Effects of Organic Substrates on Dechlorination of Aroclor 1242 in Anaerobic Sediments

LORING NIES AND TIMOTHY M. VOGEL*

*Environmental and Water Resources Engineering, Department of Civil Engineering,
The University of Michigan, Ann Arbor, Michigan 48109-2125*

The effects of different organic substrates on the abilities of anaerobic sediment enrichments to reductively dechlorinate polychlorinated biphenyls (PCBs) were studied. Sediments collected from a site previously contaminated with PCBs were dosed with additional PCBs (Aroclor 1242; approximately 300 ppm [300 µg/g], sediment dry weight) and incubated anaerobically with acetate, acetone, methanol, or glucose. The pattern of dechlorination was similar for each substrate-fed batch; however, the extents and rates of dechlorination were different. Significant dechlorination over time was observed, with the relative rates and extents of dechlorination being greatest for methanol-, glucose-, and acetone-fed batches and least for acetate-fed batches. Dechlorination occurred primarily on the *meta-* and *para-* positions of the highly chlorinated congeners, resulting in the accumulation of less-chlorinated, primarily *ortho*-substituted products. No significant dechlorination was observed in incubation batches receiving no additional organic substrate, even though identical inorganic nutrients were added to all incubation batches. In addition, dechlorination was not observed in autoclaved controls that received substrate and nutrients.

The use of polychlorinated biphenyls (PCBs) from 1930 until their manufacture was banned in 1977 led to their widespread occurrence in the environment at parts per billion levels (8). In addition, several locations have levels greater than 1,000 ppb (1,000 ng/g) and are being considered for remediation. Recent evidence of the dechlorination of PCBs in situ suggests that some transformations have occurred naturally (3). Laboratory studies have shown that many PCB congeners are aerobically degraded or anaerobically dechlorinated by microorganisms (1, 11). Anaerobic reductive dechlorination involves the loss of a chlorine substituent and its replacement by a hydrogen. The reduction potential of PCBs increases with increasing chlorine number (6, 12). However, the positions of the chlorine substituents also influence ease of reductive dechlorination, which occurs primarily at *para-* and *meta-* positions, with the subsequent accumulation of lightly chlorinated *ortho*-substituted products (11). Therefore, a general relationship between reduction potential, chlorine substituent number, chlorine substitution pattern, and dechlorination rate seems to exist. The source of electrons for the dechlorination reactions might be the reduced substrates provided for microbial growth. In the case of chlorinated solvents, evidence for chlorinated solvents undergoing fortuitous dechlorination by microorganisms growing on other electron donors and acceptors (e.g., acetate) has accumulated (14). In an attempt to examine the influence of reduced organic substrates on the microbial dechlorination of PCBs, different substrates were provided as the carbon and energy sources for naturally occurring dechlorinating microbial communities in Hudson River sediment. The substrates tested were acetate, acetone, glucose, and methanol. Methanol and acetone were tested because of their potential use as PCB solvents in laboratory studies. In order to assess the independent effects of these solvents on the dechlorinating microorganisms, a procedure to introduce PCBs into batch incubations without high concentrations of solvents was designed.

MATERIALS AND METHODS

Chemicals. Aroclor 1242 was provided by the General Electric Co. (Schenectady, N.Y.). All mineral medium chemicals were obtained from Fisher Scientific Co. (Fairlawn, N.J.). Pesticide-free hexane and acetone were obtained from J. T. Baker Chemical Co. (Phillipsburg, N.J.). Methanol was obtained from EM Science (Cherry Hill, N.J.). Octachloronaphthalene was purchased from Ultra Scientific (North Kingstown, R.I.).

Mineral medium. The anaerobic mineral medium contained the following (in milligrams per liter): $(NH_4)_2HPO_4$, 80; NH_4Cl, 1,000; K_2HPO_4, 200; sodium chloride, 10; calcium chloride, 10; magnesium chloride, 50; $CoCl_2 \cdot 6H_2O$, 1.5; $CuCl_2 \cdot 2H_2O$, 0.2; $Na_2MoO_4 \cdot H_2O$, 0.23; $ZnCl_2$, 0.19; $NiSO_4 \cdot 6H_2O$, 0.2; $FeSO_4 \cdot 7H_2O$, 1.0; $AlCl_3 \cdot 6H_2O$, 0.4; H_3BO_3, 0.38; biotin, 0.02; folic acid, 0.02; pyridoxine hydrochloride, 0.1; riboflavin, 0.05; thiamine, 0.05; nicotinic acid, 0.05; panthothenic acid, 0.05; vitamin B_{12}, 0.001; and *p*-aminobenzoic acid, 0.05. Resazurin (1 mg/liter) was added as an oxygen indicator. The preparation was adapted from that of Owen et al. (9). Sodium sulfide (500 mg/liter) was used as a reducing agent. Sodium bicarbonate (500 mg/liter) was added, the pH was adjusted to 7.0 with oxygen-free nitrogen-carbon dioxide gas (70%/30%), and the preparation was stoppered.

Experimental batch preparation. Batch incubations were prepared by adding 15 g of fresh, wet Hudson River sediment to 100-ml serum bottles (Wheaton Scientific, Millville, N.J.). The sediment had been previously contaminated with PCBs and contained approximately 20 ppm of mono-, di-, and trichlorobiphenyl congeners. The absence of more highly chlorinated congeners indicated the potential dechlorinating activity of the sediment. Additional Aroclor 1242 was then applied directly to the sediment by the following procedure. A 2.5-µl volume of acetone, followed by a 1.0-µl air gap, was drawn into the barrel of a 10-µl syringe. Then

* Corresponding author.

TABLE 1. Substrate added to and gas produced from Hudson River sediment incubations[a]

Substrate	Total dose (mg/bottle)	Total gas produced (ml)
Sodium acetate · 3H₂O	1,945	353
Glucose	1,680	1,395
Methanol	988	842
Acetone	200	242
None	0	0
Acetone (control)	200	0

[a] Initial volume of batches was 50 ml of medium plus sediment.

2.0 μl of Aroclor 1242 was drawn into the syringe, and the entire contents of the syringe were injected onto the sediment. The 2.5 μl of acetone effectively washed the Aroclor out of the syringe without the addition of large amounts of acetone (in the form of a solvent) to the bottles. PCBs were added to methanol-fed batches by being dissolved in methanol. PCBs were added to acetone-fed batches by being dissolved in acetone. Acetate and glucose were added by being dissolved in 1-ml aqueous solutions. The bottles were then flushed with nitrogen, 50 ml of anaerobic medium was added by syringe, and the bottles were then sealed with Teflon-coated stoppers (The West Co., Lancaster, Pa.) and aluminum crimp caps (Fisher Scientific). The batches then received the initial dose of the appropriate substrate. The initial doses, added by syringe, were as follows (in milligrams): sodium acetate · 3H₂O, 390; glucose, 280; and methanol, 200. The acetone-fed batches received only an initial dose of 200 mg. The other substrate-fed batches received four additional substrate doses whenever gas production ceased during the first 10 weeks of incubation. The glucose-fed batches also received a sixth dose after 12 weeks. The total mass of substrates added to batches is shown in Table 1. The experimental batches were then inoculated with a 2-ml slurry from an actively dechlorinating enrichment growing on the appropriate substrate. The inoculation of organisms acclimated to substrate accelerated the onset of methanogenesis. The substrate enrichments used for inoculating the experimental batches all contained Aroclor 1242, which was added by using acetone as a solvent. Each bottle was shaken vigorously after the addition of the substrate and enrichment inoculum but was then incubated statically at 30°C. Batches receiving no additional substrate were inoculated with a slurry from acetone-fed batch enrichments. Controls were prepared identically and autoclaved twice, the second time 24 h after the first.

Gas monitoring. Gas production by the batch incubations was measured by inserting the needle of a wetted glass-barreled syringe (10 or 30 ml) through the stopper and measuring gas volume. The gas was wasted after measurement. Gas composition was determined with a gas chromatograph (Gow Mac Instrument Co., Bridgewater, N.J.) equipped with a thermal conductivity detector with a WX7 filament set at 130 mA. A 3-m Porapack QS stainless steel 3.175-mm-diameter packed column, with helium carrier gas at 25 ml/min and an oven temperature of 80°C (isothermal), was used for separation. Injector and detector temperatures were both 105°C. The experimental gas sample composition was identified and quantified with an external standard, i.e., 65% methane–35% carbon dioxide (Air Products, Tamaque, Pa.).

Sample extraction. Subsamples of experimental batch incubations were taken for extraction of PCBs. Each subsample contained a distribution of PCB congeners that was

representative of the whole batch. Glass tubes (cutoff Pasteur pipettes) were fitted to disposable syringes with bored-out tips to prevent clogging during sample removal. Batches were shaken, Teflon stoppers were removed, headspaces were flushed with nitrogen, approximately 7 ml of sample slurry was removed by syringe, and the incubation bottles were recapped. The sample slurry was added to a 20-ml serum bottle containing 10 ml of acetone, 4 ml of hexane, and 20 μg of octachloronaphthalene as the internal standard. The PCB extraction and cleanup procedure was adapted from the method of Quensen et al. (11).

PCB analysis. The PCBs were analyzed on a 5890A gas chromatograph (Hewlett-Packard Co., Palo Alto, Calif.) equipped with an electron capture detector and a 7673A autosampler. The data were collected and processed with a 300 computer (Hewlett Packard) and a 5895A GC Chemstation. A 50-m SE-54 fused-silica capillary column (0.2-mm inside diameter, 0.11-μm-thick film; Hewlett Packard) was used. Injection was split 10:1, with column flow at 0.7 ml/min. The injector and detector temperatures were 240 and 325°C, respectively. The oven program consisted of an initial temperature of 100°C, an increase rate of 2°C/min to 240°C, and maintenance of that temperature for 25 min. Helium carrier gas and argon-methane (95%/5%) detector makeup gas were conditioned with a heated high-capacity gas purifier (Supelco, Bellefonte, Pa.).

Aroclor peak identification. The 44 largest peaks were numbered in order of elution from the gas chromatography column. Congener identifications and quantitative detector responses, relative to those of the internal standard, octachloronaphthalene, were provided by John Quensen III, Department of Crop and Soil Sciences, Michigan State University, East Lansing. The congener assignments for each peak are shown in Table 2. Four pairs of peaks (15 and 16, 17 and 18, 25 and 26, and 32 and 33) coeluted on the 25-m column used for calibration. These peak pairs were resolved on the 50-m column used in this study. Peaks 16, 17, 26, and 33 were calibrated according to the response factors published by Mullin et al. (7). The final results are presented as moles percent of each peak, with the total PCB peaks in each chromatogram summing to 100%. Complete dechlorination to biphenyl was assumed not to occur, and, therefore, total moles of PCBs were conserved. Peaks 7, 14, 30, 32, 38, 40, 42, and 44 may contain congeners of two homologs. In determining the distribution of homologs in these peaks, the total micromoles found in each peak were equally proportioned among all potential congeners in that peak. The percent removal of chlorine was calculated from the change in homolog distribution at 22 weeks relative to that of the controls (Table 3, footnote b).

RESULTS

The relative rate and the extent of dechlorination of Aroclor 1242 varied among batches that were fed different substrates. As dechlorination proceeded, a decrease in the highly chlorinated congeners and a corresponding increase in the less-chlorinated congeners occurred (Table 3). Batches receiving no substrate showed little change in congener distribution through 22 weeks of incubation (Fig. 1a). Comparison of glucose-fed batches (Fig. 1b) with the batches receiving no substrate (Fig. 1a) illustrates the shifting of the congener pattern toward the less-chlorinated PCBs over time with the addition of substrate. An indication of the extent of dechlorination is the percent removal of chlorine as chloride (Cl⁻) relative to that of control batches (Table 3,

TABLE 2. PCB congener identification by using chlorine substituent location

Peak no.[a]	Congener(s)[b] in peak			
1 ...2				
2 ...2,2'	2,6			
3 ...2,5	2,4			
4 ...2,3'				
5 ...2,4'	2,3			
6 ...2,6,2'				
7 ...2,5,2'	4,4'			
8 ...2,4,2'				
9 ...2,6,3'	2,3,6			
10 ...2,3,2'	2,6,4'			
11 ...2,5,3'				
12 ...2,4,3'				
13 ...2,5,4'				
14 ...2,4,4'	2,4,6,2'			
15 ...2',3,4	2,3,3'	2,3,4		
16 ...2,5,2',6'				
17 ...2,4,2',6'				
18 ...2,3,4'				
19 ...2,3,6,2'				
20 ...2,3,2',6'				
21 ...2,5,2',5'	3,5,2',6'			
22 ...2,4,2',5'				
23 ...2,4,2',4'				
24 ...2,4,5,2'	2,4,6,4'			
25 ...2,3,2',5'				
26 ...3,4,4'				
27 ...2,3,2',4'	2,3,6,3'			
28 ...2,6,3',4'	2,3,6,4'	2,3,4,2'	2,5,3',5'	
29 ...2,3,2',3'				
30 ...2,4,5,4'	2,3,5,2',6'			
31 ...2,5,3',4'	2',3,4,5			
32 ...2,4,3',4'	2,4,5,2',6'			
33 ...2,3,6,2',5'				
34 ...2,3,6,2',4'				
35 ...2,3,3'4'	2,3,4,4'			
36 ...2,4,5,2',5'	2,3,5,2',4'			
37 ...2,3,6,3',5'				
38 ...2,4,5,2',3'	2,3,4,5,2'	2,3,5,6,2',6'		
39 ...2,3,4,2',5'	2,3,4,6,4'			
40 ...3,4,3',4'	2,3,6,3',4'			
41 ...2,3,5,6,2',5'				
42 ...2,4,5,3',4'	2,3,4,5,3'	2,3,6,2',4',5'		
43 ...2,4,5,2',4',5'				
44 ...2,3,4,3',4'	2,3,4,2',3',6'			

[a] Peak number refers to elution order of peaks in the gas chromatogram.
[b] Numbers represent carbon with a chlorine substituent and the prime refers to a second phenyl ring.

TABLE 3. Homolog distribution for Aroclor 1242 in substrate-fed Hudson River sediment incubation batches under anaerobic conditions

Substrate and type of chlorobiphenyl	Mean mol% ± SD for controls[a]	Distribution (mol%) of chlorobiphenyls at week:					
		4	6	10	16	19	22[b]
Acetate							
Mono	4.2 ± 0.96		5.4	5.6		4.9	4.5
Di	24 ± 0.55		32	31		30	30
Tri	33 ± 0.60		34	37		38	38
Tetra	31 ± 0.58		23	23		25	24
Penta	6.7 ± 0.14		4.7	3.0		3.0	3.0
Hexa	0.94 ± 0.05		0.71	0.30		0.31	0.30
Acetone							
Mono	4.2 ± 0.96	5.6		4.5	6.3		9.1
Di	24 ± 0.55	26		30	38		47
Tri	33 ± 0.60	32		37	39		33
Tetra	31 ± 0.58	29		24	15		9.1
Penta	6.7 ± 0.14	6.2		3.9	1.8		1.2
Hexa	0.94 ± 0.05	0.88		0.53	0.27		0.19
Glucose							
Mono	4.2 ± 0.96	5.8		6.7	7.1		9.1
Di	24 ± 0.55	29		37	42		44
Tri	33 ± 0.60	34		36	36		36
Tetra	31 ± 0.58	26		17	13		8.7
Penta	6.7 ± 0.14	5.0		2.4	1.7		1.2
Hexa	0.94 ± 0.05	0.76		0.32	0.20		0.17
Methanol							
Mono	4.2 ± 0.96	5.4		8.6	5.4		5.6
Di	24 ± 0.55	32		40	37		38
Tri	33 ± 0.60	32		33	38		38
Tetra	31 ± 0.58	24		14	16		15
Penta	6.7 ± 0.14	5.9		3.0	3.2		3.0
Hexa	0.94 ± 0.05	0.85		0.46	0.48		0.45

[a] Control values are averages of values from five samples.
[b] The percent Cl^- removed at 22 weeks for each substrate was as follows: acetate, 7; acetone, 22; glucose, 21; methanol, 13.

footnote b). In general, after 22 weeks in substrate-fed batches, peaks 15 through 44 (see Table 2 for peak identification) were reduced by dechlorination, which is reflected by the decrease in tetra-, penta-, and hexachlorobiphenyls relative to levels in controls (Table 3). In addition, peak 7 (2,5,2'; 4,4') was also significantly reduced. Peaks 1 through 13 increased in moles percent as a result of the dechlorination of the highly chlorinated congeners, which was reflected by the increase in mono- and dichlorobiphenyls relative to levels in controls (Table 3). The individual congeners that underwent dechlorination were similar for all substrate-fed batches. The control values shown in Table 3 are an average of results from five different controls, sampled at weeks 0, 4, 10, 19, and 22. The standard deviation of the controls was less than 1 mol% for each homolog, giving an indication of the variability of the analyses. All other PCB datum points were from single subsamples from different batches.

Acetate-fed batches had the least-extensive dechlorination

of all the batches receiving substrate. The volume of gas produced was less than expected for the acetate-fed batches on the basis of the stoichiometric conversion of acetate to methane and carbon dioxide.

The methanol-fed batches were similar to the acetate-fed batches in both initial rates and initial extents of dechlorination (after 4 weeks). Dechlorination and measurable gas production slowed considerably when substrate addition was stopped in both acetate- and methanol-fed batches. However, the final extent of dechlorination in methanol-fed batches was greater after 22 weeks than in the acetate-fed batches, as illustrated by the higher final levels of mono-, di-, and trichlorobiphenyls and the 13% removal of chlorine as chloride (Table 3).

The glucose-fed batches dechlorinated faster and more extensively than the acetate- or methanol-fed batches. Significant dechlorination continued even after substrate addition was stopped at 12 weeks.

Initially (over the first 10 weeks), the rates and extents of dechlorination in the acetone-fed batches were the slowest for all substrate-fed batches. This correlated to the 10- to 14-week lag period in measurable gas production in acetone-fed batches. Rates of dechlorination appeared to accelerate rapidly after gas production began, although gas was shown not to significantly strip PCBs from solution. After 22 weeks, the extent of dechlorination in the acetone-fed batches was
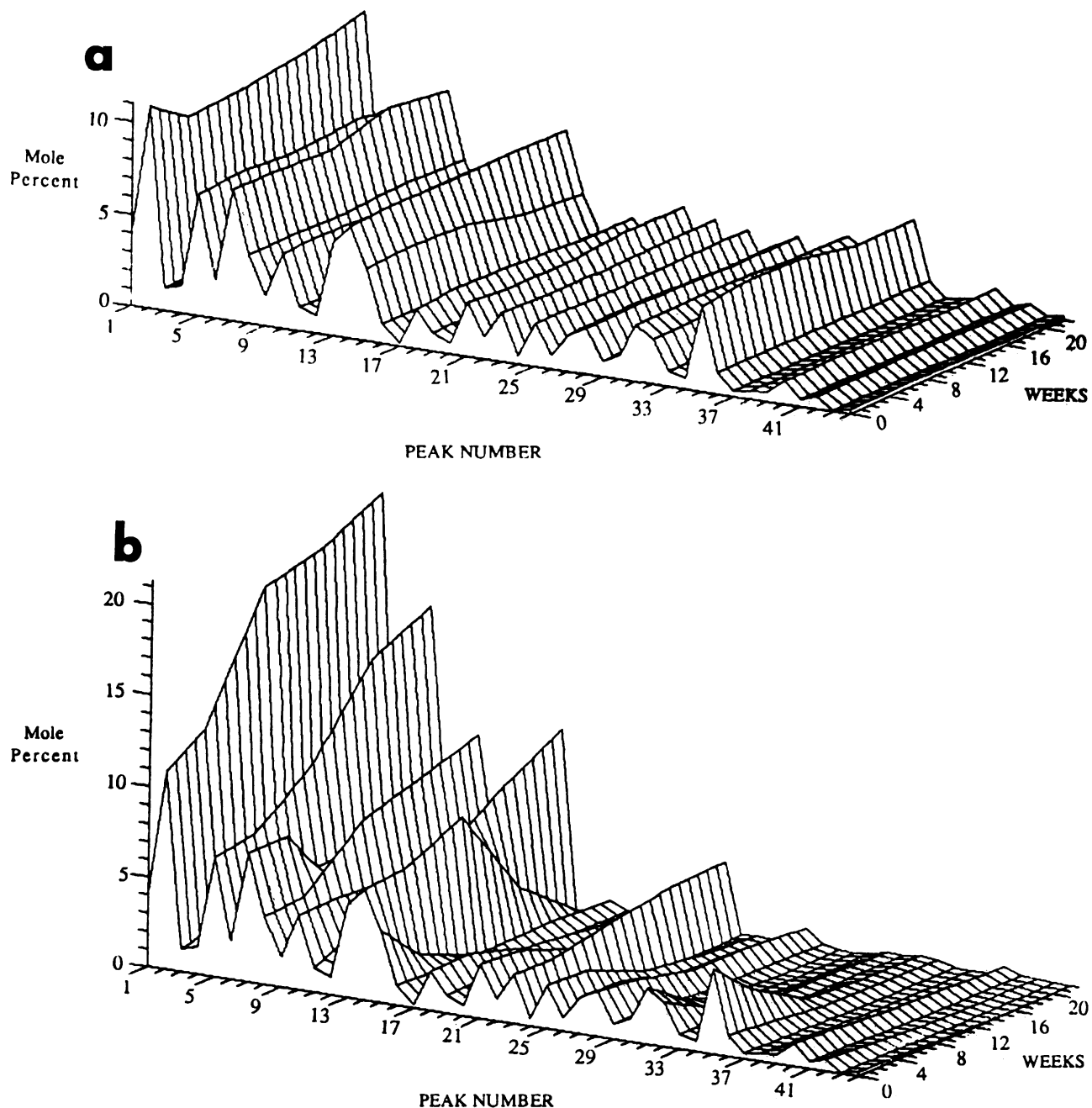
FIG. 1. Congener distribution of PCBs in batches receiving no additional organic substrate (a) and in glucose-fed batches (b) during 22 weeks of incubation.

comparable to that of glucose-fed batches, with 22 and 21% removal of chlorine as chloride, respectively (Table 3). The notable dechlorination activity between weeks 16 and 22 in the acetone-fed batches, which was rapid relative to that in glucose-fed batches, coincided with the onset of measurable gas production. Given the small amount of acetone added per batch, acetone appears to be one of the more efficient substrates for enhancing dechlorination.

In both acetone- and glucose-fed batches, more than 50% of the total PCBs were represented by mono- and dichloro-substituted congeners after 22 weeks. The primary products of dechlorination were 2,2' and 2,6 (peak 2), 2,4' and 2,3 (peak 5), and 2 (peak 1). The toxic coplaner congener

3,4,3',4' coeluted with 2,3,6,3',4' (peak 40). This peak decreased by 61 mol% in glucose-fed batches and by 71 mol% in acetone-fed batches over the 22-week incubation period.

DISCUSSION

Organic substrates, such as acetate, acetone, glucose, and methanol, appear to accelerate the dechlorination of PCBs in Hudson River sediment. A relationship also exists between substrate type and the rate and extent of dechlorination, although the congener pattern was similar in all batches. In experiments in which acetone or methanol (compounds often used as solvents for the addition of PCBs) was used as

an organic substrate, significant dechlorination was observed. In batches in which the organic substrate added was glucose or acetate, significant dechlorination was also observed. A new technique (described in Materials and Methods) for adding PCBs with only 2.5 µl of solvent prevented the solvent from being a significant supply of substrate for dechlorinating activity. In batches in which only 2.5 µl of solvent, the identical inorganic nutrients used in the substrate-fed batches, and no additional organic substrates were added, significant dechlorination was not observed after 22 weeks. Thus, differences in dechlorinating activity were observed with different substrates, although all of the substrates induced at least some dechlorination.

In the natural environment, organic compounds derived from the decomposition of detritus (13) may provide an organic substrate for the dechlorination of PCBs. In the laboratory, natural organics solubilized from sediments by autoclaving (5) might also provide sufficient substrates for dechlorinating microbes. After 54 weeks of incubation, slight dechlorination in batches receiving no additional organic substrate had occurred, indicating that the dechlorinating organisms were limited by the availability of substrate derived from organic matter in sediment. As indicated by active methanogenesis in all batches, the microbial environment was reduced; therefore, potential electron donors would have been available for mediating reductive dechlorination. The relative change in the proportion of a congener is a function of the rate of congener dechlorination and the rate of congener formation from the dechlorination of more highly chlorinated homologs. Peaks that initially increased and then decreased in later weeks illustrate this phenomenon (e.g., peak 14 in Fig. 1b). Currently, the long-term fate of the accumulated ortho-substituted products under anaerobic conditions is unclear. These lightly chlorinated congeners may eventually undergo dechlorination, or the process could stop with the accumulation of lightly chlorinated, ortho-substituted products under anaerobic conditions.

The organic substrates used in these experiments were the electron donors in a reduced methanogenic environment. The reductive dechlorination process requires an electron donor provided by a transfer of electrons from an organic substrate to PCBs through a microbial system. These substrates, or their intermediate metabolites, may be electron donors coupled with PCBs as terminal electron acceptors. The efficiencies of the organic substrates for inducing reductive dechlorination activity varied. For example, acetone-fed batches received the smallest amounts of substrate on an electron-mole basis and produced the least gas, and yet the extent of dechlorination was nearly equivalent to that of glucose-fed batches that received several substrate doses and produced more gas. Three possible explanations, each suggesting different mechanisms of dechlorination activity, exist. (i) Substrate enrichment selects for a specific dechlorinating organism(s), or a consortium of organisms, which is able to supply potential electron donors to the dechlorinating organism. (ii) The variation in apparent efficiencies is related to the thermodynamics of the electron donor-PCB redox couple. (iii) Dechlorination is a detoxication mechanism.

PCBs could possibly be used as terminal electron acceptors by microorganisms in an energy-yielding metabolic process. In reduced anaerobic sediments there is often a deficiency of potential electron acceptors (O_2, NO_3^-, SO_4^{2-}) relative to the supply of electron donors. In PCB-contaminated sediment, microorganisms might have adapted the ability to use PCBs as electron acceptors in the absence of other suitable acceptors and, hence, would potentially have

a competitive advantage over other microbes (4). If adapted organisms are using PCBs as electron acceptors, organisms from non-PCB-contaminated sites would not have the ability to use PCBs as electron acceptors and immediate reductive dechlorination would not be observed if PCBs were introduced. Another possibility is that PCBs are fortuitously dechlorinated by low-potential electron donors in anaerobic environments as electrons flow from organic substrates through the microbial electron transport processes to terminal electron acceptors. The organisms would derive no energetic benefit from this reaction, and if this is true, dechlorination should be observed in actively methanogenic sediments into which PCBs had not been previously introduced.

Regardless of the mechanism of dechlorination, the rates in this laboratory study appear much more rapid than those in the environment. For example, hexa- and pentachlorobiphenyls were reduced by 80% and tetrachlorobiphenyls were reduced by 70% in both glucose- and acetone-fed batches after 22 weeks. The in situ dechlorination of PCBs apparently occurs more slowly in some contaminated sediments (3). Thus, in situ organic substrate addition to these PCB-contaminated sites might accelerate the anaerobic dechlorination of the more-toxic highly chlorinated congeners.

Anaerobic dechlorination could potentially be used as the first step in a remediation process, both in reactors and in situ. The decrease in highly chlorinated congeners resulting from anaerobic reductive dechlorination would increase the nonchlorinated positions on the biphenyl ring. The lightly chlorinated ortho-substituted congener products would be less toxic (10). Reductively transformed PCB congeners are more amenable to aerobic degradation. All lightly chlorinated congeners that are products of reductive dechlorination are biodegraded by aerobic PCB-degrading microorganisms (1, 2). Aerobic degradation could be used as the second step in a bioremediation process. Anaerobic sites where significant dechlorination has occurred could be aerated to complete a sequential anaerobic-aerobic bioremediation process. This in situ mineralization of hazardous compounds might be preferable to their retrieval and ultimate storage in landfills.

The PCBs dechlorinated in this study were added to sediments in the laboratory. Microbial transformation of PCBs bound in historically contaminated sediments may be controlled by physical-chemical desorption mechanisms as well as rates of microbial dechlorination. Therefore, although these results hold promise for inducing PCB dechlorination, understanding of other environmental factors is required for any attempted remediation.

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