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Microbial transformation of chlorinated aromatics in sediments

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Microbial transformation of chlorinated aromatics in sediments

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1. Microbiële dehalogenering van chlooraromaten in waterbodems resulteert in een relatief geringe verandering van de chemische structuur van deze verontreinigingen, met daarentegen grote milieuhygiënische consequenties.

2. Bij de prognose van de toekomstige waterbodemkwaliteit met behulp van modellen wordt veelal ten onrechte de microbiële omzetting buiten beschouwing gelaten.

3. De berekening van de invloed van verontreinigde baggerspecie op de grondwaterkwaliteit over een termijn van 10.000 jaar, zoals vereist bij de aanleg van speciedepots, verliest aan betekenis als men bedenkt dat 10.000 jaar geleden de ijstijd eindigde.
   Beleidsstandpunt Verwijdering Baggerspecie, Tweede Kamer stuk nr. 23 450, 1993.

4. Omvangrijke saneringen van de waterbodem van de grote nederlandse sedimentatiebekkens, gebaseerd op modelleringen van "worst-case" stofgedrag, behoeft, gezien de hoge saneringenkosten, een tussenstap: het vaststellen van het feitelijk gedrag van verontreinigingen in de betreffende waterbodem qua afbreekbaarheid en verspreiding naar de omgeving.

5. Bij natuurontwikkelingsprojecten in verontreinigde uiterwaarden blijken de toxische stoffen vooralsnog meer problemen te veroorzaken bij beleidsmakers dan bij de zich ontwikkelende natuur.
   Creemers, R.C.M. 1991. Amfibieën in uiterwaarden.

6. Milieuchemisch procesonderzoek bij rijksinstituten, waarin de afstand tussen laboratorium- en veldsituatie wordt overbrugd, is essentieel voor een adequaat milieubeleid en kan vooralsnog niet geheel door universiteiten worden ingevuld.

7. Overgaan tot uitvoering van milieubeleid is midden negentiger jaren een paars devies; de beperkte middelen maken prioritering noodzakelijk en vereisen een afwegingskader dat de traditionele grenzen tussen de milieucompartimenten water, bodem en lucht wegneemt en het rendement van ingrepen inzichtelijk maakt.

8. Het onttrekken en reinigen van verontreinigd grondwater is geen saneringstechniek, maar een isolatie- of beheerstechniek.

9. Het inzicht dat verdeling van arbeid realistischer is dan het streven naar 100.000-den nieuwe arbeidsplaatsen moet nog groeien.

10. Het hoger aandeel tweelinggeboorten in Flevoland ten opzichte van de Veluwe, onderstreept de vruchtbaarheid van de polderbodem.
    Tas, R.F.J., 1990. Meerlinggeboorten regionaal bezien. CBS Mndstat. bevolk.

Stellingen behorend bij het proefschrift "Microbial transformation of chlorinated aromatics in sediments", J.E.M. Beurskens, Wageningen, 13 juni 1995.

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CHAPTER 1

GENERAL INTRODUCTION

Pollutants may enter the aquatic environment directly as aqueous emissions or indirectly via atmospheric deposition or runoff from urban and agricultural areas. Chemicals that are not readily degraded may be further dispersed, and become of particular concern if they exert toxic effects. Examples of such compounds are hexachlorbenzene, polychlorinated biphenyls and chlorinated dioxins. These hydrophobic chlorinated aromatics have a tendency to associate with organically rich phases such as suspended solids and biological tissues. In downstream stretches of rivers the suspended solids with the associated pollutants settle, leading to an accumulation of pollutants in the river bed. Many man-made chlorinated aromatics are now identified as priority pollutants that disturb ecological systems and pose a direct threat to human health if aquatic organisms are consumed or polluted water is used as drinking water.

Three main waterways, the rivers Rhine, Meuse and Scheldt, enter the North Sea from the Netherlands. These rivers drain a highly industrialized part of northwestern Europe. In the downstream areas of these three rivers enormous quantities (> 100 million m^3) of highly polluted sediments have been deposited during the last five decades. On the basis of the elevated levels in sediments and accumulation in organisms, chlorinated aromatic compounds are generally considered to be highly persistent in the aquatic environment. The concentrations of chlorinated aromatics frequently exceed the environmental quality objectives, a reason for the development of an extensive sediment dredging, treatment and confined disposal policy in the Netherlands. On the other hand, chlorinated aromatic compounds have been found to be transformed microbially under anaerobic conditions in laboratory studies (Tiedje et al., 1987). Indications that these reactions are not restricted to laboratory incubations, but also occur in polluted sediments were first reported for PCBs in the Hudson River (US) (Brown et al., 1987). Knowledge on the long-term fate of hydrophobic organic pollutants is of great importance for the assessment of the impact of pollution episodes on human health and ecosystems. However, insight into microbial

transformations of halogenated aromatics in Dutch polluted anaerobic sediments was lacking. Moreover, extrapolation of the findings from the Hudson River to Dutch sediments is complicated, since site-specific conditions determine whether microbial reactions occur. For example, PCB concentrations in Rhine sediments are much lower than the concentrations in Hudson sediment. The aim of this research was to verify whether microbial transformations of chlorinated aromatics occur in Dutch sediments and, if so, to characterize and quantify the reactions.

Chapter 1 provides information on chlorinated aromatics in sediments and describes general aspects of microbial transformation. The research questions and thesis outline are presented in the last section.

Anthropogenic chlorinated aromatic compounds: structures, sources and toxicities

Four compound groups were selected to study microbial transformation reactions in sediments: chlorinated benzenes (CBs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). These compound groups as a whole or representatives of these groups have been classified by the European Community as priority pollutants (Jones and Wild, 1991); they also were listed as priortity pollutants by the Rhine border states (IKSR, 1989).

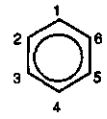
The chemical structure of benzene is given in Fig. 1.1. Each numbered carbon atom may contain a chlorine atom. Depending on the number of chlorine atoms and their place on the ring, twelve chlorinated isomers can be distinguished within the compound group of chlorinated benzenes. CBs were or are still used as industrial solvents, dielectric fluids, deodorants, pesticides and chemical intermediates. In addition, CBs may be produced unintentionally as waste products of the chemical manufacture of, for example, chlorinated aliphatics (Evers, 1989) or in aluminium foundries (Vogelgesang et al., 1986). The higher chlorinated benzenes in particular tend to accumulate in biota (Oliver and Niimi, 1983). CBs have a nonspecific, narcotic mode of toxic action in fish (Van Leeuwen et al., 1990).

Polychlorinated biphenyls are manufactured by chlorination of biphenyl (Fig. 1.1), which results in technical mixtures containing a given chlorine content. For example, Aroclor 1254 and Clophen A 30 contain 54 and 42% chlorine, respectively (de Voogt et al., 1990). Theoretically 209 different PCB congeners exist but only a few dozen are abundant. Commercial production of PCBs in the United States started in 1929. The world production reached a maximum in the early 1960s, when PCBs became widely used as dielectric fluids, heat transfer fluids, hydraulic fluids, plasticizers and flame retardants. More than

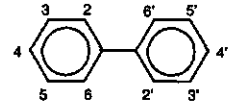
$2x10^9$ kg have been produced worldwide (Tanabe, 1988). Because of elevated levels found in a range of environmental samples and their suspected toxicity, their industrial use in much of Europe and the US has been restricted since the mid-1970s (Rapaport and Eisenreich, 1988). PCBs accumulate in biota due to their hydrophobicity. Various toxic effects are associated with PCBs and include, for example, adverse reproductive effects in fish-eating mammals and birds (Reijnders, 1986; Boon et al., 1987; van den Berg et al., 1994; Kubiak et al., 1989) and carcinogenicity (Silberhorn et al., 1990). The toxicity of PCBs depends on the place of the chlorines on the biphenyl molecule. Congeners with none or only one ortho, two para, and two or more meta chlorines, resemble 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (see below) in their biological and toxic effects (Safe, 1990). Although these so-called non-ortho and mono-ortho congeners were present at trace levels in the original PCB mixtures (Hong et al., 1993), they are now considered to be mainly responsible for the toxicity associated with PCBs (de Voogt et al., 1990).

Opposite to the CBs and PCBs, that were produced commercially, chlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) are formed as unintentional by-products in a variety of combustion and chemical manufacturing processes (Evers et al., 1993). Examples are the incineration of municipal and chemical wastes, fossil fuel combustion, and industrial production of chlorinated aliphatics and aromatics. The total number of possible PCDD- and PCDF-congeners is 75 and 135, respectively (Fig. 1.1).

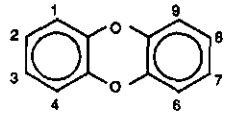
Chlorobenzenes
CBs



Polychlorobiphenyls
PCBs



Polychlorodibenzo-p-dioxins
PCDDs



Polychlorodibenzofurans
PCDFs

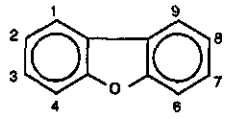


Fig. 1.1. Chemical structures and numbering of carbon atoms that may contain chlorine atoms in four classes of chlorinated aromatics.

The congeners with the highest acute toxicity are those having 4 to 6 chlorine atoms and all 2,3,7, and 8 positions substituted (Safe, 1990). These congeners induce a wide range of toxicological effects (mortality, reduced reproduction) as well as a number of biochemical and physiological responses (Kimbrough and Jensen, 1989). Biochemical effects include induction of cytochrome P450-dependent mono-oxygenases and effects on the regulation of vitamin A (Brouwer et al., 1989; van der Weiden et al., 1993).

Chlorinated aromatic compounds in sediments

Sediment pollution data from industrialized areas were selected from the literature. Studies that describe a widespread sediment pollution, preferably not related to a single emitter, were chosen to demonstrate the ubiquitous character of chlorinated aromatics in sediments. Sampling and analytical methods varied per site; the values have therefore been presented to give only an indication of the concentrations at different sites. Emissions of chlorinated aromatics showed maximum levels in the 1960s and 1970s. Contemporary emissions for hexachlorobenzene and PCBs reach levels close to those in the mid-1940s (Alcock et al., 1993; Jones et al., 1992; Rapaport and Eisenreich, 1988). In order to summarize recent pollution levels in sediments, a selection of concentrations of chlorinated aromatics in sediment top-layers has been made from the literature since 1980. However, this does not exclude the possibility of reporting contamination levels of several decades ago. Surficial sediments may reflect earlier levels due to changes in sedimentation and erosion patterns.

There have been relatively few reports on the lower chlorinated benzene concentrations in contaminated sediments. However, those available indicate that contamination levels of dichlorinated benzenes are generally the highest within this compound group and reach up to several hunderds of µg/kg (Table 1.1). 1,4-Dichlorobenzene shows the highest concentrations for the listed locations; 1,2,3-tri- and 1,2,3,4-tetrachlorobenzene the lowest concentrations. Hexachlorobenzene concentrations in sediments are more frequently reported (e.g. Watanabe et al., 1986) and concentrations are generally below the 100 µg/kg level (Table 1.1).

Table 1.1. Mean concentrations of chlorinated benzenes in surficial sediments (µg/kg d.w.).

Chlorobenzene	Locations				
	Hamburg Harbor[1] (*n*=32)	Elbe River[2] (*n*=8)	Lake Ketelmeer[3] (*n*=5)	Scheldt River[4] (*n*=4)	Lake Ontario[5] (*n*=11)
mono-		355			
1,2-di-	111	249	220	21	11
1,3-di-	132	181	110	18	74
1,4-di-	539	536	210	62	94
1,2,3-tri-	5	5	2	6	7
1,2,4-tri-	84	54	70	43	94
1,3,5-tri-	54	13	50	1	60
1,2,3,4-tetra-	6	5	5	6	33
1,2,3,5-tetra-	3		2	<dl	6
1,2,4,5-tetra-	8	11*	20	<dl	52
penta-	5	6	10	3	32
hexa-	92	50	40	23	97

[1]Hamburg Harbor (Germany) is located in the downstream area of the Elbe River, data from Götz et al., 1990b.

[2]Sampling points mainly upstream of Hamburg Harbor; samples consisted of bed sediment and sediment chamber material, data from Götz et al., 1993.
[3]Lake Ketelmeer (The Netherlands) is a downstream sedimentation area of the Rhine River, sediment deposited around 1985, data from Beurskens et al., 1994.
[4]Data from Oliver and Nicol, 1982.
[5]Suspended solids, sampled in 1987-1989, data from van Zoest and van Eck, 1991.
*Sum of 1,2,3,5- and 1,2,4,5-tetrachlorobenzene.

Since PCB contamination levels can be expressed as concentrations of the individual congeners as well as total concentrations of specific commercial mixtures (Aroclor, Clophen), comparisons of contamination levels are complicated. The concentrations of six individual PCB congeners, generally reported in European studies (i.e. 2,4,4'-trichlorobiphenyl (PCB 28), 2,2',5,5'-tetrachlorobiphenyl (PCB 52), 2,2',4,5,5'-pentachlorobiphenyl (PCB 101), 2,2',3,4,4',5'-hexachlorobiphenyl (PCB 138), 2,2'4,4',5,5'-hexachlorobiphenyl (PCB 153), and 2,2',3,4,4',5,5'-heptachlorobiphenyl (PCB 180)), are listed in Table 1.2. To what extent these six congeners represent the total PCB concentration in sediments depends on the original mixtures used and the possible changes in PCB composition due to differences in environmental behaviour of the individual congeners. The selected congeners are major constituents of PCBs generally detected in the environment. An estimation of total PCB concentrations was obtained by assuming that the sum of the six congeners represents 20% of the total PCB concentration. In the US, PCB contamination is generally reported as the total PCB

concentration of a specified Aroclor mixture. Environmental concentrations of non-ortho and mono-ortho PCBs are still seldomly reported and therefore not included in Table 1.2. There are no systematic differences in the concentrations of the individual congeners in sediments listed in Table 1.2. Total PCB concentrations in sediments are generally around or below the 1000 µg/kg level at the northwestern European locations (Table 1.2). On the other hand, PCB concentrations in some American rivers equal or exceed the 10,000 µg/kg level. The US rivers mentioned in Table 1.2 have a widespread and high PCB contamination that makes these river sediments 1-2 orders of magnitude more contaminated than sediments from rivers and lakes in northwestern Europe (Table 1.2) and sediments from other rivers in the US (Dennis, 1976).

Table 1.2. Mean concentrations of polychlorinated biphenyls (PCBs) in surficial sediments (µg/kg d.w.)

PCBs	Locations							
	Hamburg Harbor[1] (n=32)	Elbe River[2] (n=6)	Lippe River[3] (n=16)	Lake Ketelmeer[4] (n=18)	Scheldt River[5]	Lake Ontario[6] (n=38)	Hudson River[7] (n=97)	Sheboyg. River[8] (n=20)
28		15	47	52	4	17		
52		29	35	44	7	25		
101		14	20	39	10	27		
138		18	16	34	18	15		
153		14	36	34	15	25		
180		3	12	21	10	13		
Σ6PCB	507	93	166	224	64	122		
totalPCBs*	2,535	465	830	1,120	320	570	10,000	150,000

[1]Data from Götz et al., 1990b.
[2]Sampling locations mainly downstream of Hamburg Harbor, samples consisted of suspended solids, data from Sturm and Gandrass, 1988.
[3]The Lippe River (Germany) is a tributary of the Rhine River, data from Friege et al., 1989.
[4]Lake Ketelmeer (The Netherlands) is a downstream sedimentation area of the Rhine River, data from Winkels et al., 1993.
[5]Concentration in suspended solids, collected in the period 1984-1991, 5% organic carbon content; B. van Eck, Tidal Waters Devision, Rijkswaterstaat, Middelburg, The Netherlands, personal communication.
[6]Data from Oliver and Niimi; 1988, total PCB concentration is the sum of 67 identified congeners.
[7]Total PCB measured as Aroclor 1242, data from Bopp et al., 1981.
[8]Sheboygan River, total PCB measured as Aroclor 1248, data from Blasland et al., 1992.
*Total PCBs = 5xΣ6PCBs

Sediment pollution with PCDDs and PCDFs has been frequently reported in recent years but, unfortunately, the information has been expressed in many different ways (sum of homologues, toxicity equivalences, etc.), not always allowing the reconstruction of the

concentrations of the individual toxic congeners. Generally, the concentrations of the highly toxic, less chlorinated dioxins and furans are 1-2 orders of magnitude below the concentrations of the hepta and octachlorinated isomers (Table 1.3).

Table 1.3. Mean concentrations of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in surficial sediments (ng/kg d.w.)

PCDD/Fs	Locations			
	Hamburg Harbor[1] (n=5)	Elbe River[2] (n=8)	Lake Ketelmeer[3] (n=5)	Scheldt Estuary[4] (n=2)
dioxins				
2,3,7,8-tetra-	375	2	10	<4
1,2,3,7,8-penta-	77	3	<10	<6
1,2,3,4,7,8-hexa-	100	5	10	2
1,2,3,6,7,8-hexa-	386	26	10	6
1,2,3,7,8,9-hexa-	560	24	40	6
1,2,3,4,6,7,8-hepta-	1906	771	300	66
octa-	7560	4156	3600	372
furans				
2,3,7,8-tetra-	86	70	35	13
1,2,3,7,8-penta-	328	109	35	11
2,3,4,7,8-penta-	126	24	12	9
1,2,3,4,7,8-hexa-	587	210	80	15
1,2,3,6,7,8-hexa-	250	180	45	11
1,2,3,7,8,9-hexa-	21	9	<10	3
2,3,4,6,7,8-hexa-	78	20	23	8
1,2,3,4,6,7,8-hepta-	1378	1391	390	85
1,2,3,4,7,8,9-hepta-	267	124	50	<8
octa-	2712	5933	3500	245

[1]Data from Götz et al., 1990a.
[2]Data from Götz et al., 1993.
[3]Lake Ketelmeer (The Netherlands) is a downstream sedimentation area of the Rhine River, sediment deposited around 1985, data from Beurskens et al., 1994.
[4]Downstream area of the Scheldt, data from Evers et al., 1993.

Naturally occurring halogenated organic compounds

It is apparent that the contribution of humans to the pool of halogenated compounds is exerting a major impact on the environment. However, this contribution should be set against the background of natural abiotic (Symonds et al., 1988) and biotic production (Petty, 1971; Lovelock, 1975; de Jong et al., 1994). Natural sources such as forest fires

and decomposition of seaweed release 10 to 100 times more chloromethane than that manufactured by the chemical industry (Leisinger, 1983). The chemical diversity of naturally occurring halogenated compounds is impressive (Fig. 1.2). Some commercially important chlorinated antibiotics are produced by bacteria, for example, chlortetracycline and chloramphenicol (Neidleman, 1975). Common wood- and forest litter-degrading fungi produce chlorinated anisyl metabolites (Fig. 1.2). These compounds occur in the environment at concentrations up to 180 mg/kg dry weight (De Jong et al., 1994). Chlorinated anisyl metabolites can be detoxified by microbial mineralization and incorporation into humus (de Jong et al., 1994). Besides these detoxification reactions, microbial metabolism of chlorinated anisyl metabolites may result in potentially more toxic compounds, for example, chlorinated dioxins, as recently postulated by de Jong et al. (1994). The presence of dioxins in the environment may therefore not be of exclusive anthropogenic origin. The production of chlorinated dioxins from chlorophenols during wastewater treatment and composting suggests a similar mechanism (Öberg et al., 1993).

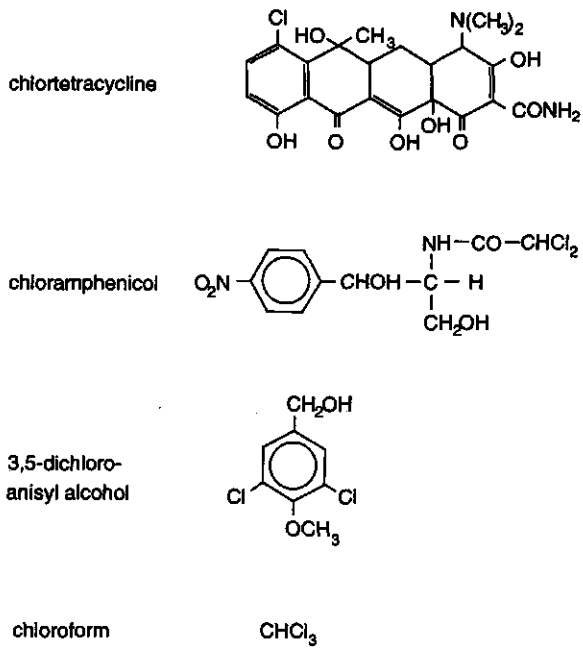


Fig. 1.2. Structures of some chlorinated metabolites produced by microorganisms.

The presence of natural halogenated compounds in the environment, in many instances as agents produced to inhibit the growth of competing or pathogenic species, acts as a major selective pressure for the evolution of detoxification mechanisms. This may be exemplified by the biodehalogenation of 2,4-dichlorophenol observed in marine sediments that contain natural sources of halophenols produced as bactericidal agents (King, 1986; King, 1988). The early exposure to natural haloaromatic and aliphatic substrates resulted in the evolution of organisms capable to metabolize those compounds. There is little doubt that this is important factor in determining the fate of both natural and anthropogenic halogenated substances in the environment.

Microbial transformation

The transformation of chemical compounds by the action of living microorganisms is one of the major processes determining the fate of organic chemicals in aquatic and terrestrial environments. Microorganisms play a major role in the degradation of chemicals because of their abundance, species diversity, catabolic versatility and high metabolic activity (Alexander, 1981). Furthermore, microbial degradation is unique in contrast to nonbiological processes, such as hydrolysis or photochemical degradation, in that mineralization, or the complete conversion of organic compounds to inorganic products (H_2O, HCl, CO_2, or CH_4), is almost always due to microbial activity. This does not always occur; however, processes may be restricted to transformation reactions in which only small structural changes in the substrate take place. Nevertheless, these transformation reactions may be of great environmental significance if the toxicity or the reactivity of the compound is affected.

Microbial transformation of organic pollutants in the environment is influenced by many factors that can be grouped into three classes: compound properties, and microbial and environmental factors. The impact some of these factors have, will be mentioned briefly. These aspects are discussed in more depth in a number of reviews (Klecka, 1985; Mohn and Tiedje, 1992; Neilson, 1990; Wilson and Jones, 1993).

Compound properties like molecular size and structure determine the susceptibility of a compound for microbial attack. The aqueous solubility determines the availability of a compound for microbial uptake. Chlorinated aromatics are hydrophobic and tend to sorb to sediments, such that only a small fraction of the compound may actually be in the water-phase. It is generally assumed that freely dissolved substrates are readily available for microorganisms. Consequently, mass transport and desorption from sediment may limit the

overall degradation rate, as recently demonstrated for the aerobic biodegradation of α-hexachlorocyclohexane in soil slurries (Rijnaarts et al., 1990). *Microbial factors* include aspects like species composition and microbial densities in a specific environment. In polluted environments the degradation capabilities of the native populations may be more diverse and degradation rates higher than in unpolluted environments, as demonstrated for polycyclic aromatic hydrocarbon degradation (Heitkamp and Cerniglia, 1987). *Environmental factors* that affect microbial transformation are, for example, pollutant concentration, redox potential, temperature and pH. Substrate concentration generally influences the rate of metabolic reactions, and is therefore of particular significance. At extreme high or low concentrations anomalies may exist. High concentrations of pollutants may be toxic even to the organisms which can metabolize them. At low concentrations a threshold may exist below which rates of biotransformation are extremely slow or non-existent (Hoover et al., 1986). The presence or absence of molecular oxygen determines the activity of microbial communities with distinct metabolic pathways (Zehnder & Svensson, 1986). Consequently, anaerobic microbial populations may have degradative abilities that are not found in aerobic microbes.

Microbial transformation of chlorinated aromatics under anaerobic conditions

Higher chorinated aromatic compounds that have been released into waterways are largely resistant to biotic and abiotic transformation reactions in the aerobic water column. Finally, these compounds partition into aquatic sediments due to their hydrophobic properties. Only a thin sediment layer (maximally a few cm) at the sediment-water interphase in most eutrophic rivers and lakes in northwestern Europe contains oxygen. In the deeper layers oxygen is depleted and anaerobic conditions prevail. Under these conditions, reductive transformations (i.e. reactions that involve the transfer of electrons to the substrate) may occur. These reactions can be catalyzed by anaerobic bacteria using the halogenated compounds as external electron acceptors. This makes reductive dehalogenation potentially a very important transformation reaction for halogenated aromatic compounds in sediments. Four reaction types in which reductive processes are involved can be distinguished (Holliger, 1992). The first reaction, the hydrogenolysis, replaces a halogen substituent in a molecule with a hydrogen atom (Fig. 1.3). Removal of alkyl and aryl halogens has been demonstrated to occur by this process. The next three reaction types have been found for the removal of alkyl halogens only. Since halogenated aliphatic compounds were not considered in this research, these reaction types are only mentioned

briefly. The second reaction type is a vicinal reduction, also known as dihalo-elimination. This reaction encompasses the removal of two halogen substituents from adjacent carbon atoms and the formation of a double bond between the carbon atoms (Fig. 1.3). The third type is a coupling and may occur when free radicals are present. The fourth type, a hydrolytic reduction, involves the formation of a carbenoid followed by hydrolysis (Holliger, 1992).

Reductive dehalogenation has been demonstrated in the laboratory in recent years for a variety of halogenated aromatic compounds, like chlorinated benzoates, phenols, benzenes, anilines and biphenyls (Mohn and Tiedje, 1992, including references cited). Most of these studies were conducted with undefined sediment slurries or enriched mixed cultures obtained from freshwater sediments, indicating the microbial potential to mediate these reactions in polluted sediments.

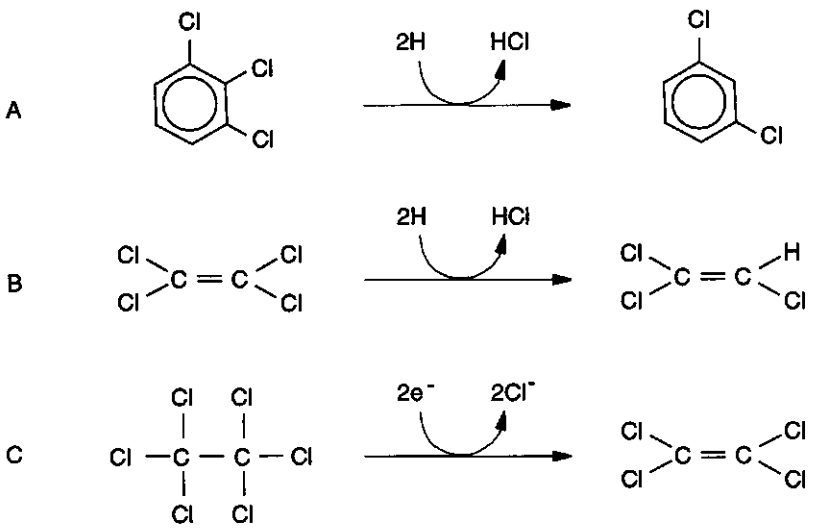


Fig. 1.3. Examples of reductive dechlorination reaction types: aryl hydrogenolysis of 1,2,3-trichlorobenzene to 1,3-dichlorobenzene (A); alkyl hydrogenolysis of tetrachloroethylene to trichloroethylene (B), and alkyl dihaloelimination of hexachloroethane to tetrachloroethylene (C).

Research questions and outline of this thesis

The presence of polychlorinated benzenes, biphenyls, dibenzo-*p*-dioxins and furans in sediments is generally interpreted as evidence for a long-term persistence. On the other hand, laboratory studies indicate significant microbial dechlorination of chlorinated benzenes and biphenyls under anaerobic conditions. In addition, *in situ* dechlorination of

polychlorinated biphenyls has been demonstrated in polluted sediments in the US. On the basis of structural similarities between chlorinated biphenyls and chlorinated dibenzo-*p*-dioxins, microbially mediated dechlorination of the latter compound group is expected to be possible. The question then arises: To what extent are chlorinated aromatics subject to microbial dechlorination in the polluted, anaerobic sediment layers in the Netherlands?

The presumed long-term persistence of chlorinated aromatics in sediments is rarely verified quantitatively. Recent environmental policies, however, are based on the assumption of an absolute persistence of these priority pollutants even if time scales of centuries are applied, for example, in risk assessments of pollutant dispersal from sediments. In fact, the time period that these chemicals are in the environment has been restricted to the last 3 to 4 decades. Thus extrapolation periods in environmental models exceed the period that these compounds are actually present in the environment many times. Taking into account that analytical techniques and research methodologies to study the environmental behaviour of chlorinated aromatics have become fully available only since the early 1980s, we must appreciate the high uncertainty of these modelled results.

In this research the microbial dechlorination of chlorinated aromatics (CBs, PCBs, PCDDs and PCDFs) in polluted anaerobic sediments has been studied in detail. A dual approach was used, in which both field and laboratory studies were essential to verify the occurrence of *in situ* microbial dechlorination. The following research questions will be addressed in this thesis:

- Are there any indications that dechlorination of chlorinated aromatics in anaerobic sediment layers occurs in the Netherlands and, if so, what are the dechlorination rates?
- Are the native, anaerobic microbial populations capable of mediating dechlorination reactions; which reactions take place and what are the products?
- Does a general reaction pattern in dechlorination reactions exist and can it be rationalized?
- What are the consequences, from an environmental point of view, of a possible *in situ* microbial dechlorination in sediments?

Chapter 2 describes the levels of some chlorinated biphenyls, dioxins, furans and other priority pollutants in dated sediment cores from Lake Ketelmeer, a sedimentation area of the Rhine River. The persistence of the chlorinated aromatics was verified with the aid of stored sediment top-layer samples collected in 1972. The same approach was applied to construct profiles of chlorinated benzenes (Chapter 3). In addition, sediment incubations were conducted to verify the dechlorination capabilities of the anaerobic microbial populations towards hexachlorobenzene. In Chapter 4 it is shown that an anaerobic

enrichment culture from Lake Ketelmeer sediment preferentially mediates the thermodynamic most profitable chlorobenzene dechlorinations. The same enrichment culture was incubated with several toxic PCB congeners and selective dechlorination (Chapter 5), in agreement with a selective disappearance observed in the sediment cores (Chapter 2),was demonstrated. Dechlorination of a model dioxin, 1,2,3,4-tetrachloro-dibenzo-*p*-dioxin by the same enrichment from Lake Ketelmeer sediment is shown in Chapter 6. From an estuarine sediment (Delfzijl harbour) a hexachlorobenzene dechlorinating microbial population was obtained under sulfate-reducing conditions (Chapter 7). In Chapter 8 the toxicological consequences and implications for environmental policies are discussed for *in situ* PCB dechlorination. Finally, in Chapter 9 the results obtained in the preceding chapters are discussed in relation to recent data from the literature. Estimates for half-lives of chlorinated aromatics in sediments, which may provide a helpful overview for modelling the fate of chlorinated aromatics in sediments, are listed. With the aid of thermodynamics a first attempt is made to rationalize the selectivity in dechlorination reactions. Prospects for biological treatment technologies that include reductive dehalogenation are briefly discussed.

References

Alcock, R.E., A.E. Johnston, S.P. McGrath, M.L. Berrow, K.C. Jones, 1993. Long-term changes in the polychlorinated biphenyl content of United Kingdom soils. Environ. Sci. Technol., 27, 1918-1923.

Alexander, M., 1981. Biodegradation of chemicals of environmental concern. Science 211, 132-138.

Beurskens, J.E.M., H.J. Winkels, J. de Wolf, C.G.C. Dekker, 1994. Trends of priority pollutants in the Rhine during the last 50 years. Water Science and Technology, 29, 77-85.

Berg, M. van den, B.L.H.J. Craane, T. Sinnige, S. van Mourik, S. Dirksen, T. Boudewijn, M. van der Gaag, I.J. Lutke-Schipholt, B. Spenkelink, A. Brouwer, 1994. Biochemical and toxic effects of polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) in the cormorant (Phalacrocorax carbo) after in ovo exposure. Environ. Toxicol. Chem., Environ. Toxicol. Chem., 13, 803-816.

Blasland & Bouck Engineers, 1992. Sheboygan river and harbour biodegradation pilot study work plan. Internal Report.

Boon, J.P., P.J.H. Reijnders, J. Dols, P. Wensvoort, M.T. Hillebrand, 1987. The kinetics of individual polychlorinated biphenyl congeners in female harbour seals (Phoca vitulina), with evidence for structure related metabolism. Aq. Toxicol., 10, 307-324.

Bopp, R.F., H.J. Simpson, C.R. Olsen, N. Kostyk, 1981. Polychlorinated biphenyls in sediments of the tidal Hudson River, New York. Environ. Sci. Technol., 15, 210-216.

Brouwer, A., P.J.H. Reijnders, J.H. Koeman, 1989. Polychlorinated biphenyl (PCB)-contaminated fish induces vitamin A and thyroid hormone deficiency in the common seal (Phoca vitulina). Aquat. Toxicol., 15, 99-106.

Brown, J.F. Jr., R.E. Wagner, H. Feng, D. Bedard, M.J. Brennan, J.C. Carnahan, R.J. May, 1987. Environmental

dechlorination of PCBs. Environ. Toxicol. Chem. 6, 579-593.

Dennis, D.S., 1976. Conf. Proc. National Conference of Polychlorinated Biphenyls 1975.

Evers, E.H.G., 1989. The formation of polychlorinated dibenzofurans, polychlorinated dibenzo-p-dioxins and related compounds during oxy-hydrochlorination of ethylene. Technical report MTC/89EE. University of Amsterdam, Amsterdam, The Netherlands.

Evers, E.H.G., H.J.C. Klamer, R.W.P.M. Laane, H.A.J. Govers, 1993. Polychlorinated dibenzo-p-dioxin and dibenzofuran residues in estuarine and coastal North Sea sediments: sources and distribution. Environ. Toxicol. Chem., 12, 1583-1598.

Friege, H., W. Stock, J. Alberti, A. Poppe, I. Juhnke, J. Knie, W. Schiller, 1989. Environmental behaviour of polychlorinated mono-methyl-substituted diphenyl-methanes (Me-PCDMs) in comparison with polychlorinated biphenyls (PCBs). II Environmental residues and aquatic toxicity. Chemosphere, 18, 1367-1378.

Götz, R., E. Schumacher, L.O. Kjeller, P.-A. Bergqvist, C. Rappe, 1990a. Polychlorierte dibenzo-p-dioxine (PCDDs) und polychlorierte dibenzofurane (PCDFs) in sedimenten und fischen aus dem Hamburger Hafen. Chemosphere, 20, 51-73.

Götz, R., E. Schumacher, K. Roch, W. Specht, R.D. Weeren, 1990b. Chlorierte kohlenwasserstoffe (CKWs) in Hamburger hafensedimenten. Vom Wasser, 75, 393-415.

Götz, R., P. Friesel, K. Roch, O. Päpke, M. Ball, A. Lis, 1993. Polychlorinated-p-dioxins (PCDDs), dibenzofurans (PCDFs), and other chlorinated compounds in the river Elbe: results on bottom sediments and fresh sediments collected in sedimentation chambers, Chemosphere, 27, 105-111.

Heitkamp, M.A., C.E. Cerniglia, 1987. Effects of chemical structure and exposure on the microbial degradation of polycyclic aromatic hydrocarbons in freshwater and estuarine ecosystems. Environ. Toxicol. Chem., 6, 535-546.

Holliger, C., 1992. Reductive dehalogenation by anaerobic bacteria. Thesis Agricultural University Wageningen, The Netherlands.

Hong, C.-S, B. Bush, J. Xiao, H. Qiao, 1993. Toxic potential of non-ortho and mono-ortho coplanar polychlorinated biphenyls in Aroclors, seals, and humans. Arch. Environ. Contam. Toxicol., 25, 118-123.

Hoover, D.G., G.E. Borgonovi, S.H. Jones, M. Alexander, 1986. Anomalies in mineralization of low concentrations of organic compounds in lake water and sewage. Appl. Environ. Microbiol., 51, 226-232.

IKSR, 1989. 10. Rheinministerkonferenz, Brussels, 29-30 November 1989.

Jones, K.C., G. Sanders, S.R. Wild, V. Burnett, A.E. Johnston, 1992. Evidence for a decline of PCBs and PAHs in rural vegetation and air in the United Kingdom. Nature, 356, 137-140.

Jones, K.C., S.R. Wild, 1991. Organic chemicals entering agricultural soils in sewage sludges: screening for their potential to transfer to crop plants and livestock. Foundation for Water Research Report no. FR0169, Water Research Centre, Medmenham, UK.

Jong, E. de, J.A. Field, H.-E. Spinnler, J.B.P.A. Wijnberg, J.A.M. de Bont, 1994. Significant biogenesis of chlorinated aromatics by fungi in natural environments. Appl. Environ. Microbiol. 60, 264-270.

Kimbrough, R.D., A.A. Jensen, 1989. Halogenated biphenyls, terphenyls, naphthalenes, dibenzodioxins and related products. Elsevier, Amsterdam.

King, G.M., 1986. Inhibition of microbial activity in marine sediments by a bromophenol from hemichordate, Nature, 323, 257-259.

King, G.M., 1988. Dehalogenation in marine sediments containing natural sources of halophenols. Appl. Environ. Microbiol., 54, 3079-3085.

Klecka, G.M., 1985. Biodegradation, In: Environmental exposure from chemicals, Vol. I, W. Brock Neely and G.E. Blau (Eds.), CRC Press Inc. Boca Raton, Florida, pp 109-155.

Kubiak, T.J., H.J. Harris, L.M. Smith, T.R. Schwartz, D.L. Stalling, J.A. Trick, L. Sileo, D.E. Docherty, T.C. Erdmann, 1989. Microcontaminants and reproductive impairment of the Forster's tern on Green Bay, Lake Michigan. Arch. Env. Contam. Toxicol., 18, 706-727.

Leeuwen, C.J. van, D.M.M. Adema, J. Hermens, 1990. Quantitative structure-activity relationships for fish early life stage toxicity. Aquat. Toxicol., 16, 321-334.

Leisinger, T., 1983. Microorganisms and xenobiotic compounds. Experientia, 39, 1183-1191.

Lovelock, J.E., 1975. Natural halocarbons in the air and in the sea, Nature, 256, 193-195.

Mohn, W.W., J.M. Tiedje, 1992. Microbial reductive dehalogenation. Microbiol. Rev., 56, 482-507.

Neidleman, S.L., 1975. Microbial halogenation. CRC Crit. Rev. Microbiol., 5, 333-358.

Nellson, A.H., 1990. The biodegradation of halogenated organic compounds, a review. J. Appl. Bacteriol., 69, 445-470.

Öberg, L., R. Andersson, N. Wagman, C. Rappe, 1993. Formation of polychlorinated dibenzo-p-dioxins and dibenzofurans from chloroorganic precursors in activated sewage sludge and garden compost. Presented at the International Conference on Naturally Produced Organohalogens, 19 to 24 September 1993, Delft, The Netherlands.

Oliver, B.G., K.D. Nicol, 1982. Chlorobenzenes in sediments, water, and selected fish from lakes Superior, Huron, Erie, and Ontario. Environ. Sci. Technol., 16, 532-536.

Oliver, B.G., A.J. Niimi, 1983. Bioconcentration of chlorobenzenes from water by rainbow trout: correlations with partition coefficients and environmental residues. Environ. Sci. Technol., 17, 287-291.

Oliver, B.G. and A.J. Niimi, 1988. Tropodynamic analysis of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in Lake Ontario ecosystem. Environ. Sci. Technol., 22, 388-397.

Petty, M.A., 1961. An introduction to the origin and biochemistry of microbial halometabolites. Bact. Rev., 25, 111.

Rapaport, R.A., S.J. Eisenreich, 1988. Historical atmospheric inputs of high molecular weight chlorinated hydrocarbons to eastern North America. Environ. Sci. Technol., 22, 931-941.

Reijnders, P.J.H., 1986. Reproductive failure in common seals feeding on fish from polluted coastal waters. Nature, 324, 456-457.

Rijnaarts, H.H.M., A. Bachmann, J.C. Jumelet, A.J.B. Zehnder, 1990. Effect of desorption and intraparticle mass transfer on the aerobic biomineralization of alpha-hexachlorocyclohexane in a contaminated calcareous soil. Environ. Sci. Technol., 24, 1349-1354.

Safe, S. 1990. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit. Rev. Toxicol. 21, 51-88.

Silberhorn, E.M., H.P. Glauert, L.W. Robertson, 1990. Carcinogenity of polyhalogenated biphenyls: PCBs and PBBs. Crit. Rev. Toxicol., 20, 439-496.

Sturm, R., J. Gandrass, 1988. Verhalten von schwerflüchtigen chlorkohlenwasserstoffen an schwebstoffen des Elbe-ästuars. Vom Wasser, 70, 265-280.

Symonds, R.B., W.I. Rose, M.H. Reed, 1988. Contribution of Cl- and F bearing gases to the atmosphere by volcanoes. Nature, 334, 415-417.

Tanabe, S., 1988. PCB problems in the future: foresight from current knowledge. Environ. Pol. 50, 5-28.

Tiedje, J.M., S.A. Boyd, B.Z. Fathepure, 1987. Anaerobic degradation of chlorinated aromatic hydrocarbons. Dev. Indus. Microbiol. 27, 117-127.

Vogelgesang, J., K. Kypke-Hutter, R. Malisch, P. Binneman, W. Dietz, 1986. The origin of a contamination of fish from the river Neckar with hexachlorobenzene, octachlorostyrene and pentachlorobenzene: Formation in an industrial process. II The formation of contaminants in the degassing of an aluminium foundry with chlorine. Z. Lebensm. Unters. Forsch. 182, 471-474.

Voogt, P. de, D.E. Wells, L. Reutergardh, U.A.T. Brinkman, 1990. Biological activity, determination and occurrence of planar, mono- and di-ortho PCBs. Intern. J. Environ. Anal. Chem. 40, 1-46.

Watanabe, I., T. Kashimoto, R. Tatsukawa, 1986. Hexabromobenzene and its debrominated compounds in river and estuary sediments in Japan. Bull. Environ. Contam. Toxicol., 36, 778-784.

Weiden, M.E.J. van der, H.J.H. Tibosch, R. Bleumink, T.L. Sinnige, C. van de Guchte, W. Seinen, M. van den Berg, 1993. Cytochrome P450 1A induction in the common carp (Cyprinus carpio) following exposure to contaminated sediments with halogenated polyaromatics. Chemosphere, 27, 1297-1309.

Wilson, S.C., K.C. Jones, 1993. Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): a review. Environ. Pollut., 81, 229-249.

Winkels, H.J., J.P.M. Vink J.E.M. Beurskens, S.B. Kroonenberg, 1993. Distribution and geochronology of priority pollutants in a large sedimentation area, River Rhine, The Netherlands. Applied Geochem., Suppl. Issue, 2, 95-101.

Zehnder, A.J.B., B.H. Svensson, 1986. Life without oxygen: what can and what cannot? Experientia 42, 1197-1205.

Zoest, van R., G.T.M. van Eck, 1991. Occurrence and behaviour of several groups of organic micropollutants in the Scheldt estuary. The Science of the Total Environment, 103, 57-71.

CHAPTER 2

GEOCHRONOLOGY OF PRIORITY POLLUTANTS IN A SEDIMENTATION AREA OF THE RHINE RIVER

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ABSTRACT

Eight sediment cores were taken from Lake Ketelmeer, a sedimentation area of the Rhine River, located in the central part of the Netherlands. Priority pollutants (8 metals, 6 planar and mono-ortho polychlorinated biphenyls, 7 polychlorinated dibenzo-*p*-dioxins, 10 polychlorinated dibenzofurans and 8 polycyclic aromatic hydrocarbons) were determined in all or in a selected number of cores. Present-day and historical levels of pollutants since the late 1930s were established through the use of radionuclide time tracers (^{137}Cs, ^{134}Cs) and area-specific geological time markers. Postdepositional redistribution of pollutants and possible transformations were evaluated by analyzing sediment top-layer samples that were taken in 1972. Disappearance in the anaerobic sediment was observed for several chlorinated biphenyls, dioxins, and furans. Disappearance of the chlorinated compounds may be caused by microbial dechlorination reactions in the anaerobic lake sediment. For the persistent metals and polycyclic aromatic hydrocarbons as well as for the somewhat changed concentrations of chlorinated aromatics, trends in the concentration profiles during the last five decades are described. Rather low concentrations of almost all studied chlorinated compounds were observed in the early 1940s. These low levels were in contrast to the metal and PAH concentrations, which were already high in the late 1930s and were lowered during the second world war. For all studied compounds, maximum concentrations were found between 1955 and 1975. Cadmium and nickel levels remained high until 1980. The highly toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin reached concentrations up to 400 ng/kg in the mid 1960s. Recently deposited sediments showed lower pollutant levels. The levels of lead, arsenic, and all studied PAHs were the lowest observed in the past five decades.

INTRODUCTION

A wide range of chemical substances, including heavy metals, radionuclides, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polycyclic aromatic hydrocarbons (PAHs) can be detected in natural aquatic environments. These pollutants originate from a variety of direct and indirect sources. The high affinity of these pollutants for particles within the water column results in relatively high pollution levels of the suspended solids. Where stream velocities decrease, for example in the lower stretches of rivers, the suspended solids settle with the associated pollutants. Once delivered to the bottom sediments, particle and pollutant burial will be affected by resuspension and bioturbation. Sediment mixing by zoobenthos at the sediment-water interface may lower pollutant concentration peaks and distribute pollutants vertically, as can be observed in sediment cores from areas with high biological activity and relatively low sedimentation rates [1]. Other processes such as molecular diffusion, transport with infiltrating water, and biotransformation of the organic pollutants, may also alter the input history of a pollutant recorded in the sediment profile.

Dated sediment cores have the potential for providing detailed chronologies of pollutant input as long as bioturbation, molecular diffusion, transport with water and biotransformation are (or may be considered to be) negligible. Based on dated sediment cores, the depositional history of metals, PCBs, PCDDs, PCDFs, and PAHs has been documented for the North American Great Lakes [2-5]. On the other hand, information on the pollution history of European rivers and lakes is relatively scarce. For the Rhine River, which drains a large industrialized area in Europe, no extensive sediment core studies are available. Only for a limited period did water-quality monitoring programs provide some historical information. These programs started at the end of the 1970s and became fully equipped during the 1980s. Decreasing concentrations of many priority pollutants in Rhine water have been observed since [6]. However, the maximum levels and their periods of occurrence in the past remain unknown. These high levels may still exist in deeper sediment layers of sedimentation areas. The objectives of the present study are to quantify the pollution levels of sediments in a sedimentation area of the Rhine River that have been deposited during the last 50 years, and to verify the occurrence of post-depositional transport and transformation processes that may change the pollution levels in the sediment.

The Rhine, one of the major European rivers, has a drainage basin that covers large areas of Switzerland and Germany and smaller areas of Austria, France, Belgium, Luxemburg, and the Netherlands (Fig. 2.1). In the Netherlands several side branches can be

distinguished in the lower course of the Rhine before it reaches the North Sea. The northern Rhine branch, the IJssel River, flows to Lake Ketelmeer. No specific point sources of pollutants are known along the IJssel River and Lake Ketelmeer. The geological history of the lake is well documented. It was created as the indispersable outlet for the IJssel water between two polders, that were reclaimed from Lake IJsselmeer between 1930 and 1960. Since the completion of the lake in the early 1950s, it has acted as a major sedimentation area for the entering IJssel water.

In the present study, concentration profiles were constructed for numerous priority pollutants in Lake Ketelmeer sediment. The cores contained sediment layers from the late 1930s onward. The possible influences of molecular diffusion, contaminant transport with infiltrating water, biotransformation, and bioturbation on the pollutant profiles were evaluated in the following manner: (a) Top-layer samples from 1972 were analyzed with present-day techniques. If pollutant levels of these samples fit in the constructed profiles, no serious alterations have occurred in the sediment. (b) Pollutant levels were determined in the originally unpolluted sea bottom directly beneath the recently deposited sediment. Elevated levels of pollutants in these layers would indicate the occurrence of downward pollutant transport. (c) Information from literature on biotransformation under anoxic conditions was taken into account.

This paper presents long-term changes in Rhine River pollution, including eight metals, six planar and mono-ortho PCBs, seven PCDDs, ten PCDFs and eight PAHs. The results give insight into pollution levels that occurred, for example, in the early 1970s, when undiluted Rhine water caused acute toxic effects in laboratory tests [7] but appropriate analytical methods were lacking. In addition, indications can be obtained for the pollution levels in areas where sedimentation patterns have changed and for example sediment layers from the 1960s remain uncovered.

MATERIALS AND METHODS

Study area

Lake Ketelmeer is a shallow, freshwater lake (surface area: 38 km^2) in the central part of The Netherlands. The lake has an open connection with Lake IJsselmeer (Fig. 2.1). Both lakes formed part of a coastal sea (the Zuiderzee) until the closure of a barrier dam in 1932, which created the Lake IJsselmeer. Land reclamation created two polders within Lake IJsselmeer, with Lake Ketelmeer as the outlet of the IJssel River situated between them. The northern dike of Lake Ketelmeer was completed in 1938 and the southern dike

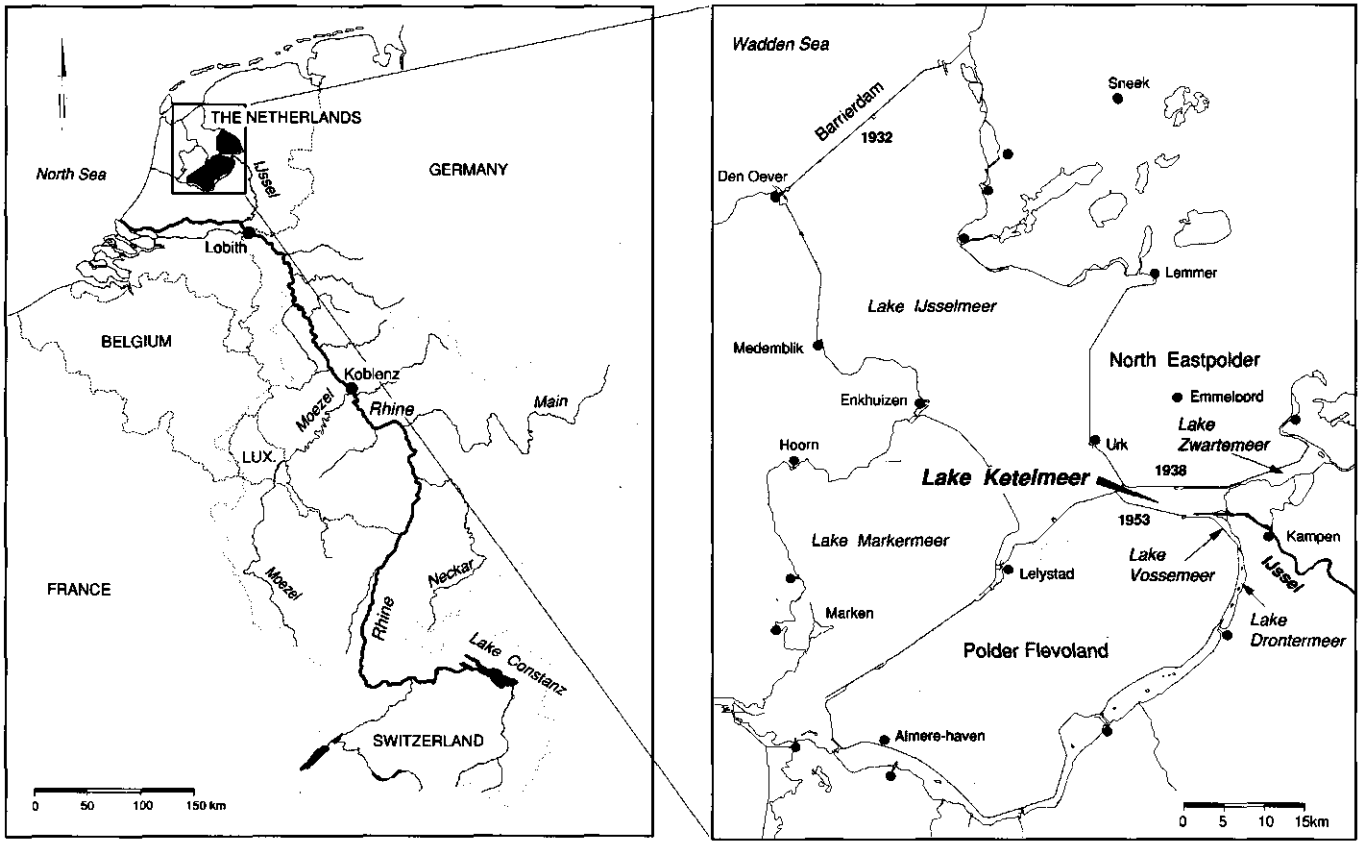

Fig. 2.1. The Rhine River drainage basin and the northern branch, the IJssel River, leading to Lake Ketelmeer. The years of completion of the different dikes are indicated.

in 1953. The most recent geological layer in this area, the IJsselmeer (IJm) deposit, is definedas the silty sediment deposited since 1932. The underlying Zuiderzee (Zu) deposit, a saltwater clay deposit, can be identified by the presence of shellfragments (*Mya arenaria*). The average thickness of the IJm-deposit is 0.45 m. The net sedimentation rate (presently approximately 0.01 m per year) has doubled since 1953 and accelerated deposition occurs in deeper zones, for example in the sandpits (approximately 0.15 m per year) [8]. The sediment cores were taken at locations known for their thick IJm-deposits. The majority of Lake Ketelmeer sediment is anaerobic; only the top- layer (1-3 cm) at the interface with the water is aerobic. Chemical characterization of the IJm-deposit indicates that illite is the dominant clay mineral (60% w/w), with smaller contributions of montmorillonite (20% w/w) and kaolinite (20% w/w) [9].

Sample collection and treatment

In 1988 and 1990 eight undisturbed sediment cores (0.15 m in diameter and average length of approximately 1 m) were taken with an open auger. The cores, containing mainly the IJm-deposit, were sectioned into 0.05 to 0.10 m intervals. One core, taken from a former sandpit, was sectioned into 0.25 m intervals. At this location the thickness of the IJm-deposit was about 4.2 m. Samples were put in glass jars with screw caps, refrigerated at 4°C and transported. Before subsamples were taken for the different chemical analyses, samples were freeze dried and homogenized.

The sediment top-layer samples (0.05 m) from 1972 were collected by the Institute for Soil Fertility Research, Haren, the Netherlands. The 10 samples taken in 1972 originate from the same areas as the eight sediment cores taken for this study. After collection in 1972, the top-layer samples were dried overnight at 40°C and stored in jars with screw caps at room temperature in the dark. Losses of organic pollutants during the drying procedure were negligible [10]. In these top-layer samples only some heavy metals were determined soon after collection. All organic pollutants were recently measured together with the core samples.

Sediment dating

The age of the different layers in the sediment cores was estimated by several methods. First, the well known geological history of this area offers some valuable recognition points in the cores. The interface between Zu-deposit and IJm-deposit is visually recognizable and indicates the early 1930s. Until 1953, when the southern dike of Lake Ketelmeer was

completed, sedimentation occurred over a much larger area. As a result, only a thin sediment layer represents the period of 1930 to 1955, except for one location, a former sandpit, that was created for the construction of the northern dike. After completion of this dike (1938), the sandpit acted as a sediment trap with high sedimentation rates (approximately 0.15 m per year). Second, ^{137}Cs and ^{134}Cs gamma activities were determined on 25 to 250 cm^3 of freeze dried sediment by counting up to 1000 min with a coaxial G detector (P-type) coupled to a multichannel analyzer. The Canberra S 340 DOS/SPECTRAN-AT application software package was used for operation of the system and analysis of the recorded gamma spectra. Third, heavy metal concentrations in sediment layers were related to the well known metal pollution history of the Rhine River. The use of the ^{210}Pb-dating technique [11] proved to be problematic in these cores, probably due to high and variable discharges of ^{210}Pb or mother nuclides (^{226}Ra) in the Rhine River.

Analysis of organic carbon and heavy metals

The organic carbon content of the sediment samples was measured by an element analyser (Carlo Elba NA 1500, Milan, Italy) after removal of carbonates with phosphoric acid. Heavy metal contents of the freeze-dried sediment core samples were determined after sample treatment with strong acids: hydrochloric acid for Cd and Pb, a mixture of sulfuric acid, nitric acid and hydrogen peroxide for As, Cr, Cu, Ni, and Zn; and a mixture of sulfuric acid, nitric acid, and potassium persulfate for Hg. Cadmium, Cr, Cu, Ni and Pb were analyzed by graphite furnace atomic absorption spectrometry (Perkin Elmer 5000, Norwalk, CT). Zinc was analyzed by flame atomic absorption spectrometry (Perkin Elmer 5000). Arsenic was analyzed using the hydride technique (Perkin Elmer 5000 + MHS1) and Hg by a mercury monitor (Milton Roy, HGM 2300, Rochester, NY). Zinc, Cu, Cd, Pb and Cr contents in the top-layer samples from 1972 were determined at the Institute for Soil Fertility Research by similar analytical methods [10].

Analysis of PCBs, PCDDs and PCDFs

The planar and mono-ortho PCBs, PCDDs, and PCDFs were determined in all layers of three selected cores and in five top-layer samples from 1972. The analyses of 3,3',4,4'-tetrachlorobiphenyl (PCB 77), 2,3,3',4,4'-pentachlorobiphenyl (PCB 105), 2,3',4,4',5-pentachlorobiphenyl (PCB 118), 3,3',4,4',5-pentachlorobiphenyl (PCB 126), 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156), and 3,3',4,4',5,5'-hexachlorobiphenyl (PCB 169) were

performed in combination with the analyses of PCDDs and PCDFs. Sediment sample cleanup and analytical procedures for PCDDs and PCDFs determinations were adopted from the literature [12,13], however, some minor modifications were applied. After spiking the freeze dried samples with ^{13}C-labeled internal standards (Cambridge Isotope Laboratories, Woburn, MA) representing each tetra- through octachlorinated homolog of PCDDs and PCDFs, a Soxhlet extraction with toluene was applied for 48 h. Evaporation was used to reduce the volume of the toluene extract and *n*-decane was added as a carrier solvent. Extract cleanup consisted of a passage through six chromatographic columns in sequence. The fifth column contained carbon dispersed on Celite® (545-AW, Supelco, Bellefonte, PA). The PCDDs, PCDFs, and PCBs were removed from this column by reverse elution with toluene. All other columns contained silica or alumina with different pretreatments. The eluate volume was further reduced and a solvent exchange to tetradecane was performed.

The extracts were analyzed by GC/MS (Hewlett Packard 5890-5971, Avondale, PA) operating in the selective ion monitoring (SIM) mode. The planar and mono-ortho PCBs and hepta- and octachlorinated dioxins and furans were analyzed with a 60 m capillary column (SE 30, 0.25 mm i.d., film thickness 0.25 μm). The oven temperature program employed was as follows: injection temperature 140°C, 10°C/min to 180°C, 5°C/min to 250°C, 10°C/min to 320°C, and 5 min isothermal at 320°C. The tetra- through hexachlorinated dioxins and furans were analyzed with a 60 m column (SP 2331, 0.25 i.d, film thickness 0.22 μm) and an oven temperature program as follows: injection temperature 150°C, 20°C/min to 220°C and 6°C/min to 260°C. Helium was used as a carrier gas. Identification was based on the retention times compared to those of the authentic standard compounds and on the intensity ratios of the molecular ions equal to the theoretical values. Quantification was based on the response factors obtained from the GC-SIM of standard mixtures and the ^{13}C-labeled internal standards. Method validation included an interlaboratory calibration experiment for PCDDs and PCDFs. In Table 2.1, dioxin and furan concentrations of the most toxic isomers measured by the above procedure in a sediment sample from Lake Ketelmeer are compared with results obtained by the Department of Environmental and Toxicological Chemistry of the University of Amsterdam (The Netherlands). The methods applied at this laboratory are described by Evers et al. [14]. Although the extraction, cleanup, and mass spectrometric techniques are different, the results, with the exception of octachlorinated dibenzofuran, agree within the measurement error of the procedures.

Table 2.1. Interlaboratory comparison of dioxin and furan concentrations (in ng/kg) in a sediment sample from Lake Ketelmeer.

	This work	ETC[1]
2,3,7,8-TCDD	450	378
1,2,3,7,8-PeCDD	<10	<15
1,2,3,4,7,8-HxCDD	30	22
1,2,3,6,7,8-HxCDD	28	33
1,2,3,7,8,9-HxCDD	150	73
1,2,3,4,6,7,8-HpCDD	500	431
OCDD	3,000	3,295
2,3,7,8-TCDF	75	126
1,2,3,7,8-PeCDF	110	131
2,3,4,7,8-PeCDF	85	85
1,2,3,4,7,8-HxCDF	310	357
1,2,3,6,7,8-HxCDF	170	211
1,2,3,7,8,9-HxCDF	<10	54
2,3,4,6,7,8-HxCDF	90	125
1,2,3,4,6,7,8-HpCDF	2,700	2,116
1,2,3,4,7,8,9-HpCDF	200	133
OCDF	16,000	8,225

[1]Department of Environmental and Toxicological Chemistry, University of Amsterdam, The Netherlands.

Analysis of PAHs

The PAH compounds evaluated in this study included fluoranthene (Flu), benzo[k]-fluoranthene (BkF), benzo[a]pyrene (BaP), fluorene (Fle), benzo[b]fluoranthene (BbF), anthracene (Ant), phenanthrene (Phen), and benzo[ghi]perylene (Bghi). Sediment samples were extracted twice with acetone for 15 min. Acetone was removed by mixing the extracts with petroleum ether and washing them with water. After separation, the aqueous phase was extracted with a second portion of petroleum ether. The combined petroleum ether extract was dried with Na_2SO_4 and concentrated with a Kuderna-Danish condensor (Technoglas, Voorhout, the Netherlands) to a volume of 5 ml. The extract volume was further reduced with a gentle stream of clean nitrogen to 1 ml. Extract cleanup consisted of passing the extract through a column of 2 g 11% (w/w) deactivated alumina (alumina W200, Super I Woelm, ICN, Enschwede, Germany) and through a column of 2 g 6% (w/w) deactivated silica (Merck 7754, Darmstadt, Germany). After sample cleanup a solvent exchange to acetonitrile was carried out. The sample extracts were injected into an HPLC (Perkin Elmer pump 250 and Spark Marathon autosampler, Emmen, the Netherlands) fitted with a 25 cm Vydac 201 TP-5 column (4.6 mm i.d.). The extracts were eluted isocratically for 5 min with 50% (v/v) acetonitrile in water and subsequently with a linear gradient to

100% acetonitrile in 15 min. The mobile-phase flow was 1.5 ml/min. The column effluent was monitored with a fluorescence detector (Perkin Elmer LS40) and a UV-detector (Kratos 783, Kratos Analytical, the Netherlands).

All concentrations of radionuclides and pollutants are reported on a per-dry-weight-of-sediment basis. For purposes of numerical calculation and graphical display, all concentrations below detection levels were assumed to be one-half the detection level.

RESULTS AND DISCUSSION

Radiocesium activities

In all cores, activity of ^{137}Cs ($t_{1/2}$=30.17 years) showed two maxima. One ^{137}Cs maximum, in the samples from near the surface, always correlated with elevated activities of ^{134}Cs ($t_{1/2}$=2.06 y), indicating the fallout from the nuclear power plant accident in Chernobyl in April 1986. The second ^{137}Cs maximum, found in the deeper layers, was related to the fallout from nuclear weapon testing in the early 1960s. Besides the radiocesium activities, several other markers were used to estimate the age of the different layers. The visually recognizable interface between the IJm- and Zu-deposits indicated the year 1932. In the core taken from the former sandpit, this interface represented the year 1938. Heavy metal concentrations in sediment layers were compared to pollution levels in dated sediment samples from other locations in the Rhine [15]. Based on this information, the sediment core layers were dated into periods that varied between two and ten years. The depth of the IJm-deposit in the various sediment cores varied between 25 and 420 cm, indicating highly variable sedimentation rates.

If sedimentation rates are not identical, graphic presentation of radionuclide activities or pollutant concentrations plotted against depth is possible only for the individual cores. However, after age estimation of the different layers, data from all cores can be combined into one graph plotted to the estimated year of deposition instead of depth. This method of data handling can introduce some inaccuracy. Bioturbation or transport with infiltrating water will have less effect on sediment core pollutant profiles at a location with high sedimentation rates than locations with low sedimentation rates [16].

In figure 2.2, Cs activities are plotted against estimated year of deposition. A certain amount of variation in activities can be observed, especially in the ^{137}Cs activity of the near surface samples. Nevertheless, a clear pattern in Cs activities during the last five decades can be distinguished and is indicated by the visually fitted curves.

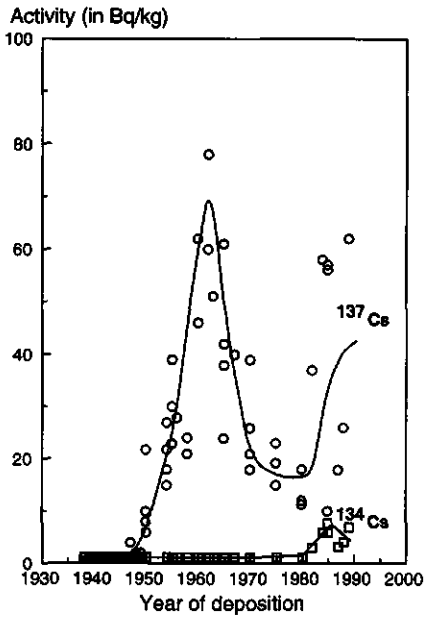

Fig. 2.2. Cesium activities in sediment layers from 8 cores vs. the estimated years of deposition. Average activities are indicated by visually fitted curves.

Organic carbon

The organic carbon (OC) content of suspended solids and sediment plays an essential role in the behavior and fate of pollutants in the aquatic environment [17]. Mineralization processes affect the organic matter in sediments. If a constant input of OC has occurred in the past, a decreasing OC content may be expected at increasing depth in the sediment. In Lake Ketelmeer the OC content in the layers of the sediment cores varied widely (Fig. 2.3A). Surprisingly, the recently deposited layers have the lowest OC content. The highest levels are found in layers that were deposited between 1950 and 1970. The OC content of the top-layer samples taken in 1972 is also shown in Figure 2.3A. Because these 5 cm top-layer samples probably reflect an average sediment composition for a four to six year period, they are put in the graph at 1969. The average OC content of these samples is somewhat higher than the OC content in the core layers dated around 1970. This difference indicates a small decrease in the carbon content of the sediment, which is probably the result of mineralization. However, other processes like resuspension and bioturbation cannot be excluded (see below). The steady decrease in the OC content since 1960 is directly related to a decrease of the total OC load in the Rhine and IJssel rivers

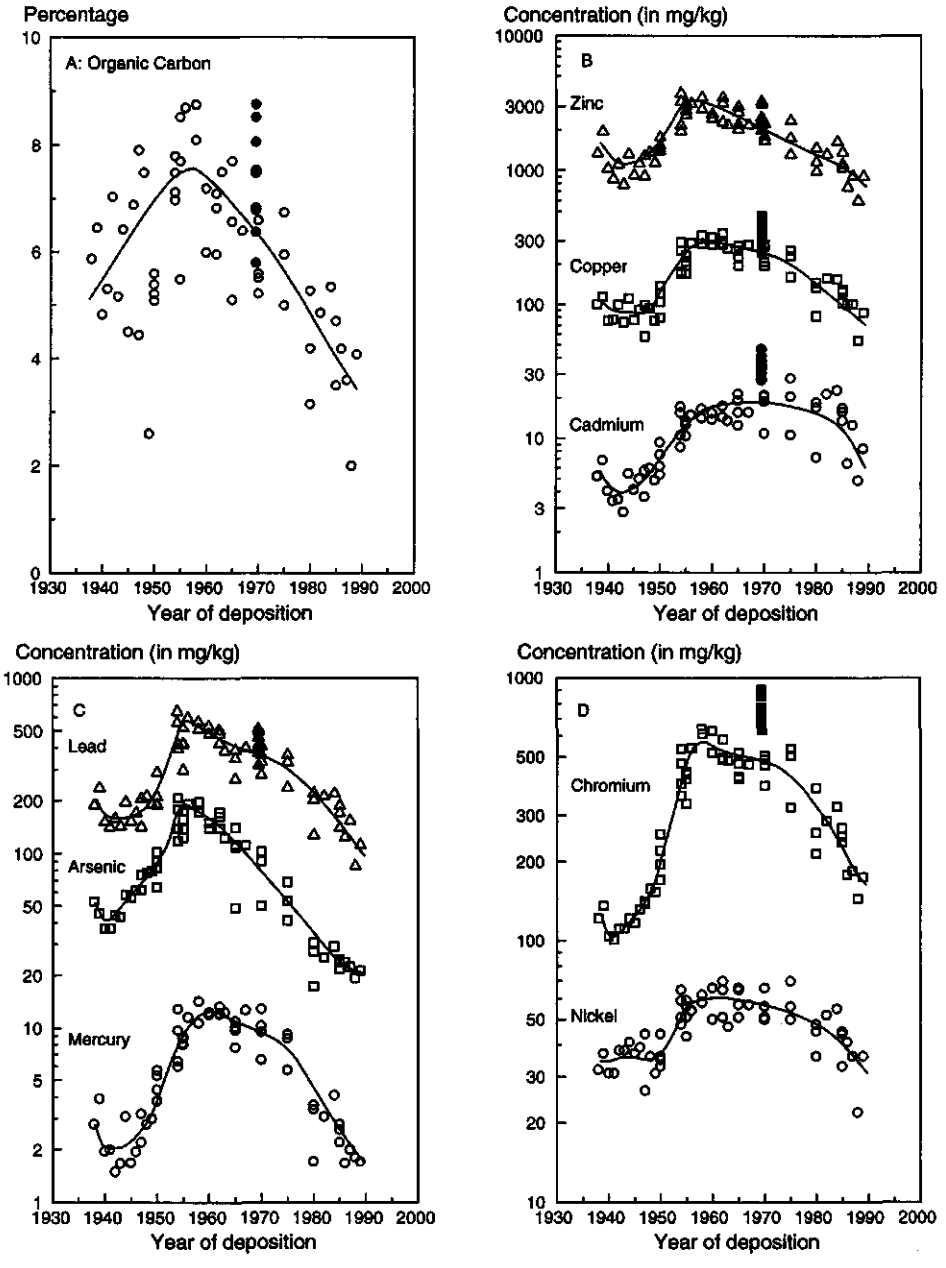

Fig. 2.3. Organic carbon content (A) and metal concentrations (B,C,D) in sediment core samples (○,□,△) and in top-layer samples from 1972 (●,■,▲) vs. the estimated years of deposition. The average concentrations in sediment core samples are indicated by the visually fitted curves.

[18]. This decline probably results from the construction of wastewater treatment plants in the drainage basin of the Rhine since the 1960s.

Heavy metals

The concentration profiles of eight heavy metals are also shown in Figure 2.3. Under the anoxic conditions prevailing in Lake Ketelmeer sediments, heavy metals are relatively immobile and, therefore, heavy metal profiles are likely to reflect the historic inputs. The 1972 top-layer samples offered an opportunity to test this assumption. The concentrations of some metals in these old top-layer samples are shown in Figure 2.3. The levels in the top-layer samples from 1972 were somewhat higher, especially for Cd and Cr, than the levels found in the core layers dated around 1970. This difference may have been caused by small deviations in the analytical methods, as heavy metal concentrations in sediment cores and in top-layer samples from 1972 were not determined by the same laboratory. In addition, the decrease in metal concentrations in the sediment cores may have been caused by resuspension and bioturbation. The 1972 top-layer samples reflected levels that may have been lowered by these processes before consolidation occurred. It became clear that these processes had introduced only limited changes, and, therefore, the profiles reflected the historic inputs without serious alterations.

All heavy metal levels were very low between 1940 and 1950. The concentrations at the end of the 1930s appeared to be higher than the concentrations in the early 1940s (second world war). From 1950 to 1965 all the studied heavy metals showed a steady increase in concentration. For all metals except Cd and Ni, a clear decrease from about 1965 was observed. Cadmium and Ni levels had only begun to decrease since the early 1980s. The recently deposited sediment had heavy metal levels that were either similar (Zn, Cu, Hg, and Ni) or below (Pb and As) the levels observed in the 1940 to 1950 period. Presently, Cd and Cr levels are still somewhat higher than the levels in the 1940 to 1950 period. The observed patterns in metal concentrations are in agreement with concentrations found in Rhine River floodplain samples over the last three decades [10].

The data from the nondegradable, relatively immobile metals indicate that resuspension and bioturbation have had only limited effects on the pollutant profiles. Consequently, combination of data from all dated cores into one graph appeared to be a feasible and elegant method to interpret the results. However, other processes, such as diffusion and transport with infiltrating water, may have affected the concentration profiles of organic pollutants. Therefore, pollutant levels were determined in the Zu-layer just beneath the IJm-deposit. The concentrations of the selected PCBs were all below the detection limit (10

ng/kg), indicating that no downward transport had occurred.

Polychlorinated biphenyls

The concentrations of four PCBs are shown in Figure 2.4. The average PCB 77 and PCB 118 levels in the samples from 1972 are somewhat higher than the levels found in the sediment cores. This difference between the stored 1972 samples and sediment cores is even more pronounced for PCB 105 and PCB 156. The difference in concentrations between top-layer samples from 1972 and core layers that had been deposited around 1970 were tested for the six studied PCB congeners (Table 2.2). The differences proved to be significant at a 0.05 level for all tested congeners, except for PCB 77 and PCB 118. Significant reductions varied between 70 and 88%. These reductions indicated that PCBs have disappeared from the anaerobic lake sediment, which may be the result of microbial dechlorination processes in the anoxic sediment. It has been established that disappearance of higher chlorinated biphenyls in the Hudson sediment [19,20] is a result of microbially mediated reductive dechlorination reactions [21,22]. Unlike the studies in the Hudson, ours was not able to demonstrate an accumulation of less chlorinated biphenyls as the reaction products in Lake Ketelmeer sediment, probably due to the limited number of congeners analyzed in this study.

Table 2.2. Comparison of mean PCB concentrations in top-layer samples (n=5) collected in 1972 and mean concentrations in recently sampled core layers (n=5) deposited around 1970.

PCB (IUPAC no.)	Concentration (ng/kg)		Reduction[1] (%)
	Top-layers collected in 1972	Sediment core layers from ±1970	
77	3,240	1,540	
105	140,000	33,600	76
118	135,000	95,000	
126	157	19	88
156	46,000	13,600	70
169	83	10	88

[1]Numerical values are given for decreases that are significant by the *t*-test at the 0.05 confidence level.

If the findings in Lake Ketelmeer can be attributed to microbial processes, this type of dechlorination seems to alter the concentrations of the individual congeners with different rates; PCB 77 and PCB 118 seem to be the most recalcitrant congeners. The other four PCBs show an average reduction of 80% during 20 years of "environmental incubation".

Assuming first-order kinetics for the microbial processes, a half-life of nine years can be estimated for these PCBs in the anaerobic Lake Ketelmeer sediment.

Although the origin of the observed reduction remains uncertain, it has been demonstrated clearly that the constructed concentration profiles of the planar and mono-ortho PCBs in Lake Ketelmeer sediment underestimated the historic inputs. Despite the substantial reductions, peaks in PCB profiles could still be recognized. The present pollution levels in the sediment layers are characterized as follows (shown partly in Fig. 2.4). The concentrations of the planar PCBs (77, 126, and 169) were below or just above the detection limit (10 ng/kg) in sediment layers from the early 1940s. On the other hand, the mono-ortho PCBs (105, 118, and 156) had elevated levels in these layers. In general, PCB concentrations showed a rapid increase from sediment layers dated around 1950 and reached maximum concentrations in layers from the 1960s and 1970s. A similar pattern has been reported for PCBs in stored Rhine River floodplain samples, with highest concentrations in the early 1970s [10]. In recently deposited sediments, concentrations of PCB 126 and PCB 169 were again below the detection limit. Recent pollution levels of PCB 77 were still elevated, as compared to the pollution levels in layers from the 1940s. The recent concentrations of the mono-ortho PCBs (105, 118, and 156) were relatively low and similar to the concentrations in layers from the early 1940s. Concentrations started to decrease after about 1970. This decrease started before an official PCB ban was introduced in European countries. The production of PCBs in the Rhine area stopped in 1983, and usage in German coal mines has been prohibited since 1985 [23].

Polychlorinated dibenzo-p-dioxins and dibenzofurans

Figure 2.5 shows the concentration profiles based on sediment core data and the levels in 1972 top-layer samples of four typical PCDDs. Differences between concentrations in the stored top-layer samples from 1972 and the concentrations in core layers deposited around 1970 are not as clear as those of some PCBs. The *t*-test revealed significant losses for only 1,2,3,4,7,8-HxCDD (not shown) and 1,2,3,6,7,8-HxCDD in anaerobic lake sediment. Although the differences for the other PCDDs are not significant, a striking tendency is observed. Almost all other PCDDs show some reduction in core layers, as compared to the top-layer samples from 1972. On the other hand, 2,3,7,8-TCDD and 1,2,3,7,8,9-HxCDD (not shown) seem to increase in the anaerobic lake sediment. The relationship between these observations and possible microbial dechlorination of PCDDs in anaerobic lake sediment is speculative. Only one study has reported microbial dechlorination of PCDDs in the laboratory [24]. Based on the results of the two HxCDDs that show significant losses in

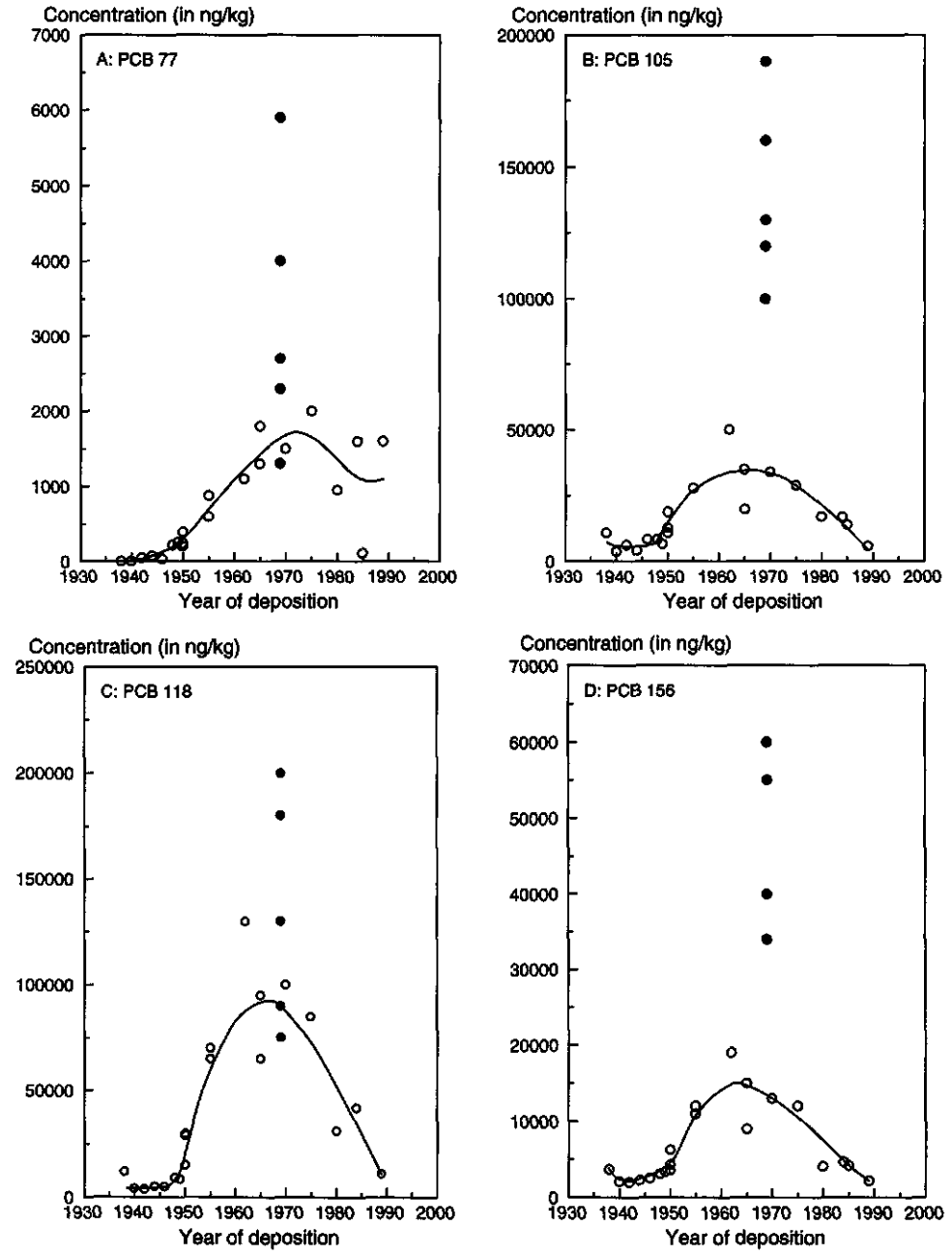

Fig. 2.4. Concentrations of 3,3',4,4'-tetrachlorobiphenyl (A), 2,3,3',4,4'-pentachlorobiphenyl (B), 2,3',4,4',5-pentachlorobiphenyl (C) and 2,3,3',4,4',5-hexachlorobiphenyl (D) in sediment core samples (○) and in top-layer samples from 1972 (●) vs. the estimated years of deposition. The average concentrations in sediment core samples are indicated by visually fitted curves.

anaerobic Lake Ketelmeer sediment, an average half-life of 13 years can be estimated for their disappearance.

Changes in concentrations of PCDDs in the anaerobic sediment since the moment of deposition in the past cannot be excluded. Therefore, the constructed PCDD concentration profiles do not necessarily reflect the original, unchanged pollution history. The concentrations of 1,2,3,7,8-PeCDD were below the detection limit (10 ng/kg) in all samples. A conspicuous difference in concentration levels between the different congeners in the sediment cores can be observed (Fig. 2.5). Before 1945, 2,3,7,8-TCDD, 1,2,3,4,7,8-HxCDD, and 1,2,3,6,7,8-HxCDD were below the detection limit (10 ng/kg). From the early and mid-1940s, a steady increase in dioxin levels was evident. The highest levels were found in sediments deposited between 1960 and 1980. Unexpected high concentrations of the highly toxic 2,3,7,8-TCDD were found, reaching up to approximately 400 ng/kg, in the mid-1960s. Highest levels of OCDD (approximately 5,000 ng/kg) were found in layers deposited in the mid-1970s. In recently deposited sediment, the 2,3,7,8-TCDD and 1,2,3,6,7,8-HxCDD showed a clear drop in concentrations, resulting in levels below the detection limit. Recently deposited sediment contains still elevated levels of the other dioxins, by comparison with the levels found before 1945.

Four typical concentration profiles of furans are shown Fig. 2.6. The profiles of furans show patterns similar to those of the PCBs and dioxins with the highest concentrations appearing beween 1960 and 1975. The concentrations in top-layer samples from 1972 are generally somewhat higher than the levels in core layers that were deposited around 1970. This difference is significant (*t*-test, 0.05 level) for 1,2,3,7,8-PeCDF and 2,3,4,7,8-PeCDF (not shown). For these two compounds an average half-life of 12 years can be estimated for the disappearance in anaerobic lake sediment. Similar to dioxins, the constructed concentration profiles of furans may reflect a somewhat lowered concentration level as compared to the original historical input. The present pollution levels in sediment layers from the last 50 years can be summarized as follows. In nearly all samples, the 1,2,3,7,8,9-HxCDF concentrations were below the detection limit. All other furans had concentrations above the detection limit since the early 1940s. Surprisingly, the concentration of 2,3,7,8-TCDF in the late 1930s was higher than in the early 1940s, which indicates an early high level of 2,3,7,8-TCDF that temporarily was reduced during the second world war. Highest levels were found in the 1960s and 1970s; maxima for 2,3,7,8-TCDF and OCDF were 200 and 15,000 ng/kg, respectively. For all studied furans the concentrations in the recently deposited sediments were in the same order of magnitude as the levels found in the early 1940s.

Transboundary pollution of the Rhine River is the main source of dioxins and furans in the

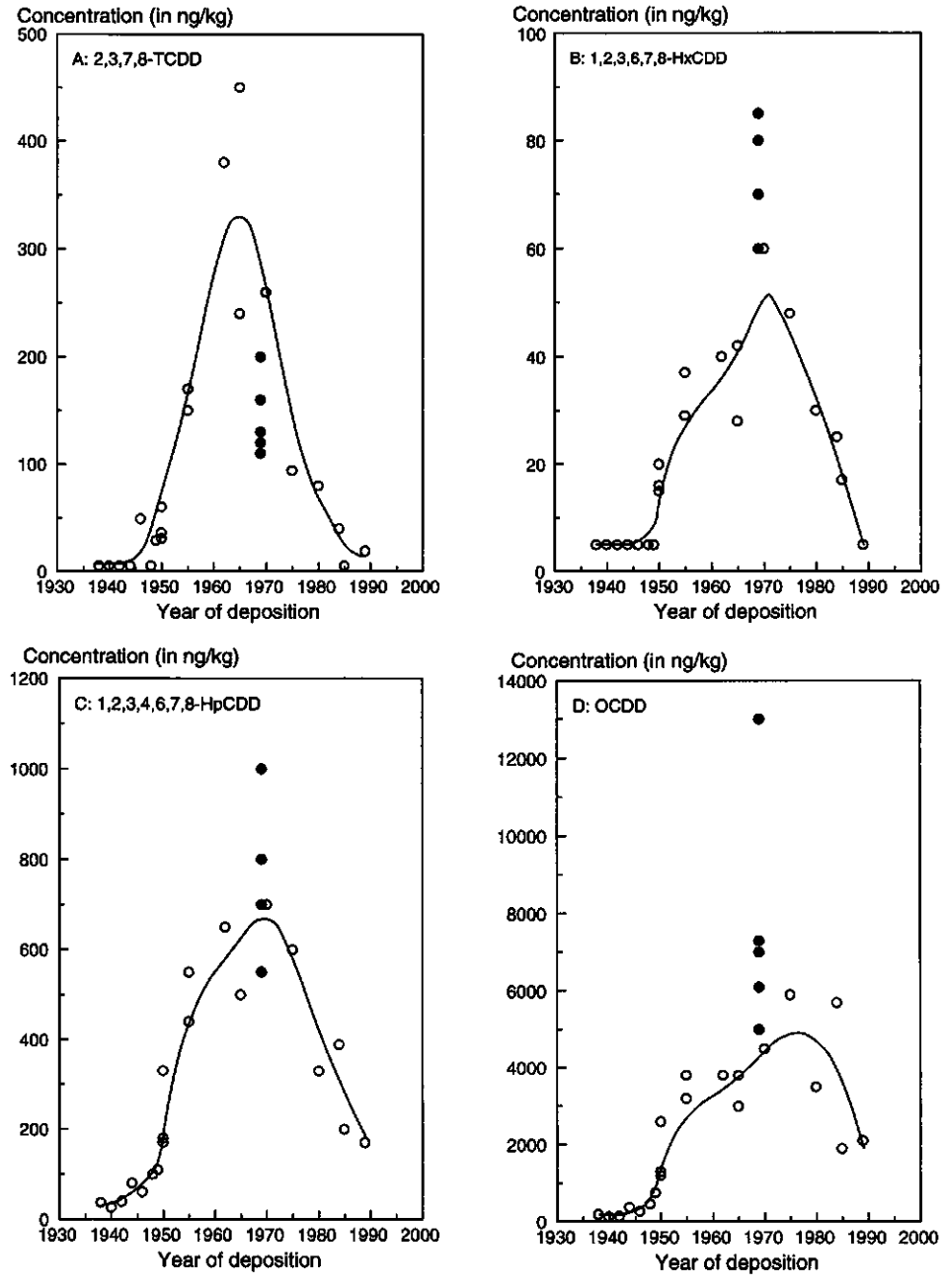

Fig. 2.5. Dioxin concentrations in sediment core samples (○) and in top-layer samples from 1972 (●) vs. the estimated years of deposition. The average concentrations in sediment core samples are indicated by visually fitted curves.

Dutch Rhine delta [25,26]. Because a variety of direct emissions from industrial processes and waste incineration as well as indirect atmospheric depositions contribute to the pollution of the Rhine with PCDDs and PCDFs, only slight indications for specific sources can be obtained from this study. The high level of 2,3,7,8-TCDD between 1960 and 1975 might be related to 2,4,5-T (2,4,5-trichlorophenoxy acetic acid) production. It has been established that 2,3,7,8-TCDD is a major contaminant of sodium 2,4,5-trichlorophenate, which is used for the manufacture of 2,4,5-T [27,28]. A German chemical manufacturing facility located at the Rhine River produced 2,4,5-T until 1983. Production capacity of 2,4,5-T at this plant was still 1,000 tons in 1980 [29]. In recently deposited sediments the higher chlorinated dioxins and furans predominate. This congener composition might be related to direct industrial sources like waste water from chlorinated aliphatics production and indirect sources like waste incineration processes. Both sources have been shown to contain high levels of the higher chlorinated dioxins and furans [30,31].

The pollution history of Lake Ketelmeer shows three characteristic periods. The first period, from the late 1930s until about 1950, seems to be relatively unpolluted. The second period, which lasted until about 1975, is characterized by maximum concentrations of PCBs, PCDDs, and PCDFs. Decreasing concentrations are observed in the third, most recent period. The periods can be represented by the years 1945, 1965, and 1985, respectively.

Toxicity equivalency factors (TEFs) have been developed as provisional methods of risk assessment for complex mixtures of PCBs, PCDDs, and PCDFs. Based on Dutch TEFs [32,33], the concentrations of PCBs, dioxins, and furans from the three characteristic years were used for calculations of the 2,3,7,8-TCDD Toxicity Equivalency Concentrations (TECs) (Table 2.3). From the calculations it appears that the total 2,3,7,8-TCDD TEC around 1945 was approximately 60 ng/kg. The furans (especially 2,3,4,7,8-PeCDF and 1,2,3,4,7,8-HxDF) were the dominant contributors (60%) to this total TEC value. The highest total TEC value was found around 1965 (650 ng/kg). The dioxins (particularly the 2,3,7,8-TCDD) were the main contributors (65%) to this total TEC value. In recently deposited sediment (1985) the calculated total TEC is approximately 80 ng/kg, and the furans are again the main contributors to this total value (60%). The levels of all compound groups in 1985 remained elevated when compared to the levels in 1945. The Σ-TEC for the selected PCBs in 1985 was still twice the value found in 1945. Dutch TEF values for one planar and the three mono-ortho PCBs differ significantly from values proposed by Safe [34]. Consequently, the contribution of PCBs changes greatly if these TEFs are used. The sumTECs of PCBs become 14.45, 167.75, and 57.75 for 1945, 1965, and 1985, respectively. Based on these TEFs, the sum of TECs for PCBs in 1985 is eight times higher than the value based on the Dutch TEFs. In addition, the contribution of the PCBs to the total TEC value in 1985

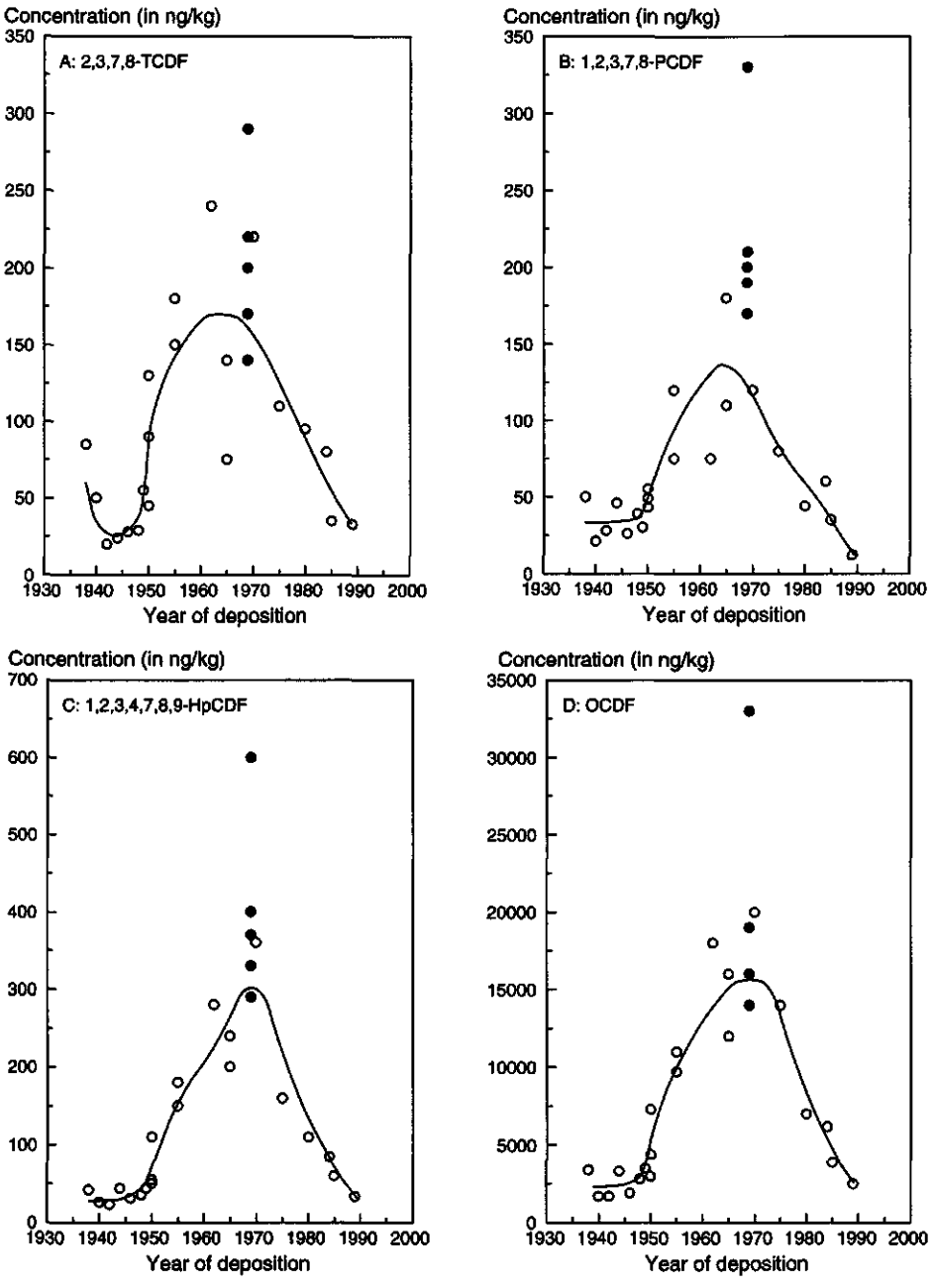

Fig. 2.6. Furan concentrations in sediment core samples (○) and in top-layer samples from 1972 (●) vs. the estimated years of deposition. The average concentrations in sediment core samples are indicated by visually fitted curves.

becomes twice the contribution of the dioxins and even exceeds the contribution of the furans. These calculations illustrate an increasing toxicological importance of the planar and mono-ortho PCBs in relation to PCDDs and PCDFs in recently deposited sediments.

Table 2.3. The 2,3,7,8-TCDD Toxicity Equivalency Concentrations (2,3,7,8-TCDD TEC) of selected PCBs, PCDDs, and PCDFs for three years that are characteristic for the pollution history of Lake Ketelmeer sediment between 1940 and 1990.

		2,3,7,8-TCDD TEC (ng/kg)		
Compound	TEF[1]	1945	1965	1985
biphenyls				
3,3',4,4',5-PeCB (126)	0.1	0.5*	0.5*	0.5*
3,3',4,4'-TeCB (77)	0.01	0.2	10	1
3,3',4,4',5,5'-HxCB (169)	0.005	0.02	0.02	0.02
2,3,3',4,4',5-HxCB (156)	0.0005	1.25	8.5	2
2,3,3',4,4'-PeCB (105)	0.0001	0.6	4.5	1.5
2,3',4,4',5-PeCB (118)	0.00005	0.25	4.75	1.85
ΣPCBs		2.82	28.27	6.87
dioxins				
2,3,7,8-TCDD	1	10	400	5*
1,2,3,7,8-PeCDD	0.5	2.5*	2.5*	2.5*
1,2,3,4,7,8-HxCDD	0.1	0.5*	3	1.5
1,2,3,6,7,8-HxCDD	0.1	0.5*	3.5	1.7
1,2,3,7,8,9-HxCDD	0.1	1	15	8.5
1,2,3,4,6,7,8-HpCDD	0.01	0.6	6	2
OCDD	0.001	0.3	3.2	2
ΣPCDDs		15.4	433.2	23.2
furans				
2,3,7,8-TCDF	0.1	4	16	3.5
1,2,3,7,8-PeCDF	0.05	1.75	5	1.75
2,3,4,7,8-PeCDF	0.5	10	42.5	11.5
1,2,3,4,7,8-HxCDF	0.1	10	38	12.5
1,2,3,6,7,8-HxCDF	0.1	5	22	7
1,2,3,7,8,9-HxCDF	0.1	0.5*	1	0.5*
2,3,4,6,7,8-HxCDF	0.1	2	14	3
1,2,3,4,6,7,8-HpCDF	0.01	4.4	32	5
1,2,3,4,7,8,9-HpCDF	0.01	0.35	2.4	0.6
OCDF	0.001	3	20	3.9
ΣPCDFs		41	192.9	49.25
totalTEC		59.2	654.4	79.3

[1]Dutch toxicity equivalency factors (TEFs) [33, 34].
*measurement below detection limit (10 ng/kg), value is based on one-half the detection limit.

Polycyclic aromatic hydrocarbons

PAHs were determined in five sediment cores. The 1972 top-layer samples were not included in the PAH analyses, and, therefore, no information about possible transformations of PAHs in Lake Ketelmeer sediment is available. Recently, McFarland and Sims [35] presented a thermodynamic evaluation of the biodegradability of PAHs under anaerobic conditions. They indicated that microbially mediated transformation of PAHs in anaerobic environments may occur under denitrification conditions but is unlikely to occur under sulfate-reducing and methanogenic conditions. In laboratory experiments, microbial transformation of the bicyclic naphthalene and acenaphthene has been shown under denitrification conditions [36]. Because methanogenic conditions prevail in Lake Ketelmeer sediment, postdepositional biodegradation of PAHs can be excluded. Consequently the concentration profiles of PAHs in Lake Ketelmeer sediment will presumably reflect the unchanged historic inputs.

The concentration profiles of eight PAHs are shown in Fig. 2.7. The selected compounds represent a broad range in molecular weights, varying from two fused aromatic rings (fluorene) to compounds that consist of six fused aromatic rings (benzo[ghi]perylene). Despite a rather high variation in the concentrations of the individual PAHs, general trends can be recognized. The oldest IJm layers in Lake Ketelmeer, deposited in the late 1930s, have relatively high levels of the selected PAHs. These high concentrations in sediment from the 1930s are in agreement with the findings at other locations, where anthropogenic PAH levels started to rise some 100 years ago [5,37]. Somewhat lower concentrations are found in Lake Ketelmeer sediment layers from the mid-1940s. The emission of PAHs was apparently reduced during the second world war. Benzo[k]fluoranthene concentrations remain relatively constant until the 1970s. Highest concentrations for almost all studied PAHs are found in layers that were deposited around 1960. A maximum level of approximately 5 mg/kg was reached for fluoranthene in this period. Similar results with peaks from the 1950s to the 1960s have been observed in other industrialized regions [5,37]. Benzo[ghi]perylene seems to be an exception; the concentrations in the late 1930s exceed the concentrations around 1960. A decline in concentrations is observed from the mid-1960s and early 1970s. The most recently deposited sediments have the lowest PAH concentrations ever observed in Lake Ketelmeer sediment during the last five decades. Concentrations have decreased about five times between the maxima from about 1960 and the levels in the late 1980s.

The PAHs in the Rhine River originate from a variety of sources. Atmospheric deposition of PAHs, related to the combustion of fossil fuels, might be one of the major sources. Reductions in the use of coal since the 1950s and 1960s have probably caused the

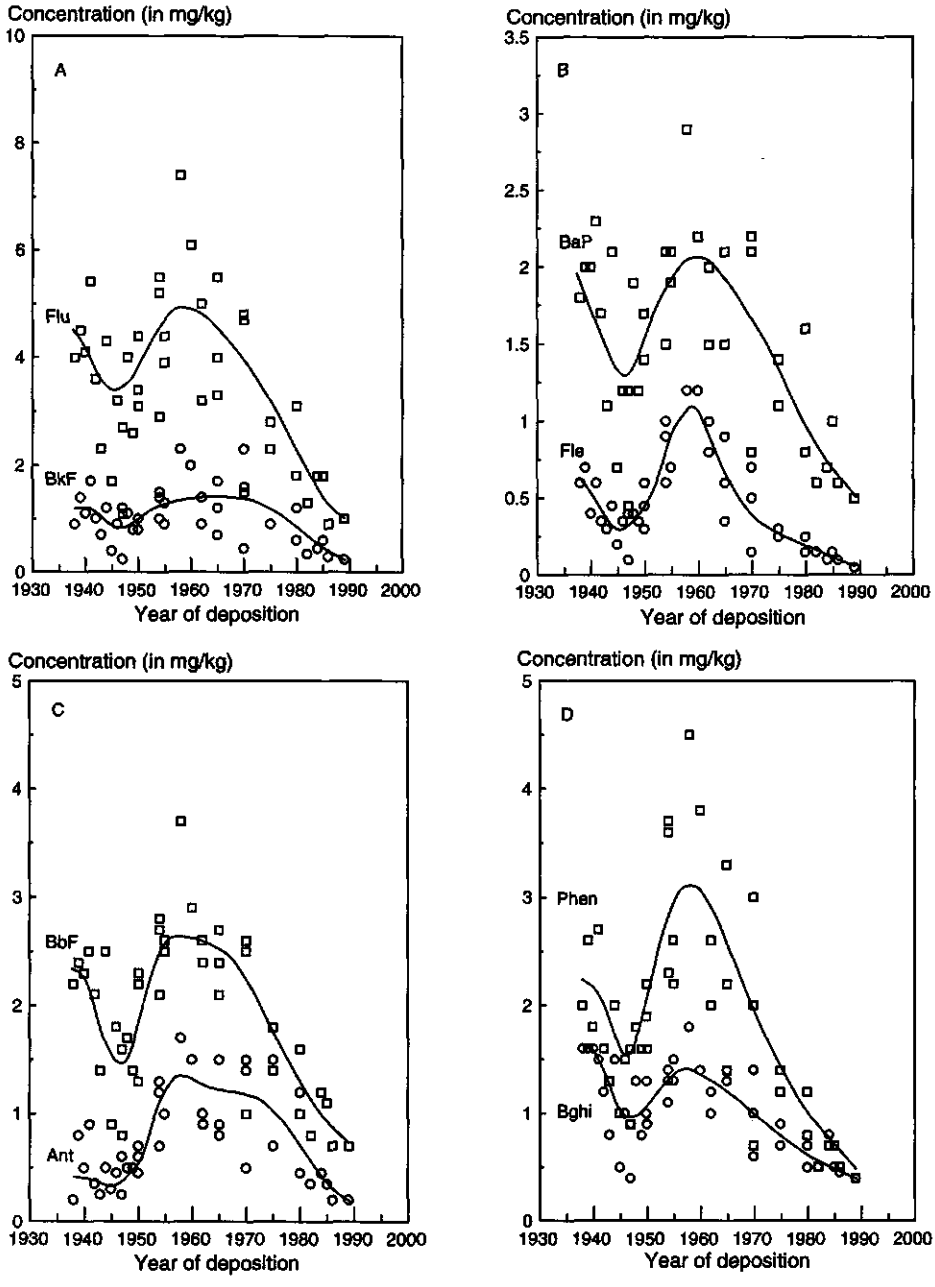

Fig. 2.7. PAH concentrations in sediment core samples vs. the estimated years of deposition. The average concentrations are indicated by visually fitted curves. Abbreviations: (A) fluoranthene (Flu) and benzo[k]fluoranthene (BkF); (B) benzo[a]pyrene (BaP) and fluorene (Fle); (C) benzo[b]fluoranthene (BbF) and anthracene (Ant); (D) phenanthrene (Phen) and benzo[ghi]perylene (Bghi).

observed reduction in the last decades. PAH concentrations in rural vegetation in the United Kingdom decreased by a factor five between the late 1960s and late 1980s [38]. This decline is clearly related to the reduction in emissions from combustion in the United Kingdom [39]. The use of other fuels, like natural gas, started in the 1960s and has become more important since that time. The change to less polluting fuels in combination with emission control technologies when coal is used, such as for electricity generation, surely contributes to the low PAH levels in the recently deposited Lake Ketelmeer sediment.

CONCLUSIONS

The concentrations of metals and PAHs in the sediment cores reflect, without any serious alterations, the historic inputs during the past five decades. The pollution history is characterized as follows:

-Low concentrations of metals were observed in the early 1940s, PAHs levels were already elevated. For both metals and PAHs, sediment cores presumably reflect a reduction in emissions during the second world war.

-Highest levels of metals and PAHs were found between 1955 and 1970. Cadmium and nickel levels remained high until 1980.

-Recently deposited sediments had rather low levels, some of which were the lowest ever observed during the last five decades (Pb, As, all studied PAHs).

Almost all chlorinated compounds showed some disappearance in the anaerobic sediment, as compared to the levels in stored 1972 top-layer samples that reflected the original pollution input. For several PCBs and some PCDDs and PCDFs this disappearance proved to be significant and may have been caused by microbial dechlorination reactions in the anaerobic sediment. Consequently the concentration profiles of the chlorinated compounds do not reflect the original pollution history. Despite these disappearances, peaks in PCB, PCDD, and PCDF concentration profiles are still observed. Presently, the following trends in concentrations of chlorinated aromatics can be observed in Lake Ketelmeer sediment:

-Almost all studied PCBs, PCDDs, and PCDFs had rather low concentrations in the early 1940s. Only some furans had elevated levels. A marked pattern was observed for 2,3,7,8-TCDF; the emissions seem to have been reduced during the second world war.

-Highest levels of chlorinated aromatics were found between 1960 and 1975. The highly toxic 2,3,7,8-TCDD reached concentrations up to 400 ng/kg during this period.

-Recently deposited sediment has elevated levels of PCBs and higher chlorinated dioxins,

as compared to the levels in the layers from the early 1940s.

The 2,3,7,8-TCDD TECs of the most toxic PCBs, PCDDs, and PCDFs were calculated for three years - 1945, 1965, and 1985 - each year representing an easily recognizable period in the pollution history of Lake Ketelmeer. The total 2,3,7,8-TCDD TEC changed dramatically over these three years: 60, 650 and 80 ng/kg, respectively. In 1945 and 1985 the dominant contributors to this total 2,3,7,8-TCDD TEC were the furans, especially the 2,3,4,7,8-PeCDF and 1,2,3,4,7,8-HxCDF. The high level of 2,3,7,8-TCDD was the main cause of the high totalTEC seen in 1965. These calculations were based on the Dutch TEFs. A much higher contribution from the PCBs is obtained if other TEFs are used.

The overall picture indicates that the recently deposited material is far less polluted than the sediment deposited in the 1960s and 1970s. These findings suggest that highly polluted sediments from the past are buried under a less polluted layer. However, the locations selected in this study have high sedimentation rates and may differ from other locations. Altered sedimentation rates occur near shipping lines where resuspension is high, or in harbors, where frequent dredging activities remove recently deposited material. At these locations the highly polluted sediment layers may remain uncovered and the aquatic organisms may still be exposed to the highly polluted sediment from the 1960s and 1970s.

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REFERENCES

1. **Robbins, J.A.** 1982. Stratigraphic and dynamic effects of sediment reworking by Great Lakes zoobenthos. *Hydrobiologia* **92**:611-622.
2. **Förstner, U.** and **G.T.W. Wittmann.** 1979. Metal pollution in the aquatic environment. Springer Verlag, Berlin, pp. 146-151.
3. **Oliver, B.G., M.N. Charlton** and **R.W. Durham.** 1989. Distribution, redistribution, and geochronoloy of polychlorinated biphenyl congeners and other chlorinated hydrocarbons in Lake Ontario sediments. *Environ. Sci. Technol.* **23**:200-208.
4. **Czuczwa, J.M.** and **R.A. Hites.** 1984. Environmental fate of combustion-generated polychlorinated dioxins and furans. *Environ. Sci. Technol.* **18**:444-450.
5. **Gschwend, P.M.** and **R.A. Hites.** 1981. Fluxes of polycyclic aromatic hydrocarbons to marine and lacustrine sediments in the northeastern United States. *Geochim. Cosmochim. Acta* **45**:2359-2367.
6. **Heymen, R.** 1990. Results of the water quality research in the Rhine River in The Netherlands between 1970 and 1989. RIZA Report 90.048, Lelystad, The Netherlands.
7. **Slooff, W.** 1983. Rijn, Lek, Waal, IJssel en uiterwaarden onder invloed van ingrepen en

verontreinigingen. In G.P. Hekstra and W. Joenje, eds., *Rijnwater in Nederland.* Oecologische Kring, Arnhem, The Netherlands, pp. 13-31.

8. **Winkels, H.J., A. van Diem** and **J. Driebergen.** 1990. Extent and degree of pollution of the sediment in the Ketellake. In D.G. Price, ed., Proceedings, Sixth international congress. International association of engineering geology, Amsterdam, The Netherlands. 6-10 August, pp. 183-188.
9. **Rijnlersce, K.** 1983. A simulation model for physical soil ripening in the IJsselmeerpolders. Research report 203, IJsselmeerpolders Development Authority, Lelystad, The Netherlands.
10. **Japenga, J., K.H. Zschuppe, A.J. de Groot** and **W. Salomons.** 1990. Heavy metals and organic micropollutants in floodplains of the River Waal, a distributary of the River Rhine, 1958-1981. *Neth. J. Agric. Sc.* **38:**381-397.
11. **Robbins, J.A.** and **D.N. Edgington.** 1975. Determination of recent sedimentation rates in Lake Michigan using Pb-210 and Cs-137. *Geochim. Cosmochim. Acta* **39:**285-304.
12. **Smith, L.M., D.L. Stalling** and **J.L. Johnson.** 1984. Determination of part per trillion levels of polychlorinated dibenzofurans and dioxins in environmental samples. *Anal. Chem.* **56:**1830-1842.
13. **Rappe, C.** 1984. Analysis of polychlorinated dioxins and furans: All 75 PCDDs and 135 PCDFs can be identified by isomer specific techniques. *Environ. Sci. Technol.* **18:**78a-90a.
14. **Evers, E.H.G., H.J.S. Klamer, R.W.P.M. Laane** and **H.A.J. Govers.** 1993. Polychlorinated dibenzo-*p*-dioxin and dibenzofuran residues in estuarine and coastal North Sea sediments: sources and distribution. *Environ. Toxicol. Chem.* **12:**1583-1598.
15. **Salomons, W.** and **A.J. de Groot.** 1977. Pollution history of trace metals in sediments, as effected by the Rhine River. Institute for Soil Fertility Research Report 184. Haren, The Netherlands.
16. **Eisenreich, S.J., P.D. Capel, J.A. Robbins** and **R. Bourbonniere.** 1989. Accumulation and diagenesis of chlorinated hydrocarbons in lacustrine sediments. *Environ. Sci. Technol.* **23:**1116-1126.
17. **Capel, P.D.** and **S.J. Eisenreich.** 1990. Relationship between chlorinated hydrocarbons and organic carbon in sediment and porewater. *J. Great Lakes Res.* **16:**245-257.
18. **International Commission for the Protection of the River Rhine against Pollution.** 1989. Measurements of physical and chemical data in the River Rhine in 1988 (in German and French). Annual report. Koblenz, Federal Republic of Germany.
19. **Brown, J.F., Jr., D.L. Bedard, M.J. Brennan, J.C. Carnahan, H. Feng** and **R.E. Wagner.** 1987. Polychlorinated biphenyl dechlorination in aquatic sediments. *Science* **236:**709-712.
20. **Brown, J.F., Jr., R.E. Wagner, H. Feng, D.L. Bedard, M.J. Brennan, J.C. Carnahan** and **R.J. May.** 1987. Environmental dechlorination of PCBs. *Environ. Toxicol. Chem.* **6:**579-593.
21 **Quensen, J.F., III, J.M. Tiedje** and **S.A. Boyd.** 1988. Reductive dechlorination of polychlorinated biphenyls by anaerobic microorganisms from sediments. *Science* **242:**752-754.
22. **Quensen, J.F., III, S.A. Boyd** and **J.M. Tiedje.** 1990. Dechlorination of four commercial polychlorinated biphenyl mixtures (Aroclors) by anaerobic microorganisms from sediments. *Appl. Environ. Microbiol.* **56:**2360-2369.
23. **Friege H., H. Klös, W. Leuchs, H.-D. Stock** and **W. Kirchner.** 1989. Contamination of sediments of the River Rhein, recent developments and perspectives. *Proceedings* The harbour, an ecological challenge, Hamburg, Federal Republic of Germany. September 11-15. pp. 128-131.
24. **Adriaens, P.** and **D. Grbic-Galic.** 1991. Anaerobic dehalogenation of highly chlorinated dioxins and dibenzofurans. *Proceedings* Dioxin '91. 11th International symposium on chlorinated dioxins and related compounds. Research Triangle Park, North Carolina, September 23-27. p. 327.
25. **Turkstra, E.** and **H.B. Pols.** 1989. PCDDs and PCDFs in dutch inland waters. *Chemosphere* **18:**539-551.
26. **Evers, E.H.G., K.C.M. Ree** and **K. Olie.** 1988. Spatial variations and correlations in the distribution of PCDDs, PCDFs and related compounds in sediments from the River Rhine-Western Europe. *Chemosphere* **17:**2271-2288.
27. **Bopp, R.F., M.L. Gross, H. Tong, H.J. Simpson, S.J. Monson, B.L. Deck** and **F.C. Moser.** 1991. A major incident of dioxin contamination: sediments of New Jersey Estuaries. *Environ. Sci. Technol.* **25:**951-956.
28. **Umbreit, T.H., E.J. Hesse** and **M.A. Gallo.** 1986. Bioavailability of dioxin in soil from a 2,4,5-T manufacturing site. *Science* **232:**497-499.
29. Umweltbundesamt. 1985. Sachstand dioxine. Umwelt Bundesamt Berichte 5. Erich Schmidt Verlag, Berlin. Federal Republic of Germany.
30. **Wenning, R.J., M.A. Harris, M.J. Ungs, D.J. Paustenbach** and **H. Bedbury.** 1992. Chemometric comparisons of polychlorinated dibenzo-p-dioxin and dibenzofuran residues in surficial sediments from Newark Bay, New Yersey and other industrialized waterways. *Arch. Environ. Contam. Toxicol.* **22:**397-413.
31. **Czuczwa, J.M.** and **R.A. Hites.** 1986. Airborne dioxins and dibenzofurans: sources and fates. *Environ.*

Sci. Technol. **20**:195-200.

32. **van Zorge, J.A., J.H. van Wijnen, R.M.C. Theelen, K. Olie** and **M. van den Berg.** 1989. Assessment of the toxicity of mixtures of halogenated dibenzo-*p*-dioxins and dibenzofurans by use of toxicity equivalency factors (TEF). *Chemosphere* **19**:1881-1895.
33. **van Zorge, J.A.** 1990. Toxicity equivalency factors for PCBs. Letter SR/1290255 Directorate-General for Environmental Protection, Directorate for Chemicals and Risk Management, Leidschendam, The Netherlands (in dutch).
34. **Safe, S.** 1990. Polychlorinated biphenyls (PCBs), dibenzo-p-dioxins (PCDDs), and related compounds: Environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). *CRC Crit. Rev. Toxicol.* **21:** 51-88.
35. **McFarland, M.J.** and **R.C. Sims.** 1991. Thermodynamic framework for evaluating PAH degradation in the subsurface. *Ground Water* **29**:885-896.
36. **Mihelcic, J.R.** and **R.G. Luthy.** 1988. Microbial degradation of acenaphthene and naphthalene under denitrification conditions in soil-water systems. *Appl. Environ. Microbiol.* **54**:1188-1198.
37. **Wakeham, S.G., C. Schaffner** and **W. Giger.** 1980. Polycyclic aromatic hydrocarbons in recent lake sediments I. Compounds having anthropogenic origins. *Geochim. Cosmochim. Acta* **44**:403-413.
38. **Jones, K.C., G. Sanders, S.R. Wild, V. Burnett** and **A.E. Johnston.** 1992. Evidence for a decline of PCBs and PAHs in rural vegetation and air in the United Kingdom. *Nature* **356**:137-140.
39. **Jones, K.C., J.A. Stratford, K.S. Waterhouse, E.T. Furlong, W. Giger, R.A. Hites, C. Schaffner** and **A.E. Johnston.** 1989. Increases in the polynuclear aromatic hydrocarbon content of an agricultural soil over the last century. *Environ. Sci. Technol.*, **23**:95-101.

CHAPTER 3

MICROBIAL DECHLORINATION OF HEXACHLOROBENZENE IN A SEDIMENTATION AREA OF THE RHINE RIVER

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ABSTRACT

In sedimentation areas of polluted rivers, microbial dechlorination of chlorinated aromatics may be of great environmental significance. This reaction may take place in the deeper, anaerobic sediment layers and involves replacement of a chlorine in the pollutant molecule by hydrogen. In this study, the microbial dechlorination of hexachlorobenzene in a sedimentation area of the Rhine River is evaluated by using Rhine water pollution data, concentrations in historical sediment samples and in recent sediment cores, and the results of anaerobic laboratory incubations with Lake Ketelmeer sediment. The various data support the conclusion that microbial dechlorination of hexachlorobenzene has occurred in the anaerobic sediment. Up to 80% of the hexachlorobenzene deposited in the early 1970s has been dechlorinated. The maximum half-life of hexachlorobenzene in the sediment is found to be 7 years.

Two limitations of microbially mediated dechlorination in the natural environment have become clear. In the first place, a residual concentration of about 40 µg/kg remains unaltered in the sediment or transformation rates of this fraction are at least extremely low. Secondly, the lower chlorinated benzenes that are produced from hexachlorobenzene appear to accumulate in the anaerobic sediment.

INTRODUCTION

The Rhine River and its tributaries drain a large, industrialized area in Europe (Fig. 2.1, page 20). The Rhine River is heavily polluted with metals (Van der Weijden & Middelburg 1989) and organic micropollutants including chlorinated benzenes (CBs) (Meijers & Van der Leer 1976). Hexachlorobenzene (HCB) was formerly in widespread use as a fungicide in agriculture and many CBs are still applied in industry and domestic products. Among the various direct and indirect sources of CBs, processes in an aluminium foundry have been identified as sources of undeliberate HCB pollution in the Rhine River (Vogelgesang et al. 1986). CBs have been found in sewage sludges (Vogelgesang et al. 1986), sediments (Kypke-Hutter et al. 1986), river water (Kühn & Brauch 1988), and fish (Kypke-Hutter et al. 1986) from the Rhine River area. Due to their hydrophobic character, CBs accumulate in sediments and biota and seem to be rather persistent in the aquatic environment, although abiotic and biotic transformations of CBs have been shown in laboratory tests. Transformations of CBs may occur in the aerobic water column as well as in the sediment.

Photochemical transformations of CBs have been demonstrated (Choudhry & Hutzinger 1984; Hirsch & Hutzinger 1989); nevertheless the overall contribution of photolysis to the transformation of CBs in the water column will probably not be very significant as a result of the limited light transmission in Rhine River water.

Under aerobic conditions, biomineralization has been shown for monochlorobenzene (Pfaender & Bartholomew 1982; Reineke & Knackmuss 1984), all dichlorobenzenes (Van der Meer et al. 1987; Schraa et al. 1986; Spain & Nishino 1987; De Bont et al. 1986; Haigler et al. 1988), 1,2,3-trichlorobenzene (Marinucci & Bartha 1979), 1,2,4-trichlorobenzene (Van der Meer et al. 1987; Pfaender & Bartholomew 1982) and 1,2,4,5-tetrachlorobenzene (Sander et al. 1991). Most of these studies were performed with organisms originating from aquatic environments, in either undefined mixed populations or pure cultures obtained by selective enrichment. The 1,3,5-trichlorobenzene and other highly chlorinated benzenes, except 1,2,4,5-tetrachlorobenzene, seem to resist aerobic biomineralization.

Since the majority of hexa-, penta- and tetrachlorobenzenes probably remains unaltered in the aerobic river water, their concentrations in water are mainly influenced by volatilization, downstream dilution and particle associated sedimentation. In the Rhine River delta, high deposition of the CBs associated with the settling particles will occur as a result of decreasing streaming velocities. Consequently, increased concentrations of the higher chlorinated benzenes can be expected in sediments of sedimentation areas. In addition, the lower chlorinated benzenes may also be found in sedimentation areas if aerobic

biodegradation processes and volatilization fail to eliminate these compounds from the water column.

Under anaerobic conditions, microbial transformations have been shown for all CBs in laboratory tests, except for mono- and 1,2,3,4-tetrachlorobenzene (Tiedje et al. 1987; Fathepure et al. 1988; Bosma et al. 1988). The anaerobic microbial transformation is a reductive dechlorination reaction. In this reaction a chlorine is replaced by a hydrogen, producing lower chlorinated benzenes. Hexachlorobenzene is mainly dechlorinated to 1,3,5-trichlorobenzene in sewage sludge (Tiedje et al. 1987; Fathepure et al. 1988). In river sediments, tri- and dichlorobenzenes are dechlorinated to monochlorobenzene (Bosma et al. 1988).

Although the origins of the anaerobic microbial consortia suggest that the results of these laboratory studies can easily be applied to the natural aquatic environment, there are some aspects that complicate the translation to field conditions: 1) long adaptation times, up to several months, are observed in laboratory tests; 2) the use of selected populations; 3) optimized incubation conditions: high temperatures, high nutrient concentrations; 4) high concentrations of the artificially added CBs. These aspects may stimulate the development of microbial populations that do not resemble the composition and capabilities of the naturally occurring populations.

Long term persistence of CBs for more than several decades has been shown in aquifers (Barber 1988) and in lake sediments (Oliver 1987). Based on CB concentration profiles in sediment cores from the Great Lakes, reported by Oliver & Nicol (1982), reductive dechlorination of CBs has been suggested by Bailey (1983). However, Oliver & Nicol (1983) did not support the suggestions of Bailey. Historic information on the concentrations of the various CBs at the time of deposition is essential to solve this disagreement. In fact, the microbial dechlorination of CBs in the natural environment, has remained uncertain.

The objective of the present study is to evaluate the possibility of reductive dechlorination of CBs in a sedimentation area of the Rhine River. The present data provide new information on the fate of CBs in sediments, taking into account the contamination levels in the Rhine River in the past, CB concentrations in the sediment at the time of deposition, the actual concentrations in sediment cores and the dechlorination capabilities of native microbial populations in the sediment, tested in the laboratory under conditions that are as close to the field situation as possible.

MATERIALS AND METHODS

Study area

Lake Ketelmeer is a shallow freshwater lake (surface area 38 km^2), located in the central part of The Netherlands. It receives water originating from the Rhine River through a northern Rhine branch, called the IJssel River. The lake has an open connection with Lake IJsselmeer (Fig. 2.1, page 20). The lakes formed part of a coastal sea (the Zuiderzee) until the closure of a barrier dam in 1932, creating the Lake IJsselmeer. Land reclamation created two polders within Lake IJsselmeer, with Lake Ketelmeer as the outlet of the IJssel River, situated between these polders. The northern dike of Lake Ketelmeer was completed in 1938 and the southern dike in 1953. The most recent geological layer in this area, the IJsselmeer (IJm) deposit, is defined as the silty sediment deposited since 1932. The underlying Zuiderzee (Zu) deposit, a saltwater clay deposit, can be identified by the presence of shell fragments (*Mya arenaria*). The average thickness of the IJm-deposit is 0.5 m. The sediment cores were taken at locations known for their thick IJm-deposits. The majority of Lake Ketelmeer sediment is anaerobic, only the top layer (1-3 cm) at the interface with the surface water is aerobic.

Sample collection and treatment

Rhine River water samples have been collected since the early 1970s at the gauging station Lobith, which is situated on the Dutch side of the German-Dutch border. Water samples are collected twice a month.

In 1988, three undisturbed sediment cores (0.15 m in diameter and with an average length of approximately 1 m) were taken with an open auger. The cores, which mainly contained IJm-deposit, were sectioned into 0.10 m layers. Samples were put in glass jars with screw caps, refrigerated at 4°C and transported to the laboratory. Before subsamples were taken for the different chemical analyses, samples were freeze dried and homogenized.

The Institute for Soil Fertility Research (Haren, The Netherlands), collected sediment top layer samples (5 cm) from 1972. The 10 historical samples used in this study originate from the same areas as the three sediment cores taken in 1988. After collection in 1972, the top layer samples were dried overnight at 40°C; since, they had been stored in jars with screw caps at room temperature in the dark.

Sediment dating

The age of the different layers in the sediment cores was estimated by several methods. The well known geological history of this area offers some valuable recognition points in cores. The interface between Zu-deposit and IJm-deposit is visually recognizable (shells) and indicates the early 1930s. The polders were under construction until the 1950s. Sedimentation occurred over a much larger area before the completion of the surrounding dikes created Lake Ketelmeer. Consequently, only a thin layer represents the 1930-1950 period. Due to the thickness of the sampled layers, no subdivision in this period could be made and therefore the 1930-1950 period has been omitted in this study. The ^{137}Cs and ^{134}Cs gamma-activities are used as historical markers. A detailed description of the applied analytical method is presented by Beurskens et al. (1993, Chapter 2). Estimations of the year of deposition were only possible for the layers of the IJm-deposit since 1950. The underlying Zu-deposit could not be dated. However, the top of the Zu-deposit has to correspond with the year 1932.

Lead as a non-volatile element in the water column and rather immobile element in anoxic sediments will serve as a tracer in this study. The long-term changes in the concentration pattern of lead in Rhine water samples is expected to be found in the sediment cores from Lake Ketelmeer. Comparison of the lead concentrations in the top layer samples from 1972 with the levels in the sediment cores offers an additional recognition point.

Chemical analyses

The contaminant concentrations in water samples from Lobith are based on data from the long term water quality research, conducted at our institute (Heymen 1990). Since the early 1970s, pollutants have been monitored in the Rhine River. As a result of the developments in the analytical methods, several analytical techniques have been applied during the past two decades. Particularly with respect to the organic micropollutants, clear improvements have been obtained. Consequently, the reported concentrations of organic micropollutants in Rhine water are probably less accurate for the 1970s than for the 1980s. The most recently applied technique for CB quantification is described below.

Water samples were extracted three times with petroleum ether. The combined petroleum ether extracts were dried with sodium sulphate and concentrated with a Kuderna-Danish condensor to a volume of 5 ml. The extract volume was further reduced to 1 ml with a gentle stream of clean nitrogen. Extract cleanup consisted of passing the extract through a column of 2 g 11% deactivated alumina. Evaporation with nitrogen concomitant with a solvent change to iso-octane was used to reduce the volume. Sample extracts were analyzed with a HP 5890A gas chromatograph, equipped with dual ^{63}Ni electron capture

detector using automated splitless injection (HP 7673A). The separations were carried out on dual capillary columns (CPsil8 and CPsil19, length 50 m, 0.22 mm i.d., filmthickness 0.12 μm). Operating conditions were as follows: temperature program of 60°C for 2 min, 10°C/min to 140°C, 5 min at 140°C, 5°C/min to 250°C, 30 min at 250°C, 5°C/min to 280°C, 15 min at 280°C; injector temperature of 250°C; detector temperature of 300°C. Helium was used as a carrier gas and nitrogen as a detector-quench gas.

CBs and polychlorinated biphenyls (PCBs) in the sediment samples have been analyzed as follows. The dried sediment samples were extracted twice with acetone for 15 min. The acetone extracts were shaken with a sodium sulphite solution to remove elemental sulphur. The extracts were mixed with petroleum ether and washed with water to remove the acetone. After separation, the aqueous phase was extracted with a second portion of petroleum ether. The petroleum ether extracts were combined and further treatment and gas chromatographic analysis were identical to the procedures for the extracts obtained from water samples, as described above. Only an additional extract cleanup through a column of 2 g 6% deactivated silica was applied.

Lead concentrations in Rhine water samples were determined after acidification of the samples to pH 2 with nitric acid. The acidified samples were analyzed by graphite furnace atomic absorption spectrometry (Perkin Elmer 5000). Lead concentrations in the sediment core samples were determined after treatment with hydrochloric acid. A graphite furnace AAS (Perkin Elmer 5000) was used for the lead analyses. Lead concentrations in the top layer samples from 1972 have been determined at the Institute for Soil Fertility Research. The applied analytical method (Japenga et al. 1990) is similar to the method described above.

All concentrations of radionuclides and pollutants in sediment samples are reported on a per-dry-weight-of-sediment basis. For purposes of numerical calculation and graphical display, all concentrations below detection levels were assumed to be one-half the detection level.

Anaerobic incubations

The dechlorination capabilities of native microbial populations in Lake Ketelmeer sediment have been tested in laboratory incubations for chlorinated compounds that already existed in the polluted sediment and for CBs and PCBs that were added to the same sediment in the laboratory. The following procedure was used, 60 ml of Lake Ketelmeer sediment slurries (30% d.w.) was added to 100 ml serum bottles, sealed with viton stoppers (Eriks b.v., Alkmaar, The Netherlands). Incubation was in the dark at 20°C. Headspace samples were taken twice a week for methane analysis. Methane was determined with a gas

chromatograph (HP 5890), equipped with a Carbosieve SII column and flame ionization detector. Helium was used as a carrier gas, gas flow was 30 ml/min. After 4 weeks of incubation, increased methane concentrations indicated that anaerobic conditions were established. At that moment, 1,2,4-trichlorobenzene (1,2,4-TCB), 1,2,4,5-tetrachlorobenzene (1,2,4,5-TeCB), hexachlorobenzene (HCB), 2,4,4'-trichlorobiphenyl (PCB 28), 2,2',5,5'-tetrachlorobiphenyl (PCB 52) and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB 153) in 100 µl of methanol were added separately to series of serum bottles. In another series of serum bottles no chlorinated compounds were added. In these bottles, the degradability of chlorinated compounds already present in the sediment, was studied. Pure methanol (100 µl) was added to these bottles, in order to obtain identical treatment for both series. Sterile controls were obtained by adding formalin to the slurries, the final concentration being 4% (v/v). Duplicates were sacrificed at each time point and 6 g (w.w.) slurry samples were extracted with acetone by the method described above in order to obtain the CB and PCB concentrations.

Dechlorination products of HCB were identified in another series of incubations with a similar experimental set-up. In order to obtain detectable changes in possible dechlorination products, the concentration level of the laboratory added HCB has been increased to 1340 nmol/kg in these experiments.

RESULTS

Pollutants in the Rhine River

A significant decrease in concentrations of HCB and lead in Rhine water near Lobith is observed during the last two decades (Fig. 3.1). The concentrations of the lower chlorinated benzenes have only been monitored after 1982. The sum of the concentrations of TCBs has decreased as well.

There are no major point sources of CBs, PCBs and lead at Lake Ketelmeer, nor along the Rhine and IJssel between Lobith and Lake Ketelmeer. In Lake Ketelmeer, sedimentation of suspended solids together with the associated pollutants occurs as a result of decreasing streaming velocities. For hydrophobic, persistent and relatively non-volatile pollutants, the changes in concentrations observed in Rhine water during the last two decades are expected to be reflected by the pollutant concentration profiles in sediment cores from Lake Ketelmeer.

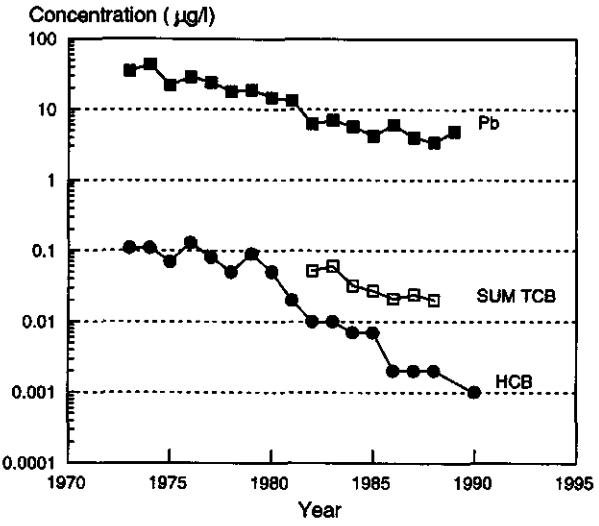

Fig. 3.1. The concentrations of lead (Pb), all three trichlorobenzenes (SUM TCB) and hexachlorobenzene (HCB) in Rhine River water, sampled at the German-Dutch border (Lobith).

Pollutants in sediment cores and stored top layer samples

The thickness of the IJsselmeer-deposit is not identical at the three sampling locations, therefore concentration profiles of pollutants are constructed by combining the data of the various cores expressed against the period of deposition, instead of depth. The cesium activities in the three cores are shown in Fig. 3.2 and clearly show the ^{137}Cs maximum in the samples from near the surface, that always correlated with elevated activities of ^{134}Cs ($t_{1/2}$=2.06 y), indicating the fallout from the nuclear power plant accident in Chernobyl in April 1986. The second ^{137}Cs maximum, found in the deeper layers, was related to the fallout from nuclear weapons testing, which had its maximum in the early 1960s. These results are in agreement with earlier sediment dating studies in this area (Beurskens et al. 1993 Chapter 2; Comans et al. 1989).

Concentration profiles of lead, PCB 52 and HCB are shown in Fig. 3.3. The PCB congener has been included as a compound that behaves rather similar as HCB in Lake Ketelmeer with regard to volatilization to the atmosphere (Henry's law constant for HCB and PCB 52 are $4 \cdot 10^{-4}$ and $1.6 \cdot 10^{-4}$ atm.m^3.mol^{-1}, respectively, Ten Hulscher et al. 1992), and with regard to sorption to suspended matter and subsequent sedimentation (Octanol/water partition constants [Log K_{ow}] for HCB and PCB 52 are 5.73 and 6.31, respectively, De Bruijn et al. 1989).

Decreasing concentrations of lead in Lake Ketelmeer sediment are observed since the

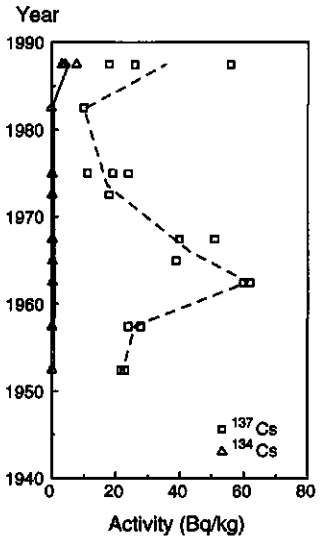

Fig. 3.2. Cesium activities in sediment layers from three cores vs. the estimated years of deposition. Average activities are indicated by visually fitted curves.

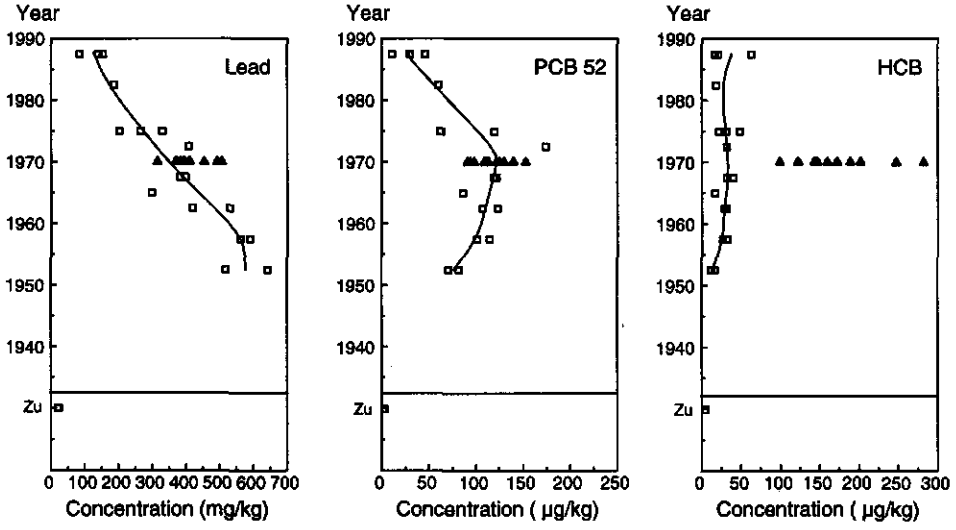

Fig. 3.3. The concentrations of lead, 2,2',5,5'-tetrachlorobiphenyl (PCB 52) and hexachlorobenzene (HCB) in sediment core layers taken in 1988 (□) vs. the estimated years of deposition. The average concentration profile in the cores is indicated by the visually fitted curve. The concentrations of the same compounds in top layer samples collected in 1972 (▲) indicate the original pollution level.

1950s (Fig. 3.3). The pattern is in agreement with the decreasing concentrations in Rhine water (Fig. 3.1). The stored top layer samples from 1972 represent the contamination levels around 1970 and are plotted in the same graphs together with the concentration profiles from the cores. The concentrations in the stored samples from 1972 confirm the age estimation, they fit rather well in the constructed lead profile. Highest concentrations of PCB 52 were found in layers deposited in the 1960-1975 period. The concentrations of PCB 52 in the samples from 1972 clearly fit in the average concentration level found in the sediment core layers around 1970. The concentration of HCB in the sediment cores is rather constant and relatively low. Surprisingly, there is no maximum in the deeper layers and the highest concentration is even found in a sample from near the surface (63 µg/kg). On the other hand, HCB concentrations in the stored samples from 1972 are two to seven times as high (range: 99-282 µg/kg) as the levels found in core layers that have been deposited around 1970 (±40 µg/kg). Even higher concentrations of HCB are found by recent analysis of stored flood plain samples of the Rhine River, taken in 1970 and 1972 (Japenga et al. 1990). Somehow HCB has disappeared in the anaerobic lake sediment. The concentrations of PCB 52 and HCB in the samples from the Zu-deposit, directly beneath the IJm-deposit, were below the detection limit (1 µg/kg). The lead concentration in the Zu-deposit (20 mg/kg) equals the natural background concentration of unpolluted Rhine sediment (Salomons, 1989).

The concentrations of pentachlorobenzene (QCB) and tetrachlorobenzenes (TeCBs) in the cores were rather low and showed only minimal losses as compared to the concentrations in the samples from 1972 (cf. Table 3.1).

The concentration profiles of the trichlorobenzenes (TCBs) are shown in Fig. 3.4. The concentrations of 1,2,3-TCB were rather low in the sediment cores and reached a maximum of 23 µg/kg in the 1960s. The concentrations in the top layer samples from 1972 were somewhat higher. For 1,2,4-TCB, high concentrations are found in the sediment cores around 1955-1965. Concentrations of 1,2,4-TCB in the top layer samples from 1972 fit rather well in the concentration profile of the cores. The 1,3,5-isomer shows highest concentrations around 1970 in the sediment cores. The concentrations in the samples from 1972 are much lower. This clear difference indicates that somehow 1,3,5-TCB has been produced in the anaerobic sediment. The concentrations of TCBs in the Zu-layers indicate some downward transport of 1,2,3- TCB (Fig. 3.4).

The concentrations of all dichlorobenzenes (DCBs) were rather high in the sediment cores and reached concentrations up to 500 µg/kg (Fig. 3.5). The concentration profile of 1,2-DCB in the sediment core has no clear maximum. Concentrations in the top layer samples from 1972 are somewhat lower than the concentrations in the cores. The concentration of

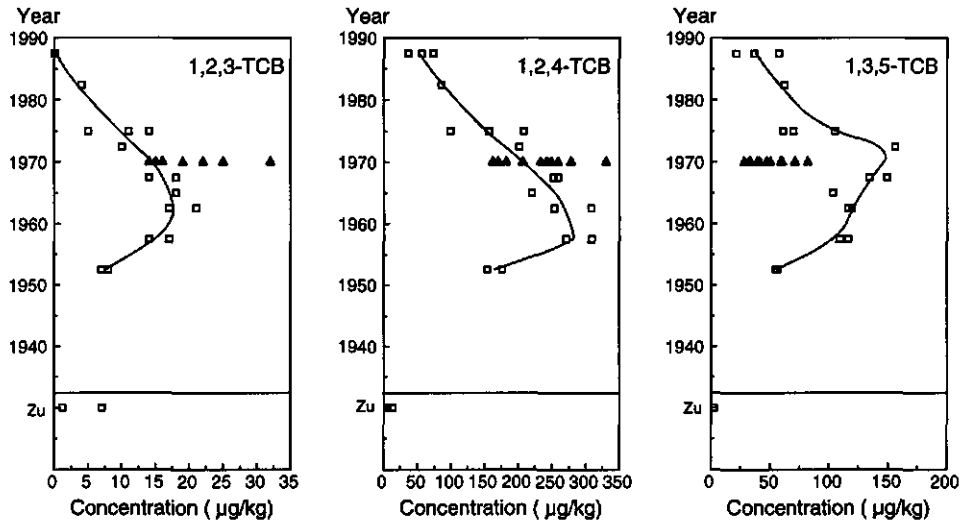

Fig. 3.4. The concentrations of the trichlorobenzenes (TCB) in sediment core layers taken in 1988 (□) vs. the estimated years of deposition. The average concentration profile in the cores is indicated by the visually fitted curve. The concentrations of the same compounds in top layer samples taken in 1972 (▲) indicate the original pollution level.

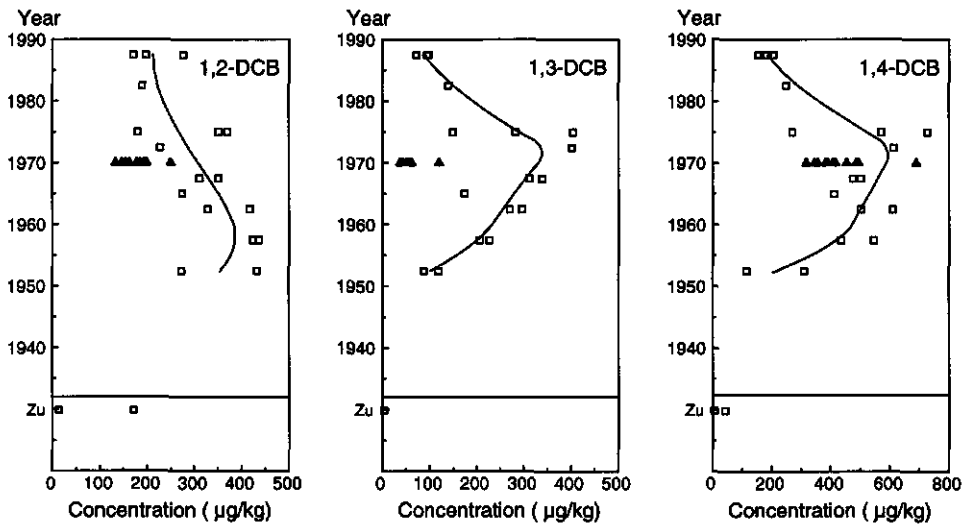

Fig. 3.5. The concentrations of the dichlorobenzenes (DCB) in sediment core layers taken in 1988 (□) vs. the estimated years of deposition. The average concentration profile in the cores is indicated by the visually fitted curve. The concentrations of the same compounds in top layer samples taken in 1972 (▲) indicate the original pollution level.

the 1,3-isomer in the cores has a maximum around 1970, and is about seven times as high as the concentration in the stored top layer samples from 1972. The concentrations of the 1,4-isomer were very high in the core layers that have been deposited between 1955 and 1975. The concentration in the top layer samples from 1972 were somewhat below the concentrations found in the sediment cores around 1970. The concentrations of the DCBs in the Zu-layers indicate that downward transport has occurred, in particular of 1,2-DCB.

As indicated by the results of Fathepure et al. (1988), the microbial dechlorination of CBs results in a stochiometric accumulation of dechlorination products. A mass balance has been calculated (Table 3.1) for the top layer samples from 1972, that reflect the original pollution level and the core layers dated around 1970. These core layers show the concentrations after almost 20 years of anaerobic environmental incubation. Only the significant differences at a 95% confidence level are considered. The loss in the total amount of HCB, QCB and 1,2,3,5-TeCB is far less than the increase in the amount of 1,3,5-TCB, 1,2-DCB and 1,3-DCB.

Table 3.1. Comparison between chlorinated benzene concentrations in top layer samples that have been taken in 1972 and recently sampled core layers that have been deposited around 1970 (s.d.=standard deviation). The differences are interpreted as disappearance (-) and production (+) of chlorinated benzenes in the anaerobic lake sediment.

Compound	Concentration (nmol/kg)				Difference[1]
	Top layers from 1972 (n=10)		Sediment core layers from ±1970 (n=3)		
	mean	s.d.	mean	s.d.	
HCB	606	205	121	16	-485
QCB	107	23	70	6	-37
1,2,3,4-TeCB	94	17	93	20	ns
1,2,3,5-TeCB	40	8	25	3	-15
1,2,4,5-TeCB	189	39	236	21	ns
1,2,3-TCB	98	21	77	22	ns
1,2,4-TCB	1226	220	1308	174	ns
1,3,5-TCB	259	90	806	63	+547
1,2-DCB	1184	159	2016	431	+832
1,3-DCB	350	64	2388	315	+2038
1,4-DCB	2759	400	3608	495	ns

[1]only significant differences at the 95% confidence level are denoted (*t*-test), ns = not significant.

Anaerobic incubations with Lake Ketelmeer sediment

The studied CBs and PCBs in the field contaminated sediment did not show any clear disappearance in the anaerobic incubations by comparison with the sterile controls (Table 3.2). HCB was the only laboratory spiked compound that showed a clear decrease by comparison with the sterile control. The disappearance of HCB occurred mainly in the first part of the experiment (half-life of HCB was 11 weeks). A rather constant residual concentration, more or less equal to the field contamination level that already existed in the sediment, remained unaltered up to 78 weeks.

Table 3.2. Anaerobic incubations of field contaminated sediment and laboratory spiked sediment (n=2).

Compound	Initial concentration (in µg/kg d.w.)	Recovery after 78 weeks (as % of initial concentration)	
		Non sterile	Sterile
Field contaminated			
1,2,4-TCB	127	98	113
1,2,4,5-TeCB	21	95	86
HCB	41	80	124
PCB 28	140	76	76
PCB 52	100	94	94
PCB 153	104	94	121
Laboratory spiked			
1,2,4-TCB	153	84	81
1,2,4,5-TeCB	63	75	154
HCB	161	37	110
PCB 28	230	83	87
PCB 52	178	99	110
PCB 153	201	106	96

In an additional experiment, a high HCB addition enabled the detection of changes in the possible dechlorination products. In the sterile controls no decrease of the HCB concentration was observed. In Fig. 3.6 the concentrations of the added HCB and produced metabolites are presented. Only compounds that showed a clear difference from the sterile controls during the 18 week incubation period are shown. An almost linear decrease of HCB resulted in a transient increase of QCB and 1,3,5-TCB concentrations and finally an increase of 1,3-DCB. The HCB dechlorination pathway can not be identified fully from this single experiment, but it became clear that 1,3,5-TCB is a major intermediate. The 1,3-DCB may be a major endproduct of the HCB dechlorination, although it remains uncertain whether a prolonged incubation would lead to a decrease of the 1,3-

DCB as well.

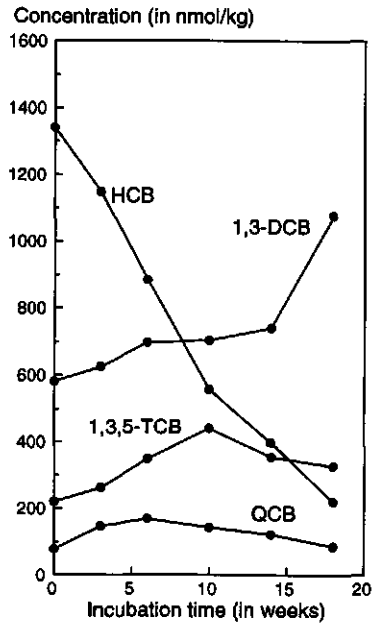

Fig. 3.6. Pattern of hexachlorobenzene dechlorination and the formation of dechlorination products during the anaerobic incubation of Lake Ketelmeer sediment after a hexachlorobenzene addition in the laboratory.

DISCUSSION AND CONCLUSIONS

Testing hypotheses for the disappearance of HCB in lake sediment

For hydrophobic, persistent and non-volatile pollutants it is expected that sediment cores from Lake Ketelmeer show concentration profiles that reflect the changes in pollutant concentrations of Rhine water during the last decades. This proved to be true for lead. For HCB the concentration profile did not reflect the decrease in concentration which is observed in Rhine water during the last two decades. Below, three hypotheses explaining the absence of a decrease in the HCB concentration in sediment cores since about 1975 will be verified. The first hypothesis is that partition processes (sorption and/or volatilization) prevent accumulation of HCB in the sediment. Secondly, the absence of a decrease is explained by the possibility of downward transport of HCB with infiltrating water. Thirdly, it is hypothesized that HCB is not persistent in the anaerobic sediment.

The first hypothesis is verified with the aid of PCB 52 concentrations in the cores. HCB and PCBs had similar discharge patterns in the Rhine River with highest levels around 1970 as demonstrated by Japenga et al. (1990) in a study using freshly deposited sediments from floodplains, sampled in 1958, 1970, 1972 and 1981. HCB and PCB 52 will behave rather similar in the water column of Lake Ketelmeer with respect to volatilization and sorption, as indicated by their water/air and water/octanol partitioning constants. For PCB 52, maximum concentrations in the sediment cores were observed in layers dated around 1970. The top layer samples from 1972 had similar PCB 52 concentrations as found in the cores around 1970 indicating that no disappearance has occurred since. Long term persistence of PCB 52 in anaerobic sediments was also found by Brown & Wagner (1990). The recalcitrance of PCB 52 in Lake Ketelmeer sediment was confirmed in the anaerobic incubations (Table 3.2). Consequently, the lack of a maximum in the HCB concentration in the sediment cores can not be explained by a low sorption to settling particles or a high volatilization to the atmosphere. In addition, analyses of top layer samples from 1972 showed that high concentrations of HCB have been deposited.

Transport of HCB to deeper sediment layers by infiltrating water is a second hypothesis for the observed relatively low and constant level of HCB in sediment cores and the high HCB concentrations in the top layer samples from 1972. Therefore the HCB levels in the Zu-layers directly beneath the IJm-deposit were also determined (Fig. 3.3). It is expected that the Zu-deposit was not polluted with chlorinated compounds at time of deposition. The Zu-layers have been deposited at least before 1932. The widespread use of chlorinated biphenyls and benzenes started somewhere in the 1940s. The concentrations of HCB in the Zu-samples were below the detection limit (1 µg/kg) and indicate that no downward transport has occurred.

The third hypothesis, HCB does not persist in the anaerobic lake sediment, is based on the findings of Fathepure et al. (1988). They showed a rapid microbial dechlorination of HCB into lower chlorinated benzenes in anaerobic sewage sludge. Sediment cores from Lake Ketelmeer show a clear loss of HCB as compared to the concentrations in the top layer samples from 1972. An increase of possibly related dechlorination products like 1,3,5-TCB is observed in the sediment cores. However, a mass balance for the CBs in sediment cores and top-layer samples (Table 3.1) indicates that not all increases of the lower chlorinated compounds in these layers can be attributed to the dechlorination of HCB. Two explanation are possible. 1) The less hydrophobic DCBs are transported downward, as indicated by the elevated levels in the Zu-deposit. This transport may result in a downward shift of the maximum in the concentration profiles. Consequently, the amount of DCBs produced within these layers will be overestimated. 2) Possible losses of CBs during sample treatment and

storage of the 1972 samples. This may result in an underestimation of the amount of HCB that has disappeared and an overestimation of the amount of lower chlorinated benzenes produced.

The anaerobic incubations with Lake Ketelmeer sediment clearly support the third hypothesis. It became clear that the native microbial population is able to dechlorinate HCB and produces lower chlorinated benzenes. Unfortunately, we were not able to demonstrate a quantitative transformation of HCB into less chlorinated benzenes in the laboratory study. This was probably caused by the rather high variation in the DCB quantification and the inability to quantify monochlorobenzene at concentrations ≤26,700 nmol/kg.

Based on the various facts it is concluded that *in situ* microbial dechlorination of HCB in the anaerobic Lake Ketelmeer sediment has occurred. The rate of HCB dechlorination in Lake Ketelmeer (a mean half-life time) is difficult to determine. Assuming first order kinetics, a maximum half-life of 7 years for HCB has been calculated for the data around 1970. Half-life times for some PCB congeners have been estimated at 8 years in anaerobic estuarine sediment (Brown & Wagner, 1990).

Implications of dechlorination in the environment.

The disappearance of HCB in Lake Ketelmeer sediment showed clearly two limitations of the microbially mediated dechlorination in the natural environment. First, dechlorination seems not to result in a complete elimination of HCB. Although after 20 years the majority of the original input has been dechlorinated (80% of the HCB deposited in the early 1970s), a residual concentration of about 40 µg/kg was observed in all sediment cores. Transformation of the residual fraction is probably very slow or even absent. No decrease of this fraction was observed in the laboratory incubations as well (Table 3.2). Nevertheless, HCB added to the same sediment in the laboratory was readily dechlorinated. This indicates that the persistence of the residual concentration is probably the result of a limited microbial availability. Secondly, the dechlorination of HCB in the environment results in the formation of lower chlorinated benzenes that seem to accumulate in the anaerobic sediment, as observed in the sediment cores. Moreover, the laboratory incubations showed the persistence of 1,2,4-TCB and 1,2,4,5-TeCB (Table 3.2) even if they are added to the sediment in the laboratory. These results indicate a high specificity of the anaerobes in Lake Ketelmeer sediment. The produced less chlorinated benzenes are less toxic than the parent compounds (van Leeuwen et al. 1990) and are less hydrophobic and therefore more mobile than HCB. Theoretically, the lower chlorinated benzenes might enter the aerobic lake water due to their increased mobility and be mineralized by aerobic microorganisms. In Lake Ketelmeer, where convective transport

through downward seepage dominates diffusive transport in the pore water, upward transport of the lower chlorinated benzenes to the aerobic upper sediment and lake water, is unlikely. Downward transport is more realistic and results in an entrance into the anaerobic ground water, probably leading to a long term persistence. Transport to the Zu-deposit has only been observed for 1,2-DCB, 1,4-DCB and 1,2,3-TCB. Although these compounds appear not to be related to the dechlorination of HCB, at least they show the potential mobility of di- and trichlorinated benzenes.

The three PCBs tested in laboratory incubations showed no significant decrease in the 78 weeks incubation period. The persistence of PCB 52 in Lake Ketelmeer sediment was confirmed by the PCB 52 concentrations in the top layer samples from 1972, that clearly fitted in the concentration profile of the sediment cores. It is concluded that PCB 52 is highly persistent in the anaerobic Lake Ketelmeer sediment. The persistence of the three tested PCBs (Table 3.2) might be the result of microbial specificity or PCB concentrations being too low to induce dechlorination, even for the laboratory spiked compounds. Quensen et al. (1988) showed that dechlorination of Aroclor 1242 strongly depended on the applied dosage. Microbially mediated reductive dechlorination of PCBs in anaerobic Hudson sediment has been indicated based on PCB congener distributions in anaerobic sediments relative to the distributions in the original mixtures (Brown et al. 1987). This was confirmed in laboratory studies using microorganisms eluted from the anaerobic sediments in which reductive dechlorination of PCBs was thought to occur (Nies & Vogel 1990; Quensen et al. 1988). Although the three tested PCBs seem to persist in Lake Ketelmeer sediment, it can not be excluded that other, more reactive, PCB congeners are transformed in Lake Ketelmeer sediment.

A complete *in situ* biological decontamination of HCB in the sediment is not feasible due to the persistence of residues and the accumulation of reaction products under anaerobic conditions. As recently demonstrated by Fathepure & Vogel (1991) a complete biomineralization of HCB can be obtained in a two stage anaerobic-aerobic biofilm reactor. However, if dechlorination takes place in the natural environment with hydrological conditions as in Lake Ketelmeer, anaerobic conditions are prolonged and the relatively mobile dechlorination products persist and may be transported. In this case, environmental dechlorination becomes an additional source of persistent and mobile compounds that are of particular concern for the ground water quality.

Acknowledgements

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REFERENCES

Bailey RE (1983) Comment on "Chlorobenzenes in sediments, water, and selected fish from Lakes Superior, Huron, Erie, and Ontario". Environ. Sci. Technol. 17: 504.

Barber LB (1988) Dichlorobenzene in ground water: evidence for long-term persistence. Ground Water 26: 696-702.

Beurskens JEM, Mol GAJ, Barreveld HL, van Munster B & Winkels HJ (1993) Geochronology of priority pollutants in a sedimentation area of the Rhine River. Environ. Toxicol. Chem. 12: 1549-1566.

Bosma TNP, van der Meer JR, Schraa G, Tros ME & Zehnder AJB (1988) Reductive dechlorination of all trichloro- and dichlorobenzene isomers. FEMS Microbiology Ecology 53: 223-229.

Brown JF Jr, Bedard DL, Brennan MJ, Carnahan JC, Feng H & Wagner RE (1987) Polychlorinated biphenyl dechlorination in aquatic sediments. Science 236: 709-712.

Brown JF Jr & Wagner RE (1990) PCB movement, dechlorination, and detoxication in the Acushnet estuary. Environ. Toxicol. Chem. 9: 1215-1233.

Choudhry GG & Hutzinger O (1984) Acetone-sensitized and nonsensitized photolyses of tetra-, penta-, and hexachlorobenzene in acetonitrile-water mixtures: photoisomerization and formation of several products including polychlorobiphenyls. Environ. Sci. Technol. 18: 235-241.

Comans RNJ, Middelburg JJ, Zonderhuis J, Woittiez JRW, de Lange GJ, Das HA & van der Weijden CH (1989) Mobilization of radiocaesium in pore water of lake sediments. Nature 339: 367-369.

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

De Bruijn J, Busser F, Seinen W & Hermens J (1989) Determination of octanol/water partition coefficients for hydrophobic organic chemicals with the slow-stirringmethod. Environ. Toxicol. Chem. 8: 499-512.

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.

Fathepure BZ & Vogel TM (1991) Complete degradation of polychlorinated hydrocarbons by a two-stage biofilm reactor. Appl. Environ. Microbiol. 57: 3418-3422.

Haigler BE, Nishino SF & Spain JS (1988) Degradation of 1,2-dichlorobenzene by a *Pseudomonas* sp. Appl. Environ. Microbiol. 54: 294-301.

Heymen R (1990) Results of the water quality research in the Rhine River in the Netherlands between 1970 and 1989. RIZA Report no. 90.048 (in dutch), Lelystad, the Netherlands.

Hirsch M & Hutzinger O (1989). Naturally occurring proteins from pond water sensitize hexachlorobenzene photolysis. Environ. Sci. Technol. 23: 1306-1307.

Japenga J, Zschuppe KH, de Groot AJ & Salomons W (1990). Heavy metals and organic micropollutants in floodplains of the Waal, a distributary of the river Rhine, 1958-1981. Netherlands Journal of Agricultural Science 38: 381-397.

Kühn W & Brauch H-J (1988) Organic micropollution in the river Rhine; determination and evaluation for the assessment of waters (in German). Fresenius Z. Anal. Chem. 330: 324-325.

Kypke-Hutter K, Vogelgesang J, Malisch R, Binnemann P. & Wetzlar H (1986) Aufklärung einer kontamination von Neckarfischen mit hexachlorbenzol, octachlorstyrol und pentachlorbenzol: entstehung bei einem industriellen prozess. I Verlauf der kontamination im Oberen Neckar. Z. Lebensm. Unters. Forsch. 182: 464-470.

Marinucci AC & Bartha R (1979) Biodegradation of 1,2,3- and 1,2,4-trichlorobenzene in soil and in liquid enrichment culture. Appl. Environ. Microbiol. 38: 811-817.

Meijers AP & van der Leer PC (1976) The occurrence of organic micropollutants in the river Rhine and the river Maas in 1974. Water Research 10: 597-604.

Nies L & Vogel TM (1990) Effects of organic substrates on dechlorination of Aroclor 1242 in anaerobic sediments. Appl. Environ. Microbiol. 56: 2612-2617.

Oliver BG (1987) Fate of some chlorobenzenes from the Niagara River in lake Ontario. In: Hites RA & Eisenreich SJ (Eds) Sources and fates of aquatic pollutants. Advances in chemistry series, 216 (pp 471-489). American Chemical Society, Washington.

Oliver BG & Nicol KD (1982) Chlorobenzenes in sediments, water, and selected fish from lakes Superior, Huron, Erie, and Ontario. Environ. Sci. Technol. 16: 532-536.

Oliver BG & Nicol KD (1983) Authors reply on comments of RE Bailey. Environ. Sci. Technol. 17: 505.

Pfaender FK & Bartholomew GW (1982) Measurement of aquatic biodegradation rates by determining heterotrophic uptake of radiolabeled pollutants. Appl. Environ. Microbiol. 44: 159-164.

Quensen JF III, Tiedje JM & Boyd SA (1988) Reductive dechlorination of polychlorinated biphenyls by anaerobic microorganisms from sediments. Science 242: 752-754.

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

Salomons W (1989) Fate and behaviour of trace metals in a shallow eutrophic lake. In: Boudou A & Ribeyre F (Eds) Aquatic ecotoxicology: fundamental concepts and methodologies, Vol 1 (pp 185-199). CRC Press, Boca Raton, Fl, USA.

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.

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.

Spain JC & Nishino SF (1987) Degradation of 1,4-dichlorobenzene by *Pseudomonas* sp. Appl. Environ. Microbiol. 53: 1010-1019.

Ten Hulscher TEM, van der Velde LE & Bruggeman WA (1992) Temperature dependence of Henry's law constants for selected chlorobenzenes, PCBs and PAHs. Environ. Toxicol. Chem. 11: 1595-1603.

Tiedje JM, Boyd SA & Fathepure BZ (1987) Anaerobic degradation of chlorinated aromatic hydrocarbons. Dev. Ind. Microbiol. 27: 117-127.

Van der Meer JR, Roelofsen W, Schraa G & Zehnder AJB (1987). Degradation of low concentrations of dichlorobenzenes and 1,2,4-trichlorobenzene by *Pseudomonas* sp. strain P51 in nonsterile soil columns. FEMS Microbiology Ecology 45: 333-341.

Van der Weijden CH & Middelburg JJ (1989) Hydrogeochemistry of the river Rhine: long term and seasonal variability, elemental budgets, base levels and pollution. Water Research 23: 1247-1266.

Van Leeuwen CJ, Adema DMM & Hermens J (1990) Quantitative structure-activity relationships for fish early life stage toxicity. Aquatic Toxicology 16: 321-334.

Vogelgesang J, Kypke-Hutter K, Malisch R, Binneman P & Dietz W (1986) The origin of a contamination of fish from the river Neckar with hexachlorobenzene, octachlorostyrene and pentachlorobenzene: formation in an industrial process. II The formation of contaminants in the degassing of an aluminium foundry with chlorine. Z. Lebensm. Unters. Forsch. 182: 471-474.

CHAPTER 4

DECHLORINATION OF CHLORINATED BENZENES BY AN ANAEROBIC MICROBIAL CONSORTIUM THAT SELECTIVELY MEDIATES THE THERMODYNAMIC MOST FAVORABLE REACTIONS

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ABSTRACT

A chlorinated benzene dechlorinating anaerobic microbial consortium was obtained by selective enrichment with hexachlorobenzene (HCB) and lactate from a freshwater sediment sample that originated from an area with proven *in situ* HCB dechlorination. The consortium was used to determine compound selectivity and relative dechlorination rates by incubation with the individual chlorinated benzenes under methanogenic conditions. The dechlorinating activity was restricted to benzenes with at least three adjacent chlorines, except for a relative slow transformation of 1,2,4,5-tetrachlorobenzene to 1,2,4-trichlorobenzene. Optimal temperature for dechlorination was around 30°C, significant dechlorinating activity was still observed at a temperature of 3°C. The selectivity of the enrichment culture showed an interesting correlation with the thermodynamics of the various dechlorination steps: from the 19 dechlorination reactions possible with benzenes that contain at least two chlorines, only the seven reactions with the highest energy release took place.

INTRODUCTION

Chlorinated benzenes (CBs) can enter the aquatic environment as solvents, pesticides, dielectric fluids, deodorants and chemical contaminants or intermediates. They are prevalent in both solid and liquid industrial effluents and in atmospheric discharges. As a result of their widespread use during several decades, CBs have become ubiquitous in the aquatic environment; they have been detected in water, sediments, and aquatic biota (1, 2). Elimination of CBs from the aquatic environment may be caused by volatilization, photodegradation, and biodegradation. Volatilization seems to be a major loss mechanism for mono- (MCB) and dichlorobenzenes (DCBs) (3, 4). Photochemical transformation of some CBs has been demonstrated (5, 6). Microbial mineralization of the lower chlorinated benzenes in the aerobic water column may occur (7-14), although reported degradation rates are slow in surface waters (15). Due to the incompleteness of the above-mentioned removal processes and the relative hydrophobic character of CBs, a substantial amount ends up in sediments.

A long-term persistence of CBs in anaerobic sediments was assumed until reductive dechlorination of hexachlorobenzene (HCB) was first reported in 1987 (16). Since then, laboratory studies have demonstrated the microbial dechlorination of any CB that contains at least two chlorines, resulting in the accumulation of less chlorinated isomers (17-19). Calculated half-lifetimes of HCB dechlorination, based on laboratory studies (17, 20), range between a few days to several weeks.

In situ reductive dechlorination of CBs in sediments was subject to speculation in the past (21-23). Only recently, dechlorination of HCB in natural sediments was demonstrated unequivocally (24, Chapter 3). Comparison of HCB concentrations in recently collected core layers deposited around 1970 with 20-year-old stored sediment top layers that reflected the original input revealed an 80% loss of HCB and increases in 1,3,5-trichlorobenzene (1,3,5-TCB) and 1,3-dichlorobenzene (1,3-DCB) during the 20 years of "environmental incubation". Laboratory incubations with sediment from the same area (Lake Ketelmeer, a sedimentation area of the Rhine River (western Europe)), demonstrated that the native anaerobic microbial population was capable of catalyzing this reaction. On the basis of the sediment core data, a maximum half-life of HCB in the anaerobic sediment was estimated to be 7 years (24, Chapter 3). Two major differences between laboratory studies and field observations can be derived. First, the compound spectrum subject to dechlorination in the environment is not as broad as observed in laboratory studies. Laboratory studies indicate the possibility of a sequential dechlorination leading to the accumulation of MCB as the only end product (17-19), instead of 1,3,5-TCB and 1,3-DCB accumulation as observed in

the environment. Second, dechlorination rates in the environment seem to be rather slow. The objective of the present study was to determine compound selectivity, relative dechlorination rates of the different CBs and the influence of temperature on the dechlorination rate carried out by an anaerobic microbial consortium from Lake Ketelmeer sediment where *in situ* dechlorination was observed. In order to make experiments less time consuming and simplify analytical procedures, an anaerobic enrichment culture was obtained from Lake Ketelmeer sediment and all experiments were carried out in liquid media without sediment.

EXPERIMENTAL SECTION

Chemicals

HCB and pentachlorobenzene (QCB) were obtained from Aldrich Chemie N.V. (Brussels, Belgium). 1,2,3,4-tetrachlorobenzene (1,2,3,4-TeCB), 1,2,4,5-TeCB and all three DCBs were purchased from Janssen Chimica (Beerse, Belgium). All trichlorobenzenes (TCBs) were obtained from Merck (Amsterdam, The Netherlands), 1,2,3,5-TeCB was obtained from Promochem (Wesel, Germany). Gases were from Hoekloos (Schiedam, The Netherlands).

Source and preparation of the inoculum

CB-contaminated sediment was collected from Lake Ketelmeer, a shallow freshwater lake in the central part of The Netherlands. The lake acts as a sedimentation area for the suspended solids from the Rhine River. Microbial dechlorination of HCB in sediment slurries, containing 30% sediment (wt/wt, on a dry weight basis), from Lake Ketelmeer has been demonstrated earlier (24, Chapter 3). With one of these sediment slurries, an enrichment procedure was started by shaking the slurry and taking a 80 mL sample to inoculate a 1 L serum bottle with 720 mL of anaerobic medium (see below), containing lactate (17.5 mM) and HCB (70 nM, added as a methanolic solution). The transfer of 80 mL samples into 720 mL fresh medium was repeated eight times after completion of the HCB dechlorination in order to get a highly active microbial consortium in the absence of sediment. In order to maintain a highly active culture for the inoculation of several experiments, repeated additions of HCB (approximately 100 nM) immediately after depletion were carried out, up to a maximum of six times in a single bottle. Methane was produced in these bottles, confirming the methanogenic conditions, but was not proportional to HCB dechlorination rates (data not shown).

Anaerobic medium

The phosphate buffered mineral medium (pH 7.0) was prepared after Holliger et al. (19) with a small modification, 0.50 g/L instead of 0.24 g/L $Na_2S \cdot 9H_2O$. The headspaces were flushed with O_2-free N_2 and sealed with Viton stoppers (Eriks b.v., Alkmaar, The Netherlands) before autoclaving. Filter-sterilized stock solutions of trace elements (25) and vitamins (19) were added, both 1 mL/L, to the autoclaved mineral medium.

Incubation methods

All experiments were carried out in 1 L serum bottles, containing 800 mL of the methanogenic medium. The general incubation protocol consisted of three phases: (A) Addition of sodium lactate (2 mL of 60% syrup; initial concentration in medium, 17.5 mM), HCB dissolved in methanol (200 µL, initial HCB concentration in medium, approximately 70 nM) and 50 mL of inoculum. In this phase, encompassing a period of about 3 weeks, growth of organisms took place and HCB dechlorination was generally slow. (B) When the concentration of HCB fell below 4 nM, a second amount of HCB was added. This amount was readily dechlorinated as a result of increased cell densities. This phase was used to characterize rate differences between bottles. (C) The actual experimental phase started when the concentration of HCB again fell below 4 nM. CBs were added to determine activity toward chlorobenzenes other than HCB, or bottles were given HCB and incubated at different temperatures in order to determine temperature influences. All tests consisted of singular incubations for the individual CBs or for HCB at different temperatures.

Sterile controls were prepared by adding formalin (final concentration 4%) to the bottles at the end of phase B, prior to the third CB addition. All incubations were in the dark at 25°C, unless otherwise stated.

Incubations with the 11 individual CBs (monochlorobenzene was excluded) to determine compound selectivity, dechlorination pathways, and rate constants followed the general protocol as described above. However, the added amounts of CBs in phase C were elevated to obtain an initial concentration of approximately 200 nM. The dechlorination rate of HCB was determined in phase B, and the dechlorination rate of the specific test compound was determined in phase C. Subsequently the rate of the test compound was standardized with the aid of the HCB dechlorination rate in phase B. Dechlorination rate constants and half-lifetimes were determined by nonlinear regression analyses of CB disappearance curves assuming first-order kinetics.

Chemical analysis

At intervals, singular 10 mL samples were removed aseptically from each active and control

incubation while swirling the medium to ensure a uniform suspension. The samples were combined with 2 mL of isooctane and shaken for 2 h. Separation of water and isooctane fraction was obtained by mixing on a vortex mixer and subsequent storage for at least 2 h at -20°C. The extraction efficiency was highly reproducible and ranged between 55 and 66% for the individual isomers. Samples of the isooctane fraction were analyzed with a HP 5890A gas chromatograph, equipped with a ^{63}Ni electron capture detector using automated splitless injection (HP 7673A). Compound separation was accomplished with a fused silica wall coated open tubular capillary column (50 m x 0.25 mm i.d.) CP Sil 19CB (0.20 µm film thickness of dimethyl- (86%), phenyl- (7%), cyanopropyl- (7%)silicone polymer, Chrompack, Bergen op Zoom, The Netherlands). Helium was used as a carrier gas, and nitrogen was used as a detector-quench gas. The operating temperatures of the injector and detector were 225 and 300°C, respectively. The oven temperature program was as follows: 90°C initially for 2 min, increase to 140°C at 2°C/min and 5 min isothermal period at 140°C before temperature increased to 225°C at 5°C/min, with an isothermal period of 90 min at the end. Retention times and peak areas were determined by using the HP 3365 ChemStation software. Peaks were identified and quantified by comparing injections with authentic external standards prepared in isooctane.

RESULTS AND DISCUSSION

Repeated additions of HCB

The maintenance of dechlorinating activity during the successive additions of CBs to the same bottles was verified in order to demonstrate the suitability of the selected experimental procedure. For that purpose, the dechlorination kinetics were determined for six consecutive HCB additions to a single bottle, with only one lactate addition at the beginning of the experiment. The first amount of HCB disappeared slowly, the subsequent additions of HCB disappeared rapidly (Figure 4.1). Disappearance of HCB coincided with an accumulation of lower chlorinated benzenes (see below). A sterile control showed neither any significant loss of HCB nor any formation of lower chlorinated benzenes. This indicates that the disappearance of HCB is mediated by an anaerobic microbial consortium that carried out a dechlorination reaction. After growth of the dechlorinating population in phase A, a stable consortium seemed to be active and a constant dechlorination capacity was observed during several successive additions. The dechlorination rate constants for periods B - F were rather constant: the mean dechlorination rate constant (k) was 0.071 h^{-1} ± 0.015 (standard deviation). The coefficient of variance was 21%, which is acceptable for

a repeated reaction in a batch system.

Repeated lactate additions combined with HCB additions in each period lowered the successive dechlorination rates, resulting in a *k* value of 0.030 h^{-1} in period F (results not shown). Therefore, lactate was added only once at the start of all following experiments.

A substantial amount of methanol, yielding an initial concentration of 6 mM, was introduced with each addition of HCB, since HCB was dissolved in methanol. Methanol may serve as a source of reducing equivalents, carbon, and energy for the dechlorinating consortium in these incubations. Stimulation of dechlorinating activity by methanol has been reported for PCBs (26).

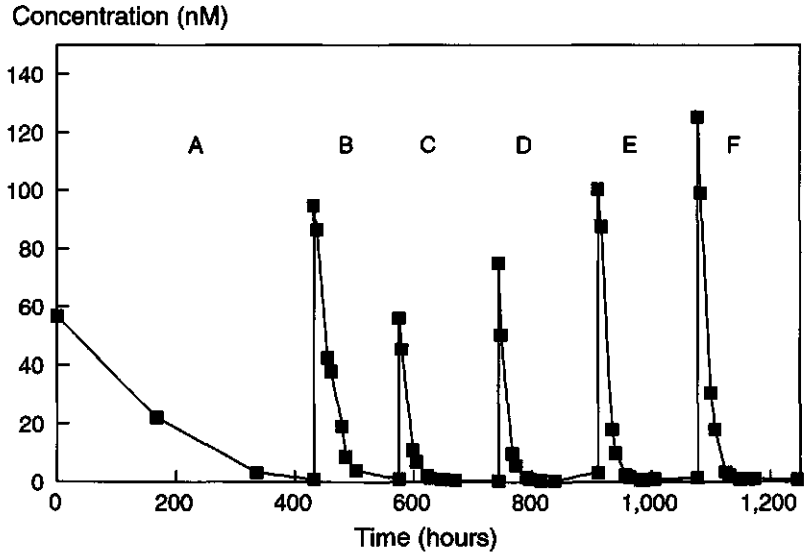

Fig. 4.1. Repeated additions of HCB to a single serum bottle with subsequent removal by the enrichment culture.

Dechlorination of the individual compounds

Sterile controls with individual compounds did not show any significant loss of the test compounds or any product formation. Therefore, transformations in the biologically active incubations can be attributed to microbial activity. The transformation of HCB and resulting product formation is shown in Figure 4.2A. The initial concentrations of 1,2,4- and 1,3,5-TCB were approximately 40 nM in period C as a result of HCB dechlorination in the preceding periods A and B. The transformation of HCB resulted in a transient accumulation of QCB, 1,2,3,5-, and 1,2,4,5-TeCB. After 380 h of incubation, a substantial amount of

1,2,4,5-TeCB was still present. The subsequent transformation of 1,2,4,5-TeCB was less rapid than the transformation of 1,2,3,5-TeCB. 1,3,5- and 1,2,4-TCB accumulated further within the experimental period. At the end of the experiment, 87% of the loss in HCB was recovered as lower chlorinated benzenes.

The incubation with QCB showed a rapid dechlorination to 1,2,3,5- and 1,2,4,5-TeCB (Figure 4.2B). Again 1,2,4,5-TeCB disappeared more slowly than 1,2,3,5-TeCB. The 1,3,5- and 1,2,4-TCB accumulated in a ratio of 60:40 after 380 h of incubation. The contribution of 1,2,4-TCB as an end product is clearly higher when produced from QCB than from HCB. About 75% of the parent QCB was recovered as lower chlorinated benzenes at the end of the experiment.

The incubation of 1,2,3,4-TeCB showed an exclusive transformation into 1,2,4-TCB (78% recovery) that was resistent to further dechlorination (Figure 4.2C).

The incubation of 1,2,3,5-TeCB resulted in a rapid accumulation of 1,3,5-TCB (Figure 4.2D). The amount of 1,2,4-TCB seemed to increase slightly during the experiment, but this increase was insufficient to support a clear conclusion regarding the dechlorination of 1,2,3,5-TeCB to 1,2,4-TCB. At the end of the experiment 105% of the loss in 1,2,3,5-TeCB was recoverd as lower chlorinated benzenes.

The incubations with 1,2,4,5-TeCB, all TCBs, and DCBs did not show any substantial substrate disappearance or any product formation within the experimental period of 380 h. An extended incubation period only showed a slow dechlorination of 1,2,3-TCB. After a lag phase of about one month, 1,3-DCB was produced. The lack of mass balance in three incubations that showed reactivity does not dramatically exceed the variation in HCB quantitation in sterile controls (≤15%). Formation of DCBs can not explain the lack of mass balance, since detection limits for these compounds were 2.3, 2.3, and 4 nM for 1,3-, 1,4-, and 1,2-DCB, respectively.

For parent compounds that showed an instantaneous reactivity (HCB, QCB, 1,2,3,4-, and 1,2,3,5-TeCB) half-lifetimes did not differ significantly and were in the range of 35 - 63 h (data not shown), indicating a high and similar affinity of the consortium for these compounds.

A striking phenomenon was observed with 1,2,4,5-TeCB. If this compound was produced from QCB, it was clearly dechlorinated (Figure 4.2B), while 1,2,4,5-TeCB added as the parent compound resisted dechlorination. Thus, there is a difference in dechlorination reactivity between compounds that are formed as an intermediate during the dechlorination and compounds that are present as a parent substrate.

The observed dechlorination pathways are summarized in Figure 4.3. HCB and QCB were dechlorinated via 1,2,3,5- and 1,2,4,5-TeCB and as final dechlorination products 1,3,5- and

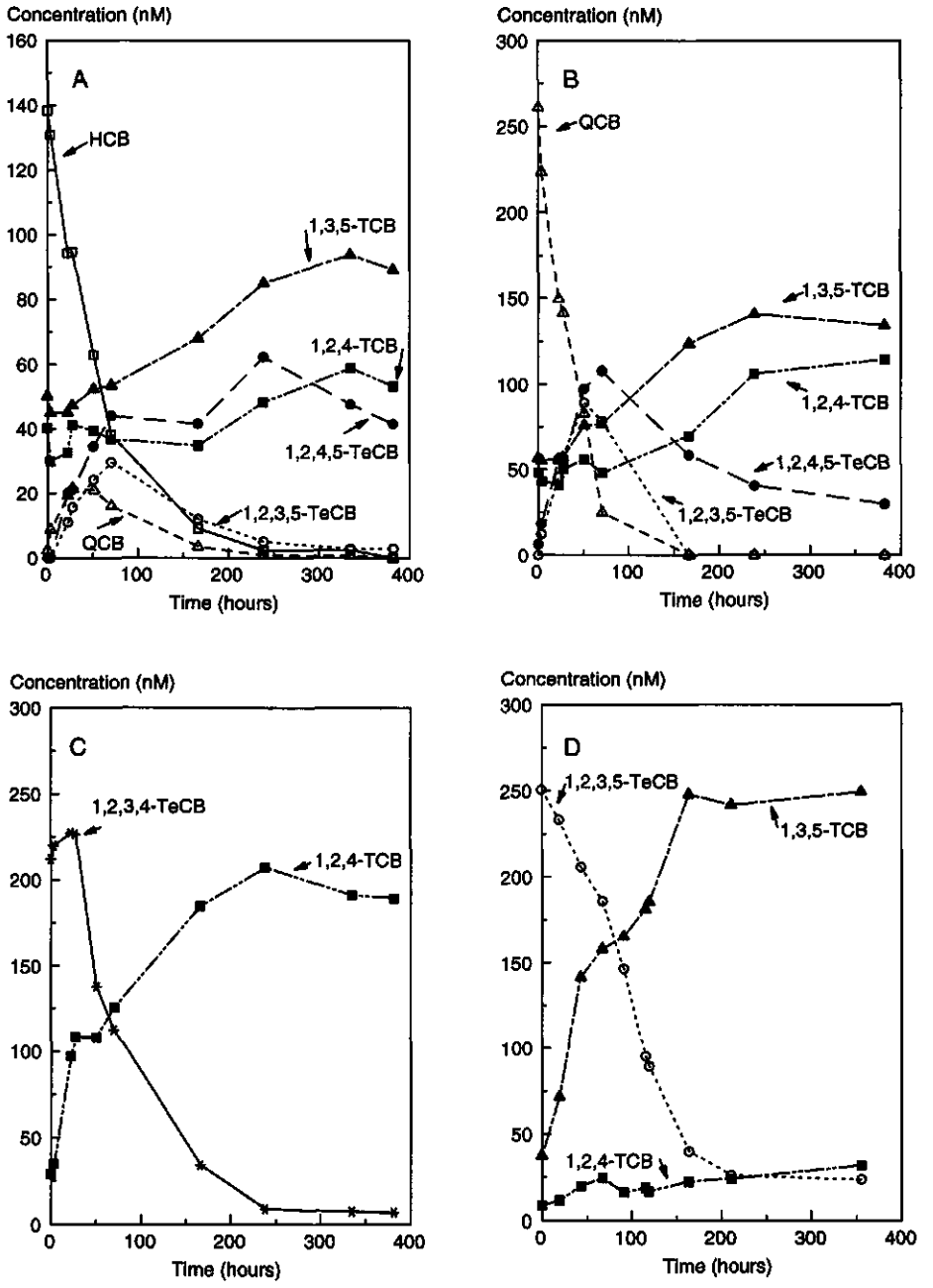

Fig. 4.2. Dechlorination of hexachlorobenzene (A), pentachlorobenzene (B), 1,2,3,4-tetrachlorobenzene (C) and 1,2,3,5-tetrachlorobenzene (D) by the enrichment culture.

1,2,4-TCB accumulated. The latter contributed at least 30% to the total amount of TCB products. The ratio between these two products deviates from earlier reports, where 1,2,4-TCB did not exceed the 10% level (17,19). In the HCB and QCB incubations, 1,2,3,4-TeCB was not detected as an intermediate, in agreement with the results of Fathepure et al. (17) and Holliger et al. (19). In incubations with Lake Ketelmeer sediment, HCB dechlorination resulted in an accumulation of 1,3-DCB (24, Chapter 3). That same sediment served as the source of the enrichment culture used in the present study. During the enrichment procedure somehow the ability to continue dechlorination of HCB to the DCB level was lost. From the dechlorination pathways of HCB, 1,2,3,4-TeCB, and 1,2,3-TCB it becomes clear that the enrichment culture from Lake Ketelmeer sediment preferentially removes the chlorine that is surrounded by chlorines on both sides. Consequently, the dechlorinating activity of this enrichment culture seems to be restricted to benzenes with at least three adjacent chlorines. The slow transformation of 1,2,4,5-TeCB into 1,2,4-TCB is the only exception.

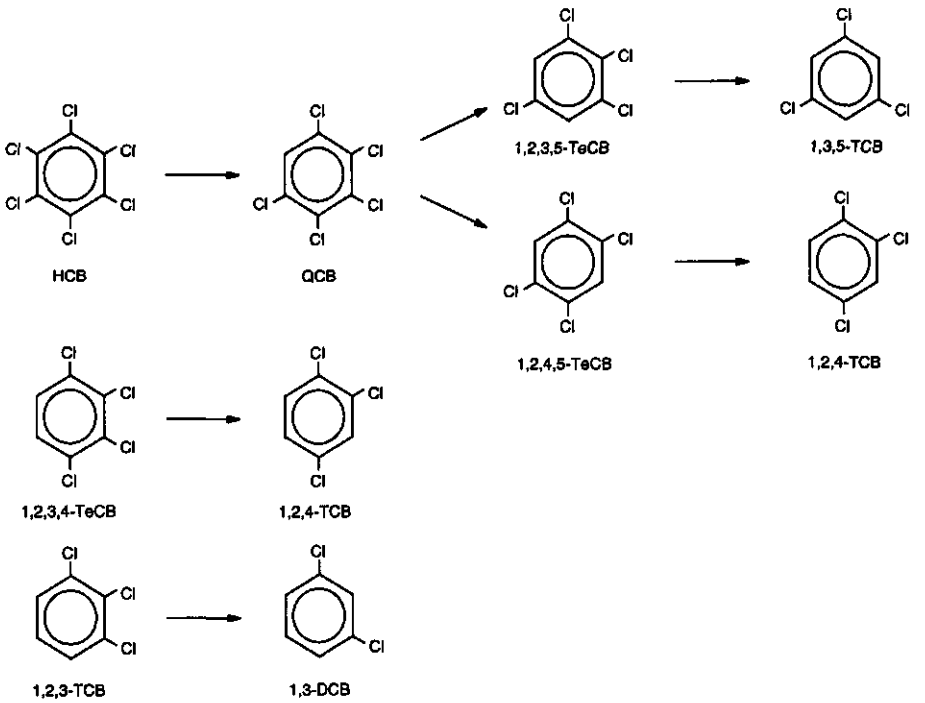

Fig. 4.3. Reductive dechlorination pathways of hexachlorobenzene, 1,2,3,4-tetrachlorobenzene, and 1,2,3-trichlorobenzene catalyzed by the anaerobic enrichment culture.

Influence of temperature

The relationship between temperature and the rate of HCB dechlorination is shown in Figure 4.4. Possible differences between bottles due to differences in cell densities or activities were taken into account by determing HCB dechlorination rates in phase B at an identical temperature for all bottles (25°C). This enabled us to calculate normalized rate constants for the HCB dechlorination in phase C at different temperatures. An optimum was observed around 30°C, with a half-life of 8 h. At temperatures relevant for field conditions during the summer, 8 - 15°C, dechlorination rates were two to six times below the rates at temperatures generally applied in laboratory research (20 - 25°C). Holliger et al. (19) found a similar optimum between 25 and 30°C for the dechlorination of 1,2,3-TCB. These authors, however, did not detect dechlorinating activity below 10°C. We still observed significant dechlorination at 3°C. This is relevant because such low temperatures occur during the winter months in Rhine sediments.

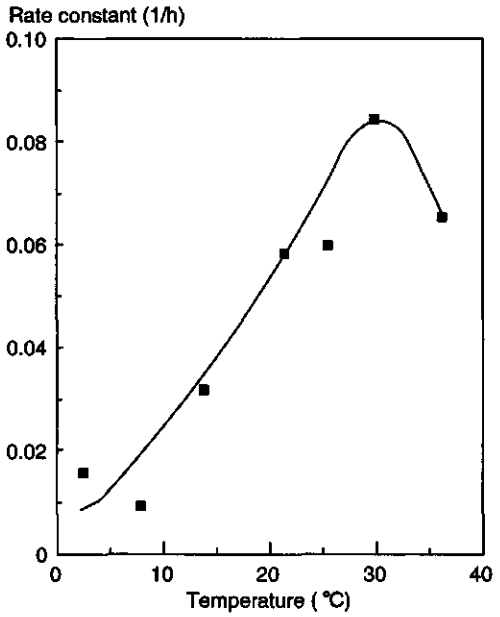

Fig. 4.4. Temperature dependence of relative dechlorination rate of hexachlorobenzene by the enrichment culture. Rate constants were calculated for HCB disappearance curves at the selected temperatures. The curves consisted of at least 6 measurements (correlation coefficients (r^2) ≥ 0.99).

Relationship with thermodynamics of dechlorination reactions

It is well-established that chlorinated aromatics can serve as electron acceptors in anaerobic environments and that dechlorinating organisms may conserve energy from this

reaction (18,19,27,28), as demonstrated for *Desulfomonile tiedjei* (29). Holliger et al. (19) indicated that for their mixed culture, capable to dechlorinate 1,2,3-TCB, H_2 served as the source of reducing equivalents. Although our incubations started with N_2 as headspace gas, fermentation of lactate in the medium apparently resulted in a sufficient H_2 production to serve as the source of reducing equivalents. Holliger et al. (19) demonstrated that 1,2,3-TCB dechlorination under N_2 headspace was obtained in the presence of lactate. Estimates for the Gibbs free energies of CB redox couples with H_2 as the electron donor have recently been reported (27) and are listed in decreasing order in Table 4.1. The dechlorinations observed with the HCB-grown enrichment culture from Lake Ketelmeer sediment are also indicated in Table 4.1. The seemingly arbitrariness of the selectivity in the catalyzed dechlorination reactions turns out to have certain systematics; from the 19 possible dechlorination reactions with benzenes that contain at least two chlorines, only the seven reactions with the highest energy release under standard conditions took place. The energy release for those dechlorination steps under standard conditions is more than 161.1 kJ per reaction. There is no immediate explanation for this observation. The energy release of those steps that are not catalyzed by the enrichment are highly exergonic too, and some of them are indeed catalyzed by other CB-dechlorinating consortia (17,18,30).

Table 4.1. Gibbs free-energy values ($\Delta G°'$) for chlorobenzene dechlorination reactions with hydrogen as electron donor (data from ref 27) and the presence (+) or absence (-) of this reaction in anaerobic incubations with enrichment culture.

$\Delta G°'^{a}$ (kJ/reaction)	Parent compound	Product	Dechlorination
-171.4	HCB	QCB	+
-167.7	QCB	1,2,3,5-TeCB	+
-166.5	1,2,3,4-TeCB	1,2,4-TCB	+
-164.3	1,2,4,5-TeCB	1,2,4-TCB	+
-163.5	1,2,3,5-TeCB	1,3,5-TCB	+
-163.4	QCB	1,2,4,5-TeCB	+
-161.2	1,2,3-TCB	1,3-DCB	+
-161.1	QCB	1,2,3,4-TeCB	–
-159.9	1,2,3,5-TeCB	1,2,4-TCB	–
-158.6	1,2,3-TCB	1,2-DCB	–
-155.2	1,2,3,4-TeCB	1,2,3-TCB	–
-153.4	1,2,4-TCB	1,4-DCB	–
-153.2	1,2-DCB	MCB	–
-150.6	1,3-DCB	MCB	–
-149.9	1,2,4-TCB	1,3-DCB	–
-148.6	1,2,3,5-TeCB	1,2,3-TCB	–
-147.3	1,2,4-TCB	1,2-DCB	–
-147.1	1,4-DCB	MCB	–
-146.2	1,3,5-TCB	1,3-DCB	–

[a]Values under standard conditions, pH=7, 25°C; H_2 in the gaseous state at a partial pressure of 100 kPa, all other compounds in aqueous solution at 1 mol/kg activity.

The Gibbs free-energy values for chlorobenzene dechlorination in Table 4.1 are for standard conditions. Under the actual conditions of the consortium, however, concentrations of substrates and products were much lower than the assumed 1 mol/kg under standard conditions and even changed during the experiment. Nevertheless, our observations are consistent with the theory behind the Gibbs free-energy of formation values. These values represent potential energy present in a compound. Location of chlorine substituents in close proximity to each other is energetically unfavorable, and it will be energetically most favorable to remove those substituents first that are close to each other. This is indeed what was observed. By enriching a consortium on HCB we have enriched for organisms/enzymes that are tailored to removing chlorine substituents in close proximity to other chlorine substituents from benzene rings with multiple adjacent chlorine substituents.

Absence of dechlorinating capabilities toward TCBs and DCBs in our enrichment may be caused by factors like, for example, the source of inoculum or the enrichment technique that consisted of repeated HCB additions. A closer look to all published CBs dechlorination studies may reveal clues as to why HCB dechlorination generally results in TCB accumulation (16,17,19,24) instead of MCB accumulation as recently reported by Ramanand et al. (30). HCB dechlorination studies that result in an accumulation of 1,3,5-TCB as the main end product were conducted with HCB (16,17,24, this study) or 1,2,3-TCB (19) in the enrichment procedure. Selecting the energetic most profitable reactions (Table 4.1) HCB dechlorination proceeds via QCB, 1,2,3,5-TeCB, to 1,3,5-TCB. Dechlorination of 1,3,5-TCB to 1,3-DCB is the least attractive step of all reactions listed in Table 4.1. Dechlorination of 1,3,5-TCB has been demonstrated (18), but started only after a lag of 6 months and was preceded by dechlorination of 1,2,3-TCB and 1,2,4-TCB present in the same column. This sequence is in agreement with the order of TCB dechlorination steps in Table 4.1 and illustrates the recalcitrance of 1,3,5-TCB. Ramanand et al. (30) used a culture acclimatized to 1,2,4-TCB to study the dechlorination of HCB. The 1,2,4-TCB was dechlorinated to MCB mainly via 1,4-DCB and in a lesser extent via 1,3-DCB. This is in accordance with the order of 1,2,4-TCB dechlorination steps in Table 4.1. Incubations with other CBs indicated that their culture preferentially removed chlorines that possess only one chlorine adjacent to the removed chlorine. This culture was simultaneously incubated with a high concentration of 1,2,4-TCB and a low concentration of HCB (concentration ratio 40:1), and dechlorination of HCB only started after depletion of 1,2,4-TCB. The HCB dechlorination proceeded via QCB, 1,2,3,4-TeCB, 1,2,3- and 1,2,4-TCB, and 1,2- and 1,4-DCB to MCB. Reactions that involved the removal of chlorines with only one adjacent chlorine dominated over reactions that are thermodynamic more profitable. Dechlorination

of HCB continued to the monochlorinated level, presumable as a result of the avoidance of 1,3,5-TCB formation. Under the described specific conditions, microorganisms mediate dechlorination steps that deviate from the thermodynamic most profitable reactions and exhibit a sequential HCB dechlorination that results in MCB accumulation. Thus, with the aid of thermodynamics, the most likely dechlorination pathway and occurrence of possible "dead-end" metabolites can be rationalized, but this does not necessarily mean that this is the only pathway that can be observed.

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REFERENCES

(1) Kühn, W.; Brauch, J.-H. *Fresenius Z. Anal. Chem.* **1988,** *330*, 324-325.

(2) Kypke-Hutter, K.; Vogelgesang, J.; Malisch, R.; Binnemann, P; Wetzlar, H. *Z. Lebensm. Unters. Forsch.* **1986,** *182*, 464-470.

(3) Mackay, D. In *Aquatic pollutants: transformation and biological effects;* Proceedings of the Second International EEC Symposium, Noordwijkerhout, The Netherlands, 1977, Hutzinger, O., van Lelyveld, L.H., Zoeteman, B.C.J., Eds.; Pergamon Press Oxford, 1977, pp 175-185.

(4) Schwarzenbach, R.P.; Molnar-Rubica, E.; Giger, W.; Wakeham, S.G. *Environ. Sci. Technol.* **1979,** *13*, 1367-1373.

(5) Choudry, G.G.; Hutzinger, O. *Environ. Sci. Technol.* **1984,** *18*, 235-241.

(6) Hirsch, M.; Hutzinger, O. *Environ. Sci. Technol.* **1989,** *23*: 1306-1307.

(7) Reineke, W.; Knackmuss H.-J. 1984. *Appl. Environ. Microbiol.* **1984,** *47*, 395-402.

(8) Van der Meer, J.R.; Roelofsen, W.; Schraa, G; Zehnder, A.J.B. *FEMS Microbiol. Ecol.* **1987,** *45*, 333-341.

(9) Schraa, G.; Boone, M.L.; Jetten, M.S.M.; Van Neerven, A.R.W.; Colberg, P.J.; Zehnder, A.J.B. *Appl. Environ. Microbiol.* **1986,** *52*, 1374-1381.

(10) Spain, J.C.; Nishino, S.F. *Appl. Environ. Microbiol.* **1987,** *53*, 1010-1019.

(11) De Bont, J.A.M.; Vorage, M.J.W.; Hartmans, S.; Van den Tweel, W.J.J. *Appl. Environ. Microbiol.* **1986,** *52*, 677-680.

(12) Haigler, B.E.; Nishino, S.F.; Spain, J.S. *Appl. Environ. Microbiol.* **1988,** *54*, 294-301.

(13) Marinucci, A.C.; Bartha, R. *Appl. Environ. Microbiol.* **1979,** *38*, 811-817.

(14) Sander, P.; Wittich, R.-M.; Fortnagel, P.; Wilkes, H.; Francke, W. *Appl. Environ. Microbiol.* **1991,** *57*,

1430-1440.

(15) Bartholomew, G.W.; Pfaender, F.K. *Appl. Environ. Microbiol.* **1983,** *45*, 103-109.

(16) Tiedje, J.M.; Boyd, S.A.; Fathepure, B.Z. *Dev. Ind. Microbiol.* **1987,** *27*, 117-127.

(17) Fathepure, B.Z.; Tiedje, J.M.; Boyd, S.A. *Appl. Environ. Microbiol.* **1988,** *54*, 327-330.

(18) Bosma, T.N.P.; Van der Meer, J.R.; Schraa, G.; Tros, M.E.; Zehnder, A.J.B. *FEMS Microbiol. Ecol.* **1988,** *53*, 223-229.

(19) Holliger, C.; Schraa, G.; Stams, A.J.M.; Zehnder, A.J.B. *Appl. Environ. Microbiol.* **1992,** *58*, 1636-1644.

(20) Fathepure, B.Z.; Vogel, T.M. *Appl. Environ. Microbiol.* **1991,** *57*, 3418-3422.

(21) Oliver, B.G.; Nicol, K.D. *Environ. Sci. Technol.* **1982,** *16*, 532-536.

(22) Bailey, R.E. *Environ. Sci. Technol.* **1983,** *17*, 504.

(23) Oliver, B.G.; Nicol, K.D. *Environ. Sci. Technol.* **1983,** *17*, 505.

(24) Beurskens, J.E.M.; Dekker, C.G.C.; Jonkhoff, J.; Pompstra, L. *Biogeochemistry* **1993,** *19*, 61-81.

(25) Houwen, F.P.; Dijkema, C.; Schoenmakers, C.H.H.; Stams, A.J.M.; Zehnder, A.J.B. *FEMS Microbiol. Lett.* **1987,** *41*, 269-274.

(26) Nies, L.; Vogel, T.M. *Appl. Environ. Microbiol.* **1990,** *56*, 2612-2617.

(27) Dolfing, J.; Harrison, B.K. *Environ. Sci. Technol.* **1992,** *26*, 2213-2218.

(28) Dolfing, J.; Tiedje, J.M. *Arch. Microbiol.* **1987,** *149*, 102-105.

(29) Dolfing, J. *Arch. Microbiol.* **1990,** *153*, 264-266.

(30) Ramanand, K.; Balba, M.T.; Duffy, J. *Appl. Environ. Microbiol.* **1993,** *59*, 3266-3274.

CHAPTER 5

MICROBIAL DECHLORINATION OF POLYCHLORINATED BIPHENYLS BY A HEXACHLOROBENZENE ACCLIMATED ENRICHMENT CULTURE

J.E.M. Beurskens, J. de Wolf, H. van den Heuvel, and J. Dolfing

ABSTRACT

A dechlorinating microbial enrichment culture was obtained from a Rhine River sedimentation area (Lake Ketelmeer, The Netherlands) with proven *in situ* dehalogenation of hexachlorobenzene (HCB) and disappearance of some polychlorinated biphenyls (PCBs), by enrichment with HCB and lactate. Incubations with various PCB congeners, especially some mono-ortho substituted congeners that exert dioxin-like toxic effects, were conducted to determine whether this culture would also exhibit dechlorinating activity towards PCBs. These experiments showed the ability of the enrichment to dechlorinate PCBs selectively. Only chlorines that were flanked by chlorine atoms on both sides were removed, ortho chlorines persisted. The observed rates of PCB dechlorination by the enrichment culture were 1 to 2 orders of magnitude lower than the rate at which HCB was dechlorinated by the same culture. Taking into account that ortho dechlorination did not occur in our enrichment, we were, with the aid of thermodynamics, able to rationalize the observed PCB dechlorination pathways.

INTRODUCTION

Sediments act as an important sink for PCBs in the aquatic environment. In general, PCB pollution levels in sediments of the major western European rivers (1-5) are 1 to 2 orders of magnitude below the levels in some severely polluted rivers in the USA, for example, the Hudson River, on which major studies on the environmental fate of PCBs are based (6-8). Nevertheless, PCB levels, which exceed sediment quality standards in, for example, Rhine River sediments, are a matter of public concern (9, 10).

PCB congeners differ in their physico-chemical and biological characteristics, leading to congener-specific toxicity. The planar or non-ortho PCBs, which are substituted in both para positions and at least two meta positions, exert dioxin-like toxic effects (11, 12). Introduction of one chlorine atom in the ortho position to the central phenyl-phenyl bond diminishes, but does not necessarily eliminate, certain biochemical activities inherent in planar compounds (11, 12). From a toxicological point of view, the non-ortho and mono-ortho substituted congeners can be regarded as the most important representatives of this compound group.

In the last decade, an increasing number of laboratory studies demonstrated reductive dechlorination of PCBs in sediments under anaerobic conditions (7, 8, 13-19). In this reaction, mediated by mixed anaerobic microbial populations, chlorines are replaced by hydrogen atoms in the PCB molecule, yielding less chorinated congeners. Microbial dechlorination of PCBs primarily results in removal of chlorines from para and meta positions; ortho dechlorination has been reported only twice (20, 21). Incubations over a broad range of PCB concentrations have shown a positive correlation between PCB concentration and dechlorination rate (8, 17). From 20 to 200 ppm, a linear relationship was observed (8). Generally, laboratory studies with PCB mixtures are conducted at ppm levels and higher. At high concentrations (700 ppm), rapid dechlorination of Aroclor 1242 was observed by Quensen et al. (22), but dechlorination did not occur at 14 mg/kg. Thus, at low concentrations a threshold may exist. In a sediment study by Rhee et al. (16), dechlorination of 2,4,5-trichlorobiphenyl occurred until a concentration of approximately 10 µg/kg was reached, indicating a much lower threshold for a single congener.

The occurrence of *in situ* microbial dechlorination of PCBs in some highly polluted sediments in the USA is now commonly accepted (6, 23-26). Dechlorination of PCBs in the polluted environment has several significant consequences: (i) dechlorination yields less hydrophobic congeners and consequently reduces the bioaccumulation potential of PCBs in the environment. (ii) since *in situ* dechlorination involves mainly the removal of meta and para chlorines, significant reduction of the toxicity associated with PCBs is obtained. (iii)

furthermore, the microbial dechlorination of PCBs might be the first step in achieving a biological decontamination of polluted sediments. The less chlorinated congeners produced are, in contrast to their parent compounds, suitable substrates for aerobic microbial mineralization. Such a sequential anaerobic-aerobic biological decontamination of sediments has recently been demonstrated for PCBs (26).

In situ microbial dechlorination of PCBs in polluted western European river sediments has not, to our knowledge, been reported yet. We recently reported significant disappearance of PCBs in Lake Ketelmeer, a sedimentation area of the Rhine River in the Netherlands. This suggests dechlorination in the environment at even substantially lower concentrations than in the USA (1, Chapter 2). This observation was based on a comparison of PCB concentrations in sediment core layers from around 1970, collected either recently or 20 years ago. Both sample series were dated and analyzed with now available techniques. Of the three planar and three mono-ortho PCBs that were quantified, four (viz. PCB 105, 126, 156 and, 169) showed significant disappearances, ranging from 70 to 88%. Concentrations of PCB 77 and PCB 118 were not significantly reduced during the 20 years of "environmental incubation", indicating a selective removal mechanism (1, Chapter 2).

In Lake Ketelmeer sediment, disappearance of hexachlorobenzene was observed as well. Sediment incubations in the laboratory under conditions as close to the field situation as possible demonstrated that the native microbial population was capable of dechlorinating hexachlorobenzene (27, Chapter 3). From this sediment an enrichment culture capable of dechlorinating some but not all chlorinated benzenes was obtained (28, Chapter 4). This provided the opportunity to test whether this culture would also exhibit dechlorinating activity towards PCBs and, if so, to verify whether the PCB dechlorination would match the observed selectivity in disappearances in sediment cores. Single congener incubations conducted enabled us to determine PCB dechlorination pathways and rates.

EXPERIMENTAL SECTION

Culture origin and incubation method

The culture was obtained from the Lake Ketelmeer sediment by enrichment with hexachlorobenzene (HCB) and lactate as described previously (28, Chapter 4). Batch incubations were prepared by dispensing 800 mL reduced synthetic medium (28, Chapter 4) to 1-L serum bottles. The phosphate-buffered mineral medium (pH 7.0) was reduced by sodium sulfide (0.24 g/l), and contained resazurin (0.0002%) as redox indicator. The headspaces were flushed with O_2-free N_2 and sealed with viton stoppers (Eriks b.v.,

Alkmaar, The Netherlands) before autoclaving. All bottles were inoculated with a 50-mL aliquot of the sediment-free enrichment culture. The general incubation protocol consisted of three phases: (A) Addition of sodium lactate (2 mL, initial concentration in medium: 17.5 mM) and HCB dissolved in methanol (200 µL, initial concentration in medium: approximately 70 nM). In this phase, encompassing a period of about three weeks, growth of organisms took place and HCB dechlorination was generally slow. (B) When the concentration of HCB fell below 4 nM, a second amount of HCB was added. This amount was readily dechlorinated as a result of increased cell densities. This phase was used to characterize differences between bottles in order to compensate differences in cell densities or dechlorinating activities. (C) The actual experimental phase started when the concentration of HCB again fell below 4 nM and a single chlorobiphenyl congener was added as a methanolic solution. All PCB congeners were purchased from Promochem (Wesel, Germany). Sterile controls were prepared by adding formalin (4%) to the bottles at the end of phase B, prior to the chlorobiphenyl addition. All cultures were incubated statically at 25°C in the dark.

Analytical procedures

At intervals, 10-mL samples were removed aseptically from each active and control incubation while swirling the medium to ensure a uniform suspension. The samples were combined with 2 mL iso-octane (nanograde, Promochem, Wesel, Germany) and shaken for 2 h. Separation of water and the iso-octane fraction was obtained by mixing on a vortex mixer and subsequently storing for at least 2 h at -20°C. PCB analysis was performed on a Hewlett-Packard (Model 5890) gas chromatograph equipped with a CP-Sil 8CB-for-PCB fused silica column (50 m x 0.25 mm i.d., film thickness 0.25 µm; Chrompack, Bergen op Zoom), a ^{63}Ni electron capture detector, and a HP7673A automatic sampler. Helium was the carrier gas and nitrogen the make-up gas. The injector and detector temperatures were 225°C and 300°C, respectively. The column was held at 90°C for 2 min, ramped at 10°C per min to 140°C, 5°C per min to 225°C, and finally held for 76 min. Compounds were identified and quantified by comparing with external standards obtained from Amchro Chromatographie GmbH (Sulzbach, Germany) using the HP3365 ChemStation software.

Calculations

The rate constants for dechlorination were calculated by linear regression analysis of the logarithmically transformed concentration curves assuming first-order kinetics.

Gibbs free-energies for all possible dechlorination reactions with the PCBs studied under standard conditions; i.e. PCBs present at equimolar concentrations, H_2 at 1 atm, 25°C,

pH=7, and [Cl^-]=1 M, were calculated with H_2 as electron donor using the equation:

$$\Delta G^{\circ\prime} = (\Delta G_f^\circ \text{BiphenylCl}_{n-1}\text{H} + \Delta G_f^\circ H^+ + \Delta G_f^\circ Cl^-) - (\Delta G_f^\circ \text{BiphenylCl}_n + \Delta G_f^\circ H_2)$$

Estimates for the Gibbs free-energies of formation (ΔG_f°) of chlorinated biphenyls were taken from Holmes et al. (29), with $H_{2\ gas}$ (0 kJ/mol) and Cl^- (-131.3 kJ/mol) taken from Stumm and Morgan (30). The estimate for the Gibbs free-energy of formation of H^+ at pH=7 (-39.87 kJ/mol) was taken from Thauer et al. (31).

RESULTS

Dechlorination pathways

Since the enrichment culture had been obtained with hexachlorobenzene, our first PCB incubations were performed with 2,3,4,5,6-pentachlorobiphenyl (PCB 116), a congener with a structure highly similar to hexachlorobenzene. The disappearance of PCB 116 in the biologically active incubations resulted in a transient accumulation of 2,3,4,6-tetrachlorobiphenyl (PCB 62) and a final accumulation of 2,3,5,6-tetrachlorobiphenyl (PCB 65), and 2,4,6-trichlorobiphenyl (PCB 30) (Figure 5.1A). These metabolites were produced via para- and meta-dechlorination. After 66 days of incubation only 20% of the total molar decrease of PCB 116 was recovered as metabolites. A substantial disappearance of PCB 116 in the sterile controls without the apparent formation of less chlorinated PCB congeners indicates that loss mechanisms other than dechlorination also occurred. A similar deficit in the mass balance for incubations with the same compound and with 2,4,5-trichlorobiphenyl has been observed by Rhee and coworkers (16, 19). Separate incubations with 2,3,5,6-tetrachlorobiphenyl (PCB 65) confirmed the inability of our enrichment to dechlorinate this compound during an extended experimental period of 162 days (data not shown).

Three mono-ortho substituted congeners were selected for laboratory incubations. The 2,3,4,3',4'-pentachlorobiphenyl (PCB 105) was nearly stoichiometrically transformed to 2,4,3',4'-tetrachlorobiphenyl (PCB 66) by meta dechlorination (Figure 5.1B). No substantial losses in the sterile controls were observed for the parent compound. Singular para- and meta-chlorine removal from 2,3,4,5,3',4'-hexachlorobiphenyl (PCB 156) was also observed (Figure 5.2A), producing the respective compounds 2,3,5,3',4'-pentachlororbiphenyl (PCB 107) and 2,4,5,3',4'-pentachlorobiphenyl (PCB 118). The PCB 156 concentration in sterile controls remained unaltered. During the 66 days of incubation, PCB 118 showed some

decrease in concentration, but no dechlorination products were observed in the biologically active incubations. A comparable decrease in concentration was observed in the sterile controls, indicating another loss mechanism than microbial dechlorination (Figure 5.2B).

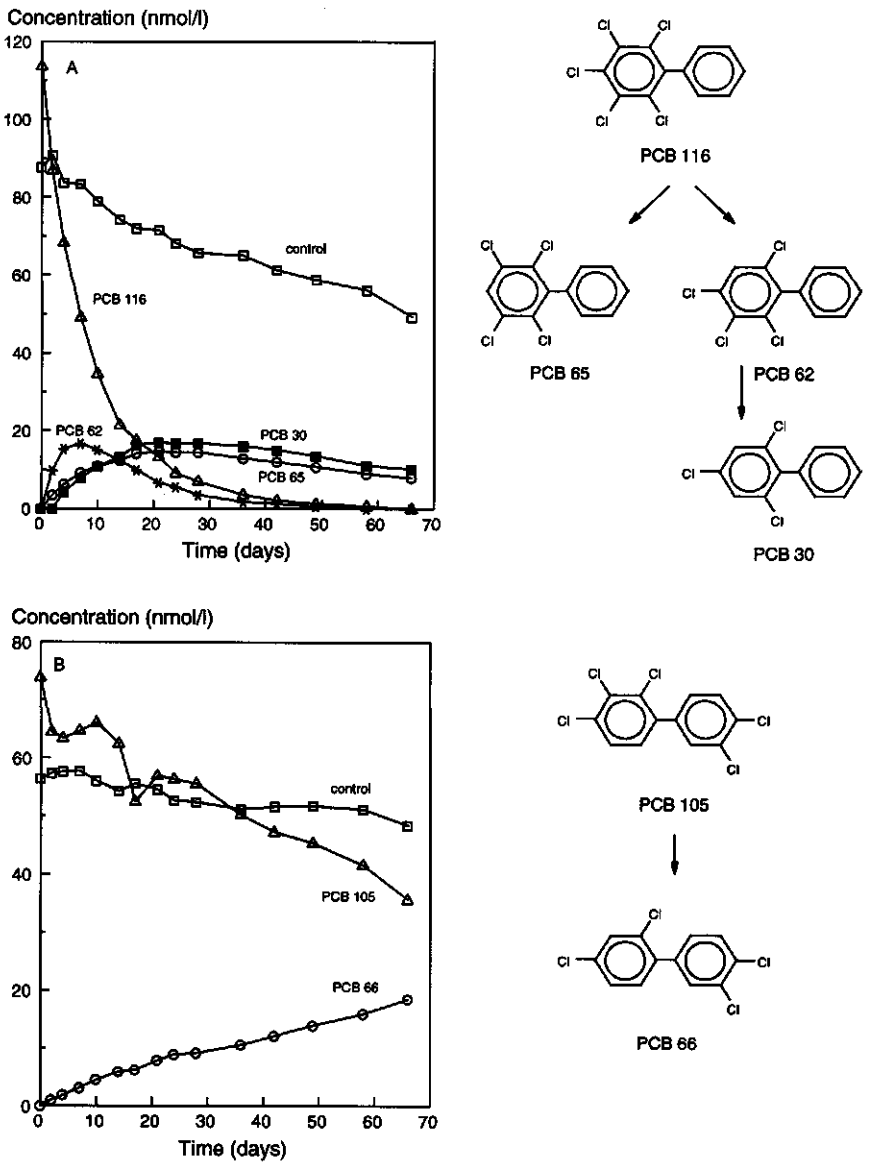

Fig. 5.1. Reactivity of 2,3,4,5,6-pentachlorobiphenyl -PCB 116- (A) and 2,3,4,3',4'-pentachlorobiphenyl -PCB 105- (B) in biologically active and sterile control incubations with the enrichment culture.

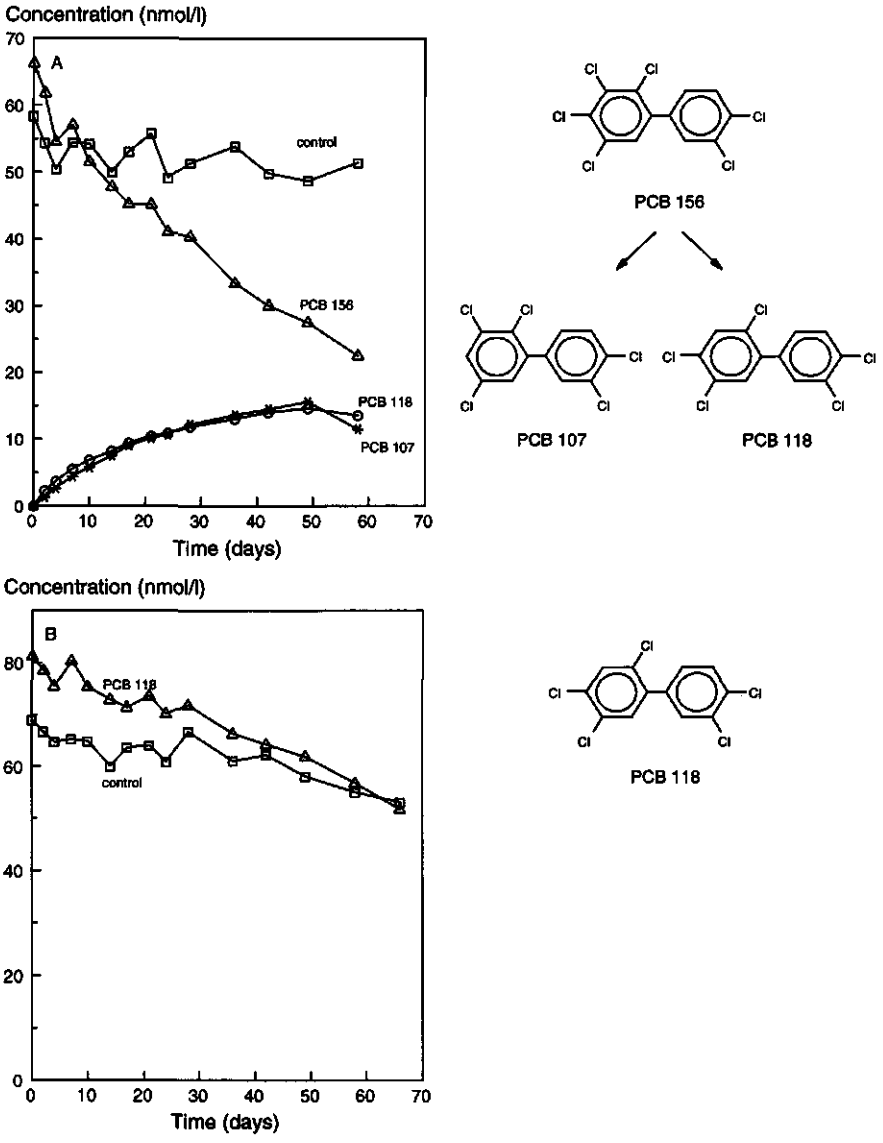

Fig. 5.2. Reactivity of 2,3,4,5,3',4'-hexachlorobiphenyl -PCB 156- (A) and 2,4,5,3',4'-pentachlorobiphenyl -PCB 118- (B) in biologically active and sterile control incubations with the enrichment culture.

Dechlorination kinetics

The experimental protocol applied was identical to the one we used in a previous study with chlorinated benzenes and the same enrichment culture (28, Chapter 4). Originally the enrichment was obtained with hexachlorobenzene; for this reason this compound and some

other chlorinated benzenes were included in the evaluation of PCB dechlorination kinetics. Since incubations were conducted in a series of separate bottles, cell densities and dechlorinating activities may have varied somewhat between bottles. Such differences between the bottles were taken into account via normalization factors. We added HCB to all bottles (phase B) prior to addition of test compounds in phase C. Based on the dechlorination rate constants for HCB in phase B, normalization factors were calculated to obtain identical HCB dechlorination rate constants, equal to the mean of all incubations in this phase (Table 5.1). These factors were used to correct the dechlorination rate constants of the individual test compounds in phase C (Table 5.1). Calculated half-lives for PCB 116, 105, and 156 were 11, 120, and 53 days, respectively. Similar half-lives, of several weeks to several months, are commonly reported in the literature based on sediment incubations in the laboratory (8, 14, 17). The half-life for HCB dechlorination, the original substrate used for the enrichment of the culture, was about one day. Thus, the reactivity of PCBs is 1 to 2 orders of magnitude below the reactivity of chlorinated benzenes, as exhibited by our enrichment culture.

Table 5.1. Relative dechlorination-rate constants (k') and half-lives ($t_{1/2}$, in days) of the individually incubated chlorinated biphenyls and benzenes (Phase C), instantaneously dechlorinated after hexachlorobenzene (HCB) dechlorination in phase B.

Phase B				Norm. factor	Phase C				Relative rates phase C	
Compnd	k (d^{-1})	n	r^2		Compnd	k (d^{-1})	n	r^2	k' (d^{-1})	$t_{1/2}$ (d)
HCB	2.04	6	0.999	0.755	PCB 116	0.0878	14	0.993	0.066	10.5
HCB	2.30	6	0.996	0.670	PCB 105	0.0086	14	0.918	0.006	120
HCB	2.06	6	0.999	0.748	PCB 156	0.0174	14	0.988	0.013	53.3
HCB[1]	1.27	5	0.967	1.213	HCB	0.408	8	0.998	0.495	1.4
HCB[1]	1.22	5	0.946	1.262	QCB	0.528	7	0.997	0.666	1.0
HCB[1]	0.84	5	0.987	1.833	1234-TeCB	0.336	8	0.978	0.616	1.1
HCB[1]	1.06	5	0.944	1.453	1235-TeCB	0.264	9	0.948	0.384	1.8
mean	1.54									

Norm. factor = normalization factor, see text at top of this page
n = number of data points
[1]Incubations with chlorinated benzenes as test compounds in phase C; data from ref 28 (Chapter 4)

DISCUSSION

Cross-adaptation to PCBs

The term cross-adaptation refers to the ability of a microbial community to degrade compounds from compound classes to which the community had not been exposed previously. The removal of aryl chlorines from both, PCBs and chlorinated benzenes by our hexachlorobenzene-adapted enrichment culture is one of the few examples that reductive dehalogenation activity is not necessarily compound-group specific. The dechlorination of chlorophenols by chlorobenzoate-adapted *D. tiedjei* (32) is the only example of cross-adaptation to chlorinated aromatics of a pure culture. For mixed microbial communities, ther is also only one example of cross-adaptation reported, viz. the dechlorination of dichloroanilines in sediment slurries acclimated to dichlorophenols (33). It is not clear why cross-adaptation related to dehalogenation of aromatics is seldomly reported. The paucity of information on cross-adaptation may be the result of the limited number of experiments conducted to search for cross-adaptation. In that case, cross-adaptation might be more widespread than current literature suggests. On the other hand, cross-adaptation may have been tested fairly frequently, but with negative results, and may therefore not have been included in literature reports. This would imply that cross-adaptation is a rare phenomenon. Cultures able to dehalogenate several compound classes are of particular importance for the development of broad-spectrum biotechnological remediation technologies.

Selectivity in dechlorination steps

The PCB dechlorination reactions catalyzed by our enrichment share one common feature: only para or meta chlorines are removed if surrounded by chlorines on both sides. This selectivity is similar to the dechlorination pattern found with hexachlorobenzene in the same culture, where we found the formation of 1,3,5-trichlorobenzene as the main dechlorination product (28, Chapter 4). This suggests that the instantaneous activity of the HCB-enrichment culture to PCBs, as tested in this study, is simply the result of an enzyme system triggered to catalyze reactions with substrates that meet a specific spatial configuration, namely three adjacent chlorine atoms. However, sediment slurries directly prepared from three different PCB-contaminated locations in the USA demonstrated that the doubly-flanked meta and para chlorines are the most reactive at these three locations as well (21). Thus, the pre-adaptation of our culture toward HCB has not resulted in an exceptional dechlorination pattern; the preferential removal of doubly-flanked meta and para chlorines appears to be generally seen in PCB dechlorination.

Previously we found that the selectivity in dechlorination steps of chlorinated benzenes

correlated with the yield in free energy (28, Chapter 4). From the 19 dechlorination reactions possible with benzenes that contain at least two chlorines, only the seven reactions with the highest energy release took place. A thermodynamic analysis of HCB dechlorination pathways by Dolfing and Harrison (34) supports the hypothesis that the first steps in the main dechlorination pathway of HCB are those with the highest energy release under standard conditions.

We therefore also looked for a possible relationship between the catalyzed PCB dechlorination steps and thermodynamics. Table 5.2 lists the possible reactions according to the corresponding release in free energy under standard conditions; to complete the picture, some structural features of the dechlorination steps are also included. The dechlorination reactions mediated by our enrichment culture are among the reactions with the highest energy release under standard conditions (Table 5.2). Reactions that involve an ortho chlorine removal were, despite a higher energetic profit than the catalyzed reactions in certain cases (e.g. in PCB 105 dechlorination), not observed. Reactions that did occur (removal of para or meta chlorines surrounded by chlorines on both sides) are in these cases the second most energetically favorable. Thus, taking into account that ortho dechlorination is not catalyzed, the observed PCB dechlorination steps correlate with the amount of energy released. This finding made it tempting to evaluate the available PCB dechlorination pathways in the literature in the same manner; it was found that PCB dechlorination patterns could also be rationalized with the aid of thermodynamics (35, Chapter 9). Nevertheless, one should keep in mind that the difference in Gibbs free energy between parent compound and products is one of the many factors that determine whether or not a specific reaction will occur (36). The stability of ortho chlorines, frequently observed, indicates that factors other than thermodynamics are indeed also involved in determining which dechlorination pathway is followed in the environment. Steric hindrance could be one of these factors.

The enrichment culture used in this study was obtained from a sedimentation area of the Rhine River where significant disappearance of hexachlorobenzene, PCB 105, 126, 156, and PCB 169 was observed in the deeper and anaerobic sediment layers (1, Chapter 2, 27, Chapter 3). On the other hand, PCB 118 and PCB 77 concentrations in this sediment were not significantly altered as compared to the documented historical input (1, Chapter 2). The laboratory incubations with PCB 105, 116, and 156 showed the ability of the enrichment culture to dechlorinate PCBs, while PCB 118 resisted dechlorination in the laboratory incubations. The laboratory results agree with the field observations and indicate that the selective disappearance of PCBs in Rhine sediments is most likely caused by *in situ* microbial dechlorination.

Table 5.2. Gibbs free-energy of formation ($\Delta G_f°$) and gibbs free-energy values for the reductive dechlorination ($\Delta G°'$) of PCBs with hydrogen as electron donor with the spatial position of the substituted chlorine and number of adjacent chlorines listed along with the presence (+) or absence (-) of the reaction in laboratory incubations with the enrichment culture.

Substrate			Product			$\Delta G°'$	Pos.[1]	Adj.Cl	Dechlor.
no.	structure	$\Delta G_f°$ kJ/mol	no.	structure	$\Delta G_f°$ kJ/mol	kJ/mol			
62	2,3,4,6	203.4	30	2,4,6	215.9	-158.7	m	2	+
			29	2,4,5	218.0	-156.5	o	1	-
			24	2,3,6	220.5	-154.1	p	1	-
			21	2,3,4	226.3	-148.3	o	0	-
65	2,3,5,6	204.7	23	2,3,5	218.1	-157.8	o	1	-
			24	2,3,6	220.5	-155.4	m	1	-
105	2,3,4,3',4'	181.6	77	3,4,3',4'	188.7	-164.1	o	1	-
			66	2,4,3',4'	190.5	-162.3	m	2	+
			56	2,3,3',4'	198.8	-154.0	p	1	-
			55	2,3,4,3'	199.9	-152.9	p	1	-
			60	2,3,4,4'	200.7	-152.1	m	1	-
116	2,3,4,5,6	197.1	62	2,3,4,6	203.4	-164.9	m	2	+
			65	2,3,5,6	204.7	-163.6	p	2	+
			61	2,3,4,5	210.2	-158.1	o	1	-
118	2,4,5,3',4'	173.6	66	2,4,3',4'	190.5	-156.1	m	1	-
			77	3,4,3',4'	188.7	-156.1	o	0	-
			70	2,5,3',4'	190.8	-154.0	p	1	-
			67	2,4,5,3'	191.3	-153.5	p	1	-
			74	2,4,5,4'	192.8	-152.0	m	1	-
156	2,3,4,5,3',4'	157.0	126	3,4,5,3',4'	171.6	-156.6	o	1	-
			107	2,3,5,3',4'	173.6	-154.6	p	2	+
			118	2,4,5,3',4'	173.6	-154.6	m	2	+
			105	2,3,4,3',4'	181.6	-146.6	m	1	-
			106	2,3,4,5,3'	186.4	-141.8	p	1	-
			114	2,3,4,5,4'	187.8	-140.4	m	1	-

[1]Positions of the removed chlorine are indicated as follows: o = ortho; m = meta; p = para.

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REFERENCES

(1) Beurskens, J.E.M.; Mol, G.A.J.; Barreveld, H.L.; Van Munster, B.; Winkels, H.J. *Environ. Toxicol. Chem.* **1993,** *12*, 1549-1566.

(2) Winkels, H.J.; Vink, J.P.M.; Beurskens, J.E.M.; Kroonenberg, S.B. *Appl. Geochem. Suppl. Issue* **1993,** *2*, 95-101.

(3) Chevreuil, M.; Chesterikoff, A.; Létolle, R. *Wat. Res.* **1987,** *4*, 427-434.

(4) Eder, G.; Sturm, R.; Ernst, W. *Chemophere* **1987,** *16*, 2487-2496.

(5) Galassi, S.; Migliavacca, M. *Ecotoxicol. Environ. Safety* **1986,** *12*, 120-126.

(6) Brown, J.F.; Wagner, R.E.; Feng, H.; Bedard, D.L.; Brennan, M.J.; Carnahan, J.C.; May, R.J. *Environ. Toxicol. Chem.* **1987,** *6*, 579-593.

(7) Quensen, J.F., III; Boyd, S.A.; Tiedje, J.M. *Appl. Environ. Microbiol.* **1990,** *56*, 2360-2369.

(8) Abramowicz, D.A.; Brennan, M.J.; Van Dort, H.M.; Gallagher, E.L. *Environ. Sci. Technol.* **1993,** *27*, 1125-1131.

(9) De Boer, J.; Stronck, C.J.N.; Traag, W.A.; Van der Meer, J. *Chemosphere* **1993,** *26*, 1823-1842.

(10) Hendriks, A.J.; Pieters, H. *Chemosphere* **1993,** *26*, 817-836.

(11) De Voogt, P.; Wells, D.E.; Reutergardh, L.; Brinkman, U.A.T. *Intern. J. Environ. Anal. Chem.,* **1990,** *40*, 1-46.

(12) Safe, S. *CRC Crit. Rev. Toxicol.* **1990,** *21*, 51-88.

(13) Morris, P.J.; Mohn, W.W.; Quensen, J.F. III; Tiedje, J.M.; Boyd, S.A. *Appl. Environ. Microbiol.* **1992,** *58*, 3088-3094.

(14) Alder, A.C.; Häggblom, M.M.; Oppenheimer, S.R.; Young, L.Y. *Environ. Sci. Technol.* **1993,** *27*, 530-538.

(15) Rhee, G.-Y.; Sokol, R.C.; Bush, B.; Bethoney, C.M. *Environ. Sci. Technol.* **1993,** *27*, 714-719.

(16) Rhee, G.-Y.; Sokol, R.C.; Bethoney, C.M.; Bush, B. *Environ. Sci. Technol.* **1993,** *27*, 1190-1192.

(17) Rhee, G.-Y.; Bush, B.; Bethoney, C.M.; Denucci, A.; Oh, H.-M.; Sokol, R.C. *Environ. Toxicol. Chem.* **1993,** *12*, 1025-1032.

(18) Rhee, G.-Y.; Bush, B.; Bethoney, C.M.; Denucci, A.; Oh, H.-M.; Sokol, R.C. *Environ. Toxicol. Chem.* **1993,** *12*, 1033-1039.

(19) Rhee, G.-Y.; Sokol, R.C.; Bethoney, C.M.; Bush, B. *Environ. Toxicol. Chem.* **1993,** *12*, 1829-1834.

(20) Van Dort, H.M.; Bedard, D.L. *Appl. Environ. Microbiol.* **1991,** *57*, 1576-1578.

(21) Williams, W.A. *Environ. Sci. Technol.* **1994,** *28*, 630-635.

(22) Quensen, J.F.III; Tiedje, J.M.; Boyd, S.A. *Science* **1988,** *242*, 752-754.

(23) Brown, J.F.; Wagner, R.E. *Environ. Toxicol. Chem.* **1990,** *9*, 1215-1233.

(24) Abramowicz, D.A. *Crit. Rev. Biotechnol.* **1990,** *10*, 241-251.

(25) Mohn, W.W.; Tiedje, J.M. *Microbiol. Rev.* **1992,** *56*, 482-507.

(26) Harkness, M.R.; McDermott, J.B.; Abramowicz, D.A.; Salvo, J.J.; Flanagan, W.P.; Stephens, M.L.; Mondello, F.J.; May, R.J.; Lobos, J.H.; Carroll, K.M.; Brennan, M.J.; Bracco, A.A.; Fish, K.M.; Warner, G.L.; Wilson, P.R.; Dietrich, D.K.; Lin, D.T.; Morgan, C.B.; Gately, W.L. *Science* **1993,** *259*, 503-507.

(27) Beurskens, J.E.M.; Dekker, C.G.C.; Jonkhoff, J.; Pompstra, L. *Biogeochem.* **1993,** *19*, 61-81.

(28) Beurskens, J.E.M.; Dekker, C.G.C.; Van Den Heuvel, H.; Swart, M.; De Wolf, J.; Dolfing, J. *Environ. Sci. Technol.* **1994,** *28*, 701-706.

(29) Holmes, D.A.; Harrison, B.K.; Dolfing, J. *Environ. Sci. Technol.* **1993,** *27,* 725-731.
(30) Stumm, W.; Morgan, J.J. *Aquatic Chemistry,* 2nd ed.; Wiley: New York, 1981.
(31) Thauer, R.K.; Jungermann, K.; Decker, K. *Bacteriol. Rev.* **1977,** *41,* 100-180.
(32) Mohn, W.W.; Kennedy, K.J. *Appl. Environ. Microbiol.* **1992,** *58,* 1367-1370.
(33) Struijs, J.; Rogers, J.E. *Appl. Environ. Microbiol.* **1989,** *55,* 2527-2531.
(34) Dolfing, J.; Harrison, B.K. *FEMS Microbiol. Ecol.* **1993,** *13,* 23-30.
(35) Dolfing, J.; Beurskens, J.E.M. *Advances in Microbial Ecol.* **1995,** *15,* in press.
(36) Dolfing, J.; Harrison, B.K. *Environ. Sci. Technol.* **1992,** *26,* 2213-2218.

CHAPTER 6

DEHALOGENATION OF CHLORINATED DIOXINS BY AN ANAEROBIC MICROBIAL CONSORTIUM FROM SEDIMENT

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ABSTRACT

Anaerobic microorganisms enriched from Rhine River sediments are able to remove chlorine substituents from polychlorinated dibenzo-*p*-dioxines (PCDDs). A model PCDD, 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (1,2,3,4-TeCDD) was reductively dechlorinated to both 1,2,3- and 1,2,4-trichlorodibenzo-*p*-dioxins (1,2,3- and 1,2,4-TrCDD). These compounds were further dechlorinated to 1,3- and 2,3-dichlorodibenzo-*p*-dioxins and traces of 2-monochlorodibenzo-*p*-dioxin. This is the first report in the literature of the anaerobic microbial dechlorination of PCDDs. The same enrichment culture was previously found to dechlorinate chlorinated benzenes (CBs) and polychlorinated biphenyls (PCBs). An anaerobic culture able to remove aryl chlorines from three classes of compounds has not been reported before. The rate at which the culture dechlorinates 1,2,3,4-TeCDD ($t_{1/2}$=15.5 days) was between those observed for CBs and PCBs. This study shows that reductive dechlorination may have an effect on PCDDs in sediments, as has been demonstrated for CBs and PCBs. The formation of metabolites with a conserved 2,3-substitution pattern from 1,2,3,4-TeCDD indicates that dechlorination of highly chlorinated dibenzo-*p*-dioxins may result in metabolites that are potentially more toxic than the parent compounds.

INTRODUCTION

Aquatic sediments act as important sinks for polychlorinated dibenzo-*p*-dioxins (PCDDs) that are discharged either directly, or indirectly via atmospheric deposition, into the aquatic environment [1-3]. Elevated levels of PCDDs are observed in sediments from industrial areas [1-5] compared to those in remote areas [6]. Accumulation of PCDDs in aquatic biota and subsequent transfer to organisms at higher trophic levels occur as a result of their hydrophobic and recalcitrant character [7-9]. PCDDs have significant biochemical and toxicological effects in fish and fish-eating birds [10-13].

Generally, PCDDs are considered to be rather persistent in the environment. However, photochemical decomposition has been demonstrated and may occur in the atmosphere [14], hydrosphere [15], and the upper layer of soils [16]. Biodegradation under aerobic conditions appears to be restricted to congeners with four or fewer chlorines [17-19]. The observed accumulation of PCDDs in sediments indicates that input rates exceed the rates of the above-mentioned elimination reactions. Once deposited in sediments, PCDDs will be buried and will eventually exist under anaerobic conditions. Long-term persistence of PCDDs under these conditions is generally assumed [20]. On the other hand, substantial changes in concentrations of polychlorinated biphenyls (PCBs) and chlorinated benzenes (CBs) in sediments are caused by anaerobic microbial processes [21-24]. Anaerobic microbial populations mediate reductive dechlorination reactions, i.e. the replacement of chlorines by hydrogen atoms, yielding less chlorinated compounds [25-28]. Whether PCDDs can be dechlorinated by a similar process has not been reported in the literature.

The disappearance of hexachlorobenzene (HCB) and several PCBs in sediments has been observed in Lake Ketelmeer (The Netherlands), a sedimentation area of the Rhine River [5, Chapter 2; 24, Chapter 3]. An anaerobic culture able to dechlorinate CBs and PCBs was enriched from this sediment [28, Chapter 4; Chapter 5]. A certain selectivity was observed in the dechlorination reactions carried out by this culture, as chlorines surrounded by two adjacent chlorines were removed preferentially. The objective of the present study was to determine the dechlorinating activity of this anaerobic enrichment culture toward PCDDs by incubation with 1,2,3,4-tetrachlorodibenzo-*p*-dioxin (1,2,3,4-TeCDD) as a model dioxin and to study the dechlorination pattern. Protocols similar to those used in the incubations of the same culture with CBs and PCBs [28, Chapter 4, Chapter 5] were used in order to compare the dechlorination kinetics of these three groups of compounds.

MATERIALS AND METHODS

Anaerobic incubations with Lake Ketelmeer sediment demonstrated the microbially mediated dechlorination of spiked HCB [24, Chapter 3]. One of the sediment slurries from this experiment was used to start an enrichment procedure that consisted of repetitive transfers of the liquid phase under anaerobic conditions in presence of HCB and lactate as described previously [28]. In this way a methanogenic, dechlorinating enrichment culture was obtained from which the sediment matrix was eliminated. Batch incubations were prepared by dispensing 1.7-L reduced synthetic medium [28, Chapter 4] to 2-L serum bottles. After inoculation with 100 ml of the enrichment culture, adding lactate (as an electron donor) (17.5 mM) and incubation with HCB (as an electron acceptor) (70 nM) as a solution in methanol, both population growth and a related increase in dechlorinating activity were obtained. Differences between bottles that were due to differences in cell density and activity were characterized as follows. Incubations consisted of three sequential additions: The incubation started with the addition of an inoculum, lactate and hexachlorobenzene (HCB). After depletion of HCB, a second amount of this compound was added. As a result of increased cell densities, this amount was readily dechlorinated. The third addition contained the compound of study, 1,2,3,4-TeCDD in this study, chlorinated benzenes or chlorinated biphenyls in previous studies. Dechlorination rates of these compounds were normalized to the dechlorination rate of the second addition of HCB to the same bottles in order to obtain relative dechlorination rates, independent of the different activities of the bottles. A solution of 1,2,3,4-TeCDD in acetone was added to give an initial concentration of approximately 500 nM. This concentration is much higher than the aqueous solubility of this compound (1.7 nM) [29], but was necessary to ensure the identification of dechlorination products by mass spectrometry. The sterile control was prepared by sequential pasteurization (20 min at 75°C), incubation (24 h at 23°C), and autoclaving (3 h at 121°C) [30].

Bottles were statically incubated at 23°C in the dark and were sampled at regular intervals. Samples (20 ml) were taken in triplicate from both the control and the active incubations. In each sample the bacterial activity was stopped by adding 3 ml KOH (10 N) and, after addition of 2,2',6,6'-tetrachlorobiphenyl (C.N. Schmidt, Amsterdam, The Netherlands) as an internal (recovery) standard, each sample was extracted with 30 ml of distilled pentane (Janssen Chimica, Tilburg, The Netherlands) and 1 ml of 2,2,4-trimethylpentane (TMP; Rathburn, Walkerburn, Scotland). After shaking manually for at least 2 min, the samples were centrifuged for 30 min at 2000 rpm. About 90% of the organic phase was removed and concentrated under a nitrogen flow to a volume of 1 ml. Cleanup was done with

sequential silica-NaOH (33% w/w of a 1 N solution) and silica-H_2SO_4 (44% w/w) columns in Pasteur pipettes. The samples were eluted with 20 ml pentane and 2 ml TMP. These samples were concentrated further under a nitrogen flow until the volume was reduced to 1 ml. Next, 2,2',4,4',6-Pentachlorobiphenyl (C.N. Schmidt b.v., Amsterdam, The Netherlands) was added as a second internal standard, prior to analysis by GC-ECD.

Chemical analyses were performed on an HP 5890 gas chromatograph, using a cold on-column injector, a DB-5 capillary column (J&W, 30 m x 0.32 mm), an autosampler (Carlo Erba A 200S) and a ^{63}Ni electron capture detector. Helium was used as the carrier gas and argon/methane as the make-up gas. The following temperature program was used: initial temperature was 90°C, rate: 70°C/min to 150°C, isothermal for 8 min, 3°C/min to 186°C, 20°C/min to 325°C, and isothermal for 12 min. Compounds were identified and quantified by comparing with external standards; 1,2,3-TrCDD, 1,2,4-TrCDD, and 2,3-DCDD were obtained from Amchro (Accu standard, 99% pure, Sulzbach/Taunus, Germany). 1,2,3,4-TeCDD and 2-MCDD were synthesized following the method of Pohland and Yang [31]. The 1,3-DCDD was synthesized following the method of Gray et al. [32]. All synthesized chemicals were purified by silica-gel columns eluted with distilled hexane (Janssen Chimica, Tilburg, The Netherlands) and checked for contamination by GC-MSD and GC-ECD. Metabolites were identified on a GC-MSD (HP 5890 gas chromatograph with a mass selective detector (HP 5970), DB-5 column (30 m x 0.32 mm), and cold on-column injection. The carrier gas was He, inlet pressure 3 psi He; temperature program: initial temperature 140°C, ramp 40°C/min, final temperature 300°C. Selective-ion monitoring of the $\underline{M}$, $\underline{M}$+2 and $\underline{M}$+4 masses was used. Metabolite identification was confirmed by comparison of mass spectra with those of authentic standards.

RESULTS AND DISCUSSION

Samples taken at regular time intervals from the active incubation showed the production of increasing concentrations of lower-chlorinated dioxins (Fig. 6.1A). In contrast, this was not seen in the sterile control. This indicates that the dechlorination in the active incubation is due to the presence of dechlorinating bacteria. The trichlorinated isomers 1,2,3-TrCDD and 1,2,4-TrCDD were both detected at low concentrations, but the 1,3- and 2,3-dichloro-dibenzo-*p*-dioxins (DCDDs) were found at higher concentrations. Identification of these products was confirmed at day 4 and day 10 by comparison of their retention times and mass spectra with those of authentic standards. These analyses also revealed the presence of a low concentration of 2-monochlorodibenzo-*p*-dioxin (2-MCDD) at day 10 (less

than 0.005 nM). Both 1-MCDD and unchlorinated dioxin were not detected (detection limit was 0.005 nM).

Concentrations of 1,2,3,4-TeCDD measured during the incubation varied considerably in both the biologically active culture and the sterile control (Fig. 6.1B). There was no clear evidence for a decrease in concentration of this compound in the active culture. This may be caused by the fact that the concentration of 1,2,3,4-TeCDD added was about 300 times its aqueous solubility [29], thus creating a continuous supply of dissolved 1,2,3,4-TeCDD, but making representative sampling difficult. The observed increase of 1,2,3,4-TeCDD concentration in the control incubation over time may be due to slow solubilization of 1,2,3,4-TeCDD in a super-saturated solution.

In contrast to the 1,2,3,4-TeCDD quantification, the tri- and dichlorinated congeners showed little variation in the triplicate samples (Fig. 6.1A). Concentrations of these compounds were equal to or below their aqueous solubilities [29]. In a preliminary experiment, lower concentrations of 1,2,3,4-TeCDD were dechlorinated rapidly (from 310 nM to less than 10 nM within 22 d). However, in this case the definite identification of the dechlorinated metabolites was not always possible. We therefore used a higher concentration of 1,2,3,4-TeCDD in the repeated experiment. The sum of the dechlorinated metabolites formed (Fig. 6.1B) indicates decreasing rates of transformation during the experimental period and that transformation stopped after about 12 d. The reason for this loss of activity is unknown.

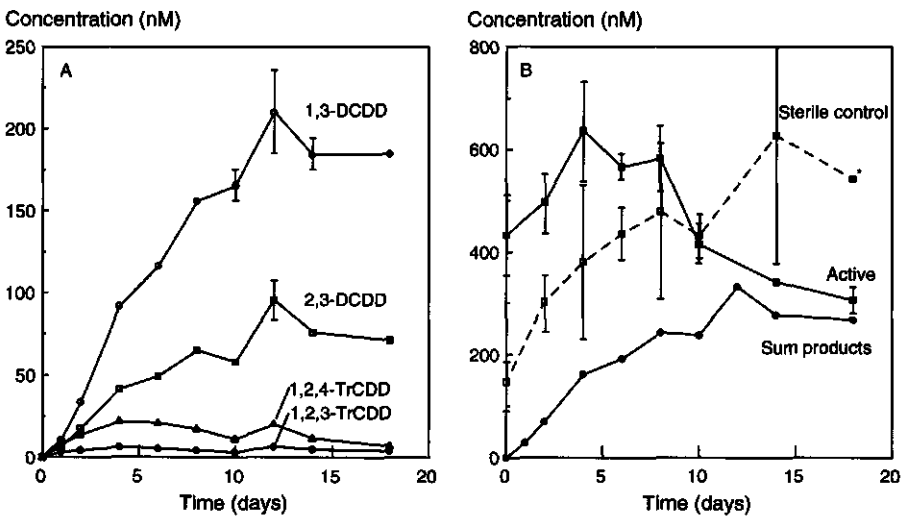

Fig. 6.1. Formation of dechlorination products in the biologically active incubations with the enrichment culture (A) and concentration course of 1,2,3,4-TeCDD in biologically active and sterile control incubations (B). The sum of the four products is included in B. Vertical bars represent ± one standard deviation of the mean based on triplicate determinations (*:single measurement); standard deviations smaller than 8 nM and 30 nM are not shown in A and B, respectively.

The possible sequential dechlorination steps are shown in Figure 6.2 and are based on the identified metabolites. Removal of the first chlorines from 1,2,3,4-TeCDD occurred from both lateral (i.e., positions 2 and 3) and non-lateral positions to give 1,2,4-TrCDD and 1,2,3-TrCDD, respectively. Subsequent chlorine removal resulted predominantly in formation of 1,3-DCDD, possibly from both TrCDDs. Removal of a non-lateral chlorine from 1,2,3-TrCDD yielded 2,3-DCDD as a minor product. The ratio of 1,3- to 2,3-DCDD concentrations was approximately 5:2. Small amounts of 2-MCDD were produced eventually. In theory this may be produced from both DCDDs, but it cannot be excluded that only one of these was the precursor.

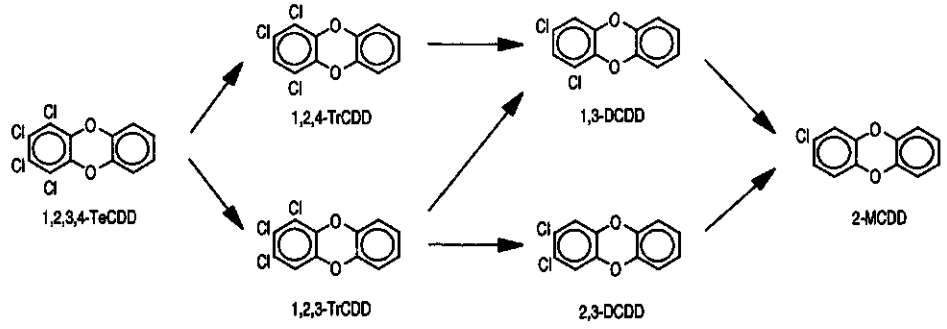

Fig. 6.2. Proposed pathways for the dechlorination of 1,2,3,4-TeCDD by the anaerobic enrichment culture.

Dehalogenation of some, but not all, chlorinated benzenes (CBs) and of some polychlorinated biphenyls (PCBs) by the same anaerobic enrichment culture was observed in previous studies [28, Chapter 4; Chapter 5]. The ability of a single consortium to remove aryl chlorines from three distinct classes of compounds has not been demonstrated before. Cross-adaptation of a dechlorinating activity between various classes of halogenated aromatic compounds is not a general phenomenon [33], but has been observed as in the cross-adaptation for chlorobenzoates and chlorophenols [34] and for dichlorophenols and dichloroanilines [35]. The application in the present study of procedures similar to previous studies [28, Chapter 4; Chapter 5] allows the comparison of relative dechlorination rate constants and half-lives, assuming first-order kinetics (Table 6.1). Half-lives of CBs in this enrichment culture, were 1 to 2 days, whereas the half-lives of PCBs varied between 10.5 and 120 d. The 1,2,3,4-TeCDD has an intermediate half-life of 15 days and is dechlorinated more rapidly than some PCBs under optimum conditions in the laboratory. Selection of the test compounds 2,3,4,5,6- pentachlorobiphenyl and 1,2,3,4,-TeCDD was based on their

structural similarity to HCB, the substrate to which our culture was pre-adapted. The higher dechlorination rates observed for 2,3,4,5,6- pentachlorobiphenyl and 1,2,3,4,-TeCDD relative to the other PCBs indicate that our enrichment does indeed most readily remove chlorines from aromatic rings with maximum chlorine substitution. PCBs, with incomplete chlorinated rings, are dechlorinated at lower rates (Table 6.1). Analogous to the PCB results, it is expected that dechlorination of other dioxins can be catalyzed by our culture, although at lower rates than found for 1,2,3,4-TeCDD.

Table 6.1. Relative half-lives for the dechlorination of chlorinated benzenes [28, Chapter 4], chlorinated biphenyls [Chapter 5], and 1,2,3,4-TeCDD by the enrichment culture in liquid media at 25°C.

Compound	Half-life (days)[a]
pentachlorobenzene	1.0
1,2,3,4-tetrachlorobenzene	1.1
hexachlorobenzene	1.4
1,2,3,5-tetrachlorobenzene	1.8
2,3,4,5,6-pentachlorobiphenyl	10.5
1,2,3,4-tetrachlorodibenzo-*p*-dioxin	15.5
2,3,4,5,3',4'-hexachlorobiphenyl	53
2,3,4,3',4'-pentachlorobiphenyl	120

[a]Half-lives were calculated by linear-regression analyses assuming first-order kinetics. Each half-life was deduced from a substrate disappearance curve based on at least 7 measurements (correlation coefficients, $r^2 \geq 0.95$) except for 1,2,3,4-TeCDD for which a disappearance curve was reconstructed by substraction of the sum of products from an initial concentration of 500 nM 1,2,3,4-TeCDD ($r^2 = 0.95$).

Estimates of dechlorination half-lives of PCDDs, PCBs, and hexachlorobenzene in the environment vary between several years to decades [5, Chapter 2; 24, Chapter 3; 36,37]. The much lower rates in the environment, compared to those observed in the laboratory may be due to various factors: lower densities of the active microbial populations, lower activities (influenced by, e.g., co-substrates, nutrients, and temperature), and lower bioavailability of sorbed chlorinated aromatics in aged contaminated sediments. Maximum bioavailability existed in our experiments as they were conducted without any sediment in super-saturated liquid media. The extreme hydrophobic properties of the higher chlorinated dioxins in particular may result in very low concentrations in the sediment pore water and consequently may limit or even completely prevent the occurrence of dechlorination in contaminated sediments. Nevertheless, the observed dechlorination of a model dioxin indicates that, for a complete assessment of the ultimate fate of PCDDs in the environment,

microbial processes cannot be ignored.

The microbially mediated dechlorination of PCBs and CBs generally results in the formation of less toxic and less bioaccumulative congeners [24, Chapter 3; 38]. In addition, the less chlorinated compounds thus produced are more suitable substrates for mineralization by aerobic microorganisms than the parent compounds are [38,39]. Whether dechlorination of PCDDs is as beneficial as that of PCBs and CBs remains uncertain. The formation of a metabolite with a 2,3-substitution pattern from 1,2,3,4-TeCDD suggests the possible formation of more toxic (2,3,7,8-substituted) products from highly chlorinated dioxins, which include a 2,3,7,8-substitution pattern. Further studies, which will include the highly toxic congeners with a 2,3,7,8-substitution pattern, and detailed field studies should reveal the environmental significance of microbial PCDD dechlorination.

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REFERENCES

1. **Evers, E.H.G., H.J.C. Klamer, R.W.P.M. Laane** and **H.A.J. Govers.** 1993. Polychlorinated dibenzo-*p*-dioxin and dibenzofuran residues in estuarine and coastal North Sea sediments: Sources and distribution. *Environ. Toxicol. Chem.* **12**:1583-1598.
2. **Wenning, R.J., M.A. Harris, M.J. Ungs, D.J. Paustenbach** and **H. Bedbury.** 1992. Chemometric comparisons of polychlorinated dibenzo-*p*-dioxin and dibenzofuran residues in surficial sediments from Newark Bay, New Jersey and other industrialized waterways. *Arch. Environ. Contam. Toxicol.* **22**:397-413.
3. **Czuczwa, J.M.** and **R.A. Hites.** 1986. Airborne dioxins and dibenzofurans: Sources and fates. *Environ. Sci. Technol.* **20**:195-200.
4. **Götz, R., P. Friesel, K. Roch, O. Päpke, M. Ball** and **A. Lis.** 1993. Polychlorinated-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and other chlorinated compounds in the river Elbe: results on bottom sediments and fresh sediments collected in sedimentation chambers. *Chemosphere* **27**:105-111.
5. **Beurskens, J.E.M., G.A.J. Mol, H.L. Barreveld, B. van Munster** and **H.J. Winkels.** 1993. Geochronology of priority pollutants in a sedimentation area of the Rhine river. *Environ. Toxicol. Chem.* **12**:1549-1566.
6. **Mosse, P.R.L.** and **D. Haynes.** 1993. Dioxin and furan concentrations in uncontaminated waters, sediments and biota of the Ninety Mile beach, Bass Strait, Australia. *Mar. Pollut. Bull.* **26**:465-468.
7. **de Boer, J., C.J.N. Stronck, W.A. Traag** and **J. van der Meer.** 1993. Non-ortho and mono-ortho substituted chlorobiphenyls and chlorinated dibenzo-*p*-dioxins and dibenzofurans in marine and freshwater fish and shellfish from the Netherlands. *Chemosphere* **26**:1823-1842.
8. **Beck, H., E.M. Breuer, A. Dross** and **W. Mathar.** 1990. Residues of PCDDs, PCDFs, PCBs and other organochlorine compounds in harbour seals and harbour porpoise. *Chemosphere* **20**:1027-1034.
9. **van den Berg, M., F. Blank, C. Heeremans, H. Wagenaar** and **K. Olie.** 1987. Presence of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans in fish-eating birds and fish from

the Netherlands. *Arch. Environ. Contam. Toxicol.* **16**:149-158.

10. **van der Weiden, M.E.J., H.J.H. Tibosch, R. Bleumink, T.L. Sinnige, C. van de Guchte, W. Seinen** and **M. van den Berg.** 1993. Cytochrome P-450 1A induction in the common carp (*Cyprinus carpio*) following exposure to contaminated sediments with halogenated polyaromatics. *Chemosphere* **27**:1297-1309.
11. **Whittle, D.M., D.B. Sergeant, S.Y. Huestis** and **W.H. Hyatt.** 1992. Foodchain accumulation of PCDD and PCDF isomers in the Great Lakes aquatic community. *Chemosphere* **25**:1559-1563.
12. **Bosveld, A.T.C., J. Gradener, M. van Kampen, A.J. Murk, E.H.G. Evers** and **M. van den Berg.** 1993. Occurrence and effects of PCBs, PCDDs and PCDFs in hatchlings of the common tern (*Sterna hirundo*). *Chemosphere* **27**:419-427.
13. **Vanden Heuvel, J.P.** and **G. Lucier.** 1993. Environmental toxicology of polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans. *Environ. Health Perspect.* **100**:189-200.
14. **Orth, R.G., C. Ritchie** and **F. Hileman.** 1989. Measurement of the photoinduced loss of vapor phase TCDD. *Chemosphere* **18**:1275-1282.
15. **Choudhry, G.G.** and **G.R.B. Webster.** 1986. Photochemical quantum yields and sunlight half-lives of polychlorodibenzo-*p*-dioxins in aquatic systems. *Chemosphere* **15**:1935-1940.
16. **Miller, G.C., V.R. Hebert, M.J. Mille, R. Mitzel** and **R.G. Zepp.** 1989. Photolysis of octachlorodibenzo-*p*-dioxin on soils: production of 2,3,7,8-TCDD. *Chemosphere* **18**:1265-1274.
17. **Quensen, J.** and **F. Matsumura.** 1983. Oxidative degradation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin by microorganisms. *Environ. Toxicol. Chem.* **2**: 261-268.
18. **Parsons, J.R.** and **M.C.M. Storms.** 1989. Biodegradation of chlorinated dibenzo-*p*-dioxins in batch and continuous cultures of strain JB1. *Chemosphere* **19**:1297-1308.
19. **Klecka, G.M.** and **D.T. Gibson.** 1980. Metabolism of dibenzo-*p*-dioxin and chlorinated dibenzo-*p*-dioxins by a *Beijerinckia* species. *Appl. Environ. Micobiol.* **39**:288-296.
20. **Bopp, R.F., M.L. Gross, H. Tong, H.J. Simpson, S.J. Monson, B.L. Deck** and **F.C. Moser.** 1991. A major incident of dioxin contamination: sediments of New Jersey estuaries. *Environ. Sci. Technol.* **25**:951-956.
21. **Brown, J.F.,Jr., R.E. Wagner, H. Feng, D.L. Bedard, M.J. Brennan, J.C. Carnahan** and **R.J. May.** 1987. Environmental dechlorination of PCBs. *Environ. Toxicol. Chem.* **6**:579-593.
22. **Brown, J.F.,Jr., D.L. Bedard, M.J. Brennan, J.C. Carnahan, H. Feng** and **R.E. Wagner.** 1987. Polychlorinated biphenyl dechlorination in aquatic sediments. *Science* **236**:709-712.
23. **Quensen, J.F.,III, J.M. Tiedje** and **S.A. Boyd.** 1988. Reductive dechlorination of polychlorinated biphenyls by anaerobic microorganisms from sediments. *Science* **242**:752-754.
24. **Beurskens, J.E.M., C.G.C. Dekker, J. Jonkhoff** and **L. Pompstra.** 1993. Microbial dechlorination of hexachlorobenzene in a sedimentation area of the Rhine river. *Biogeochemistry* **19**:61-81.
25. **Quensen, J.F.,III, S.A. Boyd** and **J.M. Tiedje.** 1990. Dechlorination of four commercial polychlorinated biphenyl mixtures (Aroclors) by anaerobic microorganisms from sediments. *Appl. Environ. Microbiol.* **56**:2360-2369.
26. **Abramowicz, D.A., M.J. Brennan, H.M. van Dort** and **E.L. Gallagher.** 1993. Factors influencing the rate of polychlorinated biphenyl dechlorination in Hudson River sediments. *Environ. Sci. Technol.* **27**:1125-1131.
27. **Fathepure, B.Z., J.M. Tiedje** and **S.A. Boyd.** 1988. Reductive dechlorination of hexachlorobenzene to tri- and dichlorobenzenes in anaerobic sewage sludge. *Appl. Environ. Microbiol.* **54**:327-330.
28. **Beurskens, J.E.M., C.G.C. Dekker, H. van den Heuvel, M. Swart, J. de Wolf** and **J. Dolfing.** 1993. Dechlorination of chlorinated benzenes by an anaerobic microbial consortium that selectively mediates the thermodynamic most favorable reactions. *Environ. Sci. Technol.* **28**:701-706.
29. **Shiu, W.Y., W. Doucette, F.A.P.C. Gobas, A. Andren** and **D. Mackay.** 1988. Physical-chemical properties of chlorinated dibenzo-*p*-dioxins. *Environ. Sci. Technol.* **22**:651-658.
30. **van Dordt, H.M.** and **D.L. Bedard.** 1991. Reductive ortho and meta dechlorination of polychlorinated biphenyl congener by anaerobic microorganisms. *Appl. Environ. Microbiol.* **57**:1576-1578.
31. **Pohland, A.E.** and **G.C. Yang.** 1972. Preparation and characterization of chlorinated dibenzo-*p*-dioxins. *J. Agric. Food Chem.* **20**:1093-1099.
32. **Gray, A.P., S.P. Cepa** and **I.J. Solomon.** 1976. Synthesis of specific polychlorinated dibenzo-*p*-dioxins. *J. Org. Chem.* **41**:2435-2437.
33. **Mohn, W.W.** and **J.M. Tiedje.** 1992. Microbial reductive dehalogenation. *Microbiol. Rev.* **56**:482-507.
34. **Mohn, W.W.** and **K.J. Kennedy.** 1992. Reductive dehalogenation of chlorophenols by *Desulfomonile tiedjei* DCB-1. *Appl. Environ. Microbiol.* **58**: 1367-1370.
35. **Struijs, J.** and **J.E. Rogers.** 1989. Reductive dehalogenation of dichloroanilines by anaerobic microorganisms in fresh and dichlorophenol-acclimated pond sediment. *Appl. Environ. Microbiol.*

55:2527-2531.

36. **Brown, J.F.,Jr.** and **R.E. Wagner.** 1990. PCB movement, dechlorination, and detoxication in the Acushnet estuary. *Environ. Toxicol. Chem.* **9**:1215-1233.
37. **Lake, J.L., R.J. Pruell** and **F.A. Osterman.** 1992. An examination of dechlorination processes and pathways in New Bedford Harbor sediments. *Mar. Environ. Res.* **33**:31-47.
38. **Abramowicz, D.A.** 1990. Aerobic and anaerobic biodegradation of PCBs: a review. *Crit. Rev. Biotechnol.* **10**:241-251.
39. **Fathepure, B.Z.** and **T.M. Vogel.** 1991. Complete degradation of polychlorinated hydrocarbons by a two-stage biofilm reactor. *Appl. Environ. Microbiol.* **57**: 3418-3422.

CHAPTER 7

REDUCTIVE DECHLORINATION OF HEXACHLOROBENZENE BY MICROORGANISMS FROM POLLUTED ESTUARINE SEDIMENT

J.E.M. Beurskens, J. de Wolf, and J. Dolfing

ABSTRACT

Reductive dechlorination of hexachlorobenzene (HCB) occurred in sediment slurries prepared with estuarine sediment from a site heavily contaminated with HCB. Disappearance of HCB was stimulated by mixing the slurries continuously or by adding lactate. Only the lactate-amended incubations yielded methane, indicating that methanogenesis is probably a minor process in this sediment. Inocula prepared from the estuarine sediment dechlorinated HCB under sulfidogenic conditions at low and high salinity with virtually no adaptation lag, and gave rise to the accumulation of 1,3,5-trichlorobenzene as the main product. Dechlorination occurred concomitantly with sulfate reduction but was not directly coupled to sulfate reduction. Dechlorinating activity under sulfidogenic conditions was only maintained if transfers were made to the saline medium. Dechlorination of HCB by an inoculum incubated under methanogenic conditions, namely, in the presence of lactate, but with no sulfate added, started after a lag phase of six days. Under sulfidogenic conditions substantial losses of HCB in sterile controls indicated the occurrence of abiotic reactions. Gaps in the mass balances revealed that such reactions or other biotic reactions in addition to dechlorination occurred in the biologically active incubations as well. Nevertheless, the formation of substantial amounts of lower chlorinated benzenes, which were absent in the sterile controls, indicates that microbial dechlorination of HCB may take place in the estuarine sediment under sulfidogenic conditions.

INTRODUCTION

Hexachlorobenzene (HCB) has been used worldwide as a fungicide and is formed as a waste product during the synthesis of several chemicals. In the aerobic aquatic environment HCB is highly persistent and tends to transfer to and accumulate in biota and sediments as a result of its hydrophobicity. After burial in sediments, where anaerobic conditions generally prevail, reductive dechlorination, i.e. the replacement of a chlorine by a hydrogen atom in the pollutant molecule, is a process of great environmental significance.
In 1987, Tiedje et al. (27) first reported the microbial dechlorination of HCB in anaerobic sewage sludge incubations. Other laboratory studies have confirmed this reaction and indicated that 1,3,5-trichlorobenzene (1,3,5-TCB) and 1,2,4-TCB are the major dechlorination products (3; Chapter 4, 13, 17). Formation of monochlorobenzene as a metabolite of HCB dechlorination has been reported once (24). Experiments with sediment columns have shown the microbial dechlorination of all trichlorobenzenes to di- and, finally, to monochlorobenzene (5). Recently, combination of field observations and laboratory incubations have indicated that *in situ* HCB dechlorination has occurred in a sedimentation area of the Rhine River resulting in an accumulation of di- and trichlorobenzenes (DCBs, TCBs)(4, Chapter 3).
To our knowledge, reductive dechlorination of HCB has been observed only under methanogenic conditions. In marine and estuarine sediments, however, sulfate is the predominant electron acceptor (29). Several studies have demonstrated the absence of dechlorinating activity in the presence of sulfate (2), or inhibition of dechlorinating activity in the presence of sulfate (1, 21, 23, 25, 26). The last group of studies was conducted with freshwater sediments that generally are methanogenic. On the other hand, dechlorination of TCBs and DCBs in a freshwater sediment column was not inhibited by sulfate concentrations up to 20 mM (5). Dehalogenation of brominated and chlorinated phenols has been shown to occur in the presence of similar sulfate concentrations (15, 19, 20), and degradation of chlorophenols can be coupled to sulfate reduction (15). The microbial dechlorination of PCBs in an estuarine environment has been recently indicated (8, 22). The seeming contradiction in the influence of sulfate on dechlorination indicates that the microbial populations involved differ and are probably site-specific.
In the Ems estuary, located in the northern part of the Netherlands, a severe contamination of harbor sediment with HCB occurred in the past. Concentrations up to 500 mg/kg dry weight are now encountered in the sediment. To determine the fate of HCB in the estuarine environment, we examined the microbial activity in relation to HCB in this sediment. In this study we report on the reductive dechlorination of pre-existing HCB in estuarine sediment

slurries and the enrichment of cultures capable of dechlorinating HCB under sulfidogenic and methanogenic conditions.

MATERIALS AND METHODS

Sediment samples

The harbor of Delfzijl is located in the Ems estuary in the northern part of the Netherlands on the border with Germany. The site is brackish with a high variation in salinity, showing a median value of 21‰. Sediment samples were collected with a box-corer from a known HCB "hot spot" in the harbor at a depth of 4.4 m. The black sediment was transported in 15-l sealed buckets and subsequently stored at 4°C in the dark. The harbor sediment has a low organic carbon content and low nitrate concentration compared to freshwater Rhine sediment with proven *in situ* HCB dechlorination (4, Chapter 3)(Table 7.1). The harbor sediment has a high sulfate concentration relative to the freshwater Rhine sediment (Table 7.1).

Table 7.1. Composition of the brackish harbor sediment and a freshwater sediment from a Rhine River sedimentation basin (Ketelmeer) with proven *in situ* HCB dechlorination (4)

	Delzijl Harbor	Ketelmeer
Organic Carbon (% d.w.)	0.9	5.3
Clay Content (% < 2 μm)	2.4	5.1
Nitrate (mg/kg)	0.41	1.4
Sulfate (mg/kg)	1340	190

Studies with sediment

The effects of an added organic substrate and mixing of sediment were studied as a first attempt to stimulate microbial dechlorination. The sediment samples were placed in an anaerobic glove box, where the sediment was sieved over 2 mm, homogenized and subsequently transferred to 100-ml serum bottles. Each bottle contained about 60 ml sediment slurry, 30% dry weight (w/w), and was sealed with viton stoppers. Sterile controls received formaldehyde to a final concentration of 4% (vol/vol). Incubations with an additional carbon source were prepared by adding sodium lactate (Baker b.v., Deventer, The Netherlands), to an initial concentration of 30 mM. The headspaces were flushed with oxygen-free N_2. All incubations were done at 20°C in the dark without shaking, except for

one test series. The bottles of this series were incubated in an end-over-end mixer (30 rpm). At regular intervals single bottles from all test series were collected and sacrificed for analyses. The bottles were homogenized by vigorous shaking before 6-g (wet weight) sediment samples were taken for analysis.

Media and chemicals

The methanogenic medium (pH 7.0) used in this study was slightly modified from Holliger et al. (17) and contained the following constituents in grams per liter of demineralized water: KH_2PO_4, 1.68; $NaHPO_4.2H_2O$, 1.64; NH_4Cl, 0.30; $CaCl_2.2H_2O$, 0.11; $MgCl_2.6H_2O$, 0.1; $NaHCO_3$, 0.82; resazurin, 0.0005; 1 ml trace element solution (18); 1 ml of vitamin solution (17). The media designed to support sulfate reducing bacteria were modified from Widdel (28). The sulfidogenic medium with low salinity had the following constituents in grams per liter of demineralized water: KH_2PO_4, 0.27; K_2HPO_4, 0.35; NH_4Cl, 0.54; $CaCl_2.2H_2O$, 0.05; $FeCl_2.4H_2O$, 0.02; $MgSO_4.7H_2O$, 0.13; Na_2SO_4, 2.5; $NaHCO_3$, 2.6; resazurin, 0.001; 2 ml of trace element solution (11); 10 ml of vitamin solution (17). The saline sulfidogenic medium contained in grams per liter of demineralized water: KH_2PO_4, 0.27; K_2HPO_4, 0.35; NH_4Cl, 1; $MgSO_4.7H_2O$, 2; Na_2SO_4, 2.5; NaCl, 16; $CaCl_2.2H_2O$, 0.3; $FeSO_4.7H_2O$, 0.003; $NaHCO_3$, 0.25; resazurin, 0.001; 2 ml of trace element solution (11); 10 ml of vitamin solution (17). The methanogenic medium as well as the sulfidogenic media were reduced by adding 0.5 g l^{-1} $Na_2S.9H_2O$. The incubations under sulfidogenic conditions were conducted a second time with one modification: the sodium sulfide was replaced by an amorphous ferrous sulfide solution. A 10-ml aliquot of a stock solution prepared as described by Brock and O'Dea (7) was used per liter medium. The vitamins, bicarbonate, and reducing agent were added from sterile stock solutions after autoclaving and cooling the medium and while gasing the medium with O_2-free N_2. The pH of the sulfidogenic media was between 6.8 and 7.2.

HCB and pentachlorobenzene (QCB) were obtained from Aldrich Chemie N.V., Brussels, Belgium. 1,2,3,4-Tetrachlorobenzene (1,2,3,4-TeCB), 1,2,4,5-TeCB, and all three DCBs were purchased from Janssen Chimica, Beerse, Belgium. All trichlorobenzenes (TCBs) were obtained from Merck (Amsterdam, The Netherlands); 1,2,3,5-TeCB was obtained from Promochem, Wesel, Germany. Gases were from Hoekloos (Schiedam, The Netherlands).

Enrichment

Sediment samples (250 g wet weight) from the HCB hot spot area in the harbor were diluted with the reduced methanogenic or sulfidogenic media (650 ml) in 1-l serum bottles. The microorganisms were eluted from the sediment by shaking the slurries for two hours.

Subsequently the slurries were allowed to settle for 15 min. Supernatants (100 ml) from these slurries were used to inoculate 1-l serum bottles that contained the corresponding media (800 ml). After addition of 2-ml aliquots of filter-sterilized sodium lactate syrup (concentration in the medium: 17.5 mM), the experiments were started by adding HCB (200 µl of a methanolic solution; final concentration in the medium approximately 100 nM). All liquid media incubations were at 25°C in the dark without shaking. Sterile controls were prepared by autoclaving at 121°C for 15 min. The incubations took place in single bottles for both the biologically active and sterile controls per test condition.

Chemical analysis

Chlorobenzene (CB) concentrations in sediment samples as well as methane production in the sediment slurry incubations were determined as described previously (4, Chapter 3), while CBs in liquid media were determined as follows. Samples of 10 ml were removed aseptically from each active and control incubation while swirling the medium to ensure a uniform suspension. Iso-octane (2 ml) was added and the mixture was shaken for 2 h. Separation of the water and iso-octane was achieved after the mixing on a vortex mixer and subsequent storage for at least 2 h at -20°C. A 200-µl sample of the iso-octane fraction was analyzed with a Hewlett Packard 5890A gas chromatograph, equipped with a ^{63}Ni electron capture detector using automated splitless injection (HP 7673A). Compound separation was accomplished with a 50 m fused silica capillary column CP Sil 8CB (0.25-mm i.d. and 0.20-µm film thickness) from Chrompack, Bergen op Zoom, The Netherlands. Helium was used as a carrier gas and nitrogen as a detector-quench gas. The operating temperatures of the injector and detector were 225°C and 300°C, respectively. The oven temperature program was: 90°C initially for 2 min, increased to 140°C at 2°C per min and 5 min isothermal at 140°C before an increase to 225°C at 5°C per min, with an isothermal period of 90 min at the end. Concentrations were determined relative to external standards using the HP 3365 ChemStation software. Sulfate concentrations in liquid media were determined after filtration of 45-ml samples over 5-µm filters and the addition of 5 ml formaldehyde (37%) to the samples and storage at -18°C. Colorimetric analyses were conducted with an autoanalyzer (Technicon, Rotterdam, The Netherlands).

RESULTS

Dechlorination of chlorinated benzenes present in sediment

The total volume of severely HCB polluted sediment in the harbor of Delfzijl is

approximately 10,000 m^3. A characteristic example of the chlorobenzene composition in the sediment of this hot spot is given in Table 7.2. High concentrations of HCB, 1,3- and 1,4-dichlorobenzene (DCB) dominate the pollution pattern.

Table 7.2. Concentrations of chlorinated benzenes in Delfzijl Harbor sediment.

Compound	Concentration (µmol/kg)
Hexachlorobenzene	1756
Pentachlorobenzene	10
1,2,3,4-Tetrachlorobenzene	<0.2[a]
1,2,3,5- and 1,2,4,5-Tetrachlorobenzene	1.6
1,2,3-Trichlorobenzene	<0.3[a]
1,2,4-Trichlorobenzene	4
1,3,5-Trichlorobenzene	17
1,2-Dichlorobenzene	<3[a]
1,3-Dichlorobenzene	306
1,4-Dichlorobenzene	136
Monochlorobenzene	80

[a]Indicated values are the detection limits

The influence of several manipulations (mixing and lactate addition) on the concentrations of pre-existing chlorinated benzenes in the sediment was studied during a 67-week period. The concentration of HCB decreased substantially during the first 10 weeks in all test series, including the formaldehyde-treated controls (Fig. 1A). After these 10 weeks, however, the HCB concentration in the control remained constant, or appeared to increase slightly, while the HCB concentration in the biologically active series decreased further. This apparent increase might be a result of differences between individual bottles of one series, since individual bottles were sacrificed for each measurement point. The series with lactate additions or mixing showed the lowest HCB concentrations after 67 weeks. The 1,3,5-TCB concentration showed a rapid increase in the continuously mixed bottles, reaching a maximum of 513 µmol kg^{-1} after 29 weeks (Fig. 7.1B). A relatively small and transient accumulation of 1,3,5-TCB was observed in the static incubations during the first 29 weeks. No increase in 1,3,5-TCB concentrations was observed in the lactate-amended and formaldehyde-treated control incubations. The 1,3-DCB concentration increased slowly in the bottles with lactate amendments during the first 30 weeks (Fig. 7.1C). The static incubated bottles showed a slow increase of 1,3-DCB, resulting in a final concentration of 133 µmol kg^{-1}, whereas the continuously mixed bottles showed a substantial increase in 1,3-DCB at the end of the experiment. The 1,3-DCB concentration in formaldehyde-treated

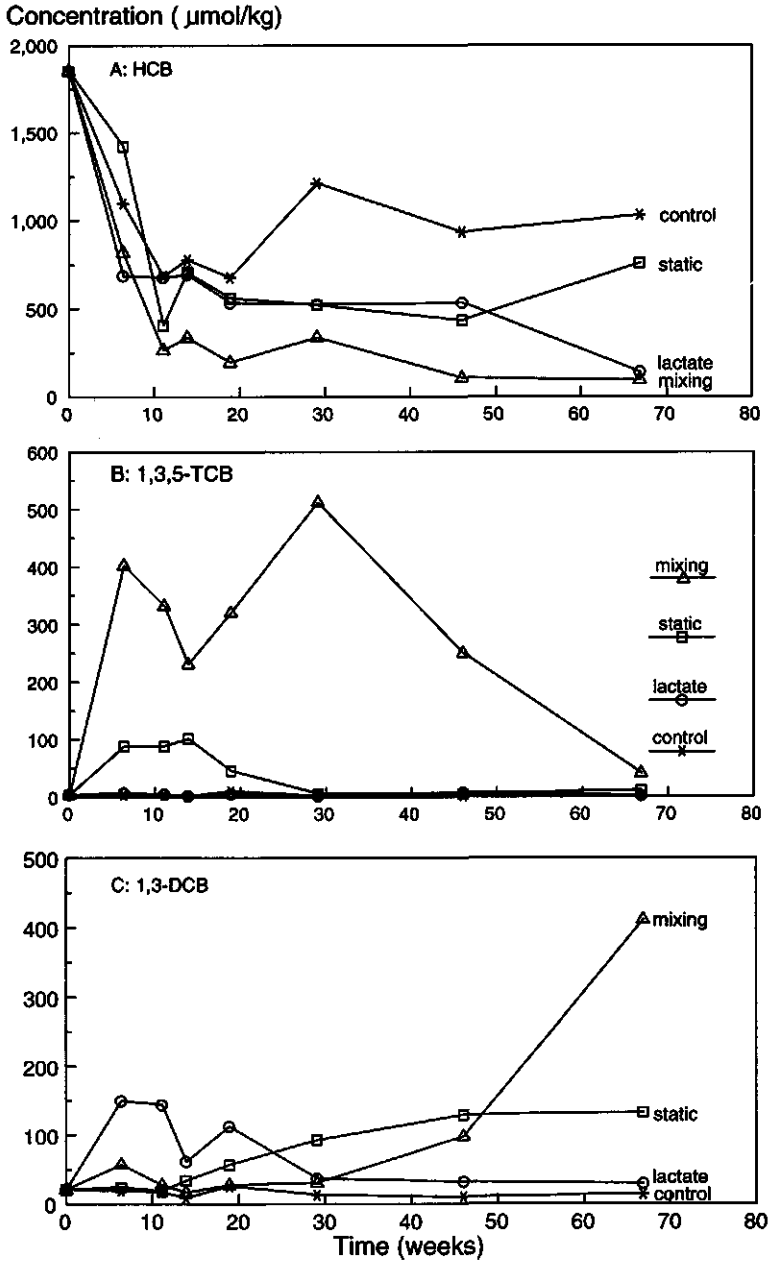

Fig. 7.1. Pattern of disappearance of pre-existing hexachlorobenzene (A) and formation of 1,3,5-trichlorobenzene (B) and 1,3-dichlorobenzene (C) in anaerobic sediment slurries under four incubation conditions: static, end-over-end mixing, lactate-amended and sterile controls.

controls remained constant throughout the experiment. The concentration of all other chlorinated benzenes did not change. Monochlorobenzene (MCB) was not present at concentrations above its detection limit (25 μmol kg^{-1}). Methane production was only observed in the lactate-amended incubations (data not shown). The absence of methane production in the sediment slurries to which no additional carbon source was added indicates that methanogenesis is probably a minor process in the harbor sediment. For all test series the total molar loss in HCB is only partially accounted for by the formation of lower chlorinated benzenes. The recoveries were 22%, 7%, <1%, and <1% for mixed, static, lactate-amended, and sterile controls, respectively, at the end of the incubation period. A maximum recovery of about 35% was observed after 29 weeks of incubation under mixing conditions.

Dechlorinating enrichments

Attempts to enrich dechlorinating microorganisms from the harbor sediment were made under various conditions by extracting the microorganisms from the sediment and incubation in liquid media. The first series of incubations was made with sodium sulfide as reducing agent.

In order to determine the influence of salinity on the dechlorinating activity, incubations under sulfidogenic conditions were conducted at low and high salinity. No substantial dechlorination was observed under these conditions (data not shown). Subsequently, sulfidogenic incubations were repeated with amorphous ferrous sulfide as reducing agent. Repeated additions of HCB under sulfidogenic conditions at high salinity in the presence of ferrous sulfide resulted in an increasing rate of HCB disappearance in the biologically active incubations on each addition. The sulfate concentration decreased to 5 mM just before the second amount of HCB was added and remained constant throughout the experiment (Fig. 7.2A). This indicates that sulfate reduction occurred in the biologically active incubations, but was not coupled to the HCB dechlorination. Sulfate concentrations in the sterile controls remained constant. On the other hand, HCB concentrations in the sterile controls decreased substantially, but did not result in an accumulation of lower chlorinated benzenes. Formation of lower chlorinated benzenes from HCB in the biologically active incubations showed a somewhat different pattern as compared to the previous sediment incubations. Besides 1,2,3,5-TeCB, 1,2,4,5-TeCB was now produced in greater quantities and after the third addition even exceeded the 1,2,3,5-TeCB concentration (Fig. 7.2B). As a result of successive chlorine removal, 1,2,4-TCB emerged as a second end product, along with 1,3,5-TCB. Concentrations of DCBs did not increase during the experiment. At the end of the experiment 40% of the total loss of HCB was

recovered as lower chlorinated benzenes.

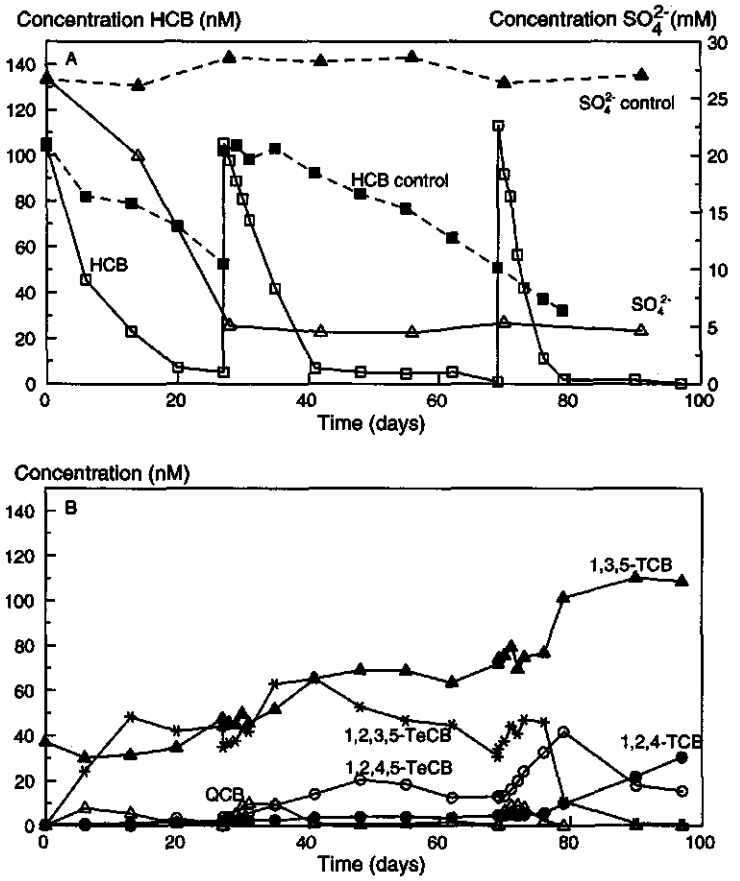

Fig. 7.2. Hexachlorobenzene (HCB) and sulfate (SO_4^{2-}) concentrations during sulfidogenic incubation at high salt concentrations of eluted microorganisms from harbor sediment under biologically active and sterile control conditions (A). HCB was added three times in the biologically active bottle and twice in the sterile controls. Lower chlorinated benzenes formed in the biologically active incubations (B): pentachlorobenzene (QCB), 1,2,4,5-tetrachlorobenzene (1,2,4,5-TeCB), 1,2,3,5-tetrachlorobenzene (1,2,3,5-TeCB), 1,2,4-trichlorobenzene (1,2,4-TCB), and 1,3,5-trichlorobenzene (1,3,5-TCB).

At low salinity, HCB concentrations decreased rapidly after the first and second HCB additions; after the third addition the disappearance ceased (Fig. 7.3A). In sterile controls, a substantial decrease in HCB was observed after both the first and second HCB addition (Fig. 7.3A), though this disappearance did not result in any detectable product formation. The sulfate concentration decreased from about 20 to 6 mM in the biologically active incubations and remained constant in the autoclaved controls, indicating sulfate reduction in

the biologically active incubations (Fig. 7.3A). The transformation of the first amount of HCB added resulted in formation of 1,2,3,5-TeCB and a small amount of 1,3,5-TCB (Fig. 7.3B). The second HCB addition resulted in a small peak of QCB and a rapid increase of 1,2,3,5-TeCB. In addition, a small amount of 1,2,4,5-TeCB was produced (Fig. 7.3B). 1,2,3,5-TeCB concentrations subsequently decreased, but this decrease did not result in a stoichiometric accumulation of 1,3,5-TCB. No increase in DCB concentrations was observed. The molar recovery of 1,3,5-TCB and both TeCBs accounted only for 50% of the loss in HCB. Particularly, the decrease in 1,2,3,5-TeCB did not result in the expected increase in 1,3,5-TCB. Sterile incubations indicated abiotic disappearance at a considerable rate, about 2 to 3 times as high as the loss in sterile controls under methanogenic conditions (see below).

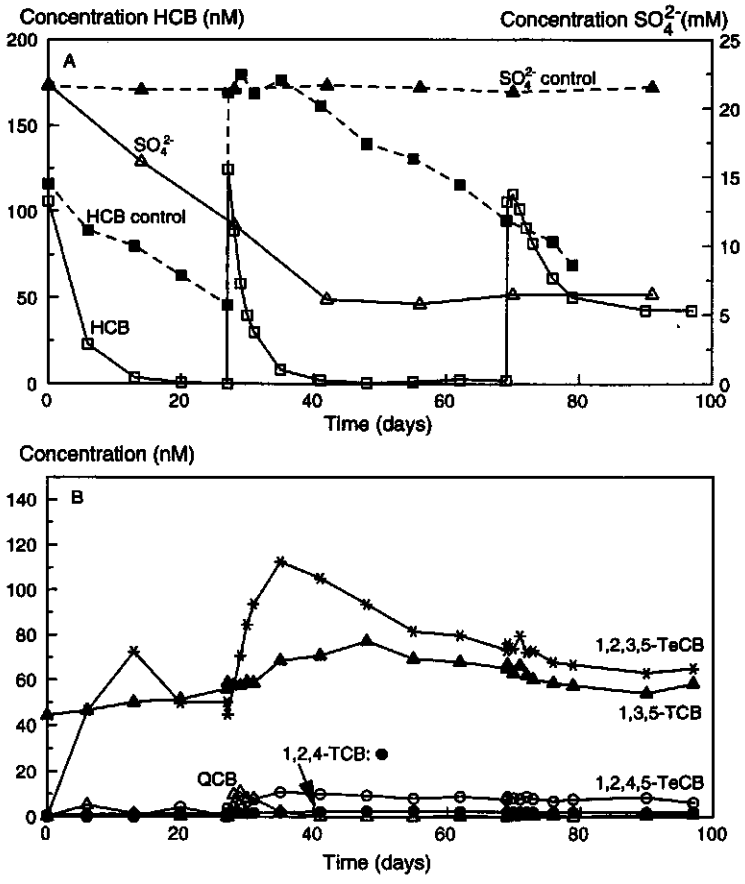

Fig. 7.3. Hexachlorobenzene (HCB) and sulfate (SO_4^{2-}) concentrations during sulfidogenic incubation at low salt concentrations of eluted microorganisms from harbor sediment under biologically active and sterile control conditions (A). HCB was added three times in the biologically active bottle and twice in the sterile controls. For lower chlorinated benzenes formed in the biologically active incubations (B) see legend Fig 7.2.

Under methanogenic conditions (at low salinity), with sodium sulfide as reducing agent, the first amount of HCB was transformed within 27 days after a lag phase of about six days. A second addition disappeared within six days without a lag phase (Fig. 7.4A). A slow decrease in HCB concentration was observed in the autoclaved controls (Fig. 7.4A), but formation of lower chlorinated benzenes was not detected in these controls. The transformation of HCB resulted in a transient accumulation of 1,2,3,5-TeCB and an accumulation of 1,3,5-TCB (Fig. 7.4B). The elevated level of 1,3,5-TCB at the beginning of the experiment was the result of high concentrations of 1,3,5-TCB in the inoculum. Accumulating levels of QCB were only observed after the second HCB addition. Formation of DCBs was not observed (detection limit 4 nM). During the first 26 days, an almost

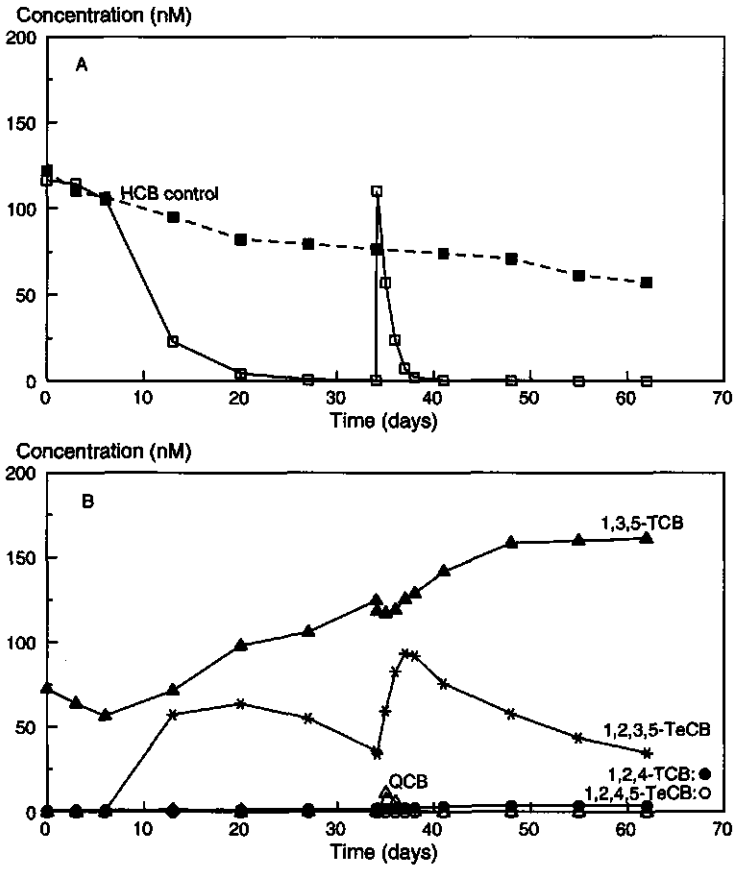

Fig. 7.4. Hexachlorobenzene (HCB) concentrations during methanogenic incubation of eluted microorganisms from harbor sediment under biologically active and sterile control conditions (A). HCB was spiked twice to the biologically active bottle and once to the sterile control. For lower chlorinated benzenes formed in the biologically active incubations (B) see legend Fig 7.2.

stoichiometric conversion of HCB to 1,2,3,5-TeCB and 1,3,5-TCB was observed. However, the total molar amount of 1,3,5-TCB and 1,2,3,5-TeCB produced at the end of the experiment is only 60% of the HCB molar loss that was spiked twice. No unidentified peaks were observed in the chromatograms.

Transfers

Since sulfidogenic conditions probably dominate in the estuarine sediment, transfers (10%) of enrichment cultures were only made with the sulfate-reducing enrichments in the presence of lactate. Transfers under sulfidogenic conditions showed HCB dechlorination at high salinity, but failed with the culture obtained at low salinity (Fig. 7.5). In the biologically active incubations no QCB peak was observed, indicating a rapid subsequent dechlorination to tetrachlorinated benzenes. Again, 1,2,4,5-TeCB concentrations were higher than 1,2,3,5-TeCB concentrations; 1,3,5-TCB and 1,2,4-TCB tended to accumulate at the end of the experiment.

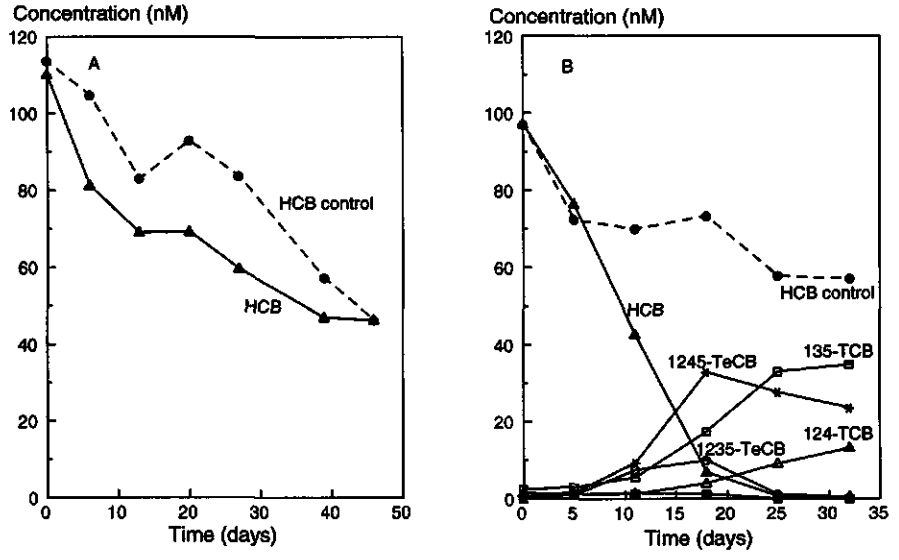

Fig. 7.5. Chlorinated benzene concentrations under sulfidogenic conditions with low (A) and high (B) salt concentrations after inoculation with the corresponding enrichments. For legends see Fig 7.2.

DISCUSSION

The concentration of the pre-existing HCB in the sediment slurries decreased in all test series. In the static and mixed test series, but not in the formaldehyde-treated series, substantial increases in 1,3,5-TCB and 1,3-DCB coincided with the HCB disappearance. These results support the conclusion that microbial dechlorination of HCB occurred in the sediment slurries. The loss of HCB in formaldehyde-treated controls did not result in a build-up of lower chlorinated benzenes and must be attributed to a mechanism other than microbial dechlorination. On the basis of the small amount of lower chlorinated benzenes produced in the lactate-amended bottles, microbial dechlorination of HCB in these bottles appeared to be a minor removal mechanism, although 92% of HCB had disappeared. In contrast to earlier reports under methanogenic conditions (13, 17), we did not observe a stoichiometric conversion of HCB into lower chlorinated benzenes.

The sterile incubations showed an abiotic loss of HCB. This disappearance is not an artefact caused by sorption to the viton stoppers or to the glass wall, since no disappearance was observed in identical sterile freshwater sediment incubations in a previous study (4, Chapter 3). Therefore the occurrence of abiotic transformations is the most plausible explanation for the loss of HCB in the sterile incubations. Abiotic reactions have presumably also taken place in the biologically active incubations and contributed to the lack of stoichiometric conversions observed in these incubations. In the biologically active incubations, formation of metabolites other than CBs may have led to the incomplete stoichiometry as well. Alternative metabolites may be produced in addition to microbial dechlorination, for example, methylthio metabolites. This type of metabolite has been reported for PCBs in anaerobic lake sediment, presumably formed by microbial action in the anaerobic sediment (9). Formation of methanethiol from the anaerobic transformation of monochloromethane has been demonstrated (6).

Dechlorination of HCB by an inoculum from the estuarine sediment was demonstrated under sulfidogenic conditions with ferrous sulfide as reducing agent. Under sulfidogenic conditions, HCB dechlorination resulted in substantial amounts of 1,2,4-TCB, along with 1,3,5-TCB, as the main product. Saline conditions appear to be essential for subculturing the enrichment under sulfidogenic conditions. In the liquid media high losses of HCB in sterile controls were again observed, indicating abiotic transformations. The reason sodium sulfide failed as a reducing agent for the establishment of sulfidogenic enrichments remains uncertain. Several possible explanations can be put forward. Firstly, iron is needed by the microbial community mediating the dechlorination reactions. Secondly, ferrous sulfide forms, in contrast to sodium sulfide, a precipitate in the medium and may offer a suitable

template for interspecies interactions involved in the microbial dechlorination (10). Thirdly, sodium sulfide results in higher freely dissolved S^{2-} concentrations that may exert toxic effects.

High concentrations of HCB, 1,3- and 1,4-DCB are present in the harbor sediment. A chlorinated aliphatic production facility at Delfzijl emitted HCB to the harbor up to the late 1980s, when emissions were eliminated. Besides HCB, lower chlorinated benzenes may have been present in the discharges from the chlorinated alifatics plant (12). However, the elevated concentrations of 1,3,5-TCB, 1,3- and 1,4-DCB, which are known dechlorination products of HCB (3, 4, 13, 17) could indicate *in situ* microbial dechlorination. Assuming that dechlorination of HCB is the single source of all the chlorinated benzenes (mono- through penta-), 24% of the original HCB input in the sediment has been transformed. This percentage is, of course, highly speculative and further elaboration of the currently available field data makes no sense as long as the original composition and concentration levels of the emissions in the past remain unknown. Nevertheless, the observation that HCB dechlorination in liquid sulfidogenic media began with virtually no adaptation lag (Fig. 7.4A), indicates the high likelihood of this process occurring naturally at the contaminated harbor site.

A complete biomineralization of HCB can be obtained by a sequential anaerobic dechlorination and aerobic biomineralization of the dechlorination products, as recently demonstrated in a two-stage biofilm reactor (14). These sequential processes were demonstrated to occur *in situ* for PCBs and were stimulated successfully in Hudson River sediments (16). Manipulations were directed to stimulate the aerobic processes, after PCBs had already undergone extensive *in situ* dechlorination in the Hudson River sediment. In the estuarine sediment in Delfzijl, however, HCB is still present at high concentrations, indicating low *in situ* dechlorination rates. This was confirmed in the laboratory, where HCB showed the least reactivity in the static incubations that most closely resemble the field conditions. Thus, stimulation of HCB dechlorination rates is the first goal for achieving a biotechnological decontamination of the estuarine sediment. Mixing the sediment slurries continuously resulted in the highest 1,3-DCB formation and therefore mixing is shown to be appropriate for stimulating HCB dechlorination. The dechlorination products, except 1,3,5-TCB, are generally suitable substrates for aerobic mineralization. Although 1,3,5-TCB accumulated in the liquid-media incubations, dechlorination proceeded to 1,3-DCB in the sediment slurries, indicating that during the enrichment procedure part of the dechlorinating capacity was lost. The challenge for the anaerobic part of biotechnological remediation will be to achieve substantial dechlorination rates and avoid formation of 1,3,5-TCB. Both aspects are currently being further investigated in an on-site pilot treatment facility that will

allow the creation of aerobic conditions in future. In addition, special attention is being given to the formation of other unknown metabolites that may complicate the efficacy of the anaerobic processes.

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REFERENCES

1. **Adrian, N.R., and J.M. Sulflita.** 1990. Reductive dehalogenation of a nitrogen heterocyclic herbicide in anoxic aquifer slurries. Appl. Environ. Microbiol. **56**:292-294.
2. **Alder, A.C., M.M. Häggblom, S.R. Oppenheimer, and L.Y. Young.** 1993. Reductive dechlorination of polychlorinated biphenyls in anaerobic sediments. Environ. Sci. Technol. **27**:530-538.
3. **Beurskens, J.E.M., C.G.C. Dekker, H. van den Heuvel, M. Swart, J. de Wolf, and J. Dolfing.** 1994. Dechlorination of chlorinated benzenes by an anaerobic microbial consortium that selectively mediates the thermodynamic most favorable reactions. Environ. Sci. Technol. **28**:701-706.
4. **Beurskens, J.E.M., C.G.C. Dekker, J. Jonkhoff, and L. Pompstra.** 1993. Microbial dechlorination of hexachlorobenzene in a sedimentation area of the Rhine River. Biogeochem. **19**:61-81.
5. **Bosma, T.N.P., J.R. van der Meer, G. Schraa, M.E. Tros, and A.J.B. Zehnder.** 1988. Reductive dechlorination of all trichloro- and dichlorobenzene isomers. FEMS Microbiol. Ecol. **53**:223-229.
6. **Braus-Stromeyer, S.A., A.M. Cook, and T. Leisinger.** 1993. Biotransformation of chloromethane to methanethiol. Environ. Sci. Technol. **27**:1577-1579.
7. **Brock, T.D., and K. O'Dea.** 1977. Amorphous ferrous sulfide as a reducing agent for culture of anaerobes. Appl. Environ. Microbiol. **33**:254-256.
8. **Brown, J.F. Jr., and R.E. Wagner.** 1990. PCB movement, dechlorination, and detoxication in the Acushnet estuary. Environ. Toxicol. Chem. **9**:1215-1233.
9. **Buser, H.-R., and M.D. Müller.** 1986. Methylthio metabolites of polychlorobiphenyls identified in sediment samples from two lakes in Switzerland. Environ. Sci. Technol. **20**:730-735.
10. **Dolfing, J., and J.M. Tiedje.** 1986. Hydrogen cycling in a three-tiered food web growing on the methanogenic conversion of 3-chlorobenzoate. FEMS Microbiol. Ecol. **38**:293-298.
11. **Edwards, E.A., L.E. Wills, M. Reinhard, and D. Grbić-Galić.** 1992. Anaerobic degradation of toluene and xylene by aquifer microorganisms under sulfate-reducing conditions. Appl. Environ. Microbiol. **58**:794-800.
12. **Evers, E.H.G.** 1989. The formation of polychlorinated dibenzofurans (PCDFs), polychlorinated dibenzo-p-dioxins (PCDDs) and related compounds during oxyhydrochlorination of ethylene. Technical Report MTC/89EE. University of Amsterdam, The Netherlands.
13. **Fathepure, B.Z., J.M. Tiedje, and S.A. Boyd.** 1988. Reductive dechlorination of hexachlorobenzene to tri- and dichlorobenzenes in anaerobic sewage sludge. Appl. Environ. Microbiol. **54**:327-330.
14. **Fathepure, B.Z., and T.M. Vogel.** 1991. Complete degradation of polychlorinated hydrocarbons by a two-stage biofilm reactor. Appl. Environ. Microbiol. **57**:3418-3422.
15. **Häggblom, M.M., and L.Y. Young.** 1990. Chlorophenol degradation coupled to sulfate reduction. Appl. Environ. Microbiol. **56**:3255-3260.
16. **Harkness, M.R., J.B. McDermott, D.A. Abramowicz, J.J. Salvo, W.P. Flanagan, M.L. Stephens, F.J. Mondello, R.J. May, J.H. Lobos, K.M. Carroll, M.J. Brennan, A.A. Bracco, K.M. Fish, G.L. Warner, P.R. Wilson, D.K. Dietrich, D.T. Lin, C.B. Morgan, and W.L. Gately.** 1993. In situ stimulation of aerobic PCB biodegradation in Hudson River sediments. Science **259**:503-507.

17. **Holliger, C., G. Schraa, A.J.M. Stams, and A.J.B. Zehnder.** 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.
18. **Houwen, F.P., C. Dijkema, C.H.H. Schoenmakers, A.J.M. Stams, and A.J.B. Zehnder.** 1987. ^{13}C-NMR study of propionate degradation by a methanogenic coculture. FEMS Microbiol. Lett. **41**:269-274.
19. **King, G.M.** 1988. Dehalogenation in marine sediments containing natural sources of halophenols. Appl. Environ. Microbiol. **54**:3079-3085.
20. **Kohring, G.-W., X. Zhang, and J. Wiegel.** 1989. Anaerobic dechlorination of 2,4-dichlorophenol in freshwater sediments in the presence of sulfate. Appl. Environ. Microbiol. **55**:2735-2737.
21. **Kuhn, E.P., G.T. Townsend, and J.M. Sulflita.** 1990. Effect of sulfate and organic carbon supplements on reductive dehalogenation of chloroanilines in anaerobic aquifer slurries. Appl. Environ. Microbiol. **56**:2630-2637.
22. **Lake, J.L., R.J. Pruell, and F.A. Osterman.** 1992. An examination of dechlorination processes and pathways in New Bedford harbor sediments. Mar. Environ. Res. **33**:31-47.
23. **Morris, P.L., W.W. Mohn, J.F. Quensen III, J.M. Tiedje, and S.A. Boyd.** 1992. Establishment of a polychlorinated biphenyl-degrading enrichment culture with predominantly *meta* dechlorination. Appl. Environ. Microbiol. **58**:3088-3094.
24. **Ramanand, K., M.T. Balba, and J. Duffy.** 1993. Reductive dehalogenation of chlorinated benzenes and toluenes under methanogenic conditions. Appl. Environ. Microbiol., **59**:3266-3272.
25. **Sharak Genthner, B.R., W.A. Price, and P.H. Pritchard.** 1989. Anaerobic degradation of chloroaromatic compounds in aquatic sediments under a variety of enrichment conditions. Appl. Environ. Microbiol. **55**:1466-1471.
26. **Sharak Genthner, B.R., W.A. Price, and P.H. Pritchard.** 1989. Characterization of anaerobic dechlorinating consortia derived from aquatic sediments Appl. Environ. Microbiol. **55**:1472-1476.
27. **Tiedje, J.M., S.A. Boyd, and B.Z. Fathepure.** 1987. Anaerobic degradation of chlorinated aromatic hydrocarbons. Dev. Ind. Microbiol. **27**:117-127.
28. **Widdel, F.** 1980. Anaerobe Abbau von Fettsäuren und Benzoesäure durch neu isolierte Arten Sulfat-Reduzierender Bacterien. Ph.D. Thesis, Göttingen, Germany.
29. **Widdel, F.** 1988. Microbiology and ecology of sulfate- and sulfur-reducing bacteria. *In* A.J.B. Zehnder (ed.), Biology of anaerobic microorganisms. John Wiley & Sons, Inc., New York.

CHAPTER 8

MICROBIAL TRANSFORMATION OF PCBs IN SEDIMENTS: WHAT CAN WE LEARN TO SOLVE PRACTICAL PROBLEMS?

J.E.M. Beurskens and P.B.M. Stortelder

ABSTRACT

The disappearance of several polychlorinated biphenyls (PCBs) was observed in the deeper anaerobic sediment layers of Lake Ketelmeer, a sedimentation area of the Rhine River. Laboratory studies with an anaerobic consortium from this area demonstrated the capabilities for transforming PCBs. The field and laboratory observations indicate that disappearances of PCBs in the sediment might be caused by *in situ* microbial dechlorination. These processes appear to have lowered the PCB toxicity of four reactive congeners by 75% during the last two decades. In addition, dechlorination reactions are the essential first steps in the only natural pathway that eventually may lead to a complete elimination of PCBs from the aquatic environment. This can only be achieved if the dechlorination products are subsequently mineralized under aerobic conditions. Several implications for environmental policies with regard to contaminated sediments are discussed. Long-term prognoses on sediment quality generated with mathematical models that include these reactions may lead to a distinct, probably less costly, sediment policy.

INTRODUCTION

Three main waterways, the Rivers Rhine, Meuse and Scheldt, enter the North Sea from the Netherlands. These three rivers drain highly industrialized areas of northwestern Europe. Enormous quantities of highly polluted sediments (> 100 million m^3) were deposited in the downstream areas during the past decades. Inventories of the sediment quality in Dutch sedimentation areas indicated a widespread contamination with polychlorinated biphenyls (PCBs), which are found among other contaminants like metals and polycyclic aromatic hydrocarbons (PAHs) (Rijkswaterstaat, 1993a; Rijkswaterstaat 1993b).

In the last decade clear improvements in water quality particularly for the Rhine River have taken place (Heymen, 1992). As a consequence, the recently deposited sediment layers are less polluted (Beurskens *et al.*, 1993a [Chapter 2]; Beurskens *et al.*, 1994a). However, in the deeper sediment layers high pollution levels from the past can still be found. Based on the toxicological properties and presumed persistence of the hydrophobic pollutants, an extensive sediment dredging, treatment and confined disposal policy for the forthcoming decades has been developed (VROM, 1993). This policy should eliminate toxicological risks and allow the development of multifunctional water systems. Newly deposited sediment will be less contaminated in future due to reduced discharges. The long-term objective is disposal of uncontaminated dredged sediment from sites where dredging is still necessary for navigation purposes, on land or at sea, without any stringent restrictions.

At present, sediment dredging in the Netherlands is undertaken for two reasons. First, maintenance of minimum water depths in fairways and harbors; second, removal of contaminated sediments that pose direct environmental risks in order to restore these sites. However, an increasing number of studies indicate that microbial processes in sediments may contribute to a natural decontamination, which is particularly relevant for the latter type of dredging locations. The objectives of the present study are: 1) to summarize the present knowledge on microbial PCB degradation, 2) to verify its significance for a Dutch Rhine sedimentation area, and 3) to evaluate the possible consequences for the environmental policy.

MICROBIAL DEGRADATION OF PCBs IN SEDIMENTS

PCBs consist of a biphenyl that has been chlorinated in one or more sites, resulting in as many as 209 different congeners containing 1 to 10 chlorine atoms (Fig. 8.1A). Of these, about 100 are considered most probably to arise during industrial synthesis. The toxicity of

PCBs depends on the spatial configuration of the chlorines. Congeners with no or only one ortho, two para, and two or more meta chlorines, resemble 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) in their biochemical and toxic effects. Based on this similarity, 2,3,7,8-TCDD toxic equivalency factors (TEFs) have been proposed that relate PCB toxicity to the 2,3,7,8-TCDD toxicity (Table 8.1) (Safe, 1990). Multiplication of the individual PCB concentrations by these factors gives the so-called 2,3,7,8-TCDD toxic equivalency concentrations.

Table 8.1. IUPAC numbering of non-ortho and mono-ortho PCBs and their toxic equivalence factors (TEF), as proposed by Safe (1990).

no.	structure	type	TEF
77	3,4,3',4'	non-ortho	0.01
105	2,3,4,3',4'	mono-ortho	0.001
114	2,3,4,5,4'	mono-ortho	0.001
118	2,4,5,3',4'	mono-ortho	0.001
123	3,4,5,2',4'	mono-ortho	0.001
126	3,4,5,3',4'	non-ortho	0.1
156	2,3,4,5,3',4'	mono-ortho	0.001
157	2,3,4,3',4',5'	mono-ortho	0.001
167	2,4,5,3',4',5'	mono-ortho	0.001
169	3,4,5,3',4',5'	non-ortho	0.05
189	2,3,4,5,3',4',5'	mono-ortho	0.001

The toxic non-ortho and mono-ortho congeners were present at trace levels in the original PCB mixtures (Hong *et al.*, 1993), but are now considered to be mainly responsible for the adverse effects of PCBs (de Voogt *et al.*, 1990). In accordance with this recent toxicological point of view, we will concentrate on the biodegradability of non-ortho and mono-ortho PCB congeners.

Knowledge on the long-term fate of PCBs in sediments is still in its infantry. This is no surprise since congener-specific analytical methods have only been available since the mid-1980s (Mullin *et al.*, 1984). The first report of PCB mixtures in the natural environment was, however, published in 1966 (Jensen, 1966). Aerobic microbial degradability of PCBs was first reported in the 1970s (Ahmed and Focht, 1973). Contrary to this, microbial metabolism under anaerobic conditions that prevail in sediments has recently been demonstrated (Quensen *et al.*, 1990).

Under anaerobic conditions reductive dechlorination of PCBs can be mediated by microorganisms in polluted sediments (Quensen *et al.*, 1990; Alder *et al.*, 1993; Abramowicz *et al.*, 1993). In this reaction chlorines are replaced by hydrogen atoms in the PCB molecule, yielding less chlorinated PCB isomers (Fig. 1). It is now well established that this reaction

has significantly altered the PCB composition in river sediments, e.g. in the severely polluted Hudson River (US) (Brown *et al.*, 1987; Lake *et al.*, 1992). Various patterns of PCB dechlorination are observed at different sites (Mohn and Tiedje, 1992) and may lead to an accumulation of mono- and dichlorobiphenyls. Dechlorination of PCBs has several significant consequences: (i) it yields less hydrophobic congeners and consequently reduces the bioaccumulation potential of PCBs in the environment; (ii) since dechlorination involves mainly the removal of meta and para chlorines, significant reduction in the TCDD-like toxicity associated with PCBs can be obtained; (iii) dechlorination might be the first step in achieving a biological decontamination of polluted sediments. The less chlorinated congeners produced are, in contrast to their parent compounds, suitable substrates for aerobic microbial mineralization (Abramowicz, 1990).

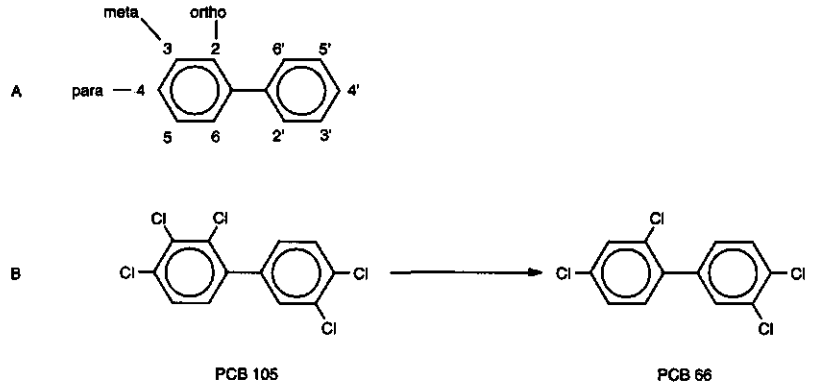

Fig. 8.1. Spatial numbering and terms for chlorine substituents in biphenyl molecule (A) and an example of meta dechlorination of the mono-ortho substituted PCB 105 (B).

FATE OF PCBs IN A SEDIMENTATION AREA OF THE RHINE

In order to quantify the occurrence of this process in the polluted Rhine sediment, field studies with sediment cores as well as laboratory studies were conducted. Several sediment cores were collected in Lake Ketelmeer, a sedimentation area of the Rhine River. The core layers were dated and priority pollutants were analyzed . Three non-ortho and three mono-ortho PCBs were determined (Beurskens *et al.*, 1993a [Chapter 2]; Beurskens *et al.*, 1994a). We were able to test the long-term persistence of pollutants in the sediment by using sediment top-layer samples that were collected in 1972. These samples, dried and

subsequently stored for almost 20 years, were analyzed together with the sediment core samples. The stored top-layer samples from 1972 reflect the original input around 1970 with either no or only limited transformation process influences.

The differences in mean concentrations between top-layer samples from 1972 and core layers that had been deposited around 1970 were tested for the six PCB congeners studied (Table 2.2, page 29). The differences proved to be significant at a 0.05 confidence level for all tested congeners, except for PCB 77 and PCB 118. Significant reductions vary between 70 and 88%.

These reductions indicate that PCBs have disappeared in the anaerobic lake sediment. Disappearance may be caused by transport or transformation processes. PCB concentration profiles in the sediment cores were not affected by downward transport with infiltrating water as demonstrated in a previous study (Beurskens et al., 1994a). Thus disappearance may be the result of microbial dechlorination processes in the anoxic sediment as mentioned above. Unlike what was found in the studies on the Hudson, an accumulation of less chlorinated biphenyls representing the reaction products, could not be demonstrated in Lake Ketelmeer sediment. This is due to the limited number of congeners analyzed in our study.

If the findings in Lake Ketelmeer are attributed to microbial processes, this type of dechlorination will seem to alter the concentrations of the individual congeners at different rates; PCB 77 and PCB 118 seem to be the most recalcitrant congeners. The other four PCBs show an average reduction of 80% during 20 years of "environmental incubation". Assuming first-order kinetics for the microbial processes a maximum half-life of approximately nine years can be estimated for these PCBs in the anaerobic Lake Ketelmeer sediment.

MICROBIAL CAPABILITIES IN RHINE SEDIMENT FOR TRANSFORMING PCBs

An anaerobic dechlorinating microbial consortium capable of dechlorinating some, but not all, chlorinated benzenes was obtained from Lake Ketelmeer sediment. Similar to that for some PCBs, hexachlorobenzene disappearance in Lake Ketelmeer sediment was observed too (Beurskens *et al.*, 1993b [Chapter 3]). The enrichment procedure consisted of repeated transfers of sediment slurries in the presence of hexachlorobenzene and lactate (Beurskens *et al.*, 1994b). This enrichment culture was incubated individually with several PCB congeners, including some of the congeners studied in the sediment cores, in order to verify whether this culture would also exhibit dechlorinating activity toward PCBs, and if so,

to verify whether the PCB dechlorination would match the observed *in situ* selectivity. These experiments showed the ability of the enrichment to dechlorinate PCBs selectively. Only chlorines that were surrounded by chlorines on both sites were removed; ortho chlorines persisted (Beurskens *et al.*, 1994c [Chapter 5]). The results were in agreement with field observations from the area where the enrichment was derived, and therefore indicate that the selective disappearance of PCBs in the deeper layers of the sediment was likely caused by *in situ* microbial dechlorination of PCBs. A general rule in dechlorination capabilities was observed: chlorines that are surrounded by other chlorines on both sites are preferentially removed. This rule was observed for both chlorinated benzenes and chlorinated biphenyls (Beurskens *et al.*, 1994b [Chapter 4]; Beurskens *et al.*, 1994c [Chapter 5]), and enables us to predict the occurrence of dechlorination and possible metabolites formed from non-ortho and mono-ortho PCBs in Lake Ketelmeer sediment (Table 8.2). Dechlorination of all non-ortho and mono-ortho congeners, except for PCB 77 and PCB 118, by the enrichment from Lake Ketelmeer sediment may occur. The observed persistence of PCB 77 and PCB 118 in the field study confirms the applicability of the general rule for dechlorination.

Table 8.2. The possibility of planar and mono-ortho PCB dechlorination by the enrichment culture and their occurrence in the field (F) and laboratory (L).

Parent		Possibility[a]	Products[b]			Observation[c]	
no.	structure		no.	structure		F	L
77	3,4,3',4'	–				–	n.t.
105	2,3,4,3',4'	+	66	2,4,3',4'		+	+
114	2,3,4,5,4'	+	63	2,3,5,4'		n.t	n.t.
			74	2,4,5,4'		n.t.	n.t.
118	2,4,5,3',4'	–				–	–
123	3,4,5,2',4'	+	68	2,4,3',5'		n.t	n.t.
126	3,4,5,3',4'	+	79	3,4,3',5'		+	n.t.
156	2,3,4,5,3',4'	+	107	2,3,5,3',4'		+	+
			118	2,4,5,3',4'		+	+
157	2,3,4,3',4',5'	+	108	2,3,4,3',5'		n.t.	n.t.
			123	3,4,5,2',4'		n.t.	n.t.
167	2,4,5,3',4',5'	+	120	2,4,5,3',5'		n.t.	n.t.
169	3,4,5,3',4',5'	+	127	3,4,5,3',5'		+	n.t.
189	2,3,4,5,3',4',5'	+	159	2,3,4,5,3',5'	n.t.	n.t.	
			162	2,3,5,3',4',5'	n.t.	n.t.	
			167	2,4,5,3',4',5'	n.t.	n.t.	

n.t.=not tested

[a]Based on the requirement for 3 adjacent chlorines, dechlorination is predicted to occur (+) or not (–).

[b]When the occurrence of dechlorination is predicted, the chlorine to be removed is expected to be the one directly surrounded by two other chlorines.

[c]Observation in field and laboratory studies as reported by Beurskens et al. (1993a, 1994c [Chapters 2 and 5]).

For almost all congeners considered in Table 8.2, dechlorination will produce compounds that have no TCDD-like toxicity, i.e. dechlorination yields congeners with a Toxic Equivalence Factor (TEF) equal to zero and not listed in Table 8.1. Exceptions are the transformations of PCBs 156, 157, and 189 that result in congeners with a similar toxicity to the parent compound as well as congeners with no dioxin-like toxicity (Table 8.2).

RISK REDUCTION DUE TO *IN SITU* MICROBIAL DECHLORINATION

In Lake Ketelmeer where a slow dechlorination of PCBs seems to occur, only congeners with three adjacent chlorines appear to be reactive. Three distinct beneficial results are related to the occurrence of microbial dechlorination of PCBs as mentioned before. In order to evaluate the toxicological significance of the observed dechlorinating activity, the reduction in 2,3,7,8-TCDD Toxic Equivalency Concentrations (TEC) was calculated for the four significantly reduced congeners (Table 8.3).

Table 8.3. Comparison of mean PCB concentrations and corresponding toxic equivalence concentrations (TEC) in top-layer samples (n=5) collected in 1972 to concentrations in recently sampled core layers (n=5) deposited around 1970.

PCB no.	Top-layer samples taken 1972 and stored for almost 20 years		Sediment core layers from ±1970 collected around 1990	
	concentr. ng/kg	TEC	concentr. ng/kg	TEC
105	140,000	140	33,600	33.6
126	157	15.7	19	1.9
156	46,000	46	13,600	13.6
169	83	4.2	10	0.5
Total TEC		205.9		48.6

The selective microbial dechlorination of four congeners has resulted in a TEC reduction of 75% in the polluted sediment during 20 years of "environmental incubation" (Table 8.3). The extent of dechlorination exhibited by the enrichment culture from the Rhine sedimentation area is less than the dechlorination extent reported for enrichments from Hudson sediment where dechlorination resulted in an accumulation of mono-, di-, and trichlorinated congeners (Harkness *et al.*, 1993). Nevertheless, the toxicological significance of the rather limited dechlorination capabilities of the microbial population in Rhine

sediments is high.

This study illustrates that even slow transformation reactions are of great importance and should be included in the risk assessment of PCBs in the environment. Unfortunately, these slow processes cannot be recognized in the generally applied inventories. Consequently, the misleading impression that PCBs are highly persistent in sediments simply because they can still be detected dominates the policy development. Research efforts in polluted European sediments should focus on detecting changes in concentrations over long time-scales and changes in PCB composition, i.e. decrease of higher chlorinated and increase of lower chlorinated congeners. This may lead to a reassessment of the fate of PCBs in sediments and allow the development of environmental policies based on half-lives of PCBs instead of PCB century-long persistence.

Complete microbial degradation of PCBs can be achieved in a two-step reaction: anaerobic dechlorination of the higher chlorinated congeners followed by mineralization of the dechlorination products under aerobic conditions (cf. Fig. 9.2, page 144). This sequential biodegradation has been demonstrated for hexachlorobenzene and PCBs in the laboratory (Fathepure and Vogel, 1991; Anid and Vogel, 1991). The first step may take place spontaneously in anaerobic sediment layers and result in an accumulation of lower chlorinated congeners. The occurrence of these reactions in polluted rivers has been studied extensively in the Hudson River (US). Aerobic mineralization of dechlorination products, stimulated in Hudson River sediments, proved to be succesful (Harkness *et al.*, 1993). Recently, evidence for naturally occurring aerobic mineralization of lower chlorinated PCBs has been reported (Flanagan and May, 1993). It was highly likely that the intermediate metabolites (chlorobenzoic acids) detected orginated from PCBs. This illustrates that the two-step microbial elimination of PCBs may occur naturally in polluted rivers.

IMPLICATIONS FOR ENVIRONMENTAL POLICY

The recent developments in the knowledge of the long-term fate of PCBs in aquatic environments may affect the development of environmental policy. Four options will be discussed in detail: 1) prevention of PCB emissions; 2) not dregding PCB contaminated sediments; 3) dredging and *ex situ* treatment; 4) removal and disposal.

1. The fact that PCBs appear to be degradable in the environment should not alter the absolute priority given to the prevention of any PCB emission. Microbial degradation is the only pathway that may eventually eliminate PCBs from the environment, therefore even

slow processes are relevant. Time-scales associated with adverse effects of PCBs, like bioaccumulation and toxic action, are much shorter than the half-lives for microbial decomposition. Consequently, any emission should be prevented. The policy on compounds with regard to production, use, and disposal of the priority pollutant PCB are therefore not affected.

2. On the other hand the policy development on management and restoration of sediments contaminated with PCBs may benefit from the recent insights into the long-term fate of PCBs. *In situ* restoration of sediments that exceed the quality objectives for PCBs may be considered as an alternative approach to dredging. The initial reactions in anaerobic sediment result in a reduced bioaccumulation potential, reduced toxicity and an increased biomineralization potential of PCBs. However, some limitations of anaerobic dechlorination reactions can be mentioned too. Transformation generally does not result in a complete disappearance of the parent compounds; residual concentrations remain unaltered or are transformed at even lower rates (Beurskens *et al.,* 1993b [Chapter 3]). In addition, dechlorination products are less hydrophobic and consequently more mobile. Transport of dechlorination products with infiltrating water may occur, as recently indicated for chlorinated benzenes (Beurskens *et al.,* 1993b [Chapter 3]). A complete elimination of PCBs requires a second, aerobic phase for mineralization of dechlorination products. This may occur spontaneously or can be stimulated by manipulating the conditions in the sediment. The only information available about the realization of the second phase is based on the Hudson. The processes in this phase clearly need more attention in future research efforts.

3. The approach to remove PCB-contaminated sediment and apply *ex situ* treatment in a confined treatment facility has several clear advantages. Firstly, removal of PCBs from the environment diminishes the risks of exposure of aquatic biota and further distribution of PCBs in the environment. Moreover, the conditions in the treatment facility can be optimized for the sequential anaerobic and aerobic steps. The first attempts for an *ex situ* treatment have been recently initiated in the US for PCBs and in the Netherlands for hexachlorobenzene using a similar two-step approach.

4. Looking at the removal of PCB-contaminated sediment and disposal, the most likely large-scale disposal sites in the Netherlands are pits in river and lake beds. In these pits anaerobic conditions will prevail and consequently dechlorination may take place spontaneously. For temporary disposal this will be profitable if combined with a subsequent aerobic phase. For permanent disposal, an accumulation of less chlorinated biphenyls is expected to occur. These compounds will probably persist under the prolonged anaerobic conditions. Since deep pits do not allow an effective alteration in conditions (for example,

aeration) this option will not contribute to an elimination of PCBs from the environment. In the Netherlands one large-scale disposal site for storage of sediments from various locations has been realized and the development of two additional sites is planned. If sediments with organochlorines are combined with sediments that are extremely contaminated with oil or heavy metals in a single disposal site, microbial dehalogenation might be inhibited by the latter classes of contaminants, as recently demonstrated by Morris *et al.* (1993).

At present, option 4 is common practice in the Netherlands. The maintenance of minimum water depths in fairways and harbors, often contaminated with a mixture of priority pollutants, requires solutions that will be available within a few years. Realization of option 2 or 3 is only possible if one important precondition is met: i.e. that chlorinated aromatics are the predominant contaminants, or in other words, that other contaminants like heavy metals and PAHs do not exceed the environmental quality standards. Thus options 2 and 3 are only alternatives for certain locations. Acceptance of these alternatives by decision-makers demands a more detailed characterization of the self-purifying capacity. There is a clear need for quantitative information on these reactions, and their occurrence should be verified in different rivers. Once this information is available, it can be incorporated into mathematical models that generate long-term prognoses and give insight into the consequences. Environmental quality prognoses incorporating reactions that contribute to a natural decontamination of sediments may lead to distinct, probably less costly, sediment policies.

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REFERENCES

Abramowicz, D.A. (1990). Aerobic and anaerobic biodegradation of PCBs: a review. *Crit. Rev. Biotechnol., 10,* 241-251.

Abramowicz, D.A., Brennan, M.J., van Dort, H.M., and Gallager, E.L. (1993). Factors influencing the rate of polychlorinated biphenyl dechlorination in Hudson river sediments. *Environ. Sci. Technol., 27,* 1125-1131.

Ahmed, M. and Focht, D.D. (1973). Degradation of polychlorinated biphenyls by two species of *Achromobacter. Can. J. Microbiol., 19,* 47-52.

Alder, A.C., Haggblom, M.M., Oppenheimer, S.R. and Young, L.Y. (1993). Reductive dechlorination of polychlorinated biphenyls in anaerobic sediments. *Environ. Sci. Technol., 27,* 530-538.

Anid, P.J. and Vogel T.M. (1991). In: Proceedings: *On-site reclamation-Processes for xenobiotic and hydrocarbon treatment*; R.E. Hinchee and R.F. Olfenbuttel (Eds.), Butterworth-Heinemann, Boston, pp. 428-436.

Beurskens, J.E.M., Mol, G.A.J., Barreveld, H.L., van Munster B. and Winkels, H.J. (1993a). Geochronology of priority pollutants in a sedimentation area of the Rhine River. *Environ. Toxicol. Chem., 12,* 1549-1566.

Beurskens, J.E.M., Dekker, C.G.C., Jonkhoff, J. and Pompstra, L. (1993b). Microbial dechlorination of hexachlorobenzene in a sedimentation area of the Rhine River. *Biogeochemistry, 19,* 61-81.

Beurskens, J.E.M., Winkels, H.J., de Wolf, J. and Dekker, C.G.C. (1994a). Trends of priority pollutants in the Rhine during the last 50 years. *Wat. Sci. Technol., 29,* 77-85.

Beurskens, J.E.M., Dekker, C.G.C., van den Heuvel, H., Swart M. de Wolf, J. and Dolfing, J. (1994b). Dechlorination of chlorinated benzenes by an anaerobic microbial consortium that selectively mediates the thermodynamic most favorable reactions. *Environ. Sci. Technol., 28,* 701-706.

Beurskens, J.E.M., de Wolf, J., van den Heuvel, H. and Dolfing, J. (1994c). Microbial dechlorination of polychlorinated biphenyls by a hexachlorobenzene acclimated enrichment culture. *submitted.*

Brown, J.F.Jr., Wagner, R.E., Feng, H., Bedard, D.L., Brennan, M.J., Carnahan, J.C., and May, R.J. (1987). Environmental dechlorination of PCBs. *Environ. Toxicol. Chem., 6,* 579-593.

De Voogt, P., Wells, D.E., Reutergardh, L. and Brinkman, U.A.T. (1990). Biological activity, determination and occurrence of planar, mono- and di-ortho PCBs. *Intern. J. Environ. Anal. Chem., 40,* 1-46.

Fathepure, B.Z. and Vogel, T.M. (1991). Complete degradation of polychlorinated hydrocarbons by a two-stage biofilm reactor. *Appl. Environ. Microbiol., 57,* 3418-3422.

Flanagan, W.P. and May, R.J. (1993). Metabolite detection as evidence for naturally occurring aerobic PCB biodegradation in Hudson River sediments. *Environ. Sci. Technol., 27,* 2207-2212.

Harkness, M.R., McDermott, J.B., Abramowicz, D.A., Salvo, J.J., Flanagan, W.P., Stephens, M.L., Mondello, F.J., May, R.J., Lobos, J.H., Carrol, K.M., Brennan, M.J., Bracco, A.A., Fish, K.M., Warner, G.L, Wilson, P.R., Dietrich, D.K., Lin, D.T., Morgan, C.B. and Gately, W.L. (1993). In situ stimulation of aerobic PCB biodegradation in Hudson River sediments. *Science, 259,* 503-507.

Heymen, R. (1992). Resultaten van het waterkwaliteitsonderzoek in de Rijn in Nederland 1972-1991. RIZA nota nr. 92.047, Lelystad, The Netherlands.

Hong, C.-S., Bush, B., Xiao, J. and Qiao, H. (1993). toxic potential of non-ortho and mono-ortho coplanar polychlorinated biphenyls in Aroclors, seals and humans. *Arch. Environ. Contam. Toxicol., 25,* 118-123.

Jensen, S. (1966). A new chemical hazard. *New Scientist, 32,* 612.

Lake J.L., Pruell, R.J. and Osterman, F.A. (1992). An examination of dechlorination processes and pathways in New Bedford Harbor sediments. *Mar. Environ. Res., 33,* 31-47.

Mohn, W.W. and Tiedje, J.M. (1992). Microbial reductive dehalogenation. *Microbiol. Rev., 56,* 482-507.

Morris, P.J., Quensen J.F. III, Tiedje J.M. and Boyd S.A. (1993). An assessment of the reductive debromination of polybrominated biphenyls in the Pine River Reservoir. *Environ. Sci. Technol., 27,* 1580-1586.

Mullin, M.D., Pochini, C.M., McCrindle, S, Romkes, M., Safe, S.H. and Safe, L.M. (1984). High-resolution PCB analysis: synthesis and chromatographic properties of all 209 PCB congeners. *Environ. Sci. Technol., 18,* 468-476.

Quensen, J.F. III, Boyd, S.A., Tiedje, J.M. (1990). Dechlorination of four commercial polychlorinated biphenyl mixtures (Aroclors) by anaerobic microorganisms from sediments. *Appl. Environ. Microbiol., 56,* 2360-2369.

Rijkswaterstaat (1993a). Saneringsprogramma waterbodem Rijkswateren 1994-2010. Directoraat-Generaal Rijkswaterstaat, Hoofddirectie, The Hague.

Rijkswaterstaat (1993b). Evaluatienota Water, aanvullende beleidsmaatregelen en financiering 1994-1998. Ministerie van Verkeer en Waterstaat, The Hague.

Safe, S. (1990). Polychlorinated biphenyls (PCBs), dibenzo-*p*-dioxins (PCDDs), dibenzofurans (PCDFs), and related compounds: environmental and mechanistic considerations wich support the development of toxic equivalency factors (TEFs). *Crit. Rev. Toxicol., 21*, 51-88.

VROM (1993). Beleidsstandpunt verwijdering baggerspecie. Tweede Kamer der Staten-Generaal, 23450, Nr 1., VROM, The Hague.

CHAPTER 9

GENERAL DISCUSSION

Determining *in situ* microbial dehalogenation

The demonstration and characterization of naturally occurring microbial reactions that alter pollution levels in sediment has gained relatively little attention. This may be explained by the fact that the determination of *in situ* biodegradation is plagued by major methodological limitations. In order to demonstrate *in situ* biodegradation, information should include (Madsen, 1991): a) laboratory studies that demonstrate the biodegradation potential of the native microbial population; b) field studies that show concentration profiles at the site suggesting contaminant losses which exceed those expected for abiotic processes and changes in reactants or products indicative for microbial metabolism; and c) an unequivocal distinction between biotic and abiotic processes.

Nevertheless, there is an increasing need for information on *in situ* processes for the assessment of the impact of pollution episodes on human health and ecosystems. The simulation of environmental processes, which is an important tool in impact assessment and in environmental policy development (RIVM, 1992), demands quantitative information of the relevant processes. Mathematical models generate long-term environmental quality prognoses over several decades to centuries (Mol and Benoist, 1994; Minderhoud and Angeletti, 1989). Within these time scales even slow *in situ* reactions are significant and should be included. Transformation kinetics are generally described as pseudo first-order reactions in models that simulate the behaviour and fate of pollutants in the environment (Schwarzenbach et al., 1993):

$$d[S] / dt = -k [S] \qquad (1)$$

The rate of transformation (d[S]/dt) is assumed to be solely proportional to its actual concentration ([S]), k is referred to as the first-order rate constant. The prefix pseudo indicates that in reality more variables may govern the reaction rate, for example the number of actively involved microorganisms, but these variables are assumed to remain

constant. The half-life, $t_{1/2}$, of a compound is independent of concentration and is deduced from equation 1:

$$t_{1/2} = (\ln 2) / k \qquad (2)$$

Whether this is the most appropiate way to describe the kinetics of microbially catalyzed dehalogenation reactions in the environment may be subject to discussion, but information for estimating additional parameters is lacking in the scarce studies that describe *in situ* reactions. In fact, even the estimation of first-order rate constants is frequently hampered. These estimations are often based on two time points; comparison of concentrations in the past with present concentrations. The resulting rate "constants" are minimum values. Reactions completed in a shorter time interval are not recognized in such studies, therefore the calculated rate constants may underestimate the realistic rates. Consequently, the estimated half-lives are maximum values.

The use of pseudo first-order rate constants may be outlandish to microbiologists who are more used to Monod and Michaelis-Menten type kinetics, but the approach certainly has its merits in environmental microbiology. In a recent study Peijnenburg et al. (1992), for example, showed that after adaptation periods of various lengths the dechlorination rates of a wide range of halogenated aromatic compounds could be described adequately by first-order rate constants. Furthermore it should be kept in mind that at concentrations that are well below the half saturation constants of the enzymatic machinery of the cells, degradation rates also are pseudo first-order.

In situ removal rates of aryl halides

The demonstration of *in situ* dehalogenation has gained little attention yet. To characterize this process quantitatively it is worthwhile to include also other halogenated aromatics than those that are primarily considered in this thesis.

The influence of environmental factors on the dechlorination rates of dichlorophenols (DCPs) to monochlorophenols in pond sediment was studied by Hale et al. (1991) by repititive field sampling. Half-lives of the various DCPs varied between 6 and 215 days. Sediment pH, redox potential, and concentration of sulfate and nitrate accounted for 83% of the variation in half-lives for 2,5- and 3,4-DCP. The number of 2,4-DCP-dechlorinating microorganisms present in sediments at the time of substrate addition was not correlated with the half-lives of this isomer. These results indicate that environmental factors may

influence DCP dechlorination to a greater extent than chlorine substitution pattern on the aromatic ring or densities of the microbial population.

Hexabromobenzene (HBB) and lower brominated benzenes were detected in river sediments in Japan (Watanabe et al., 1986). It was suggested by these authors that the lower brominated benzenes were the decomposition products of HBB in the sediments, although the actual mechanism, biotic or abiotic debromination, was not verified.

A comparison of recent and historic sediment core samples indicated a disappearance of hexachlorobenzene (HCB) in anaerobic Lake Ketelmeer sediment and an increase of tri- and dichlorobenzenes (Beurskens et al., 1993a [Chapter 3]). The maximum half-life for HCB disappearance in lake sediment was estimated to be 7 years. Sediment collected from this area and incubated with freshly spiked HCB demonstrated that the microbial population in this sediment dechlorinated the spiked HCB with a half-life of 11 weeks (Beurskens et al., 1993a). Fathepure et al. (1988) reported a similar half-life for HCB dechlorination on sewage sludge incubations in the laboratory. In another sedimentation area of the Rhine River, Hollands Diep, sediment core data were related to long-term trends of HCB concentrations in the water column; mathematical modeling of sedimentation and transformation processes suggested a half-life for HCB of two years in the anaerobic sediment (Zwolsman, 1993).

Recently, a limited *in situ* debromination of polybrominated biphenyls (PBBs) was demonstrated by Morris et al. (1993) by combining field observations with laboratory incubations. In Pine River sediment an average concentration of approximately 40 mg/kg of PBBs was found. The data indicated a 10% loss of parent compounds during 16 years of "environmental incubation", maximum half-lives of 100 years for the penta-, hexa-, and heptabromobiphenyls were estimated from these results.

Four field studies provide information that enable the calculation of half-lives for individual PCB congeners (Table 9.1). Congener distribution patterns for PCBs in sediments of the highly polluted Hudson River (Brown et al. 1987a, 1987b) and Acushnet Estuary (Brown & Wagner, 1990) suggested that reductive dechlorination of PCBs was a microbial process in these sediments. For the Hudson River this was confirmed in the laboratory when microorganisms eluted from PCB-contaminated Hudson River sediments were shown to dechlorinate commercial PCB mixtures (Quensen et al., 1990). The observations made in the extensive field study in Acushnet Estuary strongly suggest that here too anaerobic microbial populations were involved in changing the congener composition of PCBs in sediments, but this has not been verified in laboratory experiments. For two other locations, New Bedford Harbour and Lake Ketelmeer, laboratory experiments did confirm the role of native anaerobic microorganisms in PCB dechlorination (Alder et al, 1993; Chapter 5).

Table 9.1. Estimated half-lives (years) for PCB congeners in sediments from several sites.

IUPAC no	Structure	Hudson River[1]	Acushnet Estuary[2]	New Bedford Harbor[3]	Lake Ketelmeer[4]
6	2,3'	>150*			
8	2,4'	>150*			
13	3,4'	15*			
16	2,2',3		<20		
17	2,2',4	<15*			
18	2,2',5	<15*			
20	2,3,3'		8		
22	2,3,4'		8		
28	2,4,4'	10			
31	2,4',5	10		13, 465	
33	2',3,4	3	7		
35	3,3',4		7		
37	3,4,4'	3	7		
42	2,2',3,4'	4	10, >40		
44	2,2',3,5'	4	10, >40		
45	2,2'3,6	21	20, >200		
46	2,2',3,6'	15	>200		
47	2,2',4,4'	150*			
49	2,2'4,5'	150*			
52	2,2',5,5'	150*			
57	2,3,3',5		7, 20		
60	2,3,4,4'	3			
63	2,3,4',5		7, 20		
66	2,3',4,4'	3	10		
67	2,3',4,5		10		
70	2,3',4',5	3	10		
71	2,3',4'6	15	>200		
74	2,4,4',5	3	10		
82	2,2',3,3',4	6			
84	2,2'3,3',6	50			
85	2,2',3,4,4'	8	6		
87	2,2',3,4,5'	8	6		
91	2,2',3,4',6	30	>200		
95	2,2',3,5',6	30	>200		
96	2,2',3,6,6'		>200		
97	2,2',3',4,5	8			
99	2,2',4,4',5	10	20		
101	2,2',4,5,5'	10	20		
105	2,3,3',4,4'	4		4, 8	9
110	2,3,3',4',6	15	>200		
114	2,3,4,4',5	4			
118	2,3',4,4',5			7	

IUPAC no	Structure	Hudson River[1]	Acushnet Estuary[2]	New Bedford Harbor[3]	Lake Ketelmeer[4]
123	2',3,4,4',5		10		
124	2',3,4,5,5'		10		
126	3,3',4,4',5				6
128	2,2',3,3',4,4'	10			
133	2,2',3,3',4,6'		10		
135	2,2',3,3',5,6'		>200		
136	2,2',3,3',6,6'		>200		
137	2,2',3,4,4',5	8	7		
138	2,2',3,4,4',5'	10			
139	2,2',3,4,4',6		7		
141	2,2',3,4,5,5'	8	7		
144	2,2',3,4,5',6		7		
149	2,2',3,4',5',6	>30	>200		
153	2,2',4,4',5,5'	10		19	
156	2,3,3',4,4',5				10
162	2,3,3',4',5,5'		20		
163	2,3,3',4',5,6		>200		
167	2,3',4,4',5,5'		20		
169	3,3',4,4',5,5'				6
170	2,2',3,3',4,4',5	21	10		
172	2,2',3,3',4,5,5'		10		
174	2,2',3,3',4,5,6'		10		
175	2,2',3,3',4,5',6		7		
176	2,2',3,3',4,6,6'		10		
177	2,2',3,3',4',5,6		10		
180	2,2',3,4,4',5,5'	21	10		
181	2,2',3,4,4',5,6	15	10		
183	2,2',3,4,4',5',6		7		
185	2,2',3,4,5,5',6	15	10		

*Dechlorination half-lives may be underestimated by simultaneous formation of these congeners via dechlorination of higher congeners.
[1]Half-lives based on data from Brown et al., 1987.
[2]Half-lives based on data from Brown and Wagner, 1990, two half-lives for a single congener refer to different dechlorination patterns.
[3]From Lake et al., 1992; two half-lives for a single congener refer to two separate locations.
[4]From Beurskens et al., 1993a.

PCB half-lives for individual congeners range from a few to more than 100 years; there is no clear relationship between the observed half-lives and the chlorination level of the congeners (Table 9.1). PCB dechlorination rates can differ considerably between sites. For example, the half-life for PCB 71 was 15 years in Hudson River sediment versus more than 200 years in Acushnet Estuary sediment. For certain other PCB congeners, on the other

hand, half-lives were very similar at different sites (e.g. PCBs 85, 105, 137).
In situ microbial dechlorination of PCDDs and PCDFs has not been demonstrated unequivocally yet. The ability of microorganisms from sediment to dechlorinate PCDDs is demonstrated in laboratory studies (Beurskens et al., 1995 [Chapter 6]; Adriaens and Grbic-Galic, 1991). Extrapolation of laboratory results to field conditions is complicated since factors like low bioavailability and competition with other chlorinated compounds may limit the microbial PCDD dechlorination in the environment. Verification of the long-term fate of PCDDs in sediments indicated very slow disappearance processes, if it took place at all, in polluted sediments (Beurskens et al., 1993b, chapter 2; Bopp et al., 1991).
In general, the available information indicates that *in situ* microbial dehalogenation of CBs, PBBs, and PCBs is a relatively slow process that proceeds with half-lives of at least several years. On the other hand, DCPs seem to be dechlorinated in the environment with half-lives less than a year. The low reaction rates in the environment may be related to the low aqueous solubilities of these compounds. The higher chlorinated aromatic compounds reach the anaerobic sediment layers due to their hydrophobic properties and resistence to aerobic transformation reactions. The time interval between the moment of emission into a waterway and the entry into the anaerobic sediment layers may encompass several months or even years. During this period the initially surficial adsorption of a pollutant to particulate matter may change into absorption or, in other words, incorporation of the pollutant in the organic matrix of sediment particles. Consequently, the rate at which the pollutant can transfer from the deeper, anaerobic sediment layers, through (pore) water, to another phase (e.g. microorganisms) may limit the biological transformation rate (Bosma, 1994). Recently, Allard et al. (1994) showed that endogenous chlorocatechols in contaminated sediment were not accessible to microorganisms with dechlorinating activity, demonstrating the important role that bioavailability plays in contaminated sediment.

Microbial dehalogenation in marine sediments

Clear distinctions exist between the heterotrophic bacteria of freshwater and marine sediments. These distinctions are a result of differences in ionic composition and sources and types of sedimenting organic material of marine and freshwater environments. Little information exists on the microbial dehalogenation in marine environments. Dehalogenation of brominated and chlorinated phenols in marine sediments was described by King (1988). Dehalogenation with the consequent production of phenol appeared to initiate anaerobic degradation of 2,4-dibromophenol (DBP). Sulfate-reducing bacteria did not dehalogenate

DBP but appeared to degrade phenol or end products of phenol fermentation. Recently, chlorophenol dechlorination and subsequent substrate oxidation in marine sediment was shown to be coupled to sulfate reduction (Häggblom & Young, 1990). Microbial dechlorination of hexachlorobenzene under sulfate reducing conditions (20 mM sulfate) by microorganisms from estuarine sediments is described Chapter 7. Brown and Wagner (1990) describe the dechlorination in an estuarine sediment but unfortunately detailed information about salinity and sulfate concentrations in the sediment are lacking. Under sulfate reducing conditions PCB dechlorinating activity was demonstrated in sediment that originates from an estuarine area, New Bedford Harbor, however, two freshwater sediments showed no PCB dechlorination when incubated under sulfate reducing conditions (Alder et al., 1993). The influence of sulfate on dehalogenation reactions has been studied frequently and gave contradictory results. Several reports conclude that reductive dehalogenation by anaerobic microbial communities is inhibited by sulfate (Kuhn et al., 1990; Genthner et al., 1989; Allard et al., 1992; Mohn and Tiedje, 1992). However, exceptions to the above findings exist (Bosma et al., 1988; Kohring et al., 1989; Häggblom and Young, 1990). The inhibitory effects were observed with freshwater sediments or inocula obtained from freshwater sediments that generally are methanogenic. Introduction of high sulfate concentrations in these incubations means a drastic change in environmental conditions. One of the changes will involve the concentrations, the nature, and the flux of the reducing equivalents which consequently may become less available to the dechlorinating organisms. Adding sulfate to methanogenic sediments may give insight into the diversity of the dehalogenating microbial population. Unfortunately these experiments have lead to the impression that microbial dehalogenation in marine environments might be rare. In fact, the demonstration of microbial dehalogenation in marine sediments requires different research efforts, viz. incubations with originally sulfidogenic, marine sediments. Additional subjects like the influence of salinity on microbial dehalogenation can than be addressed too. Currently, only a few studies with marine or estuarine sediments are available, this type of research clearly needs more attention in future.

Selectivity and extent of dechlorination

Microbial dechlorination of chlorinated phenols and benzoates may result in a complete removal of chlorines from these molecules (Gibson & Suflita, 1986; Suflita et al., 1982). Removal of the chlorine from monochlorinated benzene or monochlorinated biphenyl has not been reported yet. Microbial dechlorination seems to be restricted to benzenes and

biphenyls that contain at least two chlorines. These higher chlorinated compounds differ considerably in reactivity, even when only small structural differences exist. For example, if all trichlorinated benzenes are incubated simultanously, the 1,3,5-trichlorobenzene appeared to be the least reactive isomer (Bosma, 1988). Moreover, certain higher chlorinated compounds may resist dechlorination at a given location (Quensen et al., 1990). Studies in the US indicate that the extent of *in situ* PCB dechlorination, i.e. the number of successive chlorine removals, varies considerably and appears to be location specific (Brown et al., 1987b; Quensen et al., 1990; Brown and Wagner, 1990; Quensen, 1992). The dechlorination patterns can be reproduced by eluting the microorganisms from each sediment and culturing them in the presence of PCBs and an originally unpolluted sediment. For example, dechlorination by Hudson River organisms resulted in the accumulation of mainly mono- and di- ortho substituted congeners (Quensen et al., 1990; Quensen, 1992). Organisms from the Acushnet River sediment, on the other hand, accumulated mostly trichlorinated biphenyls (Mohn and Kennedy, 1992). The dechlorination by a consortium from Rhine sediments (Chapter 5) resembles the dechlorination pattern that also dominates in the Acushnet Estuary (Brown and Wagner, 1990). Factors that may influence the microbial capabilities and consequently limit the extent of dechlorination at a particular site are divers and include, for example, PCB pollution history, redox potentials as well as co-contaminants. For example, the limited *in situ* debromination of the commercial PBB mixture in Pine River sediment was attributed to inhibitory effects of organic co-contaminants, petroleum products and heavy metals present in the sediment (Morris et al., 1993). Thus, it should be appreciated that the extent of microbial dehalogenation in polluted sediments may exhibit a compound and location specific character.

Residual levels

Dehalogenation studies in the laboratory are mostly conducted with sediments that originally were unpolluted and the studied compound is freshly added to series of batches. Generally, the added amount of a halogenated compound disappears to levels below the detection limit due to microbial metabolism. However, a complete disappearance of reactive compounds is generally not observed in anaerobic environments; residual concentrations remain unaltered or are transformed at much lower rates (Beurskens et al., 1993a). If field sediments with residual levels are brought into the laboratory and spiked with chlorinated aromatics, dechlorination is generally observed. However, no or only limited additional

dechlorination of the preexisting, residual levels can be demonstrated (Beurskens et al., 1993a [Chapter 3]; Alder et al., 1992). These results suggest that a limited availability of the residual levels prevent the continuation of the dechlorination process. Attempts to enhance the availability of chlorinated aromatics with surfactants are reported in the literature (see below). One may question whether residual levels, that are not available for microorganisms, still pose toxic risks for other organisms.

Toxicity of haloaromatics for anaerobic microbial activity

Data on the toxicity of chlorinated organic compounds towards anaerobic microorganisms are scarce (Sierra-Alvarez and Lettinga, 1991; van Beelen et al., 1991; Davies-Venn et al., 1992; Madsen and Aamand, 1992; Renard et al., 1993). Sierra-Alvarez and Lettinga (1991) have shown that for homologous series of chlorinated benzenes and phenols a positive correlation exists between the hydrophobicity of these compounds and their inhibitory effect on the methanogenic degradation of acetate. Such a relationship is plausible when factors such as partitioning and transport control the toxic effects of the compound. In such cases, when reductive dechlorination reduces the hydrophobicity, it also results in a decrease of the toxicity of chlorinated compounds, at least in theory. Whether such a reduction also occurs in practice remains to be seen: less chlorinated dechlorination products sorp less strongly to hydrophobic materials present in the natural environment, so it is conceivable that the effect of dechlorination is that the actual concentration of toxicants in the aqueous phase increases.
From the data that are available, some indication of the toxicity can be obtained but the question immediately rises what the ecological meaning of this toxicity is. It is well known that microbial ecosystems can adapt to stress in various ways, and chlorinated compounds are merely another source of stress. The most elegant way in which microbial ecosystems can deal with the stress exerted by halogenated compounds is by simply eliminating these compounds, i.e. by developing the appropriate enzymatic machinery to dehalogenate the stress factor.

Consequences of dehalogenation in the environment

The lower chlorinated compounds formed during microbial dehalogenation are less bioaccumulative, generally less toxic and more suitable substrates for aerobic

biomineralization. These beneficial consequences of microbial dehalogenation are generally emphasized in the literature. Formation of lower chlorinated compounds means however also an increase in water solubility relative to the water solubility of the parent compound. Consequently, reductive dehalogenation in sediments may result in the formation of mobile metabolites. At many sites in Western Europe river water infiltrates through sediments into aquifers and transport of mobile metabolites may have adverse effects on ground water quality (Zoeteman et al., 1980; McCarty et al., 1981; Schwarzenbach et al., 1983; Heij, 1989). Transport of di- and trichlorobenzenes from polluted sediments to deeper, originally unpolluted, soil layers was demonstrated by Beurskens et al. (1993a [Chapter 3]). Although the transported isomers were probably not related to the dechlorination of HCB, they demonstrate the potential mobility of lower chlorinated benzenes. Long-distance transport of dichlorobenzenes over 3.5 km in ground water illustrates the mobility of these compounds when present in groundwater (Barber et al., 1988). The rate of downward seepage of lower chlorinated compounds is determined by the sediment-water partition coefficient besides site-specific conditions like water infiltration rates and organic carbon content of the sediment. The octanol-water partition coefficient ($K_{o/w}$) can be used as an indication for the sediment-water partition coefficient and the related mobility (Schwarzenbach et al., 1993). The plot of log $K_{o/w}$ versus the number of chlorines in chlorinated benzenes, biphenyls and dioxins, as shown in Figure 9.1, illustrates that the potential mobility of chlorinated compounds increases with decreasing chlorination levels. From Fig. 9.1 it is clear that microbial dechlorination of PCBs and PCDDs results in metabolites that are more hydrophobic than di- and trichlorobenzenes (log $K_{o/w} < 4$) which proved to be mobile within a time scale of several decades. Winters and Lee (1987) determined the *in situ* mobility of monochlorobenzene and monochlorobiphenyl with field columns. As expected, monochlorobenzene was mobile and monochlorobiphenyl was immobile. It can be concluded that dechlorination of PCBs and chlorinated dioxins will not result in the formation of metabolites that are transported easily in the environment.

Di-and trichlorobenzenes are not necessarily persistent in anaerobic groundwater, as suggested by Barber et al. (1988). Bosma et al. (1988) have described the dechlorination of these compounds to monochlorobenzene in studies with methanogenic Rhine sediment columns. Biomineralization of dichlorobenzenes and monochlorobenzene may occur if, after long-distance transport, aerobic or denitrifying conditions are reached (Bosma, 1994). In this way, the sequential redox conditions that mobile contaminants encounter may eventually result in complete elimination of toxicants (Zitomer and Speece, 1993).

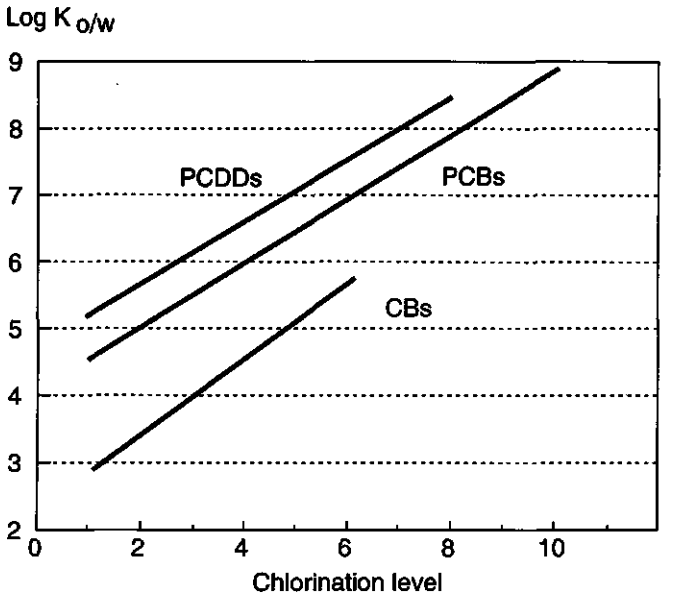


Fig. 9.1. The relationship between the average octanol-water partitioning coefficient($K_{o/w}$) and the number of chlorines present in chlorinated benzenes (CBs), polychlorinated biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins (PCDDs). Log K_{ow} values from de Bruijn et al. (1989) and Shiu et al. (1988).

Thermodynamics and the predictability of dechlorination reactions

The observation that reductive dechlorination of polychlorinated compounds is a sequential process and results in the development of distinct dechlorination patterns gives rise to the question whether these patterns can be rationalized. The redox potentials, or Gibbs free-energies, of the various redox couples may be useful to explain and predict microbial dehalogenation patterns in anaerobic environments.

Estimates for the Gibbs free-energies of formation ($\Delta G_f°$) of chlorinated aromatics were recently reported (Dolfing and Harrison, 1992; Holmes et al., 1993) and enable the calculation of energy releases in the individual dechlorination steps under standard conditions (see also chapter 5). If the complete dechlorination pathway of a compound is considered up to the halogen free molecule, no differences in the total yield of Gibbs free-energies exist, regardless of the pathway followed. This means that thermodynamics can only be applied for predicting pathways if microbial dehalogenation is a stepwise process that involves the removal of single halogens at a time. On the basis of the available data this precondition is met for the dehalogenation of halogenated aromatics. The potential energy present in halogenated compounds can be gained in dehalogenation reactions;

catalyzing reactions with the highest yield in Gibbs free-energy would be most profitable to microorganisms. The ability of microorganisms to conserve energy from a dehalogenation reaction has been demonstrated for *Desulfomonile tiedjei* (Dolfing and Tiedje, 1987; Dolfing, 1990). The hypothesis is therefore that the microbially catalyzed dehalogenation pathway will follow the redox potential of the various redox couples, i.e. that the couples with the highest energy yield will be used preferentially.

The number and position of chlorines on the aromatic ring determine the energy level of a compound. In general, removal of a chlorine from a highly chlorinated compound yields more energy than chlorine removal from a lower chlorinated compound (Dolfing and Harrison, 1992). Within a chlorination level, the position of chlorines on the ring result in differences in energy yield if a chlorine is removed. Removal of a chlorine flanked by two other chlorines will release more energy than removal of a chlorine with no or only one neighbouring chlorine. Thus, a chlorine flanked on both sides by chlorines is a better leaving group than an "isolated" chlorine. The electron withdrawing property of chlorine atoms influences the electron distribution in the aromatic nucleus during the two electron additions that are involved in the reductive dechlorination reaction. Stabilization of the negative charge after the first electron addition appears to be better if multiple chlorines are present and located in close proximity.

The differences in energy yield between the various dechlorination steps that involve the removal of a single chlorine from, for example, 2,3,4,3',4'-pentachlorobiphenyl (PCB 105) are relatively small (Table 9.2). The idea that these small differences can govern dehalogenation pathways is not self-evident. Moreover, the application of thermodynamics to dehalogenation reactions in the environment is complicated by the fact that environmental conditions deviate from the standard conditions. Substrates and products are at much lower concentrations in the environment than the assumed 1 mol/kg under standard conditions. Concentrations can vary from site to site and from moment to moment and affect the actual yield in Gibbs free-energy.

Nevertheless, with the aid of thermodynamics certain systematics in the dechlorination pathways of hexachlorobenzene were observed and the pathway could be rationalized (Dolfing and Harrison, 1993; Beurskens et al., 1994, [Chapter 4]). It should be noted that these observations are based on only a few dechlorination steps with a few enrichments cultures, so there is clearly a need for more experimental data.

The apparent applicability of the idea that thermodynamics can be used to rationalize the dechlorination pattern of chlorinated benzenes makes it tempting to apply this concept also to the dechlorination pathways of PCBs. The paucity of single congener studies makes this however a somewhat risky undertaking. The search for patterns in the dechlorination

Table 9.2. Gibbs free-energy values for the reductive dechlorination ($\Delta G^{o'}$) of PCBs with hydrogen as electron donor listed along with: the spatial position of the substituted chlorine (o: ortho; m=meta; p=para), the number of adjacent chlorines, and the presence (+) or absence (-) of the reaction as reported in the literature.

Substrate		Product		$\Delta G^{o'}$ kJ/reaction	Pos.	Adj. Cl	Dechlor.
no.	structure	no.	structure				
Abramowicz et al., 1993:							
105	2,3,4,3',4'	77	3,4,3',4'	-164.1	o	1	-
		66	2,4,3',4'	-162.3	m	2	+
		56	2,3,3',4'	-154.0	p	1	-
		55	2,3,4,3'	-152.9	p	1	-
		60	2,3,4,4'	-152.1	m	1	-
66	2,4,3',4'	37	3,4,4'	-156.0	o	1	-
		25	2,4,3'	-152.5	p	1	+
		28	2,4,4'	-151.1	m	1	-
		33	2',3,4	-146.4	p	0	-
25	2,4,3'	13	3,4'	-157.3	o	0	-
		6	2,3'	-147.7	p	0	+
		7	2,4	-145.0	m	0	-
6	2,3'	2	3	-154.9	o	0	-
		1	2	-144.3	m	0	+
Rhee et al., 1993:							
21	2,3,4	12	3,4	-167.6	o	1	-
		7	2,4	-162.1	m	2	+
		5	2,3	-155.8	p	1	-
29	2,4,5	12	3,4	-159.3	o	0	-
		9	2,5	-153.8	p	1	+
		7	2,4	-153.8	m	1	+
Williams, 1994:							
21	2,3,4	12	3,4	-167.6	o	1	-
		7	2,4	-162.1	m	2	+
		5	2,3	-155.8	p	1	-
23	2,3,5	14	3,5	-164.1	o	1	-
		9	2,5	-153.9	m	1	+
		5	2,3	-147.6	p	0	-
24	2,3,6	9	2,5	-156.3	o	1	-
		10	2,6	-152.3	m	1	+
		5	2,3	-150.0	o	0	-
29	2,4,5	12	3,4	-159.3	o	0	-
		9	2,5	-153.8	p	1	+
		7	2,4	-153.8	m	1	-
30	2,4,6	7	2,4	-151.7	o	0	-
		10	2,6	-147.7	p	0	+
38	3,4,5	14	3,5	-163.1	p	2	+
		12	3,4	-158.4	m	1	-

pathway started as soon as the first reports on dechlorination of PCBs in the environment were published. Brown et al. (1987a,b) described a series of patterns, but there was no obvious way to rationalize these patterns. The only generalisation that could be made at that time was that ortho dechlorination was seldom observed, and that dechlorination of higher chlorinated congeners appeared easier than dechlorination of lower chlorinated PCBs (Quensen et al., 1988). The observation that ortho dechlorination is rare has been made by several groups. In fact the observation of biologically mediated ortho dechlorination was reported only recently (Van Dort and Bedard, 1991; Williams, 1994) in sediment incubations that orginate form the same site. If we accept that ortho dechlorination does not generally occur, it is worthwhile to verify the relation between thermodynamics and PCB dechlorination pathways observed in single congener studies that have been reported so far (Abramowicz et al., 1993; Rhee et al., 1993; Williams, 1994).

Abramowicz et al. (1993) recently reported that PCB 105 was dechlorinated via a selective removal of all the meta and para chlorines in Hudson River sediment incubations. Identified products were PCB 66, 25, 6 and the monochlorobiphenyl PCB 1 (Abramowicz et al., 1993). Indeed all para and meta chlorines were removed, and the ortho chlorine proved to be resistent. However, removal of the ortho chlorine would have yielded the highest energy of the dechlorination steps possible at the different chlorination levels (Table 9.2). Based on the energy release under standard conditions, and taking into account the resistence of ortho chlorines, the exact dechlorination pathway can be predicted by the selection of those dechlorination steps that have the highest energy release under standard conditions (Table 9.2). The resistence of ortho chlorines indicates that other factors than thermodynamics are also involved in determining which dechlorination pathway is followed in the environment. Steric hindrance could be one of those factors. Brown et al. (1987b) have postulated a concept that explains the recalcitrance of ortho chlorines on the basis of steric hindrance at the active site of enzymes.

Recently, Rhee and co-workers have stressed that PCB dechlorination is not only determined by the position of the leaving chlorine, but was influenced by the total chlorination pattern of a congener (Rhee et al., 1993). This statement was based on sediment incubations with an enrichment culture that transformed 2,3,4-trichlorobiphenyl exclusively to 2,4-dichlorobiphenyl (meta dechlorination), while after several transfers in presence of 2,4,5-trichlorobiphenyl only 2,5-dichlorobiphenyl was produced (para dechlorination). When this para dechlorinating culture was subsequently exposed to 2,3,4-trichlorobiphenyl, again only meta dechlorination was observed. This selectivity can completely be predicted with the thermodynamic information and it is evident that the

enrichment culture of Rhee et al. (1993) simply catalyzed the thermodynamic most profitable reactions (Table 9.2). Thus, with the aid of thermodynamics, the observation of Rhee becomes explicable.

Williams (1994) incubated six trichlorobiphenyls in slurries with Hudson River sediment in an attempt to understand how the number and arrangement of chlorines on a phenyl ring affect PCB dechlorination. Combination of his observations with the free energy values for the various dechlorination steps (Table 9.2) makes clear that these data also support the hypothesis that the reactions with the highest energy release are preferably catalyzed, excluding ortho chlorines.

The cultures that were used in the aforementioned dechlorination studies were taken directly from the environment. In a cross acclimation study, a hexachlorobenzene adapted enrichment was incubated with individual PCB congeners, dechlorination patterns were in agreement with thermodynamics too (Chapter 5).

The available PCB dechlorination data indicate that dechlorination pathways can be rationalized with the aid of thermodynamics. Taking into account the fact that ortho dechlorination generally does not occur, thermodynamics appear to provide a framework that can be used in predicting PCB dechlorination pathways.

Prospects for the treatment of chlorinated aromatics in sediments

Complete microbial degradation of halogenated aromatics can be achieved in a two-step reaction; anaerobic dechlorination of the higher chlorinated isomers and mineralization of the dechlorination products under aerobic conditions (Fig. 9.2) (Fathepure and Vogel, 1991; Anid and Vogel, 1991). The first step may take place spontaneously in the deeper anaerobic sediment layers and result in an accumulation of di- and trichlorinated isomers, like for PCBs in the Hudson (Harkness et al., 1993). Biological clean up of polluted sediments demands detailed information with regard to: a) capabilities of the native microbial population; b) influence of environmental conditions on the specific reactions, e.g. temperature, pH, nutrients, carbon sources, other pollutants present, etc.; c) the availability of the chlorinated aromatics for the microbial population. If one of these factors causes a serious limitation for a successful biological decontamination, specific manipulations may be helpful. Complete degradation of HCB, PCBs and 2,3,6-trichlorobenzoic acid in a two-step reaction has been demonstrated on laboratory scale (Fathepure and Vogel, 1991; Anid and Vogel, 1991; Gerritse and Gottschal, 1992), full-scale application will be complex and generally time consuming but not unrealistic, as recently demonstrated by Harkness et al.

(1993).

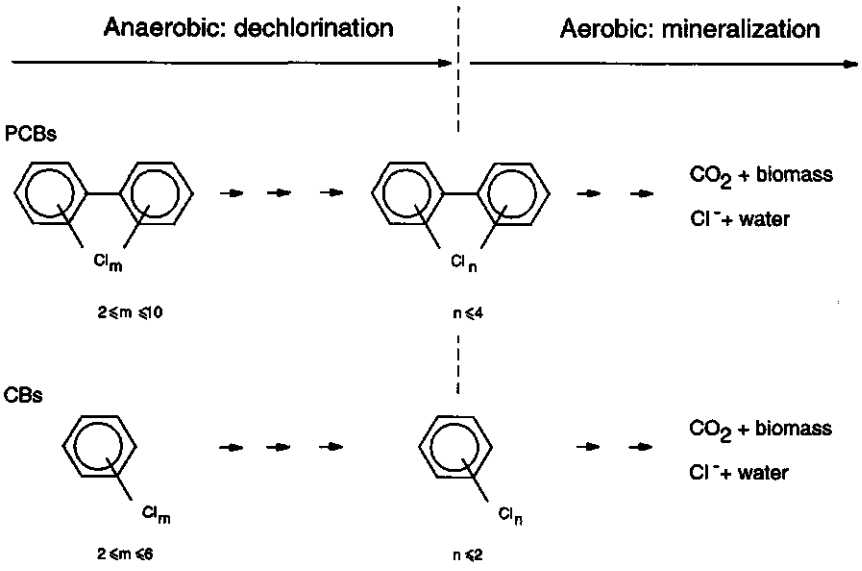


Fig 9.2. Two-step reaction scheme to eliminate biologically chlorinated benzenes and biphenyls from sediment.

For the first step, anaerobic dehalogenation, several attempts to stimulate its occurrence, that might take place *in situ,* have been reported in literature, sometimes with contradictory results. The addition of organic substrates to stimulate the overall anaerobic microbial activity is a frequently applied concept. The addition of fatty acids enhanced PCB dechlorinating activity in Hudson River sediment, but did not stimulate the PCB dechlorination in the New Bedford Harbor and Silver Lake sediments (Alder et al., 1993). Addition of butyrate to aquifer slurries capable to dehalogenate chlorinated anilines resulted in an additional dechlorination pathway besides stimulation of the overall reaction rate (Kuhn et al., 1990). Addition of various nutrients resulted in only minor rate accelerations of PCB dechlorination in Hudson sediment (Abramowicz et al., 1993). The addition of halogenated analogs in order to stimulate the dehalogenation reaction is reported only once. In laboratory experiments 2,6-dibromobiphenyl (2,6-DBB) was shown to stimulate the dechlorination of Aroclor 1260 (Bedard et al., 1992). 2,6-DBB was readily dehalogenated to biphenyl by the native microbial activity in pond sediment, however application in a field

trial did not result in the expected dichlorobiphenyl formation (Bedard, personal communication 1993).

Stimulation of the second step, aerobic mineralization, has been described as an *in situ* treatment for PCBs only once (Harkness et al., 1993). The PCBs produced by *in situ* dechlorination, were shown to undergo aerobic degradation in a large-scale field study. All other reports that deal with stimulation of the aerobic degradation of chlorinated aromatics are *ex situ* or laboratory studies. Hill et al. (1989) demonstrated that the aerobic mineralization of 4-chlorobiphenyl in lysimeters was little influenced by inoculation of a known 4-chlorobiphenyl-degrader. Treatments with water, minerals and yeast extract stimulated resident microorganisms in noninoculated lysimeters that degraded 4-chlorobiphenyl as efficiently as inoculated lysimeters.

Hydrophobic chlorinated aromatics sorb onto sediments. Over long contact time, sorbing pollutants slowly diffuse into the organic and inorganic matrix. Only a small fraction of the compound is present in the water phase. Depletion of this fraction by microbial metabolism induces a release of compounds from the sorbed fraction to the water phase. Due to extreme slow intraparticle diffusion rates this proceeds slowly and may limit the overall biodegradation rate. Intraparticle diffusion and desorption may therefore be the rate-limiting factor, controlling transformation rates of higher chlorinated aromatics in soils or sediments (Bosma, 1994). Surfactants have been shown to enhance desorption and solubilisation of PCBs that are freshly added to soil (Viney and Bewley, 1990; Samson et al., 1990; Abdul et al., 1992). However, the efficacy of surfactants to enhance the release of PCBs that are present for many years in soil or sediment remains doubtful. Stimulation of the intraparticle diffusion by surfactants might be not as easy as stimulation of the desorption of freshly added PCBs. Freshly added PCBs are mainly adsorbed at the outer regions of particles and relatively easy accessible for surfactant micelles. Many other problems still exist with a combined use of surfactants and biological degradation of pollutants. For example, surfactants may inhibit microbial activity (Viney and Bewley, 1990), or the surfactants may be preferentially used as the parent substrate. Other aspects that determine the usefullness of surfactants are the costs and their degradability.

Since the two-step biological treatment is the only natural pathway that eventually may lead to an elimination of higher chlorinated aromatics from the environment, it is worthwhile to be considered, although a two-step approach is complex. Moreover, taking into account the slow release of chlorinated aromatics from sediments, bioremediation will probably take years to decades. Measures to stimulate microbial degradation rates probably have to be repeated for being effective during such time periods. An extensive approach in which contaminated sediments are stored under conditions that support the naturally occurring

processes (anaerobic and aerobic), without applying costly stimulation measures, seems to be a feasible option. This approach claims pieces of land for many years and would be most effective if the "treatment facility" could function as a "natural ecosystem" with alternating anaerobic and aerobic conditions like, for example, in lagoons or frequently inundated river flood-plains. Attention should be given to an adequate prevention of pollutant dispersal from these sites. In this way, confined disposal and extensive treatment by using natural processes may lead to a biological decontamination of sediments in the long term.

References

Abdul, A.S., T.L. Gibson, C.C. Ang, J.C. Smith, R.E. Soczynski, 1992. In situ surfactant washing of polychlorinated biphenyls and oils from a contaminated site. Ground Water, 30, 219-231.

Abramowicz, D.A., M.J. Brennan, H.M. van Dort, E.L. Gallager, 1993. Factors influencing the rate of polychlorinated biphenyl dechlorination in Hudson river sediments. Environ. Sci. Technol., 27, 1125-1131.

Adriaens, P, D. Grbic-Galic, 1991. Anaerobic dehalogenation of highly chlorinated dioxins and benzofurans. Proceedings, Dioxin '91, 11th International Symposium on chlorinated dioxins and related compounds, Research Triagle Park, NC, p. 327.

Alder, A.C., M.M. Haggblom, S.R. Oppenheimer, L.Y. Young, 1993. Reductive dechlorination of polychlorinated biphenyls in anaerobic sediments. Environ. Sci. Technol., 27, 530-538.

Allard, A.-S., P.-A. Hynning, M. Remberger, A.H. Neilson, 1992. Role of sulfate concentration in dechlorination of 3,4,5-trichlorocatechol by stable enrichment cultures grown with coumarin and flavanone glycones and aglycones. Appl. Environ. Microbiol., 58, 961-968.

Allard, A.-S., P.-A. Hynning, M. Remberger, A.H. Neilson, 1994. Bioavailability of chlorocatechols in naturally contaminated sediment samples and of chloroguaiacols covalently bound to C2-guaiacyl residues. Appl. Environ. Microbiol., 60, 777-784.

Anid, P.J., T.M. Vogel, 1991. In: Proceedings: On-site reclamation-Processes for xenobiotic and hydrocarbon treatment; Hinchee, R.E., R.F. Olfenbuttel (Eds.), Butterworth-Heinemann, Boston, pp. 428-436.

Barber, L.B., II, 1988. Dichlorobenzene in groundwater: evidence for long term persistence. Ground Wat., 26, 696-702.

Beelen, P. van, A.K. Fleuren-Kemila, M.P.A. Huys, A.C.P. van Montfort, P.L.A. van Vlaardingen, 1991. The toxic effects of pollutants on the mineralization of acetate in subsoil microcosms. Environ. Toxicol. Chem., 10, 775-789.

Beurskens, J.E.M., C.G.C. Dekker, J. Jonkhoff, L. Pompstra, 1993a. Microbial dechlorination of hexachlorobenzene in a sedimentation area of the Rhine River. Biogeochem. 19, 61-81.

Beurskens, J.E.M., G. Mol, H.L. Barreveld, B. van Munster, H.J. Winkels, 1993b. Geochronology of priority pollutants in a sedimentation area of the Rhine River. Environ. Toxicol. Chem., 12, 1549-1566.

Beurskens, J.E.M., C.G.C. Dekker, H. van den Heuvel, M. Swart, J. de Wolf, J. Dolfing, 1994. Dechlorination of chlorinated benzenes by an anaerobic microbial consortium that selectively mediates the thermodynamic most favorable reactions. Environ. Sci. Technol., 28, 701-706.

Beurskens, J.E.M., M. Toussaint, J. de Wolf, J.M.D. van der Steen, P.C. Slot, L.C.M. Commandeur, J.R. Parsons, 1995. Dehalogenation of chlorinated dioxins by an anaerobic microbial consortium from sediment. Environ. Toxicol. Chem., 14, in press.

Bedard, D.L., H.M. van Dort, S.C. Bunnell, J.M. Principe, K.A. DeWeerd, R.J. May, L.A. Smullen, 1992. Simulation of reductive dechlorination of Aroclor 1260 contaminant in anaerobic slurries of Woods pond sediment. In: Anaerobic dehalogenation and its environmental implications; Abstracts of ASM conference, Athens, Georgia, August 30-September 4, 1992; Rogers, J.E., Abramowicz, D.A., Eds.; US-EPA, Athens, Georgia; pp 19-21.

Bopp, R.F., M.L. Gross, H. Tong, H.J. Simpson, S.J. Monson, B.L. Beck, F.C. Moser, 1991. A major incident of dioxin contamination: sediments of New Jersey estuaries. Environ. Sci. Technol., 25, 951-956.

Bosma, T.N.P., 1994. Simulation of subsurface biotransformation. Thesis Agricultural University Wageningen, The Netherlands.

Bosma, T.N.P., J.R. van der Meer, G. Schraa, M.E. Tros, A.J.B. Zehnder, 1988. Reductive dechlorination of all trichloro- and dichlorobenzene isomers. FEMS Microbiol. Ecol., 53, 223-229.

Brown, J.F., D.L. Bedard, M.J. Brennan, J.C. Carnahan, H. Feng, R.E. Wagner, 1987a. Polychlorinated biphenyl dechlorination in aquatic sediments. Science 236, 709-712.

Brown, J.F., R.E. Wagner, H. Feng, D.L. Bedard, M.J. Brennan, J.C. Carnahan, R.J. May, 1987b. Environmental dechlorination of PCBs. Environ. Toxicol. Chem., 6, 579-593.

Brown, J.F. R.E. Wagner, 1990. PCB movement, dechlorination, and detoxification in the Acushnet Estuary. Environ. Toxicol. Chem., 9, 1215-1233.

Bruijn, J. de, F. Busser, W. Seinen, J. Hermens, 1989. Determination of octanol/water partition coefficients for hydrophobic organic chemicals with the "slow-stirring" method. Environ. toxicol. Chem., 8, 499-512.

Davies-Venn, C., J.C. Young, H.H. Tabak, 1992. Impact of chlorophenols and chloroanilines on the kinetics of acetoclastic methanogenesis. Environ. Sci. Technol., 26, 1627-1635.

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., B.K. Harrison, 1992. Gibbs free energy of formation of halogenated aromatic compounds and their potential role as electron acceptors in anaerobic environments. Environ. Sci. Technol., 26, 2213-2218.

Dolfing, J., B.K. Harrison, 1993. Redox and reduction potentials as parameters to predict the degradation pathway of chlorinated benzenes in anaerobic environments. FEMS Microbiol. Ecol., 13, 23-30.

Dolfing, J., J.M. Tiedje, 1987. Growth yield increase linked to reductive dechlorination in a defined 3-chlorobenzoate degrading methanogenic coculture. Arch. Microbiol., 149, 102-105.

Dort, H.M. van, D.L. Bedard, 1991. Reductive ortho and meta dechlorination of a polychlorinated biphenyl congener by anaerobic microorganisms. Appl. Environ. Microbiol., 57, 1576-1578.

Fathepure, B.Z., J.M. Tiedje, S.A. Boyd, 1988. Reductive dechlorination of hexachlorobenzene to tri- and dichlorobenzenes in anaerobic sewage sludge. Appl. Environ. Microbiol., 54, 327-330.

Fathepure B.Z., T.M. Vogel, 1991. Complete degradation of polychlorinated hydrocarbons by a two-stage biofilm reactor. Appl. Environ. Microbiol., 57, 3418-3422.

Genthner, S.B.R., W.A. Price, P.H. Pritchard. 1989. Characterization of anaerobic dechlorinating consortia derived from aquatic sediments. Appl. Environ. Microbiol., 55, 1466-1471.

Gerritse, J., J.C. Gottschal, 1992. Mineralization of the herbicide 2,3,6-trichlorobenzoic acid by a co-culture of

anaerobic and aerobic bacteria. FEMS Microbiol. Ecol., 101, 89-98.

Gibson, S.A. & J.M. Suflita, 1986. Extrapolation of biodegradation results to groundwater aquifers: reductive dehalogenation of aromatic compounds. Appl. Environ. Microbiol.,52, 681-688.

Häggblom, M.M., L.Y. Young, 1990. Chlorophenol degradation coupled to sulfate reduction. Appl. Environ. Microbiol., 56, 3255-3260.

Hale, D.D., J.E. Rogers, J. Wiegel, 1991. Environmental factors correlated to dichlorophenol dechlorination in anoxic freshwater sediments. Environ. Toxicol. Chem. 10, 1255-1265.

Harkness, M.R., McDermott, J.B., Abramowicz, D.A., Salvo, J.J., Flanagan, W.P., Stephens, M.L., Mondello, F.J., May, R.J., Lobos, J.H., Carrol, K.M., Brennan, M.J., Bracco, A.A., Fish, K.M., Warner, G.L, Wilson, P.R., Dietrich, D.K., Lin, D.T., Morgan, C.B. and Gately, W.L., 1993. In situ stimulation of aerobic PCB biodegradation in Hudson River sediments. Science, 259, 503-507.

Heij, G.J., 1989. River-groundwater relationships in the lower parts of the Netherlands. J. Hydrol., 108, 35-62.

Hill, D.L., T.J. Phelps, A.V. Palumbo, D.C. White, G.W. Strandberg, T.L. Donaldson, 1989. Bioremediation of polychlorinated biphenyls. Degradation capabilities in field lysimeters. Appl. Biochem. Biotechnol., 20/21, 233-243.

Holmes, D.A., B.K. Harrison, J. Dolfing, 1993. Estimation of Gibbs free energies of formation for polychlorinated biphenyls. Environ. Sci. Technol., 27, 725-731.

King, G.M., 1988. Dehalogenation in marine sediments containing natural sources of halophenols. Appl. Environ. Microbiol., 54, 3079-3085.

Kohring, G.-W., X. Zhang, J. Wiegel, 1989. Anaerobic dechlorination of 2,4-dichlorophenol in freshwater sediments in the presence of sulfate. Appl. Environ. Microbiol., 55, 2735-2737.

Kuhn, E.P., G.T. Townsend, J.M. Suflita, 1990. Effect of sulfate and organic carbon supplements on reductive dehalogenation of chloroanilines in anaerobic aquifer slurries. Appl. Environ. Microbiol., 56, 2630-2637.

Lake, J.L., R.J. Pruell, F.A. Osterman, 1992. An examination of dechlorination processes and pathways in New Bedford Harbor sediments. Mar. Environ. Res., 33, 31-47.

Madsen, E.L., 1991. Determining in situ biodegradation, facts and challenges. Environ. Sci. Technol., 25, 1663-1673.

Madsen, T., J. Aamand, 1992. Anaerobic transformation and toxicity of trichlorophenols in a stable enrichment culture. Appl. Environ. Microbiol., 58, 557-561.

McCarty, P.L., M. Reinhard, B.E. Rittmann, 1981. Trace organics in groundwater. Environ. Sci. Technol., 15, 40-51.

Minderhoud, A., G. Angeletti (Eds.), 1989. Incorporation of physico-chemical processes in models describing the environmental fate of chemicals. Proceedings of a workshop of the European Community, 20-21 April 1989, Bilthoven, The Netherlands. Water Pollution Research Report 19.

Mohn, W.W., K.J. Kennedy, 1992. Reductive dehalogenation of chlorophenols by *Desulfomonile tiedjei* DCB-1. Appl. Environ. Microbiol., 58, 1367-1370.

Mohn, W.W., J.M. Tiedje, 1992. Microbial reductive dechlorination. Microbiol. Rev., 56, 482-507.

Mol, G.A.J., A.P. Benoist, 1994. Prediction of sediment quality in catchment areas of the River Rhine. Operation and use of the HORIZON model. Wat. Sci. Technol., 29, 115-120.

Morris, P.J., J.F. Quensen III, J.M. Tiedje, S.A. Boyd, 1993. An assessment of the reductive debromination of

polybrominated biphenyls in the Pine River Reservoir. Environ. Sci. Technol., 27, 1580-1586.

Peijnenburg, W.J.G.M., M.J. 't Hart, H.A. den Hollander, D. van de Meent, H.H. Verboom, N.L. Wolfe, 1992. Reductive transformation of halogenated aromatic hydrocarbons in anaerobic water-sediment systems: kinetics, mechanisms and products. Environ. Toxicol. Chem., 11, 289-300.

Quensen, J.F., 1992. Factors affecting in situ dechlorination and the expected toxicity reduction. In: Anaerobic dehalogenation and its environmental implications; Abstracts of ASM conference, Athens, Georgia, August 30-September 4, 1992; Rogers, J.E., Abramowicz, D.A., Eds.; US-EPA, Athens, Georgia; pp 24-25.

Quensen, J.F. III, S.A. Boyd, J.M. Tiedje, 1990. Dechlorination of four commercial polychlorinated biphenyl mixtures (Aroclors) by anaerobic microorganisms from sediments. Appl. Environ. Microbiol., 56, 2360-2369.

Renard, P, C. Bouillon, H. Naveau, E.-J. Nyns, 1993. Toxicity of a mixture of polychlorinated organic compounds towards an unacclimated methanogenic consortium. Biotechnol. Lett., 15, 195-200.

Rhee, G.-Y., R.C. Sokol, C.M. Bethoney, B. Bush, 1993. Dechlorination of polychlorinated biphenyls by Hudson river sediment organisms: specificity to the chlorination pattern of congeners. Environ. Sci. Technol., 27, 1190-1192.

RIVM, 1992. National environmental outlook 1990-2010. Report of the National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands.

Samson, R., T. Cseh, J. Hawari, C.W. Greer, R. Zaloum, 1990. Biotechnologies appliquees a la restauration de sites contamines avec exemple d'application d'une technique physico-chimique et biologique pour les sols contamines par des PCB. Sciences et Techniques de l'eau, 23, 15-23.

Schwarzenbach, R.P., W. Giger, E. Hoehn, J.K. Schneider, 1983. Behaviour of organic compounds during infiltration of river water to groundwater. Field studies. Environ. Sci. Technol., 17, 472-479.

Schwarzenbach, R.P., P.M. Gschwend, D.M. Imboden, 1993. Environmental organic chemistry. John Wiley & Sons, New York.

Shiu, W.Y., W. Doucette, F.A.P.C. Gobas, A. Andren, D. Mackay, 1988. Physical-chemical properties of chlorinated dibenzo-p-dioxins. Environ. Sci. Technol., 22, 651-658.

Sierra-Alvarez, R, G. Lettinga, 1991. The effect of structure on the inhibition of acetoclastic methanogenesis in granular sludge. Appl. Microbiol. Biotechnol., 34, 544-550.

Suflita, J.M., A. Horowitz, D.R. Shelton, J.M. Tiedje, 1982. Dehalogenation: a novel pathway for the anaerobic biodegradation of haloaromatic compounds. Science, 218, 1115-1117.

Viney, I, R.J.F. Bewley, 1990. Preliminary studies on the development of a microbiological treatment for polychlorinated biphenyls. Arch. Environ. Contam. Toxicol., 19, 789-796.

Watanabe, I., T. Kashimoto, R. Tatsukawa, 1986. Hexabromobenzene and its debrominated compounds in river and estuary sediments in Japan. Bull. Environ. Contam. Toxicol. 36, 778-784.

Williams, W.A., 1994. Microbial reductive dechlorination of trichlorobiphenyls in anaerobic sediment slurries. Environ. Sci. Technol., 28, 630-635.

Winters, S.L., D.R. Lee, 1987. In situ retardation of trace organics in groundwater discharge to a sandy stream bed. Environ. Sci. Technol., 21, 1182-1186.

Zitomer, D.H. & R.E. Speece, 1993. Sequential environments for enhanced biotransformation of aqueous contaminants. Environ. Sci. Technol., 27, 227-244.

Zoeteman, B.C.J., K. Harmsen, J.B.H.J. Linders, C.F.H. Morra, W. Slooff, 1980. Persistent organic pollutants in river water and ground water of The Netherlands. Chemosphere 9, 231-249.

Zwolsman, G.J., H.L.A. Sonneveldt, E.F.W. Ruijgh, 1993. Onderzoek Noordelijk Deltabekken, Zuidrand. Toepassing accumulatiemodel waterbodem Hollands Diep, onzekerheidsanalyse en calibratie. Delft Hydraulics report no. T 262 (in Dutch).

SUMMARY

Numerous contaminants like heavy metals, polycyclic aromatic hydrocarbons (PAHs), chlorinated benzenes (CBs), polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated furans (PCDFs) are detected in the major rivers in the Netherlands. These contaminants have relatively low aqueous solubilities and bind substantially to the suspended solids in river water. Due to decreasing stream velocities in the downstream stretches of a river suspended solids will settle to the river bed. Thus, the deposition of suspended solids in the downstream stretches results in transport of contaminants from the water column to the sediments.

In the past decade widespread contamination of sediments with heavy metals, PAHs, and chlorinated aromatics (CBs, PCBs, PCDDs and PCDFs) has been identified in the Netherlands. The extent and seriousness of sediment contamination is most pronounced in the sedimentation areas of the rivers Rhine, Meuse and Scheldt. The total amount of heavily contaminated sediments in these areas is estimated at more than 100 million m^3. The reduced fertility of cormorants in the Biesbosch, a sedimentation area of the Meuse, can be put forward as an example of the adverse ecological impact of contaminated sediments.

Microorganisms (bacteria and fungi) play an important role in the decomposition of organic matter in sediments. The diversity of metabolic processes in microorganisms enables them to degrade a variety of organic contaminants as well. Two degradation processes can be distinguished: transformation and mineralization. Transformation is the process by which the chemical structure of the organic substrate (or contaminant) is altered and organic products are formed. Mineralization is the process whereby, besides biomass, only inorganic products (CO_2, CH_4, H_2O, chemical elements) are formed during microbial metabolism.

In the presence of oxygen, as in the water column of rivers, the microbial degradation of chlorinated aromatic compounds decreases with an increasing number of chlorine atoms in the molecule. The relative persistence of higher chlorinated aromatics in the water column,

combined with the hydrophobic properties of the aromatics, results in deposition of these contaminants in sediments. The upper few centimeters of sediment contain oxygen; most of the sediment is anoxic. Higher chlorinated compounds can, particularly under these anoxic conditions, be transformed by anaerobic microorganisms into lower chlorinated compounds. In the last 5 to 10 years microbial dechlorination reactions, i.e. the replacement of chlorines in the contaminant molecule with hydrogen atoms, have been demonstrated in laboratory tests for a variety of chlorinated aromatics. Some of these tests, conducted with anaerobic sediment, suggest that dechlorination reactions may occur in contaminated sediments.

Determining the occurrence of *in situ* microbial dechlorination was the general objective of the research described from which the following issues were deduced for application to the compound classes selected, i.e.: CBs, PCBs, PCDDs and PCDFs:

1) What are the *in situ* dechlorination rates?
2) What types of reactions occur and what are the reaction products?
3) Does a general reaction pattern in dechlorination reactions exist?
4) What are the consequences of *in situ* microbial dechlorination in sediments from an environmental point of view?

A dual approach, including field studies and laboratory experiments, was followed to determine *in situ* reactions.

Field studies

The hypothesis that *in situ* dechlorination would lead to a shift from higher to lower chlorinated compounds in anaerobic sediments was verified in the field studies, which consisted of sediment cores collected from Lake Ketelmeer, a sedimentation area of the Rhine River. The cores were sectioned into thin layers and the year of deposition of each layer was determined using radiochemical analyses. In addition, the concentrations of the chlorinated aromatics were determined in each layer. The contaminant concentrations were plotted against the year of deposition of the individual sediment layers (chapters 2 and 3). The possibility of a shift from higher to lower chlorinated compounds was verified with the aid of historical samples, collected in 1972 from the Lake Ketelmeer sediment top layer, and subsequently dried and stored for almost 20 years. The historical samples were analyzed together with the sediment core layers, thus applying presently available, identical analytical techniques. The historical samples reflect the contaminant concentration at time of deposition in the past (around 1972), either without or with only limited

influences of degradation processes. The concentrations of chlorinated aromatics in the historical samples were compared to the concentrations found in sediment core layers deposited around 1970. In this way, the chlorinated aromatic concentrations at the time of deposition are compared with the concentrations after 20 years of "environmental incubation". Six highly toxic PCBs, representing the so-called non-ortho and mono-ortho PCBs that cause dioxin-like toxic effects, were selected and compared with the concentrations in the historical samples. Four of the six PCB congeners showed a significant disappearance in the sediment core layers relative to the historical samples. Increased concentrations of lower chlorinated congeners were not observed, probably due to the limited number of congeners analyzed. Maximum half-lives ($t_{1/2}$) for the four PCB congeners showing significant disappearances were estimated at about 10 years. Of the 17 chlorinated dioxins and furans studied, only four congeners showed significant disappearances, indicating slow or complete absence of reactions ($t_{1/2} \geq$ 12 years). Compared to historical input, significant disappearances of hexachlorobenzene ($t_{1/2} \leq$ 7 years), pentachlorobenzene and 1,2,3,5-tetrachlorobenzene were found. On the other hand, the concentrations of 1,3,5-tri-, 1,2-di-, and 1,3-dichlorobenzene increased significantly as compared to the historical input, indicating their formation in the anaerobic sediment.

Laboratory studies

Transformations in Lake Ketelmeer sediment

The field studies make the occurrence of selective disappearances of chlorinated aromatics in Lake Ketelmeer sediment clear, but these observations do not reveal the responsible mechanisms. Therefore laboratory experiments were conducted using Lake Ketelmeer sediment under conditions resembling the field conditions (chapter 3). These experiments demonstrated that the indigenous anaerobic microbial population in Lake Ketelmeer sediment is able to dechlorinate hexachlorobenzene (HCB) to 1,3,5-tri-, and 1,3-dichlorobenzene. Based on the field and laboratory observations it was concluded that *in situ* microbial dechlorination of HCB in Lake Ketelmeer had occurred, resulting in an 80% loss of HCB. The tri-, and dichlorobenzenes produced are less toxic and have a lower bioaccumulation potentials than the parent compound HCB. Moreover, tri-, and dichlorobenzenes are, opposite to HCB, suitable substrates for aerobic mineralization. The fact that the transformation products are less hydrophobic and therefore can be

transported more easily with infiltrating water than HCB can be regarded as a disadvantage of dechlorination reactions in the environment.

An enrichment culture from Lake Ketelmeer sediment that readily dechlorinates HCB under methanogenic conditions was obtained (chapter 4). Substantial dechlorinating activity was still present at low temperatures (3°C), whereas the optimum temperature for HCB dechlorination was around 30°C. The dechlorinating capabilities of the enrichment culture were verified with a variety of chlorinated aromatics. Individual incubations with 11 chlorinated benzenes demonstrated that from the 19 dechlorination reactions possible, only the seven reactions with the highest energy release took place.

The enrichment culture was also incubated with a selected number of PCB congeners in accordance with those selected in the field study. The results show a selective removal of chlorines from para and meta positions if the departing chlorine atom was surrounded by chlorines on both sides (chapter 5). PCB 118 does not meet this structural prerequisite and was not dechlorinated by the enrichment. This experimental result is in agreement with the field observation where no significant disappearance of PCB 118 is observed. The structural prerequisite can also be deduced from the observed selectivity in chlorobenzene dechlorination. The correlation with thermodynamics, as observed for chlorinated benzenes, appeared to be valid for PCBs as well. Only the energetically most favourable dechlorination steps are catalyzed by the enrichment, except ortho-dechlorinations.

The dechlorinating capacity of the enrichment culture for chlorinated dioxins was tested using 1,2,3,4-tetrachloro-*p*-dioxin (1,2,3,4-TeCDD) as a model compound (chapter 6). Microbial dechlorination of 1,2,3,4-TeCDD resulted in the formation of 1,3- and 2,3-dichlorodibenzo-*p*-dioxins as the main products. The results suggest that microbial dechlorination of PCDDs in Lake Ketelmeer sediment might be possible. However, the field data indicate that *in situ* reactivity of PCDDs is extremely slow or absent. This may be due to extremely low concentrations of PCDDs in porewater and the slow desorption of PCDDs from sediments, resulting in a low microbial availability.

The similar experimental protocols used for the laboratory incubations with CBs, PCBs and PCDDs allow the calculation of relative dechlorination rates for these three compound classes. Dechlorination half-lives for tetra-, penta-, and hexachlorobenzene are between 1 and 2 days for 1,2,3,4-TeCDD 15.5 days, and for the tested reactive PCBs, between 10 and 120 days. Remarkable is the difference between half-lives determined in the laboratory and half-lives estimated in the field study (up to a decade). In the contaminated sediments densities of the actively dechlorinating microbial populations may be smaller and activities may be lower due to lower concentrations of co-substrates and/or nutrients and lower temperatures than in the laboratory incubations. In addition, in the laboratory interference

of sediment was eliminated; experiments were conducted in liquid media. Binding of the hydrophobic chlorinated aromatics to sediments reduces the concentration in the sediment pore water substantially and the slow release of bound compounds to the pore water may limit *in situ* dechlorination rates.

Transformation in coastal sediments

The microbial dechlorination of HCB in sediment from the Ems estuary is described in chapter 7. Under sulfate-reducing conditions, prevailing in the estuarine sediment, an HCB dechlorinating-culture was obtained. The dechlorination of HCB occurred concomitantly with sulfate reduction but was not directly coupled to sulfate reduction. These results indicate that microbial dechlorination of chlorinated aromatics is not necessarily restricted to fresh water sediments but may also occur in estuarine sediments.

Environmental significance

The PCB data from the field and laboratory study support the conclusion that the occurrence of *in situ* dechlorination of PCBs in Lake Ketelmeer sediment is highly likely. The environmental significance of *in situ* dechlorination, particularly for those congeners that cause dioxin-like biochemical and toxic effects is the subject of chapter 8. The significant disappearance of four PCB congeners in Lake Ketelmeer sediment, as observed in the field study, has resulted in a 75% decrease in the dioxin-like toxicity during the last 20 years. The selectivity in the dechlorinating activity of the enrichment culture from Lake Ketelmeer was used to predict the reactivity of all non-ortho and mono-ortho PCBs. All congeners, except PCB 77 and PCB 118, appear to be reactive. Dechlorination could result almost exclusively in products that have no dioxin-like toxicity. Other advantages of PCB dechlorination, besides decreased toxicities, are a lowered bioaccumulation potential and dechlorination products being more suitable substrates for aerobic mineralization than the parent compounds.

The results of this study have been included in an overview of currently available *in situ* half-lives of chlorinated aromatics (chapter 9). Although the available data are limited, it is clear that *in situ* microbial dehalogenation is a relatively slow process that may proceed with half-lives of several years to decades in contaminated sediments. Despite the low rates, it is important to include these transformation processes in long-term sediment-

quality prognoses. After all, these transformations with their significant toxicological implications are the essential first steps that may eventually lead to a complete elimination of PCBs from the environment. The correlation between energy yield and catalyzed dechlorination steps is further verified with data from the literature. The thermodynamically most profitable dechlorination steps appear to be catalyzed preferentially, except for ortho-dechlorinations. The correlation with thermodynamics appears to be an appropiate instrument for predicting microbial dechlorination pathways.

New prospects for the biotechnological clean-up of sediments contaminated with halogenated aromatics have emerged from the results of this study. The clean-up process should include two steps; an anaerobic dehalogenation and a subsequent aerobic mineralization of dehalogenation products. The stimulation of dehalogenation rates and the application of the two-step process on pilot scale are two examples of future challenges.

SAMENVATTING

MICROBIËLE OMZETTING VAN GECHLOREERDE AROMATISCHE VERBINDINGEN IN WATERBODEMS

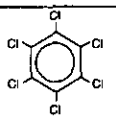
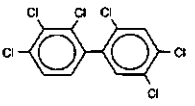
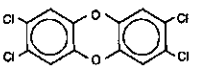
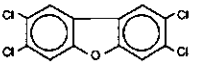
Tal van verontreinigingen zoals zware metalen, polycyclische aromatische koolwaterstoffen, chloorbenzenen (CB's), polychloorbifenylen (PCB's), polychloordibenzo-*p*-dioxinen (PCDD's) en polychloordibenzofuranen (PCDF's) worden in onze Nederlandse rivieren aangetroffen. Deze verontreinigingen zijn relatief slecht oplosbaar in water en binden in hoge mate aan het zwevende stof in het rivierwater. Het zwevende stof zakt naar de bodem in de benedenloop van een rivier als gevolg van lagere stroomsnelheden. Door de binding van verontreinigingen aan het zwevende stof gaan de verontreinigingen vanuit het water naar de waterbodem.

De afgelopen decennia is gebleken dat in Nederland veel waterbodems verontreinigd zijn met zware metalen, polycyclische aromatische koolwaterstoffen en gechloreerde aromatische stoffen (CB's, PCB's, PCDD's, PCDF's, zie intermezzo 1). Vooral in de grote sedimentatiegebieden van de Rijn, Maas en Schelde is de problematiek ernstig en omvangrijk. De totale hoeveelheid sterk verontreinigd sediment in deze gebieden wordt geschat op meer dan 100 miljoen m^3.

Micro-organismen (bacteriën en schimmels) spelen een belangrijke rol bij de afbraak van organisch materiaal in de waterbodem. Een grote verscheidenheid aan omzetttings-processen zorgt ervoor dat micro-organismen ook tal van organische verontreinigingen kunnen afbreken. Hierbij kan onderscheid worden gemaakt tussen omzetting en mineralisatie. Bij omzetting (transformatie) wordt de oorspronkelijke verbinding afgebroken tot organische omzettingsprodukten (metabolieten). Bij mineralisatie wordt een stof volledig afgebroken tot anorganische produkten (kooldioxide, water, elementen e.d.).

Onder zuurstofrijke (aërobe) condities, zoals in de waterkolom van een rivier, verloopt de microbiële afbraak van gechloreerde aromatische stoffen langzamer bij een toenemend

***Intermezzo 1.* Wat zijn gechloreerde aromatische verbindingen?**

Stofgroep	Aantal*	Voorbeeld	Bron/Toepassing
Chloorbenzenen (CB's)	12	 hexachloorbenzeen	-bestrijdingsmiddel -bijprodukt in chemische processen
Polychloorbifenylen (PCB's)	209	 PCB 138	-transformator vloeistof -hydraulische vloeistof
Polychloordibenzo-p-dioxinen (PCDD's)	75	 2,3,7,8-TeCDD	-bijprodukt in verbrandings- en chemische processen
Polychloordibenzofuranen (PCDF's)	135	 2,3,7,8-TeCDF	-bijprodukt in verbrandings- en chemische processen

*Het aantal en de plaats van chlooratomen aan de ring-structuren kan sterk varieren en bepaalt hoeveel verschillende verbindingen er binnen één stofgroep kunnen worden onderscheiden.

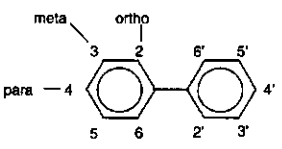
Korte karakteristiek van deze stoffen:

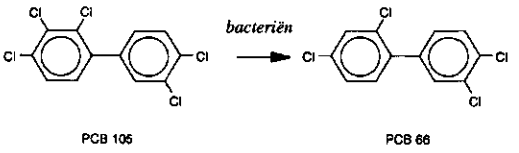
- De grootste lozingen van deze stoffen hebben in het verleden plaatsgevonden.
- Momenteel nog hoge gehalten in sedimenten en vetweefsels van bijvoorbeeld vissen.
- De plaats van de chlooratomen in het dioxine of furaan molecuul bepaalt de giftigheid van deze verbindingen. De meest giftige verbinding is 2,3,7,8-tetrachloordibenzo-p-dioxine (2,3,7,8-TeCDD), zie bovenstaande figuur.

-Er zijn 17 dioxinen en furanen met een hoge toxiciteit, inclusief 2,3,7,8-TeCDD.

-11 PCB's veroorzaken vergelijkbare effecten als 2,3,7,8-TeCDD, bijvoorbeeld PCB 105 (zie onderstaande figuur). Deze PCBs hebben hooguit één chlooratoom op de ortho posities en aan beide ringen tenminste 2 chlooratomen op de meta en para posities, hierdoor vertonen zij een grote structurele gelijkenis met 2,3,7,8-TeCDD.

-Onder zuurstofloze condities kunnen bacteriën de chlooratomen in deze verbindingen vervangen door waterstofatomen (reductieve dechlorering) en de toxiciteit veranderen (zie onderstaande reactie).





PCB 105 PCB 66

aantal chlooratomen in de af te breken stof. Deze meervoudig gechloreerde aromatische verbindingen zijn relatief persistent in de waterkolom en eindigen, gebonden aan het zwevend materiaal, in de waterbodem. Slechts de bovenste centimeters van de waterbodem bevatten zuurstof, het overgrote deel van de waterbodem is zuurstofloos (anaëroob). Onder dergelijke zuurstofloze condities zouden meervoudig gechloreerde aromatische verbindingen onder invloed van micro-organismen kunnen worden omgezet in lager gechloreerde verbindingen (zie intermezzo 1). In laboratoriumstudies is deze microbiële dechloreringsreactie, waarbij chlooratomen in het verontreinigingsmolecuul worden vervangen door waterstofatomen, voor tal van gechloreerde aromatische verbindingen vastgesteld. Enkele van deze laboratoriumstudies, uitgevoerd met anaëroob sediment, suggereerden dat dechloreringsreacties ook in waterbodems kunnen optreden.
Of deze reacties ook feitelijk optreden in verontreinigde waterbodems, kortweg *in situ* reacties genoemd, is de centrale vraagstelling van het onderzoek beschreven in dit proefschrift. Een aantal concrete onderzoeksvragen is hiervan afgeleid met betrekking tot de stofgroepen CB's, PCB's, PCDD's en PCDF's:

1) Wat is de snelheid van *in situ* reacties?
2) Welke typen reacties treden op, wat zijn de omzettingsprodukten?
3) Zijn er algemene wetmatigheden af te leiden voor de reactiepatronen?
4) Wat zijn de consequenties van *in situ* dechloreringsreacties vanuit milieuhygiënisch oogpunt?

Om *in situ* reacties vast te stellen is een gecombineerde aanpak gevolgd van veldonderzoek en laboratoriumexperimenten.

Veldonderzoek

Als *in situ* dechlorering optreedt dan zal er in de diepere, anaërobe sedimentlagen een verschuiving optreden van hoog gechloreerde verbindingen naar laag gechloreerde verbindingen. Deze hypothese is in het Ketelmeer, een sedimentatiegebied van de Rijn, getoetst. In 1972 zijn monsters van de toplaag van het sediment genomen, gedroogd en opgeslagen. Deze historische monsters bevatten gehalten van chlooraromaten ten tijde van de afzetting in 1972 met geen of minimale invloed van afbraakprocessen. In 1990 zijn boorkernen van het Ketelmeersediment genomen. Deze kernen zijn in laagjes verdeeld en elk laagje is gedateerd met behulp van radiochemische technieken en geanalyseerd op het voorkomen van metalen en organische verontreinigingen. De historische toplaagmonsters zijn tezamen met de boorkernmonsters geanalyseerd, met dezelfde hedendaagse analyse-technieken. De gehalten van chlooraromaten in de historische monsters zijn vergeleken

met de gehalten in boorkernlagen die zijn afgezet rond 1970, maar zijn bemonsterd in 1990. Deze vergelijking geeft inzicht in het optreden van verdwijningen in de periode van bijna twintig jaar dat de chlooraromaten in de Ketelmeerbodem hebben gezeten (zie intermezzo 2).

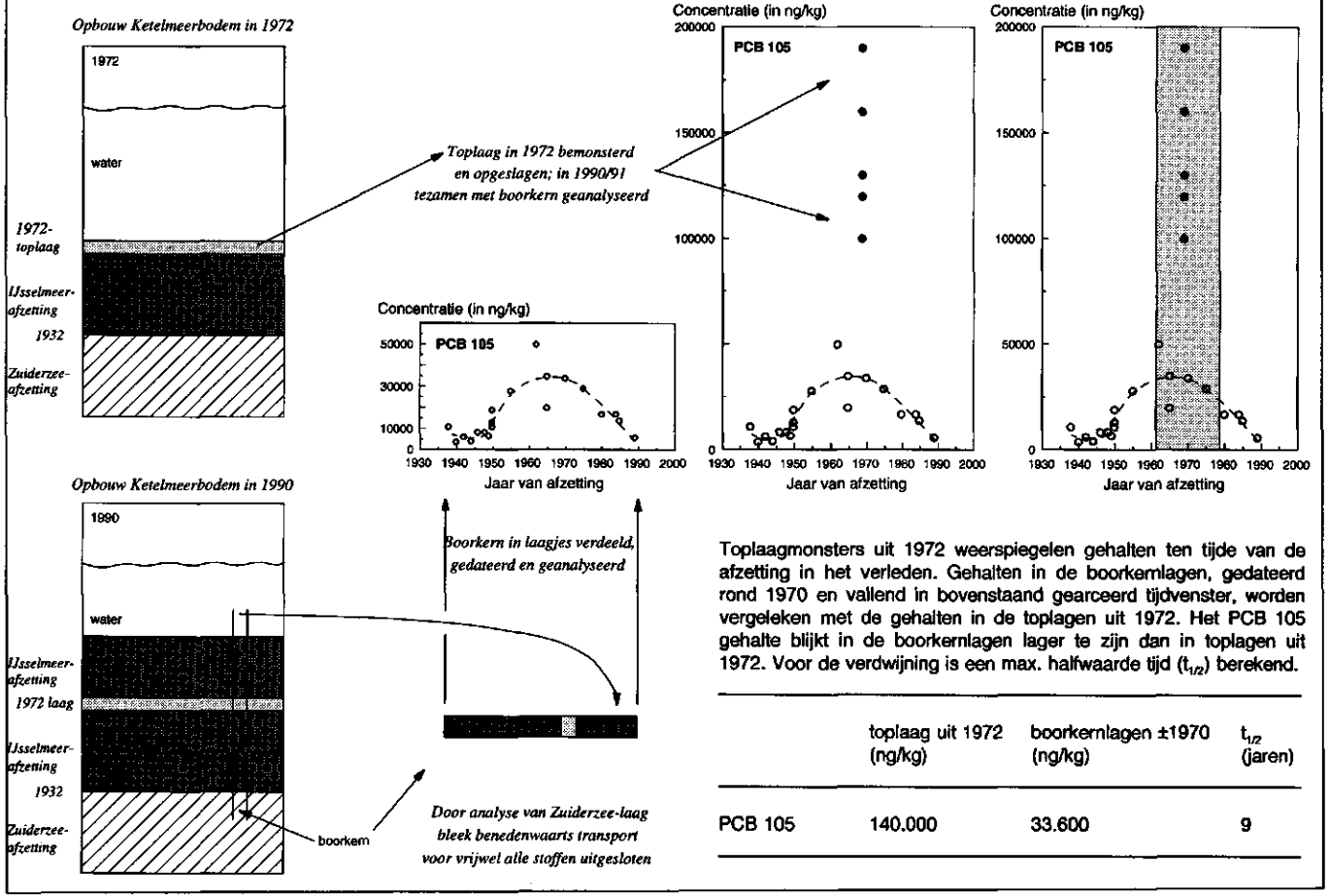
Er zijn zes PCB verbindingen geselecteerd met een dioxine-achtige toxiciteit, de zogenaamde non-ortho en mono-ortho gesubstitueerde PCB's. Voor vier van de zes bestudeerde PCB's is een significante verdwijning vastgesteld (hoofdstuk 2). Als deze verdwijning het gevolg is van microbiële dechlorering, dan is een toename van lager gechloreerde PCB's te verwachten. Dit is echter niet vastgesteld, mogelijk als gevolg van het beperkte aantal PCB's dat is geanalyseerd. Op basis van de vastgestelde verdwijningen is een maximale halfwaardetijd ($t_{1/2}$) voor de vier PCB's in het Ketelmeersediment afgeleid van circa 10 jaar. Van de 17 bestudeerde gechloreerde dioxinen en furanen is voor vier congeneren een significante verdwijning vastgesteld ($t_{1/2} \leq$ 12 jaar). Voor de chloorbenzenen is een significante verdwijning van hexachloorbenzeen ($t_{1/2} \leq 7$ jaar), pentachloorbenzeen en 1,2,3,5-tetrachloorbenzeen vastgesteld (hoofdstuk 3). De gehalten van 1,3,5-tri-, 1,2-di- en 1,3-dichloorbenzeen vertoonden daarentegen een significante toename ten opzichte van de gehalten in de historische monsters, dit duidt erop dat deze verbindingen in het anaërobe sediment *in situ* gevormd zijn, mogelijk als transformatieprodukten. De waargenomen verdwijningen zouden door benedenwaarts transport met infiltrerend water kunnen worden veroorzaakt. Dit is nader geverifieerd en bleek uitsluitend voor di- en trichloorbenzenen te zijn opgetreden.

Laboratoriumonderzoek

Omzettingen in Ketelmeersediment

De veldwaarnemingen maken duidelijk dat er sprake is van selectieve verdwijningen van gechloreerde aromaten in het anaërobe Ketelmeersediment. Voor het identificeren van het mechanisme, dat hieraan ten grondslag ligt, is aanvullend laboratoriumonderzoek verricht (hoofdstuk 3). Als aan sediment hexachloorbenzeen (HCB) werd toegevoegd en vervolgens enige tijd werd bewaard, is vastgesteld dat anaërobe micro-organismen, die van nature in het Ketelmeersediment voorkomen, in staat zijn HCB te dechloreren tot 1,3,5-tri- en 1,3-dichloorbenzeen. Op basis van de veld- en laboratoriumgegevens kan worden geconcludeerd dat *in situ* microbiële dechlorering van HCB in het Ketelmeersediment is opgetreden, resulterend in een 80% verlaagd HCB gehalte. De

Intermezzo 2. Hoe is verdwijning van PCB 105 in Ketelmeerbodem vastgesteld?



Toplaagmonsters uit 1972 weerspiegelen gehalten ten tijde van de afzetting in het verleden. Gehalten in de boorkernlagen, gedateerd rond 1970 en vallend in bovenstaand gearceerd tijdvenster, worden vergeleken met de gehalten in de toplagen uit 1972. Het PCB 105 gehalte blijkt in de boorkernlagen lager te zijn dan in toplagen uit 1972. Voor de verdwijning is een max. halfwaarde tijd ($t_{1/2}$) berekend.

	toplaag uit 1972 (ng/kg)	boorkernlagen ±1970 (ng/kg)	$t_{1/2}$ (jaren)
PCB 105	140.000	33.600	9

geproduceerde tri- en dichloorbenzenen zijn minder toxisch en minder bioaccumulerend dan de uitgangsverbinding HCB. Bovendien zijn tri-en dichloorbenzenen onder aërobe condities volledig mineraliseerbaar, in tegenstelling tot HCB. Een mogelijk nadelig effect kan zijn, dat de omzettingsprodukten mobieler zijn dan HCB en in principe gemakkelijker kunnen worden verspreid met infiltrerend water.

In het laboratorium zijn de micro-organismen, die in staat zijn HCB te dechloreren, van het Ketelmeersediment gescheiden. De verkregen cultuur is onder methaan vormende condities, in medium zonder sediment, verder gekweekt (hoofdstuk 4). Ook bij lage temperaturen (3°C) zijn deze micro-organismen actief, al verloopt de dechlorering optimaal bij circa 30°C. Het dechlorerend vermogen van deze cultuur is getest voor een breed scala aan chlooraromaten. Afzonderlijke incubaties met 11 verschillende chloorbenzenen maakten duidelijk, dat alleen de benzenen met drie of meer chlooratomen in het molecuul werden omgezet, waarbij chlooratomen die aan beide zijden omgeven werden door eveneens chlooratomen het gemakkelijkst werden verwijderd door de cultuur. Om deze selectiviteit te kunnen begrijpen is gezocht naar een mogelijke relatie met de energie die vrijkomt bij de verschillende dechloreringsreacties. Immers, in elke organische verbinding zit een hoeveelheid energie die ten dele vrij kan komen bij omzettingsreacties en kan worden benut door de micro-organismen. Hieruit bleek, dat van de 19 mogelijke dechloreringsreacties, alleen de 7 reacties die de meeste energiewinst opleveren werden gekatalyseerd. De cultuur is ook geincubeerd met PCB congeneren die in de veldstudie waren opgenomen. Uit deze incubaties blijkt een selectieve dechlorering op de para- en meta-posities (zie intermezzo 1) indien het vertrekkend chlooratoom aan beide zijden is omgeven door chlooratomen (hoofdstuk 5). De invloed van de plaats van chlooratomen aan de ring op het optreden van de dechloreringsreactie is vergelijkbaar met de waarnemingen bij de chloorbenzenen. Indien de energieopbrengsten van verschillende PCB dechloreringsstappen op een rijtje worden gezet dan blijken de waargenomen dechloreringsstappen de meeste energiewinst op te leveren. Ortho-dechloreringen vormen hierop een uitzondering; ondanks een relatief hoge energieopbrengst in sommige gevallen, zijn deze reacties niet waargenomen. In PCB 118 zijn geen drie chlooratomen op een rij beschikbaar en dit molecuul voldoet niet aan de "ideale" plaatsing van chlooratomen aan de ring voor dechlorering door de cultuur. Deze PCB verbinding werd niet gedechloreerd in de laboratorium incubaties; ook in de veldstudie werd geen significante daling van deze congeneer vastgesteld.

De relatie tussen energieopbrengst en het wel of niet optreden van bepaalde dechloreringsstappen is tevens geverifieerd met dechloreringsroutes van individuele PCB-congeneren gepubliceerd in de literatuur (hoofdstuk 9), dit in aanvulling op de eigen

bevindingen met PCB's (hoofdstuk 5). De dechloreringsroutes die de meeste winst in Gibbs vrije energie opleveren blijken bij voorkeur te worden gekatalyseerd, met uitzondering van ortho-dechloreringen. Vooralsnog lijkt de relatie tussen energieopbrengst en dechloreringsstappen een eerste middel om anaërobe microbiële omzettingsroutes van chloorbenzenen en PCB's te voorspellen.
De dechloreringscapaciteit van de cultuur ten aanzien van gechloreerde dioxinen is getest met 1,2,3,4-tetrachloordibenzo-*p*-dioxine (1,2,3,4-TeCDD) als modelverbinding (hoofdstuk 6). Dechlorering van 1,2,3,4-TeCDD resulteerde in de ophoping van 1,3- en 2,3-dichloordibenzo-*p*-dioxinen als de belangrijkste omzettingsprodukten. Dechlorering van dioxinen is in principe dus mogelijk in de Ketelmeerbodem. Op basis van de veldgegevens moet echter worden geconcludeerd dat *in situ* dechlorering van dioxinen niet of uitermate langzaam verloopt. De bijzonder lage concentraties van PCDD's in het poriewater van het sediment en de langzame desorptie vanuit het sediment beperken mogelijk het optreden van dechloreringen in de waterbodem.
De incubaties van de cultuur met CB's, PCB's en PCDD's zijn op dezelfde wijze verricht en maken het mogelijk relatieve dechloreringssnelheden voor de drie stofgroepen te berekenen. Voor tetra-, penta- en hexachloorbenzeen is de dechloreringshalfwaarde tijd 1 à 2 dagen, voor 1,2,3,4-TeCDD 15,5 dag en voor de geteste, reactieve gechloreerde bifenylen tussen 10 en 120 dagen. Opmerkelijk is het grote verschil tussen halfwaarde tijden waargenomen in het laboratorium en halfwaarde tijden afgeleid uit veldonderzoek (oplopend tot een decennium). De laboratorium incubaties zijn verricht onder optimale condities in vloeistof-cultures zonder sediment en daarmee samenhangende sorptieprocessen. In verontreinigde waterbodems zijn de dechlorerende micro-organismen in lagere dichtheden aanwezig en minder actief door lagere concentraties van co-substraten, nutriënten en lagere temperaturen. Door de binding van gechloreerde aromaten aan het sediment is de vrij beschikbare concentratie voor dechlorering in het poriewater van verontreinigde waterbodems vele malen kleiner dan in de laboratoriumexperimenten: de biologische beschikbaarheid van chlooraromaten kan dus limiterend zijn.

Omzettingen in sediment van de kustzone

In het sediment van zoete wateren, zoals het Ketelmeer, overheersen methaan vormende condities. In de bodem van zeeën en estuaria overheersen daarentegen sulfaat reducerende omstandigheden. Om na te gaan of microbiële dechlorering van

chlooraromaten ook in verontreinigd sediment van zoute wateren kan optreden is het onderzoek uitgebreid tot de kustzone. Uit incubaties met sediment van het Eems estuarium blijkt dat de van nature aanwezige micro-organismen in staat zijn HCB te dechloreren (hoofdstuk 7). Onder zowel methanogene als sulfaat reducerende condities is een HCB dechlorerende cultuur verkregen uit het sediment. De dechlorering van HCB onder sulfaat reducerende condities blijkt niet gekoppeld te zijn aan de sulfaat reductie. Deze experimenten tonen aan dat dechlorering van chlooraromaten niet beperkt is tot waterbodems van zoete wateren, maar ook kan plaatsvinden in het estuariene sediment.

Milieuhygiënische consequenties

De gegevens uit het veldonderzoek en laboratoriumonderzoek met betrekking tot PCB's leiden tot de conclusie dat *in situ* dechlorering van PCB's in het Ketelmeersediment naar alle waarschijnlijkheid heeft plaats gevonden. De milieuhygienische consequenties van *in situ* dechlorering van PCB-congeneren met dioxine-achtige biochemische en toxische effecten zijn nader uitgewerkt in hoofdstuk 8. Door de significante afname van vier PCB congeneren in het Ketelmeersediment, zoals vastgesteld in de veldstudie, is de toxiciteit met 75% gedaald gedurende 20 jaar. De dechloreringsreacties van alle non-ortho en mono-ortho PCB's zijn voorspeld op basis van de waargenomen selectiviteit in de dechlorering van PCB's door de Ketelmeercultuur. Voor vrijwel al deze congeren geldt dat dechlorering kan optreden, PCB 77 en PCB 118 zijn de enige uitzonderingen, en in de meeste gevallen resulteren de dechloreringen in produkten die geen dioxine-achtige toxiciteit bezitten. Naast de verlaging in toxiciteit leidt dechlorering van PCB's tot de vorming van produkten die minder bioaccumulerend zijn en gemakkelijker mineraliseerbaar zijn door aërobe micro-organismen dan de uitgangsverbindingen.
De *in situ* dehalogeneringshalfwaarde tijden verkregen in dit onderzoek zijn vervolgens vergeleken met literatuurgegevens (hoofdstuk 9). Hieruit komt naar voren dat *in situ* microbiële dehalogenering een relatief langzaam verlopend proces in vervuilde waterbodems is met halfwaarde tijden van jaren tot decennia. Ondanks de lage snelheden van deze processen, moet er bij lange termijn prognoses van de waterbodemkwaliteit toch rekening mee worden gehouden. Immers, deze omzettingen hebben belangrijke toxicologische consequenties en vormen de essentiele eerste stap naar een biologische eliminatie van chlooraromaten uit het milieu.
Dit werpt ook nieuw licht op de mogelijkheden van biotechnologische reiniging van waterbodems verontreinigd met chlooraromaten. Een twee-staps proces is daarbij vereist,

bestaande uit een anaërobe dehalogenering gevolgd door een aërobe mineralisatie van de dehalogeneringsprodukten. Het stimuleren van de dehalogeneringssnelheid en de realisatie van het tweestaps reinigingsproces op grote schaal zijn twee uitdagingen voor de toekomst.

Publications

Beurskens, J.E.M., A.J.M. Stams, A.J.B. Zehnder & A. Bachmann, 1991. Relative biochemical reactivity of three hexachlorocyclohexane isomers. Ecotoxicol. Environ. Safety 21, 128-136.

Winkels, H.J., J.P.M. Vink, J.E.M. Beurskens & S.B. Kroonenberg, 1993. Distribution and geochronology of priority pollutants in a large sedimentation area of the River Rhine. Applied Geochemistry, Suppl. Issue 2, 95-101.

Beurskens, J.E.M., J. de Wolf & H. van den Heuvel, 1993. Microbial dechlorination of hexachlorobenzene in anaerobic sediments: formation of mobile metabolites. In: Ter Meulen et al. (eds.) Proceedings of the Chemical Time Bombs European state-of-the-art conference, September 2-5, 1992, pp. 157-162, Published by Foundation of Ecodevelopment, Hoofddorp, The Netherlands.

Beurskens, J.E.M., G.A.J. Mol, H.L. Barreveld, B. van Munster & H.J. Winkels, 1993. Geochronology of priority pollutants in a sedimentation area of the Rhine River. Environ. Toxicol. Chem., 12, 1549-1566 (*chapter 2*).

Beurskens, J.E.M., C.G.C. Dekker, J. Jonkhoff & L. Pompstra, 1993. Microbial dechlorination of hexachlorobenzene in a sedimentation area of the Rhine River. Biogeochem., 19, 61-81 (*chapter 3*).

Beurskens, J.E.M., J. de Wolf & H. van den Heuvel, 1993. High levels of chlorinated aromatic compounds in deep Rhine sediments with special reference to microbial transformations. Land Degradation & Rehabilitation 4, 373-380.

Beurskens, J.E.M., C.G.C. Dekker, H. van den Heuvel, M. Swart, J. de Wolf & J. Dolfing, 1994. Dechlorination of chlorinated benzenes by an anaerobic microbial consortium that selectively mediates the thermodynamic most favorable reactions. Environ. Sci. Technol. 28, 701-706 (*chapter 4*).

Beurskens, J.E.M., H.J. Winkels, J. de Wolf & C.G.C. Dekker, 1994. Trends of priority pollutants in the Rhine during the last 50 years. Wat. Sci. Technol., 29, 77-85.

Beurskens, J.E.M., J. de Wolf, H. van den Heuvel & J. Dolfing. Microbial dechlorination of polychlorinated biphenyls by a hexachlorobenzene acclimated enrichment culture. Submitted (*chapter 5*).

Beurskens, J.E.M., M. Toussaint, J. de Wolf, J. van der Steen, P. Slot, L.C.M. Commandeur, J.R. Parsons, 1995. Dehalogenation of chlorinated dioxins by an anaerobic microbial consortium from sediment. Environ. Toxicol. Chem., 14, in press (*chapter 6*).

Beurskens, J.E.M., J. de Wolf & J. Dolfing. Reductive dechlorination of

hexachlorobenzene by microorganisms from polluted estuarine sediment. Submitted (*chapter 7*).

Beurskens, J.E.M. & P.B.M. Stortelder, 1995. Microbial transformation of PCBs in sediments: what can we learn to solve practical problems? Wat. Sci. Technol., in press (*chapter 8*).

Dolfing, J. & J.E.M. Beurskens, 1995. The microbial logic and environmental significance of reductive dehalogenation. Advances in Microbial Ecology, in press. (*including parts of chapter 1 and 9*).

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Dit proefschrift omvat de resultaten van onderzoek dat in de periode 1988-1993 is uitgevoerd bij het RIZA te Lelystad. Dankzij de samenwerking met vele personen en instanties is dit onderzoek tot stand gekomen. Allereerst wil ik iedereen hartelijk danken die aan dit onderzoek heeft bijgedragen.

Tijdens mijn studie heb ik kennis gemaakt met een buitengewoon boeiend onderzoeksterrein, microbiële afbraak van verontreinigingen bij de vakgroep microbiologie van de Landbouwuniversiteit (LUW), die bovendien een bijzonder stimulerende atmosfeer uitstraalde. De keuze tussen een promotiebaan bij deze vakgroep of een baan bij het RIZA was moeilijk. Bij het RIZA bestond de mogelijkheid om biodegradatieonderzoek in relatie tot verontreinigde waterbodems op te starten. Willem Bruggeman was de grote inspiratie- en kennisbron van het eerste uur. Samen met Paul Stortelder heeft hij het vertrouwen in mij gesteld en de ruimte geboden dit onderzoek gestalte te geven. Gelukkig leidde dit werk tot het aanhalen van de contacten met de vakgroep microbiologie van de LUW in de loop van 1988. De gesprekken met Prof. Alex Zehnder en deelname aan het promovendioverleg vormden een belangrijke toets voor mijn onderzoeksplannen en resultaten, en waren altijd bijzonder stimulerend.

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Curriculum Vitae

Koos (Jacobus Engelbertus Maria) Beurskens is op 6 oktober 1960 geboren te Helden. In 1980 behaalde hij het Atheneum B diploma aan het Bisschoppelijk College te Roermond. In september van dat jaar begon hij zijn biologie studie aan de toenmalige Landbouwhogeschool, nu Landbouwuniversiteit (LUW), te Wageningen. Met veel plezier heeft hij de klassieke biologische vakken als dier- en plantentaxonomie in de kandidaatsfase gevolgd. Uiteindelijk heeft hij gekozen voor een milieuchemische specialisatie, waartoe organische milieuchemie, chemische analysemethoden en bodemmicrobiologie als extra vakken zijn gevolgd. Het kandidaatsexamen is behaald in mei 1984. De doctoraalfase bestond uit drie hoofdvakken: entomologie, toxicologie en microbiologie. Achtereenvolgens werd onderzoek verricht naar de effekten van metalen op bodemarthropoden (Vakgroep Ecologie en Ecotoxicologie van de Vrije Universiteit Amsterdam), de neveneffekten van bestrijdingsmiddelen in de koolzaadteelt (Toxicologie, LUW) en de afbreekbaarheid van hexachloorcyclohexaan isomeren (Microbiologie, LUW). In januari 1987 heeft hij het doctoraal examen afgelegd en is het getuigschrift verleend met het predikaat "met lof". In datzelfde jaar werd hij aangesteld als projectleider milieuchemisch onderzoek bij de Dienst Binnenwateren/RIZA (thans RIZA) van Rijkswaterstaat te Lelystad. Belangrijke onderzoeksprojecten waren:
-verspreiding van verontreinigingen vanuit de IJssel naar Ketelmeer en IJsselmeer;
-microbiële omzetting van chlooraromaten in anaerobe sedimenten, mede in het kader van het Speerpuntprogramma Bodemonderzoek; dit proefschrift omvat de resultaten van dit project;
-mineralisatie van verontreinigingen in oppervlaktewater, waartoe overigens het diploma Veilig werken met radionucliden (niveau 4B) is behaald (juni 1988, Universiteit Utrecht);
-biologische reiniging van polycyclische aromatische koolwaterstoffen in baggerspecie, in het kader van het Programma Ontwikkeling Saneringsprocessen Waterbodems (POSW);
-afbraak van chlooraromaten in baggerspeciedepots, eveneens in het kader van het POSW.
Sinds januari 1994 is hij werkzaam als coördinator waterbodems bij het Rijksinstituut voor Volksgezondheid en Milieuhygiëne (RIVM) te Bilthoven.