Original Research Article

In Vitro Degradation and Transformation of aromatic hydrocarbons by Marine Bacteria Isolated from Contaminated Marine Environments of Niger Delta

ABSTRACT

Aims: To determine the *in vitro* degradation and transformation of aromatic hydrocarbons by marine bacteria isolated from contaminated marine environments of Niger Delta.
Study Design: Nine treatments and the controls designs were set up in triplicates containing 100 mL of sterile modified mineral basal medium in 500 mL conical flasks supplemented with 1 mg /L of xylene, anthracene and pyrene each; nine marine hydrocarbon degraders and incubated at 24 °C for 24 days study. The nine treatments and control set ups designated as ANT1, XYL2, PYR3, ANT4, PYR5, ANT6, XYL7, XYL8, PYR9 and CTRL (Without hydrocarbons) were used to determine the aromatic hydrocarbons degradability and transformation by the marine bacteria.
Place and Duration of Study: Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University, Uli Nigeria between September, 2014 to March, 2017.
Methodology: A laboratory scale study was carried on six composite samples of the sediment and water samples from the three studied areas using enrichment, screening, selection, morphological, biochemical, degradation and TLC techniques.
Results: The findings revealed that the three sampling sites harbour a lot of efficient aromatic degrading bacterial strains belonging to the genera: *Providencia, Alcaligenes, Brevundimonas, Myroides, Serratia, and Bacillus* able to significantly (*P* = .05) tolerate and grow on the aromatic hydrocarbons. The bacterial strains especially *Serratia marcescens* XYL7 significantly (*P* = .05) removed 99.50 ± 0.05 % and 60.00 ± 0.02 % in weights of xylene and pyrene, respectively while *Alcaligenes faecalis* PYR5 significantly (*P* =.05) degraded 97.40 ± 0.01 % in weight of anthracene. TLC result revealed evidences of very small low intensity spots visualized under UV illumination at 235 nm of test samples compare to control samples with large high intensity spots.
Conclusion: Thus, the excellent degradative abilities of these bacterial strains especially

Keywords: Aromatic hydrocarbons, aquatic pollution, marine bacteria, bioremediation, Niger Delta.

1. INTRODUCTION

Exploration and production of crude oil in Nigeria is carried out in the oil-rich Niger Delta region. Over 80% of the country's oil comes from this ecological zone and its surrounding offshore areas. Within the Delta, the numerous oil fields, tank farms, flow stations, pipelines, tankers and loading jetties constantly provide potential sources of oil pollution [1].

Large scale pollutions of both the terrestrial and aquatic environment in the area, consequent on activities of the oil industry have been documented [2, 3, 4]. It is therefore important to assess all remediation options on the basis of their ability to remove organic contaminants successfully. This is because most of these product especially the polycyclic aromatic hydrocarbons (PAHs), benzene, toluene, and xylene (BTX) are toxic, mutagenic and carcinogenic [5]. The physical and chemical methods like volatilization, photooxidation, chemical oxidation, and bioaccumulation are rarely successful in rapid removal and cleaning up and also these methods are not safe and cost effective when compared to microbial bioremediation [6]. A better way is to use biodegradation.

Thus, much research has focused on the biological degradation of aromatics hydrocarbons (Ahs) through metabolism and co-metabolism. The degradation process involves enzyme machinery: dehalogenases, dehydrogenases, oxygenases and hydrolases system [7]. Bioremediation is a cost-effective and sustainable biotechnology for the treatment of contaminated coastal and marine sites [8]. Over twenty genera of bacteria of marine origin have been documented to be hydrocarbon degrading. Bacteria belonging to subphyla α -, β -, and δ - proteobacteria are well established to be of such nature [6]. The bacterial genera include *Pseudomonas, Serratia, Marinobacter, Providencia, Alcaligenes, Pseudomonas, Salmonella, Nocardia, Mycobacterium, Cunninghamella, Rhodococcus, Beijerinckia, Lysinibacillus, Corynebacterium, Diaphorobacter, Pseudoxanthomonas, Bacillus* and *Sphingomonas* have been found highly capable of degrading xylene, anthracene and pyrene hydrocarbons and well documented [9,10,11,12,13,14].

Microbiological activity is affected by many environmental factors including energy source, donors and acceptors of electrons, nutrients, pH, and temperature. These parameters influence how quickly microorganisms adapt to the environment. Hydrocarbon degradation by microbial population in natural environment is influenced by physical, chemical and biological factors that contribute to the degradation of petroleum and individual hydrocarbons [15]. Several studies have reported that the growth rates of aromatic hydrocarbon degrading bacteria were affected by the concentration of xylene compounds,

temperature, pH of the medium and other factors [16]. Irshaid and Jacob [16] reported that the growth rate of isolates X1 to X4 were less when the temperature was reduced from 30 - 25 °C, whereas, at 45 °C, the growth rate almost completely ceased. The growth rate was higher at pH 6.5 than at pH 5.5 or 8.5. The shortest generation times were found to be 8 hrs for *Bacillus firmus*, followed by 9 hrs for *Pseudomonas stutzeri*, 10 hrs for *Citrobacter amalonaticus* and 11 hrs for *Pseudomonas aeruginosa* under 1 % m-xylene at 30 °C and pH 6.8.

The increased growth in urbanization, industrialization and intensive navigation in the Niger Delta will definitely lead to increases in production, use, discharge and bioaccumulation of aromatic hydrocarbons in soil and groundwater with their concomitant health impacts/risks on humans and animals population through food chain link. These facts prompted us to search for the presence of aromatic degrading microorganisms in in crude oil - impacted Niger Delta aquatic ecosystem in particular for xylene, anthracene and pyrene degraders as better options to physical and chemical agents owing to greater advantages over them. In this work, we report the *in vitro* degradation and transformation of aromatic hydrocarbons by marine bacteria isolated from contaminated marine environments of Niger Delta, Nigeria.

2. MATERIALS AND METHODS

2.1 Description of the Sampling Sites

The studied areas were Abonema Wharf Water Front in Akuku-Toru Local Government Area, Nembe Water-side in Port Harcourt Local Government Area and Onne Light Flow Terminal Seaport located in Eleme Local Government Area of Rivers State. Abonema town is 53 km and Abonema Wharf Water Front is 3 - 5 km from Port Harcourt capital city; Nembe water side is located within Port Harcourt capital city of Rivers State, while Onne Light Flow Terminal is about 35 km east from Port Harcourt capital city of Rivers State and 7 km from Onne town. These sites were geo - referenced using Handheld Global Positioning System (GPS) GPSMAP 76 sc with the coordinates obtained from the sampling points or positions Abonema Wharf Water Front, Nembe Water-side and Onne Light Flow Terminal Seaport were located between latitude 4°46'15.82"N to latitude 4°46'38.01"N and longitude 7°0'0.54"E to longitude 7°0'34.82"E

with average elevation of 4.1 m , latitude 4°45'8.72"N to latitude 4°45'26.42"N and longitude 7°1'11.37"E to longitude 7° 2'14.54"E with average elevation of 2.7 m and latitude 4°41'32.58"N and 4°41'58.18"N and longitude 7°9'26.34"E and 7°10'48.82"E with average elevation of 2.3 m, respectively. These water - ways are subjected to human - induced pressures resulting from urbanization, industrialization and intensive navigation. Abonema Wharf Water Front community is a popular and busy commercial but dangerous jetty area close to Portharcourt city inhabiting tens of thousands of different families living close to petroleum tank farms and tankers queue up daily to load refined petroleum products. Nembe Waterside is situated very close to Creek road market, Port Harcourt, Nigeria. It shares boundary with Bayelsa and links Port Harcourt city with Bonny Island where most of the oil installations in Rivers State are. It also links the Island directly with the Atlantic ocean through which crude oil is exported by massive oil tankers [1]. Onne Light Flow Terminal Seaport is a port of Nigeria and the largest oil and gas free zone in the world supporting exploration and production for Nigerian activities. It is situated on the Bonny River Estuary along Ogu creek and account for over 65 % of the export cargo through the Nigerian Sea Port.

2.2 Sample Collection and Processing

Ten samples were collected randomly at each designated points in the three particular sampling sites (Figures 1, 2 and 3) and mixed together after which a total of six representative sediment and water samples were taken for the analysis. The surface aerobic sediment samples were collected with a 95 % ethanol - sanitized plastic spatula at 5 cm depth inside 95 % ethanol - sanitized wide mouthed plastic containers. The water samples were collected at the air-water interface by hand dipping the 95 % ethanol - sanitized cylindrical shaped 2 L plastic containers. The containers were rinsed with the sediment and water samples before collecting the samples. All the composite or representative sediment and water samples containers were placed into a sterile polythene bag and then transported to the laboratory for microbiological analyses [3, 17, 18].

2.3 Enrichment, Culturing and Isolation of Aromatic Hydrocarbon Bacterial Strains

The hydrocarbon degraders were isolated from sediment and water samples of the three sampling sites using modified mineral basal agar (4 g K_2HPO_4, 1.0 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4$, 1.8 g KH_2PO_4, 0.1 g

$FeSO_4$, 0.1 g NaCl, 0.2 g $CaCl_2$, 15 g Agar agar and distilled water 1,000 mL at pH 7.00 ± 0.20) enriched with xylene, anthracene and pyrene as sole carbon and energy source. The medium was sterilized by autoclaving at 121 $^{\circ}$C and 15 psi^2 for 15 minutes. Thereafter, 0.2 mL acetone solution containing 0.1 % w/v of the selected hydrocarbons (xylene, anthracene and pyrene) were aseptically pipetted and uniformly spreaded on the agar surface of the pre - dried Petri dish plates. The acetone was allowed to evaporate under sterile condition and 0.1 mL aliquots of the 10^{-3} dilutions were spread plated on the surfaces of the solidified media with the aid of a glass spreader. The spreader was sterilized after each successive spreading by dipping it in 70 % ethanol and then passing it through flame of a Bunsen burner. The inoculated plates were sealed using adhesive tape and foil to prevent contamination and photolysis and later placed in black polythene bags, and then incubated in the dark at 28.00 ± 0.20 °C for 14 days [15,18].

2.4 Purification and Maintenance of Cultures

Colonies that developed on hydrocarbon - coated plates were replicated onto fresh hydrocarbon - coated agar plates and incubated for 14 days. Isolates that grew on these plates were selected as xylene, anthracene and pyrene degraders and sub - cultured on Bjou bottles where they are preserved at 4 °C in refrigerator [18].

2.5 Screening and Selection Test

In order to screen and select the best and strongest degrading strains, growth of the different organisms were tested by growing 5 mL of each desired isolates in large test tubes containing 25 mL of the modified mineral basal medium with 100 mg /L of xylene, anthracene and pyrene hydrocarbons which were dissolved in acetone and added to each tube after autoclaving. Thereafter, the test tubes were incubated at room temperature (28.00 ± 2.00 °C) for five days. Bacteria that started growing fast with high turbidity in the vicinity of the medium containing aromatic compounds measured at 600 nm using a UV - VIS spectrophotometer (Astell, UV - Vis Grating, 752 W) were selected as the candidate of xylene, anthracene and pyrene degrading bacteria [18,19, 20].

2.6 Characterization and identification of selected hydrocarbon utilizing bacterial isolates

2.6.1 Morphological characteristics

2.6.1.1 Colonial morphology

After sub - culturing and incubation, culturing morphological properties such as shape, elevation, margin, optic, texture, colour, size and surface characteristics of the selected bacterial strains were observed and noted [21].

2.6.1.2 Microscopic morphology

The standard methods of Gram staining and endospore staining were carried out on the selected bacterial strains as described in Cheesbrough [22] and Health Protection Agency [23].

2.6.2 Biochemical characteristics

The standard methods of testing for catalase, indole, motility, methyl red – Voges Proskauer, citrate, urease, starch hydrolysis, gelatin, nitrate reduction, coagulase, Hydrogen sulphide production, Sugar fermentation, Oxidase and Casein hydrolysis tests were carried out as described in Willey *et al*. [21] and Cheesbrough [22].

2.6.3 Identification of the marine bacterial isolates

Following morphological and biochemical characterization, the isolates were identified using Bergey's manual for determinative bacteriology, as described in Holt *et al.* [24].

2.7 Degradation Assay

Following the methods of Bennet *et al*. [25] and John and Okpokwasili [15] as modified in this study, the degradation rates of bacterial isolates were determined using hydrocarbon supplemented modified mineral basal medium (4 g K_2HPO_4, 1.0 g $(NH_4)2SO_4$, 0.1 g $MgSO_4$, 1.8 g KH_2PO_4, 0.1 g $FeSO_4$, 0.1 g NaCl, 0.2 g $CaCl_2$, 15 g Agar agar and distilled water 1,000 mL at pH 7.00 ± 0.20). Precisely, 1 ml of 48 hrs old cultures of each bacteria was introduced into 28 sterile 200 ml capacity conical flasks (4 sets of 7 flasks) in triplicates containing 100 ml of sterile modified mineral basal medium supplemented with 100

mg /L of xylene, anthracene and pyrene hydrocarbons respectively as source of carbon at 24 °C for 24 days. During incubation, representative samples from the four sets of flasks were withdrawn at intervals of 0, 4, 8, 12, 16, 20 and 24 days and the temperature, pH and optical densities ($OD_{600\ nm}$) of the media in different flasks were measured. The residual hydrocarbons were determined spectrophotometrically using ethyl acetate as the extraction solvent. For each sample, 5 ml ethyl acetate was added and vigorously shaken manually. The organic and aqueous layers from the media were separated by centrifugation at 5000 rpm for 20 mins. The aqueous layers were discarded while the organic layers were analyzed with UV - VIS spectrophotometer at 240 nm wavelength (Astell UV - Vis Grating, 752 W). The percentages of biodegradation of the hydrocarbons were determined as follows:

$$\% \text{ degradation } = \frac{a - b}{a} \times \frac{100}{1}$$

Where a = the absorbance of the medium before incubation; b is the maximum absorbance of the medium after each 4th day of the incubation period.

2.8 Instrumental Analysis for the Identification of the Degraded Products

2.8.1 Thin layer chromatographic (TLC) analysis

This was carried out using the method of Bennet *et al*. [25] as modified in this study. On a clean glass chamber, mixture of hexane/benzene: methanol at 25 ml and 5 ml proportion was prepared as solvent system for the chromatogram. One microliter (1 µl) of the organic phase was spotted and marked at the lower portion of the TLC plate. Similarly, control spots of xylene, anthracene and pyrene solutions with medium and standard hydrocarbons were placed. Plates were placed inside the glass chamber and were again covered. Once the solvent front reached the top layer of the plate, the developed chromatogram was removed and dried naturally on ambient temperature. It was then visualized first with UV light (235 nm) and lastly with iodine crystals. Test and control samples were compared from one another following the criteria of light intensity and spot sizes as a clear proof of biodegradation loss of some hydrocarbon components.

2.9 Data Analysis

The data were analyzed using Graph-Pad Prism statistical software version 7.00 (GraphPad software Inc. San Diego, California). All values were expressed as mean ± standard deviation. Ordinary one-way analysis of variance (ANOVA) followed by post Tukey's, multiple comparison test was performed on the data obtained. The results were considered statistically significant if the probability is less than .05 (P = .05) [19, 26].

3. RESULTS

3.1 Isolation and Selection Test

The result of the growth performance (OD_{600} nm) of the aromatic hydrocarbon-degraders isolated from Abonema sampled location is presented in Table 1. From the result, 13 isolates were obtained with strains XYL2, ANT4 and PYR3 having the highest significant *(*P = .05) absorbance values of 0.952 ± 0.004, 0.775 ± 0.007 and 1.041 ± 0.008 on xylene, anthracene and pyrene hydrocarbons. The result of the growth performance (OD_{600} nm) of the aromatic hydrocarbon-degraders isolated from Nembe sampled location is presented in Table 2. From the result, 17 isolates were obtained with strains XYL7, ANT1 and PYR5 having the highest significant (P = .05) absorbance values of 1.055 ± 0.002, 0.816 ± 0.007 and 0.933 ± 0.007 on xylene, anthracene and pyrene hydrocarbons. The result of the growth performance (OD_{600} nm) of the aromatic hydrocarbon-degraders isolated from Onne sampled location is presented in Table 3. From the result, 18 isolates were obtained with strains XYL8, ANT6 and PYR9 having the highest significant (P = .05) absorbance values of 0.741 ± 0.007, 1.433 ± 0.013 and 0.871 ± 0.001 on xylene, anthracene and pyrene hydrocarbons. On the basis of these results, strains ANT1, XYL2, PYR3, ANT4, PYR5, ANT6, XYL7, XYL8 and PYR9 were selected as the best and strongest degraders of xylene, anthracene and pyrene hydrocarbons.

3.2 Morphological and Biochemical Characteristics of Bacterial Isolates

The result of the colonial morphology of aromatic hydrocarbon degrading bacteria is presented in Table 4. From the result, most colonies were circular and irregular in shape, flat in elevation, undulate in margin, translucent in optic, smooth in texture, creamy in colour, 4 mm in size and glistering in surface description.

The result of the morphological and biochemical properties of the aromatic hydrocarbon degrading bacterial isolates is presented in Table 5. From the result, most bacterial isolates were Gram negative in Gram reaction, rod shaped arranged in single or pair, negative to spore, indole, methyl red, Voges Proskauer, urease, gelatin, nitrate reduction, coagulase, hydrogen sulphide production, xylose, lactose, arabinose, maltose and casein hydrolysis tests while positive to catalase, motility, citrate, starch hydrolysis, mannitol, glucose, sucrose, saccharose and oxidase tests.

3.3 Degradation Assay

The result of the changes in temperature (°C), pH and optical density (OD_{600} nm) of medium during aromatic hydrocarbons degradation by marine bacterial isolates are presented in Tables 6, 7 and 8 while the results of the weight losses from xylene, anthracene and pyrene resulting from the growth of marine bacterial isolates are presented in Tables 9, 10 and 11. From the Tables 6- 8 results, temperature fluctuated between 25.5 and 26 °C for xylene and anthracene; and between 25 and 26 °C for pyrene respectively while the control had 26 °C throughout the 24 days' period of the study. The pH fluctuated between 6.85 and 7.15 for xylene; 6.97 and 7.11 for anthracene; 6.95 and 7.19 for pyrene respectively. The pH remained almost neutral (7.05 – 7.00) in the control. The pH also changed a little within the 24 days' period of the study.

The optical density (OD_{600} nm) increased ranging from 0.01 – 0.35 for xylene; 0.11 – 0.36 for anthracene and 0.17 – 0.54 for pyrene respectively as well as ranged from 0.10 – 0.15 in the control. The optical density (OD_{600} nm) increase from 0.01 – 0.54 within the 24 days' period of the study. Similarly, from the Tables 9 - 11 results, *Serratia marcescens* XYL7 exhibited the highest capabilities to degrade the aromatic hydrocarbons with 99.50 ± 0.05 % and 60.00 ± 0.02 % in weights of xylene and pyrene respectively while *Alcaligenes faecalis* PYR5 degraded anthracene with 97.40 ± 0.01 % reduction in weight after 24 days biodegradation study.

3.4 Analysis of Degraded Products

The results of the thin layer chromatograms of xylene, anthracene and pyrene hydrocarbon degradations in the control set-up and highest degraders are shown in Plates 1a – b, 2a – b and 3a – b. The results

revealed that the control set ups possess large high intensity spots while the highest degraders *Serratia marcescens* XYL7 and *Alcaligenes faecalis* PYR5 had small low intensity spots when visualized under UV illuminator at 235 nm. The result of the retention factor of the aromatic hydrocarbons degraded by marine isolates as presented in Table 12 ranged from 0.27 ± 0.08 – 0.84 ± 0.01, 0.74 ± 0.08 – 1.00 ± 0.12 and 0.06 ± 0.03 – 1.00 ± 0.12 compared to controls which were 0.5 ± 0.01, 0.63 ± 0.02 and 0.38 ± 0.01 for xylene, anthracene and pyrene hydrocarbons.

4. DISCUSSION

The application of microbial bioremediation as a cost effective and eco-friendly treatment tool for the cleaning of certain oil -contaminated estuaries, shoreline, seas and oceans has received much attention since mechanical, physical and chemical treatments have limited effectiveness [8]. Many of the microorganisms proposed for biodegradation and bioremediation have been isolated from contaminated soils and waters [13].

In this study, a total of nine (9) isolates ANT1, XYL2, PYR3, ANT4, PYR5, ANT6, XYL7, XYL8 and PYR9 out of the 48 isolates (9/48) representing 18.75 % of the isolates were screened and selected as best and strongest degraders of xylene, anthracene and pyrene hydrocarbons which they significantly (P = .05) utilize as source of carbon and energy and is indicated by absorbance values below of each isolates (Tables 1, 2 and 3). This study agrees with the explanation of Mao *et al*. [28] that the most important aspect of microbial degradation of PAH is enrichment and isolation of indigenous PAH degraders because the indigenous PAH degrading bacteria are already adapted to utilizing PAH. Pathak and Bhatnagar [29], argued that enrichment culturing is important for the success of hydrocarbon bioremediation because the process leads to selection of microorganism accustomed to hydrocarbon degradation. Esedafe *et al*. [30] reported that an occurence of 3/41 representing 7.32 % isolates from refinery effluent were capable of utilizing phenanthrene and anthracene as sole carbon and energy sources. It also indicates that only these isolates had the physiological capabilities to metabolize the aromatic hydrocarbons.

The result in Table 4 showed that most colonies were circular and irregular in shape, flat in elevation, undulate in margin, translucent in optic, smooth in texture, creamy in colour, 4 mm in size and glistering in surface description. The results of this research agree with the research carried out by Al -Thani *et al*. [31] who reported that a diverse microbial population can be isolated from hydrocarbon contaminated samples. In agreement with research carried out by Arulazhagan *et al*. [32] and Akinbankole *et al.* [14] who reported that bacteria isolated from pyrene and anthracene enriched medium are known to utilize pyrene and anthracene as their sole carbon source for growth and energy. The result in Table 5 showed that most of the marine bacterial isolates were Gram negative in Gram reaction, rod shaped arranged in single or pair, with variable reactions to different biochemical tests. The marine bacteria were identified as *Providencia vermicola* strain ANT1, *Alcaligenes faecalis* strain XYL2, *Brevundimonas diminuta* strain PYR3, *Alcaligenes faecalis* strain ANT4, *Alcaligenes faecalis* strain PYR5, *Myroides odoratus* strain ANT6, *Serratia marcescens* strain XYL7, *Providencia* sp. strain XYL8 and *Bacillus cereus* strain PYR9 using Bergey's manual for determinative bacteriology by Holt *et al.* [24]. These findings agree with the reports of Mrozik *et al*. [33], Okerentugba and Ezeronye [34], Chikere *et al*. [1] John *et al.* [18), Irshaid and Jacob [16], Wanjohi *et al*. [35], Isiodu *et al.* [26] and Fagbemi and Sanusi [11] that two - third of most petroleum hydrocarbon degraders are Gram negatives with one - third being Gram positives but contradict the findings of Akinbankole *et al*. [14], Kafilzadeh *et al*. [36] and Kafilzadeh and Pour [19], who reported that more of Gram positive bacteria were isolated than Gram negative bacteria. A lot of rod - shaped bacteria have also been implicated in hydrocarbon degradation studies (Okoh [37], Perfumo *et al*. [38], Chikere *et al*. [1], Alfreda and Ekene [39]; John *et al.*, [18], Akinbankole *et al*. [14], Irshaid and Jacob [16], Wanjohi *et al*. [35], Isiodu *et al.* [26] and Fagbemi and Sanusi [11] and similar result was obtained in this study.

The result in Table 6 revealed that the temperature remained in optimal level of 26 °C conducive for these bacteria activities which is also observable in the control. The strains isolated in this study were mesophilic in nature and proved that mesophilic bacteria can degrade hydrocarbons. Similar result were observed by Athar *et al.* [27]. The result contradicts with the report of Irshaid and Jacob [16] that the growth rates of the xylene degrading bacteria from gasoline contaminated soil sites were less when the

temperature was reduced from 30 – 25 °C whereas at 45 °C, the growth rates almost completely ceased. This contradiction might be due to the nature of the ecosystem as marine environment is generally characterized with lower temperatures unlike the terrestrial ecosystem with higher temperatures. Bennett *et al.* [25] highly suggested that 35 °C was the optimum temperature inoder for the isolate *Mycobacterium* sp. GIPAH- 01 to grow best on anthracene hydrocarbon. The result in Table 7 showed that the pH also fluctuates slightly with optimum of 7.05 within the 24 days' period of the research. These drops in pH are probably because the degradation of the hydrocarbons by these bacteria resulted in the release of acidic substances and intermediates (organic acids and other metabolic products) which reduces the pH of the medium and is in agreement with the researches carried out by John and Okpokwasili [15], Bennett *et al.* [25], Singh *et al.* [40], Irshaid and Jacob [16] and Fagbemi and Sanusi [11]. The result in Table 8 revealed that the optical density (OD_{600} nm) increased from 0.01 – 0.54 within the 24 days' period of the research. The degradation and utilization of these compounds resulted in increase in optical density (cell mass) of the organisms and corroborates with findings of John *et al*. [18]. Akinbankole *et al.* [14] reported that cell growth analysis of anthracene and pyrene metabolizing bacteria shows that only KLA1022 (*B. toyonesis*) and JIP1005 (*S. enterica*) could utilize anthracene and pyrene as source of nutrient for their growth because there was an increase in the amount of cells for the both. A reduction in cell amount can be seen as the PAH concentration (sole carbon source) is reduced. According to peer-reviewed articles, the variations in microbial population density and diversity were affected by the levels of petroleum contamination, suggesting that highly polluted areas appear to have a greater quantity of these microbes. The variations could be attributed to adaptation of these bacterial species to utilize and flourish in the presence of crude oil or might be partly due to variations among several physicochemical environmental factors, including differing ambient environmental conditions, soil compositions and organic carbons in the soil [16]. The results in Tables 9, 10 and 11 revealed that the degree of weight losses was observed to increase with increase in incubation period but varied with different microbial species tested. *Serratia marcescens* XYL7 significantly exhibited the highest capabilities to degrade the aromatic hydrocarbons with 99.50 ± 0.05 % and 60.00 ± 0.02 % in weights of xylene and pyrene respectively while *Alcaligenes faecalis* PYR5 significant degraded anthracene with 97.40 ± 0.01 % reduction in weight after 24 days biodegradation study with evidence of increasely low optical density ($OD_{600\ nm}$) against their controls.

However, the level of xylene, anthracene and pyrene degradations also included 5.20 ± 0.03 %, 4.60 ± 0.01 %, 11.10 ± 0.06 % degradation by abiotic factor as observed in controls with no bacterial inocula. After deduction of pyrene degradation by abiotic factor, 94.30 ± 0.02 % and 49.90 ± 0.06 % of xylene and pyrene degradation was in fact contributed by *Serratia marcescens* XYL7 and 92.80 ± 0.02 % by *Alcaligenes faecalis* PYR5 during this period. Differences in xylene, anthracene and pyrene degradation abilities of bacterial strains were clearly reflected even after 4 days of incubation which was further magnified during incubation periods (4 – 24 days) with very strongly significant positive correlation (r = 0.897 – 0.996). Thus, xylene, anthracene and pyrene were degraded by all nine bacterial strains, but they differed widely in their inherent abilities. The differences might be due to high molecular weight PAHs that are more recalcitrant and hard to microbial attack and the sequence of degradation is xylene > anthracene > pyrene. Biodegradation of PAHs is depends on their chemical structure and corresponding physiochemical properties and low molecular weight PAHs degrade rapidly than high molecular weight PAHs [41]. Similar results are obtained in the present study. Akpe *et al.* [5] reported that the lower the optical density value, the higher the percentage of hydrocarbons degraded. Singh *et al.* [40] reported that *Rhodococcus pyridinvorans* NJ2 was the highest degrader (60 %) of pyrene, followed by *Pseudomonas* sp. BP10 (44 %) and the least was *Ochrobactrum intermedium* P2 (42 %) in MSM with pyrene (50 µg /mL) in 8 days. Swaathy *et al.* [13], reported that the marine isolate, *Bacillus licheniformis* MTCC 5514 degraded > 95 % of 300 ppm anthracene in an aqueous medium within 22 days and the degradation percentage reduced significantly when the concentration of anthracene increased to above 500 ppm. Akinbankole *et al.* [14] reported that pyrene and anthracene utilizing bacteria (*Salmonella enterica* and *Bacillus toyonesis*) isolated from water and used engine oil contaminated soil degraded 99 % of the PAH within seven days. Arulazhagan *et al*. [32] in their report in which bacteria they isolated from contaminated sites also degraded over 99 % of flourene and anthracene in seven days. Poornachander *et al*. [42] reported that *Bacillus cereus* CPOU13 degraded phenanthrene about to 73.46 % and its initial concentration declined from 216.32 µg to 56.57 µg; anthracene to 85.76 % and its initial concentration reduced from 209.20 µg to 32.63 µg and pyrene to 47.88 % and its initial concentration reduced from 230.14 µg to 119.95 µg. Qi *et al*. [43] reported that *Gordonia* sp. nov. Q8 could remove 73.8 % and 53.4 % of anthracene and pyrene hydrocarbons in 7 days. Lily *et al*. [12] reported that *Brachybacterium*

paraconglomeratum strain BMIT637C was the efficient degrader of anthracene being capable of degrading 70.32 % of anthracene within 10 days showing 2 x 10^{45}- fold increase in the CFU number that indicated anthracene utilization as a sole source of carbon and energy. Pandey *et al.* [44] reported that PAH (phenanthrene, flourene, anthracene, pyrene) dissipation level ranged between 38.7 to 99.7 % with highest depletion recorded in phenanthrene within seven days. The degradation rate of 3 - ring PAH was higher as compared to 4 - ring PAH (pyrene) by *Serratia marcescens* L - 11. The findings of these authors supported the results of this study.

The results in Plates 1a – b, 2a – b and 3a – b revealed that the aromatic hydrocarbons found in the control set ups showed large developed spots with high intensities compared with that of the highest degraders *Serratia marcescens* XYL7 and *Alcaligenes faecalis* PYR5 that displayed small developed spots with low intensities when visualized under UV illuminator at 235 nm with the iodine crystal spray evidently showing clear confirmations of biodegradation losses of some aromatic hydrocarbon components. The result in Table 12 revealed that the retention factor of the aromatic hydrocarbons degraded by marine isolates ranged from 0.27 ± 0.08 – 0.84 ± 0.01, 0.74 ± 0.08 – 1.00 ± 0.12 and 0.06 ± 0.03 – 1.00 ± 0.12 compared to controls which were 0.5 ± 0.01, 0.63 ± 0.02 and 0.38 ± 0.01 for xylene, anthracene and pyrene hydrocarbons. These losses or disappearances could be attributed to the utilization of aromatic hydrocarbons by marine isolates during the 24 days incubation period under optimum culture conditions of temperature and pH as sole carbon sources. Moreover, the results upheld the results of the degradation kinetics of the hydrocarbons by the marine bacterial isolates at day 24 with increasingly low OD monitored at 240 nm and is in agreement with published work of Bennet *et al*. [25] who reported that the thin layer chromatography of test anthracene showed small developed spots with low intensities when compared with control visualized at 235 nm OD - UV illuminator. Also, Teh and Hadibarata [45] reported that the thin layer chromatographic analysis of the extracted metabolites after the pyrene degradation process showed the existence of a single spot with Rf value of 0.65, which is similar to the standard Rf value of protocatechuic acid and conformed with the pyrene Rf value in this study. In agreement with Akinbankole *et al.* [14], the disappearance of pyrene and anthracene in the medium indicates that the pyrene and anthracene in the medium have been metabolized by the bacteria.

Table 1. Growth performance of the aromatic hydrocarbon - degraders isolated from Abonema sampled location

Isolate	Optical density (OD_{600} nm)		
	Xylene	Anthracene	Pyrene
AB1	0.657 ± 0.008	0.657 ± 0.001	0.580 ± 0.003
ANT4*	0.676 ± 0.004	0.775 ± 0.007	0.822 ± 0.002
AB3	0.701 ± 0.021	0.467 ± 0.029	0.666 ± 0.003
AB4	0.715 ± 0.004	0.598 ± 0.024	0.841 ± 0.001
PYR3*	0.598 ± 0.005	0.511 ± 0.003	1.041 ± 0.008
AB6	0.641 ± 0.001	0.494 ± 0.002	0.653 ± 0.001
AB7	0.687 ± 0.004	0.690 ± 0.002	0.803 ± 0.016
AB8	0.618 ± 0.001	0.638 ± 0.007	0.782 ± 0.003
AB9	0.457 ± 0.002	0.475 ± 0.001	0.573 ± 0.004
XYL2*	0.952 ± 0.004	0.312 ± 0.002	0.838 ± 0.021
AB11	0.793 ± 0.014	0.495 ± 0.002	0.970 ± 0.003
AB12	0.647 ± 0.002	0.446 ± 0.001	0.621 ± 0.005
AB13	0.328 ± 0.001	0.415 ± 0.001	0.451 ± 0.001

** = Isolates with highest degradability; values are mean ± standard deviation of triplicate determination.*

Table 2. Growth performance of the aromatic hydrocarbon - degraders isolated from Nembe sampled location

Isolate	Optical density (OD_{600} nm)		
	Xylene	Anthracene	Pyrene
NW1	0.885 ± 0.003	0.236 ± 0.005	0.708 ± 0.008
PYR5*	0.710 ± 0.003	0.216 ± 0.005	0.933 ± 0.007
NW3	0.466 ± 0.007	0.201 ± 0.000	0.806 ± 0.004
NW4	0.893 ± 0.002	0.356 ± 0.008	0.827 ± 0.008
NW5	0.750 ± 0.004	0.132 ± 0.005	0.767 ± 0.008
NW6	0.644 ± 0.004	0.246 ± 0.004	0.724 ± 0.008
NW7	0.561 ± 0.003	0.193 ± 0.005	0.808 ± 0.001
NW8	0.628 ± 0.008	0.472 ± 0.001	0.826 ± 0.008
XYL7*	1.055 ± 0.002	0.588 ± 0.005	0.927 ± 0.001
NW10	0.809 ± 0.002	0.785 ± 0.002	0.881 ± 0.004
NW11	0.826 ± 0.001	0.444 ± 0.002	0.891 ± 0.001
NW12	0.625 ± 0.005	0.563 ± 0.001	0.728 ± 0.006
NW13	0.374 ± 0.008	0.775 ± 0.001	0.760 ± 0.001
NW14	0.701 ± 0.001	0.622 ± 0.003	0.788 ± 0.007
NW15	0.705 ± 0.008	0.529 ± 0.004	0.830 ± 0.002
NW16	0.769 ± 0.002	0.380 ± 0.001	0.822 ± 0.001
ANT1*	0.804 ± 0.003	0.816 ± 0.007	0.583 ± 0.001

** = Isolates with highest degradability; values are mean ± Standard deviation of triplicate determination.*

Table 3. Growth performance of the aromatic hydrocarbon - degraders isolated from Onne sampled location

Isolate	Optical density (OD_{600} nm)		
	Xylene	Anthracene	Pyrene
ON1	0.721 ± 0.001	0.884 ± 0.007	0.500 ± 0.001
ON2	0.204 ± 0.001	0.660 ± 0.011	0.454 ± 0.001
ON3	0.473 ± 0.003	0.476 ± 0.036	0.561 ± 0.013
ON4	0.207 ± 0.001	0.766 ± 0.001	0.565 ± 0.033
ON5	0.477 ± 0.002	0.457 ± 0.001	0.378 ± 0.005
ON6	0.409 ± 0.005	0.489 ± 0.100	0.562 ± 0.021
ON7	0.251 ± 0.003	0.428 ± 0.014	0.728 ± 0.001
ON8	0.111 ± 0.005	0.429 ± 0.014	0.425 ± 0.021
ON9	0.463 ± 0.008	0.357 ± 0.011	0.281 ± 0.006
PYR9*	0.106 ± 0.001	0.335 ± 0.001	0.871 ± 0.001
ON11	0.700 ± 0.001	0.901 ± 0.005	0.417 ± 0.002
ANT6*	0.511 ± 0.006	1.433 ± 0.013	0.568 ± 0.009
ON13	0.273 ± 0.002	0.386 ± 0.002	0.527 ± 0.001
ON14	0.278 ± 0.005	0.553 ± 0.022	0.684 ± 0.003
ON15	0.291 ± 0.003	0.748 ± 0.009	0.522 ± 0.010
ON16	0.662 ± 0.001	0.919 ± 0.002	0.494 ± 0.002
XYL8*	0.741 ± 0.007	0.510 ± 0.013	0.602 ± 0.004
ON18	0.354 ± 0.002	1.004 ± 0.001	0.478 ± 0.001

** = Isolates with highest degradability; values are mean ± standard deviation of triplicate determination.*

Table 4. Colonial morphology of the aromatic hydrocarbon degrading bacterial isolates

Isolate	Colonial description						
	Shape	Elevation	Margin	Optics	Texture	Colour	Size	Surface
ANT1	Circular	Flat	Undulate	Translucent	Smooth	Creamy	4.0 mm	Dull
XYL2	Irregular	Raised	Erose	Translucent	Smooth	Creamy	3.0 mm	Dull
PYR3	Circular	Flat	Undulate	Translucent	Smooth	Creamy	2.0 mm	Glistering
ANT4	Circular	Flat	Erose	Translucent	Smooth	Creamy	5.2 mm	Glistering
PYR5	Circular	Flat	Undulate	Translucent	Smooth	Creamy	4.0 mm	Dull
ANT6	Rhizoid	Flat	Lobate	Translucent	Rough	Yellow	4.2 mm	Glistering
XYL7	Irregular	Flat	Undulate	Translucent	Rough	Red	4.0 mm	Glistering
XYL8	Irregular	Flat	Undulate	Translucent	Smooth	Creamy	4.2 mm	Glistering
PYR9	Irregular	Raised	Undulate	Translucent	Smooth	Creamy	4.0 mm	Glistering

Table 5. Morphological and biochemical properties of the aromatic hydrocarbon degrading bacterial isolates

Property	Isolate								
	ANT1	XYL2	PYR3	ANT4	PYR5	ANT6	XYL7	XYL8	PYR9
Gram reaction	–	–	–	–	–	–	–	–	+
Cellular morphology	Paired short rods	Singled long rods	Singled long rods	Singled short rods	Singled long rods	Single longer rods and long chains	Paired short rods	Single paired long Rods	Singled short rods
Spore test	–	–	–	–	–	–	–	–	+
Catalase	+	+	+	+	+	+	+	+	+
Indole	+	–	–	–	–	–	–	+	+
Motility	+	+	+	+	+	–	+	+	+
Methyl red	–	–	–	–	–	–	–	–	+
Voges-Proskauer	–	–	–	–	–	–	+	–	–
Citrate	–	+	–	+	+	+	+	–	+
Urease	+	–	–	–	–	–	–	+	+
Starch hydrolysis	+	+	–	+	–	+	–	+	+
Gelatin hydrolysis	–	–	–	–	–	–	+	–	–
NO_3 reduction	+	–	–	–	–	–	+	+	+
Coagulase test	+	–	–	–	–	–	+	+	–
H_2S production	+	–	–	–	–	+	–	+	+
Mannitol	+	–	–	–	–	+	+	+	+
Glucose	+	+	–	+	+	+	+	+	+
Xylose	–	–	–	–	–	–	+	–	+
Lactose	–	–	–	–	–	–	–	–	+
Sucrose	–	+	–	+	+	–	+	–	+
Arabinose	+	–	–	–	–	+	+	–	–
Maltose	–	–	+/–	–	–	–	–	–	+
Saccharose	+	+	–	+	+	–	+	+	+
Oxidase	–	+	+	+	+	+	+	–	+
Casein hydrolysis	–	–	–	–	–	–	+	–	–

NO_3 = Nitrate; H_2S = Hydrogen sulphide; – = Negative result; + = Positive result

Table 6. Changes in temperature (°C) of medium during aromatic hydrocarbons degradation by marine bacterial isolates

Isolate	Ahs																	
	Xyl						Ant						Pyr					
	4	8	12	16	20	24	4	8	12	16	20	24	4	8	12	16	20	24
ANT1	26.00	26.00	25.50	26.00	26.00	26.00	26.00	26.00	26.00	25.50	26.00	26.00	26.00	25.00	25.50	26.00	26.00	26.00
XYL2	26.00	26.00	25.50	26.00	26.00	26.00	26.00	26.00	26.00	25.50	26.00	26.00	26.00	25.00	25.50	26.00	26.00	26.00
PYR3	26.00	26.00	25.50	26.00	26.00	26.00	26.00	26.00	26.00	25.50	26.00	26.00	26.00	25.00	25.50	26.00	26.00	26.00
ANT4	26.00	26.00	25.50	26.00	26.00	26.00	26.00	26.00	26.00	25.50	26.00	26.00	26.00	25.00	25.50	26.00	26.00	26.00
PYR5	26.00	26.00	25.50	26.00	26.00	26.00	26.00	26.00	26.00	25.50	26.00	26.00	26.00	25.00	25.50	26.00	26.00	26.00
ANT6	26.00	26.00	25.50	26.00	26.00	26.00	26.00	26.00	26.00	25.50	26.00	26.00	26.00	25.00	25.50	26.00	26.00	26.00
XYL7	26.00	26.00	25.50	26.00	26.00	26.00	26.00	26.00	26.00	25.50	26.00	26.00	26.00	25.00	25.50	26.00	26.00	26.00
XYL8	26.00	26.00	25.50	26.00	26.00	26.00	26.00	26.00	26.00	25.50	26.00	26.00	26.00	25.00	25.50	26.00	26.00	26.00
PYR9	26.00	26.00	25.50	26.00	26.00	26.00	26.00	26.00	26.00	25.50	26.00	26.00	26.00	25.00	25.50	26.00	26.00	26.00
Control	26.00	26.00	26.00	26.00	26.00	26.00	26.00	26.00	26.00	26.00	26.00	26.00	26.00	25.00	26.00	26.00	26.00	26.00

Xyl = Xylene; Ant = Anthracene; Pyr = Pyrene; AHs = Aromatic hydrocarbons; 4 - 24 represents days of incubation

Table 7. Changes in pH of the medium during aromatic hydrocarbons degradation by marine bacterial isolates

Isolate	AHs																	
	Xyl						Ant						Pyr					
	4	8	12	16	20	24	4	8	12	16	20	24	4	8	12	16	20	24
ANT1	7.05	7.11	7.05	7.04	7.15	7.02	7.06	6.98	7.00	7.07	7.02	7.01	7.06	6.97	7.00	7.00	7.03	6.99
XYL2	7.06	7.00	7.07	7.06	7.05	7.02	7.00	6.97	7.02	6.99	7.02	7.00	7.08	6.97	7.08	7.08	7.02	6.98
PYR3	6.88	6.86	6.85	6.87	6.95	6.96	7.03	7.01	7.06	7.00	7.03	7.02	7.09	6.97	7.05	7.05	7.01	7.01
ANT4	7.03	7.00	7.03	7.05	7.01	7.02	7.03	7.01	7.09	7.05	7.02	7.01	7.06	6.96	7.03	7.03	7.02	6.98
PYR5	7.02	6.97	7.01	7.08	7.02	7.04	7.03	6.98	7.10	7.09	7.04	7.01	7.07	6.99	7.19	7.07	7.00	6.97
ANT6	7.05	6.97	7.03	7.06	7.04	7.05	7.04	7.00	7.06	7.05	7.01	7.02	7.09	6.98	7.01	7.06	7.00	6.98
XYL7	7.06	7.02	7.03	7.03	7.02	7.03	7.04	7.00	7.06	7.05	7.01	7.02	7.06	6.95	7.11	7.01	7.00	7.03
XYL8	7.06	7.03	6.99	7.03	7.01	7.04	7.02	6.99	7.11	7.00	6.99	6.97	7.06	6.95	7.13	7.04	6.98	7.04
PYR9	6.99	6.96	6.97	7.01	6.99	7.02	7.05	7.04	7.07	7.02	7.01	6.98	7.04	6.95	7.16	7.02	6.99	6.99
Control	7.05	7.05	7.05	7.05	7.05	7.05	7.03	7.03	7.03	7.03	7.03	7.03	7.00	7.00	7.00	7.00	7.00	7.00

Xyl = Xylene; Ant = Anthracene; Pyr = Pyrene; AHs = Aromatic hydrocarbons; 4 - 24 represents days of incubation

Table 8. Changes in optical density (OD_{600} nm) of the medium during aromatic hydrocarbons degradation by marine bacterial isolates

Isolate	AHs																	
	Xyl						Ant						Pyr					
	4	8	12	16	20	24	4	8	12	16	20	24	4	8	12	16	20	24
ANT1	0.17	0.18	0.19	0.20	0.21	0.22	0.20	0.21	0.23	0.24	0.25	0.26	0.17	0.19	0.20	0.23	0.25	0.27
XYL2	0.03	0.05	0.06	0.08	0.09	0.10	0.14	0.15	0.19	0.20	0.22	0.23	0.25	0.26	0.27	0.30	0.44	0.54
PYR3	0.01	0.02	0.05	0.07	0.08	0.09	0.25	0.26	0.27	0.28	0.29	0.30	0.19	0.20	0.23	0.24	0.26	0.51
ANT4	0.11	0.12	0.13	0.14	0.15	0.35	0.15	0.16	0.19	0.20	0.25	0.28	0.21	0.22	0.24	0.26	0.28	0.34
PYR5	0.20	0.25	0.28	0.30	0.32	0.34	0.11	0.13	0.15	0.17	0.19	0.21	0.18	0.20	0.22	0.24	0.26	0.28
ANT6	0.11	0.14	0.17	0.20	0.23	0.35	0.24	0.25	0.27	0.30	0.35	0.36	0.18	0.19	0.20	0.21	0.23	0.25
XYL7	0.11	0.12	0.13	0.14	0.15	0.16	0.11	0.14	0.17	0.19	0.20	0.21	0.30	0.31	0.35	0.37	0.40	0.51
XYL8	0.13	0.14	0.15	0.17	0.18	0.20	0.16	0.17	0.19	0.21	0.22	0.23	0.30	0.30	0.35	0.37	0.40	0.45
PYR9	0.18	0.20	0.22	0.23	0.24	0.25	0.14	0.16	0.18	0.20	0.21	0.23	0.25	0.27	0.30	0.35	0.40	0.45
Control	0.10	0.10	0.10	0.10	0.10	0.10	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15

Xyl = Xylene; Ant = Anthracene; Pyr = Pyrene; AHs = Aromatic hydrocarbons; 4 - 24 represents days of incubation

Table 9. Weight loss of xylene resulting from the growth of marine bacterial isolates

– Isolates with highest degradability, Values are mean ± standard deviation of triplicate determination.

		Days of incubation				
Isolate code	0	4	8	12	16	20	24
ANT1	0.248	0.026	0.024	0.021	0.018	0.016	0.015
		89.50 ± 0.02 %	90.30 ± 0.02 %	91.50 ± 0.01 %	92.70 ± 0.08 %	93.50 ± 0.01%	93.90 ± 0.06 %
XYL2	0.248	0.031	0.028	0.026	0.024	0.020	0.018
		87.50 ± 0.06 %	88.70 ± 0.01 %	89.50 ± 0.01 %	90.30 ± 0.02 %	91.90 ± 0.02 %	92.70 ± 0.06 %
PYR3	0.248	0.070	0.068	0.065	0.062	0.060	0.057
		71.80 ± 0.01 %	72.60 ± 0.03 %	73.80 ± 0.02 %	75.00 ± 0.08 %	75.80 ± 0.01 %	77.00 ± 0.01 %
ANT4	0.248	0.057	0.055	0.053	0.051	0.044	0.040
		77.00 ± 0.02 %	77.80 ± 0.01 %	78.60 ± 0.02 %	79.40 ± 0.06 %	82.20 ± 0.01 %	83.90 ± 0.02 %
PYR5	0.248	0.075	0.072	0.070	0.067	0.064	0.060
		69.80 ± 0.01 %	70.90 ± 0.07 %	71.70 ± 0.04 %	73.00 ± 0.08 %	74.10 ± 0.01%	75.80 ± 0.01 %
ANT6	0.248	0.061	0.059	0.057	0.054	0.052	0.050
		75.40 ± 0.02 %	76.20 ± 0.05 %	77.00 ± 0.06 %	78.00 ± 0.01 %	79.00 ± 0.08 %	79.80 ± 0.02 %
XYL7	0.248	0.019	0.018	0.017	0.015	0.010	0.001
		92.30 ± 0.02 %	92.70 ± 0.05 %	93.10 ± 0.02 %	93.90 ± 0.03 %	96.00 ± 0.02 %	99.50 ± 0.05 %
XYL8	0.248	0.046	0.044	0.042	0.040	0.038	0.035
		81.50 ± 0.04 %	82.30 ± 0.07 %	83.10 ± 0.31 %	83.90 ± 0.05 %	84.70 ± 0.06 %	85.90 ± 0.08 %
PYR9	0.248	0.057	0.053	0.050	0.048	0.046	0.043
		77.00 ± 0.02 %	78.60 ± 0.01 %	79.80 ± 0.05 %	80.70 ± 0.03 %	81.20 ± 0.01 %	83.90 ± 0.01 %
Control	0.248	0.246	0.243	0.241	0.240	0.237	0.235
		0.80 ± 0.02 %	2.00 ± 0.01 %	2.80 ± 0.05 %	3.20 ± 0.02 %	4.40 ± 0.05 %	5.20 ± 0.03 %

Table 10. Weight loss of anthracene resulting from the growth of marine bacterial isolates
** = Isolates with highest degradability; values are mean ± standard deviation of triplicate determination.*

		Days of incubation					
Isolate	0	4	8	12	16	20	24
ANT1	0.386	0.089	0.088	0.086	0.085	0.085	0.084
		76.90 ± 0.05 %	77.20 ± 0.02 %	77.70 ± 0.01 %	77.90 ± 0.01 %	78.00 ± 0.01 %	78.20 ± 0.02 %
XYL2	0.386	0.098	0.090	0.084	0.075	0.060	0.031
		74.60 ± 0.08 %	76.70 ± 0.07 %	78.20 ± 0.08 %	80.60 ± 0.09 %	84.40 ± 0.02 %	91.20 ± 0.01 %
PYR3	0.386	0.098	0.096	0.094	0.093	0.092	0.091
		74.60 ± 0.02 %	75.10 ± 0.05 %	75.60 ± 0.06 %	75.90 ± 0.08 %	76.10 ± 0.02 %	76.40 ± 0.02 %
ANT4	0.386	0.105	0.104	0.103	0.102	0.101	0.100
		72.70 ± 0.05 %	73.00 ± 0.02 %	73.30 ± 0.06 %	73.60 ± 0.07 %	73.80 ± 0.03 %	74.00 ± 0.07 %
PYR5*	0.386	0.060	0.050	0.040	0.030	0.020	0.010
		84.50 ± 0.03 %	87.00 ± 0.06 %	89.60 ± 0.07 %	92.20 ± 0.00 %	94.80 ± 0.02 %	97.40 ± 0.01 %
ANT6	0.386	0.070	0.066	0.054	0.050	0.043	0.046
		81.80 ± 0.02 %	82.90 ± 0.06 %	86.00 ± 0.09 %	87.00 ± 0.08 %	87.60 ± 0.04 %	88.00 ± 0.06 %
XYL7	0.386	0.124	0.122	0.120	0.116	0.111	0.109
		67.90 ± 0.08 %	68.40 ± 0.08 %	68.90 ± 0.04 %	69.90 ± 0.01 %	71.20 ± 0.04 %	71.80 ± 0.07 %
XYL8	0.386	0.055	0.050	0.045	0.040	0.035	0.030
		85.80 ± 0.02 %	87.00 ± 0.06 %	88.30 ± 0.09 %	89.60 ± 0.01 %	90.90 ± 0.01 %	92.20 ± 0.01 %
PYR9	0.386	0.033	0.031	0.027	0.024	0.020	0.016
		91.50 ± 0.01 %	92.00 ± 0.04 %	93.00 ± 0.01 %	93.80 ± 0.08 %	94.80 ± 0.07 %	95.90 ± 0.01%
Control	0.386	0.383	0.380	0.376	0.374	0.370	0.368
		0.70 ± 0.023 %	1.50 ± 0.04 %	2.60 ± 0.06 %	3.10 ± 0.08 %	4.10 ± 0.07 %	4.60 ± 0.01 %

Table 11. Weight loss from pyrene resulting from the growth of marine bacterial isolates

Isolate	Days of incubation											
	0	4		8		12		16		20		24
ANT1	0.117	0.078 33.00 0.08 %	±	0.075 35.90 0.23 %	±	0.073 37.60 0.07 %	±	0.071 39.30 0.21 %	±	0.069 41.00 0.06 %	±	0.068 41.90 0.04 %
XYL2	0.117	0.098 16.20 0.03 %	±	0.086 26.50 0.02 %	±	0.083 29.00 0.06 %	±	0.081 30.80 0.07 %	±	0.079 32.50 0.08 %	±	0.077 34.20 0.02 %
PYR3	0.117	0.103 12.0 0.07 %	±	0.100 14.5 0.09 %	±	0.099 15.3 0.03 %	±	0.097 17.0 0.02 %	±	0.094 19.7 0.08 %	±	0.090 23.0 0.03 %
ANT4	0.117	0.097 17.00 0.05 %	±	0.090 23.00 0.02 %	±	0.089 23.90 0.01 %	±	0.086 26.50 0.01 %	±	0.084 28.20 0.05 %	±	0.080 31.60 0.02 %
PYR5	0.117	0.067 40.00 0.06 %	±	0.064 45.30 0.03 %	±	0.062 47.00 0.01 %		0.060 48.70 0.04 %	±	0.057 51.30 0.03 %	±	0.053 54.70 0.03 %
ANT6	0.117	0.091 22.00 0.06 %	±	0.087 25.60 0.06 %	±	0.084 28.00 0.06 %	±	0.080 31.60 0.09 %	±	0.078 33.30 0.02 %	±	0.075 35.90 0.02 %
XYL7*	0.117	0.064 45.30 0.01 %	±	0.061 47.90 0.05 %	±	0.059 49.60 0.05 %	±	0.057 51.30 0.09 %	±	0.054 53.80 0.04 %	±	0.052 60.00 0.02 %
XYL8	0.117	0.090 23.00 0.01 %	±	0.087 25.60 0.05 %	±	0.085 27.40 0.03 %	±	0.082 29.90 0.01 %	±	0.080 31.60 0.06 %	±	0.078 33.30 0.06 %
PYR9	0.117	0.087 25.60 0.02 %	±	0.081 30.80 0.07 %	±	0.079 32.50 0.02 %	±	0.077 34.20 0.03 %	±	0.075 35.90 0.05 %	±	0.070 40.00 0.05 %
Control	0.117	0.115 1.70 0.03 %	±	0.113 3.40 0.02 %	±	0.111 5.10 0.02 %	±	0.109 7.70 0.06 %	±	0.107 8.60 0.02 %	±	0.104 11.10 0.06 %

** = Isolates with highest degradability; values are mean ± standard deviation of triplicate determination.*

Table 12. Retention factor of the aromatic hydrocarbons degraded by marine bacterial isolates

Isolate	AHs		
	Xylene	Anthracene	Pyrene
ANT1	0.27 ± 0.08	0.74 ± 0.08	0.34 ± 0.08
XYL2	0.69 ± 0.07	0.86 ± 0.01	0.24 ± 0.02
PYR3	0.76 ± 0.08	0.86 ± 0.01	1.00 ± 0.12
ANT4	0.82 ± 0.08	0.98 ± 0.08	0.83 ± 0.08
PYR5	0.82 ± 0.08	1.00 ± 0.12	0.92 ± 0.01
ANT6	0.71± 0.01	0.41 ± 0.01	0.59 ± 0.01
XYL7	0.84 ± 0.01	0.82 ± 0.08	0.38 ± 0.01
XYL8	0.32 ± 0.01	1.00 ± 0.12	0.79 ± 0.01
PYR9	0.30 ± 0.01	0.88 ± 0.01	0.06 ± 0.03
Control	0.50 ± 0.01	0.63 ± 0.02	0.38 ± 0.01

Retention factor (R_F) = $\frac{\text{distance travelled by solute}}{\text{distance travelled by solvent}}$ AHs= Aromatic hydrocarbons

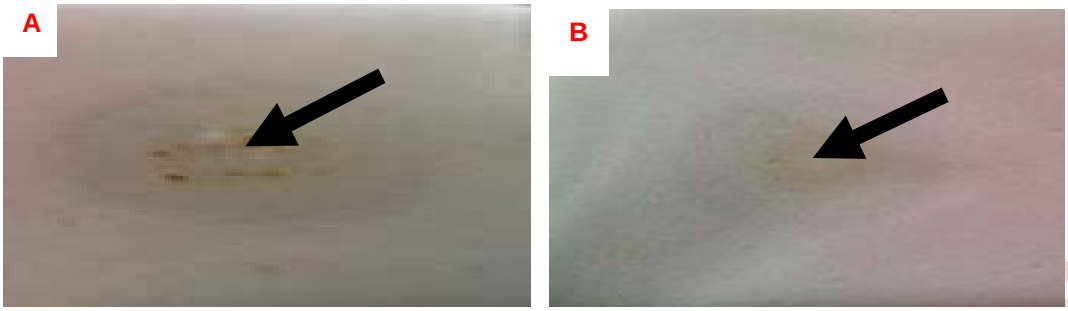

Plate 1a – b. Thin layer chromatograms of xylene hydrocarbon degradation

A. Thin layer chromatogram of xylene hydrocarbon degradation in control sample with arrow showing large high intensity spot visualized under UV illuminator at 235 nm B. Thin layer chromatogram of Serratia marcescens XYL7 on xylene hydrocarbon degradation with arrow showing small low intensity spot visualized under UV illuminator at 235 nm.

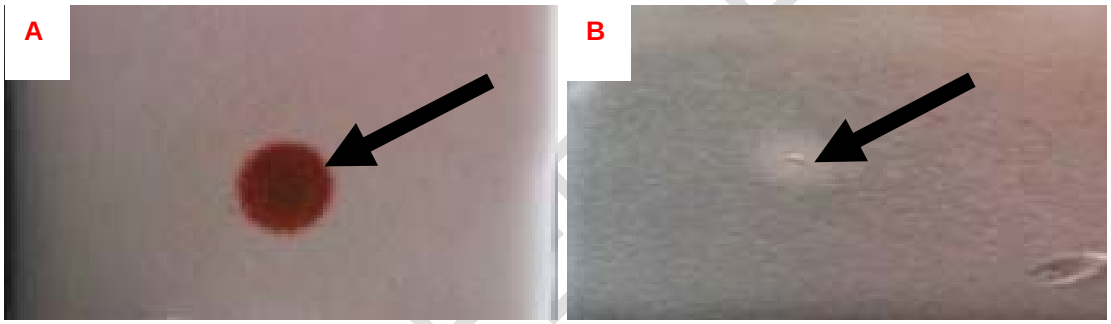

Plate 2a – b. Thin layer chromatograms of anthracene hydrocarbon degradation

A. Thin layer chromatogram of anthracene hydrocarbon degradation in control sample with arrow showing large high intensity spot visualized under UV illuminator at 235 nm B. Thin layer chromatogram of Alcaligenes faecalis PYR5 on anthracene hydrocarbon degradation with arrow showing almost cleared low intensity spot visualized under UV illuminator at 235 nm.

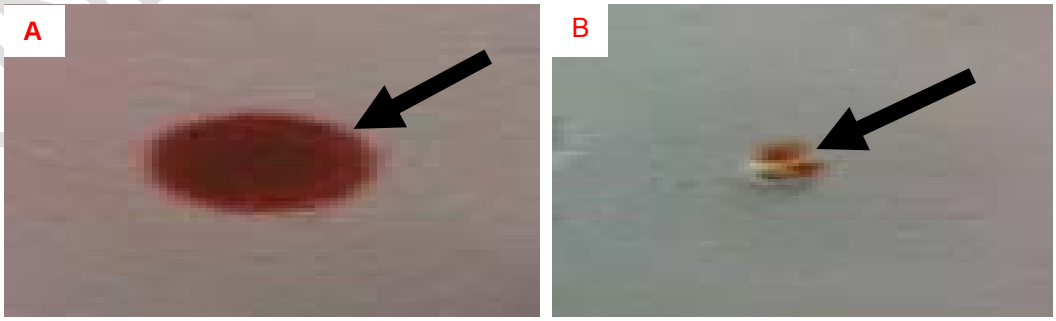

Plate 3a – b: Thin layer chromatograms of pyrene hydrocarbon degradation

A. Thin layer chromatogram of pyrene hydrocarbon degradation in control sample with arrow showing large high intensity spot visualized under UV illuminator at 235 nm B. Thin layer chromatogram of Serratia marcescens XYL7 on anthracene hydrocarbon degradation with arrow showing small low intensity spot visualized under UV illuminator at 235 nm.

Fig 1. Geoeye satellite image (2016) showing the Abonema sample points

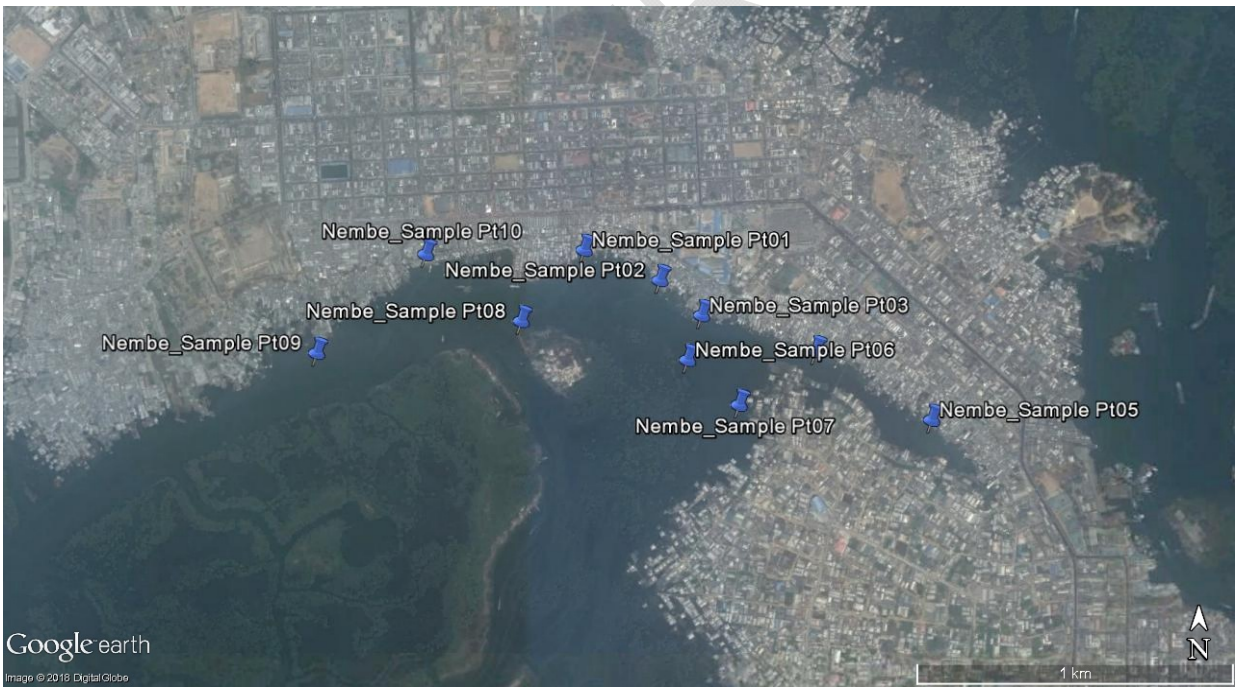

Fig 2. Geoeye satellite image (2016) showing the Nembe sample points

Fig 3. Geoeye satellite image (2016) showing the Onne sample points

5. CONCLUSION

The whole study revealed that the three sampling sites harbour a lot of efficient aromatic hydrocarbon degrading bacterial strains belonging to the genera: *Providencia, Alcaligenes, Brevundimonas, Myroides, Serratia, and Bacillus.* It also revealed that the isolated bacteria especially *Serratia marcescens* XYL7 were able to significantly (*P* =.05) degrade simple, low and high molecular weights aromatic hydrocarbons. The growth rates for these isolates were affected by concentration of aromatic hydrocarbons, the

temperature and pH of the medium. There were clear confirmations of biodegradation losses or disappearances of some aromatic hydrocarbon components indicating that the aromatic hydrocarbons in the medium have been metabolized by the bacteria. Thus, the excellent degradative abilities of these

bacterial strains especially *Serratia marcescens* XYL7 could be exploited in bioremediation campaigns in Nigeria.

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