Observing The Degradative Abilities Of Different Bacterial Isolates Using Combined Method (Dcip, Turbidity, Ph And Spectrophotometric Approach)

[1]Okorhi-Damisa F.B, [2]Tudararo -Aherobo, [3]Laurelta

Department of Environmental Management and Toxicology,
Federal University of Petroleum Resources Effurun Delta State Nigeria.

ABSTRACT: **The petroleum industry supplies a substantial quantity of world's energy demands in addition to popular petro-chemical intermediates required for production of extensive range of goods like solvents, dye stuffs, pharmaceuticals, polymers, and new chemicals etc. All these tend to generate environmental pollution when discharged in the environment; however the aim of this study is to ascertain the degradative abilities of the organisms isolated from the impacted site by monitoring their degradative abilities, the hydrocarbon-degrading potential of eight bacterial isolates were monitored using spectrophotometer, turbidity and redox indicator dye 2,6-dichlorophenolindophenol (DCIP).These isolates were identified based on their macroscopic, microscopic, biochemical and molecular characteristics, they were identified as:*Bacillus thuringiensis, Bacillus velezensis, Lysinibacillus fusiformis, Citrobacter specie, Poor Sequence quality, Stenotrophomonas specie,and Lysinibacillus pakistanensis.* **The screening was carried out for a period of 24-360 hours monitoring their TPH, Turbidity and pH with various control alongside, however the results obtained from the study shows that the pH were between 5.11± to 6.11± and control having the pH of 5.29± and 6.59 ± respectively, while the TPH were between the range of 1204.88mg/l and 136.72mg/l and control recorded the values within 1204.88mg/l and 644.24mg/l while for turbidity the values were between 1109.4*NTU* and 2532.0*NTU* and the values of the control were within 640.02*NTU* and 1215.0*NTU* . Thus considering the easiness and conciseness of sample preparation, high through put screening using Turbidometric and spectrophotometric approach could be considered as an efficient, accurate and rapid method of quantifying the level of hydrocarbon present in a sample. This study shows that the quantity of hydrocarbon utilizing bacterial produced by the strains has the potential to biodegrade and cleans up any toxic substance present in the environment.**

Key words: Tph, Turbidity, Spectrophotometer

INTRODUCTION

Petroleum oil is an important strategic resource for which all countries compete fiercely (Sun, 2009; Xu *et al*., 2018). Indeed, anthropogenic activity is reliant on oil to meet its energy demands, which causes the petrochemical industry to flourish. However, petroleum use, results in environmental deterioration (Umar *et al.*, 2021).

Total petroleum hydrocarbon (TPH) is a term used to represent petroleum (fossil oil) that consists of a blend of thousands of compounds. They are referred to as hydrocarbons because almost all consist of hydrogen and carbon. Petroleum hydrocarbons account for 50–98% of fossil oil and are considered an important component depending on the source of petroleum (Al-Dhabaan, 2019).

Fossil oil is primarily a natural, sticky and flammable liquid. The crude oils vary greatly in chemical composition. It is usually dark brown or black (though it may be yellow or green in color). From an engineering point of view, crude oils are usually classified according to their sources, gravity of the American Petoleum Institute (API) and amount of Sulphur (S). Crude oil is considered "light" when its density is low and "heavy" when it is dense. Crude oils with relatively low sulphur content are called "mild" crudes, while those containing significant amounts of sulphur are called "acid" crudes. Crude oil is a blend of various organic substances, mostly hydrocarbons, organic compound. (Varjani & Upasani, 2017).

The petroleum industry supplies a substantial quantity of world's energy demands in addition to popular petro-chemical intermediates required for production of extensive range of goods viz. solvents, dye stuffs, pharmaceuticals, polymers, and new chemicals etc. All these goods generate environmental pollution when discharged in the environment. (De la Huz *et al.*, 2018).Due to the formation of water or gas, or liquids and chemicals extracted during operations, inorganic salts like sodium chloride, magnesium chloride and other inorganic salts often follow crude oil from wells. Heavy crude oils produce large quantities of difficult to process complex hydrocarbons, such as polynuclear aromatic compounds (PNA), PAHs, alkyl aromatic compounds, heteroatoms, and metal materials. Sulphur, oxygen, nitrogen, and metal atoms are typical heteroatoms in hydrocarbons (Al-Sayegh, *et al*., 2016). Pollutants in soil mainly include heavy metals and organic compounds, such as Cd, Pb, Cr, pesticides, antibiotics, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), etc. (Masindi &Muedi 2018;Altaf.*et.al*.,2021,). These pollutants not only affect the decline of crop yield and quality, resulting in further deterioration of the atmospheric and water environment quality, but also have carcinogenic, teratogenic, mutagenic effects, and genotoxicity, which endanger human health through the food chain (Blundell *et al*., 2020; Tandzi &Mutengwa, 2020).

The largest group of environmental pollutants worldwide is produced from crude oil-based hydrocarbons (Ławniczak *et al*., 2020). Processing activities in the hydrocarbon oil industry releases hazardous aromatic organic compounds such as polyaromatic hydrocarbons (PAHs), phenolic substances that are barely degradable by nature, chlorophenols and cresols toxins from hydrocarbons are all released into the environment (Zhang *et al.*, 2019).

The continuous development and improvement of microbial remediation technology has also provided a new method for the remediation of petroleum hydrocarbon pollution, which has attracted much attention (Dombrowski *et al*., 2016)
Most petroleum hydrocarbons encountered in the environment are ultimately degraded or metabolized by indigenous bacteria because of their energetic and carbon needs for growth and reproduction, as well as the requirement to relieve physiological stress caused by the presence of petroleum hydrocarbons in the microbial bulk environment (Hazen *et al*., 2010; Kleindienst *et al*., 2015a). In general, a single bacterial strain can degrade only a few specific hydrocarbons or to certain stage due to the complexity of petroleum composition and the difficulty in degrading. However, if multiple oil-degrading bacterial strains with different enzyme activities are mixed the degradation of oil contaminates, its degrading efficiency will be significantly enhanced compared to that of the single microorganism. due to the synergism of the creature. For instance (Xiaofang Luo, *et al*., 2020) showed that mixed bacterial strains can efficiently degrade normal paraffin and transform organics 'unstable spatial configuration to stable configurations.
Many normal and extreme bacterial species have been isolated and utilized as biodegraders for dealing with petroleum hydrocarbons. The degradation pathways of a variety of petroleum hydrocarbons (e.g., aliphatics and polyaromatics) have been shown to employ oxidizing reactions; however, these pathways differ greatly because of the specific oxygenases found in different bacterial species. For instance, some bacteria can metabolize specific alkanes, while others break down aromatic or resin fractions of hydrocarbons. Recent studies have identified bacteria from more than 79 genera that are capable of degrading petroleum hydrocarbons (Tremblay *et al*., 2017); several of these bacteria such as *Achromobacter*, *Acinetobacter*,*Alkanindiges*, *Alteromonas*, *Arthrobacter*, *Burkholderia*, *Dietzia*, *Enterobacter*, *Kocuria*, *Mari nobacter*, *Mycobacterium*, *Pandoraea*, *Pseudomonas*, *Staphylococcus*, *Streptobacillus*, *Streptococcus*, and *Rhodococcus* have been found to play vital roles in petroleum hydrocarbon degradation (Jin *et al*., 2012; Varjani& Upasani, 2016; Sarkar *et al*., 2017; Varjani, 2017; Xu *et al*., 2017; Boto *et al*., 2021).

The development of microbial biotechnology and high-throughput sequencing technology, such as micro fluidic techniques (Jiang *et .al*., 2016; Guerra *et .al*., 2018), is beneficial for screening and identifying functional microorganisms from petroleum hydrocarbon-contaminated environments. Indeed, many studies have revealed that there is a large number of hydrocarbon-degrading bacteria in oil-rich environments, such as oil spill areas and oil reservoirs (Hazen *et al*., 2010; Yang *et al*., 2015), and that their abundance and quantity are closely related to the types of petroleum hydrocarbons and the surrounding environmental factors (Fuentes *et al*., 2015; Varjani & Gnansounou, 2017).
However the essence of this study is to monitor the biodegradation of fossil oil through combined screening approach using molecular characterized (hydrocarbon utilizing bacteria (HUB)) organisms isolated from an impacted site.

MATERIALS AND METHOD

Soil characterization

The earth (soil) used in this study was sourced from Hydrocarbon Pollution Research/Training site, located in Obi-Ayagha, Ughelli South, Delta State,in affiliation with integrated institute of Environment and Development (IIED), Federal University of Petroleum Resources Effurun Warri.
Soil samples from the surface horizons (0-15m) were collected from the impacted site with the history of Fossil-oil contamination using a soil auger (polluted soil sample). Composite sample was also collected (0-15m) (unpolluted soil)which is the contol sample from the College of science premises in Federal University of Petroleum Resources Effurun Warri.
Fossil oil was sourced from Bonny (Light crude)

The coordinates of the locations are:

Sample Collection Site	Type of Sample	Latitude	Longitude	Temperature
Obi-Ayagha,	Hydrocarbon Contaminated Soil	5.3674330	5.8499400	Loamy/clay 28.3^0C-29.0^0C
FUPRE Garden	Natural soil	5.570334.5	5.840970	Loamy soil 28.1^0C-29^0C

EXPERIMENTAL DESIGN

Laboratory isolation of hydrocarbon degraders using cultural methods

Ten fold serial dilution method was used, one gram of soil sample (polluted soil sample was weighed and dispensed into the first test tube containing 9mls of physiological saline which is 10^{-1}Each tube was shaken for thorough homogeneity of the mixture, One (1)ml was pipetted into another test tube containing 9mls of physiological saline to give a 10^{-2} dilution and the samples were diluted serially up to 10^{-10} .The test tube were covered with foil paper exactly 1ml of the dilution of 10^{-3}, 10^{-4} and 10^{-5} was inoculated into a duplicate sterile petri dishes containing Mineral Salt Agar (MSA). Bushnell & Hass (1941). MSA comprises of dipotassium hyrdrogen phosphate (KH_2PO_4) (1g), potassium dihydrogen phosphate (K_2HPO_4) (1g), Ammonium nitrate (NH_4NO_3(1g), Magnessium sulphate heptahydrate ($MgSO_4$) (0.2g), Ferrous sulphate heptahydrate (Feso4+7H20 (0.05g), Calcium chloride (CaCl (0.02g), agar agar (15g) all in one litre of distilled water.
The vapour phase method of Okerentugba *et al*. (2016) was adopted for the isolation of the microorganisms, after solidification of the media, a sterile filter paper (whatman No.1) saturated with sterilized crude oil was then placed inside the cover of the Petri dish, as this served as a sole carbon source, it was closed, inverted and incubated at temperature range of 30^0C for 7 days in an incubator. The colonies obtained from the Mineral salt(agar) media (MSM) for bacteria were further purified by sub culturing on nutrient agar

as this was done twice and transferred into agar slant for preservation and for further studies (subcultured every 14days to preserve the isolates).

Microbiological Analysis

All media were prepared according to manufacturer's specifications and autoclave at 121^{0}C for 15 minutes at 15 psi (pounds per square inch).

MOLECULAR IDENTIFICATION OF THE TEST ISOLATES

Pure cultures of the potential strains were preserved on the McCartney bottles,containing Nutrient agar in a slant shape. However Genomic deoxyribonucleic acid (DNA) extraction, sequencing and bioinformatics were done in International Institute of Tropical agriculture (IITA) a federal research institute located at Ibadan, in Nigeria.

DNA EXTRACTION PROCEDURE (USING ZYMO KIT)

Extraction of DNA using soil genomic DNA isolation kit

Add 50-100mg (wet weight) bacterial cells that have been resuspended in up to 200ul of water or isotonic buffer (e.g., PBS) or up to 200mg of tissue to a **ZR Bashing™ Lysis Tube**. Add 750ul **Lysis Solution** to the tube.

PROCEDURE

Secure in a bead fitted with 2 ml tube holder assembly and process at maximum speed for≥ 5 minutes. Then Centrifuge the **ZR BashingBead™ Lysis Tube** in a microcentirifuge at > 10,000 x g for 1 minute.Transfer up to 400 ul supernatant to a **Zymo-Spin™ IV Spin Filter** (orange top) in a **Collection Tube** and centrifuge at 7,000 x g for 1 minute.

Note: *Snap off the base of the **Zymo-Spin ™ Spin filter** prior to use.*

Then add 1,200 ul of **/Bacterial DNA Binding Buffer** to the filtrate in the collection tube transfer 800 ul of the mixture from Step 5 to a **Zymo-Spin™ IIC Column** in a **Collection Tube** and centrifuge at 10,000 x g for 1 minute.

Note: *The Zymo-Spin™ IIC Column has a maximum capacity of 800 ul.*

Discard the flow through from the **Collection Tube** and repeat Step Add 200 ul**DNA Pre-Wash Buffer** to the Zymo-Spin™ IIC Column in new Collection Tube and centrifuge at 10,000 x g for 1 minute Add 500 ul **Bacterial DNA wash buffer** to the Zymo-Spin™ IIC Column and centrifuge at 10,000 x g for 1 minute,Transfer the Zymo-Spin™ IIC Column to a clean 1.5 ml microcentrifuge tube and add 100ul (35 ul minimum) **DNA Elution Buffer** directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the DNA. DNA is now suitable for PCR and other downstream applications.

PCR Cocktail mix

The DNA is subjected to the following cocktail mix and condition for the PCR

10× PCR buffer	1.0
25mM Mgcl2	1.0
5pMol forward primer	0.5
5pMol reverse primer	0.5
DMSO	1.0
2.5Mm DNTPs	0.8
Taq 5u/ul	0.1
10ng/µl DNA	2.0
H2O	3.1
	10µL

PCR PRODUCT PURIFICATION

Add 2vol (20ul) of absolute ethanol to the PCR product then Incubate at room temperature for 15minutes Spin down at 10000rpm for 15minutes Decant supernatant Spin down at10000rpm for 15minutes Add 2vol (40ul) of 70% ethanol Decant supernatant Air dry Add about 10ul of ultrapure water Check for amplicon on 1.5% agarose then the PCR product is ready for sequence reaction. As displayed below

PRIMER: 27F: AGAGTTTGATCCTGGCTCAG
1492R: GGTTACCTTGTTACGACTT

PCR CONDITION

Initial den.	Den.	Ann. Tempt	Extension	No. of circles	Final extension	Hold tempt
94˚c	94˚c	56˚c	72˚c	35	72˚c	4˚c
5mins	45sec	30sec	45sec		7mins	∞

Degradative potential of the Isolates through screening

To test the potential of isolates to degrade PAHs, isolates were subjected to screening using the redox dye (2,6- Dichlorophenol indophenols), adopting the method of (Roy *et al.*, 2002). The suspension contains 2.5ml of carbon free Bushnell Haas broth suspension and 1.0 g/L is selected as the concentration of 2, 6-DCIP.((3 drop each to 200ml of Bushnell Haas broth). A high concentration may produce a dark blue colour and thus reduction may not easily detected and observed (Rodríguez-Fernández et al., 2002).A loop full of 48hours culture of the isolated bacteria and 2ml of fossil oil was introduced and cultured into the above suspension, and the pH of the medium was adjusted to pH 6.5. The reaction mixture was left in room temperature at 25- 30^{0}C using the orbital shaker (120 rpm) the degradative ability of the isolates was observed by recording a discoloration of the medium from

blue to colorless. Control(positive with fossil oil and redox dye no microorganism and negative with just the redox dyeand just mineral salt media) were also set up, Colour change was monitored (Purple/blue to colourless) which was a primary means of identifying the best degraders. However the pH, turbidity and total petroleum hydrocarbon (TPH), were further monitored using pHmeter (multifunction water quality tester), Turbidometer (2100P Hach) and Spectrophotometer (with model 752N series.Uv/vi's) (Youssef *et al.,* 2010).Isolates with the best degrading capability were selected for further studies.

DAYS(24-120hours)

DAY (240-360hours)

RESULTS AND DISCUSSION

Most petroleum hydrocarbon encountered in the environment are ultimately degraded or metabolized by indigenous bacteria because of their energetic and carbon needs for growth and reproduction, as well as the requirement to relieve physiological stress caused by the presence of petroleum hydrocarbons in the microbial bulk environment (Hazen *et al*., 2010; Kleindienst *et al*., 2015a).

Table 1 shows the **estimation of degradation by Isolates upon exposure during screening using the indicator dye** (DCIP)

Table 1: **Estimation of degradation by Isolates upon exposure during screening using the indicator dye** (DCIP)

Exposure duration	Isolates								
Exposure duration(days)	*1*	*II*	*III*	*IV*	*V*	*VI*	*VII*	*VIII*	*Control*
0	++	+	++	+++	+++	+++	+	++	+++
24	++	+	++	+++	++	+++	+	++	+++
48	++	+	++	+++	++	++	+	++	+++
72	+	_	++	+++	++	++	+	++	+++
96	+	_	+	++	++	++	+	+	+++
120	+	_	+	++	++	++	+	+	+++
144	_	_	+	++	++	++	_	+	+++

168	_	_	+	+	++	++	_	+	+++
192	_	_	+	.	+	+	_	+	+++
216	_	_	+	_	+	+	_	_	+++
240		_	_	_	+	_	_	_	+++
264	_	_	_	_	+	_	_	_	+++
288	_	_	_	_	_	_	_	_	+++
312	_	_	_	_	_	_	_	_	+++
336	__	_	_	_	_	_	_	_	+++

Key: +++ - Not degraded
++ - Partial degradation
- - Complete degradation

Upon exposure of the isolate to the dye, within 24hour some isolates were able to degrade them but as the period increases, they fade out completely apart from the control that still retained the dye, the positive symbols in triplicate indicate that they are either partially or not degraded, while the negative symbol connote partial or complete degradation. Therefore, it is possible to determine the ability of a microorganism to utilise a hydrocarbon substrate with the incorporation of DCIP as the terminal electron acceptor into the experiment by simply monitoring the colour change from blue (oxidised) to colourless (reduced). The principle of the discoloration can be described by the molecular conformation of the DCPIP indicator. As bacterial cells utilise the hydrocarbon substrates, electrons are liberated to the environment. Molecular conformation of the indicator will then take place and reflects the light in a different angle, turning its colouration from blue to colorless Nakamura et al., 2007. Mariano et al., 2008.

Spectrophotometric method using a redox dye such as Dichlorophenolindophenol (DCIP) is valuable due to their fast detection of microbial metabolism occurrence in both aerobic and anaerobic studies, due to its low resource output and it is cheap. DCIP is an enzyme-catalysed redox electron acceptor that is blue colour in oxidised state and colourless in its reduced form. In line with the method of Yoshida et al. (2001; Habib *et al*., 2017), loss of DCIP colour is observed at its peak wavelength (600 nm). Hydrocarbon oxidation processes by microbes involve redox reactions, in which electrons are transferred to electron acceptors, such as O2, nitrates, and sulfate Rabinowitz *et al*., (1998). A high concentration may produce a dark blue colour thus any reduction may not easily detect and observed by Rodríguez-Fernández et al., 2002. In summary, all the isolates showed a different range of catabolic metabolism, both isolates exhibited a degradation potential upon exposure to the fossil oil. The ability of isolate to utilise the fossil oil rapidly might occur due to its composition itself, which tends to have a higher percentage of aliphatic components. Habib et al., 2017; Knothe, et al.,2010

In this study, a total of ten bacteria were isolated from the sample gotten from the impacted site, and eight were identifiedas; *Bacillus thurigiensis, Bacillus velezensis, Lysinibacillus fusiformis, Citrobacter specie, Stenotrophomonas specie, Stenotrophomonas specie,and one poor quality sequence* using the mineral salt media composed by Bushnell & Haas,(1941). which might be due to contamination or impurity of the isolate. The result from the predicted organisms as displayed on the GenBank showed that they are all hydrocarbon utilizing Bacteria (HUB), as displayed in Table.1

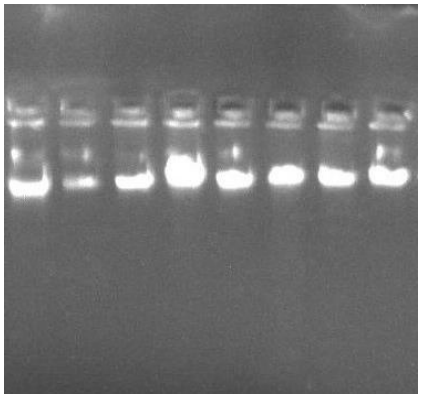

Plate 1.: Agarose gel electrophoresis showing the DNA gel

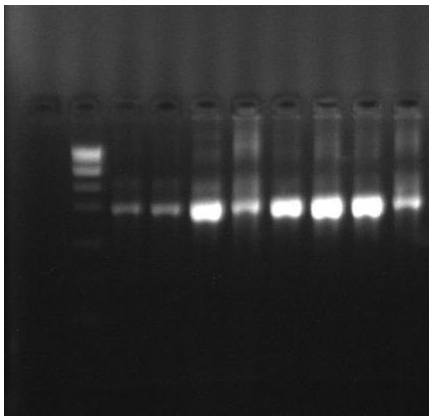

Plate 2: **Polymerase chain reaction (PCR) gel showing the amplified 16s fragment bands (1-8) of the bacterial isolates. 1500bp.**

Table 2: PREDICTED ORGANISM TABLE INCLUDING THEIR PERCENTAGE ID ON GenBank HITS

S/N	SAMPLE IDENTITY	PERCENTAGE	**GenBank ACCESSION NUMBER**
1	***Bacillus thuringiensis***	*97.18%*	CP044978.1
2	***Bacillus velezensis***	*93.06%*	Cp1016121
3	***Lysinibacillus fusiformis***	*94.84%*	FJ418643.1
4	***Citrobacter specie***	*88.47%*	CPO49739.1
5	***Poor Sequence quality***	*Poor Sequence Quality*	-
6	***Stenotrophomonas specie***	*98.30%*	JNOOO347.1
7	***Bacillus thuringiensis***	*99.21%*	CPO44978.1
8	***Lysinibacillus pakistanensis***	*84.83% c*	CP045835.1

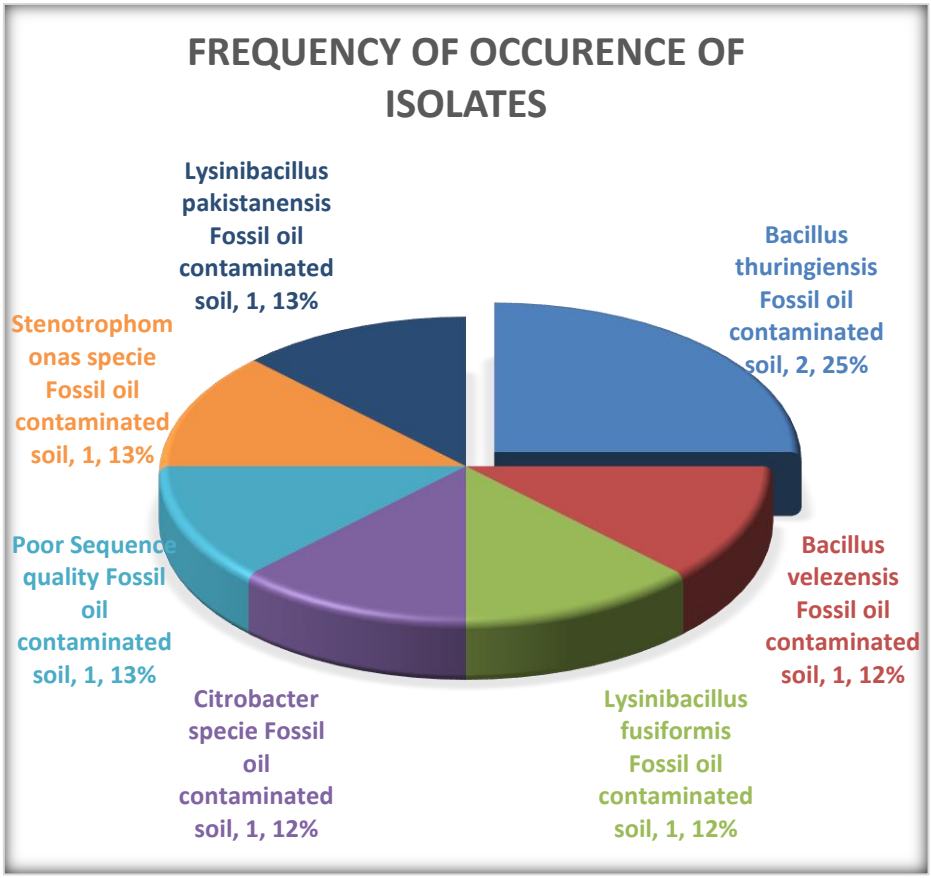

Figure 1:. Showing the percentage and frequency of occurrence of the isolates

Figure 1 shows the frequency of occurrence of the isolates with their percentage all isolates except occurred once from the result obtained from the gen bank except isolate one *Bacillus thurigiensis* occurred twice. Figure 1-3 shows the degradative abilities of microbial isolates on degradation of 200ml mineral salt broth containing 2ml of fossil oil and drops of the redox dye (0.5ml of the redox dispensed into 100ml of distil water with a loop full of 48hour cultured isolates, with control (the control with the fossil oil without the test isolate). Their abilities were scored by increase and decrease in pH, TPH and turbidity, as the day progresses, their emulsification and colour change from deep purple/blue to colourless was in line with the report of Okerentugba *et al*., (2016).

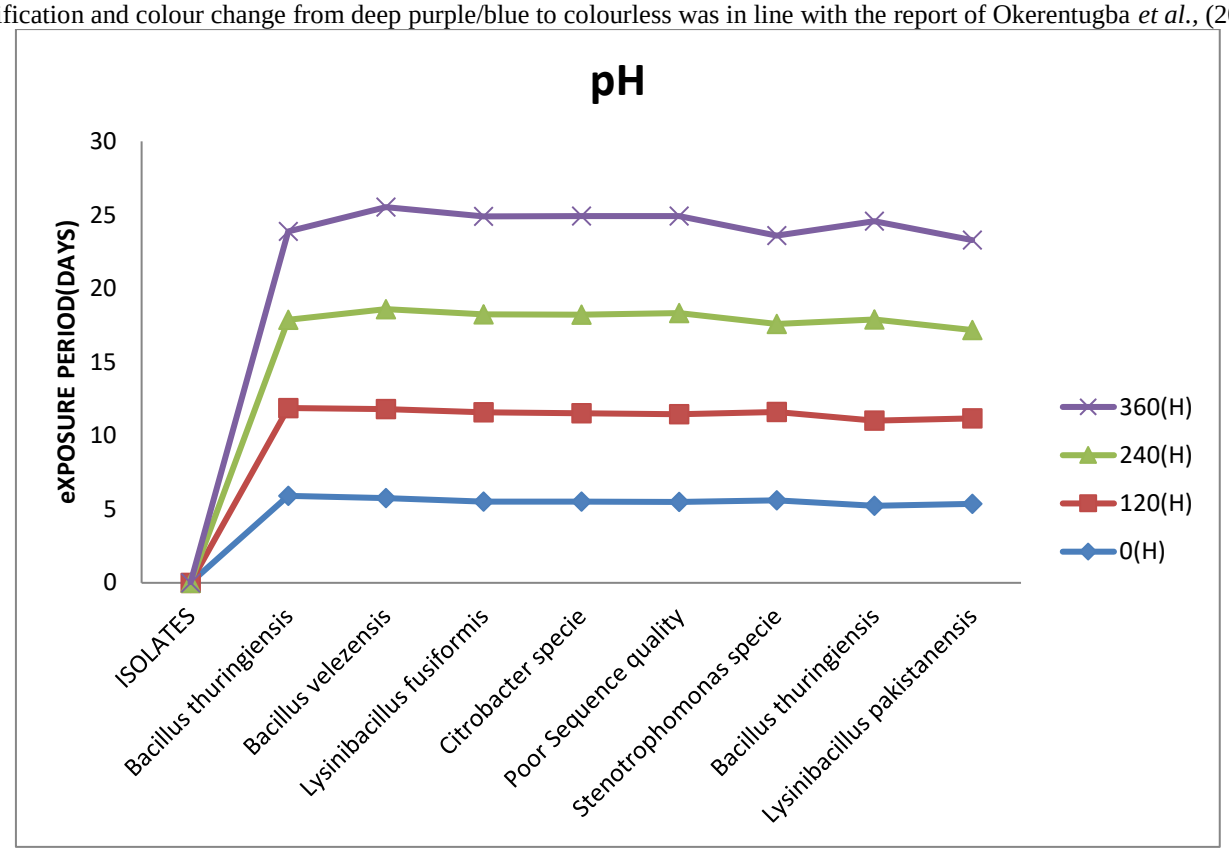

Figure 2: pH changes during screening

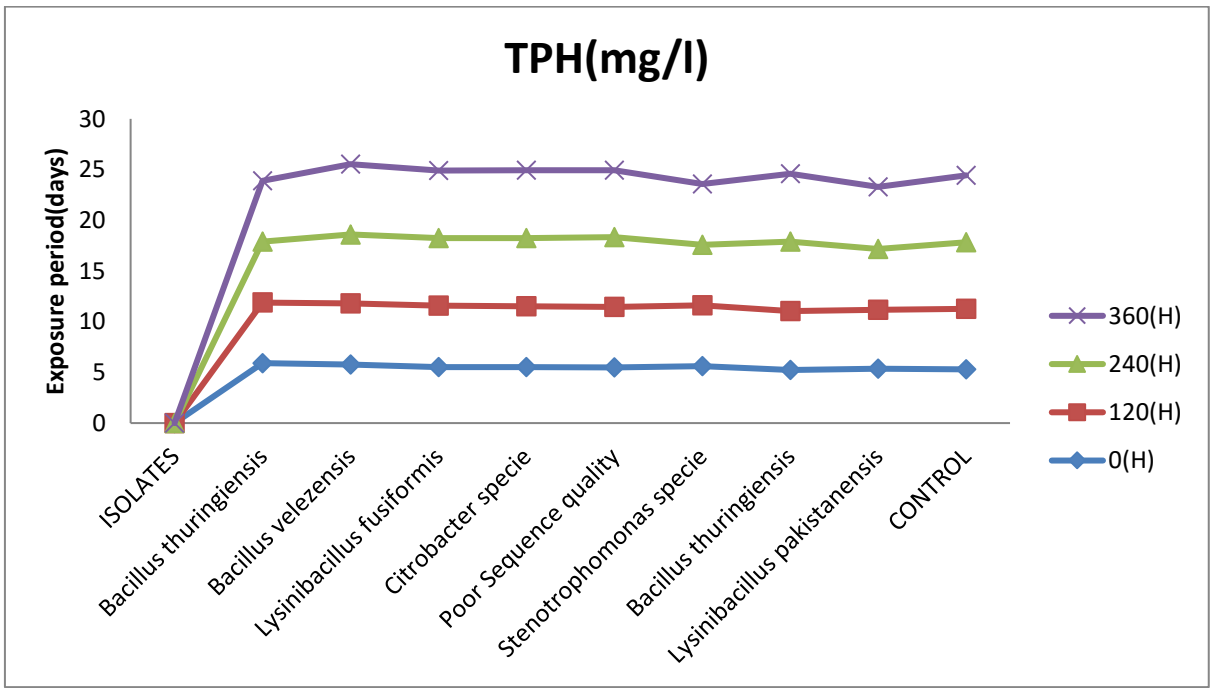

Figure.3: TPH changes during screening

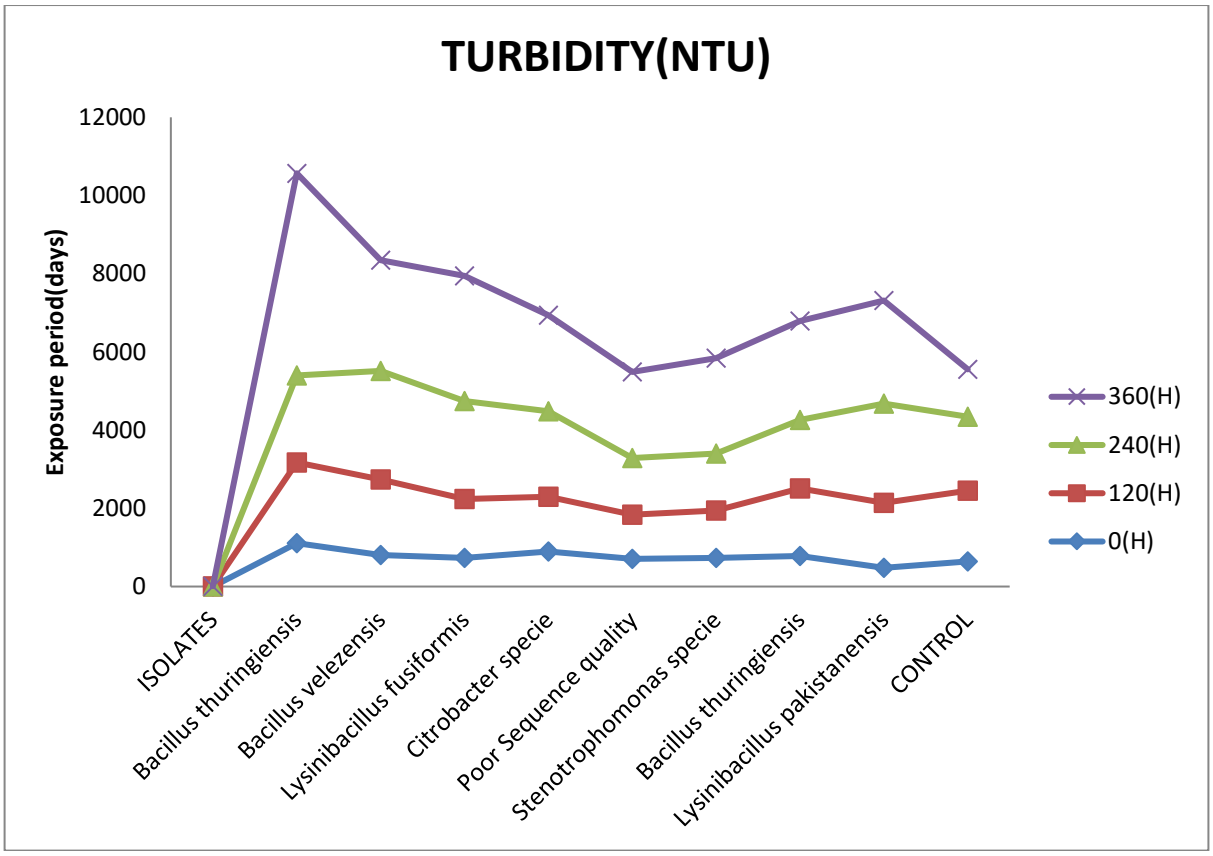

Figure 4: Turbidity of the medium during the period of screening

For The TPH, the screening result recorded on the fifteenth day(360hours), ***Stenotrophomonas specie*** had the highest TPH followed closely by *Bacillus thuringiensis*, the least were recorded for isolate 5 and 1 ***Bacillus thuringiensis.and the poor quality sequence)*** for the turbidity test *Lysinibacillus fusiformis* recorded the highest followed closely by ***Bacillus velezensis*** **then** ***Citrobacter specie*** **,organism (1 and 7)** ***Bacillus thuringiensis andBacillus thuringiensis*** recorded the lowest turbidity.

In the screening process it was observed that all the isolates except isolate 8 and the control had the ability to grow on fossil oil by utilizing it as a carbon and energy source, however their abilities were scored by turbidity, pH and total petroleum hydrocarbon most of the isolates were decolorized i.e colour gradually faded out (1,2,4,7and 6 from day zero to the last day of the experiment as incubation period progress, however few of the isolates(,3,5 and 8) retained slight colour of the indicator dye, hence the ability of the isolates to produce colour change in the medium during the screening for biodegradation potential is due to the reduction of the indicator agent 2,6 dichlorophenol indophenols by the oxidized products of hydrocarbon degradation. The colour change supports the fact that the isolates are potential hydrocarbon utilizers (Youssef *et al.*, 2010)), In a related work also observed a change in colour from deep blue to colourless(Brown *et al*.,2017), said that another criterion to determine isolates biodegradative potential is the rupture of oily surface of the culture medium which we also observed during the study, however the reduction in the total hydrocarbon content (THC) during the screening suggests that isolates are hydrocarbon utilizers.

The exposure of microorganisms to hydrocarbon makes them better suited to degrade the hydrocarbon through higher growth and replication and more efficient metabolism; however they can as well be used to maximise the rate of removal of hydrocarbon from the soil.

pH (6.5) which was favourable for the growth of the organism as the period progress, there was light increase and decrease in the various setup, including the control. Decrease and increase was recorded for the control as the period progress, The decrease in pH value may be due to increase in degradation of fossil oil by microorganisms in the soil resulting in accumulation of acidic metabolites (Ejileugha, 2015).

The highest pH value recorded in the day fifteen (360hours) of the screening shows that (7.31) was recorded for the negative control soil sample which is neutral/slightly alkaline because there is no fossil oil added, followed closely by the positive control because there was no organism to degrade it, while the lowest pH (6.0) was recorded for isolate six. The gradual decrease in pH value of other isolates may be due to increase in degradation of crude oil by microorganisms resulting in accumulation of acidic metabolites (Ejileugha, 2015). As shown in figure 1, A maximum decline in soil pH was recorded. With increasing incubation period, pH was changed according to the nature of metabolites formed during the degradation process. The pH range for better growth of the majority bacteria lies between pH 6.5 and 8.5. Thus, an optimal pH range for biodegradation of fossil oil might be considered between pH 6.0 and 8.5. Satishkumar et al. (2008) found that pH ranging between 7 and 8 was more favourable.

As expected the concentrations of Total Petroleum Hydrocarbon for isolate 1,2 and control were (1204.88mg/l,1456.64mg/l and 1204.88mg/l) measured in the fossil oil set up were high in the first day of exposure, due to hydrocarbon contents of the fossil oil that can lead to increased toxicity and destroy the ecosystem. (Cermak *et al.*, 2010).However as the incubation period increases there was drastic reduction in TPH level ranging from , 222.55mg/l,338.04mg/l,436.54mg/l and the positive control 644.24mg/l sample respectively

For the turbidity, there were gradual increase in all the various stages of the screening and slight decrease noticed with some of the isolates which is an indication that the organisms present in the medium has degradative abilities as they slowly metabolize the fossil oil.

The primary factors restricting the biodegradation efficiency of hydrocarbons are as follows: (1) limited bioavailability of petroleum hydrocarbons to bacteria, and (2) the fact that bacterial cell contact with hydrocarbon substrates is a requirement before introduction of molecular oxygen into molecules by the functional oxygenases (Vasileva-Tonkova et al., 2008; Hua and Wang, 2014). However, bacteria have evolved counter measures against petroleum contaminants, such as improving the adhesion ability of cells by altering their surface components and secreting bio emulsifier to enhance their access to target hydrocarbon substrates. Bacteria with such functions are often screened for use as environmental remediation agents, accelerating the removal of petroleum hydrocarbon pollutants from the environment (Kaczorek et al., 2012; Krasowska & Sigler, 2014 The continuous development and improvement of microbial remediation technology has also provided a new method for the remediation of petroleum hydrocarbon pollution, which has attracted much attention over the years. (Dombrowski et al., 2016; Dvořák et al., 2017).

CONCLUSION

Microorganisms are exceptionally petroleum hydrocarbon degraders, these organisms are available in the entire ecosysytem.However, the isolation and identification of these minute organisms are fundamental in proposing for a bioremediation tool. Spectrophotometric assessment proved to be the quickest method for the detection of microbial metabolism from carbon sources such as hydrocarbons. Although the detection of microbial ability to degrade hydrocarbon can be observed by this remarkable (Turbidity, Spectrophotometric) approach, thus could be considered as an efficient, accurate and rapid method of quantifying the level of hydrocarbon present in a sample. This study shows that the quantity of hydrocarbon utilizing bacterial produced by the strains has the potential to biodegrade and cleans up any toxic substance present in the environment.

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