Biodegradation of Three Agricultural Wastes by a White-rot Fungus *Pleurotus pulmonarius* (Fries) Quetlet

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Abstract: Studies were carried out on the degradation of cotton waste, rice straw and cocoa pod husks using *Pleurotus pulmonarius* in cultures incubated for 0-60 days. The proximate composition, percentages of nitrogen, carbon, potassium and phosphorus, pH, lignin contents, organic matter and enzyme production were determined using the substrates degraded by *P. pulmonarius*. Crude protein increased significantly throughout the incubation period from 1.27% in the control to 12.63% in cotton waste, 6.65% to 14.82% in rice straw and in cocoa pod husk from 7.04% to 13.82%. Crude fibre decreased significantly in cotton waste and cocoa husk from 5.88% to 5.31% and from 39.88% to 34.95% respectively but an increase was observed in rice straw from 18.42% in control to 28.08% after 60days of incubation period. The nutrient contents, pH values, organic matter showed significant differences of ($P \leq 0.05$) in the three substrates as the days of fermentation increased. Lignin contents of treated cotton waste and cocoa husk were degraded by *Pleurotus pulmonarius* but an increase was observed in the rice straw. Cellulase activities decreased significantly in contrast to the lignase activities where a consistent increase was observed on the degraded substrates as the incubation period increased.
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4

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1. Introduction

Pleurotus pulmonarius, commonly known as the Indian oyster, Phoenix mushroom, or the Lung oyster, is a mushroom very similar to *Pleurotus ostreatus*, the pearl oyster, but with a few noticeable differences; the caps of *P. pulmonarius* are paler and smaller than in *P. ostreatus* (Stamets, 2000).
P. pulmonarius in nature grow in clusters on dead wood and can be grown throughout the year. Owing to the toleranceof *Pleurotus pulmonarius*to high temperature, it has been reported that its speedy fruiting and yield efficiencies attract many cultivators to the mushroom (Trudell and Ammirati, 2009). The white-rot fungus *P. pulmonarius*, is a wood decaying basidiomycetes which is capable of degrading not only lignin but also variable recalcitrant environmental pollutants due to its ability to secrete lignolytic enzymes such as lignin peroxidase, manganese peroxidase and laccases which aid the degradation process (Ogbo et al., 2006).

Waste, generated from the mechanical processing of raw cotton prior to spinning, provides an ideal substrate for the growth of some edible mushrooms notably *Volvariella volvacea* the Chinese or straw mushroom and the oyster mushrooms (*Pleurotus spp*). Cotton waste culture widely variable amounts of total nitrogen from 0.25-1.45% for *Pleurotus* (Chang, 1999). It has a biological efficiency of 56-86% for *Pleurotus.* Rice straw consists predominantly of cell walls, comprised of cellulose, hemicellulose, and lignin. To break down these components cellulase, hemicellulase and ligninase are required (Schiere and Ibrahim, 1989). Huge quantities of agro-industrial biomass including about 900 million tons of rice straw are produced worldwide annually, more than 90% being produced in Asia (Jahromi et al., 2011). Cocoa husk is considered a valuable source of dietary fibre and is gaining considerable interest in economically advanced countries. The knowledge of the quality of the protein fraction in the husk is imperative in developing nutritional formulations in new products (Makkar et al., 1997).

Mushroom hyphae secrete large amounts of extracellular enzymes which bring about the degradation of macromolecules such as cellulose, hemicellulose, lignin and protein in the substrates (Narsi et al., 2006; Kuforiji and Fasidi, 2008). Adenipekun and Okunlade (2012) investigated the potential of *Pleurotus ostreatus* in degradation of maize stovers and rattan wood. Recycling of these unfavorable materials through mushroom culture can increase agricultural efficiency and enhance degradation process of ligno-cellulose sources (Obodai et al., 2003). Therefore, this study was undertaken to determine the potential ability of cotton waste, rice straw and cocoa husks being degraded by *Pleurotus pulmonarius.*

2 Methods

2.1 The fungus

Pure cultures of *Pleurotus pulmonarius* was obtained from the Plant Physiology Laboratory,

Department of Botany, University of Ibadan, Nigeria. Fresh cultures were obtained by repeated sub-culturing on Potato Dextrose Agar (PDA).

2.2 Degradation of Substrates by *P. pulmonarius*

The substrates used for this study were cotton waste, rice straw and cocoa pod husk. Cotton waste (*Gosspypium hirsutum*) was obtained from Textile Mills, Ibadan. Freshly harvested rice straw was collected from International Institute of Tropical Agriculture (I.I.T.A) in Ibadan. It was sun-dried for two weeks to prevent decomposition. Fresh cocoa husks were obtained and processed at Cocoa Research Institute of Nigeria (CRIN) in Ibadan.

2.3 Preparation of substrates

The method of Adenipekun and Fasidi (2005) was employed. Twenty-five grams (25g) of each dry waste were weighed into each sterile bottle, squeezed out with a muslin cloth and 75ml distilled water were added. The bottles were immediately covered with aluminum foil and sterilized in the autoclave at 121^0 C for 15 minutes. The substrates were prepared in three replicates.

2.4 Inoculation

Each of the bottles was inoculated at the centre of the substrate with two agar plugs (7mm in diameter) of vigorously growing mycelia disc and covered immediately. They were kept in a clean dark cupboard in the laboratory at 30^0C and 100% relative humidity. The controls were dried in the oven at 100^0C for 48hours to determine the initial dry weight of the substrates (Bhargava and Orskov, 1987). The experiment was replicated three times and the bottles were harvested after 30 and 60 days and their dry weights determined.

2.5 Analytical methods

pH determination: The pH of each substrate was measured with a glass electrode of an electronic pH meter by adding 100ml distilled water to 1g substrate in clean bottles. After 18 hrs at room temperature, the pH of the suspension was determined (Zadrazil and Brunnert, 1982). The experiment was done in triplicate.

Lignin determination: One gram (1g) of sample was weighed into a 250ml conical flask, to which 20ml of freshly prepared 72% H_2SO_4 at 20^0C was added and carefully mixed then allowed to stand for 2 hours. It was later refluxed with 238mls of distilled water for 4hours. The insoluble lignin was allowed to settle overnight and filtered. The residue was then transferred into a crucible of known weight and dried in the oven at 60^0C to a constant weight in a desiccator and weighed.

Percentage lignin was obtained using the formula:

$$\% \text{ Lignin} = \frac{\text{weight of insoluble lignin}}{\text{weight of oven dried sample}} \times \frac{100}{1}$$

Nutrient content analysis: Crude fibre (CF), Crude protein (CP) and pH were determined as described by Zadarazil and Brunnert (1982).The method of Association of Official Agricultural Chemists (A.O.A.C. 2003) were used to determine percentage organic carbon, organic matter, percentage nitrogen, phosphorus, potassium and enzyme assay.

Enzyme assay: Cellulase was assayed (Berridge, 1955) using 3,5-dinitrosalicyclic acid (DNSA) and the amount of reducing sugar formed was determined. Lignase was also assayed by the method of Berridge (1955).

2.6 Experimental design and Statistical analysis

A randomised complete block design was used showing a white-rot fungus, three substrates and three incubation periods were used. The white rot fungus was *P pulmonarius*, the three substrates were cotton waste, rice straw and cocoa pod husk while the incubation period were 0, 30 and 60 days. An ANOVA table was prepared for each, following the Duncan's multiple range test and least mean significant difference was carried out on lignin-degrading abilities of white-rot fungus. Each treatment was replicated three times.

3 Results

3.1 Effects of *P. pulmonarius* on proximate composition (g/100gdm) of degraded substrates

Table 1 shows the proximate composition (g/100gDM) of cotton waste, rice straw and cocoa pod husk incubated for 0-60 days. The dry matter decreased with increase in incubation period in treated cotton waste from 5.46% in the control to 3.70%, it also decreased in rice straw from 3.90% in control to 3.19% and cocoa pod husk from 5.10% in control to 3.75%. The crude fibre decreased significantly compared to untreated substrates in cotton waste and cocoa pod husk but an increase was observed in rice straw as the rate of incubation increased. Cotton waste treated with *P. pulmonarius* decreased from 5.88% in the control to 5.31% after 60 days; cocoa pod husk treated with the fungus also decreased significantly from 39.88% to 34.95% but an increase from 18.42% in control to 28.08% was observed in rice straw after 60 days. The crude protein contents of the fungal treated substrates increased significantly throughout the incubation period, from 1.27% in the control to 12.63% in cotton waste, also in rice straw from 6.65% (control) to 14.82% and in cocoa pod husk from 7.04% in control to 13.82%.

3.2 Effects of *P. pulmonarius* on nutrient contents of degraded substrates

The percentages of nitrogen, carbon, potassium and phosphorus of the three agricultural substrates are shown in Table 2. With increasing incubation period, the percentage nitrogen of treated cotton waste increased from 0.24% in the control to 2.48%, in rice straw from 1.19% to 2.56% but in cocoa pod husk it decreased from 1.26% to 0.22%. Carbon content of treated cotton waste decreased from 47.37% in the control to 44.5% and in cocoa pod husk from 37.22% to 31.87% while in rice straw, a significant increase was observed from 27.03% in the control to 41.69%. The potassium content in treated cotton waste decreased from 3.22% in control to 1.21%, but increased significantly in rice straw from 1.63% to 2.49% in control to 60 days respectively while that of cocoa pod husk increased from 1.2% to 4.73%. Percentage phosphorus increased significantly in the treated substrates. Phosphorus increased from 0.0017% in the control to 0.0022% after 2months of incubation in cotton waste, 0.0024% to 0.0027% in rice straw and 0.0014% to 0.0016% in cocoa husk but of the three substrates treated with *P. pulmonarius*, rice straw had the highest recorded value.

3.3 Effects of *P. pulmonarius* on organic matter and pH on degraded substrates

Table 3 shows the pH values of the treated substrates as the incubation period increased. The pH of cotton waste decreased from 5.86 to 5.53 but in rice straw, the pH increased from 5.77 to 6.42 and in cocoa pod husk from 6.54 to 6.76 after 60 days incubation. The percentage organic matter increased with increase in incubation period of rice straw from 68.04% to 72.04% while a decrease was observed in cotton waste and cocoa pod husk from 81.91% in the control to 76.76% and from 64.36% in the control to 55.10% respectively.

3.4 Effect of *P. pulmonarius* on lignin content of degraded substrates

The percentages of lignin content of the substrates are shown in Table 4. With increasing incubation period, the lignin content of treated cotton waste degraded by the fungus reduced from 1.90% in the control to 1.45%, for cocoa pod husk it also reduced from 28.76% to 22.33% but in rice straw there was an increase from 13.59% to 22.33%.

3.5 Effects of *P. pulmonarius* on degraded substrates for enzyme production

Table 5 shows the enzyme activities of *P. pulmonarius* on degraded cotton waste, rice straw and cocoa husk over an incubation period of 0-60 days. It was observed that cellulose activity decreased significantly all through the period of incubation on the three substrates from 9.07% to 8.47%, 9.50% to 7.27% and 3.78% to 2.9% in cotton waste, rice straw and cocoa pod husk respectively as compared with their controls. On the other hand, a consistent increase was observed in the lignase activity of the treated substrate. Cotton waste increased from 2.40% in the control to 2.97% after 2months, similarly, rice straw increased significantly from 2.83% to 5.03% and cocoa husk also increased from 7.4% to 9.30% as compared with their control.

4. Discussion

One goal of biological delignification using lignin degrading fungi is for the effective digestibility of the substrate carbohydrate. This can be achieved by a rapid lignin degradation (Adenipekun and Fasidi, 2005). In this study, *Pleurotus pulmonarius* degraded cotton waste, rice straw and cocoa pod husk. There was a consistent decrease in the dry matter of the treated substrate compared with the untreated one between 0-60 days. This is in line with (Jonathan et al., 2010) who reported that dry matter reduced significantly from 88.74% in control to 86.80% in *Lentinus subnudus* and 86.55% in *Pleurotus tuber-regium*.

There was significant ($P \leq 0.05$) increase in the crude protein (CP) contents of the substrates treated with *P. pulmonarius* with an increase in fermentation days. Similar result was observed in rice straw degraded by *P. pulmonarius* from 4.50% recorded in the control to 4.60% at day 10, 4.78% at day 20 and 9.36% after forty days fermentation (Jonathan et al., 2012). Similar trend was also observed by Akinfemi et al., (2010) who reported that the crude protein contents of the fungal treated substrates (sorghum stover) increased for *Pleurotus ostreatus* and *P. pulmonarius*. The increase in crude protein contents might be due to the secretion of certain proteinous extra cellular enzymes into the waste during their breakdown and subsequent metabolism of the products (Kadiri, 1999).

The decrease in crude fibre (CF) of *Pleurotus pulmonarius* on degraded cotton waste and cocoa husk could be due to cellulose enzymes being secreted by cellulolytic fungi. Isikhuemhen and Nerud (1999) observed that white rot fungi produced extracellular lignin modifying enzymes, the best characterized of which were laccase, lignin peroxidase and manganese peroxidases. Safari et al. (2005) also reported crude fiber loss in wheat, barley and rice straw incubated with *P. chrysosporium*. Belewu and Belewu (2005) as well reported that degradation of banana leaves decreased in fibre contents or fractions due to the production of various enzymes during the vegetative and reproductive phases with lignocelluloses degrading properties. In the opinion of Abd-Allah (2007) the high CP contents and decrease CF fractions of biodegraded substrates could be the result of the fungal

consumption of part of the produced fermentable sugar produced by the action of hydrolyzing enzyme of the fungus on the substrate which then stimulated the fungus to produce high enzyme activities.

A significant increase in nitrogen percentage was observed in cotton waste and rice straw and a decrease was observed in cocoa husk incubated with *Pleurotus pulmonarius*. This is similar to the findings of Anyakorah and Olatunji (2001) who reported higher nitrogen content (5.67%) in CW than in CP (4.44%).

Biological treatment reduces the pH of the fermented samples (Jahromi et al., 2011). The present study showed that in cotton waste there was a reduction in pH over an incubation period of 2months as compared with the control but an increase was observed in rice straw and cocoa pod husk over the same period. The change in pH value may be associated with the increase in amino nitrogen content and the presence of metabolic waste products within the substrates. Fungi are generally known to carry out their metabolic activities at acidic pH (Fasidi, 1996).

The present results show that the organic matter content was higher (P≤0.05) in rice straw and this corresponds to the findings of Oziel et al., 2008 for *Pleurotus*-treated wheat straw. In another study, *P. ostreatus* and *P. florida* cultivated on barley straw, resulted in higher organic matter digestibility for treated straw (Montañez et al., 2004). Escalona et al., (2001) reported that ash concentration increased 60 days after barley straws were incubated with *P. ostreatus*, possibly due to higher organic matter used by the fungus.

Lignin content decreased consistently (P≤0.05) on *Pleurotus*-treated cotton waste and cocoa husk in the present work. Removal of lignin contents also has a contribution in the digestibility of lignocellulosic substrates (Zadrazil and Brunnert, 1982). Lignin, cellulose and hemicellulose fractions form the bulk of CPH fibre. During the fermentation process, the weekly changes observed in the fibre fractions indicated the degree of lignocellulose biodegradation as well as the enzyme activities exhibited by *P. ostreatus* on CPH (Alemawor et al., 2009).

Cellulase activities decreased significantly at (P≤ 0.05) over an increasing incubation period. This result agrees with the findings of (Adenipekun and Okunlade, 2012) where they noted that the depletion in enzymes production as the incubation period increased might be due to metabolic activities during the process of degradation where enzymes were being used up to aid the process. On the other hand, a significant increase was observed in the lignase activities of the treated substrates over the same incubation period. This observation is similar to that of Kuforiji and Fasidi (2008) who reported that higher activities of proteinase, cellulase, lipase and catalase were observed in the fruit bodies compared to the sclerotia. These enzymes were found to affect the shelf life, food nutrient and flavor of the mushroom.

TABLE 1: Effects of *P. pulmonarius* on proximate composition (g/100gdm) of degraded substrates

Substrates	Incubation day period (days)	Dry matter	Crude fibre	Crude protein
Cotton waste	0	5.46^{a}	5.88^{a}	1.27^{b}
	30	3.81^{b}	3.92^{a}	7.37^{ab}
	60	3.70^{b}	5.32^{a}	12.63^{a}
Rice straw	0	3.90^{ab}	18.42^{c}	6.65^{b}
	30	4.14^{a}	32.07^{a}	15.20^{a}
	60	3.19^{b}	28.08^{b}	14.82^{a}
Cocoa pod husk	0	5.10^{a}	39.88^{a}	7.04^{ab}
	30	4.03^{ab}	36.02^{b}	13.82^{a}
	60	3.75^{b}	34.95^{b}	1.27^{b}

Each value is a mean of 3 replicates. Means with different superscripts in each column are significantly different at P≤0.05 according to Duncan's multiple range test

Table 2: Effects of *Pleurotus pulmonarius* on nutrient contents of degraded substrates

Substrates	Incubation Period (days)	Organic carbon (%)	Nitrogen (%)	Potassium (%)	Phosphorus (%)
Cotton waste	0	47.37[a]	0.24[b]	3.22[a]	0.0017[a]
	30	45.54[ab]	1.31[ab]	1.02[a]	0.0019[a]
	60	44.50[b]	2.28[a]	1.21[a]	0.0022[a]
Rice straw	0	39.35[b]	1.19[b]	1.63[b]	0.0024[b]
	30	43.73[a]	2.49[a]	2.32[a]	0.0034[a]
	60	41.69[b]	2.56[a]	2.49[a]	0.0027[b]
Cocoa pod husk	0	37.22[a]	1.26[ab]	1.20[c]	0.0014[a]
	30	33.76[b]	2.41[a]	4.03[b]	0.0013[a]
	60	31.87[b]	0.22[b]	4.73[a]	0.0016[a]

Each value is a mean of 3 replicates. Means with different superscripts in each column are significantly different at P ≤ 0.05 according to Duncan's multiple range test.

Table 3: Effects of *P. pulmonarius* on organic matter and pH of degraded substrates

Substrates	Incubation period (days)	Organic matter (%)	pH
Cotton waste	0	81.91[a]	5.86[a]
	30	78.74[ab]	5.54[b]
	60	76.76[b]	5.53[b]
Rice straw	0	68.04[b]	5.77[b]
	30	72.07[ab]	6.03[b]
	60	75.61[a]	6.42[a]
Cocoa pod husk	0	64.36[a]	6.54[a]
	30	58.38[b]	5.98[b]
	60	55.10[b]	6.76[a]

Each value is a mean of 3 replicates. Means with different superscript in each column are significantly different at P≤ 0.05 according to Duncan's multiple range test

Table 4: Effect of *P. pulmonarius* on lignin content of degraded substrates

Substrates	Incubation period (days)	Lignin content
Cotton waste	0	1.90[a]
	30	1.53[a]
	60	1.45[a]
Rice straw	0	13.59[b]
	30	20.47[a]
	60	20.17[a]
Cocoa pod husk	0	28.76[a]
	30	24.04[b]
	60	22.33[b]

Each value is a mean of 3 replicates. Means with different superscripts in each column are significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

Table 5: Effects of *P. pulmonarius* on degraded substrates for enzymes production

Substrates	Incubation period(days)	Lignase Unit/ml	Cellulase Unit/ml
Cotton waste	0	2.40[a]	9.07[a]
	30	2.93[a]	8.63[a]
	60	2.97[a]	8.47[a]
Rice straw	0	2.83[a]	9.50[a]
	30	3.43[a]	8.30[ab]
	60	5.03[a]	7.27[b]
Cocoa pod husk	0	7.40[b]	3.77[a]
	30	9.03[a]	2.73[a]
	60	9.30[a]	2.90[a]

Each value is a mean of 3 replicates. Means with different superscripts in each column are significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

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