Bacterial Degradation of Polychlorinated Biphenyls II. Rate Studies

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Polychlorinated biphenyls (PCB's) recently have received considerable attention. Interest in these compounds has arisen because of their widespread commercial use (NISBET and SAROFIN, 1972) and wide distribution in the environment (RISEBROUGH et al., 1968; HAMMOND, 1972).

Knowledge of the effects of PCB's on biological systems is still limited. PCB's have been shown to alter various enzyme activities (GUSTAFSON, 1970; CUTKOMP et al., 1972), to inhibit photosynthesis and growth of marine phytoplankton communities (MOORE and HARRIS, 1972) and diatoms (KEIL et al., 1971), and to stimulate the growth of a bacterium Escherichia coli (KEIL et al., 1972). Only a few reports are available on the degradation of PCB's by microorganisms. VEIGHT (1970) observed that the concentration of Aroclor® 1242 in lake water decreased at a faster rate than that of Aroclor 1260. He concluded that Aroclor 1260 was more resistant to microbial degradation than Aroclor 1242. AHMED and FOCHT (1973) described the degradation of PCB's by two species of Achromobacter.

In our previous paper (KAISER and WONG, 1974), we described metabolic products of Aroclor 1242 degradation by bacterial isolates. In this investigation, we report on the toxicity of three Aroclors to lake water bacteria and the relative rates of degradation of Aroclor 1221, biphenyl, 2-chlorobiphenyl and 4-chlorobiphenyl by these bacteria.

EXPERIMENTAL

Bacterial growth studies

Five 500 ml water samples from Hamilton Harbour, Lake Ontario were dispensed into individual 2-liter Erlenmeyer flasks and were capped with rubber stoppers wrapped with aluminum foil. Five ml each of 5% Aroclor 1221 (lot no. AB-1001), Aroclor 1242 (lot no. KB-05-415), and Aroclor 1254 (lot no. KB-05-612) in acetone were added to three of the flasks to give 0.05% solutions.

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An equal volume of acetone was added to one flask to give an acetone control. One flask was kept as lake water control. The samples were incubated at 20°C in a rotary shaker. After incubation, aliquots of the samples were plated onto nutrient agar plates (Difco) for the determination of bacterial counts.

For the degradation studies of the Aroclor 1221, 100 ml aliquots of the incubated samples were transferred to a 125 ml separatory funnel and extracted with 3 ml hexane each. Gas chromatograms were run with 1-ul aliquots of the hexane extracts.

For the degradation studies of the PCB isomers, Aroclor 1221 was repeatedly injected into an automated, preparative Pye-Unicam 105 gas chromatograph and the three major components (biphenyl, 2-chloro-, and 4-chloro-biphenyl) were condensed in separate collectors. Purity of the isomers was at least 95%, as determined by GC. Identification of the isomers was made by comparison of GC retention times, melting points and mass spectra with values in the literature (FISHBEIN, 1972). The isomers were added as 5% solutions in acetone to lake water samples to give initial concentrations of 0,05%. Before and after incubation, aliquots were removed, extracted with hexane and quantitated by gas chromatography.

The determination of the Aroclor mixtures was done under the same gas chromatographic conditions as for the previous study (KAISER and WONG, 1974). For the PCB isomers, isothermal GC conditions at 180°C were employed.

Taxonomic identification

For the taxonomic identification, bacterial colonies appearing on agar plates containing 0.1% Aroclor 1221 were purified by repeatedly streaking onto new Aroclor-agar plates. The ability of the purified cultures to use Aroclor for growth was further tested by growing them in Aroclor-mineral salt medium (KAISER and WONG, 1974). Those bacteria which used agar or contaminants in the agar medium would not be able to use Aroclor for growth. The bacteria were identified according to the taxonomic scheme of SKERMAN (1967).

RESULTS AND DISCUSSION

Aroclor as bacterial nutrients

The effects of Aroclor 1221, 1242 and 1254 on the growth of lake water bacteria in 1% glucose medium was examined. No inhibition of bacterial growth was observed in the presence of up to 0.1% Aroclor. In fact, there was a slight stimulation by Aroclor 1221 and 1242. The stimulation could be due to the ability of the bacteria to degrade these polychlorinated biphenyls. FIGURE 1

shows that bacteria could use 0,05% Aroclor 1221 and 1242 as a source of carbon and energy for growth. The ability of the bacteria to degrade PCB's decreased with an increase in percent of chlorination. No stimulation of bacterial growth was observed in Aroclor 1254 medium. Aroclor 1260 has also been shown to resist bacterial degradation (OLOFFS et al., 1972).

The number of lake water bacteria capable of growing on Aroclor agar plates was enumerated. In this experiment, aliquots of lake water were plated onto agar plates containing either Aroclor 1221 or nutrient agar. Less than 1% of the bacteria appeared on Aroclor 1221 as compared with the number on nutrient agar plates. This would indicate that only a small number of bacteria in lake water could use PCB's for growth.

Seven isolates from the Aroclor agar plates were chosen for taxonomic studies. Five isolates were identified as Achromobacter sp. and the remaining as Pseudomonas sp. according to the taxonomic scheme of SKERMAN (1967). Interestingly, two bacterial isolates from sewage effluent using biphenyl and p-chlorobiphenyl respectively as sole carbon sources also have been identified as Achromobacter species (AHMED and FOCHT, 1973).

Kinetics of Aroclor degradation

The rate of degradation of Aroclor 1221 by lake water bacteria was followed by analyzing the PCB's in the growth medium at different time intervals. FIGURE 2 shows the gas chromatograms of the PCB's, extracted from the medium with hexane. At zero time (A), three peaks were detected. After 4 days incubation (B), the bacteria began to degrade the first peak into several lower molecular weight products. After 7 days, the concentrations of the products increased and after one month incubation, the Aroclor was completely degraded into several metabolites of low molecular weight. A similar phenomenon was also observed with Aroclor 1242 and several products were identified as aliphatic and aromatic hydrocarbons (KAISER and WONG, 1974).

FIGURE 3 demonstrates the ability of bacteria to degrade 0.05% solutions of biphenyl, 2-chlorobiphenyl and 4-chlorobiphenyl isomers. These compounds are utilized under similar conditions as the Aroclor 1221 mixture. The results show that not only chlorination of the biphenyl retards the bacterial degradation, but also the position of the chlorine atom in the benzene ring is a determining factor for the rate of degradation of the two mono-chlorobiphenyls. The degradation rates were found to decrease in the order biphenyl >2-chlorobiphenyl >4-chlorobiphenyl. This observation may be related to the bacterial breakdown of Aroclor 1242 (KAISER and WONG, 1974), where a preferred formation of alkane and alkyl substituted benzene metabolites was observed. The bacterial degradation of PCB

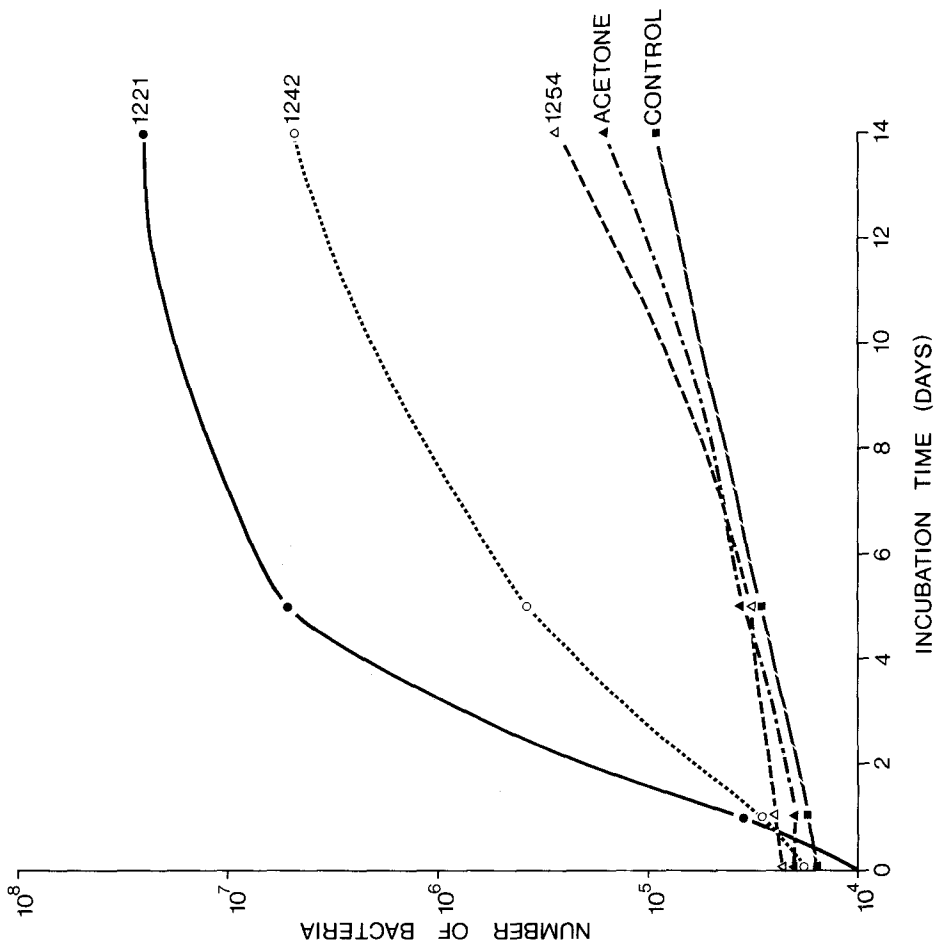

FIGURE 1. The time/growth curve of bacteria in 0.05% Aroclor 1221, Aroclor 1242 and Aroclor 1254 as carbon and energy sources. Samples with equivalent volumes of acetone/lake water and lake water only as controls.

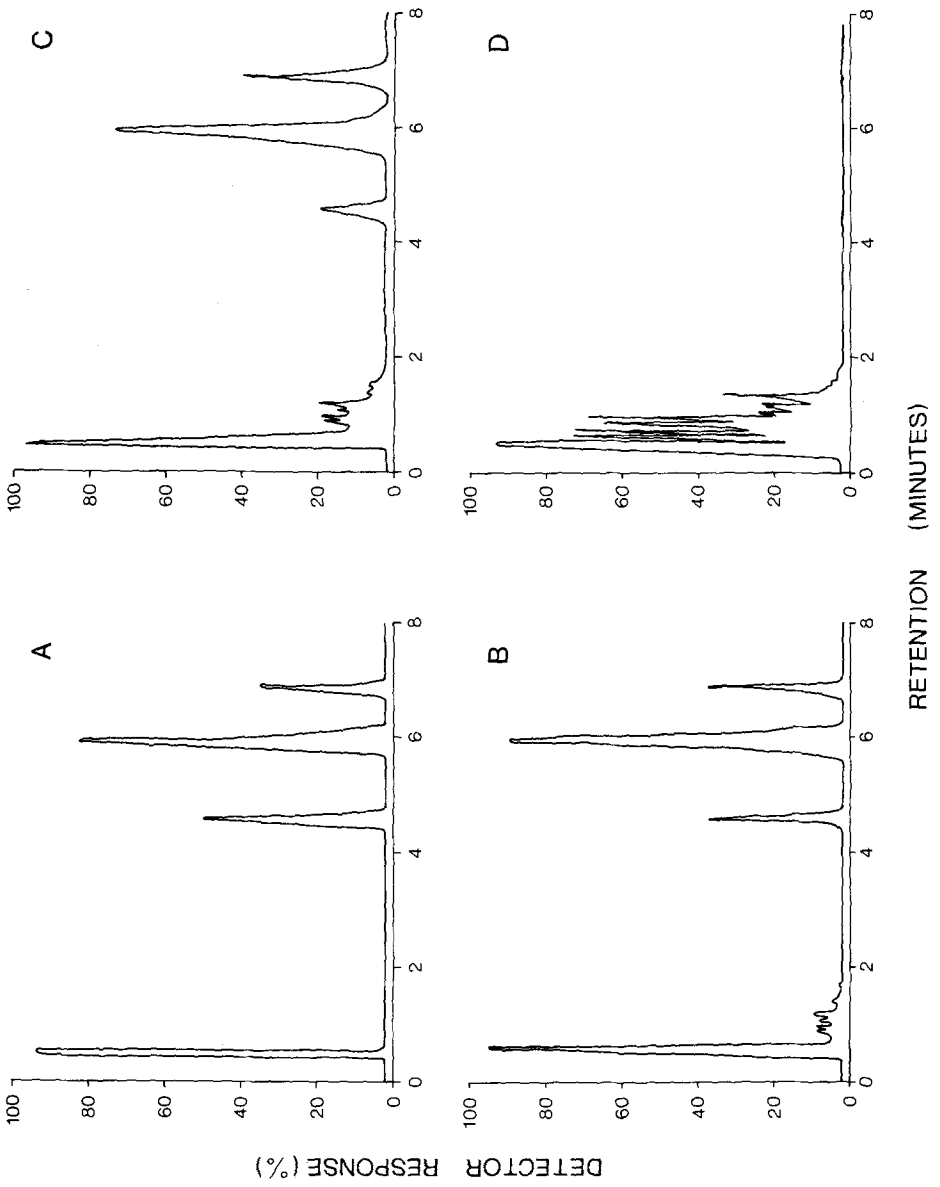

FIGURE 2. Gas chromatograms of hexane extracts of Aroclor 1221 at zero hour (A), after 4 days (B), after 7 days (C), and after one month (D) incubation with lake water bacteria.

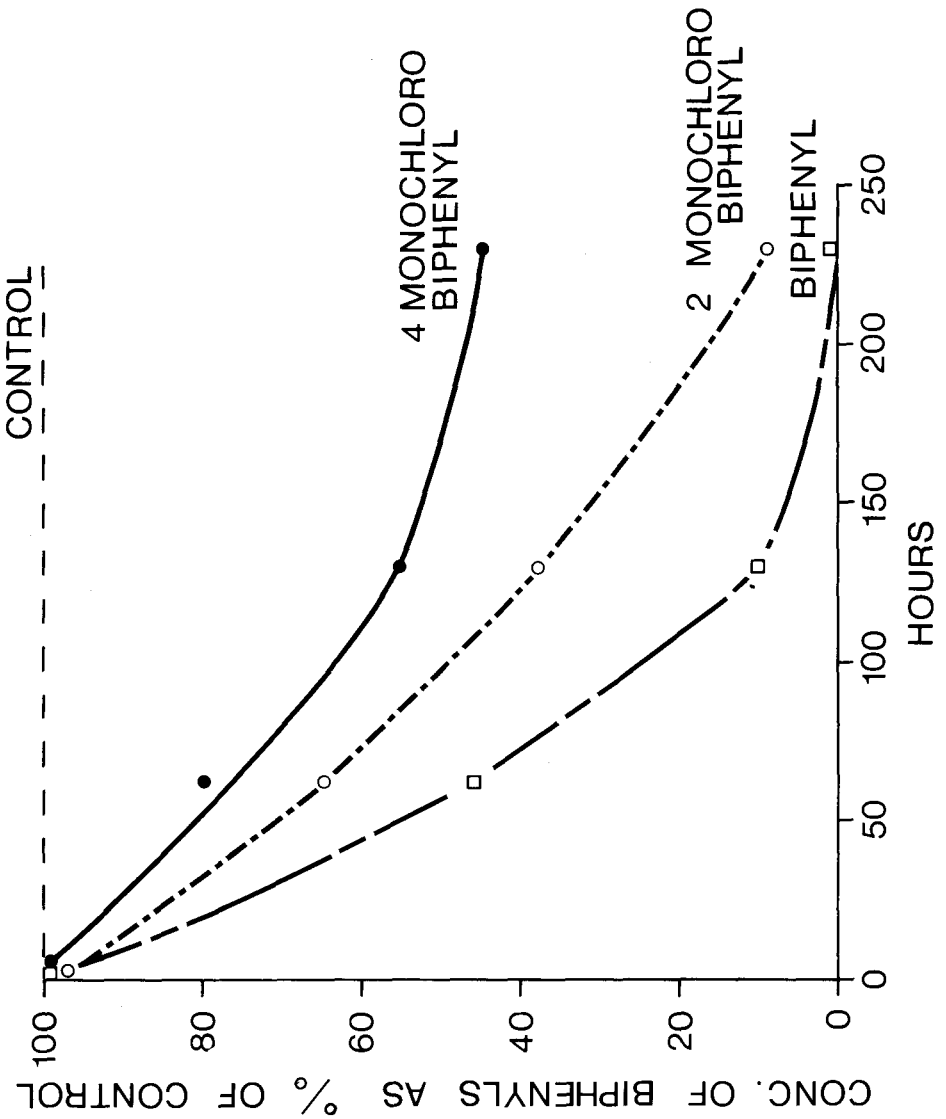

FIGURE 3. Concentrations of biphenyl, 2-chlorobiphenyl and 4-chlorobiphenyl in the growth medium after bacterial degradation during three incubation periods.

isomers is dependent on the degree of chlorine substitution of the biphenyl as well as on the stereo-chemical and electronic structure of the substrate molecule.

SUMMARY

Polychlorinated biphenyls (Aroclor 1221, 1242 and 1254) at concentrations up to 0.1% in glucose did not inhibit the growth of lake water bacteria. The bacteria used Aroclor 1221 and 1242 but not 1254 as sole carbon and energy sources for growth. Less than 1% of lake water bacteria, however, possess this ability. Seven bacterial isolates from Aroclor agar plates were identified; five belonged to Achromobacter sp. and two were Pseudomonas sp. The metabolic breakdown of Aroclor 1221 was followed. The mixture was completely degraded into several low molecular weight compounds after one month incubation. Unchlorinated biphenyl was degraded at a faster rate than 2-chlorobiphenyl and 4-chlorobiphenyl isomers.

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