APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Sept. 1983, p. 666–672
0099-2240/83/090666-07$02.00/0

Vol. 46, No. 3

Degradation and Total Mineralization of Monohalogenated Biphenyls in Natural Sediment and Mixed Bacterial Culture

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Received 3 February 1983/Accepted 24 June 1983

Mixed bacterial cultures obtained from polychlorinated biphenyl-contaminated river sediments are capable of degrading monohalogenated biphenyls under simulated natural conditions. Culture conditions include river water as supportive medium and mixed bacterial cultures obtained from river sediments. Degradation occurs when the substrates are supplied as the sole carbon source or when added together with glucose. The degradation rates of 2-, 3-, and 4-chlorobiphenyl, at 30 $\mu g\ ml^{-1}$, were 1.1, 1.6, and 2.0 $\mu g\ ml^{-1}\ day^{-1}$, respectively. Monobrominated biphenyls, including 2-, 3-, and 4-bromobiphenyl, were degraded at rates of 2.3, 4.2, and 1.4 $\mu g\ ml^{-1}\ day^{-1}$, respectively. Metabolites, including halogenated benzoates, were detected by high-performance liquid chromatography and mass spectrometry. By using chlorophenyl ring-labeled monochlorobiphenyls as substrates, total mineralization (defined as CO_2 production from the chlorophenyl ring) was observed for 4-chlorobiphenyl but not for 2-chlorobiphenyl. Rates of total mineralization of 4-chlorobiphenyl (at 39 to 385 $\mu g\ ml^{-1}$ levels) were dependent on substrate concentration, whereas variation of cell number in the range of 10^5 to 10^7 cells ml^{-1} had no significant effects. Simulated sunlight enhanced the rate of mineralization by ca. 400%.

Polychlorinated biphenyls (PCBs) are ubiquitous anthropogenic pollutants (10, 13). Polybrominated biphenyls, although not as widespread as PCBs, have also been identified as environmental pollutants (3). The high physical and chemical inertness of halogenated biphenyls places them among the most persistent pollutants; however, several reports have demonstrated microbial degradation of PCBs (1, 2, 7, 15). Degradation pathways of PCBs have been proposed, indicating that chlorobenzoates are the principal stable metabolites of PCBs (5, 6, 12, 18). Sylvestre and Fauteux (17) have suggested that 4-chlorobenzoate is the terminal degradative product of 4-chlorobiphenyl. However, in uncontaminated freshwater environments, chlorophenylglyoxylic acid in addition to chlorobenzoates can accumulate as stable environmental biotransformation products of 2-chlorobiphenyl (16).

Mineralization of PCBs has been the focus of several research groups. Reichardt et al. (12) reported mineralization of the nonchlorinated phenyl ring of monochlorinated biphenyls. Fries and Marrow (Abstr. Annu. Meet. Soc. Environ. Toxicol. Chem., p. 29; 1982) also observed the production of CO_2 from uniformly ^{14}C-labeled PCBs. Little attention has been paid specifically to the degradative fate of the chlorophenyl ring of the monochlorinated biphenyl molecule. In the present paper, we investigated the potential of microbial degradation and total mineralization, including the chlorophenyl ring, of monohalogenated biphenyls under simulated natural conditions in aquatic environments.

In this report, total mineralization is defined as the production of CO_2 from the halogenated ring of the halogenated biphenyls to differentiate it from mineralization (CO_2 production) of the nonhalogenated ring (precedent to the metabolization of the entire molecule).

MATERIALS AND METHODS

Abbreviations. The following abbreviations are used in this paper: 2CB, 2-chlorobiphenyl; 3CB, 3-chlorobiphenyl; 4CB, 4-chlorobiphenyl; 3BB, 3-bromobiphenyl; 4BB, 4-bromobiphenyl; HPLC, high-performance liquid chromatography.

Chemicals. 2CB, 3CB, 4CB, 2-bromobiphenyl, and 3BB were purchased from Analab, Inc., North Haven, Conn. 4BB was purchased from Fluka Chemical Corp., Hauppauge, N.Y. Monochlorinated benzoates were obtained from Pfaltz and Bauer, Inc., Stamford, Conn. 4-bromobenzoate was obtained from Aldrich Chemical Co., Milwaukee, Wis. Florisil and organic solvents (HPLC grade) were obtained from Fisher Scientific Co., Fairlawn, N.J.

Radioactive compounds. 4CB and 2CB labeled with ^{14}C on the chlorophenyl ring (positions 1, 2, 3, 4, 5,

and 6) were purchased from Pathfinder Laboratories Inc., St. Louis, Mo. Specific activities of 2CB and 4CB are 11.09 and 18.05 Ci mol^{-1}, respectively. Purities of the radioactive compounds are 98% or higher.

Bacterial inocula, culture conditions, and quantitation of monohalogenated biphenyl substrates. Sediment samples were obtained from river sediments, known to be contaminated with PCBs, at the Little River embayment of Fort Loudon Reservoir, Knoxville, Tenn., and vicinity. A typical river sediment sample weighed 1.4 g ml^{-1} and contained ca. 10^6 bacterial cells ml^{-1}. Acclimation of bacterial cells to 4CB was performed by repeated transfer in river water supplemented with 60 ppm ($\mu g\ ml^{-1}$) of substrate.

A 0.1-ml inoculum was put into 2 ml of filter-sterilized river water supplemented with a predetermined amount of substrate. The 2.1-ml cultures were kept airtight in 8-ml screw-capped test tubes and incubated at 24°C. After various time intervals, an equal volume of ethyl acetate was added to extract the substances. More than 99% of the substrate partitioned in the ethyl acetate phase. A 20-μl amount of the ethyl acetate phase was then injected into an HPLC system (series 2 liquid chromatograph; The Perkin-Elmer Corp., Norwalk, Conn.) equipped with a C-18/10 reverse phase column (4.6 by 250 mm). The elution solvent consisted of 92% methanol and 8% water. A variable wavelength UV detector (model LC75; Perkin-Elmer) with wavelength set at 245 nm was used to monitor the eluent. The area of the substrate peak, determined with a Hewlett-Packard model 3390A integrator, was compared with standard curves to obtain substrate concentration.

Mineralization of monochlorinated biphenyl. Monochlorinated biphenyls labeled with ^{14}C only at the chlorophenyl ring were used as substrates. Cultures, with volumes of 1.3 ml or less, were kept in 2-ml glass vials with Teflon-lined rubber caps (gas chromatography vials marketed by Fisher Chemical) and incubated at 24°C. For the river sediment cultures, air in the head space of the vials was replenished daily with syringe needles through the rubber cap. Exit air from the vials was tested for content of radioactive CO_2 (technique described below). At the end of incubation, the cultures were acidified with 0.1 ml of 1 N HCl. CO_2 gas, driven out of the solution by acidification, was sparged from the vial with compressed air through 1-ml tuberculin syringes and trapped by bubbling through 0.2 N NaOH. Before the exit gas entered the NaOH trap, the gas was passed through a Florisil column (3 by 30 mm) for the removal of volatile substrates. Trapped $^{14}CO_2$ was quantitated by liquid scintillation techniques. The authenticity of CO_2 was determined by infrared spectrometry.

Effect of simulated sunlight. Sunlight was simulated by a sunlamp (model RSM100; Sylvania) positioned 21.5 in. (56.6 cm) from the culture vials. The cultures, supplemented with 0.7 ppm of 4CB, were irradiated continuously for 12 h each day for up to 3 days. Incubation temperature was maintained at 24°C by a refrigeration unit.

Spectra of sunlight and sunlamp. The spectrum of sunlight was derived from a computer program (EFFRAD.FOR) generated by the National Photobiology Research team of the U.S. Environmental Protection Agency. A spectroradiometer (model 742; Optronic Laboratories, Inc., Silver Spring, Md.) was used to determine the spectrum of the sunlamp.

RESULTS

Degradation of monochlorinated biphenyls. To simulate natural aquatic environments, we employed river water as the supportive culture medium and low bacterial concentrations (10^3 to 10^6 cells ml^{-1}). River water and synthetic basal salts medium are equally effective in our hands (data not shown). The term 4CB-acclimated cells indicates those bacterial cells which had been repeatedly transferred in 4CB medium for a period of ca. 2 months. Nonacclimated cells were obtained directly from river sediments. With abundant 4CB available in the cultures at 60 ppm, both 4CB-acclimated cells and nonacclimated cells degraded approximately half the substrate within 6 days of incubation (Fig. 1). Nonacclimated cells showed an initial lag of ca. 3 days. When 60 ppm of glucose was added with the 4CB substrate, the degradation rate of 4CB decreased to one-half of that in the culture containing no glucose, probably owing to the preference of cells for glucose over 4CB. After 30 days of incubation, more than 99% of the 60-ppm dose of 4CB was removed by all cultures.

An early indication of the degradation of 4CB was a change in the color of the culture from colorless to bright yellow. The yellow color appeared only transiently. The duration of the yellow color varied among cultures, generally longer for nonacclimated cells (about 4 days) and shorter for 4CB-acclimated cells (about 1 day or less). This yellow pigment is probably 2-hydroxy-6-oxo-6-(4-chlorophenyl)-hexa-2,4-

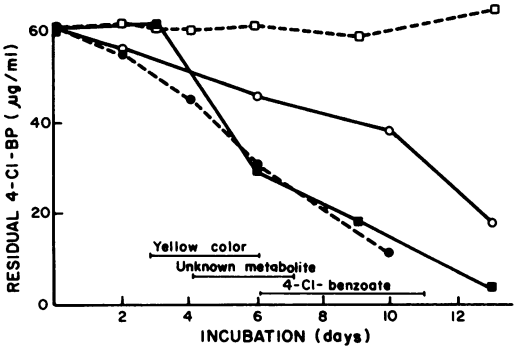

FIG. 1. Biodegradation of 4CB in sediments and mixed cultures. Each of the 2-ml cultures was inoculated with 0.2 ml of natural river sediment. 4CB-acclimated cells were inoculated at 2,000 ml^{-1}. Glucose at 60 ppm was dosed with the acclimated cells. Brackets indicate the transient appearance of biotransformation products. Symbols: □, sterile control; ■, natural sediment; ●, acclimated cells only; ○, acclimated cells plus glucose.

dienoic acid, as has been reported by Furukawa and Matsumura (5).

With ^{14}C-labeled 4CB as substrate, several radioactive and UV-absorbing metabolites were detected with HPLC (Fig. 2). A peak at 11 ml of elution volume was identified as 4-chlorobenzoate by means of mass spectrometry and cochromatography with authentic 4-chlorobenzoate (data not shown). The appearance of 4-chlorobenzoate in the 4CB culture was transient during the course of incubation; it achieved highest concentration at ca. 7 days, then gradually decreased, and finally became undectectable. When authentic 4-chlorobenzoate at 30 ppm was tested in the culture system, it was readily degraded by 4CB-acclimated cells at the rate of 17 μg ml^{-1} day^{-1}; unacclimated cells (obtained from PCB-contaminated sediment) degrade 4-chlorobenzoate at a slower rate (Table 1).

Both 2CB and 3CB were also degraded by the same bacterial consortium. Under the same culture conditions, degradation rates of 3CB and 2CB were 20 to 45% slower than 4CB (Table 2).

Degradation of monobrominated biphenyls. The bacterial consortium that degraded monochlorinated biphenyls was also capable of degrading monobrominated biphenyls. The average degradation rates of monobrominated biphenyls are comparable to those of monochlorinated biphenyls (Table 1). From the 4BB culture, we identified the major metabolite as 4-bromobenzoate by means of cochromatography with an authentic compound in HPLC (data not shown). Like the 4-chlorobenzoate in cultures of 4CB, the 4-bromobenzoate in the 4BB culture also appeared transiently. When tested with the same bacterial consortium, 4-bromobenzoate at 30 ppm was readily degraded at the rate of ca. 4 μg ml^{-1} day^{-1}.

Total mineralization of 4CB. After prolonged incubation of the 4CB cultures, we observed that all of the HPLC-detectable metabolites gradually diminished and finally disappeared. Authentic 4-chlorobenzoate was also degraded by the bacterial culture. These observations prompted us to investigate the potential of total mineralization of 4CB. Since the chlorophenyl ring in 4CB is supposedly degraded after the nonchlorinated phenyl ring, we employed 4CB with ^{14}C label only on the chlorophenyl ring as the substrate. [^{14}C]4-chlorobenzoate was detected in the 4CB culture (Fig. 2), and as it was diminishing, more $^{14}CO_2$ was detected. This observation points to the fact that the entire 4CB molecule, including the chlorophenyl ring, was mineralized by the bacterial culture.

For simulation of natural environment, river sediment freshly obtained from polluted sites was used as the source of bacterial cells. Detectable 4CB mineralization was observed as early as after 1 day of incubation (Fig. 3). Mineralization steadily increased over a period of 3 days. The nonacclimated cells in the presence of river sediment mineralized about 6 ng of 4CB at the end of 3 days. Since physical conditions in natural environments vary spatially and temporally, we examined the total mineralization of 4CB under the influence of simulated sunlight supplied by a sunlamp. The spectra of natural sunlight and the sunlamp are shown in Fig. 4. Approximately fourfold enhancement was observed when the same culture was exposed to irradiation from the sunlamp (Fig. 3). When 4CB-acclimated cells were combined with sterilized river sediment, the mineralization of 4CB was observed (Fig. 5). From an experiment conducted under similar conditions (4CB-acclimated mixed culture with river sediment), ca. 25% of the total 4CB could be converted to CO_2 (Table 3).

Effect of concentration of 4CB on mineralization. With an abundant supply of 4CB at 18 ppm (because the solubility of 4CB in water is ca. 5 ppm, the 18-ppm dosage produced at suspen-

TABLE 1. Mixed culture removal of halogenated benzoates

Substrate[a]	Condition of cell inoculum[b]	Removal rate (μg ml^{-1} day^{-1})
4-Chlorobenzoate	Acclimated to 4CB	17
4-Chlorobenzoate	Not acclimated	2.5
4-Bromobenzoate	Not acclimated	4

[a] Initial substrate concentrations were 30 μg ml^{-1}.
[b] All inocula were mixed cultures obtained from PCB-contaminated sediment. Acclimation indicates cultivation in river water containing 60 μg of 4CB ml^{-1} for 2 months before inoculation. Halogenated benzoates were determined by HPLC as described in the legend to Fig. 2.

TABLE 2. Average degradation rates of monochlorinated and monobrominated biphenyls

Substrate	Degradation rate[a] (μg ml^{-1} day^{-1})
2CB	1.1 (0.58)[b]
3CB	1.6 (0.11)
4CB	2.0 (0.28)
2BB[c]	2.3 (0.29)
3BB	4.2 (0.30)
4BB	1.4 (0.16)

[a] Fresh river sediment (0.1 ml) was inoculated into 2 ml of culture dosed with 30 ppm of substrate. Incubation time was 6 or 9 days.
[b] Standard deviation in parentheses.
[c] 2BB, 2-Bromobiphenyl.

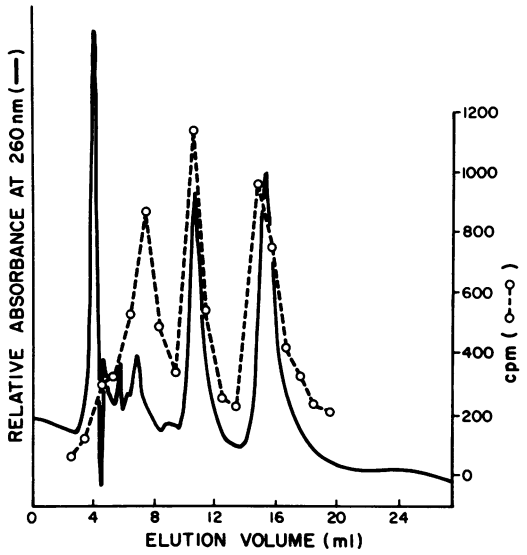

FIG. 2. Elution profile of 4CB biodegradation products detected by HPLC and liquid scintillation spectrometry. The sample contained 90 μl of filtered bacterial culture, taken from a 5-day 4CB-acclimated mixed culture as described in the legend to Fig. 1 and supplemented with [^{14}C]chlorophenyl ring-labeled 4CB. Conditions of the HPLC system are: solvent flow rate, 2 ml min^{-1}; solvent composition, 13% methanol, 87% water. The 4CB substrate was not eluted from the column under this solvent composition. Each of the radioactive peaks contains at least one metabolite; the peak at 11 ml is identified as 4-chlorobenzoate.

sion-solution), 1.6 ppm was completely mineralized after 6 days of incubation. Therefore, at relatively high 4CB concentration, the average mineralization rate is ca. 270 ng ml^{-1} day^{-1}. Since many industrially polluted aquatic sites contain parts per billion levels of PCBs, we proceeded to test the mineralization rates of 4CB at parts per billion levels (Fig. 6). The mineralization rate, in terms of micrograms of 4CB per milliliter per day was highly dependent on the substrate concentration and very slightly affected by bacterial concentration. This observation reflects the high efficiency of the 4CB-acclimated bacterial cells (i.e., the oversupply of mineralizing enzymes relative to the amount of substrate). Based on Fig. 6, the first-order rate constant (mineralization rate = k·[4CB]), k, is ca. 0.06 day^{-1}, indicating a turnover time of about 17 days.

The potential for total mineralization of 2CB was also examined in our culture system. With [^{14}C]chlorophenyl ring-labeled 2CB as the substrate, radioactive metabolites were observed; however, no radioactive CO_2 originating from the chlorophenyl ring was detected. When 2-chlorobenzoate was tested, it was nondegradable by the bacterial consortium.

DISCUSSION

Biodegradation experiments in this study were designed for the simulation of natural environments. River water was used as supportive culture medium. Mixed bacterial cultures, freshly obtained from river sediments or preacclimated to 4CB, were used at realistic concentrations. The use of mixed culture is more representative of natural environments. We have shown that all of the monochlorinated biphenyls and monobrominated biphenyls are biodegradable. We will report in a future paper that bacteria are responsible for the biodegradation of these compounds.

The results of this investigation indicate that natural mixed microbial populations degrade monohalogenated biphenyls through halogenated benzoate intermediates. It appears that in contaminated sediments these intermediates are not the terminal or ultimate biodegradation products as has been suggested (5, 6, 16, 17). The results of this study support the conclusion that the halogenated benzoates may be subject to rapid biodegradation with the resulting production of CO_2 as the terminal decomposition product. Although other studies have reported the mineralization of a portion of the 4CB (12;

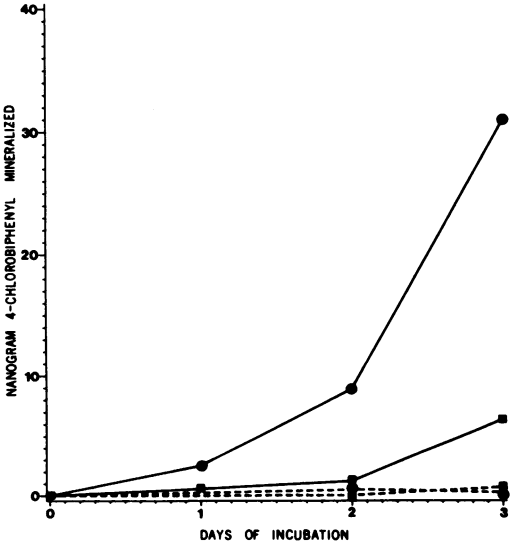

FIG. 3. Mineralization of 4CB in natural river sediment. Each 0.6-ml culture contained 0.1 ml of river sediment, 0.5 ml of river water, and 420 ng (0.7 ppm) [^{14}C]chlorophenyl ring-labeled 4CB. Data are means of triplicate measurements. Symbols: ----, sterile controls; —, test samples; ●, with simulated sunlight irradiation; ■, without simulated sunlight irradiation.

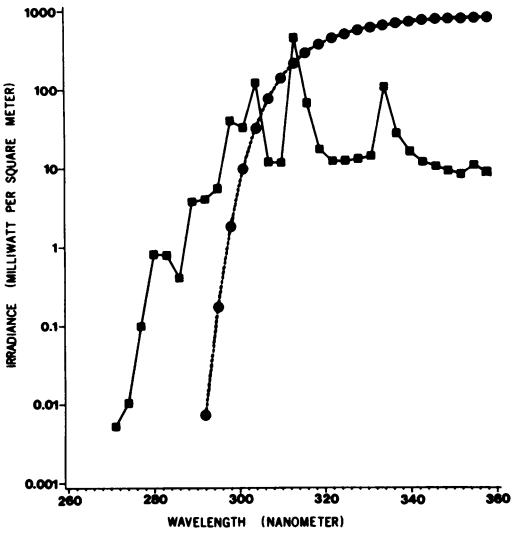

FIG. 4. Comparative intensity spectra of natural (----) and simulated (—) solar irradiation.

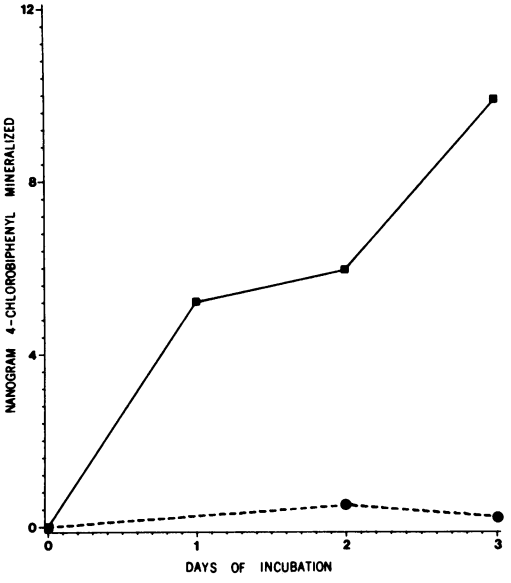

FIG. 5. Mineralization of 4CB by acclimated cells. Each 0.6-ml culture contained 0.1 ml of heat-sterilized river sediment, 420 ng (0.7 ppm) of [^{14}C]chlorophenyl ring-labeled 4CB, 0.4 ml of filter-sterilized river water, and a 0.1-ml suspension of 4CB-acclimated cells (ca. 10^5 cells). (----), Sterile control. Data are means of triplicate measurements.

G. F. Fries and G. S. Marrow, Abstr. Annu. Meet. Soc. Environ. Toxicol. Chem., p. 29, 1982), our results conclusively demonstrate that natural bacterial populations can promote the complete oxidation of the chlorinated ring of 4CB.

A 65-megadalton plasmid coding for the conversion of 4CB to 4-chlorobenzoate, but not the mineralization, has been identified in *Klebsiella* sp. (11). In more recent studies, Furukawa and Charkrabarty (4) reported the total degradation of 4CB by the combined effort of two bacterial strains under laboratory conditions. The first strain, an *Acinetobacter* sp., converts 4CB to 4-chlorobenzoate. The second strain, a *Pseudomonas putida* strain, was genetically constructed by means of introducing a TOL plasmid into the cell to enable it to utilize 4-chlorobenzoate. Obviously, two sets of degradative genes are required for the total mineralization of 4CB: the first set of genes for the conversion to 4-chlorobenzoate and the second set for the utilization of 4-chlorobenzoate. The mineralization pathway of chlorobenzoates has been proposed (8). In this study, the two sets of genes are present in the bacterial population obtained from river sediments. Natural selection, under the PCB-contaminated situation, may have effected the coexistence of these two sets of degradative genes within an aquatic ecosystem. If environmental variables such as solar irradiation can sensitize higher halogenated biphenyls to microbial degradation (via photodechlorination or other reactions), it is possible that sunlight and environmental bacteria can synergistically affect the degradation of a variety of halogenated biphenyl substrates.

Data from this study show that, with 4CB-acclimated bacteria at above 10^5 cells ml^{-1}, the mineralization rate of 4CB is substrate dependent. The pseudo-first-order rate constant, with regard to 4CB concentration, was 0.06 day^{-1}. Since the solubility of 4CB is 5 ppm, the maximum mineralization rate can be estimated as 300 ng ml^{-1} day^{-1}. In this study, with an abundant

TABLE 3. Distribution of radioactivity in ^{14}C-labeled 4CB culture[a]

Source	Radioactivity	
	cpm	% of input
4CB in solution	22,317	24.2
Metabolites	8,055	8.7
$^{14}CO_2$	23,095	25.1
Adsorbed to reactor[b]	6,597	7.2
Total recovered	60,064	65.2
Unaccounted for[c]	32,096	34.8

[a] 4CB-acclimated culture was dosed with ^{14}C-labeled 4CB (370 ng [equivalent to 92,160 cpm] was added) and incubated for 3 days.

[b] Associated with Teflon rubber reactor seal.

[c] Most likely due to volatilization loss.

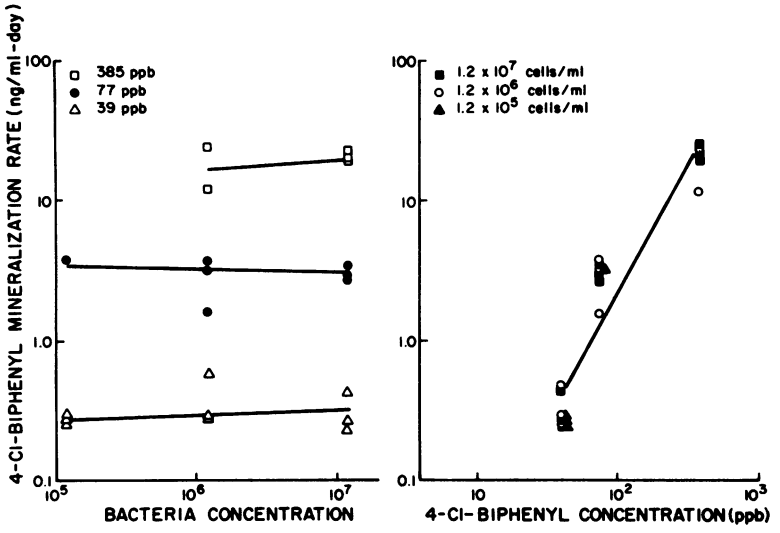

FIG. 6. Effect of 4CB and bacterial concentration on mineralization rate. (A) Mineralization rate versus bacterial concentration; (B) mineralization rate versus 4CB concentration. (—), Trend of changes. Volume of culture was 1.3 ml; incubation time was 2 days.

supply of 4CB (at 18 ppm), the mineralization rate was 270 ng ml^{-1} day^{-1}, very close to the expected value. Therefore, the mineralization rate constant of 0.06 day^{-1} seems to be applicable to the upper limit of the solubility of 4CB.

4BB, with a chemical structure similar to that of 4CB, appears to follow the same degradative fate of 4CB. The transient appearance and the ready degradability of 4-bromobenzoate indicate that the degradation of 4BB did not stop at the point of 4-bromobenzoate. Most likely, 4BB was also completely mineralized by the bacterial culture.

Photochemical degradation has been shown to be a possible route of environmental breakdown of PCBs (9, 14). In this study, we observed that irradiation from a sunlamp enhanced the rate of total mineralization of 4CB. Very likely, the irradiation altered the parent 4CB molecule or its metabolites, making it more susceptible to microbial mineralization. The precise mechanism of this enhancement effect is still under our investigation.

Since nutritional conditions in natural environments are not constant, the stability of the degradative genes and gene functions during fluctuation of various carbon sources is of importance. Our data suggest that 4CB-degradative cells are able to retain their degradative function during a temporary abundance of glucose. However, the stability of the degradative function upon long-term exposure in nonselective environments is still undetermined.

These results indicate that monohalogenated biphenyls are biodegraded and mineralized in natural aquatic ecosystems. Since the river sediments in this study were obtained from PCB-contaminated areas, it is very possible that more efficient PCB-degrading and PCB-mineralizing bacterial strains will be evolved from various polluted sites.

ACKNOWLEDGMENTS

This investigation was supported by research grant R808457010 from the U.S. Environmental Protection Agency, Office of Exploratory Research. G.S.S. was supported by a U.S. Public Health Service Research Career Development award from the National Institute of Environmental Health Sciences.

LITERATURE CITED

1. **Ahmed, M., and D. D. Focht.** 1973. Degradation of polychlorinated biphenyls by two species of Achromobacter. Can. J. Microbiol. 19:45–52.
2. **Clark, R. R., E. S. K. Chian, and R. A. Griffin.** 1979. Degradation of polychlorinated biphenyls by mixed microbial cultures. Appl. Environ. Microbiol. 37:680–685.
3. **Cordle, F., P. Corneliussen, C. Jelinek, B. Hackley, R. Lehman, J. McLaughlin, R. Rhoden, and R. Shapiro.** 1978. Human exposure to polychlorinated biphenyls and polybrominated biphenyls. Environ. Health Perspect. 24:157–172.
4. **Furukawa, K., and A. M. Chakrabarty.** 1982. Involvement of plasmids in total degradation of chlorinated biphenyls. Appl. Environ. Microbiol. 44:619–626.
5. **Furukawa, K., and F. Matsumura.** 1976. Microbial metabolism of polychlorinated biphenyls. Studies on the relative degradability of polychlorinated biphenyls components by Alkaligenes sp. J. Agric. Food Chem. 24:251–256.
6. **Furukawa, K., N. Tomizuka, and A. Kamibayashi.** 1979. Effect of chlorine substitution on the bacterial metabolism of various polychlorinated biphenyls. Appl. Environ. Microbiol. 38:301–310.
7. **Furukawa, K., K. Tonomura, and A. Kamibayashi.** 1978. Effect of chlorine substitution on the biodegradability of polychlorinated biphenyls. Appl. Environ. Microbiol. 35:223–227.

8. **Hartmann, J., W. Reineke, and H. J. Knackmuss.** 1979. Metabolism of 3-chloro-, 4-chloro-, and 3,5-dichlorobenzoate by a pseudomonad. Appl. Environ. Microbiol. **37**:421–428.
9. **Herring, J. L., E. J. Hannan, and D. D. Bills.** 1972. UV irradiation of Aroclor 1254. Bull. Environ. Contam. Toxicol. **8**:153–157.
10. **Kalmaz, E. V., and G. D. Kalmaz.** 1979. Transport, distribution, and toxic effects of polychlorinated biphenyls in ecosystems. Rev. Ecol. Model. **6**:223–251.
11. **Kamp, P. F., and A. M. Chakrabarty.** 1979. Plasmids specifying p-chlorobiphenyl degradation in enteric bacteria, p. 275–285. *In* K. N. Timmis and A. Puhler (ed.), Plasmids of medical, environmental and commercial importance. Elsevier/North-Holland Publishing Co., Amsterdam.
12. **Reichardt, P. B., B. L. Chadwick, M. A. Cole, B. R. Roberton, and D. K. Button.** 1981. Kinetic study of the biodegradation of biphenyl and its monochlorinated analogues by a mixed marine microbial community. Environ. Sci. Technol. **15**:75–79.
13. **Risebrough, R. W., P. Rieche, D. B. Peakall, S. G. Herman, and M. N. Kirven.** 1969. Polychlorinated biphenyls in the global ecosystem. Nature (London) **220**:1098–1102.
14. **Safe, S., and O. Hutzinger.** 1971. Polychlorinated biphenyls: photolysis of 2,4,6,2′,4′,6′-hexachlorobiphenyl. Nature (London) **232**:641–642.
15. **Sayler, G. S., M. Shon, and R. R. Colwell.** 1977. Growth of an estuarine *Pseudomonas* sp. on polychlorinated biphenyl. Microb. Ecol. **3**:241–255.
16. **Shiaris, M. P., and G. S. Sayler.** 1982. Biotransformation of PCB by natural assemblages of freshwater microorganisms. Environ. Sci. Technol. **16**:367–369.
17. **Sylvestre, M., and J. Fauteux.** 1982. A new facultative anaerobe capable of growth on chlorobiphenyls. J. Gen. Appl. Microbiol. **28**:61–72.
18. **Yagi, O., and R. Sudo.** 1980. Degradation of polychlorinated biphenyls by microorganisms. J. Water Pollut. Control Fed. **52**:1035–1043.