Wat. Res. Vol. 23, No. 8, pp. 957–964, 1989
Printed in Great Britain.

0043-1354/89 $3.00 + 0.00

ANAEROBIC BIODEGRADATION OF POLYCHLORINATED BIPHENYLS IN HUDSON RIVER SEDIMENTS AND DREDGED SEDIMENTS IN CLAY ENCAPSULATION

G-Y. RHEE[1], B. BUSH[1], M. P. BROWN[2], M. KANE[1] and L. SHANE[1]

[1]Wadsworth Center for Laboratories and Research, New York State Department of Health and School of Public Health, State University of New York at Albany, Albany, NY 12201–0509 and [2]Bureau of Technical Services and Research, New York State Department of Environmental Conservation, Albany, NY 12233–3502, U.S.A.

(First received September 1988; accepted in revised form January 1989)

Abstract—Anoxic Hudson River sediments and clay-encapsulated dredged sediments were investigated to determine whether anaerobic biodegradation of polychlorinated biphenyls (PCBs) occurred. Measured as a decrease in congener concentrations, clear evidence for anaerobic biodegradation of ambient PCBs was found in untreated Hudson River sediments incubated in the laboratory under an N_2 atmosphere. Of 10 different sediment treatments, which included amendments with biphenyl, yeast extract/trypticase mixture, live and sterilized anaerobic sewage sludges and inoculation with a mixed culture obtained with Aroclor 1221 enrichment, under either an N_2 or CO_2/H_2 atmosphere, biphenyl enrichment in N_2 was most effective for both sediments. About 53% of the total PCBs (375 mg/kg sediment dry wt), mainly mono- to pentachlorobiphenyls, was degraded in the biphenyl-amended Hudson River sediments and 30% (281 mg/kg sediment) in biphenyl-amended Moreau sediments after 7 months, with the spectrum of congeners degraded much broader in Hudson River than Moreau sediments. Biphenyl amendment enhanced the disappearance of highly chlorinated congeners. Inoculation with the mixed culture showed positive results in Moreau sediments but not in Hudson River sediments. Regardless of treatments, no biodegradation occurred in a CO_2/H_2 atmosphere. Moreau sediments incubated *in situ* (from November to June), showed little change in congener concentrations in all treatments, suggesting temperature as an important factor. The accumulation of less-chlorinated congeners as a result of reductive dechlorination of highly chlorinated ones was not observed.

Key words—polychlorinated biphenyls, biphenyl, xenobiotics, biodegradation, anaerobic biodegradation, Hudson River, sediments

INTRODUCTION

The Hudson River in New York State is heavily contaminated by polychlorinated biphenyls (PCBs) from the discharge by two capacitor plants of General Electric Co. located in the upper basin. Although the discharge was discontinued in 1977, PCB levels in biota and river water have been sustained at a high level due to their release from sediments. Since only the top few cm of sediments are generally aerobic, the largest reservoir of PCBs in the river is in an anaerobic environment. Thus, any microbial processes in these layers which may effect their degradation or transformations are of critical importance for the long-term health of the river. The fate of the substantial mass of PCBs presently confined to dredged sediment landfills also depends on the same anaerobic processes.

Although there is a considerable amount of evidence for aerobic degradation of PCBs [for reviews, see Furukawa (1983) and Safe (1984)], only limited experimental work has been carried out to determine the long-term effect of anaerobic deposition. Earlier studies of anaerobic fermentation of Aroclor 1254 in silage (Fries, 1972) and a tetrachlorobiphenyl in marine sediments (Carey and Harvey, 1978) failed to show any change in their concentrations. Also, no evidence for anaerobic decomposition of Aroclor 1242 was found in soil over a 210-day period (Brunner *et al.*, 1985). However, partial degradation of a monochlorobiphenyl was observed in anaerobic sediments, probably by facultative anaerobes (Sylvestre and Fauteux, 1982; Sayler *et al.*, 1984).

Brown *et al.* (1987a, b) recently reported that the congener pattern in various layers of Hudson River sediments was different from the pattern presumed to have been discharged into the river, with low-molecular-weight congeners and *ortho*-substituted congeners predominant. They attributed this difference to anaerobic reductive dechlorination. This conclusion was later disputed due to several quantitative problems with the method of interpretation (Brown *et al.*, 1988; Brown, 1988). However, Quensen *et al.* (1988) found convincing evidence for anaerobic dechlorination in Hudson River sediments incubated in the laboratory. Our recent studies (Chen *et al.*, 1988) demonstrated the strongest evidence yet for the anaerobic biodegradation of PCBs by mixed bacterial populations obtained by Aroclor 1221 enrichment of Hudson River sediments (Chen *et al.*, 1988).

In the present study, we investigated whether anaerobic biodegradation of PCBs occurred in

Hudson River sediments and dredged sediments at the Moreau encapsulation site and also attempted to identify optimum conditions to induce and/or enhance anaerobic microbial degradation.

MATERIALS AND METHODS

Throughout the experiments stringent anaerobic techniques have been employed. To take sediment samples from the Moreau site, the soil cover was first removed with a shovel and a PVC pipe (1″ dia) was driven into the sediments with a hammer. The pipe filled with compacted sediment was then removed using a portable winch. This technique allowed us to take sediment core samples from depths as deep as 2.0 m from the surface. The pipe was cut at both ends to a length to fit a GasPack jar with disposable CO_2/H_2 generator envelopes and was transported to the laboratory in the air. The jar was opened in an anaerobic hood with an $N_2/CO_2/H_2$ atmosphere (85:5:10) in the laboratory. The core tube was cut in half, and a desired amount of sediment was taken from the middle portion and added to a cystine sulfide-reduced (0.025%) synthetic mineral medium containing the anaerobic indicator resazurin. Hudson River sediments were taken and processed using similar techniques except for the use of a hand corer fitted with a Plexiglas tube (2.5″ dia).

While the sediment slurries were maintained homogeneous by mixing with a magnetic stirrer inside the anaerobic hood, 25-ml portions were pipetted to 50-ml capacity serum vials for various treatments. The treatments were sediments only under N_2; biphenyl under N_2 or CO_2/H_2; biphenyl plus yeast extract/trypticase (YE/T) under N_2 or CO_2/H_2; biphenyl, YE/T mixture and mixed bacterial populations under N_2 or CO_2/H_2; and biphenyl plus heat-sterilized or live anaerobic sewage sludges. Biphenyl (final concentration, 3.5 g/kg) was added without carrier solvents as described earlier (Lederman and Rhee, 1982). Cystine sulfide-reduced solutions of YE/T mixture (0.1%), sterilized or live sewage sludges (0.5 ml) or bacterial inoculum were then introduced into the vials as required. The bacterial inoculum was the mixed culture obtained from Hudson River sediments by repeated enrichment with Aroclor 1221 (Chen *et al.*, 1988). The control sediments contained formalin (1.3%) in an N_2 atmosphere. Each treatment, including the control, was set up in triplicate for statistical evaluation. Vials were sealed with Teflon-lined self-sealing butyl rubber stoppers with crimped aluminum caps. The vials requiring an N_2 atmosphere were flushed with high-purity N_2 gas using two hypothermic needles as a gas inlet and outlet after sealing.

One set of the triplicate Moreau sediments including the control was incubated in the laboratory at room temperature (25°C) on a rotary shaker (150 rpm). Another identical set was buried in a Styrofoam box at the Moreau sampling site about 1 m below the surface. Hudson River sediments were incubated only in the laboratory. They were incubated from November 1986 to early June 1987. After the incubation period, the vials were kept in a cold room (4°C) until extraction.

When the bottles were opened the voltage of a platinum electrode vs a silver/silver chloride electrode was measured to determine the reductive status of the slurry. The supernatant liquid was then decanted off using a Pasteur pipette. Preliminary work has shown that the supernatant contained a negligible amount of PCBs. Acetone (10 ml) was added to the vial and shaken for 1 min. The supernatant was then transferred to a 250 ml separatory funnel. The process was repeated once with acetone and twice with hexane. The remaining sediment was dried at 110°C and weighed to determine the dry weight of sediment. Distilled water (50 ml) was added to the funnel to remove acetone and the aqueous layer was collected. More water was added to the funnel and after separation the aqueous extracts were combined before back-extraction with hexane. The combined hexane extracts were dried over granular sodium sulfate and evaporated to approx. 2 ml in a Kuderna–Danish evaporator with a 3-ball Snyder column on a steam bath. Mercury (1.5 ml) was added to the extract and shaken for 1 min to remove elemental sulfur. It was then rinsed onto a 2% deactivated Flurisil column (1 cm dia) and eluted with hexane and 40 ml collected. The eluate was made up to 50 ml in a graduated flask. It was analyzed on an automated Hewlett-Packard 5840A gas chromatograph with a 5880 splitless injector, electron capture detector, and an ASCII communications board. The soda glass column (50 m × 0.25 mm) was coated with Apiezon L and temperature programmed so that 79 PCB congeners could be separated and quantitated from the standard mixture which consisted of a 1:1:1:1 mixture of Aroclors 1221, 1016, 1254 and 1260 (200 ng/ml). Recovery, accuracy and precision of the method have been reported previously (Bush *et al.*, 1987).

The formaldehyde-treated control was extracted and analyzed at the same time as other treatments in a random order and congener concentration in each treatment was expressed relative to the control value. The relative congener concentrations of the triplicate controls showed a high reproducibility of extraction and GC analysis [Fig. 1(A, B)]. Not only was the standard deviation of the mean small,

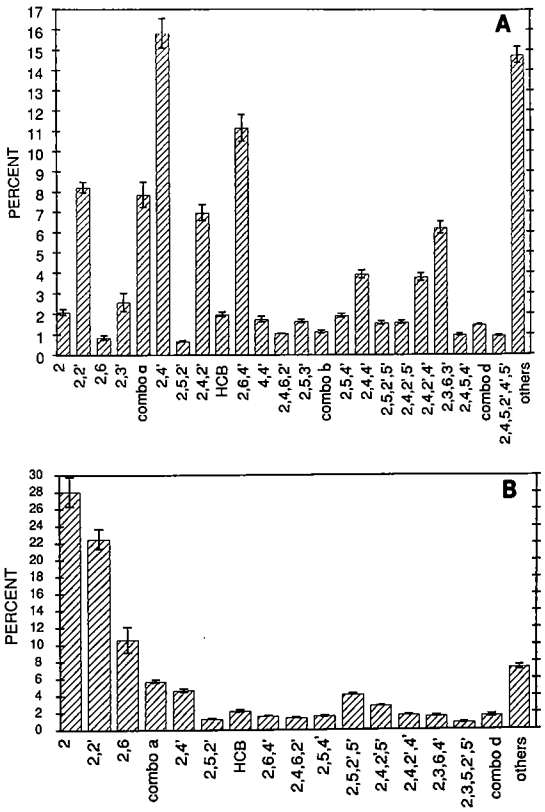

Fig. 1. Concentrations of individual congeners in the control sediments as a percentage of the total PCBs. (A) Concentrations as a percentage of the total PCB (936 mg/kg sediment dry wt) in the dredged sediments encapsulated at the Moreau disposal site and (B) concentrations as a percentage of the total (707 mg/kg sediment dry wt) in Hudson River sediments. Range bars indicate the standard deviation (SD) of triplicate sediments. The congeners which comprised >1% of the total are represented. HCB = hexachlorobenzene. Combo a consists of 2,3′ and 2,6,2′; combo b of 2,4,3′ and 2,4,2′,6′; and combo d of 2,3,4,2′,4′, 2,3,4,2′,3′ and 2,3,6,3′,4′.

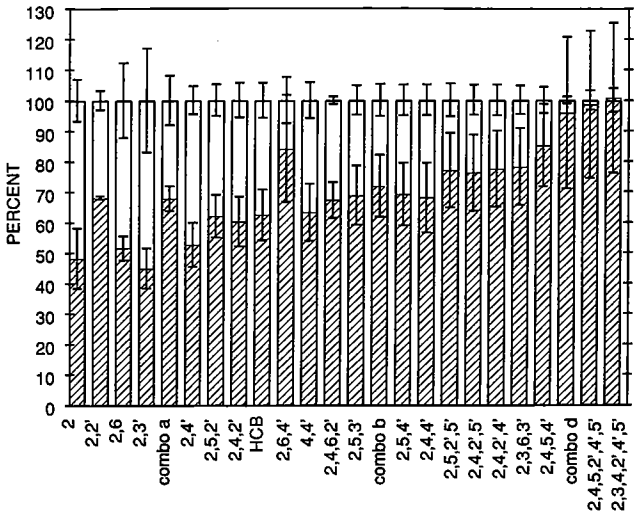

Fig. 2. Differences in congener concentrations between biphenyl-enriched Moreau sediments in N_2 (▨) and the control sediments (□) after a 7-month incubation. The total PCB concentration in the control was 936 mg/kg sediment. The concentration of individual congeners is shown in Fig. 1(A). Range bars indicate the SD of triplicate sediments.

but the values for the laboratory and *in situ* controls of the Moreau sediments were almost identical. The control concentrations after a 7-month incubation did not differ from those at time 0.

The *t*-test was used to determine the difference between the means of the triplicate control and treated sediments. The congener concentrations after incubation are presented in figures as the percentage of the control values with their standard deviations, and also as percentage degradation where degradation in two sediments or two treatments was being compared.

RESULTS

Figure 1(A, B) shows the results of the analysis of triplicate preparations of the two sediments inactivated with formaldehyde. Congeners comprising >1% of the total residue are plotted, with the final bar representing the sum of the remaining congeners. The 24 congeners >1% of the residue in the Moreau sediment comprised 87% of the total and the Hudson River sediment had 17 such congeners which together accounted for >94%. The Moreau sediment contained total PCB concentrations of 936 ± 15 mg/kg dry wt. The river sediment contained 707 ± 14 mg/kg dry wt. The mean dry weight of sediments in serum vials was 48 ± 0.8 mg for the Moreau sediment and 57 ± 1 mg for the river sediment. The reducing potential (indicated by a platinum electrode vs a silver/silver chloride electrode) of the preparations varied from -210 to -310 mV.

Moreau sediments incubated in the laboratory

A peak-by-peak comparison of untreated sediments incubated under N_2 to the control (*t*-test) showed little difference at the $P < 0.05$ level (figure not shown). This was mainly due to the large standard deviation of the mean for the untreated sediments which was derived from only two values. One of the triplicate untreated sediments was lost during extraction steps.

Biphenyl-enriched sediments under N_2 showed a clear decrease in many congeners (Fig. 2). The decrease relative to the control was highly significant ($P < 0.01$) in those with chlorination at: 2 (52%); 2,2′ (32%); 2,4′ (47%); 2,5,2′ (38%); 2,4,2′ (40%); and 2,4,6,2′ (33%). The difference was also significant ($P < 0.05$) for: 2,6 (48%); 2,3′ (55%); combination a (2,3′ + 2,6,2′) (32%); hexachlorobenzene (37%); 2,6,4′ (37%); 4,4′ (16%); 2,5,3′ (31%); combination b (2,4,3′ + 2,4,2′,6′) (28%); 2,5,4 (31%); and 2,4,4′ (32%). The decrease was generally less for the congeners with greater chlorine substitution. However, little difference was noted in biphenyl-enriched sediments incubated under CO_2/H_2.

Significant decreases in congeners were also observed in the sediments which received biphenyl, YE/T mixture and the mixed enrichment culture and incubated under N_2. The decrease of 2-monochloro- (76% of the control) and 2,6-dichlorobiphenyl (54%) were highly significant ($P < 0.01$) and 2,2′-(43%), 2,3′- (67%) and 2,4′-dichlorobiphenyl (54%) were also significantly ($P < 0.05$) reduced. In these treatments again, the decrease was generally less in congeners with higher degrees of chlorination. However, no decrease was noted in the sediments which received biphenyl and YE/T but not bacterial inoculation both under N_2 and CO_2. Thus, the bacterial inoculation must have caused the biodegradation. However, under CO_2/H_2 atmosphere, no change occurred even with the bacterial inoculation as with the biphenyl-enriched sediments under CO_2/H_2.

There was little difference between the pattern of biodegradation in the biphenyl-added sediments and the bacteria-inoculated sediments with the exception of 2,5,2′-trichlorobiphenyl (Fig. 3). The disappear-

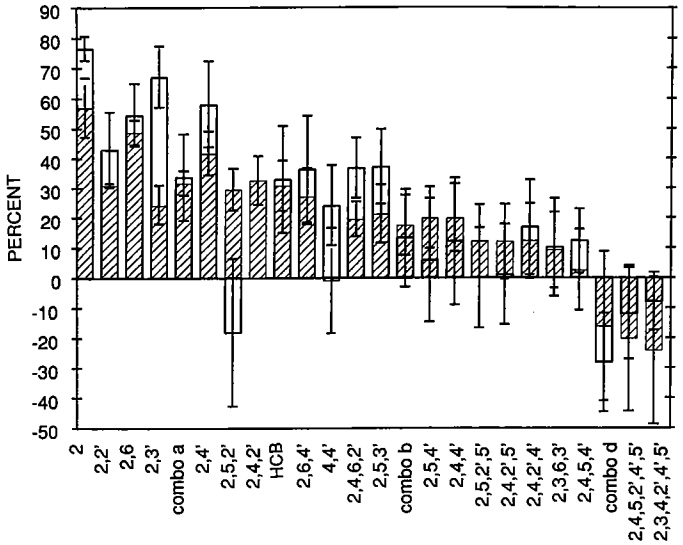

Fig. 3. The amount of biodegradation in a 7-month period in biphenyl-enriched Moreau sediments (▨) and Moreau sediments enriched with biphenyl and YE/T and inoculated with the mixed culture (□) in N_2. The values are a percentage of the control. Range bars are the SD of triplicate sediments.

ance of this congener was significant ($P < 0.05$) in the former but it remained almost intact in the latter. Although 2,6,4′-dichlorobiphenyl appeared to show a reverse pattern, its reduction in the bacteria-inoculated sediments was not statistically significant. Bacterial inoculation with concomitant addition of biphenyl and YE/T mixture seemed to enhance biodegradation more than biphenyl addition alone, especially of those congeners with <3 chlorines, but the observed difference in the congener pattern between the two treatments was not statistically significant (Wilcoxon signed rank test, $P < 0.05$).

The addition of biphenyl with YE/T mixture had no effects on congener concentrations under either N_2 or CO_2/H_2, nor did the inoculation of the bacterial-enrichment cultures to the sediments with heat-sterilized or live anaerobic sewage sludge.

Moreau sediments incubated in situ

The sediment samples were incubated about 1.0–1.5 m below the soil surface at the Moreau encapsulation site from late November 1986 to early June 1987. There was little change in congener concentration in untreated sediments under N_2 during this period. The results of biphenyl-amended sediments were difficult to evaluate, because two of the triplicate sediments were lost due to extraction mishaps. Based on a single value, they also seemed to show no decrease in congener concentrations, possibly with an exception of 2-monochlorobiphenyl. All other treatments also failed to show any significant change.

Hudson River sediments incubated in the laboratory

Untreated Hudson River sediments incubated under N_2 atmosphere showed a significant decrease ($P < 0.05$) in congeners with chlorination at: 2 (63% of the control); 2,2′ (49%); 2,4′ (49%); 2,6,4′ (38%); 2,4,6,2′ (33%); 2,5,2′5′ (49%); and 2,4,2′,5 (45%). The sediments with biphenyl amendment under N_2 showed highly significant decreases ($P < 0.01$) in: 2 (55%); 2,2′ (46%); 2,4′ (55%); 2,4,6,2′ (51%); 2,5,2′,5′ (65%); and 2,5,2′,4′ (63%). Significant reduction ($P < 0.05$) was also observed in: 2,6 (53%); combination a (2,3′ + 2,6,2′) (33%); 2,6,4′ (42%); 2,5,4′ (39%); 2,4,2′,4′ (49%); 2,3,6,4′ (44%); 2,3,5,2′,5′ (59%); and combination d (2,3,4,2′,4′ + 2,3,4,2′,3′ + 2,3,6,3′,4′) (52%) (Fig. 4). When biodegradation was compared between the untreated and biphenyl-amended sediments on a congener basis (Fig. 5), there was little difference in mono- and dichlorobiphenyls, suggesting little effects of biphenyl on them. However, a significant difference was found in congeners with higher chlorine substitutions. Thus, the biphenyl appeared to enhance the biodegradation of highly chlorinated congeners. The pattern of degradation was significantly different between the untreated and the biphenyl-amended sediments ($P < 0.05$, Wilcoxon signed rank test).

When biphenyl-enriched sediments were incubated under CO_2/H_2, no change in congener concentrations was found as with Moreau sediments. Unlike Moreau sediments, however, the treatment with biphenyl, YE/T mixture and the mixed enriched culture under N_2 showed negative results. All other treatments also failed to show any change in congener concentrations.

Pattern of degradation in Moreau and Hudson sediments

When the decrease in congener concentrations was compared between the biphenyl-amended sedi-

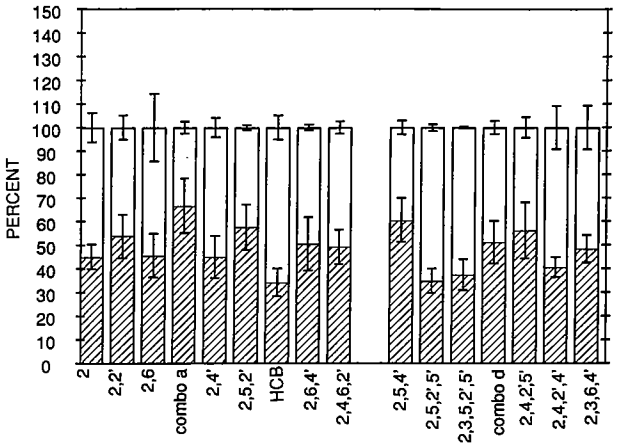

Fig. 4. Differences in congener concentrations between biphenyl-enriched Hudson River sediments (▨) in N_2 and the control (□) after a 7-month incubation. The total PCB concentration in the control was 707 mg/kg sediment. The concentration of individual congeners is shown in Fig. 1(B). Range bars are the SD of triplicate sediments.

ments from the Moreau and Hudson River for the congeners common to both sediments (Fig. 6), the relative decrease was generally much greater in the Hudson sediments. The difference was more pronounced for the congeners with higher chlorine substitutions because degradation was more sensitive to the degree of chlorination in Moreau than Hudson River sediments. The absolute concentrations of the congeners common to both sediments were different especially for mono- and dichlorobiphenyls, but those of most tri- and tetrachlorobiphenyls were not greatly different. Figure 7 compares the pattern of degradation between the biphenyl-amended Moreau and the untreated Hudson sediments which was similar to that with biphenyl-enriched Hudson sediments except that degradation was less pronounced.

The congener pattern in the Moreau sediments amended with biphenyl and YE/T mixture and inoculated with the enrichment cultures was quite different from that in Hudson River sediments with (Fig. 8) or without (not shown) biphenyl amendment. The percentage degradation of mono- and dichlorobiphenyls was comparable but in Moreau sediments no change was found in 2,5,2′-trichloro-, and 2,5,2′,5′- and 2,4,2′,5′-tetrachlorobiphenyls, unlike in the Hudson sediments.

DISCUSSION

Our findings reported here are strong evidence of anaerobic biodegradation of PCB congeners by indigenous bacteria. Several lines of evidence indicate that the decrease of PCB congeners in the present study was mediated by anaerobic microbial processes: (a) congener concentrations showed a significant decrease compared to the control in untreated, biphenyl-amended, or bacteria-inoculated

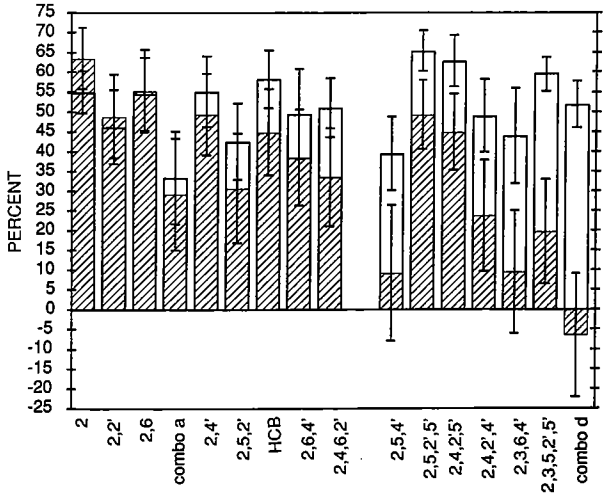

Fig. 5. The amount of biodegradation in untreated Hudson River sediments (▨) and biphenyl-enriched Hudson River sediments (□) in N_2 after a 7-month incubation. The values are a percentage of the control. Range bars are the SD of triplicate sediments.

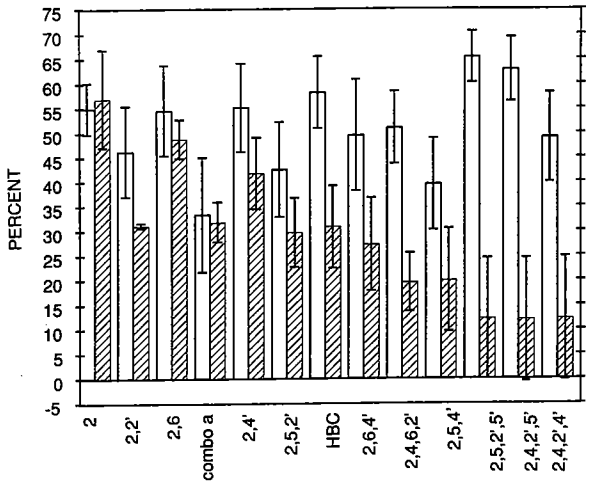

Fig. 6. The amount of biodegradation in biphenyl-enriched Moreau sediments (▨) and Hudson River sediments (□) in N_2. The values are a percentage of their respective controls. Range bars are the SD of triplicates.

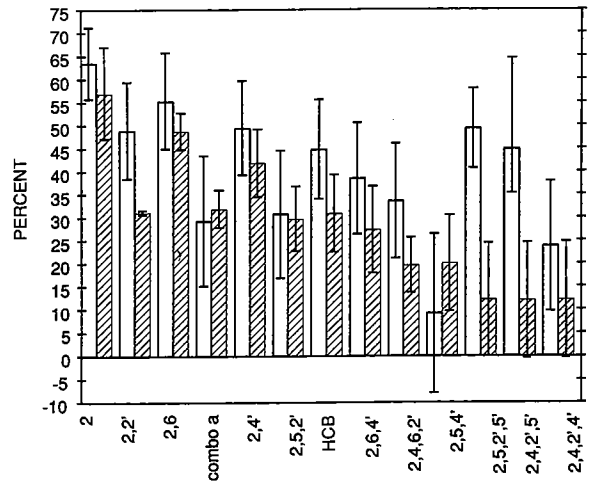

Fig. 7. The amount of biodegradation in biphenyl-enriched Moreau sediments (▨) and untreated Hudson River sediments (□) in N_2. The values are a percentage of their respective controls. Range bars are the SD of triplicates.

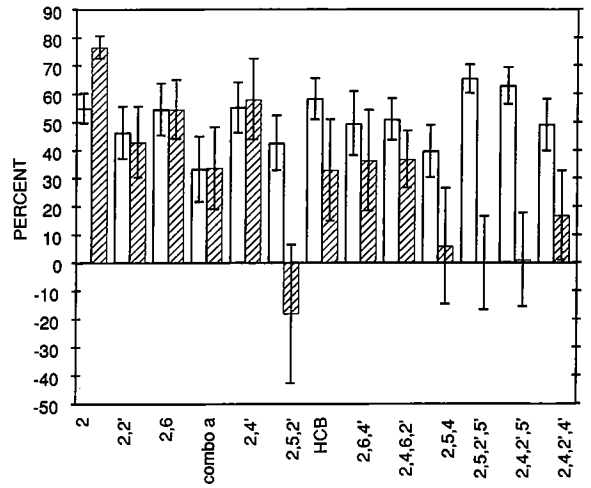

Fig. 8. The amount of biodegradation in Moreau sediments with biphenyl and YE/T, and inoculated with the mixed culture (▨) and biphenyl-enriched Hudson River sediments (□). The values are a percentage of their respective controls.

sediments; (b) the decrease of PCB congeners was significantly enhanced by amendment with the PCB analog, biphenyl under N_2; and (c) a significant reduction in congeners occurred in the mixed culture-inoculated Moreau sediments amended with biphenyl and YE/T mixture but not in the identical sediments with no bacterial inoculation.

The pattern of congener degradation indicated that indigenous populations in the Hudson sediments were capable of attacking a broader spectrum of congeners (including pentachlorobiphenyls) than those in Moreau sediments. It is unclear whether the difference was due to the qualitative difference in bacterial populations present or the difference in the relative proportion of the same populations. In a 7-month period approx. 53% of the original total PCB concentration, or 347 mg/kg, was reduced in the Hudson sediments amended with biphenyl and about 30% or 280 mg/kg in the biphenyl-amended Moreau sediments incubated in the laboratory under N_2.

In both Hudson and Moreau sediments, a common characteristic of all the biodegraded congeners was the absence of adjacent chlorines in both of the biphenyl rings. The congeners which showed highly significant degradation ($P < 0.01$) did not have adjacent chlorines in either ring. However, not all the congeners without adjacent chlorines were degraded. Otherwise, no clear degradation pattern could be recognized with respect to the chlorine position. This might suggest the nature of mixed bacterial populations present in sediments.

We were unable to characterize the degradation products because of insufficient volume of samples. At present we are investigating them as unequivocal evidence for biodegradation and also to evaluate the environmental impact of anaerobic breakdown, since the metabolic products of some haloaromatics are more toxic than the parent compounds (Reineke, 1984).

We failed to find any evidence for the accumulation of less-chlorinated congeners as a result of reductive dechlorination of highly chlorinated ones, contrary to the findings by Quensen *et al.* (1988) and the assertion by Brown *et al.* (1987a, b). However, the lack of the accumulation of less-chlorinated congeners in our study does not either prove or disprove the occurrence of reductive dechlorination. If low-molecular-weight congeners were degraded faster than they were produced by dechlorination, their accumulation would not have been observed. However, no significant decreases in highly chlorinated congeners were detected, suggesting that, if reductive dechlorination occurred, the fraction of the low-molecular-weight congeners generated by the process was quite small. It is also possible that the apparent absence of dechlorination in the present study is related to the difference in sampling sites, similar to the case in which the substrate biodegradation capability of sewage sludges varied with their sources (Shelton and Tiedje, 1984).

Reductive dechlorination has also been reported on many other chlorinated aromatic xenobiotics. For instance, γ-hexachlorocyclohexane was reductively dechlorinated followed by the production of pentachlorohexane, CO_2, γ-tetrachlorocyclohexane and some unidentified partly volatile compounds as the final products (Fries, 1972; Haider, 1977; Ohisa and Yamaguchi, 1979; Vonk and Quirijns, 1979). Chlororesorcinol was dechlorinated to resorcinol (1,3-dihydroxybenzene) (Fathepure *et al.*, 1987) which should be metabolized readily (Tschech and Schnik, 1985). Chlorophenols were also degraded anaerobically following reductive dechlorination, with some yielding CH_4 and CO_2 as final products (Boyd *et al.*, 1983; Boyd and Shelton, 1984; Guthrie *et al.*, 1984; Hrudey *et al.*, 1987; Krumme and Boyd, 1988). Halobenzoates were mineralized into CH_4 and CO_2 under strictly anaerobic conditions following dehalogenation (Suflita *et al.*, 1983; Horowitz *et al.*, 1983). It appears therefore that the reductive dechlorination of high-molecular-weight PCB congeners may require conditions different from those employed for our work including the sediment types.

It is unclear why the sediments enriched with biphenyl and those which received biphenyl, YE/T mixture and the mixed enrichment culture showed little change when incubated under CO_2/H_2 atmosphere in contrast to the same sediments under N_2, which showed significant reductions in congener concentrations. It may have been due to the competitive exclusion by incompetent species of competent organisms or consortia of organisms capable of biodegradation. Such differences in microbial populations were suggested by the methane production, which was highly significant in CO_2/H_2 but undetectable in N_2. The same reasons may also explain the failures of biodegradation in other treatments despite the positive results in untreated or biphenyl-amended sediments. Biphenyl addition appeared to be most effective for the selection of competent species. The enhancement of biodegradation by biphenyl appeared to be greater for the congeners with higher chlorine substitution (Figs 2 and 3).

The enhancement of degradation by mixed-culture inoculation was similar to that observed in our earlier work (Chen *et al.*, 1988). In the earlier studies, in which biphenyl and YE/T mixture were not used, the inoculation of the same culture to Hudson River sediments produced a significant reduction in congeners with up to 3 chlorines, whereas without inoculation no change was found after a 6-month period. However, Hudson River sediments which received an identical treatment to the Moreau sediments showed no change in congener concentrations. The reason for these negative results is unclear, but the different pattern of congener degradation in biphenyl-amended sediments between the two types of sediments is suggestive of differences in microbial composition between them and these differences could have contributed to the negative results.

The lack of changes in congener concentrations in biphenyl-enriched Moreau sediments incubated *in situ* could probably be due to the low temperature, since the identical sediments in the laboratory (25°C) exhibited a significant decrease in a number of congeners. Another reason, although less likely, may be the absence of mechanical agitation *in situ*.

It is difficult to extrapolate laboratory studies to nature. The results of untreated Hudson River sediments seem to suggest that biodegradation might occur *in situ*, however slow it might be. The results were obtained, however, at an artificially elevated temperature in the laboratory. It is unclear whether the negative result of *in situ* incubation at the Moreau site was due to slower reaction rates, which might have been positive should the incubation period have been longer, or simply the absence of necessary enzymes or insignificant levels of enzyme activities at ambient temperatures. However, our results indicate the potential for the bioremediation with artificial manipulation, such as biphenyl addition, at a specially-designed encapsulation site engineered to provide an optimal temperature. If highly chlorinated congeners can be reductively dechlorinated to those with low molecular weight, all PCB congeners would then become amenable for bioremediation. Therefore, it is highly important to clearly characterize and understand the conditions required for biodegradation and dechlorination.

Acknowledgements—This work was supported by a grant from the Hudson River Foundation (02186B015) to G-Y. R. We thank Linda Carter for her technical assistance.

REFERENCES

Boyd S. A., Shelton D. R., Berry D. and Tiedje J. M. (1983) Anaerobic biodegradation of phenolic compounds in digested sludge. *Appl. envir. Microbiol.* **46,** 50–54.

Boyd S. A. and Shelton D. R. (1984) Anaerobic biodegradation of chlorophenols in fresh and acclimated sludge. *Appl. envir. Microbiol.* **47,** 272–277.

Brown J. F. Jr, Bedard D. L., Brennan M. J., Carnahan J. C., Feng H. and Wagner E. (1987a) Polychlorinated biphenyl dechlorination in aquatic sediments. *Science* **236,** 709–712.

Brown J. F. Jr, Wagner R. E., Feng H., Bedard D. L., Brennan M. J., Carnahan J. C. and May R. J. (1987b) Environmental dechlorination of PCBs. *Envir. Toxic. Chem.* **6,** 579–593.

Brown M. P. (1989) Dechlorination of PCB in the Hudson River. *Envir. Toxic. chem.* **8,** 277–282.

Brown M. P., Bush B., Rhee G-Y. and Shane L. (1988) PCB dechlorination in Hudson River sediment. *Science* **240,** 1674–1675.

Brunner W., Sutherland F. H. and Focht D. D. (1985) Enhanced biodegradation of polychlorinated biphenyls in soil by analog enrichment and bacterial inoculation. *J. envir. Qual.* **14,** 324–328.

Bush B., Shane L. A., Wahlen M. and Brown M. P. (1987) Sedimentation of 74 PCB congeners in the upper Hudson River. *Chemosphere* **16,** 733–744.

Carey A. E. and Harvey G. R. (1978) Metabolism of polychlorinated biphenyls by marine bacteria. *Bull. envir. contam. Toxic.* **20,** 527–534.

Chen M., Hong C. S., Bush B. and Rhee G-Y. (1988) Anaerobic biodegradation of polychlorinated biphenyls by bacteria from Hudson River sediments. *Ecotoxic. envir. Saf.* **16,** 95–105.

Fathepure B. Z., Tiedje J. M. and Boyd S. A. (1987) Reductive dechlorination of 4-chlororescorcinol by anaerobic microorganisms. *Envir. Toxic. Chem.* **6,** 929–934.

Fries G. F. (1972) Degradation of chlorinated hydrocarbons under anaerobic conditions. *Adv. Chem. Ser.* **111,** 256–270.

Furukawa K. (1983) Microbial degradation of polychlorinated biphenyls (PCBs). In *Biodegradation and Detoxification of Environmental Pollutants* (Edited by Chakrabarty A. M.), pp. 59–84. CRC Press, Boca Raton, Fla.

Guthrie M. A., Kirsch E. J., Wukasch R. F. and Grady C. P. L. (1984) Pentachlorophenol biodegradation—II. Anaerobic. *Wat. Res.* **18,** 451–461.

Haider K. (1977) Degradation and metabolism of Lindane and other hexachlorocyclohexane isomers by anaerobic and aerobic soil microorganisms. *Z. Naturf.* **34,** 1066–1069.

Horowitz A., Suflita J. M. Tiedje J. M. (1983) Reductive dehalogenation of halobenzoates by anaerobic lake sediment microorganisms. *Appl. envir. Microbiol.* **45,** 1459–1465.

Hrudey S. E., Knettig E., Daignault S. A. and Fedorak P. M. (1987) Anaerobic biodegradation of monochlorophenols. *Envir. Technol. Lett.* **8,** 65–76.

Krumme M. L. and Boyd S. A. (1988) Reductive dechlorination of chlorinated phenols in anaerobic upflow bioreactors. *Wat. Res.* **22,** 171–177.

Lederman T. C. and Rhee G-Y. (1982) Bioconcentration of hexachlorobiphenyl in Great Lakes planktonic algae. *Can. J. Fish. Aquat. Sci.* **39,** 380–387.

Ohisa N. and Yamaguchi M. (1979) Clostridium species and gamma-BHC degradation in paddy soil. *Soil Biol. Biochem.* **11,** 645–649.

Quensen J. F. III, Tiedje J. M. and Boyd S. A. (1988) Reductive dechlorination of polychlorinated biphenyls by anaerobic microorganisms from sediments. *Science* **242,** 752–754.

Reineke W. (1984) Microbial degradation of halogenated aromatic compounds. In *Microbial Degradation of Organic Compounds* (Edited by Gibson D. T.), pp. 319–360. Dekker, New York.

Safe S. H. (1984) Microbial degradation of polychlorinated biphenyls. In *Microbial Degradation of Organic Compounds* (Edited by Gibson D. T.), pp. 361–369. Dekker, New York.

Sayler G. S., Kong H.-L. and Shields M. S. (1984) Plasmid-mediated biodegradative fate of monohalogenated biphenyls in facultatively anaerobic sediments. In *Genetic Control of Environmental Pollutants* (Edited by Omenn G. S. and Hollander A.), pp. 117–135. Plenum Press, New York.

Shelton D. R. and Tiedje J. M. (1984) General method for determining anaerobic biodegradation potential. *Appl. envir. Microbiol.* **47,** 850–857.

Suflita J. M., Robinson J. A. and Tiedje J. M. (1983) Kinetics of microbial dehalogenation of haloaromatic substrates in methanogenic environments. *Appl. envir. Microbiol.* **45,** 1466–1473.

Sylvestre M. and Fauteux J. (1982) A new facultative anaerobe capable of growth on chlorobiphenyls. *J. gen. appl. Microbiol.* **28,** 61–72.

Tschech A. and Schink B. (1985) Fermentative degradation of resorcinol and resorcyclic acids. *Archs Microbiol.* **143,** 52–59.

Vonk J. W. and Quirijns J. K. (1979) Anaerobic formation of alpha-hexachlorocyclohexane from gamma-hexachlorocyclohexane in soil by *Escherichia coli*. *Pest. Biochem. Physiol.* **12,** 68–74.