

2,6-Dibromobiphenyl Primes Extensive Dechlorination of Aroclor 1260 in Contaminated Sediment at 8–30 °C by Stimulating Growth of PCB-Dehalogenating Microorganisms

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We applied the most probable number (MPN) method to test the hypothesis that 2,6-dibromobiphenyl (26-BB) primes polychlorinated biphenyl (PCB) dechlorination by stimulating the growth of microorganisms that dehalogenate 26-BB and PCBs. The experiments were conducted in anaerobic microcosms of Aroclor 1260-contaminated sediment from Woods Pond (Lenox, MA). We estimate that the number of microorganisms capable of dehalogenating 26-BB and PCBs increased approximately 1000-fold (from $3\text{--}4.9 \times 10^5$ to $2\text{--}5.8 \times 10^8$ cells/g of sediment [dry weight] or from $0.7\text{--}1.2 \times 10^5$ to $0.5\text{--}1.4 \times 10^8$ cells/mL of wet sediment) after priming for 48 days with 26-BB (1050 $\mu\text{mol/L}$ of slurry) in the presence of 10 mM malate at 22 °C. All MPN samples that showed debromination of 26-BB also dehalogenated Aroclor 1260 even in the high dilutions. These results demonstrate for the first time that halogenated biphenyls prime PCB dechlorination by stimulating the growth of PCB-dechlorinating microorganisms. 26-BB primed exclusively *meta*-dechlorination of the PCBs (Process N), which effected extensive decreases (75–88%) in the hexa-through nonachlorobiphenyls in only 5–8 months at temperatures as low as 8 °C. The highest observed rates of primed dechlorination of Aroclor 1260 ranged from ~ 250 to ~ 1150 pmol of Cl mL⁻¹ day⁻¹ at 8 and 25 °C, respectively.

Introduction

Microbial reductive dechlorination of PCBs in aquatic sediments is an important environmental process because it decreases the toxicity of PCBs and increases their degradability (1–7). Unfortunately, dechlorination of PCBs in situ proceeds very slowly in sediments at some locations. Recently, it was demonstrated that PCB dechlorination can be primed in such sediments by the addition of certain brominated biphenyls (5). One of the most effective primers identified

was 2,6-dibromobiphenyl (26-BB), which primed rapid and extensive dehalogenation of the Aroclor 1260 residue in sediment from Woods Pond (Lenox, MA) at room temperature (5). Previous studies have shown repeatedly that no measurable dechlorination of the Aroclor 1260 residue could be detected when this sediment was incubated without a primer for up to 1 year at any temperature from 8 to 66 °C (5, 8).

Reductive dehalogenation of haloaromatic compounds can lead to energy conservation (9–13). For example, Mackiewicz and Wiegel recently demonstrated that the molar growth yields from the reductive dehalogenation of 3-chloro-4-hydroxyphenylacetate yielded energy for growth equivalent to that obtained from the reduction of nitrate, sulfite, or fumarate (11). Several studies have proposed that PCB-dechlorinating microorganisms also derive energy by transferring electrons to PCBs (2, 14). It has also been proposed that high concentrations of halogenated biphenyls (e.g., 2,3,4,6-tetrachlorobiphenyl [2346-CB], 23456-CB, and 26-BB) prime PCB dehalogenation because they support the growth of PCB-dechlorinating microorganisms (5, 6, 8, 15, 16). In this study, we used the most probable number (MPN) method to test this hypothesis in microcosms of Woods Pond sediment.

Woods Pond is a shallow impoundment on the upper Housatonic River. The sediment is methanogenic and on a dry weight basis is primarily composed of about 36–46% very fine to fine sand (particle size from 50 to 300 μm) and about 50% silt (particle size 1–50 μm). The sediment moisture content is 70–85%. The sediment is extremely high in combustible organic matter (30% of the total mass), much of which is still in the process of decomposing. Thus, the sediment may actually contain as much as 45–50% organic material. The sediment is contaminated to a depth of $\sim 30\text{--}100$ cm with partially dechlorinated Aroclor 1260 (15–180 $\mu\text{g/g}$ of sediment [dry weight]) and a weathered aliphatic hydrocarbon oil (5 000–32 000 $\mu\text{g/g}$ [dry weight]) (17). Temperatures in the top 45 cm of the sediment are at 8–12 °C for 6–8 weeks in the spring and fall and at 15–22 °C in the summer. Winter temperatures drop to 1–4 °C at these depths. PCB dechlorination mediated by four different microbial dechlorination processes has been documented in microcosms of Woods Pond sediment (6, 8, 15, 18). [A microbial dechlorination process is a set or series of dechlorination reactions that determines the substrate range, the specific chlorines targeted, and the sequence of microbially mediated PCB dechlorination (7).] Although no one has yet succeeded in obtaining a pure culture or even a defined consortium that dechlorinates PCBs (ref 16 and literature cited therein), several lines of evidence indicate that discrete microbial populations are responsible for the different PCB dechlorination processes observed in Woods Pond (summarized in ref 5).

Most (~ 71 mol %) of the PCBs in the Aroclor 1260 residue in Woods Pond sediment have six or more chlorines. Therefore, the mole percent fraction of hexa- through nonachlorobiphenyls provides an effective way to measure the extent of dechlorination of the Aroclor 1260 residue. Any viable method to accelerate PCB dechlorination in situ must result in extensive dechlorination of this fraction of PCBs at field temperatures. We reported earlier that 2346-CB primes microbial dechlorination of the residual Aroclor 1260 in Woods Pond sediment by various combinations of three different microbial dechlorination processes in the temperature range of 8–34 °C (8). Temperature determined the relative dominance of these dechlorination processes and the extent and products of the dechlorination.

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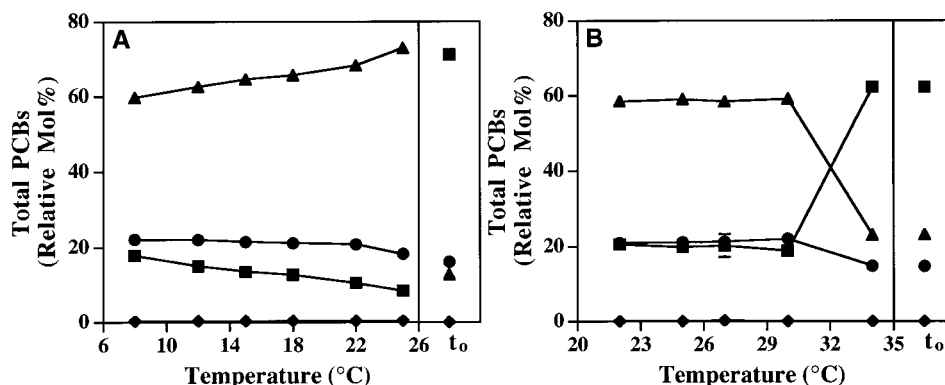


FIGURE 1. PCB homologue distribution in the Aroclor 1260 residue in samples incubated at 8–30 °C for 8–10 months after priming with 26-BB. (A) Data are from the first experiment after 8 months of incubation. (B) Data are from the second experiment after 10 months of incubation. The initial PCB homologue distribution differed for the sediments used in the two experiments (see Experimental Section). Symbols: (◆) dichlorobiphenyls, (▲) tri- and tetrachlorobiphenyls, (●) pentachlorobiphenyls, (■) hexa- through nonachlorobiphenyls. The data represent the means of triplicate samples. The bars indicate the standard deviations, otherwise the deviations were obscured by the symbols used.

The experiments with 2346-CB demonstrated that significant PCB dechlorination can be primed at field temperatures, but they also raised several concerns that must be addressed before accelerated PCB dechlorination *in situ* can become a viable option. (i) The dechlorination primed by 2346-CB was slow and ceased prematurely, resulting in only a 28% decrease of the hexa- through nonachlorobiphenyls at 8 °C and a 60–65% decrease in this fraction of PCBs at higher temperatures after 1 year of incubation. (ii) Competition of several dechlorination processes, especially at 15–22 °C, apparently decreased the efficiency of the dechlorination as manifested by suboptimal removal of the hexa- through nonachlorobiphenyls and accumulation of dead-end *ortho*-, *para*-substituted tetra- and pentachlorobiphenyls. (iii) Competition of the three dechlorination processes at 15–22 °C resulted in considerable variability in product distribution among replicates, indicating that the sequence of dechlorination was not under control. In the worst case, 2346-CB primed a very weak *para*-dechlorination of Aroclor 1260 at 18 °C, resulting in minimal dechlorination (11% decrease of the hexa- through nonachlorobiphenyls) as compared with extensive dechlorination (~60% decrease of the same fraction of PCBs) at 15 and 20 °C in the same experiment when Process N dechlorination dominated (8). We proposed earlier that 18 °C may be a transition point at which members of the community supporting *para*-dechlorination become more dominant (19). Unfortunately, 18 °C is also a prevailing summer temperature in the sediment.

We hypothesized that all three concerns could be addressed by using a primer that would allow us to control the sequence of dechlorination at 8–22 °C. Priming *meta*-dechlorination (Process N) first would have the greatest initial impact because this dechlorination process has a broader PCB congener substrate range than any of the other dechlorination processes observed in Woods Pond (6, 8, 15, 18) and because there are more *meta* than *para* chlorines in the target substrate. (The partially dechlorinated Aroclor 1260 residue contains about 2.22 *meta* chlorines per biphenyl but only 1.29 *para* chlorines per biphenyl.) Process N dechlorination of Aroclor 1260 is characterized by an almost exclusive loss of flanked *meta* chlorines to generate *ortho*-, *para*-substituted tri- through pentachlorobiphenyls (7, 18, 20a). We have already demonstrated that Woods Pond sediment also contains a population of microorganisms that can subsequently dechlorinate the products of Process N dechlorination converting them to *ortho*-substituted di- and trichlorobiphenyls (6, 8, 20b), which can be degraded aerobically. Hence, controlling the initial sequence of dechlorination would permit subsequent development of a two-stage anaerobic

dechlorination strategy that would maximize total chlorine removal. 26-BB primed a rapid and exclusive Process N dechlorination of Aroclor 1260 of Woods Pond sediment incubated at room temperature (22–25 °C) (5). The priming activity of 26-BB was not previously tested at other temperatures, but we hypothesized that it would exclusively prime Process N dechlorination over the entire temperature range of 8–30 °C because our previous experiments showed that Dechlorination Process N is active in this temperature range (8). To test this hypothesis, we determined the pattern, rate, and extent of microbial dechlorination of Aroclor 1260 primed by 26-BB over the temperature range of 8–34 °C.

In addition to the benefits described above, the finding that 26-BB activates only Process N dechlorination in a temperature range where several dechlorination processes were activated simultaneously by 2346-CB (8) would provide strong support for the hypothesis that the microorganisms that mediate Dechlorination Process N are distinct from the microorganisms that mediate the other dechlorination processes observed in Woods Pond. It would also provide the opportunity to measure the effects of temperature on a single microbial dechlorination process. That was not possible in our earlier study with 2346-CB (8).

In this paper, we demonstrate for the first time that priming with halogenated biphenyl congeners stimulates the growth of PCB-dechlorinating microorganisms. We also show that 26-BB primes extensive and exclusive Process N dechlorination of Aroclor 1260 over the entire temperature range of 8–30 °C, and we report the rates of primed PCB dechlorination at most of these temperatures.

Experimental Section

Preparation of Slurries and Incubation. Methanogenic sediment was collected from the west side of Woods Pond (Lenox, MA) (17) as described previously (21), shipped to Athens, GA, and then stored in glass containers at 4–7 °C until use. The sediment was stored for 5 days prior to the first experiment and for 1 year prior to the second experiment. Different containers of sediment were used in these experiments, and even though the sediment was collected from the same location at the same time, the dry weight (0.2 and 0.17 g dry sediment/mL wet sediment) and the PCB homologue distribution differed for the two containers of sediment due to variations in the extent of dechlorination that had occurred *in situ* (See t_0 values, Figure 1). The Aroclor 1260 in the sediment used for the second experiment was more highly dechlorinated than that used for the first experiment. (The average numbers of chlorines per biphenyl

for the residual PCBs in the sediments used in these experiments were 5.93 and 5.75 for the first and second experiments, respectively). This is typical of the variability seen throughout Woods Pond (17).

Sediment slurries were prepared under a stream of O₂-free nitrogen gas by mixing wet sediment (3 vol) with K₂HPO₄/KH₂PO₄ buffer (2 vol, pH 6.8). The final concentration of potassium phosphate buffer was 10 mM, and the resulting slurries for the two experiments contained, respectively, 0.12 and 0.10 g of sediment (dry weight)/mL. The concentrations of Aroclor 1260 in the sediment used for the two experiments were 97 and 105 μg/g of sediment (dry weight), respectively. The 26-BB was synthesized at GE (99.93% purity by gas chromatography analysis using a flame ionization detector), and the malate was from Aldrich (Milwaukee, WI). Triplicate sediment slurries were amended with 26-BB (final concentration 350 μmol/L of slurry) added from a concentrated stock in acetone and sodium L-malate (pH 6.8; final concentration 10 mM) and then incubated for 8 months in the dark without shaking at 8, 12, 15, 18, 22, or 25 °C. The final concentration of acetone was 0.33%. Malate accelerates the onset of the dehalogenation when added with a primer but has no effect when added without a primer (5, 22, 23). A second experiment included incubations at 22, 25, 27, 30, or 34 °C for 10 months. In both experiments we maintained a pH of 6.8 ± 0.2, the pH range usually observed in Woods Pond sediment, by periodic adjustment with anaerobically prepared 1 N NaOH or 1 N HCl. Sterile controls were autoclaved twice for 1 h at 121 °C on each of two consecutive days to kill presumptive spores, then amended with 26-BB, and incubated at 22 °C

Enumeration (MPN Determinations) of Dehalogenators.

Freshly collected Woods Pond sediment from a third container was used for these experiments. The concentration of Aroclor 1260 in this batch of sediment was 92 μg/g of sediment (dry weight), and the average number of chlorines per biphenyl was 5.81. We prepared a sediment slurry under a stream of O₂-free nitrogen gas by mixing 2 vol of wet sediment with 1 vol of potassium phosphate buffer (pH 6.8). The slurry contained 0.16 g of sediment/mL on a dry weight basis, and the final concentration of potassium phosphate buffer was 10 mM. The slurry was homogenized in a blender and then kept in an anaerobic chamber at room temperature for 2–4 days before use to ensure that it was completely anaerobic. A portion of the slurry was used to prepare MPN medium (see below). The remainder was used for the growth experiment.

Experiment To Determine If 26-BB Stimulates Growth of Dehalogenators. Previous experiments established that no detectable dechlorination of PCBs occurs after 1 year of incubation (at 22 °C) of unamended Woods Pond sediment slurries (8) or slurries amended with acetone (0.33% vol/vol) or acetone plus malate (10 mM) (5) but that extensive PCB dechlorination occurs in sediments amended with 26-BB or 26-BB plus malate (5, 23). We designed our experiment to test the hypothesis that 26-BB primes PCB dechlorination by stimulating the growth of microorganisms that dehalogenate PCBs. To determine the number of dehalogenators present in the sediment before exposure to 26-BB, we prepared serial dilutions of the unamended sediment slurry in MPN assay medium (see details below). We tested two different conditions in the growth experiment: amendment with only 26-BB (350 μM) and amendment with both 26-BB (350 μM) and malate (10 mM). The samples were incubated at 22 °C in the dark without shaking. After 35 days, most of the 26-BB and 56–74% of its intermediate, 2-BB, had been dehalogenated. To further enhance the priming effect of 26-BB, the samples were spiked again, this time with 700 μM 26-BB alone or 700 μM 26-BB plus 10 mM malate, respectively. The samples were incubated for 13 more days until most of the 26-BB and about 50–90% of the 2-BB had been

dehalogenated. At this time, serial dilutions of each sample were prepared in MPN assay medium to determine if the number of dehalogenating microorganisms had changed as a result of priming.

Preparation of Sterile Medium for MPN Assays. A portion of the initial unamended sediment slurry was sterilized to prepare incubation medium for the MPN assays. Aliquots of the slurry were dispensed into tubes, crimp-sealed with Teflon-coated butyl rubber stoppers, and then autoclaved for 1 h at 121 °C. The tubes were incubated for 1 day at 22 °C to permit germination of any spores and then autoclaved again. The MPN assay medium was then amended with 26-BB (350 μM) plus malate (10 mM). Previous experiments have repeatedly shown that no detectable dehalogenation occurs within 1 year in slurries that have been sterilized in this manner (8, 23).

Dilutions into MPN Medium, MPN Assay, and Criteria for Scoring. Dehalogenation activity in our MPN assay indicates the presence of dehalogenating microorganisms. To determine the reproducibility of the data, we prepared two independent series of dilutions in MPN assay medium to estimate the number of dehalogenating microorganisms. The initial dilutions for the two series were 5 × 10⁻¹ and 1 × 10⁻¹, respectively. For each of the two dilution series of the samples before priming with 26-BB, we made seven 10-fold dilutions (five replicates each) into the MPN assay tubes. After priming for 48 days with 26-BB, we made two series of nine 10-fold dilutions (five replicates each) into MPN assay tubes to determine whether the number of debrominating and dechlorinating microorganisms had increased as a result of priming. The MPN assay tubes were incubated at 22 °C, pH 6.8, for 8 months to allow ample time for low numbers of dehalogenators to multiply and dehalogenate the 26-BB and the PCBs in the MPN medium. The individual MPN dilution tubes were scored as positive for the presence of 26-BB-debrominating microorganisms if at least 60% (210 μmol/L of slurry) of the supplemented 26-BB was dehalogenated, and for PCB dechlorinator(s) when the number of meta chlorines per biphenyl for the Aroclor 1260 residue decreased by at least 15% (i.e., a drop from an initial value of 2.15 ± 0.02 to a final value of 1.83). These parameters were chosen to ensure unequivocal scoring for the two processes that exhibit different microbial-mediated dehalogenation rates. The MPNs were calculated using MPN tables developed for examination of water and wastewater samples (24).

Sample Extraction and Analysis. In this paper, individual PCB or bromobiphenyl congeners are described by listing the substituted positions on each ring, separated by a hyphen, and followed by the designation -CB or -BB. Bromobiphenyls, PCBs, and biphenyl were extracted by anhydrous diethyl ether and analyzed using a gas chromatograph (GC) equipped with a DB-1 poly(dimethylsiloxane) fused silica capillary column (J&W Scientific) and a ⁶³Ni-electron capture detector (ECD) as previously described (21). 26-BB and 2-BB were identified by matching their GC retention times with those of authentic standards (99% purity, AccuStandard). Each congener was quantified using a third-order calibration curve (CA-Cricket Graph III 1.01; 1992) generated from a calibration mixture containing 26-BB and 2-BB at 10 calibration levels ranging from 0.2 to 80 μM. We confirmed at the end of the experiment that biphenyl was the final debromination product by GC/mass spectrometric analysis. PCB congeners in the Aroclor 1260 residue were identified using congener assignments reported by Frame et al. (25) and then quantified by congener-specific analysis using a PCB standard that was customized for quantification of both unaltered and dechlorinated Aroclor 1260 as previously discussed in detail (5, 26). We used a six-point calibration curve (total PCB concentration = 1.7–55 μM, which is equivalent to 0.57–18.6 μg/mL) for quantifica-

tion of the dechlorinated Aroclor 1260. All PCB congeners in the samples were within the calibration range.

Results

Effects of Temperature on the Dehalogenation of Aroclor 1260 Primed by 26-BB. No dehalogenation of 26-BB or PCBs was detected in autoclaved controls incubated at 22 °C or in live samples incubated at 34 °C for 8–10 months. Furthermore, incubation of sediment samples without the addition of primers such as 26-BB did not lead to any dehalogenation of the Aroclor residues within 1 year. At 8–30 °C, dehalogenation of 26-BB commenced within 6–54 days in live samples. As previously reported (5, 22), 26-BB was stoichiometrically dehalogenated to biphenyl. After 3 months, 95–99% of the 26-BB and its product, 2-BB, had been dehalogenated to biphenyl in samples incubated at 12 °C or higher. At 8 °C, complete dehalogenation took ~8 months. 26-BB primed extensive dechlorination of the Aroclor 1260 residue in the sediment at all tested temperatures from 8 to 30 °C, resulting in large decreases in hexa- through nonachlorobiphenyls and corresponding increases in tri- and tetrachlorobiphenyls, even at 8 and 12 °C (Figure 1). Concurrently, the total chlorines per biphenyl for the Aroclor 1260 residue decreased by 18–26% (Supporting Information, Figure 1). This decrease resulted almost exclusively from the loss of *meta* chlorines, which decreased by 51–67%. No significant *ortho*- or *para*-dechlorination of the Aroclor 1260 residue was observed.

The observed maximal rate of dechlorination of the Aroclor 1260 residue in the sediment increased almost linearly from 8 °C (252 pmol of Cl mL⁻¹ day⁻¹) to 25 °C (1144 pmol of Cl mL⁻¹ day⁻¹) and paralleled the rate of dehalogenation of 26-BB (Figure 2A). The rate of debromination was substantially higher than the rate of dechlorination, but several factors must be considered in making this comparison. First, the rate of dehalogenation is known to be influenced by the concentration of available substrate (see ref 7 for a review). In this case, we measured the dehalogenation of 26-BB to 2-BB, so the concentration of bromine available for dehalogenation was 350 nmol/mL of slurry. Only *meta* chlorines were substrates for the PCB dechlorination that occurred, and the concentration of *meta* chlorines was 72 nmol/mL of slurry. When normalized to the available halogen substrate, the rate of debromination was, on average, ~5.4 times higher than that of the PCB dechlorination. For example, at 15 °C, the sediment microorganisms removed 4% of the available bromines and 0.8% of the *meta* chlorines per day, and at 22 °C the microorganisms removed 8.4% of the available bromines and 1.5% of the *meta* chlorines per day. These are not large differences, especially considering that (i) the 26-BB was freshly added (and may therefore have been more bioavailable) while the PCBs have been associated with the sediment for decades; (ii) 26-BB is more soluble than most of the congeners in Aroclor 1260 (based on the octanol–water partition coefficients, K_{ow} (27, 28); the log K_{ow} calculated for 26-BB is 5.46; (iii) aryl bromines are more easily removed than aryl chlorines (see ref 22 for a discussion); and (iv) all of the bromine substituents were dehalogenation substrates, but only a subset of *meta* chlorines (namely, those that are flanked by another chlorine) were substrates for dehalogenation (see text below).

Figure 2B compares the effect of temperature on the incubation time required for 50% losses of 26-BB and of hexa-through nonachlorobiphenyls (t_{50}). This measure incorporates the effects of temperature on acclimation time and rate of dehalogenation. The most rapid acclimation and dehalogenation occurred at 22–30 °C. The t_{50} for both dehalogenations gradually increased as the incubation temperature was lowered to 12 °C but increased sharply when the temperature was lowered to 8 °C. The extent of dechlorination

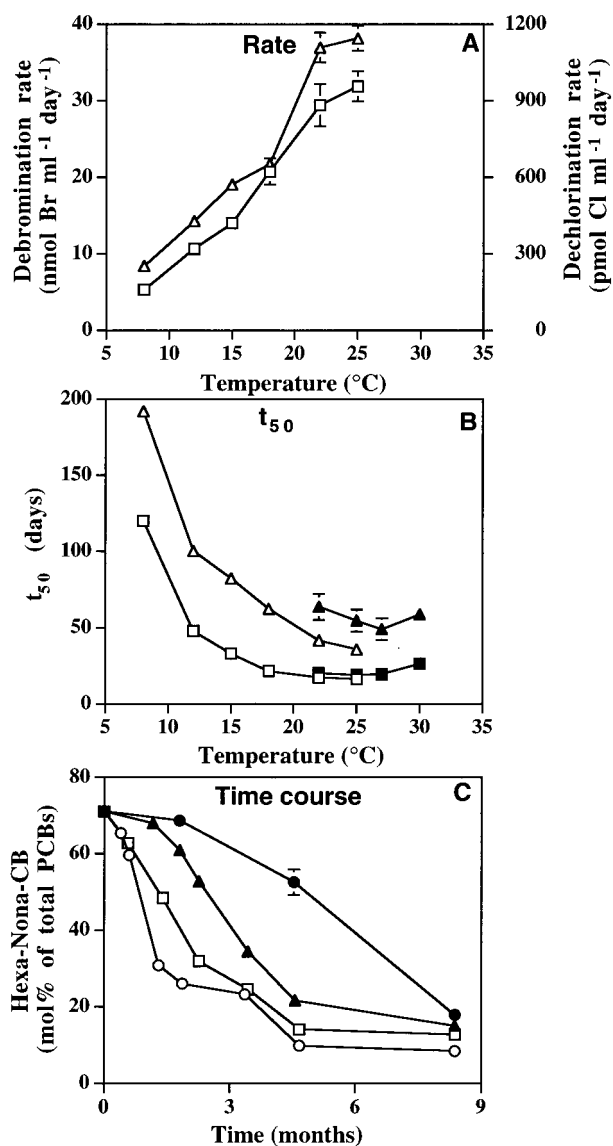


FIGURE 2. Effect of temperature on the maximal rates of dehalogenation, the time required for 50% dehalogenation, and the time course of PCB dechlorination. All data represent the means of triplicate samples. The bars indicate standard deviations, otherwise the deviations were obscured by the symbols used. (A) The maximal observed rates of dehalogenation of 26-BB to 2-BB (◻) and of PCBs (▲). Only data from the first experiment were used because there were not enough time points taken to estimate rates of dehalogenation for the second experiment. The rate of chlorine removal was calculated from the PCB concentration in the slurry and the changes in the total number of chlorines per biphenyl as a function of time. (B) Time required for 50% losses (t_{50}) of 26-BB (squares) and hexa- through nonachlorobiphenyls (triangles). Open symbols indicate data from the first experiment, and closed symbols indicate data from the second experiment. (C) Time course of dechlorination of hexa- through nonachlorobiphenyls in Woods Pond sediment samples primed with 26-BB and incubated at various temperatures. Symbols: (●) 8 °C, (▲) 12 °C, (◻) 18 °C, (○) 25 °C.

increased throughout the 8 month incubation. Within 5 months, extensive dechlorination had occurred at temperatures as low as 12 °C (Figure 2C). By 8 months, the PCBs in samples incubated at 8 °C were dechlorinated almost as extensively as at 25 °C. The total decrease in hexa- through nonachlorobiphenyls ranged from a 75% decrease at 8 °C to an 88% decrease at 25 °C (Figures 1 and 2C).

Our experiments demonstrate that 26-BB primed only Process N dechlorination at all temperatures from 8 to 30 °C.

TABLE 1. Mole Percent of Selected PCB Congeners in the Aroclor 1260 Residue in 26-BB from Sediment Microcosms Incubated at 8–34 °C for 8–10 Months after Priming with 26-BB^a

Peak #	PCB Congener ^b	IUPAC #	$t_0^{c,d}$		t_i at temperature (°C) ^d									
					8	12	15	18	22	25		27	30	34
			1st	2nd						1st ^c	2nd ^c			
17	26-4, 23-2	32, 16	0.29 ± 0.02	0.52 ± 0.06	3.17 ± 0.04	3.62 ± 0.07	3.73 ± 0.12	3.78 ± 0.03	3.89 ± 0.07	4.05 ± 0.11	2.39 ± 0.07	2.37 ± 0.16	2.34 ± 0.43	0.50 ± 0.08
24	24-4	28	1.17 ± 0.02	1.31 ± 0.05	4.48 ± 0.03	2.11 ± 0.16	3.45 ± 0.18	2.08 ± 0.32	2.79 ± 0.12	3.42 ± 0.30	3.07 ± 0.05	3.13 ± 0.11	2.99 ± 0.19	1.34 ± 0.06
25	25-26	53	1.38 ± 0.01	1.86 ± 0.03	4.67 ± 0.02	5.26 ± 0.10	5.37 ± 0.18	5.64 ± 0.13	5.76 ± 0.17	5.85 ± 0.11	4.94 ± 0.11	4.88 ± 0.19	5.03 ± 0.23	1.52 ± 0.02
26	24-26	51	1.15 ± 0.03	2.28 ± 0.05	9.10 ± 0.06	10.47 ± 0.21	10.79 ± 0.37	11.20 ± 0.32	11.53 ± 0.34	11.93 ± 0.23	12.45 ± 0.60	12.33 ± 0.81	13.51 ± 0.94	2.16 ± 0.10
31	25-25	52	1.38 ± 0.02	1.90 ± 0.03	2.40 ± 0.01	2.76 ± 0.07	2.85 ± 0.13	3.11 ± 0.09	3.17 ± 0.10	3.16 ± 0.11	1.69 ± 0.00	1.67 ± 0.04	1.76 ± 0.21	1.46 ± 0.02
32	24-25	49	2.01 ± 0.04	3.57 ± 0.03	7.94 ± 0.03	8.81 ± 0.18	9.01 ± 0.36	9.74 ± 0.27	9.95 ± 0.29	10.01 ± 0.32	7.19 ± 0.05	7.00 ± 0.14	7.23 ± 0.46	3.54 ± 0.04
33	24-24	47	4.77 ± 0.03	5.75 ± 0.02	20.51 ± 0.09	22.12 ± 0.16	22.19 ± 0.59	22.92 ± 0.37	23.18 ± 0.46	23.75 ± 0.23	21.91 ± 0.46	21.32 ± 0.83	20.74 ± 0.76	5.27 ± 0.04
44	246-24	100	0.42 ± 0.01	0.48 ± 0.00	5.59 ± 0.03	6.31 ± 0.08	6.45 ± 0.25	6.70 ± 0.20	7.18 ± 0.20	3.76 ± 0.13	3.98 ± 0.20	3.92 ± 0.34	3.86 ± 0.41	0.56 ± 0.01
49	236-24	91	1.52 ± 0.02	1.81 ± 0.03	3.06 ± 0.02	2.86 ± 0.04	2.67 ± 0.06	2.78 ± 0.06	2.68 ± 0.03	2.64 ± 0.04	3.62 ± 0.16	3.58 ± 0.12	2.78 ± 0.32	2.00 ± 0.05
53	235-24, 245-25	90, 101	2.92 ± 0.05	2.71 ± 0.03	4.79 ± 0.02	4.60 ± 0.08	4.29 ± 0.04	4.01 ± 0.14	3.77 ± 0.11	4.01 ± 0.23	5.01 ± 0.10	4.92 ± 0.15	6.59 ± 0.32	2.99 ± 0.05
67	2356-24, 234-35 235-34	147, 107 109	0.92 ± 0.03	0.96 ± 0.03	3.84 ± 0.03	3.73 ± 0.05	3.47 ± 0.07	3.50 ± 0.05	3.37 ± 0.12	3.15 ± 0.05	3.45 ± 0.11	3.27 ± 0.09	3.13 ± 0.19	1.11 ± 0.02
69	245-34, 236-245	118, 149	6.98 ± 0.07	5.95 ± 0.08	0.77 ± 0.02	0.62 ± 0.03	0.57 ± 0.15	0.48 ± 0.06	0.41 ± 0.18	0.21 ± 0.03	0.69 ± 0.10	0.83 ± 0.17	0.74 ± 0.16	6.12 ± 0.09
75	245-245	153	9.37 ± 0.10	7.33 ± 0.07	0.68 ± 0.02	0.56 ± 0.03	0.49 ± 0.05	0.44 ± 0.05	0.31 ± 0.08	0.19 ± 0.01	0.79 ± 0.09	1.02 ± 0.33	0.75 ± 0.12	7.25 ± 0.09
82	234-245, 2356-34 236-345	138, 163 164	8.15 ± 0.08	7.50 ± 0.07	1.35 ± 0.03	1.05 ± 0.03	0.90 ± 0.03	0.84 ± 0.08	0.69 ± 0.08	0.60 ± 0.04	1.88 ± 0.12	2.08 ± 0.22	1.82 ± 0.21	7.56 ± 0.06
88	2356-245	187	4.93 ± 0.03	3.17 ± 0.02	1.03 ± 0.03	0.81 ± 0.02	0.71 ± 0.03	0.71 ± 0.07	0.51 ± 0.04	0.36 ± 0.00	1.05 ± 0.09	1.09 ± 0.19	1.25 ± 0.29	3.31 ± 0.02
90	2346-245	183	3.70 ± 0.02	1.78 ± 0.02	0.42 ± 0.01	0.32 ± 0.00	0.28 ± 0.01	0.27 ± 0.03	0.18 ± 0.02	0.12 ± 0.00	0.39 ± 0.01	0.43 ± 0.09	0.25 ± 0.04	1.75 ± 0.01
93	2345-236	174	3.31 ± 0.03	2.40 ± 0.03	0.52 ± 0.01	0.35 ± 0.00	0.28 ± 0.03	0.25 ± 0.03	0.15 ± 0.03	0.09 ± 0.00	0.32 ± 0.03	0.39 ± 0.09	0.29 ± 0.04	2.46 ± 0.02
102	2345-245	180	6.19 ± 0.03	7.56 ± 0.04	1.39 ± 0.02	1.02 ± 0.02	0.80 ± 0.03	0.77 ± 0.09	0.43 ± 0.03	0.27 ± 0.00	1.83 ± 0.21	1.97 ± 0.51	1.06 ± 0.12	7.52 ± 0.04
106	2345-234	170	3.12 ± 0.02	2.81 ± 0.02	0.63 ± 0.01	0.46 ± 0.01	0.40 ± 0.06	0.35 ± 0.03	0.23 ± 0.05	0.10 ± 0.00	0.58 ± 0.06	0.64 ± 0.19	0.34 ± 0.02	2.82 ± 0.02
110	23456-245 2345-2346	203 196	1.69 ± 0.02	1.64 ± 0.01	0.93 ± 0.01	0.83 ± 0.01	0.81 ± 0.06	0.71 ± 0.04	0.60 ± 0.07	0.36 ± 0.01	0.80 ± 0.03	0.79 ± 0.04	0.58 ± 0.03	1.63 ± 0.02

^a Data were means from triplicate samples ± standard deviation. The total PCB concentration (100 mol %) = 30–32 μM. ^b Coeluting congeners in peaks that decreased (e.g., 82) are listed in order according to their relative contribution to the peak at the beginning of the incubation. Coeluting congeners in peaks that increased (e.g., peaks 17, 53, and 64) are listed in order to their presumed relative contribution to the peak at the end of the incubation. ^c t_0 is the value at the start of incubation. ^d 1st and 2nd refer to the first and second experiments. t_i for the first experiment, which included 8, 12, 15, 18, 22, and 25 °C (1st), was 8 months. t_i for the second incubation, which included 25 °C (2nd), 27, 30, and 34 °C, was 10 months.

TABLE 2. Most Probable Number (MPN) of 26-BB- and PCB-Dehalogenating Microorganisms in Woods Pond Sediment before and after Stimulation by the Addition of 26-BB^a

samples	MPN ($\times 10^5$ per g dry wt sediment)	
	dilution 1 ^b	dilution 2 ^c
before addition of 26-BB	3 (0.9–9.4) ^d	4.9 (1.6–11.9)
after addition of 26-BB (1050 μ M)	675 (225–1750)	2188 (750–6250)
after addition of 26-BB and malate (10 mM)	2000 (800–7250)	5750 (1875–20000)

^a All MPN assay tubes were incubated for 8 months at 22 °C before they were analyzed for dehalogenation. ^b Dilution started with 5×10^{-1} . ^c Dilution started with 10^{-1} . ^d Values in parentheses indicate 95% confidence limits.

This is evident from large increases in the unique products characteristic of this dechlorination process (24-24-CB, 24-26-CB, 24-25-CB, and 246-24-CB) and corresponding decreases in congeners with 34-, 234-, 236-, 245-, 2345-, 2346-, and 23456-chlorophenyl groups (Table 1). (For a detailed discussion of Process N dehalogenation, see refs 7 and 18.) In contrast to our results with 2346-CB as primer (8, 19, 21), there was very little variation between replicates primed with 26-BB as shown by the small standard deviations. It is clear from the congener distribution profile that virtually all of the highly chlorinated congeners were substantially *meta*-dechlorinated, even at 8 and 12 °C (Supporting Information, Figure 2).

Enumeration of Dehalogenating Microorganisms before and after Priming with 26-BB. We used MPN methods to estimate the number of microorganisms in the sediment that could dehalogenate 26-BB and Aroclor 1260. We estimated the size of both populations in unamended sediment at about $3\text{--}4.9 \times 10^5$ cells/g dry weight sediment ($0.7\text{--}1.2 \times 10^5$ cells/mL of wet sediment) (Table 2). The total absence of dehalogenation in the highest MPN dilutions of the unamended sediment confirmed that the dehalogenation seen at the lower dilutions was solely due to the presence of dehalogenating microorganisms in the inocula. MPN dilutions prepared after priming with 26-BB revealed that the size of both populations had increased significantly to about $6.8 \times 10^7\text{--}2.2 \times 10^8$ cells/g of sediment [dry weight] ($1.6\text{--}5.3 \times 10^7$ /mL of wet sediment). The number of dehalogenators was slightly higher ($2\text{--}5.8 \times 10^8$ /g of sediment [dry weight] = $0.5\text{--}1.4 \times 10^8$ cells/mL of wet sediment) in samples that had also been supplemented with 10 mM malate (Table 2), but the difference was not statistically significant even though malate has previously been shown to accelerate the onset of PCB dehalogenation when added with a primer (5, 22, 23). The estimated number of dehalogenating microorganisms varied between the two MPN dilution series (Table 2). However, the numbers are within 95% confidence limits. We assume that the difference was caused by nonuniform distribution of microorganisms in the sediment. Dechlorination of Aroclor 1260 was observed in all MPN dilution tubes in which dehalogenation of 26-BB occurred, and no dechlorination of Aroclor 1260 was observed in any MPN dilution tubes in which dehalogenation of 26-BB did not occur. Hence, the estimated numbers for 26-BB dehalogenators were the same as those for PCB dehalogenators. Regardless of the dilution, all actively dehalogenating samples exhibited the same pattern of dechlorination, namely, Process N (Figure 3). Furthermore, for the 26-BB primed samples, the extent of dechlorination in the MPN assay tubes after 8 months was the same at the highest positive dilution (10^{-9}) as at the lower dilutions (Figure 3). These findings demonstrate that the lower dilutions did not contain actively

dehalogenating microorganisms with substrate specificities different than the higher dilutions.

Discussion

26-BB Exclusively Primes Dechlorination Process N. We reported earlier that 2346-CB primed a combination of three different dechlorination processes with partially overlapping temperature ranges in Woods Pond sediment (8). Process N dechlorination (flanked *meta*-dechlorination) was primed at 8–30 °C, Process P dechlorination (flanked *para*-dechlorination) was primed at 12–34 °C, and Process LP dechlorination (unflanked *para*-dechlorination) was primed at 18–30 °C (8). The results of this study unequivocally establish that 26-BB stimulates only Process N dechlorination in Woods Pond sediment and that the temperature range for this dechlorination process is 8–30 °C. No evidence of Dechlorination Process P or LP was observed in samples primed with 26-BB at any incubation temperature. The exclusive priming of *meta*-dechlorination of PCBs by the *ortho*-substituted 26-BB is not understood, but our results are consistent with previously reported data (5). The absence of any dehalogenation of either 26-BB or PCBs at 34 °C is consistent with our previous conclusion that only Process P occurs at that temperature (8) and further substantiates our hypothesis that 26-BB specifically activates the microorganisms that mediate Process N dechlorination. These results indicate that the PCB dehalogenators primed by 26-BB exhibit high specificity and further support the hypothesis that different microorganisms containing distinct dehalogenating enzymes with different congener specificities are responsible for the various dechlorination processes that have been identified (7). Collectively, our results indicate that the addition of 26-BB affects the growth and dehalogenation activity of a single population of PCB-dechlorinating microorganisms, namely, the population that mediates Process N dechlorination.

Effect of Temperature on the Rate, Time Course, and Extent of Dechlorination of Aroclor 1260 Mediated by Dechlorination Process N. Because 26-BB stimulates only Dechlorination Process N, we were able to determine the effects of temperature on this single dechlorination activity (Figures 1 and 2). Even though the rate of dechlorination decreased significantly with temperature, 26-BB primed extensive dechlorination of the Aroclor 1260 residue within 5 months at 12–25 °C and within 8 months at 8 °C (Figure 2C). As we had hoped, the dechlorination primed by 26-BB had a greater impact on the most highly chlorinated PCBs than the dechlorination primed by 2346-CB, especially at the lowest temperature. The total decrease in hexa- through nonachlorobiphenyls primed by 26-BB at 8 months at 8 °C was 75% whereas the decrease in this fraction of PCBs primed by 2346-CB in 1 year was only 28% (8). These findings have important implications for the development of bioremediation strategies because they demonstrate that extensive dechlorination of Aroclor 1260 can be achieved in reasonable periods of time even in sediments at the low temperatures that prevail in northern climates.

26-BB Stimulates 1000-Fold Growth of PCB-Dehalogenating Microorganisms. Our MPN data show that the addition of 26-BB resulted in approximately 1000-fold growth of the PCB-dehalogenating microorganisms in Woods Pond sediment. This is the first direct demonstration of an increase in the population of PCB-dechlorinating microorganisms as a result of priming. It is in agreement with the recent observation that a high concentration of Aroclor 1248 stimulated a 180-fold increase in a PCB-dechlorinating population (29) and further supports the hypothesis that reductive dehalogenating microorganisms may use PCBs as electron acceptors under anaerobic conditions (2, 14). Recently, Cutter et al. (30) reported that a PCB-dechlorinating

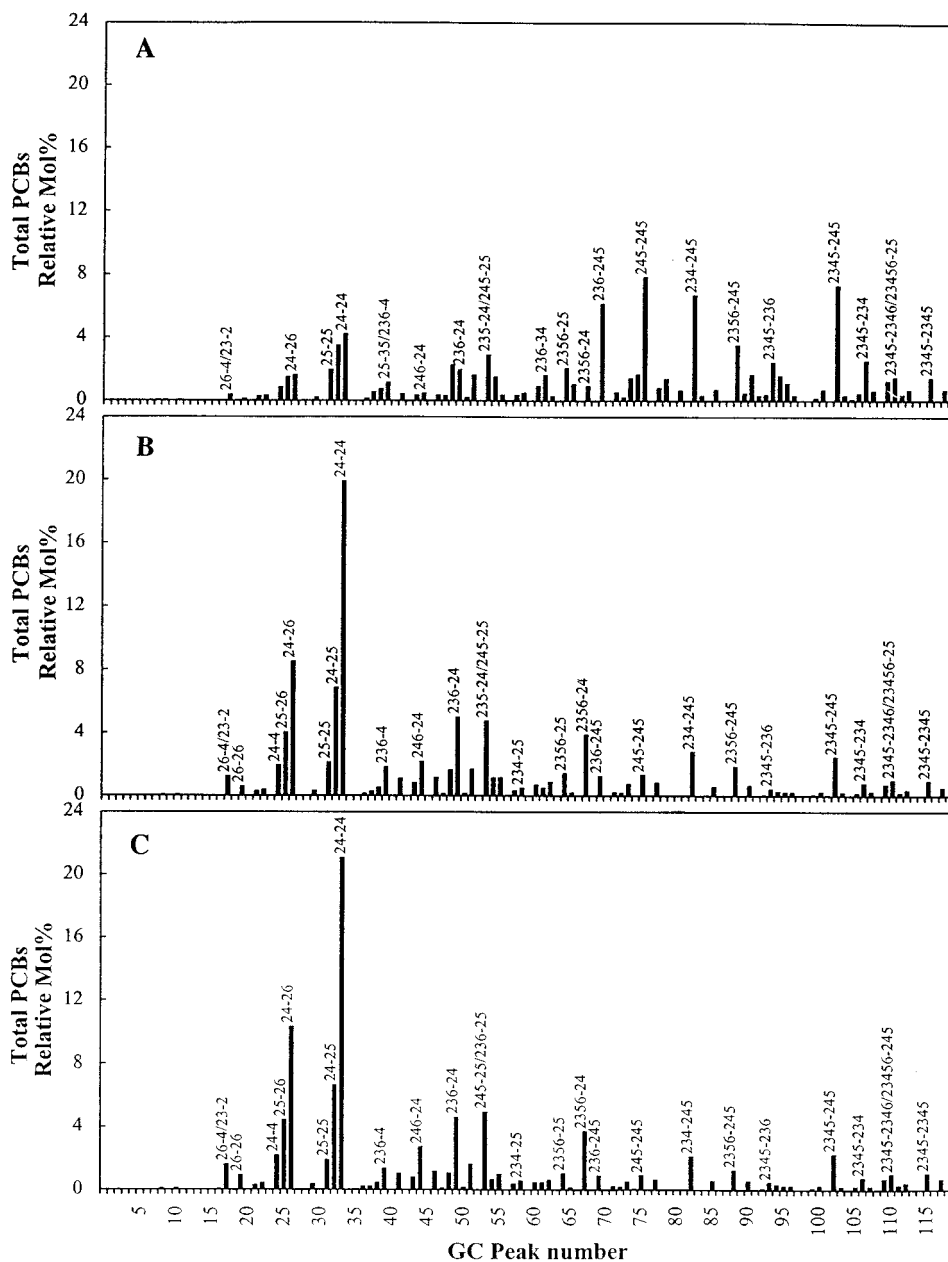


FIGURE 3. PCB congener distributions in the MPN assay medium before inoculation (A). The PCB congener distribution from averaged data of positive MPN assay tubes incubated for 8 months after inoculation with a sediment slurry primed with 26-BB plus malate, (B) the 10^{-1} dilution, and (C) the 10^{-9} dilution. The virtually identical extent and pattern of PCB dechlorination in panels B and C show that no dehalogenation specificity was lost at the highest positive dilutions. PCB congener designations denote the positions of the chlorine atoms on each phenyl ring and the hyphen represents separation of the rings. See refs 18 and 25 for a complete list of congener assignments.

enrichment culture derived from estuarine sediment could be maintained without loss of dechlorination activity in sediment-free medium by using a mixture of fatty acids as a source of carbon and electrons. Thus, it may eventually be possible to identify appropriate electron donors for the PCB dechlorination primed by 26-BB. This would allow us to determine whether reductive PCB dechlorination is coupled to the oxidation of specific electron donors in an energy-yielding process such as electron transport phosphorylation.

Our estimation of the number of PCB dechlorinators may be low. MPN is a relatively conservative method, and several factors could result in underestimation of the number of PCB dehalogenators including the fact that our MPN estimates are based on Aroclor dechlorination assays. If the presence of more than one population is necessary for dehalogenation of 26-BB and PCBs (e.g., if nondehalogenating microorganisms are required to provide cross-feeding), the

MPN method may give an underestimate of 26-BB and PCB dehalogenators because a critical member of the consortium may be present in concentrations lower than the dehalogenators.

Proposed Relationship of the Microorganisms Responsible for Dehalogenating 26-BB and Aroclor 1260. Our data indicate (i) that the number of 26-BB dehalogenators in the sediment was the same as the number of primed PCB dechlorinators carrying out Process N dechlorination and (ii) that the temperature effects on the rate and t_{50} of dehalogenation of 26-BB and Process N dechlorination of PCBs were closely correlated. Although we cannot rule out the possibility mentioned above of the presence of a critical nondechlorinating member in the microbial population, our findings suggest that the same PCB-dechlorinating microorganisms dehalogenated both 26-BB and Aroclor 1260. This interpretation is further supported by the finding that a stable

and highly specific 246-CB-dehalogenating enrichment culture derived from Woods Pond sediment could be subcultured more than 5 times (using 5% transfer inocula) with 246-BB without loss of its ability to dechlorinate 246-CB or reduction in the rate of dechlorination (23). This suggests that the same microorganisms dehalogenated both 246-CB and 246-BB. Pure cultures of PCB-dechlorinating microorganisms will be required to further substantiate the hypothesis that PCB-dechlorinating microorganisms can also dehalogenate brominated biphenyls.

Summary. We have demonstrated for the first time that priming with 26-BB stimulates the growth of PCB-dechlorinating microorganisms in PCB-contaminated sediment leading to extensive dechlorination of PCBs, even at temperatures as low as 8 °C. This means that the successful identification of environmentally acceptable primers could potentially lead to an effective method to accelerate PCB dechlorination in situ.

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Supporting Information Available

Two figures showing (1) the effect of temperature on the number and distribution of chlorines per biphenyl and (2) the PCB congener distribution pattern at 8 °C, 8 months after priming with 26-BB (2 pages). Ordering information is given on any current masthead page.

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