



Polycyclic Aromatic Hydrocarbon (PAH) Degrading Potential of Bacteria Isolated from Iko River Sediment

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Polycyclic aromatic hydrocarbon (PAH) degrading potential of bacteria isolated from Iko River sediment, Akwa Ibom State, Nigeria was investigated. The mean total heterotrophic bacteria obtained from the sediment samples was 6.4×10^4 cfu/g while 9.8×10^3 cfu/g hydrocarbon utilizing bacteria was recorded. Preliminary screening of the hydrocarbonoclastic bacterial isolates revealed that among the 12 bacterial isolates, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Alcaligenes sp* exhibited the strongest ability to utilize crude oil. The result also revealed *Bacillus subtilis* and *Pseudomonas aeruginosa* as the best PAH degraders. A higher microbial cells count in the 2-ring PAH (naphthalene) supplemented –MSM was recorded. The levels of attenuation however varied with the test organism and were accompanied by fluctuations but decreasing pH levels and slight changes in temperature of the culture medium. *In vitro* degradation study carried out for the 21 days showed that the degradation of PAH when augmented with *Pseudomonas aeruginosa* and *Bacillus subtilis* respectively was faster than when un-augmented. The PAH content was reduced by *Pseudomonas aeruginosa* and *Bacillus subtilis* from 10.42 to 9.03 and 9.56 respectively. The hydrocarbon degradation by augmented cultures of *Pseudomonas aeruginosa* and *Bacillus subtilis* implies that bioaugmentation can be harnessed for bioremediation purposes.

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1. INTRODUCTION

Increasing crude oil exploitation in the Niger Delta has led to widespread contamination of most of its creeks, rivers, swamps soils, underground water and coastal regions. Contaminations result from crude oil spills, improper disposal of drilling mud and cuttings dispersant application and other wastes resulting from crude oil extraction, transportation and refining processes [1,2]. The contaminant presents serious environmental problems which may affect physiological processes, population and behavioural profiles of organisms [3]. These chemical pollutants can be accommodated in three basic reservoirs, water, sediment and biota.

Polycyclic aromatic hydrocarbons (PAHs) are a group of ubiquitous environmental pollutants and some of them are known to be mutagenic or carcinogenic [4]. PAHs are released to the environment through anthropogenic activities such as the production and combustion of fossil fuels, and biomass [5] and enter surface waters through different pathways including atmospheric fallout, urban runoff and municipal/industrial effluents [6]. PAHs are present as natural constituents in fossil fuels, are formed during the incomplete combustion of organic material, and are therefore present in relatively high concentrations in products of fossil fuel refining [7]. Petroleum refining and transport activities are major contributors to localized loadings of PAHs in the environment. Such loadings may occur through the discharge of industrial effluents and through the accidental release of raw and refined products. Anthropogenic and natural sources of PAHs in combination with global transport phenomena results in their worldwide distribution.

The environmental fate of PAH molecules involves its chemical properties are dependent in part upon both molecular size, i.e. the number of aromatic rings, and molecule topology or the pattern of ring linkage. Ring linkage patterns in PAHs may occur such that the tertiary carbon atoms are centres of two or three interlinked rings, as in the linear kata-annelated PAH, anthracene or the peri-condensed PAH pyrene. However, most PAHs occur as hybrids encompassing various structural components, such as in the PAH benzo(a)pyrene (BaP). Due to their lipophilic nature, PAHs have a high

potential for biomagnifications through trophic transfers [8]. PAHs are also known to exert acutely toxic effects and/or possess mutagenic, teratogenic, or carcinogenic properties [9].

Higher molecular weight PAHs through degradable, are quite recalcitrant and may persist indefinitely. Microbial biotransformation is a major environmental process affecting the fate of PAHs in both terrestrial and aquatic ecosystems. Although the microbial degradation of PAHs having two or three rings is well documented [10], only within the last decade have a number of bacteria that metabolize larger PAH molecules been isolated. These include *Alcaligenes denitrificans*, several *Pseudomonas* sp. [11], *Rhodococcus* sp., strain UW1 [12], and various *Mycobacterium* species [13]. Singer and Finnerty [14] reported that different derivative genes are involved in the metabolism of aromatic and aliphatic hydrocarbons. The genes coding for the enzymes involved in the degradation of alkanes and naphthalene have been extensively characterized [15]. Assessment of hydrocarbon utilization capacity for a large number of environmental isolates has indicated that microbial strains are capable of mineralizing either aromatic or aliphatic hydrocarbon compounds. Although Foght et al. [16] postulated that bacteria having multi-degradative capacity might exist, no strains were found with the capacity to degrade both classes of compounds. However, a more recent report has established that the ability to degrade aliphatic and aromatic hydrocarbons are not necessarily mutually exclusive (Sotsky et al., 1994). Two *Pseudomonas* strains that can degrade both naphthalene and alkanes have been isolated. This *Pseudomonas* possesses both the alkane and naphthalene catabolic pathways for biodegradation [17].

Recent reports that both naphthalene and alkanes can be degraded by single bacterial species could have raised the question whether bacteria capable of degrading larger (three-or four-ring) PAHs could also metabolize alkanes and, if so, whether they would possess well-characterized hydrocarbon degradation genes, such as nahAc and alkB. An understanding of the fate of PAHs in coastal environments is important, because high PAH levels in coastal ecosystems may pose a threat to human public health and the wellbeing of the marine

environment. However, there must be an understanding of the interaction of PAHs with sediments, the responses of the microbial population to the presence of weathered PAHs, and the factors that limit the microbes' ability to degrade the PAHs to carbon dioxide and water. This study, therefore, seeks to establish the role of bacteria with strong PAH degrading potential in the remediation of PAHs in estuarine mudflats.

2. MATERIALS AND METHODS

2.1 Study Area

The study area was the mangrove ecosystem of Iko River Estuary. Iko is located within the petroleum belt of the Niger Delta, Nigeria (7°30' N and 7°45'N, and longitude 7°30' E and 7°40'E.). The Iko River estuary has semi-diurnal tides and a shallow depth ranging from 1 to 7 m at flood and ebb tide. The estuary is more than 20 km long with an average width of about 5 m. The area is characterized by a humid tropical climate with rainfall reaching about 3,000 mm per annum.

2.2 Sampling

Three sites in Iko River Estuary were chosen to represent a wide range of PAH contamination. Intertidal sediment samples were obtained with a gravity corer (6.5 cm diameter and length of 100 cm) to a depth of 10cm.

2.3 Sediment Characterization

The total carbon of the sediments was determined as described by Olajire et al. [18]. Sediment pH was measured potentiometrically in 0.01M CaCl₂ with sediment extractant ratio of 1:2 while the salinity was determined from silver thiourea (AgTU) extracts and 0.1 M silver nitrate (AgNO₃) titration using potassium chromate as the indicator and calculated as total soluble salts (chlorides + sulphates).

2.4 Determination of Naphthalene Content of Sediment

2.4.1 Sediment extraction and fractionation

The epipellic sediment samples were extracted and fractionated as described [18]. The dried and sieved samples (50 g) was weighed and spiked with pre-deuterated PAH Cocktail as internal standard and extracted with dichloromethane (DCM) using temperature-programmed Soxhlet

extractor at 65°C for 24 h. The extracts were reduced to dryness and re-dissolved in n-hexane. The extract was then fractionated on a glass column packed with 30 g of alumina deactivated with 4.5% water. Aliphatic and polycyclic aromatic hydrocarbons were eluted with 50 ml of hexane: DCM (95/5%, w/v) and the polar fractions were eluted with DCM. The PAH fractions were then concentrated by rotary evaporation. Before GC/MS analysis, fractions were dried under nitrogen and re-dissolved in DCM.

2.5 Microbiological Analysis of Sediment Samples

2.5.1 Estimation of bacterial load of sediment

Sediment samples for bacteriological analysis were aseptically obtained and analyzed within 24 hrs of collection. Precisely 10 g of each sample was weighed out, added to 90 ml of sterile distilled water and vigorously shaken for 1 minute with the aid of a vortex shaker. Ten-fold serial dilution of the sediment samples was carried out with the known volume of sterile distilled water before the enumeration of oil-degrading bacteria and yeasts.

Heterotrophic bacteria (HTB) and hydrocarbon utilizing bacteria (HUB) in the sediment samples were enumerated by the pour plate technique [19] using diluents prepared with deionised water and cultured in nutrient agar (Difco) and oil agar-mineral salt medium (MSM). Precisely 1 ml quantities of each dilution were inoculated and incubated at 28 ± 2°C for 5 days.

2.5.2 Identification of the bacterial isolates

Pure isolates obtained after the primary culture media that supported the growth of specific organisms were examined for morphology, cultural and biochemical characteristics using the earlier methods described [20].

2.5.3 Determination of hydrocarbonoclastic potential of sediment bacteria and yeast

Pure bacterial isolates obtained were inoculated or streaked onto oil agar. Visible bacterial growth and production of the zone of clearing around the colonies were regarded as evidence of the ability to degrade hydrocarbons. This was graded as strong (+++), moderate (++) , weak (+) and no (-) degrading potential as described by Ekundayo and Obire [21].

3. RESULTS AND DISCUSSION

3.1 Background Load and Profile of Polycyclic Aromatic Hydrocarbons (PAHs) in the Sediment

The suite profiles and load of PAHs in the intertidal sediments from Iko River Estuary mangrove ecosystem are presented in Table 1. The results have shown that the 2-3 rings PAHs were the dominant suite of PAHs in mudflats from Iko River Estuary. The concentration of 2-3 rings PAHs ranged from 4.20 to 5.27 mg/kgdw, while 4-rings and 5-rings PAHs ranged from 1.13 to 4.87 and from 2.14 – 2.47 mg/kgdw. The 6-rings PAHs were the least detected with a range of 0.20 – 1.04 mg/kgdw.

Fig. 1 shows the group profiles of PAHs normalized by the total sum of all the PAHs in each sample. Their ratios (Table 2) however varied from the sample locations. 2-3rings PAHs had the highest ratio in all the samples (IB₁, IB₂ and IB₃) with 0.39, 0.47 and 0.49 respectively. However, high molecular PAHs which were known to be persistent with chronic toxicity in the environment were more than 61% of total PAHs in all sediments analysed (Fig. 1). Among them, phenanthrene, fluoranthene and pyrene were the most dominant compounds with concentration of 1.27, 2.48 and 1.46 mg/kgdw respectively, which account for about 38.8% of the total PAHs in IB₁; while benzo(b)fluoranthene, benzo(k) fluoranthene, indeno (1,2,3, cd) pyrene and benzo(g,h, i,)perylene also showed high contribution in these sediments. Naphthalene and Acenaphthelyen were predominant among the low molecular weight PAHs with a concentration of 1.00 and 1.00mg/kgdw respectively.

Table 3 shows the parent PAH ratios of sediments examined in this study. This was to characterize PAHs with respect to sources. A ratio of low to high molecular weight PAHs was examined and we obtained a range of 0.65 – 0.96 for the Iko River sediments. The values are less than unity (<1) in all the sediment study. Most samples analyzed satisfied the criteria of Phen/Anth<10, Flut/Pyr>1. The ratio of naphthalene/phenanthrene was also found to be greater than unity (>1).

3.2 Bacteriological Properties of the Estuarine Sediment Samples

The results presented in Table 4 revealed the rich microbial assemblage of the tidal mudflats in

Iko River Estuary. Mean total heterotrophic bacterial counts of 6.4×10^7 cfug⁻¹, was obtained from the sediment samples. Bacterial isolates from the mudflats were characterized and found to comprise mainly of 11 genera of heterotrophic bacteria.

3.3 Hydrocarbonoclastic Potential of Bacteria and Yeast Isolated from the Mudflat

The capability of the bacteria isolates to degrade crude oil and PAH is presented in Table 5. It reveals that after 7 days of incubation, the degrees of turbidity in the MSM broth (supplemented with 1% of crude oil) for *Pseudomonas aeruginosa*, *Alcaligenes* and *Bacillus subtilis* showed heavy growth followed by *Acetobacter* sp, *Citrobacter* sp, *Serratia* sp and *Micrococcus* sp having moderate growth. As the days increased, *Staphylococcus* sp and *Alcaligenes* sp decreased in turbidity, but *Pseudomonas aeruginosa* and *Bacillus subtilis* maintained higher growth even as the number of days increased. Among the crude oil degraders, only *Pseudomonas aeruginosa*, *Alcaligenes*, *Bacillus subtilis* and *Micrococcus* sp exhibited growth on PAH supplemented media. Among the organisms that utilized PAH for growth, *Pseudomonas aeruginosa* and *Bacillus subtilis* exhibited high or strong ability to degrade PAHs. These two bacterial species, *Pseudomonas aeruginosa* and *Bacillus subtilis* were chosen for the 2- & 3- ring PAHs utilization studies.

3.4 Growth Profile of the Selected PAH Degraders on Naphthalene and Anthracene Supplemented with Minimal Salt Medium

The effect of 2% concentrations of naphthalene and anthracene supplemented minimal salt medium (MSM) on the growth (population dynamics), pH, temperature and optical densities of test organisms are presented in Tables 6 and 7 respectively. The results revealed higher microbial cells count in the 2-ring PAH (naphthalene) supplemented -MSM (Table 6) compared with that of 3-ring PAH (Anthracene) (Table 7). There were general attenuation levels (optical densities) of medium inoculated with the test isolates. The levels of attenuation however varied with the test organism and were accompanied by fluctuations but decreasing pH levels and slight changes in temperature of the culture medium.

Table 1. Total PAHs concentration (mg/kgdw), the concentration of 16 PAHs and total organic carbon (%) in sediments from Iko River Estuary

PAH weight (Ring)	Analyte	Sediment samples		
		IB ₁	IB ₂	IB ₃
2-3rings PAHs	Naphthalene	1.00	1.00	1.00
	2methylnaphthalene	0.20	0.20	0.20
	Acenaphthylene	1.00	1.00	1.00
	Acenaphthalene	0.70	0.70	0.70
	Flourene	0.70	0.70	0.70
	Phenanthrene	1.27	0.20	0.20
	Anthracene	0.40	0.40	0.40
Σ2-3rings		5.27	4.20	4.20
4rings PAHs	Flouranthene	2.48	0.20	0.20
	Pyrene	1.46	0.20	0.20
	Benzo(a)anthracene	0.53	0.33	0.56
	Chrysene	0.40	0.40	0.75
Σ4rings		4.87	1.13	1.71
5rings PAHs	Benzo(b)flourenthene	0.58	0.74	0.91
	Benzo(k)fluranthene	0.47	0.25	0.46
	Benzo(a)pyrene	0.39	0.20	0.31
	Dibenzo(a,h)anthracene	0.20	0.47	0.59
	Benzo(g,h,i)perylene	0.60	0.48	0.20
Σ5rings		2.24	2.14	2.47
6ring PAHs	Indeno(1,2,3,cd)pyrene	1.04	1.53	0.20
TOTAL PAHs		13.4	9.00	8.58
% organic carbon ^(a)		6.18	5.90	5.48
pH ^(a)		6.40	6.15	6.10
Salinity (%) ^(a)		2.86	3.15	3.12
ΣPAHs/OC (mg/kgdw)		144.3	152.5	156.5

Values are mean of triplicate determinations ($\pm 5\%$ of the mean). Σ PAHs/OC is the total PAHs concentration normalized to organic carbon content. IB₁, IB₂, IB₃ are Iko intertidal sediment samples collected

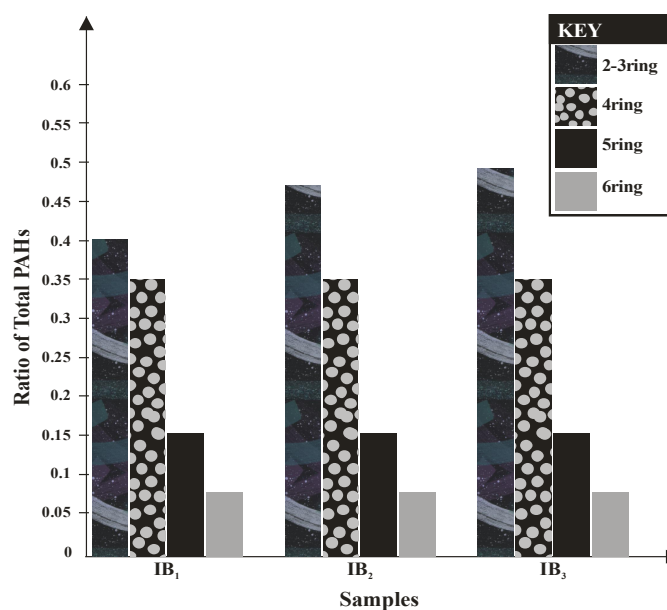
**Fig. 1. Group profile of sedimentary PAHs normalized by total PAHs in the sediments of Iko river estuary**

Table 2. Group ratios of PAHs in sediment samples from Iko river estuary

PAH weight (Ring)	IB ₁	IB ₂	IB ₃
2 – 3 rings PAHs	0.39	0.47	0.49
4- ring PAHs	0.36	0.13	0.20
5- ring PAHs	0.14	0.24	0.27
6- ring PAHs	0.08	0.17	0.02

Table 3. PAH ratio in the sediment samples from Iko River estuary mangrove ecosystem

Sample	PAH ratio			
	LMW/HMW	Phen/Anth	Flut/Pyr	Naph/Phen.
IB ₁	0.65	3.18	1.7	0.79
IB ₂	0.88	0.5	1.0	5.0
IB ₃	0.96	0.5	1.0	5.0

N/B: Naph – Naphthalene; Anth – Anthracene; Phen – Phenanthrene; Flut – Flouranthene; Pyr – Pyrene

Table 4. Bacteriological properties of the tidal mudflats from Iko river estuary

Total bacterial load (cfug ⁻¹)	Hydrocarbon utilizing bacteria (cfug ⁻¹)
6.4 x 10 ⁷	9.8 x 10 ³
Bacteria:	<i>Pseudomonas aeruginosa</i>
<i>Acetobacter sp</i>	<i>Acetobacter</i>
<i>Alcaligenes sp</i>	<i>Citrobacter</i>
<i>Bacillus subtilis</i>	<i>Bacillus subtilis</i>
<i>Bacillus sp</i>	<i>Serratia</i>
<i>Chromatium sp</i>	<i>Flavobacterium sp</i>
<i>Citrobacter sp</i>	<i>Micrococcus sp</i>
<i>Flavobacterium sp</i>	<i>Pseudomonas sp</i>
<i>Micrococcus sp</i>	<i>Alcaligenes sp</i>
<i>Norcadia sp</i>	
<i>Pseudomonas sp</i>	
<i>Serratia sp</i>	
<i>Staphylococcus sp</i>	

Table 5. Crude oil and PAH degrading capabilities of bacteria and yeasts isolated from the mangrove sediment of Iko river estuary

Isolate code	Isolate	Growth on crude oil after 7 days	Growth on crude oil after 14 days	PAH degradability
FFM21	<i>Pseudomonas aeruginosa</i>	+++	+++	+++
FFM24	<i>Acetobacter sp.</i>	++	++	+
FFM25	<i>Citrobacter sp.</i>	++	+	-
FFM26	<i>Bacillus subtilis</i>	+++	+++	+++
FFM29	<i>Serratia sp.</i>	++	+	-
FFM31	<i>Flavobacterium sp.</i>	+	+	-
FFM32	<i>Micrococcus sp.</i>	++	++	++
FFM33	<i>Staphylococcus sp.</i>	-	-	-
FFM34	<i>Pseudomonas sp.</i>	++	++	+
FFM36	<i>Alcaligenes sp.</i>	+++	++	++

Table 6. Growth profile of test organisms on naphthalene supplemented minimal salt medium

Test isolate	Incubation period (days)	Cell count (cfu/ml)	pH	OD	Temp. (°C)
<i>B. subtilis</i>	0 (inoculum)	2.0×10^2	-	-	
	3	3.1×10^4	8.0	0.230	28.5
	6	4.3×10^4	7.6	0.238	28.0
	9	5.0×10^5	7.5	0.340	28.0
	12	6.4×10^6	7.0	0.360	27.5
	15	7.1×10^6	7.2	0.480	27.5
	18	8.0×10^5	7.0	0.490	28.4
	21	9.0×10^4	6.8	0.520	28.0
<i>P. aeruginosa</i>	0 (inoculum)	2.1×10^2	-	-	
	3	2.1×10^3	8.0	0.250	28.0
	6	3.2×10^4	8.0	0.250	27.5
	9	4.8×10^4	7.8	0.350	27.8
	12	5.1×10^5	7.6	0.450	28.0
	15	7.6×10^6	7.6	0.488	27.5
	18	9.0×10^5	7.5	0.520	26.8
	21	8.9×10^5	7.2	0.535	27.5

Table 7. Growth profile of test organisms on anthracene supplemented minimal salt medium

Test isolate	Incubation period (days)	Cell count (cfu/ml)	PH	OD	Temp. (°C)
<i>B. subtilis</i>	0 (inoculum)	2.0×10^3	-	-	
	3	7.4×10^4	8.0	0.220	28.0
	6	8.2×10^4	7.6	0.230	27.5
	9	5.0×10^5	7.4	0.340	27.8
	12	6.8×10^6	7.0	0.355	27.0
	15	7.0×10^5	7.0	0.420	27.2
	18	9.4×10^4	6.8	0.460	28.5
	21	8.2×10^4	6.5	0.470	27.0
<i>P. aeruginosa</i>	0 (inoculum)	2.1×10^2	-	-	
	3	3.6×10^2	8.0	0.225	28.0
	6	4.3×10^3	7.5	0.232	28.5
	9	5.0×10^4	7.2	0.348	28.0
	12	6.1×10^5	7.0	0.400	27.5
	15	7.8×10^5	7.0	0.435	27.0
	18	8.8×10^5	6.5	0.468	26.8
	21	9.6×10^4	6.2	0.472	26.5

3.5 Profile of PAHs Degradability in Estuarine Mudflat

Among the PAH degrading isolates, only *P. aeruginosa* and *B. subtilis* exhibited strong capabilities to utilize PAHs for growth. The capabilities of *P. aeruginosa* to degrade PAHs embedded in mudflats are depicted in Table 8.

3.6 Discussion

The intertidal sediment samples from the Iko River Estuary contained a detectable level of PAHs. The PAHs representing 2–3-, 4-, and 5-

ring PAH groups are presented in Table 1. Among them, phenanthrene, fluoranthene, and pyrene were the most dominant compounds, with mean concentrations of 1.27, 2.48 and 1.46 mg/kgdw respectively which account for about 38.8% of the total PAHs in the mudflat used in this investigation, while benzo(b)fluoranthene, benzo(k)fluoranthene, indeno (1,2,3-cd) pyrene, and benzo (g, h, i) perylene also showed high contribution in the intertidal sediment. Naphthalene and acenaphthalene with a mean concentration of 1.00 mg/kgdw were predominant among the low molecular weight PAHs detected in the mudflat of Iko River Estuary.

Table 8. Summary of PAHs degradability of the test isolates

Parameter	Initial PAH concentration	After 21 days exposure (mg/kg)	
	Control	<i>P. aeruginosa</i>	<i>Bacillus subtilis</i>
Naphthalene	1.00	0.95	1.00
2-methylnaphthalene	0.20	0.20	0.20
Acenaphthylene	1.00	1.00	1.00
Acenaphthene	0.70	0.70	0.70
Flourene	0.70	0.70	0.70
Phenanthrene	0.20	0.20	0.20
Anthracene	0.40	0.35	0.40
Flouranthene	0.20	0.20	0.20
Pyrene	0.48	0.26	0.48
Benzo(a)fluoranthene	0.57	0.48	0.57
Chrysene	0.65	0.60	0.65
Benzo(b)fluoranthene	0.95	0.85	0.95
Benzo(k)fluoranthene	0.45	0.45	0.44
Benzo(a)pyrene	0.20	0.20	0.20
Dibenzo(a,h)anthracene	0.67	0.49	0.66
Benzo(g,h,i)perylene	0.51	0.25	0.51
Indeno(1,2,3-d)pyrene	1.54	1.33	1.00
Total PAH	10.42	9.21	9.56

The observed total PAHs concentration in the sediment may be due to from industrial sources via runoff and/or of biogenic origin. This is despite the higher microbial and worms activities commonly found in the "oxic" epipelagic sediment which normally enhances sediment-mixing activities that simulate PAHs degradation in sediments [22]. Formation of the dibenzo (a,h) anthracene from other PAHs by indigenous microorganism have been reported by Zuberer [23] and may have contributed to the total PAH load of the mudflat.

The isolation of microbes with crude oil and PAH degradability from the mudflat is an indication that tropical mangrove ecosystems have strong capability to recover from hydrocarbons pollution impacts, evidence in support of the process of bio-attenuation in the studied area [24,25]. The recovery of the PAH contaminated ecosystem could also partly be attributed to transformation, sequestration in sediment, and volatilization of low molecular weight PAHs and could be stimulated by high nutrient availability (bio-attenuation) or sediment transport, which may wash out the sediment to the sea [26].

Mangrove sediment provides a unique ecological environment for a wide range of microbial ecology. Bacteria form a good percentage of niches and are of necessity to the survival of their habitats. They are particularly important in controlling the chemical environment of the mangrove ecosystem. The present study has

revealed the rich microbial assemblage of the tidal mudflats samples from Iko River Estuary. Twelve genera of heterotrophic bacteria were isolated from the tidal mudflats. The diverse species of bacteria isolated from the sediments could be attributed to variation in PAHs contents of the estuary. All the bacteria genera isolated from the Iko River Estuary mangrove sediment except *Staphylococcus* sp exhibited the ability to utilize hydrocarbons as carbon and energy source for growth. However, few species of the bacteria isolates could effectively degrade PAHs. The PAHs degraders were *Pseudomonas aeruginosa*, *Acetobacter*, *Bacillus subtilis*, *Micrococcus* sp, *Pseudomonas* sp and *Alcaligenes* sp. Among the PAH-utilizing microorganisms, only *P. aeruginosa* and *B.subtilis* demonstrated strong capabilities to degrade PAHs and were selected for the *ex-situ* and "simulated *in-situ*" PAH-degradation studies.

The 2 & 3 ring PAHs utilization and degradation by the selected mangrove sediment bacteria and yeast from Iko River estuary has been evaluated. Evidence of the ability of the selected PAH degraders to utilize Naphthalene and Anthracene was established using the culture technique. *Pseudomonas aeruginosa* and *Bacillus subtilis* strains were identified as PAHs degraders based on the ability to form clearing zones and subsequent growth on Naphthalene and Anthracene supplemented MSM as sole carbon and energy source. Based on the ability of the isolates to utilize the PAH suite for microbial cell

proliferation within 4-day interval it is apparent that *B. subtilis* and *P. aeruginosa* with an initial microbial cell count of 3.5×10^4 cfu respectively were able to proliferate over time. *Pseudomonas aeruginosa* exhibited a faster initial growth of 5.9×10^6 cfu after 4 days as against 3.8×10^6 cfu/g recorded for *B. subtilis*. This is in agreement with the report of Ekpo and Umoh [27], using the carbon dioxide evolution technique to monitor hydrocarbon biodegradation; they noted that *P. aeruginosa* had the highest cumulative CO₂ production in a bioremediation experiment. It has also been reported that *Pseudomonas* species, because of their ability to degrade a wide range of pollutants, exhibited an increased rate of removal of the pollutant trichloroethylene (TCE) from groundwater [28].

The growth indices (microbial load, pH, temperature and optical densities) of the test isolates in PAHs supplemented minimal salt medium revealed that *Pseudomonas aeruginosa* had the highest growth in the sterilized minimal salt medium supplemented with PAHs. This was followed by *Bacillus subtilis*. The highest growth exhibited by *P. aeruginosa* was surprising not because it was isolated from PAHs polluted environment but also because it is known to possess a more competent and active hydrocarbon-degrading enzyme than other biodegrades [29].

The results of profiles of the naphthalene and anthracene degrading activities of the test organisms revealed that the PAH degraders utilized the PAH suites for growth. On 2-ring PAH (naphthalene) supplemented MSM *Pseudomonas aeruginosa* recorded the highest optical density of 0.535 after 21 days of incubation. This was followed by *Bacillus subtilis* with an attenuation of 0.520, and then *Saccharomyces estuary* with an attenuation of 0.515. When cultured on 3-ring PAH (anthracene) supplemented MSM, *S. estuary* recorded the highest OD of 0.475 after 21 days of incubation. This was followed by *P. aeruginosa* and *B. subtilis* with attenuation levels of 0.472 and 0.470 respectively after incubation for the same period of time.

The pH and temperature of the PAH supplemented medium was affected by the biodegradation process. The naphthalene and anthracene utilization by the organisms resulted not only to growth but also to concomitant production of acidic metabolic products. The acidic metabolites are responsible for the

decreased in the pH of the growth media of the test isolates. In this study pH reductions from 8.0 to 6.5 and 6.2 were observed for *B. subtilis* and *P. aeruginosa* respectively. Similar results have earlier been reported by Itah and Essien [30] on the biodegradation of tarballs by hydrocarbonoclastic bacteria isolated from the Bight of Bonny in Nigeria.

Many microorganisms capable of degrading petroleum components have been isolated. However, few of them seem to be important for petroleum and its PAHs component degradation in natural environments [31]. The activities of the PAH degraders in the simulated mudflat microcosm has shown that elevated PAH concentrations in tidal mudflats and the associated toxic substances including may affect the densities of microorganisms. A similar observation has earlier been reported by Omar et al., [32]. Results of the microbial growth dynamics in contaminated sediments revealed that time was a critical factor determining the effect of PAHs on microbial response. A substantial decrease in PAHs concentration and heterotrophic microbial counts was discovered within 21 days of exposure. The decrease in the number of the heterotrophic microorganisms may not be unconnected to selective toxicity. The decline in microbial counts may be partly due to nutrient exhaustion and or PAH toxicity. The survival of resistant strain and substantial PAH degradation might be responsible for the reduction in the toxic concentration of the sediment. Microbial biodegradation of PAHs in the environment can be very slow because it is influenced by a number of factors which include the population of the hydrocarbon bio degraders, temperature and nutrient availability [33].

4. CONCLUSION

Bacteria are known to utilize hydrocarbons as carbon sources and hydrocarbon-utilizing bacteria have been shown to respond to crude oil pollution. Organic contaminants in terrestrial and aquatic environments persist because they are either unavailable or not accessible to degrading microorganisms or products, or the degrading microorganisms are not able to carry out the necessary catabolic reactions, or the physicochemical environmental conditions are not adequate for degradation or removal. This investigation has revealed that tropical mangrove ecosystems have strong capability to recover from hydrocarbons pollution impacts because of the crude oil and PAH degradability by the

indigenous bacterial assemblage. The high growth rate and wide distribution of HUB in sediments under study is an indication of exposure of the ecosystem to contamination with hydrocarbons. The present study has revealed that Iko River estuary is contaminated with PAHs of more pyrolytic sources than petrogenic sources. The high PAH levels in sediment from Iko River Estuary with the disappearance of PAHs or many PAHs suites in the sediment after treatment with indigenous and strong PAH degrading species of *P. aeruginosa* and *B. subtilis*. The significant PAH degradability of *P. aeruginosa*, *B. subtilis* and *S. estuary* in both *in-situ* and *ex-situ* is a confirmation of their potential use in bioremediation of contaminated sites especially dredging wastes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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