

Biodegradation of Engine Oil by *Agaricus campestris* (A White Rot Fungus)

Adongbede EM* and Sanni RO

Department of Botany, University of Lagos, Nigeria

Abstract

The white rot fungus *Agaricus campestris* collected from the wild in Lagos State Nigeria was cultured in minimal salt medium (MSM) contaminated with engine oil with the aim of investigating its degradative potential. The Indiscriminate disposal of spent engine oil by motor mechanics and other workers in Nigeria necessitated this research. The mushroom was grown in minimal salt medium for 30 days with the engine oil as its sole carbon source. The optical densities at 530 and 620 nm of engine oil contaminated MSM seeded with *A. campestris* and engine oil contaminated MSM not seeded with the mushroom was recorded for 30 days. There was significant difference between the optical densities of the two treatments with the contaminated MSM seeded (with *A. campestris*) been higher ($p < 0.05$) at day 25. Total residual petroleum hydrocarbons were extracted at the end of 30 days with n-hexane and analyzed with gas chromatography attached to a flame ionizer detector (GC-FID). *Agaricus campestris* significantly reduced the total petroleum hydrocarbons from 2744.72 mg/l in control to 503.08 mg/l in the contaminated minimal salt solution ($p < 0.05$) (i.e. over 90% of petroleum hydrocarbons were degraded). There was formation of secondary metabolites shown by repeats of some carbon atoms not found in the control. The fungus was able to mineralize long chain carbon compounds within the C24-C28 range as some of them disappeared from the seeded contaminated MSM. This research work concludes that *Agaricus campestris* has potential for mycoremediation of engine oil contaminated sites. There is need for more research to be done to establish the growth of the fungus in soils contaminated with engine oil.

Keywords: White rot fungus; Engine oil; Minimal salt medium; Mycoremediation

Introduction

Engine oil or motor oil is used around the world in cars and other engines like power generating sets. The contamination of soil by used engine oil is on the increase due to increased use of engines that use petroleum products [1]. In developing countries like Nigeria, improper disposal of spent engine oil during manual changing of used oil by automobile and power generating sets mechanics into runoffs, gutters, water drains, open vacant plots and even farmlands used as workshops is quite frequent. The spilled oil is left unattended for several years and contamination of soil, water and even ground water is left unabated causing serious environmental hazards. The consequences of leaving spent oil in the soil creates poor conditions of growth for plants because of poor aeration, immobilization of soil nutrients, loss of water holding capacity, lowering of pH and reduction in soil catalase enzyme activity [2]. Used engine oil is similar to unused one except that the used one contains additional chemicals that may have built up when engine runs. The presence of petroleum hydrocarbons in the environment is undesirable as quite a number of PAHs are toxic, mutagenic and carcinogenic [3,4]. Used motor oil contains metals like aluminum, chromium, copper, iron, lead, manganese, nickel, silicon and tin that comes from engine parts as they wear down. These metals also pose different health challenges from the ones caused by unused engine oil, as they can cause ailments like mutation and cancer.

The effects on plants range from chlorosis, bleaching, spotting of leaves, necrosis, malformations of epidermal cells and mesophyll layers, yield reduction and impaired fertility. Quinones-Aquilar et al. [5] also reported that root stress reduces leaf growth through stomata conductance. White rot fungi are defined by their physiological capacity to degrade lignin, a complex and heterogenous polymer [6]. The white rot fungi's enzyme system is non-specific, and this enables the degradation of compounds that resemble or have similarities with lignin. These fungi have been implicated in the degradation of a wide variety of xenobiotics, including polychlorinated biphenyls,

pentachlorophenols and various groups of pesticides [6]. The use of mushroom for biodegradation has attractive prospects because of their ability to degrade lignin and compounds that resemble the lignin structure. In previous researches involving white rot fungi and their degradative potential for petroleum hydrocarbons, results have been quite promising. Hence the need to look at *Agaricus campestris* for biodegradation purposes in this study. It is quite common in Nigeria and found in soils near contaminated sites.

Materials and Methods

The test organism - *Agaricus campestris* was collected among leaf litters in soil. Engine oil was purchased from AP filling station (University of Lagos, Akoka) Lagos, Nigeria. The tissues of the fungus *Agaricus campestris* was inoculated into the malt extract agar prepared following manufacturer's instructions. The pure culture of the fungus was incubated at room temperature for 6 days in the dark. Sub culturing into freshly prepared malt extract agar was done to get more inocula.

Biodegradative potentials of *Agaricus campestris*

Agaricus campestris was grown in petroleum utilizing fungi medium prepared according to methods adopted by Adekunle and Adebambo [7]. The Minimal Salt Medium used contained-1.25 g of NaH_2PO_4 , 0.29 g of KCl, 10.0 g of NaCl, 0.42 g of NaNO_3 , 0.83 g of KH_2PO_4 , 0.42 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 5.0 g of Agar dissolved in 1000 ml of distilled water.

***Corresponding author:** Adongbede EM, Department of Botany, University of Lagos, Nigeria, Tel: +2348033990469; E-mail: erute70@yahoo.com, eadongbede@unilag.edu.ng, e.adongbede@gmail.com

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The pH and temperature of the medium was monitored throughout the experiment.

The contaminated minimal salt medium was prepared by pouring 8 ml of the minimal salt medium into a test tube and adding 2 ml of engine oil to get a 20% level of contamination. Mycelium from pure culture plates measuring 5 x 5 cm (2 pieces) was added to the contaminated medium. The experiment was repeated randomly six times by assigning numbers 1-6 to each experimental unit. The control experiment had the minimal salt medium with contaminant -engine oil but no fungus. The experimental units were allowed to stand or incubate for 30 days. Readings were taken using the photoelectric colorimeters at an interval of 5 days at two wavelengths (530 nm and 620 nm).

Percentage degradation of petroleum hydrocarbon by *Agaricus campestris*

Extraction of Petroleum hydrocarbons from the variable *Agaricus campestris* and control MSM was done using analytical grade n-hexane after 30 days. GC-FID was used to determine the total petroleum hydrocarbons present after growth of the fungi in the medium. The gas chromatography equipment used was Hewlett Packard HP and the analytical conditions were as follow: carrier gas, helium, makeup nitrogen gas (flow rate -22 ml/min), fuel-air flow rate 45 ml/min, fuel-H₂ flow rate 45 ml/min, injector temperature at 220°C, initial and final oven temperature 70-200°C. The detector was flame ionized

at 250°C. The amount of PAH before and after were seen at the peak heights of the chromatographic run.

Experimental design and data analysis

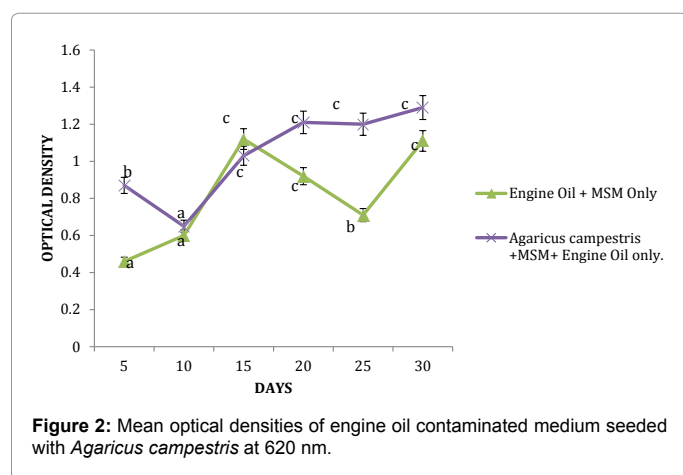
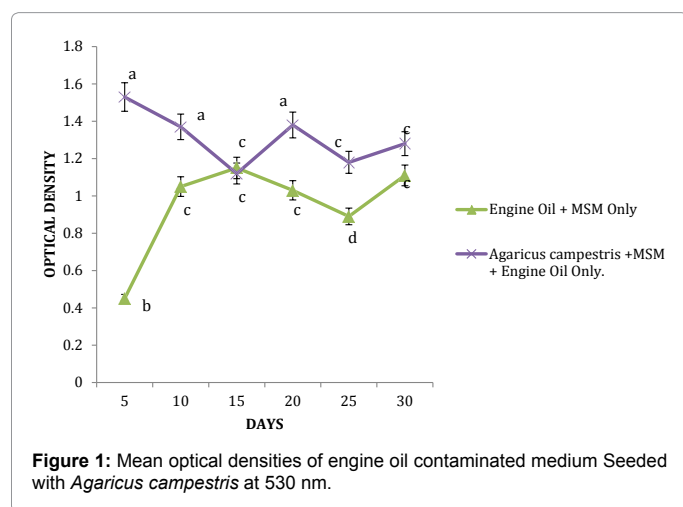
The experimental design adopted was the Randomized block design with one treatment and one variable. Engine oil represents the substrate and the independent variable was the absence or presence of *Agaricus campestris*. There were two microcosms, the control-engine oil contaminated MSM that was not seeded, and a seeded engine oil contaminated MSM. Replication was done six times for each experimental unit. Data was analyzed using SPSS statistical package version 16.0. Data collected were subjected to analysis of variance and the significant means were separated with the Duncan's multiple range tests

Results and Discussion

There was significant difference between the optical densities of engine oil contaminated MSM seeded with the mushroom and engine oil contaminated MSM not seeded with the mushroom at $p < 0.05$ (Figure 1) on days 20-25 at 530 nm. There was also significant difference between the two treatments at 620 nm on the 25th day. Here the seeded treatment was significantly higher than that not seeded (Figure 2). At the end of the experiment the optical density at 530 and 620 nm was not significantly higher in seeded treatment this could be due to senescence already setting in (Figures 1 and 2). The presence and growth of the fungus in the treatments caused noticeable increase in the optical densities as these treatments recorded significantly higher optical densities than the control that had only the minimal salt medium and engine oil at 530 nm and 620 nm (Figure 1).

The GC-FID results indicates that there was decontamination as there was reduction in the concentration of total petroleum hydrocarbons present from 27444.72 mg/l in control to 503.08 mg/l in the treatment that had both the contaminant and the mushroom therefore recording 98.17% degradation. The fungus however formed secondary metabolites with compounds having the following number of carbon atoms C1, C3, C4 (x2), C5, C7, C9 (x2) C11 (x2), C13 (x2), C14, C15 (x2) C17 C18 and C19, as these were not present or duplicated in the control but found in the treatment with the fungus and the contaminant (Figures 3 and 4). There were carbon atoms that were present in the control but duplicated in the treatment and these include C9, C11, C15 and C18 (Figures 3 and 4). Six compounds of C20 were present in the treatment while four C20 compounds were present in control. The C17 compounds were three in the control but one in the treatment indicating that two C17 compounds were completely removed and not just reduced in concentration (Figures 3 and 4). Other compounds with C24, C25, C26 and C28 were also present in the control but not seen in the treatment indicating that there were completely removed or metabolized (Figures 3 and 4). The GC-FID also shows that the fungus has more affinity for long chained petroleum hydrocarbons even within the C24 to the C28 carbon atom range.

The fungus *Agaricus campestris* successfully degraded over 90% of the petroleum hydrocarbons present in engine oil using it as its substrate and sole carbon source. *Agaricus campestris* is a white rot fungus, is known for its ability to degrade lignin the world's most recalcitrant compound and as such behaved true to type here. In other experiments *Agaricus campestris* was not so good in degrading pesticides like Diurion as it degraded only 32.90% of the pesticide [8]. Other white rot fungi like *Polyporus* spp degraded over 90% of petroleum hydrocarbons present in crude oil as recorded here in this study [9]. The use of white rot fungi



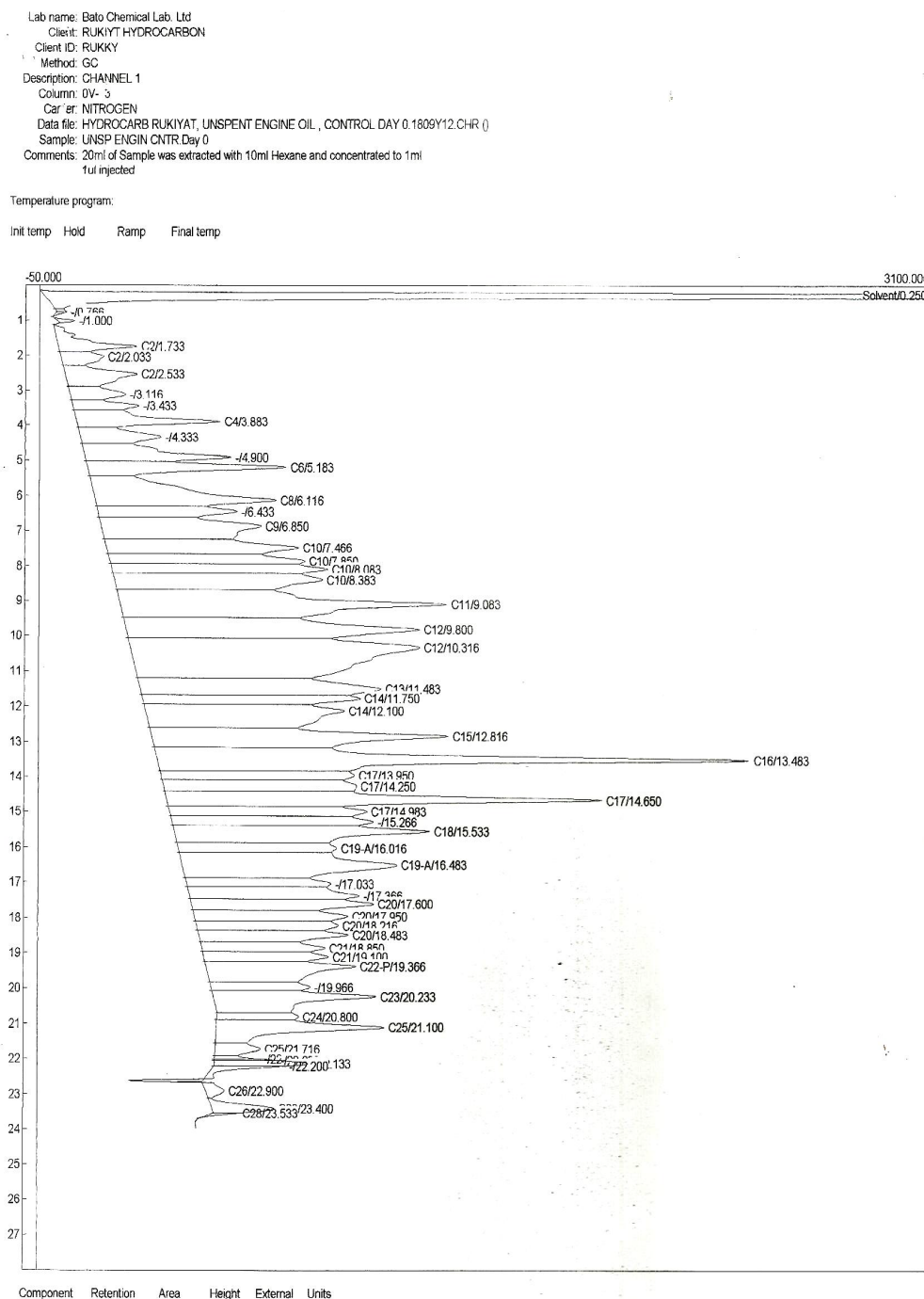


Figure 3: Chromatogram showing total petroleum hydrocarbon content in msm+ engine oil (control).

for decontamination of petroleum hydrocarbon polluted sites is not new and previous researches have shown that this group of fungi hold a lot of promise in this regard. *Phanerochaete chrysosporium* has been used successfully for remediation of media/sites polluted with petroleum hydrocarbons [10,11]. Other white rot fungi like *Lentinus squarrosulus*, *Pleurotus tuber-regium*, *Pleurotus pulmonarius*, *Pleurotus florida* have been successfully cultivated or grown in petroleum hydrocarbon contaminated sites or media with noticeable decontamination [12-

15]. One of the drawbacks of fungal remediation is also recorded in the current research that is the formation of secondary metabolites. Compounds not present in the control were noticed in the treatment. The metabolites could be undesirable and could also be harmless. According to Strauss [16], the secondary metabolites formed could be more harmful than the contaminants themselves. Previous researchers had recorded the formation of metabolites during fungal remediation. Hidayat and Tachibana [17] concluded that fungal remediation ends in

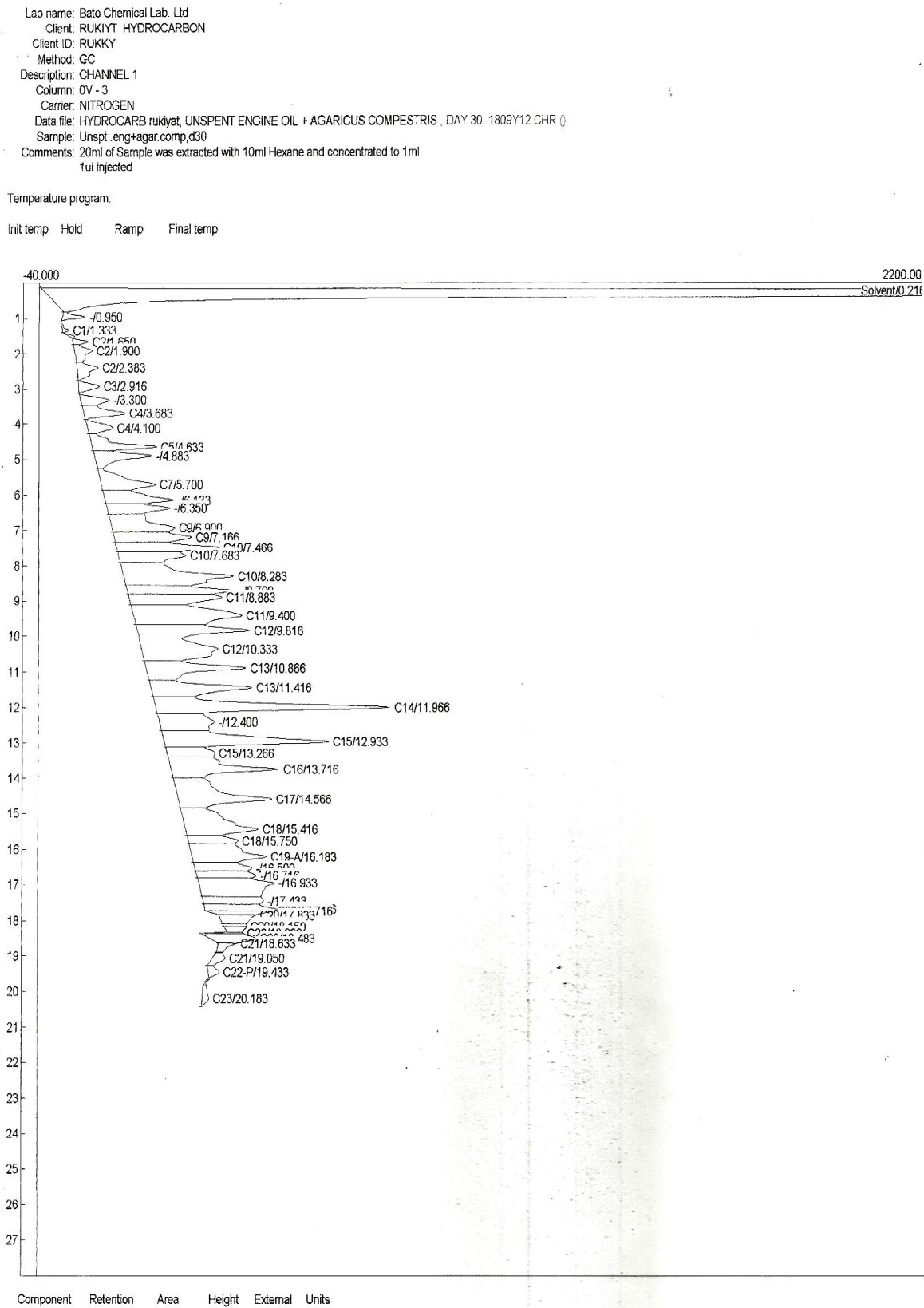


Figure 4: Chromatograms showing total hydrocarbon petroleum content in msm + engine oil seeded with agaricus campestris.

the contaminant been converted to CO₂ or secondary metabolites when metabolism is not complete.

Conclusion

The mushroom *Agaricus campestris* utilized engine oil as its sole carbon source. The reduction of petroleum hydrocarbons by 98.17% by this fungus indicates that it is a promising mycoremediation agent and should be properly harnessed for that purpose.

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