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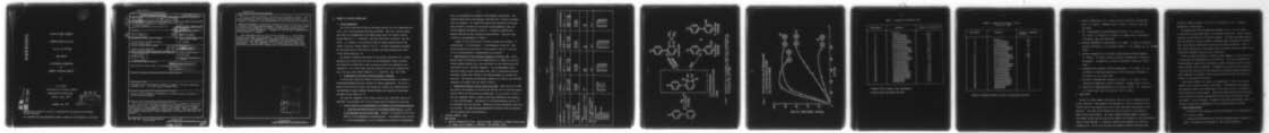
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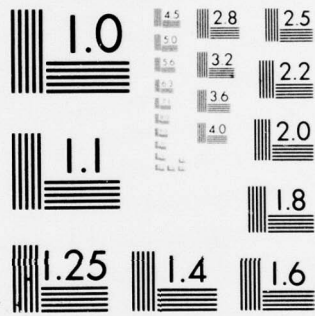
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OFFICE OF NAVAL RESEARCH

✓ CONTRACT N00014-76-C-0102

Task No. NR. 205-008

FINAL REPORT

THE MICROBIAL DEGRADATION
OF
AROMATIC PETROLEUM PRODUCTS

by

D.T. Gibson

The University of Texas at Austin

✓ Department of Microbiology

Austin, Texas 78712

November 3rd, 1977

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Pseudomonas Pxy was shown to oxidize para- and meta-xylene to the corresponding toluic acids. The latter compounds were oxidized to 4- and 3-methylcatechols prior to the enzymatic fission of the aromatic nucleus. Pathways are proposed for the degradation of both xylene isomers. The genes specifying the enzymes responsible for xylene degradation are carried on a transmissible plasmid.		

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→ A *Nocardia* species oxidized *ortho*-xylene to 3,4-dimethyl catechol. The latter compound was further metabolized to propionaldehyde and pyruvic acid. A pathway is proposed for the biodegradation of *ortho*-xylene by this organism.

→ Dibenzothiophene is oxidized by a mutant strain of *Beijerinckia* to *cis*-1,2-dihydroxy-1,2-dihydrodibenzothiophene. Enzymes present in the parent organism oxidized this metabolite to 3-hydroxy-2-formylbenzothiophene which was resistant to further degradation. A pathway for the microbial degradation of dibenzothiophene is proposed.

The same mutant strain of *Beijerinckia* oxidized 2-, 3-, and 4-chlorobiphenyl to analogous *cis*-dihydrodiols. Each metabolite was isolated and characterized. The components of a commercial polychlorinated biphenyl, Aroclor 1221, were identified and quantified by gas chromatography - mass spectrometry. The only components in this mixture that were significantly oxidized by *Beijerinckia* were biphenyl and its monochlorinated derivatives. ↑

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A. SUMMARY OF RESEARCH ACCOMPLISHED

1. Xylene Degradation

The initial reactions utilized by different bacteria for the biodegradation of *ortho*-, *meta*- and *para*-xylene have been elucidated. Both *meta*- and *para*-xylene were shown to be metabolized at the methyl group and at the aromatic nucleus (Annual Report No. 1, J. Bacteriol. 119, 923 (1974) and J. Bacteriol. 119, 930 (1974)). In contrast, *ortho*-xylene was shown to undergo hydroxylation of the aromatic ring only (Annual Reports 2 and 3). The whole biodegradative pathway for the conversion of *ortho*-xylene to pyruvate and propionaldehyde was established (Annual Report No. 3).

The genetic information that codes for the enzymes responsible for *p*-xylene degradation was shown to be carried on an extrachromosomal element (plasmid). The plasmid was transferred to other *Pseudomonas* species by transduction and conjugation. As a result these organisms acquired the ability to degrade both *para*- and *meta*-xylene (Annual Report No. 2, J. Bacteriol. 127, 1217 (1976)).

2. Biodegradation of Sulfur-Containing Aromatic Compounds

A *Beijerinckia* species was shown to oxidize dibenzothiophene to 3-hydroxy-2-formylbenzothiophene which was resistant to further degradation. The use of a mutant strain and cell extracts facilitated the isolation and identification of the degradation products between dibenzothiophene and 3-hydroxy-2-formylbenzothiophene (Annual Report No. 2).

This work was completed in the current contract year (1976-1977) and will be published in the December 1977 issue of Applied and Environmental Microbiology.

3. Initial Studies on the Biodegradation of Biphenyl and Chlorinated Biphenyls

a. Isolation and identification of the products formed from 2-, 3-, and 4-chlorobiphenyl by *Beijerinckia* B836. Biphenyl is oxidized by *Beijerinckia* B836 to *cis*-2,3-dihydroxy-2,3-dihydrobiphenyl. This organism also oxidized 3-, and 4-chlorobiphenyl to *cis*-2,3-dihydroxy-2,3-dihydro-3'-chlorobiphenyl

and *cis*-2,3-dihydroxy-2,3-dihydro-4'-chlorobiphenyl respectively. The products formed from 2-chlorobiphenyl indicated that a mixture of dihydrodiols was produced. All identified products were characterized from the data provided by infrared and ultraviolet spectrophotometry, mass and nuclear magnetic spectrometry (Table 1). In addition acetone and phenolic derivatives were isolated and characterized. These are illustrated for 4-chlorobiphenyl in Figure 1.

Studies on the relative ease of oxidation showed that biphenyl > 3-chlorobiphenyl > 4-chlorobiphenyl > 2-chlorobiphenyl (Figure 2). When chlorine substitution was present in both aromatic rings as in the case of 4,4'-dichlorobiphenyl no biodegradation was observed.

b. Identification of the individual components in Aroclor 1212. Before studies could be initiated on the biodegradation of a commercial polychlorinated biphenyl methods had to be established for the identification and quantitation of the individual components. Gas chromatography using a capillary column and mass spectrometry were used in conjunction with peak enrichment studies with individual chlorinated biphenyls to achieve this objective. The identity and percentage composition of some of the individual components of Aroclor 1221 are shown in Table 2.

c. Metabolism of Aroclor 1221 by *Beijerinckia* B836. When *Beijerinckia* B836 was grown in the presence of succinate and Aroclor 1221 for 80 hours approximately 30% of the PCB mixture was metabolized. As can be seen in Table 3 the only components shown to undergo significant degradation were biphenyl, 2-, and 4-chlorobiphenyl. Degradation of 2,4-dichlorobiphenyl and 2,4'-dichlorobiphenyl was also observed. Metabolism of high chlorinated isomers was not observed. The same percentage metabolism of Aroclor 1221 was observed with the parent strain of *Beijerinckia*.

B. TECHNICAL REPORTS - None

C. PUBLICATIONS

1. Bacterial Metabolism of *para*- and *meta*-xylene: Oxidation of a Methyl Substituent
J.F. Davey and D.T. Gibson, J. Bacteriol. 119, 923-929, (1974).

TABLE 1

Physical Properties of the *cis*-dihydrodiols Produced by *Beijerinakia* B836 in the presence of Biphenyl and 4-, 3-, and 2-chlorobiphenyl

Substrate	Biphenyl	4-chlorobiphenyl	3-chlorobiphenyl	2-chlorobiphenyl
Melting Point	93° C	90° C	96-98° C	115° C
UV Absorbance (λ_{max}^{MeOH})	303nm = 13,600 223nm = 9,200	305nm = 13,700 229nm = 7,000	303nm = 12,900 230nm = 6,000	290nm = 11,600 222nm = 7,000
Mass Spectral Data: Calculated mass Found mass Number of chlorine	188.0831 188.0837 0	222.0451 222.0448 1	222.0451 222.0449 1	222.0451 222.0453 1
%Yield Spectrophotometric Estimate Actual Recovery	81% - -	59% 27% +251	71% 25% +251	13% 4% +173
Optical rotation of dihydro- ciol [δ] ₅₈₉ in methanol	-	-	-	-
PMR spectrum bands solvent hydroxyl hydroxymethine olefinic aromatic aromatic aromatic	chloroform 2H, 2.75 δ 2H, 4.5 δ 2H, 5.9 δ 5H, 7.4 δ - -	pyridine 2H, 6.53 δ 2H, 4.94 δ 2H, 6.20 δ 2H, 7.38 δ 2H, 7.74 δ -	pyridine 2H, 6.50 δ 2H, 4.92 δ 2H, 6.22 δ 2H, 7.27 δ 1H, 7.56 δ 1H, 7.88 δ	pyridine 2H, 6.80 δ 2H, 4.84 δ 2H, 6.26 δ 3H, 7.40 δ 2H, 7.77 δ -

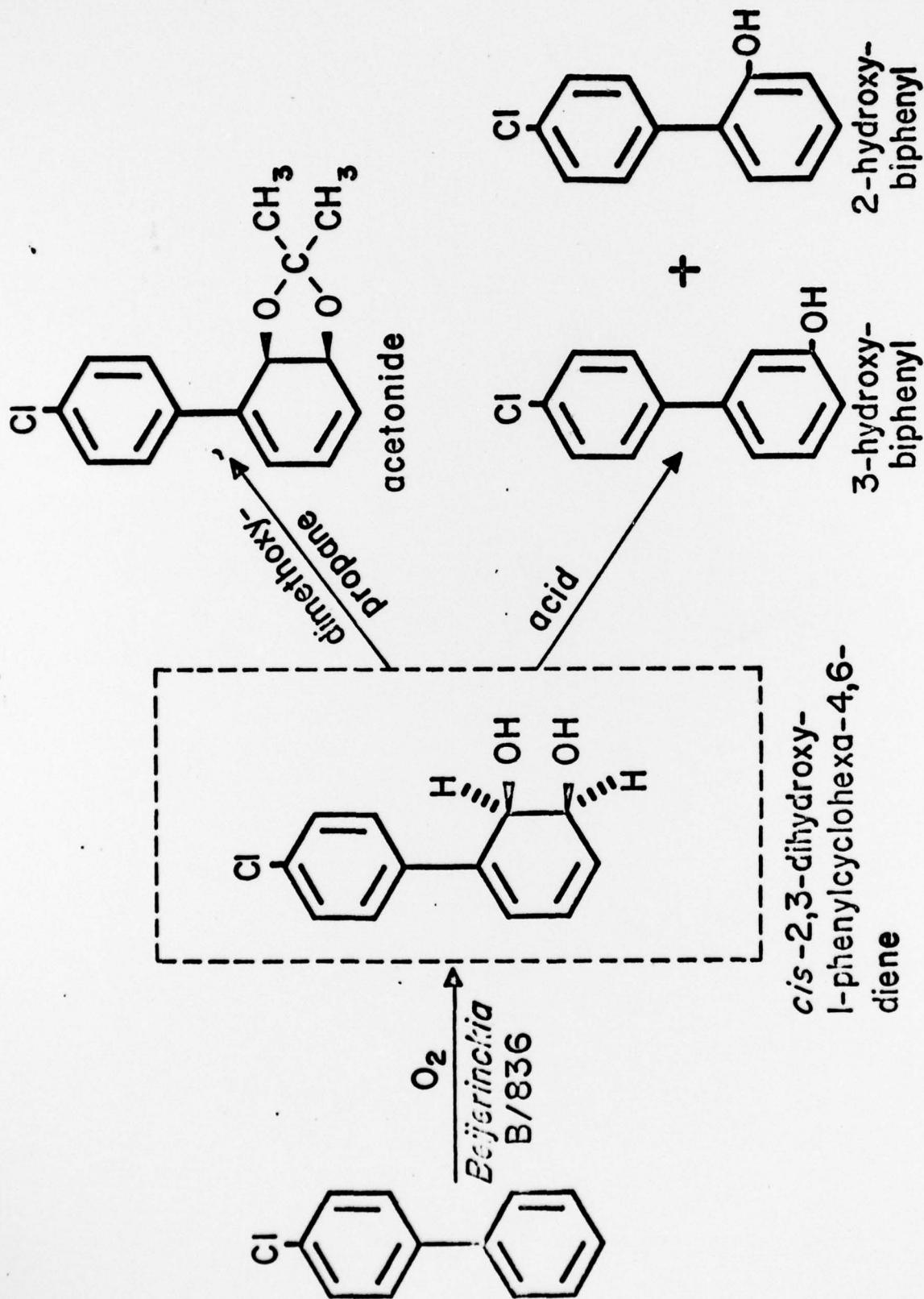


FIGURE 1. STRUCTURE OF THE *CIS*-DIHYDRODIOL FORMED FROM 4-CHLOROBIPHENYL BY *BEIJERINCKIA* B836. ACETONIDE AND PHENOL DERIVATIVES FORMED FROM THE METABOLITE ARE ALSO SHOWN.

FIGURE 2. Percent Conversion of Biphenyl and Monochlorobiphenyls to Diols By *Ecijerinclia* B/836

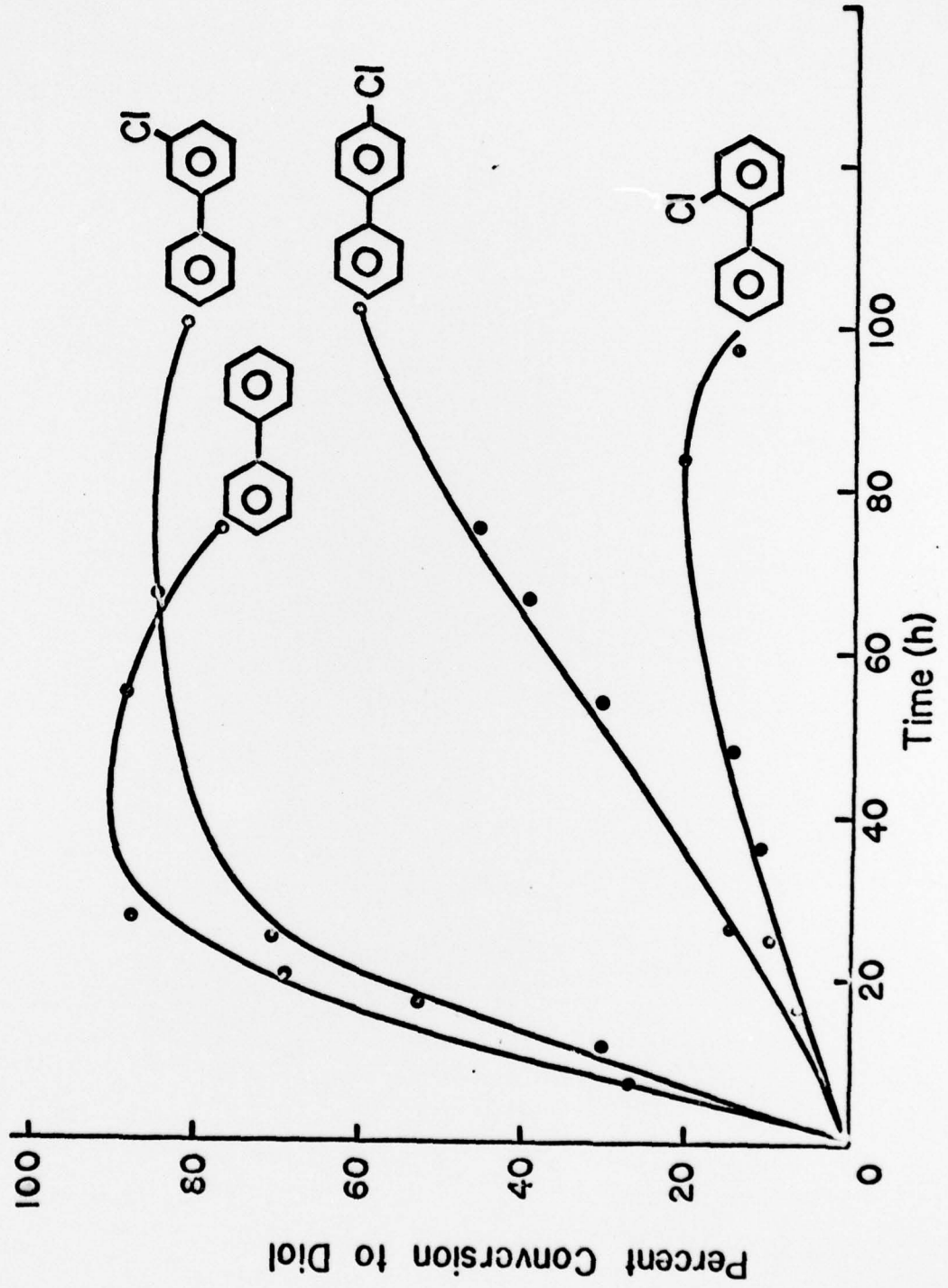


TABLE 2. Composition of Aroclor 1221

Peak Number ^a	Component	Percent in Aroclor 1221 ^b
1	biphenyl	13.2
2	2-chlorobiphenyl	35.6
3	3-chlorobiphenyl	1.7
4	4-chlorobiphenyl	19.4
5	2,6-dichlorobiphenyl	0.2
6	2,2'-dichlorobiphenyl	5.2
7	dichlorobiphenyl	1.2
8	dichlorobiphenyl	1.7
9	2,4-dichlorobiphenyl	2.8
10	2,4'-dichlorobiphenyl	11.6
11	2,3'-dichlorobiphenyl	< 0.1
12	trichlorobiphenyl	1.1
13	trichlorobiphenyl	0.5
14	3,4-dichlorobiphenyl	0.5
15	4,4'-dichlorobiphenyl	1.5
16	trichlorobiphenyl	0.7
17	trichlorobiphenyl	-
18	trichlorobiphenyl	0.5
19	trichlorobiphenyl	0.4
20	trichlorobiphenyl	0.4
21	trichlorobiphenyl	0.2
22	tetrachlorobiphenyl	< 0.2
23	tetrachlorobiphenyl	< 0.1
24	tetrachlorobiphenyl	< 0.1
25	tetrachlorobiphenyl	< 0.1
26	Unidentified	< 0.1
27	Unidentified	< 0.1

^a Numbers refer to peaks in gas chromatogram

^b Based on peak enrichment and GC-MS

TABLE 3. Oxidization of Aroclor 1221 by
Beijerinckia B836

Peak Number	Component	Percent of Component Oxidized
1	biphenyl	91%
2	2-chlorobiphenyl	18%
3	3-chlorobiphenyl	*
4	4-chlorobiphenyl	35%
5	2,6-dichlorobiphenyl	*
6	2,2'-dichlorobiphenyl	0
7	dichlorobiphenyl	0
8	dichlorobiphenyl	0
9	2,4'dichlorobiphenyl	~ 10%
10	2,4'-dichlorobiphenyl	~ 12%
11	2,3'-dichlorobiphenyl	*
12	trichlorobiphenyl	0
13	trichlorobiphenyl	0
14	3,4'-dichlorobiphenyl	0
15	4,4'-dichlorobiphenyl	0
16	trichlorobiphenyl	0
17	trichlorobiphenyl	0
18	trichlorobiphenyl	0
19	trichlorobiphenyl	0
20	trichlorobiphenyl	0
21	trichlorobiphenyl	0
22	tetrachlorobiphenyl	0
23	tetrachlorobiphenyl	0
24	tetrachlorobiphenyl	0
25	tetrachlorobiphenyl	0
26	Unidentified	0
27	Unidentified	0

* Amount of component present too small to quantitate accurately.

2. Bacterial Metabolism of *para* - and *meta*-xylene: Oxidation of the Aromatic Ring. D.T. Gibson, V. Mahadevan and J.F. Davey, J. Bacteriol. 119, 930-936 (1974).
3. XYL, a Nonconjugative Xylene-Degradative Plasmid in *Pseudomonas Pxy*. D.A. Friello, J.R. Mylroie, D.T. Gibson, J.E. Rogers and A.M. Chakrabarty, J. Bacteriol. 127, 1217-1224 (1976).
4. Microbial Degradation of Hydrocarbons, D.T. Gibson. In "Nature of Seawater". Physical and Chemical Sciences Research Report 1. E.D. Goldberg, ed., pp. 667-696 (1975).
5. Microbial Degradation of Carcinogenic Hydrocarbons and Related Compounds, D.T. Gibson. In "Sources, Effects and Sinks of Hydrocarbons in the Aquatic Environment". The American Institute of Biological Sciences, pp. 224-238 (1976).
6. Biodegradation of Aromatic Petroleum Hydrocarbons. D.T. Gibson, In, "Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms. D.A. Wolfe, ed., pp. 36-46 (1977).
7. Microbial Transformations of Aromatic Pollutants. D.T. Gibson, In, 2nd International Symposium on Aquatic Pollutants. Pergamon Press (in press).
8. Metabolism of Dibenzothiophene by a *Beijerinckia* species. A.L. Laborde and D.T. Gibson, Appl. Environ. Microbiol. (1977, in press).

D. CONCLUSIONS

Xylenes are simple aromatic hydrocarbons that are readily biodegradable under aerobic conditions. Microorganisms that degrade *meta*- and *para*-xylene are easily isolated from the environment and usually oxidize the methyl substituent of these molecules. This leads to the formation of toluic acids which are oxidized to methyl substituted catechols. The latter compound undergo enzymatic cleavage of the aromatic nucleus and the ring-fission products are converted to cell constituents, carbon dioxide and water. In contrast, organisms that degrade *ortho*-xylene are extremely rare. As far as we are aware the organism isolated during the period

of contract N0014-76-C-0102 is the first to be reported in terms of complete degradation of ortho-xylene.

The demonstration that the degradative pathway for *meta*- and *para*-xylene is specified by plasmid-borne genes is an important observation. Several catabolic sequences are now known to be carried by plasmids. This means that these organisms have the potential to exchange genetic information under environmental conditions thus increasing the biodegradative capacity of polluted environments. Also, it adds further information to the ongoing studies in genetic engineering where strains of multiple degradative capacity can be constructed.

Crude oil contains thousands of aromatic sulfur compounds. At this time little is known about the biodegradation of such molecules. The results obtained with dibenzothiophene suggest that microorganisms convert the parent molecule to water soluble aromatic sulfur derivatives. Although only one substrate was examined it is possible that the ability of microorganisms to completely degrade heterocyclic sulfur compounds is extremely limited and these molecules would then become candidates for environmental pollution.

Chlorinated biphenyls are subject to biodegradation if only one of the two aromatic nuclei contains chlorine substituents. Microorganisms that degrade such molecules produce chlorinated benzoic acids which can act as substrates for other organisms in the environment. Highly chlorinated biphenyl molecules appear to be resistant to microbial decomposition and will tend to accumulate in the environment. The only compounds in commercial polychlorinated biphenyls that are subject to significant biodegradation are the monochlorinated derivatives

E. MAJOR ACCOMPLISHMENTS

1. Elucidation of the pathways used by microorganisms for the degradation of *meta*-, and *para*-xylene.
2. Isolation and identification of the first organism known to degrade *ortho*-xylene. Elucidation of the pathway of *ortho*-xylene degradation.

3. Demonstration that the genetic information for the degradation of *meta*- and *para*-xylene is carried on a plasmid (extrachromosomal DNA).
4. Isolation and identification of the metabolic products formed from 2-, 3- and 4-chlorobiphenyl.
5. Identification of the components in Arochlor 1221 by capillary column gas chromatography and mass spectrometry.
6. Demonstration of the microbial metabolism of the lower chlorinated isomers in a commercial polychlorinated biphenyl mixture.