APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1992, p. 3249–3256
0099-2240/92/103249-08$02.00/0

Vol. 58, No. 10

Reductive Debromination of the Commercial Polybrominated Biphenyl Mixture Firemaster BP6 by Anaerobic Microorganisms from Sediments

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Received 12 May 1992/Accepted 28 July 1992

Anaerobic microorganisms eluted from three sediments, one contaminated with polybrominated biphenyls (PBBs) and two contaminated with polychlorinated biphenyls, were compared for their ability to debrominate the commercial PBB mixture Firemaster. These microorganisms were incubated with reduced anaerobic mineral medium and noncontaminated sediment amended with Firemaster. Firemaster averages six bromines per biphenyl molecule; four of the bromines are substituted in the *meta* or *para* position. The inocula from all three sources were able to debrominate the *meta* and *para* positions. Microorganisms from the Pine River (St. Louis, Mich.) contaminated with Firemaster, the Hudson River (Hudson Falls, N.Y.) contaminated with Aroclor 1242, and Silver Lake (Pittsfield, Mass.) contaminated with Aroclor 1260 removed 32, 12, and 3% of the *meta* plus *para* bromines, respectively, after 32 weeks of incubation. This suggests that previous environmental exposure to PBBs enhances the debromination capability of the sediment microbial community through selection for different strains of microorganisms. The Pine River inoculum removed an average of 1.25 bromines per biphenyl molecule during a 32-week incubation period, resulting in a mixture potentially more accessible to aerobic degradation processes. No *ortho* bromine removal was observed. However, when Firemaster was incubated with Hudson River microorganisms that had been repeatedly transferred on a pyruvate medium amended with Aroclor 1242, 17% of the *meta* and *para* bromines were removed after 16 weeks of incubation and additional debromination products, including 2-bromobiphenyl and biphenyl, were detected. This suggests the possibility for *ortho* debromination, since all components of the Firemaster mixture have at least one *ortho*-substituted bromine. These results demonstrate that microorganisms found in the PBB-contaminated Pine River sediments have the capability to debrominate the PBB congeners in Firemaster.

Polybrominated biphenyls (PBBs) are industrial compounds that were manufactured as fire retardants for thermoplastic applications. The commercial production of PBBs began in 1970, and approximately 13.3 million pounds (ca. 6.03×10^9 g) was produced in the United States between 1970 and 1976 (9). The Michigan Chemical Corp. (later Velsicol Chemical Corp.) (St. Louis, Mich.) manufactured PBBs under the trade name Firemaster BP6 between 1970 and 1974. In 1973, between 500 and 1,000 pounds (ca. 2.27×10^5 and 4.54×10^5 g, respectively) of the flame retardant was mistaken for the dairy feed additive magnesium oxide, resulting in contamination of animal feeds, animals, and soils in Michigan (5). The Michigan Chemical Corp. manufacturing site is located adjacent to the Pine River in St. Louis, Mich. Sediments at this site are contaminated with PBBs and a number of other compounds, including DDT [1,1-bis(4-chlorophenyl)-2,2,2-trichloroethane], hexabromobenzene, chlordane, petroleum products, and heavy metals (12, 13, 19).

Commercial PBBs were produced by bromination of biphenyl with elemental bromine in the presence of a catalyst; this process yielded a product less complex than commercial polychlorobiphenyl mixtures (Aroclors). More than 50% of the PBB mixture is composed of a single congener, 2,4,5-2′,4′,5′-hexabromobiphenyl (2,4,5-2′,4′,5′-BB; BB is used as an abbreviation throughout for specific PBB congeners) (24). The other major congeners are 2,4,5-2′,5′-BB, 2,4,5-3′,4′-BB, 2,4,5-3′,4′,5′-BB, and 2,3,4,5-2′,4′,5′-BB. In comparison, commercial Aroclors commonly consist of up to 90 polychlorinated biphenyl (PCB) congeners. PBBs, like PCBs, were considered to be highly recalcitrant in the environment, and there is evidence for their persistence in the environment (16). Their fate in soils is characterized by low leachability (11), lack of uptake by plants (6, 14), and absence of biological degradation (14, 15).

Recently, Brown and colleagues (3, 4) provided evidence for in situ reductive dechlorination of PCBs in anaerobic sediments from the Hudson River. Quensen et al. (21, 22) subsequently demonstrated that anaerobic microorganisms eluted from PCB-contaminated sediments could dechlorinate PCBs (Aroclor mixtures) that were added to previously uncontaminated sediments. This anaerobic reductive dechlorination of PCBs renders the Aroclor mixtures less toxic and more susceptible to aerobic degradation processes.

Although reductive dehalogenation has been shown to occur with PCBs, no studies have been conducted with PBBs. In general, bromine is lost from haloaliphatic compounds more readily than chlorine (26), and enriching for microorganisms with PBB-dehalogenating activity may lead to microorganisms with enhanced PCB dechlorination ability. In this report, we compare the reductive debromination of PBBs by anaerobic microorganisms from three sites; a Firemaster (PBB)-contaminated sediment from the Pine River (St. Louis, Mich.), an Aroclor 1242 (PCB)-contaminated sediment from the Hudson River (Hudson Falls,

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N.Y.), and an Aroclor 1260 (PCB)-contaminated sediment from Silver Lake (Pittsfield, Mass.). The PBB debromination activity of an anaerobic PCB-degrading enrichment culture (18) was also evaluated.

MATERIALS AND METHODS

Sediments. Aroclor 1242 (PCB)-contaminated sediments were collected in August 1988 from the Hudson River at river mile 193.5 near Hudson Falls, N.Y. (site H7 in reference 4). Non-PCB-contaminated Hudson River sediments were collected upstream at river mile 205. Aroclor 1260-contaminated sediments were collected from Silver Lake (near Pittsfield, Mass.) in September 1989 (3, 4). The Silver Lake sediments additionally contained high concentrations of oil and polyaromatic hydrocarbons from a coal gasification plant. Firemaster (PBB)-contaminated sediments were collected from the Pine River reservoir (St. Louis, Mich.) adjacent to the Michigan Chemical Corp. site just upstream from the hydroelectric dam (12, 13). In addition to PBBs, these sediments contained a variety of cocontaminants, including hexabromobenzene, DDT, heavy metals, and petroleum products (12, 13, 19). Aroclor 1242- and 1260-contaminated sediments were collected with a post hole digger to a depth of approximately 25 cm and transported to the laboratory in tightly sealed paint cans. Firemaster-contaminated sediments were collected by pushing 3-in. (ca. 7.5-cm) polyvinyl chloride pipes into the sediments to a depth of approximately 25 cm. The pipes containing the sediment cores were then capped, withdrawn from the river, and transported to the laboratory.

Preparation of anaerobic inoculum. Contaminated and noncontaminated sediments were placed in 2-liter Erlenmeyer flasks and flushed with O_2-free N_2-CO_2 (80:20, vol/vol) with a Hungate apparatus. Equal volumes of sediment and reduced anaerobic mineral medium (RAMM [23]) were placed in the flasks, which were then sealed with butyl rubber stoppers. The flasks were shaken by hand for approximately 2 min and allowed to settle for approximately 10 min. The supernatants were then withdrawn under a N_2-CO_2 atmosphere and used as inocula for dehalogenation experiments conducted with noncontaminated sediments amended with PCBs or PBBs and mineral medium (see below). This procedure reduced the PCB or PBB background and other chromatographic interferences, allowing quantification of the dehalogenation of the added PCBs or PBBs. The use of noncontaminated sediments known to support dehalogenation also provides experimental conditions that are nearly identical to those used previously for dehalogenation assays (21, 22).

A pyruvate and Aroclor 1242 enrichment from Hudson River sediment organisms was also used as an inoculum; this enrichment was described previously (18).

Assay vessels. Two dehalogenation assays were used in this study. For experiments conducted with Hudson River, Pine River, and Silver Lake microorganisms that were incubated with RAMM (see below) alone, glass Balch tubes (28 ml) were used. For experiments utilizing amended mineral medium (see below) and microorganisms from a pyruvate and Aroclor 1242 enrichment culture(s), organisms were incubated in glass serum bottles (160 ml).

Medium. RAMM was used for all experiments set up in glass Balch tubes. For experiments set up in serum bottles, RAMM was amended as follows: 1 mM cysteine instead of Na_2S, 10 mM instead of 4 mM potassium phosphate buffer (pH 7.0), $NaHCO_3$ omitted, and vitamins and 0.1 mM titanium(III) citrate added. Vitamins, titanium(III) citrate, and sodium pyruvate (20 mM) were added from filter-sterilized stock solutions after the mineral medium was autoclaved. Vitamins included a mixture generally used by anaerobes (27) plus 450 μg of nicotinamide per liter and 200 μg of naphthoquinone per liter (8). Titanium(III) citrate was prepared by the method of Zehnder and Wuhrmann (28). The pH of the medium was adjusted to 7.0 before the medium was autoclaved.

Cultures. Incubations were carried out in the presence of sediment because dehalogenation is not observed in their absence. Non-PCB-contaminated Hudson River sediment was air dried and sieved through a 2-mm-mesh screen. For experiments with Balch tubes, 1 g of these sediments was weighed into each glass Balch tube, and the tubes were flushed with O_2-free N_2 with a Hungate apparatus. The sediments were preincubated to ensure strict anaerobic conditions by adding 2 ml of inoculum eluted from clean Hudson River sediments and ethanol (1 μl/ml) to each tube. Tubes were sealed with butyl rubber stoppers and incubated at 37°C in the dark until methane was detected in the headspace (see below). After methane production was observed, indicating anaerobic conditions, the sealed tubes were autoclaved for 1 h at 121°C. Then 5 ml of inoculum from PCB-contaminated or PBB-contaminated sediments was added to each tube. All experiments in tubes were set up in triplicate. Controls were autoclaved as described above after inoculation.

For experiments with the pyruvate plus Aroclor 1242 enrichments (18), 25 g of sieved, air-dried, non-PCB-contaminated Hudson River sediment was added to each glass serum bottle (160 ml) along with 40 ml of amended RAMM and 10 ml of inoculum; the bottles were then sealed with butyl rubber stoppers. In these experiments, the preincubation step (for methane production) was eliminated. All experiments utilizing serum bottles were set up in duplicate.

Firemaster and Aroclor addition. A 10% (wt/vol) solution of Aroclor 1242 or 1260 (Monsanto Co., St. Louis, Mo.) or Firemaster (obtained from M. Zabik, Department of Entomology, Michigan State University) in acetone was added to each tube (or serum bottle) while flushing with O_2-free N_2 to give a final concentration of 500 or 50 μg of PCB or PBB per g of sediment. The PBB congener 2,4,5-2′,4′,5′-BB (Ultra Scientific, Hope, R.I.) was added to separate tubes at 250 or 25 μg per g of sediment. After PCB or PBB addition, the tubes were resealed with Teflon-coated rubber stoppers (West Co., Phoenixville, Pa.) and incubated at 25°C in the dark.

Analyses. The entire contents of tubes and 2-ml samples from bottles were extracted as described by Quensen et al. (21). Headspace gas analysis (primarily for CH_4 detection) was determined with a Carle model AGC-111 gas chromatograph with a 6-m Porapak Q column and microthermistor detector with argon as the carrier gas. Congener-specific PCB analysis was performed with a gas chromatograph and an electron capture detector as described previously (21). Conditions for PBB identification were the same as for PCBs with the following modifications: the detector temperature was 350°C; the column temperature was held at 160°C for 1 min, increased 2°C/min to 300°C, and then held for 30 min. Retention times and structures for the PBBs quantified are given in Table 1.

Retention times and response factors for most of the PBBs in Table 1 were based on the chromatographic analysis of all commercially available authentic standards (Ultra Scientific) and standards purified from the Firemaster mixture (7).

TABLE 1. Retention time and identification of each chromatographic peak

Peak no.	Retention time (min)	IUPAC[a] no.	Structure
1	7.481	1	2-BB[b]
2	9.556	2	3-BB[b]
3	9.834	3	4-BB[b]
4	12.693	4	2-2'-BB[b]
5	12.960	10	2,6-BB[b]
6	14.889	9	2,5-BB[b]
7	15.107	8	2,4-BB[b]
8	20.301	15	4-4'-BB[b]
9	21.892	30	2,4,6-BB[b]
10	22.114	18	2,5-2'-BB[b]
11	22.727	17	2,4-2'-BB[c]
12	25.935	29	2,4,5-BB[b]
13	26.438	26 25	2,5-3'-BB[b] or 2,4-3'-BB[c]
14	26.913	31	2,5-4'-BB[b]
15	27.252	28	2,4-4'-BB[c]
16	30.184	53	2,6-2',5'-BB[b]
17	30.653	51	2,4-2',6'-BB[c]
18	31.371	38	3,4,5-BB[b]
19	32.869	52	2,5-2',5'-BB[b]
20	33.633	49	2,4-2',5'-BB[b]
21	34.797	47	2,4-2',4'-BB[c]
22	40.381	103 80	2,4,6-2',5'-BB[b]
23	44.705	101	2,4,5-2',5'-BB[b]
24	45.631	110	2,3,6-3',4'-BB
25	47.638	155	2,4,6-2',4',6'-BB[b]
26	51.138	118	2,4,5-3',4'-BB
27	53.262	133	2,3,5-2',3',5'-BB[c]
28	55.625	146	2,3,5-2',4',5'-BB[c]
29	56.112	153	2,4,5-2',4',5'-BB[b]
30	57.499	141	2,3,4,5-2',5'-BB[c]
31	58.778	138	2,3,4-2',4',5'-BB[d]
32	59.476	158 178	2,3,4,6-3',4'-BB[c] or 2,3,5,6-2',3',5'-BB[c]
33	61.660	167	2,4,5-3',4',5'-BB[d]
34	64.086	156	2,3,4,5-3',4'-BB[d]
35	68.245	180	2,3,4,5-2',4',5'-BB[d]
36	82.638	194	2,3,4,5-2',3',4',5'-BB[d]

[a] IUPAC, International Union of Pure and Applied Chemistry.
[b] Obtained from Ultra Scientific.
[c] The identities of these debromination products, which were not present in the calibration mixture, were inferred from their theoretical retention times (a linear relationship was derived for retention times of PCBs and PBBs in the standard mixture) and confirmed by mass spectroscopy.
[d] Obtained from S. Aust (from purification of congeners found in Firemaster).

However, several additional peaks, identified as PBBs by mass spectrometry, appeared in our samples. For each of these PBBs the number of bromines present was determined by mass spectrometry. Their most probable identities (substitution patterns) were determined from their retention times based on the assumption that corresponding PBB and PCB congeners have the same relative retention times. Linear regression analysis between the observed retention times of known PBBs in Table 1 and the known relative retention times of corresponding PCBs (20) indicated that this was a valid assumption, except that PBBs with a 2,4,6-substituted ring had relative retention times slightly greater than those of the corresponding PCB congeners. Identification was aided in cases where the unknown PBB congener was obviously a debromination product, because the unknown PBB had to be a congener that could result from the debromination of congeners present in Firemaster. Response factors for these additional PBB peaks were estimated from the relative response factors of the corresponding PCBs (20) by assuming that PBB and PCB response factors were directly proportional. In each case, a constant of proportionality was calculated as the ratio between the response factors of the most closely eluting known PBB and the corresponding PCB. This was necessary because we used a split injection technique; with this technique the actual response is in part a function of the elution time.

RESULTS

Dehalogenation of Firemaster and Aroclor 1242 by Hudson River microorganisms. We compared the dechlorination of Aroclor 1242 (PCB) to the debromination of Firemaster (PBB) by Hudson River microorganisms previously shown to be capable of PCB dechlorination (22). Hudson River microorganisms removed 29% of the *meta* and *para* bromines from Firemaster and 59% of the *meta* and *para* chlorines (for an average of one chlorine removed per biphenyl molecule) from Aroclor 1242 during a 40-week incubation (Fig. 1). No clear asymptote was reached in the progress curve for PBB dehalogenation; hence, additional debromination may have occurred during a longer incubation. No *ortho* chlorine removal was observed.

Dehalogenation of Firemaster and Aroclor 1260 by Silver Lake microorganisms. The dehalogenation of Firemaster and Aroclor 1260 (both at 500 μg/g of sediment) by Silver Lake microorganisms was determined. These organisms were eluted from sediments contaminated with Aroclor 1260. Chlorines from the *meta* and *para* positions of Aroclor 1260 decreased 18% (Fig. 2), and no *ortho* chlorine removal was observed. There was a 24-week acclimation period before dechlorination for Aroclor 1260. There was no evidence of debromination of Firemaster by Silver Lake microorganisms.

Reductive debromination of Firemaster by Pine River, Hudson River, and Silver Lake microorganisms. The abilities of microorganisms eluted from PCB-contaminated sites (Hudson River and Silver Lake) and a PBB-contaminated site (Pine River) to debrominate Firemaster at 50 and 500 μg/g of sediment were compared directly. Debromination was observed at the higher Firemaster concentration (Fig. 3), but no significant debromination by microorganisms from the three sites tested was observed at 50 μg of Firemaster per g of sediment. In this second attempt with Silver Lake microorganisms, 3% of the average number of *meta* plus *para* bromines per biphenyl was removed in the first 20 weeks of incubation (Fig. 3). This loss, although small, could be distinguished statistically from the control value. Furthermore, debromination products that were not present in the control were detected. Debromination activity by the Hudson River microorganisms resulted in removal of 12% of the *meta* plus *para* chlorines. Among the three inocula, organisms from the PBB-contaminated Pine River sediments showed the highest debromination activity, resulting in removal of 32% of the *meta* plus *para* chlorines. The Pine River microorganisms removed an average of 1 bromine per biphenyl molecule from Firemaster between 8 and 20 weeks of incubation, and a total of 1.25 bromines per molecule were removed during the 32-week experiment.

The appearance of products from Firemaster debromination by Pine River microorganisms after 32 weeks of incubation included 2,4-2',4'-BB (peak 21), 2,4-2',5'-BB (peak 20), 2,5-2',5'-BB (peak 19), 2,4-2'-BB (peak 11), 2,5-2'-BB (peak 10), and a small amount of 2-2'-BB (peak 4) (Fig. 4).

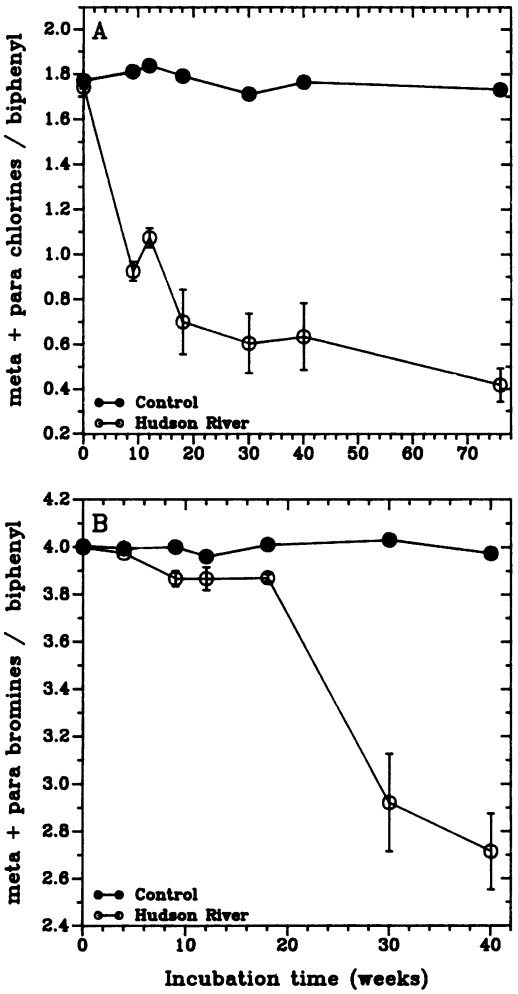

FIG. 1. Reductive dehalogenation of PCBs and PBBs (500 μg/g of sediment), expressed as the average number of *meta* plus *para* halogens per molecule, by Hudson River microorganisms. (A) Dechlorination of Aroclor 1242; (B) debromination of Firemaster. Data are means of triplicates. The vertical bars represent ± 1 standard error; error bars that are smaller than the symbols are not visible.

The major debromination products observed in incubations of Firemaster with the Hudson River microorganisms included 2,5-2'-BB (peak 10) and 2-2'-BB (peak 4) (Fig. 5). Although Silver Lake microorganisms showed limited debromination of Firemaster, several debromination products were observed, including 2,4-2'-BB (peak 11) and 2-2'-BB (peak 4) (Fig. 6).

Reductive debromination of 2,4,5-2',4',5'-BB. One PBB congener, 2,4,5-2',4',5'-BB (peak 29; Table 1) represents roughly 53% (on a molar basis) of Firemaster, and this congener (as a component of Firemaster at 500 μg/g of sediment) decreased 58% after 32 weeks of incubation with Pine River microorganisms (Fig. 4). This congener decreased 33 and 16% after 32 weeks of incubation with Hudson River and Silver Lake microorganisms, respectively (Fig. 5 and 6). Other congeners that were debrominated by the Pine River microorganisms included 2,4,5-3',4'-BB (peak 26), 2,3,4-2',4',5'-BB (peak 31), and 2,4,5-3',4',5'-BB (peak 33). Most of the debromination products of Firemaster could

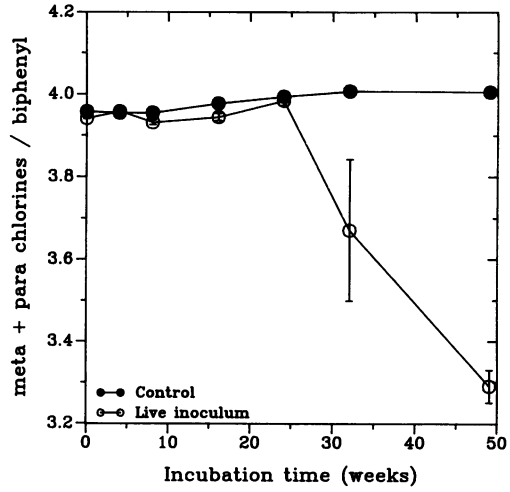

FIG. 2. Reductive dehalogenation of Aroclor 1260 (500 μg/g of sediment), expressed as the average number of *meta* plus *para* halogens per molecule, by Silver Lake microorganisms. Data are means of triplicates. The vertical bars represent ± 1 standard error; error bars that are smaller than the symbols are not visible.

be accounted for by the debromination of 2,4,5-2',4',5'-BB. When 2,4,5-2',4',5'-BB (at 250 or 25 μg/g of sediment) was incubated alone with Hudson River microorganisms, no measurable debromination was observed after 32 weeks of incubation (data not shown).

Reductive debromination by a pyruvate enrichment culture. Hudson River microorganisms that had been transferred repeatedly on Aroclor 1242 and modified RAMM medium containing pyruvate as an added electron donor and carbon source (18) were evaluated for their ability to debrominate Firemaster. These studies were conducted in serum bottles in the presence of nonautoclaved, clean Hudson River sediment. At week 16, the average number of *meta* and *para* bromines of Firemaster had decreased 17%, resulting in a

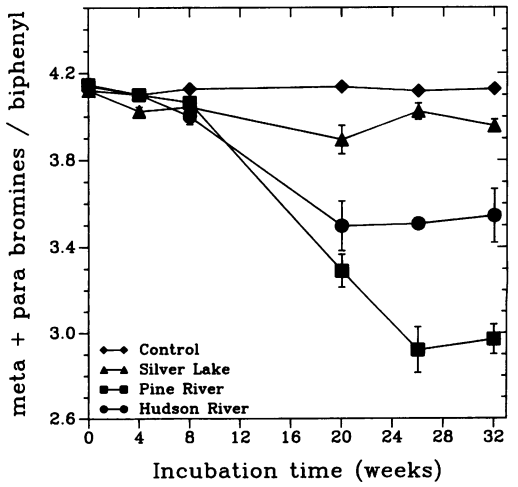

FIG. 3. Reductive debromination of Firemaster (500 μg/g of sediment), expressed as the average number of *meta* plus *para* halogens per molecule, by Silver Lake, Pine River, and Hudson River microorganisms. Data are means of triplicates. The vertical bars represent ± 1 standard error; error bars that are smaller than the symbols are not visible.

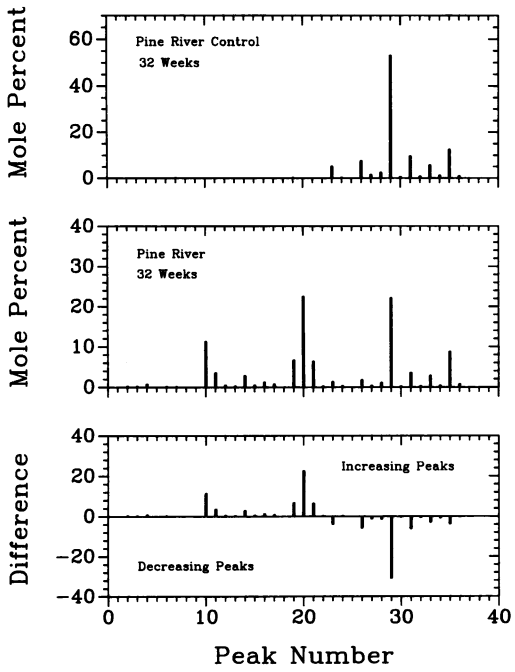

FIG. 4. Mole percentage of PBBs represented by each chromatographic peak after 32 weeks of incubation of Firemaster with microorganisms eluted from Pine River sediments. The bottom histogram shows the mole percent increase and decrease in PBBs.

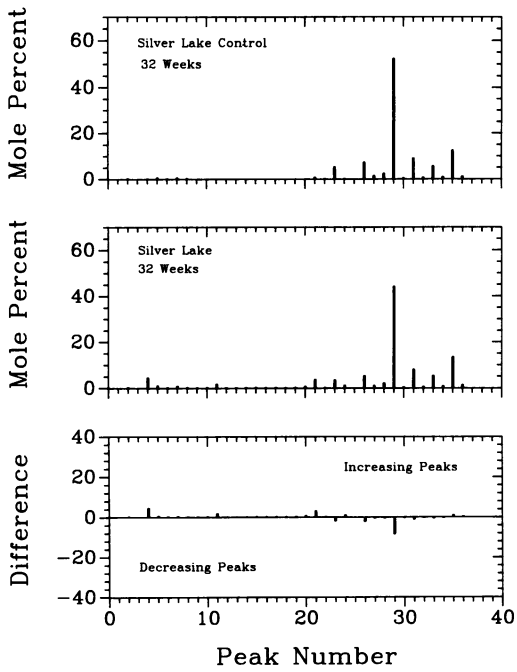

FIG. 6. Mole percentage of PBBs represented by each chromatographic peak after 32 weeks of incubation of Firemaster with microorganisms eluted from Silver Lake sediments. The bottom histogram shows the mole percent increase and decrease in PBBs.

decrease of 0.7 bromine molecule per biphenyl molecule (data not shown). The major debromination products then detected included 2,5-2',5'-BB, 2,4-2',5'-BB, 2,4-2',4'-BB, and 2,5-2'-BB (Fig. 7). Also observed after 16 weeks of incubation were 2-2'-BB (peak 4) and 2-BB (peak 1). When week 16 samples were analyzed on a mass selective detector, biphenyl was detected (Fig. 8). Biphenyl was not observed in samples analyzed before week 16. This enrichment culture

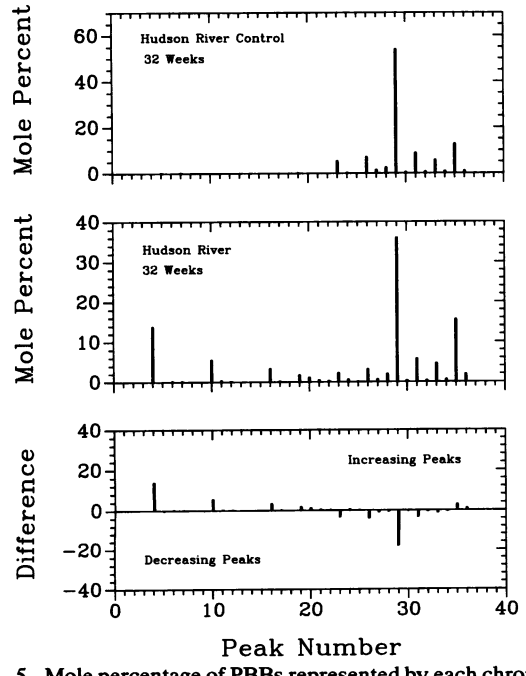

FIG. 5. Mole percentage of PBBs represented by each chromatographic peak after 32 weeks of incubation of Firemaster with microorganisms eluted from Hudson River sediments. The bottom histogram shows the mole percent increase and decrease in PBBs.

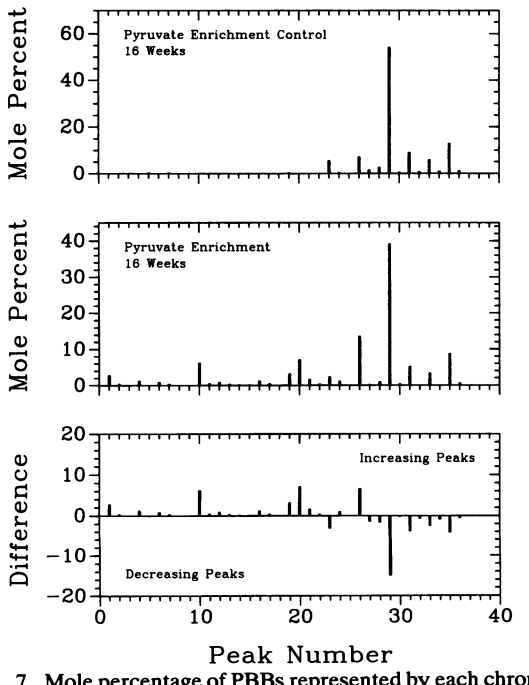

FIG. 7. Mole percentage of PBBs represented by each chromatographic peak after 16 weeks of incubation with the pyruvate enrichment culture. The bottom histogram shows the mole percent increase and decrease in PBBs.

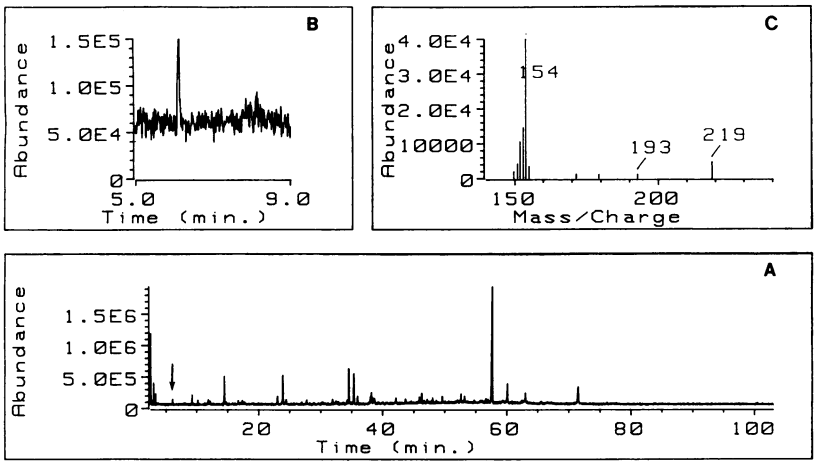

FIG. 8. Gas chromatographic-mass spectrometric analysis of PBBs after 16 weeks of incubation with the pyruvate enrichment culture. (A) Total ion chromatogram of PBBs in Firemaster after 16 weeks of incubation; (B) detail of region containing biphenyl; (C) molecular ion spectrum of the peak eluting at 6.136 min.

exhibited a shorter acclimation period and more debromination products than did the Hudson River inoculum.

DISCUSSION

The extent of dehalogenation of Firemaster, Aroclor 1242, and Aroclor 1260 by microorganisms from the three study sites is summarized in Table 2. The Hudson River inoculum dechlorinated Aroclor 1242 to a greater extent than it debrominated Firemaster. Similarly, the Silver Lake inoculum dechlorinated Aroclor 1260 to a greater extent than it debrominated Firemaster. The extent of debromination of Firemaster by the Pine River inoculum was greater than that occurring when inoculum from the Hudson River or Silver Lake was used. Thus, in each of the three experiments summarized in Table 2, the maximum extent of dehalogenation occurred when the inoculum was dehalogenating the same PBB or PCB mixture present in the inoculum source, i.e., the contaminated sediment. The only possible exception to this trend is that in one experiment the Hudson River inoculum debrominated Firemaster to an extent similar to that of the Pine River inoculum in a separate experiment. In general, organisms with prior exposure to a specific halogenated biphenyl mixture (e.g., Firemaster, Aroclor 1242, or Aroclor 1260) seem to have a greater capability for dehalogenating that mixture (21) than do organisms exposed to other halogenated biphenyl mixtures. Microorganisms eluted from Pine River sediments upstream of the site of PBB contamination were not able to dehalogenate Firemaster or Aroclor 1242 (19).

TABLE 2. Extent of dehalogenation of Firemaster (PBBs) or Aroclors (PCBs) by microorganisms collected from contaminated sites

Inoculum source	Contaminant	Incubation period (wk)	% *meta* plus *para* halogens removed
Hudson River	Aroclor 1242	40	59
	Firemaster	40	29
Silver Lake	Aroclor 1260	50	18
	Firemaster	50	0
Pine River	Firemaster	32	32
Hudson River	Firemaster	32	12
Silver Lake	Firemaster	32	3

In this study, the Silver Lake inoculum supported very limited debromination activity on Firemaster despite the fact that both Aroclor 1260 and Firemaster average about six halogens per biphenyl. Although a lack of debromination was observed with Firemaster, the same inoculum did dechlorinate Aroclor 1260 (18%). Apparently, previous exposure to Aroclor 1260 (PCB) did not predispose these microorganisms to Firemaster (PBB) debromination.

The dehalogenation products of Firemaster can be accounted for by the debromination of the major PBB congener, 2,4,5-2',4',5'-BB. When this congener was present as a component of the Firemaster mixture, 33% of it was debrominated by Hudson River microorganisms; when it was incubated alone, no measurable debromination occurred during 32 weeks of incubation. This suggests that the PBB congener behaves differently when it is a component of the PBB mixture than when it is alone. Perhaps the presence of one or more of the other congeners could be required to elicit a response (e.g., enzyme induction) that triggers reductive dehalogenation. This observation does emphasize that the behaviors of complex mixtures and single components from these mixtures may be quite different.

At a comparatively lower concentration of Firemaster (50 ppm), PBB debromination did not occur. In this instance, the concentrations of the necessary congeners may be too low to induce dehalogenation activity. The concentration dependence for reductive debromination of Firemaster was also observed previously with PCB mixtures. Dechlorination of PCBs by Hudson River microorganisms has been shown to occur more extensively at higher concentrations (i.e., 700 ppm), whereas at lower concentrations (i.e., 14 ppm) dechlorination was not observed (22). A similar response has also been observed in sediments of Woods Pond (Lenox, Mass.), which is contaminated with Aroclor 1260. Bedard and colleagues (2) observed only slight *meta* and *para* dechlorination of endogenous PCBs in sediments of Woods Pond. However, slurries of Woods Pond sediment could be stimu-

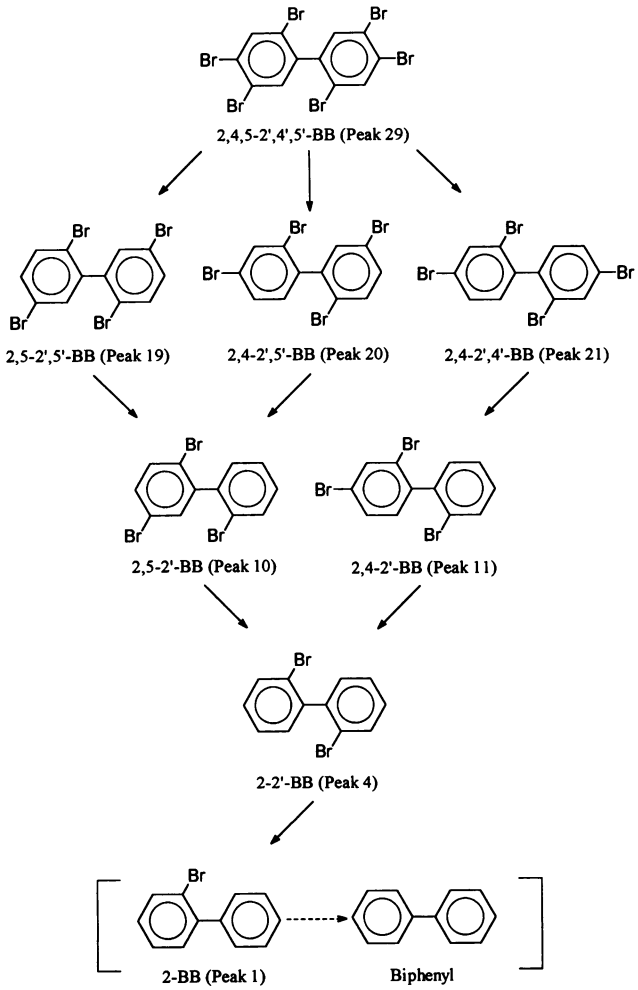

FIG. 9. Proposed pathway for the reductive debromination of 2,4,5-2',4',5'-BB in Firemaster by Pine River and Hudson River sediment microorganisms. Debromination of 2,2'-BB was only observed for a pyruvate plus Aroclor 1242 enrichment obtained from Hudson River sediment microorganisms (18).

lated to dechlorinate endogenous PCBs by adding a high concentration of a single PCB. It is unknown whether specific congeners in the PBB or PCB mixtures behave as dechlorination inducers or provide the dehalogenating microorganisms with energy for growth. It has been demonstrated that an anaerobic microorganism can obtain energy for growth from reductive dehalogenation (10, 17).

The detection of biphenyl as a debromination product of Firemaster in incubations with the pyruvate enrichment indicates the potential for complete debromination of some PBB congeners. There are no components of Firemaster without at least one *ortho*-substituted bromine present. Three congeners, 2,4,5-3',4'-BB (peak 26), 2,4,5-3',4',5'-BB (peak 33), and 2,3,4,5-3',4'-BB (peak 34), are the only possible sources of 2-BB if debromination occurs strictly at the *meta* and *para* positions. Hudson River microorganisms accumulated 2,5-2'-BB and 2-2'-BB. Pine River microorganisms accumulated several tetrabromobiphenyls (2,5-2',5'-BB, 2,4-2',5'-BB, 2,4-2',4'-BB), trichlorobiphenyls (2,5-2'-BB, 2,4-2'-BB), and a small amount of 2-2'-BB. The pyruvate enrichment with Hudson River microorganisms showed the accumulation of several tetrachlorobiphenyls (2,5-2',5'-BB, 2,4-2',5'-BB, 2,4-2',4'-BB). In addition, this enrichment resulted in the accumulation of 2,5-2'-BB and small amounts of 2-2'-BB, 2-BB, and biphenyl. A possible pathway for the complete debromination of the major component of Firemaster to biphenyl would be the sequential debromination of 2,4,5-2',4',5'-BB to 2,4-2',5'-BB and 2,5-2',5'-BB, to 2,5-2'-BB, to 2-2'-BB, to 2-BB, and then potentially to biphenyl (Fig. 9). Although we did not previously report PCB dechlorination from the *ortho* positions (21, 22), there is evidence of in situ *ortho* dechlorination of Aroclor 1260 in sediments from Silver Lake (3). In the laboratory, *ortho* and *meta* chlorine removal from a single PCB congener, 2,3,5,6-CB, during 37 weeks of incubation with methanogenic sediment from Woods Pond was observed (25).

Microorganisms eluted from sediments contaminated with PBB (Firemaster) and PCB (Aroclor 1242) were capable of reductive debromination of the commercial PBB mixture Firemaster. The decrease in acclimation period before debromination and the presence of additional debromination products when the pyruvate enrichment was incubated with Firemaster suggest that it is possible to optimize reductive dehalogenation of heavily halogenated biphenyls. Although aerobic PCB degradation of Aroclor 1260, averaging 6 chlorines per biphenyl molecule, has not been observed (1), dechlorination of the mixture would enhance the potential for aerobic biodegradation. In this study, an average of 1.25 bromines per biphenyl were removed by Pine River microorganisms. In a previous study, 0.75 chlorine per biphenyl was removed from Aroclor 1260 by Silver Lake microorganisms and 1.5 chlorines per biphenyl were removed from Aroclor 1242 by Hudson River microorganisms (21). Like PCBs, PBBs were considered to be recalcitrant in anaerobic environments. However, reductive dehalogenation of PBBs, shown to occur under strict anaerobic conditions in the laboratory, may provide a route to PBB bioremediation in natural environments.

ACKNOWLEDGMENTS

We thank Linda Schimmelpfennig and Dave Kosal for excellent technical assistance. We also thank William W. Mohn for his contribution to the development of the Aroclor 1242-pyruvate enrichment medium.

This work was supported by the Michigan Agricultural Experiment Station, the Michigan Research Excellence Fund, and the General Electric Co.

ADDENDUM IN PROOF

Bedard and Van Dort have recently observed the reductive debromination of several mono-, di-, and tribrominated biphenyls by anaerobic slurries prepared from PCB-contaminated sediments from Woods Pond, Mass. Debromination occurred at the *ortho*, *meta*, and *para* positions, resulting in the production of biphenyl (D. L. Bedard and H. M. Van Dort, Abstr. 92nd Gen. Meet. Am. Soc. Microbiol., p. 339, 1992).

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