Hydrocarbon degradation potential of heterotrophic bacteria isolated from oil-polluted sites in sakpenwa community in rivers state.

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, and analyzed for physicochemical and microbiological properties using standard techniques. Hydrocarbon utilizing bacteria (HUB) were isolated by vapour phase transfer method using the mineral salt medium. They were subjected to constant shaking in 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 bacterial isolates exhibited high hydrocarbon degradability. The degradation study was determined by monitoring colour change, Optical density (OD), pH, Total Petroleum Hydrocarbon (TPH), Total Culturable Heterotrophic Bacterial Counts (TCHBC) and Total Hydrocarbon Contents (THC) respectively for each isolate. The mean total culturable heterotrophic bacterial counts ranged from 1.65×10^{7} to 2.27×10^{8}cfu/ml while the mean total culturable hydrocarbon utilizing bacterial counts ranged from 1.09×10^{4} to 3.9×10^{5}. The optical density varied between 0.09±0.02 - 2.57±0.00 and pH ranged from 2.98±0.09 to 6.98±0.09.

KEYWORDS: hydrocarbon, heterotrophic bacteria, polluted soil, degradation potentials

INTRODUCTION

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 (Abu and Chikere, 2006), substances like gaseous emissions, oil spills, effluents and solid waste are discharged into the environment, thus, polluting the environment (Nweke and Okpokwasili, 2004). From statistics, the biotic component of the soil occupied not greater than 5% soil space; living microbes including bacteria, archaea and fungi 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 (Nannipieri *et al*. 2003). Since microorganisms in the soil are involved in various biogeochemical processes, soil activities largely depend on them (Mikkonen, 2008).

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). A study on the degradation of hydrocarbon by aerobic heterotrophic bacteria by Ichor *et al.,* (2014), took cognisance of some of these factors.

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. Soil samples were collected 500m and 1000m 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 soil sampler. The collected soil samples were transported in plastic nylon bags from the polluted sites to the Department of

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Microbiology, University of Port Harcourt laboratory for analysis within 24 hours (Iheanacho *et al*., 2014).

2.2 Samples preparation

The soil samples collected were passed through a mesh sieve (2mm pore size) to remove large particles and were thoroughly mixed. Thereafter, 5g 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± 2^0C) for 90 min to liberate the organisms into the liquid medium (Iheanacho *et al*., 2014). The pH of the samples was also noted.

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 (Odokuma and Okpokwasili, 1992). Serial dilutions of the samples were made and a 0.1ml aliquot of the 10^{-1} to 10^{-4} dilutions of each sample was transferred onto well dried, sterile nutrient agar plates (in triplicate) and incubated at 37°C for 24. 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 4^0C as a stock culture for preservation and identification (Odokuma and Ibor, 2002).

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, (2004). Appropriate diluent of 0.5ml of the samples collected from the two different set up labelled A and B respectively were inoculated into modified Mineral Salt Agar medium (MSA). The medium was made of 0.42g $MgS0_4.7H_2O$; 0.297g KCl; 0.85g KH_2PO_4; 0.424g $NaNO_3$; 1.27g K_2HPO_4; 20.12g NaCl; 250mg Amphotericin B (sold as Fungizone) and 20g agar powder. These were weighed out and hydrated in 1000mL of sterile distilled water in a conical flask. The media was sterilized by autoclaving at 121°C, 15Psi for 15min, before dispensing into sterile Petri dishes. The gelled Mineral Salt Agar (MSA) was inoculated with 0.5ml of all 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 (Chikere and Chijioke-Osuji, 2006). The plates were incubated at 28°C±2°C for seven (7) days. Colonies were counted from triplicates and mean values were record and colonies formed were stored as stock cultures at a temperature of 4^0C for preservation and identification.

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 decolourized with alcohol and washed immediately with clean water before counterstaining with safranin for one minute. The stain was washed off with clean water and the smear air-dried and observed under the microscope using oil immersion objective lens. Gram-positive bacteria stained purple while gram-negative bacteria stained red or pink (Cheesbrough, 2005).

2.6 Motility test

Twenty-four hour (24hr) culture of the isolates in peptone broth was used for motility test. Five drops of each isolate were placed on a coverslip and the concave shaped slide smeared with Vaseline at the edge of the concavity, gently on the coverslip. This slide was carefully inverted and the drop on the coverslip observed under high power objective lens (40×) (Cheesbrough, 2005).

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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 (Chikere and Okpokwasili, 2003; Oboh *et al*., 2006).

2.7.1 Catalase test

Three millilitres (3ml) 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 (Cheeshrough, 2005).

2.7.2 Urease test

Christensen's solid medium was prepared by dissolving 1g peptone; 5g NaCl; 2g K_2HPO_4 and 10g agar in 1000 ml of distilled water. Phenol red (6ml) was also added and the pH adjusted to 7.0. This was sterilized at 121^0C for 20 minutes at 15psi and allowed to cool to 50^0C. 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 (Cheeshrough, 2005).

2.7.3 Sugar fermentation test

The test was carried out to determine the ability of the isolates to ferment various sugars which are indicated by the production of acids/gas. The following sugars were used: maltose, glucose and lactose. From each sugar, 0.5g was dissolved in 50ml of peptone water and sterilized by membrane filtration. A pinch of phenol red was added as the indicator and 5ml aliquots were aseptically dispensed into the sterile test tube containing sterile Durham tubes which were inverted in the sterile broth. The broth was inoculated with the isolates using a sterile wire loop and incubated at 30^0C for 48 hours. The content was observed for change in colour and/or the production of gas (Cheeshrough, 2005).

2.7.4 Citrate utilization test

Simon's citrate agar was prepared, dispensed into test tubes, autoclaved and allowed to solidify in a slanting position. The isolates were streaked from freshly prepared cultures and incubated at 37^0C for 48 hours. The content of the test tubes was observed for the development of growth with blue colour as opposed to the original green colour of the medium which signifies citrate utilization (Cheeshrough, 2005).

2.7.5 Voges –proskauer test

The medium used for this test is the 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 37^0C. After incubation, 1.5ml of 5% alcoholic alpha-naphthol 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 (Cheeshrough, 2005).

2.7.6 Methyl red test

About 5 drops of methyl red solution were added to 2ml of a five- day old culture of the isolates inoculated in glucose-phosphate broth. Red colouration indicated a positive test while the yellow colour indicated negative text (Cheeshrough, 2005).

2.8 Statistical analysis

Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS, Version 20.0). Analysis of variance (ANOVA), P- values test of significance, was carried out at 95% level of confidence, P - values were used to determine the significance levels between various treatments and data obtained during the study.

RESULTS AND DISCUSSION

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The prevailing soil bacteria present in the various study sites including the control were ascertained following standard microscopic, cultural and biochemical methods. The various identification techniques used for the characterization of the bacterial isolates were as shown in Table 1. The bacterial diversity present in the Control soil, Site A (500m) and Site B (1000m) of this study were as represented in Table 2 These isolated bacteria were subjected to hydrocarbon degradation test in order to ascertain those that possess the potential to degrade the hydrocarbon component of the crude oil Table 3.

Table 1: Characterization of bacteria isolated from the study sites.

Strain	1	2	3	4	5	6
Gram	–	–	+	–	–	–
stainin	+	+	+	+	+	+
g	+	+	+	+	+	+
Motility	+	–	–	+	–	+
Catalase	–	–	–	–	–	–
Glucose	AB	BB	BB	AB	BB	AB
Lactose	+	–	–	+	–	–
Butt stant	–	–	+	–	–	+
Gas	+	–	+	+	–	+
H_2S	–	+	+	+	+	–
Citrate	+	+	–	–	–	–
Urease	–	–	+	+	+	+
Methyl red	AG	A	A	A	A	A
V.P	–	–	–	–	–	–
Glucose						
Sucrose	*Alcaligenes*	*Pseudomonas*	*Bacillus*	*Acinetobacter*	*Serratia*	*Citrobacter*
Probable	sp.	sp.	sp.	sp.	sp.	sp.
Organism						

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

Table 2: Culturable bacterial diversity presents in the various study sites

Control	Polluted site (500m) away	Polluted site (1000m) 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.

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

174
175 In Table 4, the physical parameter for *Pseudomonas sp* was recorded for 30 days at 5 days
176 interval. The colour change means ranged from 2536.67±234.79Pcu on day 0 to 6745.00
177 ±17.30Pcu for 30 days measured in Cobalt per Unit, while Optical Density ranged from
178 0.88±0.00 in day 0 to 1.77±0.00 in 30 days. The pH was drastically changing to acidic in the
179 experiment as it ranged from 6.18±0.02 to 5.26±0. 05. The Total Culturable Heterotrophic
180 Bacterial Count (TCHBC) measured in Cfu/ml increases from 1646.67±55.08 on the previous
181 day to 42666.67±3055.05 for day 30. (TPH) decreased from 4.58±0.01 Mg/ml to
182 0.02±0.00Mg/ml, this show that *Pseudomonas sp.* is active in the degradation process. Then
183 the Total Hydrocarbon Content (THC) decrease in percentage to 68.33 ±2.01%.
184 Similarly, the physical parameter for *Bacillus sp*. was recorded for 30 days at 5 days interval,
185 the colour change means ranged from 2536.67±243.79Pcu on day 0 to 2416.67 ±35.12Pcu for
186 30 days measured in Cobalt per Unit, while Optical Density ranged from 0.88±0.00 from day
187 0 to for 30 days 2.39±0.01. There was a slight increase in pH as it ranged from 6.18±0.02 to
188 6.30±0.08. The Total Culturable Heterotrophic Bacterial Count (TCHBC) measured in
189 Cfu/ml increases from on the first day of 1180.00±20.00 to 34666.6±7577.35 for 30 days.
190 The Total Petroleum Hydrocarbon (TPH) decreased from 4.58±0.00 Mg/ml to 0.11±0.06
191 Mg/ml, this show that *Bacillus sp.* is really degrading the hydrocarbon but at a slow rate.
192 Then the Total Hydrocarbon Content THC) decrease in percentage to 53.33± 4.04%.
193 Furthermore, the physical parameter results for *Acinetobacter sp.* were recorded for 30 days
194 at 5 days interval, the colour change means ranged from 2536.67±243.79Pcu on day 0 to
195 5406.67±268.58 Pcu for 30 days measured in Cobalt per Unit, while Optical Density ranged
196 from 0.88±0.00 from day 0 to for 30 days 2.50±0.01. There was a drastic increase in pH from
197 6.18±0.02 to 8.21±0.14. The Total Culturable Heterotrophic Bacterial Count (TCHBC)
198 measured in Cfu/ml increases from on the first day 1230.00±36.06 to 63333.33±28867.51 for
199 30 days. The Total Petroleum Hydrocarbon (TPH) decreased from 4.58±0.00 Mg/ml to
200 0.05±0.07 Mg/ml, this shows that *Acinetobacter sp.* is really degrading the hydrocarbon but
201 at a slow rate. Then the Total Hydrocarbon Content (THC) decrease in percentage to
202 52.67±1.53 %.
203 Similarly, the physical parameter for *Alcaligenes* sp. was recorded for 30 days at 5 days
204 interval, the colour change means ranged from 2536.67±243.79Pcu on day 0 to 4786.67
205 ±32.15Pcu for 30 days measured in Cobalt per Unit, while Optical Density ranged from
206 0.88±0.00 from day 0 to for 30 days 1.33±0.01. There was a slight decrease in pH as it
207 ranged from 6.18±0.02 to 5.30±0.08. The Total Culturable Heterotrophic Bacterial Count
208 (TCHBC) measured in Cfu/ml increases from on the first day 131.00±45.83 to 51000.00
209 0±165.15 for 30 days. The Total Petroleum Hydrocarbon (TPH) decreased from 4.58±0.00
210 Mg/ml to 0.04±0.00 Mg/ml, this shows that *Alcaligenes sp.* is really degrading the
211 hydrocarbon. Then the Total Hydrocarbon Content (THC) decrease in percentage to 71.67±
212 0.58%.
213 However, the strain identifies as *Pseudomonas* sp. possess a steady increase in optical density
214 (OD) from 0.88 to 1.29, which raises the pH from 4.59 to 5.26 and increase in colour change
215 from 2536.67pcu to 6745.00pcu after 30 days of degradation monitoring As shown in Table
216 4.5. OD increases slowly as seen in the second strain identified as *Bacillus* sp. from 0.88 to

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217 2.39, pH of 5.57 – 6.30 and shows a decrease in a colour change from 4393.67pcu to
218 2416.67pcu during the degradation study. There was a steady increase in OD as seen in
219 *Acinetobacter* sp. which increases from 0.88 to 2.50 for 30 days which raises the pH from
220 5.46 to 8.21 and an increase in a colour change of 2536.67pcu to 5406.67pcu. Fourth Strain
221 identified as *Alciligenes* sp. showed an increase in OD from 0.88 to 1.33, while pH increases
222 from 2.98 to 5.30 and an increase in colour change shows a wide range of 2536.67pcu to
223 4786.67pcu.
224
225 Table 4: Responses of selected hydrocarbon utilizing bacteria as used in biodegradation
226 studies
227

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
	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

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	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
30	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

228 **Mean ± standard deviation of triplicate determination**
229
230
231 The information from the degradation studies as shown in the table above (Table 4) were
232 analysed and represented in bar charts. Changes in colour (pcu) against Time(Days) (Figure
233 1), Changes in total hydrocarbon content(%) against Time(Days) (Figure 2), Changes in
234 Optical Density (OD) against Time(Days) (Figure 3), Changes in total petroleum
235 hydrocarbon in (mg/ml) against Time (Days) (Figure 4) Changes in pH against Time(Days)
236 (Figure 5), Changes in total culturable heterotrophic bacteria counts (cfu/ml) against
237 Time(Days) caused by some of the isolates during the degradation studies were represented.
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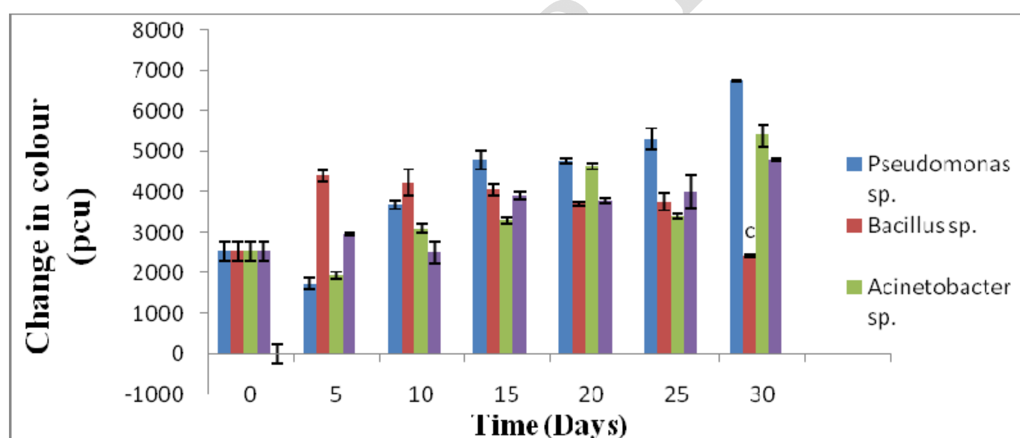

242
243 a
244
245 Figure 1: Changes in colour (pcu) against Time(Days) caused by some of the isolates during
246 the degradation studies (Data are mean ±S.D of triplicate determinations)
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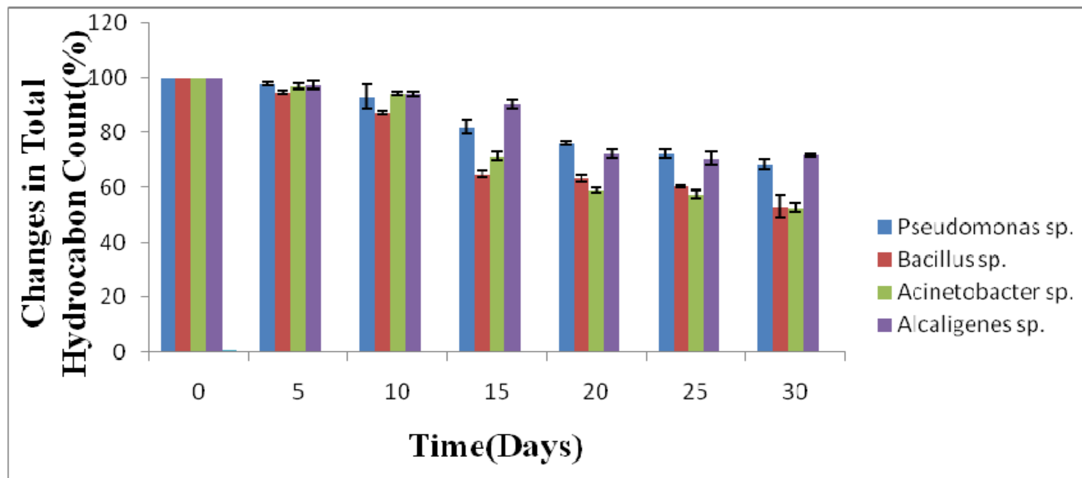
249



250 Figure 2: Changes in Total Hydrocarbon Content(%) against Time(Days) caused by some of
251 the isolates during the degradation studies (Data are mean ±S.D of triplicate
252 determinations)
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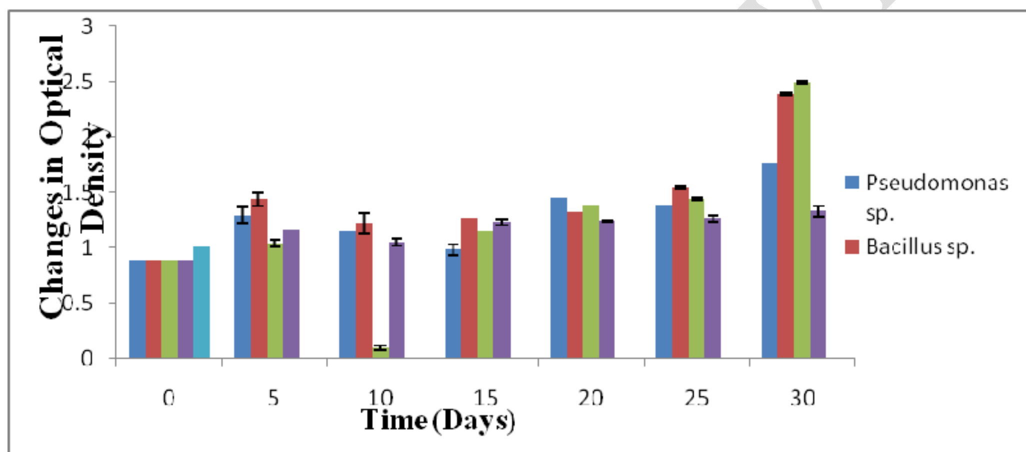

255 Figure 3: Changes in Optical Density (OD) against Time in (Days) caused by some of the
256 isolates during the degradation studies (Data are mean ±S.D of triplicate
257 determinations)
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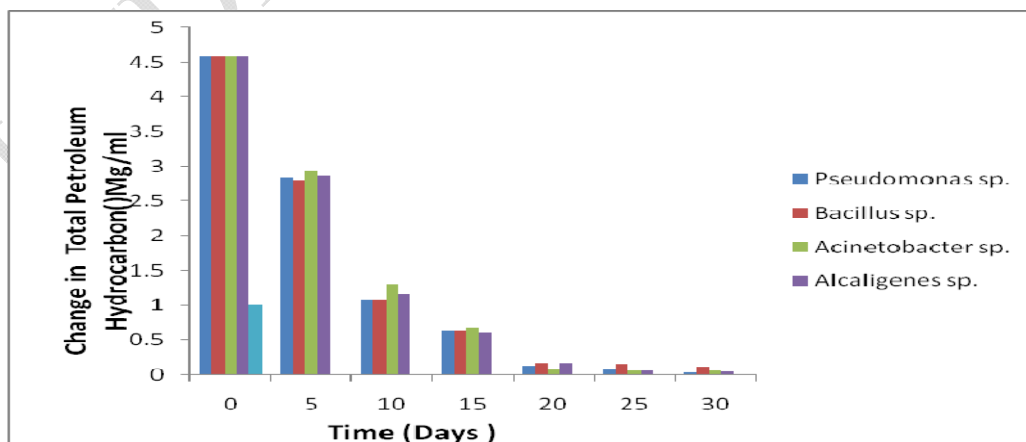
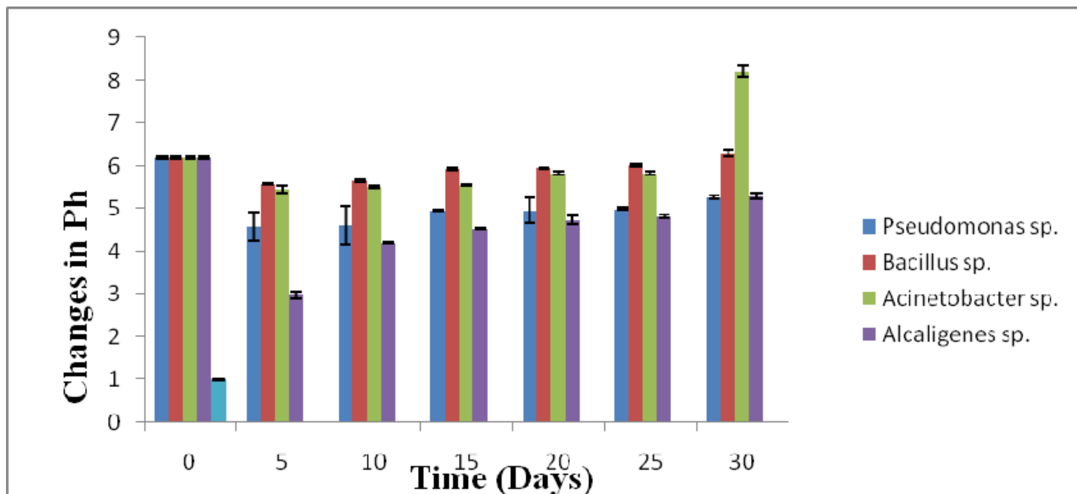


261 Figure 4: Changes in total petroleum hydrocarbon in (mg/ml) against Time in (Days) caused
262 by some of the isolates during the degradation studies (Data are mean ±S.D of
263 triplicate determinations)



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266 Figure 5: Changes in pH against Time in (Days) caused by some of the isolates during the
267 degradation studies (Data are mean ±S.D of triplicate determinations.
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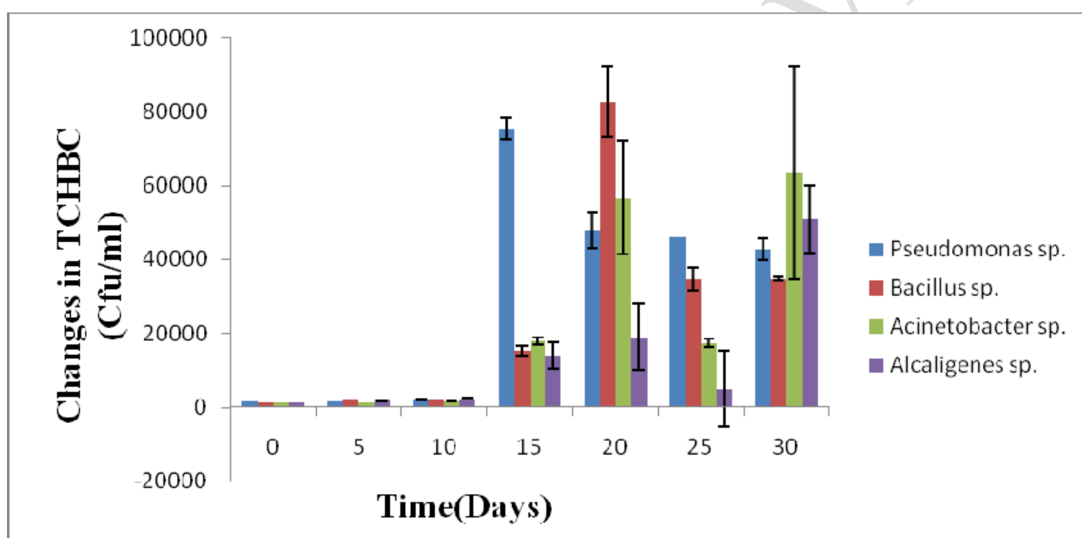

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270 Figure 6: Changes in total culturable heterotrophic bacteria counts (cfu/ml) against Time in
271 (Days) caused by some of the isolates during the degradation studies (Data are mean
272 ±S.D of triplicate determinations)
273
274 The countability of these bacteria to be predominantly Gram- negative is contrary to Austin *et*
275 *al.* (1997) who isolated predominantly gram-negative hydrocarbon utilizing bacteria from soil
276 and the aquatic environment. The isolation of *Bacillus* species is in agreement with the work
277 of Okpokwasili and Okorie (1990), who found that *Bacillus* species could also be one of the
278 predominant Gram-positive organisms found in oil-polluted areas
279 These findings showed that the biodegradation of complex hydrocarbons in nature usually
280 required the cooperation of more than a single species. The microbial populations consisting
281 of the strains belonging to various genera have been detected in petroleum-contaminated soil
282 (Ilori *et al,* 2006; Kim and Crowley 2007). This suggested that the strains from various
283 genera have their roles in the hydrocarbon transformation processes (Ghazali *et al,* 2004;
284 Cunliffe and Kertesz 2006). Individual microorganisms can metabolize only a limited range
285 of hydrocarbon substrates; hence assemblages of the mixed populations with overall broad
286 enzymatic capacities would be required to achieve considerable biodegradation of petroleum
287 hydrocarbons.

288 Statistical analysis of the result shows that there is a significant difference between various
289 heterotrophic bacteria and soil samples.
290
291 **CONCLUSION**
292 Environmental pollution caused by the release of a wide range of compound as a
293 consequence of industrial progress has assumed serious proportions. To prevent the
294 development of hazardous waste the process of bioremediation has been followed. Our
295 present study follows the isolation of hydrocarbon-degrading bacteria from oil-polluted sites.
296 The sample was collected from contaminated sites of Sakpenwa Community, Tai L.G.A,
297 Rivers State, These were brought them to the laboratory and isolation was done on the basis
298 of gram staining. Biochemical tests were performed with isolates. The isolates were screened
299 for their oil-degrading capacity.
300 Having knowledge of the processes and factors involves in the biodegradation experiment is
301 of great ecological significance in society. The elimination of oil spilt in the environment can
302 be achieved by microbial degradation when added up with some physical and chemical
303 methods
304 Further scale-up studies as the applications need to be carried out in increasing the degrading
305 ability and stability of the crude oil degrading isolate and its usage as a possible commercial
306 strain
307 We concluded that the use of consortium species makes biodegradation study more effective.
308
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