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Physico-chemical and Culture-dependent Microbiological Characterization of Spent *Pleurotus* Composts from Three Different Agro-based Wastes

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Authors' contributions

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

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Original Research Article

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ABSTRACT

Aim: Physico-chemical and culture-dependent microbiological properties of spent mushroom compost (SMC) were investigated.
Study Design: The physico-chemical and microbiological properties of spent mushroom composts were determined using standard methods.
Place and Duration of the Study: The study was conducted at the Department of Biotechnology, Federal Institute of Industrial Research, oshodi, Lagos-Nigeria between September to December, 2012.
Results: The CW-SMC, SD-SMC, and BSG-SMC, had a total nitrogen content of 30.52 mg/Kg,

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22.54 mg/Kg, and 14.20 mg/Kg, respectively while total phosphorus was observed as 14.10 mg/Kg, 12.45 mg/Kg, and 6.45 mg/Kg, respectively. In all the SMC's studied, Ca had the highest concentration (5.30-6.60 meq/100 g) among other exchangeable cations. The concentrations of Pb, Vn, Fe, Cr, and Ni were within the range of 0.02 mg/kg-8.93 mg/kg but BSG-SMC had the least concentrations of heavy metals. Quantitative compost microbiological analyses showed heavy loads of total culturable hydrocarbon utilizing bacteria (1.8-11 x 10^4cfu/g) and fungi (4.4-8.5 x 10^4cfu/g) counts. Hydrocarbon utilizing microorganisms associated with the SMC's were: *Penicillium* sp., *Aspergillus niger, Pleurotus ostreatus*, *Bacillus amyloliquefaciens, Bacillus subtilis, Bacillus licheniformis*, and *Burkholderia* species.

Conclusion: SMC's are good sources of limiting nutrients and hydrocarbon degrading microorganisms.

Keywords: Petroleum hydrocarbon; Hydrocarbon degraders; spent mushroom compost; agro-allied waste.

1. INTRODUCTION

Composting is a widely recognized remediation strategy for treating soils contaminated with petroleum hydrocarbon [1]. Generally, composting is defined as aerobic degradation of solid domestic organic and agro-based wastes into a form that can be used as amendment material for soil [2,3]. The main purpose of composting is to prepare a substrate in which the growth of mushroom is promoted to the practical exclusion of the microorganisms [4]. The first detailed record of mushroom cultivation occurred between 17th -18th Century during the Louis XIV (14 May 1643 – 1 September 1715) when Tournefort described a successful method of growing mushroom (*Agaricus bisporus*) in stable manure [4]. A good mushroom compost should have a suitable physical condition that will provide good anchorage for mushroom as well as maintain good aeration and water holding capacity, a good chemical condition that will release some nutrients from the raw materials during fermentation [5].

Several reports have emphasized that the microorganisms associated with mushroom compost after flushing/harvesting (SMC) are quite lignolytic, and usually posses xenobiotic degrading genes [3,6]. Composting matrices and composts are rich sources of xenobiotic degrading bacteria, actinomycetes, and lignolytic fungi. These microorganisms degrade pollutants to innocuous compounds such as carbon (iv) oxide and water [7].These microorganisms can also bio-transform pollutants into less toxic substances/and or lock-up pollutants within the organic matrices thereby reducing pollutant bioavailability [7].

In Nigeria, the identities of bacteria and fungi that colonize spent mushroom composts are not well documented. The objectives of this present study were to identify the petroleum hydrocarbons degrading microorganisms and physico-chemical compositions of three different SMC's of *Pleurotus ostreatus.*

2. MATERIALS AND METHODS

2.1 Source of Spent Mushroom Compost

The spent mushroom composts were produced from brewers' spent grain (BSG), cotton waste, and sawdust (SD) at the Department of Biotechnology, Federal Institute of Industrial Research, Oshodi, Lagos State, Nigeria. They were sampled from the mushroom house of the Institute, and transported to the Biotechnology laboratory of the Federal Institute of Industrial Research, Oshodi for physico-chemical and microbiological analyses.

2.2 Physico-chemical Properties of the Spent Mushroom Composts

2.2.1 Determination of pH and conductivity

Five (5) grams of the properly labelled sample were diluted with 10 ml of sterile distilled water, and the electrode of the pH meter (ADWA 8000, United Kingdom) which has been previously standardized was dipped into the diluted compost sample and swirled gently, allowed to stand until a stable/constant reading was obtained. The conductivity of the *Pleurotus* compost samples were determined using five (5) grams which were dissolved in 20 ml of distilled water. The conductivity electrode (Jenway 3015, United Kingdom) was standardized using 1220

µs/cm solution. Thereafter the electrode of the conductivity meter dipped into the dissolved compost until a constant conductivity reading appeared on the display unit.

2.2.2 Determination of total nitrogen, total phosphorus, and percentage total organic carbon

The total nitrogen in the compost was determined using macrokjeldahl digestion and Distillation methods as previously by other workers [8]. Twenty five (25) grams of air dried composts were weighed in duplicates on a filter paper and placed in a dry 500 ml kjedahl flask in order to carry out the reaction. The formula below was used in the computation.

Calculation of total nitrogen =

$$\frac{T \times M \times 14 \times 100}{\text{Weight of soil sample}}$$

Where; T=Titre value, M= Molarity of HCl used for the titration

Total Phosphorus was determined using the colorimetric method as described by other workers [9]. Fifty, (50 ml) sample extract was pipetted into a clean conical flask. This aliquot was autoclaved with $K_2S_2O_8$ and H_2SO_4 for 30 minutes at 121°C, 5 ml of ammonium molydbate were added to the autoclaved mixture to form heteropoly molybdophosphoric acid and is reduced with stanneous chlorides in an aqueous sulphuric acid medium, at 30°C, to form a molybdenum blue complex. The resulting blue colour was measured spectrometrically at 660 nm and compared to identically prepared standard (water). The detection limit of this method is 0.005 mg/l/kg.

Total organic carbon was determined using the wet oxidation technique as previously reported by others [10].

$$\% \ TOC \ = \ \frac{Titre\ value\ of\ sample - sample\ titre}{sample\ weight} \times 0.2 \times 0.3$$

2.2.3 Determination of heavy metals and exchangeable cations

The methods described by other scientists were adopted with modification [11,12]. One (1) gram of the air-dried and finely pulverized SMC sample was weighed into a 100 ml of digestion flask. Five milliliters (5 ml) of 6 M Hydrochloric Acid were added. The mixture was heated to dryness on a hot plate set at 150°C. The dry mass produced was leached with 10 ml of 4 M HCl, and thereafter filtered into a flask and the residue washed with more HCl and made up to the mark. The blank was prepared in the same as described above, but without SMC. The heavy metals were analyzed by aspirating the solution of digested sample into atomic adsorption spectrophotometer at appropriate wavelength. The concentrations of K^+, and Na^+were determined by Flame spectrophotometry. The concentrations of Ca^{2+}, and $Mg^{2+,}$ in the ashed extract were determined using Atomic Absorption spectrophotometer as absorbance of 422.7 nm, and 285.2 nm wavelength respectively.

2.2.4 Enumeration of total culturable heterotrophic bacteria

Total heterotrophic bacterial count present in the three (3) different SMC's was determined using the spread plate method on nutrient agar (Oxoid) previously reported [13]. Aliquots (0.1 ml) of appropriate dilutions were spread on triplicates of sterile nutrient agar. The plates were incubated for period of 18 hours in the incubator at 28°C±2°C. Colonies that formed during this incubation period were counted using the formula;

$$\frac{No\ of\ colonies\ X\ dilution\ factor}{\text{Amount used}}$$

2.2.5 Enumeration of total culturable hydrocarbon utilizing bacteria

The enumeration of total culturable hydrocarbon utilizing bacteria was done by using the vapour phase method as previously reported [13].

2.2.6 Enumeration of total fungi counts (THF)

The medium of choice was the potato dextrose agar, (PDA) with 10% tartaric acid using the spread plate method. The medium was prepared according to the manufacturer's (oxoid, Basingstoke, Hants) instructions and sterilized at 121°C, 15 psi and 15 minutes before dispensing into sterile disposable Petri plates. A 0.1 ml aliquot of appropriate dilutions of sample was inoculated unto the media. The plates were incubated for 5-7 days at room temperature and

colonies formed were counted and expressed as cfu/gram.

2.2.7 Enumeration of total culturable hydrocarbon utilizing fungal count

Enumeration of hydrocarbon utilizing fungi was done using mineral salt agar (MSA) containing 10 grams of NaCl, 0.45 grams of $MgSO_4$ $7H_20$, 0.42 KCl, 0.29 of KH_2PO_4, 0.86 grams of Na_2HPO_4, H_2O, 0.43 grams of $NaNO_3$, 250 mg of streptomycin, and 25.0 grams of agar powder in 100 ml. The medium was sterilized by autoclaving at 121°C for 15 minutes, 15 Psi. Crude oil (Bonny light) served as both the sole carbon and energy sources. Three replicate plates inoculated by spread plating, was inverted over sterile filter papers moistened with sterile crude oil, which was placed on the lid of Petri dish covers. The plates were inverted over the dish covers containing the crude oil impregnated filter paper [14].

2.2.8 Isolate purification, and Identification of hydrocarbon utilizing microbial isolates using molecular biology tools

The hydrocarbon utilizing bacterial and fungal isolates were purified by subculture in nutrient agar and potatoes Dextrose agar respectively. Deoxyribonucleic acid (DNA) was isolated from the microorganisms using fresh culture. Deoxyribonucleic acid (DNA) isolation was done using the universal Zymo research (ZR) fungal/bacterial DNA Kit™. Fifty (50) mg (wet weight) fungal or bacterial cells from 18 hours Luria Bertani Broth (Oxoid), and potatoes dextrose broth (Oxoid), respectively that have been re-suspended in up to 200 µl of water or isotonic buffer was taken to a ZRBashing Bead™ lysis tube. The procedures adopted for DNA isolation have been previously reported extensively [15]. PCR analysis was run with 18S rRNA, and 16S rRNA Universal primers for hydrocarbon utilizing bacteria and fungi, respectively. The PCR mixture comprises of; 1 µl of 10X buffer, 0.4 µl of 50 mM $MgCl_2$, 0.5 µl of 2.5 mm dNTPs, 0.5 µl 5mM forward primer, 0.5 µlof 5 mM Reverse primer, 0.05µl of 5units/µl taq with 2 µl of template DNA, 2 ul of 5x sequencing buffer and 1 µl of 50% DMSO and 5.05 µl of distilled water to make-up 10 µl reaction mixture. Initial denaturation temperature of 94°C for 3 minutes, 30 cycles of 94°C for 60seconds, 56°C for 60seconds, and 72°C for 120 seconds were employed. For final extension a temperature of 72°C for 10 minutes was employed. Products of PCR were sequenced by electrophoresis on 1.5% agarose gel and standard markers were run in parallel. The gels were stained with ethidium bromide and UV transilluminator was used for observation of the bands. Base nucleotide blasting was done using the sequences from Basic Local Alignment Search Tool (BLAST) search of National Centre for Biotechnology Information (NCBI) data bases. The strains showing 80-100% 16s and 18s RNA gene sequence similarity were considered to be of the same species and strain where necessary

2.3 Statistical Analyses

Statistical analyses were done with Excel 2010 version and the Duncan multiple test range were done to determine variances, significances across different spent mushroom composts (BSG, SD, CW).

3. RESULTS AND DISCUSSION

The spent mushroom composts used in this study were from three (3) different sources namely, cotton waste, sawdust, and brewers' spent grain (BSG). These spent mushroom composts were analyzed to ascertain their physico-chemical and microbiological composition. The sawdust (SD) compost had a pH of 6.67.The pH of brewers spent grain (BSG) compost, and cotton waste spent mushroom composts were near neutral (7.34), and 6.64, respectively (Table 1). The alkaline nature of the BSG compost may be as a result of initial fermentation of the grains during brewing which breaks down significant amount of acids thus shifting pH towards alkalinity. Previous, work on SMC'S reported a pH range of 5.90-7.80 for several mushroom composts of which the composting matrices were not well identified [16]. Other studies also reported a pH of 5.9-6.6 for Irish spent mushroom compost [17].

The conductivities of the SD, BSG, and CW composts were found to be 197 µs/cm, 210.0 µs/cm, 195. 00 µs/cm, respectively (Table 1). Statistical analyses showed that the conductivity of the BSG compost was highest among the three SMC's studied. The conductivity of 197.4 µs/cm is relatively high when compared with un-composted sawdust. The process of mushroom production requires that the substrate be amended with salts such as calcium carbonate, Sodium chloride, and salts that will provide micronutrients such as molybdenum, zinc etc. This substrate-salt amendment process actually

led to the elevated conductivity of the studied *Pleurotus* spent composts. In the terms of previous composite studies on spent mushroom compost, it was reported that Irish spent mushroom compost had electrical conductivities level between 580 µS/cm-903µS/cm [17].

Percentage total organic carbon (%T0C) in the SD, BSG, and CW composts were 12.10%, 8.5%, and 14.74%, respectively. Total organic carbon (%) in the spent sawdust compost analyzed was 12.10% and this translates into a carbon: nitrogen ration of 12:1 in the sawdust compost.

Total nitrogen of the spent mushroom composts were 22.54 mg/Kg, 14.20 mg/kg, and 12.45 mg/Kg for the SD, BSG, and CW, respectively. Cotton waste *Pleurotus* compost had the highest amount of total nitrogen, and could suggest the reason for its high performance during *Pleurotus* ramification. Studies at Ohio state University showed that one (1) tonne of mushroom compost is equivalent to 22.271 bs/ton of nitrogen. In the terms of previous composite studies on spent mushroom compost, it was reported in a related study reported that Irish spent mushroom compost had total nitrogen content between the ranges of 21.0 mg/kg to 87 mg/kg [17].

Total phosphorus content in the SD, BSG, and CW composts were 12.45 mg/kg, 6.46 mg/kg, and 14.10 mg/kg, respectively. Studies at Ohio state University showed that one (1) tonne 13.29 bs/ton to total phosphorus (OSU, 2006). In similar phosphorus level of 0.29% was reported in Irish spent compost; which is still within the observations from studies in our spent *Pleurotus* composts of various agro-based wastes [16].

The moisture contents of 52.289%, 52.3%, and 58.65% were observed in the SD, BSG, and CW composts respectively (Table 1). In the sawdust mushroom compost, the moisture content (%) of 54% is not surprising as the mushroom production process that generated the compost involves continuous wetting with tap water.

Studies on the exchangeable ion of the sawdust (SD) compost showed it contains 5.30 meq/100 grams,1.55 meq/100 grams, 3.50 meq/100 grams and 3.50 meq/100 grams and 3.15 meq/100 grams of Ca, k, Na and Mg respectively (Table 1). Exchangeable ions analyses on the sawdust mushroom compost include: Ca, K, Na, and Mg were present in 5.84mg/kg, 2.10 mg/kg, 3.77 mg/kg, and 3.60mg/kg, respectively in 100 grams of the saw dust spent mushroom composts. In addition the cotton waste spent compost, the concentrations of Ca, K, Na, and Mg were 6.60 meq/100 g, 1.70 meq/100 g, 2.05 meq/100 g, and 4.0 meq/100 g (Table 1). Exchangeable ions were also determined in the BSG Compost, as it contained in meg/100grams of the compost 5.84, 2.10, 377, and 3.60 of Ca, K, Na, and Mg respectively (Table 1). These exchangeable ions are very adequate and can be transported by hydrocarbon utilizing microorganisms during metabolism of petroleum hydrocarbon for biomass building. They serve as source of essential nutrients required in only trace amounts, and which cannot be synthesized by the hydrocarbon utilizers.

Heavy metals analysis of the sawdust mushroom compost showed that compost contained high amount of lead (20.01 mg/Kg) while Vanadium (Vn), Iron (Fe), total chromium ($Cr+^{3}$and Cr^{+6}), and Nickel contents were 3.42, 0.33, 0.02, and 0.24mg/kg respectively (Table 1). The heavy metal contents were also studied in BSG *Pleurotus* compost, and lead, vanadium and total chromium were not detected, but iron (Fe) and nickel contents were 0.76 mg/kg, and 0.73 mg/kg respectively (Table 1). The absence of iron, and nickel could be explained by the HACCP factor where brewers buy only grains that have very little or no amount of heavy metals. The presence of heavy metals inhibits enzymatic activities during brewing. Heavy metal content was investigated in Cotton wastes *Pleurotus* composts and lead was highest (8.93 mg/kg) in concentrations whereas Iron (Fe), total chromium (Cr^{3+} and Cr^{+6}), and nickel contents were 0.83 mg/kg, 0.08 mg/kg and 0.18 mg/kg respectively (Table 1). The spent mushroom composts are not free of heavy metals, and this is actually not surprising since the sawdust, and cotton wastes for mushroom production were obtained from trees, and cottons grown in wild environment where these metals have accumulated from.

These levels of heavy metals in this mushroom substrates is quite low, and cannot on amendment in crude oil polluted soil hinder/inhibit the expression of crude oil degradative enzymes of microorganisms including lignolytic fungi such as *Pleurotus* sp. However, it is worthy to note that some Research Institutes like FIIRO as a HACCP Process takes into account amount of heavy metals in substrate before utilizing such for mushroom production. Substrates that are heavy metal inundated are never used for mushroom cultivation and production.

Table 1. Physicochemical properties of three spent mushroom composts

Parameters	SD comspost±S.D	BSG compost±S.D	CW compost±S.D
Moisture content (%)	54.433±0.912[a]	52.289±0.76[a]	58.654±398[b]
pH	6.67±0.318[a]	7.34±0.049[b]	6.64±0.049[a]
Conductivity (µs/cm)	197.4±1078[a]	210.8±15.34[ab]	195.00±10.4[a]
Percentage total organic carbon	12.10±0.14[a]	8.5±0.00[b]	14.74±0.02[a]
Total nitrogen (mg/kg)	22.54±3.20[a]	14.20±1.69[b]	30.52±13.88[c]
Total phosphorus(mg/kg)	12.45±0.49[a]	6.46±0.32[b]	14.10±0.00[c]
Exchangeable Ions			
Calcium (Ca) (meq/100grams)	5.30±0.42[a]	5.84±0.08[a]	6.60±0.20[a]
Potassium (K)(meq/100grams)	1.55±0.21[a]	2.10±0.00[a]	1.70±0.08[a]
Sodium(Na) (meq/100grams)	3.50±0.00[a]	3.77±0.28[a]	2.05±0.07[a]
Magnesium (Mg) (meq/100grams)	3.15±0.21[a]	3.60±0.07[a]	4.0±0.00[a]
Heavy Metals Concentrations			
Lead (Pb) (mg/kg)	2.01±0.00[a]	ND	8.93±1.491[a]
Vanadium (Vn) (mg/kg)	3.42±0.32[a]	ND	ND
Iron (Fe) (mg/Kg)	0.33±0.212[a]	0.76±0.064[b]	0.83±0.106[c]
Total Chromium (mg/kg)	0.02±0.00[a]	ND	0.08±0.021[b]
Nickel (Ni) (mg/kg)	0.24±0.04[a]	0.73±0.07[ab]	0.18±0.028[a]

Means followed by the same letter along the rows are not significantly different at p<0.05 (Duncan multiple test range). S.D; Standard deviation. N.D; Not detected

Microbial studies on the sawdust compost showed a total culturable heterotrophic bacterial count of 2.82 x 10^5cfug/g while total culturable heterotrophic fungal count, total culturable hydrocarbon utilizing bacterial count, and total culturable hydrocarbon utilizing fungal count, and Total culturable 5.2 x 10^4, and 8.5 x 10^4cfu/g respectively (Table 2).

The total culturable heterotrophic bacterial count of the BSG mushroom compost was 2.70 x 10^3cfug/g. the total culturable heterotrophic fungal count, Total culturable hydrocarbon utilizing bacterial and total hydrocarbon utilizing fungal counts were 6.3 x 10^4, 11 x 10^4, and 7.5 x 10^3cfu/g respectively (Table 2). The microbiological composition of the spent sawdust compost was also determined, and observation shows that total culturable heterotrophic bacterial in the SD, CW, and BSG composts where significantly lower than the total culturable heterotrophic fungal counts in the terms of bioload (Tables 2). In that same consistent pattern, the bioload of total culturable hydrocarbon utilizing bacterial isolates in the SD, CW, and BSG compost were also significantly higher than the total culturable hydrocarbon utilizing fungi. These patterns seem to be a reverse case when considering the diversity of fungi and bacteria in most studied habitat. However, the process of mushroom production involves sterilization to knock off all vegetative cells and spore before inoculating with mushroom spawns. Thus sterilization removes all cells, but the process of wetting the substrates with tap water thereafter exposure could be the source of bacterial contamination. Furthermore, the inoculation with mushroom spawns allows *Pleurotus* to grow, and this eventually have likely formed and biological quorum with other fungi. The established quorum of most fungi leads to expression of secondary metabolites such as antibiotics which inhibit the growth of bacteria. This may justify or explain the observation of lower bacteria counts and higher fungal count in all the spent mushroom composts (SD, BSG, and CW) studied. Other previous studies have assessed the bacterial diversity in different agro-based mushroom composts. Chandna et al. [18] observed an increase in mesophilic bacterial count within the first ten (10) days of composting within range of 1.7-2.84 x 10^9Cfu/g. Similarly, an increase in thermophilic bacterial count from 10^7cfu/g. to 10^8Cfu/g within 11-32 days of composting was reported [18]. The reports on SMC in Ibadan-Nigeria showed that SMC of *Pleurotus* had heterotrophic bacterial count (Total culturable heterotrophic bacteria) within the range of 1.85 x 10^4 to 6.0 x 10^6Cfu/g within mesophilic temperature [4]. This total culturable heterotrophic bacterial count co-relates with our observation on the total culturable heterotrophic bacterial loads in spent mushroom composts of cotton waste brewers' spent grain (BSG) and sawdust substrates. In addition, other studies also observed differently that bacterial counts were at all points higher than the count of fungi [19].

The identities of the microorganisms (hydrocarbon utilizing bacterial and fungal associated with these three spent mushroom compost was determined using the conventional traditional tools and Analytical index profiling tool which combined sugar fermentation, sugar-alcohol fermentation, and blasting using API software. Hydrocarbon utilizing fungi isolated from sawdust include *Pleurotus ostreatus*, *Saccharomyces cerevisae,* and *Penicillium* species at prevalence rates of 50%, 20% and 30% respectively (Table 3). The hydrocarbon utilizing bacterial isolates found in the sawdust mushroom compost were *Bacillus amyloliquefaciens* and *Bacillus subtilis* at a prevalence rate of 20% and 80% respectively. Spent cotton waste mushroom compost had similar spectrum of hydrocarbon utilizing fungi and bacteria. Amongst the hydrocarbon utilizing fungi, *Pleurotus ostreatus, Saccharomyces cerevisiae* and *Penicillium* species had prevalence rates of 45%, 25% and 30% respectively. Notably 5% of Gram-negative *Burkholderia* species were associated with spent cotton waste mushroom compost as hydrocarbon utilizers. Spent brewers grain mushroom compost has wider hydrocarbon utilizing fungal spectrum which include *Pleurotus ostreatus, Saccharomyces cerevisiae, Penicillium* sp,and *Aspergillus niger* at prevalence rate of 47.6%, 14.29%, 33.33% and 4.78%, respectively. The hydrocarbon utilizing bacteria associated with spent BSG mushroom compost were *Bacillus licheniformis, Bacillus amyloliquefaciens and Bacillus subtilis* at prevalence rate of 40%, 20% and 40% respectively (Table 3).

The microbial identities of hydrocarbon utilizing bacterial and fungal isolates associated with each of the three spent mushroom composts showed *Pleurotus ostreatus* had the highest prevalence rate of 50%, 45%, and 47% in the SD, CW and BSG composts, respectively. Again, *Penicillium* species, *Aspergillus niger* and *Saccharomyces cerevisiae* are virtually contaminants which must have come in contact with mushroom during fruiting through tap water wetting process. The hydrocarbon utilizing bacterial isolates associated with the spent mushroom compost were mainly of *Bacillus amyloliquefaciens*, *Bacillus subtilis*, and the only single gram negative species was *Burkholderia* species. In addition, these hydrocarbon utilizing bacteria probably got into the mushroom compost either through tap water, or by their spores falling on the mushroom from the air. This is only possible for spore-producing bacteria such as *Bacillus.* Previously, the use of 16S rRNA sequencing reported the isolation of wide range of Gram-Positive and Gram-negative groups of bacteria associated with the agricultural compost studied [18]. These include*: Burkholderia, Bacillus, Staphylococcus, Kocuria, Acidovorax, Comamonas, Teribacillus, Enterobacter, Serratia, Lysinibacillus* sp, *Klebsiella* [18]. Microorganisms colonizing mushroom compost have been studied by other Nigerian Scholars. Early scientists had reported different level of colonization of certain groups of bacteria such as thermophilic actinomycetes, mesophilic and thermophilic bacteria in Mushroom compost cultivated with *Agaricus bisporus*. This early study monitored the colonization pattern and reported that thermophilic actinomycetes were the first group to colonize, while mesophilic bacterial diminished during the most productive portion of the mushroom cropping season and then increased towards the end of the season [20].

Studies in Nigeria had reported that bacteria isolates are associated with compost used in cultivation of Nigerian edible mushrooms *Pleurotus tuber-regium* (Fr.) *Singer*, and *Lentinus squarrosulus* (Berk). The study acknowledged that the identities of bacteria species involved in mushroom compost used in cultivation of *Pleurotus tuber-regium,* and *Lentinus squarrosulus* have been poorly documented [4].

Table 2. Quantitative microbiology of the spent mushroom composts

Physiological group	SD	BSG	CW
Total culturable heterotrophic bacterial count (Cfug/g)	2.8×10^5cfug/g	2.70×10^3cfug/g	2.6×10^7cfug/g
Total culturable fungal count	5.78×10^6cfug/g	6.3×104^4cfug/g	45×10^7cfug/g
Total culturable hydrocarbon utilizing bacterial count	5.2×10^4cfug/g	11×10^4cfug/g	1.8×10^5cfug/g
Total culturable hydrocarbon utilizing fungi count	8.5×10^4cfug/g	7.5×10^3cfug/g	4.4×10^4cfug/g

SD; sawdust compost, BSG; brewers spent grain compost; CW; cotton waste

Table 3. Hydrocarbon utilizing microorganisms associated with spent mushroom compost

Spent mushroom compost	Hydrocarbon utilising fungi	Hydrocarbon utilising bacteria
Spent saw dust compost (SD)	*Pleurotus ostreatus* (50)	*Bacillus amyloliquefaciens* (20)
	Saccharomyces cerevisiae (20)	*Bacillus subtilis* (80)
	Penicillium species (30)	
Spent cotton waste (CW)	*Pleurotus species* (45)	*Bacillus subtilis* (40)
	Saccharomyces cerevisiae (25)	*Bacillus licheniformis* (45)
	Penicillium (30)	*Burkolderia* species (5)
Spent brewers grain compost	*Pleurotus ostreatus* (47.6)	*Bacillus licheniformis* (40)
(BSG)	*Saccharomyces* sp, (14.29)	*Bacillus amyloliquefaciens* (20)
	Penicillium species (33.33)	*Bacillus subtilis* (40)
	Aspergillus niger (4.78)	

Numbers in brackets shows percentage prevalence

Furthermore, Jonathan [4] also documented the identities of the SMC associated bacteria to include; *Enterobacter aerogenes, Bacillus Polymyxa, Micrococcus roseus, Citrobacter freundii, Bacillus Subtilis, Clostridium perferinges, Pseudomonas aeruginosa, Bacillus cereus, Bacillus licheniformis, and Escherichia coli.* These microorganisms are related to the range of organisms found to be associated with spent composts of *Pleurotus ostreatus* isolated from the cotton waste, BSG and SD substrates. The variation in identity is that no single strain of *Escherichia coli*, and *Clostridium* p*erferinges* was isolated from the BSG, SD and CW substrates/composts. Again, while [4] most other scientists reported the identities of heterotrophic bacteria isolated from spent mushroom compost, our study reports the identities of hydrocarbon utilizing bacterial species based on the aim of the study. It is worthy to mention that the spectrum identity of hydrocarbon utilizing bacteria actually was under-estimated because of the traditional method employed, which is not capable of isolating a larger preponderance of bacteria which are said to be viable but not-culturable.

It may be possible that such nutrients may not be directly available for certain microorganisms, or lack of knowledge about the microorganisms, poor understanding of the requirement for their successful cultivation, recalcitrance, stress, sub-lethal damage to cells, and preference to a particular unknown substrate. The presence of *Bacillus cereus*, *Micrococcus roseus*, and *Pseudomonas aeruginosa* in non-mushroom composts as sugar cane waste, newspapers, and sawdust respectively has been previously reported [4].

Microorganisms colonizing mushroom compost during the composting process are regarded as active agents, which determine the chemical composition of the substrate thus making it possible for the growth of mushroom [19]. The study on the phenology of fungi associated with composting of Sawdust used in cultivating oyster mushrooms in Ghana reported prevalence rates of 23%, 30%, 70% for *Aspergillus fumigatus, Mucor pusillus,* and *Paecilomyces varioti*, respectively [19]. Compost of sawdust inoculated with *Pleurotus sajor-caju* strain PSCM was shown to have higher mould colonization than substrates inoculated with *Pleurotus sajur-caju* strain PSCJ [19]. The groups fungi reported to be present in the SMC include: *Penicillium cyclopium, Cladosporium herbarum, A. fumigatus, A. niger, A. ochraceus, P. digitatum, Rhizophus oryzae, and Trichoderma viride* [19]. This report is similar to our report on the fungi isolates associated with spent mushroom (*Pleurotus ostreatus)* of sawdust, cotton waste and brewers' spent grain (BSG) except that the fungal phenology reported in [19] were higher in spectrum as compared to our observation. It was clear that Obodai and colleagues [19] focused attention on heterotrophic fungal isolates colonizing mushroom compost while this study only focused attention on hydrocarbon utilizing fungal isolates. The spectrum or diversity of heterotrophic microorganisms are always higher than that of the hydrocarbon utilizing microorganisms. This could explain or justify the lower spectrum of hydrocarbon utilizing fungi when compared with heterotrophic fungal phenology reported [19]. The work of Obodai and colleagues also reported that *Pleurotus ostreatus* strain 0T-3 was isolated 100% at all sampling point while other organisms like *Aspergillus niger* could not be isolated until after 28 days of composting, and antibiosis of the property of the *Pleurotus ostreatus* was also mentioned to reduce the colonization and growth of bacterial species [19]. Similarly in a Nigerian study, prevalence rates of 40.9%, 22.7%, 5.6%, 17.0%,

11.6%, and 2.3% for *Mucor, Penicillum, Rhizopus, and Trichoderma*, respectively were reported [21]. Microorganisms colonizing the mushroom compost are active agents aiding mineralization thereby making it possible for mushroom growth [22]. These heterotrophic fungal species reported in the study above are quite similar to the hydrocarbon utilizing fungi associated with *Pleurotus ostreatus* spent compost observed in this study except that no single strain of hydrocarbon utilizing *Fusarium* species was observed in all the three spent mushroom composts (CW, BSG, SD) under investigation. In addition, our studies on CW, BSG and SD mushroom composts isolated *Saccharomyces cerevisiae*, while no other similar report on colonization of SMC is available. The difference between this present study and the study documented in [21] is that no single species of *Rhizopus* was isolated from any of the three spent mushroom compost (CW, BSG and SD) studied. The work of Obire and colleagues acknowledged that fungal species were found to be numerous at mesophilic and thermophilic phases of composting, and their presence also helps to degrade the compost [21]. Hydrocarbon utilizing fungi isolated from the three SMC's studied are quite similar to the heterotrophic fungi isolated from different composts by other workers [23,24]. This study is the first report on hydrocarbon utilizing fungal isolates associated with spent mushroom compost of cotton waste, sawdust and brewers' spent grain matrices. The work of Obire and Amadi added that the presence of bacteria and fungi other than *Pleurotus* in pasteurized substrates is as a result of possession of spores that were heat tolerant [21]. However, the observation from this current study suggests that rather than possession of heat resistant spores, the bacterial colonization in SMC is as a result of contamination coming from water commonly used in wetting the fruiting mushroom. The ecology of thermophilic fungi in mushroom compost was previously studied [25]. The thermophilic fungi isolated from the compost of *Agaricus bisporus* were *Abisdia corymbifera*, *Rhizomucur michei, Chaetomium thermophilium*, *Emericella nidulans*, *Talaromyces emersonii, Talaromyces thermophilus*, *Aspergillus fumigatus*, *Pacilomyces variotii* etc [25]. These fungal isolates were quite different from the hydrocarbon utilizing fungal isolates from SD, BSG and CW mushroom composts. In addition, it is also important to note that this is the first report on isolation of hydrocarbon utilizing fungal isolates from spent mushroom compost. These hydrocarbon utilizing fungi which are also lignocellulolytic are good degraders of aliphatics and aromatic hydrocarbons. Thus it holds a lot of promises as the amendment of petroleum hydrocarbon soil with spent mushroom composts could act as bioaugumentation source and also as biostimulation agent since the waste product (compost) is an environmentally friendly organic fertilizer. This fertilizer supplies limiting nutrients (N and P) to autochtonous hydrocarbon degrading microorganisms. The amendment of polluted soil with SMC acts like bioaugumentation. This is because it is microbiologically established that the three (3) spent composts (cotton Waste (CW), sawdust (SD), and brewers' spent grain (BSG) have very high preponderance of lignolytic and hydrocarbon degrading/utilizing fungi and bacteria. Notably, a study observed that at laboratory-scale, petroleum hydrocarbon polluted soil amended with spent compost after cultivation with *Pleurotus* species got bioremediated within 2 months of amendment [26]. In addition, similar other studies on petroleum hydrocarbons, insecticides, and pentachlorophenol reported degradation of these chemicals of concerns [5]. However, some other authorities have expressed great concerns over the survival of this spent mushroom compost and its lignolytic fungi and bacteria under harsh environmental conditions in an in-situ bioremediation experiment. This present study has established the presence of wide range of hydrocarbon utilizing bacterial and fungal species in spent mushroom compost after *Pleurotus ostreatus* cultivation. There are existence studies that reported the presence of *thermophilic Trichoderma, Torula, Penicillium, Mucor, Epicoccum, Monilia, and Humicola* in spent mushroom compost of *Pleurotus* [20]. This report is similar to the present study as hydrocarbon degrading *Trichoderma* was isolated form CW, SD, and BSG composts of *Pleurotus ostreatus.*

4. CONCLUSION

The study has established that spent *Pleurotus* composts of cotton waste, sawdust, and brewers spent grains are good sources of limiting nutrients required for bioremediation. Thus they can serve as cheaper and environmentally–friendly organic fertilizers for bioremediation. In addition, this study also established that they are good sources of hydrocarbon utilizing microorganisms which can be used as bioaugumentation materials.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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