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***Research article***

# BIODEGRADATION OF PETROLEUM BY FUNGI ISOLATED FROM UNPOLLUTED TROPICAL SOIL

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**ABSTRACT:** Bioremediation studies on the capabilities of *Aspergillus niger* A1, *Candida sp* C10 and *Rhizopus stolonifer* R7 isolated from unpolluted soil in Minna, Niger State Nigeria was investigated and rate of degradation measured by weight loss. *Aspergillus niger* A1, exhibited the highest ability in degrading the crude oil than *Candida sp* C10, and *Rhizopus stolonifer* R7. *Aspergillus niger* A1 degraded 53.7% of the crude oil after 16 days period of incubation while *Candida sp* C10 and *Rhizopus stolonifer* R7 degraded 45.0% and 35.0% respectively over the same period of incubation.. The result obtained demonstrated that the three fungi isolates are competent petroleum degrading organisms and may be used as best approaches to restoring oil contaminated environments through bioremediation process.

**Keywords**: Pollution, Crude oil, Spillage, Bioremediation, Fungi.

## INTRODUCTION

Petroleum hydrocarbons are widely used in our daily lives as fuel and chemical compounds. As a result of this massive use, petroleum has become one of the most common contaminants of large soil surfaces and eventually is considered as a major environmental problem (Sancartier et al., 2009). There are several ways in which hydrocarbons are degraded in the environment. One mechanism through which they can be removed from the environment is bioremediation. Bioremediation can be defined as the use of soil microbes to degrade pollutants into harmless substances (Collins, 2001). Bioremediation involves either stimulating indigenous microbial populations by environmental modifications (biostimulation), or introducing exogenous microbial populations known to be efficient degraders into a contaminated site, a process known as bioaugmentation (Bento et al., 2005). Numerous microorganisms are known for their ability to degrade hydrocarbons. The biodegradation capabilities of bacteria have been recognised but fungi have been the subject of recent research (Chaillan et al., 2004, Romero et al 2002, Garon et al., 2000; Santos and Linardi, 2004, Potin et al., 2004), due to their ability to synthesize relatively unspecific enzymes involved in cellulose and lignin degradation, which are capable of degrading high molecular weight, complex or more recalcitrant compounds, including aromatic structures.

Unfortunately however, the full potential of biodegradation by filamentous fungi for bioremediation purposes has not been fully investigated. The use of filamentous fungi isolated from contaminated soil may offer advantages for several reasons. Owing to their ability to extend through the soil by hyphal elongation, fungi can access xenobiotics. In addition, fungi are capable of growing under stressful environmental conditions, such as environments with low pH or low water activity (Potin et al., 2004). The Niger-Delta area is the centre of petroleum production and processing activities in Nigeria. The Niger-Delta is also known as one of the largest wet lands, encompassing over 20000km$^2$ in Southern Nigeria (World Bank, 1995).

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Despite the tremendous natural and human resources base, the region's potential for sustainable development remains unfulfilled and its failure is being threatened by diverse environmental problems of which oil pollution is most paramount (World Bank, 1995). The aim of this study is to investigate the relative capabilities of fungi isolates from unpolluted tropical soil in degrading a Nigerian crude oil.

## MATERIALS AND METHODS

### Collection of Samples

Soil samples used were collected at a non-crude-oil-polluted site at a depth of 10-15cm in various locations in Minna, Niger State for the isolation of fungi while the crude oil sample was collected from the Kaduna Refinery and Petrochemical Company, Kaduna, Nigeria.

### Microbial Analysis

### Isolation of Fungi

Enrichment method was used in the isolation of fungi by preparing oil broth (1.2g $K_2HPO_4$, 1.8g $K_2PO_4$, 4.0g $NH_4Cl$, 0.2g $MgSO_4.7H_2O$, 0.1g NaCl, 0.01g $FeSO_4.7H_2O$ in 1000ml distilled water, pH 7.4 and 1 percent of crude oil. The medium was sterilized at 121°C for 15 minutes and cooled before 2g of soil was inoculated and incubated while shaking using an orbital shaker for seven days at room temperature (30°C). Then Oil Agar (oil broth plus 20g agar plus 500mg Chloramphenicol) was inoculated and incubated for 7 days at room temperature (Ijah, 1998). The isolates were purified by repeated culturing in Sabouraud Dextrose Agar (SDA).

### Characterization and Identification of Isolates

The mould isolates were characterized using cultural characteristics such as the type of hyphae, colour of aerial and substrate hyphae, shape and kind of spore, presence of foot cell, sporangiophore or conidiophores and the characteristics of spore head using microscopic and macroscopic methods. The identities of the mould isolates were confirmed by comparing their characteristics with those of known taxa as described by Domsch and Gams (1970).

### Screening of Fungal Isolates for Ability to Utilize Petroleum

The screening of fungi isolates for ability to utilize petroleum was done by growing the fungi isolates in potato dextrose broth for 3-5 days before they were transferred to oil broth and incubated at room temperature without shaking. The growth of the isolates was monitored in the medium by visual observation. At the end of 14 days, the oil was extracted using diethyl ether to know the extent of oil degradation (Ijah and Ukpe, 1992) and efficient oil degraders were thus selected for further studies.

### Determination of Rates and Total Extent of Petroleum Degradation by Mould Isolates

The fungi isolates were grown in 5mls of potato dextrose broth in test tubes for five days. After the five days of incubation, the fungi were introduced into 5mls of sterile oil broth in test tubes and incubated at room temperature. This was done in duplicates for each of the fungal isolates. Negative controls had no fungal isolates. Every 4 days, residual crude oil was extracted using diethyl ether. The diethyl ether was allowed to evaporate overnight, after which the weight of the residual oil was determined. Percentage biodegradation of the crude oil was calculated as stated by Ijah and Ukpe (1992) thus: % biodegradation = weight of crude oil(control) minus weight of crude oil(dgraded) /weight of crude oil (control) x 100.

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## RESULTS

### Identification of Fungi Isolates and Frequency of Occurrence

Fourteen fungi isolates were identified as species of *Mucor, Rhizopus, Aspergilus, Penicillium* and *Candida.* *Aspergillus* were more consistently isolated and constituted 42.9% of the total isolates followed by *Rhizopus* (21.4%). *Penicillium* and *Candida* had 14.2% frequency of occurrence each while *Mucor* constituted 7.1% of the total isolates.

**Table 1 Frequency of occurrence of isolates**

| Fungi | Number of Isolates | Percentage Frequency |
|---|---|---|
| *Aspergillus* | 6 | 42.9 |
| *Rhizopus* | 3 | 21.4 |
| *Candida* | 2 | 14.3 |
| *Penicillium* | 2 | 14.3 |
| *Mucor* | 1 | 7.1 |
| Total | 14 | 100% |

*****% Frequency of occurrence = number of isolates x 100 /total number of isolates

Figure 1 shows the results of the weight losses of crude oil resulting from the growth of *Aspergillus niger*A1, *Rhizopus stoloniferR7* and *Candida sp*C10. Of the three organisms investigated in this study, *Aspergillus niger*A1 caused the highest level of crude oil degradation (53.7%) as compared to 45.0% and 35.0% degradation caused by *Candida sp*C10 and *Rhizopus stoloniferR7* respectively after 16 days.

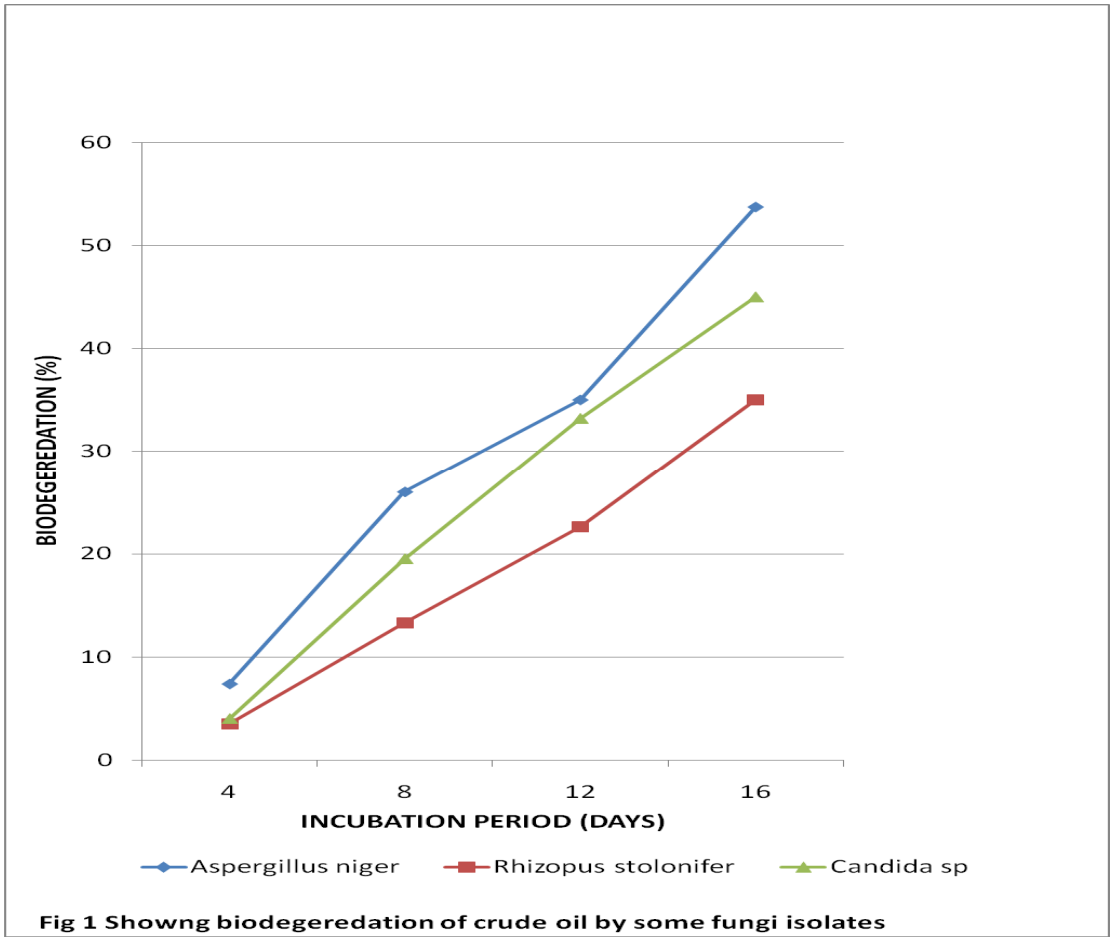


**Fig 1 Showng biodegeredation of crude oil by some fungi isolates**

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## Utilization of Petroleum by Fungi Isolates

Table 2 shows the extent of utilization of petroleum by the fungi isolates after 14 days. It was observed that nine (64.2%) out of the fourteen isolates were able to utilize the crude oil at varying rates. Three isolates (*Aspergilus niger*, 35.0% - 53.7%) utilized the oil at considerably high rate. Four isolates utilized at moderate rates (*Candida spp*, 20.5-30.5%) while two isolates utilized the oil at minimal rate (*Rhizopus stolonifer*, 10.2-15.5%).

**Table 2 Utilization of crude oil by fungi isolate**

| Oil utilization after 14 days | | |
|---|---|---|
| Coded fungi isolate | Growth in oil medium | Extent of utilization (%) |
| *Aspergillus niger* (A1) | ++ + | 40.5 |
| *Aspergillus niger* (A2) | - | - |
| *Aspergillus niger* (A3) | ++ | 25.7 |
| *Aspergillus niger* (A4) | ++ | 21.8 |
| *Aspergillus niger* (A5) | ++ | 22.6 |
| *Aspergillus niger* (A6) | - | - |
| *Rhizopus* (R7) | +++ | 32.4 |
| *Rhizopus* (R8) | - | - |
| *Rhizopus* (R9) | - | - |
| *Candida sp* (C10) | +++ | 38.5 |
| *Candida sp* (C11) | - | - |
| *Penicillium* sp (P12) | + | 10.2 |
| *Penicillium sp* (P13) | + | 10.2 |
| *Mucor sp* (M14) | + | 15.5 |

+++: maximum growth, ++: moderate growth, +: minimum growth, - : no growth

## DISCUSSION

*Aspergillus niger* A1, *Candida sp*C10 and *Rhizopus stolonifer*R7 found to degrade crude oil in this study were isolated from soil with no history of oil pollution. This finding agrees with the previous report of Ijah (1998) that crude oil degrading microorganisms are widely distributed in the Nigerian environment irrespective of whether crude oil is present in the environment or not. The efficient crude oil degrading ability of the organisms may be due to the presence of a genetic code for an inductive degradative enzyme in the organisms.

Of the three fungi species tested for their ability to degrade crude oil, *Aspergillus niger*A1 had the highest degradation of 53.7% while *Candida sp*C10 and *Rhizopus stolonifer*R7 had about 45% and 35% respectively. The high degradation ability of *Aspergillus niger*A1 may be due to its outstanding mycelial growth and also due to the higher production of extra-cellular enzymes and organic acids that enable the organism to utilize the hydrocarbon faster. This agree with the findings of Stamets (1999), that mycelial mats are used for bioremediation because they produce extra-cellular enzymes and acids that break and dismantle the long chains of hydrocarbon, the base structure common to oils, petroleum products and many other pollutants.

## CONCLUSION

The present study has revealed that *Aspergillus niger*A1 isolated from crude oil-free soil is capable of extensive degradation of crude oil. The fact that these organisms could degrade crude oil efficiently point to their potential usefulness in clearing oil spills in tropical soils.

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