Biodegradation **1**: 207–220, 1990.
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Degradation of halogenated aromatic compounds

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Key words: halobenzoates, halobenzenes, halophenols, haloanilines, halophenoxyacetates, halobiphenyls, halodibenzo-*p*-dioxins, halodibenzofurans, hydrolytic dehalogenation, oxidative dehalogenation, reductive dehalogenation

Abstract

Due to their persistence, haloaromatics are compounds of environmental concern. Aerobically, bacteria degrade these compounds by mono- or dioxygenation of the aromatic ring. The common intermediate of these reactions is (halo)catechol. Halocatechol is cleaved either intradiol (*ortho*-cleavage) or extradiol (*meta*-cleavage). In contrast to *ortho*-cleavage, *meta*-cleavage of halocatechols yields toxic metabolites. Dehalogenation may occur fortuitously during oxygenation. Specific dehalogenation of aromatic compounds is performed by hydroxylases, in which the halo-substituent is replaced by a hydroxyl group. During reductive dehalogenation, haloaromatic compounds may act as electron-acceptors. Herewith, the halo-substituent is replaced by a hydrogen atom.

Abbreviations: CBz – chlorobenzene, DCBz – dichlorobenzene, TrCBz – trichlorobenzene, TCBz – tetrachlorobenzene, PCBz – pentachlorobenzene, HCBz – hexachlorobenzene, CBA – chlorobenzoic acid, BBA – bromobenzoic acid FBA – fluorobenzoic acid, IBA – iodobenzoic acid, CP – chlorophenol, CA – chloroaniline, PCBs – polychlorinated biphenyls, CB – chlorobiphenyl, 2,4-D – 2,4-dichlorophenoxyacetic acid, 2,4,5-T – 2,4,5-trichlorophenoxyacetic acid

Introduction

Halogenated aromatic compounds have been produced industrially on a large scale for several decades. Such chemicals, particularly the chlorinated ones, have been widely used as pesticides (e.g. DDT, 2,4-D, 2,4,5-T, chlorophenols) or for other industrial uses (e.g. PCBs in electrical equipment and as hydraulic fluids). Others, such as PCDDs and PCDFs are produced unintentionally as trace contaminants during industrial syntheses and incinerations. Brominated aromatic compounds have found use as flame retardants. Fluorinated and iodinated aromatic compounds are components of pharmaceutical agents. The chemical inertness and hydrophobicity of many of these compounds has resulted in them becoming widely distributed in the environment; in particular accumulating in many terrestial and aquatic organisms. This, coupled with their toxicity, has given rise to concern about their fate in the environment.

Despite the fact that naturally occurring halogenated aromatic compounds are rare, many bacteria have been isolated which can degrade such chemicals. These bacteria are often unable to grow on these compounds, but are able to degrade them while growing on other compounds, such as their nonhalogenated analogues. This process is referred to as co-metabolism.

The pathways by which halogenated aromatic

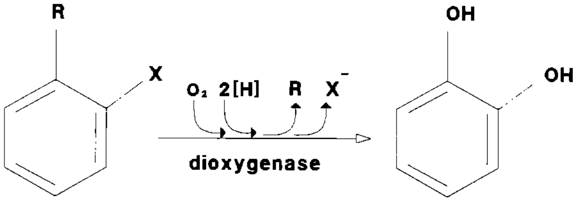

Fig. 1. Oxidative dehalogenation of haloaromatic compounds. R = e.g. COOH, H, NH_2. X = F, Cl, Br, I.

compounds are degraded by microorganisms are similar to those for the degradation of aromatic compounds in general. Under aerobic conditions aromatic compounds are transformed by mono- and di-oxygenation into dihydroxylated derivatives before ring cleavage takes place. Under anaerobic conditions, degradation follows reductive pathways. The aromaticity is lost before ring cleavage. The degradative pathways for aromatic compounds are described in detail elsewhere in this issue by Smith.

Dehalogenation of aromatic compounds

As halogen substituents of halogenated aromatic compounds are, to a large extent, responsible for their properties, removal of this substituents is a key step in their degradation. In many cases, dehalogenation of aromatic compounds occurs after the ring system is cleaved. Examples are the dehalogenation of halocatechols during ring cleavage by lactonization (Fig. 4). However, direct dehalogenation of aromatic compounds, without loss of aromaticity has been demonstrated. Three forms of such reactions are known:

1. Oxidative dehalogenation in which the halogen is lost fortuitously during oxygenation of the ring (Fig. 1). This reaction occurs only under aerobic conditions.
2. Hydrolytic dehalogenation in which a halogen (for example at the para position) is specifically replaced by a hydroxyl group (Fig. 2). The oxygen atom in the hydroxyl group is derived from water instead of from oxygen. This reaction can occur under both aerobic and denitrifying conditions.

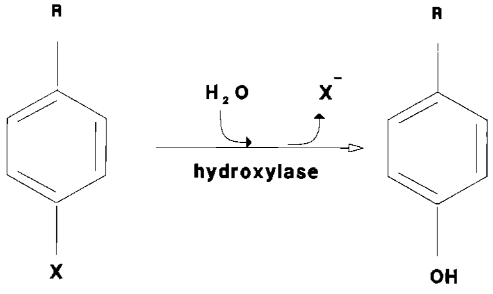

Fig. 2. Hydrolytic dehalogenation of haloaromatic compounds. R = e.g. COOH, OH, NH_2. X = F, Cl, Br, I.

3. Reductive dehalogenation in which the halogen is replaced by a hydrogen (Fig. 3). This reaction occurs almost exclusively under sulfogenic and methanogenic conditions. It has been proposed that the halogenated aromatic compound acts as a terminal electron acceptor. In one case it has been shown that reductive dehalogenation is coupled to growth and to ATP formation (see below) and thus can be referred to respiration.

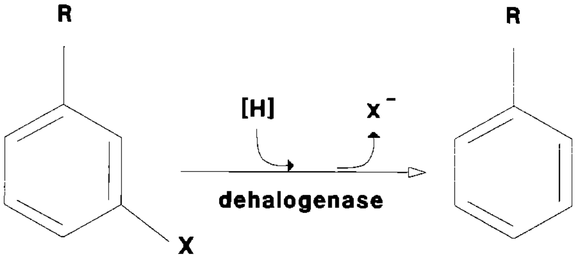

Fig. 3. Reductive dehalogenation of haloaromatic compounds. R = COOH, H, OH, NH_2, C_6H_5. X = F, Cl, Br, I.

Degradation of halogenated benzoic acids

Halogenated benzoates have been used extensively as model compounds to study the degradation of haloaromatics. The degradation of halogenated benzoate was reviewed earlier by Reineke (1984) and recently by Reineke & Knackmuss (1988).

Aerobically, halogenated benzoates are mainly degraded by dioxygenation of the aromatic ring

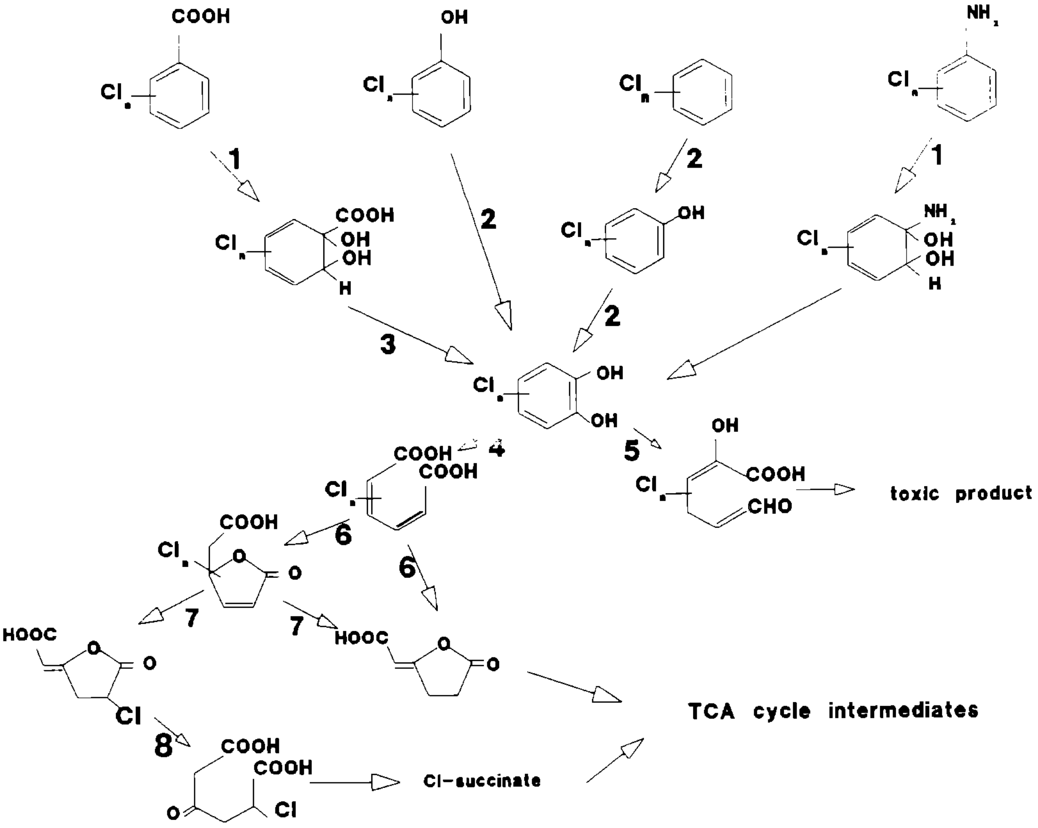

Fig. 4. Metabolic route for aerobic degradation of halobenzoates, halophenol, halobenzenes and haloanilines. 1 = 1,2-dioxygenase, 2 = monooxygenase, 3 = 3,5-cyclohexadiene-1,2-diol-carboxylate dehydrogenase, 4 = catechol-1,2-dioxygenase, 5 = catechol-2,3-dioxygenase, 6 = muconatecycloisomerase, 7 = muconolactoneisomerase, 8 = dienelactonehydrolase.

yielding halocatechols. *Ortho* substituted benzoates give 3-halocatechols, *meta* substitution gives 3- or 4-halocatechols and *para* substitution gives 4-halocatechols. Ring cleavage of these compounds takes place by the *ortho* route, yielding halo-*cis, cis*-muconates (Fig. 4). Many strains capable of degrading halobenzoates have been described. They are members of the genera *Pseudomonas* (Reineke & Knackmuss 1980; Schreiber et al. 1980; Chatterjee et al. 1981; Schmidt & Knackmuss 1984; Focht & Shelton 1987; Wyndham & Straus 1988a; Vora et al. 1988; Hartmann et al. 1989; Schlömann et al. 1990), *Alcaligenes* (Schmidt & Knackmuss 1984; Schmidt 1988; Wyndham & Straus 1988a; Schlömann et al. 1990), *Nocardia* (Cain et al. 1968; Spokes & Walker 1974) and *Azotobacter* (Walker & Harris 1970). Strain FLB 300 which is able to degrade all three monofluorobenzoates was assigned to the *Agrobacterium-Rhizobium* group (Engesser et al. 1990). Bacteria growing on these halobenzoates have catechol-1,2-dioxygenases with high activities towards substituted catechols, referred to as pyrocatechase II. The halogen substituent is eliminated from halo-*cis, cis*-muconate, halomuconolactone or even in the last catabolic step, preceeding the tricarboxylic acid cycle, from halosuccinate (Fig. 4).

Meta cleavage of halocatechols is performed by catechol-2,3-dioxygenases and gives halo-2-hydroxymuconate semialdehydes. These intermediates are toxic to the organisms.

An alternative catabolic route for 3-halobenzoate is via 5-chlorosalicylate and 2,3-dihydroxybenzoate. This route is found in *Bacillus* sp.

(Spokes & Walker 1974) and constructed in *Pseudomonas* WR1 (Lehrbach et al. 1984).

In some cases dehalogenation takes place in the first step. Halogen substitution at the *ortho* position of benzoates can lead to fortuitous oxidative dehalogenation by 1,2-dioxygenases. Oxidative dehalogenation of 2-fluorobenzoate is described for *Pseudomonas* sp. (Goldman et al. 1967; Milne et al. 1968; Vora et al. 1988), for *Acinetobacter calcoaceticus* (Clarke et al. 1975) and for *Pseudomonas* B13 (Schreiber et al. 1980). 2-Chlorobenzoate was oxidatively dehalogenated by *Pseudomonas* B300 (Sylvestre et al. 1989). *Pseudomonas putida* CLB250 oxidatively dehalogenated 2-FBA, 2-BBA and 2-CBA (Engesser & Schulte 1989). In these cases, both 1,2- and 1,6-dioxygenation took place. 1,2-Dioxygenation of 2-halobenzoates leads to catechol and 1,6-dioxygenation to 3-halocatechols. During growth the metabolic route continues via *ortho* cleavage. A mechanism in which chlorine was eliminated in the first step by 2,3-dioxygenase giving 2,3-dihydroxybenzoate as product was found in *Pseudomonas* sp. 2CBA (Fetzner et al. 1989).

Based on growth on salicylate Higson & Focht (1990) suggested hydrolytic dehalogenation yielding salicylate as the first step in 2-halobenzoate degradation by *Pseudomonas aeruginosa* 2-BBZA. However, most reports on hydrolytic dehalogenation involve the displacement of halogen by a hydroxyl group at the *para* position of benzoate. The metabolic route proceeds from 4-halobenzoate to 4-hydroxybenzoate and protocatechuate. 4-Halobenzoate dehalogenation was independent of benzoate degradation. Growing on 4-CBA, *Arthrobacter* sp. DSM 20407 showed *meta* cleavage of protocatechuate but when growing on benzoate the *ortho* cleavage route was followed (Ruisinger et al. 1976). Furthermore, a mutant strain of *Pseudomonas* sp. CBS3, which had lost the ability to grow on 4-CBA, could still grow on benzoate (Keil et al. 1981). The oxygen incorporated was demonstrated to originate from H_2O, not from O_2 (Marks et al. 1984b; Müller et al. 1984).

Genes of *Pseudomonas* sp. CBS3 specifying 4-chlorobenzoate dehalogenase were cloned in *Pseudomonas putida* KT2440. A 9.5 kilobase-pair fragment inserted in a plasmid conferred on this strain the ability to grow on 4-CBA but did not complement mutants unable to grow on 4-HBA (Savard et al. 1990). The 4-CBA dehalogenase enzyme of *Pseudomonas* CBS3 showed higher activities in alcohol than in water (Thiele et al. 1988a) and was also able to dehalogenate 4-chloro-dinitrobenzoates and 4-chloro-dinitrophenols (Thiele et al. 1988b).

Dehalogenation by hydroxylation seems very specific for the *para* position of halobenzoates. However, hydroxylation of 3-CBA was reported in 1972 by Johnston et al. Furthermore, *Acinetobacter* sp. 4CB1 showed both *para* and *meta* dehalogenation of 3,4-dichlorobenzoate, but this compound did not serve as growth substrate (Adriaens et al. 1989). Most bacteria which are capable of 4-CBA dehalogenation also dehalogenate 4-BBA and 4-IBA but not 4-FBA. These reactions are described for *Arthrobacter* sp. SU DSM 20407 (Müller et al. 1988), for *Pseudomonas* sp. CBS3 (Thiele et al. 1987) and for *Alcaligenes denitrificans* NTB-1 (Van den Tweel et al. 1986, 1987). Remarkably *Aureobacterium* sp. RHO25 dehalogenates 4-FBA but not 4-CBA (Oltmanns et al. 1989). Furthermore, Marks et al. (1984a) described a 4-chlorobenzoate dehalogenase with activity for 4-CBA, 4-BBA and 4-FBA.

Under anaerobic conditions halobenzoates are reductively dehalogenated. The first reports on this subject were in 1983 from Horowitz et al. and Suflita et al. They described the reductive dehalogenation of iodo-, bromo- and chlorobenzoates by a methanogenic bacterial consortium isolated from sewage sludge. The dechlorination of chlorobenzoates seemed specific for *meta* substituents whereas dehalogenation of iodo- and bromo-benzoates occurred at all three positions. Fluorobenzoates were never found to be reductively dehalogenated.

Several attempts were made to isolate and characterize the bacterium responsible for this reaction (Shelton & Tiedje 1984; Dolfing & Tiedje 1986; DeWeerd et al. 1986; Dolfing & Tiedje 1987; Stevens et al. 1988; Linkfield & Tiedje 1990; Mohn et al. 1990;) De Weerd et al. (1990) named this bacterium *Desulfomonile tiedjei* strain DCB-1 now catalogued as (ATCC 49306). This bacterium is a sulphate reducer and can use also 3-chlorobenzoate as terminal electron acceptor in the absence of sulphate. The reduction of 3-chlorobenzoate is coupled to ATP production (Dolfing 1990), which results in an increase of growth yield (Mohn & Tiedje 1990) and is thus a new form of anaerobic respiration.

A denitrifying consortium was also found to dehalogenate chlorobenzoates (Sharak Genthner et al. 1989). The dehalogenation occurred in presence of nitrate.

Reductive dechlorination was described for *Alcaligenes denitrificans* NTB-1Y under aerobic circumstances (van den Tweel et al. 1987). Prior to *para* hydroxylation, 2,4-dichlorobenzoate was reductively dehalogenated at the *ortho* position. Groenewegen et al. (1990) later characterized this bacterium as *Coryneform bacterium* NTB-1 and showed that ATP was necessary for the transport of 4-chlorobenzoate through the cell membrane.

Degradation of halogenated benzenes

Marinucci & Bartha (1979) described the degradation of 1,2,3- and 1,2,4-trichlorobenzenes to CO_2 in soil and cultures inoculated with soil. They identified 3,4,5-tri-, 2,6-di- and 2,3-dichlorophenol as metabolites of 1,2,3-TrCBz and 2,4-, 2,5- and 3,4-dichlorophenol in incubations with 1,2,4-TrCBz. Similarly, Ballschmiter & Scholz (1981) isolated 2,3-, 3,4- and 2,6-dichlorophenols from incubations of three *Pseudomonas putida* strains with 1,2-DCBz and 2,4,6-DCP from 1,3,5-TrCBz.

In contrast, the chlorobenzene-utilizing strain WR1306 was shown to degrade this compound via dioxygenation to form 3-chlorocatechol, which is further degraded by the *ortho*-cleavage pathway (Fig. 4) (Reineke & Knackmuss 1984). Reineke and Knackmuss suggested that the chlorophenols isolated by other workers were artefacts produced by acid-catalyzed dehydration of the *cis*-dihydrodiols formed by dioxygenation. In common with other chloroaroma-degrading bacteria, this strain appeared to contain ring cleavage enzymes showing high activity towards chlorinated substrates.

A similar pathway is utilized by two *Alcaligenes* strains which are able to grow on 1,3- and 1,4-DCBz, chlorobenzene and benzene (de Bont et al. 1986; Schraa et al. 1986), by *Pseudomonas* strain JS6 able to degrade all three dichlorobenzenes (Spain & Nishino 1987), by a 1,2-DCBz-utilising *Pseudomonas* strain (Haigler et al. 1988) and by *Pseudomonas* strain P51 which can grow on 1,2,4-TrCBz and the three dichlorobenzenes (van der Meer et al. 1987). Pyrocatechases (catechol-1,2-oxidases) with high activities towards chlorinated substrates also appear to be induced in such strains grown on chlorobenzenes. Although methyl-substituted benzenes are degraded via the *meta*-cleavage pathway, a mutant of strain JS6 degrades 4-chlorotoluene via the *ortho*-pathway (Haigler & Spain 1989).

Kröckel & Focht (1987) constructed a chlorobenzene-utilizing recombinant *Pseudomonas putida* strain from mixed cultures of toluene-grown *Pseudomonas putida* and benzoate-grown *Pseudomonas alcaligenes* strains exposed to chlorobenzene. Chromosomal DNA from the *Pseudomonas alcaligenes* strain was transferred and integrated in a TOL-like plasmid of the *Pseudomonas putida*. During insertion a 24 kB fragment was lost from the plasmid, which resulted in the loss of the ability to grow on xylene and methylbenzoates. This fragment coded for a meta-cleavage pyrocatechase with low specifity and high activity (Carney et al. 1989a, b).

Recently, bromobenzene-utilizing *Pseudomonas* strains have been isolated from chemostat cultures exposed to increasing concentrations of bromobenzene (Sperl & Harvey 1988). Bromocatechols appeared to be intermediates in the degradation of this compound.

Reductive dechlorination is the dominant degradative reaction of chlorobenzenes under anaerobic conditions. Hexachlorobenzene was dechlorinated by two pathways in anaerobic sewage sludge (Fathepure et al. 1988). The major route gave pentachlorobenzene, 1,2,3,5-TCBz and 1,3,5-TrCBz whereas the minor route yielded PCBz, 1,2,4,5-

TCBz, 1,2,4-TrCBz and dichlorobenzenes. There was no evidence for further reduction of 1,3,5-TrCBz. In contrast, Bosma et al. (1988) observed reductive dechlorination of all tri- and dichlorobenzene isomers in anaerobic sediment columns.

Degradation of halogenated phenols

Bacteria able to grow on pentachlorophenol, the most commonly used chlorophenol, were first described in the early 1970s (Chu & Kirsch 1972; Watanabe 1973). Three pathways have been identified for the biodegradation of chlorophenols and other halophenols. Mono- and di-chlorophenols are oxygenated to chlorocatechols, whereas the higher chlorinated phenols are hydroxylated to form chlorinated hydroquinones. Under anaerobic conditions chlorophenols undergo initial reductive dechlorination.

The 3-chlorobenzoate-utilizing *Pseudomonas* B 13 is also able to grow on phenol and 4-chlorophenol and to degrade other mono- and dichlorophenols (Knackmuss & Hellwig 1978). The chlorophenol-degrading bacteria show high activity of pyrocatechase II, a catechol 1,2-dioxygenase able to cleave chlorocatechols. The pathway proposed for the degradation of chlorophenols consists of initial monooxygenation to form chlorocatechols, which undergo *ortho* ring cleavage to chloromuconic acids, lactonization with loss of chloride and further degradation (the β-ketoadipate pathway). Cometabolism of 2-, 3- and 4-chloro- and 2,4- and 3,4-dichlorophenols by *Nocardia* sp. DSM 43251 follows a similar mechanism (Engelhardt et al. 1979).

Degradation of monochlorophenols was poor in defined mixed bacterial cultures containing *Pseudomonas* and *Alcaligenes* strains (Schmidt et al. 1983), due to the accumulation of toxic metabolites formed by *meta* cleavage of chlorocatechols. In the presence of *Pseudomonas* B 13, which degrades chlorocatechols via *ortho* cleavage, the chlorophenols were degraded with release of chloride. Hybrid strains were isolated from such mixed cultures which were able to grow on all three monochlorophenols, for example *Alcaligenes* strain A 7-2 (Schwien & Schmidt 1982).

The 2,4,5-trichlorophenoxyacetic acid-degrading *Pseudomonas* cepacia strain AC1100 is able to degrade a range of di-, tri-, tetra- and pentachlorophenols (Karns et al. 1983a). These chlorophenols are wholly or partly dechlorinated. Dehalogenation was also observed of 2,4-di-, 2,4,6-tri- and penta-bromophenol, but not of 2,4,6-triiodophenol. The enzymes responsible for dechlorination of 2,4,5-T, 2,4,5-trichlorophenol and PCP are induced by 2,4,5-TrCP (Karns et al. 1983b). The mechanism by which 2,4,5-TrCP is degraded by this strain has been identified as conversion of 2,4,5-TrCp to 2,5-dichlorohydroquinone, which undergoes a dehalogenation to 5-chloro-2-hydroxyhydroquinone and subsequent ring cleavage (Sangodkar et al. 1989).

Rhodococcus strains An 117 and An 213 co-metabolize monochlorophenols via the β-ketoadipate pathway (Janke et al. 1988a). Ring cleavage is catalyzed by 'ordinary' catechol-1,2-dioxygenases with low activity towards chlorocatechols. Degradation of 3- and 4-CP, but not of 2-CP, is stimulated in the presence of glucose as an extra source of energy and reducing equivalents (Janke et al. 1988b).

In contrast, the PCP-utilizing *Rhodococcus chlorophenolicus* initially attacks tri-, tetra- and pentachlorophenols by *para*-hydroxylation to produce chlorinated hydroquinones (Apajalahti & Salkinoja-Salonen 1987a). The hydroxyl group is derived from a water molecule. However, this reaction only takes place in the presence of molecular oxygen, which implies the involvement of ATP in the degradation. The tetrachlorohydroquinone formed from PCP is subsequently converted to a dichlorotrihydroxybenzene by a reaction involving both hydrolytic and reductive dechlorinations (Apajalahti & Salkinoja-Salonen 1987b) (Fig. 5). Two further reductive dechlorinations then give 1,2,4-trihydroxybenzene. Trichlorohydroquinone is degraded very slowly, suggesting that it is not an intermediate in this pathway. Similar results have been found for *Rhodococcus* strain CP-2 (Häggblom et al. 1988; 1989a, b).

A pathway involving initial hydrolytic dechlorination in the *para* position to form tetrachlorohydroquinone and further reductive dechlorinations was responsible for the degradation of PCP by an aerobic *Flavobacterium* strain (Steiert & Crawford 1986). This strain is also able to degrade and dechlorinate a range of di-, tri- and tetra-chlorophenols (Steiert et al. 1987). Chlorophenols with chlorine substituents in both *ortho* (2 and 6) positions were degraded most readily. Of these, 2,4,6-TrCP, 2,3,5,6-TeCP and PCP were inducers of the complete PCP degradation pathway.

Many microorganisms, including several strains of *Rhodococcus, Acinetobacter* and *Pseudomonas*, are able to *O*-methylate halophenols (Allard et al. 1985; Neilson et al. 1988; Häggblom et al. 1989).

Reductive dechlorination of mono-, di- and pentachlorophenols and pentabromophenol takes place in anaerobic sewage sludges (Boyd & Shelton 1984; Mikesell & Boyd 1986). Which positions are dechlorinated most rapidly depend on which monochlorophenol the sludges are adapted to. Complete reductive dehalogenation of PCP and PBP and mineralization to methane and CO_2 was observed in sludges adapted to all three monochlorophenols. Reductive dehalogenation of 2- and 3-CP, 2,4-DCP, 2,4-DBP and 2,4,6-TrBP has also been observed in anaerobic consortia enriched from aquatic sediments (King 1988; Sharak Genthner et al. 1989a,b).

Degradation of halogenated anilines

Chlorinated anilines are formed by the degradation of many pesticides in the environment. In the presence of nitrate-reducing bacteria, they undergo condensations to chlorinated azobenzenes, triazenes and biphenyls (Minard et al. 1977; Corke et al. 1979). However, these appear to be chemical reactions of diazonium cations derived from the chloroanilines. The role of the bacteria is reduction of nitrate to nitrite, which reacts with chloroanilines to form diazonium cations. Such reactions take place for a variety of substituted anilines, including mono-and dichloroanilines, but not trichloroanilines, and for monobromoanilines, but not for 2-fluoroaniline (Lammerding et al. 1982).

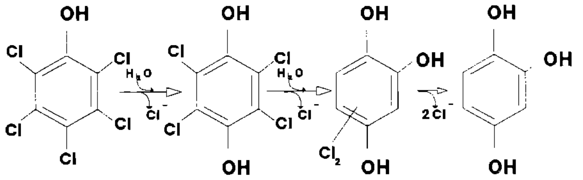

Fig. 5. Metabolic route for PCP degradation by *Rhodococcus chlorophenolicus.*

Few bacteria are known that can mineralize halogenated anilines. *Moraxella* sp. strain G is able to use aniline, 4-fluoro-, 2-chloro-, 3-chloro-, 4-chloro- and 4-bromoanilines, but not 4-iodoaniline, as sole carbon and nitrogen source (Zeyer & Kearney 1982a; Zeyer et al. 1985). This strain is also able to co-metabolize 2,4-DCA (Zeyer & Kearney 1982b; Zeyer et al. 1985). Degradation of these compounds proceeds by initial dioxygenation to form halocatechols, catalyzed by an aniline oxidase with a broad substrate specificity. Further degradation was by a modified *ortho*-cleavage pathway involving a catechol-1,2-oxidase with high activity towards substituted catechols (Zeyer et al. 1985).

Similar pathways are involved in the cometabolism of monochloroanilines by two *Rhodococcus* sp. strains (Janke et al. 1988a, b) and 3,4-DCA by a *Pseudomonas putida* strain (You & Bartha 1982). A *Pseudomonas* strain which degrades aniline via the *meta*-cleavage pathway is not able to degrade chlorinated anilines, although these compounds do induce the enzymes for aniline oxidation (Konopka et al. 1989).

Reductive dehalogenation of chloroanilines takes place under anaerobic conditions. In methanogenic, but not sulphate-reducing, aquifers sequential *ortho* and *para* dehalogenation of 2,3,4,5-TCA yielded 2,3,5-TrCA and 3,5-DCA (Kuhn & Suflita 1989). 3,4-DCA was dechlorinated to 3-CA. No further dehalogenations were detected.

Degradation of halogenated phenoxyacetic acids

The biodegradation of the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) has been investigated by various groups. In general, biodegradation of 2,4-D takes place via initial cleavage of the ether bond, followed by hydroxylation of the resulting dichlorophenol to chlorocatechols (e.g. Bollag et al. 1968a,b; Evans et al. 1971; Tiedje & Alexander 1969).

One of the best characterized 2,4-D-degrading microorganisms is *Alcaligenes eutrophus* JMP 134. This strain also degrades 4-chloro-2-methylphenoxyacetic acid, 2-methylphenoxyacetic acid and phenoxyacetic acid (Pieper et al. 1988). Ester bond cleavage of these compounds is apparently catalyzed by a monooxygenase with a wide substrate specificity. For the chlorinated compounds, the chlorocatechol intermediates are cleaved by the *ortho* mechanism but non-chlorinated compounds are cleaved by both *ortho* and *meta* routes. *Flavobacterium* strain MH degrades not only 2,4-D by this pathway, but also a range of other 2,4-dichlorophenoxyalkanoic acids (Horvath et al. 1990).

As mentioned above, the 2,4,5-T-utilizing *Pseudomonas cepacia* AC1100 initially converts this compound to 2,4,5-TrCP (Karns et al. 1983a), which is then dechlorinated to form 2,5-dichlorohydroquinone (Sangodkar et al. 1989). This strain degrades 2,4-D to chlorohydroquinone, which accumulates and inhibits 2,4,5-T degradation (Haugland et al. 1990). The 2,4-D-degrading *Alcaligenes eutrophus* strain JMP134 does not degrade 2,4,5-T. In mixed cell suspensions of strains AC1100 and JMP134 exposed to both 2,4-D and 2,4,5-T, chlorohydroquinone and chlorophenols accumulate, similarly to that in pure suspensions of AC1100. Presumably, the 2,4-D-degradation pathway of JMP134 cannot compete with that of AC1100. Conjugative transfer of the plasmid coding for 2,4-D degradation in JMP1344 to AC1100 gave a constructed strain able to simultaneously degrade 2,4-D and 2,4,5-T.

Reductive dehalogenation of chlorophenoxyacetic acids appears to be an important reaction under anaerobic conditions. 2,4,5-T was dehalogenated in methanogenc aquifer samples to form 2,4- and 2,5-dichlorophenoxyacetic acids (Gibson & Suflita 1990). Further degradation resulted in the formation of monochlorophenoxyacetic acids, chlorophenols and phenol. These reactions were inhibited by added sulphate, but stimulated by added organic substrates.

Degradation of halogenated biphenyls

The degradation of chlorinated biphenyls has been reviewed by Furukawa (1982), Parsons et al. (1983) and Safe (1984). The ability to degrade polychlorinated biphenyls (PCBs) aerobically is found in several genera of both Gram positive and Gram negative bacteria (Ohmori et al. 1973; Walia et al. 1988; Untermann et al. 1988). These are mostly members of the genera *Pseudomonas, Alcaligenes, Arthrobacter* and *Acinetobacter*.

Many reports describe the mineralization (i.e complete degradation to CO_2, often measured by the formation of $^{14}CO_2$) of individual chlorinated biphenyls (Shiaris & Sayler 1982; Kong & Sayler 1983; Bailey et al. 1983; Fries & Marrow 1984; Brunner et al. 1985) brominated biphenyls (Kong & Sayler 1983) and commercial PCB mixtures (Hankin & Sawhney 1984; Baxter & Sutherland 1984; Brunner et al. 1985). However, in many cases chlorinated benzoates accumulated.

Mineralization rates are enhanced by sunlight (Kong & Sayler 1983) and moderately aerobic circumstances (Pardue et al. 1988). Substrate enrichment and inoculation with PCB-degrading bacteria enhanced also mineralization (Brunner et al. 1985).

Mineralisation capabilities differ in different bacterial consortia due to differing metabolic abilities of the members of the population (Hiramoto et al. 1989; Pettigrew et al. 1990). One possible explanation is that biphenyls are initially degraded by *meta* cleavage whereas *meta* cleavage of the resulting chlorobenzoates leads to toxic end products (see above). Only when enzymes for *ortho* cleavage are also induced will complete mineralization be possible. *Alcaligenes* strain JB1 is able to co-metabolize both chlorobiphenyls and chlorobenzoates (Parsons et al. 1988). Complete mineralization of 4-chlorobiphenyl by a two-membered culture of *Pseudomonas* CBS3 and a facultative

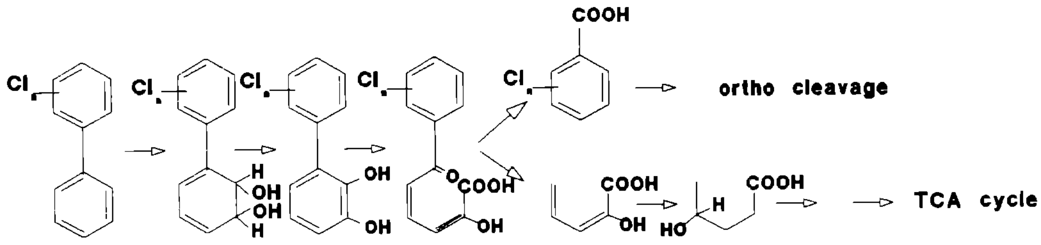

Fig. 6. Major metabolic route for aerobic degradation of PCBs.

anaerobic strain B-206 was described by Sylvestre et al. (1985). Also a coculture of *Acinetobacter* sp. strain P6 and *Acinetobacter* strain 4CB1 mineralizes 3,4-, 4,4′ DCBP and 3,3′,4,4′-TCBP by initial hydrolytic dehalogenation of the chlorobenzoate intermediates (Adriaens et al. 1989; Adriaens & Focht 1990).

The major aerobic microbial degradation pathway of PCBs, consists of 2,3-dioxygenation of the less substituted aromatic ring, *meta* cleavage and further degradation to chlorobenzoates (Fig. 6). The elimination of chlorine is thought to be a fortuitous event, which occurs in later metabolic steps. Based on studies of PCB metabolism by *Alcaligenes* sp. Y46 and *Acinetobacter* sp. P6 Furukawa (1982) proposed the following relationships between PCB structure and biodegradability.

- The less chlorinated the biphenyl, the faster aerobic degradation takes place. Biphenyls with more than 5 chlorines substituted are resistant to degradation.
- Dioxygenation takes place on the ring with the least chlorine substituents.
- Nonchlorinated vincinal *ortho* and *meta* positions favour dioxygenation.
- PCBs with chlorine substituents on both rings are more recalcitrant than isomers containing an unchlorinated ring.
- Congeners with substituted *ortho* positions are recalcitrant.

In accordance with these results *Alcaligenes* strain JB1 showed fast degradation of 2,2′,3,3′-tetrachlorobiphenyl and relatively slow degradation of 3,3′,4,4′-TCB (Parsons et al. 1988). Also cultures of *Pseudomonas* sp. KKS102 to which supernatant from a culture of a related *Pseudomonas* sp. KKS101 had been added showed relatively fast degradation of 2,2′,3,3′-TCB and slow degradation of 2,2′6,6′-TCB and 3,3′,4,4′-TCB (Kimbara et al. 1988). Similar results were described for *Corynebacterium* sp. MB1 (Bedard et al. 1986).

However, in contrast to these results *Alcaligenes eutrophus* H850 and *Pseudomonas putida* LB400 completely metabolize 2,2′,5,5′-TCB and even degrade 2,2′,4,4′,6,6′-HCB (Bedard et al. 1986). Furthermore, *Alcaligenes eutrophus* H850 does not degrade 2,2′,3,3′-TCB whereas *Pseudomonas putida* LB400 does degrade this latter compound but does not metabolize 2,2′,6,6′-TCB (Bedard et al. 1986,1987a; Bopp 1986). Dioxygenation at the 3,4-position was proposed for the degradation of 2,2′,5,5′-TCB by these organisms. All the *meta* cleavage pathway enzymes were detected in strain H850 (Unterman et al. 1988). A new metabolite, 2,4,5-trichloroacetophenone was detected in incubations of *Alcaligenes eutrophus* H850 with 2,2′,4,4′,5,5′-HCB (Bedard et al. 1987b). Some other PCB congeners may also be degraded via chloroacetophenone intermediates.

Nitration of 4-chlorobiphenyl has been reported for strain B-206 (Sylvestre et al. 1982).

The first reports on anaerobic PCB degradation involved the analysis of PCBs in sediment samples. Compared to the chlorinated aromatic mixtures originally discharged, decreased relative concentrations of highly chlorinated biphenyls were detected in river sediments. Comparison of changes in PCB concentrations in sterile and non-sterile sediments demonstrated that the bacterial community was responsible for reductive dehalogenation of PCBs (Brown et al. 1987a,b; Quensen et al. 1988,1990). Differences in congener specificity were detected in different consortia. The final products of anaerobic dechlorination are the *ortho* substituted congeners, which can be degraded aerobically. Thus, total degradation of PCBs seems possible by sequential anaerobic and aerobic treatment. However, recently it was shown that dehalogenation only occurs with biphenyls with up to seven chlorines. More highly chlorinated congeners were not dehalogenated (Quensen et al. 1990).

Degradation of halogenated dibenzo-*p*-dioxins and dibenzofurans

Very little is known of the degradation of these compounds. The co-metabolism of mono-, di- and trichlorodioxins by a biphenyl-utilizing *Beijerinckia* strain has been described (Klecka & Gibson 1980). *cis*-1,2-Dihydrodiols were isolated as the products of dioxygenation of 1-chloro- and 2-chlorodioxins. Acid-catalyzed dehydration of these compounds gives 2-hydroxylated compounds. Further metabolism of the dihydrodiols produces 1,2-dihydroxylated derivatives, but there was no evidence for ring cleavage of these compounds. In fact, the 1,2-dihydroxylated derivatives appear to inhibit the ring cleavage enzymes in this strain. The biphenyl-utilizing *Alcaligenes* strain JB1 appears to co-metabolize mono-, di- and trichlorinated dioxins by the same mechanism (Parsons & Storms 1989).

Very slow oxidative degradation of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) has been reported for a number of microorganisms, including *Pseudomonas testosteroni, Bacillus megaterium* and *Nocardiopsis* strains (Philippi et al. 1982; Quensen et al. 1983). Traces of polar metabolites, probably hydroxylated derivatives, are formed by these strains.

To date no evidence for ring cleavage of chlorinated dibenzo-*p*-dioxins has been reported. Recently, however, oxidative ring cleavage of dibenzo-*p*-dioxin by a dibenzofuran-degrading *Pseudomonas* strain was described (Harms et al. 1990).

There are almost no reports of the biodegradation of halogenated dibenzofurans. Strubel et al. (1989) stated that dibenzofuran-degrading cultures metabolized chlorinated dibenzofurans, but gave no details. Recently, degradation of 2-CDF and 2,8-DCDF by *Alcaligenes* strain JB1 was reported (Parsons et al. 1990).

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