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The biodegradation of aromatic hydrocarbons by bacteria

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Abstract

Aromatic compounds of both natural and man-made sources abound in the environment. The degradation of such chemicals is mainly accomplished by microorganisms. This review provides key background information but centres on recent developments in the bacterial degradation of selected man-made aromatic compounds. An aromatic compound can only be considered to be biodegraded if the ring undergoes cleavage, and this is taken as the major criteria for inclusion in this review (although the exact nature of the enzymic ring-cleavage has not been confirmed in all cases discussed).

The biodegradation of benzene, certain arenes, biphenyl and selected fused aromatic hydrocarbons, by single bacterial isolates, are dealt with in detail.

Introduction

For the purposes of this review aromatic compounds are restricted to benzene and compounds that resemble benzene in chemical structure. Benzene and related compounds are characterised by their possession of a large (negative) resonance energy. This results in a thermodynamic stability which manifests itself in chemical properties very different from those observed for aliphatic (including alicyclic) compounds. However, most of the hydrocarbons discussed here fall more correctly into the category of compounds classified as arenes. In this review the term 'aromatic' will be used for those hydrocarbon compounds containing an aromatic moiety which ultimately undergoes enzymatic ring-cleavage.

Aromatic hydrocarbons are ubiquitous in nature. Although there is some debate as to their origin in the environment, it is generally accepted that most are not of biosynthetic origin but are derived from the (natural) pyrolysis of organic compounds (Gibson & Subramanian 1984). Indeed, next to glucosyl residues, the benzene ring is the most widely distributed unit of chemical structure in nature (Dagley 1981). It is of little surprise therefore that micro-organisms have evolved capable of degrading aromatic compounds. Today, there is great concern regarding the occurrence of man-made aromatic hydrocarbons in the environment. Benzene, toluene, ethylbenzene, styrene and the xylenes are among the 50 largest-volume industrial chemicals produced, with production figures of the order of millions of tons per year. These compounds are widely used as fuels and industrial solvents. In addition, they and polynuclear aromatic compounds provide the starting materials for the production of pharmaceuticals, agrochemicals, polymers, explosives and many other everyday products (Gibson 1971).

The use of man-made aromatic hydrocarbons has inevitably led to their release (either accidental

or otherwise) into the environment and this problem is still escalating in spite of governmental intervention.

The biodegradation of aromatic compounds can be considered, on the one hand as part of the normal process of the carbon cycle, and as the removal of man-made pollutants from the environment, on the other.

Over the last decades this topic has been extensively reviewed (Gibson 1971; Hopper 1978; Cripps & Watkinson 1978; Gibson & Subramanian 1984; Cerniglia 1984; Dagley 1986, as examples). Since the last extensive review there have been many advances in the field at the physiological, biochemical and molecular biological levels. The majority of the recent advances made have consolidated previous findings giving a fuller, if not complete, picture.

It is impossible in such a review article to cover all the aspects of bacterial aromatic degradation of the last few years and this therefore is more of a personal view on recent developments in certain areas. It is not the intention to provide an historical account of the developments in this area as this has been more than adequately been achieved by others (Gibson & Subramanian 1984). Instead it hoped to provide the reader with some key developments in the defined areas (although some background information will be provided) and to raise some questions regarding the current inadequacies in our knowledge.

The parent member of the aromatic hydrocarbons is benzene and it is therefore logical to begin by considering the biodegradation of the 'begetter' of the other members of the series.

Benzene

There have been few reports on the bacterial biodegradation of benzene in the literature over the last five years. The excellent studies carried out in the previous three decades (Marr & Stone 1961; Gibson et al. 1968 Gibson et al. 1970; Högn & Jaenicke 1972; Axell & Geary 1975) elucidated the pathways involved, identified the intermediates and characterised the enzyme systems. Figure 1 shows the two divergent pathways employed. Both share the same initial mode of attack resulting in the formation of catechol which is further catabolised by either catechol 1,2-dioxygenase (the so called *ortho-* or intradiol-cleavage) and subsequently via the β-ketoadipate pathway or catechol 2,3-dioxygenase (the so called *meta-* or extradiol-cleavage). Both routes have been described in different benzene utilising strains.

Although there have been recent reports of the isolation of new bacterial strains which can grow on benzene (Shirai 1986; van den Tweel et al. 1988; Winstanley et al. 1987, as examples) the biodegradation routes were, not surprisingly, the same as those outlined above (Fig. 1).

Winstanley et al. (1987) described a new benzene utilising bacterium, *Acinectobacter calcoaceticus* RJE74, which carries a large plasmid (pWW174) encoding the enzymes for the catabolism of benzene via the β-ketoadipate pathway (that involving catechol 1,2-dioxygenase). This was the first report of the *ortho* pathway being plasmid encoded and was one of very few citations of plasmids in this genus.

The biodegradation of benzene has also been re-examined from a biotechnological standpoint. Interest has focused on the production of the first two intermediates in the pathways (*cis*-benzeneglycol, CBG and catechol). CBG formed biologically from benzene has been reported by various groups (Ballard et al. 1983; Ley et al. 1987; van den Tweel et al. 1988). The product (generated by mutant strains of benzene utilisers) is projected to find uses as a chiral building block for polymers and pharmaceuticals. Shirai (1986) selected a mutant strain of *Pseudomonas* sp. capable of accumulating catechol and subsequently (Shirai 1987) investigated the possibility of the use of free and immobilised cells for an industrial process. Catechol and its derivatives are important chemicals used mainly for the production of synthetic flavours such as vanillin.

Arenes

The introduction of a substituent group(s) onto the benzene ring opens up the possibility of alternative

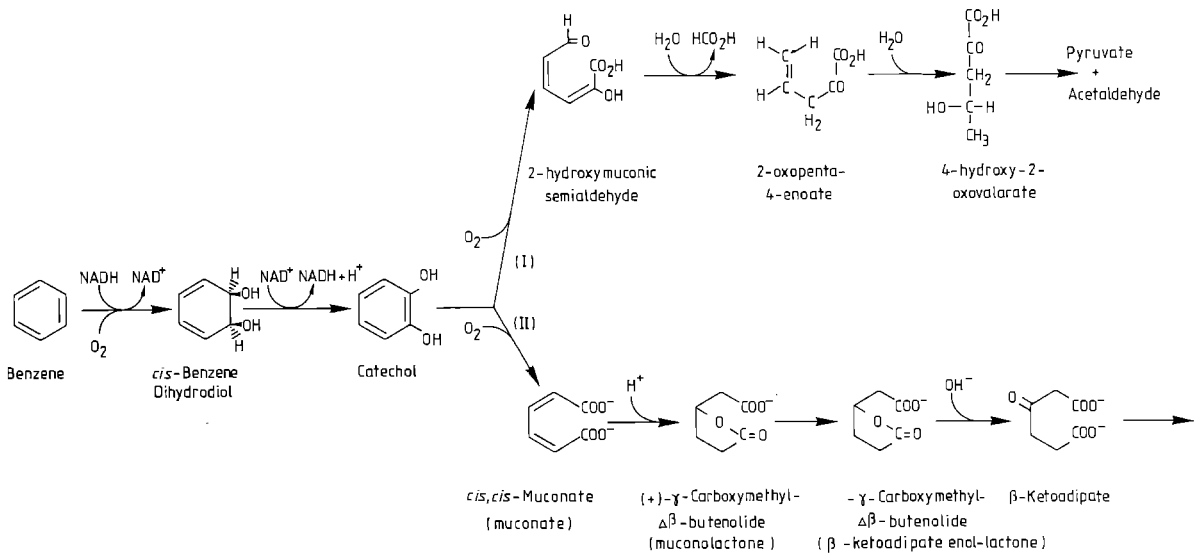

Fig. 1. The biodegradative routes of benzene. (I) *meta*-cleavage route. (II) *ortho*-cleavage route.

modes of biodegradation; either side-chain attack or ring attack. Indeed with the longer chain length alkylbenzenes the oxidation of the side chain is sufficient to support growth and the organisms may not be able to degrade the aromatic moiety. Such compounds may be regarded as substituted alkanes rather than substituted benzenes. For the purposes of this review, I will only discuss those cases where the benzene ring undergoes ring-cleavage (either before or after side chain modifications).

The biodegradation of mono-alkylbenzenes

Toluene, the simplest of these substituted benzenes, is biodegraded by both ring attack and methyl-group hydroxylation. Figure 2 gives the alternative pathways. The evidence for these routes is well established (see Hopper 1978 for review).

Over the last five years, there has been an enormous number of papers on the plasmid-encoded biodegradation of toluene. Williams and his colleagues at the University of Wales (Bangor, UK) have shown that toluene (and *m*-, *p*- xylenes, etc) is degraded via catechol and subsequently the *meta* pathway by several strains of *Pseudomonas* by enzymes encoded on plasmids, designated TOL (Bayly & Barbour 1984). These plasmids often contain two catabolic operons (Nakazawa et al. 1980; Franklin et al. 1981). The 'upper' pathway operon encodes enzymes for the successive oxidation of the hydrocarbons to the corresponding alcohol, aldehyde and carboxylic acid derivatives. The 'lower' or *meta*-cleavage pathway operon encodes enzymes for the conversion of the carboxylic acids to catechols, whose aromatic rings are then cleaved (*meta*-fission) to produce corresponding semialdehydes, which are then further catabolised through the TCA cycle (Ramos et al. 1987). Figure 3 outlines the TOL plasmid encoded pathway.

To cover the huge amount of data published in this area would require more space than is available in such a review. In a recent mini-review Burlage et al. (1989) discussed in detail the most studied of the TOL plasmids (pWWO), however it is the opinion of this author that an up to date detailed account of all of the developments in this field is now needed.

Other recent advances in the study of the biodegradation of toluene include: isolation of a novel strain (Simpson et al. 1987), studies of the growth parameters of *Pseudomonas putida* in chemostat cultures (Vecht et al. 1988) and its anaerobic biodegradation (Zeyer et al. 1990; Lovley & Lonergan 1990).

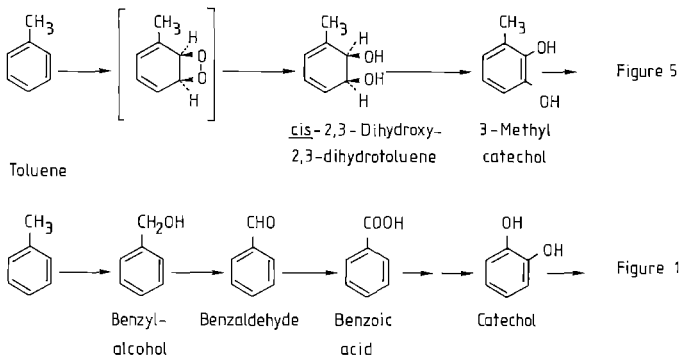

Fig. 2. The biodegradation routes of toluene.

A thermotolerant *Bacillus* sp. which grew on toluene at 50° C was isolated by Simpson et al. (1987). Biodegradation (Simpson et al. 1987) was via *cis*-toluene dihydrodiol, 3-methylcatechol and *meta*-cleavage (Fig. 2). The *cis*-toluene dihydrodiol dehydrogenase from this organism was purified and shown to possess different properties to those previously reported, most notable was a temperature optimum of 80° C (Simpson et al. 1987).

Vecht et al. (1988) recently reported on the growth of *Pseudomonas putida* in chemostat cultures with toluene as the sole source of carbon and energy (after initial growth on *m*-toluic acid). Steady states were maintained for several months with maximal biomass concentrations of 3.2 g cell dry wt/l. The maximum specific growth rate was $0.13\,h^{-1}$, with a cellular yield of 1.05 g cell dry weight/g toluene utilised.

The anaerobic biodegradation of toluene has been reported by two independent groups. A *Pseudomonas* sp. has been isolated which oxidises toluene to carbon dioxide with NO_3 or N_2O as the potential electron acceptors (Zeyer et al. 1990). It was not demonstrated whether this bacteria could obtain energy from toluene oxidation and the reduction products of nitrate and nitrous oxide were not investigated. Lovley & Lonergan (1990) isolated an unidentified bacterial strain (GS-15) which coupled the oxidation of aromatic compounds (including toluene) to the reduction of Fe(III). This was the first conclusive report of the anaerobic oxidation of an aromatic hydrocarbon. Biodegradation proceeds via the oxidation of the methyl

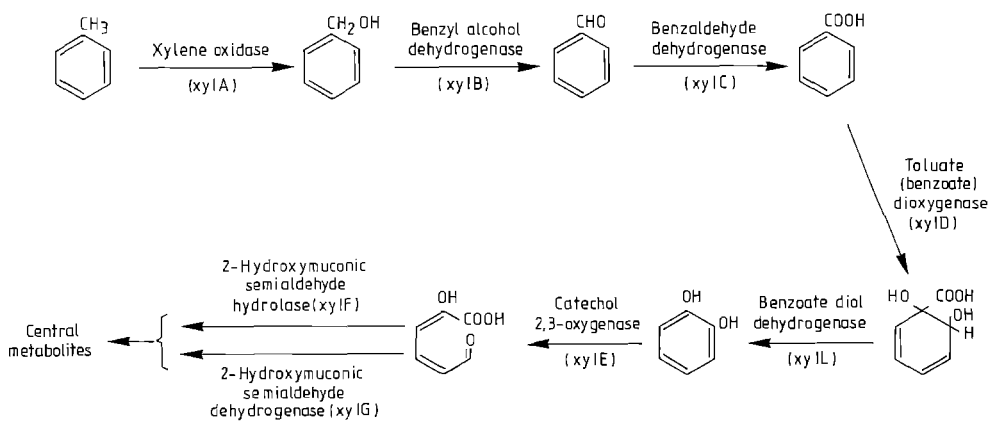

Fig. 3. The early enzymes of the TOL plasmid degradative pathway.

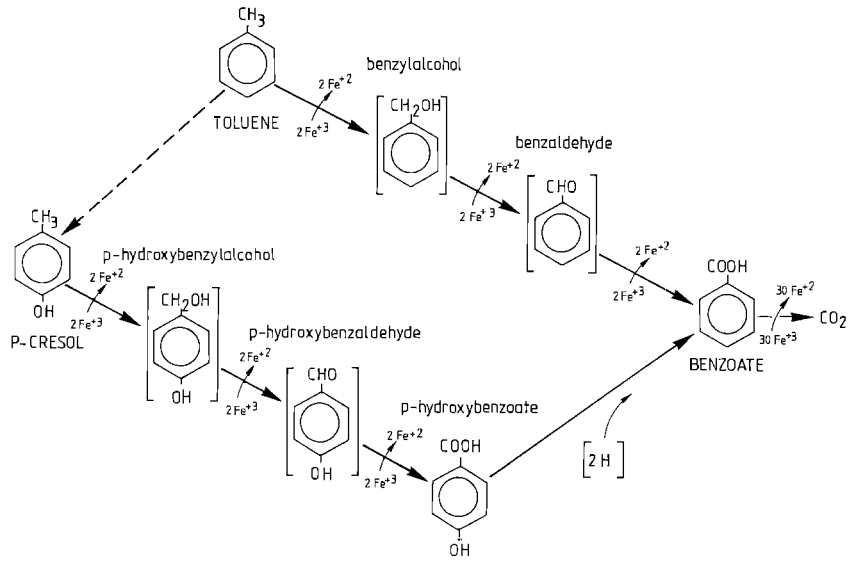

Fig. 4. The anaerobic biodegradation of toluene by strain GS-15.

group (leading to benzoate) or possibly via *p*-cresol, *p*-hydroxybenzoate and benzoate. The proposed pathway is given in Fig. 4. The anaerobic biodegradation of aromatic acids and phenols is well known (Berry et al. 1987; Evans & Fuchs 1988) and it will be interesting to see if aromatic hydrocarbons other than toluene can be anaerobically dissimilated by pure bacterial strains.

Direct cleavage of the aromatic moiety of alkylbenzenes, without prior attack of the alkyl side chain, has been demonstrated for ethylbenzene (Gibson et al. 1973), 2-phenylbutane, 3-phenylpentane (Baggi et al. 1972), n-butylbenzene (Jigami et al. 1974a), isopropylbenzene (Jigami et al. 1975; Eaton & Timmis 1986) and *tert*-butylbenzene (Catelani et al. 1977).

We recently re-examined the biodegradation of alkylbenzenes (Smith & Ratledge 1989a). *Pseudomonas* sp. NCIB 10643 grew on a range of n-alkylbenzenes (C2–C7) and on several branched species within this chain size (isopropylbenzene, isobutylbenzene, *sec*-butylbenzene, *tert*-butylbenzene and *tert*-amylbenzene). All of the alkylbenzenes were catabolised via ring attack, rather than side chain attack, proceeding via initial dioxygenase activity resulting in the corresponding 2,3-dihydro-2,3-dihydroxyalkylbenzene which underwent reduction to the corresponding 2,3-dihydroxyl- intermediate (3-alkylsubstituted catechols). The 3-substituted catechols were ring-cleaved by an extra-diol type enzyme between C1 and C2 resulting in characteristic *meta* ring-fission products. Further catabolism was by hydrolytic attack to give alkyl-chain dependent carboxylic acids and, presumably, 2-oxopenta-4-enoate.

A general pathway for the complete catabolism of these mono-alkylbenzenes is given in Fig. 5.

There have been several reports that the en-

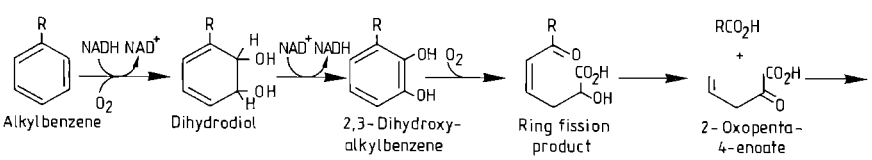

Fig. 5. The biodegradation of alkylbenzenes (C1–C7).

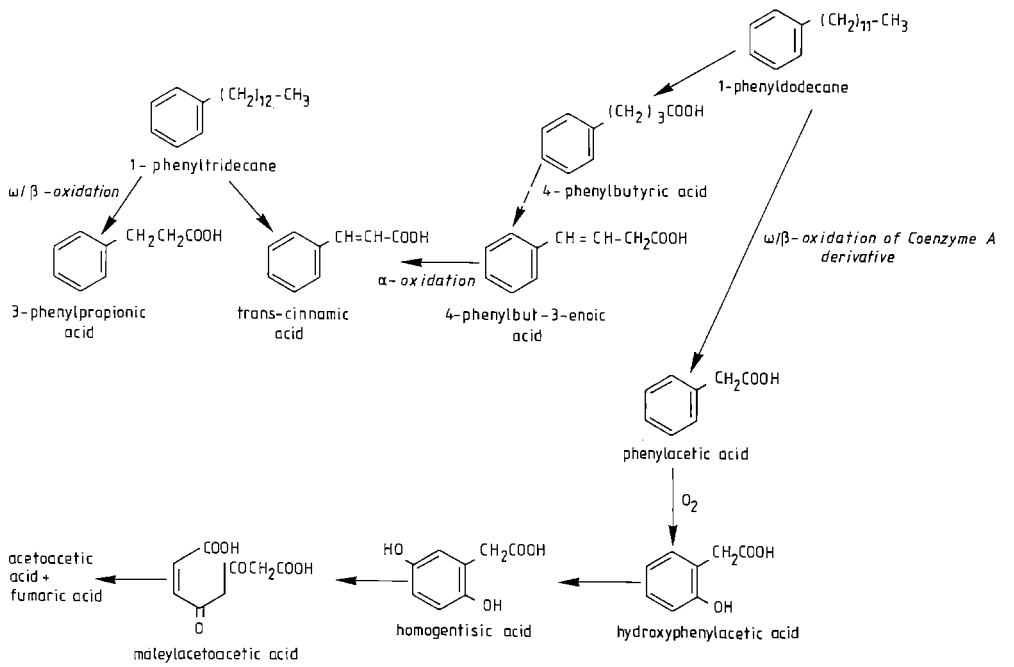

Fig. 6. The biodegradative routes of 1-phenyltridecane and 1-phenyldodecane.

zymes encoding for the biodegradation of alkylbenzenes are plasmid-borne. Bestetti & Galli (1984) showed that the genes for the catabolism of ethylbenzene (and 1-phenylethanol) in *Pseudomonas fluorescens* were sited on a 253–267 kilobase plasmid. Eaton & Timmis (1986) have demonstrated that the catabolism of isopropylbenzene by *Pseudomonas putida* RE 204 is plasmid encoded.The pathway was shown to be identical to that outlined above (see Fig. 5). Our own studies with *Pseudomonas* sp. NCIB 10643 suggested that the genes of alkylbenzene (and biphenyl) biodegradation were chromosomal (Smith & Ratledge 1989b).

When the alkyl chain length exceeds C7 the preferred route seems to be by attack on the alkyl chain. Sariaslani et al. (1974) reported on the degradation of n-dodecyl-and n-nonyl- benzenes (1-phenyldodecane and 1-phenylnonane respectively) by initial side chain attack via ω- and β-oxidation. Both compounds could be catabolised through 2,5-dihydroxyphenylacetic acid (homogentisic acid) and then ring cleaved. Amund & Higgins (1985) later confirmed this route for the degradation of 1-phenyldodecane by *Acinetobacter lwoffi* and also showed that 1-phenyltridecane was transformed to *trans*-cinnamic acids and 3-phenylpropionic acid which were not further metabolised. The proposed scheme for the biodegradation of 1-phenyldodecane and 1-phenyltridecane is given in Fig. 6.

The biodegradation of di-alkylbenzenes

Until recently only the *m*- and *p*- isomers of xylene had been shown to be biodegraded by bacteria. Both compounds are degraded by certain strains of *Pseudomonas* (notably those containing the TOL plasmid – see above) by initial oxidation of one of the methyl groups to the corresponding methyl benzylalcohols, tolualdehydes, toluic acids and methyl catechol (Davey & Gibson 1974; Davis et al. 1968). The biodegradation of *m*- and *p*-xylenes to their corresponding methylcatechols is shown in Fig. 7. The resultant catechols then undergo *meta*-cleavage. The ring-fission products of the two different methyl catechols (3-methylcatechol from *m*-xylene; 4-methylcatechol from *p*-xylene) are catabolised by different enzyme systems (Duggleby &

Williams 1986). The product from 3-methylcatechol cleavage is further degraded by a single hydrolase type enzyme (Duggleby & Williams 1986; Smith & Ratledge 1989a), whereas the product from 4-methylcatechol, an aldehyde, is converted via the enzymes of the 4-oxocrotonate branch (Sala-Trepat et al. 1972; Wigmore et al. 1974). These pathways are illustrated in Fig. 8.

An alternative mode of attack of *p*- and *m*-xylenes is via direct dioxygenase attack of the aromatic moiety yielding the corresponding *cis*-dihydrodiol with subsequent conversion to substituted catechols (3,6-dimethylcatechol from *p*-xylene; 3,5-dimethylcatechol from *m*-xylene) by dehydrogenase type enzymes (Gibson et al. 1974). However, although this is often cited as an alternative pathway for the degradation of xylenes (Baggi et al. 1987, for example) the resultant catechols are not further degraded and this route should be regarded as a biotransformation reaction.

Members of the genus *Nocardia* can co-metabolise all three of the isomers of xylene (see Gibson & Subramanian 1984 for review). Noteworthy, the *p*- and *m*-xylenes were co-metabolised (hexadecane as substrate) via *ortho* cleavage whereas *o*-xylene was attacked by *meta*-cleavage.

The first reports of the complete biodegradation of *o*-xylene as sole source of carbon and energy by pure cultures were provided by Baggi et al. (1987) and Schraa et al. (1987). Initial studies (using a strain of *Pseudomonas stutzeri*) suggested that *o*-xylene was catabolised via 3,4-dimethylcatechol with subsequent *meta*-cleavage (Baggi et al. 1987). Independently, Schraa et al. (1987) reported on the characterisation of *Corynebacterium* strain C125 able to grow on *o*-xylene as the sole source of carbon and energy. The proposed pathway was the same as that suggested by Baggi et al. (1987) and confirmed 2-dihydroxy-5-methyl-6-oxo-2,4-heptadienoate as the ring-fission product. The pathway is illustrated in Fig. 9.

The observation that none of the *p*- and *m*-xylene degrading bacteria can attack *o*-xylene (and vice versa from Baggi et al. 1987; Schraa et al. 1987) raises interesting questions regarding the evolution of these bacterial strains.

With regard to other di-alkylsubstituted benzenes, there have been no significant advances since the last review (Gibson & Subramanian 1984) was published and our knowledge is still limited to the biodegradation routes of *p*-cymene (DeFrank & Ribbons 1976; DeFrank & Ribbons 1977a, b) and 3-ethyltoluene (Jigami et al. 1974b; Kunz & Chapman 1981). In both these cases, attack begins on the smaller substituent of the ring (i.e. the methyl group), however, there is too little evidence available to judge whether this is always the case.

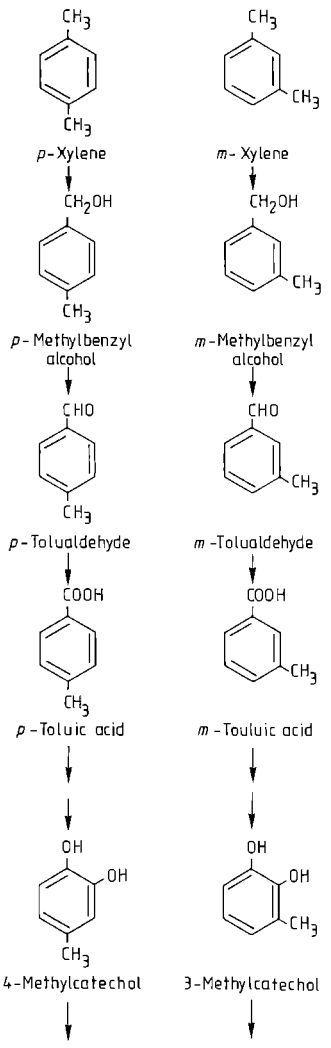

Fig. 7. The initial reactions in the bidegradation of *m*- and *p*-xylenes.

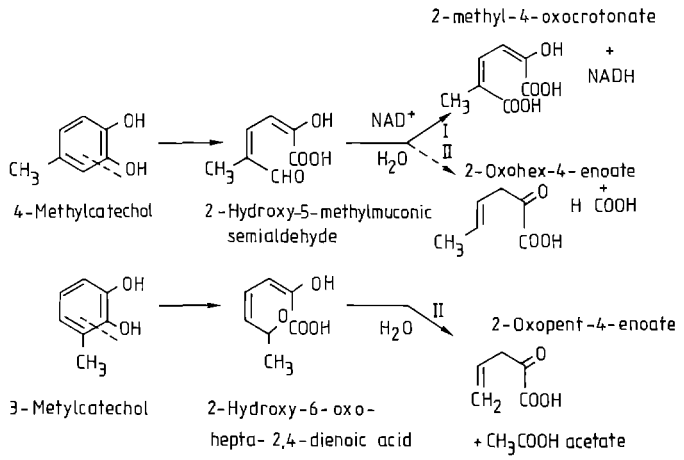

Fig. 8. The alternative pathways for the catabolism of 4- and 3-methylcatechols. (I) 4-Oxocrotonate branch. (II) Hydrolytic branch.

The biodegradation of alkenylbenzenes

Reports on the biodegradation of alkenylbenzenes are extremely scarce. This is somewhat surprising when one considers the enormous quantities styrene (the simplest member of this series) produced by the petrochemical industry (3.6 million tons in the United States in 1987 – Hartmans et al. 1989). To date there have been no detailed examinations of the complete degradation of any of the alkenylbenzenes and only a handful on the initial attack. There are however reports of bacteria able to grow on styrene (Baggi et al. 1983; Shirai & Hisatsuka 1979; van den Tweel et al. 1986) and methylstyrenes (Omori et al. 1974; Dzhusupova et al. 1985) as sole sources of carbon and energy and thus by implication these organisms must be capable of ring-cleavage.

The initial degradation of styrene by *Xanthobacter* strain 124X (a strain isolated by enrichment on styrene by van den Tweel et al. 1986) was recently reported by Hartmans et al. (1989). Although styrene oxide and 2-phenylethanol have been implicated in its degradation by other bacteria (Shirai & Hisatsuka 1979) and were shown to be oxidised by cells grown on styrene, these authors concluded that the initial step in styrene metabolism is oxygen dependent and probably involves oxidation of the aromatic nucleus. Previously, this organism was also shown to grow on ethylbenzene and toluene (van den Tweel et al. 1986).

Subsequently, this same group (Hartmans et al. 1990) isolated 14 strains of bacteria able to grow on styrene as the sole source of carbon and energy. One of these (S5) was studied in more detail and the initial reactions characterised. Styrene was converted to styrene oxide by a novel flavin monooxygenase. Further degradation proceeded via phenylacetaldehyde and phenylacetic acid. The conversion of phenylacetic acid to central metabolites was not investigated.

The biodegradation of other members of this class of aromatic hydrocarbons has received very little attention in the last few years. The biotransformation of styrenes by a strain of *Pseudomonas putida* capable of growth on α-methylstyrene has

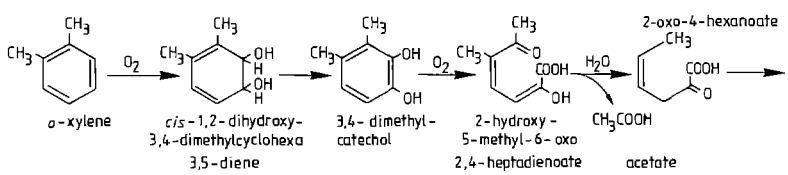

Fig. 9. The biodegradative pathway of *o*-xylene.

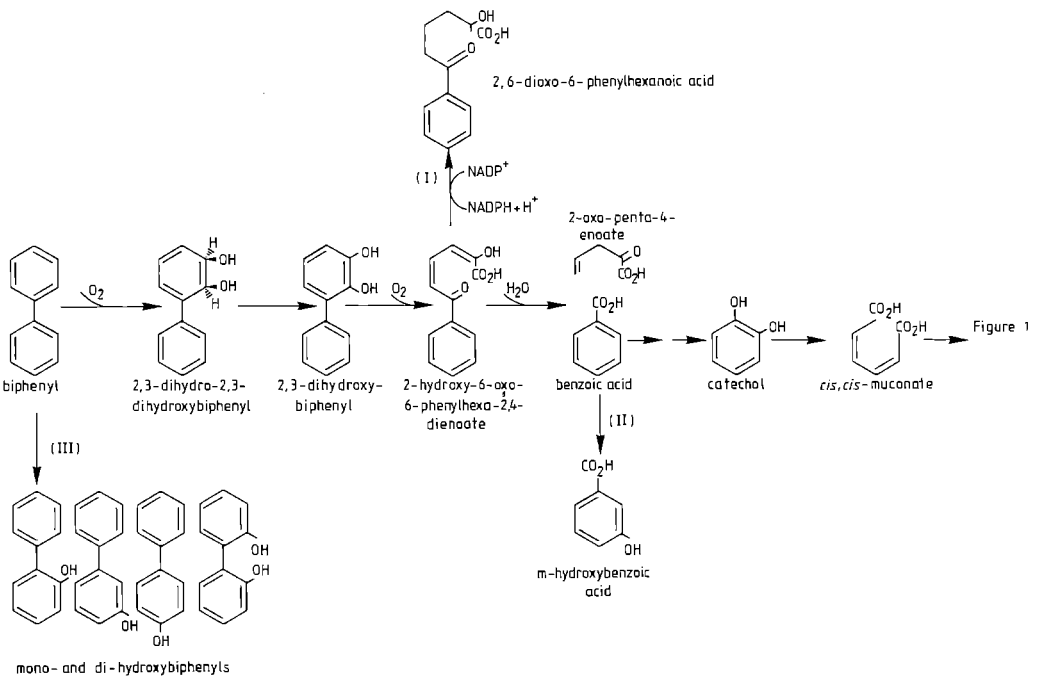

Fig. 10. The major pathway for the biodegradation of biphenyl and some alternative side reactions. (I) Reduction of the ring-fission product. (II) Conversion of benzoate to *p*-hydroxybenzoate. (III) Mono- and di- hydroxybiphenyls.

been recorded (Bestetti et al. 1989). The growth substrate was catabolised via 2-phenyl-2-propen-1-ol and 1,2-dihydroxy-3-isopropenyl-3-cyclohexene, implying a pathway different to that previously reported (Omori et al. 1974). Furthermore, the strain was also able to biotransform styrene to 1,2-dihydroxy-3-ethenyl-3-cyclohexene.

There have been several tentative reports of the involvement of *meta*-cleavage of alkenylbenzenes. Sielicki et al. (1978) observed the development of yellow culture fluids during stationary phase of growth of a mixed culture with styrene as the sole source of carbon and energy. (The appearance of yellow culture fluids often occurs during the degradation of aromatic compounds caused by the accumulation *meta*-cleavage products.) This observation was not further discussed. Hartmans et al. (1989) observed a transient accumulation of a yellow product during growth of *Xanthobacter* strain 124X on styrene and 1-phenylethanol. They reported that the compound had different spectral properties to those reported for the ring-cleavage product of the catechol of 1-phenylethanol (2,7-dihydroxy-6-oxoocta-2,4-dienoate – Cripps et al. 1978) but did not identify the product. In contrast, Dzhusupova et al. (1985) showed induced levels of protocatechuate 3,4-dioxygenase (an intra-diol cleaving enzyme) in strains of *Pseudomonas* grown on α-methylstyrene, suggesting a novel pathway, sadly without explanation.

Clearly from the foregoing discussion alkenylbenzenes are degraded by bacteria and it seems certain, from the circumstantial evidence, that ring-cleavage must occur.

Biphenyl

Biphenyl may be considered as a substituted benzene even though the substituent is in fact benzene itself; biphenyl is not a polycyclic aromatic hydrocarbon. The catabolism of biphenyl has received much attention recently due to the increasing concern over the fate of polychlorinated (PCBs) which are established as worldwide pollutants. Previous review articles have discussed the biodegradation

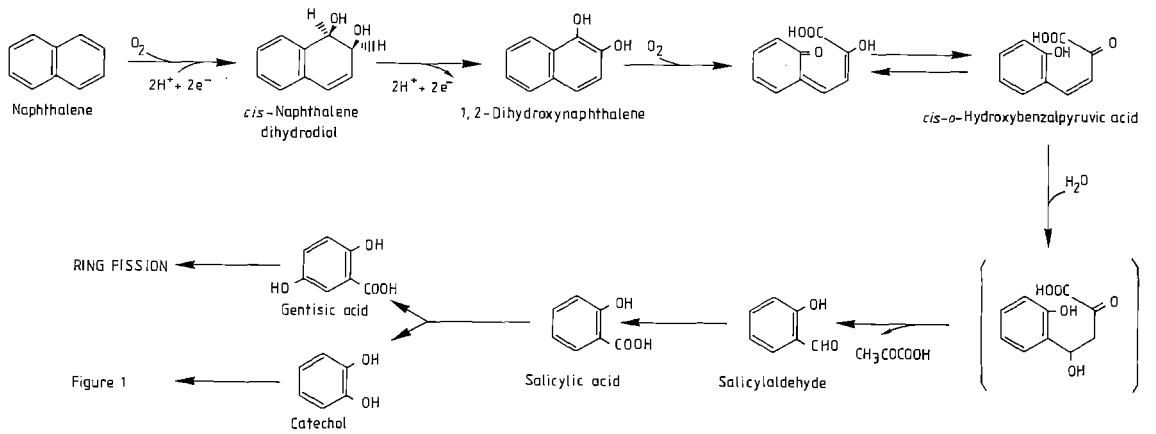

Fig. 11. The initial reactions of naphthalene biodegradation.

of biphenyl. Some of these have highlighted two key pathways for the catabolism of biphenyl (Cripps & Watkinson 1978, for exampe). Recently we (Smith & Ratledge 1989b) have re-investigated the so-called alternative pathway (Lunt & Evans 1970) and demonstrated it to be erroneous (see below).

The pathway for the biodegradation of biphenyl is given in Fig. 10. Initial attack of biphenyl proceeds via 2,3-dihydro-2,3-dihydroxybiphenyl (Catelani et al. 1971; Catelani et al. 1973; Gibson et al. 1973) and 2,3-dihydroxybiphenyl (Catelani et al. 1973; Smith & Ratledge 1989b). The catechol type compound is then ring cleaved between carbon atoms 1 and 2 to form 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate (Catelani et al. 1973; Catelani & Colombi 1974; Ishigooka et al. 1986; Smith & Ratledge 1989b). Further catabolism of this ring fission-product is via 2-oxo-penta-4-enoate and benzoate (Smith & Ratledge 1989b). This pathway is common to many species of bacteria.

Contemporary investigators have revealed some subtle differences between different bacteria. We found that benzoate was a dead-end product in the biodegradation by *Pseudomonas* sp. NCIB 10643, whereas *Nocardia* sp. NCIB 10503 catabolised the benzoate via oxidative-decarboxylation to catechol which was subsequently degraded via the β-ketoadipate pathway (Smith & Ratledge 1989b). Omori et al. (1986) demonstrated the NADPH dependent reduction of the ring-cleavage product to 2,6-dioxo-6-phenylhexanoic acid in biphenyl grown cells of *Pseudomonas cruciviae*, although no physiological significance of this enzyme step was postulated. 3-Hydroxybenzoate and cinnamic acid were also reported as intermediates in the plasmid encoded catabolism of biphenyl by a strain of *Pseudomonas putida* (Starovoitov et al. 1986). No scheme of dissimilation was proposed to account for the production of cinnamic acid. Furukawa & Suzuki (1988) transferred the plasmid encoding for the degradation of biphenyl in *Pseudomonas pseudoalcaligenes* into *Pseudomonas aeruginosa* and subsequently detected 2,3,2′,3′-tetrahydroxybiphenyl, suggesting that the initial two enzymes of biphenyl degradation had a broader substrate specificity than hitherto considered. Khan & Walia (1990) have recently cloned the genes encoding for two of the key enzymes of biphenyl biodegradation (3-phenylcatechol dioxygenase and 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase) from *Pseudomonas putida* into *Escherichia coli*.

Our own investigation with *Nocardia* sp. NCIB 10503 (Smith & Ratledge 1989b) was the first report of the complete degradation of biphenyl by an actinomycete. Previously, Schwartz (1981) reported growth of such a bacterium but only identified mono-hydroxybiphenyls and 2,2′-dihydroxybiphenyl as intermediates (Fig. 10). It remains to be seen if other members of the actinomycetes biodegrade biphenyl via mono-oxygenase type enzymes rather than the more conventional dioxygenases.

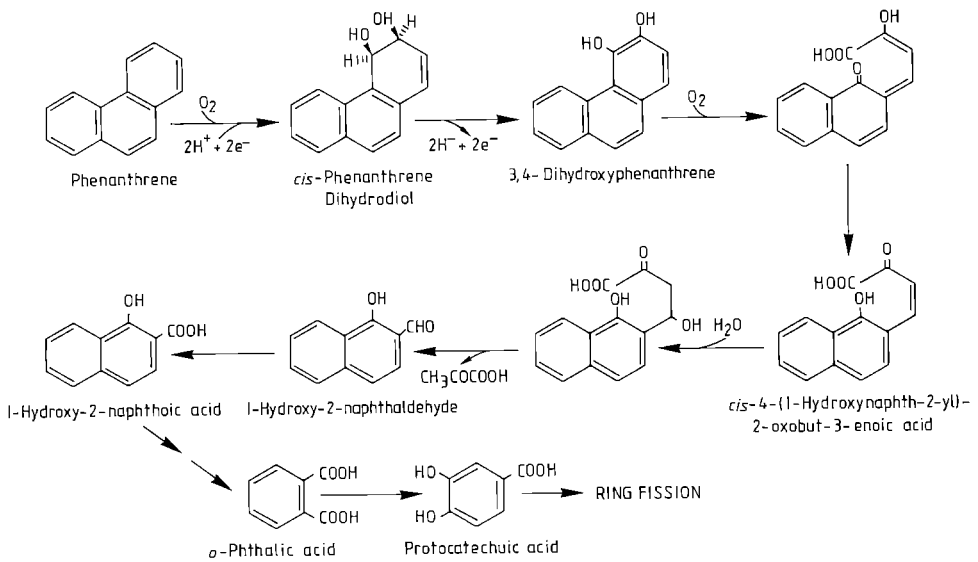

Fig. 12. Various proposed steps in the biodegradation of phenanthrene.

Fused-ring aromatic compounds

Naphthalene

Analogous with benzene, the bacterial degradation of naphthalene has frequently been reported over the last twenty years. The biodegradative route employed by the vast majority of micro-organisms is given in Fig. 11 and evidence to support this pathway can be found in previous review articles (Cerniglia 1984; Gibson & Subramanian 1984). In common with most other types of bacterial biodegradation of aromatic compounds, the initial attack proceeds via the action of dioxygenase attack. The resultant dihydrodiol is then converted to 1,2-dihydroxynaphthalene by dehydrogenase type enzymes. This catechol type compound then undergoes extradiol type cleavage between carbon atoms 1 and 9. The catabolic divergence in the catabolism of salicylate (see Fig. 11), may be somewhat misleading as it has only been reported in *Pseudomonas fluorescens*; the norm being the oxidative decarboxylation of the salicylate to yield catechol (Gibson & Subramanian 1984).

The catabolism of naphthalene by pseudomonads is often plasmid encoded as originally reported by Dunn & Gunsalus (1973). Subsequent studies have demonstrated that the genes are located on two operons (*nah* and *sal*) cited on plasmids of about 80-kilobase-pairs. The *nah* operon encodes for the genes for the conversion of naphthalene to salicylate, whilst the *sal* operon encodes for the conversion of salicylate to central metabolites (You et al. 1988). Recent reports on the genetic basis of naphthalene biodegradation have dissected the structure and function of the individual genes (Schell 1986; Schell & Wender 1986; You & Gunsalus 1986; You et al. 1988) but this work falls outside the scope of this present review.

The association and uptake of naphthalene in *Pseudomonas putida* was recently reported (Bateman et al. 1986). Neither an energised membrane or ATP were essential for the association or uptake of naphthalene. This association was not influenced by the catabolic plasmid.

Durham & Stewart (1987) proposed the recruitment of naphthalene dissimilatory enzymes of *Pseudomonas putida* for the oxidation of 1,4-dichloronaphthalene to 3,6-dichlorosalicylate (a precursor for the synthesis of the herbicide Dicamba, 3,6-dichloro-2-methoxybenzoate). However, the reported conversion was very low, being only 1% of the rate of salicylate conversion.

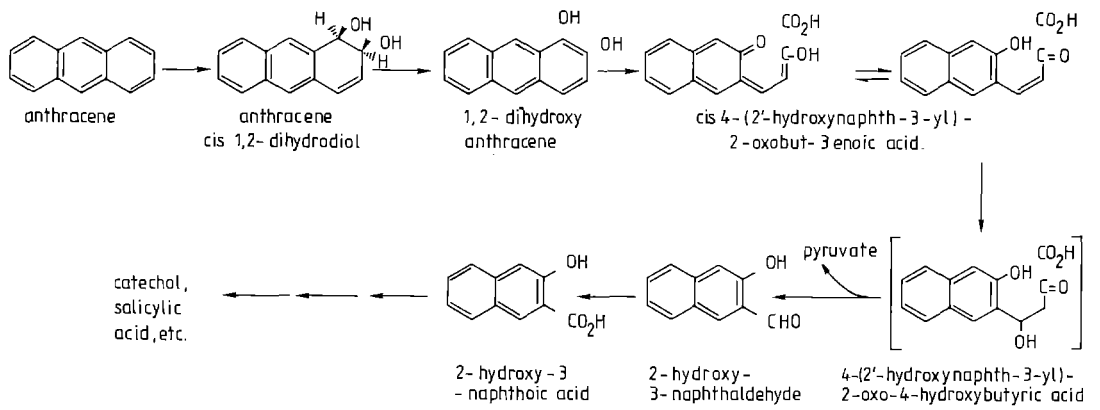

Fig. 13. Various proposed steps in the biodegradation of anthracene.

Polycyclic aromatic hydrocarbons

There is currently great concern about the levels of polycyclic hydrocarbons in the environment. The presence in the environment of large quantities may be attributed to both petrogenic and pyrogenic sources (Laflamme & Hite 1978) and these compounds are considered extremely undesirable as most are potential carcinogens, mutagens and tetragens. There has consequently been much research performed into the biodegradation of many these compounds as this is the major route through which polycyclic aromatic compounds are dissimilated. The biodegradative routes for phenanthrene and anthracene have been propsed (Figs 12 and 13, respectively) and the reader is directed to previous review articles (Gibson & Subramanian 1984; Cerniglia & Heitkamp 1989). There remain many unanswered questions regarding the biodegradation of these compounds. To date only the initial attack of these compounds has been reported and there have been no detailed investigations into the enzymes involved or the modes of ring-fission.

Foght et al. (1990) recently reported on the complete biodegradation of [^{14}C]phenanthrene in mineral oil by various different bacteria; the use of radio-labelled substrates offers conclusive evidence that the compound is biodegraded.

The biodegradation of higher molecular weight polycyclic aromatic compounds is even less well understood. There have been a few reports on the co-metabolism of these compounds (see Cerniglia & Heitkamp 1989). Until recently the only reports concerned the initial oxidation of high molecular weight polycyclic compounds by bacteria grown on alternative aromatic compounds and reflected the substrate specificity of the enzymes rather than novel modes of biodegradation. However, there have been several recent advances in this area. Heitkamp & Cerniglia (1988) isolated a single Gram-positive strain capable of the biodegradation of naphthalene, phenanthrene, fluoranthene and pyrene. The complete biodegradation of fluoranthene by pure cultures of *Pseudomonas paucimobilis* was demonstrated by Mueller et al. (1990). This was partially attributable to the use of Tween 80 in the medium to increase the bioavailability of the substrate. Fluoranthene grown cells were able to oxidise a wide range of aromatic compounds (anthraquinone, benzo[*b*]fluorene, biphenyl, chrysene and pyrene. These authors suggested that this was due to novel biodegradative routes but no route(s) have yet been postulated. Weissenfels et al. (1990) isolated various pure cultures capable of biodegrading different polycyclic aromatic compounds; *Pseudomonas paucimobilis* which grew on phenanthrene, *Pseudomonas vesicularis* capable of degrading fluorene and *Alcaligenes denitrificans* which grew on fluoranthene. No details of the biochemical pathways were included.

Concluding remarks

Although the literature abounds with reports on the bacterial biodegradation of aromatic compounds (and from this one can deduce that there exists a wealth of knowledge in this area) there do still exist gaps in our understanding.

The recent discovery of pure bacterial strains capable of anaerobic growth on toluene (Lovley & Lonergan 1990) raises the intriguing question: are there organisms that grow anaerobically on other aromatic hydrocarbons?

As outlined above, bacterial strains that degrade *p*- and *m*-xylenes cannot attack *o*-xylene (and vice versa) and this presents interesting questions regarding the evolution of these strains. It is also curious that there have only been two reports of the degradation of the *o*-isomer (Baggi et al. 1987; Schraa et al. 1987). There have been very few reports on the biodegradation of other di- and polyalkylsubstituted benzenes and this remains an area open to future studies.

With regard to alkenylbenzenes, the entire topic requires in depth studies to elucidate the pathways leading to ring-fission, the nature of the ring-fission mechanisms and the subsequent catabolism to central metabolites.

The increasing awareness of the occurrence of polycyclic aromatic hydrocarbons in the environment has provoked great research efforts into their biodegradation. The number and complexity of compounds now known to dissimilated by pure bacterial strains is rapidly increasing. As the problems, such as water solubility, are overcome, so the list is expected to grow. It is also hoped that the promising reports on the isolation of bacteria capable of growing on these compounds are followed up by detailed studies into the exact nature of the pathways employed.

An area of aromatic hydrocarbon biodegradation not covered in this review is that of mixed substrate interactions. In the environment it is unlikely that the micro-organisms are faced with single aromatic hydrocarbons; mixtures are much more likely to occur. It is well established that certain mixtures are more rapidly biodegraded than when the compounds are present singularly (McCarty et al. 1984) however, these studies are mainly based on work with non-growth-supporting (secondary) substrates, with biomass being created by one or more easily degraded primary substrate, present in high concentrations. Little is known about substrate interactions among biodegradable aromatic hydrocarbons present in growth supporting concentrations. Recently, Bauer & Capone (1988) investigated the biodegradation of mixtures of polycyclic aromatic hydrocarbons. Amongst their findings they showed that naphthalene stimulated the biodegradation of phenanthrene but not that of anthracene. Arvin et al. (1989) demonstrated the interaction of aromatic substrates during the biodegradation of benzene. The presence of toluene or xylene stimulated the degradation of benzene but that toluene and xylene had an antagonistic effect on the utilisation benzene. We (Smith et al. 1991) observed complete inhibition of growth of *Pseudomonas* sp. when presented with a mixture of biphenyl and ethylbenzene; both compounds being readily degraded when present singularly. None of these groups could explain their data and further research is required to establish if this is a commonly occurring phenomena and to elucidate the mechanisms involved.

It is possible that the answer to the above observations lies in the mechanisms of association and uptake of aromatic hydrocarbons by bacteria. This is an area of research which has been much neglected and intensive studies are needed to clarify this.

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