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Biodegradation of Pentachlorophenol in a Simulated Aquatic Environment

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Pentachlorophenol (PCP) and its salt, sodium pentachlorophenate (NaPCP), are two of the most versatile pesticides now in use in the United States and Canada (CIRELLI 1978, HOSS 1978). Because of their effectiveness against a wide spectrum of target organisms, PCP and NaPCP are used extensively as fungicide, herbicide, insecticide, bactericide, and molluscicide in forest, agriculture, textile, and paint industries. Worldwide production of PCP has been estimated to be about 200 million pounds per year and is expected to increase (DETRICK 1977).

As a result of its wide application, PCP has been detected in human and animal tissues (SHAFIK 1973, BEVENUE & BECKMAN 1967), in fishes (LANDNER et al. 1977) as well as in aquatic environments (BUBLER et al. 1973, MATSUMOTO et al. 1977), indicating this chemical's wide distribution in the total environment. Therefore, knowledge of its environmental behavior and fate is essential for effective management of its usage and waste handling.

Microbial activity affects the persistence of an organic biocide in the natural environment by means of biodegradation and biotransformation. Various microorganisms such as bacteria (REINER et al. 1978, WATANABE 1973, SUZUKI 1977) and fungi (CSERJESI & JOHNSON 1972) have demonstrated their capability of degrading PCP and other chlorophenols. However, some discrepancies appear to exist between the degradation pathways observed in natural soil and those observed in isolated microbial cultures implying the complexity involved in the study of biodegradation of biocides. For example, while PCP metabolism by isolated soil microorganisms occurs by oxidative mechanisms, its degradation in natural soil was found mainly via reductive pathway (KAUFMAN 1978). Very limited information is available regarding PCP biodegradation in aquatic ecosystem and a recent study by BOYLE et al. (1980) indicated that the oxidative pathway is the major mechanism for PCP degradation in a simulated lentic environment. The purpose of this study was to determine the rate of NaPCP biodegradation: (1) under aerobic/anaerobic conditions, (2) with/without co-metabolites and, (3) with different nitrogen sources, in laboratory environmentally-controlled fermentors employing an acclimatized bacterial culture from activated sludge.

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MATERIAL AND METHODS

Reagents and Growth Medium. Organic solvents were glass distilled as supplied by Caledon Laboratories Ltd. and the pentachlorophenol was obtained from Aldrich Chemicals, Inc., Milwaukee, WI. The growth medium developed by WATANABE (1973) was used throughout the experiments with minor modifications as follows: $NaNO_3$, 0.5 g; K_2HPO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; NaPCP, 2 mg; distilled water 1 L. Final pH of the medium was 7.0 and the medium was not sterilized.

Enrichment Technique. After many futile attempts to obtain an active PCP-degrading bacterial culture by directly using fresh activated sludge as the inoculum, an enrichment culture was started utilizing 1 L of activated sludge (MLSS 5500 ppm) as the sole growth medium plus 5 ppm of NaPCP and 20 ppm of Na 4-chlorophenate (NaMCP) as the inducers. The culture was maintained in a cyclone fermentor (LIU & TOWNSLEY 1972) and periodic examination of PCP and MCP concentrations in the culture broth was made by g.c. analysis. After approximately 6 months incubation, an active PCP-degrading bacterial culture had developed which was verified by repeatedly adding NaPCP (2 ppm) to the fermentor and following its rapid disappearance from the broth. The culture was subsequently maintained in 5% activated sludge with 2 ppm of NaPCP. Finally, the active culture was studied using the mineral medium described with NaPCP as the sole source of carbon and energy as mentioned previously.

Experimental Design. Due to the many variables involved, it is impractical to study the biodegradation of PCP in all natural environments and consequently degradation studies are conducted in the cyclone fermentors under controlled laboratory conditions which are environmentally relevant such as aeration and anoxia. Because abiotic degradation processes such as hydrolysis and photolysis sometimes occur during biological degradation, control fermentors with microbial inhibitors were also included to allow for correction for such processes. Thus six fermentors were used: three were operated under aerobic conditions and three others in an anaerobic environment. Under each of these two basic conditions, PCP was simultaneously subjected to metabolic (PCP as sole carbon and energy source), co-metabolic (with the more easily biodegradable MCP) and abiotic degradations in separate fermentors. The abiotic system was accomplished by using microbial inhibitors as follows: $HgCl_2$, 100 ppm; KCN and NaN_3 at 5 m mole L^{-1} each. The fermentors were operated at 22°C with a steady supply of air (aerobic) and nitrogen (anaerobic) at 20 mL min^{-1}. The concentrations of PCP and MCP used were 2 and 5 ppm, respectively, with mineral medium described above as the growth broth.

Extraction and Analysis. At various time intervals, 50 mL of culture broth were withdrawn from the fermentors and mixed for 1.0 h with 50 mL of 0.1 N K_2CO_3, 5 mL of acetic anhydride, and 2 mL of n-hexane in a 125-mL narrow mouth Erlenmyer flask on a magnetic stirrer. The hexane layer was analyzed for the phenolic acetates on a gas chromatograph equipped with dual FID. The dual 180 cm x 2 mm i.d. stainless steel columns contained 10% OV-1 on 80/100 mesh Chromosorb W (AW-DMCS). The injection port and the detector were maintained at 250 to 300°C, respectively, while the oven was programmed from 150 to 250°C at 8°C min^{-1}. The carrier gas was nitrogen at a flow rate of 30 mL min^{-1}. Low resolution mass spectra were used for confirmation using a Finnigan 3200 GC/MS system interfaced with PDP 8/e computer and a 180 cm x 2 mm i.d. glass column (3% OV-1 on Chromosorb W). Mass spectrometer conditions were: electron energy 70 eV; temperature of ion source and transfer line 175°C.

Measurement of Biodegradation Rate. PCP biodegradation was measured in terms of primary degradation, i.e., by following the disappearance of PCP from the fermentor broth after correction from loss due to abiotic process. Rate constants (k) were determined for the assumed first-order process in which

$$-\frac{dc_s}{dt} = kt \quad (\text{integrated form: } \ln \; C_s^o/C_s = -kt)$$

where C_s = concentration of PCP and the superscript "o" refers to time zero. The half-lives are determined from the expression $t_{\frac{1}{2}}$ 0.693/k. For all of these calculations, only values greater than 10% of control were employed. Where a lag phase was noted, these data were also omitted for the calculations.

RESULTS AND DISCUSSIONS

Various environmental factors, notably the availability of oxygen and organic nutrients among others, may affect or limit the capability of microorganisms degrading a biocide in aquatic environment. This phenomenon of environment-bacteria-biocide interaction was observed in the present investigation (Table 1). Among the factors studied, oxygenation in terms of aerobic or anaerobic condition in the growth medium was found to have a profound effect on the rate of PCP degradation (Figure 1). After 3 days, the concentration of PCP in the aerobic fermentor had decreased to a negligible amount, while 100% of the added PCP remained unchanged in the anaerobic fermentor. Further incubation of the latter to 28 days resulted in only 5% degradation, indicating the inherent persistence of PCP under anaerobic environments. The half-lives of PCP concentrations in aerobic and anaerobic metabolic fermentors were calculated to be 0.36 and 192 days, respectively.

In view of the complexity involved in the determination of a biocide's biodegradability, our results could be considered in agreement qualitatively with those of BOYLE et al. (1980). These authors indicated that PCP had a shorter half-life ($t_{\frac{1}{2}}$ = 19 days) in the oxygenated water column than that in water depleted of oxgyen ($t\frac{1}{2}$ = 80 days) in their simulated lentic environment. The difference between our results and their data may be at least partially explained by the fact that we used the acclimatized culture while natural pond water was employed in their study. Nevertheless, the role of aerobic and anaerobic environments in determining the pertistence/biodegradability of PCP is clearly demonstrated in the present study.

In Canada, as elsewhere, the assessment of biodegradability of organic substances in fresh waters is of particular concern. It is our experience, and that of others (JUNGCLAUS et al. 1978), that many of the organic substances of environmental concern are ultimately found in the sediments of lakes and rivers. It is also true that many of those (sediment) environments are anaerobic which tends to retard degradation of some of these organic contaminants. Thus it is not surprising that high concentrations (0.1 - 10 ppm) of PCP have been detected in river sediments(FOUNTAINE et al. 1976) and in lake sediments (up to 1.2 ppm), while the same lake water had a level of only 10 - 30 ppb (PIERCE et al. 1977).

It may be of interest to note that during the process of PCP degradation, MCP, the co-metabolite, was found to biodegrade under both aerobic ($t\frac{1}{2}$ = 0.29 day) and anaerobic conditions ($t\frac{1}{2}$ = 5.8 days). This observation also indicates that even the low chlorinated phenols are susceptible to conditions of oxygenation in the natural environment. This observation also illustrates the difficulty of predicting the environmental behavior of the new chemicals from other "bench mark" chemicals - the ratio of 20:1 for anaerobic; aerobic degradation for MCP would obviously not be applicable to PCP(53:1).

To further examine the impact of co-metabolism on PCP biodegradability, glucose (500 mg L^{-1}) was added to the aerobic fermentors as co-metabolite (Figure 2). Both glucose and MCP were found to suppress the rate of PCP degradation. Glucose at 100 pm has been reported to retard PCP biodegradation (WATANABE 1973). However, the repression of PCP degradation by MCP is rather surprising since low chlorinated phenols such as MCP were expected to stimulate PCP degradation as was the case with PCB cogener degradation (BAXTER et al. 1975). At this moment the reason for that is unknown.

The effect of nitrogen source in the growth medium on PCP degradation was also investigated (Table 1). Among those examined, yeast extract showed a distinct stimulation effecton PCP degradation which agreed with the literature reports (WATANABE 1973, SUZUKI 1977). Peptone, however, tended to have some inhibitory effect on PCP biodegradability. Ammonium sulfate, ammonium nitrate and urea did not exhibit any significant stimulation or inhibition effect when compared with sodium nitrate which was the nitrogen source in our basal medium.

Table 1. Degradation of PCP and MCP in Fermentors

Reaction Conditions	Induction Period (day)	Degradation $t_{1/2}$(day)	k(h^{-1})x10^2
Aerobic metabolism PCP	2.1	0.36	7.4
Aerobic co-metabolism PCP	2.5	0.52	5.1
MCP	1.3	0.29	9.1
Anaerobic metabolism PCP	13.0	190	0.014
Anaerobic co-metabolism PCP	20.0	>200	-
MCP	3.9	5.8	-
Nitrogen source*			
$NaNO_3$	1.2	0.63	4.2
$(NH_4)_2SO_4$	1.2	0.75	3.5
NH_4NO_3	1.2	0.85	3.1
Urea	1.2	0.61	1.9
Peptone	1.9	1.2	2.3
Yeast extract	1.2	0.36	7.3

* Experiments were carried out under aerobic metabolism conditions

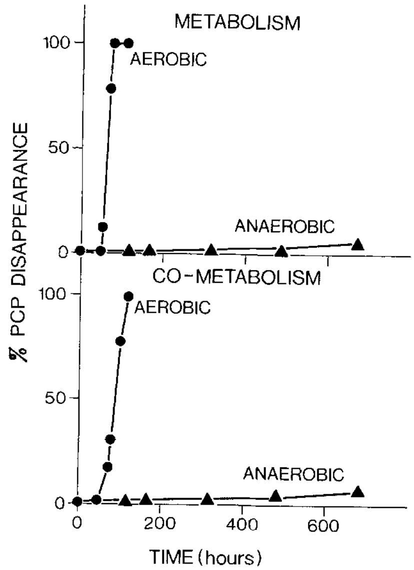

Fig. 1. Disappearance of PCP in cyclone fermentors

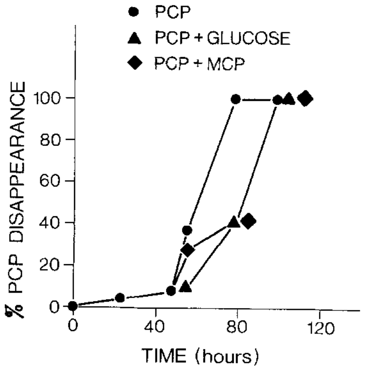

Fig.2. Effect of co-metabolites of the aerobic disappearance of PCP in cyclone fermentors

We wish to emphasize here that our laboratory biodegradation system does not pretend to be a natural aquatic ecosystem. It does, however, offer a tool for investigating the complex interactions between chemicals and different environmental conditions where rigid controls are needed in order to differentiate degradation mechanisms.

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