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Oil Spills Remediation using Native Mushroom-A Viable Option

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ABSTRACT

Oil spillage is a global threat which requires environmental friendly approach in its clean-up. The aim of this study was to assess the possibility of fungi to degrade crude oil in liquid medium. The ability of three indigenous mushroom species, *Pleurotus tuber regium*, *Pleurotus pulmonarius* and *Lentinus squarrosulus* to degrade crude oil in liquid medium was investigated. The mycelia of the mushrooms were used to inoculate a mixture of mineral salt at varying concentration of crude oil (0.5, 1.0, 1.5 and 2.0%) as the sole carbon source in triplicate. After one month of incubation at $28\pm 2^\circ\text{C}$, samples were dispensed and analyzed for residual hydrocarbon. The effect of crude oil on the growth of fungi mycelia in liquid medium was also monitored. Results showed the three indigenous mushroom degraded crude oil at different rates however, the inoculation with mycelia mixture of the three fungi was the most effective in oil degradation and was significantly different ($p = 0.05$) from other treatments. This study demonstrates the utility of three native mushroom species; *Pleurotus tuber regium*, *Pleurotus pulmonarius* and *Lentinus squarrosulus* in management of oil spills.

Key words: Indigenous mushroom, oil spillage, degradation, myco remediation, liquid medium

INTRODUCTION

The Niger Delta region of Nigeria is among the ten most important wetland and marine ecosystems in the world (Kadafa, 2012). The oil industry located within this region has contributed immensely to the growth and development of the country. However, unsustainable oil exploration activities in the Niger Delta region has made the region as one of the five most severely petroleum contaminated ecosystems in the world. Studies have shown that the quantity of oil spilled over 50 years of oil exploration in Nigeria is not less than between 9 and 13 million barrels (FME, 2006).

The livelihoods of people in the Niger Delta region are basically fishing and farming. When incidences of oil spills occur, the fertile farming lands suddenly become barren or near barren. The toxic concentration of petroleum spills causes destruction of organisms such as fish, embryonic shrimps and crabs and their populations are adversely affected (Kadafa, 2012). The consequences of oil spillage are massive pollution of land, streams and rivers.

The main causes of oil spills in the Niger Delta are vandalism, oil blowouts from the flow stations, accidental and deliberate releases and oil tankers at sea (Nwilo and Badejo, 2004, 2005). Screening of fungi for mycoremediation has been carried out by various researchers. Some of the fungi used were *Pleurotus ostreatus* (Eggen and Majcherczyk, 1998), *Trametes versicolor*, *Bjerkandera adusta*, *Pleurotus ostreatus* (Loske *et al.*, 1990; Field *et al.*, 1992), *Pleurotus subnudus*

(Adenipekun and Fasidi, 2005), *Phanerochaete chrysosporium* (Martens *et al.*, 1996), *Pleurotus tuber-regium* (Pt), *Lentinus squarrosulus* (Ls), *Pleurotus pulmonarius* (Pp) (Adedokun and Ataga, 2007, 2008), *Anthracophyllum discolor* (Acevedo *et al.*, 2011). However, reports on use of test fungi as bioremediation tools in liquid medium are limited.

Impact of oil spill spans several generations, adversely affecting environmental, health and socio-economic (livelihoods) parameters. Since these impacts are hazardous on humans and their environment, an environmental friendly control measure is expedient. This study was conducted to investigate the ability of indigenous mushrooms to degrade petroleum products in aquatic medium.

MATERIALS AND METHODS

Method used was modified from Itah and Essien (2005). Briefly, 100 mL of mineral salt was amended with varying concentration of crude oil, as sole carbon source and autoclaved at 121°C for 15 min. After cooling, sets of conical flasks were inoculated with 5 mm diameter mycelia agar disc of 10 days old culture each of *P. pulmonarius*, *P. tuber-regium* and *L. squarrosulus*. The experiment was in triplicate. Incubation was done at room temperature (28±2°C) for one month with observation and manual shaking every 2 day to break the film on the surface.

Sterile medium that did not contain any degrading fungi but only the appropriate concentration of crude oil served as control to see the natural weathering of the crude oil. Samples were withdrawn and analyzed for residual hydrocarbon. The effect of crude oil on the growth of mycelia of white rot fungi in liquid medium was quantified by weighing mycelia biomass.

Mineral salts liquid medium comprised of the basal medium and trace elements in ratio 9:1. 0.1 g streptomycin was added to the homogenized medium to prevent any form of bacterial contamination. The medium was adjusted to pH 4.5 with conc. hydrochloric acid and sterilized at 121°C for 15 min. Basal medium composition in 1 L of distilled water comprised of:

- K_2HPO_4 -1.8 mL, KH_2PO_4 -1.3 mL, NH_4Cl -40 mL, $MgSO_4 \cdot 7H_2O$ -0.2 mL, NaCl-0.1 mL, Yeast extract-0.1 mL, $FeCl_4 \cdot H_2O$ -0.05 mL

Trace element solution composition used were:

- H_3BO_3 -0.1 mL, $ZnSO_4 \cdot 7H_2O$ -0.1 mL
- $CuSO_4 \cdot 5H_2O$ -0.05 mL, $MnSO_4 \cdot H_2O$ -0.04 mL

Extraction of hydrocarbon-residual oil in biodegraded samples: Hundred milliliter biodegradation medium was acidified with 5 mL dilute hydrochloric acid (HCl: Distilled water =1:1 v/v) and poured into a separatory funnel. Each flask with medium and 0.5 g of sodium chloride was added with 10 mL Hexane.

The extraction was carried out by shaking the funnel vigorously and allowing the generated gas to escape by opening the stop-clock intermittently till all gas escaped and the two layers separated. The two layers were allowed to separate further for 20 min. The lower fraction was then extracted by opening the stop clock of the separating funnel whose bottom had been previously plugged with a glass wool. The extract was collected in a McCartney sterile bottle. The solvent was evaporated in a water bath at 80°C to obtain a constant weight. After drying and evaporation the residual crude oil was quantified by gravimetric method (Mukherji *et al.*, 2004).

RESULTS

The amount of oil degraded by test fungi and their combination after a month of incubation are presented in Fig. 1. All three fungi degraded crude oil in all concentrations. At 0.5% concentration, there was no significance difference ($p < 0.05$) in oil degradation between Pp and Ls. This was also observed between Pt and PpPtLs. There was significant difference in oil degradation between the two pairs. At 1% concentration, PpPtLs was significantly different in degradation compared to individual cultures. At 1.5% concentration, oil degradation by PpPtLs and Ls was significantly different from Pp and Pt while degradation by Ls was significantly different PpPtLs. at 2% concentration; PpPtLs was significantly different in degradation compared to individual cultures. In general, as the concentration of crude oil increases, oil degradation by PpPtLs increases, the reverse was the case with Pp and Pt while degradation by Ls varied (Fig. 1). The control set up however, showed no significant oil degradation. Table 1 shows the mycelia weight of fungi in relation to crude oil concentration. As mycelia weight increases, crude oil degradation increases.

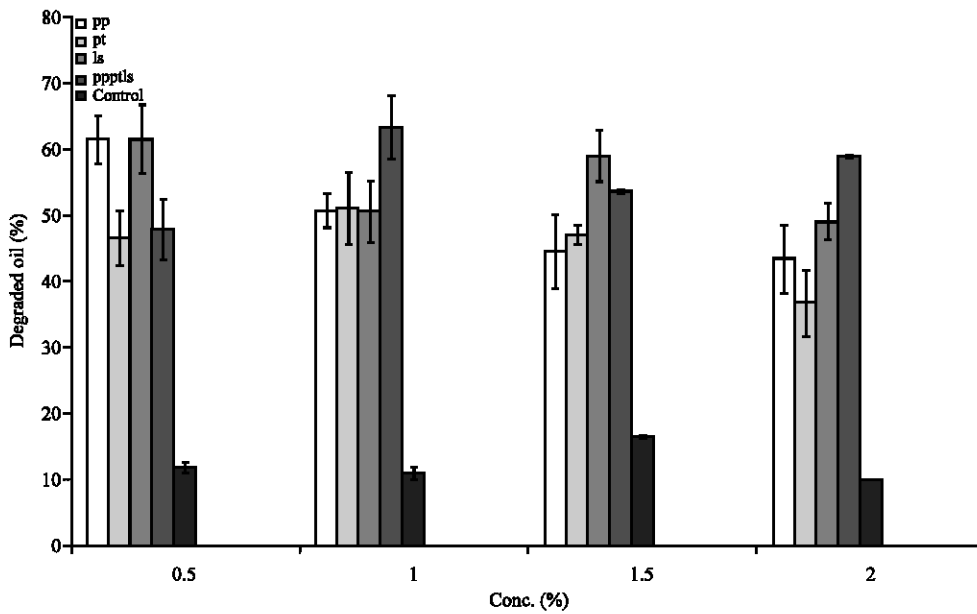


Fig. 1: Degradation of different concentration of crude oil by fungi in liquid medium. Error bar represents SD

Table 1: Growth of fungi mycelia against crude oil concentration

| Crude oil conc. (%) | % Mycelia weight (g) | | | |
|---------------------|------------------------|-----------------------|-------------------------|--------|
| | <i>P. tuber-regium</i> | <i>P. pulmonarius</i> | <i>L. squarrossulus</i> | PpPtLs |
| 0.5 | 17 | 36 | 32 | 15 |
| 1.0 | 18 | 26 | 22 | 34 |
| 1.5 | 19 | 22 | | 3428 |
| 2.0 | 16 | 24 | 25 | 32 |

Control was not treated with fungal mycelia, there was no mycelia growth observed

DISCUSSION

The ability of the fungi to degrade crude oil as sole carbon source in mineral salt medium confirms that fungi are useful not only in Solid State Fermentation (SSF) but also in aquatic medium. The different rate of degradation of crude oil by the fungi in liquid medium probably is due to the type and quantity of enzymes each of the fungus released for degradation in liquid medium. Schliephake *et al.* (2003) reported on enzymes produced by white rot fungi to which these mushrooms belong. These enzymes are responsible for the actual extent of degradation. All the fungi in single culture degraded crude oil to a certain extent probably because they all produce enzymes. Fungi mixture, comprising the three mushrooms was most effective in oil degradation with increase in crude oil concentration. This probably may be due to the different enzymes produced by these fungi which act in synergy. This observation was supported by reports from John and Okpokwasili (2012), Dave *et al.* (1994) and Ghazali *et al.* (2004) specifically stated the limitation of single cultures in bioremediation and pointed out that degradation of complex hydrocarbon will require more than single species. Other researchers who have observed degradation of toxic substances in aqueous medium by the use of fungi included (Kasinath *et al.*, 2003; Acevedo *et al.*, 2011; Loske *et al.*, 1990; Field *et al.*, 1992) also reported on the use of fungi for cleaning up oil polluted soils. The control set-ups as expected did not show any significant degradation, this was because degrading fungi were absent from the medium.

CONCLUSION

Pleurotus pulmonarius, *Pleurotus tuber-regium* and *Lentinus squarrosulus* are able to degrade crude oil. Nature has provided tremendous solution to environmental pollution in the use of microorganisms for degradation of xenobiotic compounds, waste management and bioremediation. In the near future, research should be focused on extracting fungal enzymes to enhance bioremediation in liquid medium. This may be a possible solution in remediation of aquatic polluted environment. Remediation agencies may run trials or case studies on this option.

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