APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 1997, p. 2836–2843 Vol. 63, No. 7
0099-2240/97/$04.00+0

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

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

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

Received 12 November 1996/Accepted 25 April 1997

We studied the influence of temperature (4 to 66°C) on the microbial dechlorination of 2,3,4,6-tetrachlorobiphenyl (2,3,4,6-CB) incubated for 1 year in anaerobic sediments from Woods Pond in Lenox, Mass., and Sandy Creek Nature Center Pond (SCNC) in Athens, Ga. Seven discrete dechlorination reactions were observed, four of which occurred in both sediments. These were 2,3,4,6-CB → 2,4,6-CB, 2,3,4,6-CB → 2,3,6-CB, 2,4,6-CB → 2,6-CB, and 2,3,6-CB → 2,6-CB. Three additional reactions occurred only in Woods Pond sediment. These were 2,4,6-CB → 2,4-CB, 2,4-CB → 2-CB, and 2,4-CB → 4-CB. The dechlorination reactions exhibited at least four different temperature dependencies in SCNC sediment and at least six in Woods Pond sediment. We attribute the discrete dechlorination reactions to different polychlorinated biphenyl (PCB)-dechlorinating microorganisms with distinct specificities. Temperature influenced the timing and the relative predominance of parallel pathways of dechlorination, i.e., *meta* versus *para* dechlorination of 2,3,4,6-CB and *ortho* versus *para* dechlorination of 2,4,6-CB and 2,4-CB. *meta* dechlorination of 2,3,4,6-CB to 2,4,6-CB dominated at all tested temperatures except at 18 and 34°C, where *para* dechlorination to 2,3,6-CB dominated in some replicates. The dechlorination of 2,4,6-CB was restricted to ~15 to 30°C in both sediments. Temperature affected the lag time preceding the dechlorination of 2,4,6-CB in both sediments and affected the preferred route of its dechlorination in Woods Pond sediment. *para* dechlorination dominated at 20°C, and *ortho* dechlorination dominated at 15°C, but at 18 and 22 to 30°C the relative dominance of *ortho* versus *para* dechlorination of 2,4,6-CB varied. These data indicate that field temperatures play a significant role in controlling the nature and the extent of the PCB dechlorination that occurs at a given site.

Polychlorinated biphenyls (PCBs) were used in a wide variety of applications for more than 50 years and are major pollutants in the United States. Their environmental fate is important because they accumulate in biota and have been associated with potential health effects. The major continental sink for PCBs is freshwater sediment (19); consequently, the discovery of PCB dehalogenation in freshwater and estuarine sediments has generated much interest (1, 3–5, 9–13, 15, 20, 21) because dehalogenation is expected to detoxify PCBs (5, 6).

Repeated attempts by several laboratories to isolate PCB-dechlorinating microorganisms have been unsuccessful, and dechlorination activity has been observed only in the presence of sediment (5, 14, 22, 28). Like other dehalogenating microorganisms, PCB dechlorinators probably function in syntrophic communities and may be dependent on those communities for electron donors, micronutrients, and optimal hydrogen concentration (5, 18).

At least six distinct microbial dechlorination processes can be recognized in various contaminated sediments on the basis of congener selectivity and the products observed in situ and in laboratory studies (5, 10, 13). It has been proposed that different microorganisms containing distinct dehalogenating enzymes with different congener specificities are responsible for the various identified dechlorination processes (3, 5, 7, 8, 10, 11, 21, 25, 26, 31, 32). Maximal chlorine removal appears to require the complementary action of two or more microbial dechlorination processes (5, 15). The sequence of dechlorination is very important because this determines how many chlorines are removed and which products accumulate. The effect of temperature on this phenomenon has not previously been investigated.

Our previous reports of the effect of temperature on the dechlorination of 2,3,4,6-tetrachlorobiphenyl (2,3,4,6-CB) in freshwater sediments from two different climates indicated that *ortho*, *meta*, and *para* dechlorination exhibited different temperature ranges and optima, but the effects of those differences on the sequence of dechlorination were not investigated (29, 30). Our previous data also suggested competition between *ortho* and *para* dechlorination. These data are consistent with the hypothesis that several different dechlorinating enzymes, and probably several different dechlorinating microorganisms, are involved in PCB dechlorination in these sediments.

We sought to gain a better understanding of the effects of temperature on the sequence of dechlorination in microcosms that approximate actual field conditions. For that reason, we made no attempt to control the electron donor or carbon source. However, a better understanding of the influence of

* Corresponding author. Mailing address: Department of Microbiology, 215 Biological Science Building, University of Georgia, 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.

temperature on various PCB dechlorination reactions should help to identify conditions that will facilitate the isolation of different PCB-dechlorinating microorganisms.

We studied the sequence of dechlorination of 2,3,4,6-CB at various temperatures in the two sediments used in our previous investigations: PCB-free sediment from Sandy Creek Nature Center Pond (SCNC) (Athens, Ga.) and PCB-contaminated sediment from Woods Pond (Lenox, Mass.). We chose 2,3,4,6-CB for our study because the congener itself and its products can be dechlorinated by several different routes (2) which can be followed unequivocally. Comparison of the effect of temperature on the sequence of dechlorination of this congener in sediments with different histories and from two different climates provides a potential means to differentiate between PCB dechlorination reactions that are common and those that are site specific.

Previous PCB dechlorination studies with sediment from Woods Pond demonstrated that three distinct microbial dechlorination processes can be stimulated by the addition of different PCB congeners (3, 7, 8, 26). The studies with 2,3,4,6-CB reported here were carried out, in part, to gain more insight into how environmental temperatures affect the interaction of those three dechlorination processes. We particularly sought to identify temperatures under which *ortho* and *para* dechlorination of 2,4,6-CB and 2,4-CB would occur because in situ dechlorination of PCBs in Woods Pond preferentially removes *meta*-chlorines, resulting in the accumulation of PCBs with 2,4- and 2,4,6-chlorophenyl rings. We also sought to identify temperatures that would favor the enrichment of PCB dechlorinators to carry out specific dechlorination reactions.

Sediments from SCNC have been used in the past to elucidate the reductive dehalogenation and subsequent degradation of chlorophenols in sediments without a history of contamination (16, 24, 33, 34).

Because of the difference in location, Woods Pond and SCNC differ markedly in their annual temperatures. The 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. In SCNC sediments, the summer temperatures range from 22 to 29°C at an 8-cm depth and can reach up to 35°C in the top 2 cm near the edge of the pond. From November through February, the temperatures are generally in the range of 10 to 18°C but may drop lower for a few days at a time (35).

In this study, we describe seven discrete dechlorination reactions in Woods Pond and four in SCNC. The individual reactions exhibited strong temperature dependencies in both sediments. Furthermore, temperature influenced the timing and preferred sequence of dechlorination in both sediments. Although no pure cultures have yet been isolated, the data indicate that several distinct PCB-dechlorinating microbial populations with different temperature ranges and different dechlorination specificities exist in these sediments. Most likely, each of these PCB-dechlorinating populations catalyzes only one or two of the dechlorination reactions that we observed. Furthermore, the data indicate that temperature can play a significant role in determining the nature and extent of PCB dechlorination that occurs at any given site.

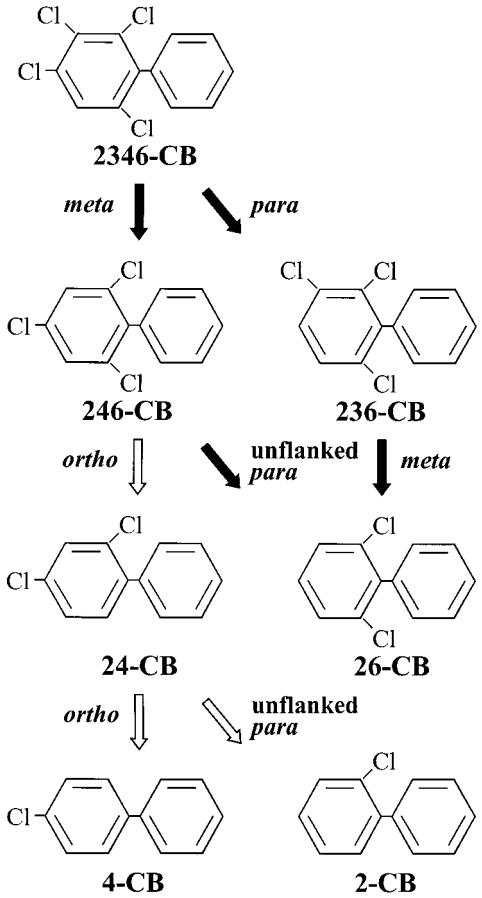

FIG. 1. Dechlorination pathways of 2,3,4,6-CB in SCNC sediment samples (reactions indicated by black arrows) and Woods Pond sediment samples (all reactions). Note that some of the reactions were observed only at specific temperatures (see text).

MATERIALS AND METHODS

Sediment collection and storage. Aroclor 1260-contaminated sediments were collected from Woods Pond, an impoundment on the Housatonic River (4), and were transported in 5-gal buckets at ambient temperatures to Athens, Ga., as described elsewhere (30). PCB-free sediments were collected from an uncontaminated pond in Sandy Creek Nature Center in Athens, Ga., as described elsewhere (17, 30). Both sediments were stored at 4 to 7°C until use.

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) but was higher than that measured in SCNC (pH 6.5 to 6.8). The final concentration of potassium phosphate was 10 mM. The slurries contained 0.15 g (dry weight) of sediment per ml. The homogenization, dispensation, and preparation of the individual incubations were as previously described (30).

The sterile controls were autoclaved twice for 1 h at 121°C on two consecutive days to eliminate viable spores before the PCB congener was added. Triplicate samples and controls were amended with 2,3,4,6-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 (Woods Pond sediment samples) and 4, 12, 18, 25, 27, 30, 34, 40, and 50°C (SCNC sediment samples). Where indicated, samples were respiked with 2,3,4,6-CB (350 μmol per liter of slurry) when at least 75% of the initial 2,3,4,6-CB had been converted to products. Most samples were incubated in water baths as described previously (30).

Sample extraction and analysis. The dechlorination of 2,3,4,6-CB in each sample was analyzed at various time points throughout 1 year of incubation. Samples were taken at intervals ranging from 2 days, for highly active samples respiked with 2,3,4,6-CB, to more than 2 months, for samples with extremely low dechlorination activities.

PCBs were extracted with anhydrous diethyl ether containing octachloronaphthalene (4 ppm) as an internal standard and were analyzed with a gas chromatograph (Hewlett-Packard, 5890 series II; 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 as previously described (30).

2,3,4,6-CB and all of its dechlorination products were identified by matching gas chromatography retention times with those of authentic standards (99%

TABLE 1. Dechlorination of 2,3,4,6-CB in SCNC sediment samples after 1 year of incubation between 4 and 50°C[a]

Temp (°C)	Sample	Congener (mol%)[b]			
		2,3,4,6-CB	2,4,6-CB	2,3,6-CB	2,6-CB
4		99.8 ± 0.1	0.2 ± 0.1	—[c]	—
12		20.2 ± 1.2	78.8 ± 1.4	0.2 ± 0.0	0.8 ± 0.2
18	A	12.1	14.1	36.4	37.4
	B	8.7	23.8	29.4	38.1
	C	11.5	44.7	3.0	40.8
25	A	7.8	12.5	0.3	79.4
	B	8.9	1.8	4.7	84.6
	C	1.1	4.1	1.3	93.5
27		5.6 ± 1.4	2.3 ± 0.4	3.3 ± 2.6	88.8 ± 3.6
30		3.1 ± 2.0	1.9 ± 1.4	1.5 ± 0.9	93.5 ± 1.2
34		4.5 ± 0.1	95.5 ± 0.1	—	—
37		99.8 ± 0.0	0.2 ± 0.0	—	—
40		99.8 ± 0.1	0.2 ± 0.1	—	—
50		97.3 ± 0.6	2.7 ± 0.6	—	—

[a] All data are the means of triplicates ± the standard deviation. The values for each of the individual triplicate samples (A, B, and C) are given when there were large differences between the triplicate samples in the product distribution.
[b] 100 mol% = 350 μM (= 700 μM at 30°C).
[c] —, not detected.

purity; AccuStandard, New Haven, Conn.) and quantified with a third-order 15-point calibration curve (30).

RESULTS

Overview of observed dechlorination pathways. We observed unequivocal dechlorination of 2,3,4,6-CB to products at 12 to 34°C and at 50°C in SCNC sediment, and at 4 to 34°C and 50 to 60°C in Woods Pond sediment (Tables 1 and 2), but not in autoclaved controls. Figure 1 summarizes all of the dechlorination pathways that were observed in either sediment. As reported previously, the first major dechlorination product was 2,4,6-CB (30). At some temperatures, 2,3,6-CB was also produced, usually in very small amounts. No *ortho* dechlorination of 2,3,4,6-CB to 2,3,4-CB was ever observed.

The 2,4,6-CB formed by dechlorination of 2,3,4,6-CB persisted at some temperatures but at most temperatures was further dechlorinated to 2,6-CB and, in Woods Pond sediment, to 2,4-CB. The 2,3,6-CB was also dechlorinated to 2,6-CB at some temperatures in both sediments. Dechlorination of 2,6-CB was never observed in either sediment. In contrast, 2,4-CB was dechlorinated to 2-CB and 4-CB in Woods Pond sediment. No biphenyl or other chlorinated biphenyls were observed in either sediment.

Effect of temperature on the initial dehalogenation of 2,3,4,6-CB. We determined from extensive time courses in both sediments (a few examples are shown in Fig. 2 to 4) that the first observed dehalogenation reaction at all temperatures except 18°C was 2,3,4,6-CB → 2,4,6-CB. The lag time preceding the dechlorination and the rate of the dechlorination varied with the sediment and the temperature (30). But in both sediments, the time course of this transformation was consistent among the replicates at any given temperature. Nearly all time

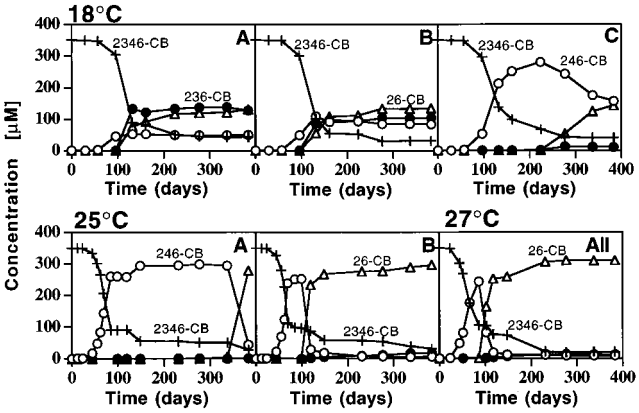

FIG. 2. Time course of dechlorination of 2,3,4,6-CB in SCNC sediment samples at 18, 25, and 27°C. A, B, and C indicate the individual bottles of the triplicate incubations. "All" indicates that all triplicates had similar dechlorination reactions; one example is shown. +, 2,3,4,6-CB; ○, 2,4,6-CB; ●, 2,3,6-CB; △, 2,6-CB.

course data revealed biphasic kinetics for this dechlorination reaction.

A second dehalogenation reaction, 2,3,4,6-CB → 2,3,6-CB, also occurred at 18 to 30°C in SCNC samples. At least trace amounts of this reaction also occurred at 12 to 34°C in Woods Pond samples, as evidenced by the transient appearance of 2,3,6-CB. The initial product distribution was 2,4,6-CB (≥95%) and 2,3,6-CB (≤5%) at all temperatures except 18°C (both sediments) and 34°C (Woods Pond sediment). At 18°C, the dechlorination specificity was much more variable and 2,3,6-CB was the major initial product in two of the three SCNC replicate samples (Fig. 2) and in triplicate Woods Pond samples (Fig. 3). However, a repeat incubation of Woods Pond sediment at 18°C resulted in the exclusive conversion of 2,3,4,6-CB to 2,4,6-CB in all three samples. Repeat incubations were also carried out at 15, 20, and 22°C, but no variation in the initial dehalogenation step occurred at any of these temperatures. We also observed variability in the initial dehalogenation of 2,3,4,6-CB at 34°C in Woods Pond sediment; in one of three replicate samples, 60% of the 2,3,4,6-CB was converted to 2,3,6-CB (Fig. 3).

The effect of readdition of 2,3,4,6-CB on the initial dehalogenation step. When 75% of the 2,3,4,6-CB had been dehalogenated, a second addition of 2,3,4,6-CB (350 μmol per liter of slurry) was made to samples at most temperatures. The freshly added 2,3,4,6-CB was dechlorinated with no lag in all instances. In most cases, the trichlorobiphenyl product distribu-

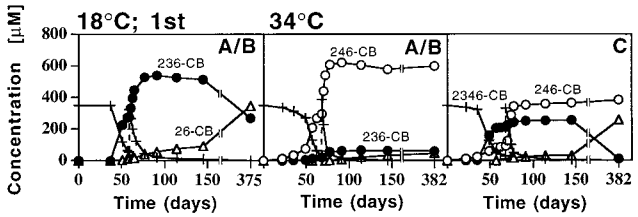

FIG. 3. Time course of dechlorination of 2,3,4,6-CB in triplicate Woods Pond sediment samples of the first incubation at 18°C and the incubation at 34°C. A/B depicts one of two replicates which had similar dechlorination reactions, while C indicates a third replicate that exhibited a different dechlorination reaction. The third replicate at 18°C (not shown) exhibited a slightly longer lag before dechlorination began, and the 2,3,6-CB was not dechlorinated to 2,6-CB within the time frame of the experiment. +, 2,3,4,6-CB; ○, 2,4,6-CB; ●, 2,3,6-CB; △, 2,6-CB. The dashed lines indicate the time of respiking with 2,3,4,6-CB.

TABLE 2. Dechlorination of 2,3,4,6-CB in Woods Pond sediment samples after 1 year of incubation between 4 and 66°C[a]

Temp (°C)	Incubation	Sample	Congener (mol%)[b]						
			2,3,4,6-CB	2,4,6-CB	2,3,6-CB	2,6-CB	2,4-CB	2-CB	4-CB
4		A	96.3	3.7	—[c]	—	—	—	—
		B	91.2	8.8	—	—	—	—	—
		C	78.3	21.7	—	—	—	—	—
8			12.3 ± 3.3	85.6 ± 4.1	—	0.2 ± 0.0	1.9 ± 0.9	—	—
12			10.6 ± 3.5	82.3 ± 0.8	—	0.1 ± 0.0	7.0 ± 3.1	—	—
15	1st[d]	A	0.3	95.7	—	0.3	3.7	—	—
		B	6.7	1.5	—	8.7	0.8	0.5	81.8
		C	—	97.6	—	0.2	2.2	—	—
	2nd[d]	A	4.1	43.0	—	1.7	3.1	—	48.1
		B	2.5	84.2	0.1	11.2	2.0	—	—
		C	7.1	46.5	0.1	2.4	4.3	8.6	31.0
18	1st	A	0.2	—	43.5	56.3	—	—	—
		B	0.2	—	21.6	78.2	—	—	—
		C	1.1	—	97.4	1.5	—	—	—
	2nd	A	0.8	3.9	0.1	91.5	2.7	1.0	—
		B	0.6	3.0	0.2	92.7	2.9	0.6	—
		C	0.7	2.6	0.1	29.3	3.9	13.7	49.7
20	1st		2.4 ± 0.7	2.9 ± 1.2	0.2 ± 0.0	89.7 ± 1.4	1.6 ± 0.5	3.2 ± 2.0	—
	2nd		0.1 ± 0.2	6.2 ± 0.5	0.2 ± 0.0	91.0 ± 1.1	2.0 ± 0.4	0.5 ± 0.0	—
22	1st	A	0.9	2.7	0.1	80.1	2.6	9.5	4.1
		B	1.1	1.5	0.1	45.7	1.6	22.9	27.1
		C	1.0	1.7	0.1	85.4	1.9	9.9	—
	2nd	A	2.4	2.7	0.2	71.7	2.4	17.5	3.1
		B	0.1	7.2	—	65.9	5.3	12.6	8.9
		C	1.6	3.7	0.1	90.1	3.7	0.8	—
25		A	2.2	1.0	0.1	9.8	3.7	32.0	51.2
		B	0.6	0.9	0.1	76.3	2.1	8.6	11.4
		C	0.6	1.4	0.1	60.9	2.6	18.1	16.3
27		A	0.5	1.1	0.1	17.2	5.1	19.8	56.2
		B	0.3	1.9	0.1	28.4	5.4	30.9	33.0
		C	0.7	1.8	0.1	68.9	3.9	21.5	3.1
30		A	—	0.7	0.2	3.6	7.6	3.9	84.0
		B	0.2	6.7	0.5	89.9	1.3	0.8	0.6
		C	—	1.1	0.3	69.1	3.0	18.6	7.9
34		A	—	84.8	8.6	6.6	—	—	—
		B	—	93.1	6.8	0.1	—	—	—
		C	0.1	59.0	1.5	39.5	—	—	—
37			99.4 ± 0.0	0.5 ± 0.0	0.1 ± 0.0	—	—	—	—
40			98.2 ± 0.5	1.8 ± 0.5	—	—	—	—	—
45			97.8 ± 2.0	2.2 ± 2.0	—	—	—	—	—
50			11.4 ± 4.7	88.5 ± 4.4	0.1 ± 0.3	—	—	—	—
55		A	35.3	64.7	—	—	—	—	—
		B	8.2	91.8					
		C	1.7	98.3					
60		A	3.7	96.3	—	—	—	—	—
		B	1.3	98.7					
		C	68.7	31.3					
66			98.9 ± 1.6	1.1 ± 1.6	—	—	—	—	—

[a] All data are the means of triplicates ± the standard deviation. The values for each of the individual triplicate samples (A, B, and C) are given when there were large differences between the triplicate samples in the product distribution.

[b] 100 mol% = 350 μM at 4 to 12, 37 to 45, and 55 to 66°C (= 700 μM at 15 to 34 and 50°C).

[c] —, not detected.

[d] 1st represents the first incubation, and 2nd represents the second incubation (see Materials and Methods for details).

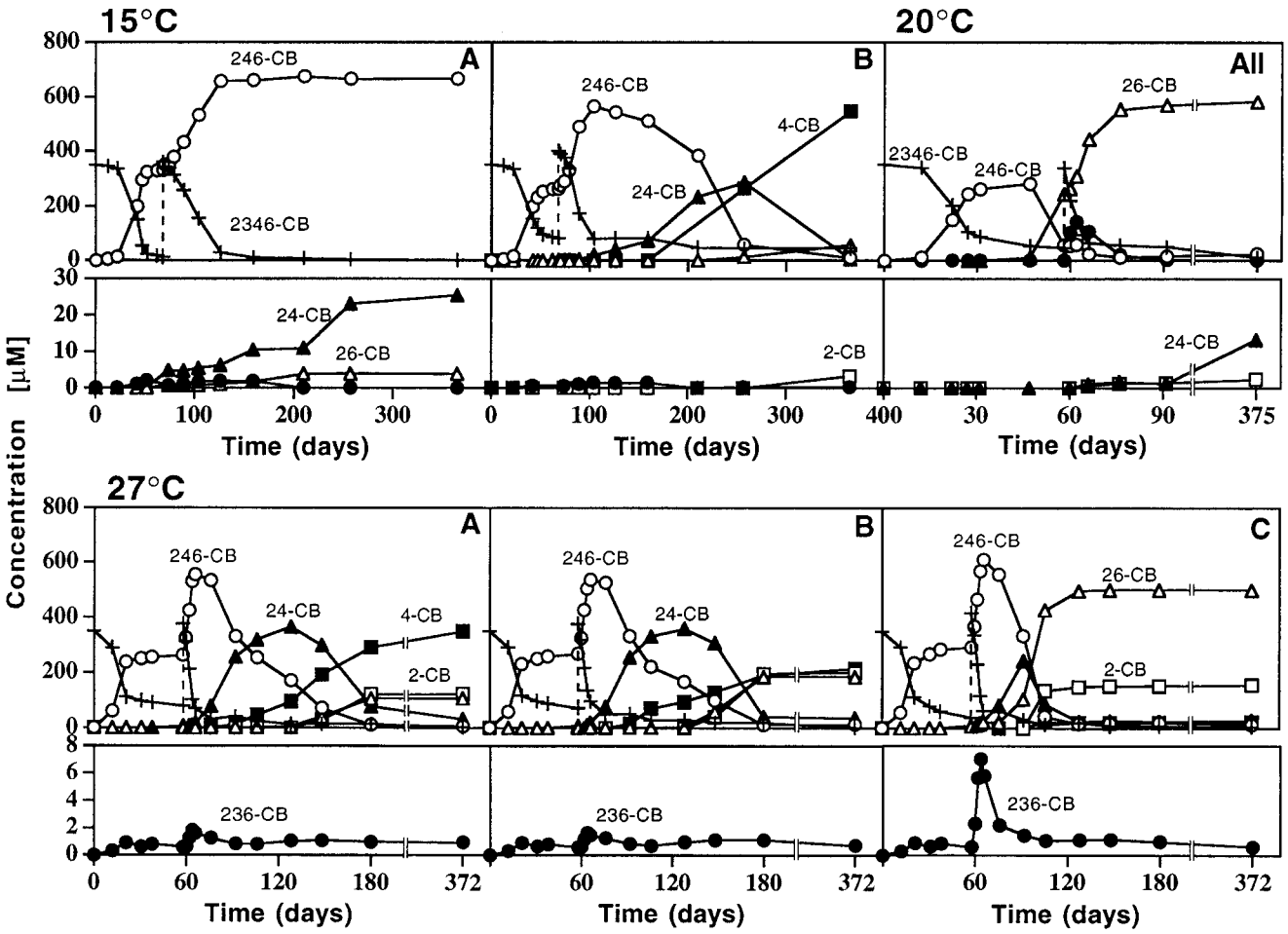

FIG. 4. Time course of dechlorination of 2,3,4,6-CB in Woods Pond sediment samples at 15, 20, and 27°C. Half of the six samples incubated at 15°C were dechlorinated as shown in panel A, and the other half were dechlorinated as shown in panel B. All six replicates at 20°C were dechlorinated as in the example shown. For 27°C, A, B, and C indicate the individual bottles of the triplicate incubations. +, 2,3,4,6-CB; ○, 2,4,6-CB; ●, 2,3,6-CB; △, 2,6-CB; ▲, 2,4-CB; □, 2-CB; ■, 4-CB. The dashed lines indicate the time of respiking with 2,3,4,6-CB.

tion from the newly added 2,3,4,6-CB matched the initial distribution, but in a few instances, the product distribution changed significantly. For example, the Woods Pond samples incubated at 20°C exhibited a change in the specificity of the initial dehalogenation reaction when 2,3,4,6-CB was readded (Fig. 4). In this case *para* dechlorination of 2,4,6-CB to 2,6-CB had already begun when the 2,3,4,6-CB was readded, and the newly added 2,3,4,6-CB was *para* dechlorinated to 2,3,6-CB.

Effect of incubation temperature on the subsequent dechlorination steps and on the accumulation of products. The subsequent dechlorination steps and the final dechlorination products of 2,3,4,6-CB (after 1 year) varied at different temperatures and in the samples from the two locations. The final distributions of 2,3,4,6-CB and its products in each sediment are presented in Tables 1 and 2. There was considerable variation between replicates in the final distribution of products at certain temperatures, especially at 18°C for SCNC samples, and at 15 through 34°C for Woods Pond samples. These variations will be described further in the sections below.

(i) SCNC sediment samples. No dechlorination products other than 2,4,6-CB were detected in any of the triplicates below 12 or above 30°C (Table 1). Between 18 and 30°C, substantial dechlorination to 2,6-CB occurred (Table 1). From 25 to 30°C, 2,4,6-CB was dechlorinated to 2,6-CB, but 2,3,6-CB was dechlorinated only at 30°C. No dechlorination of 2,6-CB was observed in any of these samples, and no other dechlorination products were formed; hence, 2,6-CB accumulated nearly stoichiometrically as the final dechlorination product between 25 and 30°C.

The time course for the conversion of 2,4,6-CB to 2,6-CB was very consistent at 27°C but was variable at 25°C (Fig. 2). A total loss of the capacity to dehalogenate 2,4,6-CB occurred between 30 and 34°C. The abruptness of this loss over a range of only 4°C was striking but was reproducible and has been observed in other independent incubations (14).

(ii) Woods Pond sediment samples. The 2,3,6-CB that was formed at 18 and 34°C persisted for 5 to ≥10 months but was ultimately dechlorinated to 2,6-CB in most samples (Fig. 3).

No dechlorination of 2,4,6-CB occurred at the lowest (4°C) or highest (34°C and above) temperatures (Table 2), and very little dechlorination of 2,4,6-CB occurred at 8°C during a year of incubation (Table 2). However, at 12°C, *ortho* dechlorination of 2,4,6-CB to 2,4-CB began to occur at low but measurable rates after 7.5 to 10 months.

Between 15 and 30°C, the 2,4,6-CB was further dehalogenated to various amounts of 2,6-CB and 2,4-CB. No dechlorination of the 2,6-CB was ever detected, but the 2,4-CB was subsequently dechlorinated to various amounts of 2-CB and

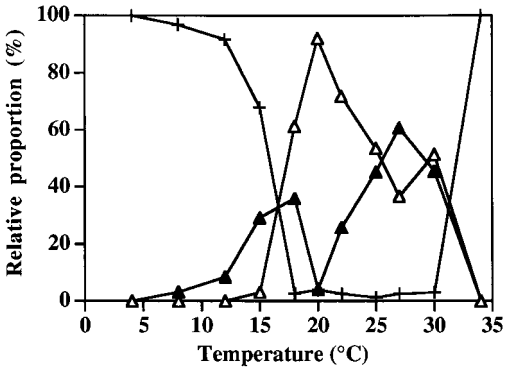

FIG. 5. Effect of temperature on dechlorination of 2,4,6-CB by loss of the *ortho* or *para* chlorine in Woods Pond sediment samples. For each incubation temperature, the 2,4,6-CB formed by dechlorination and all of its products after 1 year (Table 2) were added together. We normalized the sum to 100% and then calculated the relative proportions of 2,4,6-CB that remained unchanged (+) or that were further dechlorinated by initial removal of the *para* chlorine (△) (2,6-CB) or the *ortho* chlorine (▲) (2,4-CB and its subsequent products, 2-CB and 4-CB). The data represent averages for six replicates at 15, 20, and 22°C and for three replicates at all other incubation temperatures.

4-CB. *ortho* dechlorination of 2,4,6-CB to 2,4-CB and subsequently 4-CB was almost completely dominant at 15°C (Fig. 4). In three of six samples, this transformation converted 33 to 88% of the 2,4,6-CB to 4-CB (Table 2). However, in the remaining samples the *ortho* dechlorination occurred at such low rates (Fig. 4) that 85 to 95% of the 2,4,6-CB still persisted after 1 year.

The dechlorination of 2,4,6-CB at 20°C was highly consistent; in all six replicates, approximately 90% of the 2,4,6-CB was rapidly *para* dechlorinated to 2,6-CB, and only 2 to 5% was *ortho* dechlorinated to 2,4-CB. Some of the 2,4-CB was subsequently dechlorinated to 2-CB as shown for one sample in Fig. 4.

In sharp contrast, at 18°C, and from 22 to 30°C, the sequence of dechlorination of 2,4,6-CB was variable among replicates at the same temperature, and this is reflected in the final product distribution shown in Table 2. The time courses of the three replicates at 27°C (Fig. 4) illustrate the types of variation we observed. The 2,4,6-CB was initially *ortho* dechlorinated to 2,4-CB in all three of the replicates at 27°C. In one of the replicates (Fig. 4C), *para* dechlorination of the 2,4,6-CB to 2,6-CB began soon after the onset of *ortho* dechlorination, and subsequently *ortho* dechlorination ceased. Hence, most of the 2,4,6-CB was *para* dechlorinated to 2,6-CB in this sample, and all of the previously formed 2,4-CB was *para* dechlorinated to 2-CB. Early onset of *para* dechlorination did not occur in the other two replicates, and most of the 2,4,6-CB was *ortho* dechlorinated to 2,4-CB before *para* dechlorination began. The 2,4-CB was further dechlorinated to both 2-CB and 4-CB, but the distribution of these products was determined by the timing of the onset of *para* dechlorination.

Temperature dependence of *ortho* and *para* dechlorination of 2,4,6-CB in Woods Pond sediment samples. In order to gain a better understanding of the effect of temperature on the dechlorination of 2,4,6-CB in Woods Pond sediment samples, we compared the relative proportions of *ortho* and *para* dechlorination of this congener. This analysis of the data (Fig. 5) shows that both the dechlorination of 2,4,6-CB and the preferred site of dechlorination were strongly temperature dependent and permits us to draw a number of conclusions. (i) Little or no dechlorination of 2,4,6-CB was observed at temperatures below 12 or above 30°C. (ii) Maximal dechlorination of 2,4,6-CB occurred between 18 and 30°C. (iii) *para* dechlorination of 2,4,6-CB was optimal in the range of 18 to 22°C. (iv) *ortho* dechlorination of 2,4,6-CB extended over a broader temperature range (8 to 30°C) than *para* dechlorination and was strongest at 27°C. (v) Only minimal *ortho* dechlorination was observed at 20°C, the temperature at which *para* dechlorination was highest. (vi) *ortho* dechlorination and *para* dechlorination appeared to be equally strong in the range of 25 to 30°C.

When we compared the relative proportions of 2,4-CB and those of its *ortho* and *para* dechlorination products, 4-CB and 2-CB, respectively, the same trends were seen (data not shown). Hence, the fact that 2,4-CB was formed by *ortho* dechlorination did not influence the route of its subsequent dechlorination. Instead, the data indicate that the strongest influence on *ortho* versus *para* dechlorination of 2,4-CB was the incubation temperature.

DISCUSSION

Observed temperature dependencies of discrete dechlorination reactions and proposed PCB-dechlorinating populations responsible for these reactions in each sediment. We distinguished seven different dechlorination reactions of 2,3,4,6-CB and its products (Fig. 1). Four of these were observed in SCNC sediment, and all seven were observed in Woods Pond sediment. The data summarized in Table 3 show that the dechlorination reactions exhibited at least four different temperature dependencies in SCNC sediment and at least six in Woods Pond sediment. Substantial *meta* dechlorination of 2,3,4,6-CB occurred at thermobiotic temperatures (50, 55, and 60°C) in Woods Pond samples (Tables 2 and 3), suggesting the presence of a thermophilic PCB dechlorinator in Woods Pond. As proposed earlier (30), the observed temperature-dependent differences in dechlorination are consistent with the hypothesis that each of these sediments harbors several different PCB-dechlorinating microorganisms with distinct specificities.

We cannot exclude the possibility that a single PCB-dechlorinating enzyme catalyzes several different reactions with different temperature dependencies for the different congeners or that a single PCB-dechlorinating microorganism harbors several different PCB-dechlorinating enzymes. However, based

TABLE 3. Temperature ranges[a] for different dechlorination reactions

Dechlorination reaction	Specificity of dechlorination	Temp range (°C)		Temp at which reaction was dominant (°C)	
		SCNC	Woods Pond	SCNC	Woods Pond
2,3,4,6 → 2,4,6	*meta*	12–34 50	4–34 50–60	12–34	4–34
2,3,4,6 → 2,3,6	*para*	18–30	18 30–34	18[b]	18[b] 34[b]
2,3,6 → 2,6	*meta*	30[c]	18 30–34		
2,4,6 → 2,6	unflanked *para*	18–30	15–30		18–22
2,4 → 2	unflanked *para*		15–30		18–22
2,4,6 → 2,4	*ortho*		12–30[d]		12–15
2,4 → 4	*ortho*		12–30[d]		12–15

[a] We have included only reactions that transformed at least 2.5 mol% of the total PCB.

[b] *para* dechlorination was dominant in some, but not all, samples incubated at these temperatures.

[c] Dechlorination of 2,3,6-CB was observed unequivocally only at 30°C.

[d] *ortho* dechlorination was minimal at 20°C, but this was most likely due to competition with unflanked *para* dechlorination.

on all of the above observations and previously obtained data on chlorophenol dehalogenation in SCNC samples (16, 17, 23, 24, 33, 34), we consider it more probable that the different dechlorination reactions are due to discrete populations of PCB-dechlorinating microorganisms. This interpretation is corroborated by the substrate specificities of enrichment cultures obtained by incorporating the temperature dependencies described above (Table 3) into enrichment strategies (14, 28).

Effect of temperature on the dechlorination of 2,3,4,6-CB. In both sediments, significant dechlorination of 2,3,4,6-CB occurred over similar temperature ranges, and at most temperatures, *meta* dechlorination of 2,3,4,6-CB to 2,4,6-CB almost completely dominated (Table 3). The time of onset and rate of this dechlorination reaction varied with the temperature and sediment but were highly consistent among replicates for a given temperature and sediment with two striking exceptions. At 18°C in both sediments and at 34°C in Woods Pond sediment, the dominant reaction changed to *para* dechlorination of 2,3,4,6-CB to 2,3,6-CB in some samples. Similar sharp changes within a small temperature range have been observed previously (17). The abrupt change and the variability in the PCB dechlorination specificity at 18 and 34°C suggest that these discrete temperatures may be transition points at which members of the community supporting *para* dechlorination become more dominant. This could lead to unstable conditions in the sediment at large or to variations between microniches within the sediment and would explain the variation in dechlorination reactions observed at these temperatures. Alternatively, these temperatures might favor the *para* dechlorinators, allowing them to outcompete the *meta* dechlorinators. The variability at 18°C is particularly significant for Woods Pond because this temperature prevails in the top 15 cm of the sediment for much of the summer.

Effect of temperature on the dechlorination of 2,4,6-CB. The dechlorination of 2,4,6-CB occurred over a narrower temperature range than that of 2,3,4,6-CB in both sediments and was also more sensitive to temperature differences. In SCNC sediments, dechlorination of 2,4,6-CB to 2,6-CB was optimal and consistent among replicates at 27°C. But above and below this temperature, the timing of the onset of this reaction varied among replicates. Similar variability was observed for the *ortho* dechlorination of 2,4,6-CB to 2,4-CB at 15°C in Woods Pond sediment; *ortho* dechlorination began at about the same time in all six incubations, but there was a sharp dichotomy in the maximal rate at which the reaction occurred (Fig. 4). These observations provide further evidence of the complex and undelineated factors controlling the dechlorination of 2,4,6-CB in sediment microcosms. Apparently temperature is only one of the key parameters. Others may include spatial variations in the relative composition of both dechlorinators and nondechlorinators in the microcosms.

In Woods Pond sediment, *para* dechlorination of 2,4,6-CB to 2,6-CB was optimal and highly consistent among replicates at 20°C. But above and below this temperature, the timing and extent of *para* dechlorination were variable, most likely because of significant competition from *ortho* dechlorination (see below).

Effect of temperature on *ortho* versus unflanked *para* dechlorination of PCBs containing 2,4- and 2,4,6-chlorophenyl rings. Previous work (3) showed that *para* dechlorination of PCBs could be stimulated in Woods Pond sediment, but only *para* chlorines that were located adjacent to other chlorines were removed. Hence, the unflanked *para* chlorines on 2,4- and 2,4,6-chlorophenyl rings were not substrates. The results presented here demonstrate that Woods Pond sediments harbor microorganisms that can remove either the *ortho* or the *para* moiety from 2,4- and 2,4,6-chlorophenyl rings. Our results also indicate that a strong temperature dependence exists with regard to the preferential removal of chlorines in *ortho* and unflanked *para* positions (Fig. 5). The variations in the preferred route of dechlorination of 2,4,6-CB among replicates at some temperatures and the temperature relationships illustrated in Fig. 5 provide evidence of a competition between the *ortho* and unflanked *para* dechlorinating activities. In some cases, this resulted in less effective dechlorination. The data are consistent with the interpretation that a single PCB dechlorinator was responsible for the *ortho* dechlorination of both 2,4,6-CB and 2,4-CB and that a second PCB dechlorinator carried out unflanked *para* dechlorination of the same two substrates. This interpretation is supported by recent preliminary results with an enrichment culture obtained by repeated transfers with 2,4,6-CB as a selective dehalogenation substrate. The unflanked *para* dechlorination activity was retained, while the *ortho* dechlorination activity was eliminated (14).

Substrate specificity of *ortho* dechlorination of PCBs. There have been two previous reports of *ortho* dechlorination of PCBs in anaerobic microcosms of Woods Pond sediment. Van Dort and Bedard (25) demonstrated that the microorganisms in Woods Pond sediments dechlorinate 2,3,5,6-CB to 2,3,5-CB and then 2,5-CB, and Williams (27) demonstrated that they also dechlorinate 2,4,6-CB to 2,4-CB and 4-CB. The *ortho* dechlorination that we observed in Woods Pond exhibited substrate specificity; 2,4,6-CB and 2,4-CB were substrates for *ortho* dechlorination, but as previously reported (25), 2,3,6-CB and 2,6-CB were not. These findings are in contrast to results recently reported by Berkaw et al. (9) for estuarine sediments from Baltimore Harbor. These authors reported *ortho* dechlorination of 2,3,5,6-CB, 2,3,5-CB, and 2,3,6-CB but not 2,4,6-CB and 2,4-CB. Unfortunately, there is still no evidence that such key end products of *meta* and *para* dechlorination as 2-CB, 2-2-CB, and 2,6-CB can be dechlorinated. As reported for other locations (9, 22), no dechlorination of 2,6-CB occurred in our study despite prolonged incubation in samples that were actively *ortho* dechlorinating 2,4,6-CB and 2,4-CB. These results suggest that the microorganisms or the enzymes responsible for *ortho* dechlorination exhibit a strong substrate specificity, possibly requiring the activation of the *ortho* chlorine by an electronic effect of the *para* chlorine on 2,4,6-CB and 2,4-CB or the *meta* chlorines on 2,3,5,6-CB, 2,3,5-CB, and 2,3,6-CB.

Implications from this study. It is clear from these results that field temperatures can be expected to play a significant role in determining the extent of PCB dechlorination that occurs at a given site. Furthermore, the PCB dechlorination reactions that dominate at 25 or 30°C may differ substantially from those that occur at the lower or higher temperatures that may prevail in a given environment. Therefore, it is risky to use data collected at one temperature to predict the fate of PCBs in sediments that typically experience a different temperature range in the field. We contend that competitions similar to those described above, i.e., *meta* versus *para* dechlorination and *ortho* versus unflanked *para* dechlorination, may occur in natural sediments. This can be very important because the sequence of dechlorination will determine how many chlorines are removed and which products accumulate. The variations that we observed suggest that relatively small changes in the temperature can change the rate, extent, and pathway of dechlorination in a given sediment, both in the laboratory and in the natural environment. The development of methods to selectively "prime" (3, 8, 26) the most effective dechlorination sequences might effectively eliminate competitive reactions and lead to improved dechlorination over broader temperature

ranges. Furthermore, the use of incubation temperatures that favor specific dehalogenation reactions should facilitate the isolation of PCB-dehalogenating bacteria of interest.

ACKNOWLEDGMENT

We thank the Georgia Research Alliance for an equipment grant to J.W.

REFERENCES

1. **Abramowicz, D. A.** 1994. Aerobic PCB degradation and anaerobic PCB dechlorination in the environment. Res. Microbiol. **145:**42–46.
2. **Bedard, D. L., S. C. Bunnell, and J. M. Principe.** Unpublished data.
3. **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.
4. **Bedard, D. L., and R. J. May.** 1996. Characterization of the polychlorinated biphenyls (PCBs) in the sediment of Woods Pond: evidence for microbial dechlorination of Aroclor 1260 *in situ*. Environ. Sci. Technol. **30:**237–245.
5. **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.
6. **Bedard, D. L., and H. M. Van Dort.** The role of microbial PCB dechlorination in natural restoration and bioremediation. *In* G. S. Sayler (ed.), Biotechnology in the sustainable environment, in press. Plenum Publishing Corp., New York, N.Y.
7. **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. Office of Research and Development, U.S. Environmental Protection Agency, Athens, Ga.
8. **Bedard, D. L., H. M. Van Dort, R. J. May, and L. A. Smullen.** Enrichment of microorganisms that sequentially *meta*-, *para*-dechlorinate the residue of Aroclor 1260 in Housatonic River sediment. Submitted for publication.
9. **Berkaw, M., K. R. Sowers, and H. D. May.** 1996. Anaerobic *ortho* dechlorination of polychlorinated biphenyls by estuarine sediments from Baltimore Harbor. Appl. Environ. Microbiol. **62:**2534–2539.
10. **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.
11. **Brown, J. F., Jr., and R. E. Wagner.** 1990. PCB movement, dechlorination, and detoxication in the Acushnet estuary. Environ. Toxicol. Chem. **9:**1215–1233.
12. **Brown, J. F., Jr., R. E. Wagner, D. L. Bedard, M. J. Brennan, J. C. Carnahan, R. J. May, and T. J. Tofflemire.** 1984. PCB transformations in upper Hudson sediments. Northeast. Environ. Sci. **3:**167–169.
13. **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.
14. **Chuang, K.-S.** 1995. M.S. thesis. The University of Georgia, Athens.
15. **Fish, K. M., and J. M. Principe.** 1994. Biotransformations of Aroclor 1242 in Hudson River test tube microcosms. Appl. Environ. Microbiol. **60:**4289–4296.
16. **Hale, D. D., J. E. Rogers, and J. Wiegel.** 1992. Reductive dechlorination of dichlorophenols in anaerobic pond sediments, p. 212–222. *In* R. A. Baker (ed.), Organic substances and sediments in water, vol. III. Lewis Publishers, Inc., Boca Raton, Fla.
17. **Kohring, G.-W., J. E. Rogers, and J. Wiegel.** 1989. Anaerobic biodegradation of 2,4-dichlorophenol in freshwater lake sediments at different temperatures. Appl. Environ. Microbiol. **55:**348–353.
18. **Mohn, W. W., and J. M. Tiedje.** 1992. Microbial reductive dehalogenation. Microbiol. Rev. **56:**482–507.
19. **National Research Council.** 1979. Polychlorinated biphenyls. National Academy of Sciences, Washington, D.C.
20. **Quensen, J. F., III, J. M. Tiedje, and S. A. Boyd.** 1988. Reductive dechlorination of polychlorinated biphenyls by anaerobic microorganisms from sediments. Science **242:**752–754.
21. **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.
22. **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.
23. **Utkin, I., and J. Wiegel.** Unpublished data.
24. **Utkin, I., C. Woese, and J. Wiegel.** 1994. Isolation and characterization of *Desulfitobacterium dehalogenans* gen. nov. sp. nov., an anaerobic bacterium which reductively dechlorinates chlorophenolic compounds. Int. J. Syst. Bacteriol. **44:**612–619.
25. **Van Dort, H. M., and D. L. Bedard.** 1991. Reductive *ortho* and *meta* dechlorination of a polychlorinated biphenyl congener by anaerobic microorganisms. Appl. Environ. Microbiol. **57:**1576–1578.
26. **Van Dort, H. M., L. A. Smullen, R. J. May, and D. L. Bedard.** Priming *meta*-dechlorination of polychlorinated biphenyls that have persisted in Housatonic River sediments for decades. Submitted for publication.
27. **Williams, W. A.** 1994. Microbial reductive dechlorination of trichlorophenyls in anaerobic slurries. Environ. Sci. Technol. **28:**630–635.
28. **Wu, Q.** 1996. Ph.D. dissertation. The University of Georgia, Athens.
29. **Wu, Q., D. L. Bedard, and J. Wiegel.** 1992. Effect of temperature on the dechlorination of PCBs in methanogenic freshwater sediments, p. 491–497. *In* International Symposium on Soil Decontamination Using Biological Processes, Karlsruhe, Germany (December 1992). Dechema, Frankfurt am Main, Germany.
30. **Wu, Q., D. L. Bedard, and J. Wiegel.** 1996. Influences of incubation temperatures on the microbial reductive dechlorination of 2,3,4,6-tetrachlorobiphenyl in two freshwater sediments. Appl. Environ. Microbiol. **62:**4174–4179.
31. **Ye, D., J. F. Quensen III, J. M. Tiedje, and S. A. Boyd.** 1992. Anaerobic dechlorination of polychlorobiphenyls (Aroclor 1242) by pasteurized and ethanol-treated microorganisms from sediments. Appl. Environ. Microbiol. **58:**1110–1114.
32. **Ye, D., J. F. Quensen III, J. M. Tiedje, and S. A. Boyd.** 1995. Evidence for *para* dechlorination of polychlorobiphenyls by methanogenic bacteria. Appl. Environ. Microbiol. **61:**2166–2171.
33. **Zhang, X., and J. Wiegel.** 1990. Sequential anaerobic degradation of 2,4-dichlorophenol in freshwater sediments. Appl. Environ. Microbiol. **56:**1119–1127.
34. **Zhang, X., and J. Wiegel.** 1992. The anaerobic degradation of 3-chloro-4-hydroxybenzoate in freshwater sediments proceeds via either chlorophenol or hydroxybenzoate to phenol and subsequently to benzoate. Appl. Environ. Microbiol. **58:**3580–3585.
35. **Zhang, X., Q. Wu, G.-W. Kohring, N. Messer, and J. Wiegel.** Unpublished data.