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Crude Oil Degradation by Penicilium and Mucor Species Isolated From Fresh Water Swamp

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ABSTRACT

Two genera of fungi -Penicillium and Mucor species were screened for petroleum hydrocarbon degradation capability by subjecting them to a three-week oil degradation test in a mildly agitated mineral salts liquid medium. Growth was measured by visual estimation of mycelial tissue, while percent residual oil and pH were measured with spectrophotometer and pH meter respectively. Growth of Mucor species was profuse while Penicilium had sparse growth in the culture medium, giving a mean degradation rate (%) of 55.3 + 10.6 and 45.0 + 11.8 for Mucor and Penicilium species respectively, with an accompanying increase in pH of growth medium. Analysis of variance however indicated lack of significant difference between both fungal genera in the rate of biodegradation on one hand ((F (2, 6=0.27), p>0.05) and changes in pH of growth media on the other hand (F (2, 6 = 2.26), p>0.05). Thus, Mucor species may be a suitable candidate for preparation of probiotics for stimulating the biodegradation of oil in vulnerable environments.

Keywords: *Biodegradation, Penicilium, Mucor, Hydrocarbon, pH;*

INTRODUCTION

The prevalence of oil pollution in the world environment as a result of oil exploration and production activities brought about the need to study and find ways of safely returning the environment to status quo ante. Studies have shown that the major mechanism for removing spilled oil from the environment is by microbial degradation (Atlas and Philp, 2005; Mance a – Lopez et al, 2007; Das and Chandran, 2010). Okerentugba and Ezeronye (2003) observed that single cultures are better oil degraders than mixed cultures although most researchers advocate the use of mixed cultures of microorganisms either bacteria or fungi for bioremediation of crude oil-contaminated soils (Kaczorek and Olszanowski, 2011). Adebusoye et al (2007) and Al-Wasify and Hamed (2014) showed independently that degradation of oil was doubled when bacteria mould and yeasts were present than when bacteria alone was present.

Thus, in the use of microorganisms for cleaning an oil spill, the use of mixed culture will prove most useful, and not in the use of various species of bacteria alone but in the use of bacterial and fungal cultures due to variation in enzyme capabilities.

It has also been argued that bacterial cultures are better oil degraders than fungal cultures. Bacteria and fungi appear to have similar patterns of hydrocarbon degradation. However, in their ability to decompose organic residues, fungi are the most versatile and perhaps the most persistent of any group since they continue to decompose complex organic material after bacteria and actinomycetes have essentially ceased to function. They are especially active in acid forest soil, but they play a significant role in all soils. Venkatesagowda *et al* (2012) considered fungi to have advantages over bacteria because of their hyphae and potential hydrolytic enzymes, which can penetrate and degrade the hydrocarbons contaminated soil. Ability of fungi to degrade polycyclic aromatic hydrocarbons has also been reported by Juhasz and Naidu (2000), Ceinglia and Sutherland (2010) and Venkatesagowda *et al* (2012) and Balaji *et al* (2014). Okerentugba and Ezeronye (2003) reported the ability of three fungi such as *Penicilium* sp, *Aspergillus* sp and *Rhizopus* sp to degrade petroleum hydrocarbon which also served as sole carbon and energy sources. Furthermore, Al-Jawhari (2014) reported that the highest percentage loss of petroleum hydrocarbon by the mixed cultures of fungi were 90% with *Aspergillus niger* and *A.*

fumigatus, but the lowest loss was calculated in mixed four fungal strains (*A. niger*, *A. fumigatus*, *P. funiculosum* and *F. solani*) as 70%.

While fungi have been demonstrated to have petroleum hydrocarbon degradation potentials, the question of effectiveness or suitability of the genera and species for enhanced oil degradation for the biorestoration of a polluted environment has not been addressed. If seeding to clean oil spill in an environment is to be effective, then the use of organism with proven degradation capability is a *sine qua non*. To obtain information on the degradation efficiency of two soil mould isolates to be employed in seeding oil polluted terrestrial ecosystems in Nigeria, laboratory trials were initiated. This paper presents the results from laboratory experiments which were aimed at determining the effectiveness of two mold isolates to degrade petroleum hydrocarbon.

Materials and Methods

Soil Sampling and Isolation of *Penicillium* and *Mucor* Species

Fungi genera used in this study were isolated from fresh water swamp soil in Obrikom, Rivers State, Nigeria (Latitude: 5° 23' 41.4" (5.3948°) north, Longitude: 6° 40' 6.7" (6.6685°) east, Elevation: 22 meters), characterized by colonial appearance, micromorphology and carbohydrate fermentation patterns (Olds, 1983) and identified as *Penicilium* sp and *Mucor* species (Dirisu, 2015). They were maintained in potato dextrose agar slant (PDA) and frozen and used for biodegradation study.

Crude Oil Degradation by Isolates

The fungal isolates were tested for comparative petroleum hydrocarbon degradation using the modified mineral salts medium (mineral medium c) of Mills *et al* (1978). The medium was prepared and dispensed in 99 ml quantities into three 250ml Erlenmeyer flasks loosely covered with cotton wool and sterilized by autoclaving at 121^0C for 15 minutes and cooled. To each of these flasks was added 1ml of filter sterilized Bonny light crude oil. Each of two flasks was inoculated with 24 hr old culture of *Penicillium* or *Mucor* species in Ringers solution obtained from freshly sub-cultured pure cultures. The third flasks was not inoculated and served as control. The flasks were incubated at room temperature on a rotary shaker operated at 60 rpm for 21 days. The optical density (OD) at 560 nm, and pH of the culture in each flask were monitored at weekly intervals.

Analytical Method for Oil Degraded

The quantity of oil in the flasks was measured by toluene extraction (Odu *et al*., 1989). At weekly intervals 10 ml of the content of each flask was mixed with 10 ml of toluene and agitated for 5 minutes in a conical flask. After standing for 20 minutes, the supernatant was decanted into a cuvette and measured with Atomic Absorption Spectrophotometer (AAS) (Biotech Engineering, Phoenix 986- UK) at 560 nm. With reference to a standard curve of the absorbance of known concentrations of the petroleum hydrocarbon in the extractant, the quantity of hydrocarbon from the three flasks was determined.

Statistical Analysis

Analysis of variance (ANOVA) was conducted to determine whether there was significant difference in the rate of crude oil degradation by both fungal genera used for the study. Pearson correlation statistic (r) conducted to determine the effect of biodegradation on pH of growth medium at 95% alpha level. All analyses were performed using Microsoft Excel data analysis tool Pak 2007 and SPSS version 18.

Results

Rate of Biodegradation

At the end of the three-weeks incubation, *Penicillium* and *Mucor* species had utilized 55% and 65% of the oil respectively. However, shaking appeared to have an evaporative effect on some components of the petroleum as revealed in the results of the control (Figure 1). Analysis of variance indicate that there was no significant difference between the two fungal species in their rates of biodegradation ($F_{(2, 6=0.27)}$, $p>0.05$). The growth in hydrocarbon using it as source of energy and carbon resulted in the reduction of the residual oil as measured by AAS as shown in Figure 1: There was a drastic decrease in crude oil from initial 100% to 66%, 53% and 45% by the end of week 1, 2 and 3 respectively by *Penicillium species*. On the other hand, *Mucor species* degraded crude oil from an initial 100% to 58%, 42% and 35% by the end of week 1, 2, and 3 respectively. Control set up, which had no fungi seeded only showed a slight drop in from 100% to 92% at the end of three weeks incubation.

Effect of Biodegradation on pH of Medium

Characterization and identification of the *Pencillium* and *Mucor* species was reported in a preliminary study (Dirisu, 2015) and presented in Table 1. The changes in pH of growth medium are shown in Figure 2. The initial pH of medium was 5.5. Utilization *vis-à-vis* degradation of crude oil resulted in a slight increase in pH. Mean pH following degradation for *Penicilium* and *Mucor* are 5.47 $\pm$ 0.003 and 5.53 $\pm$ 0.02 respectively while control had pH of 5.50 $\pm$ 0.6 (figure 2). ANOVA statistic showed lack of significant difference between *Penicilium* and *Mucor* species F (2, 6 = 2.26), p>0.05) with respect of pH effects.

There was significantly negative correlations between biodegradation rates and pH of medium for both fungal genera (r (1, 3= -999), p<0.05) (Table 2). While amount of crude oil reduced (greater degradation), the pH of the medium slightly increased, although still within acidic range.

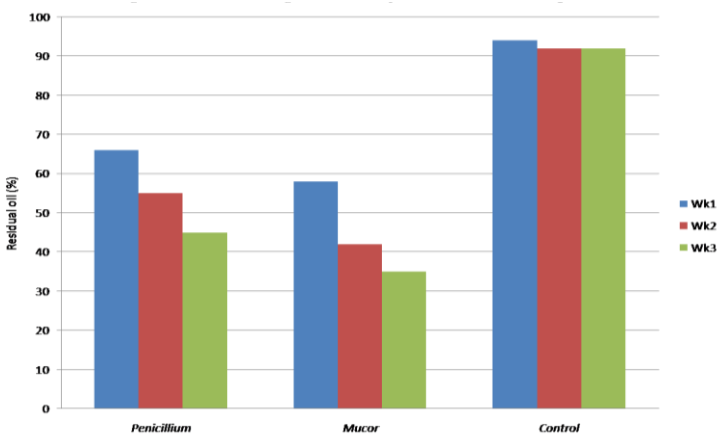

Table1. *Characterization and identification of Isolates*

Test/Morphology	*Penicillium sp*	*Mucor sp.*
Glucose	A/G	A
Fructose	A/G	A/G
Lactose	W/F	W/F
Sucrose	W/F	A
Galactose	A/G	A/G
Maltose	A	A/G
Mannose	W/F	A
Colonial Morphology	Flat concentrate colonies with radial furrows and a grey green powdery surfaces	Conidia are loose cotton wool-like aerial mycelia, grey at first, dark later
Micro-morphology	Short branches of conidiophores bearing sterigmata and produce chains of conidia, general appearances like brush.	No rhizoids, non septate mycelia, dark globose sporangia scattered over the mycelia

Key: A: acid; A/G: acid and gas; W/F: weak fermentation.

Source: *Dirisu (2015)*

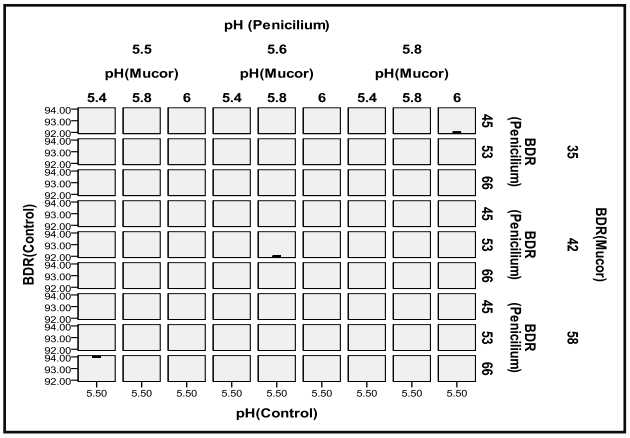

Figure2. *Plot of biodegradation and pH interactions*

Table2. *Correlation matrix for biodegradation rate and change in pH of growth medium Correlations*

		BDR (Penicilium)	pH (Penicilium)	BDR (Mucor)	pH (Mucor)	BDR (Control)	pH (Control)
BDR(Penicilium)	Pearson Correlation	1	-.947	.996	-.999*	.926	.a
	Sig. (2-tailed)		.208	.054	.034	.246	.
	N	3	3	3	3	3	3
pH (Penicilium)	Pearson Correlation	-.947	1	-.916	.929	-.756	.a
	Sig. (2-tailed)	.208		.262	.242	.454	.
	N	3	3	3	3	3	3
BDR(Mucor)	Pearson Correlation	.996	-.916	1	-.999*	.955	.a
	Sig. (2-tailed)	.054	.262		.020	.192	.
	N	3	3	3	3	3	3
pH(Mucor)	Pearson Correlation	-.999*	.929	-.999*	1	-.945	.a
	Sig. (2-tailed)	.034	.242	.020		.212	.
	N	3	3	3	3	3	3
BDR(Control)	Pearson Correlation	.926	-.756	.955	-.945	1	.a
	Sig. (2-tailed)	.246	.454	.192	.212		.
	N	3	3	3	3	3	3
pH(Control)	Pearson Correlation	.a	.a	.a	.a	.a	.a
	Sig. (2-tailed)	.	.	.	.	.	
	N	3	3	3	3	3	3

**. Correlation is significant at the 0.05 level (2-tailed).*

a. Cannot be computed because at least one of the variables is constant.

DISCUSSION

Oil degradation capability of *Penicillium* and *Mucor* species have been demonstrated using mildly agitated liquid culture. Oil degradation was accompanied by growth of the mycelial mat on the surface of the medium. Under the conditions provided, *Mucor* species grew more than the *Penicillium* species and this resulted in a corresponding reduction in the quantity of petroleum in the flasks. The biodegradation study revealed that over 50% of the oil was degraded at the end of the three-week period. *Penicillium* species had degraded 55% while *Mucor* species had degraded 65% of the oil (Figure 1). The oil-degrading abilities of these two fungi have also been previously reported (*Wemedo et al*., 2002; Obire and Anyanwu, 2009). The oil degradation rate reported in this study is also comparable to the reports of 76% rate of biodegradation by *Penicillium chrysogenum (*Farid, 2012) approximately 87% by *Penicilium funniculosum* and *Aspergillus sydowii* (Mance a-Lopez *et al*, 2007). Al-Jahwari (2014) reported a mixed fungal crude oil degradation rate of 95% after 21days of incubation. The mixed fungi included *Aspergillus* species and *Penicillum*, Al-Nasrawi (2012) reported abilities of *Aspergillus niger and P.notatum* to degrade crude oil with *Aspergillus* degrading better. Umanu and Dodo (2013) reported that pure culture of *P. chrysogenum* was the best engine oil degrader, consuming about 43.33% engine oil within 30days when compared to *Aspergillus* and *Candida* species. The greater ability of *Mucor* species to degrade a little more of the petroleum hydrocarbon than *Penicillium* species may require further studies. It was also observed that biodegradation of crude oil is usually accompanied by more growth of fungal cultures. This corroborates with Al-Nasrawi (2012) who reported that rates of fungal growth in the media containing crude oil was more than in media without crude oil. These results point to the fact that these fungi could utilize equally the hydrocarbons (Dollah, 2004; Singh, 2006; Leitao 2009; Dirisu, 2015).

The role that fungi alone play in the breakdown and degradation of petroleum hydrocarbon has not been adequately reported. Mycelial organisms can access hydrocarbon attached to rocks thus increasing the surface area available for bacterial attack. Fungi can grow in

environmentally-stressed conditions such as low pH and poor nutrient status. This initial fungal attack results in preliminary degradation and production of metabolic products which may be conducive to the growth of bacteria.

CONCLUSION

In this study, there was variation in the ability of Mucor and Penicilium species to utilize degrade petroleum hydrocarbon. It was established that Mucor degraded more oils than Penicilium species. The change in pH of growth medium may be attributed to metabolic by-products or intermediates. It does appear that *Mucor* species when grown to high number would be more suitably applied for seeding oil-polluted environments in clean-up operations than *Penicillium* species

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