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# Reductive Dechlorination of Nonachlorobiphenyls and Selected Octachlorobiphenyls by Microbial Enrichment Cultures

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The anaerobic dechlorination of four octachlorobiphenyls [2,3,4,5,6,2′,3′,4′-octachlorobiphenyl (23456−234-octaCB), 2345−2346-octaCB, 2345−2356-octaCB, and 23456−245-octaCB] and three nonachlorobiphenyls [23456−2345-nonaCB, 23456−2346-nonaCB, and 23456-2356-nonaCB] was investigated. The single congeners were added to two different previously studied microbial enrichment cultures, and the cultures were monitored over a period of 16 weeks. All seven congeners were reductively dechlorinated. The predominant dechlorination patterns for all congeners, in both cultures, showed meta-dechlorination of doubly flanked *m*-chlorines followed by *meta*-dechlorination of singly flanked *m*-chlorines. Some ortho- and para-dechlorination was also observed. All congeners had an initial concentration of 50 ppm in soil, and the extent of dechlorination over the 16-week period varied widely, from 38% dechlorination of congener 23456−245-octaCB to only 1.4% removal of congener 23456−2345-nonaCB.

## Introduction

Polychlorinated biphenyls (PCBs) are widespread environmental contaminants. One potential way of remediating contaminated areas is by using microbial degradation. Reductive dechlorination is the only biodegradation mechanism known for highly chlorinated PCB congeners, such as the majority of PCB congeners found in Aroclor 1254 and 1260 (*1*). It has been well established that microbial dechlorination of Aroclor 1260 can take place both in the environment and under laboratory conditions (*1*−*6*). Some studies have also quantified the extent of dechlorination of octa- and nonachlorobiphenyls in Aroclor 1260 mixtures (*7*−*9*). However, because of the complexity of both the starting Aroclor 1260 mixture and the product mixture formed, it is impossible to identify specific products of and degradation pathways for specific congeners in the general mixture.

Several studies have investigated the dechlorination of single PCB congeners under anaerobic conditions (*10*−*14*), but these have all focused on PCBs with six or fewer chlorine substituents. The dehalogenation of decachlorobiphenyl over time has also been reported (*15*); however, the products were only tentatively identified and not quantified.

There are no published reports on the microbial degradation of single PCB congeners with 8 or 9 chlorines showing the determination of dechlorination products. In this work, the anaerobic dechlorination of all possible nonachlorobiphenyls [23456−2345-nonaCB (IUPAC 206), 23456−2346-nonaCB (IUPAC 207), and 23456−2356-nonaCB (IUPAC 208)] and four of the most common octachlorobiphenyls found in Aroclor 1260 [(23456−234-octaCB (IUPAC 195), 2345−2346-octaCB (IUPAC 196), 2345−2346-octaCB (IUPAC 199), and 23456−245-octaCB (IUPAC 203)] was investigated.

## Experimental Section

**Enrichment Culture Preparation.** Two different enrichment cultures (BK24 and BK72) were used, both of which had previously been enriched from marine sediments with a history of PCB contamination collected from Esquimalt Harbour, BC, Canada. Enrichment cultures were developed by adding 10 mL of anaerobically eluted microorganisms from sediment to serum bottles containing 20 g of soil, 50 mg/L Aroclor 1260, and 40 mL of reduced anaerobic mineral medium medium (RAMM) prepared as described by Shelton and Tiedje (*16*) and modified according to Morris et al. (*17*). Instead of cysteine, 1 mM $Na_2S$ was used. After autoclaving, 4.8 mM sodium pyruvate was added. BK72 was enriched with 100 mg/L 4-bromobenzoate in addition to Aroclor 1260; BK24 had only Aroclor 1260 as a haloaromatic substrate. Serum bottles were crimp-sealed with butyl rubber stoppers. After an initial 11-month period, enrichment cultures were transferred at 2−3-month intervals. The enrichment cultures used in these experiments were 1 month old and had been transferred as described above four times.

**Sediment Preparation and Inoculation.** Sediment slurries were prepared in 25-mL Balch tubes by mixing 1.25 g of aerobic sand and 0.75 g of anaerobic pond sediment with 4 mL of RAMM. Tubes were crimp-sealed inside an anaerobic chamber and were autoclaved after incubation for 14 days, during which time methanogenesis was detected. Methane was identified by using a gas chromatograph equipped with a Haysep DB, 100−120 mesh, 10 in. × 1/8 in. packed column and a thermal conductivity detector. Single PCB congeners (100 $\mu$g) were added to the tubes from concentrated stock solutions in acetone, and the tubes were inoculated (10%) with an enrichment culture. Control cultures that contained extra medium instead of inoculum were also prepared, and all incubations were carried out stationary at 21 °C in the dark.

**Sampling and Analysis.** Entire cultures (one control culture and one inoculated culture) were frozen at 0, 4, 8, 12, and 16 weeks. After thawing, the cultures were vortexed for 30 s, uncapped, and amended with a known amount of decachlorobiphenyl as an internal standard. The sediment was extracted twice with 2 mL of acetone and then twice with 2 mL of hexane. Extracts were pooled and evaporated down to approximately 0.5 mL, eluted through a Florisil column, and made up to 1 mL with hexane. Samples were analyzed by using a gas chromatograph fitted with a DB5-ms column (30 m × 0.25 mm × 0.25 $\mu$m) and coupled to a mass spectrometer (Varian Saturn model 4D ion trap). The sample volume injected was 2.5 $\mu$L, the temperature of the injector was held at 260 °C, and the temperature of the transfer line was 280 °C. The column temperature program was as follows: 104 °C for 3 min, increased at 20 °C/min to 180 °C, increased at 2.5 °C/min to 278 °C, held for 3 min, increased at 22 °C/min to 300 °C, and held at 300 °C for 1 min. The

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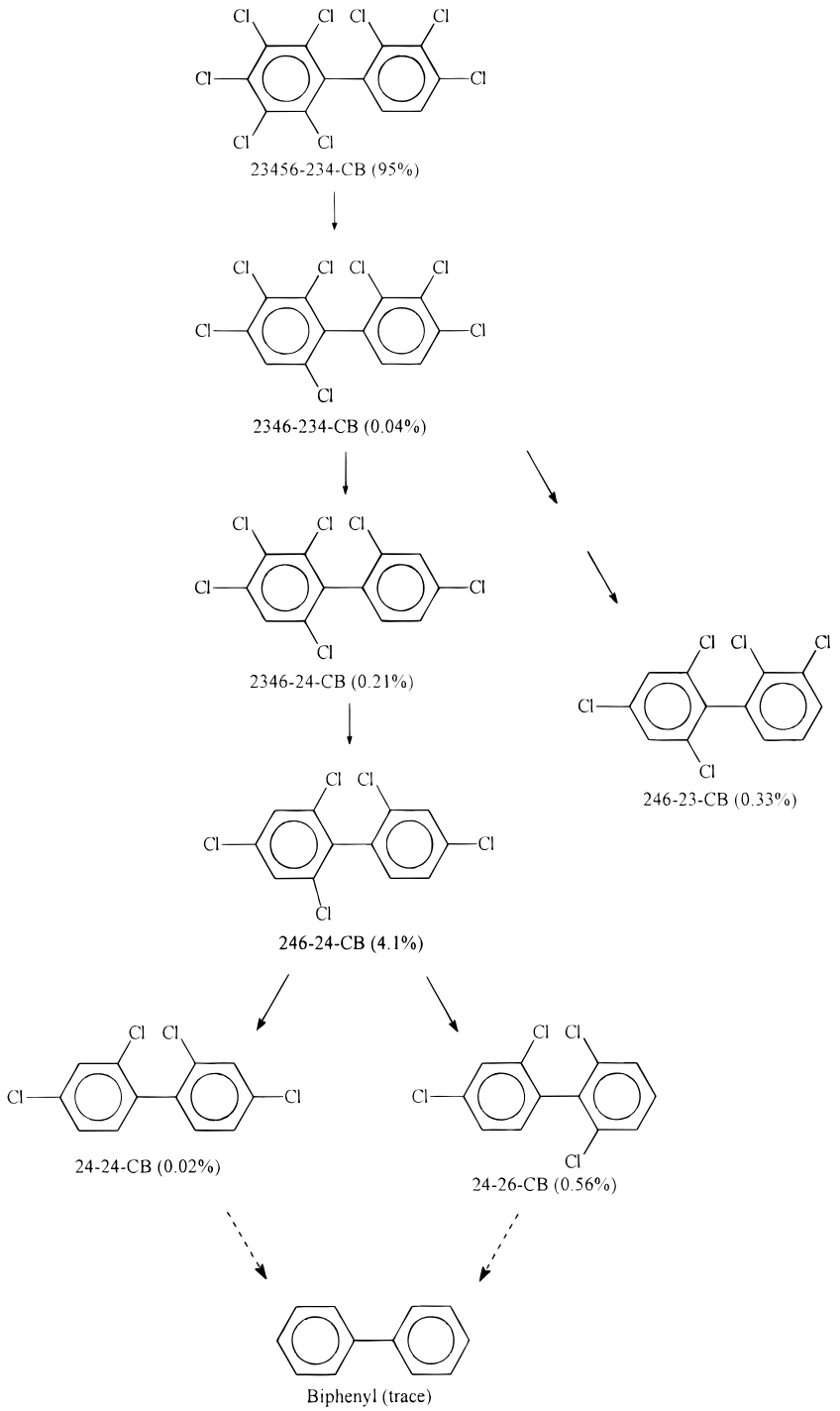



**FIGURE 1. Dechlorination of 23456−234-octaCB after 16 weeks. Relative amounts of products in a BK24 culture are given in parentheses.**

mass spectrum of each GC peak was used to determine whether the peak corresponded to a PCB, and if so, to determine the number of chlorine substituents. Congener assignments were made by comparison with Aroclor standards (Aroclors 1221, 1242, 1254, and 1260) and by comparison with published relative retention times on a DB5-ms column (*18*). The identification of major product congeners that were not present in any of the Aroclor mixtures used was confirmed by purchasing individual standards and determining their retention times and mass spectra. The identification of biphenyl was confirmed by comparison of retention times and mass spectra with those of a biphenyl standard. Linear three-point calibration curves were constructed for all congeners using either pure congeners where available or using the weight percent contributions of the components present in Aroclor standards (*19*). The detection limit for biphenyl was 8 $\mu$g/L, and the quantitation limit for biphenyl was 25 $\mu$g/L.

## Results

23456−2345-nonaCB and all four octachlorobiphenyl congeners tested yielded dechlorination products after only 4 weeks of incubation. 23456−2346-nonaCB and 23456−2356-nonaCB showed evidence of dechlorination after 12 weeks of incubation. In each case, the major product that accumulated over time resulted from removal of all *m*-chlorines. The extent of dechlorination after 16 weeks varied widely from congener to congener, although the results obtained

**TABLE 1. Major Products Formed in BK24 and BK72 Cultures after 16 Weeks**

| | | | major products (mol % of total) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | % substrate remaining | | 246−24-CB | | 246−26-CB | | 24−24-CB | | 24−26-CB | | 24−25-CB | |
| substrate | BK24 | BK72 | BK24 | BK72 | BK24 | BK72 | BK24 | BK72 | BK24 | BK72 | BK24 | BK72 |
| 23456−234 | 94.8 | 91.0 | 4.1 | 8.6 | | | | 0.4 | 0.6 | | | |
| 2345−2346 | 82.1 | 69.2 | 16.6 | 28.5 | | | 0.5 | 0.9 | | | | |
| 2345−2356 | 96.6 | 95.8 | | | | | | | 3.4 | 4.2 | | |
| 23456−245 | 61.6 | 71.1 | 37.1 | 28.0 | | | | | | | | |
| 23456−2345 | 98.1 | 98.6 | 1.0 | 0.8 | | | 0.5 | 0.4 | | | | |
| 23456−2346 | 98.4 | 96.2 | | | | | | | 1.2 | 3.1 | | |
| 23456−2356 | 95.3 | 96.4 | | | 0.9 | 0.7 | | | | 1.4 | 0.9 | 0.7 |

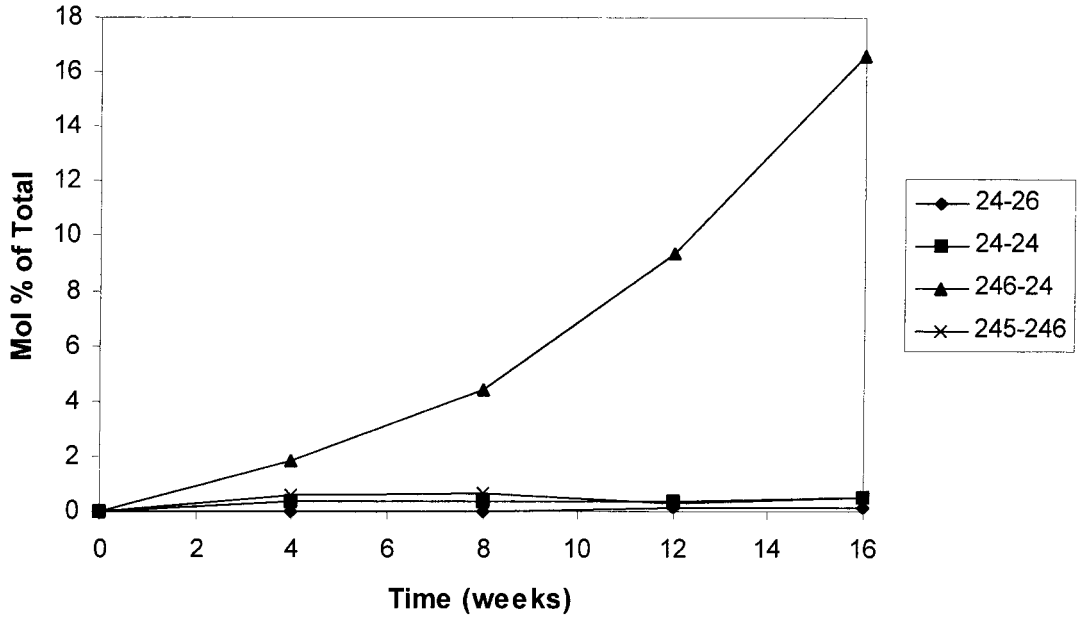



**FIGURE 2. Dechlorination products of congener 2345−2346-octaCB in a BK24 culture over time.**

for the two different cultures used were reasonably consistent for each congener (Table 1). Mass balances were calculated for the total PCBs detected in each sample, and it was found that the total recovery of PCBs ranged from 92% to 97% after 16 weeks. Since an entire culture was used for each sample, there were four replicate cultures for each treatment. The results indicate consistent dechlorination rates and specificities in each set of replicate cultures. No degradation products were detected in any of the control cultures after 16 weeks of incubation.

**Congener 195 (23456−234-octaCB).** Dechlorination products of congener 195 were detected after only 4 weeks of incubation with both BK24 and BK72. The products found after 16 weeks, and the dechlorination pathway suggested by their formation are shown in Figure 1. The numbers in parentheses show the proportions of the different products (as mol % of total PCBs present) after incubation with enrichment culture BK24. Results obtained for incubation with BK72 suggested an identical dechlorination pathway, with the exception that no 246−23-pentachlorobiphenyl was detected. The relative proportions of products were also slightly different. Trace amounts (8−25 $\mu$g/L) of biphenyl were detected after 12 weeks in BK24 cultures and after 16 weeks in BK72 cultures but were not detected in control cultures.

**Congener 196 (2345−2346-octaCB).** The amounts of the various metabolites detected for congener 196 over the time period investigated are shown in Figure 2. Results are given for incubation with enrichment culture BK24, but the relative amounts of products detected in culture BK72 are very similar. A proposed pathway for the formation of these products is shown in Figure 3. Trace amounts of biphenyl were detected after 12 weeks of incubation.

**Congener 199 (2345−2356-octaCB).** No heptachlorobiphenyl products were detected in cultures containing congener 199. Trace amounts of 2356−24-hexachlorobiphenyl were detected, and no pentachlorobiphenyl compounds were detected. The major dechlorination product found after 16 weeks was 24−26-tetrachlorobiphenyl (3.39% and 4.24% of total PCBs for cultures BK24 and BK72, respectively). Trace amounts of biphenyl were detected after 12 weeks of incubation.

**Congener 203 (23456−245-octaCB).** The time sequence of product formation from congener 203 over the 16-week sampling period is shown in Figure 4. A proposed pathway for dechlorination of congener 203, with the proportions of the various products at 16 weeks, is shown in Figure 5. Trace amounts of biphenyl were detected after 12 weeks of incubation.

**Congener 206 (23456−2345-nonaCB).** No octa-, hepta-, or hexachlorobiphenyls were detected in the cultures containing congener 206. The major metabolite detected was 246−24-pentachlorobiphenyl (1.01% and 0.81% for cultures 24 and 72, respectively, after 16 weeks). Biphenyl was also detected at trace levels after 16 weeks of incubation.

**Congener 207 (23456−2346-nonaCB).** No octa- or heptachlorobiphenyls were detected in the cultures with congener 207. 246−246-Hexachlorobiphenyl was detected after 4 weeks, and at 16 weeks this comprised 0.17% and 0.15% of the total mol % for enrichment cultures BK24 and BK72,

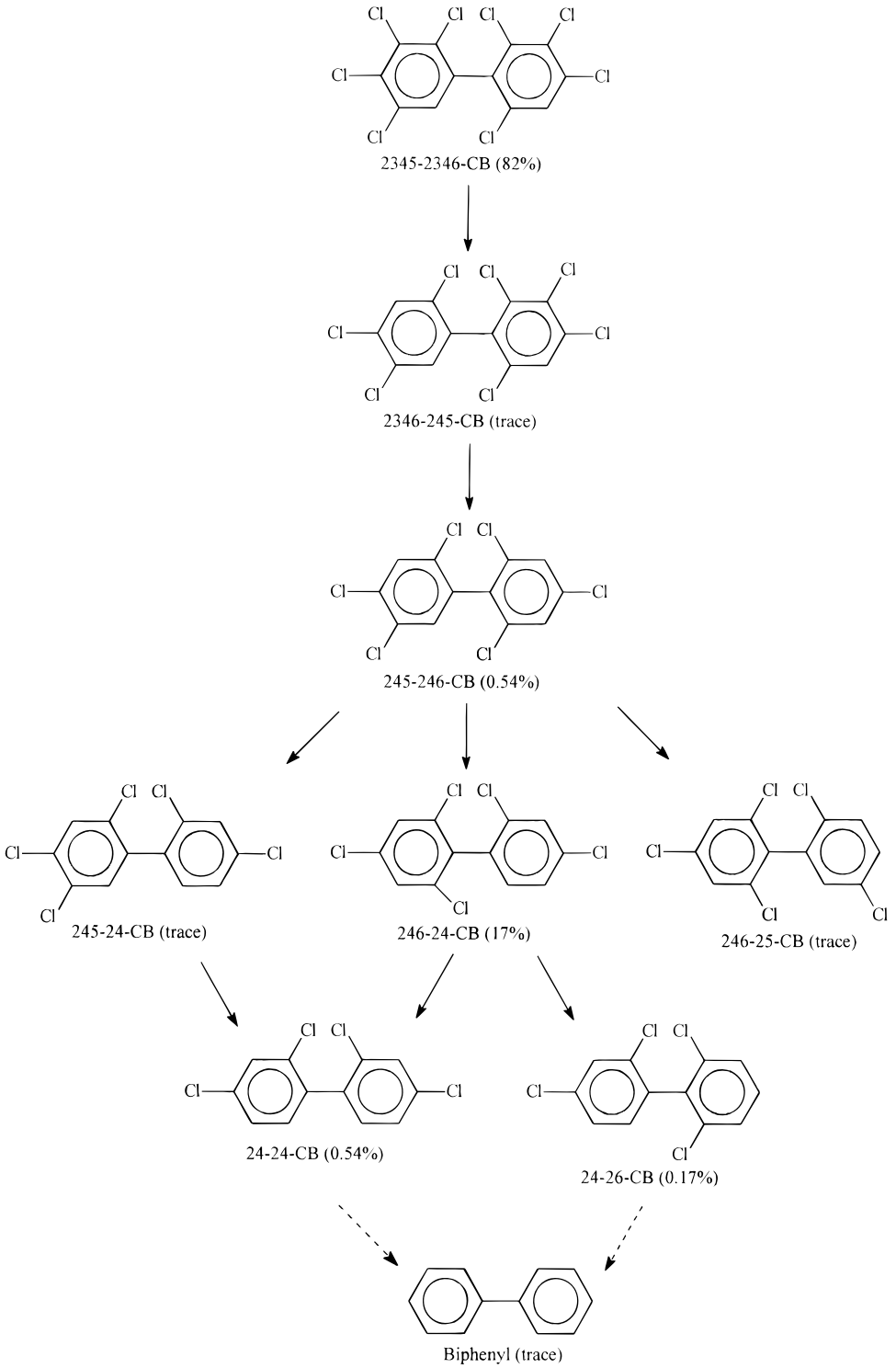



**FIGURE 3. Dechlorination of 2345−2346-octaCB after 16 weeks. Relative amounts of products in a BK24 culture are given in parentheses.**

respectively. 246−24-Pentachlorobiphenyl (0.01% and 0.02%), 24−26-tetrachlorobiphenyl (1.16%, 3.15%), 24−24-tetrachlorobiphenyl (0.22, 0.39%), and biphenyl (trace, trace) were also found.

**Congener 208 (23456−2356-nonaCB).** Cultures containing congener 208 also did not form detectable amounts of octa- or heptachlorobiphenyls. 246−236-Hexachlorobiphenyl was found in trace amounts, and 246−26-pentachlorobiphenyl (0.95%, 0.70%) was the only pentachlorobiphenyl detected. 24−24-Tetrachlorobiphenyl (1.71%, 1.69%) and 24−26-tetrachlorobiphenyl (1.39%, 0.88%) were also found. Biphenyl was detected after 12 weeks of incubation.

## Discussion

This study clearly shows, for the first time, the reductive dechlorination by anaerobic microorganisms of single octa- and nonachlorobiphenyls and the specific dechlorination products formed. When all of the proposed pathways for dechlorination are examined, dechlorination of doubly flanked *m*-chlorines is the predominant initial step of dechlorination, followed by dechlorination of singly flanked *m*-chlorines. This is consistent with Process N dechlorination as described by Bedard and Quensen (*1*), which has a dechlorination specificity for flanked and doubly flanked

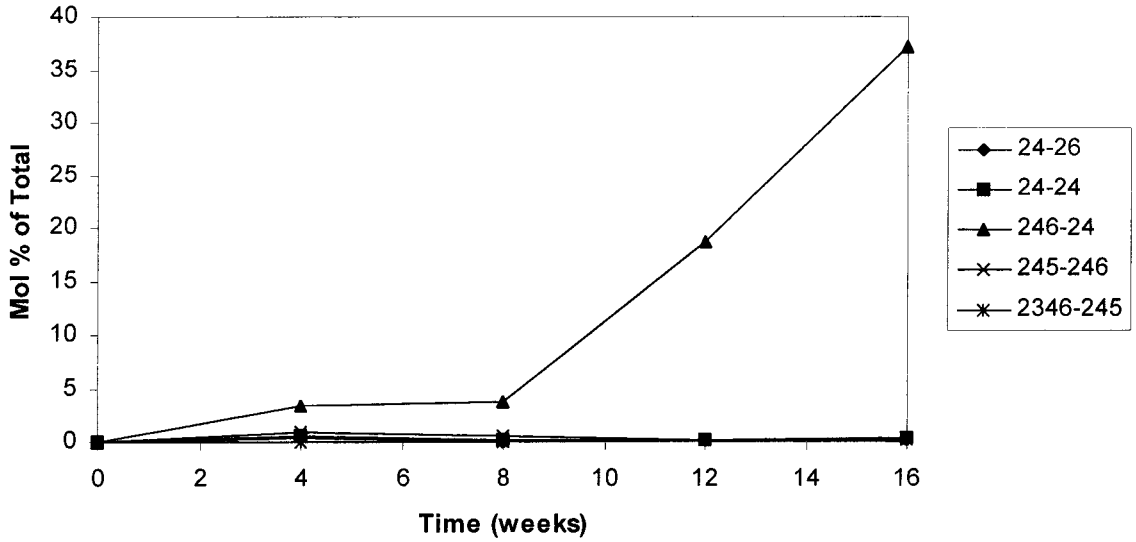



**FIGURE 4. Dechlorination products of 23456−245-octaCB in a BK24 culture over time.**

*m*-chlorines. It should also be noted that, within the different molecules containing doubly flanked chlorines, a definite sequence of dechlorination is observed depending on the pattern of chlorine substitution. For example, if one of the rings contains five chlorines, the first chlorine removed is always a *m*-chlorine on this ring. Figure 6 shows the order of removal of the three different doubly flanked *m*-chlorines in congener 195. The present results confirm suggestions that substituents on one ring affect dechlorination on the other ring (*3*). For example, when the results obtained for congeners 196 and 199 are compared, it can be seen that the first chlorine removed in both these congeners is from the same position of identically substituted rings, yet the rates of removal (as evidenced by the total removal of the congeners over time) are very different. This difference in dechlorination rates is attributed to the different chlorine configuration on the ring where dechlorination is not taking place.

Ortho- and para-dechlorination was also observed, but usually only after all *m*-chlorines were removed. For most cultures and congeners, there was more ortho-dechlorination than para-dechlorination observed. In a few instances, however, para-dechlorination was more prevalent than ortho-dechlorination. For example, for congeners 203 and 195, the tetrachlorobiphenyl products formed differ, but both are expected to come from the common 246−24-pentaCB precursor. It is possible that these differences in activity are due to differences in the anaerobic microbial populations present. The period of incubation required before ortho- and para-dechlorination occurred also varied between cultures. In some examples ortho- and para-dechlorinating activity was observed after only 8 weeks, while in the other cultures using the same single congener, ortho- and para-dechlorinating activity appeared only after 12 or 16 weeks. This once again suggests dechlorination activities of different microbial populations present in the initial inocula.

Even though some products accumulated in substantial quantities, there were also small amounts of many others present. The presence of isomeric products of one congener indicate that, even though dominant pathways are clearly observed, the dechlorination pathways are branched (i.e., the order of dechlorination sequence is varied).

In the present work no trace of any tri-, di-, or monochlorobiphenyls was found. Similar results were reported for the dechlorination of 234−234- and 236−236-hexaCB (*13*). However, several other studies that started with less highly chlorinated PCBs that were substituted with chlorines on only one of the two biphenyl rings have indicated the formation of tri-, di-, and monochlorobiphenyl products (*10*−*12*). In the present study, traces of biphenyl were found in most cultures after 12 weeks and in all cultures after 16 weeks. This suggests that, even though intermediates between tetrachlorobiphenyls and biphenyl were not detected, they were probably formed in quantities below the detection limit and were further dechlorinated to biphenyl. This is the first evidence for complete anaerobic dechlorination of nona- and octaCBs.

It is interesting to note that in the incubations with the nonachlorobiphenyls, octa- and heptachlorobiphenyl products were not detected. Also, the major penta- and hexaCB products that would be expected to accumulate, by analogy with results obtained for octaCB dechlorination (246−24-pentaCB for congener 206, 246−246-hexaCB for congener 207, and 246−26-pentaCB for congener 208), did not always accumulate. Rather, a much higher proportion of tetrachlorobiphenyl products was formed. We suggest that the initial dechlorination step of nonaCBs (e.g., 23456−2356-nonaCB) is a slower process than the subsequent dechlorination of the octa-, hepta-, hexa-, and pentaCB products, explaining the accumulation of tetraCB products rather than the penta- or hexaCB products. In contrast, for three of the octachlorobiphenyls studied, the accumulation of 246−24-pentaCB occurred, so we suggest that in this case the ortho- or para-dechlorination of this pentachlorobiphenyl is slower than the initial meta-dechlorination of the octachlorobiphenyl. With the fourth octachlorobiphenyl studied, 2345−2356-octaCB, 246−24-pentaCB did not accumulate as it was not a possible dechlorination product. In this case the product of meta-dechlorination was 24−26-tetraCB. It should be noted that, after 16 weeks, dechlorination of the congeners by the cultures was still continuing, so nothing can be concluded about the potential extent of dechlorination or the final distribution of products. It should also be noted that PCB concentrations used in these experiments greatly exceeded concentrations of octa- and nonaCBs found in most contaminated environments.

The results of other experiments investigating the dechlorination of Aroclor 1260 with the same enrichment cultures exhibit the same dechlorination patterns (e.g., predominantly Process N) as those found with the single congener experiments reported here (unpublished results). This suggests that Aroclor mixtures follow similar dechlorination pathways to the single congeners and confirms the relevance of studying single congeners as a means of elucidating dechlorination pathways in more complex mixtures. This indicates that

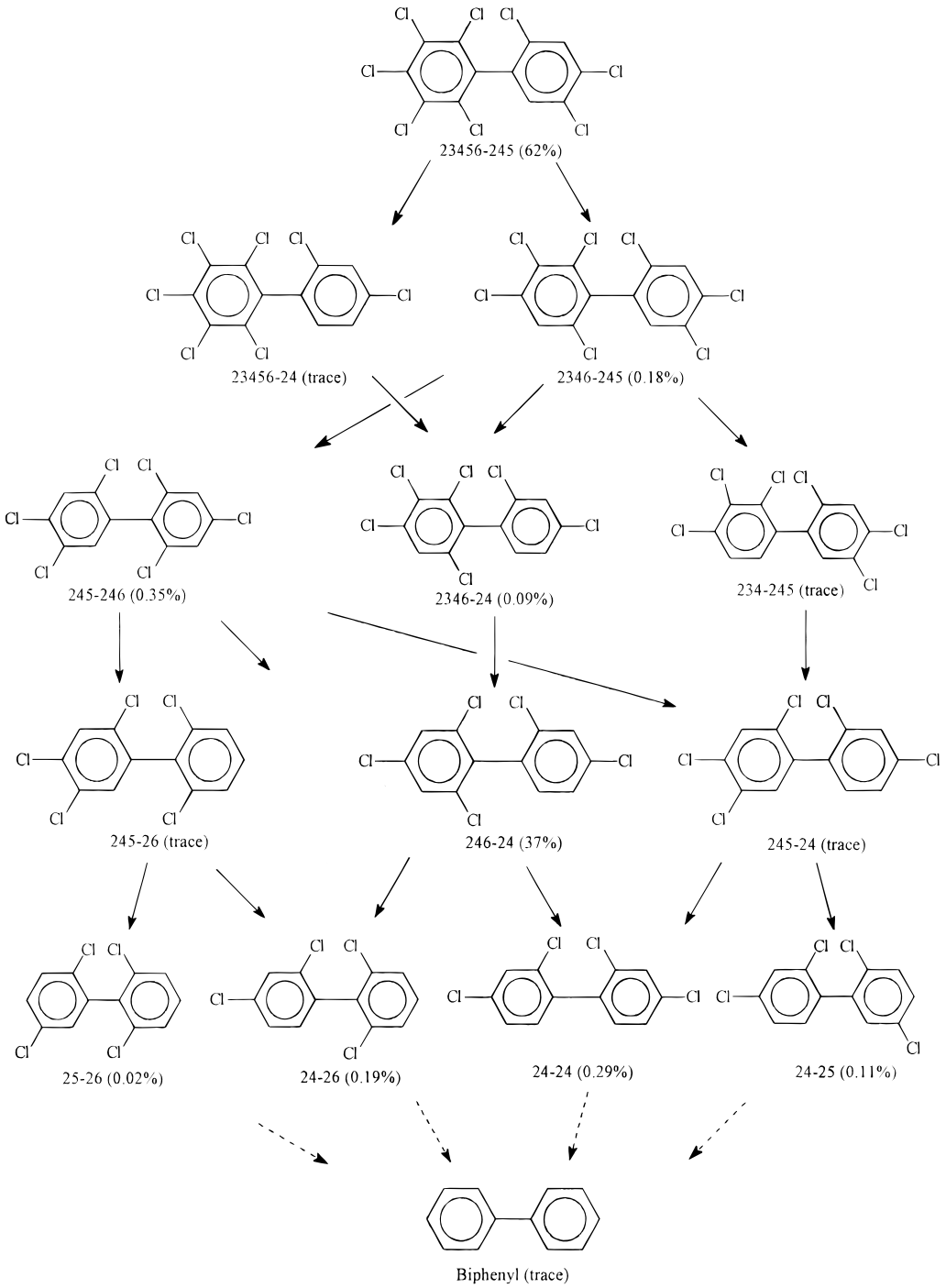
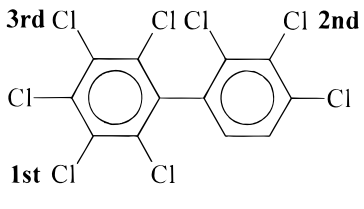



**FIGURE 5. Dechlorination of congener 23456−245-octaCB after 16 weeks. Relative amounts of products in a BK24 culture are given in parentheses.**




23456-234-octachlorobiphenyl
(Congener 195)

**FIGURE 6. Proposed order of removal of the first three chlorines from 23456 to 234-octaCB.**

competitive inhibition or other possible effects of PCB mixtures do not noticeably affect dechlorination pathways.

In conclusion, these results give a greater understanding of what may be happening to individual octa- and nona-CB congeners in anaerobic biological systems containing mixtures of different congeners (e.g., as in Aroclor 1260). The products detected suggest dechlorination pathways specific to particular congeners. For all congeners examined dechlorination was possible, and for some congeners a large fraction of the initial compound was transformed.

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