Biodegradation of Coplanar Polychlorinated Biphenyls by Anaerobic Microorganisms from Estuarine Sediments

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Abstract. In this study, we investigated the biodegradability of biphenyl and 5 congeners (one non-planar and four coplanar) of polychlorinated biphenyl (PCB). Biphenyl, the non-planar congener 2,3',4',5-tetrachlorobiphenyl (2534 CB), and the four coplanar congeners 3,3',4,4'-tetrachlorobiphenyl (34-34 CB), 3,4,4',5-tetrachlorobiphenyl (345-4 CB), 3,3',4,4',5-pentachlorobiphenyl (345-34 CB), and 3,3',4,4',5,5'-hexachlorobiphenyl (345-345 CB) were amended at a concentration of 10 mg/L into anoxic sediment slurries collected from the estuaries of the Tansui River and the Erjen River. During 2 years’ incubation under sulfidogenic conditions, biphenyl was persistent, while all other chlorinated congeners, except for 345-345 CB, were dechlorinated with or without a lag period in sediment slurries collected from both rivers. Dechlorination of coplanar and non-planar congeners began with para chlorine removal. All para chlorines from the mono-, di-, and trichlorobiphenyl groups could be removed by sediment slurries from both rivers. Microbial communities in sediment from the Erjen River additionally fostered meta-dechlorination activity, but only after removal of all the para chlorines. Addition of Tween 20 (0.05%, v/v) into sediment slurries from the Tansui River did not enhance dechlorination rates or extents, but the addition of toluene- or 3-chlorobenzoate-adapted sediments enhanced dechlorination of 34-34 CB and 345-4 CB.

Keywords: coplanar congeners, non-planar congeners, PCBs, biphenyl, dechlorination, Tween 20, anoxic estuarine sediments.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are ubiquitous contaminants of the environment (Hansen 1987;
Hutzinger and Veerkamp 1981). With an estimated 10 million tons, equivalent to one-third of the total worldwide production of PCBs, had been released into the environment (Eisenreich 1987). PCBs are highly hydrophobic and associate strongly with the organic carbons, clays, and silts that settle into the anaerobic regions of sediment (Kennedy 1984). High levels of PCBs are found in harbors, near-shore sediments, and dredge spoils. Estuarine and marine sediments are the ultimate global sinks for the worldwide accumulation of PCBs (Hansen 1987).

Although a recent study has shown that PCBs were extensively dechlorinated via a nearly complete loss of meta and para chlorines (Abramowicz et al. 1993) in the sediment of Hudson River of New York, the rates of PCB dechlorination in anaerobic environments have been slow, with major changes usually occurring only over a period of months or years. Since most PCB dechlorination studies have been conducted in freshwater sediments (Abramowicz et al. 1993; Alder et al. 1993; Bedard and Quensen 1995; Brown et al. 1987a; Quensen et al. 1990; Sokol et al. 1994), our understanding of PCB biotransformation potential in marine and estuarine sediments under anaerobic conditions is still based on only a limited number of reports (Brown and Wagner 1990; Lake et al. 1991; 1992; Ofjord et al. 1994).

209 distinct congeners of PCB are all toxic, and the toxicity of the coplanar congeners, i.e. those which have four or more chlorine atoms at both para and meta positions such as 3,3',4,4'-tetrachlorobiphenyl (34-34 CB), 3,4,4',5-tetrachlorobiphenyl (345-4 CB), 3,3',4,4',5-pentachlorobiphenyl (345-34 CB), and 3,3',4,4',5,5'-hexachlorobiphenyl (345-345 CB) is equivalent to that of 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD), which is recognized as one of the most toxic anthropogenic organic compounds (Bryan et al. 1987). Environmental coplanar PCB concentrations are generally higher than that of dioxin (Kannan et al. 1989), and they have been shown to be widespread on a global scale (Tanabe et al. 1987). For these reasons, many authors have suggested that coplanar PCBs may be a more significant environmental threat than dioxins and dibenzofurans (Klump et al. 1987; Safe 1992; U. S. Environmental Protection Agency 1993a; b).

Concern over the toxicity and bioaccumulation potential of PCBs has underlined the need to remove these compounds from the environment. In this study, we investigated the degradation potential and the degradation pathways of several coplanar congeners in sediments collected from the estuaries of the Tansui River and the Erjen River. The effects of the addition of surfactant and enrichment cultures on the dechlorination of these compounds were also investigated.

MATERIALS AND METHODS

Chemicals and reagents

All isomers of PCB (99% purity): 2 CB, 3 CB, 4 CB, 2,3 CB, 2-4 CB, 3-3 CB, 4-4 CB, 34 CB, 345 CB, 25-3 CB, 25-4 CB, 34-2 CB, 35-4 CB, 25-34 CB, 34-34 CB, 345-4 CB, 345-34 CB, 345-345 CB, were
obtained from Accustandard (New Haven, CT, USA). Toluene and biphenyl were from Yakuri Pure Chemicals (Osaka, Japan). Tween 20 was obtained from Aldrich Chemical Company (Milwaukee, WI, USA). All other chemicals were reagent or GC grade.

**Sampling sites**

Sediments were obtained from the estuaries of the Tansui River (Taipei, Taiwan) and the Erjen River (Tainan, Taiwan) in August 1995 and December 1995, respectively. The Tansui River is located in the northwest of Taiwan, while the Erjen River is located in the southwest. Salinity and pH of the overlying site water at the time of sampling are shown in Table 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Tansui River</th>
<th>Erjen River</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location (GPS)</td>
<td>N 25 07.39, E 121 27.703</td>
<td>N 22 55.107, E 120 11.124</td>
</tr>
<tr>
<td>Salinity (ppt)</td>
<td>14</td>
<td>20</td>
</tr>
<tr>
<td>pH</td>
<td>7.3</td>
<td>7.24</td>
</tr>
<tr>
<td>Organic Carbona (%)</td>
<td>1.42</td>
<td>0.48</td>
</tr>
<tr>
<td>Carbon/Nitrogenb (atom)</td>
<td>13.66</td>
<td>5.57</td>
</tr>
<tr>
<td>Total Oil and Greaseb (%)</td>
<td>0.005</td>
<td>0.007</td>
</tr>
</tbody>
</table>

*a* Organic carbon & nitrogen were measured by CHN analyzer after acidification.

*b* Dry weight basis.

**Sediment collection and treatment**

To avoid contact with atmospheric oxygen, the top sediments (0 to 5 cm) were collected by completely filling jars with sediment and site water and sealing underwater. All samples were kept in the dark and sent back to the laboratory within 12 hours. Upon arrival in the lab, sediment samples were flushed with a gas mixture of N₂: H₂ (19:1). They were then taken into an anaerobic glove box (Coy Labs, Grass Lake, MI, USA) which was filled with the same nitrogen-hydrogen gas mixture, and here they were sieved with a copper wire screen (1 by 1 mm) to remove large debris. Sieved sediment and the basal medium were used to prepare sediment slurries containing 10% solids (wt/v), as calculated on a dry-weight basis. Anoxic basal medium stock solutions were prepared as described previously (Liu and Kuo 1997). These medium stock solutions were then taken into the anaerobic glove box. The glove box was equipped with a Coy model 10 continuous readout gas analyzer to confirm the absence of O₂ during all experimental procedures. The final concentration (mg/L) of the basal medium was: KH₂PO₄, 200; KCl, 300; NH₄Cl, 300; CaCl₂-2H₂O, 150; MgCl₂-6H₂O, 300; Na₂SO₄, 3000; FeCl₃-4H₂O, 1.5; CuCl₂-2H₂O, 0.015; Na₂MoO₄-2H₂O,
0.025; NiCl₂·6H₂O, 0.025; H₃BO₃, 0.06; ZnCl₂, 0.07; MnCl₂·4H₂O, 0.1; CoCl₂·6H₂O, 0.12; Na₂SeO₃, 0.003; biotin, 0.01; p-aminobenzoic acid, 0.05; B₁₂, 0.05; and thiamin, 0.1. In addition, NaHCO₃ was added separately from a stock solution saturated with CO₂ and its final concentration was 1.2 g/L. The pH of the medium was about 7.2.

Sediment characterization

Sediment characteristics are summarized in Table 1. Organic carbon and total nitrogen content of sediments were determined by flash combustion of HCl-acidified and freeze-dried samples using a Perkin-Elmer Series II CHNS/O Analyzer model 2400. The total oil and grease content of the sediment collected from the Tansui River and the Erjen River were determined by US Environmental Protection Agency method 413.1.

All experiments were replicated and the results shown in Table 1 are the average of triplicates.

Transformation and dechlorination studies

The fates of biphenyl, one non-planar congener (25-34 CB), and each of the four PCB coplanar congeners (i.e. 34-34 CB, 345-4 CB, 345-34 CB, and 345-345 CB) were examined in the estuarine sediments. For the transformation and dechlorination studies, aliquots of sediment slurries (30 ml) were dispensed into serum bottles (50 ml) capped with butyl rubber stoppers and aluminum crimp sealed. Initial sulfate concentration of the sediment slurries was 20 mM. Sterile controls were prepared by autoclaving at 121°C for 30 min on three consecutive days. Stock solution of biphenyl was dissolved in acetonitrile to a final concentration of 20 g/L. Stock solution of each PCB congener was dissolved in acetone to a final concentration of 5 g/ml. Experiments were then initiated by adding biphenyl or PCB congeners to a final concentration of 9-12 mg/L to the active and control sediment slurries. Additional control sediment slurries were prepared by amending with acetonitrile or acetone only. All experiments were replicated, and the results are reported as the average of duplicates. The serum bottles were incubated at 23-25°C in the dark. At intervals, 1.0 ml subsamples were removed using sterile techniques from each active and control sediment slurry while swirling the slurry to ensure a uniform suspension. These subsamples were analyzed as described below. Sulfate concentration in the sediment slurries was also determined at intervals during incubation, for which purpose 1.0 ml subsamples were removed from the sediment slurries and centrifuged directly. After filtration, the supernatant was used for sulfate determination.

The effect of the addition of the surfactant Tween 20 (0.05%, v/v) on the dechlorination of 34-34 CB, 345-4 CB, and 345-345 CB in sediment slurries from the Tansui River was also determined.

Subsample preparation and analysis

Subsamples (1.0 ml) were extracted by shaking, once with 10 ml of acetone containing 40 μg of
octachloronaphthalene as the internal standard and twice more with 10 ml of hexane-acetone (9:1). The solvent extracts were combined, and the acetone was extracted with 2% NaCl in deionized water. The remaining hexane extract was extracted with 2 to 4 ml of concentrated sulfuric acid, rinsed again with 2% NaCl in deionized water, and then dried over anhydrous Na$_2$SO$_4$. Further cleanup was performed on a florisil-copper powder column. These columns were prepared by packing approximately 4 parts of 60/100-mesh florisil and 1 part of 60-mesh copper powder (to remove sulfur) in a pasteur pipette. The copper was rinsed first with 10% H$_2$SO$_4$, then deionized water, and then acetone and dried under vacuum before use. The sample was eluted from the column with hexane, and the final volume was adjusted to 25 ml before analysis on a gas chromatograph (GC).

The PCB congeners were separated and quantified by GC using a HP-5 capillary column (50 m x 0.2 mm, film thickness: 0.11 μm; Hewlett Packard, Idaho, USA). The Hewlett Packard 5890 series II GC consisted of an 18593B autosampler, a split capillary column injection port, and a $^{63}$Ni electron capture detector. Operating parameters for the GC were as follows: injector, 250°C; detector, 325°C; He carrier gas, 0.7 ml/min; and methane: argon (1:19, v/v) makeup gas, 40 ml/min. The temperature program was as follows: 200°C for 2 min; ramped to 260°C at a rate of 5°C/min; held at 260°C for 1 min; ramped to 280°C at a rate of 5°C/min; held at 280°C for 5 min. Quantification was performed by using a Hewlett-Packard 35900D Chemstation.

Parent compounds and all possible dechlorination products were initially identified by matching GC retention times with those of authentic standards (all 99% purity, Accustandard, USA) and all identifications were later confirmed by GC/mass spectrometry (GC/MS). We monitored for all possible meta- and para-dechlorination products of each parent compound. For example, following this criterion, the potential dechlorination products of 345-34 CB are 3 CB, 4 CB, 3-3 CB, 3-4 CB, 3-5 CB, 4-4 CB, 34 CB, 35 CB, 34-3 CB, 34-4, 34-5 CB, 35-3 CB, 35-4 CB, 345 CB, 345-3 CB, 345-4 CB, 34-34 CB, and 34-35 CB.

GC/MS analyses were performed with a Varian Saturn 4D GC/MS/MS. Separation of components was achieved using a DB-5 MS (J and W Scientific, CA, USA), fused-silica capillary column (30 m x 0.32 mm I.D.; 0.25μm film thickness). The column was operated under the following temperature program: 95°C for 1 min; ramped to 130°C at a rate of 4°C/min; ramped to 170°C at a rate of 15°C/min; ramped to 250°C at a rate of 2°C/min; ramped to 310°C at a rate of 30°C/min. The final temperature of 310°C was held for 2 min. The detector was programmed to scan over a mass range of 100 to 400 mass units at 1.3 scans/sec.

Four-point (5, 50, 100, and 200 μM in hexane) linear calibration curves were constructed from standards of the parent compounds and all the possible dechlorination products.

**Sulfate analysis**

Sulfate was analyzed by indirect titration (Howarth 1978).
Preparation of adapted sediment microbial communities

Large aliquots (200 ml) of the estuarine sediment slurries from the Tansui River were adapted to transform 3-chlorobenzoate or toluene by repeated additions (minimum of 2 additions) of the specific compound (10-20 mg/L) following its removal to less than 1 mg/L. The sediment slurries were considered adapted when less than 0.5 mg/L of each compound was detected within 20 days of incubation of the last addition. The adapted sediment slurries were maintained in an active state by the addition of the specific compound to the corresponding adapted sediment slurries at intervals. The rate and extent of dechlorination of each PCB congener was investigated in sediment slurries amended with or without 3-chlorobenzoate- or toluene-adapted sediment.

RESULTS

Dechlorination of 34-34 CB in anoxic estuarine sediments

As shown in Fig. 1a, 34-34 CB was removed from 44 μM to 10 μM within 600 days without a lag period in the sediment slurries collected from the Tansui River. However, 34-34 CB (30 μM) was removed within 230 days after a lag period of 63 days in the sediment slurries collected from the Erjen River. (Fig. 1b). Subsequent addition of 34-34 CB to the sediment slurries from the Erjen River resulted in an enhanced rate of 34-34 removal. There was only one intermediate product detected during incubation in the sediment slurries from both rivers, and this product was identified as 3-3 CB based on the GC and GC/MS chromatograms. Thus 2 chlorine atoms at the para positions were removed from 34-34 CB. There were no observable changes of 34-34 CB in sterile controls during the incubation period.

Dechlorination of 345-4 CB in anoxic estuarine sediments

345-4 CB (48 μM) was removed within 300 days after a lag period of 16 days in the sediment slurries collected from the Tansui River. Subsequent addition of 345-4 CB to the sediment slurries resulted in a slightly enhanced rate of 345-4 CB removal, although, the rate of 345-4 CB removal decreased after the concentration of 345-4 CB had fallen to 20 μM. Two intermediate products were detected in succession during incubation. These two products were identified as 35-4 CB and 35 CB, respectively based on the GC and GC/MS chromatograms. 35-4 CB was accumulated to 40 μM and was then remained persistent for about 180 days before it was further dechlorinated to 35 CB.

In the sediment slurries collected from the Erjen River, 345-4 CB (48 μM) was removed within 320 days without a lag period. Subsequent addition of 345-4 CB to the sediment slurries did not result in an enhanced rate of 345-4 removal, but 35-4 CB and 35 CB were again detected in succession as the intermediate products during incubation. Both 35-4 CB and 35 CB were accumulated only to a concentration of 25 μM and 18 μM, respectively. Clearly, 35 CB must have been further transformed to other compounds. However, neither 3 CB nor biphenyl was observed in the sediment slurries.
Fig. 1 Anaerobic dechlorination of 34-34 CB in sediment slurries from (a) the Tansui River and (b) the Erjen River.

Arrow: Subsequent addition of 34-34 CB to the Erjen slurries.
There were no observable changes of 345-4 CB in sterile controls during the incubation period.

**Dechlorination of 345-34 CB in anoxic estuarine sediments**

345-34 CB (50 μM) was removed to 30 μM in the first 16 days, but it then remained persistent for about 248 days before it was further removed in the sediment slurries collected from the Tansui River. There were two intermediate products detected in succession during incubation, and these were respectively identified as 35-34 CB and 35-3 CB. 345-34 CB was first transformed to 35-34 CB at the stoichiometric ratio of 1:1. 35-34 CB remained in the sediment slurry for about 200 days before it was further dechlorinated to 35-3 CB at the stoichiometric ratio of 1:1. Therefore, 345-34 CB was dechlorinated by removal of chlorines at the para position.

In the sediment slurries collected from the Erjen River, 345-34 CB (40 μM) was removed in 300 days without a lag period. Subsequent addition of 345-34 CB to the sediment slurries resulted in an enhanced rate of 345-34 removal. 35-3 CB and 3-3 CB were detected in succession, but 35-34 CB, which only appeared after all the 345-34 CB had already been removed, was not detected during incubation. Both 35-3 CB and 3-3 CB were accumulated only to a concentration of 23 μM and 15 μM, respectively. Clearly, 3-3 CB was further transformed to other compounds. However, neither 3 CB nor biphenyl was observed in the sediment slurries. Therefore, at least 2 chlorines at the para position and one chlorine at the meta position were removed from 345-34 CB.

There were no observable changes of 345-34 CB in sterile control sediment slurries during the incubation period.

**Dechlorination of 345-345 CB in anoxic estuarine sediments**

345-345 CB was persistent for up to 2 years in the sediment slurries collected from both the Tansui River and the Erjen River.

**Dechlorination of 25-34 CB in anoxic estuarine sediments**

25-34 CB (40 μM) was removed after a lag period of 165 days in the sediment slurries collected from the Tansui River. However, removal stopped at a concentration of about 22 μM. During incubation, there was one intermediate product formed at the stoichiometric ratio of 1:1, and this product was identified as 25-3 CB.

In the sediment slurries collected from the Erjen River, 25-34 CB (38 μM) was removed within 431 days without a lag period. There were two intermediate products detected in succession during incubation, and these were identified as 25-3 and 2-3 CB. 25-3 CB was quickly transformed to 2-3 CB, and the resultant transformation of 25-34 CB to 2-3 CB (via 25-3 CB) was at the stoichiometric ratio of about 1:1.

There was no observable change of 25-34 CB in sterile controls during the incubation period.
Proposed dechlorination routes

The proposed dechlorination routes for the congeners are summarized in Fig. 2.

A. $34-34\text{CB} \rightarrow 3-3\text{CB}$

B. $345-4\text{CB} \rightarrow 35-4\text{CB} \rightarrow 3\text{CB}$

C. $345-34\text{CB} \rightarrow 35-3\text{CB} \rightarrow 3\text{CB}$

D. $25-34\text{CB} \rightarrow 25-3\text{CB} \rightarrow 2-3\text{CB}$

Fig. 2 Proposed route of dechlorination for $34-34\text{CB}$, $345-4\text{CB}$, $345-34\text{CB}$ and $25-34\text{CB}$ in sediment slurries from (a) the Tansui River and (b) the Erjen River.

Effect of addition of Tween 20 on the dechlorination of $34-34\text{CB}$, $345-4\text{CB}$, and $345-345\text{CB}$

The addition of the surfactant Tween 20 (0.05%) did not enhance the rates of dechlorination of $34-34\text{CB}$, $345-4\text{CB}$, or $345-345\text{CB}$ in the sediment slurries from the Tansui River.

Effect of addition of enrichment cultures on the dechlorination of $34-34\text{CB}$ and $345-345\text{CB}$

The addition of toluene-adapted and 3-chlorobenzoate-adapted sediment decreased the lag period of dechlorination of $34-34\text{CB}$ from more than 270 days to 190 days and 170 days, respectively, in sediment slurries from the Tansui River. However, once the concentration of $34-34\text{CB}$ had fallen to about 17 µM, dechlorination ceased for almost 200 days in the sediment slurries supplemented with 3-chlorobenzoate-adapted sediment. 3-3 CB was detected as the dechlorination product in sediment slurries amended with toluene-adapted sediments and in those amended with 3-chlorobenzoate-adapted sediments.

The addition of toluene-adapted and 3-chlorobenzoate-adapted sediments also decreased the lag period of dechlorination of $345-4\text{CB}$ from more than 250 days to 170 days and 218 days, respectively, in sediment slurries from the Tansui River. $35-4\text{CB}$ was detected as the dechlorination product in both cases.

The addition of toluene-adapted or 3-chlorobenzoate-adapted sediments did not enhance dechlorination of $345-345\text{CB}$.

Biodegradation of biphenyl

Biphenyl was persistent for up to one and half years in sediment slurries collected from both the Tansui River and the Erjen River.
DISCUSSION

Bedard and Quensen (1995) indicated that there are at least six different processes (C, H, M, N, P, and Q) by which Aroclors are dechlorinated in anoxic sediments. If our proposed pathways (Fig. 2) are correct, then dechlorination of the four coplanar congeners and one non-planar congener in sediments of the Tansui River may actually result from three independent activities, namely, para-dechlorination of 4-, 34- and 345-. Our data suggest that all para chlorines from the mono-, di-, and trichlorobiphenyl groups could be removed. Therefore, the microbial communities present in sediments of the Tansui River demonstrated a preference for regiospecific removal of chlorines in the para position. This type of para-dechlorination was designated as process Q by Bedard and Quensen (1995). Transformation of Aroclor mixture by process Q alone has also been observed by Abramowicz and Brennan (1991). The rates and extent of dechlorination of these congeners were higher in sediments from the Erjen River than in sediments from the Tansui River. Dechlorination in sediments from the Erjen River may have resulted from five independent activities, namely, para-dechlorination of 4-, 34- and 345-, and meta-dechlorination of 25- and 35-. Microbial communities in sediments from the Erjen River fostered meta-dechlorination activity only after removal of all the para-chlorines. Meta-chlorine removal from a 25- was designated as process M by Bedard and Quensen (1995). However, meta-chlorine removal from a 35- does not match any known pattern of dechlorination. It therefore seems that dechlorination of the PCB congeners in sediments from the Erjen River resulted from more than two separate dechlorination processes, i.e. process Q, process M, and possibly one more type of meta-chlorine removal process. Nonetheless, process Q was the predominant and primary process in sediments from the Erjen River. Aroclor 1242 transformation by process Q (primarily) in combination with process M has also been observed in Hudson River sediment (Bedard and Quensen 1995).

Although Rhee et al. (1993) have reported the dechlorination of 34-34 CB to biphenyl by microorganisms from Hudson River sediment, in the present study we found that 34-34 CB was not dechlorinated to biphenyl even after two years incubation and that the intermediate product with the least chlorination was 3-3 CB. We also found no evidence that biphenyl was produced by the dechlorination of any of the other coplanar congeners. Furthermore, we did not observe anaerobic biotransformation of biphenyl, even though Bedard et al. (1998) have postulated that the capacity for anaerobic biodegradation of biphenyl is widespread because biphenyl is structurally related to both benzene and naphthalene, both of which have been shown to be biodegradable in estuarine or marine sediments under anaerobic conditions (Kasumi et al. 1997, Zhang and Young 1997).

We selected 25-34 CB as a non-planar congener for comparison because it is a fairly prominent component of several Aroclors and because its dechlorination has been reported in many PCB-contaminated sediments (Bedard et al. 1996, Brown and Wagner 1990; Brown et al. 1987a, 1987b). Bedard et al. (1996) reported
that after only 13 weeks of incubation, about 90% of 25-34 CB was stoichiometrically transformed to 25-3 CB in Woods Pond sediment amended with 25-34 CB. No other dechlorination products of 25-34 CB were detected. In the present study, we found that 25-34 CB (38 µM) was dechlorinated at much lower rates in both the estuarine sediments. In sediment from the Tansui River, 25-3 CB was the least chlorinated product, but an intermediate product with lower chlorination (i.e. 2-3 CB) was found in sediment from the Erjen River. We also found that although most of the coplanar congeners are more toxic than the non-planar congeners, with the exception of 345-345 CB, the rates of dechlorination of the coplanar congeners were higher than that of the non-planar congener 25-34 CB in sediments from both rivers. Probably the adjacent meta and para chlorines present in all of these coplanar congeners make them susceptible to at least partial dechlorination as described by Bedard and Quensen (1995).

Dechlorination of PCB congeners and Aroclors is generally favored by methanogenic conditions. Thus for example, in a recent study, in which anaerobic slurries from Baltimore Harbor were prepared with estuarine medium without the addition of sulfate, Wu et al. (1998) found extensive meta dechlorination and moderate ortho dechlorination of Aroclor 1260. On the other hand, studies that have investigated the effects of sulfate (10-30 mM) have reported disparate results (Alder et al. 1993; May et al. 1992; Morris et al. 1992; Ofjord et al. 1994; Rhee et al. 1993). Alder et al. (1993) for instance, found that meta and para chlorines of Aroclor 1242 were removed in the sediment from New Bedford Harbor under methanogenic conditions but not under sulfidogenic conditions. By contrast, Ofjord et al. (1994) found that chlorines were removed from Aroclor 1254 at similar rates by both methanogenic and sulfate-amended laboratory cultures enriched from marine sediments (Puget Sound, WA) in sea-salts media over a period of one year. Part of the apparent inconsistency of these results might be explained by the fact that, as we have shown here, lag periods can easily extend beyond the 100 days of incubation used by Alder et al. (1993). In any case, data from the present study provide further evidence that dechlorination can and does occur under sulfidogenic conditions, although slowly and often with long lag periods. Sulfate concentration of the Tansui and Erjen sediment slurries fell from an initial value of about 20 mM to slightly over 3 mM by the end of the incubation period (data not shown), so that PCB dechlorination occurred entirely under sulfidogenic conditions. Although the PCB dechlorination rates were low, we therefore conclude that reductive dechlorination could play a role in the bioremediation of PCBs in sulfate-containing marine and estuarine sediments. The implications of this conclusion are particularly significant because these environments are the largest reservoirs for PCB (Hansen 1987).

In this study we found that compared to the other coplanar congeners, it was relatively difficult to dechlorinate 345-345 CB and 34-34 CB. Since 345-345 CB and 34-34 CB have a more balanced steric configuration, it seems likely that for chlorinated biphenyls, and in particular the congeners of coplanar PCBs, the steric configuration might significantly affect the potential for degradation. Liu et al. (1996) found that the addition of the non-ionic surfactant Tween 20, at a concentration (0.05%,
(v/v) below the critical micelle concentration that increased PCB desorption, failed to enhance dechlorination in sediments in which biodegradation had halted. This is in agreement with the results of the present study, in which we also found that the addition of Tween 20 at 0.05% (v/v) to the sediment slurries did not enhance dechlorination of 34-34 CB, 345-4 CB or 345-345 CB, and that the extent and final congener pattern of dechlorination of 34-34 CB also remained unchanged. We therefore conclude that in the present study, the bioavailability of 34-34CB and 345-345 CB was not a factor that limited the reductive dechlorination of these congeners.

The addition of toluene- or 3-chlorobenzoate-adapted sediment did not change the routes of dechlorination for 34-34 CB or 345-4 CB. Toluene- or 3-chlorobenzoate-adapted sediment stimulated the dechlorination of coplanar PCB congeners only in the sense that it decreased the lag period of dechlorination. It seems that the addition of 3-chlorobenzoate-adapted sediment did not stimulate a selective meta-dechlorination of coplanar PCB congeners. We speculate that 3-chlorobenzoate-adapted sediment selectively enriched a population of coplanar PCB congener-dechlorinating microorganisms that can use 3-chlorobenzoate as an electron acceptor and thus "prime" the PCB dechlorination. So far, however, the specific role of the toluene-adapted sediments in the dechlorination has not been elucidated. The extensive PCB congener specificities of these enrichments were not investigated in the present study, and further research will be necessary to gain a better understanding of the basic mechanisms underlying enhancement and inhibition of microbial degradation of high concentrations of toxic compounds.

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