

Detection of Heterotrophic Bacteria That Are Able to Degrade Hydrocarbon in Soil Around IZOMBE Community of OGUTA Local Government Area, Imo State

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Abstract - This study investigated the presence and hydrocarbon-degrading capabilities of indigenous heterotrophic bacteria in oil-polluted soils from Izombe community in Oguta Local Government Area of Imo State, Nigeria. Soil samples collected at 500 m and 1000 m from major spill points were analyzed for physicochemical characteristics and microbial composition using standard microbiological and analytical methods. Results revealed diverse bacterial genera, including *Pseudomonas*, *Bacillus*, *Acinetobacter*, *Alcaligenes*, *Citrobacter*, and *Serratia*, with several strains demonstrating strong hydrocarbon-utilizing abilities. Biodegradation experiments conducted over 30 days showed significant reductions in total petroleum hydrocarbons (TPH), with *Acinetobacter sp.* exhibiting the highest degradation efficiency (99.70%), followed closely by *Pseudomonas sp.* (99.60%) and *Alcaligenes sp.* (99.20%). Physicochemical analyses indicated that crude oil contamination altered soil properties, notably reducing nitrate and phosphate levels and increasing acidity. The findings confirm that indigenous bacterial communities in Izombe possess functional genes and metabolic potential necessary for effective biodegradation of Bonny light crude oil. The study concludes that microbial consortia are critical for efficient bioremediation and recommends *Acinetobacter sp.* as a promising candidate for remediation of hydrocarbon-impacted environments in the region.

Keywords: Heterotrophic Bacteria, Degradation, Hydrocarbon, Soil Samples

I. 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 (Nannipieriet *al.* 2003). Since microorganisms in the soil are involved in various biogeochemical processes, soil activities largely depend on them (Mikkonen, 2008). Both terrestrial and aquatic ecosystems are being affected by the changes caused by xenobiotics (e.g. hydrocarbons) introduced into the environment (Rolinget *al.*, 2004; Margesinet *al.*, 2007). Researchers have shown that the bacteria are mostly the major microorganisms found in hydrocarbon polluted soil (Engelhardt *et al.*, 2001; Iwabuchiet *al.*, 2002; Kasai *et al.*, 2002). However, earlier study by Ezemonye and Ezemonye (2005) on the effect of polycyclic aromatic Hydrocarbons (PAHs) on sediment found in South-South zone of Nigeria revealed that continuous addition of petroleum-based pollutants aided the proliferation of nine (9) microbial community that possesses the requisite enzyme repertoire to degrade the pollutant, thereby making the organisms capable of surviving the harsh conditions.

A wide range of pollutants (e.g. PAHs) can be degraded by microorganisms found within the polluted environment (Barathi and Vanudevan, 2001). However, environmental factors like soil pH, moisture, temperature and nutrients affect the rate of pollutants' degradation by microorganisms in the soil. Other factors that influence pollutants' degradation are toxicity of pollutant (Kenawy *et al.*, 2007), structure of pollutant (Maier *et al.*, 2009), the dose and bioavailability of pollutant (Sims *et al.*, 2009). Studies on oil-impacted areas showed that addition of nutrient in the polluted site enhanced the isolation of microorganisms that degrade hydrocarbon (Kasai *et al.*, 2002; Rolinget *al.*, 2002;

Xuet *et al.*, 2004; Head *et al.*, 2006). The adaptation of bacteria to environmental stress normally takes place in lag phase when the organisms adapt to the changes caused by the pollutant (Chikere and Okpokwasili, 2004; Nweke and Okpokwasili, 2004). This lag phase or acclimation period enables the micro-organisms to withstand environmental condition for their survival (Head *et al.*, 2006; Yakimov *et al.*, 2007). Bacteria are primary producer in the marine food web, where their role is to recycle nutrients and breaking down of hydrocarbon (Bordenave *et al.*, 2004, Edmund and Jansson, 2006; Head *et al.*, 2006).

Petroleum hydrocarbon degradation occurs largely in mass when hydrocarbon utilizing organisms are involved (Atlas, 1981). Having a good knowledge of potential microbial degraders of hydrocarbons, helps in developing *insitu* bioremediation technique (Harayama *et al.*, 2004). Petroleum hydrocarbon degradation occurs not only in microbial genera but also in group of bacteria and fungi, together with cyanobacteria and algae. Various bacterial species isolated from soil have been proved to degrade PAHs (Aislabie *et al.*, 2004). Other hydrocarbon degraders include *Alcaligenes* sp., *Acinetobacter* sp., *Flavobacter* sp., *Cyanobacterium* sp., *Moraxella* sp. and *Bacillus* sp. (Bhattacharya *et al.*, 2002).

Hydrocarbon utilizing bacterial genera include: *Pseudomonas*, *Arthrobacter*, and *Micrococcus* (Atlas, 1981). Previous studies have found the evolution of some obligate hydrocarbon degraders (also named as obligate hydrocarbonoclastic bacteria) of indigenous marine bacterial genera. (Brooijmans *et al.*, 2009). In summary, only few number of this organism can degrade hydrocarbon *in situ* (Head *et al.*, 2006). *Alcanivorax* sp. is a good hydrocarbon degrader because it has been proved in many parts of the world including the United States to potential hydrocarbon degrader (Yakimov *et al.*, 2007). Some researchers have also tried to find out the relationship between indigenous and exogenous degraders and their conclusion measured the biodegradation rates of PAH under three anaerobic conditions, their results showed that degradation rates decreases from sulfate reducing conditions down to methanogenic conditions, and then nitrate-reducing conditions. Their results also concluded that sulfate-reducing bacteria, methanogen and eubacteria were involved in PAH biodegradation, making sulfate-reducing bacteria a major component in the PAH-adapted consortia. However, particular

microorganisms were demonstrated with anaerobic degradation capacity (Coates *et al.*, 1997). All the anaerobic hydrocarbon degrading strains were denitrifying, ferric iron-reducing, sulfate-reducing bacteria or bacteria capable of reducing proton to hydrogen (Heider *et al.*, 1999). Therefore, this study is aimed to detect heterotrophic bacteria that are able to degrade hydrocarbon in Izombe community of Oguta local government area, Imo State.

II. MATERIALS AND METHODS

Study Area

This study was carried out in Izombe community in Oguta LGA of Imo State. Izombe community is an oil-producing area and one of the communities in Oguta Local Government Area in Imo State. This community is approximately 40 minutes' drive from Owerri town is located 30 km from Owerri and 150 km North of Port Harcourt. Oil exploration and production in Izombe area started in the early 1960s with three major oil companies (Chevron Texaco, Shell and Addax Petroleum) operating in the area and producing over 70% of total oil from Oguta LGA.

Study Design and Duration

Bacteria and soil samples used for analysis of heterotrophic bacteria study and physicochemical parameters were collected according to the method of Iheanacho *et al.* (2014). The study lasted for approximately eleven months between October 2022 and August 2023.

Sample Collection

Soil samples were collected from two points 500m and 1000m away from the major spill sites. Fifty grams (50g) of the oil-polluted soil samples will be 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 New Concepts Chemical Engineering and Environmental Services, FUTU road, Obinze, Owerri for analysis within 24 hours (Iheanacho *et al.*, 2014).

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°C) for 1hr, 30 min to liberate the organisms into the

liquid medium (Iheanacho *et al.*,2014). The pH of the samples was noted.

Analysis of samples

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 0.1ml aliquot of the 10^{-1} to 10^{-4} dilutions of each sample were 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 order 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°C as a stock culture for preservation and identification (Odokuma and Ibor, 2002).

Biodegradation studies

The method proposed by Ekpo and Ekpo (2006) was used. The biodegradation study of hydrocarbons in the polluted soil was carried out using the Bushnell-Haas broth. This medium consist $MgSO_4$ 0.02g; $CaCl$ 0.2g; K_2HPO_4 100g; $KHPO_4$ 1g; NH_4NO_3 1g; F_2Cl 0.05g was autoclaved in 2 litres conical flasks. 99ml of the liquid medium (Bushnell-Haas broth) was dispensed into five (5) conical flasks into which 1 ml of sterile crude oil was added (Ekpo and Ekpo, 2006). Precisely, 5ml 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 (4°C) 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 (Okoh, 2003). Bacterial utilization of hydrocarbon was monitored using their optical density at 600nm wavelength (Ekpo and Ekpo, 2006). The total petroleum hydrocarbon was measured and changes in the hydrocarbon profile of the crude oil were monitored using Gas Chromatography-Flame Ionization Detector, GC-FID (Okoh, 2003).

Determination of Crude oil Degradation rate in Soil

The crude oil degradation rate in the soil was determined by the solvent extraction method (Chithra and Shenpagam, 2014). Five grams (5g) of soil sample was mixed with 100ml 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 (20ml) was withdrawn and put in a previously weight evaporation dish. The dish and its content were evaporated to dryness in a rotary evaporator and the dish was reweighted to obtain the difference.

The percentage (%) of the degradation was shown 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.

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

Determination of Physicochemical Parameters

The physicochemical parameters (Ca, Mg, Na, P, NH_3 , turbidity) were analyzed according to the Standard Methods (Ademoroti, 1996).

pH and Conductivity Determination (Electrometric method)

The pH of the samples were determined using Hanna digital pH meter in a sample to water ratio of 1:10 i.e. twenty (20) g soil sample of sample was weighed into a beaker. Then two hundred milliliters (200) ml of distilled water was added to it. The pH electrode was dipped into the solution and also conductivity electrode was dipped.

Nitrate (cadmium reduction method)

Nitrate was extracted using Ammonium chloride (NH_4Cl) solution by adding 250ml of Ammonium chloride to ten (10) grams of the soil sample. It was shake for one (1) hour and filtered, and then the filtrate used for the determination of nitrate.

Determination of Total Organic Carbon (Titrimetric Method)

A sample of the sludge (colloids from soil sediments) was dried to constant weight and grind to fine powder in mortar. A 5g of the sample was weighed into 500 mL ground conical flask and 10 mL 0.5M $K_2Cr_2O_7$

was added and gently swirled. 20 mL concentrated H₂SO₄ was added rapidly but with caution to avoid splashing and directing the acid into suspension. The solution was gently swirled for 1 minute until the reagents have mixed but becomes vigorous later and then allowed to stand for 30 minutes. Afterwards, 200 mL of distilled water and 10 mL of concentrated H₃PO₄ were cautiously added to avoid splashing and allowed to cool. Then 3 – 4 drops of ferrion indicator solution was added and the solution was titrated to wine – red colour end point with 0.25 M FAS (The colour change is usually from blue to deep – green as

the final end point, wine – red, is approached) noting the iron II ammonium sulphate (FAS) titre reading. A blank determination was prepared as above but without the sample and FAS titre reading noted as well.

N.B if more than 75% of the K₂Cr₂O₇ was consumed by the organic carbon, repeat the procedure using small quantity of sample because the efficiency of oxidation K₂Cr₂O₇ decreases as its concentration decrease.

Calculation

$$\text{mg total organic carbon/g sample} = \frac{V_b - V_s \times M \times 16000}{\text{weight of sample in g}}$$

OR

$$\% \text{ total organic carbon} = \frac{V_b - V_s \times M \times 1.38}{\text{weight of sample in g}}$$

Where V_b = mL FAS used for blank; V_s = mL FAS used for sample.

M = Molarity of FAS.

STATISTICAL ANALYSIS

Data obtained from the study were analyzed using the Statistical Package for Social Sciences (SPSS; version 27.0) and expressed using Tables and graphs. Analysis of variance (ANOVA) and P- values test of significance was carried out. P-value of <0.05 was considered statistically significant.

III. RESULTS & DISCUSSION

1.0 Indigenous Heterotrophic Bacteria Present in Oil-Polluted Soils

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, Sampling point A (500m) and Sampling Point B (1000m) of this study was as represented in Table 2. These isolated bacteria from Table 2 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 Sampling Points

Strain	1	2	3	4	5	6
Gramstaining	–	–	+	–	–	–
Motility	+	+	+	+	+	+
Catalase	+	+	+	+	+	+
Glucose	+	–	–	+	–	+
Lactose	–	–	–	–	–	–
Butt stant	AB	BB	BB	AB	BB	AB
Gas	+	–	–	+	–	–
H ₂ S	–	–	+	–	–	+
Citrate	+	–	+	+	–	+
Urease	–	+	+	+	+	–
Methly red	+	+	–	–	–	–
V.P	–	–	+	+	+	+
Glucose	AG	A	A	A	A	A
Sucrose	–	–	–	–	–	–

Probable Organism	<i>Alcaligenes</i> sp.	<i>Pseudomonas</i> sp.	<i>Bacillus</i> sp.	<i>Acinetobacter</i> sp.	<i>Serratia</i> sp.	<i>Citrobacter</i> sp.
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AG =acid growth, AB =acid and base, BB=base base, A= acid.

Table 2: Culturable Bacterial Diversity Found in the various Sampling Points

Control	Polluted site (500m) away	Polluted site (1000m) away
<i>Acinetobacter</i> sp.	<i>Pseudomonas</i> sp.	<i>Alcaligenes</i> sp.
<i>Alcaligenes</i> sp.	<i>Bacillus</i> sp.	<i>Citrobacter</i> sp.
<i>Pseudomonas</i> sp.	<i>Acinetobacter</i> sp.	<i>Bacillus</i> sp.
<i>Serratia</i> sp.		<i>Acinetobacter</i> sp.
<i>Bacillus</i> sp.		

Table 3: Culturable Hydrocarbon Utilizing Bacterial Isolated from the Sampling Points

Control	Polluted site (500m) away	Polluted site (1000m) away
<i>Acinetobacter</i> sp.	<i>Pseudomonas</i> sp.	<i>Alcaligenes</i> sp.
<i>Alcaligenes</i> sp.	<i>Bacillus</i> sp.	<i>Citrobacter</i> sp.
<i>Pseudomonas</i> sp.	<i>Acinetobacter</i> sp.	<i>Bacillus</i> sp.
<i>Bacillus</i> sp.		<i>Acinetobacter</i> sp.

2.0 Comparison of the Degradation Rate of Various Hydrocarbon Utilizing Bacteria

Table 4: Performance of Hydrocarbon Utilizing Bacteria in Responses to Biodegradation of Soil

Day	Parameters	<i>Pseudomonas</i> sp.	<i>Bacillus</i> sp.	<i>Acinetobacter</i> sp.	<i>Alcaligenes</i> 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
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
	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
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
	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

Mean ± standard deviation of triplicate determination

The extent of Degradation of Hydrocarbon Utilizing Bacteria are Shown in Table 5.

Table 5: The Extent of Degradation of Hydrocarbons by Selected Bacterial Degraders after 30 Days

Hydrocarbons	<i>Bacillus</i> sp.	<i>Pseudomonas</i> sp.	<i>Alcaligenes</i> sp.	<i>Acinetobacter</i> sp.
C ₈	✓	✓	✓	✓
C ₁₀	-	-	-	-
C ₁₁	✓	✓	✓	✓
C ₁₂	-	-	✓	✓
C ₁₃	✓	✓	-	-
C ₁₄	-	-	-	-
C ₁₅	✓	✓	✓	✓
C ₁₆	-	✓	✓	✓
C ₁₈	-	-	-	-
C ₂₁	✓	✓	-	-
C ₂₃	-	-	-	-
C ₂₆	✓	✓	✓	✓
C ₂₈	-	-	-	-
C ₂₉	-	-	-	-
C ₃₁	-	-	✓	✓
C ₃₂	✓	✓	-	✓
C ₃₃	✓	✓	-	-
C ₃₄	-	-	✓	✓
C ₃₅	-	-	-	-
C ₃₇	-	-	✓	-
Hydrocarbon remaining	426.15mg/ml	58.68mg/ml	111.07mg/ml	38.37mg/ml

Hydrocarbon degraded	96.90%	99.60%	99.20%	99.70%
Key: Present (remaining) = ✓ ; Complete removal/degradation = -				

3.0 Physicochemical Parameters of Polluted Soil

Table 6: Physicochemical Parameters of Polluted Soil Samples

S/N	Parameters	Control Soil	Polluted Soil 500m Away	Polluted Soil 1000m Away
1	pH	7.24	5.08	6.47
2	Conductivity(μ s/cm)	126.00	750.00	1097.00
3	Sulphate (So ₄) (mg/kg)	10.00	95.00	90.00
4	Moisture content(%)	0.20	2.20	0.04
5	Ammonia, (NO ₃) (mg/kg)	0.00	0.08	0.13
6	AmmoniaNitrogen (NH ₃ N) (mg/kg)	0.00	0.07	0.11
7	Nitrate(NO ₃) (mg/kg)	56.30	13.90	42.70
8	Nitrate-Nitrogen(NO ₃ -N) (mg/kg)	13.20	3.10	9.70
9	Calcium(Ca) (mg/kg)	0.20	0.24	0.00
10	Magnesium(Mg) (mg/kg)	0.14	0.28	0.33
11	Sodium(Na) (mg/kg)	5.00	34.00	22.00
12	Potassium(K) (mg/kg)	1.30	8.00	5.08
13	Nickel(Ni) (mg/kg)	0.00	0.00	0.00
14	Mercury (Hg) (mg/kg)	0.00	0.00	0.00
15	Lead (Pb) (mg/kg)	0.31	0.21	0.29
16	Copper(Cu) (mg/kg)	0.05	0.00	0.00
17	Iron(Fe) (mg/kg)	4.13	3.93	4.33
18	Zinc(Zn) (mg/kg)	5.24	0.49	0.01
19	CEC (mg/kg)	0.56	1.70	1.17
20	Total Organic Carbon (%)	31.60	33.00	34.70
21	Phosphate(P ₂ O ₅) (mg/kg)	31.40	4.60	9.70
22	Phosphorous (P) (mg/kg)	10.20	1.50	3.20
23	Phosphate (PO ₄ ³⁻) (mg/kg)	23.40	3.50	7.20
24	Ash content (%)	85.00	85.00	90.82
25	TPH, /kg	16.00	12984.00	184.80
26	Total Organic Matter	63.20	66.00	69.40

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 (1990) isolated similar hydrocarbon utilizing bacteria from Niger Delta aquatic systems. Chikere and Okpokwasili (2004) 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 microfloras are actively oxidizing the hydrocarbons and this is considered as another source of carbon for use in the ecosystem.

The ability of these bacteria to be predominantly Gram- negative is contrary to Austin *et al.* (1997) who isolated predominantly gram-negative hydrocarbon utilizing bacteria from soil and aquatic environment. The isolation of *Bacillus* species is in agreement with the work of Okpokwasili and Okorie (1990), who found that *Bacillus* species could also be one of the predominant Gram positive organisms found in oil polluted areas

These findings showed that the biodegradation of complex hydrocarbons in nature usually required the cooperation of more than a single species. The microbial populations consisting of the strains belonging to various genera have been detected in

petroleum-contaminated soil (Iloriet *al.*, 2006; Kim and Crowley 2007). This suggested that the strains from various genera have their roles in the hydrocarbon transformation processes (Ghazaliet *al.*, 2004; Cunliffe and Kertesz 2006). 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. Statistical analysis of the result shows that there is significant difference between various heterotrophic bacteria and soil samples.

In Table 4, the physical parameter for *Pseudomonas sp* was recorded for 30 days at 5 days interval, the colour change mean ranged from 2536.67±234.79Pcu on day 0 to 6745.00 ±17.30Pcu for 30 days measured in Cobalt per Unit, while Optical Density ranged from 0.88±0.00 from day 0 to 1.77±0.00 for 30 days. 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) measured in Cfu/ml increases from 1646.67±55.08 on the previous day to 42666.67±3055.05 for day 30. (TPH) decreased from 4.58±0.01 Mg/ml to 0.02±0.00Mg/ml, this show that *Pseudomonas sp.* is active in the degradation process. Then the Total Hydrocarbon Content (THC) decrease in percentage to 68.33 ±2.01%.

Similarly the physical parameter for *Bacillus sp.* was recorded for 30 days at 5 days interval, the colour change mean ranged from 2536.67±243.79Pcu on day 0 to 2416.67 ±35.12Pcu for 30 days measured in Cobalt per Unit, while Optical Density ranged from 0.88±0.00 from day 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 6.30±0.08. The Total Culturable Heterotrophic Bacterial Count (TCHBC) measured in Cfu/ml increases from on the first day of 1180.00±20.00 to 34666.6±7577.35 for 30 days. The Total Petroleum Hydrocarbon (TPH) decreased from 4.58±0.00 Mg/ml to 0.11±0.06 Mg/ml, this show that *Bacillus sp.* is really degrading the hydrocarbon but at a slow rate. Then the Total Hydrocarbon Content (THC) decrease in percentage to 53.33± 4.04%.

Furthermore, the physical parameter results for *Acinetobacter sp.* were recorded for 30 days at 5 days interval, the colour change mean ranged from

2536.67±243.79Pcu on day 0 to 5406.67±268.58 Pcu for 30 days measured in Cobalt per Unit, while Optical Density ranged from 0.88±0.00 from day 0 to for 30 days 2.50±0.01. There was a drastically increase in pH from 6.18±0.02 to 8.21±0.14. The Total Culturable Heterotrophic Bacterial Count (TCHBC) measured in Cfu/ml increases from on the first day 1230.00±36.06 to 63333.33±28867.51 for 30 days. The Total Petroleum Hydrocarbon (TPH) decreased from 4.58±0.00 Mg/ml to 0.05±0.07 Mg/ml, this show that *Acinetobacter sp.* is really degrading the hydrocarbon but at a slow rate. Then the Total Hydrocarbon Content (THC) decrease in percentage to 52.67±1.53 %.

Similarly, the physical parameter for *Alcaligenes sp.* was recorded for 30 days at 5 days interval, the colour change mean ranged from 2536.67±243.79Pcu on day 0 to 4786.67 ±32.15Pcu for 30 days measured in Cobalt per Unit, while Optical Density ranged from 0.88±0.00 from day 0 to for 30 days 1.33±0.01. There was a slight decrease in pH as it ranged from 6.18±0.02 to 5.30±0.08. The Total Culturable Heterotrophic Bacterial Count (TCHBC) measured in Cfu/ml increases from on the first day 131.00±45.83 to 51000.00 0±165.15 for 30 days. The Total Petroleum Hydrocarbon (TPH) decreased from 4.58±0.00 Mg/ml to 0.04±0.00 Mg/ml, this show that *Alcaligenessp.* is really degrading the hydrocarbon. Then the Total Hydrocarbon Content (THC) decrease in percentage to 71.67± 0.58%.

However, the strain identify as *Pseudomonas sp.* possess a steady increase in optical density (OD) from 0.88 to 1.29, which raises the pH from 4.59 to 5.26 and increase in colour change from 2536.67pcu to 6745.00pcu after 30 days of degradation monitoring As shown in Table 4.5. OD increases slowly as seen in the second strain indentified as *Bacillus sp.* from 0.88 to 2.39, pH of 5.57 – 6.30 and shows a decrease in colour change from 4393.67pcu to 2416.67pcu during the degradation study. There was a steady increase in OD as seen in *Acinetobactersp.* which increases from 0.88 to 2.50 for 30 days which raises the pH from 5.46 to 8.21 and increase in colour change of 2536.67pcu to 5406.67pcu. Fourth Strain identified as *Alciligenes sp.* showed an increase in OD from 0.88 to 1.33, while pH increases from 2.98 to 5.30 and increase in colour change shows a wide range of 2536.67pcu to 4786.67pcu.

Based on OD results, the two strains of *Pseudomonas* sp. and *Bacillus* sp. were considered as having the highest degradation potentials. 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 exogenous hydrocarbons (Adebusoye *et al.*, 2007). However, this result also concord with the findings of Obohet *et al.* (2006), which shows that Hydrocarbon utilization is usually accompanied with pH changes, an increase in total viable count with a decrease in pH of culture media.

The TPH present were C-8, C-10, C-11, C-12, C-13, C-14, C-15, C-16, C-18, C-21, C-23, C-26, C-28, C-29, C-31, C-32, C-33, C-34, C-35 and C-37 for the treatment on day 0-30 days as showed in Table 5. The total amount of TPH after contamination of soil with crude oil showed that the treatment had TPH of (13729.70mg/ml) for the first day and reduced significantly after 30 days by each of the bacterium as follows: *Bacillus* sp. (426.15mg/ml) which was able to degrade the Hydrocarbon by 96.90%, *Pseudomonas* sp. (58.68mg/ml), which was able to degrade the Hydrocarbon by 99.60% *Alcaligenes* sp. (111.07mg/ml) which was able to degrade the Hydrocarbon by 99.20% and *Acinetobacter* sp. (38.37mg/ml) which was able to degrade the Hydrocarbon by 99.70%

This concord with the work of Margesinet *et al.* (2003) and Quatriniet *et al.* (2008) which demonstrated that *Actinobacter* sp. play important role during petroleum hydrocarbon degradation.

C-8, C-11, C-15, and C-26, were recalcitrant to degradation while C-10, C-14, C-18, C-23, C-28, C-29, and C-35 were all degraded in the degradation study.

It is an established fact that pollutants (e.g. hydrocarbons) affect the physicochemical condition of perturbed soil ((Rolinget *et al.*, 2004; Margesinet *et al.*, 2007). Table 3 showed the level of changes in the physicochemical parameters of soil caused by the pollutant and shows how the contamination level increase, the amount of nitrate and phosphorous in the soil reduce. The control soil has a high and normal quantity of nitrate and phosphorous of 58.30mg/kg, and 10.20mg/kg respectively, after 500m away, from the polluted site the quantity of the nitrate and phosphorous were 13.90mg/kg and 1.50mg/kg and to further confirm the soil was polluted, a sample was taken from 100meter away from the polluted site for

further study and this shows a value of 42.70mg/kg and 3.2mg/kg of nitrate and phosphorous respectively. Essential nutrients in the soil were reduced as soil is contaminated, the decrease in nitrate and phosphate level is attributed to the fact that they were been used in the metabolism of organism in building biomass. There is a positive correlation in the utilization of both nitrate and phosphate and this indicate their importance in cell metabolism. It was establish that the availability of nitrogen and phosphorus limit the microbial degradation of hydrocarbon (Abu and Ogiji, 1996; Zhu *et al.*, 2001) and the pH of the soil varies from 7.24 from the control soil sample to 5.08 and 6.47, 500 and 1000meters away from the polluted site respectively, which shows that the soil sample was acidic. This concord with the work of (Amund and Adebiyi, 1991; Okpokwasili and James, 1995) which proofs that microbial utilization of hydrocarbons often leads to production of organic acids. Thus, the acids probably produced account for the reduction in pH levels.

It is an established fact that pollutants (e.g. hydrocarbons) affect the physicochemical condition of perturbed soil ((Rolinget *et al.*, 2004; Margesinet *et al.*, 2007). Table 6 showed the level of changes in the physicochemical parameters of soil caused by the pollutant and shows how the contamination level increase, the amount of nitrate and phosphorous in the soil reduce. The control soil has a high and normal quantity of nitrate and phosphorous of 58.30mg/kg, and 10.20mg/kg respectively, after 500m away, from the polluted site the quantity of the nitrate and phosphorous were 13.90mg/kg and 1.50mg/kg and to further confirm the soil was polluted, a sample was taken from 100meter away from the polluted site for further study and this shows a value of 42.70mg/kg and 3.2mg/kg of nitrate and phosphorous respectively. Essential nutrients in the soil were reduced as soil is contaminated, the decrease in nitrate and phosphate level is attributed to the fact that they were been used in the metabolism of organism in building biomass. There is a positive correlation in the utilization of both nitrate and phosphate and this indicate their importance in cell metabolism. It was established that the availability of nitrogen and phosphorus limit the microbial degradation of hydrocarbon (Abu and Ogiji, 1996; Zhu *et al.*, 2001) and the pH of the soil varies from 7.24 from the control soil sample to 5.08 and 6.47, 500 and 1000meters away from the polluted site

respectively, which shows that the soil sample was acidic. This concord with the work of (Amund and Adebiyi, 1991; Okpokwasili and James, 1995) which proofs that microbial utilization of hydrocarbons often leads to production of organic acids. Thus, the acids probably produced account for the reduction in pH levels.

IV. CONCLUSION

This study revealed that Izombe community in Oguta L.G.A, Imo State, is one of the oil exploration zone of southern Nigeria, harboring a large number of microbial hydrocarbon degraders which are able of utilizing Bonny light crude oil as carbon source when monitored. The increasing values obtained indicate that both test organisms: *Bacillus* sp. and *Pseudomonas* sp. can grow and utilizing Bonny light as carbon source but *Aciligenessp* grow at a slower rate as the days of incubation increase further. The results of the chromatographic analyses of the total petroleum hydrocarbons (TPHs) also showed that *Acinetobacter* sp. can degrade Bonny light crude oil more efficient and this evidence is shown from the reductions of both the number, sizes of the peaks and in the percentage of degradation. Therefore, it appeared that *Acinetobacter* sp. a bacterium could be more useful in the degradation study contaminated with Bonny light crude oil and is recommended for controlling oil polluted site. We concluded from the findings that this study revealed that indigenous bacterial species possess the requisite gene necessary for hydrocarbon biodegradation. This concluded that biodegradation is most often the primary mechanism for contaminant destruction including petroleum contaminants and that the use of consortium species makes biodegradation study more effective. The elimination of oil spilled in the environment can be achieved by microbial degradation when added up with some physical and chemical methods.

V. RECOMMENDATION

The following recommendations made from the study;

1. Government should enact necessary policies to prevent soil pollution and exploration from oil activities in the community.
2. Further scale-up studies as applicable need to be carried out in increasing the degrading ability and stability of the crude oil

degrading isolate and its usage as a possible commercial strain.

3. Proper awareness and sensitization programs are needed to enlighten the citizens on the dangers of oil spill and precautions to be taken when their community is contaminated.

Conflict of Interest

The authors have not declared any conflict of interest regarding funding and publication of this work.

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