Assessment of Biodegradation Ability of *Aspergillus Niger* Isolated From Mechanic Workshops Soilon Refinery Effluent and Petroleum Hydrocarbons

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Abstract- The environment is considered as man's important asset that must be protected for his life support. Regrettably the situation is different where oil refinery and petrochemical plants operate. Environmental pollution in the form of emissions and effluent discharge in these area poses serious threat to the ecosystem particularly soil, often with undesirable effects.

The study collected soil samples from three (3) different mechanic workshops soil. Soil samples collected were taken to the laboratory for isolation of Aspergillus niger using potato dextrose agar (PDA). Samples of the raw effluent was collected from the waste oil retention pond was analysed for temperature, pH, electrical conductivity ,total dissolved solids, Total suspended solids, BOD, COD.Nitrate, Phosphate were compared with FMENV maximum permissible limit.

Isolates of Aspergillus niger was confirmed for biodegradation ability of Refinery effluent augmented with glucose, Mineral Salt medium, and with nutrient. Also, the ability of Aspergillus niger to degrade petroleum hydrocarbon was confirmed using kerosene, diesel, spent engine oil, unspent engine oil, using spectrophotometer. Aspergillus niger has the percentage biodegradation in diesel, Kerosene has the least percentage biodegradation.

Index Terms- Environment, Biodegradation, fungi, refinery effluent

I. Introduction

Petroleum is at present Nigeria's and indeed the world's most important derived energy source (Moffat and Linden, 2005). Petroleum industry is one of the largest industries in Nigeria and it's the backbone of the national economy (Ojumu *et al.,* 2004).

Petroleum in its natural state is referred to as crude oil (Ukoli, 2003). The petroleum refinery industry converts crude oil into more refined products such as liquefied petroleum gas, gasoline, kerosene, aviation fuel, diesel fuel, fuel oils, lubricating oils and feed stocks for petrochemical industry (Abdulkarim *et al*., 2005). Crude oil is mainly either black or green but it can also be light yellow (Onifade *et al*., 2007). Crude oil is a complex organic compound made up of a large variety of hydrocarbons (Hidayat and Tachibana 2012). The effect of the crude oil pollution on the environment depends on the type and quantity of crude oil involved. The water soluble fraction of the oil has been reported to reduce the growth of biomass in the contaminated environment as a result of the reduction in dissolved oxygen, increase in turbidity and toxicity of the crude oil components (Edema, 2012). This problem has been amplified by the fact that a lot of the conventional treatment methods employed for the decontamination of the crude oil polluted sites are often limited in terms of application and they are not economically viable and may be only partially effective in cleaning up the contaminated site(Vasudevan and Rajaram, 2001).

Nigeria has a vast crude oil and gas deposits and attempts to explore it have left the country with unique vulnerabilities(Nduka and Orisakwe,2009). Refinery and petrochemical plants generate solid waste and sludge composed of organic, inorganic compounds including heavy metals. The process of refining crude oil consumes huge quantity of water. Consequently, large amounts of liquid effluents containing priority pollutants are released into the environment (Gargouri *et al*., 2011). Industrialization and urbanization especially in developing countries have led to the accumulation of heavy metals and petroleum hydrocarbons in the environment (Yamaso *et al.,* 2000; Adedniyi and Folabi, 2002). While petroleum Industry contributes immensely in the development of the nation, the environmental pollution from the industry is a cause of serious concern (Diya'uddeen *et al.,* 2011).

The rate of oil spillage reported in the country has been rising with a corresponding increase in petroleum production (Onifade *et al*., 2007).

The dominance of petroleum products in the world economy creates the conditions for distributing large amounts of these toxins into populated areas and ecosystems around the globe (Ojumu, 2004).

Pollution is an undesirable change in the physical, chemical and biological characteristics of all the components of an environment (Aboriba, 2001). Oil pollution are increasingly becoming a common theme in the world today and this has resulted in the degradation of the environment particularly in the oil producing areas of the world (Obahiagbon *et al*., 2009). Environmental contamination resulting from crude oil pollution typically occurs through accidental release of the crude oil and from the large quantities of oil sludge produced in refineries during the separation of oil from water as well as the oil materials present at the bottom of crude oil storage tanks (Kishore and Mukherjee, 2006).

In Nigeria, pollution of the surface and underground water by oil and solid wastes is widespread, thereby rendering water unsuitable for man's use (Bakare *et al*., 2000). The hydrocarbon molecules that make up crude oil and petroleum products are highly toxic to many living organism, including humans

(Adekunle *et al*., 2007). The pollutants in the wastewater includes: aromatics, phenols, polycyclic aromatic hydrocarbons (PAHs) and heavy metals (Bako *et al*., 2008). The wastewater generated from the petroleum industry finds its way into soil and water bodies (Domde *et al*., 2007). The wastewater may be treated by physicochemical or biological methods. Biological treatment is preferred over physicochemical as the former is cost effective, efficient and environmentally friendly (Hamza *et al*., 2008).

Large amounts of engine oil are liberated into the environment when the motor oil is changed and disposed into gutters, water drains, open vacant plots and farmlands, a common practice by motor mechanics and generators mechanics (Odjeda and Sadiq, 2002). In addition the oil is also released into the environment from the exhaust system during engine use and due to engine leaks (Anoliefo and Edegbai, 2000; Osubor and Anoliefo, 2003).

Biodegradation by microorganisms represent one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment (Okoh, 2003).
In bioremediation, degradation of toxic pollutants was carried out either through intracellular accumulation or via enzymatic transformation to less or nontoxic compounds (Brar *et al.,* 2006). However, single cultures of fungi have been found to be better than mixed cultures (Okerentugba and Ezeronye, 2003) and more recently, fungi have been found to be better degraders of petroleum than traditional bioremediation techniques including bacteria (Batelle, 2000).

Diverse fungal cultures have been investigated recently for bioremediation processes (Diaz, 2008). Filamentous fungi play an important role in degrading petroleum hydrocarbons by producing capable enzymes because of their aggressive growth, greater biomass production and extensive hyphal growth in the soil, fungi offer potential for biodegradation technology (Saadoun , 2002)

The high surface -to-cell ratio of filamentous fungi makes them better degraders under certain niches (Lawrence, 2002).

It is fungi that can especially handle breaking down some of the largest molecules present in nature (Fernande-Lugueno *et al*., 2010). Some fungi exude extracellular enzymes which allow for digestion of energy sources in their surroundings and further towards the fungus (Mai *et al*., 2004).

Aspergillus niger is a haploid filamentous fungi and is a very essential microorganism in the field of biology. The fungi is most commonly found in mesophilic environments such as decaying vegetation or soil, plants and enclosed air environment (Wu *et al*., 2000).

II. Aim and Objectives

The aim of this research work was to assess the biodegradation ability of *Aspergillus niger* isolated from mechanic workshops soil on refinery effluent and petroleum hydrocarbons**.**

(i) To isolate Aspergillus niger from mechanic workshops soil.
(ii) To determine the physicochemical properties of the refinery effluent.
(iii) To determine the biodegradation ability of *Aspergillus niger* on refinery effluents and petroleum hydrocarbons.

III. Study Area

Kaduna Refinery and Petrochemical Company(KRPC) is located in Chikun local government area of Kaduna state Nigeria. The refinery occupies an area of 2.9 square kilometers and is located on an undulating land about 700meters above sea level. The area is accessible through major roads.

IV. Materials and methods

Collection of sample

(a) Sampling site

The soil for the present study was collected from three (3) mechanic workshops. The soil was collected from top soil (0-5 cm) from the mechanic workshop. The soil was taken by tilling with a scoop and transferred into clean polythene bag. The samples were then transported to the Department of Microbiology laboratory, Ahmadu Bello University, Zaria.

(b). Effluent sample was collected from waste oil retention pond point of the petroleum refinery. Ten (10L) of the sample was collected in a plastic container with screw cap. The sample was collected and transported to the Department of Microbiology laboratory, Ahmadu Bello University, Zaria for analysis within 24h of collection. The sample was transported to the laboratory in ice box where it was refrigerated at 4^0C prior to analysis.

Isolation and Enrichment of fungus

The soil was homogeneously mixed and sieved using 2.0 mm sieve to remove unwanted soil debris. 1g of the soil sample was weighed into test tubes containing 9ml of sterile distilled water, and this was agitated for one minute using a shaker. Serial dilutions of the soil samples were made up to 10^{-5} dilution. The media used for the isolation was Potato Dextrose Agar (PDA) which was supplemented with streptomycin (50mg/ml) to prevent bacterial growth. 0.1ml of dilution 10^{-3},10^{-4},10^{-5} was inoculated in sterile petri dishes in duplicate containing 15ml of sterile Potato dextrose agar media(Guillermina *et al.,* 2002) and mineral salts medium (MSM) (Bekatorou *et al.,* 2007) (g/l): $MgSO_4 \cdot 7H2O$: 0.005; $CaCl_2 \cdot 2H_2O$: 0.005; NH_4NO_3: 0.5; K_2PO_4: 0.001; Na_2 HPO_4: 0.02; $MnSO_4$: 0.001) sterilized. The Petri dishes were then incubated at ambient temperature for 72h. Colonies suspected to be *A. niger* was sub-cultured into sterile plates containing fresh PDA and incubated for another 72h at ambient temperature. A smear of the isolated fungal hyphae was made on a clean slide and a drop of lactophenol cotton blue dye was added to it. The slides were then observed under the microscope for morphological identification of the fungi (Nagamani *et al*., 2006). Isolates was stored and maintained on PDA slants.

Physicochemical and Biochemical Parameters of the Effluent

Effluent sample was collected from waste oil retention pond point of the petroleum refinery. Ten litres (10L) of the sample was collected in a plastic container with screw cap and

transported to Zaria for analysis within 24h of collection. The sample was transported to the laboratory in ice box where it will be refrigerated at 4^0C prior to analysis.

The important parameters considered in this study include pH, temperature, total solids, total dissolved solids, total suspended solids, dissolved oxygen, Nitrates, Phosphates, chlorides, chemical oxygen demand (C.O.D), Biochemical oxygen demand (B.O.D) and electrical conductivity. Temperature and pH were measured immediately after sample collection.

DETERMINATION OF PHYSIOCHEMICAL AND BIOCHEMICAL PARAMETERS OF THE EFFLUENTS

Physiochemical Analysis of the Refinery Effluent

This was done in accordance with the methods of American Public Health Association (APHA), 2005,) Physicochemical analysis was carried out to know the physical and chemical conditions under which the effluent exist these includes temperature, pH, of the refinery effluent was recorded on the spot while the total dissolved solid (TDS), biological oxygen demand (BOD), Total suspended solids, chemical oxygen demand (COD), dissolved oxygen (DO), Chloride, Nitrates and phosphates was also analyse

1. The pH

This was done in accordance with the method of the British Standards Institution, specification for pH scale.

The pH meter was switch on and placed on stand-by for at least 15minutes.The Electrode was rinse first with distilled water, rinse again with lower standard pH buffer intended for use in calibration. The electrode was dipped into the lower standard pH buffer and the calibration button was pressed, then wait for the instrument to calibrate itself. The electrode was removed, rinsed with distilled water and rinsed again with higher standard pH buffer intended for use in calibration. The electrode was dipped into the higher standard pH buffer; the calibration button was pressed again, for the instrument to calibrate itself again. The electrode was rinsed with distilled water and rinsed again with effluent sample. The electrode was dipped into effluent sample .The pH of the effluent sample was recorded.

2. Temperature:

Temperature is the degree of hotness or coldness of a substance. The apparatus use for the measurement was the mercury thermometer. The thermometric bulb containing the mercury was vertically immersed in the effluent and allowed to stand for some minutes till the temperature reading is steady before obtaining the reading.

3. Total Solids (TS)

A clean dish of suitable size was dried at 103-105^0C in an oven until a constant weight is obtained. It was subsequently cooled to room temperature in a desiccator and later weighed. Hundred (100ml) of effluent sample was measured into a dish and evaporated to dryness on a steam bath. The outside of the dish was wiped and the residue was dried in an oven for 1h at 103-105^0C. The dish was quickly transferred to a desiccator, cooled to room temperature and weighed. The dish was dried further in an oven for 10-20 minutes, reweighed after cooling to room temperature.This was repeated until the weight of the dish plus residue is constant to within 0.05mg.

4. Total Suspended Solids (TSS)

Glass-fiber filter paper of diameter 5.5cm was inserted into the funnel assembly and clip together. A slight suction was washed with about 100ml of water and when free from excess water, the paper was carefully removed.It was then placed on a watch glass and heat in an oven at 105^0C for 1h, cooled in a desiccator and weighed. The paper was replaced in the funnel assembly and moistened with water. A suitable volume of well mixed effluent sample was measured and filter under slight suction ensuring that all solids are transferred to the paper. The residue was washed three times with about 5 to 10ml of water allowing it to drain free from water after each wash. The paper was carefully remove and placed on a watch glass and dried in an oven at 105^0C for 1h. It was then allowed to cool in a desiccator and the paper weighed.

5. Total Dissolved Solids

Effluent sample was stirred with a magnetic stirrer and a measured volume was taken into a glass fiber filter with applied vacuum. It was then washed with three successive 10ml volumes of distilled water, allowing complete drainage between washings, and suction was continued for 3minutes until filtration was completed, total filtrate with washings was transferred to a weighed evaporating dish and evaporated to dryness on a steam bath. Evaporated sample was dried in the oven for 1 h at 180±20C, cooled in a desiccator and weighed.

6. Turbidity:

The turbidity of the effluent was determined by using turbidometer.

7. Conductivity:

The conductance of effluent was determined using a conductivity set. After rinsing the cell with a portion of sample, it was then filled with sample and filtered. Upon a press of the test button, read out, which was automatically correct to 25°C was recorded as micro Siemens per centimetre (µs/cm).

8. PHOSPHATE

Hundred (100ml) of sample was transfer into volumetric flask. Ten(10ml) of vanadate-molybdate reagent was added and make up to 50ml mark. This was then allowed to stand for 10minutes. Colour change was read at 600nm.mgPO_4 1= MgPO_4 in 50ml vol. flask x1000/ml of sample

9. CHLORIDE

Hundred (100ml) of sample was transferred into conical flask. 2-3 drops of potassium chromate was then added and content was swirled for few minutes then titrated against silver nitrate until dirty reddish precipitate was obtained.
ClOmg/l=volume of $AgNO_3$ x 10

10. NITRATE

Hundred (100ml) of effluent sample was poured into a clean dried metallic crucible and kept in an oven at 100^0C till dryness. It was then removed and allowed to cool after which 2ml of

phenol disulphonic acid was added and swirled round uniformly. It was left to stand for 10 minutes and 10ml of distilled water was added, after which 5ml of ammonia solution was added and allowed to cool. Coloured change was read at 430nm wavelength.

11. OIL AND GREASE (O and G)

Hundred (100ml) of effluent sample was taken into a separating funnel, 5ml of conc. H_2SO_4 was added. Ten (10ml) of petroleum ether was added into the separating funnel, it was then agitated for 2minutes, drained off. Additional 20ml of petroleum ether was added and agitated, it was then put in an oven for 30minutes, then put in a desiccator and allowed to cool then weighed.

Chemical Parameters:

1. Dissolve Oxygen (DO):

Effluent sample was poured into 300ml BOD bottle. 2ml Manganese sulphate solution (MnSO4) and 2ml alkali-iodide azide reagent was also added. It was then stoppered with care to exude air bubbles. It was mixed gently by inverting the bottle a number of times until a clear supernatant was obtained. It was allowed to settle for two minutes after which 2ml Conc. H_2SO_4 was then added by allowing the acid to run down the neck of the bottle.

It was stoppered again and mixed by gently inversion until dissolution was complete. Hundred (100ml) of the prepared solution was transferred into conical flask, titrated with 0.0125N of $Na_2S_2O_3.5H_2O$ solution to a pale straw/yellow colour. Two (2ml) of starch solution was added and the colour becomes blue. Titration was then continued by adding thiosulphate drop wise until the blue colour disappeared.

DO (mg/l) = 16000 x M x V/V2/V1 (V1-2)

Where

M= molarity of the thiosulphate solution

V= volume of thiosulphate used for titration

V1= volume of the bottle with stopper in place

V2 = volume of aliquot taken for titration

2.Biological Oxygen Demand (BOD)

Two hundred (200ml) of the effluent sample was placed in a 300ml standard BOD bottle and covered carefully to exude air bubbles. The bottle was kept in an incubator for five days. After 5 days in incubator, bottle was brought out and 2ml of manganese sulphate solution was then added followed by 2ml of alkali-iodide azide reagent. The bottle was stoppered carefully to exude air bubbles and then mixed thoroughly by inverting the bottles several times. The precipitate was allowed to settle leaving clear supernatant after which 2ml of conc. H_2SO_4 was then added. The bottle was stoppered and mixed with gentle inversion. 100ml of the prepared solution was transferred into conical flask and 2ml of freshly prepared starch indicator was also added. The solution was then titrated with 0.0125Nof sodium thiosulphate solution until the disappearance of the blue colour.

BOD was calculated using the formular

$(BOD)_5$ in mg/l=DO_1-DO_5

D_1= DO sample before incubation

D_2= DO sample after incubation

3. Chemical Oxygen Demand (COD)

Anti-bumping granules were inserted into influx flask. Ten (10ml) of the sample was measured into the flask. 1ml of 20 % m/v mercuric sulphate solution was added and swirled to mix. 5mls of 0.021M potassium dichromate was added. Using a dispensing pipette, 15mls of 1 % m/v silver sulphate was added. The content in the flask was fit to a condenser and boiled gently for 2h. After 2h, the flask was removed to cool for approximately 10minutes and then 25mls of distilled water was added. Two drops of ferroin indicator was added to the content in the flask and the residual dichromate was titrated with standardized ferrous ammonium sulphate.

The COD was obtained using the formula

COD= 800M (Vb-Va) mg/l

Where

Vb= average number of ml of ferrous ammonium sulphate used in titrating the appropriate blank

Va= Molarity of standard ferrous ammonium sulphate solution.

Biodegradation of petroleum refinery effluent by *Aspergillus niger*

Degradation of petroleum refinery effluent was carried out in three different conditions. Erlenmeyer flasks (250 ml) containing 100 ml of effluent, in first set of experiment only effluent was taken. In second set of experiment, sterilized glucose solution was added in effluent aseptically to maintain the final concentration 1.0 % (w/v) as carbon source (Zaied*et al*., 2008), in another set, 20 % (v/v) of mineral salts medium (MSM) (Bekatorou*et al.,* 2007) (g/l): $MgSO4{\cdot}7H_2O$: 0.005; $CaCl_2{\cdot}2H_2O$: 0.005; NH_4 NO_3: 0.5; Na_2 HPO_4: 0.001; K_2HPO_4: 0.02; MnSO4: 0.001) sterilized separately and then mixed with effluent (80 ml effluent+ 20 ml MSM) aseptically. The flasks was inoculated with *A. niger* grown in PDA plates and incubated at room temperature on a rotary shaker 150rpm for 12 days and readings was taken at regular interval of 72h for total fungal counts, pH, optical density, growth was measured by optical density of microbial growth using spectrophotometer at 325nm. Control sample was maintained separately without inoculation of fungal. All experiments was carried out in duplicates. Other physicochemical parameters like pH, TS, TDS, TSS, COD, chlorides, Nitrates, phosphate was carried out before and after treatment.

Heterotrophic fungal count during degradation of petroleum refinery effluent by *Aspergillus niger*

A stock solution containing mineral media, petroleum refinery effluent and the test isolate (*Aspergillus niger*) was made by transferring 1ml aliquot from the experimental set up described above into 9ml of normal saline and a mixed aliquot of 1ml of the first dilution was then be transferred to a 9ml normal saline until a dilution of 10^{-5} will be reached. Appropriate dilutions was streaked on solid media (PDA) using spread plate technique and incubated at room temperature for a period of 2-4 days. Colonies observed after the incubation period was counted and recorded.

Confirmatory Test for Hydrocarbon Utilization Potential of the Isolated Fungus

The enrichment procedure as described by (Nwachukwu, 2000) was used in the estimation of hydrocarbon utilizer. A minimal salt broth containing 2.0g of NA_2HPO_4, 0.17g of K_2SO_4, 4.0g of NH_4NO_3, 0.53g of KH_2PO_4, 0.10g of $MgSO_4$, $7H_2O$ was dissolved in 1000ml of distilled water was prepared. The solution was sterilized by autoclaving. Twenty-six (26) conical flasks were sterilized, then 100ml of the minimal salt medium (MSM) was measured into each of the first set of four flasks. 20ml of kerosene or diesel or unspent engine oil or spent engine oil was also measured and added to the 100ml of minimal salt medium in the first four flasks, inoculated with the isolated fungus respectively, in another set of four flasks 100ml of minimal salt medium in four flask with 20ml of each hydrocarbon which served as positive control and a negative control having mineral salt medium with the isolated fungus. Each of the flasks was plugged with sterile cotton wool so as to ensure maximum aeration and prevent cross contamination. All the flasks were then incubated at room temperature (28^oC - 31^oC) for 40 days. The flasks were shaken constantly throughout the duration of the experiment to facilitate oil (cell phase contract. The ability to degrade the petroleum products (based on the growth rate of the organisms in the MSM) was measured every 5days using the visual method which is based on the turbidity of the MSM. The turbidity was measured using the spectrophotometer. All experiments were carried out in duplicates.

5. Result

Characterization of the Fungus Isolate

Cultural Characteristics	Microscopic characteristics	Inferences
Black colony with granular surface and black reverse	Septate hyphae. Dark brown large globose conidial heads. Hyaline smooth-walled conidiophores which turn dark toward the vesicle. Conidial heads are biseriate.	*Aspergillus niger*

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Physicochemical Parameters of the Untreated Refinery Effluent

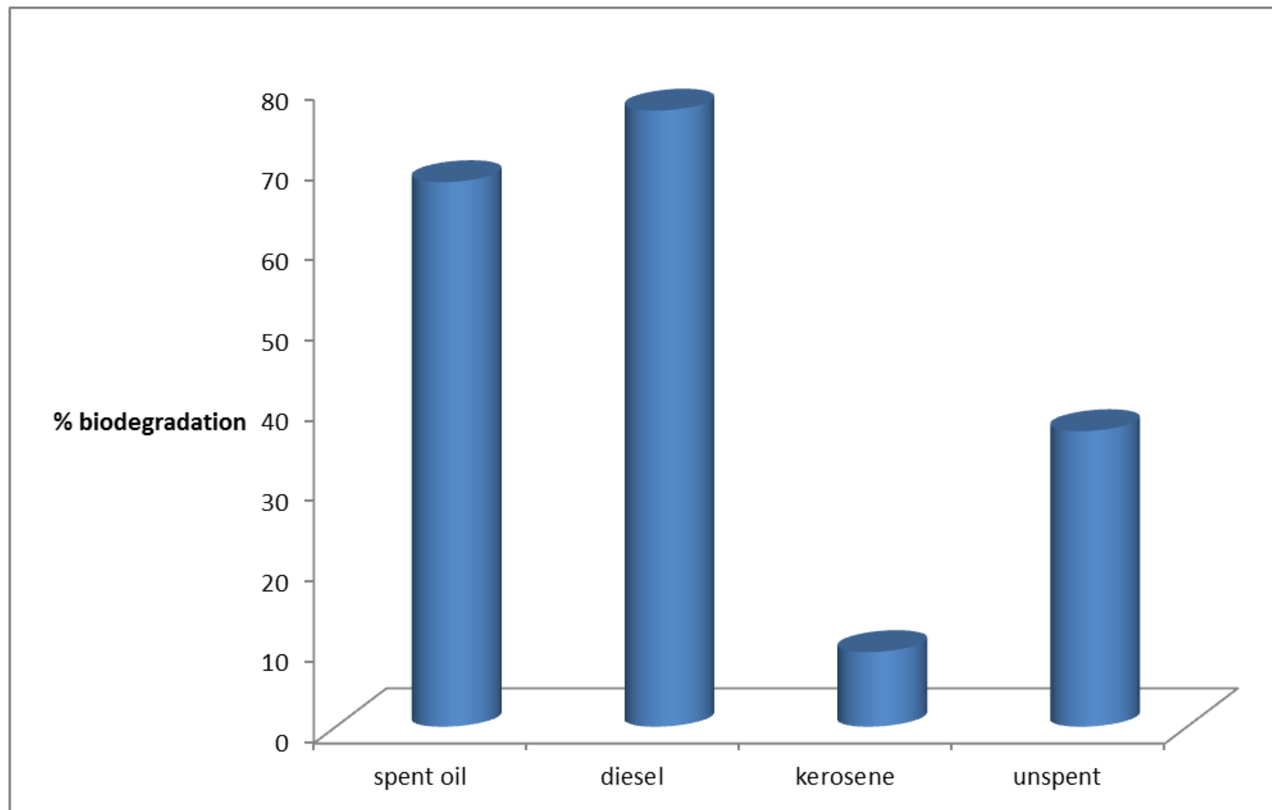
Parameters	Untreated Effluent
PH	6.8
Temperature	25.3
Turbidity	5.26
Electrical Conductivity	18.10
Phosphate (mg/l)	8.30
Chloride (mg/l)	341.8
Nitrate (mg/l)	60
Dissolve Oxygen (mg/l)	0.45
COD (mg/l)	173.41
BOD(mg/l)	70.30
Total Solid (mg/l)	732
Total Suspended Solids(mg/l)	1270
Total Dissolved Solids(mg/l)	6050
Oil and Grease (mg/l)	20.10

Key: **(mg/l)**=milligram per litre, **(µs/cm)**= microsiemens per centimeter,
NTU= Nephelometric turbidity unit.

Physicochemical parameters of the treated refinery effluent with and without nutrient source inoculated with *Aspergillus niger*

Parameters	Effluent +*A.niger*	Effluent + *A.niger* + Glucose	Effluent + *A.niger* + MSM
pH	8.9	9.1	6.6
Temperature(^oC)	25.3	25.2	25.2
Turbidity (NTU)	4.74	1.81	2.47
E.C. (µ s/cm)	0.72	0.29	0.50
Phosphate (mg/L)	2.60	1.98	0.96
Chloride (mg/L)	86.1	72.7	75.60
Nitrate (mg/L)	26.60	18.86	16.62
Dissolve Oxygen(mg/L)	3.91	9.45	9.40
COD (mg/L)	14.80	12.00	9.64
BOD (mg/L)	12.00	10.84	0.62
Total Solids (mg/L)	12.00	5.30	8.20
T. S.S. (mg/L)	11.64	4.71	7.95
T. D.S. (mg/L)	0.36	0.59	0.25
O and G(mg/L)	9.80	6.64	4.78

Key: **(mg/l)** = milligram per litre, **(µs/cm)** = microsiemens per centimeter,
NTU= Nephelometric turbidity unit.



Percentage biodegradation of petroleum hydrocarbons.

V. DISCUSSION

Pure culture of *Aspergillus niger* was isolated from soil of mechanic workshop and was identified with reference to appropriate taxonomic guides (Nagamani *et al.,* 2006).

The physicochemical analyses of the refinery effluent collected from the study site indicate that the acidic pH (6.8) of the untreated refinery effluent was within the FMENV permissible limit. The pH increased to alkaline after treatment indicating effective utilization of some organic acids by the fungi present in the effluent and by products of the fungus. This is in agreement with the reasons proffered by George *et al*. (2003). Nwaichi *et al.*(2012) reported that the alkaline nature of the effluent maybe due to the presence of soluble organic and inorganic alkalis. The increase in the pH values observed suggests that the fungus in the effluent samples was degrading the effluent to less toxic and less acidic products. This view was also suggested by some researchers (Sanyaolu *et al*., 2012). Kessington *et al*. (2009) reported that the degradation of refinery effluent into intermediate products might actually have an effect on the pH of the remediation medium. While Andrew *et al.* (2014) reported that the pH of the contaminated wastewater undergoing remediation could increase with time if the population of the remediating microbial population is allowed to grow and thrive. They reported a similar increase in the pH of the samples in the course of bioremediation. Andrew *et al*. (2014) investigated the effect of nutrient supplementation, aeration and mechanical agitation on the bioremediation of contaminated waste water. According to them, the increase in pH might have resulted from the conversion of the refinery effluent waste water into less acid products such as CO_2, H_2O and many intermediates like organic acids, lipids, esters, complex alcohol and microbial proteins in form of enzymes (Otokunefor and Obiukwu, 2010). This result is in agreement with the findings of Ogunlaja and Ogunlaja (2007) and Nduka and Orisakwe (2009).

The temperature of the untreated effluent was within the set down standard and therefore suitable for aquatic environment. It is also an indication that fungi tolerate range of temperature within the environment from which they are taken. The value obtained was in agreement with the report of Siu *et al*. (2012).

The ability of the organism to grow effectively and utilize compounds responsible for the turbidity was greatly enhanced by the augmentation with glucose and to a lesser extent mineral salts medium. Thus similar decrease in turbidity of waste water was reported by Cosa and Anthony, (2014). Similarly, the turbidity decreased generally with increase in microbial grow for all the three sets of samples throughout the remediation period.

Nutrient and phosphate are essential nutrients to plant life, but when found in excess quantities, stimulates excessive plant growth such as algae bloom (Igbinosu and Oko, 2009). The higher Phosphate level in untreated effluent may account for algal bloom observed in many discharge point and the attendant increase in high COD and BOD.

High level of nitrate in drinking water may result in health problems such as cyanosis among children and cancer of the alimentary canal. Infants, drinking ground water that has high nitrate concentration may develop cyanosis and blue baby syndrome, which is a potentially fatal condition (Haugen *et al.,* 2001).

Chloride(s) are generally considered as one of the major pollutants in the effluents which are difficult to be removed by

conventional biological treatment methods. The deployment of *Aspergillus niger* could be a viable option for remediation with high level of Chlorides as seen in this work.

The level of chemical oxygen demand (COD) was greater than the permitted limit but was efficiently reduced by the fungus to the permitted limit this could be as a result of breakdown of This is in agreement with the work of keyan *et al., (*2010). This was ascribed to the uptake of organic matter as a carbon source by the fungus (Achi *et al*., 2001). In similar studies, (Miranda *et al.,* 1996) reported 52.5-95% COD in distillery waste water treatment with *A. niger,*while Vimala, (2012), reported 60% decrease in COD by the same isolate during treatment of poplar alkaline peroxide mechanical pulping wastewater.

High level biological oxygen demand (BOD) was analysed in the untreated effluent. The BOD level was reduced significantly. The better performance observed can be explained by noting that biodegrading fungi need oxygen, carbon and hydrogen to function optimally and these are provided by added nutrients (Alwan *et al.,*2013).Other workers have also established the effectiveness of mineral salt in bioremediation (Obahiagbon and Aluyor, 2009).

Total dissolved solids effluent augmented with MSM was reduced to 0.25mg/l which is below the permissible limit of FMENV. This is supported by the work of Srivastava and Thakur (2006). This reduction may be due to several physiochemical reactions such as sedimentation, coagulation, fixation, amongst other factors like oxidation and precipitation (Wasserman *et al*., 2006). A certain level of these ions in water is essential nutrients for aquatic life (Galbrand *et al.,* 2008).

The level of total suspended solids (TSS) in the untreated effluent was 1270mg/l.Samples augmented with glucose had greater reduction (4.71mg/l) than sample augmented with MSM and effluent alone. Reduction in TSS occurs because filamentous fungi entrap the suspended solid particles in the wastewater (Alam *et al*., 2001).

Oil and Grease the value obtained from the untreated effluent (20.10mg/l) was above the acceptable limit of FMENV. The sample augmented with MSM had greater reduction than sample augmented with glucose and effluent alone. This findings is in agreement with the report of Uzoekwe and Oghosanine, (2011) and Otokunefor and Obiukwe, (2005).

Oboh *et al.* (2006) have reported the abilities of fungi species such as *Aspergillus sp*, *Penicillum* and *Rhizopus* species to grow on crude petroleum as the sole carbon and energy source when screened for hydrocarbon utilization. Also, Uzoamaka *et al*. (2009) reported that some eight isolates of fungi that showed potentials for hydrocarbon biodegradation include *A. niger*, *A.flavus,., Trichoderma spp., Rhizopus a* and *Mucor spp.*

Diesel had the highest percentage degradation of 76.7%, 67.8% in spent engine oil, 36.8% in unspent engine oil and kerosene had the least percentage degradation of 9.3% (figure Generally, *Aspergillus niger* had better degradation ability in diesel and the least degradation in kerosene this may be due to availability of ideal environment such as dissolved water for germination, carbon for food, oxygen and sulphur for respiration and trace elements for growth and propagation. Similar work has been reported by Adekunle and Adeniyi, (2015) in the biodegradation of petroleum oil by fungi, *Aspergillus niger* had 20.93% biodegradation contribution for diesel and the least percentage contribution in kerosene was 14.08%. (Amanchukwu *et al*., 1989) observed that most microorganism find it difficult biodegrading kerosene, attributing this to its type of hydrocarbon chain.

VI. Conclusion and Recommendation

Conclusion

Aspergillus niger was isolated from soil of mechanic workshop this is an indication that soil contaminated with hydrocarbons can be exploited for oil depredating organisms. Petroleum refinery generates large volume of effluents as a result of the use of chemicals and water. The discharge of untreated effluent which contain Total suspended solids (TSS), Dissolved oxygen (DO), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Nitrate, phosphate into water bodies can affect the standard of living in the environment in a negative way.

The physicochemical analysis carried out on the effluent revealed that BOD, COD, TSS, TDS, Chloride, Nitrate, Phosphate were above the acceptable limit of Federal Ministry of Environment (FMENV)

Traditional methods of clean-up of pollutants are usually inadequate. The biodegradation is cost effective technology for the treatment of pollutants. *Aspergillus niger*was found to be effective in the degradation of refinery effluent to acceptable limit and petroleum hydrocarbons.

This indicates that the fungus could be employed in the treatment of refinery effluents and bioremediation of water bodies polluted as well as biodegradation of toxic carbon pollutants.

Recommendations

1. There is need for proper treatment of effluent to meet standard guidelines for waste water discharge with regards to physicochemical parameter such as TSS, COD, BOD, TDS, oil and grease.
2. There is need for FMENV to impose all petroleum refinery industries in the country to abide to the existing regulations.
3. There is need to carry out biodegradation in the already contaminated sites, where effluent are discharge without any other treatment.
4. Common Effluent treatment Plant(CEPT) should be constructed in the refinery to minimize cost of treatment and as well as to meet the standard guideline for waste water discharge.
5. All Fungi isolates tested obtained from bioremediation studies should be further used in large scales as an alternative treatment system for industries before discharging their effluent to appropriate channels.

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