APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Dec. 1997, p. 4826–4832 Vol. 63, No. 12
0099-2240/97/$04.00+0

Two Anaerobic Polychlorinated Biphenyl-Dehalogenating Enrichments That Exhibit Different *para*-Dechlorination Specificities

QINGZHONG WU† AND JUERGEN WIEGEL*

Department of Microbiology and Center for Biological Resource Recovery, University of Georgia, Athens, Georgia 30602

Received 9 June 1997/Accepted 2 October 1997

Two anaerobic polychlorinated biphenyl (PCB)-dechlorinating enrichments with distinct substrate specificities were obtained: a 2,3,4,6-tetrachlorobiphenyl (2346-CB) *para*-dechlorinating enrichment derived from Aroclor 1260-contaminated Woods Pond (Lenox, Mass.) sediment and a 2,4,6-trichlorobiphenyl (246-CB) unflanked *para*-dechlorinating enrichment derived from PCB-free Sandy Creek Nature Center (Athens, Ga.) sediment. The enrichments have been successfully transferred to autoclaved soil slurries over 20 times by using 300 to 350 μM 2346-CB or 246-CB. Both enrichments required soil for successful transfer of dechlorination activity. The 2346-CB enrichment *para* dehalogenated, in the absence or presence of 2346-CB, only 4 of 25 tested *para* halogen-containing congeners: 234-CB, 2345-CB, 2346-CB, and 2,4,6-tribromobiphenyl (246-BrB). In the presence of 246-CB, the 246-CB enrichment *para* dehalogenated 23 of the 25 tested congeners. However, only three congeners (34-CB, 2346-CB, and 246-BrB) were dehalogenated in the absence of 246-CB, indicating that these specific congeners initiate dehalogenation in this enrichment culture. The addition of the 2346-CB (*para*)-dechlorinating enrichment did not further stimulate the 2346-CB-primed dechlorination of the Aroclor 1260 residue in Woods Pond sediment samples. Compared to the addition of the primer 246-CB or the 246-CB unflanked *para*-dechlorinating enrichment alone, the addition of both 246-CB (300 μM) and the 246-CB enrichment stimulated the unflanked *para* dechlorination of the Aroclor 1260 residue in Woods Pond sediments. These results indicate that the two enrichments contain different PCB-dechlorinating organisms, each with high substrate specificities. Furthermore, bioaugmentation with the enrichment alone did not stimulate the desired dechlorination in PCB-contaminated Woods Pond sediment.

Reductive dechlorination of polychlorinated biphenyls (PCBs) under anaerobic conditions has been unequivocally demonstrated in various sediments (1, 2, 4, 10, 11, 24, 25). It is assumed that the reductive dechlorination of chlorophenols and chlorobenzoate, an anaerobic respiration process, supports ATP production in dechlorinating organisms (13, 15, 19, 21, 27). It has been proposed (10, 11, 24) that PCB-dechlorinating microorganisms using PCBs as electron acceptors may also derive energy from reductive PCB dechlorination. If this is true, it should be possible to selectively enrich microorganisms able to carry out a specific PCB dechlorination reaction by supplying the corresponding PCB congener(s) at elevated concentrations as an electron acceptor (3, 5, 6, 29). The isolation of organisms able to reductively dechlorinate PCBs is necessary to further elucidate reductive PCB dechlorination and to study the substrate specificity and the regulation of dehalogenation. However, to date, all attempts have been unsuccessful. Only a few enrichments have been obtained (8, 9, 20, 22, 34, 35), and none have been obtained for unflanked *para* dehalogenation.

In Woods Pond (WP; Lenox, Mass.), a shallow impoundment on the Housatonic River contaminated with the PCB mixture Aroclor 1260 (4), dechlorination of the Aroclor 1260 residue can be primed by adding high concentrations of specific PCB congeners (e.g., 350 μM 2,3,4,6-tetrachlorobiphenyl [2346-CB]) or other halogenated aromatic compounds (3, 5–7, 12, 14, 29, 31) but not by changes in the temperature or pH of sediment incubations in the absence of priming congeners (12, 31). The addition of halogenated compounds to the environment to stimulate in situ dehalogenation is not desirable. Thus, besides obtaining data on the substrate specificities of enriched bacteria, we hoped to obtain an actively dehalogenating enrichment that could be used to seed desired dehalogenations in situ by adding the enrichment to the sediment. For WP, the stimulation of the dehalogenation of congeners containing an unflanked *para* chlorine (no adjacent *meta* chlorines) is especially important since these congeners accumulate during dehalogenation primed by addition of bromobiphenyls (5–7) or other PCB congeners (3, 5, 6, 29). Subsequently, we focused on flanked and unflanked *para* dechlorination and investigated whether distinct bacteria are responsible for these two activities.

We describe here the substrate specificities of two stable enrichments, one obtained from WP sediment dechlorinating 2346-CB to 236-CB and the other from PCB-free Sandy Creek Nature Center (SCNC; Athens, Ga.) pond sediment dechlorinating 246-CB to 26-CB. Furthermore, we describe the effects of the addition of these enrichments on the dehalogenation of the Aroclor 1260 residue in WP sediment samples.

* Corresponding author. Mailing address: Department of Microbiology, University of Georgia, 215 Biological Sciences Building, Athens, GA 30602-2605. Phone: (706) 542-2651. Fax: (706) 542-2674. E-mail: JWIEGEL@uga.cc.uga.edu.

† Present address: Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC 29425.

MATERIALS AND METHODS

Chemicals. PCB congeners and 2,4,6-tribromobiphenyl (246-BrB) were purchased from AccuStandard Inc., New Haven, Conn. (99% purity). Other chemicals were obtained from Sigma Chemical Co. (St. Louis, Mo.). In this report, each individual PCB or bromobiphenyl congener is described by using an abbre-

viation which lists the chlorine substitution pattern on each ring as a prefix separated by a hyphen.

Preparation of soil and media. Since sediments or soils were required to maintain the dehalogenation activity, PCB-free marsh soil was obtained from Stony Creek, N.Y., for this purpose. The marsh soil was dried in a fume hood, homogenized, and sieved (mesh no. 80) before use. The medium used for enrichment studies contained, per liter of distilled water, 20 mmol of potassium-sodium phosphate buffer (pH 6.9 to 7.0), 1 g of yeast extract, 0.5 g of NH_4Cl, 90 mg of $MgCl_2$, 25 mg of $CaCl_2$, 0.25 g of cysteine-HCl, 5 ml of a trace element solution (36), and 0.5 ml of vitamin solutions (36). The trace element solution was modified by substituting sulfate and nitrate salts with the corresponding chloride salts to minimize the occurrence of sulfate and nitrate reduction (12). The medium containing 2% (wt/vol) air-dried soil was prepared by the Hungate technique (18) and autoclaved on each of two consecutive days at 121°C for 1 h. Soil slurries were prepared as previously described (32).

Enrichment cultures. The 2346-CB enrichment was derived from a sediment grab sample from WP (4) supplemented with 2346-CB. The 246-CB enrichment was derived from sediment samples from the SCNC pond (32) by using 246-CB as an electron acceptor.

Enrichments were obtained by repeated transfers of actively dechlorinating sediment (between 20 μl and 1 ml) into autoclaved soil-containing enrichment media (20 ml) after dechlorination of more than 50% of 300 to 350 μM supplemented congeners had occurred. The subcultures were transferred successfully more than 20 times over 8 to 12 months. Duplicate enrichments were incubated at 30°C for the 2346-CB enrichment or at room temperature (22 to 27°C) for the 246-CB enrichment. The temperatures were chosen according to preliminary findings on the temperature optima for these reactions (33).

Congener specificity of enrichment cultures. Twenty-milliliter samples of the PCB-free soil slurries (pH 6.8) were dispensed in 50-ml serum bottles and autoclaved twice at 121°C for 1 h on each of two consecutive days. A 400-μl transfer inoculum of the 2346-CB enrichment and 100 μM 2346-CB or a 1-ml transfer inoculum of the 246-CB enrichment and 100 μM 246-CB were then added to the soil slurries. The bottles were crimp sealed with Teflon-lined butyl rubber stoppers. After about 50% of the supplemented 2346-CB or 246-CB had been dechlorinated to 236-CB or 26-CB, respectively, the cultures were amended separately with one of the tested congeners (100 μM) (Table 1). To test whether the individual congeners were dehalogenated in the absence of 2346-CB or 246-CB, a second series of soil incubations was tested by using cultures not supplemented with 2346-CB or 246-CB. These soil incubations were prepared as follows. After about 50% of the supplemented 2346-CB or 246-CB (100 μM) had been dechlorinated to 236-CB or 26-CB, respectively, in the preculture, 2% (vol/vol) of the 2346-CB-*para*-dechlorinating enrichment or 5% of the 246-CB unflanked *para*-dechlorinating enrichment was transferred to autoclaved, PCB-free soil. The residual concentrations of 2346-CB and 246-CB in those inoculated samples were below 0.5 and 2.5 μM, respectively. The inoculated soil slurries were then amended with the test congeners at 100 μM (Table 1).

Priming of the dehalogenation of Aroclor 1260 residue in WP sediment with enrichment cultures. The influence of 2346-CB enrichment on the priming of the dechlorination of the Aroclor 1260 residue was tested in WP sediment that had been stored in glass containers at 4 to 7°C for 15 months. To test the suitability of the 246-CB enrichment, we used WP sediments (20 ml of sediment at pH 6.8 in 50-ml serum bottles) which had undergone extensive 26-BrB-primed *meta* dechlorination (5–7) of the Aroclor 1260 residue for 10 months which led to the formation of the required unflanked *para* chlorine-substituted congeners, e.g., 24-24-CB, 24-25-CB, and 24-26-CB. Dehalogenation was tested under the following conditions: (i) no additions (control for added sediment), (ii) addition of one of the cultures mentioned above (1 ml of sediment or soil slurries in which more than 50% of the supplemented 350 μM 2346-CB or 300 μM 246-CB had been dechlorinated, respectively), (iii) addition of 350 μM 2346-CB or 300 μM 246-CB, (iv) a combination of conditions ii and iii, (v) the same as condition iv but supplemented with 20 mM pyruvate, (vi) addition of 300 μM 246-BrB, and (vii) a combination of conditions ii and vi.

Sample extraction and analysis. PCBs were extracted by anhydrous diethyl ether and analyzed with a gas chromatograph (Hewlett-Packard 5890 series II) equipped with a DB-1 polydimethysiloxane phase capillary column (30 m by 0.25 mm [inside diameter] by 0.25 μm) and a Ni^{63} electron capture detector (32). All dechlorination products of the tested congeners and Aroclor 1260 residue in WP sediment were identified by matching their gas chromatographic retention times with a customized PCB standard prepared by supplementing Aroclor 1260 with the dechlorination products observed in WP (28) or a reference standard composed of Aroclors 1242, 1254, and 1260 (70:20:10) and congener assignments reported by Frame et al. (17). In quantification of dechlorination products of the Aroclor 1260 residue, each congener was quantified by use of a second-order calibration curve generated from PCB standards at six-point calibration levels. Since our goal was to determine which congeners were substrates and not to obtain kinetic data, we elected to use a semiquantitative method for the products from the individual supplemented congeners to approximate the extent of conversion. We determined the area percentage of the individual dechlorination products by dividing the area of each observed product by the total areas of the supplemented compound remaining and all of the products observed. Since the electron capture detector is more sensitive to the higher-chlorinated parent compounds than it is to the more dechlorinated products, this is a conservative estimate.

TABLE 1. Products of dechlorination of tested PCB and PBrB congeners by 2346-CB and 246-CB enrichments

Added congener	Formed congener(s)			
	2346-CB *para*-dechlorinating enrichment		246-CB unflanked *para*-dechlorinating enrichment	
	Without 2346-CB	With 2346-CB	Without 246-CB	With 246-CB
PCBs				
4	—[b]	—	—	—
4-4	—	—	—	—
24	—	—	—	2 (23)[a]
34	—	—	3 (30)	3 (50)
234[c]	NT[d]	23 (26)	NT	23 (72)
246	—	—	26 (90)[e]	26 (89)
25-4	—	—	—	—
24-24	—	—	—	24-2 (21)
24-25	—	—	—	25-2 (27)
24-26	—	—	—	26-2 (38)
2345[c]	NT	235 (12)	NT	235 (21)
2346	236 (94)[e]	236 (95)	236 (91)	236 (93)
2356[c]	NT	—	NT	—
246-4[c]	—	—	—	26-4 (14)
246-24[c]	—	—	—	26-2 (7), 24-26 (14)
246-25[c]	—	—	—	25-26 (35)
246-35[c]	—	—	—	26-35 (34)
234-245[c]	NT	—	NT	23-25 (2), 234-25 (2), 245-23 (1)
234-246[c]	—	—	—	23-26 (3), 234-26 (6), 246-23 (3)
245-246[c]	—	—	—	25-26 (7), 246-25 (14)
246-246[c]	—	—	—	246-26 (2)
246-345[c]	—	—	—	26-35 (1), 246-35 (2)
2345-234[c]	NT	—	NT	—
2345-246[c]	—	—	—	235-246 (2)
2346-246[c]	—	—	—	236-246 (2)
PBrBs (246-BrB)	26 (89)	26 (87)	26 (85)	26 (90)

[a] Values in parentheses are concentrations (in mole percent). 100 mol% = 100 μM.
[b] —, no or little (<0.5 mol%) dechlorination product.
[c] Dechlorination products from the congener were estimated (see Materials and Methods for details).
[d] NT, not tested.
[e] Control experiment with an active culture.

RESULTS

Establishment of enrichment cultures. A 2346-CB *para*-dechlorinating enrichment was derived from WP sediments. We previously reported the *meta* and *para* dechlorination of 2346-CB in WP sediment samples (12, 32). To enrich for *para*-dechlorinating activity, the WP sediment slurries (pH 6.8) were incubated with 350 μM 2346-CB at 30°C, since under these conditions *para* dechlorination activity was expected to be sustained (33). After 1 month of incubation, however, 80% (280 μmol/ml) of the supplemented 2346-CB was *meta* dechlorinated to 246-CB, while only 7 mol of 236-CB per ml (~2 mol%) was formed by *para* dechlorination (data not shown). These results were similar to those obtained previously (33). At this point, 20 μl of actively dechlorinating sediment slurries was inoculated into 20 ml of a sterile soil slurry. With repeated transfers, the specificity changed until 236-CB became the sole dechlorination product and the *meta* dechlorination

activity was eliminated. Subsequently, the enrichment culture was transferred (0.1% transfer inoculum) over 20 times into autoclaved soil or medium containing 2% (wt/vol) soil while retaining its *para* dechlorination activity. When using 0.1% (vol/vol) transfer, approximately 50% of the added 2346-CB (175 μM) was usually dechlorinated to 236-CB within 10 days of transfer. With sterilized soil slurries (see Materials and Methods), the *para* dechlorination activity of 2346-CB was retained in dilutions of up to 10^{-7}, indicating that at least 10^7 cells of the responsible organism(s) per ml were present in the enrichment and the *meta*-dechlorinating organisms had been diluted out. All attempts to transfer the dechlorinating activity into liquid media without soil failed, regardless of whether site water from various lakes, including WP, tap water, or distilled water was used to prepare the medium.

A 246-CB unflanked *para*-dechlorinating enrichment was obtained from SCNC sediments. Slurries of the sediment were supplemented with 300 μM 246-CB and incubated at 30°C and pH 6.8. After about 6 months of incubation, 70% of the supplemented 246-CB was converted to 26-CB. We transferred (5% transfer inoculum) the culture into soil-containing medium (see Materials and Methods) more than 20 times, retaining its unflanked *para* dechlorination activity. The incubation temperature was reduced from 30°C to room temperature (22 to 27°C) since analysis of the influence of temperature (20, 22, 25, 27, 30, and 34°C were tested) on the dechlorination activity in the enrichment showed that the highest activity occurred between 25 and 27°C (data not shown). The maximal dilution for retaining the unflanked *para* dechlorination activity of 246-CB was 5×10^{-4} (vol/vol), indicating the possibility that the organism(s) was present at a very low concentration in the enrichment cultures or that it required the presence of other, nondehalogenating bacteria, which were present only in low numbers, for growth or activity. Again, the culture could not be transferred in the absence of soil.

Substrate specificity. The substrate specificities of the enrichment cultures were studied because all attempts to obtain pure cultures by extinction dilution and plating, including that on soil-containing medium, had failed.

(i) 2346-CB enrichment. After 10 transfers, the substrate specificity of the enrichment was tested in the presence of 2346-CB (100 μM). 234-CB (26%), 2345-CB (12%), and 246-BrB (87%) were dehalogenated to 23-CB, 235-CB, and 26-BrB, respectively, after 3 months of incubation. However, most of the congeners tested were not dechlorinated even though most contained *para* chlorines (Table 1). Interestingly, congeners containing 234- or 2345-chlorophenyl groups were not dehalogenated when the second ring was also substituted. In the absence of 2346-CB, only 246-BrB (the only brominated congener tested) was dehalogenated.

(ii) 246-CB enrichment. After 3 months in the presence of 246-CB, most of the congeners tested were *para* dehalogenated, although to different extents (Table 1). However, in the absence of 246-CB, the only congeners dehalogenated to a great extent were 34-CB (~30%), 2346-CB (~91%), and 246-BrB (~85%). The corresponding products, 3-CB, 236-CB, and 26-BrB, were formed by removal of a flanked *para* chlorine or an unflanked *para* bromine, respectively.

Priming dehalogenation of Aroclor 1260 residue in WP sediment with enrichment cultures. Since the addition of PCB congeners to the environment is not reasonable, we tested the use of our enrichments for stimulating the dechlorination of the Aroclor 1260 residue in WP sediments.

(i) 2346-CB enrichment. After 6 months of incubation of WP sediment in the presence of the 2346-CB *para*-dechlorinating enrichment (5%, vol/vol), about 92 mol% of the supplemented 2346-CB or 246-BrB was *para* dehalogenated to 236-CB and 26-BrB, respectively, which were not further dehalogenated. Unfortunately, no dechlorination of the Aroclor 1260 residue occurred in the presence of either the enrichment (no congener added) or the enrichment plus 2346-CB or 246-BrB. In samples with the addition of 2346-CB alone (no enrichment), *meta* dechlorination of both 2346-CB and the Aroclor 1260 residue was similar to previously obtained results (31). Surprisingly, when 246-BrB was added alone, no debromination was observed within 6 months.

(ii) 246-CB enrichment. After 3 months of incubation, 90 mol% of the supplemented congener 246-CB (or 246-BrB) was dehalogenated to 26-CB (or 26-BrB) in all sediment samples except the unamended control. Unflanked *para* dechlorination of the *meta*-dechlorinated Aroclor 1260 residue was observed in the incubations containing either 246-CB or 246-BrB both in the absence and in the presence of the enrichment culture (Fig. 1 and 2). The conclusion that unflanked *para* dechlorination of the *meta*-dechlorinated Aroclor 1260 residue occurred is based on the significant decreases in 24-24-CB and 24-26-CB and increases in 25-2-CB and 26-2-CB. The decrease in 24-24-CB, however, did not result in a corresponding increase in 24-2-CB. This could be due to lack of sufficient resolution of 25-2-CB and 24-2-CB on the DB-1 column, resulting in an apparent appearance of a greater increase in 25-2-CB than in 24-2-CB, thus providing an underestimation of 24-2-CB. The other possibility is that 24-2-CB was further dehalogenated to 2-2-CB (7), but this could not be confirmed because 2-2-CB was not resolved from the 26-CB produced by dechlorination of the added 246-CB. Consequently, the unflanked *para* dechlorination of 24-24-CB might be underestimated. More unflanked *para* dechlorination occurred in the incubations containing both 246-CB and the enrichment culture than in those supplemented only with 246-CB.

DISCUSSION

We obtained two stable, reproducibly transferable, soil-requiring, *para*-dechlorinating enrichments, a 2346-CB flanked *para*-dechlorinating enrichment from WP sediment which has a very limited substrate range and does not remove unflanked *para* chlorines and a 246-CB unflanked *para*-dechlorinating enrichment from SCNC pond sediment. A few PCB-dechlorinating enrichments have been derived from Hudson River sediment (9, 20, 22, 34, 35) and Baltimore Harbor sediment (8) by using either Aroclor 1242 or a single congener as a selective electron acceptor. These enrichments dechlorinate PCBs mainly by removing *meta*, *ortho*, and flanked *para* chlorines. Similar to those described here, all of these enrichments require sediment to sustain their dechlorination activities. So far, the specific role of sediments in the dehalogenation has not been elucidated. No extensive PCB congener specificities of those enrichments have been reported.

Comparison of the specificities of the observed dechlorination activities. Both of our enriched cultures exhibited a high degree of specificity for relatively few PCB congeners. This observation supports the hypothesis that different microorganisms with distinct dehalogenating enzymes and congener specificities are responsible for the various identified dechlorination processes (5, 6, 10, 11, 23). The 2346-CB enrichment dechlorinated PCB congeners with flanked *para* chlorines such as 234-CB, 2345-CB, and 2346-CB at the *para* position but did not remove *para* chlorines from congeners substituted on both rings. The enrichment also did not remove *meta* chlorines from PCBs like 2356-CB or unflanked *para* chlorines from 24-CB and 246-CB (Table 1). This dem-

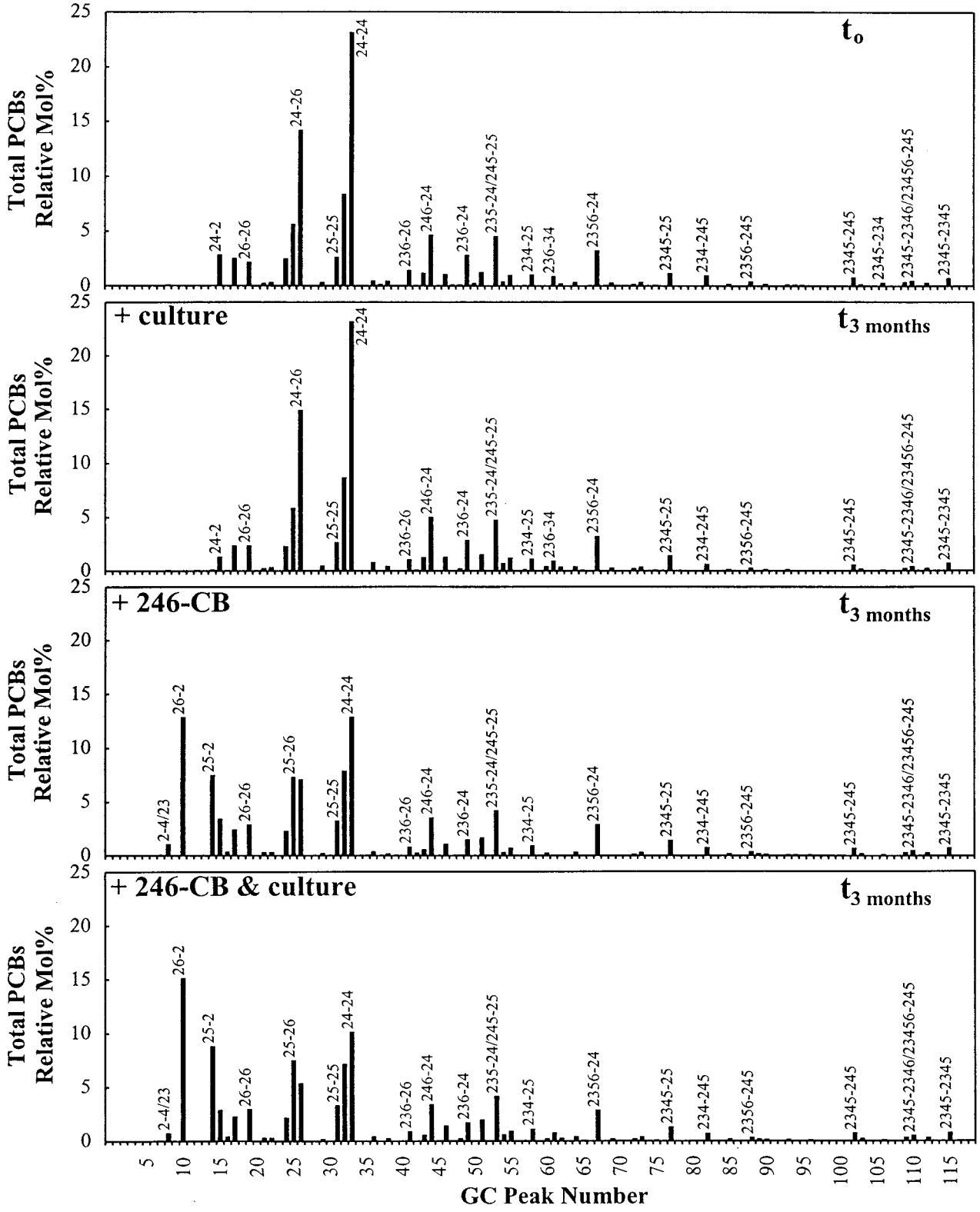

FIG. 1. Congener distribution at time zero (t_0) and 3 months ($t_{3\ months}$) of *meta*-dechlorinated Aroclor 1260 residue in WP sediment samples supplemented with the 246-CB enrichment, 300 μM 246-CB, or 300 μM 246-CB and the 246-CB enrichment.

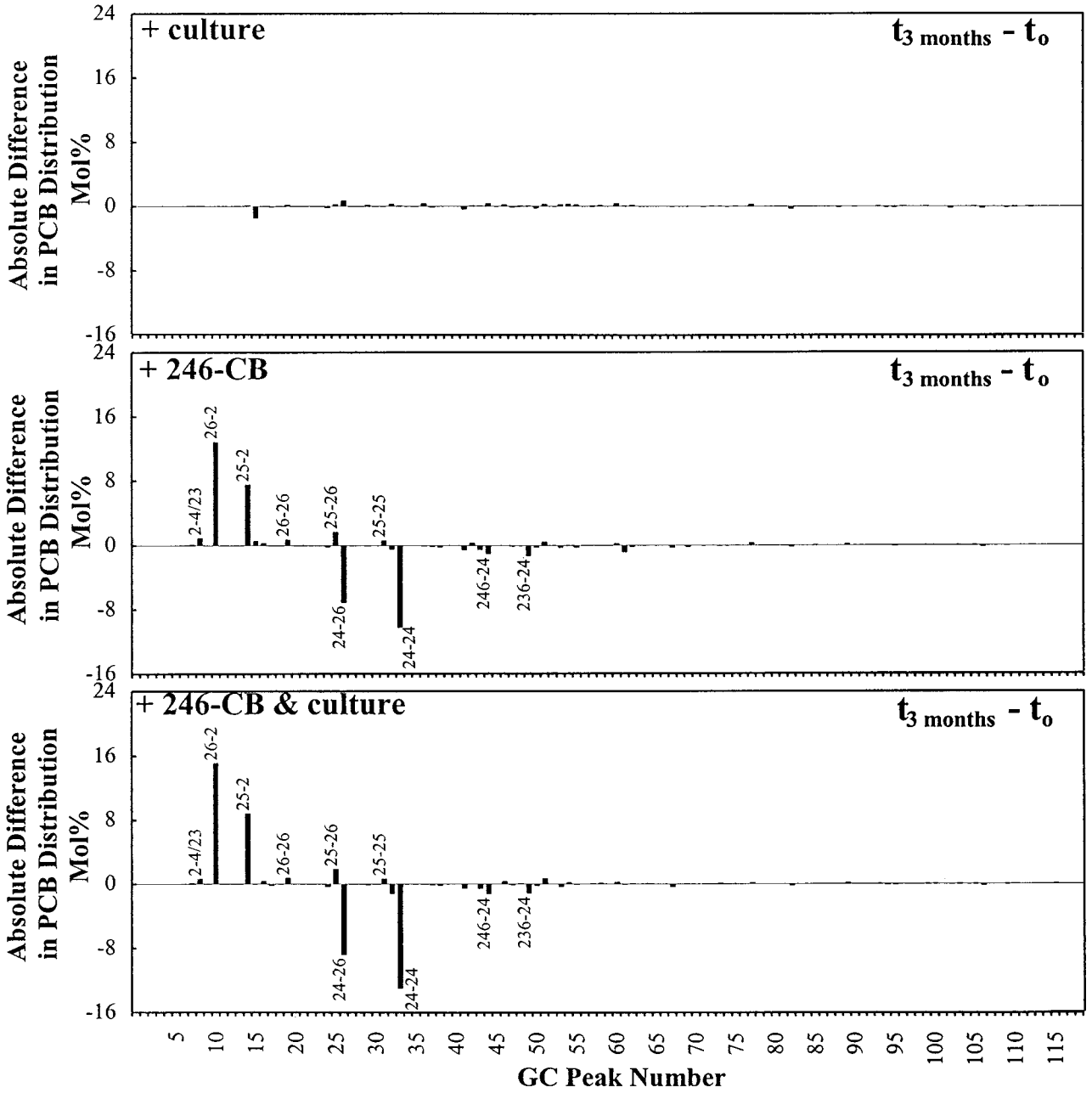

FIG. 2. Differences in congener distribution of *meta*-dechlorinated Aroclor 1260 residue between time zero and 3 months ($t_{3\ months} - t_0$) in sediment samples supplemented with the 246-CB enrichment, 300 μM 246-CB, or 300 μM 246-CB and the 246-CB enrichment.

onstrates that the dechlorinating bacteria that were enriched have a high substrate specificity and suggests that different bacteria are responsible for the *para* dechlorination that others have reported (3, 23, 35). In contrast to the 2346-CB enrichment, the 246-CB enrichment was able to dehalogenate PCBs with both flanked and unflanked *para* chlorines but not to *meta* dechlorinate 2356-CB (Table 1).

The results described here indicate that in addition to the incubation temperatures (33), the choice of the PCB congeners and concentrations of inocula can be used for selective enrichments of desirable PCB-dehalogenating cultures. We do not know whether similar conditions will yield similar substrate specificities in enrichments from other sources. *meta* and *para* dehalogenation activities have been previously separated for upper Hudson River sediment by using different approaches, such as pasteurization and ethanol treatment (34) and antibiotics (35).

Impact of congener structure on microbial dechlorination. Besides the assumed presence of several microbial populations (or enzymes) with distinct dechlorination activities, the microbial dechlorination activities are also influenced by the reduction potential of a PCB congener, i.e., the tendency of a PCB congener to act as an electron acceptor to release the chloro substituent as a chloride anion, and by its steric conformation (11, 30). The reduction potential of PCBs increases with increasing chlorine numbers (16, 26). Williams (30) compared

the dechlorination of 234-CB, 235-CB, 236-CB, 245-CB, 246-CB, and 345-CB (90 μg/ml) in slurries of Hudson River, Silver Lake (Pittsfield, Mass.), and WP sediments. In each slurry, the chlorine between two other chlorines was removed first from the trichlorobiphenyls tested. He concluded that the reactivity of a *meta* or *para* chlorine of a PCB is dependent on the number and position of the chlorines on the phenyl ring involved. He proposed the following order for the anaerobic dechlorination sequence of PCBs, from more reactive to less reactive: doubly flanked chlorines, singly flanked chlorines, unflanked chlorines, and a single chlorine. Our data support the conclusion that microbial dechlorination is influenced by these two factors. For example, the order of extent of *para* and unflanked *para* dechlorination in the 246-CB unflanked *para*-dechlorinating enrichment was 2346-CB > 246-CB > 234-CB > 34-CB > 24-CB > 4-CB (Table 1). Substitution on the second phenyl ring strongly lowered the extent of dechlorination. The 2346-CB *para*-dechlorinating enrichment dechlorinated 2346-CB by removal of flanked *para* chlorine but not 246-CB via loss of unflanked *para* chlorine. However, some differences were observed. For instance, the order of the extent of *para* dechlorination by the 2346-CB *para*-dechlorinating enrichment was 2346-CB > 234-CB > 2345-CB (Table 1). The reduction potential (16) of 2345-CB (−1.679 V) is higher than that of 2346-CB (−1.784 V) or 234-CB (−1.852 V); 2345-CB contains doubly flanked *para* chlorine, while 2346-CB and 234-CB have singly flanked *para* chlorine. The extent of dechlorination of 246-CB having an unflanked *para* chlorine in the 246-CB enrichment was higher than that of 234-CB containing a flanked *para* chlorine. These results suggest that, in addition to the molecular structure of PCBs, other factors can affect the specificity of the microbial dechlorination of PCBs. It is noteworthy that the substitutions on the second phenyl ring rendered the corresponding congeners inactive for the 2346-CB enrichment culture, i.e., 2346-CB was a highly active congener, whereas 2346-246-CB was not dechlorinated and the less active compounds 234-CB and 2345-CB became inactive when both rings were substituted (234-2345-CB).

Impact of adding enrichment cultures on the dechlorination of Aroclor 1260 residues. In contrast to the addition of 2346-CB alone, which stimulated *meta* dechlorination of the Aroclor 1260 residue, the addition of both the priming congener 2346-CB and the 2346-CB enrichment did not stimulate Aroclor residue dechlorination. This unexpected result suggests that the condition of the WP sediment is unfavorable for the growth or the dechlorinating activity of the 2346-CB enrichment or that non-PCB dechlorinators in WP sediment may compete successfully for carbon and energy sources although the 2346-CB enrichment can use 2346-CB as an additional and selective electron acceptor. No stimulation of the dechlorination of Aroclor 1260 residue was seen when the 246-CB enrichment was added alone without the 246-CB primer. Therefore, primers may be required to sustain the dechlorination activity and the 246-CB enrichments alone cannot be used to stimulate the dehalogenation of Aroclor 1260 residue in WP sediment. The results suggest the possibility that some factors (e.g., concentration of electron acceptors) other than the number of dehalogenating organisms are responsible for the difficulties in further dehalogenation of the Aroclor 1260 residue in WP sediment. Further experiments, including the enumeration of dehalogenating organisms before and after priming of the dehalogenation with specific congeners, are needed.

ACKNOWLEDGMENTS

This work was supported by a grant to J.W. from the General Electric Company and an equipment grant from the Georgia Research Alliance.

We thank Donna L. Bedard and William Jack Jones for helpful discussion.

REFERENCES

1. **Abramowicz, D. A.** 1994. Aerobic PCB degradation and anaerobic PCB dechlorination in the environment. Res. Microbiol. **145:**42–46.
2. **Alder, A. C., M. M. Häggblom, S. R. Oppenheimer, and L. Y. Young.** 1993. Reductive dechlorination of polychlorinated biphenyls in anaerobic sediments. Environ. Sci. Technol. **27:**530–538.
3. **Bedard, D. L., S. C. Bunnell, and L. A. Smullen.** 1996. Stimulation of microbial *para*-dechlorination of polychlorinated biphenyls that have persisted in Housatonic River sediment for decades. Environ. Sci. Technol. **30:**687–694.
4. **Bedard, D. L., and R. J. May.** 1996. Characterization of the polychlorinated biphenyls (PCBs) in the sediment of Woods Pond: evidence for microbial dechlorination of Aroclor 1260 *in situ*. Environ. Sci. Technol. **30:**237–245.
5. **Bedard, D. L., and J. F. Quensen III.** 1995. Microbial reductive dechlorination of polychlorinated biphenyls, p. 127–216. *In* L. Y. Young and C. Cerniglia (ed.), Microbial transformation and degradation of toxic organic chemicals. Wiley-Liss Division, John Wiley & Sons, Inc., New York, N.Y.
6. **Bedard, D. L., H. M. Van Dort, S. C. Bunnell, L. M. Principe, K. A. DeWeerd, R. J. May, and L. A. Smullen.** 1993. Stimulation of reductive dechlorination of Aroclor 1260 contaminant in anaerobic slurries of Woods Pond sediment, p. 19–21. *In* Anaerobic dehalogenation and its environmental implications. Abstracts of the 1992 American Society for Microbiology Conference. Office of Research and Development, U.S. Environmental Protection Agency, Washington, D.C.
7. **Bedard, D. L., H. M. Van Dort, and K. A. DeWeerd.** Unpublished data.
8. **Berkaw, M., K. R. Sowers, and H. D. May.** 1996. Anaerobic *ortho* dechlorination of polychlorinated biphenyls by estuarine sediments from Baltimore Harbor. Appl. Environ. Microbiol. **62:**2534–2539.
9. **Boyle, A. W., C. K. Blake, W. A. Price II, and H. D. May.** 1993. Effects of polychlorinated biphenyl congener concentration and sediment supplementation on rates of methanogenesis and 2,3,6-trichlorobiphenyl dechlorination in an anaerobic enrichment. Appl. Environ. Microbiol. **59:**3027–3031.
10. **Brown, J. F., Jr., D. L. Bedard, M. J. Brennan, J. C. Carnahan, H. Feng, and R. E. Wagner.** 1987. Polychlorinated biphenyl dechlorination in aquatic sediments. Science **236:**709–712.
11. **Brown, J. F., Jr., R. E. Wagner, H. Feng, D. L. Bedard, M. J. Brennan, J. C. Carnahan, and R. J. May.** 1987. Environmental dechlorination of PCBs. Environ. Toxicol. Chem. **6:**579–593.
12. **Chuang, K.-S.** 1995. M.S. thesis. The University of Georgia, Athens.
13. **Cole, J. R., A. L. Cascarelli, W. W. Mohn, and J. M. Tiedje.** 1994. Isolation and characterization of a novel bacterium growing via reductive dehalogenation of 2-chlorophenol. Appl. Environ. Microbiol. **60:**3536–3542.
14. **DeWeerd, K. A.** 1994. Stimulation of microbially mediated PCB dechlorination in Woods Pond sediment slurries with analogs of bromobenzoic acid, abstr. Q-325, p. 445. *In* Abstracts of the 94th General Meeting of the American Society for Microbiology 1994. American Society for Microbiology, Washington, D.C.
15. **Dolfing, J.** 1990. Reductive dechlorination of 3-chlorobenzoate is coupled to ATP production and growth in an anaerobic bacterium, strain DCB-1. Arch. Microbiol. **153:**264–266.
16. **Farwell, S. O., F. A. Beland, and R. D. Geer.** 1975. Interrupted-sweep voltammetry for the identification of polychlorinated biphenyls and naphthalenes. Anal. Chem. **47:**895–903.
17. **Frame, G. M., J. W. Cochran, and S. S. Bøwadt.** 1996. Complete PCB congener distributions for 17 Aroclor mixtures determined by 3 HRGC systems optimized for comprehensive, quantitative, congener-specific analysis. J. High Resolut. Chromatogr. **19:**657–668.
18. **Ljungdahl, L. G., and J. Wiegel.** 1986. Working with anaerobic bacteria, p. 84–96. *In* A. L. Demain and N. A. Solomon (ed.), Manual of industrial microbiology and biotechnology. American Society for Microbiology, Washington, D.C.
19. **Mackiewicz, M., and J. Wiegel.** Comparison of growth and energy yields for *Desulfitobacterium dehalogenans* during utilization of chlorophenol and various traditional electron acceptors. Appl. Environ. Microbiol., in press.
20. **May, H. D., A. W. Boyle, W. A. Price II, and C. K. Blake.** 1992. Subculturing of a polychlorinated biphenyl-dechlorinating anaerobic enrichment on solid media. Appl. Environ. Microbiol. **58:**4051–4054.
21. **Mohn, W. W., and J. M. Tiedje.** 1991. Evidence for chemiosmotic coupling of reductive dechlorination and ATP synthesis in *Desulfomonile tiedjei*. Arch. Microbiol. **157:**1–6.
22. **Morris, P. J., W. W. Mohn, J. F. Quensen III, J. M. Tiedje, and S. A. Boyd.** 1992. Establishment of a polychlorinated biphenyl-degrading enrichment culture with predominantly *meta* dechlorination. Appl. Environ. Microbiol. **58:**3088–3094.

23. **Quensen, J. F., III, S. A. Boyd, and J. M. Tiedje.** 1990. Dechlorination of four commercial polychlorinated biphenyl mixtures (Aroclors) by anaerobic microorganisms from sediments. Appl. Environ. Microbiol. **56:**2360–2369.
24. **Quensen, J. F., III, J. M. Tiedje, and S. A. Boyd.** 1988. Reductive dechlorination of polychlorinated biphenyls by anaerobic microorganisms from sediments. Science **242:**752–754.
25. **Rhee, G.-Y., B. Bush, C. M. Bethoney, A. DeNucci, H.-M. Oh, and R. C. Sokol.** 1993. Reductive dechlorination of Aroclor 1242 in anaerobic sediments: pattern, rate, and concentration dependence. Environ. Toxicol. Chem. **12:**1025–1032.
26. **Rusting, J. F., and C. L. Miaw.** 1989. Kinetic estimation of standard reduction potentials of polyhalogenated biphenyls. Environ. Sci. Technol. **23:**476–479.
27. **Sanford, R. A., J. R. Cole, F. E. Löffler, and J. M. Tiedje.** 1996. Characterization of *Desulfitobacterium chlororespirans* sp. nov., which grows by coupling the oxidation of lactate to the reductive dechlorination of 3-chloro-4-hydrobenzoate. Appl. Environ. Microbiol. **62:**3800–3808.
28. **Smullen, L. A., K. A. DeWeerd, D. L. Bedard, W. A. Fessler, J. C. Carnahan, and R. E. Wagner.** 1993. Development of a customized congener specific PCB standard for quantification of Woods Pond sediment PCBs, p. 45–66. *In* Research and Development Program for the Destruction of PCBs. Twelfth progress report. General Electric Co. Corporate Research and Development, Schenectady, N.Y.
29. **Van Dort, H. M., L. A. Smullen, R. J. May, and D. L. Bedard.** 1997. Priming *meta*-dechlorination of polychlorinated biphenyls that have persisted in Housatonic River sediments for decades. Environ. Sci. Technol. **31:**3308–3313.
30. **Williams, W. A.** 1994. Microbial reductive dechlorination of trichlorobiphenyls in anaerobic sediment slurries. Environ. Sci. Technol. **28:**630–635.
31. **Wu, Q.** 1996. Ph.D. dissertation. The University of Georgia, Athens.
32. **Wu, Q., D. L. Bedard, and J. Wiegel.** 1996. Influences of incubation temperatures on the microbial reductive dechlorination of 2,3,4,6-tetrachlorobiphenyl in freshwater sediments. Appl. Environ. Microbiol. **62:**4174–4179.
33. **Wu, Q., D. L. Bedard, and J. Wiegel.** 1997. Effect of incubation temperature on the route of microbial reductive dechlorination of 2,3,4,6-tetrachlorobiphenyl in polychlorinated biphenyl (PCB)-contaminated and PCB-free freshwater sediments. Appl. Environ. Microbiol. **63:**2836–2843.
34. **Ye, D., J. F. Quensen III, J. M. Tiedje, and S. A. Boyd.** 1992. Anaerobic dechlorination of polychlorobiphenyls (Aroclor 1242) by pasteurized and ethanol-treated microorganisms from sediments. Appl. Environ. Microbiol. **58:**1110–1114.
35. **Ye, D., J. F. Quensen III, J. M. Tiedje, and S. A. Boyd.** 1995. Evidence for *para* dechlorination of polychlorobiphenyls by methanogenic bacteria. Appl. Environ. Microbiol. **61:**2166–2171.
36. **Zhang, X., and J. Wiegel.** 1990. Isolation and partial characterization of a *Clostridium* species transforming *para*-hydroxybenzoate and 3,4-dihydroxybenzoate and producing phenol as the final transformation products. Microb. Ecol. **20:**103–121.