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Biodegradation of Nigerian wood wastes by *Pleurotus tuber-regium* (Fries) Singer

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Abstract

Studies were carried out for 90 days on the degradation of wood wastes of four economically important Nigerian trees; *Terminalia superba, Mansonia altissima, Holoptelia grandis* and *Milicia excelsa* by white rot fungus, *Pleurotus tuber-regium* a Nigerian edible mushroom. The pH of the wastes dropped to 4.0/4.2, 90 days after incubation. On the contrary, amino nitrogen content of the wastes increased consistently during this period of solid-state fermentation. Lignin degradation also increased with the increase in incubation days. The greatest lignin reduction was observed in *H. grandis* followed by *T. superba, M. altissima* and *M. excelsa*.

Digestibility of spent substrates by ruminants increased during fermentation as follows: *M. excelsa* > *M. altissima* > *T. superba* > *H. grandis*. These results are discussed in relation to the use of fermented wood wastes as feeds for ruminants.

Keywords: *Pleurotus tuber-regium*; Biodegradation; Wood wastes; Digestibility; Fermentation

1. Introduction

Pleurotus tuber-regium (Fries) Singer, is an edible tropical mushroom that is common in West Africa especially in Nigeria. This species is given this name because it always forms tubers known as sclerotia during unfavourable weather conditions (Zoberi, 1973; Oso, 1977; Okhuoya and Etugo, 1993; Jonathan, 2002). *P. tuber-regium* has been reported to be a very good source of protein, sugars, lipids and essential mineral elements (Fasidi and Ekuere, 1993; Kuforiji et al., 2003).

When the spores of this fungus settle on a suitable substratum, they germinate and form mycelium, which colonizes wood and produces sclerotia, which may be buried within the soil. *P. tuber-regium* usually produces sclerotia towards the end of the rainy season from where new fruits are formed at the beginning of new growing season (Fasidi and Olorunmaiye, 1994; Jonathan, 2002).

Different Nigerian tribes have specific and interesting ways of using *P. tuber-regium*. The Yoruba people of South Western Nigeria utilize both the sclerotia and sporophores of this fungus as an article of food. Besides, their native doctors use sclerotia of this mushroom as powerful medicinal component. When mixed with other herbs, this fungus is used to cure headache, stomach pain and fever. The Yoruba traditionalists also use the concoction of *P. tuber-regium* to neutralize effect of harmful charms invoked by their enemies (Oso, 1977, 1981).

Urobo, a tribe in the mid-western Nigeria uses the fruit bodies of *P. tuber-regium* as teeth cleaner and in the treatment of chest pain, dropsy and small pox. The Hausas in the Northern part of Nigeria use this fungus with other herbal preparation in the treatment of constipation, high blood pressure and asthma.

The wastes produced from industries and agricultural farmlands constitute a major problem to our environment.

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Such wastes include cereal straws, corn cobs, wood pulp, sawdust, cotton wastes and many others. To get rid of these wastes, they are usually burnt in heaps thereby releasing offensive odour and gases into the atmosphere. Some are even thrown into the rivers and streams thereby endangering aquatic life. These wastes could be put into appropriate use in order to reduce environmental hazard and pollution. This present study was therefore undertaken to determine the digestibility of wood wastes of some selected economically important Nigerian trees degraded by *P. tuber-regium*.

2. Methods

2.1. The fungus

The sporophores of *P. tuber-regium* growing in the wild were collected from Ibadan University Botanical Gardens. These were tissue cultured to obtain fungal mycelia (Jonathan and Fasidi, 2001). The pure culture obtained was maintained on plates of potato dextrose agar (PDA).

2.2. The substrates

The substrates used for this study were wood wastes (sawdust) of *Holoptelia grandis, Mansonia altissima, Terminalia superba* and *Milicia excelsa*. These substrates were collected from Bodija Plank Market, Bodija, Ibadan, Nigeria and sun dried for 5 days.

2.3. Vegetative growth of *P. tuber-regium* on different wood wastes

Ten grammes (10.0 g) of each substrate was separately mixed with 25.0 ml of distilled water and dispensed into 10.0 cm (diameter) Petri dishes. These plates were covered with aluminum foil and sterilized at 1.02 kg/cm^2 pressure at 121 °C for 15 min. After cooling, each Petri dish was inoculated with a vigorously growing mycelia disc (10.0 mm) of 5-day-old culture of *P. tuber-regium* and incubated at 30 ± 2 °C for 7 days. Each treatment was replicated 3 times. Vegetative growth rates were determined by measuring radial colony diameter after the 7th day of incubation.

3. Degradation of wood wastes by *P. tuber-regium*

3.1. Preparation of substrates

The jam bottles used for this experiment were thoroughly washed, dried for 10 min at 100 °C and weighed. Twenty-five grammes (25.0 g) of the dry substrates were weighed into each jam bottle and 70 ml distilled water were added. The bottles were immediately covered with aluminum foil. Fresh weight of the bottles were determined and the bottles were sterilized in the autoclave at 121 °C for 15 min. Each treatment was replicated 3 times.

3.2. Inoculation

Each bottle was inoculated at the center of the substrate with 2, 10.0 mm mycelial discs and covered immediately. They were kept in a clean dark cupboard in the laboratory at 30 °C and 100%RH. The controls were dried at 100 °C for 48 h to determine initial dry weight of the substrate. After 30, 60 and 90 days of incubation, the experimented bottles were harvested dried at 100 °C for 48 h and weighed for dry weight determination.

4. Digestibility test

In vivo digestibility test was carried out at International Livestock Research Institute (ILRI) of the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

The degraded wood wastes (spent composts) were ground to about 1 mm square particle size. Three grammes (3.0 g) of the ground sample were added into a nylon bag of a mess of about 40 μm square and 50 mm × 130 mm size and weighed. Samples were introduced into the rumen of 3 Ndama steers adopting the method of Bhargava et al. (1988). They were incubated in the rumen of these animals for 48 h after which the bags were withdrawn and washed thoroughly with cold water to prevent further digestion. They were dried at 60 °C to a constant weight after 48 h. The weight of the residue (undigested sample) was thus obtained.

$$\% \text{ Undigested} = \frac{\text{weight of residue}}{3} \times 100$$

$$\% \text{ Digested} = 100 - \% \text{ undigested}$$

5. Analytical methods

Ph Determination: Four grammes (4.0 g) of substrate were soaked in 80 ml of distilled water for 18 h at 30 ± 2 °C. The pH was determined using microprocessor based Bench pH Mv meter (Hanna Instruments Inc Rhode Island, USA).

Amino nitrogen: Amino nitrogen determination was carried out using the method of AOAC (1995).

Lignin determination: One gram (1.0 g) of the substrate was mixed with 20 ml of freshly prepared 72% H_2SO_4 at 15.20 °C for 2 h. It was later refluxed with 238 ml of distilled water for 4 h. 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 °C to a constant weight in a desiccator and weighed. Percentage lignin was obtained using this formula:

$$\%\text{Lignin} = \frac{\text{Weight of insoluble lignin}}{\text{Oven dried weight of the sample}} \times 100$$

Moisture content: The loss in weight after oven drying fresh samples at 80 °C for 72 h was taken as the moisture content.

Loss in dry matter (LDM): Loss in dry matter (LDM) was obtained using the formula below:

$$\%\mathrm{LDM} = \frac{\text{Initial dry weight of substrate} - \text{dry weight of spent substrate}}{\text{Initial dry weight}} \times 100$$

Statistical analysis: The data obtained were subjected to analysis of variance (ANOVA) and tests of significance were carried out using Pearson chi-square on SPSS computer package.

6. Results

All the substrates used in this study were found to enhance vegetative growth of *P. tuber-regium* (Table 1). The best mycelial growth was observed on the wood wastes of *Holoptelia grandis* followed in order by *Milicia exelsa* and *Terminalia superba* ($P \leqslant 0.05$). The growth of this white rot fungus caused decrease in the pH of the wastes (Table 2). The pH of *T. superba*, which was initially 6.2 dropped to 4.2 after 90 days of incubation. This change was observed in the other substrates with pH values reduced to 4.6 and 4.0. In *H. grandis* and *T. superba*, the pH values decreased as the incubation period increased but for *M. altissima* and *M. excelsa* there was no change in pH after 30 days of incubation.

Generally, it was observed that the amount of nitrogen in the substrates increased with incubation time (Table 2). The greatest amount of nitrogen was found in sawdust of *M. altissima* (4.83 mg) followed by *T. superba* (4.27 mg). Although there was amino nitrogen accumulation in all the substrates with time, there were however no statistically difference in the nitrogen contents of the different substrates used after 90 days ($P \leqslant 0.05$). During the fermentation of the wood wastes by *P. tuber-regium*, water loss occurred. The amount of water lost from the substrates was also observed to increase as the incubation period increased (Table 2).

As the fungus degraded the wood wastes, lignin content decreased as the incubation period increased (Table 3). At zero day, *M. altissima* had the highest lignin content (87.67 g), which reduced significantly to 41.00 g after 90 days. The greatest lignin reduction was noticed in *H. grandis* followed in order by *T. superba, M. altissima* and *M. excelsa* ($P \leqslant 0.05$).

The digestibility of the spent substrates increased with time in all the substrates except in *H. grandis* that had the highest dry matter degradability value after 30 days and decreased thereafter at 60 days (Table 4). There was no significance in the values obtained for digestibility of the substrates as the incubation period increased.

Table 1
Mycelial growth of *P. tuber-regium* on wood wastes of some selected Nigerian economic trees

Substrates	Mycelial extension (cm)	Mycelia density
T. superba	6.2 ± 0.01c	1^{+}
H. grandis	8.4 ± 0.6a	2^{+}
M. excelsa	6.7 ± 0.3b	3^{+}
M. altissima	5.3 ± 0.2d	4^{+}

Each value is the mean of 3 readings ± SE taken over a period of 7 days. Values followed by the same letter(s) are not significantly different by Duncan's multiple range test ($P \leqslant 0.05$).

Table 2
Amino nitrogen, water loss and pH changes during fermentation of wood wastes by *Pleurotus tuber-regium*

Substrate	Incubation period (days)	Amino nitrogen (mg)	Water loss (g)	pH values
H. grandis	0	2.24 ± 0.6c	66.56 ± 0.1	5.1 ± 0.1b
	30	3.15 ± 2.0b	70.00 ± 0.3	4.4 ± 1.7cd
	60	3.25 ± 1.5b	81. 67 ± 4.8	4.1 ± 0.1d
	90	4.20 ± 1.3a	94.40 ± 0.4	4.0 ± 0.1d
M. altissima	0	2.24 ± 2.3c	48.12 ± 2.9	5.2 ± 0.1b
	30	3.39 ± 1.6b	70.80 ± 0.3	4.0 ± 0.1d
	60	4.27 ± 2.1a	73.67 ± 0.2	4.0 ± 0.1d
	90	4.83 ± 2.6a	76.17 ± 0.2	4.0 ± 0.1d
M. excelsa	0	2.38 ± 0.9c	67.83 ± 0.9	6.3 ± 0.1a
	30	3.96 ± 1.0a	71.47 ± 2.5	4.0 ± 0.5d
	60	4.12 ± 1.5a	73.31 ± 0.7	4.0 ± 0.1d
	90	4.24 ± 0.7a	75.29 ± 1.4	4.0 ± 0.1d
T. superba	0	1.47 ± 2.2d	66.27 ± 0.1	6.2 ± 0.1a
	30	1.56 ± 0.8d	69.17 ± 0.1	4.6 ± 0.1c
	60	2.96 ± 2.3b	73.31 ± 0.7	4.3 ± 0.1cd
	90	4.27 ± 2.6a	74.30 ± 0.1	4.2 ± 0.1cd

Each value is the mean of 3 readings ± SE taken during the period of growth. Values followed by the same letter(s) along each column are not significantly different by Duncan's multiple range test ($P \leqslant 0.05$).

Table 3
Effect of fungal growth on the lignin content of wood wastes of selected Nigerian economic trees

Substrate	Incubation period (days)	Lignin value (g)	Lignin reduction
H. grandis	0	83.33 ± 7.1a	59.33
	30	81.33 ± 1.3a	
	60	28.33 ± 8.3de	
	90	24.00 ± 3.1c	
M. altissima	0	87.67 ± 5.4a	46.67
	30	57.67 ± 1.5b	
	60	55.67 ± 2.3b	
	90	41.00 ± 3.7c	
M. excelsa	0	81.67 ± 6.0a	35.17
	30	79.00 ± 3.0a	
	60	74.67 ± 2.3c	
	90	46.50 ± 2.3c	
T. superba	0	85.00 ± 1.5a	48.33
	30	55.00 ± 6.5b	
	60	44.33 ± 7.1c	
	90	35.67 ± 7.8d	

Each value is the mean of 3 readings ± SE taking during the period of growth. Values followed by the same letter(s) are not significantly different by Duncan's multiple range test ($P \leqslant 0.05$).

Table 4
Dry matter degradability of the spent substrates by ruminants after fermentation by *P. tuber-regium*

Substrate	Incubation period (days)	% Deg.
H. grandis	0	18.36 ± 9.5a
	30	21.70 ± 4.5a
	60	20.60 ± 3.0a
	90	20.46 ± 0.6a
M. altissima	0	15.91 ± 1.0ab
	30	16.71 ± 2.8ab
	60	24.41 ± 1.0a
	90	27.98 ± 1.4a
M. excelsa	0	15.85 ± 2.9ab
	30	19.63 ± 6.8a
	60	21.61 ± 2.1a
	90	29.96 ± 2.2a
T. superba	0	10.84 ± 0.1b
	30	10.53 ± 0.9b
	60	18.06 ± 1.5ab
	90	22.08 ± 1.5a

Each value is the mean of 3 readings ± SE taken during the period of growth. Values followed by the same letter(s) are not significantly different by Duncan's multiple range test ($P \leqslant 0.05$).

7. Discussion

In this study, all the fresh wood wastes investigated were found to support the growth of *P. tuber-regium*. This result is in agreement with findings of Jandaik (1974), Chang (1980) and Fasidi and Ekuere (1993) that *Pleurotus* species as a group of basidiomycetes have high saprophytic ability to grow on variety of agro industrial wastes. The ability of this fungus to flourish on different wastes may be linked to its ability to secrete hydrolyzing and oxidizing enzymes, which could aid the decomposition of recalcitrant compounds in the wastes into utilizable compounds (Kadiri, 1990; Brau et al., 2000; Jonathan, 2002). The rapid colonization of *P. tuber-regium* mycelia on selective substrates such as wood wastes of *H. grandis* and *M. excelsa* as observed in this study will considerably reduce the growth of other competitive microorganisms thereby reducing spawn contamination.

Likewise, the sawdust of *H. grandis, M. excelsa, M. altissima* and *T. superba*, which are nuisance in our environment, could be successfully utilized as substrates for cultivation of *P. tuber-regium* and other Nigerian edible mushrooms. The result of this study also reveals that composting of agricultural substrates for the growth of *P. tuber-regium* is not necessary since luxuriant growth of mycelial was obtained in uncomposted wood wastes. The change in pH value of the different substrates as the incubation period increased may be linked with the increase in amino nitrogen content and the presences of metabolic waste products within the substrates. Similar pH changes were observed by Jonathan et al. (2004) for the growth of *V. esculenta* in submerged medium. The increase in amino nitrogen content may be due to hydrolysis of protein within the substrates.

Digestibility of lignocellulosics has been known to be connected with lignin content (Zadrazil, 1982, 1985). As it could be seen from this study, *P. tuber-regium* did not only degrade lignin with increased incubation period, but also improved digestibility of substrates by ruminants. This observation is similar to that observed by Zadrazil (1985). The digestibility of wood wastes by *P. tuber-regium* as obtained in this study was low as compared with that of Zadrazil (1985). This could be due to the fact that digestibility depends on fungal species, substrates and incubation period. There is a need to screen other Nigerian basidiomycetes for their lignin decomposing abilities using many readily available agro industrial wastes.

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