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Biodegradation of Hydrocarbon by Micro-organisms Isolated from Crude Oil Contaminated Soil in Niger Delta Area of Nigeria

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ABSTRACT

Soil that was artificially polluted with Escravos light crude oil was degraded with hydrocarbon degrading micro-organisms isolated from crude oil contaminated soil in Niger Delta area. The micro-organisms isolated and applied included *Enterobacter aerogenes*, *Serratia marcescens* and *Proteus myxofaciens* immobilized in coconut fibre as a carrier. The results of the laboratory analysis showed that the immobilized micro-organisms bacteria had good self-life with micro-organisms load of 5.43 x 10^8 cfu/ml and 2.52 x 10^{19} cfu/ml on the first and 28 days respectively. The results gotten from immobilized micro-organisms system are good for the biodegradation of hydrocarbons. At the expiration of 28 days of applying the immobilized micro-organisms system in the laboratory scale degrading of Escravos light crude oil, the remaining concentration of total hydrocarbon decreased to 14.36 % for one of the samples compared to the remaining concentration of total hydrocarbon of 85.31 % in the control sample. Therefore, the immobilized micro-organisms system using coconut fibre as a carrier can serve as an effective and fast biodegradation tool for cleaning up petroleum hydrocarbon polluted soil at low cost.

Introduction

Assessment of petroleum hydrocarbon polluted sites is an investigative process to determine the nature and extent cum impact of the pollution on the environment. Site assessment is a crucial early stage in understanding what has happened at the pollution site. This stage leads to find the answers to such questions as how far has the release traveled, what is the hydrogeology of the site, etc. On other hand, remediation deals with the removal of pollutants or contaminants from environmental media such as soil, groundwater, sediment, or surface water for the general protection of human health and the environment. Biodegradation is the chemical dissolution of materials by bacteria or other biological means.

According to Onwurah, *et al.*, (2007) [1], bioremediation or biodegradation is a technology that exploits the abilities of microorganisms and other natural habitat of the biosphere to improve environmental quality for all species, including man. Remediation techniques are eventually employed in a bid to restore the polluted area to a status as near as possible to the original. It has been known for many years that the major constituents of most crude oils are biodegradable (Prince, 1997). Most of the petroleum which enters the environment is degraded naturally because many species of bacteria in the environment are endowed with the ability to oxidize petroleum hydrocarbons[(Zobell, 1964). However the rate at which natural degradation occurs is slow and does not provide immediate or rapid relief when accidents occur or to the problem of chronic pollution (Atlas and Bartha, 1992). Many reasons account for why degradation is slow in the natural environment, these include: low counts of hydrocarbon-degrading microbes, toxicity of some components, limited oil/water or soil interface, insufficiency of oxygen and lack of essential mineral nutrient(Atlas and Bartha, 1992). Many bacteria are capable of degrading the constituents of oil and the oil degrading bacteria are the most important input in bioremediation technique (Gimsing, *et al.*, 2010) [6]. Interest has arisen in the use of immobilized bacteria technology to treat oil pollution to enhance the rate of degradation.

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. Oil exploration and production equally did not spare the environment so long they have the potentials for a variety of impacts on the environment. These impacts depend upon the stage of process, size and complexity of the project, nature of sensitivity of the surrounding environment, the effectiveness of planning, pollution and control techniques.

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. Some of these spillages occur as a result of equipment failure, operational mishap or intentional damage to facilities.

Oil and gas activities have caused damage in several forms to the Niger Delta region of Nigeria. In exploration, seismic lines have cleared significant forest areas and seismic crews have generated thousands of tons of wastes, all disposed untreated directly into the ecosystem. In production, there is a considerable amount of dredging and filling of the water ways, siltation, and sulfidic dredge spoils leading to acidification of water bodies, erosion, spills (well blowouts and facility failures), pollution from gas and associated oil flaring, discharge of huge amount of production water containing significant quantities of hydrocarbons and drilling mud discharge. In transportation, laying of several thousand miles of oil and gas pipelines across Niger Delta habitats have resulted in significant habitat damage and loss, pipeline, tanker and storage tank spills. In refining, toxic sludge discharge and process spills pollute waterways, flaring and stack emissions pollute the atmosphere, and refined

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products (particularly petrochemicals) further enter the ecosystem. Niger Delta is one of the most petroleum hydrocarbon polluted ecosystem in the world [7](UNEP report 2011). Waterways and marine system have been badly polluted. Residents, particularly the rural people, have been badly affected by environmental damage and pollution from the oil industry. Fishing, the mainstay of the Delta rural economy, has been reduced in both fresh water and marine eco-system. Forests and agricultural activities have been damaged by chronic spills and atmospheric pollution.

Materials And Methods

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. Surface soil was collected by means of trowel from each of the selected sites into separate polyethene bags (about 15 litres) that were immediately taken to the laboratory.

Isolation of crude oil degrader (bacteria)

Petroleum hydrocarbon bacteria were isolated from each of the contaminated soil samples by the methods outlined below:

This involved the cultivation of hydrocarbon degraders in mineral salt broth (with crude oil as carbon source) by adopting an enrichment technique by Schlegel (1993) [8].

Mineral salt media (MSM) solution was prepared according to the composition formulated by Mills et. al. (1978) [9] as modified by Okpakwasili and Okorie (1988) [10] as follows: Ten grams of NaCl , 0.42g of $MgSO_4.7H_20$,0.29g of KCl ,0.83g of $KHPO_4$,1.25g of Na_2HPO_4 , 0.42g of $NaNO_3$, and one litre of deionised/distilled water .

Trace element solution of 0.28g of $C0S0_4.7H_2O$, 2.0g of $CaC0_3$, 0.25g of $CuS0_4.5H_20$, 0.06g of H_3B0_3 ,1.44g of $ZnSO4.7H_2O$, 1.12g of $MnSO_4.H_2O$, 4.5g of $FeSO_4.7H2O$, 10.75g of MgO , 51.30ml of Concentrated HCL , and one litre of deionised/ distilled water was also prepared.

MSM solution and trace element solution were sterilized in separate containers using autoclave at 0.14mpa for fifteen minutes. Two millilitres of trace element solution was added to one litre of MSM solution and the final MSM preparation was adjusted to pH 7.3 before it was used for cultivating the crude oil degrader bacteria. One hundred (100ml) of the final MSM preparation was taken in a sterilized Erlenmeyer flask. 10ml of crude oil contaminated soil suspension (1g soil in 10ml 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 WRPC. 1ml of crude (Escravos light , sterilized by means of mem3brane filter 0.45um and added to each flask as a sole carbon source. All flasks were incubated at 30^0C in an incubator for 10 days. After growth was observed for 10 days, 1ml of the M6SM broth culture was transferred from each of the growth flask into separate fresh 100ml MSM (with 1ml of crude oil, Escravos light added) in sterile conical flask. These were again incubated for 10days at 30^0C 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.

Transfer to nutrient agar media to obtain colonies of crude oil degrading bacteria

28g of nutrient agar powder was weighed and added to 1 litre of deionized water. This was allowed to soak for 10 minutes, swirl to mix then sterilized by autoclaving for 15 minutes and allowed to cool to 47^oC and mixed well before pouring to petri plates. A 10^{-3} dilution of each MSM growth was prepared. 1ml of this dilution from each growth flask was transferred into petri plates as inoculums and nutrient agar plates were prepared from them and labeled G and S. Each inoculated nutrient agar plate was incubated at 30^0C for 24 hours to obtain growth colonies of the bacteria. The growth on each nutrient agar plate was observed for cultural characteristic and noted down for further use in identification. Three pure bacteria culture were obtained from the different growth plates by transfer to sterile nutrient agar slants which were incubated at 30^0C for 24 hours. The pure culture slants isolates were prepared in duplicates and labeled SA, SB and GA. One set of pure culture agar slant bacteria isolate was stored in the refrigerator as stock culture for future use. The second set of pure culture agar slants bacteria isolates was used for characterization of the isolated sets of bacteria (SA,SB, and GA) and also for inoculating fresh MSM (with crude oil) for the purpose of determining the potential of each isolate (SA, SB and GA) to utilize crude oil as carbon source (ie potential to biodegrade).

Monitoring isolates (SA, SB, GA) potential to utilize crude oil as carbon source. (potential to biodegrade crude oil).

The procedure adopted measured the ability of isolates to grow in MSM broth with crude oil as a source of carbon as reflected in turbidimetric readings using turdidimeter [11] (Vasileva-Tankova and Gesheva, 2004). Four (4) sterile conical flask containing 500ml of MSM and 5ml of sterile crude oil (Escravos light) were prepared and labeled SA, SB, GA and control. Each flask was covered with cotton wool and the opening end of the flask wrapped with aluminium foil. Using a sterile wire loop, pure culture of each bacteria isolate (SA,SB,GA) were transferred into the appropriate flask laballed for it except the control and was incubated at 30^0C 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 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.

Microbiological methods for characterizing and identifying bacteria isolates

Bacterial isolates were examined for colonial morphology as well as cell micro-morphology and biochemical characteristics. Tests were carried out based on specified methods [12,13,14] described by Carpenter (1977), Cruickshank *et al.* (1975) and Gerhadt *et al.* (1981). Identification of the bacteria to the generic level was performed following the schemes of MacFaddin (1979) [15] and Holt (1982)[16].

Gram's staining

A smear of pure bacterial culture was prepared on a clean glass slide. This was air dried and heat fixed by passing the slide through a flame several times. It was then covered with crystal violet solution for 0ne (1) minute. The slide was washed with distilled water and immediately immersed in Lugol,s iodine solution for one minute. The stained slide was washed in water, blotted dry and decolourized with ethyl alcohol (95%) for 30 seconds until the dye no longer ran off the smear. This was then washed with water, blotted dry and finally counter stained with Safranin dye solution for 30 seconds, washed, dried and observed under the light microscope with the oil immersion objective. Microorganisms with blue or violet colour were termed positive, while those with pink or red colour were termed

negative. Results were recorded in terms of color of isolates, shape and arrangement that were observed.

Acid-fast stain

The modified Ziehl-Neelsen staining method [17] was used. A smear of the test isolate was prepared on a glass slide, air dried and heat fixed fixed. The smear was covered with strong carbol fuchsin solution and heated until steam rose. The preparation was allowed to stain for 5minutes heat being applied at intervals to keep the stain hot.After about 6 minutes, the slide was washed with water and decolourized in 20% (w/v) sulphuric acid. It was again washed with water. The process of decolourization was repeated several times until all traces of red disappeared from the smear. The slide was washed with water and then counter-stained with loeffler's methylene blue for one (1) minute. It was washed, blotted dry and mounted for examination under the oil immersion objective of a light microscope. Acid fast organisms stained bright red, other organisms stained blue.

Motility test

This test is used to determine if the isolate is motile or not. Motility agar with the following composition; Glucose: 20g, Agar:2g, one litre of distilled water was prepared, dispensed in 15ml amounts into MacCartney bottles and sterilized at 121^0C for 15minutes. On cooling, the solidified agar was stab inoculated with the pure culture of the isolate using a straight platinum wire and incubated at 28 ± 2^0C for 24hr. Spreading growth of the isolate out of the line of stab that renders the medium slightly opaque indicate a positive result. A negative result is indicated by growth confined to the stab.

Biochemical test (catalase production)

One (1) ml of hydrogen peroxide solution was discharged into a clean glass slide and a sterile inoculating loop was used to collect the colonies of the test organism which were subsequently immersed in the hydrogen peroxide solution. A positive result was indicated by the production of gas bubbles, while its absence was regarded as a negative result.

Citrate utilization test

Five millilitres (5ml) of simmon citrate broth was inoculated with the test organism. The broth was incubated at 37°C for 48 hours. A positive reaction was indicated by a change in the colour of the medium from green to blue color. Negative tubes were observed daily for 4 days to detect any delayed reaction.

Methyl red and voges-proskauer tests (MR-VP)

Five (5ml) of a 48 hours culture of the test organism was put into glucose phosphate medium and incubated at 37^0C for 48 hrs. The following specific tests were conducted as follows:

- **Methyl Red Test**: Five drops of methyl red indicator was then cultured in a tube, mixed and the results were observed for results. Positive cultures were bright red and negative cultures were yellow. An orange colour indicate that the isolate is variable.
- **Voges-Proskauer test: One (1)** ml of potassium hydroxide (KOH) and 3 ml of 5% alpha-naphthol was added to the culture and the reaction observed. A positive result was indicated by the production of a pink colour within 10 minutes which later turned crimson after 30 minutes. A negative result showed no colour change.

Procedure for bacteria count

One gram of soil was weighed out and poured out into a sterile maccartney bottle. Nine millitre (9ml) of sterile distilled water was added to the soil sample in (1) above, covered and mixed by shaking. With the aid of a sterile pipette, 1ml of the mixture was transferred under asceptic conditions to another Maccartney bottle containing 9ml of sterile distilled water to obtain a dilution of 10^{-2} Subsequent transfers were made until a dilution of 10^{-5} was obtained.

A loopful of the various dilutions was plated out by streaking on sterile nutrient Agar plates and incubated at room temperature (25-28^o c) for 24 hours.

The plate discrete colonies (less than or equal to 300 per plate) was selected after incubation.

The total bacteria count per gram of soil was obtained by multiplying the number of colonies on the plate by the dilution factor.

Collection and Preparation of Locally Sourced Carrier

Locally sourced agricultural harvest by-product (Coconut fibre) was collected and prepared to serve as carrier for crude oil degrader microorganisms.

Coconut fibre was obtained from the fibrous mesocarp of the coconut fruit obtained from Eku, Delta state. The fibre 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 (65^0C) for another seven days and ground in a grinding mill. The brown coloured powder was sieved through 0.48mm (ASTM Sieve) and stored in a plastic container at room temperature in the laboratory.

Characterization of cellulosic material

The agricultural harvest by-product (Coconut fire) was characterized by its water content, nitrogen content, and phosphorus content.

Determination of water content of cellulosic material. (Coconut fibre)

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 percentage. A crucible previously cleaned and oven dried was weighed (w_1). The crucible was then filled with the dried mill of the coconut fibre and weighed (w_2). The crucible containing the cellulosic material was then kept in an oven at a temperature between 105^0C to 11^0C for 24 hours. The final constant weight (w_3) of the container with the dried sample was then determined.

Determination of total nitrogen

In a typical determination, about 2.00g of the ground coconut fibre was weighed. 9ml of conc. Sulphuric acid was added and the mixture was gently heated on a hot plate until white fumes was observed. It was allowed to cool, filtered and the filtrate was made up to 100ml in volumetric flask. 25ml of the digest was taken from the flask and made up to 50ml with distilled water. 5ml of 12M potassium hydroxide was added and the solution was filtered. 25ml of the filtrate was taken and 1ml of 10% sodium tartarate and 5ml 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 15minutes for colour development. Absorbance was read at 460nm with a direct reading spectrophotometer (Hach Direct reading 2000 Spectrophotometer).

Determination of phosphorus

One gram (1g) of the dried and ground coconut fibre was weighed into a 250ml conical flask and 4ml of perchloric acid, 2ml 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.

Table 1: Results of Characterization/ Identification of Bacteria Isolate SA, SB, and GA Probable Identity of Isolates.

Parameters	Isolate GA	Isolate SA	Isolate SB
CULTURAL CHARACTERISTICS			
Shape	Round	Round	Round
Colour	Creamy	Creamy	Creamy
Size	Large	Small	Small
Elevation	Flat	Flat	Flat
Transparency	Transparency	Opaque	Opaque
MORPHOLOGY			
Gram stain	Negative	Negative	Negative
Cell type	Rod	Rod	Rod
Cell arrangement	Single	Cluster	Cluster
Acid fast	Negative	Negative	Negative
BIOCHEMICAL TEST			
Citrate utilization	+	+	+
Catalase production	+	+	+
Motility	+	+	+
Methyl red	-	-/+	+
Vogues prauskeur	+	+	+
Oxidase test	-	-	-
Oxidative test			
Glucose	+	+	+
Lactose	+	-	-
Fermentation test			
Glucose	+	+	+
Lactose	+	+	+
Probable identity	*Enterobacter aerogenes*	*Serratia marcescens*	*Proteus myxofaciens*

On cooling the solution was filtered with a Whatman No 42 filter paper made up to 100ml in a volumetric flask. 0.2112g 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. To 5ml of the digest was added 10ml of distilled water, 4ml of reagent B and made up to 25ml 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 882nm with a direct reading spectrophotometer (Hach Direct reading 200 Spectrophotometer).

Testing effectiveness of selected individual oil-degrader bacteria isolates (SB,GA, SBGA) 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. Fifty gram (50g) of sterile sharp sand was contaminated with 10%(v/w) sterile crude oil (Escravos light) in 100ml capacity Erlenmeyer flasks with loosely placed screw caps. Sterile stock solution (NPK 15-15-15 and MSM) was used as nutrient amendment to the (16%v/w) in all flasks containing oil-contaminated soil except in the control flask. Carrier with immobiled isolate, when used, was added to soil in ratio 1:5. Five (5) flasks were set-up in the 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 30^0c and agitated twice daily for aeration and mixing to increase contact between the isolates and oil contaminated soil. Sample from flasks were analysed at day zero and subsequently every 14 days for following parameters:

Bacteria count using plate-count method on nutrient agar to detect any relative increase in quantity which could be due to bioremediation activity.

Total petroleum Hydrocarbon (TPH) in soil using Gas Chromatography (GC) Technique to find o.3ut how much of the crude oil in the contaminated soil is being removed in each flask.

PH to detect any relative changes in pH during bioremediation activity.

Percentage clean-up determination.

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 in total petroleum hydrocarbon (TPH) which is also the extent of clean –up. The percentage reduction in TPH was calculated.

Results and Discussions

Table 1 shows the results of characterization/identification of bacteria isolates SA, SB, and GA probable identity. Three bacteria were isolated, characterized and identified as follows: isolate GA as Enterobacter aerogenes, SA as Serratia marcescens and SB as proteus myxofaciens which are all members of oil degrading microbes as contained in the Bergey`s Manual of Determinative Bacteriology[13].

Table 2 shows the results of monitoring of isolates (SA,SB,GA,CONTROL) for potential to utilize crude oil as carbon source (potential to biodegrade crude oil) over the period of 28 days. The results which measure the ability of isolates to grow in MSM broth with crude oil as a source of carbon as reflected in the Turbidimetric readings using Turbidimeter show that after a period of 28 days, there were growth in all the samples including the control. The growth in the control can be

attributed to presence of external bacteria in the laboratory since it is an open laboratory and other laboratory activities were taking place during the time of the research even though the growth in the control sample is very small when compared to the rest. 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 (29555) 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.

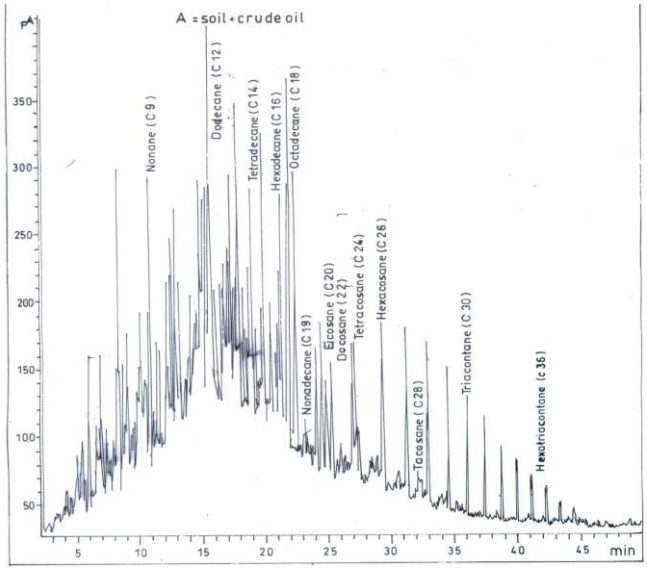

Figure 1 : Chromatogram for sample A on day 7

Table 2: Turbidity Readings of Isolates (NTU)

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

KEY: SA=Serratia Marcescens, **SB=** Proteus Myxofaciens, **GA=** Enterobacter aerogenes, **Control**

Table 3 shows the results of percentage total petroleum hydrocarbon clean up by individual bacteria isolates and the consortium (GASB). It shows remarkable clean up 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 (GASB) has cleaned the total petroleum hydrocarbon from zero percent to 52.45 percent, `D` set up which contains bacteria isolate SB has cleaned the total petroleum hydrocarbon from zero percent to 49.5 percent, `C` which contains bacteria isolate GA has cleaned the total petroleum hydrocarbon from zero percent to 42.34 percent, `B` cleaned from zero percent to 35.02, `A` set up which is the control has a zero cleanup. On day 14, the consortium (GASB) has cleaned the total petroleum hydrocarbon from 52.45 percent to 75.11 percent, isolate SB cleaned the total petroleum hydrocarbon from 49.50 percent to 68.27 percent, GA cleaned the total petroleum hydrocarbon from 42.34 percent to 65.03 percent, `B` set up cleaned from 35.02 percent to 59.12 percent and `A` which is the control cleaned from zero percent to 2.09 percent. On the 28th day, the consortium cleaned the total petroleum hydrocarbon from 75.11 percent to 86 percent, isolate SB cleaned the total petroleum hydrocarbon from 68.27 percent to 84.73 percent, isolate GA cleaned the total petroleum hydrocarbon from 65.03 percent to 82.56 percent. `B` set up has 73.35percent clean up and `A` which is the control has 14.7 percent clean up. From the above results the consortium has the highest clean up of total petroleum hydrocarbon while the control has the least clean up. The noticed cleaned up in the control set up is as a result of presence of external bacteria in the laboratory.

Table 3: Percentage total petroleum hydrocarbon clean up

Set up		% TPH Clean up	
	DAY 7	DAY 14	DAY 28
A Control	0	2.09	14.70
A	35.02	59.12	73.35
C	42.34	65.05	82.56
D	49.50	68.27	84.73
E	52.45	75.11	86.00

KEY: 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.

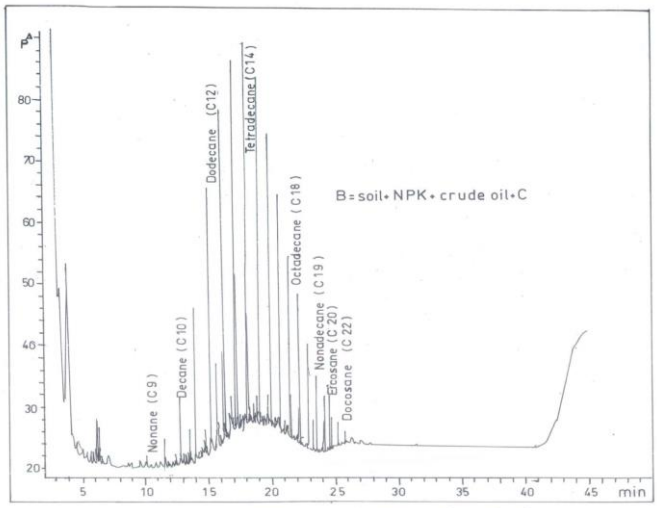

Figure 2: Chromatogram for sample B on day 7

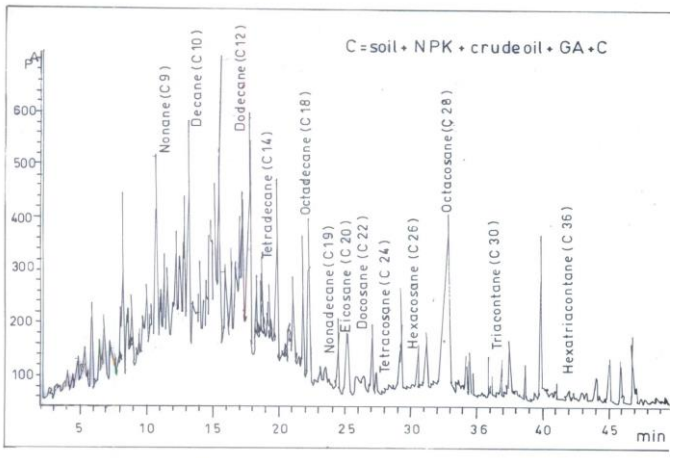

Figure 3 Chromatogram for sample C on day 7

In the results of the bacteria count (Table 4 below), on day one , both 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/ml respectively. On day 14, A showed no growth while B showed a growth of $2.6\ x10^4$ cfu/ml which could be attributed to external bacteria. C showed a growth of 7.68 x1011, D ($1.42\ x10^{12}$) and E showed a growth of $1,96\ x\ 10^{12}$ cfu/ml. By the end of 28 days period, all set up showed bacteria growth. `A` showed a growth of $1.2\ x\ 10^3$cfu/ml, `B` showed a growth of $5.22\ x\ 10^{11}$cfu/ml, `C` showed a growth of $9.30\ x\ 10^{14}$cfu/ml , `D` showed a

growth of 1.79 x10^{17}cfu/ml and `E` showed a growth of 2.52 x.10^{19} cfu/ml.

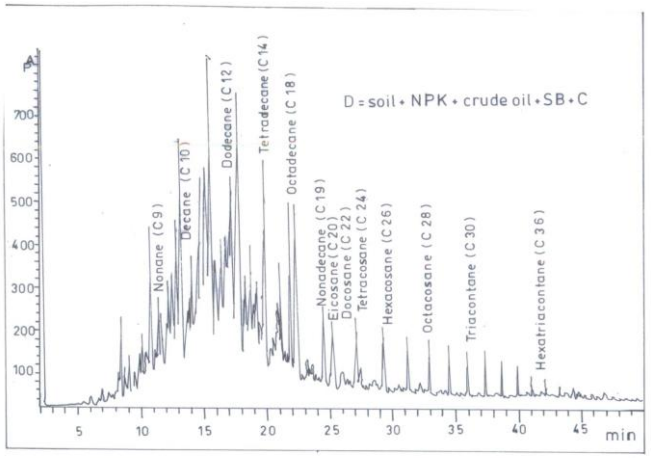

Figure. 4 : Chromatogram for sample D on day 7

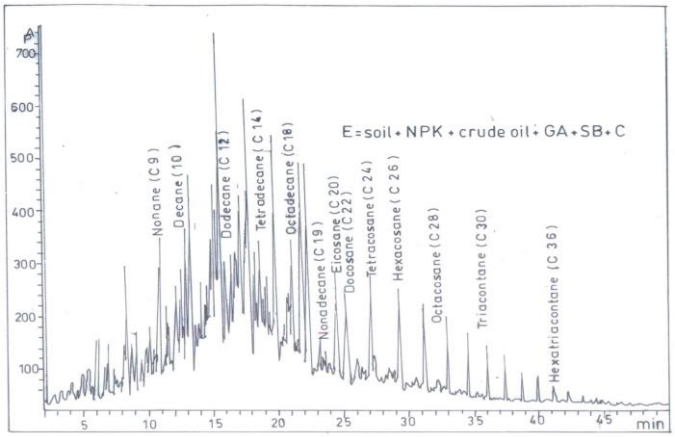

Figure. 5 : Chromatogram for sample E on day 7

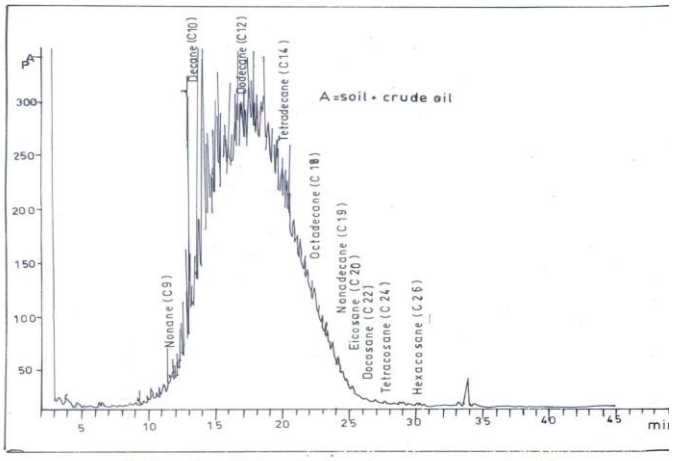

Figure. 6 : Chromatogram for sample A on day 28

There were growth in `A` and in `B` but not as much as we have in C, D, and E. Bacteria in `A` and `B` are external bacteria that entered during sampling. Looking at the results, there was remarkable increase in bacteria population in C, D and E from day zero to day 28. At the end of day 28, `E` showed the highest bacteria population of 2.52 x10^{19} cfu/ml, followed by `D` with a bacteria population of 1.79 x 10^{17} cfu/ml, `C` showed a population of 9.30 x 10^{14}cfu/ml, `B` showed a bacteria population of 5.22 x 10^{11}cfu/ml while `A` which is the control showed the least growth of bacteria of 1.2 x 10^{3} cfu/ml. Comparing the results of the bacteria count and that of percentage in total petroleum hydrocarbon clean up, it shows that the consortium which has the highest population of bacteria at the end of 28 days period (2.52 x 10^{19} cfu/ml) also showed highest clean up in total petroleum hydrocarbon.

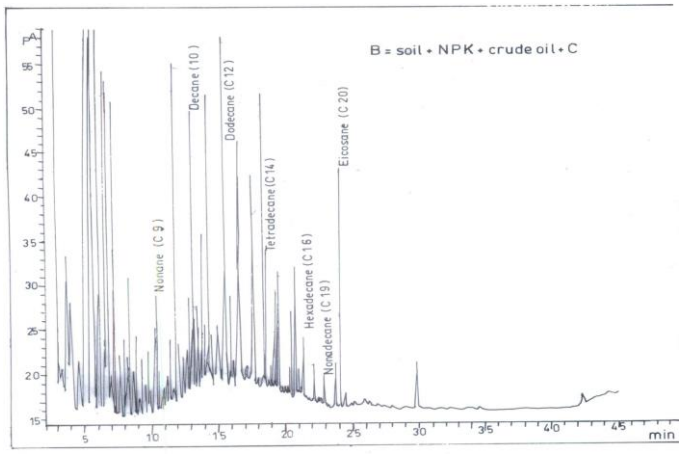

Figure. 7 : Chromatogram for sample B on day 28

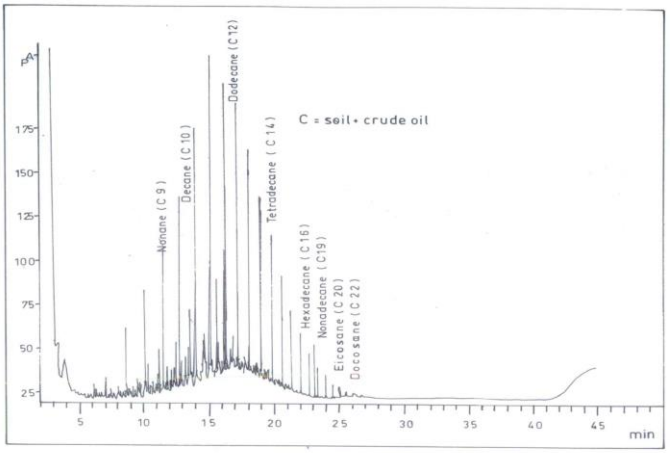

Figure. 8 : Chromatogram for sample c on day 28

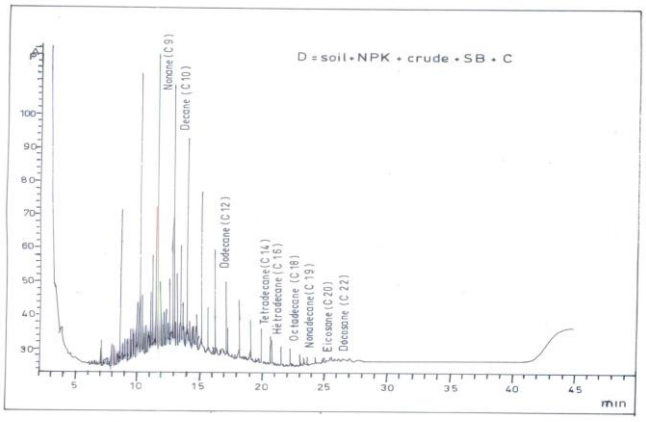

Figure. 9 : Chromatogram for sample D on day 28

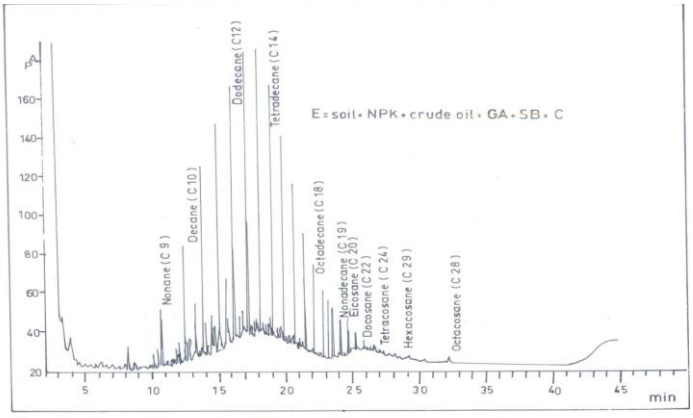

Figure. 10 : Chromatogram for sample E on day 28

The control `A` with least bacteria population of 1.2 x 10^3 cfu/ml also showed the least clean up in total petroleum hydrocarbon at the end of 28 days period. It follows that the increase in bacteria population is as result of their ability to ultilize crude oil as a source of carbon (Bio-remediate).

Table 4: Bacteria Count of Laboratory Bioremediation Experiment.

		Bacteria Load (cfu/ml)	
Set Up			
	Day 1	Day 14	Day 28
A	0	0	1.2 x 10^3
B	0	2.6 x 10^4	5.22 x 10^{11}
C	1.56 x10^8	7.68 x 10^{11}	9.30 x10^{14}
D	2.37 x 10^8	1.42 x 10^{12}	1.79 x 10^{17}
E	5.43 x 10^8	1.96 x 10^{12}	2.52 x 10^{19}

KEY: 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.

Conclusions

In conclusion, this paper on Biodegradation of Hydrocarbon by Micro-organisms Isolated from Crude Oil Contaminated Soil in Niger Delta Area of Nigeria has succeeded in developing a bacteria product that could be rapidly deployed for bio-remediation of oil polluted soil. The remediation removed up to 86% of the oil added to the soil. The products developed have great potential for use as product of choice to remediate oil polluted soil. Both the bacteria consortium and individual bacteria isolates were able to bio-remediate oil polluted soil. The research also succeeded in immobilizing crude oil degrading micro- organisms on the selected local cellulosic material (coconut fibre carrier) for ease deployment of oil degrader into petroleum hydrocarbon polluted soil.

References

Adebanwi, W, (2001): Nigeria: Shell of a State. Dollars and Sense Magazine, http://www.thirdworldtraveler.com/Africa/Nigeria_Shell_State.html.

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 in press

Atlas, R.M. and R. Bartha (1972) Degradation and mineralization of petroleum in seawater. Limitation by nitrogen and phosphorus. *Biotechnology and Bioengineering* 14, 309-318.

Atlas, R.M. (1981) Microbial degradation of Petroleum hydrocarbons. An environmental perspective. *Microbiol. Reviews* 45, 180-209.

Ayotamuno, M.J., Akor, A.J.,& Igho, T.J (2002). Effluent Quality and Wastes from Petroleum- Drilling Operations in the Niger Delta, Nigeria. Environment Management and Health, 13(2), pp 207-216.

Broome, D.C., and Wadelin, F.A. (1973). *Criteria for Quality of Petroleum Products*. J.P. Allinson (Editor). John Wiley & Sons, New York. Chapter 10.

Chen, K.C., J.J. Chen, and J.Y. Houng (2000) Improvement of nitrogen removal efficiency using immobilized microorganisms with oxidation-reduction potential monitoring. *J. Ind. Microbiol. Biotechmol.* 25, 229-234.

Chenier, P.J. (1992). *Survey of Industrial Chemistry*, 2nd Revised Edition. VCH Publishers, New York. Chapter 7.

Cunningham, C.J., I.B. Ivshina, V.I. Lozinsky, M.S. Kuyukina, and J.C. Philip (2000) Bioremediation of diesel-contaminated soil by microorganisms immobilized in polyvinyl alcohol. *International Biodeterioration and Biodegradation* vol. 54 issues 2-3, 167-174.

Díaz, M.P., K.G. Boyd, S.J.W. Grigson, and J.G. Burgess. (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.

Diya`uddeen,B.H., Mohammed, I.A., Ahmed, A.S., & Jibril, B.Y.(2008). Production of Activated Carbon from Corncobs and its Utilization in crude oil spillage. Agricultural Engineering International: cigr Ejournal Manuscript EE08 004, vol .X April 2008.

Egwaikhide, P.A., Akporhonor, E.E & Okieimeh, F.E (2007). Utilization of coconut Fibre in the removal of soluble Petroleum Fraction Polluted water.International Journal of Physics Sciences, 2(2), 47-49.

Fleming, G. (1991) *Recycling Derelict Land Institution of Civil Engineers*. Thomas Telford Ltd., London. 218 pp.

Gardea-Torresdey, J.L., J.L. Arenas, N.M.C. Francisco, K.J. Tiemann, and R. Webb. (1998) Ability of immobilized cyanobacteria to remove metal ions from solution and demonstration of the presence of metallothionein genes in various strains. *J. Hazardous Substance Research.* **1** (2), 1-8.