Isolation and Identification of Indigenous Hydrocarbon Tolerant Fungi from Soil Contaminated with Biodiesel in Benin City, Nigeria

*[1]Igiebor F.A., [2]Osarumwense J.O., [3]Obinyan, B.O., [4]Okoye, P.C.
*[1,4]*Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, PMB 1154, Benin City, Nigeria*
[2,3]*Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin, PMB 1154, Benin City, Nigeria*

Abstract

Environmental pollution by hydrocarbons is a serious problem all around the world. Scientists have identified various microorganisms that are effective degraders of these hydrocarbons in natural environments. This study was aimed at isolating and characterizing hydrocarbon degrading fungi from soil contaminated with biodiesel effluent. Five types of indigenous fungi were isolated and identified from the contaminated soil: they were Aspergillus flavus, Aspergillus niger, Aspergillus carmari, Mucor spp., Penicillium notatum. Their biodegradability capability was measured by UV spectroscopy at wavelength of 600nm for 14 days under optimal temperatures at 280C. The isolate with best degradation ability was Aspergillus carmari which has the potentials to be used as hydrocarbon degrading fungus in remediating biodiesel effluent contaminated site.

Keywords: *bioremediation, Biodiesel, fungi, effluent, hydrocarbon*

I. INTRODUCTION

Biodiesel which is a mixture of fatty acid methyl esters, have been developed as one of the most accomplished substitute fuel to fossil fuels in view of limited resources of fossil fuels and the environment concerns (Aksoy and Becerik, 1990). Petroleum products which are extensively prevalent all over the planet and their demanding use is powerfully connected to the anthropogeneous expulsion these hydrocarbons into the environment (Winkelmann *et al*., 2009). Biological and chemical processes are frequently used in remediating sites contaminated (Matsumiya and Kubo, 2007). The clean-up process can be achieved with bioremediation, a technique based on the exploit of microorganisms, which turn harmful contaminants into harmless substances such as water, carbon(iv)oxide and biomass.

Microorganisms capable of surviving these polluted environments are those have developed specific enzymatic and physiological responses that allow them to use hydrocarbon as a substrate (Saroj and Keerti, 2013). Fungi have great ability to synthesize moderately unspecified enzymes involved in cellulose and lignin decay that can degrade high molecular weight, complex and more recalcitrant toxic compound which include aromatic structures. It has been reported that single cultures of fungi have been found to be better than mixed cultures. Recently, fungi have been found to be better degraders of hydrocarbons than traditional bioremediation techniques including bacteria (Saroj and Keerti, 2013). Microbial isolates have shown good potentials in biodiesel effluent treatment, biochemical oxygen demand and lipid degradation after 12 days (Kanu *et al*., 2011).

These biodiesel presents advantages, since studies have reveal that biodiesel is more easily biodegraded and harmless than hydrocarbon contaminants (Mariano *et al*., 2008). Furthermore, some of these studies also reveal that biodiesel can support and speed up the biodegradation of diesel by means of co-metabolism (Mariano *et al*., 2008).

This study is aimed at isolating and characterizing fungi capable of degrading biodiesel effluent in a contaminated site.

II. MATERIALS AND METHODS

A. Materials

The Potato Dextrose Agar, Potato Dextrose Broth, lactophenol, Petri Dishes, Conical flask, Measuring cylinder, wire loop, spirit lamp, test tubes, microscope, autoclave, colony counter, auger, weighing balance, biodiesel effluents and so on.

B. Sample Collection

Soil samples were collected from a site in Ugbowo Campus of the University of Benin in Ovia North-East Local government of Edo State, Nigeria with an auger from the biodiesel effluent contaminated site measuring 5m × 5m. They were

collected in sterile plastic containers and taken to the laboratory for analysis.

C. *Isolation of Fungi from Soil Sample*

Fungi species were isolated from the collected soil samples by serial dilution and agar plating method wherein the soil sample was diluted from 10^{-1} to 10-3 dilutions and the diluted soil samples was inoculated into a sterile Petri dish before pouring Potato Dextrose agar. The inoculated plates were incubated at room temperature for 72hours fungi. Mixed cultures obtained after incubation were labeled accordingly and were purified by streaking on sterile potato dextrose plates

D. *Staining and Biochemical Activities of Purified Cultures*

Fungal isolates were identified by cultural and microscopic features (Richa et al., 2013). To identify isolated fungi, firstly morphological studies, i.e. examination of the size, shape, colour, spore formation and the number of days taken for the fungus to reach maximum diameter of the petri plate and texture of the fungal growth were observed. After 3-5 days of growth of the fungi, the spore bearing mycelia were then carefully sectioned, teased out and stained on a slide using lactophenol cotton blue stain and then observed with a light microscope.

E. *Biodegradation and Growth Studies*

Growth and degradation studies over a time course were carried out using biodiesel effluent. In this study, fungi were inoculated into 10ml of Mineral Salt Medium (MSM) containing 10ml biodiesel effluent and 5ml of the innoculum. While, for control preparation, 10 ml of biodiesel effluent (biodiesel effluent was added into 10ml MSM without inoculum. After that, the culture was incubated at 30°C for 14 days. At 24 hours interval during the incubation, microbial growth in culture tubes was determined spectrophotometrically by measuring absorbance at wavelength 600nm with UV-visible spectrophotometer.

III. RESULT AND DISCUSSION

A. *Result*

Table 1: Total Fungi Count

Sample	Total Fungi count ($\times 10^3$)
	28^0C for 72hours
1	1.0×10^3
2	3.0×10^3

Table 2: Fungi Identification

Isolates	Colour	Hyphae	Spore	Surface	Lactose	Maltose	Sucrose	Suspected fungi
A	Black	Septated	+ve	smooth	-ve	-ve	-ve	*Aspergillus niger*
B	Light green	Septated	+ve	smooth	-ve	-ve	-ve	*Penicillium notatum*
C	Brownish	Non-Septated	+ve	smooth	-ve	-ve	-ve	*Aspergillus carmari*
D	Light green	Septated	+ve	Smooth	-ve	-ve	-ve	*Penicilliun notatum*
E	White	Non-Septated	+ve	Rough	-ve	-ve	-ve	*Mucor spp*
F	Dark green	Septated	+ve	Smooth	-ve	-ve	-ve	*Aspergillus flavus*

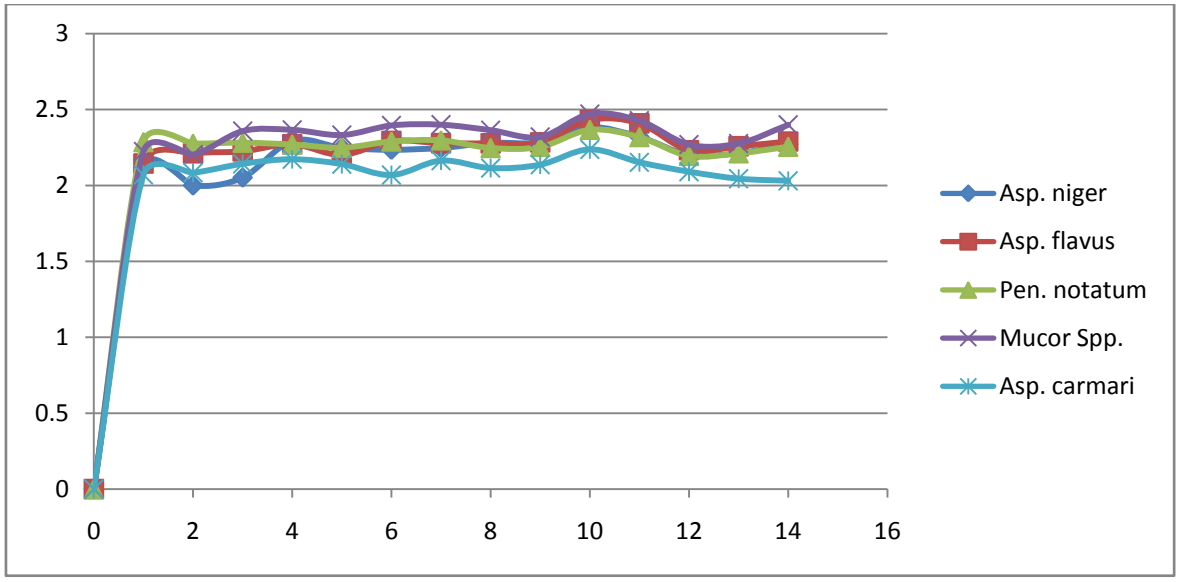

Figure 1: Optical Density at 600nm of Fungi Isolates During Biodegradation of Biodiesel Effluents

B. Discussion

Fungi have distinctive bioremediation properties which have currently being implicated not only physical and chemical treatment, but also biological processes.

The fungi isolated from the biodiesel effluent polluted site were Aspergillus flavus, Aspergillus niger, Aspergillus carmari, Mucor spp. and Penicillium notatum. The growth may be attributed to the enzymes being stable and optimally metabolically active at 28^0C. There was no significant difference in the growth of the fungal isolates at the various temperatures they were exposed to. The results of this study indicate that as shown in Figure 1, the cell density was relatively low on the first day, second and third day then increased markedly from the fourth day to the seventh day. In the following three days, the cell density remains relatively constant and decreased in the ninth day. These results were consistent with those of other studies (Shah et al., 2008).

Previous works related to the biodegradation of biodiesel mainly focused on the water contamination (Zhang et al., 1998; Makareviciene and Janulis, 2003; Pasqualino et al., 2006) with the exception of the work by Lapinskiené et al. (2006), which evaluated the microbial transformation of these compounds in soil where it demonstrated that biodiesel are more easily and faster biodegraded.

Zhang et al. (1998) further explained that biodiesel is more easily metabolized because it consist of pure fatty acids that are hydrocarbon chains with two oxygen atoms attached at one end which are biologically active, being recognized and attacked immediately by enzymes such as acetyl-CoA dehydrogenase. The biodegradation of diesel, which consists of a large amount of alkanes without oxygen attached, demands adapted microorganisms able to produce enzymes that recognise these molecules.

Pasqualino et al. (2006) confirmed that biodiesel can also promote the biodegradation of hydrocarbons by means of co-metabolism, whereby microorganisms use a second substrate which is readily degradable as source of carbon (energy) to breakdown the substrate which is scarcely attacked by the microorganisms when it is the sole carbon source. Researchers have demonstrated that in some cases biodiesel can be applied in contaminated areas as an enhancement agent to bioremediation processes (Mudge and Pereira, 1999; Taylor and Jones, 2001; Obbard et al., 2004 and FernándezÁlvarez et al., 2006, 2007).

IV. CONCLUSION

This study demonstrated that, hydrocarbon degrading organism could be isolated from hydrocarbon polluted area and Aspergillus carmari was found to be the highest performance among the fungi isolated. This could be suggested that, Aspergillus carmari has a potential to be used as hydrocarbon degrading organism in bioremediation for hydrocarbon contaminated areas.

ACKNOWLEDGEMENT

We are thankful to the God Almighty for his unending grace and being the source of our inspiration.

We are also thankful to the following technologist; Mr. B. Enebeli, (Plant Biology and Biotechnology) and Mr. John (Microbiology) for their kind support throughout the research work.

REFERENCES

[1] Aksoy, H. and Becerik,Y. (1990). Utilization prospects of Turkish raisin seed oil as an alternative engine fuel. Fuel 69: 600–603.

[2] Fernández-Álvarez P, Vila J, Garrido-Fernández JM, Grifoll M, Lema JM (2006). Trials of bioremediation on a beach affected by the heavy oil spill of the Prestige. J. Hazard. Mater., 137: 1523–1531.

[3] Fernández-Álvarez P, Vila J, Garrido-Fernández JM, Grifoll M, Lema JM (2007). Evaluation of biodiesel as bioremediation agent for the treatment of the shore affected by the heavy oil spill of the Prestige. J. Hazard. Mater. 147(3): 914-922.
[4] Kanu, I. and Achi, O. K. (2011). Industrial Effluents and Their impact on Water Quality of Receiving River in Nigeria. Journal of applied technology in Environmental al Sanitation 1(1): 75–86.
[5] Lapinskiené A, Martinkus P, Rebzdaite V (2006). Eco-toxicological studies of diesel and biodiesel fuels in aerated soil. Environ. Poll. 142: 432-437.
[6] Makareviciene V, Janulis P (2003). Environmental effect of rapeseed oil ethyl ester. Renewable Energy 28: 2395–2403.
[7] Mariano, A. P., Tomasella, R. C., Oliveira, L. M., Contiero, J. and Angelis, D. F. (2008).Biodegradability of diesel and biodiesel blends. African Journal of Biotechnology 7 (9): 1323-1328.
[8] Obbard JP, NG, KL, XU, R (2004). Bioremediation of petroleum contaminated beach sediments: use of crude palm oil and fatty acids to enhance indigenous biodegradation. Water, Air Soil Pollut. 157: 149- 161.
[9] Pasqualino JC, Montané D, Salvadó J (2006). Synergic effects of biodiesel in the biodegradability of fossil-derived fuezls. Biomass Bioenergy 30: 874-879.
[10] Richa, S., Chandra, S. and Singh, A. (2013). Isolation of microorganism from soil contaminated with degraded paper in Jharna village. Journal of Soil Science and Environmental Management 4(2): 23-27.
[11] Saroj, A. and Keerti, D. (2013). Isolation and characterization of hydrocarbon degrading microorganisms from petroleum oil contaminated soil sites. Bulletin of Environmental and Scientific Research 2 (4): 5-10
[12] Taylor LT, Jones DM (2001). Bioremediation of coal tar PAH in soils using biodiesel. Chemosphere 44: 1131-1136.
[13] Winkelmann, M., Hunger, N., Hüttl, R. and Wolf, G. (2009). Calorimetric investigations on the degradation of water insoluble hydrocarbons by the bacterium Rhodococcus opacus 1CP. Thermochimica Acta. 482:12–16.
[14] Zhang X, Peterson CL, Reece D, Haws R, Moller G (1998). Biodegradability of biodiesel in the aquatic environment. Trans. ASAE. 41: 1423-1430.