomicrobium album BG8 purified pMMO[†]	CO_2 (20%)	l
Burkholderia cepacia G4 purified To-2-MO[†]	Carbon monoxide (41%), formic acid (21%), glyoxylic acid (10%), covalently bound to proteins (12%)	m
Pseudomonas putida F1 semipurified toluene dioxygenase	Glyoxylic acid and formate	N, o

[†] MO, monooxygenases; sMMO, soluble methane monooxygenase; pMMO, membrane bound methane monooxygenase; ToMO, toluene/ortho-xylene monooxygenases; To-2-MO, toluene-2-monooxygenase.

[*] References: a- Hashimoto *et al.* 2002; b- Malachowsky *et al.* 1994; c- Shurtliff *et al.* 1996; d- Chauhan *et al.* 1998; e- van Hylckama Vlieg *et al.* 1996; f- Oldenhuis *et al.* 1989; g- Saeki *et al.* 1999; h- Newman & Wackett 1991; i- Jahng & Wood 1994; j- Lontoh *et al.* 2000; k-Fox *et al.* 1990; l- Lontoh *et al.* 2000; m- Newman & Wackett 1997; n- Lange & Wackett 1997; o- Li & Wacket 1992.

Table 13: Summary of kinetic data on trichloroethylene (TCE) biodegradation available in the literature (definition abbreviations see Table 3)

Cmpnd	Role	Redox	Cosubstr	Culture	Growth Rate d^{-1}	Pseudo 1^0 k L mg^{-1} dwt d^{-1}	ks or km mg L^{-1}	Activity mg g^{-1} dwt d^{-1}	Refs.*
TCE	CoM	AN M		Methanogenic mixed culture				0.034	a
TCE	CoM	AN M		Methanogenic biofilm				0.004	b
TCE	EA	AN H		Enrichment culture		0.027			c
TCE	EA	AN H		Enrichment culture			0.184	189.2	d
TCE	EA	AN H		*Desulfitobacterium* sp. strain Y51				28740	e
TCE	CoO	A	Formate	Methane-grown mixed culture		0.930	8.2	7600	f
TCE	CoO	A	Formate	Methane-grown mixed culture		1.600	6.2	9600	g
TCE	CoO	A	Formate	Methane-grown mixed culture		0.600	7.0	4200	h
TCE	CoO	A	Formate	Methane-grown mixed culture		0.080	1.9	150	i
TCE	CoO	A		Phenol-grown mixed culture				330	j
TCE	CoO	A		Phenol-grown mixed culture		0.030	11.0	330	k
TCE	CoO	A		Propane grown mixed culture		0.086	5.2	450	h
TCE	CoO	A		Toluene-grown mixed culture		0.020	8.6	170	g
TCE	CoO	A	Phenol	*Burkholderia cepacia* G4		3.840	0.394	757	l
TCE	CoO	A	Phenol	*Alcaligenes eutrophus* JMP134(pJP4)				19	m
TCE	CoO	A	Toluene	*Burkholderia cepacia* G4		1.200	0.790	940	n
TCE	CoO	A	Toluene	*Burkholderia cepacia* G4				568	o
TCE	CoO	A	Toluene	*Ralstonia pickettii* PKO1				454	o
TCE	CoO	A	Toluene	*Pseudomonas putida* B2		0.200	6.4	1300	p
TCE	CoO	A	Toluene	*Pseudomonas putida* F1				95	o
TCE	CoO	A	Toluene	*Pseudomonas mendocina* KR1				454	o
TCE	CoO	A		Propane-grown *Mycobacterium vaccae* JOB5		0.098	0.580	57	q
TCE	CoO	A	Ammonia	*Nitrosomonas europaea*		1.000	1.6	1599	r
TCE	CoO	A	Formate	*Methylosinus trichosporium* OB3b sMMO		2.900	19	55000	s
TCE	CoO	A		*Methylosinus trichosporium* OB3b sMMO		0.530	7.2	3800	t
TCE	CoO	A	Formate	*Methylosinus trichosporium* OB3b PP358 sMMO		1.400	11.0	21000	u
TCE	CoO	A	Formate	*Methylomonas methanica* 68-1 sMMO		0.120	29.6	3666	v
TCE	CoO	A	Formate	*Methylosinus trichosporium* OB3b pMMO		0.374	1.0	388	w
TCE	CoO	A	Formate	*Methylomicrobium album* BG8 pMMO				117	w

* References: a- Long *et al.* 1993; b- van Eekert *et al.* 2001; c- Nielsen & Keasling 1999; d- Haston & McCarty 1999; e- Suyama *et al.* 2001; f- Alvarez-Cohen & McCarty 1991b; g- Chang & Alvarez-Cohen 1996; h- Chang & Alvarez-Cohen 1995; i- Chang & Criddle 1997; j- Hopkins *et al.* 1993; k- Shurtliff *et al.* 1996; l- Folsom *et al.* 1990; m- Harker & Kim 1990; n- Landa *et al.* 1994; o- Leahy *et al.* 1996; p- Kelly *et al.* 2000; q- Wackett *et al.* 1989; r- Ely *et al.* 1997; s -Oldenhuis *et al.* 1991; t- Tsien *et al.* 1989; u- Aziz *et al.* 1999; v- Koh *et al.* 1993; w -Lontoh & Semrau 1998.

Table 14: Summary of kinetic data on perchloroethylene (PCE) biodegradation available in the literature (definition abbreviations see Table 3).

Cmpnd	Role	Redox	Cosubstr	Culture	Growth Rate d^{-1}	Pseudo 1^0 k L mg^{-1} dwt d^{-1}	ks or km mg L^{-1}	Activity mg g^{-1} dwt d^{-1}	Cell Yield g dwt g^{-1}	Refs.*
PCE	CoM	AN M		Methanogenic biofilm				0.006		a
PCE	CoM	AN M		Methanogenic mixed culture				4.5		b
PCE	CoM	AN M		Anaerobic biofilm			0.009	4.8		c
PCE	CoM	AN M		*Methanosarcina* spp.				0.040-0.090		d, e
PCE	CoM	AN M		*Methanosarcina* strain FR				6.3		f
PCE	CoM	AN F		*Sporomusa ovata*				19.5		g
PCE	CoM	AN F		*Acetobacterium woodii*				7.1		h
PCE	CoM	AN H		Enrichment culture (3-chlorobenzoate)				7.4		i
PCE	EA	AN H		Enrichment culture				687		j
PCE	EA	AN H		Enrichment culture		0.055	41.8	2288		k
PCE	EA	AN H		Enrichment culture				4378		l
PCE	EA	AN H		Enrichment culture			0.018	298		m
PCE	EA	AN H		Enrichment culture				8656		n
PCE	EA	AN H		*Clostridium bifermentans*				856		o
PCE	EA	AN H		*Dehalobacter restrictus* PER-K23	0.58			3648	0.051	p
PCE	EA	AN H		*Dehalospirillum multivorans*				8955		q
PCE	EA	AN H		*Dehalospirillum multivorans*	6.65			5970	0.034	r
PCE	EA	AN H		*Desulfitobacterium* sp. strain PCE1	2.04			37312		s
PCE	EA	AN H		*Desulfuromonas michiganensis*				16596		t
PCE	EA	AN H		*Desulfitobacterium frappieri* TCE1	5.76			4776	0.094	u
PCE	EA	AN H		*Desulfitobacterium* sp. strain PCE1	3.36			16716	0.041	u
PCE	EA	AN H		*Desulfitobacterium* sp. strain Y51				24488		v
PCE	EA	AN H		*Desulfuromonas chloroethenica* TT4B	0.23				0.004	w, x
PCE	EA	AN H		*Dehalococcoides ethenogens* 195				2746	0.174	y
PCE	CoO	A	Toluene	*Pseudomonas stutzeri* OX1			34	298		z

* References: a- van Eekert *et al.* 2001; b- Long *et al.* 1993; c- Carter & Jewell 1993; d-Fathepure & Boyd 1988a; e-Fathepure & Boyd 1988b; f- Cabirol *et al.* 1998; g- Terzenbach & Blaut 1994; h- Egli *et al.* 1988; i- Fathepure & Tiedje 1994; j- Tandol *et al.* 1994; k- Nielsen & Keasling 1999; l- Kengen *et al.* 1999; m- Haston & McCarty 1999; n- Gerritse *et al.* 1997; o- Chang *et al.* 2000; p- Holliger *et al.* 1993; q- Neumann *et al.* 1994; r- Scholzmuramatsu *et al.* 1995; s- Gerritse *et al.* 1996; t- Sung *et al.* 2003; u- Gerritse *et al.* 1999; v- Suyama *et al.* 2001; w- Krumholz 1997; x- Krumholz *et al.* 1996; y- Maymo-Gatell *et al.* 1997; z- Ryoo *et al.* 2000.

Table 15: Abbreviations of CFC and HCFC discussed in this section

Name	Formula	Abbreviation
Dichlorodifluoromethane	CCl_2F_2	CFC-12
Trichlorofluoromethane	CCl_3F	CFC-11
Dichlorofluoromethane	$CHCl_2F$	HCFC-21
Chlorodifluoromethane	$CHClF_2$	HCFC-22
Chlorofluoromethane	CH_2ClF	HCFC-31
1,1,1,2-tetrachlorodifluoroethane	CCl_3CClF_2	CFC-112a
1,1,2-trichloro-trifluoroethane	CCl_2FCClF_2	CFC-113
1,1,1-trichloro-trifluoroethane	CCl_3CF_3	CFC-113a
1,1,2-trichloro-2,2-difluoroethane	$CHCl_2CClF_2$	HCFC-122
1,1,2-trichloro-1,2-difluoroethane	$CCl_2FCHClF$	HCFC-122a
1,1-dichloro-2,2,2-trifluorethane	$CHCl_2CF_3$	HCFC-123
1,2-dichloro-1,2,2-trifluoroethane	$CHClFCClF_2$	HCFC-123a
1-chloro-1,2,2,2-tetrafluoroethane	$CHClFCF_3$	HCFC-124
1,1,2-trichloro-2-fluoroethane	CCl_3CH_2F	HCFC-131
1-chloro-1,2,2-trifluoroethane	$CHClFCHF_2$	HCFC-133
1-chloro-2,2,2-trifluoroethane	CH_2ClCF_3	HCFC-133a
1-chloro-1,1,2-trifluoroethane	$CClF_2CH_2F$	HCFC-133b
1,1-dichloro-1-fluoroethane	CCl_2FCH_3	HCFC-141b
1-chloro-1,1-difluoroethane	$CClF_2CH_3$	HCFC-142b
Trichlorofluoroethene	CCl_2CClF	CFC-1111
1,1-dichloro-difluoroethene	CCl_2CF_2	CFC-1112a
Chlorotrifluoroethene	$CClFCF_2$	CFC-1113
Cis-1,2-dichlorofluoroethene	$CHClCClF$	HCFC-1121
(E)-1-chloro-2-fluoroethene	$CHClCHF$	HCFC-1131

Table 16: Products formed from the anaerobic cometabolism of CFC and HCFC by natural mixed cultures or pure cultures

Consortium/ Microorganism	Substrate	Products/Intermediates (% product in mol)	Refs.[&]
Natural Mixed Cultures			
Sulfate-reducing enrichment culture	CFC-11	HCFC-21 (>100%)	a
Simulated landfill	CFC-11	HCFC-21	b
Municipal solid waste	CFC-11	HCFC-21	c
Anaerobic compost	CFC-11	HCFC-21, HCFC-31	d
Simulated landfill	CFC-12	HCFC-22	b
Anaerobic compost	CFC-12	HCFC-22	d
Contaminated aquifer	CFC-113	HCFC-123a[*], HCFC-122a[*]	e
Landfill leachate	CFC-113	HCFC-123a (100%), CFC-1113 (7%)[†]	f
Anaerobic freshwater sediments	HCFC-123	HCFC-133a (29%)	g
Landfill leachate	HCFC-123a	HCFC-133, HCFC-133b, CFC-1113	f
Contaminated aquifer	CFC-1111	*cis*-HCFC-1121, (*E*)-HCFC-1131	h
Contaminated aquifer	CFC-1111	*cis*-HCFC-1121, *trans*-HCFC-1121, (*E*)-HCFC-1131	i
Pure Culture			
Methanosarcina barkeri	CFC11	HCFC-21 (28%), HCFC-31 (trace), CO (6%), F^- (1%)	j
Pseudomonas putida G786	CFC-112a	HCFC-121, CFC-1112a	k

[†] temporal intermediate
[*] tentative identifications
[&] References. a - Sonier *et al.* 1994; b - Deipser & Stegmann 1997; c - Deipser & Stegmann 1994; d - Deipser 1998; e - Lesage *et al.* 1990; f - Lesage *et al.* 1992; g - Oremland *et al.* 1996; h - Vancheeswaran *et al.* 1999; i - Hageman *et al.* 2001; j - Krone & Thauer 1992; k - Hur *et al.* 1994.

Table 17: Summary of kinetic data on chlorofluorohydrocarbon biodegradation available in the literature (definition abbreviations see Table 3)

Compound	Role	Redox	Cosubstrate	Culture	Pseudo 1^0 k L mg^{-1} dwt d^{-1}	Activity mg g^{-1} dwt d^{-1}	Refs.*
CFC-11	CoM	AN M		*Methanosarcina barkeri*		165	a
CFC-12	CoM	AN F		*Clostridium pasteurianum*		0.005	b
HCFC-21	CoO	A	Methane	*Methylosinus trichosporium*		119	c
HCFC-22	CoO	A	Methane	Methanotrophic mixed culture	0.014		d
HCFC-142b	CoO	A	Methane	Methanotrophic mixed culture	0.0096		d
HCFC-123	CoO	A	Methane	Methanotrophic mixed culture	0.00054		d
CFC-1113	CoO	A	Methane	Purified sMMO *Methylosinus trichosporium*		2374	e

* References: a - Krone & Thauer 1992; b - Lovley & Woodward 1992; c - Deflaun *et al.* 1992; d - Chang & Criddle 1995; e - Fox *et al.* 1990

Table 18: Summary of kinetic data on chlorinated acetic acid biodegradation available in the literature (definition abbreviations see Table 3)

Compound	Role	Redox	Culture	Growth Rate d^{-1}	Pseudo 1^0 k L mg^{-1} dwt d^{-1}	Activity mg g^{-1} dwt d^{-1}	Cell Yield g dwt g^{-1}	Refs.*
CAA	ED	AN M	Anaerobic biofilm				0.042	a
CAA	ED	A	*Ancylobacter aquaticus* AD20	1.68				b
CAA	ED	A	CFE^+ of *Pseudomonas* strain DCA1			123425		c
CAA	CoO	A	*Pseudomonas putida*		$6.3X10^{-6}$			d
DCAA	ED	A	CFE^1 of *Xanthobacter autotrophicus* GJ10			389915		e
DCAA	ED	A	*Xanthobacter autotrophicus* GJ10	1.28				f
TCAA	EA	AN H	*Trichlorobacter thiogenes*	2.77				g
TCAA	ED	A	*Acinetobacter calcoaceticus*	0.65			0.027	h

$^+$ CFE = cell free extract, and activity expressed as mg compound converted (g protein)$^{-1}$ d^{-1}

* References: a- Egli *et al.* 1989; b - van den Wijngaard *et al.* 1992; c - Hage & Hartmans 1999; d - Castro 1993; e - Meusel & Rehm 1993; f - Janssen *et al.* 1985; g - De Wever *et al.* 2000; h - Yu & Welander 1995.

Table 19: Products observed from the cooxidation of chloropropanes.

Compound	Strain	Primary Substrate(s)	Intermediates	Refs.*
1-CPrpA	*Methylosinus trichosporium* OB3b	Cyclopropanol	Propanol, l-chloro-2-propanol, 1-chloro-3-propanol	a
1-CPrpA	*Methylosinus trichosporium* OB3b	Formate	1-chloro-2-propanol, chloride	b
1-CPrpA	*Nitrosomonas europaea*	Ammonia	Propionaldehyde, 3-chloropropanol, 1-chloro-2-propanol	c
2-CPrpA	*Methylosinus trichosporium* OB3b	Cyclopropanol	2-chloro-propanol, acetone	a
1,2-DCPrpA	*Pseudomonas* sp. strain DCA1	Chloroacetic acid or 1,2-DCA	2,3-dichloro-1-propanol, 2- chloroethanol.	d
1,2-DCPrpA	*Methylosinus trichosporium* OB3b	Methane	2,3-dichloropropanol, chloride	e
1,2-DCPrpA	*Methylosinus trichosporium* OB3b	Formate	1-chloro-2-propanol, 2-chloro-1-propanol, 2,3-dichloro-1-propanol, chloride	b
1,3-DCPrpA	Methylosinus trichosporium OB3b	Formate	Chloride	b
1,3-DCPrpA	*Acinetobacter* sp. GJ70	1,6-dichlorohexane	Chloride	f
1,3-DCPrpA	*Xanthobacter autotrophicus* GJ-10 haloalkane dehalogenase	1,2-DCA		g
1,2,3-TCPrpA	*Methylosinus trichosporium* OB3b	Formate	2-chloro-1-propanol, 2,3-dichloro-1-propanol, 1,3-dichloro-2-propanol, chloride	b

* References: a - Shimoda *et al.* 1993; b - Bosma & Janssen 1998; c - Rasche *et al.* 1990; d - Hage *et al.* 2001; e - Oldenhuis *et al.* 1989; f - Janssen *et al.* 1987; g - Keuning *et al.* 1985.

Table 20: Summary of kinetic data on chlorinated propanoid and chlorinated butadiene compound biodegradation available in the literature (definition abbreviations see Table 3)

Compound	Role	Redox	Cosubstrate	Culture	Growth Rate d^{-1}	Pseudo 1st order L mg^{-1} dwt d^{-1}	Activity mg g^{-1} dwt d^{-1}	Refs.*
1-CPrpA	ED	A		*Xanthobacter autotrophicus GJ10*	2.97			a
1-CPrpA	CoO	A	Formate	*Methylosinus trichosporium OB3b*		1.483		b
1,2-DCPrpA	EA	AN H		Enrichment culture			407	c
1,2-DCPrpA	CoO	A	Formate	*Methylosinus trichosporium OB3b*		0.518		d
1,3-DCPrpA	ED	A		*Xanthobacter autotrophicus GJ11*	2.13			e
1,3-DCPrpA	CoO	A	Formate	*Methylosinus trichosporium OB3b*		0.835		d
1,2,3-DCPrpA	CoO	A	Formate	*Methylosinus trichosporium OB3b*		0.101		d
HeCPrpA[&]	CoM	An H		*Desulfitobacterium* sp. strain Y51			3943	f
Epichlorohydrin	ED	A		*Pseudomonas sp. strain AD1*	3.02			g
1,3-DCPrpE	ED	A		Biofilm			16646	h
1,3-DCPrpE	ED	A		*Pseudomonas* sp. strain EK1			20214	h
1,3-DCPrpE	ED	A		*Rhodococcus erythropolis* EK5			54810	h
1,3-DCPrpE	CoO	A		*Delftia* sp. strain EK3			15340	h
HCBD[&]	CoM	AN M		Anaerobic biofilm			1.3	i

[&] HeCPrpA = 1,1,1,2,2,3,3-heptachloropropane; HCBD = hexachlorobutadiene

* References: a - Janssen *et al.* 1985; b - Bosma & Janssen 1998; c - Loffler *et al.* 1997; d - Bosma & Janssen 1998; e -Janssen *et al.* 1985; f- Suyama *et al.* 2001; g - van den Wijngaard *et al.* 1989; h - Katsivela *et al.* 1999; i - Booker & Pavlostathis 2000.

Table 21: Summary of biodegradability of chlorinated solvents and chlorinated aliphatic compounds covered in this review article

Compound	Biodegradability[1]					Highest Values Reported in this Review Article					
						Growth Rate		Vol. Load Bioreactors		Environmental 1^0 $k^{\dagger}$	
	Aerobic		Anaerobic			Aerobic[2]	Anaerobic[2]	Aerobic	Anaerobic	Aerobic	Anaerobic
	ED	CoM	ED	EA	CoM	d^{-1}	d^{-1}	$g\ m^{-3}\ d^{-1}$	$g\ m^{-3}\ d^{-1}$	d^{-1}	d^{-1}
CM	++	++	+	?	?	2.88^{D}	0.55^{D}				
DCM	++	++	++	?	?	3.65^{D}	0.89^{D}	40000	1250		
CF	−	++	−	?	++				78		
CT	−	−	−	?	++				31		0.700
CA	?	++	−	?	+						
1,1-DCA	?	++	−	?	++						
1,2-DCA	++	++	−	++	++	4.56^{D}		51200	71.8		
1,1,1-TCA	−	++	−	++	++				9.6		0.015
1,1,2-TCA	−	+	−	?	++						
1,1,1,2-TeCA	−	++	−	?	++						
1,1,2,2-TeCA	−	−	−	?	++						
PCA	−	−	−	?	++						
HCA	−	−	−	?	++				30		
VC	++	++	-/+	++	++	0.96^{D}	0.4^{H}	30		0.009	
1-DCE	?	++	-	++	++						
cDCE	+	++	-/+	++	++	0.23^{D}		104		0.002	
tDCE	?	++	−	+	++						
TCE	−	++	−	++	++				20		0.003
PCE	−	-/+	−	++	++		6.65^{H}		558		0.019

[1] ED = growth linked to use of compound as electron donor; EA = growth linked to use of compound as electron acceptor (halorespiration); CoM = cometabolism (no growth), under aerobic conditions typically cooxidation
(-) no biodegradation observed; (-/+), very frequently no biodegradation observed, however biodegradation reported (by one research group); (+), biodegradation observed in one study; (++), biodegradation observed in two or more studies; (?), not studied.

[a] aerobic bacteria incubated anaerobically

[g] genetically-modified microorganisms

[m] evidence based on observations with mammalian cells

[2] D = growth rate with compound as electron donor; H = growth rate with compound as electron acceptor (halorespiration)

[†] first-order rate constant in environmental samples

[c] first-order rate constant from a sediment column acclimated to HCBD

Table 21 (cont.): Summary of biodegradability of chlorinated solvents and chlorinated aliphatic compounds covered in this review

Compound	Biodegradability[1]					Highest Values in Reported in this Review Article					
						Growth Rate		Vol. Load Bioreactors		Environmental 1^0 k^f	
	Aerobic		Anaerobic			Aerobic[2]	Anaerobic[2]	Aerobic	Anaerobic	Aerobic	Anaerobic
	ED	CoM	ED	EA	CoM	d^{-1}	d^{-1}	g m^{-3} d^{-1}	g m^{-3} d^{-1}	d^{-1}	d^{-1}
CFC-12	−	−	−	−	++				0.02		0.055
CFC-11	−	−	−	−	++				0.17		0.122
HCFC-21	−	++	−	−	+						
HCFC-22	−	++	−	−	?			0.24			
CFC-112a	−	−	−	−	+[ag]						
CFC-113	−	−	−	−	++				0.03		
CFC-113a	−	−	−	−	+[ag]						
HCFC-123	−	−	−	−	+						
HCFC-123a	−	?	−	−	+						
HCFC-131	−	+	−	−	?						
HCFC-141b	−	+	−	−	?						
HCFC-142b	−	+	−	−	?						
CFC-1111	−	−	−	−	++						
CFC-1112a	−	+[g]	−	−	?						
HCFC-1121	−	+[g]	−	−	?						
CAA	++	++	++	−	?	1.68[D]			1700	0.193	
DCAA	++	++	?	−	++	1.28[D]		25100		0.866	
TCAA	++	++	?	+	++	0.65[D]	2.77[H]			0.048	
1-CPrpA	+	++	-	-	-	2.97[D]					
2-CPrpA	?	+	?	?	?						
1,1-DCPrpA	?	?	?	?	+						
1,2-DCPrpA	−	++	?	++	+				79		
1,3-DCPrpA	+	++	?	?	?	2.13[D]					
1,2,3-TCPrpA	+[g]	+	?	?	+						
HeCPrpA	−	−	−	?	+						
1,3-DCPrpE	++	+	?	?	?			399		1.39	
Epichlorohydrin	++	+	?	?	?	3.02[D]					
2-CBD	−	++[m]	?	?	?						
HCBD	−	−	?	?	++				12		8.64[c]

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Euro Chlor • Avenue E. Van Nieuwenhuyse 4, box 2 • B-1160 Brussels, Belgium
Tel: +32 2 676 72 11 | Fax: +32 2 676 72 41
Email: eurochlor@cefic.be | www.eurochlor.org