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ORIGINAL RESEARCH

Biodegradation of Escravos light crude oil by three indigenous bacteria, isolated from mechanic workshop in Lagos State, Nigeria

Bolanle Opere, Ahmeed Ashade, Muibat Fashola, Taiwo Dawodu, Yewande Banjo, Adijat Amoda, Haleema Adenle

Department of Microbiology, Lagos State University, Ojo, Lagos, Nigeria

Correspondence
Bolanle Olaitan Opere, Department of Microbiology, Faculty of Science, Lagos State University, Nigeria
Email : bolanle.opere@gmail.com

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Abstract:

Introduction: Petroleum hydrocarbons can be degraded by microorganisms such as bacteria, fungi, yeast and microalgae
Aims: The aim of this study is to isolate hydrocarbon degrading bacteria from hydrocarbon polluted site (mechanic workshops) in Lagos State.
Materials and Methods: The physicochemical and microbiological properties of the three-soil sample were analyzed using standard procedure. Hydrocarbon degraders were isolated on 1% crude oil mineral salt medium via enrichment technique. The best hydrocarbon degraders were identified on the basis of their colonial morphology, biochemical characteristics and complemented with analytical profile index (API) kit. Hydrocarbon degradation were monitored using total viable count and residual oil was determined by Gas Chromatography equipped with Flame Ionized detector (GC-FID).
Results: Alkaline pH were observed for all soil samples. The total nitrogen detected were 0.07, 0.04 and 0.1. The total heterotrophic bacteria (THB) estimated were 3 x 1010, 6.5 x 109 and 1.7 x 1010cfu/ml while the total hydrocarbon utilizing bacteria estimated via vapour phase were 1.89 x 106, 4x104 and 9.62 x 107cfu/ml for Ikeja, Iyana-Iba and Mushin sample. The organisms had a generation time of 5.56, 9.09 and 10.9, the degradation half-life was 3.85, 6.3 and 7.5 respectively. Over 60% hydrocarbon degradation were determined within 10 days for all three isolates. The GC-FID prints show reduction in peak area of various hydrocarbon fractions with a decrease in pH of the medium.
Conclusion: The study showed that hydrocarbon degraders are present in the oil polluted soil. Their degradation potential can be optimized so as curtail the adverse effect of petroleum in the environment.
Keywords: Hydrocarbon, Biodegradation, Bioaccumulation, Environmental pollution and oil spillage.

All co-authors agreed to have their names listed as authors.

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1. INTRODUCTION

Environmental pollution due to petroleum and its product is a major challenge to fight climate change in the world [1]. Petroleum is an essential material for the production of fuel such as diesel, kerosene and gasoline. Other important chemical product derived from petroleum include, pharmaceutical solvent, plastics and fertilizer [2]. Nigeria being an oil producing nation and member of organization of petroleum exporting country (OPEC) generates most of its income from sales of petroleum and petroleum products. Petroleum being an important mineral treasure, due to its high global demand is sometimes referred to as black gold. As a result of global demand activities including exploration, extraction, refining and transportation are carried out [3, 4].

Contamination of natural environment with petroleum may occur due to leakages, spill from oil well, vandalization of pipeline, drilling rigs and accident during transportation of petroleum product. It has been reported globally that the amount of petroleum seepage was estimated to be 600,000 metric tons per year with a range of uncertainty of 200,000 metric tons per year [5]. Environmental contamination with hydrocarbons may cause damages to local system since the contaminants may bioaccumulate within plants and animal tissue leading to death or mutation [6, 7]. Loss of biodiversity can also occur due to environmental contamination with petroleum. Mechanical burying, dispersion, evaporation are technologies use for remediation but quite expensive but lead to incomplete decomposition of contaminants [7]. Bioremediation which is defined as the use of organisms or its products to reduce the concentration of hazardous pollutants in the environment, is considered safe and not too expensive. One of the major mechanisms to remediate environment polluted with petroleum hydrocarbon is by the use of competent microorganism [8, 9 and 10].

Several authors have reported different organism that have the capacity to grow on petroleum hydrocarbon, utilizing it as sole carbon and energy source. Xu *et al.* [11] reported that there are over 79 genera of bacteria capable of degrading crude oil. Some of the reported hydrocarbon degrading bacteria include *Alcanivorax* spp., *Cycloclasticus* spp., *Oleiphilus* spp., *Oleispira* spp., *Thalassolituus* spp., *Planomicrobium* that all utilizes crude oil as sole carbon and energy source.

Head *et al.* [12] also reported different bacterial isolates capable of growing on different saturated hydrocarbon which include *Thalassolituus* spp. *Planomicrobium* spp., *Alcanivorax* spp., *Oleiphilus* spp., *Oleispira* spp., *Alkanoclasticum* MAE2, *Cycloclasticus* spp were equally reported to mineralise polycyclic aromatic hydrocarbon. *Burkholderia-Paraburkholderia*, *Luteibacter*, and *Acinetobacter* that degrade petroleum hydrocarbon under optimal condition were also reported by Rontani and Bonin [13]; Cui *et al.* [14] Obayori *et al.* [15], reported the isolation of *Enterobacter cloaca* strain LG1 and *Burkhoderia cepacia* strain LG2 on weathered crude oil. *Pseudomonas*, *Flavobacterium*, *Achromobacter*, *Alcaligenes, Bacillus*, *Micrococcus*, *Nocardia*, *Corynebacterium*, and *Proteus* are some of the examples of commonly isolated degraders reported in literature [16, 17]. Several genera of fungal such as *Talaromyces*, *Neosartorya*, *Amorphoteca*, and *Graphium. Y*easts namely, *Candida*, *Yarrowia*, and *Pichia* have been isolated from petroleum, contaminated soil and proved to be potential organisms for hydrocarbon degradation [18].

This paper reported the isolation of three Gram negative bacteria with potential to mineralise petroleum hydrocarbon.

2. MATERIAL AND METHODS

Sample collection

Soil samples were collected from hydrocarbon polluted soil (mechanic workshop) at three locations in Lagos State, Nigeria. The sample locations include: Ikeja, Iyana-Iba and Mushin axes of Lagos State. All the mechanic workshops were located within motor parks and had long history of contamination with hydrocarbon contaminants for more than four decades. Composite surface soil samples for microbiological analysis were collected into a sterile universal bottle using hand trowel after clearing the surface debris. Samples for physicochemical analysis were collected into a black polythene bag and transported to the laboratory on ice.

Physicochemical analysis

The physicochemical characteristics of the composite soil samples were determined using standard analytical procedures. The pH was determined using mercury in-glass electrode (ADWA pH meter model 2000) in ratio 1:1 soil-water mixture [19]. Moisture content was determined using hot air oven at 105 ºC, total organic contents were determined using dichromate oxidation as described by Walkley and Black 1934 [19]. Nitrogen content was determined using Kjeldahl method. Total hydrocarbon contents were determined using gravimetric method by refluxing 90 g of the sample in 100 ml of distilled methanol containing 3.0 g of potassium hydroxide (KOH). Other parameters such as electrical conductivity, phosphate, and potassium contents were determined using standard procedures. Heavy metals contents were analysed using atomic absorption spectrophotometry (Perkin-Elmer, Canada)

Microbiological analysis

Total heterotrophic bacteria and fungi count were determined using standard plate count method.

Bacterial enumeration was done by dissolving 10 g of sample in 90 ml of normal saline and serially diluting 1ml of the sample, after which 0.1 ml aliquot were plated on nutrient agar that has been fortified with nystatin. Total hydrocarbon utilizing bacteria (THUB) were determined using vapour phase method described by Raymond *et al.* [20]. The hydrocarbon degrading bacteria were isolated by continuous enrichment technique on mineral salt medium (MSM) described by Kastner *et al.* [21]. The medium contains per litre Na_2HPO_4 (2.13 g), KH_2PO_4 (1.3 g), NH_4Cl (0.5 g), $MgSO_4.7H_2O$ (0.2 g) and sterilized by autoclaving after which 1 ml of trace element solution was filter sterilized separately and added as described by Bauchop and Elsden [22]. Fifty ml of MSM was transferred into 250 ml Erlenmeyer flask and 0.5 ml (1%) of escravos light crude oil was added as the sole carbon and energy source before sterilization. Also, 5 grams of soil sample were added and agitated in the dark using a gyratory shaker at 150 rpm, following visual observation of turbidity and disappearance of hydrocarbon in the flask. The diluent was transferred into a fresh MSM containing crude oil flask. After four consecutive transfer, hydrocarbon degraders were isolated on Luria Bertani agar (LB) and subcultured ones on nutrient agar. Total viable count (TVC) of hydrocarbon degraders were monitored on crude oil mineral salt medium for 10 days and plated out on nutrient agar. Hydrocarbon degradation was monitored using Gas Chromatography equipped with flame ionized detector GC-FID.

Identification of biochemical characteristics of isolates

Hydrocarbon degraders were identified on the basis of their cultural, morphological and biochemical characteristics using the taxonomic scheme of Cowan and Steel [23]. Identifications were further complemented by analytical profile index (API 20 E V4.0 and 20NE V6.0) identification kit system.

Extraction of residual oil

Residual oil was extracted by adding to the culture broth 20 ml of hexane and shaking thoroughly as described by Adebusoye *et al.* [24]. After removing the aqueous phase with separating funnel, the residual oil concentration was determined by gas chromatography.

Analytical method

Hexane extracts (0.1µL) were analyzed with Hewlett Packard 5890 series II gas chromatography equipped with flame ionization detector (FID) and 30m ling HP-5 column (internal diameter, 0.25mm; film thickness, 0.25µm). The carrier gas was nitrogen. The injector and detector temperatures were maintained at 250°C and 350°C, respectively. The column was programmed at an initial temperature of 70°C; this was held for 2 min, then ramped at 10°C/min to 320°C and held for 10 min.

3. RESULTS

The physicochemical and biological characteristics result of the polluted soil samples are shown in **Table 1.** The result revealed that all the three sampled sites had alkaline pH with value 9.22, 8.55 and 8.62 representing Ikeja, Iyana-Iba and Mushin site respectively. Low nitrogen content was detected in all the samples with value 0.07, 0.004 and 0.1 respectively. The phosphorus content detected for Ikeja and Iyana-Iba sample was 7.76 and 8.12 while Mushin sample had 10.79. The total heterotrophic bacteria count obtained was 3.8×10^{10}, 6.5×10^{9} and 1.73×10^{11} cfu/ml while the total hydrocarbon utilising bacteria obtained were 1.8×10^{6}, 4.0×10^{4} and 9.62×10^{7}cfu/ml for Ikeja, Iyana-Iba and Mushin respectively.

Table 1. Physicochemical and biological characteristics of the soil samples

Parameters	Ikeja	Iyana-iba	Mushin
pH	9.22	8.85	8.62
Electrical conductivity (µS/cm)	342.00	372.00	439.00
Total Nitrogen content (%)	0.07	0.04	0.10
Total Organic content (%)	0.80	0.47	1.46
Organic matter (%)	1.50	0.81	1.62
Average percentage phosphorus (mg/kg)	7.76	8.12	10.79
Exchangeable potassium (cmol/kg)	0.30	0.32	0.22
Exchangeable sodium (cmol/kg)	0.39	0.33	0.30
Cation Exchange Capacity (cmol/kg)	12.93	9.81	6.64
Moisture Content (%)	0.52	9.75	2.07
Water holding capacity (g/g)	269.40	281.30	358.60
THB (cfu/ml)	3.8×10^{10}	6.5×10^{9}	1.73×10^{11}
THUB (cfu/ml)	1.89×10^{6}	4.0×10^{4}	9.62×10^{7}

Key: THB: Total heterotrophic bacteria

THUB: Total hydrocarbon utilizing bacteria

The morphological and biochemical characteristics of the isolates are shown in Table 2. Strain HMA2 had a creamy smooth surface, Gram negative rod, catalase and oxidase positive but tested negative to indole production. The isolate was able to reduce nitrate but could not ferment lactose and glucose and was putatively identified as *Alcaligenes faecalis* Strain IA07 with creamy smooth and shining surface. It is a Gram-negative rod, catalase negative, arginine dehydrolase positive, could utilize citrate and does not produce hydrogen sulphide. The isolate could ferment glucose, mannitol and saccharose but could not ferment sorbitol, inositol, and arabinose and were putatively identified as *Aeromonas hydrophilia*. Strain IWY1 had creamy soft translucent surface with big colonies. It is a Gram negative rod, non-glucose and sorbitol fermenter but could ferment mannitol, inositol, and rhamnose and was suggested to be *Enterobacter intermedius.*

Table 2: Cultural and biochemical characteristics of isolates

Biochemical reaction	HMA2	IA07	IWY1
Cultural characteristic	Creamy Soft smooth surface, entire and colonies.	Creamy smooth surface, convex with shining surface	Creamy soft surface with translucent big colonies
Gram reaction	-	-	-
Spore staining	+	+	+
Catalase test	-	-	-
2-Nitrophenyl-BD-Galacto pyranoside		+	-
Arginine Dihydrolase	-	+	+
Lysine Decarboxylase		+	-
Nitrate reduction	+		
Ornithine DeCarboxylase		-	-
Hydrolysis of *B* Galactosidase	-		
Citrate utilization		+	+
H_2S production		-	-
Urease	-	-	-
Mannose	-		
Tryptophane Deaminase		-	-
Indole production	-	+	-
Acetoin production (Voges proskauer)		+	-
N-acetyl Glucosamine	-		
GELatinase	-	+	-
Glucose fermentation	-	+	-
MANnitol	-	+	+
INOsitol		-	+
SORbitol		-	-
Assimilation of maltose	-		
RHAmnose		-	+
SACcharose		+	+
Melibiose		-	+
Potassium gluconate assimilation	+		
AMYgdalin		+	+
ARAbinose	-	-	+
Malate assimilation	+		
Cytochrome oxidase	+	+	-
Probable identity	*Alcaligenes faecalis*	*Aeromonas hydrophilia*	*Enterobacter intermedius*

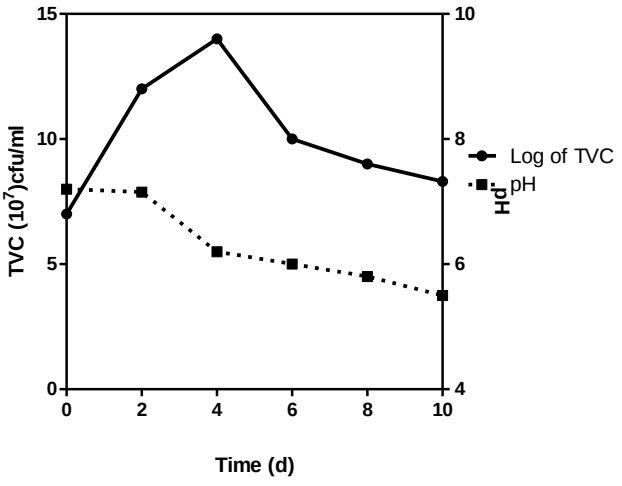


Fig 1. Growth dynamics of *Pseudomonas mandocina* HMA2 on crude oil mineral salt medium"

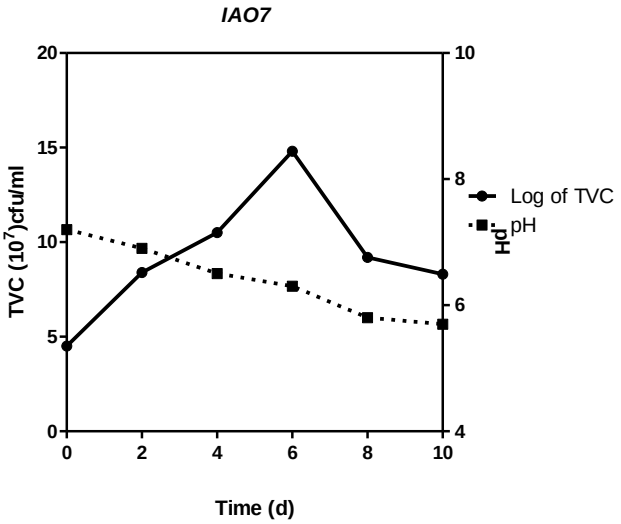


Fig 2. Growth profile of *Aeromonas hydrophilia* IAO7 on crude oil mineral salt medium

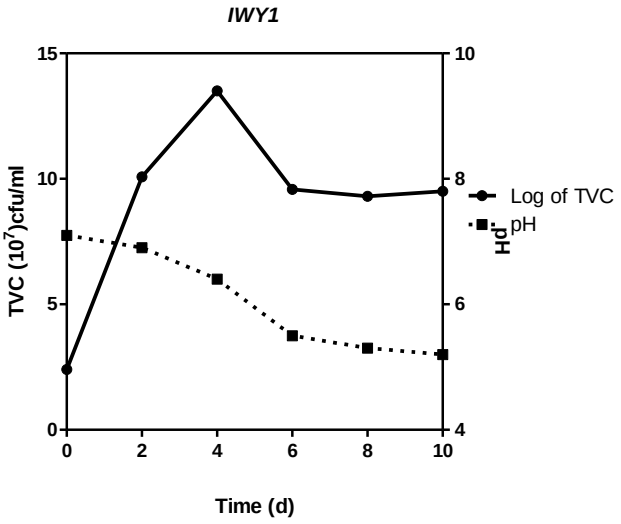


Fig 3. Growth profile of *Enterobacter intermedius* IWY1 on crude oil mineral salt medium"

The kinetics of degradation of hydrocarbon degrading bacteria is shown in Table 3. Degradation was monitored for 10 days. The percentage hydrocarbon degraded by strain HMA2 after 10 days on escravos light crude oil was 65.91 while strain IAO7 and IWY1 had 66.5 and 60.43 respectively. The growth rate for the isolate were 0.18, 0.11 and 0.092 while a mean generation time of 5.56 were calculated for strain HMA2 and 9.09, 10.98 for IAO7 and IWY1 respectively. The degradation half-life was 3.85, 6.3 and 7.53 respectively. The degradation constant depicted by Kd were also calculated as shown in the Table 3.

Table 3: Growth dynamics of isolate on hydrocarbon

Isolate	HMA2	IAO7	IWY1
Parameters			
K(/d)	0.18	0.11	0.092
T(d)	5.56	9.09	10.98
PD %	65.91	66.5	60.43
K_d (/d)	0.12	0.08	0.062
$T_{1/2}$ (d)	3.85	6.30	7.53

Key:

K: growth rate; T: mean generation time; PD: percentage degradation; Kd: degradation constant; $t_{1/2}$: degradation half-life.

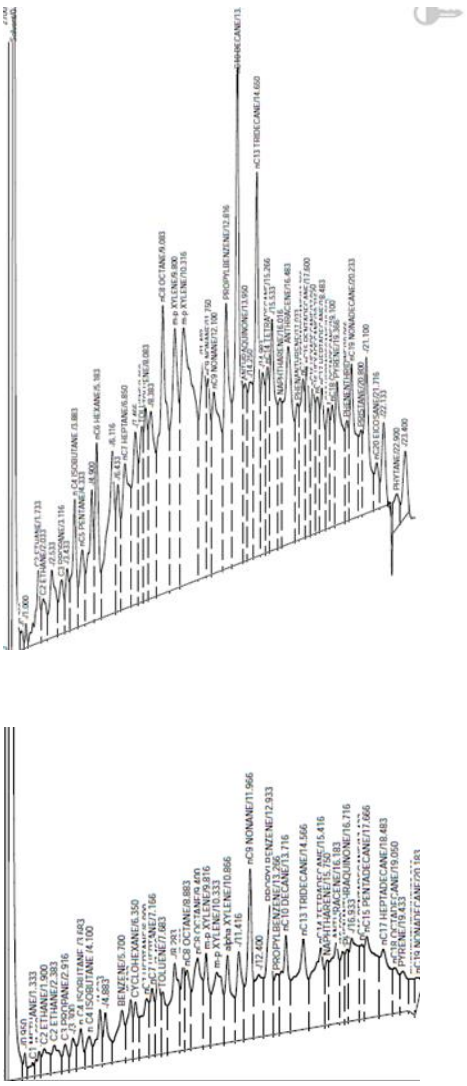
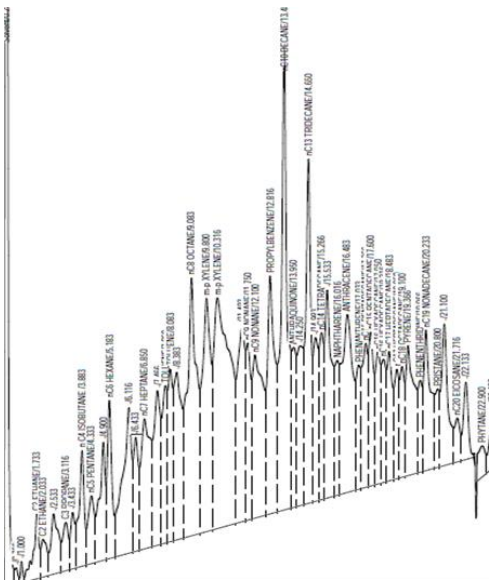


Fig 4a



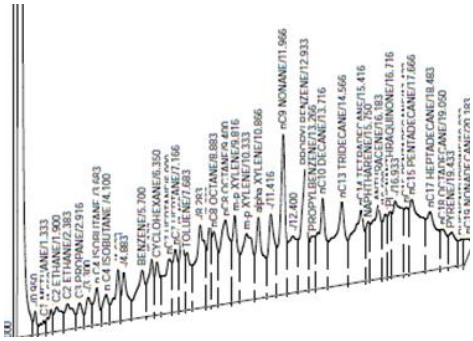


Fig 4b

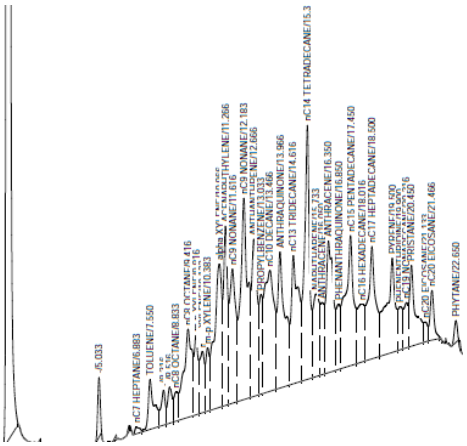
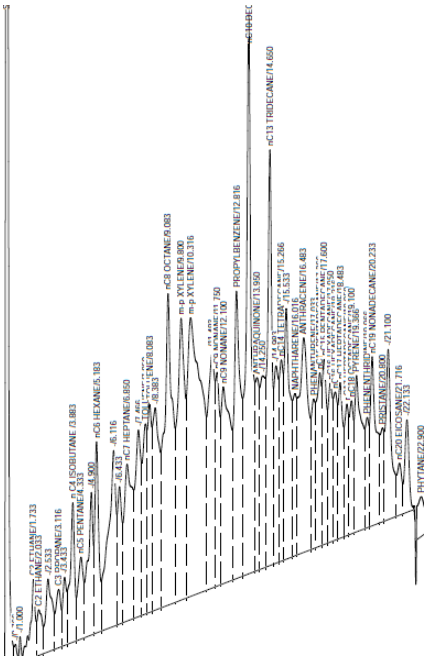


Fig 4c

Figure 4: Gas chromatographic traces of n-hexane extract of recovered fresh crude oil from culture fluids of Strain HMA2 (4a) day 0 and 10, IAO7 (4b) day 0 and 10 and IWY1 (4c) day 0 and 10 on 1% crude oil mineral salt medium and Day 0, 5 and 10 after 10 days of incubation at room temperature. The oil components were separated on 30 m long HP-5 column (internal diameter, 0.25 mm; film thickness, 0.25 µm) in a Hewlett Packard 5890 Series II gas chromatograph equipped with flame ionization detector (FID)



DISCUSSION

Spillage of petroleum compounds into the environment has a negative impact on the ecological system. Continous exposure of microorganisms to this contaminant may trigger certain mechanisms by the indigenous microbes to detoxify the pollutant.

Over time, the population of non-hydrocarbon utilizers may reduce as compared to those bacterial isolate capable of using the hydrocarbon as sole carbon and energy source [25, 26]. The physical, chemical and microbiological characteristics of the polluted soil samples are shown in Table 1. As depicted in the table, the nitrogen content of the samples was low, which may be attributed to the continuous requirement of the macronutrient for protein and nucleic acid synthesis, low potassium content noticed may also be linked to the organism requirement for active cellular respiration and membrane synthesis. The total heterotrophic bacteria, fungi and total hydrocarbon utilizing bacteria were determined. A remarkably difference between total heterotrophic count (THC) and total hydrocarbon utilizing (THUB) bacteria were observed which corroborate the work of Fagade and Oso [27]. The low value of THUB could be due to toxic components of petroleum hydrocarbon which inhibit the growth of other organism that cannot utilize it as carbon and energy source [28]. Isolating these organisms from the hydrocarbon polluted soil point to the fact that they could survive the unfavorable condition and utilizing the contaminant as carbon and energy [26].

Presence of oil degrading bacteria and fungi from the hydrocarbon polluted soil signify that the indigenous microbes were carrying out metabolic activities that may lead to bioremediation of the environment [29]. The hydrocarbon degraders isolated via enrichment technique in this study were *Alcaligenes faecalis* strain HMA2, *Aeromonas hydrophilia* IAO7 and *Enterobacter intermedius* IWY1 (Table 2). Isolating these organisms is not surprising because of their broad enzymatic activities towards mineralization of petroleum contaminant. Obayori *et al.* [30]; Chikere and Ekwuabu [31] Ogbonna *et al.* [32] reported species of *Pseudomonas*, *Bacillus*, *Alcaligenes*, *Acinetobacter*, *Flavobacterium*, *Micrococcus* and *Corynebacterium* to have crude oil degradative capability and have been isolated from hydrocarbon polluted soils. Akani and Obire [33], also reported *Staphylococcus*, *Chryseobacterium*, *Enterobacter*, *Klebsiella*, *Proteus*, *Serratia* and *Escherichia* to also have been associated with hydrocarbon degradation.

The growth curve and dynamics of the isolates on crude oil are shown in Figure 1 and Table 3. The growth dynamics increase observed is similar to that obtained by Obayori *et al.* [34] in their study of degradation of weathered crude oil by *Pseudomonas* strain. The growth dynamics may be attributed to constitutive nature of hydrocarbon assimilating capabability of the organism or could be as a result of previous exposure to the hydrocarbon contaminant leading to its utilization as carbon and energy source [35]. The generation time (T) of the isolates over 10 days on crude oil mineral salt medium were 5.56, 9.09, 10.98 and degradation half-life ($T_{1/2}$) were 3.85, 6.3 and 7.53 respectively for strain HMA2, IAO7 and IWY1. The percentage hydrocarbon degraded calculated over 10 days incubation at 28±2ºC were above 60 percent for all isolates which signifies the isolates were good candidates for bioremediation experiment.

The reduction in the peak areas of the various hydrocarbon fractions observed (Figure 2) could be due to rapid utilization of the contaminant as sole carbon and energy source as observed in the differences between the chromatographic prints over 10 days incubation period. It is noteworthy to state that a gradual reduction in the pH of the crude oil mineral salt medium were observed over 10 days of continuous shaking in the gyratory shaker. The reduction in the pH could be due to organic acid production by hydrocarbon degrading bacteria thus accounting for reduction in the pH level [36, 37]. We have reported the isolation of three bacteria with strong degradation potential for petroleum hydrocarbon from Lagos State, Nigeria.

5. CONCLUSION

Three hydrocarbon degraders were isolated using continuous enrichment technique on escravos light crude oil. They were putatively identified as *Alcaligenes faecalis*, *Aeromonas hydrophilia* and *Enterobacter intermedius* using biochemical test complemented with analytical profile index. They exhibited degradation capacity towards mineralization of crude oil. Hydrocarbon degradation were monitored using GC-FID and it revealed reduction in various hydrocarbon peak fractions.

Competing interest

No conflict of interest declared

Authors' contribution

BO conceived the idea, experimental design, overseeing of execution of experiment and manuscript preparation, AA participated in the overseeing the execution of experiment, wrote the methodology, result and discussion section, collation and interpretation of data. MF participated in experimental design, experimentation and manuscript preparation. TD participated in data collection and writing of the introduction section. YB participated in sample collection and experimentation. AA participated in sample collection and experimentation. AH participated in sample collection and experimentation.

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REFERENCES

1. Waikhom D, Ngasotter S, Devi LS, Devi MS, Singh AS. Role of Microbes in Petroleum Hydrocarbon Degradation in the Aquatic Environment: A Rev. Internatl. J. Curr. Microbiol. Appl. Sci.2020;9 (5): 2319-7706
2. Hedlund BP, Staley J T. *Vibrio cyclotrophicus* sp. nov., a polycyclic aromatic hydrocarbon (PAH)-degrading marine bacterium. *Int J Syst Evol Microbiol*. 2001; 51:61–66
3. Poland JS, Riddle MJ, Zeeb BA.Contaminants in the Arctic and the Antarctic: a comparison of sources, impacts, and remediation options. *Polar Record*. 2003; 39:369–383
4. Obayori OS, Ilori MO, Adebusoye, SA, Amund OO, Oyetibo, GO. Microbial population changes in tropical agricultural soil experimentally contaminated with crude petroleum. African Journal of Biotechnology. 2008; 7 (24): 4512-452
5. Kvenvolden K A, Cooper CK. "Natural seepage of crude oil into the marine environment," Geo-Marine Letters.2003; 23: (3-4) 140–146.
6. Alvarez PJJ, Vogel TM, Substrateinteractionsof benzene, toluene, and para-xylene during microbial degradation by pure cultures and mixed culture aquifer slurries," Applied and Environmental Microbiology. 1991; 57:10 2981– 2985
7. Das N, Chandran, P. Microbial degradation of petroleum hydrocarbon contaminants: an overview. *Biotechnol res. Intl.*2011; 1-13.
8. Amund OO, Nwokoye N. "Hydrocarbon potentials of yeast isolates from a polluted Lagoon," *Journal of Sci. Res. Develop.*1993; 1: 65–68.
9. Lal B, Khanna S. "Degradation of crude oil by Acinetobacter calcoaceticus and Alcaligenes odorans," *J. Appl. Bacteriol*. 1996; 81(4): 355–362
10. Obayori OS, Salam LB. Ogunmakinwa OO Ogunleye, MA. Degradation of crude oil (Escravos light) by Pseudomonas strains isolated from poultry droppings and cow dung. *J. Res. Rev. Sci.* – JRRS, Lagos State University 2018.
11. Xu X, Liu W, Tian S, Wang W, Qi Q, Jiang P, Gao, X, Li F, Li H, Yu H. Petroleum hydrocarbon degrading bacteria for the remediation of oil pollution under aerobic conditions: a perspective analysis. *Frontiers in Microbiology*. 2018; 9:1-11.
12. Head IM, Jones DM, Röling, WF Marine microorganisms make a meal of oil. *Nature Rev Microbiol.*2006;.4(3): 173-182.
13. Rontani, JF, Bonin P. Production of pristane and phytane in the marine environment: role of prokaryotes. *Res in microbiol,*2011;162(9): 923- 933.
14. Cui J, Chen H, Sun M, Wen J. Comparison of bacterial community structure and function under different petroleum hydrocarbon degradation conditions. *Bioprocess and Biosystems Engineering,*2020; 43(2): 303-313.
15. Obayori OS, Salam, LB, Omotoyo IM. Degradation of weathered crude oil (Escravos Light) by bacterial strains from hydrocarbons-polluted site. *Afri. J. Microbiol. Res.*2012; 6(26): 5426-5432 DOI: 10.5897/AJMR10.026
16. Atlas RM. Petroleum Microbiology. In: J Lederberg (ed) Encyclopedia of Microbiology. Academic Press, Baltimore. pp.1992; 363- 369
17. Okoh AI, Trejo-Hernandez M. Remediation of petroleum hydrocarbon polluted systems: exploiting the bioremediation strategies.2006;(5):2520-2525
18. Singh, H. Mycoremediation: Fungal Bioremediation, Wiley-Interscience, New York, NY, USA, 2006
19. Walkley A, Black, I.A. An examination of Degtjareff method for determining soil organic matter, and proposed modification of the chromic acid tritation method. *Soil Science*. 1934; 37:29-38.
20. Raymond, RL, Hudson JO, Jamison VW. Oil degradation in soil. *Appl Environ Microbiol.*1976;31:522–53.
21. Kastner, M, Breuer-Jammali, Mahro B. Enumeration and characterisation of the soil microflora from hydrocarbon- contaminated soil sites able to mineralise polyclic aromatic hydrocarbons. Appl. Microbiol. Biotechnol. 1994;41: 267–273.
22. Bauchop T, Elsden SR. The growth of microorganisms in relation to their energy. *J GenMicrobiol*.1960; 23:457-459.
23. Barrow GI, Feltham RKA (1995). Cowan and Steel's manual for identification of medical bacteria, 3rd edn. Cambridge University, Cambridge.
24. Adebusoye SA, Ilori MO, Amund OO, Teniola OD, Olatope SO. Microbial degradation of petroleum hydrocarbons in a polluted tropical stream. *World J. Microbiol. Biotechnol.*2007;23: 1149-1159.
25. Okerentugba PO, Ezeronye OU. Petroleum degrading potentials of single and mixed microbial cultures isolated from rivers and

refinery effluent in Nigeria. *Afr. J. Biotechnol.*2003. 2(9): 288 – 292.
26. Boboye B, Olukunle, OF, Adetuyi FC. Degradative activity of bacteria isolated from hydrocarbon-polluted site in Ilaje, Ondo State, Nigeria. Afr. J. of Microbiol. Res. 2010; 4(23): 2484-2491
27. Fagade, OE, Oso BA. Bacteria species diversity in three crude-oil-impacted water-bodies in the Niger Delta of Nigeria. *J. Sci. Res.*2000; 6: 20-24.
28. Labud V, Garcia C. Hernandez T. Effect of hydrocarbon pollution on the microbial properties of a sandy and a clay soil. *Chemosphere*2007; 66: 1863-1871.
29. Ojo, OA. Petroleum- utilization by native bacterial population from a wastewater carnal Southwest Nigeria. *Afr. J. Biotechnol.* 2006;5(4): 333- 337.
30. Obayori OS, Salam LB, Omotoyo IM. Degradation of weathered crude oil (Escravos Light) by bacterial strains from hydrocarbons-polluted site. *Afri. J. Microbiol. Res.*2012; 6(26): 5426-5432 DOI: 10.5897/AJMR10.026
31. Chikere CB, Ekwuabu CB.Molecular characterization of autochthonous hydrocarbon utilizing bacteria in oil- polluted sites at Bodo Community, Ogoni land, Niger Delta, Nigeria. *Nig. J. Biotechnol.*2014; 27:28–33.
32. Ogbonna DN, Nrior RR, Ezinwo FE. Bioremediation efficiency of *Bacillusamyloliquefaciens* and *Pseudomonasaeruginosa* with nutrient amendment on crude oil *polluted soil. Microbiol. Res. J. Intl.*2019; 29(5):1-13.
33. Akani NP ObireO. Bacterial population of Clarias gariepinus (Burchell 1822) exposed to an oilfield wastewater in Rivers State, Nigeria. *Asian J. Biol. Sci.*2014; 7(5):208-216.
34. *Obayori OS, Salam, LB,Omotoyo IM.*Degradation of weathered crude oil (Escravos Light) by bacterial strains from hydrocarbons-polluted site. *Afri. J. Microbiol. Res.*2012.6(26): 5426-5432 DOI: 10.5897/AJMR10.026
35. Adebusoye, SA, Ilori, MO, Amund OO. Teniola OD. Olatope SO. Microbial degradation of petroleum hydrocarbons in a polluted tropical stream. *World J. Microbiol. Biotechnol.*2007; 23: 1149-1159.
36. Amund OO. Adebiyi AG. Effect of viscosity on the biodegradability of automotive lubricating oils. *Tribol. Intern.*1991; (24): 235-237.
37. Okpokwasili GC, James WA. Microbial contamination of kerosene, gasoline, and crude oil and their spoilage potentials. Material. *u. Organismen.* 1995; (29): 147-156.