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Anaerobic *ortho* Dechlorination of Polychlorinated Biphenyls by Estuarine Sediments from Baltimore Harbor

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Reductive dechlorination of the *ortho* moiety of polychlorinated biphenyls (PCBs) as well as of *meta* and *para* moieties is shown to occur in anaerobic enrichments of Baltimore Harbor sediments. These estuarine sediments *ortho* dechlorinated 2,3,5,6-chlorinated biphenyl (CB), 2,3,5-CB, and 2,3,6-CB in freshwater or estuarine media within a relatively short period of 25 to 44 days. *ortho* dechlorination developed within 77 days in marine medium. High levels of *ortho* dechlorination (>90%) occurred when harbor sediments were supplied with only 2,3,5-CB. Incubation with 2,3,4,5,6-CB or 2,3,4,5-CB resulted in the formation of the *ortho* dechlorination product 3,5-CB; however, *para* dechlorination of these congeners always preceded *ortho* chlorine removal. *ortho* dechlorination of PCBs is an exceedingly rare event that has not been reported previously for marine or estuarine conditions. The activity was reproducible and could be sustained through sequential transfers. In contrast, freshwater sediments incubated under the same conditions exhibited only *meta* and *para* dechlorinations. The results indicate that unique anaerobic dechlorinating activity is catalyzed by microorganisms in the estuarine sediments from Baltimore Harbor.

Because of their widespread use, stability, improper disposal, and potential toxicity, polychlorinated biphenyls (PCBs) remain a ubiquitous environmental concern (8, 12, 25, 26, 30) with an estimated 10 million tons (1 ton = ca. 906 kg), equivalent to one-third of the total worldwide production, having been released into the environment (9). PCBs are highly hydrophobic and strongly associate with organic carbon, clays, and silt that settle into the anaerobic regions of sediments. Estuarine and marine sediments are the ultimate global sinks for worldwide accumulation of PCBs sorbed to particulate material (13), and environmental transformations of PCBs in estuarine sediments have been documented (6, 15, 16). However, our understanding of the biological PCB transformation potential in marine and estuarine environments, particularly in anaerobic sediments where these compounds would be prevalent, is limited. The nature of estuarine and marine environments should make them particularly well suited for transformations of halogenated xenobiotics, including PCBs. Biogenically synthesized halogenated compounds, primarily in the form of brominated aliphatic and aromatic hydrocarbons, are ubiquitous among marine organisms ranging from eubacteria and algae to metazoans and hemichordates (10). Some species in the class *Rhodophyceae* are reported to accumulate organohalides at concentrations of up to 5% (dry weight) (11). Although brominated hydrocarbons are more prolific, many chlorinated substitutions have also been reported (10). Since these halogenated organic compounds do not continue to accumulate in the environment, it is likely that some processes must be transforming them.

In freshwater sediments, anaerobic reductive dechlorination of PCBs at all positions on the biphenyl ring has been reported (for a review of anaerobic dechlorination of PCBs, see reference 4). However, reductive *ortho* dechlorination under freshwater conditions has rarely been observed, and sustaining such activity is reported to be difficult (32, 33). Anaerobic PCB dechlorination has been shown to occur in estuarine sediments (6) and has been demonstrated in the laboratory under estuarine and marine conditions (2, 21), but the dechlorination is slow and not extensive. *ortho* dechlorination has not been reported to occur in estuarine or marine sediments. Herein, anaerobic PCB-dechlorinating activities of Baltimore Harbor (BH) sediments are characterized in marine, estuarine, and freshwater enrichment media. Dechlorination of the *ortho* positions in addition to *para* and *meta* positions of PCBs is described.

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MATERIALS AND METHODS

Sediment sample. Core samples (41 by 5 cm) of sediment were taken 8 m below the surface water in the Inner Harbor of Baltimore, Md. BH sediments were black in color, gelatinous in texture, and had a strong petroleum odor. The salinity of the water column immediately above the sediments was 10 ppt at the time of sampling. The lower 30 cm of sediment was immediately transferred to a glass container that had been purged with nitrogen. Sodium sulfide nonahydrate was added to a final concentration of 0.018% (wt/vol), and the vessel was sealed under nitrogen with a butyl rubber stopper. The sediment sample was stored at room temperature in the dark prior to use. Hudson River H7 sediments were graciously supplied by General Electric Co. (Schenectady, N.Y.) and stored as described above.

Culture conditions. All media in these experiments included modified basal medium (29) composed of the following components in grams per liter (final concentration) of demineralized water: Na_2CO_3, 3.0; Na_2HPO_4, 0.6; NH_4Cl, 0.5; cysteine-HCl · H_2O, 0.25; $Na_2S \cdot 9H_2O$, 0.25; resazurin, 0.001. In addition, 1% (vol/vol) each of vitamin and trace element solutions was added (34). Estuarine medium without sulfate (E-Cl medium) contained the following components in grams per liter (final concentration) of basal medium: NaCl, 8.4; $MgCl_2 \cdot 6H_2O$, 3.95; KCl, 0.27; $CaCl_2 \cdot 2H_2O$, 0.05. Estuarine salts medium with sulfate (E medium) contained the following components in grams per liter (final concentration) of basal medium: NaCl, 8.4; $MgSO_4 \cdot 7H_2O$, 4.44; KCl, 0.27; $CaCl_2 \cdot 2H_2O$, 0.05. Marine salts medium with sulfate (M medium) contained the following components in grams per liter (final concentration) of basal medium: NaCl, 23.38; $MgSO_4 \cdot 7H_2O$, 12.32; KCl, 0.76; $CaCl_2 \cdot 2H_2O$, 0.14. Sterile media were prepared anaerobically in an atmosphere that contained N_2-CO_2 (4:1) by a modification of the Hungate technique (3). All gasses were passed through a column of reduced copper turnings at 350°C to remove traces of O_2. Media (8 ml) were dispensed into culture tubes (16 by 160 mm) and sealed with Teflon-lined butyl stoppers (The West Co., Lionville, Pa.) secured by aluminum crimp collars (Bellco Glass, Inc., Vineland, N.J.).

BH sediments (20% [vol/vol]) were inoculated into media and incubated with individual PCB congeners at the following final concentrations in micromoles per

TABLE 1. Moles percent and recovery data for BH sediment incubated with 2,3,4,5-CB in E-Cl medium

Enrichment[a]	Day	Mol% (nmol of congener)[b]							% Total recovery (nmol)[c]
		2,3,4,5-CB	2,4,5-CB	2,3,5-CB	3,5-CB	2,4-CB/2,5-CB	3-CB	4-CB	
1	0	100 (92)	0 (0)	0 (0)	0 (0)	0/0 (0/0)	0 (0)	0 (0)	53 (92)
	30	58–56 (64)	4 (4)	12–11 (13)	19–18 (21)	8/11 (9/12)	0 (0)	0 (0)	64–66 (111–114)
	66	32–29 (36)	1 (1)	1 (1)	41–36 (45)	25/33 (28/41)	0 (0)	0 (0)	64–72 (111–124)
	128	11–9 (10)	<1 (<0.5)	<1 (<0.5)	53–46 (49)	36/45 (33/48)	0 (0)	0 (0)	53–62 (92–107)
2	0	100 (113)	0 (0)	0 (0)	0 (0)	0/0 (0/0)	0 (0)	0 (0)	65 (113)
	35	41–38 (39)	2 (2)	5 (5)	35–33 (34)	17/23 (16/24)	0 (0)	0 (0)	55–60 (96–104)
	35-R	77–74 (283)	<1 (1)	2 (7)	13 (49)	8/11 (28/42)	0 (0)	0 (0)	106–110 (368–382)
	54	50–45 (102)	2 (5)	2 (4)	23–21 (48)	23/31 (47/70)	0 (0)	0 (0)	60–66 (206–229)
	54-R	67–63 (264)	2–1 (6)	1 (5)	16–15 (63)	15/19 (55/81)	0 (0)	0 (0)	76–81 (393–419)
	71	50–45 (114)	4–3 (8)	1 (2)	15–14 (35)	26/34 (58/85)	2 (4)	2 (5)	44–49 (226–253)
	154	20–16 (57)	6–5 (16)	1 (2)	10–9 (30)	45/55 (131/192)	6–5 (16)	13–10 (36)	55–67 (288–349)

[a] Enrichment 1 (fatty acids replenished) received 173 μM 2,3,4,5-CB on day 0 and was replenished with the fatty acid mixture at each sampling (see Materials and Methods for concentrations). Enrichment 2 (PCB and fatty acids replenished) received 173 μM 2,3,4,5-CB on day 0 and was replenished with the same amount of PCB at times designated with R; fatty acids were added to enrichment 2 at each sampling.

[b] Data are in moles percent with the total nanomoles for each congener recovered from a 1-ml sediment sample shown in parentheses. Congeners 2,4-CB and 2,5-CB could not be chromatographically resolved. Therefore, values for 2,4-CB and 2,5-CB are calculated for both congeners as described in Materials and Methods. All other moles percent values are a range based on values calculated for 2,4-CB or 2,5-CB.

[c] Total recovery is expressed in a percentage with the nanomoles recovered shown in parentheses (range once again dependent upon 2,4-CB or 2,5-CB).

liter: monochlorobiphenyls, 266; dichlorobiphenyls, 225; trichlorobiphenyls, 195; tetrachlorobiphenyls, 173; pentachlorobiphenyls, 154. Because of their low solubility in water, the congeners were solubilized in acetone before addition to the sediments. The final concentration of acetone was 0.1% (vol/vol). Sodium acetate, propionate, and butyrate were added to a final concentration of 2.5 mM each. Cultures were incubated at 30°C in the dark. Sterile controls (sterilized sediments) were autoclaved at 121°C for 3 h.

Sterilized controls with 2,3,4,5-, 2,3,5,6-, 2,3,6-, or 2,3,5-chlorinated biphenyl (CB) in estuarine medium without sulfate showed no activity for up to 128 to 154 days. The percent recoveries of total PCBs from all of these controls (calculated from seven incubations) were 53 ± 14 at day 0, 60 ± 17 at day 30, 50 ± 12 at day 60, 43 ± 11 at day 91, and 42 ± 10 at day 128. Similar recoveries were found in live cultures (see Tables 1 to 4).

Culture sampling and sample preparation. Replicate enrichments were sampled once at each time point. Cultures were sampled under O_2-free N_2, and samples were extracted as described previously (17, 22). Briefly, culture tubes were shaken, and a 1-ml slurry sample was immediately removed from the bottom of the tube with the reverse end of a 2-ml glass pipette. PCBs in the sample were extracted by shaking overnight with 10 ml of ethyl acetate in a 15-ml glass vial sealed with a Teflon-lined screw cap. After extraction, the organic phase was passed through a Florisil-copper column.

PCB analysis. PCB congeners were identified with a Hewlett-Packard 5890A gas chromatograph equipped with an electron capture detector (GC-ECD) and a RTX-1 capillary column (0.25 mm by 30 m; Restek Corp., Bellefonte, Pa.) as described by May et al. (17). PCB congeners were identified by retention time, and the relative molar distribution of congeners was determined from standard curves for individual congeners. Sixteen-point standard curves were individually developed for each congener. Congeners 2,4- and 2,5-CB could not be separated by the GC methods employed. In cases where 2,4- or 2,5-CB could be present, two calculations were made, one assuming all of the product as 2,4-CB and the other assuming all of the products as 2,5-CB. Congener 3-CB would shoulder onto 4-CB when both were present. A tangential integration was used to quantitate both. Identification of PCBs and biphenyl was confirmed by GC-mass spectrometry (GC-MS) with a Hewlett-Packard 5970 mass selective detector coupled to a Hewlett-Packard 5890A GC. The GC conditions were identical to those described above. In addition to the retention times expressed in the total ion chromatographs, mono-, di-, and trichlorobiphenyls were identified by their respective molecular ions (*m/z* 188, 222, and 256) and fragmentation patterns. Biphenyls, monochlorobiphenyls, and dichlorobiphenyls were assayed by selective ion monitoring at *m/z* values of 154, 188, and 222. The minimum detection limit for biphenyl was <10 pg for a 1-μl injection.

Chemicals. All PCBs were obtained at >99% purity from Accustandard Inc., New Haven, Conn. High-performance liquid chromatography (HPLC)-grade ethyl acetate was purchased from Fisher Scientific, Pittsburgh, Pa. All other chemicals were of reagent grade.

RESULTS

***ortho* dechlorination in estuarine media.** Reductive dechlorination of *ortho*-positioned chlorine atoms was observed when BH sediment was incubated anaerobically in estuarine medium without sulfate. GC-ECD analysis showed dechlorination of 2,3,4,5-CB and subsequent formation of the *ortho* dechlorination product 3,5-CB within 25 to 35 days. Identification of 3,5-CB was based on the retention time of 3,5-CB and the fact that no other dichlorinated biphenyl from the dechlorination of 2,3,4,5-CB has a retention time near that of 3,5-CB. In addition, analysis on a GC-MS confirmed that the peak with the same retention time as 3,5-CB had the same molecular ion (*m/z* 222) and fragmentation pattern as 3,5-CB.

The moles percent distribution of PCB congeners was monitored in two separate BH sediment enrichments (Table 1). Each enrichment culture was incubated with 2,3,4,5-CB, but only one of the cultures was replenished with 2,3,4,5-CB. Congener 3,4,5-CB was not detected in either enrichment. Therefore, *para* dechlorination likely preceded *ortho* dechlorination and 2,3,5-CB then became the *ortho* dechlorination substrate. In the non-PCB-replenished enrichment (enrichment 1), approximately 90% of the 2,3,4,5-CB was transformed within 128 days. Congeners 2,3,5-CB and 2,4,5-CB appeared to be transient, with nearly half of the parent congener (2,3,4,5-CB) eventually being converted to 3,5-CB. No monochlorobiphenyl was detected in the non-PCB-replenished enrichment. In the PCB-replenished culture (enrichment 2), there was significant production of 3,5-CB during the first 35 days. These data suggest that *ortho* dechlorination (3,5-CB production) was sustained. However, this interpretation is inconclusive because it was difficult later to assess the production of the 3,5-CB due to its conversion to 3-CB.

The accumulation of 4-CB late in the incubation of the enrichment 2 culture (Table 1) is indicative of a second *ortho* dechlorination that likely results from 2,4-CB. A single separate incubation of BH sediment with 2,3,4-CB also resulted in *ortho* dechlorination with the production of both 2,4-CB and 4-CB (data not shown). However, separate incubations of BH sediment with 2,4-CB and 2,4,5-CB for 154 days did not result in the formation of 4-CB. Congener 2-CB was never detected in any of these enrichments. Selective ion monitoring at *m/z* 154 by GC-MS did not detect biphenyl in any of the enrichments, indicating that complete dechlorination also did not occur. No activity was observed in sterile controls after 154 days.

TABLE 2. Moles percent and recovery data for BH sediment incubated with 2,3,5-CB in E-Cl medium

Enrichment[a]	Mol% (nmol of congener)[b]					% Total recovery[c]
	Day	2,3,5-CB	3,5-CB	2,5-CB	3-CB	
1	0	100 (141)	0 (0)	0 (0)	0 (0)	72 (141)
	30	49 (75)	51 (79)	0 (0)	0 (0)	79 (154)
	66	2 (3)	98 (125)	0 (0)	0 (0)	66 (128)
	128	0 (0)	9 (10)	1 (1)	90 (97)	55 (108)
2	0	100 (168)	0 (0)	0 (0)	0 (0)	86 (168)
	35	21 (33)	79 (123)	0 (0)	0 (0)	80 (156)
	35-R	51 (212)	49 (203)	0 (0)	0 (0)	106 (415)
	54	15 (30)	58 (117)	2 (5)	24 (49)	52 (201)
	54-R	62 (388)	27 (171)	1 (6)	10 (64)	107 (629)
	71	14 (50)	35 (120)	30 (105)	20 (70)	59 (345)
	154	9 (32)	12 (42)	60 (205)	18 (62)	58 (341)

[a] Enrichment 1 (fatty acids replenished) received 195 μM 2,3,5-CB on day 0 and was replenished with the fatty acid mixture at each sampling (see Materials and Methods for concentrations). Enrichment 2 (PCB and fatty acids replenished) received 195 μM 2,3,5-CB on day 0 and was replenished with the same amount of PCB at times designated with R; fatty acids were added to enrichment 2 at each sampling. Replenishment was identical to that described in Table 1.

[b] Data are in moles percent with the total nanomoles for each congener recovered from a 1-ml sediment sample shown in parentheses.

[c] Total recovery is expressed as a percentage with the nanomoles recovered shown in parentheses.

The data from dechlorination of 2,3,4,5-CB suggest that 2,3,5-CB is a substrate for *ortho* dechlorination. Moles percent analyses of two separate enrichments show that *ortho* dechlorination of 2,3,5-CB is heavily favored in the reductive dechlorination of this congener (Table 2). *ortho* dechlorination was sustained in an enrichment replenished with 2,3,5-CB (enrichment 2). Only when an enrichment was replenished did *meta* dechlorination of 2,3,5-CB to 2,5-CB develop. This was confirmed in enrichment 1 by the addition of 2,3,5-CB after 128 days (data not shown). Congener 3-CB accumulated in both enrichments after extended incubation. Selective ion monitoring for *m/z* 188 by GC-MS confirmed the presence of a monochlorinated biphenyl that elutes at the retention time of 3-CB. The formation of 3-CB may have resulted from *meta* dechlorination of 3,5-CB or *ortho* dechlorination of 2,5-CB. However, separate incubations of BH sediment with 2,5-CB showed no dechlorination after 154 days, and incubations with 3,5-CB resulted in the formation of 3-CB. Biphenyl was not detected by selective ion monitoring. These observations suggest that the formation of 3-CB from 2,3,5-CB, and possibly from 2,3,4,5-CB, results from the sequential dechlorination of the *ortho* moiety followed by *meta* dechlorination. The accumulation of high amounts of 2,5-CB in enrichment 2, after 2,3,5-CB had been depleted to low levels and high amounts of 3,5-CB had previously accumulated, is an anomaly that cannot be explained at this time. Total extraction of the entire enrichment culture (two ethyl acetate and two hexane acetone extractions) after 328 days of incubation recovered 42% of the added PCBs and the moles percent distribution (2,3,5-CB, 2; 3,5-CB, 14; 2,5-CB, 62; 3-CB, 22) remained relatively the same as that at 154 days.

Expanding the survey to include incubations of BH sediments with other individual PCB congeners resulted in the discovery of two other *ortho* dechlorinations. The tetrachlorobiphenyl 2,3,5,6-CB was both *meta* and *ortho* dechlorinated. Table 3 shows the moles percent distributions from two separate enrichment cultures. The accumulation of *ortho* dechlorination products 2,3,5- and 3,5-CB was dominant early on in the non-PCB-replenished enrichment. Congener 3-CB was the major product at day 128 in this enrichment culture. Such products were also present in the PCB-replenished enrichment, but more of the *meta* dechlorination products 2,3,6- and 2,6-CB accumulated. The formation of 2,5-CB could have been due to *ortho* or *meta* dechlorination. The data from Table 2 shows sustained *ortho* activity. However, this is once again difficult to assess later on because of the production of 2,5- and 3-CB.

Another congener observed to be *ortho* dechlorinated was 2,3,6-CB. Moles percent analysis of BH sediment incubated with only 2,3,6-CB showed that the ratio of 2,6-CB to 2,5-CB was nearly 3:1 in duplicate enrichments (Table 4). Replenishing the cultures with 2,3,6-CB had no effect on the ratio of 2,6-CB to 2,5-CB. However, *ortho* dechlorination was sustained in the replenished enrichments. Although the amount of 2,5-CB produced is significant, it does not appear that *ortho* dechlorination of 2,3,6-CB is as extensive as that of 2,3,5-CB. A small amount of 2,3-CB which represents another *ortho* dechlorination, that of position 6 of 2,3,6-CB, also appeared in both enrichments.

TABLE 3. Moles percent and recovery data for BH sediment incubated with 2,3,5,6-CB in E-Cl medium

Enrichment[a]	Day	Mol% (nmol of congener)[b]							% Total recovery (nmol)[c]
		2,3,5,6-CB	2,3,6-CB	2,3,5-CB	3,5-CB	2,5-CB	2,6-CB	3-CB	
1	0	100 (98)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	57 (98)
	30	99 (112)	<1 (<0.5)	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	65 (113)
	66	9 (15)	1 (1)	3 (4)	56 (88)	18 (28)	4 (7)	9 (15)	91 (158)
	128	1 (1)	<1 (<0.5)	1 (1)	6 (6)	22 (24)	6 (6)	65 (71)	63 (109)
2	0	100 (129)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	75 (129)
	35	89 (109)	1 (2)	7 (8)	2 (2)	1 (1)	0 (0)	0 (0)	71 (122)
	35-R	97 (277)	<1 (1)	2 (6)	1 (2)	0 (0)	0 (0)	0 (0)	83 (286)
	54	32 (77)	13 (31)	2 (5)	3 (7)	29 (69)	22 (52)	0 (0)	70 (241)
	54-R	63 (335)	8 (41)	1 (5)	2 (11)	15 (77)	12 (62)	0 (0)	102 (531)
	71	22 (68)	26 (80)	3 (10)	4 (11)	29 (88)	16 (49)	0 (0)	59 (306)
	154	18 (57)	14 (44)	2 (8)	5 (15)	31 (102)	26 (85)	4 (14)	63 (325)

[a] Enrichment 1 (fatty acids replenished) received 173 μM 2,3,5,6-CB on day 0 and was replenished with the fatty acid mixture at each sampling (see Materials and Methods for concentrations). Enrichment 2 (PCB and fatty acids replenished) received 173 μM 2,3,5,6-CB on day 0 and was replenished with the same amount of PCB at times designated with R; fatty acids were added to enrichment 2 at each sampling. Replenishment was identical to that described in Table 1.

[b] Data are in moles percent with the total nanomoles for each congener recovered from a 1-ml sediment sample shown in parentheses.

[c] Total recovery is expressed as a percentage with the nanomoles recovered shown in parentheses.

TABLE 4. Moles percent and recovery data for BH sediment incubated with 2,3,6-CB in E-Cl medium

Enrichment[a]	Day	Mol% (nmol of congener)[b]					% Total recovery (nmol)[c]
		2,3,6-CB	2,3-CB	2,5-CB	2,6-CB	3-CB	
1	0	100 (176)	0 (0)	0 (0)	0 (0)	0 (0)	90 (176)
	30	97 (126)	0 (0)	1 (1)	2 (3)	0 (0)	67 (130)
	66	25 (38)	<1 (<0.5)	16 (25)	58 (89)	0 (0)	78 (152)
	128	5 (6)	<1 (<0.5)	20 (27)	75 (99)	0 (0)	68 (132)
2	0	100 (83)	0 (0)	0 (0)	0 (0)	0 (0)	43 (83)
	35	72 (59)	0 (0)	7 (6)	21 (17)	0 (0)	42 (82)
	35-R	92 (263)	0 (0)	2 (6)	6 (18)	0 (0)	74 (287)
	54	14 (49)	0 (0)	23 (81)	63 (225)	0 (0)	91 (355)
	54-R	54 (407)	0 (0)	11 (85)	34 (255)	0 (0)	128 (747)
	71	21 (70)	0 (0)	19 (65)	60 (199)	0 (0)	57 (334)
	154	12 (17)	<1 (<0.5)	18 (26)	71 (103)	0 (0)	25 (146)

[a] Enrichment 1 (fatty acids replenished) received 195 μM 2,3,6-CB on day 0 and was replenished with the fatty acid mixture at each sampling (see Materials and Methods for concentrations). Enrichment 2 (PCB and fatty acids replenished) received 195 μM 2,3,6-CB on day 0 and was replenished with the same amount of PCB at times designated with R; fatty acids were added to enrichment 2 at each sampling. Replenishment was identical to that described in Table 1.

[b] Data are in moles percent with the total nanomoles for each congener recovered from a 1-ml sediment sample shown in parentheses. Some of the 2,3,6-CB used in these experiments was contaminated with 0.4% 2,3,4,6-CB. All of the 2,3,4,6-CB was transformed and a corresponding amount of 2,4,6-CB accumulated. These two congeners are not included in the data shown.

[c] Total recovery is expressed as a percentage with the nanomoles recovered shown in parentheses.

Congener 2,3,4,5,6-CB was also dechlorinated when incubated with BH sediments. Congener 2,3,4,5-CB was never observed in cultures incubated with 2,3,4,5,6-CB. Therefore, similar to the dechlorination of 2,3,4,5-CB, *meta* and/or *para* dechlorinations must precede *ortho* dechlorination of this pentachlorinated biphenyl. Significant amounts of 2,3,5-CB and 3,5-CB from 2,3,4,5,6-CB were detected over time, but 2,4,6-CB was the most prevalent product. Replicate enrichments gave similar results.

Several other congeners were tested individually in separate enrichments of BH sediment, but none were *ortho* dechlorinated. The following congeners were not dechlorinated at all: 2,2′,6,6′-CB, 2,4,6-CB, 2,2′-CB, 2,4-CB, 2,5-CB, 2,6-CB, 2-CB, 3-CB, and 4-CB (minimum of 145 days of incubation). No loss of a monochlorinated biphenyl was ever observed, and biphenyl was not detected by GC-MS in the enrichments incubated with monochlorobiphenyls. The following transformations were observed: 2,4,5-CB to 2,4- or 2,5-CB; 3,4,5-CB to 3,4-CB, 3,5-CB, and 3-CB; 2,3-CB to 2-CB; 3,4-CB to 3-CB; and 3,5-CB to 3-CB. Although 2,3,5-CB and 2,3,6-CB were *ortho* dechlorinated, it is interesting to note that 2,4,5-CB, 2,4,6-CB, and all of the *ortho*-chlorinated biphenyls tested, at least in individual incubations, were not *ortho* dechlorinated. These results suggest that *ortho* dechlorination occurs when the biphenyl ring is sufficiently chlorinated and contains a *meta* chlorine adjacent to the *ortho* chlorine.

Supernatants from several of the enrichments described above have been serially transferred in fresh E-Cl medium plus sterile BH sediment, coal-based humic acids, or Hudson River Spier Falls sediment (non-PCB contaminated). Dechlorination has been observed as early as 7 days in these transferred cultures, and *ortho* dechlorination develops within 21 days. Activity in these transfers has been observed with 0.05 to 1.0% (wt/vol [dry weight]) sediment in the medium.

***ortho* dechlorination in other estuarine, marine, and nonmarine media.** Sulfate is prevalent in marine and estuarine environments at concentrations that are reported to inhibit dechlorination of PCBs in freshwater sediments (2, 6, 31). However, PCB dechlorination has been shown to occur anaerobically with estuarine sediments in the presence of high concentrations of sulfate (21). To determine the effects of sulfate on PCB dechlorination in BH sediments, enrichments were incubated with 2,3,4,5-CB in estuarine (E) and marine (M) media that contained 18 and 50 mM $MgSO_4 \cdot 6H_2O$, respectively. For enrichments in both of these media, the moles percent distribution of congeners was very similar to that observed with the E-Cl medium, but activity in the M medium lagged (no dechlorination at 44 days, dechlorination including *ortho* at 77 days). Marine medium without sulfate ($MgCl_2 \cdot 6H_2O$ substituted for $MgSO_4 \cdot 6H_2O$) also supported *ortho* dechlorination but with less of a lag than enrichments with sulfate in the medium. These results demonstrate that anaerobic PCB dechlorination including *ortho* dechlorination develops in BH sediments inoculated into media containing relatively high sulfate and solute concentrations associated with estuarine and marine conditions. It is possible that the sulfate is consumed before dechlorination sets in. However, since sulfate was not monitored in these enrichments, the effect of sulfate on PCB dechlorination is inconclusive at this time.

Reduced anaerobic mineral medium (RAMM) (27) has been used with sediments from freshwater sites such as the upper Hudson River (1, 5, 19, 23). *ortho* dechlorination has never been reported with Hudson River sediments in RAMM. Our incubations of Hudson River H7 sediment (supplied by General Electric) with 2,3,4,5-CB in RAMM did result in *meta* and *para* dechlorination within 22 days, and no *ortho* dechlorination was observed over a period of 124 days. No *ortho* dechlorination was observed with Hudson River sediment in E, M, E-Cl, or M-Cl medium. Incubations of BH sediment in RAMM with 2,3,4,5-CB did result in *ortho* dechlorination. Once again, the transformations observed were qualitatively similar to those observed with E-Cl medium. However, the BH enrichments showed high levels of 2,3,5-CB early during incubation followed by high accumulations of 3,5-CB with no production of 3-CB. Congener 2,4,5-CB was produced only in trace amounts. These activities suggest a shift from dechlorination of the *meta* moiety to that of the *para* and *ortho* moieties when the BH sediments are incubated in RAMM, a nonmarine medium.

DISCUSSION

A review of all of the experiments presented here demonstrates that a rare and unique type of anaerobic PCB dechlorination (*ortho*) arises rapidly in enrichments containing BH sediment. The major *ortho* dechlorination pathways observed are summarized in Fig. 1. Since BH is part of the Chesapeake Bay, which is an extensive drainage basin, there is likely a gradation of freshwater, estuarine, and marine microbial communities along the length of the bay. Sediments in BH may contain components of the three communities. While *ortho*-dechlorinating activity might be attributed to populations of estuarine and marine microorganisms, wide differences in reductively dechlorinating populations have been reported among sites of close proximity in the St. Lawrence River. These differences have been attributed to sediment characteristics (28). It is therefore possible that undefined conditions in BH select for PCB-dechlorinating populations that include *ortho* dechlorination. PCBs have been reported to be associated with the particulate fraction of the water column of the Chesapeake Bay (14), and PCB contamination of BH sediment has been documented (20). PCBs were not detected in BH sediments by

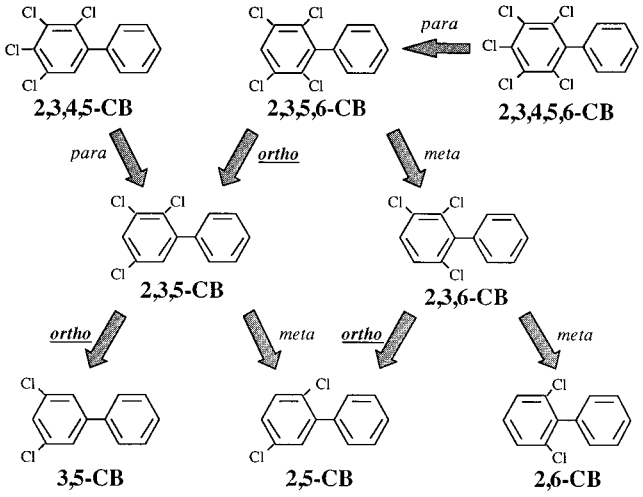

FIG. 1. *ortho* dechlorination pathways observed in this study. Not all dechlorinations are represented. The figure depicts the most prevalent *ortho* reactions and the necessary precursor reactions. Predominance of pathway or step is not indicated since this may vary with the PCB mixture and environmental conditions.

methods described in this study. However, past contamination of the harbor with PCBs or other chlorinated organics may have promoted in situ selection of dechlorinating organisms.

The removal of *ortho* chlorines from PCBs has not been demonstrated previously with estuarine or marine sediments. In addition, reports on *ortho* dechlorination with anaerobes from any environment have been infrequent. The activity has always required several months to develop and has been difficult to repeat or maintain. The best-documented report of *ortho* dechlorination was published by Van Dort and Bedard (32) and showed *ortho* dechlorination of 2,3,5,6-CB to 2,5-CB, via either 2,3,6-CB or 2,3,5-CB, in one freshwater sediment culture from Woods Pond (Lenox, Mass.) after 21 weeks of incubation. After 37 weeks, 19.4% of the PCB had been converted to 2,5-CB and 58.2% had been converted to 2,6-CB. The balance of PCB consisted of 2,3,6-CB, a trace of 2,3,5-CB, and residual 2,3,5,6-CB. Congener 2,3,5-CB never accumulated to high levels, and 3,5-CB was not detected. The *ortho*-dechlorinating activity by the Woods Pond culture ceased after 28 weeks and was subsequently followed by *meta* dechlorination. The *ortho*-dechlorinating activity did not return, and the authors attributed the loss of *ortho* activity to a change in dechlorinating populations. Williams (33) also reported that a culture from Woods Pond and one sediment culture from Silver Lake (Pittsfield, Mass.) *ortho* dechlorinated 2,4,6-CB to 2,4-CB and 4-CB after 24 weeks and more than 1 year of incubation, respectively. Sustainability was not addressed in this study. Montgomery and Vogel (18) reported that 2,3,5,6-CB was *ortho* dechlorinated to 2,3,5-CB and 3,5-CB over a 14-month period by sediment cultures under anaerobic phototrophic conditions. The investigators reported dechlorination in the dark to be nonexistent or negligible. Unfortunately, the authors did not prepare and monitor a killed-cell (sterilized-sediment) control, which would have been useful in interpreting the results since PCBs have been shown to be photochemically dechlorinated, particularly at the *ortho* position (7, 24). All of the experiments reported here involved incubation in the dark.

In contrast with *ortho*-dechlorinating activity observed with freshwater sediments, the *ortho* dechlorination observed with BH sediments is different in that 2,3,5-CB and 3,5-CB are very prevalent products in BH sediments when 2,3,4,5-CB, 2,3,5,6-CB, or 2,3,5-CB is present. Another congener specificity difference is that 2,4,6-CB was not dechlorinated in BH sediments, although this congener may require a lengthier incubation. In addition, the *ortho* dechlorination observed in BH sediment develops relatively quickly and can be very extensive with acclimation times of less than 1 month and greater than 90% *ortho* transformation of 2,3,5-CB to 3,5-CB in some enrichments. The *ortho* dechlorination is also readily maintained and reproduced. The enrichments described here were inoculated with sediments collected on 19 July 1992. *ortho*-dechlorinating enrichments have also been developed with fresh BH sediment collected from the same site on 11 July 1995. Activity has been maintained for more than 6 months by replenishment with media and 2,3,4,5-CB, and *ortho* dechlorination has been observed in five different media, including those with high solute concentrations. Transfer of the activity to fresh media has also been successful, with serial transfers demonstrating *ortho* dechlorination within 21 days.

The *ortho* dechlorination by BH sediments also appears to be broad in that a variety of congeners, including tetra-, tri-, and dichlorobiphenyls, are attacked (*ortho* dechlorination of dichlorobiphenyls appeared to have occurred only when tetra- or trichlorobiphenyls were present). The lower levels of dichlorobiphenyls and 3-CB in enrichments replenished with 2,3,5,6-CB (Table 3) suggest that the tetrachlorobiphenyl is more readily *ortho* dechlorination than lesser-chlorinated congeners and perhaps can act as a more attractive electron acceptor for an *ortho* PCB-dechlorinating anaerobe. This is consistent with previous observations that more extensively chlorinated PCBs are more readily *meta* and *para* dechlorinated in anaerobic freshwater sediment enrichments (4).

The demonstration of *ortho*-dechlorinating activity with BH sediments suggests that previously undescribed estuarine or marine anaerobic microorganisms present in these sediments are capable of unique activity or that environmental conditions enhance a biological activity rarely observed. Other marine and estuarine sites are being investigated by the methods described here to determine the prevalence of *ortho* dechlorination. Environmental conditions such as solute concentration, carbon sources, pH, and sediment, etc., are also under investigation. Additionally, the dechlorination of commercial mixtures of PCBs (Aroclors) is being examined. BH sediments amended with Aroclor 1242 (400 ppm) or Aroclor 1242 plus 2,3,4,5-CB (172.5 μM) have been analyzed after 2 months of incubation. Thus far, the 2,3,4,5-CB was *ortho* dechlorinated to 3,5-CB within 1 month, indicating that the Aroclor mixture does not inhibit *ortho* dechlorination of this congener. Congeners belonging to Aroclor 1242 were transformed after 2 months of incubation. A complete analysis of Aroclor dechlorination requires further incubation time and PCB analysis. In addition, the investigations presented here deal primarily with the dechlorination of congeners that are chlorinated on only one ring. Incubations of BH sediments with PCBs chlorinated on both rings, especially *ortho*-substituted and more heavily chlorinated congeners, is needed to further define the dechlorination potential of the anaerobic microorganisms in these sediments. The results presented in this study suggest that the development of *ortho* dechlorination in conjunction with activity specific for other chlorine substitutions could be combined for more extensive reductive dechlorination of PCBs in anaerobic environments.

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