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BIODEGRADATION OF POLYNUCLEAR AROMATIC HYDROCARBON POLLUTANTS BY SOIL AND WATER MICROORGANISMS

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ABSTRACT

BIODEGRADATION OF POLYNUCLEAR AROMATIC HYDROCARBON POLLUTANTS BY SOIL AND WATER MICROORGANISMS

Polynuclear aromatic hydrocarbons (PNAs) are widely distributed in natural soils and waters and further are introduced into the environment by, e.g., oil spills and coal conversion processes. Since these toxic chemicals not only persist in nature but also can be converted into a carcinogenic agent in the animal, their complete removal or transformation to a harmless species is important. This study (1) delineated the structural limits of PNA degradability by measuring initial rates of aromatic hydrocarbon oxidation by soil and water microorganisms and (2) determined the persistence of selected PNAs by measuring percent remaining hydrocarbon in the presence of pure and mixed microbial cultures as a function of time and by demonstrating the appearance of metabolic products. Extensive removal of potentially carcinogenic PNAs can be effected even by the very dilute microbial suspensions found in natural waters. Since bacterial degradation of aromatic hydrocarbons does not produce a carcinogenic species, it may be that in the natural environment microbes exert a protective effect on higher organisms by continuously removing these potentially harmful chemicals from the biosphere. It is anticipated that an assessment of the biodegradability of PNAs will be of assistance to bioengineers responsible for waste management in coal conversion plants and others concerned with abatement of PNA pollution of the environment.

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INTRODUCTION

Objectives

This project was developed to investigate the persistence and fate of an environmentally significant class of compounds: the polynuclear aromatic hydrocarbons (PNAs). The studies were planned to: (a) delineate the structural limits of biodegradability of polynuclear aromatic nuclei, (b) determine the type and amount of substitution which can be tolerated and still retain a biodegradable hydrocarbon molecule and (c) measure the persistence of selected PNAs in the presence of pure and mixed microbial cultures.

Background

Polynuclear aromatic hydrocarbons (PNAs) are ubiquitous pollutants in the biosphere. The relative chemical inertness of these compounds allows their accumulation in the environment; and since some of the larger molecular weight species are carcinogenic, their occurence to any appreciable extent in water supplies and the atmosphere, for example, could be hazardous to human health.

Occurence and Formation of PNAs

A wide spectrum of PNAs (more than 70 compounds having from two to seven rings) have been identified in airborne particulate matter[1]. Many of these aromatic hydrocarbons along with series of their alkyl homologs have been detected and identified in a wide range of soils as well as in recent and ancient sediments[2,3,4].

From the atmosphere and soil these compounds are transferred into surface waters. Studies on the Charles River Basin[5] have demonstrated the presence of several PNAs and suggest a multiplicity of sources ranging from indigenous soil biological materials to automobile exhaust condensates. The incidence and significance of PNAs

in aquatic environments have been the subject of a critical review by Andelman and Snodgrass[6] while analytical techniques available for monitoring PNAs and the effects of water and wastewater treatment processes upon PNA levels have been evaluated by Harrison et al[7]. Significant amounts of PNAs (often measured as benzpyrene) are present in both industrial and domestic effluents and thus present problems in wastewater treatment[8].

Hydrocarbons from petroleum spills both natural[9] and manmade[10] further pollute marine and coastal waters and find their way into biological food chains. The aliphatic and alicyclic hydrocarbon constituents of these petroleum spills are degraded more rapidly than the aromatic fraction; some of the highly condensed aromatic compounds apparently remain unaltered and, being more dense than sea water, sink and become permanent pollutants of the ocean floor.

Atmospheric PNAs particulate pollutants arise through incomplete combustion of organic materials. Estimated annual benzpyrene emissions in the United States amount to 44 tons from gasoline- and diesel fuel- powered vehicles, 475 tons from heat and power generation sources, 548 tons from refuse burning, and 207 tons from industrial emissions. Coking operations alone account annually for some 200 tons of the industrial emissions[11,12].

In addition to coking operations, other coal conversion processes also generate PNAs as by-products. The benzene-soluble tars resulting from coal gasification procedures such as the synthane process contain many condensed-ring aromatic structures of up to five rings, as well as sulfur and nitrogen heterocycles[13]. Swansiger et al.[14] have reported a compositional analysis of coal liquefaction products and list some seventeen major aromatic and hydroaromatic components.

In addition to being formed as a result of human activities, PNAs have a natural origin. Diterpenes, triterpenes, sterols and plant quinone pigments are reduced and aromatized over long periods of geological time to form highly condensed aromatic hydrocarbons[15]. Plant lignins are transformed into humic substances which become an increasingly larger fraction during peat maturation. As the coalification of peat

proceeds, aromatic clusters are formed[16]. These aromatic nuclei in bituminous vitrinites are linked through hydroaromatic ring systems such as 9,10-dihydrophenanthrene[17]. The size distribution of aromatic clusters has not been established with finality; however, polarographic studies of coal extracts indicate the presence of many biphenyl, naphthalene, phenanthrene and triphenylene structures[16].

It has been shown that several species of bacteria synthesize carcinogenic PNAs in glycerine-fructose agar initially devoid of hydrocarbons[18] and that algae can synthesize several PNAs[19,20]. Graf and Diehl[21] have demonstrated that higher plants (rye, wheat and lentils) can form these compounds during germination and growth. So, unlike many of the organic materials resulting from today's technology, e.g., polymers and pesticides, PNAs have in addition a geological and biological origin.

Metabolism of PNAs

The wide distribution of PNA-degrading organisms in the environment was noted by Sisler and Zobell[22] who found that several PNAs were oxidized by large populations of mixed cultures of marine and soil bacteria. Hydrocarbon-utilizing organisms possess a unique class of enzymes, known as oxygenases, which carry out the initial step in substrate transformation by incorporating atmospheric oxygen into the molecule[23]. In contrast to mammalian systems which transform aromatic hydrocarbons by means of monooxygenases into arene oxides[24], bacterial systems carry out the dioxygenation of the aromatic nucleus to form a cis-glycol as the first stable intermediate[25,26]. Thus naphthalene is converted by liver microsomes to naphthalene-1,2-oxide which is then hydrated to the trans-glycol (trans-1,2-dihydroxy-1,2-dihydronaphthalene); microbes, on the other hand, form the unstable cyclic peroxide which is reduced to the cis-glycol[27]. Other microbial dioxygenases play an important role in aromatic degradation in subsequent ring cleavage steps (as in Figures 1 and 2).

Carcinogenic Aspect of PNAs

Over the years there has been much interest in the mechanism of carcinogenesis by polyaromatic hydrocarbons. Daly, Jerina and Witkop[28] have reviewed the evidence that the K-region epoxide formed by microsomal oxidation of so-called carcinogenic hydrocarbons is the actual carcinogenic species. In addition to inducing malignant transformations in rodent cells[29], epoxides act as mutagens in strains of Salmonella[30], in bacteriophage[31] and in clones of Chinese hamster cells[32]. Ames

et al.[30] have shown that, in Salmonella, these epoxides function as frameshift mutagens, i.e., they alter the reading frame during transcription of genetic information from deoxyribonucleic acid to ribonucleic acid so that faulty proteins are synthesized.

Useful tests have been proposed[33,34] for the screening of human urines in order to detect exposure of human populations to carcinogens. Following activation of the precarcinogen to the active epoxide by liver homogenates, sensitive bacterial strains are used to detect the mutagenic metabolites.

Since epoxides are not intermediates in bacterial metabolism of polyaromatic hydrocarbons, bacterial degradation of PNAs conceivably could prove a useful agency for the removal of potentially carcinogenic hydrocarbons from the biosphere.

The present study employs bacterial strains (isolated using aromatic hydrocarbons found in the biosphere for enrichment substrates, e.g., naphthalene and phenanthrene) as a system in which to test the limits of fused-ring aromatic structure that can allow oxidative degradation to take place in natural environments. The persistence and fate of selected PNAs also is examined.

MATERIALS AND METHODS

Elective enrichment techniques using Illinois soil and water samples provided several microbial strains which grew well on naphthalene and/or phenanthrene as a carbon source. No organisms could be isolated which could grow on a PNA structure possessing more than three fused rings, however. The organisms which preferentially grew on naphthalene appeared to belong to the genus Pseudomonas; while the organisms which preferred phenanthrene as a growth substrate, all Gram-negative bacteria, tentatively were classified as belonging to the genus Flavobacterium.

The aromatic hydrocarbons chosen for study were among those commonly encountered as by-products of coal gasification and liquification procedures (Figure 3) and were the purest grade commercially available.

Resting cell suspensions used to delineate structural limits of degradability of aromatic compounds were obtained by growth of the microbial strain in Stanier's basal mineral medium[35] in the presence of 0.1% naphthalene (for 16 hours) or 0.05% phenanthrene (for 36 hours). The cells were harvested by centrifugation, washed twice with dilute (0.02M) potassium phosphate buffer, pH 7.2, and finally suspended in the same medium.

In a typical experiment Pseudomonas putida cells (11 mg) were assayed for oxygen consumption by conventional manometric techniques. Substrate-dependent oxygen uptake in the presence of the growth substrate naphthalene (5.0 μ moles) was typically 56.2 mμ moles O_2/min/mg cells. The initial rate of oxygen uptake in the presence of other aromatic compounds was compared with the uptake rate in the presence of growth substrate (naphthalene) set at 100 percent. In a similar manner Flavobacterium sp. cells (8 mg) exhibited an initial substrate-dependent oxygen uptake rate equivalent to a specific activity of 30.7 mμ moles O_2/min/mg cells in the presence of the growth substrate phenanthrene.

Cell-free preparations of naphthalene-grown P. putida were obtained by subjecting a cell suspension to sonic oscillation. After removal of the cellular debris by centrifugation, the aromatic hydrocarbon oxygenase system was concentrated by precipitation with ammonium sulfate; the precipitate so obtained was dissolved in phosphate buffer and stored frozen. Activity of the preparation (1.7 mg protein/ml assay mixture) was assayed by monitoring substrate-dependent oxygen uptake in the presence of naphthalene (0.5 μ mole) with a Clark oxygen electrode. Initial oxygen uptake rates in the presence of other aromatic hydrocarbons were compared, as before, with that of naphthalene set at 100 percent.

In studies designed to measure the persistence of selected PNAs, the bacterial strains employed in the respirometry experiments were exposed to simulated 'shock' loads of 10 mg pyrene, 3,4-benzpyrene, 1,2-benzanthracene, or 1,2,5,6-dibenzanthracene per 100 ml Stanier's medium in the presence and absence of the appropriate growth substrate for varying periods of time. A control which contained both hydrocarbons (growth and non-growth) but no organisms was run to check for completeness of extraction. The flasks were incubated at ambient temperature on a gyrorotary shaker away from direct light

(to prevent photooxidation).

Culture flasks were removed from the shaker at 0-, 1-, 2-, 3-, and 4-week intervals, and the contents of each flask was extracted exhaustively with benzene. The volume of the extract was reduced by vacuum distillation, and the resulting concentrate was adjusted to a volume of 4.0 ml.

One- μl portions of these samples were analyzed by standard gas chromatographic techniques in an F&M model 810 gas chromatograph equipped with a dual flame ionization detector. OV-1 (3% on 80/100 mesh GasChrom W) in a 6 ft x 1/4 in. column was employed as the liquid phase; nitrogen, at an outflow rate of 50 ml/min, was used as the carrier gas. Results of the analyses were quantitated by a digital integrator, and the data so obtained were analyzed by linear regression techniques.

Thin-layer chromatography of PNA metabolites was carried out on Eastman silica gel Chromagram sheets (No. 6061). The spotted chromatogram was developed in chloroform-acetone (4:1), and the separated compounds were visualized by means of an ultra-violet handlamp.

RESULTS AND DISCUSSION

Structural Limits of Biodegradability of Aromatic Compounds

When resting cell suspensions of P. putida and a Flavobacterium sp. (both grown to late log or early stationary phase) were examined manometrically for the effect of the number of fused rings on aromatic oxidation, the results obtained were similar to those of the growth experiments: no significant rate of oxidation of compounds containing more than three rings occurred (Table Ia). While naphthalene and phenanthrene underwent a relatively rapid rate of oxidation by both test organisms, anthracene was oxidized at a moderate rate by only the Flavobacterium. The four-membered ring systems tested were oxidized at negligible rates. In addition, three five-membered ring compounds were tested also - 3,4-benzpyrene, 1,2,5,6-dibenzanthracene and perylene - and none underwent oxidation under these test conditions.

A study of the effect of alkyl and phenyl substituents on the rate of naphthalene oxidation revealed (Table Ib) that naphthalene nuclei bearing a single, small alkyl group (methyl, ethyl or vinyl) were oxidized at a comparatively moderate to rapid rate while those bearing a phenyl substituent underwent oxidation at a negligible rate at best. A given substituent at position 2 allowed a faster rate of oxidation than one at position 1. This finding is not unexpected and agrees with reports (cited in reference 36) that the unsubstituted ring is preferentially attacked since a substituent at position 2 would offer less steric hindrance at an enzymatic binding site than would a substituent at position 1. When other methyl-substituted ring systems were tested and compared with the substituted naphthalenes (Table Ic), most of these compounds were oxidized at at least a comparatively moderate rate. While the Pseudomonas sp. exhibited a faster oxidation rate with compounds substituted in position 2, no clear cut position preference was observed for the Flavobacterium sp. Also, the Pseudomonas sp. was less tolerant to substitution than the species of Flavobacterium was.

Substituted naphthalenes containing more than one alkyl or phenyl group were examined also (Table Ib). In the series of methyl-substituted naphthalenes tested, with one exception (1,3-dimethylnaphthalene oxidation by the Flavobacterium), compounds containing one or two methyl groups on one ring supported at least a significant rate of oxidation. In agreement with Rogoff and Wender[37] who found that soil pseudomonads oxidized 2,3-dimethylnaphthalene at a more rapid rate than 2,7-dimethylnaphthalene, Table Ib shows that methyl substitution on both rings led to a decreased oxidation rate. Addition of a third methyl substituent and increase in the size of the alkyl substituent also lowered the oxidation rate.

That oxygen uptake observed in this experiment was actually a measure of ring oxidation and cleavage for this series of mono-, di-, and trimethyl-substituted naphthalenes was indicated by the demonstration of cis-glycol formation by thin-layer chromatography[38] and of split-ring products by their characteristic absorption spectra[39]. The idea that the unsubstituted ring was attacked was supported by the isolation of a compound possessing the characteristics of 7-methylnaphthalene cis-glycol from cultures of P. putida exposed to 2-methylnaphthalene.

The last structural feature to be examined was ring saturation (Table Id). These studies showed that an increased degree of saturation in a given parent hydrocarbon resulted in a decreased rate of oxidation. As long as one aromatic ring was available, the structure supported a readily measurable rate. The perhydro compounds - cis- and trans-decalin, perhydrophenanthrene, hexahydroindane, and perhydrofluorene - were relatively resistant to oxidation.

To complete the study several single-ring hydrocarbons were tested also; the results are given in Table II. Although benzene and cyclohexane were not oxidized very rapidly, toluene and the xylenes were oxidized at a moderately fast initial rate. The rather surprisingly good rates for oxidation of the xylenes may perhaps be explained by their ability to mimic naphthalene as they sit on the enzymatically active site of the aromatic oxygenase. Biphenyl was oxidized by neither organism, although reports exist that biphenyl[40,41] and even halogenated biphenyls[42] are degraded oxidatively by microorganisms.

To summarize the structural limits of degradability of fused ring hydrocarbons by whole resting cells, it was found that the number of fused rings, the size, position and number of ring substituents, and the degree of saturation all influenced initial rates of aromatic oxidation. In general, increasing number of fused rings, size and number of hydrocarbon substituents, and degree of ring saturation led to decreased initial oxidation rates. The effect of substituent position on oxidation rates appeared to be a reflection of enzymatic specificity in the case of the substituted naphthalenes.

Persistence and Fate of PNAs

What do these results indicate in an environmental context? During this manometric study oxygen uptake was monitored for about one hour and rates were calculated on the basis of the oxidation rate for the initial 20 minutes or for as long a period as the rate of oxygen consumption was strictly linear. Clearly, in the biosphere microbial removal of PNAs is much slower than in a controlled laboratory experiment. In the environment any oxidative rate which results in a removal rate greater than the rate of formation and accumulation of PNAs is significant. One experimental means of detecting these slower oxidative rates is the employment of co-oxidation (co-metabolism) techniques, i.e., the measurement of oxidation of a non-growth substrate during growth of an organism on an appropriate carbon and energy source.

Four refractory polyaromatic compounds, selected from those non-growth substrates not measurably oxidized by bacterial cells in the course of 1-2 hours, were examined by the above mentioned co-oxidation technique for persistence. The compounds chosen were pyrene and the carcinogens 3,4-benzpyrene, 1,2-benzanthracene and 1,2,5,6-dibenzanthracene.

A short preliminary experiment in which pyrene was used as the test compound showed that whereas P. putida was unable to degrade pyrene, some 30% (2.9 mg) of this hydrocarbon was utilized in 2 weeks by the Flavobacterium sp. in the presence of its growth substrate phenanthrene. In an experiment where 3,4-benzpyrene was employed as the substrate to be co-metabolized (Figure 4), it was found that in the presence of the Flavobacterium sp. less than half of the added 3,4-benzpyrene remained whereas the pseudomonad was unable to degrade the benzpyrene even in the presence of an appropriate growth substrate during the course of four weeks. In a similar experiment employing 1,2-benzanthracene (Figure 5), 63% of this hydrocarbon remained in the presence of the Flavobacterium and phenanthrene. Lastly, only 38% of the added 1,2,5,6-dibenzanthracene remained after four weeks in the presence of the Flavobacterium under growth conditions (Figure 6). The pseudomonad effected negligible utilization of the test hydrocarbon in the case of all four PNAs tested by the co-metabolism technique.

In an experiment similar to the above series a mixed culture was examined for PNA utilization. The inoculum used for this experiment (Table III) was water from a polluted stream in Urbana which contained approximately 3×10^6 organisms/ml. Culture flasks were prepared to contain 100 ml unsterile creek water instead of sterile distilled water. For each PNA tested one flask received naphthalene as a growth substrate while a second flask received phenanthrene as the growth substrate; the flasks were incubated on a gyrorotary shaker for four weeks before analysis. Both pyrene and 1,2-benzanthracene were degraded in the presence of either growth substrate by this mixed culture. The results obtained with 3,4-benzpyrene and 1,2,5,6-dibenzanthracene in the presence of creek water were similar to those obtained with these hydrocarbons in the presence of pure cultures.

When samples from the persistence studies were subjected to thin-layer chromatography on silica gel, the samples obtained from the flasks containing the Flavobacterium species and 3,4-benzpyrene were found to contain readily discernable

metabolites (Figure 7). Control 1 contained both hydrocarbons (benzpyrene and phenanthrene) but no organisms; control 2 contained the Flavobacterium and 3,4-benzpyrene but no growth substrate. A third control flask containing only microorganisms and the growth substrate phenanthrene also accumulated no polar products. By the end of 28 days three metabolites had accumulated in the experimental flasks, however. Although these compounds are unidentified as yet, the appearance of polar metabolites indicates that microorganisms can degrade PNAs extensively in the absence of growth on these compounds.

In the environment many factors in addition to hydrocarbon chemical structure contribute to the persistence of PNAs. Determination of initial rates of oxidation under optimum laboratory conditions tends to eliminate such environmental factors as toxicity of substrate or products, lack of an essential nutrient (e.g., oxygen or a mineral), inaccessibility of substrate or enzyme inactivation. Hegeman[43] has put forth the intriguing idea that some synthetic organic materials persist in nature because no organism has evolved yet that possesses the enzymatic capability to degrade these compounds. But nonetheless, this method allows one to observe the effect of such important factors as cell membrane permeability, enzyme specificity and water solubility of the hydrocarbon.

If lack of utilization of a given structure is due to lack of permeability of the cell membrane rather than to a recalcitrant chemical structure, compounds which are not attacked by whole cell suspensions should be oxidized by cell-free preparations of the same organism. Table IV lists results obtained when initial oxidation rates of cell-free extracts of P. putida were compared with those rates obtained with resting whole-cell suspensions of the same organism. Comparable amounts of cytoplasmic protein were used in each instance. In general, initial oxidation rates decreased (Table IVa) with increasing molecular weight (and decreasing water solubility) of the hydrocarbon substrate. However, anthracene and pyrene were oxidized at a much faster rate by the cell-free preparations, in the case of pyrene some 70 times as fast as whole cell rates. Cell membrane permeability may be a factor here.

When the effect of number and position of methyl groups on naphthalene oxidation by P. putida (Table IVb) was examined, a similar pattern of initial rates was found for the two preparations. The cell-free preparation in general possessed the ability to

oxidize the methyl-substituted naphthalenes at a relatively faster rate than did the whole cell suspensions. Likewise in the naphthalene saturation series (Table IVc) faster initial rates were accomplished by the cell-free preparation. In the case of cis-decalin, the very slow rate perhaps may be ascribed to enzymatic specificity for a more planar configuration as found in trans-decalin or more preferably a planar, aromatic ring as found in 1,2-dihydronaphthalene and tetralin.

That the relative water insolubility of PNAs may influence initial oxidation rates was indicated by a comparison of oxidation rates of cell-free extracts with water solubilities of the compounds listed in Table IVa. Anthracene, phenanthrene and pyrene, possessing a range of water solubilities one to two orders of magnitude greater than that of the remaining compounds (see data of Klevens, ref. 44), were oxidized at almost correspondingly greater initial rates.

Although persistent, fused-ring aromatic hydrocarbons can be degraded extensively by microorganisms. The foregoing study has shown that factors contributing to structural limits of aromatic degradability include: number of fused rings; size, number and position of substituents on the ring; and degree of ring saturation. Factors contributing to aromatic persistence most probably include: lack of cell membrane permeability, lack of substrate solubility and enzymatic specificity.

Potential Applications of the Research to Water Resources Problems

It is anticipated that an assessment of the biodegradability of PNAs will be of assistance to bioengineers responsible for waste management in coal conversion plants and others concerned with abatement of PNA pollution of the environment.

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TABLE I

Oxidation of Polynuclear Aromatic and Saturated Ring Hydrocarbons by Resting Cell Suspensions

Compound	Rate of Oxidation	
	Pseudomonas putida	Flavobacterium sp.
	Naphthalene = 100%	Phenanthrene = 100%
a. Effect of number of fused rings on oxidation.		
Naphthalene	100	79.8
Anthracene	9.8	36.0
Phenanthrene	67.0	100
1,2-Benzanthracene	0	10.1
2,3-Benzanthracene	2.7	1.1
Chrysene	0	7.9
Pyrene	1.1	10.1
Triphenylene	0	0
b. Effect of alkyl and phenyl substituents on naphthalene ring oxidation.		
1-Methylnaphthalene	41.7	60.0
1-Ethylnaphthalene	39.0	36.3
1-Phenylnaphthalene	0	0
2-Methylnaphthalene	81.0	85.3
2-Ethylnaphthalene	70.3	45.0
2-Vinylnaphthalene	78.8	40.0
2-Phenylnaphthalene	1.4	8.9
1,3-Dimethylnaphthalene	39.1	0
1,4-Dimethylnaphthalene	8.8	19.9
1,5-Dimethylnaphthalene	8.8	0
1,6-Dimethylnaphthalene	1.4	14.4
2,3-Dimethylnaphthalene	88.0	84.2
2,6-Dimethylnaphthalene	15.5	62.8
2,6(and 2,7)-Di-tert.-butylnaphthalene	0	0
2,3,5-Trimethylnaphthalene	9.0	17.5
2,3,6-Trimethylnaphthalene	7.0	9.6
1,2,3,4-Tetraphenylnaphthalene	0	0

TABLE I (Continued)

Oxidation of Polynuclear Aromatic and Saturated Ring Hydrocarbons by Resting Cell Suspensions

Compound	Rate of Oxidation: Pseudomonas putida, Naphthalene = 100%	Rate of Oxidation: Flavobacterium sp., Phenanthrene = 100%
c. Effect of position of methyl substituent on oxidation.		
Naphthalene	100	79.8
1-Methylnaphthalene	41.7	60.0
2-Methylnaphthalene	81.0	85.3
Phenanthrene	67.0	100
1-Methylphenanthrene	5.0	107
2-Methylphenanthrene	23.5	88.0
3-Methylphenanthrene	21.8	74.7
Fluorene	32.4	36.7
1-Methylfluorene	0	75.6
2-Methylfluorene	5.1	41.9
d. Effect of saturation on oxidation of polynuclear hydrocarbons.		
Naphthalene	100	79.8
1,2-Dihydronaphthalene	32.3	59.8
Tetralin	15.1	15.2
cis-Decalin	4.4	0
trans-Decalin	4.4	0
Phenanthrene	67.0	100
9,10-Dihydrophenanthrene	12.0	23.3
1,2,3,4,5,6,7,8-Octahydrophenanthrene	9.3	25.5
Perhydrophenanthrene	1.2	0.7
Indene	32.4	14.9
Indane	24.6	13.0
Hexahydroindane	6.8	1.0
Fluorene	32.4	36.7
Perhydrofluorene	0	3.4

TABLE II

Oxidation of Single-Ring Hydrocarbons
By Resting Cell Suspensions

Compound	Rate of Oxidation	
	Pseudomonas putida	Flavobacterium sp.
	Naphthalene = 100%	Phenanthrene = 100%
Benzene	6.7	0
Cyclohexane	0	0
Toluene	30.2	13.9
o-Xylene	32.9	31.0
m-Xylene	29.8	38.1
p-Xylene	36.0	25.0
Biphenyl	0	0

TABLE III

Persistence of Polynuclear Aromatic Hydrocarbons
in Natural Waters

Non-Growth Substrate	Growth Substrate	Amount Non-Growth Substrate Remaining After Four Weeks
Pyrene	Naphthalene	36.7%
	Phenanthrene	47.2
3,4-Benzpyrene	Naphthalene	83.5
	Phenanthrene	38.3
1,2-Benzanthracene	Naphthalene	58.3
	Phenanthrene	33.8
1,3,5,6-Dibenzanthracene	Naphthalene	92.7
	Phenanthrene	32.9

TABLE IV

Comparison of the Oxidation of Polynuclear Aromatic and Saturated Ring Hydrocarbons by Resting Cell Suspensions and Cell-Free Extracts of Pseudomonas putida

Compound	Rate of Oxidation: Whole Cell Suspension	Rate of Oxidation: Cell-Free Preparation
a. Effect of number of fused rings on polynuclear aromatic hydrocarbon oxidation.		
Naphthalene	100.0%	100.0%
Anthracene	9.8	61.9
Phenanthrene	67.0	60.6
2,3-Benzanthracene	2.7	3.0
1,2-Benzanthracene	0	2.5
Chrysene	0	1.9
Triphenylene	0	6.4
Pyrene	1.1	81.9
Perylene	0	0
3,4-Benzpyrene	0	0
1,2,5,6-Dibenzanthracene	0	0
b. Effect of number and position of methyl groups on naphthalene oxidation.		
1-Methylnaphthalene	41.7	60.9
2-Methylnaphthalane	81.0	97.9
1,3-Dimethylnaphthalene	39.1	61.4
1,4-Dimethylnaphthalene	8.8	40.8
1,5-Dimethylnaphthalene	8.8	37.1
1,6-Dimethylnaphthalene	1.4	42.8
2,3-Dimethylnaphthalene	88.0	87.2
2,6-Dimethylnaphthalene	15.5	36.3
2,3,5-Trimethylnaphthalene	9.0	38.1
2,3,6-Trimethylnaphthalene	7.0	37.6
c. Effect of saturation on naphthalene oxidation.		
1,2-Dihydronaphthalene	32.3	77.2
Tetralin	15.1	61.9
cis-Decalin	4.4	1.9
trans-Decalin	4.4	28.4

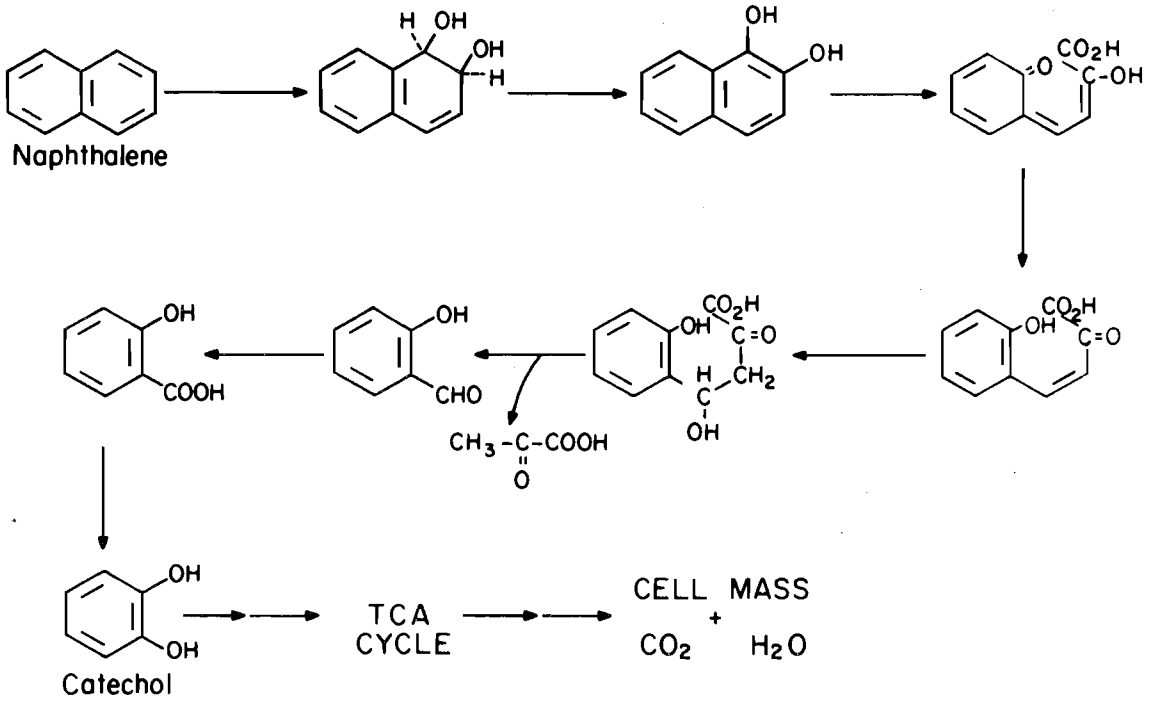

Figure 1. Microbial metabolism of naphthalene (adapted from ref. 45).

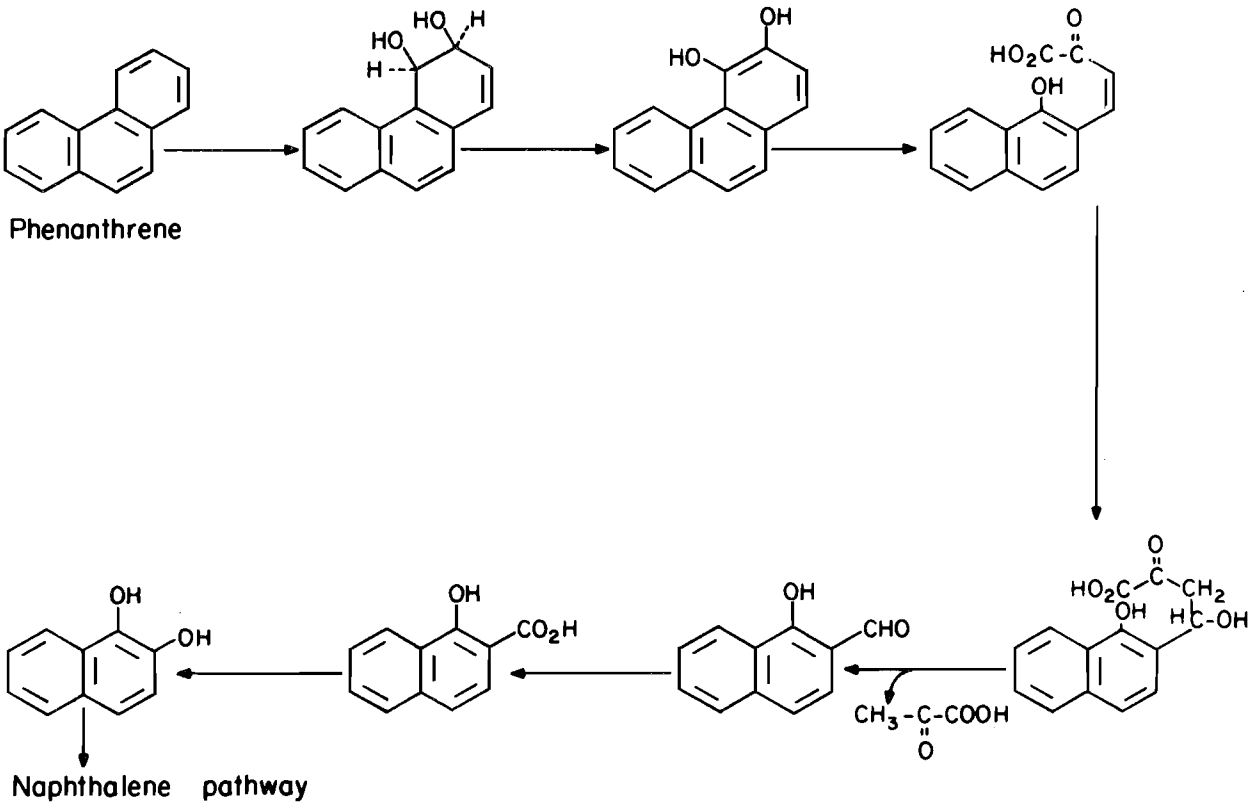

Figure 2. Microbial metabolism of phenanthrene (adapted from ref. 45).

FUSED - RING AROMATIC HYDROCARBONS IN
COAL PRODUCTS AND BY-PRODUCTS

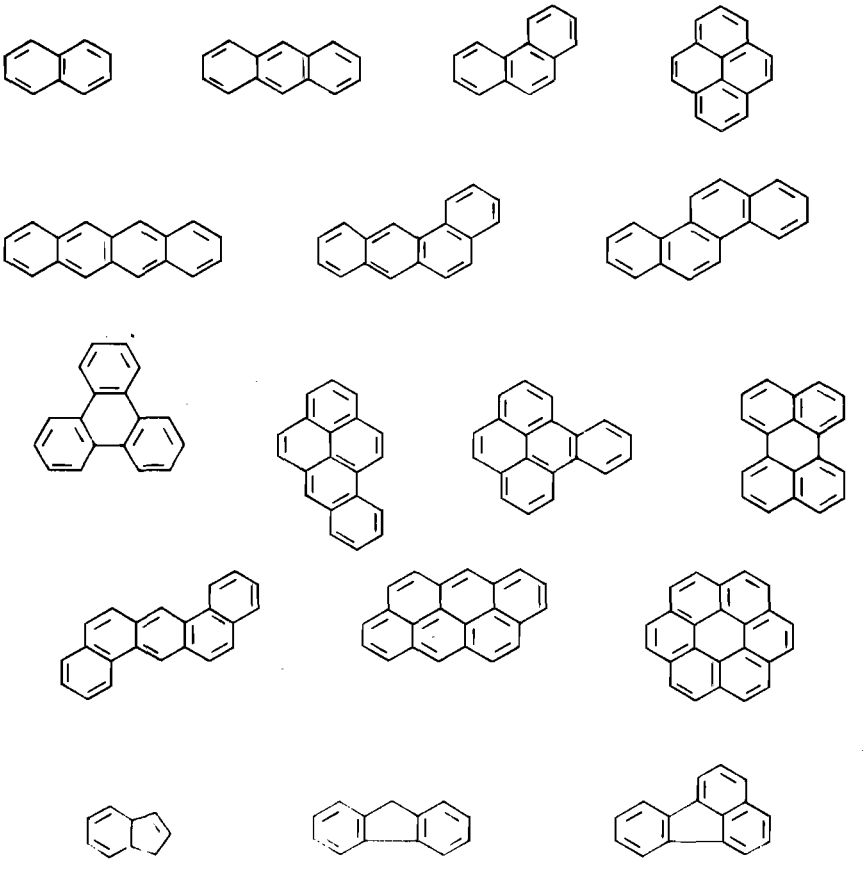

Figure 3. Fused-ring aromatic hydrocarbons commonly encountered as products and by-products of coal conversion processes: naphthalene, anthracene, phenanthrene, pyrene; 2,3-benzanthracene, 1,2-benzanthracene, chrysene; triphenylene, 3,4-benzpyrene, 1,2-benzpyrene, perylene; 1,2,5,6-dibenzanthracene, anthanthrene, coronene; idene, fluorene, fluoranthene.

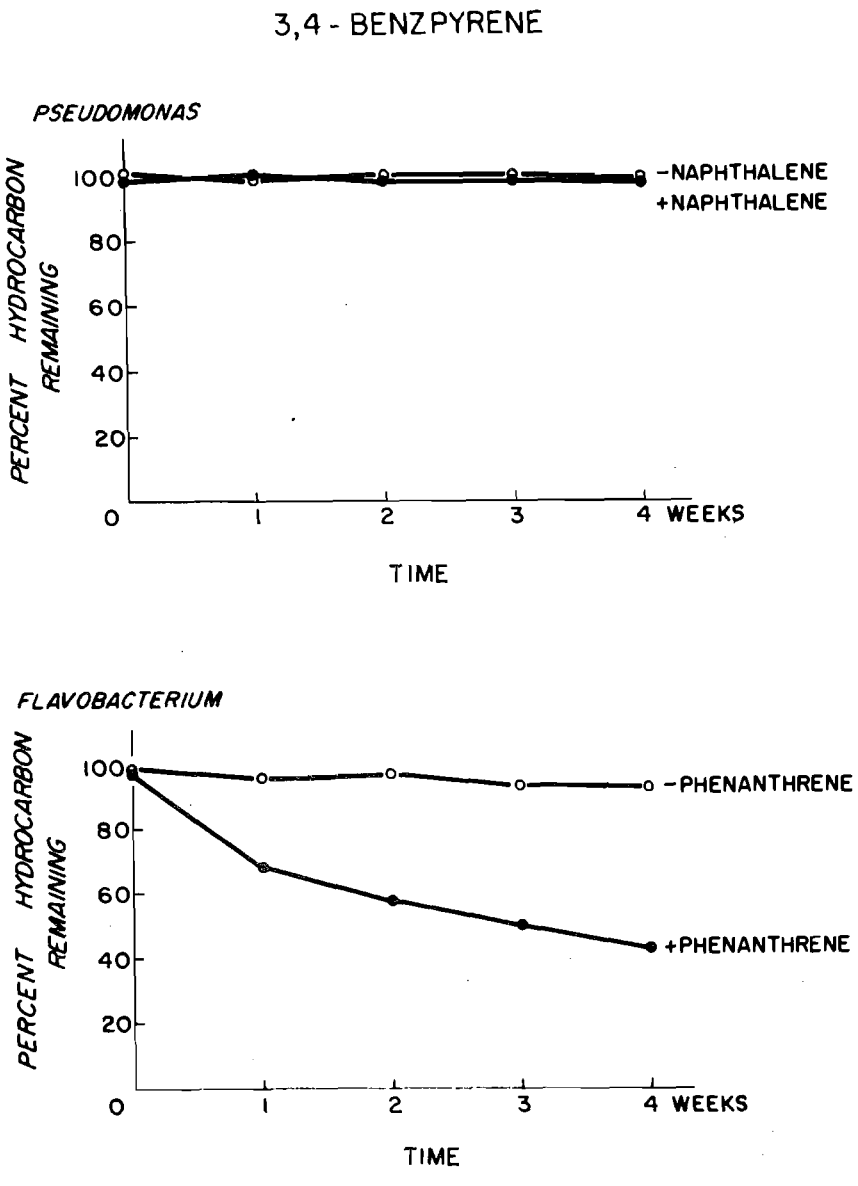

Figure 4. Persistence of 3,4-benzpyrene.

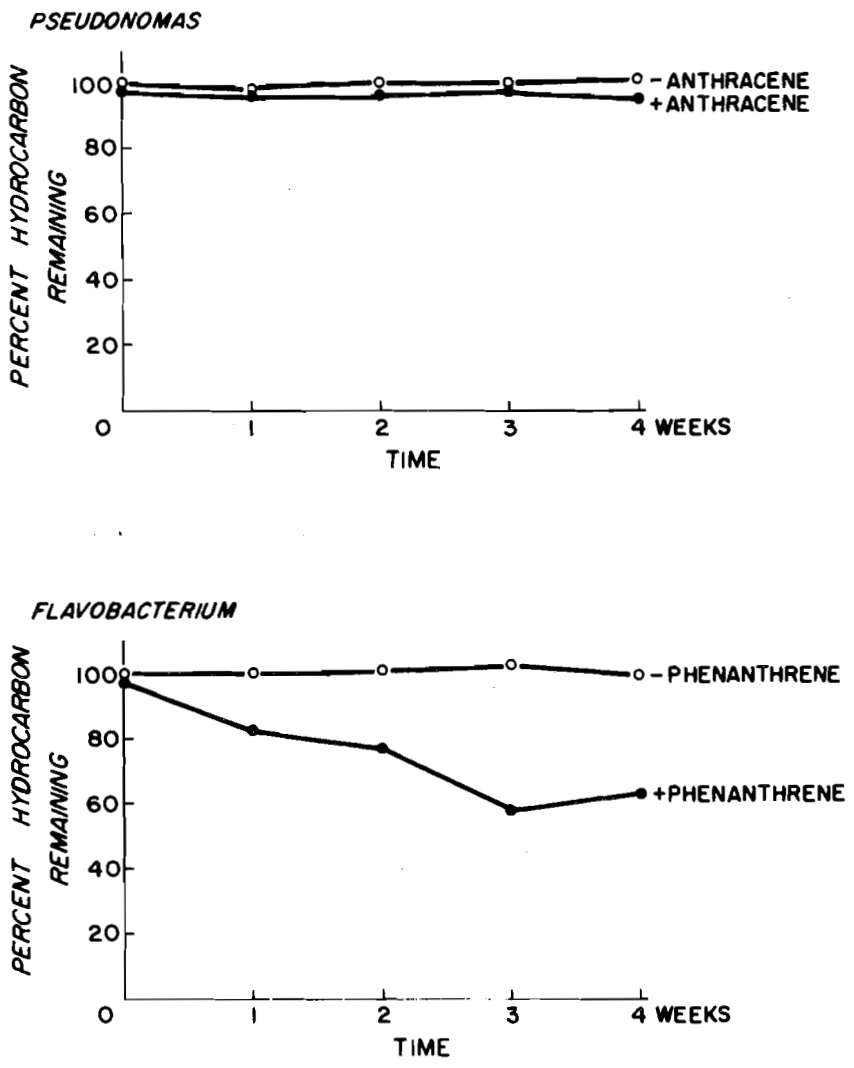

Figure 5. Persistence of 1,2-benzanthracene.

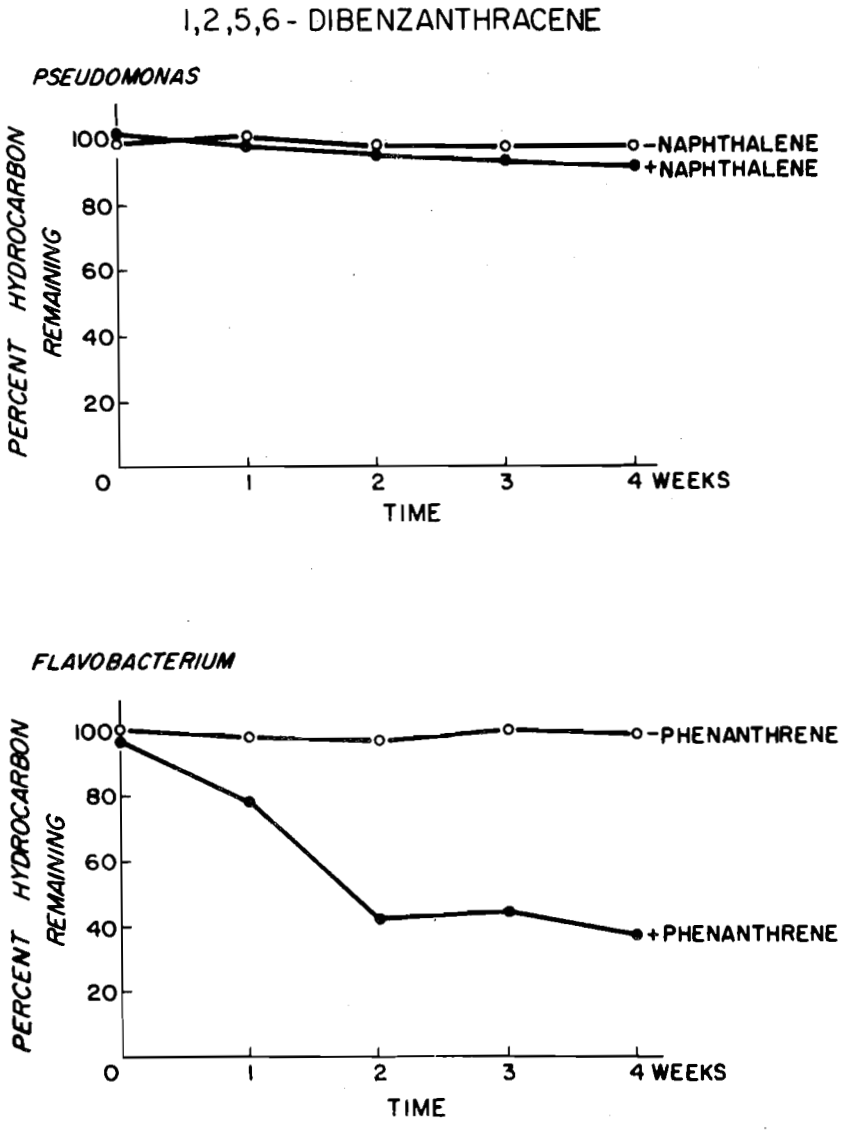

Figure 6. Persistence of 1,2,5,6-dibenzanthracene.

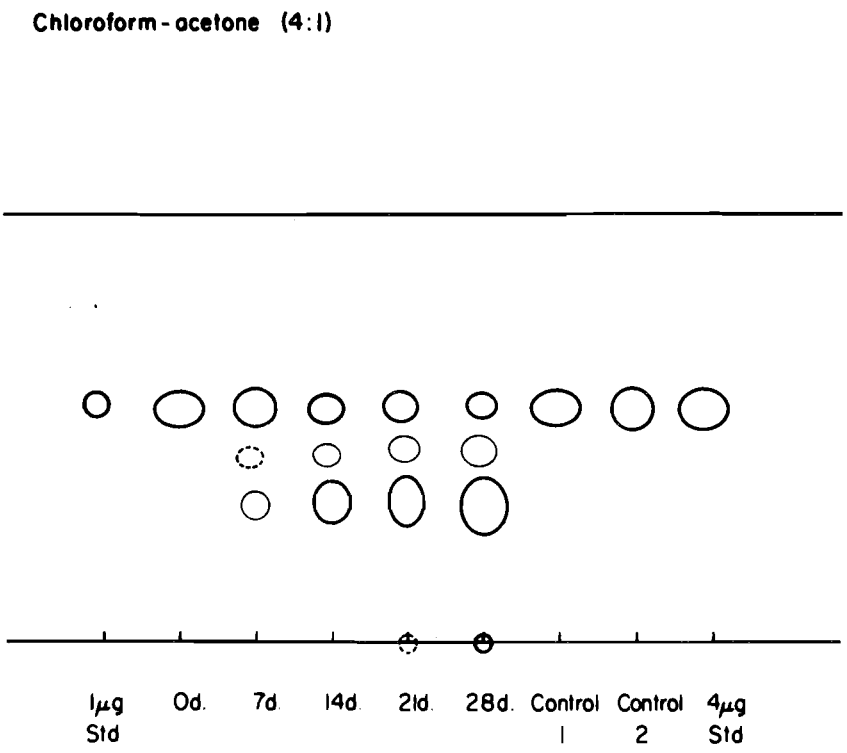

Figure 7. Time course of 3,4-benzpyrene degradation.