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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-μm 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

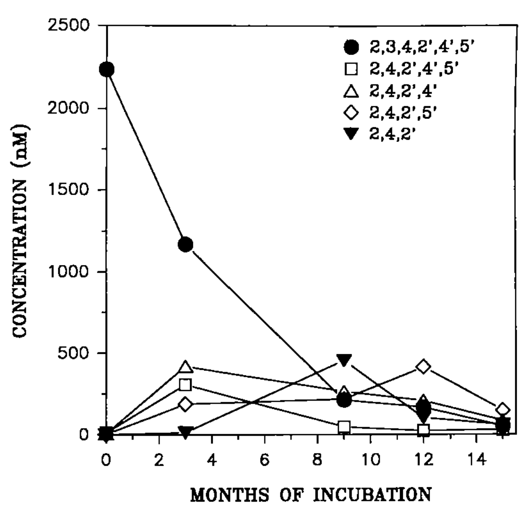

Fig. 1. The time course of 2,3,4,2′,4′,5′-hexachlorobiphenyl (●) dechlorination and the accumulation of products.

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

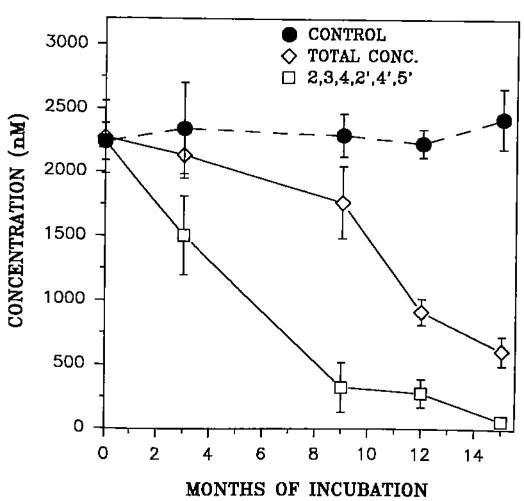

Fig. 2. Concentration changes in the parent congener 2,3,4,2′,4′,5′-hexachlorobiphenyl (□), the sum of all congeners (◇), and the control (●) over time. Each point represents the mean (±SD) of six replicate vials.

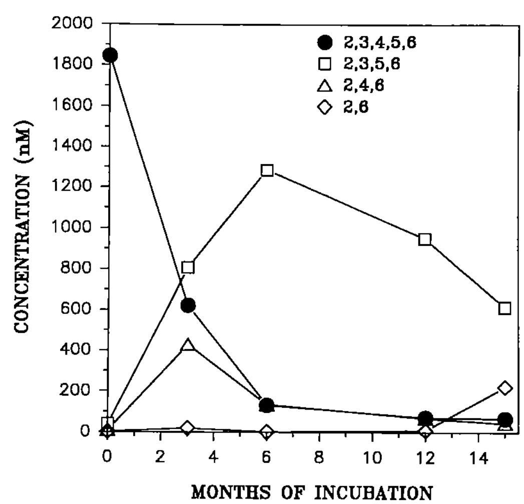

Fig. 3. The time course of 2,3,4,5,6-pentachlorobiphenyl (●) dechlorination and the accumulation of products.

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.

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.

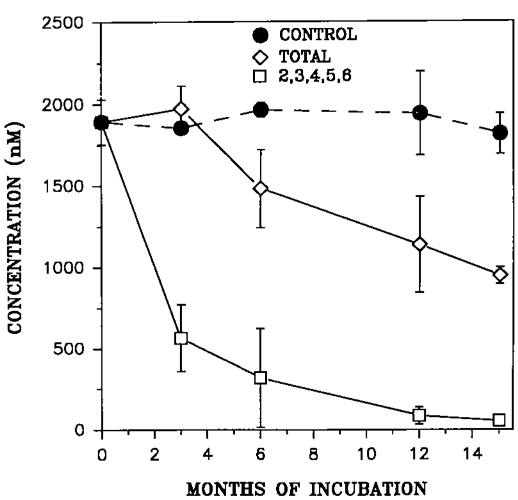

Fig. 4. Concentration changes in the parent congener 2,3,4,5,6-pentachlorobiphenyl (□), the sum of all congeners (◇), and the control (●) over time. Each point represents the mean (±SD) of five replicate vials.

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.

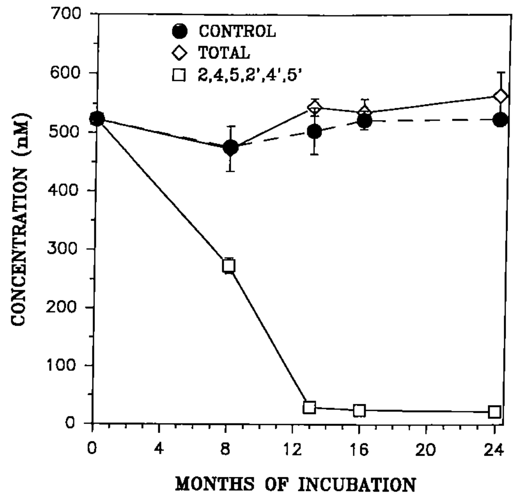

Fig. 6. Concentration changes in the parent congener 2,4,5,2′,4′,5′-hexachlorobiphenyl (□), the sum of all congeners (◇), and the control (●) over time. Each point represents the mean (±SD) of three replicate vials.

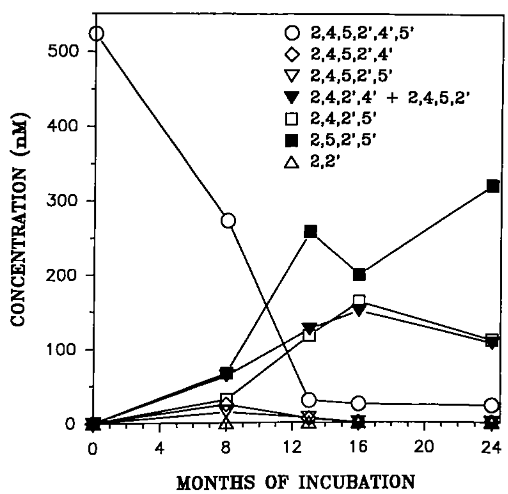

Fig. 5. The time course of 2,4,5,2′,4′,5′-hexachlorobiphenyl (○) dechlorination and the accumulation of products.

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