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# Biodegradation of hydrocarbon by bacteria isolated from crude oil contaminated soil

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ABSTRACT

Crude oil contaminated soil has posed major environmental problems in recent years. Hence, this work was designed to evaluate the potential of microorganisms isolated from crude oil contaminate soil, to degrade hydrocarbon. Samples of crude oil contaminated soil were collected from Warri Refining and petrochemical Company in Delta State and characterized using standard microbiological procedure. Isolates were assayed for their ability to degrade hydrocarbon using mineral salt medium containing crude oil as source of carbon. Soil samples were treated as follows: A – untreated/control soil plus sterile crude oil, B – untreated soil plus crude oil and carrier, C – treated soil (with *Enterobacter aerogenes*) plus crude oil and carrier, D – treated soil (with Actinomycetes) plus crude oil and carrier, E – treated soil (with *Enterobacter aerogenes* and Actinomycetes) plus crude oil and carrier. After 14 days treatment, A had no growth while B, C, D and E had bacterial count of 2.6 $x10^4$ cfu/g, 7.68$x10^{11}$, 1.42$x10^{12}$ and 1.96$x10^2$ cfu/g respectively. At the end of 28 days period, A, B, C, D and E had count of 1.2 x $10^3$cfu/g, 5.22 x $10^{11}$cfu/g, 9.30 x $10^{14}$cfu/g, 1.79 $x10^{17}$cfu/g and 2.52 $x.10^{19}$ cfu/g respectively. *Serratia marcescens, Actinomycetes* and *Enterobacter aerogenes* showed crude oil reduction of 44.3 %, 79.26 % and 61.69 % respectively. The results showed that Actinomycetes had the highest reduction rate of hydrocarbon content of soil more than other bacterial isolates.

## 1. Introduction

The world today is experiencing a rapid change with varying social, economic, and political impacts on the environment cum the totality of the surroundings including air, land and water [1]. Oil exploration and production equally did not spare the environment and have the potentials for a variety of impacts on the environment. These impacts depend upon the stage, size and complexity of the project, nature of sensitivity of the surrounding environment, the effectiveness of planning, pollution and control techniques [1]. The Niger Delta of Nigeria which covers a land mass of over 70,000 square kilometers with about 800 oil producing communities has become vulnerable to massive oil spillages [1]. Some of these spillages

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occur as a result of equipment failure, operational mishap or intentional damage to facilities. Oil and gas activities have caused damages in several forms to the Niger Delta region of Nigeria [3]. Methods devised for cleaning such crude oil contaminated site are usually cumbersome and costly. However an improved biological method which is simple, cost effective and easily practicable is bioremediation, in which microorganism are used to degrade recalcitrant chemical compounds restoring the environment [4]. While the economic significance of hydrocarbons as the primary source of fuel and its versatile application in downstream industries are obvious, the product may also have major environmental consequences. Oil exploration, production and processing represent prime sources of exposure to petroleum hydrocarbons. Though, there are other possible sources, such as vehicle and generator emissions, burning of vegetation and trash (including domestic waste), food processing and use of cooking fuels [3]. All these activities are common in Niger Delta. In looking at the environmental consequences of hydrocarbons, it is important to remember that 'hydrocarbons' is an umbrella term used for hundreds of different organic compounds [5] Secondly, hydrocarbons can cause environmental consequences due to their chemical properties (e.g. toxicity) or physical properties (e.g. smothering) [5]. Hydrocarbon pollution of soil can occur in several ways, from natural seepage of hydrocarbons in areas where petroleum is found in shallow reservoirs, to accidental spillage of crude oil on the ground [4]. Regardless of the source of contamination, once hydrocarbons come into contact with the soil, they alter its physical and chemical properties. In the least damaging scenario, such as a small spill of a volatile hydrocarbon onto dry sand, the hydrocarbons evaporate fast, causing no chemical or physical damage to the soil. In other situations, for example a spill of heavy crude oil onto clay soil, the chemicals can remain within the soil for decades, altering its permeability, causing toxicity and lowering or destroying the quality of the soil [6]. In such circumstances, the soil itself will become a source of pollution. Contaminated soil can affect the health of organisms through direct contact or via ingestion or inhalation of soil contaminants which have been vaporized [3]. Hydrocarbons can enter water through direct spills or from a spill originally occurring on land and subsequently reaching water bodies through the effects of wind, rain, surface or sub-surface flow. Hydrocarbons can cause both physical and chemical effects in water; even very small quantities of hydrocarbon can prevent oxygen transfer in the water column, thus affecting aquatic life-support systems. The presence of mere traces of a highly toxic hydrocarbon, such as benzene, may render water unfit for human consumption [7]. Benzene, for example, is a known carcinogen, in addition to having numerous other short-term effects. PAHs are potent pollutants that occur in crude oil, as well as in wood or coal. They are also produced as by-products of fuel burning particularly at low temperatures leading to incomplete combustion (whether fossil fuel or biomass). As pollutants, they are of concern because some compounds have been identified as causing cancer, changing genetic structures and affecting embryos and fetuses [6]. The aim of the present research is to carry out an assessment of petroleum hydrocarbon polluted site in Delta State as well as carry out bioremediation process on the assessed hydrocarbon polluted soil in Delta State.

## 2. Materials and methods

### *2.1. Collection of petroleum hydrocarbon contaminated soil samples*

Land area that over a long period of time had constantly received crude oil contamination (through crude oil tank bottom sludge) at the Warri Refinery premises was identified. About 15 kg surface soil was collected by means of trowel from each of the selected sites into separate polyethene bags that were immediately taken to the laboratory.

### *2.2. Isolation of crude oil degrader (bacteria)*

The hydrocarbon degraders in mineral salt broth (with crude oil as carbon source) by adopting an enrichment technique by Agiri *et al*., [6]. Mineral salt media (MSM) solution and trace element solution were prepared according to manufacturer's instruction. 2 ml of trace element solution was added to 1 l of MSM solution and the final MSM preparation was adjusted to a pH of 7.3

before it was used for cultivating the crude oil degrader bacteria. 100 ml of the final MSM preparation was taken in a sterilized Erlenmeyer flask. 10 ml of crude oil contaminated soil suspension (1g soil: 10 ml deionized water) of soil was added to the final MSM solution. Two growth flasks were prepared using crude contaminated soil obtained from different tank sludge of Warri Refining and Petrochemical Company (WRPC). 1ml of crude (Escravos light, sterilized by means of membrane filter 0.45um and added to each flask as a sole carbon source. All flasks were incubated at 30°C in an incubator (Plate III) for 10 days. After growth was observed for 10 days, 1ml of the MSM broth culture was transferred from each of the growth flask into separate fresh 100 ml MSM (with 1ml of crude oil, Escravos light added) in sterile conical flask. These were again incubated for 10 days at 30°C to obtain bacteria acclimatized to utilizing crude oil as carbon source for metabolism. The two flasks incubated showed growth at the end of the 10 days incubation period and all the isolates were sub-cultured to obtain pure culture. The procedure adopted was that which measured the ability of isolates to grow in MSM broth with crude oil as a source of carbon as reflected in turbidimetric readings using turbidimeter [5]. 4 sterile conical flask containing 500 ml of MSM and 5ml of sterile crude oil (Escravos light) were prepared and labeled. Using a sterile wire loop, pure culture of each bacteria isolate were transferred into the appropriate flask labelled for it except the control and was incubated at 30°C in a laboratory incubator over a period of 28 days. Each flask was agitated at least once daily to enhance homogenization and aeration throughout this period. Over this period of incubation, 20 ml of each samples was at a regular interval of seven days was taken from each culture flask using sterile pipettes and monitored for growth. This was measured by transferring the broth into cuvettes and the turbidity was read directly with turbidimeter.

### *2.3. Microbiological analysis*

Bacterial isolates were identified and characterized with their biochemical characteristics and according to standard microbiological procedure [4].

### *2.4. Collection and preparation of locally sourced carrier*

Locally sourced agricultural harvest by-product (Coconut fiber) was collected and prepared to serve as a carrier for crude oil degrader microorganisms [8]. The coconut fiber was obtained from the fibrous mesocarp of the coconut fruit obtained from Eku, Delta state. The fiber was pulled out from the mesocarp and reduced in size by cutting. The cuttings were then air dried for ten days and further dried in the oven (65 °C) for another seven days and ground in a grinding mill. The brown colored powder was sieved through 0.48 mm (ASTM Sieve) and stored in a plastic container at room temperature in the laboratory. The reasons for selecting agriculture harvest bye- product (coconut fiber) as local cellulosic material to serve as carrier are;

i. The agricultural material harvest bye-product selected is locally available in large quantity.
ii. The cellulosic material is believed not to serve as a nutrient source for the microorganism thus helping to prolong the shelve lives of the immobilized microorganisms.
iii. The cellulosic carrier is easily biodegraded and thus will not constitute waste problems after the microorganism are spent.

### *2.5. Characterization of cellulosic material*

The agricultural harvest bye-product (Coconut fire) was characterized based its water content, nitrogen content, and phosphorus content [9.

### *2.6. Determination of water content of the coconut fiber*

The water content also called the natural moisture content is the ratio of the weight of water to the weight of the solids in a given mass of the material. This ratio is usually expressed as a percentage. A crucible previously cleaned and oven dried was weighed ($w_1$). The crucible was then filled with the dried mill of the coconut fiber and weighed ($w_2$). The crucible containing the cellulosic material was then kept in an oven at a temperature between 105°C to 110°C for 24 hours. The final constant weight ($w_3$) of the container with the dried sample was then determined. The water (moisture)

content w (%) was determined from the relationship.

$$\text{water (moisture) content w (\%)} = \left[\frac{(w_2 - w_3)}{(w_3 - w_1)}\right] \times 100 \quad (1)$$

*2.7. Determination of total nitrogen*

2 g of the ground coconut fiber was weighed. 9 ml of concentrated Sulphuric acid was added and the mixture was gently heated on a hot plate until white fumes was observed. It was then allowed to cool, filtered and the filtrate was made up to 100 ml in volumetric flask. 25 ml of the digest was taken from the flask and made up to 50 ml with distilled water. 5 ml of 12 M potassium hydroxide was added and the solution was filtered. 25 ml of the filtrate was taken and 1 ml of 10 % sodium tartarate and 5 ml of Nessler's reagent were added. A blank sample with distilled water as the test sample was also prepared. Sample was allowed to stand for 15 minutes for colour development. Absorbance was read at 460 nm with a direct reading spectrophotometer (Hach Direct reading 2000 Spectrophotometer) [9].

*2.8. Determination of phosphorus*

1 g of the dried and ground coconut fiber was weighed into a 250 ml conical flask and 4 ml of perchloric acid, 2 ml of entreated nitric acid and 2ml entreated sulphuric acid were added in a fume chamber. The mixture was heated using a hot plate until dense white fumes were observed. It was then heated from medium to high heat for 30 seconds and then allowed to cool. 50 ml of distilled water was then added and the solution was boiled for 30 seconds. On cooling the solution was filtered with a Whatman No 42 filter paper made up to 100 ml in a volumetric flask. 0.2112 g of ascorbic acid was weighed into a beaker and phosphate reagent B was prepared by adding 40 ml of reagent A to the Ascorbic acid. 5 ml of the digest was added 10 ml of distilled water, 4 ml of reagent B and made up to 25 ml with distilled water. A blank with distilled water as the test sample was similarly prepared. Both were allowed to stand for 15 minutes for colour development and absorbance was read at 882 nm with a direct reading spectrophotometer (Hach Direct reading 200 Spectrophotometer) [9].

*2.9. Testing effectiveness of selected individual oil-degrader bacteria isolates immobilized in selected cellulosic carrier to bioremediate oil contaminated soil using laboratory scale set up*

The focus at this stage is to find out whether the individual immobilized oil degrader bacteria in cellulosic carrier will be effective in bioremediating oil- contaminated soil using laboratory scale set up. 50 g of sterile sharp sand was contaminated with 10 % (v/w) sterile crude oil (Escravos light) in 100 ml capacity Erlenmeyer flasks with loosely placed screw caps. Sterile stock solution (NPK 15-15-15 and MSM) and used as nutrient amendment to the (16 %v/w) in all flasks containing oil-contaminated soil except in the control flask. Carrier with immobilized isolate, where used, was added to soil in ratio 1:5. 5 flasks were set up in this laboratory scale test in the following arrangements:

A (control) = Sterile soil + sterile crude oil.

B = sterile soil + sterile crude oil + sterile nutrient +sterile carrier.

C = sterile soil +sterile crude oil+ sterile nutrient +GA +sterile carrier.

D = sterile soil +sterile crude oil+ sterile nutrient + SB + sterile carrier.

E (consortium) = sterile soil + sterile crude+ sterile nutrient + SB + GA + sterile carrier.

Each flask in the experimental set up was incubated at $30^0$C and agitated twice daily for aeration and mixing to increase contact between the isolates and oil contaminated soil. Sample from flasks were analyzed at day zero and subsequently every 14 days for the following parameters:

i. Bacteria count using plate-count method on nutrient agar to detect any relative increase in quantity which could be due to bioremediation activity.
ii. Total petroleum Hydrocarbon (TPH) in soil using Gas Chromatography (GC) Technique to find out how much of the crude oil in the contaminated soil is being removed in each flask.
iii. pH meter to detect any relative changes in pH during bioremediation activity.

*2.10. The assessment of the artificially petroleum polluted soil*

The assessment of the artificially polluted soil involves sample collection, extraction and analysis. This was done to know the total petroleum hydrocarbon present in the crude oil contaminated soil before introduction of the individual bacteria isolates. This was done for both soil and water. Gas Chromatograph (HP 5890 series) was the analytical equipment used for analyzing the polluted samples for total petroleum hydrocarbon [5].

*2.11. Procedure for the extraction of the soil sample for TPH analysis*

A solvent of a 50:50 mixture of acetone and methylene chloride was prepared. 10 g of crude oil polluted soil was measured into a beaker solvent rinsed with fifty (50 ml) of the solvent mixture was added into the samples. This was placed on a heating mantle with an inbuilt magnetic stirrer for 15-20 minutes. 10 g of anhydrous Sodium sulphate was added to the sample until a clear extract was formed. The extract solvent was concentrated and was further re-concentrated with the addition of 1 to 3ml of hexane. This was put in a small corked bottle. The extract sample was further analyzed using the Gas chromatography.

*2.12. Determination of percentage cleanup*

Percentage reduction in total petroleum hydrocarbon (TPH) was determined after the application of the bacteria isolates. The collected sample from the polluted soil at intervals of 7, 14, and 28 days were analyzed to check the reduction TPH which is also the extent of cleanup. The percentage reduction in TPH was calculated using equation below;

$$\%C = 100 - \left(\frac{y}{x}\right)100 \quad (2)$$

## 3. Results and discussion

The results of the bacterial count on day zero , both A and B set up showed no growth while C,D and E showed growth of 1.56 x $10^8$, 2.37 x $10^8$ and 5.43 x$10^8$ cfu/g respectively. On day 14, A showed no growth while B showed a growth of 2.6 x$10^4$ cfu/g which could be attributed to external bacteria. C showed a growth of 7.68 x$10^{11}$, D (1.42 x$10^{12}$) and E showed a growth of 1.96 x $10^{12}$ cfu/g. At the end of day 28, `E` showed the highest bacteria population of 2.52 x$10^{19}$ cfu/g while `A` which is the control showed the least growth of bacteria of 1.2 x $10^3$ cfu/g (Table 1). Three bacteria were isolated, characterized and identified as follows: isolate GA as *Enterobacter aerogenes*, SA as *Serratia marcescens* and SB as *Actinomycetes*. Preliminary screening of the biodegradative ability of isolated bacteria revealed high potential for crude oil biodegradation by these organisms. From the results of percentage cleanup determination in soil, the consortium had 85.64 % cleanup, SB isolate 84.73 % cleanup, GA isolate 82.56 % cleanup, B set up 73.35 % and control had 14.69 %.

The turbidity reading of the isolates are presented in Table 2. It shows that the turbidity for *Serratia marcescens*, *Actinomycetes* and *Enterobacter aerogenes* ranged from 56- 2955 NTU, 65-5056 NTU and 78-5011 NTU respectively.

**Table 1.** Bacterial count of treated soil sample.

| Flask code | Content | CFU/g | | |
|---|---|---|---|---|
| | | 0 day | 14 days | 28 days |
| A | Sterile soil + sterile crude oil | Nil | Nil | 1.2 x $10^3$ |
| B | Sterile soil+ sterile crude oil +sterile nutrient + carrier | Nil | 2.6 x $10^4$ | 5.22 x $10^{11}$ |
| C | Sterile soil+ sterile crude oil+ sterile nutrient +GA+ carrier | 1.56x$10^8$ | 7.68 x $10^{11}$ | 9.30 x $10^{14}$ |
| D | Sterile soil + sterile crude oil + sterile nutrient + SB + carrier | 2.37 x $10^8$ | 1.42 x $10^{12}$ | 1.79 x $10^{17}$ |
| E | Sterile soil + sterile crude oil + sterile nutrient +GA +SB + carrier | 5.43 x $10^8$ | 1.96 x $10^{12}$ | 2.52 x $10^{19}$ |

**Table 2.** Turbidity Readings of Isolates (NTU).

| Isolates | Day 0 | Day 7 | Day 14 | Day 21 | Day 28 |
|---|---|---|---|---|---|
| Control | 35 | 312 | 380 | 420 | 508 |
| SA | 56 | 356 | 909 | 1702 | 2955 |
| SB | 65 | 105 | 804 | 3063 | 5056 |
| GA | 78 | 430 | 967 | 4815 | 5011 |

**SA=** *Serratia marcescens*, **SB=** *Actinomycetes* **GA=** *Enterobacter aerogenes*, and Control

The ubiquitous distribution of oil-degrading microorganisms has already been established. Oil-degrading microbial abundance is high especially in the contaminated site. It has been reported that before contamination, hydrocarbon degrading microorganisms comprised less than 2% of the total microbial load. However after contamination, the population of degraders increases up to 10% [10]. Bacterial count on day zero of A and B set up showed no growth while C, D and E showed growth of $1.56x10^8$, $2.37x10^8$, and $5.43\ x10^8$ cfu/g respectively. On day 14, A showed no growth while B, C, D and E showed growth of $2.6\ x10^4$ cfu/g, $7.68\ x10^{11}$, $1.42\ x10^{12}$ and $1,96\ x\ 10^{12}$ cfu/g respectively. At the end of 28 days period, all set up A, B, C, D and E showed bacteria growth of $1.2\ x\ 10^3$cfu/g, $5.22\ x\ 10^{11}$cfu/g, $9.30\ x\ 10^{14}$cfu/g, $1.79\ x10^{17}$cfu/g and $2.52\ x.10^{19}$ cfu/g respectively. Increased bacterial growth observed in the different set up justifies the ability of the individual bacteria or consortium of bacteria to utilize crude oil and thereby carrying out bioremediation activities. Significant bacterial counts was observed in the set up at the 14th day. This shows that the individual or consortia of bacteria used were able to utilize the crude oil as a carbon source, thereby enabling them to proliferate. Bacteria growth significantly increased at the 28th day. This shows that bacteria utilization and biodegradation of crude oil is time dependent. Sang-Haw *et al*., [11] made a similar observation and concluded that hydrocarbon degrading bacterial populations increased rapidly during the first 30 days of 105 days testing period. They proposed this finding that it may be considered as an indicator for the feasibility of oil-polluted soils bioremediation. But, with increasing of time, due to the oil resistant components with high chain and within less remaining nutrients, the bacteria growth and oil degradation decreased [12]. Indigenous bacteria isolated from crude oil contaminated sites were *Enterobacter aerogenes* (GA), *Serratia marcescens* (SA) and *Actinomycetes* (SB) and these bacteria have been reported to be members of oil degrading microbes. Presence of these bacteria in crude oil contaminants suggests their potential role in the biodegradation of crude oil contaminated site. These isolates were subjected to different component of hydrocarbon to test their biodegrading ability as illustrated in Table 3.

**Table 3.** Isolates biodegrading ability of crude oil in soil.

| Component | Day 0<br>No degrader | Day 28<br>GA | Day 28<br>SB | Day 28<br>SA |
|---|---|---|---|---|
| Nonane | 1075.879 | 787.026 | 361.793 | 458.148 |
| Decane | 1365.008 | 429.481 | 312.777 | 266.501 |
| Dodecane | 1171.406 | 186.364 | 135.587 | 1094.497 |
| Tetradecane | 1071.614 | 380.768 | 116.513 | 932.926 |
| Hexadecane | n/d | n/d | n/d | n/d |
| Octadecane | 804.170 | 458.335 | 145.301 | 717.736 |
| Nonadecane | 892.662 | 381.739 | 154.302 | 376.730 |
| Eicosane | 1167.452 | 371.613 | 178.236 | 806.582 |
| Docosane | 446.313 | 297.325 | 281.069 | 93.556 |
| Tetracosane | 580.125 | 126.128 | 165.434 | 122.849 |
| Hexacosane | 349.361 | 0.000 | 0.000 | 103.274 |
| Octacosane | n/d | n/d | n/d | n/d |
| Triacontane | n/d | n/d | n/d | n/d |
| Hexatriacontane | n/d | n/d | n/d | n/d |
| Total (mg/L) | 8923.992 | 3418.778 | 1851.011 | 4972.799 |

Table 3 shows that the isolates were able to degrade all the hydrocarbon components except hexadecane, octacosane, triacontane and hexatriacontane. Table 4 describes the total content of the aliphatics (components of the sterile soil with the sterile crude oil).

**Table 4.** Assessment of artificially petroleum hydrocarbon in polluted soil before introduction of each bacterial isolates.

| Component | Sterile soil + Sterile crude oil |
|---|---|
| Nonane | 4598.319 |
| Decane | 5277.319 |
| Dodecane | 8487.395 |
| Tetradecane | 12605.042 |
| Hexadecane | 0.000 |
| Octadecane | 4994.621 |
| Nonadecane | 1657.815 |
| Eicosane | 2376.302 |
| Docosane | 409.537 |
| Tetracosane | 690.504 |
| Hexacosane | 380.420 |
| Octacosane | 521.084 |
| Triacontane | 815.126 |
| Hexatriacontane | 51.000 |
| Total liphatics(mg/kg) | 42864.484 |

Table 5a shows the ability of *Enterobacter aerogenes* (GA) and *Actinomycetes* (SB) to degrade crude oil in an artificial petroleum hydrocarbon polluted soil at 7 days. Table 5b shows the ability of *Enterobacter aerogenes* (GA) and *Actinomycetes* (SB) to degrade crude oil in an artificial petroleum hydrocarbon polluted soil at 14 days. Table 5c shows the ability of *Enterobacter aerogenes* (GA) and *Actinomycetes* (SB) to degrade crude oil in an artificial petroleum hydrocarbon polluted soil at 28 days. The percentage cleanup by the bacterial isolates in the soil include 61.69%, 44.3% and 79.26% for Enterobacter aerogenes, Serratia marcescens *and* Actinomycetes respectively. While the percentage reduction rate / cleanup by the bacterial isolates in total petroleum hydrocarbons in the soil include 14.69%, 73.35% and 82.56%, 84.73%, 85.64% for flask codes A (control), B, C, D and E respectively. The results of monitoring isolates potential to utilize crude oil as a source of carbon showed that after a period of 28 days, there were growths in all the samples including the control. The growth in the control can be attributed to presence of external bacteria in the laboratory. Comparing the growth shown by the isolates, SA (2955), SB (5056), and GA (5011), SB showed highest growth of 5056 followed by GA 5011, SA 2955 while the control showed the least growth of 508. Based on the above results, it is evident that isolate SB and GA performed better than SA, hence isolate SA was not introduced into the soil for bioremediation. The results also showed that it took isolate SB more time to get acclimatized to utilizing crude oil as source of carbon when compared to others. This means that different oil degrading bacteria have different times of acclimatization but does not affect their performance to utilize the oil as source of carbon. Gas chromatography analysis showed that the isolate has reduced the total petroleum hydrocarbon in soil (MSM) after a period of 28 days. It showed that isolate SA reduced the total petroleum hydrocarbon from 8924.207 mg/L to 4973.020 mg/L, isolate SB reduced the total petroleum hydrocarbon from 8924.207 mg/L to 1851.13 mg/L and isolate GA reduced the total petroleum hydrocarbon from 8924.207 mg/L to 3418.941 mg/L. Comparing the above results, isolate SB performed better followed by GA and SA which is in agreement with the turbid metric results. The hydrocarbon content of the crude oil was observed to reduce during biodegradation studies. Microorganisms have been reported to be able to utilize crude oil components as carbon sources. This is the primary mechanism behind bacteria remediation of crude oil or petroleum contamination in soil or any other samples. The majority of petroleum-degrading bacteria can degrade only few kinds of hydrocarbons [6, 9]. The middle-chain and long-chain normal alkane can be degraded by most petroleum degrading bacteria. However, the short-chain hydrocarbons and aromatic hydrocarbons can only be degraded by few petroleum-degrading bacteria. For the majority of bacteria it is difficult to digest short-chain and aromatic hydrocarbons, which can even be toxic. The results of the monitoring of individual isolates and the consortium (SB,GA) ability to utilize crude oil as source of carbon on soil showed remarkable reduction in total petroleum hydrocarbon except in `A` set up which is the

control. On day 7, `E` set up which contains a consortium of bacteria (GA,SB) has reduced the total petroleum hydrocarbon from 42867.573 mg/kg to 20348.065 mg/kg, `D` set up which contains bacteria isolate SB has reduced the total petroleum hydrocarbon from 42867.573 mg/kg to 21647.944 mg/kg, GA reduced the total petroleum hydrocarbon from 42867.573 mg/kg to 24716.462 mg/kg, `B`set up reduced from 42867.373 mg/kg to 27853.666 mg/kg and `A` set up which is the control had no reduction in total petroleum hydrocarbon. On the day 14, the consortium (GA,SB) has the total petroleum hydrocarbon from 20348.065 mg/kg to 10669.809 mg/kg, isolate SB has reduced the total petroleum hydrocarbon from 21647.944 mg/kg to 13602.694 mg/kg, `C` set up was reduced from 24716.462 mg/kg to 14992.376mg/kg, `B` set from 27716.462 mg/kg to 17525.182 mg/kg, and `A` set up which is the control has a reduction in total petroleum hydrocarbon from 42867.573 mg/kg to 41972.646 mg/kg. On the 28$^{th}$ day, the consortium has reduced the total petroleum hydrocarbon from 10669.807 mg/kg to 6154.378 mg/kg, isolate SB reduced the total petroleum hydrocarbon from 13602.694 mg/kg to 6547.481mg/kg, isolate GA reduced the total petroleum hydrocarbon from 14992.694 mg/kg to 7475.336 mg/kg, `B` set up reduced from 17525.182 mg/kg to 11423.915 mg/kg and the control had a reduction in TPH from 41972.646 mg/kg to 36566.192 mg/kg. From the above results, the consortium had the highest reduction in total petroleum hydrocarbon while the control had the least reduction. The noticed reduction in TPH in the control is as a result of external bacteria in the laboratory. From the results of percentage cleanup in soil by the bacteria isolates, isolate SA recorded 44.3 % cleanup, isolate SB recorded 79.26 % cleanup and isolate GA recorded 61.69 % cleanup. These records showed that isolate SB cleaned more than the rest followed by GA and SA hence the isolate SA was not used in the bioremediation as a result of its poor performance in terms of potential to utilize crude oil as source of carbon. From the results of percentage cleanup determination in soil, the consortium had 85.64 % cleanup, SB isolate 84.73 % cleanup, GA isolate 82.56 % cleanup, B set up 73.35 % and control had 14.69 %. From the above, the consortium had the highest percentage of cleanup while the control had the least. Comparing the results of the bacteria count and that of percentage total petroleum hydrocarbon reduction, consortium which has the highest population of bacteria at the end of the 28 days period (2.52 x $10^{19}$ cfu/ml) also showed highest reduction in total petroleum hydrocarbon. The control `A` with least bacteria population of 1.2 x103 cfu/ml also showed the least reduction in total petroleum hydrocarbon at the end of 28 days period. The increase in bacteria population is as a result of their ability to utilize crude as a source of carbon. Lastly, at end of 28 days period, the chromatogram of the control has the highest peak size while that of the consortium has the lowest peak size which is an indication of the total petroleum hydrocarbon left after the remediation period of 28 days. Percentage cleanup of crude oil from soil was observed to be highest in bacteria consortium than in than in individual bacterial species. Observation from this study reveals that bacterial consortium has higher potential for bioremediation than individual bacterium. It was also revealed that use up of total petroleum hydrocarbon was highest in the bacteria consortium compared to individual bacterial specie. This showed that the hydrocarbon content in crude oil act as carbon source to the indigenous bacterial isolates.

**Table.5a.** Monitoring of isolates (SB, GA) for potential to biodegrade crude oil in an artificial petroleum hydrocarbon polluted soil (Day 7).

| Component | A Soil+crude oil | B Soil +NPk+ crude oil+ carrier | C Soil+NPk +crude oil+GA+ carrier | D Soil+NPk +crude oil +SB+carrie | E Soil+ NPk+ Crude oil +GA+ SB+ Carrier |
|---|---|---|---|---|---|
| Nonane | 4598.319 | 2076.218 | 2333.952 | 515.310 | 3291.106 |
| Decane | 5277.319 | 5255.606\za | 1809.538 | 924.368 | 944.257 |
| Dodecane | 8487.395 | 6873.949 | 2016.806 | 885.154 | 703.114 |
| Tetradecane | 12605.042 | 3342.857 | 11980.677 | 11622.080 | 6361.345 |
| Hexadecane | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| Octadecane | 4994.621 | 4470.588 | 1842.352 | 4374.957 | 4535.014 |
| Nonadecane | 1657.815 | 1384.285 | 1641.176 | 1011.428 | 1035.084 |
| Eicosane | 2376.302 | 2094.117 | 992.560 | 815.966 | 1484.383 |
| Docosane | 409.537 | 384.056 | 334.434 | 368.303 | 352.521 |
| Tetracosane | 690.504 | 683.529 | 629.212 | 294.957 | 455.665 |
| Hexacosane | 380.420 | 311.764 | 251.711 | 317.647 | 344.607 |
| Octacosane | 521.084 | 506.112 | 224.089 | 386.462 | 500.336 |
| Triacontane | 815.126 | 443.090 | 636.167 | 98.319 | 266.106 |
| Hexatriacontane | 51.000 | 27.332 | 23.669 | 32.772 | 34.313 |
| Total aliphatics (mg/kg) | 42864.484 | 27853.503 | 24716.343 | 21647.723 | 20347.851 |
| Total PAH | 3.089 | 0.169 | 0.119 | 0.221 | 0.214 |
| TPH (mg/kg) | 42867.573 | 27853.666 | 24716.462 | 21647.944 | 20348.065 |

**Table 5b.** Monitoring of isolates (SB, GA) for potential to biodegrade crude oil in artificially petroleum hydrocarbon polluted soil (Day 14).

| Component | A Soil+crude oil | B Soil +Npk+crude oil+ carrier | C Soil+Npk +crude oil+GA+ carrier | D Soil+Npk +crude oil +SB+carrier | E Soil+Npk+ Crude oil +GA+SB+ Carrier |
|---|---|---|---|---|---|
| Nonane | 7893.170 | 1306.340 | 1468.498 | 324.263 | 2070.711 |
| Decane | 5320.377 | 4565.055 | 1138.553 | 581.630 | 94.144 |
| Dodecane | 9340.060 | 3366.588 | 1268.960 | 556.958 | 442.424 |
| Tetradecane | 10930.760 | 1819.097 | 6720.007 | 8783.140 | 3373.243 |
| Hexadecane | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| Octadecane | 3142.511 | 3071.146 | 970.447 | 1381.807 | 2853.341 |
| Nonadecane | 2043.093 | 1008.079 | 1850.545 | 636.406 | 1251.290 |
| Eicosane | 1495.144 | 1128.850 | 730.159 | 487.593 | 33.975 |
| Docosane | 257.714 | 167.516 | 210.462 | 194.688 | 251.011 |
| Tetracosane | 434.490 | 393.019 | 384.678 | 185.624 | 66.528 |
| Hexacosane | 239.395 | 159.115 | 158.415 | 162.817 | 216.862 |
| Octacosane | 327.896 | 244.310 | 41.036 | 243.197 | 8.760 |
| Triacontane | 512.899 | 278.825 | 40.303 | 61.906 | 7.472 |
| Hexatriacontane | 32.133 | 17.242 | 10.313 | 2.665 | 0.046 |
| Total aliphatics (mg/kg) | 41969.642 | 17525.182 | 14992.376 | 13602.694 | 10669.807 |
| Total PAH | 3.004 | 0.128 | 0.102 | 0.206 | 0.146 |
| TPH (mg/kg) | 41972.646 | 17525.310 | 14992.478 | 13602.900 | 10669.953 |

**Table 5c.** Monitoring of isolates (SB, GA) for potential to biodegrade crude oil in artificially petroleum hydrocarbon polluted soil (Day 28).

| Component | A Soil+crude oil | B Soil+Npk+ crude oil+ carrier | C Soil+Npk +crude oil+GA+ carrier | D Soil+Npk+ crude oil + SB+ carrie | E Soil+ Npk+ Crude oil +GA+SB+ Carrier |
|---|---|---|---|---|---|
| Nonane | 5390.610 | 1627.926 | 705.865 | 155.908 | 995.309 |
| Decane | 2595.939 | 2194.173 | 547.282 | 279.607 | 285.621 |
| Dodecane | 8566.666 | 2473.960 | 609.960 | 267.749 | 212.700 |
| Tetradecane | 11811..843 | 345.678 | 3229.915 | 3212.195 | 1621.349 |
| Hexadecane | 0.00 | 0.000 | 0.000 | 0.000 | 0.000 |
| Octadecane | 2510.452 | 1956.784 | 466.485 | 1625.465 | 1371.466 |
| Nonadecane | 1501.401 | 1388.446 | 889.490 | 305.934 | 313.088 |
| Eicosane | 918.672 | 542.619 | 254.868 | 186.346 | 448.956 |
| Docosane | 623.922 | 176.697 | 101.211 | 141.693 | 409.080 |
| Tetracosane | 1008.887 | 237.017 | 281.072 | 89.273 | 32.031 |
| Hexacosane | 615.117 | 124.596 | 76.196 | 126.375 | 104.287 |
| Octacosane | 457.654 | 213.606 | 107.843 | 116.944 | 265.196 |
| Triacontane | 546.572 | 134.069 | 192.455 | 29.810 | 80.549 |
| Hexatriacontane | 15.501 | 8.343 | 12.596 | 9.988 | 14.574 |
| Total aliphatics (mg/kg) | 36563.236 | 11423.915 | 7475.238 | 6547.287 | 6154.206 |
| Total PAH | 2.956 | 0.113 | 0.098 | 0.194 | 0.172 |
| TPH (mg/kg) | 36566.192 | 11424.028 | 7475.336 | 6547.481 | 6154.378 |

## 4. Conclusions

This research work has reviewed the potentials of indigenous soil bacteria to biodegrade oil polluted soil. It also showed that bioremediation removed up to 87 % of the oil added to the soil. Both the bacteria consortium and individual bacteria isolates were able to bio-remediate oil polluted soil. Finally, the research also succeeded in immobilizing crude oil degrading microorganisms on the selected local cellulosic material (coconut fiber carrier) for ease of deployment of oil degrader into petroleum hydrocarbon polluted soil.

## References

[1] Ayotamuno, M. J., Akor, A. J. and Igho, T. J. (2002). Effluent quality and wastes from petroleum- Drilling operations in the Niger Delta, Nigeria. *Environment management and health, 13*(2), 207-216

[2] Okoh, A. I. (2006). Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants. *Biotechnology and molecular biology reviews*, *1*(2), 38-50.

[3] United nations environmental programme (UNEP). (2011). In-situ environmental assessment of Ogoniland, Rivers state. www.unep.org, Accessed: 19th January, 2020.

[4] Ojo, O. A. (2005). Petroleum hydrocarbon utilization by native bacteria population from a wastewater canal southwest Nigeria. *African journal of biotechnology*. *5*(4), 333-337.

[5] James, G. S. (2002). Hand book of petroleum product analysis, John Wiley and Sons, Inc. New York. 3-7pp.

[6] Agiri, G. O., Akumagba, P. E., Adimula, H. A. and Edoga, M. O. (2010). Isolation and characterization of petroleum hydrocarbons degrading bacteria from soils around Warri, Delta State, Nigeria. *Journal of environmental science, 23*(4), 132-139.

[7] Miyazaki, H. (2005) Method of remedying contaminated soil by microorganisms. US Patent, 6,905,288B2.

[8] Díaz, M.P., Boyd, K. G., Grigson, S. J. W Burgess, J. G. (2002). Biodegradation of crude oil across a wide range of salinities by an extremely halotolerant bacterial consortium MPD-M, immobilized onto polypropylene fibers. *Biotechnology and bioengineering*. *79*,145-153.

[9] Chen, K. C., Chen, J. J. and Houng. J. Y. (2000). Improvement of nitrogen removal efficiency

using immobilized microorganisms with oxidation-reduction potential monitoring. *Journal of industrial microbiology and biotechnology*. *25*, 229-234.

[10] Delille, D., Delille, B. and Pelletier, E. (2002). Effectiveness of bioremediation of crude oil contaminated subantartic intertidal sediments: the microbial response. *Microbial ecology, 44*, 118-126

[11] Sang-Hwan, l., Seokho, l., Dae Yaeon, k., jeong- gyu, k., (2007). Degradation characteristics of waste lubricants under different nutrient condition. *Journal of hazardous materials 143*, 65-72.

[12] Schaefer, M. and Juliane, F. (2007). The influence of earthworms and organic additives on the biodegradation of oil contaminated soil. *Applied soil ecology 36*(2), 53-62.