5
The Bioremediation of Polychlorinated Biphenyls (PCBs): Problems and Perspectives

G.K. ROBINSON AND M.J. LENN*

Research School of Biosciences, University of Kent, Canterbury, Kent CT2 7NJ, UK

Introduction

MANUFACTURE AND HISTORY

Polychlorinated biphenyls (PCBs) were synthesized between the early 1920s and 1978 by a variety of manufacturers in the US, Europe and Japan. They were manufactured for their excellent thermal and electrical properties and were used in a wide variety of commerical applications. These varied from closed-system applications, such as dielectric fluids (capacitors and transformers) and industrial fluids (hydraulic systems and gas turbines), to open-system applications, such as in the manufacture of adhesives, textiles and printing. They were made as commercial mixtures of polychlorinated biphenyls and were manufactured by the direct chlorination of biphenyl using anhydrous chlorine. This was done at temperatures above 150°C, keeping the biphenyl molten to prevent clogging, in the presence of a catalyst such as iron filings or ferric chloride. The crude product was then purified with 0.3% alkali and distilled under vacuum. The degree of chlorination is dependent upon the contact time of the chlorine and biphenyl, which is usually between 12 and 36 hours. Theoretically, there are 209 possible congeners of polychlorinated biphenyls, but due

Abbreviations: APEG, alkali metal polyethylene glycolate; 2,4- D, 2,4-dichlorophenoxyacetic acid; DRE, destruction and removal efficiency; ECD, electron capture detector; EI, electron impact; EPA, Environmental Protection Agency; FID, flame ionization detector; GC, gas chromatography; GCMS, gas chromatography–mass spectrometry; HECD, Hall electrolytic conductivity detector; HOPDA,2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid; HPLC, high-pressure liquid chromatography; ICES, International Council for Exploration of the Sea; ICRCL, Interdepartmental Committee on the Reclamation of Contaminated Land; LC, liquid chromatography; MS, mass spectrometry; OECD, Organization for Economic Co-operation and Development; PCBs, polychlorinated biphenyls; RRT, relative retention time; SEC, steric effect coefficient; TLC, thin-layer chromatography; TOC, total organic carbon; TSCA, Toxic Substances Control Act; UV, ultraviolet.

* *Current Address: Viridian Bioprocessing Ltd, 114–116 John Wilson Business Park, Thanet Way, Chestfield, Whitstable, Kent CT5 3QT, UK.*

Biotechnology and Genetic Engineering Reviews — Vol. 12, December 1994
0264–8725/94/12/139–188 $20.00 + $0.00

to mechanistic and statistical constraints 20 PCBs are absent in commercial preparations (Hutzinger, Safe and Zitko, 1974).

Commercial production of PCBs started in 1929. Since that time 57×10^7 kg have been made in the US alone. About an equal amount was manufactured in Europe, Russia and Japan. Major manufacturers over this time period were Monsanto (US), Mitsubishi-Monsanto (Japan), Prodelec (France), Bayer (Germany) and Caffaro (Italy). The primary reason for cessation of manufacture was the persistence and recalcitrance of PCBs in the environment. Jensen had shown this as early as 1966 (Jensen, 1972). Under the Toxic Substances Control Act (1976) the US Congress banned the manufacture of PCBs although Monsanto had restricted PCB use to closed-system applications in 1971. Finally, in 1978 PCB manufacture in the US was halted and dioctyl phthalate was introduced as a replacement. In the UK PCB usage has been progressively restricted from the early 1970s until their use in new plant and equipment was banned in 1986. After the US ban, Prodelec (France) continued to manufacture Chloralkylene 12, a product containing 20% dichlorinated biphenyls. In addition, Caffaro (Italy) manufactured Apirollio 1531C and 14812, which contained 60% PCBs and 40% trichlorobenzenes, albeit for closed-system application.

In 1990, at the Third International Ministerial Conference on the Protection of the North Sea, the UK agreed, along with the other participating states, to phase out and destroy the remaining PCBs in use by 1999.

NOMENCLATURE

Polychlorinated biphenyls contain two benzene rings and, using IUPAC convention, are labelled according to ring and position. One ring is labelled clockwise and the other counterclockwise with prime numbers as shown in *Figure 1*. Additionally, each of the possible congeners has been ordered by Ballschmiter and Zell (1980) to give a further system of nomenclature. Of the commercial mixtures, the Aroclor series were the most widely used and are named according to the number of carbon atoms in the biphenyl nucleus and the percentage by weight of chlorine (for example, Aroclor 1242 contains 42% chlorine). They also differ in physical appearance, with Aroclor 1242–1248 being clear liquids, 1254–1262 light-coloured oils and 1268 an off-white powder.

SOLUBILITY

The problems associated with PCBs are largely due to the diversity of congeners

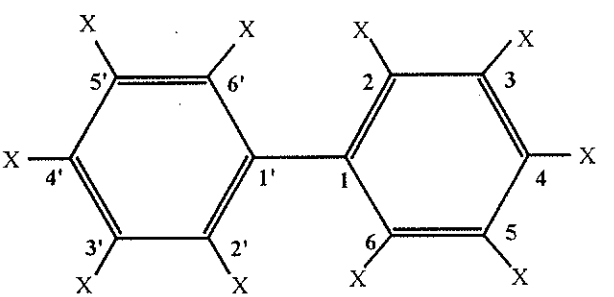

Figure 1. Conventional numbering of substituent positions in the PCB molecule X = H or Cl.

present in commercial mixtures and their associated chemical inertness. The Aroclor series of PCBs is perhaps the most widely cited preparation, largely due to the scale of its manufacture. The preparations manufactured vary in composition as outlined elsewhere. Of those available, 1242 was the most widely used Aroclor and contained a range of chlorobiphenyls containing up to five chlorine molecules. Approximately 60–80 congeners have been identified in the mixture and the properties of the Aroclor are dependent upon the congener composition. They are remarkably hydrophobic compounds and mixtures, solubility in water being governed by the percentage of higher chlorinated congeners present and the position of the chlorine atoms. Aroclor 1242 is soluble at 200 p.p.b. whereas 1260 is much less soluble, at 25 p.p.b. Of the individual congeners, 2-chlorobiphenyl is the most soluble (6 p.p.m.) and decachlorobiphenyl (0.015 p.p.m.) the least soluble in water (Hutzinger, Safe and Zitko, 1974). From the additional data shown in *Table 1*, it can be seen that even within the dichlorobiphenyls, the predicted solubility varies between 1.1 p.p.m. (2,4-dichlorobiphenyl) and 0.64 p.p.m. (2,4'-dichlorobiphenyl). Solubility is known to be a function of the total organic carbon (TOC) present in the aqueous phase. It is well known that they adsorb well to soils and sediments, with specific adsorption capacity governed by the particulate surface area and the total organic carbon content of the soil or sediment (Griffin and Chian, 1979; Lee *et al.*, 1979)

Table 1. Predicted solubilities of individual PCB congeners in water (data adapted from Dunnivant *et al.*, 1992)

PCB Congener	Solubility (p.p.m.)
2-MCB	5.8
3-MCB	4.8
4-MCB	0.76
2,2'-DCB	1.3
2,3-DCB	1.4
2,4-DCB	1.1
2,4'-DCB	0.64
2,5-DCB	1.5
2,6-DCB	2.6
3,3'-DCB	0.71
3,4-DCB	0.40
3,5-DCB	0.65
4,4'-DCB	0.02

ENVIRONMENTAL ACCUMULATION

It is estimated that 10×10^7 kg of PCBs still reside in the biosphere in the soil, air, rivers and waste streams, and lipoidal components of plants and animals (Boyle *et al.*, 1992). The average concentration of PCBs throughout US soils is 100 p.p.b., although this hides the true picture, with only a few p.p.b. present in rural areas, and up to 100 p.p.m. present in urban areas (Pal, Weber and Overcash, 1980). Of PCB-contaminated sludges disposed of in Indiana, only 15% exceeded the Environmental Protection Agency (EPA) 50 p.p.m. guidelines (Bergh and People, 1977). While this may seem to undermine the problem of PCB contamination, it must be realized that hot-spots of PCB contamination are a central concern and, due to the estimated capacitor life span of

20 years, incidents are going to increase. The PCB Spill Policy has data relating to frequency and magnitude of PCB transformer leaks; it has determined that 3.3% of such transformers will leak or spill every year and each spill comprises 66 lb of PCB (EPA Federal Register, Vol. 52). Furthermore, the EPA has recently determined that 'of the 1.6 million PCB capacitors in use in electrical substations (in US), EPA expects that 12 000 leak each year . . . Unlike PCB transformer spills, the majority of PCB capacitor spills involve the violent rupture of the capacitor and the spraying of PCBs . . . Available data indicates for over 80 percent of capacitor spills, PCBs are distributed as far as 11 feet from the centre of the spill.' (EPA Federal Register, Vol. 52).

The Organization for Economic Co-operation and Development (OECD, 1987) estimated that there were 12 000 tonnes of PCB waste in the UK. This figure is agreed in the revised Waste Management Paper No. 6 (Department of the Environment, 1994) and it is suggested that 4000–4500 tonnes have already been destroyed. Moreover, the most recent study into the deposition of PCBs, using archived vegetation samples covering the period 1965–89, showed a significant reduction of air contamination of PCBs. However, it was also noted that, in soils, the rate of deposition exceeds that of removal (Jones *et al.*, 1992).

TOXICITY

The toxicity of PCBs is a subject of much debate and research, and is related to many of the aforementioned factors which ultimately govern bioavailability. It is well known that PCBs bind covalently to DNA, both *in vivo* and *in vitro*. Discernible mutagenic activity is shown in rodents when PCBs containing 57% chlorine are studied. However, the acute toxicity may not reside in the PCBs *per se* but may be due to either the manufacture or transformation of by-products from use (Stone, 1992). Such supertoxic species have largely evaded detection due to the difficulties in analysing them (Storr-Hansen, 1991). However, it is well known that co-planarity of the chlorinated biphenyl is considered very important in acute toxicity (Huang and Gibson, 1992). This occurs in congeners which have no substitution at either of the *ortho* carbons (2,6 and 2',6'), allowing the molecule to assume a co-planar configuration. Chronic human toxicity has been well chronicled, with chloracne, pruritus, liver problems and reduced vital capacity of the respiratory system amongst the common symptoms (Kimbrough, 1980). Irrespective of the symptoms and whatever the modes of toxicity, the hydrophobicity of the congeners facilitate their accumulation in biological systems and potentiate the concern about their ultimate fate.

Bioaccumulation has been studied in bacteria (Hamdy and Lin, 1983), yeast (Cole, Reichart and Button, 1979) and fungi, but perhaps the most dramatic evidence of bioaccumulation comes from studies using oysters and fish. Aroclor 1254 (1 mg^{-1l}) has been shown to be concentrated 101 000-fold in oysters (Lowe *et al.*, 1972). Fish from the Hudson River, where GE manufactured transformers and purchased 75% of total US PCB output between 1966 and 1974, were able to concentrate Aroclor 1016 15 000-fold in only 14 days (Skea *et al.*, 1979). Bioaccumulation, like adsorption, is probably dependent upon the log P of the congeners (a measure of their hydrophobicity) and the steric effect coefficient (SEC), which is an indicator of degree and position of chlorine substitution. Obviously, this has more pronounced deleterious effects as you ascend the food chain, and the ultimate fate of the PCB is

largely dependent upon the potential of the organism to detoxify the compounds. This has been dealt with in relation to human toxicity in the submission made to the Welsh Affairs Committee (Johnston and Simmonds, 1989). Of the information currently available, detoxification requires two adjacent unsubstituted carbon atoms for the formation of the intermediate arene oxide (Matthews and Anderson, 1975).

ENVIRONMENTAL LEGISLATION REGARDING CONTAMINATED LAND AND WATER

PCBs are, along with dioxins, some of the worst of all contaminants. This is not necessarily directly related to human toxicity, although it may be regarded as such for dioxins. The main problems stem from the course of remedial action which must be taken when such pollutants are present. PCBs are extremely hydrophobic molecules and it may be agued that their health risk is not significant in terms of the possibility of getting into the water course.

The use of PCBs in the UK is restricted through the Control of Pollution (Supply and Use of Injurious Substances) Regulations 1986 SI No. 902, which defined PCBs at the 50 p.p.m. concentration level (as amended by the Environmental Protection [Controls on Injurious Substances] Regulations 1992 SI No. 31). PCBs are dealt with under the legislation on waste and special waste contained within the Control of Pollution Act (1974), Control of Pollution (Special Waste) Regulations (1980) and the Control of Pollution (Licensing of Wastes Disposal Regulations) (1976) (OECD, 1987). Additionally, a technical memorandum and Code of Practice for the disposal of PCBs and PCB-contaminated wastes has been published and is due for revision and update in 1994 (Department of the Environment, 1994). This forms part of the measures necessary to implement the European Community Directive for the disposal of PCBs and PCTs (76/403/EEC).

Legislation regarding contaminated land in the UK is extremely patchy for several reasons. Remediation is inextricably linked with end-use, such that final use as a children's playground demands more clean-up than if it were to be used as a car park. Therefore, it might be expected that trigger values have been assigned to such environmental pollutants, but this is not the case. Unlike The Netherlands (Moen, 1988), which favours multifunctionality (the remediation of land permitting *any* end-use), it is not clear what values must be attained in the UK. A value of 10 p.p.m. is suggested in Waste Management Paper No. 6 (1994) although the Department of Environment's Interdepartmental Committee on the Reclamation of Contaminated Land (ICRCL) does not have clear trigger values for PCBs related to end-use (ICRCL, 1987). Although this may seem an oversight, it must be realized that the setting of trigger values is somewhat arbitrary at the present time. Ideally, one would hope to have as full a toxicological profile as possible, but this is itself dependent upon many variables, including soil type, analytical systems employed and the presence of co-contaminants.

Treatment methods

INCINERATION

The incineration of PCBs and other classes of chemicals is an established disposal

technique. They are destroyed by high-temperature thermal oxidation. In the US the Toxic Substances Control Act (TSCA) requires a DRE (destruction and removal efficiency) of 99.9999% for thermal treatment systems. In the UK, the guidelines are set by the Department of the Environment through their Waste Management Paper No. 6. High temperatures and sufficient residence time are required to conform to the guidelines and destroy all organic components. Strict adherence to the guidelines is required to ensure that thermal oxidation to the even more problematic dioxins does not result.

Apart from liquid PCB waste, most decontaminations are probably point-source depositions in the soil. If these are large spillages, the cost of removal and disposal will be increased by the transportation costs to the incinerator. Unlike in the US, no transportable incinerators able to remove PCBs are operational in the UK. Presently there are three incinerators able to deal with PCBs in the UK. The largest of these is operated by Rechem in Panteg, South Wales. Disposal costs are estimated at approximately £1000 per tonne with the incinerator able to destroy 100–400 kg PCB per hour. The operating temperature of the kiln is 950°C, with an afterburner temperature of 1100°C. In the US, satisfactory destruction of PCBs is suggested to be achieved by incineration for 2 seconds at 1100°C with 3% excess oxygen. The incinerator (8 m × 4 m) operated by Rechem fulfils these requirements, operates with 6% excess oxygen and gives a final concentration of PCB in the stack of 2 μg m^{-3}, well below the occupational exposure limit of 1 mg m^{-3} (Health and Safety Executive, 1993). It was estimated that manufacturing waste arisings were 400 tonnes per year when the Department of the Environment produced Waste Management Paper No. 6 (1976). Within the past few years there has been considerable increase in the UK capacity to deal with chlorinated wastes and the total available capacity is now approximately 7000 tonnes per year (Waste Management Paper No. 6; Department of the Environment, 1994).

CHEMICAL METHODS

Since 1978 a family of alkali metal polyethylene glycolate (APEG) reagents have been developed which are reported to dechlorinate PCBs effectively and rapidly in soils and liquids. Reagents may be prepared as either the potassium or sodium derivatives. The potassium derivative is reportedly more effective for PCBs. The reagent is prepared by reacting a polyethylene glycol with potassium hydroxide. The resulting alkoxide reacts with a chlorine on the aryl ring, most efficiently at 60–100°C, to produce an ether and potassium chloride. Substitution of the chlorine with the ether-linked polyethylene glycol detoxifies the PCB (Amend and Lederman, 1992).

This would seem to oversimplify the problems of PCB degradation as commercial mixtures may be heavily substituted and not all chlorine atoms would be expected to be removed using this methodology. Moreover, addition of the polyethylene glycol will simply increase the water solubility of the derivatized PCB, aiding its removal from the contaminated media but still leaving an ether-derivatized chloroaromatic. Despite this, the method has been implemented in the US to treat soils contaminated with up to 3500 p.p.m. PCBs. It is not known whether the soils are destroyed in the process, but the removal of PCBs is of the order of 99%.

The problems of bioavailability of the PCBs have been alluded to and it is possible that this sort of methodology could be a useful initial step for other processes. Several

vendors offer this service (Kokozka and Flood, 1985) but, to the authors' knowledge, it is not available for use in the UK.

BIOLOGICAL METHODS

Historically, biological treatment has been favoured by industry for dilute aqueous solutions. Although incineration is cheaper for particular waste streams, and this is largely based upon the heating value, no environmental considerations are presently made. If a waste has an organic content of over 10%, it is usual for it to be incinerated. However, waste treatment costs are based on weight or volume and not on the actual waste content. Therefore, dilution of a waste may permit its treatment by biological systems, but this is not usually favoured because of the increased cost implications. Despite this, it has been estimated that if there is a choice between incineration and biological treatment, biodegradation costs are perhaps one-half to one-tenth those of incineration (League, 1990). Moreover, it was recognized in the First Report on Contaminated Land in the UK that bioremediation is 'the only true clean-up technique in use in the UK' (House of Commons, 1990).

The methodology used in biodegradation has developed rapidly since micro-organisms were first used for wastewater treatment approximately 100 years ago (Visscher and Brinkman, 1989). Although bioengineering has improved dramatically over the years, the number of reactor subgroups that exist are still relatively small. Up until 1980 the aerobic reactor option was much favoured but more recent research has switched focus to the better potential offered by anaerobic systems.

Water is the medium routinely used in biological treatment methodologies, whether it be in activated-sludge systems, anaerobic digesters, trickling filters or oxygenated lagoons. Such systems have been investigated for their potential to degrade xenobiotics. Irrespective of the system that is ultimately applied, it is the biodegradative capacity of the micro-organism that is the starting point for the development of a process.

Traditional biodegradation relied on the use of complex uncharacterized communities of organisms. This bioenrichment approach is still applied in most processes but it has sometimes been supplemented with a policy of bioaugmentation. This process differs from the former by the addition of one or more microbial strains which are specifically able to degrade a number of chemical components. Despite these additions, there is still overlap between enrichment and augmentation because the ultimate goal is mineralization, a process requiring a diversity of phenotypes. With this in mind, several processes have been formulated which use different units in series, or recycle the waste (Ng, Yap and Sivadas, 1989; Shivaraman and Parhad, 1990).

The immobilization of micro-organisms has facilitated the development of many processes. Such biofilm reactors include trickling filters (Alkins, 1981), submerged filters, rotating discs (Howard, 1990) and fluidized beds (Dillon, 1981). All of these are well suited to aerobic application, whereas downflow and upflow filters are more amenable to anaerobic operation (Krumme and Boyd, 1988). Much work has also concentrated on the support materials used in these operations. Activated sludge has little support and consists of acclimated organisms in a non-defined matrix. Use of a trickling filter allows the adhesion of the micro-organism to a variety of supports. A very thin film of waste is sprayed on to the filter bed and this facilitates good aeration and aerobic degradation.

The problems with bioremediation of contaminated soils are different in terms of the matrix but the problem of chemical recalcitrance is still pre-eminent. Many laboratory experiments have shown the degradation of target pollutants in soil microcosms and in field lysimeters (Mikesell and Boyd, 1988; Hill *et al.*, 1989; Havel and Reineke, 1993b). Such experiments have explored both bioaugmentation and bioenrichment as feasible options for field application. Additionally, freeze-dried bacterial cultures which are designed to degrade specific waste types are commercially available (Haley *et al.*, 1990). On the larger scale, one of the EPA site programme demonstrations includes a liquid/solid contact digestion system in which either sludges or soils containing between 2000 and 800 000 p.p.m. total organic carbon, from a wood-preserving facility, are degraded biologically in several stages. In this process, an acclimated bacterial seed is used along with the option to incorporate emulsifiers (EPA, 1989).

In the degradation of PCBs, the carbon–chlorine bond is the target. Although halocarbons exist in nature, they are not common (Suida and DeBarnardis, 1973; Lovelock, 1975; King, 1986). Despite this, it has been shown repeatedly that chlorinated xenobiotics may be broken down by microbial populations. The advancement of carbon–chlorine degradation has not been facilitated by the usual approach of indexing degradative pathways simply against the disappearance of the parent compound. This is best illustrated by studies of chlorinated solvents in ground water. Although such solvents are degraded by micro-organisms (Galli and McCarty, 1989a,b; Oldenhuis *et al.*, 1989) degradation may result in the formation of recalcitrant chloro-organics such as trichloroethylene and vinyl chloride.

The biodegradation of many haloaromatic species has been investigated and reviewed by Hardman (1991). More specifically, halogenated aliphatics (Vogel, Criddle and McCarty, 1987), substituted benzenes (Goulding, Gullin and Bolton, 1988), pentachlorophenol (Topp and Hanson, 1990), PCBs (Abramowicz, 1990; Boyle *et al.*, 1992), 2,4-dichlorophenoxyacetic acid (2,4-D) (Kelly, Hallberg and Tuovinen, 1989) and chloroguaiacols, chloroveratroles and chlorocatechols (Neilson *et al.*, 1987) have all been studied and reviewed. Despite the vast array of studies undertaken, it must be stated that the problems encountered in the degradation of low-level waste/chloroaromatic compounds are related to questions of bioavailability and the build-up of intermediates.

Obviously, metabolic versatility is a key attribute when isolating organisms able to remediate a particular waste stream. To this end, pseudomonads are often cited as remarkably catholic organisms. This has been further embraced by the work of Reineke and colleagues (Hartmann *et al.*, 1989; Brunsbach and Reineke, 1993; Havel and Reineke, 1993b). Other organisms attracting a great deal of attention are the white rot fungi (most notaby *Phanerochaete chrysosporium*). These organisms have been reviewed extensively by Bumpus *et al.* (1990) and their use in appropriate bioreactors for hazardous waste remediation has been chronicled (Lewandowski, Armenants and Pak, 1990).

Analysis of PCBs

As has already been stated, PCBs were sold as mixtures of congeners. The largest manufacturer was Monsanto with their Aroclor range. The latter were sold and

characterized according to the percentage of chlorine (by weight). The most popular was Aroclor 1242 which contains 42% chlorine. Other products contained as much as 60% or as little as 21% chlorine. As many as 70–80 congeners may exist within these mixtures and these differ only in the number and position of the chlorine substituents. These factors hinder the complete elucidation of metabolic events that may occur in aerobic or anaerobic systems. This is further compounded by the heterogeneous nature of the matrices from which many of these PCBs are extracted. New developments in thermal desorption chromatography permit the PCBs to be concentrated on to selective adsorbents using steam distillation and then to be thermally desorbed on to the analytical column (Brown *et al.*, 1991). This facilitates clean-up and reproducibility but is not commonly used at the present time.

When analysing the metabolic events occurring in the degradation of PCBs by micro-organisms, two main parameters are usually quantified. Both the PCBs and the released chloride are usually measured. Despite this, the problems inherent in the analysis of PCBs have yet to be completely overcome. Two systems, gas chromatography (GC) and high-pressure liquid chromatography (HPLC), are commonly employed for the routine analysis of PCBs and these have recently been supplemented with the wider use of gas chromatography–mass spectroscopy (GCMS) for both routine and in depth analysis.

Gas chromatography is by far the most common method of analysis used at the present time. The development of capillary columns has facilitated the resolution of complex chemical mixtures including the PCBs. However, the major benefit in the use of GC is the ability to couple the increased resolving power with the tried and tested detectors that are available. Two main detectors, excluding mass spectrometry (MS), are routinely used and each has advantages and disadvantages.

By far the most common general-purpose detector is that employing flame ionization (FID). The response from this detector is related to the thermally excited ions produced in a hydrogen/air flame and is directly related to the number of burnable carbon atoms. A fundamental problem with the use of the FID is the necessity to undertake extensive clean-up when sampling directly from the environment. This is necessary as soils and sediments contain many organic species that will give a response in the FID and are likely to give erroneous results when analysing the full spectrum of PCB congeners. Although the FID gives a response related to the number of carbon atoms in the nucleus, and hence gives a theoretically equal response per biphenyl molecule, it has been shown that the sensitivity is a thousand-fold less than either the electron capture (EC) or Hall electrolytic conductivity (HEC) methods of detection.

The most widely used method of detection of PCBs, or halogenated compounds in general, is the electron capture detector (ECD). It is specific for sample constituents which exhibit an affinity for electrons and a low response to other types of sample material. It contains a radioactive source (^{63}Ni) which ionizes the carrier gas and the electrons are collected at the detector electrode. Obviously, if the chemical species under investigation has an affinity for electrons it will reduce those being detected at the collector electrode. It differs from the Hall electrolytic conductivity detector (HECD) which measures the HCl formed from the parent molecule. The HECD is not widely used but does give a response directly related to the number of chlorine atoms present on the parent molecule. This is not true of the ECD which gives a dispropor-

tionate response that is not related to the degree of chlorination. Moreover, the ECD response is also a function of the position of the chlorine atoms, which is not predictable.

Despite the availability of detectors which are sensitive to PCBs there are several fundamental problems:

1. What to choose as the internal standard when using ECD.
2. How to resolve co-eluting peaks.
3. Distinguishing contaminants from true PCBs.
4. Identifying the non-*ortho* substituted, and hence the most toxic, congeners.

The choice of reference standards is of crucial importance. The disproportionality in ECD response is quite marked over the congener range, as illustrated in *Figure 2*. Furthermore, the choice of internal standard is restricted to those 'like-compounds' which are not metabolized by the organism or consortia under investigation. Octachloronaphthalene has been used extensively but gives a marked difference in response compared to the mono- and dichlorinated biphenyls. Currently, the pentachlorinated biphenyl 246-24 is used widely because it is not metabolized, is absent from the commercial mixtures, and does not co-elute with any other congener. This allows it to be used without interference from constituents, in the knowledge it will not be degraded, but does not circumvent the problems associated with disproportionality of response.

In reality, the problems of variable congener response and co-elution can only be tackled by establishing the linearity of response for each congener or by approximation using a multi-point calibration curve and internal standards. Fava, Cinti and Marchetti (1993) have used this approach with a four-point calibration plus the use of octachloronaphthalene and lindane as internal standards. Additionally, the efficiency of soil extraction was assessed using PCB 75 (246-4). The standard methodology employed in clean-up and analysis is that adopted by Quensen, Boyd and Tiedje (1990). Soils or sediments are extracted in the presence of octachloronaphthalene and are analysed relative to an autoclaved control. In this case it was an anaerobic dechlorination that was studied and was assessed by the removal of *meta* and *para* chlorines. The assumption was made that co-eluting isomers occur in equal proportions, and all co-eluting congeners increase or decrease to the same extent. The effect of this assumption was evaluated using two worst case scenarios and was shown to result in a maximum 10% error when studying heavily chlorinated mixtures (e.g. Aroclor 1260).

Although quantification of individual congeners relies on certain assumptions being made, it is well established that the order of congener elution and composition of the mixtures is fairly reproducible. *Table 2* outlines a selection of the congener profiles and peak assignments for Aroclor 1242 that have recently appeared in the literature. It can be seen that, apart from that outlined by May *et al.* (1992), they are fairly consistent, with co-eluting peaks largely unchanged. The surprising omission of the trichlorinated biphenyl 25-2, which forms 9% (w/w) of 1242, is unexplained. Despite this, the literature available on PCB traces permits a predictive determination of the qualitative presence of individual congeners without a requirement for the use of individual standards in the first instance. Obviously, complete quantification of the events occurring when PCBs are degraded does require individual standards, most of

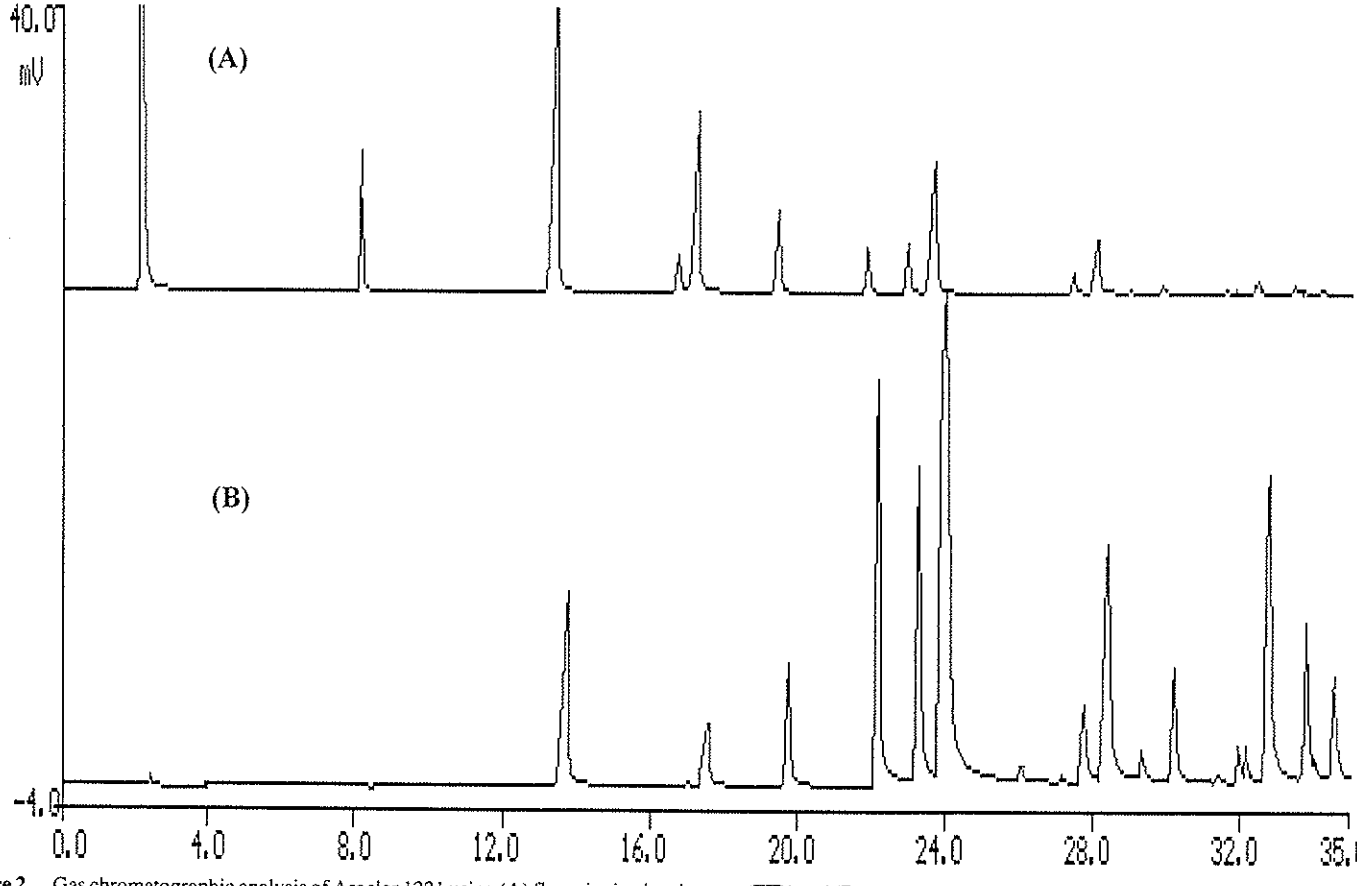

Figure 2. Gas chromatographic analysis of Aroclor 1221 using (A) flame ionization detector (FID) and (B) an electron capture detector (ECD). Using FID analysis, the relative peak areas for the different congeners reflect the relative proportions of the congeners in the mixture. The ECD does not respond to biphenyl, and generally the response increases with increasing chlorination. Note that the trace contaminants in the mixture, eluting between 29 and 35 minutes, produce a very strong signal with the ECD.

Table 2. Summary of peak assignments given in the literature

	A Pk assignation		B IUPAC No.	C Congener	D % in 1242	
1	2	2	4*	2–2	3.99	
1	2	2	10	26	0.13	2.7
2	3	3	7*	24	1.04	
2	3	3	9	25	0.31	0.9
3	4	4	6	2–3	1.24	1.2
4	5	5	5	23	0	
4	5	5	8*	2–4	8.97	5.8
5	6	6	19	26–2	0.97	0.9
6	7	Absent	18	25–2	9.36	8.7
7	7	Absent	15	4–4	0.99	
7	8	8	18*	24–2	2.92	4.3
8	9	9	24	236	0	
8	9	9	27	26–3	0.54	0.7
9	10	10	16	23–2	3.25	
9	10	10	32	26–4	2.15	4.8
10	Absent		34	2–35	0	
11	Absent		29	245	0	
12	11	11	26	25–3	0.55	1.1
13	12	12	25	24–3	1.68	7.0
14	14	13	28*	24–4	13.30	6.5
14	13	13	31	25–4	4.53	7.0
15	15	15	20*	23–3	3.64	
15	15	15	33*	2–3.4	2.83	
15	16	15	53	25–26	0.97	3.3
16	18	16	22*	23–4	2.64	
16	17	16	51	24–26	0	0.9
17	19	17	45	236–2	0.90	0.5
18	20	18	46	23–26	0.31	0.31
19	21	19	52	25–25	4.08	3.1
20	23	21	47	24–24	1.65	1.1
20	22	20	49	24–25	3.28	2.6
21	24	22	48	245–2	1.33	1.4
22	25	Absent	44	23–25	1.08	3.4
23	26	24	37*	34–4	1.62	
23	27	24	42	23–24	0	3.7
24	28	25	41*	234–2	1.67	
24	28	25	64	236–4	0	
24	28	25	71	26–34	0	3.0
25	29	26	40	23–23	0.15	0.9
26	Absent		67	245–3	0	
27	Absent		58	23–35	0	
27	Absent		63	235–4	0	
28	30	27	74	245–4	2.02	1.9
29	31	28	70	25–34	1.11	3.9
30	32	29	66	24–34	0.81	
30	33	29	95	236–25	0.53	4.8
31	34	30	91	236–24	Trace	1.3
32	35	31	56*	23–34	0.60	
32			60	234–4	0.21	2.9
32			89	234–26	0	
33			90	235–24	0	
33			101	245–25	0.27	1.1
34			99	245–24	0.55	0.55
35			112	2356–3	0	
36			83	235–23	0	
37			97	245–23	0	
38			87	234–25	0.09	1.0

39	85	235–24	0	0.7
40	110	236–34	0	1.1
41	82	234–23	0	0.7
41	151	2356–25	0	
42	106	2345–3	0	
42	123	345–24	0.36	
42	149	236–245	0	0.7
43	105	234–34	0.25	1.0
44	138	234–245	0	
44	160	23456–3	0	
44	163	2356–34	0	

Column A refers to the elution order. Each of the digits relates to the peak assigned in each of three references. The first figure is the order assigned by Barriault and Sylvestre (1993), the second is the order assigned by Nies and Vogel (1991), the third is the order assigned by May *et al.* (1992). Column B refers to the IUPAC no. assigned to each congener with the position of the chlorines outlined in column C (* = major peak). Column D indicates the % (wt) of each congener in Aroclor 1242. The first figures are those of Kimbrough (1980) and the second figures are those of Lajoie *et al.* (1993). Shading relates to co-elution of congeners according to Lajoie *et al.* (1993).

which are commercially available. Of the intermediates expected to be formed from polychlorinated biphenyls, chlorobenzoates have been detected using GC after derivatization (Gerritse, van der Wande and Gottschal, 1992).

Liquid chromatography (particularly HPLC) has been used extensively for the detection of PCBs, chlorobenzoates and chlorocatechols. Resolution of the congeners is achieved on the basis of column selection and solvent system. The most common detection system employs an ultraviolet (UV) detector to analyse the signal from the aromatic nucleus. Although sensitive, the samples must be clean and it does not offer the same predictable resolution outlined for gas chromatography. The main benefit is in the detection of chlorobenzoates and chlorocatechols, but problems of co-elution persist with these groups of compounds.

Chloride release is an excellent indicator of PCB degradation but requires a high degree of sensitivity and little background interference. Many methods are available, ranging from simple colorimetric systems to complex liquid chromatography systems. The methods are surveyed in *Table 3* and each has advantages and disadvantages. None of the methods outlined should be used in isolation, but when coupled with GC or liquid chromatography (LC) they are invaluable in the elucidation of metabolic pathways involving chloro-organics.

The definitive identification of individual PCB congeners within mixtures of congeners would aid considerably the quest for methods of eradicating PCBs. As

Table 3. Summary of some of the methods available for the detection of chloride

Basis of assay	Sensitivity
End-point titration with silver ions[1]	0.1–100 p.p.m.
Atomic adsorption[1]	50 p.p.b.
Modified Mohr titration[2]	1.5–15 p.p.m.
X-ray fluorescence spectrophotometry[2]	40 p.p.b.
Ion chromatography[3]	40 p.p.b.
Ion chromatography with electrical conductivity detector[3]	1 p.p.b.
Colorimetric[4]	350 p.p.m.

Sources: 1 *Analytical Chemistry* **47**, 343 (1975); 2 *Analytical Chemistry* **63**, 318 (1991); 3 *Analytical Chemistry* **61**, 284 (1989); 4 *Analytical Chemistry* **29**, 241 (1957).

outlined above, all of the analytical methods are deficient in some way. To this end, mass spectrometry, when coupled to gas chromatography (GCMS), has aided PCB research. Full use is often made of the isotopic distribution of chlorine (75.53% ^{35}Cl and 24.47% ^{37}Cl) in identifying ion clusters corresponding to the degree of chlorination. Despite this, GCMS should not be thought of as an analytical tool that has no drawbacks. Irrespective of the detector that is used, the resolution of the individual congeners determines the success of the methodology used thereafter. Therefore, although GCMS may definitively identify isolated congeners that do not co-elute, it is very difficult to identify those that do. Electron impact (EI) spectra of PCBs have been studied extensively by Safe and Hutzinger (1971, 1972) and it has been shown that co-eluting peaks have fragmentation patterns related to the expulsion of chlorine atoms. Therefore, if a peak consisted of three co-eluting congeners and these differed in the number of chlorine atoms they possessed, it would be difficult to distinguish them apart from the most chlorinated congener. Cairns and Siegmund (1981) further developed mass spectrometry with PCBs, using methane to protonate the molecular species. Only a very small percentage of ions (<2%) corresponded to the loss of HCl from the molecular species, causing a problem of identification when investigating the lower homologues. This technique permits instant analysis of each PCB mixture on the basis of the distribution of molecular weights. Therefore, it is possible to specify a particular *m/z* value and get a profile within any eluting peak. This permits the degradation of each congener to be assessed so long as the peak compositions are unequivocal. As with other methods, this again suffers from a disproportionality of response across the congener range. Highly chlorinated congeners have less sites available for protonation and sensitivity is therefore reduced.

In conclusion, the quantitative analysis of PCBs is a fundamental obstacle which must be overcome if their metabolism is to be fully understood. This is best illustrated by an American study which investigated the accuracy and precision of PCB analysis by accredited laboratories. For a prepared sample at 100 p.p.m. PCB, relatively high by environmental standards, 30% of laboratories out of a total of 120 would have classified the waste as not special, i.e. < 50 p.p.m. (Milby, Miller and Forrester, 1985).

The choice of technique, reference standard and clean-up method all have a bearing on the level of degradation which is finally reported. The advent of capillary columns has facilitated the resolution of many PCB congeners, but there are still many problems left to solve. Mullin *et al.* (1984) published an excellent paper which reported the separation of 187 PCB congeners using a 50 m capillary column. Despite this, it was reported that 11 pairs of compounds were not fully resolved and, although general trends existed within the congeners, it was not possible to predict separations. In general, relative retention time (RRT) increased with increasing chlorine content, but within each isomer group there were large variations in the magnitude of the GC response. Only when all congeners are resolved will it be possible to standardize conditions for the identification and quantification of any of the PCBs that may be present in environmental samples.

Bioavailability

The bioavailability of a compound may be defined as that fraction of the compound which may be acted upon by a biological system. For the purpose of this chapter we

will restrict the discussion to that fraction which may be available to micro-organisms. Ordinarily, microbial systems are investigated in the aqueous phase with water-soluble, readily available substrates. The limitations in such a system are the transport of the substrates across the biological membrane and their utilization by either inducible or constitutive enzyme systems.

PCBs are extremely hydrophobic molecules which adsorb strongly to virtually any type of matrix. When studied in the laboratory, either as single congeners or as mixtures, they are either supplied directly to the aqueous phase and allowed to equilibrate, or are added as a concentrated stock solution in an appropriate water-miscible solvent (acetone or methanol). Although both of these techniques are accepted as standard, each gives rise to different concentrations and compositions in the aqueous phase. Murphy, Mullin and Meyer (1987) conducted equilibration studies with PCBs after allowing them to equilibrate in the water phase, but they showed that preparation by addition of solvent results in an altered composition. Each of the components of a PCB mixture will become soluble in the aqueous phase until they are saturated. Therefore, if added as a solvent solution, the least soluble components will become saturated first and give rise to a second phase composed of insoluble PCBs. This will retard the partitioning into the aqueous phase and give rise to aqueous solubilities well below those reported by equilibration. Furthermore, the mixtures in the environment are present as sub-cooled liquids and are extremely temperature dependent. Therefore, even in the simplest laboratory study into PCB degradation, toxicity and solubility of mixtures may be problematic. As an indicator, data are available on solubility, vapour pressure and Henry's Law constants for the whole congener range (Murphy, Mullin and Meyer, 1987; Dunnivant *et al.*, 1992).

The main impetus behind the study of PCBs is their recalcitrance and toxicity in the environment. In natural water systems, toxic effects arise because of their accumulation by many biological systems. Movement into the atmosphere has not received significant attention, but data from Diskey and Andren (1981) and Rice, Eadie and Ertsfeld (1983) have suggested that in Lake Michigan there appears to be transfer from the water to the atmosphere. This has given rise to the conclusion that the atmosphere, rather than the sediments, is the major sink for PCBs in Lake Michigan and similar bodies of water (Murphy, Mullin and Meyer, 1987).

Irrespective of the above, it is generally accepted that the greatest problem associated with PCB contamination is due to soil and sediment contamination. Sorption of organics has attracted much attention but little definitive experimentation. It is widely accepted that sorption limits the availability of compounds for degradation, but studies in the field have not been unanimous in their conclusions (Gordon and Millero, 1985; Ogram *et al.*, 1985; Rijnaarts *et al.*, 1990). This is entirely understandable when one considers the multiplicity of variables that govern bioavailability. In the broadest context, and without alluding to the factors already mentioned, bioavailability must be governed by the physico-chemical structure of the sorbate, the nature of the sorbent, the mechanism of sorption, the factors governing equilibration and the properties of the degradative organisms.

PCBs have a high affinity for suspended solids, which is increased significantly in the presence of increased amounts of organic carbon (Hamelink, Waybrant and Ball, 1971). PCBs are usually associated with soils or sediments in a soil–water system and the sorption of PCBs by various earth materials is shown in *Table 4* (Griffin and

Table 4. Effect of surface area (SA), total organic carbon (TOC) and type of earth material on the sorption of PCBs

Sorbent	Sorption constant (K)	TOC (%)	SA ($m^2 g^{-1}$)
Ottawa silica sand	22	<0.1	0.4
Montmorillonite clay	172	0.93	21.1
Montmorillonite clay (LTA)	145	0.13	20.2
Catlin silt loam	532	4.73	26.5
Catlin (6 h) LTA	472	4.37	25.4
Catlin (12 h) LTA	310	3.64	24.5
Catlin (336 h) LTA	239	1.84	23.8
Medium temp. coal char 650°C	1938	74.04	253
Medium temp. coal char 650°C (LTA)	1432	64.00	214
High temp. coal char 980°C	1220	76.62	44
High temp. coal char 980°C (LTA)	1174	32.14	120

LTA denotes low-temperature ashed samples. Data from Griffin and Chian (1979).

Chian, 1979). The amount of PCBs sorbed by the earth materials could be related to the equilibrium concentration of PCBs and could be described by the simple linear relation:

$$x/m = KC$$

where x = micrograms of compound sorbed, m = weight of adsorbent (g), C = the equilibrium concentration of PCBs in solution ($\mu g\ ml^{-1}$) and K = sorption constant ($ml\ g^{-1}$). It can be clearly seen that the sorption of PCBs is directly related to the total organic carbon and that low-temperature ashing significantly reduces sorbed PCBs. As might be expected, the more hydrophobic, heavily chlorinated congeners adsorb much more than the lighter, chlorinated congeners (Haque and Schmedding, 1976; Lee *et al.*, 1979). Furthermore, the work of Lee *et al.* (1979) established a direct relationship between TOC and the sorption constant (K) as follows:

$$K = 255 + 18.5\ \text{TOC}.$$

This was further developed to include surface area (SA) as a variable, giving rise to the linear regression relation of:

$$K = 188 + 3.36\ \text{SA} + 11.4\ \text{TOC}.$$

Aroclor 1242 was also studied and the sorption constant (K_{OC}) normalized for the organic carbon content, had a value of 10 725. Moreover, it was possible to express the linear regression relationship as:

$$K = 12.43 + 4.06\ \text{SA} + 73.45\ \text{TOC}.$$

It can be seen that, of the two coefficients (SA and TOC), it is the total organic carbon content of the soil that largely governs sorption.

Chlorobenzoates and chlorocatechols, which are products of the biodegradation of PCBs, are far more soluble in water than the parent compounds. Solubility figures produced by Dolfing and Harrison (1992) suggest that, apart from 4-chlorobenzoate, all of the mono- and dichlorinated benzoates are soluble to levels > 2.5 mM. It might therefore be expected that sorption of these chemical species is insufficient to limit their bioavailability. Further data relating to the movement of these chemical species

in different soils would be beneficial when investigating the mineralization of congener mixtures.

The movement of Aroclors in soils has been studied with respect to both the soil characteristics and the mobile phase. Using soil thin-layer chromatography (TLC), Griffin and Chian (1979) have shown that Aroclors 1242 and 1254 are virtually immobile when either water or landfill leachate is the solvent. However, if an organic solvent such as acetone, methanol, benzene or carbon tetrachloride is used, the whole congener range is eluted. Several studies have established how far PCBs move within soils of different types. It can be concluded from these studies that PCBs are not significantly leached by water. Furthermore, the distance migrated may be related to the clay and/or organic carbon content (Suzuki, 1975; Tucker, Litschgi and Mees, 1975; Moza, Weisberger and Klein, 1976).

All of the factors that have been outlined above pertain to either sorption or aqueous solublity. This permits the use of predictive techniques to ascertain the bioavailability of organic compounds. Despite this, soils are very heterogeneous environments and consist of aggregates which vary in size. Most organisms exist on the outside of aggregates and in the small pore spaces between them. Electron microscopy has facilitated the study of these microenvironments and it has been estimated that micro-organisms occupy less than 1% of the total available pore space (Paul and Clark, 1989). Therefore, pore neck size and water content of the pore are also determinants of bioavailability. Furthermore, a recent study by Guerin and Boyd (1992) concluded that, all physical and physico-chemical factors aside, there are important organism-specific properties which make generalizations regarding the bioavailability of sorbed substrates inappropriate. It is therefore necessary to plan strategically with end-use being a major factor.

Microbial degradation of PCBs and their intermediates

The degradation of aromatic organochlorines occurs via four well-characterized mechanisms, as follows:

1. Oxygenolytic dechlorination

This occurs via dioxygenative attack on the aromatic nucleus and is dependent upon the position of the chlorine substituents. The oxygenolytic cleavage of the chlorine atom occurs fortuitously when both atoms of molecular oxygen are incorporated into the aromatic nucleus. PCBs are principally hydroxylated on the unsubstituted carbons at positions 2 and 3 and at unsubstituted 3,4 positions. In either case, the mechanism seems to require the presence of adjacent unchlorinated atoms.

2. Hydrolytic dechlorination

In this reaction the incorporation of a hydroxyl group leads to the concomitant release of a chlorine substituent. Unlike the above, the hydroxyl group is derived from water and the reaction is catalysed by a halidohydrolase-type dehalogenase. The requirement for water rather than molecular oxygen also permits this dechlorination to occur in anaerobic, denitrifying conditions.

3. Reductive dechlorination

Reductive dechlorination results in the removal of the chloride substituent with the concurrent addition of electrons to the molecule. Although it has been reported to occur in both aerobic and anaerobic environments, it has been shown to occur primarily in the latter.

4. Chloride release after cleavage of the aromatic ring

The present chapter will deal with all the mechanisms outlined above. Starting with anaerobic degradation, both anaerobic and aerobic degradation will be discussed. This will be related to the metabolism of the three primary classes of compounds involved in the degradation of polychlorinated biphenyls, namely PCBs, chlorobenzoates and chlorocatechols.

ANAEROBIC DEGRADATION

Polychlorinated biphenyls (PCBs)

Historically, it was anticipated that anaerobic environments were a sink for the more heavily chlorinated PCB congeners. Using both marine muds (Carey and Harvey, 1978) and silage (Fries, 1971) it was concluded that anaerobic environments did not contribute significantly to the degradation of PCBs. Since that time, many other workers have studied the anaerobic degradation of PCBs and have shown that this supposition is not valid. It must be noted that evidence of the anaerobic degradation of the biphenyl nucleus has not been demonstrated. Chen *et al.* (1988) indicated the production of $^{14}CO_2$ when Hudson River sediments were incubated anaerobically with ^{14}C-labelled monochlorobiphenyls. To the authors' knowledge, this has not been shown subsequently and has not been reported by other workers.

The role that anaerobes play in the degradation of PCBs was first shown by Brown *et al.* (1987). Again using Hudson River sediments, they showed that the congener distribution of a known PCB mixture altered radically over a long period of time. The primary finding was that highly chlorinated PCB congeners were extensively removed, giving rise to a much higher proportion of the lower-chlorinated congeners. In all sediments, the levels of 26–2, 26–3 and all dichlorinated biphenyls increased two- to six-fold, and the level of 2-chlorobiphenyl increased seven- to seventy-fold. These findings should be considered in the light of the disproportionality of congener response of the electron capture detector outlined earlier. Despite this, it is clear that the transformations observed specifically involved the removal of *meta* and *para* chlorines. Moreover, the central finding of this and subsequent work was that congener dechlorination is specific for the particular sediment from which it is derived. Three different groups have made advances in the study of anaerobic dechlorination of PCBs (Quensen *et al.*, Abramowicz *et al.* and Vogel *et al.*). Moreover, Quensen and co-workers have shown that sediment derived from the Hudson River dechlorinates a range of congeners in Aroclors 1242, 1248, 1254 and 1260. However, a consortium that is completely devoid of sediment and able to anaerobically dechlorinate PCBs has, to our knowledge, never been shown.

Four patterns of reductive dechlorination have previously been shown to occur in anaerobic sediments:

1. *meta* removal – shown in Hudson River, Sheboygan River, Lagoons and Silver Lake;
2. *para* removal – shown in Hudson River, Saginaw River;
3. *meta* and *para* removal – shown in Hudson River;
4. *ortho* removal – shown in unacclimated pond sediment.

It must be noted that the incidence of the first three classes far outweighs that of the fourth, which was only recently shown using 2356-tetrachlorobiphenyl (Van Dort and Bedard, 1991). The frequency of dechlorination (*meta* > *para* > *ortho*) follows that suggested by Tiedje *et al.* (1993) in their review of reductive dechlorination. The ability to distinguish between populations able to dechlorinate PCBs was first shown by Ye *et al.* (1992). Using Hudson River sediments, they demonstrated that heat- or ethanol-treated samples, for enrichment of spore formers, did not produce methanol and preferentially removed *meta* chlorines. Untreated samples removed both *meta* and *para* chlorines, showing that sediment treatment eliminated the population responsible for *para* dechlorination.

The range of congeners that may be degraded by the anaerobic populations cannot easily be predicted, although there appears to be some dispute between workers in the area. Quensen, Boyd and Tiedje (1990) suggested that dechlorination occurs only with up to heptachlorinated substitutions, whereas Abramowicz (1990) favoured the dechlorination of octachlorinated biphenyls. In our view, a threshold is unlikely to exist considering the phenotypic diversity of microbial populations outlined above. It is clear, however, that as the degree of chlorination increases, so does the probability of reductive dehalogenation. Moreover, as the proportion of mono- and dichlorinated biphenyls increase, the role of anaerobes is reduced.

From a toxicology standpoint, the dechlorination of *meta* and *para* chlorines is very advantageous. It will be recalled that the most toxic PCB congeners (34–34, 345–34 and 345–345) are those which are co-planar, possessing no *ortho* chlorines. These congeners are structurally similar to 2378-tetrachlorodibenzo-*p*-dioxin and exhibit dioxin-like toxicity. They are a central target for the anaerobic degradative mechanisms and consequently their removal leads to risk reduction. Those possessing *ortho* chlorines have not been extensively tested, but it is suggested in the review by Tiedje *et al.* (1993) that overall toxicity has been demonstrably reduced using both toxicity bioassays (Giesy, Jones and Mora, 1992, unpublished, quoted by Tiedje *et al.*, 1993) and by congener-specific tandem quadrapole mass spectrometry (Quensen *et al.*, 1992). However, despite the conclusion drawn by Tiedje *et al.* (1993), we believe more extensive toxicological testing is required in this area because it is quite clear that *ortho*-enriched congeners are a major component of commercial Aroclors, with Aroclor 1242 containing > 85% *ortho*-substituted congeners.

More than 50 organochlorine compounds have been shown to be reductively dechlorinated (Tiedje *et al.*, 1993). It is widely believed that anaerobes, from whichever environment, utilize chlorine as the terminal electron acceptor, with the addition of the electron to the aryl carbon–chlorine bond. Subsequently, the chloride is lost and hydrogen is abstracted. Of the potential primary electron donors (water, hydrogen or unspecified organic compound), Nies and Vogel (1991) provided

evidence that the proton source was water. Using a deuterium label, the authors showed that the dechlorination of 23456-pentachlorinated biphenyl was concomitant with the incorporation of the label. However, other sources of reducing equivalents should not be discounted. Krone *et al.* (1989) have shown that vitamin B_{12}, a known hydride-transfer agent, can catalyse the reductive dechlorination of certain chloromethanes. Mohn and Tiedje (1992) offered several possible routes to reductive dechlorination and concluded that direct electron addition and nucleophilic attack is involved. This could involve transition metal-containing coenzymes, hydride ions or NADH.

Chlorobenzoates

Chlorobenzoates are the most noted intermediates in the aerobic degradation of polychlorinated biphenyls. In many ways they also mirror the degradation of the parent compounds, with a diversity of mono-, di- and trichlorinated congeners possible. However, unlike the parent compounds (PCBs), it may be argued that they are of more importance in that the problems presented by individual congeners are magnified as they are channelled towards fewer central chlorinated intermediates.

The anaerobic degradation of chlorobenzoates was first shown by Horowitz, Suflita and Tiedje (1983) and Suflita, Robinson and Tiedje (1983). The latter studied the reductive dehalogenation of halobenzoates and demonstrated that a methanogenic consortium from sludge was able to dehalogenate iodo-, bromo- and chlorobenzoates, but not fluorobenzoates, and showed a specificity towards the *meta* position of chlorobenzoate. Shelton and Tiedje (1984) isolated, and DeWeerd *et al.* (1990) characterized an organism, *Desulfomonile tiedje* strain DCB1, from this consortium, which has become a benchmark in the field of reductive dehalogenation of aromatic compounds. With this bacterium as the key dechlorinating organism, a defined 3-chlorobenzoate-degrading methanogenic consortium was constructed and shown to be able to grow with 3-chlorobenzoate as the sole carbon and energy source (Dolfing and Tiedje, 1986). This finding established the fundamental precedent and underlying biochemical mechanism, which showed that micro-organisms can grow using chlorinated substrates as their sole electron acceptor. In the consortium, H_2 was the source of reducing equivalents and the sole function of *D. tiedje* was to furnish benzoate from 3-chlorobenzoate. It must be stressed that no energy-yielding reactions other than reductive dechlorination are known for this organism, leading to the conclusion that *D. tiedje* DCB1 is able to conserve energy from the dechlorination reaction (Dolfing and Tiedje, 1987). More recently, the isolate was used in axenic culture to show that (1) the cell yield was higher with 3-chlorobenzoate than with benzoate, and (2) the level of ATP was higher in 3-chlorobenzoate-metabolizing cells compared with benzoate-metabolizing cells (Dolfing, 1990; Mohn and Tiedje, 1990). Having established the precedent, much research has focused on the ubiquity of micro-organisms able to utilize chlorinated organics as electron acceptors in anaerobic environments. Despite theoretical studies (Dolfing and Harrison, 1992) which have established the potential of chloroaromatics to serve as electron acceptors, we are unaware of any other pure isolates that have been proven to derive energy in a manner analogous to *D. tiedje* DCB1.

As with polychlorinated biphenyls, the characterization of anaerobic

chlorobenzoate-degrading microbial consortia has lagged behind the establishment of reproducible precedents for site and congener-specific dechlorination. Gerritse, van der Wande and Gottschal (1992) have shown *ortho* dechlorination, and have outlined some of the arbiters of dechlorination. Although the *ortho* and *para* positions might be expected to be most susceptible to dechlorination, the presence of the carboxyl group favours the following order of dechlorination (*meta* > *ortho* > *para*). Despite this generality, *ortho* dechlorination is by no means commonplace. Gerritse, van der Wande and Gottschal (1992) have shown *ortho* dechlorination, but this was achieved at the expense of long incubation times (50% dechlorination of 246- to 24-dichlorobenzoate took 400 days). Moreover, this consortium was unable to further dechlorinate monochlorinated benzoates. These findings further illustrate the specificity of individual consortia and potentiate the belief that predictive ground-rules will be very difficult or impossible to formulate.

Chlorocatechols

Surprisingly, very little research appears to have been done on the anaerobic degradation of chlorocatechols. This may, in part, be due to the reliance on aerobic mechanisms of degradation for this class of compounds. Alternatively, in the context of PCB biodegradation, it may be expected that reductive, or other methods of dechlorination, will precede the incorporation of dioxygen into the aromatic nucleus. This would yield mono- or dichlorocatechols, thus restricting the opportunity to utilize anaerobes for their degradation. As with PCBs and chlorobenzoates, metabolically stable enrichment cultures are isomer specific. Allard *et al.* (1991) have studied microbial consortia from the Gulf of Bothnia and the Baltic Sea (Neilson *et al.*, 1987, 1988). A systematic examination of all possible chlorocatechols with a range of anaerobic consortia revealed that certain chlorocatechols (3,4; 3,6 and 3,4,6) were resistant to dechlorination under the test conditions. Moreover, even for the chlorocatechols which were dechlorinated, differences in both the rate of reaction and yield were observed. In the longer term, two further pitfalls in the use of these consortia were suggested: (1) over several years the rate of dechlorination was substantially reduced; and (2) the products of certain dechlorinations were altered.

In conclusion, it can be seen that the anaerobic degradation of chloroaromatics has received substantial attention. However, it must be concluded that the fate of PCBs, chlorobenzoates and chlorocatechols, under anaerobic conditions, cannot at present be resolved on a general and rational basis. Apart from the obvious environmental factors (pH, temperature, nutrient availability, bioavailability and presence of co-contaminants) several determinants are common to each class of compound:

1. The rate of anaerobic dechlorination is often, but not always, a function of the number and position of the chlorine atoms.
2. The concentration of the aryl chloride may determine the acclimation period (PCBs are very slowly degraded at < 50 p.p.m. compared with 100–1000 p.p.m.).
3. Consortia are often undefined and uncharacterized and inextricably associated with the matrix from which they originate.
4. Consortia are isomer/congener specific.

It is difficult to attribute the level of importance of each of the above and to

understand their interactions. However, it is clear that each contributes to the acclimation period. A specific study of this process, using chlorobenzoates as target compounds, has shown that the acclimation period is inversely related to the subsequent rate of dehalogenation (Linkfield, Suflita and Tiedje, 1989). These authors identified induction, genetic change, diauxy and the selection of a rare population as possible explanations. Ultimately, the authors identified induction as the most likely arbiter, in the system they studied, but it seems equally feasible that the other factors have a role to play in other cases.

AEROBIC BIODEGRADATION OF PCBS AND THEIR INTERMEDIATES

There have been many reports of aerobic microbial degradation of PCBs. However, it is hard to find evidence of single species that utilize individual, or mixtures of, PCB congener(s) as the sole source of carbon for growth. It is essential when studying PCB biodegradation to distinguish between *degradation*, which can refer to any single or multi-step bioconversion of the PCB molecule, and *mineralization*, which refers to the complete utilization of the PCB substrates to give biomass, carbon dioxide and chloride.

Aerobic PCB metabolism, whether or not it leads to mineralization of the substrates, is often based on the well-documented pathway for biphenyl catabolism. Before discussing the various routes of microbial PCB metabolism in any detail, it is useful to describe the overall route by which biphenyl is assimilated by bacteria. The biphenyl degradation pathway, shown in *Figure 3*, can be considered to consist of an *upper* pathway, for the oxidation of biphenyl to benzoic acid, and a *lower* pathway, by which benzoic acid is mineralized via oxidation to catechol.

The upper pathway has been demonstrated in a number of bacterial species (Lunt and Evans, 1970; Catelani, Sorlini and Trecanni, 1971; Catelani *et al.*, 1973; Gibson *et al.*, 1973) and is known to be the result of the action of four enzymes; biphenyl 2,3-dioxygenase, an NAD^+-dependent dihydrodiol dehydrogenase, 2,3-dihydroxybiphenyl dioxygenase and 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid (HOPDA) hydrolase. The action of this last enzyme produces benzoic acid and a five-carbon hydroxy-acid which is thought to be mineralized via the corresponding keto-acid.

In the lower pathway, benzoic acid is mineralized via oxidation to form catechol. Catechol is a central intermediate in the microbial degradation of aromatic compounds, and is readily catabolized via the *meta*- or *ortho*-cleavage pathways (Dagley, 1978).

Co-metabolism of PCBs by biphenyl degrading bacteria

In a number of bacterial strains, the enzymes of the upper pathway for biphenyl degradation have a sufficiently broad substrate specificity to accommodate the oxidation of many PCB congeners. The great majority of reports concerning aerobic bacterial PCB degradation involve co-metabolism of certain PCB congeners by cells growing or pre-grown on biphenyl. This process was first recognized by Ahmed and Focht (1973) who described the degradation of mono- and dichlorobiphenyls to the corresponding chlorobenzoates by two species of *Achromobacter*. The co-metabolism of PCBs by bacteria expressing the upper biphenyl degradation pathway has

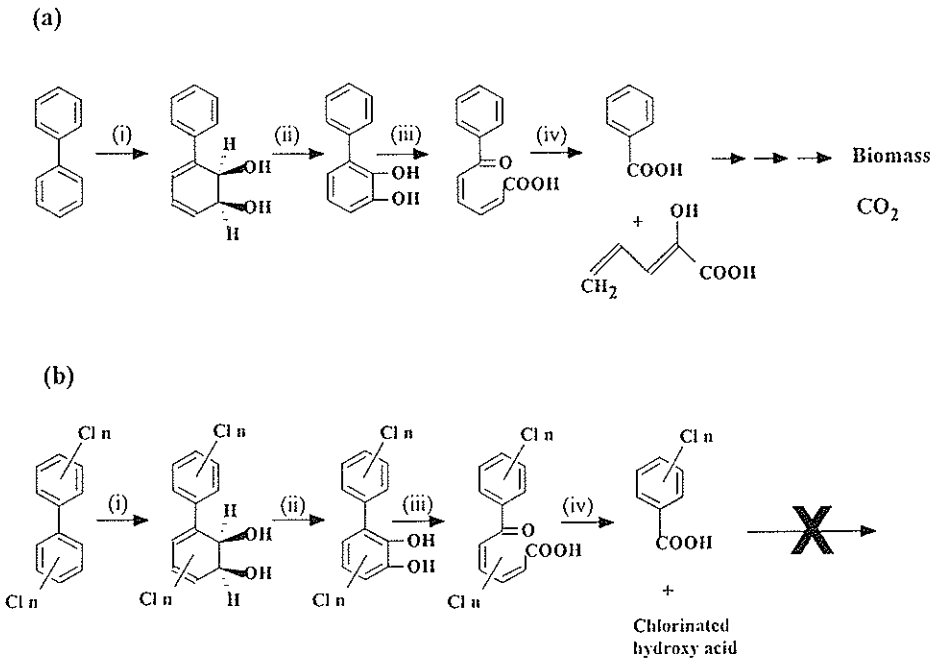

Figure 3. The upper pathway for (a) bacterial biphenyl catabolism and (b) co-metabolism of PCBs by bacteria grown with biphenyl as the sole source of carbon. (i) biphenyl 2,3-dioxygenase; (ii) dihydrodiol dehydrogense; (iii) 2,3-dihydroxybiphenyl dioxygenase; (iv) 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid hydrolase. Co-metabolism of PCBs rarely proceeds beyond the formation of chlorobenzoates.

since been extensively investigated, particularly by Furukawa, and by the General Electric Company, New York.

Furukawa and co-workers have published a number of studies into the co-metabolism of PCBs by *Alcaligenes* sp. strain Y42 and *Acinetobacter* sp. strain P6, which were originally isolated by enrichment culture using biphenyl and 4-chlorobiphenyl, respectively, as the sole source of carbon for growth and energy (Furukawa, Matsumura and Tonomura, 1978; Furukawa, Tonomura and Kamibayashi, 1978; Furukawa, Tomizuka and Kamibayashi, 1979). In these experiments, the bacteria were pre-grown using biphenyl or 4-chlorobiphenyl as the sole source of carbon before being challenged with a range of 36 PCB congeners. Of these, a total of 23 compounds were metabolized by *Alcaligenes* sp. strain Y42 and 33 by *Acinetobacter* sp. strain P6. A number of correlations were obtained between PCB structure (number and position of chlorine substitutions) and biodegradability, which can be summarized as follows:

1. Degradation decreased as the degree of chlorination increased.
2. 2,2'- and 2,6-chlorinated congeners exhibited a striking resistance to degradation.
3. Congeners possessing chlorine substitutions on both rings were less susceptible to degradation than those having the same number of chlorines on a single ring.
4. Ring fission occurred preferentially on the less- or non-chlorinated ring.
5. The formation and stable accumulation of a yellow *meta*-cleavage product was always observed in the degradation of 4'-chloro substituted congeners.

The routes of degradation of the congeners studied were generally analogous to the upper pathway for biphenyl catabolism, with most of the congeners being metabolized no further than the corresponding chlorobenzoates, and several different metabolites accumulated, as described in *Table 5*. The mineralization of the PCBs was not observed. It was notable that the co-metabolism of 2,3,2',3'- and 2,3,2',5'-chlorobiphenyls resulted in the accumulation of large amounts of unidentified dichlorinated metabolites.

Table 5. Some of the metabolites that can accumulate from the co-metabolism of PCBs by bacteria

Metabolites accumulated	Typical congeners forming the metabolites
Unidentified chlorinated compounds	2,2'-Substituted tetra/penta-chlorobiphenyls
Trihydroxylated compounds	2'-Substituted trichlorobiphenyls
Chlorinated *meta*-cleavage products (HOPDA)	4'-Substituted congeners
Chlorinated benzoic acids	Many congeners
Chlorinated acetophenones	4-Chlorobiphenyl, congeners with 3-' 2,5- or 2,4,5-chlorophenyl rings

Data compiled from Furukawa, Tomizuka and Kamibayashi (1979), Bedard *et al.* (1987a,b), Barton and Crawford (1988) and Bedard (1990).

The General Electric Company, New York, has reported the results of many studies using co-metabolic degradation of PCBs by bacteria grown on biphenyl. This group has isolated two strains, *Alcaligenes eutrophus* strain H850 and *Pseudomonas* sp. strain LB400, both of which have the ability to co-metabolize a particularly broad spectrum of PCB congeners (Bedard *et al.*, 1986, 1987a, b; Bopp, 1986). In comparison with the organisms studied by Furukawa, both of these bacteria have a superior ability to degrade *ortho*-substituted chlorophenyl rings. This is particularly noteworthy, as 2-; 2,4-; 2,5-; 2,3,6- and 2,4,5-chlorophenyl rings are found on many of the congeners present in commercial PCB mixtures (Bedard, 1990). In addition, these strains have only a very limited ability to degrade 4'-substituted congeners. On the basis of these results, Bedard *et al.* (1987b) and subsequently Nadim *et al.* (1988) proposed the presence of an additional 3,4-dioxygenation activity in these strains. 3,4-Dioxygenation results in the formation of the corresponding 3,4-dihydrodiol which cannot be dehydrogenated by these organisms. This compound undergoes a second 3,4-dioxygenase attack on the other chlorophenyl ring to generate a *bis*-3,4-diol which accumulates (Nadim *et al.*, 1988).

An additional property of *A. eutrophus* H850 and *Pseudomonas* sp. strain LB400 is that in both organisms the biphenyl 2,3-dioxygenase can, and may even show a preference for, attack at an *ortho*-chlorinated carbon (Bedard, 1990). It is proposed that in this reaction, instead of forming a dihydrodiol, a chloride ion is spontaneously lost from an unstable intermediate to form a dihydroxychlorobiphenyl, which is a normal intermediate in the upper chlorobiphenyl pathway.

It is not yet certain whether the ability of bacteria such as *A. eutrophus* H850 and *Pseudomonas* sp. strain LB400 to degrade a wide range of chlorinated biphenyls is due to the action of a single broad-specificity dioxygenase or two different enzymes, conferring a 2,3- or 3,4-dioxygenation, respectively. It does appear, however, that only one enzyme may be responsible. Gibson *et al.* (1993) have reported that homogeneous preparations of the oxygenase component of the *Pseudomonas* sp.

strain LB400 biphenyl dioxygenase system oxidize 2,5,2',5'-tetrachlorobiphenyl at the 3,4-position. Further, attempts by Mondello (1989) to demonstrate the presence of more than one biphenyl dioxygenase in *Pseudomonas* sp. strain LB400 were not successful. Whether or not one enzyme can be responsible for the different types of dioxygenase attack, it is clear that different classes of dioxygenase activity do exist. Bedard and Haberl (1990) proposed that the bacterial strains studied by the group at General Electric represent four distinct classes of PCB dioxygenase activity.

The genes encoding for enzymes of the upper PCB degradative pathway have been isolated from a number of bacteria and cloned (Furukawa and Miyazaki, 1986; Khan and Walia, 1989; Mondello, 1989; Hayase, Taira and Furukawa, 1990). Yates and Mondello (1989) conducted DNA–DNA hybridization experiments, comparing genes encoding PCB-degradative enzymes from several PCB-degrading strains. These genes were found to be closely related in *A. eutrophus* strain H850 and *Pseudomonas* sp. strain LB400, but genetically distinct from the six other strains tested. Strong conservation of restriction sites in these organisms was only found in the region of DNA encoding PCB degradation; no other sequence similarities were detected between the two genomes. Comparison of PCB-degradative genes (designated *bph A, B, C* and *D*, encoding biphenyl 2,3-dioxygenase, biphenyl dihydrodiol dehydrogenase, 2,3-dihydroxybiphenyl dioxygenase and 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid (HOPDA) hydrolase, respectively) at the nucleotide-sequence level has revealed a high degree of conservation between different species (Furukawa, Arimura and Miyazaki, 1987; Furukawa *et al.*, 1989; Kimbara *et al.*, 1989; Peloquin and Greer, 1993). These genes are chromosomally encoded, and it is not clear whether sequence homologies have arisen by DNA transfer or whether the organisms share a common phylogenetic origin.

Metabolites formed by PCB co-metabolism

The degree of PCB removal that can be obtained by co-metabolizing bacteria growing with biphenyl supplied as a source of carbon and energy is undeniably impressive. Indeed, Unterman *et al.* (1988) reported that treatment of Aroclor 1242 with a combination of *Corynebacterium* sp. strain MB1 and *Pseudomonas* sp. strain LB400, representing two different classes of PCB dioxygenase, resulted in some degradation of almost all congeners in the Aroclor mixture.

The scheme for PCB degradation outlined above, in which PCBs are co-metabolized by a pathway analogous to the upper pathway for biphenyl degradation, is very much a simplified overview. In fact, resting-cell experiments by many workers have demonstrated that a large number of different chlorinated metabolites are produced using this approach. The types of products that can accumulate are largely independent of the bacterial strain used, and are determined primarily by the chlorine substitution pattern of the reacting ring (Bedard and Haberl, 1990). One way around this problem may be to adopt the use of mixed cultures of PCB-degrading bacteria. However, Bedard and Haberl (1990) concluded that despite the presence of different classes of PCB dioxygenase, the specificities of the enzymes required for further degradation were similar in eight different strains studied. Hence it is not surprising that, in addition to chlorinated benzoic acids, chlorinated dihydroxybiphenyls and chlorinated HOPDA are major products of PCB co-metabolism.

In addition to the chlorinated intermediates from the upper chlorobiphenyl pathway, a number of other products accumulate from PCB co-metabolism. As reported above, accumulation of the corresponding *bis* diol results from 3,4-dioxygenation of PCBs. Barton and Crawford (1988) reported the generation of large quantities of 4-chloroacetophenone by *Pseudomonas* sp. MB86 when grown with 4-chlorobiphenyl as the sole source of carbon and energy. This compound was believed to be a dead-end metabolite, and growth of the organism was inhibited by the build-up of this metabolite. Following a detailed study on the degradation of 4-chlorobiphenyl by *Achromobacter* sp. strain B-218 and *Bacillus brevis* strain B-257, Massé *et al.* (1984) reported the accumulation of a large number of different chlorinated aromatic acids in the growth medium. Some of these products were postulated to have been generated via a 3,4-dihydroxylation of 4-chlorobiphenyl. Other differences in these minor degradation products were thought to be due to reduction of one or both double bonds in HOPDA.

Ring-chlorinated acetophenones can also result from 2,3-dioxygenase attack on a 3-chlorophenyl ring (Bedard, 1990), or from attack on a 2,5- or 2,4,5-chlorophenyl ring of a PCB by a route that has not yet been elucidated (Bedard *et al.*, 1987b). The degradation of 2,4,6-trichlorobiphenyl by *Acinetobacter* sp. strain P6, grown with 4-chlorobiphenyl as the sole carbon source, has been shown to result in the stable accumulation of a trihydroxy compound (Furukawa, Tomizuka and Kamibayashi, 1979). In the same paper it was reported that several 2,3-chlorinated PCBs were readily metabolized by both *Acinetobacter* sp. strain P6 and *Alcaligenes* sp. strain Y42 to give unidentified products.

It can be seen that the use of bacteria able to co-metabolize PCBs, although often providing a high degree of PCB removal, is likely to result in the production of a number of different, sometimes uncharacterized metabolites. One consequence may be that accumulation of these chlorinated aromatic compounds may inhibit the degradation of PCBs, as has been shown to be the case with chlorobenzoates (Sondossi, Sylvestre and Ahmed, 1992). When considering the use of this approach for the clean-up of contaminated sites, the consequences of the potential release of these metabolites must be a consideration. This can be seen to be even more important when one considers the large amount of information that is available about the toxicology of PCBs, whereas the toxicity of many of the potential products is largely unknown.

Microbial degradation of chlorobenzoates

Degradation of chlorobenzoates represents the lower pathway in the aerobic mineralization of PCBs. Chlorinated benzoates can be degraded aerobically by a number of routes, involving dechlorination via ring cleavage and lactonization, or by hydrolytic or oxidative dehalogenation (Reineke and Knackmuss, 1988).

The principle route of aerobic chlorobenzoate degradation involves oxygenation by benzoate dioxygenase, giving chlorinated 1,2-dihydro-1,2-dihydroxybenzoates. These products undergo an enzymic dehydrogenation to give chlorocatechols (Reineke and Knackmuss, 1978a, b). Subsequent degradation of the chlorocatechols by *ortho* cleavage produces chloromuconates. Lactonization by chloromuconate cycloisomerases subsequently results in the spontaneous elimination of hydrogen chloride (see *Figure 4*).

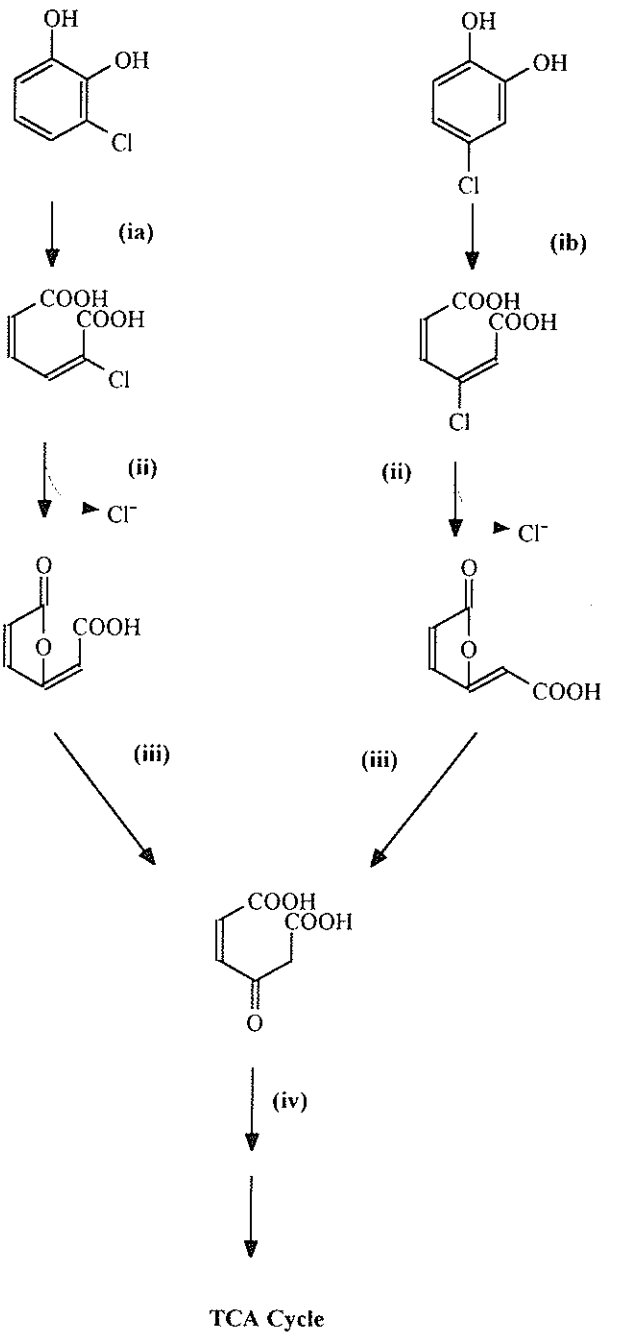

Figure 4. Mineralization of chlorocatechols by the modified *ortho*-cleavage pathway in *Pseudomonas* sp. strain B13. (ia) Catechol- 1,2-dioxygenase (pyrocatechase type I); (ib) chlorocatechol-1,2-dioxygenase (pyrocatechase type II); (ii) *cis, cis*-chloromuconate cycloisomerase; (iii) 4-carboxymethylenebut-2-en-4-olide hydrolase; (iv) maleyl acetate reductase.

Reineke and Knackmuss have studied this route extensively in *Pseudomonas* sp. strain B13. This organism was isolated by continuous enrichment using benzoate, and subsequently 3-chlorobenzoate, as the sole source of organic carbon (Dorn *et al.*, 1974). Non-selective dioxygenation of 3-chlorobenzoate gave a mixture of 3-chlorocatechol (67%) and 4-chlorocatechol (33%). The benzoate dioxygenase from *Pseudomonas* sp. strain B13 showed a high degree of substrate specificity, being able to oxidze benzoate and 3-chlorobenzoate but not 2-chloro- or 4-chlorobenzoate. Specificity of benzoate dioxygenases has also been reported for enzymes from *Nocardia* (Cain, Tranter and Darrah, 1968), *Arthrobacter* (Horvarth and Alexander, 1970), *Pseudomonas fluorescens* (Hughes, 1965) and *Azotobacter* (Walker and Harris, 1970). Degradation of 3-chlorobenzoate via dioxygenation to chlorocatechol has also been shown to occur in *Pseudomonas* H1 (Haller and Finn, 1979).

Focht and co-workers have studied chlorobenzoate metabolism extensively, and have isolated two *Pseudomonas* strains that are capable of the degradation of a wide range of chlorinated benzoic acids. *Pseudomonas aeruginosa* JB2, isolated from a PCB-contaminated soil, is able to grow with 2-chloro-, 3-chloro-, 2,3-dichloro-, 2,5-dichloro- or 2,3,5-trichloro-benzoates as the sole source of carbon, with chlorocatechols as the central intermediates in mineralization (Hickey and Focht, 1990). *Pseudomonas putida* P111 is a particularly versatile strain which was isolated by enrichment using 2,5-dichlorobenzoate as the sole source of carbon for growth (Hernandez *et al.*, 1991). This organism is able to use 2-, 3- and 4-chlorobenzoate, 2,3-; 2,4- and 2,5-dichlorobenzoate and 2,3,5-trichlorobenzoate as the sole carbon source for growth. However, growth on all *ortho*-substituted benzoates was completely inhibited by the presence of low concentrations of 3,5-dichlorobenzoate. Interestingly, the presence of this compound resulted in higher yields from cells grown with 3-, or 4-chlorobenzoate.

It is important to recognize that degradation of compounds via chlorocatechols must proceed through the modified *ortho*-cleavage pathway outlined above. *Meta* cleavage of chlorocatechols by catechol-2,3-dioxygenases can occur, but results in the formation of acid chlorides which are highly toxic, causing irreversible inactivation of the catechol-2,3-dioxygenase (Bartels, Knackmuss and Reineke, 1984). Catechol-2,3-dioxygenases are also susceptible to substrate inhibition by chlorocatechols (Klecka and Gibson, 1981).

Oxygenolytic dechlorination of 2-chlorobenzoate is a fortuitous event resulting from oxidation by benzoate-1,2-dioxygenases. Oxygen is introduced into the ring such that an *ortho* hydroxy group is attached to the same carbon as the chlorine substituent. Chloride is released spontaneously from the *cis* dihydrodiol. This has been observed as the first step in the mineralization of this compound by several bacteria (Zaitsev and Karasevich, 1982; Engesser and Schulte, 1989; Fetzner, Muller and Lingens, 1989; Sylvestre, Mailhiot and Ahmad, 1989).

Hydrolytic dechlorination has been reported as the principle route in the degradation of 4-chlorobenzoate. This mechanism results in the formation of 4-hydroxybenzoate, which is readily mineralized by a number of bacteria. This route has been shown to occur in *Acinetobacter* sp. strain 4CB1, which was isolated from a PCB-contaminated soil by Adriens *et al.* (1989). It was established that the dehalogenase enzyme uses water as the hydroxyl donor. 4-Hydroxybenzoate was oxidized by an NADH-dependent mono-oxygenase to form protocatechuate, which

was subsequently degraded by both *ortho* and *meta* cleavage. The 4-chlorobenzoate dehalogenase from *Pseudomonas* sp. strain CBS3 was purified by Elsner *et al.* (1991) and demonstrated to be a three-component complex. Ring dehalogenation to give 3-hydroxybenzoate has also been reported as the first step in the degradation of 3-chlorobenzoate by a *Pseudomonas* sp. (Johnson, Briggs and Alexander, 1972).

Mineralization of chlorobenzoates by co-metabolism and by constructed strains

Although the use of chlorobenzoates as the sole source of carbon for growth by naturally occurring bacteria appears to be fairly limited, the scope for chlorobenzoate mineralization can be broadened considerably by exploiting co-metabolic effects and by the construction of specialized hybrid strains.

The degradation of 3,4-dichlorobenzoate by *Acinetobacter* sp. strain 4-CB1 was elucidated by Adriens and Focht (1991). This organism was unable to use 3,4-dichlorobenzoate as a sole carbon source for growth. However, cells grown on 4-chlorobenzoate were able to co-metabolize 3,4-dichlorobenzoate via hydrolytic dechlorination to give 3-chloro-4-hydroxybenzoate. Subsequent degradation of 3-chloro-4-hydroxybenzoate was accomplished without the requirement for a co-substrate. *Pseudomonas putida* strain P111 is able to use 3,5-dichlorobenzoate as a growth substrate only in the presence of 3- or 4-chlorobenzoate. In this instance, the monochlorobenzoates induce the formation of a functional dihydrodiol dehydrogenase, which is not induced by 3,5-dichlorobenzoate (Hernandez *et al.*, 1991).

Pseudomonas sp. strain JB1, isolated by enrichment using biphenyl as a carbon source, is able to use benzoic acid as the sole carbon source, but cannot grow with chlorobenzoates (Parsons *et al.*, 1988). Benzoate-grown cultures of this organism were able to degrade 3-chlorobenzoate, and to a lesser extent 4-chlorobenzoate, 3,5- and 3,4-dichlorobenzoates. 2-Chlorobenzoate, 2,5- and 2,6-dichlorobenzoate were not co-metabolized. Analysis of intermediates, although not conclusive, suggested that co-metabolism of the chlorobenzoates proceeded through *meta* cleavage of chlorocatechols. As discussed above, such transformations are likely to be counter-productive, resulting in production of toxic chloro-2-hydroxymuconate semialdehydes.

Pseudomonas aeruginosa strain JB2, isolated by Hickey and Focht (1990), was detailed above as a bacterium with a broad chlorobenzoate-degrading capacity. Although this organism could not use 2,4-dichlorobenzoate as a growth substrate, cells grown on benzoate could effect the mineralization of this compound.

The growth spectrum of *Pseudomonas* sp. strain B13 was extended considerably by introduction of the TOL plasmid from *Pseudomonas putida* strain mt-2, which encodes a broad specificity benzoate-1,2-dioxygenase (Reineke and Knackmuss, 1979). Exconjugants were prepared by combining donor and recipient cultures on a filter and subsequent selection on 4-chlorobenzoate agar plates. The resultant strains were able to utilize 4-chloro- and 3,5-dichlorobenzoates. In *Pseudomonas* sp. strain B13 and its derivative strains, chlorocatechols are assimilated by an *ortho*-cleavage pathway requiring the induction of one of two sets of enzymes, depending on the position of chlorine substitution. Hence 3-chlorobenzoate-grown cells possess both pyrocatechase type I (the ring fission enzyme normally induced by catechol) and the iso-functional pyrocatechase type II, which has a high activity with chlorosubstituted catechols. In cells grown with 4-chlorobenzoate as the sole source of carbon,

induction of the type II pyrocatechase is proportionally much higher (Reineke and Knackmuss, 1980).

The degradative capacity of *Pseudomonas* sp. strain B13 was extended to include 2-chlorobenzoate by Hartmann *et al.* (1989). The 3-methylsalicylate-degrading bacterium, *Pseudomonas* sp. strain WR401 (Engelberts, Schmidt and Reineke, 1989) was mated with *Pseudomonas* sp. strain B13 to obtain transconjugants able to grow with 5-chlorosalicylate as the sole source of carbon and energy. Subsequent selection and adaption led to the isolation of strains able to mineralize 2-chlorobenzoate.

Microbial degradation of chlorinated acetophenones

The build-up of chlorinated acetophenones poses a potential problem in PCB bioremediation, as these compounds are toxic, and can inhibit the growth of PCB-degrading bacteria (Barton and Crawford, 1988; Havel and Reineke, 1993a).

Acetophenone is readily degraded by bacteria. The mineralization of acetophenone by an *Arthrobacter* sp. was investigated by Cripps (1975), and shown to occur through a reaction equivalent to the Baeyer–Villiger oxidation, giving phenyl acetate which was further degraded via phenol and catechol. This organism was unable to use chlorinated acetophenones as a carbon source for growth. Degradation of acetophenone by a *Nocardia* sp. was shown to occur by the same route (Cripps, Trudgill and Whateley, 1978). Little is known, however, about the degradation of chlorinated acetophenones. Higson and Focht (1990) showed that all monochlorinated acetophenones and 2,4- and 2,5-dichloroacetophenones can be degraded by an *Alcaligenes* sp., grown with acetophenone as the sole source of carbon. Degradation proceeded via an analogous Baeyer–Villiger type oxidation, giving chlorinated phenyl acetates which were further oxidized to the corresponding chlorophenols. Some degree of chloride release was observed with each substrate, suggesting that mineralization did occur. *Ortho*-chlorinated acetophenones were dehalogenated to a far lesser extent that other isomers. None of the chloroacetophenones supported growth of this organism, due to either feedback inhibition by chlorophenol or a lack of induction of the Baeyer–Villiger oxygenase.

In the only available report of microbial growth on, and mineralization of, a chlorinated acetohenone, Havel and Reineke (1993a) described the isolation and characterization of a mixed culture which was able to mineralize 4-chloroacetophenone. The culture consisted of an *Arthrobacter* species and a *Micrococcus* species. *Arthrobacter* sp. strain M5 was capable of the mineralization of 4-chloroacetophenone only under very carefully controlled conditions of substrate supply. This was due to the toxic effects of 4-chlorophenol which rapidly accumulated in the medium. The function of *Micrococcus* sp. strain B1 was to rapidly remove 4-chlorophenol formed by the *Arthrobacter* sp. Resting-cell experiments suggested that *Arthrobacter* sp. strain M5 might be able to grow with other chlorinated acetophenones if grown in co-culture with a bacterium capable of mineralization of the corresponding chlorophenols. These experiments suggest that the mineralization of these PCB metabolites may require a delicate control of conditions that could be difficult to achieve in environmental samples. Further complications may arise from the stable accumulation of chloro-1-phenylethanols which were reported to result from the reduction of the ketone.

Mineralization of PCBs by micro-organisms

Athough bacteria able to use biphenyl as the sole source of carbon for growth are ubiquitous, no polychlorinated biphenyl has been shown to support the growth of a naturally occurring micro-organism in pure culture. Indeed, Focht (1993) suggests that bacteria that use PCBs as sole carbon sources probably do not exist in nature. The only chlorobiphenyl that has been reported to support the growth of naturally occurring micro-organisms in pure culture is 4-chlorobiphenyl. Growth on 4-chlorobiphenyl appears to be usually limited to breakdown of the non-chlorinated ring, with the corresponding accumulation of 4-chlorobenzoate. This has been shown in *Achromobacter* (Ahmed and Focht, 1973; Massé *et al.*, 1984), *Pseudomonas* sp. (Barton and Crawford, 1988), *Acinetobacter* sp. (Furukawa, Tonomura and Kamibayashi, 1978) and *Bacillus brevis* (Massé *et al.*, 1984). It is also worth noting that the five-carbon hydroxy-acid resulting from fission of the non-chlorinated ring has not been isolated from cultures, and its degradation has not been unequivocally established.

Shields, Hooper and Sayler (1985) isolated strains of *Alcaligenes* and *Acinetobacter* species from a mixed culture degrading monochlorinated biphenyls. Both strains harboured a plasmid encoding the complete pathway for mineralization of 4-chlorobiphenyl, which was degraded via 4-chlorobenzoate. Mineralization was demonstrated by measurement of $^{14}CO_2$ production from *U*-^{14}C-4-chlorobiphenyl.

Some mineralization of lower-chlorinated biphenyls by microbial consortia certainly occurs in the environment. However, only a few PCB-degrading mixed cultures have been successfully demonstrated in the laboratory. Even with microbial consortia, mineralization is generally limited to *para*-chlorinated biphenyls. Chlorinated benzoic acids have a role in regulating bacterial PCB degradation; hence for significant degradation of PCBs to occur in the environment there is the requirement for the presence of organisms possessing genes encoding both the upper pathway (PCB degradation) and the lower pathway (chlorobenzoate degradation). In an attempted simulation of a natural river environment, Kong and Sayler (1983) showed that all monochlorinated and monobrominated biphenyls were degraded at low concentrations. Degradation occurred through the corresponding halogenated benzoates. However, only in the case of 4-chlorobiphenyl was mineralization demonstrated. Further work by this group identified a freshwater bacterial consortium which was able to mineralize both 4-chlorobiphenyl and 4,4'-dichlorobiphenyl, when either compound was provided as the sole source of carbon (Pettigrew *et al.*, 1990). This consortium consisted of *Pseudomonas testosteroni*, which facilitated the degradation of 4-chlorobiphenyl and 4,4'-dichlorobiphenyl to 4-chlorobenzoate, and an *Arthrobacter* sp., which was able to mineralize 4-chlorobenzoate. The successful mineralization of these compounds by this consortium depended on the presence of a third organism, a strain of *Pseudomonas putida*, for which a role in the consortium was not established.

Furukawa and Chakrabarty (1982) achieved a 98% release of chloride from 4-chloro- and 3,5-dichlorobiphenyl by co-culturing *Acinetobacter* sp. strain P6 with pseudomonads able to use chlorobenzoates. The mineralization of 3-chlorobiphenyl has also been achieved by an aerobic bacterial consortium (Fava and Marchetti, 1991). This consortium consisted of two pseudomonads capable of degrading the

substrate to 3-chlorobenzoate, and a strain of *Pseudomonas fluorescens* which was able to co-mineralize 3-chlorobenzoate when supplied with benzoic acid.

Fava *et al.* (1994) obtained a three-membered bacterial consortium capable of mineralizing all three monochlorobiphenyls as well as 2,5-, and 3,4-dichlorobiphenyl when cultured in shake-flasks. Significant degradation and dechlorination of several other di- and trichlorinated biphenyls was also observed. *Pseudomonas* sp. strain CPE1 was isolated from this co-culture and its ability to mineralize 4-chlorobiphenyl was demonstrated when supplied as the sole carbon source in pure culture. Degradation was routed through 4-chlorobenzoic acid. Mineralization was demonstrated by the measurement of near-stoichiometric amounts of chloride released into the medium.

With the development of specialized strains able to effect the mineralization of several chlorinated benzoic acids, it is theoretically possible to devise co-cultures able to mineralize lowly chlorinated PCBs. However, Havel and Reineke (1991) demonstrated that the stability of co-cultures of chlorobiphenyl- and chlorobenzoate-degrading strains can be difficult to achieve. *Pseudomonas* sp. strain B13 was cultured with biphenyl-degrading isolates and incubated in the presence of 3-chlorobiphenyl. Chloride release was never greater than 50% due to the inhibitory effect of black-coloured products that accumulated in the medium. The build-up of black-coloured products, which has been investigated more fully by Haller and Finn (1979) was due to an accumulation of chlorocatechols, which undergo auto-oxidation to give chloroquinones which readily polymerize. For the same reason, no greater than 20% chloride release was attained with co-cultures theoretically able to completely mineralize 2-chlorobiphenyl.

PCB biodegradation by fungi

Dmochewitz and Ballschmiter (1988) demonstrated that *Aspergillus niger* is able to degrade the lower-chlorinated PCBs present in Clophen A30 (a commercial PCB preparation equivalent to Aroclor 1242). Aroclors 1254 and 1260 were not degraded. *Para*-chlorination appeared to inhibit attack; 4,4'-dichlorination essentially preventing attack by the fungus.

Phanerochaete chrysosporium has been shown to degrade many PCBs. It is believed to utilize the same enzymes involved in lignin degradation. Dechlorination of PCBs is thought to occur via the production of hydroxy radicals. *Phanerochaete chrysosporium* mineralizes highly chlorinated congeners and Aroclor 1254 (Bumpus *et al.*, 1985, 1988); however, only very low PCB concentrations can be tolerated (e.g. 250 p.p.b. Aroclor 1254; 5.5 p.p.b. 3,4,3',4'-chlorobiphenyl). Eaton (1985) reported 7% mineralization of Aroclor 1254 (0.3 p.p.m.) after 22 days' incubation with nitrogen-limited cultures of *P. chrysosporium*. Sustained rates of degradation require the addition of complex carbohydrate carbon sources (Bumpus *et al.*, 1988). Another limitation to the use of this organism is that growth is unlikely to occur at temperatures below 15°C (Lamar *et al.*, 1988; Viney and Bewley, 1988). The successful application of this white-rot fungus to PCB degradation will require demonstration of activity at much higher concentrations than those reported to date.

CONSTRUCTED STRAINS

The development of individual strains possessing both the upper (chlorobiphenyl)

and lower (chlorobenzoate) pathways has been achieved by a number of workers, and may offer the greatest opportunities for PCB degradation. This approach has a number of potential advantages over the use of mixed cultures. In a co-culture system, the organism responsible for the oxidation of PCBs to chlorobenzoates derives no carbon or energy from the process and requires either another carbon source or must successfully compete with other bacteria for the water-soluble five-carbon acid released from ring cleavage. Furthermore, the requirement for the addition of biphenyl as a substrate to initiate and maintain activity can be obviated if the organism can also assimilate carbon from chlorobenzoate degradation.

A number of workers have used the principles of *in vivo* genetic exchange to construct strains able to mineralize monochlorinated biphenyls when grown on these compounds as the sole source of organic carbon. Much of this work can be regarded as an extension of the development of chlorobenzoate-degrading organisms detailed earlier in this report. Mokross, Schmidt and Reineke (1990) obtained a number of 3-chlorobiphenyl-degrading bacteria by mating *P. putida* strain BN10 and *Pseudomonas* sp. strain B13. *Pseudomonas putida* strain BN10 was isolated for its ability to mineralize biphenyl and to convert 3-chlorobiphenyl to 3-chlorocatechol. Mating with strain B13, which is able to transfer 3-chlorobenzoate-degradative ability to other pseudomonads (Reineke *et al.*, 1982), produced *P. putida* BN210, which mineralized 3-chlorobiphenyl when grown with this compound as the sole source of organic carbon. Growth of this organism on 3-chlorobiphenyl induced the following enzyme activities; phenyl catechol-2,3-dioxygenase, benzoate dioxygenase, catechol -1,2-dioxygenase, chloromuconate cycloisomerase and 4-carboxymethylenbut-2-en -4-olide hydrolase. This organism grew on 2- or 4-chlorobiphenyl, with accumulation of the corresponding chlorobenzoate.

Using similar techniques, this group has also obtained a hybrid strain of *P. cepacia* (JHR22) that mineralized 2-, 3- or 4-chlorobiphenyl and 2,4- or 3,5-dichlorobiphenyl when grown with any of these compounds as the sole source of carbon (Havel and Reineke, 1991). To a large extent, use of this hybrid strain overcame the problem of black-coloured products that were found in co-cultures. Growth with 4-chlorobiphenyl resulted in only 50% chloride release, and the formation of 50% 4-chloro-dihydrodihydroxybenzoate. The organism was able to tolerate high concentrations of 4-chlorobiphenyl. Stoichiometric chloride release from 2-chlorobiphenyl was observed when this substrate was supplied in low concentrations, but growth was inhibited by the formation of black-coloured products when concentrations greater than 4 mM were provided in the medium. *Pseudomonas cepacia* JHR22 was also able to grow with the commercial PCB mixture Aroclor 1221 as the sole source of carbon, with corresponding mineralization of several congeners.

Another approach to obtaining hybrid organisms able to mineralize chlorinated biphenyls is the use of multi-chemostat enrichment. By this process, Adams *et al.* (1992) constructed a 3-chlorobiphenyl- mineralizing recombinant (*Pseudomonas* sp. strain CB15) by intergenic mating of a biphenyl utilizer and a chlorobenzoate utilizer. The accumulation of coloured products from polymerization of chloroquinones was noted in these cultures. Combination of the upper and lower pathways in this single strain allowed some interesting studies on inhibition of the enzymes responsible for ring cleavage in both pathways. 3- Chlorocatechol was found to be a potent inhibitor of 2,3-dihyroxybiphenyl dioxygenase, causing 98% inhibition when present at a

concentration of 0.33 mM. Similarly, 2,3-dihydroxybiphenyl was found to competitively inhibit the *ortho* fission of chlorocatechol. 2,3-Dihyroxybiphenyl dioxygenase was also subject to substrate inhibition.

This group also obtained an organism that mineralized 2-chloro- and 2,5-dichlorobiphenyl by mating *P. aeruginosa* strain JB2, which mineralizes chlorobenzoates, with *Arthrobacter* sp. strain B1Barc, which degrades 2-chlorobiphenyl to 2-chlorobenzoate (Hickey, Brenner and Focht, 1992). In contrast to the hybrid strains obtained by Reineke and co-workers, this strain, *P. aeruginosa* strain UCR2, spontaneously lost the ability to mineralize 2-chlorobiphenyl when cultured in the absence of 2-chlorobiphenyl or 2-chlorobenzoate, instead accumulating near-stoichiometric amounts of 2-chlorobenzoate from 2-chlorobiphenyl. Genetic analysis was not successful in demonstrating the genetic events involved during strain construction, and suggested that the pathway by which strain UCR2 was derived was more complex than a single unilateral genetic exchange.

APPLICATION OF BIODEGRADATION TO THE CLEAN-UP OF PCB-CONTAMINATED SOILS AND SEDIMENTS

Given the range of microbial processes leading to PCB degradation (anaerobic reductive dechlorination, co-metabolism by cells grown on biphenyl, mineralization of 4-chlorobiphenyl, the development of constructed strains and fungal dechlorination), one might expect that bioremediation of PCB-contaminated sites was a feasible option. This may prove to be the case, but to date no truly successful PCB bioremediation process has been demonstrated. In the preceding discussion we have shown that anaerobic reductive dechlorination of higher-chlorinated PCBs results in the accumulation of predominantly mono-, di-, and tri-chlorinated congeners, and that these lowly chlorinated congeners are more amenable to aerobic biodegradation. Recently, the application of a sequential anaerobic–aerobic process for complete mineralization of PCBs in environmental samples has been considered.

Any claims to effect the bioremediation of hydrophobic pollutants such as PCBs must be carefully examined, as problems arising from sorption to soils or bioreactor materials, or by leaching, can produce false-positive results. Such difficulties have been discussed in detail by Shannon and Unterman (1993). Another important factor in the assessment of bioremediation processes, which we believe tends to be overlooked in the literature, is the formation of metabolites which may be unidentified, could be far more mobile (more hydrophilic) than the target compounds, and which have less-defined toxicological characteristics.

Brunner, Sutherland and Focht (1985) undertook a study to determine likely methods for the stimulation of PCB biodegradation in soil. Importantly, mineralization of PCBs was determined by measuring release of $^{14}CO_2$ from [^{14}C]Aroclor 1242 in addition to GC analyses. [^{14}C]Aroclor 1242 was added to soil to a concentration of 100 p.p.m. Over a period of 210 days, improving the nutrient status of the soil by sludge or straw amendment showed little significant mineralization of Aroclor 1242. Similarly, incubation under anaerobic conditions had no effect. Inoculation with the PCB co-metabolizing *Acinetobacter* sp. strain P6 (Furukawa, Tomizuka and Kamibayashi, 1979) resulted in significant depletion of the lower-chlorinated congeners from Aroclor 1242, but little mineralization was observed. Application of

biphenyl to the soil (3.66 g biphenyl per kg soil) was far more successful and effected the mineralization of many of the congeners present. This process, whereby a structurally similar non-chlorinated analogue is used as a growth substrate and to stimulate co-metabolism of the target molecule(s) has been termed 'analogue enrichment'. Clearly, the indigenous microbial population was able to co-metabolize PCBs when the required enzyme activities were induced by growth on biphenyl. This effect could be stimulated further by addition of both *Acinetobacter* sp. strain P6 and biphenyl. That biphenyl amendment was by far the most important factor was illustrated by the fact that repeated biphenyl additions immediately increased the rate of $^{14}CO_2$ evolution.

Researchers at the General Electric Company have performed degradation trials with PCBs bound to soils using their PCB co-metabolizing strains. Unterman *et al.* (1986) reported initial experiments using soil spiked with Aroclor 1242. Aqueous buffer and washed cells pre-grown on biphenyl were added to give a soil slurry culture (10% v/v soil/water). The slurries were incubated with agitation. Using *Pseudomonas* sp. strain LB400 and *Corynebacterium* sp. strain MB1, 95% of 50 p.p.m. Aroclor 1242 was degraded in 24 hours. When the study was repeated by adding solid biphenyl and inoculating soil slurries with LB400 (1% inoculum) the organism grew well, but significantly less degradation of the PCBs occurred. This contrasts with studies by Kohler, Kohler-Staub and Focht (1988), who showed that growing cultures of *Arthrobacter* sp. strain B1B and *Acinetobacter* sp. strain P6 were far superior to resting-cell suspensions in terms of the extent of PCB degradation and the range of congeners that were transformed. Bioremediation was also investigated using soil from an actual PCB-contaminated site (Unterman *et al.*, 1988). In this case, cells were pre-grown using biphenyl but were not washed prior to addition to the soil. By this means, biphenyl and bacteria were simultaneously added to the soil. The soil was inoculated three times per week for 15 weeks with *Pseudomonas* sp. strain LB400 applied to the surface. Significant depletion of PCB congeners only occurred in the top 1 cm of the soil. When the soil system was also regularly mixed, 35% depletion of PCB congeners was achieved after 23 weeks. Improtantly, it was noted that the addition of nutrients and biphenyl alone was sufficient to stimulate some PCB degradation by the indigenous microflora.

Barriault and Sylvestre (1993) studied the degradation of Aroclor 1242 in soils using *Pseudomonas testosteroni* strain B-356. Again, repeated additions of biphenyl were found to be the most effective way to maintain co-metabolic activity. The viable cell count of the introduced bacteria remained fairly constant throughout the 3 week experiment, whereas addition of biphenyl resulted in a large increase in the native soil population. This demonstrates the need to account for the contribution of the indigenous population to any effects observed in bioremediation studies. Also noted was the importance of the bioavailability of the PCBs in determining their degradation in soil. Inoculation with a biosurfactant-producing strain in addition to *P. testosteroni* B-356 and repeated biphenyl additions was the most successful treatment, providing for degradation of approximately 30% of added Aroclor 1242. One important conclusion from this work was that the accumulation of toxic metabolites from biphenyl and PCBs inhibit the biodegradation process. Such effects have also been noted by Sondossi, Sylvestre and Ahmad (1992), with regard to the accumulation of chlorobenzoates and their degradation products. Chlorobenzoates were found

to inhibit biphenyl and chlorobiphenyl oxidation by *P. testosteroni* B-356; effects were notable at concentrations of 3-chlorobenzoate of only 160 μM. Chlorocatechols and muconic semi-aldehydes resulting from chlorobenzoate degradation were also found to inhibit the HOPDA dehydrogenase in this organism; only 0.5 μM 3-chlorocatechol was required to inhibit 99% of this enzyme activity. The practical implications with regard to PCB bioremediation are illustrated in work by Hickey, Searles and Focht (1993) who demonstrated that PCB mineralization in soils was enhanced by inoculation with chlorobenzoate-degrading bacteria. Inoculation with only pseudomonad strains able to mineralize chlorobenzoates was more effective at stimulating PCB mineralization than inoculation with PCB co-metabolizers or chlorobiphenyl-mineralizing strains in combination with chlorobenzoate-degrading strains.

Difficulties with multiple inoculation of soils were also found by Havel and Reineke (1992). Soil microcosms were inoculated with three bacterial strains which were theoretically able to mineralize several congeners present in Aroclor 1221. In pre-sterilized soil, about 70% of the congeners were degraded. However, in non-sterilized soil 4-chlorobenzoate was metabolized by the indigenous bacteria, giving toxic metabolites which rapidly inhibited the growth of the introduced strains. Use of the constructed hybrid strain *Pseudomonas cepacia* strain JHR22, which was able to mineralize components of Aroclor 1221 in axenic culture, was more successful (Havel and Reineke, 1993b). The organism survived in non-sterile soil for several weeks and mineralized all congeners in Aroclor 1221 which were chlorinated on only one phenyl ring. The hybrid pathway for PCB mineralization, which originated from three different strains was more successful than consortia because 4-chlorobenzoate was degraded before it accumulated sufficiently to be metabolized to toxic products by the indigenous soil population.

A range of different approaches to the bioremediation of PCB-contaminated soil was investigated by Viney and Bewley (1988, 1990). The use of surfactants applied to the soil to increase the bioavailability of the PCBs was investigated, but all surfactants examined were shown to have some inhibitory effect on PCB metabolism by the selected bacteria and fungi. The most effective treatment in large-scale soil systems (10 kg horticultural sand) involved inoculation with a mixture of bacterial strains (including *A. eutrophus* H850, *Pseudomonas* sp. strain LB400 and *Corynebacterium* sp. strain MB1 from GEC) and *P. chrysosporium*. Soil systems were incubated at 25°C, turned frequently, and received repeated additions of biphenyl and phosphate-buffered mineral salts. Under these conditions 42% of the total PCB from Aroclor 1242 was depleted. The more highly chlorinated congeners from this mixture were not degraded.

Apart from the limitations in the degree of PCB degradation that can be achieved by exploiting co-metabolism by biphenyl utilizers, it must be recognized that biphenyl is itself fairly insoluble, toxic and costly; the addition of biphenyl to large-scale soil environments may itself not prove to be feasible. Viney and Bewley (1988) calculated a biphenyl requirement of 1% (w/w) of the total soil to be treated.

Harkness *et al.* (1993) conducted a study of the *in situ* aerobic biodegradation of PCBs in sediment in the Hudson River. The PCBs present in the Hudson River sediment at the time of the experiment had previously undergone considerable transformation, presumably by microbial reductive dechlorination, since their release

into the river (the date of PCB release is not given). Although the original PCB mixture was predominantly Aroclor 1242, PCBs in the sediment samples were mainly mono- and di-chlorobiphenyls (these constitute approximately 9% of the congeners in Aroclor 1242). Addition of inorganic nutrients, biphenyl and oxygen resulted in the loss of 35–55% of the PCBs present, with the resultant formation of chlorobenzoates, over a period of 73 days. Repeated inoculation with a PCB-degrading bacterium (*A. eutrophus* strain H850) failed to improve biodegradative ability of the system. Approximately 50% of the PCBs in the sediment were resistant to degradation due to their sorption to the organic components of the sediment matrix. In small-scale laboratory experiments, Anid, Ravest-Webster and Vogel (1993) investigated the stimulation of aerobic PCB degradation in anaerobic Hudson River sediments by addition of hydrogen peroxide as an oxygenation agent. Again, the PCB congener distribution of the sediment indicated that considerable reductive dechlorination had occurred previously. In this study, Aroclor 1242 was added to the sediment to give a final concentration of 300 p.p.m. Sediments were incubated under anaerobic conditions for 76 weeks before oxygenation by addition of hydrogen peroxide. Analysis demonstrated that the anaerobic treatment reduced the levels of di-, tri-, tetra-, penta- and hexachlorinated congeners by 11%, 73%, 66%, 73% and 94%, respectively: correspondingly the amount of monochlorobiphenyls increased by 76%. At the highest dose of hydrogen peroxide (1.7 g H_2O_2 per litre sediment/water), subsequent oxygenation resulted in a 95% reduction in the total mass of PCBs present in the sediment. As no chloride measurements were reported during these experiments it is not possible to determine the extent to which mineralization of the PCBs occurred. This group also noted that a certain proportion of the PCBs were resistant to degradation, and this was attributed to sorption of PCBs on the sediment matrix.

Although it is recognized that the extreme hydrophobicity of PCBs limits their bioavailability both in soils and aqueous systems, few efforts have been made to overcome this problem. The hydrophobicity of these compounds may also influence materials that can be used to construct bioreactors, as the PCBs adhere strongly to a wide range of surfaces.

The use of surfactants is well established in oil recovery and hydrocarbon degradation; however, the implications for PCB degradation are not clear. Aronstein and Alexander (1993) have recently reported that addition of non-ionic surfactants can enhance biodegradation of biphenyl and phenanthrene in laboratory soil columns, even at concentrations lower than those required to desorb the compounds from soil, suggesting that physiological factors may also be involved. Similarly, Guerin and Jones (1988) found that the addition of anionic surfactants to liquid cultures of *Mycobacterium* sp. strain BG1 enhanced the rate of degradation of phenanthrene, but also that the order of enhancement did not correlate with increased solubility. Surfactants have also been shown to enhance significantly ligninase production by *P. chrysosporium* (Asther *et al.*, 1987; Lestan *et al.*, 1993). The potential of surfactants to facilitate the biodegradation of PCBs merits considerable further attention. Kiu (1980) found that the rate of degradation of PCBs by *Pseudomonas* sp. strain 7509 could be enhanced by growing the cells in a stable PCB–ligninsulfonate emulsion. Biodegradation took place at the PCB–water interface.

There is clearly scope for the use of *in vitro* DNA manipulation in PCB bioremediation. Mondello (1989) achieved the expression of the *P. putida* LB400

bphABCD genes in *Escherichia coli*. The recombinant strains were able to effect PCB degradation in liquid culture without the requirement of growth on biphenyl. However, *E. coli* is unlikely to be a suitable host, since this is not a native soil organism and does not grow readily at ambient temperatures. Lajoie *et al.* (1993) have recently exploited genetic engineering techniques in an elegant attempt at overcoming both the insolubility of PCBs and the need for a growth substrate to allow co-metabolism of PCBs. Broad-substrate-specificity PCB-degrading genes were cloned from a naturally occurring *Pseudomonas* species into a plasmid (pRK293). The plasmids were transferred to a host (*Pseudomonas paucimobilis* 1IGP4) which uses a non-toxic, water-soluble, non-ionic surfactant as a selective growth substrate. Treatment of soil contaminated with Aroclor 1242 in the presence of the surfactant (Igepal CO-720, 1% w/w) with the recombinant strain resulted in the degradation of many PCB congeners in the absence of biphenyl. The authors proposed that the ability of the surfactant to enhance the bioavailability of the PCBs could be better exploited in a soil–slurry bioreactor.

Conclusions

In this chapter we have attempted to discuss all the factors relevant to the development of strategies for PCB bioremediation. The rational design of processes for the degradation of PCBs have been impeded by the very physico-chemical characteristics which made them attractive chemicals. The extreme hydrophobicity of PCBs and their high sorption coefficients provide the first obstacle to degradation, namely the very low bioavailability of PCBs. Typical bioremediation strategies rely on the target compounds being at least partially soluble in the aqueous phase in order to employ conventional biotreatment processes. One option is to pretreat the soil or sediment to give a PCB-rich liquor. Surfactant or solvent soil washing processes that are efficient, inexpensive and compatible with microbial PCB degradation have not yet been demonstrated. While not precluding this approach, it seems unlikely that suitable surfactants or solvents are available which would permit their use in a bioreactor. They are likely to be both inhibitory to any microbial population and to create a soil or sediment which, though PCB free, is unable to be returned to source without further treatment. The complexity of commercial PCB mixtures, which contained up to 70 congeners, has proved a major obstacle to their biodegradation. Much research has consequently focused upon single congeners or defined congener mixtures which are not representative of real contaminated sites.

The first step in any bioremediation process must be to consider the nature of the problem and the desired outcome within the framework of legislative guidelines. Legislation varies between countries and it is outside the scope of this chapter to compare global guidelines for PCBs. However, the situation in the UK serves to illustrate several factors. At present we have no system for measuring PCBs in which the individual congeners are assigned a weighted toxicity equivalent, thus enabling a standardized total figure to be reported. This has been recommended by the International Council for Exploration of the Sea (DoE, 1994). However, even the projected ICES guidelines recommend the use of only seven congeners (Ballschmiter numbers 7, 28, 52, 101, 118, 136, 153 and 180). Each of these are *ortho* substituted, and thus co-planar PCBs are not considered, even though it is known that these are the most

toxic congeners (Safe, 1990). In the UK, guidelines for PCB-contaminated soils and sediments are presently related to end-use for the particular site. While appreciating that this is preferable to multifunctionality, target values must be more clearly defined and be related to potential toxicity.

Whether setting guidelines or attempting to attain them, the validity and reproducibility of the analyses is of fundamental importance. We have attempted to present a review of the methods currently employed, each of which has inherent problems. It is worth reiterating that validation of results is of primary concern and the findings of Milby, Miller and Forrester (1985) and Holden (1986) show that even accredited laboratories are unable to provide reliable determination of PCBs. When this is coupled to the multiplicity of matrices, the inability to resolve clearly all congeners, the disproportionality of detector responses and the variety of internal standards used, it is not surprising that objective criticism of such data is difficult.

When developing bioremediation strategies, and assuming targets are known and measurable, the major task is to obtain isolates and consortia that are able to degrade, and ultimately mineralize, PCBs. Mineralization has to be the ultimate target in remediation strategies as it obviates further problems caused by known and unknown breakdown products. To achieve this requires both anaerobic and aerobic microorganisms for the reasons explained above. In both cases it seems clear that the origin of the isolate(s), the congener range, the presence of co-contaminants and the matrix characteristics are primary determinants of initial attack. Moreover, many of these determinants are site specific, and this should be borne in mind during laboratory investigations. Alongside these factors, the findings of key workers in the field have provided us with invaluable information relating to the microbial catalysts.

By far the least well-understood catabolic route is that which occurs under anaerobic conditions. Reductive dechlorination of PCBs by either an axenic culture or consortia free from sediment has never been shown. This has hampered the study of anaerobic PCB biodegradation and left many questions, such as the role of the sediment, unanswered. Despite this, it is clear that different consortia can have different congener specificites and the rate of dechlorination is often a function of the number and position of the chlorine atoms. One clear trend is that *meta* and *para* dechlorination of PCBs occurs far more frequently than *ortho* dechlorination. This is also true of the *ortho* chlorobenzoates, impeding the possible degradative routes of commercial PCB mixtures.

Much greater effort has been expended on the aerobic degradation of PCBs and their intermediates. Axenic cultures, consortia and hybrid strains have all been isolated and well characterized. Common rules relating to catabolism have been formulated, but the application of such strains has been limited. This is partly due to the same site-specific constraints applied to the anaerobes. Additionally, our understanding of the biochemistry and regulation of the processes involved in aerobic haloaromatic catabolism has alerted us to the limitations of these organisms. Cometabolic degradation of PCBs has been studied extensively and shown to facilitate the degradation of many congeners. However, although PCBs have been shown to be removed, the production of a number of different, and sometimes uncharacterized, metabolites has been shown. Chlorobenzoates, a major bottleneck in PCB mineralization, have been shown to be inhibitory to PCB biodegradation. It would therefore seem likely that these and other intermediates may inhibit or retard PCB biodegrada-

tion. The requirement for addition of biphenyl to stimulate co-metabolism may prove to be unfeasible on a large scale. This requirement may be obviated by developments in strain construction and genetic engineering, but fully competent strains have not yet been developed. In addition, legislation concerning the release of genetically engineered organisms into the environment does not currently favour this approach.

In conclusion, many factors influence the biodegradation of PCBs. Once clear on the legislative guidelines and the analytical methodology, a number of hurdles remain to be overcome. Despite the availability of extensive literature, clear ground-rules on the isolation and employment of micro-organisms for PCB mineralization have yet to be formulated. It seems unlikely that this will ever be possible, site investigation always being a prerequisite to bioremediation. This aside, current research will hopefully provide a basis for the sequential anaerobic and aerobic biotreatment of PCB-contaminated soils and sediments. Clearly, for such a process the challenge is as great in terms of engineering as it is for development of competent microbial consortia. Unlike many studies to date, success must not be determined solely by PCB disappearance, a yardstick too often used to promote incomplete bioremediation strategies. The problems associated with PCBs are by no means exclusive to these compounds and any rational remediation strategy is likely to be a blueprint for bioremediation of other hydrophobic pollutants.

Acknowledgements

The authors gratefully acknowledge the European Community who are funding the studies into PCB biodegradation in the laboratories of Prof. C.J. Knowles at the University of Kent. This is being done in collaboration with the laboratories of Prof. W. Reineke (Wuppertal, Germany), Dr G. Schraa (Wageningen, Holland) and Dr D. van der Lelie (Mol, Belgium). The authors acknowledge Prof. C.J. Knowles for his advice in the preparation of this chapter.

References

ABRAMOWICZ, D.A. (1990). Aerobic and anaerobic biodegradation of PCBs: a review. *Critical Reviews in Biotechnology* **10**, 241–251.

ADAMS, R.H., HUANG, C.M., HIGSON, F.K., BRENNER, V. AND FOCHT, D.D. (1992). Construction of a 3-chlorobiphenyl utilising recombinant from an intergenic mating. *Applied and Environmental Microbiology* **58**, 647–654.

ADRIENS, P. AND FOCHT, D.D. (1991). Cometabolism of 3,4-dichlorobenzoate by *Acinetobacter* sp. strain 4-CB1. *Applied and Environmental Microbiology* **57**, 173–179.

ADRIENS, P., KOHLER, H.-P.E., KOHLER-STAUB, D. AND FOCHT, D.D. (1989). Bacterial dehalogenation of chlorobenzoates and coculture biodegradation of 4,4'-dichlorobiphenyl. *Applied and Environmental Microbiology* **55**, 887–892.

AHMED, M. AND FOCHT, D.D. (1973). Degradation of polychlorinated biphenyls by two species of *Achromobacter*. *Canadian Journal of Microbiology* **19**, 47–52.

ALKINS, P.R. (1981). The pesticide manufacturing industry – Current waste treatment and disposal practices. EPA Project No. 12020-FYE NTIS Cat No. PB-211129. Jan. 1972. In *Pesticide Destruction and Detoxification Processes and Techniques* (A.P. Dillon, Ed.). Noyes Data Corp., Park Ridge, NJ.

ALLARD, A.S., HYNNING, P.A., LINDGREN, C., REMBERGER, M. AND NIELSON, A.H. (1991). Dechlorination of chlorocatechols by stable enrichment cultures of anaerobic bacteria. *Applied and Environmental Microbiology* **57**, 77–84.

AMEND, L.J. AND LEDERMAN, P.B. (1992). Critical evaluation of PCB remediation technologies. *Environmental Progress* **11**, 173–177.

ANID, P.J., RAVEST-WEBSTER, B.P. AND VOGEL, T.M. (1993). Effect of hydrogen peroxide on the biodegradation of PCBs in anaerobically dechlorinated river sediments. *Biodegradation* **4**, 241–248.

ARONSTEIN, B.N. AND ALEXANDER, M. (1993). Effect of a non-ionic surfactant added to the soil surface on the biodegradation of aromatic hydrocarbons within the soil. *Applied Microbiology and Biotechnology* **39**, 386–390.

ASTHER, M., CORRIEU, G., DRAPRON, R. AND ODIER, E. (1987). Effect of Tween 80 and oleic acid on ligninase production by *Phanerochaete chrysosporium* INA-12. *Enzyme and Microbial Technology* **9**, 245–249.

BALLSCHMITER, M. AND ZELL, M. (1980). Analysis of PCBs by glass capillary gas chromatography. *Fresenius Zeitschrift für Analytische Chemie* **302**, 20.

BARRIAULT, D. AND SYLVESTRE, M. (1993). Factors affecting PCB degradation by an implanted bacterial strain in soil microcosms. *Canadian Journal of Microbiology* **39**, 594–602.

BARTELS, I., KNACKMUSS, H.-J. AND REINEKE, W. (1984). Suicide inactivation of catechol-2,3-dioxygenase from *Pseudomonas putida* mt-2 by 3-halocatechols. *Applied and Environmental Microbiology* **47**, 500–505.

BARTON, M. AND CRAWFORD, R. (1988). Novel biotransformation of 4-chlorobiphenyl by a *Pseudomonas* sp. *Applied and Environmental Microbiology* **54**, 594–595.

BEDARD, D. (1990). Bacterial transformation of polychlorinated biphenyls. *Advances in Biotechnology* **4**, 369–387.

BEDARD, D.L. AND HABERL, M.L. (1990). Influence of chlorine substitution pattern on the degradation of PCBs by eight bacterial strains. *Microbial Ecology* **20**, 87–102.

BEDARD, D.L., UNTERMAN, R., BOPP, L.H., BRENNAN, M.J., HABERL, M.L. AND JOHNSON, C. (1986). Rapid assay for screening and characterizing microorganisms for the ability to degrade polychlorinated biphenyls. *Applied and Environmental Microbiology* **51**, 761–768.

BEDARD, D.L., WAGNER, D.L., BRENNAN, M.J., HABERL, M.L. AND BROWN, J.F. (1987a). Extensive degradation of Aroclors and environmentally transformed polychlorinated biphenyls by *Alcaligines eutrophus* H850. *Applied and Environmental Microbiology* **53**, 1094–1102.

BEDARD, D.L., HABERL, M.L., MAY, R. AND BRENNAN, M.J. (1987b). Evidence for novel mechanisms of polychlorinated biphenyl metabolism in *Alcaligines eutrophus* H850. *Applied and Environmental Microbiology* **53**, 1103–1112.

BERGH, A.K. AND PEOPLE, R.S. (1977). PCB distribution in sewage waste and their environmental and community effects p 4–6. *Proceedings of the National Conference on the Treatment and Disposal of Industrial Wastewaters and Residues, Houston.*

BOPP, L.H. (1986). Degradation of highly chlorinated PCBs by *Pseudomonas* strain LB400. *Journal of Industrial Microbiology* **1**, 23–29.

BOYLE, A.W., SILVIN, C.J., HASSETT, J.P., NAKAS, J.P. AND TANENBAUM, S.W. (1992). Bacterial PCB biodegradation. *Biodegradation* **3**, 285–298.

BROWN, J.F., JR, WAGNER, R.E., FENG, H., BEDARD, D.L., BRENNAN, M.J., CARNAHAN, J.C. AND RAY, R.J. (1987). Environmental dechlorination of PCBs. *Environmental and Toxicological Chemistry* **6**, 579–593.

BROWN, R.S., PETTIT, K., PRICE, D. AND JONES, P.W. (1991). Thermal desorption gas chromatography: a quick screening technique for polychlorinated biphenyls. *Chemosphere* **23**, 1145–1150.

BRUNNER, W., SUTHERLAND, F.H. AND FOCHT, D.D. (1985). Enhanced biodegradation of polychlorinated biphenyls in soil by analogue enrichment and bacterial inoculation. *Journal of Environmental Quality* **14**, 324–328.

BRUNSBACH, F.R. AND REINEKE, W. (1993). Degradation of chlorobenzoates in soil slurry using special organisms. *Applied Microbiology and Biotechnology* **39**, 117–122.

BUMPUS, J.A., TIEN, M., WRIGHT, D. AND AUST, S.D. (1985). Oxidation of persistent environmental pollutants by a white rot fungus. *Science* **228**, 1434–1436.

BUMPUS, J.A., FERNANDO, T., MILESKI, G.J. AND AUST, S.D. (1988). Biodegradation of organopollutants by *Phanerochaete chrysoporium*: practical considerations. *Proceedings*

of the 13th Annual Research Symposium on Land Disposal Remedial Action, Incineration and Treatment of Hazardous Waste, pp. 411–418. US Environmental Protection Agency, EPA/600/2–86/040.

BUMPUS, J.A., MILESKI, G., BROCK, B., ASHBAUGH, W. AND AUST, S.D. (1990). Biological oxidations of organic compounds by enzymes from a white rot fungus. In *Innovative Hazardous Waste Treatment Technology*. Vol. 3: *Biological Processes* (H.M. Freeman and P.R. Sferra, Eds), pp. 47–54. Technomic Publishing.

CAIN, R.B., TRANTER, E.K. AND DARRAH, J. (1968). The utilisation of some halogenated aromatic acids by *Nocardia*. *Biochemical Journal* **106**, 211–227.

CAIRNS, T. AND SIEGMUND, E.G. (1981). Determination of polychlorinated biphenyls by chemical ionization mass-spectrometry. *Analytical Chemistry* **53**, 1599–1603.

CAREY, A.E. AND HARVEY, G.R. (1978). Metabolism of polychlorinated biphenyls by marine bacteria. *Bulletin of Environmental Contamination and Toxicology* **20**, 527–534.

CATELANI, D., SORLINI, C. AND TRECANNI, V. (1971). The metabolism of biphenyl by *Pseudomonas putida*. *Experientia* **27**, 1173–1174.

CATELANI, D., COLOMBI, A., SORLINI, C. AND TRECANNI, V. (1973). Metabolism of biphenyl. *Biochemical Journal* **134**, 1063–1066.

CHEN, M., HONG, C.S., BUSH, B. AND RHEE, G.Y. (1988). Anaerobic biodegradation of polychlorinated biphenyls by bacteria from Hudson River sediments. *Ecotoxicology and Environmental Safety* **16**, 95–105.

COLE, M.A., REICHART, P.B. AND BUTTON, D.K. (1979). Reversible bioconcentration of monochlorinated biphenyls by *Rhodotorula rubra*, correlations with aqueous solubility. *Bulletin of Environmental Contamination and Toxicology* **23**, 44.

CRIPPS, R.E. (1975). The metabolism of acetophenone. *Biochemical Journal* **152**, 233–241.

CRIPPS, R.E., TRUDGILL, P.W. AND WHATELEY, J.G. (1978). The metabolism of 1-phenylethanol and acetophenone by *Nocardia* T5 and an *Arthrobacter* species. *European Journal of Biochemistry* **86**, 175–186.

DAGLEY, S. (1978). Pathways for the utilisation of organic growth substrates. In *The Bacteria*, Vol. 6 (L.N. Ornston and J.R. Sokatch, Eds), pp. 305–388. Academic Press, New York.

DEPARTMENT OF THE ENVIRONMENT (1976, 1994). *Polychlorinated biphenyls*. Waste Management Paper No. 6. HMSO, London.

DEWEERD, K.A., MANDELCO, L., TANNER, R.S., WOESE, C.R. AND SUFLITA, J.M. (1990). *Desulfomonile tiedje* gen. nov. and sp. nov., a novel anaerobic, dehalogenating, sulfate-reducing bacterium. *Archives of Microbiology* **154**, 23–30.

DILLON, A.P. (1981). *Pesticide Disposal and Detoxification Processes and Techniques*. Noyes Data Corp., Park Ridge, NJ.

DMOCHEWITZ, S. AND BALLSCHMITER, K. (1988). Microbial transformation of technical mixtures of polychlorinated-biphenyls (PCB) by the fungus *Aspergillus niger*. *Chemosphere* **17**, 111–121.

DOLFING, J. (1990). Reductive dechlorination of 3-chlorobenzoate is coupled to ATP production and growth in an anaerobic bacterium, strain DCB-1. *Archives of Microbiology* **153**, 264–266.

DOLFING, J. AND HARRISON, B.K. (1992). Gibbs free energy of formation of halogenated aromatic compounds and their potential role as electron acceptors in anaerobic environments. *Environmental Science and Technology* **26**, 2213–2218.

DOLFING, J. AND TIEDJE, J.M. (1986). Hydrogen cycling in a three- tiered food web growing on the methanogenic conversion of 3-chlorobenzoate. *FEMS Microbial Ecology* **38**, 293–298.

DOLFING, J. AND TIEDJE, J.M. (1987). Growth yield increase linked to reductive dechlorination in a defined 3-chlorobenzoate degrading methanogenic culture. *Archives of Microbiology* **149**, 102–105.

DORN, E., HELLWIG, M., REINEKE, W. AND KNACKMUSS, H.J. (1974). Isolation and characterisation of a 3-chlorobenzoate degrading pseudomonad. *Archives of Microbiology* **99**, 61–70.

DOSKEY, P.V. AND ANDREN, A.W. (1981). Concentrations of airborne PCBs over Lake Michigan. *Journal of Great Lakes Research* **7**, 15–20.

DUNNIVANT, F.M., ELZERMAN, A.W., JURS, P.C. AND HASON, M.N. (1992). Quantitative

structure–property relationships for aqueous solubilities and Henry's Law constants of polychlorinated biphenyls. *Environmental Science and Technology* **26**, 1567–1573.

EATON, D.C. (1985). Mineralisation of polychlorinated biphenyls by *Phanerochaete chrysosporium*, a lignolytic fungus. *Enzyme and Microbial Technology* **7**, 194–196.

ELSNER, A., LOFFLER, F., MIYASHITA, K., MULLER, R. AND LINGENS, F. (1991). Resolution of 4-chlorobenzoate dehalogenase from *Pseudomonas* sp. strain CBS3 into three components. *Applied and Environmental Microbiology* **57**, 324–326.

ENGELBERTS, K., SCHMIDT, E. AND REINEKE, W. (1989). Degradation of *O*-toluate by *Pseudomonas* sp. strain WR401. *FEMS Microbiology Letters* **59**, 35–38.

ENGESSER, K.H. AND SCHULTE, P. (1989). Degradation of 2-bromo, 2- chloro- and 2-fluorobenzoate by *Pseudomonas putida* CLB 250. *FEMS Microbiology Letters* **60**, 143–148.

EPA (1989). The Superfund Innovative Technology Evaluation Programme. Technology Profiles Report No. EPA/540/5–89/013. EPA Risk Reduction Engineering Laboratory, Cincinnati, Ohio.

EPA FEDERAL REGISTER Vol. 52, 63/P 10695.

FAVA, F. AND MARCHETTI, L. (1991). Degradation and mineralisation of 3-chlorobiphenyl by mixed aerobic bacterial culture. *Applied Microbiology and Biotechnology* **36**, 240–245.

FAVA, F., CINTI, S. AND MARCHETTI, L. (1993). Dechlorination of Fenchlor 54 and of a synthetic mixture of PCBs by anaerobic microorganisms. *Applied Microbiology and Biotechnology* **38**, 808–814.

FAVA, F., DI GIOIA, D., CINTI, S., MARCHETTI, L. AND QUATTRONI, G. (1994). Degradation and dechlorination of low-chlorinated biphenyls by a three-membered bacterial co-culture. *Applied Microbiology and Biotechnology* **41**, 117–123.

FETZNER, S., MULLER, R. AND LINGENS, F. (1989). A novel metabolite in the microbial degradation of 2-chlorobenzoate. *Biochemical and Biophysical Research Communications* **161**, 700–705.

FOCHT, D.D. (1993). Microbial degradation of chlorinated biphenyls. *Soil Biochemistry* **8**, 341–407.

FRIES, F.G. (1971). Degradation of chlorinated hydrocarbons under anaerobic conditions. *Advanced Chemistry Series* **111**, 256–270.

FURUKAWA, K. AND CHAKRABARTY, A.M. (1982). Involvement of plasmids in total degradation of chlorinated biphenyls. *Applied and Environmental Microbiology* **44**, 619–626.

FURUKAWA, K. AND MIYAZAKI, T. (1986). Cloning of a gene cluster encoding biphenyl and chlorobiphenyl degradation in *Pseudomonas pseudoalcaligenes*. *Journal of Bacteriology* **166**, 392–398.

FURUKAWA, K., ARIMURA, M. AND MIYAZAKI, T. (1987). Nucleotide sequence of the 2,3-dihydroxybiphenyl dioxygenase gene of *Pseudomonas pseudoalcaligenes*. *Journal of Bacteriology* **169**, 427–429.

FURUKAWA, K., MATSUMURA, F. AND TONOMURA, K. (1978). *Alcaligines* and *Acinetobacter* strains capable of degrading polychlorinated biphenyls. *Agricultural and Biological Chemistry* **42**, 543–548.

FURUKAWA, K., TOMIZUKA, N. AND KAMIBAYASHI, A. (1979). Effect of chlorine substitution on the bacterial metabolism of various polychlorinated biphenyls. *Applied and Environmental Microbiology* **38**, 301–310.

FURUKAWA, K., TONOMURA, K. AND KAMIBAYASHI, A. (1978). Effect of chlorine substitution on the biodegradability of polychlorinated biphenyls. *Applied and Environmental Microbiology* **35**, 223–227.

FURUKAWA, K., HAYASE, N., TAIRA, R. AND TOMIZUKA, N. (1989). Molecular relationship of chromosomal genes encoding biphenyl/polychlorinated biphenyl catabolism; some soil bacteria possess a highly conserved *bph* operon. *Journal of Bacteriology* **171**, 5467–5472.

GALLI, R. AND MCCARTY, P.L. (1989a). Biotransformation of 1,1,1-trichloroethane, trichloromethane and tetrachloromethane by a *Clostridium* sp. *Applied and Environmental Microbiology* **55**, 837–844.

GALLI, R. AND MCCARTY, P.L. (1989b). Kinetics of the biotransformation of 1,1,1-trichloromethane by *Clostridium* sp. strain TCA 11B. *Applied and Environmental Microbiology* **55**, 845–851.

GERRITSE, J., VAN DER WANDE, B.J. AND GOTTSCHAL, J.C. (1992). Specific removal of chlorine from the *ortho* position of halogenated benzoic acids by reductive dechlorination in anaerobic enrichment cultures. *FEMS Microbiology Letters* **100**, 273–280.

GIBSON, D.T., CRUDEN, D.L., HADDOCK, J.D., ZYLSTRA, G.J. AND BRAND, J.M. (1993). Oxidation of polychlorinated biphenyls by *Pseudomonas* sp. strain LB400 and *Pseudomonas pseudoalcaligines* KF707. *Journal of Bacteriology* **175**, 4561–4564.

GIBSON, D.T., ROBERTS, R.L., WELLS, M.C. AND KOBAL, V.M. (1973). Oxidation of biphenyl by a *Beijerinckia* species. *Biochemical and Biophysical Research Communications* **50**, 211–219.

GORDON, A.S. AND MILLERO, F.J. (1985). Adsorption mediated decrease in the biodegradation rate of organic compounds. *Microbial Ecology* **11**, 289–298.

GOULDING, C., GULLIN, C.J. AND BOLTON, E. (1988). Biodegradation of substituted benzenes. *Journal of Applied Bacteriology* **65**, 1–5.

GRIFFIN, R.A. AND CHIAN, E.S.K. (1979). Attenuation of water soluble PCBs by earth materials. *EGN 86 Illinois State Geological Survey*, Champaign, p. 98.

GUERIN, W.F. AND BOYD, S.A. (1992). Differential bioavailability of soil-sorbed naphthalene to two bacterial species. *Applied and Environmental Microbiology* **58**, 1142–1152.

GUERIN, W.F. AND JONES, G.E. (1988). Mineralisation of phenanthrene by a *Mycobacterium* sp. *Applied and Environmental Microbiology* **54**, 937–944.

HALEY, M.V., VICKERS, E.J., CHEG, T.-U., DEFRANK, J., JUSTUS, T.A. AND LANDIS, W.G. (1990). Biodegradation and aquatic toxicity reduction of the persistent riot control material, 1,4-dibenz-oxazephine. In *American Society for Testing and Materials ASTM STP 1096* (W.G. Landis and W.M. van der Scahlie, Eds).

HALLER, H.D. AND FINN, R.R. (1979). Biodegradation of 3-chlorobenzoate and the formation of black colour in the presence and absence of benzoate. *European Journal of Applied Microbiology and Biotechnology* **8**, 191–205.

HAMDY, N.K. AND LIN, Y.C. (1983). Biodegradation of chlorinated hydrocarbons. *83rd AGM of the American Society of Microbiology, New Orleans, 6 March.*

HAMELINK, J.L., WAYBRANT, R.C. AND BALL, R.C. (1971). A proposal: exchange equilibria control the degree chlorinated hydrocarbons are biologically magnified in Lentic environments. *Transactions of the American Fish Society* **100**, 207.

HAQUE, R. AND SCHMEDDING, D. (1976). Studies on the adsorption of selected polychlorinated biphenyls and isomers on several surfaces. *Journal of Environmental Science and Health* **B11**, 129.

HARDMAN, D.J. (1991). Biotransformation of halogenated compounds. *Critical Reviews in Biotechnology* **11**, 1–40.

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 degradation in Hudson River sediments. *Science* **259**, 503–507.

HARTMANN, J., ENGELBERTS, K., NORDHAUS, B., SCHMIDT, E. AND REINEKE, W. (1989). Degradation of 2-chlorobenzoate by *in vivo* constructed hybrid pseudomonads. *FEMS Microbiology Letters* **61**, 17–22.

HAVEL, J. AND REINEKE, W. (1991). Total degradation of various chlorobiphenyls by cocultures and *in vivo* constructed hybrid pseudomonads. *FEMS Microbiology Letters* **78**, 163–170.

HAVEL, J. AND REINEKE, W. (1992). Degradation of Aroclor 1221 and survival of strains in soil microcosms. *Applied Microbiology and Biotechnology* **38**, 129–134.

HAVEL, J. AND REINEKE, W. (1993a). Microbial degradation of chlorinated acetophenones. *Applied and Environmental Microbiology* **59**, 2706–2712.

HAVEL, J. AND REINEKE, W. (1993b). Degradation of Aroclor 1221 in soil by a hybrid pseudomonad. *FEMS Microbiology Letters* **108**, 211–218.

HAYASE, N., TAIRA, K. AND FURUKAWA, K. (1990). *Pseudomonas putida* KF715 *bphABCD* operon encoding polychlorinated biphenyl degradation: cloning, analysis, and expression in soil bacteria. *Journal of Bacteriology* **172**, 1160–1164.

HEALTH AND SAFETY EXECUTIVE (1993). Occupational Exposure Limits 1993, EH40/93.

HERNANDEZ, B.S., HIGSON, F.K., KONDRAT, R. AND FOCHT, D.D. (1991). Metabolism of and inhibition of chlorobenzoates in *Pseudomonas putida* P111. *Applied and Environmental Microbiology* **57**, 3361–3366.

HICKEY, W.J. AND FOCHT, D.D. (1990). Degradation of mono, di, and trihalogenated benzoic acids by *Pseudomonas aeroginosa* JB2. *Applied and Environmental Microbiology* **56**, 3842–3850.

HICKEY, W.J., BRENNER, V. AND FOCHT, D.D. (1992). Mineralisation of 2-chloro and 2,5-dichlorobiphenyl by *Pseudomonas* sp. strain UCR2. *FEMS Microbiology Letters* **98**, 175–180.

HICKEY, W.J., SEARLES, D.B. AND FOCHT, D.D. (1993). Enhanced mineralisation of polychlorinated biphenyls in soil inoculated with chlorobenzoate degrading bacteria. *Applied and Environmental Microbiology* **59**, 1194–1200.

HIGSON, F.K. AND FOCHT, D.D. (1990). Bacterial degradation of ring chlorinated acetophenones. *Applied and Environmental Microbiology* **56**, 3678–3685.

HILL, D.L., PHELPS, T.J., PALUMBO, A.V., WHITE, D.C., STRANDBERG, G.W. AND DONALDSON, T.L. (1989). Bioremediation of polychlorinated biphenyls: degradation capabilities in field lysimeters. *Applied Biochemistry and Biotechnology* **20/21**, 233–243.

HOLDEN, A.V. (1986). The reliability of PCB analysis. In *PCBs and the Environment*, Vol. 1 (J.S. Waid, Ed.), pp. 66–78. CRC Press, Boca Raton.

HOROWITZ, A., SUFLITA, J.M. AND TIEDJE, J.M. (1983). Reductive dehalogenations of halobenzoates by anaerobic lake sediment microorganisms. *Applied and Environmental Microbiology* **45**, 1459–1465.

HORVARTH, R.S. AND ALEXANDER, M. (1970). Cometabolism of *m*-chlorobenzoate by an *Arthrobacter*. *Applied Microbiology* **20**, 254–258.

HOUSE OF COMMONS SELECT COMMITTEE ON THE ENVIRONMENT (1990). First Report on Contaminated Land. Vol. I. xliv (143).

HOWARD, H.K. (1990). Biodegradation of aqueous hazardous waste leachates in a pilot-scale rotating biological contractor. In *Innovative Hazardous Waste Treatment Technology*. Vol. 3: *Biological Processes* (H.M. Freeman and P.R. Sferra, Eds), pp. 1–6. Technomic Publishing.

HUANG, S. AND GIBSON, G.G. (1992). Species and congener specific induction of hepatic cytochrome P450 4a by PCBs. *Biochemical Pharmacology* **43**, 637–639.

HUGHES, D.E. (1965). The metabolism of halogen substituted benzoic acids by *Pseudomonas fluorescens*. *Biochemical Journal* **96**, 181–188.

HUTZINGER, O., SAFE, S. AND ZITKO, V. (1974). *The Chemistry of PCBs*. CRC Press, Cleveland, Ohio.

ICRCL (1987). *Guidance on the Assessment and Redevelopment of Contaminated Land*. Guidance Note ICRCL 59/83, (2nd Edn). ICRCL, DoE, Romney House, 43 Marsham St, London SW1 3PY, UK.

JENSEN, S. (1972). The PCB story. *Ambio* **1**, 123–131.

JOHNSON, H.W., BRIGGS, G.G. AND ALEXANDER, M. (1972). Metabolism of 3-chlorobenzoic acid by a pseudomonad. *Soil Biology and Biochemistry* **4**, 187–190.

JOHNSTON, P.A. AND SIMMONDS, M.P. (1989). *A brief overview of the health and environmental effects of PCBs*. Submission to the Welsh Affairs Committee (12/12/89).

JONES, K.C., SANDERS, G., WILD, R., BURNETT, V. AND JOHNSTON, A.E. (1992). Evidence for a decline of PCBs and PAHs in rural vegetation and air in the United Kingdom. *Nature* **356**, 137–140.

KELLY, M.P., HALLBERG, K.B. AND TUOVINEN, O.H. (1989). Biological degradation of 2,4-dichlorophenoxyacetic acid: chloride mass balance. *Applied and Environmental Microbiology* **55**, 2717–2719.

KHAN, A. AND WALIA, S. (1989). Cloning of bacterial genes specifying degradation of 4-chlorobiphenyl from *Pseudomonas putida* OU843. *Applied and Environmental Microbiology* **55**, 2717–2719.

KHAN, A. AND WALIA, S. (1989). Cloning of bacterial genes specifying degradation of 4-chlorobiphenyl from *Pseudomonas putida* OU843. *Applied and Environmental Microbiology* **55**, 798–805.

KIMBARA, R., HASHMOTO, T., FUKUDA, M., ROANA, T., TAKAGI, M., OISHI, M. AND YANO, R. (1989). Cloning and sequencing of two tandem genes involved in degradation of 2,3-dihydroxybiphenyl to benzoic acid in the polychlorinated biphenyl-degrading soil bacterium *Pseudomonas* sp. strain KKS102. *Journal of Bacteriology* **171**, 2740–2747.

KIMBROUGH, R.D. (1980). Occupational exposure. In *Halogenated Biphenyls, Terphenyls, Naphthalenes, Dibenzodioxins and Related Products* (R.D. Kimbrough, Ed.), Chapter 9c. Topics in Environmental Health, Vol. 4. Elsevier/North-Holland Biomedical Press, Amsterdam.

KING, G.M. (1986). Inhibition of microbial activity in marine sediments by a bromophenol from a hemichordate. *Nature* **323**, 257.

KLECKA, G.M. AND GIBSON, D.T. (1981). Inhibition of catechol-2,3- dioxygenase from *Pseudomonas putida* by 3-chlorocatechol. *Applied and Environmental Microbiology* **41**, 1159–1165.

KOHLER, H.-P.E., KOHLER-STAUB, D. AND FOCHT, D.D. (1988). Cometabolism of polychlorinated biphenyls: enhanced transformation of Aroclor 1254 by growing bacterial cells. *Applied and Environmental Microbiology* **54**, 1940–1945.

KOKOZKA, L. AND FLOOD, J. (1985). A guide to EPA-approved PCB disposal methos. *Chemical Engineering*, 8 July, 41–43.

KONG, H.-L. AND SAYLER, G.S. (1983). Degradation and total mineralisation of monohalogenated biphenyls in natural sediment and mixed bacterial culture. *Applied and Environmental Microbiology* **46**, 666–672.

KRONE, U.E., LAUFER, K., THAUER, R.K. AND HOGENKAMP, H.P.C. (1989). Reductive dehalogenation of chlorinated C^1-hydrocarbons mediated by corrinoids. *Biochemistry* **28**, 4908–4914.

KRUMME, M.L. AND BOYD, S.A. (1988). Reductive dechlorination of chlorinated phenol in anaerobic upflow reactors. *Water Research* **22**, 171–177.

LAJOIE, C.A., ZYLSTRA, G.J., DEFLAUN, M.F. AND STROM, P.F. (1993). Development of Field application vectors for bioremediation of soils contaminated with polychlorinated biphenyls. *Applied and Environmental Microbiology* **59**, 1735–1741.

LAMAR, R.T., LARSEN, M.J., KIRK, T.K. AND GLASER, J.A. (1988). Growth of the white-rot fungus *Phanerochaete chrysosporium* in soil. *Proceedings of the 13th Annual Research Symposium on Land Disposal Remedial Action, Incineration and Treatment of Hazardous Waste*, pp. 411–418. US Environmental Protection Agency, EPA/600/2-86/040.

LEAGUE, J. (1990). Liquid solids contact (LSC) or how to bug hazardous waste to death. In *Innovative Hazardous Waste Treatment Technology*. Vol. 3: *Biological Processes* (H.M. Freeman and P.R. Sferra, Eds), pp. 19–24. Technomic Publishing.

LEE, M.C., GRIFFIN, R.A., MILLER, M.L. AND CHIAN, E.S.K. (1979). Adsorption of water soluble PCB Aroclor 1242 and used capacitor fluid by soil materials and coal chars. *Journal of Environmental Science and Health* **A14**, 415.

LESTAN, D., CERNILEC, M., STRANCAR, A. AND PERDIH, A. (1993). Influence of some surfactants and related compounds on ligninolytic activity of *Phanerochaete chrysosporium*. *FEMS Microbiology Letters* **106**, 17–22.

LEWANDOWSKI, G.A., ARMENANTS, P.M. AND PAK, D. (1990). Reactor design for hazardous waste treatment using a white rot fungus. *Water Research* **24**, 75–82.

LINKFIELD, T.G., SUFLITA, J.M. AND TIEDJE, J.M. (1989). Characterisation of the acclimation period before anaerobe dehalogenation of halobenzoates. *Applied and Environmental Microbiology* **55**, 2773–2778.

LIU, D. (1980). Enhancement of PCBs biodegradation by sodium ligninsulfonate. *Water Research* **14**, 1467–1475.

LOVELOCK, J.E. (1975). Natural halocarbons in the air and in the sea. *Nature* **256**, 193.

LOWE, J.I., PARRISH, P.R., PATRICK, J.M., JR AND FORRESTER, J. (1972). Effects of the PCB Aroclor 1254 on the American oyster *Crassastrea virginica*. *Marine Biology* **17**, 209.

LUNT, D. AND EVANS, C. (1970). The microbial metabolism of biphenyl. *Biochemical Journal* **118**, 54–55.

MASSÉ, R., MESSIER, F., PELOQUIN, L., AYOTTE, C. AND SYLVESTRE, M. (1984). Microbial degradation of 4-chlorobiphenyl: a model compound of chlorinated biphenyls. *Applied and Environmental Microbiology* **47**, 947–951.

MATTHEWS, H.B. AND ANDERSON, M. (1975). PCB dechlorinated versus PCB distribution and excretion. *Proceedings of the National Conference on PCBS* (F.A. Ayer, compiler), p. 50. US EPA Office of Toxic Substances, US Dept. Commerce, Washington, DC.

MAY, H.D., BOYLE, A.W., ALLENPRICE II, W. AND BLAKE, C.K. (1992). Subculturing of a polychlorinated biphenyl – dechlorinating anaerobic enrichment on solid media. *Applied and Environmental Microbiology* **58**, 4051–4054.

MIKESELL, M.D. AND BOYD, S.A. (1988). Enhancement of pentachlorophenol degradation in soil through induced anaerobiosis and bioaugmentation with anaerobic sewage sludge. *Environmental Science and Technology* **22**, 1411–1414.

MILBY, T.H., MILLER, P.E. AND FORRESTER, T.L. (1985). PCB-containing transformer fires: decontamination guidelines based on health consideration. *Journal of Occupational Medicine* **27**, 351–356.

MOEN, J.E.T. (1988). Soil protection in the Netherlands. In *Contaminated Soil '88*, Vol. 2. (W. Wolf, W.J. van der Brink and F.J. Colon, Eds), p. 1495. Kluwer Academic Publishers, Dordrecht.

MOHN, W.W. AND TIEDJE, J.M. (1990). Strain DCB-1 conserves energy for growth from reductive dechlorination coupled to formate oxidation. *Archives of Microbiology* **153**, 267–271.

MOHN, W.W. AND TIEDJE, J.M. (1992). Microbial reductive dehalogenation. *Microbiology Reviews* Sept., 482–507.

MOKROSS, H., SCHMIDT, E. AND REINEKE, W. (1990). Degradation of 3- chlorobiphenyl by *in vivo* constructed hybrid pseudomonads. *FEMS Microbiology Letters* **71**, 179–186.

MONDELLO, F.J. (1989). Cloning and expression in *Escherichia coli* of *Pseudomonas* strain LB400 genes encoding polychlorinated biphenyl degradation. *Journal of Bacteriology* **171**, 1725–1732.

MOZA, P., WEISBERGER, I. AND KLEIN, W. (1976). Fate of 2,2'-dichlorobiphenyl- ^{14}C in carrots, sugarbeets and soil under outdoor conditions. *Journal of Agricultural and Food Chemistry* **24**, 881.

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. *Environmental Science and Technology* **18**, 468–476.

MURPHY, T.J., MULLIN, M.D. AND MEYER, J.A. (1987). Equilibration of polychlorinated biphenyls and toxaphene with air and water. *Environmental Science and Technology* **21**, 155–162.

NADIM, L.M., SCHOCKEN, M.J., HIGSON, F.K., GIBSON, D.T., BEDARD, D.L., BOBB, L.H. AND MONDELLO, F.J. (1988). Bacterial oxidation of polychlorinated biphenyls. *Proceedings of the 13th Annual Research Symposium on Land Disposal Remedial Action, Incineration and Treatment of Hazardous Waste*, pp. 395–402. US Environmental Protection Agency, EPA/600/2-86/040.

NEILSON, A.H., ALLARD, A.S., LINDGREN, C. AND REMBERGER, M. (1987). Transformations of chloroguaiacols, chloroveratroles, and chlorocatechols by stable consortia of anaerobic bacteria. *Applied and Environmental Microbiology* **53**, 2511–2519.

NEILSON, A.H., ALLARD, A.S., HYNNING, P.A. AND REMBERGER, M. (1988). Transformations of halogenated aromatic aldehydes by metabolically stable anaerobic enrichment cultures. *Applied and Environmental Microbiology* **54**, 2226–2236.

NG, W.J., YAP, M.G.S. AND SIVADAS, M. (1989). Biological treatment of a pharmaceutical wastewater. *Biological Wastes* **29**, 299–311.

NIES, L. AND VOGEL, T.M. (1991). Identification of the proton source for the microbial reductive dechlorination of 2,3,4,5,6-pentachlorobiphenyl. *Applied and Environmental Microbiology* **57**, 2771–2774.

OECD (1987). *Control of PCB Waste*. Environmental Monograph No. 12.

OGRAM, A.V., JESSUP, R.E., LOU, L.T. AND RAO, P.S.C. (1985). Effects of sorption on biological degradation rates of (2,4-dichlorophenoxy) acetic acid in soils. *Applied and Environmental Microbiology* **49**, 582–587.

OLDENHUIS, R., VINCK, R.L.J.M., JANSSEN, D.B. AND WITHOLT, B. (1989). Degradation of chlorinated aliphatic hydrocarbons by *Methylosinus trichosprorium* OB3b expressing soluble methane monooxygenase. *Applied and Environmental Microbiology* **55**, 2819–2826.

PAL, D., WEBER, J.B. AND OVERCASH, M.R. (1980). The fate of PCBs in soil plant systems. *Residue Reviews* **74**, 45–98.

PARSONS, J.R., SIJM, D.T.H., VAN LAAR, A. AND HUTZINGER, O. (1988). Biodegradation of chlorinated biphenyls and benzoic acids by a *Pseudomonas* strain. *Applied Microbiology and Biotechnology* **29**, 81–84.

PAUL, E.A. AND CLARK, F.E. (1989). *Soil Microbiology and Biochemistry*. Academic Press, San Diego.

PELOQUIN, L. AND GREER, C.W. (1993). Cloning and expression of the polychlorinated biphenyl-degradation gene cluster from *Arthrobacter* M5 and comparison to analogous genes from Gram-negative bacteria. *Gene* **125**, 35–40.

PETTIGREW, C.A., BREEN, A., CORCORAN, C. AND SAYLER, G.S. (1990). Chlorinated biphenyl mineralisation by individual populations and consortia of freshwater bacteria. *Applied and Environmental Microbiology* **56**, 2036–2045.

QUENSEN, J.F., BOYD, S. AND TIEDJE, J. (1990). Dechlorination of four commercial polychlorinated biphenyl mixtures (Aroclors) by anaerobic microorganisms from sediments. *Applied and Environmental Microbiology* **56**, 2360–2369.

QUENSEN, J.F., TIEDJE, J.M., BOYD, S.A., ENKE, C., LOPSHIRE, R., GIESY, J., MORA, M., CRAWFORD, R. AND TILLIT, D. (1992). Evaluation of the suitability of reductive dechlorination for remediating PCB-contaminated soils and sediments. In *Soil Decontamination using Biological Processes*. DECHEMA, Frankfurt, Germany.

REINEKE, W. AND KNACKMUSS, H.-J. (1978a). Chemical structure and biodegradability of halogenated aromatic compounds. Substituent effects on 1,2-dioxygenation of benzoic acid. *Biochimica et Biophysica Acta* **542**, 412–423.

REINEKE, W. AND KNACKMUSS, H.-J. (1978b). Chemical structure and biodegradability of halogenated aromatic compounds. Substituent effects on dehydrogenation of 3,5-cyclohexadiene-1,2-diol-1-carboxylic acid. *Biochimica et Biophysica Acta* **542**, 424–429.

REINEKE, W. AND KNACKMUSS, H.-J. (1979). Construction of haloaromatics utilising bacteria. *Nature* **277**, 385–386.

REINEKE, W. AND KNACKMUSS, H.-J. (1980). Hybrid pathway for chlorobenzoate metabolism in *Pseudomonas* B13 derivatives. *Journal of Bacteriology* **142**, 467–473.

REINEKE, W. AND KNACKMUSS, H.-J. (1988). Microbial degradation of Haloaromatics. *Annual Reviews of Microbiology* **42**, 263–287.

REINEKE, W., WESSELS, S.W., RUBIO, M.A., LATORRE, J., SCHWEIN, U., SCHMIDT, E., SCHLOMANN, M. AND KNACKMUSS, H.-J. (1982). Degradation of monochlorinated aromatics following transfer of genes encoding chlorocatechol catabolism. *FEMS Microbiology Letters* **14**, 291–294.

RICE, C.P., EADIE, B.J. AND ERTSFELD, K.M. (1983). Enrichment of PCBs in Lake Michigan surface films. *Journal of Great Lakes Research* **8**, 265–270.

RIJNAARTS, H.H.M., BACHMANN, A., JUMELET, J.C. AND ZEHNDER, A.J.B. (1990). Effect of desorption and intraparticle mass transfer on the aerobic biomineralization of α-hexachlorocyclohexane in a contaminated calcareous soil. *Environmental Science and Technology* **24**, 1349–1354.

SAFE, S.H. (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). *Critical Reviews in Toxicology* **21**, 51–88.

SAFE, S. AND HUTZINGER, O. (1971). Chlorine randomization between phenyl groups in the electron impact-induced fragmentation of polychlorinated biphenyls. *Journal of the Chemical Society. Chemical Communications* 446–448.

SAFE, S. AND HUTZINGER, O. (1972). Mass spectra of polychlorinated biphenyls. *Journal of the Chemical Society. Perkin Transactions* **1**(5), 686–691.

SHANNON, M.J.R. AND UNTERMAN, R. (1993). Evaluating bioremediation: distinguishing fact from fiction. *Annual Reviews in Microbiology* **47**, 715–738.

SHELTON, D.R. AND TIEDJE, J.M. (1984). Isolation and partial characterisation of an anaerobic consortium that mineralizes 3-chlorobenzoic acid. *Applied and Environmental Microbiology* **48**, 840–848.

SHIELDS, M.S., HOOPER, S.W. AND SAYLER, G.S. (1985). Plasmid-mediated mineralisation of 4-chlorobiphenyl. *Journal of Bacteriology* **163**, 882–889.

SHIVARAMAN, N. AND PARHAD, N.M. (1990). Biological treatment of cyanide wastewaters. In *Innovative Hazardous Waste Treatment Technology*. Vol. 3: *Biological Processes* (H.M. Freeman and P.R. Sferra, Eds), pp. 39–46. Technomic Publishing.

SKEA, J.C., SIMONIN, H.A., BEAN, H.J., COLQUHOUN, J.R., SPAGNOLI, J.J. AND VEITH, G.D. (1979). Bioaccumulation of Aroclor 1016 in Hudson River fish. *Bulletin of Environmental Contamination and Toxicology* **22**, 332.

SONDOSSI, M., SYLVESTRE, M. AND AHMAD, D. (1992). Effects of chlorobenzoate transformation on the *Ps. testosteroni* biphenyl and chlorobiphenyl degradation pathway. *Applied and Environmental Microbiology* **58**, 485–495.

STONE, R. (1992). Swimming against the PCB tide. *Science* **255**, 798–799.

STORR-HANSEN, E. (1991). Comparative analysis of 30 PCB congeners and two capillary columns of different polarity with non-linear multi-level calibration. *Journal of Chromatography* **558**, 375–391.

SUFLITA, J.M., ROBINSON, J.A. AND TIEDJE, J.M. (1983). Kinetics of microbial dehalogenation of haloaromatic substrates in methanogenic environments. *Applied and Environmental Microbiology* **45**, 1466–1473.

SUIDA, J.F. AND DE BERNARDIS, J.F. (1973). Naturally occurring halogenated organic compounds. *Lloydia* **36**, 107.

SUZUKI, M. (1975). Translocation of polychlorobiphenyls in soil into plants. *Archives of Environmental Contamination and Toxicology* **5**, 343.

SYLVESTRE, M.V., MAILHIOT, K. AND AHMAD, D. (1989). Isolation and preliminary characterisation of a 2-chlorobenzoate degrading *Pseudomonas*. *Canadian Journal of Microbiology* **35**, 439–443.

TIEDJE, J.M., QUENSEN, J.F., CHEE-SANFORD, J., SCHIMEL, J.P. AND BOYD, S.A. (1993). Microbial reductive dechlorination of PCBs. *Biodegradation* **4**, 231–240.

TOPP, E. AND HANSON, R.S. (1990). Degradation of pentachlorophenol by a *Flavobacterium* species grown in continuous culture under various nutrient limitations. *Applied and Environmental Microbiology* **56**, 541–544.

TUCKER, E.S., LITSCHGI, W.J. AND MEES, W.M. (1975). Migration of polychlorinated biphenyls in soil induced by percolating water. *Bulletin of Environmental Contamination and Toxicology* **13**, 86.

UNTERMAN, R., BRENNAN, M.J., BROOKS, R.E. AND JOHNSON, C. (1986). Biological degradation of polychlorinated biphenyls. In *Biotechnology for Degradation of Toxic Chemicals in Hazardous Wastes* (R.J. Scholze, Ed.), Noyes Data Corp, Park Ridge, NJ.

UNTERMAN, R., BEDARD, D.L., BRENNAN, M.J., BOPP, L.H., MONDELLO, F.J., BROOKS, R.E., MOBLEY, D.P., MCDERMOTT, J.B., SCHWARTZ, C.C. AND DIETRICH, D.K. (1988). Biological approaches for polychlorinated biphenyl degradation. In *Environmental Biotechnology: Reducing Risks from Environmental Chemicals Through Biotechnology* (G.S. Omenn, Ed.), pp. 253–269. Plenum Press, New York.

VAN DORT, H.M. AND BEDARD, D.L. (1991). Reductive *ortho* and *meta* dechlorination of a polychlorinated biphenyl congener by anaerobic microorganisms. *Applied and Environmental Microbiology* **57**, 1576–1578.

VINEY, I. AND BEWLEY, R.J.F. (1988). *Microbial degradation of polychlorinated biphenyls*. Welsh Office Report. 0802883.

VINEY, I. AND BEWLEY, R.J.F. (1990). Preliminary studies on the development of a microbiological treatment for polychlorinated biphenyls. *Archives of Environmental Contamination and Toxicology* **19**, 789–796.

VISSCHER, K. AND BRINKMAN, J. (1989). Biological degradation of zenobiotics in waste management. *Hazardous Waste and Hazardous Materials* **6**, 201–212.

VOGEL, T.M., CRIDDLE, C.S. AND MCCARTY, P.L. (1987). Transformation of halogenated aliphatic compounds. *Environmental Science and Technology* **21**, 722–736.

WALKER, N. AND HARRIS, D. (1970). Metabolism of 3-chlorobenzoic acid by *Azotobacter* sp. *Soil Biology and Biochemistry* **2**, 27–32.

YATES, J.R. AND MONDELLO, F.J. (1989). Sequence similarities in the genes encoding PCB degradation by *Pseudomonas* strain LB400 and *Alcaligines eutrophus* H850. *Journal of Bacteriology* **171**, 1733–1735.

YE, D.Y., QUENSEN, J.F., TIEDJE, J.M. AND BOYD, S.A. (1992). Anaerobic dechlorination of polychlorobiphenyls (Aroclor 1242) by pasteurized and ethanol-treated microorganisms from sediments. *Applied and Environmental Microbiology* **58**, 1110–1114.

ZAITSEV, G.M. AND KARASEVICH, Y.N. (1982). Utilisation of 2-chlorobenzoic acid. *Mikrobiologiya* **53**, 75–80.