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Dechlorination of Four Commercial Polychlorinated Biphenyl Mixtures (Aroclors) by Anaerobic Microorganisms from Sediments†

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The rate, extent, and pattern of dechlorination of four Aroclors by inocula prepared from two polychlorinated biphenyl (PCB)-contaminated sediments were compared. The four mixtures used, Aroclors 1242, 1248, 1254, and 1260, average approximately three, four, five, and six chlorines, respectively, per biphenyl molecule. All four Aroclors were dechlorinated with the loss of *meta* plus *para* chlorines ranging from 15 to 85%. Microorganisms from an Aroclor 1242-contaminated site in the upper Hudson River dechlorinated Aroclor 1242 to a greater extent than did microorganisms from Aroclor 1260-contaminated sediments from Silver Lake, Mass. The Silver Lake inoculum dechlorinated Aroclor 1260 more rapidly than the Hudson River inoculum did and showed a preferential removal of *meta* chlorines. For each inoculum the rate and extent of dechlorination tended to decrease as the degree of chlorination of the Aroclor increased, especially for Aroclor 1260. The maximal observed dechlorination rates were 0.3, 0.3, and 0.2 μg-atoms of Cl removed per g of sediment per week for Aroclors 1242, 1248, and 1254, respectively. The maximal observed dechlorination rates for Hudson River and Silver Lake organisms for Aroclor 1260 were 0.04 and 0.21 μg-atoms of Cl removed per g of sediment per week, respectively. The dechlorination patterns obtained suggested that the Hudson River microorganisms were more capable than the Silver Lake organisms of removing the last *para* chlorine. These results suggest that there are different PCB-dechlorinating microorganisms at different sites, with characteristic specificities for PCB dechlorination.

Aroclor is the trade name given to the complex mixtures of polychlorinated biphenyls (PCBs) that were manufactured in the United States by Monsanto between 1929 and 1978 for a variety of commercial purposes. The various Aroclors produced differed in the percentage of chlorine by weight. Most were given a numerical designation beginning with 12 for 12 carbon atoms and ending with two digits expressing the percentage by weight of chlorine. Thus, Aroclor 1242 is 42% chlorine by weight and averages 3.2 chlorines per molecule. Aroclors 1248, 1254, and 1260 average approximately four, five, and six chlorines per molecule, respectively.

The Aroclors are therefore complex mixtures, because each of the 10 positions on the biphenyl molecule may be substituted with either chlorine or hydrogen. Theoretically, 209 different chlorinated biphenyls are possible. Homologs, differing in the number of chlorines, and isomers, differing in the distribution of the same number of chlorines, are collectively referred to as congeners.

The aerobic biodegradation of PCBs is generally limited to congeners with five or fewer chlorines and two adjacent unsubstituted carbon atoms (1, 10). The aerobic degradation of most components of Aroclor 1242 (2, 6, 8, 9, 14), and some of Aroclor 1254 (2, 11) have been demonstrated, but aerobic degradation requires an additional growth substrate (e.g., biphenyl) and is generally less effective as the degree of chlorination increases. There is no convincing evidence for the aerobic biodegradation of Aroclor 1260. The biologically mediated process of reductive dechlorination of PCBs, first proposed by Brown et al. (3–5) and confirmed by Quensen et al. (12), is therefore of great interest to the development of a biotreatment system for PCBs. The removal of the chlorines renders the PCB mixture both less toxic and more readily degraded aerobically.

In this report we compare the dechlorination of Aroclors 1242, 1248, 1254, and 1260 by microorganisms from the upper Hudson River, N.Y. (HR), and Silver Lake, Mass. (SL), with regard to the rate, extent, and congener selectivity observed.

MATERIALS AND METHODS

Sediment collection sites. Aroclor 1260-contaminated sediments were collected from SL near Pittsfield, Mass. (3, 5). In addition to the PCBs, these sediments contained high concentrations of oil and polyaromatic hydrocarbons from a coal gasification plant. Aroclor 1242-contaminated sediments were collected on two occasions (January and August 1988) from the upper HR at River Mile 193.5 near Hudson Falls, N.Y. (site H7 in reference 4). "Clean" (non-PCB-contaminated) sediments were collected upstream at River Mile 205. Sediments were collected with a post hole digger to a depth of approximately 25 cm and transported to the laboratory in completely filled and tightly sealed paint cans to minimize exposure to oxygen.

Preparation of assay vessels. PCB-free, sieved, air-dried sediments from the HR were added to serum tubes (1 g per 28-ml tube) or bottles (25 g per 160-ml bottle, nominally 125 ml), and the tubes or bottles were sealed with butyl rubber stoppers in an anaerobic glove box. The sediments were then moistened with reduced anaerobic mineral medium (13) (2 ml per tube or 25 ml per bottle) containing anaerobic microorganisms eluted from the clean HR sediments and a small quantity of ethanol (1 μl/ml). The ethanol served as a substrate allowing aerobic bacteria to consume all residual oxygen. The tubes or bottles were then incubated at 37°C until methane was detected in the headspace (about 1 week). The detection of methane confirmed that the sediments were

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completely anaerobic. The tubes were then sterilized by autoclaving for 1 h at 121°C.

Inoculation. The contaminated sediments were placed in Erlenmeyer flasks that were flushed with oxygen-free nitrogen and carbon dioxide (80:20) by using a Hungate apparatus. An equal volume of sterile reduced anaerobic mineral medium was then added to the sediments, and the flask was sealed. The flask was then shaken by hand for approximately 2 min, and the contents were allowed to settle. The supernatant, which contained microorganisms eluted from the sediments, was then used to inoculate the assay tubes (5 ml per tube unless stated otherwise) or bottles (50 ml) prepared as above. Triplicate serum bottles were used for the experiments set up with the HR inoculum and Aroclors 1248, 1254, and 1260. Serum tubes were used for the experiments with the SL inoculum and with the HR inoculum and Aroclor 1242. An additional experiment was performed with serum tubes by using Aroclor 1260 and an HR inoculum (2 ml per tube) prepared from sediments collected in January 1988. All other HR inocula were prepared from the sediments collected in August 1988. Controls were then autoclaved at 121°C for 1 h before addition of the PCBs.

PCB addition. A 10% (wt/vol) solution of one of the Aroclors (Monsanto Co., St. Louis, Mo.) in acetone was added to each assay vessel (5 μl/g of sediment) while it was flushed with filter-sterilized O_2-free N_2-CO_2 (80:20, vol/vol) by using a Hungate gassing apparatus. This gave a PCB concentration of 500 μg/g on a sediment dry weight basis. The vessels were resealed with sterile Teflon-coated rubber stoppers (West Co., Phoenixville, Pa.) and shaken thoroughly to ensure dispersal of the PCBs.

Incubation and sampling. The vessels were incubated in the dark at 25°C. Bottles were sampled periodically by shaking thoroughly, removing the stoppers, and then withdrawing approximately 2 ml of the sediment slurry with a sterile filed-off Pasteur pipette while flushing with filter-sterilized N_2-CO_2 by using the Hungate apparatus. The bottles were resealed after sampling. This procedure is preferable to withdrawing samples through the stoppers with a syringe because the sediments would clog the syringe needle and would therefore not be represented in the sample and because the punctured stoppers would allow O_2 to enter the bottles. In the experiments in which tubes were used as the incubation vessels, triplicate samples were taken at each time interval except for the HR experiment with Aroclor 1260 (duplicate samples). The use of serum tubes allowed the total PCB recovery to be determined because the entire contents were extracted for a single observation.

Sample preparation and analysis. Samples were extracted by shaking once with 10 ml of acetone containing 40 μg of octachloronaphthalene as the internal standard and twice more with 10 ml of hexane-acetone (9:1). The solvent extracts were combined, and the acetone was extracted with 2% NaCl in deionized water. The remaining hexane extract was extracted with 2 to 4 ml of concentrated sulfuric acid, rinsed again with 2% NaCl in deionized water, and then dried over anhydrous Na_2SO_4. Further cleanup was performed on a Florisil-copper powder column. These columns were prepared by packing approximately 4 parts of 60/100-mesh Florisil and 1 part of 60-mesh copper powder (to remove sulfur) in a Pasteur pipette. The copper was rinsed with 10% H_2SO_4, deionized water, and acetone and dried under vacuum before use. The sample was eluted from the column with hexane, and the final volume was adjusted to 25 ml before analysis on a gas chromatograph.

A congener-specific analysis was performed by using a gas chromatograph (no. 5890; Hewlett-Packard Co., Palo Alto, Calif.) with a Hewlett-Packard Ultrabond 1 capillary column (25 m by 0.2 mm [inner diameter]; SE-54 equivalent) and electron capture detector. Quantitation on a molar basis was performed by using a Hewlett-Packard 5895A Chemstation and a three-point calibration table with a mixture of 2-chlorobiphenyl (2-CB), 4-CB, Aroclor 1242, and Aroclor 1260 as the standard. Peak identities and amounts were based on analysis of the standard mixture performed by R. E. Wagner, General Electric (GE) Research and Development Center (5).

Data summation. For the purpose of comparing the dechlorination patterns exhibited by the two inocula, the mole percentage of all PCBs recovered was calculated. Increasing and decreasing peaks were readily identifiable by subtracting the values for an autoclaved control from those for the corresponding live treatment and plotting the results as histograms.

To compare the rate and extent of dechlorination, it was necessary to calculate the average number of chlorines per biphenyl molecule. The dechlorination rate was defined as the microgram-atoms of chlorine released per gram of sediment per week. The dechlorination rates were calculated from the change in the average number of chlorines; it was assumed that there was no loss of the biphenyl moiety. On the basis of the results of the experiments in the serum tubes, this was a valid assumption. The extent of dechlorination was defined as the percentage of *meta* and *para* chlorines removed. The values used for the molecular weight and the number of *ortho* and *meta* chlorines per biphenyl for each peak are given in Table 1. The average molecular weight for coeluting homologs was based on mass-spectrometric analyses performed by R. J. May, General Electric Research and Development Center. The relative proportions of coeluting homologs were calculated from these average molecular weights. Coeluting isomers were assumed to occur in equal proportions. We further assumed that all coeluting congeners increased or decreased to the same extent as a result of dechlorination.

The effects of violations of these two simplifying assumptions were evaluated by assuming two worse-case scenarios. In the first scenario, we assumed that all peaks representing coeluting congeners came to contain only the congener(s) with the smallest number of *ortho* and total chlorines as a result of the dechlorination process. In the other scenario, we assumed that each peak representing coeluting congeners came to contain only the congener(s) with the greatest number of *ortho* and total chlorines per biphenyl. It is unlikely that either of these two extremes would occur in reality. We then calculated the average number of chlorines by position given each of these cases.

The results depended on the Aroclor used in an experiment. For Aroclors 1242, 1248, and 1254, estimates of the number of chlorines by position in a worse-case scenario differed from those obtained by using our simplifying assumptions by only a few hundredths of a chlorine. These results can be explained because all of the predominant dechlorination products formed from the dechlorination of these Aroclors occur in peaks in which, if there are coeluting congeners, they have the same number of *ortho* and total chlorines. In the case of Aroclor 1260, violation of our simplifying assumptions would have greater impact, but estimates of the average number of chlorines by position would still be in error by fewer than 0.2 chlorine, or less than 10%. Given that this is the maximum error (bias) to be expected from a violation of our assumptions, and that the

TABLE 1. PCB structure, average molecular weight, and number of chlorines associated with each chromatographic peak

Peak no.	IUPAC no.[a]	Structure	Avg no. of Cl		Avg mol wt
			ortho	Total	
1	1	2	1	1	188.7
2	3	4	0	1	188.7
3	4, 10	2-2, 2,6	2	2	223.1
4	7, 9	2,4, 2,5	1	2	223.1
5	6	2-3	1	2	223.1
6	8, 5	2-4, 2,3	1	2	223.1
7	19	2,6-2	3	3	257.5
8	12, 13	3,4, 3-4	0	2	223.1
9	18, 15	2,5-2, 4-4	1.51	2.75	249.0
10	17	2,4-2	2	3	257.5
11	27, 24	2,6-3, 2,3,6	2	3	257.5
12	16, 32	2,3-2, 2,6-4	2	3	257.5
13	34 (54)	3,5-2 (2,6-2,6)	1	3	257.5
14	29	2,4,5	1	3	257.5
15	26	2,5-3	1	3	257.5
16	25	2,4-3	1	3	257.5
17	31	2,5-4	1	3	257.5
18	28 (50)	2,4-4 (2,4,6-2)	1	3	257.5
19	33, 21, 20, 53	3,4-2, 2,3,4, 2,3-3, 2,5-2,6	1.12	3.06	259.5
20	22 (51)	2,3-4 (2,4-2,6)	1	3	257.5
21	45	2,3,6-2	3	4	292.0
22	46	2,3-2,6	3	4	292.0
23	52, 73	2,5-2,5, 2,6-3,5	2	4	292.0
24	49	2,4-2,5	2	4	292.0
25	47	2,4-2,4	2	4	292.0
26	48, 75	2,4,5-2, 2,4,6-4	2	4	292.0
27	35	3,4-3	0	3	257.5
28	44	2,3-2,5	2	4	292.0
29	42, 59, 37	2,3-2,4, 2,3,6-3, 3,4-4	0.84	3.42	272.4
30	71, 41, 64, 72	2,6-3,4, 2,3,4-2, 2,3,6-4, 2,5-3,5	1.75	4	292.0
31	96	2,3,6-2,6	4	5	326.4
32	40	2,3-2,3	2	4	292.0
33	57, 67, 100, 103	2,3,5-3, 2,4,5-3, 2,4,6-2,4, 2,4,6-2,5	1.40	4.20	298.9
34	58, 63	2,3-3,5, 2,3,5-4	1	4	292.0
35	74 (94)	2,4,5-4 (2,3,5-2,6)	1	4	292.0
36	70, 76	2,5-3,4, 3,4,5-2	1	4	292.0
37	95, 102, 66	2,3,6-2,5, 2,4,5-2,6, 2,4-3,4	1.08	4.04	293.5
38	55, 91	2,3,4-3, 2,3,6-2,4	2.90	4.95	324.7
39	56, 60 (84, 92)	2,3-3,4, 2,3,4-4 (2,3,6-2,3, 2,3,5-2,5)	1	4	292.0
40	101, 90	2,4,5-2,5, 2,3,5-2,4	2	5	326.4
41	99	2,4,5-2,4	2	5	326.4
42	150, 112, 119	2,3,6-2,4,6, 2,3,5,6-3, 2,4,6-3,4	2.67	5	326.4
43	97, 86 (152)	2,4,5-2,3 2,3,4,5-2 (2,3,5,6-2,6)	2	5	326.4
44	87, 115, 111	2,3,4-2,5, 2,3,4,6-4, 2,3,5-3,5	1.67	5	326.4
45	85	2,3,4-2,4	2	5	326.4
46	136	2,3,6-2,3,6	4	6	360.9
47	77, 110	3,4-3,4, 2,3,6-3,4	1.38	4.69	315.8
48	151	2,3,5,6-2,5	3	6	360.9
49	135, 124, 144	2,3,5-2,3,6, 3,4,5-2,5, 2,3,4,6-2,5	2.74	5.87	356.3
50	118, 149, 106	2,4,5-3,4, 2,3,6-2,4,5, 2,3,4,5-3	1.64	5.32	337.5
51	134, 143, 114	2,3,5,6-2,3, 2,3,4,5-2,6, 2,3,4,5-4	2.24	5.62	347.8
52	122, 131, 133	3,4,5-2,3, 2,3,4,6-2,3, 2,3,5-2,3,5	1.45	5.30	336.8
53	146, 161	2,3,5-2,4,5, 2,3,4,6-3,5	2	6	360.9
54	153	2,4,5-2,4,5	2	6	360.9
55	132, 105	2,3,4-2,3,6, 2,3,4-3,4	2.24	5.62	347.8
56	141	2,3,4,5-2,5	2	6	360.9
57	179	2,3,5,6-2,3,6	4	7	395.3
58	137	2,3,4,5-2,4	2	6	360.9
59	176	2,3,4,6-2,3,6	4	7	395.3
60	138, 163	2,3,4-2,4,5, 2,3,5,6-3,4	2	6	360.9
61	158	2,3,4,6-3,4	2	6	360.9
62	178	2,3,5,6-2,3,5	3	7	395.3
63	175	2,3,4,6-2,3,5	3	7	395.3
64	187, 182	2,3,5,6-2,4,5, 2,3,4,5-2,4,6	3	7	395.3
65	183	2,3,4,6-2,4,5	3	7	395.3
66	167	2,4,5-3,4,5	1	6	360.9

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TABLE 1—*Continued*

Peak no.	IUPAC no.	Structure	Avg no. of Cl		Avg mol wt
			ortho	Total	
67	185	2,3,4,5,6-2,5	3	7	395.3
68	174, 181	2,3,4,5-2,3,6, 2,3,4,5,6-2,4	3	7	395.3
69	177	2,3,5,6-2,3,4	3	7	395.3
70	171, 156, 202	2,3,4,6-2,3,4, 2,3,4,5-3,4, 2,3,5,6-2,3,5,6	2.41	6.71	386.4
71	173, 200, 204	2,3,4,5,6-2,3, 2,3,4,6-2,3,5,6, 2,3,4,5,6-2,4,6	3.87	7.87	425.4
72	172, 192	2,3,4,5-2,3,5, 2,3,4,5,6-3,5	2	7	395.3
73	180	2,3,4,5-2,4,5	2	7	395.3
74	193	2,3,5,6-3,4,5	2	7	395.3
75	191	2,3,4,6-3,4,5	2	7	395.3
76	199	2,3,4,5,6-2,3,6	4	8	429.8
77	170	2,3,4,5-2,3,4	2	7	395.3
78	190	2,3,4,5,6-3,4	2	7	395.3
79	201	2,3,5,6-2,3,4,5	3	8	429.8
80	196, 203	2,3,4,5-2,3,4,6, 2,3,4,5,6-2,4,5	3	8	429.8
81	189	2,3,4,5,-3,4,5	1	7	395.3
82	195	2,3,4,5,6-2,3,4	3	8	429.8
83	208	2,3,4,5,6-2,3,5,6	4	9	464.2
84	194	2,3,4,5-2,3,4,5	2	8	429.8
85	205	2,3,4,5,6-3,4,5	2	8	429.8
86	206	2,3,4,5,6-2,3,4,5	3	9	464.2
87	OCN	Internal standard			
88	209	2,3,4,5,6-2,3,4,5,6	4	10	498.6

[a] Congeners that are enclosed in parentheses are not present in the standard in greater than trace amounts and were therefore ignored in estimating the average number of chlorines.

same bias applies to all samples, we can have great confidence in our comparisons of the rates and extent of dechlorination between treatments.

RESULTS

Dechlorination by HR microorganisms. The rate of dechlorination by HR microorganisms was similar for Aroclors 1242 and 1248 (Table 2), but the extent of dechlorination decreased with increasing degrees of chlorination. Aroclors 1242 and 1248 showed extensive dechlorination from the *meta* and *para* positions within 8 weeks (Fig. 1), but little subsequent dechlorination. By the end of 12 weeks, 85 and 75% of the chlorines in the *meta* plus *para* positions had been removed for Aroclors 1242 and 1248, respectively.

TABLE 2. Maximal observed dechlorination rates of the Aroclors tested for microorganisms collected from the two sites

Site	Aroclor	Mean rate ± SD (μg-atoms of Cl^- removed/g of sediment per week)[a]	Period (weeks)	% *meta* and *para* chlorine removed
HR	1242	$0.31^{AB} \pm 0.03$	0–8	85[b]
	1248	$0.34^{A} \pm 0.01$	0–8	75[b]
	1254	$0.22^{C} \pm 0.02$	0–8	63[c]
	1260[d]	$0.00^{D} \pm 0.03$	0–25	0[c]
	1260[e]	$0.04^{E} \pm 0.005$	16–24	15[f]
SL	1242	$0.30^{B} \pm 0.02$	0–4	46[g]
	1260	$0.21^{C} \pm 0.01$	12–16	19[g]

[a] Significant differences between rates (least significant difference test, 0.05 confidence level) are indicated by different capital letters next to means.
[b] After 12 weeks.
[c] After 25 weeks.
[d] Serum bottle experiment; sediments collected in August 1988.
[e] Serum tube experiment; sediments collected in January 1988.
[f] After 50 weeks.
[g] After 16 weeks.

There was no apparent dechlorination from the *ortho* positions. Aroclor 1254 was dechlorinated at a somewhat lesser rate (Table 2), but continuously throughout the 25 weeks of the experiment (Fig. 1). By then, an average of two *meta* plus *para* chlorines, or 63% of the chlorines in these positions, had been removed. The percent decrease for individual peaks in each Aroclor is given in Table 3.

Two attempts to dechlorinate Aroclor 1260 by using HR microorganisms were made. In one, the inoculum was pre-

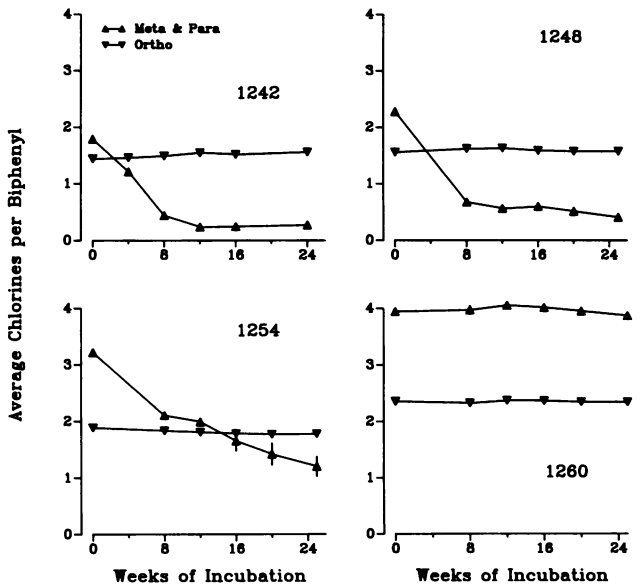

FIG. 1. Removal of chlorines by position from each Aroclor by microorganisms eluted from the HR sediments collected in August 1988. Where not shown, standard error bars are smaller than the symbols.

TABLE 3. Percent decrease of each peak in each Aroclor for both HR and SL inocula

Peak no.	Mean % decrease ± SD for[a]:					
	HR				SL	
	Aroclor 1242[b]	Aroclor 1248[b]	Aroclor 1254[b]	Aroclor 1260[c]	Aroclor 1242[b]	Aroclor 1260[b]
1	+	+	+		+	
2	+				37 ± 3	
3	+	+	+		+	
4	88 ± 1	+	+		+	
5	68 ± 9	+	+		81 ± 8	
6	72 ± 8	+	+		+	
7	+	+	+		+	+
8	63 ± 15	+			+	
9	97 ± 1	87 ± 1	+	+	98 ± 0	
10	86 ± 2	70 ± 16	+		+	+
11	+	+	+		+	+
12	93 ± 1	61 ± 16	+	+	23 ± 2	+
13	+	+	+		(+)	
14	80 ± 3	+			100 ± 0	+
15	76 ± 4	+	+	+	44 ± 14	
16	51 ± 8	+	+		+	+
17	89 ± 2	(−)	+		+	
18	95 ± 1	(−)	+	+	+	+
19	96 ± 1	100 ± 0	100 ± 0	+	95 ± 1	+
20	98 ± 0	87 ± 2	(−)		94 ± 2	
21	95 ± 1	96 ± 0	+		78 ± 3	
22	99 ± 2	97 ± 4	+		94 ± 1	
23	92 ± 0	91 ± 1	51 ± 14	+	91 ± 3	+
24	92 ± 0	84 ± 2	(+)	+	81 ± 3	+
25	79 ± 9	72 ± 12	+	+	100 ± 0	+
26	NR[d]	NR	NR	NR	100 ± 0	NR
27	+				83 ± 11	+
28	98 ± 0	98 ± 1	80 ± 14	+	99 ± 0	
29	97 ± 0	96 ± 2	(−)		89 ± 3	+
30	97 ± 1	97 ± 1	75 ± 2	+	78 ± 2	+
31						+
32	95 ± 1	84 ± 2	+	+	92 ± 2	+
33	94 ± 1	43 ± 12	+	+	85 ± 6	+
34	76 ± 18	(−)	(+)		(+)	+
35	93 ± 1	81 ± 6	69 ± 13	+	88 ± 6	
36	98 ± 1	95 ± 1	90 ± 3		99 ± 0	88 ± 12
37	95 ± 1	93 ± 2	81 ± 5	(−)	95 ± 1	61 ± 5
38	89 ± 2	97 ± 5	50 ± 13	+	71 ± 3	+
39	96 ± 1	94 ± 1	62 ± 6	+	95 ± 1	+
40	91 ± 1	92 ± 2	72 ± 5	(+)	87 ± 4	+
41	87 ± 2	74 ± 7	54 ± 7	+	84 ± 7	+
42						
43	86 ± 4	94 ± 3	70 ± 11	(−)	(−)	+
44	90 ± 2	85 ± 3	68 ± 13	(−)	78 ± 18	+
45	88 ± 2	87 ± 4	65 ± 13		91 ± 5	
46		91 ± 15	61 ± 5	(+)		(−)
47	90 ± 1	91 ± 3	69 ± 16	(+)	83 ± 4	45 ± 5
48	92 ± 1	87 ± 2	65 ± 5	(+)	90 ± 2	(−)
49	96 ± 8	74 ± 6	63 ± 10	(+)	75 ± 8	63 ± 1
50			60 ± 10	(−)		62 ± 2
51				+		+
52		100 ± 0	62 ± 9	+		+
53		59 ± 17	47 ± 12	(+)		37 ± 4
54	71 ± 3	66 ± 5	63 ± 8	71 ± 5	77 ± 7	65 ± 3
55	89 ± 0	100 ± 0	90 ± 18	(−)	99 ± 2	73 ± 4
56		58 ± 6	60 ± 3	77 ± 11		74 ± 3
57			(+)	(+)		(−)
58		(+)	(+)	(+)		+
59			44 ± 11	38 ± 1		61 ± 3
60	55 ± 11	(−)	54 ± 10	22 ± 3	72 ± 5	57 ± 4
61			52 ± 16	72 ± 9		65 ± 4
62			60 ± 7	+		+
63			53 ± 1	50 ± 1		55 ± 5
64		(+)	35 ± 5	(−)		60 ± 3

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TABLE 3—*Continued*

Peak no.	Mean % decrease ± SD for[a]:					
	HR				SL	
	Aroclor 1242[b]	Aroclor 1248[b]	Aroclor 1254[b]	Aroclor 1260[c]	Aroclor 1242[b]	Aroclor 1260[b]
65			42 ± 14	71 ± 6		58 ± 4
66			(−)	77 ± 9		74 ± 4
67			54 ± 17	(−)		56 ± 5
68			42 ± 8	(−)		48 ± 4
69			(−)	(−)		55 ± 4
70			48 ± 11	(−)		47 ± 5
71			49 ± 13	(−)		35 ± 6
72			(−)	(−)		38 ± 5
73			33 ± 7	(−)		44 ± 5
74				(−)		36 ± 4
75						52 ± 5
76				(−)		31 ± 3
77			28 ± 9	(−)		44 ± 5
78				(+)		
79				(−)		(−)
80				38 ± 5		(−)
81						34 ± 10
82				28 ± 1		(−)
83						(−)
84				(−)		(−)
85						30 ± 8
86				(+)		(−)

[a] Data are indicated only for peaks representing at least 0.1 mol% of either the total PCBs in the Aroclor or the total PCBs recovered. Numerical values are given for decreases that are significant by the *t* test at the 0.05 confidence level. Symbols: +, significant net increase; (+), insignificant net increase; (−), insignificant net decrease.

[b] Decrease after 16 weeks of incubation.

[c] Decrease after 50 weeks of incubation.

[d] NR, Not resolved.

pared from sediments collected in January 1988 and the incubation was carried out in serum tubes (2 ml of inoculum per tube). In the second, the inoculum was prepared from the same batch of sediments (collected in August 1988) as the experiments described above for Aroclors 1242, 1248, and 1254, and the incubation was carried out in serum bottles.

For the latter experiment, carried out with bottles, there was no evidence of Aroclor 1260 dechlorination after 25 weeks of incubation (Fig. 1). However, for the former experiment, performed with tubes, Aroclor 1260 dechlorination was first evident at 24 weeks and continued slowly until the end of the experiment at 50 weeks. These data show that the dechlorination of Aroclor 1260 followed a longer lag phase than did that of the less highly chlorinated Aroclors and occurred more slowly (Table 2). After 50 weeks of incubation in serum tubes, 15% of the *meta* plus *para* chlorines had been removed. The percent decrease for each Aroclor 1260 peak due to dechlorination by HR microorganisms in the first (serum tube) experiment is given in Table 3.

The dechlorination patterns for Aroclors 1242, 1248, and 1254 by HR microorganisms were similar in that in all cases 2-chlorobiphenyl (2-CB) (peak 1) and 2-2-CB/2,6-CB (peak 3) were the major products (Fig. 2 through 4). 2-3-CB (peak 5) and 2-4-CB/2,3-CB (peak 6) exhibited small decreases relative to most other peaks in Aroclor 1242, whereas 2,6-2-CB (peak 7) and 2,6-3-CB (peak 11) showed net increases. All of these congeners (peaks 5, 6, 7, and 11) accumulated during the dechlorination of Aroclors 1248 and 1254. (Peaks 8 [3,4-CB and 3-4-CB] and 13 [3,5-2-CB] were in all cases very small and were neglected in these comparisons of dechlorination patterns.) For Aroclor 1254, several other peaks also showed net increases, but this may have resulted from the lower dechlorination rate and the greater proportion of more highly chlorinated congeners in Aroclor 1254 that serve as the sources of these products. There are no apparent differences in microbial selectivity for congeners among these three Aroclors.

The dechlorination of Aroclor 1260 by the HR microorganisms, however, did follow a different pattern. Far more 2,5-2,5-CB (peak 23) accumulated than did 2,4-2,4-CB (peak 25) (Fig. 5); this is uncharacteristic of the dechlorination of the less highly chlorinated Aroclors by the HR microorganisms in the above experiments. Peak 39 (probably mainly 2,3,5-2,5-CB) was also a major product, and the only trichlorobiphenyls exhibiting net increases were 2,5-3-CB and 2,4-3-CB (peaks 15 and 16). These and other features were clearly different from those of the transformation patterns exhibited by the less highly chlorinated Aroclors (Fig. 2 through 4), indicating dechlorination by a different microbial system.

Dechlorination by Silver Lake microorganisms. The initial dechlorination rate of Aroclor 1242 by SL microorganisms was similar to that by the HR microorganisms (Table 2), but little dechlorination occurred after the first 4 weeks (Fig. 6). Therefore, dechlorination was less extensive than with HR microorganisms, as only 46% of the *meta* plus *para* chlorines were removed even after 16 weeks.

In dechlorinating Aroclor 1242, the SL microorganisms left several congeners besides the congeners substituted at only *ortho* positions, which were characteristic of dechlorination by the HR microorganisms. The most prominent of these were 2-4-CB/2,3-CB (peak 6), 2,4-2-CB (peak 10), and 2,4-4-CB/2,4,6-2-CB (peak 18) (Fig. 7). At the 8-week time point there was also a substantial amount of 2,4-2,4-CB

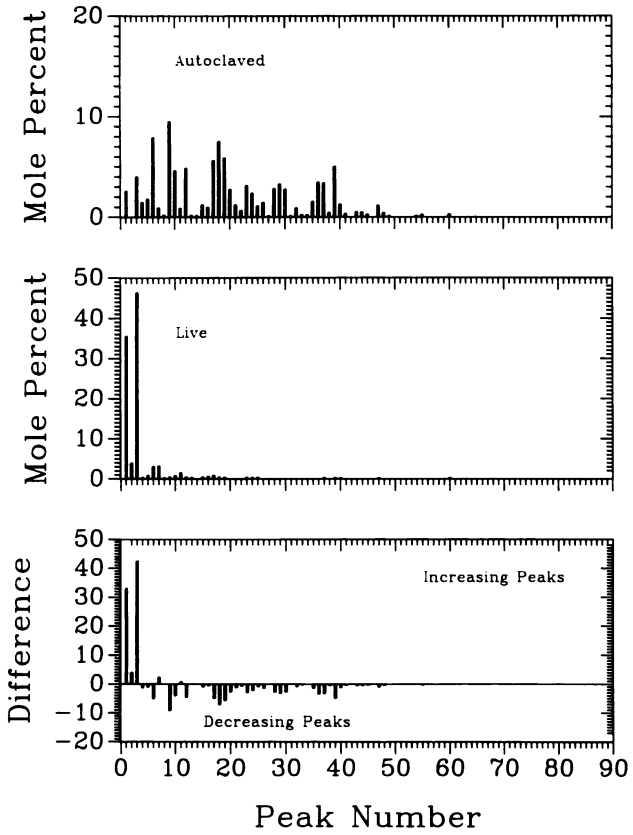

FIG. 2. Mole percentage of PCBs represented by each chromatographic peak before and after 16 weeks of incubation of Aroclor 1242 with microorganisms eluted from the HR sediments collected in August 1988. Pattern C dechlorination is indicated.

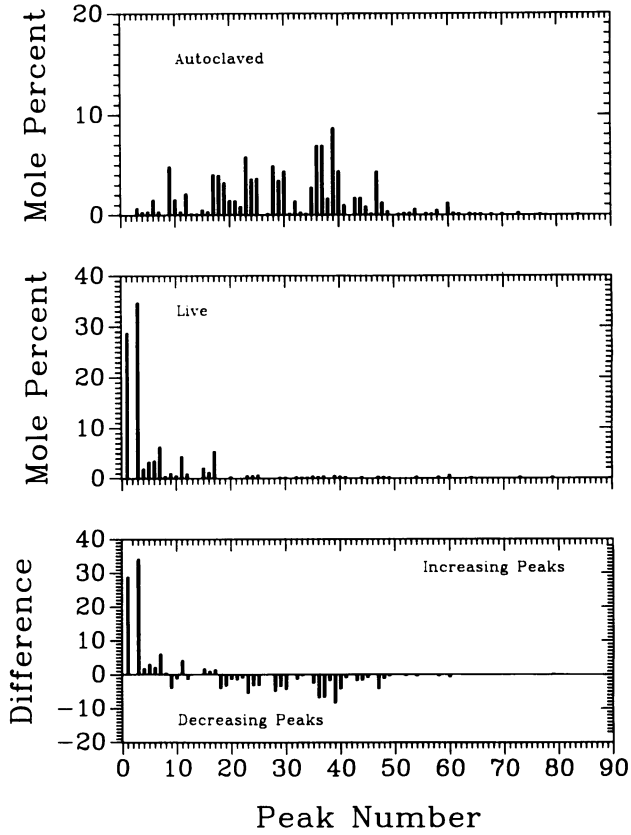

FIG. 3. Mole percentage of PCBs represented by each chromatographic peak before and after 16 weeks of incubation of Aroclor 1248 with microorganisms eluted from the HR sediments collected in August 1988. Pattern C dechlorination is indicated.

(peak 25), which subsequently diminished. All of these peaks represent congeners having 2-4-substitution patterns (peaks 10 and 18 may also be considered to have a 2,4- substitution pattern [Table 1]).

Dechlorination of Aroclor 1260 by the SL microorganisms was first detectable after 8 weeks (Fig. 6), and the rate increased to 0.21 μg-atoms of Cl^- removed per g of sediment per week between 12 and 16 weeks (Table 2). A total of 19% of the *meta* plus *para* chlorines were removed by the end of 16 weeks.

The most remarkable aspect of the Aroclor 1260 dechlorination pattern exhibited by the SL microorganisms was the high accumulation of 2,4-2,4-CB (peak 25) (Fig. 8). This single congener represented 18% (on a molar basis) of all of the PCBs recovered after 16 weeks of incubation. Because di- and trichlorinated congeners with 2-4- or 2,4- substitution patterns also accumulated from the dechlorination of Aroclor 1242, it appears very likely that both Aroclors were dechlorinated by the same microbial system.

DISCUSSION

These results demonstrate that the more heavily chlorinated Aroclors (Aroclors 1248, 1254, and 1260) can also be dechlorinated biologically. As observed previously for Aroclor 1242 (12), dechlorination occurred almost exclusively from the *meta* and *para* positions. However, the proportion of *meta* plus *para* chlorines removed within a given time interval tended to decrease with increasing degrees of chlorination. Similarly, whereas the maximum observed dechlorination rates were approximately equal for Aroclors 1242 and 1248, the rates decreased with higher levels of chlorination. A longer lag time was evident only for Aroclor 1260.

It was previously noted that greater dechlorinating activity was associated with PCB-contaminated sediments (12). This implies that there has been selection at contaminated sites for microorganisms capable of effecting dechlorination. The responsible selective pressures may arise from the ability to use PCBs as terminal-electron acceptors and/or from the ability to use the energy that is potentially available from dechlorination. Strain DCB-1 apparently obtains energy from the dechlorination of *meta*-chlorobenzoate (6a, 7, 11a). Electron acceptors are generally the factors limiting metabolism in anaerobic environments. Therefore, any microorganisms that could use PCBs as terminal-electron acceptors would be at a selective advantage (5).

It now appears that different dechlorination activities may be selected depending on the particular Aroclor present at a site. The SL inoculum exhibited both a shorter lag time and more rapid dechlorination of Aroclor 1260 than the HR inoculum. This may have been the result of previous exposure, since the SL site was contaminated primarily with Aroclor 1260. On the other hand, although the maximal observed dechlorination rates for Aroclor 1242 were similar for the two inocula, the HR microorganisms were able to

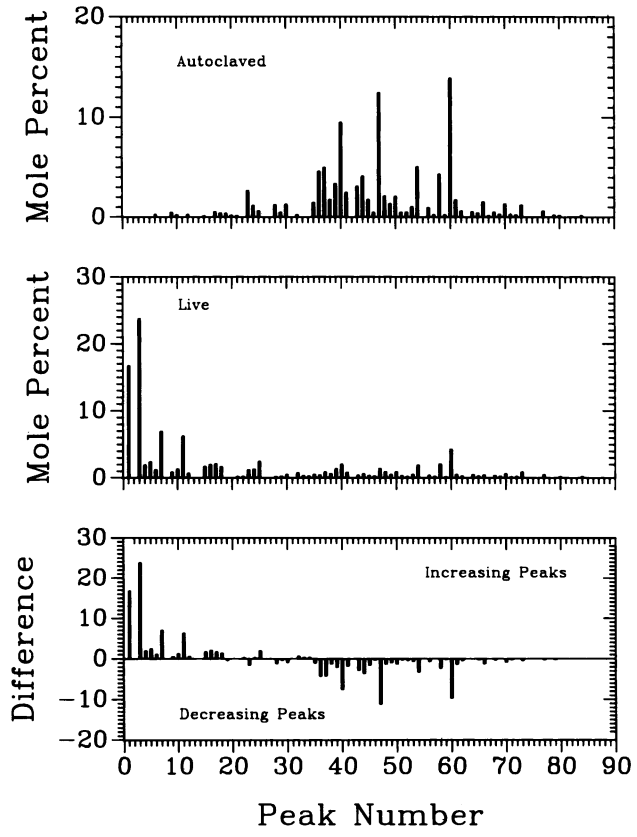

FIG. 4. Mole percentage of PCBs represented by each chromatographic peak before and after 16 weeks of incubation of Aroclor 1254 with microorganisms eluted from the HR sediments collected in August 1988. Pattern C dechlorination is indicated.

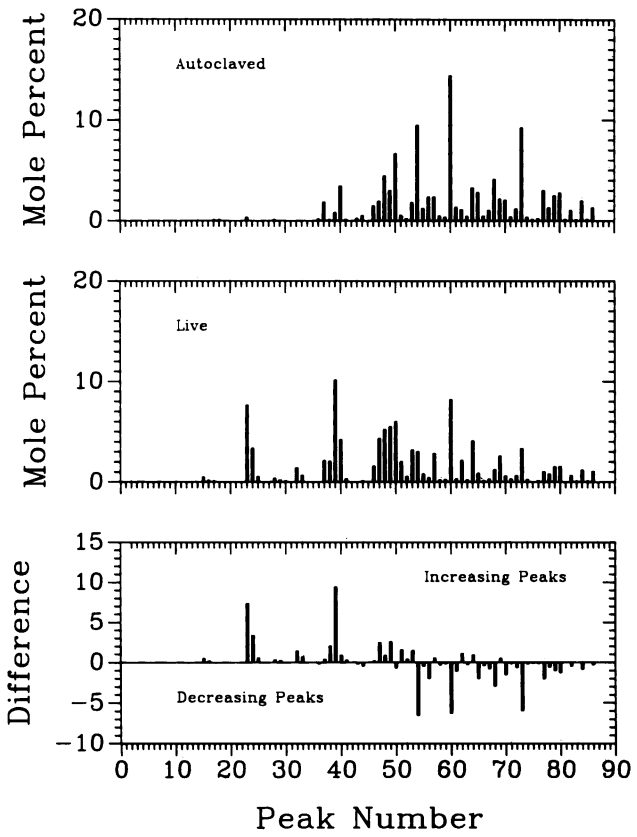

FIG. 5. Mole percentage of PCBs represented by each chromatographic peak before and after 50 weeks of incubation of Aroclor 1260 with microorganisms eluted from the HR sediments collected in January 1988. Pattern H dechlorination is indicated.

more extensively dechlorinate Aroclor 1242, the PCB mixture to which they were exposed for many years.

Four dechlorination patterns have been described for sediment samples taken from the upper HR (3–5). Three of these can be distinguished by the predominant dichlorobiphenyls that accumulate. The pattern obtained in these experiments from the dechlorination of Aroclor 1242 by the HR microorganisms closely resembles pattern C. In this pattern, 2-2-CB and/or 2,6-CB (coeluting isomers) are the only dichlorobiphenyls that accumulate. Thus, both *meta* and *para* chlorines are almost completely removed.

In these experiments the dechlorination of Aroclors 1242, 1248, and 1254 by HR microorganisms apparently followed the same type C dechlorination pattern, but dechlorination of Aroclor 1260 did not. The dechlorination of Aroclor 1260 by HR microorganisms in the serum tube experiment occurred only after a long lag time and then produced more 2,5-2,5-CB than 2,4-2,4-CB, with 2,5-3-CB and 2,4-3-CB as the only trichlorobiphenyls showing net increases. The observed pattern of PCB congener decreases and increases was essentially identical to dechlorination pattern H, which has been observed in sediments from New Bedford Harbor, Mass., Escambia Bay, Fla., the Housatonic River, Mass., and the upper portion of the HR estuary, N.Y., as well as in some laboratory cultures that were inoculated with upper HR sediments (*Research and Development Program for the Destruction of PCBs*, GE Corporate Research and Development, Schenectady, N.Y., 1989 [henceforth GE Report, 1989]; J. F. Brown, Jr., and R. E. Wagner, Environ. Toxicol. Chem., in press).

It appears from other data that dechlorination pattern C is the result of two separate and partially complementary dechlorination activities. The first of these to be discovered (12), and subsequently designated pattern Q (GE Report, 1989), is characterized by the accumulation of the dechlorination products found in pattern C plus several *meta*-substituted products such as 2-3-CB, 2,5-2-CB, and 2,5-2,5-CB. Under similar culture conditions, HR inocula have also yielded a second dechlorination pattern (pattern M, GE Report, 1989; G. D. Griffith, J. F. Quensen, S. A. Boyd, and J. M. Tiedje, unpublished data) characterized by the accumulation of the congeners found in pattern C plus several *para*-substituted products such as 2-4-CB, 2,4-2-CB, 2,6-4-CB, and 2,4-2,4-CB. The existence of these two distinct dechlorinating activities suggests that two PCB-dechlorinating populations may exist in the HR sediments. Culture conditions and the particular batch of sediment used may both contribute to determining whether one or both dechlorination patterns are expressed in a particular experiment.

The dechlorination of Aroclor 1260 by the HR microorganisms resulted in the accumulation of *ortho*- and *meta*-substituted products. In this respect, the congener specificity appears to be different from that obtained on the other Aroclors. This may have been because the HR microorganisms showing Aroclor 1260 dechlorination were eluted from sediments collected at a different time or because only a

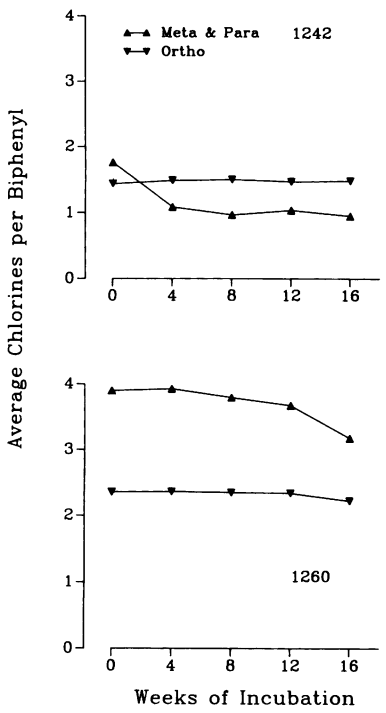

FIG. 6. Removal of chlorines by position from Aroclors 1242 and 1260 by microorganisms eluted from the SL sediments. All standard error bars were smaller than the symbols.

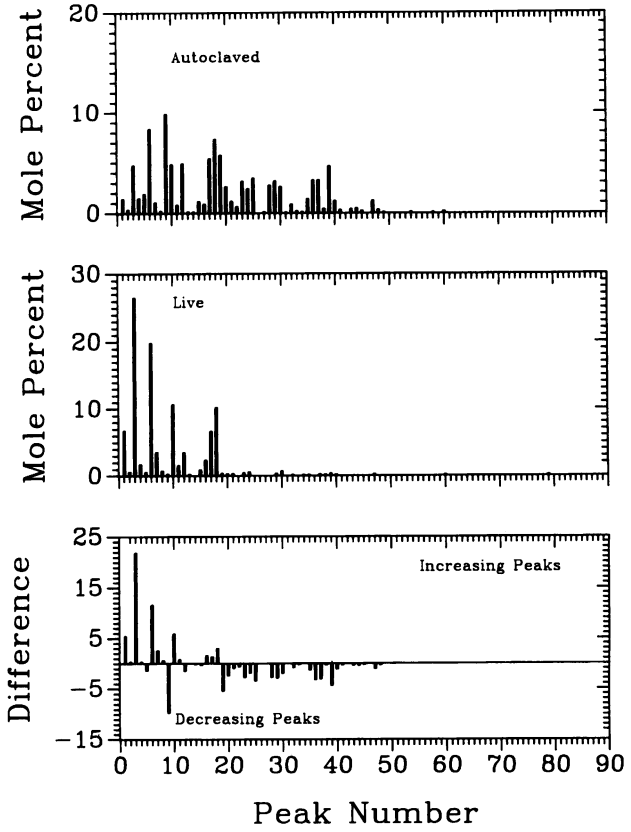

FIG. 7. Mole percentage of PCBs represented by each chromatographic peak before and after 16 weeks of incubation of Aroclor 1242 with microorganisms eluted from the SL sediments. This is probably pattern N dechlorination.

subset of the PCB-dechlorinating microorganisms present in the HR sediments are able to dechlorinate Aroclor 1260.

The dechlorination pattern obtained with the SL inoculum in these experiments is unlike either of the environmental dechlorination patterns (F and G) described for Aroclor 1260-contaminated SL sediments (3, 5). Instead it resembles pattern N found in Wood's Pond, a dammed stillwater on the Housatonic River a few miles below SL (J. F. Brown, Jr., personal communication). Pattern N has also been obtained in laboratory experiments using Wood's Pond sediments (D. L. Bedard, personal communication). Pattern N differs from patterns F and G in that it does not show the accumulation of 2,5-3-CB, 2,4-3-CB, or 2,5-2,5-CB. Instead, it tends to accumulate 2,6-4-CB, 2,4-4-CB, 2,4-2-CB, and 2,4-2,4-CB. Also characteristic of pattern N is the accumulation of 2,3,5,6-2,4-CB. This isomer eluted between peaks 49 and 50 (Table 1), increased over time in the chromatograms of our SL-Aroclor 1260 experiment, but was not quantitated because it does not exist in our calibration mixture. These products are all uncharacteristic of both patterns F and G. Although pattern N as described resulted from the dechlorination of Aroclor 1260, there are enough similarities in the dechlorination products formed from Aroclors 1242 and 1260 in our experiments to conclude that both Aroclors were probably dechlorinated by the same system.

The pattern N activity exhibited by the SL inoculum tended to preferentially remove *meta* rather than *para* chlorines, as does the pattern M component of the HR sediment. For Aroclor 1242 both patterns M and N give mostly *ortho*- and *para*-substituted di- and trichlorobiphenyls as dechlorination products, and both show the accumulation of 2,4-2,4-CB and the elimination of 2,5-2,5-CB. Pattern N activity, however, like patterns F and G, exhibited almost indiscriminant activity on the more highly chlorinated PCB congeners and was especially effective at dechlorinating the tetra- through hepta-CBs with 2,4,5-trichlorophenyl groups, most of which are not attacked by system M (Brown, personal communication).

There are several possible reasons why Aroclor 1260 is not dechlorinated as readily as Aroclor 1242. Although the SL microorganisms had been previously exposed to Aroclor 1260, they still dechlorinated Aroclor 1242 more rapidly. This may have been because Aroclor 1260 is less biologically available because of its lower water solubility, or there may be some congeners that are toxic or inhibit the dechlorination of others. Alternatively, certain less highly chlorinated congeners not present in Aroclor 1260 may stimulate dechlorination (for example, by supporting growth of the dechlorinating organisms).

The demonstration of Aroclor 1260 dechlorination is significant because the biological degradation of this particular Aroclor has not been previously demonstrated (at least by product formation) under controlled experimental conditions. It indicates that more PCB congeners are potentially degradable by a sequential anaerobic-aerobic treatment system. Even for many congeners reported to be aerobically degradable, a sequential anaerobic-aerobic system has an additional advantage. Aerobically, many of the more highly chlorinated Aroclor components are only hydroxylated, not actually mineralized. Because the anaerobic step removes chlorines that limit aerobic degradation, giving products that are aerobically degradable, an anaerobic-aerobic sequence has the potential to mineralize PCBs.

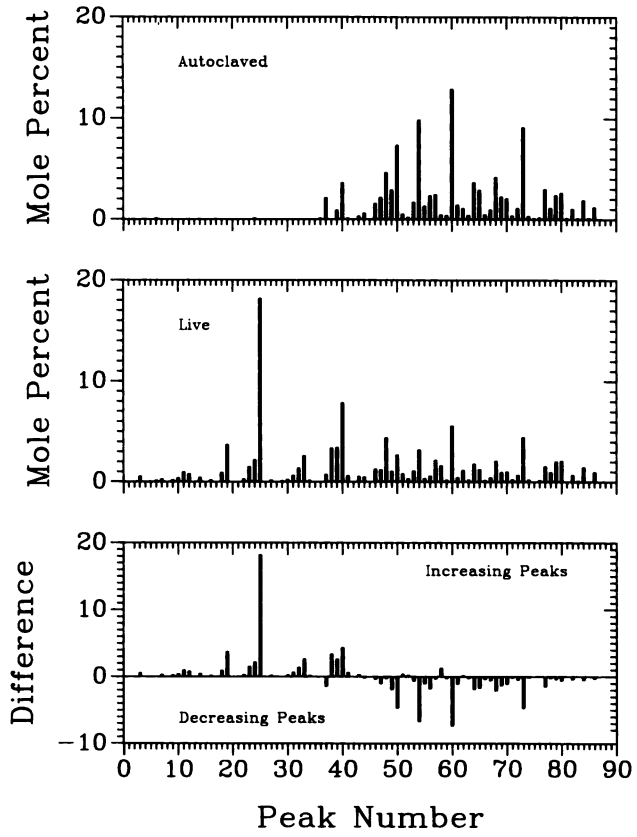

FIG. 8. Mole percentage of PCBs represented by each chromatographic peak before and after 16 weeks of incubation of Aroclor 1260 with microorganisms eluted from the SL sediments. Pattern N dechlorination is indicated.

The more PCB congeners that can be dechlorinated by the anaerobic step, the more completely all Aroclors can be degraded by a sequential system. The SL inoculum more readily dechlorinated the more highly chlorinated congeners present in Aroclor 1260 but accumulated *para*-substituted products. The HR inoculum more extensively dechlorinated Aroclor 1242 and did not accumulate *para*-substituted products. The existence of these different dechlorination patterns implies that there are different species or strains of microorganisms capable of dechlorinating PCBs in the two inocula, each with its own specificity. The complementary nature of the specificities observed for the HR and SL sediments suggests that greater overall dechlorination might be achieved by combining the two cultures.

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