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Population Dynamics of Polychlorinated Biphenyl-Dechlorinating Microorganisms in Contaminated Sediments

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The growth dynamics of polychlorinated biphenyl (PCB)-dechlorinating microorganisms were determined for the first time, along with those of sulfate reducers and methanogens, by using the most-probable-number technique. The time course of Aroclor 1248 dechlorination mirrored the growth of dechlorinators; dechlorination ensued when the dechlorinating population increased by 2 orders of magnitude from 2.5×10^5 to 4.6×10^7 cells g of sediment^{-1}, at a specific growth rate of 6.7 day^{-1} between 2 and 6 weeks. During this period, PCB-dechlorinating microorganisms dechlorinated Aroclor 1248 at a rate of 3.9×10^{-8} mol of Cl g of sediment^{-1} day^{-1}, reducing the average number of Cl molecules per biphenyl from 3.9 to 2.8. The growth yield was 4.2×10^{13} cells mol of Cl dechlorinated^{-1}. Once dechlorination reached a plateau, after 6 weeks, the number of dechlorinators began to decrease. On the other hand, dechlorinators inoculated into PCB-free sediments decreased over time from their initial level, suggesting that PCBs are required for their selective enrichment. The numbers of sulfate reducers and methanogens increased in both PCB-free and contaminated sediments, showing little difference between them. The maximum population size of sulfate reducers was about an order of magnitude higher than that of dechlorinators, whereas that of methanogens was slightly less. Unlike those of dechlorinators, however, numbers of both sulfate reducers and methanogens remained high even when dechlorination ceased. The results of this study imply that PCB concentrations may have to exceed a certain threshold to maintain the growth of PCB dechlorinators.

Reductive dechlorination of polychlorinated biphenyls (PCBs) is a natural bioremedial process that transforms PCBs into lower-chlorinated congeners (26). Despite clear evidence for the microbial reductive dechlorination of PCBs in both the laboratory and natural sediments during the past decade (see references in references 5 and 26), efforts to isolate PCB-dechlorinating microorganisms have been unsuccessful and little is known about the microbial dynamics of PCB dechlorination. Although sulfate reducers and methanogens have been implicated in certain types of PCB dechlorination (31, 32), their exact role in the dechlorination process also is not clearly understood.

PCB dechlorination studies have focused mainly on determining quantitative shifts in congener profiles at PCB-contaminated sites and confirming PCB-dechlorinating activities through laboratory incubation experiments (see references in reference 5). Although selection of dechlorinating microorganisms in PCB-contaminated sediments has been speculated about (1, 24), investigations have failed to directly demonstrate enrichment in relation to the in situ PCB dechlorination potential in the sites. While laboratory investigations have shown that the rate of PCB dechlorination depends on the total PCB concentration (1, 14, 27, 29), it remains unclear whether the dependence was due to different biomasses of dechlorinating microorganisms or the affinity of dechlorinating enzymes.

To enhance PCB dechlorination through the enrichment of dechlorinating populations, various carbon and energy sources have been added to sediments (1, 2, 6, 16, 20, 21). Although an increased rate or a reduced lag time has been observed, attempts to enhance the extent of PCB dechlorination have generally failed. The nutritional requirement for PCB dechlorination thus remains unclear.

It has been proposed that dechlorinating microorganisms can use PCBs as electron acceptors in anaerobic respiration (7). Thermodynamic calculations also show that energy available from reductive dechlorination of PCBs is sufficient to support the growth of dechlorinators (15). If dechlorinating microorganisms compete for electron donors with other populations of the community in highly reduced sediments where terminal electron acceptors may be limiting, the opportunity to use PCBs as alternative electron acceptors would provide some selective advantage for the dechlorinators (7, 24). Thus, priming with halogenated compounds has been tried to stimulate PCB dechlorination by selectively enriching dechlorinating populations, only to find enhancement for a limited number of congeners (4).

To enhance and optimize PCB-dechlorinating microbial activities in situ or ex situ, it is essential to understand the factors controlling the growth of PCB dechlorinators and their interactions with other populations of the community. In the present study, we investigated the population dynamics of PCB dechlorinators, sulfate reducers, and methanogens in laboratory cultures with and without PCB amendment. We used the most-probable-number (MPN) technique to estimate the numbers of the above microorganisms in the cultures.

MATERIALS AND METHODS

Culture preparation. Laboratory cultures were set up in triplicate as follows: PCB-free sediments collected from Owasco Lake, N.Y., were air dried and sifted through a 150-μm sieve. Sediments were spiked with PCBs by mixing with Aroclor 1248 in hexane to yield a concentration of 300 μg g (dry weight) of sediment^{-1} and evaporating the hexane. PCB-free and Aroclor 1248-spiked sediments were separately made into slurries by adding reduced synthetic minimal medium (3) in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, Mich.) with an N_2-CO_2-H_2 atmosphere (85:5:10). The sediment slurries contained 20% (wt/vol) sediment on a dry-weight basis. Batch incubation vials were prepared by dispensing 60-ml aliquots of sediment slurry into 150-ml serum

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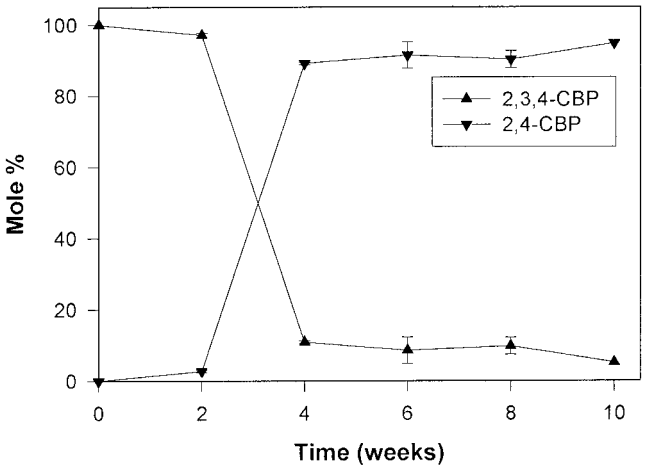

FIG. 1. Dechlorination of 2,3,4-CBP to 2,4-CBP as a function of time in test vials inoculated with a 10^{-2} dilution of the inoculum. Each point represents the mean (± the standard deviation) of duplicate vials.

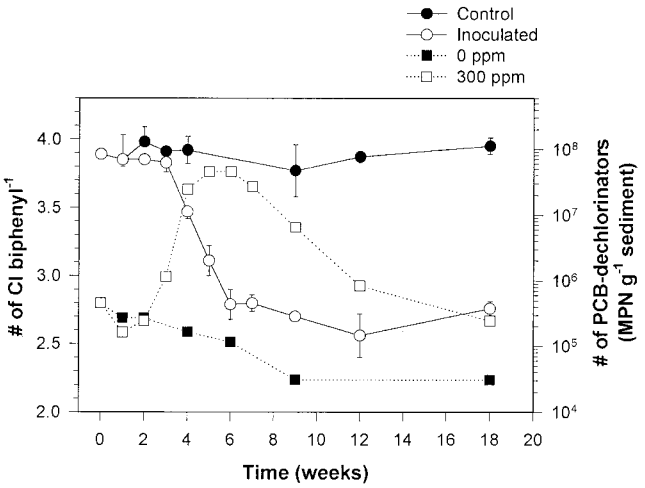

FIG. 2. Aroclor 1248 dechlorination, expressed as the number of Cl molecules per biphenyl, in PCB-amended, inoculated (○) and autoclaved control (●) sediments and the MPN of PCB dechlorinators in inoculated, PCB-amended (□) and unamended (■) sediments with the time course of incubation. While each number of Cl molecules per biphenyl represents the mean (± the standard deviation) of triplicate vials, the values of PCB dechlorinators were determined from one of the triplicate vials by using a five-tube MPN procedure.

vials and sealing them with Teflon-lined rubber septa and aluminum crimp seals. The vials were autoclaved (121°C, 40 min) three times on 3 successive days. To minimize variation between inoculations, 2 ml of sediment slurry from a single batch of previous enrichment cultures (16) was inoculated into each of the vials containing PCB-free and Aroclor 1248-spiked sediments, except for controls. After inoculation, the cultures were incubated statically at room temperature in the dark. The initial enrichment cultures had been set up with microorganisms eluted from the General Motors (GM) site in the St. Lawrence River and successively transferred (10%, vol/vol) into fresh, Aroclor 1248-spiked sediments.

Sampling. At predetermined time intervals, 1-ml portions of sediment slurry from the triplicate vials of Aroclor 1248-amended cultures were sampled for PCB analysis, while another 1 ml was taken from one of the triplicate (both PCB-free and Aroclor 1248-amended) vials and transferred into a diluent vial for MPN determination. For lipid analysis, 2-ml samples were taken from duplicate vials and placed in 50-ml test tubes. All of the sampling and transfer was done with a sterile syringe after vortex mixing in the anaerobic chamber. Immediately after sampling, the punctured Teflon-lined rubber septa and aluminum crimp seals were replaced with new ones.

MPN determination. A five-tube MPN procedure was used to enumerate PCB dechlorinators, sulfate reducers, and methanogens from one of the triplicate culture vials. MPN test or diluent vials were prepared with the sieved Owasco Lake sediments as described above, except by using 30-ml serum vials. The diluent vials contained 9 ml of PCB-free sediment slurry with a sediment content of 2.5% (wt/vol), while the MPN test vials had sediments spiked with a mixture of 2,3,4- and 2,5,3′,4′-chlorobiphenyls (CBPs) (150 and 100 μg g (dry weight) of sediment^{-1}, respectively). A dilution series (10^{-1} to 10^{-9}) of each sample was prepared by serially transferring a 1-ml portion of sediment slurry by using a sterile syringe with a 20-gauge needle immediately after vortex mixing. The MPN test vials, five vials per dilution, were inoculated with 1 ml of sediment slurry from each dilution in the 10^{-3} to 10^{-9} range and incubated statically at room temperature for 16 weeks.

The number of PCB dechlorinators was estimated by assaying the dechlorination of 2,3,4,- and 2,5,3′,4′-CBPs in the MPN test vials. Vials were considered dechlorinator positive if dechlorination occurred after 16 weeks of incubation; in dechlorination-positive cases, more than 50% of the parent congeners was dechlorinated with concomitant increases of daughter congeners. A comparison of the experimental samples to those of autoclaved controls showed no molar loss of PCB congeners after 16 weeks. Figure 1 shows the dechlorination of 2,3,4-CBP by a 10^{-2} dilution of the inoculum over time; dechlorination occurred mostly between 2 and 4 weeks, producing 2,4-CBP. The growth of sulfate reducers was determined by the blackening of sediments in the MPN test vials with the production of FeS (22). Vials with black sediments (compared to the brownish original color) were counted as positive. Autoclaved controls or sediments with molybdate showed the original sediment color with no black appearance. Methane formation was determined after 16 weeks by analyzing headspace samples of the MPN test vials on a gas chromatograph with a thermal conductivity detector as described by Pavlostathis et al. (23). Vials were considered positive if methane was present regardless of its concentration, although the amount of methane produced in the MPN vials, in general, decreased with increasing dilutions. The MPNs of PCB dechlorinators, sulfate reducers, and methanogens were calculated from the numbers of positive vials in consecutive dilutions by using an MS-DOS QBASIC program (18) and normalized as the MPN (of dechlorinators, sulfate reducers, or methanogens) per gram (dry weight) of sediment.

Microbial biomass determination. Lipids were extracted from samples with chloroform and methanol by the method of Bligh and Dyer as described by Dobbs and Findlay (10). The lipid extracts were fractionated into neutral lipids, glycolipids, and phospholipids on a silicic acid column (10), and the phospholipid fraction was collected and dried under a stream of nitrogen. The inorganic phosphate was released from the phospholipids by potassium persulfate digestion (95°C, 18 h) and quantitated colorimetrically (10, 13). The phospholipid phosphate concentration was normalized as nanomoles of phosphate per gram (dry weight) of sediment.

PCB extraction and analysis. Sediments from the culture vials were extracted with acetone and hexane by ultrasonication as described previously (27). PCBs in the MPN test vials were extracted in the following manner. First, the aqueous phase was pipetted off into a 150-ml Erlenmeyer flask. The sediments were then extracted twice, first with acetone-hexane (1:1, vol/vol) overnight and then with hexane for 4 h by shaking on an orbital shaker. The solvent extracts were combined with the aqueous phase. Distilled water (50 ml) was added to the flask for phase separation, and the hexane layer was placed into a flask containing sodium sulfate. All of the hexane extracts were concentrated in a Kuderna-Danish condensing apparatus, treated with a tetrabutylammonium sulfite reagent to remove elemental sulfur, and cleaned up on a 4% deactivated Florisil column (27). Quantitative congener-specific PCB analysis was performed by using one of two gas chromatographs (Hewlett-Packard [HP] 5890A or 5890II), each equipped with a ^{63}Ni electron capture detector, an HP 7673 autosampler, and an HP 3396 integrator. Aroclor 1248 samples were analyzed on an Rtx-5 fused silica capillary column (60 m by 0.25 mm [inside diameter] by 0.1 μm; Restek Corporation), while an Apiezon-L column (30 m by 0.25 mm [inside diameter] by 0.2 μm; Restek Corporation) was used to analyze 2,3,4- and 2,5,3′,4′-CBP samples. The gas chromatography conditions used were described elsewhere (30). An equal-amount mixture of Aroclors 1016, 1221, 1254, and 1260 (0.2 μg of each ml^{-1} in *n*-hexane) was used as a calibration standard to quantitate Aroclor 1248 samples. The Apiezon column was calibrated with a mixture of single-congener standards (AccuStandard, New Haven, Conn.) (30). The standard mixtures were also used for quality control of the gas chromatography analysis as described previously (27). All of the chromatographic data were collected and processed on a microcomputer by using a ChromPerfect chromatography data system (Justice Innovations, Mountain View, Calif.). The moles percent of PCB congeners and the average number of Cl molecules per biphenyl were calculated based on the concentration of each congener. Coeluting congeners were assumed to be present in equal proportions for the calculations (25, 30).

RESULTS

PCB dechlorination versus growth dynamics of PCB dechlorinators. Dechlorination of Aroclor 1248 by the GM sediment microorganisms occurred mostly between 3 and 6 weeks, after a 3-week lag period (Fig. 2). Analysis after 6 weeks showed that the average number of Cl molecules per biphenyl was reduced

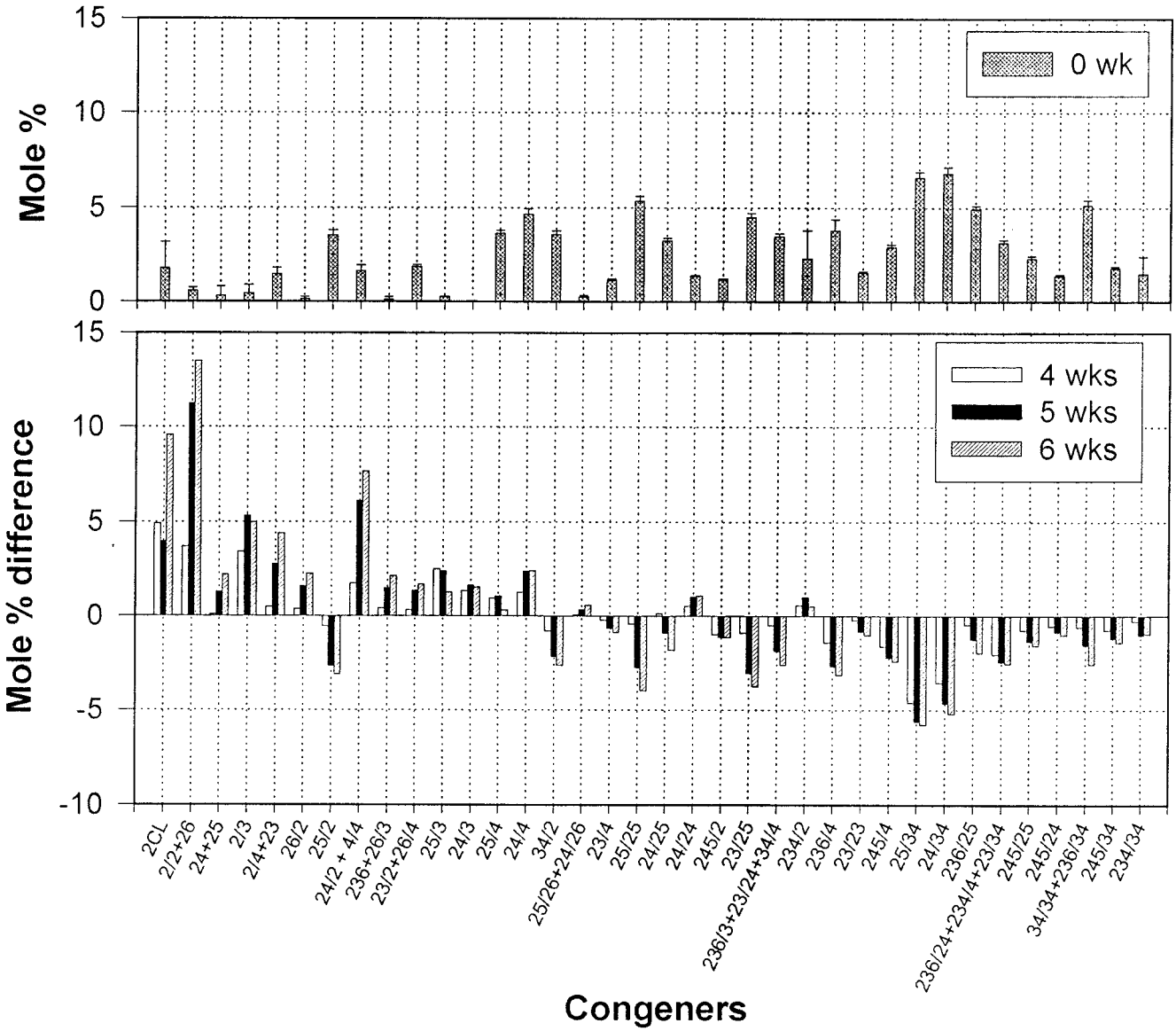

FIG. 3. Moles percent (mean ± standard deviation) of Aroclor 1248 congeners in week 0 controls (top) and changes in the moles percent of Aroclor 1248 congeners after 4, 5, and 6 weeks of incubation (bottom). Congeners of which the moles percent were higher than 1% at any time point were selected for the graph.

from 3.9 to 2.8, or an overall 28% reduction from the original Aroclor 1248. Congeners were dechlorinated from both the *meta* and *para* positions (Fig. 3). After 6 weeks, dechlorination reached a plateau with no further changes out to 18 weeks; this level was considered to represent the apparent endpoint of dechlorination (Fig. 2). The molar concentration of total PCBs remained constant throughout the incubation periods.

When the number of PCB dechlorinators was estimated in these sediments over time by the MPN technique, the growth of dechlorinators mirrored the time course of dechlorination (Fig. 2); dechlorination ensued when the population increased by 2 orders of magnitude, from 2.5×10^5 to 4.6×10^7 cells g of sediment^{-1} or a growth rate of 1.6×10^6 cells g sediment^{-1} day^{-1} between 2 and 6 weeks. During the exponential phase of growth (2 to 6 weeks), the specific growth rate and the doubling time of dechlorinators were 6.7 day^{-1} and 0.1 day, respectively. For this period, the rate of dechlorination was 1.0×10^{-8} mol (Aroclor 1248) g of sediment^{-1} day^{-1} or 3.9×10^{-8} mol of Cl g of sediment^{-1} day^{-1}, based on the changes in the average number of Cl molecules per biphenyl. The growth yield of dechlorinators was calculated as 1.6×10^{14} cells mol (Aroclor 1248) dechlorinated^{-1} or 4.2×10^{13} cells mol of Cl dechlorinated^{-1}. (A value of 288.55 was used as the average molecular weight of Aroclor 1248 [$C_{12}H_{6.11}Cl_{3.89}$]). When dechlorination apparently ceased at the low plateau, the number of dechlorinators also began to decrease, to a level of 2.5×10^5 cells g of sediment^{-1} after 18 weeks.

In the dechlorination-positive MPN test vials, there was no difference in the extent of 2,3,4-CBP dechlorination between different dilution levels; more than 85% of the 2,3,4-CBP was dechlorinated after 16 weeks, and 2,4-CBP was the only product. Dechlorination of 2,5,3′,4′-CBP also occurred in the vials which were positive for 2,3,4-CBP. The product of 2,5,3′,4′-CBP dechlorination was 2,5,3′-CBP in most of the test vials, whereas some vials of dilutions lower than the critical dilution level (where both positive and negative vials can be counted)

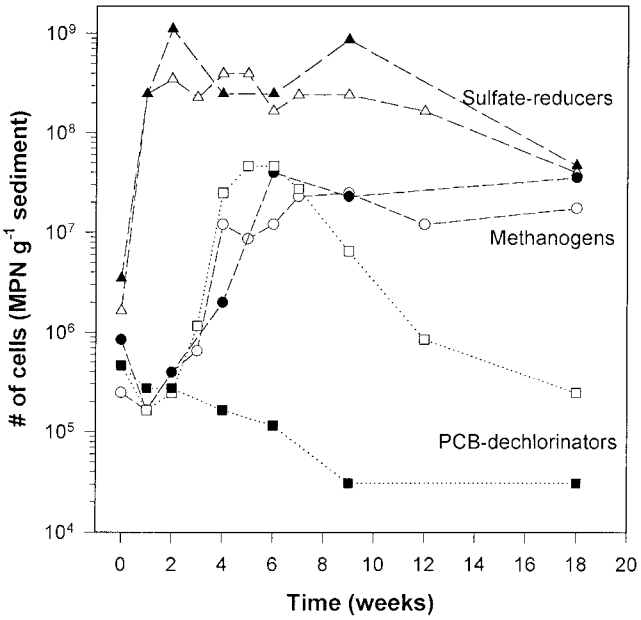

FIG. 4. Comparison of growth dynamics of PCB dechlorinators (squares), sulfate reducers (triangles), and methanogens (circles) in cultures with (blank symbols) and without (black symbols) PCB amendment. Symbols: □, dechlorinators, 300 ppm; ■, dechlorinators, 0 ppm; △, sulfate reducers, 300 ppm; ▲, sulfate reducers, 0 ppm; ○, methanogens, 300 ppm; ●, methanogens, 0 ppm. The values were determined from one of the triplicate vials by using a five-tube MPN procedure.

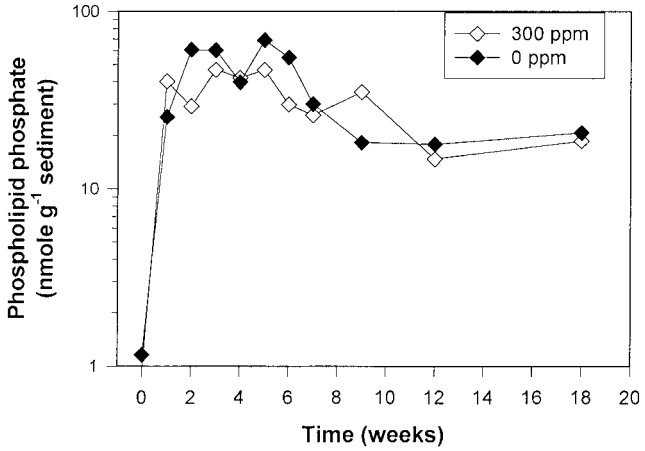

FIG. 5. Time course changes in the phospholipid phosphate contents of cultures with (◇) and without (◆) PCB amendment.

produced mainly 2,5,4′-CBP along with a small amount of 2,5,3′-CBP, suggesting the presence of different populations.

The results of the MPN analysis showed marked differences in the growth dynamics of PCB dechlorinators between the cultures with and without PCB amendment (Fig. 2). With the same inoculum from the GM site, the number of PCB dechlorinators in the cultures decreased in PCB-free sediments; the initial population size of 4.6×10^5 cells g of sediment^{-1} decreased to 3.1×10^4 cells g of sediment^{-1} after 9 weeks and remained at this level up to 18 weeks. These results indicate that PCBs are needed for the selection of dechlorinators or dechlorination activity.

Detailed examination of chromatographic pattern changes showed that initial decreases (observed after 4 weeks) involved 2,4,5,2′-, 2,4,5,4′-, 2,5,3′4′-, 2,4,3′,4′-, and 2,3,6,2′,4′- plus 2,3,4,4′- plus 2,3,3′,4′-CBPs with concomitant increases in 2,2′- plus 2,6-, 2,5,4′-, 2,5,3′-, 2,4,3′-, 2,4,4′-, and 2,3′-CBPs (Fig. 3). By 5 weeks, there were additional decreases in *meta*-rich congeners such as 2,5,2′,5′-, 2,4,2′,5′-, 2,3,2′,5′-, 2,3,6,3′- plus 2,3,2′,4′-, plus 3,4,4′-, and 2,5,2′-CBPs with an accumulation of 2,2′- plus 2,6-, 2,4,2′- plus 4,4′-, 2,3,6- plus 2,6,3′-, and 2,3,2′- plus 2,6,4′-CBPs.

Growth dynamics of sulfate reducers and methanogens. In Aroclor 1248-spiked sediments, sulfate reducers grew rapidly during the first week, reaching their highest level earlier than dechlorinators (Fig. 4). The sulfate-reducing population increased from an initial level of 1.7×10^6 to 2.5×10^8 cells g of sediment^{-1} after 1 week; at its maximum, the population size was about an order of magnitude higher than that of dechlorinators (4.0×10^8 versus 4.6×10^7 cells g of sediment^{-1}). After the exponential growth phase, the number of sulfate reducers remained high up to 9 weeks and then began to decrease, to a level of 4.0×10^7 cells g of sediment^{-1} after 18 weeks, which was still about 2 orders of magnitude higher than that of dechlorinators (Fig. 4). Sulfate reduction was observed in all dechlorinator-positive MPN test vials.

On the other hand, the growth of methanogens had a lag time similar to that of the dechlorinators, and the highest number of methanogens was 2.5×10^7 cells g of sediment^{-1} after 7 weeks, which was slightly less than that of dechlorinators. However, unlike dechlorinators, the size of the methanogenic population changed little after reaching the upper plateau, maintaining its maximum level up to 18 weeks (Fig. 4). Interestingly, the number of sulfate reducers and methanogens in PCB-free sediments showed little difference from that in PCB-amended sediments (Fig. 4).

Dechlorination of 2,3,4- and 2,5,3′,4′-CBPs was observed in some MPN test vials in which no methane production was detected, indicating that methanogenesis was not a requisite for dechlorination of the two congeners. In these vials, the dechlorination products of 2,3,4- and 2,5,3′,4′-CBPs were 2,4-, and 2,5,3′-CBPs, respectively.

Total eubacterial biomass. The changes in microbial biomass over time, measured as the phospholipid phosphate content in samples, were also similar between cultures with and without PCB amendment ($P = 0.21$; paired t test) (Fig. 5). Therefore, the proportion of dechlorinators in the total populations appears to be rather small. In Aroclor 1248-spiked sediments, the phospholipid phosphate content increased from −0.1 (value subtracted with controls) to 40.2 nmol g of sediment^{-1} after 1 week, which decreased to 18.6 nmol g of sediment^{-1} after 18 weeks. With a conversion factor of 3.43×10^7 cells nmol of phospholipid phosphate^{-1} (13), the phospholipid content corresponded to a total eubacterial biomass of 1.4×10^9 cells g of sediment^{-1} after 1 week.

DISCUSSION

Although the MPN method may be tedious and rather imprecise, it is the only method available for measuring the abundance of PCB dechlorinators because, as far as we know, no molecular probes or biochemical markers have been developed for the population. For the present study, we assumed that dechlorination activity in the MPN test vials depended on the presence of dechlorinating microorganisms or the number of dechlorinating consortia. If dechlorination was not expressed in sediments due to the lack of nondechlorinating members of consortia, the MPN method would have counted them instead of dechlorinators. Nonetheless, the MPN reflects the number

of a PCB-dechlorinating consortium as a unit. However, if dechlorinating populations changed over the course of incubation, the MPN technique could not have differentiated them. If there were dechlorinators which were unable to dechlorinate the two congeners (2,3,4- and 2,5,3′,4′-CBPs), their use might have underestimated dechlorinators; however, both congeners were dechlorinated in dechlorination-positive vials and dechlorination of 2,5,3′,4′-CBP was observed in all different types of dechlorination (5).

It is possible that some PCB dechlorinators are also methanogens and/or sulfate reducers (and vice versa). If they are, however, they appear to represent only a small part of the total population of methanogens or sulfate reducers, because (i) there was no clear difference in the growth dynamics of methanogens and sulfate reducers between PCB-spiked and PCB-free sediments, and (ii) unlike dechlorinating organisms, the total number of methanogens and sulfate reducers did not decline with the apparent cessation of dechlorination. These observations also indicate that methanogens and sulfate reducers, in general, may not gain any advantage from interactions with dechlorinators. However, it is possible that the interactions are essential for the growth of dechlorinators.

The results of this study demonstrate that the growth of PCB dechlorinators requires the presence of PCBs. They also suggest that "dechlorinatable" PCBs are, indeed, limiting for their growth. Dechlorinating microorganisms can derive energy in anaerobic respiration, as indicated by reductive dechlorination of 3-chlorobenzoate (11, 12).

The growth yield (4.2×10^{13} cells mol of Cl dechlorinated^{-1}) of PCB dechlorinators during the exponential phase in the present study was comparable to the growth yield of a 3-chlorobenzoate-degrading consortium (1.9 g of protein mol^{-1}, which is equivalent to the 9.5×10^{12} cells mol^{-1} obtained by applying 0.2 pg of protein *E. coli* cell^{-1} [9]) (11), that of *Desulfomonile tiedjei* on 3-chlorobenzoate (6.6 g of protein mol^{-1}) (12), or that of strain 2CP-1 on 2-chlorophenol (2.6 g of protein mol^{-1}) (8). The growth dynamics of PCB dechlorinators with and without PCB amendment were also similar to those of *D. tiedjei* growing in a defined 3-chlorobenzoate-degrading consortium added with 3-chlorobenzoate and benzoate (11).

Stimulation of dechlorination activities has been observed in various laboratory cultures (4, 6, 20, 21) and attributed to the increased enrichment by PCB-dechlorinating populations and/or induction of corresponding enzymes (4, 21). The results of other studies (7, 24) suggest that enrichment of dechlorination activity in the present study is probably due to the increased number of dechlorinators. However, the fact that PCB-free sediments did not show any growth of dechlorinators supports the assumption that the dechlorinators require PCBs as their electron acceptors and are not in competition with other organisms for electron acceptors. The decline of the dechlorinating populations may be due to the diminishing level of suitable congeners that the populations could use as electron acceptors. Earlier studies have shown that the removal of Cl from PCBs is determined not only by its substitution position, as in the case of *ortho* Cl, but also by the pattern of Cl substitution on the biphenyl ring (27, 28). The incomplete dechlorination of *meta* and *para* Cls at the plateau level was found to be due to the Cl substitution patterns of products that dechlorinating microorganisms could not use further; it was not because of PCB bioavailability, carbon or energy limitation, the accumulation of intermediate metabolic products, or end product inhibition (19). Therefore, the presence of environmental PCBs does not necessarily indicate the presence of competent dechlorinating organisms in effective numbers. However, the size of the dechlorinating population can suggest the potential for further dechlorination of the site. In this regard, it is important to find any correlation between the PCB concentration and the biomass of dechlorinators.

Different dechlorination patterns have been attributed to different dechlorination competence, indicating different species of dechlorinators (7, 25, 30). When we assayed 2,5,3′,4′-CBP dechlorination in the test vials, either 2,5,3′-CBP alone or both 2,5,4′- and 2,5,3′-CBPs were produced, depending on the vials. In dechlorination processes M and N, 2,5,3′,4′-CBP is dechlorinated to 2,5,4′- and 2,4′-CBPs, while *para* dechlorination of 2,5,3′,4′-CBP occurs in other processes producing 2,5,3′-CBP (5). Similarly, when we measured the number of PCB dechlorinators in an enrichment culture of GM sediments with a mixture of 2,3,4- and 2,3,2′,5′-CBPs, the critical dilution level for 2,3,4-CBP dechlorination was about 10- to 10^2-fold higher than that of 2,3,2′,5′-CBP dechlorination; that is, the number of 2,3,4-CBP dechlorinators might be higher than that of 2,3,2′,5′-CBP dechlorinators, although 2,3,2′,5′-CBP dechlorinators might also dechlorinate 2,3,4-CBP (17). These findings suggest the presence of at least two different PCB-dechlorinating species in the cultures and/or interactions between these species.

The primary *para* dechlorination observed after 4 weeks in Aroclor 1248-dechlorinating cultures is comparable to dechlorination activities H and H′ (5). Additional decreases in *meta*-rich congeners thereafter may imply activity similar to that of dechlorination process M (5). Although sulfate-reducing spore formers and methanogens have been proposed to mediate dechlorination processes M and H, respectively (5, 31, 32), it is still possible that those dechlorination processes are mediated by several different microorganisms. The observation that *para* dechlorination of 2,5,3′,4′-CBP to 2,5,3′-CBP could occur independently of methanogenesis in the test vials suggests the involvement of microorganisms other than methanogens in the dechlorination reaction and possibly *para* dechlorination processes such as H and H′.

For the biotechnological applications of this anaerobic process, it is necessary to further define the roles of nondechlorinating species, as well as methanogens and sulfate reducers, in a microbial community on PCB dechlorination. It is still unclear whether dechlorinators may survive in cultures in which dechlorination has ceased and/or in cultures with negligible amount of PCBs.

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