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Extensive Degradation of Aroclors and Environmentally Transformed Polychlorinated Biphenyls by *Alcaligenes eutrophus* H850

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We have isolated and characterized a strain of *Alcaligenes eutrophus*, designated H850, that rapidly degrades a broad and unusual spectrum of polychlorinated biphenyls (PCBs) including many tetra- and pentachlorobiphenyls and several hexachlorobiphenyls. This strain, which was isolated from PCB-containing dredge spoils by enrichment on biphenyl, grows well on biphenyl and 2-chlorobiphenyl but poorly on 3- and 4-chlorobiphenyl. Capillary gas-chromatographic analysis showed that biphenyl-grown resting cells of H850 degraded the components of 38 of the 41 largest peaks of Aroclor 1242 and 15 of the 44 largest peaks of Aroclor 1254, resulting in an overall reduction of PCBs by 81% for Aroclor 1242 (10 ppm) and 35% for Aroclor 1254 (10 ppm) in 2 days. Furthermore, H850 metabolized the predominantly *ortho*-substituted PCB congeners that resulted from the environmental transformation of the more highly chlorinated congeners of Aroclor 1242 by the upper Hudson River anaerobic *meta*-, *para*-dechlorination agent system C (J. F. Brown, R. E. Wagner, Jr., D. L. Bedard, M. J. Brennan, J. C. Carnahan, R. J. May, and J. J. Tofflemire, Northeast Environ. Sci. 3:167–179, 1984). The congener selectivity patterns indicate that a two-step process consisting of anaerobic dechlorination followed by oxidation by H850 can effectively degrade all of the congeners in Aroclor 1242 and possibly all those in Aroclor 1254.

Polychlorinated biphenyls (PCBs) are pollutants that have attracted concern because of their worldwide distribution, their persistence in the environment, and their possible health effects. PCB molecules consist of a biphenyl nucleus carrying 1 to 10 chlorines; hence there are 209 possible PCB congeners that differ in the number and position of the chlorines. Because the commercial PCB products commonly used (e. g., Aroclors [Monsanto] and Kanechlors [Kanegafuchi]) contain more than 60 to 80 congeners at analytically detectable levels, they pose a particularly difficult challenge as candidates for microbial degradation. Nevertheless, there have been several studies of the bacterial degradation of commercial PCBs. Tucker et al. (23) reported the degradation of mono- and dichlorobiphenyls and several trichlorobiphenyls in Aroclors 1221 and 1242 by activated sludge, but found no degradation of the more highly chlorinated Aroclor 1254. Similarly, Kaneko et al. reported that even very low concentrations (1 ppb) of Kanechlor 500 (which is equivalent to Aroclor 1254) were not degraded by activated sludge (18). *Nocardia* and *Pseudomonas* spp. were reported to selectively degrade mono- through tetrachlorobiphenyls in Aroclor 1242 (5 ppm) after 10 to 30 days of incubation (2), and three mixed cultures were reported to degrade most of the mono-, di-, and trichlorobiphenyls and several tetrachlorobiphenyls in water saturated with Aroclor 1242 (7). A kinetic analysis of the degradation of PCBs by one of these mixed cultures showed wide differences in the rate of degradation of isomers as well as congeners that differed in chlorine number (7). More recently, Furukawa et al. demonstrated that *Acinetobacter* sp. strain P6 selectively degraded congeners containing one to four chlorines in Kanechlors 300 and 400 (equivalent to Aroclors 1242 and 1248) but did not significantly alter Kanechlor 500 (13).

In contrast to the studies above, there have been two reports of extensive bacterial degradation of Aroclor 1254 (19, 22). However, because extensive depletion of highly chlorinated Aroclors can result from physical losses due to adsorption (R. Unterman, M. J. Brennan, R. E. Brooks, and C. Johnson, *in* Proceedings of the 1986 International Conference on Innovative Biological Treatment of Toxic Wastewaters, in press), these results may not be entirely due to biodegradation.

In the present study, we have examined the congener-specific degradation of Aroclors 1242, 1248, and 1254 and of an environmentally dechlorinated Aroclor by *Alcaligenes eutrophus* H850 and, in one case, by *Corynebacterium* sp. strain MB1. The data presented demonstrate that *A. eutrophus* H850 can degrade at least 44, and perhaps as many as 53, of the congeners in these Aroclors, including many of the pentachlorobiphenyls and several hexachlorobiphenyls.

MATERIALS AND METHODS

Bacterial strains. We used three strains of *A. eutrophus* in these studies. Two strains that are facultatively chemolithotrophic in an atmosphere consisting of H_2, O_2, and CO_2, were obtained from the American Type Culture Collection, Rockville, Md. The first is the type strain, ATCC 17697, which has been extensively characterized for dissimilation of aromatic compounds (17). The second strain, ATCC 29597, has been characterized only by its ability to grow autotrophically. The third strain, H850, was isolated from PCB-contaminated soil as described below and has been

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deposited with the U.S. Department of Agriculture as strain NRRLB 15940.

Corynebacterium sp. strain MB1 was isolated from a culture of *Acinetobacter* sp. strain P6 (10, 11) obtained from K. Furukawa and A. M. Chakrabarty.

Enrichment, isolation, and maintenance of bacterial cultures. A mixed culture, designated H8, was derived from PCB-containing dredge spoils from the upper Hudson River, N.Y., by repeated transfer in a phosphate-buffered minimal salts medium (PAS) containing biphenyl as a carbon source and supplemented with 0.005% yeast extract (5). Six isolates capable of growth on biphenyl were obtained from the mixed culture and were identified by the American Type Culture Collection as four slightly different strains of *A. eutrophus*. Preliminary characterization of PCB-degradative competence (5) showed that the six isolates were very similar; hence we selected one strain, H850, for further characterization.

Frozen stocks of H850 and MB1 were prepared every 6 months by mixing equal volumes of glycerol and a culture freshly grown on PAS-biphenyl. These stocks were maintained at −20°C. Cell cultures were inoculated from frozen stocks and transferred twice on PAS-biphenyl prior to being assayed for PCB-degradative ability.

Chemicals. Monochlorobiphenyls were purchased from Pfaltz and Bauer, Inc., Stamford, Conn., and other pure PCB congeners were obtained from Foxboro Analabs, Inc., North Haven, Conn., or Ultra Scientific, Hope, R.I. Biphenyl was purchased from Aldrich Chemical Co., Inc., Milwaukee, Wis.

Growth substrate utilization. Cultures were incubated with shaking (250 rpm) at 30°C in flasks containing 100 ml of PAS supplemented with 0.005% yeast extract and containing 100 mg of biphenyl or the monochlorobiphenyl being tested. Growth was monitored over a period of 3 days by measuring optical density at 615 nm with a Spectronic 20 (Bausch & Lomb, Inc., Rochester, N.Y.). An optical density of 0.3 or greater was considered to be evidence of growth.

Resting-cell assays to measure PCB-degradative competence. Cells were grown on PAS-biphenyl, washed, and suspended in 0.05 M sodium phosphate buffer (pH 7.5) at an optical density at 615 nm of 1.0 (approximately 3.8×10^9 cells per ml for H850 and 1.2×10^9 cells per ml for MB1). Aroclors, environmentally transformed Aroclor, and defined mixtures of PCB congeners were added to the cell suspensions as concentrated acetone solutions. This facilitates adsorption of the PCBs to the cells. In defined congener assays, each congener was present at 5 μM, and in Aroclor assays, the total PCB concentration was 10 ppm (10 μg/ml). Cells were incubated for up to 72 h at 30°C in a gyratory shaker (250 rpm). Control cells were inactivated before the addition of PCBs by lowering the pH to 1 or 2 with perchloric acid (final concentration, 0.7%) or by heating at 70°C for 20 min. In all other respects, controls were treated in the same way as experimental samples. Additional details of the assay methods have been published previously (5).

Extraction of environmentally transformed PCBs from river sediment. Sediment core samples were originally obtained from the upper Hudson River to profile and characterize PCB deposition and congener alteration. A polycarbonate tube (2.7 m by 45 mm [inner diameter]) was used to obtain the core sample, which was then frozen and cut into ca. 1-in. (2.54-cm) sections. The sediment from each section was air dried and passed through a 28-mesh sieve to remove large debris and produce a homogeneous sample. A 5-gram portion of the sediment was extracted by gentle boiling for 18 to 20 h with a 1:1 mixture of acetone and hexane in a Soxhlet extractor. The extract was concentrated to approximately 5 ml, and sulfur (which interferes with the electron capture detector) was removed by shaking with 1 ml of mercury. Concentrated sulfuric acid (approximately 2 ml) was added to remove the precipitated mercury-sulfur complex and to remove coextracted water, hydrocarbons, and colored biogenic acids. The organic layer was concentrated to 1 or 2 ml and applied to a Florisil Sep-pak column (Waters Associates, Inc., Milford, Mass.). Coextracted polar materials and certain chlorinated and phosphated pesticides were retained by the column, and the purified PCBs were eluted with hexane. From this point, the sample was ready for aerobic degradation studies.

Extraction of PCBs from cells. Resting-cell incubations were stopped by heating at 70°C for 20 min or by adding perchloric acid to a final concentration of 0.7%. Triton X-100 was added (final concentration, 1%), and the PCBs were extracted from the cells with 4 volumes of hexane as previously described (5).

GC analysis. (i) Defined congener mixtures. Samples were analyzed isothermally (190°C) as previously described (5) on a 5880A gas chromatograph (GC) (Hewlett-Packard Co., Calif.) with an automatic sampler, an electron capture detector, and a glass column (6 ft [1.83 m] by 4 mm) packed with 1.5% SP-2250/1.95% SP-2401 on 100/120 Supelcoport (Supelco, Inc., Bellefont, Pa.).

(ii) Aroclors. Samples were analyzed as previously described (5) on a Vista 4600 GC (Varian, Sunnyvale, Calif.) with an electron capture detector (operated at 300°C) and a fused silica capillary column (30 m by 0.25 mm [inner diameter]) coated with a 0.25-μm bonded liquid phase of DB-1 (polydimethylsiloxane) (J & W Scientific, Inc., Folsom, Calif.).

We established the peak positions of 70 PCB congeners in Aroclors by coelution with commercially available congeners. Additional peak assignments were based on relative retention times published for all 209 PCB congeners on a slightly different column phase (SE-54) (20) and on assignments published previously by Ballschmiter and Zell (1). Because certain substitution patterns of chlorophenyl rings (e.g. 3,5- and 2,4,6-) are known to be quite rare in Aroclors (16), we have listed only congeners believed to be the major constituents of each peak. Further details and a complete listing of PCB congener assignments for Aroclors will be published elsewhere (J. F. Brown, Jr., R. E. Wagner, H. Feng, D. L. Bedard, M. J. Brennan, J. C. Carnahan, and R. J. May, Environ. Toxicol. Chem., in press).

Chromatograms of experimental samples were normalized by matching the area of peaks that were not degraded, e.g., Aroclor 1242 peaks 40 and 41, with the area of the same peaks in a control. Percent degradation was calculated for each Aroclor peak on the basis of peak area. We also calculated the weight percent of each peak in Aroclor standards and in the dechlorinated Aroclor sample by a modification of the method of Webb and McCall (24), that is, by determination of the weight of chlorine per unit area of peak with a Hall electrolytic conductivity detector (Tracor Instruments, Austin, Texas) combined with mass-spectrometric determination of the isomer class(es) present in each peak. This information allowed us to calculate the molar concentration of the PCB components of each peak, which in turn permitted us to calculate the total reduction of PCBs for each of our experimental samples. The numerical data presented represent single samples; hence the absolute amount of degradation would be expected to vary slightly

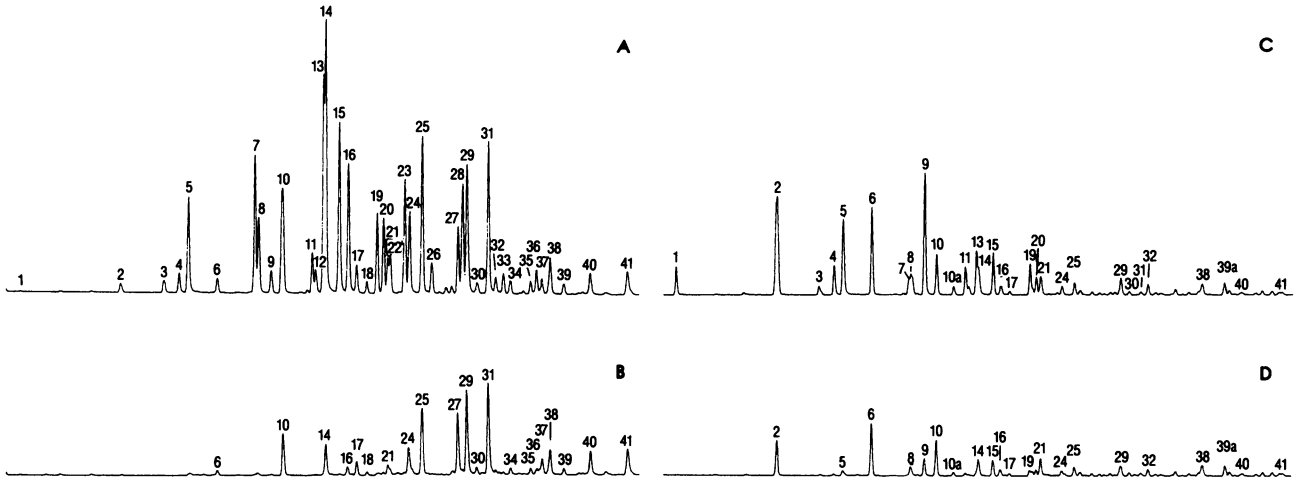

FIG. 1. Biodegradation of Aroclor 1242 and of environmentally dechlorinated Aroclor 1242 by biphenyl-grown resting cells of *A. eutrophus* H850. (A) Aroclor 1242 incubated with heat-inactivated cells (control for panel B). (B) Aroclor 1242 (10 ppm) after 48 h of incubation at 30°C with resting cells of *A. eutrophus* H850. (C) Environmentally dechlorinated Aroclor 1242 incubated with heat-inactivated cells (control for panel D). (D) Environmentally dechlorinated Aroclor 1242 (10 ppm) after 48 h of incubation at 30°C with resting cells of *A. eutrophus* H850. The GC profiles of the controls were indistinguishable from those of the original PCB mixtures, thus demonstrating the efficiency of our extraction procedures. Chromatogram C was scaled to represent the same molar concentration of PCBs as that in chromatogram A. Chromatograms of aerobically degraded PCBs were matched with those of controls on the basis of peak area of undegraded peaks (panels A and B, peaks 40 and 41; panels C and D, peak 38). Congener assignments and percent degradation for each Aroclor 1242 peak are presented in Table 1. The concentration of PCBs in each peak is given for all four samples in Table 3.

because of biological variation. However, a defined congener assay on the cells used in these experiments established that their biodegradative activity was the same as that normally observed (Table 1 in reference 4). The experimental error due to extraction and GC analysis was estimated at ±5% on the basis of a comparison of 14 nondegraded peaks of the biodegraded sample of Aroclor 1254 and its control (see Fig. 2 and Table 4). Percent degradation for individual Aroclor peaks was rounded to the nearest 5% as follows: 83 to 87% was rounded to 85%, 88 to 92% was rounded to 90%, etc.

RESULTS AND DISCUSSION

Taxonomic identification. H850 has been identified by the American Type Culture Collection as a strain of *A. eutrophus* according to *Bergey's manual* (15). The identification was based on an extensive analysis and comparison with the type strain. The species *A. eutrophus* is commonly thought to belong to a group called the hydrogen bacteria and is facultatively autotrophic in an atmosphere consisting of H_2, O_2, and CO_2. Strain H850, however, was unable to grow autotrophically under any of four sets of conditions tested in which the proportion of O_2 was varied from 2 to 15%. H850 harbors a single cryptic plasmid that was estimated by restriction analysis to be approximately 65 kilobases. Further characteristics of the strain have been published (3).

Strain MB1 has been classified as a *Corynebacterium* sp. by the American Type Culture Collection according to *Bergey's manual* (8). Although the characteristics of *Arthrobacter* and *Corynebacterium* spp. are very similar, these genera can be distinguished by cell wall analysis. Such an analysis demonstrated the presence of mesodiaminopimelic acid, thereby establishing that MB1 is a *Corynebacterium* sp. However, we cannot rule out the possibility that the *Arthrobacter* sp. strain M5 of Furukawa and Chakrabarty (10), which was also isolated from a culture of *Acinetobacter* strain P6, is identical to *Corynebacterium* sp. strain MB1. Additional characteristics of *Corynebacterium* sp. strain MB1 have been published (3).

Utilization and Degradation of PCBs by *A. eutrophus* strains. H850 utilized biphenyl and all three monochlorobi-

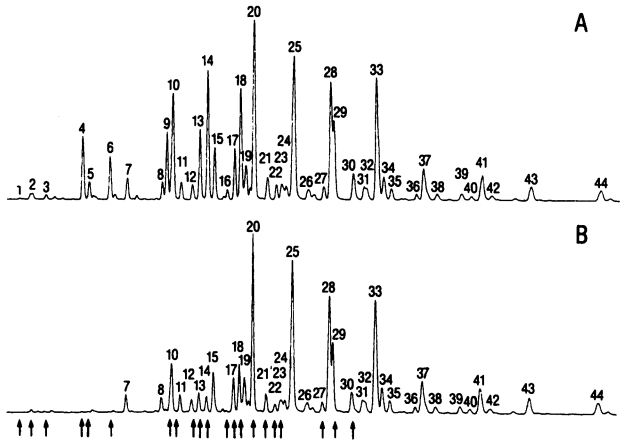

FIG. 2. Biodegradation of Aroclor 1254 by *A. eutrophus* H850. Biphenyl-grown resting cells of *A. eutrophus* H850 were incubated with Aroclor 1254 (10 ppm) for 48 h at 30°C and then extracted and analyzed by capillary GC. (A) Aroclor 1254 incubated with heat-inactivated cells (control). (B) Aroclor 1254 after incubation with resting cells of *A. eutrophus* H850. Arrows indicate Aroclor peaks that were significantly decreased following biodegradation. Chromatograms were matched on the basis of four undegraded peaks: peaks 28, 33, 37, and 41. Congener assignments and percent degradation for each Aroclor peak are presented in Table 1. The PCB concentration in each peak is given for both samples in Table 4.

TABLE 1. Analysis of the degradation of Aroclors 1242, 1248, and 1254 by *A. eutrophus* H850[a]

Retention time (min)[b]	Peak no.			Congener identification[c]	% Degradation[d]		
	Aroclor 1242	Aroclor 1248	Aroclor 1254		Aroclor 1242	Aroclor 1248	Aroclor 1254
18.29	1			2[e]			
20.54	2			**2,2'** 2,6	95		
21.51	3			2,4 2,5	100		
21.85	4			2,3'	100		
22.05	5			2,3 **2,4'**	95		
22.70	6			2,6,2'	60		
23.54	7	1		2,5,2'	100	100	
23.61	8	2		2,4,2' 4,4'	95	100	
23.90	9			2,3,6 **2,6,3'**	90		
24.15	10	3		2,3,2' 2,6,4'	70	70	
24.81	11			2,5,3'	100		
24.89	12			2,4,3'	100		
25.07	13	4	1	2,5,4'	100	100	100
25.11	14	5	2	2,4,4'	90	95	45
25.43	15	6	3	**2',3,4** 2,5,2',6'	100	100	100
25.62	16	7		**2,3,4'** 2,4,2',6'	95	85	
25.81	17	8		2,3,6,2'	55	60	
26.05	18	9		2,3,2',6'	75	85	
26.27	19	10	4	2,5,2',5'	95	100	100
26.41	20	11	5	2,4,2',5'	95	95	95
26.51	21	12		2,4,2',4'	65	80	
26.56	22	13		2,4,5,2'	85	85	
26.89	23	14	6	2,3,2',5'	95	100	100
27.15	24	15		3,4,4' 2,3,2',4'	60	85	
27.28	25	16	7	2,3,4,2' 2,3,6,4' 2,6,3',4'	55	60	15[f]
27.50	26	17		2,3,2',3'	95	100	
28.08	27	18	8	2,4,5,4'	0	15[f]	0
28.18	28	19	9	2,5,3',4'	95	95	95
28.27	29	20	10	2,4,3',4' 2,3,6,2',5'	45	50	50
28.51	30	21	11	2,3,6,2',4'	40	0	0
28.65	31	22	12	2,3,3',4' 2,3,4,4'	35	35	20
28.76	32	23	13	2,3,6,2',3' 2,3,5,2',5'	70	75	70
29.02	33	24	14	2,3,5,2',4' **2,4,5,2',5'**	85	80	85
29.11	34	25	15	2,4,5,2',4'	40	55	20
29.45			16	2,3,5,2',3'			85
29.56	35	26	17	**2,4,5,2',3'** 2,3,5,6,2',6'	40	50	25
29.72	36	27	18	2,3,4,2',5'	70	70	60
29.85	37	28	19	2,3,4,2',4'	0	0	0
29.97	38	29	20	2,3,6,3',4' 3,4,3',4'	25	40	20
30.30	39	30	21	2,3,4,2',3'	40	35	25
30.46			22	2,3,5,6,2',5'			40
30.65			23	2,3,5,2',3',6' 2,3,4,6,2',5' 3,4,5,2',5'			15[f]
30.75			24	2,3,5,3',4' 2,3,5,6,2',4'			15[f]
30.89	40	31	25	2,3,6,2',4',5' 2,4,5,3',4'	0	0	0
31.25			26	2,3,4,5,4' 2,3,5,6,2',3' 2,3,4,5,2',6'			0
31.60			27	2,3,5,2',4',5'			25
31.75	41	32	28	2,3,4,3',4' 2,3,4,2',3',6'	0	0	0
31.90			29	2,4,5,2',4',5'			20
32.25			30	2,3,4,5,2',5'			20
32.50			31	2,3,4,2',3',5'			0
32.55			32	2,3,4,5,2',4'			0
32.75		33	33	2,3,4,2',4',5' 2,3,5,6,3',4'		0	0
32.95			34	2,3,4,6,3',4'			0
33.10			35	2,3,4,5,2',3'			0
33.65			36	2,3,4,5,2',4',6' **2,3,5,6,2',4',5'**			0
33.80			37	2,3,4,2',3',4'			0
34.10			38	2,4,5,3',4',5'			0
34.70			39	2,3,4,5,2',3',6' **2,3,4,5,2',4'**			0
34.90			40	2,3,5,6,2',3',4'			0
35.10			41	**2,3,4,5,3',4'** 2,3,4,6,2',3',4'			0
35.85			42	2,3,4,5,2',3',5'			0
36.20			43	2,3,4,5,2',4',5'			0
37.75			44	2,3,4,5,2',3',4'			0

[a] Aroclors (10 ppm) were added separately to biphenyl-grown resting cells of *A. eutrophus* and incubated with shaking (250 rpm) at 30°C for 48 h.
[b] This represents retention time on a fused silica capillary column coated with DB-1.
[c] Boldface indicates major congener(s) in the peak.
[d] Percent degradation was determined by direct comparison with a control consisting of heat-killed cells (70°C, 20 min) but otherwise identical to the experimental sample. Degradation less than 15% is not considered significant and is not reported.
[e] This congener is barely detectable in Aroclor 1242 but is quite prominent in Aroclor 1242 dechlorinated by upper Hudson River system C (Fig. 2C).
[f] Degradation of 15% is considered questionable unless confirmed by other data.

TABLE 2. Identification of tri- and tetrachlorobiphenyls in Aroclor 1242 that show reduced susceptibility to degradation by *A. eutrophus* H850[a]

Aroclor 1242 peak	PCB congener identification	% Degradation
6[b]	2,6,2'	60
10[b]	2,6,4' 2,3,2'	70
17[b]	2,3,6,2'	55
18[b]	2,3,2',6'	75
25[b]	2,3,6,4' 2,3,4,2' 2,6,3',4'	55
21[c]	2,4,2',4'	65
24[c]	3,4,4' 2,3,2',4'	60
27[c]	2,4,5,4'	0
29[c]	2,4,3',4' 2,3,6,2',5'	40
31[c]	2,3,3',4' 2,3,4,4'	35

[a] Data are taken from Table 1.
[b] The common structural characteristic of these congeners is two *ortho* chlorines (i.e., positions 2, 6), on one ring.
[c] The common structural characteristic of these congeners is that both rings are substituted in the *para* positions (i.e., position 4 or 4').

phenyls as growth substrates, but neither of the two autotrophic strains of *A. eutrophus* tested (ATCC 17697 and ATCC 29597) could utilize these substrates. When grown on biphenyl, H850 had a generation time of 6 h and reached cell densities in excess of 10^{10} cells per ml. Growth on 2-chlorobiphenyl (2-CB) was considerably slower, with a maximum cell density of 10^9 cells per ml, and growth on 3-CB and 4-CB was minimal. Nevertheless, we successfully maintained H850 for more than 1 year (more than 30 transfers) by using a mixture of the three monochlorobiphenyls as the sole source of carbon and energy.

All three strains were assayed for the ability to degrade two defined mixtures of PCBs (5) after growth on succinate (0.2%) in the presence of biphenyl. Under these conditions, H850 still degraded many congeners as well as when grown on biphenyl but exhibited a markedly decreased ability to degrade certain congeners, in particular, 2,3,4,2',5'-CB, 2,4,4'-CB, 4,4'-CB, 2,4,2'4'-CB, 2,4,5,2',3'-CB, and 2,4,5,2',4',5'-CB (data not shown). However, neither of the other *A. eutrophus* strains was capable of degrading any of the PCB congeners in this assay. Therefore, it appears that the PCB-degradative ability of H850 is not shared by all strains of *A. eutrophus*.

Degradation of commercial PCB mixtures (Aroclors). When tested on the commercial PCB products that are common environmental contaminants, *A. eutrophus* H850 oxidized virtually all of the detectable di-, tri-, and tetrachlorobiphenyls and most of the pentachlorobiphenyls in 48 h, thus effecting extensive degradation of Aroclors 1242 and 1248 and partial degradation of Aroclor 1254 (Fig. 1 A and B, Fig. 2, and Table 1).

The major components of the largest peaks resolved by capillary gas chromatography for each Aroclor have been identified, and the amount of degradation of these components has been calculated for each peak (Table 1). This permitted a detailed chemical characterization of the biodegradation of all three Aroclors. The results demonstrate the remarkable versatility of the PCB-degradative enzyme(s) in *A. eutrophus* H850.

At least 39 and perhaps as many as 49 of the 60 congeners identified in Aroclor 1242 were degraded, albeit to different extents. Most of the di-, tri- and tetrachlorobiphenyl peaks of Aroclor 1242 were completely eliminated (95 to 100% decreased). Those that were not (0 to 75% decreased) could be divided into two groups on the basis of common structural features (Table 2). Each of the Aroclor peaks in the first group contained a congener chlorinated at positions 2 and 6, while each of the peaks in the second group contained a congener chlorinated in both *para* positions, 4 and 4'. The recalcitrance of these congeners was clearly not related to their concentration, since related congeners present at higher concentrations were degraded much more efficiently (Table 3, compare peaks 6 and 7, peaks 18 and 23, and peaks 19 and 21). In fact, the peaks listed in Table 2 represent only 21% of the total PCBs in Aroclor 1242, but they represent 53% of the residue after 48 h degradation (Table 3). Thus it appears that chlorines at positions 2,6 or 4,4' may negatively influence PCB biodegradation by H850.

The degradation of 2,3,2',3'-CB, 2,4,2',4'-CB, and 2,5,2',5'-CB (Tables 1 and 3) indicates that 2,3-, 2,4-,and 2,5-chlorophenyl rings can all be metabolized by H850. These chlorination patterns are quite common in the Aroclors and probably account for the susceptibility of many of the tetra- and pentachlorobiphenyls to oxidation by H850. Indeed, 10 of the 11 congeners making up the pentachlorobiphenyl peaks that were depleted by H850 (Aroclor 1242 peaks 30, 32 through 36, 39, and Aroclor 1254 peak 16) contain a 2,3-, 2,4-, or 2,5-chlorophenyl ring. The single exception, 2,3,6,3',4'-CB, contains a 2,3,6-chlorophenyl ring that is now also known to be attacked by H850 (4). On the other hand, three of the four pentachlorobiphenyls that were not degraded (Aroclor 1242 peaks 40 and 41 and Aroclor 1254 peak 24) contain a 3,4-chlorophenyl ring. This suggests that 3,4-chlorophenyl rings are not metabolized by H850.

The complexity of the rules governing the chlorine configurations that permit biodegradation in H850 can be best

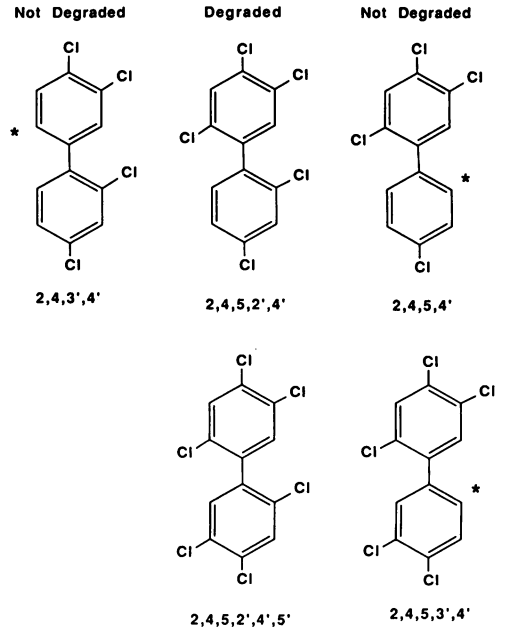

FIG. 3. Structures of several PCB congeners that are degraded by *A. eutrophus* H850 and of several closely related homologs that are not. Note that in each case, the nondegradable congener can be converted to the adjacent degradable homolog by the addition of a second *ortho* chlorine at the position indicated by the asterisk. This observation does not hold for all sets of homologs; for example, 2,3,4,4'-CB and 2,3,4,2'4'-CB are both recalcitrant to degradation by H850.

TABLE 3. Composition of Aroclor 1242 and environmentally dechlorinated Aroclor 1242 before and after oxidation by *A. eutrophus* H850[a]

Aroclor 1242 peak no.	Congener identification[b]	PCB concn (pmol/ml)[c]			
		1242 control	H850-oxidized 1242	Dechlorinated 1242 control	H850-oxidized Dechlorinated 1242
1	(2)	0	0	15,320	0
2	**2,2'** 2,6	1,240	90	9,170	1,020
3	2,4 2,5	420	0	500	70
4	2,3'	520	0	1,380	100
5	2,3 **2,4'**	2,610	80	3,620	350
6	2,6,2'	320	120	2,860	1,870
7	2,5,2'	3,390	20	600	130
8	2,4,2' 4,4'	1,680	70	590	490
9	2,3,6 **2,6,3'**	260	20	2,050	330
10	2,3,2' 2,6,4'	1,850	530	830	740
10a	(2',3,5) (2,6,2',6')	0	0	250	130
11	2,5,3'	430	0	560	50
12	2,4,3'	280	0	170	20
13	2,5,4'	2,740	0	1,110	0
14	2,4,4'	2,730	330	420	330
15	**2',3,4** 2,5,2',6'	2,550	30	940	400
16	**2,3,4'** 2,4,2',6'	1,290	80	180	130
17	2,3,6,2'	330	150	70	50
18	2,3,2',6'	190	50	0	0
19	2,5,2',5' (2,6,3',5')	1,090	60	800	160
20	2,4,2',5'	880	40	360	130
21	2,4,2',4'	370	130	410	370
22	2,4,5,2'	480	70	0	0
23	2,3,2',5'	1,150	40	40	0
24	3,4,4' 2,3,2',4'	1,330	550	240	150
25	2,3,4,2' 2,3,6,4' 2,6,3',4'	1,030	470	140	110
26	2,3,2',3'	280	10	70	60
27	2,4,5,4'	670	640	50	60
28	2,5,3',4'	1,360	60	60	30
29	2,4,3',4' 2,3,6,2',5'	1,650	950	360	270
30	2,3,6,2',4'	420	250	190	180
31	2,3,3',4' 2,3,4,4'	900	600	40	40
32	2,3,6,2',3' 2,3,5,2',5'	300	90	310	200
33	2,3,5,2',4' **2,4,5,2',5'**	340	50	70	60
34	2,4,5,2',4'	310	190	100	90
35	**2,4,5,2',3'** 2,3,5,6,2',6'	280	160	0	0
36	2,3,4,2',5'	280	80	60	60
37	2,3,4,2',4'	240	260	50	0
38	**2,3,6,3',4'** 3,4,3',4'	340	250	220	220
39	2,3,4,2',3'	220	130	0	0
39a	(2,3,5,6,2',5')[d]	0	0	160	140
40	2,3,6,2',4',5' 2,4,5,3',4'	240	260	60	70
41	2,3,4,3',4' 2,3,4,2',3',6'	290	280	40	50

[a] Experimental samples and conditions are described in the legend to Fig. 1. GC profiles for these samples are also shown in Fig. 1.

[b] Boldface represents the major congener(s) present in this Aroclor 1242 peak. This may not be the major congener in dechlorinated 1242. Parentheses indicate congeners which are present at significant concentrations in dechlorinated 1242 but not in the unaltered Aroclor.

[c] PCB concentration of each Aroclor peak in 10 ppm of Aroclor 1242 (column 3) and environmentally dechlorinated Aroclor 1242 (column 5), and for each of these substrates following a 48-h incubation with *A. eutrophus* H850. (columns 4 and 6, respectively). The total concentrations for columns 3 and 4 are 37,280 and 7,190 pmol/ml (amount degraded, 30,090 pmol/ml) and for columns 5 and 6, 44,450 and 8,660 pmol/ml (amount degraded, 35,790 pmol/ml). Because of its altered congener composition, 10 ppm of the dechlorinated PCB actually contains a higher concentration of PCB than Aroclor 1242 (44.45 versus 37.28 μM). Therefore, although H850 oxidized both samples to comparable levels, more PCB was degraded in the dechlorinated sample than in Aroclor 1242.

[d] This congener is most probably due to traces of Aroclor 1260 and its dechlorination products in the environmental sample.

illustrated by an example. Two tetrachlorobiphenyls, 2,4,3',4'-CB (Table 1 in reference 4) and 2,4,5,4'-CB (Table 1) were unusually resistant to degradation by H850. Remarkably, a pentachlorobiphenyl (2,4,5,2',4'-CB) and a hexachlorobiphenyl (2,4,5,2',4',5'-CB) having chlorines in all of the positions occupied in the recalcitrant tetrachlorobiphenyls (Fig. 3) were degraded under comparable conditions (Tables 1, 3, and 4). Each of the recalcitrant congeners can be converted to a degradable homolog by the addition of an *ortho* chlorine at the position indicated. Significantly, the degradable congeners thus formed have a single *ortho* chlorine on each ring, i.e., at positions 2,2'. Further examples of accelerated degradation of more highly chlorinated homologs are described in the accompanying paper (4).

It is apparent from the data described above that the chlorination pattern on both rings determines whether a PCB congener can be degraded by H850. Congeners with chlorines in both *para* positions, 4,4', or chlorines at both *ortho* positions, 2,6, of either ring are generally not degraded as well as congeners lacking these characteristics. On the

TABLE 4. Composition of Aroclor 1254 and Aroclor 1254 oxidized by *A. eutrophus* H850[a]

Aroclor 1254 peak no.	Congener identification[b]	PCB concn (pmol/ml)[c]		
		Aroclor 1254 control	H850-oxidized Aroclor 1254	Amt degraded[d]
1	2,5,4′	220	0	220
2	2,4,4′	160	90	70
3	2′,3,4 2, 2,5,2′6′	210	0	210
4	2,5,2′,5′	1,660	20	1,640
5	2,4,2′,5′	290	10	280
6	2,3,2′,5′	660	10	650
7	2,3,4,2′ 2,3,6,4′ 2,6,3′,4′	190	160	
8	2,4,5,4′	280	270	
9	2,5,3′,4′	1,010	40	970
10	2,4,3′,4′ 2,3,6,2′,5′	2,370	1,130	1,240
11	2,3,6,2′,4′	320	300	
12	2,3,3′,4′ 2,3,4,4′	240	190	50
13	2,3,6,2′,3′ 2,3,5,2′,5′	1,040	300	740
14	2,3,5,2′,4′ **2,4,5,2′,5′**	2,460	310	2,150
15	2,4,5,2′,4′	830	660	170
16	2,3,5,2′,3′	240	30	210
17	**2,4,5,2′,3′** 2,3,5,6,2′,6′	640	470	170
18	2,3,4,2′,5′	1,180	490	690
19	2,3,4,2′,4′	400	410	0
20	**2,3,6,3′,4′** 3,4,3′,4′	2,840	2,290	550
21	2,3,4,2′,3′	310	240	70
22	2,3,5,6,2′,5′	240	140	100
23	2,3,5,2′,3′,6′ 2,3,4,6,2′,5′ 3,4,5,2′,5′	440	370	70[e]
24	2,3,5,3′,4′ 2,3,5,6,2′,4′	510	430	80[e]
25	2,3,6,2′,4′,5′ **2,4,5,3′,4′**	3,030	3,210	0
26	2,3,4,5,4′ 2,3,5,6,2′,3′ 2,3,4,5,2′,6′	820	820	0
27	2,3,5,2′,4′,5′	220	170	50
28	2,3,4,3′,4′ 2,3,4,2′,3′,6′	1,700	1,740	0
29	2,4,5,2′,4′,5′	1,060	870	190
30	2,3,4,5,2′,5′	250	200	50
31	2,3,4,2′,3′,5′	120	120	0
32	2,3,4,5,2′,4′	110	90	
33	**2,3,4,2′,4′,5′** 2,3,5,6,3′,4′	1,640	1,620	
34	2,3,4,6,3′,4′	270	270	0
35	2,3,4,5,2′,3′	200	200	0
36	2,3,4,5,2′,4′,6′ **2,3,5,6,2′,4′,5′**	160	140	
37	2,3,4,2′,3′,4′	420	430	
38	2,4,5,3′,4′,5′	200	190	
39	**2,3,4,5,2′,3′,6′** 2,3,4,5,2′,4′	180	170	
40	2,3,5,6,2′,3′,4′	160	160	0
41	**2,3,4,5,3′,4′** 2,3,4,6,2′,3′,4′	410	410	0
42	2,3,4,5,2′,3′,5′	210	200	
43	2,3,4,5,2′,4′,5′	180	180	0
44	2,3,4,5,2′,3′,4′	170	170	0

[a] Experimental details and GC profiles are given in Fig. 2 and its legend.
[b] Boldface indicates the major congener present in this Aroclor peak.
[c] PCB concentration in 10 ppm of Aroclor 1254 (column 3) and in the residue of this Aroclor following 48 h of incubation with H850 (column 4). The difference is given in column 5. The total concentrations are 30,250 and 19,720 pmol/ml for columns 3 and 4, respectively; the total amount degraded (column 5) is 10,620 pmol/ml.
[d] A loss of less than 15% of the original PCB in any peak is not considered significant and is not reported.
[e] These values represent approximately a 15% loss and may not be significant.

other hand, congeners that are chlorinated on both rings are more likely to be degraded if each ring has an *ortho* chlorine (positions 2,2′). The last observation is particularly significant, because congeners containing either combination of two *ortho* chlorines are strikingly resistant to degradation by most bacteria (5, 14).

Aroclor 1248 differs from Aroclor 1242 primarily by virtue of its higher proportions of the same tetra- and pentachlorobiphenyls and was degraded nearly as well as Aroclor 1242 (Table 1). Aroclor 1254, on the other hand, contains penta-, hexa-, and heptachlorobiphenyls, the penta- and hexachlorobiphenyls being present at the highest concentrations. Roughly 35% of the total PCB in Aroclor 1254, including most of the tri-, tetra-, and pentachlorobiphenyls and several hexachlorobiphenyls, was degraded by H850 (Fig. 2; Table 4). The hexachlorobiphenyl peaks that were decreased following incubation with H850 (peaks 22, 23, 27, 29, and 30) all contained congeners with a 2,5- or a 2,4,5-chlorophenyl ring. Congeners with these chlorination patterns are abundant in Aroclors and are degraded particularly well by H850 (4). In fact, it is quite possible that other hexachlorobiphenyls containing 2,5- and 2,4,5-chlorophenyl rings would be degraded in experiments involving lower concentrations of Aroclor 1254 or longer incubations. Indeed, we have observed that several highly chlorinated congeners that are components of Aroclor 1254 (2,3,5,6,2′,5′-CB, peak 22; 2,4,5,2′,4′,5′-CB, peak 29; and 2,3,4,5,2′,5′-CB, peak 30) were substantially degraded by H850 (83, 93, and 50%,

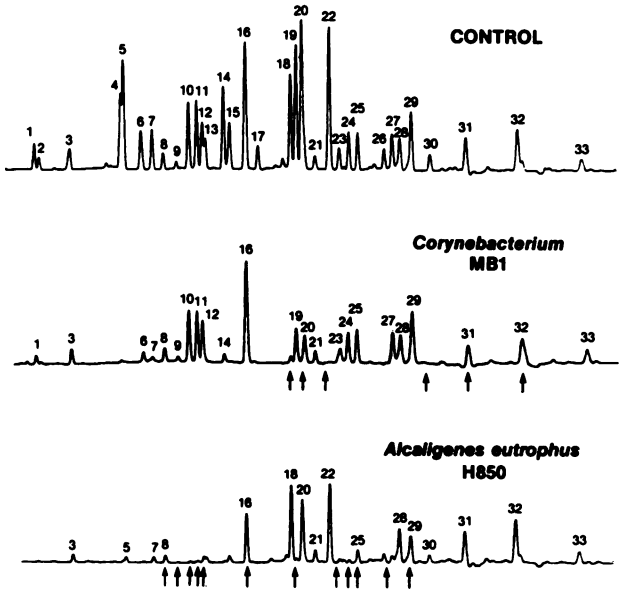

FIG. 4. Biodegradation of Aroclor 1248 by resting cells of *A. eutrophus* H850 and *Corynebacterium* sp. strain MB1. Top panel: Aroclor 1248 incubated with heat-inactivated cells. Middle and bottom panels: Aroclor 1248 after incubation at 30°C for 72 h with resting cells of *Corynebacterium* sp. strain MB1 and *A. eutrophus* H850, respectively. The arrows indicate Aroclor peaks that were decreased to a greater extent by one of the two organisms.

respectively) in 48 to 72 h when assayed at a concentration of 5 μM (approximately 1.8 ppm) in defined mixtures with only one or two other congeners present (4).

Degradation of environmentally transformed Aroclor 1242. We have recently shown that PCBs in aquatic sediments may undergo reductive dechlorination which is probably mediated by anaerobic bacteria (6; Brown et al., in press). These findings prompted us to subject a sample of environmentally dechlorinated PCBs to oxidative degradation. (See reference 4 for evidence that H850 oxidizes PCBs.) The GC profile of Aroclor 1242 transformed by upper Hudson River dechlorination system C (6) is shown in Fig. 1C. Figure 1D shows the same sample after incubation with H850. Table 3 compares the composition of Aroclor 1242 with that of the Aroclor residues following oxidation by H850, environmental dechlorination, and combined dechlorination-oxidation. The removal of chlorines from *meta* and *para* positions by reductive dechlorination, particularly from congeners with four or more chlorines, has resulted in a decrease in highly substituted congeners and in the generation of predominantly *ortho*-substituted mono-, di-, and trichlorobiphenyls: 2-CB, 2,2'-CB, 2,6-CB, 2,3'-CB, 2,6,2'-CB, 2,3,6-CB, and 2,6,3'-CB (6; Brown et al. in press). Moreover, all four of the Aroclor 1242 peaks that were not decreased following incubation with H850 (peaks 27, 37, 40, and 41) were largely removed by environmental dechlorination. Most of the PCBs in the dechlorinated sample were rapidly oxidized by H850 (81% removal in 48 h). Furthermore, one-third of the residual PCB following dechlorination-oxidation is accounted for by two congeners, 2,6-CB (peak 2) and 2,6,2'-CB (peak 6). Both of these congeners are slowly degraded by H850, so it appears that a longer incubation might remove even more PCB.

Thus, the constituents of every congener peak in Aroclor 1242 were transformed by dechlorination, oxidation, or a combination of both. These data indicate that Aroclor 1242 could be completely degraded by the combination of both transformations. Furthermore, since dechlorination is more effective on highly chlorinated congeners, such a dual attack should also degrade Aroclor 1254 and Aroclor 1260.

Comparison of the degradation of Aroclor 1248 by *A. eutrophus* H850 and *Corynebacterium* sp. strain MB1. The major route of PCB degradation in most bacteria that have been characterized appears to involve a 2,3-dioxygenase, and, despite subtle individual differences, the overall patterns of congener specifity exhibited by these strains are quite similar (5, 9, 21). This is especially true for *Alcaligenes* sp. strain Y42, *Acinetobacter* sp. strain P6, and *Corynebacterium* sp. strain MB1 (5, 12). Therefore, to determine how the PCB congener selectivity pattern of *A. eutrophus* H850 compares with those of these strains, we examined the degradation of Aroclor 1248 by both H850 and *Corynebacterium* sp. strain MB1. Analysis of the GC profile revealed major differences in congener specificity (Fig. 4). Both strains degraded most of the trichlorobiphenyls but exhibited selective oxidation of the tetra- and pentachlorobiphenyls and little degradation of the hexachlorobiphenyls. Twelve of the tetra- and pentachlorobiphenyl peaks were eliminated or substantially decreased after incubation with H850 but not MB1 (Fig. 4). Conversely, six other tetra- and pentachlorobiphenyl peaks were removed more completely by MB1 than by H850. Thus these strains show distinct differences in their ability to degrade the constituents of nearly half of the congener peaks in Aroclor 1248. This complementary specificity suggests that a biodegradative process involving both organisms, or the enzymes from both organisms, would oxidize Aroclor 1248 even more completely than a process involving either strain alone.

Table 5 identifies the major constituents of the Aroclor 1248 peaks that were decreased to a greater extent by either H850 or MB1. Three peaks (peaks 10, 11, and 12) that were

TABLE 5. PCB congeners in Aroclor 1248 selectively degraded by *A. eutrophus* H850 or *Corynebacterium* sp. strain MB1[a]

Aroclor 1248 peak no.[b]	Congener identification[c]	% Degradation[d]	
		H850	MB1
8	2,3,6,2'	60	0
9	2,3,2',6'	85	40
10	2,5,2',5'	100	30
11	2,4,2',5'	95	30
12	2,4,2',4'	80	15
16	2,3,4,2' 2,3,6,4' **2,6,3'4'**	60	25
19	2,5,3',4'	95	75
23	2,3,6,2',3' 2,3,5,2',5'	75	35
24	**2,4,5,2',5'** 2,3,5,2',4'	80	35
25	2,4,5,2'4'	55	30
27	2,3,4,2',5'	70	25
29	**2,3,6,3',4'** 3,4,3',4	40	25
18	2,4,5,4'	15	90
20	2,4,3',4' 2,3,6,2',5'	50	85
22	**2,3,3',4'** 2,3,4,4'	35	100
30	2,3,4,2',3'	35	100
31	**2,4,5,3',4'** 2,3,6,2',4',5'	0	60

[a] Data were taken from an experiment identical to that described in the legend to Fig. 4, except that the incubation was stopped after 48 h. In this experiment, peak 32 was not decreased by MB1.
[b] Peak assignments are as listed in Table 1.
[c] Boldface type denotes the major congener in this Aroclor 1248 peak.
[d] Degradation of 15% is considered questionable unless confirmed by other data.

eliminated by H850 but not by MB1 contain congeners composed exclusively of 2,4- and 2,5-chlorophenyl rings. In fact, 8 of the 12 peaks that were more completely removed by H850 contain congeners that have a 2,4- or 2,5-chlorophenyl ring (Table 5). These results suggest that the PCB-degradative system in H850 differs significantly from that in most other PCB-degrading bacteria by virtue of its unusual ability to oxidize 2,4- and 2,5-dichlorophenyl rings in PCBs.

It is more difficult to interpret the differences in selectivity responsible for the superior removal of five tetra- and pentachlorobiphenyl peaks by MB1 (Table 5). Three of these peaks contain multiple components, and H850 partially decreased all but peak 31. However, it is notable that four of the five peaks contain congeners chlorinated at both *para* positions, 4 and 4', and three contain congeners that have a 3,4-chlorophenyl ring. This suggests that the enzymes responsible for oxidizing PCBs in MB1 (and possibly other bacteria that degrade PCBs via a 2,3-dioxygenase) can tolerate chlorines at carbon positions 4,4' and 3,4 more easily than does the PCB-degradative system in H850.

The accompanying paper examines the metabolism of individual PCB congeners in H850 in detail. The results provide evidence for novel dioxygenase activity and biodegradative products, suggesting that H850 may degrade many PCB congeners via a new pathway (4).

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