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BIODEGRADATION OF DIESEL FUEL USING *Talaromyces flavus* (Klöcker) ISOLATED FROM THE RHIZOSPERE OF *Helianthus annuus* L.

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ABSTRACT

Petroleum derived contaminants constitute one of the most prevalent sources of environmental degradation in the industrialized world. The hydrocarbon molecules present in petroleum products when spilled in the soil or groundwater are highly toxic which can be dangerous to human health. This research was carried out to find out the degradative ability of *Talaromyces flavus* isolated from the rhizosphere of *Helianthus annuus.* The degradative activity of *Talaromyces flavus*on diesel fuel was carried out for a period of 30days in test tubes containing enrichment medium (Minimal salt Medium) with the change in optical density measured on a photo electric colorimeter at 620nm. The optical density (Turbidity) was used to analyze the growth rate of the fungus in the enrichment medium. A Gas Chromatography analysis (GC- FID) was carried out after 30days of incubation to know the degradation of diesel. The GC analysis after 30days of incubation (enrichment medium, diesel fuel and *Talaromyces flavus*) was compared with that of control (enrichment medium, diesel fuel no organism) and it evidently showed that 80.76% of diesel fuel was degraded by *Talaromyces flavus*. The fungus isolated from the root zone of *Helianthus annuus* have high degradative ability and is as such responsible for phytoremediation recorded for this plant.

Keywords: *Talaromyces flavus,* Optical density, diesel, Gas Chromatography

Introduction

Nigeria is Africa's energy giant, and the most prolific oil producing country in the continent (Isa *et al.*, 2013). Diesel is a heavy oil used as fuel for internal combustion in diesel engines, as a burner fuel in heating installations, such as furnaces and for enriching water gas to increase its luminosity. While Petroleum industry contributes immensely in the development of the nation, the environmental pollution from the industry is a cause for serious concern. The toxicity of aromatic compounds has been widely documented and their disastrous effects towards human and the environment are greatly concerned (Cherukupalle *et al.*, 2012). Diesel spills can inhibit photosynthesis, invariably threatens life and productivity. Hydrocarbon enters into the environment through accidental spills, waste disposal, as pesticides and during transportation, usage and storage of petroleum. When this happens, there is immediate need to repair the soil because the hydrocarbon molecules that makes up the petroleum products is highly toxic and can enter the food chain. The extensive use of Petroleum products leads to contamination of almost all compartments of the environment, and biodegradation of the hydrocarbon by natural populations of microorganism has been reported to be the main process acting in the depuration of hydrocarbon polluted environment (Chaillan *et al.,* 2004). Different microorganisms with different ability to utilize hydrocarbon as their sole source of carbon as energy for metabolic activities and this organism are ubiquitous in the environment. White rot fungi are capable of using their mycelia to bio-remediate hydrocarbon products through their high production of organic acids, reduction of pH, chelators, oxidative enzymes and extracellular enzymes (Nwinyi *et al.,* 2014). The microbial utilization of hydrocarbon depends on the chemical nature of thecompounds within the petroleum mixture and on environmental determinants (Adeline *et al.,* 2009). Extensive hydrocarbon exploration often result in the pollution of the environment which could lead to disastrous consequences for the biotic and abiotic components of the ecosystem if notresolved. Remediation of hydrocarbon contaminated system could be achieved by either physicochemical or biological methods (Jyothi *et al.,* 2012). Microorganisms are equipped with metabolic machinery to use petroleum products as a carbon and energy source (Udochukwu *et al.,* 2014). Fungi can achieve the degradation of organic compounds by the production of extracellular and intracellular enzymes that catalyze various reactions in the degradation (Paszczynski and Crawford 2000). Bio-remediation processes rely on the ability of microorganism present naturally which are highly efficient due to their simplicity and cost effectiveness when compared to other technologies (Jyothi *et al.,* 2012). It is important to know the microorganisms around the root zone of plants that show phytoremediation potential and also be able to determine the extent to which these organisms can degrade a particular contaminants. This will help in determining exactly what organisms should be used for bio-augmentation of the

plants to enhance optimal removal of contaminants (Adongbede and Majekodunmi, 2016). This research focuses on the biodegradation of diesel fuel using *Talaromyces flavus*.

MATERIALS AND METHODS

SAMPLE

The plant (*Helianthus annuus*) was collected from the University of Ibadan, Oyo state, located on latitude 7° 23' 28.19" N and longitude 3° 54' 59.99" E. The soil was collected around the plant rhizospere of *Helianthus annuus* from the same location as the plant. Petroleum hydrocarbon (Diesel) was collected from NNPC (Ogun State, Abeokuta).

MICROORGANISM

Serial dilution was used for this experiment. One Gram (1g) of soil was added into a sterile test tube containing 10ml of distilled water, soil and water was mixed thoroughly by shaking the water-soil mixture vigorously for 3minutes. One ml was drawn from the solution using a sterile pipette and transferred to the second water blank and mixed as above. This method was done the same way to give 10^{-2} 10^{-3} 10^{-4} and 10^{-5} dilutions. 0.1ml from 10^{-2} 10^{-3} 10^{-4} and 10^{-5} dilutions was pipette into separate petri-dishes containing the Malt Extract Agar (MEA). MEA was prepared according toGalloway and Burgess (1952) description. The plates were then incubated at room temperature (28^0C-31^0C) in an incubator and observed daily for fungal growth.
The fungus was identified based on its morphological study, that is, 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.

Each test tube was sterilized and plugged with non-absorbent cotton wool which was wrapped with aluminum foil to prevent cross contamination and then arranged on test tube rack. Each test tube contained 10ml of the enrichment medium (MSM). 2ml of diesel was added into test tube containing MSM and then inoculated with *Talaromyces flavus*. *Talaromyces flavus* was again inoculated into test tube containing MSMonly, that is, not containing diesel to serve as control.2ml of diesel was then added into test tube containing MSM without inoculating 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.

GAS CHROMATOGRAPHY

After 30days, the extent of Petroleum hydrocarbon degraded by *Talaromyces flavus* 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 220°C, initial and final oven temperature70-200°C. The detector type was flame ionized with temperature at 250°C. 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

The microorganism isolated from the rhizosphere of *Helianthus annuus* was identified to be *Talaromyces flavus. T. flavus* showed degradation of diesel fuel to its lowest possible fractions of carbon at different rates after 30days of incubation. Degradation is measured by the optical density at 620nm wavelengths and Gas Chromatography analysis

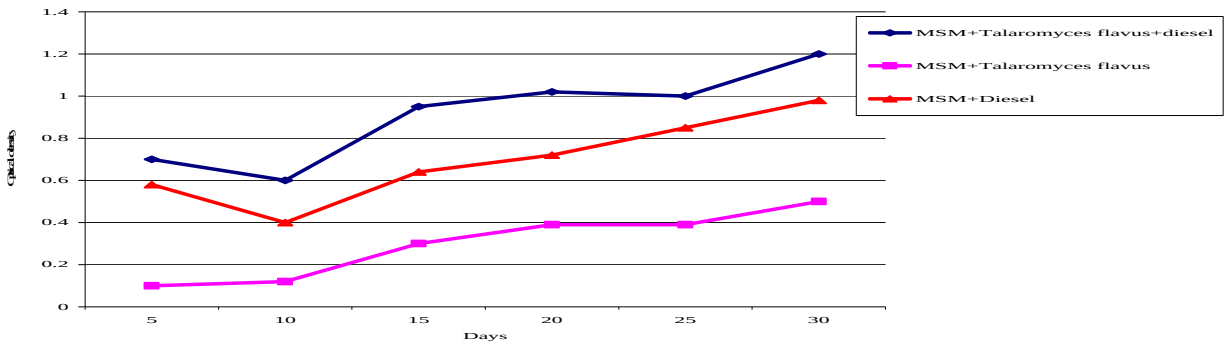

Fig 1: Growth Pattern of *Talaromyces flavus* In Diesel at 620nm

During the biodegradation study, optical density was used to monitor the growth pattern of the fungus in the enrichment medium (MSM) and it was observed that degradation rate by the fungus differed from day 5 to day 30. *T. flavus* had its highest degrading rate on the 30th day of incubation with an optical density of 1.21 and its least degrading rate was observed on the 10th

day with a density of 0.60. It was observed in this study that mycelium of the fungus grew much faster in test tube containing *Talaromyces flavus*, enrichment medium and diesel than in the control (enrichment medium and *Talaromyces flavus*), showing that the environment is quite suitable for their growth and degradation process.

GAS CHROMATOGRAPHY

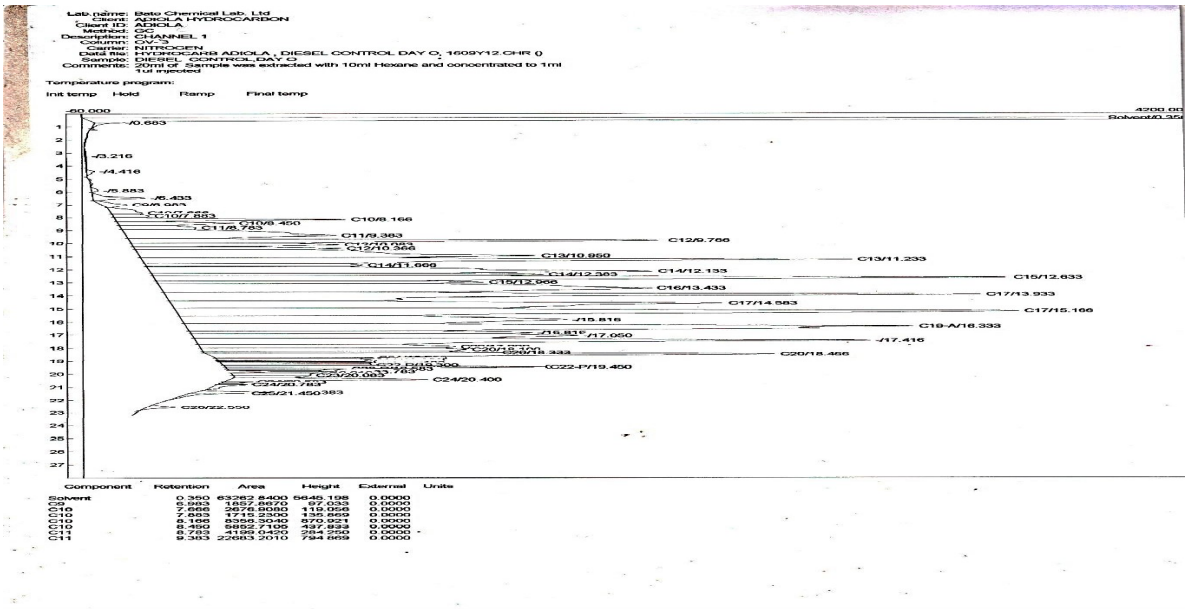

FIG2a: Chromatogram of minimal salt solution with Diesel fuel (Day 0)

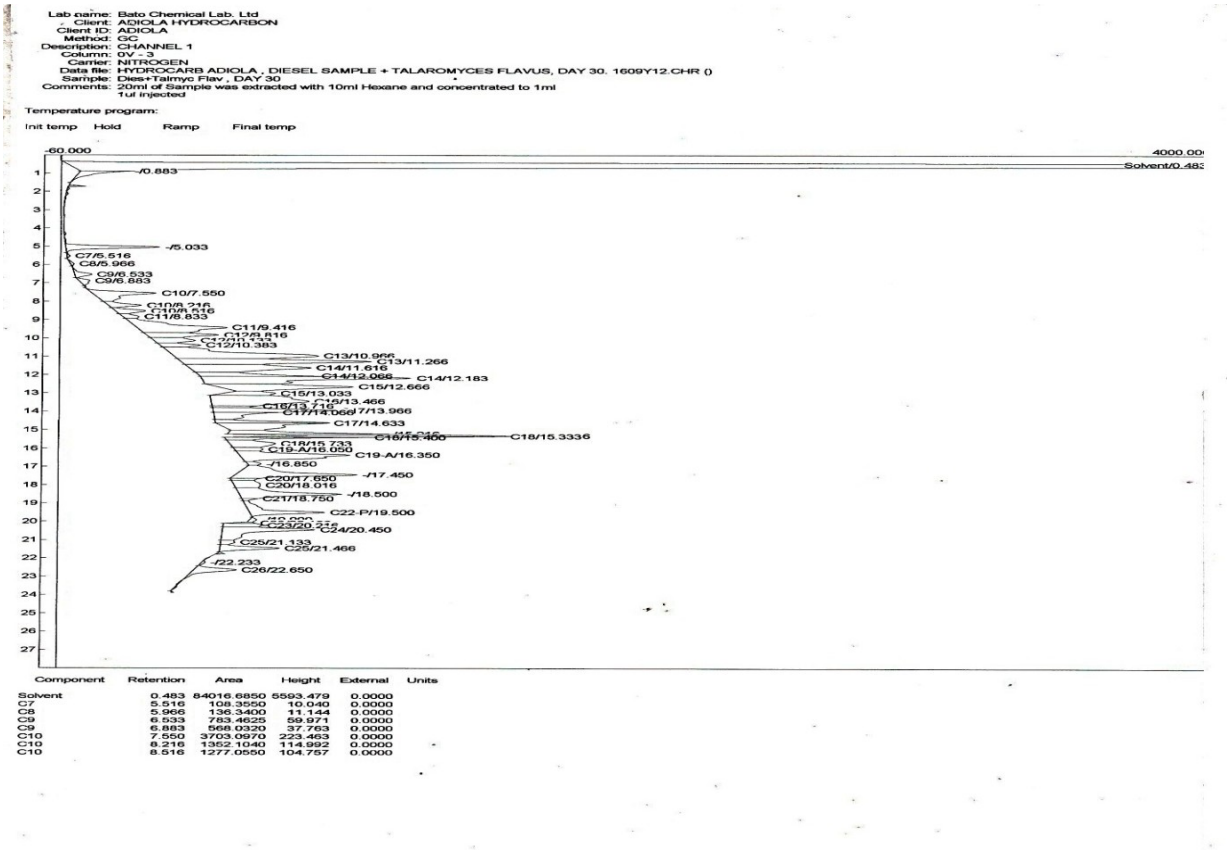

FIG 2b: Chromatogram of minimal salt solution, *Talaromyces flavus* with diesel fuel (Day 30)

In the chromatogram for enrichment medium and diesel fuel (DAY 0), which was the control and prepared on the day of Gas chromatography analysis, carbon 9-26 were detected at various peaks. The chromatogram for *Taloromyces flavus*, enrichment medium and diesel fuel (DAY 30) showed that carbons (C_7 and C_8) have been metabolized. Two chains of C_{16} appeared in the control and only a chain was found in the sample after 30days of incubation. C_{17} also had two chains which had been degraded to a single chain of C_{17}. The 6 chains of C_{21} appearing in the control had been degraded to a single chain of C_{21}. C_{24} had been degraded from 4 chains to a single chain of carbon after 30days of incubation. The gas chromatography of the biodegradation experiment after 30days of incubation shows reduction in concentration of residual hydrocarbon present in the media.

Table 1: Percentage degradation of Diesel fuel by *Talaromyces flavus*

PETROLEUM PRODUCT/ORGANISM	Values
CONTROL (DAY 0)	3399.98mg/L
MSM+*Talaromycesflavus*+diesel (DAY30)	654.13mg/L
PERCENTAGE	80.76%

The total concentration of diesel present in the control was 3399.98mg/L while the residual diesel after 30days of incubation was 654.13mg/L. The Gas chromatography analysis evidently showed that *Talaromyces flavus* degraded 80.76% of diesel.
The percentage degradation of samples was calculated as follow;

$$\text{Concentration of sample} = \frac{\text{Peak area of sample} \times \text{Concentration of the standard}}{\text{Standard peak area}}$$

NOTE: The result divided by 10 (Dilution Factor)

Standard concentration= 10,000mg\L

Standard peak area= 210660.85

DISCUSSION

After looking at the list of fungi that can degrade different PAHs, one could imagine that there is a fungus out there to degrade every type of persistent pollutant, and each one only has to be found (Brito *et al.*, 2006). Molds belonging to the genera *Aspergillus, Penicillium, Fusarium, Amorphoteca, Neosartorya, Paecilomyces, Talaromyces, Graphium* and the yeasts *Candida, Yarrowia*and *Pichia* have been used in hydrocarbon degradation (Chaillan*et al.*, 2004).The results obtained from this research work evidently shows that *Talaromyces flavus* was able to degrade diesel fuel to the lowest possible fraction. Singh and Lin (2009) reported that the significant enhancement of diesel degradation was observed soon after the supplementation of fertilizers in soil. Fungi and bacterial are fast growing organisms and hence degradation or breakdown of compounds by them is faster. Fungi are peculiar because they secrete extracellular enzymes and breakdown compounds externally and most often indiscriminately with recorded success in mineralizing some of the largest molecules on earth (Adongbede and Majekodunmi, 2016). The supplementation of fertilizer stimulates diesel degradation rate in contaminated soil.

The enrichment medium used in this research served as a source of nutrient in which the fungus was able to grow on and breakdown the carbon atoms of diesel fuel. Mycelia growth of *Talaromyces flavus* was observed in the test tubes containing diesel fuel and the enrichment medium. This indicates that *Talaromyces flavus* was able to utilize the diesel as its carbon source. The reason for the increased growth of the organism may be due to the presence of nitrogen, phosphorus and potassium in the enrichment medium which is necessary for biodegradative activity. Nutrients are very important ingredients for successful biodegradation of hydrocarbon pollutants, especially nitrogen, phosphorus and in some cases iron (Cooney, 1988). Hence the additions of nutrients are necessary to enhance the biodegradation of oil pollutants (Choi *et al.*, 2002; Kim *et al.*, 2005). Optical density (or turbidity) has been used to predict concentration and mass of bacterial suspension (Koch 1970), fungal growth in nutrient broth (Wells and Spalding 1975). Optical density measured at 620nm was used to monitor the growth rate of *Talaromyces flavus* in diesel fuel and this is clearly represented in Fig 1. The measure of degradation or utilization of diesel was indicated by turbidity in color which was equivalent to increase in optical density (Nwinyi *et al.*, 2014). Jyothi (2012) reported that the optical density readings based on the turbidity of MSM broth at regular intervals of 2days shows that *Micrococcus lateus* and *Bacillus megaterium* had the greatest ability to degrade petrol while *Bacillus megaterium* and *Corynebacterium xerosis* showed the greatest ability to degrade diesel.

Kerosene and diesel fuel are mixtures of carbon chains containing 6-16 and 8-21 carbon atoms respectively. The most direct way to measure bioremediation efficacy is to monitor hydrocarbon disappearance rates (Atlas and Bartha 1992). In this study, GC- FID analysis of diesel fuel (control) and residual after 30days of incubation showed that degradation of diesel was significant, with the result clearly showing metabolism of carbon chains (Fig 2). This result showed the content of diesel fuel prepared on the day of gas chromatography analysis to be 3399.50mg/L and the residual diesel fuel after 30days of incubation to be 654.13mg/L. GC-FID showed that *Talaromyces flavus* after 30days of incubation has degraded 80.76% of diesel.

CONCLUSION

Results of research showed that *Talaromyces flavus*was capable of producing enzymes that was able to disintegrate the hydrocarbon chains thereby rendering it harmless. Hence, *Helianthus annuus*can be considered as an alternative in selection of plants to be used for clean-up strategy for the treatment of contaminated soil.

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