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Full Length Research Paper

Degradation of Aroclor 1221 by microbial populations of the Lagos lagoon

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Samples obtained from three locations in the Lagos lagoon were studied for the effect of Aroclor 1221 on their population dynamics. In all three cases, both control and experimental microcosms showed slight increases between day 5 and day 10 with the highest value of 4 × 10^{10} cfu/ml in control and 1.2 × 10^{11}, 8 × 10^{10} and 9 × 10^{10} cfu/ml for Iddo, Apapa and Tin Can samples respectively. Three isolates namely, *Bacillus subtilis*, *Alcaligenes eutrophus* and *Pseudomona aeruginosa* were obtained from the microcosms after successive enrichment. All the isolates grew readily on 100 ppm of Aroclor 1221 concomitant with production of yellow metabolites in mineral salts medium. Whereas maximal growth was observed at day 12 on biphenyl, that of the polychlorinated biphenyl (PCB) mixture was on day 15. Generally, growth dynamics were similar irrespective of the substrate while typical generation times, with the exception of *B. subtilis* on Aroclor 1221, ranged insignificantly ($P < 0.05$) from 6.86 to 8.35 day, thus, suggesting that chlorine substitution has little or no effect on catabolic potentials of the organisms. The degradative capability of these strains suggest that they contribute immensely to the self-purification processes occurring in the lagoon, and this could be exploited for decontamination of PCB polluted aquatic ecosystems.

Key words: Aroclor 1221; biodegradation; polychlorinated biphenyls (PCBs); Lagos lagoon, bacterial strains.

INTRODUCTION

Polychlorinated biphenyls (PCBs) are ubiquitous and very recalcitrant environmental pollutants (Kim and Picardal, 2000). They are generally released into the environment from landfills containing PCB waste materials and by improper or illegal disposal of PCB materials (such as spent transformer fluid) to open areas and by environment cycling process of PCBs previously introduced into the environment (Wiegel and Wu, 2000). There is great concern about their presence in the environment due to reported human and animal toxicity (Kimbrough, 1980; Robinson and Lenn, 1994) and ease of bioaccumulation in environmental compartments.

PCBs do not occur naturally in the environment; their commercial production by direct chlorination of biphenyl with anhydrous chlorine gas started in 1929 till around 1986. PCBs were marketed under different names such as Aroclor, Kanechlor and Clophen. Aroclor 1221, which is the focus of this paper, contains biphenyl (11%), monochlorobiphenyls (51%), dichlorobiphenyl (32%), trichlorobiphenyl (4%), tetrachlorobiphenyl (2%) and pentachlorobiphenyl (0.5%) (Erickson, 2001). With the exception of monochlorobiphenyls, PCBs are rarely metabolized as growth substrates because biphenyl utilizers are generally not able to utilize either the chlorobenzoic acid or chlorinated 5-carbon aliphatic acid (2-hydroxy-2,4-pentadienoic acid) that are produced as metabolic intermediates following initial cleavage (Kim and Picardal, 2000). Aroclor 1221, however, appears to be reasonably biodegradable in the environment. In water, significant volatilization of Aroclor 1221 may occur rapidly but adsorption to sediments and suspended matter would also frequently occur. Aerobic bacterial degradation of congeners of Aroclor 1221 and chlorobenzoate intermediates have been reported (Kim and Picardal, 2000; Adebusoye et al., 2007a). Organisms so far reported include strains of *Alcaligenes* sp., *Acinetobacter* sp., *Pseudomonas* sp. and *Ralstonia* sp.

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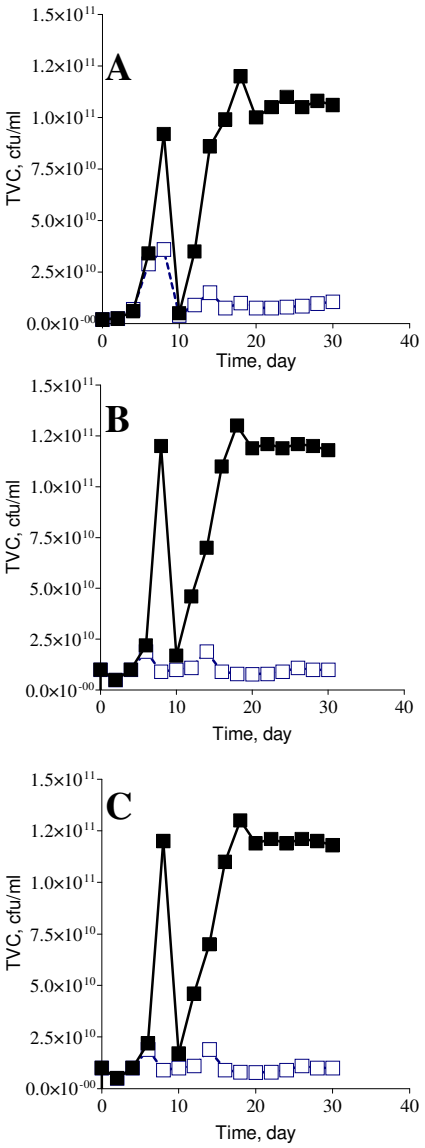

Figure 1. Aroclor 1221 Degradation by Microbial Communities in lagoon water (■) samples from Tin Can (A), Apapa (B) and Iddo (C). Corresponding control samples without substrate are represented with open symbols. Data represent the averages of three replicate determinations. Error bars were eliminated for clarity. The PCB commercial mixture was supplied at 100 ppm concentration.

(Furukawa et al., 1979; Arensdorf and Focht, 1995; Kim and Picardal, 2000; Adebusoye, et al. 2007a).

The Lagos lagoon is large, highly abused brackish water, which serves as dump for a wide variety of pollutants of both domestic and industrial effluents and unregulated disposal of automobile and electrical wastes. However, it is also a fisheries resource for over 10 million inhabitants of the mega-city (Ilori, 1999). The impacts of some of these wastes, particularly the petroleum hydrocarbons and heavy metals on the ecology of the lagoon have been reported (Amund and Nwokoye, 1993; Amund and Igiri, 1990). However, not much is known about the PCB biodegradation status of the lagoon microbial community. In this paper, we report the isolation of three bacterial strains from microcosms of lagoon water and their growth profiles on biphenyl and Aroclor 1221.

MATERIALS AND METHODS

Heterotrophic growth in Aroclor 1221 supplemented lagoon water

Water samples (100 ml) were collected in sterile conical flasks (250 ml) at three sites on the Lagos lagoon characterized by high level of indiscriminate pollution with domestic and industrial wastes namely; Tin Can, Apapa and Iddo. Experimental flasks were supplemented with 100 ppm of Aroclor 1221, while unsupplemented flasks served as control. The flasks were stoppered with Teflon-coated seals and incubated at room temperature (29 ± 2°C) with shaking at 150 rpm. Total heterotrophic bacterial counts were determined by plating out aliquots on nutrient agar at regular intervals for 30 days. Total fungal populations were also determined by plating out aliquots on potato dextrose agar.

Physico-chemistry of Lagos lagoon water

The physoco-chemical composition of lagoon water samples was determined by standard methods described by Eaton et al. (1995). Parameters assayed were pH, salinity, dissolved oxygen, phosphorus content, organic carbon and total hydrocarbon content.

Isolation and characterization of PCB-degrading bacteria

Isolation of bacteria capable of growth on chlorobiphenyls as sole carbon and energy sources was carried out by traditional enrichment methods after several repeated transfers in mineral salts (MS) supplemented with Aroclor 1221 as described by Adebusoye et al. (2007a). Pure cultures were isolated from enrichments by plating out appropriate dilutions onto Luria Bertani (LB) agar plates. Isolates were further screened for the ability to grow on Aroclor 1221 by inoculating them individually into MS medium supplemented with Aroclor 1221. The MS medium described by Kästner et al. (1994) was used. The medium was fortified with trace elements solution described by Bauchop and Elsden (1960). Isolates were identified on the basis of their cultural, cellular morphology and biochemical characteristics according to the scheme of Barrow and Feltham (1995).

Growth studies

The growth of isolates on Aroclor 1221 and Biphenyl was monitored by inoculating pure isolates into sterile MS medium amended with 100 ppm of the respective substrate as a sole carbon source and incubating in the dark at room temperature. The inoculum was grown in LB agar. Developed colonies were scooped, suspended in sterile MS medium and inoculated to give a final concentration of 0.05 (OD_{500}). Growth was measured by total viable count (TVC) at regular time intervals for 20 - 25 days. Uninoculated flasks were set up similarly to serve as control.

RESULTS

Degradation of Aroclor 1221 by indigenous microflora

In the three microcosms, both control and experimental setups did not show visible sign of growth until day 5. The

Table 1. Microbial populations of lagoon water samples.

Sample source	Bacteria			Fungi[a]	
	Total heterotrophs (×10^8 cfu/ml)	Total PCB-utilizers (× 10^4 cfu/ml)	% PCB-utilizers	Total yeasts (× 10^4 cfu/ml)	Total mould (× 10^4 cfu/ml)
Tincan	6.5	10	0.015	80	5
Apapa	5	9	0.018	28	60
Iddo	7	8	0.012	21	2

[a]No PCB-utilizing yeasts and moulds were detected in all three lagoon water samples.

Table 2. Physico-chemical properties of lagoon water samples.

Sample source	pH	Dissolved oxygen (mg/l)	Salinity (%)	Phosphorus (mg/l)	Organic carbon (%)	Total hydrocarbon content (mg/l)
Tincan	7.49	1.0	1.37	N/D[a]	2.68	8.0
Apapa	7.80	1.8	1.52	0.042	2.86	90.0
Iddo	7.73	2.4	1.34	0.107	2.72	80.0

[a]Not detected.

control samples showed slight increases between day 5 and day 10 of upward of 4 × 10^{10} cfu/ml, after which it dropped and fluctuated below 2 × 10^{10} cfu/ml for the next 20 days (Figure 1). In the experimental flasks, increases were noticed between day 5 and day 10, with peaks 1.2 ×10^{11} cfu/ml, 8 × 10^{10} cfu/ml and 9 × 10^{10} cfu/ml for Iddo, Apapa and Tin Can, respectively, on day 8. After slight drops, the heterotrophic populations rose sharply again till about day 18 in all the three cases. The highest values were 1.4 × 10^{11} cfu/ml, 1.2 × 10^{11} cfu/ml and 1.2 × 10^{11} cfu/ml for Iddo, Apapa and Tin Can, respectively. The populations fluctuated slightly below these values till the end of the experiment.

Initial population counts showed that Iddo water had the highest heterotrophic bacteria with Apapa, having the lowest (5 × 10^8 cfu/ml). However, results from fungal counts showed that Iddo samples had the lowest counts (Table 1). The proportions of bacteria that are PCB utilizers generally fell below 0.1%. Interestingly, none of the fungal communities in the three samples was found to utilize PCB as sources of carbon and energy.

Physico-chemical compositions of lagoon water

The physico-chemical properties of the lagoon water samples from various sampling points are summarized in Table 2. The pH fell within the neutral range with the highest recorded for Apapa. The salinity ranged from 1.34 to 1.52%, indicating that the ecosystem is brackish. The phosphorus content fell below 0.1% while, this parameter was below detection limit in Tin Can sample (see Table 2).

Isolation and characterization of Aroclor 1221 degraders

Three isolates were obtained from the microcosms following the enrichment protocol. The organism from Iddo was Gram-positive, spore-forming rod, catalase- and oxidase-positive. It liquefied gelatin, fermented sugars including mannitol and salicin and was subsequently tentatively identified as *Bacillus subtilis*. The isolates from Tin Can were identified as *Alcaligenes eutrophus*. Phenotypic properties showed that it was urease-, catalase-, oxidase- and indole-positive, motile but negative for most sugars except glucose and lactose. From the Apapa microcosm, a Gram-negative motile rod that failed to ferment most sugars tested, produced yellow pigment that diffuses easily into the medium and grew at 42°C was isolated. The isolate was identified as *Pseudomonas aeruginosa*.

Growth profile of isolates on Aroclor 1221 and biphenyl

All three isolates exhibited logarithmic population increases when grown on MS medium amended with biphenyl or Aroclor 1221 as sole source of carbon and energy. There was appearance of yellow colour 2 weeks after incubation with Aroclor 1221. It is noteworthy that no colour change was observed in media supplemented with biphenyl. The growth dynamics are illustrated in Table 3, and Figures 2 and 3. During growth on biphenyl, typical generation times ranged insignificantly from 6.86 to 7.74 day at $P > 0.05$ level of probability (Table 3). However, *P. aeruginosa* utilized the substrate best with the highest

Table 3. Growth kinetics of bacterial strains on biphenyl and Aroclor 1221.

Bacterial species	Biphenyl		Aroclor 1221	
	Generation time (d)	Specific growth rate (d^{-1})	Generation time (d)	Specific growth rate (d^{-1})
Alcaligenes eutropus	7.51	0.09	8.30	0.08
Bacillus subtilis	7.74	0.09	11.61	0.06
Pseudomonas aeruginosa	6.86	0.10	8.35	0.08

Mean generation times and specific growth rates were calculated using nonlinear regression growth curves from the first 12 days on biphenyl and 15 days on Aroclor 1221 during which growth rates were maximal.

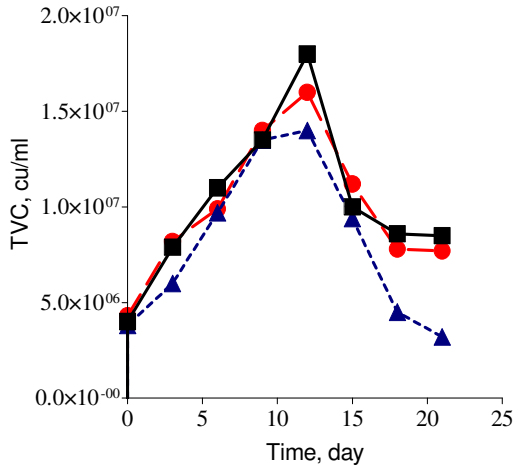

Figure 2. Growth dynamics of *Pseudomonas aeruginosa* (black squares), *Alcaligenes eutrophus* (blue triangles) and *Bacillus subtilis* (red circles) on 100 ppm of biphenyl. Data points represent the averages of triplicate determinations.

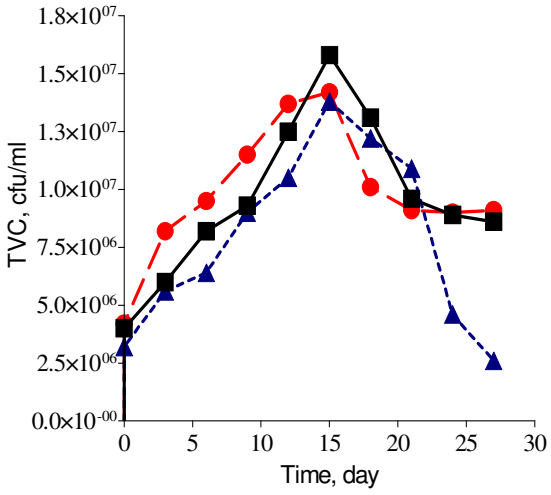

Figure 3. Growth dynamics of *Pseudomonas aeruginosa* (black squares), *Alcaligenes eutrophus* (blue triangles) and *Bacillus subtilis* (red circles) on 100 ppm of Aroclor 1221. Data points represent the averages of triplicate determinations.

population density of 1.59 × 10^7 cfu/ml with the least generation time. Generally, growth peaked at day 12 for all the isolates after which sharp decreases were observed (Figure 2). In the case of *P. aeruginosa* and *B. subtilis*, growth later stabilized from day 18.

The growth dynamics of the isolates on Aroclor 1221 were relatively similar to biphenyl, although it took longer time to achieve maximal population densities. Generation times obtained were 8.3, 11.61 and 8.35 day respectively for *A. eutrophus*, *B. subtilis* and *P. aeruginosa* (Figure 3). Population increases were observed from the onset of experiment till day 15 before declining sharply, while those of *B. subtilis* and *P. aeruginosa* stabilized from day 21 similar to obtained profiles on biphenyl.

DISCUSSION

Micro-organisms capable of growth on polychlorinated biphenyls and their individual congeners have been well reported (Walia et al., 1988; Bedard et al., 1987; Kim and Picardal, 2000). Recent reports showed that a number of tropical bacteria with this ability exist in Nigerian soils (Adebusoye et al., 2007a, b)

Consistent increase and decrease in the TVCs of indigenous organisms in the lagoon water samples occurred in both test and control experiments when amended with either biphenyl or Aroclor 1221. The initial increase in population of heterotrophic bacteria observed in control flasks between day 5 and day 10 (Figure 1) could be attributed to utilization of other organic nutrients present in the water. Depletion of the carried-over nutrients therefore, led to permanent decline of the growth of the organisms in the control set-ups. The presence of such nutrient may be due to the fact that the three points from which the samples were obtained on the lagoon namely Iddo, Apapa and Tin Can are areas of intense discharge of untreated industrial and domestic wastes and other anthropogenic activities. Interestingly, the organic matter content of between 2.68 and 2.86% and hydrocarbon content of 8 – 90 mg/l detected in the waters (Table 2) would seem to authenticate this. Relatively high number of PCB-degraders was obtained in all three samples analyzed even though the percent occurrence was generally less than 0.1. It is not a surprise therefore, that

the indigenous microflora was able to grow and multiply in the presence of Aroclor 1221 (Figure 1). The presence of xenobiotic-degraders in the lagoon waters could be traceable to continual pollution of the environment resulting from indiscriminate discharge of PCBs, PCB-containing wastes and herbicides, and the selective pressure exerted by these pollutants over time leading to the ability to produce catabolic phenotypes (Hickey and Focht, 1990; Wackett and Hersberger, 2001; Adebusoye et al., 2007b). The three bacteria isolated in this study were *P. aeruginosa*, *A. eutrophus* and *B. subtilis*. Interestingly, these genera of organisms have been implicated in the catabolism of PCBs and chlorobenzoic acids (a primary metabolite of PCB and pesticide catabolism) by several workers. For instance, Nawaz and Chapatawala (1991) reported a strain of *P. aeruginosa* that transformed commercial mixtures of Aroclor 1242 and 1254. Similarly, *A. eutrophus* strains H850 capable of degrading broad and unusual spectrum of PCB congeners was demonstrated to extensively degrade Aroclors 1242 and 1254 (Bedard et al., 1987).

The growth dynamics of the isolates on Aroclor 1221, as depicted in Figure 3, showed a stepwise curve before attainment of peak. The growth profiles seem reasonable considering the steric effects of the various congeners making up the mixture, the sequential metabolism of these congeners and/or acclimation prior to further degradation of toxic intermediates, usually chlorobenzoic acids. This inference is further reinforced by the smoother exponential growth curves observed for the three isolates on biphenyl (Figure 2) on which growth peaked at about day 11 as against 15 for Aroclor 1221. It has been suggested that the slow rate of removal of PCBs from the environment could be attributed to the non-productive metabolism of chlorobenzoic acids to the antibacterial dead-end product, protoanemonin, which kills PCB degrader (Abraham et al., 2002). Thus, apart from exhaustion of nutrient, accumulation of toxic dead-end product may account for the post peak sharp drop in cell counts. However, the appearance of yellow colour in the enrichment cultures after several days and of course in the Aroclor cultures indicated *meta*-cleavage. This is in consonance with earlier reports (Hernandez et al., 1991; Adebusoye, 2007a, b) and is incontrovertible evidence of biodegradation of the substrate by the bacterial isolates. The yellow colour could have resulted from the *meta*-cleavage of chlorobiphenyl nucleus or most probably from *meta*-cleavage of (chloro) catechol resulting from further metabolism of chlorobenzoic acids.

In this study, we have demonstrated for the first time in the Lagos lagoon, aerobic microbial communities exhibiting Aroclor 1221 catabolic phenotypes. Similarly, we have shown the existence of three bacterial isolates from this ecosystem with capacity to utilize Aroclor 1221 as a sole source of carbon and energy. The growth curves of axenic cultures of these isolates showed a stepwise pattern suggesting sequential catabolism of individual components of the PCB mixture and/or further degradation of the primary metabolites of PCB catabolic pathway. Kinetic data obtained during growth on Aroclor 1221 were very similar to that of biphenyl (Table 3) suggesting that chlorine substitution has little or no effect on catabolic potentials of the organisms. The metabolic potentials of these organisms are indication of the contribution to the self-purification processes occurring in the lagoon, which could exploited for the cleanup of sites contaminated with PCBs or allied xenobiotic compounds.

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