South Asian Journal of Research in Microbiology

4(2): 1-12, 2019; Article no.SAJRM.49904

Hydrocarbon Degradation Potential of Heterotrophic Bacteria Isolated from Oil Polluted Sites in Sakpenwa Community in Rivers State

R. N. Akwukwaegbu[1*], P. O. Okerentugba[1], G. C. Okpokwasili[1], H. O. Stanley[1] and C. J. Ugboma[2]

[1]Department of Microbiology, University of Port Harcourt, Port Harcourt, Nigeria.
[2]Department of Microbiology, Rivers State University, Nkpolu Oroworukwo, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Author RNA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors POO and GCO managed the analyses of the study. Authors HOS and CJU managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SAJRM/2019/v4i230102
Editor(s):
(1) Dr. Mehdi Razzaghi-Abyaneh, Department of Mycology, Pasteur Institute of Iran, Iran.
(2) Dr. Osunsanmi Foluso Oluwagbemiga, Department of Biochemistry and Microbiology, University of Zululand, South Africa.
Reviewers:
(1) P. Saravana Kumari, Rathnavel Subramaniam College of Arts and Science, India.
(2) Daniel Delille, University Pierre and Marie Curie, France.
(3) Haruna Yahaya Ismail, University of Maiduguri, Nigeria.
Complete Peer review History: http://www.sdiarticle3.com/review-history/49904

Original Research Article

Received 14 May 2019
Accepted 24 July 2019
Published 30 July 2019

ABSTRACT

In this study, hydrocarbon degradation potentials of heterotrophic bacteria isolated from oil-polluted soil were examined. Samples were collected from Sakpenwa, an oil producing community in Tai LGA of Rivers State, Nigeria and analyzed for physicochemical and microbiological properties using standard techniques. Hydrocarbon utilizing bacteria (HUB) were isolated by vapour phase transfer method using mineral salt medium. The biodegradation study was carried out on a standard laboratory shaker for 30 days in Bushnell -Haas agar supplemented with 5% of crude oil. Fifteen (15) bacterial isolates were screened for hydrocarbon degradation potentials of which five isolates exhibited high hydrocarbon degradability. The following parameters were monitored using each of the five isolates and a consortium during the biodegradation study: Colour change, Optical density (OD), pH, Total Petroleum Hydrocarbon (TPH), Total Hydrocarbon Contents (THC) and

**Corresponding author: E-mail: adannerose91@gmail.com;*

Total Cuturable Heterotrophic Bacterial Counts (TCHBC). The mean TCHBC ranged from 1.65×107 to 2.27×108cfu/ml while the mean Total Culturable Hydrocarbon Utilizing Bacterial Counts ranged from 1.09×104 to 3.9×105. The optical density varied from 0.09±0.02 - 2.57±0.00 and pH ranged from 2.98±0.09 - 6.98±0.09. The optical density varied to .09±0.02 - 2.57±0.00 and pH ranged from 2.98±0.09 -0.98±0.09. The gravimetric analysis showed that *Bacillus* sp., *Pseudomonas* sp. *Alcaligenes* sp. and *Acinetobacter* sp. were able to degrade 96.90%, 99.60%, 99.20% and 99.70% of the hydrocarbons respectively. This study demonstrated that indigenous bacterial species were highly efficient in the biodegradation of petroleum hydrocarbons.

Keywords: Hydrocarbon; heterotrophic bacteria; polluted soil; degradation potentials.

1. INTRODUCTION

The world depends on petroleum, and its use as fuel has led to intensive economic development worldwide. The great need for this energy source has led to the gradual exhaustion of natural oil reserves and alteration in the balance of the ecosystem. The threat caused by environmental pollution due to the continuous release of petroleum and petrochemical products has been recognized as a significant and serious problem to man and his environment [1,2]. In Nigeria, 80% of the crude oil used is supplied from the South-South region of the country. Therefore, as a result of high oil exploration activities going on in this part of the country over years [3], substances like gaseous emissions, oil spills, effluents and solid waste are discharged into the environment, thus, polluting the environment [4].

The biotic component of the soil which occupied not greater than 5% soil space includes living microbes; bacteria, archaea and fungi which are responsible for 80-90% of soil processes and formation such as recycling of nutrients, transformation of organic matter, and maintenances of soil structure in microbial decomposers [5,6]. Since microorganisms in the soil are involved in various bio-geochemical processes, hydro-carbon pollutants entering the environment are susceptible to their activities and thus bioremediation largely depend on them. The degradation of hydrocarbons is influenced by many factors (temperature, relative humidity, soil structure, soil moisture, soil pH, soil biota, pollutant's structure, dose, toxicity, and bioavailability [7].

The aim of this study was to ascertain the hydrocarbon degradation potential of heterotrophic bacteria isolated from oil polluted sites in Sakpenwa community in Rivers State, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area and Sample Collection

The study site was located at the oil polluted sites in Sakpenwa community in Ogoni land, Tai Local Government Area, Rivers State, Nigeria. Soil samples were collected 500 m and 1000 m away from the major spill sites. Fifty grams (50g) of the oil-polluted soil samples were collected from each of the sampling points using a spade like soil sampler to excavate soil 7-10 cm depth from the surface of the soil. The collected soil samples were transported in plastic nylon bags from the polluted sites to the Department of Microbiology, University of Port Harcourt laboratory for analysis within 24 hours.

2.2 Samples Preparation

The soil samples collected were passed through a mesh sieve (2 mm pore size) to remove large particles and were thoroughly mixed. Thereafter, 5 g of each soil sample was suspended in 45 ml of distilled water. The suspended samples were mixed properly in a rotary shaker at 100 rpm at room temperature (28± 2ºC) for 90 minutes to liberate the organisms into the liquid medium [8].

2.3 Isolation and Enumeration of Total Heterotrophic Bacteria

The total culturable heterotrophic bacterial count for each degradation set-up was enumerated using the streak plate method [9]. Serial dilutions of the samples were made and 0.1ml aliquot of the 10^{-1} to 10^{-4} dilutions of each sample were transferred onto well dried, sterile nutrient agar plates composed of peptone 5.0 g; Beef extract 3.0 g; agar 15.0 g; Nacl 5.0g; in 100ml of distilled water and incubated at 37ºC for 24 hours (in triplicate). After incubation, the bacterial colonies that grew on the plates were counted and sub-cultured onto fresh nutrient agar plates using the streak-plate method in other to obtain pure

cultures of each colony. Discrete colonies on the plates were then transferred into nutrient agar slants, properly labelled and stored at 4ºC as a stock culture for preservation and identification [10].

2.4 Enumeration of Total Culturable Hydrocarbon Utilizing Bacteria (TCHUB)

The enumeration of Total Culturable Hydrocarbon Utilizing Bacteria (TCHUB) was done by applying the vapour phase method described by Atuanya and Ibeh [11]. Appropriate diluent of 0.5 ml of the samples collected from the two different set up (1000 m and 500 m away from the polluted site) labelled A and B respectively were inoculated into modified Mineral Salt Agar medium (MSA). The medium was made of 0.42 g $MgS0_4.7H_2O$; 0.297 g KCl; 0.85 g KH_2PO_4; 0.424 g $NaNO_3$; 1.27 g K_2HPO_4; 20.12 g NaCl; 250 mg Amphotericin B (sold as Fungizone) and 20 g agar powder. These were weighed out and hydrated in 1000 mL of sterile distilled water in a conical flask. The media was sterilized by autoclaving at 121ºC, 15Psi for 15 min, before dispensing into sterile Petri dishes. The gelled MSA was inoculated with 0.5 ml of serial dilutions of the polluted soil sites A and B sample respectively. Filter paper (Whatman No 1) was saturated with Bonny light crude oil, and the crude oil impregnated papers were aseptically placed onto the covers of Petri dishes and inverted. The hydrocarbon saturated filter papers supply hydrocarbon by vapour-phase transfer to the inoculums. The plates were incubated at 28ºC±2ºC for seven (7) days. Colonies were counted from triplicates and mean values were record.

2.5 Gram Staining

Isolates were subjected to Gram's reaction to check whether they are Gram-positive or Gram negative. A thin smear was made on a clean grease-free glass slide. The smear was air dried and heat-fixed by rapidly passing it over the flame of a Bunsen burner three times .The fixed smear was covered with crystal violet stain for 30 to 60 seconds and rapidly washed off with clean water and then flooded with Lugol's iodine for one minute. The iodine was washed off with clean water and the smear was decolorized with alcohol and washed immediately with clean water before counter staining with safranin for one minute. The stain was washed off with clean water and the smear air-dried and observed under microscope using oil immersion objective lens. Gram positive bacteria stained purple while gram negative bacteria stained red or pink [12].

2.6 Motility Test

Twenty-four hour (24 hr) culture of the isolates in peptone broth was used for motility test. Five drops of each isolate was placed on a cover slip and the concave shaped slide smeared with Vaseline at the edge of the concavity, gently on the cover slip. This slide was carefully inverted and the drop on the cover slip observed under high power objective lens (40×) [12].

2.7 Biochemical Tests

The bacterial isolates obtained were characterized and identified based on their cultural, morphological and biochemical characteristics using the scheme of Bergey's Manual of Determinative Bacteriology [13,14].

2.7.1 Catalase test

Three millilitres (3 ml) of 3% hydrogen peroxide solution was poured into different test tubes and sterile glass rod was used to introduce some colonies of the isolates into the test tubes containing the hydrogen peroxide. The contents of the test tubes were observed for gas bubbling. Catalase positive bacteria showed active bubbles while catalase negative did not [12].

2.7.2 Urease test

Christensen's solid medium was prepared by dissolving 1 g peptone; 5 g NaCl; 2 g K_2HPO_4 and 10 g agar in 1000 ml of distilled water. Phenol red (6 ml) was also added and the pH adjusted to 7.0. This was sterilized at 121ºC for 20 minutes at 15psi and allowed to cool to 50ºC. 10 ml of 10% solution of glucose and 100 ml of 20% urea solution were sterilized by filtration. The glucose and urea were mixed aseptically with the agar medium and dispensed in 5ml amounts into bijou bottles and allowed to solidify in the slope position. Urease positive bacteria indicated pink colour while urease negative bacteria did not show pink colour [12].

2.7.3 Sugar fermentation test

The test was carried out to determine the ability of the isolates to ferment various sugars which is indicated by the production of acids/gas. The following sugars were used: maltose, glucose

and lactose. From each sugar, 0.5 g was dissolved in 50 ml of peptone water and sterilized by membrane filtration. A pinch of phenol red was added as indicator and 5ml aliquots were aseptically dispensed into sterile test tube containing sterile Durham tubes which were inverted in the sterile broth. The broth was inoculated with the isolates using sterile wire loop and incubated at 30ºC for 48 hours. The content was observed for change in colour and/or the production of gas [12].

2.7.4 Citrate utilization test

This test was used to study the ability of organisms to utilize citrate present in Simon's medium as a sole source of carbon for growth. Simon's citrate agar was prepared, dispensed into test tubes, autoclaved and allowed to solidity in a slanting position. The isolates were streaked from freshly prepared cultures and incubated at 37ºC for 48 hours. The content of the test tubes were observed for the development of growth with blue colour as opposed to the original green colour of the medium which signifies citrate utilization [12].

2.7.5 Voges –proskauer test

The medium used for this test is glucose phosphate medium. After sterilization, the medium was allowed to cool and the test organism was inoculated into the broth and incubated for five days at 37ºC. After incubation, 1.5 ml of 5% alcoholic alpha napthtol and 0.5 ml of 40% aqueous KOH were added. The test tubes were shaken vigorously and allowed to stand for 5 minutes. The content was observed for the development of pink or red colour [12].

2.7.6 Methyl red test

About 5 drops of methyl red solution was added to 2 ml of a five- day old culture of the isolates inoculated in glucose-phosphate broth. Red colouration indicated a positive test while yellow colour indicated negative [12].

2.8 Biodegradation Studies

The method proposed by Ekpo and Ekpo [15] was used. The biodegradation study of hydrocarbons in the polluted soil was carried out using the Bushnell-Haas broth. This medium consisting of $MgSO_4$ 0.02 g; CaCl 0.2 g; K_2HPO_4 100 g; $KHPO_4$ 1 g; NH_4NO_3 1 g; F_2Cl 0.05 g was autoclaved in 2 litres conical flasks, and 99ml of the medium was dispensed into five (5) conical flasks into which 1 ml of sterile crude oil was added. Precisely, 5 ml of each of the bacterial isolates (in liquid broth) were inoculated into five (5) different conical flasks containing the liquid medium. The concentration of day zero was use as control to the other subsequent days. The bacterial cultures were incubated at ambient temperature (4ºC) in an electric shaker of 100 strokes per minute for 30 minutes each day. Sampling period was set for every 5 days for 30 days. Bacterial utilization of hydrocarbon was monitored using their optical density at 600nm wavelength.

2.9 Determination of Total Hydrocarbon Content (THC)

The total hydrocarbon content in the soil was determined by the solvent extraction method described in Chithra and Hema [16]. Five grams (5 g) of soil sample was mixed with 100 ml of normal hexane in a flask and corked. The mixture was shaken using a mechanical shaker for 1hr, and then allowed to settle. With the use of a sterile syringe, an aliquot of the oil extract in the solvent solution (20 ml) was withdrawn and put in a previously weighed evaporation dish. The dish and its content were evaporated to dryness in a rotary evaporator and the dish was reweighed to obtain the difference.

The percentage (%) of the degradation was obtained as follows:

Weight of residue crude oil = weight of beaker containing extracted crude – weight of empty beaker.

Amount of crude oil degraded= weight of crude oil added to the media – weight of residual crude oil.

$$\%\ Degradation = \frac{amount\ of\ crude\ oil\ degraded}{amount\ of\ crude\ oil\ added\ to\ the\ media} \times 100$$

2.10 pH, Optical Density and Colour Change Determination

HANNA HI983903 digital pH meter was use to determined the pH in a sample to water 1: 10. That is (20 g) of each sample was weighed into a beaker then 200ml of distilled water was added to it. The pH electrode was dipped into solution. The spectrumlab 7553 was used to measure the optical density by preparing (20 g) of each sample weighed into a beaker then 200 ml of

distilled water was added to it and placed in a test tube at a blank space of 600nm. Furthermore, 4 ml of the sample from biodegradation set up flask and diluted samples were mixed in a conical flask and colour change was monitored using the millimetre reading machine. Sampling period was set for every 5 days for 30 days. All analyses were carried in three replicates, and the mean values obtained were recorded.

2.11 Determination of Total Petroleum Hydrocarbon (TPH)

One gram (1 g) of the dried sample was weighed into a digestion flask and 20 ml of the acid mixture (650 ml of con.HNO_3 and 80 ml of perchoric acid and 20 ml of conc.H_2SO_4) added, before the flask was heated until a clear digestion is obtained. It was dilute with distilled water to the 25 ml mask of a conical flask, appropriate dilutions are then made for each experimental set up.

2.12 Statistical Analysis

Analysis of variance (ANOVA) was carried out to determine the significance levels between various treatments at 95% level of confidence, using Statistical Package for Social Sciences (SPSS, Version 20.0).

3. RESULTS

The bacteria present in the various study sites including the control as ascertained following standard microscopic, cultural and biochemical methods are shown in Table 1. The bacterial diversity present in the control soil, Site A (500 m) and Site B (1000 m) of this study are as represented in Table 2. The hydrocarbon utilizing bacterial isolates from the study sites are presented in Table 3.

The information from the degradation studies as shown in Table 4 were analysed and represented in bar charts. Changes in colour (Pcu) against Time(Days) (Fig. 1). Changes in total hydrocarbon content(%) against Time(Days) (Fig. 2). Changes in Optical Density (OD) against Time (Days) (Fig. 3). Changes in total petroluem hydrocarbon in (mg/ml) against Time (Days) (Fig. 4). Changes in pH against Time(Days) (Fig. 5), Changes in total cuturable heterotrophic bacteria counts (cfu/ml) against Time (Days) (Fig. 6).

4. DISCUSSION

Environment pollution caused by the release of a wide range of compounds as a consequence of industrial progress has assumed serious dimensions. To prevent development of hazardous waste the process of bioremediation has been suggested an excellent intervention measure. This present study screened for the hydrocarbon degrading potentials of bacteria isolated from oil polluted sites in Sakpenwa Community, Tai L.G.A, Rivers State, Nigeria.

Acinetobacter sp., *Alcaligenes* sp., *Pseudomonas* sp., Serratia sp., *Bacillus* sp. and Citrobacter sp. were isolated from the polluted soil. The isolation of *Bacillus* sp. is in agreement with the work of Okpokwasili and Okorie [17],

Table 1. Characterization of bacteria isolated from the study sites

Strain	1	2	3	4	5	6
Gram staining	-	-	+	-	-	-
Motility	+	+	+	+	+	+
Catalase	+	+	+	+	+	+
Butt stant	AB	BB	BB	AB	BB	AB
Citrate	+	+	+	+	-	+
Urease	-		+	+	+	-
Methlyred	+	-	-	-	-	-
V.P	-	-	+	+	+	+
Glucose	AG	A	A	A	A	A
Sucrose	-	-	-	-	-	-
Probable Organism	*Alcaligenes* sp.	*Pseudomonas* sp.	*Bacillus* sp.	*Acinetobacter* sp.	*Serratia* sp.	*Citrobacter* sp.

AG =acid growth, AB =acid and base, BB=base base, A= acid

who reported that *Bacillus* species are one of the predominant Gram positive organisms found in oil polluted areas. The microbial populations consisting of the various genera in this study have been detected in petroleum-contaminated soil in other studies as well [18,19]. Various strains of *Acinetobacter* sp. *Alcaligenes* sp. *Pseudomonas* sp., *Serratia* sp., *Bacillus* sp. and *Citrobacter* sp. play active roles in the hydrocarbon transformation processes [20,21].

The hydrocarbon-utilizing bacterial genera isolated from the oil contaminated soil (Tables 2 and 3) were *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Alcaligenes* and *Citrobacter*. Okpokwasili and Okorie [17] isolated similar hydrocarbon utilizing bacteria from Niger Delta aquatic systems. Chikere and Okpokwasili [19] also made similar findings on petroleum effluents. It has also been observed that some microorganisms are more abundant in areas of high concentration of hydrocarbons. These micro floras are actively oxidizing the hydrocarbons and this is considered as another source of carbon for use in the ecosystem. Individual microorganisms can metabolize only a limited range of hydrocarbon substrates; hence assemblages of the mixed populations with overall broad enzymatic capacities would be required to achieve considerable biodegradation of petroleum hydrocarbons, as is obtainable in a natural environment.

Table 2. Culturable bacterial presents in the various study sites

Control	Polluted site (500 m) away	Polluted site (1000 m) away
Acinetobacter sp.	*Pseudomonas* sp.	*Alcaligenes* sp.
Alcaligenes sp.	*Bacillus* sp.	*Citrobacter* sp.
Pseudomonas sp.	*Acinetobacter* sp.	*Bacillus* sp.
Serratia sp.		*Acinetobacter* sp.
Bacillus sp.		

Table 3. Culturable hydrocarbon utilizing bacterial isolates from the study sites

Control	Polluted site (500 m) away	Polluted site (1000 m) away
Acinetobacter sp.	*Pseudomonas* sp.	*Alcaligenes* sp.
Alcaligenes sp.	*Bacillus* sp.	*Citrobacter* sp.
Pseudomonas sp.	*Acinetobacter* sp.	*Bacillus* sp.
Bacillus sp.		*Acinetobacter* sp.

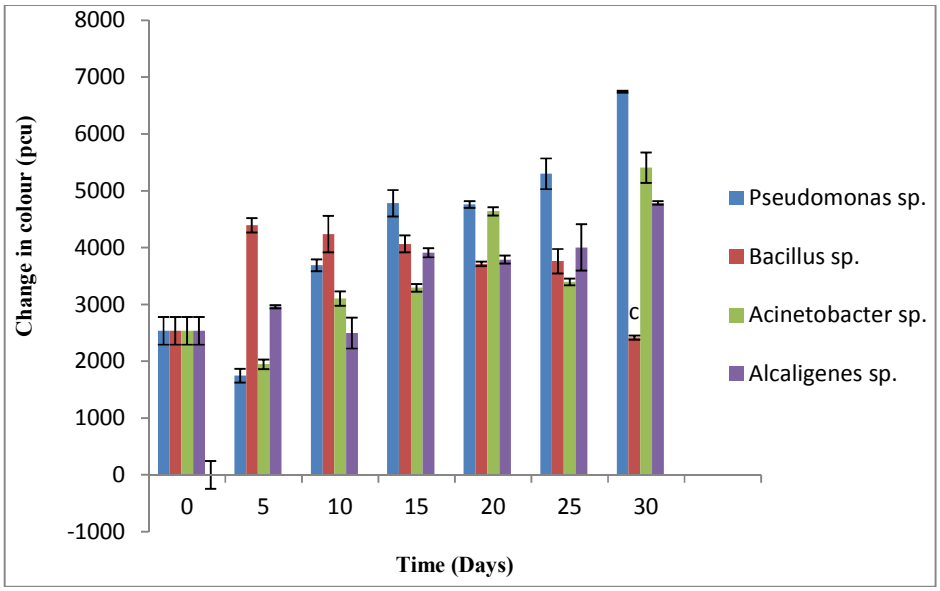

Fig. 1. Changes in colour (pcu) against time(days) caused by some of the isolates during thedegradation studies (data are mean ±s.d of triplicate determinations)

Table 4. Responses of selected hydrocarbon utilizing bacteria as used in biodegradation studies

Day	Parameters	*Pseudomonas* sp.	*Bacillus* sp.	*Acinetobacter* sp	*Alcaligenes* sp
0	Colour (pcu)	2536.67±234.79	2536.67±234.76	2536.67±243.79	2536.67±243.79
	OD	0.88±0.00	0.88±0.00	0.88±0.00	0.88±0.00
	pH	6.18±0.02	6.18±0.02	6.18±0.02	6.18±0.02
	TCHBC(cfu/ml)	1646.67±55.08	1180.00±20.00	1230.00±36.06	1310.00±45.83
	TPH (mg/ml)	4.58±0.00	4.54±0.01	4.58±0.00	4.58±0.00
	THC (%)	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
5	Colour (pcu)	1746.67±122.20	4393.33±125.03	1946.67±83.27	2060.00±30.00
	OD	1.29±0.07	1.44±0.06	1.04±0.03	1.16±0.03
	pH	4.59±0.34	5.57±0.01	5.46±0.08	2.98±0.08
	TCHBC(cfu/ml)	1656.67±65.06	2190.00±101.49	1230.00±20.00	1773.00±61.10
	TPH (mg/ml)	2.84±0.00	2.80±0.02	2.93±0.00	2.86±0.00
	THC (%)	97.67±0.58	94.67±0.58	97.00±1.00	97.33±1.53
10	Colour (pcu)	3690.00±105.83	4240.67±320.78	3103.33±127.41	2496.67±271.54
	OD	1.15±0.00	1.22±0.09	0.91±0.02	1.05±0.03
	pH	4.61±0.45	5.64±0.03	5.51±0.02	4.20±0.02
	TCHBC(cfu/ml)	2030.00±170.00	2273.33±70.24	1600.00±163.71	2346.67±50.33
	TPH (mg/ml)	1.08±0.00	1.08±0.00	1.29±0.00	1.15±0.01
	THC (%)	93.00±4.36	87.33±0.58	94.33±0.59	94.00±1.00
15	Colour (pcu)	4780.00±233.02	4060.67±148.44	3293.33±66.58	3910.00±80.00
	OD	0.25±0.05	1.27±0.00	1.15±0.00	1.23±0.01
	pH	4.99±0.02	5.93±0.04	5.55±0.01	4.53±0.01
	TCHBC(cfu/ml)	35333.33±305.05	15266.67±305.12	18000.00±964.37	13866.67±583.15
	TPH (mg/ml)	0.62±0.00	0.62±0.00	0.68±0.00	0.65±0.01
	THC (%)	82.00±2.31	65.00±1.00	71.33±1.53	90.33±1.53
20	Colour (pcu)	4760.00±60.83	3715.00±37.75	4640.00±72.86	3790.00±69.28
	OD	1.45±0.00	1.32±0.00	1.38±0.00	1.24±0.03
	pH	4.83±0.29	5.95±0.02	5.83±0.05	4.73±0.10
	TCHBC(cfu/ml)	48000.00±458.58	82666.67±541.63	56666.67±527.25	19000.00±590.00
	TPH (mg/ml)	0.12±0.00	0.16±0.00	0.08±0.00	1.16±0.00
	THC (%)	76.33±0.58	63.33±1.15	59.00±1.00	72.33±1.53
25	Colour (pcu)	5300.00±270.14	3760.00±216.56	3396.67±56.86	4003.00±408.57
	OD	1.38±0.00	1.54±0.01	1.44±0.01	1.26±0.05

Day	Parameters	*Pseudomonas* sp.	*Bacillus* sp.	*Acinetobacter* sp	*Alcaligenes* sp
	pH	4.99±0.03	6.01±0.02	5.83±0.05	4.81±0.04
	TCHBC(cfu/ml)	46000.00±0.00	34666.67±305.05	17366.67±105.87	50000.00±480.89
	TPH (mg/ml)	0.07±0.00	0.14±0.00	0.05±0.00	0.05±0.00
	THC (%)	72.33±1.53	60.33±0.58	57.33±1.53	70.67±2.52
	Colour (pcu)	6745.00±17.30	2416.67±35.12	5406.67±268.58	4786.67±32.15
	OD	1.77±0.00	2.39±0.01	2.50±0.01	1.33±0.01
	pH	5.26±0.04	6.30±0.08	8.21±0.14	5.30±0.08
	TCHBC(cfu/ml)	42666.67±305.05	34666.60±577.35	63333.33±287.51	51000.00±165.15
	TPH (mg/ml)	0.02±0.00	0.11±0.06	0.05±0.07	0.04±0.00
	THC (%)	68.33±2.01	53.33±4.04	52.67±1.53	70.10±0.58

Mean ± standard deviation of triplicate determination

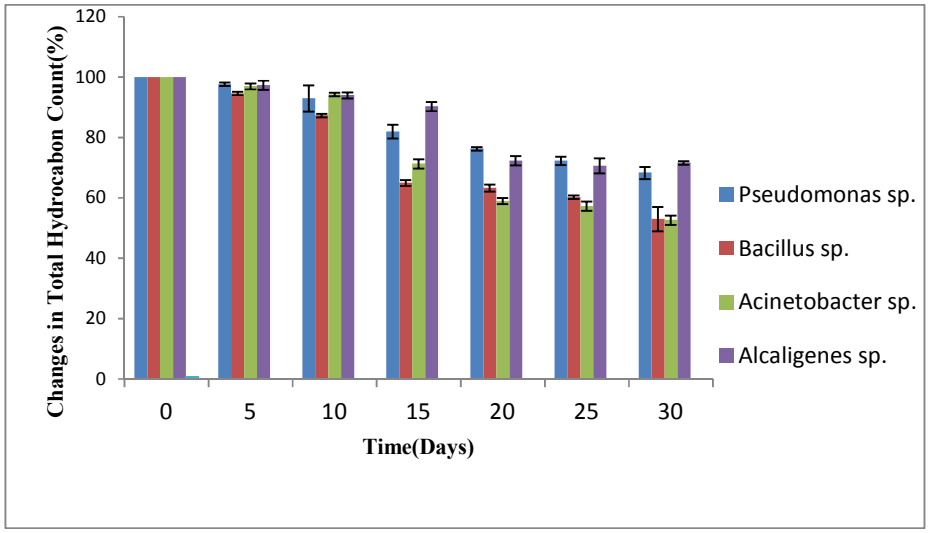

Fig. 2. Changes in total hydrocarbon content (%) against time(Days)caused by some of the isolates during the degradation studies (Data are mean ±S.D of triplicate determinations)

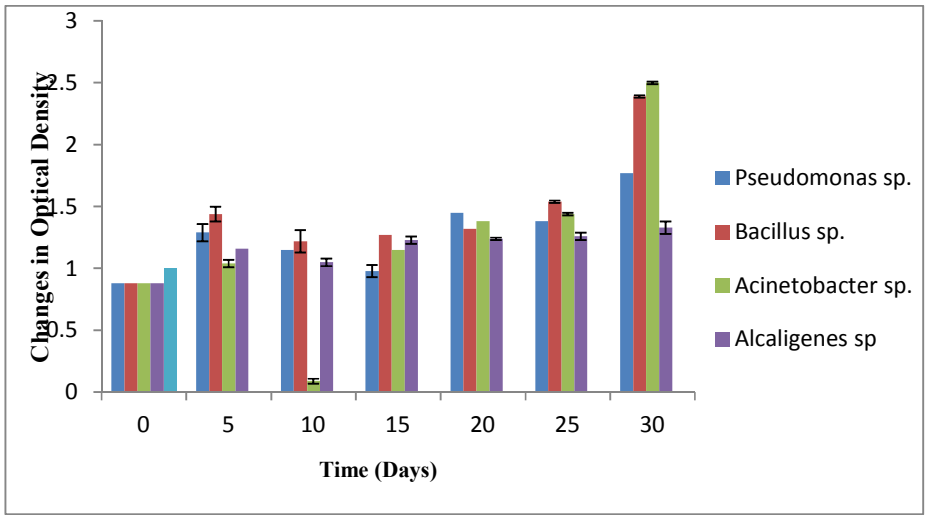

Fig. 3. Changes in optical density (OD) against time in (Days)caused by some of the isolates during the degradation studies (Data are mean ±S.D of triplicate determinations)

The physical parameters of the soil during the biodegradation study varied with the bacteria isolate used. For *Pseudomonas* sp. the colour change mean ranged from 2536.67±234.79Pcu to 6745.00 ±17.30Pcu, while Optical Density (OD) ranged from 0.88±0.00. The pH was drastically changing to acidic in the experiment as it ranged from 6.18±0.02 to 5.26±0. 05. The Total Culturable Heterotrophic Bacterial Count (TCHBC) increased from 1646.67±55.08 Cfu/ml to 42666.67±3055.05 Cfu/ml. Total petroleum Hydrocarbon (TPH) decreased from 4.58±0.01 Mg/ml to 0.02±0.00 Mg/ml, representing 99.6% reduction, which shows that *Pseudomonas* sp. is active in the degradation process. Similarly for *Bacillus* sp. colour change ranged from 2536.67±243.79Pcu to 2416.67 ±35.12Pcu, OD ranged from 0.88±0.00 to 2.39±0.01, pH from 6.18±0.02 to 6.30±0.08, TCHBC increased from 1180.00±20.00 Cfu/ml to 34666.6±7577.35 Cfu/ml, and TPH decreased from 4.58±0.00 Mg/ml to 0.11±0.06 Mg/ml, which is slightly lesser that for *Pseudomonas* sp. albeit statistically insignificant. For *Acinetobacter* sp. colour change ranged from 2536.67±243.79Pcu to 5406.67±268.58 Pcu; OD ranged from

0.88±0.00 to 2.50±0.01; pH from 6.18±0.02 to 8.21±0.14; TCHBC increased from 1230.00±36.06 Cfu/ml to 63333.33±28867.51 Cfu/ml while TPH decreased from 4.58±0.00 Mg/ml to 0.05±0.07 Mg/ml.For *Alcaligenes* sp. colour change ranged from 2536.67±243.79Pcu to 4786.67 ±32.15Pcu; OD ranged from 0.88±0.00 1.33±0.01; pH ranged from 6.18±0.02 to 5.30±0.08; TCHBC increased from 131.00±45.83 Cfu/ml to 51000.00 0±165.15 Cfu/ml. There was no statistical difference ($p>0.05$) in the utilization rate for the organisms.

The four isolates were considered as having the high degradation potentials. This report agrees with the works of Roseberg [22] which reported utilization of hydrocarbon as carbon source by hydrocarbon utilizers. The growth dynamics observed might either be due to their constitutive nature of hydrocarbon assimilating capabilities or adaptation of the strains as a result of previous exposure to exoge nous hydrocarbons [23]. This result also concord with the findings of Oboh et al. [14], which reported that hydrocarbon utilization is usually accompanied with pH

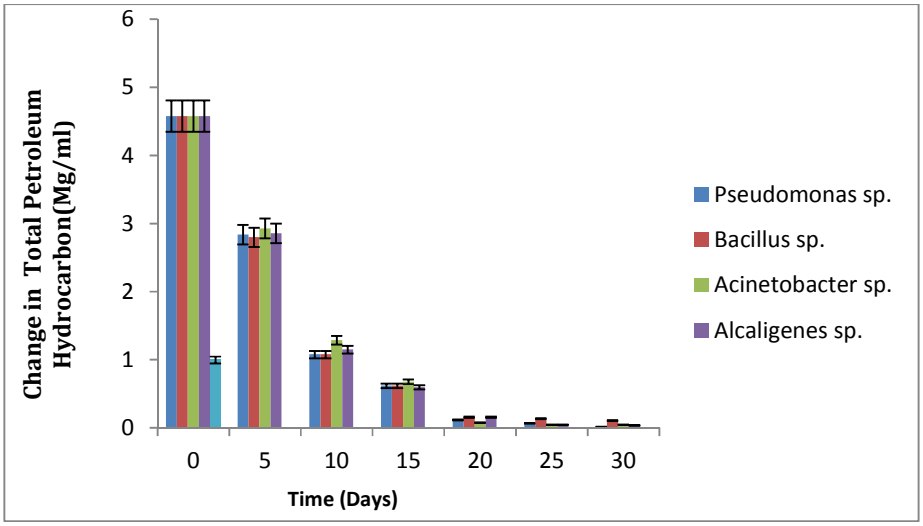

Fig. 4. Changes in total petroluem hydrocarbon in (mg/ml) against time in (Days)caused by some of the isolates during the degradation studies (Data are mean ±S.D of triplicate determinations)

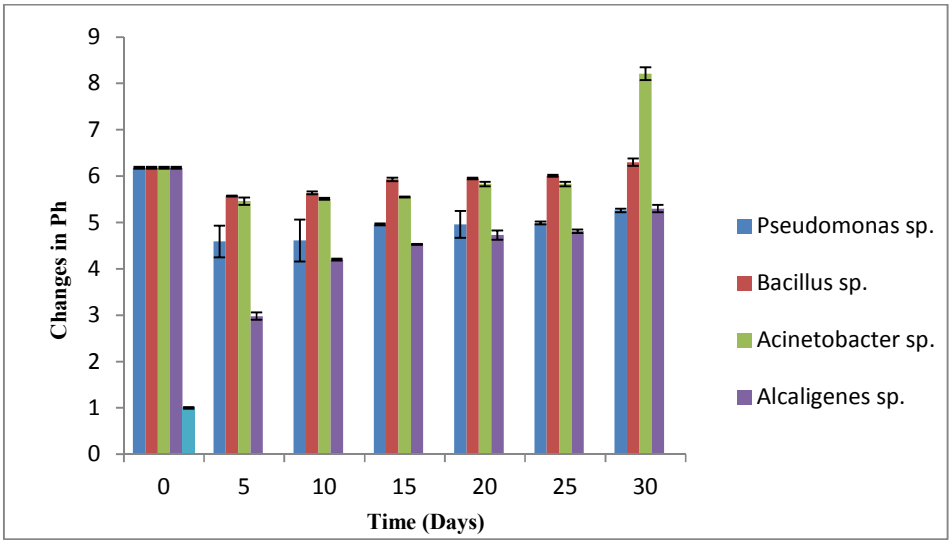

Fig. 5. Changes in pH against time in (Days) caused by some of the isolates during the degradation studies (Data are mean ±S.D of triplicate determinations

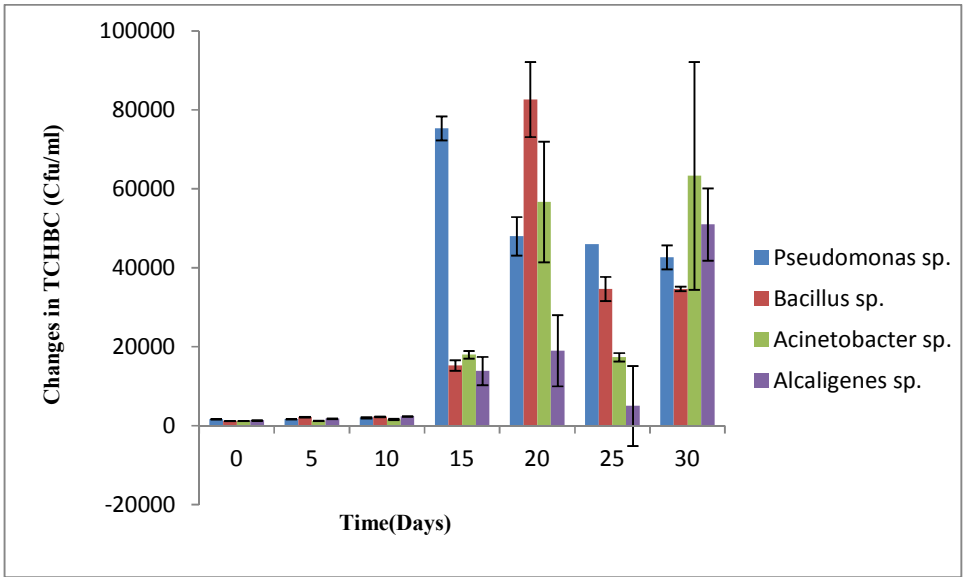

Fig. 6. Changes in total culturable heterotrophic bacteria counts (cfu/ml) against Time in (Days) caused by some of the isolates during the degradation studies (Data are mean ±S.D of triplicate determinations)

changes, an increase in total viable count with a decrease in pH of culture media. Colour change observed in the degradation screening test was as a result of increase in microbial population. The utilization of the hydrocarbons by these organisms leads to an increase in cell densities population [14].

These results reflect the need for further research and intense prospecting for novel organisms and genes with high crude oil (hydrocarbon) degrading potential in the southern region of Nigeria.

5. CONCLUSION

The elimination of petroleum hydrocarbon pollutants in the environment can be achieved by microbial degradation. Indigenous bacterial species were able degrade considerable amounts petroleum hydrocarbons in polluted soil. Further scale-up studies as applicable need to be carried out to ascertain the suitability of the crude oil degrading isolates for field application.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Okoh AI, Trejo-Hernandez MR. Remediation of petroleum hydrocarbon polluted systems: Exploiting the bioremediation strategies. Afr. J. Biotechnol. 2006;5:2520-2525.
2. Nwilo PC, Badejo OT. Impacts of Oil spill along the Nigerian Coast. Proceedings of the 1st International Congress on Petroleum Contaminated Soils, Sediments and Water, (PCSSW' 01), Imperial College, London; 2001.
3. Abu GO, Chikere BO. Cell surface properties of hydrocarbon–utilizing bacterial isolated from Port Harcourt marine environment. Nigeria Journal of Microbiology. 2006;20:809-816.
4. Nweke CO, Okpokwasili GC. Effects of bioremediation treatments on the bacterial populations of soil at different depths. Nigeria Journal of Microbiology. 2004;18: 363-372.
5. Nannnipperi AG, Rota R. Combined slurry and solid –phase bioremediation of diesel contaminated soils. Journal of Hazardous Materials. 2003;100(1):79–94.
6. Mikkonen A. Master thesis. Length heterogeneity PCR fingerprinting–A technique to monitor bacterial population dynamics during rhizoremediation of fuel oil contaminated soil: 12-60. Department of Applied Chemistry and Microbiology, University of Helsinki, Finland. 2008;1:1-129.

7. Ichor T, Okerentugba PO, Okpokwasili GC. Molecular characterization of aerobic heterohophic bacteria isolated from petroleum hydrocarbon polluted brackish waters of bodo creeks, Rivers State Nigeria. Open Journal of Ecology. 2014; 4:715-722.
DOI: org/10.4236/oje.2014.412061
8. Iheanacho CC, Okerentugba PO, Orji FA, Ataikiru TL. Hydrocarbon degradation potentials of indigeneous fungal isolates from a petroleum hydrocarbon contaminated soil in Sakpenwa community, Niger Delta. Global Advanced Research Journal of Environmental Science and Toxicology. 2014;3(1):006-011.
9. Odokuma LO, Okpokwasili GC. Role of composition in the biodegradation of dispersants. Waste Manage. 1992;12:39 – 43.
10. Odokuma LO, Ibor MN. Nitrogen fixing bacteria enhanced bioremediation of crude oil polluted, Global Journal of Environmental Sciences. 2002;8(4),455-468.
11. Atuanya EI, Ibeh IN. Bioremediation of crude oil contaminated loamy-sand and clay soils, Nigerian Journal of Microbiology. 2004;18:6373-6386.
12. Cheeshrough M. District Laboratory Manual for Tropical Countries, Part 2, Cambridge University Press, UK. 2005; 156.
13. Chikere BO, Okpokwasili GC. Enhancement of biodegradation of petrochemicals by nutrient supplementation. Nigerian Journal of Microbiology. 2003;17(2):130-135.
14. Oboh BO, Ilori MOI, Akinyemi JO, Adebusoye SA. Hydrocarbon degrading potentials of bacteria isolated from a bitumen (tarsand) deposit. Nature and Science. 2006;4(5):51-57.
15. Ekpo MA, Ekpo EI. Utilization of bonny light and bonny medium crude oil by microorganisms isolated from qua iboe River Estuarine. Nigeria Journal of Microbiology. 2006;20(1):832 – 839.
16. Chithra S, Hema SN. Biodegradation of crude oil by gravimetric analysis International Journal of Advanced Technology in Engineering and Science. 2014;(2):2348 – 7550.
17. Okpokwasili GC, Okorie BB. biodegradation potentials of microorganisms isolated from car engine lubricating oil. Tribulogy international. 1988;21:215-220.
18. Ilori MO, Amund OO, Ezeani CJ, Omoijiahina S, Adebusoye SA. Occurrence and growth potentials of hydrocarbon degrading bacteria on the phylloplane of some tropical plants. Africa Journal of Biotechnology. 2006;5(7):542-454.
19. Chikere BO, Okpokwasili GC. Enhancement of biodegradation of petrochemicals by nutrient supplementation. Nigerian Journal of Microbiology. 2003;17(2):130-135.
20. Cunliffe M, Kertesz MA. Effect of Sphingobium yanoikuyae B1 inoculation on bacterial community and polycyclic aromatic hydrocarbon degradation in aged and freshly PAH-contaminated soils. Environmental Pollution. 2006;144:228-237.
21. Ghazali FM, Rahman RNA, Saleh AB, Basri M. Biodegradation of hydrocarbons in soil by microbial consortium. Journal of International biodeterioration and biodegradation. 2004;54:61-67.
22. Roseberg E. Hydrocarbon oxidizing bacteria.
Available:http://en. Httpmall/1023/9334
23. Adebusoye SA, Ilori MO, Amund OO, Teniola OD, Olatope SO. Microbial degradation of petroleum hydrocarbons in a polluted tropical stream. Journal of Microbiology Biotechnology. 2007;23: 1149-1159.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/49904