APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1997, p. 4818–4825 Vol. 63, No. 12
0099-2240/97/$04.00+0

Temperature Determines the Pattern of Anaerobic Microbial Dechlorination of Aroclor 1260 Primed by 2,3,4,6-Tetrachlorobiphenyl in Woods Pond Sediment

QINGZHONG WU,[1,2]† DONNA L. BEDARD,[3] AND JUERGEN WIEGEL[1,2]*

Department of Microbiology[1] and Center for Biological Resource Recovery,[2] University of Georgia, Athens, Georgia 30602, and Characterization and Environmental Technology Laboratory, GE Research and Development Center, Schenectady, New York 12301[3]

Received 4 June 1997/Accepted 10 October 1997

Reductive dechlorination of the Aroclor 1260 residue in Woods Pond (Lenox, Mass.) sediment samples was investigated for a year at incubation temperatures from 4 to 66°C. Sediment slurries were incubated anaerobically with and without 2,3,4,6-tetrachlorobiphenyl (2346-CB; 350 μM) as a primer for dechlorination of the Aroclor 1260 residue. Dechlorination of the Aroclor residue occurred only in live samples primed with 2346-CB and only at 8 to 34°C and 50 to 60°C. The extent and pattern of polychlorinated biphenyl (PCB) dechlorination were temperature dependent. At 8 to 34°C, the dechlorination resulted in 28 to 65% decreases of the hexa- through nonachlorobiphenyls and corresponding increases in the tri- and tetrachlorobiphenyls. At 12 to 30°C, 30 to 40% of the hexa- through nonachlorobiphenyls were dechlorinated in just 3 months. The optimal temperature for overall chlorine removal was 20 to 27°C. We observed four different microbial dechlorination processes with different but partially overlapping temperature ranges, i.e., Process N (flanked *meta* dechlorination) at 8 to 30°C, Process P (flanked *para* dechlorination) at 12 to 34°C, Process LP (unflanked *para* dechlorination) at 18 to 30°C, and Process T (a very restricted *meta* dechlorination of specific hepta- and octachlorobiphenyls) at 50 to 60°C. These temperature ranges should aid in the development of strategies for the enrichment and isolation of the microorganisms responsible for each dechlorination process. The incubation temperature determined the relative dominance of the four PCB dechlorination processes and the extent and products of dechlorination. Hence, understanding the effects of temperature on PCB dechlorination at contaminated sites should assist in predicting the environmental fate of PCBs or planning bioremediation strategies at those sites.

Polychlorinated biphenyls (PCBs) are ubiquitous contaminants that remain a public concern because of their persistence and bioaccumulation in the environment and their potential toxicity to humans and wildlife (13, 19, 25). PCBs can be oxidatively degraded under aerobic conditions, but the process generally occurs only with PCB congeners with five or fewer chlorines (6, 10). Recently, it has been recognized that highly chlorinated PCB congeners with six to nine chlorines can be dehalogenated under anaerobic conditions (2, 7, 8, 11, 14, 16, 22). However, there are substantial differences in the extent of environmental dechlorination at different PCB-contaminated sites. For example, extensive microbial dechlorination of PCBs has occurred in the upper Hudson River via substantial *meta* and *para* dechlorination (14, 16), while the PCBs in Woods Pond (Lenox, Mass.) have been only slightly dechlorinated, via limited *meta* and *para* dechlorination (7).

The sediments of the Housatonic River are contaminated with PCBs from storm sewer discharge and drainage from a transformer-manufacturing operation located in Pittsfield, Mass. Some of the PCB-contaminated sediments have accumulated in Woods Pond, a shallow impoundment located 11 miles downstream of Pittsfield. The pond sediments are contaminated with a PCB mixture (15 to 180 μg/g of sediment [dry weight]) composed of tri- to octachlorobiphenyls, the residue from partially dechlorinated Aroclor 1260, and with weathered hydrocarbon oil (5,000 to 32,000 μg/g of sediment [dry weight]) (7). Recent data reveal that extensive dechlorination of the PCBs has occurred in a few locations in Woods Pond and in the Housatonic River upstream of the pond (9). However, in many locations, the dechlorination is far less extensive. Reasons for limited environmental dechlorination of PCBs may include unfavorable growth conditions for PCB-dechlorinating microorganisms such as temperature, nutrients, bioavailability, and cocontaminants (1–3, 5, 20, 21, 23, 24, 26, 27).

We sought to investigate how temperature affects dechlorination of the Aroclor 1260 in Woods Pond sediment. Summer temperatures in Woods Pond sediments range from 15°C at a 45-cm depth to 18 to 20°C at a 10- to 15-cm depth. Winter temperatures drop to 1 to 4°C at all depths. One objective of our study was to determine whether temperature effects are responsible for the limited dechlorination that has occurred in Woods Pond and, conversely, whether raising the temperature would accelerate dechlorination.

A second objective was to study how temperature affects the pattern of PCB dechlorination in sediment. A microbial dechlorination process is a set or series of dechlorination reactions that determines which PCB congeners are substrates, which chlorines will be removed from those congeners, and the order in which they will be removed (8). In each of the PCB-

* Corresponding author. Mailing address: Department of Microbiology, University of Georgia, 215 Biological Science Building, Athens, GA 30602-2605. Phone: (706) 542-2651. Fax: (706) 542-2674. E-mail: JWIEGEL@uga.cc.uga.edu.

† Present address: Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC 29425.

contaminated sites that have been studied, several different PCB dechlorination processes appear to be responsible for the microbial dechlorination that has occurred (7, 8, 14, 15, 16, 26). Maximal chlorine removal appears to require the complementary action of two or more dechlorination processes (8, 22).

It has been proposed that discrete dechlorinating microorganisms harboring dehalogenases with different regiospecificities are responsible for the various dechlorination processes that have been described (5, 8, 11, 12, 14–16, 22). Since microorganisms exhibit different temperature ranges for growth, it is likely that temperature influences which dechlorination processes are active within a sediment, but this topic has not yet been investigated. Three distinct microbial PCB dechlorination processes, Processes N, P, and LP, can be primed in Woods Pond sediment by the addition of elevated concentrations (200 to 500 μM) of certain PCB congeners (5, 8, 11, 12, 28). For the study reported here, we chose to use 2,3,4,6-tetrachlorobiphenyl (2346-CB) as a primer because the congener itself and its products, which can be unequivocally identified, can be dechlorinated by several different routes, including *ortho*, *meta*, and *para* dechlorination (4, 29, 30), and because 2346-CB can prime extensive dechlorination of Aroclor 1260 by at least two distinct but complementary microbial dechlorination processes (4).

Previously, we showed that the discrete dechlorination reactions of 2346-CB and its intermediate products are strongly temperature dependent (30). In this study, we sought to understand how temperature, especially the environmentally relevant temperature range of 8 to 22°C, affects the extent and pattern of microbial dechlorination of PCBs. We discovered that temperature determines the relative dominance of the three major dechlorination processes that occur in Woods Pond, and we established temperature ranges for each dechlorination process. This information should aid in developing strategies to enrich and isolate the microorganisms responsible for each dechlorination process. We also determined that substantial *meta* dechlorination (Process N) of the Aroclor residue can be primed at temperatures as low as 8°C. Furthermore, raising the temperature alone without the addition of a primer did not stimulate PCB dechlorination. Hence, temperature effects alone cannot explain the limited dechlorination observed in Woods Pond.

MATERIALS AND METHODS

Sediment collection and storage. Methanogenic sediment from the western shore of Woods Pond was collected, stored, and prepared for the experiments as described previously (29).

Preparation of slurries and incubation. Sediment slurries were prepared under a stream of O_2-free nitrogen gas by mixing wet sediment (8.5 volumes) with K_2HPO_4-KH_2PO_4 buffer (1.5 volumes, pH 6.9). Hence, the sediment pH approximated the original pH of the sediment as measured in Woods Pond (pH 6.9 to 7.2). The final concentration of potassium phosphate was 10 mM. The slurries contained 0.15 g of sediment (dry weight) per ml. The homogenization, dispensation, and preparation of the individual incubations were done as described previously (29).

The controls included sterilized sediment slurries and sediment slurries without the addition of 2346-CB. The sterile controls were autoclaved twice for 1 h at 121°C on 2 consecutive days to eliminate viable spores before the PCB congener was added. Triplicate samples and sterile controls were amended with 2346-CB (350 μmol per liter of slurry) and incubated in the dark without shaking at the following temperatures: 4, 8, 12, 15, 18, 20, 22, 25, 27, 30, 34, 37, 40, 45, 50, 55, 60, and 66°C. Most samples were incubated in water baths as described previously (29). Thermometers were calibrated with a U.S. Environmental Protection Agency-certified water analysis thermometer (Fisher Scientific; a National Institute of Standards and Technique traceable certificate showed actual readings at seven calibration temperatures with ±0.02°C). Temperatures in the water baths were checked at least weekly and maintained at the desired temperature with a variation of less than ±1°C.

The samples incubated at temperatures from 15 to 34°C and at 50°C were respiked with 2346-CB (350 μM) when at least 75% of the congener had been dechlorinated (30). The first observed dechlorination product of 2346-CB was 2,4,6-trichlorobiphenyl (246-CB) at all temperatures except 18°C. At 18°C, however, the major dechlorination product of 2346-CB was 236-CB. Thus, repeat incubations were carried out at 15, 18, 20, and 22°C.

Sample extraction and analysis. PCB extraction and analysis were performed as described previously (29). Briefly, PCBs were extracted with anhydrous diethyl ether containing octachloronaphthalene (4 ppm) as an internal standard and analyzed with a gas chromatograph (5890 series II; Hewlett-Packard, Wilmington, Del.) equipped with a DB-1 polydimethylsiloxane-phase capillary column (30 m by 0.25 mm [inside diameter] by 0.25 μm; J & W Scientific, Folsom, Calif.) and a Ni^{63} electron capture detector.

In this paper, the individual PCB congeners will be identified by listing the substituted positions on each ring, separated by a hyphen followed by the designation -CB (chlorobiphenyl). 2346-CB and its dechlorination products were identified and quantified as described previously (29). The PCBs in the Aroclor 1260 residue were identified and quantified by use of a calibration standard consisting of Aroclors 1242, 1254, and 1260 (70:20:10) that had been previously characterized by Northeast Analytical, Inc., Schenectady, N.Y., with the weight percent PCB congener distributions published by Frame et al. (18). Congener assignments for Aroclor peaks that are composed of coeluting congeners include only those congeners determined to be significant peak components in Aroclor 1260 (18) and its in situ dechlorination products verified by qualitative gas chromatography-mass spectrometry (7). The PCBs in each peak were quantified by use of a second-order calibration curve generated from standards at six calibration levels. The PCB congener distribution and homolog distribution for each sample were calculated and reported in units of mole percent after the peaks corresponding to 2346-CB and its dechlorination products were subtracted. The homolog distribution of the Aroclor residue and the number of *ortho*, *meta*, and *para* chlorines per biphenyl were calculated on the basis of the assumption that there was no loss of the biphenyl moiety in the dechlorination process and that all coeluting congeners increased or decreased to the same extent as a result of dechlorination (22).

The concentration of the 2346-CB primer (350 μM = 681 μg/g of sediment [dry weight]) was more than 18-fold higher than the total concentration of the PCBs in the sediment (~37 μg/g of sediment [dry weight]); hence, the peaks containing 2346-CB and all of its dechlorination products (peaks 2, 4, 5, 11, 14, and 35) were deleted in our analyses. Consequently, all congeners that coelute with 2346-CB and its products were also excluded from the analyses. Only 1 to 2 mol% of the PCBs found in typical Woods Pond sediment samples was lost by this modification of the analysis (28), but several potential dechlorination products could not be measured, e.g., 2-2-CB, which coeluted with 26-CB, and 26-3-CB, which coeluted with 236-CB. Thus, quantifying the Aroclor residues in terms of mole percent led to a conservative evaluation of the dechlorination since it did not take into consideration several potential dechlorination products.

RESULTS AND DISCUSSION

Dechlorination of the Aroclor 1260 residue primed by 2346-CB: temperature range and extent of the dechlorination. Significant dechlorination of the Aroclor 1260 residue occurred in samples primed with 2346-CB (350 μM) but only at temperatures from 8 to 34°C and, to a more limited extent, from 50 to 60°C (Fig. 1A and 2A and Table 1). The observed temperature range for dechlorination of the Aroclor residue paralleled that of the 2346-CB primer (Fig. 1B and 2B). The dechlorination was selective and progressed with the incubation time. No dechlorination occurred in autoclaved controls. Furthermore, no PCB dechlorination was detected for over a year (380 days) of incubation at any temperature in samples not primed with 2346-CB. These data indicate that the dechlorination is biological and that changes in the incubation temperature alone (i.e., raising it above the ambient temperature) cannot be used to initiate dehalogenation of PCBs in these sediments.

The extent of dechlorination of the Aroclor residue was temperature dependent. At 8 to 34°C, the 2346-CB-primed dechlorination of the Aroclor residue resulted in large decreases in the hexa- through nonachlorobiphenyls and increases in the tri- and tetrachlorobiphenyls (Fig. 1A). The total concentration of pentachlorobiphenyls stayed fairly constant, most likely due to a net balance between increases in pentachlorobiphenyls formed from dechlorination of hexa- through nonachlorobiphenyls and decreases in pentachlorobiphenyls dechlorinated to tri- and tetrachlorobiphenyls. At 3 months, 30 to 40% of the hexa- through nonachlorobiphenyls were

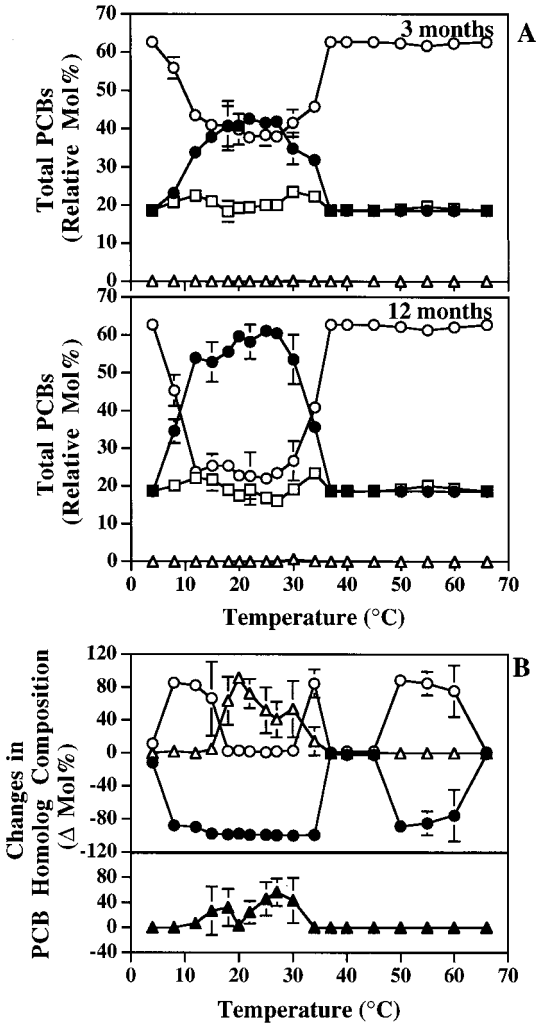

FIG. 1. Changes observed in the homolog distribution of PCBs in Woods Pond sediment samples at temperatures from 4 to 66°C. (A) Homolog distribution of the Aroclor 1260 residue after 3 and 12 months of incubation. Symbols: △, dichlorobiphenyls; ●, tri- plus tetrachlorobiphenyls; □, pentachlorobiphenyls; ○, hexa- through nonachlorobiphenyls. (B) Homolog distribution of 2346-CB and its dechlorination products after 12 months of incubation. Symbols: ▲, monochlorobiphenyls (2-CB plus 4-CB); △, dichlorobiphenyls (24-CB plus 26-CB); ○, trichlorobiphenyls (236-CB plus 246-CB); ●, tetrachlorobiphenyl (2346-CB). The data represent averages for the triplicate samples. Standard deviations are represented by vertical bars; if no bar is evident, the deviation was smaller than the size of the symbol.

dechlorinated at all temperatures from 12 to 30°C. This dechlorination increased throughout the 1-year incubation (Fig. 1A). After a year, the extent of dechlorination ranged from a 28% decrease of the hexa- through nonachlorobiphenyls at 8°C to a 63 to 65% decrease at 20 to 27°C (Fig. 1A).

At 50 to 60°C, 31 to 89 mol% of the 2346-CB primer was dechlorinated to 246-CB (Fig. 1B) (30). However, dechlorination of the Aroclor residue was restricted to *meta* dechlorination of specific hepta- and octachlorobiphenyls (e.g., 2345-245-CB, 2345-234-CB, and 2345-2345-CB), which were converted to hexachlorobiphenyls (e.g., 245-245-CB and 234-245-CB). The data for 55°C is shown in Table 1. Overall, the hepta- and octachlorobiphenyls at 50 to 60°C showed significant decreases of 13.9 to 14.4% (compared to a standard deviations of ±3%). These were matched by corresponding increases of 13.5 to 14.4% in the hexachlorobiphenyls. No significant dechlorination of di- through pentachlorobiphenyls was observed.

Temperature-dependent changes in *ortho*, *meta*, and *para* dechlorination of the Aroclor residue. The dechlorination of the Aroclor residue was more restricted than that of 2346-CB but generally followed the same trend. The major difference is that no *ortho* dechlorination of the Aroclor 1260 residue was detected at any temperature, although strong *ortho* dechlorination of 246-CB (the first product of 2346-CB) to 24-CB and then 4-CB was observed from 8 to 30°C (Fig. 2B) (30). Thus, we assume that the microorganisms capable of *ortho*-dechlorinating 246-CB or 24-CB exhibit a high substrate specificity for these lower-chlorinated congeners.

A comparison of the chlorine distributions of the Aroclor

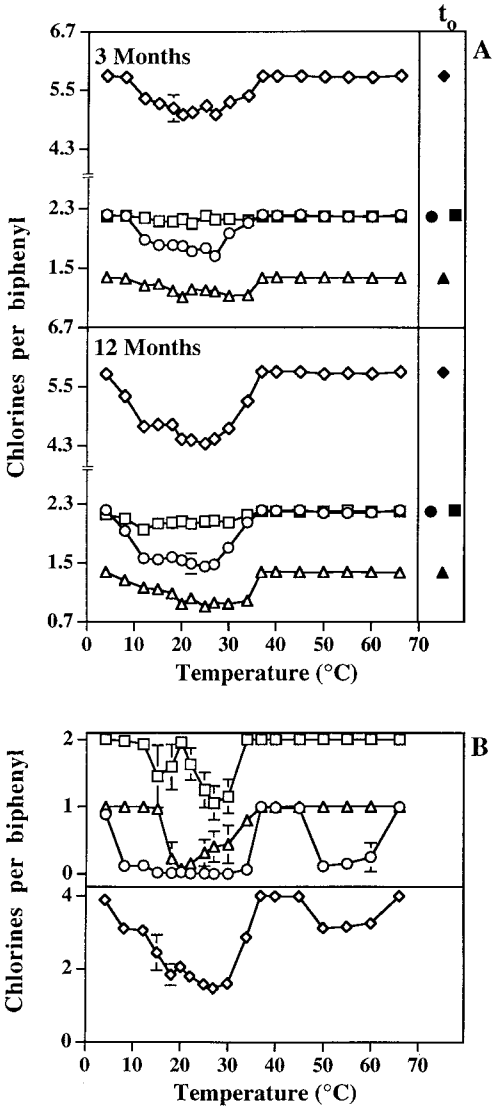

FIG. 2. Residual chlorines of PCBs in Woods Pond sediment samples at temperatures from 4 to 66°C. (A) Numbers of *ortho*, *meta*, *para*, and total chlorines per biphenyl of the Aroclor 1260 residue after 3 and 12 months of incubation; (B) numbers of chlorines of 2346-CB after 12 months of incubation. Symbols: □, *ortho* chlorines; ○, *meta* chlorines; △, *para* chlorines; ◇, total chlorines. The corresponding closed symbols indicate the values at the start of incubation. The data represent averages for the triplicate samples. Standard deviations are represented by vertical bars; if no bar is evident, the deviation was smaller than the size of the symbol. Data in panel B are from reference 29.

TABLE 1. Dechlorination of selected PCB congeners of the Aroclor 1260 residue in 2346-CB-amended Woods Pond samples at various temperatures after 1 year of incubation[a]

Peak no.	PCB congener	Mol% at t_0[b]	Mol% of congener at temp (°C) of:														
			8	12	15		18		20		22		25	27	30	34	55
					1st[c]	2nd[c]	1st	2nd	1st	2nd	1st	2nd					
10	26-2	0.00	0.00 ± 0.00	0.06 ± 0.04	0.48 ± 0.68	0.34 ± 0.08	0.35 ± 0.48	2.49 ± 0.09	4.56 ± 0.18	2.95 ± 0.09	2.60 ± 1.59	2.28 ± 1.62	4.94 ± 0.21	3.91 ± 0.65	1.11 ± 0.72	0.10 ± 0.14	0.00 ± 0.00
14	25-2, 4-4	0.08	0.08 ± 0.03	0.08 ± 0.00	0.08 ± 0.01	0.46 ± 0.09	1.06 ± 0.45	1.79 ± 0.09	7.39 ± 0.36	3.06 ± 0.05	1.68 ± 1.02	2.30 ± 1.12	4.59 ± 1.54	2.95 ± 0.55	2.27 ± 1.07	0.08 ± 0.01	0.08 ± 0.01
15	24-2	0.14	0.40 ± 0.08	0.14 ± 0.01	1.33 ± 1.89	1.95 ± 0.58	0.10 ± 0.07	2.29 ± 0.30	—[d]	2.02 ± 0.17	1.95 ± 1.23	1.82 ± 0.62	2.34 ± 1.28	3.23 ± 0.12	1.11 ± 0.52	1.72 ± 2.43	0.14 ± 0.02
17	23-2, 26-4	0.29	1.17 ± 0.23	1.47 ± 0.05	2.68 ± 0.51	2.25 ± 0.16	0.34 ± 0.01	1.76 ± 0.11	2.25 ± 0.08	1.92 ± 0.06	2.74 ± 0.90	2.05 ± 0.02	2.21 ± 0.20	2.10 ± 0.09	1.22 ± 0.16	0.36 ± 0.00	0.64 ± 0.15
26	24-26	1.60	3.55 ± 0.77	7.98 ± 0.11	6.29 ± 0.49	5.64 ± 0.59	0.77 ± 0.53	4.10 ± 0.21	4.11 ± 0.25	4.14 ± 0.22	5.46 ± 2.64	6.32 ± 1.67	4.21 ± 0.48	4.30 ± 0.76	3.24 ± 0.98	1.29 ± 0.19	1.83 ± 0.10
31	25-25	1.62	2.74 ± 0.13	3.04 ± 0.12	4.56 ± 1.32	5.35 ± 0.38	4.91 ± 1.44	5.96 ± 0.25	6.57 ± 0.41	5.57 ± 0.05	5.86 ± 1.03	6.58 ± 0.21	7.97 ± 0.04	7.72 ± 0.16	10.39 ± 2.05	8.55 ± 2.32	1.63 ± 0.23
32	24-25	2.42	5.12 ± 0.21	6.02 ± 0.15	7.87 ± 2.74	8.38 ± 1.53	2.58 ± 0.24	7.95 ± 0.79	7.08 ± 0.47	7.77 ± 0.13	8.69 ± 1.13	10.96 ± 1.33	10.93 ± 0.74	10.40 ± 1.15	10.08 ± 2.79	4.60 ± 0.50	2.54 ± 0.12
33	24-24	3.10	7.82 ± 1.19	13.29 ± 0.08	12.66 ± 0.93	12.81 ± 0.98	2.09 ± 0.82	8.15 ± 0.21	8.30 ± 0.32	12.98 ± 0.60	10.75 ± 3.54	15.69 ± 2.98	8.39 ± 0.86	8.79 ± 1.25	5.89 ± 1.51	4.09 ± 0.09	3.42 ± 0.42
37	23-25	0.53	0.48 ± 0.05	0.29 ± 0.01	0.19 ± 0.01	0.36 ± 0.05	0.41 ± 0.13	0.22 ± 0.02	0.23 ± 0.12	0.31 ± 0.06	0.21 ± 0.11	0.23 ± 0.05	0.18 ± 0.01	0.21 ± 0.08	1.31 ± 0.17	1.71 ± 0.16	0.54 ± 0.41
44	246-24	0.85	3.25 ± 0.64	4.58 ± 0.24	2.72 ± 0.27	4.20 ± 0.37	0.32 ± 0.03	4.16 ± 0.17	3.22 ± 0.57	3.61 ± 0.24	4.21 ± 2.00	4.68 ± 0.12	4.23 ± 0.31	6.75 ± 0.34	4.63 ± 0.47	0.36 ± 0.00	1.58 ± 0.76
49	236-24	1.24	3.11 ± 0.19	3.37 ± 0.04	3.87 ± 0.05	3.09 ± 0.31	1.29 ± 0.02	3.05 ± 0.06	2.47 ± 0.12	3.04 ± 0.07	3.11 ± 0.52	3.46 ± 0.58	2.90 ± 0.16	3.10 ± 0.39	3.36 ± 0.82	1.24 ± 0.10	1.32 ± 0.10
51	235-25, 236-23	1.18	1.21 ± 0.07	1.23 ± 0.05	1.11 ± 0.55	1.05 ± 0.11	1.71 ± 0.23	1.07 ± 0.05	1.02 ± 0.15	1.11 ± 0.07	0.98 ± 0.35	0.94 ± 0.12	1.00 ± 0.12	1.03 ± 0.02	1.05 ± 0.51	5.29 ± 0.55	1.23 ± 0.25
53	245-25, 235-24	3.22	3.27 ± 0.30	5.35 ± 0.10	3.98 ± 0.56	3.60 ± 0.33	2.40 ± 0.37	1.70 ± 0.56	3.79 ± 0.44	3.73 ± 0.08	3.33 ± 2.01	1.20 ± 0.64	0.97 ± 0.06	1.44 ± 0.54	2.29 ± 0.96	1.85 ± 0.18	3.36 ± 0.11
56	235-23	0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.68 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.51 ± 0.72	0.50 ± 0.23
67	234-35, 235-34, 2356-24	1.04	2.49 ± 0.36	4.37 ± 0.05	4.37 ± 0.31	3.66 ± 0.08	1.12 ± 0.02	3.69 ± 0.04	4.74 ± 0.03	3.79 ± 0.08	4.84 ± 0.50	3.88 ± 0.16	4.92 ± 0.15	4.46 ± 0.23	3.30 ± 0.24	0.91 ± 0.02	1.12 ± 0.14
69	236-245, 245-34	6.92	3.87 ± 0.54	1.40 ± 0.01	1.89 ± 0.20	1.73 ± 0.09	5.75 ± 0.71	2.07 ± 0.04	1.55 ± 0.01	1.57 ± 0.07	1.51 ± 0.42	1.30 ± 0.28	1.69 ± 0.36	1.76 ± 0.12	3.08 ± 0.51	6.30 ± 0.24	6.56 ± 0.20
75	245-245	7.88	5.01 ± 0.79	1.57 ± 0.03	1.97 ± 0.26	1.80 ± 0.14	6.61 ± 0.70	2.58 ± 0.03	1.69 ± 0.02	1.60 ± 0.10	1.54 ± 0.62	1.09 ± 0.06	1.39 ± 0.16	1.66 ± 0.10	2.23 ± 0.10	0.71 ± 0.10	9.99 ± 0.38
82	234-245, 236-345, 2356-34	7.64	4.63 ± 0.41	2.47 ± 0.04	2.65 ± 0.65	2.53 ± 0.12	5.62 ± 0.96	3.08 ± 0.17	2.39 ± 0.05	1.87 ± 0.01	2.44 ± 0.40	1.76 ± 0.50	1.89 ± 0.14	1.98 ± 0.17	2.48 ± 0.88	3.80 ± 0.14	8.51 ± 1.20
88	2356-245	4.53	4.03 ± 0.28	2.06 ± 0.06	2.82 ± 0.40	2.75 ± 0.19	4.79 ± 0.12	3.09 ± 0.02	2.56 ± 0.07	2.05 ± 0.10	2.34 ± 0.72	1.44 ± 0.07	2.67 ± 0.22	2.96 ± 0.07	4.10 ± 0.18	5.59 ± 0.12	4.57 ± 0.20
90	2346-245	2.03	1.74 ± 0.20	0.94 ± 0.03	0.81 ± 0.30	0.72 ± 0.09	2.41 ± 0.13	1.19 ± 0.06	0.85 ± 0.06	0.77 ± 0.03	0.68 ± 0.42	0.49 ± 0.02	0.48 ± 0.05	0.52 ± 0.02	0.68 ± 0.18	1.86 ± 0.20	2.47 ± 0.11
93	2345-236	3.16	2.12 ± 0.21	0.69 ± 0.02	0.67 ± 0.19	0.57 ± 0.09	3.31 ± 0.12	1.22 ± 0.05	0.54 ± 0.01	0.62 ± 0.08	0.39 ± 0.28	0.31 ± 0.04	0.29 ± 0.04	0.36 ± 0.02	0.48 ± 0.14	0.84 ± 0.10	3.13 ± 0.12
102	2345-245	6.90	4.78 ± 0.49	2.22 ± 0.04	1.50 ± 0.70	1.45 ± 0.17	7.44 ± 0.35	3.21 ± 0.21	1.44 ± 0.04	1.27 ± 0.04	0.81 ± 0.52	0.72 ± 0.12	0.64 ± 0.07	0.79 ± 0.02	0.76 ± 0.28	1.95 ± 0.18	4.89 ± 1.72
106	2345-234	2.53	1.49 ± 0.12	0.53 ± 0.02	0.49 ± 0.17	0.41 ± 0.06	2.05 ± 0.11	1.07 ± 0.11	0.43 ± 0.02	0.55 ± 0.09	0.24 ± 0.12	0.32 ± 0.18	0.12 ± 0.01	0.12 ± 0.01	0.16 ± 0.11	0.64 ± 0.17	1.34 ± 0.32
110	2345-2346, 23456-245	2.00	1.84 ± 0.07	1.18 ± 0.04	1.08 ± 0.19	0.84 ± 0.09	1.70 ± 0.02	0.88 ± 0.05	1.01 ± 0.02	0.93 ± 0.05	0.82 ± 0.26	0.69 ± 0.05	0.88 ± 0.08	0.97 ± 0.05	0.92 ± 0.30	1.26 ± 0.02	1.78 ± 0.10
115	2345-2345	1.28	1.08 ± 0.04	0.73 ± 0.04	0.72 ± 0.03	0.53 ± 0.05	1.20 ± 0.04	0.52 ± 0.02	0.64 ± 0.02	0.55 ± 0.05	0.56 ± 0.05	0.49 ± 0.03	0.63 ± 0.07	0.64 ± 0.07	0.65 ± 0.08	0.64 ± 0.08	0.85 ± 0.09

[a] For reference, 100 mol% equals 15 μM. All data are the means of triplicate samples ± standard deviations. The standard deviation of each peak in three analyses of the same sample was ±4%.
[b] The mole percent value at the start of incubation.
[c] 1st, the first incubation; 2nd, a repeated incubation started at a later time (for further details, see reference 30).
[d] Not resolved from peak 14.

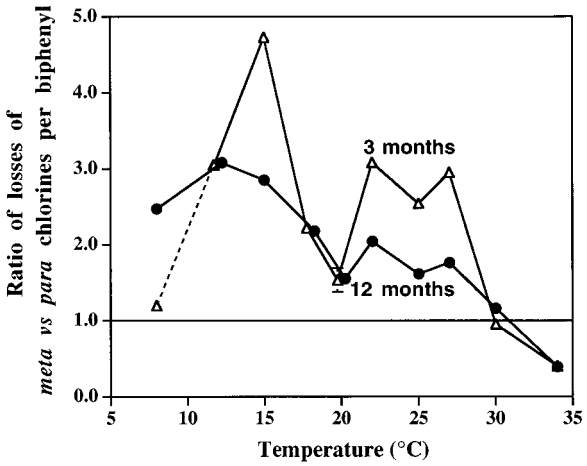

FIG. 3. Ratio of losses of *meta* chlorines to losses of *para* chlorines per biphenyl as a function of temperature after 3 and 12 months of incubation. The *meta* and *para* chlorine losses at 8°C after 3 months were extremely low; hence, a dotted line is used to indicate uncertainty in this data point. The data represent averages for the triplicate samples. Standard deviations are represented by vertical bars; if no bar is evident, the deviation was smaller than the size of the symbol.

1260 residue throughout the incubation time indicated significant decreases in *meta* and/or *para* chlorines at temperatures between 8 and 34°C (Fig. 2A). A high degree of *meta* dechlorination occurred from 12 to 27°C, while less *meta* dechlorination occurred at temperatures beyond this range. In contrast, the final extent of *para* dechlorination was fairly constant within the higher temperature range of 20 to 34°C and decreased below 20°C. As shown in Fig. 3, more *meta* than *para* chlorines were removed at all temperatures except 30 and 34°C. The progressive decrease in the relative proportions of *meta* and *para* dechlorination between 12 and 34°C reflects temperature-dependent shifts in the relative activity of three major microbial dechlorination processes (see below).

Temperature-dependent changes in the dechlorination pattern of the Aroclor residue. We consider the analysis of the qualitative temperature-dependent changes in the dechlorination pattern to be more important than the overall quantitative changes in the major congeners documented in Table 1 because they demonstrate that temperature can alter the route of dechlorination and hence the final products. The difference in the dehalogenation reactions occurring at the various temperatures is evident from the changes in the final concentrations of the various congeners (Fig. 4 and 5).

We concluded earlier (30) that temperature determines the relative proportions of *meta* and *para* dechlorination of 2346-CB and whether 246-CB will be dechlorinated. From a comparison of the dechlorination products of 2346-CB and 246-CB with the dechlorination patterns of the Aroclor 1260 observed between 8 and 34°C (see below), we now conclude that the specific dechlorination reactions of 2346-CB or 246-CB that occur at a particular temperature determine which dechlorination process(es) will be primed. Specifically, *meta* dechlorination of 2346-CB primes the onset of flanked *meta* dechlorination (Process N), *para* dechlorination of 2346-CB primes flanked *para* dechlorination (Process P), and unflanked *para* dechlorination of 246-CB primes unflanked *para* dechlorination (Process LP). Temperature determines the relative activity of each dechlorination Process.

(i) Dechlorination Process N. At temperatures from 8 to 15°C, the major dechlorination products were 24-24-CB, 24-25-CB, 24-26-CB, 25-26-CB, and 2356-24-CB (Table 1 and Fig. 4B and 5A). This pattern of dechlorination is characterized by an almost exclusive loss of flanked *meta* chlorines, i.e., *meta* chlorines that have neighboring *ortho* or *para* chlorines (8, 22, 28), and is known as Process N. The *meta* dechlorination of the Aroclor residue parallels the predominant *meta* dechlorination of 2346-CB to 246-CB observed at these temperatures (Fig. 2B) (29, 30).

(ii) Dechlorination Process LP. At temperatures from 18°C (the second incubation) to 30°C, the pattern of dechlorination exhibited the products characteristic of Process N but also exhibited dechlorination products characteristic of Process LP (Table 1 and Fig. 4C and 5B). Process LP removes flanked *para* chlorines from many congeners and unflanked *para* chlorines from congeners containing 24- and 246-chlorophenyl groups and hence is characterized by strong increases of 26-2-CB, 25-2-CB, and 24-2-CB (12). Compared to the results from Process N, the combination of Processes N and LP resulted in smaller increases in 24-24-CB and 24-26-CB. This is because the 24-24-CB and 24-26-CB formed by Process N dechlorination were converted to 24-2-CB and 26-2-CB, respectively, by dechlorination Process LP. 2-2-CB was probably also formed from further dechlorination of 24-2-CB (12), but this could not be confirmed because our analysis did not resolve 2-2-CB from the 26-CB produced by dechlorination of the 2346-CB primer. Process LP is particularly important because it further dechlorinates the products of Process N dechlorination. The sequential *meta* and then *para* dechlorination of the residual Aroclor 1260 was analogous to dechlorination of 2346-CB to 246-CB and then to 26-CB that occurred in the same temperature range (30). Unfortunately, little or no unflanked *para* dechlorination of the Aroclor residue was observed at temperatures below 18°C.

(iii) Dechlorination Process P. At 34°C, a significant change in the pattern of dechlorination occurred again: 25-25-CB, 24-25-CB, 235-25-CB, and 235-23-CB showed the largest increases (Table 1 and Fig. 4D and 5C), while most congeners containing 234-, 245-, and 2345-chlorophenyl rings decreased. These changes indicate that Process P dechlorination predominates at 34°C. Process P is characterized by the highly selective removal of *para* chlorines flanked by at least one *meta* chlorine and by a more limited dechlorination than Process N (5, 8, 11). The strong occurrence of Process P and the abrupt cessation of Process N at this temperature were somewhat unexpected.

Substantial increases in 25-25-CB were also observed at 30°C and, to a lesser extent, at 12 to 27°C, suggesting that Process P may have also occurred at these temperatures. In addition, the especially high increases of 24-25-CB that occurred at 22 to 30°C (Table 1) indicate that Process P contributed to the formation of this congener. Dechlorination Processes P and N both produce 24-25-CB, but for Processes P (Fig. 4D and 5C) (5) and N (Fig. 4B and 5A) (22, 28), the relative increase of 24-25-CB is lower than that of 25-25-CB or 24-24-CB, respectively. Hence, the proportionally higher increases in 24-25-CB relative to those of 25-25-CB and 24-24-CB that occurred at 22 to 30°C indicate that this congener was formed by the concerted action of both dechlorination processes at these temperatures.

(iv) Dechlorination Process T. At the thermobiotic temperatures of 50 to 60°C, dechlorination of the Aroclor residue was restricted to a highly specific *meta* dechlorination of 2345-chloro-substituted hepta- and octachlorobiphenyls (Fig. 5D). This was evident from decreases in 2345-2345-CB, 2345-245-CB, 2345-234-CB, and 2345-2346-CB and corresponding increases in 245-245-CB, 245-234-CB, and 2346-245-CB (Table 1 and Fig. 5D). No significant dechlorination of hexachlorobiphenyls

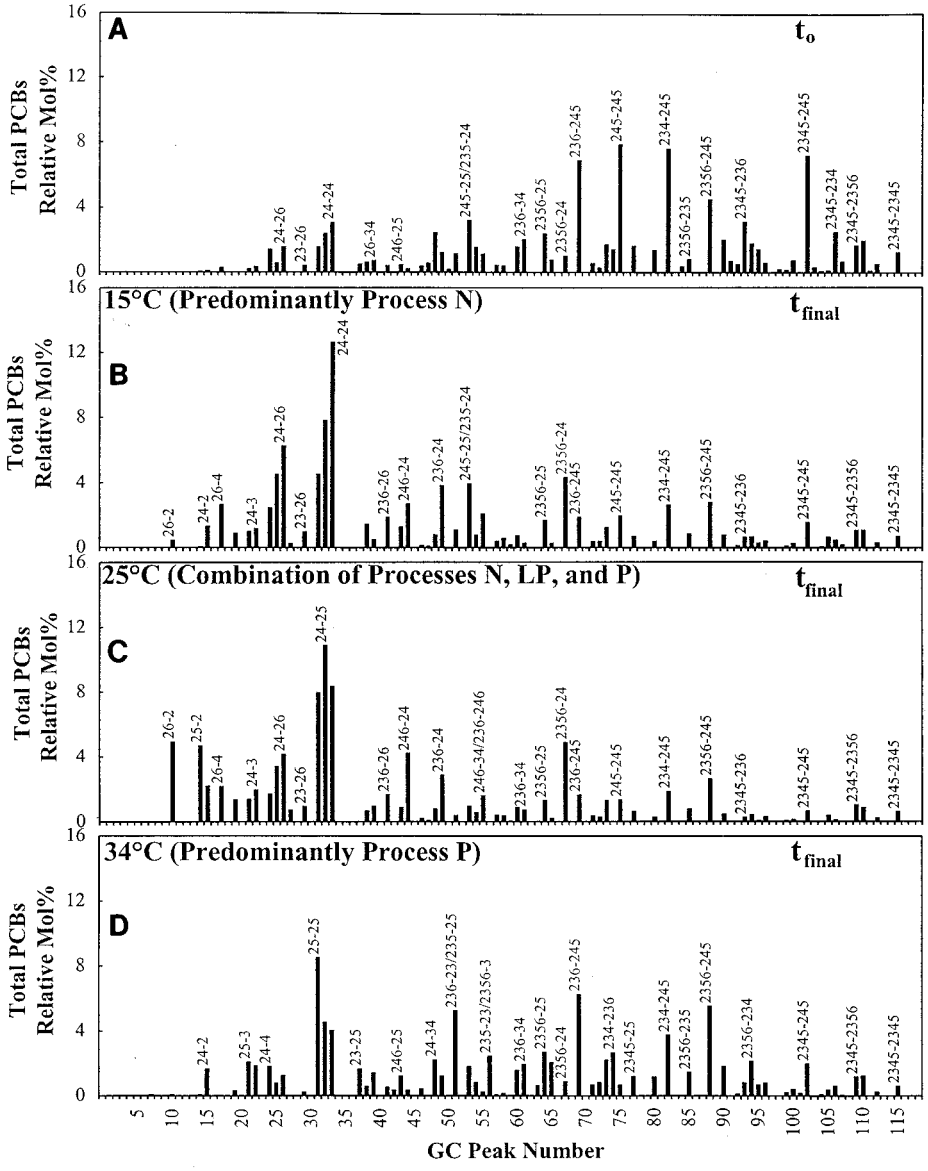

FIG. 4. Congener distribution of the Aroclor 1260 residue in Woods Pond sediment samples at the start (A) (t_0) of the incubation and after 365 to 372 days (t_{final}) of incubation at 15 (B), 25 (C), and 34°C (D). The dechlorination was predominantly Process N at 15°C (B); a combination of Processes N, LP, and P at 25°C (C), and predominantly Process P at 34°C (D). See reference 5 for a complete list of PCB congener assignments. The data represent averages for the triplicate samples. See Table 1 for standard deviations of values for major congeners. GC, gas chromatography.

was observed, and no tri- or tetrachlorobiphenyls were formed. This restricted dechlorination pattern apparently represents a new microbial dechlorination process, which we designate Process T. The same hepta- and octachlorobiphenyls are also dechlorinated by Process N, but the following two properties of Process N make it clear that the thermobiotic dechlorination is a separate activity. (1) The first congeners to decrease in Process N dechlorination are penta- and hexachlorobiphenyls, not hepta- and octachlorobiphenyls (data not shown). (2) Process N dechlorination of 2345-2345-CB, 2345-245-CB, and 2345-234-CB does not result in even-transient increases of 245-245-CB or 234-245-CB (data not shown). This is apparently because Process N dechlorinates these intermediates more rapidly than their parent congeners.

Temperature ranges for the microbial dechlorination processes. Our data show that the four dechlorination processes we observed in Woods Pond sediment have different temperature ranges and provide further support for the hypothesis that discrete PCB-dechlorinating populations catalyze these dechlorination processes. Process N occurred at temperatures from 8 to 30°C, Process LP occurred from 18 to 30°C, and Process T occurred from 50 to 60°C. Process P could be clearly identified only at 34°C, but the relative increases in 25-25-CB and 24-25-CB indicate that it also contributed to the *para* dechlorination that occurred from 22 to 30°C and, to a lesser extent, from 12 to 20°C.

Temperature-related variations among replicate samples. Variations among triplicate samples were generally restricted to quantitative differences and time-dependent differences that were consistent over the 1-year incubation period and did not change the observed dechlorination pattern. In agreement with the data on the dechlorination of 2346-CB (30), the variations

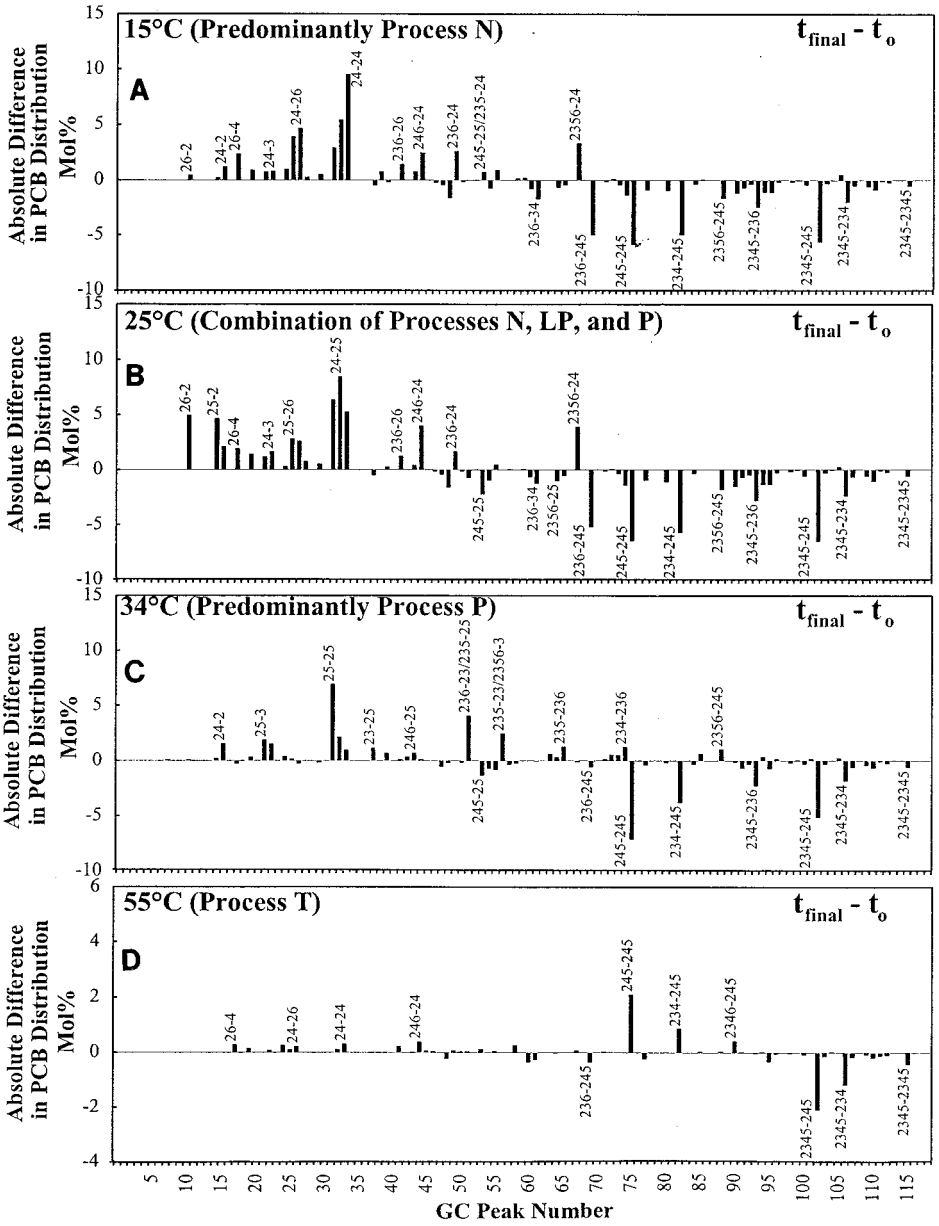

FIG. 5. Absolute difference in the congener distribution of the Aroclor 1260 residue between (t_0) and (t_{final}) ($t_{final} - t_0$) at 15 (A); a combination of Processes N, LP, and P) at 25°C (B); Process P at 34°C (C); and Process T at 55°C. The data represent averages for the triplicate samples. See reference 5 for a complete list of PCB congener assignments. The data represent averages for the triplicate samples. GC, gas chromatography.

were highest at incubation temperatures at which several dechlorination processes occurred together, i.e., from 18 to 30°C, indicating that small differences in the samples intensified the temperature-induced effects.

The greatest variation at a single temperature occurred at 18°C. The extent and pattern of dechlorination of the Aroclor residue at 18°C were so different from those observed at slightly higher or lower temperatures that repeat incubations were done at 15, 18, 20, and 22°C. In the first incubation at 18°C, the major trichlorobiphenyl formed from 2346-CB was 236-CB (*para* dechlorination), instead of 246-CB, the *meta* dechlorination product found at most tested temperatures, and the Aroclor 1260 residue was only slightly dehalogenated compared to the results at higher or lower temperatures. Significant increases of 26-2-CB, 25-2-CB, 25-25-CB, and 235-25-CB occurred and were accompanied by decreases in 24-26-CB, 24-24-CB, and 245-25-CB. The dechlorination pattern showed modest removal of both flanked and unflanked *para* chlorines and may represent a weak LP dechlorination. The results of the second set of incubations at 15, 20, and 22°C matched those of the initial incubations (Table 1), but the results at 18°C were comparable to those of the incubations at 20 and 22°C and not to the results of the first incubation. This time, the 2346-CB primer was dechlorinated to 246-CB. The extent and pattern of dechlorination of the Aroclor residue were also comparable to those for the incubations at 20 and 22°C, namely, a combination of Processes N, LP, and P (Table 1 and Fig. 4C and 5B). We proposed earlier that 18°C may be a transition point at

which members of the community supporting *para* dechlorination become more dominant (30). This might explain the absence of Process N activity in the first incubation at 18°C. However, regardless of the reason, these findings clearly demonstrate that differences in the onset of the dechlorination can have strong effects on the overall dechlorination.

Implications from this study. The addition of 2346-CB primed *meta* and/or *para* dechlorination of the Aroclor 1260 residue in Woods Pond sediment samples over a wide range of temperatures, including the environmentally relevant temperatures of 8 to 22°C and the thermobiotic temperatures of 50 to 60°C. Dechlorination of 246-CB, a dechlorination product of 2346-CB, further extended the dechlorination of the Aroclor residue by priming removal of unflanked *para* chlorines at temperatures from 18 to 30°C. Temperature determined which PCB dechlorination processes were active and strongly affected the relative dominance of the different dechlorination processes and, hence, the extent and specificity of the dechlorination. These data indicate that understanding the effects of temperature on dechlorination at a given site will assist in predictions of environmental fate and in development of bioremediation strategies. To our knowledge, this is the first detailed report of the influence of temperature on the pattern of PCB dechlorination. However, we believe, based on our published data (29, 30) comparing the influence of temperature on the dechlorination of 2346-CB in Woods Pond sediment and a PCB-free sediment (Sandy Creek Nature Park Pond) and on Fish's findings on the effects of temperature on the dechlorination of Aroclor 1242 in Hudson River sediment (17), that the kind of effects we observed are common at other sites. The strong dominance of Process N dechlorination at 8 to 20°C, temperatures that prevail in Woods Pond from spring through fall, is consistent with previous observations of the dechlorination that has occurred in situ (7). However, temperature effects do not explain the limited dechlorination at this site. The temperature ranges that we established for each of the four dechlorination processes should aid in the development of strategies for the enrichment and isolation of the microorganisms responsible for each dechlorination process.

ACKNOWLEDGMENT

This work was supported by an equipment grant to J.W. from the Georgia Research Alliance.

REFERENCES

1. **Abramowicz, D. A., M. J. Brennan, H. M. Van Dort, and E. L. Gallagher.** 1993. Factors influencing the rate of polychlorinated biphenyl dechlorination in Hudson River sediments. Environ. Sci. Technol. **27:**1125–1131.
2. **Alder, A. C., M. M. Häggblom, S. R. Oppenheimer, and L. Y. Young.** 1993. Reductive dechlorination of polychlorinated biphenyls in anaerobic sediments. Environ. Sci. Technol. **27:**530–538.
3. **Assaf-Anid, N., L. Nies, and T. M. Vogel.** 1992. Reductive dechlorination of a polychlorinated biphenyl congener and hexachlorobenzene by vitamin B_{12}. Appl. Environ. Microbiol. **58:**1057–1060.
4. **Bedard, D. L., S. C. Bunnell, and J. M. Principe.** Unpublished data.
5. **Bedard, D. L., S. C. Bunnell, and L. A. Smullen.** 1996. Stimulation of microbial *para* dechlorination of polychlorinated biphenyls that have persisted in Housatonic River sediment for decades. Environ. Sci. Technol. **30:**687–694.
6. **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.
7. **Bedard, D. L., and R. J. May.** 1996. Characterization of the polychlorinated biphenyls (PCBs) in the sediment of Woods Pond: evidence for *in situ* dechlorination of Aroclor 1260. Environ. Sci. Technol. **30:**237–245.
8. **Bedard, D. L., and J. F. Quensen III.** 1995. Microbial reductive dechlorination of polychlorinated biphenyls, p. 127–216. *In* L. Y. Young and C. Cerniglia (ed.), Microbial transformation and degradation of toxic organic chemicals. Wiley-Liss Division, John Wiley & Sons, Inc., New York, N.Y.
9. **Bedard, D. L., L. A. Smullen, and R. J. May.** 1996. Microbial dechlorination of highly chlorinated PCBs in the Housatonic River, p. 117. *In* Abstracts of the 1996 International Symposium on Subsurface Microbiology.
10. **Bedard, D. L., R. Unterman, L. H. Bopp, M. J. Brennan, M. L. Haberl, and C. Johnson.** 1986. Rapid assay for screening and characterizing microorganisms for the ability to degrade polychlorinated biphenyls. Appl. Environ. Microbiol. **51:**761–768.
11. **Bedard, D. L., H. M. Van Dort, S. C. Bunnell, L. M. Principe, K. A. DeWeerd, R. J. May, and L. A. Smullen.** 1993. Stimulation of reductive dechlorination of Aroclor 1260 contaminant in anaerobic slurries of Woods Pond sediment, p. 19–21. *In* Anaerobic dehalogenation and its environmental implications. Abstracts of the 1992 American Society for Microbiology Conference, Athens, Ga. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.
12. **Bedard, D. L., H. M. Van Dort, R. J. May, and L. A. Smullen.** 1997. Enrichment of microorganisms that sequentially *meta*-, *para*-dechlorinate the residue of Aroclor 1260 in Housatonic River sediment. Environ. Sci. Technol. **31:**3308–3313.
13. **Borlakoglu, J. T., and K. D. Haegeles.** 1991. Comparative aspects in the bioaccumulation, metabolism and toxicity with PCBs. Comp. Biochem. Physiol. C **100:**327–338.
14. **Brown, J. F., Jr., D. L. Bedard, M. J. Brennan, J. C. Carnahan, H. Feng, and R. E. Wagner.** 1987. Polychlorinated biphenyl dechlorination in aquatic sediments. Science **236:**709–712.
15. **Brown, J. F., Jr., and R. E. Wagner.** 1990. PCB movement, dechlorination, and detoxication in the Acushnet estuary. Environ. Toxicol. Chem. **9:**1215–1233.
16. **Brown, J. F., Jr., R. E. Wagner, H. Feng, D. L. Bedard, M. J. Brennan, J. C. Carnahan, and R. J. May.** 1987. Environmental dechlorination of PCBs. Environ. Toxicol. Chem. **6:**579–593.
17. **Fish, K. M.** Personal communication.
18. **Frame, G. M., R. E. Wagner, J. C. Carnahan, J. F. Brown, Jr., R. J. May, L. A. Smullen, and D. L. Bedard.** 1996. Comprehensive, quantitative, congener-specific analyses of eight Aroclors and complete PCB congener assignments on DB-1 capillary GC columns. Chemosphere **33:**603–623.
19. **Kimbrough, R. D.** 1995. Polychlorinated biphenyl (PCBs) and human health: an update. Crit. Rev. Toxicol. **25:**133–163.
20. **Morris, P. J., W. W. Mohn, J. F. Quensen III, J. M. Tiedje, and S. A. Boyd.** 1992. Establishment of a polychlorinated biphenyl-degrading enrichment culture with predominantly *meta* dechlorination. Appl. Environ. Microbiol. **58:**3088–3094.
21. **Nies, L., and T. M. Vogel.** 1990. Effects of organic substrates on dechlorination of Aroclor 1242 in anaerobic sediments. Appl. Environ. Microbiol. **56:**2612–2617.
22. **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.
23. **Quensen, J. F., III, M. J. Zwiernik, and S. A. Boyd.** 1993. Effects of high concentrations of oil and heavy metals on PCB dechlorination, abstr. Q-146, p. 373. *In* Abstracts of the 93rd General Meeting of the American Society for Microbiology 1993. American Society for Microbiology, Washington, D.C.
24. **Rhee, G.-Y., B. Bush, C. M. Bethoney, A. DeNucci, H.-M. Oh, and R. C. Sokol.** 1993. Reductive dechlorination of Aroclor 1242 in anaerobic sediments: pattern, rate, and concentration dependence. Environ. Toxicol. Chem. **12:**1025–1032.
25. **Safe, S. H.** 1994. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. Crit. Rev. Toxicol. **24:**87–149.
26. **Sokol, R. C., O.-S. Kwon, C. M. Bethoney, and G.-Y. Rhee.** 1994. Reductive dechlorination of polychlorinated biphenyls in St. Lawrence River sediments and variations in dechlorination characteristics. Environ. Sci. Technol. **28:**2054–2064.
27. **Tiedje, J. M., J. F. Quensen III, J. Chee-Sanford, J. P. Schimel, J. A. Cole, and S. A. Boyd.** 1993. Microbial reductive dechlorination of PCBs. Biodegradation **4:**231–240.
28. **Van Dort, H. M., L. A. Smullen, R. J. May, and D. L. Bedard.** 1997. Priming *meta*-dechlorination of polychlorinated biphenyls that have persisted in Housatonic River sediments for decades. Environ. Sci. Technol. **31:**3300–3307.
29. **Wu, Q., D. L. Bedard, and J. Wiegel.** 1996. Influences of incubation temperatures on the reductive dechlorination of 2,3,4,6-tetrachlorobiphenyl in two freshwater sediments. Appl. Environ. Microbiol. **62:**4174–4179.
30. **Wu, Q., D. L. Bedard, and J. Wiegel.** 1997. Effect of incubation temperature on the route of microbial reductive dechlorination of 2,3,4,6-tetrachlorobiphenyl in polychlorinated biphenyl (PCB)-contaminated and PCB-free freshwater sediments. Appl. Environ. Microbiol. **63:**2836–2843.