Research Journal of Agriculture and Environmental Management Vol. 5(4), pp. 132-137, May, 2016
Available online at http://www.apexjournal.org
ISSN 2315-8719© 2016 Apex Journal International

Full Length Research

Biodegradation of crude oil using *Aspergillus niger* isolated from the rhizospere of *Helianthus annuus*

Majekodunmi, A.O.[1] and Adongbede E.M.[2]

[1]Forestry Research Institute of Nigeria, Jericho Hill, Ibadan, Nigeria.
[2]Department of Botany and Microbiology, University of Lagos, Akoka, Nigeria.

Accepted 20 May, 2016; Published 30 May, 2016

Biodegradation of crude oil using *Aspergillus niger* strain isolated from the rhizosphere of *Helianthus annuus* (sunflower) was investigated over a period of 30days. The ability of the fungus to degrade crude oil in test tubes containing minimal salt medium was done by measuring the change in optical density read on a photo colorimeter. *A. niger* in the minimal salt medium showed degradation of crude oil at different rates over the period of 30days. To further investigate the rate of degradation, gas chromatography was done. The Gas Chromatography analysis (GC-FID) after 30days of incubation (Minimal salt medium, *A. niger* and crude oil) and control (Minimal salt medium, crude oil without fungus) were investigated upon to know the percentage degradation of the petroleum product. In this research work, *A. niger* degraded 86.94% of crude oil.

Key word: Biodegradation, *Aspergillus niger,* rhizosphere, *Helianthus annuus,* Gas Chromatography.

INTRODUCTION

The biodegradation of petroleum and other hydrocarbons in the environment is a complex process (Leahy and Colwell, 1990). Biodegradation of oil contaminated soils, which uses the ability of microorganisms to degrade and/or detoxify organic contamination has been established as one of the efficient, economic, versatile and environmentally sound treatment (Margesin and Schinner, 1997). The effectiveness of bioremediation is often a function of the extent to which a microbial population or consortium can be enriched and maintained in environment. When few or no indigenous degradative microorganisms exist in a contaminated area and practically does not allow time for the natural enrichment of suitable population, inoculation may be a convenient option (Kalyuzhnyi, 2000). Some compounds in hydrocarbons may not be degraded by organisms (Atlas and Brag, 2009). Others may be degraded and broken down into carbon dioxide, water and fatty acids (Anene and Chika, 2011) while others may be transformed into other compounds. Additions of nutrients are necessary to enhance the biodegradation of oil pollutants (Choi *et al.,* 2002; Kim *et al.,* 2005). However, excessive nutrient concentrations can inhibit the biodegradation activity (Challain *et al.,* 2006), and several authors have reported the negative effect of a high NPK levels on the biodegradation of hydrocarbons (Oudot *et al.,* 1998; Chaıneau *et al.,* 2005) and more especially on the aromatics (Carmichael and Plaender, 1997). To remediate soils contaminated with petroleum products, bioremediation had been proven to be the most reliable and effective strategy to cleaning up contaminated soils. Most mechanical methods to reduce hydrocarbon pollution is expensive, time consuming and depends mainly on excavating of the soil, treating in separate area or better treatment facilities. These treatments include incineration and/or burial in secure landfills (USEPA 2001). These are effective treatments but after burning, the soil loses most of its nutritional value and structure. These methods do not remove the contamination but only

*Corresponding author. Email: *adheholar@yahoo.com, e.adongbede@gmail.com*

relocate the problem (Lageman *et al.,* 2005). Bioremediation processes have been shown to be effective methods that stimulate the biodegradation of contaminated soil (Swannell *et al.,* 1996)

MATERIAL AND METHODS

Sample

The plant (*Helianthus annuus*) was collected from the University of Ibadan, Oyo state. The soil was collected from the plant rhizospere of *H. annuus.* Petroleum hydrocarbon (Crude oil) was collected from Chevron (Portharcourt, River State).

Microorganism

Serial dilution was used for this experiment. 1g of soil was added into a sterile test tube containing 10ml of distilled water, then 1ml was drawn from the solution using a sterile pipette which was added into another sterile test tube containing 9ml distilled water. This method was done the same way to give the 10^{-3} dilution. 0.1ml from the 10^{-3} dilution was pipette into Petri dishes containing the Malt Extract Agar (MEA). MEA was prepared according to manufacturers' description. The plates were then incubated at room temperature (28ºC-31ºC) in an incubator and observed daily for fungal growth.

The fungus was identified based on its morphological study i.e. the shape, size, and spore formation after 72hour under a light microscope.

Biodegradation assay

An enrichment medium for petroleum utilizing fungi was prepared and this was prepared according to the composition of Adekunle and Adebambo (2007). A Minimal Salt Medium containing 1.25g of $NaHPO_4$, 0.29g of KCl, 10.0g of NaCl, 0.42g of $NaNO_3$, 0.83g of KH_2PO_4, 0.42g of $MgSO_4.7H_2O$ and 5.0g of Agar was dissolved in 1000ml of distilled water.

Three test tubes were sterilized, plugged with non-absorbent cotton wool wrapped with aluminum foil, and arranged on test tube rack. Each of the test tubes contained 10ml of minimal salt medium (MSM). Two of the test tubes contained 2ml of crude oil leaving the last test tube as control. *Aspergillus niger* which was isolated from the rhizosphere of *H. annuus* was then inoculated into the test tube containing MSM + crude oil and MSM onlyleaving one test tube containing the petroleum hydrocarbon to serve as control (not inoculated with fungi). Each of the test tubes was plugged with sterile nonabsorbent cotton which was wrapped with aluminum foil so as to prevent cross contamination. All the test tubes were then incubated at room temperature in an incubator for 30days. Constant shaking of the test tubes was ensured.

Percentage degradation of petroleum hydrocarbon by fungi

After 30days, the extent of Petroleum hydrocarbon degraded by *A. niger* was determined by Gas Chromatography. The quantity of hydrocarbon present after growth of the fungus in the medium was determined using GC-FID. The gas chromatography equipment used was Hewlett Packard HP. The analytical conditions were as follow: carrier gas, helium, makeup nitrogen gas (flow rate -22ml/min), fuel-air flow rate 45ml/min, fuel-H2 flow rate 45ml/min, injector temperature at 220ºC, initial and final oven temperature 70-200^{0}C. The detector type was flame ionized with temperature at 250C. The amount of PAH before and after were seen at the peak height of the chromatographic run.The concentration and peak area of the standard was then used to quantify the concentration of the test sample with respect to the peak area.

RESULTS

On the 25th day of incubation, *A. niger* was found to have the highest optical density of 1.61. On the 20th day of incubation, a decrease in optical density was recorded and this may be a due to the fact that the fungal mycelium was interfering with the light intensity of the photocolorimeter because during this study the growth of fungi in test tubes containing them was visible to the eye. The growth pattern of the fungus in the minimal salt medium increased from the 5th day till the 30th day of incubation (Figure 1).

Aspergillus niger showed its least degrading rate on the 10th day of incubation with an optical density of 0.65. From the 15th day of incubation, *Aspergillus niger* had an increasing rate in optical density till the 30th day of incubation. This clearly shows that *Aspeillus niger* degraded crude oil to its lowest possible fraction (Figure 2).

In the chromatogram for minimal saltand crude oil (Figure 3a), which was the control and prepared on the day of Gas chromatography analysis, carbon 2- 28 were detected at various peaks. The chromatogram of *A. niger* (Figure 3b) in minimal salt medium and crude oil showed that C_6, C_8, C_{22}, C_{23}, C_{24}, C_{25}, C_{26}, C_{27} and C_{28} had all been degraded after 30 days of incubation. The gas chromatography of the biodegradation experiment after 30days of incubation shows reduction in concentration of residual hydrocarbon present in the media.

The percentage degradation of samples were calculated as follows;

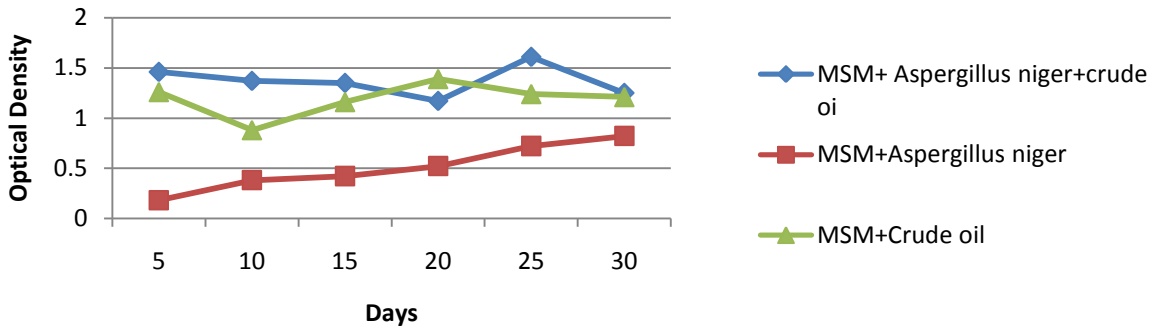


Figure 1. Growth pattern of *Aspergillus niger* in crude oil at 530nm.

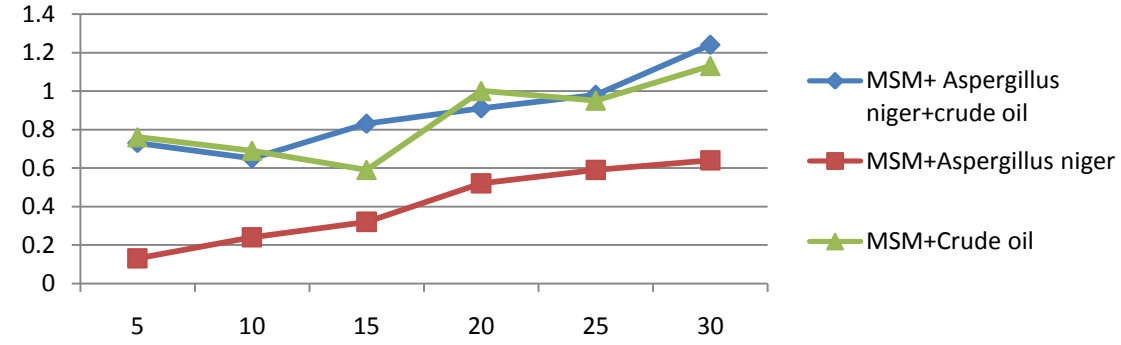


Figure 2. Growth pattern of aspergillus niger in crude oil at 620nm.

Sample peak area=Total peak area – Solvent peak area

$$\text{Concentration of sample} = \frac{\text{Sum total (Peak area of sample)} \times \text{Conc. of the standard}}{\text{Sum total (Peak area of standard)}}$$

NOTE: The result divided by 10(Dilution Factor)
Standard concentration= 10,000mg\L
Standard peak area= 210660.85

The concentration of crude oil present in the control was 3867.44mg/L while the residual crude oil after 30days of incubation was 504.99mg/L. The Gas chromatography analysis evidently showed that *A. niger* degraded 86.94% of crude oil (Table 1).

DISCUSSION

Petroleum degrading microorganisms are ubiquitously distributed in the natural environment, such as in aquatic and terrestrial ecosystem.While they often are found functioning together with bacteria and an array of microorganisms, it is fungi that can especially handle breaking down some of the largest molecules present in nature (Fernandez-Luqueno *et al*., 2010). The result of this research showed that *A. niger* isolated from the rhizosphere of *H. annuus* was capable of degrading crude oil. George-Okafor (2009) reported that 8 isolates showed potentials for hydrocarbon biodegradation and identified them has *Aspergillus versicolor, A. niger, Aspergillus flavus, Syncephalastrum* spp., *Trichoderma* spp., *Neurospora sitophila, Rhizopus arrhizus* and *Mucor* spp. Of these eight isolates, *A. versicolor* and *A. Niger* exhibited the fastest onset and highest extent of biodegradation. Both isolates exhibited above 98% degradation efficiency for polycyclic aromatic hydrocarbon .This research show that over 80% of crude oil was degraded by *A. niger.* There was fluctuation in the growth pattern of the fungus which explains that *A. niger* was able to grow and use the crude oil as its carbon source. This research also showed that the growth of the fungus was significant in the test tubes containing minimal salt and crude oil than in test tubes containing only minimal salt medium, the reason for this is because the test tubes containing the minimal salt and crude oil comprises of adequate nutrients which stimulated the growth of the fungus.

Efeovbokhan (2012) reported that a minimum of 45 days is required for microorganisms to biodegrade hydrocarbons in water to an acceptable level and turn it

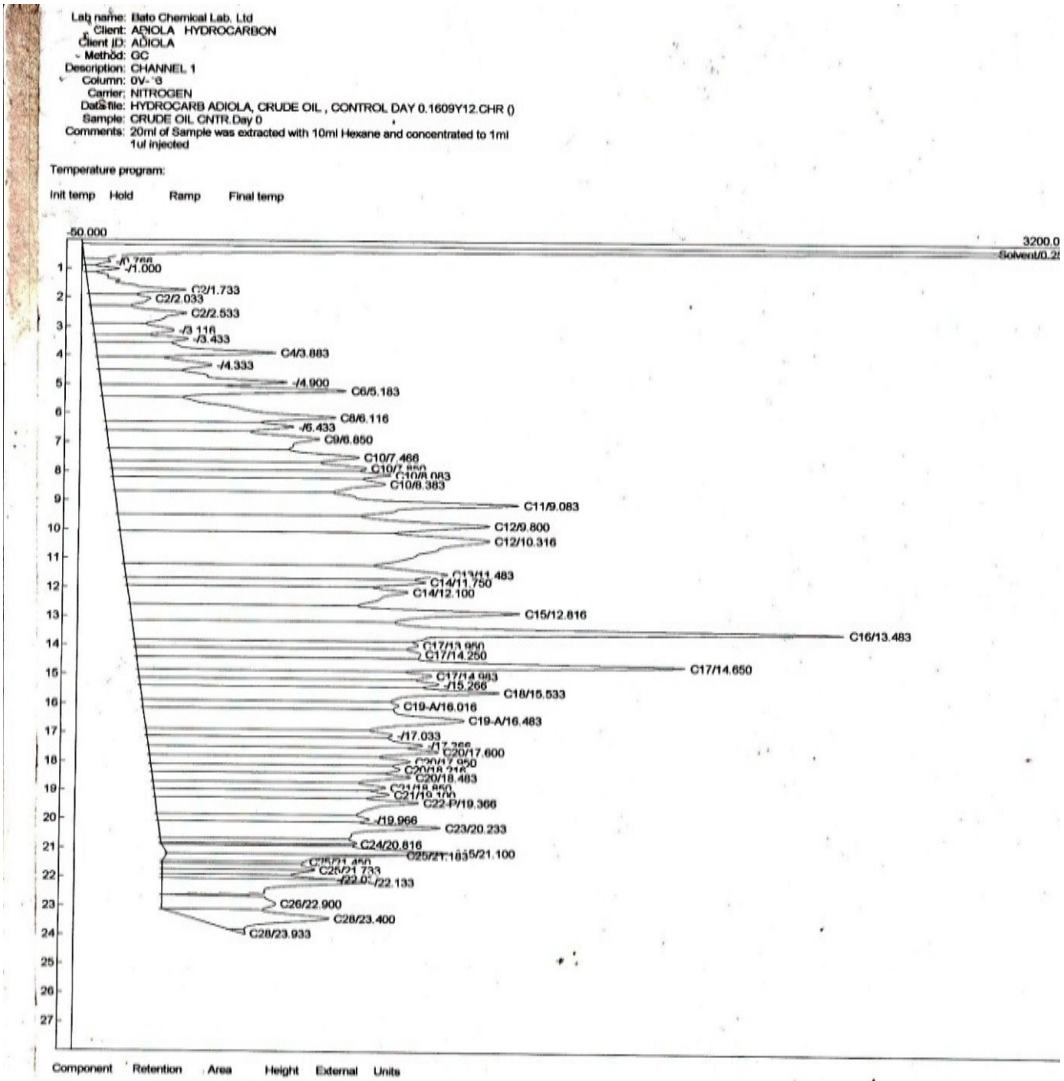
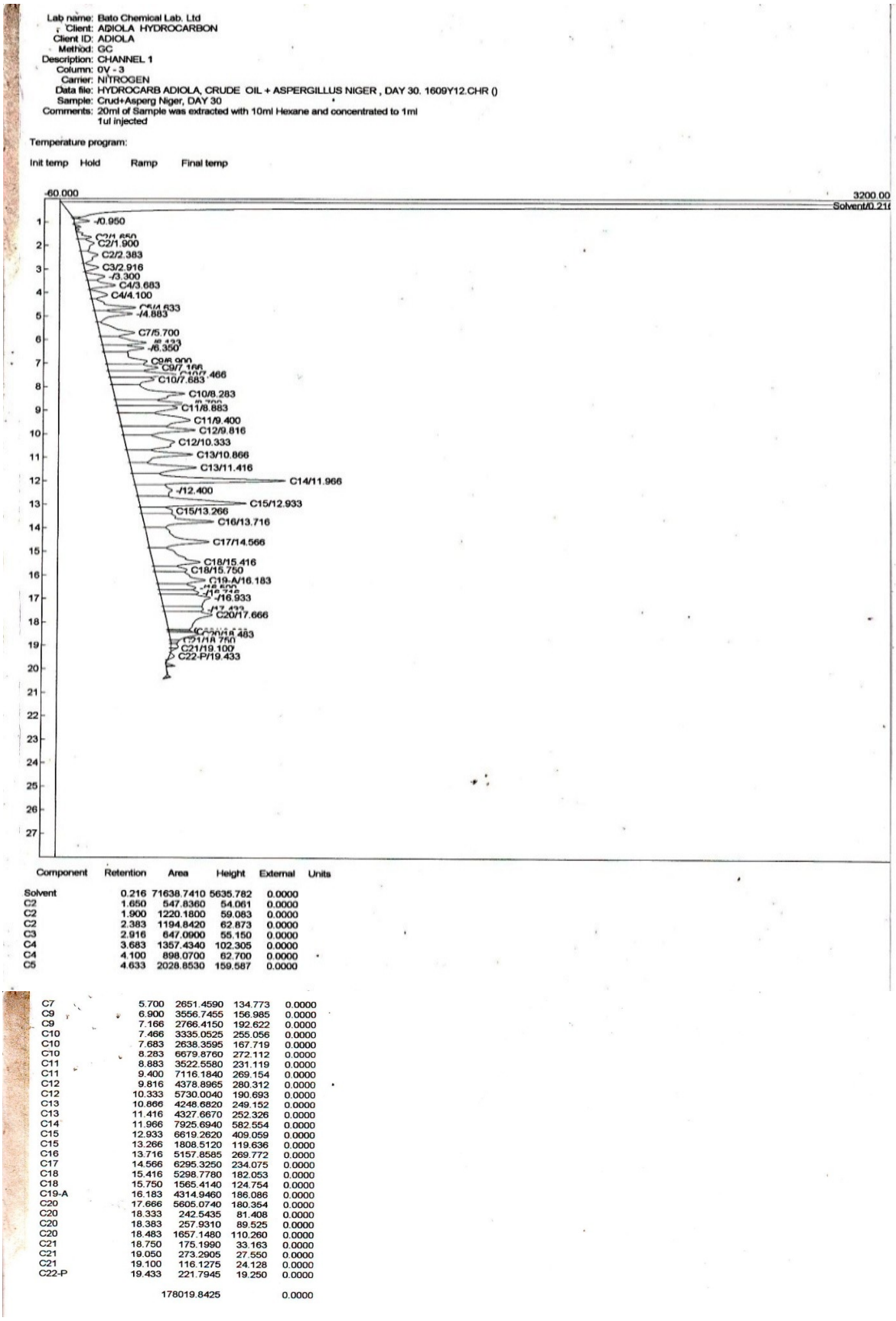


Figure 3. Chromatogram of minimal salt solution with crude oil.



Component	Retention	Area	Height	External	Units
Solvent	0.216	71638.7410	5635.782	0.0000	
C2	1.650	547.8360	54.061	0.0000	
C2	1.900	1220.1800	59.083	0.0000	
C2	2.383	1194.8420	62.873	0.0000	
C3	2.916	647.0900	55.150	0.0000	
C4	3.683	1357.4340	102.305	0.0000	
C4	4.100	898.0700	62.700	0.0000	
C5	4.633	2028.8530	159.587	0.0000	
C7	5.700	2651.4590	134.773	0.0000	
C9	6.900	3556.7455	156.985	0.0000	
C9	7.166	2766.4150	192.622	0.0000	
C10	7.466	3335.0525	255.056	0.0000	
C10	7.683	2638.3595	167.719	0.0000	
C10	8.283	6679.8760	272.112	0.0000	
C11	8.883	3522.5580	231.119	0.0000	
C11	9.400	7116.1840	269.154	0.0000	
C12	9.816	4378.8965	280.312	0.0000	
C12	10.333	5730.0040	190.693	0.0000	
C13	10.866	4248.6820	249.152	0.0000	
C13	11.416	4327.6670	252.326	0.0000	
C14	11.966	7925.6940	582.554	0.0000	
C15	12.933	6619.2620	409.059	0.0000	
C15	13.266	1808.5120	119.636	0.0000	
C16	13.716	5157.8585	269.772	0.0000	
C17	14.566	6295.3250	234.075	0.0000	
C18	15.416	5298.7780	182.053	0.0000	
C18	15.750	1565.4140	124.754	0.0000	
C19-A	16.183	4314.9460	186.086	0.0000	
C20	17.666	5605.0740	180.354	0.0000	
C20	18.333	242.5435	81.408	0.0000	
C20	18.383	257.9310	89.525	0.0000	
C20	18.483	1657.1480	110.260	0.0000	
C21	18.750	175.1990	33.163	0.0000	
C21	19.050	273.2905	27.550	0.0000	
C21	19.100	116.1275	24.128	0.0000	
C22-P	19.433	221.7945	19.250	0.0000	
		178019.8425		0.0000	

Figure 3b. Chromatogram of A*spergillus niger* in minimal salt solution with crude oil.

Table 1. Percentage degradation of crude oil by *Aspergillus niger.*

PETROLEUM PRODUCT /ORGANISM	
CONTROL(DAY 0)	3867.44mg/L
MSM + *Aspergillus niger*+ crude oil (DAY 30)	504.99mg/L
PERCENTAGE	86.94%

to less toxic substances (CO_2 and H_2O). The petroleum product used in this research was added to minimal salt medium and the growth of the organism on the medium proved that fungi has the ability of utilizing and breaking down hydrocarbon to a nontoxic form. Also, the organism was able to use the hydrocarbon as an environment for growth thereby producing enzymes which were able to disintegrate the chains of hydrogen and carbon to simpler form that can be used for itsnutrition and growth. George-Okafor (2009), reported that *A. versicolor* and *A. niger* displayed the fastest onset and highest extent of biodegradation. It was seen on the chromatogram that after 30 days of incubation, the peaks had been reduced. This may be attributed to increased biodegradation rate (Susarla *et al*., 2002). Gas chromatography analysis showed that *A. niger* degraded 86.94% of crude oil. George-Okafor (2009), reported that *A. versicolor* and *A. niger* exhibited high biodegradation efficiency (>98%) within 7days of incubation.

Conclusion

Results from research work carried out shows that *A. niger* is capable of breaking down and utilizing hydrocarbon and using the environment for growth by producing enzymes which are able to disintegrate the chains of hydrogen and carbon to simpler form that can be used for their growth and nutrition. Hence, *H. annuus* can be effectively used as an agent for the cleanup of oil contaminated sites.

REFERENCES

Adekunle, A.A. and Adebambo, O.A., (2007). Petroleum Hydrocarbon Utilization by Fungi Isolated From *Detarium senegalense* (J. F Gmelin) Seeds. *J. Am. Sci*. 3(1): 25-31.

Anene, M. and Chika, N. (2006). Studies on the bioutililization of some Petroleum Hydrocarbons by single and mixed cultures of some bacterial species. *Afr. J. Microbiol. Res.* 5(12): 1457-1466.

Atlas, R. and Brag, J. (2009). Bioremediation of marine oil spills; when and when not- the Exxon valdex experience. *Microbial Biotechnol*. 2: 213- 221.

Carmichael, L.M., and Plaender, F.K. (1997). The effect of inorganic and organic supplements on the microbial degradation of phenanthrene and pyrene in soils. *Biodegradation* 8:1-13.

Chaillan, F., Chaıneau, C.H, Point, V., Saliot, A., and Oudot, J. (2006). Factors inhibiting bioremediation of soil contaminated with weathered oils and drill cuttings. *Environ. Pollut.* 144: 255-265.

Chaıneau, C.H, Rougeux, G, Yepremian, C and Oudot, J. (2005). Effects of nutrient concentration on the biodegradation of crude oil and associated microbial populations in the soil. *Soil Biol. Biochem.* 37(8):1490-1497.

Choi, S.C, Kwon, K.K, Sohn, J.H and Kim, S.J. (2002). Evaluation of fertilizer additions to stimulate oil biodegradation in sand seashore mesocosms. *J. Microbiol. Biotechnol*. 12(3):431-436.

Efeovbokhan, V. E., Hymore, F.K., Oyakhire, C., and Taiwo, G. (2012). The Effects of *Pseudomonas aeroginosa* and *Aspergillus niger* on the Bioremediation of Raw and Treated Crude Oil Polluted Water. *Int. J. Sci. Technol.* 2(6):1-8.

Fernández-Luqueño, F., Valenzuela-Encinas, C., Marsch, R., Martínez-Suárez, C., Vázquez-Núñez, E. and Dendooven, L. (2010). Microbial communities to mitigate contamination of PAHs in soil—possibilities and challenges: a review. *Environ. Pollut. Sci. Res*. 10:11-30.

George-Okafor, U.O. (2009). Hydrocarbon Degradation Potentials of Indigenous Fungal Isolates from Petroleum Contaminated Soils. *J. Phys. Nat. Sci.* 3(1):1-6.

Leahy, J.G., and Colwell, R.R (1990). Microbial degradation of hydrocarbons in the environment. *Microbiol. Rev.* 54(3): 305-315.

Margesin, R., and Schinner, F. (1997). Bioremediation of diesel-oil contaminated alpine soils at low temperatures. *Appl. Microbiol. Biotechnol*. 47:462–468.

Oudot, J, Merlin, F.X, and Pinvidic, P. (1998). Weathering rates of oil components in a bioremediation experiment in estuarine sediments. *Mar. Environ. Res.* 45: 113-125.

Stephen, E., Emmanuel, O.E., Okpanachi, O.S., Emmanuel, S., Temola, O.T, Musa, K. and Ebiloma, I.P. (2013). Invitro study of biodegradation of spent lubricating oil by *Aspergillus niger. Nat. Sci*.11(10): 40-44.

Susarla, S., Medina, V.F. and McCutcheon, S.C. (2002). Phytoremediation: An Ecological Solution to organic chemical contamination. *Ecol. Engg*.18: 662-668.