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Degradation of Polychlorinated Biphenyl Mixtures (Aroclors 1242, 1254, and 1260) by the White Rot Fungus *Phanerochaete chrysosporium* as Evidenced by Congener-Specific Analysis

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Evidence for substantial degradation of polychlorinated biphenyl mixtures Aroclor 1242, 1254, and 1260 by the white rot fungus *Phanerochaete chrysosporium*, based on congener-specific gas chromatographic analysis, is presented. Maximal degradation (percent by weight) of Aroclors 1242, 1254, and 1260 was 60.9, 30.5, and 17.6%, respectively. Most of the congeners in Aroclors 1242 and 1254 were degraded extensively both in low-N (ligninolytic) as well as high-N (nonligninolytic) defined media. Even more extensive degradation of the congeners was observed in malt extract medium. Congeners with varying numbers of *ortho*, *meta*, and *para* chlorines were extensively degraded, indicating relative nonspecificity for the position of chlorine substitutions on the biphenyl ring. Aroclor 1260, which has not been conclusively shown to undergo aerobic microbial degradation, was shown to undergo substantial net degradation by *P. chrysosporium*. Maximal degradation of Aroclor 1260 was observed in malt extract medium (18.4% on a molar basis), in which most of the individual congeners were degraded.

Polychlorinated biphenyls (PCBs) are a family of compounds with a wide range of industrial applications in heat transfer fluids, dielectric fluids, hydraulic fluids, flame retardants, plasticizers, solvent extenders, and organic diluents. In the United States and the United Kingdom, complex PCB mixtures were manufactured under the trade name Aroclor. Three of the commonly used Aroclors are 1242, 1254, and 1260, which contain 42, 54, and 60% chlorine by weight, with an average of 3, 5, and 6 chlorines per biphenyl molecule, respectively. Aroclors consist of a number of congeners which differ in the number and distribution of chlorines on the biphenyl nucleus. About 150 congeners have been reported in the environment. PCBs have entered into soil and sediment environments as a result of improper disposal of industrial PCB wastes and leakage of PCBs from electric transformers. Their chemical inertness, due to a stable molecular structure and hydrophobicity, is believed to be responsible for their low biodegradation in ecosystems, leading to their persistence in the environment.

Some PCB congeners have been shown to be transformed by both aerobic and anaerobic bacteria (1, 12). The aerobic biodegradation of PCBs is generally limited to less-chlorinated congeners (5 or fewer chlorines per biphenyl molecule) by a mechanism involving dioxygenation of the aromatic ring (2, 3). The more-chlorinated congeners are generally recalcitrant to aerobic degradation. For instance, the aerobic microbial degradation of most of the congeners of Aroclor 1242 and that of some of the congeners of Aroclor 1254 have been demonstrated in the past, but there has been no convincing evidence to date for aerobic microbial degradation of Aroclor 1260. In contrast to aerobic biodegradation, PCBs undergo reductive dechlorination under anaerobic conditions, leading to the transformation of the more-chlorinated congeners to less-chlorinated congeners, usually leaving the biphenyl ring intact. Also, chlorines substituted in the *ortho* position are generally recalcitrant to anaerobic dechlorination (8, 12).

Phanerochaete chrysosporium, a lignin-degrading white rot fungus, is known to mineralize a wide range of chloroaromatic environmental pollutants to CO_2 (4, 9). Degradation of many of these pollutants was shown to be mediated by the lignin-degrading enzyme system of this organism. Major components of the lignin-degrading enzyme system include lignin peroxidases (LIPs), Mn(II)-dependent peroxidases (MNPs), and the H_2O_2-producing system, which are induced during secondary metabolism, under nutrient-limiting culture conditions but not under nutrient-rich conditions (13). More recently, a number of chloroaromatic pollutants were shown to be degraded by *P. chrysosporium* under nonligninolytic culture conditions (such as in defined high-N medium or nutrient-rich malt extract medium) when LIPs and MNPs are not produced (10, 16, 18, 19). Previous studies (5, 15) indicated low levels of mineralization of 0.9 to 1.1% for individual PCB congeners, such as 3,3′,4,4′-chlorobiphenyl (CB), 2,2′,4,4′-CB, and 2,2′,4,4′,5,5′-CB when these were added to cultures at a low concentration (0.036 to 1.55 ppm). Eaton (7) reported ≤9% mineralization when 0.25 ppm of Aroclor 1254 was added to the cultures. Although these data suggested mineralization of selected PCBs and PCB mixtures by *P. chrysosporium*, some skepticism of the reported PCB-degrading potential of this organism was expressed because of the low concentrations tested (1). There are no convincing data on the ability of the fungus to show substantial degradation of PCB mixtures at environmentally relevant concentrations or at levels degraded by known bacterial systems. Such data are needed to evaluate the PCB bioremediation potential of *P. chrysosporium*. Furthermore, relative degradation of individual PCB congeners in different Aroclor mixtures is not known. Such information is useful for comparing the nature and extent of degradation of various PCB congeners in fungal versus bacterial systems (1).

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Also, degradation of the highly chlorinated PCB mixture Aroclor 1260 has not been demonstrated before in bacterial or fungal systems (1). Moreover, previous studies have focused on PCB degradation by *P. chrysosporium* in nitrogen-limited cultures and have not examined the PCB-degrading ability of the fungus under nutrient-rich conditions that prevail at some contamination sites. In this study, we investigated degradation of relatively high concentrations of Aroclors 1242, 1254, and 1260 by *P. chrysosporium* under varied culture conditions, using congener-specific gas chromatographic (GC) analysis. Our results show (i) extensive degradation of most of the PCB congeners in Aroclor 1242 and many of the congeners in Aroclor 1254, including the *ortho*-substituted congeners under both nutrient-limited and nutrient-rich culture conditions, and (ii) for the first time, degradation of Aroclor 1260 by *P. chrysosporium.*

MATERIALS AND METHODS

Strain. *P. chrysosporium* ME-446 (ATCC 34541) was used in the present study and was maintained on malt extract agar slants as previously described (17).

Chemicals. Aroclors 1242, 1254, and 1260 were obtained from Ultra Scientific (North Kingstown, R.I.). A stock solution (10%, wt/vol) of each of the Aroclors was prepared in acetone and stored in a freezer (−20°C) until use.

Media and inoculum. Low-nitrogen basal III medium (LN medium) and high-N medium (HN medium) were described previously (17). Malt extract medium (ME medium) contained 2% malt extract (Difco Laboratories, Detroit, Mich.), 2% glucose, and 0.1% Bacto Peptone, pH 4.5. A blended mycelial inoculum was prepared in LN medium (without Tween 80) as described previously (17) and was used at the 10% (vol/vol) level.

Culture conditions. The organism was grown in static liquid cultures (10 ml) in 160-ml glass serum bottles sealed with Teflon-coated rubber stoppers. The three different media used were LN medium (2.4 mM N and 56 mM glucose), HN medium (24 mM N and 56 mM glucose), and ME medium (8 mM N and 112 mM glucose plus additional C as malt extract). LN medium allows the expression of the lignin-degrading enzyme system, including LIPs and MNPs, while HN and ME media do not allow expression of LIPs and MNPs (13, 17). The media were spiked with Aroclor 1242, 1254, or 1260 to a final concentration of 10 ppm, followed by incubation at 37°C for 15 or 30 days. Heat-killed controls were prepared, using parallel autoclaved cultures which were pregrown for 7 days under identical conditions. Mycelial mass in these control cultures was equivalent to that in experimental cultures. The cultures were oxygenated (17) at 3-day intervals for the first 15 days and at 6-day intervals thereafter. Parallel heat-killed controls were similarly oxygenated to correct for any vapor loss of PCBs in experimental cultures. However, our results showed that vapor loss is not a problem, as the actual PCB recoveries in our study were very similar to those obtained in earlier anaerobic studies (i.e., closed system) on PCB biodegradation (12). All data are means of samples from triplicate bottles for each treatment. The standard deviations for the congeners were within the limits of experimental error found in previous studies (12).

Analysis. At the end of the incubation period, the cultures and controls were acidified and blended in a stainless steel blender (Sorvall Omni-mixer) and the residual PCBs were extracted with a mixture of hexane and acetone, using a modification of the procedure previously described for river sediments (12) with the following modifications. The ratio of hexane to acetone in the extraction solvent was changed to 7:3, and two centrifugation steps (3,000 rpm at room temperature) were included to separate the solvent and the aqueous phase containing the fungal mycelium. The florisil column which was used to clean up the hexane extract containing PCBs in the above-described procedure was not required in our extraction protocol in this study. We obtained 80 to 95% extraction efficiency, which was consistent with previously published reports on PCB extraction from river sediments (12). Congener-specific PCB analysis was done by using capillary GC (Hewlett Packard model 5890 chromatograph with a 50-m Hewlett-Packard Ultra-2 capillary column, electron capture detector, and data system), as previously described (12). Percent degradation was calculated by determining the net percent difference between the residual PCB concentration in experimental cultures and that in parallel heat-killed controls.

RESULTS

Degradation of Aroclors 1242 and 1254. Degradation of Aroclor 1242 and that of Aroclor 1254 by *P. chrysosporium*, as determined by congener-specific GC analysis, were compared in LN, HN, and ME media. Values for net reduction (percent decrease by weight) in total PCB content for Aroclor 1242 were comparable (56.3 to 60.9%) for all the media. However, maximal degradation (30.5%) of Aroclor 1254 was observed with ME medium, while relatively low levels of degradation (21.6 and 10%, respectively) were seen with LN and HN media (Fig. 1).

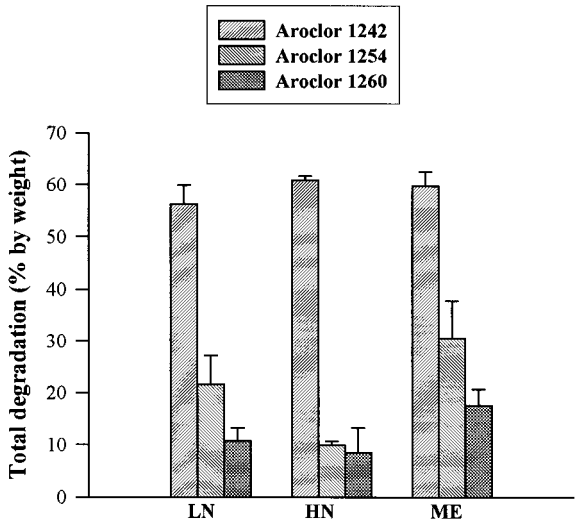

FIG. 1. Loss of total PCB content in Aroclors 1242, 1254, and 1260 by *P. chrysosporium* when grown in different media. Cultures were incubated for 30 days. Values presented are means of triplicate samples. LN, 2.4 mM N; HN, 24 mM N.

The extent of degradation of individual PCB congeners in Aroclor 1242 in LN medium and that in HN medium are presented in Table 1. The number and positions of chlorines on each congener are also given in Table 1. It should be noted that a few of the peaks represent more than one congener, as the GC technique used does not distinguish these congeners. The results (Table 1 and Fig. 2) show that almost all the congeners in Aroclor 1242 were degraded, although the amount of degradation for each congener varied in the three media (LN, HN, and ME). Relatively high levels of degradation were seen for congeners eluting in peaks 1 to 23, while the congeners represented by peaks 24 and higher were degraded to a lower extent, suggesting that the relative degradability of a congener decreases with the increase in the number of chlorine substitutions on the biphenyl nucleus (Table 1 and Fig. 2). The data further show that *P. chrysosporium* is capable of transforming a variety of congeners with varying degrees and positions of chlorine substitutions. For instance, congeners with *ortho*, *meta*, and/or *para* substitutions were all degraded. Interestingly, *ortho*-substituted congeners with as many as three *ortho* chlorines were degraded, though the extent of their degradation was different under different culture conditions. For example, 2,2′,3,6′-CB (GC peak 23) underwent 70.2% degradation in LN medium, compared with 61.0% degradation in HN medium and 81.5% degradation in ME medium (Table 1 and Fig. 2). Many of the congeners in Aroclor 1254 were decreased substantially under both nitrogen-limited and nitrogen-sufficient culture conditions (Table 1). However, 50% of the congeners showed maximal degradation in ME medium (compared with that in HN and LN media), and this accounts for the highest loss in total PCB content in ME medium.

The results in Fig. 3 show time-dependent degradation of different PCB congeners in Aroclors 1242 and 1254 when incubated for 15 and 30 days in LN medium. Most of the congeners in both the Aroclors showed a higher level of degradation at the end of 30 days of incubation than at the end of 15 days. Degradation of some of the more-chlorinated congeners

TABLE 1. Comparative degradation of Aroclors 1242 and 1254 by *P. chrysosporium* in different media[a]

Peak no.	Congener structure[b]	No. of chlorines/ biphenyl		Mol wt (avg)	Degradation (%) of congeners in different culture media[c]					
		Total (avg)	Ortho (avg)		Aroclor 1242			Aroclor 1254		
					LN	HN	ME	LN	HN	ME
1	2	1	1	188.7	100 (0)	100 (0)	100 (0)			
2	3	1	0	188.7						
3	4	1	0	188.7	100 (0)	100 (0)	100 (0)			
4	2-2, 26	2	2	223.1	93 (1)	96 (0)	97 (0)			
5	24, 25	2	1	223.1	88 (1)	93 (2)	93 (1)			
6	2-3	2	1	223.1	89 (1)	95 (1)	98 (0)			
7	2-4, 23	2	1	223.1	85 (1)	92 (1)	95 (0)			
8	26-2	3	3	257.5	93 (1)	93 (2)	95 (0)			
9	34, 3-4	2	0	223.1	82 (1)	78 (1)	83 (3)			
10	25-2, 4-4	2.75	1.51	249	78 (0)	85 (3)	81 (2)			
11	24-2	3	2	257.5	79 (1)	85 (3)	83 (2)			
12	26-3, 236	3	2	257.5	81 (0)	86 (1)	91 (1)			
13	23-2, 26-4	3	2	257.5	82 (0)	89 (2)	94 (1)			
14	35-2 (26-26)	3	1	257.5	68 (3)	85 (21)	62 (5)			
15	245	3	1	257.5	64 (4)	72 (2)	52 (5)			
16	25-3	3	1	257.5	58 (5)	66 (2)	49 (5)			
17	24-3	3	1	257.5	65 (4)	73 (3)	78 (4)			
18	25-4	3	1	257.5	54 (5)	62 (2)	40 (5)			
19	24-4 (246-2)	3	1	257.5	69 (3)	71 (2)	76 (4)			
20	34-2, 234, 23-3, 25-26	3.06	1.12	259.5	65 (4)	71 (2)	77 (3)			
21	23-4 (24-26)	3	1	257.5	57 (6)	65 (2)	71 (4)			
22	236-2	4	3	292	66 (2)	67 (2)	54 (4)			
23	23-26	4	3	292	70 (4)	61 (2)	81 (2)			
24	25-25, 26-35	4	2	292	40 (5)	44 (1)	26 (4)	45 (3)	28 (1)	33 (5)
25	24-25	4	2	292	43 (5)	48 (1)	31 (4)	58 (2)	32 (2)	53 (4)
26	24-24	4	2	292	47 (6)	52 (0)	54 (6)			
27	245-2, 246-4	4	2	292	49 (6)	54 (0)	49 (5)			
28	34-3	3	0	257.5						
29	23-25	4	2	292	48 (5)	56 (1)	58 (5)	81 (0)	75 (2)	94 (0)
30	23-24, 236-3, 34-4	3.42	0.84	272.4	43 (6)	48 (3)	52 (4)	72 (1)	67 (3)	88 (1)
31	26-34, 234-2, 236-4, 25-35	4	1.75	292	46 (6)	48 (1)	47 (3)	57 (2)	39 (2)	70 (7)
32	236-26	5	4	326.4						
33	23-23	4	2	292	38 (7)	32 (4)	26 (3)			
34	235-3, 245-3, 246-24, 246-25	4.2	1.4	298.9	13 (10)	0 (0)	0 (0)			
35	23-35, 235-4	4	1	292						
36	245-4 (235-26)	4	1	292	23 (7)	23 (8)	16 (4)	30 (4)	14 (0)	35 (11)
37	25-34, 345-2	4	1	292	25 (7)	29 (9)	18 (3)	26 (4)	8 (0)	31 (7)
38	236-25, 245-26, 24-34	4.04	1.08	293.5	34 (7)	35 (5)	46 (6)	0 (0)	20 (1)	33 (5)
39	234-3, 236-24	4.95	2.9	324.7	31 (6)	31 (6)	24 (3)	67 (1)	36 (4)	45 (11)
40	246-246-CB (surrogate)	6	4	360.9						
41	23-34, 234-4	4	1	292	26 (7)	27 (6)	28 (4)	22 (4)	6 (1)	24 (7)
42	245-25, 235-24	5	2	326.4	14 (7)	18 (8)	0 (0)	28 (4)	13 (2)	31 (7)
43	245-24	5	2	326.4						
44	236-246, 2356-3, 246-34	5.33	2.67	337.8						
45	245-23, 2345-2 (2356-26)	5	2	326.4	18 (7)	18 (7)	17 (3)	35 (3)	19 (1)	51 (5)
46	234-25, 2346-4, 235-35	5	1.67	326.4	6 (10)	11 (6)	0 (0)	44 (2)	20 (1)	62 (3)
47	234-24	5	2	326.4				74 (0)	58 (4)	87 (1)
48	236-236	6	4	360.9				16 (6)	0 (0)	4 (4)
49	34-34, 236-34	4.69	1.38	315.8	10 (6)	11 (10)	13 (2)	24 (5)	8 (2)	41 (7)
50	2356-25	6	3	360.9	12 (7)	14 (11)	11 (1)	13 (5)	0 (0)	9 (5)
51	235-236, 345-25, 2346-25	5.87	2.74	356.3				21 (7)	27 (1)	63 (5)
52	245-34, 236-245, 2345-3	5.32	1.64	337.5				18 (4)	0 (0)	0 (0)
53	2356-23, 2345-26, 2345-4	5.62	2.24	347.8				8 (8)	0 (0)	10 (5)
54	235-245, 2346-35	6	2	360.9				6 (7)	0 (0)	0 (0)
55	245-245	6	2	360.9	0 (0)	22 (10)	37 (26)	1 (5)	0 (0)	0 (0)
56	234-236, 234-34	5.62	2.24	347.8	6 (9)	4 (10)	6 (1)	19 (4)	0 (0)	16 (7)
57	2345-25	6	2	360.9				8 (6)	0 (0)	14 (9)
58	2356-236	7	4	395.3						
59	2345-24	6	2	360.9				14 (7)	0 (0)	28 (16)
60	2346-236	7	4	395.3						
61	234-245, 2356-34	6	2	360.9				10 (4)	0 (0)	17 (8)
62	2346-34	6	2	360.9				4 (6)	0 (0)	6 (10)
63	2356-235	7	3	395.3				0 (0)	0 (0)	0 (0)
64	2346-235	7	3	395.3						

Continued on following page

TABLE 1—*Continued*

Peak no.	Congener structure[b]	No. of chlorines/ biphenyl		Mol wt (avg)	Degradation (%) of congeners in different culture media[c]					
		Total (avg)	Ortho (avg)		Aroclor 1242			Aroclor 1254		
					LN	HN	ME	LN	HN	ME
65	2356-245, 2345-246	7	3	395.3						
66	2346-245	7	3	395.3						
67	245-345	6	1	360.9				2 (6)	0 (0)	2 (8)
68	23456-25	7	3	395.3						
69	2345-236, 23456-24	7	3	395.3				6 (10)	0 (0)	0 (0)
70	2356-234	7	3	395.3						
71	2346-234, 2345-34, 2356-2356	6.71	2.41	386.4				4 (6)	0 (0)	3 (10)
72	23456-23, 2346-2356, 23456-246	7.87	3.87	425.4						
73	2345-235, 23456-35	7	2	395.3						
74	2345-245	7	2	395.3				0 (0)	0 (0)	6 (11)
75	2356-345	7	2	395.3						
76	2346-345	7	2	395.3						
77	23456-236	8	4	429.8						
78	2345-234	7	2	395.3				0 (0)	0 (0)	4 (11)
79	23456-34	7	2	395.3						
80	2356-2345	8	3	429.8						
81	2345-2346, 23456-245	8	3	429.8						
82	2345-345	7	1	395.3						
83	23456-234	8	3	429.8						
84	23456-2356	9	4	464.2						
85	2345-2345	8	2	429.8						
86	23456-345	8	2	429.8						
87	23456-2345	9	3	464.2						
88	OCN (internal standard)									

[a] Initial concentration of Aroclor added, 10 ppm; incubation period, 30 days.

[b] The numbering scheme used for the congener structure was adapted from the system of Quensen et al. (12). For example, the structure of peak number 43 is shown as 245-24-CB for ease of presentation and readability rather than the more conventional designation, 2,2′,4,4′,5-CB. Note that some of the peaks represent more than one congener, as these are not resolved by the GC technique used. Congeners in parentheses are present in trace amounts in the Aroclors used and were therefore ignored in estimating the average number of chlorines. OCN, octachloronaphthalene.

[c] Percent degradation represents the net decrease (percent by weight) in experimental cultures, compared with that of the heat-killed controls. Minor peaks representing a concentration of <0.1 μmol/liter were ignored for calculation of percent degradation, to avoid confusion. Values presented are means of triplicate samples and are standardized on the recovery of the surrogate PCB congener 246-246-CB. Values in parentheses represent standard deviations as percentages of the means. Blank space across from a peak number represents absence of that peak in the given Aroclor.

in Aroclor 1254 (e.g., peaks 51 through 78) was observed only in cultures incubated for 30 days. Similar effects of incubation were observed in HN medium. However, in ME medium, there was relatively little difference in the extents of degradation of a majority of the congeners for the 15- and 30-day incubation periods (data not shown).

Degradation of Aroclor 1260. It was of particular interest that *P. chrysosporium* showed substantial degradation of Aroclor 1260, a highly chlorinated mixture of PCB congeners (Fig. 1; Table 2). Degradation was observed in both nutrient-limited (ligninolytic) and nutrient-rich (nonligninolytic) conditions. Among the three media used, the highest level of degradation was achieved in ME medium (17.6% by weight) and somewhat lower levels of degradation were observed in LN (10.7%) and HN (8.4%) media (Fig. 1). Most of the components of Aroclor 1260 were degraded (Table 2), though to various extents (1.6 to 66.5%), depending on the number and positions of chlorine substitutions on the biphenyl ring. The degradability of a given congener varied with the medium used (Table 2). Length of incubation (15 versus 30 days) affected degradation of Aroclor 1260, in that many of the more-chlorinated congeners were degraded in 30 days but not in 15 days of incubation (data not shown).

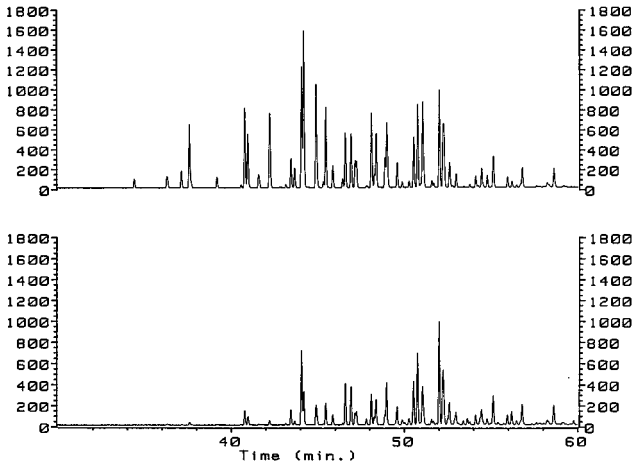

FIG. 2. Biodegradation of Aroclor 1242 in ME medium by *P. chrysosporium* as shown by the congener-specific GC analysis of residual PCBs in heat-killed control (top panel) and in experimental culture (bottom panel) after 30 days of incubation. *y*-axis values represent detector responses, while *x*-axis values correspond to the retention times for different congener peaks in the GC run. The order and the congener structures of the GC peaks are the same as those presented in Table 1.

DISCUSSION

P. chrysosporium is known to degrade a wide range of aromatic pollutants (see references 4 and 9). Published reports (5,

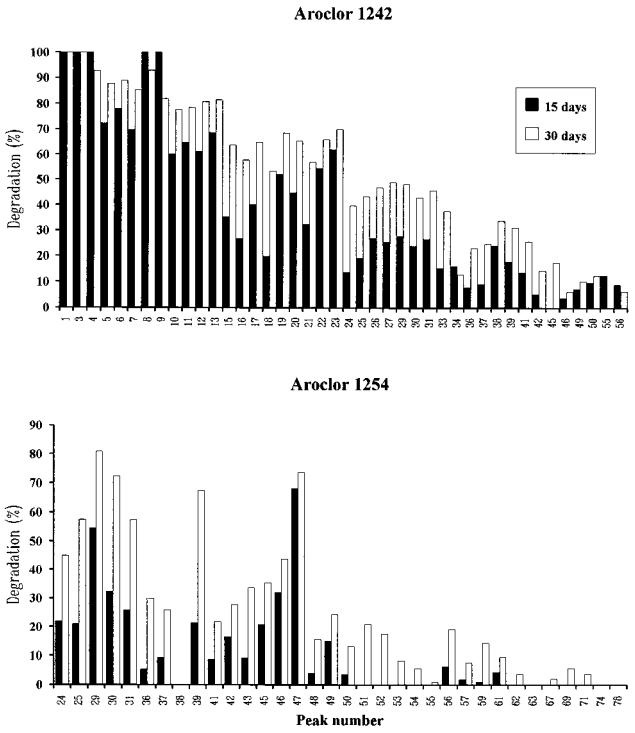

FIG. 3. Effect of length of incubation period on the percent degradation of different PCB congeners in Aroclors 1242 and 1254 by *P. chrysosporium* in LN medium. Peak numbers (*x* axis) represent different congeners in each Aroclor, as defined in Table 1. Values presented are means of triplicate samples.

7, 15) on the degradation of PCBs by *P. chrysosporium* indicated mineralization of very low concentrations of radiolabeled PCBs to $^{14}CO_2$, primarily in ligninolytic cultures, in which LIPs and MNPs are known to be produced. For example, Eaton (7) reported about 9% mineralization of ^{14}C-labeled Aroclor 1254 to $^{14}CO_2$ when a low concentration (0.25 ppm) was added to low-N (2.2 mM N) cultures of *P. chrysosporium*. In comparison, a 20 to 30% loss of 10 ppm of Aroclor 1254 was observed in our study, as determined by GC analysis (Fig. 1). Moreover, we have demonstrated substantial degradation of individual congeners in Aroclors 1242 and 1254 not only under nutrient-limited conditions but also under nutrient-rich conditions. These observations are significant from the standpoint of a potential application of *P. chrysosporium* for the degradation of PCBs in the environment, where nutrient concentrations might vary from site to site. As nutrient limitation at certain contamination sites would lead to poor growth of the fungus, supplementation with nutrients might be needed for obtaining a higher mycelial mass of *P. chrysosporium*. In this context, our results indicate that nutrient-rich conditions do not adversely affect degradation of PCBs by this organism. Our data show that some of the common congeners in Aroclors 1242 and 1254 are not degraded (on a percent basis) to the same extent in the two mixtures (Table 1). For instance, disappearance of peak 38 is lower in Aroclor 1254 than in Aroclor 1242 (Table 1). One reason for these observed differences is that the two Aroclor mixtures vary quantitatively in their congener composition. In the specific example given above, peak 38 has three congeners, namely 236-25, 24-34, and 245-26, whose concentrations (percent weight of the total PCBs) are 2.87, 1.66, and <0.05 in Aroclor 1242 but 6.02, 0.59, and <0.05 in Aroclor 1254 (14). The observed difference in percent weight reduction in peak 38 in the two Aroclors can, therefore, be explained if the 24-34 congener in each Aroclor is degraded but not the 236-25. This explanation also holds true for many of the peaks common to the two Aroclors. Furthermore, the two Aroclors also differ qualitatively in their congener composition; this may affect the relative reactivity of a given congener in the two mixtures. Also, as the degradation proceeds in a PCB mixture, the metabolites released from some of the congeners might further affect the relative reactivity of a given congener, and this metabolite effect on relative reactivity may vary in the two Aroclor mixtures and in different media.

In previous studies using aerobic bacterial isolates, significant degradation of most of the congeners in Aroclor 1242 and some of the congeners in Aroclor 1254 have been reported (2, 3, 11). However, unlike *P. chrysosporium*, these isolates required the addition of biphenyl for inducing the degradation activity and showed a preference for congeners with unsubstituted 2,3 positions on the PCB moiety (2, 3). Substantial degradation of PCB mixture Clophen A 30 (which is considered equivalent to Aroclor 1242 in chlorine content) by *Aspergillus niger* (6) was observed, but congeners with substitutions in the 4 or 2,5(3,6) positions tended to persist. Moreover, *A. niger* was found to be incapable of transforming the more-chlorinated PCB mixtures Clophen A 50 and Clophen A 60 (which are equivalent to Aroclors 1254 and 1260, respectively). In con-

TABLE 2. Degradation of individual congeners in Aroclor 1260 by *P. chrysosporium*[a]

Peak no.	Congener structure	Amt added (μmol/liter)	Degradation (%) of congeners		
			LN	HN	ME
38	236-25, 245-26, 24-34	0.4	18 (13)	35 (9)	38 (7)
41	23-34, 234-4	0.1	19 (6)	32 (5)	33 (15)
42	245-25, 235-24	0.8	26 (8)	21 (6)	34 (6)
46	234-25, 2346-4, 235-35	0.1	42 (4)	46 (4)	67 (10)
48	236-236	0.3	21 (8)	11 (6)	16 (8)
49	34-34, 236-34	0.3	31 (6)	24 (5)	57 (5)
50	2356-25	0.9	12 (6)	8 (6)	11 (6)
51	235-236, 345-25, 2346-25	0.7	19 (5)	8 (5)	18 (5)
52	245-34, 236-245, 2345-3	1.4	23 (4)	17 (6)	33 (3)
54	235-245, 2346-35	0.3	6 (2)	7 (6)	10 (5)
55	245-245	2.0	7 (4)	4 (6)	9 (2)
56	234-236, 234-34	0.2	31 (3)	17 (5)	34 (9)
57	2345-25	0.4	16 (3)	10 (6)	29 (1)
58	2356-236	0.5	6 (3)	2 (6)	9 (2)
61	234-245, 2356-34	3.0	18 (2)	11 (6)	29 (1)
62	2346-34	0.3	7 (1)	7 (7)	18 (2)
63	2356-235	0.2	3 (1)	2 (7)	2 (3)
65	2356-245, 2345-246	0.6	4 (2)	4 (7)	8 (2)
66	2346-245	0.4	4 (1)	4 (7)	8 (3)
68	23456-25	0.2	1 (1)	2 (10)	4 (4)
69	2345-236, 23456-24	0.8	9 (1)	6 (7)	17 (2)
70	2356-234	0.4	4 (1)	3 (7)	9 (3)
71	2346-234, 2345-34, 2356-2356	0.4	10 (2)	8 (8)	15 (3)
73	2345-235, 23456-35	0.2	2 (1)	4 (9)	5 (4)
74	2345-245	1.7	3 (1)	4 (7)	9 (3)
78	2345-234	0.6	2 (0)	3 (8)	7 (3)
79	23456-34	0.2	0 (0)	0 (0)	5 (8)
80	2356-2345	0.5	2 (1)	4 (8)	7 (3)
81	2345-2346, 23456-245	0.4	0 (0)	2 (9)	5 (4)
83	23456-234	0.2	1 (4)	1 (10)	7 (4)
85	2345-2345	0.4	0 (0)	2 (10)	5 (4)
87	23456-2345	0.2	0 (0)	1 (12)	9 (4)

[a] Experimental conditions and other details were as described in the footnotes for Table 1.

trast, our results show that *P. chrysosporium* is capable of substantial degradation of various congeners in all of the three Aroclors tested. Moreover, *P. chrysosporium* does not show any noticeable specificity for congeners with *ortho*, *meta*, or *para* chlorine substitutions in Aroclors. The relative nonspecificity towards degradation of differentially substituted congeners observed in this study might be due to a free radical mechanism of attack. Such a free radical mechanism of attack has previously been reported to have a role in the degradation of a wide range of other aromatic pollutants such as dioxins and chlorophenols by this fungus (9). However, our data show a higher rate of degradation of less-chlorinated congeners (peaks 1 through 23) than of the more-chlorinated congeners (peaks 24 and higher) in Aroclor 1242 by this organism (Table 1). This suggests that chlorine substitution does, to some extent, affect oxidative attack by the fungal enzyme system but to a lesser extent than for the bacterial dioxygenases (1).

Degradation of Aroclor 1260 by *P. chrysosporium* is particularly significant. To our knowledge, this is the first conclusive demonstration of the degradation of PCBs in Aroclor 1260 by any microorganism. Although reductive dechlorination of certain components of Aroclor 1260 has been reported in earlier work (8, 12), there was no net decrease in the molar quantity of PCBs in those anaerobic transformations. Using this measure, we observed up to an 18.4% decrease in the molar quantity of PCBs in Aroclor 1260 with *P. chrysosporium.*

In conclusion, *P. chrysosporium* substantially degrades both less-chlorinated and more-chlorinated PCB mixtures (Aroclors 1242, 1254, and 1260). Net degradation of most of the individual PCB congeners in these formulations was observed. The fungus does not appear to show specificity for *ortho*-, *meta*-, or *para*-substituted PCB congeners.

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REFERENCES

1. **Abramowicz, D. A.** 1990. Aerobic and anaerobic biodegradation of PCBs: a review. Crit. Rev. Biotechnol. **10:**241–251.
2. **Bedard, D. L., R. E. Wagner, M. J. Brennan, M. L. Haberl, and J. F. Brown, Jr.** 1987. Extensive degradation of Aroclors and environmentally transformed polychlorinated biphenyls by *Alcaligenes eutrophus* H850. Appl. Environ. Microbiol. **53:**1094–1102.
3. **Boyle, A. W., C. J. Silvin, J. P. Hassett, J. P. Nakas, and S. W. Tanenbaum.** 1992. Bacterial PCB degradation. Biodegradation **3:**285–298.
4. **Bumpus, J. A., and S. D. Aust.** 1987. Biodegradation of environmental pollutants by the white rot fungus *Phanerochaete chrysosporium*: involvement of the lignin degrading system. Bioassays **6:**116–120.
5. **Bumpus, J. A., M. Tien, D. Wright, and S. D. Aust.** 1985. Oxidation of persistent environmental pollutants by a white rot fungus. Science **228:**1434–1436.
6. **Dmochewitz, S., and K. Ballschmiter.** 1988. Microbial transformation of technical mixtures of polychlorinated biphenyls (PCB) by the fungus *Aspergillus niger*. Chemosphere **17:**111–121.
7. **Eaton, D. C.** 1985. Mineralization of polychlorinated biphenyls by *Phanerochaete chrysosporium*: a ligninolytic fungus. Enzyme Microb. Technol. **7:**194–196.
8. **GE Progress Report.** 1993. General Electric Company Research and Development program for the destruction of PCBs: twelfth progress report for the period Aug. 1, 1992–July 31, 1993. General Electric Co. Corporate Research and Development, Schenectady, N.Y.
9. **Hammel, K. E.** 1992. Oxidation of aromatic pollutants by lignin-degrading fungi and their extracellular peroxidases, p. 41–60. *In* H. Sigel and A. Sigel (ed.), Metal ions in biological systems, vol. 28. Degradation of environmental pollutants by microorganisms and their metalloenzymes. Marcel Dekker, Inc., New York.
10. **Köhler, A., A. Jäger, H. Willershausen, and H. Graf.** 1988. Extracellular ligninase of *Phanerochaete chrysosporium* Burdsall has no role in the degradation of DDT. Appl. Microbiol. Biotechnol. **29:**618–620.
11. **Köhler, H.-P. E., D. Kohler-Staub, and D. D. Focht.** 1988. Cometabolism of polychlorinated biphenyls: enhanced transformation of Aroclor 1254 by growing bacterial cells. Appl. Environ. Microbiol. **54:**1940–1945.
12. **Quensen, J. F., III, S. A. Boyd, and J. M. Tiedje.** 1990. Dechlorination of four commercial polychlorinated biphenyl mixtures (Aroclors) by anaerobic microorganisms from sediments. Appl. Environ. Microbiol. **56:**2360–2369.
13. **Reddy, C. A.** 1993. An overview of the recent advances on the physiology and molecular biology of lignin peroxidases of *Phanerochaete chrysosporium*. J. Biotechnol. **30:**91–107.
14. **Schulz, D. E., G. Petrick, and J. C. Duinker.** 1989. Complete characterization of polychlorinated biphenyl congeners in commercial Aroclor and Clophen mixtures by multidimensional gas chromatography-electron capture detection. Environ. Sci. Technol. **23:**852–858.
15. **Thomas, D. R., K. S. Carswell, and G. Georgiou.** 1992. Mineralization of biphenyl and PCBs by the white rot fungus *Phanerochaete chrysosporium*. Biotechnol. Bioeng. **40:**1395–1402.
16. **Yadav, J. S., and C. A. Reddy.** 1992. Non-involvement of lignin peroxidases and manganese peroxidases in 2,4,5-trichlorophenoxyacetic acid degradation by *Phanerochaete chrysosporium*. Biotechnol. Lett. **14:**1089–1092.
17. **Yadav, J. S., and C. A. Reddy.** 1993. Degradation of benzene, toluene, ethylbenzene, and xylenes (BTEX) by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **59:**756–762.
18. **Yadav, J. S., and C. A. Reddy.** 1993. Mineralization of 2,4-dichlorophenoxyacetic acid (2,4-D) and mixtures of 2,4-D and 2,4,5-trichlorophenoxyacetic acid by *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **59:**2904–2908.
19. **Yadav, J. S., R. E. Wallace, and C. A. Reddy.** 1995. Mineralization of mono- and dichlorobenzenes and simultaneous degradation of chloro- and methyl-substituted benzenes by the white rot fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. **61:**677–680.