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In vitro Degradation and Reduction of Aromatic Hydrocarbons by Marine Bacteria Isolated from Contaminated Marine Environments of Niger Delta

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Authors' contributions

This work was carried out in collaboration among all authors. Authors BOU and EIC designed the study. Authors BOU and UCA performed the statistical analysis, wrote the protocol and authors BOU and ELO wrote the first draft of the manuscript while authors BOU, OU, COC and UCA managed the analyses of the study. All authors managed the literature searches, read and approved the final manuscript.

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ABSTRACT

Aims: To determine the *in vitro* degradation and reduction of aromatic hydrocarbons by marine bacteria isolated from contaminated marine environments of Niger Delta.
Study Design: Nine treatments and nine 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

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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 reduction by the marine bacteria.
Place and Duration of Study: The studied sites 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, Nigeria between September, 2014 and 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, phenotypical, degradation and TLC techniques.
Results: The results showed that the three studied areas harbour numerous promising aromatic degrading bacterial strains belonging to the genera: *Providencia, Alcaligenes, Brevundimonas, Myroides, Serratia,* and *Bacillus*. The bacterial strains especially *Serratia marcescens* XYL7 significantly (P = .05) had 99.50 ± 0.05 % and 60.00 ± 0.02 % degradations 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 large spots size reductions or losses of test samples compare to control samples with minor spot sizes.
Conclusion: Thus, the outstanding degradative abilities of these strains could be exploited in bioremediation campaigns in Nigeria.

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

1. INTRODUCTION

The oil - rich Niger Delta region is the centre of exploration and production of petroleum in Nigeria. Over 80 - 90 % of the Nigeria's crude oil originated from this important ecological area and its adjacent offshore regions. Within the region, numerous causes of petroleum contamination may arise from transport vehicles and mishap from tankers, oil fields and stations, pipelines and storage tanks [1]. As a result of the activities mentioned above and from the petroleum companies, large scale contaminations of both the terrestrial and aquatic ecosystems in the area have been reported and documented [2-4]. It is thus, necessary to consider all remediation alternatives on the basis of their capacity to eliminate organic pollutants effectively as most of these waste products especially the benzene, toluene, and xylene (BTX) and polycyclic aromatic hydrocarbons (PAHs) are generally considered as toxicants [5]. The physical and chemical methods like volatilization, photooxidation, chemical oxidation, advanced oxidative processes and bioaccumulation are not safe and cost effective when compared to microbial bioremediation [6]. A healthier and economical way is to use enhanced biodegradation.

Thus, much research has focused on the biological degradation of aromatics hydrocarbons (Ahs) through metabolism and co-metabolism [7]. Mao et al. [8] and Pathak and Bhatnagar [9], in their studies, recommended that supplementation and isolation of native aromatic hydrocarbon degraders is the most significant part of microbial degradation or bioremediation of aromatic hydrocarbons as the degraders have been well acclimatized leading to selection of better utilizers and diverse degraders of the hydrocarbons. Esedafe et al. [10] reported that an occurrence of 3/41 signifying 7.32 % of the isolates from refinery effluent were capable of utilizing phenanthrene and anthracene as sole carbon and energy sources.

Microbiological activity is affected by many environmental factors including energy source, donors and acceptors of electrons, nutrients, pH, and temperature. Most microbes use respiration to transform biological energy sources (organic and inorganic fuels, light) into ATP. Microorganisms especially prokaryotes employed a wide range of electron donors both organic carbon and inorganic compounds such as H_2O, H_2, as well as numerous alternative electron acceptors such as organic carbon, O_2, CO_2 or oxygen substitutes for respiration in the absence of molecular oxygen, which makes them exceptionally adaptable with regard to energy [11]. Hydrocarbon degradation by microbial population in natural ecosystem is influenced by physical, chemical and biological factors that contribute to the degradation of petroleum and individual hydrocarbons [12]. Amongst the physical factors, temperature play a significant role, firstly by directly changing the chemistry of

pollutants and then secondly, by influencing the physiology and diversity of the microbial environment [13]. Amongst the chemical factor, nutrients are very essential requirements for effective biodegradation process, especially nitrogen, phosphorus and in some cases iron [14]. Also, among the biological factor, the kind of microorganisms, their genomes and their earlier contact to hydrocarbons affects the capacity with which the hydrocarbons could be degraded [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 such as microbial number and nutrient availability [16]. The previous study reported that the growth rate of bacterial strains X1, X2, X3 and X4 under 1 % of m-xylene decreased as the temperature reduced from 30 - 25 °C, whereas at 45 °C, the growth rate was practically ceased. The optimum growth rate of the strains was obtained at pH 6.5 but had fluctuating growth rate at pH 5.5 or 8.5 [16].

The advancement in industrial and physical development as well as the enormous transportation activities in the Niger Delta will definitely lead to increases in exploration, production, use, release and bioaccumulation of aromatic hydrocarbons in the terrestrial and aquatic ecosystems 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 crude oil - impacted Niger Delta aquatic ecosystem in particular for xylene, anthracene and pyrene hydrocarbon potent degraders as alternative to physical and chemical agents owing to greater advantages over them. In this work, we report the *in vitro* degradation and reduction 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 chosen because high anthropologic and industrial acivities and are 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.

2.2 Sample of Collection

Ten samples of marine sediment and water were collected randomly at ten (10) designated points in the three sampling sites (Figs. 1, 2 and 3). They were mixed together after which a total of six representative sediment and water samples were taken for the analysis. The surface aerobic sediment samples were obtained 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 wetted 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 in ice packed coolers to keep them under a temperature not more than 4.00 °C. They were transported to the laboratory for microbiological analyses and stored at 4°C in refrigerator for further processing [3,17,18].

2.3 Isolation of Aromatic Hydrocarbon Degrading Bacterial Strains

The hydrocarbon degraders were isolated from sediment and water samples of the three sampling sites using modified mineral basal agar (MBA) (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) supplemented with

xylene, anthracene and pyrene as sole carbon and energy sources. The medium was sterilized by autoclaving at 121 °C and 15 psi for 15 minutes. Thereafter, 0.2 mL acetone solution containing 0.1 % w/v of the representative aromatic hydrocarbons (xylene, anthracene and pyrene) were aseptically pipetted and uniformly spread on the agar surface of the Petri dish plates. After the acetone evaporation, 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 under aseptic conditions. The inoculated plates were sealed using adhesive tape and foil to prevent contamination and photolysis and later placed in black polythene bags. They were incubated in the dark at 28.00 ± 2.00 °C for 14 days [15,18].

2.4 Preservation of Cultures

Colonies that developed on MBA plates were sub-cultured onto new MBA plates and incubated for another 14 days. Forty - eight isolates that grew on these plates were selected as xylene, anthracene and pyrene degraders. They were later sub - cultured on Bijou bottles and preserved at 4.00 °C in refrigerator [18].

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

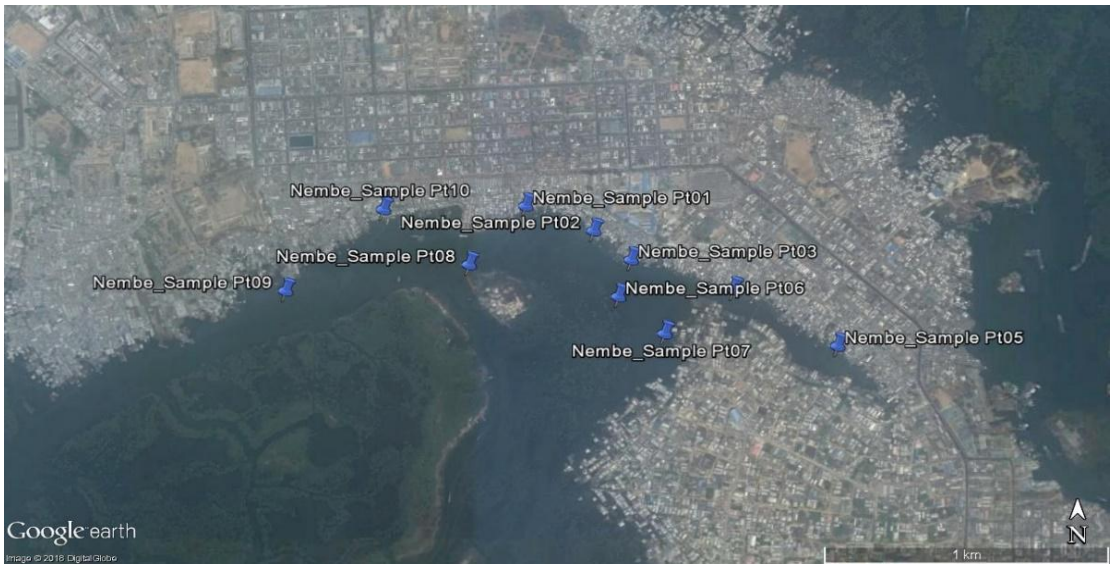


Fig. 2. Geoeye satellite image (2016) showing the Nembe sample points

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

2.5 Screening Test for the Most Potent Marine Degrading Bacterial Isolates

In order to screen and chose the most potent degrading bacterial strains, the growths of the forty-eight isolates was tested by growing 5 mL of each isolates in large test tubes containing 25 mL of the modified mineral basal medium (MBM) augmented 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 28.00 ± 2.00 °C for five days. After incubation, growths of bacterial cultures as indicated by turbidity were measured at 600 nm using a UV - VIS spectrophotometer (Astell, UV - Vis Grating, 752 W) and the cultures with the highest optical densities on each hydrocarbon in three studied sites were chosen as the most potent xylene, anthracene and pyrene degrading bacteria [18-20].

2.6 Phenotypical Identification of Selected Hydrocarbon Utilizing Bacterial Isolates

2.6.1 Morphological characterization

2.6.1.1 Colonial characteristics

Following sub - culturing and incubation, the colonial features such as shape, elevation, margin, optic, texture, colour, size and surface descriptions of the nine chosen bacterial strains were observed and noted [21].

2.6.1.2 Microscopic characteristics

The standard methods of Gram staining and endospore staining were carried out on the chosen 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 Biodegradation Study and Analytical Technique

Following the methods of Bennet et al. [25] and John and Okpokwasili [12] as modified in this study, the degradation rates of bacterial isolates were determined using hydrocarbon supplemented MBM as previously stated.

Precisely, 1 mL of 48 hrs old cultures of each bacterial strains was inoculated into 28 sterile 200 mL capacity conical flasks (4 sets of 7 flasks) in triplicates comprising 100 mL of sterile MBM augmented with 100 mg /L of xylene, anthracene and pyrene aromatic hydrocarbons, respectively. The treatment set ups were incubated at 24.00 °C for 24 days. During incubation, 5 mL representative samples from the different sets of flasks were pipetted at intervals of 0, 4, 8, 12, 16, 20 and 24 days and the temperature, pH and bacterial growth (optical densities ($OD_{600\ nm}$)) of the media in different flasks were measured. The residual aromatic hydrocarbons were determined spectrophotometrically using ethyl acetate as the extraction solvent. For each sample, 5 mL of ethyl acetate was added and vigorously shaken manually. The organic and water layers from the media were separated by centrifugation at 5000 rpm for 20 mins. The water layers were disposed off 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 various hydrocarbons were determined as follows:

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

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

2.8 Thin Layer Chromatographic (TLC) Analysis for the Degraded Aromatic Hydrocarbons

This was carried out according to the method of Bennet et al. [26] as modified in this study in order to check whether the 100 mg /L of the aromatic hydrocarbons from the MBM as stated above were degraded and reduced into lesser and minor forms and parts by the existence of either small spots or by reduction in sizes in comparison to the controls. 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 phases of the three strains with the highest percentage of degradation on each of the three aromatic hydrocarbons was spotted and marked at the lower end of the TLC plate. Similarly, control spots of xylene, anthracene and pyrene solutions with medium were placed alongside. 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 taken out and air - dried at 28.00 ± 2.00ºC. 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 principles of light intensity and spot sizes.

2.9 Analysis of Data

All values were expressed as mean ± standard deviation and analyzed using Graph-Pad Prism statistical software version 7.00. Test of significance was performed on the data obtained using ordinary one-way analysis of variance (ANOVA) followed by post Tukey's, multiple comparison test. The results were considered statistically significant (P = .05) at 95 % confidence interval [19,26].

3. RESULTS

3.1 Screening Test

The result of the optical density (OD_{600} nm) determination of the aromatic hydrocarbon bacterial degraders isolated from Abonema sampled location is presented in Fig. 4. From the result, strains XYL2, ANT4 and PYR3 out of the 13 strains isolated were found to have the highest significant *(P* = .05) optical densities of 0.952 ± 0.004, 0.775 ± 0.007 and 1.041 ± 0.008 on xylene, anthracene and pyrene aromatic hydrocarbons. The result of the optical density (OD_{600} nm) determination of the aromatic hydrocarbon bacterial degraders isolated from Nembe sampled location is presented in Fig. 5. From the result, strains XYL7, ANT1 and PYR5 out of the 17 strains isolated were found to have the highest significant (P = .05) optical densities of 1.055 ± 0.002, 0.816 ± 0.007 and 0.933 ± 0.007 on xylene, anthracene and pyrene aromatic hydrocarbons. The result of the optical density (OD_{600} nm) determination of the aromatic hydrocarbon bacterial degraders isolated from Onne sampled location is presented in Fig. 6. From the result, strains XYL8, ANT6 and PYR9 out of the 18 strains isolated were found to have the highest significant (P = .05) optical densities 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 chosen as the most

potent degraders of xylene, anthracene and pyrene aromatic hydrocarbons.

3.2 Taxon of the Degrading Bacterial Isolates

The result of the macroscopic description of degrading bacterial isolates is presented in Table 1. 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 shiny in surface description. The result of the microscopic and biochemical features of the degrading bacterial isolates is presented in Table 2. From the result, most bacterial isolates were Gram negative, 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 Metabolism and Kinetics of Degradation

The result of the changes in temperature (°C), pH and optical density (OD_{600} nm) of medium during aromatic hydrocarbon degradation by marine bacterial isolates are presented in Tables 3, 4 and 5 while the results of the percentage weight reductions of xylene, anthracene and pyrene hydrocarbons by marine bacterial isolates are presented in Figs. 7, 8 and 9. From the Tables 3 - 5 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 Figs. 7 - 9 results, *Serratia marcescens* XYL7 had the maximum percentage degradations and reductions in weight of 99.50 ± 0.05 % and 60.00 ± 0.02 % for residual xylene and pyrene hydrocarbons, respectively while *Alcaligenes faecalis* PYR5 had highest percentage degradation and reduction in weight of 97.40 ± 0.01 % for residual anthracene hydrocarbons after 24 days biodegradation study.

3.4 Thin Layer Chromatography Study of the Organic Layer

The results of the thin layer chromatograms of xylene, anthracene and pyrene hydrocarbon degradations in the control set-up and their highest degraders are shown in Plates 1a – b, 2a – b and 3a – b. The results revealed that all the control set ups possess high and large intensity spots while the highest degraders *Serratia marcescens* XYL7 and *Alcaligenes faecalis* PYR5 had low and small intensity spots with evidence of spot size reductions 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 6 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, respectively.

4. DISCUSSION

The use of microbial bioremediation as an economical and environmental - friendly treatment tool for the protection of certain petroleum – polluted oceans, seas, estuaries and shoreline has gained extensive and growing interest since mechanical, physical and chemical alternatives are inadequate and ineffective [8]. Petroleum polluted soils and waters are potent sources of efficient hydrocarbon degraders as they are recommended for biodegradation and bioremediation treatment options [27]. In this study, a total of nine (9) strains ANT1, XYL2, PYR3, ANT4, PYR5, ANT6, XYL7, XYL8 and PYR9 out of the 48 isolates (9/48) suggesting 18.75 % of the isolates screened were chosen as the best adapted and potent degraders of xylene, anthracene and pyrene aromatic hydrocarbons which they use as sources of carbon and energy and are indicated by the highest optical densities for each strains and hydrocarbons (Figs. 4, 5 and 6). The result in Table 1 and Table 2 showed that most of the marine bacterial isolates were Gram negative, 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 Isiodu et al. [26] and Fagbemi and Sanusi [28] that two - third of most petroleum hydrocarbon degraders are Gram negatives with one - third being Gram positives. Previous studies have reported that a lot of rod - shaped bacteria have been implicated in hydrocarbon degradation studies and similar result was obtained in this study [26,28,29].

The result in Table 3 revealed that the temperature remained in optimal level of 26 °C conducive for the bacteria activities which is also observable in the control. The strains isolated in this study were mesophilic in nature thereby proving that mesophilic bacteria can degrade hydrocarbons. Similar result was observed by Athar et al. [30]. The result contradicts the report of Irshaid and Jacob [16] that the decrease in temperature from 30 – 25°C led to decrease in growth rates of xylene degrading bacteria from gasoline contaminated soil sites while whereas the growth rates almost terminated at 45 °C,. 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. The result in Table 4 showed that the drops in pH are probably because the degradation of the hydrocarbons by these bacteria resulted in the discharge 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 previous studies [16,25,31]. The result in Table 5 revealed that the degradation and utilization of these compounds resulted in increase in optical density (cell mass) of these bacteria and corroborates with the findings of John et al. [18] and Akinbankole et al. [32]. The previous study reported that cell growth study of anthracene and pyrene metabolizing bacteria revealed that only two strains KLA1022 (*B. toyonesis*) and JIP1005 (*S. enterica*) could utilize anthracene and pyrene hydrocarbons as source of nutrient for their growth and metabolism because there was substantial rise in the quantity of cells for the both strains. A decline in cell quantity was realized as the PAH concentration (sole carbon source) was decreased [32]. These differences could be attributed to the acclimatization of these bacterial species to exploit and thrive in the presence of petroleum or might be partly due to discrepancies among several physicochemical environmental factors, including divergent ambient environmental conditions, sediment organic carbons and structures [16].

The results in Figs. 7, 8 and 9 revealed that the degree of weight reductions was observed to rise with increase in incubation period but varied with different microbial species tested. *Serratia marcescens* XYL7 significantly demonstrated the highest abilities to degrade the aromatic hydrocarbons with 99.50 ± 0.05 % and 60.00 ± 0.02 % weight reductions in xylene and pyrene, respectively while *Alcaligenes faecalis* PYR5 significant degraded anthracene with 97.40 ± 0.01 % weight reduction after 24 days biodegradation study with proof of increased low optical density ($OD_{600\ nm}$) against their controls. However, the levels 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 detected in controls with no bacterial inocula. After deduction of xylene, anthracene and pyrene degradation by the abiotic factor, 94.30 ± 0.02 % and 49.90 ± 0.06 % of xylene and pyrene degradation was in fact aided by *Serratia marcescens* XYL7 while 92.80 ± 0.02 % of anthracene was aided by *Alcaligenes faecalis* PYR5 during this period. Discrepancies in xylene, anthracene and pyrene degradation abilities of the bacterial strains were evidently revealed even after 4 days of incubation which was further intensified during incubation periods (4 – 24 days) with very strongly significant positive relationship (r = 0.897 – 0.996). Thus, xylene, anthracene and pyrene were degraded by all the nine bacterial strains, but they varied extensively in their innate capabilities. These disparities could be due to high molecular weight aromatic hydrocarbons that are resistant to microbial attacks and the order of degradation is xylene > anthracene > pyrene. Biodegradation of aromatic hydrocarbons is depended on their chemical configurations and equivalent physiochemical properties and low molecular weight aromatic hydrocarbons are rapidly degrade rapidly than high molecular weight aromatic hydrocarbons [33]. Similar results are obtained by Akpe et al. [5] who observed that the higher the percentage of hydrocarbons degraded, the lower the optical density values. Previous study 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 [31]. In another previous study, it was 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 [34]. The findings of these authors supported the results of this study.

The results in Plates 1a – b, 2a – b and 3a – b evidently revealed that the 100 mg /L of xylene, anthracene and pyrene hydrocarbons from the assay medium were degraded and reduced into lesser, smaller and lighter fragments/sizes with clear confirmations of biodegradation losses of some aromatic hydrocarbon components. These losses, disappearances or reduction in spot sizes could be ascribed to the usage of aromatic hydrocarbons by marine isolates during the 24 days incubation period under ideal cultural settings of pH and temperature as sole carbon sources. Moreover, the findings upheld the results of the kinetics of degradation 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 the test anthracene showed small fragments with low intensities when compared with control having larger fragments with high intensities as visualized at 235 nm OD - UV illuminator. Previous study reported that the thin layer chromatographic study of the purified metabolites after the pyrene degradation course showed the presence of a single spot with Rf value of 0.65, which is similar to the reference Rf value of protocatechuic acid [35] and conformed with the pyrene Rf value in this study (Table 6). Also, in agreement with another previous study, the losses of pyrene and anthracene in the medium showed that they have been metabolized by the degrading bacteria [32].

Table 1. Macroscopic description of the degrading bacterial isolates

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

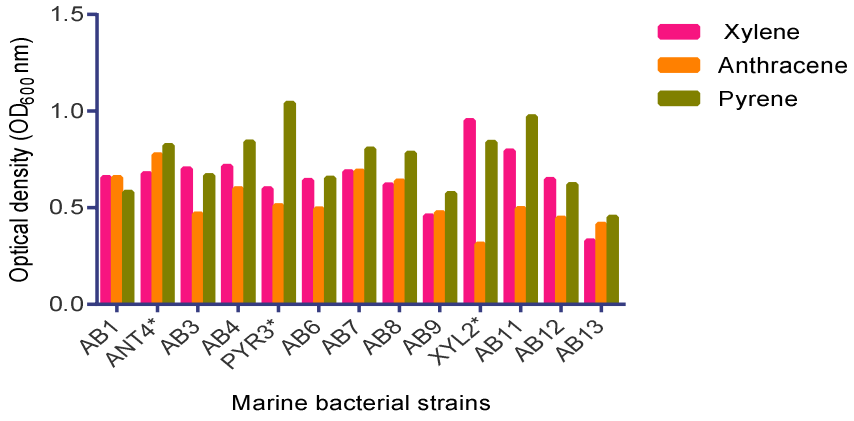


Fig. 4. Optical density (OD_{600} nm) determination of the aromatic hydrocarbon bacterial degraders isolated from Abonema sampled location

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

Table 2. Microscopic and biochemical features of the 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	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 3. Changes in temperature (°C) of medium during aromatic hydrocarbon 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 4. Changes in pH of the medium during aromatic hydrocarbon 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 5. Changes in the optical density (OD_{600} nm) of the medium during aromatic hydrocarbon 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 6. 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

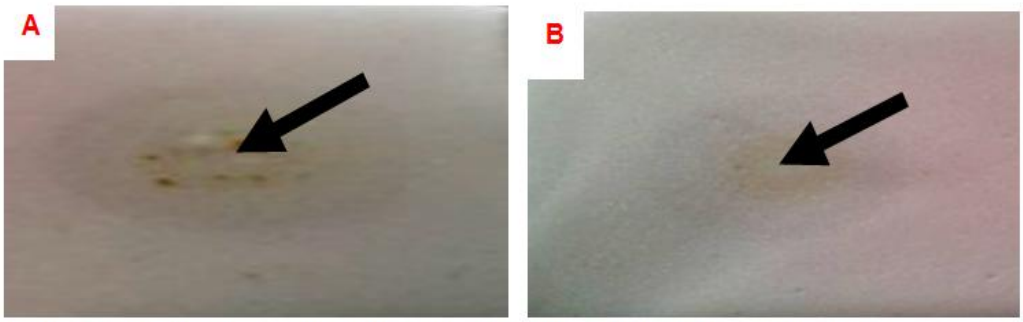


Plate 1a – b. Thin layer chromatograms of xylene hydrocarbon degradation

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.

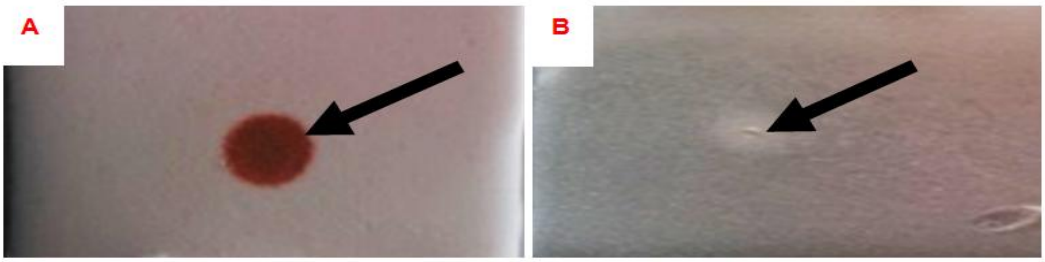


Plate 2a – b. Thin layer chromatograms of anthracene hydrocarbon degradation

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.

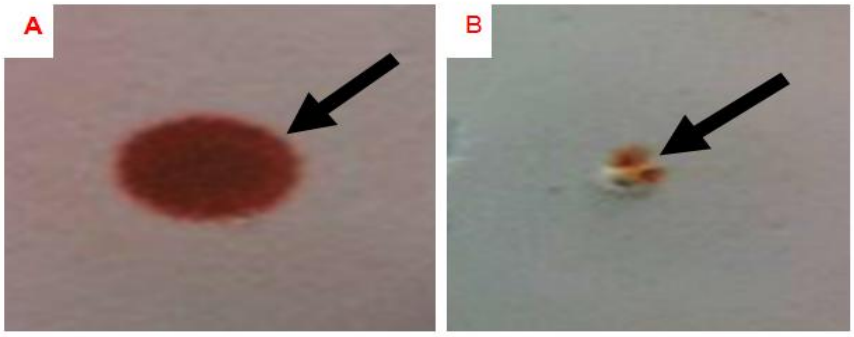


Plate 3a – b. Thin layer chromatograms of pyrene hydrocarbon degradation

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.

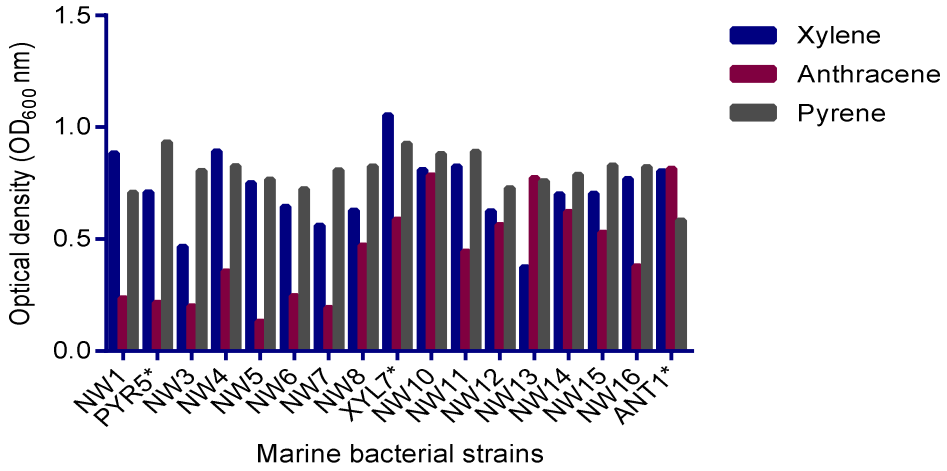


Fig. 5. Optical density (OD_{600} nm) determination of the aromatic hydrocarbon bacterial degraders isolated from Nembe sampled location

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

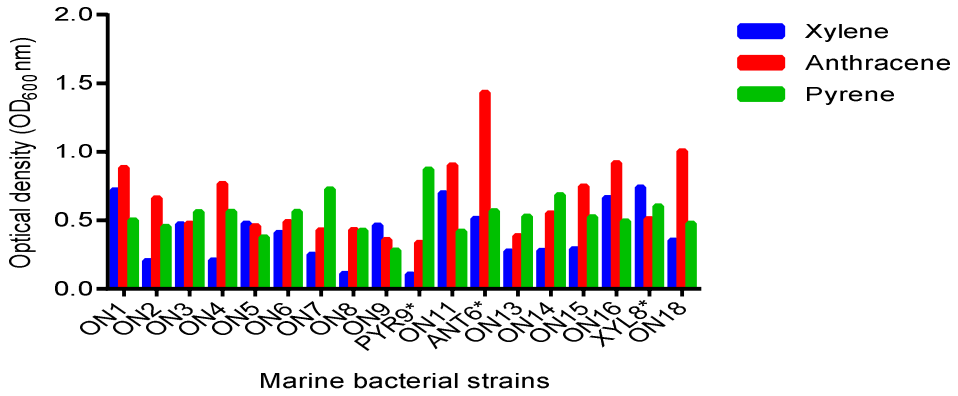
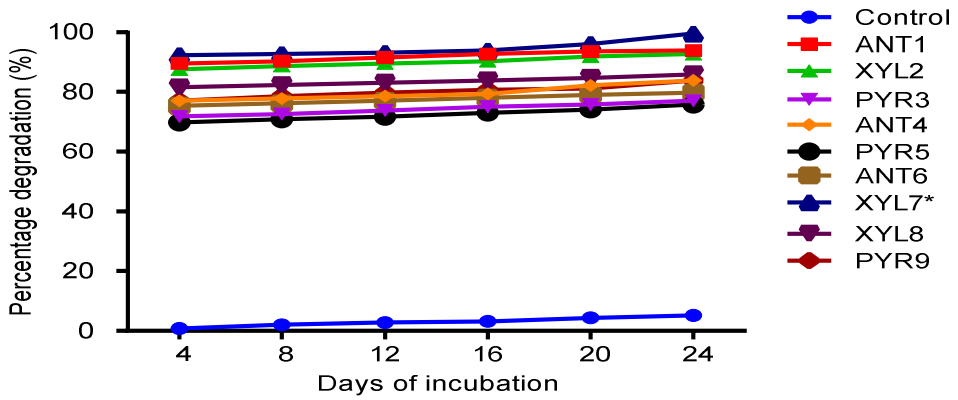


Fig. 6. Optical density (OD_{600} nm) determination of the aromatic hydrocarbon bacterial degraders isolated from Onne sampled location

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



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

Fig. 7. Percentage weight reductions of xylene hydrocarbons by marine bacterial isolates

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

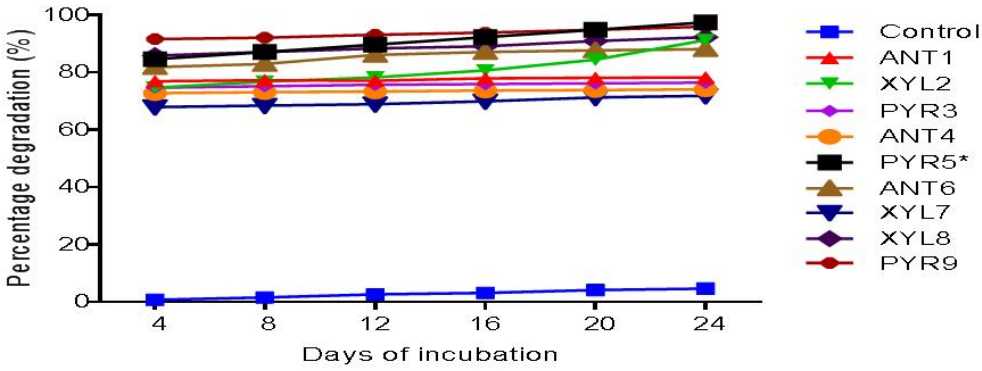


Fig. 8. Percentage weight reductions of anthracene hydrocarbons by marine bacterial isolates

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

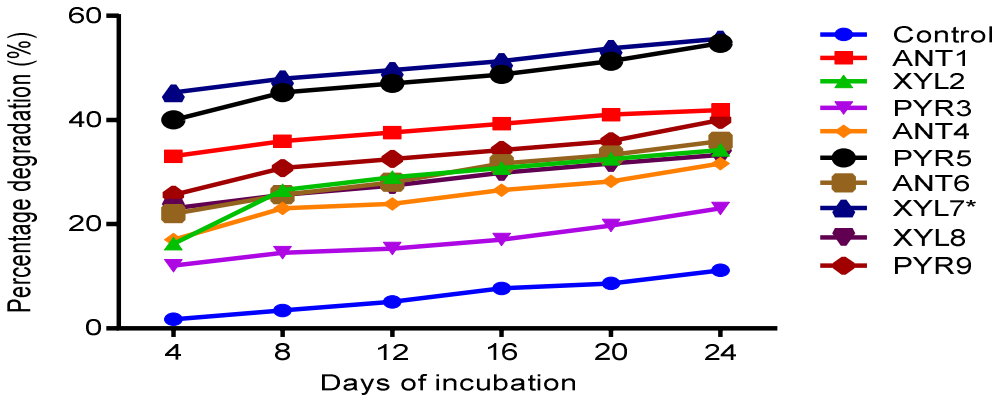


Fig. 9. Percentage weight reductions of pyrene hydrocarbons by marine bacterial isolates
** = Isolates with highest percentage degradability; values are mean ± standard deviation of triplicate determination.*

5. CONCLUSION

This study has shown that all the studied sites are sources of potent aromatic hydrocarbon degrading bacterial strains belonging to the genera: *Providencia, Alcaligenes, Brevundimonas, Myroides, Serratia, and Bacillus.* It also showed that the isolated bacteria especially *Serratia marcescens* XYL7 were able to significantly (*P* =.05) degrade mono and polyaromatic hydrocarbons. The growth rates for these isolates were moderately affected by temperature, pH and concentration of aromatic hydrocarbons. There were clear confirmations of biodegradation losses, disappearances or reductions in spot sizes of the aromatic hydrocarbon components indicating that the hydrocarbons in the medium have been reduced into smaller, lesser and lighter fragments by these strains of bacteria. Thus, the promising potentials of these bacterial strains especially *Serratia marcescens* XYL7 with regards to *in vitro* degradations and reductions of these pollutants could be exploited in bioremediation campaigns in Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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