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# Efficiency of *Pluerotus ostreatus* in Bioremoval of Total Petroleum Hydrocarbon from Refinery Effluent

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author CMI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HOS and OMI managed the analyses of the study. Author OMI managed the literature searches. All authors read and approved the final manuscript.*

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## ABSTRACT

Refinery effluent requires treatment to reduce residual hydrocarbons before discharging into the environment, due to their toxicity. This study was conducted to investigate the efficiency of *Pluerotus ostreatus* to bioremove Total Petroleum Hydrocarbons (TPH) in effluent discharged from the Port Harcourt refinery. Effluent treatment for TPH removal was conducted in the laboratory with *Pluerotus ostreatus*, sawdust and rice bran amendments. Five treatment options were used: Raw sample (Treatment A), which served as the positive control to monitor natural attenuation; filtered sample (Treatment B), which served as the negative control; effluent seeded with spawn and rice bran (Treatment C); effluent seeded with spawn and sawdust (Treatment D) and effluent seeded with spawn, sawdust and rice bran (Treatment E). Treatment E was efficient in removing 95.29% of TPH. Treatment B was least efficient in TPH removal with 10.10% reduction. The one-way analysis of Variance (ANOVA) revealed that there was no significant difference between the treatments C, D and E at 5% ($p<0.05$) level of significance. However, a Post Hoc analysis revealed that there exist significant differences between treatments A, B, C, D and E in the reduction of the concentration of

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TPH in the effluent samples. *Pleurotus ostreatus* indeed has bioadsorbent potential that can be exploited in the treatment of environments polluted with effluent containing significant levels of TPH.

*Keywords: Total petroleum hydrocarbon; Pleurotus ostreatus; bioadsorbent; effluent.*

## 1. INTRODUCTION

Total Petroleum Hydrocarbons (TPH) is a term used to describe a broad family of several hundred chemical compounds originating from crude oil. Hydrocarbon contamination of the environment may originate from crude oil extraction and refining, pipeline vandalism, runoff, oil accidents, illegal discharges and shipping [1,2]. Crude oil and petroleum products vary in chemical composition and impact on the environment, with contamination from gasoline, kerosene, fuel oil, mineral oil, and asphalt potentially widespread.

Wastes from petroleum industry pollute the environment when they are not properly treated before discharge. Untreated effluents discharged into the environment are known to be toxic to aquatic and terrestrial organisms. Results of various environmental studies carried out in oil spill areas show shocking levels of pollution and adverse effects on biota due to the hazardous nature of the hydrocarbon components [2]. Measurement of the total amount of TPH in impacted media is important because there are several constituents, and it is not practical to measure each one separately [2]. Chemicals that occur in TPH range from light to heavy molecular-weight hydrocarbons, including the more familiar hexane, benzene, toluene, xylenes, naphthalene, and fluorene. Petroleum hydrocarbon ranges are monitored at various levels depending on the state and testing site.

The search for new technologies involving the removal of petroleum hydrocarbons from wastewaters has directed attention to bioremoval, based on metal binding capacities of various biological materials. Isikhuemhen et al. [3] reported that white-rot fungi are increasingly being investigated and used in bioremediation because of their ability to degrade an extremely diverse range of very persistent or toxic environmental pollutants. White-rot fungi digest lignin in wood by the secretion of extracellular enzymes, giving wood a bleached appearance, hence there name [4,5]. Lignin decomposing white-rot fungi show extraordinary abilities to transform recalcitrant pollutants like polycyclic aromatic hydrocarbons (PAHs) [6]. They have also been found to be involved in mineralization, biodegradation, transformation and co-metabolism [7]. It is a cost-effective approach based on the fact that the technology involves the use of waste from farms or they are very easy to regenerate as is the case with *Pleurotus ostreatus* and other harvested biomass. Many fungal species have been extensively studied for their heavy metals removal efficiency [8-10]. *Pleurotus ostreatus* an edible white rot fungus commercially known as oyster mushroom, whose substrate for cultivation are easily available agricultural wastes [11,12], and other mushrooms have been investigated for their heavy metal biosorbent potential. There is, however, the paucity of information on the use of *Pleurotus ostreatus* for TPH removal from refinery effluent. The present study examined the efficiency of *Pleurotus ostreatus* in bioremoval of TPH in effluent obtained from the Port Harcourt refinery Eleme, Rivers State, Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Sample Collection

The mushroom, *Pleurotus ostreatus* used was obtained from NDDC/RSUST/DIPLOMAT mushroom/spawn production and research centre of the Faculty of Agriculture Teaching and Research Campus, Rivers State University of Science and Technology, Nkpolu, Port Harcourt. A 10 litre container sterilized with 70% ethanol was used to collect the effluent from the refinery drains and taken to the laboratory for immediate analysis. The physiochemical parameters of the effluent were analysed for baseline study. The substrates, sawdust and rice bran were fermented and pasteurized using heat at NDDC/RSUST/DILOMAT mushroom/spawn production and research centre.

### 2.2 Substrate for Spawn Preparation

Guinea corn was used for spawn preparation. The colonized spawn was bought at NDDC/RSUST/DILOMAT mushroom/spawn production and research centre of the Faculty of

Agriculture Teaching and Research Campus, Rivers State University of Science and Technology, Nkpolu, Port Harcourt.

### 2.3 Experimental Design

Five different treatment options were set up labeled A-E in a plastic container with cover. Treatment A: Containing 1.6 litres of the original sample to monitor the natural process, served as the positive control. Treatment B: Containing 1.6 litres of the filtered sample, served as the negative control. Treatment C: Containing 1.6 litres of the effluent seeded with 0.1 g of spawn and fermented 0.5 g rice bran. Treatment D: Containing 1.6 litres of the effluent seeded with 0.1 g of spawn and 0.5 g of fermented sawdust. Treatment E: Containing 1.6 litres of the effluent, seeded with 0.1 g of spawn, 0.5 g of fermented sawdust and 0.5 g of fermented rice bran. The samples were kept at room temperature for 60 days in an airtight cupboard to prevent contamination. Fifty millilitres (50 ml) of each sample was used for analysis. The concentration of the TPH was measured every 15 days.

### 2.4 Baseline Physicochemical Characteristic of Effluent

Turbidity, temperature, pH, Dissolved Oxygen (DO), Total Dissolved Solids (TDS) were analysed in situ with a HORIBA, U-51 series Multi-parameter water quality checker. Total organic carbon (TOC) was determined using TOC analyzer (Hach). Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), phosphate and nitrate were determined as described by the American Public Health Association [13]. Total petroleum hydrocarbon (TPH) was determined using the ASTM D5765-95 method.

### 2.5 Statistical Analyses

Experimental data were subjected to one-way analysis of variance (ANOVA) using SPSS 20 and Post Hoc Tests LSD ALPHA (0.05).

## 3. RESULTS AND DISCUSSION

### 3.1 Baseline Physicochemical Parameters

The baseline physicochemical parameters of the effluent are shown in Table 1. It was observed that the initial temperature of the effluent was 27.0°C with pH at 6.87 and differed from that of filtered effluent which was 26.90°C at pH 7.05. There were variations in the physicochemical parameters, with filtered effluent showing lower levels.

**Table 1. Baseline physicochemical parameters**

| Parameter | Effluent | Filtered effluent |
|---|---|---|
| pH-value | 6.87 | 7.05 |
| Temp. °C | 27.10 | 26.90 |
| Total dissolved solids, mg/l | 231.28 | 208.88 |
| Turbidity, NTU | 29.60 | 19.50 |
| DO, mg/l | 5.01 | 5.16 |
| Nitrate, mg/l | 4.22 | 4.14 |
| Phosphate, mg/l | 0.63 | 0.54 |
| BOD, mg/l | 6.25 | 6.01 |
| COD, mg/l | 10.05 | 8.88 |
| TOC, mg/l | 3.50 | 1.66 |
| TPH, mg/l | 5.09 | 2.97 |

### 3.2 Total Petroleum Hydrocarbon Removal

Total petroleum hydrocarbon concentrations with various treatments are presented in Fig. 1. The TPH removal efficiency of the various treatments showed variations. Treatment option A (positive control) which is the natural process showed the presence of TPH and treatment option B (negative control) which is the filtered sample showed a reduction in the concentration of the TPH, an indication that not all the TPH were eliminated by filtration.

Concentrations of total organic carbon, nitrate and phosphate in the medium were able to sustain the growth of *P. ostreatus* in the effluent for the 60-day study period, in the presence of rice bran and sawdust biomass substrates. As the fungus grew their mycelia and cell mass entrapped the petroleum hydrocarbons in the effluent samples. *Pleurotus ostreatus* is a two-phased biosorbent that can take care of both petroleum hydrocarbons and also heavy metals in the contaminated matrix. This is because white rot fungi produce extracellular enzymes with low substrate specificity that enables degradation of a wide array of aromatic compounds including petroleum hydrocarbons [5]. The spawn grew easily on sawdust and rice bran which are a low-cost agricultural waste. This is important because the prolific growth of the mushroom enhances the bioremoval process by providing more binding sites for the pollutants.

Fungi have excellent biosorbent capability due to their fast growth rate, the minimal nutrient requirement as well as large biomass production [10]. In the study of Kirk et al. [4], it was reported that production of appropriate enzyme system and increase in cell population are apparent basis for pollutant reduction following bioremediation. A similar reason was adjudged in this study as significant decreases were observed from day 30 in treatments C, D and E, after sustained biomass production. The composition of the fungal cell wall and the filamentous morphology of fungi add to their potential for bioremoval.

The one-way analysis of Variance (ANOVA) revealed that there was no significant difference between the treatments C, D and E at 5% ($p<0.05$) level of significance. However, a Post Hoc analysis revealed that there exist significant differences between treatments A, B, C, D and E in the reduction of the concentration of TPH in the effluent samples.

Regulations and recommendations are stipulated for TPH in air, water and soil based on levels that affect humans and animals. Therefore, this research work was extended to longer bioadsorption days for the spawn to utterly remove the petroleum hydrocarbons or to reduce the concentration to below permissive level in the effluent. Presence of TPH even in small amount can be injurious to health. Humans are exposed to TPH from many sources, like gasoline fumes at the pump, spilled crankcase, oil on pavement, chemicals at home or work, or certain pesticides that contain TPH components as solvents [1]. Exposure to TPH can also come during extraction and refining of crude, manufacturing of petrochemicals or use these products.

The impact of TPH release into the environment can be dire, affecting the health of organisms that come in contact with the compounds [14]. Effluents are discharged into water bodies, altering its chemistry with potential to adversely impact aquatic lives [15,16]. It has been reported that TPH levels in seawater are on the increase [17]. It can end up in the food chain through the aquatic routes, a reason why treatment to permissible limit if outright removal is not possible, is recommended. Therefore, mushrooms used for the bioremoval of TPH from effluent should be properly discarded. They can be incinerated and the ash not deposited in soils or water bodies but recycled to get back the petroleum hydrocarbons or removed to dump sites that are not used for agricultural purposes.

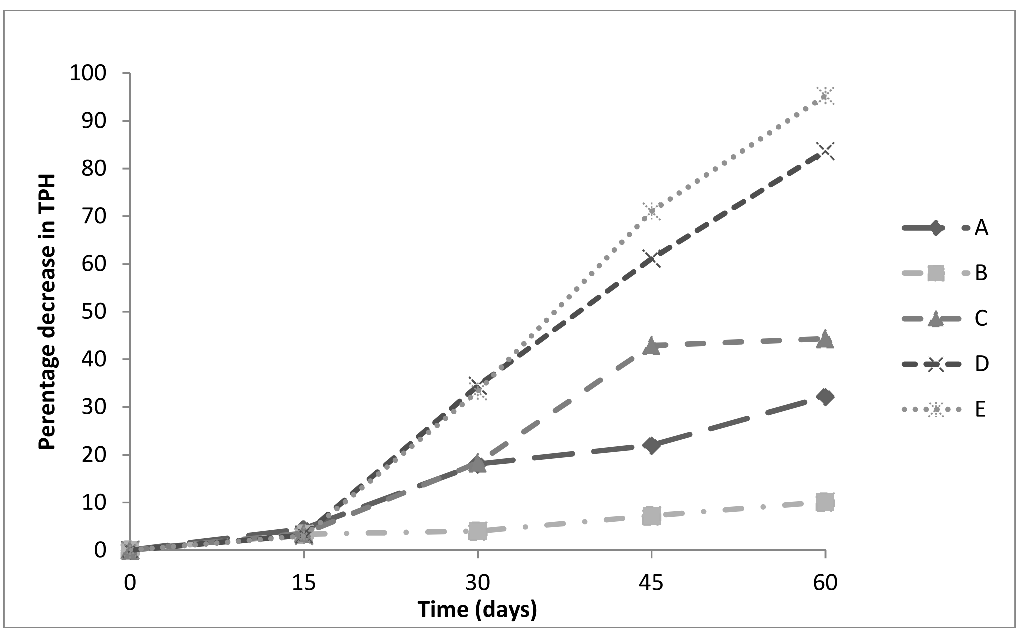


**Fig. 1. Changes in concentration of TPH**

## 4. CONCLUSION

*Pleurotus ostreatus* can biodegrade and bind petroleum hydrocarbons in associated medium thereby aiding their removal. This can be attributed to the fungus capability to grow fast and produce large biomass with minimal nutrient requirement, in addition to its cell wall and filamentous morphology. The growth substrate is therefore critical to *Pleurotus ostreatus* biosorbent potential, where, rice bran and sawdust can be found useful.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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