Frontiers in Environmental Microbiology
2016; 2(6): 38-44
http://www.sciencepublishinggroup.com/j/fem
doi: 10.11648/j.fem.20160206.13
ISSN: 2469-7869 (Print); ISSN: 2469-8067 (Online)

Hydrocarbon Degradation Potentials of Fungi Associated with Oil-Contaminated Soil from Selected Mechanic Workshops in Awka, Anambra State, Nigeria

Edna Ifeoma Chukwura, Nwando Miriam. Ojiegbu, Amechi Sampson Nwankwegu*

Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria

Email address:

smetchie@yahoo.com (A. S. Nwankwegu)

*Corresponding author

To cite this article:

Edna Ifeoma Chukwura, Nwando Miriam. Ojiegbu, Amechi Sampson Nwankwegu. Hydrocarbon Degradation Potentials of Fungi Associated with Oil-Contaminated Soil from Selected Mechanic Workshops in Awka, Anambra State, Nigeria. *Frontiers in Environmental Microbiology.* Vol. 2, No. 6, 2016, pp. 38-44. doi: 10.11648/j.fem.20160206.13

Received: October 29, 2016; **Accepted:** November 21, 2016; **Published:** December 20, 2016

Abstract: Biodegradation potentials of soil mycobiota isolated from five automobile mechanic workshops and a farmland in Awka town on engine oil were investigated using standard methods. The place has variable climatic condition with peak of annual rainfall in August and temperature range of 32 - 42°C. Heterotrophic fungal counts from the soil samples in colony forming unit per gram (cfu/g) ranged from 2.0 x 10^3 to 3.0 x 10^3. Hydrocarbon utilizing fungal counts ranged from 0.7 x 10^3cfu/g to 1.2 x 10^3cfu/g. The statistical analysis of the mean values using analysis of variance showed that, there was no significant difference at $P < 0.05$. Fungal isolates identified as engine oil degraders using cultural, morphological and biochemical characteristics include; *Aspergillus niger, Aspergillus fumigatus, Penicillium xingjiangense, Mucor racemosus,* and *Rhodotorula* sp. Biodegradation ability of the selected fungi on engine oil was studied in a shake- flask culture and the residual hydrocarbon content was measured. The culture fluid for the biodegradation assay was Bacto Bushnell-Haas broth with 10% (^{V}/v) engine oil and 0.1% (^{V}/v) Tween 80. Incubation was at 25°C for 28 days on an incubator-shaker. The results showed decrease in the pH values and hydrocarbon content. Mixed culture had higher percentage hydrocarbon reduction (83.4%) than the axenic cultures: *Aspergillus niger* (79.3%); *Penicillium xingjiangense* (73.7%); *Aspergillus fumigatus* (71.7%) and *Mucor racemosus* (69.1%). They were subjected to gas chromatography on specific polycyclic aromatic hydrocarbons biodegradation. It was observed that *Aspergillus niger* and *Penicillium xingjiangense* were more effective in PAHs reduction when compared with the control sample. Hence, further studies need to be done to confirm that *Aspergillus niger* and *Penicillium xingjiangense* can be used for bioremediation of engine oil contaminated soil.

Keywords: Biodegradation, Soil, PAHs, Engine Oil, Mechanic Workshops

1. Introduction

Crude oil (petroleum) is a naturally occurring complex mixture of hydrocarbon and non- hydrocarbon compounds which at appropriate concentration, possesses a measurable toxicity towards living system. The toxicity of petroleum products varies widely, depending on their composition, concentration, environmental factors and the biological state of the organisms at the time of the contamination [1]. Generally, soil conditions of agricultural land, microorganisms as well as plants are damaged or altered by any contact with crude oil and beyond 3% concentration; it becomes increasingly toxic to soil biota and crop growth [2] [3]. Crude oil spillage endangers public health, contaminate drinking water, devastate natural resources, and disrupt the economy [4].

The consumption of engine oil in Nigeria has been on the increase in recent years due to the upsurge in the number of vehicles, power plants, and generators that make use of these lubricants [5]. This directly affects the rate at which spent engine oil enters and pollutes the environment. The indiscriminate disposal of this waste oil increases pollution

incidents in the environment [5]. Some of the pollution effects of used oil in the environment include: reduction in oxygen supply to microorganisms, pollution of ground and surface waters and accumulation of metal ions which are toxic to plant.

Bioremediation process is a microorganism mediated transformation or degradation of contaminants into non-hazardous or less hazardous substances. It is an attractive approach for cleaning up of hydrocarbons from environment because it is a simple technique, easy to maintain, applicable over large areas, cost effective and leads to complete destruction of the contaminant [6] [7]. Main reason for this concept is that the majority of the molecules in the crude oil and refined product are biodegradable. Many physical, chemical and environmental factors like temperature, nutrients, oxygen, biodegradability, photoxidation, bioavailability, soil moisture, soil acidity and alkalinity etc. affect the process of biodegradation of hydrocarbons [8].

Bacteria and fungi are known to be the principal agents of biodegradation of hydrocarbons. Fungi have a higher tolerance to the toxicity of hydrocarbons due to their physiology and adaptation to such variations in the environment and have the mechanism for the elimination of spilled oil from the environment. Fungi have been found to be better degraders of petroleum than traditional bioremediation techniques with bacteria [9]. Fungi have also demonstrated the ability to degrade and mineralize phenols, halogenated phenolic compounds, petroleum hydrocarbons, polycyclic aromatic compounds and polychlorinated-biphenyls [10].

Many researchers studied the role of fungi in biodegradation process of petroleum products and the most common fungi which have been recorded as biodegraders belong to the following genera: *Alternaria, Aspergillus, Candida, Cephalosporium, Cladosporium, Fusarium, Geotrichum, Gliocladium, Mucor, Pleurotus, Paecilomyces, Penicillium, Polyporus, Rhizopus, Rhodotorula, Saccharomyces, Talaromyces and Torulopsis* [11]. The advantages associated with fungal bioremediation are primarily in the versatility of the technology and its cost efficiency compared to other remediation technologies such as incineration, thermal desorption and extraction. The use of fungi is expected to be relatively economical as they can be grown on a number of inexpensive agricultural or forest wastes such as corncobs and sawdust. More so, their utilization is a gentle non-aggressive approach.

Increased proliferation of automobile workshops within Nigeria has contributed markedly to the problem of soil contamination and this has resulted in the concomitant exposure of the surrounding soil within the vicinity of these workshops to high levels of spent engine oil and lubricating oils.

Automobile workshops abound within Awka town and disposal of the spent engine oil into drainage systems is a common practice among mechanics. Hence, the aim of this study is to evaluate the hydrocarbon degradation potentials of fungi associated with these oil-contaminated soils.

2. Materials and Methods

2.1. Sources of Soil Samples

Oil contaminated soil samples were taken from five automobile mechanic workshops located within different quarters in Awka with the aid of a hand auger. A control soil sample was also collected from a fallow farmland at Nkwelle in Awka town. Samples from each site (0-10cm depth) were collected aseptically into sterile bottles using sterile spatula and sent to the laboratory for microbiological analysis [12].

2.2. Media and Chemicals

The medium used for enumeration of heterotrophic fungi was Sabouraud dextrose agar (SDA); for isolation of engine oil (hydrocarbon) utilizing fungi, modified mineral salt agar prepared by adding 1% engine oil was used. For testing biodegradation capability of indigenous fungal isolates, Bacto Bushnell- Haas broth with Tween 80 (0.1%), redox reagent (2%) and engine oil (1%) was used.

2.3. Enumeration of Heterotrophic and Engine Oil (Hydrocarbon) Utilizing Fungi in Soil Samples

Each sample was homogenously mixed and carefully sorted to remove stones and other unwanted soil debris. Isolation and enumeration of heterotrophic fungi was done by serial dilution agar plating method. Sabouraud Dextrose Agar (SDA) culture media was used to isolate the fungal species. Sterile saline, i.e., 0.85% w/v sodium chloride, was used as diluent for inoculum preparation. A 10-fold serial dilution was made using 1g of each sample. Then 1.0ml aliquot of 10^{-3} dilution of each sample was cultured on plates of SDA by pour plate method. An oil agar medium was also used for preliminary isolation of engine oil utilizing mycoflora. Plating was done in duplicates and all the media were supplemented with chloramphenicol (500mg/L) to inhibit bacterial growth. The culture plates were swirled, allowed to solidify and incubated at room temperature (25°C) for 5days (SDA plates) and 9days (oil agar plates). The resulting fungal colonies were enumerated and recorded as colony forming units (CFU) per 1.0 g of each soil sample [13].

2.4. Fungal Characterization

The cultural characteristics of the purified isolates were noted and the microscopic features of both the filamentous fungal and yeast isolates were observed using the wet mount technique [14]. Purified cultures were stored on SDA slants for further characterization. Lactophenol cotton blue stain was used as mordant. The microscopic structures observed were recorded and compared to those stated in previous studies [15]. Several biochemical tests such as urea hydrolysis, sugar fermentation tests and assimilation of carbon compounds were conducted to further characterize the yeast isolate.

2.4.1. Slide Culture Preparation

An already prepared and autoclaved SDA was pipetted with a sterile syringe and transferred aseptically to a sterile slide in drops. A pure culture of the fungal isolate was inoculated to each slide and covered with a cover slip. The slide was laid on a Petri dish, covered and incubated without inverting the position at a temperature of 30°C for 5 days to allow the organism to grow very well.

2.4.2. Microscopic Examination of the Slide Culture

A fresh slide was flooded with few drops of lactophenol cotton blue and covered with a cover slip. The slide culture itself was also flooded with few drops of lactophenol cotton blue stain and covered with a fresh slip. Both were thereafter viewed under x40 objective lens.

2.4.3. Urease Test

The test is performed to demonstrate organism that can decompose urea by means of the enzyme urease to form ammonia. Briefly, a portion of a yeast colony was inoculated on a slant of Christensen's urea agar, which contains urea. The slant was incubated at 28°C and examined daily for 4 days. Urease production was indicated by the change in the amber colour of the medium to pink.

2.4.4. Carbohydrate Assimilation

This test is used to detect organisms that produce both acid and gas or acid alone when glucose or a given sugar is fermented. Procedurally, 1.0g of each sugar, 100ml of peptone water and 1.5ml of bromothymol-purple indicator were put together into different beakers. These were dispensed into test tubes containing Durham's tubes in inverted positions. The tubes were sterilized at 115°C for 10mins and allowed to cool. The isolate was inoculated into the tubes and incubated for 4 days at 28°C. Acid production was indicated by change of medium from purple to yellow colour then gas production was indicated by the presence of gas in Durham's tube.

2.5. Screening Test

Ability of fungal isolates to utilize engine oil as Sole Carbon Source was determined. A screening test was utilized for confirming biodegradation ability of fungal isolates [16]. Two agar plugs (1cm^2 each) of a pure growth of each isolate were inoculated into Bacto Bushnell-Haas broth (50ml/250 Erlenmeyer flask) incorporated with sterile engine oil (1% v/v), redox indicator 2,6-dichlorophenol indophenols (2% w/v) and Tween 80 (0.1% v/v). The control flask had no organism. Incubation was done at room temperature 25°C with constant shaking at 180rev/min for 7days. The aliquots in the flasks were monitored daily for color change from deep blue to colorless. After 7days incubation, 5ml aliquots were collected from each flask and centrifuged (5000rpm) at room temperature for 5minutes and the supernatant read using a HACH 2010 portable data logging spectrophotometer at 600nm.

2.6. Preparation of Cell Suspension for Biodegradation Assay

Four organisms with high utilization ability were chosen for the degradation test. The cell suspensions were prepared by inoculating two agar plugs each of the purified fungal mycelia from the respective SDA culture plates unto 5ml of mineral salt medium [16]. Incubation was done at 150 rpm in a shaker at 25°C for 96 h. Thereafter, 2ml of a 96 h cell suspension of each of the selected potential strains were inoculated into the Bacto Bushell-Haas broth (100ml/500ml Erlenmeyer flask) containing 0.1%(v/v) Tween 80 and 10% (v/v) engine oil without redox indicator. Control flask without the organism was prepared accordingly. Incubation was done at 25°C for 28 days at 180rpm. At 4days interval during the incubation, samples were drawn from each flask for measurement of pH and residual hydrocarbon [17] [18].

2.7. Determination of pH

The pH of each culture flask was determined at 96hr interval for 28 days with the aid of SUNTEX pH meter SP-701. 10ml of sample was poured into a small beaker and the calibrated pH meter dipped into it, then the reading was taken from the meter display.

2.8. Determination of Residual Hydrocarbon

The residual hydrocarbon content of the respective culture flask at 96h interval for a period of 28 days was determined. 1ml of sample was dissolved in 5ml of chloroform; the mixture was shaken vigorously and read at 450nm against a blank, then concentration interpolated from the working curve using the absorbance readings [19].

2.9. Hydrocarbon Quantification by Gas Chromatography

After 28days, samples from the two best potential strains; *Aspergillus niger* and *Penicillium xingjiangense* as well as the control were subjected to gas chromatography analysis to determine the extent of polycyclic aromatic hydrocarbons (PAHs) degradation at Springboard Research Laboratories, Udoka Housing Estate, Awka, Anambra State. The chromatographic equipment used was Buck 530 equipped with an on-column, automatic injector, flame ionization detector, HP 88 capillary column (100m x 0.25µm film thickness) CA, USA. Detector temperature: 250°C, integrator chart speed: 2cm/min. inlet temperature 180°C and initial-final oven temperature at 70°C - 280°C. The chromatograph was then attached to an integrator.

The specific procedure for the assay is as follows: 1ml of sample was dissolved in 5ml carbon tetrachloride; the mixture was shaken and centrifuged. Top solvent phase was transferred to a small test tube for injection. Caution was taken in collecting only the organic layer because of the risk of injecting water into the GC column [19]. The percentage degradation of the hydrocarbon content of the engine oil by fungal was calculated [7] [19]. Amount degraded = Initial concentration – final concentration. % degradation of

hydrocarbon content = (amount degraded/initial concentration) x100.

2.10. Statistical Analysis

Each set of data in the experiments was collected in three replicates and the analytical result was the mean of three measurements. The standard deviations (error bars) and statistical significance (5% level of significance) was analyzed with GraphPad Prism 6® software (Trial version 6.1) and IBM SPSS statistics version 21 using One way analysis of variance (ANOVA) and Turkey test.

3. Results

3.1. Microbial Growth Dynamics

The mean count of total heterotrophic fungi and total engine oil utilizing fungi (Table 1) in soil samples using general and enriched mycological media varied considerably. The heterotrophic fungal counts observed for the soil samples collected from the respective auto-mechanic workshops ranged from 2.0 x 10^3 CFU/g to 2.7 x 10^3 CFU/g. The total engine oil utilizing fungal count for the auto-mechanic soil samples ranged from 1.0 x 10^3 CFU/g to 1.2 x 10^3 CFU/g. The control soil sample gave a mean count of 3.0 x 10^3 CFU/g of the total heterotrophic fungi and 0.7 x 10^3 CFU/g of the total engine oil utilizing fungi.

Table 1. *Total heterotrophic (THF) and hydrocarbon utilizing fungi (HUF) in the contaminated soil.*

Sample	10^3 CFU/g soil THF	10^3 CFU/g soil HUF
A	2.4 ± 0.66^b	1.1 ± 0.16^a
B	2.2 ± 0.66^b	1.2 ± 0.56^b
C	2.7 ± 0.56^b	1.0 ± 0.42^b
D	2.0 ± 0.15^a	1.1 ± 0.13^a
E	2.2 ± 0.16^a	1.0 ± 0.20^a
Control	3.0 ± 0.14^a	0.7 ± 0.11^a

Values are mean of replicate analyses ±S.D.
A – E (designations of different engine oil - contaminated soil sample)
Control (agricultural soil free from hydrocarbon contamination)
Mean superscripts with different letter differ significantly ($P < 0.05$)

3.2. Fungal Characterization

The cultural and microscopic characteristics of the filamentous fungal isolates (Table 2) that were able to utilize engine oil (hydrocarbons) revealed that most fungi were molds. The isolates were further identified using ITS rDNA sequencing analysis as *Aspergillus. niger, Aspergillus. fumigatus, Mucor racemosus,* and *Penicillium xingjiangense* respectively.

Characterization the yeast, *Rhodotorula* sp isolate (Table 3) reported peach, elevated and mucoid colonies on SDA plates with oval shaped, budding cells in lacto phenol cotton blue staining. The isolate tested positive to urease activity and was able to assimilate both glucose and sucrose but fermented only glucose.

Table 2. *Fungal cultural and microscopic characteristics.*

Cultural characteristics	Microscopic characteristics	Identity
Colonies are fast growing, white becoming deep brown on the surface and pale yellow on the reverse	Chains of round conidia with smooth- walled conidiophores. The phialides are biserate and conidial heads radiate	*Aspergillus niger*
Colonies are fast growing, grey green on the surface with yellow on the reverse side.	Chains of round condia with conidial heads in the form of compact columns. Phialides uniserate, concentrated on the upper surface of the vesicle.	*Aspergillus fumigatus*
Colonies have very rapid growth with wooly texture. Color is grayish on the surface and reverse is pale.	Non septate broad hyphae, branched sporangiophores with round sporangiospores. Stolons and rhizoids are absent.	*Mucor racemosus*
Colonies have moderately rapid growth. Color is white on the surface with pale on the reverse side.	Septate hyphae, phialides grouped in brush like clusters at the ends of the conidiophores. Chain of unicellular conidia.	*Penicillium xingjiangense.*

Table 3. *Yeast (Rhodotorula sp) Characteristics.*

Test	Characteristic
Morphology on SDA	Peach, elevated, mucoid colonies.
Lacto Phenol Blue staining	Oval shaped, budding yeast cells.
Urease activity	Positive
Assimilations:	
Glucose	Positive
Maltose	Negative
Sucrose	Positive
Lactose	Negative
Fermentation:	
Glucose	Positive
Maltose	Negative
Sucrose	Negative
Lactose	Negative

3.3. Fungal Hydrocarbon Utilization Index

The result of the screening test (Table 4) shows that *Aspergillus niger, Penicillium xingjiangense, Aspergillus fumigatus,* and *Mucor racemosus* had the best biodegradation potential with optical densities of 0.928, 0.844, 0.686, and 0.576 respectively. *Rhodotorula* sp. (optical density of 0.219) had the least biodegradation potential.

Table 4. *Engine oil utilizing capabilities of the fungal species.*

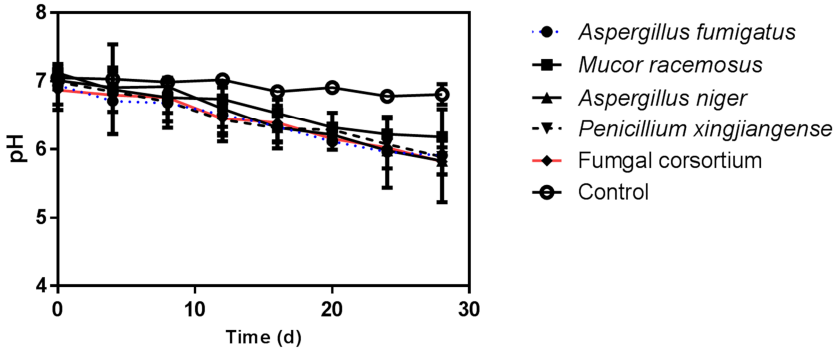
Fungal hydrocarbon dragraders	Optical density (600 nm)
Aspergillus niger	0.928 ± 0.30
Aspergillus fumigatus	0.686 ± 0.02
Penicillium xingjiangense	0.844 ± 0.13
Rhodotorula sp	0.219 ± 0.41
Mucor racemosus	0.576 ± 0.43

Values are mean of replicate analyses ±S.D.

3.4. Effect on pH and Hydrocarbon Degradation

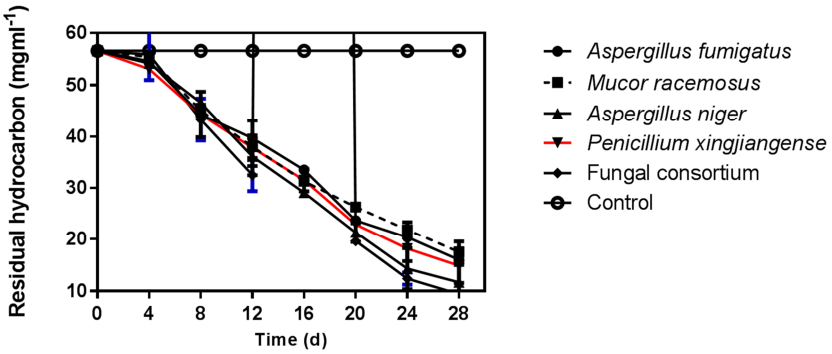
Reduction in pH (Fig. 1) and the extractable hydrocarbon content (Fig. 2) during the biodegradation assay of the selected fungal isolates showed a total pH variations and oil utilization potentials in all microcosms. Amongst the axenic and mixed

cultures, mixed culture had the lowest pH (5.82) recorded on the 28th day. The control flask had the highest pH reading (7.03) recorded on the 4th day of the growth profile study. A slight increase in pH was observed in *Aspergillus niger* from 4th to the 8th day. Mixed culture (consortium) exhibited the highest reduction in hydrocarbon content (9.425mg/L) after 28 days incubation. Then amongst the axenic cultures, *A. niger* effected the highest reduction in the hydrocarbon content (11.742mg/L). No hydrocarbon reduction was recorded in the control flask during the shake flask study.



Values are mean of replicate analyses: Error bars (± S.D)

Fig. 1. *Effect of fungal microcosms' pH on hydrocarbon degradation for 28 d incubation.*



Values are mean of replicate analyses: Error bars (± S.D)

Fig. 2. *Residual extractable hydrocarbon for the 28 d study period.*

3.5. Fungal Hydrocarbon and PAH Removal Efficiencies

Percentage degradation of the hydrocarbon content (Table 5) and the extent of utilization of the polycyclic aromatic hydrocarbons (PAHs) differed significantly. Mixed culture (fungal consortium) of the four isolates recorded the highest percentage degradation of 83.36%, followed by *Aspergillus* - 79.27%; *Penicillium xingjiangense* - 73.69%; *Aspergillus fumigatus* - 71.71% and *Mucor racemosus* with 69.05%. The result of PAH reduction by *A. niger* and *P. xingjiangense* shows total depletion in some of the PAH fractions. *Aspergillus niger* recorded 100% reduction of phenanthrene, fluoranthene, pyrene and benzo (k) fluoranthene with partial reduction of benzo (a) pyrene and dibenzyl (a-h) anthracene. *Penicillium xingjiangense* exhibited 100% reduction of phenanthrene, fluoranthene and benzo (k) fluoranthene and up to 99.1% reduction of benzo (a) pyrene with partial reduction of pyrene and dibenzyl (a-h) anthracene. The pH and hydrocarbon degradation profiles of the axenic and mixed fungal isolates from 0-28 days incubation in engine oil are also varied based on hydrocarbon utilization by species.

Table 5. *Microbial hydrocarbon content removal efficiency.*

Microorganism	C_o (mgml^{-1})	C_f (mgml^{-1})	A_d (mgml^{-1})	% d
A. fumigatus	56.60 ± 0.11	16.03 ± 0.15	40.60 ± 0.25	71.71 ± 0.23
M. racemosus	56.60 ± 0.20	17.50 ± 0.03	39.10 ± 0.02	69.05 ± 0.25
A. nigger	56.60 ± 0.24	11.70±0.28	44.90 ± 0.47	79.27 ± 0.20
P. xingjiangense	56.60 ± 0.29	14.90 ± 0.20	41.70 ± 0.26	73.69 ± 0.13
Consortium	56.60 ± 0.13	9.20 ± 0.19	47.20 ± 0.43	83.36 ± 0.14
Control	56.60 ± 0.17Aa	56.60 ± 0.19	0.00±0.00	0.00±0.00

Values are mean of triplicate analyses ± SD
C_o (mgml^{-1}) = Initial hydrocarbon content,
C_f (mgml^{-1}) = Final hydrocarbon content
A_d (mgml^{-1}) = Amount of hydrocarbon degraded
% d = percentage degradation/removal efficiency

4. Discussion

The non-contaminated reference soil sample collected has zero residual oil content, confirming that the reference sample was not polluted with oil. The viable heterotrophic fungal counts obtained from the auto mechanic workshop soils could be reflective of the adaptive abilities of those fungal isolates to thrive even in the event of deliberate anthropogenic intermittent discharges of various types and quantities of petroleum products on these soil surfaces over periods of time. The morphological and biochemical characterization of the engine oil (hydrocarbon) utilizing fungal isolates obtained from the soil samples revealed the following; *Aspergillus niger*, *Aspergillus fumigatus, Penicillium xingjiangense, Mucor racemosus,* and *Rhodotorula* sp. Some of these organisms have earlier been reported as hydrocarbon biodegraders [20]. During the primary step for confirming biodegradation potential of fungal isolates, all the isolates utilized the engine oil as the sole source of carbon and scored for optical density with *Aspergillus niger* scoring the highest 0.928, thus, best degradation potential amongst the isolates while *Rhodotorula* sp. scored the lowest 0.219. The ability of these isolates to produce a color change in the Bacto Bushnell-Haas broth medium is presumably due to the reduction of the indicator by the oxidized products of hydrocarbon degradation.

The growth profiles of the single and mixed fungal cultures revealed a continuous drop in pH values of the culture fluid 6.94-5.91 for *A. fumigatus*, 7.12-6.18 for *Mucor racemosus*, 7.01-5.83 for *A. niger*, 6.99-5.89 for *Penicillium xingjiangense,* and 6.87-5.82 for the mixed culture with a concomitant decrease in the hydrocarbon content of the inoculated engine oil 56.646-16.027 $mgml^{-1}$ for *A. fumigatus*, 56.646-17.531 $mgml^{-1}$ for *Mucor racemosus*, 56.646-11.742 $mgml^{-1}$ for *A. niger*, 56.646-14.903 $mgml^{-1}$ for *Penicillium xingjiangense*, and 56.646-9.425 $mgml^{-1}$ for the mixed culture. Microbial degradation of hydrocarbon often leads to production of organic acids and other metabolic products [21]. Thus, organic acids probably produced account for the reduction in pH levels [2] [12] [17] [18] [22]. Hydrogen ion concentration is a major variable governing the activity and composition of fungi [23]. Thus, in spite of the acidic pH of the surrounding medium, the growth profile isolates were able to assimilate the petroleum hydrocarbon and also effect a reduction in the residual hydrocarbon content of the engine oil. Amongst the axenic isolates, *Aspergillus niger* effected the highest reduction in residual hydrocarbon content of the inoculated engine oil during the growth profile test.

The high rate of engine oil (hydrocarbon) degradation by the four fungi could emanate from their massive growth and enzyme production responses during their growth phases. The utilization of 0.1% of Tween 80 during the assay and the implication of these four organisms in hydrocarbon degradation from the present study is similar to the findings of previous investigations [19]. *Aspergillus niger* and *Penicillium xingjiangense* displayed the highest extent of degradation within 28days among the single species microcosms. Thus, they were selected for further study on specific polycyclic aromatic hydrocarbons (PAHs) degradation. However, the mixed culture containing all the isolates exhibited more degradation ability than any of the individual isolates and this is in line with other works [1] [24] [25]. In a mixed culture, some species utilize intermediates of degradation of the original hydrocarbon produced by other members of the culture leading to a complete degradation of the oil, thus, a mixed culture (microbial consortium) is effective seeders for oil spill clean-up responses.

The biodegradation of engine oil by the fungal isolates was on a very wide scale, where on one hand 71.71% of the hydrocarbon content was degraded by *Aspergillus fumigatus*, 69.05% by *Mucor racemosus*, 79.27% by *Aspergillus niger*, 73.69% by *Penicillium xingjiangense*, and 83.36% by the mixed culture. The results showed that the 10% engine oil added to the medium and the culture conditions were very appropriate for their growth and biodegradation.

The gas chromatography analysis on PAHs degradation before and after treatment with *Aspergillus niger* and *Penicillium xingjiangense* shows that the two isolates exhibit biodegradation efficiency and also confirmed their high degradation potentials. The fact that the isolates were able to degrade PAHs effectively suggests that the degradation of the aliphatic moieties could be easier and faster than their polycyclic aromatic moieties [26]. The chromatogram report shows the difference in retention time and peak height between the control and experimental samples, and it was observed that *A. niger* and *Penicillium xingjiangense* were efficient in PAHs reduction when compared with the control.

5. Conclusion

The result revealed that soils within the premises of automobile workshops are good sources of hydrocarbonclastic fungi notably; *Aspergillus niger*, *Penicillium xingjiangense*, *Aspergillus fumigatus*, and *Mucor racemosus*. It was observed that oil degrading fungi could also be isolated from soil not directly contaminated with engine oil or similar pollutants. Mixed culture of fungi had the highest ability to degrade engine oil (83.4%), followed by *Aspergillus niger* (79.3%), *Penicillium xingjiangense* (73.7%), *Aspergillus fumigatus* (71.7%), and *Mucor racemosus* (69.1%). These fungal isolates can be effectively utilized in bioaugmentation aimed at the removal of hydrocarbon pollutants from contaminated environments especially those located within the residential vicinity. Hence, further studies need to be done to confirm that *Aspergillus niger* and *Penicillium xingjiangense* can be used for bioremediation of engine oil contaminated soil.

Acknowledgments

We immensely thank Springboard Research Laboratory, Udoka Estate, Awka, Anambra State, Nigeria, for gas chromatographic analysis.

References

[1] Obire, O and Ayanwu E. C. Impact of Various Concentrations of Crude Oil on Fungal Populations of Soil. International Journal of Environmental Science and Technology. 2009, Vol. 6, pp. 211–218.

[2] Onuoha, C. I., Arinze, A. E., Ataga, A. E. 2003. Evaluation of Growth of Some Fungi in Crude Oil Polluted Environment. Global Journal of Agricultural Sciences. 2003, Vol. 2, pp. 80–81.

[3] Ojumu, T. V., Bello, O. O., Sonibare, J. A., Solomon, B. O. 2004. Evaluation of microbial systems for bioremediation of petroleum refinery effluents in Nigeria. African Journal of Biotechnology. 2004, Vol. 4, pp. 31–35.

[4] Gesinde, A. F., Agbo, E. B., Agho, M. O., Dike, E. F. C. Bioremediation of some Nigerian and Arabian crude oil by Fungal Isolates. International Journal of Physical and. Applied Sciences. 2008, Vol. 2, pp. 37–44.

[5] Odjegba, V. J. and Atebe, J. O. The Effect of Used Engine Oil on Carborhydrate, Mineral Content and Nitrate Reductase Activity of Leafy Vegetable. (*Amaranthus hybridus* leaf). Journal of Applied Science and Environmental Management. 2007, Vol. 11, pp. 191–196.

[6] Achal, V., D. Kumari, and. Pan, X. Bioremediation of chromium contaminated soil by a brown-rot fungus, Gloeophyllumsepiarium. Research Journal of Microbiology. 2011, Vol. 6, pp. 166–171.

[7] Nwankwegu, A. S., Orji, M. U., Onwosi, C. O. Studies on organic and in-organic biostimulants in bioremediation of diesel-contaminated arable soil. Chemosphere. 2016, Vol. 162, pp. 148–156.

[8] Rahman, K. S. M., Rahman, T. J., Kourkoutas, Y., Petsas, I., Marchant, R., Banat, I. M. Enhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid and micronutrients. Bioresource Technology. 2003, Vol. 90, pp. 159–168.

[9] Ojo, O. A. Petroleum-hydrocarbon utilization by nature bacterial population from a Wastewater canal Southwest Nigeria. African Journal of Biotechnology. 2005, Vol. 5, pp. 333–337.

[10] Singh, H. Mycoremediation: Fungal Bioremediation. New York: Wiley Interscience, 2006, pp. 592.

[11] Adekunle, A. A., and Adebambo, O. A. Petroleum Hydrocarbon Utilization by Fungi Isolated From Detarium Senegalense (J. F Gmelin) Seeds. Journal of American Sciences. 2007, Vol. 3, 69–76.

[12] Nwankwegu, A. S., Onwosi, C. O., Orji, M. U., Anaukwu, C. G., Okafor, U. C., Azi, F., Marins, P. E. Reclamation of DPK hydrocarbon polluted agricultural soil using a selected bulking agent. Journal of Environmental Management. 2016, Vol. 172, pp. 136–142.

[13] Harley, J. P., Prescott, L. M. Laboratory Excercises in Microbiology. 5th edn. New York: MacGraw- Hill. 2002, pp 449.

[14] Sharma, P. Manual of Microbiology: Tools and Techniques. 2nd edn. New Delhi: Ane books, 2009, pp. 405.

[15] Aneja, K. R., 2005. Experiments in Microbiology, Plant pathology and Biotechnology. 4th edn. New Delhi: New Age Publication, pp. 606.

[16] George- Okafor, U., Tasie, F., Moutoe- Okafor, F. Hydrocarbon degradation potentials of indigenous fungal isolates from petroleum contaminated soils. Journal of Physical and Natural Sciences. 2009. Vol. 3, pp. 1–6.

[17] Nwankwegu, A. S., Onwosi, C. O., Azi, F., Azumini, P., Anaukwu, C. G. Use of rice husk as bulking agent in bioremediation of automobile gas oil impinged agricultural soil. Soil and Sediment Contamination 2016, http://dx.doi.org/10.1080/15320383.2017.1245711.

[18] Nwankwegu, A. S. Sawdust assisted bioremediation of PMS hydrocarbonimpacted agricultural soil in Niger Delta Nigeria. International Journal of Pharma and Biosciences. 2016, Vol. 7, pp. 47–57.

[19] Lambert, P. Fingas, M., Goldthorp, M. An evaluation of field total petroleum hydrocarbon (TPH) systems. Journal of Hazardous Materials. 2001, Vol. 83, pp. 65–81.

[20] April, T. M., Foght, J. M., Currah, R. S. Hydrocarbon-degrading filamentous fungi isolated from flare pit soils in Northern and Western Canad. a Canad. Journal of Microbiology. 2000, Vol. 46, 38–49.

[21] Qi, B., Moe, W. M., Kinney, K. A. Biodegradation of volatile organic compounds by five fungal species. Applied Microbiology and Biotechnology. 2002, Vol. 58, pp. 684–689.

[22] Oboh, B. O., Ilori, M. O., Akinyemi, J. O., Adebusoye, S. A. Hydrocarbon Degrading Potentials of Bacteria Isolated from a Nigerian Bitumen (Tarsand) Deposit. Natural Sciences. 2006, Vol. 4, 51–57.

[23] Sebiomo, A., Awosanya, A. O., Awotodu, A. D. Utilization of crude oil and gasoline by ten bacterial and five fungal isolates. Journal of Microbiology. 2011.

[24] Bento, F. M and Gaylarde, C. C. Biodeterioration of stored diesel oil: studies in Brazil. International Biodeterioration and Biodegradation. 2001, Vol. 47, pp. 107–112.

[25] Facundo, J. M. R., Vanessa, H. R., Teresa, M. L. Biodegradation of Diesel oil in Soil by a Microbial Consortium. Water, Air and Soil Pollution. 2001, Vol. 128, pp. 313–320.

[26] Kanaly, R. A and Harayama, S. Biodegradation of high molecular-weight polycyclic aromatic hydrocarbons by bacteria. Journal of Bacteriology. 2000, Vol. 182, pp. 2059–2067.