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Assessment of Continuous Biodegradation of Commercial PCB Formulations

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Polychlorinated biphenyls (PCBs) pose great environmental concern, largely because of their behaviour in terms of biodegradability. Improper handling of PCBs and poor disposal procedure have both contributed to their wide-spread occurrence in the environment (PEAKALL 1975; FRANK et al. 1977), and accumulation in the food chains (WARE & ADDISON 1973; BRAESTRUP et al. 1974; MUSIAL et al. 1974; VEITH et al. 1979). Thus, recently, many of the world's industrial nations have begun to establish legislation which requires that all new chemicals, and existing chemicals of possible environmental concern, should be subjected to hazard evaluation in which a chemical's biodegradability is used as one of the major criteria.

Biodegradation studies are still in their infancy with many new methods and techniques needing to be developed. This is evidenced by the recent initiation of the ring test programmes on chemical's biodegradability by OECD (Organization for Economic Co-operation and Development) in 1978 and by ISO (International Standards Organization) in 1979, indicating that an increased research activity is required in this area.

Most studies on PCB degradation have been conducted in the laboratory, on a relatively small scale, employing either rotary shaker culture (AHMED & FOCHT 1973; WONG & KAISER 1975; FURUKAWA & MATSUMURA 1976) or glass-stoppered bottled culture (REICHARDT et al. 1981). Although such an approach is a useful means of providing information on the metabolic pathways of PCB degradations (HUTZINGER et al. 1974), it fails to include any consideration of effects which may limit the applicability of laboratory results in the natural environment.

The objective of the present study has been to assess continuous biodegradation of commercial PCB formulations under a simulated wastewater treatment process, which is more typical of environmental conditions. The studies are based on the use of a large bench scale test.

MATERIALS AND METHODS

Chemicals: The commercial PCB formulations (Aroclor 1221, Lot No. KD-07-101; Aroclor 1016, Lot No. KD-06-761; Aroclor 1254, Lot

0007-4861/82/0029-0200 $01.60

No. KD-11-631) were courteously provided by the Monsanto Co., St. Louis, MO. All pure PCB isomers were obtained from Analab, North Haven, CT. Sodium ligninsulfonate was acquired from Matheson Coleman and Bell, Cincinnati, OH. Organic solvents were glass distilled, as supplied by Caledon Laboratories Ltd., Georgetown, ONT.

PCB Emulsion: A small stainless steel sonic cup, containing 1,000 mg of PCB, 1 mL of sodium ligninsulfonate solution (50 mg mL^{-1}) and 10 mL of distilled water, was placed in a model 350 ultrasonic apparatus (Heat Systems Ultrasonics Inc., Plainsview, N.Y.) and the contents were subjected to a pulse sonification (50%) for 2 min. The resultant emulsion was very uniform and stable.

Bacterial Culture and Medium: A PCB-degrading Pseudomonas sp. 7509, originally isolated from activated sludge (LIU 1980) was used in the present study. The culture was maintained on a mineral medium with Aroclor 1221 as the sole carbon and energy source. Fresh municipal raw sewage (BOD_5 = 140 to 170 ppm), fortified with 20 mg L^{-1} each of nitrogen (as NH_4Cl) and phosphorus (as KH_2PO_4 + K_2HPO_4), was employed as the biodegradation medium in the fermentor.

Continuous Biodegradation Test: The bench scale test was carried out in a 14-L microferm fermentor (New Brunswick Scientific Co., Edison, N.J.) equipped with automatic temperature, dissolved oxygen and agitation speed controls. The level of the medium in the fermentor was controlled by a glass level controller, which was interfaced with a constant speed control unit (Cole Parmer Co., Chicago, IL) and a model E650-048 peristaltic pump (Electro Craft Corp., Hopkins, MN). The inflow of the growth medium was also controlled by a similar but separate unit. The stock tank was a 20-L glass carboy, which contained 15 L of fortified sewage plus ligninsulfonate and Aroclor at concentrations of 50 and 100 mg L^{-1}, respectively. In a typical continuous operation, the fermentor was charged with 9 L of fortified raw sewage and 1 L of inoculum (P. sp. 7509). The temperature of the fermentor was kept at 20°C, with the impeller speed at 400 rpm and the dissolved oxygen at 2 mg L^{-1} level. After a period of three days (for equilibrium), the growth medium containing Aroclor was added to the fermentor, at a flow rate of 26 mL h^{-1}. The duration of each experiment varied according to the type of Aroclor used. Details of the fermentor operation, in batch mode, are provided in a previous publication (LIU 1981).

Determination of Degradation: At appropriate time intervals, 20 mL of broth were withdrawn from the fermentor for the determinaton of the primary biodegradability; i.e., following the disappearance of PCBs from the growth medium. All samples were run in duplicate. Upon acidification with two drops of 12 N H_2SO_4, 5 mL of the fermentor broth and 1 mL of n-hexane were vigorously mixed in a 15-mL conical glass centrifuge tube for 1 min on a vortex mixer. The emulsion was broken down by centrifugation at

2,000 × g for 5 min, and the clear hexane extract was used for g.c. analysis. A gas chromatograph, equipped with dual FID and interfaced with a Spectra Physics SP 4000 chromatographic data system was employed for such analysis. The 1.8 m × 2 mm i.d. stainless steel columns, containing 10% OV-1 on Chromosorb W(AW-DMCS), were temperature programmed from 150 to 250°C at 8°C min^{-1}, while the injector and detector were kept at 250 and 280°C, respectively. The carrier gas was nitrogen, at a flow rate of 30 mL min^{-1}.

RESULTS AND DISCUSSIONS

One difficulty, encountered in the study of biodegradation of commercial PCB formulations, is that the formulations themselves are not single compounds, but rather a complex mixture of different isomers (TUCKER et al. 1975). This not only complicates the analytical procedure, but also presents an intricate degradation mechanism due to the presence of a multi-component substrate in the growth medium. This complexity is not well reflected in experiments using the shaker culture technique because progressive deterioration of the growth meidum, from nutrient exhaustion and accumulation of toxic metabolic waste in the shaker culture, tends to overshadow such complexity (MALEK & FENEL 1966). Table 1 demonstrates the usefulness of the continuous culture technique, in evaluating the biodegradation process of multi-component Aroclor 1221. Apparently, the medium flow rate had a profound effect on the pattern of Aroclor 1221 degradation. At low flow rates of 13 and 26 mL h^{-1} (retention times equal to 32.1 and 16.0 days, respectively), all the seven major components in Aroclor 1221 (biphenyl, 2-chlorobiphenyl, 4-chlorobiphenyl, 2,2'-dichlorobiphenyl, 2,4,-dichlorobiphenyl, 2,4'-dichlorobiphenyl, and 4,4'-dichlorobiphenyl), were degraded by P. sp. 7509. This was expected, since only limited amounts of substrate (31.2 and 62.4 mg Aroclor 1221 d^{-1}) were supplied to the fermentor. Consequently, the bacteria had no alternative but to degrade all components in the Aroclor 1221 to obtain enough energy for growth.

However, with higher medium flow rates, excess amounts of Arochlor 1221 were made available to P. sp. 7509 and the bacteria were, thus, capable of exhibiting a preference for a given component among the mixture of substrates. This is indeed, what happened under the situation of unlimited substrate supply (Aroclor 1221); from Table 1 it can be seen that some accumulation of 2,2'-dichlorobiphenyl, 2,4'-dichlorobiphenyl and 4,4'-dichlorobiphenyl occurred in the fermentor broth at higher flow rates (39 to 91 mL h^{-1}). It should also be noted that, despite the highest flow rate of 91 mL h^{-1} (retention time = 4.6 days), 70 to 75% of these three isomers (above), had been degraded in the fermentor under the process of continuous biodegradation. The four other components (biphenyl, 2-chlorobiphenyl, 4-chlorobiphenyl and 2,4-dichlorobiphenyl), in the Aroclor 1221 appeared to be degraded soon after their addition to the fermentor. Thus, it appears that, in the continuous process of Aroclor 1221 biodegradation, not only are the preferred components (such as biphenyl and monoclorobiphenyls) degraded first, but that the other more persistent isomers are also degraded, though at a lesser rate.

Table 1. Effect of flow rate on Aroclor 1221 degradation*

Peak No.	Components	% in Aroclor 1221 Before Degradation	Flow Rate (mL/h) 13	26	39	52	65	91
1	Biphenyl	13.95	0	0	0	0	0	0
2	2-PCB	34.21	0	0	0	0	0	0
3	4-PCB	21.29	0	0	0	0	0	0
4	2,2'-PCB	5.74	0	0	10.73	12.90	26.57	28.79
5	2,4-PCB	2.61	0	0	0	0	0	0
6	2,4'-PCB	13.36	0	0	5.12	8.75	33.53	28.28
7	4,4'-PCB	5.42	0	0	22.95	22.25	20.59	23.55
TOTAL PCBs build-up			0.44	0.60	1.80	2.10	4.31	6.10

* The fermentor was at 20°C with impeller at 400 rpm and DO at 2 ppm.

Table 2 shows the degradation course of each individual isomer in Arochlor 1221, at the medium flow rate of 52 mL h^{-1} (retention time = 8.0 days). Biphenyl was apparently metabolized more rapidly than any of the monochlorobiphenyls and dichlorobiphenyls by P. sp. 7509. In addition, there were significant differences in the degradation rates of the individual monochlorobiphenyl and dichlorobiphenyl isomers. While this situation is consistent with the generalization that the more highly chlorinated biphenyls are more resistant to biodegradation (REICHARDT et al. 1981), the results in this paper also demonstrate that the position of chlorine substitution on the biphenyl molecule is one of the major factors determining its biodegradability. Thus, caution must be exercised when labortory experimental results, based on pure substrate studies, are used to predict the environmental behaviour of commercial formulations, which may contain a various mixture of isomers.

The biodegradation potential of P. sp. 7509 to oxidize Aroclor 1221 under the continuous culture growth, is further demonstrated in Figure 1. The number above each peak of the gas chromatograms corresponds to the peak number identified in Table 1. Rapid degradation of Arochlor 1221, at the very high medium flow rate of 91 mL h^{-1}, was observed in the fermentor. For example, 88% of 2-chlorobiphenyl (peak No. 2) in the broth was eliminted between the time intervals of 42 to 50 h, indicating the ability of P.

sp. 7509 to degrade the monochlorobiphenyl in Aroclor 1221. To further examine the effect of the flow rate on Aroclor 1221 degradation, the build-up and disappearance of 2,4'-dichlorobiphenyl in the fermentor broth was followed (Figure 2). There was no apparent accumulation of this isomer, at low flow rates of 13 to 26 mL h^{-1} at the end of the experiments. A slight build-up (2 to 2.5%) was observed at a higher flow rate (39 to 52 mL h^{-1}). However, an increase of the medium flow rate from 52 mL h^{-1} to 65 to 91 mL h^{-1}, resulted in a significant amount (7.0 to 12.5%) of 2,4'-dichlorobiphenyl accumulation in the fermentor broth. The above findings indicate that complex degradation mechanisms are involved in the oxidation of multi-component substrates such as Aroclor 1221. The data presented in this study do not characterize the details of such degradation mechanisms, but they do suggest the value of using a continuous culture technique.

Table 2. Percentage of Aroclor 1221 degradation at the flow rate of 52 mL h^{-1}

Time (h)	Peak Numbers						
	1	2	3	4	5	6	7
0	0	0	0	1.1	0	0	18.5
20	0	4.1	0.7	8.0	6.1	7.0	24.3
41	0	0.3	0	14.1	0	9.0	23.3
49	0	0.2	0	14.6	0	8.2	21.5
66	0	0.1	0	14.9	0	5.5	20.9
73	0	0.1	0	15.0	0	4.3	22.2
138	0	0	0	2.4	0	4.5	11.4
162	0	0	0	2.5	0	4.3	8.3
186	0	0	0	3.9	0	3.4	8.1
210	0	0	0	4.7	0	3.3	8.6

The biodegradation of higher chlorinated commercial PCB formulations was also investigated using a continuous culture technique. In these experiments, the medium flow rate was kept at 26 mL h^{-1}, with dissolved oxygen at 2 mg L^{-1} and the impeller speed at 400 rpm. The data in Tables 3 and 4 indicated that Aroclor 1016 and 1254 were more resistant to microbial degradation than Aroclor 1221. This was expected since the major components in Aroclor 1221 are a mixture of monochlorobiphenyls, while Aroclor 1016 and 1254 are primarily composed of trichlorobiphenyls and pentachlorobiphenyls, respectively (TUCKER et al. 1975). It should be noted here that the packed columns, used in the analytical procedure, were incapable of resolving all the isomers in Aroclor 1016 and 1254. Consequently, the results of Tables 3 and 4 should be considered only qualitative, since more than one isomer could appear under a single peak. The inability of P. sp.7509 to rapidly degrade the higher chlorinated PCBs,

Table 3. Degradation of Aroclor 1016 under continuous culture growth

Time (h)	% Individual Peak Build-up											
	1	2	3	4	5	6	7	8	9	10	11	12
0	3.7	52.8	0	0	13.0	0	0	0	0	0	0	0
21	18.2	46.5	4.2	0	16.4	0	4.2	0	1.4	1.3	0	2.8
45	21.5	39.2	5.0	10.4	14.5	11.2	8.5	0	5.1	3.3	4.4	3.0
100	18.4	36.6	8.7	16.0	17.6	16.4	15.4	0	10.5	7.7	12.0	9.4
165	8.2	31.9	9.6	19.5	18.0	18.0	10.5	13.3	13.7	11.0	17.6	14.6
214	7.1	29.3	10.4	20.1	18.3	18.7	12.2	14.5	15.8	12.4	19.7	17.4
358	5.4	40.2	13.2	28.3	35.4	38.3	32.1	31.2	35.5	30.8	34.3	21.4
460*	7.8	33.7	3.5	28.5	38.1	41.4	42.6	**	38.9	33.5	40.2	28.5
618	0	11.3	0	9.1	12.3	16.7	10.6		20.6	17.3	23.2	10.8

* After 460 h the fermentor was switched to batch operation.

** Peak No. 8 started to integrate with peak No. 7.

Table 4. Degradation of Aroclor 1254 under continuous culture growth

Time (h)	% Individual Peak Build-up								
	1	2	3	4	5	6	7	8	9
0	0	0	0	0	0	0	0	0	0
17	7.4	0	0	1.2	0	0	0	0	0
48	13.0	19.0	38.9	4.5	3.4	2.5	2.3	2.2	2.3
140	16.8	17.6	39.4	8.3	7.0	5.5	5.5	5.2	4.3
162	17.6	22.1	38.0	9.5	8.2	6.4	6.4	6.0	5.0
190	17.0	22.7	38.1	9.9	8.6	6.8	6.6	6.2	5.1
214	20.0	24.8	40.8	12.2	10.7	8.5	8.1	7.7	6.3
329	25.0	29.3	40.0	18.6	17.6	16.7	15.2	14.2	10.7
476*	34.0	36.6	47.3	30.6	30.9	30.1	29.4	29.2	26.8
720	27.7	30.8	51.5	21.8	23.8	20.3	19.4	20.0	20.0

* After 476 h the fermentor was switched to batch operation.

under continuously cultured growth, is reflected by the significant amount of Aroclor 1016 and 1254 accumulation in the fermentor broth. However, a change in the fermentor operation, from continuous process to batch operation at 460 h, resulted in the

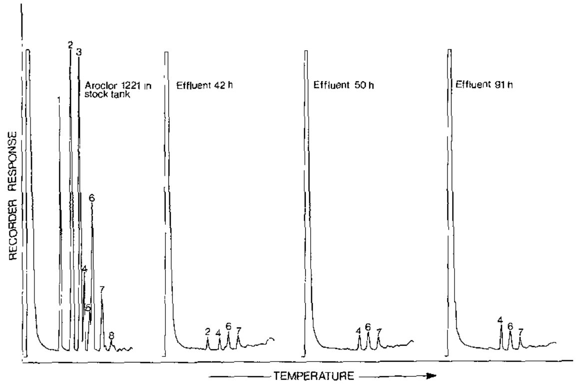

Figure 1. Gas chromatograms showing the biodegradation of Aroclor 1221 at a flow rate of 91 mL h^{-1}

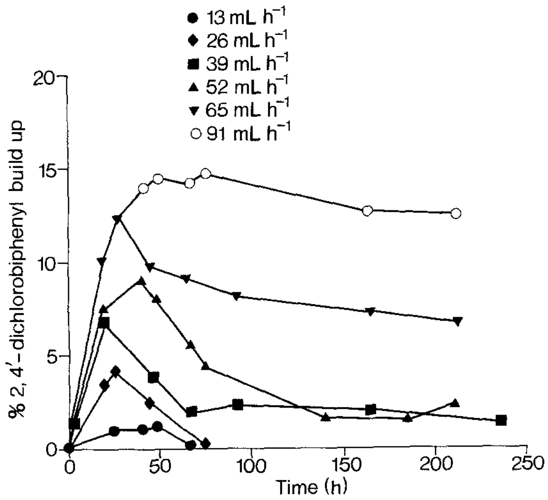

Figure 2. Effect of medium flow rate on the degradation pattern of 2,4'-PCB in Aroclor 1221.

degradation of Aroclor 1016 within the next 6.6 days (Table 3), while only 30% more of the Aroclor 1254 was degraded, after the fementor had been in batch operation for 10.1 days.

CONCLUSION

The bench scale test demonstrates that substrate availability in the growth medium can significantly effect the biodegradation pattern of commercially formulated PCBs. Under limited substrate supply, all seven major components of Aroclor 1221 were degraded by P. sp. 7509. With excessive amounts of nutrient, however, some preferences for given components were observed. The position of chlorine substitution on the biphenyl molecule appears to be one of the major factors determining an isomer's biodegradability. The biodegradation rate of higher chlorinated PCB formulations was much slower under the continuous loading. However, significant degradation of such formulations did occur, when the fermentor was switched to batch operation.

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