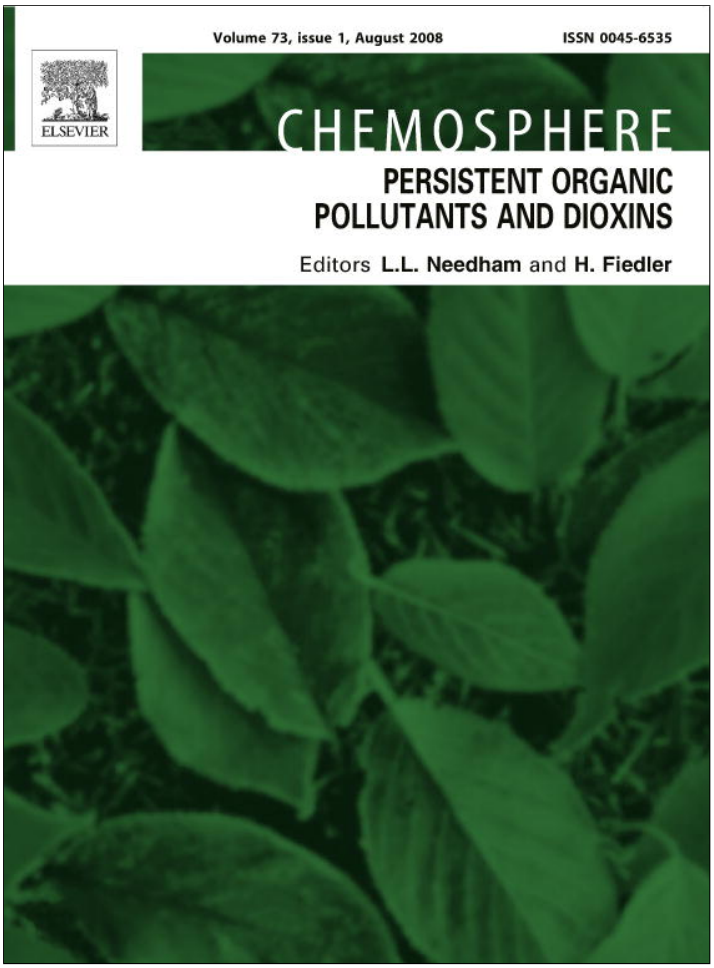

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Extensive biodegradation of polychlorinated biphenyls in Aroclor 1242 and electrical transformer fluid (Askarel) by natural strains of microorganisms indigenous to contaminated African systems

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ABSTRACT

Evidence for substantial aerobic degradation of Aroclor 1242 and Askarel fluid by newly characterized bacterial strains belonging to the *Enterobacter*, *Ralstonia* and *Pseudomonas* genera is presented. The organisms exhibited degradative activity in terms of total PCB/Askarel degradation, degradation of individual congeners and diversity of congeners attacked. Maximal degradation by the various isolates of Askarel ranged from 69% to 86% whereas, Aroclor 1242, with the exception of *Ralstonia* sp. SA-4 (9.7%), was degraded by 37% to 91%. PCB analysis showed that at least 45 of the representative congeners in Aroclor 1242 were extensively transformed by benzoate-grown cells without the need for biphenyl as an inducer of the upper degradation pathway. In incubations with Aroclor 1242, no clear correlation was observed between percentage of congener transformed and the degree of chlorination, regardless of the presence or absence of biphenyl. Recovery of significant but nonstoichiometric amounts of chloride from the culture media showed partial dechlorination of congeners and suggested production of partial degradation products. Addition of biphenyl evidently enhanced dechlorination of the mixture by some isolates. With the exception of *Ralstonia* sp. SA-5, chloride released ranged from 24% to 60% in the presence of biphenyl versus 0.35% to 15% without biphenyl.

1. Introduction

Polychlorinated biphenyls (PCBs) were commercially produced as mixtures containing various levels of chlorination, and were sold under several trade names, including Aroclor, Clophen, Fenchlor, Kanechlor, Phenoclor, and Pyralene (Erickson, 2001). PCBs are considered ubiquitous pollutants that are well known to have a range of toxic effects and, in some cases, to be carcinogenic and mutagenic, making them a serious environmental problem (Larsen, 2006; Simon et al., 2007). For these reasons, their environmental fate and microbial degradability are of great importance.

Much of the environmental contamination by PCBs is in the form of complex commercial mixtures such as Aroclor 1242, which contains an average of 42% chlorine by weight, with approximately 13% dichlorobiphenyls (diCBs), 45% trichlorobiphenyls (triCBs), 31% tetrachlorobiphenyls (tetraCBs) and 10% pentachlorobiphenyls (pentaCBs) (USDHEW, 1972; Abramowicz, 1990; Erickson, 2001). Aroclor 1242 was commonly used in the commercial formulation of dielectric transformer fluids including Askarel. Askarel is a blend of PCBs and chlorinated benzenes usually added to reduce viscosity. Because commercial mixtures such as Aroclors and Askarel consist of numerous congeners, differing in the number and distribution of chlorines on the biphenyl nucleus, they pose a particularly difficult challenge for microbial degradation. Biodegradation by single isolates requires enzymes with broad specificity, able to attack a wide range of congeners with different chlorination patterns. Nevertheless, there have been several studies of the degradation of commercial PCBs. Degradation rates differ from organism to organism, and it is known that the rates of the individual congeners can vary significantly depending on the initial concentrations and on the composition of the mixture (Bedard et al., 1986; Billingsley et al., 1997). Baxter et al. (1975) reported 88% and 76% degradation of Aroclor 1242 during 52 days incubation with *Nocardia* and *Pseudomonas* spp., respectively in the presence of biphenyl. Analysis of the mixture showed selective degradation of monothrough tetrachlorobiphenyls. In another study, microbial communities in activated sludge were shown to degrade 81%, 33%, 26%, and 15% of Aroclors 1221, 1016, 1242 and 1254, respectively (Tucker et al., 1975). With Aroclor 1221, only biphenyl and the monochlorobiphenyls (monoCBs) were biodegraded; the di-, tri-, tetra-, and pentaCB congeners were not degraded (Tucker et al.,

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1975). Clark et al. (1979) demonstrated that the gas chromatographic (GC) profile of Aroclor 1242 was greatly altered after 5 days of incubation with mixed cultures including predominantly *Alcaligenes* spp.

Some aerobic bacterial strains with exceptional ability to cometabolically degrade an even larger range of congeners including penta- and hexachlorobiphenyls have also been documented. Using a pure culture of *Acinetobacter* sp. P6, Furukawa et al. (1983) showed selective degradation of congeners containing 1–4 chlorines in Kanechlors 300 and 400 (equivalent to Aroclors 1242 and 1248). In a related study, Bedard et al. (1987) reported extensive degradation of most of the congeners in 10 ppm of Aroclors 1242, 1248 and 1254 by resting cells of *Alcaligenes* sp. H850 during a 72-h incubation period. Similarly, *Burkholderia* sp. LB400 (Mondello, 1989) and *Rhodococcus* sp. RHAI (Seto et al., 1995) have also demonstrated the capacity to degrade more than 90% of the PCBs present in the mixture of Aroclors 1242, 1248, and Kanechlors 200, 300, 400 and 500. Furthermore, Commandeur et al. (1996) reported that a more extensive removal of some congeners was observed when Aroclor 1242 was presented as an artificial mixture inoculated with *Alcaligenes* sp. JBI.

In all these studies, either biphenyl was used to enhance biodegradation or very low concentrations (5–50 ppm) of the commercial product were used. More importantly, there are no convincing data on the ability of these strains to substantially mineralize some congeners of the mixtures or cleave the carbon–chlorine bond as evidenced by chloride release. Although recent studies have demonstrated growth by some isolates on dichlorobiphenyls (diCBs) (Kim and Picardal, 2001; Adebusoye et al., 2007a, 2008a), most microorganisms so far characterized can only grow on monochlorobiphenyls (CBs) and their use in aerobic PCB bioremediation may not be possible without the addition of biphenyl. Unfortunately, biphenyl is toxic, often subject to regulatory restrictions, and not easily dispersed in contaminated soils or sludges. Since biphenyl addition to a contaminated soil or sediment could be problematic, repeated addition of capable microorganisms (bioaugmentation) might be required. Several researchers have suggested genetic manipulation of PCB-degraders by adding genes for the utilization of chlorobenzoic acid (CBA) to enable these strains grow well on PCBs (Furukawa, 1994; Hrywna et al., 1999; Rodrigues et al., 2006). Apart from potential genetic instability, use of such a construct in the environment might have licensing difficulties. Therefore, isolation of organisms that can utilize a broad spectrum of PCB congeners including *ortho*-substituted diCBs as sole carbon and energy sources, while cometabolizing other non-growth congeners in commercial PCB mixtures without the need of biphenyl, could be a valuable step in development of a viable and robust PCB bioremediation technology.

Recently, we described growth of tropical bacterial strains indigenous to Nigerian contaminated soils on *ortho*-substituted diCB congeners (Adebusoye et al., 2007a) and on di- and trichlorobenzenes (Adebusoye et al., 2007b). The organisms have the unusual ability to grow aerobically on diverse chlorinated compounds and PAHs as sole sources of carbon and energy when the substrates are presented singly (Adebusoye et al., 2008b). In this study, we investigated degradation of the more complex PCB mixtures present in Aroclor 1242 and Askarel fluid by these isolates. Since almost all environmental contamination consists of such commercial mixtures rather than single congeners, we also desired to determine if degradation was inhibited by non-growth congeners or their partial metabolites. Our results demonstrated substantial degradation of Aroclor 1242, and, for the first time, extensive degradation of Askarel dielectric fluid under aerobic conditions, in the presence and absence of biphenyl as a primary substrate. To our knowledge, this is the first report of degradation of commercial PCB mixtures by bacterial strains obtained from African systems where PCB contamination is widespread. Included in our isolates is a strain of *Enterobacter*, a genus not usually associated with biodegradation of chlorinated organic compounds.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The bacterial isolates used for this investigation, namely *Enterobacter* sp. SA-2, *Ralstonia* sp. SA-3, *Ralstonia* sp. SA-4, *Ralstonia* sp. SA-5, and *Pseudomonas* sp. SA-6 were isolated from a landfill containing PCB-contaminated dredge spoils or from a transformer-fluid-contaminated soil and enriched separately by repeated transfer on Askarel fluid and Aroclor 1221 as previously described (Adebusoye et al., 2007a,b, 2008b). Detail phenotypic characterization and 16S rRNA gene analysis as well as substrate diversity of the isolates has been described elsewhere (Adebusoye et al., 2007c). Briefly, all the strains utilize the following as sole carbon and energy sources: benzoate, biphenyl, 2-, 3- and 4-CB (Adebusoye et al., 2008b); 2,2′-, 2,4′- and 2,3-diCB (Adebusoye et al, 2007a); 3,3′- and 3,5-diCB (Adebusoye et al., 2008a); and various di- and trichlrobenzenes (Adebusoye et al., 2007b). The organisms were grown aerobically at 25 °C in an orbital shaker (Labline Instrument Inc.). The medium used for maintenance of cultures for degradation studies was a phosphate-buffered mineral salts (MS) medium containing 2.5 mM sodium benzoate or 100 ppm (0.65 mM) biphenyl as a carbon source. The MS medium was prepared as previously described (Adebusoye et al., 2007b). The purity was controlled by frequent plating on nutrient agar and/or MS agar supplemented with 2.5 mM benzoate.

2.2. PCB degradation studies

Cells were cultivated in MS medium with benzoate (2.5 mM) as sole carbon source and incubated aerobically at 25 °C on an orbital shaker (100 rpm). After 16–18 h of incubation, the cultures were centrifuged for 20 min at 4 °C (Sorval RC 5C Plus). The pelleted cells were washed twice in MS medium lacking benzoate, recentrifuged and finally resuspended in MS medium without benzoate at an optical density (OD) of 0.5–0.6 at 600 nm. One milliliter (approximately 10^5–10^6 cells/ml) of this washed-cell suspension was used to inoculate 6 ml of MS medium already supplemented with either 100 ppm Aroclor 1242 or Askarel fluid. To another set of tubes used in cometabolic studies, replicate tubes containing Aroclor 1242 or Askarel were supplemented with 100 ppm (0.65 mM) biphenyl as a primary substrate. Because PCBs and biphenyl are relatively insoluble in water, the compounds were added to the MS medium in 10 μl concentrated heptamethylnonane (HMN) stock solutions to a final concentration of 100 ppm as previously described (Kim and Picardal, 2000; Adebusoye et al., 2007a). The tubes were crimp-sealed with Teflon-lined rubber stoppers and incubated horizontally on an orbital shaker at 25 °C. Three replicate tubes were used for all treatments. In order to evaluate any possible loss of the xenobiotics during the incubation, these analyses were also performed on replicate uninoculated controls, which were prepared and incubated under the same conditions of the samples. Transformation reactions were stopped after 12 days by adding 5 ml of hexane, vortexing for 1–2 min and thereafter, mixed continuously on a tube rotator for 12 h. Previous experiments in our laboratory have shown that hexane extraction lyses cells and that identical congener recoveries are obtained using uninoculated or killed-cell controls. Since no solid phase was present other than biomass, our extraction method allowed almost complete PCB recovery without the need for Soxhlet extraction. The organic phase was collected in 2-ml glass vials, crimp-sealed with Teflon-lined septa, and stored at

−20 °C for GC analysis. The aqueous phase remaining after removal of the hexane layer was centrifuged (Hermle Z 180 M Labnet) at 13 000g, and filtered through HPLC-grade filters (0.25 μm pore size). The filtered sample was similarly collected in glass vials and frozen for subsequent analysis of chloride.

2.3. Analytical procedures

Hexane extracts were analyzed on an HP 5890 Series II GC (Hewlett–Packard Co., Palo Alto, CA, USA) fitted with an HP 3396 Series II Integrator, and a ^{63}Ni electron capture detector (ECD) and flame ionization detector (FID) as previously described (Adebusoye et al., 2008b). Extracts (1 μl) were injected using a 10-μl Hamilton syringe and separated on a 30-m, DB-5 megabore fused-silica capillary column (J & W Scientific, Folsom, CA, USA; 0.53 mm id, 2.5 μm film thickness).

Percent degradation of each peak was calculated by comparison of the area under individual peaks in inoculated reactors with areas of corresponding peaks in uninoculated controls (Sylvestre and Sondossi, 1994) The total degradation of Aroclor 1242 and Askarel was calculated from the decrease in total summation of all ECD area counts. Unambiguous identification of all individual peaks was not always possible with available instrumentation. Where possible, the assignment of congener identity or homolog class to eluting peaks was based on comparisons of peak retention indexes of samples to those of pure congeners for various homologs run during analysis of the samples as well as comparison of the GC fingerprints with those of Bedard et al. (1987), Quensen et al. (1990), Yadav et al. (1995) and Gilber and Crowley (1997).

Chloride released into the culture fluids was quantified using HPLC (Waters Corp. Milford, Mass. USA) equipped with an auto sample injector (10-μl loop) and conductivity detector (Model 432). Separation was performed on an IonPac AS17 analytical column (4 × 250 mm; Dionex) which was preceded by an AG17 guard column at a flow rate of 1.2 ml/min. The eluent composition was 5 mM NaOH and the injection volume was 10 μl. Calibrated standards and blanks were run with each sample set. The percentage of chloride released from the Aroclor mixture was determined based on percent composition of chlorine in the mixture. For instance, Aroclor 1242 was assumed to contain chlorine content of 42%.

2.4. Chemicals

Aroclor 1242 (>98.6% purity) was purchased from AccuStandard Inc. (New Haven, CT. USA). Biphenyl (99+%), was acquired from Sigma–Aldrich Corp. (St Louis, MD. USA). Sodium benzoate (99+% purity), 2,2,4,4,6,8,8-heptamethylnonane (HMN), and all other organic solvents (HPLC grade) were obtained from Fisher Scientific Co. (Springfield, NJ. USA). Askarel oil (a blend of PCBs and chlorobenzenes) was generously supplied by the Power Holding Company of Nigeria (PHCN) formerly the National Electric Power Authority (NEPA) Transformer Workshop, Ijora, Lagos, Nigeria.

2.5. Statistical analysis

Analyses of variance and *t*-tests were performed using the Prism 5.0 computer software program (GraphPad Software, San Diego CA, USA).

3. Results

3.1. Degradation of Aroclor 1242

Degradation of Aroclor 1242 was evaluated using washed, benzoate-grown cells. Since the highly-branched HMN used as a carrier is not known to be biologically degradable, no carbon substrates other than the PCB commercial mixture were provided. Values for net reduction (percent reduction in total ECD area counts) in total PCB content are presented in Fig. 1. The lack of significant disappearance of PCBs in replicate, uninoculated control tubes (data not shown) demonstrated that the removal was not due to physical–chemical losses. Since our prolonged hexane extraction lyses cells and also extracts congeners sorbed to cells, disappearance of PCB commercial mixture was not a result of cell sorption. The greatest degradation in the absence of biphenyl was observed with strains SA-5, 90.5% and SA-2, 91.4%, while the lowest was seen in tubes inoculated with SA-4. In fact, degradation activity of the latter was almost negligible (9.7%). Since Aroclor 1242 contains a negligible amount of biphenyl, some of the observed disappearance may have resulted from cometabolic degradation by cells growing on biphenyl. Our isolates were able to grow on all three monochlorobiphenyls (CBs) and some diCBs as a sole source of carbon and energy, therefore it is more likely that degradation occurred during growth on the CBs (1%) and diCBs (13%) present in Aroclor 1242. This inference is further corroborated by extensive depletion of Askarel dielectric fluid, which contains very high concentrations of pentaCBs, hexaCBs and hepataCBs (see Fig. 2). The extensive degradation of PCBs was not mirrored by stoichiometric release of Cl^-, suggesting that PCB congeners were only partially degraded in many cases. In incubations with SA-5, for example, less than 10% of the expected Cl^- was recovered even though 90.5% of PCBs were removed.

To better understand the PCB degradation capabilities of the organisms, an experiment to monitor transformation activities in the presence of 100 ppm (0.65 mM) biphenyl as primary growth substrate was performed. The results obtained were not comparable with incubations in the absence of biphenyl, at least for some of the organisms. The data in Fig. 1 show that transformation in the presence of biphenyl was greatly enhanced only with strains SA-4 and SA-6. However, elimination of chloride from Aroclor 1242 was significantly promoted in the presence of biphenyl. Chloride released ranged significantly ($P < 0.05$) from 24% to 60% when biphenyl was present as against 0.35% to 15% released without biphenyl (Fig. 1).

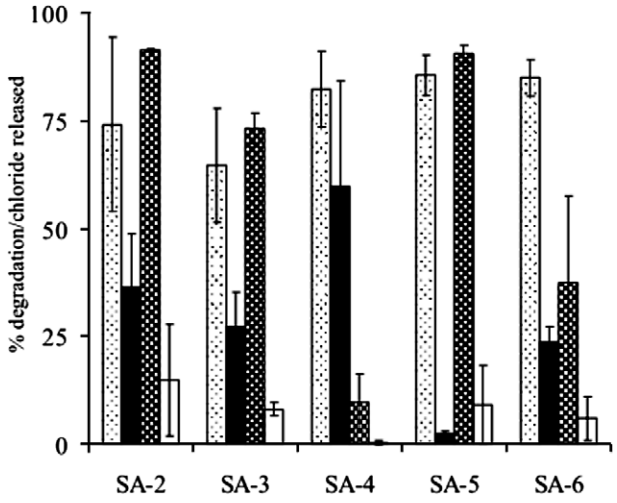

Fig. 1. Transformation of Aroclor 1242 with biphenyl, ▩, and without biphenyl, ▩, and chloride eliminated in tubes with biphenyl, ■, or without biphenyl, □. Percent degradation represents the net decrease (in ECD area counts) in experimental cultures, compared with that of the non-inoculated controls. No chloride was detected in control tubes. Values presented are means ± standard deviations for triplicate cultures. Aroclor 1242 was supplied with/without biphenyl amendment at a concentration of 100 ppm for both compounds and incubated with each organism for 12 days.

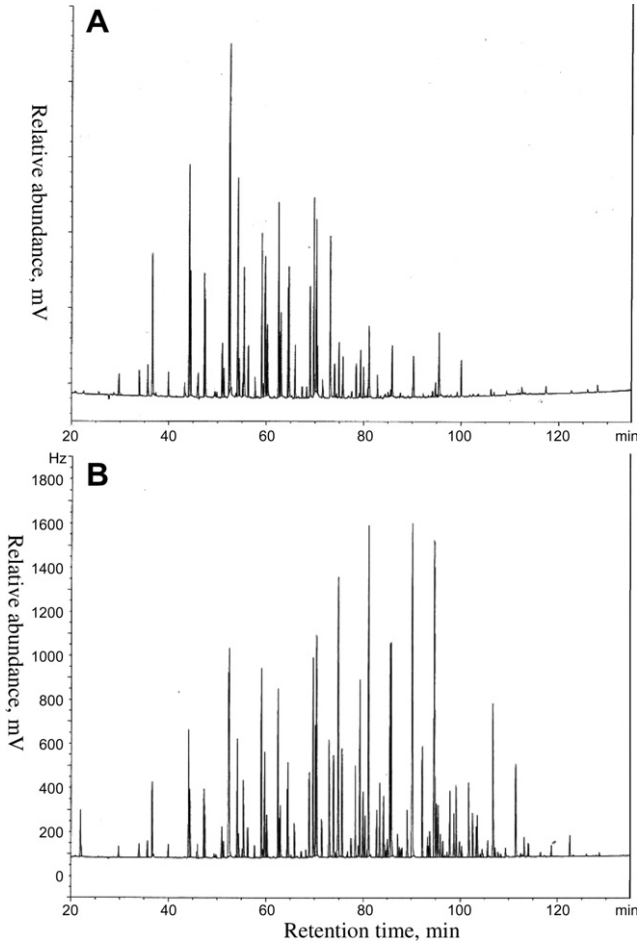

Fig. 2. GC profiles of 0.1 μg/ml of fresh undegraded Aroclor 1242 (A) and Askarel fluid (B). Note the high chlorination of Askarel fluid.

Analysis of transformation of individual PCB congeners in Aroclor 1242 in the presence and absence of biphenyl for tubes inoculated with SA-2, SA-3 and SA-4 are tabulated on Table 1. Results showed that majority of component peaks were significantly degraded albeit to varying extents. Some peaks were not reproducible from one GC run to another and in those cases, the percentage of degradation was not reported in Table 1. It is noteworthy that relatively similar levels of degradation were observed for all the congeners, suggesting that increasing number of chlorine substitution on biphenyl nucleus had limited effect on the pattern of degradation exhibited by the isolates. Strains SA-2 and SA-3 eliminated virtually all the detectable peaks assigned to three, four, five and possibly six (peak 57) chlorine substitutions without the need for biphenyl, thus effecting extensive transformation of the mixture. With the exception of enhanced degradation in tubes incubated with SA-4, addition of biphenyl to culture media yielded no significant increase in congener transformation. Since our isolates are able to grow on monoCBs and some diCBs present in Aroclor 1242, biphenyl was apparently unnecessary as a growth substrate and enzyme inducer. A one-way variance analysis for results of PCB degradation revealed that the results varied significantly at the $P < 0.05$ confidence level. Similarly, statistical analyses showed that the data obtained from congener analysis of tubes without biphenyl amendment differ quite significantly ($P < 0.05$) from those obtained in biphenyl supplemented cultures with the exception of strain SA-3. In the case of strain SA-3, even though the differences were not statistically significant, critical analysis of the data tabulated in Table 1 showed that the consumption of many congeners in the absence of biphenyl was higher than in its presence, similar to the results obtained from SA-2 incubations. The only exception was strain SA-4.

For a majority of the congeners, the extent of degradation was identical at both 12 and 18 days; thus, most of the degradation was considered to have occurred in the less than 12 days. Critical examination of congener analysis revealed that only peaks 9 and 11 were significantly enhanced by biphenyl supplementation during incubation with SA-3. In the case of SA-2, such enhancement was noticed with peak numbers 2, 24 and 32. Generally, in terms of PCB degradation competence in the absence of biphenyl, SA-2 was the best degrader of the three strains analyzed (Table 1).

3.2. Degradation of Askarel dielectric fluid

Gas chromatographic analysis of Askarel fluid utilized in this study revealed a mixture of both PCB congeners and highly chlorinated benzenes with 3–4 chlorine substituents. Comparison of the GC profiles of Askarel with that of Aroclor 1242 revealed that the Askarel fluid used in our study contained constituents with an average degree of chlorination that was greater than that of Aroclor 1242. (Fig. 2). GC–MS further revealed that the Askarel contained: 1,2,3-tri-, 1,3,5-tri-, 1,2,3,4-tetra-, 1,2,3,5-tetra- and 2,3,4,5-tetrachlorobenzene; 2,3,4,6-, 2,2′,5,6-tetraCB; 2,3,3′,4,6-, 2,3,4,5,5′-pentaCB-, 2,2′,3,3′,5,5′-, 2′,2,3,3′,4,5′-hexaCB; and 2,3,3′,4,5,5′,6-, 2,2′,3,4,5,6,6′-heptaCB (Ilori and Robinson, University of Kent at Canterbury, UK, unpublished data). In addition to these congeners, GC–FID also showed that the Askarel contained significant quantities of unidentified, non-chlorinated hydrocarbons either as constituents or as contaminants. Transformation of Askarel fluid was studied in the presence and absence of biphenyl with the five isolates and found to be considerably similar in terms of utilization of the substrate (Fig. 3). This inference was further corroborated by variance analysis, which shows that the degradation abilities of the organisms did not differ significantly ($P < 0.05$), irrespective of whether or not biphenyl was supplied as a substrate. All the organisms exhibited extensive degradation of Askarel fluid. Between 69% and 85% was transformed during the 12-day cultivation period, coupled with intense turbidity of the culture media. Using an acridine-typical cell density increases observed were in the range of four orders of magnitude compared with 2–3 orders of magnitude obtained for Aroclor 1242 (data not shown).

4. Discussion

Biodegradation of commercial mixtures of PCBs have been reported to require biphenyl as a primary substrate and inducer of PCB-degradative activity (Clark et al., 1979; Brunner et al., 1985; Kohler et al., 1988; Boyle et al., 1992). Biphenyl, however, is toxic, highly hydrophobic, expensive and can be subject to regulatory restrictions as well. Microorganisms that grow on environmental contaminants without the need of an additional primary substrate help to obviate this problem. Quite significantly, we have demonstrated for the first time, a more extensive degradation of total PCBs and individual congeners in a relatively high concentration (100 ppm) of Aroclor 1242 by recently characterized axenic cultures of bacterial strains isolated from tropical African contaminated systems with or without the need for biphenyl. Our results indicate that biphenyl may be unnecessary for inducing enzymes of the upper pathways, at least for *Enterobacter* sp. strain SA-2 and *Ralstonia* sp. strain SA-3. However, the cometabolic studies showed greatly enhanced degradation of virtually all the isomers during incubation with *Ralstonia* sp. SA-4. The data in Fig. 1 suggest that our isolates may have more effective degradation ability than other bacteria previously described in the literature. Different

Table 1
Analysis of the transformation of Aroclor 1242 components by microbial strains

Peak no.	No. of chlorine	Congener assignment	Percent degradation[a]					
			SA-2		SA-3		SA-4	
			Without biphenyl	With biphenyl	Without biphenyl	With biphenyl	Without biphenyl	With biphenyl
1	1	3	67.5 (46.2)	98.5 (7.5)	100 (0)	100 (0)	80.1 (11.6)	82.4 (24.9)
2	1	4	41.3 (3.9)	100 (0)	40.3 (12.4)	100 (0)	78.2 (9.1)	78.7 (15.4)
3	2	2,2'; 4,4'	92.3 (3.4)	73.9 (9.3)	82.1 (10.6)	70.4 (17.8)	33.0 (3.4)	87.7 (7.7)
4	2	2,5; 2,4	87.2 (11.2)	81.0 (10.7)	100 (0)	100 (0)	–	–
5	2	2,3'	91.7 (1.7)	78.8 (15.8)	80.2 (11.5)	70.3 (16.0)	43.3 (3.5)	86.7 (7.6)
6	2	2,4'; 3,4	94.6 (1.6)	80.3 (18.0)	–[b]	–	38.6 (5.0)	87.5 (7.5)
7	2	2,3; 2,6	96.4 (1.9)	94.0 (3.3)	82.7 (15.0)	54.7 (10.8)	97.5 (1.2)	94.0 (3.1)
8	2,3	2,2',4	92.6 (0.9)	75.9 (20.4)	81.1 (8.5)	70.5 (15.6)	8.9 (6.7)	87.1 (6.8)
9	2,3	3,3'; 2,4,6	100 (0)	75.0 (15.3)	9.3 (3.5)	56.2 (1.1)	20.8 (7.3)	69.9 (15.6)
11	3	2,'2,5	66 (6.4)	68 (10.5)	36 (3.4)	63 (12.5)	28.2 (4.5)	73 (10.4)
12	3	2,3',6	94.3 (1.2)	88.0 (5.2)	80.8 (15.1)	65.4 (14.3)	16.6 (4.2)	85.9 (7.0)
14	3	2,2',3	91.4 (2.0)	76.6 (19.7)	81.2 (11.0)	70.5 (16.7)	11.7 (6.8)	87.7 (7.7)
15	3	2',3,5	94.1 (1.0)	88.5 (4.3)	81.2 (14.5)	66.7 (13.8)	53.4 (12.3)	86.6 (7.0)
16	3	2,3,4	94.0 (10.4)	75.8 (16.3)	85.7 (9.2)	72.5 (9.0)	17.6 (6.6)	92.3 (6.8)
17	3,4	2,4,5; 2,2',4,6	53.1 (7.3)	49 (4.1)	63.6 (17.3)	69 (15.1)	–	–
18	3	2,3'4	95.0 (0.8)	90.2 (2.9)	83.6 (11.5)	70.8 (12.5)	15.3 (6.3)	88.6 (5.4)
19	3,4	2,4,4'	94.1 (0.8)	78.5 (21.1)	83.8 (10.6)	72.0 (12.3)	13.6 (7.0)	89.2 (5.5)
20	3	2',3,4; 2,3,3'	91.8 (0.2)	74.8 (22.3)	–	–	13.7 (5.2)	85.7 (7.4)
21	3	2,2',3,6	96.0 (0.7)	89.0 (3.2)	82.6 (16.1)	66.6 (12.9)	27.3 (3.8)	86.8 (6.2)
22	3	2,2',3',6	91.2 (0.7)	71.8 (19.6)	71.3 (14.7)	72.1 (11.3)	14.5 (4.2)	82.8 (6.4)
23	3,4	2,2'5,5'; 2,3',6,5'	93.9 (0.2)	90.1 (2.3)	82.2 (11.5)	69.8 (12.4)	10.7 (6.5)	88.0 (5.8)
24	4	2,2',4,5'	53.6 (4.1)	67 (12.3)	–	–	36 (4.7)	76.3 (15.4)
25	4	2,2',4,4'	–	–	55.1 (7.4)	63.2 (15.3)	23.7 (7.1)	82.1 (11.9)
27	4	3,3',4	92.1 (1.4)	86.3 (3.4)	80.3 (11.2)	67.4 (12.7)	11.3 (6.3)	86.5 (6.8)
28	4	2,2',3,6	91.4 (1.4)	86.4 (3.0)	80.2 (10.7)	67.9 (12.5)	10.9 (6.4)	86.6 (6.8)
30	4	2,2',4,5	93.4 (0.3)	74.5 (17.2)	80.3 (12.7)	67.8 (14.8)	10.3 (5.7)	86.7 (7.7)
32	4	2,2', 3,5'	45 (3.2)	57.2(11.2)	67.9 (11)	57.4 (11.4)	17.9 (13.3)	77.4 (6.4)
34	3,4	3,4,4'; 2,2',3,4'	52.5 (13.2)	53.2 (6.2)	74.3 (15.1)	72.2 (16.6)	23.2 (5.1)	67.4 (6.4)
35	4	2,2'3,5'	92.5 (1.4)	88.4(3.4)	82.1 (10.6)	67.9 (9.2)	11.2 (7.2)	85.8 (4.1)
36	4	2,2',3,4	67.2 (10.6)	60.3 (9.5)	59.3 (5.5)	53.4 (3.6)	12.3 (2.3)	61.7 (20.8)
37	4	2,2',3,3'	65.3 (15)	63.4 (6.6)	61.3 (11.5)	56.4 (4.6)	32.6 (5.3)	67.3 (17.8)
38	4	2,4,4',5	91.5 (0.1)	87.8 (2.3)	81.1 (10.8)	68.3 (12.7)	9.3 (7.2)	87.0 (6.3)
39	4	2,3',4',5	91.7 (0.3)	75.5 (18.7)	66.5 (14.2)	71.3 (15.2)	9.2 (6.7)	85.9 (7.0)
41	4	2,3',4,4'	67.8 (6.3)	65.3(13.1)	56.1 (6.4)	53.3 (4.4)	36.1 (9.2)	71.5 (8.4)
43	4,5	2,2'3,4',6	91.7 (0.1)	76.6 (19.9)	–	–	8.2 (5.7)	87.0 (6.2)
45	4,5	2,2',4,4',5	47.9 (11)	47.4 (6.4)	50.5 (7.3)	51.2 (8.1)	–	–
46	5	2,2',3',4,5	87.2 (6.3)	72.8 (15.3)	73.1 (13.2)	69.4 (11.2)	9.9 (5.7)	83.8 (9.3)
47	4,5	2,2',3,4,5'	72.3 (41.2)	59.4(21.5)	90.3 (13.7)	77.6 (19.7)	–	–
48	5	2,2',3,4,4'	50.0 (21.1)	50.0 (13.3)	50 (9.3)	50.0 (5.7)	50.0 (29.7)	50.0 (11.8)
50	4,5	3,3',4,4'; 2,3,3',4',6	43.8 (7.6)	46.2 (10.2)	60 (12.3)	61.0 (10.7)	17.4 (7.4)	60.4 (9.6)
52	5	2,2',3,3',4	91.2 (0.3)	85.8(1.9)	80.9 (10.7)	67.0 (11.6)	9.8 (4.5)	85.4 (5.3)
53	4,5	2,2',3,5,5'6	45.4 (9.7)	50.2 (11.1)	62.1 (5.5)	73.7 (14.6)	37.2(3.3)	57.2 (12.5)
55	5	2,2',4,4'5,5'	40 (4.3)	37.2 (5.5)	57.2 (10)	55.2 (10.5)	57.2 (10)	65.6 (9.3)
56	5,6	2,2',3,3'4,6	87.0 (1.1)	63.5 (21.4)	75.7 (10.7)	67.5 (18.1)	92.5 (10.7)	77.8 (3.4)
57	5,6	2,3,3',4,4'; 2,2',3,3',4,6'	91.2 (0.4)	86.1 (1.8)	80.8 (10.1)	72.0 (15.6)	46.8 (23.4)	86.4 (6.2)

Values presented are means of triplicate samples. Values in parentheses represent standard deviations as percentages of the means. The PCB commercial mixture was supplied with or without biphenyl amendment at an equal concentration of 100 ppm and incubated with each organism for 12 days. Congener number assignment was based on retention time on a DB-5 megabore capillary column as well as comparison with published data (Bedard et al., 1987; Quensen et al., 1990; Yadav et al., 1995; Gilber and Crowley, 1997).

[a] Percent degradation represents the net decrease (in ECD area counts) in experimental cultures, compared with that of the non-inoculated controls.

[b] Peaks that were not reproducible from one GC run to another.

experimental conditions used in the different studies make it difficult, however, to unequivocally compare microbial degradation abilities. According to Barriault and Sylvestre (1993), B-356 was only able to degrade approximately 50% of Aroclor 1242 after 21 days incubation with biphenyl as co-substrate, whereas, 37–91% transformation of Aroclor 1242 was achieved by all of our isolates in 12 days without biphenyl amendment, except for strain SA-4. In an earlier study, Boyle et al. (1992) reported between 12.8% and 24.59% loss in total congener concentration when *Comamonas testosteroni* and *Rhodococcus rhodochrous* were inoculated with either 5 ppm or 10 ppm of Aroclor 1242, respectively. No degradation was observed above 25 ppm.

At least 45 of the congeners in Aroclor 1242 were degraded to varying extents (Table 1). Results depicted in Table 1 show most of the di-, tri-, tetra-, penta-, and perhaps hexachlorobiphenyl peaks were greatly reduced. In incubations with SA-4, however, transformation of many congeners was limited in the absence of biphenyl. The usual pattern reported in the literature is that monoCBs and diCBs are more easily degraded, whereas, triCBs, tetraCBs, pentaCBs and hexaCBs are less readily attacked. Interestingly, in most cases, we observed greater loss of the highly chlorinated congeners than the lightly substituted components, suggesting that the degree of chlorine substitution may not solely always control the pattern of PCB transformation. Our findings support the hypothesis of Arnett et al. (2000) that the position rather than the number of chlorine substituents has a greater influence on the rates of oxidation of PCBs by enzymes of the upper pathway.

Generally, there is a paucity of information on the release of chloride during metabolic transformation of PCB mixtures by biphenyl-oxidizing bacteria, possibly because transformation may sometimes involve only initial ring hydroxylation without subsequent Cl^- release. While our Cl^- recovery was much less than stoichiometric, significant amounts of chloride were recovered from culture fluids (Fig. 1). This would mean that some congeners were

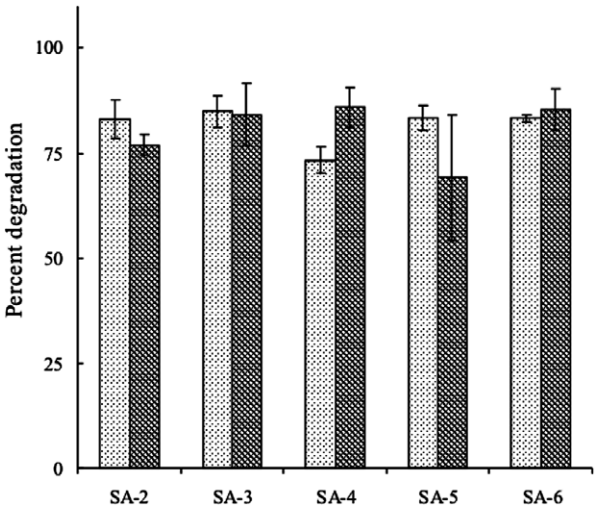

Fig. 3. Degradation of Askarel with, ▫, or without, ▪, biphenyl as a growth substrate. Percent degradation represents the net decrease (in ECD area counts) in experimental cultures, compared with that of the non-inoculated controls. Values presented are means of triplicate samples. Both the cometabolic substrate (Askarel) and the growth substrate (biphenyl) were supplied in equal proportion at 100 ppm and incubated with each organism for 12 days.

either completely mineralized or, more likely, partially transformed to CBAs or other products. In addition to Cl^-, we also tentatively identified, based on HPLC retention times, various CBAs including mono-, di- and trichlorobenzoates in aqueous extracts (data not shown). We did not attempt to positively identify individual CBAs. These endpoint products distributions are in keeping with those found earlier by Yagi and Sudo (1980), Boyle et al. (1992) and Commandeur et al. (1996). More importantly, these results further corroborate our recent findings (Adebusoye et al., 2007a, b, 2008a, b) where we have unambiguously shown the abilities of these microorganisms to utilize individual monoCBs, diCBs and trichlorobenzenes as sole sources of carbon and energy with the evidence of chloride release and CBA production.

Degradation of Askarel dielectric transformer fluid by our isolates is particularly significant for a number of reasons. To our knowledge, this is the first substantial demonstration of degradation of this recalcitrant xenobiotic pollutant by any microorganism. We observed greater cell densities in incubations with Askarel fluid than Aroclor 1242. Recently, we documented growth of these strains on di- and trichlorobenzenes, some of which are components in Askarel (Adebusoye et al., 2007a). We hypothesize that growth in Askarel incubations occurred on some of the PCB congeners, polychlorinated benzenes, and possibly non-chlorinated hydrocarbons present in the Askarel. Growth on these various substrates may have resulted in extensive cometabolism of other constituents in the Askarel fluid.

The extensive degradative capability reported in this study is an indication of novel or more extensive degradative pathways that may be evolving in response to the selective pressure of long-term PCB exposure, or as we previously opined (Adebusoye et al., 2007a, b), it could be that the tropical soils from where these organisms were isolated contained bacteria with multifunctional dioxygenases with relaxed substrate specificities. It is evident from Table 1 that *Enterobacter* sp. SA-2 was the most versatile strain. This is quite remarkable as this organism belongs to the family Enterobacteriaceae which are mainly regarded as inhabitants of animal guts (Diaz et al., 2001). Although many genera of microorganisms have the ability to degrade PCBs and possibly use monoCBs as a sole source of carbon and energy, such phenomena are not commonly encountered in enteric bacteria. *Enterobacter* species have been reported to cause a few pathogenic manifestations (though some are free-living) and to have metal resistance genes, but there have been no reports indicating that this bacterium is capable of extensive degradation of PCB mixtures. Although we documented growth of this strain on monoCBs (Adebusoye et al., 2008b) and diCBs (Adebusoye et al., 2007a), it should be remembered that enteric organisms have not been commonly described in chemically-polluted systems. Many studies involving the use of molecular tools to evaluate community structure changes in contaminated sites have yet to implicate this group of bacteria as active microflora (Nogales et al., 2001). Therefore, the ability of this group to degrade Aroclor 1242 and highly chlorinated Askarel fluid appears to be an unusual finding. Additionally, comparison of the data obtained from tubes inoculated with SA-2 with those of SA-3 or SA-4 revealed that the values differed just significantly ($P < 0.05\%$) thus suggesting to some extent that the catabolic functions inherent in SA-2 are quite different from the other isolates.

Since the isolates described are able to grow on select chlorobenzenes and PAHs in addition to transforming Aroclor 1242 and Askarel without the need for biphenyl, they could be useful tools in bioaugmentation strategies for bioremediation of chemically ill-defined contaminated sites with multiple contaminants without the need for repeated inoculation of PCB-cometabolizing microorganisms unable to grow on the contaminant.

The isolation of these bacteria from a soil still contaminated with PCBs highlights the importance of other factors, e.g., toxicity of co-contaminants, competition with other microorganisms, and limited bioavailability due to poor aqueous solubility and sorption to soil solids, that may effectively limit biotransformation of these pollutants even if capable bacteria are present. Therefore, even though our isolates were able to achieve significant levels of transformation under ideal conditions in the laboratory, their effectiveness in a contaminated soil remains to be ascertained.

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