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Dechlorination of Polychlorinated Biphenyls by Hudson River Sediment Organisms: Specificity to the Chlorination Pattern of Congeners

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Reductive dechlorination of polychlorinated biphenyls (PCBs) by Hudson River sediment organisms was investigated using the single congeners 2,3,4-, 2,4,5-, and 2,3,4,5,6-chlorobiphenyls (CBPs). In repeated enrichments, the sediment culture produced only 2,4-CBP from 2,3,4-CBP and was unable to remove para-Cl. However, the same organisms yielded 2,3,5,6-CBP from 2,3,4,5,6-CBP by para dechlorination as one of the products. In 2,4,5-CBP they produced primarily 2,4-CBP and a small amount of 2,5-CBP. However, with repeated transfers, the proportion of 2,5-CBP increased, becoming the only product in the fifth transfer. When this culture was transferred back to 2,3,4-CBP, it was only capable of meta-dechlorination, yielding 2,4-CBP. Therefore, dechlorination was not specific to the chlorine substitution position *per se* but was determined by the chlorination pattern on congeners. These enrichment experiments also suggested the existence of a threshold concentration for dechlorination. With 2,4,5-CBP, more than 35 mol % of the initial parent congener concentration was not accounted for, indicating that there might be anaerobic biotransformations other than dechlorination.

Introduction

Polychlorinated biphenyls (PCBs) are reductively dechlorinated by sediment microorganisms in the anaerobic environment (*1–7*). Reductive dechlorination of PCBs by microorganisms from the Hudson River occurs through the removal of meta- and para-chlorines (Cls) with no apparent loss of ortho-chlorines (*4–7*). In the dechlorination of Aroclor 1242, meta-Cls were found to be removed at a much faster rate than chlorines from the para position (*6*). However, a closer examination of the dechlorination sequence of Aroclor 1242 indicated that the first chlorines removed were from para not meta positions in 2,3,3′,4′-, 2,4,3′,4′-, and 2,3′,4′-chlorobiphenyls (CBPs). An attempt to enrich dechlorinating organisms using 2,3,4-CBP also showed that its dechlorination produced only 2,4-CBP. These results suggested that dechlorination might not be specific to the substitution position *per se* (*6*). Therefore, the dechlorination specificity of Hudson River sediment cultures was investigated using various single congeners. This paper reports that dechlorination is not specific to the substitution position of the chlorine relative to the biphenyl bridge, but is determined by the pattern of chlorine substitution on the phenyl ring.

Materials and Methods

Congener-spiked sediment slurries were prepared as follows without introducing carrier solvents. PCB-free sediments were collected from Owasco Lake, NY. They were air-dried and then sifted through a sieve (pore size 600 μm). The organic content was 90 mg·g^{-1} or 9% by dry weight. The dry sediments were spiked with 2,3,4-CBP or other congeners (AccuStandard, New Haven, CT) in hexane to yield a concentration of 300 μg/g on a sediment dry weight basis. After the hexane was evaporated, the spiked sediments were made into slurries by adding biologically reduced synthetic minimal medium (*8*) in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, MI). To ensure the homogeneous distribution of PCBs, the sediments slurry was stirred overnight with a magnetic stirrer. The slurry was further reduced by adding cysteine sulfide (0.025%), and the redox indicator resazurin (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 CO_2/N_2 (80:20, vol/vol) atmosphere. Each vial contained 0.5 g of sediment on a dry weight basis. The vials were then autoclaved and, except for the controls, inoculated with organisms eluted from Hudson River sediments. These sediments were collected from the upper Hudson River near Fort Edward, NY. All treatments, including controls, were set up in either duplicate or triplicate. Vials were incubated at room temperature.

Transfers. The initial enrichment culture was inoculated into sediments containing 2,3,4-CBP. Every 2 months, one of the duplicate vials were used to transfer organisms into fresh sediments (see Figure 1). A 0.5-mL aliquot of the supernatant of this vial was used as the inoculum into fresh 2,3,4-CBP. The remainder of the slurry was analyzed to determine dechlorination. The remaining duplicate vial was further incubated for a period ranging from 4 to 13 months.

At each sampling, the entire contents of each vial, including a control, was extracted, as described previously (*6*). Extracts were analyzed on Apiezon L column (30 m, Restek, Bellefonte, PA) by GC with an EC detector (Hewlett-Packard 5890 Series II). Helium was used as the carrier gas and argon–methane (95:5%) as the makeup gas. The injector and detector temperatures were 250 and 300 °C, respectively. The oven program consisted of an initial temperature of 90 °C increased at a rate of 10 °C/min to 150 °C and then increased at a rate of 3 °C/min up to 220 °C, which was maintained for 25 min. Data were collected and analyzed by Chrom Perfect chromatography data system (Justice Innovations, Palo Alto, CA). GC was calibrated using individual congeners corresponding to all potential dechlorination products (AccuStandard, New Haven, CT). Dechlorination products were identified by GC and confirmed by GC/MSD (Hewlett-Packard 5970 series).

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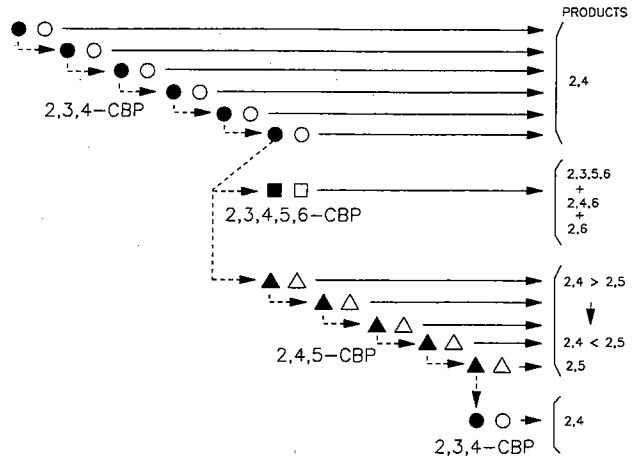

Figure 1. Schematic diagram for the transfer of enrichment cultures to sediments containing various congeners. Transfers were made in duplicate into sediments containing congeners listed on the left. Symbols represent different congeners. Dark symbols indicate vials used for transfers and congener analysis to confirm dechlorination at the time of transfer. Empty symbols indicate vials remaining in incubation of periods ranging from 4 to 13 months (length of solid line is not an indication of the length of the incubation period). Dechlorination products found under the extended incubation are listed on the right.

Table I. Dechlorination of 2,4,5-Trichlorobiphenyl by Enrichment Culture in 2,3,4-Trichlorobiphenyl at Various Transfer Steps with Different Incubation Periods

transfer no.	incubation period (months)	congeners (nmol) 2,4,5	2,4	2,5	2	total
1[a]	9–10	48 (7)[b]	531 (59)	16 (3)		595 (53)
2	8	31	446	86		563
3	7	31	238	279		548
4	6	40	9	342		391
5	5	42	0	342		384

[a] Culture from the fifth transfer in 2,3,4-trichlorobiphenyl. [b] Mean of three values with standard deviation in parentheses.

Results

The 2,3,4-CBP enrichment culture was transferred every 2 months for a total of six transfers (Figure 1). The only dechlorination product found at the time of each transfer was 2,4-CBP. When the duplicate vials were incubated for periods ranging from 4 to 13 months, 2,4-CBP was still the only product, accounting for an average of 94 mol % of the initial parent congener. No 2,3-CBP, 2-CBP or biphenyl were detected. Therefore, the sediment culture appeared to be capable of only removing the meta-chlorine from 2,3,4-CBP.

The dechlorination specificity of the organisms in 2,3,4-CBP sediments was examined further using other congeners. A subset of the organisms from the sixth 2,3,4-CBP transfer was inoculated into sediments containing 2,3,4,5,6- or 2,4,5-CBP (Figure 1). After 4 months of incubation with 2,3,4,5,6-CBP, the organisms produced 2,4,6- and 2,3,5,6-CBP via meta- and para-dechlorination, respectively. Further incubation yielded 2,6-CBP.

In 2,4,5-CBP sediments, the initial dechlorination product was predominantly 2,4-CBP (89 mol %), but a small amount of 2,5-CBP (3 mol %) was also detected (Table I). Thus, the organisms that were unable to remove the para-Cl from 2,3,4-CBP were now able to remove it from 2,4,5-CBP, similar to what was found with 2,3,4,5,6-CBP. With successive enrichment in 2,4,5-CBP sediments, the proportion of the two products gradually reversed so that by the fifth transfer 2,5-CBP was only dechlorination product; 2,4-CBP was no longer detected (Table I). This para-dechlorinating culture was subsequently inoculated back into 2,3,4-CBP, from which it was initially unable to remove para-Cl (Figure 1). The culture produced only 2,4-CBP from 2,3,4-CBP sediments by meta-dechlorination, as was found previously. After 5 months of incubation, more than 96 mol % of the parent congener was transformed into 2,4-CBP, with no 2-CBP or biphenyl detected. Therefore, dechlorination does not appear to be specific to chlorine substitution position but is determined by the pattern of chlorine substitution on the phenyl ring.

As the transfer was repeated in 2,4,5-CBP, the total molar concentration recovered steadily decreased from 595 to 384 nmol (Table I). However, neither 2-CBP nor biphenyl was detected. Their absence was further confirmed by sediment spiking–recovery experiments in which known amounts of all potential dechlorination products, including biphenyl, were quantitatively recovered by the same analytical procedure after similar time periods (data not shown). Therefore, the decrease in total molar concentration did not appear to be due to dechlorination.

It was also interesting to note that the residual concentration of the parent congener, 2,4,5-CBP, remained constant regardless of the number of transfers or the length of incubation (Table I). The concentration did not vary even when the total congener concentration decreased and the dechlorination products shifted from 2,4- to 2,5-CBP.

Discussion

Dechlorination of individual PCB congeners 2,3,4-, 2,4,5-, and 2,3,4,5,6-CBP was not specific to the chlorine substitution position *per se* but was determined by the chlorine substitution pattern. This was clear from the different dechlorination characteristics of para-chlorine. The removal of meta-chlorine also showed pattern specificity; the fifth transfer in 2,4,5-CBP was unable to remove the meta-chlorine, thus yielding 2,5-CBP as a the final product. However, when the same culture was inoculated into 2,3,4-CBP, they could remove only meta-chlorine, producing 2,4-CBP. Our 2-year incubation study of Aroclor 1254 (*9*) also showed the pattern dependence of dechlorination; specific congeners such as 2,4,2′,4′-, 2,4,2′,5′-, 2,4,4′-, and 2,4′-CBPs accumulated despite the evidence that the organisms were capable of removing both meta- and para-Cls from other congeners.

If dechlorination is specific to ring substitution position only, the terminal products of PCB dechlorination by Hudson River organisms would be congeners with only ortho substitutions, since meta- and para-chlorines are readily removed (*4–6*). Consequently, the presence of congeners containing meta- or para-chlorines would suggest the potential for further dechlorination. However, if dechlorination is not determined by substitution position, as suggested by the present study, the terminal products may include meta- and para-substituted congeners. Therefore, their presence would not necessarily indicate the potential for further dechlorination.

Pattern-specific dechlorination may explain in part why some Hudson River sediments, which show signs of advanced dechlorination, contain concentrations of non-ortho-chlorines that are much higher than the concentrations of purely ortho-substituted congeners (2; 2,2′ +

2,6; 2,6,2′). For example, the combined concentration of 2,3′-CBP, 2,4′ + 2,3-CBP, and 2,4,4′ + 2,5,4′-CBPs in sediments from the Thompson Island pool was about 40 mol % whereas that of ortho congeners was approximately 25 mol %. Interestingly, these same congeners are among the most resistant congeners in the dechlorination of Aroclors 1242 and 1254 (*6*, *9*). However, the accumulating products may vary from site to site. There is evidence that the dechlorination competence varies with contamination sites or the composition of sediment organisms. For example, a transfer study of contaminated sediments from the St. Lawrence River near Massena, NY, showed that sediment organisms yielded only 2,5-CBP from 2,4,5-CBP throughout all transfers unlike the organisms in Hudson River sediments. Van Dort and Bedard (*10*) reported that sediment organisms in the Housatonic River could dechlorinate even the ortho-chlorine from 2,3,5,6-CBP. Such different microbial characteristics suggest that it may be possible to anaerobically bioremediate PCBs using either a mixture of or sequential treatment with organisms having complementary characteristics.

The results of the present single-congener study may not be directly extrapolated to the natural environment, because it is possible that the dechlorination products may vary in the presence of other congeners. Different contamination areas may also harbor microbial populations with different dechlorinating characteristics. Even with the same sediment organisms, it is also possible that dechlorination products could vary with different sediment environments. However, single-congener studies are indispensable for understanding the mechanism of reductive dechlorination.

The product shift of 2,4,5-CBP dechlorination from mostly 2,4- and 2,5-CBP with successive transfers suggests that the repeated transfers may have enriched populations that could dechlorinate the para-Cl of 2,4,5-CBP. However, this culture was specific in removing the para-Cl only in this substitution configuration but not in the 2,3,4-CBP configuration, since the microorganisms were unable to yield 2,3-CBP. Ye et al. (*11*) reported two dechlorinating populations characterized by their dechlorinating activity following heat and/or ethanol treatments. Treated cultures lost some of the para-dechlorination activity present in the fresh untreated inoculum. Recent experiments have also shown that the dechlorination products of 2,3,4-CBP were different under H_2/CO_2 (80/20 vol/vol) atmosphere; the inoculum from the same sediments produced both 2,3- and 2,4-CBPs, eventually yielding 2-CBP in 7 months (Rhee, unpublished results). This change in products may also involve different microbial populations, although it is possible that the change was produced by the same organisms using H_2 as an electron donor.

The residual concentration of 2,4,5-CBP did not vary with either the length of incubation or the number of transfers. It also remained the same even when the total congener concentration decreased and the dechlorination product shifted from 2,4- to 2,5-CBP. It appears, therefore, that the residual concentration may represent a threshold level below which no dechlorination could take place. It is possible that the residual concentration might also be due to certain factors that become limiting. However, similar constant residual concentrations have been observed in the dechlorination of Aroclor 1254 for certain congeners such as 2,4,3′,4′-, 2,4,5,2′,5′-, and 2,3,4,2′,4′,5′ + 2,3,4,6,3′,4′-CBPs whereas many other parent congeners became undetectable (*9*). If the residual level represents a threshold, it is important to find whether this concentration is subject to modification by sediment characteristics and other environmental variables, since this may set the limit to which natural microbial populations can bioremediate in-place PCBs.

The mass balance of 2,4,5-CBP dechlorination showed a significant deficit in the total molar concentration. Similar reduction was also found with 2,3,4,2′,4′,5′- and 2,3,4,5,6-CBPs (*12*). Such a loss appears to be congener specific, since it was not found with 2,4,2′,4′- and 2,4,5,2′,4′,5′-CBP in the same study. To confirm full recovery of dechlorination products in these studies, sediments were spiked with the commercial preparations of all potential products. Some of the congeners were incubated as long as 2 years to determine if the length of incubation affected their recovery. All of the congeners were quantitatively recovered, and the incubation period had no effect (*12*). Taken together, these results indicate that the loss may be due to an as yet unknown type of biodegradation.

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Literature Cited

(1) Brown, J. F., Jr.; Bedard, D. L.; Bernnan, M. J.; Carnahan, J. C.; Feng, H.; Wagner, R. E. *Science* **1987**, *236*, 709–712.
(2) Brown, J. F., Jr.; Wagner, R. E.; Feng, H.; Bedard, D. L.; Brennan, M. J.; Carnahan, J. C.; May, R. J. *Environ. Toxicol. Chem.* **1987**, *6*, 579–593.
(3) Nies, L.; Vogel, T. M. *Appl. Environ. Microbiol.* **1990**, *56*, 2612–2617.
(4) Quensen, J. F., III; Boyd, S. A.; Tiedje, J. M. *Appl. Environ. Microbiol.* **1990**, *56*, 2360–2369.
(5) Quensen, J. F., III.; Tiedje, J. M.; Boyd, S. A. *Science* **1988**, *242*, 752–754.
(6) Rhee, G.-Y.; Bush, B.; Bethoney, C. M.; DeNucci, A.; Oh, H.-M.; Sokol, R. C. *Environ. Toxicol. Chem.*, in press.
(7) Rhee, G.-Y.; Bush, B.; Bethoney, C. M.; DeNucci, A.; Oh, H.-M.; Sokol, R. C. *Environ. Toxicol. Chem.*, in press.
(8) Balch, W. E.; Fox, G. E.; Magrum, L. J.; Woese, C. R.; Wolfe, R. S. *Microbial. Rev.* **1979**, *43*, 260–296.
(9) Rhee, G.-Y.; Sokol, R. C.; Bush, B.; Bethoney, C. M. *Environ. Sci. Technol.* **1993**, *27*, 714–719.
(10) Van Dort, H.; Bedard, D. A. *Appl. Environ. Microbiol.* **1991**, *57*, 1576–1578.
(11) Ye, D.; Quensen, J. F., III; Tiedje, J. M.; Boyd, S. A. *Appl. Environ. Microbiol.* **1992**, *58*, 1110–1114.
(12) Rhee, G.-Y.; Sokol, R. C.; Bethoney, C. M.; Bush, B. *Environ. Toxicol. Chem.*, in press.

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