Microbial Metabolism of Polychlorinated Biphenyls. Studies on the Relative Degradability of Polychlorinated Biphenyl Components by *Alkaligenes* sp.

Kensuke Furukawa and Fumio Matsumura*

A bacterial strain belonging to the genus *Alkaligenes* was isolated from a lake sediment by using biphenyl as a sole carbon source. Metabolic degradation of various polychlorinated biphenyls (PCB) by this organism was studied. The bacterium was capable of metabolizing various PCB components, including a highly chlorinated one such as 2,4,5,2',5'-pentachlorobiphenyl, through an oxidative route. The organism appears to preferentially degrade the less chlorinated (ring II) of the two rings. Degradation takes place in two major steps. The first step produces metabolic intermediates which are considered to be chlorinated derivatives of 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid, which often has a yellow color with an absorption around 400 nm. In the second step these colored metabolic intermediates are degraded to corresponding chlorobenzoic acids. Among the colored intermediates, the ones derived from PCB components with a chlorine substitution at the 4' position on ring II particularly exhibited a long lasting yellow color indicating the stability of the intermediates. Among chlorinated benzoic acids, meta chloro substitutes derived from PCB components with a 3- or 3'-chlorine substitution appeared to be further metabolized by this organism. PCB components with all the chlorines only on one ring degraded relatively fast as compared to those possessing an equivalent number of chlorines on both rings. Generally speaking, degradation of PCBs becomes increasingly difficult as the degree of chlorination increases.

Polychlorinated biphenyls (PCBs) have been shown to be serious environmental contaminants. They are a very complex and heterogeneous series of chemical mixtures, and are known to accumulate in biological systems, particularly in aquatic ecosystems. Despite the importance of PCBs in the field of environmental toxicology, not much is known about the metabolic fates of these chemicals in the environment.

Ahmad and Focht (1973) studied the pattern of degradation of several monochloro- and dichlorobiphenyls in two species of *Achromobacter* and found that chlorinated benzoic acids formed as a result of oxidative degradation activities on these PCB components. These *Achromobacter* species were isolated from sewage effluent by using biphenyl and *p*-chlorobiphenyl as sole carbon sources. Based upon previous studies on oxidative ring opening mechanisms on aromatic compounds (e.g., Catelani et al., 1973, on biphenyl) these workers hypothesized that such an oxidative metabolic process proceeds through the formation of a catechol intermediate on one of the rings and then a muconic acid.

In the present study we have made attempts to further investigate the nature of such metabolic activities in microbial species by using many other PCB components including commercially used PCB preparations. The major purpose of the study was to establish the relative rates of degradability among the PCB components, and thereby assess the change in PCB compositions in the environment.

MATERIALS AND METHODS

Method of Isolation of Microorganism. The organism was obtained by preparing an enrichment culture from an aquatic sediment sample at Lake Mendota (Wisconsin). Such an enrichment was set up by adding 1–2 g of aquatic sediments to a 250-ml flask which contained 100 ml of an organic medium (medium A): glucose, 0.5 g; peptone, 0.5 g; KH_2PO_4, 0.5 g; distilled water, 1 l. (pH 7.0). This was then placed on a rotary shaker at 250 rpm, and was incubated for 3 days at 26°C. Isolation was carried out in a second 250-ml flask which contained 100 ml of a mineral salt solution (medium B): $(NH_4)_2SO_4$, 1 g; KH_2PO_4, 0.2 g; K_2HPO_4, 1.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; NaCl, 0.1 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; $CaCl_2 \cdot 2H_2O$, 0.02 g (pH 7.5). This was autoclaved, and 0.1 g of well-ground biphenyl was added to the medium. An inoculum of the enrichment culture (1 ml) was then transferred to this second flask. It was then placed likewise on a rotary shaker and incubated at 26°C until good growth was observed.

To isolate the organism an inoculum of the above liquid culture was streaked on the surface of the medium B agar prepared with excessive biphenyl in petri dishes. After incubation several yellowish single colonies were observed. A portion of a single colony was picked up and subcultured in medium B to yield a pure culture. Occasionally penicillin (20 to 30 units per ml) was added to the culturing medium to avoid contamination by other organisms.

Incubation and Extraction Method. After the organism grew, the culture fluid was filtered through glass wool once to remove the remaining solid biphenyl, and the cells were harvested by centrifugation at 8000*g* for 10 min, and washed twice with 0.05 *M* phosphate buffer (pH 7.0). The washed cells were suspended (to make optical density = 2.2 at 680 nm) in the same buffer containing a PCB component or Aroclor mixture (commercially used PCB mixture). The incubated mixture was shaken on the rotary shaker. After incubation for a predetermined period, the reaction mixtures were acidified with concentrated HCl to make pH 1–2, and were extracted twice with ethyl acetate or ethyl ether. For purification of the yellow, metabolic intermediates of PCBs, an equal amount of 2 N NaOH was added to the ethyl acetate layer. The yellow color was found to transfer to the aqueous alkali phase. The water layer was then acidified with concentrated HCl, and reextracted with fresh ethyl acetate. The ethyl acetate phase containing the yellow metabolic products was concentrated and applied to thin-layer chromatography (TLC).

Spectrophotometric Measurements. A Varian Techtron spectrophotometer, Model 635, was used for all uv–visible spectrophotometric measurements. A Finnigan GC/MS Model 1015 was used for mass spectrometric studies. Infrared spectra were obtained on KBr pellets of purified compounds by using a Beckman IR-33 spectrophotometer.

Chromatographic Techniques. Silica gel HF thin-

Department of Entomology, University of Wisconsin, Madison, Wisconsin 53706.

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layer plates (0.25 mm thick activated at 100°C for 2 h) were used for all analytical thin-layer chromatography (TLC). For preparative TLC, plates with 0.5 mm thickness of the same silica gel were used. Solvent systems used for analysis of metabolites were *n*-hexane–ethanol–acetic acid, 10:2:1 or 5:2:1. For ^{14}C-labeled PCB components autoradiograms were prepared by exposing the developed TLC plates on x-ray films for 2 to 4 weeks. Gas chromatographic (GC) analysis was performed on a Model 5750 F&M Scientific, Hewlett-Packard equipped with a ^{63}Ni-electron capture detector. The column used in analytical work was an SE-30 (at 3% on Chromosorb G, 80–100 mesh) packed in 1.8 m × 3 mm i.d. glass column. The column operating conditions were: injection temperature, 250°C; column temperature, 200°C; detector temperature, 240°C; argon–methane (95:5) carrier gas flow rate at 45 ml/min.

Chemicals. 2-Monochlorobiphenyl; 2,2'-, 2,3'-, 2,4'-, 3,3'-, 4,4'-, 2,4-, 2,5-, 2,6-, and 3,4-dichlorobiphenyl; 2,5,2'-, 2,5,3'-, 2,5,4'-, and 2,4,4'-trichlorobiphenyl; 2,3,2',3'-, 2,-4,2',4'-, and 2,4,2',5'-tetrachlorobiphenyl; and 2,4,5,2',5'-pentachlorobiphenyl were obtained from Analabs, Inc. (North Haven, Conn.). Biphenyl and 3-monochlorobiphenyl were obtained from *Pfaltz & Bauer, Inc. (Flushing, N.Y.).* Aldrich Chemical Co. (Milwaukee, Wis.) was the source of 4-monochlorobiphenyl and 2,5-dichlorobenzoic acid. Aroclor 1242, 1254, and 1260 were obtained from Monsanto Co. (St. Louis, Mo.). 2,5,2'-Trichlorobiphenyl-^{14}C (2'-chlorophenyl-*U*-^{14}C) and 2,4,5,2',5'-pentachlorobiphenyl-^{14}C (2,4,5-trichlorophenyl-*U*-^{14}C) were purchased from California Bionuclear Co. (Sun Valley, Calif.).

RESULTS

The organism isolated by the enrichment method with biphenyl as a sole carbon source was a gram-negative rod, motile with one to two subpolar "degenerately peritrichous" flagella, and was identified as belonging to the genus *Alkaligenes*. The microbiological properties of this organism will be reported elsewhere. This organism is designated as *Alkaligenes* Y-42 in the present paper.

Metabolism of 2,5,2'-Trichlorobiphenyl-^{14}C and 2,4,5,2',5'-Pentachlorobiphenyl-^{14}C by Y-42 Strain. More than 20 strains of stock cultures, including some *Pseudomonas* sp., and isolates from soil were investigated for the metabolism of 2,4,5,2',5'-pentachlorobiphenyl-^{14}C (0.1 μCi, 3.3 μg). None of them were found to be capable of metabolizing these PCB components. *Alkaligenes* Y-42 was, however, found to be capable of metabolizing both 2,5,2'-trichlorobiphenyl-^{14}C (0.1 μCi, 2.6 μg) and 2,4,5,-2',5'-pentachlorobiphenyl-^{14}C (0.1 μCi, 3.3 μg). After incubating for 20 h, the reaction mixture was centrifuged to separate the cells and the supernatant. They were separately extracted by ether, and developed on TLC plates. Autoradiographic results of the TLC plate showed some spots other than the starting material, representing metabolic products derived from tri- and pentachlorobiphenyl-^{14}C (Figure 1). A large amount of radioactivity was recovered in the supernatant rather than in the cell fraction. Under the experimental conditions the original 2,5,2'-trichlorobiphenyl-^{14}C disappeared completely, but a large amount of original 2,4,5,2',5'-pentachlorobiphenyl-^{14}C remained, indicating that the degradation of pentachlorobiphenyl was much slower than that of trichlorobiphenyl in the Y-42 strain. These metabolites were scraped from the TLC plate and the radioactivity in each R_f position was measured. Table I shows the radioactivity and percentages of each metabolite in relation to the total radioactivity recovered. Almost all the radioactivity (87.8%) was observed in the region designated as M-3 (see Figure 1) in the case of ^{14}C-labeled 2,5,2' metabolism. On the other hand, in the case of ^{14}C-labeled 2,4,5,2',5' metabolism 59.5% of the radioactivity was found in M-3 and 32.7% in M-1.

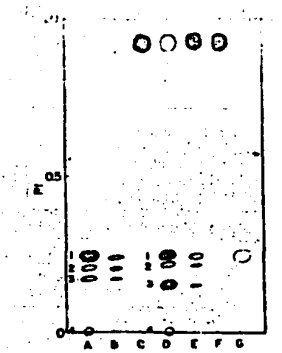

Figure 1. Representation of the autoradiograms of metabolites from 2,5,2'-trichlorobiphenyl-^{14}C and 2,4,5,2',5'-pentachlorobiphenyl-^{14}C as separated on a silica gel HF thin-layer chromatogram (TLC). The TLC plate was developed by using *n*-hexane–ethanol–acetic acid (10:2:1). Metabolic products were arbitrarily designated as M-1, -2, -3, and -4 according to the position on the chromatogram (also see Table I): (A) 2,5,2'-trichlorobiphenyl-^{14}C supernatant extract; (B) 2,5,2'-trichlorobiphenyl-^{14}C cell extract; (C) 2,5,2'-trichlorobiphenyl-^{14}C; (D) 2,4,5,2',5'-pentachlorobiphenyl-^{14}C supernatant extract; (E) 2,4,5,2',5'-pentachlorobiphenyl-^{14}C cell extract; (F) 2,4,5,2',5'-pentachlorobiphenyl-^{14}C; (G) 2-chlorobenzoic acid (detected under uv light).

Table I. Radioactivity and Percentages of Metabolites from 2,5,2'-Trichlorobiphenyl-^{14}C and 2,4,5,2',5'-Pentachlorobiphenyl-^{14}C[a]

Metabolites	Radioact. found, cpm	% of total
2,5,2'-Trichlorobiphenyl-^{14}C		
M-1	3 831	87.8
-2	163	3.7
-3	356	8.2
-4	11	0.3
Total	4 361	100
2,4,5,2',5'-Pentachlorobiphenyl-^{14}C		
M-1	3 874	32.7
-2	766	6.5
-3	7 054	59.5
-4	149	1.3
Total	11 843	100

[a] Each metabolite from 2,5,2'-trichlorobiphenyl-^{14}C supernatant extract and 2,4,5,2',5'-pentachlorobiphenyl-^{14}C supernatant extract was scraped and the radioactivity was measured. Metabolite numbering corresponds to the numbering of spots in Figure 1.

Action of Y-42 Strain on Aroclor 1242. The Y-42 strain was incubated in the mineral salt medium containing 2-, 3-, or 4-monochlorobiphenyl as a sole carbon source. Growth was slow, however. In spite of the slow growth, this organism was capable of cometabolizing various PCB components. The washed cells were incubated with Aroclor 1242 (100 μg) for 20 h, and the whole mixture was extracted with ethyl acetate as described under Incubation and Extraction Method. The ethyl acetate solution was

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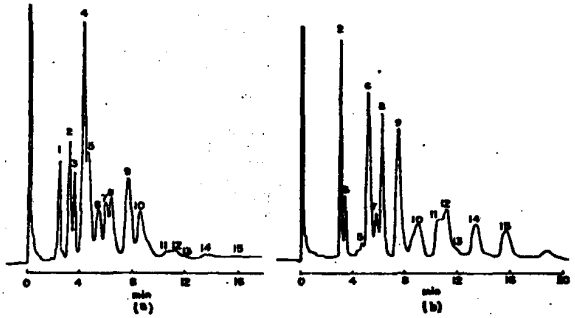

Figure 2. Changes of gas chromatographic pattern of Aroclor 1242 after incubation. Analyzed on an SE30 (3 mm x 1.7 m) column by using a ^{63}Ni electron capture detector: (a) Aroclor 1242 standard (before incubation); (b) ethyl acetate extract after 20 h incubation.

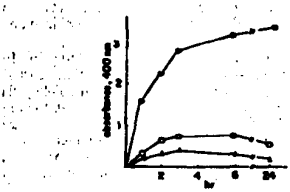

Figure 3. The rate of yellow color production from Aroclor 1242, 1254, and 1260 by Y-42 strain. The reaction mixture was centrifuged after incubation for the indicated periods, and the yellow color of the supernatant was measured at 400 nm: (•) Aroclor 1242; (○) Aroclor 1254; (▲) Aroclor 1260.

then analyzed on the gas chromatograph with an EC detector. The result is shown in Figure 2. The gas chromatographic pattern of the extract after incubation was greatly different from standard Aroclor 1242; the two main peaks of Aroclor 1242, peaks 1 and 4 (see Figure 2), disappeared and peaks 3, 5, and 7 were greatly decreased. On the other hand, the relative intensities of other peaks such as 2, 6, and 8–15 were increased. A bright yellow colored product with an absorption maximum at 398 nm appeared during the incubation period. Aroclor 1254 and Aroclor 1260 were incubated in the same manner, and the production of a yellow color was measured at 400 nm. The production of yellow color occurred in the following order: Aroclor 1242 >> Aroclor 1254 > Aroclor 1260 (Figure 3). The results of the experiments shown in Figure 2 and Figure 3 led us to confirm that Y-42 strain degrades PCB components differently depending on the numbers of chlorines and their position.

Decomposition and the Metabolism of 2-, 4,4'-, 2,5-, 2,4,4'-, 2,5,4'-, and 2,4,5-Chlorobiphenyl. 2-Monochloro-, 4,4'- and 2,5-dichloro-, 2,4,4'-, 2,5,4'-, and 2,4,5-trichlorobiphenyl were used for further detailed studies. Two hundred micrograms each of these compounds was incubated with washed *Alkaligenes* Y-42 cells in 20 ml of 0.05 M phosphate buffer (pH 7.0) with shaking. Efforts were made to control the number of cells constant per tube by adjusting the cellular preparation to give an optical density of 2.2 at 660 nm. The yellow color appeared in all

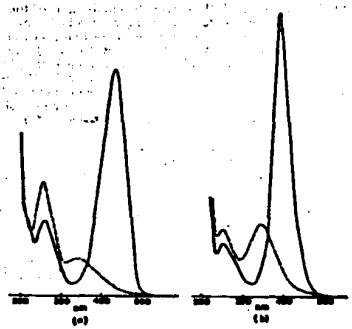

Figure 4. The absorption spectra of yellow compounds from 2,5,4'-trichlorobiphenyl and 4,4'-dichlorobiphenyl: (a) yellow compound from 4,4'-dichlorobiphenyl; (b) yellow compound from 2,5,4'-trichlorobiphenyl; in alkali (–); in acid (- - -).

reaction mixtures to varying extents and it generally intensified with time. The color faded in 2-mono-, 2,5-di-, and 2,4,5-trichlorobiphenyl upon further incubation, but such fading did not occur in the incubates where 2,4,4'- or 2,5,4'-trichlorobiphenyl were the substrate. Figure 4 shows the absorption spectra of the purified yellow compounds derived from 4,4'-di- and 2,5,4'-trichlorobiphenyl. Absorption maxima were 434 nm in 4,4'-dichloro- and 399 nm in 2,5,4'-trichlorobiphenyl at neutral and alkaline pH. The yellow color disappeared in acidic pH (pH 1–2) and the absorption maxima shifted to around 340 nm in both compounds. The yellow compounds from other PCB components had the absorption maxima between 398 and 415 nm in neutral and alkaline pH. After incubation for 20 h, the reaction mixtures were acidified and extracted twice with ethyl acetate. The concentrated ethyl acetate extracts were then subjected to TLC (Figure 5). Dark main spots which correspond to the R_f value (0.33) of chlorobenzoic acids (2-chloro- and 2,5-dichlorobenzoic acid) were detected under uv light from the extracts of 2-

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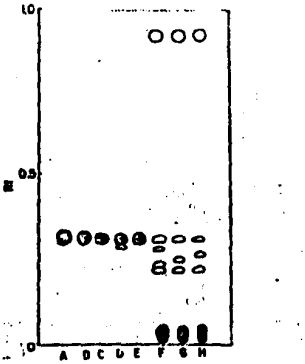

Figure 5. Silica gel HF thin-layer chromatograms of the incubates containing metabolites from certain PCB isomers. Solvent system used was *n*-hexane–ethanol–acetic acid (5:2:1). Each spot was detected under uv light, except yellow compounds (indicated by hatch marks): (A) 2-chlorobenzoic acid; (B) 2,5-dichlorobenzoic acid; (C) 2-chlorobiphenyl extract; (D) 2,5-dichlorobiphenyl extract; (E) 2,4,5-trichlorobiphenyl extract; (F) 2,5,4'-trichlorobiphenyl extract; (G) 4,4'-dichlorobiphenyl extract; (H) 2,4,6'-trichlorobiphenyl extract.

monochloro- and 2,5-dichlorobiphenyl. A spot with an identical R_f also appeared in the extract of the incubation products from 2,4,5-trichlorobiphenyl. On the other hand, four or five spots were observed in the extracts of 4,4'-di-, 2,4,4'-, and 2,5,4'-trichlorobiphenyl. One of the spots from these three compounds had the identical R_f value (0.33) with chlorobenzoic acid. It was observed that the bright yellow spots had some tailing to the origin of the chromatograms as shown in Figure 5. These yellow colored compounds were scraped from the TLC plate, extracted with ethyl acetate, and stocked for further analytical purposes. The ethyl acetate extracts from 2-mono-, 4,4'-di-, and 2,4,5-trichlorobiphenyl corresponding to each chlorobenzoic acid on TLC were subjected to mass spectrometry. The mass spectra of the metabolites from 2-mono- and 4,4'-dichlorobiphenyl showed a parent peak at m/e 156, and the intensity of the peak corresponding to the parent + 2 (35%) m/e indicated the presence of a single chlorine atom in the molecule. The base peaks at m/e 139 and 141 (3:1 ratio) were diagnosed to represent the $M^+ - OH$ fragment, and those at m/e 111 and 113 (3:1) represent the $M^+ - COOH$ fragment. The mass spectrum of the metabolite of 2,5-dichlorobiphenyl showed a parent ion peak at m/e 190, and the ratio of parent ion:parent + 2:parent + 4 was about 10:7:1, indicating the presence of two chlorine atoms in the molecule. The base peaks at m/e 173, 175, and 177 occurring in the ratio of 10:7:1 indicated fragmentation of –OH, and those at m/e 145, 147, and 149 (10:7:1) indicated fragmentation of –COOH. The mass spectrum of 2,4,5-trichlorobiphenyl metabolite showed a parent peak at 224. The ratio of parent/parent + 2/parent + 4/parent + 6 was 100:95:30:4, indicating the presence of three chlorine atoms in the molecule: the major peaks at m/e 207, 209, 211, and 213 (100:98:30:4) and at m/e 179, 181, 183, and 185 (100:98:30:4) indicating the fragmentation of –OH and –COOH, respectively. The infrared spectra of these metabolites were identical with authentic 2-chlorobenzoic acid for 2-chlorobiphenyl metabolite, 4-chlorobenzoic acid for 4,4'-dichlorobiphenyl metabolite, 2,5-dichlorobenzoic acid for 2,5-dichlorobiphenyl metabolite, and 2,4,5-trichlorobenzoic acid from 2,4,5-trichlorobiphenyl.

The yellow compounds obtained from the metabolites of 4,4'-, 2,4,4'-, and 2,5,4'-chlorobiphenyl were methylated with diazomethane in ether and subjected to mass spectrometry. Although the parent ion peaks of these yellow compounds were not observed, some fragment ions were recognized. The yellow compound from 4,4'-dichlorobiphenyl showed peaks at m/e 139 and 141 as well as 111 and 113 (both at 3:1 ratios). These fragments can be considered as $C_6H_4ClCO^+$ and $C_6H_4Cl^+$. The yellow compounds from 2,4,4'- and 2,5,4'-trichlorobiphenyl showed peaks at m/e 173, 175, and 177 (10:7:1) and m/e 145, 147, and 149 (10:7:1), indicating that these fragments were $C_6H_2Cl_2CO^+$, and $C_6H_2Cl_2^+$, respectively.

The infrared spectrum of the yellow metabolic product (see Figure 5) on 2,4,4'-trichlorobiphenyl showed the presence of peaks at 3400–3600 cm^{-1} (weak) for –OH, 1730 cm^{-1} (strong) for ketonic carbonyl, 1690 cm^{-1} (medium) for α,β-unsaturated carbonyl of acid, and 1000, 1478, and 1420 cm^{-1} (all medium) for phenyl C—C skeletal in-plane vibrations.

Decomposition of Various PCBs by Y-42 Strain. In order to assess the ease of microbial degradation 21 PCB components (200 μg each) were incubated with Y-42 cells and the rate of color production was measured at 400 nm (at 434 nm for 4,4'-dichlorobiphenyl). These results are shown in Figure 6. The yellow color production was hardly recognized from three monochlorobiphenyls, 2,2'-, 2,3'-, 3,3'-, 2,6-, and 3,4-di-, 2,4,2',4'- and 2,4,2',5'-tetra-, and 2,4,5,2',5'-pentachlorobiphenyl. On the other hand, with 2,5,2'- and 2,5,3'-tri- or 2,3,2',3-tetrachlorobiphenyl as a substrate, a small level of color production was observed. A strong yellow color was produced from 2,4- and 2,5-di- and 2,4,5-trichlorobiphenyl, but the color markedly declined, and it almost disappeared in 7 h in 2,4-di- and 2,4,5-trichlorobiphenyl as shown in Figure 6. On the other hand, the bright yellow color produced from 2,4'-di-, 4,4'-di-, 2,5,4'-tri-, and 2,4,4'-trichlorobiphenyl did not fade and rather intensified in the case of 2,5,4'- and 2,4,4'-trichlorobiphenyl during the incubation period (7 h). After 7 h incubation, the reaction mixtures were acidified and extracted with ethyl acetate twice. The ethyl acetate extracts were then subjected to GC analyses for the determination of original PCB residues. Levels of 80–85% recovery of some PCBs were obtained from the control tubes which were extracted immediately after the addition of PCB to the organism. The decomposition ratio of various PCBs was calculated from the results of GC analysis as shown in Table II. No original PCBs were detected by GC from three monochlorobiphenyls (2-, 3-, and 4-), 2,3'-, 2,4'-, 3,3'-, 2,4-, 2,6-, and 3,4-dichlorobiphenyl, 2,4,5- and 2,4,4'-trichlorobiphenyl. These compounds were completely degraded in 7 h by Y-42 strain. 2,2'- and 2,4-di- and 2,5,3'-trichlorobiphenyl were also easy to degrade, although a small amount of residue was detected after 7 h. A 20–30% portion of original compound was retained as 4,4'-di- or 2,5,4'- or 2,5,2'-trichlorobiphenyl, while about 50% of 2,3,2',3'-tetrachlorobiphenyl was degraded. However, the degradations of two other tetrachlorobiphenyls, 2,4,2',4'- and 2,4,2',5'-, and 2,4,5,2',5'-pentachlorobiphenyl were very slow.

The ethyl acetate extracts of 7-h incubates were methylated by using diazomethane, and applied to GC to determine the amount of chlorobenzoic acid formed in

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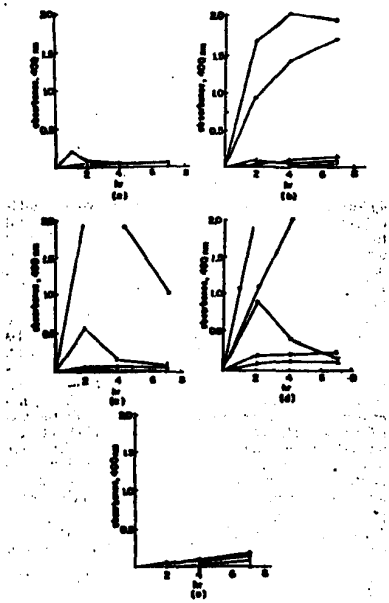

Figure 6. Patterns of the production of yellow colored compounds from various PCB compounds. The color production was measured at 400 nm except for 4,4'-dichlorobiphenyl which was measured at 434 nm: (a) monochlorobiphenyl: (○) 2-; (△) 3-; (X) 4-; (b) dichlorobiphenyl: (X) 2,2'-; (△) 2,3'-; (○) 2,4'-; (◇) 3,3'-; (○) 4,4'-; (c) dichlorobiphenyl: (△) 2,4-; (○) 2,5-; (○) 2,6-; (X) 3,4-; (d) trichlorobiphenyl: (○) 2,4,4'-; (△) 2,5,3'-; (X) 2,5,3'-; (○) 2,5,4'-; (○) 2,4,5-; (◇) tetra- and pentachlorobiphenyl: (○) 2,3,3',5'-; (○) 2,4,2',4'-; (X) 2,4,2',5'-; (△) 2,4,5,3',5'-.

relation to other metabolites. Monochlorobenzoic acid was detected in the extracts of 2-mono-, 4-mono-, 2,2'-, 2,4'-, and 4,4'-dichlorobiphenyl incubates with the amounts shown in Table II. A small peak of monochlorobenzoic acid was also observed in 2,5,2'-, 2,5,3'-, 2,4,4'-, and 2,5,4'-trichlorobiphenyl incubates. Dichlorobenzoic acid was determined in 2,4-, 2,5-, and 3,4-di-, 2,5,2'-, 2,5,3'-, 2,4,4'-, and 2,5,4'-tri-, and 2,3,2',3'-tetrachlorobiphenyl. Trichlorobenzoic acid was determined in 2,4,5-trichlorobiphenyl extract. However, no chlorobenzoic acid was detected in the extracts from 3-mono- and 2,3'-, 3,3'-di-, and 2,6-dichlorobiphenyl incubates, despite the complete degradation of these substances by the microorganisms. Some other peaks (with retention times (t_R) of 1.60, 2.72, and 3.12 min) besides that for monochlorobenzoic acid (t_R 0.96 min) were detected in the extracts of 2,2'-, 3,3'-, 2,4'-, and 4,4'-dichlorobiphenyl incubates. The identities of these metabolites have not been established.

DISCUSSION

We may be able to divide PCB components into four groups according to the pattern of degradation by Y-42 strain.

Table II. Degradation of Various PCB Isomers and Formation of Yellow Compounds and Chlorobenzoic Acids

PCB components	% of starting material remaining after 7 h	Yellow compd formation	Chlorobenzoic[a] acid formation, μg
2-	0	+	167
3-	0	+	0
4-	0	+	147
2,2'-	2	+	41
2,3'-	0	+	0
2,4'-	0	++++	91
3,3'-	0	+	0
4,4'-	16	++++	46
2,4-	0	++	128
2,5-	2	++++	161
2,6-	0	−	0
3,4-	0	−	74
2,4,5-	0	+++	++++
2,5,2'-	22	+	9
2,5,3'-	2	++	12
2,5,4'-	0	++++	58
2,4,4'-	0	+++++	74
2,3,2',3'-	45	+	29
2,4,2',4'-	88	+	1
2,4,2',5'-	96	−	2
2,4,5,3',5'-	86	−	0

[a] In each case 200 μg of the starting PCB component was incubated with Y-42 washed cells for 7 h.

The first group consists of 2-, 3-, 4-, 2,2'-, 2,3'-, 3,3'-, 2,6-, and 3,4-chlorobiphenyl. These compounds are easily decomposed to monodichlorobenzoic acids by the organisms. Yellow compounds are probably the intermediates to produce chlorobenzoic acids. However, the reaction speed is so quick that the yellow color is not clearly recognized during the incubation. Failure to detect chlorobenzoic acid in 3-mono- and 2,3'-, 3,3'-, and 2,6-dichlorobiphenyl extracts may indicate that these chlorobenzoic acids are degraded faster than they are produced by the microorganisms. Biphenyl may also be included in this group. Except for 2,6-dichlorobiphenyl these compounds have a 3-(or 3'-)chlorophenyl in common. Thus it is likely that *m*-chlorobenzoic acid (i.e. 3-chlorobenzoic acid) is further degraded by this organism.

The second group consists of 2,4-, 2,5-, and 2,4,5-chlorobiphenyl. The yellow colored compounds appear as intermediates and become maximum in a very early stage of incubation and the color fades gradually upon further incubation. The compounds of this group are also decomposed easily to accumulate dichloro- or trichlorobenzoic acids.

The third group consists of 2,4'- and 4,4'-di- and 2,5,4'- and 2,4,4'-trichlorobiphenyl. In this group, the yellow color appears very strongly and the color does not fade even after a long incubation indicating the stability of these metabolic intermediates. It should be noted that the compounds of this group have 4'-chlorophenyl in common in the molecule. The presence of such a moiety must prevent the yellow compound from being readily metabolized to chlorobenzoic acids.

The compounds belonging to the fourth group are 2,-3,2',3'-, 2,4,2',4'-, and 2,4,2',5'-tetra- and 2,4,5,2',5'-pentachlorobiphenyl. As in the case with many other highly chlorinated biphenyls these analogues are generally considered to be very resistant to microbial degradation. Y-42 strain, however, is able to degrade 2,3,2',3'-tetra- and even 2,4,5,2',5'-pentachlorobiphenyl, although the rate is very slow.

There are a few compounds which are difficult to

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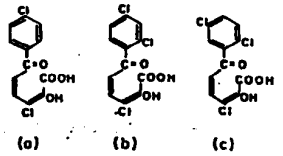
classify: 2,5,2'- and 2,5,3'-trichlorobiphenyl should be included in the first group, but the degradation rate is much slower than for other compounds in this group. Also, a slight yellow color is observed during the incubation period, thus making them a little different from the rest of the components of the first group.

There have been some reports to indicate the chemical nature of the compounds which have a yellow color with absorption maxima around 400 nm in neutral and basic pH and 320 nm in acidic pH. Dagley et al. (1960) reported that the intermediate derived from microbial degradation of catechol has a bright yellow color with absorption maxima at 373 nm in neutral and alkaline pH and 317 nm in acidic pH. The compounds were produced by cleavage of catechol by catechol 2:3-dioxygenase and identified as α-hydroxymuconic semialdehyde. Lunt and Evans (1970) reported that gram-negative bacteria produced α-hydroxy-β-phenylmuconic semialdehyde [mp 122°C, λ_{max} 350 nm (acid) and 430 nm (alkali)] from biphenyl.

Catelani et al. (1973) revealed that an oxidative metabolic process of biphenyl in *Pseudomonas putida* proceeds through formation of catechol-type intermediates on one of the rings and then a derivative of muconic semialdehyde (2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoate). There are other excellent works on the metabolic pathways of aromatic compounds (Gibson, 1968) and halogenated aromatics (Smith et al., 1968) via formation of catechols (Dagley and Gibson, 1965) which indicate that the ring opening takes place by the formation of muconic acid analogs which often exhibit yellow color. These facts suggest that yellow colored compounds derived from aromatic compounds with absorption maxima around 400 nm in neutral and alkaline pH are indeed α-hydroxymuconic acid or semialdehyde derivatives. The keto forms have absorption maxima around 320–350 nm. Our mass spectrometric data also support this view inasmuch as the fragmentations corresponding to C_6H_4ClCO or $C_6H_3Cl_2CO$ were derived from the yellow compounds of 4,4'-di- and 2,4,4'- and 2,5,4'-trichlorobiphenyl. The infrared spectra of 2,4,4' and 2,5,4' derived metabolites indicate the presence of ketonic carbonyl, hydroxyl, and carboxylic acid moiety in the molecule. The compounds thus appear to be the chlorinated derivatives of α-hydroxymuconic acid with structures a, b, and c (a) (from



4,4'-dichlorobiphenyl) 3-chloro-2-hydroxy-6-oxo-6-(4-chlorophenyl)hexa-2,4-dienoic acid (or sometimes referred to as α-hydroxy-β-chloro-6-(4-chlorophenyl)muconic acid); (b) (from 2,4,4'-trichlorophenyl) 3-chloro-2-hydroxy-6-oxo-6-(2,4-dichlorophenyl)hexa-2,4-dienoic acid; (c) (from 2,5,4'-trichlorophenyl) 3-chloro-2-hydroxy-6-oxo-6-(2,5-dichlorophenyl)hexa-2,4-dienoic acid.

These results clearly indicate that microorganisms are likely to degrade the less chlorinated members of PCBs first, leaving highly chlorinated PCBs in the environment. Among different PCB preparations less chlorinated ones (e.g., Aroclor 1242) degrade faster than the highly chlorinated preparations such as Aroclor 1260. Also, microorganisms appear to preferentially degrade the less chlorinated of the two aromatic rings. It would be of great interest and concern for environmental toxicologists to see whether there will be such a change in the residue composition of PCBs in nature in the next few years eventually leading to accumulation of very stable polychlorobiphenyls.

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