

Characterization of the PCB Substrate Range of Microbial Dechlorination Process LP

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We have characterized the substrate range of Process LP, a PCB dechlorination activity mediated by anaerobic bacteria, in Housatonic River sediment (Lenox, MA). Process LP has the rare ability to remove unflanked *para* chlorines from polychlorinated biphenyls (PCBs). We used 2,6-difluoro-4-chlorobiphenyl (DFCB) to activate Process LP in anoxic sediment microcosms and tested its ability to dechlorinate 34 potential PCB substrates, all of which are significant components of PCB mixtures found in contaminated sediments. We used gas chromatography–mass spectrometry to monitor the dechlorination of DFCB and PCBs and the appearance of products. The preferred substrates for Process LP were PCB congeners in which the target *para* chlorines were flanked by *meta* chlorines, such as those having 3,4- and 2,4,5-chlorophenyl rings. The unflanked *para* chlorines on PCBs with 2,4-, 2,4,6-, and sometimes 4-chlorophenyl rings, were also substrates. Furthermore, the data revealed that Process LP can also *meta*-dechlorinate 2,3-, 2,3,4-, and 2,3,5-chlorophenyl groups on some congeners. A single *ortho* chlorine on the unattacked ring generally enhanced dechlorination activity, but the presence of 3 or 4 *ortho* chlorines or a 4-chlorophenyl group decreased the dechlorination efficiency. PCBs with 2,4-, 2,4,6-, 2,3-, and 2,3,5-chlorophenyl rings are often terminal dechlorination products of other microbial dechlorination activities. Since these PCBs are substrates for Process LP, this dechlorination activity works especially well in tandem with other dechlorination activities and further reduces the toxicity and persistence of PCBs. The data presented here will facilitate the construction of accurate models to interpret in situ PCB dechlorination and predict PCB fate.

Introduction

Commercial mixtures of polychlorinated biphenyls (PCBs) were used worldwide for multiple applications from about 1932 to 1978. Several million kilograms of PCBs are estimated to persist in the environment, primarily in aquatic sediments including many rivers, lakes, and estuaries (1, 2). PCBs bioaccumulate and biomagnify in the food chain and are potentially toxic to both humans and wildlife.

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Commercial PCBs, sold in the United States under the trade name Aroclor, were manufactured by chlorinating biphenyl to specified weight percents of chlorine. There are 209 PCB congeners that differ in the number and position of chlorines. (Figure 1 shows the structure and nomenclature for PCBs.) Aroclors are complex mixtures, typically containing 60–90 different PCB congeners (3).

The Housatonic River runs past a site in Pittsfield, Massachusetts that produced PCB-containing electrical transformers from 1932 to 1978 (4) and the river sediment is contaminated with Aroclors 1260 and 1254 (5). Much of the contaminated sediment has accumulated in Woods Pond, a shallow impoundment of the river in Lenox, Massachusetts. The 13-mile stretch of the river from Pittsfield through Woods Pond is a Superfund site on the national priority list (4). Exposure to the accumulated PCBs at this site has promoted the development of microbial communities that are capable of dechlorinating many of the PCBs in the sediment (5–8). However, despite the presence of these organisms, the extent to which PCB dechlorination in situ has occurred is highly variable (5, 9). Furthermore, it appears that dechlorination is no longer occurring (10, 11).

We previously identified three distinct microbial PCB dechlorination activities in Woods Pond sediment: Processes N, P, and LP. Processes N and P remove flanked *meta* and *para* chlorines, respectively, from many PCB congeners in Aroclor 1260 and have been described in detail (6, 8, 12, 13). These activities can be stimulated in sediment by the addition of specific halogenated aromatic compounds (10, 14, 15).

Dechlorination Process LP has the rare ability to remove unflanked *para* chlorines from 2,4- and 2,4,6-chlorophenyl rings. This activity has been only briefly described and the PCB substrate range was not fully characterized (7, 16). Process LP is potentially very important because it can further dechlorinate the *ortho*-, *para*-substituted terminal dechlorination products of Process N dechlorination such as 24-24-CB (Figure 1B). This means that heptachlorobiphenyls such as 2345-245-CB and 2345-234-CB, both major components of Aroclor 1260, can potentially be dechlorinated to 2-2-CB via sequential dechlorination by Processes N and LP.

The bacterial agents responsible for Processes N, P, and LP have not yet been identified. There have been two reports of highly enriched sediment-free cultures of PCB-dechlorinating bacteria (17, 18), but neither of those cultures has been demonstrated to dechlorinate any of the congeners that occur in significant concentrations in environmental samples. To date, only microcosm studies with sediments have succeeded in sustaining broad range dechlorinating activity against the PCBs that actually persist in the environment.

Our laboratory previously discovered that Process LP dechlorination can be specifically stimulated in sediment slurries by the addition of 2,6-difluoro-4-chlorobiphenyl (DFCB) (19). This discovery facilitated our study of LP dechlorination in microcosms and enabled us to investigate its PCB substrate range.

Dechlorination Process Q, which occurs in the Upper Hudson River (New York), is the only other microbially mediated dechlorination activity known to target unflanked *para* chlorines (12, 20, 21). Several years ago, Zwiernik and colleagues reported that the addition of ferrous sulfate to sediment microcosms promoted Process Q dechlorination (22). On the basis of their evaluation of the effects of various amendments including ferrous sulfate, sodium sulfate, and ferrous chloride, they proposed that the bacteria carrying out Process Q are sulfate reducers that are sensitive to toxicity

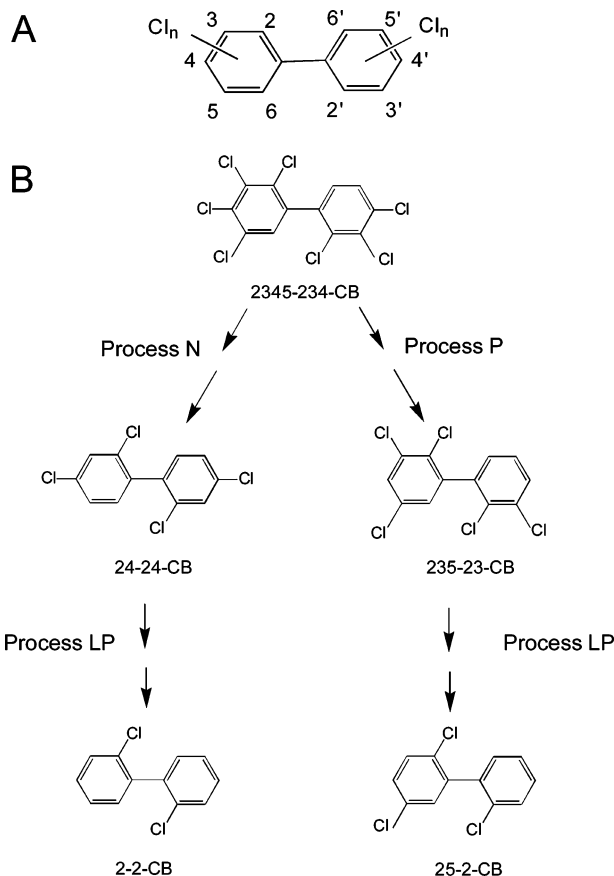


FIGURE 1. (A) PCB structure showing numbering system. *Ortho* positions are at 2, 2', 6, and 6'. *Meta* positions are at 3, 3', 5, and 5', and *para* positions are at 4 and 4'. (B) Dechlorination of a major component of Aroclor 1260 by Processes N, P, and LP. In this paper we shall refer to PCB congeners by listing the substituted positions on each ring separated by a hyphen. Thus 2345-234-CB is the congener substituted at positions 2, 2', 3, 3', 4, 4', and 5.

from soluble sulfides. They proposed that ferrous sulfate promoted Process Q because the ferrous ion (Fe^{2+}) removes sulfide from solution by forming the insoluble precipitate FeS . Given the similarities of Processes Q and LP, we reasoned that these activities might be identical and might be mediated by the same bacterial agent.

Our objectives were (1) to characterize the PCB substrate range of Process LP and (2) to determine whether ferrous sulfate promotes the activity of Process LP. We report here that Process LP removes flanked and unflanked *para* chlorines from a wide range of di- through pentachlorobiphenyls. In addition, Process LP removes *meta* chlorines that are flanked by an *ortho* chlorine such as those on 2,3-, 2,3,4-, and 2,3,5-chlorophenyl rings. These data will facilitate modeling to interpret PCB dechlorination in contaminated sediments and to predict the fate of the PCBs (23–25). The chlorophenyl ring substrate specificity of Process LP is very similar to that of Process Q (12). However, ferrous sulfate strongly inhibited Process LP activity. Hence, our data suggest that the bacteria that mediate Process LP are not the same as those that mediate Process Q.

Experimental Section

Sediment Collection and Storage. Sediments were collected by repeated core sampling near the western shore of Woods Pond, then transferred to one-gallon glass jars. The sample jars were then filled to the top with site water, sealed, and stored at 4 °C.

Reagents. PCB congeners (99.9% purity) were purchased from AccuStandard (New Haven, CT). DFCB and 2,6-difluorobiphenyl (DFB) (99.9% purity) were gifts from GE Corporate Research and Development (Niskayuna, NY). Malic acid was purchased from Fisher Scientific and converted to the disodium salt by adjusting to pH 7.0 with sodium hydroxide.

Design of Experiments Using Multiple PCB Substrates in Mixtures. On the basis of our previous research, we identified 27 PCB congeners ranging from mono- to pentachlorobiphenyls that were potential substrates for LP dechlorination. Each of these is a significant component of a commercial PCB mixture or a significant microbial dechlorination product of commercial PCBs. Previous observations of microbial PCB dechlorination (26) demonstrated that flanked *para* chlorines (such as those on 3,4-, 2,3,4-, and 2,4,5-chlorophenyl rings) would be removed first, unflanked *para* chlorines (such as those on 2,4-, and 2,4,6-chlorophenyl rings) would be removed next, and isolated *para* chlorines on 4-chlorophenyl rings would be removed last. On the basis of these guidelines, we predicted the dechlorination products of the 27 PCB congeners. From previous research (3) we knew the elution order for all PCB congeners on our DB-1 capillary gas chromatograph (GC) column and used this information to design PCB congener substrate mixes such that all substrates and potential products would be fully resolved by capillary GC. We designed five separate substrate mixes, A–E, and corresponding product mixes, the contents of which are listed in Table 1. Each substrate mix also contained the congener 35-35-CB (not listed in table) as an internal standard because it is not a substrate for any of the microbial dechlorination activities present in Woods Pond sediment. Two congeners, 24-25-CB and 25-34-CB, were included in each of two substrate mixes.

Concentrated stock solutions of the five PCB substrate mixes were prepared in GC grade acetone (OmniSolv, EM Science). PCBs were added to microcosms to a nominal final concentration of $5\ \mu\text{M}$ for each congener. Concentrated stock mixes of probable product congeners were prepared in GC grade hexane (OmniSolv, EM Science).

Experimental Setup. Microcosms were prepared in an anaerobic chamber (Coy Laboratories) in an atmosphere of 95–97% N_2 and up to 5% H_2 . Woods Pond sediment was sieved to remove debris, then combined with anoxic sterile ultrapure H_2O to form a slurry (40% wet sediment/60% water). The slurry was dispensed into 60-mL serum bottles in 30-mL aliquots. We added sterile disodium malate (pH 7.0) to a final concentration of 10 mM from a 1 M stock solution, and DFCB to a final concentration of $700\ \mu\text{M}$ from a 140 mM stock in GC grade methanol (OmniSolv, EM Science). Each PCB substrate mix was incubated with triplicate live samples and one autoclaved control. The total PCB concentration in each microcosm varied from 20 to $50\ \mu\text{M}$ depending on the number of congeners in the substrate mix. Microcosms were sealed with Teflon-faced butyl rubber septa (West Pharmaceutical Services) and aluminum crimp caps, and were incubated in the dark at 22–24 °C. Sterile controls were prepared by pasteurizing the microcosms at 75 °C for 10 min, incubating them at 22–24 °C for 24 h, and finally autoclaving them at 121 °C for 3 h.

Experiments Testing PCB Congeners Individually. On the basis of the observations from the initial experiments, we carried out further experiments testing selected PCB congeners individually at concentrations of either 50 or $100\ \mu\text{M}$. Some of these experiments included 35-35-CB (10 or $20\ \mu\text{M}$) as an internal standard. Others did not and were quantified by external calibration standards (see PCB analysis).

Experiments Investigating the Effect of Ferrous Sulfate on Process LP. We replicated the treatments described by

TABLE 1. Composition of Substrate Mixes and Corresponding Product Mixes

Substrate and Product Mix A			Substrate and Product Mix B			Substrate and Product Mix C			Substrate & Product Mix D			Substrate and Product Mix E		
DB1 Pk No. ^a	expected products	DB1 Pk No. ^a	substrates	expected products	DB1 Pk No. ^a	substrates	expected products	DB1 Pk No. ^a	substrates	expected products	DB1 Pk No. ^a	substrates	expected products	DB1 Pk No. ^a
4	biphenyl	7	24-CB	2-CB	15	24-2-CB	2-2-CB	5	22	24-3-CB	7	4-4-CB	4-CB	4
8	2-CB	17	26-4-CB	26-CB	20	245-CB	25-CB	6	33	24-24-CB	15	234-CB	23-CB	8
11	26-CB	24	24-4-CB	2-4-CB	23	25-4-CB	25-CB	6	38	34-4-CB	13	246-4-CB	26-4-CB	17
24	26-2-CB	26	24-26-CB	26-2-CB	25	34-2-CB	2-3-CB	7	26	246-24-CB	26	246-4-CB	26-CB	5
32	25-2-CB	32	24-25-CB	25-2-CB	44	246-24-CB	246-2-CB	24	19	235-CB	19			
36	3-3-CB	34	245-2-CB	25-2-CB	45	235-4-CB								
38	23-2-CB	37.1	246-26-CB	26-26-CB	19									
47	25-3-CB	47	25-34-CB	25-3-CB	21									
		43	246-25-CB	25-26-CB	25									

^a Our DB-1 GC column resolves the 209 PCB congeners into 118 peaks. Some congeners co-elute. The DB1 peak number refers to elution order of the peaks.

Zwiernik and colleagues (22) in our sediment microcosms to determine if ferrous sulfate promotes Process LP dechlorination as it does Process Q. For these experiments, microcosms were set up with 700 μ M DFCB and 50 μ M 24-2-CB. No malate was added. Additional treatments were as follows: molybdate (Na_2MoO_4 , 20 mM) (to inhibit sulfate reduction), FeSO_4 (10 mM), Na_2SO_4 (10 mM), FeCl_2 (10 mM), FeSO_4 plus Na_2MoO_4 , and Na_2SO_4 plus FeCl_2 .

Extraction of PCBs, DFCB, and DFB. We sampled the microcosms at intervals of 7–10 days for PCB/DFCB/DFB extraction and analysis during the course of the incubations (up to 152 days). Aliquots (1 mL) of each microcosm were aseptically removed using a cut off pipet tip under a stream of sterile, O_2 -free, N_2 gas and transferred to 8-mL glass vials fitted with Teflon-lined screw caps. Halogenated biphenyls were extracted with 5 mL of anhydrous diethyl ether (Mallinckrodt) by vigorous horizontal shaking on a platform shaker for a minimum of 16 h. Acid-reduced copper filings (~50 mg) were added to each extract to remove sulfur. Quantitative comparisons of samples extracted by this simple procedure and by rigorous Soxhlet procedure (EPA 3540) (27) showed no difference.

Quantitative DFCB/DFB Analysis. We used a Hewlett-Packard 5890/5971A gas chromatograph/mass spectrometer (GC/MS) equipped with a DB-1 capillary column (J & W Scientific) (30 m \times 0.25 mm \times 0.25 μ m phase thickness) to analyze the sample extracts. For all analyses, the injection temperature was 250 $^\circ\text{C}$ and the detector temperature was 280 $^\circ\text{C}$.

We used selected ion monitoring to quantify DFCB dehalogenation. The ions monitored were: m/z 224, 226, and 188 for DFCB, and m/z 190, 189, and 188 for DFB. The GC oven was programmed from 50 to 150 $^\circ\text{C}$ at 20 $^\circ\text{C}/\text{min}$, then to 210 $^\circ\text{C}$ at 4 $^\circ\text{C}/\text{min}$, then to 270 $^\circ\text{C}$ at 20 $^\circ\text{C}/\text{min}$, and held for 10 min. DFCB dehalogenation was quantified using a linear four-point calibration curve forced through zero. Calibration standard concentrations for DFB and DFCB were 10, 50, 100, and 200 μM .

Semi-quantitative PCB Analysis. We used a scan method to analyze PCB dechlorination. The mass ranges scanned were m/z 100 to 350 at 1.7 scans per second. For analysis of samples containing substrate mixes B–E, we used a multi-step temperature program as follows: 50 to 160 $^\circ\text{C}$ at 25 $^\circ\text{C}/\text{min}$, 160 to 200 $^\circ\text{C}$ at 2 $^\circ\text{C}/\text{min}$, 200 to 270 $^\circ\text{C}$ at 8 $^\circ\text{C}/\text{min}$, and hold for 15 min. The temperature program was modified for the analysis of Substrate A to resolve 246-CB and 3-3-CB. After the initial increase to 160 $^\circ\text{C}$, the temperature was raised to 180 $^\circ\text{C}$ at 0.5 $^\circ\text{C}/\text{min}$, then to 270 $^\circ\text{C}$ at 20 $^\circ\text{C}/\text{min}$, and held for 15 min.

Analytical PCB standards contained each PCB substrate mix and its corresponding PCB product mix (all congeners at 5 μM). PCB products in the samples were identified by matching the retention times of the congeners in the appropriate standards with those in the sample. Product identification was confirmed by checking the mass spectrum. In instances where a product that formed was not in the mix of predicted products for that substrate, the product was identified by matching its retention time to that of other possible products in other substrate or product mixes.

We calculated the amount of PCB dechlorination for each congener by using a ratio method. The ratio of the abundance of each PCB substrate to that of the internal standard, 35-35-CB, before dechlorination of the substrate began (R_i) was compared to the ratio after dechlorination was completed (R_f), or at the end of the experiment. The percent dechlorination was calculated by the formula $(1 - (R_f/R_i)) \times 100\%$.

Quantitative PCB Analysis. PCBs were analyzed by the scan method described above. Calibration standards of 1, 5, 10, and 20 μM were prepared for each PCB congener and its

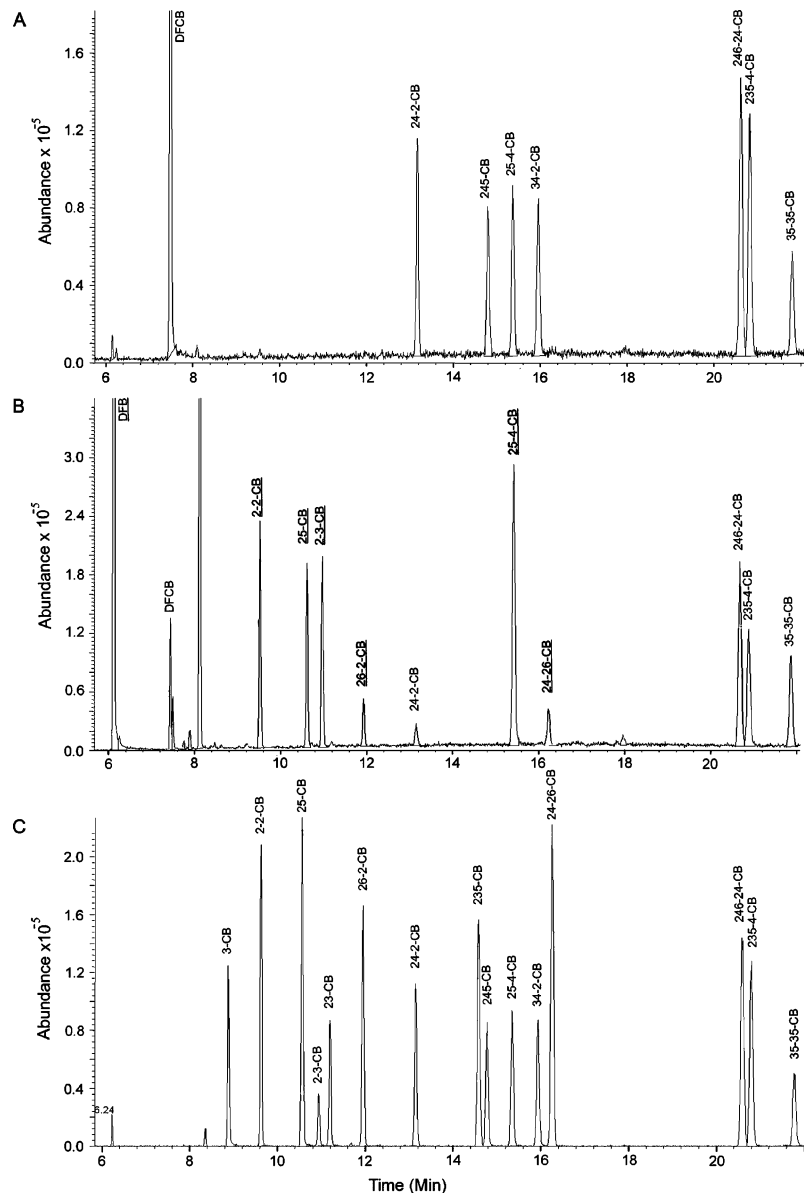


FIGURE 2. Dechlorination of Substrate Mix C by Process LP. (A) Extract from sample at day 8, before dechlorination has begun. (B) Extract from the same sample at day 118, after dechlorination is complete. Dechlorination products are underscored. (C) Standard showing all PCB substrates and products.

TABLE 2. Grouping of PCB Congeners Dechlorinated by Process LP^a According to Dechlorination Efficiency

Best Substrates		Good Substrates		Low Substrates		Poor or Non Substrates	
congener	mean ± SD	congener	mean ± SD	congener	mean ± SD	congener	mean ± SD
34-2-CB	100 ± 0	24-2-CB	71 ± 31	24-25-CB ^b	28 ± 8	246-26-CB ^d	2 ± 2
34-3-CB	100 ± 0	235-4-CB	55 ± 6	246-2-CB	24 ± 14	24-4-CB ^c	1 ± 1
25-34-CB	100 ± 0	24-CB	57 ± 21	24-3-CB	23 ± 1	26-4-CB ^d	1 ± 0
234-CB	100 ± 0	246-CB	53 ± 38	246-24-CB ^c	21 ± 19	246-4-CB ^d	0 ± 0
245-CB	100 ± 0	24-24-CB	47 ± 7	246-25-CB	18 ± 15	4-CB	0 ± 0
245-2-CB	95 ± 4	23-24-CB	46 ± 15	24-26-CB ^c	18 ± 17		
34-4-CB	85 ± 13			2-4-CB ^c	13 ± 12		
				4-4-CB	11 ± 2		

^a The chlorine(s) removed from each congener is/are underscored. In all cases dechlorination was confirmed by the appearance of a dechlorination product. ^b The value reported is from substrate mix A. Dechlorination in substrate mix B was observed in all three samples, but averaged only 10%. ^c Dechlorination was observed in only 2 of the 3 samples. ^d Dechlorination was observed in only 1 of the 3 samples.

expected products. The concentrations of each congener and all of its products were quantified using a linear four-point calibration curve forced through zero. The total mole percent for each congener and its products was then calculated.

Results

Substrate Mix Experiments. In all cases DFCB was dechlorinated to DFB and this commenced before PCB dechlori-

TABLE 3. PCB Congeners^a with 2,3-, 2,4-, 2,3,5-, and 4-Chlorophenyl Rings Grouped by Efficiency of Dechlorination by Process LP

Best Substrates		Good Substrates		Poor Substrates		Nonsubstrates	
congener	mean % dechlorination	congener	mean % dechlorination	congener	mean % dechlorination	congener	mean % dechlorination
23-23-CB	>90	23-CB	71	26-4-CB	>15	4-CB	0
235-CB	>90	24-CB	71	2-4-CB	<15	4-4-CB	0
				23-4-CB	<15	25-4-CB	0
				24-4-CB	≤15		

^a PCB congeners were tested individually at a final concentration of 100 μM. DFCB and 35-35-CB were added to final concentrations of 700 μM and 10–20 μM, respectively. Results are semi-quantitative, based on the ratio of the area of the congener peak to that of the internal standard, 35-35-CB. In many of the samples the 35-35-CB was undetectable so the dechlorination was estimated based on comparison of the area of the substrate at T_{zero} and T_{final} . Except for nonsubstrates, dechlorination was always confirmed by appearance of the expected dechlorination product. The chlorines removed are underscored. No dechlorination occurred in autoclaved controls.

nation began. Figure 2 shows the results of one replicate incubated with substrate mix C. Visual inspection of the chromatogram showed decreases in DFCB, 24-2-CB, 245-CB, 34-2-CB, 246-24-CB, and 235-4-CB relative to the internal standard, 35-35-CB. For all except 235-4-CB, the expected products were also seen: DFB, 2-2-CB, 25-CB, 2-3-CB, and 24-26-CB and 26-2-CB, respectively. Further inspection of the chromatograms revealed that 25-4-CB was elevated relative to 35-35-CB, apparently because 235-4-CB was dechlorinated by loss of the *meta* chlorine.

Table 2 summarizes the results of the substrate mix experiments. Congeners are grouped into four categories based on their relative ease of dechlorination. All but five congeners were significantly dechlorinated in at least two replicates. The congeners that showed the highest level of dechlorination all had adjacent *meta* and *para* chlorines, as seen in 3,4- and 2,4,5- chlorine substitution patterns. We had expected these congeners to readily dechlorinate, since dechlorination of flanked *para* chlorines is observed more often than dechlorination of unflanked *para* chlorines, and it is generally assumed that flanked *para* chlorines are more readily removed than unflanked *para* chlorines.

We observed four additional trends. First, for some congeners, e.g., 24-2-CB and 246-CB, the proportion of the congener that was dechlorinated differed substantially in the three replicate samples, resulting in high standard deviations. In a few cases dechlorination of a particular PCB congener was observed in only two of the replicates (designated by footnote c in Table 2.) However, because dechlorination was confirmed by the appearance of the appropriate dechlorination product in all cases, the dechlorination is real despite the variation among replicates in the proportion of the congener that was dechlorinated. Second, congeners with three or more *ortho* chlorines, such as 246-2-CB and 24-26-CB were dechlorinated far less efficiently than their homologues with fewer *ortho* chlorines. Third, the 4-chlorophenyl ring was a poor substrate as illustrated by the very modest dechlorination of 2-4-CB and 4-4-CB and the lack of dechlorination of 4-CB and 26-4-CB. Fourth, a 4-chlorophenyl ring adversely affected dechlorination of the opposite ring. For example, 24-CB, 24-2-CB, and 246-CB were dechlorinated by an average of 53–71%, but 24-4-CB and 246-4-CB were not significantly dechlorinated.

Unexpectedly, we also observed *meta* dechlorination of two congeners, 234-CB and 235-4-CB. 234-CB was dechlorinated to 24-CB and then to 2-CB; 235-4-CB was dechlorinated to 25-4-CB. The observation of *meta* dechlorination raised the question of whether the *meta* chlorine of 23-24-CB was also removed. We were unable to determine that from the substrate mix experiments because both of the expected products, 24-2-CB and 2-2-CB, coeluted with other products from the congeners in the same substrate mix.

Experiments Testing Selected PCB Congeners Individually. We carried out additional experiments with eleven PCB

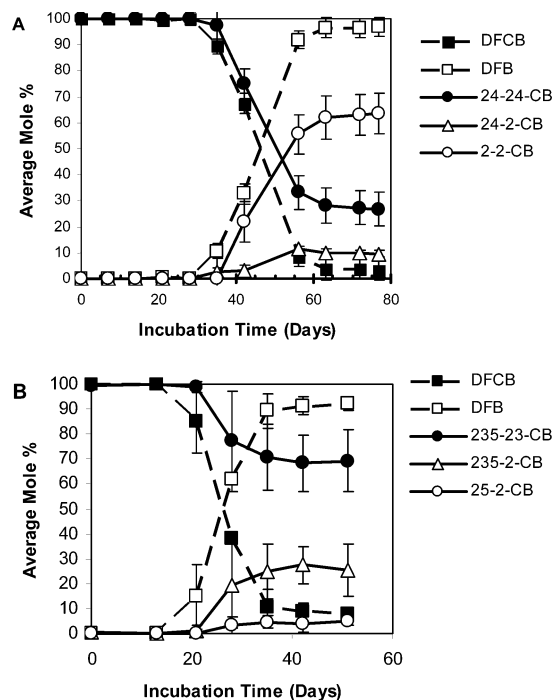


FIGURE 3. Time course of dechlorination of DFCB and two PCB congeners by Process LP. (A) Dechlorination of 24-24-CB. (B) Dechlorination of 235-23-CB. The data are the means of triplicates. Standard deviations are plotted for all data except DFCB and DFB at day 28 in Figure 3B. The standard deviation for those data was 51 mol % due to a slight difference in the timing of dechlorination among replicates.

congeners incubated individually to further investigate the dechlorination of *meta*-chlorinated congeners and of congeners with 4-chlorophenyl rings. The results (Table 3) confirm that Process LP removes *meta* chlorines adjacent to *ortho* chlorines such as those on the 2,3- and 2,3,5-chlorophenyl rings. The results also confirm that 4-chlorophenyl rings are poor or nonsubstrates, and that they adversely affect the dechlorination of the opposite ring. For example, compare the dechlorination of 23-CB and 24-CB with that of 23-4-CB and 24-4-CB.

Time Course of Dechlorination of Tetra- and Pentachlorobiphenyls. A final set of quantitative experiments compared the dechlorination of six tetra- and pentachlorobiphenyls composed primarily of 2,3-, 2,4-, and 2,3,5-chlorophenyl rings and quantified their dechlorination products. Figure 3 compares the time course of dechlorination of DFCB with that of 24-24-CB and 235-23-CB in two sets of microcosms. The data shown are the mean of triplicates. These illustrate several observations that applied to all experiments. First, there was a variable lag time preceding dechlorination of DFCB. Second, PCB dechlori-

TABLE 4. Quantitative Dechlorination^a of Tetra- and Pentachlorobiphenyls by Process LP

Substrate		1st Product		2nd Product	
congener	mean % dechlorination	congener	mean % of total products	congener	mean % of total products
23-23-CB	82.6 ± 3.5	23-2-CB	1.7 ± 0.4	2-2-CB	98.3 ± 0.4
23-25-CB	72.8 ± 17.8	25-2-CB	100.0 ± 0.0		
24-24-CB	70.2 ± 3.4	24-2-CB	13.1 ± 1.0	2-2-CB	86.9 ± 1.1
23-24-CB	36.5 ± 9.0	23-2-CB	4.4 ± 2.2	2-2-CB	93.6 ± 3.7
		24-2-CB	2.0 ± 1.6		
235-23-CB	30.7 ± 12.4	235-2-CB	83.5 ± 1.8	25-2-CB	16.5 ± 1.8
235-24-CB	10.0 ± 4.3	Tetra-CB ^b	93.4 ± 11.5	25-2-CB	6.6 ± 11.5

^a PCB congeners were tested individually, each at a final concentration of 50 μM. DFCB was added to a final concentration of 700 μM. Results are quantitative, based on calibration curves of all substrates and products. Data are presented as means of triplicates ± standard deviation. No dechlorination occurred in autoclaved controls. ^b The two possible products, 235-2-CB and 24-25-CB could not be resolved.

nation commenced with, or slightly after, DFCB dechlorination. Third, the dechlorination kinetics of DFCB varied from a rapid decline in 10–15 days (Figure 3B), to a more gradual decline over 30 days or more (Figure 3A). Typically, more dechlorination of the PCB was observed when the dechlorination of DFCB was more gradual. Fourth, as illustrated for 235-23-CB (Figure 3B), PCB dechlorination ceased when DFCB dechlorination ceased or was completed.

Table 4 summarizes the results of the quantitative experiments. Three of the tetrachlorobiphenyls were efficiently dechlorinated to 2-2-CB with little accumulation of the trichlorobiphenyl intermediate. The fourth, 23-25-CB, was dechlorinated to 25-2-CB, confirming that the 2,5-chlorophenyl ring is not a substrate. The pentachlorobiphenyls were more difficult substrates. A small proportion of each was dechlorinated to 25-2-CB, but the major products of the pentachlorobiphenyls were tetrachlorobiphenyls. There were two potential dechlorination intermediates for 235-23-CB: 235-2-CB and 23-25-CB. 235-2-CB accumulated to high levels, but 23-25-CB was never detected. In the case of 235-24-CB, there were also two possible intermediates: 235-2-CB and 24-25-CB. We were unable to determine whether one or both were intermediates because these two congeners co-elute on our GC column. It is not clear why the pentachlorobiphenyls were poorer substrates than the tetrachlorobiphenyls, or why a larger proportion of them was not converted to 25-2-CB.

Experiments Investigating the Effect of Ferrous Sulfate on Process LP. We chose to use the dechlorination of 24-24-CB to assess the effect of ferrous sulfate on Process LP. 24-24-CB is the single most important substrate that Process LP dechlorinates because it is the product of several major components of Aroclor 1260 and may represent 25 mole percent or more of the final dechlorinated PCBs (13, 14).

Figure 4 summarizes the results of our experiments which were designed to replicate those described for Process Q in Hudson River sediment (22). Sodium sulfate inhibited dechlorination of both DFCB (data not shown) and 24-24-CB. Sulfate typically inhibits PCB dechlorination because it is a competing electron acceptor, but the sulfate is usually depleted in 2–4 weeks and then PCB dechlorination begins (12, 22). In our experiments the inhibition caused by sulfate was not overcome in 78 days which should be more than sufficient time for sulfate to be depleted. The addition of ferrous sulfate instead of sodium sulfate did not alleviate the inhibition of DFCB or PCB dechlorination, even though the ferrous ion should have precipitated any sulfide formed, thereby eliminating sulfide toxicity. The combined addition of molybdate, which inhibits sulfate reduction (28, 29), and FeSO₄ was expected to have no effect, but in fact both DFCB (data not shown) and PCB dechlorination were completely inhibited (data in Figure 4 are obscured by data for NaSO₄ plus FeCl₂). Molybdate alone actually hastened the onset of

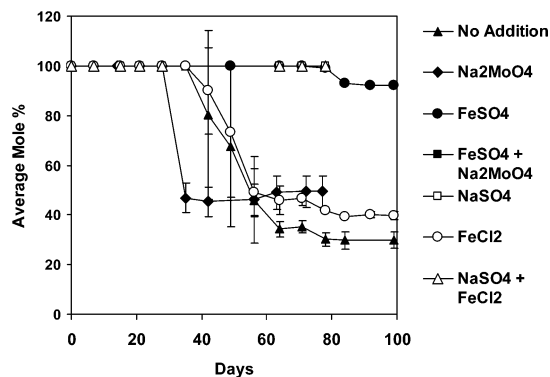


FIGURE 4. Effect of various additives on the dechlorination of 24-24-CB. Data are the means of triplicates ± standard deviation. Large standard deviations between days 40 and 60 are due to slight differences in the timing of the onset of dechlorination among replicates

dechlorination, but the final extent of dechlorination was less than that in untreated controls. FeCl₂ caused only slight inhibition.

Discussion

Process LP Complements Other Microbial Dechlorination Processes. Twenty-five of the 34 PCB congeners that we tested proved to be at least moderately good dechlorination substrates for Process LP. The best substrates were PCB congeners with adjacent *meta* and *para* chlorines, such as those on 3,4-, 2,3,4-, and 2,4,5-chlorophenyl rings. But 2,3-, 2,4-, 2,4,6-, and to a lesser extent, 2,3,5-chlorophenyl rings were also good substrates. This is especially significant because this latter group of chlorophenyl rings comprise the terminal dechlorination products of other microbial dechlorination processes such as H, H', and M, which occur in the Hudson River, and N and P which occur in the Housatonic River (12). In fact, 24-24-CB comprises up to 25 mole % of the total PCBs after dechlorination of Aroclor 1260 by Process N (14). Figure 1B shows the dechlorination of a heptachlorobiphenyl, which is a major component of Aroclor 1260, by Processes N, P, and LP. Clearly Process LP complements the action of Processes N and P. The action of Process LP in tandem with either N or P will result in further decreases in the toxicity and persistence of PCBs (30–33).

Effect of Multiple Ortho Chlorines on Dechlorination. Each of the four PCB congeners with three *ortho* chlorines, 246-2-CB, 246-24-CB, 246-25-CB, and 24-26-CB, was only moderately dechlorinated (18 to 24%), and each showed considerable variation among triplicates as evidenced by the high standard deviations. The single congener tested with four *ortho* chlorines, 246-26-CB, was not significantly dechlorinated. The phenyl rings of congeners with three or more

ortho chlorines are not free to rotate, and this might impair dehalogenase access to these congeners. Also, because of their inability to rotate, each of these congeners exists as two distinct stereoisomers. It may be that two distinct dehalogenases are required to dechlorinate the two enantiomeric forms (34).

Effect of 4-Chlorophenyl Ring on LP Dechlorination.

Only two congeners, 25-4-CB and 4-CB, yielded absolutely no dechlorination products. We detected traces of dechlorination products for 246-26-CB, 246-4-CB, and 24-4-CB, demonstrating that they can be dechlorinated by Process LP, but they are clearly poor substrates. Four other congeners, 2-4-CB, 4-4-CB, 23-4-CB, and 26-4-CB, were also poor substrates. All but one of the nine worst substrates had a 4-chlorophenyl ring. We conclude from these data that the 4-chlorophenyl ring itself is a poor substrate, and furthermore, the presence of a 4-chlorophenyl ring has an adverse effect on the dechlorination of the opposite ring. This is apparent from comparing the dechlorination of 23-4-CB, 24-4-CB, and 246-4-CB with that of other congeners containing 2,3-, 2,4-, and 2,4,6-chlorophenyl rings (Tables 2 and 3).

Effect of PCB Mixtures on Dechlorination of Individual Components. We included 24-25-CB and 25-34-CB in two substrate mixes, A and B. The results for 25-34-CB were the same for both substrate mixes, but the mean dechlorination of 24-25-CB differed in the two substrate mixes, $28.1 \pm 8.4\%$ in Mix A vs $9.9 \pm 8.4\%$ in Mix B. A likely reason for this is that the PCB composition of the two substrate mixes influenced the efficiency of the dechlorination of 24-25-CB. There were two pentachlorobiphenyls in Mix B, but none in Mix A. Furthermore, three of the congeners in Mix B had three or four *ortho* chlorines, while only one in Mix A did. From these observations it appears that the efficiency of dechlorination for the congeners as a whole in a PCB mixture may affect the efficiency of dechlorination of any given individual congener.

Dechlorination of *Ortho*-Flanked *Meta* Chlorines. Ten of the congeners that we studied were dechlorinated through the loss of a *meta* chlorine from a 2,3-, 2,3,4-, or 2,3,5-chlorophenyl group. This indicates that LP dechlorination is not restricted to *para* dechlorination, as previously thought, but can also remove *meta* chlorines that are flanked by an *ortho* chlorine. Research done on Process Q in the Hudson River, which also dechlorinates flanked and unflanked *para* chlorines, has shown similar results in that *meta* dechlorination of 2,3-, 2,3,4-, and 2,3,6-chlorophenyl groups occurs (12).

Value of the Data for Modeling Dechlorination. Our in-depth characterization of the substrate range of this PCB dechlorination activity will facilitate the development of accurate models to interpret PCB dechlorination in situ and to predict PCB fate. Convincing models have been published recently for in situ PCB dechlorination in sediments of the Ashtabula River (OH), the Fox River (WI), and the Sheboygan River (WI) (23–25). Because the accuracy of such models depends on the accuracy of the descriptive data fed into them, it stands to reason that detailed substrate data such as those presented here will further improve these models.

Effects of Ferrous Sulfate on Processes LP and Q. Our experiments testing the effect of ferrous sulfate on Process LP differed substantially from the results of similar experiments testing Process Q in Hudson River sediment. Sodium sulfate inhibited Process Q dechlorination while ferrous sulfate stimulated it, and the combination of molybdate and ferrous sulfate had no effect (22). Those results supported the interpretation that Process Q is mediated by sulfate reducers that are sensitive to sulfide toxicity. Since molybdate inhibits sulfate reduction, it blocked the positive effect of ferrous sulfate when added simultaneously. Our results showed that both ferrous sulfate and sodium sulfate inhibit Process LP and do not support a role of sulfate reducers for

Process LP. Our data suggest that sulfate inhibits Process LP by some other mechanism that is not clear.

Summation. In conclusion, Process LP can remove flanked and unflanked *para* chlorines on di- through pentachlorobiphenyls. It can also remove *meta* chlorines flanked by an *ortho* chlorine. It is particularly well suited to dechlorinating the terminal dechlorination products of Processes H, H', M, N, and P, and is most effective when acting in tandem with one or more of these activities. The combined activity of Process LP with any of these microbial dechlorination processes can dechlorinate many hexa- and heptachlorobiphenyls to di- and trichlorobiphenyls. The resulting PCBs are less toxic and less persistent (30–33). Thus, Process LP will be important for the development of PCB remediation technologies. Our detailed characterization of the PCB substrate range of Process LP will facilitate modeling in situ dechlorination reactions in contaminated sediments (23–25) and prediction of PCB fate.

Acknowledgments

This work was supported by National Science Foundation Award 0077837 and by a grant from GE Corporate Environmental Programs. We thank Greta Van Slyke Jerzak for technical assistance.

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Received for review February 7, 2005. Revised manuscript received May 24, 2005. Accepted June 7, 2005.

ES050255I