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# ANAEROBIC DECHLORINATION OF AROCLOR 1242 AS AFFECTED BY SOME ENVIRONMENTAL CONDITIONS

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**Abstract**—The effect of electron acceptors and biphenyl on reductive dechlorination was investigated using the commercial polychlorinated biphenyl mixture Aroclor 1242 and sediment microorganisms from the Hudson River. Polychlorinated biphenyl- (PCB-) free sediments spiked with the Aroclor at 700 $\mu$g/g and anaerobically incubated for six months, with and without biphenyl enrichment, showed that dechlorination was most advanced with $CO_2$ without biphenyl enrichment. A small amount of methane was detected. However, methane production per se was not essential for dechlorination, because addition of the inhibitor of methane production, 2-bromoethane sulfonate, did not affect dechlorination. On the other hand, dechlorination was inhibited partly by sulfate and completely by nitrate. Biphenyl enrichment initially inhibited dechlorination under both methanogenic and sulfidogenic conditions.

**Keywords**—Polychlorinated biphenyls Reductive dechlorination Electron acceptors Biphenyl Anaerobic sediments

## INTRODUCTION

Biological reductive dechlorination has been reported for a wide variety of haloaromatic compounds [1]. In aquatic sediments, it is more important than aerobic degradation, because anoxic conditions prevail in most subsurface layers. Reductive dechlorination can also transform even the highly chlorinated congeners that aerobes are unable to attack. Although it has received wide attention in recent years, the environmental conditions necessary for it to occur are yet unclear. This information is essential to assess its potential importance in contaminated environments and utilize it for bioremediation in controlled environments. Reductive dehalogenation has been investigated, mostly with undefined mixed natural microbial populations from contaminated sediments, aquifers, or sewage, in undefined media or sediments. The responsible organism or consortium of microorganisms was characterized only in a limited number of cases with a few simple compounds [2–4].

In general, highly reducing methanogenic conditions seem to favor reductive dehalogenation [5,6]. Chlorobenzoates, chlorophenols, 2,4-dichlorophenoxyacetate, and 2,4,5-trichlorophenoxyacetate (2,4,5-T) were dechlorinated by microbiota from a methanogenic aquifer, but not by those from a sulfate-reducing site [7]. The heterocyclic herbicide bromacil (5-bromo-3-*sec*-butyl-6-methyluracil) was dehalogenated under methanogenic conditions but not under sulfate-reducing conditions [8]. Anaerobic degradation of chlorophenols and chlorobenzoates presumably involving dehalogenation also was observed most often with methanogenic enrichments compared with sulfate or nitrate enrichments in a variety of freshwater sediments [9]. The biodegradation of halobenzoates involving dechlorination was tightly coupled to methanogenesis [10].

On the other hand, 2,4-chlorophenol and 4-chlorophenol were dechlorinated stoichiometrically with the reduction of sulfate by organisms from anaerobic lake sediments [11]. 2,3,4,5-Tetrachloroaniline was dechlorinated in sulfate-enriched methanogenic aquifer slurries, although the rate was slower and its pathway was also different [12]. Similarly, the sulfate enrichment of methanogenic aquifer slurries exhibited slower dechlorination of 2,4,5-T, probably due to delays in acclimation [13]; at a high level, however, sulfate inhibited aryl halide removal [7]. The reductive dechlorination of aromatic compounds has not been reported to date under denitrifying conditions.

A comparison of our earlier incubation studies

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of Hudson River sediments in the laboratory [14] with that of other laboratories [15] suggested that polychlorinated biphenyl (PCB) biotransformation might be affected by experimental conditions. Our studies failed to show dechlorination of in-place PCBs [14], and further investigations with sediments from other sites in the river also failed to show the dechlorination of in-place PCBs for up to two years when the study was terminated (G.-Y. Rhee, unpublished data). On the other hand, the experimental details of these studies were different from those of the other study, in which the dechlorination was clearly demonstrated [15]. The difference in experimental details included the substrate for dechlorination (i.e., in-place PCBs vs. Aroclor 1242), the gas atmosphere of incubation, and the sediment composition. The present study investigated the effects of different electron acceptors, the nonchlorinated structural analog biphenyl, and the initial reducing potential, using Aroclor 1242 as the substrate for dechlorination.

## MATERIALS AND METHODS

Dechlorination of Aroclor 1242 was investigated with $CO_2$, $SO_4^{2-}$, and $NO_3^-$ as electron acceptors. The sediments amended with each acceptor were incubated with or without biphenyl amendment, and with or without chemical reduction of sediment slurries by cysteine sulfide following gaseous reduction in an anaerobic chamber. The individual congener 2,3,4-trichlorobiphenyl was used to investigate the effect 2-bromoethane sulfonate had on dechlorination.

Sediment slurries were prepared using PCB-free sediments from Owasco Lake, New York, as described elsewhere [1]. The sediment was spiked with Aroclor 1242 (AccuStandard, Inc., New Haven, CT) in hexane to yield a total PCB concentration of 700 $\mu$g/g on a sediment dry-weight basis. Biphenyl (AccuStandard, Inc., New Haven, CT) (1,000 $\mu$g/g) was added to those sediments requiring it at the time of PCB contamination. The hexane solvent was subsequently evaporated before a sediment slurry was made with biologically reduced synthetic medium [16]. The sediment slurry was stirred on a magnetic stirrer overnight in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI) to ensure the homogeneous distribution of the PCBs. When required, $KNO_3$ or $K_2SO_4$ was added to sediment slurries to yield a final concentration of 16 and 10 mM (pH approximately 6.8), respectively. A 20-ml portion of the slurries was transferred into a 50-ml serum vial (Wheaton Scientific, Millville, NJ) and sealed with a Teflon®-lined rubber cap and aluminum crimp. When needed, the slurry was further reduced by adding cysteine sulfide (0.025%). The redox indicator resazurin (Aldrich Chemical Co., Milwaukee, WI) (0.0001%) was also added. The initial reducing potential with and without the chemical reduction (indicated by a platinum electrode vs. a silver/silver chloride electrode) was approximately −300 and −150 mV, respectively. The gas atmosphere of the vials was replaced with $CO_2/N_2$ (80:20 v/v) or $N_2$, and the vials were autoclaved. All vials, except the controls, were inoculated with a 0.5-ml aliquot of the supernatant of a slurry prepared from Hudson River sediment collected near Fort Edward, New York. Control vials consisted of the sterilized sediments without the inoculum. Each treatment, including the controls, was set up in triplicate for each sampling period. The vials were shaken on rotary shaker at room temperature for six months. The incubation was terminated by placing vials in a cold room (4°C) until their extraction. The sediments were extracted and analyzed as described earlier [1].

## RESULTS AND DISCUSSION

Analysis of the PCB congener pattern after six months of incubation showed clear evidence of dechlorination in sediments under methanogenic conditions. There was a decrease in the highly chlorinated congeners and a corresponding increase in the less chlorinated congeners, when compared to the control sediments (Fig. 1). However, a comparison of the congener patterns under sulfidogenic and nitrate-reducing conditions showed that dechlorination was partially inhibited by sulfate and completely inhibited by nitrate enrichment (Fig. 1). An analysis of the gas phase showed that methane was produced in sediments with carbon dioxide but not in either sulfate- or nitrate-enriched sediments.

In the methanogenic sediments, the penta- and tetrachlorinated biphenyls decreased by 80 and 50%, respectively. There was a corresponding small (but not significant) increase in the trichlorinated biphenyls and a >200% increase in the dichlorinated biphenyls (Fig. 2).

The ratio of the gram-atoms (g-at) of Cl per mole of biphenyl in experimental sediments to that in the control (dechlorination index) showed that overall 12% of Cl was removed under methanogenic conditions (Table 1). The dechlorination index showed that the chemical reduction of sediments and initial reducing potential of sediments had little influence on the extent of dechlorination, be-

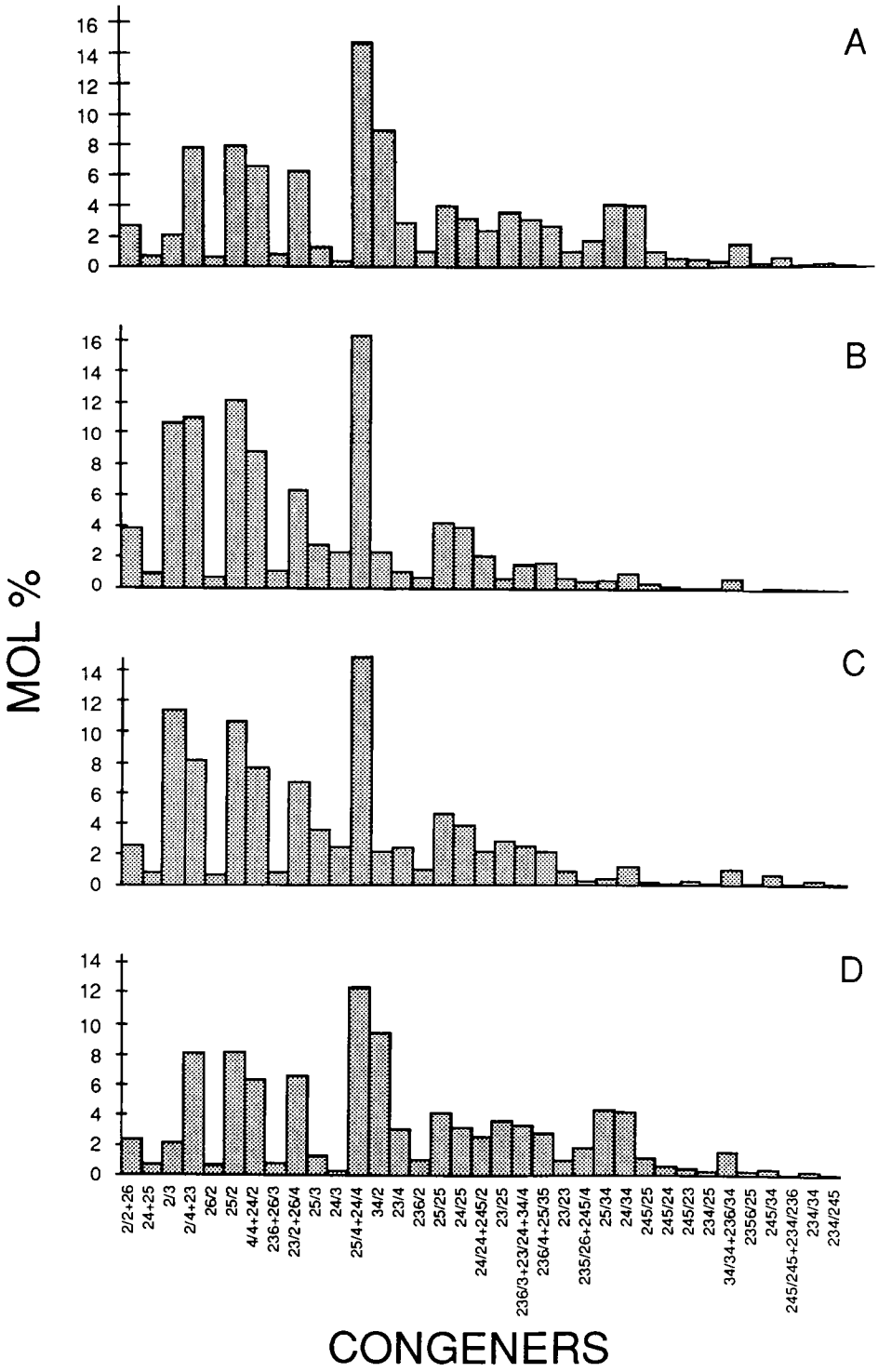


Fig. 1. Relative concentrations of Aroclor 1242 congeners after a six-month incubation under (**B**) methanogenic, (**C**) sulfidogenic, and (**D**) denitrifying conditions. The control (**A**) is autoclaved sediments.

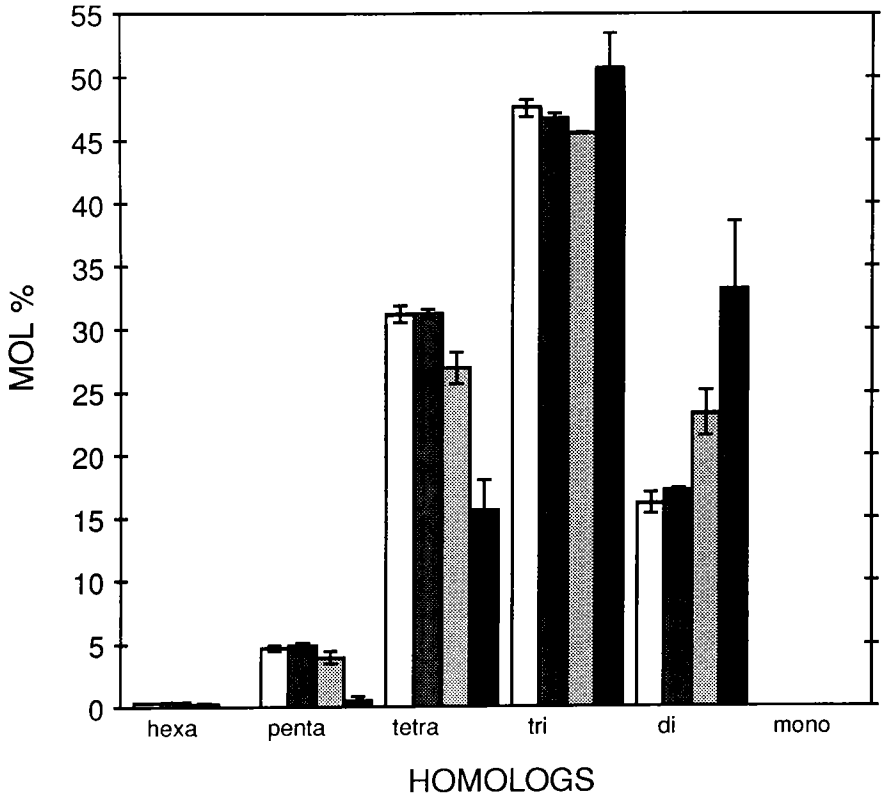


Fig. 2. Relative concentration of homologs under methanogenic (■), sulfidogenic (▨), and denitrifying (▩) conditions. The control (□) is autoclaved sediments.

cause the total amounts of Cl removed from sediments with and without cysteine sulfide amendment were very similar at 12 and 13%, respectively (Table 1). Biphenyl amendment appeared to inhibit dechlorination, although dechlorination was observed in one of the triplicate sediment vials (contributing to the large SD; Table 1). However, other experiments suggested that biphenyl enrichment may only delay the onset of dechlorination. In these experiments, which lasted 10 months, dechlorination in the biphenyl-amended sediments eventually started after a long lag phase (data not shown). In our earlier studies, a biphenyl enrichment of Hudson River sediments enhanced the decrease of ambient PCBs, but no evidence for dechlorination was detected [14].

Under sulfidogenic conditions, the penta- and tetrachlorinated biphenyls decreased by 20 and 16%, respectively, and the dichlorinated biphenyls increased by 40% following six months of incubation. The total extent of dechlorination was much smaller under sulfate-reducing conditions, where only 7% of the Cl was removed compared to the 12% removed under methanogenic conditions (Table 1). However, the congeners involved in dechlorination under sulfidogenic conditions were not qualitatively different from those under methanogenic conditions. Similar to the methanogenic conditions, there was no difference in dechlorination with or without cysteine sulfide amendment (Table 1). With biphenyl enrichment, no dechlorination was detected for at least six months, irrespective of the initial redox value (Table 1).

With other halogenated compounds, sulfate-reducing conditions were also inhibitory for dehalogenation. In 2,4,5-T and a number of haloaromatic compounds [7,13], high sulfate levels or sulfate enrichments suppressed dehalogenation or reduced its rate [13]. With 2,4,5-T, biological removal of sulfate relaxed the inhibitory effect of dehalogenation, but stimulation of methanogenesis without sulfate removal did not. In the present study, it was not possible to determine whether the smaller extent of dechlorination was an indication of steady sup-

Table 1. Gram-atoms chlorine per mole of biphenyl after a six-month incubation of Aroclor 1242 with Hudson River microorganisms and various electron acceptors and sediment amendments

| | Cl | Control | No amendment | Biphenyl amendment | CS reduction | Biphenyl + CS amendment |
|---|---|---|---|---|---|---|
| $CO_2$ | *o* | 1.46 (0.01) | 1.43 (0.03) | 1.45 (0.01)[a] | 1.41[b] | 1.41 (0.06)[a] |
| | *m* | 0.95 (0.02) | 0.72 (0.05) | 0.87 (0.13)[a] | 0.69[b] | 0.87 (0.13)[a] |
| | *p* | 0.83 (0.02) | 0.67 (0.07) | 0.76 (0.12)[a] | 0.70[b] | 0.80 (0.03)[a] |
| | Total | 3.23 (0.03) | 2.83 (0.08) | 3.07 (0.26)[a] | 2.81[b] | 3.03 (0.26)[a] |
| $SO_4^{2-}$ | *o* | 1.45 (0.01) | 1.43 (0.003) | 1.45 (0.03) | 1.43 (0.03) | 1.43 (0.02) |
| | *m* | 0.95 (0.02) | 0.89 (0.01) | 0.94 (0.03) | 0.86 (0.06) | 0.98 (0.02) |
| | *p* | 0.83 (0.02) | 0.68 (0.003) | 0.86 (0.01) | 0.75 (0.09) | 0.85 (0.01) |
| | Total | 3.23 (0.03) | 3.00 (0.01) | 3.24 (0.07) | 3.03 (0.15) | 3.26 (0.04) |
| $NO_3^-$ | *o* | 1.45 (0.01) | 1.45 (0.01) | 1.46[b] | 1.45 (0.01) | 1.45 (0.01) |
| | *m* | 0.95 (0.02) | 0.99 (0.04) | 1.00[b] | 0.94 (0.05) | 0.97 (0.01) |
| | *p* | 0.83 (0.02) | 0.84 (0.01) | 0.86[b] | 0.88 (0.06) | 0.87 (0.05) |
| | Total | 3.23 (0.03) | 3.28 (0.06) | 3.32[b] | 3.27 (0.003) | 3.29 (0.04) |

Values are the mean of three replicates with SD in parentheses.
CS = cysteine sulfide.
[a]One triplicate showed dechlorination.
[b]Single value.

pression or whether it merely represented a longer acclimation phase, with the dechlorination rate increasing with further incubation to eventually compensate for the initial lag.

Nitrate-reducing conditions were completely inhibitory to reductive dechlorination. The congener pattern (Fig. 1), homolog distribution (Fig. 2), and dechlorination index (Table 1) in the nitrate-enriched sediments were not different from the control values. In anaerobic environments, nitrate, sulfate, and $CO_2$ are used in this order of preference as alternate electron acceptors, probably because the free energy yield decreases in the same order. Because the reaction yielding most energy is favored in microbial succession and dissimilatory nitrate reduction can occur at higher Eh than sulfate reduction or methanogenesis, nitrate-reducing organisms are generally found nearer the sediment surface, followed by sulfate reducers and then methanogens [17]. Although there is still no clear evidence, PCBs may be used as the terminal electron acceptor in reductive dechlorination and therefore may compete with other potential acceptors for electrons.

Although dechlorination was greatest under methanogenic conditions, methane production per se was not required for PCB dechlorination. Inhibition of methanogens with 10 mM 2-bromoethane sulfonate (BES) had little effect on the dechlorination of sediments containing the single congener 2,3,4-trichlorobiphenyl (Table 2). The concentrations of the parent congener, 2,3,4-trichlorobiphenyl, and its dechlorination product, 2,4-dichlorobiphenyl, at each sampling interval were similar in sediments with and without BES. Thus, methane production was not necessary for PCB dechlorination. By contrast, the biotransformation of other haloaromatic

Table 2. Time course for dechlorination of 2,3,4-trichlorobiphenyl (2,3,4-CBP) to 2,4-dichlorobiphenyl (2,4-CBP) by Hudson River microorganisms with and without the addition of 10 mM 2-bromoethane sulfonate (BES)

| | Concn. (mol %) of congeners[a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Week 6 | | Week 8 | | Week 10 | | Week 12 | |
| Congeners | No BES | BES | No BES | BES | No BES | BES | No BES | BES |
| 2,3,4-CBP | 26 (17.0) | 17 (5.0) | 3 (0.1) | 3 (0.1) | 2 (0.3) | 4 (1.3) | 3 (0.6) | 3 (0.2) |
| 2,4-CBP | 74 (17.0) | 84 (5.0) | 97 (0.1) | 97 (0.1) | 98 (0.3) | 96 (1.3) | 97 (0.6) | 97 (0.2) |

[a]Values are the mean from three replicates with SD in parentheses; average total molar concentration was 627 ± 86.

compounds was adversely affected by BES. For example, the anaerobic biodegradation of 2,4,5-trichlorophenoxyacetic acid, which involved dechlorination steps, was inhibited by BES in a methanogenic aquifer [13]; so was the anaerobic degradation of chloroaromatic compounds in diverse aquatic sediments under various conditions that probably involved dechlorination [9].

The effect of electron acceptors on dechlorination may be highly significant in estuarine and marine environments, because high sulfate concentrations there would inhibit or slow dechlorination. This effect may in part explain the congener pattern of PCBs showing less dechlorination in the downstream area of the Hudson River than the upper stretch of the river (R. Bopp, personal communication). It also appears that dechlorination is less likely to occur in anaerobic layers near the sediment surface and nitrate-rich areas such as sewage discharge points. The extent of denitrification in these sediments is not well known due to technical difficulties of in situ measurements [18].

Although reductive dechlorination has been demonstrated in the laboratory [1,15] and in the natural environment [19], it is possible that many sites may not undergo biotransformation, as the immense environmental heterogeneity of sediment microsites, with their widely varying compositions, may not render every site conducive to dechlorination. Results from the present study suggest that any PCB bioremediation strategy involving reductive dehalogenation should avoid sulfidogenic or nitrate-reducing conditions. In this connection, it is interesting to note that the inhibitory effect of sulfate on dehalogenation was mitigated by the suppression of sulfate reduction by molybdate or biological removal of sulfate [13].

Little is known about nutritional requirements for the dechlorination of chlorobiphenyls or other halogenated aromatic pollutants. However, the addition of reduced organic carbons appeared to stimulate dechlorination. With the biodegradation of 2,4,5-trichlorophenoxyacetic acid, short-chain organic acids or alcohols stimulated the onset and rate of its dehalogenation and decreased the amount of the parent substrate still detectable as halogenated intermediates at the end of the experiments [13]. For the dechlorination of PCBs, acetone, acetate, methanol, or glucose enhanced the rate [20]. Supplements of yeast extract and peptone were essential for the dechlorination of 2-chlorophenol [21], and casamino acids were supplemented in a basal medium used for the dechlorination of halogenated aromatic aldehydes and chlorocatechols by a consortium of marine sediment bacteria [22,23].

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# A LONG-TERM STUDY OF ANAEROBIC DECHLORINATION OF PCB CONGENERS BY SEDIMENT MICROORGANISMS: PATHWAYS AND MASS BALANCE

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**Abstract**—Reductive dechlorination of PCBs by Hudson River sediment microorganisms was investigated using individual congeners, 2,3,4,2′,4′,5′-, 2,4,5,2′,4′,5′-, 2,3,4,5,6-, 2,4,2′,4′-, and 3,4,3′,4′-chlorobiphenyls (CBPs) in long-term studies lasting 15 to 20 months. The dechlorination of 2,3,4,2′,4′,5′-CBP yielded 2,4,5,2′,4′-, 2,4,2′,4′-, 2,4,2′,5′-, and 2,4,2′-CBPs; notably absent was 2,2′-CBP. Yet, the total molar concentration of all congeners decreased with time and at 15 months accounted for only 25% of the initial concentration of the parent compound. 2,3,4,5,6-CBP produced 2,3,5,6-, 2,4,6-, and 2,6-CBPs. At 15 months the sum of all congeners accounted for only about 50% of the initial amount of the parent congener. On the other hand, 2,4,5,2′,4′,5′-CBP yielded six daughter products, including 2,2′-CBP, and did not show any decrease in total molar concentration even at 20 months. 2,4,2′,4′-CBP did not show any change at 15 months. These results indicate that anaerobic PCB biotransformation may include mechanisms other than dechlorination and that the mechanisms are congener dependent. Biphenyl was detected with 3,4,3′,4′-CBP, indicating complete dechlorination; however, it accounted for <10% of the total molar loss. $^{14}$C-labeled tracer of this congener showed that all radioactivity was in the hexane fraction, suggesting that transformation products were hydrophobic.

**Keywords**—PCB congeners Reductive dechlorination Mass balance Biodegradation

## INTRODUCTION

In anaerobic sediments, microbial removal of aryl halides modifies the congener composition of polychlorinated biphenyls (PCBs). In Hudson River sediments, the removal occurs at the *m*- and *p*- but not at the *o*-position. This was experimentally demonstrated with Aroclor 1242 in the laboratory [1–5]. There is also indirect evidence that the same process has occurred in natural sediments [6,7]. It was thus predicted that the final products of dechlorination would be congeners with only *o*-substitutions.

Recent findings showed that Cl removal was determined by the pattern of Cl substitution on the biphenyl ring rather than the substitution position itself [4]. When Hudson River sediment organisms were incubated in sediments spiked with 2,3,4-chlorobiphenyl (CBP), they quantitatively converted the congener into 2,4-CBP and were unable to remove the *p*-Cl during one-year experiments. When the enrichment culture in 2,3,4-CBP was transferred into sediments spiked with 2,4,5- or 2,3,4,5,6-CBP, however, they were able to dechlorinate *p*-Cl. Furthermore, when these *p*-dechlorinating organisms were transferred back into 2,3,4-sediments following several transfers in the tri- or penta-CBP, again only 2,4-CBP was produced [8]. These results suggested that terminal products of dechlorination in the Hudson River might not necessarily be only *o*-substituted congeners and might include those with Cl substitution at the *m*- and/or *p*-positions as well.

The mass balance of anaerobic transformation has so far received little attention, probably in part because reductive dechlorination has been considered as the only mechanism. This assumption, however, is not supported by any rigorous mass-balance studies. The paucity of such studies may stem from the fact that an accurate quantitation is difficult for Aroclor biotransformations because, among other problems, many congeners with varying response

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factors coelute in the routine GC analysis of PCBs with a capillary column. This coelution problem can be overcome by multidimensional GC [9], but it is not suited for a routine application.

To predict the fate of PCBs in anaerobic sediments, it is necessary to understand the pathway and products of dechlorination. It is also essential to determine whether there may be biotransformation mechanisms other than dehalogenation in long-term studies. Therefore, we investigated the pathway and mass balance of the anaerobic transformation of PCBs using selected single congeners.

## MATERIALS AND METHODS

Dechlorination was investigated with the individual congeners 2,3,4,2′,4′,5′-, 2,3,4,5,6-, 2,4,2′,4′-, 3,4,3′,4′-, and 2,4,5,2′,4′,5′-CBPs, obtained from AccuStandard, Inc. (New Haven, CT). $^{14}$C-labeled congeners were obtained from Sigma Chemical Co. (St. Louis, MO). PCB-free sediments from Owasco Lake, New York, were spiked with each congener without carrier solvents, as described previously [4]. The sediments were inoculated with the supernatant of a slurry prepared from Hudson River sediments collected near Fort Miller, New York. They were then incubated at room temperature in 50-ml serum vials, which were sealed with Teflon®-lined rubber caps and aluminum crimps [4]. Sterilized sediments served as the control. Vials were not shaken during incubation, because no difference had been noted between shaken and unshaken sediments (G.-Y. Rhee et al.; unpublished results).

The experiments were set up in triplicate. Each sampling consisted of extracting the sediments of each triplicate vial, including a set of controls, as described earlier [4]. The extracts were then analyzed on a Hewlett Packard (HP; Avondale, PA) Ultra II® fused-silica capillary column (0.33-$\mu$m film thickness, 0.25-mm i.d.) and also on a C-87 (Apolane; Restek Corp., Bellefonte, PA) column by a GC (HP 5890A and HP 5890II) with a $^{63}$Ni electron-capture detector (ECD), as described earlier [4]. Dechlorination products were identified by matching GC retention times with the pure chemical standards (AccuStandard, Inc.) for all possible congeners that could be produced. When necessary, a mass selective detector (MSD; Hewlett Packard 5970) interfaced with an HP Ultra II column was used for further confirmation of products. All PCB concentrations were determined in the linear range of the ECD response. Biphenyl was determined by a GC with capillary column and flame ionization detector (FID). Qualitative confirmation was carried out using a GC-MSD. $^{14}$C radioactivity was counted in a liquid scintillation counter (LKB Rackbeta, Finland).

## RESULTS AND DISCUSSION

### *2,3,4,2′,4′,5′-hexaCBP*

The biotransformation of this congener was initially followed every three months in a 15-month incubation study. However, the analysis of the first sample showed three different congener products, suggesting that the initial sequence of dechlorination was missed. To investigate this initial stage, short-term experiments were carried out concurrently in which samples were analyzed every three weeks for 18 weeks. Although the two experiments were inoculated with the supernatant of slurries made of the same sediment cores, the inocula might not have necessarily been identical, as they were made at different times.

In the short-term study, 2,4,2′,4′,5′- and 2,4,2′,4′-CBP were detected simultaneously at 18 weeks. These congeners were the product of *m*-dechlorination, and it appeared that two *m*-dechlorinations occurred simultaneously or in a rapid succession. On the other hand, the first analysis of the long-term experiments at three months showed 2,4,2′,5′-CBP in addition to these two congeners (Fig. 1; the temporal difference in dechlorination between the two experiments probably stemmed from differences in the inoculum). The concentration of 2,4,2′,5′- was almost twice that of 2,4,2′,4′-CBP. Although the concentration of 2,4,2′,4′- appeared to decline over time (Fig. 1), the decrease was not statistically

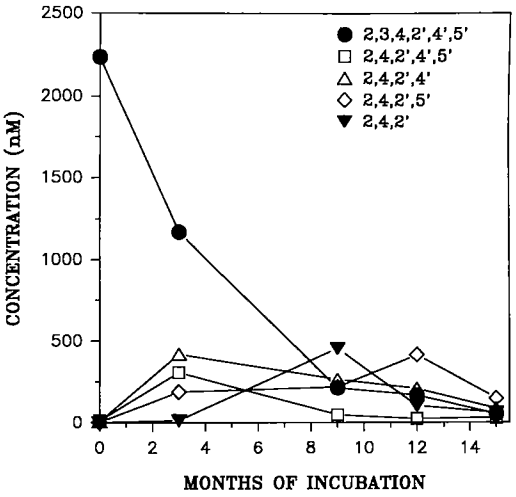


Fig. 1. The time course of 2,3,4,2′,4′,5′-hexachlorobiphenyl (●) dechlorination and the accumulation of products.

significant. Further incubation yielded only one additional product, 2,4,2′-CBP, which was first detected at nine months. No 2,2′-CBP was detected, even after 15 months of incubation (Fig. 1). The absence of 2,2′-CBP was not due to the efficiency of its recovery, because this congener was quantitatively recovered from 2,2′-CBP-spiked sediments incubated for a comparable period in another experiment.

The molar stoichiometry of dechlorination revealed a significant decrease in the total concentration of congeners after six months of incubation (Fig. 2). The total concentration in the figure is the mean of six replicate sediment values. The sum of congeners at six months was about 78% of the parent control; at 12 months, 41%; and at 15 months, only 27%. To confirm a full recovery of dechlorination products, sediments were spiked with the commercial preparations of the dechlorination products as well as 2,2- and 2-CBPs. All of the congeners were quantitatively recovered following the same extraction and analytical procedures. Furthermore, when one of the products, 2,4,2′,4′-CBP was incubated separately for a similar length of time as 2,3,4,2′,4′,5′-CBP, the original concentration of 2,4,2′,4′-CBP was consistently recovered in the control and experimental sediments, demonstrating no biodegradation and, at the same time, the time independence of its recovery (see below). Similarly, in a separate 20-month incubation study of 2- and 2,2′-CBP, these two congeners were quantitatively recovered from both the control and the experimental sediments, regardless of the length of incubation (G.Y. Rhee et al., unpublished results).

The significant decrease in the total molar concentration suggested that there might be another pathway leading possibly to non-PCB products. The nature of the products has not been identified, but they seemed to be hydrophobic (see discussion of 3,4,3′,4′-CBP below). Recently, similar decreases in total PCB concentration have been observed in other laboratories (L. Nies and D. Abramowicz, personal communications).

### *2,3,4,5,6-pentaCBP*

The biotransformation of this congener was also carried out in the same manner as 2,3,4,2′,4′,5′-CBP, using both short- and long-term experiments. In these experiments, the first dechlorination products, 2,3,5,6- and 2,4,6-CBPs, were detected simultaneously at three months. The molar concentration of the former was nearly twice that of the latter (Fig. 3). It appeared, therefore, that *m*- and *p*-dechlorination occurred concurrently, but the removal of *p*-Cl was the dominant pathway. Interestingly, a study of 3,4,5-triCBP using the same inoculum showed that the first step of its transformation also involved the *p*-Cl, yielding 3,5-diCBP. These results suggested that when *p*-Cl was surrounded by two adjacent *m*-Cls, the *p*-Cl was removed first.

The production of 2,4,6-CBP, although very low, demonstrated a pathway that involved *m*-dechlorination. However, its precursor, 2,3,4,6-CBP, was

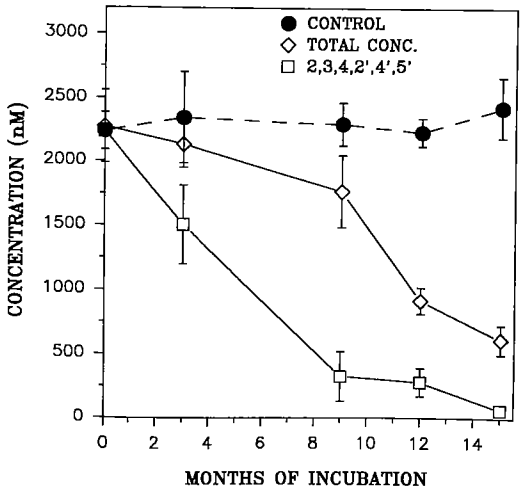


Fig. 2. Concentration changes in the parent congener 2,3,4,2′,4′,5′-hexachlorobiphenyl (□), the sum of all congeners (◇), and the control (●) over time. Each point represents the mean (±SD) of six replicate vials.

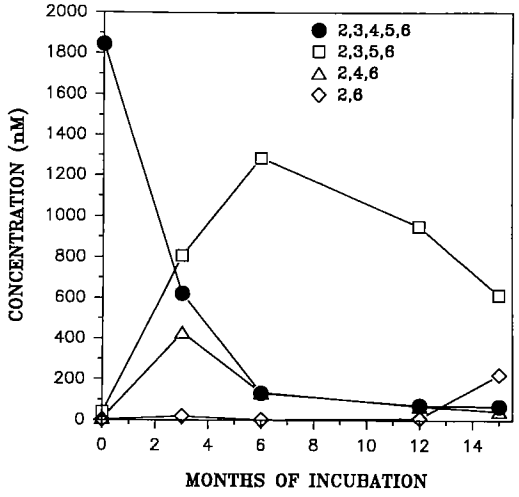


Fig. 3. The time course of 2,3,4,5,6-pentachlorobiphenyl (●) dechlorination and the accumulation of products.

not detected (its absence was determined with an Apolane column, which, unlike the Ultra II column, separates it from 2,3,5,6-CBP). These results suggested that both *m*-Cls were removed simultaneously or in rapid succession.

The long-term experiment yielded one additional product, 2,6-CBP, which was detected in a small quantity at 12 months (approximately 10% of the initial parent congener concentration). Its concentration increased a little further with time, but no biphenyl was detected.

The lag period for the dechlorination of 2,3,4,5,6-CBP was much shorter than that for 2,3,4,2',4',5'-CBP, even though the same inoculum was used (12 vs. 16 weeks). Thus, it appeared that the lag time was congener specific.

A molar stoichiometry of dechlorination in the long-term experiments showed that there was a steady decline in the total PCB concentration with time after the first three months of incubation (Fig. 4). The decline was highly significant, especially when total PCB concentrations were compared between the controls and 12- or 15-month experimental sediments. At 12 months the total was approximately 60% of the initial concentration of the parent compound, and at 15 months it was only about 50%.

2,3,5,6-CBP increased in mirror image to the decline of the parent compound until six months, after which it also started to decrease (Fig. 3). Despite the decline, its concentration still remained highest among the dechlorination products. 2,4,6-CBP produced at three months also began to decrease to a low level with further incubation. Therefore, the observed decrease of total PCB concentration appeared to involve all congeners except the di-*o*-CBP (2,6-), which was detected only at a very low level after 12 to 15 months of incubation.

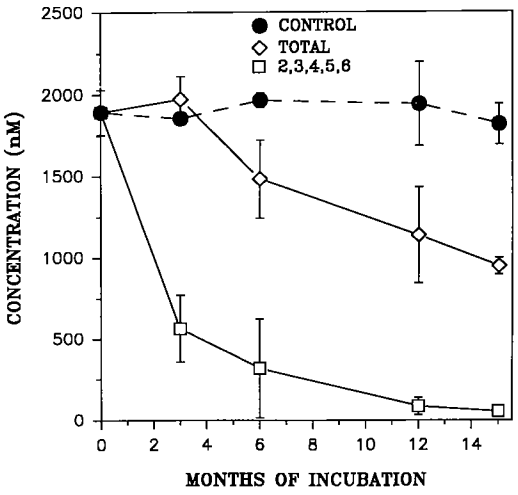


Fig. 4. Concentration changes in the parent congener 2,3,4,5,6-pentachlorobiphenyl (□), the sum of all congeners (◇), and the control (●) over time. Each point represents the mean (±SD) of five replicate vials.

As in the hexaCBP study, spiking and extraction experiments with commercial preparations of the dechlorination products showed quantitative recovery. Therefore, the decrease in the total PCB concentration suggested that 2,3,4,5,6-CBP might be anaerobically degraded into non-PCB compounds. The nature of the product(s) is currently under investigation.

### *2,4,2',4'-, 3,4,3',4'-, and 2,4,5,2',4',5'-CBPs*

In an effort to determine degradation products, biodegradation was investigated using three commercially available $^{14}$C-labeled congeners, 2,4,2',4'-, 3,4,3',4'-, and 2,4,5,2',4',5'-CBPs. 2,4,2',4'-CBP did not show any dechlorination, even after 15 months of incubation. This congener was also found to be among the most recalcitrant congeners in Aroclor 1242 [4] and Aroclor 1254 [10]. Such a pattern of change also suggested the importance of the overall Cl substitution pattern for dechlorination, rather than substitution position itself.

In 3,4,3',4'-CBP dechlorination, only one intermediate product, 3,3'-CBP, was detected. Obviously, its production involved *p*-dechlorination. Interestingly, this pattern of dechlorination was consistent with that observed with Aroclor 1242, in which the first dechlorination was also the removal of *p*-Cl on a ring that had three and four substitutions with no *o*-Cl [4]. These results confirmed that dehalogenation was determined by the chlorination pattern of congeners rather than the substitution position per se.

Biphenyl was also detected as a product in the 3,4,3',4'-CBP experiment, suggesting a complete dechlorination. Biphenyl was confirmed and quantified by a GC with a FID and a GC-MSD. Its concentration, however, accounted for <10% of the parent congener loss. It is not clear whether the disappearance of 3,4,3',4'-CBP was through a complete dechlorination to biphenyl or through an as yet unknown degradation pathway of CBPs. A transformation study using $^{14}$C-labeled 3,4,3',4'-CBP showed that even after most of the parent compound and 3,3'-CBP had disappeared at 15 months, all the radioactivity was in the hexane fraction of the extract, suggesting that transformation

products were hydrophobic. These results may suggest that the unidentified non-PCB transformation products of 2,3,4,5,6- and 2,3,4,2′,4′,5′-CBPs could be hydrophobic and not completely mineralized.

Dechlorination of 2,4,5,2′,4′,5′-CBP yielded six products, including 2,2′-CBP, which was not among the products of 2,3,4,2′,4′,5′-CBP dechlorination (Fig. 5). In contrast to the 2,3,4,2′,4′,5′- and 2,3,4,5,6-CBP experiments, the 2,4,5,2′,4′,5′-CBP experiment showed biotransformation without the loss of total PCB, even after 20 months of incubation (Fig. 6). These results suggest that the loss of total PCB, through an as yet unidentified biotransformation pathway, may be congener specific. Such biotransformation characteristics might explain the present congener profile in the Hudson River, which includes congeners containing *m*- and/or *p*-Cl at significant concentrations along with 2,2′- or 2,6-CBP, although it could be argued alternatively as an incomplete stage of dechlorination. In addition, these results also support the conclusion that the loss of total PCBs observed in the 2,3,4,2′,4′,5′- and 2,3,4,5,6-CBP was not due to recovery efficiency.

Our recent long-term study of 2,3,4- and 2,4,5-CBPs has demonstrated decreasing PCB concentration, along with the production of new congeners by dechlorination [8]. It is possible that any decrease in sediment PCB concentrations does not necessarily mean the disappearance of pollutants; they may simply be transformed into other recalcitrant products, just as DDT is converted to DDE. Therefore, it is urgent and crucial to characterize the nature of non-PCB biotransformation products so that their potential impact on the environment and further biotransformation potential can be evaluated.

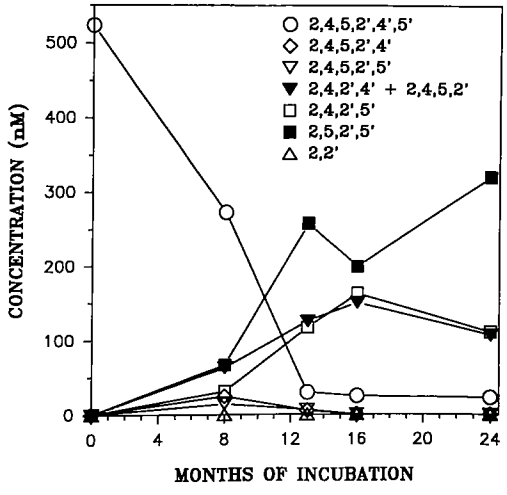


Fig. 5. The time course of 2,4,5,2′,4′,5′-hexachlorobiphenyl (○) dechlorination and the accumulation of products.

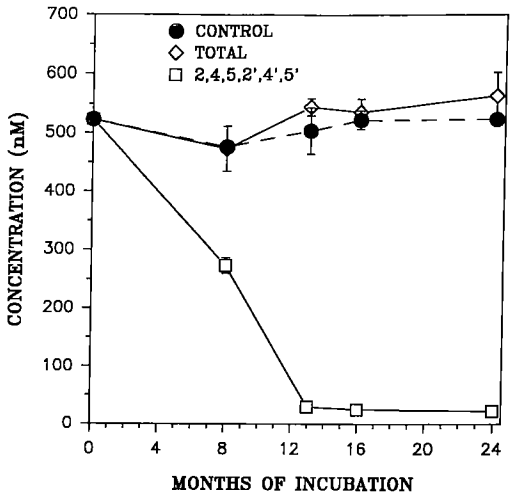


Fig. 6. Concentration changes in the parent congener 2,4,5,2′,4′,5′-hexachlorobiphenyl (□), the sum of all congeners (◇), and the control (●) over time. Each point represents the mean (±SD) of three replicate vials.

*Acknowledgement*—This study was supported by the Hudson River Foundation (00988A024 and 02186B015), the New York Center for Hazardous Waste Management (150W009), and the U.S. Environmental Protection Agency (R816903010). We thank Dr. R. Bopp for his helpful discussion during the course of this study. Excellent technical support by Alan DeNucci, Rebecca Slezak, and Bryan Michon is also acknowledged.

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# REDUCTIVE DECHLORINATION OF AROCLOR 1242 IN ANAEROBIC SEDIMENTS: PATTERN, RATE AND CONCENTRATION DEPENDENCE

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**Abstract**—Anaerobic biotransformation of polychlorinated biphenyls of Hudson River sediment microorganisms was investigated using the commercial mixture Aroclor 1242 in the laboratory at six different concentrations: 120, 300, 500, 800, 1,000, and 1,500 μg/g (on a sediment dry-weight basis). Dechlorination was concentration dependent. No change in congener composition was found at 1,000 and 1,500 μg/g during seven months of incubation, but significant shifts were observed in sediments with concentrations below 800 μg/g. A mass balance of the transformation indicated that, despite the shifts, the total molar concentration remained the same. An optimum concentration, based on the decrease of Cl per biphenyl, was 500 μg/g, but based on Cl removed per gram sediment it had a range from 500 to 800 μg/g. Dechlorination (total Cl removed per biphenyl) at 300 and 500 μg/g appeared to be first order, with rate constants of −0.039 and −0.059 per month, respectively. The rate also varied with the substitution position; it was faster for *m*-Cl, followed by *p*-Cl, but no *o*-Cl was removed. However, the faster rate of *m*-dechlorination in Aroclor 1242 was probably due to a high concentration of congeners in the Aroclor with Cl substitution patterns favoring its removal, rather than the meta-position itself.

**Keywords**—Polychlorinated biphenyls Biotransformation Reductive dechlorination Hudson River sediment microorganisms

## INTRODUCTION

The aquatic dynamics of polychlorinated biphenyls (PCBs) in general are similar to those of particulates, owing to the hydrophobicity of these xenobiotics. Therefore, their accumulation in sediments follows, by and large, the chronology of sedimentation. In the Hudson River, PCBs are found as deep as 1 m from the sediment surface [1]. In productive or polluted aquatic environments, highly reducing conditions prevail in subsurface layers due to the depletion of $O_2$ by aerobic microorganisms on the surface. Therefore most sediment PCBs are found in anaerobic layers.

In recent years, a new type of anaerobic respiration involving dehalogenation by microbial consortia has been observed in a variety of structurally dissimilar compounds; they include halobenzoates [2-8], halobenzenes [9,10], chloroanilines [11,12], chlorophenols [13-16], chlorocatechols [17-19], and haloaromatic aldehydes [20]. In the absence of molecular oxygen, the aryl halogens of homocyclic aromatic compounds are biologically removed by reduction [12,18].

Laboratory investigations [21-24] showed that reductive dechlorination also occurred in PCBs. A comparative study of the congener patterns between contemporary sediments in the Hudson River and the Aroclors presumed to have been initially discharged [25-27] strongly suggested that PCBs were also anaerobically dechlorinated in nature. However, when ambient PCBs from Hudson River sediments and from dredged sediments under clay encapsulation were incubated in the laboratory, no sign of dechlorination was observed; rather, the total concentration decreased significantly [28].

The present study was carried out using Aroclor 1242, a mixture composed mainly of tri- and tetrachlorobiphenyls, to investigate dechlorination and its rate, and the effects of PCB concentration on the biotransformation.

## MATERIALS AND METHODS

PCB-free sediments were collected from Owasco Lake, New York. They were air dried and then

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sifted through a sieve with a pore size of approximately 600 μm (organic content was 90 mg $g^{-1}$ or 9% by dry weight). The dry sediments were spiked with various amounts of Aroclor 1242 in hexane to give desired concentrations of 120, 300, 500, 800, 1,000, and 1,500 μg/g on a sediment dry-weight basis. [$^{14}$C]Aroclor 1242 (Amersham Corp., Arlington Heights, IL) (0.005 μCi) was added as a tracer. After the hexane was evaporated, the spiked sediments were made into slurries by adding biologically reduced synthetic minimal medium [29] in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI). To ensure the homogeneous distribution of PCBs, the sediment slurry was stirred overnight with a magnetic stirrer. The slurry was further reduced by adding cysteine sulfide (Sigma Chemical Co., St. Louis, MO) (0.025%), and the redox indicator resazurin (Aldrich Chemical Co., Milwaukee, WI) (0.0001%) was also added.

Batch incubations were prepared by dispensing 20 ml of the sediment slurry to 50-ml serum vials (Wheaton Scientific, Millville, NJ) and crimp sealing the vials with a Teflon®-lined rubber septum under $N_2$ atmosphere in the anaerobic chamber. The vials were then autoclaved and, except for the controls, inoculated with the supernatant of Hudson River sediment slurries (0.5 ml). The slurry was made from core samples of sediment collected from the upper Hudson River near Fort Edward, New York. Each PCB concentration, including controls, was set up in triplicate. These sediments were incubated at room temperature. To minimize any possible temporal variations in PCB extraction efficiency and analytical conditions, a set of control vials were analyzed at each sampling interval.

*Sample extraction*

To analyze for PCBs, an entire vial was extracted in the following manner. First, the aqueous layer was pipetted off the sediment into a beaker and set aside. Sediments were extracted twice for 3 min with acetone (10 ml) under ultrasonication (Sonics & Materials, Inc., Danbury, CT) in a water-jacketed cooling system. The sediments were then further extracted twice with hexane with the same procedure. The solvent extracts and aqueous phase were placed together in a 250-ml separatory funnel and shaken vigorously for 1 min, then allowed to separate. The aqueous layer was decanted. To remove acetone and any residual sediments from the solvent layer, distilled water (50 ml) was added to the funnel. The funnel was shaken and the aqueous layer was aspirated. The solvent layer was decanted into a flask containing 5 to 10 g of sodium sulfate (Sigma Chemical Co., St. Louis, MO) to remove any residual water. The aqueous layer was then placed back into the separatory funnel and extracted twice with hexane; the solvent was placed with the rest of the previously extracted solvent now in the sodium sulfate solution.

Sulfur, a major interfering substance in sediment PCB analysis, was removed by treating the extracts with a tetrabutylammonium hydrogen sulfate (Sigma Chemical Co., St. Louis, MO) (TBAHS) solution. The TBAHS solution was prepared by dissolving 3.39 g of TBAHS in 100 ml distilled water. The solution was washed three times with 20 ml of hexane. Twenty-five grams of anhydrous sodium sulfite (Sigma Chemical Co., St. Louis, MO) was added, and the solution was shaken until clear. To remove sulfur, a 2-ml volume of sediment extract was mixed with 2 ml TBAHS solution and 4 ml 2-propanol, then shaken for 1 min. Then 10 ml of distilled water was added and shaken for 1 min. After separating, a 1-ml sample was taken from the hexane layer and placed into a chromatographic column containing 10 g 4% deactivated Florisil (Sigma Chemical Co., St. Louis, MO; 60–100 mesh) topped with 1 g of anhydrous sodium sulfate. The column was eluted with 65 ml of hexane, the first 50 ml of which was collected. When necessary, the eluent was concentrated in a Kuderna–Danish condensing apparatus. The radioactivity counts in the solvent and extracted sediments showed an extraction efficiency of >96%. The extraction efficiency did not change with the length of sediment incubation.

*PCB analysis*

Samples were analyzed on a Hewlett Packard (HP; Avondale, PA) Ultra II® fused-silica capillary column (0.33-μm film thickness, 0.25-mm i.d.) and, when necessary, also on a C-87 (Apolane) column (Restek Corp., Bellefonte, PA), by GC (HP 5890A and HP 5890II) with a $^{63}$Ni electron-capture detector. If needed, an HP 5970 mass selective detector (MSD), interfaced with an HP Ultra II column, was used for further confirmation of peaks. GC quality control was maintained according to Bush et al. [30]. When individual congener analyses varied by more than ±10%, recalibration was carried out, but, as a matter of routine, recalibration was done every sixth sample with a QC/QA sample. The QC/QA solution contained a mixture of Aroclors 1221, 1016, 1254, and 1260 at 0.2 μg/mL of each in hexane. The Aroclors were supplied by the U.S. Environmental Protection Agency (EPA) pesticide repository.

## RESULTS

The first analysis after three months of incubation demonstrated unequivocal evidence for dechlorination. It also clearly showed its dependence on initial Aroclor 1242 concentrations. The molar concentration of 36 major peaks showed a dramatic change in the profile involving a shift from high- to low-molecular-weight congeners, with the change most pronounced at 500 μg/g, followed by 300, then 120 and 800 μg/g (Fig. 1; the values are an average of triplicate sediments, and the standard error was <10% of the mean). No change was observed at the two highest concentrations, 1,000 and 1,500 μg/g, up to the end of the experiment at seven months.

A mass balance of the transformation showed that the total molar concentration of PCBs remained unchanged ($P > 0.05$) during the seven months. Therefore, all transformation during this period involved dechlorination without any loss of PCB molecules. A time-course plot of homologs in mole percentages showed increases in mono- and dichlorinated biphenyls (CBPs) with concomitant decreases in congeners with three or more chlorines (Fig. 2).

The time course of dechlorination expressed in terms of milligram-atoms (mg-at) of Cl per mole of biphenyl was fastest at 500 μg/g, followed by 300 μg/g, then 120 and 800 μg/g (Fig. 3). In the first three months, 25% of the total Cl was dechlorinated in the 500-μg/g sediments and 21% in 300-μg/g sediments, but only 8 and 3% were removed in 120- and 800-μg/g sediments, respectively. The change in the 300- and 500-μg/g sediments could be described by first-order kinetics, with rate constants of −0.039 and −0.059 per month ($P < 0.05$), respectively.

Dechlorination rate also varied widely with the substitution position; *m*-Cl was removed the fastest, followed by *p*-Cl, with no *o*-Cl removal. Dechlorination at each position was also concentration dependent, similar to total dechlorination. At the optimal concentration of 500 μg/g, the time course of *m*- and *p*-dechlorination appeared to be first order, with rate constants of −0.150 and −0.098 per month ($P < 0.05$), respectively (Fig. 4). Approximately 54% of the total *m*-Cl was dechlorinated during the first three months; in the ensuing 1.5 months, the dechlorination progressed to 59, then to 65% after seven months. A similar time course of *m*- and *p*-Cl dechlorination was observed in the 300-μg/g sediments. By contrast, in the suboptimal concentrations of 120 and 800 μg/g, dechlorination began only after a long lag. In the 800-μg/g sediments, for example, only 4% of the *m*-Cl was removed after three months. This then advanced to 26 and 46% after 4.5 and seven months, respectively. An initial lag period in the 300- and 500-μg/g sediments was also possible, but it was not detected because the sampling schedule was spaced at an interval longer than the lag.

When dechlorination in the seven-month period was expressed on a sediment dry-weight basis, there was little difference between the 500- and 800-μg/g sediments, although the latter exhibited a long initial lag (Fig. 5). At seven months, the total amount of Cl removed per gram of sediments was 1.8 mol in both sediments. Therefore, in terms of the concentration of Cl removed from PCBs, the optimum concentration ranged from at least 500 to 800 μg/g, with the upper threshold concentration for the inhibition of dechlorination between 800 and 1,000 μg/g.

The sequence of dechlorination could be discerned when a composite of chromatograms was made, disregarding sediment PCB concentrations and incubation time, to show advancing stages of dechlorination. The composite revealed that the peaks for congeners with 3′,4′-substitution, 2,3′,4′-, 2,5,3′,4′-, and 2,4,3′,4′-CBP, were first to decrease and this decrease was accompanied by the concomitant increase of the peaks for 2,3-,2,5,3′-, and 2,4,3′-CBPs. These results indicated *p*-dechlorination. It is most interesting to note that the first dechlorination involved *p*-Cl, despite the fact that the rate was fastest for *m*-Cl. These results implied that it was not the *p*-position per se that was responsible for the slower dechlorination rate at that position.

The next major decreases occurred in 2,3,2′,5′, 2,3,4′, (2,3,6,3′ + 2,5,3′,5′), and (2,3,6,3′ + 2,3,2′,4′ + 3,4,4′) peaks, followed by 2,4,5,4′, then 2,5,2′,5′ with 2,5,2′ peaks. At the same time, major increases were found in (2,5,4′ + 2,4,4′), (2,4′ + 2,3), (4,4′ + 2,4,2′) peaks, and, finally, in 2,6,2′ and 2. The further dechlorination of earlier dechlorination products then ensued. It was interesting that (2,3,2′ + 2,6,4′) and (2,4,2′,4′ + 2,4,5,2′) peaks were most resistant to dechlorination, because they seemed to decrease only when dechlorination of the products was taking place near the end of the experiments. In Aroclor 1242, about two-thirds of the former peak comprised 2,3,2′, and the latter peak contained almost equal proportions of the two coeluters [31]. However, the coeluters were not separately determined in experimental samples.

In sediments with the most advanced dechlorination (the 500-μg/g sediments at seven months), mono- or di-ortho-congeners made up approximately