University of Groningen

Microbiological aspects of the removal of chlorinated hydrocarbons from air

Dolfing, Jan; Wijngaard, Arjan J. van den; Janssen, Dick

Published in:
Biodegradation

DOI:
10.1007/BF00695974

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
1993

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Dolfing, J., Wijngaard, A. J. V. D., & Janssen, D. B. (1993). Microbiological aspects of the removal of chlorinated hydrocarbons from air. Biodegradation, 4, 261-282. DOI: 10.1007/BF00695974

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 11-02-2018

Biodegradation **4**: 261–282, 1993.
© 1993 *Kluwer Academic Publishers. Printed in the Netherlands.*

Microbiological aspects of the removal of chlorinated hydrocarbons from air

Jan Dolfing, Arjan J. van den Wijngaard & Dick B. Janssen
Department of Biochemistry, University of Groningen, 9747 AG Groningen, The Netherlands

Received 6 January 1993; accepted in revised form 5 July 1993

Key words: chlorinated hydrocarbons, biodegradation, biotransformation, cometabolism, gaseous emissions, waste gas

Abstract

Chlorinated hydrocarbons are widely used synthetic chemicals that are frequently present in industrial emissions. Bacterial degradation has been demonstrated for several components of this class of compounds. Structural features that affect the degradability include the number of chlorine atoms and the presence of oxygen substituents. Biological removal from waste streams of compounds that serve as a growth substrate can relatively easily be achieved. Substrates with more chlorine substituents can be converted cometabolically by oxidative routes. The microbiological principles that influence the biodegradability of chlorinated hydrocarbons are described. A number of factors that will determine the performance of microorganisms in systems for waste gas treatment is discussed. Pilot plant evaluations, including economics, of a biological trickling filter for the treatment of dichloromethane containing waste gas indicate that at least for this compound biological treatment is cost effective.

Introduction

Chlorinated aliphatic hydrocarbons are industrially produced in very large amounts (Table 1). Dichloroethene and vinylchloride rank fourth and sixth on the list of bulk organics produced in the USA in 1990, after ethene, propene, and urea (Anonymous 1992). Both compounds serve as precursors for the synthesis of PVC, and are as such not purposely emitted into the atmosphere. Tetrachloroethene and trichloroethene (which was not listed in the aforementioned overview), on the other hand, serve as solvents and degreasers in the dry and metal cleaning industry, and a large part of the annual production eventually ends up in the environment where these compounds are degraded slowly if it all (Alexander 1981; Hutzinger & Veerkamp 1981). The most plausible explanation for the observed persistence of many chlorinated aliphatics is their xenobiotic structure. They are not synthesized naturally, and nature has not (yet) developed enzyme systems tailored to deal with these compounds.

The carbon-chlorine bond per se is, however, not xenobiotic. Chloramphenicol is a well known example of a compound containing a carbon-halogen bond. In marine environments algae produce large amounts of methyl iodide (Lovelock et al. 1973). Other compounds that occur naturally in marine environments are compounds like carbon tetrachloride, methyl chloride, chloroform, and bromoform, which were previously considered to be typical industrial products but are now known to also have significant natural sources (Pearson & McConell 1975; Gschwend et al. 1985). Asplund & Grimvall (1991) recently presented evidence that halogenated compounds are also produced naturally in

Table 1. Production* and use of chlorinated aliphatic compounds.

	10^6 kg/year	Major use
Chloromethane	350	Blowing agent
Dichloromethane	210	Solvent
Trichloromethane	220	Solvent, degreaser
Tetrachloromethane	190	Synthetic rubber
Chloroethane	70	Intermediate
1,2-Dichloroethane	6300	Vinylchloride
1,1,1-Trichloroethane	360	Dry cleaning, metal cleaning
Chloroethene	4800	PVC
Tetrachloroethene	170	Dry cleaning, metal cleaning

* : US production in 1990; source: International Trade Commission as cited in: Facts and figures for the chemical industry, Chemical & Engineering News (1992) 70 (26), p. 36.

terrestrial environments, probably by the action of chloroperoxidases, and that they are are more widespread than previously thought.

It still seems reasonable to assume that most microorganisms are not routinely exposed to significant amounts of halogenated aliphatics. Nevertheless, in the last decades several microorganisms have been described that can degrade these compounds, and sometimes can even use them as carbon and/or energy source (Tables 2 and 3). The fundamental question then arises how these pathways have evolved. This problem has been reviewed recently (Van der Meer et al. 1992) and falls beyond the scope of the present paper. Here we will outline what is known about the organisms that are described, and how we can apply this knowledge for the removal of chlorinated hydrocarbons from air. The last few years there has been a strong drive to develop systems that can remove chlorinated hydrocarbons from air, because there is legislative pressure to diminish the amounts of chlorinated hydrocarbons released into the atmosphere, both in the EEC and in the USA. Table 4 lists some of the standards that must be met in western Europe.

The isolates capable of growth on chlorinated aliphatic compounds mostly can do this only with substrates that have no more than one or two chlorine substituents. The available data may be summarized as follows (Table 2).

- Most industrial hydrocarbons with a single halogen substituent per molecule can be used as a sole carbon source by specific microbial cultures. Compounds with two or more halogens become more refractile, but dichloromethane, 1,2-dichloroethane, and the dichlorobenzenes can also be degraded by pure cultures (Brunner et al 1980; Stucki et al. 1981; Janssen et al. 1985; De Bont et al. 1986; Schraa et al. 1986). Dichloroethenes and most trichloro-compounds (but see Sander et al. (1991) for growth on tri- and tetrachlorobenzenes) do not support aerobic microbial growth.
- Cometabolic conversions that rely on non-specific enzymes are possible for most compounds possessing at least one carbon-hydrogen bond. The enzymes involved are ususally monooxygenases or dioxygenases that do not specifically cleave carbon-halogen bonds, but produce unstable intermediates that decompose and release

Table 2. Biodegradation of chlorinated hydrocarbons.

Aerobic	growth substrates
	chloromethane
	dichloromethane
	chloroethene
	chloroethane
	1,2-dichloroethane
	1,3-dichloropropane
	1-chlorobutane
	3-chloro-1,2-epoxypropane
	chlorobenzene
	dichlorobenzenes
	1,2,4-trichlorobenzene
	chlorotoluene
Aerobic	decomposition by cometabolism
	trichloromethane
	dichloroethenes
	trichloroethene
	1,1-dichloroethane
	1,1,1-trichloroethane
	chloropropenes
Anaerobic	growth substrates
	methylchloride
	dichloromethane
Anaerobic	metabolism
	chloromethanes
	chloroethenes
	trichlorobenzene
	hexachlorobenzene

halides by chemical decomposition (Janssen & Witholt 1992).

- Methylchloride can be degraded under anaerobic conditions, presumably by acetogens (Traunecker et al. 1991).
- Highly chlorinated compounds can fortuitously be reductively dehalogenated (Krone et al. 1989, 1991; Schanke & Wackett 1992). Reduced cofactors or coenzymes, specifically involved in oxidation-reduction reactions, are involved in these transformations. Examples are factor F_{430} from methanogens, and cobalamins from acetogens or methanogens. Reduced cytochome P_{450}, as it occurs in some aerobes, can also catalyze reductive conversions (Janssen & Witholt 1992).
- At least one compound (tetrachloroethene, Holliger 1992) can serve as a physiological electron acceptor, just as found with 3-chlorobenzoate (Dolfing & Tiedje 1987; Dolfing 1990; Mohn & Tiedje 1990). The biochemistry of these processes is not understood.

The present review will be focused on aerobic conversions. Currently, there are no effcient process technologies for transfer of contaminants from the gas phase to an anaerobic compartment, as would be required for the anaerobic conversions.

Aerobic biodegradation

Bacterial utilization of halogenated aliphatic compounds as growth substrate is dependent on the capacity of microorganisms to cleave or labilize the carbon-halogen bond and form intermediates that can be chanelled into energy-generating oxidative routes.

Table 3 lists a number of well-characterized bacterial cultures capable of growth on simple chlorinated hydrocarbons. The growth rates (μ_{max}) of these cultures are of the same order of magnitude as found with other simple aliphatic or aromatic compounds (0.5–0.05 h^{-1}). Several specific dehalogenation mechanisms have been identified in the organisms that grow on these compounds (Janssen et al. 1989). The enzymes involved have been classified as glutathione transferases, hydrolytic dehalogenases, and haloalcohol dehalogenases (Fig. 1).

Table 3. Examples of aerobic bacterial growth on volatile halogenated compounds.

Compound	Organism	μ (h^{-1})	Reference
Chloromethane	*Hyphomicorbium* sp.	0.09	Hartmans et al. 1986
Dichloromethane	*Hyphomicrobium* DM2	0.07	Stucki et al. 1981
	strain DCM11	0.22	Scholtz et al. 1988
1,2-Dichloroethane	*Xanthobacter autotrophicus* GJ10	0.11	Janssen et al. 1985
	Ancylobacter aquaticus AD20	0.08	van den Wijngaard et al. 1992
1-Chloropropane	*Xanthobacter autotrophicus* GJ10	0.12	Janssen et al. 1985
1,3-Dichloropropane	*Xanthobacter autotrophicus* GJ10	0.09	Janssen et al. 1985
1-Chlorobutane	*Xanthobacter autotrophicus* GJ10	0.10	Janssen et al. 1985
	strain GJ70	0.15	Janssen et al. 1987, 1988
1-Chloropentane	strain GJ70	0.21	Janssen et al. 1987, 1988
1,6-Dichlorohexane	strain GJ70	ND*	Janssen et al. 1987, 1988
Chloroethene	*Mycobacterium aurum* L1	0.04	Hartmans & de Bont 1992
Epichlorohydrin	*Pseudomonas* AD-1	0.13	van den Wijngaard et al. 1989
Chlorobenzene	strain WR1306	0.55	Reineke & Knackmuss 1984
1,4-Dichlorobenzene	*Alcaligenes* A175	0.09	Schraa et al. 1986
3-Chloroaniline	*Pseudomonas acidovorans* CA28	0.09	Loidl et al. 1990
4-Chloroaniline	*Pseudomonas acidovorans* CA28	0.04	Loidl et al. 1990
4-Chloroaniline	*Moraxella* sp.	0.12	Zeyer et al. 1985
Chlorotoluenes	*Pseudomonas cepacia* HCV	ND*	Vandenbergh et al. 1981

* : ND = not determined.

Table 4. Off-gas standards for volatile organic compounds in western Europe.

	mg/m^3		
	NL*	FRG**	DK***
Tetrachloroethene	100	100	100
Trichloroethene	100	100	100
1,1-Dichloroethene	20	20	1 –5
1,2-Dichloroethene	150	150	–
Chloroethene	5	5	1 –5
Ethene	150	–	–
Tetrachloromethane	20	20	0.1–5
Trichloromethane	20	20	1 –5
Dichloromethane	150	150	100
Chloromethane	20	20	100
Methane	–	–	–
1,1,2,2-Tetrachloroethane	20	20	–
1,1,1-Trichloroethane	100	100	300
1,1,2-Trichloroethane	20	20	–
1,1-Dichloroethane	100	100	–
1,2-Dichloroethane	5	20	0.1–0.5
Chloroethane	150	150	300
Ethane	–	–	–

* : Stafbureau NER (1992) Nederlandse Emissie Richtlijnen, RIVM, Bilthoven.
** : Anonymous (1986).
*** : Stafbureau NER Personal Communication 31-1-1992.

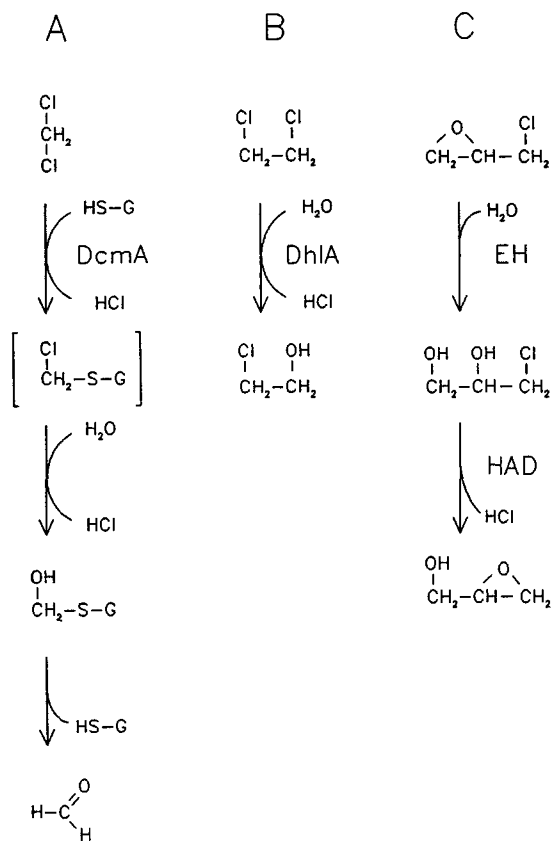


Fig. 1. Mechanisms for the dehalogenation of chlorinated hydrocarbons by bacterial cultures. *(A)* Dichloromethane dehalogenase (DcmA) converts its substrate in a glutathione dependent reaction; *(B)* 1,2-dichloroethane is hydrolyzed to 2-chloroethanol by haloalkane dehalogenase (DhlA); *(C)* epichlorohydrin is dehalogenated by a haloalcohol dehalogenase (HAD) after cleavage of the epoxide ring by an epoxide hydrolase (EH).

Glutathione transferases

Bacterial growth on dichloromethane has been shown in the beginning of the 1980s by Leisinger and co-workers (Stucki et al. 1981). Methylotrophs of the genera *Hyphomicrobium* and *Methylobacterium* dehalogenate dichloromethane to formaldehyde by a glutathione transferase reaction catalyzed by the dichloromethane dehalogenase (Kohler-Staub & Leisinger 1985). The inducible enzyme is a hexameric protein composed of 6 identical subunits with a molecular mass of 32 kD. Via a nucleophilic displacement reaction, glutathione and dichloromethane form chloromethylglutathione, an unstable intermediate that is hydrolyzed to hydroxymethylglutathione. This hemimercaptal was proposed to be cleaved to form formaldehyde and glutathione, but it can also be used as a substrate by some formaldehyde dehydrogenases (Harrington & Kallio 1960).

The dehalogenase constitutes about 16% of the total soluble protein of induced cells. It has an extremely low turnover number of 0.5 mol of dichloromethane per mol of enzyme subunit per second. The apparent K_m value for dichloromethane is 30 μM, so the low efficiency of the enzymatic process is not due to incompetence in substrate binding, but rather to a low reaction rate. In *Hyphomicrobium* DM2 growing on dichloromethane, the low specific activity of the dehalogenase is compensated by the high intracellular concentration of the enzyme. This strategy of the organism may indicate that dichloromethane dehalogenase represents a recently evolved enzyme whose catalytic activity is still in

the process of being optimized (Kohler-Staub & Leisinger 1985).

Induction of dichloromethane dehalogenase by its substrate is based on negative control by means of autoregulation of repressor synthesis. This mechanism ensures high levels of repressor protein under inducing conditions, thereby preparing the cell for immediate shut-down of enzyme synthesis when the supply of dichloromethane is depleted. In view of the high level of dichloromethane dehalogenase in induced cells such a mechanism seems indispensable (La Roche & Leisinger 1991).

In various dichloromethane-utilizing bacteria the enzyme is immunologically cross-reactive (Kohler-Staub et al. 1986). A six times higher turnover rate for dichloromethane was found with a new glutathione transferase isolated from a faster growing dichloromethane utilizer, strain DM11. The purified enzyme was only weakly cross-reactive immunologically with earlier studied glutathione transferases. In spite of the observed structural and kinetic differences, however, DNA probe methodology indicated potentially evolutionary relatedness between both groups of dichloromethane dehalogenases (Scholtz et al. 1988).

Hydrolytic dehalogenases

Halogenated aliphatics can be dehalogenated by two different types of haloalkane dehalogenase dehalogenases. The first one described was discovered in *Xanthobacter autotrophicus* GJ10, a N_2-fixing hydrogen bacterium isolated on 1,2-dichloroethane (Janssen et al. 1984, 1985; Keuning et al. 1985). The enzyme, a single polypeptide with a molecular mass of 35.1 kD, converts 1,2-dichloroethane into chloroethanol with formation of inorganic chloride. The enzyme has broad substrate specificity: more than 24 different halogenated aliphatics can be converted by the enzyme, among them environmentally important compounds such as 1,2-dibromoethane and 1,2-dichloropropane. Recently, three different *Ancylobacter aquaticus* strains able to grow on 1,2-dichloroethane were isolated (van den Wijngaard et al. 1992). In each of these strains haloalkane dehalogenase was synthesized constitutively. In strain AD25 this enzyme comprised about 35% of the total soluble protein. The nucleotide sequences of the haloalkane dehalogenase (*dhlA*) genes of these strains were the same as the sequence of *dhlA* from *X. autotrophicus* GJ10. Thus, until now only one enzyme that efficiently converts dichloroethane to 2-chloroethanol has been found. This, and the low turnover number with dichloroethane suggest that this dehalogenase is still in an evolutionary primitive state (van den Wijngaard et al. 1992).

One of the intermediates in the degradation pathway of 1,2-dichloroethane is 2-chloroacetate (Janssen et al. 1985). This and other (2-)halocarboxylic acids are converted by a second class of hydrolytic dehalogenases, the so-called halocarboxylic acid dehalogenases (Motosugi & Soda 1983). These enzymes hydrolyse compounds such as chloroacetic and 2-chloropropionic acid to yield glycolate and lactate, respectively. The latter compounds are excellent growth substrates, and can be degraded further by the central metabolism of most cells (Schlegel 1986).

Other hydrolytic haloalkane dehalogenases have been found in Gram-positive bacteria (Janssen et al. 1988; Scholtz et al. 1987; Yokata et al. 1987). These enzymes have activity for longer chain halogenated aliphatic compounds, but not for 1,2-dichloroethane. Furthermore, these enzymes are immunologically and biochemically different from the dehalogenases obtained from Gram-negative bacteria (Scholtz et al. 1987). The observation that the haloalkane dehalogenases in Gram-positive bacteria are inducible, while the haloalkane dehalogenases in Gram-negative bacteria are constitutively expressed suggests that these systems are futher evolved in Gram-positive bacteria than in their Gram-negative counterparts.

Haloalcohol dehalogenases

Aliphatic chloroalcohols such as chloroethanol are oxidized to chloroacetic acids before the dechlorination step occurs (Stucki & Leisinger 1983). With epichlorohydrin-utilizing bacteria, however, the pathway can proceed via a fundamentally different

route (van den Wijngaard et al. 1989). With epichlorohydrin (3-chloro-1,2-epoxypropane) the dechlorination takes place at the level of 3-chloro-1,2-propanediol by an intramolecular substitution reaction catalyzed by a haloalcohol dehalogenase, an enzyme also identified in other halopropanol utilizing organisms (Castro & Bartnicki 1968; Bartnicki & Castro 1969). The enzymes catalyzing these reactions are specific for vicinal haloalcohols and haloketones.

Immunological diferences between haloalcohol dehalogenases in Gram-positive and Gram-negative bacteria indicate that at least two different types of these enzymes are present in nature (van den Wijngaard et al. 1991). The haloalcohol dehalogenase involved in epichlorohydrin degradation is also known as halohydrin hydrogen-halide-lyase (Nagasawa et al. 1992). *Corynebacterium* N-1074 forms two non homologous forms of this enzyme, one of which offers potential for the biological production of optically active (*R*)-3-chloro-1,2-propanediol, an attractive intermediate with industrial applications (Nakamura et al. 1992).

Aerobic cometabolism

In cases of cometabolism (Horvath 1972), organisms do not grow on the compound of interest, but transform it fortuitously with enzymes having a broad substrate range. Cometabolic conversions catalyzed by bacterial mono- and dioxygenases have been described for several halogenated hydrocarbons (Table 2). Most work has been done with methanotrophic bacteria and with toluene oxidizers. From the results of these studies, a number of important conclusions can be drawn:

- Growth on a number of different carbon sources may be coupled to the cometabolic degradation of chlorinated hydrocarbons. Methane (Oldenhuis et al. 1989), propane (Wackett et al. 1989), propene (Ensign et al. 1992), phenol (Nelson et al. 1987), toluene (Nelson et al. 1987; Wackett & Gibson 1988), ammonia (Arciero et al. 1989), isoprene (Ewers et al. 1990), and isopropylbenzene (Dabrock et al. 1992) have been suggested. Table 5 lists the reported degradation rates observed with these substrates.
- The growth stimulating substrate must induce the synthesis of a suitable oxygenase enzyme. This can be a monooxygenase, as with methanotrophs (Little et al. 1988; Oldenhuis et al. 1989) and some toluene degraders (Shields et al. 1991), or a dioxygenase as with other toluene degraders (Wackett & Gibson 1988). Constitutive mutants

Table 5. Degradation of trichloroethylene by bacterial pure cultures that produce oxygenases.

Culture	V_{max}[1]	Conc.[2]	K_s[2]	V_{max}/K_s[3]	Substrate[4]	Reference
Alcaligenes eutrophus	0.2	25			phenol	Harker & Kim 1990
Escherichia coli with toluene	1–2	–	–	–	LB + IPTG	Winter & Ensley 1989
oxidation genes	1	100	–	–		Zylstra et al. 1989
Methylosinus trichosporium OB3b	580	–	145	4	methane	Oldenhuis et al. 1989
Methylomonas GJ6	0.001	100			methane	Oldenhuis 1992
Methylomonas MM2	12		4	3.2	methane	Henry & Grbic-Galic 1991
Nitrosomonas europaea	0.8	12	–	–	ammonia	Arciero et al. 1989
Pseudomonas cepacia G4	8	–	3	2.7	phenol	Folsom et al. 1990
Pseudomonas putida F1	1.8	80	–	–	toluene	Wackett & Gibson 1988
Rhodococcus erythropolis JE77	0.15	–	19	0.008	isoprene	Ewers et al. 1990
Strain 46-1	0.3	1–2	–	–	methane	Little et al. 1988
Xanthobacter Py2	8.6	100	–	–	propene	Ensign et al. 1992

[1] nmol/min/mg protein.
[2] μM.
[3] l/min/g protein.
[4] substrate for growth of the culture and induction of the catabolic enzyme.
LB = Luria broth; IPTG = isopropyl-b d-thiogalactopyranoside.

(Shields & Reagin 1992) or recombinant strains (Winter et al. 1989) could also be applied.

- The monooxygenase reactions require electrons which must be generated from an added cosubstrate. It is preferable not to use the natural substrate for the generation of these reducing equivalents, because competition of the chlorinated hydrocarbon with the growth substrate for the active site of the oxygenase may inhibit degradation. Thus, with methanotrophs, formate was found to be more efficient than methane or methanol (Oldenhuis et al. 1989). Recently it was proposed that lipid storage granules may serve as an endogenous source of electrons for the cometabolic oxidation of halogenated compounds in methanotrophs (Henry & Grbic-Galic 1991). The microbial ability to form endogenous reserves may have significance in the development of air treatment systems where the source of electrons is added intermittently, or in a two-stage system with cell recycle.
- Cometabolism gives no selective advantage, therefore other conditions to specifically stimulate the required organisms are needed. This may be the addition of growth substrate. However, both with toluene (Nelson et al. 1988) and methane (Oldenhuis et al. 1989) oxidizing organisms it has been found that the capacity to degrade chlorinated compounds is not a general phenomenon but a property specific for some strains. In fact, the organisms that catalyze cometabolic conversions of halogenated compounds are at a disadvantage because of the aforementioned consumption of reducing equivalents by the monooxygenase reaction. Thus, in the presence of e.g. trichloroethene there will be a selective advantage for organisms that do not catalyze cometabolic reactions, such as methanotrophs that do not form soluble methane monooxygenase (see below) or toluene oxidizers that degrade toluene via a pathway in which toluene is first oxidized to benzoate.
- Mineralization cannot always be expected. The fate of compounds that are converted by cometabolism is dependent on the chemistry of the degradation products. As a consequence, toxic products may be formed in the cell and accumulate. Product toxicity thus has been found in several cases (Oldenhuis et al. 1989; Wackett & Householder 1989). In the case of product toxicity due to trichloroethene metabolism in *Pseudomonas putida* F1, this problem could be overcome by transferring the genes encoding for the toluene dioxygenase to *E. coli*, where trichloroethene was subsequently converted without any toxic effects to the host organism (Winter et al. 1989). There are hopes (Hoyle 1992) that this organism will be the first genetically engineered organism to obtain EPA approval for field-testing in contained reactors.

The above factors strongly influence the process design of cometabolic transformation processes to specific applications. With compounds such as chloroform and trichloroethene, product toxicity is very high, limiting the amount of chlorinated hydrocarbon that can be removed by methane oxidizing bacteria to about 0.5 mmol/g of newly cultivated cells (Oldenhuis et al. 1991). A model to describe the effects of products toxicity and competitive inhibition on the removal of these substrates in batch and continuous culture has been described (Alvarez-Cohen & McCarty 1991a,b). The results indicate that cometabolic transformation may be useful for the removal of low concentrations, but less applicable for high concentrations of waste components, such as often occur in industrial effluents.

In methanotrophic bacteria, at least two classes of methane monooxygenases (MMOs) can be distinguished on the basis of their intracellular location (Dalton et al. 1984). All methanotrophs tested are able to form a particulate or membrane-bound enzyme (pMMO), whereas only some cultures such as *Methylosinus trichosporium* OB3b are capable of producing soluble type of MMO (sMMO) that has a broader substrate range when copper becomes limiting. Trichloroethene is only degraded at significant rates by *M. trichosporium* cells when sMMO is expressed (Oldenhuis et al. 1989; Tsien et al. 1989). Repression of the formation of sMMO by elevated levels of copper may affect the applicability of this type of conversion for bioremediation of polluted groundwater (Phelps et al. 1992), but is less problematic for its applicability in air treatment systems.

The kinetically best studied example of toluene

and phenol degrading organisms with potential for the degradation of halogenated aliphatics is strain G4, identified as a *Pseudomonas cepacia* (Nelson et al. 1986; Folsom et al. 1990; Shields et al. 1991). Its V_{max} values are not as high as those in *M. trichosporium* OB3b cells that contain sMMO, but the value of V_{max}/K_m (or first order rate constant), which determines conversion at low substrate concentration ($[S] << K_m$), is about the same. The higher V_{max} of methanotrophs is not of very high practical significance, since conversion of trichloroethene will be extremely lethal for these organisms. In *P. cepacia* G4 this apparently poses less of a problem (Dolfing et al. manuscript in preparation). Shields and Reagin (1992) have obtained a stable *P. cepacia* G4 mutant that constitutively expresses the toluene monooxygenase that is responsible for the cooxidation of trichloroethene. The availability of this strain offers a solution to many of the problems listed above.

Another promising line of research is the cloning of the toluene monooxygenase gene (TMO) from *Pseudomonas mendocina* into *E. coli* where it resides under the control of the powerful *E. coli* stress promotor groEL. The groEL promotor is induced in reponse to a variety of stress conditions including carbon, nitrogen, and phosphorus starvation. When grown under nitrogen, phosphor, or glucose limitation *E. coli* strain AMS66:pDL3(pGroEL:TMO) showed significant biodegradation. Little and coworkers (1991) report that the TCE degradation rates were comparable to the highest rates published for strains containing toluene catabolic genes. In related research it was observed that various halogenated compounds elicit the synthesis in *E. coli* of (unique) sets of stress proteins (Blom et al. 1992). A better understanding of this phenomenon may further the possibilities to construct strains where the halogenated compounds themselves induce the monooxygenase genes that degrade them.

Ammonia monooxygenase containing cells like *N. europaea* are less suitable for application in biological treatnment systems because the rates of conversion of halogenated aliphatic compounds are extremely low (Arciero et al. 1989).

Table 6 lists the degradation products of a number of chlorinated aliphatic hydrocarbons detected after exposure of these compounds to cells of *M. trichosporium* OB3b and *Methylomonas* strain GJ6 (Oldenhuis et al. 1989; Newman & Wackett 1991; Oldenhuis 1992). More than 90% of the substrate was dechlorinated in these experiments. The nature of the degradation products depends on the type of enzyme to which the chlorinated compounds are exposed. Exposure of trichloroethene to *Methylosinus* or *Methylomonas* results in the formation of chloride, trichloroethanol, trichloroacetaldehyde and trichloroacetate (Table 6), while exposure to *Methylocystis* results in the formation of trichloroacetate and dichloroacetate as chlorinated organic products (Nakajima et al. 1992). Little et al. (1988) on the other hand, using strain 46–1 found mainly dichloroacetate as the chlorinated product, and glyoxylate and CO_2 as non-chlorinated products. These results are again different from those obtained by Li and Wackett (1992) who found formic acid and glyoxylic acid as the major oxidation products, and could not detect chlorinated organics as degradation products of trichlorethene oxidation by the toluene dioxygenase of *P. putida* F1, overproduced in *E. coli*. The observations point to the formation of TCE-dioxetane and 1,2-dihydroxy-trichloroethane in dioxygenase catalyzed oxidation versus the formation of TCE-epoxide and trichloroacetaldehyde in monooxygenase catalyzed oxidation reactions. These differences are not only of academic interest but have also implications for the safe use of bacteria in bioremediation systems,

Table 6. Products identified after incubation of chlorinated hydrocarbons with *M. trichosporium* OB3b or *Methylomonas*GJ6*.

Substrate	Product
Trichloroethene	chloride, trichloroethanol, trichloroacetaldehyde, trichloroacetic acid,
cis-1,2-Dichloroethene	chloride, *cis*-1,2-dichloroethene epoxide
trans-1,2-Dichloroethene	chloride, *trans*-1,2-dichloroethene epoxide
1,2-Dichloropropane	chloride, 1,2-dichloro-3-propanol
1,1,1-Trichloropropane	trichloroethanol

* Data from Oldenhuis et al. 1989, Newman & Wackett (1991), and Oldenhuis 1992.

where complete mass balances are essential, and complete dechlorination is preferred.

Anaerobic biodegradation

Ambient air at a temperature of 25 °C contains about 8 mmoles of oxygen per liter. Gaseous waste streams will seldom contain more than 32 mmoles of reducing equivalents (about 1 mmol of benzene or toluene) per liter of air and therefore aerobic degradation will prevail in most treatment processes. Mass transfer limitation can however cause oxygen limited zones in complex biofilms even though the biofilms occur in otherwise aerobic environments (Dalsgaard & Revsbech 1992), and it is tempting to speculate that the right combination of differences in diffusion coefficients and microbial activities can cause and indeed be used to create anaerobic conditions in aerobic waste gas treatment systems. Currently no proven techniques are available for effective separation of aerobic and anaerobic zones, which would be required for effective reductive dechlorination processes in an air treatment system. Such techniques could be applied for the biodegradation of e.g. tetrachloroethene containing air streams.

As stated before, tetrachloroethene has never been found to be biodegradable under aerobic conditions, but complete anaerobic dechlorination of tetrachloroethene to ethene and ethane has now been reported by two groups (de Bruin et al. 1992; DiStefano et al. 1992). Ethene and ethane can subsequently be degraded under aerobic conditions (Hartmans et al. 1989). It should, however, not be forgotten that reductive dechlorination processes require reducing equivalents (Dolfing & Tiedje 1986). If no additional carbon source is added the availablity of reducing equivalents could be a problem for the anaerobic organisms in a hypothetical anaerobic-aerobic degradation of tetrachloroethene, because ethene and ethane are not known to be biodegradable under anaerobic conditions, and it is not advantageous for aerobic bacteria to excrete intermediates from the degradation pathway of ethene and ethane that could serve as source of reducting equivalents for the anaerobic dechlorinating organisms.

Other interesting substrates for combined anaerobic/aerobic degradation would be the highly chlorinated benzenes. These compounds too have never been shown to be biodegradable under aerobic conditions, but they are biodegradable under anaerobic conditions (Fathepure et al. 1988; Holliger et al. 1992). Benzene can be mineralized under both aerobic and anaerobic conditions (Edwards & Grbic-Galic 1992), and the reducing equivalents that are liberated by the anaerobic 'food chain' can probably be recycled back to the organisms that perform the dechlorination reaction. For the time being, these schemes are still theoretical.

Above we have stressed the (im)possibilities to anaerobically degrade chlorinated compounds via microbially catalyzed reductive dechlorination mechanisms. In these processes the carbon-halogen bond serves as electron acceptor (Vogel et al. 1987; Dolfing & Harrison 1992). The current idea is that these reactions are carried out by specialized anaerobes that catalyze only the dechlorination steps, and require an exogenous source of reducing power. In this perception, complete mineralization of the halogenated compound requires the presence of a series of microorganisms that sequentially carry out the various steps in consortia that resemble food chains and food webs (Dolfing & Tiedje 1986; Dolfing 1988). Recently, however, a variation on this theme has been described in that a bacterium (strain MC) was isolated that can grow on the fermentation of methyl chloride plus CO_2 to acetate and HCl (Traunecker et al. 1991). Thus, no external supply of reducing equivalents is necessary for the growth of this homoacetogen. The growth rate of the organism ($\mu = 0.023\ h^{-1}$) is in the same range as the growth rate of its aerobic counterpart ($\mu = 0.09\ h^{-1}$).

Another example of cultures that can grow with a chlorinated aliphatic compound as sole significant source of carbon and energy are the dichloromethane-grown enrichment cultures described by Stromeyer et al. (1991) and by Freedman and Gossett (1991). Reductive dechlorination is probably of marginal importance in these cultures. The hypothesis is that dichloromethane is hydrolysed to for-

maldehyde (Stromeyer et al. 1991) in a glutathione-dependent reaction, analogous to the reaction catalyzed by dichloromethane dehalogenase in aerobic organisms (La Roche & Leisinger 1990).

Causes of recalcitrance

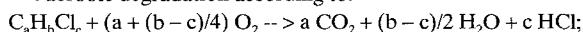
Thermodynamics

As already indicated above, the carbon-halogen bond per se is not xenobiotic. Thus, there must be other reasons why certain halogenated compounds

Table 7. Gibbs free energy of formation values of selected halogenated aliphatic compounds, and the amount of energy released when these compounds are completely mineralized with either O_2 or CO_2 as final electron acceptor*.

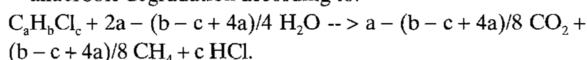
Compound		ΔGf° (kJ)**	ΔG°′ (kJ/reaction)	
			aerobic	anaerobic
methanes				
CH_4		- 50.83	- 812.7	-
CH_3Cl		- 58.45	- 738.6	- 126.0
CH_2Cl_2		- 68.87	- 661.7	- 253.3
$CHCl_3$		- 70.12	- 594.0	- 389.8
CCl_4		- 53.56	- 544.1	- 544.0
ethanes				
C_2H_6		- 32.80	- 1456.4	- 435.4
C_2H_5Cl		- 60.50	- 1362.1	- 545.3
$C_2H_4Cl_2$	(1,1)	- 73.30	- 1282.8	- 670.2
	(1,2)	- 73.85	- 1282.3	- 669.7
$C_2H_3Cl_3$	(1,1,1)	- 76.19	- 1213.5	- 805.1
	(1,1,2)	- 77.49	- 1212.2	- 803.8
ethenes				
C_2H_4		68.24	- 1319.7	- 67.5
C_2H_3Cl		51.51	- 1236.5	- 215.5
$C_2H_2Cl_2$	(*trans*)	26.57	- 1145.0	- 328.2
	(*cis*)	24.35	- 1142.7	- 325.9
	(1,1)	24.18	- 1143.1	- 326.3
C_2HCl_3		19.87	- 1071.8	- 459.2
C_2Cl_4		20.50	- 1006.0	- 597.6

* : aerobic degradation according to:



$C_aH_bCl_c + (a + (b - c)/4)\ O_2 \rightarrow a\ CO_2 + (b - c)/2\ H_2O + c\ HCl$;

anaerobic degradation according to:



$C_aH_bCl_c + 2a - (b - c + 4a)/4\ H_2O \rightarrow a - (b - c + 4a)/8\ CO_2 + (b - c + 4a)/8\ CH_4 + c\ HCl$.

** : The free energy of formation values of the halogenated compounds are from Dean (1985), the values for the other compounds are from Thauer et al. (1977) and Stumm & Morgan (1981).

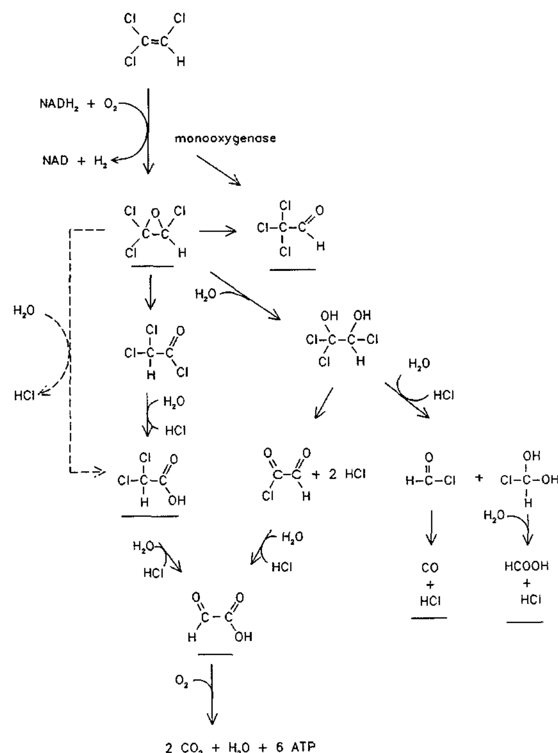


Fig. 2. Conversions of trichloroethene by a monooxygenase reaction. The primary product is trichloroethene epoxide, which decomposes chemically to a range of products. The ratio of the various products is pH dependent. The underlined compounds have been identified in bacterial and eukaryotic monooxygenase reactions (Henschler 1985; Little et al. 1988; Nakajima et al. 1992). The broken arrow indicates a hypothetical conversion of trichloroethene epoxide to dichloroacetic acid, a compound that can be used as a carbon and energy source by various bacteria. Such a conversion could thus detoxify the reactive epoxide, prevent formation of reactive intermediates, and allow growth on TCE.

are resistent to biodegradation, and why growth at the expense of compounds such as trichloroethene has never been observed. One explanation that is sometimes given is the thermodynamics of the degradation process. It has been argued that the degradation of compounds like tetra- and trichloroethene does not yield sufficient energy to allow microbial growth with these compounds as sole carbon and energy source (Witholt et al. 1989). Table 7 shows that this is not necessarily the case. Aerobic mineralisation of these compounds yields amounts of energy that are comparable to the amount of energy available from the degradation of vinylchlo-

ride, a compound on which growth is possible (Hartmans & de Bont 1992). Aerobic growth on other halogenated compounds is also thermodynamically feasible, and recalcitrance probably is caused by the absence of biochemical pathways that allow fixation of the energy in cell components. There is, however, selective pressure towards the development of organisms that can use, for example, trichloroethene (TCE) as sole carbon and energy source. This can be illustrated by the following line of reasoning (Fig. 2). Dichloroacetate is one of the products that has been found during the degradation of trichloroethene, and microbial growth on dichloroacetate has been reported. The conversion of TCE to dichloroacetate by established routes requires the investment of one NADH for the conversion of TCE to TCE-epoxide, while the steps from TCE-epoxide to dichloroacetate do not need additional 'energy'. Dichloroacetate has been shown to be degradable by halidohydrolases (Hardman & Slater 1981; Weightman & Slater 1980). The reaction product is glyoxylate. According to Schlegel (1986) two moles of glyoxylate can be activated to one mole of 3-phosphoglycerate. This costs 2[H] and 1 ATP. The subsequent conversion of 3-phosphoglycerate into acetylCoA results in the formation of 1 ATP and 1 NADH. Thus the conversion of two moles of glyoxylic acid results in the formation of one mole of acetylCoA. Oxidation of acetylCoA results in the formation of 3 NAD (P)H, 1 ATP and 1 FAD. This is equivalent to 12 ATP. The conclusion is that the dichloroacetate pathway of TCE degradation can lead to the formation of 3 moles of ATP per mole of TCE mineralized, which should be enough to support microbial growth with TCE as carbon and energy source, even when one takes into consideration that these 3 moles of ATP per mole of TCE are a maximum, as some glyoxylate and acetylCoA are required for the synthesis of the tricarboxylic acid cycle intermediate malate (Schlegel 1986). Obviously, this pathway will only occur in bacteria which have the enzymes of the glyoxylate pathway.

Clearly, there are some obstacles that microorganisms must overcome before growth on trichloroethene via a dichloroacetate pathway will be practically feasible. For example, dichloroacetate (in this example) must be the major, or even better, the only product from trichloroethene metabolism. Also, the formation of toxic intermediates must be minimized in order to avoid excessive high costs of the repair of damaged cell components. There are, however, no fundamental reasons why microorganisms should not be able to overcome problems like these under the right selection pressure.

Enzymology

Apart from unfavorable environmental conditions as they can occcur in nature, the simplest reason why a compound would be recalcitrant is probably the absence of enzymes to degrade these compounds. The chemical structure of compounds such as tetrachloroethene and carbon tetrachloride do not offer an enzyme a 'weak spot' in their structure where oxidative enzymes can easily attack.

Tetrachloroethene and carbon tetrachloride are also not known to be natural compounds and hence it is not likely that catabolic enzymes have evolved that allow organisms to use these compounds as growth substrates. This line of reasoning does not always hold, however. 1,2-Dichloroethane is also not a natural compound. Industrial production on a large scale started only some 70 years ago, but organisms that can degrade 1,2-dichloroethane can be isolated from the environment (van den Wijngaard et al. 1992). Apparently, there was sufficient selective pressure towards the development of enzymes that can degrade this compound and funnel the products into existing catabolic routes. An alternative, less likely explanation is that 1,2-dichloroethane is degraded fortuitously by an existing pathway. Interestingly, the dehalogenase that is responsible for the first dehalogenation step in the degradation pathway of 1,2-dichloroethane is identical in all the organisms tested so far. Genetic studies suggest that these organisms may have obtained the *dhlA* gene encoding for the haloalkane dehalogenase by horizontal gene transmission (van den Wijngaard et al 1992). There are indications that in *Xanthobacter autotrophicus* GJ10 this gene is localized on a large plasmid with a size of about 200 kb (Tardif et al. 1991).

A second example where the line of reasoning that a halogenated compound is not known to be formed in nature and is therefore not biodegradable does not hold is the anaerobic degradation of tetrachloroethene: Holliger (1992) recently described a bacterium, *Dehalobacter restrictus*, that couples growth to the reductive dechlorination of tetrachloroethene to trichloroethene and *cis*-1,2-dichloroethene. These examples illustrate that it is the structure of the compound rather than the question whether or not a compound is xenobiotic that determines its biodegradability.

A cause of the absence of specific catabolic enzymes can be the lack of induction by the xenobiotic compound. Examples are trichloroethene and other compounds that are degraded cometabolically. Cometabolic degradation of trichloroethene by methanotrophs or toluene degraders is true cometabolism in that this conversion is completely fortuitous and does not result in the formation of biomass. On the contrary, it costs the organisms energy in the form of reducing equivalents needed by the monooxygenase.

There is, however, also a possible scenario in which enzymes, induced by one substrate, allow the organism to derive carbon and energy from a second compound that cannot serve as a growth substrate. It has been argued that this may be common in natural ecosystems in which a variety of related molecules are present at low concentrations. In such situations, evolution of a specific induction system for the degradation of each compound would be inefficient, whereas induction of a non-specific enzyme by one component of the mixture would allow the organism to derive carbon and energy from a suite of related compounds (Haigler et al. 1992). This hypothesis can be useful in the design of a strategy to identify and isolate bacteria that can degrade halogenated compounds without using them as growth substrate.

Nothing is known about a third potential factor that may also cause recalcitrance, viz. failure of an organism to transport chlorinated hydrocarbons into the cell. It is generally tacidly assumed that uptake of this class of lipophilic compounds proceeds by passive diffusion, although it is known that the cell membrane may act as a barrier for the permeation of hydrophobic compounds (Parsons et al. 1987). The only well studied example of microbial uptake of a chlorinated compound is the energy dependent uptake system for 4-chlorobenzoate in the coryneform bacterium NTB-1 (Groenewegen et al. 1990).

Toxic intermediates

Some chlorinated aliphatic compounds are recalcitrant because of the formation of toxic intermediates. A well-known example is the degradation of trichloroethene by methanotrophic bacteria. Here, conversion products, probably TCE epoxide or acylchlorides produced after hydrolysis of TCE epoxide, are formed that react with other cell components (Oldenhuis et al. 1991).

Henschler (1985) has reviewed the chemical and biological reactivity of halogenated aliphatics and their proposed degradation products. Based on these studies it is currently not possible to answer the question whether the observed toxicity of TCE degradation products is caused by the epoxide or by acylchloride(s). A way around the toxicity problem would be to work with organisms that have enzymes to degrade these toxic intermediates at high rates. This line of reasoning was followed by Knackmuss and coworkers (Ewers et al. 1990) who worked with isoprene degrading organisms for the removal of TCE, since isoprene shows some structural resemblance to TCE. The idea was that these organisms probably possess an epoxidase to remove the toxic chlorinated epoxides that are formed in the initial step of the degradation pathway of trichloroethene. This idea worked indeed in that isoprene degraders do oxidize TCE without poisoning themselves with toxic intermediates (Ewers et al. 1991). It is not clear, however, whether this is because of the presence of an active epoxidase or because the cells degrade TCE at such a low rate that the formation of new cell material more than makes up for the cell material that is destroyed by the chloroepoxide.

Another example of the formation of toxic intermediates that hinder degradation is the conversion of chlorobenzene via the meta cleavage pathway of chlorocatechol. Productive chlorocatechol degra-

dation proceeds via the so-called ortho cleavage pathway where the chlorocatechol intermediates undergo an intradiol cleavage by a nonspecific catechol 1,2-dioxygenase (Reineke & Knackmuss 1984). Since chloroaromatic compounds cannot be efficiently metabolized via the meta cleavage routes, toxic chlorocatechols accumulate and inhibit the ring cleavage enzyme (Bartels et al. 1984; Klecka & Gibson 1981). Thus, the induction of the appropriate route is important for efficacious degradation of chloroaromatics. This can be a problem when mixtures of chlorinated and nonchlorinated aromatics are present that induce the expression of the two pathways simultaneously.

Treatment systems

Biofilters

The oldest and most simple treatment method for contaminated gases is the biofilter. This is composed of a container filled with a suitable packing material such as compost and amendments. Addition of microorganisms may be useful for rapid acclimation and startup. Treatment of halogenated compounds is more complicated than treatment of biologically produced odorous substances, such as occur in wastewater treatment emmissions or the food processing industry, for which these systems are traditionally used.

An essential feature of the biodegradation of chlorinated compounds is the production of inorganic chloride. In unbuffered systems, this will cause a drop of the pH, whereas buffering with alkali will cause accumulation of salt. A static biofilter is not mixed, and therefore acid or salt (in case of the presence of solid buffer) will accumulate. Although rinsing may temporary relieve the inhibitory effect caused by this, it is clear that the biofilter is not the optimal treatment system for components that leave non-volatile products behind (Ottengraf et al. 1986; Ottengraf 1987).

The use of a liquid phase in the reactor allows mixing, pH control, and removal of waste products, including a surplus of biomass if it is produced. There are currently two systems on the market that can do this: the trickling filter and the bioscrubber.

Trickling filters

In trickling filters, gas to liquid mass transfer and biodegradation take place in the same compartment, allowing strong gradients of the compound to be degraded between both phases. Therefore, trickling filters are the system of choice for poorly water soluble components, which are otherwise difficult to transfer to the liquid phase. In a trickling filter microorganisms are immobilized on a carrier material much like in a compost filter (Ottengraf 1987; Kirchner et al. 1991, 1992). The main difference is that water is continuously trickled over the filter bed. Excess chloride and detaching cells are continuously removed from the filter bed. This system has proven its reliablity in waste water treatment, and has been sucessfully adapted for air treatment. The essential difference between the two fields of application is, of course, waste water versus air, although in waste water treatment too a gaseous substrate, oxygen, has to make a phase transfer step from the air into the liquid phase. Phase transfer can easily become rate limiting in such systems, especially for compounds with a high Henry coefficient (the Henry coefficient is defined as the equilibrium ratio of the concentration of a compound in the gas phase versus the water phase). Generally, compounds are deemed suitable for biodegradation in a trickling filter if the dimensionless Henry coefficient is below 0.01–0.05. Table 8 gives the Henry coefficients for a number of chlorinated hydrocarbons. Hartmans & Tramper (1991) and Diks (1992) have shown that even a compound with a Henry coefficient of 0.1, dichloromethane, can be efficiently degraded in a trickling filter. Diks (1992) inoculated a trickling filter with a dichloromethane grown culture of *Hyphomicrobium* GJ21. The biological degradation process in the biolayer rather than the gas-liquid mass transfer was the rate limiting step in the overall elimination process. An evaluation of the economic aspects of the system showed that the costs of the trickling filter are in the same range as those re-

Table 8. Dimensionless Henry's law constants and solubilities of some organic compounds.

	H_c^1	S^2	S^3
Benzene	0.22	22.8	
Ethylbenzene	0.32	1.4	
Toluene	0.27	5.6	
o-Xylene	0.20	1.6	
m-Xylene	0.28	1.5	
p-Xylene	0.29	1.7	
Tetrachloroethylene	0.72	0.8	5
Trichloroethylene	0.39	8.4	8
1,1-Dichloroethylene	1.07	4.2	25
cis-1,2-Dichloroethylene	0.17	36.1	35
trans-1,2-Dichloroethylene	0.38	65.0	31
Chloroethylene	1.14	43.2	13
Ethylene	8.75	0.8	11
Tetrachloromethane	1.24	7.5	
Trichloromethane	0.15	66.2	
Dichloromethane	0.09	228	
Chloromethane	0.36	106	
Methane	27.2	1.5	
1,1,2,2-Tetrachloroethane	0.019	17.9	
1,1,1-Trichloroethane	0.70	5.4	
1,1,2-Trichloroethane	0.048	33.1	
1,1-Dichloroethane	0.23	51.5	
1,2-Dichloroethane	0.044	87.9	
Chloroethane	0.46	88.0	
Ethane	20.4	2.0	

[1] Dimensionless Henry's constant at 25° C (Gossett 1987; Mackay & Shiu 1981).
[2] Aqueous solubility in mM at 25° C (Mackay & Shiu 1981).
[3] Aqueous solubility in mM at 23° C (Fox et al. 1990).

ported for other waste gas purification techniques (Diks 1992).

There are a number of ways to enhance mass transfer from the gas phase to the liquid phase, the simplest being a high recirculation rate of liquid medium through the reactor. The objective of a high recirculation rate is to promote phase transfer processes via a high turbulence. Of course this recirculation process costs energy so there is an optimum somewhere. Interestingly it was recently proposed to recirculate the liquid intermittently (Wolff 1992). The idea is that as a result of this approach most of the time only a very thin liquid layer is present on the surface of the biomass layer, thus shortening the distance that gases have to diffuse from the gas phase to the surface of the biolayer.

Another factor that can be varied is the way the gas is led through the reactor: counter-current to the liquid stream or co-current with the liquid stream. Theoretically co-currently should be better, because of the liquid recycle system employed on trickling filter systems. In a counter-current system just before the liquid comes into contact with the inlet gas just before it is recycled to the top of the reactor. Thus the liquid leaving the filter bed will be in equilibrium with the (high) inlet gas concentration. In the upper part of the filter it then comes into contact with the treated gas stream with only a low amount of substrate left. Thus the liquid will return some of the substrate to the effluent gas (Diks & Ottengraf 1991a).

Numerical modeling as well as experimental results show, however, that at least for dichloromethane the conversion effficieny of a trickling filter is virtually unaffected by the relative flow direction of the mobile phases, probably because the recirculation of the liquid phase has a smoothing effect on the axial liquid gradients (Diks & Ottengraf 1991a,b). The liquid recycle rate in these experiments was 1000 to 8000 times the bed volume per hour! Hartmans & Tramper (1991), on the other hand, studied dichloromethane removal in a trickling filter in which the recirculation rate was only 3 to 15 times per hour the bed volume.

Bioscrubbers

An alternative to the biofilter systems are the biowashers or bioscrubbers. These systems consist of two units: a scrubber where the contaminants are absorbed in the water phase, and a bioreactor, generally an activated sludge unit, where the compounds are biodegraded. Bioscrubbers are especially well suited for the treatment of waste streams with higher concentrations of chlorinated hydrocarbons, since the mass transfer and the degradation occur at different places. Thus, the wash column and the bioreactor can be optimized separately (Kok 1992). Bioscrubbers are especially well suited for the removal of compounds with a relatively low partition coefficient ($H < 0.01$–0.05) (Diks 1992).

Modifications of these techniques

An interesting variation on the conventional biowashers is the addition of a solvent to enhance the solubility of the waste gas in the liquid phase (Schippert 1989). For a succesful application, the organic solvent must be inmiscible with water, have a low equilibrium gas concentration, and of course a high partition coefficient for the pollutants. Organic solvents used for this purpose are mostly silicone fluids or phthalates (Diks 1992).

A three-phase fluidized bed reactor has been used succesfully for the removal of dichloromethane from waste gas. In this system microorganisms are immobilized on inert carrier material which is kept fluidized by an injected gas flow. Very high volumetric loading rates can be applied in such systems, but fluidization of the carrier material costs too much energy to be economically atttractive (Stucki 1989).

Contaminants with a relatively low water solubility can also be effficiently removed with membrane bioreactors. Here the idea is to use hydrophobic microporous membranes for efficient mass transfer from the gas phase to the liquid phase (Hartmans et al. 1992). The organic compounds as well as oxygen are transferred from the gas phase to the membrane. After penetrating the membrane, both compounds are degraded by microorganisms which are either immobilized on the membrane or present in the bulk of the liquid.

Behaviour of microorganisms in treatment systems

Microorganisms are the biocatalysts in waste gas treatment systems. Their presence and activity determine to a large extent the overall performance of the treatment system and hence it is vital to engineer the system in such a way that the microorganisms can do the job optimally. The most obvious requirement for an efficient bioreactor is that the microorganisms are present at sufficient cell densities to degrade the added contaminants. Immobilisation is the most widely used technique to achieve this objective and waste gas treatment is no exception to this. In trickling filters, a wide variety of carrier materials are used to obtain microbial immobilisation. Experience shows that microorganisms will generally stick to most materials, although the time needed to develop a biolayer can vary widely. In the last decades much research has been done into the factors and the mechanisms that govern colonisation and biolayer formation. For applied purposes, we nevertheless still have to rely on trial and error approaches (Diks 1992).

Similarly, it has been said that the behavior of immobilized microorganisms is frequently different from the behavior of the same microorganisms in free living form (van Loosdrecht et al. 1990; Fletcher 1991). Currently, not enough is known about the ecophysiology of microorganisms in waste gas treatment systems to make any pertinent predictions on the relevance of this phenomenon. It is clear that microorganisms in biolayers experience different conditions from those in free living form. Steep gradients in pH and oxygen concentration are two of the most obvious factors (Kuenen et al. 1986; Revsbech & Jørgensen 1986). Important factors in this context are the thickness of the biolayer and the specific activity of the cells. The latter determines the steepness of the substrate gradient and thus determines whether the organisms at the inside of the layer encounter the substrate. Overall biofilm effectivity will also be influenced by local pH gradients in the biofilm, but pH measurements by De Beer (Ottengraf & Diks 1992) in a dichloromethane degrading biofilm of *Hyphomicrobium* suggest that this problem is merely academic as long as the bulk pH is kept close to the optimal pH of the system.

One of the consequences of biolayer formation is that it can cause clogging of the reactor. Especially when the size of the carrier material is too small, the microorgansims can bridge the gap between particles and glue them together. This causes an increase in the pressure drop over the filter bed and a decrease in the surface of the biolayer, resulting in an increase in the mass resistance through the biolayer. Systematic knowledge on these phenomena is lacking (Diks 1992). The growth rate of microorganisms in trickling filters is determined by the rate at which biomass is wasted and microorganisms are sloughed of. This sloughing is determined by the liq-

uid recirculation rate. The potential biomass density in the reactor is determined by the loading rate of the system and the growth yield of the organism. Currently, no models are available where the interplay between these parameters are worked out for a few model compounds. For practical purposes, it may be interesting to operate systems where clogging problems are experienced under a limitation other than carbon. Under such conditions, the microorganisms may exhibit high carbon conversion activities without forming much biomass.

The development of bioreactors for air purification is a relatively young branch of bioprocess engineering. As recently as 1989 the group of Rehm felt compelled to state that 'purification plants are generally designed on the basis of experiments which are carried out on site using pilot plants. There are either insufficient design and material data or no data at all'. This prompted Rehm and co-workers to study the fundamentals of a trickle-bed bioreactor. These studies were focussed on the concentration ranges where separation efficiency was dominated by external mass transfer (Kirchner et al. 1989). In their experiments the pollutant concentration in the liquid phase was nearly zero at lower mass flow rates (< 350 $g/m^3/h$), indicating that mass transfer through the liquid film was the rate-determining step (Kirchner et al. 1992).

Most of the work published on the removal of halogenated compounds from off gases has been done with dichloromethane and trichloroethene. Trickle-bed bioreactors operated with dichloromethane turn out to be very robust. A reactor system with a volume of 66 l operated by Hartmans & Tramper (1991) was inoculated with *Hyphomicrobium* strain DM20 and run for a period of three months with dichloromethane loading rates varying from 1.9 to 4.8 g h/h, corresponding to inlet dichloromethane concentrations of 0.7 to 1.8 g/m^3. Under these conditions dichloromethane conversions of 80 to 95% were achieved. In the course of the experiments a new unidentified pink methylotrophic dichloromethane degrading bacterium, designated strain DM21, gradually became the dominant organism in the reactor. Interruptions of several days in the dichloromethane supply had hardly any effect on the system. Once the dichloromethane supply was restored, the original dichloromethane-elimination efficiency was regained very rapidly. Furthermore, the system was able to recover from a pH shock of pH 3.5 within several days. Variations in the temperature between 18 and 30 °C hardly affected the dichloromethane elimination efficiency under conditions where degradation was mass transfer limited. Apparently, the temperature dependency of the diffusion coefficient which results in a higher mass transfer resistance at lower temperatures is compensated by the increased solubility of dichloromethane at lower temperatures, resulting in an increased driving force for mass transfer. The system as operated by Hartmans was able to bring the dichloromethane concentration in (synthetic) waste gas down to below the maximal allowable concentration of 150 mg/m^3 required by German and Dutch laws.

Diks & Ottengraf (1991a,b) also studied the removal of dichloromethane from waste gases using a biological trickling filter. These authors state that 'it is commonly thought that the application of trickling filters is restricted to compounds which show at least moderate water solubility'. In their paper it is shown that for dichloromethane, in spite of being poorly water-soluble, the biological reaction instead of the gas-liquid mass transfer is the rate-limiting step. The trickling filter was inoculated with *Hyphomicrobium* strain GJ21, but after several months of operation the biomass in the reactor consisted also of several other strains of bacteria and 'a multitude of higher organisms like flagellates, ciliates, nematodes etc.' (Diks & Ottengraf 1991b). Possibly as a result of the establishment of this true food chain, production and consumption of biomass in the filter were in equilibrium and no biomass had to be discharged. The biomass in the trickling filter had a specific activity for dichloromethane (DCM) of 0.08 g DCM/g TSS h/h^{-1} (TSS, total suspended solids), versus 0.64 g DCM/g TSS h^{-1} for *Hyphomicrobium* in pure culture. Just like Hartmans & Tramper (1991), Diks & Ottengraf found that the temperature hardly affects the overall performance of the system up to a gas inlet concentration of 8 g/m^3.

TCE is only degraded cometabolically. This obviously has consequences for the design and oper-

ation of bioreactors for TCE removal. A cosubstrate must be supplied to keep the cells induced and active, and in some cases especially with methanotrophs, the accumulation of potentially lethal intermediates must be prevented. Simply feeding TCE continuously without a cosubstrate to a methanotroph will result in exhaustion of the cells due to a lack of reducing power (Uchiyama et al. 1992). A number of different strategies to overcome these problems can be envisaged. One of the strategies that have been proposed is to incorporate two different compartments in the reactor system, one (the 'breeder') in which the growth substrate e.g. methane is supplied, and one (the 'degrader') in which TCE is fed to the cells. In this way competition between methane and TCE is avoided. When methanotrophs are used for TCE degradation supplying formate to the degrader as a source of reducing equivalents for the mono-oxygenase system responsible for TCE-oxidation considerably improves the degradation kinetics of the system (Alvarez-Cohen & McCarty 1991a). Since methanotrophic bacteria poison themselves by oxidizing TCE the question then rises what to do with the cells that have been exposed to TCE. Alvarez-Cohen & McCarty (1991b) simply wasted their methanotrophic biomass after exposure to TCE, while McFarland et al. (1992) recirculated their methanotrophs to the breeder compartment.

Oldenhuis et al. (1991) have estimated that conversion of 1 mmol of TCE by *Methylosinus trichosporium* OB3b results in the inactivation of 0.48 g biomass. At a yield of 0.5 g of cells per gram of methane oxidized, this corresponds to a methane consumption requirement of 60 mol methane per mol TCE converted. If it is indeed possible to regenerate spent cells from the degrader in the breeder, then it would be possible to reduce this requirement for methane. This requirement was even more significant in the mixed methanotrophic cultures used by Alvarez-Cohen and McCarty (1991a), who estimated a methane consumption of 630 mol methane per mol TCE converted. Phelps et al. (1990) reported a substrate conversion ratio of 50 to 150 moles of substrate (methane plus propane) per mol TCE converted in continuous-recycle expanded-bed bioreactors. In these reactors the TCE concentration could not be reduced to less than 500 μg/l, while others have observed a lower limit of 50–100 μg/l in a methanotrophic fixed-film packed-bed bioreactor. These levels are substantially higher than the 'threshold' for TCE degradation observed with cell suspensions (Garbarini & Lion 1986). Tros et al. (1992) have made a systematic study of this threshold phenomenon and found that the 'threshold' concentration is not an intrinsic property of bacteria, but that it is determined by the specific characteristics of the system in which biodegradation takes place.

Folsom & Chapman (1991) used recirculation with *Pseudomonas cepacia* G4, but these authors did not state why they recirculated the cells, or whether TCE degradation by *P. cepacia* was toxic to the cells. A two-compartment system was used to avoid stripping of TCE by not sparging with air in the degrader, where the cells reside only temporarily, and to avoid the simultaneous presence of the potentially competitive substrates phenol and TCE.

It has been argued that competitive inhibition will be less of a problem in biofilm reactors than in suspended cultures, because diffusional resistance in the biolayer will allow existence of microenvironments in which the competitive substrate is depleted (Strand et al. 1991). It is not clear, however, how this would be beneficial to the overall performance of the system, since the addition of the cosubstrate has a function, otherwise it could be left out completely.

An alternative would be to have two or more compartments with immobilized cells that are intermittently either exposed to TCE or regenerated with the primary substrate or another appropriate source of reducing power. This approach was taken in a multiple segment trickling filter developed by the group of Knackmuss for the degradation of TCE with isoprene-grown cells of *Rhodococcus erythropolis* JE77 (Ewers et al. 1991). This was the first reactor study in which gaseous TCE was biodegraded. In the studies of McFarland et al. (1992) and of Folsom & Chapman (1991) the experiments were done with dissolved TCE, while the work of Alvarez-Cohen & McCarty (1991a,b) was based on batch experiments and computer simulations.

Conclusions

Biological waste gas treatment is rapidly becoming a grown-up technology, that can also be applied successfully for the removal of chlorinated hydrocarbons from air. In the last decade microbiologists have isolated and characterized a wide variety of microorganisms that can grow on some of these compounds. The development of suitable treatment systems for such growth substrates is now in the hands of engineers who have to find ways to minimize mass transfer limitation. For some of the chlorinated hydrocarbons that so far cannot be used as microbial growth substrates cometabolic systems may be a viable alternative. Here, detailed kinetic and biochemical knowledge is required to be able to manipulate the conditions in such a way that the formation of toxic intermediates and inhibitory interactions between primary and seondary substrate are minimal. Currently, various alternative approaches are under scrutiny with trichloroethene as model compound. Finally, there are those halogenated aliphatics that are so far not known to be biodegradable under aerobic conditions. Since there are no intrinsic thermodynamic or biochemical reasons why this should be the case it is conceivable that there are ongoing evolutionary processes leading to the development of organisms that can aerobically degrade, for example, perchlorinated aliphatics. In the meantime it is worthwile to develop systems that contain anaerobic microenvironments, where such compounds are known to be less resistant to biodegradation.

Whether biodegradation will eventually be a viable waste treatment technology for the removal of chlorinated hydrocarbons from air will depend on the performance and cost effectiveness of such systems. Pilot plant evaluations, including economics, of a bioreactor for the treatment of dichloromethane containing waste gas indicate that at least for this compound biological treatment is cost effective.

References

Anonymous (1986) TA-Luft. Bundesministerium für Umweltschutz, Germany.

Anonymous (1992) Facts and figures for the chemical industry. Chem. Eng. News 70(26): 36

Alexander M (1981) Biodegradation of chemicals of environmental concern. Science 211: 132–138

Alvarez-Cohen L & McCarty PL (1991a) A cometabolic transformation model for halogenated aliphatic compounds exhibiting product toxicity. Environ. Sci. Technol. 25: 1381–1387

Alvarez-Cohen L & McCarty PL (1991b) Two-stage dispersed growth treatment of halogenated aliphatic compounds by cometabolism. Environ. Sci. Technol. 25: 1387–1393

Arciero D, Vanelli T, Logan TM & Hooper AB (1989) Degradation of trichloroethylene by the ammonia-oxidizing bacterium *Nitrosomas europaea*. Biochem. Biophys. Res. Commun. 159: 640–643

Asplund G & Grimvall A (1991) Organohalogens in nature. Environ. Sci. Technol. 25: 1346–1350

Bartels I, Knackmuss H-J & Reineke W (1984) Suicide inactivation of catechol 2,3-dioxygenase from *Pseudomonas putida* mt-2 by 3-halocatechols. Appl. Environ. Microbiol. 47: 500–505

Bartnicki EW & Castro CE (1969) Biodehalogenation. The pathway of transhalogenation and the stereochemistry of epoxide formation from halohydrins. Biochemistry 8: 4677–4680

Blom A, Harder W & Matin A (1992) Unique and overlapping pollutant stress proteins of *Escherichia coli*. Appl. Environ. Microbiol. 58: 331–334

Brunner W, Staub D & Leisinger T (1980) Bacterial degradation of dichloromethane. Appl. Environ. Microbiol. 40: 950–958

Castro CE & Bartnicki EW (1968) Biodehalogenation. Epoxidation of halohydrins, epoxide opening, and transhalogenation by a *Flavobacterium* sp. Biochemistry 7: 3213–3218

Dabrock B, Riedel J, Bertam J & Gottschalk G (1992) Isopropylbenzene (cumene) – a new substrate for the isolation of trichloroethylene-degrading bacteria. Arch. Microbiol. 158: 9–13

Dalsgaard T & Revsbech NP (1992) Regulating factors of denitrification in trickling biofilms as measured with the oxygen/nitrous oxide microsensor. FEMS Microbiol. Ecol. 101: 151–164

Dalton H, Prior SD, Leak DJ & Stanley SH (1984) Regulation and control of methane monooxygenase. In: Crawford RL & Hanson RS (Eds) Microbial Growth on C_1 Compounds (pp 75–82). American Society for Microbiology, Washington, DC

Dean JA (1985) Lange's Handbook of Chemistry, 13th Edition. McGraw-Hill, New York

De Bont JAM, Vorage MJAW, Hartmans S & Van den Tweel WJJ (1986) Microbial degradation of 1,3-dichlorobenzene. Appl. Environ. Microbiol. 52: 677–680

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

Diks RMM (1992) The removal of dichloromethane from waste gases in a biological trickling filter. Ph.D. Thesis, Eindhoven University of Technology, The Netherlands

Diks RMM & Ottengraf SPP (1991a) Verification studies of a simplified model for the removal of dichloromethane from waste gases using a biological trickling filter (Part I). Bioprocess Engineering 6: 93–99

Diks RMM & Ottengraf SPP (1991b) Verification studies of a simplified model for the removal of dichloromethane from waste gases using a biological trickling filter (Part II). Bioprocess Engineering 6: 131–140

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

Dolfing J (1988) Acetogenesis. In: Zehnder AJB (Ed) Biology of Anaerobic Microorganisms (pp 417–468). Wiley, New York

Dolfing J (1990) Reductive dechlorination of 3-chlorobenzoate is coupled to ATP production and growth in an anaerobic bacterium, strain DCB-1. Arch. Microbiol. 153: 264–266

Dolfing J & Tiedje JM (1986) Hydrogen cycling in a three-tiered food web growing on the methanogenic conversion of 3-chlorobenzoate. FEMS Microbiol. Ecol. 38:.293–298

Dolfing J & Tiedje JM (1987) Growth yield increase linked to reductive dechlorination in a defined 3-chlorobenzoate degrading methanogenic coculture. Arch. Microbiol. 149: 102–105

Dolfing J & Harrison BK (1992) The Gibbs free energy of formation of halogenated aromatic comounds and their potential role as electron acceptors in anaerobic environments. Environ. Sci. Technol. 26: 2213–2218

Edwards EA & Grbic-Galic (1992) Complete mineralization of benzene by aquifer microorganisms under strictly anaerobic conditions. Appl. Environ. Microbiol. 58: 2663–2666

Ensign SA, Hyman MR & Arp DA (1992) Cometabolic degradation of chlorinated alkanes by alkene monooxygenase in a propylene-grown *Xanthobacter* strain. Appl. Environ. Microbiol. 58: 3038–3046

Ewers J, Clemens W & Knackmuss H-J (1991) Biodegradation of chloroethenes using isoprene as co-substrate. In: Proceedings of the International Symposium on Environmental Biotechnology (pp 77–83), April 22–25, 1991, Oostende, Belgium

Ewers J, Freier-Schröder D & Knackmuss H-J (1990) Selection of trichloroethylene (TCE) degrading bacteria that resist inactivation by TCE. Arch. Microbiol. 154: 410–413

Fathepure BZ, Tiedje JM & Boyd SA (1988) Reductive dechlorination of hexachlorobenzene to tri- and dichlorobenzenes in anaerobic sewage sludge. Appl. Environ. Microbiol. 54: 327–330

Fletcher M (1991) The physiological activity of bacteria attached to solid surfaces. Adv. Microbial Physiol. 32: 53–85

Folsom BR, Chapman PJ & Pritchard PH (1990) Phenol and trichloroethylene degradation by *Pseudomonas cepacia* G4: kinetics and interactions between substrates. Appl. Environ. Microbiol. 56: 1279–1285

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

Freedman DL & Gossett JM (1991) Biodegradation of dichloromethane and its utilization as a growth substrate under methanogenic conditions. Appl. Environ. Microbiol. 57: 2847–2857

Fox BG, Borneman JG, Wackett LP & Lipscomb JD (1990) Haloalkene oxidation by the soluble methane monooxygenase from *Methylosinus trichosporium* OB3b: mechanistic and environmental implications. Biochemistry 29: 6419–6427

Garbarini DR & Lion W (1986) Influence of the nature of soil organics on the sorption of toluene and trichloroethylene. Environ. Sci. Technol. 20: 1263–1269

Gossett JM (1987) Measurement of Henry's law constants for C_1 and C_2 chlorinated hydrocarbons. Environ. Sci. Technol. 21: 202–208

Groenewegen PEJ, Driessen AJM, Konings WN & De Bont JAM (1990) Energy-dependent uptake of 4-chlorobenzoate in the coryneform bacterium NTB-1. J. Bacteriol. 172: 419–423

Gschwend PM, Macfarlane JK & Newman KA (1985) Volatile halogenated organic compounds released to seawater from temperate marine macroalgae. Science 227: 1033–1035

Haigler BE, Pettigrew CA & Spain JC (1992) Biodegradation of mixtures of substituted benzenes by *Pseudomonas* sp. strain JS150. Appl. Environ. Microbiol. 58: 2237–2244

Hardman DJ & Slater JH (1981) Dehalogenases in soil bacteria. J. Gen. Microbiol. 123: 117–128

Harker AR & Kim Y (1990) Trichloroethylene degradation by two independent aromatic degrading pathways in *Alcaligenes eutrophus* JMP134. Appl. Environ. Microbiol. 56: 1179–1181

Harrington AA & Kallio RE (1960) Oxidation of methanol and formaldehyde by *Pseudomonas methanica*. Can. J. Microbiol. 6: 1–7

Hartmans S & De Bont JAM (1992) Aerobic vinyl chloride metabolism in *Mycobacterium aurum* L1. Appl. Environ. Microbiol. 58: 1220–1226

Hartmans S, De Bont JAM & Harder W (1989) Microbial metabolism of short-chain unsaturated hydrocarbons. FEMS Microbiol. Rev. 63: 235–264

Hartmans S, Leenen EJTM & Voskuilen GTH (1992) Membrane bioreactor with porous hydrophobic membranes for waste-gas treatment. In: Dragt AJ & Van Ham J (Eds) Biotechniques for Air Pollution Abatement and Odour Control Policies (pp 103–106). Elsevier Science Publishers, Amsterdam, The Netherlands

Hartmans S, Schmuckle A, Cook AM & Leisinger T (1986) Methyl chloride: naturally occurring toxicant and C-1 growth substrate. J. Gen. Microbiol. 132: 1139–1142

Hartmans S & Tramper J (1991) Dichloromethane removal from waste gases with a trickle-bed bioreactor. Bioprocess Engineering 6: 83–92

Henry SM & Grbic-Galic D (1991) Influence of endogenous and exogenous electron donors and trichloroethylene oxidation toxicity on trichloroethylene oxidation by methanotrophic cultures from a groundwater aquifer. Appl. Environ. Microbiol. 57: 236–244

Henschler D (1985) Halogenated alkenes and alkynes. In: Anders MW (Ed) Bioactivation of Foreign Compounds (pp 317–347). Academic Press, New York

Holliger HC (1992) Reductive dehalogenation by anaerobic bacteria. Ph.D. Thesis, Agricultural University, Wageningen, The Netherlands

Holliger C, Schraa G, Stams AJM & Zehnder AJB (1992) Enrichment and properties of an anaerobic mixed culture reductively dechlorinating 1,2,3-trichlorobenzene to 1,3-dichlorobenzene. Appl. Environ. Microbiol. 58: 1636–1644

Horvath RS (1972) Microbial cometabolism and the degradation of organic compounds in nature. Bacteriol. Rev. 36: 146–155

Hoyle R (1992) Crumbling obstacles to engineered bugs. Bio/technol. 10: 8

Hutzinger O & Veerkamp W (1981) Xenobiotic chemicals with pollution potential. In: Leisinger T, Cook A, Hutter R & Nuesch J (Eds) Microbial Degradation of Xenobiotic and Recalcitrant Compounds (pp 3–45). Academic Press, London

Janssen DB, Gerritse J, Brackman J, Kalk C, Jager D & Witholt B (1988) Purification and characterization of a bacterial dehalogenase with activity toward halogenated alkanes, alcohols, and ethers. Eur. J. Biochem. 171: 67–72

Janssen DB, Jager D & Witholt B (1987) Degradation of *n*-haloalkanes and α,ω-dihaloalkanes by wild-type and mutants of *Acinetobacter* sp-strain GJ70. Appl. Environ. Microbiol. 53: 561–566

Janssen DB, Oldenhuis R & van den Wijngaard AJ (1989) Degradation of xenobiotic compounds by microorganisms. VDI Berichte 735: 25–39

Janssen DB, Scheper A, Dijkhuizen L & Witholt B (1985) Degradation of halogenated aliphatic compounds by *Xanthobacter autotrophicus* GJ10. Appl. Environ. Microbiol. 49: 673–677

Janssen DB, Scheper A & Witholt B (1984) Biodegradation of 2-chloroethanol and 1,2-dichloroethane by bacterial cultures. Prog. Ind. Microbiol. 20: 169–178

Janssen DB & Witholt B (1992) Aerobic and anaerobic degradation of halogenated aliphatics. In: Sigel H & Sigel A (Eds) Metal Ions in Biological Systems, Vol 28, Degradation of Environmental Pollutants by Microorganisms and Their Metalloenzymes (pp 299–327). Marcel Dekker, New York

Keuning S, Janssen DB & Witholt B (1985) Purification and characterization of hydrolytic haloalkane dehalogenase from *Xanthobacter autotrophicus* GJ10. J. Bacteriol. 163: 635–639

Kirchner K, Gossen CA & Rehm H-J (1991) Purification of exhaust air containing organic pollutants in a trickle-bed reactor. Appl. Microbiol. Biotechnol. 35: 396–400

Kirchner K, Schlachter U & Rehm H-J (1989) Biological purification of exhaust air using fixed bacterial monocultures. Appl. Microbiol. Biotechnol. 31: 629–632

Kirchner K, Wagner S & Rehm H-J (1992) Exhaust gas purification using biocatalysts (fixed bacteria monocultures) – the influence of biofilm diffusion rate (O_2) on the overall reaction rate. Appl. Microbiol. Biotechnol. 37: 277–279

Klecka GM & Gibson DT (1981) Inhibition of catechol 2,3-dioxygenase from *Pseudomonas putida* by 3-chlorocatechol. Appl. Environ. Microbiol. 41: 1159–1165

Kohler-Staub D, Hartmans S, Gälli R, Suter F & Leisinger T (1986) Evidence for identical dichloromethane dehalogenases in different methylotrophic bacteria. J. Gen. Microbiol. 132: 2837–2843

Kohler-Staub D & Leisinger T (1985) Dichloromethane dehalogenase of *Hyphomicrobium* sp. strain DM2. J. Bacteriol. 162: 676–681

Kok HJG (1992) Bioscrubbing of air contaminated with high concentrations of hydrocarbons. In: Dragt AJ & van Ham J (Eds) Biotechniques for Air Pollution Abatement and Odour Control Policies (pp 77–82). Elsevier Science Publishers, Amsterdam, The Netherlands

Krone UE, Thauer RK & Hogenkamp HPC (1989) Reductive dehalogenation of chlorinated C_1-hydrocarbons mediated by corrinoids. Biochemistry 28: 4908–4914

Krone UE, Laufer K, Thauer RK & Hogenkamp HPC (1989) Coenzyme F_{430} as a possible catalyst for the reductive dehalogenation of chlorinated C_1 hydrocarbons in methanogenic bacteria. Biochemistry 28: 10061–10065

Krone UE, Thauer RK, Hogenkamp HPC & Steinbach K (1991) Reductive formation of carbon monoxide from CCl_4 and FREONs 11, 12, and 13 catalyzed by corrinoids. Biochemistry 30: 2713–2719

Kuenen JG, Jørgensen BB & Revsbech NP (1986) Oxygen microprofiles in trichling filter biofilms. Water Res. 20: 1589–1598

La Roche SD & Leisinger T (1990) Sequence analysis and expression of the bacterial dichloromethane dehalogenase structural gene, a member of the glutathione S-transferase supergene family. J. Bacteriol. 172: 164–171

La Roche SD & Leisinger T (1991) Identification of *dcm*R, the regulatory gene governing expression of dichloromethane dehalogenase in *Methylobacterium* sp. strain DM4. J. Bacteriol. 173: 6714–6721

Li S & Wackett LP (1992) Trichloroethylene oxidation by toluene dioxygenase. Biochem. Biophys. Res. Commun. 185: 443–451

Little CD, Palumbo AV, Herbes SE, Lidstrom ME, Tyndall RL & Gilmer PJ (1988) Trichloroethylene biodegradation by a methane-oxidizing bacterium. Appl. Environ. Microbiol. 54: 951–956

Little CD, Keyhan M, Fraley CD, McCann MP & Matin A (1991) Use of stress-induced promoter to enhance trichloroethylene biodegradation in nutrient-limited recombinant *E. coli*. Abstracts ASM Q–109

Loidl M, Hinteregger C, Ditzelmüller G, Ferschl A & Streichsbier F (1990) Degradation of aniline and monochlorinated anilines by soil-born *Pseudomonas acidovorans* strains. Arch. Microbiol. 155: 56–61

Lovelock JE, Maggs RJ & Wade RJ (1973) Halogenated hydrocarbons in and over the Atlantic. Nature (London) 241: 194–196

Mackay D & Shiu WY (1981) A critical review of Henry's law constants for chemicals of environmental interest. J. Phys. Chem. Ref. Data 10: 1175–1199

McFarland MJ, Vogel CM & Spain JC (1992) Methanotrophic

cometabolism of trichloroethylene (TCE) in a two stage bioreactor system. Water Research 26: 259–265

Mohn WW & Tiedje JM (1990) Strain DCB-1 conserves energy for growth from reductive dechlorination coupled to formate oxidation. Arch. Microbiol. 153: 267–271

Motosugi K & Soda K (1983) Microbial degradation of synthetic organochlorine compounds. Experientia 39: 1214–1220

Nagasawa T, Nakamura T, Yu F, Watanabe I & Yamada H (1992) Purification and characterization of halohydrin hydrogen-halide lyase from a recombinant *Escherichia coli* containing the gene from a *Corynebacterium* sp. Appl. Microbiol. Biotechnol. 36: 478–482

Nakajima T, Uchiyama H, Yagi O & Nakahara T (1992) Novel metabolite of trichloroethylene in a methanotrophic bacterium, *Methylocystis* sp. M, and hypothetical degradation pathway. Biosci. Biotech. Biochem. 56: 486–489

Nakamura T, Nagasawa T, Yu F, Watanabe I & Yamada H (1992) Resolution and some properties of enzymes involved in enantioselective transformation of 1,3-dichloro-2-propanol to (*R*)-3-chloro-1,2-propanediol by *Corynebacterium* sp. strain N-1074. J. Bacteriol. 174: 7613–7619

Nelson MJK, Montgomery SO, Mahaffey WR & Pritchard PH (1987) Biodegradation of trichloroethylene and involvement of an aromatic biodegradative pathway. Appl. Environ. Microbiol. 53: 949–954

Nelson MJK, Montgomery SO, O'Neill EJ & Pritchard PH (1986) Aerobic metabolism of trichloroethylene by a bacterial isolate. Appl. Environ. Microbiol. 52: 383–384

Nelson MJK, Montgomery SO & Pritchard PH (1988) Trichloroethylene metabolism by microorganisms that degrade aromatic compounds. Appl. Environ. Microbiol. 54: 604–606

Newman LM & Wackett LP (1991) Fate of 2,2,2,-trichloroacetaldehyde (chloral hydrate) produced during trichloroethylene oxidation by methanotrophs. Appl. Environ. Microbiol. 57: 2399–2402

Oldenhuis R (1992) Microbial degradation of chlorinated compounds. Ph.D. Thesis, University of Groningen, The Netherlands

Oldenhuis RJ, Oedzes JY, Van der Waarde JJ & Janssen DB (1991) Kinetics of chlorinated hydrocarbon degradation by *Methylosinus trichosporium* OB3b and toxicity of trichloroethylene. Appl. Environ. Microbiol. 57: 7–14

Oldenhuis R, Vink RLJM Janssen DB & Witholt B (1989) Degradation of chlorinated aliphatic hydrocarbons by *Methylosinus trichosporium* OB3b expressing soluble methane monooxygenase. Appl. Environ. Microbiol. 55: 2819–2826

Ottengraf SPP (1987) Biological systems for waste gas elimination. Trends Biotechnol. 5: 132–136

Ottengraf SPP & Diks RMM (1992) Process technology of biotechniques. In: Dragt AJ & van Ham J (Eds) Biotechniques for Air Pollution Abatement and Odour Control Policies (pp 17–31). Elsevier Science Publishers, Amsterdam, The Netherlands

Ottengraf SPP, Meesters JJP, Van den Oever AHC & Rozema HR (1986) Biological elimination of volatile xenobiotic compounds in biofilters. Bioprocess Engineering 1: 61–69

Parsons JR, Opperhuizen A & Hutzinger O (1987) Influence of membrane permeation on biodegradation kinetics of hydrophobic compounds. Chemosphere 16: 1361–1370

Pearson CR & McConell G (1975) Chlorinated C_1 and C_2 hydrocarbons in the marine environment. Proc. R. Soc. London B 189: 305–332

Phelps PA, Agarwal S, Speitel GE Jr. & Georgiou G (1992) *Methylosinus trichosporium* OB3b mutants having constitutive expression of soluble methane monooxygenase in the presence of high levels of copper. Appl. Environ. Microbiol. 58: 3701–3708

Phelps TJ, Niedzielski JJ, Schram RM, Herbes SE & White DC (1990) Biodegradation of trichloroethylene in continuous-recycle expanded-bed bioreactors. Appl. Environ. Microbiol. 56: 1702–1709

Reineke W & Knackmuss H-J (1984) Microbial metabolism of haloaromatics: isolation and properties of a chlorobenzene-degrading bacterium. Appl. Environ. Microbiol. 47: 395–402

Revsbech NP & Jørgensen BB (1986) Microelectrodes: Their use in microbial ecology. Adv. Microb. Ecol. 9: 293–352

Sander P, Wittich R-M, Fortnagel P, Wilkes H & Francke W (1991) Degradation of 1,2,4-trichloro- and 1,2,4,5-tetrachlorobenzene by *Pseudomonas* strains. Appl. Environ. Microbiol. 57: 1430–1440

Schanke CA & Wackett LP (1992) Environmental reductive elimination reactions of polychlorinated ethanes mimicked by transition-metal coenzymes. Environ. Sci. Technol. 26: 830–833

Schippert E (1989) Das Biosolv-Verfahren von Keramchemie zur Absorption von schwer wasserlöslichen Lösemitteln. In: Biologische Abgasreinigung (pp 161–177) VDI-Verlag, Dusseldorf

Schlegel HG (1986) General Microbiology (p 253). Cambridge University Press, Cambridge, UK

Scholtz R, Leisinger T, Suter F & Cook AM (1987) Characterization of 1-chlorohexane halidohydrolase, a dehalogenase of wide substrate range from an *Arthrobacter* sp. J. Bacteriol. 169: 5016–5021

Scholtz R, Wackett LP, Egli C, Cook AM & Leisinger T (1988) Dichloromethane dehalogenase with improved catalytic activity isolated from a fast-growing dichloromethane-utilizing bacterium. J. Bacteriol. 170: 5698–5704

Schraa G, Boone ML, Jetten MSM, van Neerven ARW, Colberg PJ & Zehnder AJB (1986) Degradation of 1,4-dichlorobenzene by *Alcaligenes* sp. strain A175. Appl. Environ. Microbiol. 52: 1374–1381

Shields MS, Montgomery S, Cuskey SM, Chapman PJ & Pritchard PH (1991) Mutants of *Pseudomonas cepacia* G4 defective in catabolism of aromatic compounds and trichloroethylene. Appl. Environ. Microbiol. 57: 1935–1941

Shields MS & Reagin MJ (1992) Selection of a *Pseudomonas cepacia* strain constitutive for the degradation of trichloroethylene. Appl. Environ. Microbiol. 58: 3977–3983

Stafbureau NER (1992) Nederlandse Emissie Richtlijnen, RIVM, Bilthoven, The Netherlands

Strand SE, Wodrich JV & Stensel HD (1991) Biodegradation of

chlorinated solvents in a sparged, methanotrophic biofilm reactor. J. Wat. Poll. Control Fed. 63: 859–867

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

Stucki G (1989) Biologische Entzorgung von Methylenchlorid aus Abluft und Abwaser. Swiss Chem. 11: 35–38

Stucki GR, Gälli R, Ebershold H-R & Leisinger T (1981) Dehalogenation of dichloromethane by cell extracts of *Hyphomicrobium* DM2. Arch. Microbiol. 130: 366–371

Stucki G & Leisinger T (1983) Bacterial degradation of 2-chloroethanol proceeds via 2-chloroacetic acid. FEMS Microbiol. Lett. 16: 123–126

Stumm W & Morgan JJ (1981) Aquatic Chemistry, 2nd edition (pp 749–756). Wiley, New York

Tardif G, Greer CW, Labbé D & Lau PCK (1991) Involvement of a large plasmid in the degradation of dichloromethane by *Xanthobacter autotrophicus*. Appl. Environ. Microbiol. 57: 1853–1857

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

Traunecker J, Preuß A & Diekert G (1991) Isolation and characterization of a methyl chloride utilizing, strictly anaerobic bacterium. Arch. Microbiol. 156: 416–421 Tros ME, Schraa G & Zehnder AJB (1992) Biodegradation of xenobiotic compounds at low concentrations: role of threshold concentrations. In: Abstracts of the Sixth International Symposium on Microbial Ecology (p 131), 6–11 September 1992, Barcelona Tsien H-C, Brusseau GA, Hanson RS & Wackett LP (1989) Biodegradation of trichloroethylene by *Methylosinus trichosporium* OB3b. Appl. Environ. Microbiol. 55: 3155–3161

Uchiyama H, Oguri K, Yagi O & Kokufuta E (1992) Trichloroethylene degradation by immobilized resting cells of *Methylocystis* sp. M in a gas-solid bioreactor. Biotechnol. Lett. 14: 619–622

Vandenbergh PA, Olsen RH & Colaruotolo JF (1981) Isolation and genetic characterization of bacteria that degrade chloroaromatic compounds. Appl. Environ. Microbiol. 42: 737–739

Van den Wijngaard AJ, Janssen DB & Witholt B (1989) Degradation of epichlorohydrin and halohydrins by bacterial cultures isolated from freshwater sediment. J. Gen. Microbiol. 135: 2199–2208

Van den Wijngaard AJ, Reuvekamp PTW & Janssen DB (1991) Purification and characterization of haloalcohol dehalogenase from *Arthrobacter* sp. strain AD2. J. Bacteriol. 173: 124–129

Van den Wijngaard AJ, Van der Kamp KWHJ, Van der Ploeg J, Pries F & Janssen DB (1992) Degradation of 1,2-dichloroethane by *Ancylobacter aquaticus* and other facultative methylotrophs. Appl. Environ. Microbiol. 58: 976–983

Van der Meer JR, De Vos WM, Harayama S & Zehnder AJB (1992) Molecular mechanisms of genetic adaptation to xenobiotic compounds. Microbiol. Rev. 56: 677–694

Van Loosdrecht MCM, Lyklema J, Norde W & Zehnder AJB (1990) Influence of interfaces on microbial activity. Microbiol. Rev. 54: 75–87

Vogel TM, Criddle CS & McCarty PL (1987) Transformations of halogenated aliphatic compounds. Environ. Sci. Technol. 21: 722–736

Wackett LP, Brusseau GA, Householder SR & Hanson RS (1989) Survey of microbial oxygenases: trichloroethylene degradation by propane-oxidizing bacteria. Appl. Environ. Microbiol. 55: 2960–2964

Wackett LP & Gibson DT (1988) Degradation of trichloroethylene by toluene dioxygenase in whole-cell studies with *Pseudomonas putida* F1. Appl. Environ. Microbiol. 54: 1703–1708

Wackett LP & Householder SR (1989) Toxicity of trichloroethylene to *Pseudomonas putida* F1 is mediated by toluene dioxygenase. Appl. Environ. Microbiol. 55: 2723–2725

Weightman AJ & Slater JH (1980) Selection of *Pseudomonas putida* strains with elevated dehalogenase activities by continuous culture growth on chlorinated alkanoic acids. J. Gen. Microbiol. 121: 187–193

Winter RB, Yen K-M & Ensley BD (1989) Efficient degradation of trichloroethylene by a recombinant *Escherichia coli*. Biotechnol. 7: 282–285

Witholt B, Janssen DB & Keuning S (1989) Biodegradation of xenobiotics by specific bacteria: research and applications. In: Biotreatment. The Use of Microorganisms in the Treatment of Hazardous Materials and Hazardous Wastes (pp 109–117). Proceedings of the 2nd National Conference, November 27–29, 1989, Washington, DC, The Hazardous Materials Control Research Institute

Wolff F (1992) Biologische Abluftreinigung mit einem intermittierend befeuchteten Tropfkorper. In: Dragt AJ & van Ham J (Eds) Biotechniques for air pollution abatement and odour control policies (pp 49–62). Elsevier, Amsterdam, The Netherlands

Yokota T, Omori T & Kodama T (1987) Purification and properties of haloalkane dehalogenase from *Corynebacterium* sp. strain m15-3. J. Bacteriol. 169: 4049–4054

Zeyer JA, Wasserfallen A & Timmis KN (1985) Microbial mineralization of ring-substituted anilines through an *ortho*-cleavage pathway. Appl. Environ. Microbiol. 50: 447–453

Zylstra GJ, Wackett LP & Gibson DT (1989) Trichloroethylene degradation by *Escherichia coli* containing the cloned *Pseudomonas putida* F1 toluene dioxygenase genes. Appl. Environ. Microbiol. 55: 3162–3166