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Biodegradation of Aroclor 1221 Type PCBs in Sewage Wastewater

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Highly valued for their electric insulating and other properties, polychlorinated biphenyls (PCBs) have, until recently, found extensive use in industry as dielectric fluids in transformers, hydraulic fluids in heavy machinery, and in formulations for paint and ink (BROADHURST 1972). It has been estimated that the total world production of PCBs during the past 45 years has exceeded one million tons, of which about 40% has been released to the environment (Interdepartmental Task Force 1972). Unfortunately, their stability also increases their persistence, and indeed they do not break down easily in the natural environment including the lower chlorinated ones (DUINKER et al. 1980). As a result, high concentrations of PCBs have accumulated at various levels in the food-chains in our ecosystem. Thus PCBs have been detected in both fresh water and sea water (PEAKALL 1975), in sediments (FRANK et al. 1977), plankton (WARE & ADDISON 1973), fishes (VEITH et al. 1979), birds (BRAESTRUP et al. 1974), and in human tissues (MUSIAL et al. 1974) indicating that they are ubiquitous contaminants in the total environment.

The literature now abounds with reports concerning the metabolism of PCBs in living organisms and several excellent works regarding microbial degrdation of PCBs have been published (AHMED & FOCHT 1973; HUTZINGER et al. 1974; WONG & KAISER 1975; FURUKAWA & MATSUMURA 1976). Studies on microbial degradation of PCBs are essential to understand the behaviour of PCBs in the environment. One difficulty encountered in the study of the interaction between PCBs and biota is that PCBs are not single compounds, but complex mixture of different isomers (TUCKER et al. 1975), which greatly complicates the assessment of the processes of interaction with microorganism such as biodegradation and biotransformation. For example, PCBs mixtures were reported to biodegrade much faster than the pure components (BAXTER et al. 1975) implying that different mechanisms could be involved in the degradation of commercial PCBs formulations and their correponding single isomer. As significant amounts of PCBs enter the aquatic environment via the route of the wastewater effluents (BERGH & PEOPLES 1977), there is a need for information concerning the fate and behaviour of PCBs in the sewage effluent. The objective of this study was to assess the biodegradation of commercial PCBs formulations in municipal sewage including the factors affecting the degradation.

MATERIALS AND METHODS

Chemicals: The commercial PCBs mixture (Aroclor 1221, lot no. KD-07-101) was courteously provided by the Monsanto Co., St. Louis,

0007-4861/81/0027-0695 $01.80

MO. All pure PCB isomers were obtained from Analab, North Haven, CT. Sodium ligninsulfonate was acquired from the Matheson Coleman and Bell, Cincinnati, OH. Organic solvents were glass distilled as supplied by Caledon Laboratories Ltd., Georgetown, ON.

PCBs Emulsion: A small stainless steel sonic cup containing 1000 mg of PCBs, 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 with an average PCBs droplet size of 0.5-1 um.

Bacterial Culture and Media: The bacterial culture used in the present study was a PCB-degrading Pseudomonas sp. 7509 originally isolated from the activated sludge (Liu 1980). The culture was maintained on the following basal medium with Aroclor 1221 as the sole carbon and energy source (g L^{-1}): K_2HPO_4, 1.3; KH_2PO_4, 0.82; $(NH_4)_2SO_4$, 1.0; $MgSO_4.7H_2O$, 0.05; $FeSO_4.7H_2O$, 0.01; $CuSO_4.5H_2O$, 0.01; $CoCl_2.6H_2O$, 0.01; $MnCl_2.4H_2O$, 0.01; NaCl, 0.05. The final pH of the medium was 6.9 and was sterilized at 121°C for 15 min. The PCBs emulsion and ligninsulfonate were subsequently added to the medium at concentrations of 100 and 50 ppm, respectively. For the bench scale biodegradation test, fresh municipal raw sewage fortified with nitrogen (as NH_4Cl) and phosphorus (as KH_2PO_4 + K_2HPO_4) at 20 ppm each was employed as the growth medium. The raw sewage was not sterilized with a typical BOD concentrations of 140-170 ppm.

Determination of Degradation: In all experiments, only the primary biodegradation of PCBs was followed, i.e., the disappearance of PCBs from the growth media or by measurement of the oxygen uptake rate. Upon acidification with 2 drops of 12 N H_2SO_4, 5 mL of the cultural 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 x g for 5 min and the clear hexane extract was used for g.c. analysis. Gas chromatographic analyses were carried out on a Hewlett-Packard 5730A gas chromatograph equipped with dual FID and interfaced with a Spectra Physics SP 4000 chromatographic data system. The 1.8 m x 2 mm i.d. stainless steel columns containing 10% OV-1 on Chromosorb W(AW-DMCS) were temperature programmed from 150-250°C at 8°C min^{-1}, while the injector and detector were kept at 250 and 280°C, respectively. The carried gas was nitrogen at a flow rate of 30 mL min^{-1}.

Manometry: Oxygen uptake was determined at 20°C using a Gilson differential respirometer. Resting cell suspensions were prepared from culture grown in the basal medium with Aroclor 1221 as the carbon and energy source as reported above. The cells were harvested by centrifuging at 15,000 g for 20 min. The cells were washed three times in cold 0.05 M (pH 7.0) phosphate buffer and resuspended in the same buffer. The cell suspensions were so adjusted that 1 mL of suspension contained 3-4 mg cell (dry wt.). The

standard incubation mixtures for the assay were: 1 mL of cell suspension, 1 mL of 0.05 M phosphate buffer (pH 7.0), 0.8 mL of distilled water, 0.2 mL of substrate (in the side arm) and 0.15 mL of 20% KOH in the center well for CO_2 absorption.

Bench Scale Test: The bench scale test of PCBs biodegradation was carried out in a 14-L microferm fermentor (New Brunswick Scientific Co., Edison, N.J.) equipped with automatic temperature, pH, dissolved oxygen and agitation speed controls. In a typical batch operation, the fermentor was charged with 10 L of fresh raw sewage which was fortified with 20 ppm of phosphorus and nitrogen each. The temperature was automatically controlled at 20°C at 400 rpm and dissolved oxygen (D.O.) in the fermentor broth at 2 ppm.

RESULTS AND DISCUSSIONS

The results presented in this and a previous paper (LIU 1980) suggested that P. sp. 7509 could oxidize commercial PCBs formulation (Aroclor 1221) at a rapid rate. If this bacterium is to be considered as a seeding culture for wastewater treatment, it is essential that the bacterium be able to degrade PCBs in the presence of other rich organic nutrients normally found in wastewater. Investigations in this area could also provide useful information on the possible interference from other organic compounds in the removal of PCBs by wastewater treatment process. As shown in Figure 1, P. sp. 7509. oxidized the Aroclor 1221 at a rate approximately 10 times faster than sewage. There are at least two possible explanations for this observation. First, the sewage contained some toxic materials that inhibited the bacterial respiration. This was unlikely, because addition of Aroclor 1221 (emulsion) to the sewage after 80 min resulted in an immediate increase in oxygen consumption. The second possibility was that P. sp. 7509 preferred Aroclor 1221 to the other organic substrates. This preference was demonstrated in the same Figure 1. Glucose, a substrate used readily by most bacteria, was poorly oxidized by this bacterium.

Since the temperature of sewage treatment plants in Canada vary considerably with seasons, it was of interest to test the ability of P. sp. 7509 to oxidize PCBs at different temperatures. Figure 2 shows the effect of temperature on the oxidation rate of Aroclor 1221 emulsion by this bacterium. From 15 to 35°C, the bacterium could rapidly oxidize Aroclor 1221 with the corresponding Qo_2 of 81 (15°C), 182 (25°C) and 338 (35°C). Decreasing the temperature to 4-10°C drastically retarded the microbial oxidation rate of Aroclor 1221 (Qo_2 = 15 at 4°C, 32 at 10°C). Similarly, a raise of temperature from 10 to 15°C resulted in an increase of Aroclor 1221 oxidation rate by 2.5 times. Nevertheless, even at such a low temperature of 4°C, this bacterium was still able to oxidize the Aroclor 1221. To provide a more realistic and optimal degradation temperature, 20°C was therefore used for all experiments including the bench scale biodegradation test.

Nitrogen and phosphorous which are required for bacterial growth are present in limited amounts in certain wastewater. Bench scale

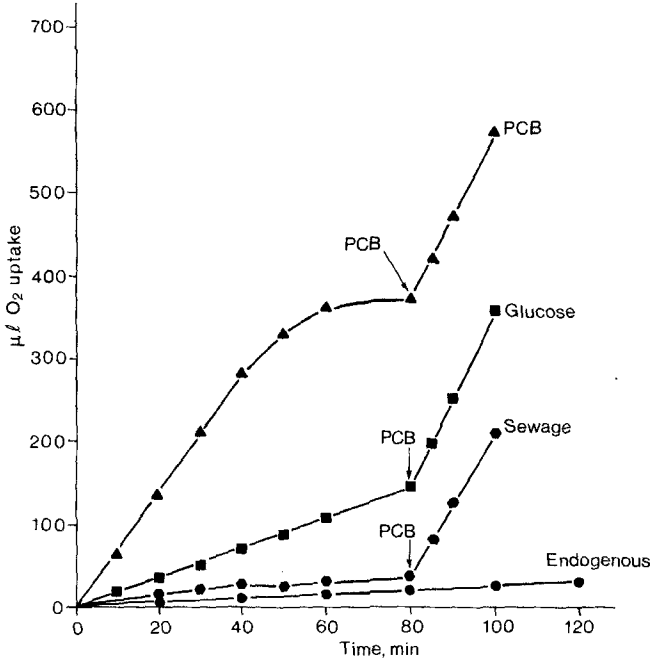

Figure 1. Comparison of raw sewage, glucose and Aroclor 1221 oxidation by P. sp. 7509. Additional 200 ul of Aroclor emulsion (2 mg) were added after 80 min, as indicated in the figure.

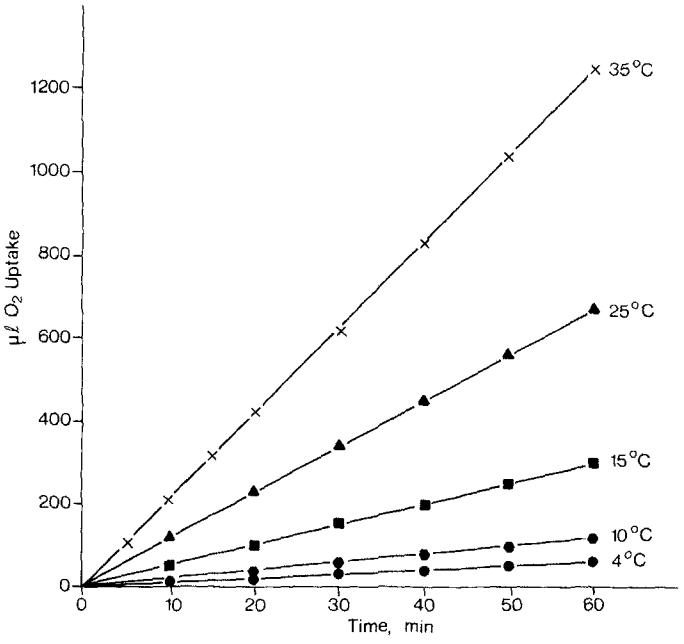

Figure 2. Effect of temperature on the oxidation of Aroclor 1221 by P. sp. 7509. Data have been corrected for endogenous respiration.

experiments were conducted to study the effect of nitrogen and phosphorous concentrations on the degradation rate of Aroclor 1221 by P. sp. 7509 in raw sewage. Addition of nitrogen and phosphorous concentrations ranging from 2 mg to 20 mg L^{-1} to sewage (the minimal value roughly equivalent to that found in municipal raw sewage) did not significantly affect the biodegradation rate of Aroclor 1221. However, raw sewage with N and P fortifications constantly yielded more reproducible results implying that the concentrations of N and P in raw sewage were on the border of rate limiting. Consequently, fortified raw sewage (N and P at 20 mg L^{-1} each) was used in all bench scale tests.

Although several studies on the relative rate of PCBs biodegradation have been reported (AHMED & FOCHT 1973; WONG & KAISER 1975; REICHARDT et al. 1981), the effect of dissolved oxygen on the degradation rate of PCBs by bacteria has not received any attention. Bench scale tests were thus initiated to study this effect. In these experiments, the fermentor was charged with 10 L of fortified raw sewage seeded with P. sp. 7509. The fermentor's impeller was kept at 400 rpm with the dissolved oxygen concentrations in the fermentor broth varying from 0 to 4 ppm level depended on the experimental conditions desired. Data presented in Figure 3 indicates that the rate of Aroclor 1221 biodegradation occurred without limitation at dissolved oxygen concentrations of 1 ppm and above. At dissolved oxygen concentrations less than 1 ppm, a strong reduction of the PCBs degradation rate resulted. These data indicated that P. sp. 7509 could degrade Aroclor 1221 under the operational conditions of the sewage treatment plant where the dissolved oxygen in the activated sludge reactor is generally maintained about 2-3 ppm level (ECKENFELDER & O'CONNOR 1961). Thus, the level of dissolved oxygen in the growth medium does not appear to be the rate-limiting factor for microbial degradation of PCBs. This is consistent with results by MACLENNAN & PIRT (1970) who investigated the effect of dissolved oxygen on the rate of hydrocarbon degradation. They reported that the biodegradation rate was not affected until the dissolved oxygen in the growth medium fell below 0.3 ppm level.

Perhaps the most interesting observation noted in the present study was that the rate of Aroclor 1221 degradation was intimately depended on the agitation speed of the fermentor's impeller. In these batch exerpiments, the dissolved oxygen in the fermentor broth was maintained at 2 ppm, while the impeller speed was varied from 0 to 800 rpm. Data in Figure 4 indicated that the rate of Aroclor 1221 degradation (expressed in terms of half-life) was a logarithmic function of the impeller speed between zero and 800 rpm. This excellent correlation ($r = 0.96$; $n = 5$) implies that good mixing is essential in the study of PCBs biodegradation.

The effect of impeller speed on Aroclor 1221 degradation is further demonstrated by the gas chromatograms of Figure 5. After 66 h incubation, 86.3% of the added PCBs was degraded by P. sp. 7509 at 800 rpm, while only 16.9% PCBs was reduced at 100 rpm. The results

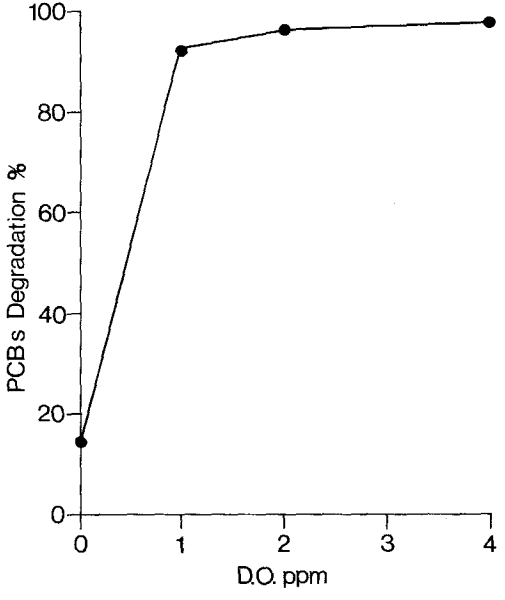

Figure 3. Effect of dissolved oxygen on the rate of Aroclor 1221 degradation in the fermentor at 20°C.

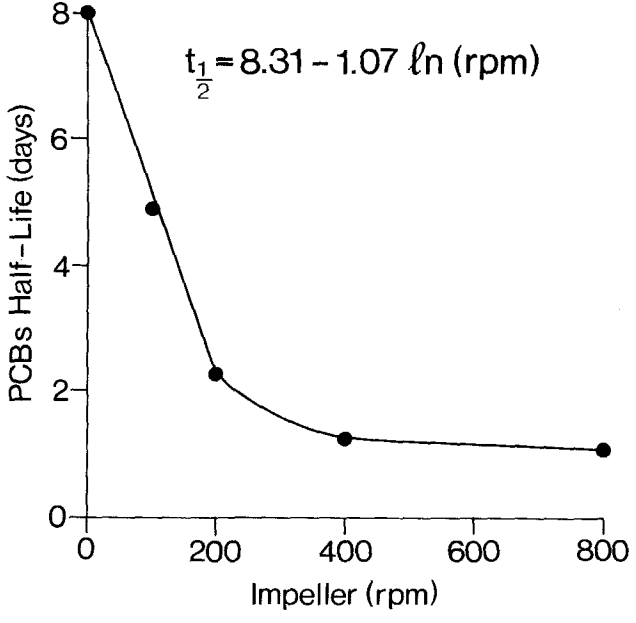

Figure 4. Effect of impeller speed on the rate of Aroclor 1221 degradation. The initial concentration of Aroclor 1221 was 100 ppm with dissolved oxygen at 2 ppm.

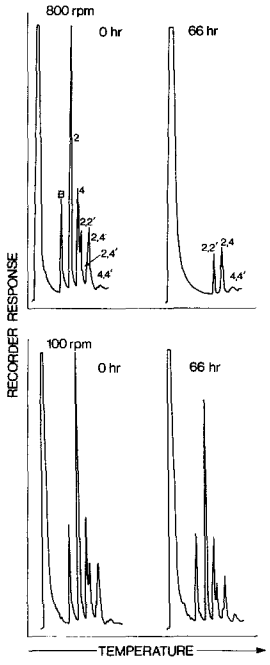

Figure 5. FID gas chromatograms of hexane extracts of Aroclor 1221 at 0 h and after 66 h incubation with P. sp. 7509 in fortified raw sewage.

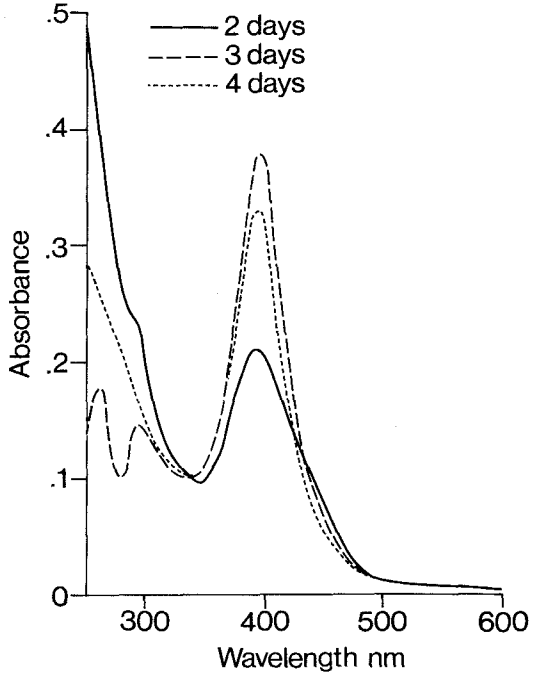

Figure 6. Production of yellow color from fermentor broth.

in the present investigation and the earlier study (LIU 1980) suggested that the chance or frequency of contact between bacteria and PCBs droplet is one of the major factors in determining the rate of microbial degradation of PCBs. To verify the observation that the rapid loss of Aroclor 1221 from the fermentor broth at high impeller speed was not due to volatilization, microbial inhibitors ($HgCl_2$ at 100 mg L^{-1}, KCN and NaN_3 at 5 m moles L^{-1} each) were added to the fermentor broth in a separate experiment. With dissolved oxygen at 2 ppm level and impeller speed at 400 rpm, the loss of Aroclor 1221 from the fermentor due to physical processes amounted to only 8% after 10 days incubation. The inclusion of sodium ligninsulfonate in the fermentor broth was probably responsible for preventing the rapid loss of Aroclor 1221 from the fermentor by lowering the PCBs vapor pressure. Other evidence for microbial degradation of PCBs in fermentor was also noted by following the bright yellow color production in the supernatant of the fermentor broth (Figure 6). The yellow color product had an absorption maximum at 395 nm during the early stage of incubation and rapidly disappeared from the broth. The yellow color compound appeared to be the chlorinated derivatives of α-hydroxymuconic acid as suggested by FURUKAWA and MATSUMURA (1976). The results in the present study clearly indicated that P. sp. 7509 was capable of degrading the lower chlorinated Aroclor 1221 in municipal wastewater under the batch operational conditions. Experiments are being carried out to assess the feasibility of degrading higher chlorinated PCBs in wastewater under the continuously operational conditions.

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REFERENCES

AHMED, M. and D.D. FOCHT: Can. J. Microbiol. 19, 47 (1973).
BAXTER, R.A., P.E. GILBERT, R.A. LIDGETT, J.H. MAINPRIZE and H.A. VODDEN: Sci. Total Environ. 4, 53 (1975).
BERGH, A.K. and R.S. PEOPLES: Sci. Total Environ. 8, 197 (1977).
BRAESTRUP, L., J. CLAUSEN and O. BERG: Bull. Environm. Contam. Toxicol. 11, 326 (1974).
BROADHURST, M.G.: Environ. Health Prosp. 1, 81 (1973).
DUINKER, J.C., M.T.J. HILLEBRAND, K.H. PALMORK and S. WILHELMSEN: Bull. Environm. Contam. Toxicol. 25, 956 (1980).
ECKENFELDER, Jr. W.W. and D.J. O'CONNOR: Biological Waste Treatment. 1st ed. London-New York: Pergamon Press 1961.
FRANK, R., M. HOLDRINET, H.E. BRAUN, R.L. THOMAS, A.L.W. KEMP and J.M. JAUUET: Sci. Total Environ. 8, 205 (1977).
FURUKAWA, K. and F. MATSUMURA: J. Agri. Food Chem. 24, 251 (1976).
HUTZINGER, O., S. SAFE and V. ZITKO: The Chemistry of PCB's. Cleveland: CRC Press 1974.

Interdepartmental Task Force on PCBs: Polychlorinated Biphenyls and the Environment. U.S. National Technical Information Service, Springfield, VA. 1972.
LIU, D.: Wat. Research 14, 1467 (1980).
MACLENNAN, D.G. and S.J. PIRT: J. Appl. Bact. 33, 390 (1970).
MUSIAL, C.J., O. HUTZINGER, V. ZITKO and J. CROCKER. Bull. Environm. Contam. Toxicol. 12, 258 (1974).
PEAKALL, D.B.: Crit. Rev. Environ. Control 5, 469 (1975).
REICHARDT, P.B., B.L. CHADWICK, M.A. COLE, B.R. ROBERTSON and D.K. BUTTON: Environ. Sci. Technol. 15, 75 (1981).
TUCKER, E.S., V.W. SAEGER and O. HICKS: Bull. Environm. Contam. Toxicol. 14, 705 (1975).
VEITH, G.D., D.W. KUEHL, E.N. LEONARD, F.A. PUGLISI and A.E. LEMKE: Pestic. Monitor. J. 13, 1 (1979).
WARE, D.M. and R.F. ADDISON: Nature 246, 519 (1973).
WONG, P.T.S. and K.L.E KAISER: Bull. Environm. Contam. Toxicol. 13, 249 (1975).

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